Appendix E

Derivation of a Site-Specific Water Column Selenium Standard for Lake Koocanusa.

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EXECUTIVE SUMMARY

This document provides the Montana Department of Environmental Quality's scientific framework and recommendations for site-specific selenium water quality standards for Lake Koocanusa. The proposed standards are designed to protect fish as the most sensitive ecological endpoint, including federally listed threatened species, from effects of elevated levels of selenium. The standards described herein reflect the latest science on the toxicological effects of selenium. This document considered the United States Environmental Protection Agency's 2016 304(a) National Recommended Water Quality Criteria to develop site-specific selenium criteria, whenever feasible, and their guidance to states on developing site-specific criteria as described in Appendix K of, *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*.

The proposed criteria for Lake Koocanusa consist of a site-specific water column value and 304(a) recommended fish tissue values. The site-specific water column value is based on biodynamic selenium modeling using the United States Geological Survey Ecosystem-Scale Selenium Model. The proposed values are presented below.

Proposed Selenium Water Quality Criteria for Lake Koocanusa, Montana.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

ACKNOWLEDGEMENTS

This work was guided and directed by the collaborative state and provincial efforts of the Lake Koocanusa Monitoring and Research Working Group; in particular, the collective knowledge of the Monitoring and Research Committee and the expertise of the scientists recognized for their work in selenium who participated on the Selenium Technical Subcommittee. For the coordinated monitoring efforts, we would like to thank Montana Fish Wildlife and Parks, United States Army Corps of Engineers, United States Geological Survey, United States Fish and Wildlife Service, British Columbia Ministry of Environment and Climate Change Strategy, and Teck Resources Limited. We would like to thank the United States Geological Survey Selenium Modeling Team. We acknowledge and appreciate the British Columbia Ministry of Environment and Climate Change Strategy for the staff support and overall collaboration and participation throughout this multi-year long process. Without the joined efforts between the Department of Environmental Quality and the British Columbia Ministry of Environment staff this work would not have been possible.

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ACRONYMS

Acronym	Definition
ABMP	Area Based Management Plan
ARM	Administrative Rules of Montana
BAF	Bioaccumulation factor
BC	British Columbia
BC-ENV	Ministry of Environment and Climate Change Strategy
CWA	Clean Water Act
CSKT	Confederated Salish and Kootenai Tribes
DEQ	Department of Environmental Quality (Montana)
dw	Dry weight
EF	Enrichment factor
EO	Egg-ovary
EPA	United States Environmental Protection Agency
ESA	Endangered Species Act
EVWQP	Elk Valley Water Quality Plan
K_d	Partitioning coefficient
Kg	Kilogram
KNC	Ktunaxa Nation Council
KTOI	Kootenai Tribe of Idaho
LKMRC	Lake Koocanusa Monitoring and Research Committee
LKMRWG	Lake Koocanusa Monitoring and Research Working Group
LOEC	Low Effect Concentration
MT	Montana
ppb	Parts per billion (equivalent to micrograms per liter in aqueous solutions)
ppm	Parts per million (equivalent to milligrams per liter in aqueous solutions)
Se	Selenium
SOP	Standard Operating Procedure
SETSC	Selenium Technical Subcommittee (of the LKMRC)
SOP	Standard Operating Procedure
TAC	Technical Advisory Committee
TTF	Trophic transfer factor
TSI	Trophic state index
USACE	United States Army Corps of Engineers
USGS	United States Geological Survey
µg/L	Micrograms per liter
WQG	Water Quality Guideline
WQPB	Water Quality Planning Bureau
WQSM	Water Quality Standards and Modeling Section

1.0 INTRODUCTION

This document presents the scientific basis used for the development of the site-specific water column selenium standard for Lake Koocanusa, MT. This work was a collaborative effort between the Montana Department of Environmental Quality (DEQ), the British Columbia (BC) Ministry of Environment and Climate Change Strategy (BC-ENV), the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), and a Selenium Technical Subcommittee (SeTSC).

1.1 BACKGROUND

In 2010, a Memorandum of Understanding and Cooperation (MOUC) between BC and MT was drafted to end the decades-long dispute over transboundary mining in the Flathead Valley. The MOUC expanded collaboration on environmental protection and assessments that have bi-national significance. Coordinated efforts began between BC-ENV and DEQ to address regional transboundary water quality issues including those in the Elk Valley, BC.

In 2012, DEQ added Lake Koocanusa to the 303(d) list of impaired or threatened waterbodies, as threatened by selenium (DEQ, 2012). In April 2013, the BC Minister of Environment issued a Ministerial Order (No. M113) under the Environmental Management Act to remediate water quality effects of past mining activities and to guide environmental management of future mining activities in the Elk Valley, including the Canadian portion of Lake Koocanusa.

This Order mandated the development of an area-based management plan (ABMP) due to evidence of increasing concentrations of water quality constituents of potential concern, including but not limited to selenium (Se), from numerous sources related to mining activity in the Elk Valley watershed https://www2.gov.bc.ca/gov/content/environment/waste-management/industrial-waste/mining-smelting/teck-area-based-management-plan. As the sole operator of the five coal mines in the Elk Valley, Teck Resources Limited (Teck) was required by the Order to develop the Elk Valley ABMP according to requirements outlined in the Order. Agencies from Canada and the US participated in a Technical Advisory Committee (TAC)—established as a condition under the Order—to provide science-based technical advice to Teck during the development of the Elk Valley Water Quality Plan (EVWQP). The EVWQP was submitted by Teck to BC-ENV in July 2014 and revised by BC-ENV in 2019. The EVWQP established short, medium, and long-term water quality targets for Se (and other constituents) at specific order stations in the Elk Valley, including a station in Lake Koocanusa (LK2) where the target for Se was set at 2 µg/L (equivalent to the BC Water Quality Guideline (WQG)). These water quality targets were incorporated into enforceable limits under Permit No. 107517, issued to Teck on November 19, 2014 (**Table 1-1**).

Table 1-1. Effluent limits for Order Stations in the Elk Valley (Teck, 2014).

Management Unit	Order Stations	Selenium (µg/L)
1	FR4	57
2	FR5	40
3	ER1	19
4	ER2	19
5	ER3, ER4	19
6	LK2	2

At the conclusion of the EVWQP development process, the TAC recommended that a site-specific ecological effects assessment be completed to evaluate whether the BC WQG set at 2 µg/L for Se is protective for Lake Koocanusa. Although BC WQGs are designed to be protective of aquatic life and wildlife, WQGs do not account for site-specific factors, so the TAC recommended that an ecological effects assessment be conducted for Lake Koocanusa. The TAC concluded that Se bioaccumulation in organisms is affected by site-specific factors and has potentially irreversible consequences (i.e. extirpation of species). In addition, current concentrations of Se in Lake Koocanusa were recorded above the BC alert threshold of 1 µg/L promulgated by BC-ENV during the same time period that draft EPA 304(a) Se criteria—released in 2015 and finalized in 2016—showed that a protective Se water quality concentration for aquatic life in lentic waters was 1.5 µg/L (**Table 1-2**). EPA also provided a recommendation that site-specific Se criteria be developed whenever possible.

TAC recommendations on Lake Koocanusa and government-to-government discussions on transboundary impacts resulted in formal commitments by BC-ENV to DEQ and the EPA to establish a process to assess whether a Se target of 2 µg/L at Lake Koocanusa is protective, and to provide a forum for discussing other water quality issues relevant to Lake Koocanusa. To meet this commitment, BC-ENV proposed the establishment of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG).

As directed by the Steering Committee of the LKMRWG, a Selenium Technical Subcommittee (SeTSC) was established in 2015 with selenium experts from both the US and Canada. In 2019, technical representatives from the Ktunaxa Nation Council (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI) joined the SeTSC. The SeTSC multi-agency, multi-institutional, international team of experts worked with DEQ and BC-ENV to develop the scientific basis for a site-specific Se criterion in Lake Koocanusa. The multi-year collaborative effort included research goals and methodology, coordinated monitoring plans, Se biodynamic modeling, and Se criteria recommendations. The technical work of the SeTSC was routinely reported to the Monitoring and Research Committee (MRC) and the Steering Committee. **Figure 1-1** describes the structure of the LKMRWG.

The goal of this coordinated effort, is a MT and BC co-developed site-specific Se criteria for Lake Koocanusa detailed in this document.

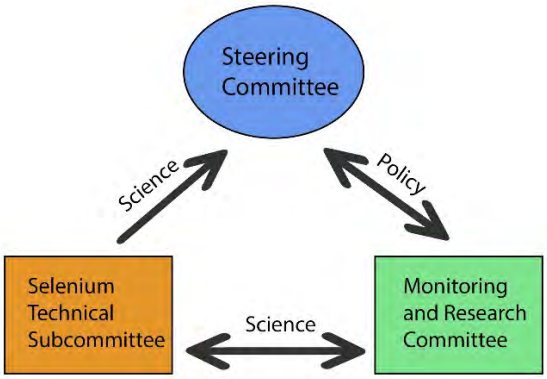

Figure 1-1. Structure and information flow of the LKMRWG.

1.2 PURPOSE

The purpose of this document is to provide the technical framework for the derivation of a site-specific selenium standard for Lake Koocanusa, MT. Montana is required under section 303(c)(2)(B) of the federal Clean Water Act (CWA) to establish water quality criteria for toxic pollutants, for which selenium is listed in section 307(a)(1). In adopting criteria, Montana is authorized to establish numeric values based on CWA Section 304(a) guidance modified to reflect site-specific conditions.

1.3 EXISTING SELENIUM WATER QUALITY STANDARDS

This section details the existing Se water quality standards for Montana, the 304(a) criteria, and British Columbia's Se WQG's defined in **Table 1-1**.

Table 1-2. Selenium water column thresholds applicable to Lake Koocanusa.

Agency	Target (µg/L)	Description
EPA	1.5	2016 updated criterion for dissolved Se in lentic systems
ENV	2	2014 Established guideline for aqueous Se
ENV	1	2014 Established Alert level for aqueous Se
DEQ	5	Chronic standard for dissolved Se established based on 1987 EPA guidance
DEQ	20	Acute standard for dissolved Se established based on 1987 EPA guidance

1.3.1 Montana's Surface Water Quality Standards for Selenium

Existing Se water quality standards are found in Department Circular DEQ -7 (June 2019 edition) which is incorporated by reference into the Administrative Rules of Montana (ARM) title 17, chapter 30, subchapter 6. The current acute (20 µg/L) and chronic (5 µg/L) water column standards were established based upon the 1987 EPA Ambient Water Quality Criteria for Selenium (U.S. EPA, 1987).

Water quality standards are designed to protect the beneficial uses of a given waterbody. The state of Montana has classified waterbodies based on the beneficial uses they are expected to support, as found in ARM 17.30.621 through 17.30.629. Lake Koocanusa is classified as a B-1 waterbody (ARM 17.30.609). State law requires that waterbodies in a B-1 use class be suitable for drinking, culinary, and food processing purposes after conventional treatment; bathing, swimming, and recreation; growth and propagation of salmonid fishes and associated aquatic life, waterfowl and furbearers; and agricultural and industrial water supply. The most sensitive beneficial use for Se in Lake Koocanusa is growth and propagation of salmonid fishes and associated aquatic life.

Section 305(b) of the federal CWA requires states to assess waterbodies to determine whether the waterbodies are supporting their beneficial uses. In 2012, DEQ listed Lake Koocanusa as threatened by Se. Montana defines a threatened waterbody as currently meeting water quality standards and supporting the beneficial uses, but standards are likely to be exceeded and beneficial uses threatened if current trends continue.

1.3.2 National Ambient Water Quality Criteria for Selenium

The CWA section 304(a) requires the EPA to develop water quality criteria using the best available science. While the EPA requires states to consider their recommendations (40 CFR part 131) when adopting water quality standards, it is a recommendation only and the state must adopt the criterion into state water quality standards for it to become a regulation.

The science on Se toxicology has significantly advanced over the last thirty years. The EPA first issued recommended water quality criteria for Se in 1980, with revised criteria issued in 1987. In 1996, the acute criterion was updated to account for toxicity of two Se species, selenite and selenate. In 2004, EPA released the first draft update to the 1996 Se criterion which included fish tissue criteria to account for a dietary exposure pathway. In 2015, EPA released a draft update to the 2004 criteria, and it was finalized in 2016 as *Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater* (U.S. EPA, 2016).

The 2016 document considers the latest scientific information and provides guidance to states and tribes to protect freshwater aquatic life from toxic effects of Se. It is based on dietary exposure and accounts for reproductive effects in fish. The 2016 recommended criteria—which are derived for the protection of 95% of species nation-wide—is comprised of four elements (**Figure 1-2**). Two elements are fish tissue concentrations and two are water column concentrations. The elements are defined below:

1) a fish egg-ovary element,
2) a fish whole-body or muscle element,
3) a water column element (one for lentic and one for lotic) and
4) an intermittent element for short term exposures

EPA's 2016 guidance document recognizes that selenium bioaccumulation and toxicity in an ecosystem are based on site-specific environmental factors, therefore, the EPA provides additional guidance and methodology in Appendix K for states and tribes to follow when deriving site-specific criteria.

Media Type	Fish Tissue[1]		Water Column[4]	
Criterion Element	**Egg/Ovary [2]**	**Fish Whole Body or Muscle [3]**	**Monthly Average Exposure**	**Intermittent Exposure[5]**
Magnitude	15.1 mg/kg dw	8.5 mg/kg dw whole body or 11.3 mg/kg dw muscle (skinless, boneless filet)	1.5 µg/L in lentic aquatic systems 3.1 µg/L in lotic aquatic systems	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
Duration	Instantaneous measurement[6]	Instantaneous measurement[6]	30 days	Number of days/month with an elevated concentration
Frequency	Not to be exceeded	Not to be exceeded	Not more than once in three years on average	Not more than once in three years on average

1. Fish tissue elements are expressed as steady-state.
2. Egg/Ovary supersedes any whole-body, muscle, or water column element when fish egg/ovary concentrations are measured.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. Water column values are the applicable criterion element in the absence of steady-state condition fish tissue data.
5. Where WQC30-day is the water column monthly element, for either a lentic or lotic waters; C_{bkgrnd} is the average background selenium concentration, and fint is the fraction of any 30-day period during which elevated selenium concentrations occur, with f_{int} assigned a value ≥0.033 (corresponding to 1 day).
6. Fish tissue data provide instantaneous point measurements that reflect integrative accumulation of selenium over time and space in fish population(s) at a given site.

Figure 1-2. Selenium water quality criteria (U.S. EPA, 2016).

1.3.3 British Columbia's Water Quality Guideline for Selenium

BC-ENV is responsible for developing province-wide ambient WQGs. Per BC-ENV Policy 6.10.03.02, WQG's are defined as:

"A maximum and/or minimum value for a physical, chemical or biological characteristic of water, sediment or biota, applicable province-wide, which should not be exceeded to prevent specified detrimental effects from occurring to a water use, including aquatic life, under specific environmental conditions."

BC's aquatic WQGs are science-based and intended for provincial-wide application. BC-ENV applies an appropriate assessment factor determined on a case-by-case basis, according to the toxicity and bioaccumulation potential of the constituent (B.C. ENV, 2014). The assessment factor is the best estimate of a no-effect concentration, for the protection of 100% of species during all life stages. WQGs are not legally enforceable themselves but are used to provide scientific basis for best management practices and may be incorporated into permits (such as Elk Valley Permit No. 107517).

BC-ENV updated their Se Water Quality Guidelines (WQGs) in 2014 which includes an assessment factor of 2 pertaining to Se. (**Table 1-3**).

Table 1-3. BC Provincial WQG for selenium (B.C. ENV, 2014).

Water Use		Updated 2012 BC Se WQG	2001 Approved BC Se WQG	Guideline Derivation Method/Approach
Source Drinking Water		10 µg/L	10 µg/L	*Source Drinking Water:* Adopted from Health Canada; a maximum acceptable concentration of 10 µg/L to protect against adverse effects in humans from excessive exposure.
Human Consumption Screening Values				
High fish intake (0.22 kg/day)		1.8 µg/g (ww), 7.3 (dw)[1]	None proposed	*Tissue Consumption*: Values were derived using Health Canada's recommended equation for ingestion of Se-contaminated fish and the dietary reference value's tolerable upper intake.
Moderate fish intake (0.11 kg/day)		3.6 µg/g (ww), 14.5 (dw)	None proposed	
Low fish intake (0.03 kg/day)		18.7 µg/g (ww), 75.0 (dw)	None proposed	
Aquatic Life				*Water column*: Review of previous WQG (uncertainty factor (UF) applied to toxicity threshold); weight of evidence including food web modelling and reported relationships between impacts and Se concentrations in water.
Water column freshwater & marine				
Alert concentration	1	µg/L	None proposed	
Guideline	2	µg/L	2 µg/L	
Sediment - Alert concentration		2 µg/g (dw)	None proposed	*Sediment*: Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time.
Dietary				*Dietary:* Weight of evidence; lowest published toxicity thresholds, no UF applied; insufficient data for full guidelines at this time. Invertebrate tissue as surrogate for aquatic dietary tissue.
Invertebrate tissue (interim)		4 µg/g (dw)	2 µg/g (dw)	
Tissue (fish)				*Egg/ovary:* Combination weight of evidence and mean of published effects data with an UF of 2 applied; *Whole-body*: previous WB guideline compared with published literature, mean of published effects data with UF (2) applied and weight of evidence; *Muscle*: WB translation to derive muscle WQG, no additional UF applied to muscle guideline.
Egg/ovary		11 µg/g (dw)	None proposed	
Whole-body (WB)		4 µg/g (dw)	4 µg/g (dw)	
Muscle/muscle plug (interim)		4 µg/g (dw)	None proposed	
Wildlife				The *water column* guideline for aquatic life (fish) is adopted for wildlife since dietary accumulation is most critical. *Bird eggs* were used as surrogate for all wildlife; weight of evidence; egg Se most direct/sensitive measure; mallard EC10 with UF of 2 applied.
Water column		2 µg/L	4 µg/L (maximum)	
Bird egg		6 µg/g (dw)	7 µg/g (dw)	
Recreation and Aesthetics		None proposed	None proposed	No data
Irrigation Water				Not updated at this time
2001 guideline not updated		10 µg/L	10 µg/L	
Livestock Watering				Not updated at this time
2001 guideline not updated		30 µg/L	30 µg/L	
Industrial Water		None proposed	None proposed	No data

1.4 SELENIUM

Selenium is a member of Group 16, the chalcogen group on the periodic table of elements. Classified as a nonmetal, Se has properties of both metals and nonmetals. Selenium is considered chemically similar to other nonmetals in Group 16, for example sulfur (S) (Chapman et al., 2010). Selenium, a naturally occurring trace element essential for life, has a narrow margin between the amount necessary for proper functioning of organisms and the amount considered toxic (Janz, 2011). The toxicological potential of Se is strongly related to its chemical form (speciation).

1.4.1 Physical-chemical properties

Selenium exists in four oxidation states; elemental Se (0), selenite (+4), selenate (+6), and selenide (-2) (**Table 1-4**). Three primary transformation mechanisms occur for Se; 1) oxidation/reduction, 2) mineralization/immobilization, and 3) volatilization with Se speciation, with microbial activity and pH-redox conditions driving the kinetics of each function.

In aquatic ecosystems there exists three main fates for Se; it can 1) remain in solution, 2) be assimilated by organisms, and 3) it can be sorbed to suspended sediment. The oxyanions, selenate and selenite, are the dissolved inorganic forms of Se most commonly present in waters. It is generally understood that

selenite is less soluble, more easily transformed, assimilated by organisms, and considered to have a greater toxicity in aquatic systems than selenate. When taken up by organisms, Se can replace S in two amino acids, thought to contribute to toxicity in organisms particularly in oviparous (egg-laying) vertebrates (EPA, 2016). Finally, selenate and more commonly selenite can sorb to organic matter, clays, and oxides and hydroxides most frequently associated with the ions of iron (Fe), manganese (Mn), and sodium (Na). EPA (2016) provides significant detail on both the biochemical and the geochemical pathways of selenium in aquatic systems. It is clear that an understanding of the biogeochemical transformations of selenium is essential to understanding the fate, transport, and toxicological effects of selenium in the environment.

Table 1-4. Examples of the forms of selenium found in the environment (B.C. ENV, 2014).

Name	Valence/ Oxidation State	Forms/Se Species	Occurrence
Selenides	-II, Se^{II-}, Se^{2-}	Inorganic selenides, (Se^{2-}, HSe^-)	Found in reducing environments, sorbed onto soil/mineral particles, e.g., ferroselite ($FeSe_2$), chalcopyrite ($CuFeSe_2$)
		Hydrogen selenide, H_2Se	Unstable highly toxic gas, converts to Se^0 in H_20
		Organic selenides, R_2Se	
		Volatile organic selenides:	
		dimethyl selenide (DMSe), $(CH_3)_2Se$;	Gas, volatilization from soil/sediment bacteria and fungi
		dimethyl diselenide (DMDSe), $(CH_3)_2Se_2$;	Gas, volatilization from soil/sediment plants
		dimethyl selenone $(CH_3)2SeO_2$	Volatile metabolite, intermediate form between DMSe and DMDSe
		Biochemical intermediates, amino acids	Many forms, but most common are the amino acids selenomethionine (SeMet) and selenocysteine (SeCys)
Elemental selenium	0, Se^0		Insoluble, fairly stable, unweathered mineral form of Se, found in water, soil, sediment and biological tissue
Selenium dioxide	+II, Se^{+II}, Se^{+2}	SeO_2	Gas, not a naturally occurring form, product of fossil fuel combustion (coal, oil, gas), and smelting, soluble, forms selenous acid with water
Selenites/selenous acid	+ IV, Se^{+IV}, Se^{+4}	SeO_3^{2-}	Soluble, found in mildly oxidizing conditions in air, water, soil/sediment,
		Hydrogen selenite ($HSeO_3^-$)	Common form of selenites in soils, easily sorbed onto iron(hydr)oxide minerals $Fe(OH)SeO_3$, or other ions e.g., sodium selenite Na_2SeO_3, highly mobile and available to plants
		Selenous acid (H_2SeO_3)	
Selenates/selenic acid	+ VI, Se^{+VI}, Se^{+6}	SeO_4^{2-}	Common form of Se in surface water and soils, very soluble in water, stable in well-oxygenated water, not easily transformed biologically to more reduced forms, reduction reactions slow. In plants, selenate is actively transported against electrochemical potential gradient.
		Hydrogen selenate $HSeO_4^-$	
		Selenic acid H_2SeO_4	

1.4.2 Selenium Toxicity to Wildlife

Selenium bioaccumulates in wildlife primarily through a dietary pathway with egg-laying vertebrates determined to be the most sensitive. EPA (2016) outlines the differences in bioaccumulation between lentic (lake-like) and lotic (flowing-water) systems. Retention time, dissolved oxygen, and carbon content result in greater bioaccumulation in lentic systems. It is understood that while Se is nutritionally required in small quantities, it becomes highly toxic in slightly greater amounts with the potential to cause rippling effects through both aquatic and terrestrial food webs (Naslund et al., 2020). **Figure 1-3** describes selenium toxicity for fish and birds. Fish are considered the most sensitive ecological end point in Lake Koocanusa as determined by the SeTSC (see **Section 3.7**), therefore, fish are the focus of this report and the development of the Se standards for Lake Koocanusa.

Selenium toxicity in fish is most severe at the reproductive stage where newly hatched larval fish may experience teratogenic deformities and death while feeding off yolk sacs enriched in Se (Lemly 1993, Skorupa 1998). Extremely high concentrations of Se can be lethal to adult fish but this is not common; rather, more commonly, fish are exposed to various sublethal effects (**Figure 1-3**).

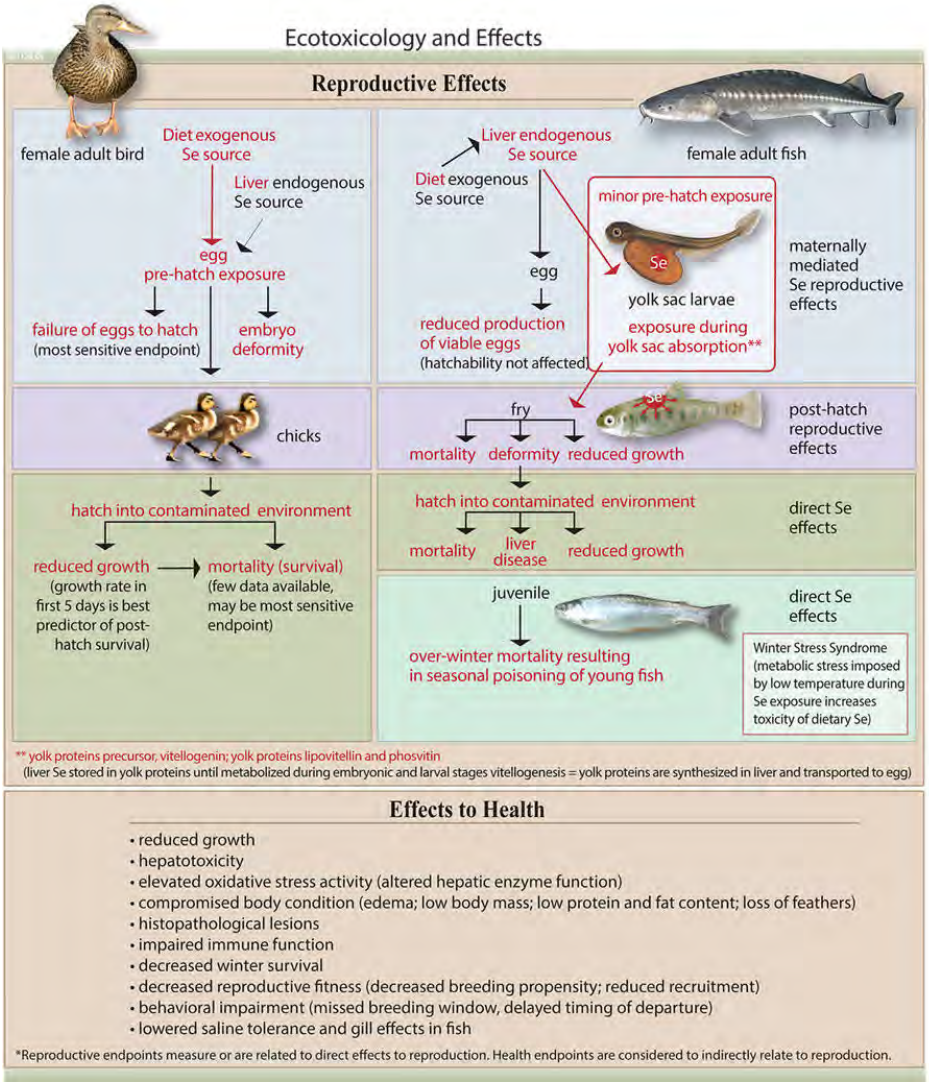

Figure 1-3. Details of general ecotoxicological pathways of selenium for fish and birds (top) and effects of concern for selenium (bottom). As represented here, birds and fish differ in how selenium is taken up in the diet and distributed among tissues (Presser and Skorupa, 2019).

1.4.3 Sources of Selenium

The primary source of Se to Lake Koocanusa is anthropogenic release to the environment from historic and present-day mining operations in the Elk Valley, BC. Coal in the Elk Valley belongs mainly to the Mist Mountain Formation of the Jurassic-Cretaceous Kootenay Group and is part of the East Kootenay coalfields (Grieve, 1952). Carbonate bedrock in the Elk Valley is excavated to access coal seams underneath for metallurgical steelmaking coal production. The excavation process creates a by-product

called overburden (waste rock) which becomes exposed to oxidation, increasing selenium mobilization through infiltration and runoff to nearby groundwater and surface water including the Elk River, BC.

Currently, Teck is the sole operator of five active coal mines in the Elk Valley and permitted under Elk Valley Permit No. 107517 (**Figure 1-4**). That permit authorizes Se concentrations at sites throughout the Valley (**Table 1-1**). **Figure 1-5** illustrates the location of Teck's existing coal mines in relation to coal bearing strata in the Kootenai (Kootenay) River Watershed.

Figure 1-4. Teck's Operations in the Elk Valley Watershed (Teck, 2017).

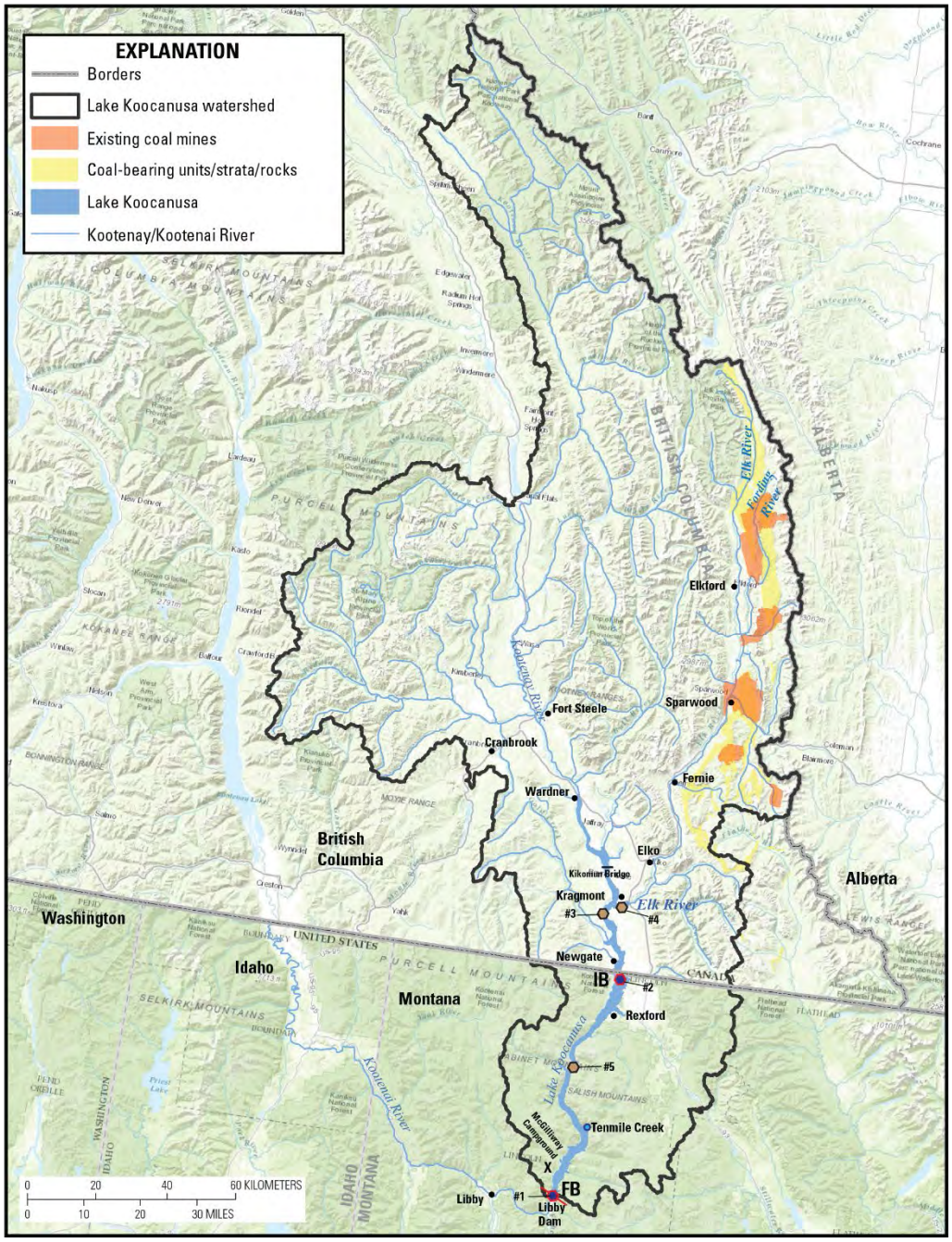

Figure 1-5. Teck's existing coal mines and location of coal bearing strata within the Kootenai (Kootenay) River Watershed (Jenni et al., 2017).

From 1984 to 2019, concentrations ranging from below detection level (DL) to greater than 8 µg/L have been recorded 2.2 miles upstream from Lake Koocanusa on the Elk River, a tributary (**Figure 1-6**). Selenium contributions from the Kootenay River are minimal and described in **Figure 1-7**, and **Table 1-5**.

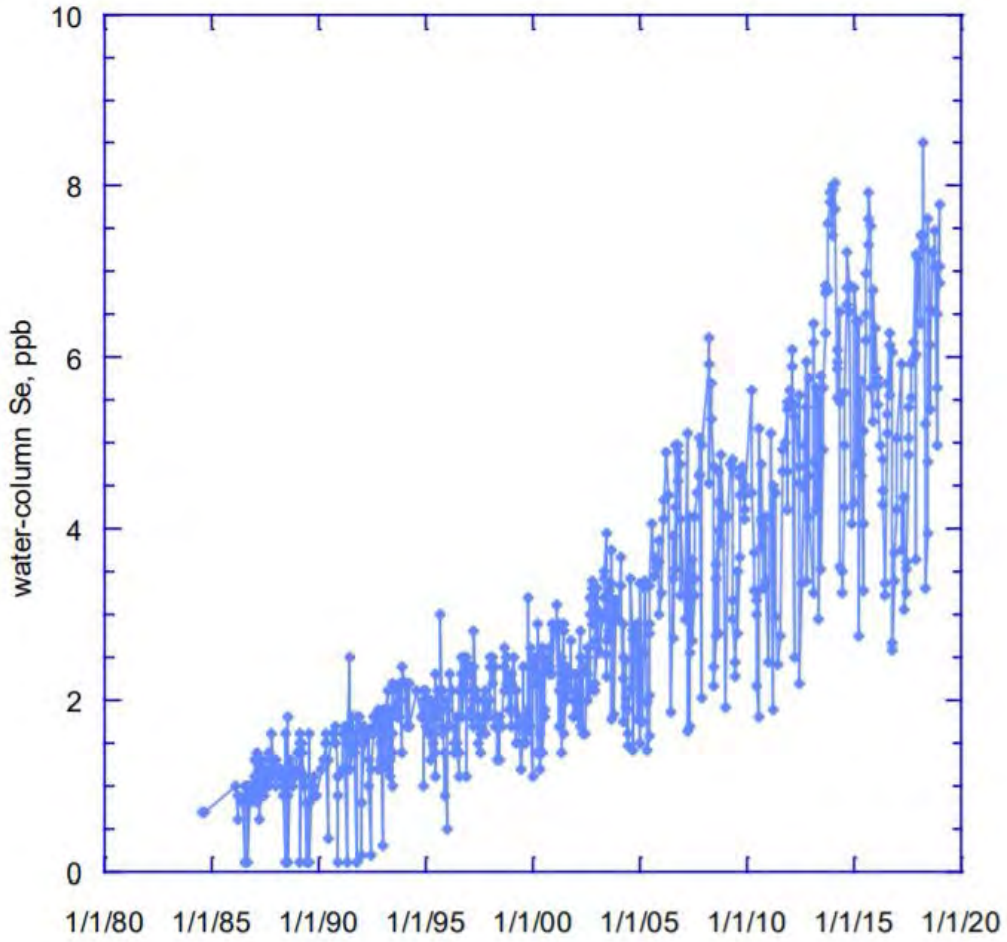

Figure 1-6. Se concentrations from 1984-2019 recorded at station BC08NK003 on the Elk River, a tributary to the Kootenay River located 2.2 miles upstream of its confluence with Lake Koocanusa (Presser and Naftz, 2020).

The Elk River contributes over 95% of the selenium to Lake Koocanusa with the Kootenay River and Bull River delivering together less than 5%. McDonald (2009) reported that in 2008 the total selenium loads were 23,720 lb/yr (10,759 kg/yr). The Elk River contributed 22,450 lb (10,183 kg) Se, the Kootenay River provided 1,078 lb (489 kg) Se, and the Bull River added only 192 (87 kg) Se. In 2012, it was determined that from 1992 to 2012, the amount of Se entering the lake each year increased fivefold, from 5,732 lb (2,600 kg) in 1992 to more than 28,660 lb (13,000 kg). **Figure 1-7** shows the comparison of Se load estimates between the Kootenay River and Elk River. The values presented in **Figure 1-7** were calculated and incorporate scaled flow values, and do include some uncertainty and are not direct measurements of load or water quality and quantity at the reported locations. **Table 1-5** describes the loading comparisons and Se concentration comparisons between upstream and downstream sites as reported in Teck's 2019 Monitoring report.

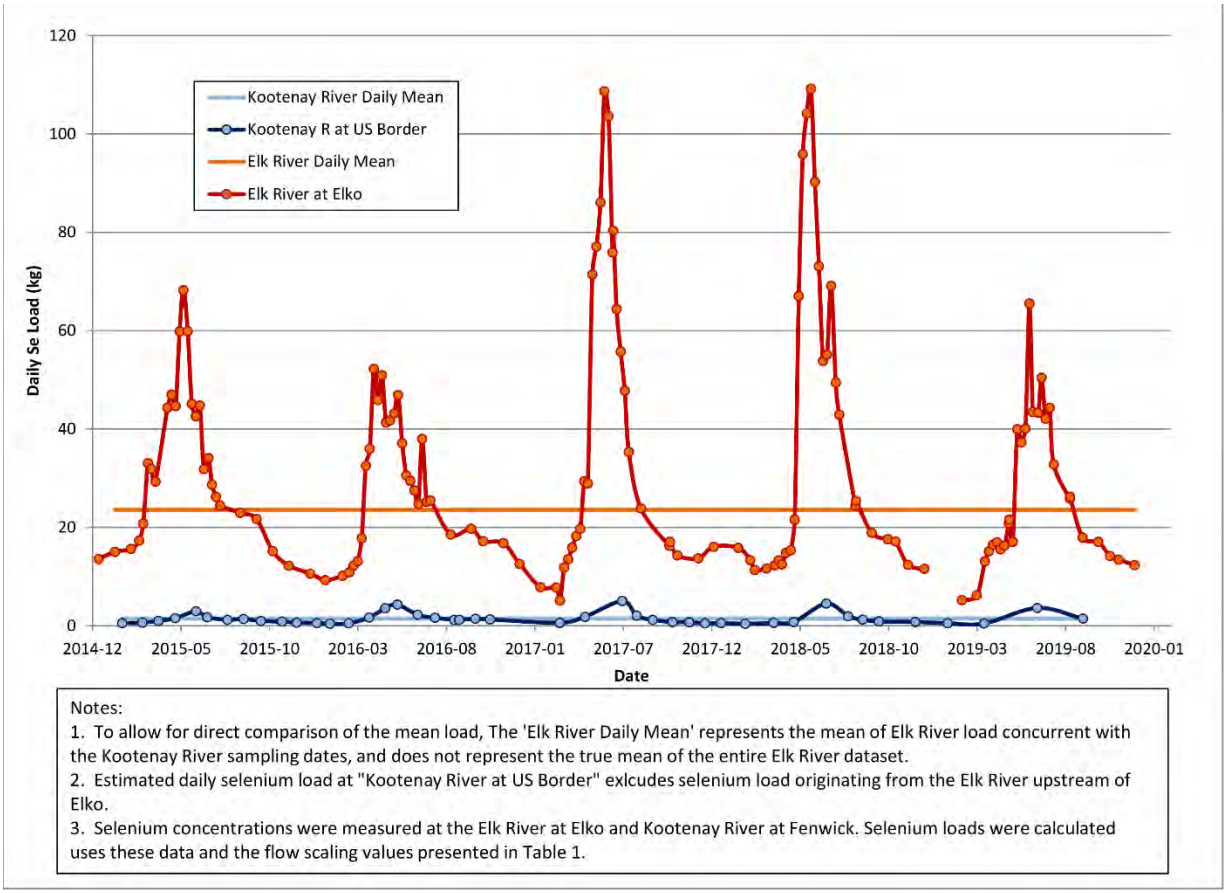

Figure 1-7. Selenium loads from the Kootenay River and Elk River (Sheldon Reddekopp, BC-ENV, personal communication, 8/4/2020).

Table 1-5. Average monthly selenium concentrations, selenium loadings, and total water volume at two Teck monitoring sites; 1) the mouth of the Elk River (RG_ELKMOUTH), and 2) the upstream site on the Kootenay River (RG_WARDB) (Minnow, 2019).

Source	Month	Average Selenium µg/L	Total Volume (m^3)	Selenium Loadings (kg/day)
Elk River (RG_ELKMOUTH)	January	6.66	41,006,976	7
	February	7.43	25,089,073	6
	March	7.21	46,239,045	11
	April	5.62	106,686,508	18
	May	3.84	276,188,619	32
	June	3.57	421,070,214	51
	July	4.64	297,267,032	46
	August	5.92	149,747,763	30
	September	6.1	98,566,165	20
	October	6.62	82,005,592	19
	November	6.54	54,291,842	14
	December	6.57	60,114,499	12
Kootenay River (RG_WARDB)	January	0.14	112,819,052	0
	February	0.14	77,486,057	0
	March	0.12	116,675,008	0
	April	0.11	267,887,635	1
	May	0.09	947,360,464	3
	June	0.08	1,370,615,445	4
	July	0.11	941,856,710	3
	August	0.11	482,055,684	2
	September	0.1	359,498,669	1
	October	0.1	267,152,361	1
	November	0.11	171,424,041	1
	December	0.15	147,008,197	1

2.0 SITE DESCRIPTION

Lake Koocanusa, sometimes referred to as Koocanusa Reservoir, is a 90-mile long transboundary body of water that lies in northwest Montana and southeast British Columbia (**Figure 2-1**). It is within the international Kootenai (Kootenay) River watershed, draining an area of approximately 19,420 square miles (50,298 km^2). Around 70% of the watershed is located within BC with 23% in Montana and 6% in Idaho (USFWS, 2006). The Kootenai River is the second largest tributary to the Columbia River in volume and third in drainage area (USACE, 1972). The total river length is 485 miles (781 km), originating in southeast British Columbia, extending through Montana and Idaho, returning back into BC where it flows through Kootenay Lake, and finally reaches the Columbia River at Castlegar, BC. (**Figure 2-2**).

Lake Koocanusa was formed by the impoundment of the Kootenai (Kootenay) River upon construction of Libby Dam, approximately 17 miles (27 km) upstream of Libby, Montana, and was completed in 1972. The reservoir occupies lands on the territories of the Ktunaxa First Nations (KNC), Confederated Salish and Kootenai Tribes (CSKT), and the Kootenai Tribe of Idaho (KTOI). Lake Koocanusa was created under the Columbia River Treaty (CRT) between the United States and Canada to provide power and flood control (Storm et al., 1982). Construction of the Libby dam resulted in the inundation of approximately 90 miles (145 km) of the Kootenay River and 40 miles (65km) of low-gradient tributary habitat. The reservoir operations of Lake Koocanusa are managed by the U.S. Army Corps of Engineers (USACE) outlined in the CRT and hydroelectric power is sold by Bonneville Power Administration (BPA).

Figure 2-1. Transboundary Lake Koocanusa in northwest Montana.

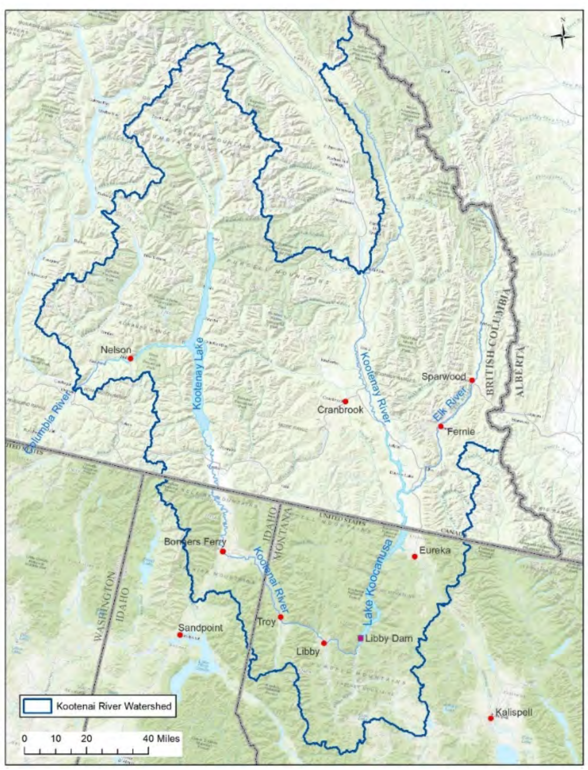

Figure 2-2. Kootenai (Kootenay) River Watershed.

2.1 RESERVOIR HYDROLOGY

A complete hydrological description of the Kootenai (Kootenay) River watershed, Lake Koocanusa and its tributaries, hydrology, climate, and physical properties can be found in the Lake Koocanusa Data Compilation Report (Lotic, 2019).

The construction of the Libby Dam in 1972 converted the Kootenai (Kootenay) river from a lotic to a lentic system. The aquatic community, dryland ecosystems, waterfowl species, and other human uses and values were affected.

Three major rivers supply water to Lake Koocanusa. The mean annual flow contribution from the Kootenay River is 56%, the Elk River provides 22%, and the Bull River contributes 11%. The Tobacco River provides 2% while the remaining 9% coming from ungauged flows (Lotic, 2019).

Lake Koocanusa water-level elevations are managed primarily for power and flood control purposes (Storm et al., 1982). **Table 2-1** describes the hydrological characteristics of Lake Koocanusa. Maximum surface area is 46,500 acres (188 km^2) with 28 sq. miles (72km^2), or 38% within BC. Maximum depth at full pool is approximately 350 ft (107 m) and mean bulk water retention time is 6 months. Typical draw down is 98 ft (30 m) with a maximum draw down capacity of 170 feet (52 m).

Table 2-1. Lake Koocanusa hydrological characteristics (Chisholm, 1989)

Surface elevation		
	maximum pool	2,459 ft (749.5 m)
	minimum operational pool	2,287 ft (697.1 m)
	minimul pool (dead storage)	2,222 ft (671.2 m)
Area		
	maximum pool	46,500 acres (188 km^2)
	minimum operational pool	14,487 acres (58.6 km^2)
Volume		
	maximum pool	5,869,400 acre-ft (7.24 km^3)
	minimum operational pool	890,000 acre-ft (1.10 km^3)
Maximum legnth		90 mi (145 km)
Maximum depth		350 ft (107 m)
Mean depth		126 ft (38 m)
Shoreline length		224 mi (360 km)
Shoreline development		4.6 mi (7.4 km)
Drainage Area		8,985 sq. mi (23,271 sq. km)

Water levels in Lake Koocanusa are generally lowest in late winter and early spring (i.e., February through April) and highest in summer and early fall (i.e. August through October; Minnow, 2014). Power generation drawdown begins in November with maximum draws during April. Flow is dependent on hydro-related demands, where spring flows have been attenuated, winter flows are above normal, and daily and hourly flows can change drastically (FWP, 2019b). Residence times have been reported as

variable. Presser and Naftz (2020) describe reports ranging from 1.7-7.5 months during initial construction (1972-1980). While more recent reports range between 5.5 and 9 months.

The USACE manages Libby Dam with a selective withdrawal system to provide flows through Libby Dam for downstream Kootenai River fish. Specifically, flows are provided for federally listed white sturgeon (*Acipenser transmontanus*), bull trout (*Salvelinus confluentus*), and salmon (*Oncorhynchus nerka*) during spring; for salmon during summer, and for bull trout and resident fish in September (BPA, 2018). The selective withdrawal system was installed on Libby Dam to control temperature of water releases from the dam. This system is intended to maximize the probability of allowing significant white sturgeon recruitment, provide a year-round thermograph that approximates normative conditions, while also meeting flood damage reduction objectives (BPA, 2018). The ecological flow plan followed by USACE was developed to restore ecological function in support of Kootenai River white sturgeon recovery while also maintaining flood control (USFWS, 2006). These functional normative flows (ecological flows) are designed to more closely mimic pre-dam hydrographs (**Figure 2-3**). Annual variability for flow and elevation for the reservoir and river from 2006 to 2019 are shown in **Figure 2-4**.

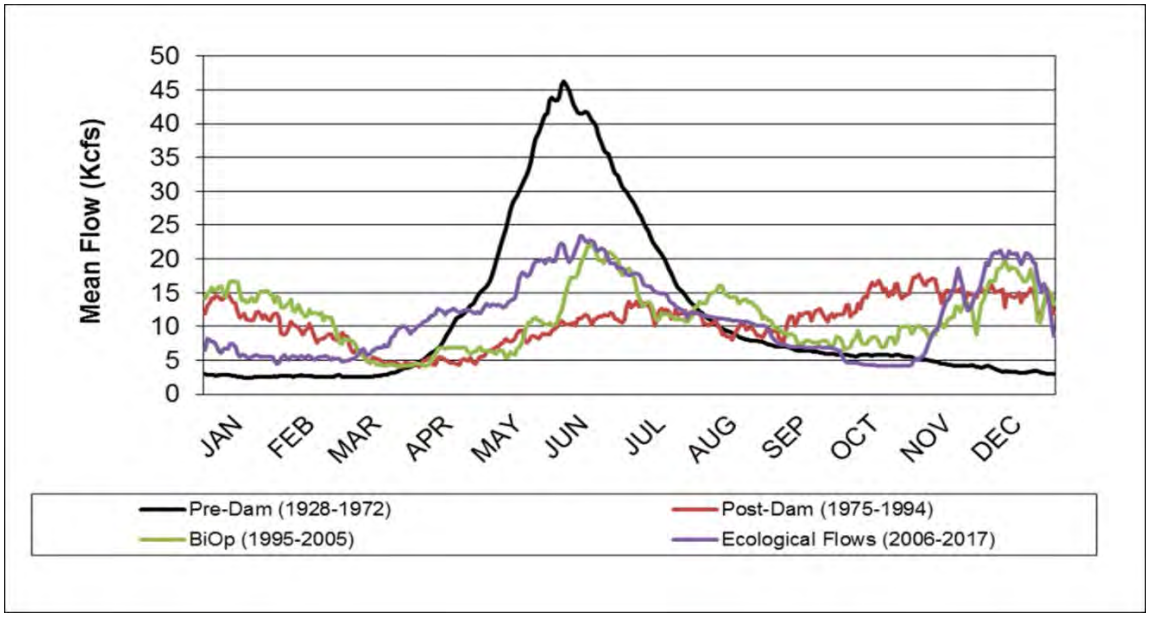

Figure 2-3. USACE stream flow management out of Libby dam from 1976-2017. Ecological flows are displayed in purple and are designed to more similarly mimic pre-dam flows displayed in black (U.S. ACE, 2019).

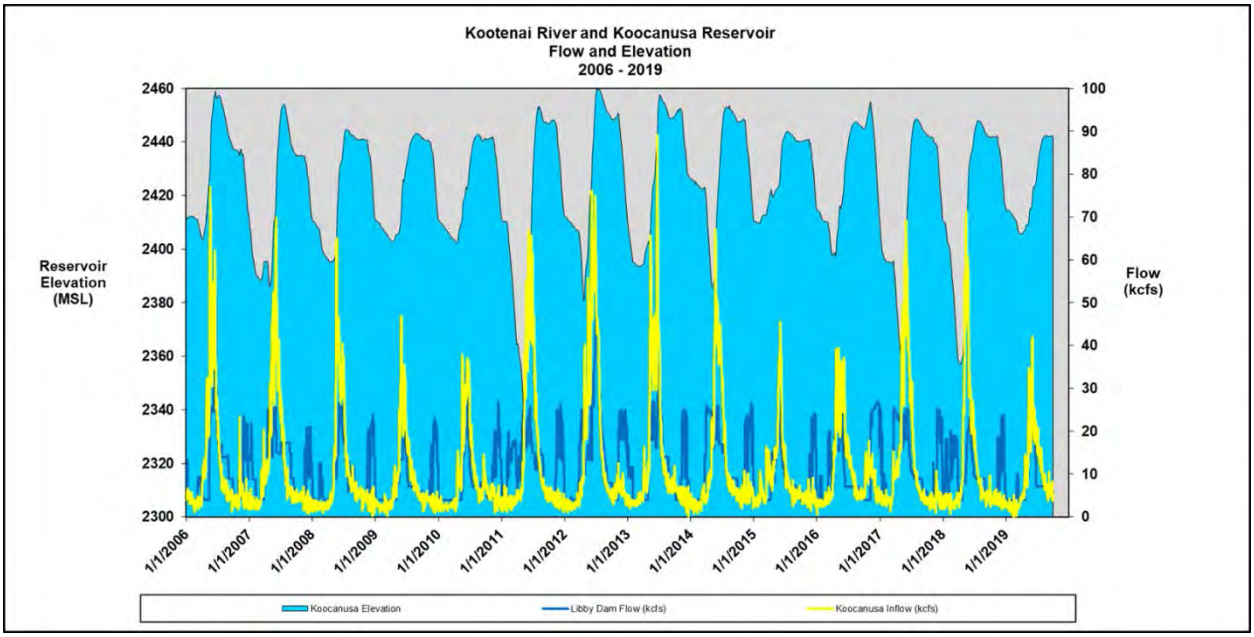

Figure 2-4. Fluctuations in reservoir elevation and flow from 2006-2018 (U.S. ACE, 2019).

The seasonal fluctuation of water levels affects the hydrology of the waterbody and associated aquatic life by impeding the establishment of riparian vegetation or aquatic macrophytes which has left the composition of the littoral zone to be that of cobble, mud, and sand substrates with limited habitat structures. Variable water levels have also affected bank stability and can impact spawning success of certain fish such as burbot (Lotic, 2019).

2.2 PHYSICOCHEMICAL CHARACTERISTICS

Routine monitoring has been conducted on the US portion of the reservoir beginning in the early 1970s with detailed summaries included in reports by USACE found at https://www.nws.usace.army.mil/About/Offices/Engineering/Hydraulics-and-Hydrology/Water-Quality/Water-Quality-Documents/ and USGS publicly available data found at https://www.waterqualitydata.us/provider/NWIS/USGS-MT/USGS-12301919/. The USACE maintains three main monitoring stations in Lake Koocanusa (**Figure 2-5**).

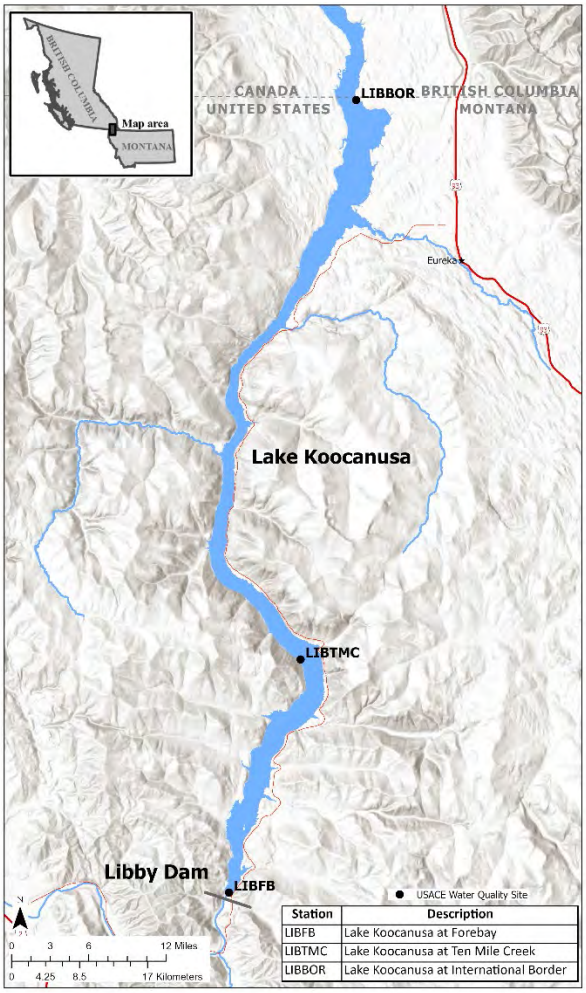

Figure 2-5. Locations of USACE's routine monitoring stations in Lake Koocanusa.

In BC, routine physicochemical monitoring is done by the federal department of Environment and Climate Change Canada (ECCC) which has a long-term monitoring station (BC08NK0003) on the Elk River approximately 2.2 miles upstream from the confluence with the Kootenay River. Additionally, under the EVWQP, Teck is required to conduct comprehensive physico-chemical and biological monitoring which first began in 2014. Monitoring sites for Teck are located both upstream and downstream of the Elk River. Monitoring reports by Teck are published annually and are vetted by BC-ENV scientists, the KNC, and independent scientists. Reports from 2014-2019 can be found on at https://www.teck.com/responsibility/sustainability-topics/water/water-quality-in-the-elk-valley/.

2.2.1 Nutrients & chlorophyll *a*

Nutrients and chlorophyll *a* routinely monitored by the USACE and Teck and are detailed extensively in USACE reports found at https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/ and Teck reports found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Briefly, Lake Koocanusa in Montana is considered oligotrophic and at times mesotrophic as defined by Lake Koocanusa's trophic State Index (TSI). TSI measures transparency and presence of nutrients and chlorophyll *a* (Carlson, 1977). The Canadian section of the reservoir experiences periods of eutrophic conditions in spring (April – June) but qualifies as oligotrophic the remainder of the year. The majority

of the phosphorus (P) entering the reservoir is P sorbed to soil particles, entering the reservoir during spring runoff which is likely not biologically available. The years with the highest runoff are associated with greater total phosphorus (TP). The P-bound sediment settles from the water column to the sediment layer as it travels through the 90-mile reservoir towards the dam. As a result, Lake Koocanusa acts as a nutrient sink, trapping an estimated 63% of incoming phosphorus (Wood, 1982). Soluble Reactive Phosphorus (SRP) is consistently lower than the 1 µg/l detection limit (DL). While low levels of TP and SRP are recorded in the reservoir, increasing concentrations of nitrogen (N) have been detected (**Figure 2-6 and Figure 2-7**). Elevated nitrate concentrations entering the reservoir are linked to blasting practices during coal production (Mahmood et al., 2017). The high N concentrations and low P concentrations have resulted in a N:P ratio far from what is considered healthy for an aquatic system and has had deleterious effects below Libby Dam by encouraging the presence of the nuisance diatom *Didymosphenia geminata* (didymo) (Dunn et al., 2015).

Chlorophyll *a* concentrations are greatest during the spring and early summer with similar concentrations recorded at all three USACE monitoring stations. Higher concentrations of chlorophyll *a* are associated with high flow years. Chlorophyll *a* measurements supported the stratification trends identified with dissolved oxygen, temperature and pH (Minnow, 2018).

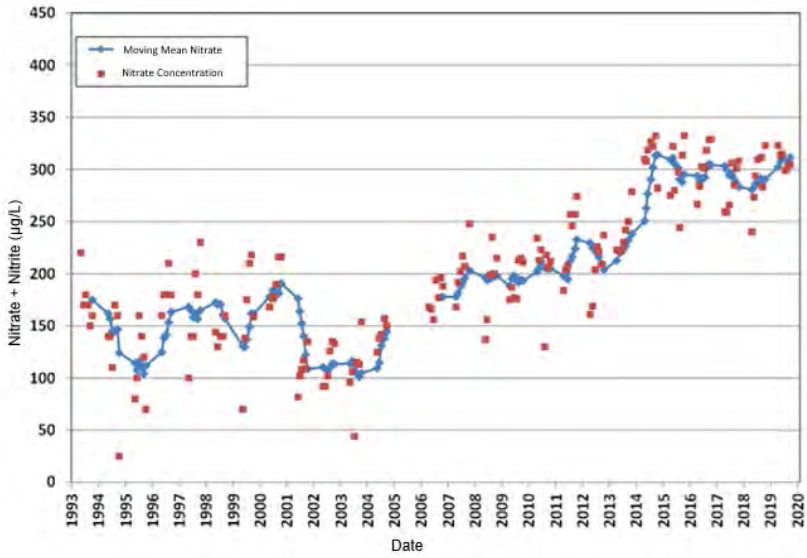

Figure 2-6. Nitrate + nitrite concentrations in the hypolimnion at the LIBFB (Forebay) monitoring station (USACE, 2018).

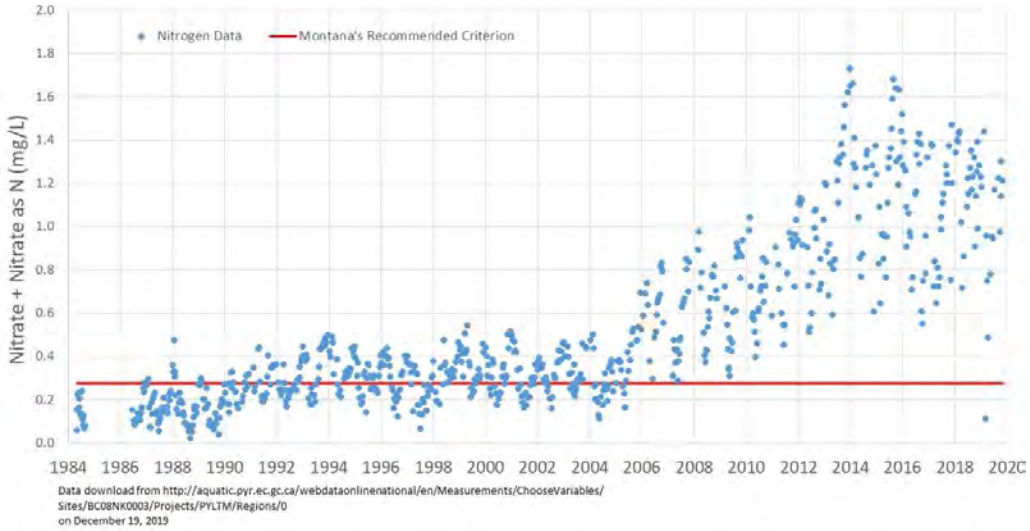

Figure 2-7. Nitrate + nitrite from 1984-2019 recorded at station BC08NK003 located 2.2 miles upstream of Lake Koocanusa (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020).

2.2.2 Metals and Metalloids

Lake Koocanusa has been monitored for a suite of major and trace metals and metalloids since the 1970s by USACE and USGS. Trend data for each metal and metalloid can be found in the USACE reports https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/. In BC, metal(loid) data are included in the Teck monitoring reports found in at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

The MT data show below DL or low levels of metals with the exception of the metalloid selenium. Selenium has been routinely monitored on the US side of the reservoir since 2013. Dissolved selenium concentrations in MT range from 0.04-2.29 µg/L from 2013-2018.

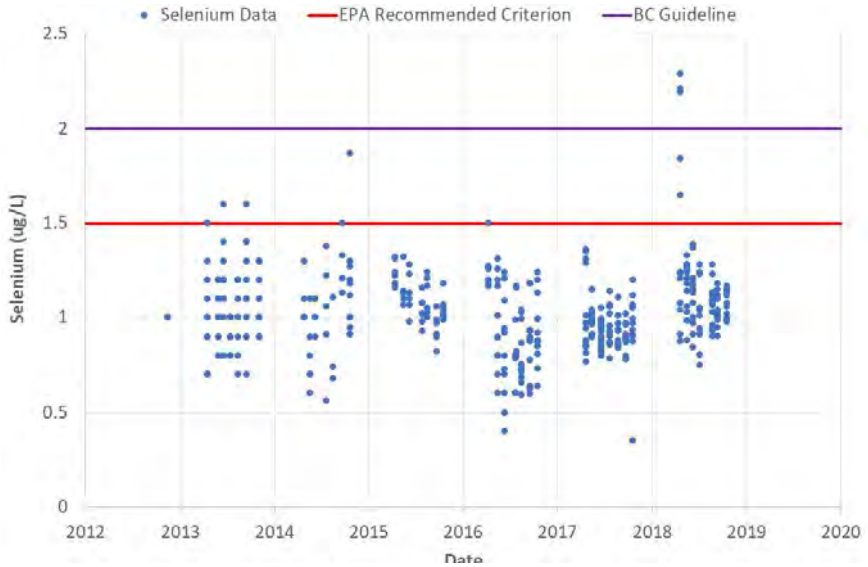

Figure 2-9. Selenium Data in Lake Koocanusa, U.S. Stations, all depths 2012-2018. (Jason Gildea, USEPA Region VIII, personal communication, 7/10/2020)

2.3 BIOLOGICAL CHARACTERISTICS

This section details baseline biological characteristics of Lake Koocanusa to provide a basic understanding of the system.

2.3.1 Phytoplankton

USACE has included phytoplankton in their routine monitoring from 2006-present. Phytoplankton densities vary from year to year and by location within the reservoir. A more detailed report of USACE's findings can be found in https://usace.contentdm.oclc.org/digital/collection/p266001coll1/id/8870/.

Peak concentrations occur between May and August. The dominant algal group are Diatoms followed by Chrysophytes. Cyanobacteria have been measured since 2015 with *Planktolynbia* documented as the dominant type of cyanobacteria. The diatom species dominance has changed since sampling began in 2006. The most abundant diatoms in the reservoir include: *Asterionella, Cyclotella, Fragilaria, Stephanodiscus, Syndedra,* and *Nitzschia*. From 2008-2012 diatoms were recorded at low density with diverse composition (**Figure 2-10 and Figure 2-11**). In 2014 a shift was recorded in which *Cyclotella* and *Fragilaria* dominated. In 2017 USACE recorded high density and low diversity with near total dominance of *Cyclotella* and *Fragilaria*. There have been no differences in the phytoplankton community compositions between upstream and downstream monitoring locations on the Elk River (Minnow, 2019).

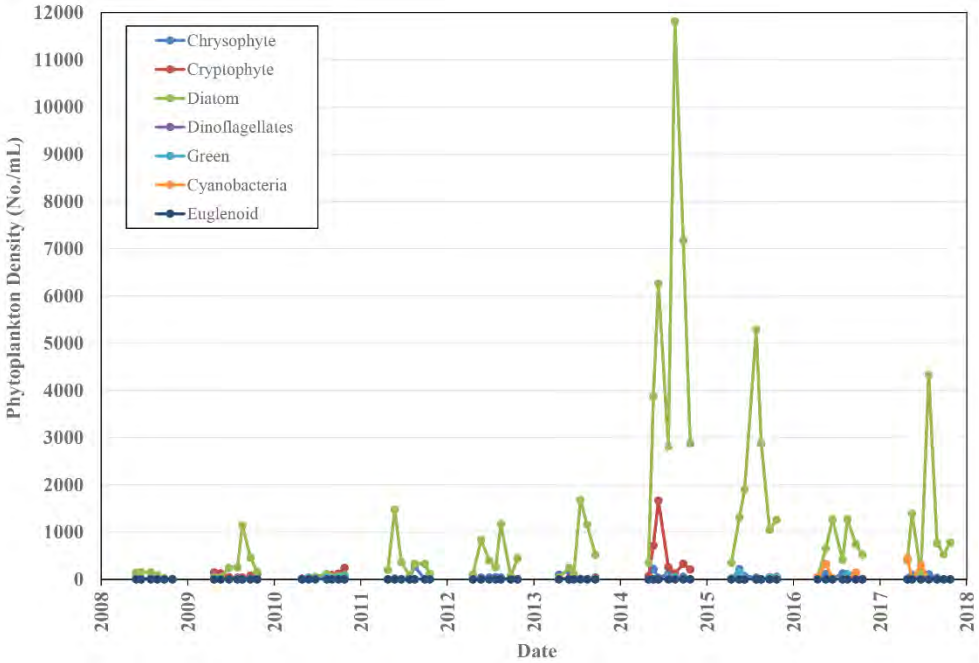

Figure 2-10. Phytoplankton density at LIBFB (Forebay) from 2008-2017 (U.S. ACE, 2019).

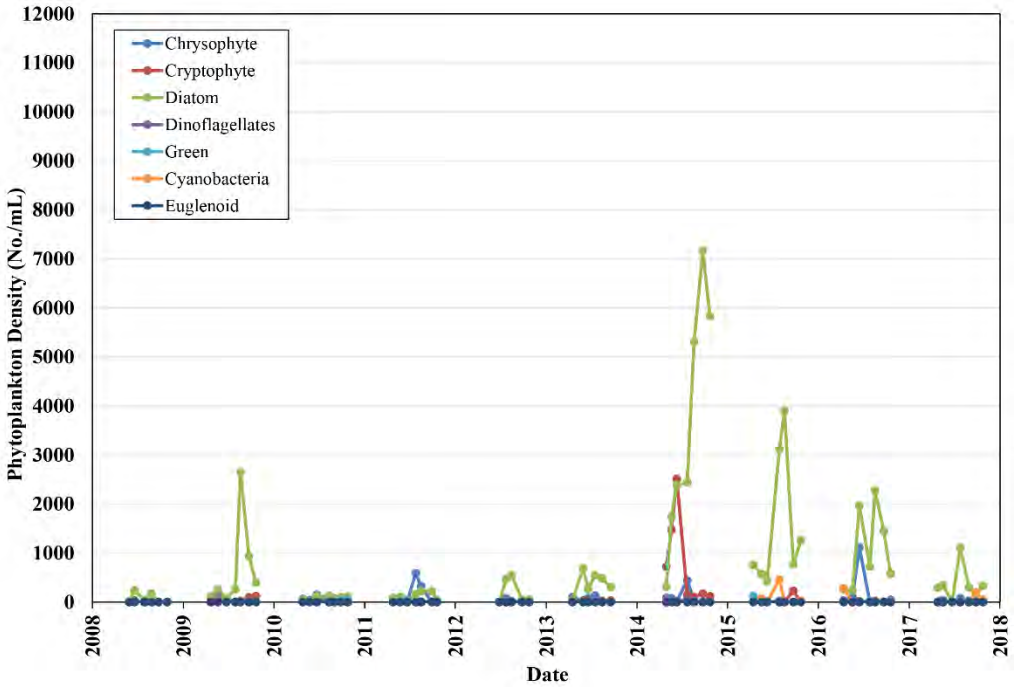

Figure 2-11. Phytoplankton density at LIBBOR (International Border) from 2008-2017 (U.S. ACE, 2019).

2.3.2 Zooplankton

Zooplankton abundance and composition recorded in Montana by USACE has been variable between the years of record 2006-2017. Copepods dominated between 2006 and 2010 while Rotifers dominated between 2011 and 2017 (**Figure 2-12 and Figure 2-13**). Within Rotifers, *Keratella* and *Keilicottia*

dominate. Cyclopoid and *Diacyclops* are the dominant copepods. *Daphnia* and *Bosmina* are the dominant Cladocerans. Seasonal succession in zooplankton community is likely in response to food source availability, changes in temperature, and grazing pressure by fish.

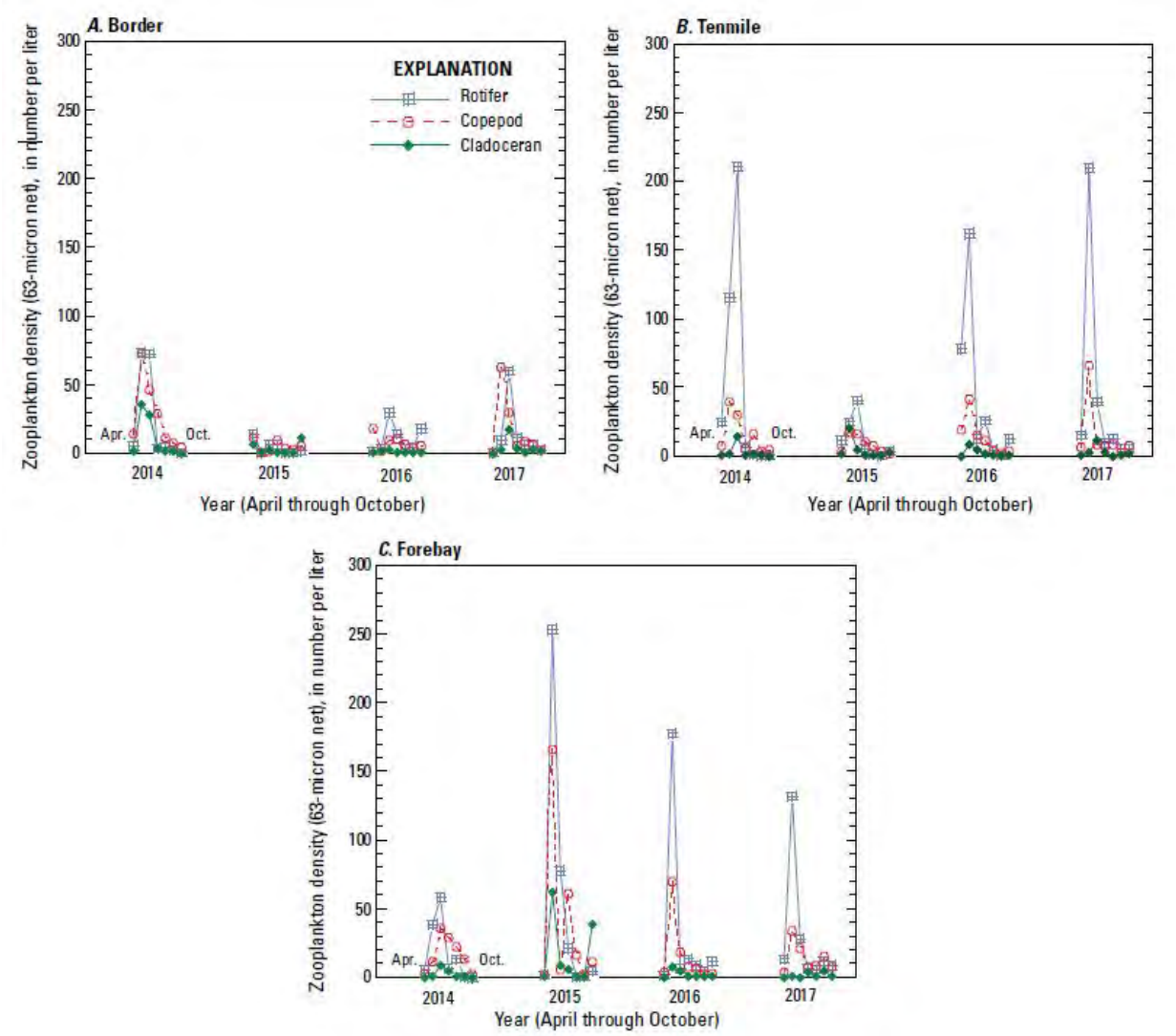

Figure 2-12. Zooplankton densities 2014-2017 (63 micron net) at sites A) Border, B) Tenmile, and C) Forebay (Presser and Naftz, 2020).

Figure 2-13 describes the Se concentrations in Zooplankton collected by Teck, USACE, and USGS between 2008 and 2019.

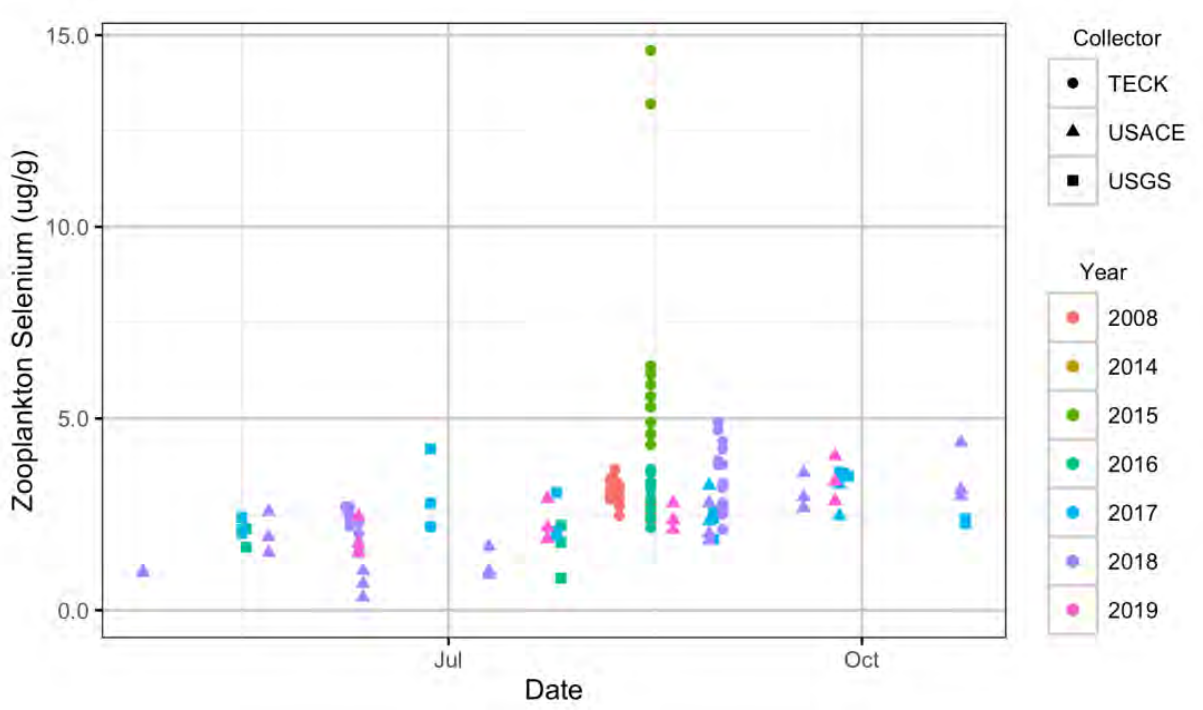

Figure 2-13. Zooplankton selenium concentrations by date, collector, and year (Thorley, 2020).

2.3.3 Macroinvertebrates

Routine data on richness and abundance for macroinvertebrates on the Montana portion of Lake Koocanusa do not exist. Montana Fish Wildlife and Parks (FWP) in collaboration with DEQ conducted benthic and surface macroinvertebrate sampling during 2018 (see **Section 3.0**). The results for Se concentration in macroinvertebrates showed chironomid dominance.

In BC, Teck conducted benthic community data collection and detailed reports on richness and abundance can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf. Dominant taxa found at their sampling sites were *Chironomus, Procladius* and *Tanytarsus*. Additionally, Teck has monitored for Se in macroinvertebrate tissues as required by their permit. Their 2019 monitoring report showed Se concentrations in benthic tissues were higher at the mouth of the Elk River compared to upstream (Kootenay River) and downstream locations. Moreover, average selenium concentrations in invertebrate tissues collected both upstream and downstream of the Elk River have oscillated between being below BC guidelines (4 µg/g dw) during spring samples and above guidelines in summer and fall samples (Minnow, 2019). Between sites, there were significant differences found in selenium concentrations from Kootenay River upstream concentrations (lower Se) to downstream concentrations (higher Se) regardless of season.

2.3.4 Fish

Numerous biological inventories have been conducted on the MT portion of the reservoir both pre-and-post dam construction. Reports detailing fish monitoring results can be located in Libby Mitigation Reports located at http://fwp.mt.gov/fwpDoc.html?id=95385 and results from fish monitoring conducted by Teck can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

Figure 2-13 shows the relative abundance of the following fish species found in Lake Koocanusa: bull trout, burbot (*Lota lota*), Kokanee (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), westslope cutthroat trout (*Oncorhynchus clarkia lewisi*), mountain whitefish (*Prosopium williamsoni*), northern pikeminnow (*Ptychocheilus oregonensis*), redside shiner (*Richardsonius balteatus*), peamouth chub (*Mylocheilus caurinus*), longnose sucker (*Catostomus catostomus*), and largescale sucker (*Catostomus macrocheilus*) (Presser and Naftz, 2020). An accidental release of 250,000 kokanee fry into Lake Koocanusa from Kootenay Trout Hatchery in the late 1970s (Richards, 1997) led to the establishment of Kokanee in the reservoir. MT FWP stocks rainbow trout under their fisheries management program.

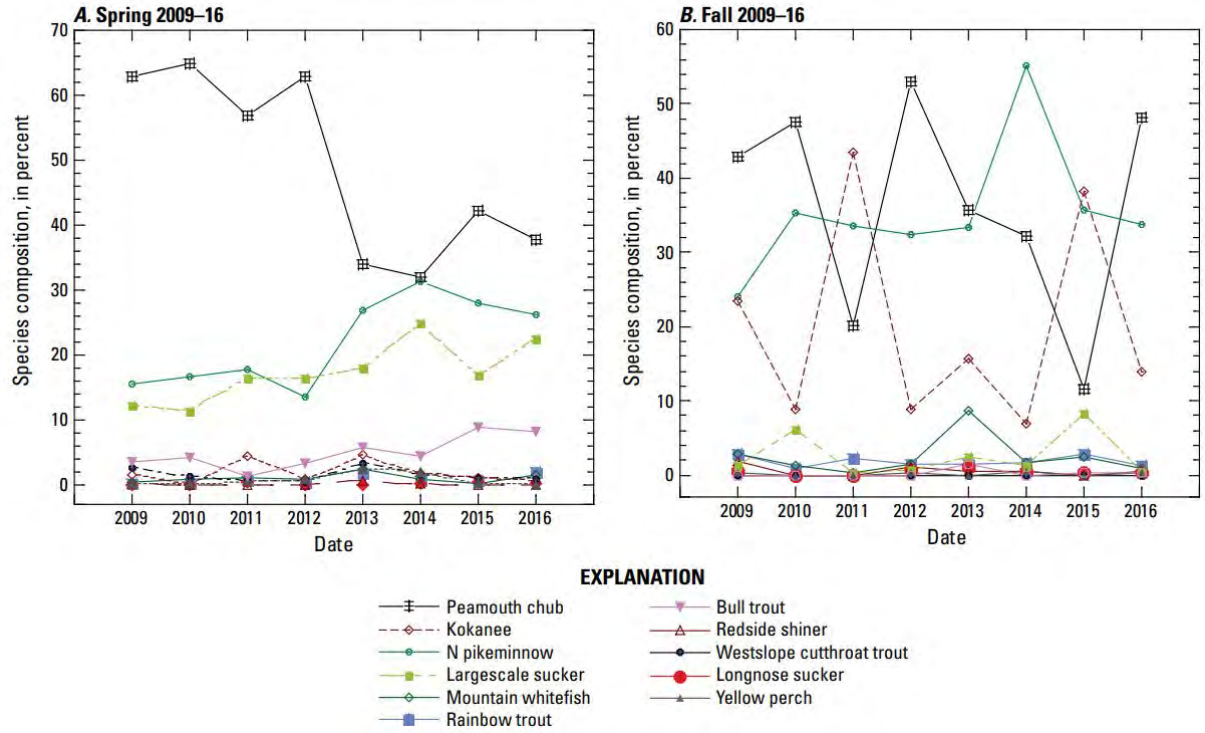

Figure 2-13. Fish species composition during A) Spring 2009-2019 and B) Fall 2009-2016 (Presser and Naftz, 2020).

Figures 2-14 and 2-15 show the results of MT FWP's 2018 fish sampling. All three redside shiner egg/ovary samples exceeded the EPA 304(a) criterion of 15.1 mg/kg dw along with one sample from peamouth chub. The other samples all remained below the EPA criterion. The 2018 fish muscle tissue samples were found to be at a comparable concentration as MT FWP's 2008 results. All samples were below the EPA fish muscle criterion of 11.3 mg/kg dw. Fish tissue samples from the BC portion of the reservoir vary in concentrations for egg/ovary ranging from 4-5 mg/kg dw for kokanee and yellow perch to higher levels for redside shiner, peamouth chub, and northern pikeminnow, ranging between 5 mg/kg to 40 mg/kg with one red side shiner reaching 80 mg/kg. The BC WQG for egg/ovary is 11 mg/kg dw.

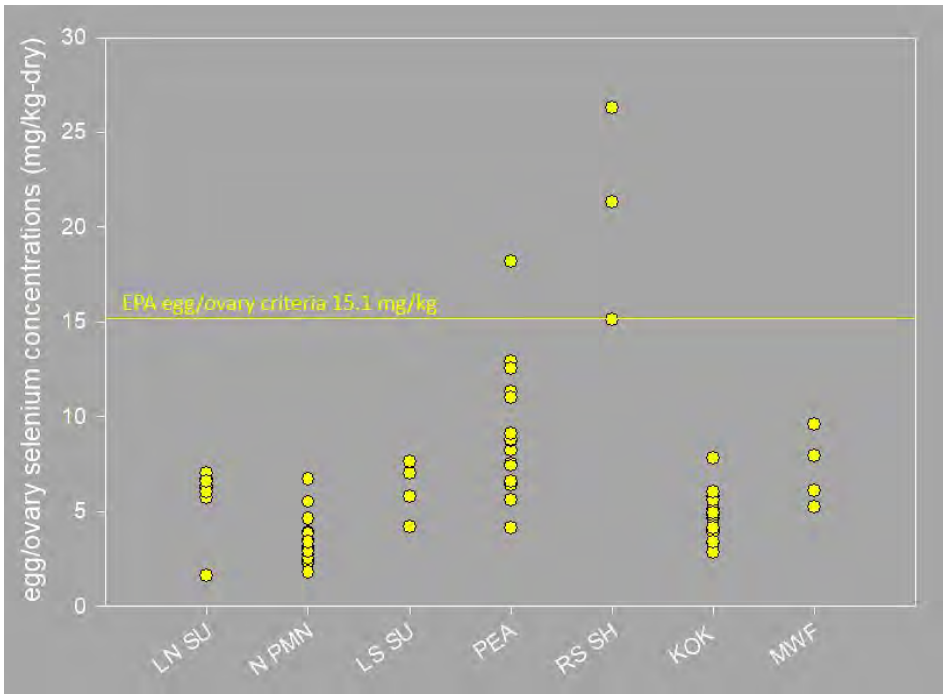

Figure 2-14. Montana Fish Wildlife and Parks 2018 egg/ovary Se concentrations found in LN SU (longnose sucker), N PMN (northern pikeminnow), LS SU (largescale sucker), PEA (peamouth chub), RS SH (redside shiner), KOK (kokanee), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

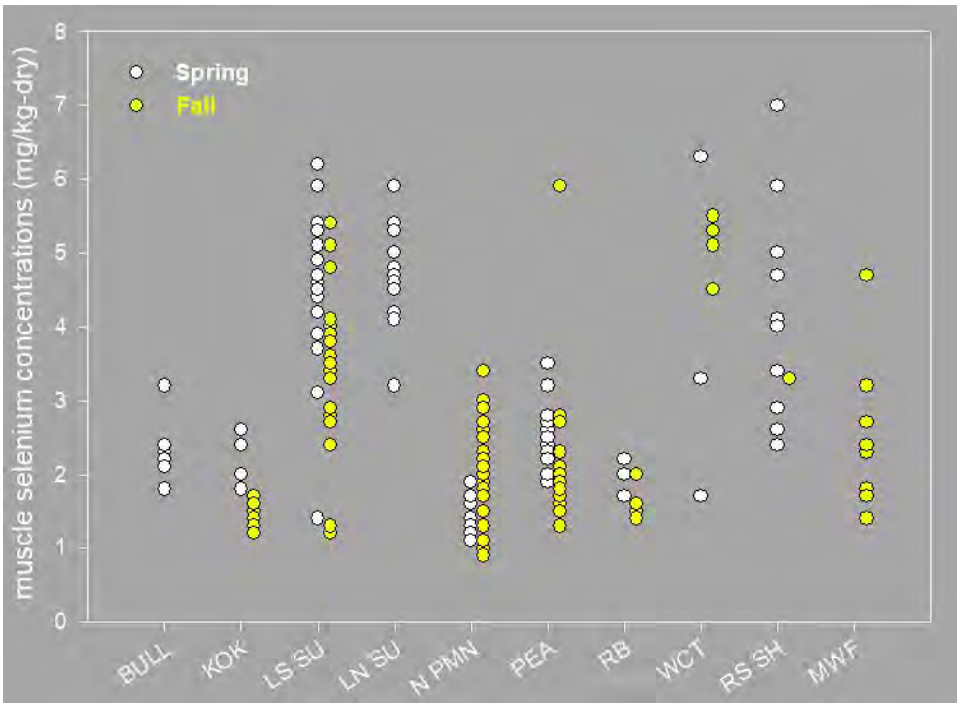

Figure 2-15. Montana Fish Wildlife and Parks 2018 muscle selenium concentrations found in BULL (bull trout), KOK (kokanee), LS SU (largescale sucker), LN SU (longnose sucker), N PMN (northern pikeminnow), PEA (peamouth chub), RB (rainbow trout), WCT (westslope cutthroat trout), RS SH (redside shiner), and MWF (mountain whitefish) (Trevor Selch, MT FWP, personal communication, 9/19/2019).

2.3.5 Birds

Lake Koocanusa encompasses transboundary migratory routes and the Pacific Flyway. On the Montana portion of the reservoir, the most common shore bird on Lake Koocanusa is spotted sandpiper (*Actitis macularius*). Less common during the summer months along the shore are killdeer (*Charadrius vociferus*) but they are regulars on the mud flats at Rexford in fall and into winter. Other shore birds are limited on Lake Koocanusa because of the steep shoreline, a result of dam management. Presser and Naftz (2020) reports that the common resident birds on Lake Koocanusa are Bald Eagle (*Haliaeetus leucocephalus*) and Osprey (*Pandion haliaetus*), Common Loon (*Gavia immer*), Ring-billed Gull (*Larus delawarensis*), Canada Goose (*Branta canadensis*), Mallard (*Anas platyrhynchos*), Common Goldeneye (*Bucephala clangula*), Common Merganser (*Mergus merganser*), Western Grebes (*Aechmophorus occidentalis*), and swallow species (*Hirundinidae spp.*). They further detail avian species which pass through Lake Koocanusa during migration, including Eared Grebe (*Podiceps nigricollis*), Lesser Yellowleg (*Tringa flavipes*), Wilson's Phalarope (*Phalaporpus tricolor*), and several species of ducks including American Wigeon (*Mareca americana*), Gadwall (*Mareca strepera*), Redhead (*Aythya americana*), Ring-necked Duck (*Aythya collaris*), Green-winged Teal (*Anas carolinensis*), Blue-winged Teal (*Anas discors*), Hooded Merganser (*Lophodytes cucullatus*), and Lesser Scaup (*Aytha affinis*).

3.0 DATA COLLECTION FOR CRITERIA DEVELOPMENT

This section presents details on enhanced Se data collection efforts conducted to inform criteria development. In addition to routine monitoring (see **Section 2.0**), the state, federal, provincial, and industry monitoring entities followed recommendations provided by the SeTSC to the extent feasible. Specifically, coordinated cross-border monitoring protocols were developed, additional parameters collected, and monitoring sites added. **Table 3-1** describes the parameters collected, years of record, responsible monitoring entity, and if the sampling was associated with routine monitoring or added.

Significant advances in coordinated monitoring efforts occurred between 2015-2019, but as a result of the transboundary nature of the reservoir and dual jurisdictions, perfectly matched datasets for target parameters were not possible. Presser and Naftz (2020) detail data collection efforts specific to the biodynamic modeling and utility of data while explanation on sample collection and analysis are described in the Presser and Naftz (2020) data release.

Table 3-1. Monitoring data, years of collection, and monitoring entities.

Data	Sampling (routine or added for criteria development)	USGS	USACE	Teck	ENV	FWP	USFWS
phyiscochemical parameters	routine		2015-2019	2015-2019			
dissolved selenium	routine	2012-2019	2015-2019	2015-2019	2017		
selenium speciation	added	2015	2016				
particulate selenium	added	2015-2019	2017-2019	2017-2019	2017		
sediment selenium	routine			2015-2019			
periphyton tissue selenium	added				2017	2017	
invertebrate tissue selenium	added			2016; 2018-2019		2018-2019	
zooplanktoon selenium	added	2016-2017	2016-2019	2018-2019			
fish muscle tissue selenium	routine			2015-2019		2015-2019	
fish whole body tissue selenium	routine			2015-2019		2015-2019	
fish egg/ovary tissue selenium	routine			2015-2019		2015-2019	
fish food habits	added					2017-2018	
bird egg selenium	added						2016

3.1 DISSOLVED AND PARTICULATE SE

The ratio of Se concentration of suspended particulate matter (SPM) to dissolved Se in the water is the environmental partitioning coefficient (K_d). For the most accurate understanding of the K_d, it is necessary to measure SPM and dissolved Se values across multiple years and multiple seasons. Throughout spring-fall months, large volume water samples were collected at two depths (epilimnion and hypolimnion), centrifuged, and analyzed for Se. Dissolved Se samples, defined as passing through a 0.45 µm filter, were collected at the same time and location as SPM samples (these are considered matched samples). USGS and USACE each collected matched dissolved and SPM samples at the main MT monitoring sites, the International Boundary, Tenmile, and Forebay. Teck and BC-ENV collected dissolved and SPM Se samples on the BC portion of the reservoir (**Figure 3-1**). A more robust matched dataset exists for the Montana portion of the reservoir.

Biogeochemical processes have the potential to influence Se distribution between particulate and dissolved phases. At each matched sampling site, high resolution vertical profile data was collected including, temperature, pH, specific conductance, DO, and fluorescent dissolved organic matter (fDOM). Details on these parameters are located in Presser and Naftz (2020).

A concerted effort was made to analyze SPM samples for Se speciation but due to limited sample mass as a result of low lake productivity, the laboratory was unable to complete Se speciation on all SPM data. Only a small subset of the SPM data includes Se speciation.

Figure 3-1. Location of sites where water quality and (or) SPM samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3. 2 PERIPHYTON

DEQ and FWP conducted a pilot study to determine how Se moves through the food chain at the periphyton level, subsequently transferred to periphyton-associated macroinvertebrates and, if present, periphyton feeding fish. Sampling of shoreline periphyton occurred at Tenmile and Rexford sites in MT

during September 2017. Teck additionally sampled for periphyton on the BC portion of the reservoir. Full details on these sampling methods are found in Lotic (2019).

3.3 ZOOPLANKTON

Zooplankton is routinely sampled by USACE and Teck for density and identification. In addition, zooplankton selenium concentrations were measured to help understand the trophic transfer function from SPM to zooplankton. Zooplankton was sampled concurrently with water and SPM samples in MT and BC. **Figure 3-2** details the transboundary zooplankton sampling sites.

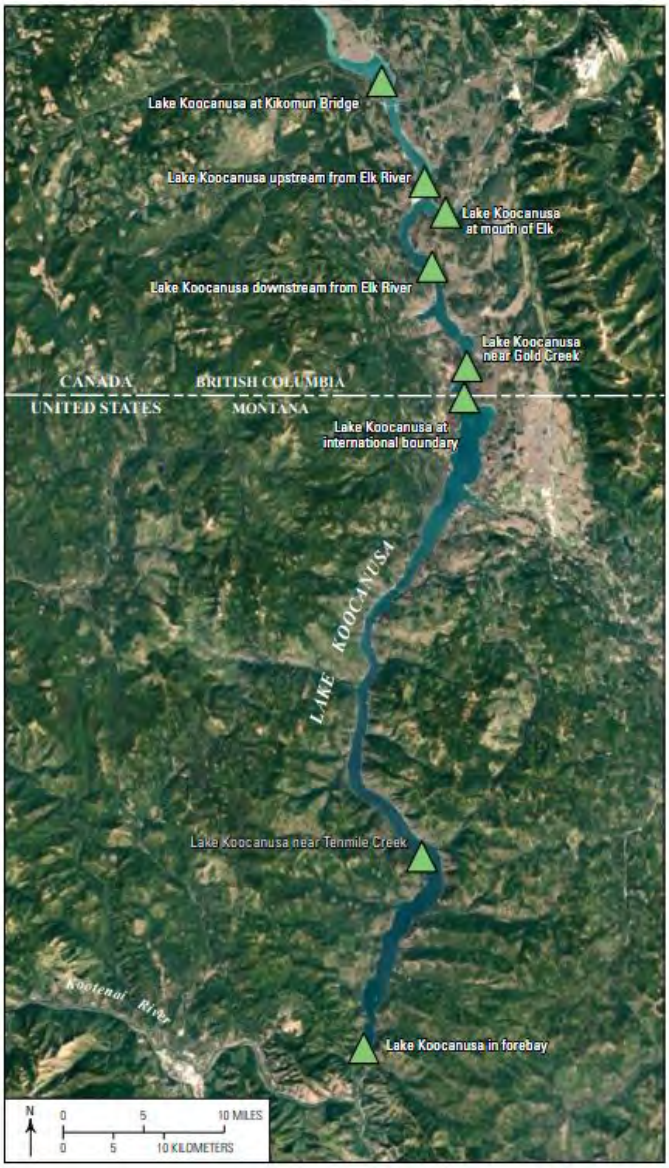

Figure 3-2. Location of sites where zooplankton samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.4 INVERTEBRATES

To better understand the trophic transfer of selenium, FWP collaborated with DEQ to collect and analyze surface and benthic invertebrates in Lake Koocanusa. As part of Teck's monitoring requirements, sampling for invertebrates for selenium analysis continued as routine monitoring on the BC portion of the lake. **Figure 3-3** displays the macroinvertebrate sampling sites in MT and BC.

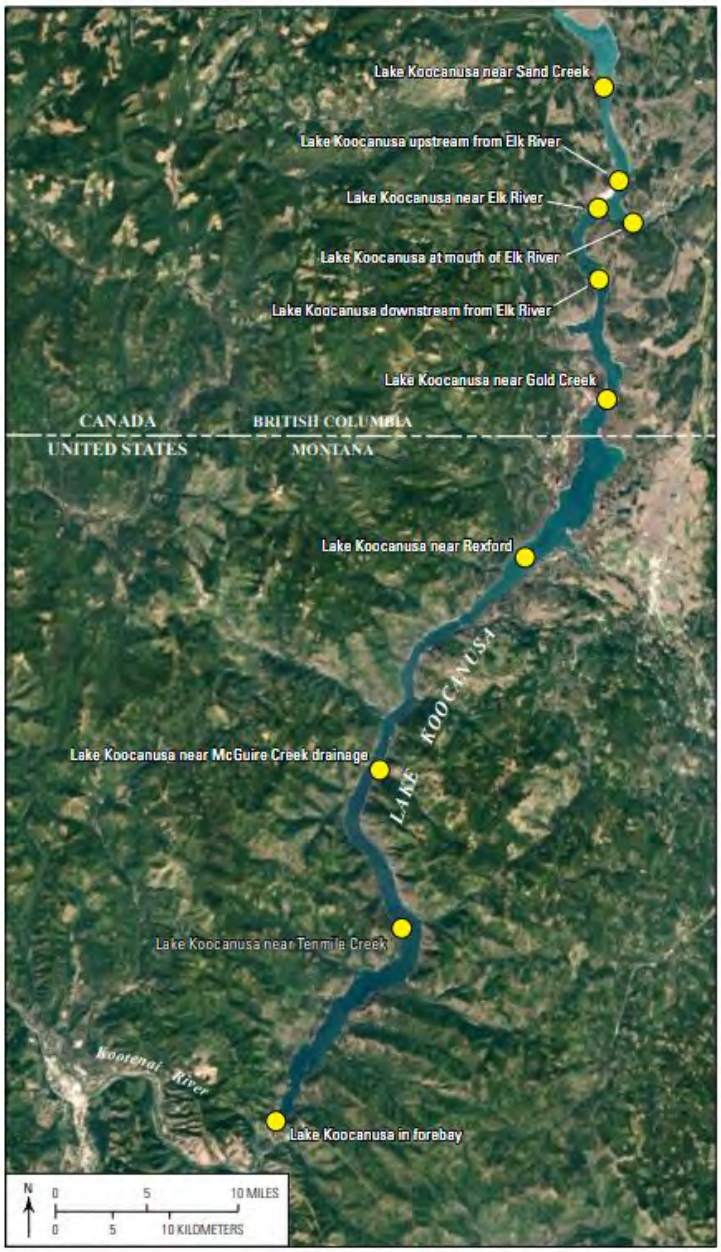

Figure 3-3. Locations of sites where invertebrate samples were collected in Montana and British Columbia (Presser and Naftz, 2020).

3.5 FISH

FWP and DEQ worked collaboratively to sample fish tissue and continue baseline monitoring of fish tissue Se in Lake Koocanusa and evaluate concentration trends. Since 2008, fish tissues have been collected and analyzed for Se. In 2008, 2013, and 2018 bull trout, longnose sucker, northern pikeminnow, kokanee, peamouth, rainbow trout, and westslope cutthroat trout were targeted by FWP for tissue and opportunistic egg/ovary sampling. The three locations for this effort were; 1) near the mouth of the Elk River in Canada, 2) Rexford, and 3) McGillivray (Tenmile). Egg/ovary samples were taken if ovaries were with eggs, but the stage of development was not noted. In 2018-2019 FWP expanded their Tenmile site further south to the Forebay to give a more complete spatial representation of the lower portion of the reservoir.

Figure 3-4. Fish collection sites for Montana and British Columbia. Explanations of collections within area 1 (South of Elk), area 2 (International Boundary), area 3 (Tenmile), and area 4 (Forebay) are further defined in Presser and Naftz, 2020.

A fish food habit study was conducted by FWP in 2017 to determine any differences from previous food habit information collected from fish in Lake Koocanusa from 1983-1992. Target species for the food habit study were westslope cutthroat trout, rainbow trout, longnose suckers, kokanee, and burbot. Stomach contents were analyzed and no major differences in food habits were determined.

Teck conducted fish sampling and analysis for Se as outlined in their permit requirement. Similarly, Teck sampled whole body, muscle, and fish egg/ovary tissue. Detailed information can be found at https://www.teck.com/media/Koocanusa-Reservoir-Monitoring-2018-Report.pdf.

3.6 BIRDS

USFWS conducted a preliminary risk assessment for avian exposure to Se in Lake Koocanusa. Data were collected in June 2016 for killdeer. Methods and results are described in Skorupa and Nelson (2018). The concentrations found for killdeer were well below the known toxicity for this species. The results of this study suggest birds in the MT portion of Lake Koocanusa are not currently experiencing Se-induced reproductive impairment. The SeTSC determined that fish are the most sensitive endpoint for consideration for modeling, and so fish became the focus for future data collection efforts to inform criteria development. No additional bird studies were conducted on the US portion of the reservoir, although Teck continues to monitor birds, primarily spotted sandpiper in BC, as part of their permit requirement.

4.0 SELENIUM MODELING

This section provides an overview of information on the USGS biodynamic selenium modeling utilized for derivation of a protective water column criterion for Lake Koocanusa. Consistent with the approach used by EPA in developing 304(a) criteria, DEQ partnered with the USGS to employ the mechanistic Ecosystem-Scale Selenium modeling approach for Lake Koocanusa (Presser and Luoma, 2010). The work by Presser and Naftz (2020) tailors the Presser and Luoma (2010) model to the Lake Koocanusa ecosystem. The peer-reviewed report and data release can be found at https://pubs.er.usgs.gov/publication/ofr20201098.

DEQ worked with the SeTSC and the USGS while concurrently following the EPA guidance in Appendix K for criteria derivation.

4.1 ECOSYSTEM-SCALE MODEL OVERVIEW

Presser and Luoma (2010) describe the Ecosystem-Scale Model and its use in understanding bioaccumulation and trophic transfer as essential to managing ecological risks from Se. The modeling process, using key components outlined below, provides a basis for understanding and quantifying dietary uptake and linkages among food webs. **Figure 4-1** illustrates the processes regulating movement of Se through an ecosystem.

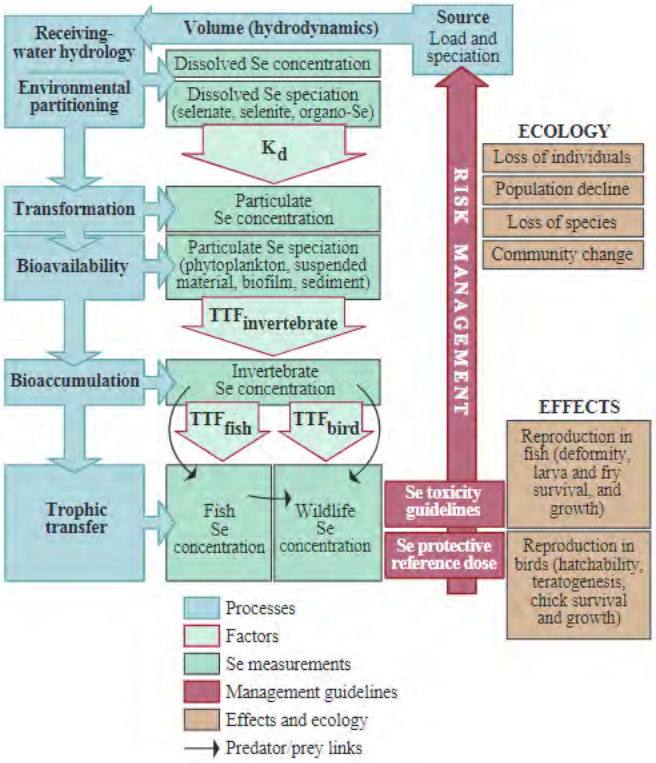

Figure 4-1. Conceptual illustration of the Selenium Ecosystem Scale Model (Presser and Luoma, 2010).

Jenni et al. (2017) first described tailoring the Presser and Luoma (2010) conceptual model to the Lake Koocanusa ecosystem. The key factors of the modeling include a tissue criterion element, a trophic transfer factor (TTF), and a partitioning coefficient (K_d). All of these are required to derive a protective dissolved water column number, as described by the equation below:

$$C_{target} = \frac{C_{tissue\ criterion\ element}}{TTF^{composite} X\ K_d}$$

Where,

C_{target}	= translated site-specific water criterion element (µg/L)
$C_{tissue\ criterion\ element}$	= fish tissue criterion element (mg/kg dw)
$TTF^{composite}$	= product of the species-specific trophic transfer factor (TTF) values in each trophic level of the food web of the target fish species related to the tissue criterion element (no units of measurement)
K_d	= environmental partitioning factor

If the desired tissue criterion element is for an egg-ovary concentration, Jenni et al. (2017) prescribe translating this into a whole body concentration using either EPA guidelines or species-and site-specific data. Each of the key modeling factors incorporated as model inputs are described in more detail below.

4.2 FISH TISSUE CRITERION ELEMENT

It is widely understood that Se toxicity is manifested through chronic dietary exposure to Se. A growing body of research has described egg-laying vertebrates as the most sensitive ecological endpoint for selenium (see **Section 1.4.2**). The SeTSC scientific recommendation was to consider fish as the most

sensitive endpoint in the Lake Koocanusa ecosystem (see **Section 3.7**). Limited toxicity data exists for fish species in Lake Koocanusa, only species in the genus *Onchorhynchus* (rainbow trout and westslope cutthroat trout) and species within *Salvelinus* (bull trout) have known Se toxicity thresholds. **Table 4-1** describes the toxicity thresholds based on 10% effect concentrations (EC10's) described in EPA (2016). Laboratory analysis whereby the effect concentrations results in 10% mortality is referred to as an EC10. This is also referred to as a low effect concentration (LOEC).

Table 4-1. Tested reproductive-effect whole body (WB) concentrations measured directly or converted to WB concentrations from egg-ovary (EO) concentrations (EPA, 2016). Taxon resident in Lake Koocanusa *are Salvelinus*, *O. mykiss*, *Onchorhynchus*.

Taxon*	EO Chronic Value	EO/WB CF	Direct or Calculated WB Repro Chronic Value	Direct Calculation or Basis for EO/WB CF (from Appendix B)
Salvelinus	56.2	1.61	34.9	Dolly Varden EO/M (1.26) x all fish M/WB (1.27)
Esox	34.0	2.39	14.2	Northern pike EO/M (1.88) x all fish M/WB (1.27)
Cyprinodon	27.0	1.20	22.6	Desert pupfish EO/WB
O. mykiss	24.5	2.44	10.0	Rainbow trout EO/M (1.92) x all fish M/WB (1.27)
Rudolph et al. 2008	24.7	1.96	12.6	*Oncorhynchus* EO/WB
Nautilus 2011	27.7	1.96	14.1	*Oncorhynchus* EO/WB
O. clarkii	26.2	NA	13.3	Geometric mean of two studies
Oncorhynchus	25.3	NA	11.6	Geometric mean of *O. mykiss* and *O. clarkii* WB SMCVs
Micropterus	26.3	1.42	18.5	*Micropterus* EO/WB
Salmo	21.0	NA	13.2	Directly calculated EC_{10}
Coyle et al. 1993	26.3	NA	8.6	Directly calculated EC_{10}
Doroshov et al. 1992a	22.6	2.13	10.6	Bluegill sunfish EO/WB
Hermanutz et al. 1992, 1996	14.7	NA	10.6	Directly calculated EC_{10}
Lepomis	20.6	NA	9.9	Geometric mean of three studies
Acipenser	15.6	1.69	9.2	White sturgeon EO/M (1.33) x all fish M/WB (1.27)

EPA (2016) describes Se concentrations in egg or ovaries as the best predictors of Se toxicity but explains the vulnerability of a species is the product of its propensity to accumulate Se from its environment through diet and transfer the Se from its body into the eggs. Therefore, EPA includes guidance in Appendix K to use whole body tissue as a reasonable alternative for modeling site specific criteria derivation. Presser and Naftz (2020) provide details outlining the necessity to model from whole body rather than eggs or ovaries in Lake Koocanusa. Moreover, given the limited toxicity data for the resident species in Lake Koocanusa, the EPA guideline for whole body calculated to 8.5 mg/kg dw was used for the criterion element in modeling (Presser and Naftz, 2020). This value was calculated by EPA using OLS regression based on the known four most-sensitive species in the nation, including the most sensitive species, white sturgeon, which is resident in the downstream Kootenai River. A criterion element of 8.5 mg/kg dw is considered protective of white sturgeon across the US. While Presser and Naftz (2020)

applied the 8.5 mg/kg 304(a) whole body criterion in the modeling, they describe how other values may be applied depending on the level of protection desired and the goals of the modeling.

4.3 TROPHIC TRANSFER FACTORS

Selenium trophic transfer factors (TTFs) describe uptake and efflux of prey and predator species (Presser, 2013). Chapman et al. (2010) discusses the similarity of TTFs found within groups of related species and species with a similar trophic status (level). Chapman further describes an important concept in understanding Se TTFs is that the majority of Se enrichment occurs at the lowest trophic levels through particulates and primary consumers. The implication of this is that secondary and tertiary consumers may not always experience substantially higher Se exposure than lower trophic levels. This differs from contaminants such as mercury (Hg) that consistently bio-magnify at higher trophic levels.

Presser and Naftz (2020) utilized TTFs established from laboratory experiments. Field derived TTFs have greater uncertainty than laboratory derived TTFs and Lake Koocanusa had limited data; therefore, USGS determined it most appropriate to model with laboratory derived TTFs as described in Presser and Luoma (2010). The following TTFs were applied; 2.8 (aquatic insects), 1.5 (zooplankton), and 1.1 (fish). Presser and Naftz (2020) present two choices for bioavailability (100% and 60%). The 60% bioavailability effectively reduces the TTF's by 60% to match observed data.

4.4 FOOD WEB MODELS

Two primary food web models were presented in Presser and Naftz (2020) and described in Tables 6 and 7 of their report. Included are the invertebrate to fish model (IFM) and trophic fish model (TFM).

The IFM model is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish guideline wb}/TTF_{fish}\ /[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

The TFM modeling is summarized by the following equation:

$$\text{predicted protective } C_{Se\ dissolved} = \text{fish tissue guideline wb}/TTF_{fishTL4}/TTF_{fishTL3}[(TTF_{invert1}*\text{invert fraction1}) + (TTF_{invert2}*\text{invert fraction2})]/\text{SPM \% bioavailability}/(K_d/1{,}000)$$

Focal fish, previously selected by the SeTSC, were grouped into categorized food webs representative of variations in diet of modeled fish. The categorized food webs described for the IFM model are located in **Table 4-2** and for the TFM model are in **Table 4-3**. These tables present different model scenario options, in which the invertebrate fraction (invert faction) in the above equations are modified based on the percentage consumed. The adjustment to the food web, in turn, modifies the bioaccumulation potential (BAP). The BAP is the combined effect of diet and TTFs. The food web consisting of 100% chironomids (aquatic insects) has the greatest bioaccumulation potential.

Table 4-2. Invertebrate to fish (IFM) model: fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
RBT, WCT, RSS, LNS	100% chironomid	3.08	1.85
PMC, LSS, MWF	50% chironomid 50% zooplankton	2.37	1.42
rainbow trout: Dec-Mar	25% chironomid 75% zooplankton	2.01	1.20
kokanee	100% zooplankton	1.65	0.99

Table 4-3. Trophic level fish model (TFM): fish species, categorized food webs, and associated bioaccumulation potential (BAP) applying both the 100% SPM bioavailability and 60% bioavailability.

fish species	food web	food web	bioaccumulation potential w 100% SPM bioavailability	bioaccumulation potential w 60% SPM bioavailablity
BT, burbot (winter, benthic), NPM	100% TL3 fish species (100% insectivores)	100% chironomid or aquatic insect	3.39	2.03
BT, burbot, NPM	100% TL3 species (50% IV; 50% PV)	50% chironomid 50% zooplankton	2.60	1.56
[1)]BT, burbot (summer fish), NPM	100% TL3 species (100% planktivores)	100% zooplankton	1.82	1.09

4.5 PARTITIONING COEFFICIENT (K_D)

The partitioning coefficient (K_d) describes the relationship between Se concentrations in particulate and dissolved phases (Presser, 2010). The term K_d has been used interchangeably with enrichment factor (EF), a term more commonly used by EPA. The K_d is a simple ratio described below which could be expanded to include a more complex enrichment function incorporating saturation kinetics. In this modeling process the equation below was used. The collection of matched particulate Se and dissolved Se is described in **Section 3.1** using the terminology suspended particulate matter (SPM) and particulate interchangeably.

$$K_d = [\text{particulate Se}]/[\text{dissolved Se}]$$

K_d values calculated from 2015-2019 ranged from 424.2 to 7,474.5 L/g. Rather than statistically reducing the K_d dataset down to a single representative value to use in the model equation, Presser and Naftz (2020) present each K_d calculation as an independent scenario (n=87). The result of this is that each model scenario includes 87 predicted dissolved selenium concentrations.

4.6 MODELING CONCLUSIONS

Presser and Naftz (2020) present a report and accompanying data release which provide the data, rationale, food web modeling structure, and interactive spreadsheets for the quantitative derivation of a site-specific selenium guideline for Lake Koocanusa.

Model predictions of protective dissolved selenium concentrations were specific to the EPA national guideline of 8.5 mg/kg whole body criterion, while recognizing that this whole-body concentration could be changed to meet the protection goals of BC-ENV. Modeling choices and assumptions used for the modeled scenarios were guided by the goals described in the report, and previously defined by the SeTSC.

Those goals are summarized here as:

- Consideration of ecologically significant species and those important to stakeholders,
- Protection of ecosystems during maximum dietary exposure (i.e., feeding within a benthic food web),
- Protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding in an ecosystem that functions as a lentic reservoir, and
- Long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all Se loading profiles, and all water years.

The IFM model based on the food web with maximum BAP (maximum dietary exposure) was through a 100% chironomid (aquatic insect) diet and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice. As noted in **Section 4.5**, Presser and Naftz (2020) recommend that model runs be undertaken for each measured K_d, in order to provide the full range of candidate criteria for a specified model scenario.

The TFM model was based on the food web with maximum BAP (TL3: trophic level 3) was through a piscivorous diet of 100% forage fish which had a diet of 100% chironomid (aquatic insect), and two choices of bioavailability (100% and 60%). The model provided 87 predicted, protective dissolved selenium concentrations for each bioavailability choice.

Presser and Naftz (2020) did not provide a final recommended protective water column Se concentration. Rather, as described above, the report provided the foundation from which DEQ and BC-ENV were able to co-develop a protective water column Se standard.

5.0 CRITERIA DEVELOPMENT AND IDENTIFICATION

The scientific expertise of the SeTSC guided the development of Lake Koocanusa's site specific water column Se standard, from the early stages of data collection to the final recommendations for ecosystem-scale model factors. The USGS modeling results described in **Section 4.0** describes the foundation from which a protective water column value would be co-developed between DEQ and BC-ENV. This section presents the scientific recommendations of the SeTSC and analysis by DEQ and BC-

ENV to co-develop a numeric water column standard that prevents impairment of the aquatic life beneficial uses of Lake Koocanusa.

5.1 SeTSC Recommendations

As part of the criteria development process and different protection goals between BC and the US, the LKMRWG Steering Committee (comprised of a BC and DEQ representative) solicited individual SeTSC recommendations on model input parameters (whole body tissue criterion, food web, TTF, and K_d). The SeTSC was additionally requested to provide recommendations on a final protective water column Se standard for Lake Koocanusa. Recommendations were discussed at length throughout a half-day teleconference held August 25, 2020. A subset of the members submitted additional written recommendations (**Appendix A**). The three USGS SeTSC members recused themselves from providing recommendations beyond what is provided in Presser and Naftz (2020) and Jenni and Schmidt (2020).

5.1.1 Fish Tissue Criterion Element

Presser and Naftz (2020) applied the US EPA criterion of 8.5 mg/kg whole body tissue threshold, yet describe that modification of this value may be appropriate depending on the level of protection desired and modeling goals. The derivation of the national whole body fish tissue threshold includes white sturgeon, the species with the known greatest sensitivity to selenium (EPA, 2016). In spite of that, five out of seven participating SeTSC members recommended applying a lower whole body value. Their recommendations ranged from 4.6 – 7.0 mg/kg dw. Primary rationale for selecting a lower whole body value included; 1) consistency with the lower British Columbia guideline which is considered protective of 100% of species at all life stages, 2) uncertainty around potentially sensitive species and species of cultural importance for which no toxicity data exists, 3) providing assurance for sensitive aquatic dependent wildlife which may become resident in the future, 4) based on selenium toxicity expertise and understanding of the reservoir.

5.1.2 Trophic Transfer Factors (TTFs)

Recommendations for TTFs are intertwined with the bioavailability. As described in **Section 4.3** Presser and Naftz (2020) included a 2.8 TTF for aquatic insects and 1.5 for zooplankton. Additionally, Presser and Naftz (2020) present two bioavailability choices, 100% and 60% bioavailability. The 60% bioavailability was recognized to be the option better tailored to Lake Koocanusa. The 60% bioavailability effectively reduces the 2.8 TTF (aquatic insects) to 1.7 and the 1.5 TTF (zooplankton) to 0.9. However, there was general agreement among the SeTSC that the 2.8 and 1.5 TTFs at the 60% bioavailability may still be over predictive. The SeTSC individual members had varying recommendations for approaching TTFs including; 1) applying the 2.8 and 1.5 at 60% as a conservative measure particularly if using a less conservative whole body threshold (8.5 mg/kg dw), 2) use site specific TTFs (recommendations ranged from 1.1 – 1.2 (aquatic insects) and 0.52-0.85 (zooplankton), 3) combine the Presser and Luoma (2010) and EPA (2016) TTF data to produce a more robust dataset from which to derive a TTF based on the central tendency.

5.1.3 Food Web Model

Presser and Naftz (2020) included two models, the IFM and TFM. Within each are categorized food webs representative of variations in diet of modeled fish. Recommendations focused on using a piscivorous food web at 100% aquatic insects. This food web is referred to as TFM with TL3 100% Aquatic Insects (**Section 4.4**). The rationale for TFM with TL3 100% aquatic insects, the most

conservative food web, was for protection for potentially sensitive piscivorous species and species of cultural importance.

5.1.4 Partitioning Coefficient (Kd)

Two of the SeTSC members recommended additional analysis be done to sub-set the K_d dataset to include only K_d values from the epilimnion. The epilimnion showed slightly greater K_d values overall, and the rationale to include only epilimnion K_d data was to include a conservative approach which may be more representative of Se entering the food chain, specifically through primary producers.

Ultimately, a protective water column value must be selected from the distribution of observed K_d values. Recommendations ranged from the 50th percentile (median) to the 90th percentile depending on the model assumptions (model inputs). The recommendations as to which K_d percentile to select was reliant upon the level of conservatism incorporated into other model parameters, particularly the whole body tissue criterion. There was overall agreement that if applying a lower (more conservative) whole body value, then a median K_d would be protective of the beneficial use. However, if a less conservative whole body value was used, such as the 8.5 mg/kg, then a more protective percentile from the distribution would be recommended to ensure adequate protection.

5.1.5 Water Column Concentration Recommendations of the SeTSC

Recommendations on a final Se water column criterion were presented from four of the seven participating SeTSC members. Final values presented ranged from 0.6 μg/L to 1.5 μg/L, with one committee member describing a range for consideration between 0.73 μg/L to 0.8 μg/L. Three of the four SeTSC members proposed criteria recommendations less than 0.9 μg/L with one recommendation of 1.5 μg/L. The 1.5 μg/L recommendation was proposed with the rationale to follow the EPA 304(a) criteria, rather than utilize the work presented in Presser and Naftz (2020).

5.2 DEQ & BC-ENV SUPPLEMENTAL ANALYSIS

As previously stated, the goal of this work was to co-develop a site-specific water column standard for Lake Koocanusa. A challenge of that work has been the differing protection goals between BC-ENV and DEQ. However, the two agencies worked collaboratively, giving consideration to SeTSC recommendations and the protection goals of each agency, to co-develop three additional model scenarios to consider in conjunction with the model assumptions presented in Presser and Naftz (2020). This section describes the collaborative analysis and the DEQ proposed dissolved Se standard. **Table 5-1** describes model inputs (model assumptions) representative of a balance between the SeTSC recommendations and BC-ENV and DEQ agency goals for the water body.

The scenarios described in **Table 5-1** apply a whole body tissue value of 5.6 mg/kg. As this work was a collaborative process between BC-ENV and DEQ, the 5.6 value was incorporated to remain consistent with BC-ENV's more stringent guidelines. This value falls within the range recommended by five out of seven SeTSC members (see **Section 5.1.1**). This value was derived by applying a westslope cutthroat (a resident species within the known most Se sensitive genus) egg-ovary to whole body conversion factor of 1.96 (EPA, 2016) to the BC provincial egg ovary guideline of 11.0 mg/kg dw.

Based upon recommendations by some SeTSC members to include site specific TTFs, BC-ENV and DEQ applied a bioavailability of 45%. This work was guided both by analysis by Thorley (2020) and the

recommendations by two of the seven SeTSC members. While the 45% bioaccumulation incorporates less conservatism, it was found to be more representative of the observed concentrations. It was determined by BC-ENV and DEQ that this 45% bioavailability would only be appropriate if a more conservative whole body tissue value was also applied (5.6 mg/kg). The 45% effectively reduces the aquatic insect TTF from 2.8 to 1.26 and the zooplankton from 1.5 to 0.68. For aquatic insects, this value is very close to site-specific TTFs recommended by two SeTSC members which ranged from 1.1-1.2 and is within the range of site specific zooplankton TTFs recommended that ranged from 0.56-0.85. The live Excel spreadsheets for the three model scenarios presented in **Table 5-1** are available from DEQ upon request (please contact DEQ's Water Quality Standards & Modeling Section).

Table 5-1. Three additional model scenarios developed by BC-ENV and DEQ incorporating SeTSC recommendations.

Scenario	Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
1	5.6	IFM	100% Aquatic Insects		2.8		45%	50th (median)	0.89
2	5.6	TFM	75% Aquatic Insects/ 25% Zooplankton	1.1	2.8	1.5	45%	50th (median)	0.91
3	5.6	TFM	100% Aquatic Insects	1.1	2.8		45%	50th (median)	0.8

With consideration of the SeTSC recommendations and supplemental DEQ and BC-ENV analysis, DEQ determined Scenario 3 from **Table 5-1,** resulting in a dissolved water column numeric standard of 0.8 µg/L, to be protective of the aquatic life beneficial uses of Lake Koocanusa. Scenario 3 ensures all ecosystem food webs are protected, a stated goal of the SeTSC.

Additionally, DEQ considered the SeTSC recommendation to use the 8.5 mg/kg tissue threshold. Through working collaboratively with BC-ENV, the following model assumptions as described in Presser and Naftz (2020) were applied; IFM 100% Aquatic Insects, 60% bioavailability, and DEQ selected a more conservative percentile from the upper quartile of the K_d distribution (75th percentile). This modeling scenario also meets the protection goals defined by the SeTSC and DEQ. This scenario resulted in a water column Se value of 0.8 µg/L protective of the aquatic life beneficial uses of Lake Koocanusa.

Table 5-2. Model inputs DEQ considered following the two SeTSC member recommendations to apply 8.5 mg/kg and following the recommendations to then identify a more conservative K_d percentile.

Whole body tissue thresdhold (mg/kg dw)	Food Web	Diet	TTF Fish	TTF Aquatic Insects	TTF Zoo-plankton	Bio-availability	Kd percentile	Predicted dissolved water column Se (µg/L)
8.5	TFM	100% Aquatic Insects	1.1	2.8		60%	75th	0.8

6.0 PROPOSED CRITERIA FOR LAKE KOOCANUSA

Proposed Se standards for Lake Koocanusa contain two classes of selenium standards; fish tissue standards, which limit the amount of Se allowed to accumulate in different fish tissues, and a water column standard which was derived from bioaccumulation modeling also intended to limit Se accumulation in fish tissue.

Table 6-1. Proposed water column and fish tissue Se standards for Lake Koocanusa.

Parameter	Se Concentration
Dissolved selenium (μg/L)	0.8
Egg/ovary (mg/kg dw)	15.1
Muscle (mg/kg dw)	11.3
Whole body (mg/kg dw)	8.5

The national fish tissue standards have a hierarchy of importance; the egg/ovary standard is the most takes precedence because those data are the most indicative of selenium toxicological effects on fish at the reproductive stage. However, fish egg/ovary tissue is not always available. Fish muscle or whole body tissue standards can be used in the absence of fish egg/ovary tissue. The fish tissue standards supersede the water column standard only when the lake or river is in steady-state, referring to conditions whereby there are no activities resulting in new, increasing, or changing selenium loads to the lake, and selenium concentrations in fish have stabilized. Lake Koocanusa is not currently in steady state (Presser and Naftz, 2020). Therefore, both the fish tissue standards and water column standards are applicable standards for Lake Koocanusa. The department will determine when Lake Koocanusa reaches steady state after review and analysis has been carried out by the department during triennial review. The proposed water column standards are chronic values. There is no acute selenium standard included since the greatest toxicity risk to aquatic life is from chronic dietary exposure.

6.1 FREQUENCY AND DURATION

The proposed recommendation for return frequency is consistent with EPA's current 304(a) guidance based on the 1985 Guidelines for water column criteria. The proposed return frequency for a water column criteria exceedance of not more than once in three years, on average, is based on EPA's determination of the resiliency of the aquatic ecosystem to recover from a toxin when the impacts are associated exclusively with a water column exposure (Stephan et al., 1985).

The duration component of the criteria describes the exposure time-period and restricts the length of time that the concentration in the receiving water can be continuously above a criterion concentration, in order to protect aquatic life. The proposed durations are consistent with the current 304(a) guidance such that the numerical fish tissue criterion elements are specified as instantaneous. The use of an instantaneous measurement (duration) not to be exceeded (frequency) is because fish tissue data provide point measurements that reflect integrative accumulation of selenium over time and space in the fish population(s) at a given site. Selenium concentrations in fish tissue are generally expected to only change gradually over time in response to environmental fluctuations. The duration for the chronic water column standard is a 30-day average.

6.2 PROTECTION OF DOWNSTREAM WATERS

Federal regulation at 40 CFR 131.10(b) requires the State to consider and ensure the attainment and maintenance of downstream (intra-and interstate waters) WQS. The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream Endangered Species Act (ESA) listed, white sturgeon.

The Kootenai River is in a B-1 use class, identical to what was outlined for Lake Koocanusa (**Section 1.3.1**). The designated use class for the Kootenai River in Idaho is outlined in Idaho's regulations found at 58.01.02.100 as, "*water quality appropriate for the protection and maintenance of a viable aquatic life community for cold water species*." Idaho defines viable aquatic life as communities that are functioning and intact. Additionally, the Kootenai River Native Fish Conservation Program includes a Tribal Sturgeon Hatchery managed by the Kootenai Tribe of Idaho (KTOI) to prevent extinction, preserve the existing gene pool, and rebuild a healthy age class of the ESA listed endangered white sturgeon.

The proposed Se standards for Lake Koocanusa are considered protective of downstream use including the protection of downstream ESA listed, white sturgeon. DEQ modeled white sturgeon using the bioaccumulation model by Presser and Naftz (2020). Applying the white sturgeon whole body sensitivity of 9.2 mg/kg, the food web of TFM with TL3 100% aquatic insects, a TTF of 2.8, and a bioavailability of 60%, the results showed 0.8 μg/L to be protective at the 90th percentile of the Kd distribution. As white sturgeon are not resident within Lake Koocanusa, DEQ finds this to meet the protection of downstream use including protection of white sturgeon.

6.5 PROTECTION OF FEDERALLY LISTED SPECIES

The bull trout was listed as threatened under the ESA on November 1, 1999 (64 FR 58910). Bull trout are native to Lake Koocanusa and the Kootenai river, representing a geographically distinct and important population within the broader bull trout range. In Montana, the management of fisheries including that of bull trout is executed by FWP. Montana FWP biologists monitor spawning sites (redds) annually as a metric for measuring fish reproduction, recruitment, and fisheries management. The monitoring takes place during the fall as most bull trout spawn between late August and early November. Current trends for bull trout in Lake Koocanusa are common abundance with a stable population. The 2002 FWP Bull Trout Report lists current threats to bull trout in Lake Koocanusa (above Libby Dam) as identified by the Montana Bull Trout Scientific Group as including introduced fish species, rural residential development, forestry, mining, agriculture, water diversions, and illegal harvest.

At present, there are no known selenium toxicity studies for bull trout. There are toxicity tests for the taxonomically similar, dolly varden, both in the genus *Salvelinus*. Research shows dolly varden to be among the most tolerant fish species to Se. **Table 4-1** describes the whole body and egg ovary selenium toxicity thresholds for dolly varden being 34.9 (whole body) and 56.4 (egg ovary). DEQ modeled bull trout using the bioaccumulation modeling by Presser and Naftz (2020) by substituting the whole body guideline for the *Salvelinus* whole body toxicity threshold of 34.9 to determine that the proposed standards including the site specific water column standard of 0.8 μg/L is protective such that no individual bull trout will be harmed.

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Threatened Bull Trout (Salvelinus confluentus). U.S. Fish and Wildlife Service, Pacific Region, Portland, Oregon

APPENDICES

1. Appendix A – Selenium Technical Subcommittee written recommendations

28th August, 2020

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Sean Moore| Director, Watershed Science and Adaptation, Environmental Sustainability and Strategic Policy Direction, BC Ministry of Environment| LKMRC Co-Chair
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Dear LK MRC Co-Chairs and Members,

Please accept this recommendation on behalf of the Confederated Salish and Kootenai Tribes (CSKT) and the Kootenai Tribe of Idaho (KTOI), constituent governments of the transboundary Ktunaxa Nation. You will find herein our scientific justification and rationale, regarding the request to provide written recommendation on the inputs to the model developed by US Geological Survey (USGS), in support of a site-specific selenium criteria for Koocanusa Reservoir.[1]

The Transboundary Kootenai watershed sits entirely within the transboundary Ktunaxa Nation Territory and provides critical habitat for rare and threatened fish species including bull trout, burbot, westslope cutthroat trout, and endangered Kootenai River white sturgeon. Unabated selenium inputs from the Elk Valley mines into Koocanusa Reservoir demonstrate a clear, increasing trend dating back to 1984.[2] Selenium leaching from the Teck Ltd. mines in the Elk Valley of British Columbia is resulting in degradation of water quality and presenting unacceptable impairment and risks to Ktunaxa Territory resources. As noted in our previous

[1] Presser, T.S., Naftz, D.L. Naftz, 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A. and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020-1098, 40 p., https://doi.org/10.3133/ofr20201098.

[2] Unpublished data from 2019 collected by the U.S. Environmental Protection Agency, U.S. Geological Survey and Kootenai Tribe of Idaho for the Kootenai River and tributaries. 2019.

letters, we are specifically concerned about impacts on the water quality, fish and fish habitat, species at risk, impacts to other species and resources that depend on those waters and fish, and traditional cultural values, including human health impacts from consumption of contaminated fish, in the entire transboundary Kootenai watershed.

Based on historical and recent data for water quality and fish tissue, it is imperative that Montana work now to adopt a site-specific selenium criteria for the health and protection of all fish species in Koocanusa Reservoir and downstream in the Kootenai watershed. We recognize that existing data documents increasing selenium in several species of fish in Koocanusa Reservoir, including three species that exceed the 2016 EPA recommended criteria for selenium in fish tissue. Further, Koocanusa Reservoir is currently unprotected, given that Montana did not adopt the national recommended selenium criteria, as revised and released by EPA in 2016.[3] The best available science, including the 2020 USGS model and report, demonstrates that there are historical, on-going, and projected future inputs of selenium into Koocanusa Reservoir, and it is the responsibility of the State of Montana to adopt a selenium criteria that is sufficiently protective to ensure the immediate and long-term protection and restoration of Koocanusa Reservoir, and downstream uses in the Kootenai River, from the ecological impacts of selenium contamination. Given the current impacts and risk to Ktunaxa territory resources, the KTOI and CSKT are in full support of the commitment by the State of Montana to adopt a site-specific selenium criterion by December, 2020, including initiation of the formal rule-making process in September, 2020.

In addition, we support the scientifically defensible and peer-reviewed report and model developed by USGS in support of criteria development, including the approach of the USGS to base the model on a conservative and protective approach. The authors of the model are among the top selenium experts in North America, with decades of experience in the field of selenium toxicology, and the model they have developed is peer-reviewed and capable of generating a defensible, protective criterion for the reservoir, based on the factors that influence selenium in the reservoir.

Given that Koocanusa Reservoir is already degraded due to input of contaminants from mining in the Elk Valley of British Columbia, we support a criterion that manages the reservoir to improve and restore from the already degraded condition. Current levels of selenium contamination caused by Elk River coal mining above and below Libby Dam is with high probability already causing, and threatens to continue, negative physiological effects to organisms dependent on aquatic resources, including birds, and possibly humans. A conservative site-specific criterion is needed to support management that improves and restores the water quality and aquatic life in the reservoir.

[3] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

There is evidence of significant bioaccumulation of selenium already occurring across the Kootenai ecosystem, including the Idaho and BC portions of the Kootenai.[4] This bioaccumulation has been occurring and will continue even at current water column selenium concentrations that are below the current criteria/exceedance limits. Literature provides evidence that body burden concentrations found in Kootenai River white sturgeon, burbot, mountain whitefish, and freshwater mussels are likely already having significant physiological effects. This is a critical concern to the Ktunaxa Nation governments, given the cultural significance of these species, as well as the tremendous effort and resources dedicated to ecosystem restoration.

The selection of a conservative and protective site-specific selenium criterion is necessary to, at minimum; prevent further increases in selenium into the Kootenai ecosystem. Current data is showing increasing concentrations of selenium in larger portions of the reservoir, which in turn will increase selenium concentrations below Libby Dam.[5] This trend will continue until effective mine impact mitigation is implemented at an appropriate scale.

The overall selenium loading into the reservoir from the Elk River needs to be stabilized and reduced in order to prevent near-future partitioning and release of selenium into the reservoir and also the downstream Kootenai River.

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L. Therefore, based on the specific framework of the USGS model W6, Model run #2, the CSKT and KTOI are specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Based on the attached background, modeling recommendations and rationale, the KTOI and CSKT recommends using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates.

In summary, we are recommending a conservative site-specific criterion for selenium in Koocanusa Reservoir, based on the following uncertainties;

1. Koocanusa Reservoir currently demonstrates system degradation and impairment. This is demonstrated by the following:
    a. Fish tissue concentrations (muscle, whole body, and/or egg ovaries) at times exceed USEPA and B.C. recommend thresholds.

[4] https://governmentofbc.maps.arcgis.com/apps/webappviewer/index.html?id=0ecd608e27ec45cd923bdcfeefba00a7

[5] Presser, TS, and DL Naftz. 2020. Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, USA, and British Columbia, Canada: US Geological Survey Open-File Report 2020-1098, 40 p. https://doi.org/10.3133/ofr20201098.

b. The reservoir has increasing pollutant loads, as demonstrated by B.C. long-term monitoring station on the Elk River at HWY 93.
c. The reservoir has an increasing mass of selenium over an increasing reservoir area (Presser and Naftz, Figure 17).
d. The reservoir has declining burbot populations.
e. Fish populations demonstrate gonadal disfunction and dysfunctional selenium dietary bioaccumulation.

2. Water quality monitoring data indicate the Koocanusa Reservoir is a dynamic system and it is possible that current monitoring efforts have not defined nor captured critical time periods or critical portions of the reservoir.
3. A delay or lag in uptake of selenium into the food web, from the water column, is highly likely and at a magnitude that presents a significant risk. The outcome is increasing and perpetuated bioaccumulation of selenium in benthos and fish above elevated levels.
4. To return to a restored condition, MT DEQ must avoid normalizing current degraded conditions and strive for a condition that is improved from current conditions.
5. On-going revisions to the modeling in the Elk and Fording River, including the Implementation Plan Adjustment to the Elk Valley Water Quality Plan, that increases the observed and modeled future contaminant delivery into Koocanusa Reservoir from the Elk Valley Mines.[6]

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

Sincerely,

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[6] 2019 Implementation Plan Adjustment Annex B - Regional Water Quality Model Modifications
https://www.teck.com/media/Annex-B-Regional-Water-Quality-Model-Modifications.pdf

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August 28, 2020

Dear SeSTC Committee Members and Co-Chairs,

Selenium Technical Sub-Committee members were requested to submit written recommendations to the SeTSC Co-Chairs for the site-specific selenium criteria. Below you will find our recommendations, serving as a representatives of the Kootenai Tribe of Idaho (KTOI) and the Confederated Salish and Kootenai Tribes (CSKT). Please see below for background, recommendations and rationale for the site-specific criteria.

We based on our recommendation on a site-specific criterion that protects burbot (*Lota lota*), the fish species that are most sensitive to selenium bioaccumulation in Koocanusa Reservoir. Burbot have been functionally extirpated from the reservoir and are culturally important to the Ktunaxa Nation community. Burbot populations declined over two decades ago when the ambient reservoir Se concentrations were below what is currently seen today. In published literature, burbot have been shown to be particularly sensitive and susceptible to the bioaccumulation of selenium.[1] Muscatello and Janz observed significant bioaccumulation in burbot (10 ug/g dw WB) at low aqueous (<0.5 µg/L) and benthic invertebrate (0.5-3 µg/g) selenium concentrations.[2] This is reinforced with the general knowledge that the burbot population decline[3] and eventual functional-extirpation in Koocanusa Reservoir coincides with the Elk River Coal Mines operational history and subsequent water pollution caused by those coal mines; and severely complicates the restoration of burbot above Libby Dam.[4]

The burbot population declined when the ambient reservoir Se concentrations were below the aqueous concentrations that are currently seen today. Limited KTOI data is also showing that burbot in the mainstem Kootenai River are accumulating selenium at rates that are known to cause significant negative physiological effects on other fish species. Those effects include reproductive failure, reduced growth, and mortality (KTOI, unpublished data).
Further, mining contaminant inputs into Koocanusa Reservoir present a critical uncertainty in the Kootenai River Ecosystem Restoration program[5], and will continue to act in synergy with

[1] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[2] Muscatello, JR, and DM Janz. 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci Tot Environ 407:1318-1325.
[3] Dunnigan, J., J. DeShazer, T. Ostrowski, M. Benner, J. Lampton, L. Garrow, and M. Boyer. 2018. Mitigation for the Construction and Operation of Libby Dam, 1/1/2017 – 12/31/2017 Annual Report, 1995-004-00. 252 pp.
[4] Cope, A. 2018. Upper Kootenay River Burbot Conservation Strategy, Draft Report. 59 pp.
[5] www.http://restoringthekootenai.org

the habitat alterations perpetuating white sturgeon and burbot recruitment failure below Libby Dam.

In addition to burbot, it is critically important that the criterion is based on considerations for protection and restoration of the Kootenai River white sturgeon (*Acipenser transmontanus*) downstream of Libby Dam given their sensitivity to reproductive impacts from selenium toxicity. We note that white sturgeon are the most toxicologically sensitive fish as ranked by the US EPA in its national guidance.[6]

With respect to birds and wildlife, the Kootenai River Basin was once one of the more ecologically productive inter-montaine ecosystems, supporting resident and migratory bird populations; however, Koocanusa Reservoir currently does not support robust shorebird populations. Shorebirds are particularly vulnerable to selenium toxicity, as they are highly sensitive to selenium exposures.[7] Skorupa et al found reproductive failure in aquatic birds with 3.0 µg/g selenium concentrations in their eggs.[8] Birds have been shown to be particularly sensitive to selenium exposures due to their feeding habits that are linked to the aquatic environment.[9] Stanley et al found that a 7 mg Se/kg dietary exposure in mallard ducks caused a >30% embryo mortality.[10]

Hamilton reviewed approximately 40 different studies investigating selenium toxicity for fish, aquatic birds, phytoplankton, and zooplankton.[11] Several tables within this paper provided a comprehensive compilation of species tested, tissues sampled, selenium concentrations tested for effects, corresponding physiological effects, and study citations. The physiological effects concluded by the individual studies listed throughout the review tables are “Mortality”, “Reduced Growth”, “Reproductive Failure”, “Reduced Weight”, and “Reduced Cell Replication”.

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[6] U.S. Environmental Protection Agency [USEPA], 2016a, Aquatic life ambient water quality criterion for selenium—Freshwater: Washington, D.C., U.S. Environmental Protection Agency, EPA 822–R–16–006), 807 p., accessed May 2020 at https://www.epa.gov/ sites/ production/ files/ 2016- 07/ documents/ aquatic_ life_ awqc_ for_ selenium_ - _ freshwater_ 2016.pdf.

[7] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[8] Skorupa, JP, HM Ohlendorf, and RL Hothem. In press. Interpretive guidelines for selenium-exposed waterbirds. J. Wildlife Management.

[9] Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In Ecological assessment of selenium in the aquatic environment; proceedings. SETAC Workshop on Ecological Assessment of Selenium in the Aquatic Environment (2009: Pensacola, FL) Ed. by Pellston M. Chapman et al. CRC Press. 339 pages.

[10] Stanley, TR Jr, GJ Smith, DJ Hoffman, H Heinz, and R Rosscoe. 1996. Effects of boron and selenium on mallard reproduction and duckling growth and survival. Environ Toxicol Chem 15:1124-1132

[11] Hamilton, SJ. 2003. Review of residue-based selenium toxicity thresholds for freshwater fish. Ecotoxicology and Environmental Safety 56:201-210.

For several fish and aquatic bird studies listed, the selenium toxicity levels causing mortality, reduced growth, reproductive failure, and/or reduced weight were whole body tissue and/or egg concentrations as low as 1-4 ppm.

Thorley cites data collected from water and fish tissue (whole body and egg/ovary) Se concentrations for Koocanusa Reservoir.[12] Water concentrations ranged 0.5 -1.5 µg/L, and corresponding fish tissues from several fish species ranged from 1.0 – 6.0 ppm for whole body, and ~2.0 to 80.0 for egg/ovary. Even if the 80.0 µg/g observation is an outlier, results from peamouth chub (*Mylocheilus caurinus*), redside shiner (*Richardsonius balteatus*), and Northern pikeminnow (*Ptychocheilus oregonensis*) were predominantly 10.0 – 40.0 µg/g for egg/ovary samples. These are tissue concentrations at water concentrations of 0.5-1.5 µg/L.

Thorley also presents data collected from zooplankton and benthic macroinvertebrate Se concentrations for sample sites located within Koocanusa Reservoir.[13] Zooplankton selenium concentrations ranged between <1 to 5 µg/g, with some samples upwards of 14 µg/g Se. Benthic macroinvertebrate tissue concentrations ranged between <1 to 12.5 µg/g Se, with the mean Se concentration near 5 µg/g Se.

The EPA whole-body threshold of 8.5 mg/kg dw is based upon the known sensitivity of white sturgeon. This is scientifically defensible and appropriate on the national level. However, the 8.5 mg/kg dw whole-body criterion does not account for other potentially sensitive and susceptible fish species or protection of the most sensitive designated use, which includes tribal harvest treaty rights. Whitefish (*Prosopium williamsoni*) and burbot are culturally important fish species that are consumed by Ktunaxa citizens from all three Ktunaxa Nation governments. A minimum whole-body threshold of 5.6 mg/kg dw should be considered. Using the BC MOE egg/ovary guideline of 22 mg/kg dw, and factoring in the safety/assessment factor of 2, and using the EC10 egg/ovary to whole-body conversion for rainbow trout of 1.9, this leads to a more conservative 5.6 mg/kg dw whole-body recommendation. The KTOI and CSKT recommend using a 5.6 mg/kg dw whole-body threshold. The 5.6 mg/kg dw whole-body threshold accounts for the potentially sensitive fish species of mountain whitefish and burbot and incorporates the Ktunuxa Nation Council's preferred fish consumption rates. The KTOI and CSKT recommend a conservative site-specific criterion for Koocanusa Reservoir until additional science and data collection demonstrate otherwise.

Current reservoir selenium outflows are approximately 1.0 µg/L (range between 0.8 and 1.2 µg/L, depending upon dam operations, time of year, and hydrologic conditions within the basin). Kootenai River white sturgeon egg selenium concentrations in the mainstem river

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[12] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

[13] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

below Libby Dam range between 3.0 and 6.0 mg/kg dw. Of the five whole-body burbot tissue samples collected by the KTOI, one was above the 8.5 mg/kg dw EPA threshold, and mountain whitefish egg concentrations exceed EPA's 15.1 mg/kg dw threshold, with some of these values almost double the EPA recommended criteria (KTOI 2020; unpublished data). These measurements indicate that, like Koocanusa Reservoir, the Kootenai River requires the development of a site-specific water column selenium criterion. KTOI and CSKT understand that this will likely require a multi-year effort to collect adequate data and develop a site-specific criterion for the Kootenai River, and we encourage DEQ to begin this effort immediately in collaboration with both Tribes. For now, KTOI and CSKT support MT DEQ setting an interim criterion for the Kootenai River that is equal to EPA's national recommended value for water column, fish tissue, and egg/ovaries. In summary, we support the adoption of a conservative site-specific criterion for Koocanusa Reservoir now, to reduce uncertainty and risk in the Kootenai River downstream, and the subsequent initiation of a rigorous, scientific process to develop a site-specific criterion for the Kootenai River.

After evaluating multiple scenarios using a reasonable range of variable values within the USGS models provided to the SeTSC, the KTOI and CSKT recommends using the 'W6. TFM with TL3 100% Aquatic Insects' model. This model is conservative and protective of the most selenium-susceptible trophic levels; and is also considered the most protective, as it incorporates whitefish and burbot.

We recognize the variability of TTF's, conversion factors, and K_d values. Given the uncertainty and wide fluctuations in K_d throughout the reservoir (values ranging between 400 and 7000), a conservative K_d should be used. In order to be protective of the reservoir ecosystem across time and location, the 90th percentile K_d should be used to capture the worst-case scenario. The use of the median K_d value is also supported in literature. The use of the 1.1 TTF is supported by literature and is scientifically defensible. To manage the uncertainty in the water concentration guideline, Jenni, Naftz, and Presser (2017) suggested triangular distributions with a TTF for invertebrates (aquatic insects and zooplankton combined) between 1 and 3.5 with a mode of 1.3, a TTF for fish between 0.6 and 1.6 with a mode of 1.1 and a Kd between 800 and 6,500 with a mode of 3,000.

Model Input Recommendations

With respect to the specific model inputs, we provide the following recommendations and rationale; Given the varying K_d values within the reservoir, and the two recommended TTF values for aquatic insects, we ran six variations of the W6 model that incorporate the different K_d and TTF values. Listed below are the outputs from the six model runs.

1. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.22 µg/L (given the model correction of 100% Se bioavailability) to 0.37 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

2. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.37 µg/L (given the model correction of 100% Se bioavailability) to 0.61 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

3. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.8 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.53 µg/L (given the model correction of 100% Se bioavailability) to 0.89 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

4. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a maximum K_d, water concentrations of 0.29 µg/L (given the model correction of 100% Se bioavailability) to 0.49 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

5. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a median K_d of 4500, water concentrations of 0.49 µg/L (given the model correction of 100% Se bioavailability) to 0.82 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

6. Model W6 (TFM with TL3 100% Aquatic Insects) with the 5.6 mg/kg dw whole-body threshold, a TTF of 1.1 for fish, a TTF of 2.1 for aquatic invertebrates, and a K_d of 3100, water concentrations of 0.71 µg/L (given the model correction of 100% Se bioavailability) to 1.18 µg/L Se are produced as the criteria (given the model correction of 60% Se bioavailability).

After reviewing the model outputs for the differing variables, CSKT and KTOI highlight that, at minimum, the recommended water column selenium criteria needs to be below 1.0 µg/L.

Based on the specific framework of the USGS model W6, Model run #2 as described above, the CSKT and KTOI is specifically recommending a water column selenium concentration criterion of 0.61 µg/L selenium.

Current whole-body fish tissue samples from Northern pikeminnow, peamouth chub, redside shiner, and largescale sucker in Koocanusa Reservoir exceed, and in many individuals sampled, greatly exceed, the EPA whole-body criteria in the current aqueous conditions in the reservoir.[14] This clearly indicates to KTOI and CSKT that to be protective of all fish species in the reservoir, the site-specific criterion should be lower than the current selenium concentrations

[14] Thorley, JL. 2020. Koocanusa Reservoir Water and Fish Tissue Selenium Concentrations 2019. A Poisson Consulting Analysis Appendix. https://www.poissonconsulting.ca/f/1298248550.

sampled in the reservoir. Also, as noted in Presser and Naftz, 2020, it is important to determine where Koocanusa Reservoir is in an impairment-restoration cycle so as not to base protection on survivor bias, the maintenance of a currently degraded ecosystem, or normalized toxicity. In a broader context, one of the overall consequences of revised selenium regulations is that their derivation is now dependent on being able to define and understand the status of the ecosystem on which protection is based. And, as described in Presser and Naftz, 2020, the Koocanusa Reservoir system demonstrates traits of a currently degraded system (see Table 1 in the report and subsequent discussions). This further illustrates to CSKT and KTOI that a protective site-specific water column selenium criterion should be lower than existing conditions in the reservoir.

Given that there may be a lag in the biological uptake and detection of selenium across the food web in the reservoir, it is important to adopt a more conservative criterion at this time, to ensure protection under unknown future selenium levels and the increasing contaminant trends. Any selenium concentrations above the background concentrations represent an increase from baseline conditions for the Kootenai Basin and are likely already having, and will perpetuate negative impacts upon the ecosystem. According to Chapman et al[15] in the Selenium Risk Characterization chapter 7, Lentic systems were identified to be at an increased risk of Se-caused adverse effects due to the maximized mobility of selenium into the food web, thereby increasing the chance for elevated exposures.

Continuing downriver into the altered lower-river ecosystem driven by Libby Dam operations, the food web in the mainstem Kootenai River is quite different than the reservoir; therefore the movement of selenium from Koocanusa Reservoir through Libby Dam and into the lower-river is relatively unknown. Water and tissue sampling in the Kootenai River below Libby Dam suggests the current selenium concentrations and loading into the river are already having negative impacts on the ecosystem.

In conclusion, the KTOI and CSKT support a conservative approach to the adoption of a site-specific selenium criteria that is protective of all species of fish and wildlife at all times of the year, throughout the reservoir, and protective of the downstream ecosystem.

Thank you very much for your consideration,

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RE: KNC Selenium Technical Sub-Committee recommendations on Presser and Naftz (2020) selenium bioaccumulation model for Koocanusa Reservoir

In response to the request from the co-chairs of the Selenium Technical Sub-Committee (SeTSC) of the Koocanusa Reservoir Monitoring and Research Working Group, the Ktunaxa Nation Council (KNC) technical representatives of the SeTSC are pleased to offer our recommendations for consideration in setting the water quality benchmark (also "criterion" or "objective") for the protection of designated uses in the Koocanusa Reservoir. Specifically, we are providing our recommendation on the requested topics, including model inputs, quantitative approaches to using the USGS model outputs (Presser and Naftz 2020), and the proposed range of water column concentrations that would be protective of designated uses.

We would highlight that the following technical recommendations were developed based on our current understanding of the Koocanusa Reservoir and the data and information available (e.g., Beaman 2020; DeForest 2020; Lotic Environmental 2018; Presser and Naftz 2020; Thorley 2020). KNC technical representatives are providing recommendations at this time to honour the Se TSC co-chair request in recognition of the timelines required by Montana Department of Environmental Quality (DEQ) and their process for rule-setting. We also must acknowledge that KNC is committed to working government to government with the Province of British Columbia in developing a water quality objective for Koocanusa Reservoir. The technical recommendations made in this memo will be provided to KNC's Lands and Resources Council in September 2020, after which KNC's formal recommendations for the water quality objective for Koocanusa Reservoir will be submitted.

Model Inputs

Target Tissue Concentration

The ecosystem-scale model ("USGS model") used by Presser and Naftz (2020) estimates the concentration of selenium in trophic levels within a food web beginning with particulate material (i.e., sediment, phytoplankton, and detritus) in the water column through primary consumers (e.g., benthic invertebrates and zooplankton) and into higher trophic levels including planktivorous and piscivorous fish. The objective of the USGS model is to derive a range of selenium concentrations in the water column that are predictive of whole-body concentrations in fish that meet the protection goal of preventing adverse effects on fish reproduction. Various tissue-based whole-body selenium guidelines have been proposed to meet this objective, including the USEPA whole-body guideline of 8.5 µg/g dry weight (dw).

KNC technical representatives, along with some other members of the SeTSC, have recommended the BCMOE (2014) tissue-based guideline of 11 µg/g dw in egg/ovary tissue be used to provide assurances that the most sensitive species at the most sensitive life-stage would be protected during indefinite exposure. To facilitate the use of the BCMOE (2014) tissue-based guideline in the USGS model, we have converted the egg/ovary tissue concentration to a whole-body tissue concentration. The BCMOE (2014) guideline is based on an egg hatchability/viability test with rainbow trout. USEPA (2016) provides an egg/ovary:whole-body conversion factor of 1.96 for rainbow trout. Applying the conversion factor results in a whole-body tissue guideline of 5.6 µg/g dw, which can be used directly in the USGS model as the target tissue concentration.

In previous communications, KNC technical representatives requested the evaluation of a tissue-based guideline applied to the diet of fish and other wildlife (i.e., birds and mammals). The threshold of 4 µg/g dw (BCMOE 2014) was recommended as a protective threshold in prey fish (e.g., redside shiner, peamouth chub). However, based on an evaluation of the data available in the reservoir, the trophic transfer factor (TTF) between prey fish (e.g., peamouth chub) and piscivorous fish (e.g., northern pikeminnow) is roughly 1, indicating that the 4 µg/g dw threshold may be lower than the site-specific data warrants.

When setting water quality objectives, it is important to understand whether the protection goal sought (i.e., fish reproduction) would be protective of other designated uses (e.g., wildlife, recreation, human health). The target tissue concentration can be compared to whole-body tissue-based guidelines for other designated uses, including wildlife consumers (e.g., birds) and human consumers. With respect to avian receptors, Ohlendorf and Heinz (2011) suggest diets greater than 5 µg/g dw could reduce egg hatchability in sensitive avian receptors, including the mallard duck (EC_{10} of 4.9 µg/g dw; Joe Skorupa, USFWS, SeTSC member; pers. comm.). J. Skorupa (pers. comm.) also stated that an

appropriate threshold for protection, considering whole-body concentrations, would likely be in the 4.5 to 6 µg/g dw range (pers. comm) when considering an egg/ovary:whole-body conversion factor for burbot (i.e., 2.3) and using either the BCMOE (2014) egg/ovary threshold of 11 µg/g dw or USEPA (2016) egg/ovary threshold of 15.1 µg/g dw, respectively.

Recognizing the importance of food security and the continuity of Ktunaxa practice including hunting, fishing, trapping, camping, ceremonial practices, and the transmission of knowledge and identity to future generations, we also evaluated whether tissue concentrations would be protective of Ktunaxa citizens at their preferred consumption rates. BCMOE (2014) provides human health screening values for low (i.e., 30 g/day) to high (i.e., 220 g/day) fish consumption. By using the approach in BCMOE (2014), we calculated a whole-body threshold that we consider to be protective of cultural practices such as harvesting and consuming fish at preferred rates. By applying the most current estimate of Ktunaxa preferred consumption rates of 245 g/day, and selecting mountain whitefish as a focal species due to our understanding of cultural practices, we estimate that a muscle concentration of 1.6 µg/g wet weight (ww) or 6.8 µg/g dw would be an appropriate screening value. Using the muscle:whole-body conversion factor for mountain whitefish of 1.27 (USEPA 2016), an appropriate whole-body tissue threshold of 5.3 µg/g dw would achieve the BCMOE (2014) screening values and protect Ktunaxa cultural practices at currently understood preferred rates. We note that the Ktunaxa preferred consumption rate is our current best-estimate based on diet surveys and likely underestimates the importance of fish in Ktunaxa diet resulting from the exclusion of anadromous salmon from Ktunaxa Territory (ʔamakʔis Ktunaxa).

Given the estimates of ecological and human health thresholds for whole-body selenium concentrations noted above, we offer the following recommendation.

> **Recommendation 1:** We recommend that the water quality objective for Koocanusa Reservoir protect ʔa·kxaṁʔis q̓api qapsin (All Living Things) and that the Kootenay ecosystem (including the reservoir) be managed in such a way that Ktunaxa rights, title, and practices are protected. Accordingly, a target tissue concentration of 5.3 ug/g dw has been identified as protective of cultural use and a target tissue concentration of 5.6 µg/g dw using the BCMOE tissue-based guideline for the protection of ecological receptors. Therefore, it is our recommendation that 5.3 µg/g dw be used as the target tissue concentration in the USGS model, as it is protective of all uses.

Food Web Model

To assist in the development of a range of selenium concentrations in the water column that are predictive of whole-body concentrations in a variety of fish species, Presser and Naftz (2020) developed a series of food web models accounting for variations in the source (e.g., aquatic insects, zooplankton, fish) and proportions of food sources in the diet of model species. From our perspective, the *TFM w TL3 100% AqIns* model, which reflects the food web of burbot, bull trout, and northern pikeminnow is considered appropriately conservative and protective of the culturally important burbot, which has shown significant population declines (Hardy and Paragamian 2013). This food web assumes a piscivorous feeder with a diet consisting of prey fish that feed on aquatic insects (e.g., juvenile rainbow trout, westslope cutthroat trout, redside shiner, and longnose sucker).

Given the protection goal of protecting all fish species in the reservoir, we offer the following recommendation.

> **Recommendation 2:** We recommend the use of *TFM w TL3 100% AqIns* to represent the food web in the USGS model.

Trophic Transfer Factors (TTFs)

Presser and Naftz (2020) rely on literature derived TTFs in the mechanistic model as presented in Presser and Luoma (2010). The TTFs used, include:

- $TTF_{Particulate\ to\ Insects}$: 2.8
- $TTF_{Particulate\ to\ Zooplankton}$: 1.5
- $TTF_{Prey\ to\ Fish}$: 1.1

To account for any site-specific differences in the TTFs resulting from differences in community structure (e.g., proportion of rotifers, Presser and Naftz 2020; or, proportion of sediment, detritus, and algae in the particulate material, Beaman 2020), Presser and Naftz (2020) applied a bioavailability factor of 60%. As an example, in a food web that includes a focal species feeding on 100% insects, the bioaccumulation factor from particulate material to fish would be 2.8 multiplied by 1.1 or 3.08. To account for site-specific bioavailability, the bioaccumulation factor of 3.08 would be adjusted downwards to 1.85, assuming 60% bioavailability. The bioavailability adjustment was derived through a validation exercise (Presser and Naftz 2020).

Members of the SeTSC and supporting consultants have proposed alternative TTFs. Beaman (2020) proposed to expand the original Presser and Luoma (2010) TTF dataset to include additional studies as presented in (USEPA 2016). Both Thorley (2020) and DeForest (2020) derived site-specific TTFs for aquatic insects/benthic invertebrates of 1.2

(Figure 1). Thorley (2020) and DeForest (2020) also derived site-specific TTFs for zooplankton. Thorley (2020) accounted for seasonality in the zooplankton selenium concentrations (Figure 2), while DeForest (2020) presented statistical estimates of the TTF based on pooled data (i.e., arithmetic mean of 0.53). When considering seasonality, the upper estimate of mean TTFs was 0.85, which corresponds to September. Conceptually, this relationship is supported with data from Woods (1982) where it is demonstrated that primary productivity in the reservoir typically peaks in August; it would therefore be expected, that peaks in primary consumers would follow soon after.

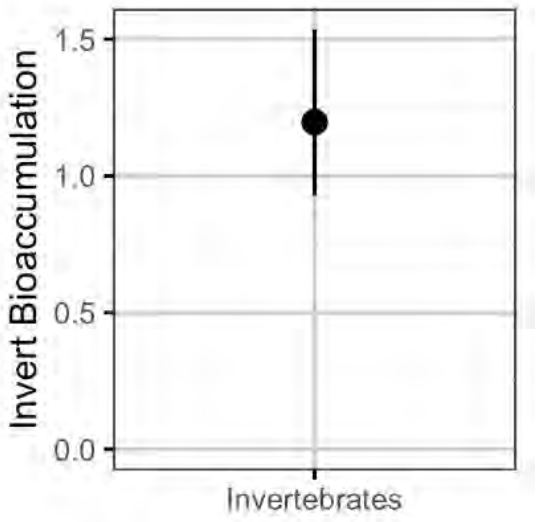

Figure 1. Estimated particulate to invertebrate selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

Presser and Naftz (2020) and Beaman (2020) acknowledged that there are sparse data available on aquatic/benthic insects and zooplankton, which results in greater uncertainty in site-specific TTFs. Secondly, an important assumption of lab- or field-derived bioaccumulation factors is that system is in steady-state with respect to exposure concentrations. Koocanusa Reservoir is not in steady state, driven largely by the dynamic nature of the operation of the Libby Dam and increasing selenium loads from the Elk River (Presser and Naftz 2020). Accordingly, Presser and Naftz (2020) and Beaman (2020) propose the use of literature-based TTFs. Despite relying on literature-based TTFs, Presser and Naftz (2020) apply a bioavailability factor of 60% to account for site-specific conditions via a validation exercise. Thorley (2020) estimated bioavailability factors of 43% and 57% for invertebrates and zooplankton, respectively, using data collected from the reservoir. KNC technical representatives also determined that the literature-based TTFs were likely too high when considering the concentrations observed in the invertebrate and zooplankton data.

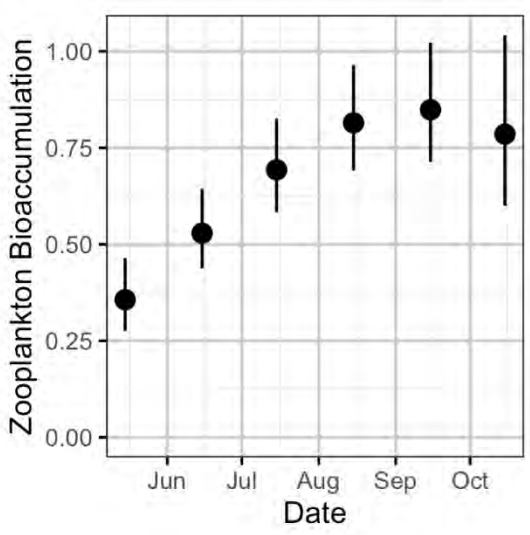

Figure 2. Estimated particulate to zooplankton selenium trophic transfer factor with 95% confidence intervals (from Thorley 2020).

We consider both approaches to estimating TTFs as reasonable and defensible methods and acknowledge the uncertainties in these estimates due to the non-steady state of the system. The implications of a temporal lag in selenium inputs and biological uptake, as well as with respect to selenium retention within the reservoir, are currently unknown. Given that we do have some information on site-specific bioaccumulation within the system from the previous 5 to 10 years, we recommend relying on the measured site-specific TTFs. It must be acknowledged that the adjusted TTFs from Presser and Naftz (2020) and the site-specific TTFs developed by Thorley (2020) are relatively similar (Table 1).

> **Recommendation 3:** We recommend using the site-specific TTFs of 1.2 for invertebrates and 0.85 for zooplankton.

Kd Results

The USGS model determines an aqueous selenium concentration that would be considered ecologically protective for each of the measured Kd estimates (n = 87) for each food web model (e.g., *TFM w TL3 100% AqIns*). The Kd estimates ranged from 424 to 7,475 with a median Kd of 4,547 (Presser and Naftz 2020). The measured Kds were typically higher in the epilimnion of the reservoir compared to the hypolimnion (Figure 3; Thorley 2020). Given the slight differences in Kd estimates between the epilimnion and hypolimnion, and the greater degree of primary productivity in the epilimnion, the Kd estimates from the epilimnetic zone should be included for any quantitative approach to application of the

Table 1. Summary of trophic transfer factors and bioaccumulation factors evaluated for use in the USGS model.

	TTFs - Presser and Naftz (2020)	TTFs - Site-Specific (Thorley 2020)	Estimated Bioavailability Factors (Thorley 2020)	
Trophic Transfer Step				
TTF_{invert}	2.8	1.2	2.8	--
$TTF_{zooplankton}$	1.5	0.85	--	1.5
TTF_{fish}	1.1	1.1	1.1	1.1
Bioavailability Adjustment	0.6	--	0.43	0.57
Bioaccumulation Factor				
BAFinvert-fish	1.85	1.3	1.3	--
BAFzooplankton-fish	0.99	0.94	--	0.94

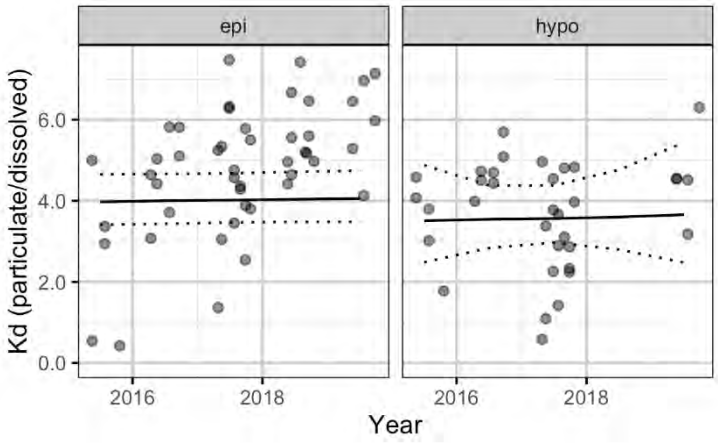

Figure 3. Estimated and observed ratio of particulate to water selenium (Kd = ratio x 1,000) in the epilimnion and hypolimnion with 95% confidence intervals (from Thorley 2020).

model results (see below). This would allow for a more conservative approach to quantifying a Kd for deriving the ecological benchmark considering the uncertainties in the degree of selenium assimilation in the water column of the hypolimnion and also considering that estimated Kd at the sediment water interface in the reservoir are more similar to the epilimnion than the hypolimnion (ranging between 2,641 and 5,812; median: 4,775; Table 2).

Recommendation #4: Kd estimates from the epilimnion (n = 50) should be carried forward into the quantitative assessment of model results below.

Table 2. Predicted Kd estimates in detritus/sediment of the reservoir proper using benthic tissue samples (collected with ponar; predominantly chironomids; Table T19 - Presser and Naftz 2020). Predicted particulate Se was calculated using the site-specific TTF for invertebrates (1.2; Thorley 2020) and associated water quality (mean concentrations by date and depth) in the reservoir (Presser and Naftz 2020).

Site Name	Sample ID	Invertebrate Se (µg/g dw)	Predicted Particulate Se (µg/g dw)	Associated Water Quality (µg/L)	Estimated Kd
Tenmile/Forebay	C3418	5.07	4.23	1.6	2,641
Tenmile/Forebay	C3421	8.37	6.98	1.2	5,812
Rexford	C345-BI-D1	4.50	3.75	1.0	3,750
Rexford	C345-BI-D2	5.73	4.78	1.0	4,775
Tenmile/Forebay	C3426-BI-D	6.43	5.36	0.93	5,761

Quantitative Approaches to Application

It is expected that some primary consumers and higher trophic level receptors (i.e., fish) would reflect reduced variability in selenium tissue burden compared to primary producers under varying selenium concentrations. Biokinetic modeling presented by DeForest *et al.* (2015) suggests that zooplankton would likely exhibit similar, but only slightly reduced variability, while benthic invertebrates and fish would have moderate and high reductions in variability, respectively when considering continuous exposure over varying Kd measurements. However, these higher trophic organisms would also be slower to respond to trends over time. Accordingly, it is reasonable to assume that fish are integrators of the varying selenium concentrations and that some measure of central tendency, with an appropriate level of conservatism to account for changes over time is reasonable.

Analysis of the Distribution

Beaman (2020) recommended a percentile, such as the median be used, to select the Kd for guideline derivation. Thorley (2020) estimated the expected Kd for the epilimnion of Koocanusa Reservoir at roughly 4,000 while the expected Kd in the hypolimnion is 3,500. The upper 95% upper confidence interval in the epilimnion is roughly 4,800 (Figure 3). The median Kd using all measurements is 4,547 while the median Kd in the epilimnetic measurements is 5,017.

> **Recommendation #5:** We recommend the range of Kd estimates for use in the model is between 4,547, the median of pooled Kd estimates (n = 87), and 5,017, the median of epilimnetic Kd estimates (n = 50).

Protective Concentrations

The USGS model was populated with the recommendations above to estimate the range of aqueous concentrations considered to be protective of all uses. It is estimated that the range of protective concentrations in Koocanusa Reservoir is 0.73 to 0.80 µg/L; model results are presented in Table 3. These results align well with the estimated 95% lower confidence interval of the mean dissolved selenium concentration (i.e., roughly 0.9 µg/L) associated with a whole-body concentration in burbot of 5.6 µg/L (Thorley 2020).

Table 3. Estimated range of ecologically protective concentrations using the recommendations for model inputs and Kd selection presented in this memorandum and the USGS model (Presser and Naftz 2020).

[1.] Kd scenario that most closely matches the pooled median of 4,574 and epilimnetic median of 5,071.

Tissue Target Concentration	Aquatic Insect TTF	Fish TTF	Bioavailability Factor	Kd[1]	Benchmark (µg/L)
5.3	1.2	1.1	1.0	5,000	0.73
5.3	1.2	1.1	1.0	4,579	0.80

Considerations for Future Activities/Monitoring

- We recommend that a mass balance analysis be conducted to quantify inputs from the Elk River (and other sources), exports (via Libby Dam) and the pool of selenium in the reservoir and the rate at which it is increasing over time.
- We recommend that fish tissue monitoring be conducted to get an accurate measure of ripe egg selenium concentrations (pre-spawn) to validate conversion factors.
- We recommend efforts focus on the estimation of assimilation efficiency factors (Kds) in periphyton (e.g., through the deployment of artificial substrates, seeded with periphyton)
- We recommend collection of additional benthic invertebrate samples for tissue analysis from the vicinity of the artificial substrates as well in the reservoir proper.
- We recommend collection of additional zooplankton samples in various seasons throughout the year at multiple locations throughout the reservoir.

Closure

The recommendations put forth in this letter provide a range of reasonable and defensible model parameters considering the data and information collected to date. Using the recommendations for model parameters in the USGS model, we estimate a range of ecologically protective benchmarks between 0.73 and 0.80 µg/L. This range is

corroborated by the work conducted by Poisson Consulting, which estimated a criterion for burbot, a high selenium accumulator, of roughly 0.9 µg/L (Thorley 2020). It is important to note that this range of estimates is lower than the current concentrations (i.e., 1 µg/L; Thorley 2020) in the reservoir. Accordingly, these analyses suggest that there is a need to stabilize and reduce loadings of selenium into Koocanusa Reservoir in order to meet protection goals as we do not have confidence that increasing selenium concentrations above these levels would be protective of aquatic life or Ktunaxa cultural practices.

We appreciate the opportunity to provide feedback and look forward to further engagement on this process.

Sincerely,

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Heather McMahon
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Ktunaxa Nation Council

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Disclaimer: *Please note the comments and recommendations contained in this document are strictly for Montana's and British Columbia's consideration. The views expressed in these comments and recommendations are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Regarding Montana's submission, the comments do not constitute approval or disapproval decisions under CWA Section 303(c). Neither are these comments a determination by the EPA Administrator under CWA Section 303(c)(4)(B) that revised or new standards are necessary to meet the requirements of the Act. These comments and recommendations do not impose any binding requirements, determine the obligations of the regulated community, change or substitute for any statutory provision or regulation requirement, represent, change or substitute for any Agency policy or guidance, or control in any case of conflict between this discussion and statute, regulation, policy or guidance.*

The Selenium Technical Subcommittee (SeTSC), was established by the Lake Koocanusa Monitoring and Research Working Group (LKMRWG), at the direction of the Steering Committee and is comprised of selenium experts from both the US and Canada and supported by the Montana Department of Environmental Quality and BC Ministry of Environment.

The overall objectives for the Se TSC are to develop selenium criteria/objectives for Lake Koocanusa that are protective of the uses of lake including, but not limited to, aquatic life, human health, recreation, wildlife, and agriculture, with the specific goal of answering the questions:

- Are the current Canadian (selenium target of 2 µg/L, as set out in the BC Water Quality Guideline) or Montana (WQS = 5 ug/L), protective of the uses in Lake Koocanusa in their respective jurisdictions?
- If not, what is an appropriate target value for selenium in Lake Koocanusa that can be adopted and implemented by Montana and British Columbia?

The SeTSC has been meeting regularly for four years through conference calls and in-person workshops with the primary goal of evaluating available data facilitating the development of a site-specific selenium criterion for Lake Koocanusa. More specifically, the process has involved the following tasks:

- Collection and analysis of existing Lake Koocanusa information to identify gaps in scientific understanding of the lake chemistry and ecology that are relevant to the stated research objectives.
- Prioritize monitoring and research activities and tasks
- Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- Definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa.

Seminal products developed during this process include the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019). These products provided critical information enhancing the SeTSC

understanding of the available data and the mechanistic model proposed for use in development of a site-specific water quality threshold protective of the designated uses of Lake Koocanusa.

The SeTSC in consideration of jurisdictional requirements, and with input from stakeholders, developed a set of constraints and considerations critical to the modeling efforts (*Proposed Workplan for Developing a Site-Specific Selenium Water-Column Criterion for Lake Koocanusa*; "the workplan") and resulting outputs including:

- The site-specific criterion will meet the regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.
- The site-specific criterion will also consider ecologically significant species and those important to stakeholders
- The definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir
- The site-specific criterion will provide long-term protection for fish in all parts of the reservoir during all phases of reservoir operation, all selenium loading profiles, and all water years (precipitation/runoff scenarios).
- The Development and/or evaluation work plans for various research projects including the development of SAPs/QAPPs.
- site-specific criterion also will protect downstream uses including protection of the endangered Kootenai River white sturgeon

Using the requirements and constraints set forth above, four main alternative levels of protection were proposed for model runs for Lake Koocanusa:

- Two scenarios based on the BCMoE egg-ovary selenium guideline of 11.0 mg/kg dw that consider individuals and populations of fish species explicitly.
- Two scenarios based on the USEPA national 304(a) egg-ovary selenium criterion of 15.1 mg/kg dw that consider individuals and populations of fish species explicitly.

An additional scenario was proposed by stakeholders as a potential no effect threshold (BCMoE tissue guideline of 4.0 mg/kg dw) protective of sensitive wildlife receptors. This value is like a "no-effect" threshold of 5.5 mg/kg dw proposed to EPA by USFWS in 2005 (J. Skorupa personal communication).

SeTSC members were charged with providing comments on the USGS modeling report "*Understanding and Documenting the Scientific Basis of Selenium Ecological Protection in Support of Site-Specific Guidelines Development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada*" Open-File Report 2020–1098.

Committee members were asked to comment on:

- Model Inputs (used to confirm or run model scenarios):

- Tissue threshold (use of whole body 8.5 ug/g, 4 ug/g, or other?)
- Model(s) to run, i.e. Invertebrate to fish model and/or Trophic level fish model and species-specific model (Table 10.)
- Food Web / diet fraction(s), e.g. %aquatic insect / %zooplankton: 100/0, 75/25, 50/50
- TTFs (invert to fish): generic option (1.1) or other?
- Bioavailability (100%, 60% or other percentage)
- Kd data set (Specific sub-sets?)

The following comments reflect my personal scientific views on the USGS model assumptions and outputs from the report. The focus of these comments is based on regulatory requirements to protect the designated uses of waterbodies under the U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

1. Model Inputs - Tissue threshold

The USGS selected the USEPA whole-body (wb) selenium criterion element 8.5 mg/kg dw, a value translated from the egg-ovary criterion of 15.1 mg/kg dw. In EPA, 2016, Species-specific whole-body values were calculated directly from whole body tissue [Se] concentrations measured in reproductive toxicity studies, or more commonly by applying an egg-ovary (EO) to whole-body (WB) conversion factor (CF) based on taxonomic relatedness. The lowest WB concentration in the available data is from the white sturgeon (9.2 mg/kg dw), the most sensitive species in the EPA national toxicity dataset. The criterion (8.5 mg/kg dw) is based on an OLS regression-based projection using an n = 15 taxa. The EPA used a generic (median of all fish [1.78]) conversion factor (CF) to convert the egg-ovary criterion to a whole-body threshold. EPA derived CFs for matched pairs of field-collected egg or ovary and whole body samples and used medians based on the available species-specific distributions of whole body and reproductive tissue data. Concern has been expressed over the EPA's use of the median, as it may not be appropriate to a specific site (i.e., Lake Koocanusa). There is a paucity of field data of sufficient quality to calculate field-based EO-WB conversion factors for resident species. Ideally, laboratory data directly measure reproductive tissues (mature eggs) and whole-body measurements would be more readily available. There are two studies available bluegill (Hermanutz, 1993, 1996) and brown trout (Formation 2009) with directly measured tissues resulting in EO-WB conversion factors of 1.38 and 1.59 respectively, providing support for the EO-WB CF of 1.78 used in the national freshwater selenium criterion EPA, 2016)

National criteria are limited to predicting sensitivity to a given contaminant based on the range of toxicity data available, and the national data set may be modified by applying the Recalculation Procedure (40CFR131.11(b)ii) to edit the species toxicity database to reflect taxonomic relatedness to the site assemblage, while including tested surrogates for untested resident species. As discussed previously (Beaman presentation to SeTSC, October 2019), the fish assemblage in Lake Koocanusa is well comprehensively represented by the EPA selenium toxicity database (EPA, 2016) augmented by other data (DeForest 2012, Teck, draft redside shiner toxicity report 2020). This database provides precise quantitative reproductive toxicity values for 5/13 resident species, as well as qualitative species or genus surrogate level tissue values for an additional 4 species (mountain whitefish, largescale and longnose sucker, and redside shiner), leaving 4/13 species unrepresented in the site-specific toxicity database. Early in the process, the SeTSC agreed that all fish species without data would be deemed equally sensitive to the white sturgeon, the most sensitive species in EPA's toxicity dataset. Therefore, the

white sturgeon whole body value of 9.2 mg/kg dw would be applied to the burbot, northern pikeminnow, peamouth chub, and yellow perch. When these 4 values are added to the censored site database (removing non-resident species), the resulting criterion is 9.2 mg/kg since the 5 most sensitive taxa are all equally sensitive. This provides valuable information demonstrating that the EPA tissue threshold of 8.5 mg/kg dw is likely sufficiently protective of the assemblage of fish species in Lake Koocanusa, including those with no toxicity data.

Recommendation: use the EPA whole body criterion element of 8.5 mg/kg dw, as it provides a protective goal for the species assemblage in Lake Koocanusa.

2. Bioavailability of selenium in Lake Koocanusa & Bioaccumulation Potential of Resident Fish Species
Selenium bioavailability at the base of the food web is impacted by the form of inorganic selenium (inorganic selenate and selenite) and its interaction with different types of particles in the aquatic environment. The relationship between the [Se] in suspended particulate matter (SPM) and the [Se] in the invertebrate (TTF) is a function of the type of particulate that the invertebrate encounters (sediment, detritus, phytoplankton) and the assimilation efficiency (AE) of the organism based on the form of selenium encountered adsorbed on (elemental Se or inorganic Se) or contained in (organic Se) the particulate.

Because selenium speciation data for SPM was not available for Lake Koocanusa, USGS addressed this uncertainty by using AEs of various species from other studies (e.g., Presser and Luoma 2010a) and using different types of particulate matter to account for the site- or species-specific bioavailability of foods likely to be consumed by invertebrates.

Two SPM bioavailability factors (100 percent and 60 percent) are used within each food-web scenario to quantify the efficiency of assimilation of SPM by invertebrates. USGS cited AEs varying from 55 to 86 percent among various invertebrate species, with smaller differences among living food types such as different species of algae. In my review of EPA 2016, I note that the saltwater copepods have AEs of ~50 – 55% whereas other invertebrates, particularly saltwater mollusks have median AEs ranging 61 – 96% (EPA 2016 Appendix B). Conversely, laboratory studies using freshwater species (including surrogates for resident macroinvertebrates:

Species	Median AE	Range AE	Taxonomic surrogacy
Water flea (D. magna)	40.6%	24.9 -57.9%	zooplankton
Blackworm (L. variegatus)	16.5%	9 – 24%	chironomid (benthic detritivore)
Mayfly	39%	38 – 40%	resident aquatic insect

Even available data for mollusks, a taxon known to have high selenium AE, are notably lower in FW (asiatic clam – 55%; zebra mussel – 26% [18-46%]) indicate that lower AE may be more appropriate.

Given the paucity of resident invertebrate data, USGS used [Se] ranges to examine the impact of bioavailability assumptions on model validation. For macroinvertebrates collected in Montana waters, the AE = 100% resulted in significant overpredictions (2X upper limit of 18.8 for predicted vs 9.1 for observed, 2018), whereas the AE = 60% resulted in more comparable results (observed [Se], 0.4–9.1 µg/g dw vs predicted 0.7–11.3 µg/g dw). Similar observations were made for validation comparisons of macroinvertebrates collected in the lake south of the Elk River, as well as zooplankton collected in US and Canadian waters. The table shows a comparison of water values calculated using the TFM or IFM model using assumed bioavailability of 60% or 100%

Table 1. Influence of Bioavailability of water column values in IFM and TFM models

Centile	TFM (100%)	IFM (100%)	TFM (60%)	IFM (60%)
median	0.55	0.61	**0.92**	**1.01**
40th centile	0.52	0.58	**0.87**	**0.96**
30th centile	0.49	0.54	**0.82**	**0.90**
20th centile	0.45	0.50	**0.75**	**0.83**
10th centile	0.40	0.44	**0.66**	**0.73**
5th centile	0.38	0.42	**0.63**	**0.70**

Recommendation: use the bioavailability of 60% based on model validation results and literature values (EPA 2016) that support freshwater AEs ≤ 60%.

Bioaccumulation Potential (BAP)
USGS developed the following scenarios for the insectivorous fish model:

- 100-percent aquatic insect (rainbow trout, Westslope cutthroat trout, redside shiner, longnose sucker),
- 50-percent aquatic insect and 50-percent zooplankton (peamouth chub, largescale sucker, mountain whitefish),
- 75-percent zooplankton and 25-percent aquatic insect (rainbow trout December–March), and
- 100-percent zooplankton (kokanee).

The following scenarios were used for piscivores. The scenarios for bull trout, burbot (winter and summer), and northern pikeminnow are as follows:

- 100-percent insectivores,
- 50-percent aquatic insect and 50-percent zooplankton, and
- 100-percent planktivores.

Given the paucity of stomach content data available to confirm older food web studies illustrated in Lotic Environmental 2017, the USGS developed insectivorous and piscivorous food webs using conservative assumptions regarding the weighting of dietary components. This is consistent with the principles discussed in the USGS workplan, that "definition of critical endpoints to be sufficiently protective of the uses of Lake Koocanusa, including protection of 100% of the fish species in the reservoir assuming a reproductive endpoint from reproductively mature females that are feeding (assuming maximum dietary exposure) in an ecosystem that functions as a lentic reservoir".

Recommendation: although some refinement of food webs would be more consistent with previous food web studies documented in the Lotic Environmental report, the use of "model food webs" are consistent with the modeling principle that assumes maximum dietary exposure.

3. Water -Particulate Partitioning Coefficient (Kd)
Kds were collected by different agencies at different times during the years 2015 -2019. Kds were not spatially and temporally comparable on a year over year basis. Due to the density, spatial and temporal inconsistencies in Kd sampling between 2015 and 2019, it is difficult to partition a subset of Kds for examination of its influence on modeled water concentrations for Lake Koocanusa. Therefore, USGS used the entire Kd datasets allowing for 87 independent scenarios within each (IFM and TFM) model. The full distribution of scenarios can be assessed statistically, with selection of a protective water value

expressed as a percent of scenarios where maximally exposed fish are protected (based on attainment of the whole-body value of ≤ 8.5 mg/kg dw using conservative food web assumptions.

Table 2. Centile Distribution of Kds and Water Values (ug/L) generated by IFM and TFM model scenarios

			Model	
Centile	Kd	Centile	IFM	TFM
median	4547	median	1.01	0.92
60th centile	4788	40th centile	0.96	0.87
70th centile	5090	30th centile	0.90	0.82
80th centile	5569	20th centile	0.83	0.75
90th centile	6311	10th centile	0.73	0.66
95th centile	6611	5th centile	0.70	0.63

This table displays the mean epilimnetic and hypolimnetic Kds by year and location.

Table 3. Annual Average Kd by Site, Sampling Year, and Layer

Location	Layer	Sampling Year				
		2015	2016	2017	2018	2019
South of Elk River	epi			4525	5482	
	hypo			2722		
US/Can Border	epi	3971	4256	3748	5745	6573
	hypo	2956	4625	3662		5012
Tenmile	epi				5222	
Forebay	epi	1446	5150	5458	5480	5518
	hypo	4188	4878	3150		3864

Kds collected in the hypolimnion were typically substantially lower than those collected in the epilimnion, however this is not unexpected. Particulate in the hypoliminion is typically composed of detritus (scenescing phytoplankton from the epilimnion or other particulates). In reducing environments such as the epilimnion, selenium released from detritus is recycled as selenite or organoselenium, but is not typically re-incorporated in particulate, resulting in lower Kds. These Kds should not be discounted, since detritivores that tolerate hypoxic conditions such as chironomids are an important macroinvertebrate food source to benthic invertivores and omnivores. These fish species are then preyed upon by demersal predators like the burbot.

Kds in the epilimnion of the lakes forebay were consistent between 2015 and 2019. Epilimnetic Kds were more variable at the international border and appear to be increasing from 2017 – 2019. This is uncertain due to the presence of an extreme value (Kd = 7139) in a small dataset (n = 3) in 2019.

A more refined species-specific approach applying more toxicological knowledge about the resident fish community could allow for examination of individual Kds using Table 10. Because we know that applying the 8.5 mg/kg dw threshold is overprotective for a number of species, I substituted the actual available toxicity data for resident species and their surrogates using data and information from EPA, 2016, DeForest, 2012, and Teck, personal communication (See attached tables, Table 10 SSC Comparison). For the species with no toxicity data (pikeminnow, yellow perch, burbot, and peamouth),

the whole-body threshold of 8.5 mg/kg dw was used as a default. The default TTF of 2.8 was used for invertebrates, and assumed TTFs were used for each fish species, however a TTF of 1.7 was used for burbot. Then, centiles of the Kd distribution (median, 60th, 70th, 80th, and 90th) were selected to represent a "steady state" exposure at the base of the food web throughout the lake. This provided a species-specific water threshold corresponding to each whole-body value from the toxicity database (n = 12 species). The median, 40th, 30th, 20th, and 10th centile values of the distributions are displayed below.

Table 4. Site Specific Water Thresholds (ug/L) for Fish Species Assemblage in Lake Koocanusa

	Lake Koocanusa Kd				
Centile	**Median**	**60th**	**70th**	**80th**	**90th**
	4547	**4788**	**5090**	**5569**	**6311**
Median	1.32	1.25	1.18	1.08	0.95
40th centile	1.16	1.11	1.04	0.95	0.84
30th centile	1.03	0.97	0.92	0.84	0.74
20th centile	0.83	0.78	0.74	0.67	0.59
10th centile	0.76	0.73	0.68	0.62	0.55

Observations:

- The maximum possible water value that might be considered protective of the fish assemblage at Lake Koocanusa is 1.32 ug/L, lower than the EPA national default lentic concentration of 1.5 ug/L.
- Approximately 50% of the water values are above 0.9 ug/L, including a water value representing a 90th centile of potential exposure based on Kd (0.95 ug/L).
- Based on the overall distribution of Kds in the dataset, the selection of a Kd between the 60th and 80th centile would provide robust temporal and spatial coverage for of the Kd distribution collected from the lake between 2015 and 2019

Sources of uncertainty related to various aspects of the toxicity dataset:

- The default WB EC10s for four species with no toxicity data.
- The median CF (EPA 2016) applied to species vs site specific CFs

The sensitivity of the untested species will likely have the largest impact on the range of protective values for species in Lake Koocanusa, so this could influence the selection of protective water value. If one or more of the untested species are tested and more sensitive, selection of a lower water value from the distribution may be more defensible particularly if this species is highly exposed to a food web with high bioaccumulation potential.

Recommendation: Selection of water values should be based on Kd, (either selection of a protective centile of the range of water threshold outputs in the IFM/TFM models or consideration of a conservative Kd based and considering the sensitivity of the species assemblage.

4. Trophic Transfer Factor (TTF)

Due to the paucity of insect data collected, and the lack of concurrently collected particulate data, USGS selected TTFs derived and used in previous studies (Presser & Luoma 2010).

The mean "all insect" TTF (2.8) that USGS is using to model Lake Koocanusa is composed of:

mayfly, caddisfly, cranefly, stonefly, damselfly, corixid (waterboatmen), and chironomid (midge)
The median "all insect" TTF used in EPA 2016 is composed of:
mayfly (match), Diptera (cranefly surrogate – match?), damselfly (match) dragonfly, waterboatmen (match), and chironomid (midge) - match

Both the P&L 2010 and EPA 2016 datasets have significant (4/7) taxonomic overlap.

The USGS model uses the mean as its central tendency estimate, whereas the EPA 2016 derivation uses the median ratio. The median ratio is not influenced by extreme values. For example, the median TTF for the waterboatmen dataset (n = 29) is 1.48, whereas the mean is 57% higher (2.32). The range of the distribution of 28 TTFs values is 0.139 – 5.62, with one extreme value, 21.0. If this extreme value was excluded, then the mean of the distribution would be 1.65, similar to the median.

Another uncertainty is the [Se] and TTFs associated with terrestrial insects. The Lotic Environmental food web report indicated that insectivorous fish species and lifestages consume a substantial proportion of terrestrial insects. For example, trout consumed between 40-50% of terrestrial insects between April and November (Table A.3.5 from Dalbey, 1996). These insect's exposure to Se is uncertain and is likely a product of local site-specific terrestrial conditions due to most insects small home range. For example, terrestrial insects from riparian habitats near the Elk River likely have higher [Se] than terrestrial insects inhabiting nearshore areas of the forebay. This makes prediction of TTFs for insectivores more complex.

Recommendation: Combine the P&L 2010, and the EPA 2016 TTF datasets to maximize the available TTF data, producing a more robust dataset from which to derive a central tendency estimate. Examine TTF distributions and select the most appropriate central tendency distribution on a species-specific basis. I Use of the median (vs the mean) of the more robust combined datasets avoids conducting additional analyses to determine censorship of extreme values (outliers).
The EPA TTF dataset (EPA 2016) has been provided to the SeTSC co-chairs upon their request.

5. Protection of Downstream Uses:
A key principle of the modeling effort is to ensure that the site-specific criterion adopted for Lake Koocanusa will protect downstream uses including protection of the endangered Kootenai River white sturgeon. This is consistent with the regulatory requirements of U.S. Clean Water Act and protection of threatened or endangered species under the U.S. Endangered Species Act.

The available data for eggs collected from white sturgeon in the Kootenai River from 2015 – 2019 indicate moderate elevation of [Se]. The maximum [Se] observed in the dataset was 5.76 mg/kg dw roughly 63% lower than the white sturgeon EC10 from Linville, 2006 (EPA, 2016).

Table 5. Selenium from white sturgeon eggs sampled in Kootenai River.

	2015	2016	2017	2018	2019
average	4.2	3.3	4.1	3.9	4.2
75th centile	4.7	3.4	4.7	4.2	4.5
95th centile	5.3	3.4	5.6	4.8	5.3

Based on current conditions, selenium concentrations in sturgeon eggs do not show an increasing trend between 2015 and 2019, however water thresholds adopted in Lake Koocanusa should ensure that this

trend does not increase over time. An important data gap is the loading of selenium (both particulate and total, and its fate in the downstream Kootenai River. Continued monitoring of sturgeon should continue to enable early detection of increasing selenium in mature eggs to insure protection of this important species. Future understanding of selenium loading may allow for refinement of the Lake Koocanusa SS water threshold, as well as water quality standards proposed for the Kootenai River by Montana.

Overall Recommendation for adoption of a water column threshold for Lake Koocanusa:
I believe that the SSC water value should strike a balance between protection of the fish assemblage in Lake Koocanusa and downstream uses (protection of white sturgeon) based on current conditions, and the opportunity to refine regulatory thresholds in the future based on future monitoring actions targeted on refining the USGS model.

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MEMORANDUM

To: Lauren Sullivan (MT DEQ) and Sheldon Reddekopp (BC ENV)

From: David DeForest

Subject: Comments on Koocanusa Reservoir Selenium Modeling and Recommendations for Site-specific Selenium Criteria

Date: August 28, 2020

This memorandum provides my comments on the Koocanusa Reservoir selenium model developed by the U.S. Geological Survey (Presser and Naftz 2020) and my recommendations for site-specific selenium criteria. Based on review of the U.S. Geological Survey (USGS) model and other lines of evidence, including empirical monitoring data for the reservoir, it is my opinion that the U.S. Environmental Protection Agency's (USEPA's) surface water quality criterion of 1.5 µg/L for lentic water bodies is protective of fish and the aquatic community in Koocanusa Reservoir. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessment. The following summarizes the lines of evidence that support this conclusion.

1 Selenium - Fate and Effects in Freshwater Aquatic Systems

There is scientific consensus that selenium concentrations in fish tissue, and particularly in fish eggs, are the strongest indicator of potential selenium toxicity to fish (Chapman et al. 2010). Fish primarily bioaccumulate selenium from their diet in the form of organic selenium. Selenium bioaccumulated by females is maternally transferred to ovaries and eggs. Adult fish are insensitive to selenium, but if concentrations are sufficiently high in the eggs, selenium may cause mortality, deformities, or edema in developing larvae as the yolk sac is absorbed (Janz et al. 2010).

Selenium is typically mobilized or released into surface waters, from both natural and anthropogenic sources, as inorganic selenium (typically selenate or selenite) (Maher et al. 2010). Inorganic selenium species are taken up at the base of the food web (e.g., algae), and transformed to organic selenium species. Site-specific surface water characteristics have a substantial influence on selenium speciation and bioaccumulation potential (Stewart et al. 2010). For example, a lentic (standing) water body with high biological

productivity, a long retention time, and strong reducing conditions has a greater selenium bioaccumulation potential then a lotic (flowing) water body with low biological productivity, a short retention time, and oxic conditions. This means that surface water selenium concentrations that may reach a toxic concentration in fish eggs may range by two orders of magnitude or more among sites.

1.1 USEPA Selenium Criteria

Based on selenium's fate and effects in aquatic systems, the USEPA derived ambient water quality criteria for selenium with the following hierarchy (USEPA 2016):

1. Fish egg selenium concentration of 15.1 mg/kg dry weight (dw)
2. Adult fish muscle selenium concentration of 11.3 mg/kg dw or adult fish whole body selenium concentration of 8.5 mg/kg dw
3. Surface water selenium concentration of 1.5 µg/L for lentic waters or 3.1 µg/L for lotic waters

The fish egg (or ripe ovary) selenium criterion of 15.1 mg/kg dw supersedes (is given priority over) the selenium criteria for muscle or whole body tissue and for surface water concentrations.

1.1.1 Fish Egg Selenium Criterion

The USEPA fish egg selenium criterion is based on maternal transfer studies in which parent females were exposed to diet-borne organic selenium in the laboratory or naturally exposed to diet-borne organic selenium in the field. Larval survival and development were assessed in the offspring of exposed parent females and selenium EC10s (10% effect concentrations) calculated based on the egg selenium concentration.

The USEPA's egg selenium criterion is based on genus mean EC10s for eight different genera. White sturgeon (*Acipenser transmontanus*) has the lowest EC10, which is 15.6 mg/kg dw. The EC10s for the remaining seven genera used to derive the USEPA's egg selenium criterion range from 20.6 mg/kg dw for bluegill (*Lepomis macrochirus*) to 56.2 mg/kg dw for Dolly Varden (*Salvelinus malma*). The fish egg selenium criterion of 15.1 mg/kg dw is based on the 5th percentile of the genus sensitivity distribution, which is extrapolated to a concentration less than the white sturgeon EC10 (Figure 1).

1.1.2 Fish Muscle and Whole Body Selenium Criteria

Muscle and whole body selenium criteria were developed using the same approach as for the egg selenium criterion. The USEPA compiled EC10s based on muscle or whole body selenium concentrations in parent females from maternal transfer studies. Direct measures of muscle and whole body selenium concentrations were used to calculate EC10s when reported. When necessary, egg-to-muscle or egg-to-whole body conversion factors were applied to estimate muscle or whole body selenium EC10s. As for the egg selenium criterion, the muscle and the whole body selenium criteria were calculated

based on the 5th percentile genus mean EC10s, and both criteria were extrapolated to concentrations less than the EC10 of white sturgeon (Figures 2 and 3).

1.1.3 Surface Water Selenium Criterion

The USEPA surface water criterion was developed to ensure protection of fish and the aquatic community for sites with high selenium bioaccumulation potential. This means that the surface water criterion may be conservative for sites with moderate to low selenium bioaccumulation potential. Simply put, the surface water selenium criterion was back-calculated from the egg criterion, which is driven by the sensitivity of white sturgeon, based on sites with high selenium bioaccumulation potential.

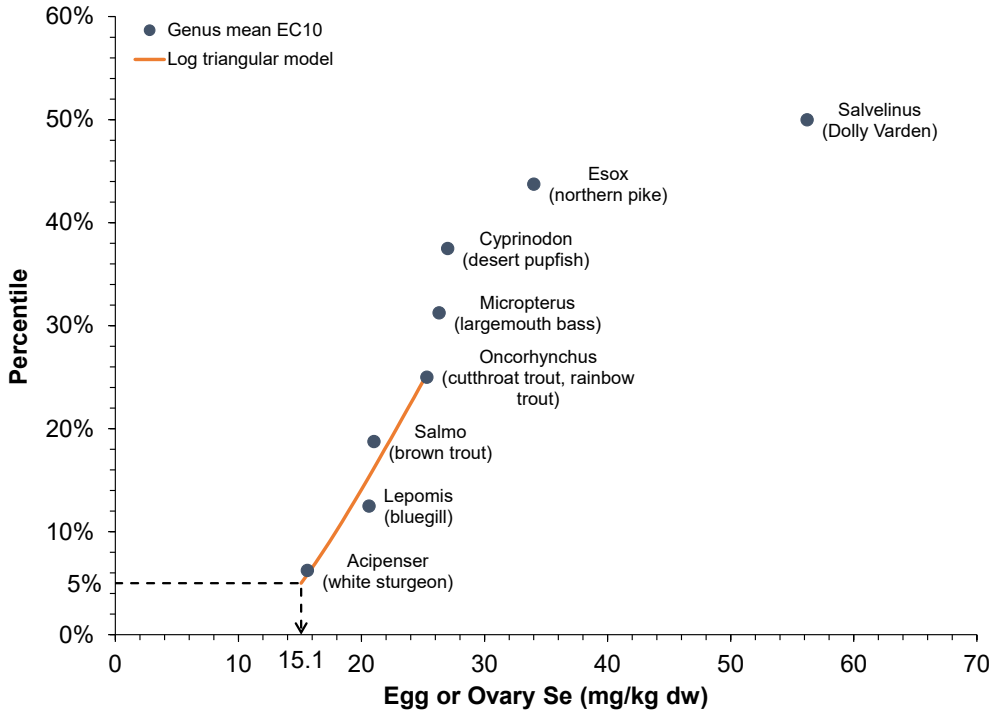

Figure 1. Derivation of the USEPA's egg/ovary selenium criterion of 15.1 mg/kg dw.
Note: Blue symbols are genus mean EC10s with the labels identifying the genus and, in parentheses, the species that comprise each genus mean EC10 (with the exception of two trout species comprising *Oncorhynchus*, all other genus mean EC10s are comprised of a single fish species). The orange curve is the log triangular distribution model that the USEPA traditionally uses to calculate the 5th percentile of the genus sensitivity distribution for criteria development. This model is fit to only the four most sensitive genera. Although the total number of genus mean EC10s is eight, the total sample size is based on an n of 15, which is why the percentiles on the y-axis do not extend to 100%. The sample size of 15 accounts for less sensitive fish genera for which definitive EC10s could not be calculated and less sensitive invertebrates.

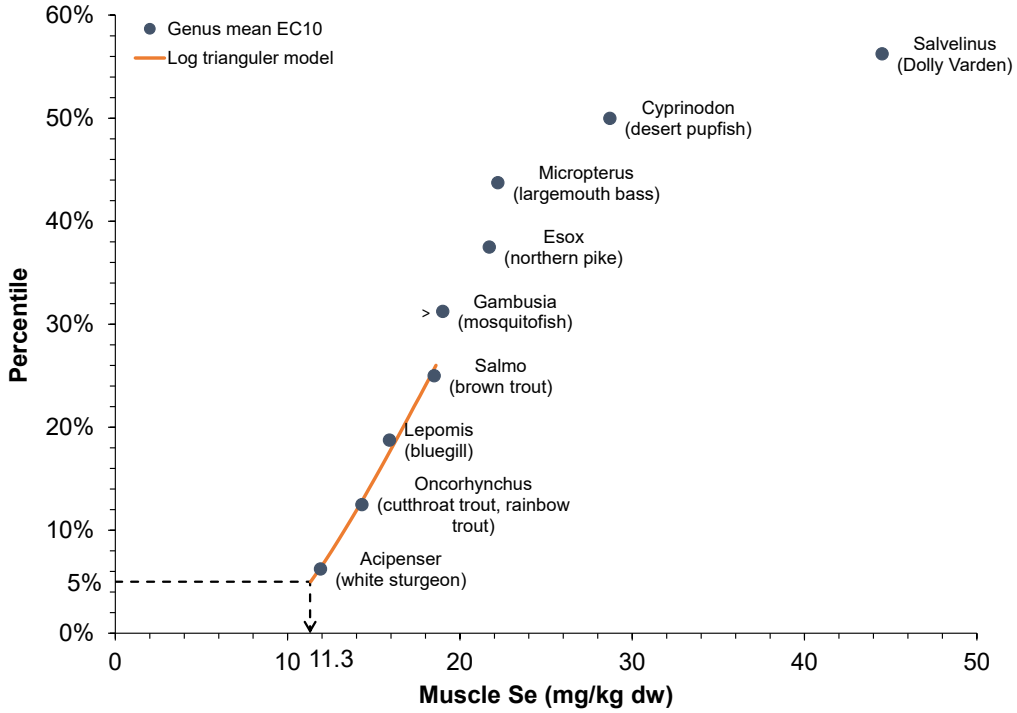

Figure 2. Derivation of the USEPA's muscle selenium criterion of 11.3 mg/kg dw.
Note: See Figure 1 for description.

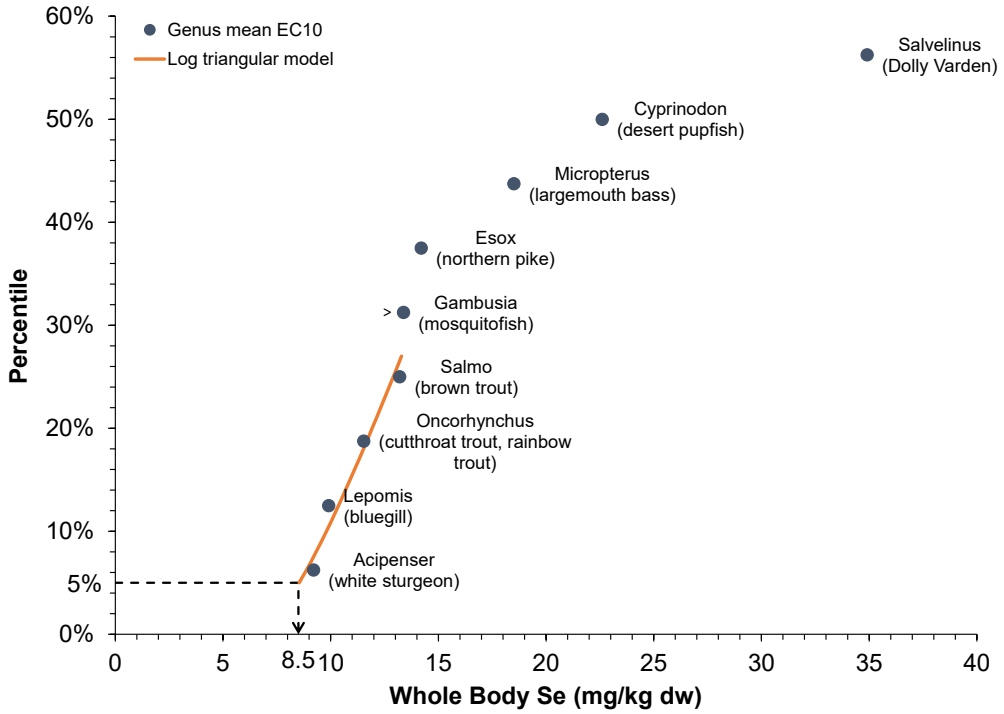

Figure 3. Derivation of the USEPA's whole body selenium criterion of 8.5 mg/kg dw.
Note: See Figure 1 for description.

1.2 BC Environment Selenium Guidelines

1.2.1 Fish Egg Selenium Guideline

BC Environment compiled selenium toxicity data for a variety of fish species, including many of the same species considered by the USEPA in deriving its selenium guideline (BCMOE 2014). As outlined within the guideline, it was determined that toxicity data for the genus *Oncorhynchus* would be a sensitive surrogate for fish in BC. Egg selenium EC10s of 22.05 and 21.97 mg/kg dw for rainbow trout and westslope cutthroat trout were compiled. An uncertainty factor of 2 was applied to each of these, which resulted in a mean of 11 mg/kg dw. The basis for the uncertainty factor of 2 was described as "a value that meets the balance between adequacy and protection, while addressing the inherent uncertainties in published toxicity threshold estimates." However, the basis for determining that it meets this balance was not described. Selenium data for comparison to the guideline should be based on the mean concentration of at least eight samples (eggs or ripe ovary from 8 individual females) (BCMOE 2014).

1.2.2 Fish Muscle and Whole Body Guideline

BC Environment derived a whole body fish selenium guideline of 4 mg/kg dw, which was developed by weighting literature-based evidence and the mean of published effects data for multiple species with an uncertainty factor of 2 (BCMOE 2014). BC Environment similarly derived an interim muscle selenium guideline of 4 mg/kg dw, which was based on low effect concentrations for rainbow trout, brown trout, and bluegill. However, the guideline was defined as interim due to uncertainty and limited primary toxicity data in their review. As for egg selenium, collection of selenium data for comparison to the whole body and muscle guidelines should be based on the mean concentration of at least eight whole body or muscle samples (BCMOE 2014).

1.2.3 Surface Water Selenium Guideline

The BC surface water selenium guideline of 2 µg/L was based on several studies that had lowest observed effect concentrations that converged around a water selenium concentration of 10 µg/L—this concentration was divided by an uncertainty factor of 5 to derive the guideline of 2 µg/L (BCMOE 2014). An alert guideline of 1 µg/L was also derived based on evidence from some studies that concentrations above this could pose a risk to aquatic life (BCMOE 2014). The water selenium concentration measured for comparison to the alert concentration and the guideline should be the mean of five evenly spaced samples over 30 days (BCMOE 2014).

1.3 USEPA Selenium Criteria are Protective of Fish

The USEPA's egg selenium criterion of 15.1 mg/kg dw is 1.4-times greater than the BC egg selenium guideline of 11 mg/kg dw, and the USEPA's muscle and whole body selenium criteria of 11.3 and 8.5 mg/kg dw are 2.8-times and 2.1-times greater, respectively, than BC's muscle and whole body guidelines of 4 mg/kg dw. The USEPA's lentic water criterion of 1.5 µg/L falls between the BC alert concentration of 1 µg/L and

guideline of 2 µg/L. The BC fish tissue guidelines are less than the USEPA's fish tissue criteria, but this difference is driven by the applied uncertainty factor of 2. In my opinion, the uncertainty factor of 2 is overly conservative and not supported by the science. As such, USEPA's fish tissue-based selenium criteria are protective of fish.

As previously noted, USEPA's fish tissue-based selenium criteria are based on the 5th percentile of genus mean EC10s (which are less than the EC10 for the most sensitive species, white sturgeon). This is a conservative approach for selenium criteria development for several reasons:

- First, EC10s cannot literally be considered 10% effect concentrations. This is because EC10s often fall within the statistical "noise" of selenium toxic effect thresholds. Most concentration-response data are inadequate to calculate concentrations associated with extremely low effects (e.g., EC0 or EC1) without having extremely high uncertainty (USEPA 2015). More importantly, very low effect concentrations are often indistinguishable from natural biological variability in data used to develop the concentration-response relationship. Concentration-response data for sensitive coldwater fish species are provided in Figure 4, which show that the USEPA's ovary selenium criterion of 15.1 mg/kg dw falls within the variability of responses at the egg selenium concentrations associated with the "flat" region of the concentration-response curve. As such, the USEPA's egg selenium criterion of 15.1 mg/kg dw and BC egg guideline of 11 mg/kg dw are equally protective.

  Another way to demonstrate this is to plot the concentration-response data for the most sensitive fish species in the USEPA's ambient water quality criteria document (USEPA 2016). Concentration-response models were fit using USEPA's Toxicity Relationship Analysis Program (TRAP) (USEPA 2015), which the USEPA used in developing its selenium criteria. TRAP defines the "control value" as Y0, which is the plateau in the concentration-response relationship before an inflection point indicating an adverse response is detected and then increasing levels of effect are fit by the model. Concentration-response data are normalized for their respective Y0 value so that data from multiple species and tests can be plotted together, as shown in Figure 5. Individual data points are given open symbols to denote those treatments that comprised Y0 (i.e., considered part of the control population) and filled symbols denote those treatments not considered part of the control population. As shown in Figure 5, the USEPA's egg selenium criterion of 15.1 mg/kg dw (i.e., the 5th percentile of genus mean EC10s) falls within the grouping of open symbols, which shows that use of EC10s to derive criteria are within the "noise" of no-effect concentrations.

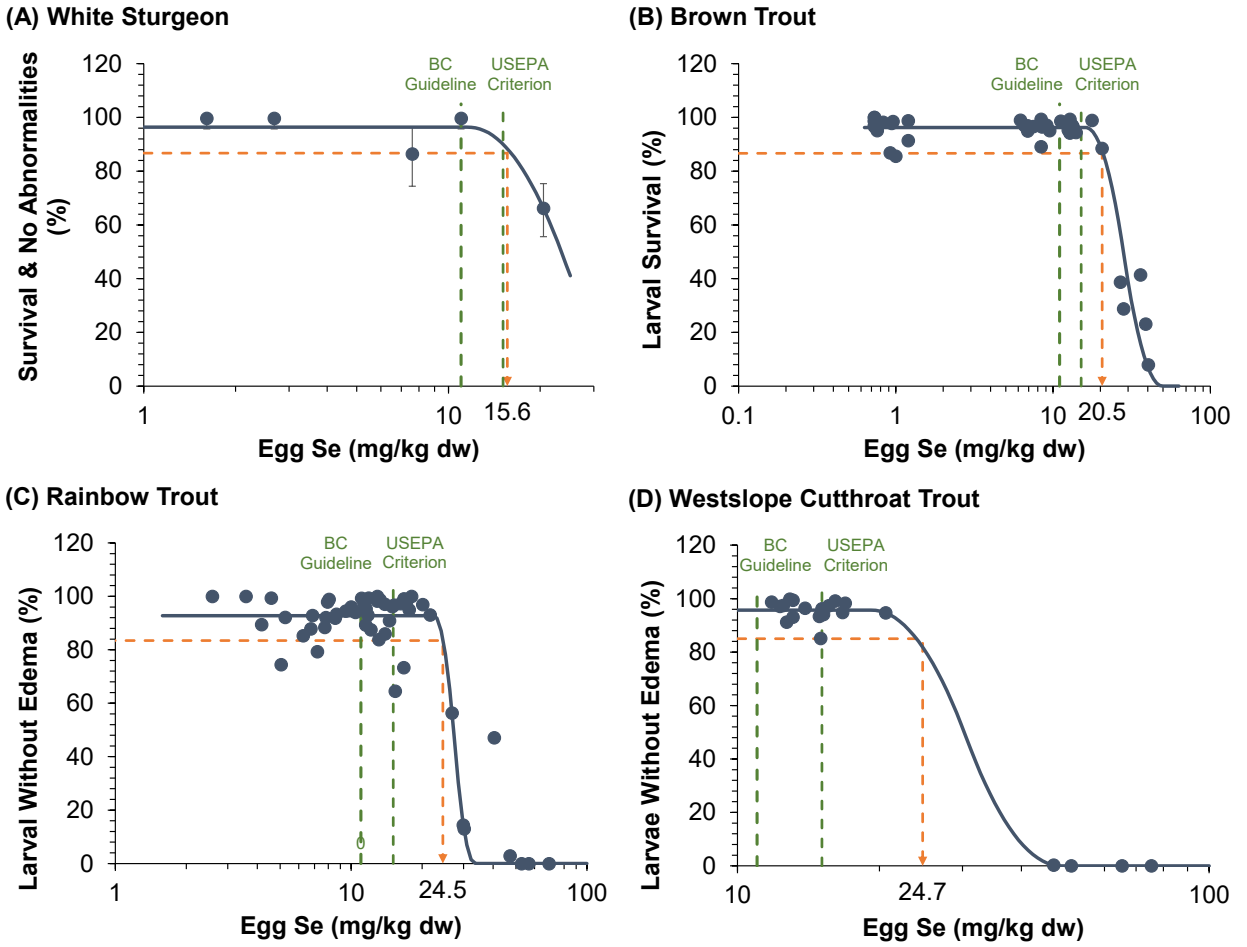

Figure 4. Examples of concentration-response relationships for (A) white sturgeon; (B) brown trout; (C) rainbow trout; and (D) westslope cutthroat trout.
Note: Orange line denotes the EC10, which falls within the variability of the biological response at low egg selenium concentrations. Green dashed lines denote the USEPA's chronic egg/ovary selenium criterion of 15.1 mg/kg dw and the BC chronic egg/ovary selenium guideline of 11 mg/kg dw.

- Second, with the exception of white sturgeon, all egg selenium EC10s compiled in the USEPA's selenium criteria document are ≥20.6 mg/kg dw. The USEPA (2016) egg selenium toxicity data for criteria development, along with data for additional species, are plotted in Figure 6 (sources of data are provided in Table 1). As shown, the first fish species for which egg selenium thresholds could be calculated were fathead minnow and bluegill in the early 1990s. As additional species have been tested, only one (white sturgeon) has been identified as being more sensitive than bluegill. In fact, there tends to be an increasing pattern of less sensitive species being tested with time. This suggests the likelihood of identifying species that are more sensitive than the most sensitive species tested to date is low.

Based on the above, the remainder of my comments focus on evaluations relative to the USEPA's fish selenium criteria.

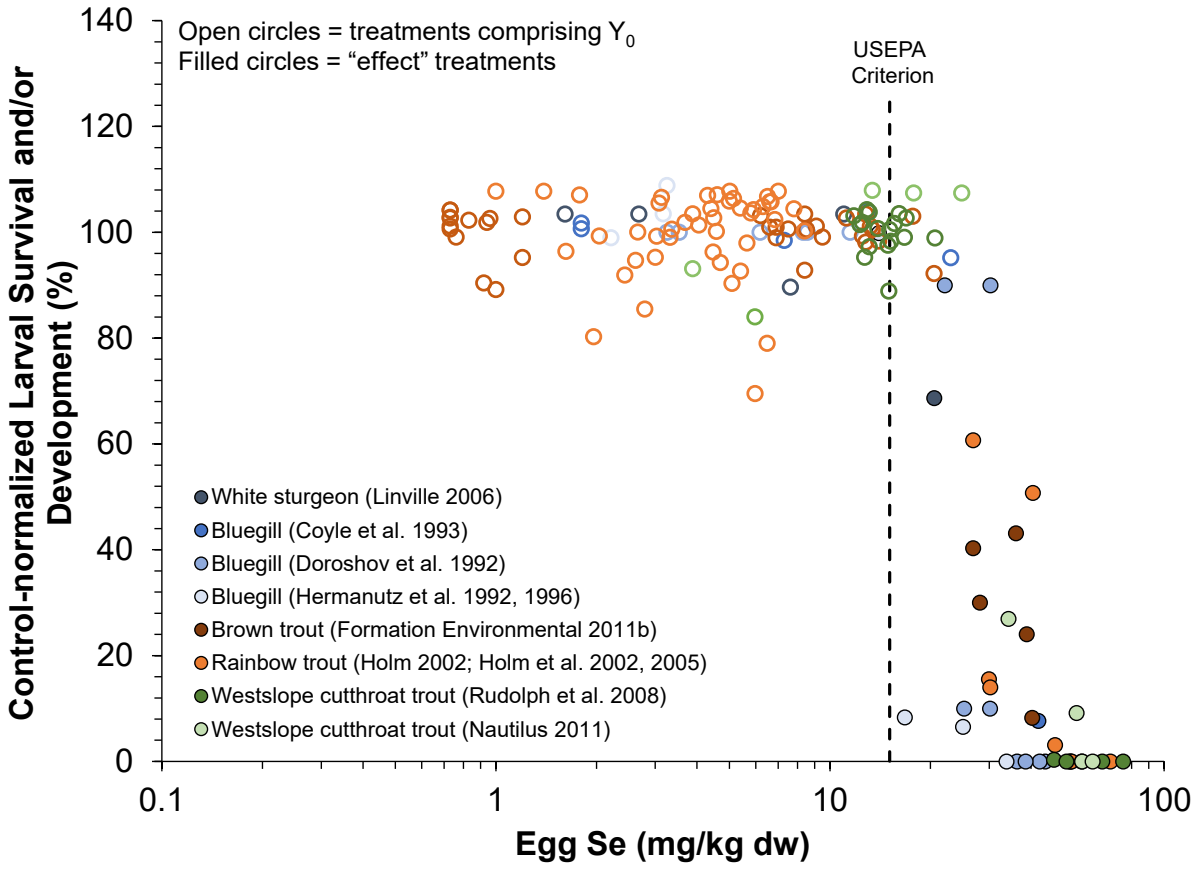

Figure 5. Concentration-response data for the four most sensitive genera in the USEPA's ambient water quality criteria document for selenium (USEPA 2016): (1) *Acipenser* (white sturgeon); (2) *Lepomis* (bluegill); (3) *Salmo* (brown trout); and (4) *Oncorhynchus* (rainbow trout, westslope cutthroat trout).
Note: Open symbols denote treatments that comprised Y0 (i.e., considered part of the control or reference population) and filled symbols denote those treatments that do not comprise Y0.

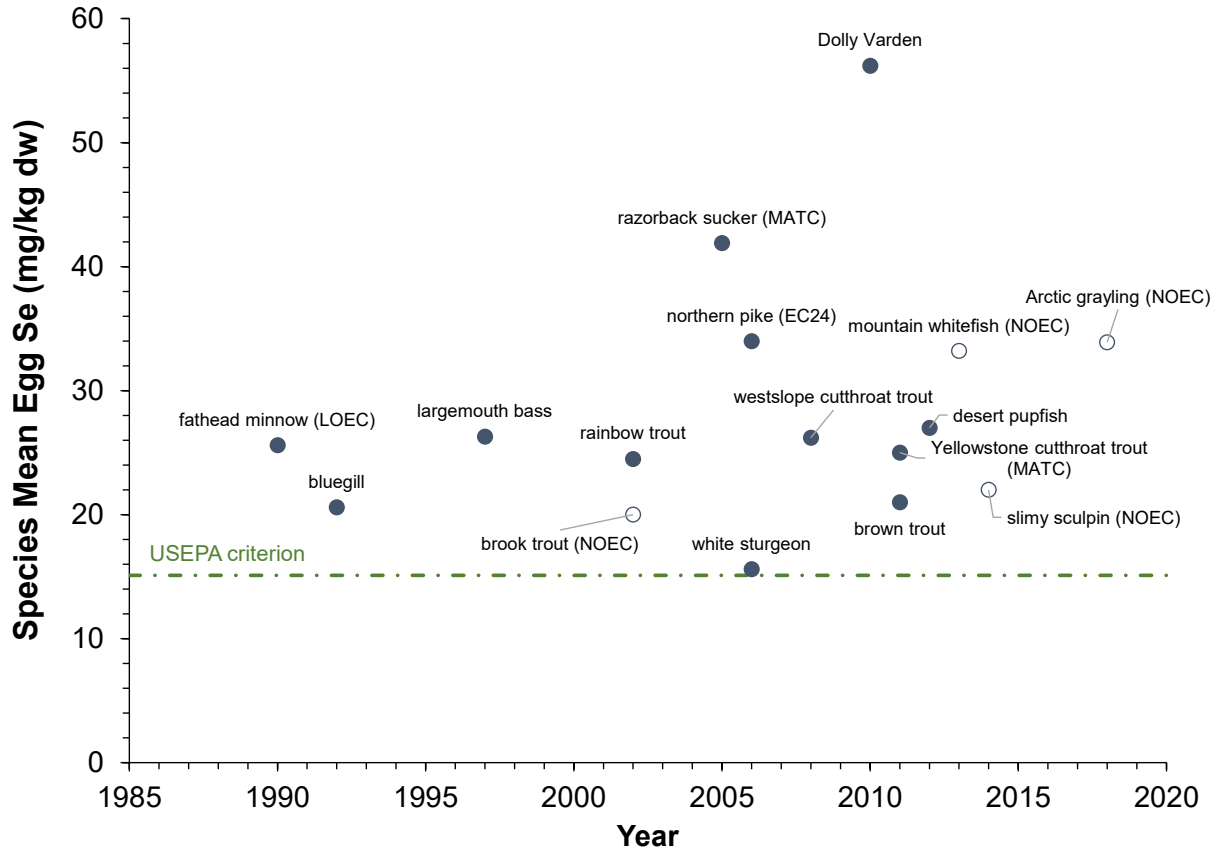

Figure 6. Relationship between fish egg selenium EC10s (and other toxicity thresholds) as a function of the year published or reported.
Note: Open symbols denote no-observed-effect concentrations (NOECs) from tests in which EC10s could not be estimated.

Table 1. Egg/ripe ovary selenium toxicity thresholds for North American freshwater fish.

Species	Statistical Endpoint	Species Mean Egg or Ovary Se (mg/kg dw)	Genus Mean Egg or Ovary Se (mg/kg dw)	Original Study Reference	Source of Toxicity Value Selected[1]
Salvelinus malma (Dolly Varden)	EC10	56.2	56.2[a]	McDonald et al. 2010	USEPA 2016
Salvelinus fontinalis (brook trout)	NOEC	>20		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Xyrauchen texanus (razorback sucker)	MATC	41.9	41.9	Hamilton et al. 2005a,b	DeForest et al. 2012
Esox lucius (northern pike)	EC24	34	34	Muscatello et al. 2006	USEPA 2016
Thymallus arcticus (Arctic grayling)	NOEC	>33.9	>33.9	Windward et al. 2018	Windward et al. 2018
Prosopium williamsoni (mountain whitefish)	NOEC	>33.2	>33.2	Nautilus 2013	Nautilus 2013
Cyprinodon macularius (desert pupfish)	EC10	27	27	Besser et al. 2012	USEPA 2016
Micropterus salmoides (largemouth bass)	EC10	26.3	26.3	Carolina Light and Power 1997	USEPA 2016
Pimephales promelas (fathead minnow)	LOEC	<25.6	<25.6	Schultz and Hermanutz 1990	USEPA 2016
Oncorhynchus clarkii lewisi (westslope cutthroat trout)	EC10	26.2	25.2[b]	Rudolph et al. 2008; Nautilus 2011	USEPA 2016
Oncorhynchus clarkii bouvieri (Yellowstone cutthroat trout)	MATC	25		Formation Environmental 2011a	DeForest et al. 2012
Oncorhynchus mykiss (rainbow trout)	EC10	24.5		Holm 2002; Holm et al. 2003, 2005	USEPA 2016
Cottus cognatus (slimy sculpin)	NOEC	>22	>22	Lo et al. 2014	Lo et al. 2014
Salmo trutta (brown trout)	EC10	21	21	Formation Environmental 2011b	USEPA 2016
Lepomis macrochirus (bluegill)	EC10	20.6	20.6	Doroshov et al. 1992; Coyle et al. 1993; Hermanutz et al. 1992, 1996	USEPA 2016
Acipenser transmontanus (white sturgeon)	EC10	15.6	15.6	Linville 2006	USEPA 2016

[1] EC10 values (or alternative statistical endpoints) were not always provided in the original study source, so source of value is provided.
[a] The genus mean value for *Salvelinus* was set equal to the EC10 for Dolly Varden, as no effects were observed in brook trout at the highest concentration tested.
[b] Although the statistical endpoint for Yellowstone cutthroat trout was an MATC, it was geometrically averaged with the EC10 values for westslope cutthroat trout and rainbow trout because values for all three species were similar.
EC10 = 10% effect concentration
EC24 = 24% effect concentration
NOEC = no-observed-effect concentration
LOEC = lowest-observed-effect concentration
MATC = maximum acceptable toxicant concentration (geometric mean of NOEC and LOEC)

2 Empirical Fish Tissue Selenium Data for Koocanusa Reservoir

This section compares empirical fish selenium concentrations to the USEPA's egg, muscle, and whole body selenium criteria. Data for non-Cyprinids and Cyprinids are discussed separately in Sections 2.1 and 2.2, respectively.

2.1 Non-Cyprinids

Out of more than 1,200 individual samples of non-cyprinid fish species in the reservoir, there have been only three measurements (0.2%) that exceeded criteria. These include two ovary selenium measurements (Figure 7) and one muscle selenium measurement (Figure 8). No exceedances of the whole body selenium criterion have been observed (Figure 9). For each of the aforementioned individual selenium criteria exceedances, mean selenium concentrations from other samples of the same species, from the same location and time did not exceed criteria:

- Rainbow trout ovary Se concentration of 19.8 mg/kg dw
  - Mean of 14.0 mg/kg dw for sampling location and time (n = 2) and less than the rainbow trout-specific EC10 of 24.5 mg/kg dw
- Longnose sucker ovary Se concentration of 21 mg/kg dw
  - Mean of 12.8 mg/kg dw for sampling location and time (n = 3)
- Yellow perch muscle Se concentration of 15 mg/kg dw
  - Mean of 4.6 mg/kg dw for sampling location and time (n = 10)

Consequently, empirical monitoring of selenium concentrations in non-cyprinids supports that these species are not adversely impacted by selenium in the reservoir.

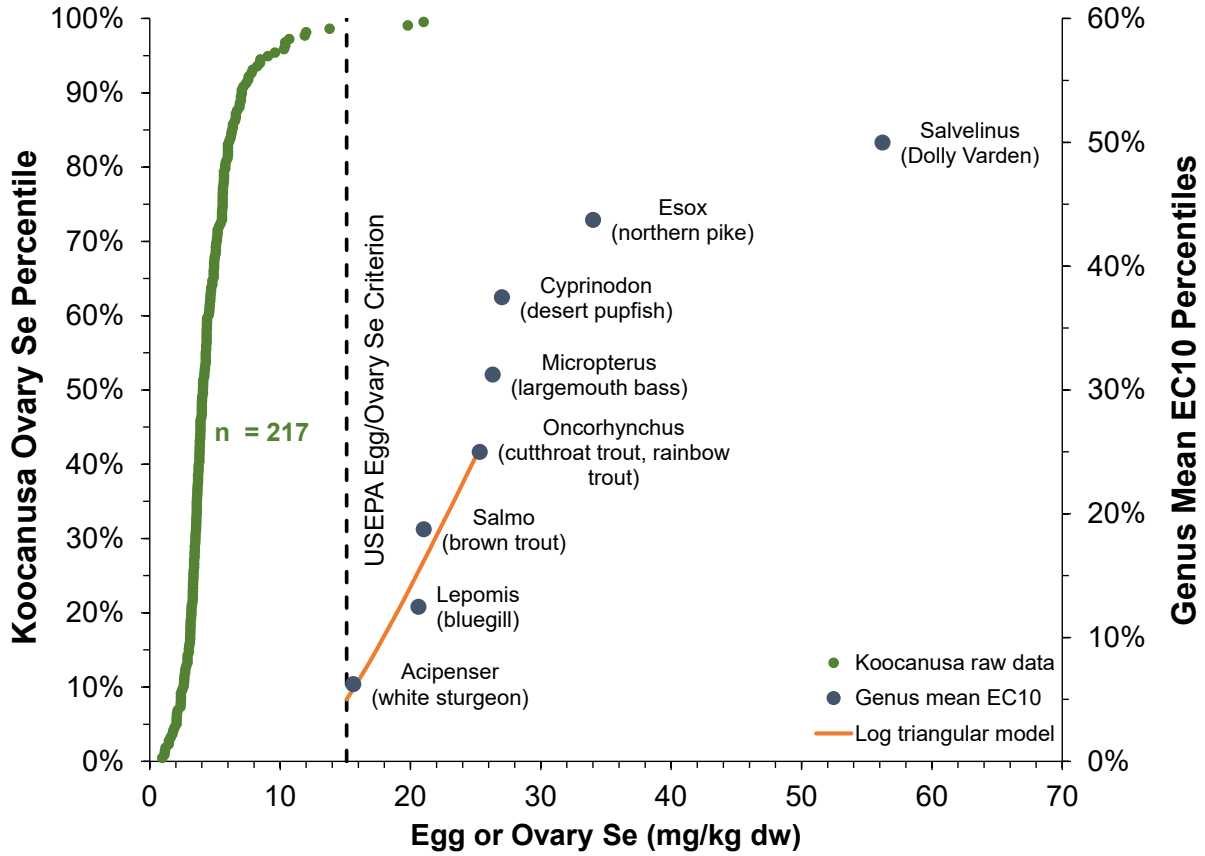

Figure 7. Cumulative distribution of empirical selenium concentrations in fish ovary samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of egg/ovary selenium concentrations used to derive the USEPA egg/ovary selenium criterion.

Note: Ovary selenium data for northern pikeminnow, peamouth chub, and redside shiner are excluded (see Section 2.2 for discussion of these cyprinids).

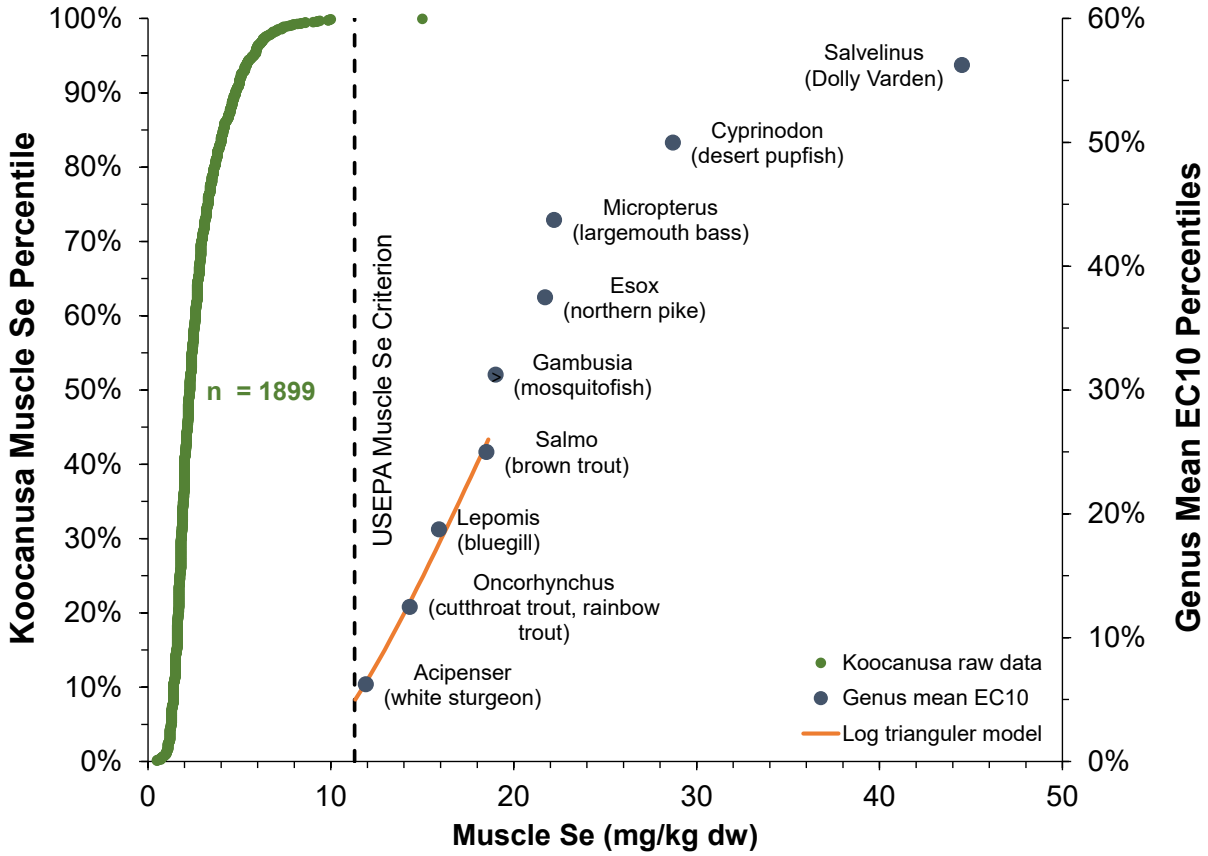

Figure 8. Cumulative distribution of empirical selenium concentrations in fish muscle samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of muscle selenium concentrations used to derive the USEPA muscle selenium criterion.

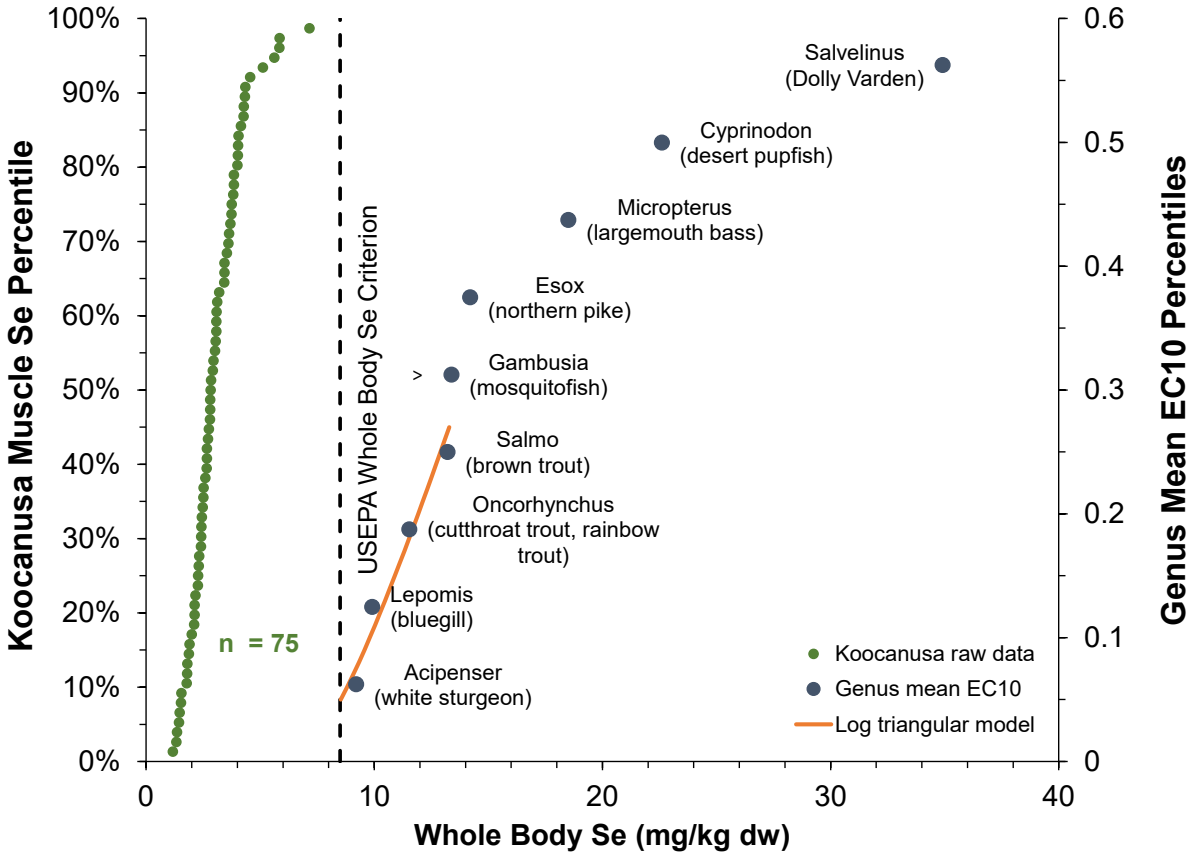

Figure 9. Cumulative distribution of empirical selenium concentrations in whole body fish samples collected from Koocanusa Reservoir and comparison to genus sensitivity distribution of whole body selenium concentrations used to derive the USEPA whole body selenium criterion.

2.2 Cyprinids

The three cyprinids in Koocanusa Reservoir are northern pikeminnow, peamouth chub, and redside shiner. Muscle and whole body selenium concentrations in these three species have never exceeded USEPA criteria. Ovary selenium concentrations for these species have exceeded the USEPA's egg selenium criterion but, as discussed further below, most of the ovary selenium data were not collected during spawning and concentrations are likely overestimated and should not be used. Further, the USEPA has compiled evidence that cyprinids are not uniquely sensitive to selenium.

2.2.1 Ovary Selenium Concentrations and Maturity

Based on routine monitoring of northern pikeminnow ovary selenium concentrations in Koocanusa Reservoir, it was previously observed that concentrations were higher and more variable in the BC portion of the reservoir, and lower and less variable on the Montana side. Further evaluation of these data revealed that fish collected in BC were

smaller than those collected in Montana, reflective of different sampling techniques. In addition, there was an indication that ovary selenium concentrations and the gonado-somatic index (GSI) were inversely related in northern pikeminnow collected in BC (GSI had not been measured in the northern pikeminnow collected in Montana). The GSI is a measure of ovary maturity, so ideally selenium would be analyzed in ovaries of fish with a GSI that is reflective of the fish's condition at the time of spawning.

These observations led to the northern pikeminnow study conducted in 2019, which included additional sampling at different times during the spawning period to collect fish with a range of sizes and GSI values. This report concluded that ovary selenium concentrations should not be used for assessing selenium risks to northern pikeminnow when the GSI is <5%, as selenium concentrations in the ovaries of these fish overestimate selenium concentrations that are relevant to the time of spawning (EcoTox et al. 2020). The study report was provided to the SeTSC and the study presented to the SeTSC by Dr. Brix in June 2020 (for completeness a copy the report is provided in Attachment 1).

A preliminary evaluation of ovary selenium concentrations and GSI for peamouth chub in Koocanusa Reservoir likewise indicates that there is a minimum GSI below which ovary selenium concentrations should not be used for assessing selenium risks. Based on a study of peamouth chub in the Columbia River (WA, USA), females had a mean GSI of about 8% during the period of spawning (Figure 10; Gray and Dauble 2001). Of the 153 peamouth chub ovary selenium concentrations measured in Koocanusa Reservoir, 29 (19%) exceed the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 11). Of those, the GSI was >8% in just three of the samples. Although this evaluation is not as robust or definitive as the northern pikeminnow study, it similarly highlights that future monitoring of selenium concentrations in peamouth chub should target spawning periods.

Although not a cyprinid, a recent evaluation of mountain whitefish data similarly found that ovary selenium concentrations are inversely related to GSI (Brix et al. 2020). Thus, there is an increasing body of information highlighting the importance of measuring selenium concentrations in ripe ovaries during spawning, as measurement of selenium in immature ovaries may overestimate selenium concentrations at spawning. The mountain whitefish evaluation is included as Attachment 2.

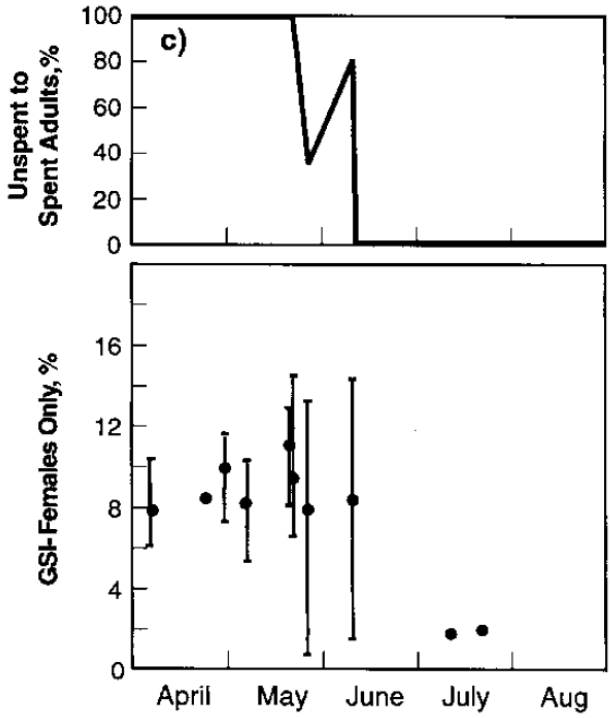

Figure 10. Spawning periods (top panel) and female GSI for peamouth chub in the Columbia River (WA, USA).
Note: Figure 3 in Gray and Dauble (2001).

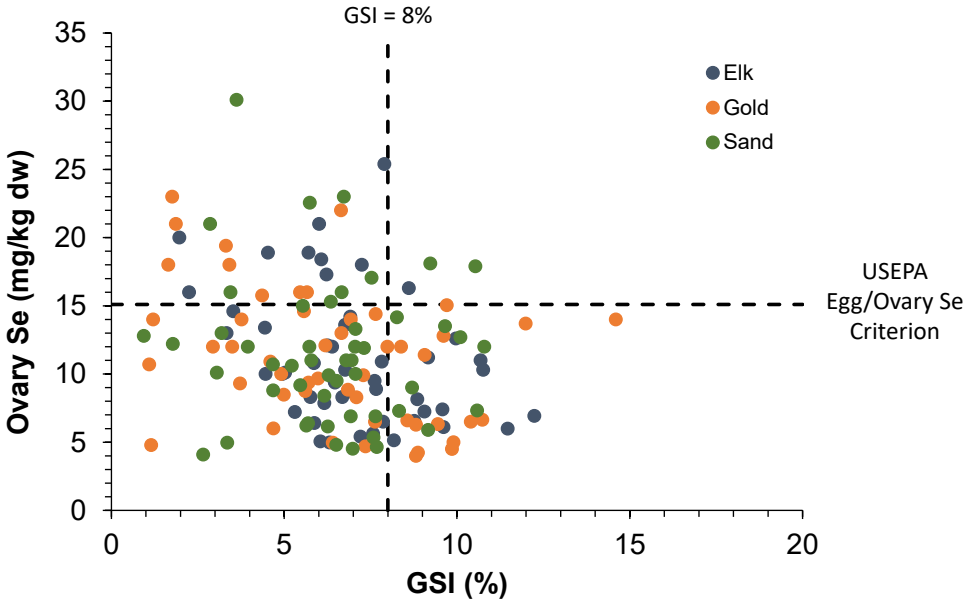

Figure 11. Relationship between ovary selenium concentrations in peamouth chub and GSI.

2.2.2 Conversion Factors for Ripe Ovaries

As noted in Presser and Naftz (2020), egg-ovary tissue can be substituted in the selenium model if the appropriate tissue-to-tissue conversion factors are available. Muscle-to-egg/ovary and whole body-to-egg/ovary conversion factors from USEPA (2016), for example, may be obtained for species of interest. Ideally, site-specific tissue-to-tissue conversion factors may be calculated. If site-specific muscle-to-ovary or whole body-to-ovary conversion factors are derived from Koocanusa Reservoir data, caution must be used to ensure that the ovary data used to calculate the conversion factors are based on ripe ovaries during the fish's spawning period. The following provides an example based on northern pikeminnow.

The northern pikeminnow study resulted in the conclusion that ovary selenium concentrations should not be used to assess selenium risk if the GSI is <5%. Based on this, I derived a site-specific northern pikeminnow muscle-to-ovary conversion factor from those muscle and ovary pairs from fish with a GSI >5% (Figure 12). Following USEPA (2016) methods, the conversion factor is calculated as the median, which is 2.5 for this dataset. This median muscle-to-ovary conversion factor of 2.5 was then applied to northern pikeminnow muscle data to estimate selenium concentrations in ripe ovaries representative of spawning conditions. Reservoir-wide annual mean estimates of selenium concentrations in ripe ovaries range from 3.3 to 7.7 mg/kg dw (Figure 13). Mean estimated selenium concentrations in ripe ovaries as a function of sampling location and sampling time range from 3.0 to 13.0 mg/kg dw, which are all still lower than the USEPA's egg selenium criterion of 15.1 mg/kg dw (Figure 14). This northern pikeminnow example demonstrates that evaluation of selenium concentrations in ripe ovaries has an important influence in evaluating potential selenium-related impacts.

2.2.3 Cyprinid Sensitivity to Selenium

In developing its ambient water quality criteria for selenium, the USEPA conducted a review of cyprinid sensitivity based on a review of field and laboratory data (Appendix E in USEPA [2016]). Based on field studies of regions in the United States with elevated selenium concentrations, there was no evidence of selenium-related impacts and the USEPA concluded that "native cyprinids appear to have a tolerance to selenium that is greater than centrarchid and salmonid species." Based on these conclusions, the USEPA's egg selenium criterion, driven by the sensitivity of white sturgeon, is conservative for cyprinids. Current studies on the sensitivity of redside shiner to selenium, and planned studies on the sensitivity of northern pikeminnow to selenium,[1] will provide additional information on the sensitivity of cyprinids.

[1] The 2019 northern pikeminnow study was intended to also test its sensitivity to selenium, but an insufficient number of ripe females were captured from which eggs could be manually expressed.

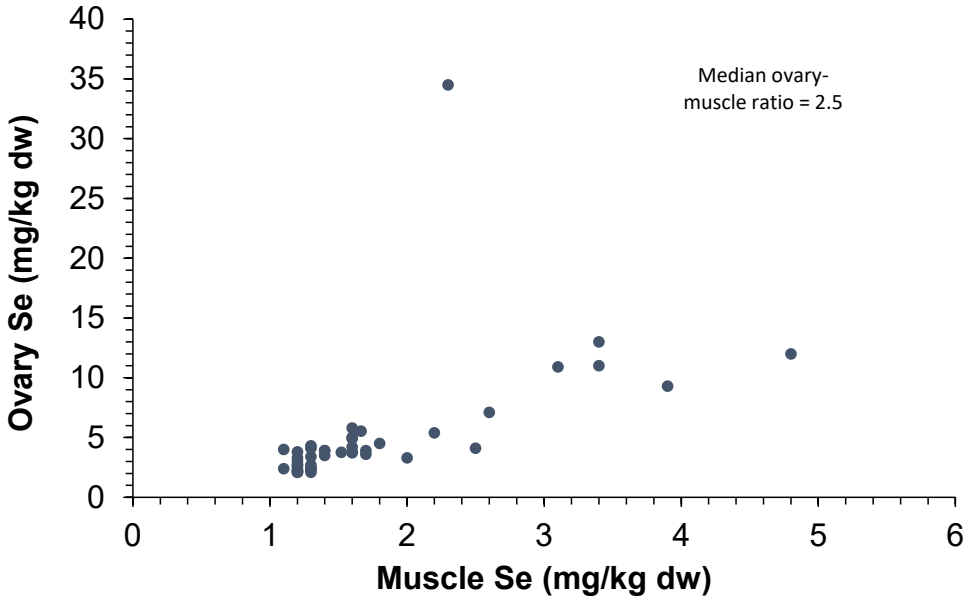

Figure 12. Site-specific relationship between ovary and muscle selenium in northern pikeminnow with a GSI >5%.
Note: The median ovary-muscle ratio is 2.5 with and without the apparent high outlier.

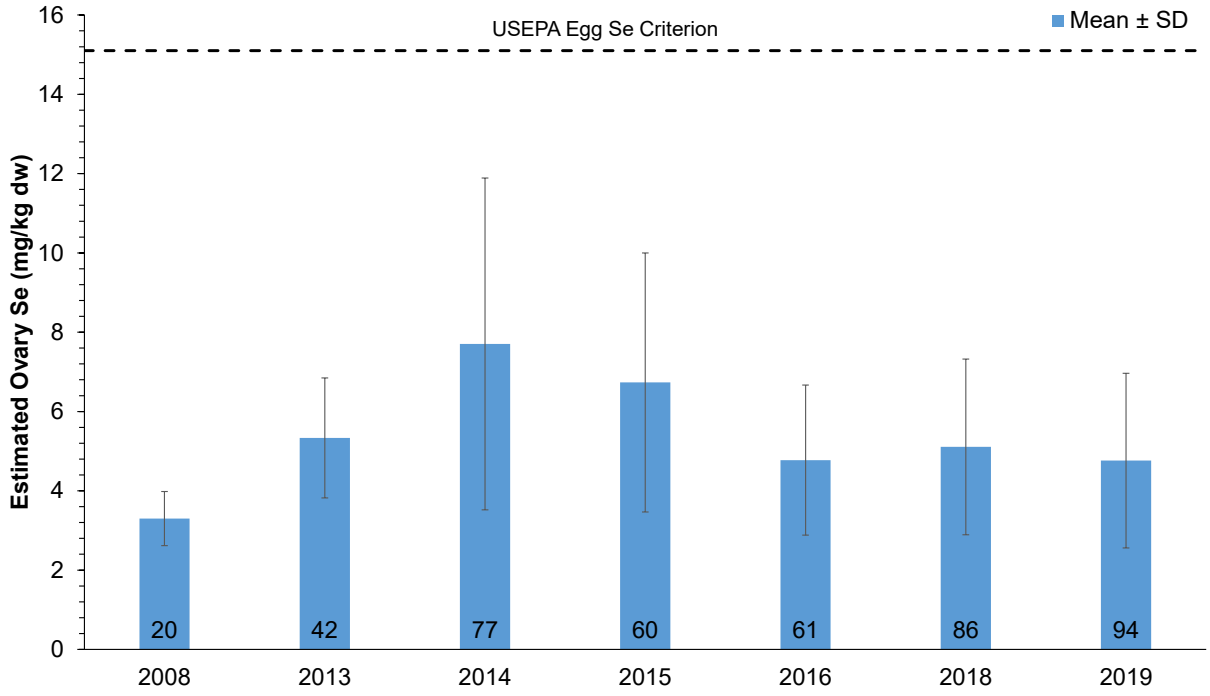

Figure 13. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow based on all samples in Koocanusa Reservoir by year.
Note: Median ovary-muscle ratio is 2.5. Numbers within base of column denote sample sizes.

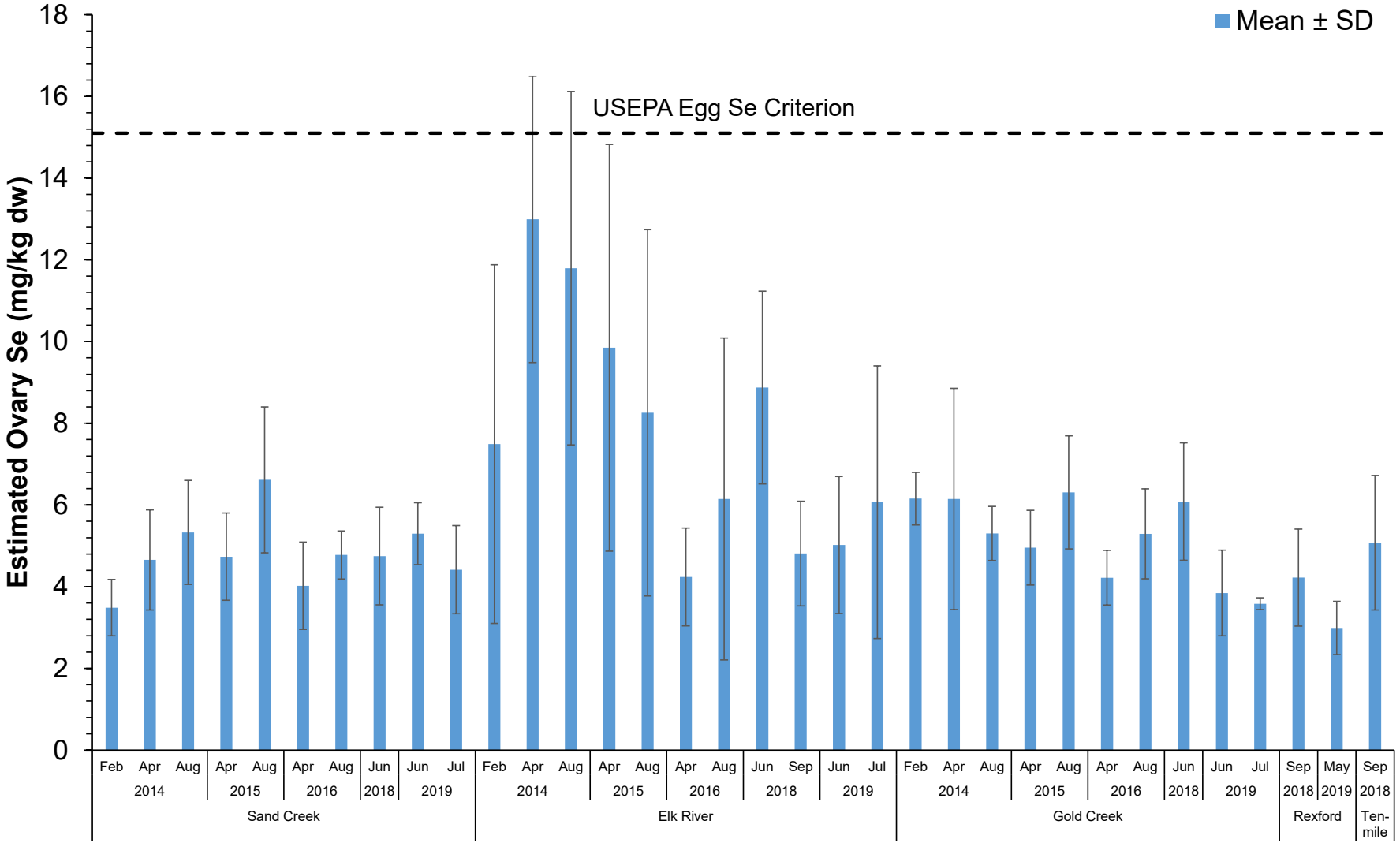

Figure 14. Mean (±SD) estimated ripe ovary selenium concentrations in northern pikeminnow by location and sampling time within Koocanusa Reservoir.
Note: Median ovary-muscle ratio is 2.5.

3 Review and Validation of Koocanusa Reservoir Selenium Bioaccumulation Models

Section 3.1 first discusses the USGS selenium model (Presser and Naftz 2020) and modifications to model inputs based on consideration of site-specific data for invertebrates are provided in Section 3.2. Validation of model outputs based on site-specific fish selenium data is then provided in Section 3.3.**USGS Model**

The USGS selenium model was developed to support derivation of a site-specific water selenium criterion for Koocanusa Reservoir (Presser and Naftz 2020). This model was based on the framework for Koocanusa Reservoir previously presented in Jenni et al. (2017), which was developed following the ecosystem-scale selenium modeling methodology described in Presser and Luoma (2010). This model uses k_d values to describe partitioning of selenium from water to particulates at the base of the food web and trophic transfer factors (TTFs) to describe partitioning of selenium between food web components (e.g., from particulates to invertebrates and from invertebrates to fish). For example:

$$C_{fish} = C_{water} \times k_d \times TTF_{invert} \times TTF_{fish} \times 0.001 \quad (1)$$

Where: C_{water} = Se concentration in water (µg/L)
C_{fish} = Se concentration in fish tissue (mg/kg dw)
k_d = ratio of Se concentration in particulates and water (L/kg dw)
TTF_{invert} = ratio of Se concentration in invertebrates and particulates
TTF_{fish} = ratio of Se concentration in fish and invertebrates
0.001 = conversion factor to convert from µg to mg

Based on k_d and TTF data or assumptions, and a target fish selenium concentration of interest (e.g., a criterion), Equation 1 can be rearranged to solve for the water selenium concentration predicted to result in the target fish selenium concentration:

$$C_{water} = \frac{C_{fish}}{k_d + TTF_{invert} + TTF_{fish}} \quad (2)$$

Equations 1 and 2 can be modified to include additional trophic level steps, such as a prey fish consumed by piscivorous fish. The equations can also be modified to include relative dietary fractions of invertebrates, such as 50% benthic invertebrates and 50% zooplankton. The following summarizes model inputs used in Presser and Naftz (2020):

- **k_d:** Selenium k_d values were calculated from 87 paired particulate and surface water selenium concentrations[2] measured in large volume suspended sediment (LVSS) samples. These samples were collected at the epilimnion and hypolimnion

[2] At the time of writing, these complete data have not been made available by the USGS and were requested on August 18, 2020.

from four locations in the reservoir: (1) south of Elk River; (2) the international boundary; (3) Tenmile (hypolimnion only); and (4) the forebay.

- **TTF_{invert}:** TTFs of 2.8 and 1.5 were assumed for benthic invertebrates and zooplankton, respectively. These TTFs were derived and reported in Presser and Luoma (2010) based on data from other studies and are not specific to Koocanusa Reservoir. Presser and Naftz (2020) incorporated a bioavailability fraction of 0.6 (60%), which effectively reduce the benthic invertebrate TTF from 2.8 to 1.7 and the zooplankton TTF from 1.5 to 0.9.

- **TTF_{fish}:** A whole body selenium TTF of 1.1 was assumed in the presentation of the model in the report, which is the mean TTF from 25 fish species as reported in Presser and Luoma (2010). It was noted, however, that species-specific TTFs could be considered and examples were provided.

- **C_{fish}:** The USEPA's whole body selenium guideline of 8.5 mg/kg dw was used in the report, but it was noted that other fish tissue selenium concentrations of interest could be considered.

For presentation and discussion of the model herein, water selenium concentrations are back-calculated from the whole body criterion of 8.5 mg/kg dw based on a k_d of 5,000 L/kg dw and benthic invertebrate, zooplankton, and fish TTFs of 2.8, 1.5, and 1.1, respectively (with and without the bioavailability factor of 0.6 for invertebrates). Water concentrations were back-calculated for four different food chain scenarios to bracket the range of exposure conditions: (1) 100% benthic invertebrates; (2) 100% zooplankton; (3) 50% benthic invertebrates and 50% zooplankton; and (4) 100% fish. These example calculations are similar to those provided in Table 10 of Presser and Naftz (2020). Presser and Naftz (2020) did not recommend selection of a single representative k_d for the reservoir, but a k_d of 5,000 L/kg dw was selected because this value was used in their Table 10 and it is also approximately the median k_d for the reservoir and thus a reasonable estimate of central tendency.

Based on the above model input assumptions and food chain scenarios, the water selenium concentrations projected to result in the whole body selenium criterion of 8.5 mg/kg dw range from 0.5 µg/L (100% fish diet) to 1.0 µg/L (100% fish diet) with a bioavailability factor of 100% (first set of green-highlighted rows in Table 2). If the bioavailability factor of 0.6 is assumed, the range in back-calculated selenium concentrations increases to 0.84 to 1.7 µg/L (second set of green-highlighted rows in Table 2).

Table 2. Examples of water selenium concentrations projected to result in whole body selenium criterion of 8.5 mg/kg dw based on different k_d, TTF, and food chain scenarios.

Scenario	Diet	TTF			SPM Bioavail-ability Fraction	k_d (L/kg dw)	WB Criterion (mg/kg dw)	Water Se (µg/L)	Mean Water Se (µg/L)
		Insects	Zoo-plankton	Fish					
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1	100% Insects	2.8	1.5	3.1*	1	5000	8.5	**0.55**	**0.70**
	100% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**1.0**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	1	5000	8.5	**0.72**	
	100% Fish	2.8	1.5	3.1*	1	5000	8.5	**0.50**	
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	100% Insects	2.8	1.5	3.1*	0.6	5000	8.5	**0.92**	**1.2**
	100% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.7**	
	50% Insects / 50% Zooplankton	2.8	1.5	3.1*	0.6	5000	8.5	**1.2**	
	100% Fish	2.8	1.5	3.1*	0.6	5000	8.5	**0.84**	
Alternative Assumptions									
Alternative Assumptions: Median k_d from USGS (2020) and median site-specific TTFs for inverts	100% Insects	1.1	0.52	1.2*	1	4547	8.5	**1.5**	**2.1**
	100% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**3.3**	
	50% Insects / 50% Zooplankton	1.1	0.52	1.2*	1	4547	8.5	**2.1**	
	100% Fish	1.1	0.52	1.2*	1	4547	8.5	**1.4**	
Alternative Assumptions: 75th percentile k_d from USGS (2020) and 75th percentile site-specific TTFs for inverts	100% Insects	1.2	0.65	1.3*	1	5268	8.5	**1.2**	**1.5**
	100% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**2.3**	
	50% Insects / 50% Zooplankton	1.2	0.65	1.3*	1	5268	8.5	**1.6**	
	100% Fish	1.2	0.65	1.3*	1	5268	8.5	**1.1**	

SPM = suspended particulate matter
TTF = trophic transfer factor
WB = whole body
*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

3.2 Alternative Inputs for Selenium Model

As discussed above, TTFs for benthic invertebrates and zooplankton in the USGS model are based on data compiled in Presser and Luoma (2010) and are not specific to Koocanusa Reservoir. As a point of comparison to those invertebrate TTFs, I developed zooplankton and benthic invertebrate TTFs based on site-specific data.

Site-specific selenium TTFs for zooplankton were developed by co-locating zooplankton and surface water selenium samples. In this paired dataset, zooplankton selenium concentrations ranged from 0.34 to 4.21 mg/kg dw and particulate selenium concentrations ranged from 2.0 to 6.6 mg/kg dw (Figure 15A). With one exception, resulting zooplankton TTFs were less than 1 and the median TTF was 0.52 (Figure 15B). The one exception was a TTF of 1.9, was calculated from the lowest particulate selenium concentration in the paired dataset. This observation is consistent with patterns in other studies, in which TTFs are greater at low selenium exposure concentrations (DeForest et al. 2007). **The median TTF of 0.52 based on site-specific data is less than the mean zooplankton TTF of 1.5 in the USGS model, as well as the TTF of 0.9 in the USGS model if the 60% bioavailability factor is applied.** As such and consistent with comments from other SeTSC members on August 25, 2020, TTFs for zooplankton as proposed by the USGS are overly conservative.

For benthic invertebrates, co-located selenium concentrations in particulates are not available. As such, a two-step approach was used to estimate site-specific selenium TTFs for benthic invertebrates. First, benthic invertebrate and surface water selenium samples were co-located. Benthic invertebrate selenium concentrations in these co-located samples range from 0.38 to 9.1 mg/kg dw and surface water selenium concentrations range from 0.15 to 2.4 µg/L (Figure 16A). These co-located data were used to develop bioaccumulation factors (BAFs), which are calculated as the benthic invertebrate selenium concentration divided by the surface water selenium concentration (multiplied by 1,000 to convert to units of L/kg dw). As observed in other selenium BAF datasets (DeForest et al. 2007), there is an inverse relationship between BAFs and water selenium concentration (Figure 16B). This relationship is described by a linear regression model in log-log scale (Figure 16C).

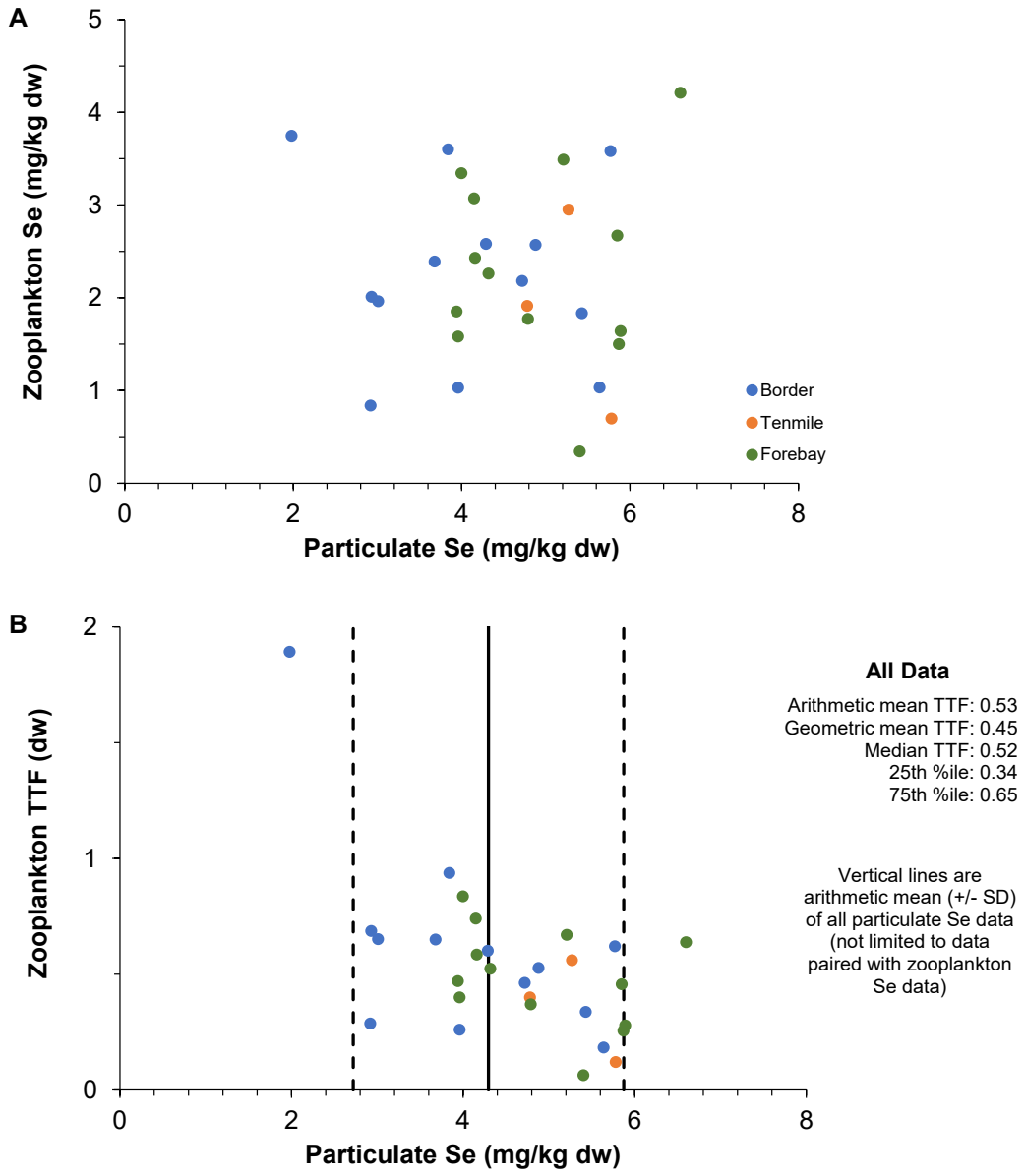

Figure 15. (A) Relationship between selenium concentrations in co-located zooplankton and particulate samples; and (B) relationship between zooplankton TTFs and particulate selenium concentrations.

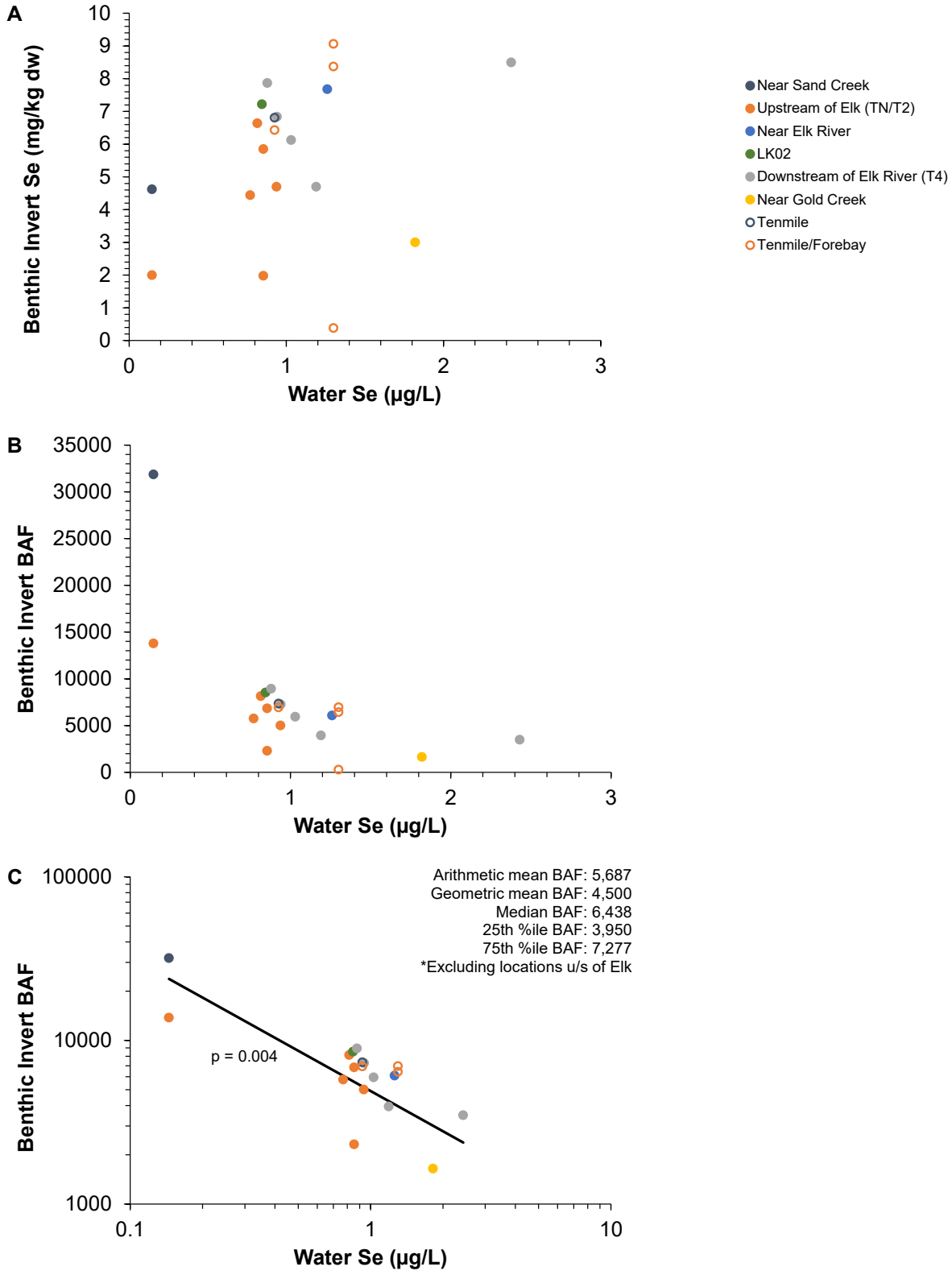

Figure 16. Relationships between (A) co-located benthic invertebrate and water selenium concentrations; and benthic invertebrate BAFs and water selenium concentrations in (B) normal scale and (C) log-normal scale.

The second step was to apply the benthic invertebrate BAF model from Figure 16C to surface water selenium concentrations used to develop the selenium k_d values. Thus, for each water selenium concentration in the k_d dataset, there is a co-located measure of particulate selenium and a co-located estimate of benthic invertebrate selenium (Figure 17A). Benthic invertebrate TTFs were then calculated based on the paired measured particulate selenium concentrations and estimated benthic invertebrate selenium concentrations. Based on the inverse relationship between benthic invertebrate BAFs and surface water, there is also an inverse relationship between estimated benthic invertebrate TTFs and particulate selenium concentrations (Figure 17B). To avoid undue influence of high TTFs at low selenium exposure concentrations and low TTFs at high selenium exposure concentrations, the benthic invertebrate TTF summary statistics were calculated over the range of the mean measured selenium concentrations plus and minus one standard deviation (Figure 17B). The median TTF within this particulate selenium range is 1.1. **The estimated TTF from site-specific data is less than the benthic invertebrate TTF of 2.8 in the USGS model, as well as the TTF of 1.7 in the USGS model if the 60% bioavailability factor is applied.**

Two additional model scenarios were then evaluated using the above-mentioned site-specific data. One model scenario considered the 50th percentile k_d and the 50th percentile TTFs of 1.1 and 0.52 for benthic invertebrates and zooplankton, respectively. The second scenario was more conservative by considering the 75th percentile k_d and 75th percentile TTFs of 1.2 and 0.65 for benthic invertebrates and zooplankton, respectively. Based on the 50th percentile assumption, the water selenium concentrations predicted to result in a whole body selenium criterion of 8.5 mg/kg dw range from 1.4 µg/L (100% fish diet) to 3.3 µg/L (100% zooplankton diet) (first set of blue-highlighted rows in Table 2). Based on the 75th percentile assumption, water selenium concentrations predicted to result in the whole body selenium criterion of 8.5 mg/kg dw range from 1.1 µg/L (100% fish diet) to 2.3 µg/L (100% zooplankton diet) (second set of blue-highlighted rows in Table 2).

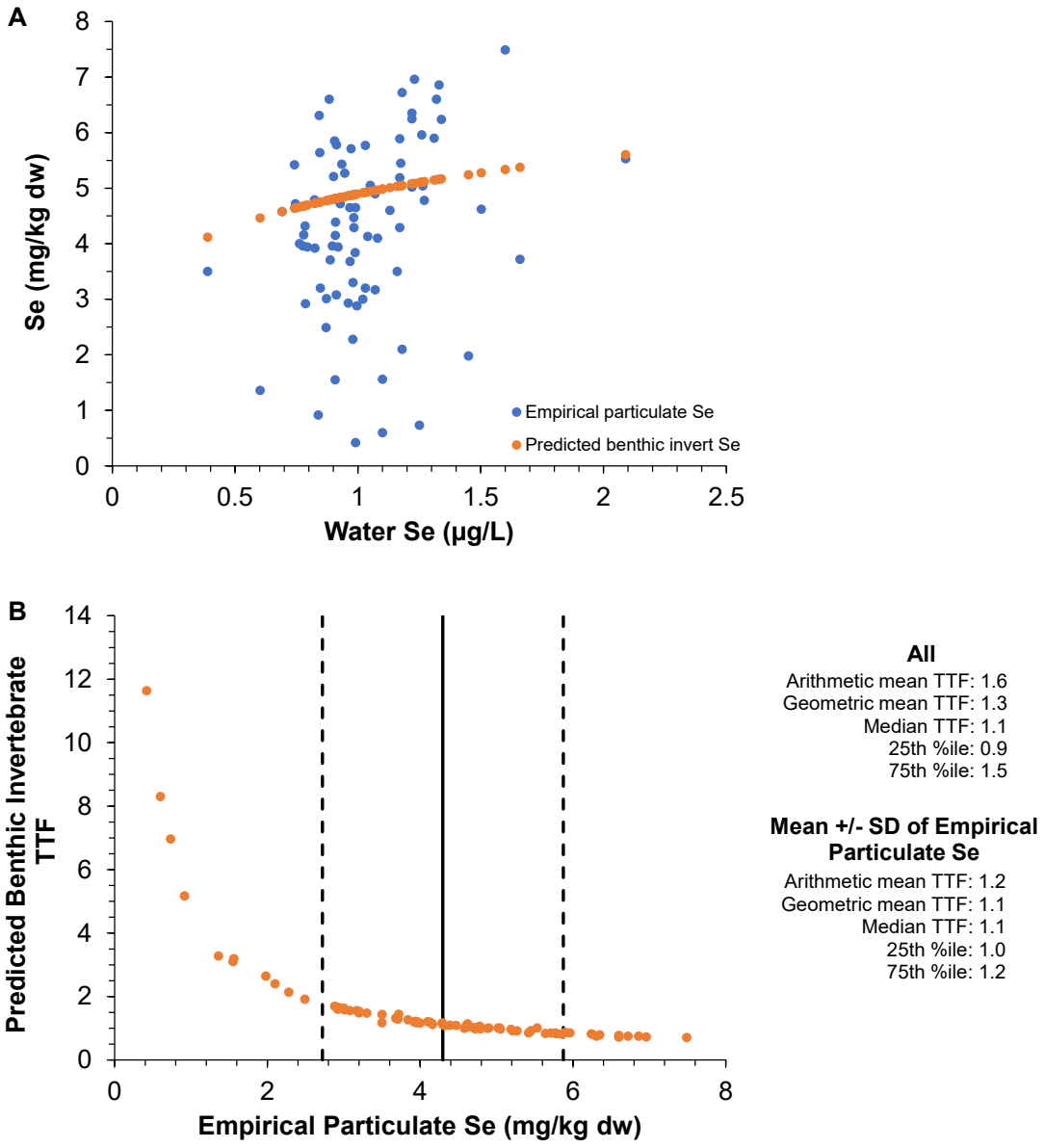

Figure 17. Relationships between (A) empirical particulate Se concentrations and predicted benthic invertebrate Se concentrations; and (B) between predicted benthic invertebrate TTFs and particulate Se concentrations.

3.3 Validation of Models

Empirical fish tissue selenium data for Koocanusa Reservoir were not considered in the development or validation of the selenium model described in Presser and Naftz (2020). It appears that empirical fish selenium data were not considered because it was assumed that the reservoir was currently degraded from the cumulative effects of various stressors and that, by inference, the suggestion appears to be that selenium concentrations in fish in the reservoir are not representative of what would be expected in an unstressed

system. A reservoir is of course a managed system and issues related to drawdown and flow-regime changes, even in the absence of any other stressors, can have a substantial influence on habitat and aquatic communities. In my opinion, consideration of empirical fish selenium data is a critical piece of evidence for evaluating selenium bioaccumulation in the reservoir. In addition, fish selenium data in the reservoir will presumably be important for determining compliance with selenium criteria in the future.

I conducted a validation of the USGS model and alternative input assumptions based on the invertebrate TTF evaluation described in Section 3.2. The first step of the validation process was to first identify paired fish tissue and water selenium concentrations in the reservoir. Fish selenium concentrations were then predicted from water selenium concentrations based on various k_d and TTF assumptions. The selection of TTFs was based on the dietary assumption for the fish species being evaluated in the validation. For example, peamouth chub are assumed to have a diet comprised of 50% benthic invertebrates and 50% zooplankton, so for this fish species the benthic invertebrate and zooplankton TTFs were each given 50% weight.

Validation of four different modeling scenarios (consistent with those described above) was evaluated:

1. USGS model with suspended particulate matter bioavailability fraction of 100%. A k_d of 5,000 L/kg was used for consistency with the model examples provided in Table 10 of Presser and Naftz (2020) (Table 3).

2. USGS model with suspended particulate matter bioavailability fraction reduced to 60% (Table 3).

3. Alternative model inputs based on the 50th percentile k_d from the USGS model and the 50th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

4. A more conservative alternative model with inputs based on the 75th percentile k_d from the USGS model and the 75th percentile TTFs for benthic invertebrates and zooplankton (Table 3).

Table 3. Model scenarios included in the validation.

Scenario	k_d (L/kg dw)	SPM Bioavail-ability Fraction	TTF		
			Insects	Zoo-plankton	Fish
USGS (2020) - Table 10: SPM Bioavailability Fraction = 1.0	5000	1	2.8	1.5	3.1*
USGS (2020) - Table 10: SPM Bioavailability Fraction = 0.6	5000	0.6	2.8	1.5	3.1*
50th %ile site-specific k_d and invert TTFs	4547	1	1.1	0.52	1.2*
75th %ile site-specific k_d and invert TTFs	5268	1	1.2	0.65	1.3*

*Assumes prey fish are feeding exclusively on insects: Insect TTF × 1.1

Predicted fish selenium concentrations were then compared to observed fish selenium concentrations. Comparisons were made using a standard selenium bioconcentration and trophic transfer figure, similar to that provided in Figure 18. This figure format visually shows the transfer of selenium from surface water to particulates, invertebrates, and fish as a function of assumed k_d values and TTFs.

Validation evaluations presented here are based on selenium concentrations in fish muscle and whole body tissue. The validation is focused on these two tissue types because of uncertainties in the ovary selenium data due to the timing of ovary sampling relative to spawning periods and ovary maturity. Further discussion of ovary selenium concentrations and maturity is provided in Sections 2 and 4.

Over the validation scenarios evaluated, measured muscle and whole body selenium concentrations were consistently over-predicted by the USGS model regardless of model assumptions (Figure 19). Based on the USGS model with the 60% bioavailability assumption, predicted fish selenium concentrations were over-predicted, on average, by a factor of 5.0 (Table 4). In most of the scenarios evaluated, this resulted in muscle and whole body selenium concentrations that were predicted to exceed the muscle selenium criterion of 11.3 mg/kg dw and whole body selenium concentration of 8.5 mg/kg dw, while measured fish selenium concentrations were generally less than 50% of criteria concentrations. Even when considering site-specific k_d summary statistics and site-specific invertebrate TTFs the USGS model predicts muscle and whole body selenium concentrations that, on average, are a factor of 2.9 greater than observed (Table 4).

In my opinion, the consistent over-prediction of selenium concentrations in fish tissue is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.

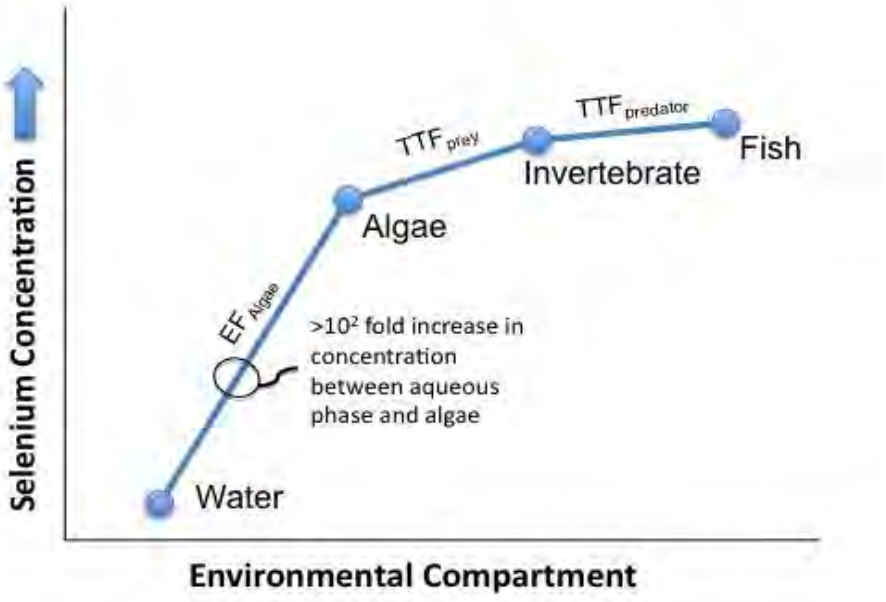

Figure 18. Example of multi-step selenium bioconcentration and trophic transfer plots in a fish food chain.
Source: Stewart R, Grosell M, Buchwalter D, Fisher N, Luoma S, Mathews T, Orr P, Wang W-X. 2010. Bioaccumulation and trophic transfer of selenium. In: Ecological assessment of selenium in the aquatic environment. Pensacola, FL:SETAC Press, 93-139.

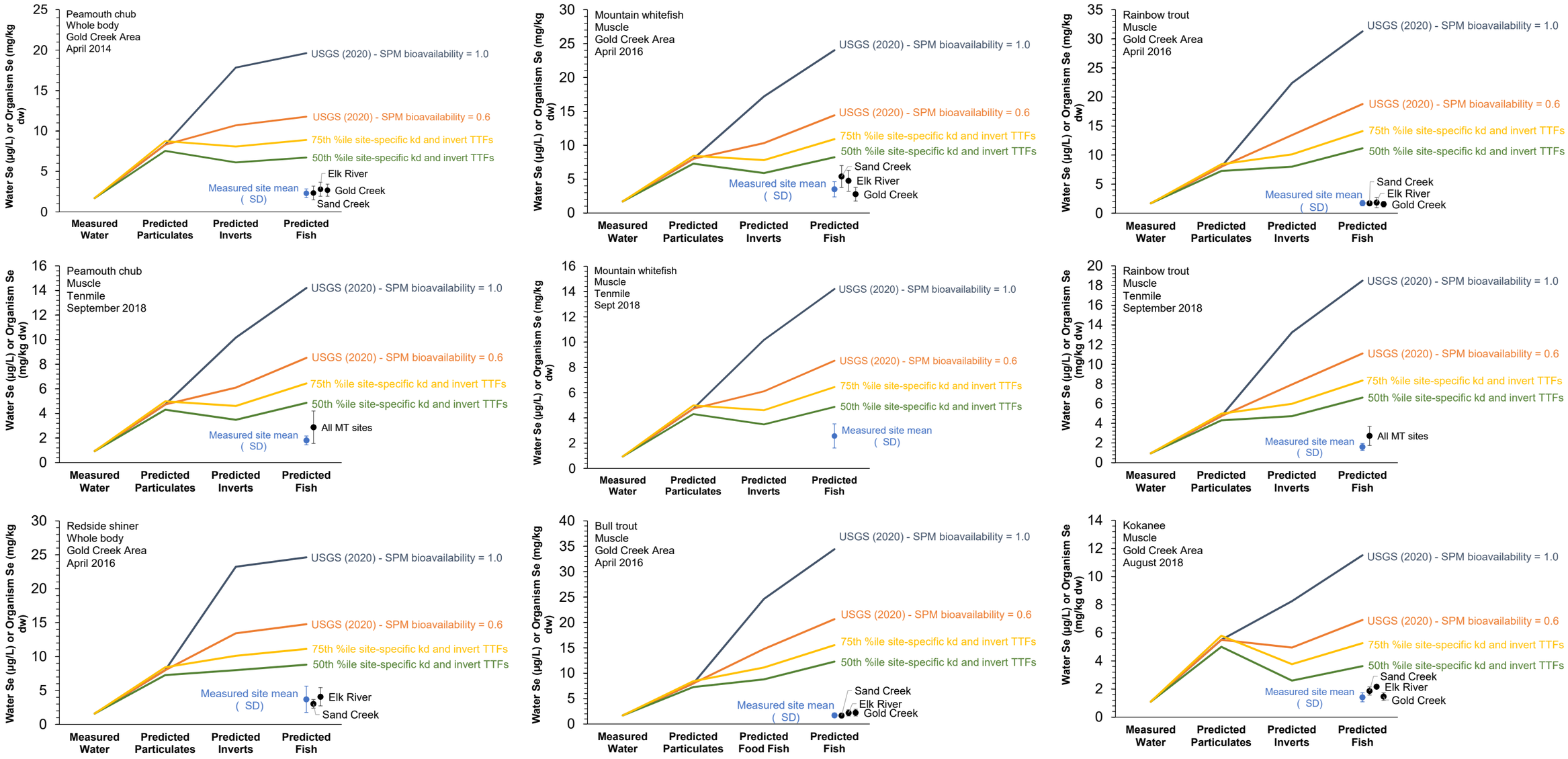

Figure 19. Comparisons of predicted and observed whole body selenium concentrations in Koocanusa Reservoir fish.
Note: See text boxes within each panel for the species, tissue type, sample location, and sampling data.

Table 4. Examples of observed and predicted fish selenium concentrations in Koocanusa Reservoir based on different modeling scenarios.

Site	Scenario	Water Se (µg/L)	kd (L/kg dw)	Predicted Particulate Se (mg/kg dw)	Bioavailability Fraction	TTF			Food Chain Se (mg/kg dw)			Fraction Diet			TTF Target Fish	Predicted Fish Se (mg/kg dw)	Mean Measured Fish Se (mg/kg dw)	SD Measured Fish Se (mg/kg dw)	Predicted-to-Observed Ratio
						Insects	Zoo-plankton	Fish	Insects	Zoo-plankton	Insect-ivorous Fish	Insects	Zoo-plankton	Fish					
Peamouth Chub - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	1	2.8	1.5	1.1	23.2	12.5	25.6	0.5	0.5	0	1.1	19.6	2.3	0.54	8.5
South of Elk (Gold Creek Area)	USGS (2020)	1.7	5000	8.3	0.6	2.8	1.5	1.1	13.9	7.5	15.3	0.5	0.5	0	1.1	11.8	2.3	0.54	5.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.7	4547	7.5	1	1.1	0.52	1.1	8.3	3.9	9.1	0.5	0.5	0	1.1	6.7	2.3	0.54	2.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.7	5268	8.7	1	1.2	0.65	1.1	10.5	5.7	11.5	0.5	0.5	0	1.1	8.9	2.3	0.54	3.9
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	1	2.8	1.5	1.1	3.6	2.0	4.0	0.5	0.5	0	1.1	3.1	1.4	0.30	2.2
North of Elk (Kikomun Area)	USGS (2020)	0.26	5000	1.3	0.6	2.8	1.5	1.1	2.2	1.2	2.4	0.5	0.5	0	1.1	1.8	1.4	0.30	1.3
North of Elk (Kikomun Area)	50th %ile site-specific k_d & invert TTFs	0.26	4547	1.2	1	1.1	0.52	1.1	1.3	0.6	1.4	0.5	0.5	0	1.1	1.1	1.4	0.30	0.8
North of Elk (Kikomun Area)	75th %ile site-specific k_d & invert TTFs	0.26	5268	1.4	1	1.2	0.65	1.1	3.8	0.6	4.2	0.5	0.5	0	1.1	2.5	1.4	0.30	1.8
Peamouth Chub - Muscle																			
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	1.8	0.36	7.8
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	1.8	0.36	4.7
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	1.8	0.36	2.7
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	1.8	0.36	3.6
Mountain Whitefish – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0.5	0.5	0	1.4	24.0	3.5	1.1	6.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0.5	0.5	0	1.4	14.4	3.5	1.1	4.1
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0.5	0.5	0	1.4	8.2	3.5	1.1	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0.5	0.5	0	1.4	10.9	3.5	1.1	3.1
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	0.5	0.5	0	1.4	14.2	2.6	0.96	5.5
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	0.5	0.5	0	1.4	8.5	2.6	0.96	3.3
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	0.5	0.5	0	1.4	4.9	2.6	0.96	1.9
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	0.5	0.5	0	1.4	6.4	2.6	0.96	2.5
Rainbow Trout – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.4	31.3	3.5	1.12	8.9
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.4	18.8	3.5	1.12	5.4
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.4	11.2	3.5	1.12	3.2
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.4	14.1	3.5	1.12	4.0
Tenmile	USGS (2020)	0.946	5000	4.73	1	2.8	1.5	1.1	13.2	7.1	14.6	1	0	0	1.4	18.5	1.6	0.35	11.6
Tenmile	USGS (2020)	0.946	5000	4.73	0.6	2.8	1.5	1.1	7.9	4.3	8.7	1	0	0	1.4	11.1	1.6	0.35	6.9
Tenmile	50th %ile site-specific k_d & invert TTFs	0.946	4547	4.3	1	1.1	0.52	1.1	4.7	2.2	5.2	1	0	0	1.4	6.6	1.6	0.35	4.1
Tenmile	75th %ile site-specific k_d & invert TTFs	0.946	5268	5.0	1	1.2	0.65	1.1	6.0	3.2	6.6	1	0	0	1.4	8.4	1.6	0.35	5.2
Bull Trout - Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	0	0	1	1.4	34.4	2.5	0.91	13.8
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	0	0	1	1.4	20.7	2.5	0.91	8.3
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	0	0	1	1.4	12.3	2.5	0.91	4.9
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	0	0	1	1.4	15.5	2.5	0.91	6.2
Redside Shiner - Whole Body																			
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	1	2.8	1.5	1.1	22.4	12.0	24.6	1	0	0	1.1	24.6	3.7	1.9	6.7
South of Elk (Gold Creek Area)	USGS (2020)	1.6	5000	8	0.6	2.8	1.5	1.1	13.4	7.2	14.8	1	0	0	1.1	14.8	3.7	1.9	4.0
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.6	4547	7.3	1	1.1	0.52	1.1	8.0	3.8	8.8	1	0	0	1.1	8.8	3.7	1.9	2.4
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.6	5268	8.4	1	1.2	0.65	1.1	10.1	5.5	11.1	1	0	0	1.1	11.1	3.7	1.9	3.0
Kokanee – Muscle																			
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	1	2.8	1.5	1.1	15.4	8.3	16.9	0	1	0	1.4	11.5	1.4	0.32	8.2
South of Elk (Gold Creek Area)	USGS (2020)	1.1	5000	5.5	0.6	2.8	1.5	1.1	9.2	5.0	10.2	0	1	0	1.4	6.9	1.4	0.32	4.9
South of Elk (Gold Creek Area)	50th %ile site-specific k_d & invert TTFs	1.1	4547	5.0	1	1.1	0.52	1.1	5.5	2.6	6.1	0	1	0	1.4	3.6	1.4	0.32	2.6
South of Elk (Gold Creek Area)	75th %ile site-specific k_d & invert TTFs	1.1	5268	5.8	1	1.2	0.65	1.1	7.0	3.8	7.6	0	1	0	1.4	5.3	1.4	0.32	3.7

4 Summary and Recommendations

- The USEPA's fish tissue selenium criteria are conservative and protective of fish in Koocanusa Reservoir. These criteria are based on the 5th percentile of genus mean EC10s, which is extrapolated to a concentration that is less than the white sturgeon EC10. There is less uncertainty in the egg selenium criterion (which supersedes the muscle and whole body selenium criteria) due to differences in muscle-to-egg and whole body-to-egg relationships among species, but conservatism in the criteria calculation method ensures that the muscle and whole body selenium criteria are still protective.
- With the exception of cyprinids (which are discussed in the next bullet), there have been just three exceedances of a fish tissue criterion based on over 2,800 measurements in individual fish within Koocanusa Reservoir. These include a rainbow trout and a longnose sucker ovary sample (see Figure 7), and a yellow perch muscle sample (see Figure 8). No whole body selenium concentrations have exceeded the USEPA's whole body selenium criterion (Figure 9). Based on evaluations of ovary selenium concentrations and ovary maturity discussed for cyprinids (as well as mountain whitefish), there is uncertainty in some of the ovary selenium data for other fish species, specifically the potential to over-estimate exposure due to immature ovaries. Despite this uncertainty, most ovary selenium concentration fall well below the USEPA's egg selenium criterion so this uncertainty is unimportant for most of the cases.
- Three cyprinids—northern pikeminnow, peamouth chub, and redside shiner—have had the highest ovary selenium concentrations in Koocanusa Reservoir and these concentration have exceeded the USEPA's egg selenium criterion. As shown in the northern pikeminnow study, however, the elevated ovary selenium concentrations are associated with immature ovaries as samples were not collected at time of spawning. A similar pattern appears to be observed in peamouth chub, while recent studies with redside shiner will soon provide more information on selenium bioaccumulation in redside shiner eggs and effects. These data on cyprinids in Koocanusa Reservoir, coupled with the USEPA's conclusion that cyprinids are not uniquely sensitivity based on evaluations of data from sites in the U.S. with high selenium concentrations, indicates it is unlikely that cyprinids in the reservoir are uniquely sensitive to selenium, and in fact may be relatively insensitive.
- Based on more than 2,800 empirical selenium concentrations in non-cyprinids that exceeded criteria in just three samples, along with an increasing level of understanding of selenium concentrations in cyprinids, there are currently no data to indicate that fish in Koocanusa Reservoir are at risk from selenium under current conditions. Surface water selenium concentrations in the reservoir over the period of fish selenium monitoring have ranged between 1 and 2 µg/L in the BC

portion of the reservoir downstream from the Elk River. Based on consideration of both empirical fish and surface water selenium concentrations, **it is my opinion that the USEPA's lentic criterion of 1.5 µg/L is protective of fish in Koocanusa Reservoir**. Furthermore, fish tissue data should supersede water quality data in terms of monitoring and assessments consistent with the USEPA criterion.

- Additional support that the USEPA's lentic criterion of 1.5 µg/L is provided by the evaluation of alternative model inputs and the model validation relative to empirical fish selenium data. As shown in Figures 19 and in Table 4, even the least conservative model scenario evaluated (use of the 50th percentile k_d and site-specific invertebrate TTFs) consistently over-predicted measured fish selenium concentrations. This conservative model translated the whole body fish selenium criterion of 8.5 mg/kg dw to surface water selenium concentrations of:
    - 1.4 µg/L for piscivorous fish (100% fish diet),
    - 1.5 µg/L for fish with a 100% insect diet,
    - 2.1 µg/L for fish with a 50% insect and 50% zooplankton diet, and
    - 3.3 µg/L for fish with a 100% zooplankton diet.

  In my opinion, the consistent over-prediction of selenium concentrations in fish tissue by the USGS model is in part driven by k_d values that are over-predicting selenium exposure in Koocanusa Reservoir. Even consideration of site-specific invertebrate TTFs, which implicitly accounts for site-specific bioavailability of selenium in particulate samples, results in consistent over-prediction of fish selenium concentrations using the USGS model. Accordingly, the multi-step modeling approach appears to have too much uncertainty to support, by itself, recommendations for a site-specific selenium criterion for Koocanusa Reservoir.
- The USEPA's lentic criterion of 1.5 µg/L would also be protective of white sturgeon in the Kootenai River downstream of the Libby Dam. Current surface water monitoring data for the forebay and in the river downstream of the dam indicate that the selenium concentrations are similar. Egg selenium concentrations in white sturgeon collected from the Kootenai River from 2015 to 2019 have ranged from 3.0 to 5.7 mg/kg dw with an average of 4.1 mg/kg dw (n = 38). Thus, even the maximum egg selenium concentration is just 38% of the USEPA's egg selenium criterion of 15.1 mg/kg dw.

5 CLOSURE

Thank you for the opportunity to provide these comments. Please do not hesitate to contact me (DavidD@windwardenv.com; 206-812-5426) if you have any questions or if there is additional information I can provide.

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ATTACHMENT 1

Evaluation of Selenium Concentrations in Ovary of Northern Pikeminnow (*Ptychocheilus oregonensis*)

FINAL REPORT

EVALUATION OF SELENIUM CONCENTRATIONS IN OVARY OF NORTHERN PIKEMINNOW (*Ptychocheilus oregonensis*)

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Table of Contents

1. INTRODUCTION

Ongoing monitoring of Koocanusa Reservoir indicates ovary Se concentrations from some northern pikeminnow (NPM; *Ptychocheilus oregonensis*) collected from the Canadian side of the Reservoir were above both the 11 mg kg^{-1} dry weight (dw) egg Se guideline established by the British Columbia Ministry of the Environment and Climate Change Strategy (BC ENV, 2014) and the 15.1 mg kg^{-1} dw egg Se criteria established by the U.S. Environmental Protection Agency (USEPA, 2016). However, the sensitivity of NPM to Se is currently unknown and so the ecological risk posed by observed egg Se concentrations is uncertain. Further, historical ovary Se concentrations were collected from unripe fish (i.e., not in spawning condition) and the influence of gonadal maturation stage on egg Se concentrations is uncertain. The following presents results from a study to characterize the influence of gonadal maturation stage, fish size, and fish sampling location on ovary Se concentrations in NPM collected from Koocanusa Reservoir.

Efforts to also conduct a toxicity test evaluating the effects of maternally transferred Se on NPM embryo-larval development were unsuccessful in 2019 due to the inability to collect a sufficient number of female fish in spawning condition. As such, this test is not discussed further in this report.

2. BACKGROUND

Northern pikeminnow are distributed throughout the Pacific drainages as far north as the Nass River drainage in BC, Canada to the Columbia River drainage in the U.S. They are most common along sandy, cobble, gravel, boulder or bedrock shorelines during summer and deeper waters during winter (Scott and Crossman 1973, Coker et al. 2001). Northern pikeminnow are late spring-summer spawners, typically spawning when water reaches 14-18 °C with males generally present in larger congregations on breeding grounds over gravel and cobble shallows (Gadomski et al., 2001). Females may have multiple spawning bouts with more than one male throughout the season. Eggs hatch after 8-10 days at 15-17 °C (Coker et al., 2001; Gadomski et al., 2001; Scott and Crossman, 1973).

Koocanusa Reservoir was formed by Libby Dam, located 30 km northeast of Libby, Montana at river mile 221.9 of the Kootenai River[1]. The reservoir is 145 km long, of which 68 km are in BC, Canada. The predominant source of water to the reservoir is the Kootenay River, of which the Elk River is a tributary. Northern pikeminnow are resident to Koocanusa Reservoir and have been sampled for ovary and muscle Se in BC, Canada and Montana (MT), U.S. over the last 11 years.

1. The Kootenay River is referred to as the 'Kootenai River' in the U.S.

Monitoring data indicate NPM ovary Se concentrations on the MT side of the reservoir have low variability within and across sampling years compared to fish collected from the BC side of the reservoir (Figure 1). Some fish collected on the BC side of the reservoir are above both the BC ENV guideline and the USEPA fish egg Se criteria of 11 and 15.1 mg kg^{-1} dw, respectively. These data also indicate ovary Se concentrations in fish collected from the BC side of Koocanusa Reservoir appear to be significantly ($p<0.05$) higher than those collected from the U.S. side.

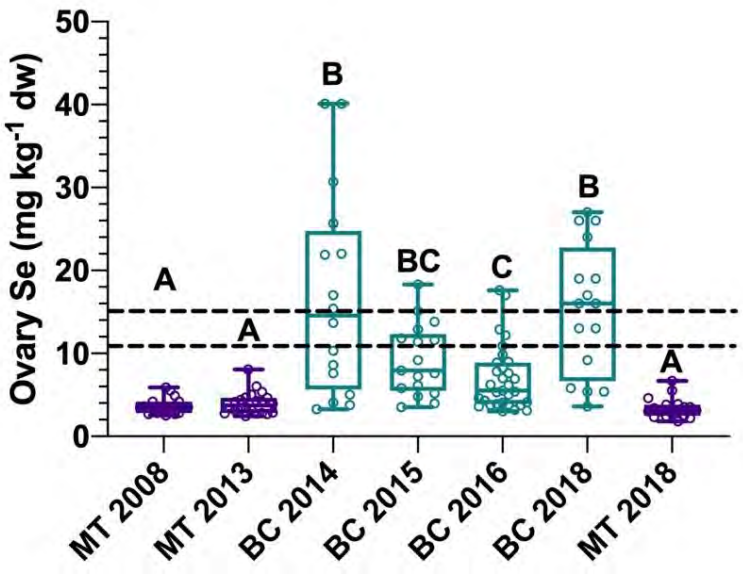

Figure 1. Ovary Se concentrations on the Montana (MT) and British Columbia (BC) sides of Koocanusa Reservoir (2008-2018). Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines. Different letters indicate significant differences ($p<0.05$).

There are several potential biases in the data collected to date that complicate the interpretation of differences in NPM ovary Se data. First, NPM typically reach spawning condition when they have a gonado-somatic index (GSI) of 8-12% (Gray and Dauble, 2001; Petersen and Ward, 1999). While GSI data are not available for fish caught in MT, only a single female on the BC side of the reservoir has been collected with a GSI in this range. The impact of collecting unripe ovaries on observed ovary Se concentrations is unknown, but much of the variability in ovary Se concentrations in the existing BC data is associated with a GSI <2%. Further 55% of ovaries collected from fish with a GSI <2% are above the BC ENV egg Se guideline of 11 mg kg^{-1} dw, while only 7% of ovaries collected from fish with a GSI >2% are above this guideline (Figure 2).

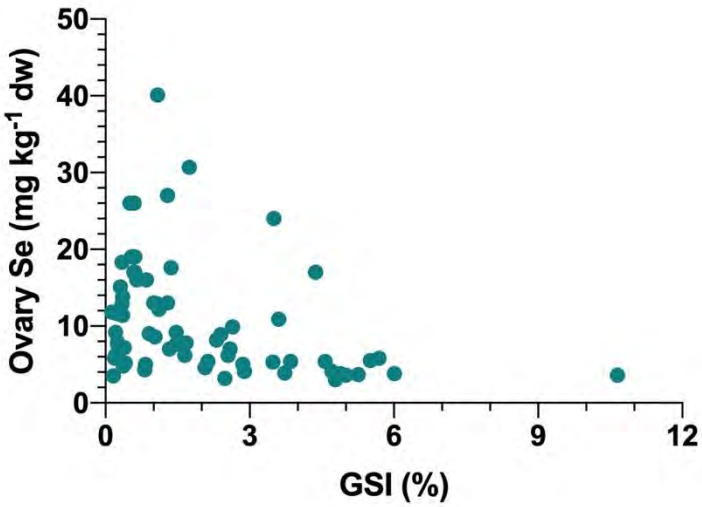

Figure 2. Relationship between ovary Se and GSI for northern pikeminnow collected on the BC side of Koocanusa Reservoir.

Second, there is a significant relationship between fish size and ovary Se concentrations (Figure 3A), and fish collected on the BC side of the reservoir tend to be smaller than those collected in MT (see Figure 3B). Collection of smaller fish on the Canadian side of the reservoir may be the result of sampling bias as fish collection has been restricted to angling, while on the MT side of the reservoir fish are collected using gill nets.

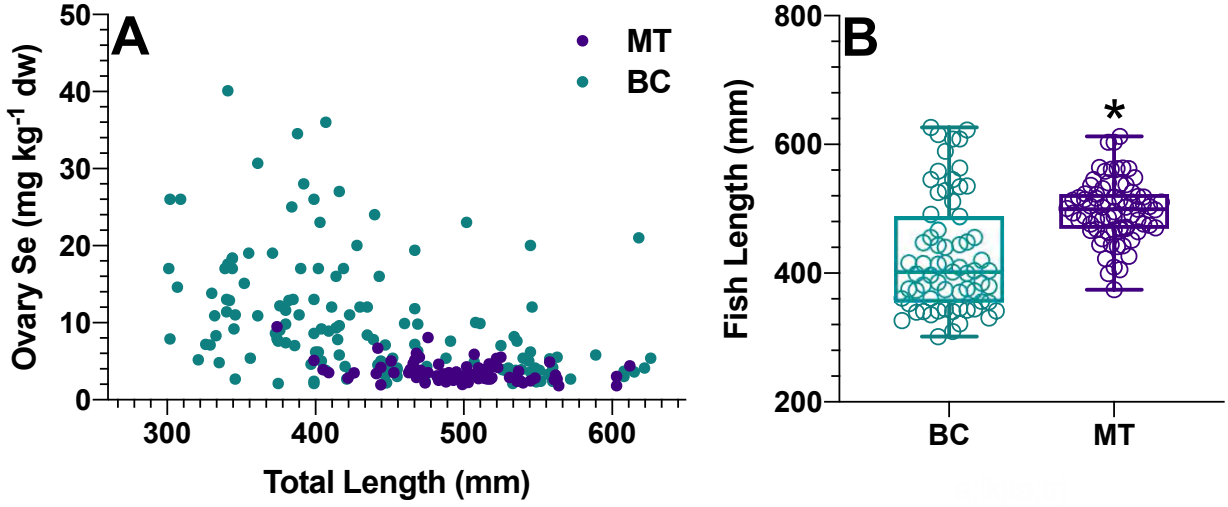

Figure 3. Relationship between ovary Se and fish length (A) and fish length distributions (B) for northern pikeminnow collected from Koocanusa Reservoir. Box plots represent mean, quartiles, maximum and minimum values. * = significant difference in the MT fish length compared to the BC fish lengths ($p<0.001$).

Overall, these observations suggest data collected to date on NPM ovary Se concentrations may be biased. However, this conclusion is uncertain due to the lack of ovary Se data in ripe fish, along with associated size and GSI data. Regardless of potential biases in historical ovary

Se sampling, the sensitivity of NPM to maternally transferred Se is not known. The original objectives of this study were to address both of these uncertainties.

3. OBJECTIVES AND STUDY DESIGN OVERVIEW

This study originally had two objectives:

1) To determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations; and
2) To evaluate the relationship between ovary Se concentrations and ovary development, fish size, and sampling location.

As discussed above, the inability to collect a sufficient number of female fish in spawning condition resulted in the first objective not being achieved. However, the extended effort to collect female fish in spawning condition lead to the collection of a large number (n=79 on the BC side of the reservoir) of samples for ovary Se analysis in support of the second objective. To achieve the second objective, the study had the following key elements:

1) Prior to NPM reaching spawning condition, unripe ovaries/eggs and muscle were collected and GSI measured in sexually mature females (30-60+ cm) to provide information on the relationships between ovary Se, GSI, fish size, and sampling location. As described earlier, historical data indicated fish size and sampling might influence egg Se concentrations, though these potential relationships may be confounded by other variables. The home range of NPM within the reservoir is unknown and so the extent to which ovary Se may reflect exposure to local Se sources (e.g., the Elk River) is also unknown. The developmental stage of a subset of ovaries were also characterized using histological techniques.
2) Attempts were made to collect a gradient of egg Se concentrations from ripe fish by collecting adult NPM of varying size (30-60+ cm) from several locations in Koocanusa Reservoir. This was ultimately unsuccessful but led to the collection of an increased number of ovary samples for Se analysis.

4. FIELD SAMPLING

Details of the field sampling strategy and methods employed are provided in the NPM Study Plan (EcoTox et al., 2019) and summarized here.

4.1 Sampling Strategy

There were originally two phases to the NPM field sampling program. In Phase 1 (beginning June 14, 2019), female NPM were collected from the BC side of the reservoir prior to reaching spawning condition to further characterize the effects of GSI, fish size, and sampling location on ovary Se concentrations as well as monitor spawning condition of the fish. Phase 2 sampling was

intended for once NPM reached spawning condition, and would involve collecting both male and female fish for the Se toxicity study. As only a few ripe fish were collected, Phase 2 sampling was never realized.

Although not explicitly part of this study, there was also an additional NPM sampling effort on the Montana side of the reservoir. In this effort (May 15, 2019), 15 female NPM were collected from Rexford in collaboration with personnel from Montana DEQ. This effort was made to ensure GSI data were collected and they represent the only fish from the Montana side of the reservoir for which GSI data are available.

Mature NPM were collected from various locations in Koocanusa Reservoir (BC side) using multiple sampling methods, consistent with scientific fish collection permit conditions and detailed in the NPM Study Plan (EcoTox et al., 2019). Six locations in Koocanusa Reservoir were initially identified in the study plan, but ultimately 10 locations were sampled in an attempt to collect additional females in spawning condition for the Se toxicity study (Figure 4). Sampling in these areas focused on inlets based on the assumption that NPM would congregate in these areas prior to moving upstream to spawn.

4.2 Sampling Methods

Northern pikeminnow were captured using multiple methods subject to and consistent with fish collection permit conditions. Short-set gill nets (starting with a maximum set time of 20 minutes) were used to reduce fish mortalities (Buchanan et al., 2002). Gill netting was anticipated to be the most efficient capture method and both cotton and monofilament 3-5” mesh nets were deployed. Short set times were used to avoid stress to both NPM and by-catch, particularly as species of concern, bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Onchorhynchus clarki lewisi*) are present in the reservoir. Three foot-diameter hoop nets were deployed and left to fish overnight (i.e., approximately 24 h). Cod pot traps were an additional capture method used, but not originally considered in the study plan. They function similarly to a minnow trap but on a larger scale (65” long x 40” wide, with 4” opening). These traps sit on the bottom substrate similar to the hoop net but sample a smaller area. Cod traps are quicker to deploy and pull; but are more difficult to transport as the metal frame cannot be collapsed. Similar to hoop nets, these traps were deployed and left to fish overnight (approximately 24 h). Angling was conducted from sampling boats. Angling was mainly employed between gill net sets as it has much lower catch per unit effort (CPUE) and often targets smaller individuals. Angling was also employed to scout the lower Elk River below the Elk River bridge at Kootenay Hwy 93. All fish captured were identified to species, enumerated, and all non-target individuals were released alive at the point of capture.

Northern pikeminnow sampled during Phase 1 were sacrificed by a decisive blow to the head. Fish processing and handling for tissue sampling was consistent with provincial guidelines (BC ENV, 2016).

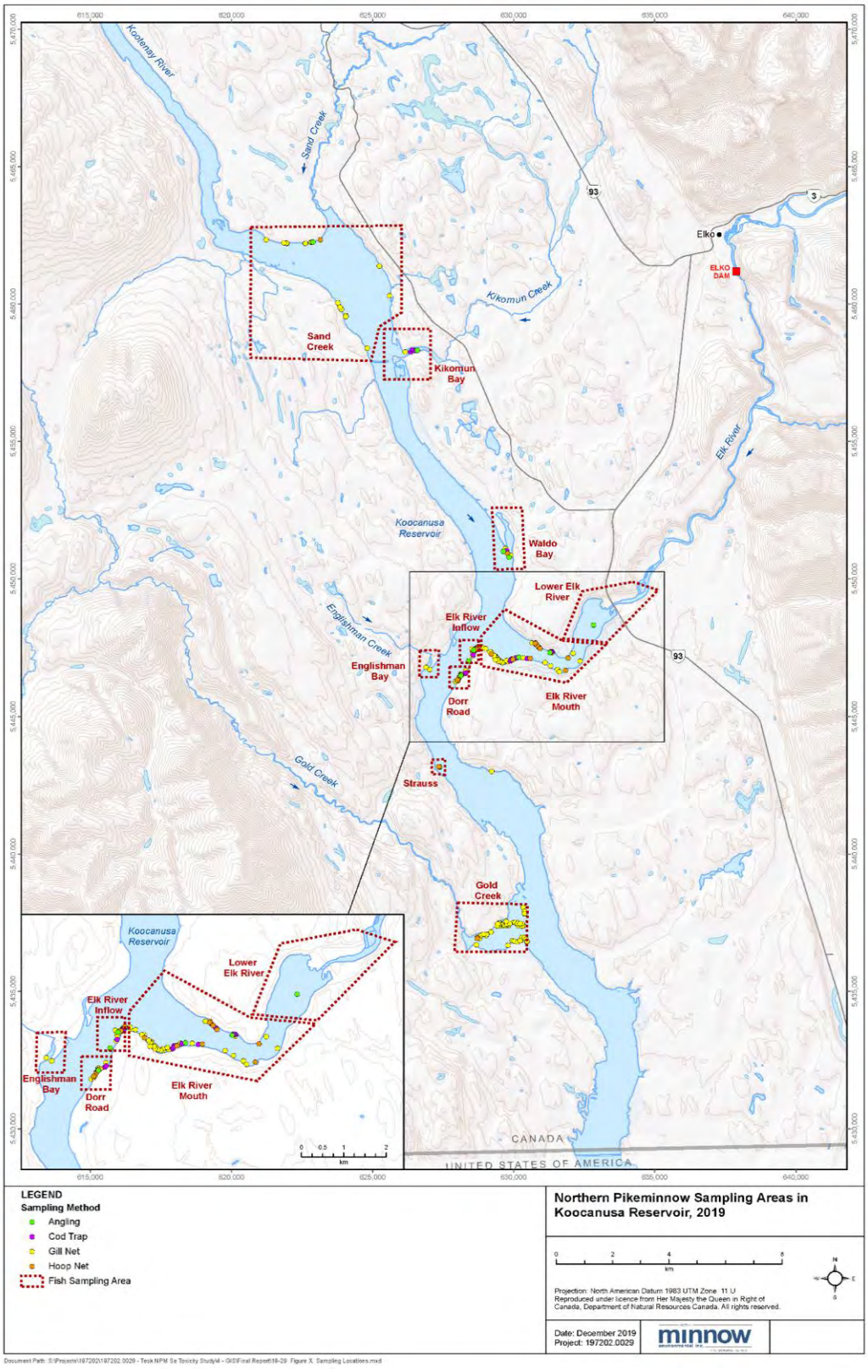

Figure 4. Northern Pikeminnow Sampling Areas on the Canadian Side of Koocanusa Reservoir.

Fish were kept on ice in coolers and transported to a dedicated field laboratory for processing as soon as possible following capture (i.e., within hours). Fork and total lengths and body weights were measured. Each fish was opened and the sex and/or sexual maturity recorded. Whole gonads and livers were removed from each fish and weighed to the nearest milligram using an analytical balance to allow for calculation of gonado-somatic indices. Whole ovaries were collected from each female and placed in separately labelled, polyethylene (Whirl-Pak®) bags. A skinless, boneless muscle fillet sample was also collected from each fish to provide supplemental data on muscle Se concentrations. Following these measures, age structures (i.e., otoliths) were removed from each fish. Each age structure was wrapped separately in waxed paper and placed inside a labelled envelope and archived for analysis. Internal or external deformities, erosions (fin and gill), lesions, or tumours (DELT) observed during processing (Sanders et al., 1999) and parasites were recorded. Tissue samples (ovaries, muscle, and age structures) were stored frozen pending shipment to the respective laboratory for analysis.

Mature female NPM retained for gonad and muscle collection were weighed and measured for total and fork length. Obvious external deformities, erosions (fin and gill), lesions, and tumours (i.e., DELT survey) and parasites observed during processing were recorded.

Ovary and muscle samples were all sent to Saskatchewan Research Council (SRC) in Saskatoon, SK for chemical analysis.

4.3 Permits

A permit for fish collection was obtained from the BC Ministry of the Environment and Climate Change Strategy (BC ENV *Application to Collect Fish for a Scientific Purpose*) and an additional permit was obtained for transport of eggs to the University of Saskatchewan (UoS) facility in Saskatoon, SK from Fisheries and Oceans Canada (License #119412), BC ENV (License #119412) and the Government of Saskatchewan (SK Import #2019-16).

5. LABORATORY METHODS

5.1 Ovarian Histology

All methods for histology preparation followed the UoS Toxicology Centre's draft standard operating procedure for histology (Appendix A). Field-collected NPM were dissected at the field laboratory and gonads excised, weighed and then immediately preserved in 10% buffered formalin. After 24 hours samples were transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming

table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

As, to the best of our knowledge, no previous studies have characterized NPM gonads histologically, oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish (OECD, 2009). Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

5.2 Analytical Chemistry

Ovary and muscle samples collected in the field were submitted for chemical analysis at SRC in Saskatoon, SK. In addition to Se, ovaries, eggs, and muscle were analyzed for 24 other elements (listed in Table 5 of the Study Plan). Results for these other elements are provided in Appendix C but are not discussed further in this report. Samples were analyzed using high resolution inductively coupled plasma mass spectrometry (HR-ICPMS). The detection limit for Se was 0.01 $\mu g\ g^{-1}$ dw. Moisture content was measured by freeze drying and results were reported on a dry weight basis along with moisture content to allow conversion to wet-weight values.

Standard quality control procedures for sample analysis were included as detailed in the Study Plan (EcoTox et al., 2019).

6. RESULTS

6.1 Fish Sampling

Four different methods were employed to capture NPM during the study: hoop nets, cod pot traps, gill nets and angling. The fish sampling was separated into 2 phases. The goal of Phase 1 was to sample approximately 36 sexually mature females of varying sizes and ranges of gonadosomatic index (GSI) values, with half being from sites directly influenced by the Elk River. This phase also allowed tracking of spawning condition within the population. When ripe fish were initially collected, Phase 2 sampling was initiated to focus on collecting fish for fertilization and assessment of larval deformities as a function of egg Se concentrations.

Different sampling methods had varying degrees of success in catching mature females and CPUE changed through the sampling period (Table 1). Monofilament gill nets were more successful than cotton mesh gill nets so after approximately two weeks remaining gill net sampling only used 3” and 4” monofilament nets. Overall, gill nets were the most successful capture method for mature females over the longest sampling period. Though gill nets had high

incidence of bycatch, survival rates were high (4 mortalities over 92 hours of effort in 9 sample areas) due to short set times. Elk Mouth, Elk Inflow and Gold Creek sites were sampled with greatest effort over the longest periods of time in response to capture success rates. Gold Creek and Elk Mouth, both at locations of tributary inflow, had the highest CPUE through the last weeks of June and tended to decrease through July. Elk Inflow area, where the Elk tributary inflow opens into the reservoir, had peak CPUE through mid-July then drastically declined moving into the last two weeks of July (Table 2).

Table 1. Female northern pikeminnow CPUE and total effort (hours) by gear type and sampling area.

CPUE	Dorr Rd.	Elk Inflow	Elk Mouth	Lower Elk	Sand Cr.	Gold Cr.	Englishman Bay	Strauss	Waldo Bay	Kikomun Bay	Total Gear CPUE	Total Gear Effort
Gill Net	5.24	60.00	55.34	-	7.78	136.61	0.00	8.11	0.00	-	273.08	
Gill Effort	7.68	18.00	19.47	-	10.85	31.45	1.70	1.50	1.20	-		91.85
Hoop Net	20.20	0.04	1.47	-	0.17	0.04	-	0.04	0.34	-	22.30	
Hoop Effort	596.25	67.68	492.08	-	46.43	93.50	-	95.18	117.37	-		1508.50
Cod Trap	0.43	0.19	0.25	-	-	-	-	-	0.00	0.04	0.91	
Cod Effort	238.43	237.76	420.62	-	-	-	-	-	24.00	99.42		1020.23
Angling	0.25	4.00	0.00	0.00	0.67	-	-	-	0.00	0.00	4.92	
Angling Effort	4.00	9.83	0.07	6.00	1.50	-	-	-	0.66	1.00		23.06
Total CPUE	26.12	64.23	57.06	0.00	8.62	136.65	0.00	8.15	0.34	0.04	**301.21**	
Total Effort	846.36	333.27	932.24	6.00	58.78	124.95	1.70	96.86	143.23	100.42		**2643.64**

Table 2. Gill net CPUE at most successful sample areas through the sampling period.

CPUE	Elk Inflow	Elk Mouth	Gold Creek
June	18.00	35.20	62.71
July 1-7	Not sampled	15.00	57.40
July 8-14	128.96	5.14	Not sampled
July 15-21	9.50	0.00	8.05
July 22-26	0.00	0.00	8.44

When sampling commenced many mature females had higher GSI than anticipated and there was difficulty capturing low GSI/large individuals and high GSI/small individuals. More fish were processed in an effort to capture the desired range of GSI and size. As the field season progressed and few ripe fish were captured, more fish were processed than originally anticipated with a total of 79 fish processed by end of the study (Table 3). This allowed inclusion of a greater GSI and size range as well as a range of egg development for histology analysis (15 fish). The high GSI/small size categories were eventually captured at Elk influenced sites but not at other sites (Table 3 and Figure 5).

Table 3. Fish GSI and total length for Rexford, Elk River and all other sites combined.

GSI/SIZE	300-400 mm	401-500 mm	501-600+ mm
Rexford, MT (n=15)			
<2%	2	6	0
2-4%	0	0	7
4-7%	0	0	0
7+%	0	0	0
Elk River, BC (n=49)			
<2%	3	6	1
2-4%	3	3	2
4-7%	2	3	3
7+%	3	11	9
Other Sites, BC (n=30)			
<2%	9	6	1
2-4%	0	2	1
4-7%	1	2	1
7+%	0	3	4

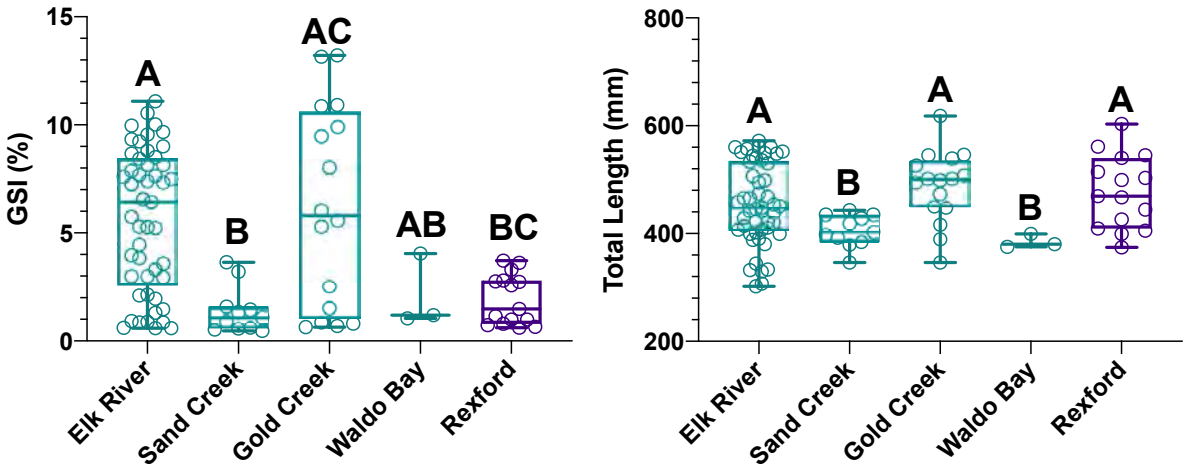

Figure 5. Northern pikeminnow GSI and total length by location for samples collected in 2019. Different letters indicate statistically significant differences ($p<0.05$) as determined by ANOVA.

6.2 Ovarian Histology

Between July 8 and 19, 2019, ovaries from 15 NPM were collected for histological analysis of maturation stages of oocytes across fish of different sizes and reproductive development (weight: 250 – 1800 g; fork length: 33.2 – 61.8 cm) and GSI (range: 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (Figure 6 and Table 4-1 in Appendix A). While there was no obvious relationship between the size of fish and GSI, there was a clear correlation between GSI and ovarian maturation stage (Figure 7) with fish having GSI >5% all being at oocyte maturation stage 3, with one exception. Similarly, there was a significant linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($r^2 = 0.81$), revealing that ovaries of mature fish with a GSI > 5% consisted of over 50% LVOs (Figure 6).

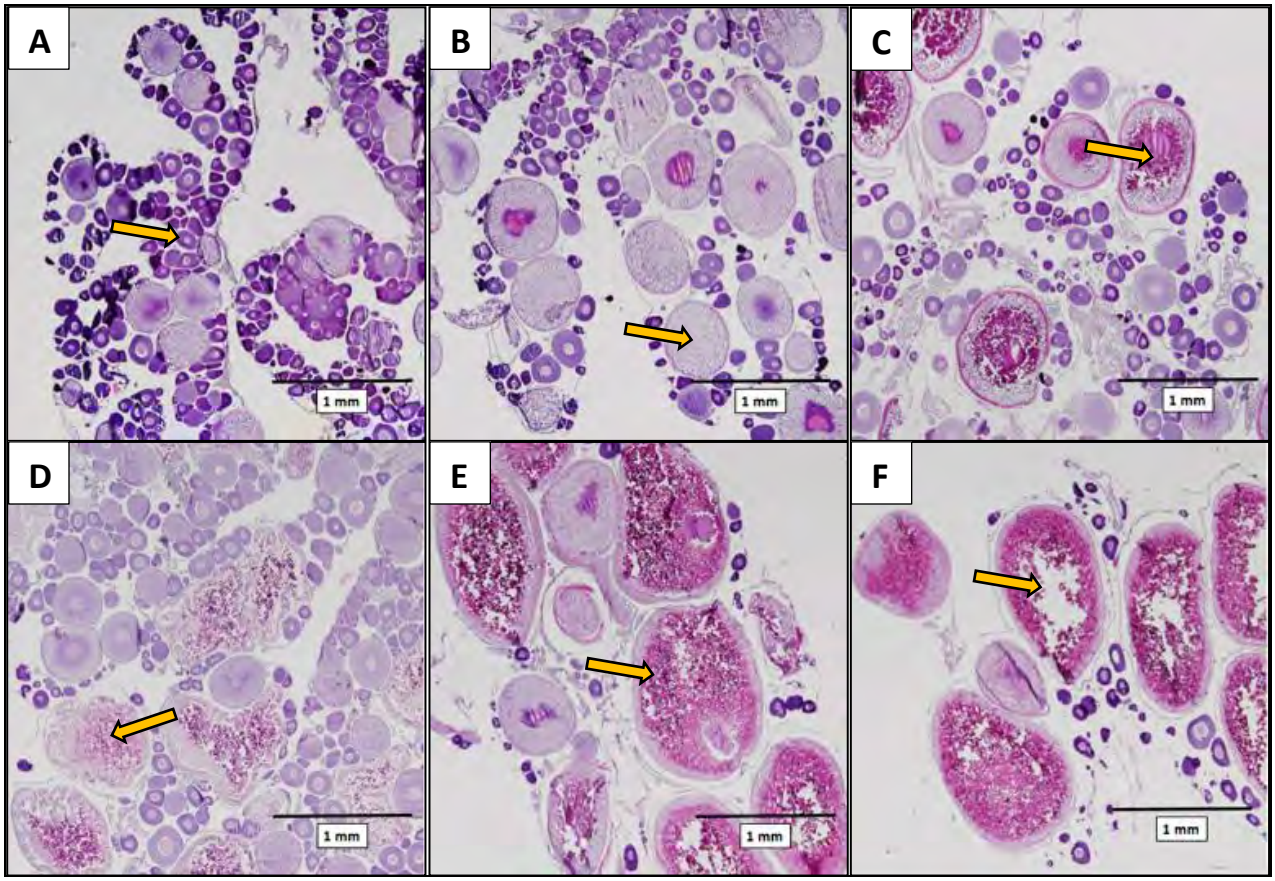

Figure 6. Histomicrographs of ovaries of northern pikeminnow representing early development stages. (Stage 1) consisting mainly of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E and F; Arrows).

Multivariate Analysis of Ovary Se Data

Analysis of historical ovary Se data for NPM suggests there are significant relationships with sampling location, fish size, and GSI (Figures 1-3). However, the historical data set lacks information on GSI for fish from the Montana side of the reservoir, may be confounded by correlations between fish size and GSI, and is limited for fish with a relatively high GSI (>5%). The sampling program for this study was designed to address these limitations and provide a robust dataset for evaluating the influence of multiple factors on ovary Se concentrations in NPM.

Historical sampling data (collected 2013-2018) were collated with data collected from 2019 (Appendix B). An initial exploratory analysis of natural log (ln)-transformed data was conducted by Principal Component Analysis (PCA) (prcomp, R) using z-scores of independent variables (total length, GSI, body weight, and gonad weight) to identify correlations among these variables and select the most appropriate variables for linear

modeling. The first two axes of the PCA with four input variables explained 99% of the variance in the four variables. Bivariate relationships among independent variables, and bivariate relationships between ovary Se and independent variables were plotted by area and year to help visualize effects of area and year on relationships. Natural log (ln) transformations of total length and body weight were highly correlated (R = 0.98), and a biplot from the first PCA with all four variables showed very similar relationship between body weight and total length and final PC scores. Because GSI includes body weight in its denominator, and body weight and total length were highly correlated, body weight was removed from the independent variables used in the MLR and total length was used as a measure of fish size in the model to reduce collinearity (Figure 8).

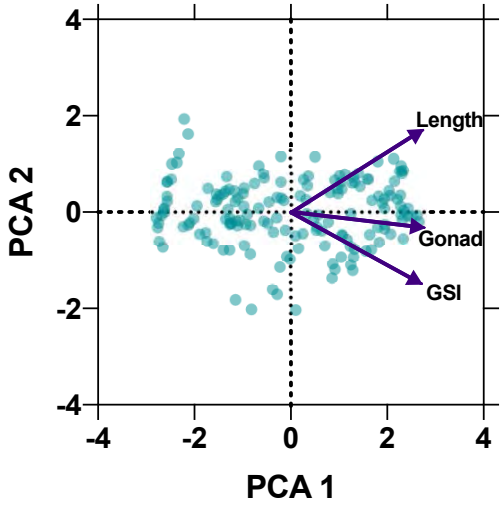

Figure 8. Biplot from PCA using z-scores of ln-transformed total length, gonad weight, and GSI.

After selecting initial independent variables (length, gonad weight, and GSI), exploratory linear and multiple linear regressions (MLR) were conducted to predict ln ovary Se for various subsets of the data. For example, models using one or more independent variables were developed for data sets for individual years, different combinations of years, individual locations, and different combinations of locations. These exploratory analyses were intended to gain a better understanding of how the data were distributed as a function of the independent variables, location, and sampling year. Based on these exploratory analyses, we concluded that the initial models should be developed using only the 2019 data because these data had been collected with a more balanced design of GSI and fish size classes than earlier data. Developing an initial model based on data from multiple years could introduce biases due to the incomplete sampling design with respect to the independent variables being evaluated.

The first model was developed to test for differences between area-specific slopes and intercepts with stepwise analysis using Bayesian Information Criterion (BIC) and to identify final models (Eq. 1). The contrasts used to test for area-specific intercepts tested for differences between Elk influenced sites and other sites.

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) +

Ln(GW) + area* Ln(GW) (Eq. 1)

where, OvSe = ovary Se, TL = total length (cm), GSI = gonadosomatic index, and GW = gonad weight. Variance inflation factors (VIFs) were relatively high for this model (>7) (Zuur et al., 2010) and gonad weight was not retained in the BIC version of the model, so gonad weight was removed and a second model was developed (Eq. 2).

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) (Eq. 2)

Area-specific slopes were not retained in the BIC model, resulting in a final model with area-specific intercepts and pooled slopes. Exclusion of area-specific slopes means that relationships between independent variables (total length and GSI) and ovary Se are statistically similar between sites. Retention of area-specific intercepts indicates that while differences in fish size and GSI between sites explains some of the observed differences in ovary Se concentrations, there are also statistically significant differences in ovary Se concentrations between some sites independent of the influence of fish size and GSI. This model performed reasonably well in terms of predicting ovary Se concentrations for the data on which it was based (Adj. R^2 = 0.72; Figure 9). Further, the predicted R^2 of 0.69 is just slightly lower than the adjusted R^2 of 0.72, indicating the model is not over-parameterized or unduly influenced by individual data points.

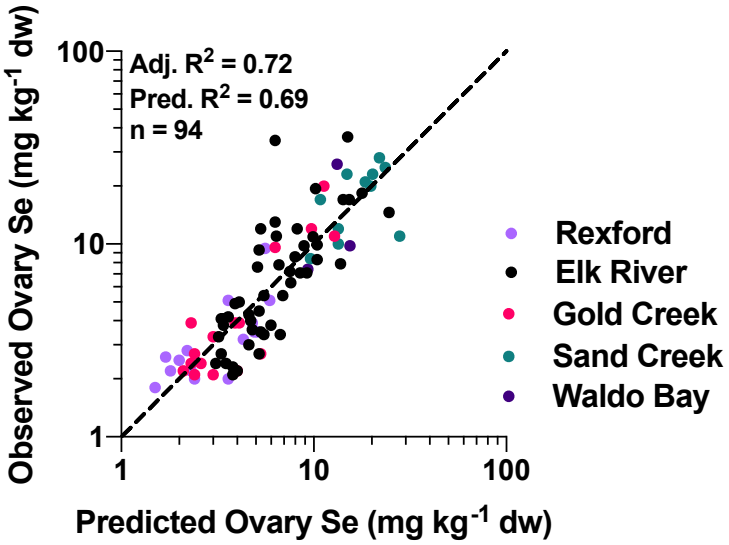

Figure 9. Ovary Se MLR model based on 2019 data only (see Eq. 2).

Once the MLR model based on data from 2019 only was developed, data from years prior to 2019 were then evaluated using the 2019 MLR model. Exploratory analyses indicated that data from 2014 and 2015 did not fit the model well. The majority of samples for 2014 were collected in February with the remaining 2014 samples and all 2015 samples collected in April. As would be expected given the sampling times, GSI was low in both data sets. The 2015 data set in particular consisted of fish with GSI <0.5%, which appears to introduce non-linearities into the overall relationship between GSI and ovary Se (Figure 10). Consequently, we opted to exclude the 2015 data from further analysis. It may have been possible to include the 2014 data set in the model, but given the limited amount of data (n=5) and limited ranged of GSI, we opted to exclude it from the analysis as well. Consequently, a model was fit using data from 2016-2019. The full model included "year" as a term to test for differences in ovary Se concentrations between years and as before was evaluated using BIC to select the most parsimonious variables for inclusion (Eq. 3).

$$\text{Ln(OvSe)} = \text{area} + \text{year} + \text{Ln(TL)} + \text{area*}\ \text{Ln(TL)} + \text{Ln(GSI)} + \text{area*}\ \text{Ln(GSI)} \quad \text{(Eq. 3)}$$

The final model selected by BIC using all data from 2016-2019 (n=141) retained the same variables as the model using only 2019 data with only slight differences in the model coefficients (Table 4). Adjusted and predicted R^2 for the BIC model were 0.67 and 0.65, respectively (Figure 11). The model tested for effects of year and area as well as area-specific slopes. Again, area-specific slopes were not retained in the BIC model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (ovary Se) across sites. Similarly, year was not retained as a factor in the model indicating there were no significant differences across the three sampling years (2016, 2018, and 2019) included in the analysis. Significant differences in area-specific intercepts were observed and retained in the model. The intercepts for both Gold Creek and Rexford were significantly lower than the Elk intercept ($p = 0.01$ and $p < 0.01$, respectively) indicating that after accounting for the influence of fish length and GSI, ovary Se concentrations in fish collected from Gold Creek and Rexford were significantly lower than those observed for fish collected near the Elk River (Table 4).

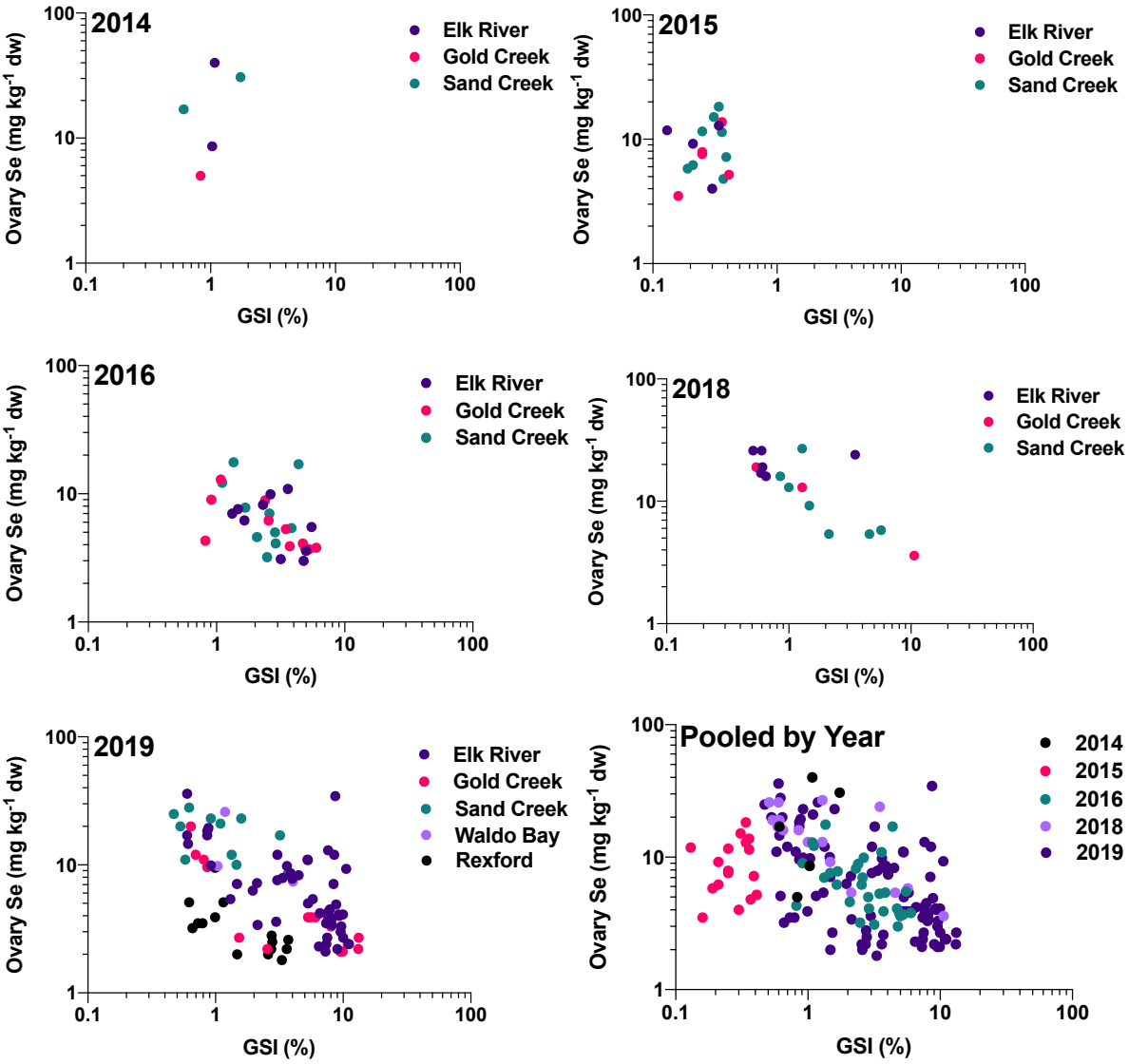

Figure 10. Relationship between GSI and Ovary Se by Year

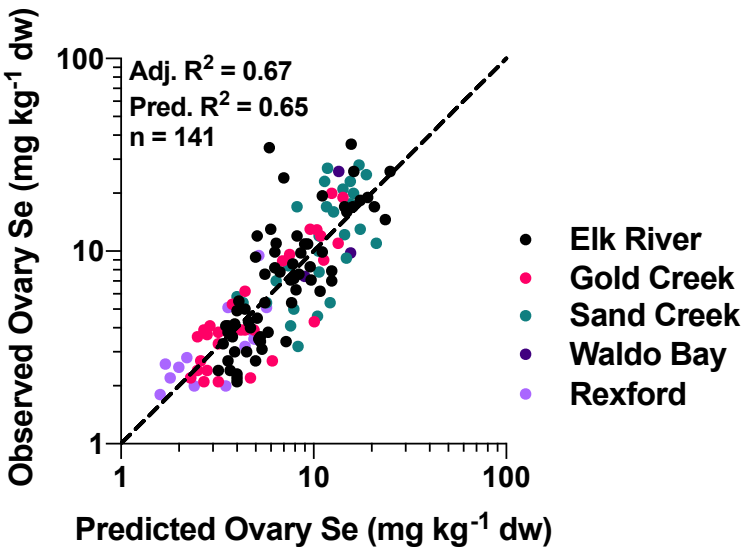

Figure 11. Final Ovary Se MLR model based on 2016-2019 data.

Table 4. Final ovary Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	7.94	0.96	8.31	-	
	Gold	7.66	0.10	-2.81	0.01	
	Sand	7.98	0.10	0.45	0.65	
	Waldo	8.02	0.26	0.33	0.75	
	Rexford	6.91	0.13	-7.62	<0.01	
Slopes	Ln Total Length	-1.45	0.26	-5.62	<0.01	-0.289
	Ln GSI	-0.39	0.05	-8.08	<0.01	-0.493

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that GSI (standardized slope = -0.49) has a stronger effect on ovary Se concentrations in the model than total length (standardized slope = -0.33) over the range sampled for these variables (Table 4). Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model appear to have equal variance (p = 0.145) but may not be normal (p = 0.031).

One potential caveat to this model is that the PCA analysis indicates a level of correlation between total length and GSI, as both variables have positive associations for PC1, though opposite associations for PC2 (Figure 8). A simple correlation analysis indicates these two variables are somewhat correlated (r=0.41; Figure 12). This correlation is primarily caused by the lack of data for fish with a total length >54 cm and GSI <3%. This observation is supported by the lack of a significant correlation (p >0.05) between total length and GSI for fish with a GSI >3%. It is unclear whether this data gap is due to sampling bias or some

mechanistic reason why fish in this category are not observed, though the former seems more likely. Regardless, this correlation introduces some uncertainty into the ovary Se model. While VIFs for the model were low (1.4 for both total length and GSI) suggesting the correlation is not unduly influencing the model, the full influence of this correlation can be difficult to characterize.

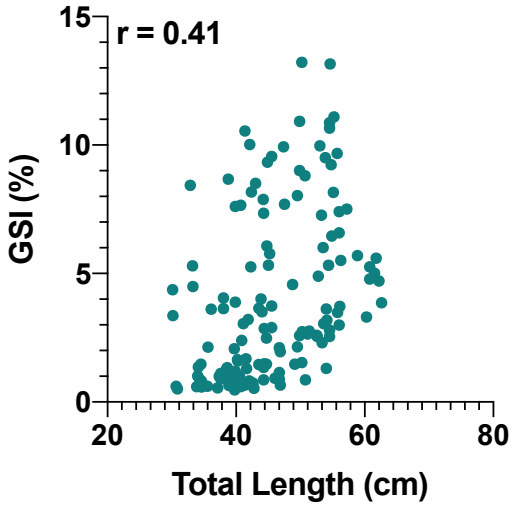

Figure 12. Correlation between total length and GSI in northern pikeminnow (2016-2019).

We further evaluated this issue by constraining the data set to only those data with GSI >3% (n = 69) where there is no correlation between fish length and GSI and re-parameterized the model. The resulting MLR model still retained both GSI and total length as variables (Adj. $R^2 = 0.55$), again supporting the premise that both variables are important predictors of ovary Se. However, the standardized model coefficients changed with total length (-0.49) now more important than GSI (-0.35). This reversal in relative importance of standardized model coefficients may simply be the result of constraining the original data set by ~50% or it could be an indication that the correlation between total length and GSI in the full data set is influencing the way variance is partitioned in the model.

Ultimately, the uncertainties associated with the correlation between total length and GSI appear to have relatively modest impacts on model predictions of ovary Se. Based on the final model using the full data set, differences in area-specific intercepts between sites would translate to predictions of ovary Se concentrations being, on average, 2.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold Creek and Elk River ovary Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.3 times higher than those from Gold Creek for a given fish length and GSI. Estimated mean ovary Se concentrations for small (30 cm) and large (60 cm) females with a

GSI of 6% (a conservatively low GSI for female fish ready to spawn) are below the BC ENV egg Se guideline (11 mg^{-1} kg dw) at all sampling locations using the MLR model based on the full data set. In comparison, the MLR model based on the data set constrained to a GSI >3% (i.e., the data set with no correlation between total length and GSI) provides higher estimates of mean ovary Se for small (30 cm) fish (Table 5), but estimates are generally within 30% of those using the MLR model based on the full data set. The somewhat larger increase in estimated mean ovary Se for Sand is the result of a higher intercept using the constrained data set.

Table 5. MLR model estimated mean ovary Se concentrations in female northern pikeminnow collected from different locations in Koocanusa Reservoir as a function of fish size and GSI. Estimated provided for the MLR model based on all data and the model based only on data where GSI was >3%.

Site	Fish Length (cm)	GSI (%)	Estimated Mean Ovary Se (mg kg^{-1} dw) All Data	Estimated Mean Ovary Se (mg kg^{-1} dw) Data with GSI >3%
Elk River	30	6	10.1	11.6
	60	6	3.7	3.5
Gold Creek	30	6	7.6	9.3
	60	6	2.8	2.8
Sand Creek	30	6	10.5	15.0
	60	6	3.8	4.6
Waldo Bay	30	6	10.9	9.0
	60	6	4.0	2.7
Rexford	30	6	3.6	5.0
	60	6	1.3	1.5

6.4 Multivariate Analysis of Muscle Se Data

Concurrent with ovary sampling, muscle samples have also been collected and analyzed for Se concentrations. The muscle Se data is a potential second line of evidence to support the observations and conclusions from the ovary Se analysis. As has been demonstrated in other species (USEPA, 2016), ovary Se and muscle Se concentrations in NPM are correlated (Figure 13). Consequently, observations based on ovary Se concentrations regarding the effects of fish size and sampling location are expected to also be observed for muscle Se data. While there is no identified mechanistic link between GSI and muscle Se concentrations, it is possible a correlation between GSI and muscle Se might be observed given the correlations between ovary Se and muscle Se, as well as total length and GSI in the data set.

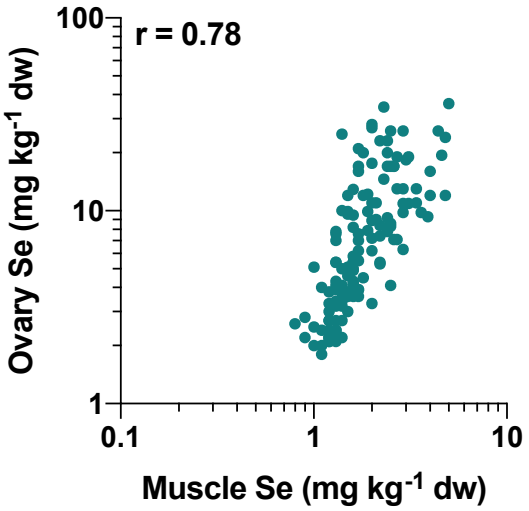

Figure 13. Correlation between muscle Se and ovary Se concentrations in NPM collected from Koocanusa Reservoir (2016-2019).

The multivariate analysis of muscle Se data used the same general multivariate approach described above for ovary Se data. The same data used in the ovary Se analysis was used in the muscle Se analysis for comparability except for a single fish collected from the mouth of the Elk River in 2016 for which no muscle data were collected (n = 140). Given the results of the ovary Se analysis, an initial model using only the 2019 data was not developed for muscle. Instead, the full data set (2016-2019) was used to evaluate the same general full model:

Ln(muscle Se) = area + year + Ln(TL) + area* Ln(TL) + Ln(GSI) +

area* Ln(GSI) (Eq. 4)

The model selected by BIC retained both total fish length and GSI as variables. Adjusted and predicted R^2 for the BIC muscle Se model were lower than for the ovary Se model at 0.47 and 0.45, respectively (Figure 14). The lower performance of the muscle Se model appears to be driven by underprediction of the relatively high muscle Se data for fish collected from the mouth of Elk River, although area-specific slopes were not retained in the model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (muscle Se) across sites (Table 6). Significant differences in area-specific intercepts were identified in the model. The intercepts for Gold Creek, Sand Creek and Rexford were all significantly lower than the Elk intercept ($p < 0.01$) indicating that after accounting for the influence of fish length and GSI, muscle Se concentrations in fish collected from all three locations were significantly lower than for fish collected near the Elk River (Table 6). Based on the final model, differences in area-specific intercepts between sites would translate to predictions of muscle Se concentrations being, on average, 1.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold and

Sand Creeks versus Elk River muscle Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.2-1.3 times higher than those from Gold and Sand Creeks for a given fish length and GSI.

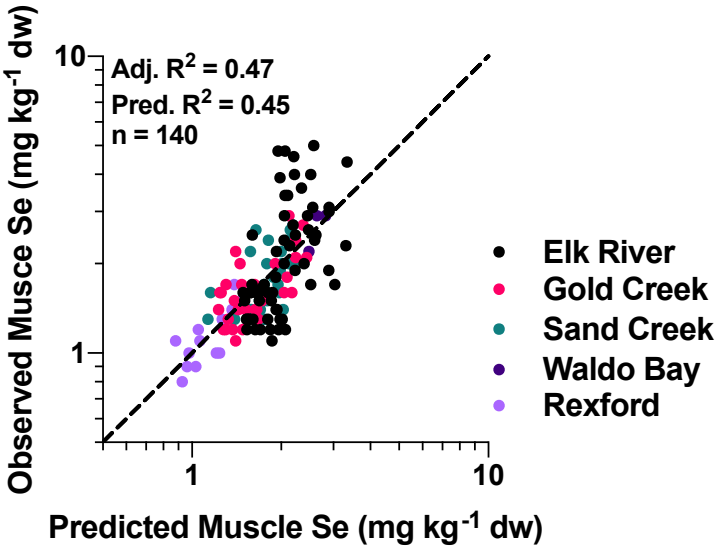

Figure 14. Final Muscle Se MLR model based on 2016-2019 data.

Table 6. Final muscle Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	4.197	0.607	6.92	-	
	Gold	4.022	0.063	-2.77	<0.01	
	Sand	3.919	0.066	-4.21	<0.01	
	Waldo	4.263	0.167	0.40	0.69	
	Rexford	3.619	0.085	-6.76	<0.01	
Slopes	Ln Total Length	-0.889	0.164	-5.41	<0.01	-0.406
	Ln GSI	-0.086	0.030	-2.81	<0.01	-0.215

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that total fish length (standardized slope = -0.41) has a stronger effect on muscle Se concentrations in the model than GSI (standardized slope = -0.21) over the range sampled for these variables (Table 5). This is the opposite of what was observed for ovary Se, but again, should be treated with caution given the correlation between total length and GSI. Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model have unequal variance (p = 0.001) and are not normally distributed (p = 0.001) again demonstrating the model has some systematic biases.

7. DISCUSSION AND CONCLUSIONS

The objectives of this study were to: 1.) determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations, and 2.) to evaluate the effects of GSI, fish size, and sampling location on ovary and muscle Se concentrations. The first objective was not achieved due to the inability to collect a sufficient number of ripe female NPM. In the remainder of this report, the success in achieving the second objective and implications of study findings are discussed.

7.1 Effects of GSI, Fish Size, and Sampling Location on Ovary Se Concentrations

Historical monitoring data suggest GSI, fish size, and sampling location may influence ovary Se concentrations, but the data are confounded by relatively small sample sizes, are unevenly distributed for some variables (e.g., GSI), and potentially auto-correlated. To address these limitations, a total of 94 additional ovary Se samples were collected in 2019 that were relatively evenly distributed across size classes and to a lesser extent GSIs.

Using 2019 data and incorporating most of the data from historical monitoring (total n = 141), an MLR model that characterizes ovary Se concentrations as a function of fish size (total length) and GSI was developed. While the model has some uncertainties related to the correlation between total fish length and GSI in the data set used for model development, the conclusion that total length and GSI are important predictors of ovary Se concentrations in NPM appears robust and predictions using a constrained data set with no correlation between independent variables are generally within 30% of the model based on the full data set.

There were several key findings from this model. First, the model indicates that fish with lower GSI have higher ovary Se concentrations independent of any other variables. This indicates that fish collected early in the year (e.g., February-May) have ovary Se concentrations that overestimate the egg Se concentrations that the fish will have at the time of spawning. The mechanism underlying this reduction in egg Se with development is currently unclear. Transfer of Se into the eggs is known to be associated with vitellogenesis (Janz et al., 2010) and the ovarian histology component of this study demonstrates vitellogenesis coincides with egg development and an increase in GSI, as is typical of most teleost fish. Consequently, an increase in egg Se rather than a decrease in egg Se would be expected with increasing GSI. However, there are many species-specific complexities to the process of vitellogenesis including variations in the use of multiple vitellogenin isoforms, variations in timing of primary and secondary vitellogenesis, and the level of processing of vitellogenin in the egg and associated level of water absorption (Hara et al., 2016). These processes could all influence how Se concentrations in eggs change during the course of egg development and to the best our knowledge, have not been studied in any detail in NPM or closely related species. However, regardless of the mechanism, the reduction in ovary Se concentrations with increasing GSI is important for assessing potential Se risks to NPM in the

reservoir as it is the ovary/egg Se concentration at the time of spawning that should be compared to an egg Se effect concentration.

Given this finding, ovary Se data from fish with low GSI (i.e., <5%) should be excluded from the data set when assessing potential risk to NPM. The oocyte maturation study demonstrated a strong positive relationship ($R^2 = 0.81$) between GSI and oocyte development. Fish where the majority of oocytes in an ovary were stage 3 (late vitellogenic) oocytes were associated with a GSI >5% (Figure 6). Consequently, only ovaries collected from fish with a GSI ≥5% should be used in assessing Se risks to NPM as these ovaries are likely to provide a relatively unbiased estimate of egg Se concentrations for comparison to egg Se toxicity thresholds. Using this data usability qualifier (GSI ≥5%) restricts the ovary Se data set. All data prior to 2016 are eliminated from assessment due to either low GSI or GSI not being reported and the distribution of ovary Se concentrations are significantly lower (Figure 15). Of all the samples collected from fish with a GSI >5% (n=45), only a single fish has exceeded the USEPA egg Se criteria of 15.1 mg kg^{-1} dw and only 4 fish have exceeded the BC ENV guideline of 11 mg kg^{-1} dw.

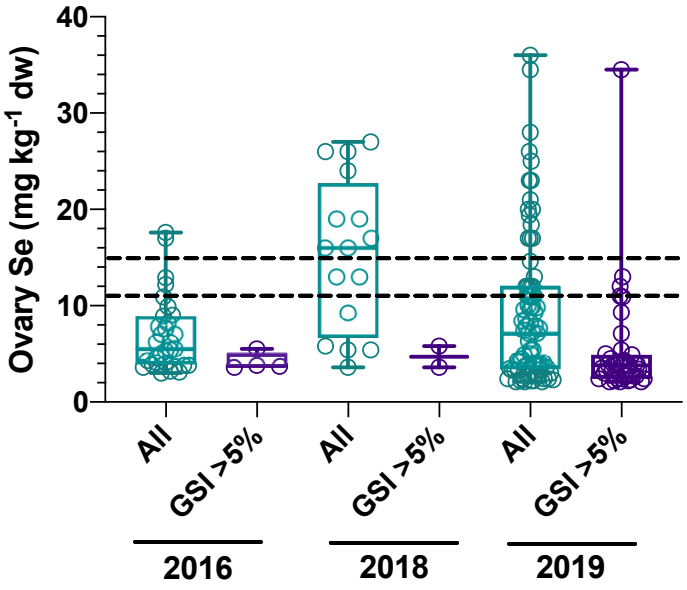

Figure 15. Comparison of ovary Se concentrations for all fish versus only fish with GSI ≥5%. Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines.

The second significant finding from development of the ovary MLR model was that fish size has a significant effect on ovary Se concentrations in NPM, with smaller fish having higher ovary Se concentrations. This is likely the result of differences in dietary preferences between small and large adult NPM. Small adult NPM (<30 cm) typically have a primarily insectivorous diet, but become increasingly piscivorous with increasing size, feeding

primarily on juvenile salmonids (Clarke et al., 2005; Petersen, 2001; Zimmerman, 1999). Whole body trophic transfer factors (TTFs) for fish (i.e., invertebrate to fish or fish to fish) are typically <1 except at very low (<1 mg kg^{-1} dw) dietary Se concentrations (DeForest et al., 2007). A consequence of TTFs <1 is that consumers at progressively higher trophic levels will have progressively lower whole body Se concentrations (i.e., biodilution). This may explain the size effect observed in the current analysis as small NPM feeding on insects would be expected to have higher Se exposure than large NPM which have a higher trophic level and are feeding on juvenile salmonids.

The third, and final, significant finding resulting from the ovary Se MLR model was identification of effects of sampling location on ovary Se concentrations. By accounting for the influence of fish size and GSI on ovary Se, the model was able to test for differences in ovary Se concentrations between sampling locations. Results from this analysis indicate that fish collected from Gold Creek and Rexford have significantly lower ovary Se concentrations than locations sampled higher in the reservoir. Locations higher in the reservoir are generally closer to the Se input from the Elk River although the Sand Creek sampling location is further from the Elk River than the Gold Creek sampling location (Figure 4).

7.2 Effects of GSI, Fish Size, and Sampling Location on Muscle Se Concentrations

The muscle Se MLR model was not as robust as the ovary Se MLR model (Figures 11 and 13). There are likely several reasons for this outcome. First, the range in muscle Se concentrations (0.8-5.0 mg kg^{-1} dw) is much less than observed for ovary Se concentrations (1.8-36 mg kg^{-1} dw). Consequently, small errors in analytical precision will introduce significantly more variance in the muscle Se MLR model. Second, although apparently not significant enough to be detected by the BIC analysis, the muscle Se data collected from the mouth of the Elk River qualitatively appear to have a systematic bias (i.e., different slope) with respect to the MLR model, over-predicting low muscle Se concentrations and under-predicting high muscle Se concentrations.

Despite the muscle Se model being less robust, it did generally support the observations of the ovary Se model. Specifically, the muscle model supports that fish size is an important variable in determining NPM Se concentrations in Koocanusa Reservoir (Table 5). It also supports observations that fish collected from the mouth of the Elk River have higher Se tissue concentrations than fish from most other locations sampled in the reservoir (Table 5).

The finding that GSI is a significant variable in the muscle Se model was somewhat unexpected. Mechanistically, there is no obvious reason why GSI would be an important determinant of muscle Se concentrations. It is possible that retention of GSI in the model is simply an artifact of GSI being an important variable in predicting ovary Se and ovary Se being generally strongly correlated to muscle Se, or that total length and GSI are somewhat correlated. Supporting this hypothesis is the observation that the standardized slope for GSI is

only half of the slope for fish size (Table 6), indicating it has proportionally less influence on muscle Se concentrations while whereas the opposite is true for ovary Se where the standardized slope for GSI is twice as steep as for fish size (Table 4).

7.3 Conclusions and Recommendations

A primary objective of this study was to determine the sensitivity of embryo-larval NPM to maternally transferred egg Se, which was not achieved due to the limited number of ripe female fish collected. However, the second objective of this study to evaluate the effects of fish size, GSI and sampling location on NPM ovary Se was successfully accomplished. Results from this effort indicates that all three variables influence ovary Se concentrations. Importantly, the study concludes that ovary Se data collected from fish with a GSI <5% should not be used to assess Se risks to NPM as these data over-estimate egg Se concentrations. However, the study also demonstrates that small adult NPM have higher egg Se concentrations than large NPM likely due to a predominantly insectivorous diet and that NPM near the Elk River and further upstream (i.e., Sand Creek) have higher egg Se concentrations than those collected further down the reservoir.

Based on these results, this sub-population (small adult fish that reside in the upper reservoir) of NPM likely exhibit higher egg Se concentrations than the overall NPM population in the reservoir, although the mean ovary Se concentration is still predicted to be below the BC ENV egg Se guideline. The relative size of this sub-population and distribution of egg Se concentrations within it is not well characterized, but current results suggests understanding the sensitivity of NPM to maternally transferred egg Se concentrations may still be important. Consequently, additional sampling to characterize the distribution of ovary Se concentrations in NPM in the upper reservoir and to conduct a toxicity study to determine their sensitivity to egg Se concentrations is recommended.

The main limitation of the 2019 Se toxicity study was an inability to capture a sufficient number of ripe female NPM. It is currently unclear why there was so much difficulty collecting a greater number of ripe females. Relatively large numbers of females were collected in the second half of June with GSIs in the range expected for ripe females (Table 2). This continued into early July, but despite relatively high GSIs, only a few fish manually expressed eggs. By mid-July, the CPUE began to drop rapidly and fish that had already spawned began to be captured. As the field season progressed, it was apparent that NPM were not continually congregating in the same areas during the presumed spawning period. Where abundant ripe males were found one day, no ripe males were present only two days later. It was expected that ripe females would be present in these congregations of males or join them days after they were located. This was not the case. Considerable effort (2,644 fishing hours) was invested using four different capture methods across a large spatial scale (~30 km reach of the reservoir). Although an abundance of mature females with high GSIs were captured in

the first four weeks of sampling, CPUE dropped drastically through the last two weeks without locating the desired numbers of ripe females.

A clearer understanding of where Koocanusa NPM populations are spawning is needed, including whether it occurs in congregations and whether tributaries are possible spawning habitat areas. Some changes in gear use, particularly setting gill nets during dusk and dawn, may increase capture rates, but this introduces new safety issues for crews, which will need to be addressed in planning. Greater capture success may result from investing efforts in tracking NPM movements within Koocanusa Reservoir. Understanding NPM movements provides possibilities for improving understanding of habitat use during spawning and allows more focused fishing efforts in suspected spawning sites.

8. CLOSURE

We trust this report provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact Kevin Brix at (305) 773-8347.

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APPENDIX A

UNIVERSITY OF SASKATCHEWAN SELENIUM TOXICITY REPORT

APPENDIX B

NORTHERN PIKEMINNOW OVARY SELENIUM, MUSCLE SELENIUM, AND GSI DATA FOR KOOCANUSA RESERVOIR: 2008-2019

APPENDIX C

NORTHERN PIKEMINNOW METAL TISSUE DATA: 2019

Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

Report

Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

Submitted to:

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Prepared by:

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Saskatoon, SK, Canada

March 2020

Table of Contents

1 Study Rationale

Ongoing monitoring in the transboundary Koocanusa Reservoir located in British Columbia (BC) indicated a range of selenium (Se) concentrations in wild northern pikeminnow (NPM; *Ptychocheilus oregonensis*) that in some cases exceeded the U.S. Environmental Protection Agency (USEPA) criterion for fish egg/ovary of 15.1 mg/Kg dry weight [dw] and the British Columbia Ministry of the Environment and Climate Change Strategy (ENV) guideline of 11 mg/Kg dw (Brix et al. 2019). Embryonic life-stages of fishes are particularly susceptible to Se exposure via maternal transfer (Janz et al. 2010). However, to the best of our knowledge no studies have investigated the sensitivity of NPM to Se. Also, recent data suggest that there is a negative correlation between relative gonad size of females (as represented by gonadosomatic index [GSI]) and ovary Se concentrations. While this trend could indicate lower exposures of embryos under the assumption that GSI is directly related to maturation stage (later maturation stages are assumed to have greater GSIs, which were reported to have lower Se concentrations), little is known about gonadal phenotypes and their correlation with GSI in this species.

Therefore, this study aimed to investigate 1) the potential effects of maternal transfer of Se to embryos of NPM collected from several sites on the BC side of Koocanusa Reservoir, representing a gradient of Se concentrations, and 2) to characterize gonadal maturation phenotypes prior to and during the reproductive season of NPM in Koocanusa Reservoir. Unfortunately, despite extensive efforts, an insufficient number of female NPM in spawning condition were collected to properly characterize the relationship between egg Se concentrations and NPM embryo-larval development. Consequently, this report only presents the methods and results of the gonadal maturation characterization.

2 Objective

The main objective of this study was to determine whether egg Se concentrations found in NPM from different locations in Koocanusa Reservoir as well as the Elk River, BC may have effects on developing embryos and larvae of NPM. The secondary objective of this study was to characterize ovarian phenotypes of NPMs prior to and during their reproductive season using histology. To accomplish this,

Specific objectives to be addressed during the 2019 NPM early life stage (ELS) studies were:

- Characterize concentrations of Se in parent fish and embryos collected from the BC portion of Koocanusa Reservoir.
- Collect gonadal tissues (representing a range of GSIs) from NPM of different sizes prior to and during the reproductive season to characterize ovarian maturation and oocyte developmental stages.
- Establish a field-fertilization, and an on-site and laboratory culture protocol for NPM embryos and fry.

- Characterize survival, growth, and development of ELS of NPM related to tissue Se concentrations in ovaries of parent fish and eggs/embryos.
- Describe (if detectable) the toxicity threshold concentration (LC_{10} [mortality] and/or EC_{10} [time to hatch, time to swim-up, teratogenicity, growth]) of maternally transferred Se in NPM embryos.

Unfortunately, an insufficient number of female NPM in spawning condition were collected during the study to allow for full development of a protocol and characterization of the effect of maternally transferred Se on developing NPM embryos and larvae. Consequently, only the methods and results for the ovarian histology assessment are provided in this report.

3 Methods

3.1 Ovarian Histology to Assess Gonadal Maturation Stages

All methods for histology preparation followed the UofS Toxicology Centre's draft standard operating procedure (Appendix A). Field-collected NPM were dissected on site and gonads were excised, weighed and then immediately preserved in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed with an automated unit by the UofS Health Sciences Histology Core Facility, to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings, and cooled for 20 minutes to allow sufficient hardening. Because the ovary samples were fragile, blocks were pre-sectioned to expose the tissues and soaked in a glycerin-ethanol solution for 24 hours before section collection. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section, and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

Oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads (2009) - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish. Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

Gonadosomatic indices (GSIs) were calculated for all fish from which gonads were collected for histological assessment as follows (Eq. 1):

GSI = gonad weight [g] / body weight [g] *100 (1)

4 Results & Discussion

4.1 Ovarian Histology to Assess Gonadal Maturation Stages

Between July 8, 2019 and July 19, 2019, ovaries from a total of 15 NPM were collected for histological analyses of maturation stages of oocytes across fish of different sizes (weight range: 250 – 1800 g; fork length range: 33.2 – 61.8 cm), and GSIs (range 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (*Figure 4-1*; ***Table 4-1***). While there was no obvious relationship between the size of fish and GSIs, there was a clear positive correlation between GSI and ovarian maturation stage (*Figure 4-1*) with fish having GSIs greater than or equal to 5% all grouping in the final maturation stage (3) with one exception. Similarly, there was a significant and linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($R^2 = 0.81$), revealing that ovaries of mature fish with a GSI greater than 5% consisted of over 50% LVOs (*Figure 4-2B*). Finally, there was a negative relationship between ovarian Se concentrations and GSI as well as oocyte development stages (*Figure 4-3*).

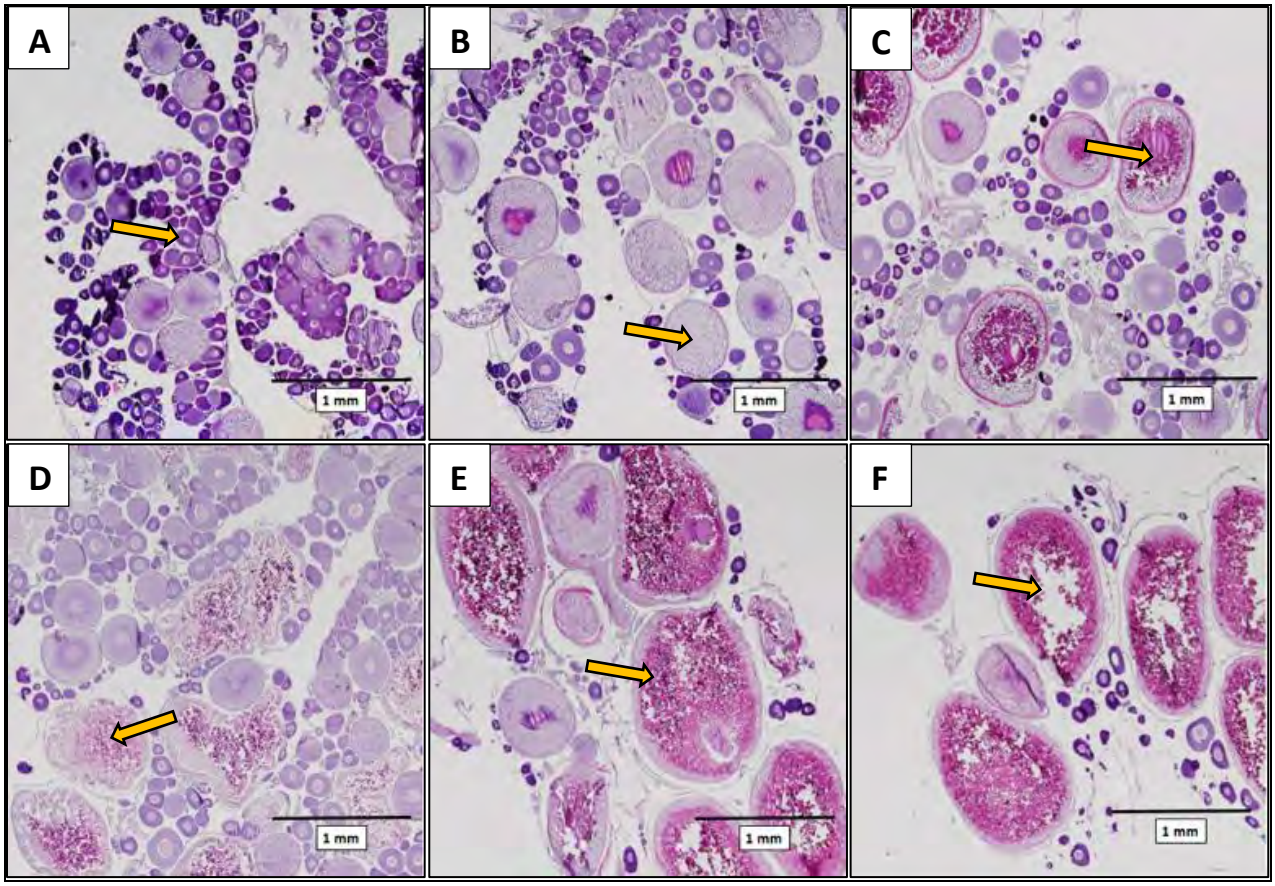

Figure 4-1. Histomicrographs of ovaries of northern pikeminnow representing early development stages (Stage 1) predominantly consisting of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E&F; Arrows).

Table 4-1. Summary of northern pikeminnow oocyte histology analysis detailing maternal morphometric characteristics, oocyte development counts, percent area covered by cell development categories, assessed gonadal developmental stage, and notes. Abbreviations are defined as follows: GSI – gonadosomatic index; PO – perinucleolar oocytes; CAO – cortical alveolar oocytes; EVO – early vitellogenic oocytes; LVO – late vitellogenic oocytes.

Sample ID	Maternal Assessment				Histological Assesment									
	Total Length (cm)	Total Weight (g)	GSI (%)	Ovary Se (µg/g)	Cell Type Count				Percent Area of Cell Type				Developmental Stage	Notes
					PO	CAO	EVO	LVO	PO	CAO	EVO	LVO		
SC-06	43.5	495	1.46	10	341	40	25	10	61.8	16.9	12.7	8.5	2	Atresia Present
GC-14	50.8	1030	0.86	9.6	364	38	4	0	67.7	28.9	3.4	0.0	1	
GC-15	61.8	1800	5.60	3.9	34	12	4	10	6.3	8.6	4.8	80.3	3	
ER-31	33.2	300	5.30	10.9	62	9	8	20	9.0	4.6	10.7	75.7	3	
ER-34	54.0	1470	1.31	5.4	156	46	10	8	37.4	27.6	13.1	21.9	2	
ER-35	40.7	530	7.65	3.8	47	9	4	18	11.2	3.7	5.3	79.7	3	
ER-36	41.4	650	10.54	9.3	19	8	5	14	3.9	6.9	9.8	79.4	3	
ER-37	45.2	750	5.77	5.4	67	8	3	3	32.5	14.2	17.6	35.7	2	
ER-38	41.1	560	3.05	12	54	11	12	9	11.5	11.6	38.5	38.4	2	
ER-39	42.1	580	10.02	2.7	42	10	4	12	10.6	7.4	7.5	74.4	3	Atresia Present
ER-40	34.4	250	0.86	18.4	367	37	0	0	73.5	26.5	0.0	0.0	1	
ER-41	42.4	530	8.17	3.4	48	6	3	10	18.3	5.2	6.8	69.7	3	
ER-42	42.3	620	5.25	11	35	7	4	5	12.0	10.9	22.8	54.3	3	
ER-44	40.7	610	0.60	36	255	64	0	0	41.7	58.3	0.0	0.0	1	
ER-45	49.9	1200	9.01	2.2	11	3	1	6	7.1	8.8	5.2	79.0	3	

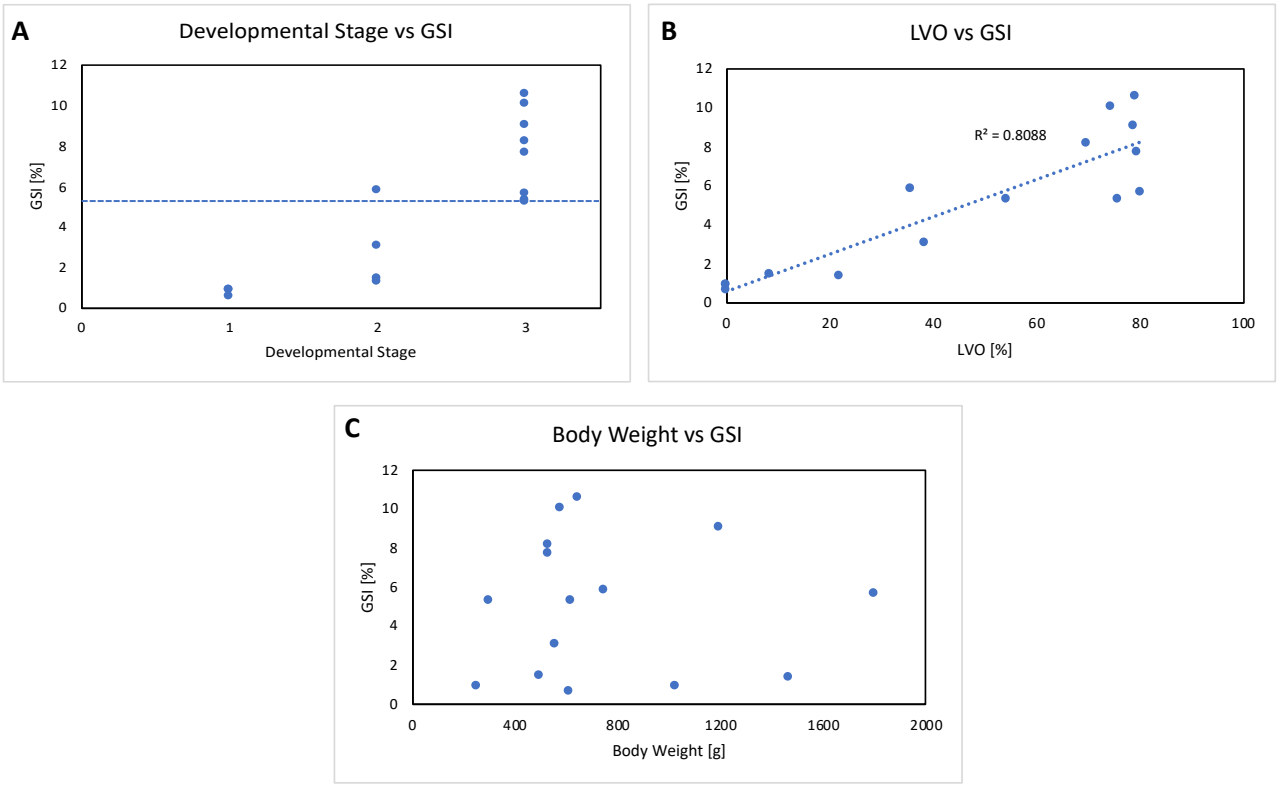

Figure 4-2. Relationships between gonadosomatic indices (GSIs; %) and A) body weight, B) LVO, and C) gonadal development stage in northern pikeminnow. Dotted line represents the 5% GSI level.

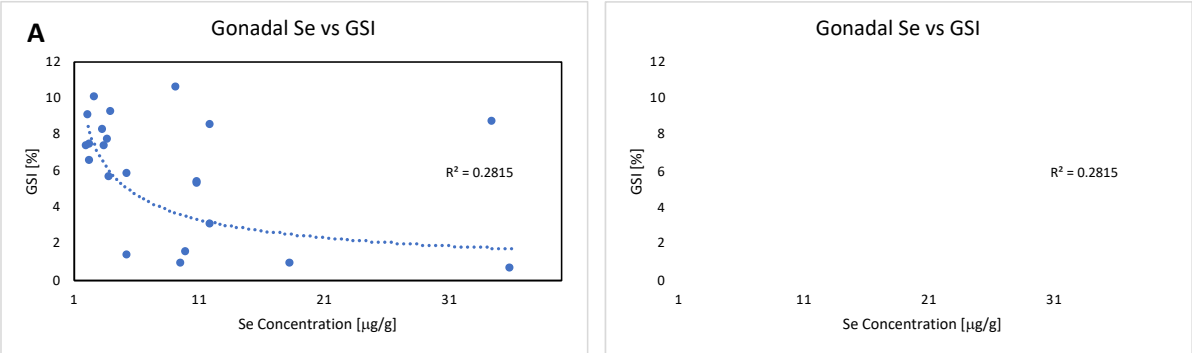

Figure 4-3. Relationship between ovarian Se concentration (µg/g d.w.) and A) gonadosomatic index (GSI), or B) Developmental Stage of oocytes in northern pikeminnow collected from the Koocanusa Reservoir. **Note:** Panel A includes data from 6 additional fish for which no histological evaluation was conducted.

5 Conclusions

This study successfully characterized, for the first time, the phenotypes of different ovarian maturation stage prior to spawning. Clear correlations between histologically determined proportion of follicular stages and GSI were described, demonstrating that fish in the final maturation stage (3) all had GSIs greater than or equal to 5%. Finally, there was significant, albeit weak, negative correlation between ovarian Se concentrations and maturation stage and GSI, indicating greater exposure of immature females. However, sample size and variability were such that future studies are required to confirm this relationship.

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Appendix A – Histological Procedures

University of Saskatchewan

Toxicology Centre

STANDARD OPERATING PROCEDURE

Histological Procedures

Draft

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DEFINITIONS AND ACRONYMS

PLEASE NOTE:

This protocol was produced for and by highly-qualified personnel of the Toxicology Centre of the University of Saskatchewan. It is therefore an in-house document and it should not be distributed without previous consent.

1.0 TISSUE SAMPLING & FIXATION

Morphometric measurements must be recorded as quickly as possible after the experimental animal is euthanized, because rapid degradation of tissues interferes with subsequent histological analyses. Record the following as applicable: individual ID#, sex, length (total length, fork length, standard length, snout-to-vent length, etc.), total weight, gonad weight, liver weight, appearance of secondary sex characteristics, and deformities or other external abnormalities.

There are several options for collecting histology samples, depending on the size of the organism:

1.1 Wole body/intact:

Fix the animal whole, and leave it intact for subsequent processing. In this case, the whole organism must be small enough to fit in a histocasette/standard paraffin block/microscope slide (e.g. fathead minnow at 30 days post-hatch);

1.2 Whole body/dissect

Fix the animal whole, and excise the tissues of interest at a later date prior to processing. For example, an adult fathead minnow or *Xenopus* metamorph can be fixed whole, transferred to 70% ethanol for storage, and then dissected to remove tissues of interest such as liver, gonad, thyroid etc. In this case, during sampling it is necessary to make an incision to expose the internal organs and allow rapid penetration of the fixative.

- Make a shallow mid-ventral incision through the body wall the entire length of the body cavity, being careful to not damage any of the internal organs;
- For adult fathead minnows, make a lateral incision up one side of the body wall to allow the fixative to penetrate the viscera. If possible, using fine forceps, gently move the viscera aside, grasp the swim bladder and discard;
- Attach an individual paper tag with ID# to the body using a needle, fishing line and a waterproof ‘rite-in-the-rain’ paper tag. Label with pencil only, because ethanol removes ink. Attach the tag by passing the needle and line through the body and tying it off.

1.3 Tissue necropsy:

Excise tissue samples from the freshly euthanized animal prior to fixation. This is done for large-bodied specimens that can’t be fixed whole;

- In some cases an entire organ can be excised completely intact and fixed whole for histological analysis. Alternately, it may be necessary to remove only a portion of the tissue of interest, which should be done in a standardised manner, e.g.:

- middle portion of the left or right gonad;
- a particular lobe of the liver;
- right 2nd gill arch;
- middle portion of the posterior kidney;

- When possible, tissue samples should not exceed 1 cm in any direction, although there are exceptions.

When collecting samples for histology, it is preferable to use chemical overdose, because physical methods of euthanasia can sometimes damage histological samples. It is possible to collect different types of samples from a given individual, e.g. remove the fresh liver for biochemical analysis, and then fix the remaining tissues for histology.

1.4 Fixation:

Proper fixation of tissues is one of the most crucial steps in routine histology, and should be kept consistent across samples. The histology samples (i.e. tissue samples in histocassettes, or whole body samples) should be placed in fixative within 2-3 minutes of euthanasia. Ensure that samples are fully submerged, using a minimum of 10 volumes of fixative to 1 volume of tissue. Use Nalgene wide-mouth leak-proof polyethylene containers.

Samples should remain submerged in fixative for 48 hours, and are then transferred to 70% ethanol for storage. Fixative cannot be re-used, and should be disposed appropriately. The 70% ethanol should be poured off and replenished two more times (minimum 1-2 days each) to remove excess fixative prior to tissue processing.

Commonly used histological fixatives include: 10% Neutral Buffered Formalin, Cal-Ex™ II (Fisher), Davidson's Fixative, and Bouin's fluid. Those containing acids have superior tissue penetration with the added advantage of de-calcifying bone, which can improve tissue sectioning. Cal-Ex is therefore preferred for whole body fixation. Davidson's Fixative is also popular; it can be prepared in advance using stock chemicals, and has a reasonable shelf life for longer-term storage.

Davidson's Fixative

Formalin	200 ml
100% Ethanol	300 ml
Glycerin	100 ml
Glacial Acetic Acid	100 ml
Distilled Water	300 ml

2.0 SPECIMEN GROSSING

Once fixed and stored in 70% ethanol, the specimens can be further trimmed if necessary prior to tissue processing. Whole body samples can be dissected to remove tissues of interest. In some cases, an entire organ can be excised (e.g. the gonad, liver), or alternately, a representative portion of the tissue of interest can be removed. Note: fixed weights and lengths can be used to generate condition factor, gonado- and hepatosomatic indices.

3.0 TISSUE PROCESSING and EMBEDDING

Fixed tissue specimens (stored in 70% ethanol) are loaded into a Vacuum Infiltration Processor (aka "**Tissue Processor**"). This programmable, automated unit contains reservoirs of various solvents as well as molten paraffin wax. The tissue processor can be programmed to control temperature, stir the solutions, and create pressure/vacuum cycles during sample processing, all of which can enhance the penetration of solutions through the tissues. The purpose of the process is: (1) **dehydration** - the tissues are bathed in a series of progressively stronger alcohols (70% up to 100%) to remove excess water from the cells, (2) **clearing** - the alcohol is flushed from the tissues and replaced with xylene or toluene (which are capable of dissolving paraffin) (3) **infiltration** - the tissues are infiltrated with molten paraffin. The final result is an intact tissue sample perfused with paraffin, which is immediately placed in a paraffin-filled mould and allowed to cool.

4.1 Tissue Processing:

It takes 14 hours to run a batch of samples through the tissues processor; this is typically done overnight, with sample embedding happening the following morning. Ensure that the samples are stored in a third rinse of 70% ethanol prior to loading them in the processor.

4.2 Embedding

1) Arrive at the Lab 20-30 minutes before the end of the processing run to prepare for embedding.

- Ensure that the Cryo station is turned on;
- Label an embedding ring for each sample to be embedded;
- Coat the embedding moulds with a thin layer of Mould Release, and place them on the warming console;

2) When the processing run is complete, remove the samples from the tissue processor and place them in the 'holding basin' full of molten paraffin wax in the embedding

console. Ensure that the samples do not cool down and solidify at this point, i.e. get the cassettes into the melted wax as quickly as possible, and keep the lid closed;

3) Using the heated paraffin dispenser, place a small amount of paraffin in the bottom of the mould. (Note that the paraffin dispenser flow rate can be adjusted);

4) Open a histocassette and spill the tissue sample out into the holding wax. Set the cassette aside.

5) Using heated forceps, gently grab the tissue and place in the bottom of the mould to attain the appropriate ***orientation for sectioning***. Place it on the cooling pad for 5-10 seconds, to ensure that the wax gels, and the tissue is held in place.

6) Place the labelled embedding ring on the mould with and fill with paraffin. The wax level should be above the rim of the embedding ring to account for shrinkage during cooling. Set it on the Cryo console to cool.

7) Repeat until all samples are embedded, working as quickly as possible.

8) Leave the blocks on the Cryo console for ~20 minutes. Gently pull the mould off and set the block on the benchtop to cool. Transport solidified blocks back to the Toxicology Centre, and let sit overnight prior to attempting any trimming or sectioning.

Table 1. Tissue processing program used at the University of Saskatchewan Histology Core Facility. Tissues are dehydrated in graded alcohols (Station 1 to 7), cleared in xylene (Station 8 to 10), and infiltrated with molten paraffin (Station 11 to 14).

Station	Reagent	Time	Temp (°C)	Pres/Vac Cycle	Mix
1	Ethanol 70%	1 hr	ambient	V	On
2	Ethanol 80%	1 hr	ambient	V	On
3	Ethanol 95%	1 hr	ambient	V	On
4	Ethanol 95%	1 hr	ambient	V	On
5	100% ethanol	1 hr	ambient	V	On
6	100% ethanol	1 hr	ambient	V	On
7	100% ethanol	1 hr	ambient	V	On
8	Ethanol/Xylene	1 hr	ambient	V	On
9	Xylene	1 hr	ambient	V	On
10	Xylene	1 hr	ambient	V	On
11	Paraffin	1 hr	60	V	On
12	Paraffin	1 hr	60	V	On
13	Paraffin	1 hr	60	V	On
14	Paraffin	1 hr	60	V	On

5.0 MICROTOMY (aka SECTIONING)

The embedding ring of the paraffin block is mounted on a rotary microtome. Ribbons of thin sections are created, and these are placed on glass microscope slides. The user can control the thickness of the sections (usually 5-7 μm, thinner is generally better), as well as the number and spacing of the sections retained on the microscope slide. There are several options for sectioning:

- **Single representative section** – one section is retained from each block, this is considered to be representative of the entire tissue;
- **Serial sectioning** - the user cuts through the entire tissue, and all sections are retained (labour-intensive);
- **Step sectioning** - the user cuts through the tissue and retains representative sections at pre-defined intervals;

5.1 General Methods for Sectioning:

- Turn on slide warming table, let it warm up to 40°C (temperature is generally pre-set, and shouldn't require adjustments);
- In a small beaker, prepare ~40mL of distilled water containing ~4 drops of Mayer's Albumin mounting medium, stored in fridge. This should be sufficient for 1 day of sectioning; fresh solution should be made up daily (1 drop Mayer's per 10 mL dH20);
- Use a razor blade to trim excess wax from the tissue blocks to within 2mm of the tissue edge. Maintain square sides on trimmed portion;
- Wipe down a fresh microtome blade with xylene to remove the oil coating, and mount it in the knife holder;
- Ensure microtome is clean and lubricated (see user manual);
- Pre-label a slide for the first paraffin block using solvent resistant marker (slides will be dipped in solvents during staining).
- Place the block firmly in the microtome chuck. Section the block according to the specific protocol (i.e. a single 'representative' section per block, step sections, or serial secions). The sections should come off the blade in continuous ribbons. Note that if the blocks are trimmed small, numerous sections and multiple rows of sections can be placed on a single slide;
- Place the labelled slide on the warming table and flood with the mounting medium. Float the sections of interest on the slide until they appear smooth and free of wrinkles;

- Once the sections are smooth, wipe away excess mounting medium from the slide (Kimwipe), and place it in a slide holder. Full racks of slides are stored in the 40C oven (minimum overnight) prior to staining;
- If scratches or nicks appear in the ribbons during sectioning, move the blade to an unused area, or replace entirely;

6.0 SLIDE STAINING - HEMATOXYLIN and EOSIN

Once the tissue sections have been allowed to dry overnight in a 40°C oven, they can be stained for light microscopy. Myriad staining techniques exist; Hematoxylin and Eosin ("**H&E**") is a common 2-part staining technique routinely used for basic paraffin sections. A rack full of slides is immersed in a series of solvents and stains, resulting in de-waxing of the sections and differential uptake of the 2 stains in various cellular components.

6.1 Staining:

- The stains and solvents can be used to stain app. 10 – 12 racks of slides, and then must be replaced. Check with other lab users regarding the status of the stain series, or if necessary check the quality of the most recently stained slides for fading or loss of contrast. Solvents can be topped up if they have evaporated down;
- Do not stain paraffin sections unless the slides have dried in 40°C oven for minimum 24 hours;
- Multiple racks can be stained at once. When the first boat is in the hematoxylin, a second rack can be started;
- It takes ~45 minutes to stain and coverglass one rack of slides;
- Staining and coverglassing are done in the fume hood;
- Before starting, check supplies of cytoseal and coverglass (use #1 thickness).

Table 2: Step-by-step staining process.

Station	Solution	Time	Notes
1	Xylene 1	2 min	
2	Xylene 2	2 min	
3	Xylene / 100% Ethanol	2 min	1:1 Ratio
4	100% Ethanol	2 min	
5	95% Ethanol	2 min	
6	70% Ethanol	2 min	
7	Tap Water	2 min	Replace often
8	Distilled Water	2 min	Replace often
9	Hematoxylin	5 min	
10	Tap water	rinse 4x	Water should run clear
11	Acid Alcohol (0.1%)	15 sec	0.1ml HCl/100ml 70% EtOH
12	Water	rinse 2x	
13	Phosphotungstic Acid (0.33%)	30 sec	(0.33 g/100ml Water)
14	Citric Acid (0.33%)	30 sec	(0.33 g/100ml Water)
15	Running Tap Water	5 min	
16	Eosin Y	2.5 min	
17	Tap Water	rinse 4x	Water should run clear
	70% Ethanol		
18	95% Ethanol	1 min	
19	100% Ethanol	2 min	
20	100% Ethanol	2 min	
21	Xylene / 100% Ethanol	2 min	1:1 Ratio
22	Xylene	2 min	
23	Xylene	Holding	

6.2 Coverglassing:

- Slides should be coverglassed as soon as possible after staining is completed. The slide rack is held in the last Xylene station until coverglassing is completed;
- Place a coverglass on a cork, add thin line of cytoseal full length;
- Remove slide from Xylene, blot slide edge on paper towel, do not allow Xylene to evaporate completely;
- Turn slide upside down, slowly lower it onto the coverglass at a slight angle, avoid trapping air bubbles in the cytoseal;
- Wipe off the back of the slide, and place flat on trays to dry, ensuring that the slide edges are not touching each other. Slides should air dry minimum 1 week before placing in slide boxes.

Appendix B. Northern Pikeminnow Ovary Selenium, Muscle Selenium, and GSI data for Koocanusa Reservoir: 2008-2019

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
MT	2008	May	14	-	Rexford	2.8	1.0	54.8	-	1973.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.5	1.0	48.3	-	1340.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.1	51.3	-	1347.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.1	51.8	-	1740.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.7	1.2	53.7	-	1708.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.2	1.2	56.2	-	1705.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.8	1.2	50.2	-	1592.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.2	50.0	-	1226.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.9	1.2	50.7	-	1306.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.9	1.3	53.1	-	1720.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.9	1.3	55.8	-	1789.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.3	49.5	-	1303.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.3	47.6	-	1183.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.4	50.9	-	1557.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.6	52.3	-	1586.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.7	51.8	-	1728.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.0	1.9	60.3	-	2259.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.5	1.9	47.0	-	1140.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.7	1.5	56.3	-	1851.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.4	1.5	48.6	-	1134.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.7	1.7	51.7	-	1297.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	1.7	47.0	-	1039.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.3	1.8	52.2	-	1465.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.4	1.8	53.8	-	1506.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.3	1.8	50.2	-	1190.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.4	1.9	61.2	-	2696.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	6.0	2.0	46.8	-	953.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	8.1	2.3	47.6	-	1043.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.1	2.3	51.0	-	1361.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.0	2.3	45.1	-	925.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	2.4	48.8	-	1052.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.5	56.2	-	1860.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.7	1.5	51.0	-	1343.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.3	1.6	50.4	-	1148.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.5	1.7	46.3	-	898.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.7	42.2	-	662.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.9	51.9	-	1134.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.2	1.9	44.4	-	776.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.9	1.9	46.4	-	1116.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.7	2.3	46.6	-	762.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	40.1	5.0	41.0	37.2	505.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	25.7	1.6	38.2	34.9	465.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	3.3	2.4	29.5	27.2	316.0	-	-	-	-	-
BC	2014	April	-	-	Elk River	21.9	4.6	39.3	35.2	440.0	-	-	-	-	-
BC	2014	April	-	ER-PM-14G-Apr-14	Elk River	40.1	6.2	34.1	30.8	312.0	-	3.4	-	-	1.08
BC	2014	April	-	ER-PM-11G-Apr-14	Elk River	8.6	2.5	37.3	33.5	438.0	-	4.5	-	-	1.03
BC	2014	February	-	-	Gold Creek	7.6	2.8	39.3	35.7	495.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	15.4	2.2	35.5	32.3	360.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	10.3	2.5	38.6	34.9	500.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	4.1	2.4	38.0	-	580.0	-	-	-	-	-
BC	2014	April	-	GC-PM-10G-Apr-14	Gold Creek	5.0	2.3	40.4	36.5	522.0	-	4.3	-	-	0.83

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2014	February	-	-	Sand Creek	13.7	1.6	35.5	32.1	320.0	-	-	-	-	-
BC	2014	February	-	-	Sand Creek	3.8	1.2	49.9	45.4	1200.0	-	-	-	-	-
BC	2014	April	-	SC-PM-10G-Apr-14	Sand Creek	17.0	1.6	34.4	31.2	300.0	-	1.8	-	-	0.61
BC	2014	April	-	SC-PM-01G-Apr-14	Sand Creek	30.7	2.9	36.1	32.8	355.0	-	6.2	-	-	1.74
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	4.0	-	35.8	32.3	380.0	-	1.1	-	-	0.30
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	12.9	4.1	34.2	30.8	320.0	13.0	1.1	4.01	315	0.34
BC	2015	April	-	ER-NSC-25-Apr-15	Elk River	9.2	3.1	40.4	36.5	560.0	13.0	1.2	7.81	551	0.21
BC	2015	April	-	ER-NSC-13-Apr-15	Elk River	11.8	6.0	46.7	42.7	885.0	13.0	1.1	12.97	871	0.13
BC	2015	April	-	GC-NSC-13-Apr-15	Gold Creek	5.2	1.9	32.1	28.9	252.0	9.0	1.0	5.50	245	0.41
BC	2015	April	-	GC-NSC-31-Apr-15	Gold Creek	13.8	2.0	33.0	30.2	261.0	12.0	0.9	2.30	258	0.36
BC	2015	April	-	GC-NSC-33-Apr-15	Gold Creek	7.9	2.3	37.4	34.0	390.0	10.0	1.0	2.41	387	0.25
BC	2015	April	-	GC-NSC-34-Apr-15	Gold Creek	7.6	1.6	37.5	34.0	390.0	9.0	1.0	2.10	387	0.25
BC	2015	April	-	GC-NSC-49-Apr-15	Gold Creek	3.5	1.3	44.8	40.1	880.0	12.0	1.4	17.22	861	0.16
BC	2015	April	-	SC-NSC-37-Apr-15	Sand Creek	7.2	1.7	32.6	29.4	261.0	9.0	1.0	3.22	257	0.39
BC	2015	April	-	SC-NSC-36-Apr-15	Sand Creek	4.8	1.9	33.5	30.0	275.0	10.0	1.0	2.51	271	0.37
BC	2015	April	-	SC-NSC-33-Apr-15	Sand Creek	11.4	1.4	34.0	30.2	300.0	8.0	1.1	3.30	296	0.36
BC	2015	April	-	SC-NSC-47-Apr-15	Sand Creek	18.3	2.4	34.4	30.8	325.0	13.0	1.1	3.66	320	0.34
BC	2015	April	-	SC-NSC-44-Apr-15	Sand Creek	15.1	2.4	35.2	31.7	370.0	13.0	1.2	5.49	363	0.31
BC	2015	April	-	SC-NSC-39-Apr-15	Sand Creek	11.6	2.0	38.0	34.0	445.0	12.0	1.1	3.34	441	0.25
BC	2015	April	-	SC-NSC-13-Apr-15	Sand Creek	6.2	1.5	40.0	36.3	492.0	14.0	1.0	8.33	483	0.21
BC	2015	April	-	SC-NSC-46-Apr-15	Sand Creek	5.8	1.7	41.6	37.5	630.0	13.0	1.2	14.58	614	0.19
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	3.1	-	54.1	49.5	1500.0		47.7			3.18
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	10.9	2.9	36.1	32.9	455.0	16.0	16.4	5.00	434	3.60
BC	2016	April	-	ER-NSC-19 O-Apr-16	Elk River	7.0	1.7	38.6	35.2	555.0	14.0	7.4	25.12	522	1.33
BC	2016	April	-	ER-NSC-17 O-Apr-16	Elk River	6.2	2.0	40.2	36.2	615.0	14.0	10.2	9.28	596	1.65
BC	2016	April	-	ER-NSC-27 O -Apr-16	Elk River	7.6	1.3	49.1	45.0	1200.0	15.0	17.6	24.42	1,158	1.47
BC	2016	April	-	ER-NSC-38 O-Apr-16	Elk River	9.9	1.5	51.1	47.0	1400.0	15.0	36.9	24.67	1,338	2.64
BC	2016	April	-	ER-NSC-16 O-Apr-16	Elk River	8.2	1.6	53.4	48.0	1540.0	14.0	35.5	17.35	1,487	2.31
BC	2016	April	-	ER-NSC-28 O-Apr-16	Elk River	5.5	1.7	56.3	50.8	1900.0	22.0	104.8	31.83	1,763	5.51
BC	2016	April	-	ER-NSC-15 O-Apr-16	Elk River	3.0	1.5	60.8	55.9	2600.0	20.0	124.3	46.89	2,429	4.78
BC	2016	April	-	ER-NSC-29 O-Apr-16	Elk River	3.6	1.6	61.5	56.7	2640.0	21.0	132.0	55.87	2,452	5.00
BC	2016	April	-	GC-NSC-17 O-Apr-16	Gold Creek	9.0	2.1	37.6	33.9	435.0	12.0	4.0	7.86	423	0.91
BC	2016	April	-	GC-NSC-01 O-Apr-16	Gold Creek	12.9	1.6	38.2	34.9	450.0	13.0	4.9	8.31	437	1.08
BC	2016	April	-	GC-NSC-14 O-Apr-16	Gold Creek	8.9	2.0	40.9	36.9	585.0	13.0	14.1	5.36	566	2.40
BC	2016	April	-	GC-NSC-16 O-Apr-16	Gold Creek	4.3	1.6	42.0	38.3	610.0	12.0	5.0	6.28	599	0.82
BC	2016	April	-	GC-NSC-12 O-Apr-16	Gold Creek	3.9	1.3	45.5	41.6	940.0	14.0	35.1	15.21	890	3.73
BC	2016	April	-	GC-NSC-26 O-Apr-16	Gold Creek	3.8	1.5	53.5	48.0	1600.0	15.0	96.2	42.73	1,461	6.01
BC	2016	April	-	GC-NSC-24 O-Apr-16	Gold Creek	3.8	1.4	52.8	48.2	1640.0	14.0	80.3	25.92	1,534	4.89
BC	2016	April	-	GC-NSC-06 O-Apr-16	Gold Creek	6.2	1.7	54.5	48.8	1400.0	15.0	35.7	22.15	1,342	2.55
BC	2016	April	-	GC-NSC-08 O-Apr-16	Gold Creek	5.3	2.2	55.8	50.6	1870.0	17.0	65.1	30.61	1,774	3.48
BC	2016	April	-	GC-NSC-13 O -Apr-16	Gold Creek	3.7	1.6	60.8	55.6	2360.0	17.0	124.2	62.56	2,173	5.26
BC	2016	April	-	GC-NSC-05 O-Apr-16	Gold Creek	4.1	1.6	62.2	57.0	2500.0	15.0	117.8	58.55	2,324	4.71
BC	2016	April	-	SC-NSC-25 O-Apr-16	Sand Creek	17.0	2.6	30.1	27.2	257.0	12.0	11.2	3.32	242	4.37
BC	2016	April	-	SC-NSC-09 O-Apr-16	Sand Creek	17.6	2.0	34.1	30.5	315.0	13.0	4.3	3.97	307	1.36
BC	2016	April	-	SC-NSC-21 O-Apr-16	Sand Creek	12.2	1.9	37.6	33.8	520.0	13.0	5.8	9.69	505	1.11
BC	2016	April	-	SC-NSC-29 O-Apr-16	Sand Creek	4.6	1.5	39.7	35.9	550.0	10.0	11.4	13.65	525	2.07
BC	2016	April	-	SC-NSC-03 O-Apr-16	Sand Creek	7.8	1.3	41.5	37.5	760.0	14.0	12.7	9.50	738	1.68
BC	2016	April	-	SC-NSC-35 O-Apr-16	Sand Creek	5.0	1.4	44.4	39.8	935.0	14.0	26.8	20.35	888	2.86
BC	2016	April	-	SC-NSC-36 O-Apr-16	Sand Creek	3.2	1.4	44.7	40.4	875.0	14.0	21.7	21.84	831	2.48
BC	2016	April	-	SC-NSC-32 O-Apr-16	Sand Creek	4.1	1.4	45.5	41.2	996.0	15.0	28.8	20.28	947	2.89
BC	2016	April	-	SC-NSC-34 O-Apr-16	Sand Creek	7.0	1.3	52.5	47.8	1420.0	15.0	36.8	19.82	1,363	2.59

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2016	April	-	SC-NSC-33 O-Apr-16	Sand Creek	5.4	1.3	62.6	57.4	2530.0	21.0	97.4	48.72	2,384	3.85
BC	2018	June	7	RG_ER_NSC06O_20180607	Elk River	26.0	4.4	30.9	27.7	205.0	6.0	1.1	2.93	201	0.51
BC	2018	June	7	RG_ER_NSC03O_20180607	Elk River	17.0	1.7	33.9	30.2	275.0	10.0	1.6	2.06	271	0.59
BC	2018	June	6	RG_ER_NSC02O_20180606	Elk River	19.0	3.1	35.5	31.8	315.0	9.0	1.9	2.70	310	0.61
BC	2018	June	7	RG_ER_NSC05O_20180607	Elk River	26.0	2.5	39.9	35.7	445.0	11.0	2.7	5.16	437	0.60
BC	2018	June	6	RG_ER_NSC01O_20180606	Elk River	16.0	4.0	41.4	37.0	545.0	10.0	3.6	6.42	535	0.65
BC	2018	June	7	RG_ER_NSC04O_20180607	Elk River	24.0	4.8	44.0	39.8	755.0	12.0	26.4	4.40	724	3.50
BC	2018	June	7	RG_GC_NSC02O_20180607	Gold Creek	19.0	2.7	37.1	33.5	350.0	9.0	1.9	4.32	344	0.54
BC	2018	June	7	RG_GC_NSC01O_20180607	Gold Creek	13.0	2.9	38.5	34.7	475.0	9.0	6.1	8.25	461	1.29
BC	2018	June	7	RG_GC_NSC03O_20180607	Gold Creek	3.6	1.7	54.5	50.1	1800.0	15.0	191.6	47.82	1,561	10.65
BC	2018	June	5	RG_SC_NSC05O_20180605	Sand Creek	13.0	2.7	34.0	31.0	280.0	8.0	2.8	2.76	274	1.00
BC	2018	June	5	RG_SC_NSC03O_20180605	Sand Creek	9.2	2.4	34.5	31.2	330.0	10.0	4.9	2.93	322	1.47
BC	2018	June	5	RG_SC_NSC04O_20180605	Sand Creek	5.4	1.6	35.6	32.4	340.0	9.0	7.2	3.65	329	2.13
BC	2018	June	10	RG_SC_NSC06O_20180610	Sand Creek	27.0	2.0	41.6	37.7	530.0	11.0	6.8	4.65	519	1.29
BC	2018	June	5	RG_SC_NSC01O_20180605	Sand Creek	16.0	1.7	44.3	40.4	685.0	12.0	5.8	10.55	669	0.85
BC	2018	June	5	RG_SC_NSC02O_20180605	Sand Creek	5.4	1.3	48.8	44.8	1140.0	13.0	52.1	24.00	1,064	4.57
BC	2018	June	10	RG_SC_NSC07O_20180610	Sand Creek	5.8	1.6	58.9	53.9	1690.0	17.0	96.2	29.16	1,565	5.69
MT	2018	May	8	-	Rexford	3.5	1.1	49.0	-	1215.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.2	1.1	47.4	-	1090.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	5.5	1.3	52.5	-	1575.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	4.6	1.4	48.3	-	1155.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.5	1.4	45.3	-	1110.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.7	1.4	51.6	-	1290.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.4	1.6	53.6	-	1360.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	6.7	1.6	44.2	-	760.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.9	1.9	51.3	-	1395.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.3	1.4	48.8	-	1220.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.5	1.1	49.3	-	1190.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.9	1.1	46.8	-	915.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.4	1.1	44.1	-	960.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.1	1.2	48.2	-	1070.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	1.8	1.2	56.4	-	1575.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.3	49.8	-	1145.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.6	48.2	-	1150.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.8	1.6	47.1	-	955.0	-	-	-	-	-
BC	2019	June	14	6/14/2019 RG_ER-NPM-01_20190614	Elk River	3.8	1.6	55.1	50.2	1680.0	-	137.0	-	-	8.15
BC	2019	June	14	6/14/2019 RG_ER-NPM-02_20190614	Elk River	2.7	1.3	57.2	53.0	1520.0	-	114.0	-	-	7.50
BC	2019	June	17	6/17/2019 RG_ER-NPM-03_20190617	Elk River	3.3	1.2	55.7	50.5	1550.0	-	149.9	-	-	9.67
BC	2019	June	17	6/17/2019 RG_ER-NPM-04_20190617	Elk River	3.0	1.2	45.5	41.6	1050.0	-	100.3	-	-	9.55
BC	2019	June	18	6/18/2019 RG_ER-NPM-05_20190618	Elk River	4.9	1.6	50.7	46.6	1140.0	-	100.3	-	-	8.80
BC	2019	June	18	6/18/2019 RG_ER-NPM-06_20190618	Elk River	5.0	1.6	54.4	49.6	1540.0	-	81.9	-	-	5.32
BC	2019	June	18	6/18/2019 RG_ER-NPM-07_20190618	Elk River	4.3	1.3	47.5	43.0	1140.0	-	87.7	-	-	7.69
BC	2019	June	18	6/18/2019 RG_ER-NPM-08_20190618	Elk River	4.2	1.6	56.0	51.2	1400.0	-	92.1	-	-	6.58
BC	2019	June	19	6/19/2019 RG_ER-NPM-09_20190619	Elk River	7.2	2.0	46.7	42.4	880.0	-	18.6	-	-	2.11
BC	2019	June	19	6/19/2019 RG_ER-NPM-10_20190619	Elk River	9.9	1.9	46.0	42.2	720.0	-	6.6	-	-	0.92
BC	2019	June	19	6/19/2019 RG_ER-NPM-11_20190619	Elk River	17.0	2.5	40.2	36.5	620.0	-	3.7	-	-	0.60
BC	2019	June	20	6/20/2019 RG_ER-NPM-12_20190620	Elk River	2.4	1.3	55.2	50.3	1950.0	-	216.3	-	-	11.09
BC	2019	June	20	6/20/2019 RG_ER-NPM-13_20190620	Elk River	3.6	1.5	56.0	51.5	1590.0	-	47.6	-	-	2.99
BC	2019	June	20	6/20/2019 RG_ER-NPM-14_20190620	Elk River	7.6	1.7	53.6	48.8	1520.0	-	46.2	-	-	3.04
BC	2019	June	20	6/20/2019 RG_ER-NPM-15_20190620	Elk River	8.6	2.5	39.9	36.2	480.0	-	18.6	-	-	3.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-16_20190620	Elk River	17.0	2.4	39.0	34.9	430.0	-	3.7	-	-	0.86

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_ER-NPM-17_20190620	Elk River	4.5	1.8	44.2	40.0	840.0	-	66.2	-	-	7.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-18_20190620	Elk River	4.1	2.5	53.0	48.0	1540.0	-	153.5	-	-	9.97
BC	2019	June	25	6/25/2019 RG_ER-NPM-19_20190625	Elk River	7.9	1.9	30.2	28.3	200.0	-	6.7	-	-	3.35
BC	2019	June	25	6/25/2019 RG_ER-NPM-20_20190625	Elk River	6.3	2.9	46.9	42.4	740.0	-	14.5	-	-	1.96
BC	2019	June	27	6/27/2019 RG_ER-NPM-21_20190627	Elk River	13.0	3.4	39.9	36.9	710.0	-	54.0	-	-	7.61
BC	2019	June	28	6/28/2019 RG_ER-NPM-22_20190628	Elk River	7.1	2.6	32.9	29.5	295.0	-	24.9	-	-	8.43
BC	2019	June	28	6/28/2019 RG_ER-NPM-23_20190628	Elk River	9.8	3.6	38.0	34.2	440.0	-	16.0	-	-	3.63
BC	2019	June	28	6/28/2019 RG_ER-NPM-24_20190628	Elk River	7.8	2.4	43.9	39.6	740.0	-	29.6	-	-	4.00
BC	2019	June	28	6/28/2019 RG_ER-NPM-25_20190628	Elk River	8.3	2.5	33.3	30.4	340.0	-	15.3	-	-	4.49
BC	2019	June	28	6/28/2019 RG_ER-NPM-26_20190628	Elk River	4.0	1.1	44.9	41.5	915.0	-	85.3	-	-	9.33
BC	2019	June	28	6/28/2019 RG_ER-NPM-27_20190628	Elk River	14.6	2.3	30.7	27.6	180.0	-	1.1	-	-	0.61
BC	2019	July	3	7/3/2019 RG_ER-NPM-28_20190703	Elk River	34.5	2.3	38.8	35.7	550.0	-	47.7	-	-	8.67
BC	2019	July	4	7/4/2019 RG_ER-NPM-29_20190704	Elk River	19.4	4.6	46.7	42.4	700.0	-	6.1	-	-	0.88
BC	2019	July	4	7/4/2019 RG_ER-NPM-30_20190704	Elk River	7.1	2.7	44.7	40.5	760.0	-	11.1	-	-	1.47
BC	2019	July	8	7/8/2019 RG_ER-NPM-31_20190708	Elk River	10.9	3.1	33.2	29.9	300.0	-	15.9	-	-	5.30
BC	2019	July	9	7/9/2019 RG_ER-NPM-32_20190709	Elk River	4.1	1.3	54.8	50.0	1550.0	-	143.1	-	20.09	9.23
BC	2019	July	9	7/9/2019 RG_ER-NPM-33_20190709	Elk River	3.5	1.4	44.3	41.6	780.0	-	57.3	-	-	7.35
BC	2019	July	9	7/9/2019 RG_ER-NPM-34_20190709	Elk River	5.4	1.6	54.0	49.0	1470.0	-	19.3	-	-	1.31
BC	2019	July	9	7/9/2019 RG_ER-NPM-35_20190709	Elk River	3.8	1.2	40.7	36.0	530.0	-	40.6	-	-	7.65
BC	2019	July	9	7/9/2019 RG_ER-NPM-36_20190709	Elk River	9.3	3.9	41.4	37.9	650.0	-	68.5	-	-	10.54
BC	2019	July	9	7/9/2019 RG_ER-NPM-37_20190709	Elk River	5.4	2.2	45.2	40.5	750.0	-	43.3	-	-	5.77
BC	2019	July	10	7/10/2019 RG_ER-NPM-38_20190710	Elk River	12.0	4.0	41.1	37.1	560.0	-	17.1	-	-	3.05
BC	2019	July	10	7/10/2019 RG_ER-NPM-39_20190710	Elk River	2.7	1.2	42.1	38.3	580.0	-	58.1	-	-	10.02
BC	2019	July	10	7/10/2019 RG_ER-NPM-40_20190710	Elk River	18.4	3.0	34.4	30.4	250.0	-	2.1	-	-	0.86
BC	2019	July	10	7/10/2019 RG_ER-NPM-41_20190710	Elk River	3.4	1.3	42.4	38.3	530.0	-	43.3	-	-	8.17
BC	2019	July	10	7/10/2019 RG_ER-NPM-42_20190710	Elk River	11.0	3.4	42.3	38.8	620.0	-	32.6	-	-	5.25
BC	2019	July	11	7/11/2019 RG_ER-NPM-43_20190711	Elk River	12.0	4.8	43.0	39.3	825.0	-	70.1	-	-	8.50
BC	2019	July	12	7/12/2019 RG_ER-NPM-44_20190712	Elk River	36.0	5.0	40.7	36.6	610.0	-	3.7	-	-	0.60
BC	2019	July	12	7/12/2019 RG_ER-NPM-45_20190712	Elk River	2.2	1.2	49.9	45.3	1200.0	-	108.1	-	-	9.01
BC	2019	July	13	7/13/2019 RG_ER-NPM-46_20190713	Elk River	2.4	1.3	56.0	50.6	1575.0	-	116.5	-	-	7.40
BC	2019	July	15	7/15/2019 RG_ER-NPM-47_20190715	Elk River	2.1	1.2	53.3	48.2	1200.0	-	87.2	-	-	7.27
BC	2019	July	16	7/16/2019 RG_ER-NPM-48_20190716	Elk River	2.3	1.3	54.9	50.5	1240.0	-	80.1	-	-	6.46
BC	2019	July	26	7/26/2019 RG_ER-NPM-49_20190726	Elk River	3.4	1.4	49.5	45.0	590.0	-	12.6	-	-	2.14
BC	2019	June	26	6/26/2019 RG_GC-NPM-01_20190626	Gold Creek	2.4	1.2	54.5	49.0	1375.0	-	149.5	-	-	10.87
BC	2019	June	26	6/26/2019 RG_GC-NPM-02_20190626	Gold Creek	2.1	1.3	53.9	49.3	1425.0	-	135.5	-	-	9.51
BC	2019	June	26	6/26/2019 RG_GC-NPM-03_20190626	Gold Creek	2.1	1.2	47.4	43.1	1075.0	-	106.7	-	-	9.93
BC	2019	June	26	6/26/2019 RG_GC-NPM-04_20190626	Gold Creek	20.0	2.4	38.9	34.9	460.0	-	2.9	-	-	0.64
BC	2019	June	26	6/26/2019 RG_GC-NPM-05_20190626	Gold Creek	3.9	1.7	44.8	40.4	915.0	-	55.5	-	-	6.07
BC	2019	June	26	6/26/2019 RG_GC-NPM-06_20190626	Gold Creek	2.4	1.1	49.9	44.9	1060.0	-	115.8	-	-	10.92
BC	2019	June	26	6/26/2019 RG_GC-NPM-07_20190626	Gold Creek	11.0	2.1	34.6	31.3	375.0	-	3.0	-	-	0.81
BC	2019	June	27	6/27/2019 RG_GC-NPM-08_20190627	Gold Creek	2.2	1.2	54.6	49.8	1600.0	-	210.4	-	-	13.15
BC	2019	June	27	6/27/2019 RG_GC-NPM-09_20190627	Gold Creek	2.2	1.4	52.6	47.8	1060.0	-	27.0	-	-	2.55
BC	2019	June	27	6/27/2019 RG_GC-NPM-10_20190627	Gold Creek	2.7	1.2	50.2	45.4	1280.0	-	169.2	-	-	13.22
BC	2019	June	27	6/27/2019 RG_GC-NPM-11_20190627	Gold Creek	12.0	1.8	41.6	37.4	600.0	-	4.2	-	-	0.70
BC	2019	June	27	6/27/2019 RG_GC-NPM-12_20190627	Gold Creek	3.9	1.4	45.0	40.5	920.0	-	48.9	-	-	5.32
BC	2019	June	27	6/27/2019 RG_GC-NPM-13_20190627	Gold Creek	3.3	2.0	49.5	44.8	1150.0	-	92.4	-	-	8.03
BC	2019	July	18	7/18/2019 RG_GC-NPM-14_20190718	Gold Creek	9.6	1.5	50.8	46.2	1030.0	-	8.8	-	-	0.86
BC	2019	July	19	7/19/2019 RG_GC-NPM-15_20190719	Gold Creek	3.9	1.4	61.8	57.0	1800.0	-	100.9	-	-	5.60
BC	2019	July	25	7/25/2019 RG_GC-NPM-16_20190725	Gold Creek	2.7	1.4	50.2	46.0	1200.0	-	18.3	-	-	1.53
BC	2019	June	20	6/20/2019 RG_SC-NPM-01_20190620	Sand Creek	8.4	2.2	43.5	39.3	740.0	-	26.9	-	-	3.64
BC	2019	June	20	6/20/2019 RG_SC-NPM-02_20190620	Sand Creek	20.0	1.8	42.8	38.0	600.0	-	3.2	-	-	0.53

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_SC-NPM-03_20190620	Sand Creek	11.0	2.0	34.6	31.6	340.0	-	2.0	-	-	0.58
BC	2019	June	20	6/20/2019 RG_SC-NPM-04_20190620	Sand Creek	17.0	2.6	41.9	38.9	640.0	-	20.5	-	-	3.20
BC	2019	June	20	6/20/2019 RG_SC-NPM-05_20190620	Sand Creek	28.0	2.0	39.2	35.5	490.0	-	3.1	-	-	0.62
BC	2019	July	24	7/24/2019 RG_SC-NPM-06_20190724	Sand Creek	10.0	1.4	43.5	34.2	495.0	-	7.2	-	-	1.46
BC	2019	July	25	7/25/2019 RG_SC-NPM-07_20190725	Sand Creek	21.0	1.7	38.4	35.0	525.0	-	5.7	-	-	1.09
BC	2019	July	25	7/25/2019 RG_SC-NPM-08_20190725	Sand Creek	23.0	2.4	40.3	37.3	540.0	-	8.6	-	-	1.59
BC	2019	July	25	7/25/2019 RG_SC-NPM-09_20190725	Sand Creek	12.0	1.5	44.3	39.9	790.0	-	10.6	-	-	1.34
BC	2019	July	26	7/26/2019 RG_SC-NPM-10_20190726	Sand Creek	25.0	1.4	39.8	35.7	510.0	-	2.4	-	-	0.47
BC	2019	July	26	7/26/2019 RG_SC-NPM-11_20190726	Sand Creek	23.0	2.2	37.9	34.2	495.0	-	4.6	-	-	0.92
BC	2019	June	21	6/21/2019 RG_WB-NPM-01_20190621	Waldo Bay	7.4	2.2	38.0	34.5	406.0	-	16.4	-	-	4.04
BC	2019	June	26	6/26/2019 RG-WB-NPM-02_20190626	Waldo Bay	26.0	2.9	39.9	35.9	480.0	-	5.7	-	-	1.19
BC	2019	June	26	6/26/2019 RG-WB-NPM-03_20190626	Waldo Bay	9.8	2.9	37.5	33.7	440.0	-	4.6	-	-	1.04
MT	2019	May	15	Rexford_NSC_01	Rexford	3.9	1.7	40.5	37.0	540.0	-	5.3	7.4	527.28	0.99
MT	2019	May	15	Rexford_NSC_02	Rexford	2.5	1.0	54.5	50.3	1785.0	-	49.7	32.04	1703.24	2.79
MT	2019	May	15	Rexford_NSC_03	Rexford	5.1	1.5	39.9	36.2	495.0	-	3.1	7.74	484.17	0.62
MT	2019	May	15	Rexford_NSC_04	Rexford	2.2	1.2	50.3	46.0	1500.0	-	41.0	29.007	1430.033	2.73
MT	2019	May	15	Rexford_NSC_05	Rexford	1.8	1.1	60.3	54.8	2060.0	-	68.2	29.69	1962.14	3.31
MT	2019	May	15	Rexford_NSC_06	Rexford	3.5	1.4	42.6	38.8	760.0	-	5.5	27.71	726.76	0.73
MT	2019	May	15	Rexford_NSC_07	Rexford	3.2	1.3	46.9	42.5	1070.0	-	7.1	20.16	1042.76	0.66
MT	2019	May	15	Rexford_NSC_08	Rexford	3.5	1.4	40.9	36.4	610.0	-	4.8	13.94	591.22	0.79
MT	2019	May	15	Rexford_NSC_09	Rexford	2.6	0.8	56.1	51.3	1620.0	-	60.3	28.73	1530.992	3.72
MT	2019	May	15	Rexford_NSC_10	Rexford	2.0	1.1	49.9	44.9	1200.0	-	30.9	14.03	1155.03	2.58
MT	2019	May	15	Rexford_NSC_11	Rexford	9.5	1.6	37.4	33.5	475.0	-	4.7	7.27	463.01	0.99
MT	2019	May	15	Rexford_NSC_12	Rexford	5.1	1.0	46.7	42.0	940.0	-	10.8	16.07	913.11	1.15
MT	2019	May	15	Rexford_NSC_13	Rexford	2.0	1.0	44.4	40.1	890.0	-	13.1	22.17	854.689	1.48
MT	2019	May	15	Rexford_NSC_14	Rexford	2.8	0.9	51.4	47.2	1540.0	-	42.4	20.822	1476.813	2.75
MT	2019	May	15	Rexford_NSC_15	Rexford	2.2	0.9	54.0	49.3	1490.0	-	53.9	21.269	1414.834	3.62

Appendix C. Northen Pikeminnow Tissue Data: 2019

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG-ER-NPM-32-BF-20190709	Eggs	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	0.01	2.2	21	<0.01	0.6	0.037	0.03	<0.05	2.8	<0.01	0.10	<0.005	<0.05	<0.2	<0.005	<0.1	66	64.02
7/9/2019 RG-ER-NPM-33-BF-20190709	Eggs	<5	<0.02	<0.05	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.6	56	<0.05	0.9	0.04	<0.05	<0.5	3.0	<0.02	0.2	<0.01	<0.2	<0.5	<0.02	<0.2	80	65.11
7/12/2019 RG-ER-NPM-43-BF-20190712	Eggs	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.02	2.9	45	<0.01	2.4	0.046	0.04	0.07	11.7	<0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	69	64.87
7/13/2019 RG-ER-NPM-46-BF-20190713	Eggs	<2	<0.01	0.11	0.13	<0.01	<1	<0.01	<0.05	0.02	2.1	30	<0.01	0.3	0.042	0.03	<0.05	2.7	<0.01	0.10	0.010	<0.05	<0.2	<0.005	<0.1	98	63.44
7/16/2019 RG-ER-NPM-48-BF-20190716	Eggs	<2	<0.01	0.10	0.36	<0.01	<1	<0.01	0.06	0.02	2.6	22	<0.01	0.8	0.051	0.04	<0.05	2.3	<0.01	0.15	0.009	<0.05	<0.2	<0.005	<0.1	77	69.18
7/3/2019 RG-ER-NPM-28-AF-20190703	Eggs	120	<0.01	0.04	1.2	<0.01	<1	<0.01	0.06	0.04	2.4	74	0.07	2.9	0.019	0.04	0.10	8.4	<0.01	2.9	<0.005	<0.05	1.0	0.031	<0.1	77	95.04
7/9/2019 RG-ER-NPM-32-AF-20190709	Eggs	260	<0.02	0.17	2.6	<0.02	<2	<0.02	<0.1	0.04	2.2	140	0.51	7.4	0.03	<0.05	0.2	2.9	<0.02	3.0	<0.01	<0.1	4.3	0.05	<0.2	68	96.25
7/13/2019 RG-ER-NPM-46-AF-20190713	Eggs	100	<0.01	0.08	3.3	<0.01	<1	<0.01	0.07	0.04	2.2	130	0.20	12	0.041	0.05	0.10	2.3	<0.01	2.6	0.011	<0.05	3.8	0.028	<0.1	88	94.15
7/15/2019 RG-ER-NPM-47-AF-20190715	Eggs	60	<0.1	<0.5	<5	<0.02	<50	<0.02	<5	<5	<5	<50	<0.5	<5	0.07	<0.5	<5	1.8	<0.02	1	<0.1	<2	<5	<0.1	<1	70	94.79
7/16/2019 RG-ER-NPM-48-AF-20190716	Eggs	560	0.01	0.31	15	0.02	<1	0.01	0.09	0.11	2.6	760	1.1	85	0.048	0.12	0.21	2.7	0.01	6.7	0.015	<0.05	24	0.14	0.4	86	91.84
7/3/2019 RG-ER-NPM-28-PF_20190703	Eggs	<5	<0.02	0.25	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.1	34	<0.05	2.3	0.02	<0.05	<0.5	9.6	<0.02	0.1	<0.01	<0.2	<0.5	<0.02	<0.2	62	66.93
6/14/2019 RG_ER-NPM-01-M_20190614	Muscle	2	<0.01	0.15	0.25	<0.01	<1	<0.01	0.24	0.01	2.0	25	<0.01	0.3	1.3	<0.02	0.05	1.6	<0.01	3.8	0.016	<0.05	<0.2	<0.005	<0.1	19	72.64
6/14/2019 RG_ER-NPM-02-M_20190614	Muscle	<2	<0.01	0.21	0.95	<0.01	<1	<0.01	<0.05	0.02	2.6	24	0.02	0.8	2.2	<0.02	<0.05	1.3	<0.01	6.2	0.014	<0.05	<0.2	<0.005	<0.1	33	72.76
6/17/2019 RG_ER-NPM-03-M_20190617	Muscle	<2	<0.01	0.22	0.92	<0.01	<1	<0.01	0.15	0.02	3.5	33	0.06	0.6	1.2	<0.02	<0.05	1.2	<0.01	3.6	0.013	<0.05	<0.2	<0.005	<0.1	28	71.78
6/17/2019 RG_ER-NPM-04-M_20190617	Muscle	<2	<0.01	0.16	0.90	<0.01	<1	<0.01	<0.05	0.02	2.6	26	<0.01	0.6	0.66	<0.02	<0.05	1.2	<0.01	3.0	0.021	<0.05	0.2	<0.005	<0.1	22	71.16
6/18/2019 RG_ER-NPM-05-M_20190618	Muscle	<2	<0.01	0.04	0.72	<0.01	<1	<0.01	0.20	0.01	0.85	9	<0.01	0.6	1.7	<0.02	<0.05	1.6	<0.01	3.6	0.010	<0.05	<0.2	<0.005	<0.1	19	77.76
6/18/2019 RG_ER-NPM-06-M_20190618	Muscle	<2	<0.01	0.07	0.58	<0.01	<1	<0.01	<0.05	0.03	0.79	8	<0.01	0.8	2.1	<0.02	<0.05	1.6	<0.01	3.0	0.008	<0.05	<0.2	<0.005	<0.1	17	78.52
6/18/2019 RG_ER-NPM-07-M_20190618	Muscle	<2	<0.01	0.17	1.2	<0.01	<1	<0.01	<0.05	0.02	2.4	23	<0.01	1.0	0.90	<0.02	0.08	1.3	<0.01	5.7	0.013	<0.05	<0.2	<0.005	<0.1	24	72.59
6/18/2019 RG_ER-NPM-08-M_20190618	Muscle	<2	<0.01	0.03	0.75	<0.01	<1	<0.01	<0.05	0.01	0.95	13	0.01	0.4	1.9	<0.02	<0.05	1.6	<0.01	3.1	0.009	<0.05	<0.2	<0.005	<0.1	22	78.06
6/19/2019 RG_ER-NPM-09-M_20190619	Muscle	3	<0.01	0.06	1.7	<0.01	<1	<0.01	<0.05	0.03	2.5	27	0.09	1.2	0.94	<0.02	0.07	2.0	<0.01	8.5	0.017	<0.05	<0.2	<0.005	<0.1	38	76.79
6/19/2019 RG_ER-NPM-10-M_20190619	Muscle	<2	<0.01	0.03	0.95	<0.01	<1	<0.01	<0.05	0.02	1.7	16	0.02	0.7	0.98	<0.02	<0.05	1.9	<0.01	3.0	0.009	<0.05	<0.2	<0.005	<0.1	29	76.48
6/19/2019 RG_ER-NPM-11-M_20190619	Muscle	<2	<0.01	0.07	0.36	<0.01	<1	<0.01	<0.05	0.01	1.5	16	0.03	0.6	0.87	<0.02	<0.05	2.5	<0.01	1.4	0.012	<0.05	<0.2	<0.005	<0.1	18	78.43
6/20/2019 RG_ER-NPM-12-M_20190620	Muscle	<2	<0.01	0.17	0.20	<0.01	<1	<0.01	<0.05	<0.01	0.96	9	0.01	0.2	1.5	<0.02	<0.05	1.3	<0.01	0.61	0.010	<0.05	<0.2	<0.005	<0.1	15	72.41
6/20/2019 RG_ER-NPM-13-M_20190620	Muscle	<2	<0.01	0.08	0.78	<0.01	<1	<0.01	<0.05	0.02	1.1	11	0.01	0.7	1.5	<0.02	<0.05	1.5	<0.01	4.5	0.006	<0.05	<0.2	<0.005	<0.1	19	75.51
6/20/2019 RG_ER-NPM-14-M_20190620	Muscle	<2	<0.01	0.14	0.65	<0.01	<1	<0.01	<0.05	0.01	1.1	11	<0.01	0.6	1.3	<0.02	<0.05	1.7	<0.01	2.6	0.010	<0.05	<0.2	<0.005	<0.1	16	74.20
6/20/2019 RG_ER-NPM-15-M_20190620	Muscle	<2	<0.01	0.02	0.85	<0.01	<1	<0.01	<0.05	0.01	0.92	14	0.02	0.9	1.1	<0.02	<0.05	2.5	<0.01	2.0	0.008	<0.05	<0.2	<0.005	<0.1	24	79.46
6/20/2019 RG_ER-NPM-16-M_20190620	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	0.09	0.03	1.9	29	0.01	1.3	1.0	<0.02	<0.05	2.4	<0.01	4.8	0.011	<0.05	<0.2	<0.005	<0.1	34	80.14
6/20/2019 RG_ER-NPM-17-M_20190620	Muscle	<2	<0.01	0.08	1.0	<0.01	<1	<0.01	<0.05	0.02	2.2	22	<0.01	1.0	1.1	<0.02	<0.05	1.8	<0.01	3.7	0.013	<0.05	<0.2	<0.005	<0.1	26	78.24
6/20/2019 RG_ER-NPM-18-M_20190620	Muscle	<2	<0.01	0.07	0.46	<0.01	<1	<0.01	<0.05	<0.01	0.83	7	<0.01	0.4	1.7	<0.02	<0.05	2.5	<0.01	1.7	<0.005	<0.05	<0.2	<0.005	<0.1	20	74.74
6/25/2019 RG_ER-NPM-19-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.02	1.8	17	<0.01	1.0	0.41	<0.02	<0.05	1.9	<0.01	2.2	0.021	<0.05	<0.2	<0.005	<0.1	38	78.49
6/25/2019 RG_ER-NPM-20-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.03	2.7	27	0.01	1.4	0.92	<0.02	<0.05	2.9	<0.01	7.1	0.018	<0.05	<0.2	<0.005	<0.1	29	76.00
6/27/2019 RG_ER-NPM-21-M_20190627	Muscle	<2	<0.01	0.04	1.3	<0.01	<1	<0.01	<0.05	0.02	2.6	35	<0.01	1.9	0.78	<0.02	<0.05	3.4	<0.01	7.1	0.011	<0.05	<0.2	<0.005	<0.1	28	76.67
6/28/2019 RG_ER-NPM-22-M_20190628	Muscle	<2	<0.01	0.02	0.84	<0.01	<1	<0.01	<0.05	0.02	0.92	9	<0.01	1.2	0.61	<0.02	0.05	2.6	<0.01	3.3	0.016	<0.05	<0.2	<0.005	<0.1	18	76.61
6/28/2019 RG_ER-NPM-23-M_20190628	Muscle	<2	<0.01	0.01	2.4	<0.01	<1	<0.01	0.08	0.02	2.3	21	<0.01	1.3	1.9	<0.02	<0.05	3.6	<0.01	3.4	0.012	<0.05	<0.2	<0.005	<0.1	25	74.79
6/28/2019 RG_ER-NPM-24-M_20190628	Muscle	<2	<0.01	0.06	2.0	<0.01	<1	<0.01	0.22	0.02	1.5	20	<0.01	1.4	0.88	<0.02	<0.05	2.4	<0.01	8.4	0.008	<0.05	<0.2	<0.005	<0.1	28	78.90
6/28/2019 RG_ER-NPM-25-M_20190628	Muscle	<2	<0.01	0.02	0.50	<0.01	<1	<0.01	0.06	<0.01	1.1	10	<0.01	0.7	1.0	<0.02	<0.05	2.5	<0.01	1.5	0.012	<0.05	<0.2	<0.005	<0.1	17	75.15
6/28/2019 RG_ER-NPM-26-M_20190628	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	0.07	0.02	1.7	21	<0.01	1.5	1.1	<0.02	<0.05	1.1	<0.01	7.1	0.010	<0.05	<0.2	<0.005	<0.1	24	77.37
6/28/2019 RG_ER-NPM-27-M_20190628	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	0.02	0.83	11	<0.01	1.2	0.35	<0.02	<0.05	2.3	<0.01	4.7	0.013	<0.05	<0.2	<0.005	<0.1	22	77.97
7/3/2019 RG_ER-NPM-28-M_20190703	Muscle	<2	<0.01	0.03	1.1	<0.01	<1	<0.01	<0.05	0.01	1.2	12	0.02	1.4	0.86	<0.02	<0.05	2.3	<0.01	4.7	0.010	<0.05	<0.2	<0.005	<0.1	19	76.85
7/4/2019 RG_ER-NPM-29-M_20190704	Muscle	<2	<0.01	0.03	0.37	<0.01	<1	<0.01	<0.05	0.01	1.7	21	<0.01	0.6	0.82	<0.02	<0.05	4.6	<0.01	0.75	0.011	<0.05	<0.2	<0.005	<0.1	21	78.32

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/4/2019 RG_ER-NPM-30-M_20190704	Muscle	<2	<0.01	0.03	0.58	<0.01	<1	<0.01	<0.05	<0.01	0.73	9	0.02	0.8	1.6	<0.02	<0.05	2.7	<0.01	2.3	0.006	<0.05	<0.2	<0.005	<0.1	15	77.17
7/8/2019 RG_ER-NPM-31-M_20190708	Muscle	3	<0.01	0.04	1.4	<0.01	<1	<0.01	13	0.05	2.3	170	0.02	1.6	0.72	<0.02	0.40	3.1	<0.01	2.7	0.019	<0.05	<0.2	<0.005	<0.1	33	78.99
7/9/2019 RG_ER-NPM-32-M_20190709	Muscle	<2	<0.01	0.12	0.77	<0.01	<1	<0.01	0.77	0.02	1.8	26	<0.01	0.5	1.2	<0.02	<0.05	1.3	<0.01	3.2	0.010	<0.05	<0.2	<0.005	<0.1	25	72.74
7/9/2019 RG_ER-NPM-33-M_20190709	Muscle	<2	<0.01	0.11	0.92	<0.01	<1	<0.01	0.11	0.02	2.2	21	<0.01	0.8	0.76	<0.02	<0.05	1.4	<0.01	3.9	0.021	<0.05	<0.2	<0.005	<0.1	26	75.45
7/9/2019 RG_ER-NPM-34-M_20190709	Muscle	<2	<0.01	0.12	1.5	<0.01	<1	<0.01	0.17	0.02	2.0	20	<0.01	1.1	1.4	<0.02	<0.05	1.6	<0.01	5.4	0.011	<0.05	<0.2	<0.005	<0.1	28	74.77
7/9/2019 RG_ER-NPM-35-M_20190709	Muscle	<2	<0.01	0.32	1.8	<0.01	<1	<0.01	0.14	0.02	2.2	24	<0.01	1.3	1.0	<0.02	<0.05	1.2	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	31	77.08
7/9/2019 RG_ER-NPM-36-M_20190709	Muscle	<2	<0.01	0.06	1.4	<0.01	<1	<0.01	0.17	0.03	2.7	25	<0.01	1.4	2.0	<0.02	<0.05	3.9	<0.01	4.3	0.020	<0.05	<0.2	<0.005	<0.1	34	78.53
7/9/2019 RG_ER-NPM-37-M_20190709	Muscle	4	<0.01	0.11	1.8	<0.01	<1	<0.01	0.07	0.03	2.2	26	0.02	2.0	0.87	<0.02	<0.05	2.2	<0.01	6.6	0.009	<0.05	<0.2	<0.005	<0.1	33	79.86
7/10/2019 RG_ER-NPM-38-M_20190710	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	0.10	0.02	2.4	25	0.02	1.4	0.80	<0.02	0.05	4.0	<0.01	4.0	0.011	<0.05	<0.2	<0.005	<0.1	29	78.21
7/10/2019 RG_ER-NPM-39-M_20190710	Muscle	<2	<0.01	0.11	0.46	<0.01	<1	<0.01	0.06	0.01	1.6	15	<0.01	0.5	0.92	<0.02	<0.05	1.2	<0.01	1.7	0.027	<0.05	<0.2	<0.005	<0.1	20	76.75
7/10/2019 RG_ER-NPM-40-M_20190710	Muscle	5	<0.01	0.09	0.74	<0.01	<1	<0.01	0.11	0.02	1.6	27	0.04	0.9	0.66	<0.02	0.09	3.0	<0.01	1.2	0.010	<0.05	<0.2	<0.005	<0.1	32	79.51
7/10/2019 RG_ER-NPM-41-M_20190710	Muscle	<2	<0.01	0.12	0.91	<0.01	<1	<0.01	0.18	0.01	1.0	11	<0.01	0.8	0.87	<0.02	<0.05	1.3	<0.01	3.1	0.024	<0.05	<0.2	<0.005	<0.1	21	76.75
7/10/2019 RG_ER-NPM-42-M_20190710	Muscle	<2	<0.01	0.03	1.5	<0.01	<1	<0.01	0.08	0.02	1.7	15	<0.01	1.4	0.92	<0.02	<0.05	3.4	<0.01	5.2	0.009	<0.05	<0.2	<0.005	<0.1	29	78.08
7/11/2019 RG_ER-NPM-43-M_20190711	Muscle	<2	<0.01	0.05	1.1	<0.01	<1	<0.01	0.13	0.02	2.6	17	0.02	1.3	0.69	<0.02	<0.05	4.8	<0.01	4.0	0.022	<0.05	<0.2	<0.005	<0.1	26	79.26
7/12/2019 RG_ER-NPM-44-M_20190712	Muscle	<2	<0.01	0.05	0.14	<0.01	<1	<0.01	<0.05	<0.01	0.73	10	<0.01	0.5	1.1	<0.02	<0.05	5.0	<0.01	0.10	0.008	<0.05	<0.2	<0.005	<0.1	17	78.91
7/12/2019 RG_ER-NPM-45-M_20190712	Muscle	<2	<0.01	0.10	0.19	<0.01	<1	<0.01	0.05	<0.01	0.77	6	<0.01	0.2	1.0	<0.02	<0.05	1.2	<0.01	0.80	0.015	<0.05	<0.2	<0.005	<0.1	14	76.31
7/13/2019 RG_ER-NPM-46-M_20190713	Muscle	2	<0.01	0.19	0.60	<0.01	<1	<0.01	0.06	0.02	2.2	23	0.01	0.4	1.3	<0.02	<0.05	1.3	<0.01	2.8	0.022	<0.05	<0.2	<0.005	<0.1	28	75.43
7/15/2019 RG_ER-NPM-47-M_20190715	Muscle	<2	<0.01	0.17	1.0	<0.01	<1	<0.01	0.07	0.02	1.6	16	<0.01	0.7	1.1	<0.02	0.12	1.2	<0.01	5.2	0.014	<0.05	<0.2	<0.005	<0.1	26	76.36
7/16/2019 RG_ER-NPM-48-M_20190716	Muscle	<2	<0.01	0.14	1.2	<0.01	<1	<0.01	<0.05	0.02	1.4	12	<0.01	0.8	1.7	<0.02	<0.05	1.3	<0.01	4.9	0.021	<0.05	<0.2	<0.005	<0.1	27	78.26
7/26/2019 RG_ER-NPM-49-M_20190726	Muscle	<2	<0.01	0.09	0.71	<0.01	<1	<0.01	0.06	0.02	1.5	15	<0.01	0.6	1.6	<0.02	<0.05	1.4	<0.01	3.6	0.014	<0.05	<0.2	<0.005	<0.1	24	77.86
6/26/2019 RG_GC-NPM-01-M_20190626	Muscle	<2	<0.01	0.18	0.17	<0.01	<1	<0.01	0.05	<0.01	0.84	8	<0.01	0.1	2.6	<0.02	<0.05	1.2	<0.01	0.51	0.007	<0.05	<0.2	<0.005	<0.1	12	75.39
6/26/2019 RG_GC-NPM-02-M_20190626	Muscle	<2	<0.01	0.07	0.07	<0.01	<1	<0.01	<0.05	<0.01	0.75	7	<0.01	0.1	1.5	<0.02	<0.05	1.3	<0.01	0.12	0.015	<0.05	<0.2	<0.005	<0.1	15	77.80
6/26/2019 RG_GC-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	0.54	<0.01	<1	<0.01	<0.05	<0.01	0.68	7	<0.01	0.5	1.1	<0.02	<0.05	1.2	<0.01	2.3	0.010	<0.05	<0.2	<0.005	<0.1	18	77.49
6/26/2019 RG_GC-NPM-04-M_20190626	Muscle	<2	<0.01	0.06	0.60	<0.01	<1	<0.01	<0.05	<0.01	0.82	12	<0.01	0.8	1.1	<0.02	<0.05	2.4	<0.01	2.3	0.011	<0.05	<0.2	<0.005	<0.1	22	78.87
6/26/2019 RG_GC-NPM-05-M_20190626	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.8	20	0.01	1.4	1.1	<0.02	<0.05	1.7	<0.01	3.4	0.009	<0.05	<0.2	<0.005	<0.1	25	77.49
6/26/2019 RG_GC-NPM-06-M_20190626	Muscle	<2	<0.01	0.12	1.0	<0.01	<1	<0.01	<0.05	<0.01	1.7	17	<0.01	0.7	0.92	<0.02	<0.05	1.1	<0.01	3.8	0.021	<0.05	<0.2	<0.005	<0.1	22	75.90
6/26/2019 RG_GC-NPM-07-M_20190626	Muscle	<2	<0.01	0.02	1.1	<0.01	<1	<0.01	0.06	<0.01	1.3	20	0.01	1.2	1.1	<0.02	<0.05	2.1	<0.01	4.6	0.015	<0.05	<0.2	<0.005	<0.1	26	79.07
6/27/2019 RG_GC-NPM-08-M_20190627	Muscle	2	<0.01	0.10	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.4	12	0.23	0.7	1.2	<0.02	<0.05	1.2	<0.01	6.6	0.022	<0.05	0.4	<0.005	<0.1	31	75.59
6/27/2019 RG_GC-NPM-09-M_20190627	Muscle	2	<0.01	0.04	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.91	12	<0.01	0.2	1.5	<0.02	<0.05	1.4	<0.01	0.78	0.017	<0.05	<0.2	<0.005	<0.1	14	78.43
6/27/2019 RG_GC-NPM-10-M_20190627	Muscle	<2	<0.01	0.09	0.92	<0.01	<1	<0.01	<0.05	<0.01	1.6	13	<0.01	0.7	1.2	<0.02	<0.05	1.2	<0.01	4.8	0.022	<0.05	<0.2	<0.005	<0.1	20	75.35
6/27/2019 RG_GC-NPM-11-M_20190627	Muscle	<2	<0.01	0.09	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.63	10	<0.01	0.3	1.1	<0.02	<0.05	1.8	<0.01	0.60	0.005	<0.05	<0.2	<0.005	<0.1	14	77.35
6/27/2019 RG_GC-NPM-12-M_20190627	Muscle	<2	<0.01	0.10	1.3	<0.01	<1	<0.01	<0.05	<0.01	2.2	21	<0.01	1.4	1.0	<0.02	<0.05	1.4	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	26	78.38
6/27/2019 RG_GC-NPM-13-M_20190627	Muscle	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	<0.01	0.82	11	<0.01	0.3	1.6	<0.02	<0.05	2.0	<0.01	0.57	<0.005	<0.05	<0.2	<0.005	<0.1	14	78.91
7/18/2019 RG_GC-NPM-14-M_20190718	Muscle	<2	<0.01	0.11	0.78	<0.01	<1	<0.01	<0.05	<0.01	2.9	34	<0.01	0.4	1.7	<0.02	<0.05	1.5	<0.01	1.3	0.014	<0.05	<0.2	<0.005	<0.1	27	76.81
7/19/2019 RG_GC-NPM-15-M_20190719	Muscle	2	<0.01	0.04	0.44	<0.01	<1	<0.01	<0.05	<0.01	0.84	6	<0.01	0.3	2.6	<0.02	<0.05	1.4	<0.01	1.8	0.011	<0.05	<0.2	<0.005	<0.1	20	80.92
7/25/2019 RG_GC-NPM-16-M_20190725	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	<0.01	1.2	9	<0.01	0.8	1.3	<0.02	<0.05	1.4	<0.01	5.6	0.016	<0.05	<0.2	<0.005	<0.1	27	77.46
6/20/2019 RG_SC-NPM-01-M_20190620	Muscle	<2	<0.01	0.05	0.70	<0.01	<1	<0.01	<0.05	<0.01	2.5	31	<0.01	0.9	1.0	<0.02	<0.05	2.2	<0.01	2.7	0.013	<0.05	<0.2	<0.005	<0.1	27	78.73
6/20/2019 RG_SC-NPM-02-M_20190620	Muscle	<2	<0.01	0.03	0.69	<0.01	<1	<0.01	1.2	<0.01	1.2	24	<0.01	0.9	0.99	0.04	0.12	1.8	<0.01	3.8	0.010	<0.05	<0.2	<0.005	<0.1	23	80.68
6/20/2019 RG_SC-NPM-03-M_20190620	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	<0.05	<0.01	2.1	21	0.02	1.6	0.55	<0.02	<0.05	2.0	<0.01	5.1	0.012	<0.05	<0.2	<0.005	<0.1	34	79.58
6/20/2019 RG_SC-NPM-04-M_20190620	Muscle	<2	<0.01	0.07	0.77	<0.01	<1	<0.01	<0.05	<0.01	2.1	25	<0.01	0.8	1.1	<0.02	<0.05	2.6	<0.01	2.9	0.016	<0.05	<0.2	<0.005	<0.1	34	79.13
6/20/2019 RG_SC-NPM-05-M_20190620	Muscle	<2	<0.01	0.04	0.93	<0.01	<1	<0.01	<0.05	0.01	1.1	13	<0.01	0.9	0.81	<0.02	<0.05	2.0	<0.01	3.4	0.011	<0.05	<0.2	<0.005	<0.1	24	79.40

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/24/2019 RG_SC-NPM-06-M_20190724	Muscle	<2	<0.01	0.01	0.79	<0.01	<1	<0.01	0.32	0.01	0.85	17	<0.01	1.3	1.4	<0.02	<0.05	1.4	<0.01	4.8	<0.005	<0.05	<0.2	<0.005	<0.1	18	79.65
7/25/2019 RG_SC-NPM-07-M_20190725	Muscle	3	<0.01	0.06	1.1	<0.01	<1	<0.01	0.06	0.01	0.95	15	0.02	1.6	1.3	<0.02	<0.05	1.7	<0.01	4.7	0.007	<0.05	<0.2	<0.005	<0.1	44	80.43
7/25/2019 RG_SC-NPM-08-M_20190725	Muscle	<2	<0.01	0.10	1.0	<0.01	<1	<0.01	0.05	0.02	1.0	15	<0.01	1.8	1.3	<0.02	0.05	2.4	<0.01	6.6	0.014	<0.05	<0.2	<0.005	<0.1	24	78.64
7/25/2019 RG_SC-NPM-09-M_20190725	Muscle	<2	<0.01	0.02	0.24	<0.01	<1	<0.01	<0.05	0.01	1.8	19	<0.01	0.5	1.2	<0.02	<0.05	1.5	<0.01	1.1	0.006	<0.05	<0.2	<0.005	<0.1	19	76.51
7/26/2019 RG_SC-NPM-10-M_20190726	Muscle	<2	<0.01	0.07	0.39	<0.01	<1	<0.01	<0.05	0.01	0.76	10	<0.01	0.6	1.0	<0.02	0.06	1.4	<0.01	1.4	0.006	<0.05	<0.2	<0.005	<0.1	17	79.18
7/26/2019 RG_SC-NPM-11-M_20190726	Muscle	<2	<0.01	0.06	0.28	<0.01	<1	<0.01	<0.05	<0.01	1.1	14	<0.01	0.4	0.90	<0.02	<0.05	2.2	<0.01	1.1	0.013	<0.05	<0.2	<0.005	<0.1	21	79.53
6/21/2019 RG_WB-NPM-01-M_20190621	Muscle	<2	<0.01	0.02	0.97	<0.01	<1	<0.01	<0.05	0.02	1.7	23	0.04	0.9	1.1	<0.02	<0.05	2.2	<0.01	2.8	0.010	<0.05	<0.2	<0.005	<0.1	28	77.69
6/26/2019 RG-WB-NPM-02-M_20190626	Muscle	<2	<0.01	0.09	2.4	<0.01	<1	<0.01	<0.05	0.02	2.6	28	0.01	1.7	0.99	<0.02	<0.05	2.9	<0.01	9.4	0.015	<0.05	<0.2	<0.005	<0.1	44	79.29
6/26/2019 RG-WB-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	1.5	<0.01	<1	<0.01	<0.05	0.02	2.5	26	<0.01	1.5	1.0	<0.02	<0.05	2.9	<0.01	3.9	0.006	<0.05	<0.2	<0.005	<0.1	32	79.01
6/14/2019 RG_ER-NPM-01-O_20190614	Ovary	<2	<0.01	0.07	0.12	<0.01	<1	<0.01	<0.05	0.02	3.2	43	<0.01	0.6	0.10	0.05	<0.05	3.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	97	64.38
6/14/2019 RG_ER-NPM-02-O_20190614	Ovary	<2	<0.01	0.07	0.14	<0.01	<1	<0.01	<0.05	0.03	3.1	38	<0.01	3.0	0.16	0.06	<0.05	2.7	0.01	0.19	0.007	<0.05	<0.2	<0.005	<0.1	110	65.57
6/17/2019 RG_ER-NPM-03-O_20190617	Ovary	<2	<0.01	0.06	0.22	<0.01	<1	<0.01	<0.05	0.03	2.8	45	0.01	1.3	0.10	0.05	<0.05	3.3	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	94	65.63
6/17/2019 RG_ER-NPM-04-O_20190617	Ovary	<2	<0.01	0.09	0.13	<0.01	<1	<0.01	<0.05	0.02	2.8	41	<0.01	0.7	0.051	0.04	<0.05	3.0	<0.01	0.11	0.009	<0.05	<0.2	<0.005	<0.1	87	61.36
6/18/2019 RG_ER-NPM-05-O_20190618	Ovary	<2	<0.01	0.04	0.21	<0.01	<1	<0.01	<0.05	0.03	3.0	37	0.01	2.2	0.12	0.04	<0.05	4.9	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	78	62.62
6/18/2019 RG_ER-NPM-06-O_20190618	Ovary	<2	<0.01	0.26	0.14	<0.01	<1	<0.01	<0.05	0.03	2.6	52	<0.01	2.2	0.14	0.07	<0.05	5.0	<0.01	0.20	0.006	<0.05	<0.2	<0.005	<0.1	120	67.92
6/18/2019 RG_ER-NPM-07-O_20190618	Ovary	<2	<0.01	0.06	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	41	<0.01	1.3	0.11	0.04	<0.05	4.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	87	63.03
6/18/2019 RG_ER-NPM-08-O_20190618	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.03	2.3	91	0.01	2.6	0.13	0.08	<0.05	4.2	<0.01	0.27	0.009	<0.05	<0.2	<0.005	<0.1	120	67.65
6/19/2019 RG_ER-NPM-09-O_20190619	Ovary	3	<0.01	0.06	0.16	<0.01	<1	<0.01	<0.05	0.03	2.8	60	0.18	2.2	0.054	0.07	<0.05	7.2	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	68.24
6/19/2019 RG_ER-NPM-10-O_20190619	Ovary	2	<0.01	0.04	0.26	<0.01	<1	<0.01	0.21	0.05	2.8	96	0.13	3.4	0.17	0.14	<0.05	9.9	<0.01	0.32	0.023	<0.05	<0.2	<0.005	<0.1	260	76.39
6/19/2019 RG_ER-NPM-11-O_20190619	Ovary	4	<0.01	0.06	0.32	<0.01	<1	0.01	0.11	0.06	4.1	130	0.18	2.7	0.096	0.12	0.06	17	<0.01	0.41	0.049	<0.05	<0.2	<0.005	<0.1	500	79.40
6/20/2019 RG_ER-NPM-12-O_20190620	Ovary	<2	<0.01	0.07	0.15	<0.01	<1	<0.01	<0.05	0.02	3.0	33	<0.01	0.5	0.081	0.04	<0.05	2.4	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	84	63.59
6/20/2019 RG_ER-NPM-13-O_20190620	Ovary	<2	<0.01	0.06	0.14	<0.01	<1	<0.01	<0.05	0.03	2.4	54	<0.01	1.9	0.23	0.06	<0.05	3.6	<0.01	0.22	0.008	<0.05	<0.2	<0.005	<0.1	120	71.25
6/20/2019 RG_ER-NPM-14-O_20190620	Ovary	<2	<0.01	0.06	0.12	<0.01	<1	<0.01	<0.05	0.04	2.6	60	<0.01	1.8	0.21	0.08	<0.05	7.6	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	72.31
6/20/2019 RG_ER-NPM-15-O_20190620	Ovary	<2	<0.01	0.04	0.36	<0.01	<1	<0.01	<0.05	0.05	2.9	95	<0.01	5.5	0.078	0.10	<0.05	8.6	<0.01	0.24	0.009	<0.05	<0.2	<0.005	<0.1	150	70.05
6/20/2019 RG_ER-NPM-16-O_20190620	Ovary	2	<0.01	0.08	0.35	<0.01	<1	0.01	0.09	0.07	3.6	150	0.02	2.5	0.13	0.12	0.06	17	<0.01	0.26	0.033	<0.05	<0.2	<0.005	<0.1	520	83.66
6/20/2019 RG_ER-NPM-17-O_20190620	Ovary	<2	<0.01	0.07	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	2.3	0.085	0.04	<0.05	4.5	<0.01	0.16	0.005	<0.05	<0.2	<0.005	<0.1	74	65.19
6/20/2019 RG_ER-NPM-18-O_20190620	Ovary	<2	<0.01	0.05	0.22	<0.01	<1	<0.01	<0.05	0.02	1.8	32	<0.01	2.5	0.14	0.04	<0.05	4.1	<0.01	0.17	<0.005	<0.05	<0.2	<0.005	<0.1	80	65.88
6/25/2019 RG_ER-NPM-19-O_20190625	Ovary	<2	<0.01	0.08	0.38	<0.01	<1	<0.01	<0.05	0.04	2.6	66	<0.01	8.3	0.024	0.07	<0.05	7.9	<0.01	0.17	0.018	<0.05	<0.2	<0.005	<0.1	120	66.61
6/25/2019 RG_ER-NPM-20-O_20190625	Ovary	<2	<0.01	0.04	0.08	<0.01	<1	<0.01	<0.05	0.06	3.2	110	<0.01	2.6	0.12	0.08	<0.05	6.3	<0.01	0.21	0.030	<0.05	<0.2	<0.005	<0.1	180	75.96
6/27/2019 RG_ER-NPM-21-O_20190627	Ovary	<2	<0.01	0.03	0.11	<0.01	<1	<0.01	<0.05	0.02	2.7	49	<0.01	2.3	0.039	0.03	<0.05	13	<0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	80	60.36
6/28/2019 RG_ER-NPM-22-O_20190628	Ovary	<2	<0.01	0.05	0.17	<0.01	<1	<0.01	<0.05	0.02	2.2	37	<0.01	2.5	0.021	0.03	<0.05	7.1	<0.01	0.09	0.008	<0.05	<0.2	<0.005	<0.1	79	61.41
6/28/2019 RG_ER-NPM-23-O_20190628	Ovary	<2	<0.01	0.01	0.32	<0.01	<1	<0.01	<0.05	0.03	2.7	44	<0.01	4.1	0.12	0.04	<0.05	9.8	0.02	0.14	0.006	<0.05	<0.2	<0.005	<0.1	100	65.42
6/28/2019 RG_ER-NPM-24-O_20190628	Ovary	<2	<0.01	0.05	0.19	<0.01	<1	<0.01	<0.05	0.04	3.1	55	<0.01	1.5	0.12	0.05	<0.05	7.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	120	67.86
6/28/2019 RG_ER-NPM-25-O_20190628	Ovary	<2	<0.01	0.02	0.22	<0.01	<1	<0.01	<0.05	0.03	3.3	51	<0.01	4.7	0.050	0.06	<0.05	8.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	97	63.20
6/28/2019 RG_ER-NPM-26-O_20190628	Ovary	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	0.02	3.0	54	<0.01	2.1	0.037	0.04	<0.05	4.0	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	77	62.47
6/28/2019 RG_ER-NPM-27-O_20190628	Ovary	<2	<0.01	0.86	1.5	<0.01	<1	0.10	0.06	0.06	1.5	47	0.07	0.4	0.023	<0.02	0.06	14.6	<0.01	0.10	0.021	<0.05	<0.2	<0.005	<0.1	300	56.47
7/3/2019 RG_ER-NPM-28-O_20190703	Ovary	4	<0.01	0.03	0.20	<0.01	<1	0.01	<0.05	0.04	2.8	170	0.06	2.8	0.088	0.08	<0.05	34.5	<0.01	0.27	0.018	<0.05	<0.2	<0.005	<0.1	180	77.60
7/4/2019 RG_ER-NPM-29-O_20190704	Ovary	6	<0.01	0.05	0.39	<0.01	<1	0.04	0.22	0.07	3.9	150	0.06	2.8	0.097	0.15	0.10	19.4	<0.01	0.57	0.034	<0.05	0.3	<0.005	<0.1	330	77.92
7/4/2019 RG_ER-NPM-30-O_20190704	Ovary	14	<0.01	0.04	0.44	<0.01	<1	0.02	0.05	0.05	4.2	110	0.15	3.7	0.11	0.07	<0.05	7.1	<0.01	0.36	0.014	<0.05	0.3	<0.005	0.1	200	76.76
7/8/2019 RG_ER-NPM-31-O_20190708	Ovary	<2	<0.01	0.04	0.20	<0.01	<1	<0.01	0.09	0.03	3.3	64	<0.01	3.5	0.068	0.05	<0.05	10.9	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	100	65.74
7/9/2019 RG_ER-NPM-32-O_20190709	Ovary	<2	<0.01	0.08	0.13	<0.01	<1	<0.01	0.10	0.04	3.3	81	0.01	1.2	0.13	0.08	<0.05	4.1	<0.01	0.36	0.016	<0.05	<0.2	<0.005	<0.1	160	74.41

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG_ER-NPM-33-O_20190709	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.6	47	<0.01	0.9	0.030	<0.02	<0.05	3.5	<0.01	0.18	0.010	<0.05	<0.2	<0.005	<0.1	97	63.66
7/9/2019 RG_ER-NPM-34-O_20190709	Ovary	3	<0.01	0.07	0.13	<0.01	<1	0.01	<0.05	0.05	3.2	77	<0.01	3.1	0.21	0.08	<0.05	5.4	<0.01	0.24	0.029	<0.05	<0.2	<0.005	0.1	260	76.24
7/9/2019 RG_ER-NPM-35-O_20190709	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.02	2.4	57	<0.01	1.1	0.056	0.04	<0.05	3.8	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	82	65.42
7/9/2019 RG_ER-NPM-36-O_20190709	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	0.02	3.0	43	<0.01	2.4	0.12	0.04	<0.05	9.3	0.01	0.10	0.006	<0.05	<0.2	<0.005	<0.1	78	62.62
7/9/2019 RG_ER-NPM-37-O_20190709	Ovary	<2	<0.01	0.06	0.23	<0.01	<1	<0.01	0.21	0.03	3.1	48	<0.01	1.5	0.050	0.05	<0.05	5.4	<0.01	0.24	<0.005	<0.05	<0.2	<0.005	<0.1	92	63.31
7/10/2019 RG_ER-NPM-38-O_20190710	Ovary	3	<0.01	0.04	0.19	<0.01	<1	<0.01	<0.05	0.04	3.6	57	<0.01	5.9	0.071	0.08	<0.05	12	0.01	0.28	0.007	<0.05	<0.2	<0.005	<0.1	130	69.92
7/10/2019 RG_ER-NPM-39-O_20190710	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.02	3.3	48	<0.01	2.0	0.038	0.04	<0.05	2.7	0.01	0.14	0.010	<0.05	<0.2	<0.005	<0.1	110	62.30
7/10/2019 RG_ER-NPM-40-O_20190710	Ovary	5	<0.01	0.06	0.35	<0.01	<1	0.02	0.12	0.04	4.0	110	0.07	1.4	0.060	0.07	0.06	18.4	<0.01	0.24	0.036	<0.05	0.2	<0.005	<0.1	540	79.88
7/10/2019 RG_ER-NPM-41-O_20190710	Ovary	<2	<0.01	0.07	0.17	<0.01	<1	<0.01	<0.05	0.02	2.6	52	<0.01	1.3	0.030	0.05	<0.05	3.4	<0.01	0.13	0.015	<0.05	<0.2	<0.005	<0.1	110	64.12
7/10/2019 RG_ER-NPM-42-O_20190710	Ovary	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.03	3.2	55	<0.01	5.1	0.079	0.07	<0.05	11	0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	100	65.47
7/11/2019 RG_ER-NPM-43-O_20190711	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.03	3.0	42	<0.01	2.5	0.049	0.04	<0.05	12	0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	78	64.30
7/12/2019 RG_ER-NPM-44-O_20190712	Ovary	3	<0.01	0.05	0.15	<0.01	<1	0.01	0.11	0.06	3.6	110	0.02	2.9	0.068	0.14	<0.05	36	<0.01	0.34	0.032	<0.05	<0.2	<0.005	<0.1	410	78.06
7/12/2019 RG_ER-NPM-45-O_20190712	Ovary	<2	<0.01	0.09	0.22	<0.01	<1	<0.01	<0.05	0.02	2.7	33	<0.01	0.5	0.049	0.04	<0.05	2.2	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	91	62.46
7/13/2019 RG_ER-NPM-46-O_20190713	Ovary	<2	<0.01	0.12	0.07	<0.01	<1	<0.01	<0.05	0.04	3.3	91	<0.01	0.6	0.17	0.09	<0.05	2.4	<0.01	0.22	0.046	<0.05	<0.2	<0.005	<0.1	210	75.90
7/15/2019 RG_ER-NPM-47-O_20190715	Ovary	<2	<0.01	0.12	0.14	<0.01	<1	<0.01	<0.05	0.02	2.5	31	<0.01	0.7	0.091	0.03	<0.05	2.1	<0.01	0.12	0.011	<0.05	<0.2	<0.005	<0.1	99	66.82
7/16/2019 RG_ER-NPM-48-O_20190716	Ovary	<2	<0.01	0.11	0.06	<0.01	<1	0.01	<0.05	0.05	3.7	69	<0.01	1.4	0.37	0.11	<0.05	2.3	<0.01	0.33	0.059	<0.05	<0.2	<0.005	<0.1	230	81.71
7/26/2019 RG_ER-NPM-49-O_20190726	Ovary	2	<0.01	0.15	0.32	<0.01	<1	0.02	0.06	0.05	3.7	240	<0.01	1.2	0.23	0.07	<0.05	3.4	<0.01	0.43	0.051	<0.05	<0.2	0.008	0.1	380	82.16
6/26/2019 RG_GC-NPM-01-O_20190626	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	41	<0.01	0.6	0.14	0.05	<0.05	2.4	<0.01	0.17	0.005	<0.05	<0.2	<0.005	<0.1	100	64.51
6/26/2019 RG_GC-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	0.6	0.096	0.06	<0.05	2.1	<0.01	0.14	0.012	<0.05	<0.2	<0.005	<0.1	110	65.08
6/26/2019 RG_GC-NPM-03-O_20190626	Ovary	2	<0.01	0.05	0.18	<0.01	<1	<0.01	0.40	0.04	3.1	47	<0.01	0.6	0.049	0.04	<0.05	2.1	<0.01	0.12	0.006	<0.05	<0.2	<0.005	<0.1	77	63.05
6/26/2019 RG_GC-NPM-04-O_20190626	Ovary	<2	<0.01	0.17	0.19	<0.01	<1	<0.01	0.07	<0.01	2.5	75	0.01	1.1	0.096	0.04	<0.05	20	<0.01	0.21	0.032	<0.05	<0.2	<0.005	<0.1	360	72.82
6/26/2019 RG_GC-NPM-05-O_20190626	Ovary	<2	<0.01	0.03	0.26	<0.01	<1	<0.01	<0.05	<0.01	2.2	46	<0.01	1.9	0.076	0.03	<0.05	3.9	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	83	63.49
6/26/2019 RG_GC-NPM-06-O_20190626	Ovary	<2	<0.01	0.06	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.7	40	<0.01	1.9	0.048	0.04	<0.05	2.4	<0.01	0.12	0.012	<0.05	<0.2	<0.005	<0.1	100	63.02
6/26/2019 RG_GC-NPM-07-O_20190626	Ovary	<2	<0.01	0.11	0.13	<0.01	<1	0.01	0.12	<0.01	3.2	120	0.01	1.4	0.082	0.08	<0.05	11	<0.01	0.19	0.047	<0.05	<0.2	<0.005	<0.1	360	77.70
6/27/2019 RG_GC-NPM-08-O_20190627	Ovary	<2	<0.01	0.15	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	30	<0.01	0.4	0.078	0.03	<0.05	2.2	<0.01	0.11	0.012	<0.05	<0.2	<0.005	<0.1	100	62.90
6/27/2019 RG_GC-NPM-09-O_20190627	Ovary	<2	<0.01	0.10	0.06	<0.01	<1	<0.01	<0.05	<0.01	3.8	68	<0.01	1.5	0.25	0.10	<0.05	2.2	<0.01	0.30	0.036	<0.05	<0.2	<0.005	<0.1	180	77.62
6/27/2019 RG_GC-NPM-10-O_20190627	Ovary	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	40	<0.01	1.1	0.093	0.04	<0.05	2.7	<0.01	0.11	0.010	<0.05	<0.2	<0.005	<0.1	90	61.14
6/27/2019 RG_GC-NPM-11-O_20190627	Ovary	<2	<0.01	0.04	0.12	<0.01	<1	<0.01	0.11	0.01	2.8	92	<0.01	2.4	0.13	0.10	<0.05	12	<0.01	0.29	0.018	<0.05	<0.2	<0.005	<0.1	340	78.10
6/27/2019 RG_GC-NPM-12-O_20190627	Ovary	<2	<0.01	0.04	0.25	<0.01	<1	<0.01	<0.05	<0.01	2.3	47	<0.01	3.3	0.070	0.03	<0.05	3.9	<0.01	0.12	<0.005	<0.05	<0.2	<0.005	<0.1	95	64.74
6/27/2019 RG_GC-NPM-13-O_20190627	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.1	33	<0.01	1.3	0.078	0.03	<0.05	3.3	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	65	61.68
7/18/2019 RG_GC-NPM-14-O_20190718	Ovary	7	<0.01	0.09	0.18	<0.01	<1	0.02	<0.05	<0.01	3.3	140	0.02	1.5	0.28	0.08	<0.05	9.6	<0.01	0.27	0.045	<0.05	0.2	<0.005	0.1	430	79.54
7/19/2019 RG_GC-NPM-15-O_20190719	Ovary	2	<0.01	0.10	0.21	<0.01	<1	<0.01	<0.05	<0.01	2.2	37	<0.01	0.5	0.27	0.04	<0.05	3.9	<0.01	0.11	0.008	<0.05	<0.2	<0.005	<0.1	120	67.63
7/25/2019 RG_GC-NPM-16-O_20190725	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.8	39	<0.01	1.1	0.083	0.03	<0.05	2.7	<0.01	0.11	0.011	<0.05	0.4	<0.005	<0.1	91	64.09
6/20/2019 RG_SC-NPM-01-O_20190620	Ovary	<2	<0.01	0.04	0.13	<0.01	<1	<0.01	<0.05	<0.01	3.1	56	<0.01	3.3	0.071	0.06	<0.05	8.4	<0.01	0.20	0.008	<0.05	<0.2	<0.005	<0.1	120	68.02
6/20/2019 RG_SC-NPM-02-O_20190620	Ovary	7	<0.01	0.06	0.26	<0.01	<1	<0.01	0.05	<0.01	2.6	130	0.03	1.2	0.12	0.06	<0.05	20	<0.01	0.84	0.041	<0.05	<0.2	<0.005	<0.1	490	80.46
6/20/2019 RG_SC-NPM-03-O_20190620	Ovary	<2	<0.01	0.09	0.07	<0.01	<1	<0.01	<0.05	<0.01	3.0	93	0.02	2.1	0.041	0.08	<0.05	11	<0.01	0.20	0.025	<0.05	<0.2	<0.005	<0.1	320	73.78
6/20/2019 RG_SC-NPM-04-O_20190620	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	<0.01	3.6	110	0.02	3.0	0.098	0.09	<0.05	17	<0.01	0.72	0.012	<0.05	<0.2	<0.005	<0.1	140	69.48
6/20/2019 RG_SC-NPM-05-O_20190620	Ovary	<2	<0.01	0.10	0.33	<0.01	<1	<0.01	<0.05	0.05	3.0	98	<0.01	1.8	0.088	0.10	0.05	28	<0.01	0.23	0.034	<0.05	<0.2	<0.005	<0.1	360	75.91
7/24/2019 RG_SC-NPM-06-O_20190724	Ovary	<2	<0.01	0.08	0.18	<0.01	<1	<0.01	<0.05	0.05	2.5	120	<0.01	1.9	0.11	0.04	0.09	10	<0.01	0.78	0.008	<0.05	<0.2	<0.005	<0.1	250	77.04
7/25/2019 RG_SC-NPM-07-O_20190725	Ovary	<2	<0.01	0.07	0.09	<0.01	<1	<0.01	<0.05	0.04	3.0	100	<0.01	8.9	0.14	0.16	<0.05	21	<0.01	0.38	0.018	<0.05	<0.2	<0.005	<0.1	280	77.16
7/25/2019 RG_SC-NPM-08-O_20190725	Ovary	<2	<0.01	0.15	0.15	<0.01	<1	<0.01	<0.05	0.05	2.8	86	<0.01	5.1	0.092	0.10	<0.05	23	<0.01	0.57	0.018	<0.05	<0.2	<0.005	<0.1	200	74.33

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/25/2019 RG_SC-NPM-09-O_20190725	Ovary	<2	<0.01	0.08	0.07	<0.01	<1	<0.01	<0.05	0.05	2.7	75	<0.01	3.5	0.18	0.12	0.08	12	<0.01	0.33	0.007	<0.05	<0.2	<0.005	<0.1	220	75.90
7/26/2019 RG_SC-NPM-10-O_20190726	Ovary	3	<0.01	0.13	0.62	<0.01	<1	<0.01	0.09	0.05	3.2	100	<0.01	1.6	0.072	0.07	0.07	25	<0.01	0.28	0.023	<0.05	<0.2	<0.005	<0.1	440	75.75
7/26/2019 RG_SC-NPM-11-O_20190726	Ovary	<2	<0.01	0.12	0.15	<0.01	<1	<0.01	0.08	0.07	4.2	160	<0.01	2.8	0.12	0.05	<0.05	23	<0.01	0.62	0.028	<0.05	<0.2	<0.005	<0.1	320	80.08
6/21/2019 RG_WB-NPM-01-O_20190621	Ovary	5	<0.01	0.02	0.23	<0.01	<1	<0.01	<0.05	0.04	3.6	77	0.06	4.5	0.069	0.09	0.06	7.4	<0.01	0.40	0.006	<0.05	<0.2	<0.005	<0.1	120	67.47
7/13/2019 RG_ER-NPM-47-O_20190713	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	45	<0.01	0.9	0.045	0.03	<0.05	3.5	<0.01	0.10	0.009	<0.05	<0.2	<0.005	<0.1	94	64.10
6/26/2019 RG-WB-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.05	3.0	83	<0.01	4.4	0.12	0.13	<0.05	26	<0.01	0.28	0.030	<0.05	<0.2	<0.005	<0.1	260	76.76
6/26/2019 RG-WB-NPM-03-O_20190626	Ovary	<2	<0.01	0.14	0.12	<0.01	<1	<0.01	<0.05	0.03	2.0	65	<0.01	1.6	0.18	0.06	<0.05	9.8	<0.01	0.14	0.007	<0.05	<0.2	<0.005	<0.1	240	70.13

ATTACHMENT 2

Analysis of Historical Mountain Whitefish Data Memorandum

EcoTox

3211 SW 19th Terrace, Miami, FL 33145
Tel: 305-773-8347

MEMORANDUM

Date: June 10, 2020

To: Mariah Arnold

From: Kevin Brix (EcoTox), David DeForest (Windward), and Lucinda Tear (Windward)

Subject: Analysis of Historical Mountain Whitefish Data

This memorandum describes the methods and results of a multivariate analysis of historical monitoring data for mountain whitefish (MW) collected in the Elk Valley. The first objective of this analysis was to determine whether factors such as gonado-somatic index (GSI), fish size (as measured by fork length), and fish sampling location influence observed ovary selenium (Se) concentrations in MW in the Elk Valley. The second objective of the analysis was to identify sampling locations and estimate the sampling intensity required (i.e., number of fish) to have a high probability of collecting MW in spawning condition with egg Se concentrations >33 mg kg^{-1} dw, the maximum egg Se concentration observed in previous efforts to conduct a selenium toxicity study with this species (Nautilus Environmental 2017).

Introduction

Historical and ongoing monitoring of MW in the Elk Valley indicate individual, and in some cases mean, ovary Se concentrations exceed the BC ENV guideline of 11 mg kg^{-1} dw, as well as the Level 1 Benchmark (18 mg kg^{-1} dw) and in some cases the Interim Screening Benchmark (29 mg kg^{-1} dw). This is true for ovaries collected from both reference and mine-exposed locations, although ovaries collected from fish in mine-exposed areas are on average higher than those collected from reference areas.

Given the ovary Se concentrations observed historically, Teck conducted three rounds (2010, 2011, and 2013) of toxicity testing in an attempt to estimate the sensitivity of the embryo-larval stage of MW to maternally transferred Se (Nautilus Environmental 2017). Combined, these studies demonstrated no effects on MW embryo-larval survival, growth, or development up to the highest egg Se concentration obtained (33 mg kg dw^{-1}). However, through 2018, ovary Se concentrations as high as 81 mg kg^{-1} dw have been observed through sampling under the Regional Aquatics Effect Monitoring Program (RAEMP) (Figure 1). Consequently, it is

important to understand whether effects are occurring in MW with egg Se concentrations >33 mg kg^{-1} dw and Teck anticipates conducting a fourth toxicity study on MW in the Fall of 2020.

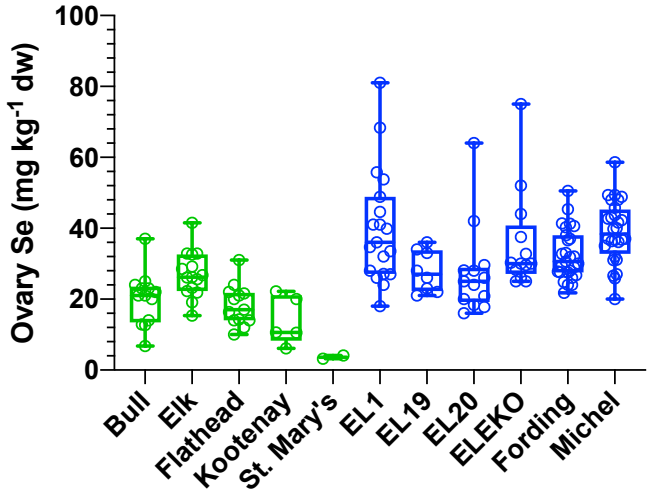


Figure 1. Ovary Se concentrations in mountain whitefish by location (2006-2018).

A preliminary review of the MW ovary Se data collected in 2018 demonstrates a negative correlation between GSI and ovary Se concentrations (Figure 2). The relationship is similar to that observed for northern pikeminnow (*Ptychocheilus oregonensis*; NPM). In NPM, females in spawning condition have a GSI >5%, while much of the historical ovary Se data were from fish with a GSI <5%, leading to an overestimation of egg Se concentrations likely to occur in NPM from Koocanusa Reservoir (EcoTox et al. 2020).

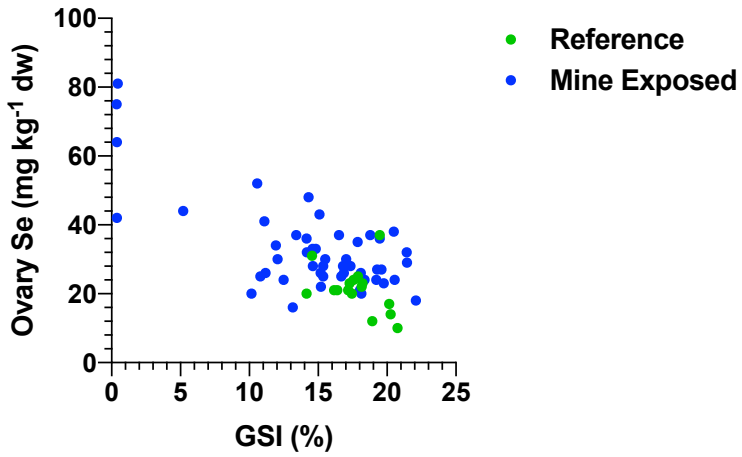


Figure 2. Relationship between GSI and ovary Se in mountain whitefish collected from Elk Valley in 2018.

Based on the preliminary analysis of the 2018 MW data, it is possible a similar bias exists in the ovary Se monitoring data for this species. This is a critical issue to understand with respect to developing a study plan for the 2020 MW Se toxicity study. If this negative relationship between GSI and ovary Se occurs across historical monitoring data, it will be important to understand the distribution of ovary Se data associated only with fish in spawning condition. This will inform the study design with respect to the sampling intensity required (i.e., number of females needing to be sampled) to have a high likelihood of collecting fish with egg Se concentrations >33 mg kg^{-1} dw. Similar to NPM, it is possible other factors (e.g., fish size and sampling location) also influence ovary Se concentrations (EcoTox et al. 2020). Understanding the importance of these factors can improve the sampling design for the 2020 MW Se toxicity study.

Data for Analysis

Historical monitoring data from 2006-2018 collected in the Elk River drainage and reference locations were considered in evaluating potential relationships between ovary Se concentrations and GSI, fish size (fork length), or sampling location. Data from 2012 were excluded from the analysis because information on GSI was not collected that year. Similarly, data from Koocanusa Reservoir were excluded from the analysis as GSI data were not available for fish collected from this location. In total, data from 156 fish were available for the analysis (Table 1).

Table 1. Ovary Se Sample Size by Location

	Year				
Location	2006	2009	2015	2018	Total
Mine Exposed Sites					
EL1	5	5		9	19
EL19				8	8
EL20			5	8	13
ELEKO			5	8	13
Fording	5	10	4	8	27
Michel	10	5	5	8	28
References Sites					
Bull River			5		5
Elk River	5	10			15
Flathead River			5	8	13
Kootenay River			5		5
St. Mary's River			2		2

Exploratory Analysis

An initial exploratory analysis of data was conducted using scatter plots and Principal Component Analysis (PCA) to provide visual and quantitative understanding of correlations among the variables of interest and how the relationships among those variables varied geographically. Fork length, GSI, and ovary Se were natural log (ln)-transformed, centered, and scaled for the PCA.

Scatter plots suggest that, similar to the 2018 data set (Figure 2), a relationship exists between GSI and ovary Se, while there does not appear to be a relationship with fish length (Figure 3).

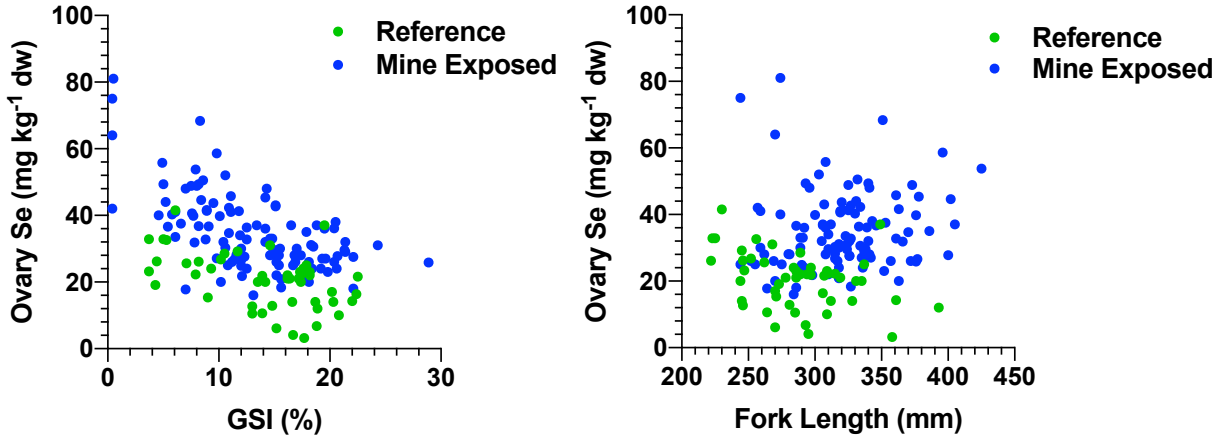


Figure 3. Relationships between GSI and fish length versus ovary Se using data summarized in Table 1.

The first two axes of the PCA explained approximately 88% of the variance. Axis 1 was most highly correlated with GSI (Figure 4) and explained 49% of the variance. Axis 2 was somewhat equally correlated with fork length and ovary Se and explained 38% of the variance. The biplot of PCA scores shows each of the variables having similar effects on the spread of the data (arrows indicating direction of increasing value of the associated variable are approximately equal length), and identifies four samples with very low GSI as quite separate from the rest of the data (far left of Axis 1).

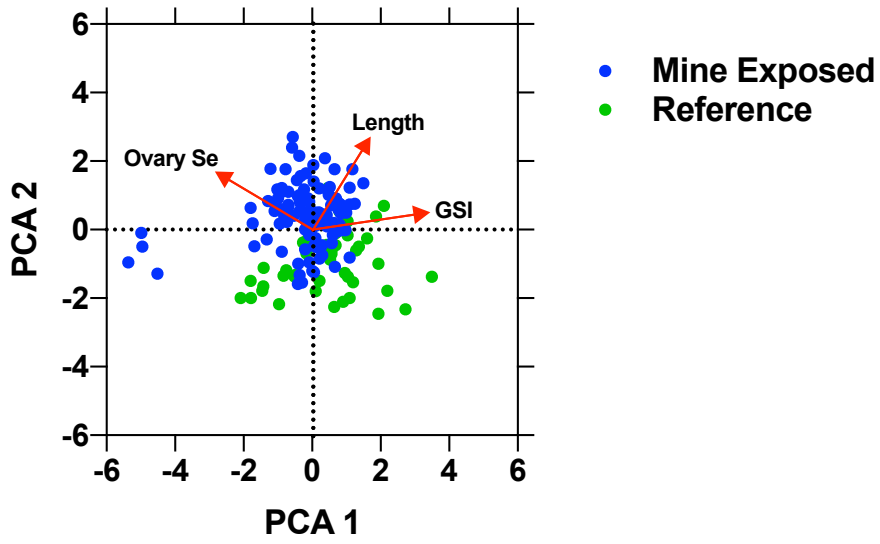


Figure 4. Biplot of Principal Component Analysis using ln-transformed fork length, GSI, and ovary Se. Arrows indicate direction of increasing values of the associated variable.

Multi-linear Regression (MLR) Analysis

Although the exploratory analyses indicated four data points differed from the bulk of the data, all data were retained in the initial MLR analyses. As in the exploratory analyses, all data were ln-transformed. A step-wise MLR model testing for linear effects of fork length and GSI with area-specific slopes and intercepts was run using the Akaike and Bayesian Information Criteria (AIC, BIC) to identify the most parsimonious models (R, stepAIC) (Equation 1).

$$Ln(OvSe) = area + Ln(FL) + area^* Ln(FL) + Ln(GSI) + area^* Ln(GSI) \quad \text{(Eq. 1)}$$

where, OvSe = ovary Se, FL = fork length (mm), and GSI = gonadosomatic index.

Model residuals were tested for normality using Shapiro Wilks (shapiro.test, R) and Nonconstant Variance (ncv, R).

The models identified by both AIC and BIC were identical. Fish size, as measured by fork length, was not retained in the model, indicating it does not have a significant influence on ovary Se. In contrast, GSI was highly significant ($p < 0.001$), but no area-specific slopes were retained, indicating the relationship between GSI and ovary Se is similar across sites. Area-specific intercepts were retained in the model, indicating significant differences in ovary Se among sites after correcting for the effect of GSI.

Two samples with low ovary Se collected from St. Mary's River (reference location) did not fit the model well. These are not part of the four samples with very low GSI identified in the PCA. The two samples from St. Mary's River were removed and the model was rerun to evaluate how these two outliers might be unduly influencing model parameterization. Results were similar to the first run, with GSI highly significant ($p < 0.001$) and area-specific intercepts with the same differences as the previous model.

Adjusted and predicted R^2 for the final model were 0.59 and 0.58, respectively (Figure 5). Model residuals were not normally distributed and did not have constant variance across the range of predicted values (Shapiro Wilks $p < 0.001$, Nonconstant Variance Test $p < 0.001$). Overall, the model appears to predict the mine-exposed sites reasonably well, but performs more poorly for the reference locations.

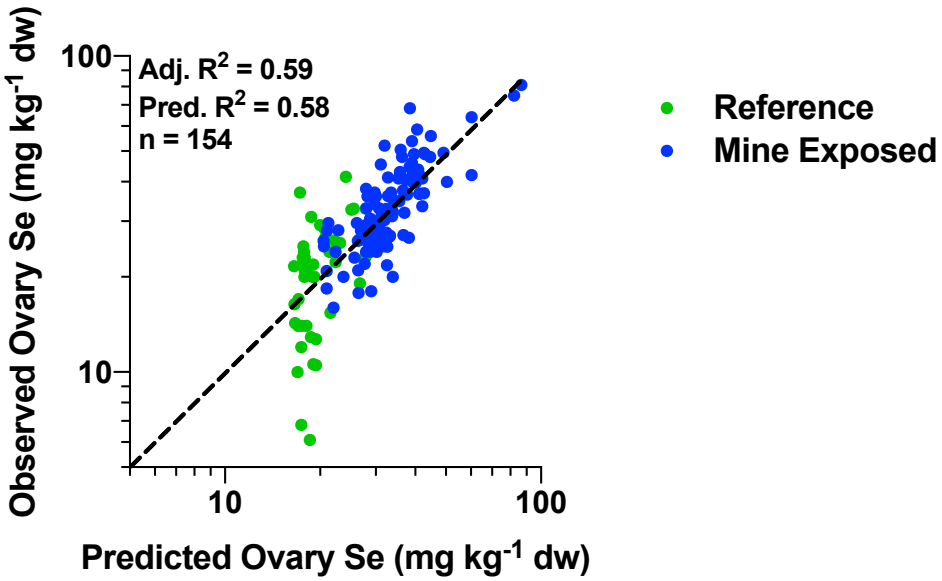


Figure 5. Final Linear Model Excluding Data from St. Mary's River.

The final model has a ln GSI slope of -0.288 and area-specific intercepts (Table 2). Treatment contrasts comparing Michel Creek to other locations indicate, with the exception of EL1, all areas have a significantly ($p < 0.05$) lower intercept than Michel Creek (Table 2). This means that after accounting for the effect of GSI on ovary Se concentrations, Michel Creek has significantly higher ovary Se concentrations than all locations except EL1.

Table 2. Final ovary Se model coefficients and significance. Note: The intercept t values and *p* values relate to testing for significant differences in intercepts relative to Michel Creek.

		Estimate	Std. Error	t Value	*p* Value
Intercepts	Reference	3.705	0.065	-10.10	<0.001
	EL1	4.261	0.081	-1.22	0.223
	EL19	4.106	0.109	-2.33	0.021
	EL20	3.836	0.092	-5.69	<0.001
	ELEKO	4.145	0.091	-2.34	0.020
	Fording	4.203	0.073	-2.15	0.033
	Michel	4.360	0.097	-	-
Slope	ln GSI	-0.288	0.033	-8.80	<0.001

Sample Size Analysis

The negative relationship between GSI and ovary Se provides evidence that this variable needs to be considered in evaluating sampling effort for future toxicity studies. Information on the GSI typically associated with MW in spawning conditioning is limited, but a recent study indicates a GSI of >15% is associated with spawning in this species (Irvine et al. 2017). The model also demonstrated that significant differences in ovary Se between sampling locations exist, with Michel Creek having the highest ovary Se on average. Given this information, we focused our sample size analysis on MW with a GSI >15% collected from Michel Creek.

The objective of the sample size analysis was to estimate the sampling intensity required to obtain egg Se concentrations with a specific level of confidence. Specifically, we wanted to estimate the number of female MW that will need to be sampled from Michel Creek to obtain eggs from at least 3 females with a given egg Se concentration with 90% confidence. Recalling that the highest egg Se concentration evaluated in previous toxicity testing efforts was 33 mg kg^{-1} dw, we estimated samples sizes needed to collect egg Se concentrations of 34, 36, 40, and 43 mg kg^{-1} dw. First, we calculated the proportion of fish captured with both a GSI >15% and specified ovary Se concentration as:

P(ovary Se >X and GSI >15%) = Count(ovary Se >X and GSI >15%)/Count(GSI >15%)

The proportions ranged from 0 to 0.50 with the highest proportions (as well as the highest number of fish) in Michel and Fording drainages. To estimate the sample size needed to collect at least 3 fish with a specific ovary Se, we assumed the proportion of fish with the specified P fit the binomial distribution.

P ~ Binomial(n,p)

where p = observed proportion of ovary Ovary Se fish = observed P, and n = sample size.

We estimated the number of samples required to be 90% confident of collecting at least 3 fish with the specified ovary Se fish for a range of p levels [P = 0.1, 0.2, 0.3, 0.4, 0.5] using the inverse of the binomial distribution [Excel function =BINOM.INV(*n, p, alpha*) or R function qbinom(1-*alpha*, *n*, *p*]. This was done by submitting a range of possible sample sizes (n = 5:100) to each function to find the minimum sample size at which 3 ovary Se samples would be collected with the desired confidence (*alpha* = 0.10). In parallel, we estimated the probability of collecting at least 3 ovary Se samples for the same range of sample sizes and p levels (Excel function =1-BINOM.DIST(2, P, *n*, cumulative = 1), pbinom(2, *n*, *alpha*, lower.tail = FALSE).

Results from this analysis indicate that even a relatively modest increase in the range of ovary Se concentrations of 5 mg kg^{-1} dw over the maximum concentration of the existing data can only be accomplished by sampling Michel Creek and would require sampling at least 28 female fish (Table 1). The sample size remains constant for ovary Se >38 mg kg^{-1} dw because the probability of capturing a fish remains constant. This is because the sample size for this analysis is small and unevenly distributed (n=11 for Michel Creek fish with GSI >15%). Consequently, there is some uncertainty in these estimates and caution should be exercised in using these values for sample design, i.e., sampling more fish than estimated by this analysis is recommended.

Table 1. Estimated Sample Size Required to Obtain 3 Female Mountain Whitefish with Specified Ovary Se Concentration with 90% Probability of Success Assuming GSI = 15%.

	Target Ovary Se Concentration				
Location	**34 mg kg^{-1} dw**	**36 mg kg^{-1} dw**	**38 mg kg^{-1} dw**	**40 mg kg^{-1} dw**	**43 mg kg^{-1} dw**
Michel Creek	9	13	28	28	28
Fording River	24	28	UC[1]	UC[1]	UC[1]

[1] Unable to calculate as no ovary samples with this Se concentration from fish with a GSI of at least 15% have been collected.

Conclusions

This analysis was conducted to provide information on the sampling design for the planned MW Se toxicity study. The objective of the toxicity study is to extend the range of concentration-response data above 33 mg kg dw^{-1}, the highest concentration observed over the course of three previous rounds of testing. This analysis demonstrated that ovary Se concentrations in MW are related to the GSI, but not the size, of female MW from which they are collected. After correcting for the effect of GSI using a linear model, differences in ovary Se between mine-

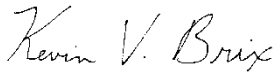
exposed and reference locations were detected, with fish collected from Michel Creek having the highest ovary Se.

A subsequent sample size analysis used historical monitoring data including only fish with a GSI >15%, the likely GSI associated with fish in spawning condition. The sample size analysis indicates that at least 28 female fish will need to be collected to have a high (90%) probability of collecting at least 3 fish that increase the range of the concentration-response data by 5-10 mg kg dw^{-1}. Based on this analysis, we recommend that at least 30 female fish from Michel Creek be sampled for the 2020 mountain whitefish toxicity study. We also recommend 5-10 fish from 2 reference locations be collected to provide controls for the study.

Closing

I trust that this analysis provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact me at (305) 773-8347.

Sincerely,



Kevin V. Brix, Ph.D.
Principal Scientist
EcoTox LLC

References

EcoTox, University of Saskatchewan and Minnow Environmental (2020). Draft Report - Evaluation of selenium concentrations in ovary of northern pikeminnow (*Ptychochelius oregonensis*). Miami, Florida, EcoTox LLC**:** 27 pp. + appendices.

Irvine, R. L., J. L. Thorley and L. Porto (2017). "When do mountain whitefish (*Prosopium williamsoni*) spawn? A comparison of estimates based on gonadosomatic indices and spawner and egg counts." Open Fish Sci. J. **10**: 12-22.

Nautilus Environmental (2017). Evaluation of the effects of selenium on early lifestage development of mountain whitefish from the Elk Valley, BC. Burnaby, British Columbia, Nautilus Environmental**:** 561 pp.

APPENDICES

2. Appendix B – Monitoring and Research Committee written recommendations

From:	Gildea, Jason
To:	Sullivan, Lauren; Reddekopp, Sheldon ENV:EX
Cc:	Myla Kelly; Epps, Deb ENV:EX; Schmit, Ayn; McGrath, Patricia; McLaughlin, Julianne; Beaman, Joe
Subject:	2020 08 28 J.Gildea-USEPA_recmds
Date:	Friday, August 28, 2020 4:10:24 PM
Attachments:	image003.jpg

Disclaimer: *Please note the comments and recommendations contained in this document are strictly for Montana's and British Columbia's consideration. The views expressed in these comments and recommendations are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Regarding Montana's submission, the comments do not constitute approval or disapproval decisions under CWA Section 303(c). Neither are these comments a determination by the EPA Administrator under CWA Section 303(c)(4)(B) that revised or new standards are necessary to meet the requirements of the Act. These comments and recommendations do not impose any binding requirements, determine the obligations of the regulated community, change or substitute for any statutory provision or regulation requirement, represent, change or substitute for any Agency policy or guidance, or control in any case of conflict between this discussion and statute, regulation, policy or guidance.*

Lauren and Sheldon,
Thank you for the opportunity to provide comments on the technical analysis for setting a site-specific criterion for Lake Koocanusa. Please consider the following as my formal submission of comments as a member of the Lake Koocanusa Monitoring and Research Committee (MRC) to the chairs of the Selenium Technical Subcommittee (SeTSC).

First, I would like to acknowledge the extensive, well vetted process for getting to this point in the criterion development. The Lake Koocanusa Monitoring and Research Working Group (LKMRWG), which includes the MRC and SeTSC, has been meeting regularly for six years with the primary goal of developing and implementing a site-specific selenium criterion for Lake Koocanusa. Multiple in-person and phone-based meetings occurred during this time period allowing committee members and other stakeholders ample opportunity to discuss technical issues, provide input, collect data, analyze data, understand reservoir hydrodynamics and chemistry, assimilate information, and to develop and understand the selenium model. Specifically during this time period, I note that the USGS report titled *Conceptual modeling framework to support development of site-specific selenium criteria for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada* (Jenni, Naftz, and Presser, 2017) and the Lotic Environmental titled *Koocanusa Reservoir Data Compilation Report Volume 2* (Lotic Environmental, 2019) were produced and significantly added to the group's understanding of the process and available data. These reports, along with all data and information from the meetings, are well organized and archived on a website maintained by the Montana Department of Environmental Quality (DEQ) and B.C. Ministry of Environment and Climate Change Strategies (ENV) (available at http://lakekoocanusaconservation.pbworks.com/

w/page/100633354/FrontPage). The six-year process has provided all stakeholders with substantial background information and has prepared us to fully comment on the site-specific criterion model in a timely manner.

I note that it is seven years since B.C. ENV issued Ministerial Order #M113, which states that, "the Minister of Environment wishes to reduce calcite formation and to manage water quality to stabilize and reverse increasing trends in the water contaminant concentrations." Despite this order, pollutant concentrations and loads continue to increase into Lake Koocanusa, as demonstrated at the B.C. water quality monitoring site located at the Elk River Hwy 93 Crossing (Figure 1). This information is alarming and highlights the need to immediately stabilize and reverse pollutant loads into Lake Koocanusa.

Given the six-year process leading up to this point, I believe that it is now imperative for DEQ and B.C. to quickly adopt and implement a site-specific selenium criterion for Lake Koocanusa. I fully support Montana DEQ's proposed schedule to propose and adopt the criterion by December 2020 and I believe that the currently available science allows you to do that. The CWA and EPA's regulations at 40 CFR Part 131 require states and authorized tribes to establish water quality criteria that are protective of the designated use and scientifically sound, and then require those states and tribes to periodically (at least once every three years) review and revise the criteria as needed. Therefore, I encourage you to use the science available to you now to develop a site-specific selenium criterion. As you know, Lake Koocanusa currently has a site-specific selenium objective of 2 µg/L in B.C. and a water column chronic criterion of 5 µg/L in Montana. The modeling results provided by USGS in their 2020 report demonstrate that protective selenium concentrations for Lake Koocanusa are likely lower than either of these values, necessitating the need for immediate action to revise the criterion to a protective value. Presser and Naftz, 2020, note that, "78 percent of predictions are <1.5 µg/L and at least 46 percent of predictions are <1 µg/L for protection of this community of core benthic feeders." Delaying criterion development in any way will exacerbate on-going degradation of the Lake Koocanusa ecosystem, particularly given that pollutant loads are increasing.

Thank you again for the opportunity to comment on the site-specific selenium criterion and I look forward to working with you in the future on Lake Koocanusa issues.

Jason Gildea
Sent via email August 28, 2020
Hydrologist, USEPA Region 8, Montana Office
Gildea.Jason@epa.gov
406-457-5028

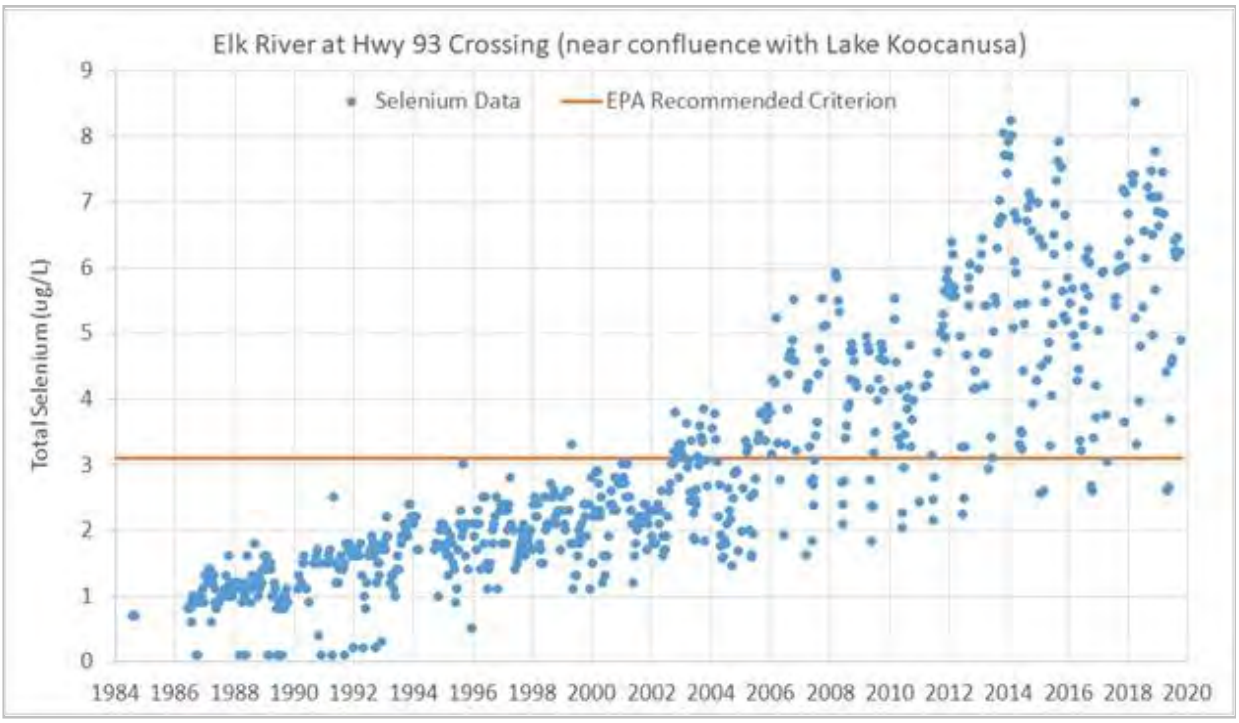


Figure 1. Selenium concentrations in the Elk River at HWY 93 crossing with EPA's recommended selenium criterion for lentic waters for comparison.

Tim Davis| Administrator, Water Quality Division, Montana Department of Environmental Quality |LKMRC Co-Chair
Delivered via email to : TimDavis@mt.gov

August 28, 2020

Dear Mr. Davis and Mr. Moore:

Headwaters Montana works in northwest Montana to protect the water, wildlife, and traditional quiet outdoor recreation. We have over 2,000 subscribers. For the past several years we have been engaged in the Lake Koocanusa Monitoring and Research Committee (MRC) and the MRC's Selenium Technical Committee (SeTC) as an observer. We have appreciated being a part of the process and send this letter to provide you with our views and recommendation for the Montana selenium standard setting process that is now in its final phase.

First, we want to thank the DEQ and your dedicated staff for stepping up and taking on the significant task of working with BC to set this standard. It has proven to be an expensive and time-consuming process, but one which was necessary. Scientists are learning more and more about the toxic effects of selenium, and it has become apparent to us that the LKMRC process was necessary. Montana could have adopted the US EPA national standard. However, it was understood by many of the SeTC that selenium in Koocanusa had potentially more harmful and toxic effects. We think that the USGS model for selenium in Koocanusa proves this point and that the US EPA national standard would not have been protective of fish and aquatic life in the reservoir, and or would have had to be adjusted downward rather quickly after adoption.

Second, we would like to acknowledge that the standard-setting process is both a scientific and political one.

With respect to the science, it is clear that more and better data would have been helpful to the USGS modeling process. The USGS model was built with the scientific expertise of the most knowledgeable selenium experts in North America. We are confident that the essential components of the model reflect good scientific judgement. Headwaters Montana has played a role in securing congressional appropriations for the water quality monitoring on Lake Koocanusa, in particular the funding for the "super sipper" buoy deployed at the border. Our continuing efforts to secure addition federal funds for water quality monitoring should help ensure that the USGS has more robust data in the future.

The important point is that the USGS model represents the best available science at this point in time, and that future data will only make the model's predictions more reliable. Given that we can now expect BC and Teck to export even higher levels of selenium in the near future, we support the model and the goal of setting a selenium standard at the border in 2020 that is protective of aquatic life throughout the reservoir and downstream in the Kootenai River, including in Idaho and beyond.

During the August 25 MRC virtual meeting, some confusion was expressed by some on the MRC as to the "placement" of the selenium standard. Both BC and MT have used the motto, "one lake, one number" to describe the goal of the six-year effort. We want to be clear that we understand and expect the Montana standard will be set for the international border and does not represent a 'reservoir average' number.

With respect to the political decisions to be made by Montana on setting a selenium standard for the border, we understand that Montana will be in communication with both BC and Teck. We can anticipate some of their arguments and would like to address and correct several of them here. We think BC and Teck will argue for as high a number as possible, and one that will not protect US/Montana downstream interests. They will:

- Argue that the USGS is not scientifically valid. That no site-specific egg-ovary data or fish data were used. That 'generic' fish data was used. They will try to argue that the 'uncertainty' of the model should require additional data collection, study and analysis…. And delay. Our response to this, should BC and Teck make these arguments, is that 'uncertainty' argues for a more protective standard rather than a weak standard or delay, and that the standard should be set in 2020 and not delayed. The US and Montana will continue to collect data and the USGS model predictions will improve over time. After six years, further delay is not needed. It is better to approve now a protective standard that protects all aquatic life than study and delay.

In closing we would like to emphasize that:

- We support the MT-BC process
- We support the USGS model
- We support the timeframe for setting a selenium standard at the border before the end of 2020
- We support a standard that is conservative and protective of all aquatic life given the uncertainties of increased selenium loading, proposed new mines in the Elk Valley, and the future opportunity to refine the model – and the standard – as time goes by.
- We also support the Confederated Salish and Kootenai Tribes, the Kootenai Tribe of Idaho, and the Ktunaxa National Council's recommended number(s), that we anticipate will be at or below 1.0 ug/l.

Thank you for the opportunity to comment.

Sincerely,

Dave Hadden, Ex. Dir.
Headwaters Montana, Inc.
406-270-3184 / info@headwatersmontana.org

Deborah Epps
British Columbia Ministry of Environment
Deb.Epps@gov.bc.ca

Myla Kelly
Montana Department of Environmental Quality
MKelly@mt.gov

August 28, 2020

RE: development of site-specific selenium criteria for Lake Koocanusa

Dear Deborah and Myla,

Montana Department of Fish, Wildlife & Parks (MFWP) has been involved in monitoring and researching selenium (Se) in Lake Koocanusa fish since 2008. MFWP has participated in the Lake Koocanusa Monitoring and Research Working Group since its inception in 2015, and has been an active member of the Monitoring and Research Committee (MRC), including the collection of fish and macro-invertebrate samples on the reservoir. To that end, MFWP has been actively involved in the effort to develop a site specific Se criteria for Lake Koocanusa and is fully supportive of the process led by British Columbia (BC) and MDEQ.

Like many large rivers in Montana, Lake Koocanusa is a complex system that has many anthropogenic stressors to fish. However, despite alterations to natural flow, temperature and sediment regimes, the reservoir still supports a largely native fish component. Burbot are a native species in the reservoir that are listed as a species of concern and have high cultural, sport-fish, and ecological values. Their populations have been declining steadily since 1989, however the reason for their decline is unclear. Burbot are difficult to capture, especially females with eggs, resulting in limited data for this species. Unfortunately, not much is known about burbot sensitivity to Se since there is no surrogate species to reference. These uncertainties warrant a conservative approach for the development of a Se criteria for Lake Koocanusa.

Due to the uncertainties surrounding the variability of the data used in the models to develop a site-specific criteria and unknown sensitivities of fish species, a conservative approach to criteria setting is imperative. The tissue concentrations of Se found in several fish species in Lake Koocanusa currently exceed EPA criteria and BC guidelines. With tissue exceedances occurring from waterborne concentration of ~1 ug/L, it is evident that a site-specific criterion would need to be less than 1 ug/L to be protective based on the best available science. Montana cannot wait any longer to adopt site specific criteria for Lake Koocanusa, and as more data becomes available this number could be revised (if needed) during triennial reviews. Waterborne concentration of Se downstream of Libby Dam have also

been observed ~1 ug/L. Therefore, applying a conservative approach for the Lake Koocanusa criteria would also provide protection of downstream fish populations where tissue exceedances have also been documented and where the most sensitive species to Se toxicity, the endangered white sturgeon, is present.

Thank you for the opportunity to comment on the adoption of site-specific criteria for Lake Koocanusa and for all the efforts MDEQ and BCMOE has made toward this process.

Trevor Selch

Fisheries Pollution Biologist
Montana Fish, Wildlife & Parks

Cc:

Lauren Sullivan (MDEQ)
Sheldon Reddekopp (BC MOE)
Tim Davis (MDEQ)
Sean Moore (BC MOE)

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August 31, 2020

Ms. Myla Kelly
Water Quality Standards Section Supervisor
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Ms. Deb Epps
A/Regional Director
Monitoring, Assessment and Stewardship
Ministry of Environment and Climate Change Strategy
PO Box 9334 Stn Prov Govt
Victoria, BC V8W 9N3

Re: Teck response to the USGS Sampling and Analysis Plan for the collection of selenium and associated water quality parameters in Lake Koocanusa

Dear Ms. Kelly and Ms. Epps,

In your roles as Co-Chairs of the Lake Koocanusa Monitoring and Research Working Group (LKMRWG) and as requested during the working group meeting on August 26, 2020, I write to provide Teck's response to the recently published USGS Sampling and Analysis Plan and the process that will inform a draft selenium criteria for aquatic life in Koocanusa Reservoir. Given the nature of Teck's response, the Co-Chairs of the Selenium Technical Subcommittee (SeTSC) have been copied on this letter.

Teck has been operating for over 100 years to become a leading Canadian diversified natural resource company, committed to responsible mining and mineral development. Strong sustainability practices are core to how we do business and Teck's leadership in this area is recognized globally. For instance, Teck was named to the Dow Jones Sustainability Index in 2019 for our 10th straight year, and we were the top ranked mining company in the world on this index. Central to this is Teck's commitment to responsible management of water resources and to protecting water quality where we operate.

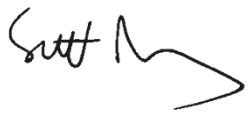
Over the last five years, Teck has made significant progress towards achieving the objectives of the Elk Valley Water Quality Plan (EVWQP), which is a long-term approach to addressing the management of selenium, nitrate and other substances released by current and historic mining activities in the Elk Valley. We forecast that our total investment in water quality management will be $1.2 billion through to 2024. This includes the water treatment facility at our Fording River Operations that is scheduled to complete in 2021. Additionally, Teck has more than 25 research and development projects underway, including the advancement of smaller, in-situ water treatment facilities that can be built closer to where treatment is needed. We are already seeing reductions in selenium and nitrate concentrations downstream of the Line Creek treatment facility, and we expect to see further significant reductions in other areas as future facilities come online. Details and information on our efforts can be found in the data and reports available online on Teck's website[1] and on the U.S. Environmental Protection Agency WQX Portal[2].

[1] Teck Resources Limited: "Responsible Mining in the Elk Valley".
[2] United States Environmental Protection Agency: "Water Quality Data (WQX)"

As members of the LKMRWG and observers to the SeTSC, we have made best efforts to understand and to provide input into the committee's work to inform a science-based and site-specific selenium recommended value for the Koocanusa Reservoir. This is a complex and multifaceted initiative and we appreciate the efforts that have gone into this work to date. It is our hope that the expertise and input of LKMRWG and SeTSC members and observers to this process is fully considered in your deliberations. We look forward to further engagement with the LKMRWG, SeTSC, Montana Department of Environmental Quality and the BC Ministry of Environment and Climate Change Strategy as this process continues to advance.

If you have any questions about our response to the USGS Sampling and Analysis Plan or Teck's approach to water quality management, please do not hesitate to contact me at Scott.Maloney@teck.com. We thank you for the level of engagement to date and for this opportunity to provide input directly to you for this process.

Sincerely,



Scott Maloney
Vice President, Environment

Cc: Tim Davis, Administrator, Water Quality Division, Montana Department of Environmental Quality
Lauren Sullivan, Water Quality Standards and Modeling, Montana Department of Environmental Quality
Kevin Jardine, Deputy Minister, Ministry of Environment and Climate Change Strategy
James Mack, Assistant Deputy Minister, Ministry of Environment and Climate Change Strategy
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Teck comments on the U.S. Geological Survey Lake Koocanusa Open-File Report 2020–1098

Overview

The comments below are Teck's response to the above-referenced open-file report developed for Lake Koocanusa by the U.S. Geological Survey (Presser and Naftz 2020). In summary, we believe there are a number of misleading and inaccurate statements within the report, and, despite the importance of site-specific fish tissue data, it was not used as a means to validate the model. As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the Koocanusa Reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion.

Misleading and inaccuracies within the report

The U.S. Geological Survey (USGS) fails to acknowledge that Lake Koocanusa has been listed under section 303(d) of the U.S. *Clean Water Act* as "impaired" since 2002 due to hydro-modifications, specifically Libby Dam[3]. By focusing on the 2012 reporting year, Presser and Naftz (2020) obscure this fact and create the potential for readers to inaccurately conclude that the listing is due to selenium concentrations. This is further exasperated by an incomplete analysis of data collected at the mouth of the Elk River by Environment and Climate Change Canada.

As noted by Presser and Naftz (2020), aqueous selenium concentrations measured at the mouth of the Elk River above Highway 93 (BC08NK0003)[4] have increased during the period of record, but incorrectly identify this trend as *continuing* to increase. Rather and as illustrated within Figure 1 below, selenium concentrations at the mouth of the Elk River have not increased since 2014. On the contrary, not only have selenium concentrations stabilized but a negative slope for the best-fit linear regression line suggests that concentrations are decreasing. This trend is wholly consistent with the goals and intentions of the Area Based Management Plan to "stabilize and reverse increasing trends in water contaminant concentrations". Furthermore, the suggestion that selenium loads are and will continue to increase is not supported by data.

[3] https://ofmpub.epa.gov/waters10/attains_waterbody.control?p_au_id=MT76D003_010&p_cycle=2002.

[4] http://aquatic.pyr.ec.gc.ca/webdataonlinenational/en/SiteDetails/BC08NK0003/Projects/PYLTM/Regions/0

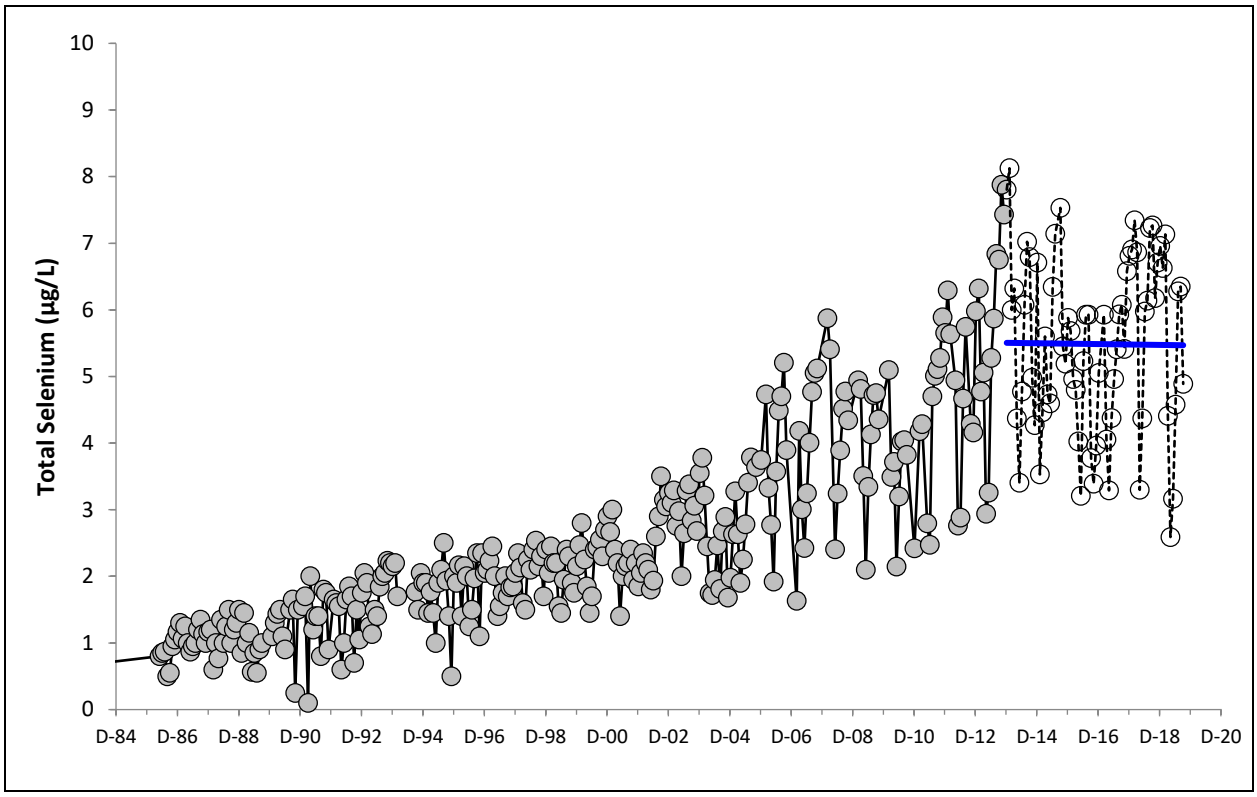

Figure 1. Monthly Average Total Selenium Concentrations as Recorded at the Mouth of the Elk River by Environment and Climate Change Canada.

Note: Data points are a monthly mean concentrations, while the blue line is a best-fit linear regression for data collected from 2014 to 2019 inclusive and has a slope of -1.7×10^{-5}.

Annual selenium loads from the Elk River into the reservoir have not been increasing and are inconsistent with gradient maps presented within the report. Presser and Naftz (2020) suggest that cumulative cross-sectional areas with selenium concentrations greater than 1 µg/L have increased three- (from 2017 to 2018) and four-fold (from 2017 to 2019) due to increasing selenium loads from the Elk River. Selenium concentrations (see Figure 1) and loads (see Figure 2) from the Elk River are inconsistent with the aforementioned suggestion.

Presser and Naftz (2020) correctly identify that the increase in proportional area of the reservoir containing selenium concentrations >1 µg/L suggests a system that is not in steady state, but fail to directly account for the significant role Libby Dam and its hydrodynamic cycles plays in this matter. Although Presser and Naftz (2020) identify Lake Koocanusa as a highly modified hydrological and ecological system, and that it experiences traditional problems associated with dam management (e.g., large elevation changes during drawdown and refilling operations), the report does not wholly consider the ramifications of such annual fluctuations particularly on the British Columbia side of the reservoir. As demonstrated by Dr. M. Sokal during the October 2016 Monitoring Research Committee meeting in Kalispell, Montana, Lake Koocanusa's hydrologic cycle significantly influences water quantity within the Canadian portion of the reservoir. During high water conditions (Figure 3, left panel) the reservoir is hydrologically like a "lake"; while under low water conditions (Figure 3, right panel) it is more like a "river" (Sokal 2016).

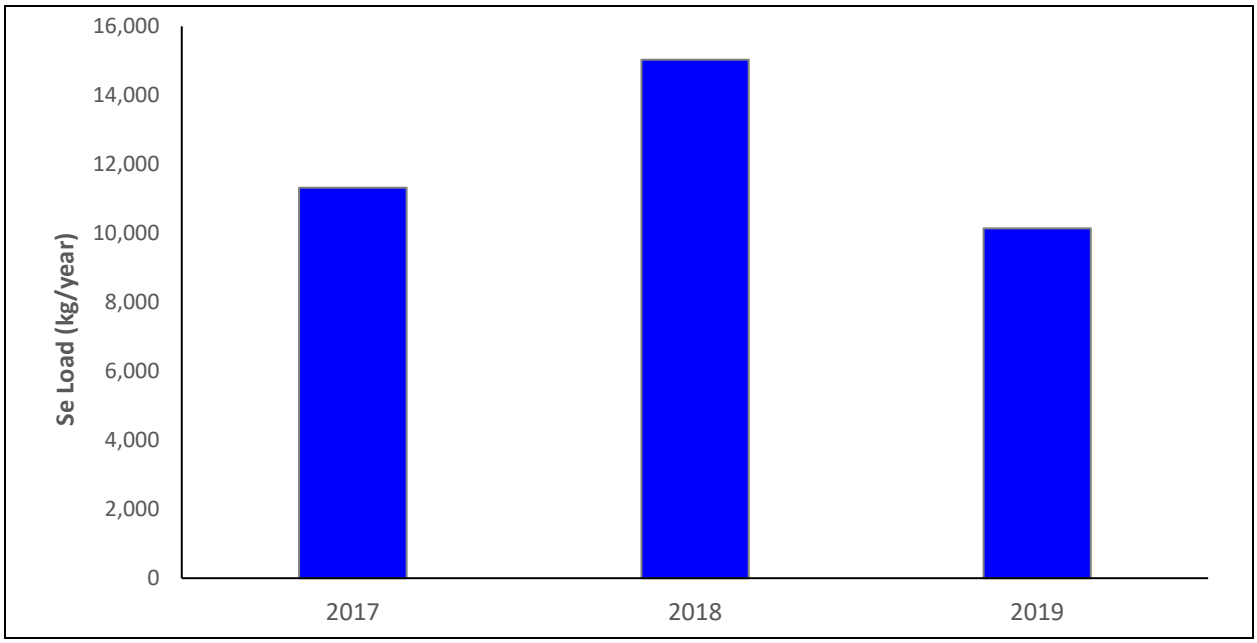

Figure 2. Selenium Loads associated with the Elk River into Lake Koocanusa.

Note: Selenium loads (2017 = 11,316 kg, 2018 = 15,035 kg, and 2019 = 10,144 kg) as determined from annual monitoring reports for the reservoir submitted to the ENV (Minnow, 2019 and 2020, and Teck 2018).

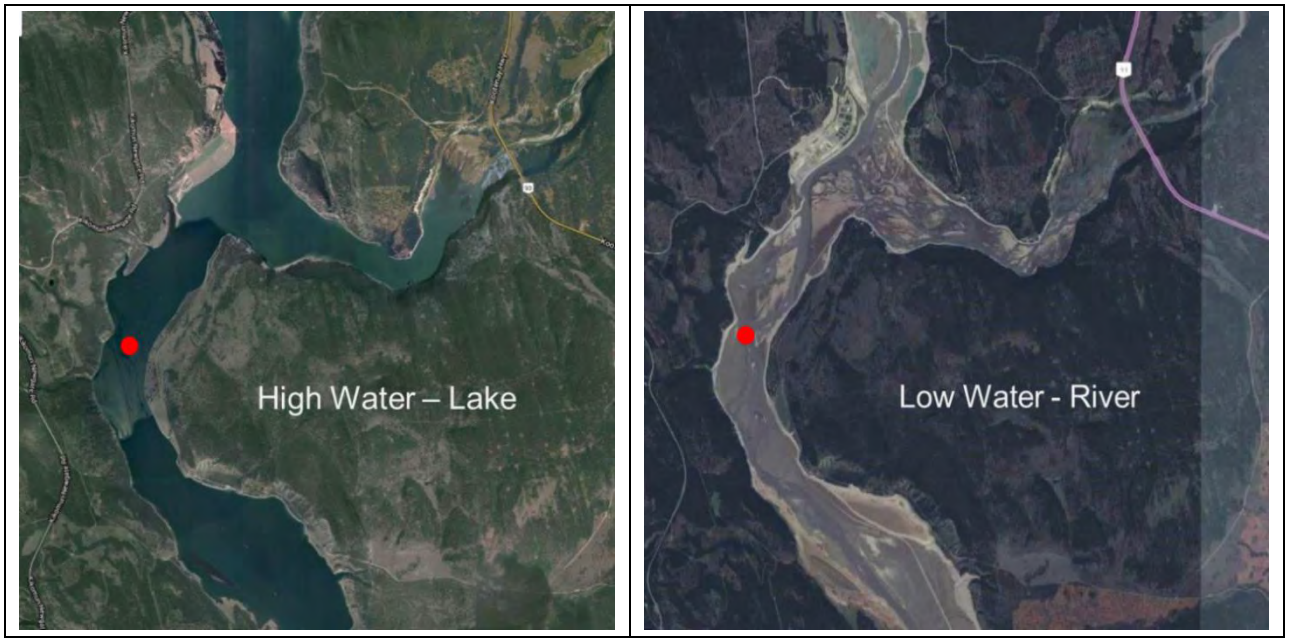

Figure 3. Aerial Imagery of Lake Koocanusa near the Confluence of the Elk River during High- (left panel) and Low-Water (right panel) Conditions.

Note: Imagery obtained from PowerPoint presentation made by Michael Sokal, PhD, Environmental Protection Division, BC ENV at the Lake Koocanusa Monitoring Research Working Group Meeting Kalispell, MT (October 17-18, 2016; Meeting No. 5). The red dot illustrated within the images reflects Order Station Environmental Monitoring System (EMS) E300230 (RG_DSELK).

Lack of model validation via fish tissue data

Model validation was limited to evaluating ranges of observed and predicted selenium concentrations in invertebrates (zooplankton and macroinvertebrates). Based on the qualitative evaluation presented within Presser and Naftz (2020), it is clear that unless an adjustment of selenium bioavailability is made, the model grossly over-predicts invertebrate concentrations. Even with a bioavailability assumption of 60 percent, the model over-estimates invertebrate concentrations. This over-estimation is carried through to the endpoint of primary concern (fish tissue).

For instance, assuming a bioavailability of 60 percent predicted whole-body fish tissue concentrations are two times greater than measured data (e.g., site-specific mean = 5.4 mg/kg dw, n = 22). Furthermore, if one considers fish muscle tissue concentrations relative to the U.S. Environmental Protection Agency's criterion which identifies a limit of 11.3 mg/kg dw for adult fish muscle this positive bias increases to a factor of three (e.g., site-specific mean = 3.8 mg/kg dw, n = 120). As a result, the ecosystem-scale model consistently over-predicts fish tissue selenium concentrations within the reservoir and is inherently too uncertain to support derivation of a water quality objective/criterion. Therefore and consistent with recommendations made to Monitoring and Research Committee Co-Chairs on December 4, 2015, selenium criteria/objectives should focus on empirical data, specifically fish tissues, and be evaluated relative to the U.S. Environmental Protection Agency (2016) fish tissue-based selenium criteria which are protective of fish.

As a result, the question at hand for the Monitoring and Research Committee is better expressed in terms of environmental monitoring data within the reservoir, in relation to existing water quality criteria/guidelines. The presumption being that existing/pending water quality criteria/guidelines are, as intended, protective of aquatic life within the reservoir. This is different than what is currently being expressed which assumes that existing/pending water quality criteria/guidelines are not protective of aquatic life within the reservoir.

Process toward a recommended value

According to the BC-MT 2020 Work Plan, revised May 1, 2020, the Co-Chairs of the SeTSC will finalize a technical assessment report and recommendations of a draft selenium criteria for consideration by Montana Department of Environmental Quality and by the British Columbia Ministry of Environment and Climate Change. It is not clear whether the Co-Chairs will include responses to comments on the USGS report submitted by SeTSC members and observers. In the interest of transparency and in recognition of the deep technical expertise on the SeTSC, we recommend that the report include how SeTSC member and observer input was considered to inform the recommended value.

Appendix: references

Presser, T.S., and Naftz, D.L., 2020, Understanding and documenting the scientific basis of selenium ecological protection in support of site-specific guidelines development for Lake Koocanusa, Montana, U.S.A., and British Columbia, Canada: U.S. Geological Survey Open-File Report 2020–1098, 40 p., https://doi.org/ 10.3133/ ofr20201098.

Minnow Environmental Inc. 2019. Koocanusa Reservoir Monitoring Program Annual Report, 2018. Prepared for: Teck Coal Limited. June 2019

Minnow Environmental Inc. 2020. Koocanusa Reservoir Monitoring Program Annual Report, 2019. Prepared for: Teck Coal Limited. June 2020

Teck Coal Limited. 2018. 2017 Summary Report of Monitoring Results in the Koocanusa Reservoir. June 28, 2018

U.S. Environmental Protection Agency. 2016. Aquatic life ambient water quality criterion for selenium – Freshwater 2016. Office of Water, Washington, D.C., USA. EPA 822-R-16-006.

wildsight

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Tim Davis, Montana DEQ
Sean Moore, BC MoE

SeTSC Co-chairs:
Sheldon Reddekopp, BC MoE
Lauren Sullivan, Montana DEQ

August 28, 2020

Thank you for the opportunity to provide input on the selection of the shared water column selenium limit to protect aquatic life for Lake Koocanusa.

Wildsight is strongly supportive of conservative, protective selenium limit for Lake Koocanusa, both for the protection of aquatic life in the reservoir and downstream in the Kootenai/Kootenay River and even in Kootenay Lake. As a general principle, we believe that modelling assumptions should be conservative and err on the side of caution in order to ensure protection for all species.

We recognize that ecological modelling will never be as precise or simple as we may wish it to be, nor will we ever have all the data we'd like; however, the USGS report and the SeTSC process that came before it have been open, transparent and comprehensive, involving detailed data collection over a number of years and peer-reviewed analysis from well-regarded experts on selenium toxicology. It is time to act on this data and modelling to set a protective selenium limit for Koocanusa, rather than allowing further increases in selenium with potentially disastrous effects on aquatic life. The USGS report makes it clear that selenium levels in Koocanusa must be reduced. Work to set a protective limit and to reduce selenium concentrations below that limit must begin immediately.

Specifically, a protective standard must be based on a protective whole-body selenium limit. We support the Ktunaxa Nation, KTOI and CSKT in calling for a 5.6 mg/kg dw whole-body limit, based on BC's 11 mg/kg egg/ovary Water Quality Guideline, which includes an uncertainty/safety factor of 2, and a conversion factor of 1.9 for rainbow trout. In this case, a median K_d of 4547 would be appropriate, as BC's Water Quality Guideline already includes an uncertainty/safety factor. However, if the limit is based on the EPA's limit of 8.5 mg/kg dw, we believe a more conservative approach is needed. In the Aquatic Life Ambient Water Quality

Criterion for Selenium, the EPA uses the 20th percentile of the translations of the egg-ovary criterion concentrations to water column concentrations to provide a "high probability of protection for most aquatic systems".[1] In this case, using a 80th percentile K_d of 5566 would be appropriate and provide an equivalent degree of protection to that intended in the EPA standard. Using a median or other lower K_d would not be appropriate if the EPA standard of 8.5 mg/kg is used.

With the very steep curve relating selenium egg/ovary concentrations to mortality, and relatively widely distributed data for selenium concentrations for individuals of the same species found in Koocanusa, it is clearly necessary to take a cautious, protective approach, which favours the lower BC whole-body limit with its uncertainty/safety factor.

We also support the 5.6 mg/kg whole-body limit to allow for preferred Ktunaxa fish consumption rates without exceeding human health limits.

We share the concerns expressed by some SeTSC members about lag times for biological effects from increasing selenium concentrations in Koocanusa. Lag times for selenium moving from water to sediment and at the various trophic levels could lead to an underestimation of the risk to aquatic life from the measured selenium concentrations. This concern should further strengthen the case for a standard with a reasonable safety margin to ensure protection.

We support the use of the W6 model (TFM w TL3 100% aquatic insects) to protect burbot and mountain whitefish. Using this model with the 5.6 mg/kg limit and a median K_d leads to a 0.6µg/L water limit, while using the model with a 8.5 mg/kg limit and a 80th percentile K_d leads to a 0.8µg/L water limit.

Additionally, a conservative approach is needed to protect downstream species in the Kootenai/Kootenay River and Kootenay Lake. Of particular concern are white sturgeon, endangered in both Canada and the US, and burbot. We note that elevated selenium levels in water above 1µg/L have been found in both the US and Canadian portions of the river and recent KTOI data shows significant selenium levels in fish tissue. With the Canadian portion of the Kootenay River between the US border and Kootenay Lake, as well as the Kootenay River delta into Kootenay Lake, designated as critical habitat for white sturgeon under Canadian SARA, it is crucial that a protective standard be set for Koocanusa that ensures protection for white sturgeon in this critical habitat. While we are not aware of any current tissue data available for sturgeon in Canada, we note the sensitivity of the species in the EPA Criterion, the recent troubling KTOI data that showed selenium in fish tissue above EPA and BC standards, and BC water quality data showing selenium exceeding 1.0µg/L in the Kootenay River at Creston and exceeding 0.6µg/L in the southern portion of Kootenay Lake[2]. Clearly, more study is needed, but these pieces of evidence already available should be highly concerning. A conservative

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[1] Aquatic Life Ambient Water Quality Criterion for Selenium - Freshwater https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf, p. 92.

[2] Data from BC EMS, Locations IDs E206587 and E216952

standard should be set immediately in Koocanusa, the only significant source of downstream selenium, while work is done to quantify the effects of selenium concentrations in the Kootenai/Kootenay River and Kootenay Lake on sturgeon, burbot and other species.

Comparing the USGS model to the EVWQP model

We have heard concerns about the validity of the USGS model and the assumptions made for inputs to the model. In this context, we believe it is constructive to compare the USGS modelling to the modelling done to support the selenium limits set under Teck's EMA discharge permit in the Elk Valley, based on the 2014 Elk Valley Water Quality Plan.

While converting from the established thresholds for egg/ovary selenium to whole body values may not be ideal, it is necessary to use the existing, available data and to accept that obtaining reliable egg/ovary data is difficult. Here we compare the USGS approach to Teck's approach for the EVWQP modelling in 2014[3]. While Teck's approach uses egg/ovary data directly, the study on which the EVWQP modelling is based gives a large range for the 95% confidence interval of 12-31µg/L for WCT reproductive EC_{10} in eggs/ovaries, with a result that the derived selenium concentrations in water for 10% effect size should be subject to large uncertainties, though no uncertainties are given. Consequently, the calculated water concentration for 10% effect size and the water limit based on this concentration should be subject to a large degree of uncertainty.

We believe the USGS approach, based on established egg-ovary criteria, is far superior to the species-specific approach taken by Teck. The conversion to whole body values may increase uncertainty to a small degree, but far less than relying on one study with eggs from a few fish to determine thresholds.

We also have heard significant concern about widely varying values used in the USGS model, especially K_d values. In Teck's EVWQP modelling, we also observe very wide ranges of values in matched samples for water to invertebrates and invertebrates to fish eggs. We believe the USGS conclusions are far better supported by the data than the conclusions in the EVWQP modelling, because the data used by the USGS is mostly in the relevant ranges of selenium concentrations, while Teck's data is largely outside the relevant ranges. The overwhelming majority of the data used by Teck is significantly below or significantly above the relevant egg/ovary and water concentrations. Teck's data is also from a mix of lentic and lotic environments.

Overall, if BC is willing to rely on the relatively weak modelling behind the EVWQP to protect fish in the Elk River Watershed, they should also be willing to immediately adopt limits based on the modelling in the stronger USGS study. In any case, uncertainty in the data or the model should

[3] This modelling work was done by Golder Associates.

lead to a more protective limit in order to keep aquatic life safe, rather than further delay while selenium in the reservoir is already above safe limits in water and in fish tissue.

Timeline for adoption

We support Montana's plan to adopt a protective limit in 2020 and urge BC to also adopt the same limit this year. Increasing selenium concentrations in recent years have already put fish and other aquatic life at risk. We cannot afford to continue to allow selenium concentrations to increase. BC must provide a regulatory framework to give strong incentives for Teck to immediately reduce selenium loadings entering Lake Koocanusa to safe levels. We cannot continue to rely on promises of future selenium reductions, while mining and selenium-leaching waste rock dumps expand daily.

Additionally, BC needs to take immediate action to characterize the full timescale of the selenium leaching problem and to evaluate proposed solutions such as active water treatment or saturated rock fills in that context. BC needs to ensure that Teck meets this Koocanusa limit not only in the immediate future, but also in the long term, after mining ends and for the centuries or longer that selenium leaching will continue. BC must not allow a situation where $100 million for operating and capital costs is required annually to maintain water treatment in perpetuity in order to meet the Koocanusa limit.[4]

With Teck's plans to nearly double the amount of selenium-leaching waste rock over the next 20 years per the 2019 Implementation Plan Adjustment, it is essential that BC provide a clear and strong regulatory signal to Teck immediately, in order to ensure the full costs of mitigating pollution from that waste rock, if such mitigation is possible, are included in Teck's plans—and selenium levels are not allowed to increase past the point where Teck is able to reduce selenium below the Koocanusa limit.

The adoption of the conservative, protective limit for Koocanusa this year would also enable effective environmental assessments over the next two years, both federally and provincially, for Teck's Castle Project and North Coal's Michel Project.

Geographic application of the limit

We urge BC to adopt the shared Koocanusa limit in concert with Montana and to apply that limit to the entire Canadian portion of Lake Koocanusa. Fish may be resident in certain areas of

[4] Teck 2019 Annual Report, p. 13 indicates an estimated long-term operating cost for water treatment of $3 per tonne and annual production of roughly 24 million tonnes, leading to an estimate of $72 million annually. Including capital costs for replacement of treatment facilities would increase this estimate significantly. However, the report states that current operating costs are $31 million ($1.30 per tonne) with only two small facilities in operation and 14 or more additional facilities are planned. This estimate appears to be highly optimistic and the true cost may be much higher.

the reservoir, especially smaller species like redside shiner and peamouth chub, which have both shown tissue concentrations well above BC and EPA limits. We oppose Teck's proposal in their 2019 EVWQP Implementation Plan Adjustment to measure selenium concentrations in Lake Koocanusa as an average of four sampling sites distributed from the Elk River to the border.

In particular, BC should adopt the Koocanusa limit at the current LK2 (South of the Elk River) permit order station. If there are concerns about full mixing at this location, BC should allow an average concentration of a reservoir transect, at various depths, to measure compliance with the limit. The limit should also apply at the border, in the Forebay, and at any other sampling locations in BC or Montana.

Sincerely,

Lars Sander-Green
Mining Lead
Wildsight