ECOTOX TOOLBOX
User's Manual

v. 1.2

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1. OVERVIEW

1.1 Toolbox Summary

The EcoTox Toolbox (the Toolbox) is a R Shiny application for modeling the bioavailability, bioaccumulation and effects of environmental stressors with a focus on inorganic stressors. Currently, the Toolbox has several models for Al, Cu, Ni, and Se and a Se Fish Tissue database (Figure 1). Details on each model are provided in Section 4:

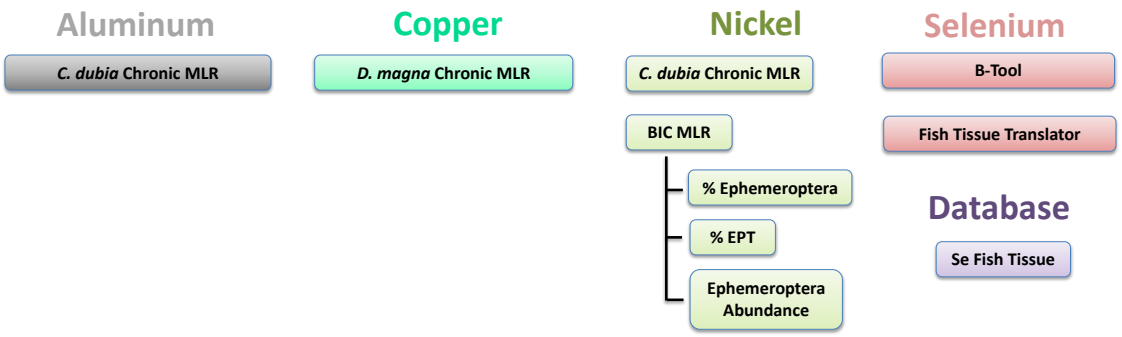

Figure 1. Overview of the EcoTox Toolbox

This manual provides information on online access, Toolbox organization and capabilities, example user workflows, a technical overview of each model, and details on the underlying equations and parameterization of each Tool.

1.2 Version 1.1 Update Summary

Version 1.2 of the Toolbox includes two updates compared to Version 1.1:

1. Addition of the Ni MLR model for *C. dubia*; and
2. Addition of Ni MLR models for benthic invertebrate community (BIC) metrics (% Ephemeroptera, % EPT, and Ephemeroptera abundance).

2. ACCESS TO THE TOOLBOX AND CITATION

The EcoTox Toolbox is an R shiny app that can be freely accessed via the EcoTox LLC website (https://www.ecotoxllc.com). If you have any issues with access or app functionality at any time, please contact feel free to contact KevinBrix@ecotoxllc.com.

If you use the Toolbox to support analyses in reports or peer reviewed publications, please consider citing it:

Brix, K.V. 2026. EcoTox Toolbox, v1.1. EcoTox LLC, Miami, Florida

As mentioned, access to the Toolbox is free and the only thing being requested in return is to fairly cite it's use. Additionally, if the Tool being used is a direct adaptation of a model

from the peer-reviewed literature, please cite the primary source for the model as well to properly acknowledge the scientists that developed the model.

3. OVERVIEW OF TOOLBOX WORKFLOWS

3.1 Selecting a Stressor and Tool from the Toolbox

When the Toolbox opens you will see a **Title Page** that includes information on the appropriate citation when the Toolbox is used in reports or papers. You can then navigate to the **Main Page** (or any of the other pages) by clicking on the tabs in the top toolbar. On the left-hand side of the **Main Page** is a sidebar user interface (UI). The first option is a dropdown window that allows you to **Select Stressor**. Depending on the stressor selected, different stressor-specific options appear below to **Select Tool**. Depending on the Tool selected additional dropdown windows may appear that allow you to navigate to the specific model you are interested in using. The remainder of Section 3 provides information on general workflows for type of Tool. Details on the technical basis for specific Tools are provided in subsequent sections.

3.2 MLR Models

Multiple linear regression models (MLRs) allow you to estimate effects for a specific metal, species, and endpoint while accounting for the effects of water quality parameters on metal bioavailability (typically, but not always, hardness, pH, and DOC (Brix et al. 2020). For each Tool, in the left sidebar UI, users will see two **Input Modes** to run the model; **Single Sample** and **Upload Excel File**. In single sample mode, enter hardness (mg L^{-1} as $CaCO_3$), pH (standard units), and DOC (mg L^{-1}) concentrations (or any other parameters that are requested) into the windows in the sidebar and press Run Model. Results will be provided in a **Model Outputs** table. Results can be exported as an Excel file via the **Download Results** button. **Upload Excel File** mode allows the user to import data for multiple samples and run them through the model simultaneously. A **Download Import Template** button allows you to export a data import template. Once data are entered click Browse and select the import file for analysis. You will see summary information on the import in **Input Summary**. It is **critical** to carefully review the data you have uploaded from a file before running the model to ensure it has properly loaded. Once you are satisfied, press **Run Model** and results will be plotted in the figure. The **X-axis Selector** allows you to plot the data as a time series (**Date**) or by **Site ID** which plots data using sites as a categorical variable. For both options, multiple sites can be plotted on the same figure. **Download Results** allows the user to export an Excel file with model inputs and results in tabular form. You can also export figures as .png files. If you place the cursor in the upper right-hand corner of each plot, it will activate a series of controls to change your view of a specific plot. Clicking the camera image allows you to export the .png file to any location you want.

Note that when you run the model you will see a green indicator saying the results are current. If you then change the data inputs, you will see this indicator turn yellow and state that inputs have changed and data export options have been disabled until the model is run again. This prevents accidental paired export of model outputs from one data set and model inputs from a data set subsequently loaded into the app but without the model being run again. The models also have guardrails establish to prevent predictions outside the environmental conditions over which it was parameterized.

3.3 Selenium B-Tool

To access the B-Tool select Se as the stressor and then **B-Tool** from the **Main Page** UI. The B-Tool is a steady-state model that allows you to estimate benthic invertebrate Se concentrations based on water Se speciation data (de Bruyn et al. 2025). The model is applicable only to lotic systems and should not be applied to lentic systems. The same general steps in terms of data upload, running the model, and plotting and exporting as previously described are available when using the B-Tool.

3.4 Selenium Fish Tissue Translator Tool

The Selenium Fish Tissue Translator Tool allows you to estimate Se concentrations in one fish tissue using data from another tissue (e.g., estimate ripe ovary/egg Se concentrations from muscle Se data). Relationships between these tissues are species-specific and so only species-specific models are available to make these estimates. Most models are based on a total least squares (TLS) regression between the two tissues of interest using log-transformed data. Models that include extrapolation to or from ripe ovary/egg (ROE) are based on carefully curated data sets where there is high confidence the data is either ripe ovaries or eggs. This is important as unripe ovaries tend to have higher Se concentrations than ripe ovaries or eggs and have the potential to systematically bias models (Brix et al. 2025, Detering et al. 2025). For this reason, it is also important that ROE data input to these models also be from either eggs or ripe ovaries. The one exception in the Fish Tissue Translator Tool are data for redside shiner (*Richardsonius balteatus*) where there are separate data for ripe ovaries and eggs. Further details on the technical basis for this species-specific model are provided in Section 4.3. The data for each model have been loaded into the **Database** and are largely from the dataset described in Detering et al. (2025) with some further refinement.

To run the Fish Tissue Translator, select it from the Toolbox on the **Main Page** UI. This will pull up a new UI. First selected the species you are interested in from the drop-down menu. Then select the source tissues (i.e., the tissue for which you have data) and the target tissue (i.e., the tissue for which you want to estimate Se concentrations). If there are data available for that species and tissue combination, the Toolbox will automatically derive the appropriate TLS regression and present a plot with summary statistics. This allows you to evaluate the model and whether it is sufficiently robust for your application. If it is, you can

upload an Excel file with your data to analyze. As with monitoring data, there is a specific data format the Toolbox is expecting, and a template file is provided in the root directory (Fish Tissue Translator Data Upload.xlsx). To upload data, you can press the **Browse** button on the **Main Page** UI and select the file where your data are located. Once uploaded press **Run translator** button to run the analysis. Output from the model can be accessed by pressing the **Download Predictions** button which provides an Excel spreadsheet of the predictions.

4. TECHNICAL OVERVIEW OF MODELS

This section provides a technical overview of the models in the EcoTox Toolbox.

4.1 Chronic Al MLR Model – *Ceriodaphnia dubia*

The *Ceriodaphnia dubia* chronic MLR model for Al predicts the EC20 for *C. dubia* reproduction while taking into account the effects of hardness, pH, and DOC on Al bioavailability. Data from 30 chronic *C. dubia* tests were used to develop the model using ln-transformed data. Several different model forms were evaluated in DeForest et al. (2020) with the final selected model having the general form:

$$\ln(EC20) = a + b \times \ln(DOC) + c \times pH + d \times \ln(Hard) - e \times \ln(Hard) \times pH$$

where, EC20 is the total Al concentration (µg L^{-1}) causing a 20% effect on *C. dubia* reproduction, and DOC (mg L^{-1}), pH, and hardness (mg L^{-1} as $CaCO_3$) represent the water quality conditions at which the EC20 is being estimated. The EC20, DOC, and hardness are ln-transformed in the model but both inputs and outputs are provided as untransformed data.

The C. dubia MLR parameterization is summarized below (Table 1).

Table 1. Summary of Chronic *C. dubia* MLR Parameterization

Parameter	*C. dubia*
Intercept	-9.272
ln DOC	0.619
pH	2.021
ln Hardness	2.581
ln Hardness x pH	-0.307
Adj. R^2	0.91
Predicted R^2	0.89

4.2 Chronic Cu MLR Model – *Daphnia magna*

The *Daphnia magna* chronic MLR model for Cu predicts the EC20 for *D. magna* reproduction while accounting for the effects of hardness, pH, and DOC on Cu bioavailability

(Brix et al. 2021). Data from 74 chronic *D. magna* tests were used to develop the model using ln-transformed data. The models has a general form of:

$$\ln(EC20) = a + b \times \ln(DOC) + c \times pH + d \times \ln(Hard)$$

where, EC20 is the dissolved Cu concentration (µg L^{-1}) causing a 20% effect on *D. magna* reproduction, and DOC (mg L^{-1}), pH, and hardness (mg L^{-1} as $CaCO_3$) represent the water quality conditions at which the EC20 is being estimated. The EC20, DOC, and hardness are ln-transformed in the model but both inputs and outputs are provided as untransformed data.

The *D. magna* Cu MLR parameterization is summarized below (Table 2).

Table 2. Summary Chronic of *D. magna* Cu MLR Parameterization

Parameter	*C. dubia*
Intercept	0.372
ln DOC	0.851
pH	0.175
ln Hardness	0.198
n	74
Adj. R^2	0.87
Predicted R^2	0.86

4.3 Nickel Multiple Linear Regression Models

The Ni MLR models are based on studies reported in Van Geest et al. (2025) and de Bruyn et al. (2025). The models use a two-step approach. First, a laboratory derived MLR accounts for the effects of hardness, DOC, and bicarbonate on chronic Ni toxicity to *Ceriodaphnia dubia*. The predicted effect on *C. dubia* reproduction is then used as the exposure variable in field calibrated models describing changes in BIC metrics. Currently the metrics available are % Ephemeroptera, % Ephemeroptera, Plecoptera, and Trichoptera (% EPT), and Ephemeroptera abundance. Consequently, the BIC models assume the same response to water chemistry effects as the *C. dubia* models.

4.3.1 *Ceriodaphnia dubia* MLR

The *C. dubia* MLR is based on compiled chronic data from previous studies and supplemental testing with Ni-spiked site and formulated waters. The combined data set includes 101 EC50s for *C. dubia* reproduction and covers a broad range of water chemistry conditions. The model predicts the dissolved Ni EC50 for *C. dubia* reproduction as:

$$log_{10}(EC50) = \beta_0 + \beta_H log_{10}(H) + \beta_B log_{10}(B) + \beta_{DOC} log_{10}(DOC)$$

where (EC50) is expressed in µg L^{-1} dissolved Ni, (H) is hardness in mg L^{-1} as CaCO3, (B) is bicarbonate in mg L^{-1}, and (DOC) is dissolved organic carbon in mg L^{-1}. Model coefficients are summarized in Table 4.

Table 4. Summary of *C. dubia* Bicarbonate MLR and Response Model Parameters

Parameter	Estimate
β_0	1.124
β_H	0.411
β_B	-0.520
β_{DOC}	0.547

A concentration-response model converts predicted EC50 into an EC_x using a mean slope of 2.05 (95% CI = 1.79 – 2.31) from all site-specific experiments. This allows for calculation of the dissolved Ni concentration associated with a specific ECx:

$$EC_x = 10^{\left[log_{10}(EC50) - \frac{log_{10}\left(\frac{1}{x}-1\right)}{s}\right]}$$

or an EC_x associated with a specified dissolved Ni concentration:

$$\%\ Effect = 100\left[\frac{1}{1+10^{s[log_{10}(EC50)\ -\ log_{10}(Ni)]}}\right]$$

The MLR was parameterized over hardness concentrations of 15–1,020 mg L^{-1} as $CaCO_3$, DOC concentrations of 0.4–40 mg L^{-1}, and bicarbonate concentrations of 8–366 mg L^{-1} HCO_3. The Toolbox does not calculate results for samples outside these ranges. This restriction avoids extrapolation beyond the experimental parameterization, although a small portion of the field data evaluated by de Bruyn et al. (2025) had bicarbonate concentrations above 366 mg L^{-1}.

4.3.2 Benthic Invertebrate Community Models

The BIC models were derived by applying the *C. dubia* MLR model to paired water quality and BIC monitoring data from Elk Valley, British Columbia. The applicability of these models to other watersheds has not been tested but is expected to be reasonably predictive for first through fourth order streams in temperate zones at a similar elevation (~1,300 – 2,300 m). The data set was screened to remove sampling locations/events with significant effects from other mine-associated stressors. The resulting data set contains 434 sampling events from 77 mine-influenced and 43 reference locations. Dissolved Ni concentrations ranged from <0.5 µg L^{-1} to 30 µg L^{-1}, corresponding to predicted effects of up to 92% on *C. dubia* reproduction.

BIC responses were evaluated using limiting factor analysis. This approach recognizes that many environmental factors can reduce BIC metrics at low Ni exposure, producing substantial variation in observed values. If Ni becomes limiting as exposure increases, the upper bound of the response distribution should decline even when variation caused by other

factors remains. Quantile regression was therefore used to characterize changes in the upper portion of the response distribution rather than changes in its mean or median.

Logistic curves were fit to quantiles 0.80–0.95 in increments of 0.01:

$$y_q(x) = \frac{Asym_q}{1 + exp\left(\frac{Mid_q - x}{Scal_q}\right)}$$

where, $y_q(x)$ is the fitted value of the BIC metric at quantile (q), (x) is the predicted effect of Ni on *C. dubia* reproduction, $Asym_q$ is the upper asymptote, Mid_q is the value of (x) at the inflection point, and $Scal_q$ is the scale parameter. Negative values of $Scal_q$ describe a declining BIC response with increasing predicted Ni toxicity.

Models were considered credible when the fitted parameters were reasonably consistent or varied monotonically among adjacent quantiles. Credible models were obtained for all 16 tested quantiles for percent Ephemeroptera, for 14 quantiles for Ephemeroptera abundance (all but q89 and q90), and for only 6 quantiles (q80–q85) for %EPT. Parameter ranges for the curves retained in the Toolbox are summarized in Table 5.

Table 5. Summary of BIC Model Parameters

BIC Metric	Credible Quantiles	Asym Range	Mid Range	Scal Range
% Ephemeroptera	q80 – q95	0.632 – 0.828	55.0 – 67.0	-34.3 – -19.7
% EPT	q80 – q85	89.5 – 94.9	82.8 – 91.3	-30.6 – -19.9
Ephemeroptera Abundance	q80 – q88, q91 – q95	3.778 – 4.057	108 – 134	-19.3 – -9.2

For each credible quantile, the model calculates the relative reduction in the fitted BIC response as it relates to the predicted effect on *C. dubia* reproduction:

$$Effect_q(x) = 100\left[1 - \frac{y_q(x)}{y_q(0)}\right]$$

where, $Effect_q(x)$ is the percent reduction in the fitted upper quantile response. The model summarizes these responses as the arithmetic mean and sample standard deviation. This output is interpreted as the mean modeled reduction in the upper quantile BIC response. Representative effects thresholds for the available BIC endpoints expressed as predicted effects on *C. dubia* reproduction are provided in Table 6.

Table 6. Summary of BIC Effect Thresholds Expressed as Percent Effect on *C. dubia* Reproduction (mean ± SD)

BIC Metric	EC10 (%)	EC20 (%)	EC50 (%)
% Ephemeroptera	21.8 ± 3.6	35.8 ± 3.3	67.4 ± 1.9
% EPT	40.1 ± 2.1	56.3 ± 0.9	86.7 ± 3.8
Ephemeroptera Abundance	56.4 ± 5.8	66.7 ± 4.2	83.0 ± 3.0

The BIC models are constrained by the same water quality parameterization ranges as the *C. dubia* MLR model. Additionally, the field data used to fit the quantile regressions extended to a maximum predicted effect of 92% on *C. dubia* reproduction. The Toolbox will therefore not calculate BIC effects for dissolved Ni concentrations predicted to exceed a 92% effect on *C. dubia* reproduction.

4.4 Selenium B-Tool

The B-Tool is a steady-state model based on a large dataset of paired analyses of water Se speciation and composite macroinvertebrate samples. The data were collected in the Elk Valley drainage of British Columbia that has elevated selenium concentrations associated with coal mining operations. The model also considers the inhibitory effects of sulphate on selenate uptake (Williams et al. 1994, Lo et al. 2015, Ponton et al. 2018). In taking this simple one-step approach and bypassing periphyton (Se concentrations in macroinvertebrates implicitly account for Se uptake by periphyton), the model eliminates much of the uncertainty associated with sampling this intermediate compartment in the food chain (Adams et al. 1998). Heterogeneity in Se speciation in water in the dataset facilitated a sequential analysis of subsets of the data to model the individual contribution of each Se species to observed invertebrate Se (de Bruyn et al. 2025). The general model form is:

$$Inv_{Se} = [SeVI]\left(a_{[SeVI]}[SeVI]^{b_{(SeVI)}}[SO_4]^{b_{SO_4}}\right) + [Se(IV)]\left(a_{[SeIV]}[Se(IV)]^{b_{Se(IV)}}\right) + [DMSeO](a_{DMSeO}) + [MeSeIV](a_{MeSeIV})$$

where, Inv_{Se} is the invertebrate Se concentration (mg kg^{-1} dw), each of the Se species are in expressed in µg L^{-1} and SO_4 is in mg L^{-1}.

The B-Tool parameterization was derived in de Bruyn et al. (2025) (Table 3).

Table 3. Summary of B-Tool Model Parameters

Se Species	Parameter	de Bruyn et al. 2025
Selenate	$a_{Se(VI)}$	6.56
	$b_{Se(VI)}$	-0.936
	b_{SO4}	-0.052
Selenite	$a_{Se(IV)}$	3.16
	$b_{Se(IV)}$	-0.373
DMSeO	a_{DMSeO}	13.1
MeSeIV	$a_{MeSe(IV)}$	104

4.5 Selenium Fish Translator Tool

The Fish Translator Tool uses available data from Elk Valley, the scientific literature, and grey literature reports on Se concentrations in fish eggs, ripe ovaries, muscle, and whole body. Data for unripe ovaries or ovaries of unknown ripeness have been excluded to avoid potential

effects of oocyte development on Se concentrations. The is evidence that in many species ovary Se concentrations are inversely related to oocyte development stage (Brix et al. 2025, Detering et al. 2025). Relationships between tissues are generally modeled using total least squares (TLS) regression (Golub and Van Loan 1980). This model structure is selected for most models over ordinary least squares (OLS) regression because from a mechanistic perspective, the Se concentration in one tissue is generally independent or only weakly dependent on the concentration in another tissue. Using log transformed data, the general form for the TLS regression models is:

$$y_{is} = a_s + b_s x_{is} + \varepsilon_{is},$$
$$x_{is} = log_{(10)}(Data_{is}),$$
$$y_{is} = log_{(10)}(Predicted_{is}),$$
$$\varepsilon_{is} \sim \mathcal{N}(0, \sigma_s^2)$$

Where x_{is} and y_{is} are the Se concentrations in the tissue with data and tissue to be predicted, respectively; a_s and b_s denote the species-specific intercept and slope, respectively; and σ represents residual variation around the fitted relationship, used here as a descriptive error term rather than the estimation criterion.

For most species, data from ripe ovaries and eggs (ROE) have been pooled on the assumption that they are equivalent representations of egg Se. This is likely to be approximately true for fish that are mass spawners as oocytes within the ovary are all at the same stage of development. However, in fractional spawners, ovaries contain multiple cohorts of oocytes at different stages of development. Consequently, ovaries may overpredict egg Se concentrations because they are dominated by oocytes are earlier stages of development. Redside shiner is the only species with paired ripe ovary and egg Se data available, and these data support the hypothesis that ripe ovaries will overpredict egg Se concentrations (de Bruyn et al. 2023, Brix et al. 2025). Consequently, for this species (and likely other fractional spawners once data become available) muscle Se is used to predict egg (only) Se and there is an additional model from predicting egg Se from ripe ovary only data (note ROE data should not be used for this model). Unlike other tissue models, there is a clear and direct mechanistic link between ripe ovary Se and egg Se concentrations. Consequently, this relationship is characterized using an OLS rather than TLS regression model. The OLS model structure is the same as the TLS model described above but the fitted relationship minimizes squared deviations in the target tissue rather than orthogonal deviations from the fitted line.

Uncertainty in tissues predictions is characterized using a bootstrap approach following standard resampling methods (Efron and Tibshirani 1993). For each bootstrap iteration, paired calibration observations are sampled with replacement (n=5,000), the applicable TLS or OLS model is refit, and the target tissue concentration is predicted for the user-supplied source tissue concentration.

Two types of uncertainty intervals are reported. The 95% confidence interval characterize uncertainty in the fitted mean tissue relationship. For each bootstrap iteration, the refitted model is used to estimate the expected target tissue concentration at the specified source tissue concentration. The confidence interval is calculated as the 2.5th and 97.5th percentiles of the resulting boostrap of fitted mean predictions. The 95% prediction interval characterizes uncertainty for an individual tissue prediction. For each bootstrap iteration, residual variation in $\log_{10}$ target tissue is add to the bootstrap model prediction. Prediction intervals are therefore wider than confidence intervals because they include uncertainty in both the fitted tissue relationship and residual variation around the relationship. Both intervals are calculated in $\log_{10}$ space and then back-transformed, so they are generally asymmetric in normal space.

The available models and summary statistics describing their performance are summarized in Table 4. Note that the R^2 for some models is low and users should carefully consider whether a selected model is sufficiently robust for the intended use.

Table 4. Summary of Fish Tissue Translator

Species	Common Name	Model	N	R^2
Acipenser transmontanus	White Sturgeon	Muscle-ROE	6	0.93
Catostomus catastomus	Longnose Sucker	Muscle-Whole Body	11	0.84
Catostomus discobolus	Bluehead Sucker	Muscle-Whole Body	10	0.95
Catostomus latipinnis	Flannelmouth Sucker	Muscle-Whole Body	12	0.60
Catostomus macrocheilus	Largescale Sucker	Muscle-Whole Body	32	0.57
Coregonus clupeaformis	Lake Whitefish	Muscle-Whole Body	16	0.26
Cyprinus carpio	Common Carp	Muscle-Whole Body	9	0.85
Esox lucius	Northern Pike	Muscle-ROE	14	0.92
Gila robusta	Roundtail Chub	Muscle-Whole Body	10	0.65
Ictalurus melas	Black Bullhead	Muscle-Whole Body	14	0.10
Ictalurus punctatus	Channel Catfish	Muscle-Whole Body	8	0.39
Lepomis macrochirus	Bluegill Sunfish	Muscle-ROE	4	0.99
Lota lota	Burbot	Muscle-Whole Body	26	0.17
Micropterus salmoides	Largemouth Bass	Muscle-ROE	11	0.21
Oncorhynchus clarkii lewisii	Westslope Cutthroat Trout	Muscle-ROE	54	0.91
Oncorhynchus mykiss	Rainbow Trout	Muscle-ROE Muscle-Whole Body	46 51	0.94 0.82
Prosopium williamsoni	Mountain Whitefish	Muscle-ROE	31	0.09
Ptychochelius oregonenis	Northern Pikeminnow	Muscle-ROE	64	0.48
Richardsonius balteatus	Redside Shiner	Muscle-Egg Ripe Ovary-Egg	56 56	0.89 0.87
Salmo trutta	Brown Trout	Whole Body-ROE	31	0.85
Salvelinus fontinalis	Brook Trout	Muscle-ROE	17	0.88
Salvelinus malma	Dolly Varden	Muscle-ROE Whole Body-ROE Whole Body-Muscle	16 10 10	0.92 0.97 0.81
Stizostedion vitreum	Walleye	Muscle-ROE	8	<0.01
Thymallus arcticus	Arctic Grayling	Muscle-ROE	34	0.76

Species	Common Name	Model	N	R^2
		Whole Body-ROE	34	0.91
		Whole Body-Muscle	34	0.93
Xyrauchen texanus	Razorback Sucker	Muscle-ROE	34	0.88

5. REFERENCES

Adams, W. J., K. V. Brix, K. A. Cothern, L. M. Tear, R. D. Cardwell, A. Fairbrother and J. E. Toll (1998). Assessment of selenium food chain transfer and critical exposure factors for avian wildlife species: need for site-specific data. Environmental Toxicology and Risk Assessment: Seventh Volume, STP 1333. E. E. Little, A. J. Delonay and B. M. Greenberg. Philadelphia, Pennsylvania, American Society for Testing and Materials**:** pp. 312-342.

Brix, K. V., D. K. DeForest, L. Tear, W. J. G. M. Peijnenburg, A. Peters, E. T. Middleton and R. J. Erickson (2020). Development of empirical bioavailability models for metals. Environ. Toxicol. Chem. **39**(1): 85-100.

Brix, K. V., L. Tear, R. C. Santore, K. Croteau and D. K. DeForest (2021). Comparative performance of multiple linear regression and biotic ligand models for estimating the bioavailability of copper in freshwater. Environ. Toxicol. Chem. **40**(6): 1649-1661.

Brix, K. V., L. M. Tear, J. Elphick, J. Ings, C. Detering, M. Carr, K. Raes, M. C. Arnold, M. Adzic, M. Hecker, A. M. H. de Bruyn and D. K. DeForest (2025). Oocyte developmental stage influences ovary selenium concentrations in fish - implications for ovary selenium monitoring. Environ. Toxicol. Chem. **44**(6): 1561-1574.

de Bruyn, A. M. H., C. B. Heddle, J. Ings, H. Gurleyuk, K. V. Brix, S. N. Luoma and M. C. Arnold (2025). Development of a bioaccumulation model for selenium oxyanions and organoselenium in stream biota. Environ. Toxicol. Chem. **44**(2): 363-374.

de Bruyn, A. M. H., B. P. Lo, J. Van Geest, D. Semeniuk, J. Elphick, J. Ings, C. Good, M. C. Arnold and K. V. Brix (2023). Effects of maternally transferred selenium on early life stage development of redside shiner (*Richardsonius balteatus*). Environ. Toxicol. Chem. **42**(11): 2350-2357.

de Bruyn, A. M. H., J. L. Van Geest, J. C. Arens, J. S. Ings, N. A. Manklow, K. V. Brix and M. C. Arnold (2025). Calibration of a laboratory-based chronic toxicity model to nickel effects on stream invertebrates in the field. Environ. Toxicol. Chem. **44**(10): 2972-2982.

DeForest, D. K., K. V. Brix, L. M. Tear, A. S. Cardwell, W. A. Stubblefield, E. Nordheim and W. J. Adams (2020). Updated multiple linear regression (MLR) models for predicting chronic aluminum toxicity to freshwater aquatic organisms and development water quality guidelines. Environ. Toxicol. Chem. **39**(9): 1724-1736.

Detering, C., K. V. Brix, M. Adzic, B. A. Fulton and D. K. DeForest (2025). Relationships in selenium concentrations among fish tissues to support selenium assessments and regulations. Environ. Toxicol. Chem. **44**(6): 1742-1757.

Efron, B. and R. Tibshirani (1993). An Introduction to the Bootstrap. New York, Chapman and Hall/CRC.

Golub, G. H. and C. F. Van Loan (1980). An analysis of the total least squares problem. SIAM J. Numer. Anal. **17**(6): 883-893.

Lo, B. P., J. R. Elphick, H. C. Bailey, J. A. Baker and C. J. Kennedy (2015). The effect of sulfate on selenate bioaccumulation in two freshwater primary producers: A duckweed (*Lemna minor*) and a green alga (*Pseudokirchneriella subcapitata*). Environ. Toxicol. Chem. **34**(12): 2841-2845.

Ponton, D. E., C. Fortin and L. Hare (2018). Organic selenium, selenate, and selenite accumulation by lake plankton and the alga *Chlamydomonas reinhardtii* at different pH and sulfate concentrations. Environ. Toxicol. Chem. **37**(8): 2112-2122.

Van Geest, J. L., J. M. Daley, J. R. Elphick, M. Gallant, K. V. Brix, N. A. Manklow, M. C. Arnold, M. Digel and A. M. H. de Bruyn (2025). Development of a multiple linear regression model for chronic nickel toxicity to *Ceriodaphnia dubia*: performance of bicarbonate vs. pH as toxicity modifying factors. Environ. Toxicol. Chem. **44**(10): 2983-2995.

Williams, M. J., R. S. Ogle, A. W. Knight and R. G. Burau (1994). Effects of sulfate on selenate uptake and toxicity in the green alga Selenastrum capricornutum. Arch. Environ. Contam. Toxicol. **27**: 449-453.