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Original Article

Hazard/Risk Assessment

Relationships in selenium concentrations among fish tissues to support selenium assessments and regulations

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Abstract

Selenium (Se) toxicity to fish is primarily manifested via maternal transfer to the eggs. Several regulatory jurisdictions have adopted Se criteria or guidelines expressed as the Se concentration in fish eggs (typically expressed as egg/ovary Se concentrations, thereby suggesting these are equivalent). Because it is not always feasible to sample fish eggs or ripe ovaries at a site, it can be necessary to sample muscle or whole-body (WB) tissue. This study evaluated whether there were consistent relationships in tissue Se concentration within and across fish species. Additionally, based on findings in a companion study, we evaluated whether ovary Se concentrations are inversely related to oocyte development in a wide range of fish species. Consistent with the companion study, the spawning status of the wide range of fish species had a significant influence on Se concentrations, with Se concentrations in eggs and ripe ovaries typically lower and less variable than those in unripe ovaries. Considering Se data for eggs|ripe ovaries, there was a consistent relationship with muscle or WB Se over a wide range of fish species. Additionally, for a majority of fish species, the relationships between egg|ripe ovary Se concentrations versus muscle and WB Se concentrations had slopes significantly different than 1, indicating that single-tissue Se ratios are not applicable over a broad range of muscle or WB Se concentrations. Considering these findings, we recommend future studies clearly report whether eggs or ovaries were sampled and provide other diagnostic measures of reproductive status such as the gonado-somatic index. We also conclude direct comparison of Se concentrations from unripe ovaries to egg Se thresholds is not appropriate.

Keywords: selenium, bioaccumulation, ecological risk assessment, regulatory guidelines, fish tissue

Introduction

Several regulatory jurisdictions in North America have adopted fish tissue-based selenium (Se) criteria or guidelines. The United States Environmental Protection Agency (USEPA), for example, recommends Se criteria of 15.1 mg/kg dry weight for fish eggs|ovaries, 11.3 mg/kg dry weight for fish muscle, and 8.5 mg/kg dry weight for whole-body (WB) fish tissue (USEPA, 2016, 2021). Several U.S. states have adopted these or comparable criteria into their water quality standards. Similarly, the Canadian federal environmental quality guidelines for Se are 14.7 mg/kg dry weight for fish eggs|ovaries, and 6.7 mg/kg dry weight for WB fish tissue (Environment and Climate Change Canada [ECCC], 2022).

Selenium toxicity to fish primarily results from maternal transfer of Se to the eggs, which can result in larval mortality, deformities, or edema in developing larvae as the yolk sac is absorbed (Janz et al., 2010). As such, Se concentrations in eggs, or by association ripe ovaries, are considered the most reliable indicator of potential Se toxicity to fish (ECCC, 2022; Janz et al., 2010; USEPA, 2016). However, sampling of fish eggs or ripe ovaries can be logistically challenging, because it can be difficult to locate and catch females in spawning condition or it may not be possible to obtain sufficient sample mass for reliable Se analyses. Further, unless eggs can be readily expressed, sampling of eggs or ripe ovaries is not always an option when there is a desire for nonlethal sampling or to coordinate sample collection with fish consumption monitoring when assessing human health risks. For these reasons, regulatory jurisdictions in the United States and Canada also developed Se criteria and guidelines based on muscle and/or WB Se tissue.

There is greater uncertainty when the muscle or WB Se criteria are implemented over the egg|ovary Se criterion, because these tissues provide an indirect measure of exposure relative to the most sensitive endpoints based on maternal transfer to eggs. This is why the USEPA's Se criterion for eggs|ovaries supersedes its recommended muscle and WB Se criteria. Although expressed as an "egg|ovary" Se criterion, it is inferred that the ovary be ripe because the criterion was derived from maternal transfer toxicity

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studies in which Se concentrations were measured in eggs or ripe ovaries. In Appendix K of USEPA's ambient water quality criteria document, it is stated that "ripe or gravid" females need to be collected from a site for comparison to the egg/ovary Se criterion (USEPA, 2016; 2021). Further, the USEPA's technical support document for fish tissue monitoring states that ovaries should be ripe, and that unripe or developing ovaries should not be used for implementing the egg/ovary Se criterion (USEPA, 2024a).

Understanding Se concentration relationships among tissues is important for implementing the USEPA's muscle and WB criteria versus the egg/ovary criterion and managing the associated uncertainty. If we assume that the egg/ovary, muscle, and WB Se criteria are applicable to all fish species, it will not matter which tissue is analyzed for determining compliance with the Se criteria. For this hypothetical assumption, the egg-to-muscle Se concentration ratio would be 1.3 (egg|ovary Se criterion divided by the muscle Se criterion), and the egg-to-WB Se concentration ratio would be 1.8 (egg|ovary Se criterion divided by the WB Se criterion). However, these ratios do not apply to all fish species. If a fish species has an average egg-to-muscle Se concentration ratio >1.3, that species could be in compliance with the muscle Se criterion but not the egg/ovary Se criterion. Conversely, if a fish species has an average egg-to-muscle Se concentration ratio <1.3, that fish species could be out of compliance with the muscle Se criterion but in compliance with the more relevant egg/ovary Se criterion.

Understanding Se concentration relationships among tissues is also important for developing site-specific Se bioaccumulation models to support site-specific Se water quality criteria or site-specific assessments. These site-specific Se bioaccumulation models are often based on WB Se concentration data, either because WB Se data are more readily available for the water body of interest or because the model was developed from literature-based trophic transfer factors that are based on WB Se concentrations (Mendes et al., 2025; Presser & Naftz, 2020; USEPA, 2024a, 2024b). Because these models are typically based on WB Se concentrations, or relationships between WB Se and muscle Se, that are then applied to an egg/ovary criterion, one or more tissue conversion factors are usually required.

The objective of this study is to develop between-tissue Se concentration relationships that can support regulatory and site-specific Se assessment needs. In part, this study represents an update to tissue Se relationships previously compiled by the USEPA, which were used to support development of updated Se criteria (USEPA, 2016). In addition to considering additional datasets that may not have been available to the USEPA at that time, this study is a companion to Brix et al. (2025), which demonstrates that oocyte development stage has an important influence on the magnitude of ovary Se concentrations, using four fish species as an example: northern pikeminnow (*Ptychocheilus oregonensis*), mountain whitefish (*Prosopium williamsoni*), peamouth chub (*Mylocheilus caurinus*), and redside shiner (*Richardsonius balteatus*). Based on the results of that study, the present study provides a meta-analysis to evaluate whether similar observations on Se concentrations relative to oocyte development apply to other fish species, and then to determine the implications of the relationship between oocyte development and Se concentration in developing Se relationships in eggs and ovaries versus muscle and WB tissue. This study also evaluates relationships between Se concentrations in muscle and WB tissue.

The first step of the study was to compile a database of Se concentrations for eggs, ovaries, muscle, and WB tissue for a suite of fish species representing several families and orders. This database was then used to test the following hypotheses: (1) Ovary Se concentrations are inversely related to oocyte development as characterized by gonado-somatic index (GSI) or qualitative descriptions of spawning status; (2) Variability in Se concentration relationships between ovaries and muscle or WB tissue among fish species is reduced when only data for eggs or ovaries of spawning females are considered; and (3) Tissue Se concentration relationships observed across fish species are influenced by phylogenetic relationships.

Methods

Terminology

In compiling data for this study, it became apparent that the terms "eggs" and "ovaries" were sometimes used interchangeably by study authors or in monitoring reports and databases. In the present study, we use the term "eggs" when eggs were clearly sampled and "ovaries" for all other samples of female reproductive tissue. If a study noted that the fish sampled was of spawning status or that the ovary was otherwise ripe, we refer to the sample as "ripe ovaries." If the ovary sample was noted as unripe or if no information on oocyte development was provided, then we refer to the sample simply as "ovaries."

Fish tissue Se database

Fish tissue Se data were obtained by directly contacting potential data holders (e.g., mine operators with Se monitoring programs), querying publicly available online databases, and through searches of peer-reviewed and grey literature. A standard data template was developed that included fields for fish species, tissue sampled, and the water body from which the fish was collected, as well as any available measurements from the sample such as the length and mass of the sampled fish, and the GSI, which can be used to infer oocyte ripeness (Brix et al., 2025; McGarvey et al., 2021). Lastly, when available, qualitative descriptions of spawning condition of the fish were tabulated. If Se tissue concentrations were reported on a wet weight basis (less than 0.01% of the samples), they were converted to dry weight concentrations based on an assumed moisture content of 61% for rainbow trout and brook trout eggs, and 80% and 76% for centrarchid muscle and ovaries, respectively (USEPA, 2016). Data were excluded from the study if there were fewer than two tissue types reported per sample out of egg, ovary, muscle, and WB, or if there were fewer than five samples for a single species. No effort was made to independently review the analytical data quality from these studies; all data were assumed to be of adequate quality.

Hypothesis 1: Ovary Se concentrations are inversely related to oocyte development

In the companion to this study, Brix et al. (2025) observed that in northern pikeminnow and mountain whitefish, two species with synchronous oocyte development, an inverse relationship between ovary Se concentrations and GSI was observed (R^2 = 0.72–0.82). They also observed in redside shiner, a species with asynchronous oocyte development, a relationship between Se concentrations in expressible eggs and the remaining ovary, with egg concentrations on average 59% of those observed in the ovary. In a fourth species, peamouth chub, where it is unclear whether oocyte development is synchronous or asynchronous, a weak relationship (R^2 = 0.08) between ovary Se and GSI was observed. To evaluate whether egg|ovary Se concentrations decrease with increasing egg|ovary ripeness for other fish species, similar analyses based either on GSI data or qualitative measures of egg|ovary ripeness were conducted. Because quantitative GSI data or qualitative information on

egg|ovary ripeness were not always reported, this evaluation was completed on a subset of species.

For species with GSI data, trends and relationships between tissue Se concentration ratios (e.g., egg|ovary-to-muscle) and GSI were tested using correlation and ordinary least squares (OLS) regression. Because fish samples were potentially collected from a range of Se exposure conditions, the egg|ovary Se concentrations were normalized to corresponding muscle Se concentrations based on the assumption that muscle Se concentrations more directly reflect Se exposure and are less influenced by the spawning status of the fish. Although muscle Se concentrations may theoretically be reduced as Se is mobilized during vitellogenesis, changes in muscle Se concentrations are less responsive to changes in spawning status than ovary Se concentrations (see additional discussion in online supplementary material S1). Correlation and OLS regression were performed for all species that had paired tissue-concentration ratios and GSI measurements for at least 10 samples, and a minimum GSI range of at least fivefold (11 species). These minimum sample size and GSI ranges were selected so that any conclusions regarding the presence or absence of statistically significant relationships were based on adequate data. These minimum sample size and GSI ranges were applied to support this meta-analysis of a suite of fish species; alternative minimum ranges may be appropriate for specific species in support of focused assessments.

For fish species with qualitative data on egg|ovary ripeness, we evaluated tissue Se concentrations for ripe females compared with other pre-spawning categories, which were grouped into a single "unripe" category. Eight species had datasets with Se concentrations for ripe/gravid females and at least one other spawning status. For each of these eight species, egg|ovary Se concentrations in ripe versus unripe females were compared using two methods: nonparametric analysis of covariance (ANCOVA) and nonparametric multiple contrasts. The nonparametric ANCOVA compared egg|ovary Se in the two groups using muscle Se concentrations as a covariate that represented Se exposure. This method was conducted in the R software environment (R Core Team, 2024) using the "fANCOVA" package (Wang, 2020) with significance evaluated using the ANOVA-type statistic ($\alpha = 0.05$; Dette & Neumeyer, 2001). This method is the more robust of the two methods; however, the number of species analyzed using nonparametric ANCOVA was reduced from eight to four as the method required that species-specific datasets have similar range and overlap of muscle Se concentrations in both the ripe and unripe categories. Nonparametric multiple contrasts were performed as a secondary analysis to corroborate the ANCOVA and test the remaining four species. Therefore, for all eight species, egg|ovary-to-muscle Se concentration ratios were compared in the ripe versus unripe spawning statuses within each species using nonparametric multiple contrast tests with simultaneous confidence intervals and the log odds asymptotic approximation method (Noguchi et al., 2020; familywise error rate = 0.05).

Hypothesis 2: Variability in Se concentration relationships between ovaries and muscle or WB tissue is reduced when only data for eggs or ovaries of spawning females are considered

Linear regression was used to compare relationships between egg|ovary Se and muscle or WB Se. All available species-specific datasets were first reduced to only samples qualitatively described as "ripe females." Individual species were first evaluated using both OLS and total least squares (TLS) regression to assess the presence of a linear relationship between egg|ripe ovary Se and muscle or WB Se. Due to concerns that a narrow concentration range in the predictive tissue (i.e., muscle or WB) for a species could interfere with the ability to determine the presence or nature of the relationship with egg|ripe ovary Se, species with a concentration range <10 mg/kg dry weight in the predictive tissue were omitted if the predictive R^2 was <0.35. Species with a significant slope and a linear relationship (qualitatively assessed using residual diagnostic plots) were included in the following multiple linear regression (MLR) models:

$$\log(\text{Egg|Ripe Ovary Se}) = \log(\text{Muscle Se}) + \text{Species} + \log(\text{Muscle Se}) * \text{Species} \quad (1)$$

$$\log(\text{Egg|Ripe Ovary Se}) = \log(\text{WB Se}) + \text{Species} + \log(\text{WB Se}) * \text{Species} \quad (2)$$

Hypothesis 3: Tissue Se concentration relationships observed across fish species are influenced by phylogenetic relationships

This hypothesis was evaluated using tissue concentration regressions tested in Hypothesis 2, and also using unadjusted tissue concentration ratios from samples in each of the three tissue pairings (i.e., egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, and WB-to-muscle). Linear models and tissue ratios were then qualitatively assessed to determine whether similar patterns were observed for fish species within taxonomic levels (genus, family, order).

Based on the conclusion from Hypothesis 1, only relationships between muscle and WB versus eggs or ripe ovaries were included in this evaluation. To evaluate relationships between WB and muscle Se, however, the dataset was not limited to data for ripe females. It has previously been hypothesized that muscle Se concentrations in females may be higher prior to vitellogenesis and lower during vitellogenesis and post-spawning as Se is mobilized to the ovaries, but evaluation of the current dataset does not support this hypothesis (see online supplementary material S1). For WB tissue in females, Se concentrations may be lower post-spawning as Se is depurated from the fish along with the eggs (although the corresponding loss in egg mass may partially mitigate the change in Se on a concentration basis). In the present study, due to paired muscle and WB Se data for ripe females only being identified for three fish species, and because the need to translate between muscle and WB Se concentrations may not be limited to ripe females, we used all paired muscle and WB Se data regardless of spawning status. In addition, this allowed use of muscle and WB Se concentration data for male fish.

Results

Fish tissue Se database

Paired egg|ovary and muscle Se concentrations were compiled for 40 species ($n = 2{,}533$), paired egg|ovary and WB Se concentrations were compiled for 30 species ($n = 428$), and paired muscle and WB Se concentrations were compiled for 24 species ($n = 497$; Table 1). For the paired egg|ovary and muscle data, GSI data were available for 19 species ($n = 1{,}143$) and data for ripe females were available for 21 species ($n = 499$; Table 1). For paired egg|ovary and WB data, GSI data were available for 16 species ($n = 112$) and data for ripe females were available for 7 species ($n = 172$; Table 1). All raw data are provided in online supplementary material S2.

Hypothesis 1: Ovary Se Concentrations are Inversely Related to Oocyte Development

Relationships between egg|ovary Se and GSI

A total of 19 fish species with paired egg|ovary and muscle Se concentrations had GSI data for at least a subset of the dataset.

Table 1. Numbers of samples and fish species represented for each tissue pairing.

			GSI data		Qualitative "ripe" data	
Tissue pair	**Total no. of samples**	**Total no. of species**	**No. of species**	**No. of samples**	**No. of species**	**No. of samples**
Eggs|ovaries and muscle	2,533	40	19	1143	21	499
Eggs|ovaries and WB	428	30	16	112	7	172
Muscle and WB	497	24	–	–	–	–

Note. *GSI*, gonadosomatic index; *WB*, whole body.

Based on our established minimum data requirements, relationships between egg|ovary-to-muscle Se ratios and GSI could be evaluated for 10 species, of which six had significant negative correlations and negative regression slopes with GSI once data were log-transformed (Figure 1). For WB tissue, a total of 17 fish species had paired egg|ovary Se concentrations and GSI data for at least a subset of the dataset, but minimum data requirements were met for only four species. All had negative correlations with GSI once data were log-transformed, but none were significant, and neither were the regression slopes (Table 2).

Qualitative evaluation of the residuals plots from the tested regressions suggested potential relationships between model residuals and GSI, so a follow-up analysis tested the relationship between the absolute value of each model's residuals against GSI as the predictor. Model residuals exhibited significant negative correlations with GSI for two species in the egg|ovary-to-muscle Se models (northern pikeminnow [$p < 0.001$] and Sacramento splittail [$p < 0.001$]), while a negative trend was observed for mountain whitefish ($p = 0.076$). Only Sacramento splittail exhibited a significant negative correlation in the egg|ovary-to-WB Se model residuals ($p = 0.01$; Table 2). The presence of model residuals that are not independent from the predictor variable violates a regression assumption necessary for testing the significance of model coefficients (i.e., slope and intercept) and fitting prediction intervals. Although this occurrence did limit the interpretability of the regressions that were modeled for the GSI analysis, it showed that for several species, as GSI increases, the variability of Se tissue concentration ratios decreases. Thus, as the spawning status approaches ripe, the relationship between egg|ovary Se and muscle or WB Se becomes more predictable.

Overall, the evaluation confirms that spawning status, as inferred by GSI, has an important influence on both the magnitude and variability in egg|ovary-to-muscle and egg|ovary-to-WB relationships for most of the species evaluated.

Egg|ovary Se concentrations as a function of spawning status

In the analysis based on qualitative descriptions of spawning status, 5 of the 8 tested species showed significant differences between egg|ovary-to-muscle Se ratios in ripe versus unripe samples (Table 3). For the four species that could be tested using both the ANCOVA and the multiple contrasts, there was agreement on the conclusion of the tests between the two methods for three species (rainbow trout, brook trout, and mountain whitefish) and disagreement for westslope cutthroat trout (nonparametric ANCOVA $p = 0.01$; nonparametric multiple contrast $p = 1$).

The ANCOVA is a more robust analysis to test this hypothesis and is capable of detecting more nuanced differences between categorical groups than multiple contrasts. A graphical representation of the ANCOVA performed on rainbow trout, a species with agreement between ANCOVA and multiple contrasts, and westslope cutthroat trout, a species with disagreement between the two methods, is provided in Figure 2. For westslope cutthroat trout, nonparametric regressions of the ripe and unripe groups diverge from one another while still exhibiting large overlap (Figure 2B). The divergence was not detected when contrasting ripe versus unripe tissue ratios (Figure 3). However, the corroboration of results for the other three species supports accepting the multiple contrast results for the four remaining species that could not be tested using ANCOVA. As with fish species that had decreased variability in residuals with increasing GSI, there was less variability in egg|ovary-to-muscle Se ratios in samples from ripe females than unripe females (Fligner-Killeen test, $\alpha < 0.05$; Figure 3).

Results of this meta-analysis are consistent with the findings from the focused study reported in the companion study (Brix et al., 2025), which is that spawning status has an important influence on the magnitude and variability in ovary Se concentrations. As such, evaluating relationships between ovary Se concentrations and muscle or WB Se concentrations must be based on Se concentration in ripe ovaries to be meaningful for comparison to egg-based criteria or guidelines.

Hypothesis 2: Variability in Se Concentration Relationships Between Ovaries and Muscle or WB Tissue Is Reduced When Only Data for Eggs or Ovaries of Spawning Females are Considered

Of the 19 species with paired egg|ripe ovary and muscle Se data, datasets for 12 species passed the regression acceptability requirements (i.e., minimum five samples per tissue pair; presence of a linear relationship based on OLS; and predictive $R^2 \geq 0.35$ when the muscle Se range was <10 mg/kg dry wt) and were included in the final pooled MLR model (Table 4; Figure 4a). Of the seven species excluded from the model, five were omitted for not exhibiting a relationship (i.e., slope) significantly different from 0 (largemouth bass, white sucker, walleye, bull trout, and mountain whitefish; see online supplementary material S3A). It is unclear whether the species omitted for lack of a significant slope had no detectable relationship due to limitations of the available dataset or because of a true absence of relationship between tissue concentrations.

Of the 12 species included in the pooled MLR, bluegill had a broad range of muscle Se concentrations and a median slope relative to the other species and was therefore selected as the baseline model species against which the other species were compared in the MLR. The bluegill regression model is:

$$\log(\text{Egg|Ripe Ovary Se}) = 0.86 \times \log(\text{Muscle Se}) + 0.29 \quad (3)$$

Of the remaining 11 species included in the MLR analysis, many did not have significantly different slopes and intercepts relative to bluegill (Table 4). The predictive R^2 for the pooled MLR model with all species retained is 0.95 (Figure 4A), and the predictive R^2 for the OLS models for individual species ranged from 0.61 to 0.94 (median predictive $R^2 = 0.84$; Table 4).

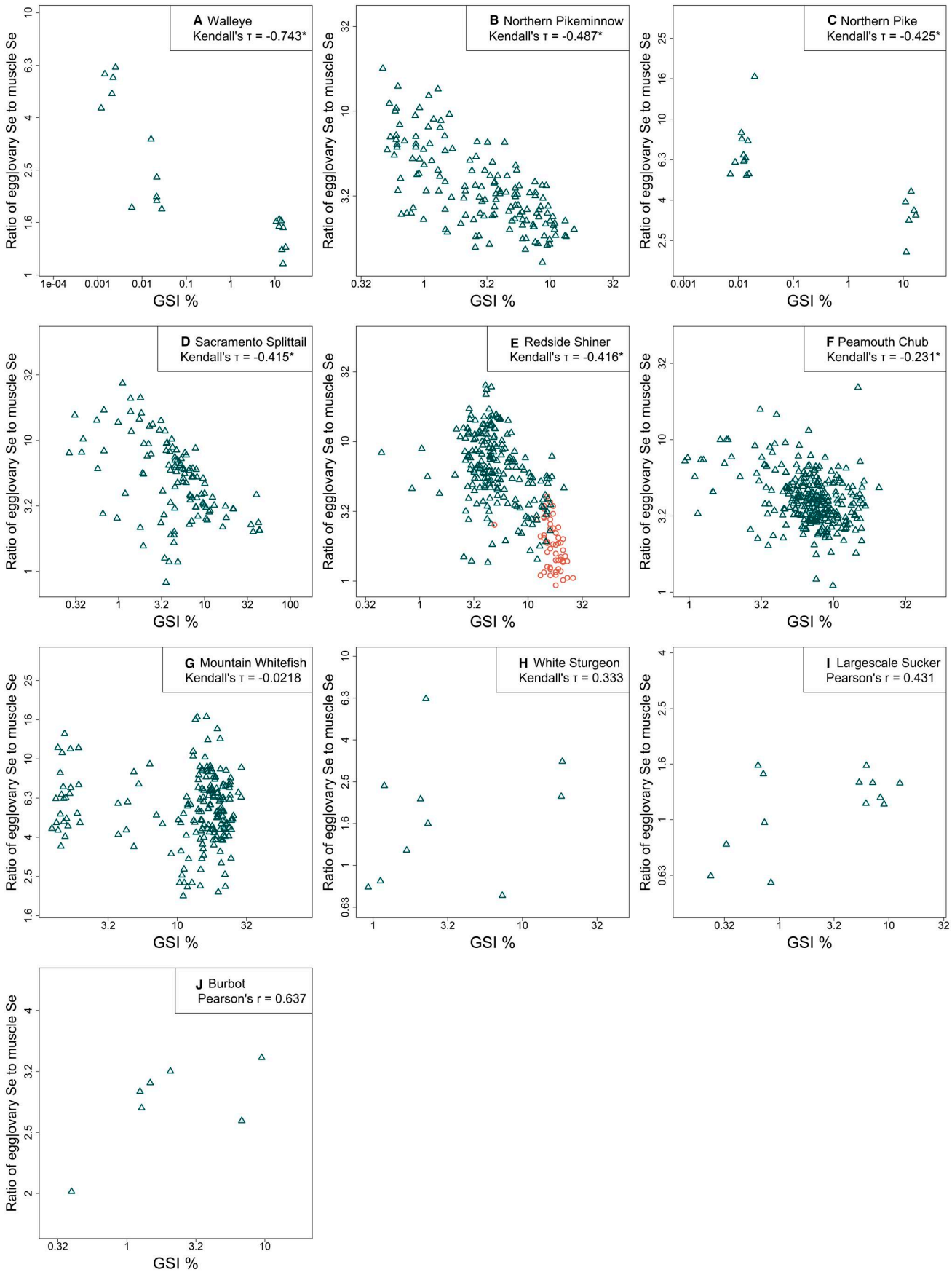

Figure 1. Relationships between egg|ovary-to-muscle selenium (Se) ratios and the gonadosomatic index (GSI) for 10 fish species. Correlation statistics were calculated using log10-transformed values and are represented as either Kendall's τ or Pearson's r and are marked with an asterisk (*) when significant ($\alpha = 0.05$). Orange circles used egg Se concentrations from samples from de Bruyn et al. (2023), which also provided paired concentrations of ovary Se.

Table 2. Summary of correlation and regression statistics between log-transformed selenium (Se) tissue ratios and log-transformed gonadosomatic index (GSI).

Se tissue ratio compared to GSI	Species	Interpretation	Correlation statistic[a]	Correlation *p*-value	Regression slope coefficient	Regression *p*-value	Regression predictive R^2	Regression residuals normality *p*-value[b]	|Residuals| correlation statistic	|Residuals| correlation *p*-value
Log10 ratio of egg|ovary to muscle	Walleye	Significant negative correlation. Nonlinear relationship in primary regression therefore follow-up residuals regression is not interpretable.	−0.743	**9.02E-07**	—[c]	—	—	—	—	—
	Northern Pikeminnow	Significant negative correlation. Significant correlation between absolute value of residuals and predictor show decreasing variability with increasing GSI.	−0.487	**1.35E-18**	−0.39	—	0.457	0.307	−0.195	**4.28E-04**
	Northern Pike	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.425	**0.0137**	−0.102	—	0.489	**0.0267**	−0.137	0.454
	Sacramento Splittail	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.415	**3.38E-11**	−0.348	—	0.245	**7.56E-04**	−0.39	**4.44E-10**
	Peamouth Chub	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.231	**7.33E-09**	−0.272	—	0.125	**6.15E-05**	−0.0226	0.572
	Redside Shiner	Significant negative correlation. Regression residuals fail normality; cannot test for coefficient significance.	−0.414	**3.07E-24**	−0.656	—	0.351	**0.0147**	−0.0402	0.323
	Mountain Whitefish	No correlation detected. Passes regression assumptions; slope coefficient is nonsignificant.	−0.0218	0.655	−0.0422	0.219	−0.0111	0.317	−0.0869	0.076
	White Sturgeon	Passes regression assumptions; slope coefficient is nonsignificant.	0.333	0.216	0.162	0.462	−0.316	0.868	−0.244	0.381
	Burbot	No significant correlation. Passes regression assumptions; slope coefficient is nonsignificant.	0.637	0.124	0.0983	0.287	−0.553	0.0553	−0.247	0.593
	Largescale Sucker	Passes regression assumptions; slope coefficient is nonsignificant.	0.431	0.124	0.127	0.124	−0.0453	0.239	−0.231	0.279
Log10 ratio of egg|ovary to WB	Creek Chub	Passes regression assumptions; slope coefficient is nonsignificant.	−0.387	0.092	−0.211	0.092	−0.0142	0.5	−0.0301	0.9
	Central Stoneroller	Passes regression assumptions; slope coefficient is nonsignificant.	−0.364	0.116	−0.338	0.0909	−0.569	0.086	−0.333	0.153
	Bluegill	Passes regression assumptions; slope coefficient is nonsignificant.	−0.286	0.399	−0.314	0.239	−0.26	0.342	−0.357	0.275
	Sacramento Splittail	No correlation detected. Significant correlation between absolute value of residuals and predictor show decreasing variability with increasing GSI.	−0.212	0.261	−0.0553	0.261	−0.0999	0.61	−0.315	**0.0143**

Note. Tests performed on the absolute value of the residuals from the original regression, "|Residuals|," test whether the relationship between the ratio and GSI changes with GSI. The Interpretation information summarizes the findings for each row. Bolded values in the correlation *p*-value columns indicate significant relationships; bolded values in the residuals normality column indicate that assumption failed.

[a] If both the log-transformed ratio and log-transformed GSI for a given species fit a normal distribution (Shapiro-Wilk test, $\alpha = 0.05$), a Pearson correlation was used and the corresponding correlation statistic is reported as Pearson's *r*. If either variable had a non-normal distribution, Kendall's correlation was used instead and the corresponding correlation statistic reported is Kendall's tau.

[b] Regression residuals tested for normal distribution using a Shapiro-Wilk test. Failure of this test ($\alpha < 0.05$) means model coefficients (i.e., slope and intercept) cannot be tested for significance (indicated with "—" in the Regression *p*-value column).

[c] If assumptions were not met to appropriately calculate a statistic, "—" indicates none was calculated.

Table 3. *p*-values resulting from comparing egg|ovary-to-muscle selenium (Se) concentrations in ripe versus unripe samples within each listed species.

Species	Scientific name	Nonparametric ANCOVA	Nonparametric multiple comparisons
Rainbow Trout	*Oncorhynchus mykiss*	**$p = 0.005$**	**$p = 5E\text{-}05$**
Brook Trout	*Salvelinus fontinalis*	**$p = 0.005$**	**$p = 0.003$**
Westslope Cutthroat Trout[a]	*Oncorhynchus clarkii lewisi*	**$p = 0.02$**	$p = 1$
Mountain Whitefish	*Prosopium williamsoni*	$p = 0.3$	$p = 1$
Bull Trout	*Salvelinus confluentus*	na	$p = 0.2$
Northern Pike	*Esox lucius*	na	**$p = 2E\text{-}05$**
Walleye	*Stizostedion vitreum*	na	**$p = 0.001$**
White Sturgeon	*Acipenser transmontanus*	na	$p = 1$

Note. The nonparametric multiple contrasts compared muscle Se-normalized versus egg|ovary Se concentrations. Bolded *p*-values are significantly different ($a = 0.05$). "na" indicates the test could not be performed for that species (data unsuitable).
[a] The nonparametric nonparametric analysis of covariance (ANCOVA) is a stronger test for this question and is more capable of detecting the subtler differences between ripe and unripe samples of this species (Figure 2B).

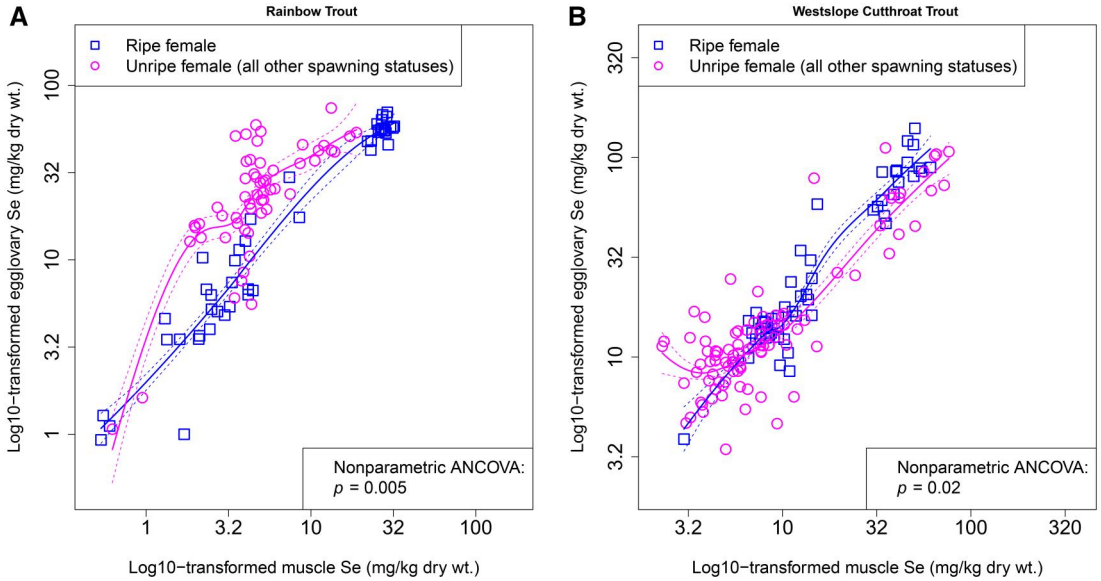

Figure 2. Egg|ovary selenium (Se) versus muscle Se concentrations fitted with weighted smoothing function for samples from ripe and unripe females for (A) rainbow trout and (B) westslope cutthroat trout.

For the pooled MLR model for egg|ripe ovary versus WB Se, all species met the criteria for inclusion in the MLR (Table 4; Figure 4B). As with the muscle Se model, the MLR predicting egg|ripe ovary Se from WB Se was evaluated relative to bluegill. The bluegill regression is:

$$\log(\text{Egg|Ripe Ovary Se}) = 0.91 \times \log(\text{WB Se}) + 0.35 \quad (4)$$

Only brown trout exhibited a significantly different slope from the bluegill model, and brown trout, desert pupfish, and Dolly Varden each had significantly different y-intercepts (Table 4). The predictive R^2 was 0.88 (Figure 4B), and the predictive R^2 for individual species OLS regressions ranged from 0.70 to 1.0 (Table 4), indicating, as for muscle Se, predictable Se tissue concentration relationships when females are in ripe spawning status.

A primary outcome of the MLR modeling analysis is demonstration that much of the variability in egg|ovary-to-muscle Se and egg|ovary-to-WB Se relationships among species is reduced when data for eggs and ripe ovaries are considered. There are, as noted, some species for which intercepts or slopes are significantly different, and some species that did not meet minimum requirements for being included in the MLR analyses, so there is not a "universal model" that can be used to predict egg|ripe ovary Se concentrations from muscle or WB Se concentrations. The primary models, however, provide a useful starting point for evaluating fish species lacking tissue Se relationship data. For species included in the pooled MLR models for predicting egg|ripe ovary Se concentrations from muscle or WB, we recommend using all species-specific model adjustments, whether or not they were significantly different from the pooled model (Table 4). It is not recommended to extrapolate relationships beyond the range of data on which the model is based.

As noted in the *Methods*, TLS regression was also tested and compared to OLS regression (results are reported in online supplementary material S3). Once the dataset was reduced to only samples with eggs|ripe ovaries, the differences between OLS and TLS models were minimal.

Hypothesis 3: Tissue Se Concentration Relationships Observed Across Fish Species are Influenced by Phylogenetic Relationships

Of the 12 species represented in the egg|ripe ovary-to-muscle MLR, five are from the order Salmoniformes, two each are from Hiodontiformes and Perciformes, and one each is from

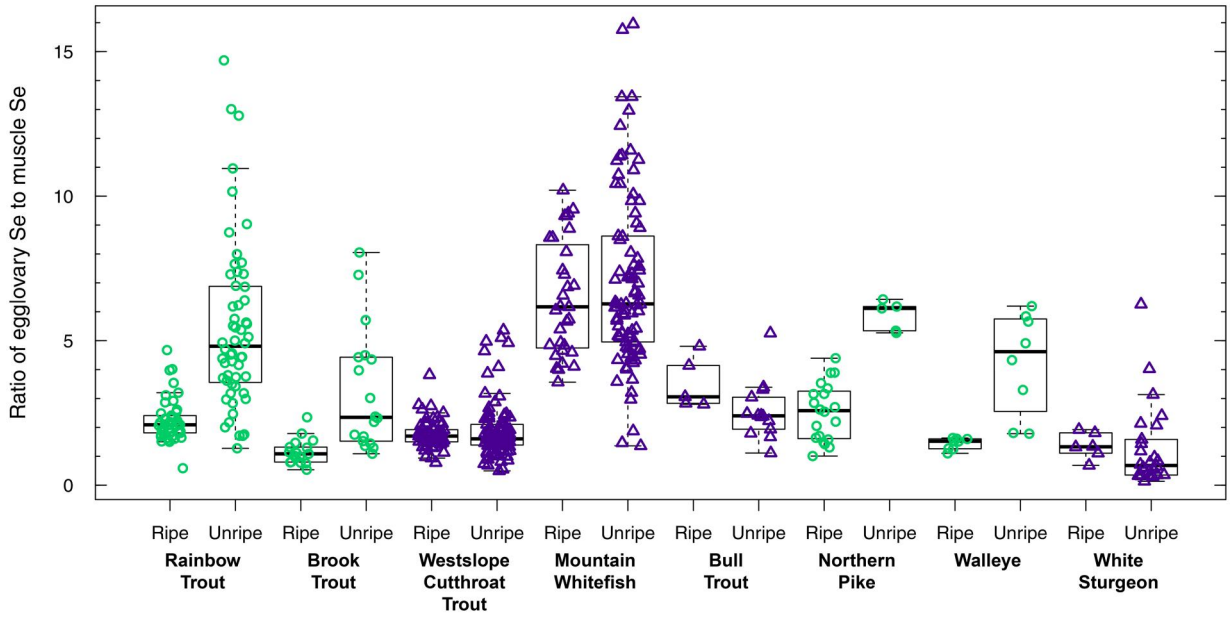

Figure 3. Comparison of egg|ovary-to-muscle selenium (Se) ratios for samples from ripe and unripe females within each species. Boxes denote interquartile range (IQR; 25th–75th percentiles) with horizontal line denoting the median. Lower whisker denotes smallest value greater than 25th percentile − 1.5 × IQR, and upper whisker denotes largest value less than 75th quantile + 1.5 × IQR. Species plotted with green circles exhibited a significant difference between the ripe and unripe samples using nonparametric multiple contrasts.

Acipenseriformes, Cypriniformes, and Esociformes. The slopes of the linear models do not indicate that any groupings or patterns are due to taxonomic relatedness; they are distributed throughout the range of slopes (Table 4). This also applies to mountain whitefish, which is a salmonid that was removed from the MLR model because its tissue concentration relationships did not exhibit a slope different from 0 (see online supplementary material S3A). The family Hiodontidae had two modeled species that also shared the same genus, but, as with taxonomic order, the models for these species did not exhibit similarities.

Because all species in the Salmoniformes order are also in the Salmonidae family, the genera were evaluated for the salmonids instead. In this instance, it was noted that, when multiple species were present within genera (*Oncorhynchus* and *Salvelinus*), they grouped together in the overall ranking of slopes. This suggests a possible phylogenetic effect in the tissue concentration relationships. The two *Salvelinus* species in particular (brook trout and Dolly Varden) have effectively the same slope albeit different intercepts, while the slopes of the two *Oncorhynchus* species (westslope cutthroat trout and rainbow trout) are less similar, differing by 0.1 on a log scale. Overall, though, within-genus comparisons were limited, and data for additional taxa are needed before a phylogenetic pattern is concluded.

The species that passed minimum data requirements for the egg|ripe ovary-to-WB MLR were fewer in number and dominated by the order Salmoniformes, making patterns or groupings difficult to evaluate (Table 4). Unlike the muscle dataset, there were no duplicate families or genera represented across the seven available species. Rather, the overall similarity of slopes exhibited across the available species indicates a possible consistency of Se partitioning.

For relationships between WB and muscle Se, an interesting pattern in taxonomic similarities emerges. The same regression requirements were applied to samples with paired WB and muscle Se concentrations; however, because there is little effect of spawning status on Se concentrations in either tissue, all available samples were included regardless of spawning status (see *Methods*). In addition, the datasets with paired muscle and WB Se concentrations were not necessarily linked only to females, so the issue of spawning status does not apply to the entire dataset. Indeed, all species that had the minimum sample size and range of muscle tissue concentrations required to test in regressions showed linear relationships with log-transformed tissue concentrations with a similar distribution of predictive R^2 as the egg|ripe ovary-to-muscle- and egg|ripe ovary-to-WB regressions (Table 4; Figure 4C).

The regression models for 10 of the 17 fish species did not have y-intercepts that were significantly different from 0 and all were from either the orders Cypriniformes or Perciformes. By contrast, 5 of the 7 remaining species with intercepts significantly greater than 0 were salmonids (0.128–0.917), exhibiting a distinct pattern in model coefficients not shown in either of the two egg|ripe ovary models. This indicates salmonids are generally characterized by greater WB Se concentrations compared to muscle, particularly at lower muscle Se concentrations. This is the opposite of that observed for the orders Cypriniformes and Perciformes, which had intercepts of 0 and slopes between 0.75 and 1, indicating their WB tissue Se concentration is consistently lower than muscle (Sacramento splittail was the only observed exception, showing again that these patterns are not universal). The slopes for the salmonids are diverse with two in the range of 0.75–1 exhibited by the other orders, two significantly lower (0.5–0.65), and one significantly higher (i.e., 1.19). The intercepts of the WB-to-muscle Se relationships for salmonids are consistently greater than for the two non-salmonid orders, but there is still high variability in the magnitudes of both intercepts and slopes among salmonid species.

Some of the same patterns seen in the regression models for each of the three tissue pairing types can also be seen when comparing tissue concentration ratios (Table 5; Figure 5). The two salmonids in the genus *Salvelinus* that had similar egg|ripe ovary-to-muscle slopes also share similar medians, but otherwise orders, families, and genera are distributed without apparent pattern throughout the ratio medians of the available species for both egg|ripe ovary-to-muscle or egg|ripe ovary-to-WB Se ratios. For the WB-to-muscle Se ratios, the taxonomic patterns can also be observed. Salmonids are

Table 4. Ordinary least squares log-log regression models for egg|ripe ovary selenium (Se) versus muscle Se and whole body (WB) Se, and WB Se versus muscle Se.

Species	Order	Family	Genus	Slope	Intercept	Adj. R^2	Pred. R^2	Muscle or WB Se range (mg/kg dry wt)[a]	Sample size
Egg|ripe ovary Se vs. muscle Se									
Redside Shiner	Cypriniformes	Cyprinidae	Richardsonius	1.27*	0.252	0.869	0.861	0.6–4.6	40
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.22*	0.579*	0.831	0.791	1.07–4.78	19
Mooneye	Hiodontiformes	Hiodontidae	Hiodon	1.12*	0.0902	0.93	0.91	4.48–12.5	12
Westslope Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	1.09*	0.126*	0.905	0.901	3–61	54
Rainbow Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.995*	0.331	0.939	0.936	0.532–32	46
Goldeye	Hiodontiformes	Hiodontidae	Hiodon	0.909	0.188	0.866	0.82	1.88–3.46	8
Bluegill	Perciformes	Centrarchidae	Lepomis	0.862	0.286	0.863	0.853	1.47–55.2	36
Brook Trout	Salmoniformes	Salmonidae	Salvelinus	0.808	0.211	0.866	0.834	1.4–34.7	17
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	0.802	0.411	0.91	0.885	2.2–117	16
White Sturgeon	Acipenseriformes	Acipenseridae	Acipenser	0.789	0.238	0.906	0.822	1.22–15.3	6
Northern Pike	Esociformes	Esocidae	Esox	0.769	0.474*	0.853	0.834	0.9–47.8	20
Yellow Perch	Perciformes	Percidae	Perca	0.517*	0.24	0.634	0.612	0.542–2.24	44
Egg|ripe ovary Se vs. WB Se									
Brown Trout	Salmoniformes	Salmonidae	Salmo	1.27*	−0.0959*	0.832	0.807	4.7–20	23
Slimy Sculpin	Scorpaeniformes	Cottidae	Cottus	1.07	0.229	0.9	0.87	1.55–10.8	20
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	1.05	−0.0186*	0.96	0.953	2.2–108	10
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.02	0.345	0.913	0.9	1.78–11	19
Yellowstone Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.966	0.16	0.725	0.704	1.2–25.7	21
Desert Pupfish	Cyprinodontiformes	Cyprinodontidae	Cyprinodon	0.916	0.126*	0.999	0.999	0.73–26.9	6
Bluegill	Perciformes	Centrarchidae	Lepomis	0.911	0.349	0.844	0.827	0.92–26.8	33
WB Se vs. muscle Se									
Arctic Grayling	Salmoniformes	Salmonidae	Thymallus	1.19*	0.231*	0.905	0.889	1.07–4.78	19
Sacramento Splittail	Cypriniformes	Cyprinidae	Pogonichthys	1.17*	0.0545*	0.65	0.618	1.02–3.15	30
Rainbow Trout	Salmoniformes	Salmonidae	Oncorhynchus	1.02	0.128*	0.817	0.808	0.674–1.97	51
Longnose Sucker	Cypriniformes	Catostomidae	Catostomus	1.01	0.0231	0.819	0.763	0.954–2.39	11
Roundtail Chub	Cypriniformes	Cyprinidae	Gila	1	−0.0599	0.601	0.506	4.34–9.84	10
Common Carp	Cypriniformes	Cyprinidae	Cyprinus	0.977	−0.112	0.823	0.687	5.06–24.2	9
Flannelmouth Sucker	Cypriniformes	Catostomidae	Catostomus	0.955	−0.138	0.561	0.465	3.56–7.28	12
Bluegill	Perciformes	Centrarchidae	Lepomis	0.892	−0.0423	0.928	0.92	1.86–55.2	34
Walleye	Perciformes	Percidae	Stizostedion	0.864	0.0195	0.478	0.451	0.939–2.74	48
Brown Trout	Salmoniformes	Salmonidae	Salmo	0.843	0.159*	0.951	0.933	3.17–25.1	9
Smallmouth Bass	Perciformes	Centrarchidae	Micropterus	0.834	0.00875	0.926	0.917	0.75–11	29
Largescale Sucker	Cypriniformes	Catostomidae	Catostomus	0.832	−0.0477	0.558	0.499	0.55–1.66	32
Bluehead Sucker	Cypriniformes	Catostomidae	Catostomus	0.817	−0.0235	0.942	0.931	1.47–8.57	10
Yellow Perch	Perciformes	Percidae	Perca	0.765	0.0154	0.719	0.639	2.2–3.1	11
Yellowstone Cutthroat Trout	Salmoniformes	Salmonidae	Oncorhynchus	0.653*	0.303*	0.747	0.726	2.44–10.9	43
Burbot	Gadiformes	Lotidae	Lota	0.645*	0.184*	0.645	0.602	0.928–5.92	31
Dolly Varden	Salmoniformes	Salmonidae	Salvelinus	0.515*	0.917*	0.784	0.683	19.5–113	10

Note. Slope and intercept values with "*" indicate that the model coefficient was significantly different from the corresponding coefficient of the bluegill model.
[a] Range of muscle or WB Se concentrations in dataset used to develop model.

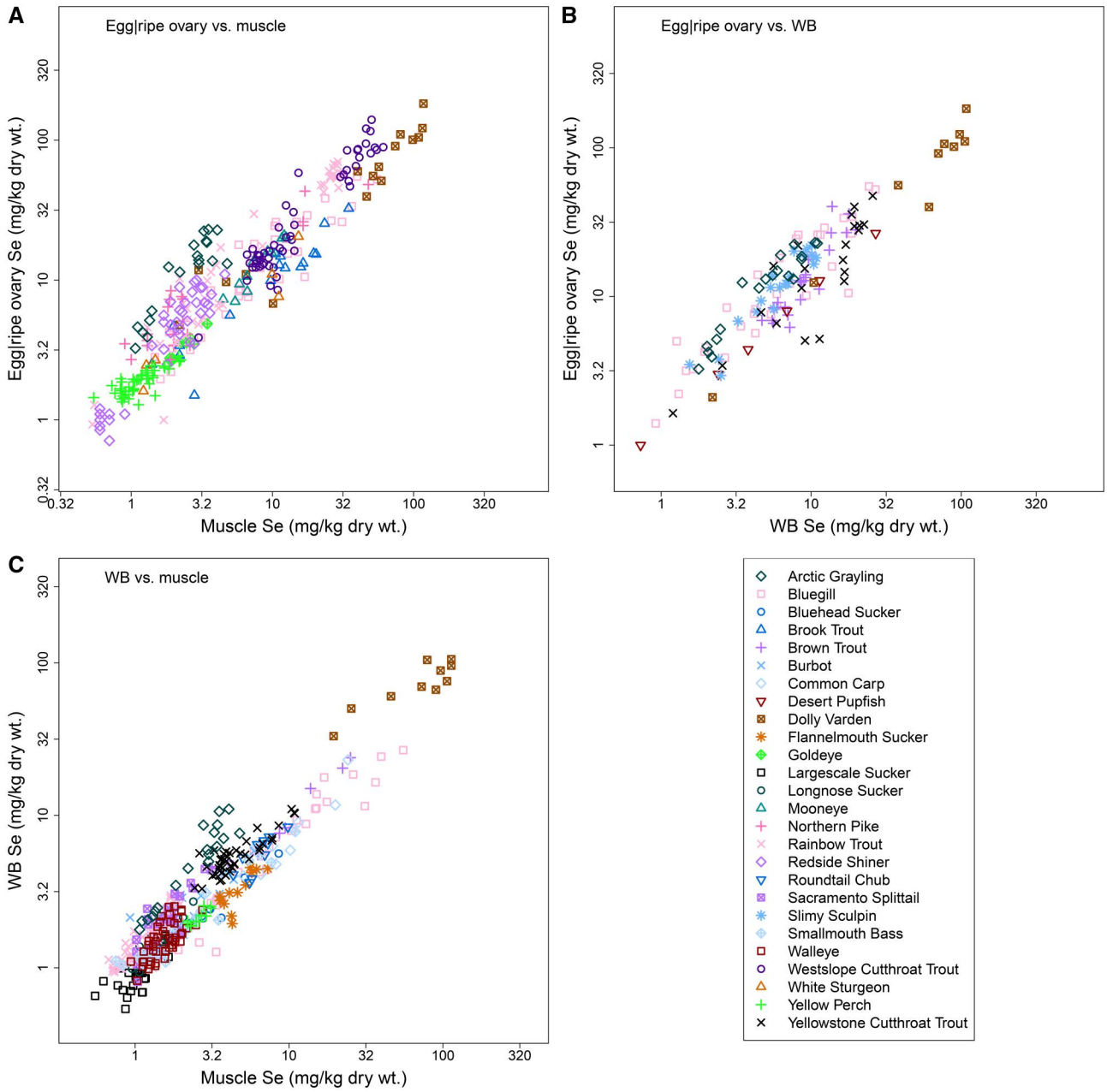

Figure 4. Relationships between egg|ripe ovary selenium (Se) concentrations and (A) muscle Se and (B) whole body (WB) Se, and between (C) WB Se and muscle Se. Multiple linear regression model statistics and coefficients can be found in Table 4.

mostly characterized by median ratios ≥1, and species in the orders Cypriniformes and Perciformes are mostly characterized by median ratios ≤1. The only exceptions are the Sacramento splittail, which was the only Cypriniformes species with either a slope or intercept significantly larger than the other Cypriniformes or Perciformes, and the salmonids Dolly Varden and brown trout, which had median ratios slightly less than 1 rather than greater than 1.

The clearest difference between the regressions and ratios is the high level of precision with which regressions can predict tissue concentrations juxtaposed against the poor predictive capacity of the ratios. The linear regressions that emerge after a log transformation of the tissue concentrations translate to wide ranges of ratios within a species. Several species exhibited as much as a fivefold difference in egg|ripe ovary-to-muscle ratios, whereas their corresponding linear regression had predictive R^2 > 0.85. For prediction of egg Se concentrations, the reported linear regressions are more accurate over a wider range of muscle or WB Se concentrations compared with the use of the tissue concentration ratio.

Discussion

Impact of spawning status on Se tissue concentration relationships

This study presents three analyses that each support the conclusion that spawning status has a significant impact on the relationships between egg|ovary Se and muscle or WB for many fish species. Correlating tissue Se concentration ratios with measured GSI showed that as oocytes develop and the ovary mass increases, the difference between the Se concentration in the eggs|ovaries and the muscle or WB tissue decreases for several species (Figure 1; Table 2). For species where this is observed, the

Table 5. Tissue concentration ratios for three tissue pairs, separated by species.

Ratio type	Sample spawning status	Species	Order	Family/Genus[a]	Median	Mean	Standard deviation	Sample size	Range of ratios
Egg|ripe ovary vs. muscle	Ripe	Mountain Whitefish	Salmoniformes	Prosopium	6.17	6.47	2	31	3.56–10.2
	Ripe	Arctic Grayling	Salmoniformes	Thymallus	4.62	4.76	1.31	19	2.74–6.85
	Ripe	Bull Trout	Salmoniformes	Salvelinus	3.06	3.53	0.901	5	2.8–4.81
	Ripe	Northern Pike	Esociformes	Esocidae	2.58	2.52	0.99	20	1.01–4.39
	Ripe	Razorback Sucker	Cypriniformes	Catostomidae	2.37	2.37	0.997	34	1.06–5.24
	Ripe	Rainbow Trout	Salmoniformes	Oncorhynchus	2.09	2.23	0.738	46	0.588–4.67
	Ripe	Redside Shiner	Cypriniformes	Cyprinidae	2.07	2.21	0.752	40	1.02–4.05
	Ripe	Northern Pikeminnow	Cypriniformes	Cyprinidae	1.86	2	0.482	43	1.39–3.65
	Ripe	Lake Whitefish	Salmoniformes	Coregonus	1.72	1.83	0.415	9	1.06–2.57
	Ripe	Westslope Cutthroat Trout	Salmoniformes	Oncorhynchus	1.7	1.77	0.509	54	0.78–3.82
	Ripe	Yellow Perch	Perciformes	Percidae	1.63	1.65	0.335	44	1.01–2.66
	Ripe	Mooneye	Hiodontiformes	Hiodontidae	1.61	1.6	0.172	12	1.25–1.85
	Ripe	Walleye	Perciformes	Percidae	1.52	1.44	0.201	8	1.1–1.63
	Ripe	Bluegill	Perciformes	Centrarchidae	1.45	1.55	0.559	36	0.623–3.09
	Ripe	Goldeye	Hiodontiformes	Hiodontidae	1.43	1.43	0.103	8	1.25–1.61
	Ripe	White Sturgeon	Acipenseriformes	Acipenseridae	1.33	1.36	0.456	6	0.686–1.92
	Ripe	White Sucker	Cypriniformes	Catostomidae	1.23	1.28	0.228	6	0.967–1.63
	Ripe	Dolly Varden	Salmoniformes	Salvelinus	1.18	1.45	0.788	16	0.673–3.93
	Ripe	Brook Trout	Salmoniformes	Salvelinus	1.09	1.16	0.445	17	0.536–2.35
	Ripe	Largemouth Bass	Perciformes	Centrarchidae	1.05	1.13	0.339	12	0.769–1.8
Egg|ovary vs. WB	Ripe	Arctic Grayling	Salmoniformes	Thymallus	2.19	2.33	0.468	19	1.72–3.57
	Ripe	Yellowstone Cutthroat Trout	Salmoniformes	Oncorhynchus	2.05	2.75	2.12	37	0.456–10.4
	Ripe	Bluegill	Perciformes	Centrarchidae	1.93	2.04	0.707	33	0.594–3.94
	Ripe	Slimy Sculpin	Scorpaeniformes	Cottidae	1.89	1.93	0.353	20	1.18–2.63
	Ripe	Brown Trout	Salmoniformes	Salmo	1.4	1.18	0.641	31	0.211–2.92
	Ripe	Dolly Varden	Salmoniformes	Salvelinus	1.23	1.21	0.289	10	0.656–1.7
	Ripe	Desert Pupfish	Cyprinodontiformes	Cyprinodontidae	1.16	1.17	0.128	6	0.989–1.37
WB vs. muscle	All	Mountain Whitefish	Salmoniformes	Prosopium	2.73	7.25	12.7	7	0.761–35.7
	All	Arctic Grayling	Salmoniformes	Thymallus	1.84	2.04	0.467	19	1.6–3.1
	All	Kokanee	Salmoniformes	Oncorhynchus	1.36	1.41	0.265	34	0.931–2.08
	All	Rainbow Trout	Salmoniformes	Oncorhynchus	1.33	1.36	0.193	51	0.769–1.77
	All	Lake Whitefish	Salmoniformes	Coregonus	1.28	1.25	0.273	16	0.832–1.79
	All	Sacramento Splittail	Cypriniformes	Cyprinidae	1.25	1.26	0.313	30	0.779–2.03
	All	Yellowstone Cutthroat Trout	Salmoniformes	Oncorhynchus	1.21	1.21	0.251	43	0.885–2.15
	All	Burbot	Gadiformes	Lotidae	1.19	1.24	0.316	31	0.801–2.31
	All	Black Bullhead	Siluriformes	Ictaluridae	1.12	1.11	0.357	14	0.22–1.69
	All	Longnose Sucker	Cypriniformes	Catostomidae	1.04	1.06	0.133	11	0.876–1.29
	All	Walleye	Perciformes	Percidae	0.965	1.02	0.222	49	0.737–1.51
	All	Brown Trout	Salmoniformes	Salmo	0.952	1.05	0.201	9	0.876–1.44
	All	Dolly Varden	Salmoniformes	Salvelinus	0.947	1.14	0.425	10	0.713–1.98
	All	Channel Catfish	Siluriformes	Ictaluridae	0.919	0.92	0.31	8	0.516–1.58
	All	Roundtail Chub	Cypriniformes	Cyprinidae	0.919	0.886	0.143	10	0.653–1.05
	All	Largescale Sucker	Cypriniformes	Catostomidae	0.903	0.901	0.169	32	0.618–1.31
	All	Smallmouth Bass	Perciformes	Centrarchidae	0.888	0.927	0.182	29	0.592–1.48
	All	Yellow Perch	Perciformes	Percidae	0.817	0.83	0.055	11	0.754–0.924
	All	Bluehead Sucker	Cypriniformes	Catostomidae	0.794	0.773	0.091	10	0.585–0.884
	All	Common Carp	Cypriniformes	Cyprinidae	0.784	0.749	0.17	9	0.579–1.04
	All	Bluegill	Perciformes	Centrarchidae	0.767	0.752	0.171	34	0.37–1.05
	All	Flannelmouth Sucker	Cypriniformes	Catostomidae	0.706	0.688	0.113	12	0.456–0.829

[a] All species in the Salmoniformes order are from the Salmonidae family; therefore, genus was substituted instead.

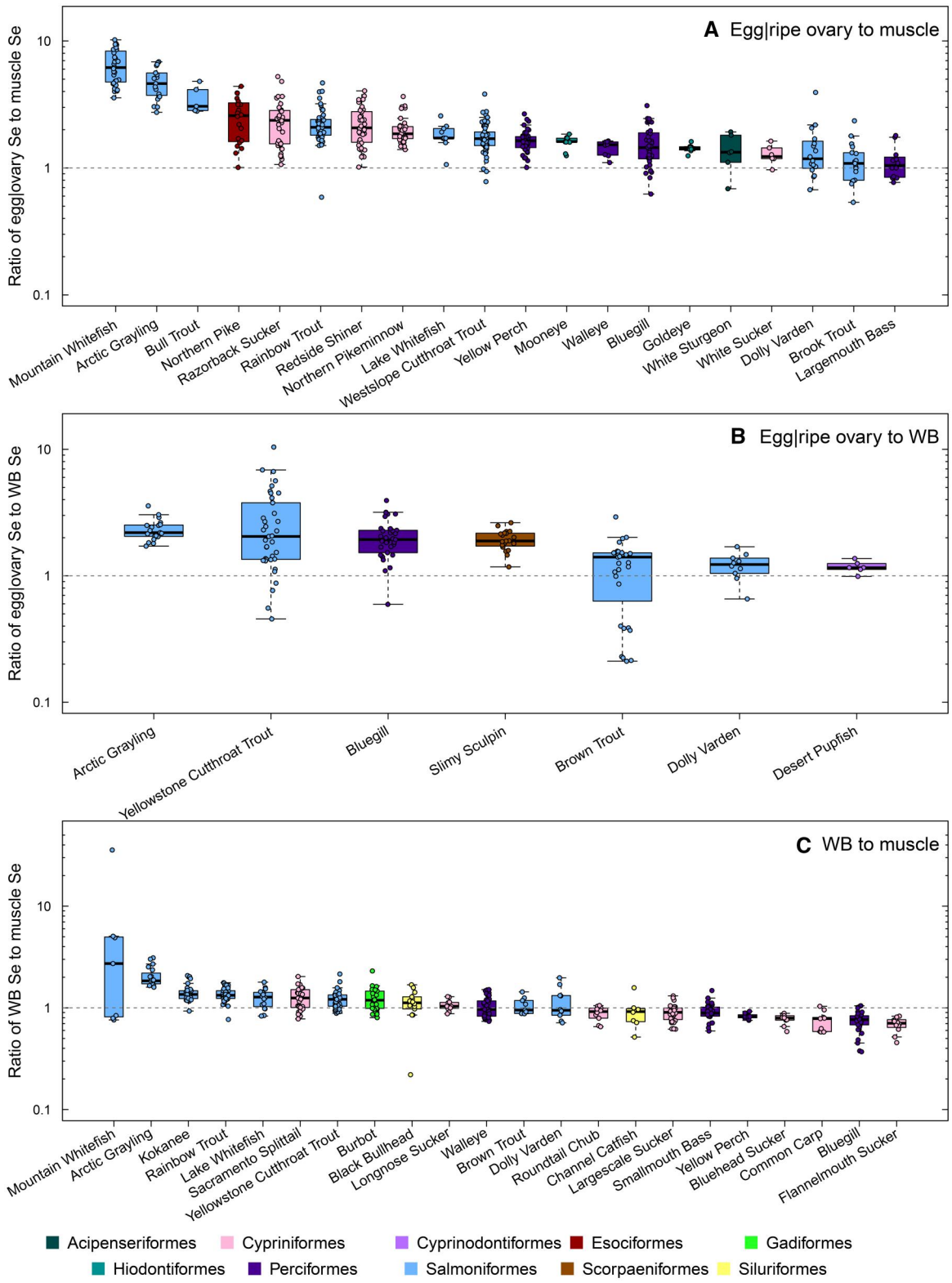

Figure 5. (A) Egg|ripe ovary-to-muscle selenium (Se) ratios; (B) egg|ripe ovary-to-whole body (WB) Se ratios; and (C) WB-to-muscle Se ratios. Box plots are as defined in Figure 3. Points with the same color are in the same taxonomic order.

pattern is driven by decreasing egg|ovary Se concentrations with increasing GSI, as muscle and WB Se concentrations tend not to vary over the GSI range. This pattern is also supported by the analysis comparing egg|ovary-to-muscle Se ratios in ripe and unripe samples (Table 3; Figure 2). Species that exhibited a difference in egg|ovary-to-muscle Se or WB Se ratios between samples from ripe and unripe females consistently had lower tissue Se concentration ratios in the ripe sample group than the unripe group (Figure 3). Although significant patterns were not detected for all species evaluated, either as correlations between egg|ovary-to-muscle Se or egg|ovary-to-WB Se ratios versus GSI or as detected differences between ripe and unripe sample

populations, for several species it is clear that spawning status influences egg|ovary Se concentrations and subsequent ratios with muscle and WB Se. As a result, analysis of Se concentrations in eggs|ovaries from unripe females or from females of uncertain ripeness may result in erroneous estimates of larval exposure to Se via maternal transfer (i.e., an overestimation of Se concentrations in ripe eggs|ovaries). The data in the present study and the companion study (Brix et al., 2025) support this observation. Possible mechanisms are discussed in Brix et al. (2025).

The analyses using GSI and spawning status both showed a decrease in variability of egg|ovary-to-muscle Se or WB Se ratios once females are ripe—even for species that did not have significantly different ratios in the ripe and unripe sample populations or significant correlations with GSI (Figure 3; Table 2). This finding shows that, not only can the ripe spawning status impact the relationship between Se concentrations in eggs|ovaries versus muscle or WB, it also impacts variability in the relationship and accuracy of predictions made based on the relationship. The relationship between egg|ovary Se for ripe and unripe rainbow trout samples (Figure 2A) demonstrates how the tissue concentration relationships become more predictable once the females become ripe. The samples in the unripe group exhibit high variability that sometimes deviate far from the weighted smoothing line, particularly around 3–5 mg/kg dry weight muscle Se where egg|ovary Se ranges from as low 5.6 mg/kg dry weight to as high as 59.3 mg/kg dry weight. By contrast, samples in the ripe group form a linear relationship.

Based on the high variance that can be introduced by including all stages of oocyte development, and that egg|ripe ovary Se concentrations tend to decrease as the fish approaches the ripe status, only ripe samples were used to generate predictive models for egg|ripe ovary Se based on muscle or WB Se.

Tissue Se ratios versus regression-based relationships

In the present study, we evaluated regression-based approaches for comparing tissue Se relationships among species and concluded that there are significant log-log linear relationships between egg|ripe ovary and muscle Se concentrations for many species. For example, in the MLR evaluation for these relationships, species with a log-log slope <1 meant that modeled egg|ripe ovary Se concentrations increased at a lower rate than corresponding increases in muscle Se concentrations when not log-transformed (Figure 6). For species with a log-log slope >1, the opposite is true: the egg|ripe ovary-to-muscle Se ratios for species in the model were not constant but increased at a higher rate than corresponding increases in muscle Se concentrations. In contrast, evaluations of median tissue Se concentrations do not consider the potential influence of varying Se exposure conditions that could influence the ratio. We evaluated both approaches in the present study, depending on the hypothesis being addressed.

For most fish species in our evaluation, the range and standard deviation of tissue Se concentration ratios (i.e., egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, WB-to-muscle) were high compared to the median or mean ratio (Figure 5; Table 5). For example, coefficients of variation for egg|ripe ovary-to-muscle, egg|ripe ovary-to-WB, and WB-to-muscle averaged 29%, 34%, and 28%, respectively. This shows that, even when only examining egg|ripe ovary samples of an individual species, the Se concentration ratio between egg|ripe ovary and muscle or WB tends to have high variability, and a single ratio is therefore an uncertain predictor of egg|ripe ovary Se concentrations over a range of muscle or WB Se concentrations. As noted above, both multiple and species-specific regression models that predict egg|ripe ovary Se from muscle or WB Se have slopes that are significantly different from zero, meaning that relationships between egg|ripe ovary and muscle or WB Se concentrations are concentration dependent. As such, to the extent that data are available for a given species and that significant models can be developed, we recommend the use of regression-based relationships for deriving tissue Se concentration ratios.

The USEPA evaluated regressions versus tissue ratios and recommended the median tissue Se ratio (USEPA, 2016). In calculating tissue Se ratios, the USEPA calculated the median if the linear regression relationship in Se concentrations between the two tissues being evaluated was positive and significant ($\alpha = 0.05$). No tissue Se ratio was calculated by the USEPA if these two requirements were not met. Although the USEPA noted that there may be cases for which the regression-based approach may better capture tissue Se relationships across the fuller range of Se concentrations, they stated that median ratios are simpler to explain and implement, and are not affected by outliers or the distribution of variance over the range of data (USEPA, 2016, 2021). However, we note that developing relationships between egg|ovary Se and either muscle or WB Se when controlling for spawning status (i.e., considering only data for ripe eggs|ovaries) reduces a significant portion of unequal variance across data ranges for many species (Figures 2 and 4). Also, when the log-log slope is not equal to 1, the use of median ratios has increasing

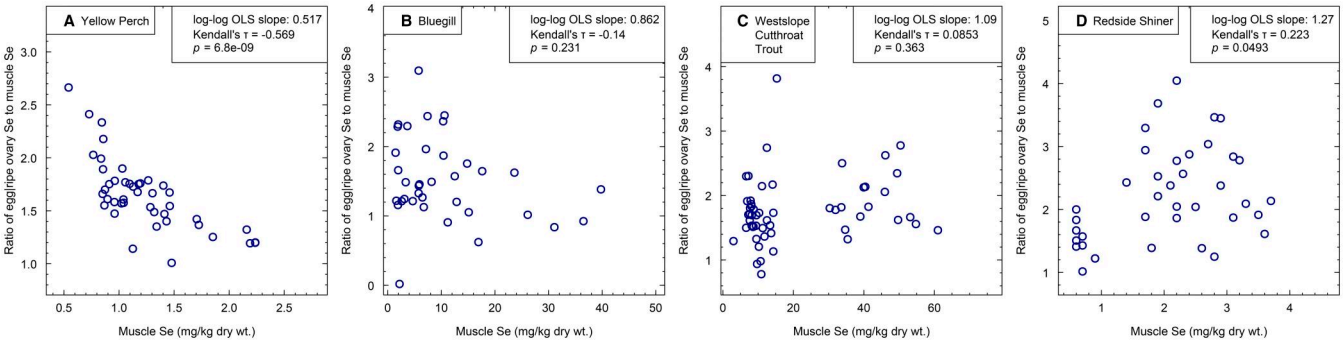

Figure 6. Ratios of egg|ripe ovary-selenium (Se)-to-muscle-Se concentrations plotted against muscle Se for species with egg|ripe ovary-to-muscle log-log slopes that are (A) considerably less than 1 (yellow perch), (B) slightly less than 1 (bluegill), (C) slightly greater than 1 (westslope cutthroat trout), and (D) considerably greater than 1 (redside shiner). The two species with log-log slopes farthest from 1 show the steepest correlations (Kendall's τ) of tissue concentration ratios with increasing muscle concentrations. The two species with log-log slopes closer to 1 (i.e., bluegill and westslope cutthroat trout) show shallower and nonsignificant correlations. Even when there is no relationship with muscle concentration, tissue concentration ratios exhibit a wide range.

uncertainty as one deviates further from the muscle or WB Se concentration from which the median ratio was derived (Figure 6). For these reasons, we consider a regression-based approach more robust than a ratio approach.

Variability in fish tissue Se relationships among fish populations

While in most cases tissue Se relationships in a species when data are available from multiple water bodies or sampling locations appear similar, our review identified a few examples of differences in tissue relationships across sampling locations for a given species. For example, Yellowstone cutthroat trout from a spawning station on Henry's Lake have much greater egg-to-WB Se ratios than wild-caught trout naturally exposed to a range of Se concentrations in lotic systems and other trout reared from hatchery-sourced eggs that were provided dietary organic Se in a 2.5-year study. The greater egg-to-WB Se ratios for the Henry's Lake hatchery samples were driven by WB Se concentrations that were much less than in the wild lotic population and in the feeding study, while egg Se concentrations were not proportionally lower. Based on this observation, data for the Henry's Lake population with divergent tissue Se ratios were excluded from our analysis (see online supplementary material S4 for further discussion).

This phenomenon of tissue concentration relationships being variable among subpopulations was also observed for Arctic grayling and redside shiner. For Arctic grayling, Brix et al. (2021) hypothesized that fish from an isolated lentic population had different tissue Se relationships than those from lotic populations due to differences in Se speciation and bioaccumulation between lentic and lotic site types, which may affect Se homeostasis among tissues. However, for redside shiner, de Bruyn et al. (2023) observed that the relationship between egg and muscle Se in fish from some but not all lentic sites was similar to the relationship in fish collected from lotic sites, (i.e., there was not a clear pattern between lentic and lotic sites for redside shiner). Regardless of the mechanisms causing this variability in fish tissue Se relationships that is sometimes observed in fish of the same species collected from different water bodies, consideration should be given to all of the available data to make an informed decision on the most relevant data for the site of interest.

Variability among fish species

When comparing relationships between egg|ripe ovary Se concentrations and corresponding muscle or WB Se concentrations, there is no clear pattern between species in the same order and family. Some similarities were observed among model coefficients between species in some of the salmonid genera (see *Results* and Table 4). Although there are a limited number of examples for which there are models for more than one species in a genus, we recommend that a model for an intra-genus surrogate be used to represent a species of interest if a species-specific model is not available. This recommendation is in part based on consistency in the egg|ripe ovary versus muscle Se models for species within the genera *Oncorhynchus* and *Salvelinus*, which are based on robust datasets spanning a wide range of muscle Se concentrations (Table 4). This recommendation is also consistent with observations that, for a wide range of toxicants, species within the same genus have similar sensitivities (USEPA, 1985). However, if the regression for a species of interest is not reported and there is not a surrogate model for another species in the same genus, the bluegill model is recommended instead based on its robust dataset and its median slope relative to the other species.

Summary and conclusions

We paired Se concentrations in eggs|ripe ovaries versus muscle and WB tissue for 21 and 7 species, respectively. Significant relationships between Se concentrations in eggs|ripe ovaries and muscle were observed for 12 of the 21 fish species, and for all 7 fish species with paired egg|ripe ovary and WB Se concentration data. In addition, paired WB and muscle Se concentrations were identified for 22 fish species, 17 of which had significant relationships. The latter represents an increase to the muscle-to-WB Se ratios derived in (USEPA, 2016), which include eight fish species in the families Catostomidae, Centrarchidae, and Cyprinidae. The additional muscle-to-WB Se relationships in the present study included species in Ictaluridae, Lotidae, Salmonidae, and Percidae. Although there is variability in egg|ripe ovary Se versus muscle or WB Se relationships among species (e.g., some species are not retained in the pooled models, or some species that are retained in the pooled model have species-specific intercept or slope adjustments), there is high agreement in the behavior of the tissue Se relationships overall. Intrafamily and -order relationships are not strong enough for making assumptions among species based on taxonomic relatedness, but we suggest that this is a feature of the precision of the species-specific models and that, were the error margins wider, there would be even fewer statistical differences between the modeled species. In the absence of a species-specific model reported herein, we recommend using the model for a species in the same genus if available, or otherwise the bluegill model.

The most important observations from this study are further confirmation that oocyte development stage influences ovary Se concentrations (Brix et al., 2025), such that ovary Se concentrations tend to decrease with increasing oocyte development and, when developing muscle-to-egg|ovary Se relationships and WB-to-egg|ovary Se relationships based only on data for eggs or ripe ovaries, much of the variability among several species is reduced. This indicates that some of the variability originally assumed to be due to species-specific differences in between-tissue Se relationships is actually due to variability in oocyte development. The USEPA's ambient water quality criteria document for Se (USEPA, 2016, 2021) includes muscle-to-egg|ovary Se conversion factors and WB-to-egg|ovary Se conversion factors that were used to support development of certain criterion elements, and these conversion factors are often used as a resource for other Se assessments. Importantly, however, the data used to develop those conversion factors are not always based on eggs or ripe ovaries. Given our findings, we suggest the conversion factors developed in our analysis using additional data and constrained to fish with ripe ovaries or eggs are more appropriate for developing tissue Se relationships in support of bioaccumulation model development and other Se assessments.

Although, for a given Se exposure, consideration of only egg or ripe ovary Se concentrations is generally shown to reduce variability when compared to Se concentrations in ovaries of variable ripeness, there can still be relatively high variability in muscle-to-egg|ovary Se conversion factors and WB-to-egg|ovary Se conversion factors because these factors may be concentration-dependent. In other words, the conversion factors are generally not constant over a range of Se exposure concentrations. For this reason, when adequate data are available for a species, more reliable conversion factors may be derived using regression equations rather than from applying a conversion factor derived from data for one set of Se exposure conditions and then applied to another. We note that this is a general recommendation, but it may not always be

possible to derive statistically significant relationships between Se concentrations in eggs or ripe ovaries versus either muscle or WB tissue (e.g., mountain whitefish).

In summary, the meta-analysis conducted in the present study emphasizes the importance of analyzing Se concentrations in eggs or ripe ovaries and using those concentrations to develop relationships between eggs|ovaries and muscle or WB tissue. Although the terms "eggs" and "ovaries" are often used interchangeably in the peer-reviewed literature, unpublished reports, and technical presentations, it is critical that these terms be applied accurately. We suggest that "eggs" and "ripe ovaries" are acceptable terms to describe samples from actively spawning fish, while the term "ovary" should be reserved for samples collected from pre-spawning fish or fish of unknown spawning status. This has important implications for determining compliance with Se criteria or guidelines based on eggs or ripe ovaries and for the development of between-tissue Se concentration relationships that may be used in the development of site-specific Se models and assessments.

Supplementary material

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

Data availability

Data, associated metadata, and calculation tools are available in the online supplementary material.

Author contributions

Claire Detering (Conceptualization, Formal analysis, Methodology, Visualization), Kevin Brix (Conceptualization, Methodology), Marko Adzic (Conceptualization, Data curation, Funding acquisition), Barry Fulton (Conceptualization, Methodology), and David DeForest (Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Visualization)

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Conflicts of interest

None declared.

Ethics statement

None.

Disclaimer

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