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Mercury, Arsenic, and Antimony in Aquatic Biota from the Middle Kuskokwim River Region, Alaska, 2010–2014

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Cover Photos

Background photo: *Red Devil Creek.*
Inset photo left: *filamentous algae/periphyton.*
Inset photo center: *slimy sculpin (Cottus cognatus).*
Inset photo right: *implanting a radio transmitter on northern pike (Esox Lucius).*
Back cover photo: *Red Devil Creek.*

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Table of Contents

List of Figures

List of Tables

Executive Summary

This report summarizes results and interpretation of mercury (Hg), arsenic (As), and antimony (As) concentrations in fish and aquatic insects collected in the Kuskokwim River and its tributaries between the Aniak and Stony rivers. It also summarizes the results and interpretation of mercury concentrations related to movements of northern pike (*Esox lucius*) and burbot (also known as lush) (*Lota lota*) throughout the watersheds of the middle Kuskokwim River, Alaska.

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). Two hundred twenty eight of the 332 cinnabar (mercury sulfide) locations in Alaska are within the Yukon-Kuskokwim region; 82 of these have been mined. 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). Remnant waste rock and processed ore are still present from early and mid-twentieth century mines (USGS 2005).

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). Elevated mercury concentrations in northern pike and burbot, heavily used subsistence fish species in the middle Kuskokwim River, warranted the State of Alaska to issue consumption guidance (http://bit.ly/2dqa1nt). The State of Alaska has no consumption guidance for arsenic or antimony in fish (Verbrugge 2007, Hamade 2014).

Fish and aquatic insects were collected in the mainstem Kuskokwim and large tributaries (referred to herein as "Rivers") and in small tributaries (referred to herein as "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 because they represented different trophic levels. Adult salmon were not sampled because they spend most of their lives at sea. All samples were analyzed for total mercury (THg), total arsenic (As), and antimony (Sb). A proportion of samples were also sampled 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, although northern pike from the George River had significantly higher mercury concentrations than other northern pike. There were no spatial differences in mercury concentrations in sheefish (inconnu), which are anadromous in the study area. Sheefish, unlike salmon, do not die after one spawning event and return to the spawning watershed for multiple years.

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, 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 the 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.

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 (Cinnabar Creek in the upper Holitna Drainage, and mines in the George River drainage). As is common with other studies, older predatory fish had greater mercury concentrations that younger fish of the same species or fish lower on the food chain. However, some results weren’t explained by either age or trophic status. Seasonal variations in mercury concentrations in burbot led to hypotheses that mercury concentrations may also vary with distance and direction of seasonal movements from point sources.

By using small muscle biopsies to determine mercury concentrations in northern pike and burbot equipped with radio transmitters, we were able to correlate concentrations to fish location and movements over one to two years. Northern pike were relatively stationary compared to burbot, and pike from watersheds with more mineralization and mines had significantly higher concentrations of mercury than pike from watersheds without those features. The exception was in the mainstem Kuskokwim watersheds that had high levels of mineralization and many mines within contributing tributaries, but lower pike mercury concentrations, perhaps due to greater flow. In contrast, burbot moved large distances throughout many watersheds, and overall had lower, but also less variable, mercury concentrations.

These data will help subsistence users identify species and areas that will minimize mercury exposure from eating fish, especially for women of childbearing age and children. In particular, northern pike caught in the George, Holitna, and Takotna watershed will likely have greater mercury concentrations than northern pike caught elsewhere, including on the mainstem Kuskokwim; larger pike will likely have greater mercury concentrations than small pike caught in the same location; and throughout the region, burbot (lush) generally have lower mercury concentrations than northern pike.

Introduction

The Kuskokwim River (Figure 1) arises in the mountains and rivers of Interior Alaska, between the Kuskokwim Mountains and the Alaska Range. The river flows west-southwest through Southwest Alaska for approximately 700 miles (11,130 kilometers; km), discharging water from its 48,000-square-mile (124,319 square kms) watershed into Kuskokwim Bay. This river's diverse populations of wildlife and resident and anadromous fish have supported a subsistence way of life for Yupik and Athabascan people for generations.

The middle section of the Kuskokwim River runs through a highly mineralized region of Alaska (middle Kuskokwim region) that contains mercury (Hg), antimony (Sb), gold (Au), silver (Ag), and polymetallic deposits (Szumigala and Swainbank 1998; USGS 2008). Much of the mineralization in southwest Alaska contains extensive deposits of cinnabar, the principal mercury mineral. Cinnabar has been found at 332 locations in Alaska, 228 within the Yukon-Kuskokwim watershed, of which 82 have been mined (USGS 2008). The prevalence of mineralization in an area where fish are a

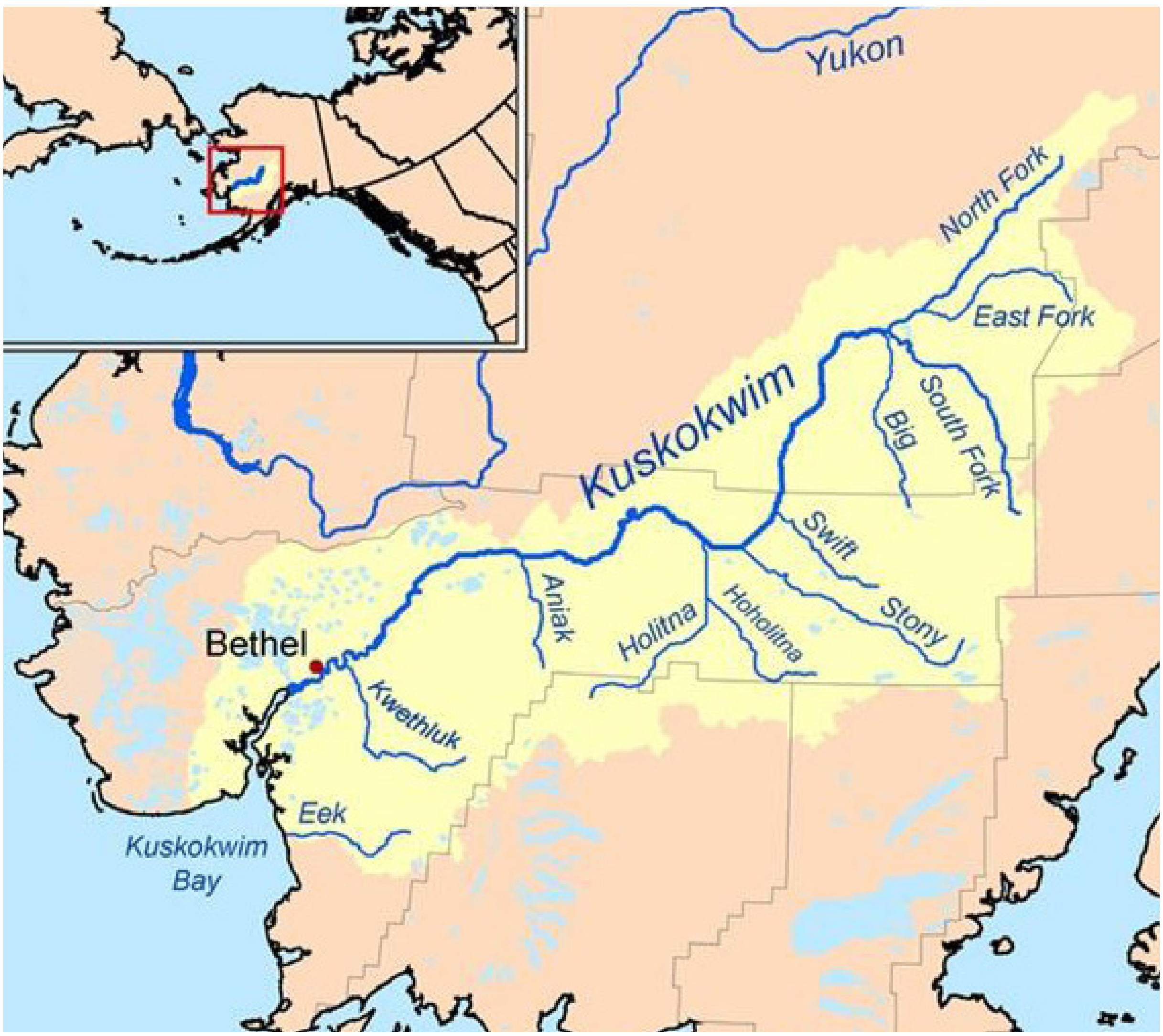


Figure 1. Map showing the Kuskokwim River Watershed, Alaska.

primary source of protein has led to several studies of mercury and other metals in fish (Gray et al. 1994, 2000; Jewett and Duffy; 2007). All previous studies, however, focused on relatively small study areas and a limited range of species.

Purpose and Objectives

The goal of this study is to establish a baseline condition of several different metals in fish tissue, including mercury and methyl mercury (MeHg; the most toxic form), along a roughly 270-mile stretch of the Kuskokwim River and selected tributaries between the Aniak and Takotna rivers. Earlier work established that inorganic mercury in the river/watershed was converted into methyl mercury, bioaccumulating in individual fish and biomagnifying up the food chain.

This study evaluated mercury concentrations in aquatic biota, especially edible fish, and related those concentrations to fish movements and distribution in different reaches of the middle Kuskokwim River watershed. Fish tissue and macroinvertebrate contaminant data were collected to assess the range of concentrations in a number of species, representing multiple trophic levels, throughout the study area. Seasonal fish movement data and tissue data in select species were collected to identify areas where fish were being exposed to metals, and to evaluate relative bioavailability of mercury in the Kuskokwim River and sampled tributaries. Surface water and streambed sediment contaminant data were also collected to establish the nature of source material in individual tributaries.

This study was central to an effort by the Bureau of Land Management (BLM) to assess contaminants in the middle Kuskokwim region (Varner 2012).

Objectives included:

- Sampling surface waters and streambed sediments for contaminants, including total mercury and methyl mercury, in the mainstem Kuskokwim and selected watersheds within the project area;
- Sampling all trophic levels from benthic macroinvertebrates to top-predatory fish species;
- Sampling resident fish species harvested most often 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 predatory fish species; and,
- Evaluating trends in concentrations of multiple metals and fish movement in relation to distance and downstream location from the Red Devil Mine and other mercury mine sites.

This document contains the results and interpretation of mercury, arsenic, and antimony 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.

The study was conducted jointly by the BLM, Alaska Department of Fish and Game (ADFG), and U.S. Fish and Wildlife Service (USFWS) from 2010–2014. Data analysis was focused primarily on the chemical and statistical analyses of metal contaminants data from fish and macroinvertebrates collected in the middle and lower Kuskokwim River.

Mining History and Metals Mobility

Active mining began along the middle Kuskokwim region in the early 20th century. The majority of mines were focused on gold recovery, and most were placer mines. A small number of lode mercury mines were developed in the region, but none have been active since the 1960's. The impact of placer mines on fish tissue concentrations has not been studied in the middle Kuskokwim region. Better understood is the influence of processed tailings associated with lode mines on local biota.

Eight mines produced elemental mercury, or quicksilver, at five locations, including the Alice and Bessie (or Parks), Kolmakof, Cinnabar Creek, Lucky Day, and Red Devil mines. The largest was Red Devil Mine, abandoned in 1971, but Cinnabar Creek also produced substantial amounts of mercury (Sainsbury and MacKevett 1965). One of three known mineral deposits in the Cinnabar Creek watershed was mined in the late 1950s, producing several hundred flasks of mercury from the on-site processing, or retort, facility (Red Devil produced 35,000 flasks) (USGS 2008).

The results of a Red Devil Mine environmental investigation have enhanced our understanding of mercury and arsenic mobility due to the presence of uncontrolled waste rock and processed tailings. Data collected in the vicinity of the tailings pile at Cinnabar Creek indicate similar conditions to those observed in Red Devil Creek. While the potential environmental impacts of the Red Devil Mine are well understood, none of the potential environmental impacts of the other mines have been studied beyond U.S. Geological Survey (USGS) work at Cinnabar Creek (Gray et al. 2000).

Trace metals from uncontrolled mine wastes or exposed mineral deposits can readily transfer to the environment through simple physical releases of sediments into flowing waters (erosion) (Hoffman et al. 2003), or through chemical processes such as dissolution or acidification of sulfide minerals (Forstner and Wittman 1983). The bedrock prevalent in the middle Kuskokwim region contains sufficient carbonate minerals to prevent acidification from being a prominent factor in metals mobility. Therefore, dissolution in water and erosion of fine-grained material containing mercury, arsenic, and antimony are the main methods by which these metals move into water and eventually into biota in the middle Kuskokwim region.

Previous Studies

Mercury in fish is a potential exposure pathway for humans along the Kuskokwim River and is a significant concern due to high fish consumption rates by subsistence consumers (e.g., Jewett and Duffy 2007). The prevalence of fish in rural diets has fueled studies of mercury in fish throughout Alaska (Gray et al. 1994, 2000; Duffy et al. 1999; Jewett et al. 2003; Jewett and Duffy 2007; Zhang et al. 2001; USFWS, unpublished data).

Gray et al. (2000) sampled sediments, water, and fish tissue both 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). In sediments, they found that total mercury was greater downstream from these mines compared to reference reaches, and that methyl mercury made up less than 1% of the total mercury in sediments (except for one site at the mouth of a beaver dam complex on Cinnabar Creek). By comparing mercury in filtered and unfiltered water, they determined that most of the mercury downstream of mines is suspended or particulate, not dissolved (Gray et al. 2000). Although these authors hypothesized that mercury from the mines would be diluted downstream, Dolly Varden (Salvelinus malma) and Arctic grayling (Thymallus arcticus) collected downstream from mines had much greater mercury concentrations (up to 620 and 420 nanograms per gram wet weight [ww] in muscle) compared to fish from regional baseline streams (200 nanograms per gram) (Gray et al. 2000). Gray et al. (2000, p. 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.”

A combination of factors determine the amount and chemical form of mercury that ends up in fish, including mercury 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 mercury from sediments or bedrock and use of mercury amalgam in gold mining); and geologic erosion. Each of these sources has spatially and temporally variable contributions to the total amount of mercury that enters Alaska’s waters. However, it is difficult to determine proportional contributions of any of these sources (Lindberg et al. 2007), especially when there are multiple potential point sources within a region or watershed.

Since most mercury in fish muscle is methyl mercury, the methylating potential of the watershed is also important. Although mercury can occur as elemental (liquid) mercury and mineral complexes (cinnabar) in the ground, inorganic mercury are converted to toxic organic complexes (e.g., methyl mercury) 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 mercury—as methyl mercury—in fish, assuming other factors are equal.

Fish are exposed to methyl mercury primarily through their diet (Weiner et al. 2003). Methyl mercury—unlike inorganic mercury—is not easily excreted and builds up (“biomagnifies”) in food chains (Figure 2). Older fish often have greater concentrations than younger fish of the same species in the same area (Evans et al. 2005) because of this.

Range size also affects mercury (total mercury and methyl mercury) concentrations in fish. Non-migratory fish with small ranges living near a mercury-containing material that is in contact with the water, such as slimy sculpin, will have greater concentrations compared to migratory fish with larger ranges such as sheefish. Fish within the middle Kuskokwim River watershed have a wide variety of range sizes and migratory habits (Figure 3).

Exposure through gills or digestive tissue to waterborne, non-organic, particulate-bound mercury (such as from an eroding mine site) can also cause elevated inorganic mercury concentrations in fish, as hypothesized by Gray et al. (2000). The ratio of methyl mercury to total mercury (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 diet, where most of the total mercury is methyl mercury. In other words, an MeHg:THg ratio in fish that is much less than 1.0 can indicate acute, short-term exposure to mercury in water (Guilherme et al. 2010), while one closer to 1.0 can indicate chronic, longer-term exposure to mercury via ingestion of methyl mercury from prey (Weiner et al. 2003; Lasorsa and Allen-Gil 1995).

Arsenic and antimony have not been studied as much as mercury, but both are ubiquitous and toxic substances that are found in elevated

[1] Where THg = MeHg + inorganic Hg

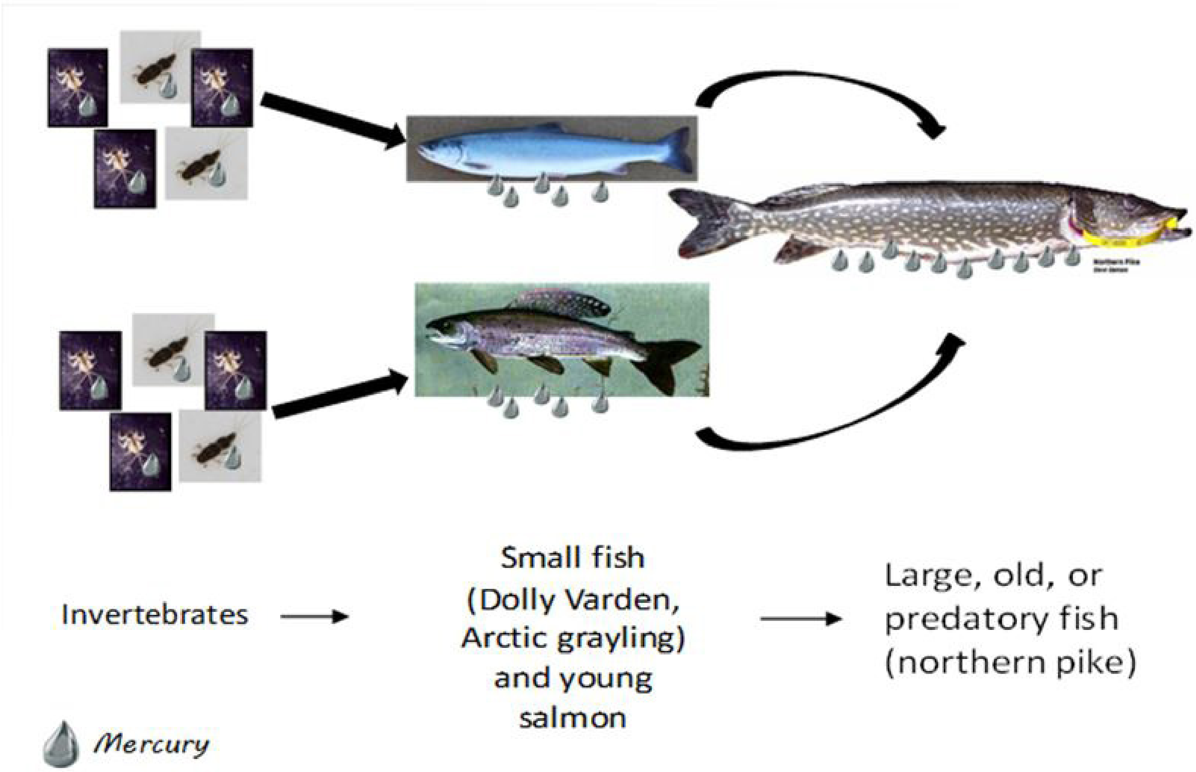


Figure 2. Generalized schematic for mercury biomagnification in the aquatic food chain of the middle Kuskokwim River watershed, Alaska.

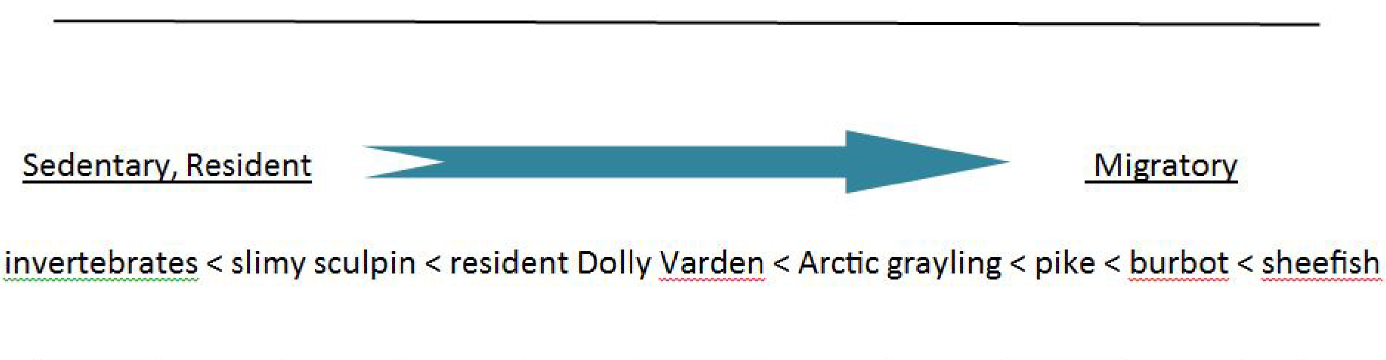


Figure 3. Relative migratory propensity of aquatic biota in the middle Kuskokwim River, Alaska. Propensity ranges from completely sedentary (macroinvertebrates) to estuarine anadromous (sheefish).

concentrations in mineralized areas such as the middle Kuskokwim region and downstream of active and abandoned mines (Eisler 1988). The amount of total arsenic and antimony that ends up in aquatic biota depends on exposure, accumulation, excretion rate. and mechanism. Organisms can be exposed through water or food uptake, but unlike mercury, arsenic and antimony do not biomagnify up food chains, and their behavior in aquatic systems and organisms is relatively transient and dynamic (Eisler 1988).

Methods

Study Area

The study area covers the middle section of the Kuskokwim River where mineralization in the vicinity of the river is most prevalent. The full length of the middle Kuskokwim and the tributaries included in this study are described in depth by Varner (2012). The 2010 fish tissue sampling area included a 117 km (73 miles) section of the Kuskokwim River from the confluence of the George River upstream to the confluence of the Stony River, with approximately 73 km (45 miles) of tributaries (Figure 4, Figure 5). The study area was expanded in 2011 to include the Kuskokwim River from Aniak to just upstream of McGrath, an approximately 410-km (255-mile) span (Figure 6).

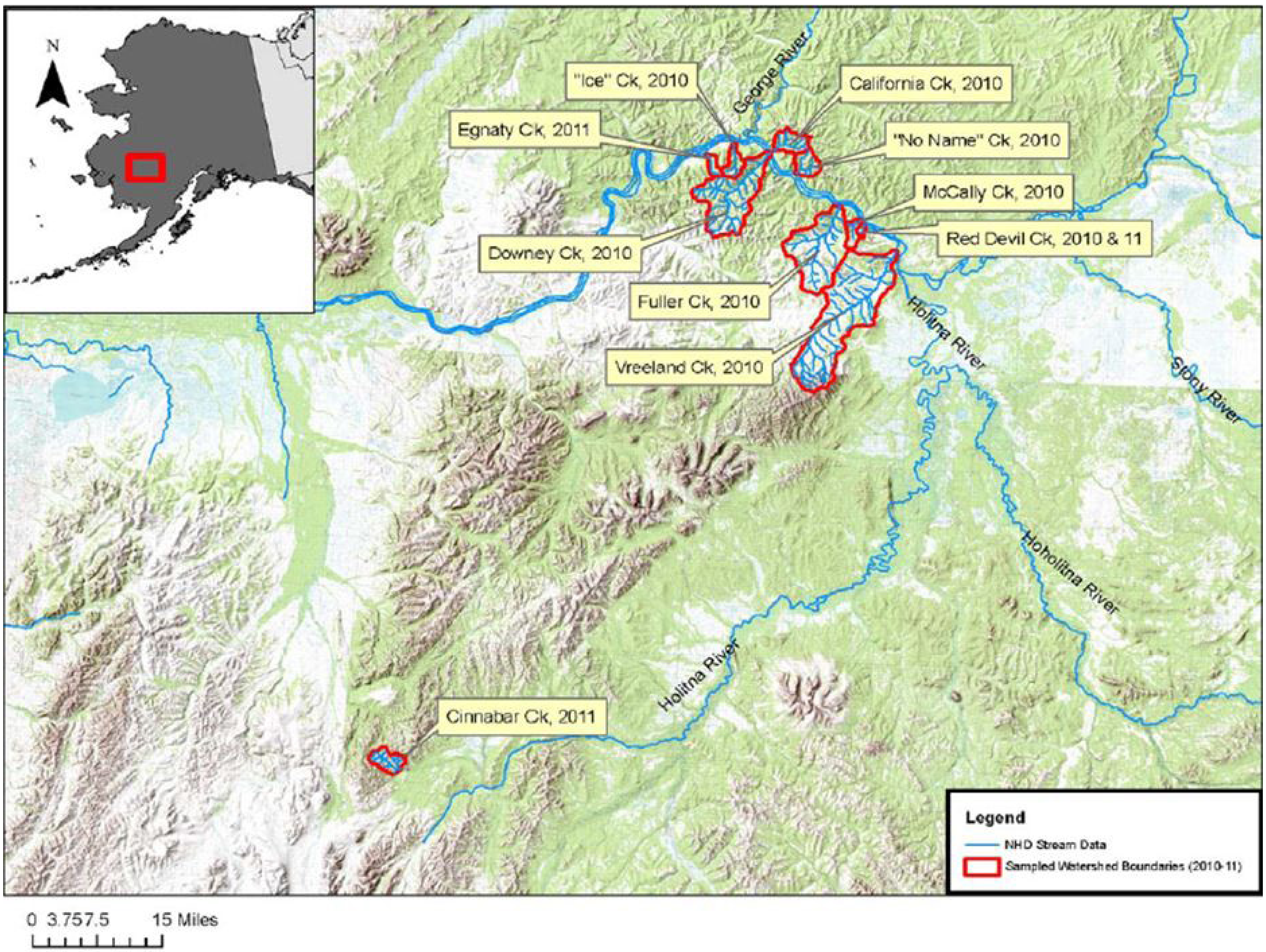


Figure 4. Tributary sample reaches for analysis of mercury in aquatic biota from the middle Kuskokwim River, Alaska, in summer 2010.

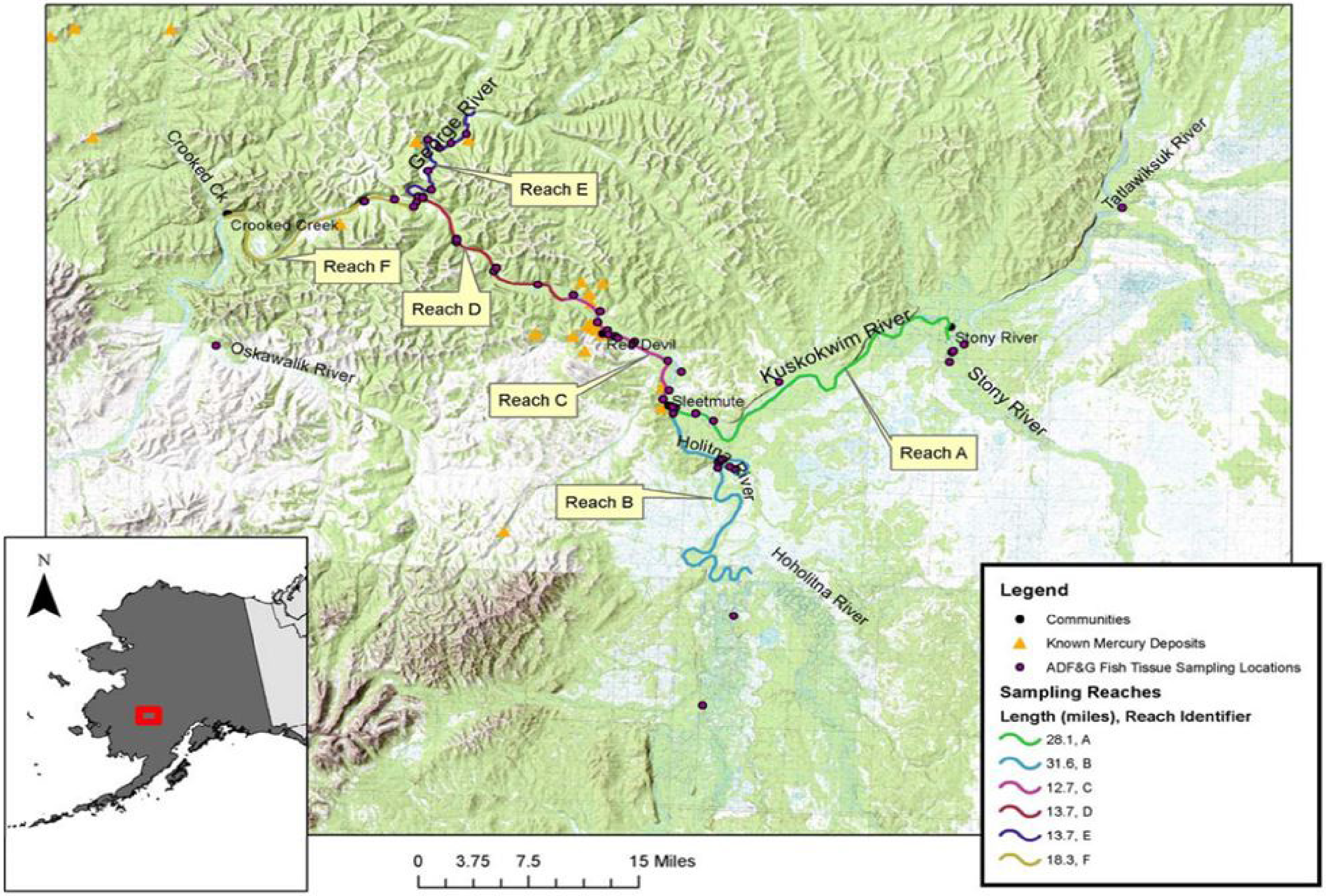


Figure 5. River sample reaches for analysis of mercury in aquatic biota from the middle Kuskokwim River, Alaska, in summer 2010.

Fish Tissue 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 mercury 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. In 2011, additional non-lethal muscle biopsy samples were collected from pike and burbot (Lota lota) that were radio-tagged for telemetry. The several separate collection events are summarized in Table 1.

Sample 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; Figure 4). Tributary sample reaches were further defined as the first 300 meters of the creek above the main river high water mark. The mouths of slough-like and low-gradient tributaries in the mainstem Kuskokwim, and some larger tributaries ("River" sample reaches; Figure 5) were sampled because they were good habitat for some target species (northern pike [*Esox lucius*]) and burbot (also known as lush). River sample reaches were delineated by hydrologic features, including large tributaries (Figure 5). Watershed characteristics of most sampling reaches were summarized by Varner (2012).

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, burbot, northern pike, and sheefish (also known as 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*

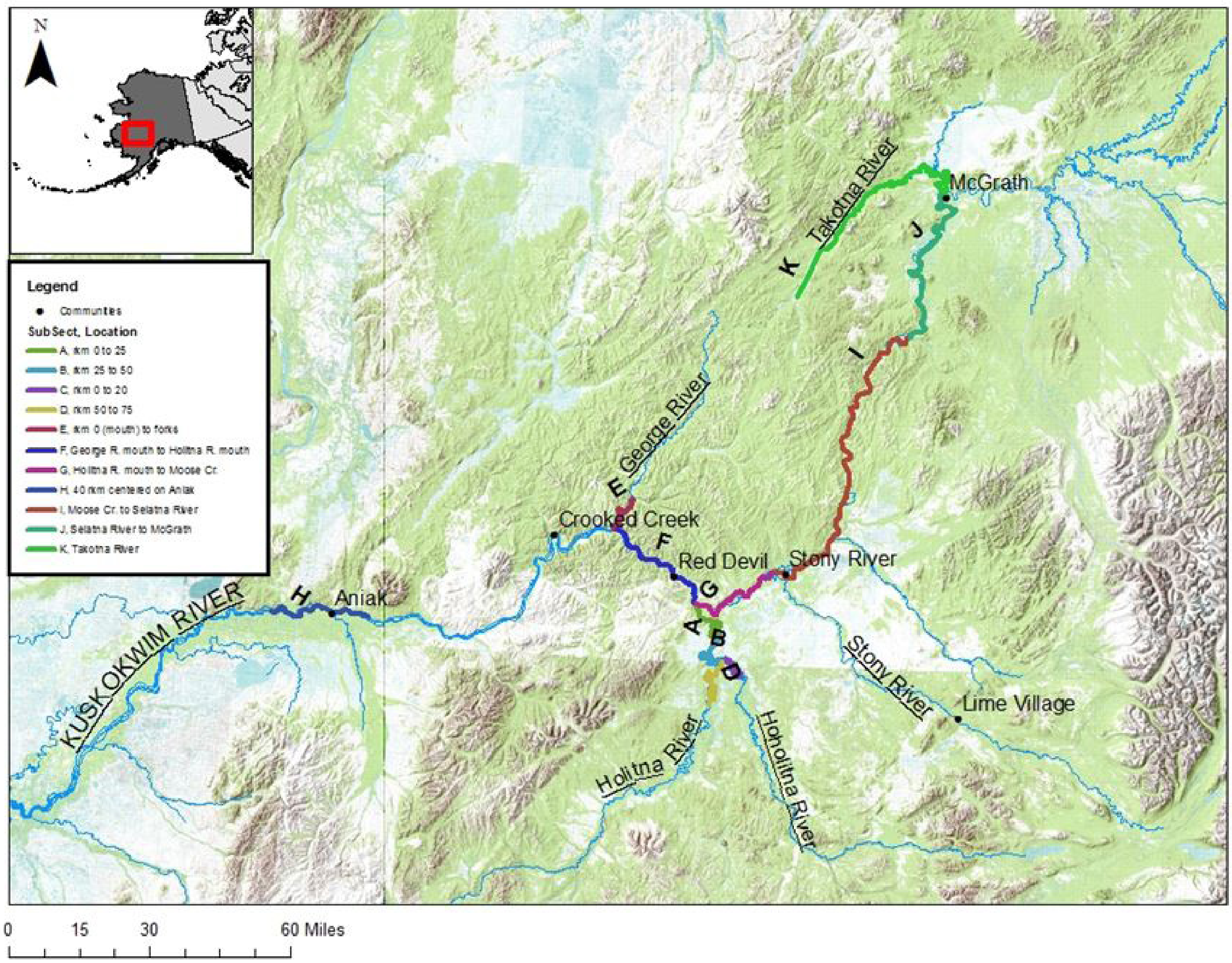
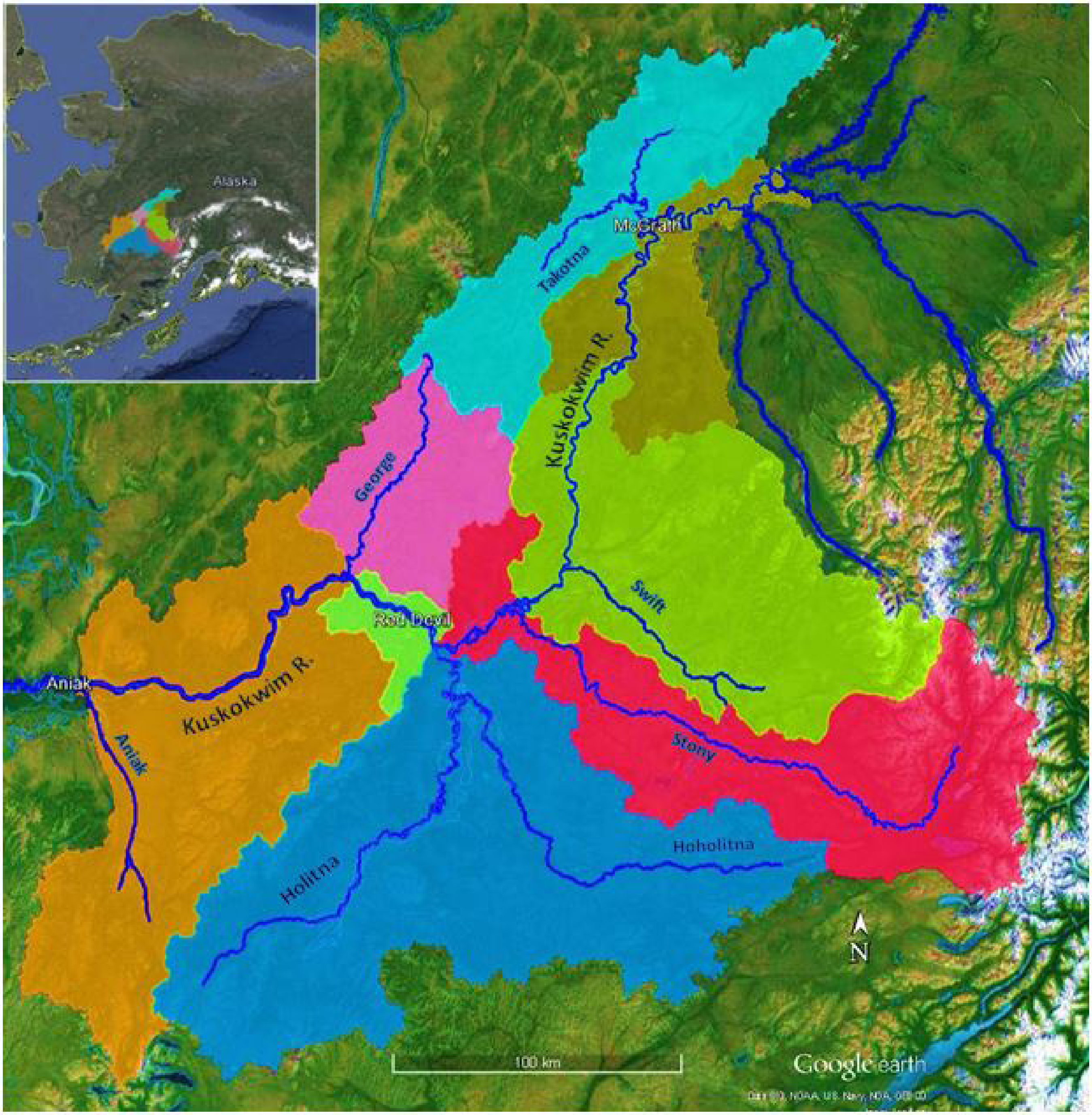


Figure 6. Stream reaches sampled for radio tag deployment and tissue sample collection in northern pike and burbot in the middle Kuskokwim River area, Alaska, 2011-12. Reaches were slightly different from those previously sampled (Figure 5). Within a subsection, radio telemetry tags and biopsy samples were apportioned among fish length strata.



Kusko-Aniak: Kuskokwim River – Aniak R. (incl.) to George R.
George: George River
Kusko above George: Kuskokwim River – George R. to Sleetmute
Holitna: Holitna and Hoholitna Rivers
Kusko-Stony: Kuskokwim River – Holitna R. to Stony R (incl.)
Kusko–Swift: Kuskokwim River –Stony River to Selatna, incl. Swift R.
Kusko above Selatna: Kuskokwim River – Selatna R. to Middle/North Forks
Takotna: Takotna River and Nixon Fork

Figure 7. Watersheds in the middle Kuskokwim River region, Alaska, USA, used to compare mercury concentrations and fish movements. Watershed boundaries were based on U.S. Geological Survey Hydrologic Unit Codes for Alaska (http://agdc.usgs.gov/data/projects/anwr/metadata/akhuc.html).

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

Date	Purpose	Species Collected	Locations[a]
June–October 2010	General sampling event in most tributary and all river reaches	All	All except Cinnabar and Egnaty creeks.
November 2010–January 2011	Characterize mercury in winter burbot subsistence fishery	Burbot (n=54)	Reaches A and C of the Kuskokwim River,
June 2011	Establish seasonal movement patterns; non-lethal muscle biopsies from radio-tagged fish	Burbot (n=118)	George River, Holitna and Hoholitna rivers, Kuskokwim River
June–September 2011	Characterize mercury concentrations in small fish from additional tributaries, including one with a former mercury mine	Pike (n=160)	Cinnabar Creek, Egnaty Creek, Red Devil Creek

[a]See Figures 4-6 n=number of specimens

cognatus), juvenile Dolly Varden, 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. Adult spawning salmon were not sampled because their muscle trace element concentrations would primarily reflect ocean exposure rather than freshwater. A very few juvenile coho salmon (*Oncorhynchus kisutch*) and juvenile 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.

Analytical Chemistry

In 2010, Physis Environmental Laboratories, Inc. (Physis) of Anaheim, California, analyzed samples for mercury, arsenic, antimony (and 16 other trace elements) and Frontier Global Sciences, Inc. (Frontier) of Seattle, Washington analyzed samples for methyl mercury. In 2011, Frontier analyzed samples for trace elements, methyl mercury, and inorganic arsenic. Their 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 Standard Operating Procedure FGS-054), methyl mercury (Frontier Standard Operating Procedure Method FGS 070), and inorganic arsenic (EPA Method 1632) in tissues. At Physis, mercury Method Detection Limits (MDLs) were 0.00001 micrograms per gram (µg/g), and Reporting Limits (RLs) were 0.02 µg/g; arsenic and antimony 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.

Analytical data reports underwent a third-party quality assurance review using EPA Validation Level IV criteria to ensure that data quality was appropriate for remedial decisions under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (EPA 2010) by Laboratory Data Consultants, Inc. of Carlsbad, California for samples analyzed by Physis in 2010, and by Physis for samples analyzed by Frontier in 2011. No data were considered invalid after the quality assurance review, so all were used in subsequent analyses.

Telemetry Procedures

Between 23 June 2011 and 13 December 2013, the project team located pike and burbot, fitted them with transmitters (LotekTM coded tags with motion sensors), and collected non-lethal muscle biopsy samples for laboratory analysis. The operational life of the northern pike and burbot transmitters was approximately 2.5 years, and the smaller transmitters used for Arctic grayling were expected to operate for approximately 14 months. Radio tags operated on five frequencies between 149.500 and 149.999 megahertz, with individual transmitters digitally coded for identification. Motion sensors indicated when there was no movement for > 24 hours, indicative of mortality or an expelled tag.

Radio-tagged fish were located using a combination of ground-based tracking stations and aerial tracking surveys. Five solar-powered tracking stations were used to collect movement information by recording fish passage, which were located on the mainstem Kuskokwim River near Aniak, Holitna River approximately 1.5 km (1 mile) upstream from its mouth, mainstem Kuskokwim river approximately 5 km (3.1 miles) downstream from the George River, mainstem Kuskokwim approximately 5 km (3.1 miles) downstream from Stony Village, and mainstem Kuskokwim at McGrath upstream from the Takotna River. Data recorded from the tracking stations was used to supplement and help interpret the aerial survey data. These tracking stations were only operational when there was sufficient solar radiation to power them from mid-March to mid-November. Recorded data were periodically downloaded using a satellite modem.

Tracking flights were conducted using a fixed wing aircraft and a Lotek SRX 600 receiver with an internal global positioning system (GPS) that recorded time and location. Approximately 11 flights were flown between early June 2012 and February 2014. As no northern pike were expected in the reach of the Kuskokwim River downstream of the Aniak River, this reach was flown less frequently. A survey of this reach was flown once each during summer and winter to determine whether major movements occurred out of the 2012 study area (i.e., downstream of Aniak River).

Tagged fish were not always located within tagging reaches (Figure 6), so the mainstrem Kuskokwim River and major tributaries within the study area were divided into watersheds based on U.S.G.S. Hydrologic Units (http://agdc.usgs.gov/data/projects/anwr/metadata/akhuc.html). These include (Figure 7):

1. **Kusko-Aniak:** the mainstem Kuskokwim River from Aniak to the George River, including the Aniak and Oskawalik rivers;
2. **George**: the George River, including the East and South forks;
3. **Kusko above George:** the mainstem Kuskokwim River upstream of the George River to Sleetmute;
4. **Holitna:** the Holitna and Hoholitna rivers;
5. **Kusko-Stony**: The mainstem Kuskokwim River from the Holitna River to the Stony River, including the Stony River and Moose Creek;
6. **Kusko-Swift:** The mainstem Kuskokwim River from the Stony River to the Selatna River, including the Swift and Tatlawiksuk rivers;
7. **Kusko above Selatna**: The mainstem Kuskokwim River from the Selatna River to the North Fork of the Kuskokwim; and,
8. **Takotna:** The Takotna River, including the Nixon Fork.

Statistical Data Analysis

A variety of parametric and non-parametric statistical methods (SYSTAT 13) was used to explore patterns and test differences in average (arithmetic mean) total mercury, methyl mercury, total and inorganic arsenic, and antimony concentrations among and between reaches, species, and tissues; among watersheds in which radio-tagged fish were captured (“capture” watersheds) or had the highest proportion of locations (“max-use” watersheds); and, whether mercury was correlated with length (if so, length was used as a covariate in subsequent analyses). These included General Linear Models (GLMs), similar to Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA), Pearson’s correlation; Kruskal-Wallis and Mann-Whitney U tests on ranks (used if the analyte was detected in 50–80% of samples). Post-hoc tests (Tukey’s or Bonferroni’s) were used to determine statistical significance for differences among reaches and species. Statistical significance equated to $\alpha < 0.05$.

Data were log-transformed when necessary to meet assumptions of normality and equal variance, but are always reported as received from the laboratory, in µg/g (microgram per gram) or milligrams per kilogram (mg/kg), which are equivalent to each other (and to parts per million), 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). Groups that had no or few samples (e.g., a particular species was not collected, or was collected in low numbers in a particular reach) were generally not included in statistical analyses but were included in discussion.

To evaluate the relationships between mercury concentrations and fish movements, only radio-tagged fish with > 3 locations were used. Locations were used if the signal was strong, or estimated if there was a strong signal at the same location immediately prior to or subsequent to the date of that flight.

Watershed characteristics were compared that might affect mercury concentrations in fish to mean mercury concentrations of radio-tagged northern pike that used each watershed the most.

The number of sites of mineral occurrences/prospects, and mines (active and inactive) in the Alaska Resource Data File (ARDF) (USGS 2008) were used as potential point sources within each watershed. In this dataset, mines have had enough development activity to actually produce minerals; occurrences and prospects have little to no development.

The subset was also evaluated that contained mercury as a main or secondary commodity. The total watershed area was used to describe each watershed’s ability to capture atmospherically deposited mercury, and the proportion of each watershed that was wetland was used to describe each watershed’s potential to transform elemental mercury into the more toxic methyl mercury. To identify the strongest explanatory watershed characteristics for mercury in northern pike, models were selected that had the lowest Akaike information criterion (corrected to account for finite sample size) (AICc) of those characteristics that were statistically significant.

Results

Statistical tests for differences among sampling sites and species require approximately similar sample sizes. Not all species, however, were collected at all sample reaches in both 2010 and 2011, resulting in varied sample sizes (Tables 2 and 3). Consequently, statistical comparisons were not always possible 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, tributaries between Red Devil Creek and the George River, and downriver of the George River. Slimy sculpin, juvenile Dolly Varden, and macroinvertebrate data from 2011 tributary reaches were compared among Red Devil, Cinnabar, and Egnaty creeks.

Mercury (Hg)

There were significant differences between mercury 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 for statistical analysis. Data was pooled for the purpose of creating study-wide summary statistics (Table 4). In the same species and tissues, data from 2010 were consistently greater than 2011 data (Figure 8). Samples from 2010 were analyzed at Physis, and 2011 samples were analyzed at Frontier, but it is unknown whether the differences were attributable to annual or inter-laboratory variation.

Mercury Differences among Tributary Samples

Small, sedentary fish and macroinvertebrates from Red Devil and Cinnabar creeks had significantly greater total mercury concentrations than the same biota in other tributaries of the middle Kuskokwim River, after accounting for unequal sample sizes, interannual or inter-laboratory variation, and body length.

Length and total mercury were significantly correlated for slimy sculpin whole body samples ($r = 0.317$, $p < 0.000$, $n=166$), and was used as a subsequent covariate. Length was not correlated with mercury for whole body Dolly Varden ($r = 0.262$, $p = 0.059$, $n=53$) or whole body Arctic grayling ($r=0.090$, $p = 0.477$, $n=64$).

In 2010, total mercury was significantly greater at Red Devil Creek compared to all other reaches in slimy sculpin (even after accounting for length; ANOVA, $F = 2.927$, $p= 0.007$) macroinvertebrates (ANOVA, $F=179.2$ 2,23, $p < 0.001$), and Dolly Varden, (ANOVA, $F= 198.1$ 2,48, $p < 0.001$) (Figure 9a,b,d). Arctic grayling did not have significantly different mercury concentrations among reaches, but Arctic grayling were also not collected at Red Devil Creek in 2010 (Figure 9b).

In 2011, total mercury 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, $F_{2,30} = 12.369$, $p<0.001$) (Figure 9a,b). Macroinvertebrates were not collected at Egnaty Creek, but like slimy sculpin and Dolly Varden, there was no significant difference in macroinvertebrate total mercury concentrations between Cinnabar and Red Devil creeks (ANOVA, $F_{1,6}=0.016$, $p=0.904$) (Figure 9d).

Mercury Differences among River Samples

Length and age were significantly correlated with muscle total mercury concentrations in

Table 2. Number of samples collected by tributary location and species for a study of trace element dynamics in aquatic biota in the middle Kuskokwim River, Alaska, 2010–2011. Reaches are listed from downstream to upstream.

Tributary (Year)	Arctic Grayling	Slimy Sculpin	Dolly Varden	Macroinvertebrates
Egnaty Creek (2011)	0	13	2	0
Ice Creek (2010)	11	24	7	3
Downey Creek (2010)	4	20	7	6
California Creek (2010)	12	23	2	3
No Name Creek (2010)	12	22	2	1
Fuller Creek (2010)	24	22	0	5
McCally Creek (2010)	0	10	22	2
Red Devil Creek (2010)	0	22	11	3
Red Devil Creek (2011)	2	24	6	4
Vreeland Creek (2010)	1	23	2	6
Cinnabar Creek (2011)	0	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 middle Kuskokwim River, Alaska, 2010–2011. Reaches are listed from downstream to upstream.

River (Reach)	Arctic Grayling		Burbot		Northern Pike		Sheefish	
	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle
Oskwalik River (F)	6	6	0	0	0	0	0	0
Kuskokwim River (F)	7	7	21	21	6	6	3	3
George River (E)	8	8	17	17	17	17	7	7
Kuskokwim River (D)	3	3	11	11	32	32	0	0
Kuskokwim River (C)[a]	1	1	16	16	33	33	4	4
Kuskokwim River (C) (winter 2011)	0	0	4	4	0	0	0	0
Holitna River (B)	0	0	2	2	35	34	12	12
Kuskokwim River (A)	0	0	19	19	2	2	0	0
Kuskokwim River (A) (winter 2011)	0	0	50	50	0	0	0	0
Stony River (A)	0	0	0	0	11	11	0	0
Tatlawiksuk River (A)	0	0	0	0	0	0	12	12

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

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

Northern pike from the George River (Reach E) had significantly greater mercury concentrations than northern pike from all other reaches (r F5,127 = 6.616, $p < 0.0001$, n=134) (Figure 10a). *Burbot* collected in Reach

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 (n)	**Year**	**Tissue**	**Minimum**	**Maximum**	**Mean**	**SD[a]**
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)[b]	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)[b]	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)[b]	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] SD = standard deviation
[b] There were statistically significant differences between data collected in 2010 and data collected in 2011.

Table 5. Pearson's correlation coefficient r, Bonferroni's p adjusting 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 middle Kuskokwim River, Alaska, 2010.

Species	**Liver Mercury and Muscle Mercury *r* (p, n)**	**Age and Muscle Mercury *r* (p, n)**	**Length and Muscle Mercury *r* (p, n)**
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)

r = Person's correlation coefficient; p = Bonferroni's p-value adjusting for multiple comparisons; and n = number of samples

C of the Kuskokwim River had significantly lower mercury 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) (Figure 10b). There were no significant differences among reaches for sheefish (ANOVA $F_{4,33}$ = 1.182, p=0.337, n=38) (Figure 10c). 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 (Figure 10d). Only one Arctic grayling was collected in Reach C, so data for this fish were not included in statistical analyses. However, that individual fish had the greatest mercury muscle concentration of all River-collected Arctic grayling (Figure 10d).

Overall, when a species had significantly different mercury concentrations among reaches (northern pike, burbot, and Arctic grayling), fish from the George River had the greatest mercury concentrations.

Mercury in All Species

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

Methyl Mercury to Total Mercury Ratios

In fish tissues, an MeHg:THg ratio much less than 1.0 can indicate acute, short-term exposure to mercury 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 mercury via ingestion of methyl mercury 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). They concluded that the methyl

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

Species and Sample	Sample Reach Type	Tissue	Number of Samples	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

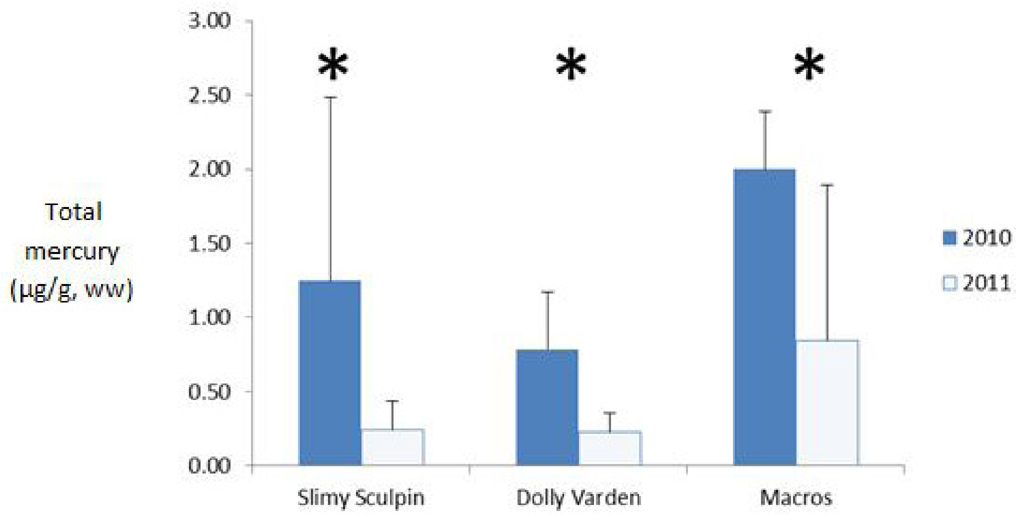


Figure 8. Mean (+ standard deviation, SD) total mercury concentrations (µg/g, ww) in small fish and macroinvertebrates collected in Red Devil Creek, Alaska, 2010 and 2011. Asterisks indicate significant differences in mercury concentrations between years; samples were also analyzed at different laboratories each year.

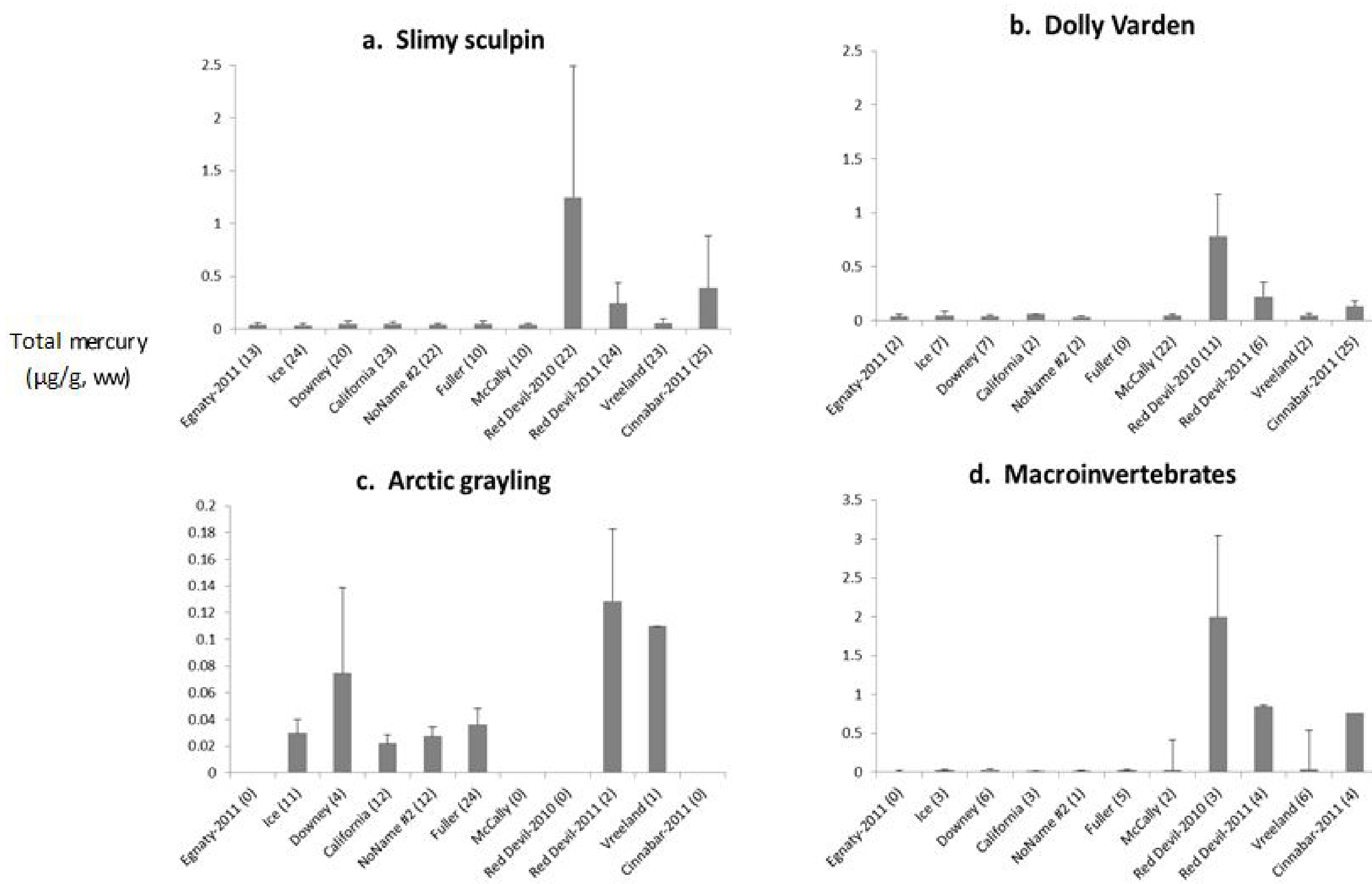


Figure 9. Mean (+ standard deviation, SD) total mercury concentrations (µg/g, ww) in muscle of (a) slimy sculpin, (b) Dolly Varden, (c) Arctic grayling, and (d) macroinvertebrates from small tributaries of the middle Kuskokwim River, Alaska, 2010-2011.

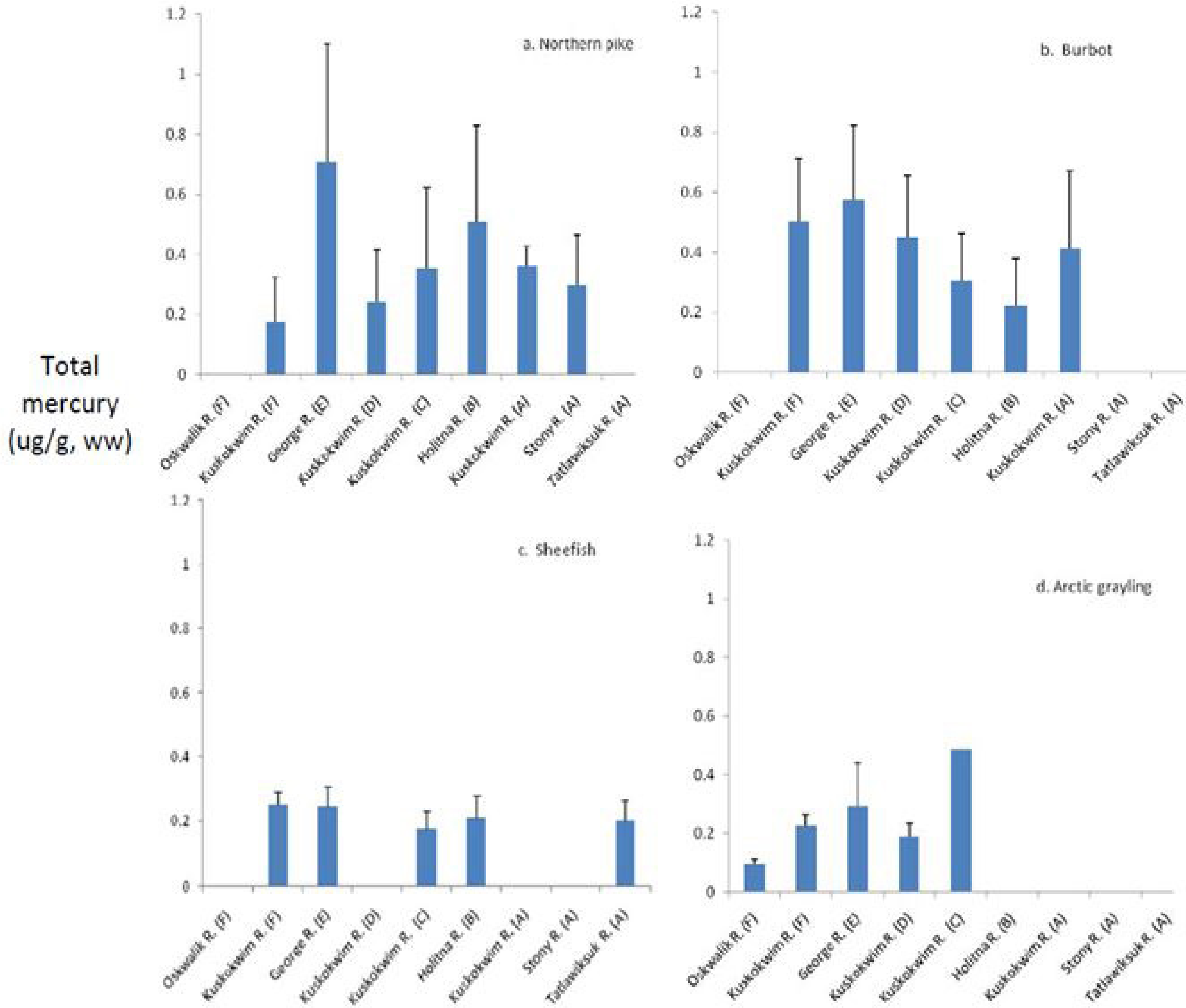


Figure 10. Mean (+ standard deviation, SD) total mercury concentrations (µg/g, ww) in muscle of northern pike (a), burbot (b), sheefish (c), and Arctic grayling (d) from the middle Kuskokwim River and its large tributaries, Alaska, 2010. Reaches are listed in upstream order from left to right.

mercury proportion was lower at the “base of the food chain” in freshwater cladoceran zooplankton (Watras and Bloom 1992, p. 1317).

Mercury in, and Movements of, Radio-tagged Fish

Northern pike and burbot are the two resident fish species that both prey on a number of smaller fish species and are an important winter food source for local communities. Because mercury concentrations often increase with trophic level (biomagnification), the telemetry component of this study focused primarily on these two top predators. A total of 210 northern pike were captured in six watersheds, and 63 burbot were captured in three watersheds Table 7, Figure 7). The median number of usable locations were 13 (range = 4–20) for northern pike and 6 (range = 4–21) for burbot.

The watershed in which a fish was captured and tagged was its “capture” watershed. The watershed in which a fish had the greatest proportion of its locations, i.e., the watershed in which it stayed, was its “max-use” watershed. Only 32% of burbot stayed within their capture watershed, but 90% of northern pike did (Figure 12). These results were expected based on local traditional ecological knowledge. Pike that did not stay in their capture watershed were almost always found in nearby watersheds; for example, of the seven pike captured in the Kusko above George watershed, three (43%) stayed there and four moved to and stayed in the adjacent Holitna watershed. Interestingly, 80% of burbot captured in the Kusko-Aniak watershed stayed there, but only 9% and 10% stayed in the Kusko above George and the Kusko-Stony capture watersheds, respectively.

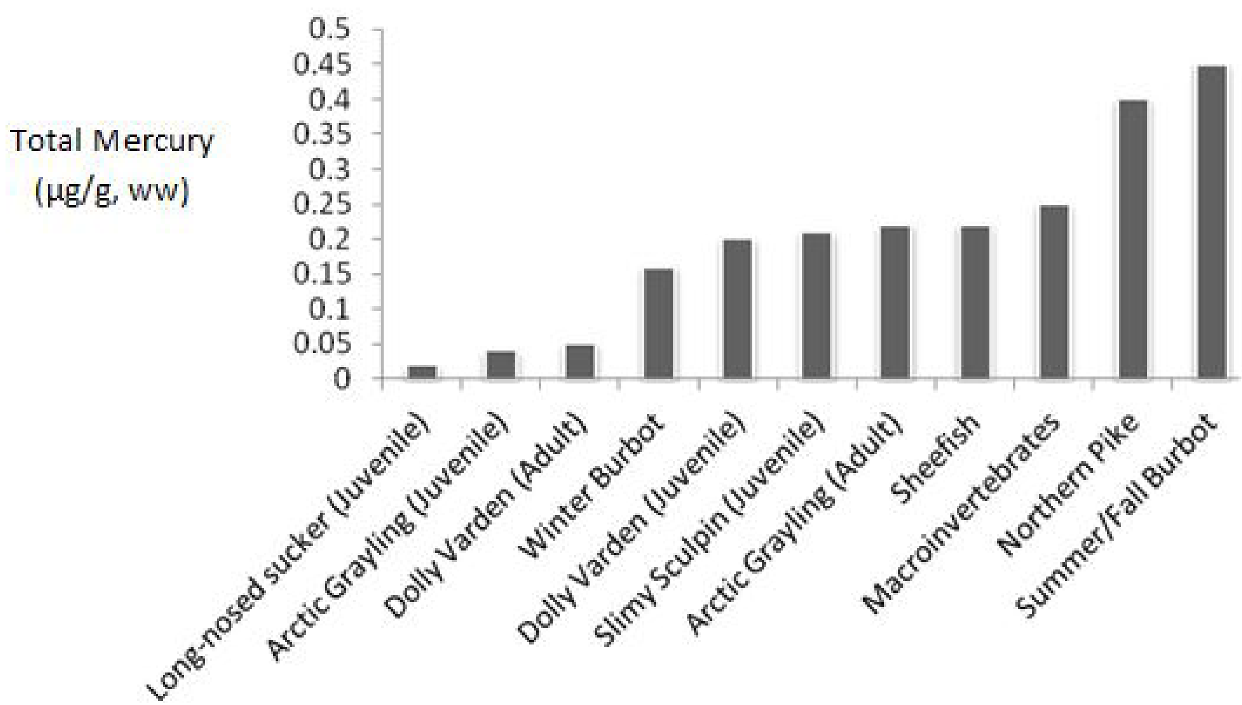


Figure 11. Mean total mercury concentrations (µg/g, ww) in fish (by species) and macroinvertebrates collected in the middle Kuskokwim River region, Alaska, 2010.

Table 7. Number of fish sampled within watersheds (see Figure 7) to study on mercury in relation to fish movements and potential point sources in the Middle Kuskokwim River region, Alaska, 2011–2013.

	Capture Watershed							
Species	**Kusko-Aniak**	**George River**	**Kusko above George**	**Holitna**	**Kusko-Stony**	**Kusko-Swift**	**Kusko above Selatna**	**Takotna**
Northern pike	0	23	7	104	18	0	26	32
Burbot	20	0	21	0	22	0	0	0

Table 8. Results of modeling to determine strength of association between watershed characteristics and mean mercury concentrations in northern pike muscle from the middle Kuskokwim River region, Alaska, 2011–2013.

Watershed characteristic	*p*-value[a]	AIC[cb]
Number of occurrences/prospects (all minerals)	0.003	1.87
Number of mines (all minerals)	0.04	9.29
Number of mercury-containing mines	0.169	12.6
Number of mercury-containing occurrences/prospects	0.813	15.7
Total area	0.842	15.7
Proportion of watershed in wetland	0.989	15.8

[a] A *p*-value < 0.05 was considered significant.
[b] Akaike Information Criterion (corrected for finite sample size); smaller values reflect better-fitting models

Using a slightly different perspective, the fish were also evaluated whether they used only one watershed, regardless of whether it was their initial capture watershed, or traveled across multiple watersheds. Northern pike traveled less than burbot; they had a significantly greater maximum proportion of locations in one watershed (often up to 1.00, i.e., they were always located there) than did burbot; conversely, burbot used significantly more watersheds than northern pike (Mann-Whitney U-test, $p < 0.001$).

Across the entire study area, and for a subset of fish that were observed most frequently in the mainstem Kuskokwim watersheds, northern pike had significantly greater mercury concentrations than burbot (Figure 13). Total mercury concentrations were not compared between species and among all watersheds together, because burbot tended to use the mainstem Kuskokwim and northern pike tended to use large tributaries.

Mercury Differences in Northern Pike and Burbot among Capture and Max-Use Watersheds

Northern pike from the George, Holitna, and Takotna capture watersheds had significantly greater total mercury concentrations than northern pike captured in the Kusko above George, Kusko-Stony, or Kusko above Selatna watersheds (Figure 14). Similarly, northern pike in the max-use George, Holitna, and Takotna watersheds had significantly greater total mercury concentrations than northern pike in other max-use watersheds, which were mostly mainstem Kuskokwim (Kusko above George, Kusko-Stony, and Kusko above Selatna) (Figure 15a). Kusko-Aniak and Kusko-Swift watersheds were excluded from this analysis due to low sample size. There were no significant differences in burbot total mercury among capture watersheds (Figure 14) or among max-use watersheds (Figure 15b).

Watershed Characteristics and Mercury in Northern Pike

This analysis was performed excluding the Kusko-Aniak and Kusko-Swift watersheds, in which no northern pike were collected. Watershed mineralization (occurrences/ prospects and mines, with and without mercury) had stronger associations with mercury in northern pike than did watershed size and proportion of watershed that was wetland (Figure 16). In particular, both the number of occurrences and prospects and the number of mines were significantly associated with mercury in northern pike, and the number of occurrences was a better fitting model (Table 8). All models had outliers, and some had data points with large leverage, which was not unexpected based on the small number of watersheds and the large variation in both mercury concentrations and explanatory variables.

Seasonal Mercury Differences in Burbot

Burbot were collected during the winter sampling season in the Kuskokwim River, Reaches A and C, to characterize mercury in this subsistence fishery. Mercury was significantly greater in burbot muscle and liver in the summer/fall sampling season compared to winter (F1,86 = 35.152 and 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 (Figure 17). Telemetry data indicate that burbot captured in the summer/fall were likely resident in their capture area, while winter-caught burbot were more likely to have come from lower down the river.

Mercury in Boiled Burbot Muscle and Eggs

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

Arsenic (As) and Antimony (Sb)

The greatest average arsenic concentrations were in macroinvertebrates (Table 9). The greatest average antimony concentrations were in whole (juvenile) Dolly Varden (Table 9).

Arsenic and Antimony Differences among Tributary Samples

Total arsenic was much greater in biota collected from Red Devil Creek than in all other tributary reaches (tested with the most complete data set, from 2010; Figure 18). Excluding Red Devil, Cinnabar Creek had the next highest concentrations (Figure 18). Differences among other creeks were relatively minor and perhaps not biologically significant, however, compared to the difference between Red Devil and all other tributaries (Figure 18).

Among species sampled in tributaries, macroinvertebrates had the greatest arsenic concentrations when Red Devil Creek samples were included (GLM; F = 14.7223,220, $p < 0.001$) than when they were not (GLM; F = 102.3573,184, $p < 0.001$) (tested within the most complete data from 2010; Figure 18). Arctic grayling had significantly lower arsenic concentrations than all other biota when Red Devil samples were included, but no Arctic

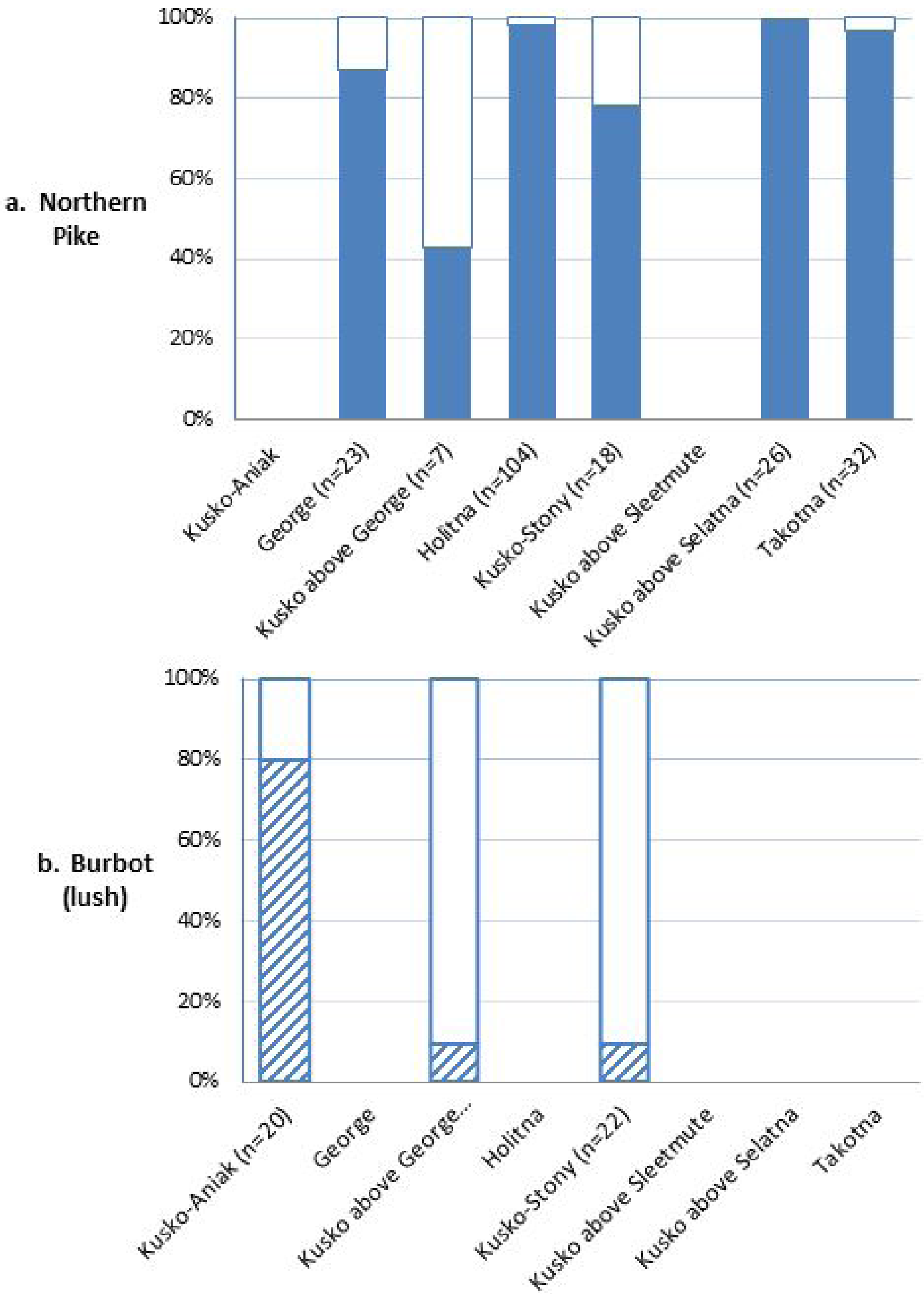


Figure 12. Percentage of (a) northern pike and (b) burbot that stayed within their capture watershed (colored bars) compared to those that did not (white bars), in the middle Kuskokwim River study area, Alaska, 2011–2013

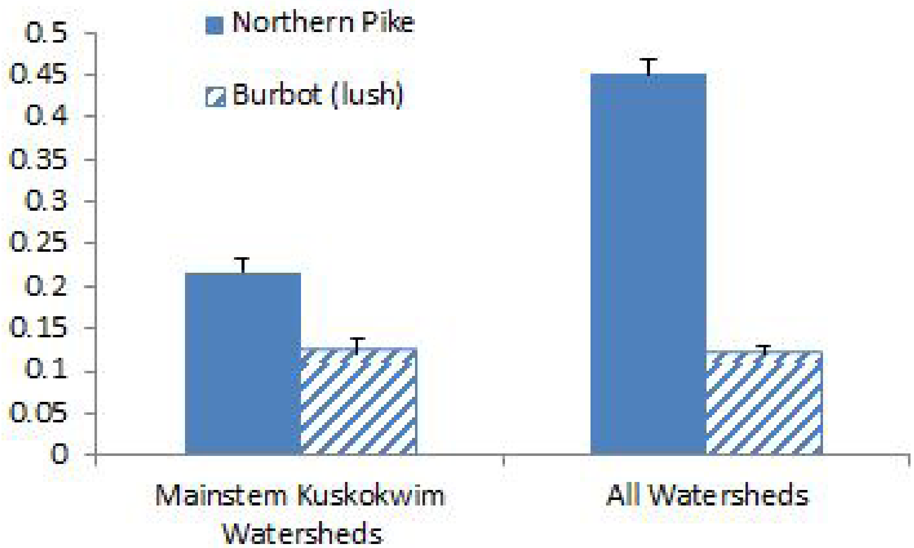


Figure 13. Mean (+ standard deviation, SD) total mercury concentrations (mg/kg, ww) in muscle biopsies of radio-tagged northern pike and burbot that used mainstem Kuskokwim watersheds, and that used all watersheds in the middle Kuskokwim River study area, Alaska, 2011–2013. Unlike superscripts indicate significant differences between species.

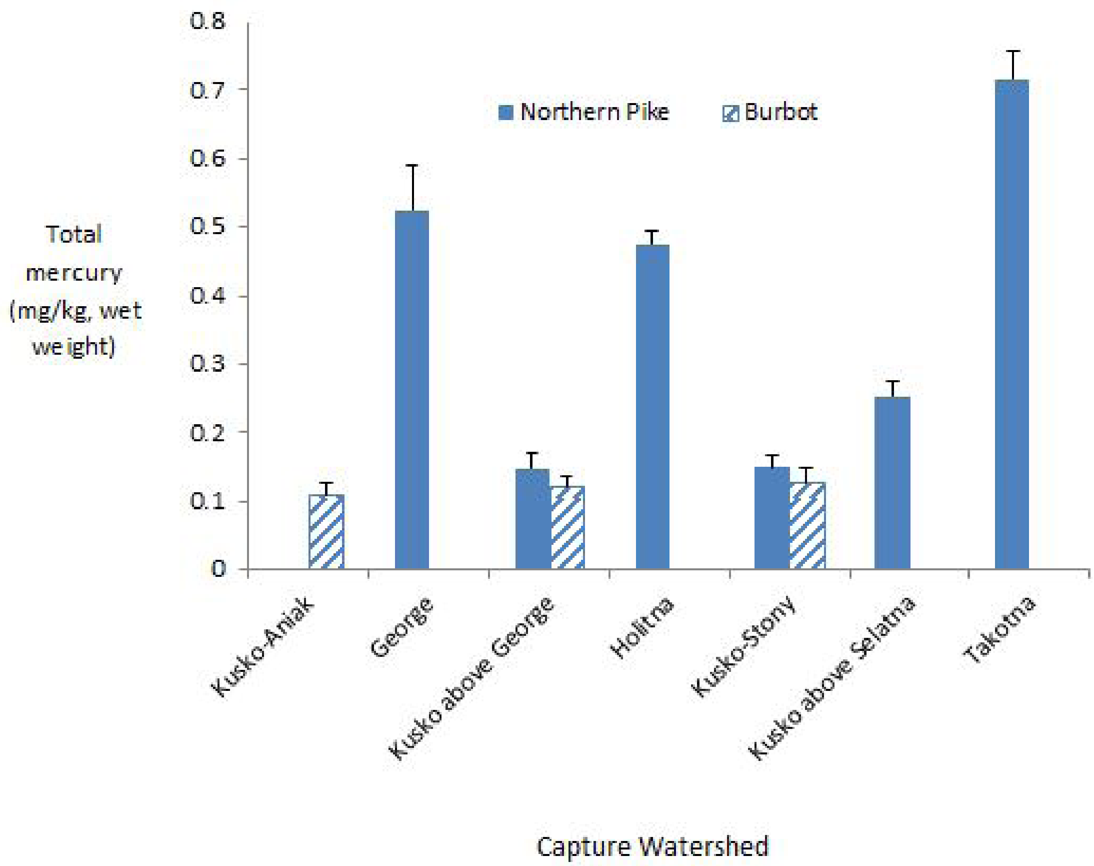


Figure 14. Mean (+ standard error, SE) total mercury concentrations (mg/kg, ww) in muscle biopsies of radio-tagged northern pike and burbot across capture watersheds in the middle Kuskokwim River region, Alaska, USA, 2011–2013.

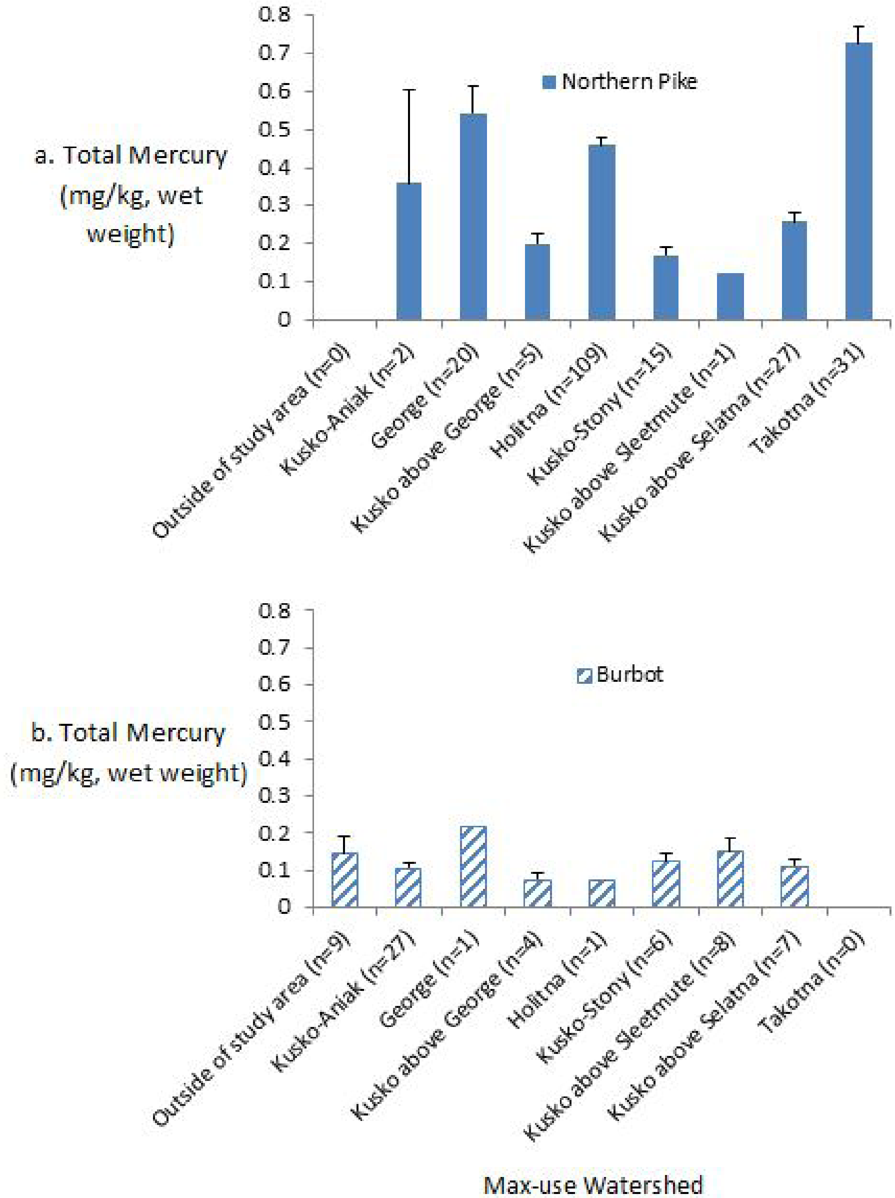


Figure 15. Mean (+ standard error, SE) total mercury concentrations (mg/kg, wet weight) in muscle biopsies of telemetered (a) northern pike and (b) burbot across watersheds with the highest proportion of locations for individual fish (max-use watersheds) in the middle Kuskokwim River region, Alaska, USA, 2011–2013.

grayling were collected at Red Devil in 2010; Arctic grayling and Dolly Varden concentrations were not different among tributaries when Red Devil samples were excluded.

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 than in all other tributaries, with no other significant differences (GLM; $F_{5,20} = 37.06$, $p < 0.001$) (Table 10). 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

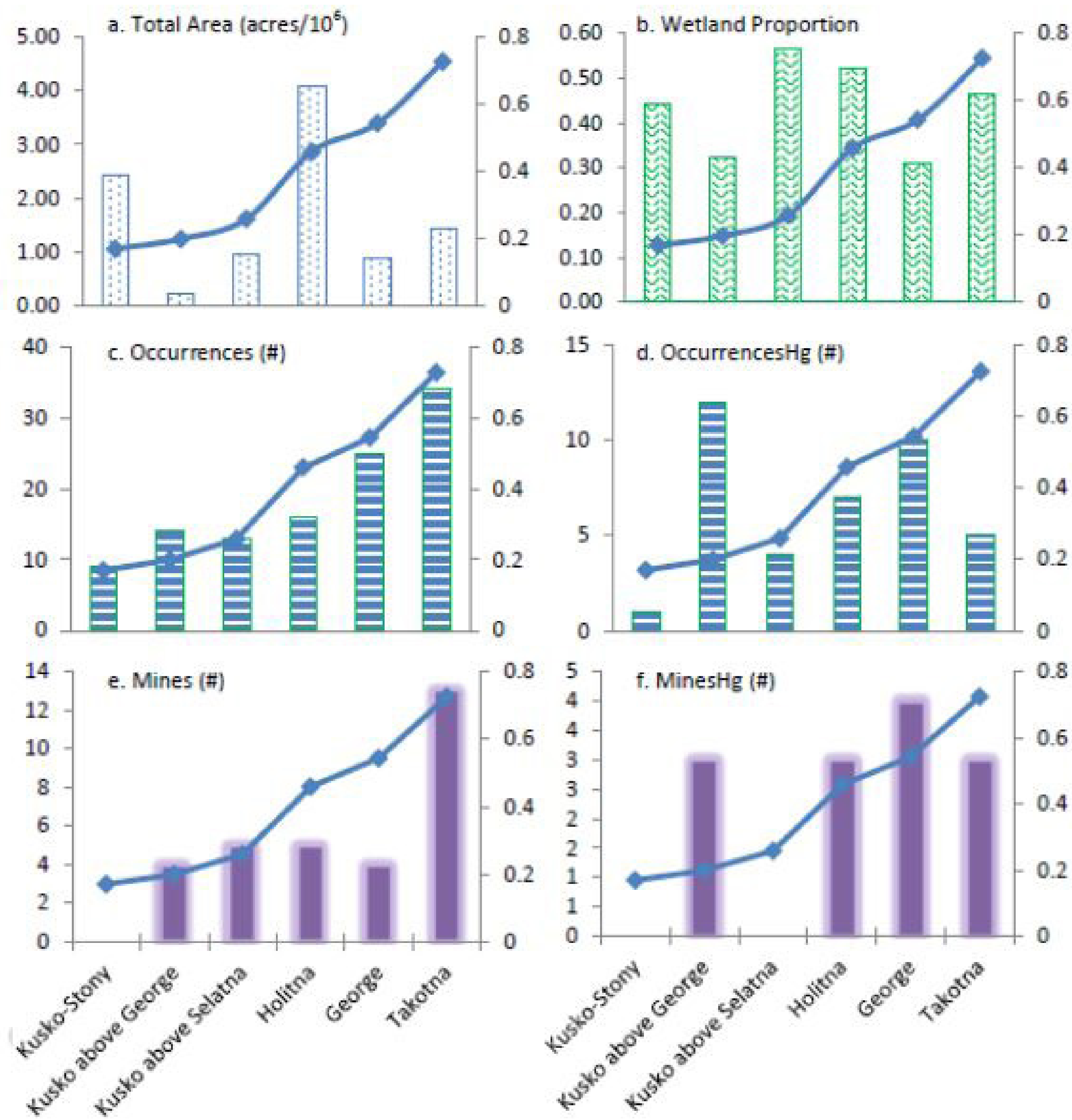


Figure 16. Watershed characteristics related to mean northern pike muscle mercury concentrations (mg/kg, right axis, for watersheds in the middle Kuskokwim River region, Alaska, USA, 2011-2013. Characteristics include: a) Wetland area (acres/106); b) Proportion of watershed in wetland; c) Number of mineral occurrences or prospects (Occurrences); d) Occurrences and prospects with mercury (OccurrencesHg); e) Number of mines (Mines); and f) Number of Mines with mercury (MinesHg). Data on mineral sites from USGS (2008); watersheds (on x-axis) are described in text, and are ordered (left-right) by increasing mercury concentrations.

Table 9. Total arsenic and antimony concentrations (mg/kg, wet weight) summary statistics for aquatic species sampled in the middle Kuskokwim River region, Alaska, 2010–2011

Species, Age, Tissue (n)	Total Arsenic (As)			Total Antimony (Sb)			Number (%) Detects[b]
	Mini-mum	Maxi-mum	Mean (SD[a])	Mini-mum	Maxi-mum	Mean (SD[a])	
Arctic Grayling, adult liver (n=23)	nd[c]	0.09	0.04 (0.02)	nd[c]	0.08	na[d]	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)

[a] SD = standard deviation [b] Method detection limits were 0.025 mg/kg, wet weight [c] nd = non-detect [d] na = not applicable

Table 10. Mean (and standard deviation, SD) antimony concentrations (mg/kg ww) from macroinvertebrate whole body samples collected from tributaries of the middle Kuskokwim River, Alaska, 2010–2011. Superscript "a" indicates a significantly different concentration among the 2010 sample sites.

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

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) antimony 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).

Arsenic and Antimony Differences among River Samples

While juvenile Chinook salmon had the greatest total arsenic concentrations among all species sampled (Table 9), the small Chinook sample size precluded robust conclusions from those data. Among other River species, sheefish and burbot had the greatest overall arsenic concentrations, in both muscle and liver (Table 9). Antimony was not detected in any muscle sample, and was detected in only five of 352 liver samples, at concentrations near the detection limit (MDL = 0.025 mg/kg

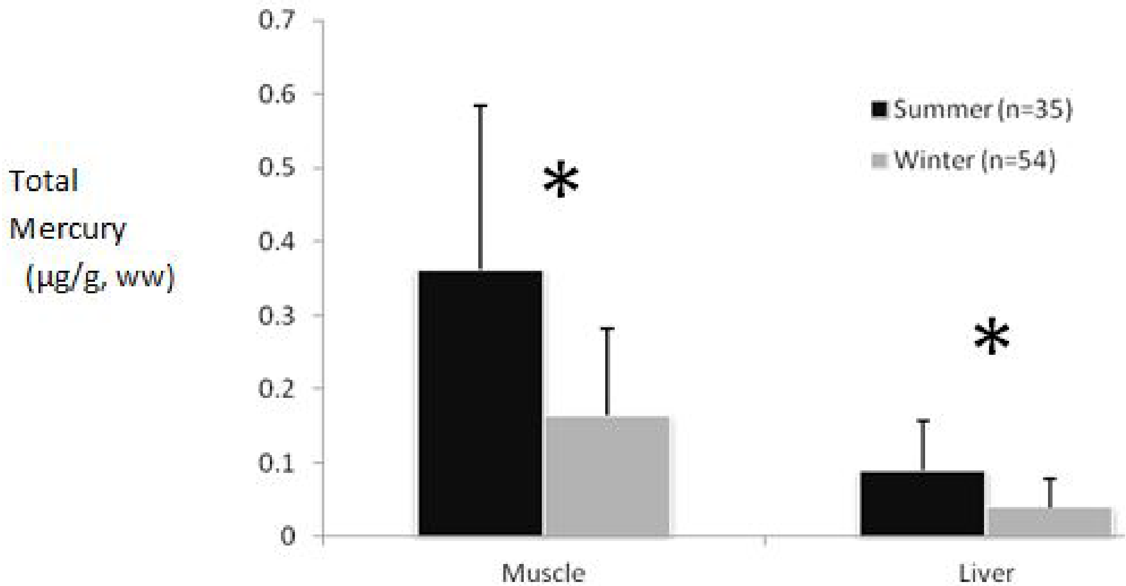


Figure 17. Mean (+ standard deviation, SD) total mercury concentrations (µg/g, ww) in burbot issues collected from June to early October (summer) and late November to early December (winter) from the middle Kuskokwim River, Alaska, 2010. Asterisks indicate significant differences between seasons with each tissue.

Table 11. Pearson's correlation coefficient *r* (p-value, number of samples *n*), with tissue means (and standard deviations, *SD*) of total arsenic in muscle and liver of fish collected from multiple reaches in the middle 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)

ww)(Table 9). In all species, liver and muscle arsenic concentrations were significantly positively correlated (Table 11). Burbot, northern pike, and sheefish were tested for arsenic differences among reaches (excluding reaches where sample sizes were < 3; Table 8). Arsenic in burbot muscle and liver clearly and significantly declined from upstream reaches to downstream reaches (Figure 19). Similar but less significant patterns were seen in northern pike and sheefish (Figure 19).

Inorganic Arsenic

A subset of 2011 Arctic grayling, Dolly Varden, macroinvertebrate, and slimy sculpin samples from Red Devil, Cinnabar, and Egnaty creeks was analyzed for inorganic arsenic (Table 12). Total arsenic concentrations in Red Devil Creek 2011 samples were similar to Red Devil 2010 samples (in the same species and matrices) (Table 12), while 2011 samples from Cinnabar and Egnaty creeks had total arsenic concentrations similar to those in samples from 2010 tributary reaches other than Red Devil (Table 12). Further, total and inorganic arsenic concentrations from Red Devil Creek were significantly greater than those in Cinnabar and Egnaty creeks (Figure 20), tested in Dolly Varden ($KW = 14.9612$, $p = 0.001$), slimy sculpin ($KW = 46.9822$, $p < 0.001$), and macroinvertebrates (total arsenic only; GLM; $F1,6 = 9.107$, $p = 0.023$). This was expected based on sediment and water data (M. Varner, pers. comm.).

Total arsenic and inorganic arsenic were significantly correlated within individuals (Pearson $r = 0.961$, $p < 0.001$). Average ratios of inorganic to total arsenic were 0.65 for Arctic grayling (n=2), 0.62 for slimy sculpin (n=17), and 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 12. Total arsenic and inorganic arsenic concentrations (mg/kj, wet weight) summary statistics for whole body fish and macroinvertebrate samples from the middle Kuskokwim River region, Alaska, 2010–2011

Species, Site	Total Arsenic					Inorganic Aresenic				
	Samples	Minimum	Maximum	Mean (SDa)	Number (%) detects[b]	*Samples*	Minimum	Maximum	Mean (SD)	Number (%) detects
Arctic Grayling, Red Devil Cr	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 Cr	25	nd[c]	0.95	0.42 (0.32)	14 (56)	3	0.03	0.12	0.08 (0.05)	3 (100)
Dolly Varden, Egnaty Cr	2	nd	nd	nd	0 (0)	–[e]	–	–	–	–
Dolly Varden, Red Devil Cr	6	2.0	16	6.1 (5.3)	6 (100)	2	0.62	2.0	1.3 (0.94)	2 (100)
Macroinvertebrates, Cinnabar Cr	4	0.70	9.7	5.4 (3.7)	4 (100)	–	–	–	–	–
Macroinvertebrates, Red Devil Cr	4	29	280	170 (110)	4 (100)	–	–	–	–	–
Slimy Sculpin, Cinnabar Cr	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 Cr	12	0.43	0.43	na[d]	1 (8)	–	–	–	–	–
Slimy Sculpin, Red Devil Cr	24	1.6	46	12 (11)	24 (100)	12	0.21	38	14 (11)	12 (100)

[a]SD = standard deviation [b] method detection limits were 0.3 mg/kg, wet weight [c] nd = non-detect [d] na = not applicable *n*=number of samples
[e] – = not analyzed

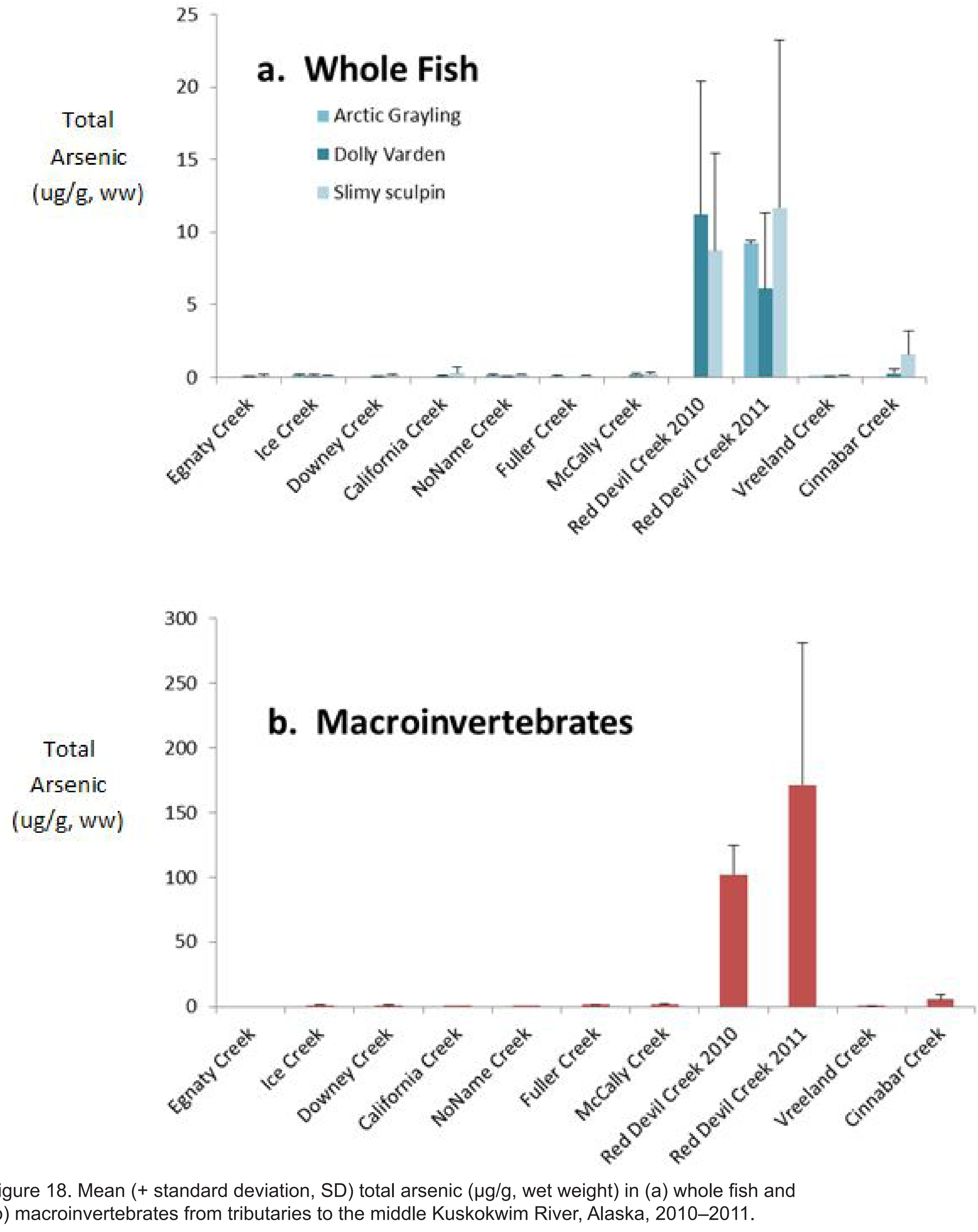


Figure 18. Mean (+ standard deviation, SD) total arsenic (µg/g, wet weight) in (a) whole fish and (b) macroinvertebrates from tributaries to the middle Kuskokwim River, Alaska, 2010–2011.

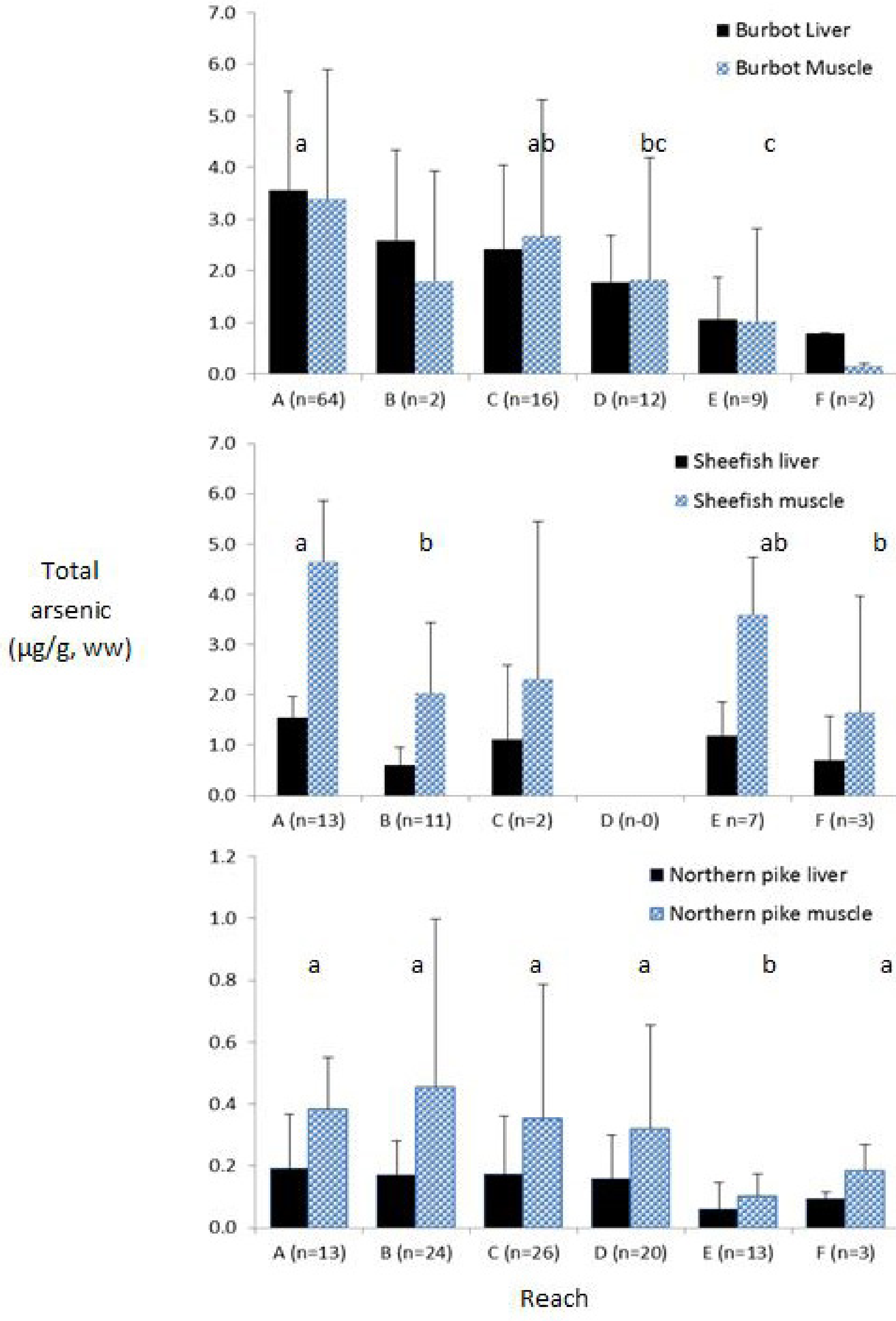


Figure 19. Mean (+ standard deviation, SD) total arsenic concentrations in burbot (lush), sheefish (inconnu), and northern pike collected from reaches of the middle Kuskokwim River, Alaska, 2010–2011. Unlike superscripts indicate significant differences among reaches in both liver and muscle concentrations. Reaches with no superscripts had low sample size and were not included in statistical tests.

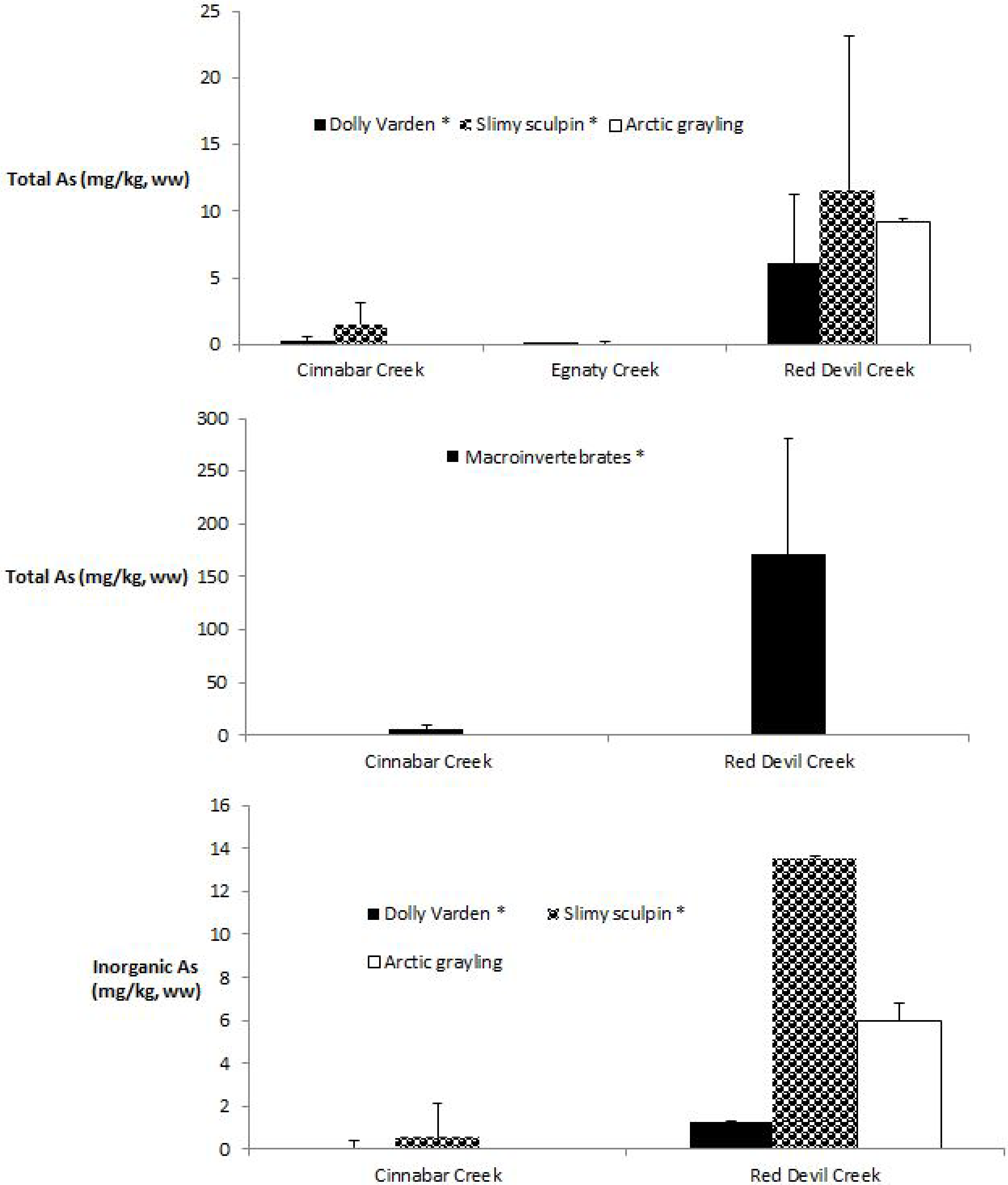


Figure 20. Mean (+ standard deviation, SD) total arsenic (As) and inorganic arsenic in Dolly Varden, slimy sculpin, Arctic grayling (Red Devil Creek only), and macroinvertebrate (total arsenic only) whole body samples from three geologically similar creeks in the middle Kuskokwim River, Alaska, 2011. Asterisks indicate significant differences among creeks within fish species or within macroinvertebrates.

Seasonal Arsenic Differences in Burbot

Burbot 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) (Figure 21). Telemetry data indicated that burbot captured in the summer/fall were likely resident in their capture area, while winter-caught burbot were more likely to have come from lower down the river.

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 true 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 arsenic in burbot eggs (n=5) was 2.45 (SO = 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.

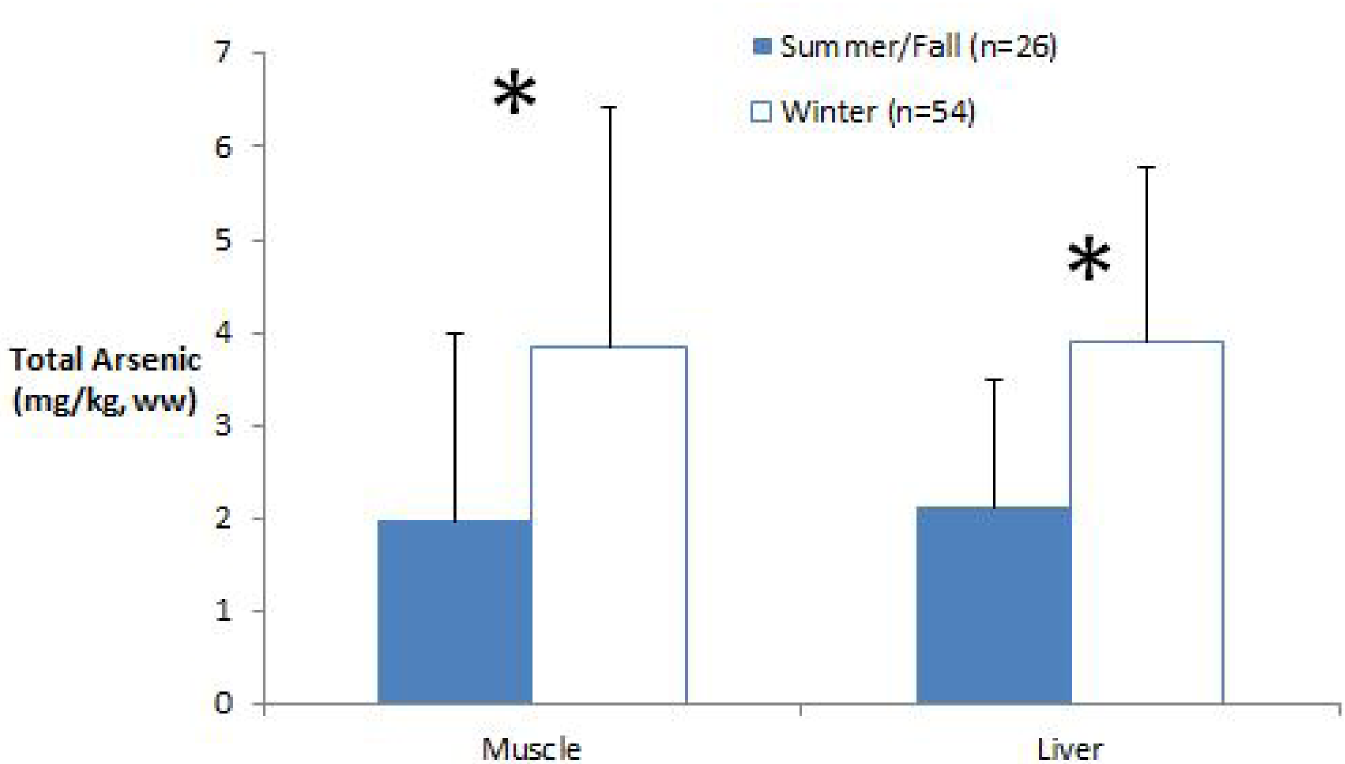


Figure 21. Mean (+ standard deviation, SD) total arsenic concentrations in burbot, tissues collected in June through early October 2010 (summer/fall), and late November 2010 through January 2011 (winter) from the middle Kuskokwim River, Alaska. Asterisks indicate significant differences between the seasons within tissues.

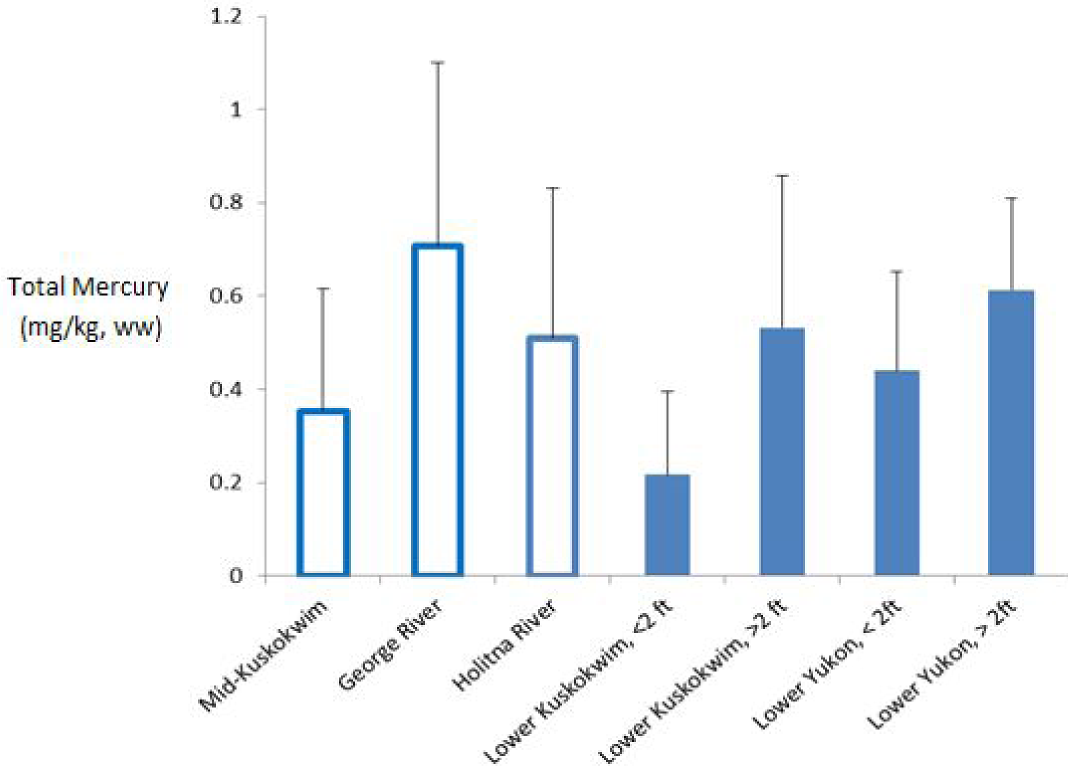


Figure 22. Mean (+ standard deviation, SD) total mercury concentrations (mg/kg ww) in northern pike from western Alaska, 2001–2011. Total mercury concentrations from the current study (white bars) were from the middle Kuskokwim River area (from Crooked Creek to the Stony River). Total mercury concentrations from USFWS (unpubl. data) (solid bars) were from the lower Kuskokwim and Yukon rivers.

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 mercury and somewhat greater arsenic concentrations compared to other tributaries. The Dolly Varden collected in Red Devil Creek were juveniles and probably only seasonal residents in the creek (K. Wuttig, Alaska Department of Fish and Game, pers. comm.). The large and significant differences in mercury and arsenic 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 River and its large tributaries showed no clear patterns in mercury and arsenic concentrations, with two exceptions. Non-anadromous fish (i.e., northern pike, burbot, and Arctic grayling) from the George, Holitna, and Hoholitna rivers had greater mercury concentrations than other fish, and total arsenic in burbot decreased with downstream sampling locations.

Mercury

Mercury concentrations in northern pike found in this study were similar to those in other datasets from the Yukon-Kuskokwim Delta and the Kuskokwim River, although without accounting for size, the comparisons are gross. Still, this study's data (northern pike average total mercury 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 unpublished data).

Northern pike mercury concentrations from this study were similar in magnitude to data collected on National Wildlife Refuges in Alaska (USFWS, unpublished data) (Figure 22). In that study, lower Kuskokwim River fish samples were collected downstream from Aniak to the Johnson River mouth, while regional Yukon River samples were collected from Holy Cross to Emmonak. The USFWS results provide a regional context for the middle Kuskokwim study and highlight the numerous factors that can contribute to fish mercury concentrations.

For example, older pike (greater than two feet in length), have greater mercury concentrations than younger pike from the same area (Figure 22). Moreover, lower Yukon pike have greater average mercury concentrations (after accounting for size and age) than middle Kuskokwim River pike (Figure 22), even though there are more known mineralized areas in the Kuskokwim River. 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). George River northern pike, which have the greatest mercury concentrations (Figure 22), tended to remain in the George River watershed for extended periods of time, and there were multiple local mercury sources and a high proportion of wetlands where mercury methylation takes place. While speculative as to source and conditions that generate the methylated form of mercury, these data do emphasize the factors that can influence mercury concentrations in fish.

There were no clear explanatory factors for seasonal differences in mercury concentrations in burbot. Telemetry data indicated that burbot captured in the summer/fall were likely resident in their capture area, while winter-caught burbot were more likely to have come from lower down the river. Lower winter concentrations could therefore reflect a different sampled population, but the underlying cause of differences was unclear. Although methyl mercury depurates from fish muscle very slowly, lower winter concentrations could be explained by slower metabolism reducing uptake through food (Forstner and Wittman 1981). Winter-caught burbot may simply have a different exposure history compared to those caught in summer.

In the absence of mercury point sources, mercury concentrations in fish tissues should closely track trophic position because mercury biomagnifies up food chains. Trophic order, as suggested by relative mercury concentrations in fish from this study (Figure 2), 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 mercury concentrations; adult Arctic grayling had greater mercury concentrations than juveniles.

Mercury concentrations do vary with point sources, however, and would confound a strict life-history explanation for differences in mercury. For example, the mean mercury for Dolly Varden juveniles was much greater than for adults (Table 4), although juveniles are lower on the food chain (K. Wuttig, Alaska Department of Fish and Game, pers. comm.). The juvenile mean was greatly influenced by the high mercury from Red Devil Creek (Figure 9); without Red Devil Creek, the juvenile Dolly Varden mean was 0.04 µg/g ww, less than the mean mercury in adult Dolly Varden. Thus, the point sources in Red Devil (and Cinnabar) Creek resulted in greater mercury concentrations in some younger and lower-trophic status organisms.

In fish tissues, an MeHg:THg ratio much less than 1.0 can indicate acute, short-term exposure to mercury 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 mercury via ingestion of methyl mercury 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 total mercury concentrations in Red Devil and Cinnabar creeks may indicate exposure to a point source of inorganic mercury, as Guilherme et al. (2010) determined for caged mullet (Liza aurata) in a mercury-contaminated system in Portugal.

Methyl mercury 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 mercury 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 mercury ratios close to or less than 1 (Table 6), but Dolly Varden had a mean liver:muscle mercury ratio of 2.28 (n=7, from Kuskokwim River reaches D and F). For all species for which muscle and liver were analyzed separately (all of which were collected in River reaches), only Dolly Varden showed evidence of potential short-term or acute exposure based on liver:muscle mercury ratios.

Toxic thresholds for mercury 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 longnosed sucker, juvenile Arctic grayling, and adult Dolly Varden had species mean mercury 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 (Figure 9). Fish sampled in river reaches all exceeded the lower thresholds, and some clearly exceeded the higher thresholds (Figure 10). Therefore, the elevated mercury concentrations within the study area may be affecting fish health and reproduction, but the magnitude of individual or population-level effects is unknown.

Burbot traveled across multiple watersheds, from the headwaters to the estuary of the Kuskokwim River, and had lower mercury concentrations than northern pike. One explanation for their lower mercury concentrations is that they simply spent less time near the potential point sources; they stayed primarily in the mainstem Kuskokwim River (Figure 12). The sampled burbot may have also been lower on the food chain than the sampled northern pike, as that affects mercury concentrations in fish, but in the middle Kuskokwim River the species are thought to have similar diets.

Northern pike, in contrast, traveled relatively short distances and often stayed within one watershed seasonally and over multiple years (Figure 13). This means they were more "resident" than burbot, and therefore better indicators of watershed-level mercury contamination. The high levels of variation in pike mercury among watersheds, even after accounting for length, may reflect characteristics of those watersheds that contribute to capturing, methylating, or liberating mercury from the geology to suspended or particulate forms in water, which could then be ingested by fish (as described in Gray et al. 2000).

Watersheds differ in their ability to capture atmospherically deposited mercury or transform it into methyl mercury, but watershed size and proportion of wetlands did not show strong relationships to northern pike mercury concentrations (Table 8, Figure 16). If these were primary drivers of mercury concentrations in northern pike, then it would be expected to see much greater mercury concentrations in the Kusko-Stony watershed, with its relatively large size and wetland proportion (Figure 16). If watershed size and proportion of wetlands were drivers, mercury concentrations in northern pike would be relatively low in the George watershed and relatively high in the Kusko-Selatna watershed. Yet, the opposite is the case in both watersheds (Figure 16).

Watershed mineralization—as indexed by the number of occurrences/prospect—had the strongest relationships to mercury in northern pike (Table 8, Figure 16). The two are related, since more mining occurs in areas of greater mineralization. The number of mercury-containing occurrences/prospects and mines did not show a strong relationship with high concentrations of mercury in northern pike. The contribution of mines to mercury in northern pike cannot be distinguished due to the imprecise nature of the data at a broad scale.

The highest northern pike mercury concentrations were observed in the George, Holitna, and Takotna watersheds (Figure 15). These watersheds also have some of the highest numbers of occurrences/prospects and mines containing mercury (Figure 16; USGS 2008), and extensive wetlands that may serve as methylation engines. One mined area in the headwaters of the Holitna has similar mercury levels in the sediments, water, and fish tissue to those observed at Red Devil Creek, which contained tailings from a mercury mine. The Takotna watershed, which has active mining and extensive wetlands, also had the highest concentrations of mercury in pike, and there was no correlation with mercury concentrations in muscle and pike length, indicating consistent mercury exposure in young fish (Guilherme et al. 2010).

The Kusko-Aniak watershed has more mines than other watersheds, but was the max-use watershed for only two northern pike, making estimation of a mean mercury value inappropriate. However, historical pike data (USFWS, unpublished data) from the Aniak River include mercury concentrations over 1 mg/kg wet weight, similar to the highest mercury values from this study. These data mirror the results and conclusions of Gray (2000) for smaller Dolly Varden and Arctic grayling.

One exception to this general pattern is the Kusko above George watershed, which also has a high number of mercury-containing mines and occurrences/prospects (Figure 16) but relatively low mercury concentrations in northern pike (Figure 15). This exception highlights differences between the mainstem Kuskokwim, a large-volume river that may dilute inputs but that also may be influenced by geology and activity in upstream watersheds, and tributaries which drain smaller watersheds and are relatively discrete. An analysis of more precise spatial information on sediment transport in relation to mercury point sources within each of our defined watersheds might better explain this exception.

Conditions that likely led to high tissue concentrations in northern pike were a source of mercury, the presence of wetlands that tend to enhance methylation, and extended residence time. The results illustrated in Figure 16 do not indicate a strong correlation between northern pike mercury concentrations and number of mineral occurrences that include mercury or the number of mines that have a mercury association. Further, while at least two mines in the area are mercury mines (Red Devil and Cinnabar Creek), the majority of mines are placer operations that target gold. Additional work focusing on how mining activity that exposes ore bodies containing cinnabar material could increase exposure of resident fish to mercury is needed. At present, it is unclear whether a relationship exists between tissue concentration and mining activity in a given watershed, or if tissue concentrations are a function of the underlying geology in the watershed.

Arsenic and Antimony

Arsenic differences in biota from Red Devil Creek 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 arsenic in freshwater fish tissues range from 1.3 to 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 arsenic concentrations.

There were no clear explanatory factors for seasonal differences in arsenic concentrations in burbot. Fu et al. (2010) noted an opposite pattern of lower arsenic and antimony concentrations (in water) in December than in July at a contaminated antimony mine site in China. They attributed the difference to greater rainfall in July causing greater local fallout of antimony and arsenic from smelting emissions. Seasonal differences in arsenic likely reflect different exposure scenarios for the sampled fish; they may be from two different sub-populations. Telemetry data indicated that burbot captured in the summer/fall were likely resident in their capture area, while winter-caught burbot were more likely to have come from lower down the river. Additional data from ongoing telemetry studies should further inform these analyses.

In a related study, Foata et al. (2009) found that environmental concentrations of total arsenic 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. Antimony was detected in samples from the Presa River 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 in Italy ranged from over 1,000 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, antimony had similar relative concentrations, although absolute antimony concentrations were about an order of magnitude lower compared to arsenic, for the same taxa. These results are similar to ours. Although antimony and lead were correlated with a reduction in macroinvertebrate biodiversity on a river downstream of an antimony 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).

Table 13. Northern pike consumption guidance, based on methyl mercury concentrations, issued by the State of Alaska for the Yukon-Kuskokwim Delta and the middle Kuskokwim River area, Alaska, for fish collected in 2010–2011.

Methyl Mercury Concentration in Fish (mg/kg, wet weight)	Recommended Meals[a] per Month	Area[b] of Capture (Data source)
0–0.15	Unlimited	Stony River, middle Kuskokwim (current study)
>0.15–0.32	up to 16	Lower Kuskokwim < 2 feet (U.S. Fish and Wildlife Service, unpubl. data)
>0.32–0.40	up to 12	Holitna River (current study)
>0.40–0.64	up to 8	Lower Yukon, Lower Kuskokwim > 2 feet (U.S. Fish and Wildlife Service, unpubl. data)
>0.64–1.2	up to 4	George River (current study)
>1.2–1.4	up to 3	George River (current study)
>1.4–2.0	up to 2	George River (current study)
>2.0–3.4	up to 1	George River (current study)

[a] A meal is a six-ounce portion of fish
[b] The middle 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.

Subsistence Concerns

Mercury concentrations in northern pike and burbot, which are used for subsistence, warranted issuing consumption guidance (http://bit.ly/2dEyuZp) under the State of Alaska's guidelines (Hamade 2014; http://bit.ly/2cO1R5i). These data enable subsistence users in the middle Kuskokwim region to reduce mercury exposure from these commonly used fish species for women of child-bearing age or children. For example, northern pike in general had greater mercury concentrations in their muscle compared to burbot, as did large northern pike compared to small. Based on the data, a subsistence consumer wanting to minimize mercury exposure would choose burbot over northern pike, and small northern pike over large ones.

Pike from watersheds other than the Kuskokwim have similarly or more restrictive guidance, such as pike from the lower Yukon River (Table 13). The most restrictive consumption guidance is for pike from the George River.

The State of Alaska has no consumption guidance for arsenic or antimony in fish used for subsistence (Verbrugge 2007, Hamade 2014). 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 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 arsenic), although the data were highly variable. Should these fish be used for subsistence, inorganic arsenic concentrations may be of concern.

Three key findings for subsistence users are:

1. Pike caught in the George, Holitna, and Takotna watersheds will likely have greater mercury concentrations than pike caught from other watersheds or the mainstem Kuskokwim. Larger pike will generally have greater mercury concentrations than small pike from the same location.
2. Larger pike will generally have greater mercury concentrations than small pike from the same location.
3. Throughout the middle Kuskokwim region, burbot (lush) have generally lower mercury concentrations than northern pike.

Conclusions

Results of this study indicate that there is measurable and biologically significant elevation of mercury and, to a lesser extent, arsenic in aquatic biota in Red Devil Creek, which has been impacted by historical lode mining and on-site ore processing. Prior to 2014, sediments with high concentrations of mercury, arsenic, and antimony were deposited in the Kuskokwim River at the mouth of Red Devil Creek. However, the turbid water conditions and swift current in the river are not conducive to northern pike habitat, nor formation of wetlands that promotes methylation. The low number of northern pike caught in the Kusko above George watershed and their relatively low mercury concentration are consistent with the marginal habitat suitability for pike (e.g., few slough or clear water off channel areas) within that portion of the Kuskokwim River.

Elevated mercury in aquatic biota is evident near another abandoned mine on Cinnabar Creek. That mine is on land managed by the State of Alaska in the upper Holitna Drainage. Cinnabar (a locally prevalent mercury ore mineral) was extracted through a lode mine and processed on-site. Conditions in Cinnabar Creek are similar to those at Red Devil Creek. The potential impact of the Cinnabar Creek mine on the larger Holitna watershed has not been studied.

Northern pike residing in both the George and Takotna watersheds displayed elevated levels of mercury, which may affect fish health or reproduction. Both watersheds contain extensive mineralization and have a long history of mining. The nature, extent, and impacts of that mining activity have not been studied in these watersheds.

Fish movements among spawning and wintering areas helped explain differences in muscle mercury concentrations between burbot and northern pike in the middle Kuskokwim region, by establishing how much time individual fish spent in proximity to potential mercury sources in this highly but variably mineralized region. Northern pike tended to stay in tributaries of the mainstem Kuskokwim and had greater mercury concentrations when they were in more mineralized watersheds. Burbot tended to travel widely over the course of a year, which meant they spent less time in proximity to mineralized areas. Individuals of both species that stayed in the mainstem Kuskokwim also had overall lower mercury concentrations in spite of being in proximity to mercury sources

The tissue concentration data collected as part of this study indicate that both mercury and arsenic may be of concern for subsistence consumers of fish along the middle section of the Kuskokwim River and its tributaries. Ongoing data collection and analyses will better inform discussions about the transfer of mercury, arsenic, antimony; their various chemical forms; and other trace elements within the middle Kuskokwim River region from mineralized areas and mines.

Addressing unanswered questions regarding the causes for elevated mercury and arsenic tissue concentrations in resident fish will require integrating additional telemetry studies on northern pike and burbot, with more detailed analysis of mining activity, a more complete understanding of the nature and extent of mineralized areas and establishing watershed characteristics that could be used as an index for mercury methylation potential.

Subsistence users catching pike in the George, Holitna, and Takotna watersheds should be aware that those pike will likely have greater concentrations of mercury than pike caught from non-mineralized watersheds or the mainstem Kuskokwim; that larger pike from the same area will generally have greater mercury concentrations than small pike; and that throughout the region burbot, also known as lush, have lower mercury concentrations than northern pike.

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Technical 61 Report