United States Department of the Interior

BUREAU OF LAND MANAGEMENT
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In Reply Refer To:
3720 (AKA000)
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Dear Reader:

As many of you are aware, the Bureau of Land Management (BLM) has collected water, sediment and fish tissue data along the middle section of the Kuskokwim River to better understand concentrations of metals in fish that live in the river year round.

The attached interim report "Mercury, Arsenic, and Antimony in Aquatic Biota from the Middle Kuskokwim River Region, Alaska, 2010-2011" reflects the efforts of multiple agencies over a 3-year period and encompasses study of the Kuskokwim River from McGrath to Aniak and numerous tributaries. Assistance from the Alaska Department of Fish and Game (ADF&G), as well as the U.S. Fish and Wildlife Service (USFWS), was essential during the design and implementation of the study. The people living along the river were also key to the study, since they provided invaluable assistance on where and when to collect fish samples for the species being targeted. The USFWS, which has extensive expertise in analyzing contaminant data, prepared the interim report for the BLM.

The natural geology of the region includes numerous mineralized areas that contain roughly 69 percent of all the known mercury deposits in Alaska. Those same mineral deposits also contain other metals, including arsenic and antimony. Other fish contaminant studies in western Alaska since the mid-1990s have shown measurable concentrations of mercury in predator fish species in both the Kuskokwim and Yukon Rivers. Based on previous research, the BLM focused this study on metals, emphasizing mercury because it is shown to accumulate in larger predatory fish that are an important subsistence resource.

Since 2010, the BLM and ADF&G have collected tissue samples of over 1,200 fish from the Kuskokwim River and 17 of its tributaries, covering roughly 730 miles of stream. Of those sampled fish, nearly 570 were tagged with radio transmitters, and their movements will be tracked over the next 1-2 years. The tracking data will show the amount of time fish spend in specific areas of the Kuskokwim Basin and allow us to identify areas that may contribute to the elevated metals in fish within the study area.

This interim report summarizes the mercury, arsenic, and antimony concentrations in fish tissue collected in 2010 and 2011 and our analysis of those results. This study found the greatest concentrations of mercury in pike were associated with the George and Holitna Rivers, two major tributaries of the Kuskokwim River but outside the influence of the Red Devil Mine. Although the next report will integrate the fish tracking results, preliminary data indicate that adult pike within these two rivers seldom move into the Kuskokwim River; therefore, their mercury levels appear to be a direct result of mercury exposure in those watersheds and not from other sources in the Kuskokwim River.

In the Holitna River, one potential source of mercury could be Cinnabar Creek. The study found that fish from Cinnabar Creek and Red Devil Creek had elevated concentrations of mercury and arsenic compared to small fish from tributaries where no mining has occurred. Detecting elevated mercury concentrations in small fish near streams with adjacent abandoned mines was expected based on past studies from the Kuskokwim and Yukon Rivers. Arsenic levels were high in small fish collected near mined areas as expected based on the local geology. The proportion of arsenic that was inorganic, the more toxic form of arsenic, was much higher than typically observed in fish. Inorganic arsenic is usually less than 10 percent of the total arsenic found in fish, yet samples from Cinnabar and Red Devil Creeks averaged 70 and 66 percent respectively.

Prior to this study, fish consumption guidance to limit mercury exposure was issued by the State of Alaska for pike in the lower Kuskokwim and lower Yukon. Information from BLM's study led the State of Alaska to issue guidance in June 2011 for the middle Kuskokwim, which included burbot (lush) and pike. Fish consumption guidance for the Kuskokwim region can be found at: http://www.epi.alaska.gov/eh/fish.

The results of this study illustrate that many natural mercury deposits and historic mine sites are adding mercury to the Kuskokwim watershed, occurring within a highly mineralized region of western Alaska. The BLM plans to present results from this fish study in February at the Alaska Forum on the Environment. In addition, the data included in the interim report will be made available to the State of Alaska, the U.S. Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry for their consideration regarding future guidance on fish consumption in the Kuskokwim River watershed.

For more information on the fish tissue study and the Red Devil Mine please go the project website at: http://www.blm.gov/ak/fisheries/rdm_fish.html. Contact Matt Varner, Fisheries Biologist, at 907-271-3348 for additional information.

Sincerely,

Karen Kelleher

Karen Kelleher
District Manager

Enclosure: Interim Report, "Mercury, Arsenic, and Antimony in Aquatic Biota from the Middle Kuskokwim River Region, Alaska, 2010-2011"

Mercury, Arsenic, and Antimony in Aquatic Biota from the Middle Kuskokwim River Region, Alaska, 2010-2011

Interim Report

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Executive Summary

This report summarizes results and interpretation of mercury, arsenic and antimony concentrations in fish and aquatic insects collected in the Kuskokwim River and its tributaries between the Aniak and Stony rivers.

The middle Kuskokwim River runs through a highly mineralized region of Alaska that contains mercury, antimony, gold, silver, and polymetallic deposits (Szumigala and Swainbank 1998). This area is referred to as Alaska's "mercury belt" because of the numerous mercury mineral deposits and mines in the watershed (Gray et al. 1994, 2000). The potential for transfer of metals and other trace elements, particularly mercury, to fish or the environment through erosion from mined and unmined areas along the Kuskokwim River has been extensively studied (Jewett and Duffy 2007; Jewett et al. 2003; Zhang et al. 2001; Gray et al. 2000, 1994; Duffy et al. 1999; U.S. Fish and Wildlife Service, unpubl. data). These studies focus on mercury because of global human health concerns over mercury levels in fish, and in particular reliance on fish in subsistence diets (e.g. Jewett and Duffy 2007). Arsenic and antimony, other elements associated with mercury deposits, can also pose human health risks.

Fish and aquatic insects were collected in the mainstem Kuskokwim and large tributaries (Rivers) and in small tributaries (Tributaries). A large study area was selected to help ensure that spatial differences in mercury concentrations could be detected if present, with individual sampling areas delineated by major hydrological changes (e.g., the confluence of Holitna and Kuskokwim Rivers) and possible mercury point sources (e.g., abandoned mines). Most sampled fish were those favored by subsistence users; other fish and insects were sampled since they represent different places on the food chain. Adult salmon were not sampled because they spend most of their lives at sea. All samples were analyzed for total mercury, total arsenic, and antimony. A proportion of samples were tested more rigorously for methyl mercury and inorganic arsenic, toxic forms that are proportions of total mercury and total arsenic, respectively.

Small, sedentary fish (slimy sculpin, juvenile Dolly Varden and juvenile Arctic grayling) and insects from Red Devil and Cinnabar Creeks had significantly greater mercury concentrations than the same fish in other Tributaries. Northern pike, burbot (lush), and Arctic grayling collected in Rivers had variable mercury levels across the area. Northern pike from the George River had significantly higher mercury concentrations compared to other pike. There were no spatial differences in mercury concentrations in sheefish, which are anadromous in the study area but unlike salmon do not die after one spawning event. Total mercury concentrations in sampled fish muscle were compared to known harmful levels for fish. In Tributaries, long-nosed sucker, juvenile Arctic grayling, and adult Dolly Varden had average total mercury concentrations below harmful levels. However, concentrations in juvenile Dolly Varden and slimy sculpin from Red Devil and Cinnabar Creeks exceeded harmful levels. Also, all fish (northern pike, burbot, adult Arctic grayling, adult Dolly Varden, and sheefish) sampled in Rivers had levels of mercury that could be considered harmful to them.

Total arsenic and antimony concentrations were higher in fish and insects collected from Red Devil Creek compared to all other Tributaries. However, the levels varied considerably among Rivers. Insects had higher arsenic and antimony concentrations compared to fish, but were not tested for inorganic arsenic. Of the fish sampled, slimy sculpin had the highest inorganic arsenic levels.

Known elevated mercury concentrations in subsistence fish species in the middle Kuskokwim River warranted consumption guidance (http://www.epi.alaska.gov/eh/fish/default.htm) under the State of Alaska's guidelines. The State of Alaska has no consumption guidance for arsenic or antimony in fish (Verbrugge 2007).

A high proportion (>90%) of arsenic found in fish has been assumed to be less toxic organic forms, e.g. arsenobetaine or "fish arsenic" (ATSDR 2007). However, in the small number of fish samples tested for this study, less than 50% of the total arsenic was organic (although the data were highly variable). Should these fish be used for human consumption, the proportion of inorganic arsenic would be of concern.

Results to date indicate that while there is a measurable and biologically significant elevation of mercury and to a lesser extent arsenic in fish and insects in Red Devil Creek, similar levels are found near other abandoned mines in the middle Kuskokwim River watershed. These other areas include Cinnabar Creek, which is on land managed by the State of Alaska in the upper Holitna Drainage, and the George River. Ongoing data collection and analyses for this study include fish movement studies on northern pike and burbot, and analysis of watershed mercury methylation potential.

Introduction

The Kuskokwim River (Fig. 1) arises in mountains and rivers of Interior Alaska, between the Kuskokwim Mountains and the Alaska Range. It flows west-southwest through Southwest Alaska for approximately 700 miles (11,130 km), discharging waters from its 48,000 sq mi (124,319 km²) watershed into Kuskokwim Bay. This river, with diverse populations of wildlife and resident and anadromous fish, has supported a subsistence way of life for Yupik and Athabascan people for generations.

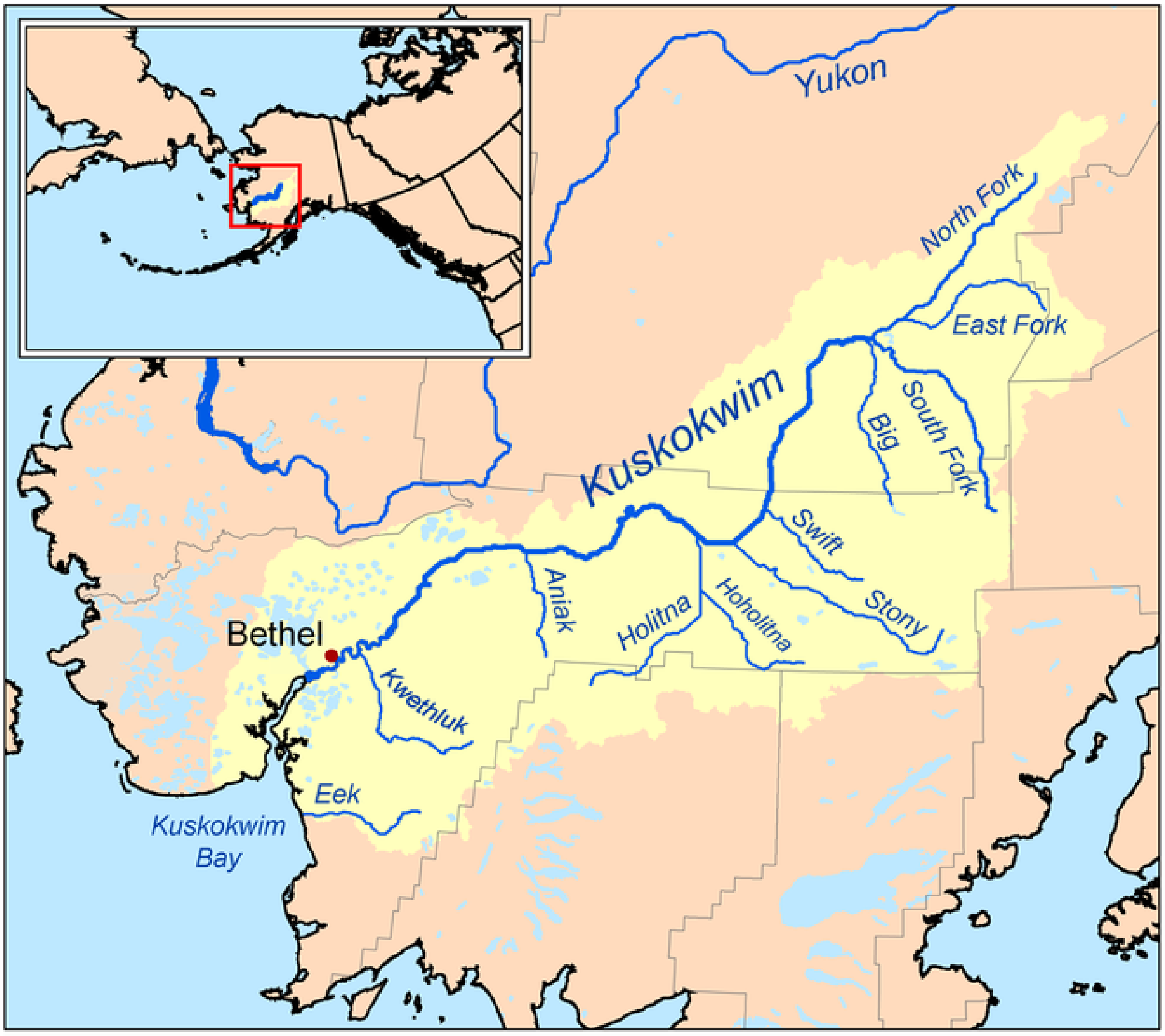

Figure 1. Kuskokwim River Watershed, Alaska, USA.

The middle Kuskokwim River runs through a highly mineralized region of Alaska that contains mercury (Hg), antimony (Sb), gold (Au), silver (Ag), and polymetallic deposits (Szumigala and Swainbank 1998, USGS 2005). Remnant waste rock and processed ore from early and mid-20th century mines are still present. Cinnabar, which contains mercury, has been found at 332 locations in Alaska (USGS 2005), 228 of which are in the Yukon-Kuskokwim watershed, and 82 of which have been mined. Many cinnabar deposits are associated with gold and other precious metals. Elemental mercury, or quicksilver, was produced from eight mines at five locations along the Kuskokwim River, including the Alice and Bessie (or Parks), Kolmakof , Cinnabar Creek, Lucky Day, and Red Devil mines. The Red Devil mine, abandoned in 1971, was the largest, but Cinnabar Creek also produced substantial amounts of mercury (Sainsbury and MacKevett 1965).

Trace elements, including metals, from uncontrolled mine wastes or exposed mineral deposits can readily transfer to the environment downstream of mines through simple physical release of sediments into flowing waters (erosion) (Hoffman et al. 2003), or through chemical processes such as acidification of sulfide minerals, which liberates metal from geologic materials (Forstner and Wittman 1983). The latter is likely not the case on the Kuskokwim River. Neutral to slightly alkaline pH levels, the insolubility of the dominant mercury mineral cinnabar (HgS), and the lack of sulfide-producing minerals led Gray et al. (1994, 2000) to conclude that there was low potential for acid mine drainage downstream of mercury mines and mineral deposits on the Kuskokwim.

Nationwide concern over Hg in fish as an exposure pathway for humans, high fish consumption rates of Alaskan subsistence consumers (e.g. Jewett and Duffy 2007), and the presence of mercury mineral deposits and mines on the Kuskokwim River and its tributaries (e.g. Gray et al. 1994, 2000) have fueled studies of Hg in fish in Alaska (Gray et al. 1994, 2000; Duffy et al. 1999; Jewett et al. 2003; Jewett and Duffy 2007; Zhang et al. 2001, U.S. Fish and Wildlife Service, unpubl. data). Gray et al. (2000) sampled sediments, water, and fish tissue upstream and downstream of three abandoned mercury mines in southwest Alaska (Red Devil and Cinnabar Creek in the Kuskokwim River watershed and Red Top on the Wood River near Dillingham). They found that total Hg (THg) was greater in sediments downstream from mines compared to reference reaches, and that methyl mercury (MeHg; a toxic form) comprised less than one percent of the THg (except for one site at the mouth of a beaver dam complex on Cinnabar Creek). By comparing Hg in filtered and unfiltered water, they determined that most of the Hg downstream of mines is suspended or particulate, not dissolved (Gray et al. 2000). Although these authors hypothesized that Hg from the mines would be diluted downstream, Dolly Varden and Arctic Grayling collected downstream from mines had much greater Hg concentrations (up to 620 and 420 ng/g ww in muscle) compared to fish from regional baseline streams (200 ng/g) (Gray et al. 2000). Gray et al. (2000, pg. 32) concluded, “elevated mercury concentrations in freshwater fish collected near the mines indicate that some biologically available mercury is taken up by the fish….through food sources or particles suspended in the water.”

Arsenic (As) and antimony (Sb) have not been studied as much as mercury (Gray et al. 1994, 2000; Duffy et al. 1999; Jewett et al. 2003; Jewett and Duffy 2007; Zhang et al. 2001), but both are ubiquitous and toxic substances that are found in elevated concentrations in mineralized areas such as the Kuskokwim, and downstream of active and abandoned mines (Eisler 1988). The amount of total As that ends up in aquatic biota depends on exposure, accumulation, and excretion rates and mechanisms. Organisms can be exposed through water or food uptake, but unlike Hg, As and Sb do not biomagnify up food chains, and their behavior in aquatic systems and organisms is transient and dynamic (Eisler 1988).

Mercury dynamics are far more complex, with a combination of factors determining the amount and chemical form of Hg that ends up in fish, including Hg sources, fish life history characteristics, and methylation rates. Mercury is delivered to Alaska's aquatic environment via atmospheric deposition from global, regional, or local sources, such as combustion of fossil fuels upwind of Alaska (e.g., coal-fired power plants in Asia); volcanoes and forest fires (Kelly et al. 2006); mining (which includes release of Hg from sediments or bedrock and use of Hg amalgam in gold mining); and geologic erosion. Each of these has spatially and temporally variable contributions to the total amount of Hg that enters Alaska's waters. However, proportional contributions of any of these sources are difficult to determine (Lindberg et al. 2007), especially when there are multiple potential point sources within a region or watershed.

Since most Hg in fish muscle is MeHg, the methylating potential of the watershed is also important. Although Hg can occur as elemental (liquid) Hg and mineral complexes (cinnabar) in the ground, these inorganic forms of Hg can be converted to toxic organic complexes (e.g., MeHg) by sulfate-reducing anaerobic bacteria in lake and river sediments. Greater anaerobic sediment volumes (e.g. behind dams or in surrounding wetlands) result in more methylating bacteria and greater concentrations of Hg – as MeHg – in fish, assuming other factors are equal.

Fish are exposed to MeHg primarily through their diet (Weiner et al. 2003). Methyl mercury is not easily excreted, and builds up in food chains (Fig. 2). Older fish often have greater concentrations than younger fish of the same species in the same area (Evans et al. 2005), because MeHg – unlike inorganic Hg - bioaccumulates within individuals. Other life history characteristics that affect Hg concentrations in fish (THg and MeHg) include range size. Non-migratory fish with small ranges living near an Hg-containing material that is in contact with the water – a source - will have greater concentrations compared to migratory fish with larger ranges, all else being equal. Aquatic biota in Kuskokwim River region have a wide variety of range sizes and migratory habits (Fig. 3).

However, exposure through gills or digestive tissue to waterborne, non-organic, particulate-bound Hg (such as from an eroding mine site) can also cause elevated inorganic Hg concentrations in fish, as hypothesized by Gray et al. (2000). The ratio of MeHg to THg (MeHg:THg)[1] may distinguish between acute exposure to inorganic mercury, absorbed through the gills or digestive tissues, compared to chronic exposure to organic mercury through the

[1] Where THg = MeHg + inorganic Hg.

diet, where most of the total mercury is methylmercury. Said another way, in fish, a MeHg:THg ratio much less than 1.0 can indicate acute, short-term exposure to Hg in water (Guilherme et al. 2010), while one closer to 1.0 can indicate chronic, longer-term exposure to Hg via ingestion of MeHg from prey (Weiner et al. 2003, Lasorsa and Allen-Gil 1995).

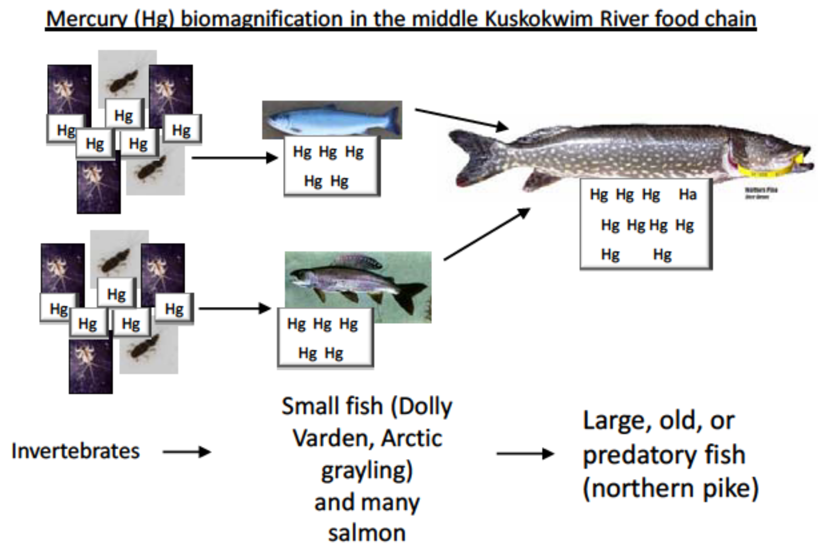

Figure 2. Generalized schematic for mercury biomagnification in the aquatic food chain of the middle Kuskokwim River, Alaska.

Sedentary, Resident → Migratory

invertebrates < slimy sculpin < resident Dolly Varden < Arctic grayling < pike < burbot < sheefish

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Figure 3. Relative migratory propensity of aquatic biota measured for mercury in the middle Kuskokwim River, Alaska. Propensity ranges from completely sedentary (macroinvertebrates) to estuarine anadromous (sheefish).

This document contains results and interpretation of Hg, As, and Sb concentrations in aquatic biota, incorporating residency, trophic status, and distance and direction from the abandoned mercury mine at Red Devil Creek (the site of a BLM environmental investigation) and other prospects and mine sites. This report is one chapter of joint reporting by the BLM and U.S. Fish and Wildlife Service (USFWS) on the chemical and statistical analysis of metal contaminants data from fish and macroinvertebrates collected in the mid- and lower Kuskokwim River.

Objectives of the larger BLM study include (from Varner, 2012):

- Sampling surface waters and streambed sediments for contaminants, including total mercury and methyl mercury (the most toxic form of mercury), in the mainstem Kuskokwim and within selected watersheds in the project area;
- Sampling all trophic levels from benthic macroinvertebrates to top-predatory fish species;
- Sampling resident fish species most often harvested by local people;
- Estimating tributary fish community composition;
- Analyzing tributary watershed health using metric analysis of benthic macroinvertebrates from selected drainages;
- Establishing the relative seasonal distributions of two top-predatory fish species; and
- Evaluating trends in concentrations of multiple metals and fish movement in relation to distance from and downstream location from the Red Devil mine and other mercury mine sites.

Methods

Study Area

The study area is described in depth by Varner (2012) and sample sites are shown in Figs. 4-6. Briefly, the 2010 sampling area included a 117 km section of the Kuskokwim River from the confluence of the George River upstream to the confluence of the Stony River, with approximately 73 km of tributaries. In 2011, the study area was expanded to include the Kuskokwim River from Aniak to just upstream of McGrath, an approximately 410 km span.

Sample Collection

Fish were collected from the Kuskokwim River and large and small tributaries as outlined in the Final Operations Plan (Varner 2010). Tissues were dissected following U.S. Geological Survey (USGS) procedures (Scudder et al. 2008) for collecting fish tissues for Hg analysis, except that all dissections were completed in a lab, not in the field. Field processing was limited to species identification, body measurements, and washing, tagging, bagging, and freezing the carcasses. Fish age was determined by otolith analysis.

Samples reaches were chosen to reflect different habitats and fish harvest locations in the study area, which was defined based upon work by John Gray (USGS) (Gray et al. 1994, 2000). Cinnabar Creek and nearly all wadeable, perennial streams between Sleetmute and Crooked Creek were sampled for invertebrates and smaller fish ("Tributary" sample reaches; Fig. 4). The mouths of slough-like and low-gradient tributaries in the mainstem Kuskokwim, and some larger tributaries ("River" sample reaches; Fig. 5) were sampled because they were good habitat for some target species (northern pike *Esox lucius* and burbot (also known as lush) (*Lota lota*). Watershed characteristics of each sampling reach are summarized by Varner (2012). "Tributary" sample reaches were further defined as the first 300 meters of the creek above the main river high water mark (Fig. 4). "River" sample reaches were delineated by hydrologic features, including large tributaries (Fig. 5). In 2011, additional non-lethal samples were collected from pike and burbot (lush) that were radio-tagged for telemetry (Fig. 6).

There were several separate collection events during 2010 and 2011 (Table 1). Target species were selected because local people subsist on them, or because they represented different trophic levels and spatial range sizes. River species included Arctic grayling *Thymallus arcticus*, burbot, northern pike, and sheefish or inconnu *Stenodus leucichthys.* Muscle and liver were sampled in River fish because subsistence users may eat these tissues separately, and the collected fish were individually large enough to provide sufficient sample volume from each tissue. Tributary species included slimy sculpin *Cottus cognatus*, juvenile Dolly Varden *Salvelinus malma*, juvenile Arctic grayling, and macroinvertebrates. These smaller Tributary fish were analyzed as whole body or composite whole body samples; separating tissues may have reduced sample volumes below amounts usable for analytical chemistry.

We did not target adult spawning salmon because their muscle trace element concentrations would primarily reflect ocean exposure rather than freshwater. A very few juvenile coho salmon *Oncorhynchus kisutch* and Chinook salmon *O. tshawytscha* were sampled. Other species sampled in very low numbers included long-nosed sucker *Catastomus catastomus* and humpback whitefish *Coregonus pidschian;* data for these species and the juvenile salmon are reported anecdotally.

Table 1. Sampling events associated with a BLM environmental investigation of the abandoned Red Devil mercury mine in the mid-Kuskokwim River watershed, 2010-2011.

Date	Purpose	Species Collected	Locations
June – Oct., 2010	General sampling event in most tributary and all river reaches	All	All except Cinnabar and Egnaty Cks.
Nov., 2010 – Jan., 2011	Characterize mercury in winter burbot subsistence fishery	Burbot (n=54)	Kuskokwim R., Reaches A and C
June, 2011	Establish seasonal movement patterns; non-lethal muscle biopsies from radio-tagged fish	Burbot (n=118) Pike (n=160)	George R. Holitna/Hoholitna R. Kuskokwim R.
June – Sept., 2011	Characterize mercury concentrations in small fish from additional tributaries, including one with a former mercury mine	Arctic grayling Dolly Varden Macroinvertebrates Slimy sculpin	Cinnabar Ck. Egnaty Ck. Red Devil Ck.

Analytical Chemistry

In 2010, samples were analyzed for Hg, As, Sb (and 16 other trace elements) by Physis Environmental Laboratories, Inc., Anaheim, CA (Physis) and for MeHg by Frontier Global Sciences, Inc., Seattle, WA (Frontier). In 2011, samples were analyzed for trace elements, MeHg, and inorganic arsenic by Frontier. Methods followed U.S. Environmental Protection Agency (EPA) methods for analysis of metals including mercury (EPA SW-846 Method 6020m, EPA Method 245.7(m), Frontier SOP FGS-054), MeHg (Frontier Geosciences SOP Method FGS 070), and inorganic arsenic (EPA Method 1632) in tissues. At Physis, Hg Method Detection Limits (MDLs) were 0.00001 µg/g and Reporting Limits (RLs) were 0.02 µg/g; As and Sb MDLs were 0.025 µg/g and RLs were 0.05 µg/g. At Frontier, the MDLs and RLs varied by sample and were reported with each sample.

Data Analysis

Data reports underwent a third-party QA review using EPA Validation Level IV criteria to assure data quality was appropriate for remedial decisions under CERCLA (U.S.E.P.A. 2010) by

Laboratory Data Consultants, Inc. (Carlsbad, CA) for samples analyzed by Physis in 2010, and by Physis for samples analyzed by Frontier in 2011. No data were considered invalid after the QA review, so all were used in subsequent analyses.

General linear models (GLM), similar to Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA), were used to explore patterns and test differences in average (arithmetic mean) tHg, MeHg, total and inorganic As, and Sb concentrations among and between reaches, species, and tissues, with a significance level set at $\alpha = 0.05$. Post-hoc tests (Tukey's or Bonferroni's) were used to determine statistical significance for differences among reaches and species. Groups that had no or few samples (e.g., a particular species was not collected, or collected in low numbers, in a particular reach) were dealt with on an individual basis. Data were often log-transformed to better meet assumptions of these parametric models, although data are always reported as µg/g (equivalent to mg/kg and parts per million, ppm) wet weight (ww). If non-detects (i.e., values less than the MDL) occurred in less than 10% of data, they were substituted with 0.5 times the MDL for descriptive or inferential parametric statistics (since a small number of such substitutions are unlikely to be influential; Gibbons 1994). Non-parametric statistical tests (based on ranks) were used if the analyte was detected in 50-80% of samples.

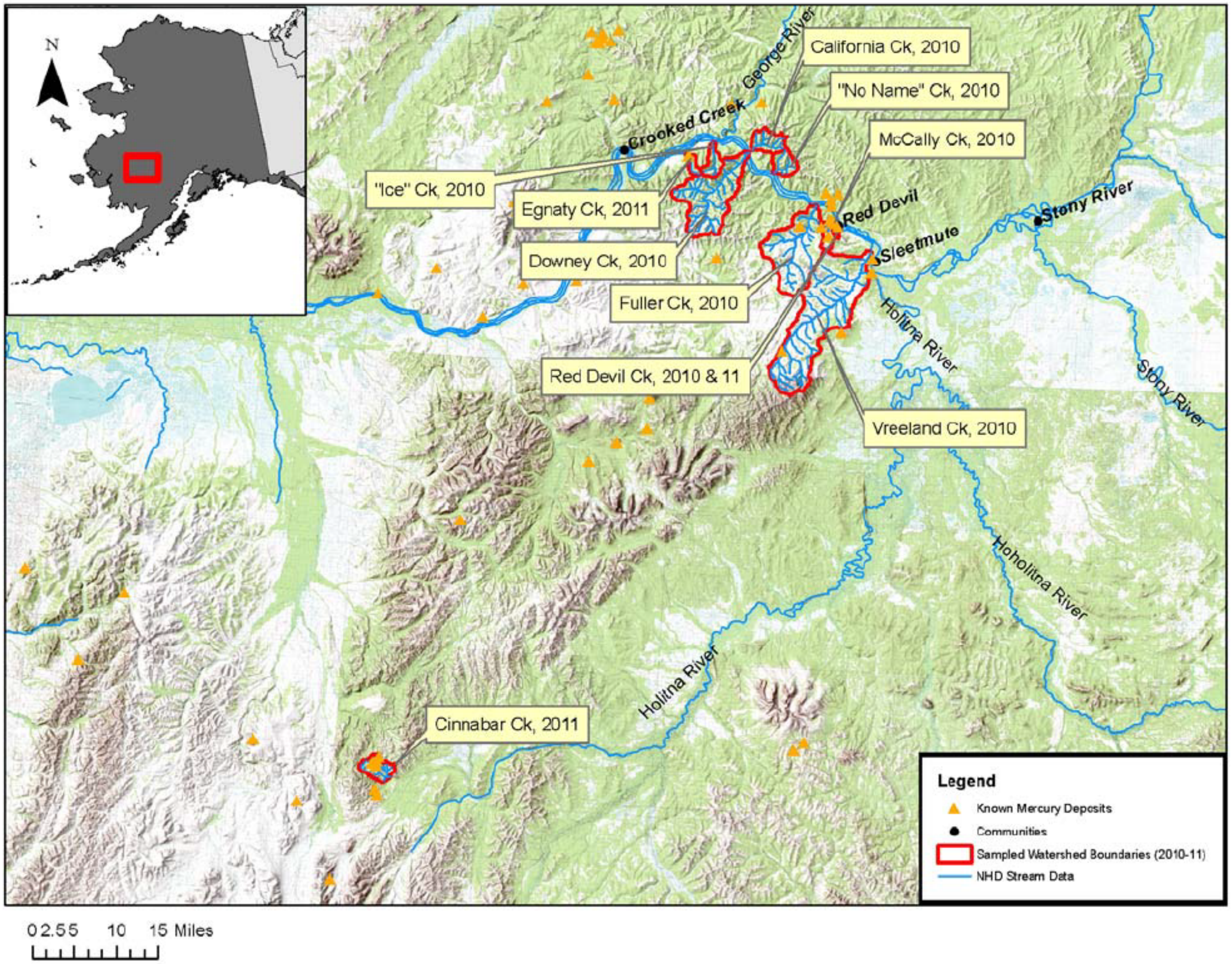

Figure 4. "Tributary" sample reaches for analysis of mercury in aquatic biota from the middle Kuskokwim River, Alaska, in summer, 2010.

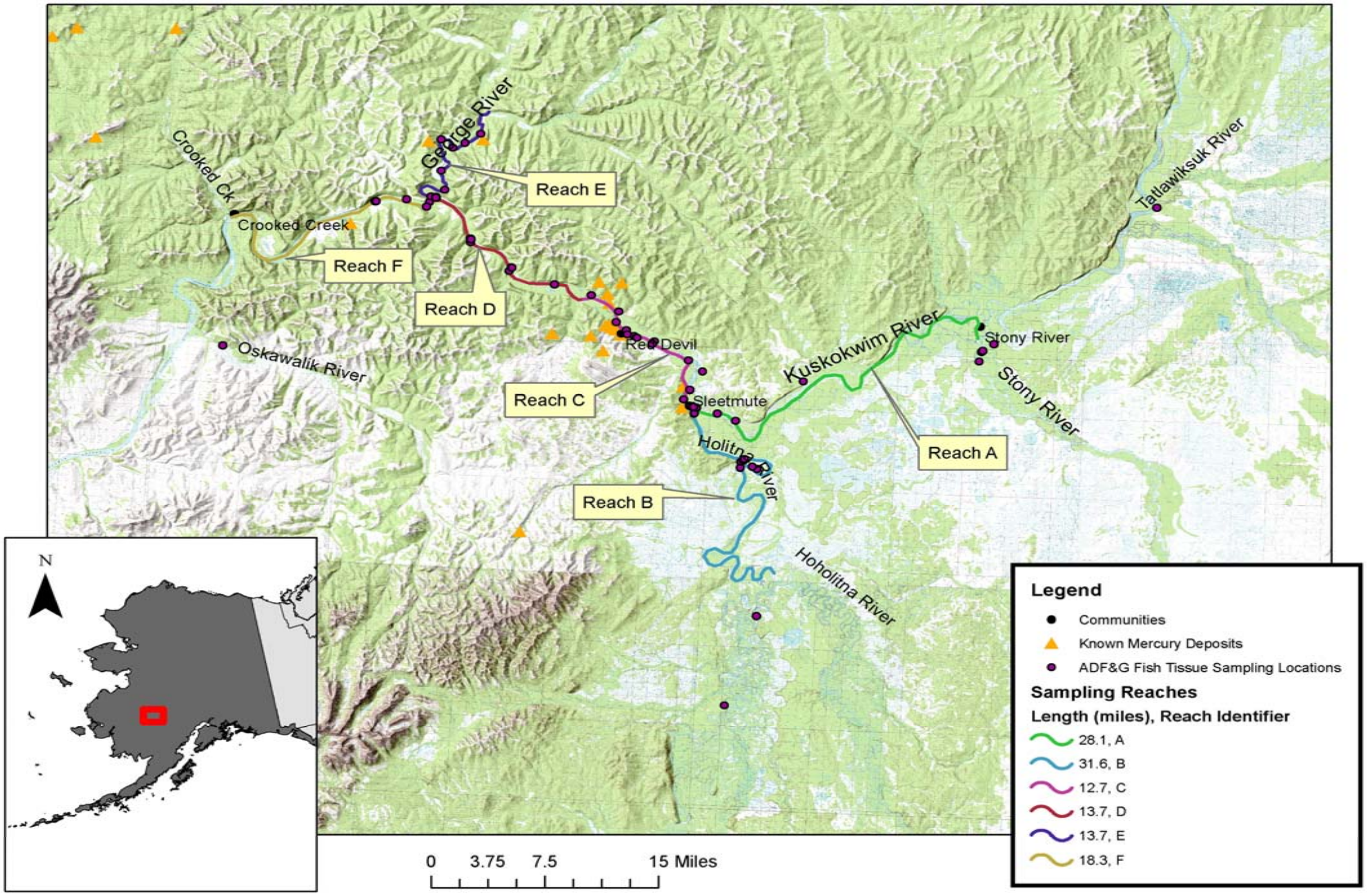

Figure 5. "River" sample reaches for analysis of mercury in aquatic biota from the middle Kuskokwim River, Alaska, in summer, 2010.

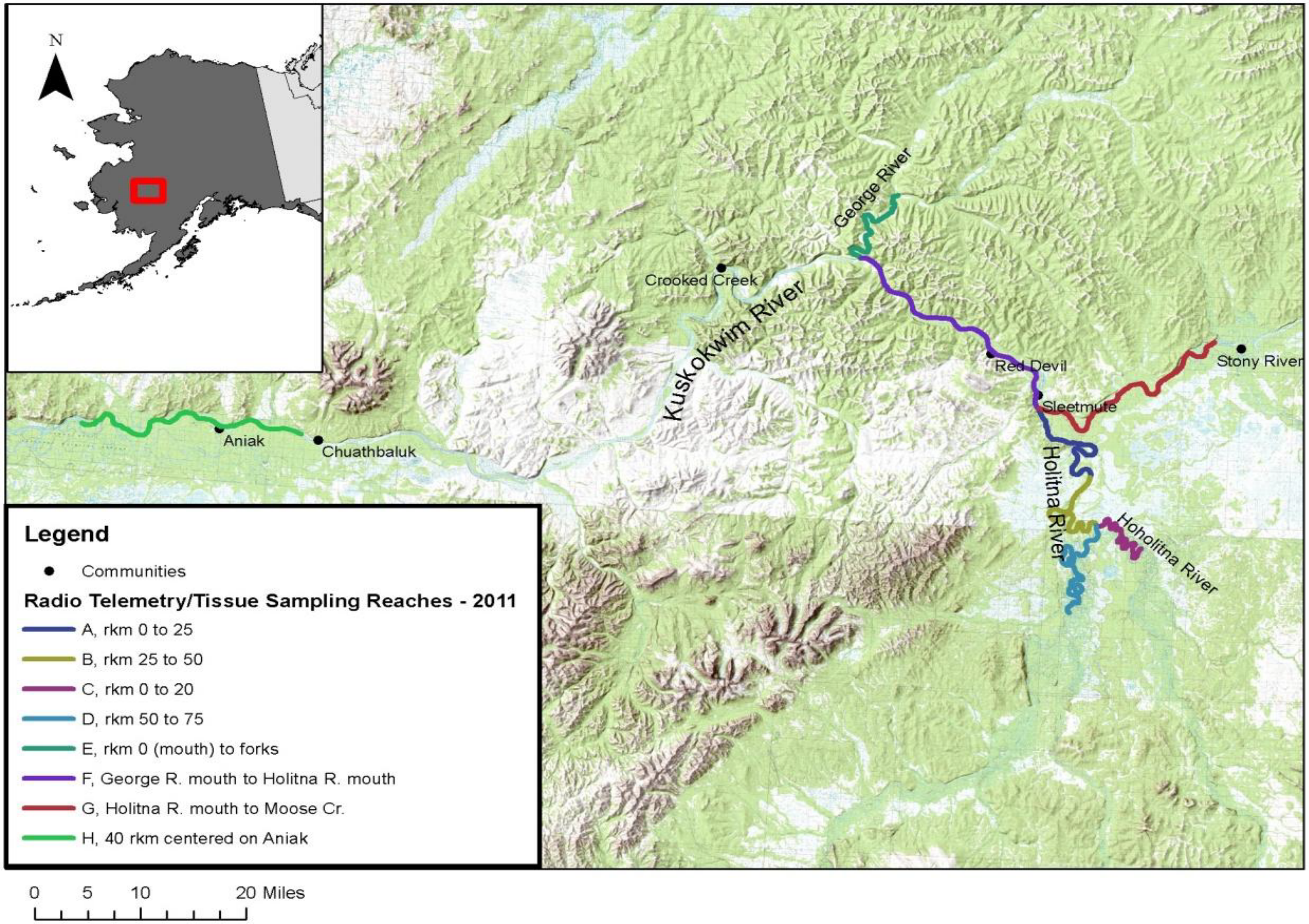

Figure 6. Stream reaches sampled for radio tag deployment and tissue sample collection in northern pike and burbot (lush) in the mid-Kuskokwim River area, Alaska, 2011. Reaches were slightly different from those previously sampled (Fig. 5). Within a subsection, radio telemetry tags and biopsy samples were apportioned among fish length strata.

Results

Statistical tests for differences among sampling sites and among species require similar sample sizes among the sites and species. However, not all species were collected at all sample reaches in both years, and sample sizes varied (Tables 2 and 3), so statistical comparisons were not always among all samples sites and species. Dolly Varden and macroinvertebrate data from tributary reaches in 2010 were grouped into tributaries upriver of Red Devil Creek, Red Devil Creek itself, tributaries between Red Devil Creek and the George River, and downriver of the George. Slimy sculpin, juvenile Dolly Varden, and macroinvertebrate data from 2011 tributary reaches were compared among Red Devil, Cinnabar, and Egaty Creeks.

Table 2. Number of samples collected by Tributary location and species for a study of trace element dynamics in aquatic biota in the mid-Kuskokwim River, Alaska, 2010 – 2011. Reaches are listed from downstream to upstream. Samples were collected in 2010 unless otherwise indicated.

Tributary	Arctic Grayling	Slimy Sculpin	Dolly Varden	Macroinvertebrates
Egnaty Ck. (2011)		13	2	
Ice Ck.	11	24	7	3
Downey Ck.	4	20	7	6
California Ck.	12	23	2	3
NoName Ck .	12	22	2	1
Fuller Ck .	24	22		5
McCally Ck .		10	22	2
Red Devil Ck.		22	11	3
Red Devil Ck. (2011)	2	24	6	4
Vreeland Ck .	1	23	2	6
Cinnabar Ck. (2011)		25	25	4

Table 3. Number of samples collected by River location and species for a study of trace element dynamics in aquatic biota in the mid-Kuskokwim River, Alaska, 2010 – 2011. Reaches are listed from downstream to upstream. Samples were collected in 2010 unless otherwise indicated.

	Arctic Grayling		Burbot		Northern Pike		Sheefish	
River (Reach)	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**	**Muscle**	**Liver**	**Muscle**
Oskwalik R. (F)	6	6						
Kuskokwim R. (F)	7	7	21	21	6	6	3	3
George R. (E)	8	8	17	17	17	17	7	7
Kuskokwim R. (D)	3	3	11	11	32	32		
Kuskokwim R. (C)[1]	1	1	16	16	33	33	4	4
Kuskokwim R. (C) (winter 2011)			4	4				
Holitna R. (B)			2	2	35	34	12	12
Kuskokwim R. (A)			19	19	2	2		
Kuskokwim R. (A) (winter 2011)			50	50				
Stony R. (A)					11	11		
Tatlawiksuk R. (A)							12	12

[1] Red Devil Creek is one of several tributaries that enter the Kuskokwim River in this reach.

Mercury

There were significant differences between Hg concentrations in 2010 and 2011 data sets (tested by comparing slimy sculpin, Dolly Varden, and macroinvertebrate samples collected in Red Devil Creek; $p = 0.001$, 0.004, and < 0.001, respectively), precluding pooling of the annual data sets. 2010 data were consistently greater than 2011 data (Fig. 7). 2010 samples were analyzed at Physis and 2011 samples were analyzed at Frontier, but it is unknown whether the differences are attributable to annual or inter-laboratory variation.

The greatest average THg concentration among all samples pooled by species and tissue type was in burbot muscle, although the greatest individual concentration was in a slimy sculpin whole body sample (Table 4).

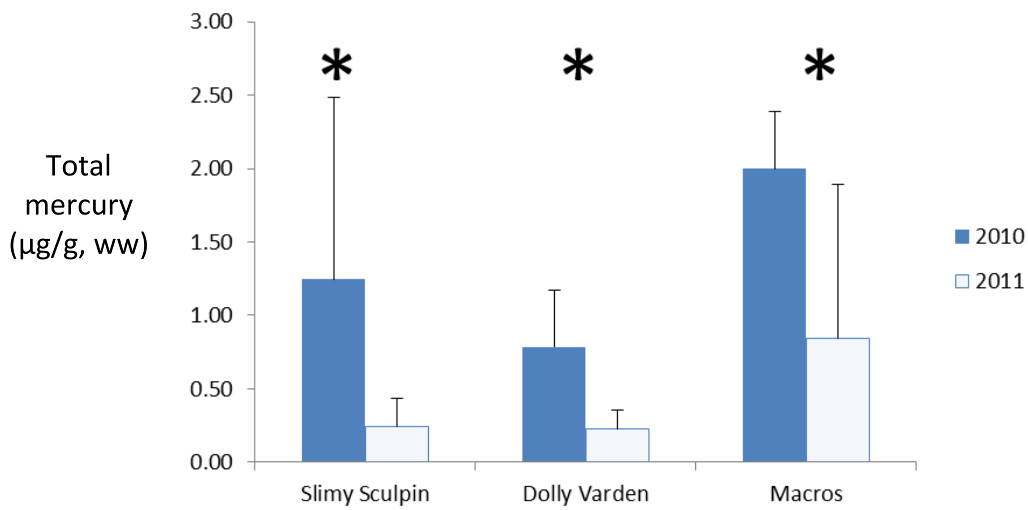

Figure 7. Mean total mercury concentrations (µg/g, ww) (SD) in small fish and macroinvertebrates collected in Red Devil Creek, Alaska, 2010-2011. Asterisks indicate significant differences in mercury concentrations between years; samples were also analyzed at different laboratories each year.

Table 4. Summary statistics for total mercury concentrations (µg/g, ww) in aquatic species sampled in the middle Kuskokwim River region, Alaska, 2010 – 2011.

Species, Age, Year (n)	Year	Tissue	Minimum	Maximum	Mean	SD
Northern pike, adult (135)	2010	Liver	0.01	1.77	0.23	0.29
		Muscle	0.02	1.37	0.40	0.31
Northern pike, adult (160)	2011	Muscle biopsy	0.06	1.14	0.41	0.23
Burbot, adult (35)	2010	Liver	0.01	0.42	0.13	0.10
		Muscle	0.09	1.05	0.45	0.23
Burbot, adult (118)	2011	Muscle biopsy	0.01	0.75	0.14	0.12
Burbot, adult, winter (54)	2010	Liver	0.01	0.20	0.04	0.04
		Muscle	0.05	0.57	0.16	0.12
Sheefish, adult (38)	2010	Liver	0.13	0.58	0.29	0.11
		Muscle	0.11	0.35	0.22	0.06
Arctic grayling, adult (25)	2010	Liver	0.09	0.82	0.26	0.18
		Muscle	0.08	0.49	0.22	0.12
Arctic grayling, juvenile (55)	2010	Whole fish	0.01	0.17	0.04	0.02
Dolly Varden, adult (7)	2010	Liver	0.03	0.38	0.11	0.13
		Muscle	0.01	0.13	0.05	0.04
Dolly Varden, juvenile (53) [A]	2010	Whole fish	0.02	1.61	0.20	0.35
Dolly Varden, juvenile (33)	2011	Whole fish	0.02	0.42	0.14	0.08
Slimy sculpin (165) [A]	2010	Whole fish	0.02	3.70	0.21	0.60
Slimy sculpin (62)	2011	Whole fish	0.01	2.69	0.26	0.36
Macroinvertebrates (27) [A]	2010	Whole body	0.01	2.38	0.25	0.64
Macroinvertebrates (8)	2011	Whole body	0.05	2.41	0.80	0.76
Long-nosed sucker (12)	2010	Whole fish	0.01	0.03	0.02	0.01

[A] There were statistically significant differences between data collected in 2010 and data collected in 2011.

Mercury Differences Among "Tributary" Samples

In 2010, length and THg were significantly correlated for slimy sculpin whole body samples (r = 0.317, p <0.000, n=166), but not for whole body Dolly Varden (r = 0.262, p = 0.059, n=53) or whole body Arctic grayling (r=0.090, p = 0.477, n=64). Length was therefore used as a covariate for testing differences among Tributaries in slimy sculpin.

In 2010, THg was significantly greater at Red Devil Creek compared to all other reaches in slimy sculpin, macroinvertebrates and Dolly Varden, even after accounting for length, (ANCOVA, F = 2.927, p= 0.007; ANOVA, $F=179.2_{2,23}$, p < 0.001; ANOVA, $F= 198.1_{2, 48}$, p < 0.001, respectively) (Fig. 8). Arctic grayling did not have significantly different Hg concentrations among reaches, but Arctic grayling were also not collected at Red Devil Creek (Fig. 8) in 2010.

In 2010, juvenile whole Arctic grayling had significantly lower Hg concentrations than juvenile whole Dolly Varden, slimy sculpin, and macroinvertebrates (Table 4). However, Arctic grayling were not sampled at Red Devil Creek in 2010, where all other Tributary species had elevated Hg concentrations.

In 2011, Total Hg was significantly greater at Red Devil and Cinnabar Creeks compared to Egnaty Creek in slimy sculpin (ANOVA, $F_{2,59} = 58.337$, p<0.001) and Dolly Varden (ANOVA, $F_{2,30} = 12.369$, p<0.001) (Fig. 8). Macroinvertebrates were not collected at Egnaty Creek, but like slimy sculpin and Dolly Varden, there was no significant difference in macroinvertebrate THg concentrations between Cinnabar and Red Devil Creeks (ANOVA, $F_{1,6}=0.016$, p=0.904) (Fig. 8).

In summary, small, sedentary fish and macroinvertebrates from Red Devil and Cinnabar Creeks had significantly greater THg concentrations than the same biota in other tributaries of the middle Kuskwim, after accounting for unequal sample sizes, interannual or inter-laboratory variation, and body length.

Mercury Differences Among "River" Samples

Northern pike from the George River (Reach E) had significantly greater Hg concentrations than northern pike from all other reaches after adjusting for length (ANCOVA $F_{5, 127} = 6.616$, p < 0.0001, n=134) (Fig. 9).

Burbot collected in Reach C of the Kuskokwim River had significantly lower Hg concentrations than burbot from the George River (Reach E) and Reach F of the Kuskokwim River (ANOVA $F_{4,79} = 3.964$, p=0.006, n=84) (Fig. 9). There were no significant differences among reaches for sheefish (ANOVA $F_{4,33} = 1.182$, p=0.337, n=38) (Fig. 9).

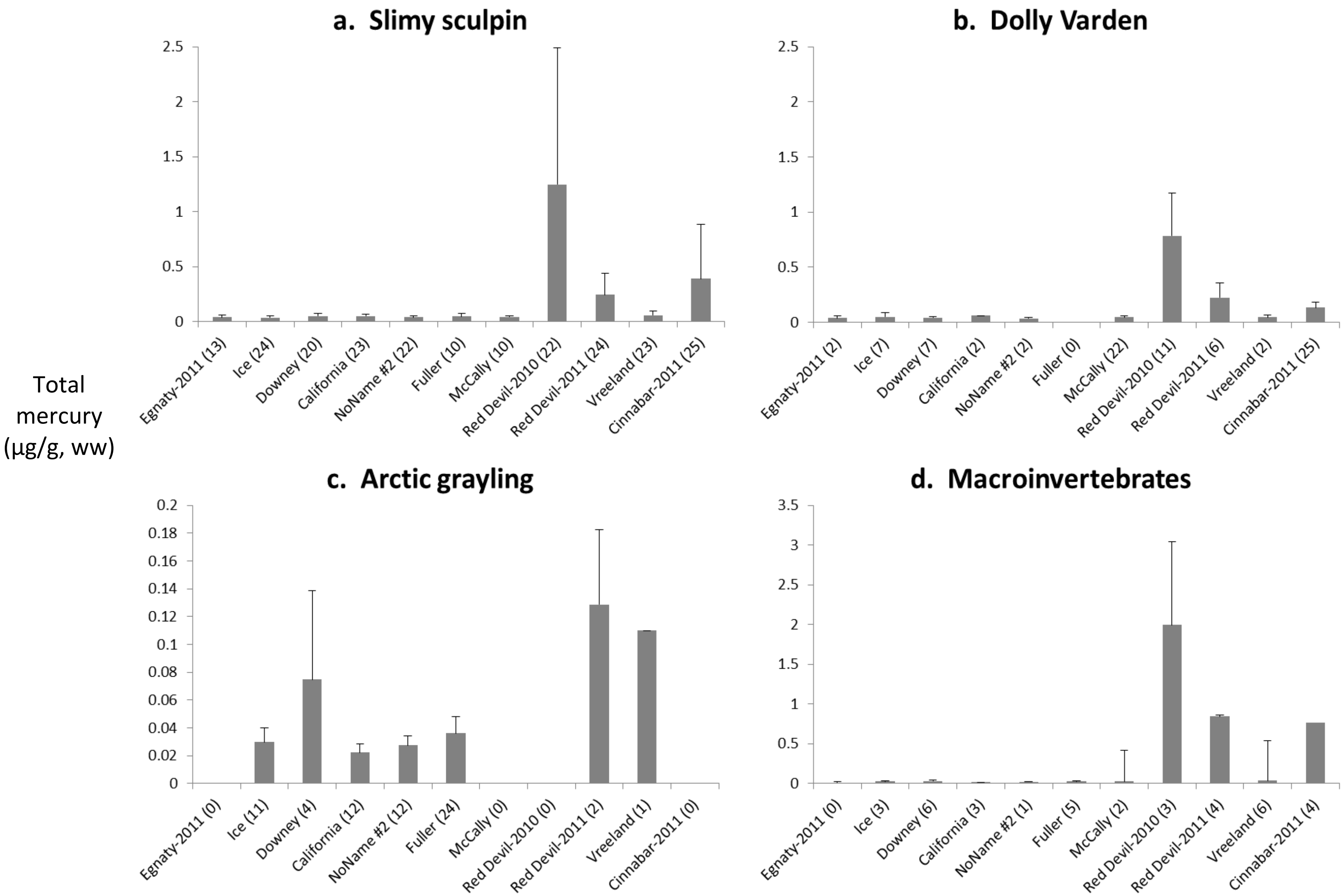

Figure 8. Mean total mercury concentrations (µg/g, ww) (SD) in muscle of (a) slimy sculpin, (b)Dolly Varden, (c) Arctic grayling, and (d) macroinvertebrates from small tributaries of the middle Kuskokwim River, Alaska, 2010-2011. Tributaries (and n) are listed in upstream order from left to right, and were sampled in 2010 unless noted otherwise.

Mercury concentrations from Arctic grayling in the Oskwalik River were lower than those from all other reaches, significantly so in the George River (Reach E) and Reach F of the Kuskokwim River (Fig. 9). Only one Arctic grayling was collected in Reach C, so data for this fish were removed from the analysis. Interestingly, that individual fish had the greatest Hg muscle concentration of all River-collected Arctic grayling (Fig. 9).

Overall, for those species that had significantly different Hg concentrations among reaches (pike, burbot, and Arctic grayling), fish from the George River had consistently high Hg concentrations.

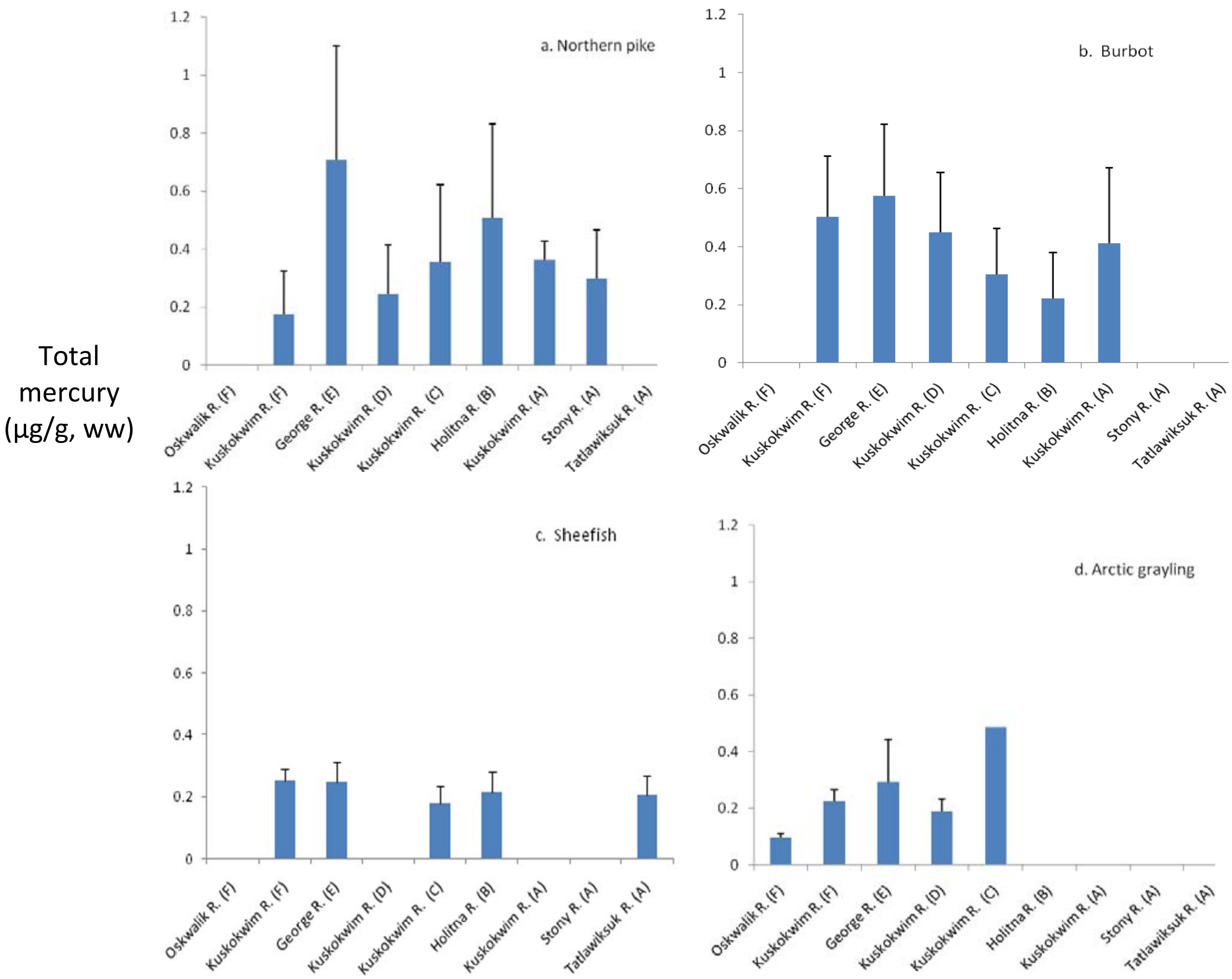

Figure 9. Mean total mercury concentrations (µg/g, ww) (SD) in muscle of northern pike (a), burbot (lush) (b), sheefish (inconnu) (c), and Arctic grayling (d) from the middle Kuskokwim River and its large tributaries, Alaska, 2010. Reaches are listed in upstream order from left to right.

Mercury Differences Between Liver and Muscle Samples

Liver and muscle Hg concentrations were significantly correlated in all (River) species (Table 5), so we avoided duplicative statistical analysis by testing only muscle Hg for differences among reaches and species. Length and age were significantly correlated with muscle THg concentrations in northern pike but not in burbot (lush), sheefish (inconnu), or Arctic grayling from River reaches (Table 5). In northern pike, the relationships between length and muscle Hg were more consistent among reaches than the age-muscle Hg relationships, making length a more useful covariate to account for differences in fish size or age when comparing differences among reaches.

Table 5. Pearson's correlation coefficient *r*, Bonferroni's *p* (adjusted for multiple comparisons), and number of samples n for testing correlations between liver and muscle total mercury, and between age, length, and total mercury in fish collected in the mid-Kuskokwim River, Alaska, in summer 2010.

Species	Liver Hg and muscle Hg	Age and muscle Hg	Length and muscle Hg
Northern pike	0.897 (0.000, 135)	0.236 (0.042,107)	0.521 (0.000, 134)
Burbot (lush)	0.865 (0.000, 86)	0.109 (0.964, 85)	-0.191 (0.239, 85)
Sheefish (inconnu)	0.807 (0.000, 38)	0.027 (1.00, 32)	0.010 (1.00, 38)
Arctic grayling	0.886 (0.000, 25)	-0.072 (1.00, 22)	0.368 (0.252, 23)

Methyl Mercury to Total Mercury Ratios

In fish tissues, a MeHg:THg ratio much less than 1.0 can indicate acute, short-term exposure to Hg in water with uptake by gill or digestive tissue (Guilherme et al. 2010), while one closer to 1.0 can indicate chronic, longer-term exposure to Hg via ingestion of MeHg from prey (Weiner et al. 2003), as in piscivorous fish (Lasorsa and Allen-Gil 1995). The MeHg:THg ratios for River fish, which also were more piscivorous, were near 1.0, but those for Tributary fish were lower (Table 6).

The MeHg:THg ratio for invertebrates is not as well studied. The macroinvertebrate MeHg:THg ratio, at 0.5, was consistent with Watras and Bloom (1992) who concluded that the MeHg proportion was lower at the "base of the food chain," (Watras and Bloom 1992: 1317) in freshwater cladoceran zooplankton.

Table 6. Average methyl mercury to total mercury ratios (MeHg:THg) in aquatic species from the middle Kuskokwim River, Alaska, 2010.

Species and Reach Type	Tissue	N	Average MeHg:THg Ratio
Northern Pike – River	Liver	6	1.0
	Muscle	6	1.1
Burbot – River	Liver	10	1.2
	Muscle	10	1.2
Sheefish – River	Liver	8	1.0
	Muscle	9	1.2
Arctic Grayling –River	Liver	3	1.0
	Muscle	3	1.2
Slimy Sculpin – Tributary	Whole fish	6	0.6
Dolly Varden – Tributary	Whole fish	4	0.8
Macroinvertebrates – Tributary	Whole body	7	0.5

Mercury in Radio-tagged Fish (2011)

Mercury concentrations in radio-tagged fish (muscle biopsy samples, collected in 2011 and analyzed by Frontier; Table 7) were similar to those collected in 2010. For example, Holitna River northern pike had mean (and standard deviation) THg of 0.479 (0.222) and 0.510 (0.320) mg/kg ww in 2010 and 2011, respectively. Length was significantly correlated with THg in northern pike ($p < 0.001$), but not in burbot ($p = 0.658$). Within this data set, northern pike from the Holitna, Hoholitna, and George Rivers all had significantly greater concentrations than those from either reach sampled in the Kuskowkim (GLM; $F_{6,153} = 22.247$, $p < 0.001$). Burbot concentrations, which were sampled only in tagging reaches F, G, and H, were only slightly statistically significant lower in tagging reach H (near Aniak) compared to tagging reach F (just downstream of the mouth of the Holitna) (GLM; $F_{2,115} = 3.192$, $p = 0.045$). Mercury was not detected in one burbot from tagging reach H, the only fish sampled to date in which this was so (MDL for this fish = 0.012 µg/g ww). For tagging reaches F and G (the two reaches where both species were sampled) total Hg concentrations were not significantly different between northern pike and burbot biopsy samples.

Table 7. Total mercury concentration (µg/g, ww) summary statistics in biopsy samples from northern pike and burbot fitted with radio transmitters in the middle Kuskokwim River region, Alaska, June, 2011. Tagging reaches are displayed in Figure 6.

Species	Tagging Reach	n	Range	Mean	SD
Northern pike	A-Holitna	30	0.057 - 0.674	0.348	0.170
Northern pike	B-Holitna	30	0.212 - 1.010	0.554	0.232
Northern pike	C-Hoholitna	30	0.141 - 0.708	0.454	0.165
Northern pike	D-Holitna	29	0.165 - 1.050	0.536	0.204
Northern pike	E-George	13	0.117 - 1.140	0.401	0.272
Northern pike	F-Kuskokwim	8	0.079 - 0.245	0.158	0.062
Northern pike	G-Kuskokwim	20	0.055 - 0.297	0.157	0.072
Burbot (lush)	F-Kuskokwim	38	0.043 - 0.748	0.163	0.147
Burbot (lush)	G-Kuskokwim	40	0.037 - 0.510	0.139	0.104
Burbot (lush)	H-Kuskokwim	40	0.026 - 0.393	0.107	0.080

Seasonal Mercury Differences in Burbot (Lush)

Burbot (lush) were collected during the winter sampling season to characterize Hg in this subsistence fishery. A multivariate analysis was used to test for differences between the sampling seasons and between Reach A and C (which were the only reaches in which winter burbot were collected), in both muscle and liver. Mercury was significantly greater in burbot tissues in the summer/fall sampling season compared to winter ($F_{1,86} = 35.152, 19.257$, $p < 0.001$ for muscle and liver, respectively), but was not significantly different between Reach C and Reach A in either summer/fall or winter (Fig. 10). Preliminary telemetry data indicate that summer/fall burbot were likely resident in their capture area, while winter-caµght burbot were more likely to have come from lower down the river (M. Varner, pers. comm.).

Mercury in Boiled Burbot Muscle and Eggs

Raw and boiled muscle samples from 13 burbot (12 from Reach A and 1 from Reach C) were analyzed. Livers and eggs (n=5) from these fish were also analyzed.There was no significant difference in Hg concentration after burbot muscle samples were boiled (paired t-test; p = 0.249). The mean concentration of Hg in burbot eggs (n=5) was 0.014 (SD = 0.007), and the mean Hg concentration in the water used to boil the muscle samples (n=13) was 0.0006 (SD = 0.005) mg/L (ppm).

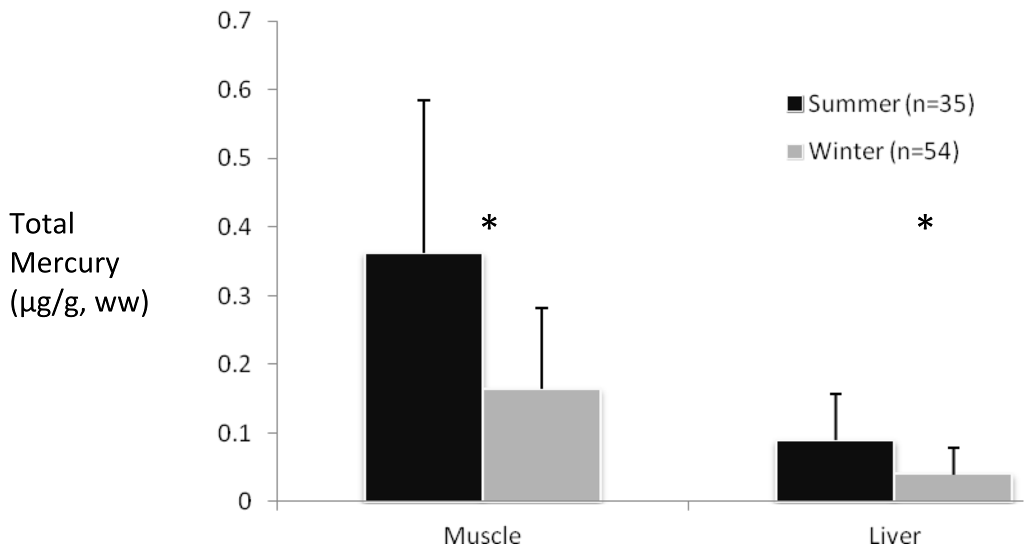

Figure 10. Mean total mercury concentrations (µg/g, ww) (SD) in burbot (lush) tissues collected in June – early October (summer) and late November – early December (winter) from the mid-Kuskokwim River region, Alaska, 2010. Asterisks indicate significant differences between the seasons.

Mercury Across All Species

Although data were insufficient to do a global comparison among all species, Hg concentrations generally tracked age and trophic status. Older fish of the same species, and more predatory fish, generally had greater average Hg concentrations (Fig. 11).

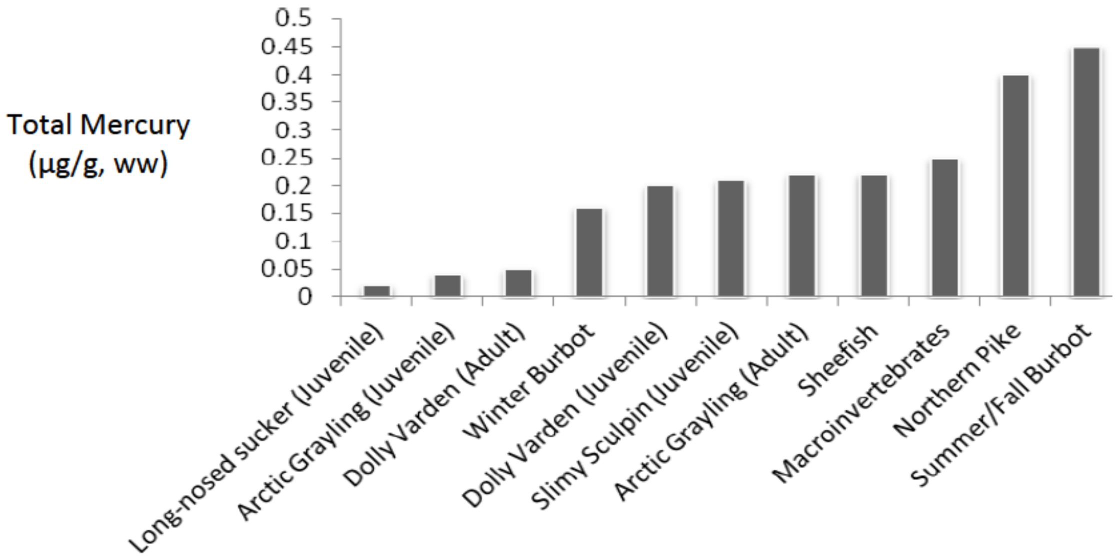

Figure 11. Mean total mercury concentrations (µg/g, ww) in fish (by species) and macroinvertebrates collected in the mid-Kuskokwim River region, Alaska, 2010.

Arsenic and Antimony

The greatest average As concentrations were in macroinvertebrates (Table 8). The greatest average Sb concentrations were in whole (juvenile) Dolly Varden (Table 8).

Arsenic Differences among "Tributary" Samples

Total As was much greater in biota collected from Red Devil Creek compared to all other tributary reaches (tested with the most complete data, from 2010; Fig. 12). Excluding Red Devil, Cinnabar Creek had the next highest concentrations (Fig. 12). Differences among other creeks were relatively minor and perhaps not biologically significant compared to the difference between Red Devil and all other tributaries, however (Fig. 12).

Arsenic Across All Species

Among species sampled in tributaries, macroinvertebrates had the greatest As concentrations when Red Devil Creek samples were included (GLM; $F = 14.722_{3,220}$, $p < 0.001$) and when they were not (GLM; $F = 102.357_{3,184}$, $p < 0.001$) (tested within the most complete data from 2010; Fig. 13). Arctic grayling had significantly lower arsenic concentrations than all other biota when Red Devil samples were included, but no Arctic grayling were collected at Red Devil in 2010; Arctic grayling and Dolly Varden concentrations were not different when Red Devil samples were excluded.

Antimony Differences Among "Tributary" Samples

Antimony, which was only measured in 2010, was detected in fewer samples than other metals of concern. It was detected in most macroinvertebrate samples (100% detections in all tributary macroinvertebrates except 83% detections from Vreeland Creek), and was significantly greater in Red Devil macroinvertebrates compared to all other tributaries, with no other significant differences (GLM; $F_{5,20} = 37.06$, $p <0.001$) (Table 9). Antimony was detected in 80% of slimy sculpin samples from McCally Creek, but at a mean concentration (0.08 mg/kg ww) significantly lower than those from Red Devil (10.2 mg/kg ww) (GLM; $F_{1,30} = 9.806$, $p=0.004$). Antimony was detected in only 0-30% of all other tributary samples, at concentrations near the detection limit (MDL – 0.025 mg/kg ww). Mean (SD) Sb concentrations (mg/kg ww) in other Red Devil Creek biota were 12.0 (19.1) in Dolly Varden (n=11), 10.2 (10.1) in slimy sculpin (n=22), 0.49 in one coho salmon, and 2.25 (0.705) in Chinook (n=2).

Table 8. Total arsenic (As) and antimony (Sb) concentrations (mg/kg, ww) summary statistics for aquatic species sampled in the middle Kuskokwim River region, Alaska, 2010-2011. Whole body data include those from Red Devil Creek, which had significantly greater As and Sb concentrations than other whole body samples. Method detection limits were 0.025 mg/kg, ww.

	Total arsenic (As)			Antimony (Sb)			
Species, age, tissue (n)	**Minimum**	**Maximum**	**Mean (SD)**	**Minimum**	**Maximum**	**Mean (SD)**	**Number (%) detects**
Arctic Grayling, adult liver (n=23)	nd	0.09	0.04 (0.02)	nd	0.08	na	1 (4)
Arctic Grayling, adult muscle (n=23)	nd	0.06	0.03 (0.02)	nd	nd	na	0 (0)
Arctic Grayling, juvenile whole body (n=64)	0.04	0.36	0.12 (0.05)	nd	0.03	na	1 (2)
Burbot, adult liver (n=140)	0.29	8.5	2.4 (1.9)	nd	nd	na	0 (0)
Burbot, adult muscle (n=140)	0.07	11	2.2 (2.5)	nd	nd	na	0 (0)
Burbot, Boiled Muscle (n=13)	0.60	6.2	2.6 (1.6)	nd	nd	na	0 (0)
Burbot, Egg (n=5)	1.3	4.4	2.5 (1.3)	nd	nd	na	0 (0)
Chinook, juvenile whole body (n=2)	6.95	7.6	7.3 (0.43)	1.8	2.8	2.3 (0.71)	2 (100)
Coho, juvenile whole body (n=2)	nd	0.07	na	nd	0.49	na	1 (50)
Dolly Varden, adult liver (n=9)	0.03	0.74	0.29 (0.25)	nd	nd	na	0 (0)
Dolly Varden, adult muscle (n=9)	nd	0.82	0.38 (0.31)	nd	nd	na	0 (0)
Dolly Varden, juvenile whole body (n=53)	0.04	35	2.4 (6.07)	nd	68	7.0 (15)	19 (36)
Humpback Whitefish, adult liver (n=1)	0.04	0.04	na	nd	nd	na	0 (0)
Humpback Whitefish, adult muscle (n=1)	nd	nd	na	nd	nd	na	0 (0)
Long-nosed sucker, whole body (n=12)	0.18	0.44	0.34 (0.07)	nd	nd	na	0 (0)
Macroinvertebrates, whole body (n=29)	0.48	130	12 (32)	nd	21	2.3 (6.4)	28 (97)
Northern Pike, adult liver (n=135)	nd	0.86	0.14 (0.15)	nd	0.05	0.04 (0.01)	4 (3)
Northern Pike, adult muscle (n=135)	0.04	2.6	0.31 (0.37)	nd	nd	na	0 (0)
Sheefish, adult liver (n=39)	0.03	2.5	1.1 (0.69)	nd	0.03	na	1 (3)
Sheefish, adult muscle (n=39)	0.04	6.8	3.1 (1.9)	nd	0.03	na	1 (3)
Slimy Sculpin, whole body (n=166)	0.07	24	1.3 (3.8)	nd	38	5.6 (9.0)	40 (24)
Water, Boiled (n=13)	0.99	8.6	3.9 (2.0)	nd	nd	na	0 (0)

Table 9. Mean (and standard deviation, SD) antimony (Sb) concentrations (mg/kg ww) from macroinvertebrate whole body samples collected from tributaries of the mid-Kuskokwim River, Alaska, 2010-11. Unlike superscripts indicate significantly different concentrations among 2010 sample sites.

Tributary	Mean (SD)
Downey Creek (n=6)	0.098 (0.083) [a]
Ice Creek (n-3)	0.118 (0.078) [a]
Red Devil Creek (n=3)	20.3 (1.25) [b]
Fuller Creek (n=5)	0.093 (0.005) [a]
Vreeland Creek (n=6)	0.138 (0.144) [a]
California Creek (n=3)	0.125 (0.007) [a]

Arsenic and Antimony Differences Among River Samples

While Chinook salmon had the greatest total As concentrations among all species sampled (Table 8), the small Chinook sample size precluded robust conclusions from those data. Among other River species, sheefish (inconnu) and burbot (lush) had the greatest overall As concentrations, in both muscle and liver (Table 8). Antimony was not detected in any muscle sample, and was detected in only 5 of 352 liver samples, at concentrations near the detection limit (MDL = 0.025 mg/kg ww) (Table 8). In all species, liver and muscle As concentrations were significantly positively correlated (Table 10).

Table 10. Results of correlation tests (Pearson's correlation coefficient *r*, Bonferroni's *p*, and number of samples n), and muscle and liver means (and standard deviations) of total arsenic in muscle and liver of fish collected from multiple reaches in the mid-Kuskokwim River, Alaska, 2010-2011.

Species	Correlation statistics	Muscle mean (SD)	Liver mean (SD)
Arctic grayling	0.486 (0.022, 22)	0.03 (0.02)	0.04 (0.02)
Burbot	0.904 (<0.001, 105)	2.80 (2.55)	2.90 (1.89)
Dolly Varden	0.893 (0.007, 7)	0.48 (0.25)	0.36 (0.24)
Northern pike	0.905 (< 0.001, 99)	0.34 (0.40)	0.15 (0.15)
Sheefish (inconnu)	0.963 (< 0.001, 36)	3.26 (1.84)	1.09 (0.67)

Burbot, northern pike, and sheefish were tested for As differences among reaches (excluding reaches where sample sizes were < 3; see Table 1). Arsenic in burbot muscle and liver clearly and significantly declined from upstream reaches to downstream reaches (Fig. 14). Similar but less significant patterns were seen in northern pike and sheefish (inconnu) (Fig. 14).

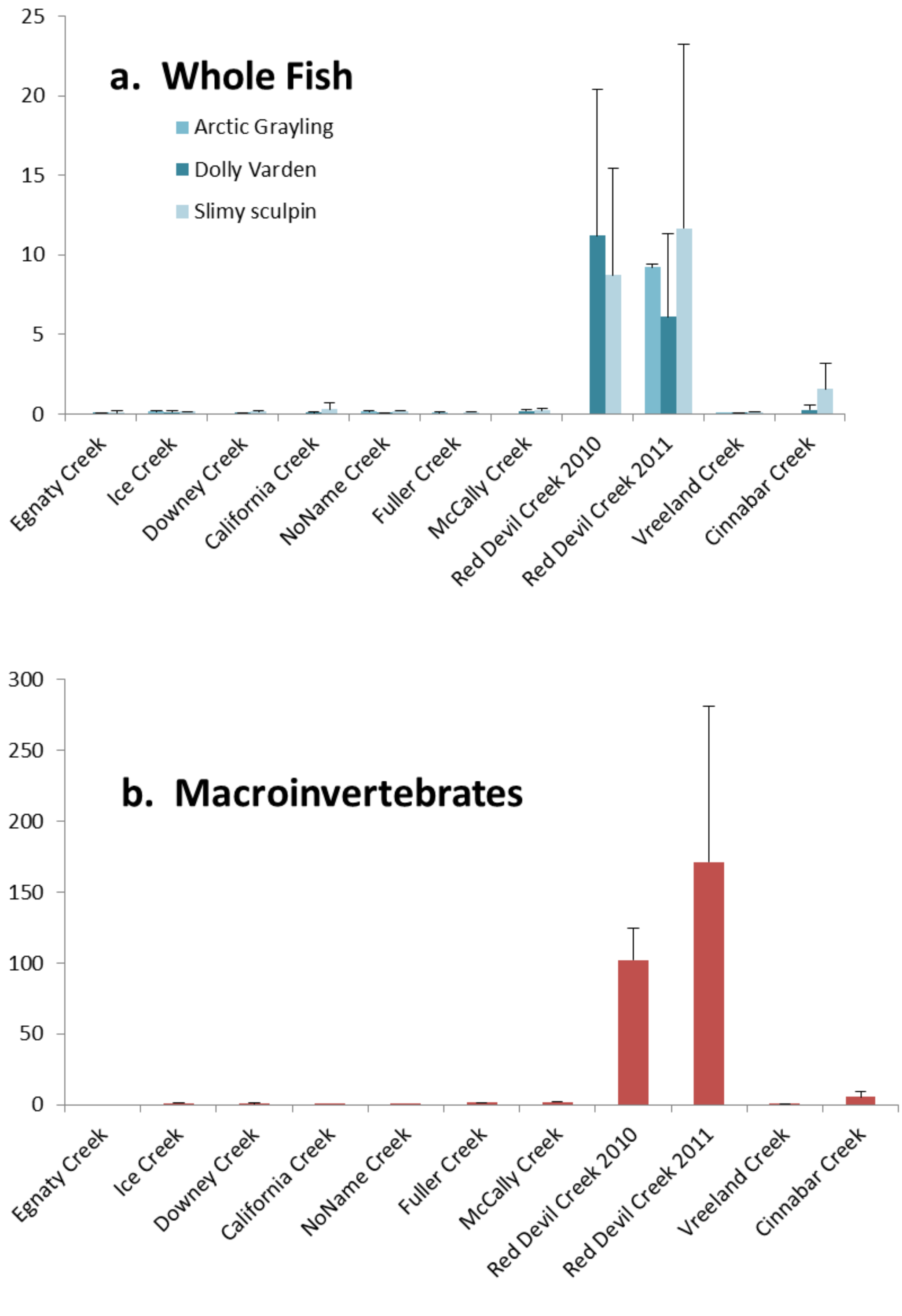

Figure 12. Total arsenic (µg/g, ww) in whole fish (a) and macroinvertebrates (b) from tributaries to the middle Kuskokwim River, Alaska, 2010-2011.

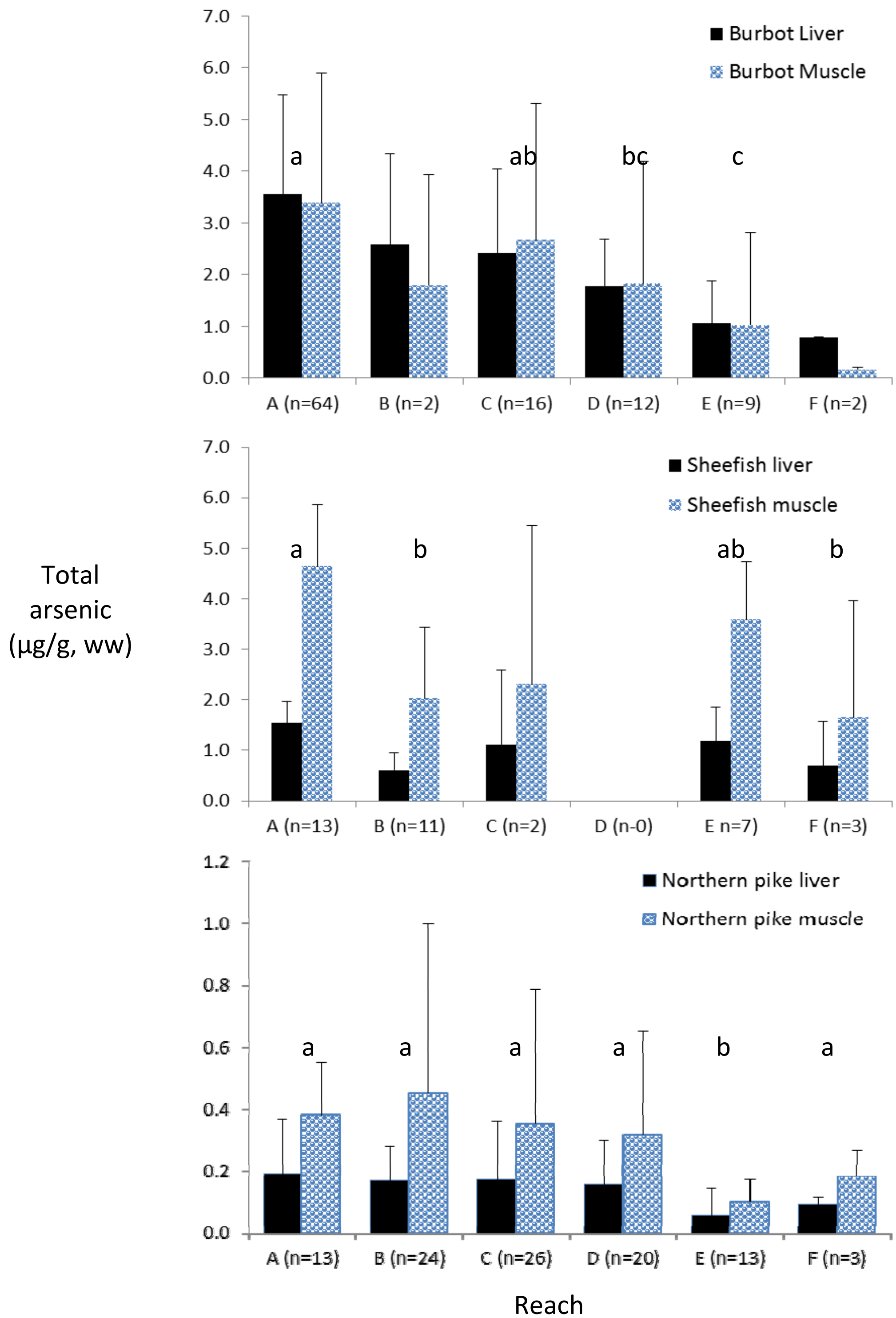

Figure 13. Mean (SD) total arsenic concentrations in burbot (lush), sheefish (inconnu), and northern pike collected from reaches of the middle Kuskokwim River, Alaska, 2010-2011. Unlike superscripts indicate significant differences among reaches in both liver and muscle concentrations. Reaches with no superscripts had low sample size and were not included in statistical tests.

Inorganic Arsenic

Arctic grayling, Dolly Varden, macroinvertebrates, and slimy sculpins were collected from Red Devil Creek, Cinnabar Creek, and Egnaty Creek in 2011. Egnaty Creek was added because of a documented occurrence of valuable minerals, and Cinnabar Creek because there was an abandoned mercury mine there. One of three known mineral deposits in the Cinnabar Creek watershed (USGS 2005) was mined in the late 1950s, producing several hundred flasks of mercury from the on-site retort facility (Red Devil produced 35,000 flasks) (USGS 2005).

A subset of samples was analyzed for inorganic arsenic (Table 11). Total As concentrations in Red Devil Creek 2011 samples were similar to Red Devil 2010 samples (in the same species and matrices) (Table 11, Fig. 13), while 2011 samples from Cinnabar and Egnaty Creeks had total As concentrations similar to those in samples from 2010 tributary reaches other than Red Devil (Table 11, Fig. 14). Further, total and inorganic As concentrations from Red Devil Creek were significantly greater than those in Cinnabar and Egnaty Creeks (Fig. 15), tested in Dolly Varden ($KW = 14.961_2$, $p = 0.001$), slimy sculpin ($KW = 46.982_2$, $p < 0.001$), and macroinvertebrates (GLM; $F_{1,6} = 9.107$, $p = 0.023$). This was expected based on sediment and water data (M. Varner, pers. comm.).

Total As and inorganic As were significantly correlated within individuals (Pearson $r = 0.961$, $p < 0.001$). Average ratios of inorganic to total As were 0.65 for Arctic grayling (n=2), 0.62 for slimy sculpin (n=17), 0.913 for Dolly Varden (n=4). By site, they were 0.70 at Cinnabar Creek (n=7) and 0.66 at Red Devil Creek (n=16).

Table 11. Mean total and inorganic arsenic (As) concentrations (mg/kg ww), standard deviation (SD), sample size, and number and percent of values over the reporting limits ("detects") by site and species in aquatic biota in the mid-Kuskokwim River, Alaska, 2011. Samples were whole bodies, "nd" indicates values below the reporting limits (i.e., non-detects), which were approximately 0.3 mg/kg ww.

	Total arsenic (As)					**Inorganic As**				
Species, site	**n**	**Minimum**	**Maximum**	**Mean (SD)**	**Number (%) detects**	**n**	**Minimum**	**Maximum**	**Mean (SD)**	**Number (%) detects**
Arctic Grayling, Red Devil Creek	2	9.1	9.4	9.2 (0.18)	2 (100)	2	5.4	6.5	6.0 (0.79)	2 (100)
Dolly Varden, Cinnabar Creek	25	nd	0.95	0.42 (0.32)	14 (56)	3	0.03	0.12	0.08 (0.05)	3 (100)
Dolly Varden, Egnaty Creek	2	nd	nd	nd	0 (0)	not analyzed				
Dolly Varden, Red Devil Creek	6	2.0	16	6.1 (5.3)	6 (100)	2	0.62	2.0	1.3 (0.94)	2 (100)
Macroinvertebrates, Cinnabar Creek	4	0.70	9.7	5.4 (3.7)	4 (100)	not analyzed				
Macroinvertebrates, Red Devil Creek	4	29	280	170 (110)	4 (100)	not analyzed				
Slimy Sculpin, Cinnabar Creek	24	0.35	8.3	1.6 (1.6)	24 (100)	5	0.34	0.95	0.58 (0.29)	5 (100)
Slimy Sculpin, Egnaty Creek	12	0.43	0.43	na	1 (8)	not analyzed				
Slimy Sculpin, Red Devil Creek	24	1.6	46	12 (11)	24 (100)	12	0.21	38	14 (11)	12 (100)

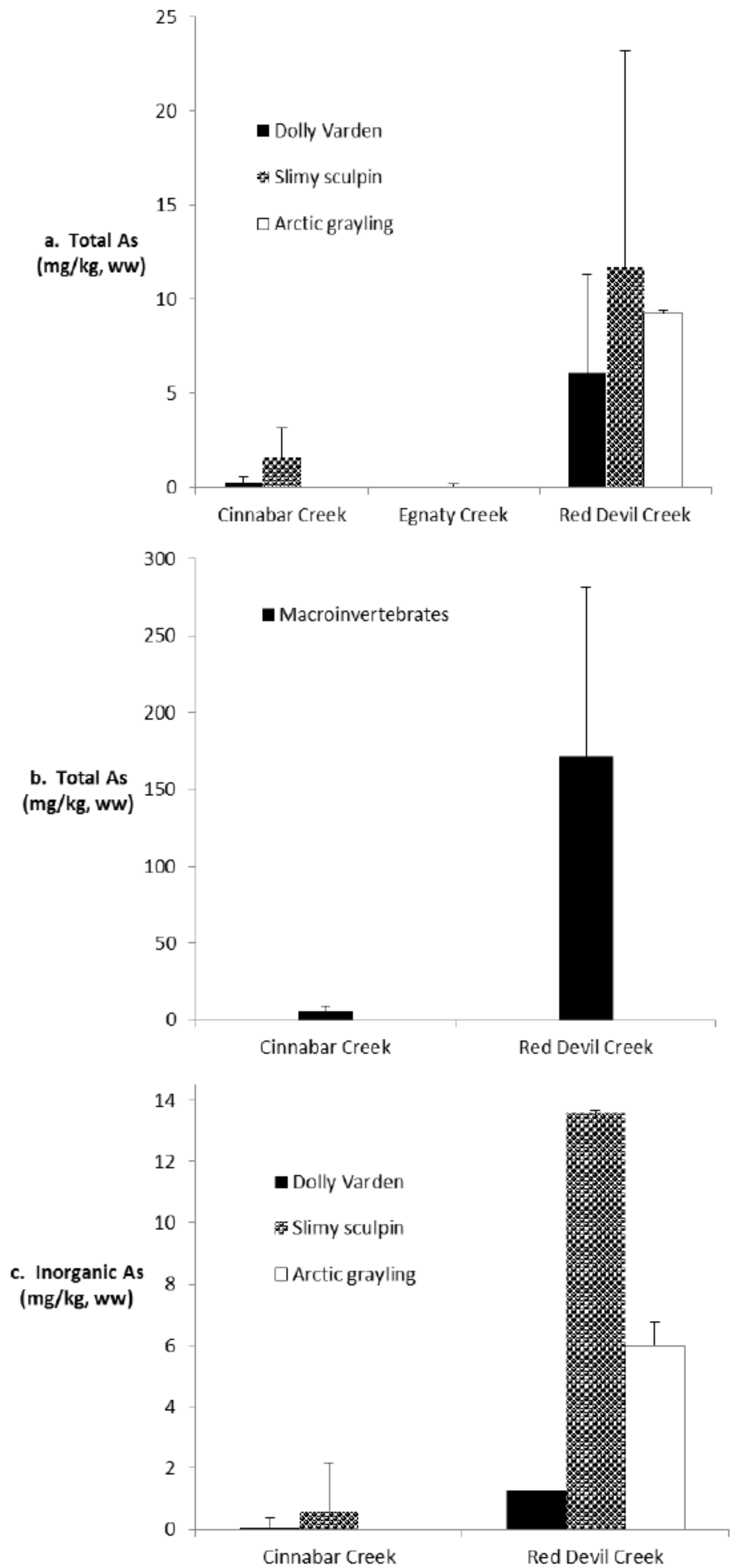

Figure 14. Mean (SD) differences in total arsenic (a,b) and inorganic arsenic (c) measured in Dolly Varden, slimy sculpin, Arctic grayling, and macroinvertebrate whole body samples from three geologically similar creeks in the mid-Kuskokwim River, Alaska, 2011.

Seasonal Arsenic Differences in Burbot (Lush)

Burbot (lush) were collected during the winter sampling season to characterize trace element concentrations in this subsistence fishery. A multivariate analysis was used to test for differences between the sampling seasons and between Reach A and C (which were the only reaches in which winter burbot were collected), in both muscle and liver. Arsenic was significantly lower in burbot tissues in the summer/fall sampling season compared to winter ($F_{1,1,77}$ = 15.537, 17.613, $p < 0.001$ for seasonal differences muscle and liver, respectively), but was not significantly different between Reach C and Reach A in either summer/fall or winter ($p=0.993$, 0.754 for muscle and liver, respectively) (Fig. 16).

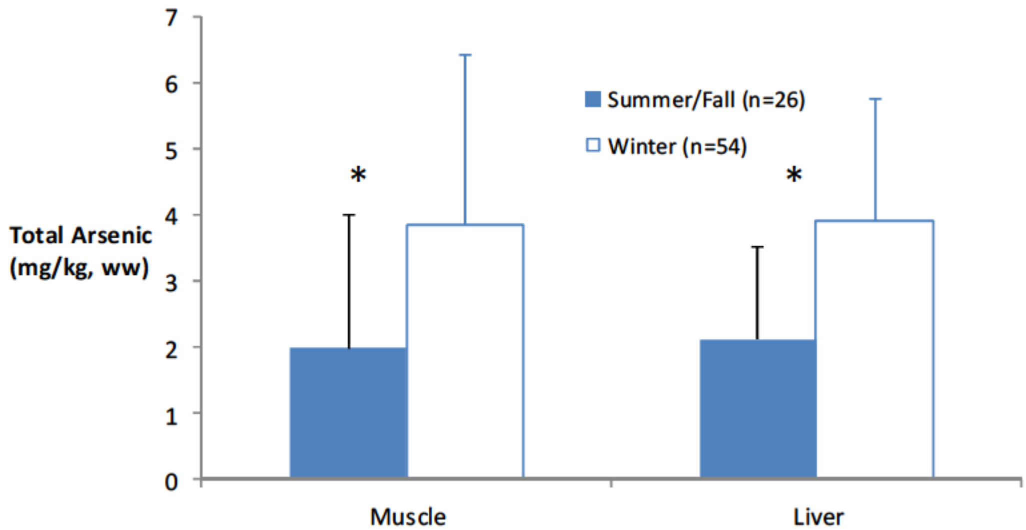

Figure 15. Mean total arsenic concentrations (SD) in burbot (*Lota lota*) also known as lush, tissues collected in June – early October, 2010 (summer/fall) and late November, 2010 – January 2011 (winter) from the mid-Kuskokwim River, Alaska. Asterisks indicate significant differences between the seasons within each tissue.

Arsenic in Boiled Burbot Muscle and Eggs

Arsenic concentrations were significantly lower in boiled burbot muscle compared to the same muscle before boiling (paired t-test; t = 3.159, p =0.008). This was so even when including a significant outlier that had much a much greater boiled muscle concentration (6.20 mg/kg ww) compared to pre-boiling (1.95 mg/kg ww). The mean concentration of As in burbot eggs (n=5) was 2.45 (SD = 1.33) mg/kg ww. Antimony was not detected in boiled burbot muscle, or burbot eggs. Inorganic arsenic was not measured in boiled muscle or egg samples.

Discussion

Sedentary organisms, such as larval benthic macroinvertebrates and apparently non-migratory (Morrow 1980) slimy sculpin in Red Devil and Cinnabar Creeks clearly had much greater Hg and somewhat greater As concentrations compared to other tributaries, indicative of active point sources within those creeks. The Dolly Varden collected in Red Devil Creek were juveniles and probably non-resident in Red Devil Creek itself (K. Wuttig, ADFG, pers. comm.), but the large and significant differences in Hg and As concentrations from clearly resident biota of Red Devil and Cinnabar Creeks compared to those of nearby tributaries likely reflected input from the mine sites in these two creeks.

In contrast, relatively mobile fish from the mainstem middle Kuskokwim and its large tributaries showed no clear patterns in Hg and As concentrations, with two exceptions. Non-anadromous fish (i.e., northern pike, burbot, and Arctic grayling) from the George, Holitna, and Hoholitna rivers had greater Hg concentrations than other fish, and total As in burbot decreased with downstream sampling location. A joint ADFG and BLM telemetry project will provide movement data for burbot and northern pike in the study area. These data, combined with tissue trace element concentrations, will be used to determine complete spatial and temporal exposure patterns, rather than the current single snapshot from the single geographic location of capture.

Mercury

Mercury concentrations in northern pike from this study were similar to those in other datasets from the Yukon-Kuskokwim Delta or the Kuskokwim River, although without accounting for size the comparisons are gross. Still, this study's data (northern pike average total Hg of 0.4 µg/g) were consistent to an order of magnitude with data from several others (0.5 to 0.7 µg/g from Jewett et al. 2003; Duffy et al. 1998, 1999; and USFWS unpubl. data).

Northern pike mercury concentrations from the current study were similar to data collected in the lower Kuskokwim River and the lower Yukon River as part of a large study evaluating mercury in northern pike on National Wildlife Refuges in Alaska (U.S. Fish and Wildlife Service, unpubl. data) (Fig. 16). Lower Kuskokwim River fish samples were collected downstream from Aniak to the Johnson River mouth. Yukon River samples were collected from Holy Cross to Emmonak. In this regional context, data for this important subsistence species speak to the numerous factors that can contribute to fish mercury concentrations.

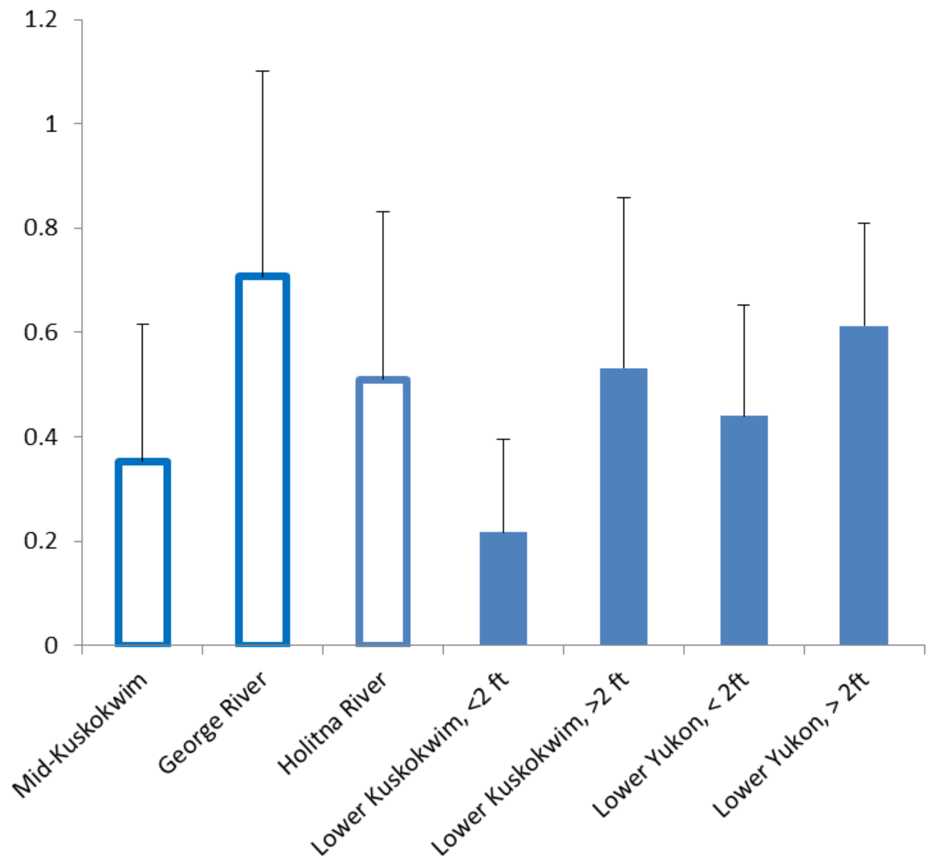

Figure 16. Average (SD) mercury or methyl mercury concentrations (mg/kg wet weight) in northern pike from western Alaska, 2001-2011. White bars are total mercury concentrations from the current study evaluating mercury in the mid-Kuskokwim River area (from Crooked Creek to the Stony River). Solid bars (methyl mercury values calculated as 97% of total mercury) were data collected for a large study evaluating mercury in northern pike (separated into less than or greater than two feet in length) on National Wildlife Refuges in Alaska (U.S. Fish and Wildlife Service, unpubl. data).

For example, older pike (greater than two feet in length), have greater mercury concentrations than younger pike from the same area (Fig. 16). Also, lower Yukon pike have greater average mercury concentrations (after accounting for size and age) than Kuskokwim River pike (Fig. 16) even though there are more identified point sources (cinnabar deposits and mercury mine sites) in the Kuskokwim. Among many potential reasons, this may be because the Yukon watershed is much larger than the Kuskokwim, providing a larger catchment area for atmospherically deposited mercury or melting permafrost (Schuster et al. 2011). The George River pike, which have the greatest mercury concentrations (Fig. 16), may reflect point sources in the mines in the headwaters plus a high proportion of wetlands – where mercury methylation takes place - in the watershed. While speculative as to source, these data do emphasize the many factors that can influence mercury concentrations in fish.

Both sets of data have been also been used to issue fish consumption advisories based on mercury by the State of Alaska (http://www.epi.alaska.gov/eh/fish/; current study at http://www.epi.alaska.gov/eh/fish/MidKuskoPikeBurbotFactSheetJune2011.pdf, previous study at http://www.epi.alaska.gov/eh/fish/YKD_Pike_Mercury_Poster.pdf). Pike from watersheds other than the Kuskokwim have similarly or more restrictive guidance, such as pike from the lower Yukon River (Table 12). The most restrictive consumption guidance is for pike from the George River.

Table 12. Fish consumption guidance based on methyl mercury concentrations issued by the State of Alaska for northern pike collected in the Yukon-Kuskokwim Delta and the middle Kuskokwim River area.

Methyl mercury concentration in fish (mg/kg, wet weight)	Recommended meals[1] per month	Area[2] of Capture (Data source)
0 - 0.15	Unlimited	
>0.15 - 0.32	up to 16	Stony River, Mid-Kuskokwim (current study) Lower Kuskokwim < 2 ft (USFWS)
>0.32 - 0.40	up to 12	
>0.40 - 0.64	up to 8	Holitna River (current study) Lower Yukon, Lower Kuskokwim > 2 ft (USFWS)
>0.64 – 1.2	up to 4	George River (current study)
>1.2 - 1.4	up to 3	
>1.4 - 2 l.0	up to 2	
>2.0 - 3.4	up to 1	

[1] A meal is a six-ounce portion of fish

[2] The mid-Kuskokwim, George, Stony, and Holitna areas were defined as in this study. The lower Kuskokwim area was from Aniak downstream to the Johnson River slightly SW of Bethel; the lower Yukon area was from Holy Cross downstream to Emmonak.

There weren't clear explanatory factors for seasonal differences in Hg concentrations in burbot. Lower winter concentrations could reflect a different sampled population, or, less likely, be explained by slower metabolism reducing MeHg uptake through food (Forstner and Wittman 1981). However, since MeHg depurates from fish muscle tissue very slowly, winter-caught burbot may simply have a different exposure history compared to those caught in summer. Additional data from ongoing telemetry studies should further inform these analyses.

In the absence of point sources, Hg concentrations in fish tissues should closely track trophic position, because Hg biomagnifies up food chains. Trophic order as suggested by relative Hg concentrations in fish from this study (Fig. 11) was slightly different from the trophic order proposed elsewhere for northern North American freshwater fishes (in Canada; Vander Zanden et al. 1997). This may be an illustration of how other life history characteristics (e.g., age) may affect Hg concentrations, as adult Arctic grayling had greater Hg concentrations than juvenile (Fig. 11). However, Hg concentrations do vary with point sources, and would confound a strict life-history explanation for differences in mercury. For example, the mean Hg for Dolly Varden juveniles was much greater than for adults (Fig. 11), although juveniles are lower on the food chain (K. Wuttig, ADFG, pers. comm.). However, the juvenile mean was greatly influenced by the high Hg from Red Devil Creek (Fig. 8); without Red Devil Creek, the juvenile Dolly Varden mean was 0.04 µg/g ww, less than the mean Hg in adult Dolly Varden. Thus, the "point sources" in Red Devil (and Cinnabar) Creek resulted in greater Hg concentrations in younger and lower-trophic status organisms.

In fish tissues, a MeHg:THg ratio much less than 1.0 can indicate acute, short-term exposure to Hg in water with uptake by gill or digestive tissue (Guilherme et al. 2010), while a ratio closer to 1.0 can indicate chronic, longer-term exposure to Hg via ingestion of MeHg from prey (Weiner et al. 2003), as in piscivorous fish (Lasorsa and Allen-Gil 1995). The low MeHg:THg ratio of tributary-dwelling species combined with their high THg concentrations in Red Devil and Cinnabar Creeks may indicate exposure to a point source of inorganic Hg, as Guilherme et al. (2010) determined for caged mullet (*Liza aurata)* in a Hg-contaminated system in Portugal. However, our MeHg measurements were limited to those necessary for establishing MeHg:THg ratios, established as a percentage of the total number of samples. To further evaluate differences in MeHG:THg ratios among reaches and species would require larger sample sizes.

Mercury concentrations within an individual fish's liver and muscle can indicate exposure timing (Guilherme et al. 2010). Much greater Hg concentrations in liver compared to muscle indicate recent high exposure, either through gill uptake or ingestion. Sheefish, Arctic grayling, burbot, and northern pike had liver:muscle Hg ratios close to or less than 1 (1.36, 1.10, 0.415, 0.248, respectively), but Dolly Varden had a mean liver:muscle Hg ratio of 2.28 (n=7, from Kuskokwim River reaches D and F). Therefore, of all species for which muscle and liver were analyzed separately (and these were all collected in River reaches) only Dolly Varden showed evidence of potential short-term or acute exposure based on liver:muscle Hg ratios.

Toxic thresholds for Hg in freshwater fish were summarized by Sandheinrich and Wiener (2011). Concentrations as low as 0.03 µg/g ww in muscle were enough to affect biochemistry (e.g., nervous system enzymes) and gene expression, and thresholds for effects on reproduction, histology, and growth were approximately 0.5 µg/g ww in muscle (Sandheinrich and Wiener 2011). Only long-nosed sucker, juvenile Arctic grayling, and adult Dolly Varden had species mean concentrations below most thresholds (Table 4). Juvenile Dolly Varden and slimy sculpin from Red Devil and Cinnabar Creeks, and Arctic grayling from several creeks, had concentrations exceeding both thresholds (Fig. 8). Fish sampled in river reaches all exceeded the lower thresholds, and some clearly exceeded the higher thresholds (Fig. 9). Therefore, the elevated Hg concentrations within the study area may be affecting fish health and reproduction, but the magnitude of individual or population-level effects is unknown.

Arsenic and Antimony

Arsenic differences in biota from Red Devil and other tributaries were similar to patterns found in water and sediment concentrations from those creeks, with Red Devil Creek having relatively high concentrations (M. Varner, pers. comm.). Background concentrations of arsenic in freshwater biota are < 1 mg/kg (Eisler 1988), in all tissues. Concentrations greater than that indicate exposure to arsenic from pesticides, herbicides, or mine wastes (Eisler 1988 and references therein). Toxic thresholds for total As in freshwater fish tissues range from 1.3 – 5 mg/kg ww (acute toxicity in bluegill *Lepomis macrochirus*) (NRCC 1978), and 5.4-11.6 mg/kg associated with reduced survival and growth in freshwater fish (Gilderhus 1966, McGreachy and Dixon 1992). Using these criteria, fish from Red Devil Creek, including two juvenile Chinook salmon, may be negatively affected by high As concentrations.

There weren't clear explanatory factors for seasonal differences in As concentrations in burbot. Fu et al. (2010) noted the opposite pattern of lower As and Sb concentrations (in water) in December compared to July at a contaminated Sb mine site in China. They attributed the difference to greater rainfall in July causing greater local fallout of Sb and As from smelting emissions. Seasonal differences in As likely reflect different exposure scenarios for the sampled fish; they may be from two different sub-populations. Additional data from ongoing telemetry studies should further inform these analyses.

Environmental concentrations of total As in brown trout (*Salmo trutta*) from a several-decades abandoned arsenic mine on the Presa River in Italy and a reference site in Corsica were 1174 and 0.044 µg/g (equivalent to mg/kg) ww in liver, and 0.997 and 0.053 in muscle (Foata et al. 2009). Antimony was detected in samples from the mine site, at relatively low concentrations (0.096 and 0.075 µg/g ww in liver and muscle, respectively) but not in reference site samples, similar to our findings. Elsewhere in Alaska, male burbot from Kotlik had the greatest whole body concentrations of arsenic in a basin-wide study of fish from the Yukon River, but those were 1.95 µg/g (Hinck et al. 2006), similar in magnitude to the 2.2 µg/g found in this study.

Arsenic concentrations in benthic invertebrates from the Presa River ranged from over 1000 µg/g dw in "shredder" species, to several hundred µg/g in "scrapers," and "collectors" (Culioli et al. 2009). The lowest concentrations were found in predatory benthos, from 23 – 130 µg/g. In that study, Sb had similar relative concentrations, although absolute Sb concentrations were about an order of magnitude lower compared to As, for the same taxa. These results are similar to ours. Although Sb and lead were correlated with a reduction in macroinvertebrate biodiversity on a river downstream of an Sb mine in Turkey (Duran et al. 2007), the BLM National Aquatic Monitoring Center was unable to detect meaningful differences in biodiversity metrics between sampled tributaries in the current study (Varner 2012).

Subsistence Concerns

Mercury concentrations in northern pike and burbot, which are used for subsistence, warranted issuing consumption guidance (http://www.epi.alaska.gov/eh/fish/default.htm) under the State of Alaska's guidelines (Verbrugge 2007).

However, the State of Alaska has no consumption guidance for arsenic or antimony in fish used for subsistence (Verbrugge 2007). Most arsenic in fish tissues is assumed to be less toxic organic forms, e.g. arsenobetaine or "fish arsenic" (ATSDR 2007), with inorganic arsenic in fish and seafood normally being 0-10%, and often < 1% (Abernathy and Morgan 2001). In the small number of whole body samples (from Arctic grayling, slimy sculpin, and Dolly Varden) and sites (Red Devil, Cinnabar, and Egnaty Creeks) tested for total and inorganic arsenic, often more than half of the total arsenic was inorganic arsenic (i.e., average ratios > 0.5 of inorganic to total As), although the data were highly variable. Should these fish be used for subsistence, inorganic arsenic concentrations may be of concern. Data from this study will be provided to the State of Alaska, Dept. of Health and Human Services, Section of Epidemiology, the federal Agency for Toxic Substances and Disease Registry, and the U.S. Environmental Protection Agency, for their guidance on subsistence consumption.

Conclusion

In conclusion, results to date indicate that while there is measurable and biologically significant elevation of mercury and to a lesser extent arsenic in aquatic biota in Red Devil Creek, similar increases are evident near other abandoned mines in the middle Kuskokwim River watershed. These include a site on Cinnabar Creek, which is on land managed by the State of Alaska in the upper Holitna Drainage, and sites on the George River. Mercury levels may affect fish health or reproduction near these sites, and both Hg and As may be of concern for subsistence consumers of fish from these sites. Ongoing data collection and analyses will better inform discussions about the transfer of Hg, As, Sb, their various chemical forms, and other trace elements within the middle Kuskokwim River region from cinnabar deposits, Red Devil, and other abandoned mines. These include the previously mentioned telemetry studies on northern pike and burbot, as well as an analysis of Hg mines and cinnabar deposits, wetland area, sediment and water Hg concentrations, and other watershed characteristics as an index for Hg methylation potential.

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