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Original Article

Environmental Toxicology

Oocyte developmental stage influences ovary selenium concentrations in fish—implications for ovary selenium monitoring

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Abstract

Monitoring selenium (Se) concentrations in fish ovaries is an important tool for evaluating the ecological risk posed by Se in aquatic systems. Most guidance recommends sampling fish ovaries as closely as possible to when fish spawn on the premise that Se is mobilized from the liver to the ovary during vitellogenesis, and therefore, sampling ovaries during the early phases of oocyte maturation may underestimate egg Se concentrations at the time of spawning. In this study, we evaluated ovary Se data from two species with synchronous oocyte development (*Ptychocheilus oregonensis* and *Prosopium williamsoni*), one species with asynchronous oocyte development (*Richardsonius balteatus*) and one where the mode of development is unclear (*Mylocheilus caurinus*). A multivariate analysis of ovary Se as a function of fish sampling location, size, and gonado-somatic index (GSI) demonstrated ovary Se was strongly negatively correlated with GSI in fish species with synchronous oocyte development but only weakly correlated in a species with asynchronous development. In *R. balteatus*, a relationship between expressible (released) egg Se and remaining ovary Se was observed, with egg concentrations approximately 54% of ovary concentrations on average. Overall, our findings suggest that current understanding of the mechanisms by which Se is maternally transferred to oocytes is not entirely correct and raises questions regarding how and when during the reproductive cycle Se is mobilized to ovaries. Further, our findings have significant implications for interpretation of ovary Se monitoring data collected from unripe fish. We developed regression-based models to correct ovary Se data that are biased by sampling females not in spawning condition and demonstrate how this bias can impact evaluation of Se risk to fish.

Keywords: tissue thresholds, fish, gonado-somatic index, ovary, selenium

Introduction

Selenium (Se) is an essential trace element but is also a developmental toxicant for oviparous vertebrates when accumulated via the diet to elevated concentrations (Janz et al., 2010). In aquatic systems, Se is accumulated from water into primary producers with bioconcentration factors that are dependent on the particular aqueous Se species present (Adams et al., 1998; de Bruyn et al., 2025; DeForest et al., 2017; Presser & Luoma, 2010) and water quality parameters that affect bioavailability (DeForest et al., 2017; Ponton et al., 2020). Once accumulated by primary producers, Se is transferred through the diet to primary consumers and then higher trophic levels, including fish (DeForest et al., 2016).

In vertebrates, the liver is the initial organ for Se metabolism and synthesis of selenoproteins, and it is the primary organ involved in Se detoxification and storage (Burk & Hill, 2009). Although it varies by species, liver Se concentrations in fish are typically approximately 3-fold higher than muscle Se concentrations (Herrmann et al., 2018; Khadra et al., 2019; Muscatello et al., 2006; Sager & Cofield, 1984; Sorensen et al., 1984). In oviparous vertebrates, Se is maternally transferred from the liver to oocytes during vitellogenesis, leading to elevated egg Se concentrations, although albumins and other proteins are also involved in maternal Se transfer in reptiles and birds (Davis & Fear, 1996; Jacobs et al., 1993; Kroll & Doroshov, 1991; Unrine et al., 2006). Elevated Se in fish eggs causes malformation and edema in developing embryos, leading to reduced embryo hatching success and embryo-larval survival (Janz et al., 2010). The exact mechanisms underlying these effects are not well studied but generally are attributed to Se substituting for sulfur (S), leading to

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misfolding of proteins and/or oxidative stress (Goldsztejn et al., 2022; Kupsco & Schlenk, 2016; Palace et al., 2004; Plateau et al., 2017).

Numerous studies have demonstrated high variability in Se accumulation into aquatic biota as a function of waterborne Se. This variability is a result of differences in Se speciation across sites, with reduced organo-Se forms much more bioavailable than selenite, which in turn is more bioavailable than selenate (Adams et al., 1998; Brix et al., 2005; de Bruyn et al., 2025; DeForest et al., 2017; Presser & Luoma, 2010). The prevalence of these reduced species is a function of the Se species released to the environment and the characteristics of the environment itself. Periodically hypoxic or anoxic environments such as wetlands and other lentic systems are more likely to have reduced Se species present, because these conditions tend to support redox conditions that ultimately initiate dissimilatory and assimilatory reduction of Se by biota at the base of the food web (Martin et al., 2022; Oremland et al., 1989, 1990).

As a result of this variability, environmental regulation of Se based on waterborne Se concentrations has proved challenging, because concentrations safe for fish can range from <1 to >20 μg L^{-1} depending on site-specific conditions (Brix et al., 2005; DeForest et al., 2017). Instead, regulatory agencies in Canada and the United States have focused on tissue-based guidelines (here and throughout we use guidelines to generically refer to criteria, standards, and guidelines), with egg/ovary Se concentrations being the preferred tissue, although other tissues such as muscle and whole body can be used as a surrogate (BCMOE, 2014; USEPA, 2016). For egg/ovary samples, both the United States Environmental Protection Agency (USEPA) and British Columbia Ministry of the Environment (BCMOE) guidelines recommend either eggs or, in cases where this is not logistically feasible, ripe ovaries be collected, although neither guidance provides details on what is considered a ripe ovary.

The rationale for collecting ripe ovaries as opposed to ovaries earlier in the reproductive cycle is based on the premise that Se transfer from the liver to the ovaries occurs during vitellogenesis. Vitellogenesis in rainbow trout (*Oncorhynchus mykiss*) in particular has been well characterized, with the majority of vitellogenin (Vtg), and presumably Se, transferred to eggs during the last two months of the reproductive cycle (Tyler et al., 1990; Figure 1). Consequently, sampling ovaries prior to this period has been assumed to underestimate egg Se concentrations and resulting potential effects on fish (USEPA, 2016). To our knowledge, this assumption has never been tested.

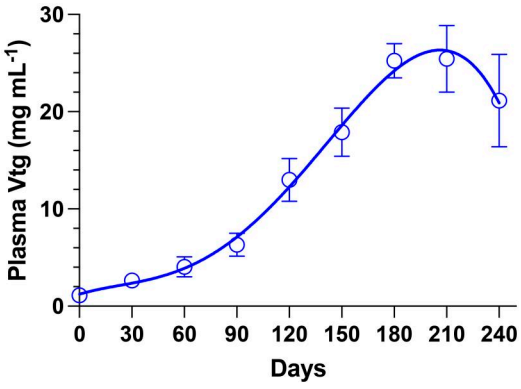

Figure 1. Plasma vitellogenin during the ovarian developmental cycle in rainbow trout. Ovulation occurs at day 240. Mean ± SEM. Tyler et al. (1990). The dynamics of oocyte growth during vitellogenesis in the rainbow trout (*Oncorhynchus mykiss*). *Biology of Reproduction*, 43, 202–209. Adapted with permission of Oxford University Press on behalf of SETAC. *Note*. Vtg = vitellogenin.

Given the above scientific and regulatory background, we evaluated fish ovary and egg Se data for four fish species—northern pikeminnow (*Ptychocheilus oregonensis*; NPM), mountain whitefish (*Prosopium williamsoni*; MWF), peamouth chub (*Mylocheilus caurinus*; PMC), and redside shiner (*Richardsonius balteatus*; RSS). Samples were collected from the Kootenay and Elk River drainages of southeastern British Columbia, as well as Koocanusa Reservoir into which they drain and join the greater Kootenay/Kootenai River drainage (see online supplementary material Figure S1 and Tables S1–S4). Koocanusa Reservoir is a transboundary water body formed by the Libby Dam in northwestern Montana.

Metallurgical coal mine operations in Elk Valley have resulted in the mobilization and release of Se, primarily as selenate, into the Elk River drainage and Koocanusa Reservoir. Routine monitoring of ovary Se concentrations in the four species has demonstrated exceedances of both the BC (11 mg kg^{-1} dry wt) and USEPA (15.1 mg kg^{-1} dry wt) egg Se regulatory values in some locations (see online supplementary material Figure S2). Ovary Se samples have been collected at different times relative to the timing of fish spawning primarily due to annual differences in logistical constraints, but also factors that affect the timing of spawning such as interannual variability in seasonal temperatures.

In this paper, we describe an evaluation of these historical ovary Se monitoring data as well as additional sampling efforts conducted during recent toxicity studies described elsewhere (de Bruyn et al., 2023). Specifically, the variable sampling times in the available monitoring data sets allowed us to test the hypothesis that sampling and analyzing unripe ovaries leads to an underestimation of egg Se concentrations in fish. In a companion to this paper (Detering et al., 2025, Companion paper), we further explore how different Se tissue ratios (egg/ovary-muscle, egg/ovary-whole body) often used in compliance monitoring are influenced by deriving the ratios from ripe versus unripe ovaries.

Methods

Fish collection and sampling

Within the study area, NPM reside primarily in Koocanusa Reservoir. This species undergoes synchronous oocyte development and typically spawns in early summer when water temperatures reach 14–18 °C (Coker et al., 2001; Gadomski et al., 2001; Scott & Crossman, 1973). Mountain whitefish also has synchronous oocyte development and resides in both Koocanusa Reservoir and the Elk River watershed, with fish spawning in late October or early November when water temperatures reach 5 °C (Boyer, 2016; Irvine et al., 2017). Redside shiner occurs in both Koocanusa Reservoir and small streams and ponds in the Elk Valley watershed. Redside shiner exhibits asynchronous oocyte development and spawns multiple times throughout the late spring and early summer once temperatures reach 10 °C (de Bruyn et al., 2023; Scott & Crossman, 1998). Peamouth chub are largely restricted to Koocanusa Reservoir within the study area. It is unclear whether this species undergoes synchronous or asynchronous oocyte development. Documented hybridization between PMC and RSS with reproductively viable offspring in several locations (not Koocanusa Reservoir) suggests a similar reproductive biology between these two species, although this is not definitive evidence of synchronous spawning (Aspinwall & McPhail, 1995).

Fish sampling to characterize ovary Se concentrations has been conducted routinely since 2009–2016 depending on the species. Sampling locations vary to some extent by species based on where they naturally occur and the specific objectives of the sampling programs but generally include locations with no waterborne Se enrichment and locations with elevated waterborne Se associated with mining activities (see online supplementary material Figure S2). Fish were collected using a variety of techniques (angling, various types of traps, short-set gill netting) appropriate for each species. On collection, fish were euthanized in accordance with provincial fish collection permits. The whole fish was weighed, and then fish ovaries were removed and weighed before being stored on ice until returned to sample holding facilities the day of collection, where they were stored frozen at −20 °C until shipment to a commercial analytical laboratory for analysis. Gonado-somatic index (GSI) was estimated (gonad weight/total fish weight × 100) and used in this study as a metric for characterizing gonad development in each species.

In addition to routine annual sampling, several supplemental sampling programs were conducted to support this study. First, in 2019, a supplemental sampling program was conducted for NPM. The objective of this program was to measure ovary Se concentrations in fish with a range of GSI and fish size ($n = 79$), because preliminary analyses of data from earlier sampling indicated both of these variables might influence ovary Se concentrations. In addition, a subset of the collected ovaries ($n = 15$) was histologically evaluated for oocyte developmental stage to relate developmental stage to GSI in this species using standard methods (see online supplementary material). Finally, efforts to conduct a study on the sensitivity of early-life stage NPM to maternally transferred Se was conducted in 2022 that included collection of expressible eggs that were analyzed for Se concentration ($n = 30$).

A second supplemental sampling program was conducted on RSS concurrent with a study characterizing the sensitivity of embryo-larval life stages of this species to maternally transferred Se (de Bruyn et al., 2023). Redside shiner has asynchronous oocyte development so that during the spawning season, ovaries continually have a mixture of oocytes at different developmental stages and ovulated eggs available for spawning. During the RSS toxicity study, eggs were expressed from females, and then the female was euthanized and the remaining ovary sampled ($n = 56$; de Bruyn et al., 2023). This allowed comparative measurement of Se concentrations in both readily expressible eggs and oocytes still developing in the ovary of the same fish.

Analytical chemistry

Ovaries (all four species) and eggs (RSS and NPM only) were frozen and shipped on ice to a commercial analytical laboratory for Se analysis. The laboratory used varied across the 13-year period and across different study programs. Similarly, the instrument used varied across laboratories and over time as new instrumentation became available. In general, samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) or high resolution (HR)-ICP-MS using modified USEPA Method 3052 and modified Method 6020B (USEPA, 1995, 1996). The exception was some eggs and ovaries from the RSS toxicity study that had low sample mass were analyzed by laser ablation ICP-MS, as detailed elsewhere (Ashby et al., 2023; de Bruyn et al., 2023). Analytical detection limits varied across years and samples (due to sample mass limitations) but were always <0.5 mg kg^{-1} dry weight and typically 0.1–0.01 mg kg^{-1} dry weight. Certified standard reference material (typically DORM-4 Fish Protein Certified Reference Material for Trace Metals) was measured concurrently with each batch of samples for total Se. Blanks, matrix blanks, and duplicate analyses were also conducted with each analytical run. Percent moisture was determined for each sample and used to convert the wet weight Se measurements to a dry weight concentration, or samples were dried prior to analysis and analyzed directly.

Data analysis

The approach to data analysis for each species was generally the same. Data describing ovary Se concentrations, GSI, and fish size (total or fork length depending on species) were collated across sampling years and locations. An initial exploratory analysis of log-transformed data was conducted by principal component analysis (prcomp, R) using Z-scores of independent variables (fish length, GSI, body weight, and gonad weight) to identify correlations among these variables and select the most appropriate variables for linear modeling. As expected, body weight, gonad weight, and GSI were strongly correlated, so fish length and GSI were retained as variables. Bivariate relationships among independent variables and between ovary Se and independent variables were plotted by area and year to help visualize effects of area and year on relationships.

After selecting independent variables, exploratory linear and multiple linear regressions (MLR) were conducted to predict ovary Se for various subsets of the data. For example, models using one or more independent variables were developed for data sets for individual years, different combinations of years, individual locations, and different combinations of locations. These exploratory analyses were intended to gain a better understanding of how the data were distributed as a function of the independent variables, location, and sampling year.

Using insights gained from these exploratory analyses, stepwise MLR models based on the Bayesian information criterion (BIC) were developed to test for statistical differences between area-specific slopes and intercepts and to identify the most parsimonious model to explain the data. Stepwise modeling was conducted in R (stepAIC). Model residuals were tested for normality using Shapiro-Wilk (shapiro.test, R) and nonconstant variance (ncv, R). The performance of each model was evaluated using adjusted R^2, predicted R^2, and root mean square error. Collinearity between variables was assessed using variance inflation factors (Zuur et al., 2010).

In addition to MLR analyses, paired data on Se concentrations in expressed eggs and the remaining ovary for RSS and oocyte development stages and GSI for NPM were examined using ordinary least squares regression to evaluate whether a relationship was observed between these variables.

Results

NPM ovarian histology

Northern pikeminnow ovaries for histological analysis of oocyte maturation stages across fish of different sizes (weight: 250–1,800 g; fork length: 33.2–61.8 cm) and GSI (range: 0.60%–10.5%) represented all stages of oocyte maturation, ranging from immature (Stage 1) to preovulation (Stage 3; Figure 2 and online supplementary material Table S5). A positive correlation was observed between GSI and ovarian maturation stage (Figure 3A), with all having GSI ≥8% being at oocyte maturation stage 3. Similarly, a significant linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($R^2 = 0.81$) revealed that ovaries of mature fish with a GSI ≥8% had >75% LVO (80% was the maximum measured; Figure 3B). This indicates there is high confidence that NPM with a GSI ≥8% are in spawning condition.

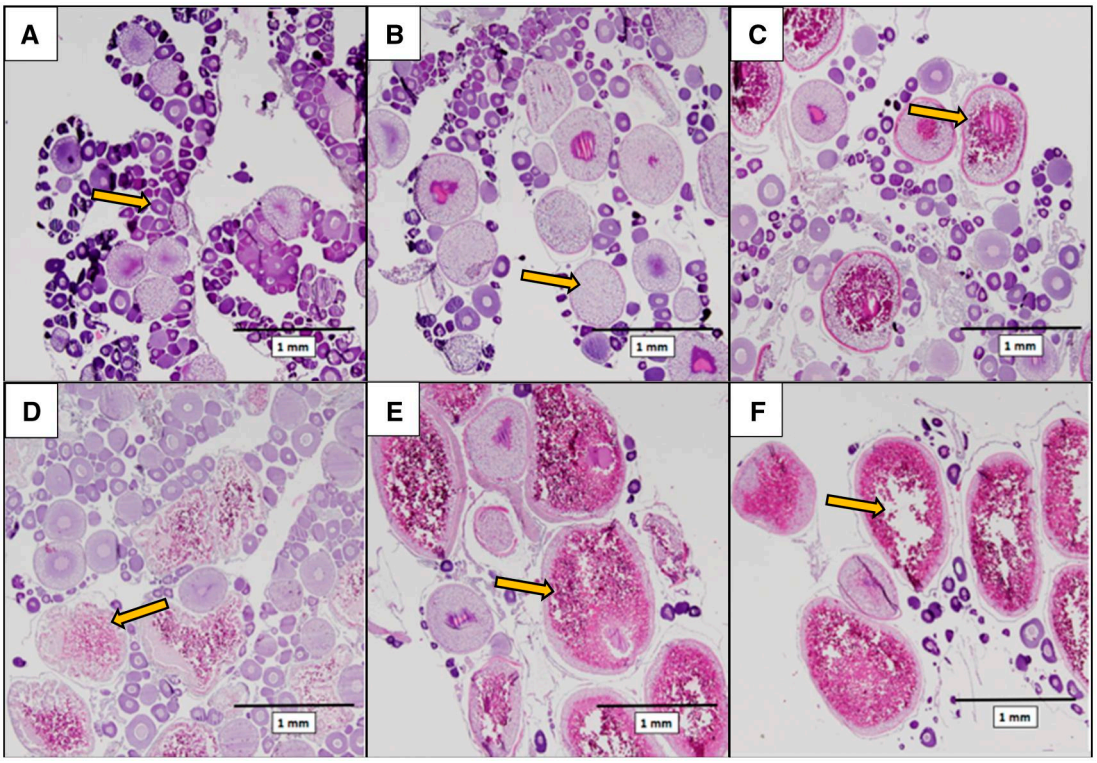

Figure 2. Histomicrographs of ovaries of northern pikeminnow representing early development stages. (Stage 1) consisting mainly of perinucleolar oocytes (A; arrows) and cortical alveolar oocytes (B; arrows), mid development stages (Stage 2) with increasing proportions of early (C; arrows) and mid-vitellogenic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

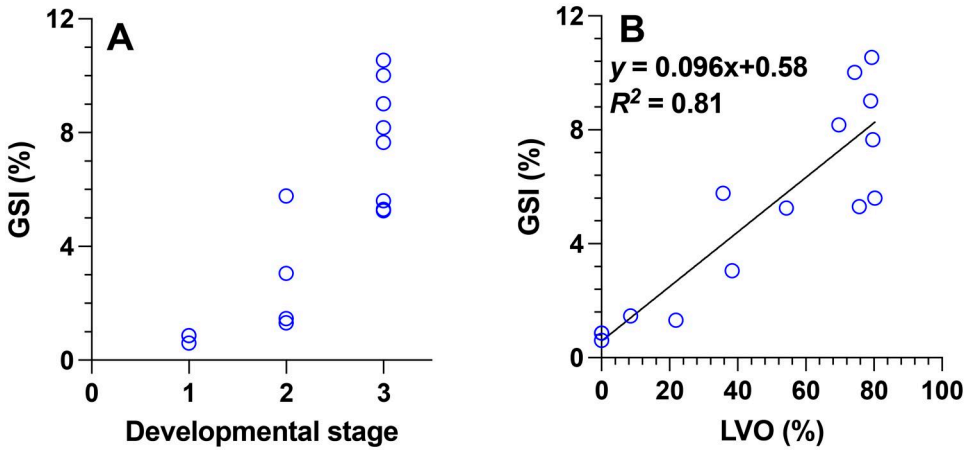

Figure 3. Relationships between gonado-somatic index (GSI) and (A) Developmental stage and (B) Late-stage vitellogenic oocytes (LVO) in northern pikeminnow.

NPM analysis

The NPM data ($n = 145$) were all collected from Koocanusa Reservoir between 2016 and 2021. Samples were associated with five different locations within the reservoir, including one (Rexford) on the Montana side of the reservoir. The MLR analysis for NPM was intended to evaluate whether a model could be developed to predict ovary Se as a function of fish size, GSI, and sampling location. The data set from 2019 was collected with a more balanced design intended to capture a relatively evenly distributed range of GSI and fish size classes, compared to earlier and subsequent sampling efforts that focused on collecting a specific number of ovary samples independent of GSI or fish size considerations. After exploratory analysis of all data, we initially focused our MLR analysis on the 2019 data set to avoid biases in data distribution that might confound model development.

The first model was developed to test for differences between area-specific slopes and intercepts (Equation 1). The contrasts used to test for area-specific intercepts evaluated differences between samples collected at the mouth of the Elk River, which is closest to the source of Se from mining activities, and other sampling locations.

$$\mathrm{Log(OvSe)} = \mathrm{area} + \mathrm{Log(TL)} + \mathrm{area} * \mathrm{Log(TL)} + \mathrm{Log(GSI)} + \mathrm{area} * \mathrm{Log(GSI)} \quad \text{(Equation 1)}$$

where OvSe is ovary Se, TL is the total length (cm), and GSI is the gonado-somatic index.

Area-specific slopes were not retained in the BIC-selected model, resulting in a final model with area-specific intercepts and pooled slopes. Exclusion of area-specific slopes means that

relationships between independent variables (total length and GSI) and ovary Se were statistically similar among sites. Retention of area-specific intercepts indicates that while differences in fish size and GSI between sites explained some of the observed differences in ovary Se concentrations, statistically significant differences exist among site-specific mean ovary Se concentrations (presumed to reflect spatial differences in dietary Se concentrations). This model performed reasonably well (adjusted $R^2 = 0.72$) in terms of predicting ovary Se concentrations for the data on which it was based (see online supplementary material Figure S3), and the slightly lower predicted R^2 of 0.69 indicates the model was not overparameterized or unduly influenced by individual data points.

Once the MLR model based on data from 2019 only was developed, data from other years were then evaluated using the 2019 MLR model structure. An initial model included a term to test for differences in mean ovary Se concentrations between years (Equation 2).

$$\text{Log(OvSe)} = \text{area} + \text{year} + \text{Log(TL)} + \text{area} * \text{Log(TL)} + \text{Log(GSI)} + \text{area} * \text{Log(GSI)} \quad \text{(Equation 2)}$$

The final model using all data from 2016 to 2021 retained the same variables as the model using only 2019 data with only slight differences in model coefficients (Table 1; see online supplementary material Table S6). Adjusted and predicted R^2 for the final model were 0.72 and 0.71, respectively (Figure 4). Neither year or area-specific slopes were retained in the model, indicating that mean ovary concentrations did not differ across the four sampling years and that the addition of data from the other years did not create differences among area-specific slopes. Significant differences in area-specific intercepts were retained in the model. The intercepts for Gold Creek ($p = 0.002$) and Rexford ($p < 10^{-5}$) were significantly lower than the Elk River intercept, indicating that after accounting for the influence of fish length and GSI, ovary Se concentrations in NPM collected from Gold Creek and Rexford were significantly lower than those observed for fish collected near the Elk River mouth (Table 1; see online supplementary material Table S6).

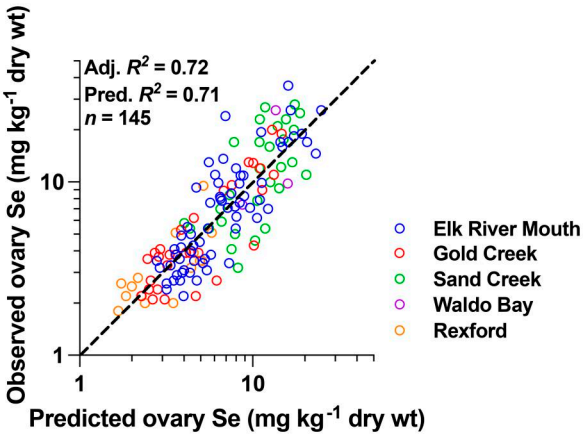

Figure 4. Final ovary Se-gonado-somatic index multiple linear regression model for northern pikeminnow based on 2016–2021 data. Dashed line represents 1:1 line of agreement.

Standardized slope coefficients, which provide a measure of the relative steepness of the slopes of multiple independent variables, indicate that GSI (standardized slope = –0.53) has a stronger effect on ovary Se concentrations than total length (standardized slope = –0.32) over the sampled range (Table 1). Residuals of the final model were normally distributed ($p = 0.912$; see online supplementary material Figure S4) but had unequal variance ($p = 0.036$). Residuals also indicate the model tends to underpredict ovary Se concentrations at low ovary Se and overpredict at high ovary Se (see online supplementary material Figure S4A).

MWF analysis

Analysis of the MWF data set ($n = 143$) proceeded as described for NPM except that no data analogous to the 2019 NPM data were available for initial model development. Instead, we used the final model structure from the NPM analysis as a baseline model for the MWF data set (Equation 2). Fish size, as measured by fork length, was not retained in the model, indicating it does not have a significant influence on ovary Se ($p = 0.97$). In contrast, GSI was significant ($p < 10^{-5}$), but no area-specific slopes were retained, indicating the relationship between GSI and ovary Se is similar across sites. Area-specific intercepts were retained in the model, indicating significant differences in mean ovary Se among sites after correcting for the effect of GSI (Table 2; see online supplementary material Table S7). This is expected because some sampling locations (e.g., Michel; see online supplementary material Figure S1) exhibited elevated Se concentrations associated with mining activity, while others are not influenced by mining.

Adjusted and predicted R^2 (0.82 and 0.79, respectively) indicate the model is not overparameterized or affected by individual data (Figure 5). Model residuals were not normally distributed, and variances differed somewhat across the range of predicted values (Shapiro-Wilk $p = 0.003$, nonconstant variance $p = 0.054$; see online supplementary material Figure S5). This is not surprising, given the much higher heterogeneity in sampling locations in terms of waterborne Se concentrations and habitat, compared to the NPM data set. Only two locations (EL1 and Michel) had a wide distribution of GSI values (see online supplementary material Figure S5). The final model had a log GSI slope of –0.109, substantially lower than the slope of –0.412 for NPM (Tables 1 and 2).

Table 1. Final ovary se model coefficients and significance for northern pikeminnow.

Model parameters		Estimate	Std. error	p	Standardized regression slope
Intercepts	Elk River Mouth	3.156	0.355	NA	
	Gold Creek Mouth	3.036	0.039	0.002	
	Sand Creek Mouth	3.178	0.040	0.60	
	Waldo Bay	3.194	0.692	0.69	
	Rexford	2.708	0.053	$<10^{-5}$	
Slopes	Log Fork Length	−1.306	0.227	$<10^{-7}$	−0.318
	Log GSI	−0.412	0.044	$<10^{-15}$	−0.527

Note. The p-values relate to testing for significant differences in intercepts relative to Elk River Mouth.

Table 2. Final ovary standard error model coefficients and significance for mountain whitefish.

		Estimate	Std. error	*p*
Intercepts	Bull Mouth	1.507	0.040	2×10^{-4}
	Bull River	1.179	0.053	$<10^{-5}$
	EL1	1.629	0.030	0.243
	EL19	1.568	0.032	0.003
	EL20	1.499	0.031	$<10^{-5}$
	ELEKO	1.606	0.037	0.123
	Flathead	1.385	0.033	$<10^{-5}$
	Fording	1.582	0.027	0.003
	Kikomun	0.754	0.045	$<10^{-5}$
	Kootenay	1.223	0.048	$<10^{-5}$
	Michel	1.663	0.031	NA
	St. Mary	0.695	0.072	$<10^{-5}$
Slope	Log GSI	−0.109	0.023	$<10^{-5}$

Note. The *p*-values relate to testing for significant differences in intercepts relative to Michel Creek. GSI = gonado-somatic index.

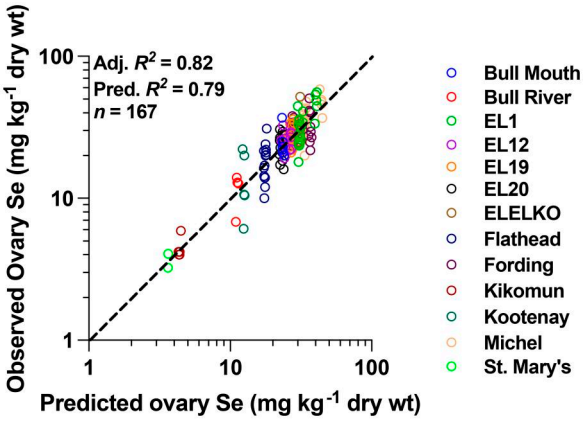

Figure 5. Final ovary Se-gonado-somatic index multiple linear regression model for mountain whitefish based on 2009–2021 data. Dashed line represents 1:1 line of agreement.

RSS analysis

Analysis of the RSS data set ($n = 274$) using the same approach described for NPM and MWF indicated GSI is a significant predictor of ovary Se ($p = 0.007$, log GSI slope = –0.158), whereas fish size is not ($p = 0.88$). However, the adjusted R^2 for the model is low (0.28) and similar to the MWF model; much of the explanatory power of the model appears to be associated with the location of specific intercepts rather than GSI (Figure 6A).

Using data collected during a maternal transfer toxicity study with RSS (de Bruyn et al., 2023), we also evaluated Se concentrations in expressible eggs that had completed development and in the remaining ovary, which represents an integrated measure of Se concentration in oocytes across the full range of development from pre-vitellogenesis to those that have completed vitellogenesis but not yet ovulated. This analysis demonstrated a strong log-linear relationship between ovary Se and egg Se ($R^2 = 0.95$), indicating that egg Se concentrations are typically approximately 54% of ovary Se concentrations in RSS during the spawning season (Figure 6B).

PMC analysis

Analysis of the PMC data set ($n = 282$) using the same approach described for MWF and RSS identified GSI as a significant ($p < 10^{-5}$) predictor of ovary Se concentrations. Neither fish size ($p = 0.17$) nor sampling location (0.12) was significant, and BIC did not retain them in the model. The log GSI slope of the final model was –0.279, but the model had a low adjusted R^2 (0.08) and predicted R^2 (0.07; Figure 7 and online supplementary material Table S9) and is not considered reliable.

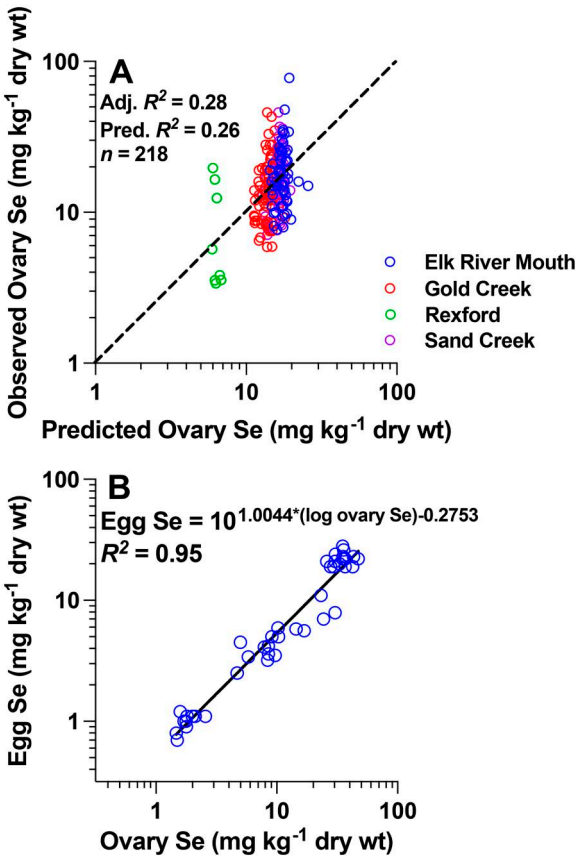

Figure 6. (A) Final ovary Se-gonado-somatic index multiple linear regression model for redside shiner based on 2015–2022 data. Dashed line represents 1:1 line of agreement. (B) Relationship between ovary Se and expressible egg Se in redside shiner.

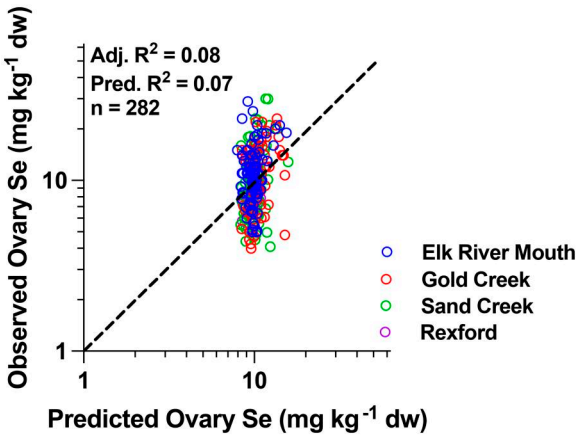

Figure 7. Final ovary Se-gonado-somatic index multiple linear regression model for peamouth chub based on 2015–2021 data. Dashed line represents 1:1 line of agreement.

Discussion

The primary objective of this study was to evaluate how ovary Se concentrations vary as a function of oocyte development. To

accomplish this, we used ovary Se monitoring data sets for four fish species collected from the Kootenay region in British Columbia and Montana from 2009 to 2022. Contrary to the prevailing paradigm that ovary Se concentrations increase as oocyte development progresses, we found that ovary Se concentrations generally decrease as oocyte development progresses. In the companion paper to this study, a meta-analysis of a more diverse group of fish species using a somewhat different modeling approach provides support that this is a widely observed, if not general, phenomenon (Detering et al., 2025, Companion paper).

Our findings suggest that current scientific understanding of the mechanisms by which Se is maternally transferred to oocytes is not entirely correct. They also have significant implications in terms of assessing regulatory compliance with egg/ovary-based environmental guidelines for Se. Here we discuss each of these topics and conclude with recommendations for improving ovary Se monitoring programs and research priorities to increase our understanding of maternal Se transfer in fish.

Conceptual mechanistic model of Se dilution in oocytes

Currently, we cannot provide a fully supported mechanistic conceptual model to explain our observations that ovary Se concentrations are inversely related to GSI in species with synchronous oocyte development and analogously that developing oocytes have higher Se concentrations than expressed eggs in species with asynchronous oocyte development. We also note that our analysis is limited to four fish species, so it should not be assumed that our observations are the result of a general phenomenon, although in a companion paper we provide further evidence this is the case for additional species (Detering et al., 2025, Companion paper). Acknowledging these limitations, we explore several potential mechanisms that may explain empirical observations (Figure 8). We readily acknowledge there may be other mechanisms we have not considered that may contribute to empirical observations.

In fish, the precursor to egg yolk protein is Vtg, which is synthesized in the liver and transported via the circulatory system to the ovary. In the ovary, Vtg is taken up by oocytes by endocytosis and subsequently proteolyzed into its three main components: lipovitellin (Lv), phosvitin (Pv), and β'-component (β'-c; Hara et al., 2016). Lipovitellin is the primary component of egg yolk and is approximately 20% lipid. Phosvitin is a phospho-protein composed of approximately 10% phosphorous and approximately 50% serine and is responsible for binding essential metals such as Ca, Fe, and Zn for transport into the oocyte. The specific role of β'-c in oocyte development is not well understood, but this molecule has a high cysteine content.

The original hypothesis that Vtg was the primary mechanism for maternal transfer of Se was developed based on measurements in white sturgeon (*Acipenser transmontanus*; Kroll & Doroshov, 1991). Because this species has low plasma Vtg concentrations even during vitellogenesis, direct isolation and measurement of Se in circulating plasma Vtg were not made. Instead, the proteolyzed components of Vtg, namely Lv and Pv, were isolated from oocytes. Based on a small sample ($n = 6$) with high variance around the mean, average Se concentrations in Pv were 3.8-fold higher than in Lv and oocyte Se was strongly related to Se concentrations in Pv. The higher Se accumulation in Pv compared to Lv is consistent with Pv being comparatively enriched in S-bearing amino acids that would allow for Se substitution (Finn, 2007). To the best of our knowledge, despite the intervening 30+ years, this remains the only study in which Se concentrations were directly measured in Vtg or its components for fish.

The vitellogenic profile over time differs between fish with synchronous versus asynchronous oocyte development. We acknowledge that this comparison is a very broad generalization and that species-specific variations exist within these two categories. However, in general, in fish with asynchronous oocyte

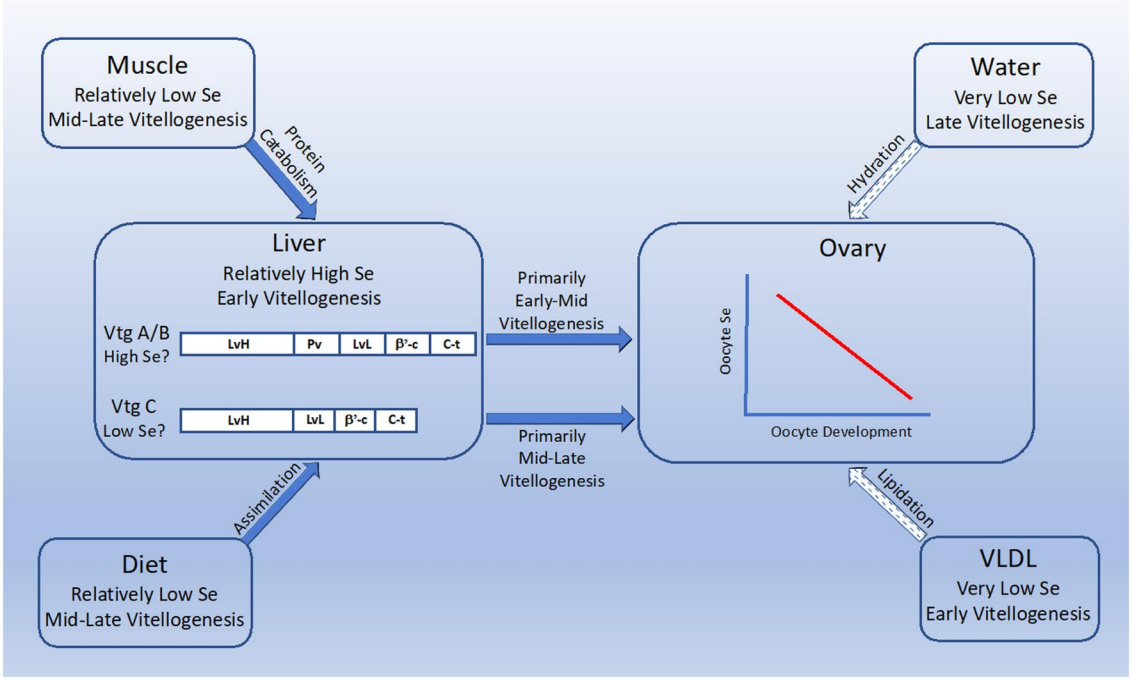

Figure 8. Conceptual model of potential mechanisms leading to reduced ovary Se during oocyte development and maturation. See text for extended description. Dashed arrows indicate mechanisms unlikely to have strong influence on ovary Se concentrations. *Note*. Vtg = vitellogenin; VLDL = very-low-density lipoprotein; LvH = lipovitellin heavy chain; Lvl = lipovitellin light chain; Pv = phosvitin; β′-c = β′ component; C-t = C-terminal coding domain.

development and multiple spawning cycles across the spawning season such as RSS, the vitellogenic cycle (Figure 1) typically lasts from a few days to perhaps a week. Multiple cohorts of oocytes in different stages of development are present within the ovary, and the amplitude of changes in circulating Vtg with each cycle is often relatively small (Cerda et al., 1996; Connolly et al., 2014; Jensen et al., 2001; Li et al., 2011). In fish with synchronous oocyte development such as NPM and MWF, vitellogenesis may occur over an extended period, with a characteristically large increase in circulating Vtg in the 1–4 months leading up to ovulation (Figure 1; Hiramatsu et al., 1997; Rinchard et al., 1997; Scott & Sumpter, 1983; Tyler et al., 1990). It is this surge in Vtg synthesis and corresponding uptake by oocytes that led to the conceptual model of Se concentrations increasing in oocytes as they approach maturation (USEPA, 2016).

We have identified four potential mechanisms to explain why empirical observations contradict this model. Conceptually, they all involve accumulation of relatively high Se materials during early-mid vitellogenesis followed by dilution of these materials in mid-late vitellogenesis and/or during oocyte maturation (Figure 8). One mechanism, preovulatory oocyte hydration (Cerda, 2009; Milla et al., 2006), is generally not applicable and will not be discussed further, because egg and ovary Se concentrations are typically evaluated on a dry weight basis. None of the potential mechanisms are mutually exclusive, and some combination of processes may be involved with species-specific variation in their relative contribution.

The first potentially significant contributor to this dilution effect is related to the synthesis and subsequent uptake of different Vtg isoforms over the course of vitellogenesis. There is considerable variability in nomenclature across the literature, but in general there are three main isoforms of Vtg in fish—Aa, Ab, and C (Hara et al., 2016). Structurally, Vtg Aa and Ab are quite similar, each composed of Lv, Pv, and β'-c. In contrast, Vtg C lacks both Pv and β'-c and is generally considered an ancestral form of Vtg (Finn, 2007; Hara et al., 2016). The absence of a Pv domain in Vtg C suggests this isoform will have relatively low Se concentrations compared to the other isoforms, because the limited available data indicate Pv has approximately 4-fold higher Se content compared to Lv in fish (Kroll & Doroshov, 1991).

The uptake dynamics of Vtg isoforms into oocytes is temporally complex, being regulated by both synthesis rates in the liver and receptor density at the oocyte surface for uptake (Andersen et al., 2017; Hara et al., 2016; Tyler et al., 1990). Further, there is considerable species-specific variability in the relative contribution of each isoform to the final yolk protein composition and in the timing of those contributions (Andersen et al., 2017; Jiang et al., 2021; Williams et al., 2014; Yilmaz et al., 2018). In general, the relative yolk protein composition is Vtg Aa≈Ab>C, with Vtg C contributing approximately 30% to <1% of total Vtg depending on the species (Andersen et al., 2017; Davis et al., 2007; Mushirobira et al., 2013). Available studies indicate that Vtg C expression is less sensitive to estrogen than Vtg Aa or Ab. This means that Vtg Aa and Ab will tend to be the predominant Vtgs during early and mid-vitellogenesis, and are then diluted to varying degrees from mid- to late vitellogenesis as estrogen concentrations increase and Vtg C synthesis is induced (Amano et al., 2010; Andersen et al., 2017; Mushirobira et al., 2013, 2018; Williams et al., 2014). Keeping in mind that Vtg C likely has comparatively low Se content, it would effectively dilute the oocyte Se concentration during the later stages of vitellogenesis. Quantitative estimates of how much this could dilute egg Se would be species-specific and quite speculative given the limited data available at this time, but we suggest this mechanism is worth further investigation.

Another potential mechanism we considered is dilution of Vtg Se by oocyte uptake of very-low-density lipoproteins (VLDL). Marine fish that spawn in pelagic environments produce large oil droplets to facilitate egg flotation (Wiegand, 1996). The lipid source for these oil droplets is VLDL rather than Vtg and represents a significant potential dilution of Se associated with Vtg for these species. In freshwater species that typically have negatively buoyant eggs, lipid content is much lower and Vtg is the primary lipid source. In these species, VLDL uptake still occurs but primarily during the early vitellogenic stage, and it is unlikely to be an important mechanism of Vtg Se dilution throughout most of vitellogenesis (Hiramatsu et al., 2015).

A final potential mechanism that may contribute to the inverse relationship between ovary Se and GSI is dilution of hepatic protein and its relatively high associated Se content with muscle and/or dietary protein and their comparatively lower Se content. As with many other metals and metalloids, Se is preferentially accumulated in the liver of teleosts, with comparatively lower concentrations in other tissues such as muscle. We have not comprehensively reviewed this issue, but an initial search of the literature revealed data for eight species with a mean Se liver: muscle ratio of 2.9 across species. We expect a similar or slightly higher Se liver: diet ratio because trophic transfer factors for fish are typically approximately 1 on a whole-body basis (Presser & Luoma, 2010) and muscle: whole body ratios are typically approximately 1.5 (USEPA, 2016).

Vitellogenin synthesis occurs almost exclusively in the liver, and the liver appears to be an important source of protein for Vtg (Rinchard & Kestemont, 2003). However, hepatic protein stores are not sufficient to support all of the protein requirements of vitellogenesis. Instead, protein from the diet and/or protein catabolism of the muscle are critical secondary protein sources that are processed by the liver to support vitellogenesis (Cleveland & Weber, 2011; Martin et al., 1993; Mommsen & Walsh, 1988). Consequently, while early vitellogenesis may be supported directly by liver protein and its relatively high Se content, as vitellogenic activity increases, other protein sources with comparatively lower Se will be required, providing a mechanism for Se dilution during mid-late vitellogenesis. This is particularly true in species with synchronous oocyte development, and the effect may be more muted in those with asynchronous development where peak Vtg concentrations and therefore protein demand are typically lower (Cerda et al., 1996; Jensen et al., 2001).

MLR models

Despite our inability to provide a well-supported mechanistic model at this time, we were able to develop simple statistical models that describe our empirical observations on the inverse relationship between ovary Se and oocyte development. We used an MLR framework for this modeling because our initial model development with NPM indicated female size was also a potential explanatory variable. While this was confirmed for NPM, size appears not to be an important variable for the other species.

We hypothesized the ovary-GSI relationship would be strongest for the two synchronous spawning species (NPM and MWF) and weaker or perhaps absent from the asynchronous spawners (RSS and likely PMC). The most robust demonstration of the relationship between ovary Se and GSI was for the NPM data set, which benefited from having a structured sampling program intended to provide relatively evenly distributed data across a range of GSI and fish sizes in contrast to the other data sets

where sampling was more ad hoc relative to the objectives of our analysis.

In NPM, the size effect is likely the result of differences in dietary preferences between small and large adult NPM. Small adult NPM (<30 cm) typically have a primarily insectivorous diet, but they become increasingly piscivorous with increasing size, feeding primarily on juvenile salmonids (Clarke et al., 2005; Petersen, 2001; Zimmerman, 1999). Whole-body trophic transfer factors (TTFs) for fish (i.e., invertebrate to fish or fish to fish) are typically <1 except at very low (<1 mg kg^{-1} dry wt) dietary Se concentrations (DeForest et al., 2007). A consequence of TTFs <1 is that consumers at progressively higher trophic levels will have progressively lower whole-body Se concentrations (i.e., biodilution). This may explain the size effect observed in the current analysis, because small NPM feeding on insects would be expected to have higher dietary Se exposure than large NPM feeding on juvenile salmonids. The importance of size in other fish species will likely be dependent on whether they also undergo dietary shifts within the size range at which they are reproductively mature.

Overall, we have relatively high confidence in the NPM model. The model benefits from a relatively well-distributed data set from the 2019 sampling program that specifically targeted females with a range of GSI and size, while data sets for the other species relied on data collected with the primary objective of meeting a target number of fish in any given year. For NPM, the ovary Se-GSI relationship is well characterized both within and across locations. Model residuals for the NPM model are relatively well distributed as a function of ovary Se, GSI, and fish length, although the model may underpredict slightly at low ovary Se concentrations and overpredict at high ovary Se concentrations.

Although the MWF model had a higher R^2 than the NPM model, we believe this model is somewhat more uncertain. Compared to NPM, more of the variance in MWF ovary Se concentrations was associated with sampling location effects rather than the effect of GSI. This is because much of the MWF data set was sampled in the Elk River drainage, where the Se exposure gradient is larger compared to locations within Koocanusa Reservoir. Additionally, a substantially larger fraction of the MWF data set is for fish near or above the GSI associated with spawning in this species, muting the GSI signal in the data set. This is not to suggest that the ovary Se-GSI relationship is not present for MWF, because there is high confidence it is present ($p < 10^{-5}$), but rather that the model would benefit from additional data, particularly fish with relatively low GSI (<5%).

The model for PMC was not very precise, with a low R^2 (0.08), and we suggest it should not be used in regulatory assessments. The PMC data set is large ($n = 282$), with a relatively wide range of GSI, suggesting the weak model performance is not the result of lack of data. Uncertainty about the mode of oocyte development in this species makes it difficult to determine whether this weak relationship is consistent or inconsistent with expectations. In other words, is this a species with synchronous oocyte development and a weak ovary Se-GSI relationship or a species that, despite having asynchronous oocyte development, exhibits a weak ovary Se-GSI relationship? The fact that PMC is known to successfully hybridize with RSC and the poor performance of the model provide weight of evidence for the latter, but uncertainty remains.

We also detected a significant relationship between GSI and ovary Se for RSC ($p = 0.007$). Similar to PMC, the model had relatively low precision (adjusted $R^2 = 0.29$), and most of the variance in the model appeared to be explained by location-specific intercepts rather than GSI (Figure 6A). However, given that RSC is an asynchronous spawner with ovaries that contain oocytes at various stages of development, we expected the ovary-GSI relationship to be muted (or absent) and explain little of the variation in ovary Se concentrations. There was a strong relationship ($R^2 = 0.95$) between expressible egg Se and remaining ovary Se (Figure 6B), and we conclude the simple linear model describing this relationship is suitable for estimating egg Se concentrations from ovary Se data when fish are sampled during the spawning season. Conceptually, this is analogous to observations for NPM and MWF in that the, on average, less developed oocytes in the ovary have higher Se concentrations than the fully developed expressible eggs. If PMC also has asynchronous oocyte development, or for other species that are known to be asynchronous spawners, it may be appropriate to use the same approach.

Regulatory implications of ovary Se-GSI relationship

The challenges associated with sample timing in environmental monitoring and effects assessment for fish are not new. In Canada, for example, the Environmental Effect Monitoring program which includes sampling fish for indications of energy storage (condition, liver size) and usage (growth, gonad size) requires sampling sentinel fish during their spawning season, which has been defined for multiple species (Barrett & Munkittrick, 2010). Our observation that ovary Se concentrations are inversely related to oocyte development is analogous and has similar implications for regulatory compliance monitoring and risk assessment.

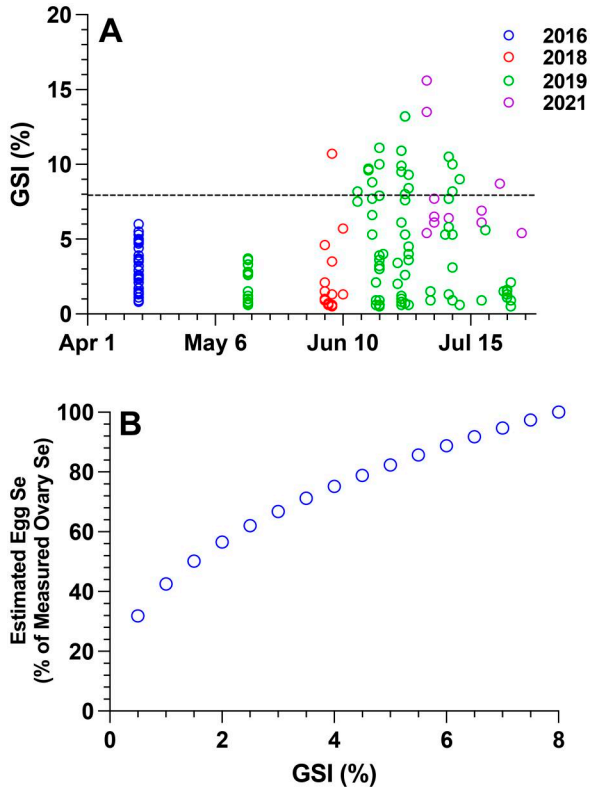

Figure 9. (A) Female northern pikeminnow gonado-somatic index (GSI) as a function of sampling date (2016–2021). Dashed line represents GSI associated with females in spawning condition (see Figure 3). (B) Estimated egg Se concentration expressed as a percentage of measured ovary Se concentration and as a function of GSI at time of sample collection. Estimates based on northern pikeminnow multiple linear regression model (Table 1).

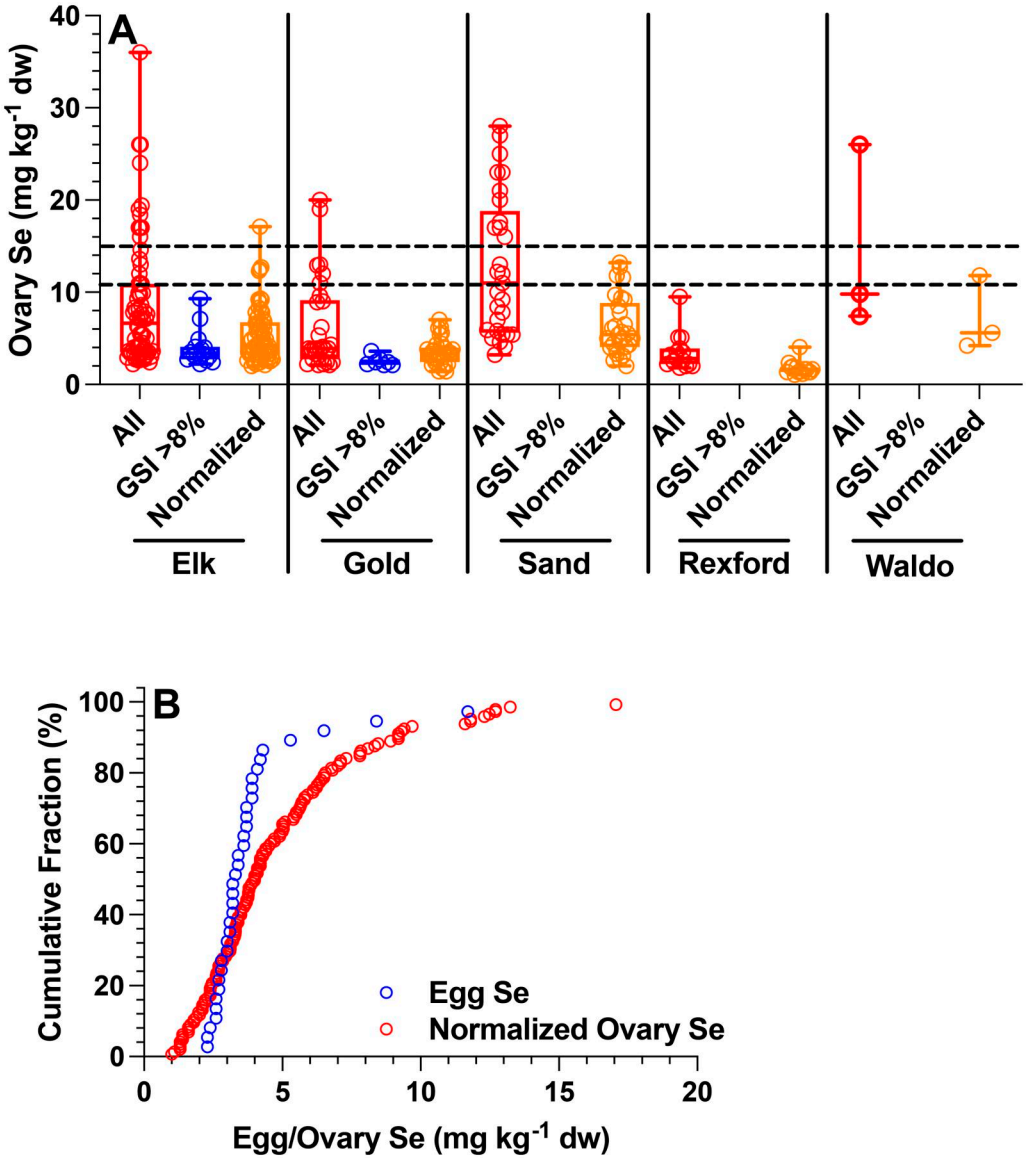

Figure 10. (A) Distribution of ovary Se concentrations in northern pikeminnow based on all data collected, only data from females with a gonadosomatic index (GSI) >8%, and all data but data from females with GSI <8% normalized to 8% using the northern pikeminnow multiple linear regression model. Dashed lines indicate egg Se environmental guidelines for British Columbia Environment (11 mg kg^{-1} dry wt) and U.S. Environmetal Protection Agency (15.1 mg kg^{-1} dry wt). (B) Cumulative frequency distributions of normalized ovary Se concentrations (2016–2021) and egg Se concentrations (2019–2022).

As a demonstration of the impact this can have, we can further evaluate the NPM monitoring data using the MLR model developed for this species. For NPM, assuming a GSI of >8% equates to fish in spawning condition (Figure 3), we observed that in Koocanusa Reservoir, this species comes into spawning condition in mid-June and finishes spawning by the end of July (Figure 9A). Importantly, unripe females with relatively low GSI are consistently collected prior to the beginning of June and can be collected throughout the spawning season. This highlights the importance of collecting GSI data rather than simply relying on a spawning window to characterize fish spawning condition. Using the MLR model, we can also generalize the effect of sampling NPM with low GSI by expressing the estimated egg Se concentration as a percentage of the measured ovary Se concentration and associated GSI (Figure 9B). At a GSI of 0.5%, estimated egg Se at the time of spawning is only 32% of the measured ovary Se when the fish was collected. In NPM, this overestimation is moderately relatively quickly with estimated egg Se 67% of measured ovary Se at a GSI of 3% and 89% of measured ovary Se at a GSI of 6%.

To further demonstrate the impacts of this effect, we comparatively plotted the ovary Se monitoring data using all data, only data from fish with a GSI >8%, and all data with those values from fish with a GSI <8% normalized to 8% using the GSI slope parameter from the MLR model in Equation 3:

$$\text{Normalized Ovary Se} = 10^{(\text{Log(Measured Ovary Se} - (-0.413 * (\text{Log(Measured GSI)} - \text{Log(Target GSI)})))} \quad \text{(Equation 3)}$$

where Target GSI is 8%.

Considering ovary Se data only from fish in or near spawning condition provides a very different perspective on the extent to which NPM are exceeding BC and U.S. egg/ovary Se guidelines and the potential risk to this species in Koocanusa Reservoir (Figure 10A). While this reduced data set provides a more accurate estimate of egg Se concentrations in NPM in Koocanusa Reservoir, 81% of historical data had a GSI <8% and were excluded, resulting in a large loss of information. Alternatively, estimating ripe ovary Se in the excluded subset of data by normalizing to a GSI of 8% allowed for retention of this information but introduced some uncertainty about the true values.

To evaluate the robustness of these predictions, we used a smaller data set ($n = 36$) of expressible eggs collected from NPM in Koocanusa Reservoir in 2019 and 2022 and compared these data to the normalized ovary Se data set ($n = 145$). The cumulative frequency distributions overlap strongly with the normalized data skewed toward slightly higher concentrations, suggesting the MLR model may be slightly conservative (Figure 10B).

While normalizing the NPM data had a large impact on the assessment of potential risk from Se in Koocanusa Reservoir to this species, this difference was driven largely by the high fraction (81%) of ovary Se data collected from fish prior to reaching spawning condition. In other scenarios, the difference may be more modest. For example, in the MWF data set, where only 28% of the data were less than the estimated GSI spawning threshold of 15% for this species (Irvine et al., 2017) and the slope of the GSI relationship was shallower, changes in the overall assessment were relatively small (see online supplementary material Figure S6).

Analogous to the GSI normalization approach for NPM and MWF, the RSS ripe ovary data can be used to estimate egg Se concentrations which are more appropriate for comparison to the egg Se toxicity threshold for this species (>28 mg kg^{-1} dry wt). Similar to NPM, this resulted in a reduction in the estimated risk to this species from Se exposure in Koocanusa Reservoir (see online supplementary material Figure S7).

Conclusions and recommendations

Our analysis suggests the existing paradigm that sampling ovary Se prior to fish reaching spawning condition will underestimate egg Se is not correct, and instead will lead to an overestimation. The mechanisms underlying these observations are not understood, but we have identified several candidates, with differential expression of Vtg isoforms and mobilization of dietary/muscle protein with relatively low Se being primary candidates. All of these mechanisms are currently speculative and in need of experimental testing, as are additional mechanisms not considered here.

Regardless of the underlying mechanism(s), the apparent inverse relationship between ovary Se and oocyte development as characterized by GSI has significant regulatory implications. While regulators generally recommend sampling ovary Se from fish in spawning condition, this is not always logistically feasible, or the spawning window is sufficiently wide due to geographic and/or interannual climate variability that the exact condition of sampled fish is uncertain. To address this issue, we recommend always determining and reporting GSI in addition to ovary Se concentrations. This will allow for development of new models, or improvement of existing models, to normalize data to an appropriate developmental stage. Fish species with asynchronous oocyte development present an additional challenge because ovary Se reflects oocytes with a range of developmental stages and, therefore, a range of Se concentrations. For these species, one approach would be to develop models relating ovary Se to expressible egg Se as we demonstrated for RSS. To that end, we recommend that when collecting expressible egg Se samples, ovary Se samples should be collected from the same fish to allow for development of these models. In the absence of these models, it is important to recognize that ovary Se is likely to overestimate egg Se concentrations, perhaps substantially.

In addition to the direct consequences of the ovary-GSI relationship on interpreting ovary Se data, we note that the ovary-GSI relationship also affects the interpretation of tissue ratios between egg/ovaries and muscle or whole-body Se, to the extent those ratios are based on unripe ovary data (USEPA, 2021). We explore this issue further in a companion paper that considers a broader range of fish species (Detering et al., 2025, Companion paper).

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

All data in this study are provided in online supplementary materials.

Author contributions

Kevin V. Brix (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Visualization, Writing—original draft, Writing—review & editing), Lucinda M. Tear (Data curation, Formal analysis, Visualization, Writing—review & editing), James R. Elphick (Data curation, Investigation, Writing—review & editing), Jennifer Ings (Data curation, Investigation), Claire Detering (Writing—review & editing), Megan Carr (Investigation, Methodology), Katherine Raes (Investigation), Mariah C. Arnold (Funding acquisition, Project administration, Resources, Supervision, Writing—review & editing), Marko Adzic (Supervision, Writing—review & editing), Markus Hecker (Investigation, Supervision), Adrian de Bruyn (Investigation, Supervision, Writing—review & editing), and David K. DeForest (Data curation, Formal analysis, Investigation, Writing—review & editing)

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Conflicts of interest

KVB, LMT, JRE, JI, CD, MC, KR, AdB, and DKD declare they have no conflicts of interest. MCA and MA are employed by Elk Valley Resources.

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