Appendix N

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Critical Review

Guidance on assessing the potential impacts of selenium in freshwater ecosystems

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EDITOR'S NOTE:

This article is part of the special series "Regulatory issues surrounding the management of selenium." This series presents a collection of views from North American experts on the aquatic behavior of selenium and environmental, physiological, and operational factors that must be considered in Se regulatory frameworks.

Abstract

Despite decades of fate and effects studies, environmental selenium (Se) contamination and management remain an issue for many freshwater systems in North America. Several regulatory bodies have promulgated updated targets or management levels for Se; however, additional guidance on best practices for monitoring Se to protect freshwater aquatic life is warranted. In this article, we describe current approaches to assessing the ecological risks of Se in impaired freshwater systems and outline recommended methods for collecting and analyzing biological and abiotic samples and interpreting data. Because reproductive impairment of fish populations is most commonly used to determine the potential impacts of Se, several biological factors that could affect Se toxicity are explored, including diet, trophic positions, reproductive biology, body size and maturity, migratory movements, and use of seasonal habitats. Measuring Se concentrations in mature eggs is the most reliable metric for estimating potential reproductive impairment in fish populations because the range of toxicity thresholds is relatively narrow for all but a few tolerant fish species. In situations where collecting mature eggs is not feasible, we review the use of alternative fish tissue for estimating potential effects. Factors affecting Se uptake from freshwater are also considered with guidance on collecting abiotic (e.g., water and sediment) and biotic components of aquatic food webs (e.g., macroinvertebrates, biofilm). *Integr Environ Assess Manag* 2024;00:1–16. © 2024 SETAC

KEYWORDS: Environmental assessment; Freshwater; Invertebrates; Selenium; Toxicity threshold

INTRODUCTION

The issue of environmental selenium (Se) pollution in North America first emerged in the 1960s (Lemly & Skorupa, 2012) and is now known to result from activities related to the irrigation of seleniferous soils, the extraction and preconsumption processing of metals and fossil fuels, coal combustion, and the management of associated waste streams, including their storage and disposal (Chapman et al., 2010). Ecosystem-scale case studies of Se contamination in the 1970s and 1980s motivated decades of field- and laboratory-based research that has contributed to a strong understanding of Se's environmental fate and distribution, biogeochemistry, environmental persistence, and toxicity (see Chapman et al., 2010; Lemly, 2002, for reviews). This research has since informed environmental regulatory structures, including the British Columbia Ministry of Environment (BC MoE), ambient water quality guidelines (Beatty & Russo, 2014), and the USEPA aquatic life ambient water quality criteria (USEPA, 2016). These approaches are distinct from those focused on other contaminants because they reflect that Se readily bioaccumulates in aquatic food webs and exposure occurs primarily through dietary intake. Accordingly, regulatory guidelines prioritize fish tissue-based Se concentration data for aquatic life protection.

Improved waste management has resulted in less environmental Se loading from some major sources, such as effluents from coal-fired power plants; however, Se contamination continues to be a pressing problem for aquatic systems in North America with active mining and irrigation of seleniferous soils, and due to legacy effects at historically contaminated sites. Habitats affected by elevated

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concentrations of Se include the Clark Fork River ecosystem (Montana, USA; Gruen, 2020), the Upper Colorado River Basin, (USA; Brandt et al., 2021; Day et al., 2020), the Elk Valley (BC, Canada; Storb et al., 2023; Wellen et al., 2015), the Upper Columbia River Basin (USA; Gruen, 2020), and the Salton Sea (USA; Hennequin et al., 2022). Therefore, the consequences of Se loading on aquatic ecosystems remain a substantial and widespread ecological concern.

In this article, we argue that there is a need for updated guidance on best practices for environmental Se monitoring for aquatic life protection. Such guidance would provide a timely response to regulatory changes aimed at applying improved Se science to managing persistent environmental Se problems in Canada and the United States. For example, Environment and Climate Change Canada (ECCC) has recently revised the Environmental Effects Monitoring (EEM) requirements for metal and diamond mines regulated under the *Metal and Diamond Mining Effluent Regulations* to include the monitoring of Se in fish tissue. In Canada, EEM is a cyclical and mandatory regulatory program that assesses the potential effects of industrial effluents, including metal and diamond mines and with recent recommendations for coal mines, on aquatic receiving environments. Environmental Effects Monitoring includes a fish survey that assesses potential effects on growth, population structure, and reproduction in resident, effluent-exposed fish compared with fish from reference areas. Environment and Climate Change Canada also proposes to develop the *Coal Mining Effluent Regulations* under the *Fisheries Act*, which will include Se effluent standards for 28 existing mines, as well as all future coal mines and coal mine expansions (ECCC, 2020a, 2020b). In the United States, Montana recently adopted an updated Se standard for Lake Koocanusa and the Kootenai River mainstem (US Geological Survey [USGS], 2022), and the USEPA is proposing distinct criteria for California's San Francisco Bay and Delta that consider unique ecological factors influencing Se uptake and toxicity (USEPA, 2022).

This article synthesizes what has been learned about assessing ecological Se risks in impaired freshwater systems in North America over more than 60 years, focusing primarily on freshwater systems. Recommended monitoring practices reflecting the current state of science are also outlined. Because many management efforts center on the need to protect sensitive fish species, we emphasize issues specific to fish biology while highlighting considerations for monitoring Se in abiotic compartments and lower trophic levels. Benthic invertebrates are not only dietary Se exposure vectors for fish but also sensitive indicators of overall aquatic environmental quality. Therefore, considerations for including invertebrates and sediment sampling in environmental monitoring programs for Se are also provided. Additionally, we review issues related to physicochemical factors that can affect Se bioaccumulation, choices of sampling locations, and sampling considerations for benthic invertebrates and sediment, as well as analytical approaches to determining Se in abiotic and biological samples. We consider methods for assessing exposure to mine effluents, appropriate sampling effort and analytical methods, and data analysis and reporting.

BACKGROUND ON THE ROLE OF DIETARY EXPOSURE IN SE TOXICITY

Dietary exposure is the primary route of Se exposure for fish species. It is widely accepted that Se enters aquatic food webs via concentration by primary producers, is subsequently biotransformed to organic selenide compounds, and transfers through dietary pathways from prey to consumer species (Ponton et al., 2020; Presser & Luoma, 2010). Selenium is an essential micronutrient for all vertebrates and is incorporated in many selenoproteins that support antioxidant function, thyroid hormone regulation, cell proliferation, and muscle metabolism (Beatty & Russo, 2014). However, at concentrations only four- to sevenfold higher than those required in the diet, Se negatively affects reproduction in vertebrates that lay eggs, including fish, which are more sensitive to the potential negative effects of Se than other aquatic organisms (Beatty & Russo, 2014; USEPA, 2016). The primary adverse effects are mediated by the dietary intake of Se by female fish and maternal transfer to their eggs (DeForest et al., 2012). Impaired population recruitment resulting from elevated exposure to Se occurs within a relatively narrow range of Se concentrations in eggs among all fish species studied to date (Janz, 2011). During embryonic development, Se is assimilated from the yolk and can replace sulfur in the amino acid methionine, yielding selenomethionine during embryonic protein synthesis. Although the underlying mechanisms are not completely understood, it is thought that higher rates of Se substitution in proteins and oxidative stress resulting from excess Se metabolism can lead to decreased hatching, elevated rates of mortality, deformities, edema, and mortality that can affect population recruitment (Janz et al., 2010; Kupsco & Schlenk, 2016).

CONSIDERATIONS FOR SELECTING SENTINEL FISH SPECIES

Elevated environmental Se concentrations in North America have spurred a critical reevaluation of how best to monitor ecological impacts and determine which wild species should be selected as sentinels for monitoring efforts. Because dietary factors are most important for mediating the effects of Se in fish, exposure to an effluent or other point source is less important than understanding dietary exposure pathways for determining risk and selecting sentinel fish species. Established or conceptualized dietary pathways of exposure can be estimated based on knowledge of prey organisms and analysis of Se concentrations in those taxa. Participation in the local food web is the first gatekeeper function that must be considered to establish a list of suitable sentinel fish species, likely in consultation with a local fish biologist (ECCC, 2012; Figure 1). Sufficient abundance at the exposed and reference sites will also need to be verified before selecting a sentinel species. If a species

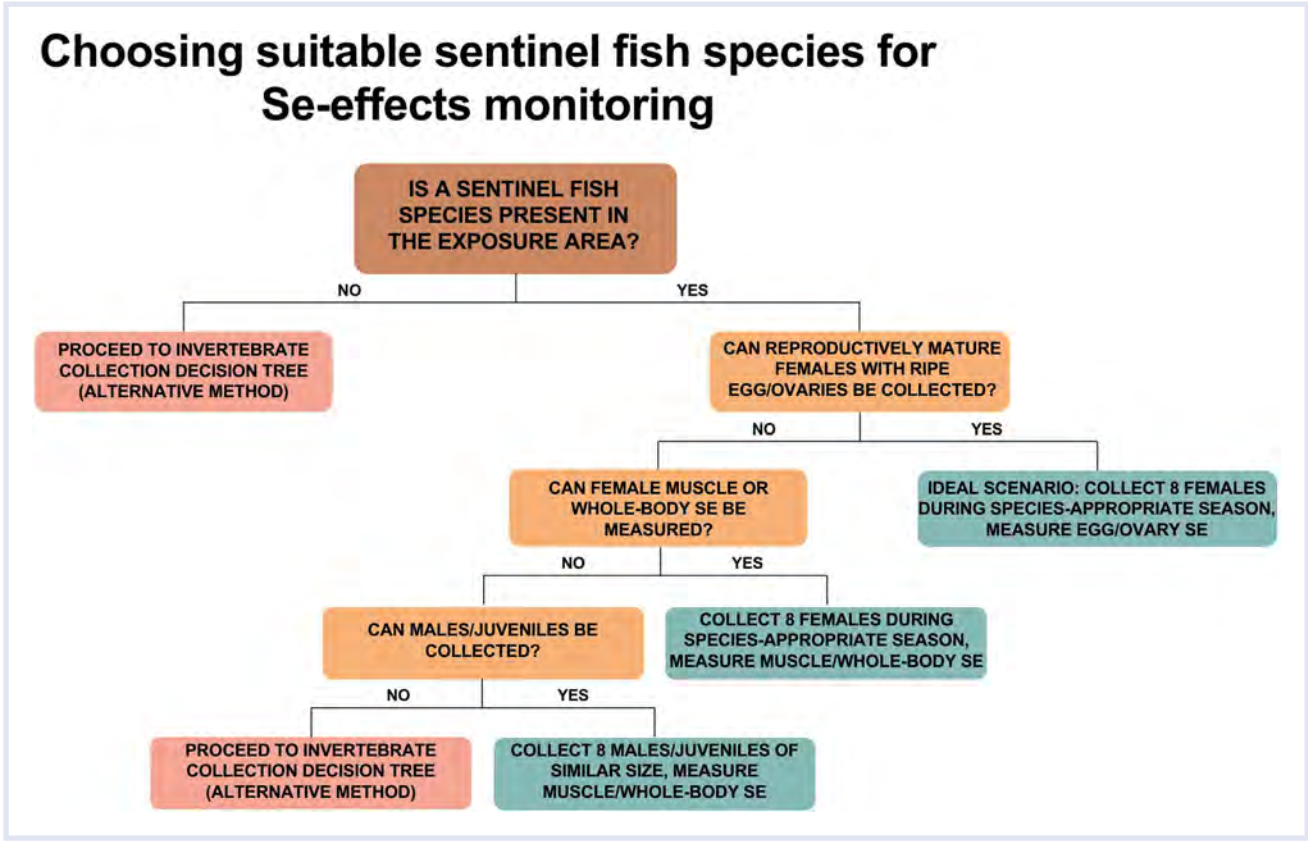

FIGURE 1 Decision tree for choosing suitable sentinel fish species for selenium (Se)-effects monitoring

has been used in prior studies, this may provide some context for temporal data comparisons and the relative abundance of the species (ECCC, 2012). Where a point source discharge is evident, immediate exposure at the discharge location is less important than residence and feeding in habitats influenced by that discharge and where conditions are favorable for Se to be incorporated into the food web. In other words, dietary Se concentrations are not necessarily greatest when they are closest to the discharge; instead, they may be elevated at sites downstream where accumulation in the local food web is facilitated (e.g., depositional zones; Brandt et al., 2021). Finally, sampling eight fish is often sufficient to detect differences between affected and reference locations, but sample sizes increase at higher Se thresholds because of heterogeneity among individual fish at higher Se exposures (Hitt & Smith, 2015).

Several fundamental tools can be used to assess the exposure of fish to potentially elevated Se concentrations in downstream habitats. To assess exposure pathways, it is often useful to construct a conceptual model of exposure that outlines potential routes of exposure to dietary Se for fish in downstream and reference habitats (Presser & Luoma, 2010). The conceptual site model should consider, for example, potential depositional zones where hotspots of Se accumulation may develop. Historical data from previous studies should be reviewed, if available, and if warranted, the conceptual site model could be refined by assessing Se concentrations in primary producers, biofilm, periphyton, algae, bacteria and fungi, and secondary consumers (i.e., benthic invertebrate taxa) commonly the prey of fish. Determining exposure also requires understanding the residence times of a given fish species in specific habitats. Quantitative and seasonal fish population and community surveys should be conducted to determine fish species assemblages, abundance, and habitat use patterns (e.g., via telemetry studies; ECCC, 2012; Cope et al., 2016). Once fish assemblages and potential exposure routes have been determined at the exposed and reference sites, candidate fish species for further exposure monitoring can be identified. In many cases, these initial steps are adequate to refine the conceptual site model so that Se exposure pathways are understood. More detailed studies may seek additional refinement, specifically for dietary exposure routes.

To further refine the conceptual site model, typical dietary items for fish species that are screened-in as potential monitoring candidates can be predicted using resources such as FishBase (https://fishbase.se/search.php). Diet items identified in the conceptual site model can then be verified using gut content analysis of fish sampled from the area. For large-bodied fish, gut contents can be obtained nonlethally using nonlethal gastric lavage techniques (Hartleb & Moring, 1995), but for small-bodied fish it may be necessary to lethally sample fish to obtain gut contents for visual identification of forage items. Analysis of $\delta^{15}N$ stable

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isotope values in representative components of the food web and in fish tissue can be used to further verify trophic relationships (e.g., Brandt et al., 2021). Finally, analysis of Se concentrations in annual growth zones of otoliths can provide additional information regarding the residence patterns of fish at a given site and the relative changes in Se exposure over time (Johnson et al., 2020). Although microchemical analyses of Se in annual growth zones are more technically challenging, they have recently become commercially available.

Trophic position and trophic transfer

Dietary exposure of a given fish species to Se is affected by several factors, including its trophic position. The most important step for determining Se accumulation in aquatic food webs is ascertaining Se uptake from water by primary producers and microbes (i.e., the particulate fraction). However, this step is mediated by species- and habitat-specific factors referred to as enrichment functions (EF), which can vary over several orders of magnitude and are difficult to predict (Ponton et al., 2020; Stewart et al., 2010). The secondary concentration of Se is described by trophic transfer factors (TTFs) that apply to specific consumer species feeding in a given environment. Trophic transfer factors incorporate the ingestion rates and the assimilation efficiency of Se (Stewart et al., 2010). Trophic transfer factors derived for fish feeding on invertebrates, and for top predator level fish feeding on forage fish, have been derived empirically for many North American fish species (Presser & Luoma, 2010). Compared with EFs, TTF values are far lower and less variable, with values for fish feeding on invertebrates generally ranging from 0.5 to 1.8 (Presser & Luoma, 2010). Fewer studies have examined TTFs for upper trophic level piscivorous fish, but the available data indicate similar values for fish feeding on invertebrates (Stewart et al., 2010). Trophic transfer factors as high as 10 have been estimated for benthivorous fish such as spottail shiner, white sucker, stickleback, and burbot, but TTFs vary depending on the specific prey consumed (Muscatello et al., 2008, Muscatello & Janz, 2009). If they were identified, higher TTFs among upper trophic level fish would indicate Se biomagnification. Historically, there has been some scientific discord about the ability of Se to biomagnify (Beatty & Russo, 2014, and references therein), but data from field studies indicate that biomagnification (i.e., TTFs consistently >1) from forage fish species to piscivores is uncommon (Brandt et al., 2021; Day et al., 2020). In other words, fish feeding at a higher trophic level do not typically have higher tissue Se concentrations than those feeding at lower trophic levels. Furthermore, in their thorough evaluation of aquatic food web data, the USEPA (2016) found little variation in Se across all trophic levels of fish, except for fish consuming mollusks. This is relevant to sentinel species selection because fish that consume bivalves as a primary prey item may accumulate higher concentrations of Se than species feeding on other organisms. Mollusks, including bivalves, have proportionally greater uptake and up to 10 times slower elimination rates for Se than other organisms (Stewart et al., 2010). Finally, if bivalves are used as a surrogate for fish, an accepted alternative in Canada's EEM for metal mines, relatively high estimates of Se bioaccumulation may be derived.

We examined the relationship between trophic status and TTFs for freshwater fish species used for EEM studies in Canada. A numeric value for trophic status was retrieved from FishBase (http://www.fishbase.org), a database of more than 8300 fish that has compiled dietary records to assess trophic level based on weighted calculations of all food items consumed by a species. These estimates agree closely with the trophic positions determined by stable isotope studies (Romanuk et al., 2011). Species-specific TTFs were obtained from a list published in USEPA (2016). It is important to recognize that the analysis is based on a single median TTF for each fish species (USEPA, 2016) despite understanding that TTFs are dynamic ranges and are influenced by context-dependent factors (Graves et al., 2019; Presser & Naftz, 2020) and that uptake functions (e.g., EFs and TTFs) are inversely related to Se exposure concentrations in aquatic systems (DeForest et al., 2017). These inverse relationships reflect that receptor-mediated uptake of selenocysteine dominates at low Se concentrations but that selenomethionine uptake increases (i.e., the proportion of Se as selenomethionine increases) as receptors become saturated, resulting in lower relative TTFs (Janz et al., 2014; Ponton et al., 2020). Paired TTF and trophic status assignments were available for 28 species that included varied feeding strategies (i.e., benthivorous, invertivorous, piscivorous, and carnivorous) and a range of TTFs (i.e., 2.4–4.7). There was no significant relationship between species-specific TTF and trophic status (Figure 2), suggesting that Se does not biomagnify and that diet type has a greater influence on Se uptake than trophic status.

Reproductive biology

After assessing dietary exposure pathways and confirming sufficient abundance within a study area, understanding a species' reproductive biology is the next most important factor in selecting a sentinel species. The adverse consequences of elevated Se exposure are associated primarily with Se assimilation from the yolk during embryological development (Janz et al., 2010). This early life exposure is a consequence of Se substitution for sulfur during vitellogenin (an egg yolk precursor phospholipoglycoprotein) synthesis in the maternal liver and subsequent transfer from maternal fish to ovary/egg tissue during the final oocyte maturation phases of egg development (Janz et al., 2010). The resulting enrichment of Se in egg yolk leads to elevated exposure during yolk resorption by embryos after egg fertilization (Janz et al., 2010). As such, Se concentrations in egg/ovary tissue—and more specifically, eggs collected from gravid (i.e., ripe and running) females after the vitellogenic phase of development—are the most reliable measure of potential reproductive effects in fish populations (Beatty & Russo, 2014; Brandt et al., 2019; Janz, 2011; USEPA, 2016).

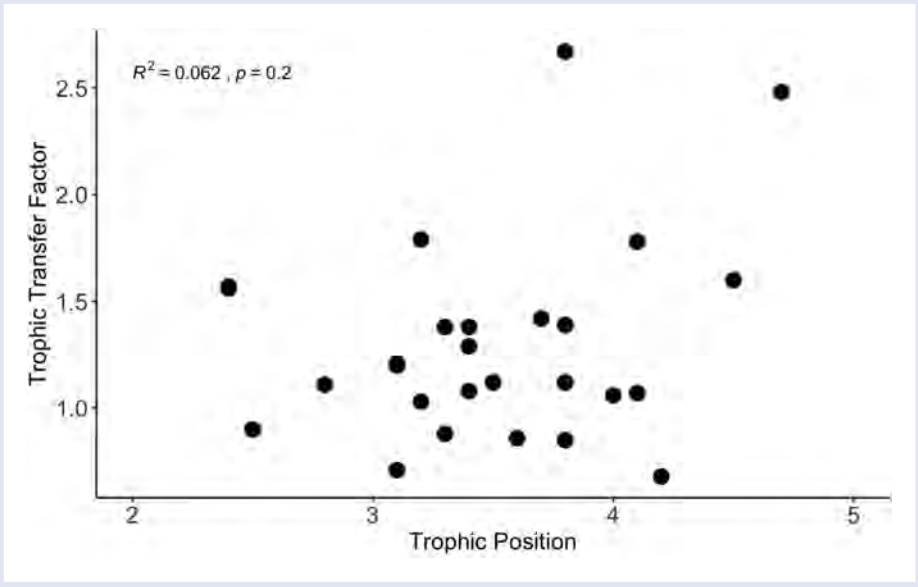

FIGURE 2 Trophic transfer factors (TTFs) vs. trophic position for fish used for Environmental Effects Monitoring (EEM) studies in Canada

Because they relate to the amounts of Se available for maternal transfer to eggs, factors related to egg development and adult spawning are important considerations when selecting sentinel species for monitoring purposes. The processes of vitellogenesis and oogenesis, and when they occur relative to spawning, vary considerably among species (Osmundson et al., 2000; Tyler et al., 1990), and maternal Se burdens immediately preceding these processes determine Se loading in eggs (Golder Associates, 2018). Furthermore, some small-bodied fish spawn several times in each reproductive cycle; therefore, clutches of eggs derived across spawning events may have varying Se concentrations reflecting maternal tissue stores and/or recent dietary intake (Driessnack et al., 2011). Egg sampling should be conducted either immediately before or during spawning for single spawning species and before the first spawning event for multiple spawners.

The selection of sentinel species should also consider the resources invested in eggs (ECCC, 2012). For a species to be a viable sentinel species candidate, it should be practical to obtain sufficient eggs for Se analysis and to determine other reproductive metrics, including the gonadal somatic index (GSI). The GSI is the proportion of a fish's weight contributed by reproductive tissue and is used to measure reproductive potential. Numbers of eggs and egg sizes may also be considered indicators of energy investment. The ecological importance and evolutionary factors governing egg numbers and sizes in fish have been linked to habitat suitability, spawning season, parental size, and energy investment (Kolm & Ahnesjö, 2005; Sargent et al., 1987). It has been suggested that species with relatively large eggs and yolk contents could deposit more selenium in their eggs than species with smaller eggs and yolks, and this may be related to developmental Se sensitivity (Osmundson & Skorupa, 2011). However, we found no significant relationships between the existing Se-toxicity thresholds (i.e., EC_{10} values) and egg volume (Figure 3A), yolk volume (Figure 3B), or the ratio of yolk:egg volume (Figure 3C) in mature eggs spawned from gravid females of 12 species previously used for EEM studies in Canada (range in *p*-values = 0.39–9.94).

Selenium developmental toxicity may have less to do with egg size or yolk:egg ratio than with Se transport between tissues during egg formation and/or the use of vitellogenin during early embryological development. For example, DeForest et al. (2017) suggested that fish with greater sensitivity to Se (i.e., low EC_{10} values) also partition more Se to eggs from muscle or whole-body Se concentrations relative to species with lower Se sensitivity. Variability in vitellogenin synthesis may also play a role in determining how Se is mobilized from the muscle and/or whole body and in differences in species-specific sensitivity to Se. There are differences among fish species in the number of VTG protein sequences produced during vitellogenesis. The protein sequence is a major factor in determining other constituents that may bind to VTG and are delivered to the egg along with the lipoprotein (Riddle & Hu, 2021). Differences in the vitellogenin sequences may dictate how strongly Se binds to the lipoprotein during egg maturation and how efficiently Se is mobilized from the tissue to the developing oocyte. Mutations in some vitellogenin subtypes can even increase the rates of edema and spinal deformities (Riddle & Hu, 2021), both hallmarks of Se developmental toxicity. Additional research is needed to examine the potential role of vitellogenin subtypes to determine differences among species in the mobilization of Se from tissue to egg and relative sensitivities to the effects of Se on embryological development.

Understanding the temporal linkages between maternal dietary Se intake, tissue deposition and oogenesis, and spawning is a key element for selecting appropriate sentinel

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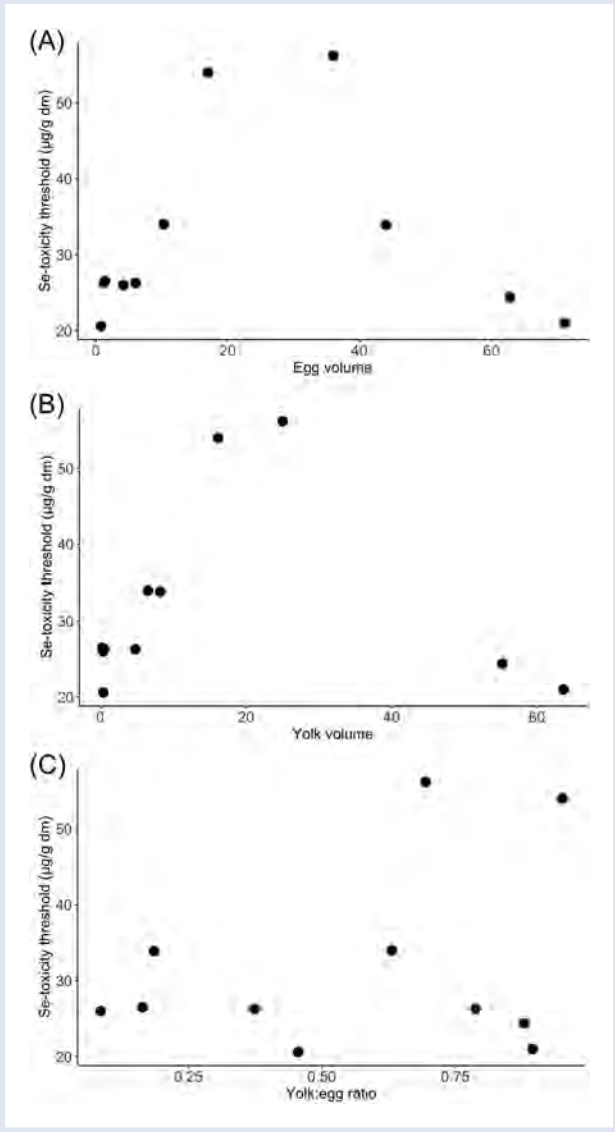

FIGURE 3 Selenium toxicity threshold concentrations µg g^{-1} dw) vs. egg volume (A), yolk volume (B), and yolk:egg volume (C) for fish species previously used for metal mining Environmental Effects Monitoring (EEM) studies in Canada

fish species to assess the potential effects of Se. Therefore, it is advantageous to select sentinel species whose reproductive timing is well characterized and for which Se-dietary exposure is also linked to the local food web before or during gonad maturation. In many cases, this may mean selecting large-bodied fish species that spawn once per year (USEPA, 2016). There are some added advantages to this approach. For example, there is more information regarding Se-toxicity thresholds for large-bodied fish, and it is more practical to estimate GSI from spawning fish using nonlethal methods. Specifically, the expressed egg volume and mass of large-bodied fish can often be measured to provide a sufficiently reliable measure of GSI in lieu of lethal sampling of gonad tissue (Bromage et al., 1990). Small-bodied fish with known reproductive biology should also be considered. For example, fathead minnow eggs can be collected on artificial spawning substrate deployed at spawning locations (Masson et al., 2006). Establishing field methods to collect eggs from other small-bodied species with less information regarding reproduction requires significant investment in field resources and time (De Bruyn et al., 2023). However, the effort is warranted if these species benefit the study design via smaller home ranges and participation in local food webs.

Environmental monitoring programs have established relatively wide sampling windows for determining GSI in many species of North American fish (ECCC, 2012; USEPA, 2016), but these times may not be suitable for determining the potential for Se exposure and effects. Sampling within larger windows of ovarian development may not impair the ability to detect relative differences in GSI for large-bodied species (ECCC, 2012), but sampling eggs before full maturation does not allow meaningful comparisons of Se concentrations in eggs with existing toxicity thresholds (USEPA, 2016). For example, it has been recommended to sample ovary tissue and assess GSI in late autumn for species such as rainbow trout that spawn synchronously in spring (ECCC, 2012). However, this sampling period occurs well before the peak of vitellogenesis when the oocyte diameter is only 40%–50% of the expected size at ovulation (Tyler et al., 1990) and before a significant portion of maternally deposited Se would be delivered to the eggs (Janz et al., 2014). Because toxicity thresholds are established based on concentrations measured in eggs, it is imperative to perform Se analysis of mature eggs to allow comparisons with egg Se guidelines or criteria or Se EC_{10} values (USEPA, 2016). Although final egg maturation before ovulation can increase the diameter and weight of each oocyte caused by hydration significantly (Milla et al., 2006), these increases will not affect reported egg Se concentrations, which are provided on a dry-weight basis (i.e., after freeze drying; Beatty & Russo, 2014; ECCC & Health Canada, 2017; USEPA, 2016).

Body size and maturity

As discussed above, trophic position is not a reliable predictor of a fish's Se burden. Accordingly, we did not find a significant relationship between maximum body size and Se TTFs for the same list of species noted above ($R^2 = 0.035$, $p > 0.33$; data not shown). Previous studies have also reported that, among 20 freshwater and marine species, size variations were not a significant factor in determining fish muscle or whole-body Se concentrations (Burger & Gochfeld, 2011; Cianciolo et al., 2020; Stewart et al., 2010). It is possible to explore whether this trend also applies to juvenile life stages. A small set of relevant studies have contrastingly reported (a) significantly higher uptake and assimilation by relatively large larval fathead minnows than by smaller fish of the same age (Bennett et al., 1986), and (b) lower feeding rates and Se bioaccumulation by larger juvenile black seabream despite higher Se assimilation efficiency (Zhang & Wang, 2007). Finally, it has been demonstrated that rapid growth does not dilute whole-body Se concentrations in fish, as it does for some bioaccumulating contaminants, because greater food

intake during growth phases can counteract growth dilution effects (Luoma & Rainbow, 2005; USEPA, 2016).

On the other hand, dietary shifts that correspond to size transitions (e.g., ontogenetic shifts) can affect Se accumulation. Changes in fish Se concentration will be context dependent and reflect whether the predator species transitions to a diet with relatively higher or lower Se concentrations (Stewart et al., 2004). In conclusion, body size should not be a factor in selecting an appropriate sentinel fish species. However, dietary shifts resulting from size transitions must be considered, especially among juvenile fish. This is considered in more detail in the Monitoring Se in juvenile life stages section.

Migratory vs. nonmigratory fish species

Federal guidance in both Canada and the United States recommends against selecting highly mobile or migratory species for Se-impact studies because their exposure to areas influenced by the effluent is uncertain (ECCC & Health Canada, 2017; USEPA, 2016). The main reason for this is that dietary intake of Se can vary significantly among habitats, and migratory species could be exposed to areas of low or elevated Se concentrations, confounding source allocation and assessment of Se risk in a given area of influence (Beatty & Russo, 2014). If migratory species are selected, it is important to understand the dietary exposure pathways, including the timing of diet exposure relative to egg yolk deposition for that species (USEPA, 2016).

Localized movement by mobile fish species has also been shown to influence dietary Se exposure. Prior research has identified the confounding effects of seasonal or biological patterns in lentic versus lotic habitat occupancy, within-habitat use patterns, and interannual variability in habitat use on Se exposure (Friedrich et al., 2011; Orr et al., 2006; Palace et al., 2007). Collectively, these studies indicate that mobile species can complicate efforts to assess environmental Se impacts on fish. Sedentary and small-bodied fish (adult size $\leq$150 mm) may be preferred choices for environmental Se monitoring studies, especially if their food chains are well characterized (Palace et al., 2005, and references therein). However, these species may be relatively understudied and incomplete understanding (e.g., about their reproductive cycles) can therefore compromise their use as sentinels (Barrett et al., 2015).

Sensitivity and species of conservation concern

The relative sensitivities among different fish species to the reproductive effects of Se have informed several recent regulatory documents (Beatty & Russo, 2014; ECCC & Health Canada, 2017; Jenni et al., 2017; USEPA, 2016, 2018). Species sensitivity distribution (SSD) curves, based on effects linked to Se concentration in eggs, were used to model the relative sensitivity to Se among different fish. Depending on the approach used to set target tissue concentrations, slightly different thresholds can be derived. For example, the USEPA (2016) calculated a chronic criterion of 15.1 mg kg^{-1} (dry weight [dw]) based on the 5th percentile of a distribution of EC_{10} values compiled from 10 fish genera. The EC_{10} estimates the concentration of Se in eggs that results in a less than or equal to 10% effect among all fish species for which data are available (USEPA, 2016). The BC Ministry of the Environment derived a final threshold value 11 mg kg^{-1} dw by incorporating a safety factor of 2 from the geometric mean of EC_{10} values from the two most sensitive resident species in the province: rainbow trout and Westslope cutthroat trout (Beatty & Russo, 2014). In Canada, a predicted no-effect concentration (PNEC) for Se in fish eggs was developed (14.7 mg kg^{-1} dw). The PNEC estimates the hazardous concentration to 5% of fish species (i.e., = HC_5; ECCC, 2022; ECCC & Health Canada, 2017). Additional models have advocated SSD approaches using only the data for species that are relevant to a given study area (DeForest et al., 2012). Regardless of the refinements included in the applied models, there is a relatively narrow range in toxicity thresholds based on egg Se concentrations for most freshwater fish species (i.e., 16.2–25 mg kg^{-1} dw for relatively sensitive species and >54 mg kg^{-1} dw for relatively tolerant species including dolly varden and mountain whitefish), although the number of species for which established toxicity thresholds based on Se concentrations in eggs is still limited (De Bruyn et al., 2023, Brix et al., 2021).

Fish species with established chronic toxicity thresholds based on Se concentrations in eggs are preferred for studies of Se effects. However, this may not always be practical given the limited number of species for which information is available. For example, species for which egg thresholds have been established may not overlap with an assessment area of interest. Ultimately, it is more important to select monitoring species based on knowledge of their dietary exposure pathways and participation in the local food web than to select only species with established Se-toxicity thresholds. Again, this is because the range of Se-toxicity thresholds in eggs is relatively narrow. Therefore, if species without known toxicity thresholds but with high site fidelity and established dietary exposure pathways available, then current toxicity thresholds derived from the SSD of EC_{10} values for eggs from all species can be conservatively applied to estimate risk based on Se concentrations in their eggs.

It is important to note that the presence of species of conservation concern may serve as specific motivation for monitoring efforts in Se-impaired habitats (i.e., where there is contaminant impairment of established critical habitat; see Brandt et al., 2021, for example). Here, the research context at hand will determine whether monitoring efforts prioritize generating data for the susceptible species in place of an alternative sentinel species. In cases where it is not feasible or advisable to sample the susceptible population, a similar species should be identified as a proxy for Se concentration monitoring. Ideally, proxy species will be like the species of conservation concern in aspects that influence their Se exposure, as described above (i.e., diet, reproductive biology, and habitat use) so that concentrations measured in the proxy species provide reliable estimates.

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It is often difficult to identify a sentinel species that meets all the criteria described above, and research managers must consider the trade-offs during species selection. When possible, monitoring several species can help account for shortcomings presented by the use of any one species. Thorough and transparent justifications of the species selected for any given monitoring effort are important for contextualizing the results of a study as they relate to the associated contamination context.

Monitoring Se in other tissue

Mature eggs remain the best tissue to sample for evaluating potential reproductive effects arising from Se exposures. Because there is a strong positive relationship between concentrations of Se in ripe ovary tissue and eggs, the toxicity values based on residues in either tissue are often assumed to be equivalent (ECCC & Health Canada, 2017). However, the log–log regression equations for Se measured in the ovary and egg vary between 0.57 and 0.97 among species (reviewed in ECCC & Health Canada, 2017), indicating that egg concentrations can sometimes be higher than those in the ripe ovary and that there are differences in the slopes of the relationship among species. Recent work also reveals that Se concentrations can be higher in ovary tissue after spawning than in ovulated eggs (De Bruyn et al., 2023). As discussed above, vitellogenin and the associated Se are incorporated during the final stages of oocyte maturation, so Se concentrations in ovary tissue will not always be comparable with those in mature eggs. Therefore, only ovary tissue containing mature eggs should be considered equivalent to eggs when comparing Se concentrations with established toxicity thresholds (USEPA, 2016).

Conversion factors for relating egg/ovary Se concentrations to muscle or whole-body Se concentrations have been established for numerous species. Such conversion factors are not only species specific but are only intended for application to adult fish (ECCC & Health Canada, 2017; USEPA, 2016). When supported by appropriate species-specific statistical models, conversion factors should be applied to tissue collected during periods appropriate to that species' corresponding reproductive cycle and phase (Casey & Siwik, 2000; Presser & Naftz, 2020; North American Metals Council [NAMC], 2009). Muscle tissues should be collected from gravid females for the best modeling of Se concentrations in eggs. Muscle tissue samples should not be collected immediately after spawning because of reproductive depuration of Se (Day et al., 2020; USEPA, 2021). The predictive relationships between egg and muscle or whole-body Se concentrations are less certain in species that do not spawn annually because tissue Se mobilization will not be synchronized across the population (Rideout & Tomkiewicz, 2011).

Whole-body samples (i.e., complete intact fish, including ovaries and eggs) can be the preferred sample type when monitoring small-bodied fish that spawn multiple times during the year. When samples are collected outside the active spawning season, this strategy can be used to avoid the inherent variability of Se concentrations in ovaries among actively spawning fish (USEPA, 2016). In Canada, there are at least 15 multiple spawning species that have been used for previous EEM studies; however, established whole-body to egg Se concentration relationships have only been established for creek chub and fathead minnows and, of these, toxicity thresholds have only been described for fathead minnows. Because females can accumulate significant concentrations of Se in eggs, it is recommended to separate whole-body tissue by sex before analysis. If male and female fish muscle samples must be pooled, the practice should be confined to periods outside oogenesis for that species (Mo et al., 2020).

Asynchronous spawning species represent a distinct challenge in establishing models of Se concentrations in eggs versus muscle. This is because ovaries contain oocytes at various stages of development, and each egg stage might contain different Se concentrations. For these species, comparisons of contaminant levels should be made between mature spawned eggs, collected separately from ovary tissue and muscle tissue obtained during the spawning period (USEPA, 2016). The relationship between ovary and egg concentrations has been established in only two asynchronous spawning species in Canada, the fathead minnow (*Pimephales promelas*) and, more recently, redside shiners (*Richardsonius balteatus*; De Bruyn et al., 2023).

Obtaining samples from gravid female fish is especially difficult for some species like burbot and shorthorn sculpin that spawn in winter under the ice (Froese & Pauly, 2021) and other early spring spawners including northern pike and pearl dace (Eklöf et al., 2023; Timlick et al., 2022) that spawn when ice conditions are unsafe. In these instances, it may be necessary to assess the risks of Se toxicity using the analysis of other tissues (i.e., muscle or whole body), obtained at times outside the spawning window.

When Se concentrations cannot be measured in eggs or reliably extrapolated from muscle tissue, assessment using generic tissue guidelines is an accepted approach to screening level assessments. This method is often used at sites that lack species with established Se-toxicity thresholds or well characterized spawning strategies and peak spawning times. Predicted no-effect concentrations have been established for egg, muscle, and whole-body tissue based on multi-SSD for reproductive toxicity thresholds in eggs (ECCC & Health Canada, 2017). Most of the species used to develop the PNEC are relevant to North America, providing a high degree of confidence in the derived PNEC. The generally applicable PNEC for egg/ovary tissue (=14.7 mg kg^{-1} dw) is based on the modeled concentration of Se in eggs that is expected to present a hazard to less than 5% of fish species. Therefore, in the absence of species-specific information, Se concentrations in eggs or ovaries can be compared with PNEC to estimate potential Se effects. Similarly, a whole-body PNEC (=6.7 mg kg^{-1} dw), developed by converting egg:ovary to whole-body concentrations using established relationships gleaned from the scientific literature, can also be used for screening purposes

(ECCC & Health Canada, 2017). The ECCC and Health Canada (2017) PNECs align well with the egg:ovary (15.1 mg kg^{-1}) and whole body (8.5 mg kg^{-1}) criterion for Protection of Aquatic Life, prescribed by the USEPA (2016), who also specified a criterion for muscle (11.3 mg kg^{-1}). Comparisons against these nonspecies-specific threshold values should be considered a screening level assessment only and not a substitute for subsequent collection of more empirical data to establish species-specific, Se-toxicity thresholds. Considerable variability in tissue–tissue relationships between fish species (Janz et al., 2010) should motivate additional data collection to refine risk assessment estimates for Se.

Monitoring Se in juvenile life stages

When adult female fish are either not appropriate for monitoring because they do not participate in the local food web or are absent (e.g., the affected site is a nursery habitat), whole-body samples of juvenile fish may be collected. These can then be analyzed for screening level comparisons with whole-body threshold concentrations of Se for the same species (ECCC, 2012; USEPA, 2021). Nonlethal surveys of juvenile life stages can also provide information about population recruitment and complement our understanding of the potential reproductive effects of Se in the study context (Gray et al., 2002).

It is most appropriate to collect whole-body samples when assessing the potential effects of Se on the growth of juvenile fish. Dietary thresholds for effects on growth among juvenile fish are less well established and less certain than for reproductive effects of Se but appear to occur at similar concentrations to those described for reproductive effects (e.g., 4–10 mg kg^{-1}, reviewed in Beatty & Russo, 2014). Based on their review of these data, the authors recommended a whole-body tissue guideline of 4 mg kg^{-1} (dw) to be protective of effects to juvenile fish growth. Study design considerations, including pooling fish of the same species, sex (where possible), and size, should also apply to juvenile collections. Juveniles of many small-bodied fish species have strikingly similar appearances, so rigorous methods must be applied to verify closely related species before pooling (ECCC, 2012). Additionally, although trophic position and body size do not appear to greatly affect the potential for Se accumulation, gape size can vary among juvenile fish of different sizes, affecting their access to certain prey items, which can significantly affect their Se accumulation potential (Bennett et al., 1986; Zhang & Wang, 2007). For this reason, an accompanying analysis of Se concentrations in dietary items of different sizes can facilitate the interpretation of juvenile whole-body Se concentrations and potential effects on their growth.

ASSESSING ECOLOGICAL SE EFFECTS WITH ALTERNATIVE ENVIRONMENTAL MEDIA

As described above, fish tissue integrates the influence of abiotic and lower trophic level variation and should be prioritized for ecosystem assessments. In cases where it is not possible to collect sufficient fish, abiotic (i.e., water and sediment) and lower trophic level sample types can be analyzed as the basis for inferring Se exposure in fish (Figure 4). Several studies have illustrated the value of such approaches (e.g., deBruyn & Chapman, 2007; Graves et al., 2021), and robust, context-dependent Se bioaccumulation models can be developed from data representing Se cycling from the water column to particulate, macroinvertebrate, and fish compartments (Brandt et al., 2021; Presser & Luoma, 2010; Presser & Naftz, 2020). Subsequent use of

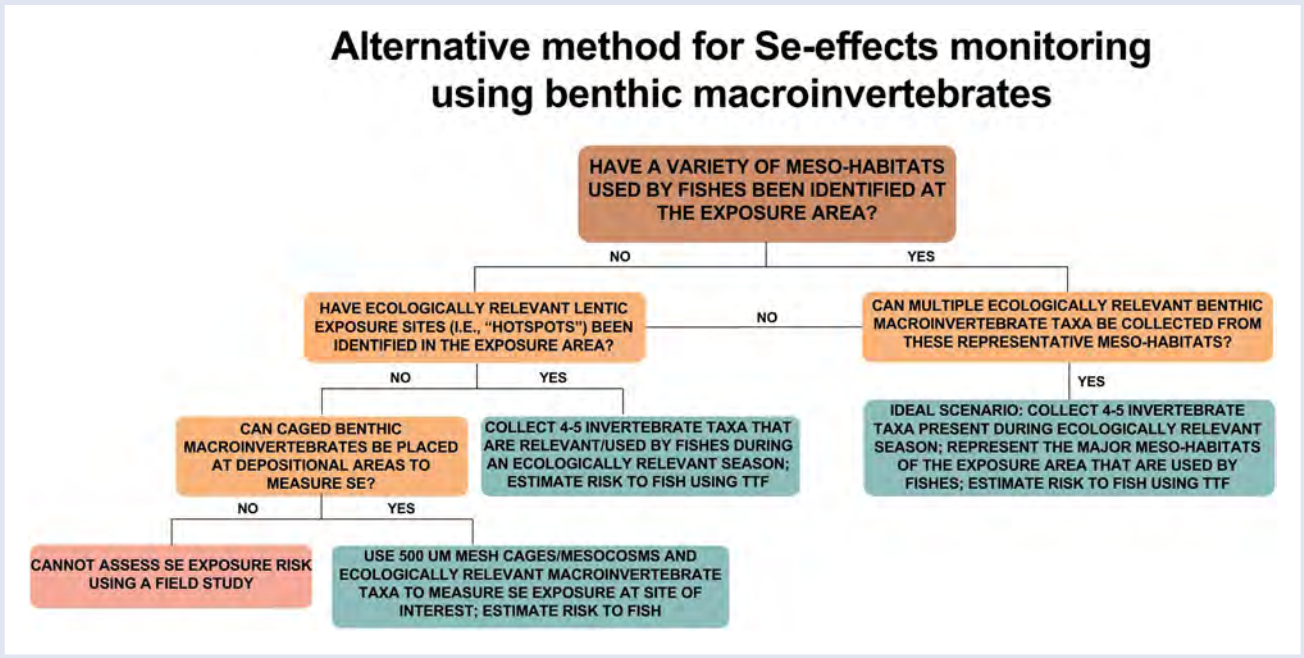

FIGURE 4 Decision tree for assessing potential reproductive effects of selenium (Se) in fish using invertebrate collections

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these models can make use of established concentration relationships to predict fish tissue Se concentrations if the accompanying uncertainty is appropriate to the monitoring effort. This section provides an overview of the sampling approaches used for these alternative sampling types, beginning with macroinvertebrates as the prey of lower trophic level fish species. Additional details are provided in Supporting Information S1 to guide sampling efforts in cases where macroinvertebrates collections are in place of, rather than complementary to, fish sampling.

Macroinvertebrates

Se variability and taxonomic resolution. Selenium concentrations in macroinvertebrates reflect the influence of abiotic and particulate Se concentrations on the food web and represent dietary exposures to resident insectivorous fish. Bioaccumulation of Se by benthic macroinvertebrates depends on both physiological factors, such as assimilation, ingestion, and efflux rates, and ecological factors such as habitat type, habitat use, and diet (reviewed in Presser & Luoma, 2010). We assessed these relationships using Se concentration data for macroinvertebrates and associated diet items collected from both lentic and lotic systems in Canada and the United States (see Supporting Information S1 for details). Relationships between Se concentrations in macroinvertebrates and their associated diets (i.e., TTFs) are highly variable both within and among taxonomic groups (Figure 5, Supporting Information Figure S2; Table 1, Supporting Information Table S1). Therefore, taxonomic, habitat, and diet-related variations in Se uptake must be considered when assessing Se exposure to higher trophic levels. It is also advised to measure macroinvertebrate $\delta^{13}C$ and $\delta^{15}N$ values to characterize the food web structure and assess the relative contribution of various prey to the fish species of interest. Sorting invertebrates to the level of family or functional feeding group before Se analysis provides a means of understanding the relative input and/or use of different habitats and different invertebrate types to fish. Including four to five representative taxa will further our understanding of the risk of exposure to fish based on invertebrate diet items and mesohabitat use.

Site selection and sample timing. Site selection and the timing and/or seasonality of sample collection should be designed to best represent food web exposure to fish. As such, a sampling design to collect representative invertebrate taxa from both erosional and depositional zones of affected and reference sites is preferred, although sampling efforts can be designed such that proportionately greater efforts are placed on potential hotspot mesohabitats. Sampling should be performed when it represents the most relevant exposure to fish relative to effluent exposure, gonad maturation, life-history characteristics, or feeding patterns most relevant to Se accumulation. Note that the relevant dietary Se exposure period for many fish species can occur well before gonad maturation. If aqueous and biotic Se concentrations are temporally consistent, one sampling event at a time relevant to fish Se exposure should suffice. If there are large seasonal fluctuations in aqueous Se concentrations (caused by seasonal changes in water flow and resulting dilution and/or concentration of effluent), a seasonal study design should be considered.

Sampling and processing. Standard operating protocols for the collection and processing of macroinvertebrates can be followed to collect organisms from a variety of habitats (ECCC, 2012; USEPA, 2016). The main adjustments and/or

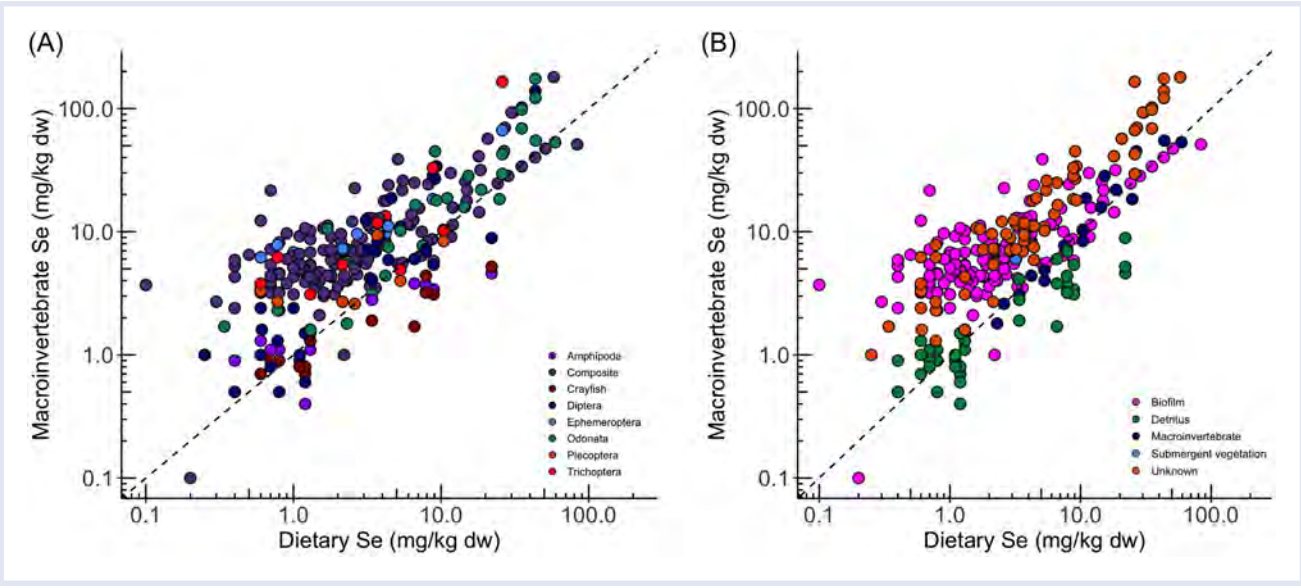

FIGURE 5 Macroinvertebrate vs. dietary selenium (Se) concentrations for several taxonomic groups collected from both lotic and lentic environments throughout Canada and the USA. Colors indicate taxonomic group (A) or diet item (B). Dashed lines mark the 1:1 concentration relationship between diet and invertebrate Se (i.e., trophic transfer factor [TTF] = 1)

TABLE 1 Summary of trophic transfer factors (TTFs) for various taxonomic groups of macroinvertebrates collected from lentic and lotic habitats throughout the USA and Canada

Invertebrate (order)	Average TTF (±SD)	Minimum TTF	Maximum TTF
Amphipoda	1.0 ± 0.6	0.2	2.2
Composite (unknown)	4.3 ± 4.6	0.5	37
Diptera	2.0 ± 1.1	0.4	4.1
Ephemeroptera	4.5 ± 2.9	2.0	10
Odonata	2.1 ± 1.4	0.7	6.2
Plecoptera	2.0 ± 1.7	0.8	5.5
Trichoptera	3.8 ± 2.4	0.9	7.9

Note: TTFs are based on assumed diet (generally detritus or biofilm Se measured from the same site). All data were obtained from previous literature reviews (DeForest et al., 2017; Presser & Luoma, 2010). See Supporting Information S1 for methods.

considerations specifically for the collection of macroinvertebrates for Se determination are avoiding contamination by using acid-cleaned plastic materials and obtaining sufficient mass of four to five taxonomic groups for Se analysis. Three replicate composite samples of each taxon collected from different subsites in a site are recommended to characterize the variation in Se (ECCC, 2012; USGS, 2008). Details on sample processing and analysis of tissue Se concentrations by ICP-MS are provided in USEPA (2014, 2015) and Vacchina and Dumont (2018).

Statistical analyses. To assess whether Se exposure raises concerns in a particular area, tissue Se concentrations in macroinvertebrates can be compared with predetermined guidelines or alert levels (for instance, the BC MoE [Ministry of Environment] interim guideline for macroinvertebrates is 4 mg kg^{-1} dw). This is the preferred method over strictly comparing means between the exposed and reference sites. It is also useful to compare affected sites with the distribution of concentrations at reference sites to understand the relative change in Se exposure between impact and reference areas. As such, an effect size is less important than establishing whether an appropriate alert and/or trigger level is exceeded. Thus, macroinvertebrate sampling plans should be designed to ensure that several reference sites are sampled and that affected site samples can be compared with the distribution of reference samples and the alert and/or trigger level of Se.

To estimate fish tissue Se concentrations from macroinvertebrate Se when fish cannot be collected, a known TTF value for a particular fish species can be applied if the data are available. If no relevant TTF data exist for a particular species, a mean TTF of 1.2 (the mean of all fish TTFs examined through meta-analysis, data from DeForest et al., 2017; Presser & Luoma, 2010) can be used to estimate fish muscle Se. Then, this estimated concentration can be compared with the fish tissue Se guidelines established by the USEPA (11.3 mg kg^{-1} dw) or BC MoE (4 mg g^{-1} dw; interim; Beatty & Russo, 2014) as described above.

Water, sediment, and particulate samples. The biogeochemical cycling of Se among abiotic and particulate compartments, including uptake by primary producers as the largest bioaccumulation step in aquatic food webs, is highly variable both within and among study sites (Maher et al., 2010; Ponton et al., 2020; Presser & Luoma, 2010). As a result, Se concentrations in these sample types have been shown to be unreliable predictors of Se concentrations in upper trophic levels (Brandt et al., 2021; Ponton et al., 2020; Presser & Luoma, 2010). However, the inclusion of water, sediment, and particulate samples is valuable for broader assessments of Se-impaired ecosystems. Furthermore, federal criteria in Canada and the United States include dissolved Se concentrations considered protective of aquatic life, such that concentrations measured above these thresholds indicate the potential for adverse environmental health consequences. Monitoring of dissolved Se concentrations can therefore help determine whether monitoring of upper trophic levels is indicated; however, the USEPA and BC MoE recommend that fish tissue data take precedence over water column data (Beatty & Russo, 2014; USEPA, 2016). There has been considerable research into the influence of abiotic and context-specific factors on Se biogeochemical cycling in aquatic systems (Besser et al., 1993; Franz et al., 2011; Ponton et al., 2020; Simmons & Wallschläger, 2005). Here, we summarize the factors that should be considered when sampling water, sediment, and particulates to determine selenium exposure for fish.

Water. Selenium speciation significantly influences Se uptake at the base of aquatic food webs (reviewed in Ponton et al., 2020, alongside other mediators of Se partitioning). However, due to analytical costs and challenges, few field studies have reported on dissolved Se speciation. Therefore, the influence of aqueous Se speciation on bioaccumulation is not completely understood. Se concentrations in surface water are generally reported as total dissolved Se, and aquatic life criteria are also based on total Se concentrations. Water collection protocols developed by the USEPA and the International Organization for Standardization (ISO) allow for

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standardized approaches and data comparison across studies (e.g., filtration at 0.45 μm and acidification).

Factors related to water column Se dynamics, including Se speciation and the hydrology of the impaired system, inform study design. Selenate (SeO_4^{2-}; SeVI) is the fully oxidized form of Se in water and is typically the predominant form of Se in fast-flowing (i.e., lotic) and oxidizing systems such as rivers and streams. The reduced forms of Se, including selenite (S; SeO_3^{2-}; SeIV) and organo-Se species, are more abundant in slow-moving waters (i.e., lentic) with higher residence times in lakes and wetlands (Sharma et al., 2015). Dominant Se species can also depend on contamination sources because coal-fired power plant and oil refinery effluents are typically dominated by selenite, whereas mine effluent and agricultural drainage tend to be mostly selenate (Maher et al., 2010; Young et al., 2010). Laboratory studies have shown that organo-Se forms are the most bioavailable, followed by selenite and selenate (Besser et al., 1993; Franz et al., 2011; Simmons & Wallschläger, 2005), suggesting that greater Se bioaccumulation is expected in environments that favor larger proportions of reduced Se species.

In general, lentic environments are associated with greater Se accumulation than lotic environments because of greater biological activity, larger proportions of reduced Se forms, and greater potential for Se remobilization and recycling from sediments (Hillwalker et al., 2006; Orr et al., 2012; Simmons & Wallschläger, 2005). Longer water residence times in lentic environments can also facilitate Se bioaccumulation (Luoma & Rainbow, 2005; Orr et al., 2006; Sharma et al., 2015). Reducing the conditions in lentic waters can lead to greater Se accumulation in sediment and greater bioavailability in water (Luoma & Rainbow, 2005; Simmons & Wallschläger, 2005; USEPA, 2016). Greater partitioning of Se in sediment and detritus in lentic systems also appears to support food web Se accumulation in lentic systems so that benthic invertebrates and their consumers in lentic systems may be exposed to higher Se concentrations (Hillwalker et al., 2006; Orr et al., 2006; Simmons & Wallschläger, 2005) As discussed above with respect to invertebrate and fish collection, the observation that habitat type (i.e., lentic vs. lotic systems) influences Se accumulation should be a key consideration in the design of monitoring studies aimed at estimating Se exposures (Martin et al., 2008, 2011). Finally, Se speciation can be useful for identifying potential sources of Se uptake into food webs. For example, anomalously high Se concentrations of organo-Se species in lotic systems can indicate influences from nearby ponds or oxbows not previously identified as contributing to the local environment.

Sediment, particulates, and biofilm. Partitioning Se to sediment and particulates, and enriching Se into the base of the food web, dictates exposure to higher trophic level organisms (Presser & Luoma, 2010). As such, colocated sediment, particulate, and/or biofilm samples collected alongside macroinvertebrates and fish are important to fully characterize Se bioaccumulation in the food web and can aid the interpretation and understanding of Se exposure in higher trophic level organisms (Luoma & Presser, 2009). Selenium concentrations in these compartments can be highly variable both spatially and temporally. Consideration of the factors influencing binding and resuspension of Se to, and from, sediment and particulates. The relevance of the compartments being sampled to the species of interest should be considered during sampling to reduce variation and understand food web Se exposure.

Selenium binds to organic matter in sediment and particulates, making these compartments an important sink for Se in the aquatic environment. Furthermore, depositional zones with greater concentrations of organic matter are probably associated with greater Se deposition. The potential for this bound Se to be resuspended from organic matter into water depends on the sediment oxidation-reduction status (Maher et al., 2010; Martin et al., 2008, 2011). Lentic environments generally have longer residence times and are associated with greater biological activity and lower oxygen, leading to reducing conditions (Simmons & Wallschläger, 2005). Reducing conditions promote the release of Se previously bound to organic carbon in sediments and kinetically favor the reduction in selenate to more bioavailable forms of Se such as selenite and organo-Se (Masscheleyn & Patrick, 1993; Masscheleyn et al., 1991), leading to greater bioavailability of Se in lentic or depositional environments.

In addition to reducing conditions that favor the remobilization of Se from sediments, greater residence times in lentic systems also promote recycling of Se and greater opportunity for Se uptake relative to systems with high flow that are constantly flushed (Sharma et al., 2015). Once Se is assimilated by primary producers, it may continue cycling through biological compartments through trophic transfer to higher trophic level organisms or by settling into sediment in dead and decaying organisms (Maher et al., 2010; Sharma et al., 2015). From there, detritivorous or microbial communities in the sediment may assimilate or biotransform Se, facilitating reuptake and trophic transfer through the food web (Orr et al., 2006). Microbial activity is a main driver of Se bioaccumulation because this biological activity leads to the biotransformation of Se to more bioavailable forms, increasing the transport of Se to sediments in bioavailable forms where benthos is exposed (Orr et al., 2006; Simmons & Wallschläger, 2005). Additionally, Se that is taken up by organisms will eventually become detritus or sediment after the organisms die, decompose, and settle out of the water column. Therefore, higher productivity and/or biomass results in more Se accumulation in bottom sediments (Maier & Knight, 1994).

Due to the influence of environmental conditions on Se binding and/or uptake in sediment, particulates, and biofilm, particular emphasis should be placed on collecting samples that represent exposure pathways most relevant to the fish species of concern. Standardizing sample collection to lentic or depositional environments within sites of interest

is advised to isolate high Se exposure samples. Collecting a consistent and/or relevant size fraction of sediment and particulates (<63 μm), and collecting biofilm taxa that are relevant to the food web are also important (Beatty & Russo, 2014). In addition to focusing on depositional zones where higher Se accumulation is expected, sampling microhabitats relevant to the fish species of interest is recommended.

CONCLUSION

- Decades of research and environmental fate and effects data have resulted in updated regulatory frameworks to manage Se in aquatic systems, but impaired systems still exist across North America.
- Selenium is incorporated into aquatic food webs by primary producers, with subsequent exposure to higher trophic levels occurring primarily via dietary intake.
- Fish are more sensitive to the potentially negative effects of Se than other aquatic organisms, and the primary negative effects are mediated by dietary intake of Se by female fish, maternal deposition into her eggs, and impaired embryo development.
- Conceptualized pathways of dietary exposure can be estimated for a given fish species based on knowledge of their prey organisms and analysis of Se concentrations in those taxa.
- Fish feeding at a higher trophic level and with greater maximum body size typically do not accumulate more Se than those feeding at lower trophic levels, suggesting that Se does not biomagnify.
- Ontogenetic diet shifts can affect the potential for fish to accumulate Se.
- Determining concentrations of Se in the water column, particulate and macroinvertebrates can augment our understanding of fish tissue concentrations or serve as proxies in cases where collection of fish tissue is not possible. Collection of these samples should be designed to best represent food web exposure to fish, including several habitat types and affected and reference sites.

AUTHOR CONTRIBUTION

Vince Palace: Conceptualization; data curation; formal analysis; funding acquisition; project administration; writing—original draft; writing—review and editing. **Stephanie Graves**: Conceptualization; data curation; formal analysis; visualization; writing—original draft; writing—review and editing. **Jessica Brandt**: Conceptualization; formal analysis; methodology; validation; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Cited data are available in publicly available formats or contained in the Supporting Information.

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SUPPORTING INFORMATION

Files ancillary to the main document but pertinent to the central theme of the discussion are provided.

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Supporting Information for

Guidance for assessing potential impacts of selenium in freshwater ecosystems

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SI part a: Considerations for selecting sentinel fish species

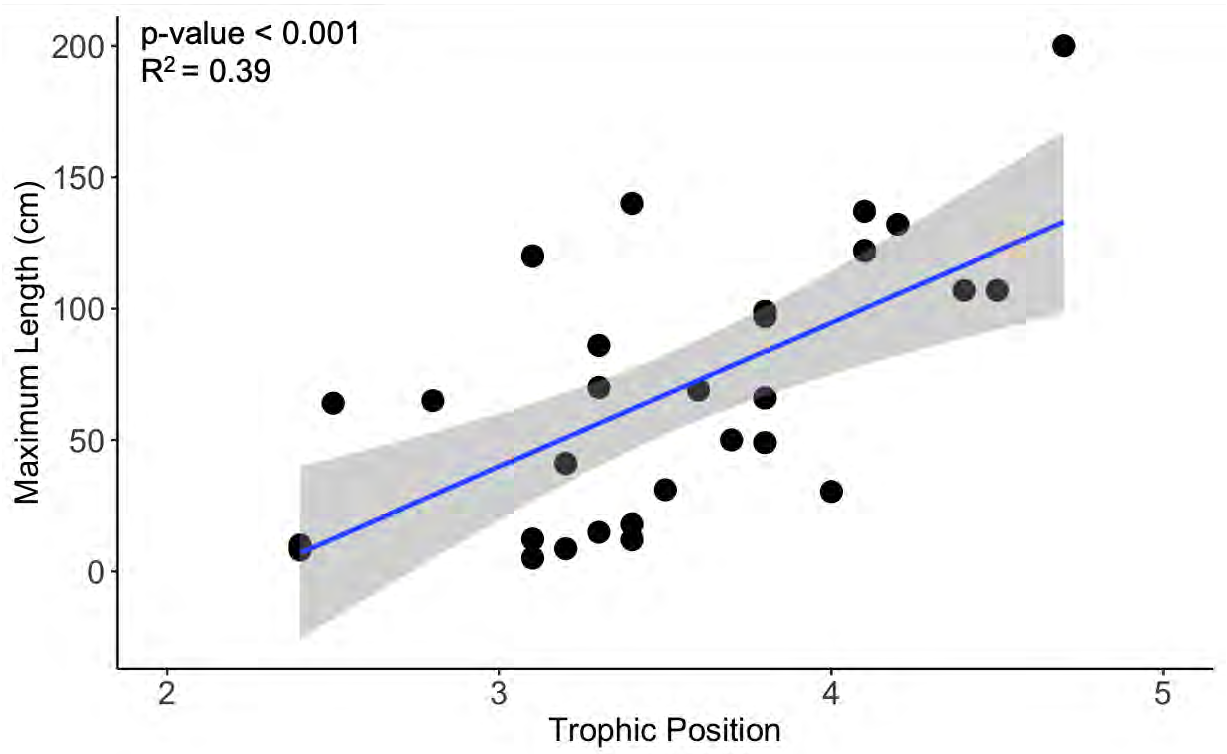

Figure S1: Maximum body length (cm) versus relative trophic position for fish species previously used for metal mining EEM studies in Canada.

Table S1: Egg and yolk volumes and egg:yolk volume ratios for fish species with known selenium toxicity thresholds that have previously been used for Environmental Effects studies in Canada.

Family Common Name *(Scientific Name)*	Established Se Toxicity Thresholds Egg/ovary (dw)	Egg Volume mm^3	Yolk volume mm^3	Ratio yolk:egg volume	Reference and Notes
Dolly varden (Salvelinus malma)	56.2 egg (a)	36	25	0.694444	Galagher et al. (2019)*
Cutthroat trout (Salmo clarki)	26.3 egg (a)	6.03	4.75	0.787728	Carim et al. (2021)**
Brown trout (Salmo trutta)	21.0 egg (a)	71.1	63.6	0.894515	Bonisławska et al. 2001
Rainbow trout (Oncorhynchus mykiss)	24.4 egg (a)	62.8	55.2	0.878981	Bonisławska et al. 2001
Arctic grayling (Thymallus arcticus)	>33.9 (b)	44	8.18	0.185909	Bonisławska et al. 2001
Mountain whitefish (Prosopium williamsoni)	>54 (d)	17	16.15	0.95	Wydoski et al. 2001, Mitz et al. 2019 and Sreethran et al. 2015
Northern pike (Esox lucius)	34.0 (E)	10.3	6.5	0.631068	Bonisławska et al. 2001
Fathead minnow (Pimephales promelas)	<26.5 egg (a)	1.4	0.23	0.164286	Wang et al. 2014, Scahill 2008
White sucker (Catostomus commersoni)	26	4.2	0.36	0.085714	Munkittrick and Dixon 1989, Fuiman and Trojnar 1980,
Bluegill (Lepomis macrochirus)	20.6 egg (a)	0.79	0.36		Oplinger and Wahl 2015
Largemouth bass (Micropterus salmoides)	26.3 ovary (a)	1.23	0.46	0.373984	Sepúlveda et al. 2003

**Proportional yolk volume estimated from other Salvelinus species from Bonisławska et al. 2001*

*** Yolk voluome estimated from average of 4 other salmonids from Bonisławska et al. 2001*

SI part b: Assessing ecological Se effects with alternative environmental media

a. Meta-analysis methods

To understand how habitat type, diet, and taxonomy influence Se trophic transfer in benthic macroinvertebrates, we conducted a meta-analysis using data reported by Presser and Luoma (2010) and by DeForest et al. (2017). Using analysis of covariance (ANCOVA), with the covariate "Order" included, selenium concentrations in benthic macroinvertebrates were related to those in diet across six orders (n=277 individual data points; Supporting Information 2, Table 1). For data points that listed diet items (biofilm, detritus, invertebrate, submergent vegetation or unknown) and habitat (lentic or lotic), ANCOVA was used to assess how diet and habitat influenced the relationship between BMI and diet Se. Trophic transfer factors (BMI Se concentration/dietary Se concentration) were calculated for the orders Amphipoda, Diptera, Ephemeroptera, Odonata, Plecoptera, Trichoptera.

b. Meta-analysis results

We observed significant positive relationships between dietary Se and invertebrate Se concentrations for several taxonomic groups (Figure 5A; Table S1). The slopes of these relationships differed among taxonomic groups, indicating differences in the trophic transfer of Se among taxa; Diptera, Odonata and Trichoptera had the highest slope estimates, while Amphipoda, Ephemeroptera, Plecoptera and Crayfish had relatively lower slope estimates (Figure 5A; Table S1). Trophic transfer factors derived from these data ranged from 0.2 in Amphipoda to 37 in a composite sample, illustrating the range in TTFs that can be observed in BMIs. Though these estimated TTFs are dependent on an assumed dietary Se concentration, it is evident that taxonomic variation in Se uptake is high and needs to be considered when assessing Se exposure to higher trophic levels. The greater variation in composite samples also demonstrates the need to sort and analyze invertebrates by finer taxonomic groups.

Benthic macroinvertebrate feeding strategies are diverse and due to the variation in Se exposure among food web pathways (for instance, greater exposure from benthic-detrital pathways versus particulate TSe in the water column), feeding habits can have a significant impact on invertebrate Se concentrations (Orr *et al.*, 2006; Muscatello *et al.*, 2008; Graves *et al.*, 2019, 2021) Invertebrate feeding strategies are classified by FFGs. The main FFGs are: collector-gatherers (feed on fine particulate organic matter (FPOM) in detritus/sediment), collector-filterers (filter FPOM from the water column), scrapers (scrape algae from surfaces), shredders (feed on coarse

particulate organic matter (CPOM) i.e., leaf litter), and predators (feed on other invertebrates). To date, no known studies have thoroughly investigated the variation in Se bioaccumulation among these FFGs, but previous studies have observed overall greater accumulation of Se in collector-gatherers, and lesser accumulation of Se in collector-filterers (Orr *et al.*, 2006; Muscatello *et al.*, 2008; Graves *et al.*, 2019, 2021). We were not able to separate taxa by functional feeding groups (FFGs) due to the coarse taxonomic identification herein, but we did observe that the slope of the relationship between invertebrate Se and dietary Se differed according to reported diet; invertebrates feeding on detritus had the lowest slope estimates, followed by biofilm, while those feeding on other invertebrates had greater slope estimates.

In contrast to the effect of receiving environment on Se uptake at the base of the food web, there was no significant difference in slopes or intercepts of the relationship between invertebrate Se and dietary Se in lentic versus lotic environments (ANCOVA, $p=0.713$, t-value=-0.368, Table S1), suggesting that the receiving environment does not impact TTFs in the same way that it affects EFs. This is unsurprising, since the majority of Se accumulated by benthic macroinvertebrates is attributed to diet.

Overall, our meta-analyses confirmed that taxonomic group and diet both influence Se bioaccumulation among macroinvertebrate BMI taxa, suggesting that these factors need to be considered in the sampling of macroinvertebrate BMI to infer Se exposure to fish.

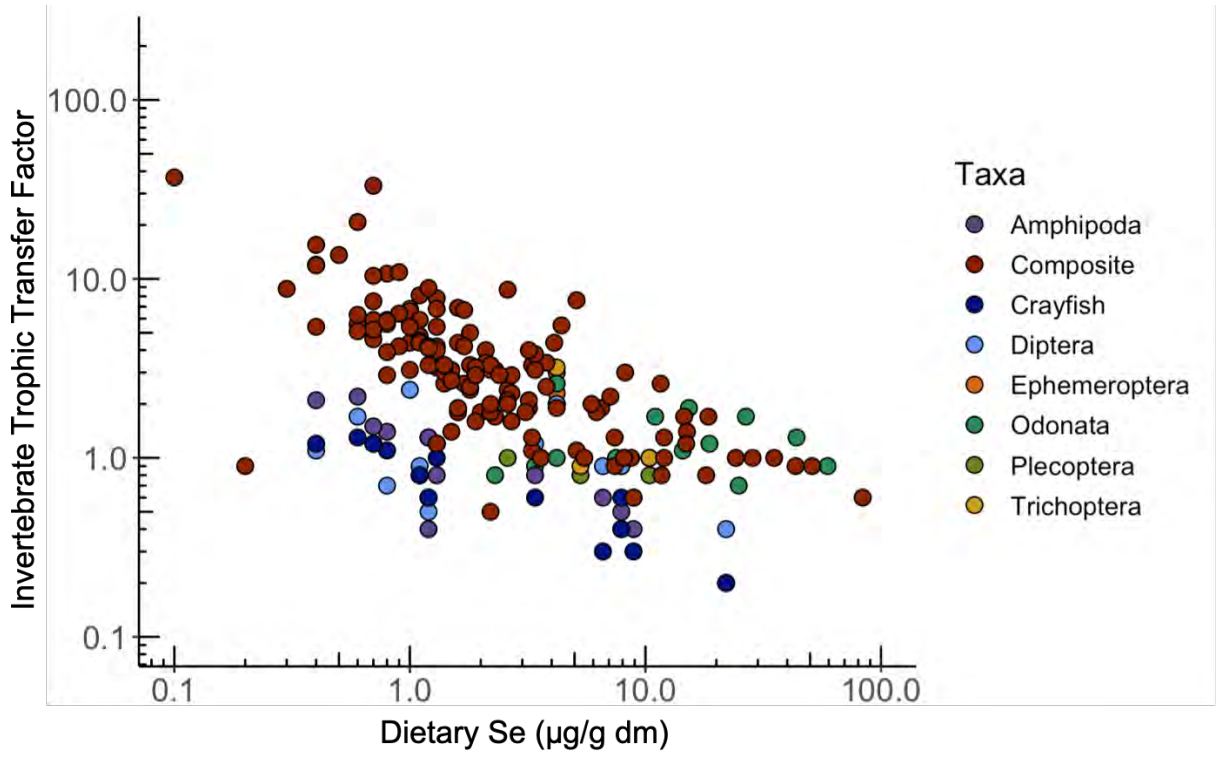

Figure S2: Invertebrate trophic transfer factors for various taxonomic groups plotted against dietary Se concentrations. Invertebrate TTFs vary widely among taxa and decrease with increasing dietary exposure.

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Table S2: Statistical analyses of relationships between invertebrate diet Se and invertebrate Se body burdens

X variable	Y variable	Covariate	Statistical test	p-value	F-statistic	df	R2	Slope	Intercept
log Diet Se	log Invert Se - Amphipoda	N/A	LR	<0.001	33.04	1,12	0.71	0.501	0.056
log Diet Se	log Invert Se - Composite	N/A	LR	<0.001	212.7	1153	0.58	0.587	0.04
log Diet Se	log Invert Se - Crayfish	N/A	LR	<0.001	120	1,12	0.9	0.579	-0.053
log Diet Se	log Invert Se - Diptera	N/A	LR	<0.001	129.2	1,30	0.81	1.056	0.177
log Diet Se	log Invert Se - Ephemeroptera	N/A	LR	<0.001	42.81	1,12	0.76	0.529	0.782
log Diet Se	log Invert Se - Odonata	N/A	LR	<0.001	124.7	1,27	0.82	0.886	0.365
log Diet Se	log Invert Se - Plecoptera	N/A	LR	0.036	7.23	1,6	0.47	0.419	0.468
log Diet Se	log Invert Se - Trichoptera	N/A	LR	0.004	15.37	1,8	0.62	0.81	0.585
log diet Se	log invert Se	Taxa	ANCOVA	<0.001	59.54	15,260	0.76	0.501 (amphipoda)	0.056
		logdiet:Composite	ANCOVA	0.515					
		logdiet:Crayfish	ANCOVA	0.659					
		logdiet:Diptera	ANCOVA	<0.001					
		logdiet:Ephemeroptera	ANCOVA	0.886					
		logdiet:Odonata	ANCOVA	0.01					
		logdiet:Plecoptera	ANCOVA	0.727					
		logdiet:Trichoptera	ANCOVA	0.128					
log diet Se	log invert Se	Lentic vs Lotic	ANCOVA	<0.001	95.95	5 and 270	0.63	0.629 (lentic)	0.36
		logdiet:lotic	ANCOVA	0.713				-0.0448	0.143
log diet Se	log invert Se	Diet	ANCOVA	<0.001	120.5	10 and 265	0.81	0.493 (Biofilm)	0.66
		logdiet:BMI	ANCOVA	0.0017				0.619	-0.756
		logdiet:Detritus	ANCOVA	0.056				0.143	-0.655
		logdiet:unknown	ANCOVA	<0.001				0.412	-0.119
		logdiet:waterboatmen	ANCOVA	0.005				0.504	-0.596

SI part c: Detailed information on the sampling and analysis of macroinvertebrates to determine Se exposure in aquatic ecosystems:

In this section we provide important details on the aspects of study design and sample collection that are necessary to determine Se concentrations in macroinvertebrates for the purpose of estimating Se exposure in higher trophic level organisms. At the end of this section we provide a table (Table S2) summarizing the sampling considerations for collecting macroinvertebrates to estimate Se exposure.

a. Taxonomic resolution

Invertebrates should be live-sorted at least to coarse taxonomic levels in the field. Once sorted, invertebrates should be stored at -20°C for transport and until further processing. Given the amount of variation in TSe among invertebrate taxonomic groups and functional feeding groups, we recommend that invertebrates be sorted to Family prior to TSe analysis. In addition, grouping invertebrates by size/similar instar stage will reduce variation caused by changes in feeding habits. Invertebrate samples should be limited to one thawing event to minimize protein breakdown and loss of Se from the sample. Collected, sorted, and identified invertebrates should then be freeze-dried for 48-72 hours (until a constant mass is obtained) and ground to a homogenous powder using an acid-cleaned mortar and pestle. Ground samples are transferred to acid-cleaned vials or containers and transported to the laboratory responsible for the analysis of TSe.

b. Site selection

Streams and rivers can be divided into types of habitats that are more, or less, likely to be subject to high Se accumulation. These habitat characteristics may be used to focus on sampling sites and invertebrate taxa that would be expected to accumulate the most Se. Lotic environments can be subdivided into erosional and depositional habitats. Erosional habitats are defined by fast-flowing water with coarse sediments like cobble, gravel, and boulders . “Riffle” and “run” meso-habitats are considered erosional where sediments do not accumulate. In these well-oxygenated meso-habitats, Se is expected to exist mainly as selenate and Se bioaccumulation is expected to be lower. Erosional habitats are characteristic of head-water and medium-sized streams but are less common in large rivers. In contrast, depositional habitats have slower-moving water where sediments accumulate. They are characterized by finer substrates like sand, silt and clay. Due to these characteristics, depositional habitats tend to be associated with greater Se accumulation.

However, the decision to focus strictly on studying Se accumulation in invertebrates from depositional habitats is complicated because generally, erosional habitats such as riffles support greater invertebrate diversity and may represent a higher proportion of a fish's preferred diet items, even though Se concentrations are expected to be lower. To best represent food web exposure to fish, a sampling design to collect representative invertebrate taxa from both erosional and depositional zones of a site is preferred, though sampling efforts can be designed such that proportionately greater efforts are placed on potential "hot-spot" meso-habitats.

c. Timing and Seasonality of sample collection

In contrast to collections of macroinvertebrates for community composition analysis, collecting invertebrates to estimate dietary exposure risk to fish does not need to coincide with the presence of the greatest number of taxa or the largest instars. Instead, sampling should be performed when it represents the most relevant exposure to fish relative to effluent exposure, gonad maturation or life history characteristics or feeding patterns most relevant to Se accumulation.

If there are large seasonal fluctuations in aqueous Se concentrations (for instance, due to seasonal changes in water flow and resulting dilution/concentration of effluent), a seasonal study design should be considered. Based on previous studies - temporal variation of Se in biota is site-specific and should be evaluated on a case-by-case basis. Golder (2018) assessed temporal variation of Se in macroinvertebrates by collecting composite samples from May to October in mine-influenced streams of the Elk River watershed. They found no seasonal differences but Se concentrations among sites within each sampling time were highly variable. This variation was likely caused by compositing many taxonomic groups and functional feeding groups into one sample.. In the same investigation, there was no evidence that accounting for lag in accumulation of Se in invertebrates relative to concentrations in water would improve performance of models relating water to invertebrate Se.

In contrast to the work by Golder (2018), Brandt *et al.* (2021) found that throughout the Lower Gunnison River, Hydropsychidae TSe concentrations were lower in October than April or August. This may be related to seasonal fluctuations in aqueous Se concentrations, or seasonal energy dynamics in the river. In fall and winter, when days are shorter and biofilm growth is slow, allochthony represents a relatively larger energy source to streams and rivers. Greater activity of shredders during this time period is expected, with terrestrial organic matter presumably

contributing significantly to Se accumulation in invertebrates. During spring and summer when autochthony dominates energy production, activity of scrapers and collector-gatherers is expected to increase, and Se concentrations may be subsidized during these times of year. No studies have investigated the impact of seasonal changes in energy sources and activity levels of different invertebrate groups in relation to Se accumulation patterns, but it should be considered in the selection of invertebrate sampling times.

d. Sampling methods

Standard protocols for the collection of macroinvertebrates in wadeable or non-wadeable streams, wetlands, or lakes have been prescribed (BC MoE, 2006; ECCC, 2012). Collecting invertebrates from natural substrates is always preferred, however, if standard collection techniques are not feasible, or if increased standardization of collection is needed for the purposes of seasonal/repeated sampling, artificial substrates such as rock baskets or Hester Dendy Samplers can be used (BC MoE, 2006). This will limit the diversity of invertebrate taxa being collected, but will increase consistency and decrease variation among sites and among sampling times (BC MoE, 2006). Collecting invertebrates for Se analysis requires enough mass from 4-5 different Families and/or FFGs). Three replicate samples of each taxa should be collected from different sub-sites within a site to characterize variation in TSe concentrations (i.e. a total of 15 invertebrate samples will need to be collected from each site) (USGS, 2008; ECCC, 2012). Due to the small size/mass of most benthic invertebrates, many individuals need to be pooled to obtain sufficient mass for Se analysis. Compositing several species and measuring the average concentration of the pooled individuals will decrease the number of samples needed within a site (by decreasing variation in results), but it should be noted that information about variability and the range of concentrations among individuals will be lost (US EPA, 2016). Composite samples are acceptable when the objective of the study is to determine if a reference site is different from an impacted site but when variation of Se among taxonomic groups is needed, compositing should be limited to genus. If the site is large and variable and there is a need to characterize Se bioaccumulation across larger spatial scales, more effort can be directed to multiple sites, rather than collecting replicate samples within one site. This recommendation is based on research by Cianciolo et al. (2020) and Brandt et al. (2021) showing that Se bioaccumulation does not necessarily decrease as the distance from a point source increases. Rather, Se can be efficiently transported downstream and there is often no

difference in bioaccumulation at sites immediately downstream of a source compared with sites as far as 20 km downstream.

e. Quality Assurance/Quality Control and Analytical Considerations

Commercial analytical laboratories typically require large dry masses for sample analysis but, samples of 0.01 to 0.1 g dry weight are often sufficient for TSe analysis using ICP-MS techniques. Analytical results for the same samples can differ between analytical laboratories due to different instruments and calibration standards. Variation in results needs to be considered during study design, the selection and analysis of calibration standards and reference materials, and in the interpretation of results (US EPA 2021). The consistent use of standardized methods for collection, handling, preservation, and analysis are recommended. With respect to analytical accuracy and precision, sufficient sensitivity in the analysis is necessary to reduce variability. For instance, analytical methods with detection limits sufficiently lower than the lowest expected concentrations are needed. For animal tissue such as invertebrates and fish, this should be achievable since most instrument detection limits are around 0.1 ppb, and most animal tissue Se concentrations are > 1 ppm.

Measuring and reporting tissue concentrations in dry weightmass is the standard and preferred method for Se analysis. Freeze-drying is preferred, rather than a drying oven, to reduce potential of Se loss by evaporation. To prepare the tissues for analysis, dried tissue is homogenized and then digested in strong acid (HNO_3) using a microwave digestion (closed-vessel) or heated digestion (open-vessel) procedure. Reporting requirements for analysis of Se concentrations should include: 1) limit of quantification, 2) limit of detection, 3) means of method and instrumental blanks, 4) percent recovery of instrumental standards and certified reference materials, and 5) variability in duplicates.

f. Power Analysis, Statistical Analysis and Considerations

Guidance for each specific sample type (compartment) may vary, but overall, the statistical design that best suits current understanding of Se accumulation and variability in aquatic ecosystems is one where concentrations in compartments (eg. macroinvertebrates, sediment, fish) at impacted sites are compared to a guideline or to a distribution of concentrations at reference sites, rather than strictly comparing means between exposed and reference sites (Figure 7.1). As such, an effect size is less important than establishing whether an appropriate alert/trigger level is exceeded.

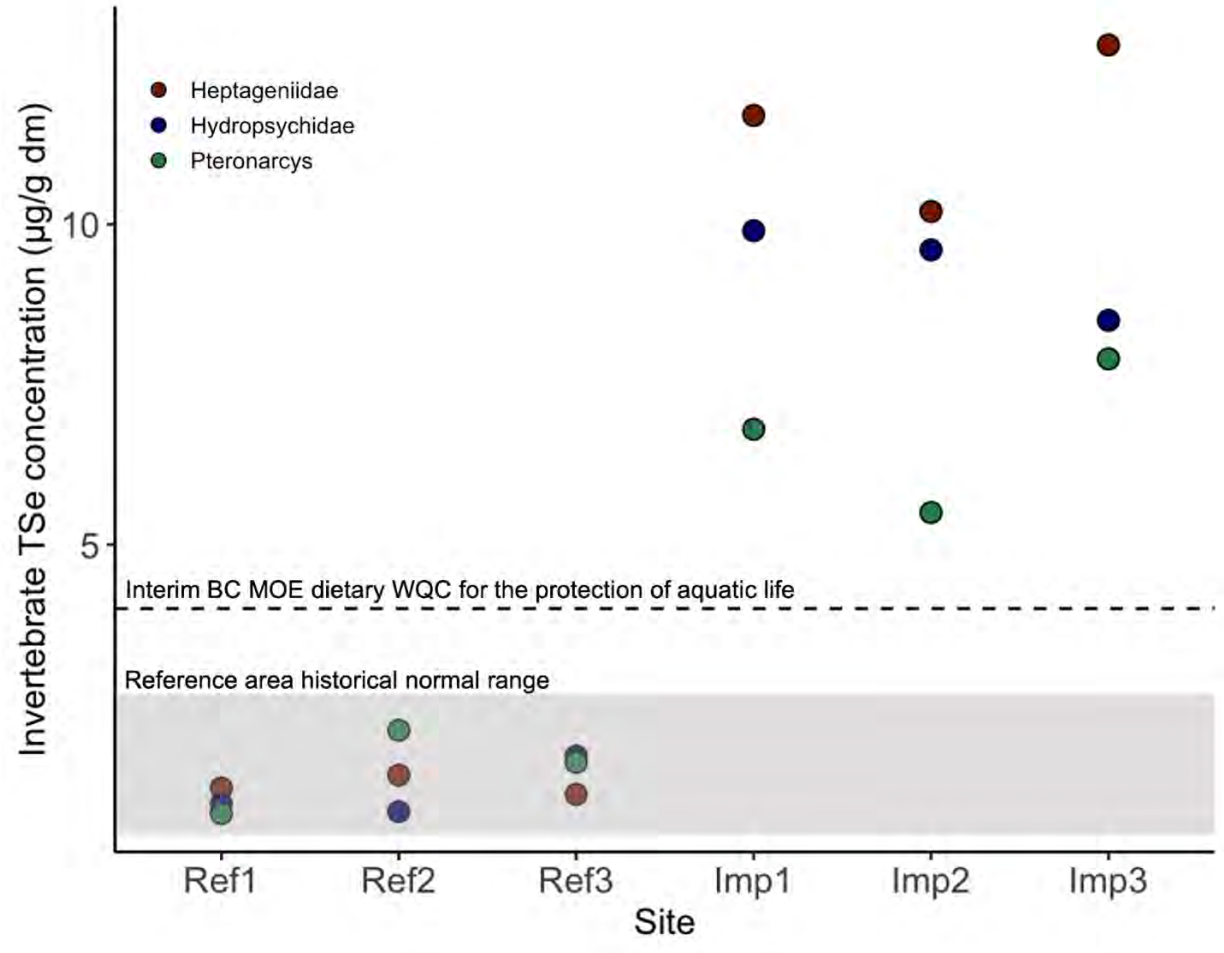

Figure S3: An example of data comparisons for the benthic invertebrate compartment from a site being investigated for potential Se effects. The BC MoE interim guideline for the protection of aquatic life was used in this case.

The sampling design for the collection of benthic invertebrates will depend on the goal of the study. Two main scenarios for sampling are presented below, because the sampling design will differ based on the goal:

A. *Characterizing invertebrate Se based on the potential diet of fishes - i.e. collection of invertebrates from all mesohabitat types.*

   This is the ideal study design because it allows the characterization of Se across all relevant areas where fish may accumulate Se. This would include riffles, runs and pools. In this

scenario, the objective would be to target invertebrate taxa that are relevant to fish diet at the site of interest, and that represent each of the five main functional feeding groups. This includes collecting scrapers, collector-gatherers, collector-filterers, shredders, and predators. If fish study species are decided *a priori*, the invertebrate taxa selected can be chosen based on the known diet of the fish of interest. Through this approach, the different pathways of food web exposure to fish and the relative importance of different dietary items to fish at the site can be evaluated.

B. *Characterizing invertebrate Se in the "worst-case scenario" - i.e. collection of invertebrates from lentic/depositional habitats only.*

   In this scenario, the focus of the study is sampling areas where Se bioaccumulation is expected to be highest. In this case, pools, backwaters, and main channel margins should be sampled for invertebrates that are expected to contain greater Se concentrations due to their location and feeding strategies (collector-gatherers feeding on fine particulate organic matter, detritivore shredders feeding on biofilm-conditioned leaf litter, and predators feeding on other invertebrates).

Though collecting three replicate samples within a site is generally recommended, power analysis can be performed using previous data from other reference and impacted sites to determine the appropriate sample size in a particular scenario. An example is presented here: The statistical power to detect changes in benthic invertebrates will be based on 1) variability in Se concentrations among sites, 2) the size of change being detected, and 3) the number of samples collected. Variability in Se concentrations among sites can be estimated using previously collected data. For instance, we can use Muscatello *et al.* (2008) data from reference lakes and lakes impacted by mining activity to estimate variability in invertebrate Se. For detritivores, mean TSe concentrations (± standard deviation) were 12.39 ± 10.89 mg kg^{-1} dm at an impacted site and 0.93 ± 0.49 mg kg^{-1} dm at a reference lake. Predator TSe was 12.74 ± 2.0 mg kg^{-1} dm at the impacted lake and 1.23 ± 0.96 mg kg^{-1} dm at the reference lake. To determine the number of samples needed to detect the increased Se in an impacted lake, the critical effect size is calculated using the mean and SD of each invertebrate type using equation 1.

Equation (1) $CES = mean_{impacted}\text{-}mean_{reference}/SD_{reference}$

For detritivores, the CES is 1.05 and for predators, the CES is 5.8. Using this information and a t-test power calculator in the "pwr" package in RStudio software environment, a sample size of 15 would be needed to detect a difference in detritivore Se and a sample size of 2 would be needed to detect a difference in predator Se.

g. Se-Toxicity to invertebrates and influence on fish Se

deBruyn and Chapman (2007) reviewed sublethal and lethal toxicity of Se to a range of invertebrate taxa, and found that lethal concentrations ranged from 10 to 1000 μg Se/g dm, while sublethal effects occurred in the range of 1 to 80 μg Se/g dm. In multiple laboratory experiments using the mayfly *Centroptilum triangulifer*, Conley et al. (2011, 2013) demonstrated that relatively low dietary Se exposure (12.8 μg/g dm) results in decreased fecundity, and that adult mayfly survivorship decreased significantly when Se body burdens were 31.7 μg/g dm. In limnocorral experiments where Se was added as selenite and the community-level effects were monitored for 63 days, zooplankton showed adverse effects at whole-body Se concentrations of 11 μg/g dm, while macroinvertebrates invertebrates showed decreased survivorship at dietary Se concentrations of 30 μg/g dm (Graves et al. 2022). These recent studies have demonstrated that invertebrate Se toxicity and community-level adverse effects can be observed at concentrations similar to those affecting fish health (Conley et al. 2011, 2013; Graves et al. 2019, 2022). In addition, similar to egg-laying vertebrates, early life-stages of invertebrate taxa appear to be more sensitive to elevated Se exposure. As a result, the toxic effects of Se on lower-trophic level organisms (i.e, zooplankton, macroinvertebrates) should be considered in the experimental design and interpretation of Se exposure data.

The toxicity of Se to invertebrates at relatively low, environmentally relevant levels is important to consider in the estimation of Se exposure to fishes. First, changes in the invertebrate community at an impacted site can change fish Se exposure due to changes in food availability, and shifts in diet to more available species. For instance, at sites where the density of preferred food items such as Ephemeroptera have decreased due to Se toxicity, the relative density of more tolerant taxa such as Chironomidae may increase. According to previous studies, detritivorous taxa such as many Chironomids may accumulate greater Se concentrations due to their habitat and

feeding preferences, and this could lead to elevated exposure in fish. In addition to the influence of Se bioaccumulation, Se toxicity to invertebrates may negatively impact fish health if preferred food sources are unavailable.

Table S3: Sampling considerations for study design and sample collection to determine Se concentrations in benthic macroinvertebrates at Se-impacted sites.

Sampling aspect	**Highlights of study design and sample collection**
Taxonomic resolution	● 4-5 Representative taxa at each site ● Identified and sorted at least to Family level prior to analysis ● Representation of multiple functional feeding groups ● Relevant to higher trophic levels
Site selection	● Ideally both erosional (lotic) and depositional zone sampled to best represent food web exposure to fish ● Sampling effort can be higher in "hot spot" meso-habitats - depositional or lentic environments generally associated with greater Se accumulation ● 3 replicates within a site x
Sampling effort	● 4-5 representative taxa x 3 replicates per site – need to pool individuals to obtain minimum sample mass for Se analyses (~0.1 g dry mass). ● If TSe variation among taxonomic groups is not known, finer taxonomic resolution is helpful (Genus level) ● At larger, more variable/diverse sites multiple sites is better than replicates from fewer sites
Sample collection - timing	● One sampling season likely sufficient but need to consider the dynamics of the system and seasonality of exposure ● Choose timing relevant to fish exposure (time of recent/significant effluent exposure, ecologically relevant to fish gonad maturation)
Sample collection - techniques	● Established SOPs for quantitative collection of macroinvertebrateBMIs can be followed ● Common methods: kick net/D net, grab samples (Ekman, dredges), sweep nets, hand picking from rocks
Sample collection - storage and preservation	● Sort to Family and group by size, freeze at -20℃ ● Freeze-dry 48-72h ● Ground to homogenous powder for TSe analysis
Analytical and QA/QC considerations	● Consistent and standardized methods of collection, handling, preserving, and analyzing samples ● ICP-MS is most common methods ● Typical instrument detection limit for animal tissue = 0.1 ppb ● Measure/report tissue Se in dry weight as mg kg^{-1} dw (or µg g^{-1} dw)
Statistical analysis considerations	● Se concentrations at impacted sites compared to guidelines – has an alert or trigger level been exceeded?

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