United States Environmental Protection Agency

Office of Water
EPA 820-R-24-003
April 2024

Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion

Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion

While this document cites statutes and regulations that contain requirements applicable to Clean Water Act (CWA) implementation programs it does not impose legally binding requirements on the EPA, states, authorized Tribes, other regulatory authorities, or the regulated community, and may not apply to a particular situation based upon the circumstances. The EPA, state, Tribal and other decision makers retain the discretion to adopt approaches on a case-by-case basis that differ from those provided in this technical support document as appropriate and consistent with statutory and regulatory requirements. The EPA may update this document as new information becomes available. In addition to this document, the EPA has related documents that provide considerations and recommendations on implementing criteria based on the national CWA section 304(a) recommended selenium criterion for freshwater, which are available at the EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium.

Table of Contents

Tables

List of Acronyms and Abbreviations

CF	Conversion Factor
CW	Cold water
CWA	Clean Water Act
EC_{10}	10% effect concentration
µM	Micron
MDL	Method detection limit
MPCA	Minnesota Pollution Control Agency
NAMC-SWG	North American Metals Council-Selenium Work Group
NCCA	National Coastal Condition Assessment
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
PBA	Performance-based approach
QA/QC	Quality assurance/quality control
QL	Quantitation limit
SETAC	Society of Environmental Toxicology and Chemistry
SSD	Species sensitivity distribution
TMDL	Total maximum daily load
TSM	Technical support materials
TTF	Trophic transfer factor
USEPA	U.S. Environmental Protection Agency
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey
WQC	Water quality criteria
WQS	Water quality standards
WW	Warm water
ww	Wet weight

Definitions[1]

Anadromous fish

Fish with a life cycle that is divided between fresh and saltwater, including fish migrating to spawn in freshwater. Migrations should be cyclical, predictable, and cover more than 100 km (FishBase 2016).

Asynchronous spawning

Eggs are released in batches over a period of time that can last days or even months (Murua and Saborido-Rey 2003).

Fecundity

The physiological maximum potential reproductive output of an individual (usually female) over its lifetime (Bradshaw and McMahon 2008).

Gravid

Having the body distended with ripe eggs (FishBase 2016).

Indeterminate fecundity

Potential annual fecundity is not fixed before the onset of spawning and eggs can develop at any time during the spawning season (FishBase 2016).

Iteroparous

Producing offspring in successive batches, for example, annual or seasonal batches, as is the case for most fishes (FishBase 2016).

Method detection limit

The minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results (USEPA 2016).

Quantitation limit

The smallest detectable concentration of analyte greater than the detection limit where the required accuracy (precision and bias) is achieved for the intended purpose (USEPA 2017).

Oocyte

Female sex cell which develops into an ovum. Oogonia become oocytes when meiosis begins, and specialized cells surround each oocyte to form a follicle. The oocyte undergoes maturation in preparation for spawning as an egg (modified from FishBase 2016).

[1]This glossary is meant to provide plain language definitions for key terms used in this document. Individuals should consult the Clean Water Act and the EPA's implementing regulations to identify whether there are legal definitions of these terms.

Potamodromous

Fish species that spend their whole life in freshwater, but generally migrate for spawning purposes, typically back to a natal upstream tributary from a mainstream river or between connected lake and river systems. Migrations should be cyclical, predictable, and cover more than 100 km (FishBase 2016).

Semelparous

Producing all offspring at one time, such as most salmon. Usually these fish die after reproduction (FishBase 2016).

Site

In the context of site-specific criteria, a “site” may be a state, region, watershed, waterbody, or segment of a waterbody. A “site” for fish sampling is a specific waterbody segment.

Synchronous spawning

Eggs are released in a single episode during each breeding season (Murua and Saborido-Rey 2003).

Vitellogenesis

The process by which the yolk is formed and accumulated in the ovum. This is also the period when nutrients stored in the liver are transferred to the developing oocytes in the ovary or ovaries (FishBase 2016).

1.0 Introduction

1.1 The EPA's National CWA Section 304(a) Recommended Chronic Aquatic Life Selenium Criterion in Freshwater

In 2016, the U.S. Environmental Protection Agency updated its national Clean Water Act (CWA) section 304(a) recommended chronic aquatic life criterion for selenium in freshwater systems to reflect the latest scientific information and, in 2021, issued an erratum, *2021 Revision to: Final Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016*.[2] The EPA's national CWA section 304(a) recommended selenium criterion still reflects the latest science and the EPA is not aware of any updated scientific information that would change this recommendation. The latest scientific information indicates that selenium toxicity to aquatic life is driven by dietary exposures and that the reproductive life stages of egg-laying vertebrates are the most sensitive to the toxic effects of selenium. The recommended criterion has four criterion elements: (1) a fish egg-ovary criterion element; (2) a fish whole-body and/or muscle criterion element; (3) a water column criterion element (one value for lentic and one value for lotic aquatic systems); and (4) a water column intermittent criterion element (to account for potential chronic effects from short-term exposures to high concentrations in lentic and lotic aquatic systems) (see Table 1). Under the EPA's 2016 national CWA section 304(a) recommended selenium criterion, the fish tissue criterion elements have primacy over water column elements, except where there are no fish, where fish tissue data are not adequate, or for waterbodies with new or increased discharges where selenium concentrations in fish tissue might not have stabilized and reached steady-state. The EPA also recommends that the egg-ovary tissue criterion element has primacy over whole-body and muscle tissue criterion elements.

Toxicity data indicate that the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse reproductive effects in aquatic organisms. Toxicity to developing embryos and larvae is directly linked to egg selenium concentration.[3] The EPA derived the whole-body, muscle tissue, and water column elements from the egg-ovary element so that states and authorized Tribes could more readily implement their water quality criteria (WQC) based on the EPA's national CWA section 304(a) recommended selenium criterion. The assessment of the available data on chronic selenium exposure for fish, invertebrates, and amphibians indicates that a criterion element derived from fish is expected to be protective of the aquatic community in a waterbody, since other taxa

---

[2] In 2021, the EPA identified that the following text was missing from the second sentence in footnote 4 in the selenium criterion table: "When selenium inputs are increasing" and issued an erratum. The EPA corrected footnote 4 to state: "4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. When selenium inputs are increasing, water column values are the applicable criterion element in the absence of steady-state condition fish tissue data."

[3] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

appear to be less sensitive to selenium than fish. The EPA did not develop an acute criterion for selenium when it updated the chronic criterion. Although selenium may cause acute toxicity at high concentrations, the most deleterious effects on aquatic organisms are due to selenium's bioaccumulative properties.

In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from chronic exposure to chemicals via the diet (through the food web pathway). Such studies also do not account for the accumulation kinetics of many bioaccumulative compounds, such as selenium, and may underestimate effects from long-term accumulation in some types of aquatic systems. Therefore, since acute studies do not address the primary exposure pathway for organisms to selenium and because chronic toxicity occurs at lower concentrations, an acute criterion was not included in the national CWA section 304(a) recommended selenium criterion. As described in the EPA's *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016 (*hereafter referred to as *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*), the EPA also included an intermittent exposure criterion element to provide protection from the most significant chronic effects of selenium toxicity, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high concentration exposure events.[4]

The EPA recommends, as stated in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016,* that states and authorized Tribes[5] adopt into their water quality standards (WQS) a selenium criterion that includes all four criterion elements.[6] For more information see the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*, which can be found at https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf.

[4] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[5] Throughout this document and in the CWA, the term "states" means the fifty states, the District of Columbia, the Commonwealth of Puerto Rico, the United States Virgin Islands, Guam, American Samoa, and the Commonwealth of the Northern Mariana Islands. The term "authorized Tribe" means those federally recognized Indian Tribes with authority to administer a CWA WQS program.

[6] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Table 1. Summary of the Recommended Freshwater Selenium Ambient Chronic Water Quality Criterion for Protection of Aquatic Life.

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

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

1.2 Selenium Technical Support Materials

The EPA has prepared a four-volume set of documents to provide recommendations to states, authorized Tribes, and other agencies for implementing their WQC based on the national CWA section 304(a) recommended selenium criterion for aquatic life.[7] These four documents constitute the Technical Support Materials for the EPA's *Aquatic Life Ambient Water Quality*

[7] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Criterion for Selenium–Freshwater 2016.[8] Each document of the set focuses on a specific aspect of implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. Together, these four EPA documents provide information to assist states and authorized Tribes with adopting WQC based on the EPA's national CWA section 304(a) recommended selenium criterion and implementing them in various CWA programs.

1) *Technical Support for Adopting and Implementing the EPA's Selenium 2016 Criterion in Water Quality Standards*: Provides recommendations for the adoption and implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, including the various flexibilities available to states and authorized Tribes using WQS tools.
2) *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*: Provides an overview of how to establish or enhance existing fish tissue monitoring programs to facilitate implementation of fish tissue criterion elements based on the national CWA section 304(a) recommended selenium criterion.
3) *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*: Provides information to help National Pollutant Discharge Elimination System (NPDES) permit writers understand what permitting guidance (i.e., state or Tribal implementation procedures) may be appropriate to implement state and authorized Tribal WQS based on the EPA's national CWA section 304(a) recommended selenium criterion. This set of FAQs also provides recommendations on how to establish water quality-based effluent limits (WQBELs) in NPDES permits.
4) *Frequently Asked Questions: Implementing the EPA's 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs*: Provides information on how to complete assessments, list impaired waters, and develop TMDLs to implement the EPA-approved[9] WQS that are based on the EPA's national CWA section 304(a) recommended selenium criterion.

1.3 Document Overview

This document, *Technical Support for Fish Tissue Monitoring for Implementing the EPA's 2016 Selenium Criterion*, is intended to provide recommendations for sampling fish tissue for the implementation of WQC based on the national CWA section 304(a) recommended selenium criterion. The recommendations provide information for states and authorized Tribes to consider so they can collect fish tissue samples that represent the selenium exposure and vulnerable species at a particular site. The recommendations are written assuming all four

[8] USEPA. 2021. *2021 Revision to: Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016.* EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

[9] Approved by the EPA includes both WQS adopted by states, territories or authorized Tribes and approved by the EPA and WQS promulgated by the EPA.

criterion elements of the national CWA section 304(a) recommended selenium criterion were adopted by a state or authorized Tribe along with the recommended hierarchical structure of the criterion. These recommendations may also be helpful to states and authorized Tribes that have not yet adopted WQC based on the national CWA section 304(a) recommended selenium criterion but are considering doing so and are interested in evaluating the concentration of selenium within their fish populations.

This document examines technical considerations for developing a robust sampling program to characterize selenium concentrations in fish tissue for a variety of CWA implementation programs (e.g., CWA section 303(d) listing and TMDLs). Some aspects of this document can also be used to support the development of site-specific water column criterion elements. Site-specific water column criterion elements can be developed by performing a site-specific water column translation from the fish tissue elements of the criterion. See Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) for site-specific approaches for translating between fish tissue and water column concentrations.

Site-specific conditions may at times impact field collection decisions, particularly conditions such as fish abundance, species availability, and site accessibility. The EPA recommends that states and authorized Tribes use the following document as a tool for developing field sampling plans with the knowledge that there may need to be deviations from these plans due to actual conditions at a site. To maintain this flexibility, the EPA recommends that states and authorized Tribes incorporate these recommendations into guidance and use them for planning purposes, rather than adopting them into a rule or other legally binding mechanism. The one exception is if a state or authorized Tribe develops a performance-based approach (PBA) for translating fish tissue criterion elements into site-specific water column criterion elements. A PBA is a WQS that is a transparent process, such as a criterion derivation methodology, rather than a specific outcome, such as a concentration of a pollutant. The approval of a PBA would also serve for CWA purposes as the approval of each outcome generated from following that process or method. States and authorized Tribes can adopt PBAs as part of their selenium WQS that must be submitted to the EPA for review and approval or disapproval, in accordance with the CWA section 303(c) and the EPA's implementing regulations at 40 Code of Federal Regulations (CFR) Part 131. Information from this technical support material can be useful in developing parts of the methodology included in a PBA. Since a PBA is used to generate values used for CWA purposes, that methodology must be legally binding. For more information on the PBA, see section 2.2.1 of *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards* (USEPA 2024c).

This document is intended to assist states and authorized Tribes in planning for and enhancing their monitoring programs to collect fish tissue samples that are representative of the selenium exposure and species that are present at a site; it is not intended to limit the fish tissue data that states and authorized Tribes could evaluate for waterbody assessments with the recommended criterion. In other words, these recommendations should not be used to exclude the consideration of data for assessment decisions, but rather could be used in planning the collection of that data. The EPA's regulations require that states and authorized Tribes evaluate all existing and readily available water quality-related data for assessment decisions (40 CFR 130.7(b)(5)), which for WQS based on this criterion would include selenium data and information for any fish species. For additional information about assessment decisions for this criterion, please refer to the *Frequently Asked Questions: Implementing the 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load Programs* (USEPA 2024a).

This document does not specifically address the site-specific modification of the fish tissue criterion elements, which can be developed using fish assemblage data and the recalculation procedure (USEPA 2013). States and authorized Tribes interested in developing site-specific fish tissue criterion elements should engage their EPA regional office early in the process to evaluate this option.

2.0 Monitoring Strategy

The EPA's national CWA section 304(a) recommended selenium criterion was designed to protect "populations of fish, amphibians, aquatic invertebrates, and plants" (USEPA 2021). As studies have shown that selenium is accumulated in organisms primarily through dietary exposure and that fish are the taxa most sensitive to selenium within the aquatic community, fish tissue criterion elements were developed as part of this criterion (USEPA 2021). When developing monitoring strategies for CWA implementation of criteria based on the national CWA section 304(a) recommended selenium aquatic life criterion, the EPA recommends that states and authorized Tribes review their existing fish tissue monitoring programs and determine how best to incorporate the collection of fish tissue samples for such implementation into their existing monitoring programs. For example, existing fish tissue monitoring programs may already be collecting data to assess the risk of fish consumption to human health. These existing monitoring programs may be designed with different objectives than monitoring activities designed to assess the attainment of an aquatic life criterion. These differences should be considered and reconciled when using an existing fish tissue monitoring program for assessing the fish tissue criterion elements of the selenium aquatic life criterion.

The following sections discuss study design and sampling considerations regarding fish tissue types, sample types, target species, and spatial and temporal variability. These topics should be considered to ensure the collection of a fish tissue sample that is representative of the selenium exposure and the species present at a site. These topics should be considered when collecting data to assess fish tissue concentrations against the fish tissue criterion elements of the states or authorized Tribes' selenium aquatic life criterion or collecting data to translate a fish tissue criterion element into a site-specific water column criterion element.

Additionally, the relationship between fish sampling locations and timing, species' habits and natural history, and the selenium source(s) should be understood and accounted for during the design of sampling plans for criterion implementation. Fish tissue sampling methods should be designed to characterize the variability of selenium in the target population, including areas of high selenium bioaccumulation.

Since the national CWA section 304(a) recommended selenium criterion establishes population-level protection, the EPA generally recommends implementing these fish tissue criterion elements with a measure of central tendency (e.g., mean, median) when the selenium concentration in fish tissue is determined to be at steady-state, as this will represent the level of exposure that the population as a whole is experiencing. When characterizing the sample population's exposure, the monitoring data set and information about the underlying distribution of the sample population should be used to determine which measure of central tendency is most appropriate. In addition, the variability around that measure of central tendency (e.g., standard deviation, standard error, confidence interval) should be quantified and considered when making implementation decisions. The EPA recommends using a measure of central tendency only for characterizing a data set that was collected during a single sampling event (identified location over a specified period of time (generally less than a week)). For implementation purposes, a measure of central tendency should not be calculated between different sampling events; instead, different sampling events should all be considered as discrete data sets.

However, there are some situations where it may be more appropriate to implement the criterion using a metric other than a measure of central tendency, such as using the upper bound of the confidence interval or using the maximum measured value of all data sets. For example, it could be appropriate to use the maximum value from a single sampling event to determine reasonable potential for an NPDES permitted discharge (USEPA 1991). For more information, see *Frequently Asked Questions: Implementing Water Quality Standards Based on the EPA's 2016 Selenium Criterion in Clean Water Act Section 402 National Pollutant Discharge Elimination System Permits*.

The EPA encourages states and authorized Tribes to have early discussions with their EPA regional office to ensure that studies capture the appropriate data. Detailed examples of field collection procedures and sampling design considerations can be found in *Sampling Protocols for Collecting Surface Water, Bed Sediment, Bivalves, and Fish for Priority Pollutant Analysis* (USEPA 1982); *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis* (the EPA's *Fish Advisory Guidance Volume 1*) (USEPA 2000); *Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue* (USEPA 2002a); the EPA's National Aquatic Resource Survey Field Operations Manuals for rivers and streams and Great Lakes and coastal waters (USEPA 2019a, 2019b, 2015); and *Protocols Manual for Water Quality Sampling in Canada* (CCME 2011). Appendix A of this document presents egg and ovary collection and sample preparation methods.

2.1 Tissue Type

The EPA recommends sampling egg-ovary tissue, when possible. From a toxicological standpoint, the most representative measure of exposure to a toxic substance is its concentration at the site of toxic action. In fish (the aquatic species most sensitive to selenium), the most ecologically relevant sites of toxic action are the mature reproductive tissues of adults (ovaries) or early life stages (vitellogenic eggs/larvae). This was a major point of consensus at the 2009 Society of Environmental Toxicology and Chemistry (SETAC) Pellston workshop on selenium risk assessment (Chapman et al. 2009). Therefore, the national CWA section 304(a) recommended selenium criterion is based on reproductive effects in fish, as represented by selenium concentrations in egg-ovary tissue.

While egg-ovary tissue of adult female fish is the most direct reflection of reproductive toxicity in fish, samples of muscle or whole-body tissue from adult fish serve as robust alternatives and may be collected for implementation purposes when egg-ovary tissue is impractical to sample.

Collection of fish samples for egg-ovary analysis poses special challenges as only gravid female fish can be sampled. Due to this constraint, the decision of what tissue type to collect should be made based on the following considerations:

- *Temporal*: Most fish species that are synchronous spawners spawn in the spring; whereas fish tissue collection for fish consumption advisories typically occurs in the late summer or early fall, when contaminant loads in the edible portion of the fish are highest. Spring sampling may also be challenging in states or on authorized Tribal lands where rivers and streams have high flows due to storm run-off and spring snow melt. Timing the collection of mature eggs from asynchronous spawners can also be challenging, as these species can have eggs in various stages of development at once.

- *Spatial*: Some fish species migrate to upstream areas to spawn, and these areas may be harder to access than higher order downstream segments that are inhabited during non-spawning seasons.

- *Size*: It is difficult to collect and analyze egg-ovary tissue samples from small fish species (e.g., certain species in the family Cyprinidae or Cyprinodontidae) due to the logistics of the collection and the small amount of tissue available for analysis (number of eggs or biomass).

Due to these considerations, states and authorized Tribes have considerable discretion when selecting the fish tissue type to collect in their sampling protocols. The flexibility provided by being able to collect data from multiple fish tissue types allows for leveraging existing monitoring capacity. A number of the species that are good target species for selenium sampling could also be commonly collected as muscle (fillet) samples in state and authorized Tribal fish tissue monitoring programs for other contaminants (e.g., trout, salmon, bass, sunfish) (see section 2.3 for more information on target species). The whole-body tissue criterion element also simplifies the collection and processing of small fish species that may be the dominant trophic level in lower order stream networks.

When developing a new–or modifying an existing–fish tissue monitoring strategy, states and authorized Tribes may want to consider funding and staff resources, opportunities to work with other fish tissue monitoring programs, existing information on the spawning habits and size of target species, and potential population level effects associated with using lethal sampling techniques when deciding what fish tissue type to sample. To keep the public, stakeholders, and the EPA well informed, it is good practice for monitoring programs to describe in their sampling protocols why they are sampling a particular tissue type. Similar considerations might also be evaluated when selecting a tissue to sample for the development of a site-specific water column criterion element. If possible, the EPA recommends that egg-ovary tissue be sampled to conduct a site-specific water column translation (see section 2.1.1). Sampling considerations associated with different types of fish tissue are presented in Table 2.

Table 2. Sampling Considerations Associated with Different Types of Fish Tissue.

Issue	Egg-ovary	Whole-body	Muscle/Fillet	Comments
Criterion Hierarchy Considerations	Has primacy in the criterion; supersedes other fish tissue criterion elements and the water column criterion elements	Supersedes water column criterion elements; equal consideration to muscle tissue	Supersedes water column criterion elements; equal consideration to whole-body tissue	While selenium concentrations in all three tissue types are significantly correlated to reproductive toxicity effects seen in fish, egg-ovary concentrations have the strongest relationship.
Ease of collection	Difficult	Easy[a]	Easy – except on small fish[a]	Egg-ovary samples are only collected from gravid females; there are seasonal and logistical considerations, and species-specific sampling windows. See Appendices A and B.
Consistency with existing state & authorized Tribal methods	Not typically collected	Sometimes collected	Primary tissue collected	Whole-body samples might be collected in special cases, such as for certain human populations that consume whole fish or for ecological risk assessments.
Sample availability	Limited–only from gravid females	Always	Always	For waterbodies with small sized species at top trophic levels, whole-body may be the only option due to issues collecting a sufficient mass of muscle tissue.

Issue	Egg-ovary	Whole-body	Muscle/Fillet	Comments
Ability to make composite sample	Yes	Yes	Yes	Compositing can be used to reduce the overall cost of an analytical program, primarily by reducing the number of samples that must be analyzed to represent an average concentration. Compositing can also ensure that enough mass is available for chemical analyses. However, by compositing samples, information on the range of selenium concentrations in individual organisms is lost.
Ability to test individual sample	Yes, on larger species; may be difficult on small species	Yes, on larger species; may be difficult on small species	Yes, on larger species; may be difficult on small species	Individual samples are valuable when sampling from waters known or suspected to be impacted by selenium discharges (see section 3.2.2); however, the need to analyze multiple individual samples versus a few composite samples can make them more resource intensive to prepare and expensive to analyze.

[a] Availability of fish may make collections challenging. If sampling will result in detrimental impacts on the fish populations of a waterbody, the EPA recommends collecting water samples instead.

2.1.1 Egg-ovary Tissue Sample

Selenium concentrations in all three tissue types are significantly correlated to reproductive toxicity effects seen in fish; however, egg-ovary concentrations have the strongest relationship. The EPA recommends sampling fish egg-ovary tissue for assessment of the selenium aquatic life criterion or for development of a site-specific water column criterion element, when possible. Egg-ovary tissue refers to mature eggs, pre-spawn ovary tissue that contains mature eggs, or both. As an oocyte grows into a mature egg, it passes through several stages of development (i.e., oogenesis, primary oocyte growth, cortical alveolus stage, vitellogenesis, maturation, and ovulation) (Tyler and Sumpter 1996). During this egg development process, the oocyte size increases dramatically as the yolk is developed. For example, the diameter of an undeveloped oocyte of the Rainbow Trout is around 20 µm and the fully developed egg is about 4 mm (Nagahama 1983). Selenium is transferred from an adult female fish to her eggs during vitellogenesis. Eggs should not be collected until after this transfer has occurred as this will more accurately represent larval exposure to selenium. Appendix A of this document presents egg and ovary collection and sample preparation methods.

Egg-ovary tissue from pre-spawn, reproductively mature (also called “gravid” or “vitellogenic”) females is the preferable tissue to collect because it will give the most accurate representation of potential selenium hazard to reproduction. Egg-ovary tissue data provide point measurements that reflect integrative dietary accumulation, transfer, and deposition of selenium over time and space in female fish at a given site. Research has shown that selenium concentrations in egg-ovary tissue is strongly correlated with selenium in the maternal diet (Janz et al. 2010).

When using egg-ovary tissue for the implementation of criteria based on the national CWA section 304(a) recommended selenium criterion, states and authorized Tribes should carefully consider the difficulty in timing egg-ovary sampling with spawning periods. Ovary tissue sampled from a female that is not gravid will not be representative of the selenium concentrations of this tissue for a gravid individual. A female is typically gravid for a small window of time for most synchronous species; the timing of the spawning season will depend on the species, geography, and a number of environmental cues (e.g., temperature, flow, photoperiod). In northern latitudes or higher elevations, spring spawning may occur slightly later and fall spawning may occur slightly earlier than in southern latitudes or lower elevations. For more information on expected spawning seasons, see Appendix B. The EPA recommends that states and authorized Tribes consult with local fish biologists, who may work at other state or authorized Tribal agencies (e.g., Departments of Natural Resources, Departments of Fish and Game), when designing sampling plans.

Reproductively mature females of most fish species, except indeterminate spawning species and viviparous species (i.e., live bearing), will produce eggs that can be sampled for selenium. However, it may be impractical to sample eggs from small-bodied fish. In this instance, the whole-body should be collected (with the eggs if the fish is female) and compared to the whole-body criterion element. Ovary tissue of synchronous spawners (e.g., species in the genus *Oncorhynchus*) typically contain oocytes that are all in the same stage of development. Fish species that spawn multiple times per season (asynchronous, e.g., some species in the family Cyprinidae) have variable cycles of oogenesis and commonly have ovary tissue that contains oocytes and eggs at all stages of development (Nagahama 1983). In these asynchronous spawners, egg maturation may occur well before, immediately prior to, or during the spawning season. For example, Green Sunfish can spawn multiple times per season (Osmundson and Skorupa 2011, Chapman et al. 2010). Thus, special care should be taken when sampling asynchronous species for egg-ovary tissue, as the pre-spawn window can be hard to predict.

Given these considerations, the EPA has the following recommendations when sampling female asynchronous spawners: (1) if the fish is too small to easily sample the egg-ovary tissue, the whole-body should be sampled (including the eggs) and the selenium concentration should be compared to the whole-body criterion element; (2) if fish are sampled during the reproductive season and they are large enough to easily sample the egg-ovary tissue, this tissue should be sampled (the 75% rule does not apply to egg-ovary composite samples (see section 2.2.1 below)); and (3) muscle tissue should not be sampled during the reproductive season as selenium may be depleted from this tissue during this time.

The egg-ovary tissue criterion element has primacy over all other criterion elements of the national CWA section 304(A) recommended selenium criterion. The EPA recognizes that many states and authorized Tribes do not currently collect egg-ovary tissue as part of their regular monitoring programs and may not have the resources to augment their existing monitoring programs to include egg-ovary tissue collection. While egg-ovary remains the preferable tissue type, whole-body or muscle tissue samples can be used as an alternative.

2.1.2 Whole-body and Muscle Tissue Samples

Whole-body and muscle tissue samples may be collected as an alternative to egg-ovary tissue. States or authorized Tribes might choose to use whole-body or muscle tissue samples because egg-ovary tissue is not available year-round and because of the difficulty with appropriately timing the collection of egg-ovary tissue. States or authorized Tribes might also choose to use whole-body samples because a small-bodied species might be the most appropriate species to sample in a particular situation, such as in a low order streams where only small-bodied species are available for sampling (Beatty and Russo 2014). In addition, states and authorized Tribes may sample whole-body or muscle tissue because existing monitoring programs can more readily incorporate such selenium analysis into their existing fish tissue monitoring strategies.

Selenium concentrations in these tissues will provide representative information on selenium bioaccumulation and ecological exposure at almost any time of the year (except pre- and post-spawn windows for females). However, there will likely be some variation across seasons due to dietary composition, temperature, depuration of selenium from tissue during vitellogenesis prior to spawning, and other factors. The only time of year that should be avoided for collecting whole-body or muscle tissue samples from female fish is directly pre- and post-spawn because they could have reduced selenium concentrations in their tissues due to the recent transfer of selenium to eggs (unless eggs are included in a pre-spawn whole-body sample) (USEPA 2021).

Summer and fall may be prime periods for whole-body and muscle tissue collection from spring spawners due to the engorgement of populations to replenish fat and energy reserves post-spawn and for over-wintering. If sampling fall spawners, spring and summer should be targeted for sampling. Winter tissue collection is discouraged, except in subtropical regions where metabolic changes due to lower water temperatures do not occur. Whole-body and muscle tissue data provide point measurements that reflect integrative dietary accumulation and deposition of selenium in fish tissues over time and space in fish population(s) at a given site.

The EPA is aware that some states and authorized Tribes use muscle plugs in their monitoring programs as a non-lethal alternative to collecting muscle fillets. States or authorized Tribes that utilize plugs should be aware of certain considerations. Contaminant concentrations can vary considerably depending on where the plug is taken from a fish. Plugs should be collected from a descaled meaty portion of the dorsal muscle tissue, between the dorsal fin and lateral line (USEPA 2019a). Waddell and May (1995) found that selenium concentrations in plugs from this location were significantly correlated to adjacent muscle tissue. Studies with mercury have also shown that this location results in a sample that has homogeneous concentrations and concentrations that were similar to mean concentrations for a muscle fillet (Cizdziel et al. 2002). Plugs provide very small tissue masses (about a gram of tissue per fish) and may not provide enough biomass for reanalysis or quality assurance/quality control (QA/QC) analysis. This may lead to difficulty confirming the quality of the sample analysis. In addition, relatively small individuals may not recover from a muscle plug biopsy punch. Care should be taken to ensure that there is enough tissue for the analytical method. States and authorized Tribes may want to consider compositing muscle plugs to attain tissue masses that are needed for analysis (see section 2.2.1 for more information on compositing samples).

In addition, states and authorized Tribes may want to establish species-specific conversion factors or regressions at the start of their sampling program that quantify the relationship between the muscle plug concentration and the muscle fillet concentration so that selenium concentrations from plugs can be appropriately compared to the muscle tissue criterion element. If taking this approach, the EPA recommends consulting a statistician about determining a sufficient sample size for establishing the regression. The EPA has also conducted a study to test the applicability of plugs for monitoring fish tissue for selenium and better define the relationship between selenium concentrations in muscle plugs and muscle fillets. The EPA found that there were no statistically significant differences between log concentrations of fillet plugs and log concentrations of homogenized fillets at the community level. However, the authors of this study did caution that when using a selenium plug monitoring alternative, samplers must employ a sufficiently sensitive analytical method and consider total solids in the tissue samples (Stahl et al. 2021).

A specific case where sampling whole-body or muscle tissue is recommended over sampling egg-ovary is for sampling anadromous juvenile Pacific salmonids. This is because the whole-body and muscle tissue of these juvenile fish are more representative of the selenium exposure of a site than the adults of these species. Anadromous fish species start their lives in freshwater, then as juveniles the fish undergo smoltification and migrate to the ocean, where they stay until adulthood before migrating back into freshwater to spawn. These species include but are not limited to Coho, Chum, and Chinook Salmon, and marine adapted Rainbow Trout (steelhead).[10] Adult anadromous females (in the genus *Oncorhynchus* – except steelhead and Brown Trout) stop eating prior to re-entering freshwater environments as part of the physiological modifications required for the migratory spawning process, and thus, lack exposure to freshwater selenium sources. They are also semelparous (except steelhead), meaning they die after spawning so there is no post-spawn residual exposure. Since adults of these species are not residents of the waterbody, the selenium concentrations will not be

[10] Before sampling these species, states and authorized Tribes should determine whether or not they are listed as threatened or endangered in the sampling location and whether a "take" permit may be required for sampling or where an alternative species should be sampled.

representative of localized freshwater selenium sources (see section 6.4.1 of the EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016*) (USEPA 2021). Given this lifecycle, it is not appropriate to sample adult semelparous anadromous fish for assessment of freshwater waterbodies, as their selenium concentrations will not be representative of selenium exposure from where they are sampled. Instead, the juvenile fish from these species should be sampled, so they will reflect local selenium concentrations. An exception are landlocked variants of Striped Bass that cannot migrate out to sea, or hybrids (e.g., "wipers" which are Striped Bass-White Bass crosses) in the Midwest. Adult fish in these landlocked populations may be representative of localized freshwater selenium concentrations, and thus appropriate for sampling. Although more uncertain, some studies indicate that selenium might affect endpoints such as juvenile growth and survival (Hamilton et al. 1990, DeForest and Adams 2011), therefore the most appropriate tissue to sample for Pacific anadromous salmon smolt is the whole-body.

Researchers are still investigating, on an ongoing basis, the impacts of reproductive strategies on fish selenium concentrations; however, it appears the reproductive strategy employed by a fish species may impact the distribution of selenium throughout a fish's tissues. This relationship, quantified by a conversion factor (CF), can be dynamic between tissue types, particularly between egg-ovary tissue and other fish tissue depending on the stage of the reproductive cycle (Conley et al. 2014). This may be especially true with asynchronous spawners, which can have oocytes in multiple stages of development at once (Conley et al 2014). Asynchronous spawners may transfer selenium more quickly to eggs and ovaries than synchronous spawners, which may make their egg-ovary to whole-body and muscle ratios more variable than synchronous spawners. In addition, the amount of selenium in muscle tissue can be limited compared to other fish tissue such as the liver (Mathews et al 2008) or egg-ovary (Herrmann et al. 2020). As selenium could be limited in muscle tissue, states and authorized Tribes may want to consider sampling whole-body tissue, rather than muscle, particularly for asynchronous spawners to capture the selenium that is distributed to other tissues.

Due to the variability in CFs within and between species, there can be times where data from one tissue type may be higher than its criterion element while data from another tissue type may be below its criterion element for the same waterbody. In these situations, the hierarchy (i.e., egg-ovary has primacy over all other tissue types) should be followed, and an implementation decision should be made based on which tissue type has primacy. This is more likely to be an issue for species that have large CFs between tissue types, such as Mountain Whitefish. If possible, egg-ovary samples should be taken from these species to make the most accurate assessment about potential selenium effects on fish.

2.2 Sample Type

States and authorized Tribes have flexibility in the type of sample that is collected to represent an instantaneous measurement of selenium in a fish population at a given site (see criterion duration footnote 6). Samples can include composites of multiple fish or the collection of individual fish in the population. The field sampling and analytical considerations for both sample types are described below.

2.2.1 Composite Samples

Composite samples are homogeneous mixtures of one type of tissue (e.g., egg-ovary, whole-body, or muscle tissue) from two or more individual organisms of the same species, collected at a particular place and time, and analyzed as a single sample. Composite samples can be useful for collecting enough tissue from small fish species to perform the appropriate analyses. Composite samples also allow for the analysis of additional target analytes if fish tissue samples are being collected as part of a broader fish tissue monitoring effort. Because chemical analytical costs are usually higher than field costs, using composite samples may be a cost-effective way to represent average selenium tissue concentrations in target species sample populations by reducing the number of individual chemical analytical samples that are needed to characterize concentrations in the sample population (Patil et al. 2011). Composite sampling may also help with the issue of determining how to incorporate a sample with a concentration below the method detection limit (MDL) into an average, as the composite represents a physical averaging of the samples (USEPA 2011). Composite samples have also been shown to provide equivalent estimates of the mean compared to individual samples (Zhou et al. 2018, USEPA 1995). However, with composite samples, extreme contaminant concentration values for individual organisms are attenuated (Patil et al. 2011). Information from each individual sample is lost, which may mean losing information about variability within a site. Also, if a set number of fish are being analyzed, compositing those fish rather than analyzing them individually will result in fewer data points, which can potentially lead to having less power in statistical analyses. However, this will be dependent on the data set and knowledge about the underlying sampling distribution. Hitt and Smith (2015) found that composites of two to four fish did not decrease power relative to individual samples when the sampling distribution was known, because the composites had greater precision for estimating the mean (but power did decrease when an empirical sampling distribution was constructed from prior research instead of being known).

The EPA's *Fish Advisory Guidance Volume 1* recommends collecting three to ten individuals for a composite sample for each target species, as availability allows (USEPA 2000). This is similar to recommendations from the National Water Quality Assessment Program administered by the U.S. Geological Survey (USGS) for collecting at least five fish, but eight, if possible, per composite (Crawford and Luoma 1993). It is also in line with the Base Monitoring Program of the Great Lakes Fish Monitoring and Surveillance Program. This program also generally includes five fish in each of its composite samples, as five represents a reasonable number of fish that also satisfy statistical requirements for that study (USEPA 2012). Section 6.1.2.7.1 ("Guidelines for Determining Sample Sizes") of the EPA's *Fish Advisory Guidance Volume 1* maintains that it is not possible to recommend a single set of sample size requirements for all fish contaminant monitoring studies (USEPA 2000). Rather, the EPA presents a more general approach to sample size determination that is both scientifically defensible and cost-effective. Table 6-1 in the EPA's *Fish Advisory Guidance Volume 1* shows the varying precision achieved by using additional numbers of individuals per composite and additional replicate composite samples. The data suggest that greater precision in the estimated standard error is gained by increasing the number of replicate composite samples than by increasing the number of fish per composite (USEPA 2000). The EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends collecting at least two composite samples at each site, and encourages a third, in order to properly estimate the site variance. An alternative sampling approach may be to collect a greater number (five or greater) of smaller composites (two to three fish), which would increase sample size and statistical power, but still minimize resource expense compared to individual sampling (USEPA 2018, Hitt and Smith 2015).

As a rule of thumb, if a state or authorized Tribe does not have previous data available for its sampling location, the EPA recommends composites of at least five fish be used for fish tissue monitoring for CWA implementation of the selenium criterion (USEPA 1982). The EPA recognizes that sometimes it might not be possible to collect a five-fish composite or collecting that many fish may impact the fish population at a location. In these cases, the EPA encourages the state or authorized Tribe to get as close to five fish as possible in the composite given resources and the fish population at a site. Given the large number of variables that go into fish collection, the EPA does not recommend including rigid minimum sample size requirements in CWA implementation programs. Rather, implementation decisions (e.g., impairment decisions) should be made based on the available data for a waterbody. The one exception is if a state or authorized Tribe adopts a PBA for translating water column criterion elements into fish tissue criterion elements. The PBA, as a criterion derivation process, includes information (such as minimum sample sizes) to ensure scientifically defensible, transparent, and repeatable results are obtained (see section 2.2.1 of USEPA 2024c).

The spatial variability of a site should also be considered when collecting composite samples. If a site is particularly large with high variability in selenium throughout, the site may need to be divided into subsites and composites collected from within each subsite to appropriately quantify the selenium impacts at that site.

The EPA recommends that fish used in a composite sample meet the following specifications:

- All the same species (USEPA 1982, USEPA 2000, Crawford and Luoma 1993, USEPA 2012);
- Of similar size (Crawford and Luoma 1993), so that the smallest individual in a composite is no less than 75% of the total length (size) of the largest individual (USEPA 2000, USEPA 2012) (the "75% rule"; this "75% rule" does not apply to fish from which egg-ovary samples are collected);
- Collected at the same time (i.e., collected as close to the same time as possible, but all samples should be collected within a week of each other) (USEPA 2000, USEPA 2012); and
- Collected in sufficient numbers to provide a composite homogenate tissue sample of at least 20 grams wet weight for selenium analysis (USEPA 2000).

The EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends including an equal number of fish in each composite sample and collecting two to three composite samples. However, when sampling fish in waters potentially impacted by selenium, the number of composite replicates may be determined on a case-by-case basis. This decision would primarily be based on the amount of variation in selenium concentration expected at the site and the number of individuals of the target species present at the site.

As species have different selenium bioaccumulation potentials and different sensitivities to selenium (USEPA 2021), it is not scientifically defensible to create a composite sample that consists of more than one species. Compositing individuals that are the same genus, but not the same species is not appropriate. Accurate taxonomic identification is essential to prevent the mixing of species in a sample.

The EPA recognizes that, in contrast to some other bioaccumulative contaminants in fish, selenium concentrations are generally conserved across fish size (May et al. 2008). But many states and authorized Tribes may add selenium monitoring to their existing fish tissue monitoring programs that assess the risk of fish consumption to human health from a number of chemicals. Many other chemicals that are assessed through this monitoring are persistent, bioaccumulative chemicals, such as mercury, polychlorinated biphenyls (PCBs), and other substances that biomagnify through the food web and build up over the lifetime of the fish, resulting in greater concentrations of chemicals in larger fish. Therefore, for these fish consumption monitoring efforts, a common guideline is that fish to be composited should be a similar size so that the smallest individual in a composite is no less than 75% of the total length (size) of the largest individual (USEPA 2000). If a state or authorized Tribe is combining their monitoring efforts for selenium and fish consumption advisories, the EPA also recommends following the "75% rule" for the sizes of individual specimens included within a composite when sampling whole-body or muscle tissue (this 75% rule recommendation does not apply to fish collected for egg-ovary samples). In addition, following the 75% rule for whole-body samples included in a composite ensures that all fish samples are making relatively equal contributions to the composite sample.

Individual fish used in a composite sample should ideally be collected at the same time (if possible) so that temporal changes in contaminant concentrations are minimized. A best practice is to collect all fish included in the composite sample within a week of each other so that the composite sample accurately reflects the selenium concentration of fish in that waterbody at that time.

The EPA recommends collecting a tissue sample of at least 20 grams wet weight (ww) for analysis, for both composite samples and individual samples. When creating composite samples from muscle tissue or egg-ovary tissue, an equal mass of homogenized tissue from each fish should be combined to create the composite, and then 20 grams ww should be sampled from the composite for analysis. To make sure that similar masses of tissue are added from each fish when creating composite samples from whole bodies, all fish included in a composite should meet the 75% rule. While the specific amount of tissue needed for analysis will be dependent on the laboratory and analytical method used for analysis, 20 grams ww is a reasonable estimate of the amount of tissue needed for typical selenium analyses. This mass allows for five grams of tissue for the selenium analysis, five grams of tissue for a matrix spike sample, five grams of tissue for dry weight analysis, and a final five grams of tissue available in case there is a problem with one of the other analyses and a procedure needs to be repeated. In addition, a sample of 20 grams ww allows for a quality control sample to be processed, which can ensure homogeneity of the tissue sample. To allow for all analyses and contingencies, 20 grams is the ideal mass to collect, although these analyses can be run on smaller quantities (6-12 grams ww). The EPA recommends discussing the mass requirements for these analyses with an analytical lab if 20 grams ww cannot be collected.

Monitoring agencies typically collect composite samples for other analytes when sampling for fish consumption advisories. If agencies currently discard or archive the composite homogenates that are in excess of their current analytical needs, the excess tissue could be used, if adequate in mass, for selenium analysis. Agencies could also collect additional tissue mass and add selenium as an analyte to their current sampling protocol. If after these analyses enough remaining tissue mass is available, agencies may want to retain tissue from the individual fish in case future analyses are needed on the individuals.

Most fish tissue samples for selenium analysis are processed as wet tissue, resulting in a selenium concentration based on wet weight. As the national CWA section 304(a) recommended selenium criterion is based on a dry weight selenium concentration, an analysis of the moisture content of the tissue needs to be performed and the wet weight concentration needs to be converted to a dry weight concentration. See Appendix C of this document for more information on how to perform a wet weight to dry weight conversion.

The EPA recognizes that if a state or authorized Tribe collects muscle plugs, they will likely be collecting sample masses of less than 20 grams ww per fish. States and authorized Tribes should ensure that the mass of tissue they are collecting and the analytical methods that they are using will allow for accurate quantification of selenium. Stahl et al. (2021) used a modification to EPA Method 200.8 that utilized an inductively coupled plasma instrument with a triple quadrupole mass spectrometer detection system (Agilent Model 8800 ICP-QQQ) to get adequate sensitivity for measuring selenium in samples with small tissue masses. Paired with the digestion method of EPA SW-846 Method 3050B (USEPA 1996), the EPA was able to effectively measure selenium in samples in the one- to two-gram ww range. This analysis will also need to include a percent moisture analysis. Compositing muscle plugs (one per fish from multiple fish) may be one way to achieve sufficient mass for analysis.

The EPA also recommends collecting an equal number of fish in each composite, as this simplifies the statistical methods needed to analyze the results from this analysis. With equal numbers of fish, the arithmetic average of the replicate composite measurements is an unbiased estimator of the population mean. When unequal numbers are used, the arithmetic average is no longer unbiased. Instead, a weighted average of the composite measurements is calculated, where the weight for each composite reflects the number of fish in each composite sample. At times, fish are lost or damaged prior to compositing. When several fish are damaged or lost, the allocation of the remaining fish to composites may be reconfigured to allow equal numbers of fish in composites. During this reconfiguration process, a sampler may be faced with the choice of either making composites of an equal number of fish or to follow the 75% rule. The EPA recommends adhering with the 75% rule over having an equal number of fish in each composite if both parameters cannot be met. If an equal number of fish cannot be included in each composite, care should be taken to adjust the statistical procedures to account for the unequal allocations (USEPA 2000).

While sampling, the EPA recommends documenting and reporting additional information about the fish samples to assist with interpretation of the data. Useful information to document includes the species, length, age (if it can be estimated), weight, and sex of the fish samples. Documenting information about the sampling site and day can also be useful for data interpretation. Samplers should note the location of collection by the Global Positioning System (GPS) if possible, sampling date, and weather. Samplers may also want to note flow rate and other characteristics about the site that may affect the concentration of selenium at the sampling sites.

2.2.2 Individual Samples

An individual sample is a discrete sample from a single fish, and can be an egg-ovary, whole-body, or muscle (fillet) tissue sample. Use of composite samples for selenium fish tissue monitoring is acceptable, but there are some instances where collecting individual fish may be desirable.

Analysis of individual fish samples may be of interest when collecting data for a site-specific study or for statistically evaluating patterns in selenium concentrations over time or space. Analysis of individual fish may also be important when evaluating selenium concentrations in critical species, where understanding potential toxicity at the individual level is important. A critical species is a resident species that (1) is commercially or recreationally important at the site; (2) is listed as threatened or endangered under section 4 of the Endangered Species Act; or (3) is a species for which there is firm evidence that its loss would yield an unacceptable impact on the site’s commercially or recreationally important species, endangered species, abundances of a variety of other species, or structure or function. Analysis of individual samples also allows for the evaluation of spatial and temporal differences among individuals of a species or across the population of a species residing in a specific waterbody.

For waterbodies or segments that are known to be impacted by selenium, individual samples describe the range of variability within a population, including characterizing extreme values. Individual samples may provide better information about selenium source-exposure relationships in large waterbodies. Individual samples may also allow for the identification of fish that are migrant or transient in a population, since those fish may have higher or lower concentrations of selenium than other fish in the area. If studies are being conducted to monitor trends, the EPA recommends sampling fish of the same species throughout the entire course of the study so that data from multiple sampling events are comparable.

If using individual samples to calculate a central tendency of selenium fish tissue concentrations, all fish should be the same species and collected from the same waterbody (or site for large waterbodies) within the same sampling period (ideally within the same week) (USEPA 1982). The samples should be of the same tissue type. Individual fish samples that are collected for the implementation of fish muscle or whole-body criterion element do not need to be constrained to the 75% rule, but samplers should be cognizant that fish may change their diets as they mature and mature fish may be migratory or more motile than small fish. Therefore, selenium concentrations may shift as fish grow due to ontogenetic shifts in fish diet (Stewart et al 2010). Thus, assessors should be cautious about calculating a central tendency from a data set with both juvenile and adult fish data.

The EPA recommends targeting at least five fish (USEPA 1982) (per waterbody or per site for larger waterbodies) for individual analysis. Greater or fewer fish samples may be needed based on the variation of selenium at a particular site. Those entities desiring greater statistical power for their analyses should collect additional fish samples. If collecting at least five individuals of one species is not possible, fewer specimens may be collected, but the statistical power of analyses will be affected (Hitt and Smith 2015). As with composite samples, the EPA recommends collecting 20 grams ww as a minimum tissue mass per individual fish for analysis and QA/QC.

Similar to the collection of composite samples, when sampling individual fish the EPA recommends documenting and reporting additional information about the fish samples to assist with interpretation of the data. Useful information to document includes the species, length, age (if it can be estimated), weight, and sex of the fish samples. Documenting information about the sampling site and day can also be useful for data interpretation. Samplers should note the location of collection by GPS if possible, and sampling date and weather. Samplers may want to also note flow rate and other characteristics about the site that may affect the concentration of selenium at the sampling sites.

2.3 Target Species

The two main factors to consider when selecting a target fish species to sample for the implementation of criteria based on the national CWA section 304(a) recommended selenium criterion are (1) a species' toxicological sensitivity to selenium; and (2) a species' bioaccumulation potential for selenium. In addition, it is important to consider a species' home range and how that will impact its ability to represent selenium exposure at a site and the species' potential for exposure to selenium within the site. The EPA recommends that states and authorized Tribes create a priority list of target species based on these factors for sampling teams. This list should identify the primary target species and alternative species if the primary species is not present, not present in sufficient numbers for sampling, or if sampling could impact the primary target species population at that site. When developing a priority list, states and authorized Tribes should also ensure that sampling does not impact any protected species (e.g., threatened, endangered) or fisheries management goals. If data cannot be acquired for a desired target species, data from any fish species can be used to make assessment decisions, as the criterion was developed for use with any fish species.

For the purposes of implementing criteria based on the national CWA section 304(a) recommended selenium criterion, a species' toxicological sensitivity to selenium, is defined as a species or a surrogate species' 10% effect concentration (EC_{10}). An EC_{10} is the concentration of a chemical that is estimated to result in a 10% effect in a measured chronic endpoint (e.g., growth, reproduction, or survival). For the national CWA section 304(a) recommended selenium criterion, a species' EC_{10} is the concentration of selenium within egg-ovary tissue that results in a 10% effect on a reproductive endpoint for that species. Based on the best available and acceptable reproductive-effect studies, as well as extensive analyses, the EPA developed a species sensitivity distribution (SSD) to support the derivation of the national CWA section 304(a) recommended selenium criterion based on a species' EC_{10} value (see Table 3.2 in USEPA 2021). The SSD for the national CWA section 304(a) selenium criterion indicates that the four most sensitive genera for fish reproductive effects (in decreasing order) are *Acipenser*, *Lepomis*, *Salmo*, and *Oncorhynchus*.

The bioaccumulation potential of a species is largely determined by its dietary composition and the exposure of its prey to selenium. Consumption of benthic invertebrates tends to drive greater selenium bioaccumulation than consumption of plankton (Schneider et al. 2015, Simmons and Wallschläger 2005, Stewart et al. 2010, Ponton and Hare 2015). Among benthic organisms, the consumption of mollusks tends to drive greater selenium bioaccumulation than consumption of other benthic invertebrates (Luoma and Presser 2009, Stewart et al. 2004). Mollusks, such as mussels and clams, can accumulate selenium to a much greater extent than planktonic crustaceans and insects due to higher ingestion rates of both suspended particulate-bound selenium (algae) and dissolved selenium from the water column through filter feeding. Mollusks can also have a lower selenium elimination rate (Johns et al. 1988, Reinfelder et al. 1997, Stewart et al. 2010). However, this tendency for greater selenium accumulation in bivalves may be more prominent in marine or estuarine habitats than in freshwater habitats (Stewart et al. 2010). The greater bioaccumulation of selenium in benthic organisms suggests that bottom feeding fish may have higher selenium levels, at least for the portion of their lifecycle that ties their energy needs to food webs with benthic organisms. Other studies (Saiki et al. 1993, Saiki and Lowe 1987) have shown that detritivores may also be exposed to high levels of dietary selenium, as high concentrations of selenium were measured in detritus.

The bioaccumulation potentials of organisms at higher trophic levels (such as piscivores) are dependent on its food chain's cumulative exposure to and bioaccumulation of selenium. Trophic transfer factors (TTFs) provide a numeric representation of bioaccumulation between a consumer and its diet. A composite TTF ($TTF^{composite}$), which is the product of TTFs at each trophic level of a consumer's food chain, represents the overall TTF for a higher trophic level organism. In addition, composite TTFs account for the proportion of different food sources in a consumer's diet. Evaluations of composite TTFs may be helpful in determining which species to target for sampling. Local influences, such as prey availability, selenium distribution, and selenium speciation, can also impact a species ultimate bioaccumulation potential at a site. The EPA recommends using site specific information to help inform the selection of target species, if available. More information about TTFs can be found in Section 3.0 of Appendix B in the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). In addition, an explanation of how composite TTFs are calculated is included in Appendix E of this document.

The sensitivity of a species indicates at what selenium concentration a species will experience reproductive effects, while bioaccumulation potential influences how much selenium a species will accumulate. The intersection of these two will determine to what extent a species is impacted by selenium at a site (i.e., the species has accumulated enough selenium to exceed its EC_{10}). As both a species' sensitivity to selenium and bioaccumulation of selenium influences whether it will be impacted by selenium, states and authorized Tribes should consider both when selecting a target species and designing fish tissue sampling plans. The EPA recommends the following prioritization scheme for selecting a target species. Other considerations, however, such as home range and habitat preferences, may also factor into a state or authorized Tribe's prioritization scheme.

1) Sample the species that has the greatest bioaccumulation potential from within the four most sensitive genera (sensitive according to the SSD from USEPA 2021).
2) If no species are present from the four most sensitive genera, sample the species with the greatest bioaccumulation potential.
3) If no species are present from the four most sensitive genera and if all species have similar bioaccumulation potential, sample the species from within the most sensitive genera present at the site.

States and authorized Tribes should begin with targeting the fish species that has the greatest bioaccumulation potential from within the four most sensitive genera. As described above, the SSD for the national CWA section 304(a) recommended selenium criterion determined that the four most sensitive genera for fish reproductive effects (in decreasing order) are *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus.* When selecting a fish species to sample from within these genera, states and authorized Tribes should consider the diet and exposure of all the species at the site that are within those four most sensitive genera and select the species that has the greatest potential to bioaccumulate selenium. For example, if a site has multiple species of *Lepomis* present (e.g., Bluegill and Redear Sunfish), the state or authorized Tribe should sample the species that has the greatest bioaccumulation potential (Redear Sunfish, depending on prey available).

When determining the bioaccumulation potential of a fish species at a site it is important to consider both the diet of that species and the prey available at the site. Many species are opportunistic feeders, so their diet will be driven by what prey are available. A species may consume mollusks if available, but they should not be considered a part of the species' diet if no mollusks are present at the sampling site. In the San Francisco Bay estuary, White Sturgeon are monitored not only because they are sensitive to the toxic effects of selenium (low EC_{10}), but also because their primary prey at that site (clams) bioaccumulate selenium very efficiently. As a result, White Sturgeon receive large doses of selenium and may be more likely to bioaccumulate selenium to levels of concern than another species. Table 3 provides a summary of these genera (highlighting information for the specific species tested), their relative sensitivity, their general habitat type (warm water (WW) or cold water (CW)), and their estimated relative bioaccumulation potential based on consideration of typical diet and trophic level. Fish that consume primarily benthic organisms will tend to exhibit greater selenium bioaccumulation than fish that feed higher in the water column at the same trophic level (Schneider et al. 2015, Simmons and Wallschläger 2005). Table 3 also provides a representative list of species that are within the same genus as the tested sensitive species and that could be considered as surrogates for tissue collection. A more comprehensive list of these surrogate species, along with details about relevant characteristics and occurrence is presented in Appendix D.

If no species from the four most sensitive genera are present at the site, then the state or authorized Tribe should target the fish species with the greatest bioaccumulation potential. As stated above, bioaccumulation potential will be predominantly determined by the diet of the species. Composite trophic transfer factors can provide a numeric representation of bioaccumulation between a consumer and its diet and help inform the decision about which species to sample.

If all species that are present at a site have similar diets and bioaccumulation potential and are not from the four most sensitive genera, then the state or authorized Tribe should target the most sensitive species. This will be the species with the lowest EC_{10}. If the EC_{10} of a particular species is unavailable, sensitivity can be estimated from the EC_{10} of a closely related taxon.

In addition to sensitivity and bioaccumulation potential, there are a number of factors that should be considered when identifying appropriate fish species for collection. The following summarizes some key points for consideration:

1) White Sturgeon (and available surrogates) are in the most sensitive genera (based on available data) and are distributed in large river systems in the U.S. These species are typically sampled by specialized monitoring programs (e.g., U.S. Fish and Wildlife Service (USFWS) and National Oceanic and Atmospheric Administration-National Marine Fisheries Service (NOAA-NMFS)). If a state or authorized Tribe would like to sample White Sturgeon, coordination with these existing programs may provide for expanded sampling opportunities or the use of existing selenium fish tissue data. Collecting and processing this species may be challenging and states and authorized Tribes should determine whether they have the appropriate resources to sample this genus. Several species and specific populations of species within the *Acipenser* genus are federally listed species under the Endangered Species Act and may not be appropriate to sample. USFWS, NOAA-NMFS, and appropriate state agencies should be consulted before sampling any federal or state listed threatened or endangered species.

2) Bluegill, and the related sunfish species in the genus *Lepomis* are widely distributed in warm water habitats, while trout species (particularly Rainbow and Brown Trout) are widely distributed in cold water habitats. These species are frequently targeted by monitoring programs in states and on authorized Tribal lands with warm water and cold water habitats, respectively, offering a possible opportunity for leveraging these existing sampling programs for the collection of tissue for selenium analysis.
3) Smaller warm and cold water systems (e.g., wadeable streams), which are not typically targeted by state and authorized Tribal fish tissue contaminant monitoring programs, are often dominated by cyprinid (minnow) species and may represent a source of fish tissue for selenium sampling in waterbodies where other species may not be present. Some of these species are shown in Table 3 and a broader range of these species are shown in Appendix D. Given the large number of minnow genera and species, and the diversity of their trophic strategies and habitats, the sensitivity and bioaccumulation potential of individual members in this diverse group should be considered when evaluating a candidate species for consideration in tissue sampling. However, state and authorized Tribal biomonitoring programs typically sample these waters for index of biological integrity metrics; therefore, their expertise and sampling program could be leveraged to target species and obtain representative samples.
4) Although generalizations can be made about the potential for bioaccumulation within fish species, when developing a sampling plan, the potential for bioaccumulation should be considered for the specific area being evaluated. Potential for bioaccumulation within any given species can vary significantly with location-specific factors, including prey type and availability, and the nature of selenium distribution in the environment.
5) There are a number of species for which toxicity data are not available, but for which dietary information could allow states and authorized Tribes to characterize the potential for selenium bioaccumulation. Some of these species are collected as target species in state and authorized Tribal monitoring programs and could be considered to characterize selenium tissue concentrations in the absence of sensitive species if available data indicate these species bioaccumulate selenium.

Along with sensitivity and bioaccumulation, it is also important to consider how a species' habitat preferences, feeding regimes, and/or home ranges will affect their selenium exposure. Species with smaller home ranges (typically smaller-bodied fishes or juveniles) may be at risk of greater selenium exposure if the elevated selenium is localized to their home range or their prey's home range. Species with larger home ranges may not represent local selenium exposure. States and authorized Tribes should target species with limited motility and home ranges that closely match the site being evaluated, so that the fish reflect exposure to selenium at that particular site. Local fish biologists may be able to help states and authorized Tribes identify species' habitat preferences, feeding regimes, motility, and home ranges. If possible, migratory species and highly motile species should be avoided. Highly motile fish species, such as potamodromous and anadromous species, could travel back and forth between areas with low and elevated selenium concentrations, resulting in variable selenium tissue concentrations (Beatty and Russo 2014). It is possible that typical selenium exposure concentrations of migratory adults would be lower than concentrations at rearing grounds; therefore, these fish

Table 3. Potential Target Species for Collection Based on Selenium Sensitivity and Bioaccumulation Potential.

Sensitivity Ranking (Based on Egg-Ovary EC_{10})	*Genus*, Common Name	Habitat (WW/CW)	Bioaccumulation			Additional Representative Surrogate Species[b]
			Expected Relative Bioaccumulation Potential	Adult Diet	Trophic Level (TL)	
1	*Acipenser,* White Sturgeon[a]	WW	Medium-High	Invertivore Piscivore Molluscivore	TL3/TL4	Shortnose Sturgeon[a], Lake Sturgeon[a]
2	*Lepomis*, Bluegill	WW	Medium	Invertivore Piscivore	TL3	Pumpkinseed[a], Redear Sunfish[a], Green Sunfish[a], Redbreast Sunfish[a], Longear Sunfish, Warmouth, Orangespotted Sunfish, Redspotted Sunfish, Bantam Sunfish, Northern Longear Sunfish, Spotted Sunfish
3	*Salmo,* Brown Trout[a]	CW	Medium-High	Invertivore Piscivore Molluscivore	TL3/TL4	None
4	*Oncorhynchus*, Rainbow Trout	CW	Medium-High	Invertivore Piscivore	TL3/TL4	None
	Oncorhynchus, Cutthroat trout	CW	Medium	Invertivore	TL3	None
5	*Micropterus* Largemouth Bass	WW	Medium – High	Invertivore Piscivore	TL4	Smallmouth Bass, Spotted Bass, Redeye Bass
Not Ranked	*Pimephales* Fathead Minnow[c]	WW	Low-Medium	Herbivore Invertivore	TL2/TL3	WW: Satinfin Shiner, Red Shiner, Golden Shiner[a], Bull Chub[a], Creek Chub, Roundtail Chub, Thicklip Chub[a], Striped Shiner, Central Stoneroller, Blacknose Dace, Speckled Dace, Cutlips Minnow[a], River Darter[a], Arkansas Darter[a]
						CW: Redside Shiner, Peamouth Chub, Mottled Sculpin

WW = warm water, CW = cold water, TL = trophic level

[a] Fish species that consume mollusks (e.g., clams, mussels, snails) as part of their diet and are anticipated to have relatively high bioaccumulation potential. For Brown Trout, molluscivory is incidental and will likely only be significant on a site-specific basis where mollusks are abundant.

[b] Species are surrogates for sensitivity based on taxonomic relationships.

[c] Fathead minnow is a surrogate for small-bodied fish species inhabiting wadeable streams.

may not reflect selenium concentrations that other fish species would experience at their time of spawning. Given this issue, resident species should be the first choice when selecting a target species. Recently stocked fish should also be avoided, regardless of species, since their residence time before sampling may be too short to provide a representative sample. The EPA recommends speaking with local fish and game authorities to understand stocking frequency in waters where fish are being sampled for selenium analysis. If stocked fish must be sampled, the EPA recommends sampling fish regularly after their addition to the waterbody to see when their selenium concentrations stabilize. Before this stabilization point, it would not be appropriate to use stocked fish for implementation purposes.

If migratory or highly motile species must be sampled, the EPA recommends that sampling plans account for the life history of these species so that the correct locations for sampling within a watershed are selected. Before sampling these species, knowledge about the migratory extent of these species and the time spent in each location should be known so that the influence of selenium at each habitat location can be accounted for. Depending on the species, it may be possible to target sampling during a non-migratory phase for the species. At times, additional studies may be needed to determine where selenium exposure occurs for these species. The EPA recommends consulting local fish biologists for information about the migratory patterns of local fish populations and considering this information in making a target species selection and determining when sampling will occur. If Pacific anadromous species are selected as target species to be used for sampling, the EPA recommends that states and authorized Tribes sample the whole-body of juveniles and compare the concentration to the whole-body criterion element. This recommendation is due to the lack of selenium exposure to adult salmonids from freshwater prior to reproduction (see section 6.4.1.1 in USEPA 2021).

One or more species that is/are sensitive to selenium (e.g., Bluegill, Rainbow Trout, Brown Trout) are commonly present in waterbodies. However, if selenium-sensitive species or species that potentially bioaccumulate high levels of selenium are not available in sufficient numbers to sample, then another species that is available in sufficient numbers may be used for fish tissue monitoring, including species known to be tolerant to selenium. These selenium tissue samples can still be compared to the appropriate tissue criterion elements (see Table 1), which are designed to be protective of the entire aquatic community and can still be used to make an assessment decision. In addition, if fish tissue data from other fish species than the ones recommended in this section were collected for an alternate purpose, those data can also be used for an assessment decision.

As there are many considerations involved in the selection of a target species, the EPA recommends that sampling teams develop a sampling priority plan before going into the field. This plan should start by identifying the species and factors that will increase selenium exposure at a site. From there, an initial target species should be selected and any decisions about sampling locations can be made. The plan can then identify a prioritized list of species that the state or authorized Tribe will sample if the primary target species is not present in sufficient numbers or if sampling could impact the primary target species population at that site. The EPA recommends that the plan also include what type of sampling gear is required for the collection of each species. In addition, the plan should specify if collection permits are required for fish tissue sampling, and if so, include instructions for acquiring them before the sampling event. Having a clear plan will ensure that the fish collected will provide the most representative data of selenium conditions at the site.

States and authorized Tribes may want to limit the number of target species that are sampled in their state or authorized Tribal waters. The use of a limited number of target species allows for the comparison of fish contaminant data across sites over a broad geographic area. It is difficult to compare contaminant monitoring results within a state or authorized Tribe or among states or authorized Tribes unless the data are from the same species because of differences in habitat, food preferences, and rate of contaminant uptake among various fish species. Limiting the number of species collected across a state or authorized Tribal waters could allow for the better comparison of contaminant data from across a state or region. Given this, the EPA recommends that states and authorized Tribes evaluate the range of sensitive species with high bioaccumulation potential across their state or Tribal waters and determine which ones may be able to be sampled at multiple locations across the state or Tribal lands.

If a state or authorized Tribe is sampling fish tissue for the development of a site-specific water column criterion element, the state or authorized Tribe only needs to sample one species but may want to consider expanding the sampling to include multiple species to better understand the system at the site. The species that is sampled should have both high bioaccumulation potential and high sensitivity to selenium (as described earlier in this section). Sampling additional species that have high bioaccumulation potential, as well as species with high sensitivity to selenium, could provide a more complete picture of selenium dynamics at the site.

Lastly, care should be taken to avoid sampling federal-, state-, and Tribal-protected, threatened, or endangered species when selecting a target species. For example, although *Acipenser*, *Salmo*, and *Oncorhynchus* are three of the four most sensitive genera, many species within these genera are listed as threatened or endangered under the federal Endangered Species Act and thus, are not suitable for sampling. Before sampling from these genera and other genera with federally listed and other protected species, the EPA recommends speaking to local fish biologists and other relevant government entities (e.g., USFWS, NOAA-NMFS, state and authorized Tribal wildlife agencies). When conducting monitoring to ensure these species are protected, states and authorized Tribes should target surrogate species that have similar taxonomy (preferably at the genus level), diets, and trophic levels. Species with similar taxonomy, diets, and trophic levels should have similar selenium sensitivity and bioaccumulation as the threatened or endangered species. If a taxonomically similar surrogate is not present, then states and authorized Tribes should target a species with a similar diet and trophic level.

2.4 Sampling Locations

Several factors should be considered when selecting where fish should be sampled (to be analyzed either individually or as a composite) to accurately characterize the concentration of selenium at a site of interest. The spatial extent of the site should be defined and the factors that may affect selenium variability throughout the site should be identified so that they can be considered in the design of the sampling plan. The selection of a site and how its boundaries are defined will be influenced by the objective of the monitoring and by past monitoring activities (see section 3.0 *Leveraging Existing Fish Tissue Monitoring Programs and Sample Design*). The factors informing the site definition and the subsequent selection of sampling locations will vary, and may include:

- Monitoring objectives (e.g., assessment, site-specific selenium studies);
- Waterbody type and site hydrology (lotic vs. lentic);
- Waterbody size;
- Selenium sources and their location;
- Aquatic habitat variability; and
- Physical barriers to fish movement.

These factors (and potentially others) will influence the definition of the site boundaries, decisions about where fish are collected from within the site, and decisions about how many fish need to be collected at the site. Sections 2.4.1 – 2.2.4 discuss a non-exhaustive set of factors that the EPA recommends states and authorized Tribes consider when selecting sites and sample locations; however, particular situations may warrant the consideration of other important factors.

2.4.1 Waterbody Type

Selenium concentrations and bioaccumulation patterns are different in lotic (flowing waters, such as rivers and streams) versus lentic (very slow moving or still waters, such as lakes and reservoirs) environments. Water residence time is typically shorter in lotic systems than in lentic systems, and subsequently, aquatic organisms living in lentic systems tend to bioaccumulate proportionately more selenium than organisms living in lotic systems (ATSDR 2003; EPRI 2006; Luoma and Rainbow 2005; Orr et al. 2006; Simmons and Wallschlägel 2005). In addition, lentic waterbodies tend to have greater reducing conditions (conditions that lead to reduction reactions and reduced ionic species of selenium, such as selenite), which create an environment where selenium accumulates in sediment more readily and may also lead to higher bioavailability in the water column (Luoma and Rainbow 2008, Simmons and Wallschlägel 2005). Benthic organisms in lentic systems can then be exposed to higher concentrations of selenium in the sediment, leading to increased bioaccumulation potential in other organisms feeding on the benthic organisms (Simmons and Wallschläger 2005, Orr et al. 2006). For example, Hillwalker et al. (2006), found that the body burden concentrations of selenium in insects within similar taxa were up to seven times greater in lentic systems than lotic systems within the same watershed.

When sampling fish, consideration should be given to the different flow characteristics of the site that is being sampled along with the locations where fish are feeding and obtaining their selenium body burdens. Some areas of a lotic site may have lentic characteristics and vice versa. For example, some rivers may have slow moving pools or backwaters that have characteristics similar to lentic environments. Human-made lakes and reservoirs may have some features that are intermediate between typical lotic and lentic systems. For example, reservoirs tend to be longer and narrower than natural lakes, and generally have a shorter water retention time than a natural lake of comparable volume (Thornton et al. 1990). When sampling sites, attempts should be made to sample all habitat types to appropriately characterize the range and distribution of selenium concentrations at a site.

2.4.2 Waterbody Size

Generally, the variability of selenium within resident fish populations would be expected to be low when the spatial and temporal variability of selenium concentrations across all compartments of the ecosystem are low (e.g., water column, sediment). As the area of a site increases, the spatial and temporal variability is expected to also increase, thus increasing the number of samples needed to characterize the selenium concentrations in the resident fish populations. If the waterbody is sufficiently large that sub-populations are expected to be present (potentially applicable to large reservoirs), it could be advantageous for the sub-populations to be represented separately in the data set.

2.4.3 Site-specific Studies for Water Column Translations

If a site-specific water column criterion element is being developed, a study should be designed that captures data that appropriately reflect the whole site (i.e., captures spatial, temporal, and habitat variability). To support the development of a site-specific water column criterion, data beyond what are necessary for other CWA implementation purposes (e.g., listing) are typically necessary (e.g., additional sampling locations, sampling times, species of fish, and/or selenium measured in multiple matrices) to ensure that the designated use is appropriately protected by the site-specific water column translation. The extent of the sampling and type of data collected will depend on the size and complexity of the site. It will also depend on whether there are any discharges of selenium into the site. A “site” may be a state, region, watershed, waterbody, segment of waterbody, category of water (e.g., ephemeral stream), etc. Regardless of how the site is defined, the site-specific water column translation should be derived to protect aquatic life for the entire site, including both areas upstream and downstream of a discharge if one is present at the site, in accordance with CWA section 303(c) and the EPA’s implementing regulations at 40 CFR Part 131. To ensure protection for aquatic life throughout the entire site, fish should be sampled from locations where selenium is expected to bioaccumulate the most (areas of the site with more lentic properties and areas where selenium may be elevated due to source contributions). In addition to sampling from the area of greatest exposure, states and authorized Tribes may sample fish from various locations at the site to understand the dynamics of selenium within that site. With that knowledge, the site-specific water column translation can generally be designed to be protective of the most vulnerable fish community. If selenium concentrations are significantly different between different locations within a site, the state or authorized Tribe may consider whether the site should be broken into two sites. In addition, a site-specific water column criterion element would need to be protective of downstream waters (40 CFR 131.10(b)).

Additional information related to sampling fish tissue to support a site-specific water column translation can be found in Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021) and in *Technical Support for Adopting and Implementing the EPA's 2016 Selenium Criterion in Water Quality Standards* (USEPA 2024c). One example of a method for conducting a site-specific water column translation can be found in the *Draft Translation of Selenium Tissue Criterion Elements to Site-Specific Water Column Criterion Elements for California Version 1, August 8, 2018* (USEPA 2018). As there are a large number of factors to consider when designing a site-specific study, the EPA strongly recommends engaging with the EPA regional offices early in the development of a site-specific water column translation to discuss study design and data needs.

2.4.4 Point Sources

When selecting sampling locations, samplers should consider where and how selenium is entering a waterbody and determine whether exposure is relatively equal throughout the waterbody or if some sections of the waterbody have greater exposure. The sampling objectives will provide direction on how known sources (e.g., discharge, tributary with elevated selenium, irrigation return canal, groundwater discharge) and any associated mixing zones (if allowed under state or authorized Tribal water quality standards) should be taken into consideration when collecting fish tissue samples. Two potential objectives and the associated sampling recommendations are explored here.

When the objective is to collect data to support waterbody assessment decisions, the goal is to measure the central tendency of the selenium concentration in the target population throughout the sampling reach. Therefore, when a point source is located within the defined sampling reach, states and authorized Tribes should not avoid sampling fish from areas of incomplete mixing resulting from a discharge or tributary. Given the mobility of many fish taxa, it is reasonable to expect that fish freely move in and out of areas of incomplete mixing when the conditions do not elicit an avoidance response (e.g., due to chemical or temperature gradients). In some discharge situations, fish can be attracted to the effluent and spend a significant portion of their time in the area of incomplete mixing. Also, depending on their life history, some fish species have a limited mobility range and may spend more time in the area of incomplete mixing if it overlaps with their territory, breeding grounds, or feeding grounds. Ultimately, states and authorized Tribes should consider and document if there are any reasons to avoid tissue collection from a location adjacent to a known point source prior to sampling (e.g., conditions are not representative of the rest of the segment). States and authorized Tribes may also want to consider whether further segmentation of an assessment unit into upstream and downstream segments is warranted due to the presence of a discharge.

When the sampling objective is to characterize the contribution of selenium that a known point source(s) is making to the waterbody, samples collected upstream and downstream of the point source(s) should be assessed independently (i.e., not composited or averaged). The downstream sampling reach should be large enough to include samples collected within and downstream of areas of incomplete mixing to characterize the range of bioaccumulation potential in the tissue samples as the water column concentrations decrease. One way to do this would be to collect fish and water samples at regular intervals from the discharge to observe how the selenium concentrations in both types of samples decrease downstream of the discharge. It is important to understand the hydrology in the system as this will influence the range and direction of transport of selenium from the discharge source(s) to other portions of the waterbody/site. It is also important to understand the ecology of that target species to understand their home range and potential for exposure. For more information on considerations related to selenium sources and the locations of those sources, see section 2.1 in *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021).

3.0 Leveraging Existing Fish Tissue Monitoring Programs and Sample Designs

3.1 Augmenting Existing Fish Tissue Monitoring Programs

Many states and authorized Tribes have existing fish tissue monitoring programs that can be leveraged to collect fish tissue data to assess against the fish tissue criterion elements of criteria based on the EPA's 2016 national CWA section 304(a) recommended selenium criterion. In 2010, 45 states monitored chemical contaminants in fish tissue to assess risks to human health. Twenty-eight states identified selenium as a contaminant in their human health monitoring programs (USEPA 2011). These states can potentially modify their current fish tissue monitoring programs to not only assess human health risks, but also assess attainment of the aquatic life selenium criterion.

The design of an agency's existing fish tissue monitoring program will likely drive its approach to selenium monitoring. Agencies should evaluate how current sampling and analytical protocols can be modified to meet both the objectives of fish tissue monitoring for risk to human health and aquatic life protection. In the following sections, the EPA provides case studies with examples of programs that might have the capacity and framework to augment their existing monitoring strategies to include fish tissue monitoring for the selenium aquatic life criterion.

3.1.1 Consistency with Existing Programs

The EPA recommends states and authorized Tribes evaluate current fish tissue monitoring programs to determine how they can be augmented to implement criteria based on the national CWA section 304(a) recommended selenium criterion. To the extent possible within a state or authorized Tribal program, the EPA recommends that fish tissue monitoring for the selenium aquatic life criterion should be consistent with the state or authorized Tribe's current fish tissue monitoring practices regarding spatial and temporal considerations, species collected, and sample type collected. In this way, logistical modifications to a state or authorized Tribe's fish tissue monitoring program can be minimized. Muscle tissue is the most common type of sample collected and analyzed by monitoring programs. Less frequently, states and authorized Tribes collect and analyze whole-body samples. To maximize efficiency, a portion of these samples can be submitted for selenium analysis. States and authorized Tribes can realize cost efficiencies by choosing to use whole-bodies or muscle tissue (fillets) that are already being collected for an existing monitoring program.

When using existing sampling programs that are designed for human health protection to assess selenium levels for protection of aquatic life, states and authorized Tribes should recognize the potential limitations of these data. Those data may not represent the area's most likely to be contaminated by selenium, the most relevant time periods for sampling, and/or the most appropriate species. State and authorized Tribal programs may need to alter their typical monitoring protocols due to spatial, temporal, or species/sample type considerations specific to selenium. To address these additional sampling needs, states and authorized Tribes may be able to leverage expertise and logistical assistance from other agencies with existing fish tissue monitoring programs. Since the EPA's national CWA section 304(a) recommended selenium criterion applies to ecological risk and not human health, monitoring agencies could evaluate their target species list and determine if they include appropriate species for assessing selenium risk to aquatic life. See the discussion in section 2.3 about selecting target species and see *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater* (USEPA 2021) for more information about species' sensitivity and bioaccumulation potential.

A survey conducted in 2010 reported that 40 state agencies conduct fish sampling at regular intervals, and several conduct statewide, rotating basin sampling programs over a multi-year period (USEPA 2011). Many states and authorized Tribes may be able to use their current fish tissue sampling programs to monitor for the selenium criterion as well. Agencies could monitor state- or basin-wide, and track progress in individual basins relative to other areas. Regular yearly sampling could be conducted, with intensified sampling in targeted basins as needed (see Table 4 for several documents that provide guidance for sampling and survey designs). Several states use a probabilistic survey design to select sampling sites. This type of sampling design can produce estimates that represent the condition of the whole watershed, and an estimate of random spatial variability (USEPA 2000). Probability-based sampling provides the basis for estimating the resource (i.e., fish population(s)) extent and condition, for characterizing trends in resource extent or condition, and for representing spatial patterns, all with known certainty (USEPA 2009). Additional or more targeted sampling approaches may be needed in areas where elevated selenium is associated with a known point of discharge. The case study below presents the Kansas Department of Health and the Environment's (KDHE) fish tissue monitoring program, which uses several designs for selecting sites. Based on the information available, it is likely that a state or authorized Tribe with a similar program could take advantage of their current sampling strategy to perform screening level selenium analysis throughout their state or Tribal area. Where selenium is already a primary parameter of interest, the state or authorized Tribe may have data to support more intensive studies in certain waterbodies.

CASE STUDY: The Kansas Department of Health and the Environment

The Kansas Department of Health and the Environment (KDHE) currently collects fish samples annually from 30-50 fixed and rotating stations. The KDHE selects sites based on targeted, census, and probability-based sampling designs. Specific sub-program objectives determine the numbers, species, and sizes of fish collected from a particular waterbody, and the tissues and parameters of interest (https://www.kdhe.ks.gov/1268/Fish-Tissue-Contaminant-Monitoring-Progr).

Highlights:

- Whole fish, muscle, muscle plugs, or other specific tissues were collected for different programs;
- Selenium was a primary parameter of interest; and
- Specific tissues (such as egg-ovary) were analyzed for specific chemicals of concern known to accumulate in certain organs.

This program is an example of an existing fish sampling program that could be enhanced to collect data for the implementation of a state or authorized Tribal criterion based on the national CWA section 304(a) recommended selenium criterion.

https://www.kdhe.ks.gov/DocumentCenter/View/11902/Fish-Tissue-Contaminant-Monitoring-Programs-Quality-Assurance-Management-Plan-PDF

3.1.2 Temporal Considerations

Various temporal considerations will influence fish tissue monitoring strategies for selenium. These can include considerations related to the ecology of the fish (e.g., species' spawning season) or to abiotic environmental factors (e.g., weather conditions and river flows). Temporal considerations will influence decisions regarding which tissue type is sampled: egg-ovary, whole-body, or muscle tissue. For example, most fish species that are synchronous spawners spawn in the spring, making spring the prime season to sample egg-ovary tissues, yet sampling for health advisories is typically done in the fall when concentrations of contaminants are highest in muscle tissue (fillets). Agencies will need to consider their resources and determine which fish tissue type they would like to sample and at what time of year. If agencies plan to sample egg-ovary tissue, they should plan to sample it right before spawning. If an agency plans to conserve resources and sample for both fish consumption advisories and the selenium criterion at the same time, whole-body or muscle tissue should be sampled outside of species-specific pre- and post-spawning windows. In this case, muscle (or whole-body) tissue can be composited and evaluated for the selenium aquatic life criterion in addition to contaminants of interest for fish consumption advisories. If the agency has information indicating that there may be seasonal differences in whole-body or muscle tissue concentrations, then agencies should plan to sample during the season when the highest selenium concentrations are expected. Agencies may want to sample spring spawners in late summer or fall to avoid the potential for underestimating selenium body burdens. Selenium body burdens can be decreased directly post-spawn due to the selenium depuration from the body via the maternal transfer of selenium to eggs and the subsequent release of eggs to the environment.

For egg-ovary tissue sampling, the EPA recommends that agencies with fish tissue monitoring responsibilities consult with local fisheries biologists to determine the appropriate time for sampling specific species in their region to capture the specimens in their pre-spawning phase. These regional experts will be familiar with the local species and are able to use their best professional judgment to determine which species are appropriate for egg-ovary sampling and the appropriate sampling period based on spawning season.

3.1.3 Spatial Considerations

Monitoring agencies generally target high-use fishing areas, areas of special concern, and areas of suspected contamination (such as waterbodies where fish advisories have been issued), when selecting sites for sampling fish tissue (USEPA 2011). As current fish tissue monitoring programs are typically designed to specifically address the risk to human health from fish consumption, these programs predominantly sample locations where fishing is common. This may lead to mostly sampling lakes and higher order streams. States and authorized Tribes using this sampling design should consider if these existing programs will adequately capture waterbodies impacted by point and non-point sources of selenium and potential areas of selenium contamination. If not, agencies may want to modify sampling designs to target such areas for sampling.

Some states and authorized Tribes may incorporate fish tissue sampling for selenium into a statistical survey designed to understand the distribution of tissue concentrations across the state or Tribal lands. The underlying geology of a region may produce elevated selenium concentrations in certain areas and make nearby waterbodies prone to selenium bioaccumulation, particularly if anthropogenic activities increase the release of selenium into the system. This should be kept in mind when selecting sites, and when analyzing data from these areas (Beatty and Russo 2014).[11]

Additional sampling locations may need to be added to a current fish tissue monitoring program that are outside of areas that are typically targeted due to fishing use when sampling for the assessment of the selenium aquatic life criterion. For example, mine runoff may elevate selenium concentrations in headwater streams, which may not be normally targeted for fish tissue monitoring. Agencies should also consider a species' home range in relation to the location of a known selenium source (e.g., the migratory patterns of a certain species versus the location of a power plant on a reservoir). It is also important to consider the relationship of an upstream source to downstream habitats, particularly when downstream habitats have characteristics that will lead to greater selenium bioaccumulation (e.g., lentic systems). Monitoring plans may need to be adjusted to reflect the species of fish available in a waterbody (e.g., small streams), temporal issues (e.g., spring flood/safety, low flow), and the types of appropriate sampling gear.

The monitoring strategy in the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) discusses two tiers of studies used to identify locations where fish consumption advisories may be needed. Information from these studies may be utilized to develop selenium specific monitoring programs for the assessment of the aquatic life criterion. Tier 1 studies are screening studies that evaluate a large number of sites for chemical contamination with few samples per site. These would be most useful for waterbodies or regions where there are no known or expected selenium problems. Screening studies can help states and authorized Tribes identify those sites where selenium concentrations are elevated relative to other waterbodies. Information from screening studies can be used to prioritize waterbodies for future fish tissue monitoring, thus enabling resources to be used more efficiently. For example, waterbodies with fish having low selenium concentrations may be monitored less frequently in the future, while waterbodies with fish having selenium concentrations at or near the tissue criterion elements may be prioritized for more frequent or more intensive monitoring. In addition, data collected during these screening studies can be used to inform assessment determinations for the waters where the samples were collected.

Tier 2 studies are intensive studies of areas identified as potential problems in screening studies. The purpose of a Tier 2 study is to determine the magnitude of chemical contamination in sensitive fish species, and to assess the geographic extent of the contamination. If a Tier 2 study is being conducted for selenium, fish species from a sensitive genus with high bioaccumulation potential should be sampled either in addition to or in place of sensitive species. Agencies will typically use Tier 2 studies to determine the overall magnitude and variability of a specific contaminant that was found at elevated levels during a Tier 1 study. In many areas, selenium sources have been well characterized; in these areas a screening study may not provide any additional information that would change the course of the investigation.

[11] See the USGS's map of selenium concentrations in soils and stream segments at https://mrdata.usgs.gov/geochem/doc/averages/se/usa.html.

At these sites, it may be most useful to move directly to an intensive study designed to capture the magnitude and geographical extent of the selenium contamination in fish tissue. These studies may be helpful as a basis for developing a site-specific water column criterion element, if relevant.

3.2 Existing Resources and Information

3.2.1 Available Expertise

Within each state or authorized Tribe, the agency that develops the WQS and the agency that typically conducts fish sampling may not be the same. When designing sampling plans to assess the selenium aquatic life criterion, agencies with experience in the development and execution of fish sampling programs can be consulted to aid in designing an effective fish tissue monitoring plan. State and authorized Tribal agencies should also determine whether there is any overlap in current sampling efforts. Various state and authorized Tribal (e.g., Department of Natural Resources) and federal agencies (e.g., EPA, USFWS, NOAA-NMFS, USGS) have expertise in fish sampling, biology, and ecology, and may be able to assist with designing a sampling plan.

All states and many authorized Tribes and interstate commissions have established biological assessment programs, and most have fisheries biologists and managers. This could provide a state or authorized Tribe with the capacity to establish or modify existing fish tissue monitoring programs to facilitate implementation of the fish tissue-based criterion elements of criteria based on the national CWA section 304(a) recommended selenium criterion. In addition to individual state and authorized Tribal agencies and local expertise, federal (e.g., USFWS) and state and authorized Tribal resource agency collaborations could be used, as necessary, to help fill in data gaps and provide supporting data. By using all available resources for information and expertise, monitoring agencies should be able to:

- Identify potential sites/locations, waterbodies, and watersheds for selenium sampling beyond the coverage of current monitoring programs;
- Design an appropriate monitoring strategy (including selection of fish tissue type and sample type (i.e., individual or composite samples));
- Select target species;
- Identify pre-spawning periods; and
- Procure analytical support.

The case study below presents Minnesota’s Fish Contaminant Monitoring Program, which is implemented through a collaborative partnership of four state agencies to maximize available expertise. Based on the available information, a state or authorized Tribe with a similar collaborative program could take advantage of their joint resources to devise the most efficient approach for adding selenium to their current fish tissue monitoring strategy. They could also use their extensive database to determine where to conduct more intensive studies.

CASE STUDY: Minnesota's Fish Contaminant Monitoring Program

Minnesota's Fish Contaminant Monitoring Program is implemented through a partnership of Minnesota Departments of Natural Resources, Health, and Agriculture and the Minnesota Pollution Control Agency (MPCA). The data are used to issue fish consumption advisories, identify impaired waters, research mercury cycling, and document long term trends for PCBs and mercury.

Highlights (MPCA 2008, P. McCann, personal communication, May 7, 2018):

- Approximately 130 lakes and river sites are sampled annually;
- The Fish Contaminant Monitoring Program database contains over 52,000 data records; and
- As of 2016, the program has sampled 1,410 lakes of the estimated 5,500 fishing lakes in the state.

This program is a robust example of how interagency cooperation can maximize available expertise, resources, and cost effectiveness.

https://www.health.state.mn.us/communities/environment/fish/techinfo/index.html

3.2.2 Existing Guidance

The EPA and other stakeholders have produced numerous documents on bioassessment techniques. Specific sections of these documents contain information that may be helpful in developing guidelines for sampling fish for selenium fish tissue analysis, particularly for species like cyprinids, which are not typically targeted by state and authorized Tribal monitoring programs. For example, *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish - Second Edition Chapter 3* (Barbour et al. 1999) provides guidance and information on the elements of biomonitoring, including seasonality and methods for fish collections.

If sampling for both the selenium criterion and for fish consumption advisories, the EPA's *Fish Advisory Guidance Volume 1* discusses study design considerations and the major protocols that must be specified for fish collection, such as site selection, analyte screening values, sampling times, sampling type, and QA/QC.

Also, the EPA's *Fish Advisory Guidance Volume 1* provides useful information on the collection of whole-body and muscle tissue samples. Specifically, section 7.2.2 of the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) includes detailed directions for preparing muscle and whole-body samples. The limitation to this guidance is that it was developed specifically for assessing human health risks associated with consumption of fish and shellfish. As a result, there are aspects of implementing the aquatic life selenium fish tissue-based criterion that are not specifically addressed by the EPA's *Fish Advisory Guidance Volume 1* (e.g., fish egg-ovary sampling, ecological risks). A selection of recommended documents for additional guidance is presented in Table 4.

Table 4. Recommended Documents for Additional Guidance.

Title	Author	Link
Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis[1]	USEPA 2000	https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf
Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish - Second Edition	Barbour et al. 1999	https://www.epa.gov/sites/production/files/2019-02/documents/rapid-bioassessment-streams-rivers-1999.pdf
Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue[1]	USEPA 2002a	http://www.epa.gov/sites/production/files/2015-07/documents/fish-study-fieldplan.pdf
The National Study of Chemical Residues in Lake Fish Tissue (Final Report)[1]	USEPA 2009	https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005P2Z.PDF?Dockey=P1005P2Z.PDF
Concepts and Approaches for the Bioassessment of Non-Wadeable Streams and Rivers	Flotemersch et al. 2006	https://nepis.epa.gov/Exe/ZyPDF.cgi/600006KV.PDF?Dockey=600006KV.PDF
Guidance on Choosing a Sampling Design for Environmental Data Collection	USEPA 2002b	http://www.epa.gov/sites/production/files/2015-06/documents/g5s-final.pdf
Spatially Balanced Survey Designs for Natural Resources. Design and Analysis of Long-Term Ecological Monitoring Studies	Olsen et al. 2012	https://www.cambridge.org/core/books/design-and-analysis-of-longterm-ecological-monitoring-studies/spatially-balanced-survey-designs-for-natural-resources/F06C6F53022E46D694D1233782D5F274
Spatially Balanced Sampling of Natural Resources	Stevens and Olsen 2004	https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.files/fileID/13339
Application of Global Grids in Environmental Sampling	Olsen et al. 1998	https://archive.epa.gov/nheerl/arm/web/html/abolsen98.html
National Rivers and Streams Assessment 2018/19: Field Operations Manual – Non-Wadeable[1]	USEPA 2019a	https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_nonwadeable_version_1.2.pdf
National Rivers and Streams Assessment 2018/19: Field Operations Manual – Wadeable[1]	USEPA 2019b	https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_wadeable_version_1.2_0.pdf

Title	Author	Link
National Coastal Condition Assessment 2015 Field Operations Manual[1]	USEPA 2015	https://www.epa.gov/sites/production/files/2016-03/documents/national_coastal_condition_assessment_2015_field_operation_manual_version_1.0_1.pdf
Biomonitoring of Environmental Status and Trends (BEST) Program: Field Procedures for Assessing the Exposure of Fish to Environmental Contaminants	Schmitt et al. 1999	https://www.cerc.usgs.gov/pubs/center/pdfDocs/91116.pdf

[1] Fishing sampling in these references are designed specifically for assessing risk to human health through fish consumption.

3.2.3 Using Existing Data to Enhance Selenium Monitoring

The EPA recommends states and authorized Tribes consider and use all available data, as appropriate, to inform and enhance selenium monitoring. According to the EPA's *2010 Fish Advisory Survey Report*, 28 states identify selenium as a contaminant in their fish monitoring program (USEPA 2011). Several states have conducted extensive statewide assessments and could have existing selenium data. Other organizations may also have selenium data available. For example, the Ohio River Valley Water Sanitation Commission collects fish tissue samples for selenium analysis as part of their Fish Consumption Advisory Program and has data available online (http://www.orsanco.org/fish-tissue). National scale data sources for selenium in fish tissue samples include the EPA's *2008-2009 National Rivers and Streams Assessment* (available at https://www.epa.gov/national-aquatic-resource-surveys/national-rivers-and-streams-assessment-2008-2009-results) and the National Listing of Fish Advisories Fish Tissue Search database (available at https://fishadvisoryonline.epa.gov/FishTissue.aspx). The EPA also has concentration data available from 100 paired mercury and selenium fish fillet samples collected in 2007 (available at http://www.epa.gov/sites/production/files/2015-07/mercury-finaldata2012.xlsx). Sample sites for this 2007 study were randomly selected from U.S. locations where mercury advisories for fish consumption were in place at the time of sampling. Available data can be used to conserve limited resources by providing baseline information which can inform future collections by indicating which areas may and may not need additional monitoring.

4.0 Sample Analysis

4.1 Analytical Chemistry

Fish tissue sampling to support implementation of criteria based on the national CWA section 304(a) recommended selenium criterion should address many of the same analytical concerns as those associated with other fish tissue monitoring programs. Various researchers have shown that analytical results on the same population of fish can differ between studies and even within studies. These uncertainties, inherent in any sampling program, can be minimized through a rigorous study design, clear data quality objectives, meticulous QA/QC protocols, and careful execution of the monitoring program in the field. Standardized sampling methods should be followed in the field to ensure the appropriate samples (that have been handled, preserved, and shipped according to protocol) are analyzed in the laboratory (Beatty and Russo 2014). Consistent analytical methods and procedures should be used across implementation programs that are utilizing fish tissue data. Analytical methods should be selected that are sufficiently sensitive to address study objectives (e.g., analytical methods with detection limits below the selenium fish tissue criterion elements after allowing for conversion to dry weight concentrations) and minimize the number of values that are below the MDL. Results should be reported to the appropriate significant figures for the precision of the analytical method.

Laboratories should be selected based on relevant laboratory accreditations, strong QA/QC protocols, and experience with using analytical methods for selenium and the fish tissue matrix. Samples should be prepared in accordance with the tissue type. Section 7.2.2 of the EPA's *Fish Advisory Guidance Volume 1* (USEPA 2000) includes detailed directions for preparing muscle and whole-body samples. Appendix A of this document includes directions for preparing egg and ovary samples.

As of the publication date of this technical support material (TSM), the EPA does not have approved 40 CFR Part 136 analytical methods for measuring selenium in fish tissue. However, states and authorized Tribes are not required to use EPA-approved analytical methods for the monitoring and assessment of criteria attainment or criteria development. Additionally, in the case of pollutants or pollutant parameters for which there are not approved analytical methods under 40 CFR Part 136 or methods are not otherwise required under 40 CFR Chapter I, subchapter N or O, monitoring for activities related to NPDES permit applications, permit limits, or permit compliance reports shall be conducted according to a test procedure specified in the NPDES permit for such pollutants or pollutant parameters.[12] In the assessment of criteria attainment and establishment of lists of waters not attaining criteria, however, states and authorized Tribes are required to assemble and evaluate all existing and readily-available water quality-related data and information (40 CFR 130.7(b)(5)). If a state or authorized Tribe has additional laws concerning data acceptability or laboratory accreditation programs, then the fish tissue analytical methods implemented by the state or authorized Tribe should follow these laws.

Before selecting an analytical method and a laboratory to conduct selenium analyses, states and authorized Tribes should discuss with laboratories their MDLs for detecting selenium in fish tissue using a particular analytical method. States and authorized Tribes should confirm whether those MDLs are for wet weight or dry weight and ensure that they are sensitive enough for the assessment of the selenium criterion or for site-specific study purposes. Table 5 presents several analytical procedures for measuring selenium in solids and biota with MDLs that are sufficiently sensitive for comparison to the tissue criterion elements. Exact MDLs and

[12] The standard conditions of a NPDES permit (40 CFR 122.41(j)(4) and 122.44(i)) require, when available, permittees use test procedures specified in 40 CFR Part 136.

quantitation limits (QL) for these analytical methods are not provided, as those values are laboratory and project specific; however, all the analytical methods listed below should be able to detect a selenium concentration of at least 1.5 mg/kg dry weight (dw). States and authorized Tribes should decide which value they want the laboratory to use for reporting, whether they would like it to be equal to the MDL, QL, or some alternative value that they have confidence in using for regulatory decisions. See section 4.2 of this document for discussion about evaluating data that are below the MDL or falls in between the MDL and the QL. Furthermore, some of the analytical methods and procedures identified in Table 5 do not include specific QC requirements and acceptance limits. Therefore, states and authorized Tribes should work closely with the laboratory to establish appropriate requirements so that data meet the selenium monitoring objectives.

Table 5. List of Test Procedures for Total Selenium in Solids and Biota.

Method	Digestion / Preparation in reference method?	Links to Analytical Methods
EPA Method 6020B[a] – Inductively Coupled Plasma - Mass Spectrometry (ICP - MS)	No – Recommended: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/esam/epa-method-6020b-sw-846-inductively-coupled-plasma-mass-spectrometry https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf
EPA Method 7742[a] – Selenium (Atomic Absorption, Borohydride Reduction)	No- Recommended: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/sites/production/files/2015-12/documents/7742.pdf (See links for digestion methods above)
USGS I-9020-05 – Determination of Elements in Natural-water, Biota, Sediment, and Soil Samples using Collision /Reaction Cell ICP – MS	No – References 3052 (total) Recommended: 3052 (total), or 3050B (total recoverable)	https://pubs.usgs.gov/tm/2006/tm5b1/P DF/TM5-B1.pdf (See links for digestion methods above)

Method	Digestion / Preparation in reference method?	Links to Analytical Methods
NOAA 140.1 - Graphite Furnace-Atomic Absorption for the Analysis of Trace Metals in Marine Animal Tissues	Yes – Teflon Bomb	https://www.nemi.gov/methods/method_summary/7185/
EPA Method 200.8, Rev 5.4[1,2] – Determinations of Trace Elements in Waters by ICP- MS (USEPA 1994)	Yes - Section 11.3 May also use: 3052 (total), or 3050B (total recoverable)	https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf

[1] These EPA methods are not included in 40 CFR Part 136 for fish tissue analysis. The EPA does not currently have any 40 CFR Part 136 methods for analyzing parameters in fish tissue.
[2] Tissue samples must be digested before using this method.

Fish tissue samples should be homogenized and digested prior to analysis using strong acid and either a closed-vessel microwave digestion or an open-vessel heated digestion procedure. If samples are to be dried before homogenization and digestion, freeze drying is a good drying technique to use to minimize selenium losses from the sample. The possibility of volatilization of selenium from the sample is more likely when oven drying so, if possible, freeze drying should be used for selenium samples (Iyengar et al 1978). However, undried tissues may be homogenized and digested, and a dry weight conversion can be determined using a separate aliquot of the homogenized tissue. The suitability of a given technique should be discussed with the individual laboratory given its capabilities and preference. The laboratory and the agency submitting the samples should mutually decide on a technique that meets the purposes of the monitoring. Care should be taken to use a process that minimizes the loss of volatile selenium. Reference materials (from a source like the National Institutes of Standards and Technology, the National Research Council of Canada, or other traceable source), analytical duplicates, and matrix spike samples are recommended to determine the applicability of the selected digestion and analysis procedures.

The North American Metals Council-Selenium Work Group (NAMC-SWG) has published comprehensive discussions of analytical concerns relevant to selenium: Ohlendorf et al. (2008) and Ohlendorf et al. (2011). An additional NAMC-SWG document, Ralston et al. (2008), presents guidance on analytical methods for selenium. Inductively coupled plasma mass spectrometry is the typical method used for analyzing selenium in tissue and other matrices; however, this method is sensitive to interferences. When using this method, these potential interferences should be addressed. Alternative methods for analyzing selenium are discussed in D'Ulivo (1997), Ohlendorf et al. (2008), and Ralston et al. (2008). States and authorized Tribes should choose an analytical method that is sufficiently sensitive to implement its WQS for selenium or meet the site-specific study objectives.

States and authorized Tribes can also consider adapting methods for analyzing selenium in water to measure selenium in fish tissue, as long as the fish tissue samples are appropriately digested. In particular, EPA Method 200.8, Rev 5.4, *Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry* (USEPA 1994) can easily be adapted to tissue analyses by the addition of an appropriate digestion procedure. Additional information regarding analytical methods for water samples can be found in Appendix L of the *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2021). Complete descriptions of analytical methods appropriate for analyzing selenium in different media can be found in the National Environmental Methods Index at http://www.nemi.gov.

4.2 Data Analysis

Generally, for implementation purposes, states and authorized Tribes can calculate a measure of central tendency (e.g., mean, median) for their selenium fish tissue data sets. The monitoring data set and information about the underlying distribution of the sample population should be used to determine which measure of central tendency is most appropriate. In addition, the variability around that measure of central tendency (e.g., standard deviation, standard error, confidence interval) should be quantified and considered when making implementation decisions. The EPA recommends using a measure of central tendency only for characterizing a data set that was collected during a single sampling event (identified location over a specified period of time (generally less than a week)). Different sampling events should all be considered as discrete data sets.

At times, states and authorized Tribes may conduct resource management studies that include the collection of fish tissue data from several locations within a region of interest or for multiple time periods (e.g., seasons or years) from a single location, or a combination of both. Data from intensive studies such as these may be used to perform spatial or temporal analyses to provide information on selenium variability in a target species population. The EPA's *Fish Advisory Guidance Volume 1* provides recommended statistical approaches for comparing contaminants measured at different locations or over time (See Appendix N of USEPA 2000). The EPA recommends that states and authorized Tribes consult a statistician to determine the specific statistical tests needed for a particular study question and data set. Consulting a statistician at the time of the study design may be useful for assuring that the appropriate data are collected to answer the desired question.

When performing these data analyses, states and authorized Tribes should consider how they will address potential data quality concerns, such as the use of analytical results that are below the MDL and/or analytical results that are in between the MDL and the QL. These results can be largely avoided with proper quality assurance project planning. The collection of sufficient tissue mass and use of a sufficiently sensitive analytical method will provide results with a minimal number of values below the MDL and between the MDL and QL. However, if a state or authorized Tribe is using a data set that includes values below the MDL or in between the MDL and the QL, it should decide how it will evaluate these values.

There are various options to deal with these measurements. The EPA notes that identifying and developing approaches to statistically analyze data sets containing non-quantified chemical concentration values (i.e., “censored data”) is an active area of research and no one method can be recommended in all circumstances (for more information see: Helsel 2005, Pleil 2016, and Singh and Nocerino 2002). The EPA’s *Fish Advisory Guidance Volume 1* (USEPA 2000) recommends using one-half of the MDL for values below the MDL in calculating mean values (section 9.1.2). The guidance also recommends that measurements that fall between the MDL and the QL be assigned a value of the MDL plus one-half the difference between the MDL and the QL. The EPA notes, however, that these conventions provide a biased estimate of the average concentration (Gilbert 1987) and, where the computed average is close to the criterion, might suggest an impairment when one does not exist or, conversely, suggest no impairment when one does exist. As an alternative to this option, some states, authorized Tribes, and laboratories may choose to apply what is called a “J” flag to any results reported at or above the MDL, but below the QL. The “J” flag would indicate that the chemical is present, but the reported value is an estimate of the true concentration since it was detected below the QL. Some states and authorized Tribes may choose to use these “J” flagged values for data analysis. The EPA used this option for the National Study of Chemical Residues in Lake Fish Tissue, including all the “J” flagged data in analyses of the fish tissue data (USEPA 2009).

States or authorized Tribes can also calculate the average of a data set that includes values below the MDL using other statistical methods (e.g., robust regression on order statistics, maximum likelihood estimation, Kaplan-Meier) (Helsel 2012, Millard 2013). George et al. (2021) have published a review of several methods for data reporting and analyzed the potential bias each can introduce into the calculation of the mean.

One approach that a state or authorized Tribe could take to ascertain the effect of what value is used to quantify samples below the MDL is to conduct a sensitivity analysis. In a sensitivity analysis, the state or authorized Tribe would compute the mean concentration by first using the value of the MDL to quantify samples below the MDL and then using a zero value for samples below the MDL. For example, if the MDL is 1.5 mg/kg dw, first the mean would be calculated with all values below the MDL being assigned the value of 1.5 mg/kg dw. Then the mean would be recalculated with the value of 0.0 mg/kg dw being assigned to all values below the MDL. If both calculated means are above or below the criterion, the choice of how to quantify samples below the MDL does not affect the decision. However, if one calculated mean is below the criterion and the other is above, the choice of how to quantify samples below the MDL does affect the decision, and a state or authorized Tribe may want to use a more sophisticated approach such as the ones presented in Helsel (2012) or Millard (2013).

All data handling options have tradeoffs. A state or authorized Tribe should understand the tradeoffs of which option it uses, especially if the choice makes a difference as to whether a waterbody is considered impaired. Furthermore, a state or authorized Tribe should be clear about which approach it used in its assessment methodology. The selected approach must be consistent with the state or authorized Tribe’s EPA-approved WQS and should generally adhere to any published assessment method associated with them. For further discussion on handling values below the MDL, see USEPA 2000 (section 9.1) and USEPA 2010 (section 4.3.1). In general, states and authorized Tribes should not have issues with measurements of selenium in fish tissue being below the MDL when a sufficient mass of tissue is collected as the method sensitivities are low enough and all fish should have selenium concentrations higher than those MDLs. Similarly, with sufficient sample mass and appropriate analytical methods, it is unlikely that many states and authorized Tribes will have selenium measurements between the MDL and QL for fish tissue.

References

ATSDR. 2003. Toxicological profile for selenium. Agency for toxic substances and disease registry. http://www.atsdr.cdc.gov/ToxProfiles/tp92.pdf

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition, chapter 3. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. https://nepis.epa.gov/Exe/ZyPDF.cgi/20004OQK.PDF?Dockey=20004OQK.PDF

Beatty, J.M. and G.A. Russo. 2014. Ambient water quality guidelines for selenium technical report update. ISBN 978-0-7726-6740-3. British Columbia Ministry of Environment, Environmental Sustainability and Strategic Policy Division, Water Protection and Sustainability Branch. https://www2.gov.bc.ca//assets/gov/environment/air-land-water/water/waterquality/water-quality-guidelines/approved-wqgs/bc_moe_se_wqg.pdf

Bradshaw, C.J.A. and C.R. McMahon. 2008. Fecundity. In: Jørgensen, E.J. and B.D. Fath, (eds). Encyclopedia of Ecology, 1st ed. Amsterdam, The Netherlands: Elsevier B.V., 1535-1543.

Chapman, P.M., W.J. Adams, M. L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, and D.P. Shaw. 2009. Ecological assessment of selenium in the aquatic environment: Summary of a SETAC Pellston Workshop. Pensacola FL: Society of Environmental Toxicology and Chemistry (SETAC). https://cdn.ymaws.com/www.setac.org/resource/resmgr/publications_and_resources/selsummary.pdf

Chapman P.M., W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A Maher, H.M. Ohlendorf, T.S. Presser, and D.P. Shaw (eds). 2010. Ecological Assessment of Selenium in the Aquatic Environment. SETAC Press, Pensacola, FL, USA.

Cizdziel, J.V., T.A. Hinners, and E.M. Heithmar. 2002. Determination of total mercury in fish tissues using combustion atomic absorption spectrometry with gold amalgamation. Water Air Soil Pollut. 135:355-370. https://link.springer.com/article/10.1023/A:1014798012212

Conley, J.M., A.T.D. Watson, L. Xie, and D.B. Buchwalter. 2014. Dynamic selenium assimilation, distribution, efflux, and maternal transfer in Japanese Medaka fed a diet of Se-enriched mayflies. Environ. Sci. Technol. 48: 2971-2978. https://pubs.acs.org/doi/10.1021/es404933t

Crawford, J.K. and S.N. Luoma. 1993. Guidelines for studies of contaminants in biological tissues for the national water-quality assessment program. Open-File Report 92-494. U.S. Geological Survey, Lemoyne, Pennsylvania. https://www.usgs.gov/publications/guidelines-studies-contaminants-biological-tissues-national-water-quality-assessment

DeForest, D.K. and W.J. Adams. 2011. Selenium accumulation and toxicity in freshwater fishes. In: Beyer, W.N., and J.P. Meador, editors. Environmental Contaminants in Biota – Interpreting Tissue Concentrations, 2nd ed. Boca Raton, FL (US): CRC Press, pp. 185-221. https://www.researchgate.net/publication/260139875_Environmental_Contaminants_in_Biota_Interpreting_Tissue_Concentrations_Second_edition_by_W_Nelson_Beyer_James_P_Meador

D'Ulivo, A. 1997. Determination of selenium and tellurium in environmental samples. Analyst 122:117R-144R. http://pubs.rsc.org/en/content/articlelanding/1997/an/a704759b/unauth#!divAbstract

EPRI. 2006. Fate and effects of selenium in lentic and lotic Systems. Electric Power Research Institute. Product ID 1005315. 104 pages.

FishBase. System Glossary. https://fishbase.mnhn.fr/search.php . Accessed March 2016.

Flotemersch, J.E., J.B. Stribling, and M.J. Paul. 2006. Concepts and approaches for the bioassessment of non-wadeable streams and rivers. EPA/600/R-06/127. U. S. EPA, Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH. https://nepis.epa.gov/Exe/ZyPDF.cgi/600006KV.PDF?Dockey=600006KV.PDF

George, B. J., L. Gains-Germain, K. Broms, K. Black, M. Furman, M.D. Hays, K.W. Thomas, J.E. Simmons. 2021. Censoring trace-level environmental data: Statistical analysis considerations to limit bias. Environ. Sci. Technol. 55: 3786–3795. https://pubs.acs.org/doi/10.1021/acs.est.0c02256

Gilbert, R.O. 1987. Statistical Methods for Environmental Pollution Monitoring. New York: VanNostrand Reinhold Company.

Hamilton, S.J., K.J. Buhl, N.L. Faerber, R.H. Wiedmeyer, and F.A. Bullard. 1990. Toxicity of organic selenium in the diet to Chinook salmon. Environ. Toxicol. and Chem. 9:347-358. http://onlinelibrary.wiley.com/doi/10.1002/etc.5620090310/abstract

Helsel D.R. 2005. Nondetects and data analysis. Statistics for censored environmental data. Hoboken, NJ: Wiley-Interscience.

Helsel, D.R. 2012. Statistics for Censored Environmental Data Using Minitab and R, 2nd ed. Denver, CO: Wiley and Sons.

Herrmann, S.J., D.W.R. Nimmo, J.S. Carsella, I.V. Melnykov, C.M. Kennedy, K.B. Rogers, L.M. Herrmann-Hoesing. 2020. Differential bioaccumulation of mercury and selenium in stomach contents and tissues of three Colorado, USA, cutthroat trout populations. Bull. Environ. Contam. Toxicol. 104: 595-601. https://pubmed.ncbi.nlm.nih.gov/32242255/

Hillwalker, W. E., P. C. Jepson, and K. A. Anderson. 2006. Selenium accumulation patterns in lotic and lentic aquatic systems. J Sci. Total. Environ. 366:367-379. http://www.sciencedirect.com/science/article/pii/S0048969706000076

Hitt, N.P. and D.R. Smith. 2015. Threshold-dependent sample sizes for selenium assessment with stream fish tissue. Integr. Environ. Assess. Manag. 11:143–149. http://onlinelibrary.wiley.com/doi/10.1002/ieam.1579/full

Iyenger, G.V., K. Kasperek, and L.E. Feinendegen. 1978. Retention of the metabolized trace elements in biological tissues following different drying procedures. J Sci. Total. Environ. 10: 1-16. https://www.sciencedirect.com/science/article/pii/0048969778900451

Janz, D.M., D.K. DeForest, M.L. Brooks, P.M. Chapman, G. Gilron, D. Hoff, W.A. Hopkins, D.O. McIntyre, C.A. Mebane, V.P. Palace, J.P. Skorupa and M. Wayland. 2010. Selenium toxicity to aquatic organisms. In: Chapman P.M., W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, D.P. Shaw(eds). Ecological Assessment of Selenium in the Aquatic Environment. SETAC Press, Pensacola, FL, USA.

Johns, C., S.N. Luoma, and V. Elrod. 1988. Selenium accumulation in benthic bivalves and fine sediments of San Francisco Bay, the Sacramento-San Joaquin Delta (USA), and selected tributaries. Estuar. Coast. Shelf Sci. 27(4): 381-396. http://www.sciencedirect.com/science/article/pii/0272771488900959

KDHE, Division of Environment, Bureau of Water, Watershed Planning, Monitoring, and Assessment Section. 2020. Division of Environment Quality Management Plan, Part III: Fish Tissue Contaminant Monitoring Program. Quality Assurance Management Plan, Revision 3. Topeka, Kansas. https://www.kdhe.ks.gov/DocumentCenter/View/11902/Fish-Tissue-Contaminant-Monitoring-Programs-Quality-Assurance-Management-Plan-PDF

Linares-Casenave, J., R. Linville, J.P.V. Eenennaam, J.B. Muguet, and S.I. Doroshov. 2015. Selenium tissue burden compartmentalization in resident white sturgeon (*Acipenser transmontanus*) of the San Francisco Bay Delta Estuary. Environ. Toxicol. and Chem. 34: 152-160. https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.2775

Luoma, S.N. and T.S. Presser. 2009. Emerging opportunities in management of selenium contamination. Environ. Sci. Technol. 43:8483-8487. http://pubs.acs.org/doi/pdf/10.1021/es900828h

Luoma, S.N. and P.S. Rainbow. 2008. Selenium: Dietary exposure, trophic transfer and food web effects. In: Metal Contamination in Aquatic Environments: Science and Lateral Management. New York, NY (US); Cambridge University Press. 573pp.

Mathews, T. and N.S. Fisher. 2008. Trophic transfer of seven trace metals in a four-step marine food chain. Mar. Ecol. Prog. Ser. 367: 23-33. https://www.jstor.org/stable/24872882

May, T.W, J.F. Fairchild, J.D. Petty, M.J. Walther, J Lucero, M. Delvaux, J. Manring, and M. Armbruster. 2008. An evaluation of selenium concentrations in water, sediment, invertebrates, and fish from the Solomon River Basin. Environ. Monit. Assess. 137: 213-232. https://link.springer.com/article/10.1007/s10661-007-9742-y

Millard, S.P. 2013. EnvStats: An R Package for Environmental Statistics. Seattle, WA: Springer.

MPCA. 2008. Minnesota's Fish Contaminant Monitoring Program. Pollution Prevention/Sustainability Fact Sheet 4.05. St. Paul, MN. https://www.ag.state.mn.us/Office/Cases/3M/docs/PTX/PTX3280.pdf

Murua, H. and F. Saborido-Rey. 2003. Female reproductive strategies of marine fish species of the North Atlantic. J. Northw. Atl. Fish. Sci. 33: 23–31. https://journal.nafo.int/Portals/0/2003-Vol33/murua.pdf

Nagahama, Y. 1983. The functional morphology of teleost gonads. In: Hoar W.S., D.J. Randall, E.M. Donaldson. (eds). Fish Physiology Academic Press, New York, 233-275 pp.

NOAA. 1998. Sampling and analytical methods of the national status and trends program mussel watch project: 1993-1996 Update. NOAA Technical Memorandum NOS ORCA 130. March 1998. Silver Spring, MD. 233pp.

NAMC-SWG. 2008. Selenium tissue thresholds: Tissue selection criteria, threshold development endpoints, and potential to predict population or community effects in the field. Washington, DC. http://www.namc.org/docs/00043675.PDF

Ohlendorf, H.M., S.M. Covington, E.R. Byron, and C.A. Arenal. 2008. Approach for conducting site-specific assessments of selenium bioaccumulation in aquatic systems. Washington DC (US): North American Metals Council. http://www.namc.org/docs/00043671.PDF

Ohlendorf, H.M., S.M. Covington, E.R. Byron, and C.A. Arenal. 2011. Conducting site-specific assessments of selenium bioaccumulation in aquatic systems. Integr.Environ. Assess. and Manag.7(3):314-324. http://onlinelibrary.wiley.com/doi/10.1002/ieam.157/abstract

Olsen, A.R., T.M. Kincaid, and Q. Payton, 2012. Spatially balanced survey designs for natural resources. In: Gitzen, R.A., J. J. Millspaugh, A.B. Cooper and D.S. Licht. (eds.) Design and Analysis of Long-Term Ecological Monitoring Studies. Cambridge, UK, Cambridge University Press, 126-150. https://www.cambridge.org/core/books/design-and-analysis-of-long-term-ecological-monitoring-studies/508A10FEE39E7E93EF07B005D06952F5

Olsen, A.R., D.L. Stevens, Jr., and D. White, 1998. Application of global grids in environmental sampling. Computing Science and Statistics 30, 279-284. https://archive.epa.gov/nheerl/arm/web/html/abolsen98.html

Orr, P.L., K.R. Guiguer, and C.K. Russel. 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. Ecotoxicol. and Environ. Safe. 63:175-188. http://www.sciencedirect.com/science/article/pii/S0147651305002277

Osmundson, B. and J. Skorupa. 2011. CO-Selenium in fish tissue: Prediction equations for conversion between whole body, muscle, and eggs. Project FFS ID: 6F50. Department of the Interior, U.S. Fish and Wildlife Service, Region #6. https://ecos.fws.gov/ServCat/DownloadFile/21636?Reference=23117Patil, G.P., S.D. Gore, and C. Taillie. 2011. Composite Sampling: A Novel Method to Accomplish Observational Economy in Environmental Studies. New York, New York (US): Springer.

Pleil, J.D. 2016. Imputing defensible values for left-censored 'below level of quantitation'(LoQ) biomarker measurements. J. Breath Res. 10(4): 045001. https://pubmed.ncbi.nlm.nih.gov/27753432/

Ponton, D.E. and L. Hare. 2015. Using sulfur stable isotopes to understand feeding behavior and selenium concentrations in yellow perch (*Perca flavescens*). Environ. Sci. Technol. 49: 7633-7640.

Ralston, N.V.C., J. Unrine, and D. Wallschläger. 2008. Biogeochemistry and analysis of selenium and its species. Washington DC (US): North American Metals Council. 61p. http://www.namc.org/docs/00043673.PDF

Reinfelder, J.R., W.X. Wang, S.N. Luoma, and N.S. Fisher. 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: A comparison of oysters, clams and mussels. Mar. Biol.129(3): 443-452. http://wwwrcamnl.wr.usgs.gov/tracel/references/pdf/MarBio_v129p443.pdf

Saiki M.K., M.R. Jennings, and W.G. Brumbaugh. 1993. Boron, molybdenum, and selenium in aquatic food chains from the Lower San Joaquin River and its tributaries, California. Arch. Environ. Contam. Toxicol. 24:307-319. https://link.springer.com/article/10.1007/BF01128729

Saiki M.K. and T.P. Lowe. 1987. Selenium in aquatic organisms from subsurface agricultural drainage water, San Joaquin Valley, California. Arch. Environ. Contam. Toxicol. 16:657-670. https://link.springer.com/article/10.1007/BF01055416

Schmitt, C.J., V.S. Blazer, G.M. Dethloff, D.E. Tillitt, T.S. Gross, W.L. Bryant Jr, L.R. DeWeese, S.B. Smith, R.W. Goede, T.M. Bartish, and T.J. Kubiak. 1999. Biomonitoring of Environmental Status and Trends (BEST) Program: field procedures for assessing the exposure of fish to environmental contaminants. U.S. Geological Survey, Biological Resources Division, Columbia, (MO): Information and Technology Report USGS/BRD-1999-0007. Iv + 35pp. + appendices.

Schneider, L., W. Maher, J. Potts, A.M. Taylor, G.E. Batley, F. Krikowa, A.A. Chariton, and B. Gruber. 2015. Modeling food web structure and selenium biomagnification in Lake Macquarie, New South Wales, Australia, using stable carbon and nitrogen isotopes. Environ. Toxicol. Chem. 34.3:608-17. http://onlinelibrary.wiley.com/doi/10.1002/etc.2847/abstract

Simmons, D.B.D. and D. Wallschläger. 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. Environ. Toxicol. Chem. 24(6):1331. http://onlinelibrary.wiley.com/doi/10.1897/04-176R.1/abstract

Singh, A. and J. Nocerino. 2002. Robust estimation of mean and variance using environmental data sets with below detection limit observations. Chemom. Intell. Lab. Syst. 60(1-2):69-86. https://www.sciencedirect.com/science/article/pii/S0169743901001861

Stahl, L.L., B.D. Snyder, H.B. McCarty, T.R. Cohen, K.M. Miller, M.B. Fernandez, and J.C. Healey. 2021. An evaluation of fish tissue monitoring alternatives for mercury and selenium: fish muscle biopsy samples versus homogenized whole fillets. Arch. Environ. Contam. Toxicol. 81: 236-254. https://link.springer.com/article/10.1007/s00244-021-00872-w

Stevens, D.L. and Olsen A.R. 2004. Spatially balanced sampling of natural resources. J. Am. Stat. Assoc. 99: 262-278. https://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.files/fileID/13339

Stewart,A.R., S.N. Luoma, C.E. Schlekat, M.A. Doblin, and K.A. Hieb. 2004. Food web pathway determines how selenium affects aquatic ecosystems: a San Francisco Bay case study. Environ. Sci. Technol. 38: 4519-4526. https://pubs.er.usgs.gov/publication/70026685

Stewart, A.R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W-X. Wang. 2010. Bioaccumulation and trophic transfer of selenium. In: Chapman P.M., W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, D.P. Shaw(eds). Ecological Assessment of Selenium in the Aquatic Environment. SETAC Press, Pensacola, FL, USA. https://www.researchgate.net/publication/313220382_Bioaccumulation_and_trophic_transfer_of_selenium

Thornton, K. W. 1990. Perspectives on reservoir limnology. Chapter 1. In: Reservoir Limnology: Ecological Perspectives. Thornton, K. W., B. L. Kimmel, and F. E. Payne. John Wiley and Sons, Inc. Hoboken, NJ. 256 pp.

Tyler, C.R., and J.P. Sumpter. 1996. Oocyte growth and development in teleosts. Rev. Fish Biol. Fisher. 6: 287-318. http://link.springer.com/article/10.1007/BF00122584

USEPA. 1982. Sampling protocols for collecting surface water, bed sediment, bivalves, and fish for priority pollutant analysis. Final Draft Report. U.S. Environmental Protection Agency, Office of Water, Washington DC. https://nepis.epa.gov/Exe/tiff2png.cgi/9101NBLQ.PNG?-r+75+-g+7+D%3A%5CZYFILES%5CINDEX%20DATA%5C81THRU85%5CTIFF%5C00002793%5C9101NBLQ.TIF

USEPA. 1994. Method 200.8. Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry, Revision 5.4. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH. https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf

USEPA. 1995. EPA Observational Economy Series, Volume 1: Composite Sampling. EPA-230-R-95-005. U.S. Environmental Protection Agency, Policy, Planning and Evaluation, August 1995. https://www.epa.gov/sites/production/files/2016-03/documents/comp-samp.pdf

USEPA. 1996a. Method 3052. Microwave assisted acid digestion of siliceous and organically based matrices, Revision 0. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). Office of Land and Emergency Management, Washington, DC, December 1996. https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf

USEPA. 1996b. Method 3050B. Acid digestion of sediments, sludges, and soil, Revision 2. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). Office of Land and Emergency Management, Washington, DC, December 1996. https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

USEPA. 2000. Guidance for assessing chemical contaminant data for use in fish advisories, Vol 1: Fish Sampling and Analysis. EPA 823-B-00-007. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf

USEPA. 2002a. Field sampling plan for the national study of chemical residues in lake fish tissue. EPA-823-R-02-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC. http://www.epa.gov/sites/production/files/2015-07/documents/fish-study-fieldplan.pdf

USEPA. 2002b. Guidance on choosing a sampling design for environmental data collection. EPA-240-R-02-005. U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC. http://www.epa.gov/sites/production/files/2015-06/documents/g5s-final.pdf

USEPA. 2009. The national study of chemical residues in lake fish tissue. EPA-823-R-09-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1005P2Z.PDF?Dockey=P1005P2Z.PDF

USEPA. 2010. Guidance for implementing the January 2001 methylmercury water quality criterion. EPA 823-R-10-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyPDF.cgi/P1007BKQ.PDF?Dockey=P1007BKQ.PDF

USEPA. 2011. Summary of responses to the 2010 national survey of fish advisory programs. EPA-820-R-12-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. Accessed on-line at https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LIPR.PDF?Dockey=P100LIPR.PDF

USEPA. 2012. Great lakes fish monitoring and surveillance program: quality assurance project plan for sample collection activities. Version 2. U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, Illinois. https://www.epa.gov/sites/default/files/2016-02/documents/glfmsp_qapp_version_2_111312_merged_508_0.pdf

USEPA. 2013. Revised deletion process for the site-specific recalculation procedure for aquatic life criteria. EPA 823-R-13-001. U.S. Environmental Protection Agency, Office of Water, Washington DC. https://www.epa.gov/sites/production/files/2015-08/documents/revised_deletion_process_for_the_site-specific_recalculation_procedure_for_aquatic_life_criteria.pdf

USEPA. 2014. Method 6020B (SW-846): Inductively coupled plasma - mass spectrometry, Revision 2. U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). Office of Land and Emergency Management, Washington, DC, July 2014. https://www.epa.gov/sites/production/files/2015-12/documents/6020b.pdf

USEPA. 2015. National coastal condition assessment: Field operations manual. EPA-841-R-14-007. U.S. Environmental Protection Agency, Office of Water Washington, DC. https://www.epa.gov/sites/production/files/2016-03/documents/national_coastal_condition_assessment_2015_field_operation_manual_version_1.0_1.pdf

USEPA. 2016. Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. EPA-821-R-16-006. U.S. Environmental Protection Agency, Office of Water, Washington D.C. https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

USEPA. 2018. Draft translation of selenium tissue criterion elements to site-specific water column criterion elements for California Version 1, August 8, 2018. U.S. Environmental

Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2018-12/documents/california_selenium_2040-af79_pba_20181121_508c_.pdf

USEPA. 2019a. National river and streams assessment 2018/19: Field operations manual-non-wadeable. EPA-841-B-17-003b. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_nonwadeable_version_1.2.pdf

USEPA. 2019b. National rivers and streams assessment 2018/19: Field operations manual – wadeable. EPA-841-B-17-003a. U.S. Environmental Protection Agency, Office of Water Washington, DC. https://www.epa.gov/sites/production/files/2019-05/documents/nrsa_1819_fom_wadeable_version_1.2_0.pdf

USEPA. 2021. 2021 Revision to: Aquatic life ambient water quality criterion for selenium–freshwater 2016. EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

USEPA. 2024a. Frequently Asked Questions*:* Implementing the EPA's 2016 selenium criterion in Clean Water Act sections 303(d) and 305(b) assessment, listing, and total maximum daily load programs. EPA-830-R-24-004. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-faq-cwa303.pdf

USEPA. 2024b. Frequently Asked Questions: Implementing water quality standards based on EPA's 2016 selenium criterion in Clean Water Act section 402 National Pollutant Discharge Elimination System programs. EPA-820-R-24-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-faq-cwa402.pdf

USEPA. 2024c. Technical support for adopting and implementing EPA's selenium 2016 criterion in water quality standards. EPA-820-R-24-001. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/system/files/documents/2024-03/selenium-adopting-tsd.pdf

USEPA. Vocabulary Catalog. https://sor.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&glossaryName=FEM%20Glossary#formTop. Last updated December 2017.

USGS. 2006. Determination of elements in natural-water, biota, sediment, and soil samples using collision/reaction cell inductively coupled plasma–mass spectrometry: U.S. Geological Survey Techniques and Methods, book 5, sec. B, chap. 1, 88 p. https://pubs.usgs.gov/tm/2006/tm5b1/

Waddell, B. and T. May. 1995. Selenium concentrations in the razorback sucker (*Xyrauchen texanus*): substitution of non-lethal muscle plugs for muscle tissue in contaminant assessment. Arch. Environ. Contam. Toxicol. 18: 321-326. https://pubs.er.usgs.gov/publication/70174185

Zhou, C., J. Pagano, B.A. Crimmins, P.K. Hopke, M.S. Milligan, E.W. Murphy, and T.M. Holsen. 2018. Polychlorinated biphenyls and organochlorine pesticides concentration patterns and trends in top predator fish of Laurentian Great Lakes from 1999 to 2014. J. Great Lakes Res. 44(4):716-724. https://www.sciencedirect.com/science/article/pii/S038013301830042X

Appendix A:
Egg and Ovary Sample Preparation

Scope

This guidance is for egg and ovary collection from freshwater fish. The egg extraction method is excerpted and adapted from a more comprehensive guidance, *Standard operating procedure for evaluating selenium-induced deformities in early life stages of freshwater fish* (Janz and Muscatello 2008), that includes gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish. The ovary dissection method was compiled from peer-reviewed literature.

1) Field collection and handling of adult fish

> "Spawning adults can be collected in the field using a wide variety of techniques, including fish traps (e.g., hoop or trap nets), electrofishing or angling in areas close to spawning areas. Gillnets are also effective in capturing fish during spawning migrations, but it is essential to monitor these nets constantly to remove fish immediately after capture. If possible, the use of passive capture methods (e.g., hoop or trap nets) is recommended since this is the least stressful capture technique of those listed above. Trap nets are usually set up in creeks, streams or narrows in lakes, although successful fish capture can also occur when these nets are set perpendicular to shore in lentic habitats. Trap or hoop nets can be purchased from fisheries suppliers, or even constructed in creeks and streams using chicken wire, baling wire and reinforcing bar." (Janz and Muscatello 2008)

Fish should be held in livewells until adult female fish are selected for egg collection.

2) Egg collection procedures

Fish should be carefully observed for signs of physical damage, mortality, or other sources of stress. Since any handling of the fish will remove the protective body layer of slime, fish should be handled as little as possible using dip nets and soft material gloves. Adult fish for egg collection should be randomly selected from livewells.

> "Eggs should not be in contact with water; thus, it is imperative to dry the area surrounding the urogenital opening with paper towels. All the material used for egg collection should be carefully cleaned and dried. Precautions to avoid fecal, blood or urine contamination should be taken. [Eggs] must be kept covered to avoid direct sun exposure. [Egg collection] should proceed after recording weight and length [of the gravid female]. Gentle pressure from behind the pectoral fins towards the anus is applied to express the eggs. This process needs to

> be repeated several times. Check that eggs are released 'clean' (e.g., without feces) before starting collection to avoid contamination of the entire egg batch. Eggs are individually collected into pre-cleaned stainless steel bowls and kept covered in a cool place until use. Collected eggs should be closely inspected and eggs with adhered feces, urine or blood discarded by using a clean plastic pipette." (Janz and Muscatello 2008)

Eggs are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate. An individual or composite homogenate tissue sample of 20 grams ww should be collected for analysis of selenium.

3) Ovary dissection procedures

Fish designated for ovary collection should be humanely euthanized, and necropsy procedures should commence immediately following euthanasia (Wolf et al. 2004). The fish should be placed in right lateral recumbency on a piece of aluminum foil. The left body wall should be removed by using fine dissecting instruments (Wolf et al. 2004). To identify female specimens for ovary collection, sex is determined by macroscopic inspection when the body cavity is opened. The ovaries are paired organs suspended from the dorsal wall, with color ranging from clear to white to yellow-orange. A yellow-orange color is indicative of a ripening or ripe adult specimen. Further, increased blood flow during the reproductive season causes the ovaries to become highly vascularized and appear reddish. In cross-section, the ovaries are round to elliptical and contain a central cavity (lumen). In young fish, the texture of the ovaries varies from smooth to slightly granular. The ovarian texture in a ripe fish will be highly granular (Fisheries Information Network 2006). If inspection of the ovaries reveals that the specimen is immature or developing, it is not recommended that the eggs/ovarian tissue be used for tissue monitoring for selenium.

After confirmation that the specimen is a ripe female, the ovaries should be excised by severing the oviducts and mesenteric attachments. All gonads are dissected in a caudal to cranial direction (Wolf et al. 2004). Ovaries are then weighed to the nearest gram using a top-loading digital scale, frozen for storage, and shipped for laboratory analysis when appropriate (Orr et al. 2012). An individual or composite homogenate sample of 20 grams ww of tissue should be collected for analysis of selenium.

4) Storing fish eggs and ovaries

"Eggs and ovaries should be kept frozen until analysis. After collection, samples should be kept in a container with ice or freezer packs until transfer to a freezer (–20°C) for storage" (Janz and Muscatello 2008). It is recommended to transfer the samples collected from each individual female into sealed resealable plastic storage bags to "prevent water (from ice melting) entering the sample" (Janz and Muscatello 2008). Recommendations for the storage, preservation and holding time for egg and ovary samples are equivalent to other tissue samples. Samples should be frozen at –20°C in plastic, borosilicate glass, quartz, or PTFE bottles. The recommended maximum holding time is six months but can be up to two years for most trace metals, including selenium (USEPA 2000).

5) Laboratory preparation of egg and tissue samples for metal analysis

> “Egg and tissue samples should be thawed, and wet weight recorded for each individual sample. To prevent cross contamination between samples, a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is required to record the moisture content for each individual sample in order to express analytical data on a dry weight basis. Trace element (e.g., selenium) analysis is routinely performed using hydride generation atomic absorption spectrophotometry (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry-weight basis.” (Janz and Muscatello 2008)

References

Fisheries Information Network. 2006. Biological Sampling Manual. Gulf States Marine Fisheries Commission. http://www.gsmfc.org/pubs/FIN/Biological%20Sampling%20Manual.pdf

Janz, D.M. and J.R. Muscatello. 2008. Standard operating procedure for evaluating selenium-induced deformities in early life stages of freshwater fish. Appendix A in Selenium tissue thresholds: Tissue selection criteria, threshold development endpoints, and potential to predict population or community effects in the field. Washington DC, USA: North America Metals Council – Selenium Working Group. http://www.namc.org/docs/00043675.PDF

Orr, P.L., C.I.E. Wiramanaden, M.D. Paine, W. Franklin, and C. Fraser. 2012. Food chain model based on field data to predict westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) ovary selenium concentrations from water selenium concentrations in the Elk Valley, British Columbia. Environ. Toxicol. Chem. 31(3):672-680. http://onlinelibrary.wiley.com/doi/10.1002/etc.1730/abstract

USEPA. 1994. Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry, Revision 5.4. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH. https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf

USEPA. 2000. Guidance for assessing chemical contaminant data for use in fish advisories, Vol 1: Fish sampling and analysis. EPA 823-B-00-007. U.S. Environmental Protection Agency, Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf

Wolf, J.C., D.R. Dietrich, U. Friederich, J. Caunter, and A.R. Brown. 2004. Qualitative and quantitative histomorphologic assessment of fathead minnow *Pimephales promelas* gonads as an endpoint for evaluating endocrine-active compounds: a pilot methodology study. Toxicol. Pathol. 32(5): 600-612. http://tpx.sagepub.com/content/32/5/600.full.pdf+html

Appendix B: Spawning Seasons for Example Fish Assemblages from Select U.S. Watersheds

This appendix contains spawning season calendars for fish assemblages from selected watersheds in six different areas of the United States. The calendars are intended to provide examples of spawning periods for fish species commonly collected in those areas. The EPA recommends that monitoring agencies use all available locally relevant resources to determine the appropriate time to collect fish for the purpose of implementing the selenium criterion, including contacting their local natural resources or fish and game agency.

References

Auer, N.A. (ed). 1982. Identification of larval fishes of the Great Lakes basin with emphasis on the Lake Michigan drainage. Great Lakes Fishery Commission, Ann Arbor, MI. Special Pub. 82 – 3:744 pp.

Boschung, H.T. and R.L. Mayden. 2004. Fishes of Alabama. Washington, D.C: Smithsonian Books.

Hendrickson, D.A. and A.E. Cohen. 2022. Fishes of Texas Project Database (Version 3.0). http://doi.org/10.17603/C3WC70. Accessed (10/4/2023).Nevada Division of Environmental Protection. 2006. Fact Sheet. Temperature Criteria for Various Fish Species as Recommended to NDEP during the 1980s. https://ndep.nv.gov/uploads/documents/recommended_temp_criteria06_1_.pdf

Page, L.M. and B.M. Burr. 1991. Peterson Field Guides: Freshwater Fishes. Boston: Houghton Mifflin Company.

Texas Parks and Wildlife. Wildlife Fact Sheets. https://tpwd.texas.gov/huntwild/wild/species/. Accessed 2015.

Scarola, J.F. 1973. Freshwater Fishes of New Hampshire. New Hampshire Fish and Game Department, Division of Inland and Marine Fisheries.

Wang, J.C.S. and R.J. Kernehan. 1979. Fishes of the Delaware Estuaries: A Guide to the Early Life Histories. Towson, MD: EA Communications.

Table B-1. Spawning Seasons for Example Fish Assemblages in the Merrimack River, MA and NH Watershed.

Family	Scientific Name	Common Name	Spawning Season
Atherinopsidae	*Menidia menidia*	Atlantic Silverside	April through August
Catostomidae	*Catostomus commersonii*	White Sucker	March through July
Centrarchidae	*Ambloplites rupestris*	Rock Bass	April through July
Centrarchidae	*Enneacanthus obesus*	Banded Sunfish	April through July
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	April through July
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	June through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through June
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	April through July
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	March through August
Cyprinidae	*Carassius auratus*	Goldfish	March through August
Cyprinidae	*Cyprinus carpio*	Common Carp	April through August
Cyprinidae	*Luxilus cornutus*	Common Shiner	May through July
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	May through July
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	May through June
Cyprinidae	*Notropis bifrenatus*	Bridle Shiner	May through August
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	May through September
Cyprinidae	*Rhinichthys atratulus*	Blacknose Dace	April through July
Cyprinidae	*Rhinichthys cataractae*	Longnose Dace	April through June
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	March through June
Cyprinidae	*Semotilus corporalis*	Fallfish	April through May
Esocidae	*Esox lucius*	Northern Pike	March through May
Esocidae	*Esox niger*	Chain Pickerel	March through May
Fundulidae	*Fundulus diaphanus*	Banded Killifish	April through August
Fundulidae	*Fundulus heteroclitus*	Mummichog	June through July
Gadidae	*Lota lota*	Burbot	January through April
Gasterosteidae	*Apeltes quadracus*	Fourspine Stickleback	April through May
Gasterosteidae	*Gasterosteus aculeatus*	Threespine Stickleback	March through June
Gasterosteidae	*Pungitius pungitius*	Ninespine Stickleback	April through August
Ictaluridae	*Ameiurus catus*	White Catfish	May through July
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through June
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	April through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through September
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through July
Ictaluridae	*Noturus insignis*	Margined Madtom	June through July
Moronidae	*Morone americana*	White Perch	May through June
Percidae	*Etheostoma fusiforme*	Swamp Darter	April through May
Percidae	*Etheostoma olmstedi*	Tessellated Darter	March through May
Percidae	*Perca flavescens*	Yellow Perch	May through July

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Sander vitreus*	Walleye	April through May
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	April through June
Salmonidae	*Salmo trutta*	Brown Trout	October through February
Salmonidae	*Salvelinus fontinalis*	Brook Trout	September through November

(Scarola 1973, Page and Burr 1991)

Table B-2. Spawning Seasons for Example Fish Assemblages in the Delaware River, DE Watershed.

Family	Scientific Name	Common Name	Spawning Season
Aphredoderidae	*Aphredoderus sayanus*	Pirate Perch	April through May
Atherinopsidae	*Membras martinica*	Rough Silverside	May through August
Atherinopsidae	*Menidia peninsulae*	Tidewater Silverside	May through August
Atherinopsidae	*Menidia menidia*	Atlantic Silverside	April through August
Catostomidae	*Catostomus commersonii*	White Sucker	March through May
Catostomidae	*Erimyzon oblongus*	Creek Chubsucker	March through May
Centrarchidae	*Acantharchus pomotis*	Mud Sunfish	May through June
Centrarchidae	*Enneacanthus chaetodon*	Blackbanded Sunfish	May through July
Centrarchidae	*Enneacanthus gloriosus*	Bluespotted Sunfish	May through September
Centrarchidae	*Enneacanthus obesus*	Banded Sunfish	June through September
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	May through June
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	May through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis annularis*	White Crappie	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through June
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through June
Cyprinidae	*Carassius auratus*	Goldfish	June through July
Cyprinidae	*Cyprinus carpio*	Common Carp	May through July
Cyprinidae	*Hybognathus nuchalis*	Mississippi Silvery Minnow	April through May
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Cyprinella analostana*	Satinfin Shiner	March through July
Cyprinidae	*Notropis bifrenatus*	Bridle Shiner	March through August
Cyprinidae	*Notropis chalybaeus*	Ironcolor Shiner	April through May
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	April through July
Cyprinidae	*Rhinichthys atratulus*	Blacknose Dace	May through June
Esocidae	*Esox americanus americanus*	Redfin Pickerel	February through March
Fundulidae	*Fundulus diaphanus*	Banded Killifish	April through August
Fundulidae	*Fundulus heteroclitus*	Mummichog	April through September
Fundulidae	*Fundulus majalis*	Striped Killifish	April through September
Fundulidae	*Lucania parva*	Rainwater Killifish	May through July
Ictaluridae	*Ameiurus catus*	White Catfish	April through July
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	May through July
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	May through July
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through July
Moronidae	*Morone americana*	White Perch	April through June
Percidae	*Etheostoma fusiforme*	Swamp Darter	April through May
Percidae	*Etheostoma olmstedi*	Tessellated Darter	March through May

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Perca flavescens*	Yellow Perch	March through April
Poeciliidae	*Gambusia affinis*	Mosquitofish	May through August
Umbridae	*Umbra pygmaea*	Eastern Mudminnow	April through June

(Wang and Kernehan 1979, Page and Burr 1991)

Table B-3. Spawning Seasons for Example Fish Assemblages in the Cahaba River, AL Watershed.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Atherinopsidae	*Labidesthes sicculus*	Brook Silverside	June through August
Catostomidae	*Carpiodes cyprinus*	Quillback	March through September
Catostomidae	*Carpiodes velifer*	Highfin Carpsucker	May through July
Catostomidae	*Erimyzon oblongus*	Creek Chubsucker	March through May
Catostomidae	*Erimyzon sucetta*	Lake Chubsucker	March through April
Catostomidae	*Erimyzon tenuis*	Sharpfin Chubsucker	March through April
Catostomidae	*Hypentelium etowanum*	Alabama Hog Sucker	April through June
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	March through April
Catostomidae	*Minytrema melanops*	Spotted Sucker	April through May
Catostomidae	*Moxostoma carinatum*	River Redhorse	April
Catostomidae	*Moxostoma duquesnii*	Black Redhorse	April through May
Catostomidae	*Moxostoma erythrurum*	Golden Redhorse	April through June
Catostomidae	*Moxostoma poecilurum*	Blacktail Redhorse	April
Centrarchidae	*Ambloplites ariommus*	Shadow Bass	May through October
Centrarchidae	*Centrarchus macropterus*	Flier	February through May
Centrarchidae	*Lepomis macrochirus*	Bluegill	March through May
Centrarchidae	*Lepomis marginatus*	Dollar Sunfish	May through August
Centrarchidae	*Lepomis megalotis*	Longear Sunfish	May through August
Centrarchidae	*Lepomis microlophus*	Redear Sunfish	March through May; September through November
Centrarchidae	*Lepomis miniatus*	Redspotted Sunfish	March through September
Centrarchidae	*Micropterus coosae*	Redeye Bass	May through July
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	March through May
Centrarchidae	*Micropterus punctulatus*	Spotted Bass	April through May
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis annularis*	White Crappie	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	February through May
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through May
Clupeidae	*Dorosoma petenense*	Threadfin Shad	April through August
Cottidae	*Cottus carolinae*	Banded Sculpin	January through March
Cyprinidae	*Campostoma oligolepis*	Largescale Stoneroller	April through May
Cyprinidae	*Cyprinella callistia*	Alabama Shiner	March through May
Cyprinidae	*Cyprinella trichroistia*	Tricolor Shiner	June through July
Cyprinidae	*Cyprinella venusta*	Blacktail Shiner	March through October
Cyprinidae	*Hybognathus nuchalis*	Mississippi Silvery Minnow	March through April
Cyprinidae	*Hybopsis winchelli*	Clear Chub	February through April
Cyprinidae	*Luxilus chrysocephalus*	Striped Shiner	April through August

Family	Scientific Name	Common Name	Spawning Season
Cyprinidae	*Lythrurus bellus*	Pretty Shiner	April through June
Cyprinidae	*Macrhybopsis storeriana*	Silver Chub	May through August
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Notropis ammophilus*	Orangefin Shiner	April through October
Cyprinidae	*Notropis asperifrons*	Burrhead Shiner	April through June
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	May through July
Cyprinidae	*Notropis baileyi*	Rough Shiner	May through October
Cyprinidae	*Notropis buccatus*	Silverjaw Minnow	March through June
Cyprinidae	*Notropis candidus*	Silverside Shiner	June through September
Cyprinidae	*Notropis chrosomus*	Rainbow Shiner	May through June
Cyprinidae	*Notropis edwardraneyi*	Fluvial Shiner	May through June
Cyprinidae	*Notropis stilbius*	Silverstripe Shiner	March through August
Cyprinidae	*Notropis texanus*	Weed Shiner	February through October
Cyprinidae	*Notropis uranoscopus*	Skygazer Shiner	May through July
Cyprinidae	*Notropis volucellus*	Mimic Shiner	April through August
Cyprinidae	*Opsopoeodus emiliae*	Pugnose Minnow	April through September
Cyprinidae	*Phenacobius catostomus*	Riffle Minnow	April through May
Cyprinidae	*Pimephales notatus*	Bluntnose Minnow	April through August
Cyprinidae	*Pimephales vigilax*	Bullhead Minnow	May through August
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	April through May
Cyprinidae	*Semotilus thoreauianus*	Dixie Chub	April through May
Elassomatidae	*Elassoma zonatum*	Banded Pygmy Sunfish	March through April
Esocidae	*Esox americanus*	Redfin Pickerel	April through May
Esocidae	*Esox niger*	Chain Pickerel	April through October
Fundulidae	*Fundulus olivaceus*	Blackspotted Topminnow	March through September
Hiodontidae	*Hiodon tergisus*	Mooneye	April through May
Ictaluridae	*Ameiurus melas*	Black Bullhead	May through August
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	April through June
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	April through August
Ictaluridae	*Ictalurus furcatus*	Blue Catfish	April through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through July
Ictaluridae	*Noturus funebris*	Black Madtom	May through June
Ictaluridae	*Noturus gyrinus*	Tadpole Madtom	May through September
Ictaluridae	*Pylodictis olivaris*	Flathead Catfish	June through July
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	May through July
Lepisosteidae	*Lepisosteus osseus*	Longnose Gar	April through August
Moronidae	*Morone chrysops*	White Bass	February through March
Percidae	*Ammocrypta beanii*	Naked Sand Darter	March through October
Percidae	*Etheostoma meridianum*	Southern Sand Darter	April through June
Percidae	*Etheostoma chlorosomum*	Bluntnose Darter	April
Percidae	*Etheostoma jordani*	Greenbreast Darter	April through May

Family	Scientific Name	Common Name	Spawning Season
Percidae	*Etheostoma nigrum*	Johnny Darter	March through May
Percidae	*Etheostoma parvipinne*	Goldstripe Darter	March through April
Percidae	*Etheostoma ramseyi*	Alabama Darter	March through May
Percidae	*Etheostoma rupestre*	Rock Darter	March through April
Percidae	*Etheostoma stigmaeum*	Speckled Darter	March through May
Percidae	*Etheostoma swaini*	Gulf Darter	March through April
Percidae	*Percina kathae*	Mobile Logperch	April through June
Percidae	*Percina maculata*	Blackside Darter	March through June
Percidae	*Percina nigrofasciata*	Blackbanded Darter	May through June
Percidae	*Percina vigil*	Saddleback Darter	February through April
Percidae	*Sander vitreus*	Walleye	March through April
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	May through June

(Boschung and Mayden 2004)

Table B-4. Spawning Seasons for Example Fish Assemblages in the Chicago River, IL Watershed.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Catostomidae	*Catostomus commersonii*	White Sucker	April through May
Centrarchidae	*Ambloplites rupestris*	Rock Bass	May through July
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	June through August
Centrarchidae	*Lepomis humilis*	Orangespotted Sunfish	May through July
Centrarchidae	*Lepomis gibbosus*	Pumpkinseed	May through July
Centrarchidae	*Lepomis gulosus*	Warmouth	May through August
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through June
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through July
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	May through July
Cyprinidae	*Campostoma anomalum*	Central Stoneroller	April through July
Cyprinidae	*Carassius auratus*	Goldfish	May through June
Cyprinidae	*Cyprinella spiloptera*	Spotfin Shiner	May through August
Cyprinidae	*Cyprinus carpio*	Common Carp	May through August
Cyprinidae	*Hybopsis dorsalis*	Bigmouth Shiner	May through June
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	May through August
Cyprinidae	*Notropis atherinoides*	Emerald Shiner	April through August
Cyprinidae	*Notropis hudsonius*	Spottail Shiner	June through July
Cyprinidae	*Notropis stramineus*	Sand Shiner	May through July
Cyprinidae	*Pimephales notatus*	Bluntnose Minnow	May through August
Cyprinidae	*Pimephales promelas*	Fathead Minnow	May through August
Cyprinidae	*Semotilus atromaculatus*	Creek Chub	April through June
Cyprinodontidae	*Fundulus notatus*	Blackstripe Topminnow	May through August
Esocidae	*Esox americanus*	Grass Pickerel	May through June; November
Esocidae	*Esox lucius*	Northern Pike	March through May
Gobiidae	*Neogobius melanostomus*	Round Goby	April through May
Ictaluridae	*Ameiurus melas*	Black Bullhead	May through June
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through June
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through August
Moronidae	*Morone americana*	White Perch	May through June
Moronidae	*Morone chrysops*	White Bass	April through June
Moronidae	*Morone mississippiensis*	Yellow Bass	April through May
Percidae	*Etheostoma nigrum*	Johnny Darter	April through June
Percidae	*Sander vitreus*	Walleye	April through May
Percidae	*Perca flavescens*	Yellow Perch	May through July
Umbridae	*Umbra limi*	Central Mudminnow	April through May

(Auer 1982, Page and Burr 1991)

Table B-5. Spawning Seasons for Example Fish Assemblages in the Truckee and Carson River, NV Watersheds.

Family	Scientific Name	Common Name	Spawning Season
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through July
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	April through July
Centrarchidae	*Lepomis macrochirus*	Bluegill	May through August
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	May through July
Ictaluridae	*Ictaluridae*	Catfish species	June through July
Moronidae	*Morone saxatilis*	Striped Bass*	April through June
Moronidae	*Morone chrysops*	White Bass	April through June
Percidae	*Sander vitreus*	Walleye	January through April
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	March through May
Salmonidae	*Salmo trutta*	Brown Trout	January through March
Salmonidae	*Prosopium williamsoni*	Mountain Whitefish	October through December

This population of striped bass is landlocked and cannot migrate out to sea.

(Nevada Division of Environmental Protection 2006)

Table B-6. Spawning Seasons for Example Fish Assemblages in the Rio Grande and Colorado River, TX Watersheds.

Family	Scientific Name	Common Name	Spawning Season
Amiidae	*Amia calva*	Bowfin	March through June
Anguillidae	*Anguilla rostrata*	American Eel	February through June
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	March through September
Catostomidae	*Ictiobus cyprinellus*	Bigmouth Buffalo	April through May
Catostomidae	*Ictiobus niger*	Black Buffalo	April through May
Centrarchidae	*Lepomis macrochirus*	Bluegill	April through September
Centrarchidae	*Lepomis cyanellus*	Green Sunfish	April through August
Centrarchidae	*Lepomis megalotis*	Longear Sunfish	May through June
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	April through October
Centrarchidae	*Lepomis microlophus*	Redear Sunfish	May through July
Centrarchidae	*Lepomis gulosus*	Warmouth	March through October
Centrarchidae	*Micropterus salmoides*	Largemouth Bass	February through May
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass	April through May
Centrarchidae	*Micropterus punctulatus*	Spotted Bass	April through June
Centrarchidae	*Micropterus treculii*	Guadalupe Bass	March through June
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie	March through May
Centrarchidae	*Pomoxis annularis*	White Crappie	March through May
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande Cichlid	March through August
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	April through June
Clupeidae	*Dorosoma petenense*	Threadfin Shad	April through September
Cyprinidae	*Ctenopharyngodon idella*	Grass Carp	April through July
Cyprinidae	*Cyprinus carpio*	Common Carp	March through June
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	April through September
Cyprinidae	*Cyprinella venusta*	Blacktail Shiner	April through September
Cyprinidae	*Notropis amabilis*	Texas Shiner	February through September
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	April through July
Cyprinidae	*Pimephales promelas*	Fathead Minnow	May through September
Esocidae	*Esox niger*	Chain Pickerel	December through February
Ictaluridae	*Ictalurus furcatus*	Blue Catfish	April through May
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	April through June
Ictaluridae	*Pylodictis olivaris*	Flathead Catfish	June through July
Ictaluridae	*Ameiurus melas*	Black Bullhead	April through June
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	May through July
Lepisosteidae	*Atractosteus spatula*	Alligator Gar	April through May
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	April through June

Family	Scientific Name	Common Name	Spawning Season
Lepisosteidae	*Lepisosteus osseus*	Longnose Gar	April through July
Lepisosteidae	*Lepisosteus platostomus*	Shortnose Gar	May through July
Moronidae	*Morone chrysops*	White Bass	March through May
Moronidae	*Morone mississippiensis*	Yellow Bass	April through June
Moronidae	*Morone saxatilis*	Striped Bass*	February through April
Percidae	*Sander vitreus*	Walleye	February through April
Polyodontidae	*Polyodon spathula*	Paddlefish	February through June
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout	November through February
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	April through June
Sciaenidae	*Sciaenops ocellatus*	Red Drum	August through October

*This population of striped bass is landlocked and cannot migrate out to sea.

(Hendrickson and Cohen 2015, Texas Parks and Wildlife Department 2016)

Appendix C:
Conversion of Wet to Dry Tissue Weight

Conversion of Wet to Dry Tissue Weight

Selenium data in fish tissues can be reported in either dry weight (dw) or wet weight (ww) concentrations. It is essential that exposure assessors be aware of this difference so that they may ensure consistency between units when comparing data. If the contaminant concentration is measured in wet weight of fish, then the concentration must be converted to dry weight units in order to be compared to the selenium criterion, which is expressed in dry weight (USEPA 2021). Wet weight may be converted to dry weight, and vice versa, using the following equations:

ww = dw x [1 - (percent moisture/100)] (USEPA 2011)

dw = ww / [1 - (percent moisture/100)] (USEPA 2011)

Measurements reported as wet weight can be converted to equivalent dry weights using available percent moisture data for the relevant species and tissue type. If percent moisture data are unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) may be used. Table C-1 lists percent moisture of some species by tissue type (USEPA 2021). Percent moisture can vary within species; therefore, the data in Table C-1 should generally be used when dealing with historical data. When using field collected data, measuring percent moisture within the field collected sample will provide the most accurate measurement of percent moisture, thus giving more accurate conversions between dry weight and wet weight data.

Table C-1. Percent Moisture by Species and Tissue Type.

Scientific Name	**Common Name**	**Average % Moisture**	**% Moisture by Tissue**			**Reference**
			Whole-body	**Muscle**	**Egg-ovary**	
Cyprinus carpio	Common Carp	75.64[a]		75.81[b]		USEPA 2014[a] Chatakondi et al. 1995[b]
Rhinichthys cataractae	Longnose Dace	73.25				USEPA 2014
Rhinichthys atratulus	Blacknose Dace	73.75				USEPA 2014
Semotilus atromaculatus	Creek Chub	76.71				USEPA 2014
Pimephales promelas	Fathead Minnow	76.64			75.3	USEPA 2014
Pimephales notatus	Bluntnose Minnow	74.8				USEPA 2014

Scientific Name	Common Name	Average % Moisture	% Moisture by Tissue			Reference
			Whole-body	Muscle	Egg-ovary	
Nocomis micropogon	River Chub	75.2				USEPA 2014
Ictalurus punctatus	Channel Catfish			81.22[a] 78.43[b]		Pinkney 2003[a] May et al. 2009[b]
Ictalurus melas	Black Bullhead	76.82				USEPA 2014
Pylodictis olivaris	Flathead Catfish			75.97		May et al. 2009
Catostomus commersonii	White Sucker	77.37				USEPA 2014
Coregonus clupeaformis	Lake Whitefish			80		Rieberger 1992
Oncorhynchus kisutch	Coho Salmon			80		Rieberger 1992
Oncorhynchus mykiss	Rainbow Trout			77.54	61.2	USEPA 2021
Sander canadensis	Sauger	77				USEPA 2014
Perca flavescens	Yellow Perch	73.98				USEPA 2014
Micropterus salmoides	Largemouth Bass	75.74[a]		79.06[b] 78.53[c]		USEPA 2014[a] Pinkney 2003[b] May et al. 2009[c]
Micropterus dolomieu	Smallmouth Bass	74.22				USEPA 2014
Pomoxis annularis	White Crappie			80.57		May et al. 2009
Pomoxis nigromaculatus	Black Crappie			79.75		May et al. 2009
Lepomis macrochirus	Bluegill		74.8	80.09	76	USEPA 2021
Ambloplites rupestris	Rock Bass	74.95				USEPA 2014
Esox lucius	Northern Pike			78		Rieberger 1992
Pylodictis olivaris	Flathead Catfish				58.97	May et al. 2009
Scaphirhynchus platorynchus	Shovelnose Sturgeon			77.13	47.18	May et al. 2009

References

Chatakondi, N., R.T. Lovell, P.L. Duncan, M. Hayat, T.T. Chen, D.A. Powers, J.D. Weete, K. Cummins, and R.A. Dunham. 1995. Body composition of transgenic common carp, *Cyprinus carpio*, containing rainbow growth hormone gene. Aquaculture 138: 99-109. http://www.sciencedirect.com/science/article/pii/0044848695010785

May, T.W., M.J. Walther, W.G. Brumbaugh, and M. McKee, 2009. Concentrations of elements in whole-body fish, fish contaminant monitoring program: U.S. Geological Survey Open-File Report 2009–1278, 11 p. https://pubs.usgs.gov/of/2009/1278/pdf/OF2009_1278.pdf

Pinkney, A.E. 2003. Investigation of fish tissue contaminant concentrations at Painted Turtle Pond, Occoquan Bay National Wildlife Refuge, Woodbridge, Virginia. Annapolis, MD: U.S. Fish and Wildlife Service.

Rieberger, K. 1992. Metal concentrations in fish tissue from uncontaminated B.C Lakes. Ministry of Environment, Lands and Parks, Province of British Columbia. www2.gov.bc.ca/assets/gov/environment/air-land-water/water/waterquality/monitoring-water-quality/wq_bc_metal_in_fish.pdf

USEPA. 2011. Exposure factors handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC; EPA/600/R-09/052F. Available from the National Technical Information Service, Springfield, VA, and online at https://www.epa.gov/expobox/about-exposure-factors-handbook

USEPA. 2014. External peer review draft aquatic life ambient water quality criterion for selenium–freshwater 2014. EPA 822-P-14-001. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. https://19january2017snapshot.epa.gov/sites/production/files/2016-07/documents/2014_draft_document_external_peer_review_draft_aquatic_life_ambient_wqc_for_se_freshwater.pdf

USEPA. 2021. 2021 Revision to: Aquatic life ambient water quality criterion for selenium–freshwater 2016. EPA 822-R-21-006. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. https://www.epa.gov/system/files/documents/2021-08/selenium-freshwater2016-2021-revision.pdf

Appendix D: Extended List of Potential Target Species for Monitoring of Selenium in Fish Tissue

This appendix is intended for use with the recommendations in section 2.3 and Table 3. This appendix provides detailed information for the target species identified in Table 3, as well as additional species that may be considered appropriate for monitoring in certain situations (e.g., when species in Table 3 are unavailable for collection).

The tables in this appendix are generally organized by taxon (with the exception of a group of molluscivores) and includes nomenclature, distribution within US states, basic habitat information (warm water [WW] or cool or cold water [CW]), presence in waterbodies (e.g., lotic, lentic), adult diet, and adult trophic level. Information is presented by the following groupings:

1) Sturgeon in the family Acipenseridae
2) Sunfish and other genera in the family Centrarchidae
3) Trout and other genera in the family Salmonidae
4) Freshwater molluscivores & related genera (*Catostomidae*)
5) Minnows in the family Cyprinidae
6) Darters in the family Percidae
7) Sculpin in the family Cottidae

Information sources for these species include NatureServe (https://explorer.natureserve.org/Search), and the USGS NAS - Nonindigenous Aquatic Species Database (https://nas.er.usgs.gov/). Users of this Appendix should consult with both as they examine available information to make decisions about target species in state and authorized Tribal waters as they develop sampling plans.

1) Sturgeon

The family Acipenseridae comprise 27 species in four genera, with two genera (*Acipenser* and *Scaphyrhynchus*) occurring in the U.S. Sturgeon in the genus *Acipenser* (e.g., White Sturgeon) include three freshwater species. Sturgeons are long-lived, late maturing bottom feeding fishes inhabiting large river systems and estuaries. Independent monitoring for these species is generally discouraged since most populations are under pressure from habitat loss and other stressors and coordination with federal agencies (USFWS or NOAA-NMFS) is therefore recommended prior to developing sampling plans that may include these species.

Table D-1. Species in the Family Acipenseridae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Genus *Acipenser*			
White Sturgeon (*Acipenser transmontanus*)	US: AK, AZ, CA, ID, MT, OR, WA	WW Lotic Estuarine	Invertivore Piscivore Molluscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=300 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100679/Acipenser_transmontanus			
Shortnose Sturgeon* (*Acipenser brevirostrum*)	US: CT, DC, DE, FL, GA, MA, MD, ME, NC, NH, NJ, NY, PA, RI, SC, VA	WW Lotic Estuarine	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105033/Acipenser_brevirostrum			
Lake Sturgeon* (*Acipenser fluvescens*)	US: AL, AR, GA, IA, IL, IN, KS, KY, MI, MN, MO, NC, ND, NE, NY, OH, PA, SD, TN, VT, WI, WV	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=299 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104232/Acipenser_fulvescens			
Other Sturgeon:			
Shovelnose Sturgeon	US: AL, AR, IA, IL, IN, KS, KY, LA, MN, MO, MS, MT, ND, NE, NM,	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
(*Scaphirhynchus platorynchus*)	OH, OK, PA, SD, TN, TX, WI, WV, WY	Estuarine	
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103361/Scaphirhynchus_platorynchus			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

2) Sunfish (genus Lepomis), and other genera in the family Centrarchidae

The Bluegill is a species of freshwater fish and a member of the sunfish family Centrarchidae of the order Perciformes. It is native to North America and lives in streams, rivers, lakes, and ponds. The centrarchid family comprises 38 species of fish and includes many recreational and sportfish familiar to North Americans, including the Rock Bass, Largemouth Bass, Pumpkinseed, and crappies. This family typically inhabits medium to large warm water river systems and all sizes of lentic waterbodies. All species in the family are native to only North America.

Table D-2. Species in the Family Centrarchidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Genus *Lepomis*			
Bluegill (*Lepomis macrochirus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=385 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101764/Lepomis_macrochirus			
Pumpkinseed* (*Lepomis gibbosus*)	US: AL, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NV, NY, OH, OR, PA, RI, SC, TN, TX, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=382 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105048/Lepomis_gibbosus			
Redear Sunfish* (*Lepomis microlophus*)	US: AL, AR, AZ, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MI, MO, MS, NC, NE, NM, NV, OH, OK, OR, PA, SC, TN, TX, VA, VT, WV	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=390 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100707/Lepomis_microlophus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Green Sunfish (*Lepomis cyanellus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NJ, NM, NN, NV, NY, OH, OK, OR, PA, SC, SD, TN, TX, UT, VA, WA, WI, WV, WY	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=380 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103917/Lepomis_cyanellus https://pubs.usgs.gov/pp/1803/pdf/pp1803.pdf			
Redbreast Sunfish* (*Lepomis auritus*)	US: AL, AR, CT, DC, DE, FL, GA, KY, LA, MA, MD, ME, MS, NC, NH, NJ, NY, OK, PA, RI, SC, TN, TX, VA, VT, WV	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=379 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101339/Lepomis_auritus			
Longear Sunfish (*Lepomis megalotis*)	US: AL, AR, FL, GA, IA, IL, IN, KS, KY, LA, MD, MN, MO, MS, NC, NJ, NM, OH, OK, PA, TN, TX, VA, WI, WV	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=388 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.885331/Lepomis_megalotis			
Warmouth (*Lepomis gulosus*)	US: AL, AR, AZ, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, NJ, NM, NV, NY, OH, OK, OR, PA, SC, TN, TX, VA, WA, WI, WV	WW Lentic Lotic	Piscivore Invertivore TL3/4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=376 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102803/Lepomis_gulosus			
Orangespotted Sunfish (*Lepomis humilis*)	US: AL, AR, CO, FL, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=383 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103679/Lepomis_humilis			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Redspotted Sunfish (*Lepomis miniatus*)	US: AL, AR, IL, IN, KY, LA, MO, MS, OK, TN, TX	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=391 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105029/Lepomis_miniatus			
Bantam Sunfish (*Lepomis symmetricus*)	US: AR, IL, IN, KY, LA, MO, MS, OK, TN, TX	WW Lentic Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103718/Lepomis_symmetricus			
Northern Longear Sunfish (*Lepomis peltastes*)	US: IL, IN, MI, MN, NY, OH, PA	WW Lentic Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.883965/Lepomis_peltastes			
Spotted Sunfish (*Lepomis punctatus*)	US: FL, GA, NC, SC, TN	WW Lentic Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100708/Lepomis_punctatus			
Other Centrarchids			
Largemouth Bass (*Micropterus salmoides*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=401 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101622/Micropterus_salmoides			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Smallmouth Bass (*Micropterus dolomieu*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=396 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104786/Micropterus_dolomieu			
Spotted Bass (*Micropterus punctulatus*)	US: AL, AR, AZ, CA, FL, GA, IA, IL, IN, KS, KY, LA, MO, MS, NC, NE, NM, NV, OH, OK, PA, TN, TX, VA, WV	WW Lentic Lotic	Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=397 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.872252/Micropterus_punctulatus			
White Crappie (*Pomoxis annularis*)	US: AL, AR, AZ, CA, CO, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=408 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106200/Pomoxis_annularis			
Black Crappie (*Pomoxis nigromaculatus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=409 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103134/Pomoxis_nigromaculatus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Rock Bass (*Ambloplites rupestris*)	US: AL, AR, AZ, CT, DC, DE, GA, IA, IL, IN, KS, KY, MA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NY, OH, OK, PA, RI, SC, SD, TN, TX, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=373 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105635/Ambloplites_rupestris			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

3) Salmonids, including Brown, Rainbow, and Cutthroat Trout and whitefish

Trout is the common name for species of freshwater fish belonging to the genera *Oncorhynchus*, *Salmo*, and *Salvelinus* in the family Salmonidae. Trout are considered cold water fish and are usually found in clear streams, rivers and lakes with temperatures not exceeding 60ºF (16ºC).

Table D-3. Species in the Family Salmonidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Family Salmonidae			
Brown Trout (*Salmo trutta*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SD, TN, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=931 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103603/Salmo_trutta			
Rainbow Trout (*Oncorhynchus mykiss*)	US: AK, AL, AR, AZ, CA, CO, CT, DE, GA, HI, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore Piscivore TL3/TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=910 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105164/Oncorhynchus_mykiss			
Cutthroat Trout (*Oncorhynchus clarkii*)	US: AK, AR, AZ, CA, CO, ID, MD, MT, ND, NM, NN, NV, OR, UT, WA, WY	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=890 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103888/Oncorhynchus_clarkii			
Dolly Varden (*Salvelinus malma*)	US: AK, NV, NM, WA, WY	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3/TL4

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=941 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104555/Salvelinus_malma			
Brook Trout (*Salvelinus fontinalis*)	US: AK, AR, AZ, CA, CO, CT, DE, GA, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OR, PA, RI, SC, SD, TN, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=939 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103972/Salvelinus_fontinalis			
Mountain Whitefish (*Prosopium williamsoni*)	US: CA, CO, ID, MT, NV, OR, UT, WA, WY	CW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=924 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104696/Prosopium_williamsoni			
Round Whitefish (*Prosopium cylindraceum*)	US: AK, CT, IL, ME, MI, MN, NH, NY, VT, WI	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=921 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102380/Prosopium_cylindraceum			
Lake Whitefish* (*Coregonus clupeiformes*)	US: AK, ID, IL, IN, ME, MI, MN, MT, ND, NH, NV, NY, OH, PA, SD, VT, WA, WI	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=887 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105498/Coregonus_clupeaformis			

*Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

4) Freshwater Molluscivores and Related Genera

Molluscivorous fish feed either preferentially or opportunistically on a variety of mollusks (e.g., clams, mussels, and snails) in freshwater systems. Although taxonomically diverse, physiologically these fish are adapted to feed on mollusks due to the presence of teeth or plates on the lower (and in some species upper) pharyngeal jaws, as well as mouth gape and jaw muscle structure that accommodates feeding on mollusks (Eastman 1977). A study by the Army Corps of Engineers (Kirk, 2001) documents at least 17 species* of North American fish that consume invasive zebra mussels and quagga mussels in the genus *Dreissena*. Several of these species are sunfish in the genus *Lepomis*; they are presented in the table addressing sunfish. Molluscivores may have elevated exposure to selenium, as mollusks bioaccumulate more selenium than other classes of aquatic invertebrates. These taxa typically inhabit larger warm water lentic and lotic systems.

Table D-4. Molluscivores and Related Genera That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Freshwater Molluscivores			
Freshwater Drum* (*Aplodinotus grunniens*)	US: AL, AR, CO, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, MT, NC, ND, NE, NM, NY, OH, OK, PA, SD, TN, TX, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore Molluscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=946 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100338/Aplodinotus_grunniens			
White Perch* (*Morone americana*)	US: CO, CT, DC, DE, GA, IN, MA, MD, ME, MI, NC, NE, NH, NJ, NY, PA, RI, VA, VT, WI	WW Lentic Lotic Estuarine	Invertivore Molluscivore Piscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=777 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100436/Morone_americana			
White bass* (*Morone chrysops*)	US: AL, AR, AZ, CA, CO, DC, FL, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, MT, NC, ND, NE, NM, NV, NY, OH, OK, PA, SC, SD, TN, TX, UT, VA, WI, WV	WW Lentic Lotic	Piscivore Invertivore Molluscivore TL4

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=779 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100951/Morone_chrysops			
Round Goby* (*Neogobius melanostomus*)	US: IL, IN, MI, MN, NY, OH, PA, WI	WW Lentic Lotic	Piscivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=713 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100501/Neogobius_melanostomus			
Brown Bullhead* (*Ameiurus nebulosus*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, VA, VT, WA, WI, WV	WW Lentic Lotic	Omnivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=734 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103081/Ameiurus_nebulosus			
Yellow Perch* (*Perca flavescens*)	US: AL, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Invertivore Piscivore Molluscivore TL4
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=820 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102985/Perca_flavescens			
Common carp* (*Cyprinus carpio*)	US: AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NN, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Omnivore Molluscivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=4 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105636/Cyprinus_carpio			
Catostomidae			
Smallmouth Buffalo (*Ictiobus bubalus*)	US: AL, AR, AZ, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, MT, NC, ND, NE, NM, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=361 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105191/Ictiobus_bubalus			
Black Buffalo (*Ictiobus niger*)	US: AL, AR, AZ, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NC, ND, NE, OH, OK, PA, SD, TN, TX, WI, WV	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=363 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101227/Ictiobus_niger			
White sucker* (*Catostomus commersoni*)	US: AL, AR, CO, CT, DC, DE, GA, IA, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NM, NN, NY, OH, OK, PA, RI, SC, SD, TN, UT, VA, VT, WI, WV, WY	WW Lentic Lotic	Herbivore Molluscivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=346 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.833297/Catostomus_commersonii			
Largescale Sucker (*Catostomus macrocheilus*)	US: ID, MT, NV, OR, WA	CW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1098871/Catostomus_macrocheilus			
Greater Redhorse* (*Moxostoma valencienni*)	US: IL, IN, KY, MI, MN, ND, NY, OH, VT, WI	WW Lentic Lotic	Invertivore Molluscivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101488/Moxostoma_valenciennesi			
Shorthead Redhorse (*Moxostoma macrolepidutum*)	US: DC, DE, IA, IL, IN, KS, MD, MI, MN, MO, MS, MT, NC, ND, NE, NY, OH, OK, PA, SC, SD, TX, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=366 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.791411/Moxostoma_macrolepidotum			
River Redhorse (*Moxostoma carinatum*)	US: AL, AR, FL, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NC, NY, OH, OK, PA, TN, VA, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106031/Moxostoma_carinatum			
Golden redhorse (*Moxostoma erythrurum*)	US: AL, AR, DC, GA, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, ND, NY, OH, OK, PA, SD, TN, TX, VA, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=365 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100778/Moxostoma_erythrurum			
Silver Redhorse (*Moxostoma anisurum*)	US: AL, AR, GA, IA, IL, IN, KY, MI, MN, MO, MS, ND, NY, OH, PA, TN, VA, VT, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2912 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100712/Moxostoma_anisurum			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

5) Minnows (Cyprinidae)

The family Cyprinidae (carps and minnows) is naturally distributed throughout most of the world and is the largest family of freshwater fishes with about 2,010 species in 210 genera. About 300 species in 50 genera are native to North America (Canada, Mexico, United States; Nelson, 2006). Cyprinids exhibit considerable variation in morphology, diet, and habitat use, and are often the only fish taxa (along with darters and sculpins) occurring in small order streams. Although cyprinids are not typically considered monitoring targets for contaminant analysis in their tissues, they are routinely collected as part of state biomonitoring programs that use the fish index of biotic integrity to assess stream health in wadeable streams.

The EPA recommends that fish tissue monitoring programs collaborate with state or authorized Tribal biomonitoring programs to leverage expertise, experience, and resources to collect cyprinids and related species in watersheds located in geographic areas of elevated selenium where anthropogenic activities may introduce selenium to surface waters if other more sensitive species are not present.

Table D-5. Species in the Family Cyprinidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Fathead Minnow (*Pimephales promelas*)	US: AL, AR, AZ, CA, CO, CT, DE, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NM, NN, NV, NY, OK, OR, PA, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	WW Lentic Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=621 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102599/Pimephales_promelas			
Bluntnose Minnow (*Pimephales notatus*)	US: AL, AR, CT, DC, GA, IA, IL, IN, KS, KY, LA, MA, MD, MI, MN, MO, MS, NC, ND, NE, NJ, NY, OH, OK, PA, SD, TN, VA, VT, WI, WV	WW Lotic Lentic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=620 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103436/Pimephales_notatus			
Bullhead Minnow (*Pimephales vigilax*)	US: AL, AR, CO, GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, NE, NM, OH, OK, PA, SD, TN, TX, VA, WI,	WW Lentic Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=623 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106123/Pimephales_vigilax			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Cutlip Minnow (*Exoglossum maxillingua*)	US: CT, DC, DE, MD, NC, NJ, NY, PA, VA, VT, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=530 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102719/Exoglossum_maxillingua			
Suckermouth Minnow (*Phenacobius mirabilis*)	US: AL, AR, CO, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, NE, NM, OH, OK, SD, TN, TX, VA, WI, WV, WY	WW Lotic	Herbivore Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=617 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104716/Phenacobius_mirabilis			
Blackstripe Topminnow (*Fundulus notatus*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MI, MO, MS, OH, OK, TN, TX, WI	WW Lentic Lotic	Herbivore Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=690 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100269/Fundulus_notatus			
Starhead Topminnow (*Fundulus dispar*)	US: AL, AR, FL, IA, IL, IN, KY, LA, MI, MO, MS, OK, TN, WI	WW Lentic Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105342/Fundulus_dispar			
Western Blacknose Dace (*Rhinichthys obtusus*)	US: AL, GA, IA, IL, IN, KS, KY, MI, MN, MO, MS, NC, ND, NE, NY, OH, PA, SC, SD, TN, VA, WI, WV	CW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.790464/Rhinichthys_obtusus			
Eastern Blacknose Dace (*Rhinichthys atratalus*)	US: CT, DC, DE, GA, MA, MD, ME, NC, NH, NJ, NY, PA, RI, VA, VT, WV	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=637 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.828296/Rhinichthys_atratulus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Longnose Dace (*Rhinichthys cataractae*)	US: CO, CT, DC, DE, GA, IA, ID, IL, IN, MA, MD, ME, MI, MN, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	CW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=638 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101847/Rhinichthys_cataractae			
Finescale Dace (*Chrosomus neogaeus*)	US: ME, MI, MN, ND, NE, NH, NY, SD, VT, WI, WY	WW Lentic Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2556 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102927/Chrosomus_neogaeus			
Speckled Dace (*Rhinichthys osculus*)	US: AZ, CA, CO, ID, NM, NN, NV, OR, UT, WA, WY	CW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=640 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100335/Rhinichthys_osculus			
Satinfin Shiner (*Cyprinella analostana*)	US: DC, DE, MD, NC, NJ, NY, PA,	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=516 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106108/Cyprinella_analostana			
Red Shiner (*Cyprinella lutrensis*)	US: AL, AR, AZ, CO, GA, IA, IL, IN, KS, KY, LA, MN, MO, MS, NC, ND, NE, NM, NN, NV, OK, SD, TN, TX,	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=518 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105504/Cyprinella_lutrensis			
Bigmouth Shiner (*Notropis dorsalis*)	US: CO, IA, IL, IN, KS, MI, MN, MO, ND, NE, NY, OH, PA, SD, TN, WI, WV, WY	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=593 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104308/Notropis_dorsalis			
Chub Shiner (*Notropis potteri*)	US: AR, LA, OK, TX	WW Lentic Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=606 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105179/Notropis_potteri			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Sand Shiner (*Notropis stramineus*)	US: AR, AZ, CO, IA, IL, IN, KS, KY, MI, MN, MO, MT, ND, NE, NM, NN, NY, OH, OK, PA, SD, TN, TX, UT, VA, VT, WI, WV, WY	WW Lentic Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=600 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104717/Notropis_stramineus			
Redside Shiner (*Richardsonius balteatus*)	US: AZ, CO, ID, MT, NV, OR, UT, WA, WY	CW Lentic Lotic	Herbivore Invertivore Molluscivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=644 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100279/Richardsonius_balteatus			
Thicklip Chub (*Cyprinella labrosa*)	US: NC, SC, VA	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101215/Cyprinella_labrosa			
Streamline Chub (*Erimystax dissimilis*)	US: AL, IN, KY, NY, OH, PA, TN, VA, WV	WW Lotic	Invertivore Molluscivore TL3
Map: & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106034/Erimystax_dissimilis			
Shoal Chub (*Macrhybopsis hyostoma*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MN, MO, MS, NE, OH, OK, TN, TX, WI, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106278/Macrhybopsis_hyostoma			
Silver Chub (*Macrhybopsis storeriana*)	US: AL, AR, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, NY, OH, OK, PA, SD, TN, TX, WI, WV	WW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101653/Macrhybopsis_storeriana			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
River Chub (*Nocomis micropogon*)	US: AL, DC, GA, IL, IN, KY, MD, MI, NC, NY, OH, PA, SC, TN, VA, WV	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=577 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101786/Nocomis_micropogon			
Bull Chub* (*Nocomis raneyi*)	US: NC, VA	WW Lotic	Herbivore Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101374/Nocomis_raneyi			
Peamouth (*Mylocheilus caurinus*)	US: ID, MT, OR, WA	CW Lentic Lotic	Invertivore Molluscivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2349 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100544/Mylocheilus_caurinus			
Creek Chub (*Semotilus atromaculatus*)	US: AL, AR, CO, CT, DC, DE, FL, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NY, OH, OK, PA, SC, SD, TN, TX, UT, VA, VT, WI, WV, WY	WW Lotic	Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=649 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104867/Semotilus_atromaculatus			
Central Stoneroller (*Campostoma anomalum*)	US: AR, CO, CT, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, ND, NE, NM, NY, OH, OK, PA, SC, SD, TN, TX, VA, WI, WV, WY	WW Lotic	Herbivore
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=506 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.844144/Campostoma_anomalum			
Largescale Stoneroller (*Campostoma oligolepis*)	US: AL, AR, GA, IA, IL, IN, KY, MN, MO, MS, ND, OK, VA, WI	WW Lotic	Herbivore

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=507 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102552/Campostoma_oligolepis			
Sacramento Splittail (*Pogonichthys macrolepidotus*)	US: CA	WW Lotic Estuarine	Herbivore Invertivore
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105438/Pogonichthys_macrolepidotus			

* Species documented to consume invasive zebra mussels and quagga mussels in the genus *Dreissena* by the Army Corps of Engineers (Kirk, 2001).

6) Darters (Percidae)

Darters are small, perch-like fish in the family Percidae and are found in freshwater streams in North America. Darters typically occur in riverine systems, inhabiting cold to cool streams and small river systems in North America. Species distributions range from single watersheds in one state to multiple watersheds in several states. Darters are typically benthic omnivores, practicing herbivory as well as preying on invertebrates and for some species, small fish and fish eggs as well.

Table D-6. Species in the Family Percidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Greenside Darter (*Etheostoma blennioides*)	US: AL, AR, DC, GA, IL, IN, KS, KY, MD, MI, MO, MS, NC, NY, OH, OK, PA, TN, VA, WV	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=808 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.790349/Etheostoma_blennioides			
Arkansas Darter (*Etheostoma cragini*)	US: AR, CO, KS, MO, OK	WW Lotic	Invertivore Molluscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=810 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103800/Etheostoma_cragini			
Iowa Darter (*Etheostoma exile*)	US: CO, IA, IL, IN, MI, MN, MT, ND, NE, NM, NY, OH, PA, SD, UT, WI, WY	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=812 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100441/Etheostoma_exile			
Fantail Darter (*Etheostoma flabellare*)	US: AL, AR, DC, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, NY, OH, OK, PA, SC, TN, VA, VT, WI, WV	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.832912/Etheostoma_flabellare			
Least Darter (*Etheostoma microperca*)	US: AR, IA, IL, IN, KS, KY, MI, MN, MO, OH, OK, WI	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103616/Etheostoma_microperca			
Johnny Darter (*Etheostoma nigrum*)	US: AL, AR, CO, IA, IL, IN, KS, KY, MD, MI, MN, MO, MS, NC, ND, NE, NY, OH, OK, PA, SD, TN, UT, VA, WI, WV, WY	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=814 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.100152/Etheostoma_nigrum			
Tessellated Darter (*Etheostoma olmstedi*)	US: CT, DC, DE, FL, GA, MA, MD, NC, NH, NJ, NY, PA, RI, SC, VA, VT, WV	WW Lotic Lentic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=816 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106063/Etheostoma_olmstedi			
Cypress Darter (*Etheostoma proeliare*)	US: AL, AR, FL, IL, KY, LA, MO, MS, OK, TN, TX	WW Lotic Lentic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101593/Etheostoma_proeliare			
Redline Darter (*Etheostoma rufilineatum*)	US: AL, GA, KY, MS, NC, TN, VA	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2886 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103471/Etheostoma_rufilineatum			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Orangebelly Darter (*Etheostoma radiosum*)	US: AR, OK, TX	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1156842/Etheostoma_radiosum			
Orangethroat Darter (*Etheostoma spectabile*)	US: AR, CO, IA, IL, IN, KS, KY, MI, MO, NE, OH, OK, TN, TX, WY	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102592/Etheostoma_spectabile			
Speckled Darter (*Etheostoma stigmaeum*)	US: AL, AR, FL, GA, KY, LA, MO, MS,	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1006953/Etheostoma_stigmaeum			
Gulf Darter (*Etheostoma swaini*)	US: AL, FL, GA, KY, LA, MS, TN	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102176/Etheostoma_swaini			
Variegate Darter (*Etheostoma variatum*)	US: IN, KY, NY, OH, PA, VA, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.102018/Etheostoma_variatum			
Banded Darter (*Etheostoma zonale*)	US: AL, AR, GA, IA, IL, IN, KS, KY, MD, MI, MN, MO, NC, NY, OH,	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=818 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106576/Etheostoma_zonale			
River Darter (*Percina shumardi*)	US: AL, AR, GA, IA, IL, IN, KS, KY, LA, MI, MN, MO,	WW Lotic	Invertivore TL3

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
	MS, ND, OH, OK, PA, TN, TX, WI, WV		
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=826 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101870/Percina_shumardi			
Slenderhead Darter (*Percina phoxocephala*)	US: AL, AR, IA, IL, IN, KS, KY, MN, MO, MS, OH, OK, SD, TN, WI, WV	WW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.104090/Percina_phoxocephala			
Shield Darter (*Percina peltata*)	US: DC, DE, MD, NJ, NY, PA, VA, WV	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2775 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.105028/Percina_peltata			
Blackbanded Darter (*Percina nigrofasciata*)	US: AL, FL, GA, LA, MS, NC, SC, TN	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=824 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.1255463/Percina_nigrofasciata			
Blackside Darter (*Percina maculata*)	US: AL, AR, IA, IL, IN, KS, KY, LA, MI, MN, MO, MS, ND, NE, NY, OH, OK, PA, SD, TN, TX, VA, WI, WV	WW Lotic	Invertivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=823 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106566/Percina_maculata			

7) Sculpins (Scorpionidae)

Sculpins are members of the family Scorpionidae (Scorpionfish), and most species in the Northern Hemisphere are saltwater fishes. Most freshwater sculpins in the U.S. are in the genus *Cottus* and are small benthic predators consuming mainly invertebrates. Sculpins typically prefer cooler, headwater streams, but can be found in larger warmer streams in some states.

Table D-7. Species in the Family Scorpionidae That May be Sampled for Implementation of the Selenium Criterion.

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Mottled Sculpin (*Cottus bairdii*)	US: AL, AZ, CO, DE, GA, IA, ID, IL, IN, KY, MD, MI, MN, MO, MS, MT, NC, NM, NN, NV, NY, OH, OR, PA, SC, TN, UT, VA, VT, WA, WI, WV, WY	CW/WW Lotic Lentic	Herbivore Invertivore Piscivore TL3
Map: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=502 Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819868/Cottus_bairdii			
Paiute Sculpin (*Cottus beldingii*)	US: CA, CO, ID, NV, OR, UT, WA, WY	CW/WW Lotic	Herbivore Molluscivore Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101884/Cottus_beldingii			
Banded Sculpin (*Cottus carolinae*)	US: AL, AR, GA, IL, IN, KS, KY, MO, MS, NC, OK, TN, VA	CW/WW Lotic	Herbivore Invertivore Piscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.819914/Cottus_carolinae			
Slimy Sculpin (*Cottus cognatus*)	US: AK, CT, IA, ID, IL, IN, MA, ME, MI, MN, MT, NH, NJ, NY, PA, VA, VT, WA, WI, WV	CW Lotic Lentic	Herbivore Invertivore Piscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.101449/Cottus_cognatus			

Name (Common, Scientific)	Distribution	Habitat (WW/CW) Lentic/Lotic	Adult Diet/ Trophic Level (TL)
Shorthead Sculpin (*Cottus confuses*)	US: ID, NV, OR, WA	CW Lotic	Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.905574/Cottus_confusus			
Riffle Sculpin (*Cottus gulosus*)	US: CA, OR, WA	CW Lotic	Invertivore Molluscivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103055/Cottus_gulosus			
Spoonhead Sculpin (*Cottus ricei*)	US: IL, MI, MN, MT, NY, OH, PA, WI	CW Lotic Lentic	Omnivore Invertivore TL3
Map & Info: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.103689/Cottus_ricei			

References

Eastman, J. 1977. The pharyngeal bones and teeth of catostomid fish. Am. Midl. Nat. 97(1): 68-88.

Kirk, J.P., K.J. Killgore, and L.G. Sanders. 2001. Potential of North American molluscivorous fish to control dreissenid mussels. U.S. Army Corps of Engineers Zebra Mussel Research Program.

NatureServe Explorer. NatureServe Explorer. https://explorer.natureserve.org/. Accessed 2020.

Nelson, J. S. 2006. Fishes of the World. 4th ed. Hoboken, New Jersey: John Wiley & Sons.

USGS. 2020. Nonindigenous aquatic species database, Gainesville, FL. nas.er.usgs.gov

Appendix E: Calculation of Composite Trophic Transfer Factors

Derivation of Trophic Transfer Factor (TTF) Values

The parameter $TTF^{composite}$ (composite trophic transfer factor) in Equation 1 quantitatively represents all dietary pathways of selenium exposure for a particular fish species within an aquatic system. The parameter is derived from species-specific *TTF* values representing the food web characteristics of the aquatic system and the proportion of species consumed. It is possible to differentiate bioaccumulative potential for different predator species and food webs by modeling different exposure scenarios. For example, where a fish species of interest is a trophic level 4 predator that primarily consumes trophic level 3 fish, the term $TTF^{composite}$ can be represented as the product of all *TTF* parameters that includes the additional trophic levels given as:

$$TTF^{composite} = TTF^{TL4} \times TTF^{TL3} \times TTF^{TL2}$$

(Equation 1)

where:

TTF^{TL2}	=	the trophic transfer factor of the trophic level 2 species
TTF^{TL3}	=	the trophic transfer factor of the trophic level 3 species
TTF^{TL4}	=	the trophic transfer factor of the trophic level 4 species
$TTF^{composite}$	=	the product of all the trophic transfer factors

The consumption of more than one species of organism at the same trophic level can also be modeled by expressing the *TTF* at a particular trophic level as the weighted average of the *TTF*s of all species consumed given as:

$$\overline{TTF}^{TLx} = \sum_i \left(TTF_i^{TLx} \times w_i\right)$$

(Equation 2)

where:

TTF_i^{TLx}	=	the trophic transfer factor of the i[th] species at a particular trophic level
w_i	=	the proportion of the i[th] species consumed

Figure 1 below describes five example food web scenarios and the formulation of $TTF^{composite}$ to model selenium bioaccumulation in each of them.

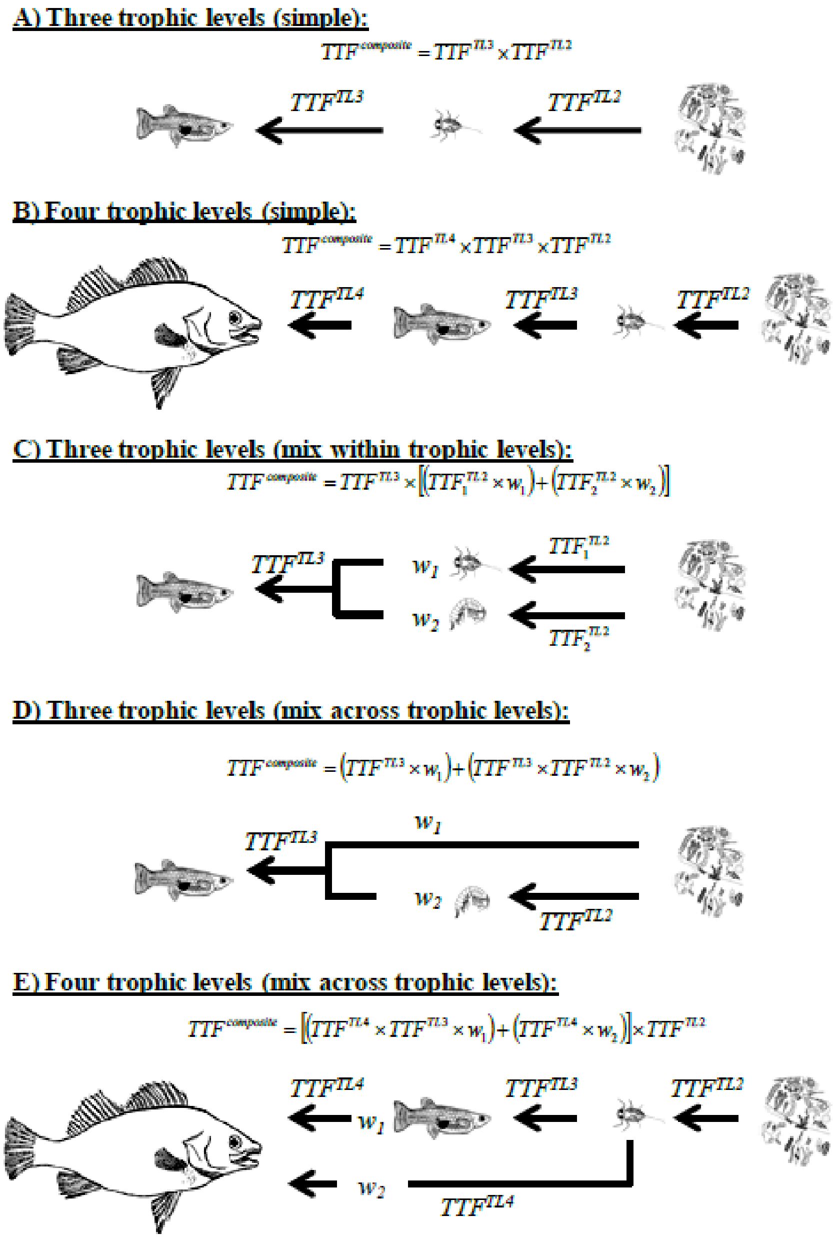

Figure 1. Example aquatic system scenarios and the derivation of the equation parameter $TTF^{composite}$.