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Technical Support for Fish Tissue Monitoring for Implementation of EPA's 2016 Selenium Criterion

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This document provides an overview on how to establish or modify existing fish tissue monitoring programs to facilitate the collection and analysis of fish tissue for the implementation of the fish tissue-based criterion elements in the 2016 selenium water quality criterion, including waterbody assessment and listing as well as development of water column-based site-specific criteria. The document does not address the development of fish-tissue-based site-specific criteria. The document does not impose legally binding requirements on EPA, states, authorized tribes, other regulatory authorities, or the regulated community, and may not apply to a particular situation based upon the circumstances. 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 where appropriate and consistent with statutory and regulatory requirements. EPA could update this document as new information becomes available. In addition to this document, EPA has other documents which provide considerations and recommendations on implementing the selenium criterion and can be found at EPA's selenium website: https://www.epa.gov/wqc/aquatic-life-criterion-selenium

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List of Acronyms

MDL	Method Detection Limit
NAMC	North American Metals Council
QA/QC	Quality assurance/quality control
SSD	Species sensitivity distribution
TMDL	Total Maximum Daily Load
TSM	Technical Support Materials
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
WQC	Water quality criterion
WQS	Water quality standard

Definitions

Anadromous fish
Types of fish whose life cycle is divided between fresh and saltwater, including migrating to spawn in freshwater. Migrations should be cyclical and predictable and cover more than 100 km. (FishBase, 2016)

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

Exogenous feeding
Nutrient acquisition in which the food source is orally ingested and digested in the intestines. (Balon, 2013)

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, e.g., annual or seasonal batches, as is the case in most fishes. (FishBase, 2016)

Potamodromous
Fish species that spend their whole life in fresh water, 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 and predictable and cover more than 100 km. (FishBase, 2016)

Synchronous spawners
Eggs are released in a single episode in 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)

Document Overview

This document is part of a series of documents prepared by the U.S. Environmental Protection Agency (EPA) Office of Water to provide an overview to states, authorized tribes, and other agencies on EPA's 2016 CWA section 304(a) recommendations for Aquatic Life Water Quality Criterion for Selenium – Freshwater (USEPA 2016a). This document is intended to be used in conjunction with three companion documents:

1) Technical Support for Adopting and Implementing EPA's Selenium 2016 Criterion in Water Quality Standards
2) Frequently Asked Questions (FAQs): Implementing WQS that Include Elements Similar or Identical to EPA's 2016 Selenium Criterion in Clean Water Act Section 402 NPDES Programs
3) Frequently Asked Questions (FAQs): Implementing the 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing, and Total Maximum Daily Load (TMDL) Programs

Collectively, these four documents comprise the Technical Support Materials (TSM) for *EPA's Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2016a). This document provides an overview on how to establish or modify existing fish tissue monitoring programs to facilitate implementation of the fish tissue-based criterion elements in the 2016 selenium water quality criterion. This includes monitoring for waterbody assessment and listing as well as development of water column-based site-specific criteria. The document does not specifically address monitoring for the development of fish-tissue-based site-specific criteria. States and authorized tribes who wish to develop fish-tissue-based site-specific criteria should engage their EPA Regional office early in the process to ensure the development of sound scientific analyses.[1]

Criteria Overview

The EPA updated its national recommended chronic aquatic life criterion for selenium in freshwater to reflect the latest scientific information, which indicates that toxicity to aquatic life is driven by dietary exposures. The criterion has four elements: (1) a fish egg-ovary element, (2) a fish whole-body and/or muscle element, (3) a water column element (one value for lentic and one value for lotic aquatic systems), and (4) a water column intermittent element to account for potential chronic effects from short-term exposures (one value for lentic and one value for lotic aquatic systems). EPA's *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* contains a recommendation that states and authorized tribes adopt into their water quality standards (WQS) a selenium criterion that includes all four elements (USEPA 2016a). The criterion document also recommends that—because the fish tissue-based concentration is a more direct measure of selenium toxicity to aquatic life than water column concentrations—fish tissue elements supersede the water column elements when both types of data are available (Table 1). All tissue elements have primacy over water element(s), except where there are no fish, or for water bodies with new discharges where tissue concentrations in fish might not have stabilized. EPA did not develop an acute criterion for selenium when it updated the chronic criterion. In the case of bioaccumulative compounds like selenium, acute toxicity studies do not address risks that result from exposure to chemicals via the diet (through the food web). Such studies also do not account

[1] 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.

for the slow accumulation kinetics of many bioaccumulative compounds such as selenium and may underestimate effects from long-term accumulation in different types of aquatic systems. Because exposure to selenium toxicity is primarily driven by organisms eating selenium-contaminated food rather than being exposed only to selenium dissolved in water, chronic exposure is a more relevant concern for aquatic life. However, as described in the criterion document, EPA included an intermittent criterion element. Application of the intermittent exposure criterion element will provide protection from the most important selenium toxicity effect, reproductive toxicity, by protecting against selenium bioaccumulation in the aquatic ecosystem resulting from short-term, high exposure events (USEPA 2016a).

The selenium aquatic life chronic criterion is unique, in part, because it is the first aquatic life criterion based on fish tissue. EPA has previously published fish tissue-based criteria for methyl-mercury, but those criteria are for protecting human health. Therefore, states and authorized tribes have experience sampling fish tissue for the purposes of issuing fish consumption advisories, thus collection of fish tissue for water quality assessment is common.

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 or 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	$WQC_{int} = \frac{WQC_{30-day} - C_{bkgrnd}(1 - f_{int})}{f_{int}}$
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.
3. Fish whole-body or muscle tissue supersedes water column element when both fish tissue and water concentrations are measured.
4. Water column values are based on dissolved total selenium in water and are derived from fish tissue values via bioaccumulation modeling. 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.

EPA derived fish tissue and water column elements from the underlying scientific studies on selenium reproductive effects in fish taking into consideration the implementation of criteria for Clean Water Act purposes (e.g., permitting, monitoring, and assessment). Available toxicity data indicate the selenium concentration in fish eggs and ovaries is the most robust and consistent measurement endpoint directly tied to adverse aquatic effects. Toxicity in developing embryos and larvae is directly linked to egg selenium concentration (USEPA 2016a). EPA derived the whole-body and muscle tissue elements from the egg-ovary element so that states and authorized tribes could more readily implement EPA's selenium criterion.

EPA recommends that states and authorized tribes adopt into their water quality standards a selenium criterion that expresses the four elements as a single criterion composed of multiple parts in a manner that explicitly affirms the primacy of the whole-body or muscle element over the water column elements, and the egg-ovary element over any other element. Adopting the fish whole-body and muscle tissue element into water quality standards ensures the protection of aquatic life when measurements from fish eggs or ovaries are not available. Adopting the water column element ensures protection when fish tissue measurements are not available. For approaches for translating between fish tissue and water column selenium concentrations, see Appendix K of *Aquatic Life Ambient Water Quality Criterion for Selenium–Freshwater 2016* (USEPA 2016a). For information on how to use the four-part criterion for the purposes of National Pollutant Discharge Elimination System (NPDES) permitting and waterbody assessment, listing, and TMDL development, see *Frequently Asked Questions (FAQs): Implementing WQS that Include Elements Similar or Identical to EPA's 2016 Selenium Criterion in Clean Water Act Section 402 NPDES Programs* (USEPA 2016b) and *FAQs: Implementing the 2016 Selenium Criterion in Clean Water Act Sections 303(d) and 305(b) Assessment, Listing and Total Maximum Daily Load (TMDL) Programs* (USEPA 2016c), respectively.

Monitoring Strategy

The following sections review study design and sampling considerations regarding fish tissue types, sample types, target species and sizes, and spatial and temporal concerns. Additional information regarding adoption of, implementation of, and compliance with the criteria can be found in the three companion documents (USEPA 2016b, USEPA 2016c, and USEPA 2016d).

When considering monitoring strategies, agencies should first review their existing fish tissue monitoring programs, if any exist, and determine how best to incorporate fish tissue sampling for selenium. The relationship between fish tissue sampling locations, species habits and natural history, and selenium sources should be understood and taken into account during sampling for implementation of the criterion. Detailed field collection procedures can be found in EPA's 2000 Fish Advisory Guidance (USEPA 2000a) and the Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue (USEPA 2002). Appendix A of this document presents egg and ovary collection and sample preparation methods.

Tissue Type

From the toxicology standpoint, the most relevant measure of exposure to a toxic substance is its concentration at the site of toxic action. Because of selenium's mode of action in fish, the most ecologically relevant sites of toxic action are the developing tissues during early life stages. This was a major point of consensus of the 2009 SETAC Pellston workshop on selenium risk assessment (Chapman et al. 2009). The 304(a) selenium aquatic life criterion is based on reproductive impacts in fish. Egg

and/or ovary tissue is the closest surrogate for measuring actual reproductive effects from maternal exposure to selenium. Therefore, selenium concentrations in egg-ovary tissue is the most useful exposure measure for estimating ecological effects.

Egg-ovary tissue of adult female fish may be the best surrogate for assessment of reproductive toxicity in fish, however some states or authorized tribes may instead sample muscle or whole-body tissue from adult fish due to the following considerations:

- *Temporal*: Most fish species that are synchronous spawners do so in the spring; whereas fish tissue collection for advisories typically occur in the late summer or early fall, when contaminant loads in the edible portion of the fish are highest.
- *Spatial*: Some fish species (e.g., salmonids) migrate to upstream areas to spawn; areas may be harder to access than larger order downstream segments that are inhabited during non-spawning seasons.
- *Size*: It is difficult to collect egg-ovary (or muscle) tissue samples from small fish species (e.g., certain species in the family Cyprinidae or Cyprinodontidae) because the amount of tissue available for analysis is small, and many of these species are asynchronous spawners that do not have a large number or biomass of eggs at any one time.

Due to these various concerns, states or authorized tribes have considerable discretion when selecting the fish tissue type to be used in their sampling protocols. The flexibility provided by having multiple fish tissue types for water quality monitoring and assessment purposes also leverages existing monitoring capacity since a number of the species that are good target species for selenium sampling may also be commonly collected as muscle (fillet) samples in state and tribal fish tissue monitoring programs (e.g., trout/salmon, bass/sunfish). The whole-body tissue criterion element also simplifies the collection and processing of small fish species that may be the dominant trophic level in smaller order stream networks. When developing a new or modifying an existing fish tissue monitoring strategy, states or authorized tribes should consider the available resources, existing information on the spawning habits and size of target species, and potential population level effects associated with lethal sampling techniques. They should also consider where the relevant exposure is, and understand where fish are feeding and obtaining their selenium body burdens. 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
Ease of collection	Difficult	Easy	Easy – except on small fish	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 & tribal methods	Not typically collected	Sometimes collected	Primary tissue collected	Whole-body might be collected in special cases, for certain populations that consume whole fish, or for eco-risk assessments.
Sample availability	Limited – only from gravid females	Always	Always	For water bodies with small species at top trophic levels, whole-body may be the only option due to issues collecting sufficient muscle tissue.
Ability to make composite sample	Yes	Yes	Yes	Composite samples are the most cost-effective way to represent average selenium tissue concentrations. However, information on elevated levels of chemical contamination in individual organisms is likely attenuated.
Ability to test individual sample	Yes on larger species; smaller species or asynchronous spawners may require composited tissue	Yes on larger species, may be difficult on small species	Yes on larger species, may be difficult on small species	Individual samples are more resource intensive to prepare and more expensive to analyze, but are valuable when sampling from waters known or suspected to be impacted by selenium discharges.

*See Appendix C for methods to convert from wet weight to dry weight and vice versa.

Egg-ovary Tissue Sample

Egg-ovary is the preferable tissue to collect because the egg-ovary tissue of pre-spawn, reproductively mature (also called "gravid" or "vitellogenic") females will give the most accurate view of potential selenium hazard to reproduction. Egg-ovary tissue (which refers to eggs, ovaries, or both) 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, which is transferred from the adult female during vitellogenesis. Buhl and Hamilton found concentrations 2-5 times higher in eggs than that in the maternal muscle tissue, indicating that dietary selenium was transferred from the female in a concentration-dependent manner (Buhl and Hamilton 2000). When using egg-ovary tissue for the implementation of the selenium criterion, states and authorized tribes must be careful to consider the difficulty in timing egg-ovary sampling with spawning periods. Timing errors related to fish reproduction may result in data that falsely indicate the selenium criterion is being met.

Monitoring programs should sample for reproductively mature females from iteroparous fish species (i.e., fish that have multiple reproductive life cycles over the course of its lifetime) that are single batch (synchronous) or multiple batch (asynchronous) spawners. Fish species that spawn multiple times per season (asynchronous; e.g., species in the family Cyprinidae) have variable cycles of oogenesis and thus special care should be taken when using these for egg-ovary monitoring as the pre-spawn window can be hard to predict. Egg maturation may occur well before, immediately prior to, or during the spawning season. For example, *Lepomis cyanellus* (Green Sunfish) can spawn multiple times per season (Osmundson and Skorupa 2011, Chapman et al. 2010). For many fish species, vitellogenesis can occur over several months prior to spawning, with a relatively large amount of yolk deposited into eggs (Osmundson and Skorupa 2011). It is also possible that species with relatively large eggs and yolks deposit more selenium in their eggs than species with smaller eggs and yolks (Osmundson and Skorupa 2011). Selenium concentrations in the eggs and ovarian tissues are expected to be at their maximum level when eggs have maximum levels of vitellogenin prior to spawning; therefore, egg-ovary tissue samples collected outside of the pre-spawn window are not suitable for assessment in comparison to the national egg-ovary fish tissue criterion element. 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. Appendix A of this document presents egg and ovary collection and sample preparation methods.

An egg-ovary tissue sample from a female that is not gravid will not be representative for monitoring and assessment when compared with gravid egg-ovary results, since the egg-ovary tissues represent the potential selenium load available to eggs and larvae through maternal transfer. Larger game species such as Rainbow Trout (*Oncorhynchus mykiss)* and Walleye (*Stizostedion vitreum)* will be logistically simpler to sample because they spawn once per year, which allows for easier collection of egg-ovary tissue since the reproductive timing and habits of these species in freshwater tend to be well understood in most areas.

Species should be sampled when females are expected to be gravid. This will depend on the species and geography, and for most species this will happen in spring but may happen later at higher latitudes. For example, different species of trout begin releasing eggs and sperm (spawning) during different times of the year. Rainbow Trout (*Oncorhynchus mykiss)* spawn in the late spring and early summer as water temperatures rise. Brown Trout (*Salmo trutta)* spawn in the fall, typically from late September to early November, and Lake Trout (*Salvelinus namaycush*) also spawn during the autumn months. See Appendix B of this document for spawning windows of different species in various regions across the US.

The egg-ovary tissue element has primacy over all other elements, thus, when available, it is the ultimate arbiter for compliance with the selenium water quality criterion. Most states and authorized tribes do not currently collect egg-ovary tissue as part of their regular monitoring programs. EPA recognizes that many states and authorized tribes 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 samples can be used as an alternative.

Whole-body and Muscle Tissue Samples

The whole-body and muscle tissue elements of EPA's selenium criterion were derived from the egg-ovary element. Whole-body and muscle tissue samples are acceptable alternatives because selenium concentrations in fish collected at any time of the year (except pre-spawn windows for females) will provide sufficient information on selenium bioaccumulation, although there will likely be some variation across seasons, due to prey availability, temperature, depuration of selenium from tissue during vitellogenesis prior to spawning, and other factors. Summer and fall may be prime periods for whole-

body and muscle tissue collection due to the engorgement of populations to replenish fat and energy reserves post-spawn and for over-wintering. Winter tissue collection is discouraged, except for subtropical regions. Whole-body and muscle fish 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 whole-body tissue element is intended to be used for whole fish for small fish species or small individuals of larger fish species. Whole-body and muscle tissue are equally preferred in the absence of egg-ovary tissue.

Whole-body and muscle tissue samples are relatively easy to collect, and do not have the same spatial considerations and temporal restrictions as egg-ovary tissue. Muscle tissue is the most common type of sample collected and analyzed by monitoring programs, and whole-body samples are sometimes submitted by states and authorized tribes for analysis. A portion of these samples already collected can be submitted for selenium analysis. States or authorized tribes will realize cost efficiencies by choosing to use whole bodies or fillets that are already being collected for an existing monitoring program. EPA is aware that some states and authorized tribes make use of muscle plugs in their monitoring programs. However, it is important to remember that contaminant concentrations can vary considerably depending on where the plug is collected. Plugs provide very small tissue quantities (about a gram of tissue per fish) and therefore not enough biomass for possible reanalysis or quality assurance/quality control considerations. In addition, relatively small individuals may not recover from a muscle plug biopsy punch. Care should be taken to ensure that the sampling protocols involving plugs have a sound scientific basis and that there is enough tissue for the analytical method.

States or authorized tribes might choose to use whole-body or muscle tissue samples because seasonal restrictions on fish sampling may prevent sampling for egg-ovary tissue, or because existing monitoring programs can incorporate selenium analysis into their existing fish tissue monitoring strategies. States or authorized tribes might also choose to use whole-body samples because juvenile or small-bodied species are the most appropriate to sample in a particular situation (Beatty and Russo 2014). In small streams and watersheds that are dominated by lower trophic level fish, it may be difficult to collect egg-ovary tissue from small fish species (e.g., species in the family Cyprinidae or Cyprinodontidae), due to the small amount of egg-ovary tissue available for analysis. In addition, most small bodied fish (i.e., minnows – cyprinids, cyprinodonts and Killifish [*Fundulus* spp.]) are asynchronous spawners, and produce eggs sporadically over the spawning season such that there is no one "best" time to collect mature eggs. Furthermore, the small body mass (even at adult stage) for many of these fish necessitates the collection of multiple individuals to ensure a sufficient tissue sample for processing and analytical chemistry analyses.

Another case where whole-body or muscle samples might be used is for Pacific anadromous juvenile (smolt) salmonids. Anadromous fish species are those spawned in freshwater, then migrate to the ocean as juveniles (e.g., smolts), where they grow into adults before migrating back into freshwater to spawn. Notable among these species are the coho, chum, and Chinook salmon, as well as marine adapted rainbow trout (steelhead). Adult anadromous females (in the genus *Oncorhynchus*) 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 representative of localized freshwater selenium sources (see Section 6.4.1 of the criterion document) (USEPA 2016a). 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), so monitoring of selenium in the whole body of Pacific anadromous salmon smolt is the most appropriate tissue to assess selenium hazard to these fish species.

Seasonal considerations are less stringent for whole-body and muscle tissue sampling. Seasonal collection of whole-body or muscle fish tissue samples should be timed to avoid the pre-spawning influence on selenium tissue concentrations, particularly for females, since enhanced depuration of selenium from tissue stores may occur during vitellogenesis prior to spawning (USEPA 2016a).

Sample Type

For fish tissue monitoring of selenium for implementing EPA's recommended selenium criterion, EPA recommends using composite samples. This is based on current EPA guidance on fish tissue monitoring which recommends using composite samples (USEPA 2000a).

Composite Samples

Composite samples are homogeneous mixtures of one type of tissue (e.g., egg-ovary sample, whole-body, or muscle) from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample. Composite samples of fish tissue are recommended for selenium analysis to help identify those sites where selenium concentrations are elevated. They are also best for small fish species where they become a logistical necessity due to small amounts of tissue per individual fish. Because the costs of individual chemical analyses are usually higher than field costs, EPA recommends using composite samples as the most cost-effective way to represent average selenium tissue concentrations in target species populations (see Table 3). Since composites represent a physical averaging of the samples, they also avoid the issue of how non-detections will be factored into averaging (USEPA 2010a). Additionally, composite samples ensure adequate sample mass to allow analyses for any additional target analytes. A disadvantage of using composite samples, however, is that elevated/extreme contaminant concentration values for individual organisms are attenuated.

Current EPA guidance on fish tissue monitoring recommends using composite samples and recommends using 3 to 10 individuals for a composite sample for each target species as availability allows (USEPA 2000a). In Section 6.1.2.7.1 of the Fish Advisory Guidance ("Guidelines for Determining Sample Sizes"), the guidance maintains that it is not possible to recommend a single set of sample size requirements for all fish contaminant monitoring studies (USEPA 2000a). Rather, EPA presents a more general approach to sample size determination that is both scientifically defensible and cost-effective. EPA provides a table in this section of the guidance that 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 samples than by increasing the number of fish per composite.

At each site, states and authorized tribes should determine the appropriate number of individuals per composite sample and number of replicate composite samples. This should be based on site-specific estimations of the population variance of the target analyte concentration, fisheries management considerations, and statistical power consideration. For example, fewer replicate composite samples and/or fewer individuals per composite sample may be required if the population variance of the selenium concentration at a site is small. In this case, it would not be cost-effective to use sample sizes that are larger than required to achieve the desired statistical power. Additionally, fish tissue monitoring for criteria implementation may be conducted on much smaller streams than those sampled for fish

consumption purposes, and there may be limited numbers of fish available in these smaller tributaries.

In EPA's National Lake Fish Tissue Study, composites were generally required to include five fish (USEPA 2002a). This composite size represented a reasonable number of fish that also satisfied statistical requirements. Based on this precedent and EPA's Fish Advisory Guidance (USEPA 2000a), EPA recommends that in most waters composites of five fish be used for fish tissue monitoring for selenium criteria implementation. However, EPA recognizes that sometimes it might not be possible to collect a five-fish composite (or, as described above, five fish might not be needed to have statistical power). In these limited cases, EPA encourages the state or tribe to use as many fish as possible in the composite. Organisms used in a composite sample should meet the following recommendations (USEPA 2000a):

- All the same species.[2]
- Of similar size so that the smallest individual in a composite is no less than 75% of the total length (size) of the largest individual (the "75% rule"; does not apply to egg-ovary samples).
- Collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart).
- Collected in sufficient numbers to provide at least 20 grams composite homogenate sample of tissue for analysis of selenium.

EPA's 2000 Fish Advisory Guidance (USEPA 2000a) provides recommendations on the number of composite samples to collect. It recommends collecting at least two composite samples at each site, and encourages a third, in order to properly estimate the site variance. For the purposes of sampling fish in potential selenium impacted waters, the number of composite replicates may be determined on a case-by-case basis. This decision would primarily be based on the presence of target species and the numbers of individuals present at the site in question.

Individual organisms used in composite samples must be of the same species, in part because of the differences in selenium bioaccumulation potential between species (USEPA 2016a). Accurate taxonomic identification is essential to prevent the mixing of species in a sample. EPA recognizes that, in contrast to other bioaccumulative contaminants in fish, selenium concentrations are generally conserved or increase incrementally at each trophic level in a food web. This is because there is relatively little variation across all trophic levels of fish since the trophic transfer factors from prey to fish are small, with some exceptions (e.g., molluscivorous fish) (USEPA 2016a). However, EPA still recommends following the "75% rule" for whole body or muscle tissue (does not apply to egg-ovary samples) for the sizes of individual specimens within a composite.

The tissue mass recommendation is based on EPA Method 200.8 for solid samples, which states that a 20 gram sample is sufficient if the sample is <35% moisture; a 50-100 gram sample is recommended if the moisture content is >35% (USEPA 1994a). Since many fish tissue samples are 70-80% moisture, monitoring agencies should consider the tissue mass as they develop their sampling and analysis plans. Monitoring agencies typically collect composite samples for other analytes in addition to selenium; additional biomass should be collected to accommodate selenium as well as standard contaminant analyses, if necessary. If agencies currently discard or archive the composite homogenates in excess of their current analytical needs, it may be easy to save the excess tissue to use an additional 20 grams (or

[2] Ensuring that a composite sample consists of the same species is particularly important for selenium as different species can have different sensitivity to selenium and have different bioaccumulation potential (see "Target Species" discussion below).

more if needed) for selenium analysis. Agencies that submit composite tissue samples for their advisory analyses could take advantage of the opportunity to add selenium as an analyte to their sampling protocol.

Individual Sample

An individual sample is a discrete sample from a single fish, and can be an egg-ovary sample, a whole body, or a muscle (fillet) sample. Although EPA recommends states or authorized tribes use composite samples for selenium fish tissue monitoring, there are some instances where collecting individual fish may be desirable.

Analysis of individual fish samples may be of interest to evaluate spatial and temporal differences among individuals of a species of similar size or across the population of a species residing in a specific water body. For water bodies or segments that are known to be impacted by selenium, individual samples may better estimate the magnitude (i.e., extreme values) of the impact and may provide information about selenium source-exposure relationships in large water bodies. Individual samples may also allow for the identification of fish that are migrant or transient in a population, since that fish may have a higher or lower concentration of selenium than other fish in the area. EPA recommends 20 grams as a minimum tissue mass required per individual fish for analysis and QA/QC (USEPA 1994a).

If using individual samples for the purposes of selenium criteria implementation, all fish should be the same species and from the same waterbody (or site for large waterbodies) within the same sampling period. Where the monitoring agency plans to arithmetically composite such individual samples or calculate an average concentration, the fish should be of similar size (within the 75% rule) and the samples should be of the same tissue type. When using individual fish tissue samples for selenium monitoring, EPA recommends targeting at least 5 individuals for analysis to achieve measurements of a reasonable statistical power (see discussion of statistical power in the "Composite Sample" discussion above). In the event that collecting at least 5 individuals of one species is not possible, fewer specimens may be sufficient to provide adequate biomass for both selenium analysis and quality assurance/quality control (QA/QC), but the statistical power of the analysis may be affected. EPA recommends 20 grams as a minimum tissue mass required per individual fish for analysis and QA/QC.

Target Species

Different species have varying sensitivity to selenium and as such, states or authorized tribes should consider selenium sensitivity, along with bioaccumulation potential, when designing fish tissue monitoring plans. EPA recommends that states or authorized tribes target species that have higher selenium sensitivity, but if this is not possible, the selenium criterion is designed to be used for any fish species (with the exception of anadromous fish species). Migratory and highly mobile fish species should be avoided for selenium sampling, if possible. 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. Since the selenium criterion applies to ecological risk and not human health, monitoring agencies could evaluate their target species list and decide if they are including appropriate species for assessing selenium risk in their regions (see Table 3). When selecting target fish species for selenium criterion monitoring, monitoring agencies should focus on species that are sensitive to selenium, that may potentially accumulate high concentrations of selenium, and that are easy to identify (USEPA 2000a).

Table 3: Target Species for Implementation of Selenium Criterion

Family	Scientific Name	Common Name
Acipenseridae	*Scaphirhynchus platorynchus*	Shovelnose Sturgeon
Acipenseridae	***Acipenser fulvescens***	**Lake Sturgeon**
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo
Catostomidae	*Ictiobus cyprinellus*	Bigmouth Buffalo
Catostomidae	***Catostomus commersonii***	**White Sucker**
Catostomidae	*Catostomus catostomus*	Longnose Sucker
Catostomidae	*Catostomus macrocheilus*	Largescale Sucker
Catostomidae	*Minytrema melanops*	Spotted Sucker
Catostomidae	*Moxostoma anisurum*	Silver Redhorse
Catostomidae	*Moxostoma congestum*	Grey Redhorse
Catostomidae	*Moxostoma duquesnei*	Black Redhorse
Catostomidae	*Moxostoma erythrurum*	Golden Redhorse
Catostomidae	*Moxostoma macrolepidotum*	Shorthead Redhorse
Catostomidae	*Moxostoma poecilurum*	Blacktail Redhorse
Catostomidae	*Carpiodes cyprinus*	Quillback
Centrarchidae	*Micropterus salmoides*	Largemouth Bass
Centrarchidae	*Micropterus dolomieu*	Smallmouth Bass
Centrarchidae	*Pomoxis annularis*	White Crappie
Centrarchidae	*Pomoxis nigromaculatus*	Black Crappie
Centrarchidae	*Lepomis macrochirus*	Bluegill
Centrarchidae	*Lepomis cyanellus*	Green sunfish
Centrarchidae	*Ambloplites rupestris*	Rock Bass
Cyprinidae	***Cyprinus carpio***	**Common Carp**
Cyprinidae	*Campostoma anomalum*	Central Stoneroller
Cyprinidae	*Rhinichthys cataractae*	Longnose Dace
Cyprinidae	*Rhinichthys atratulus*	Blacknose Dace
Cyprinidae	*Semotilus atromaculatus*	Creek Chub
Cyprinidae	*Semotilus corporalis*	Fallfish
Cyprinidae	*Pimephales promelas*	Fathead Minnow
Cyprinidae	*Pimephales notatus*	Bluntnose Minnow
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner
Cyprinidae	*Notropis atherinoides*	Emerald Shiner
Cyprinidae	*Notropis hudsonius*	Spottail Shiner
Cyprinidae	*Nocomis micropogon*	River Chub
Esocidae	*Esox lucius*	Northern Pike
Esocidae	*Esox masquinongy*	Muskellunge
Ictaluridae	*Ictalurus catus*	White Catfish
Ictaluridae	*Ictalurus punctatus*	Channel Catfish
Ictaluridae	*Ictalurus melas*	Black Bullhead
Ictaluridae	***Ictalurus nebulosus***	**Brown Bullhead**
Ictaluridae	*Ictalurus natalis*	Yellow Bullhead
Ictaluridae	*Pylodictis olivaris*	Flathead Catfish
Moronidae	***Morone chrysops***	**White Bass**
Moronidae	*Morone saxatilis*[1]	Striped Bass[1]
Moronidae	***Morone americana***	**White perch**
Percidae	*Sander vitreus*	Walleye

Family	Scientific Name	Common Name
Percidae	*Sander canadensis*	Sauger
Percidae	***Perca flavescens***	**Yellow Perch**
Salmonidae	***Coregonus clupeaformis***	**Lake Whitefish**
Salmonidae	*Oncorhynchus kisutch*[2,3]	Coho Salmon[2,3]
Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout
Salmonidae	*Oncorhynchus tschawytscha*[2,4]	Chinook Salmon[2,4]
Salmonidae	*Salvelinus namaycush*	Lake Trout
Salmonidae	*Salmo trutta*	Brown Trout
Salmonidae	*Salvelinus fontinalis*	Brook Trout
Sciaenidae	***Aplodinotus grunniens***	**Freshwater Drum**

Common molluscivorous fish species are indicated in bold. Molluscivorous fish species have a higher potential to bioaccumulate selenium, since the available data indicate that mollusks generally have a higher trophic transfer factor than other invertebrate taxa (USEPA 2016a).

[1] Adult specimens are acceptable if the population is landlocked

[2] Where Pacific anadromous fish are listed, the target species only includes juveniles (smolt stage)

[3] Endangered in Central California Coast; Threatened in Lower Columbia River, Oregon Coast, and Southern Oregon - Northern California Coast (USFWS 2016)

[4] Endangered in Sacramento River and Upper Columbia River; Threatened in California Coastal, Central Valley, Lower Columbia River, Puget Sound, Snake River, and Upper Willamette River (USFWS 2016)

Bioaccumulation of selenium by higher trophic level fish is highly influenced by diet. For example, fish that primarily consume freshwater mollusks (e.g., *Lepomis microlophus*, or redear sunfish) will exhibit greater selenium bioaccumulation than fish that consume primarily insects or crustaceans from waters with the same concentration of dissolved selenium because mollusks tend to accumulate selenium at higher concentrations than other trophic level 2 organisms (Luoma and Presser 2009; Stewart et al. 2004). Because of this, diet is an important factor to consider when selecting species to monitor. For example, in the San Francisco estuary, sturgeon are monitored not only because they are sensitive to the toxic effects of selenium, but also because their primary prey accumulates selenium very efficiently. As a result, the sturgeon receive large doses of selenium.

Based on the best available and acceptable reproductive-effect studies as well as extensive analyses, EPA developed a species sensitivity distribution (SSD) to support the derivation of the national selenium criterion (USEPA 2016a). This SSD presents the four most sensitive genera for fish reproductive effects (in decreasing order) to be *Acipenser*, *Lepomis, Salmo,* and *Oncorhynchus.* These genera have known sensitivity to selenium and should be targeted for selenium monitoring, but care should be taken to avoid sampling threatened or endangered species and anadromous species. For example, *Acipenser,* although the most sensitive, is a genus of sturgeon; many species are threatened or endangered and thus are not suitable for sampling. When selecting species from these genera, it is important to consider the diet of certain species compared to others, and select the species that best represent the potential accumulation in the waterbody. As mentioned, fish that primarily consume freshwater mollusks will exhibit greater selenium bioaccumulation than fish that consume primarily insects or crustaceans form the same waters (Luoma and Presser 2009; Stewart et al. 2004). Fish that consume primarily benthic insects will tend to exhibit greater selenium bioaccumulation than fish that feed higher in the water column (Schneider et al., 2015; Simmons and Wallschläger, 2005).

Species that are sensitive to selenium are commonly present but if they are not available in sufficient numbers, then other species that are available in sufficient numbers can be used for fish tissue monitoring. In smaller streams, cyprinids may be the only species available. Species known to be tolerant to selenium may also be appropriate to use, since their selenium tissue concentration will be compared to the tissue

element threshold (see Table 1) which is designed to be protective of the entire aquatic community. A waterbody with selenium impacts and only tolerant species should still show selenium impacts, since even tolerant fish bioaccumulate selenium. For example, there are data from West Virginia and Colorado that show some native cyprinids including blacknose dace and central stoneroller with tissue concentrations over 40 mg/kg dry weight. (USEPA, 2016a). (*Note: Research is needed to determine whether certain species are resistant to bioaccumulation of selenium versus other species.*)

When selecting target species, it is important to consider all of the organisms and trophic levels that are potentially at risk in the study area. For example, certain species will have habitat preferences that expose them to higher levels of accumulated selenium. If possible, migratory species and highly mobile species should be avoided. Highly mobile fish species such as potamodromous and anadromous species could travel back and forth between areas with low and elevated selenium concentrations, resulting in variable tissue selenium concentration data (Beatty and Russo 2014). It is possible that typical adult selenium exposure concentrations would be lower than concentrations at rearing grounds, and for these reasons resident species should be the first choice for selecting target species.

If migratory or highly mobile species must be sampled, then sampling plans should account for the life history of these species so that the correct locations for sampling within a watershed are selected. Potamodromous species vary in the extent to which they migrate for spawning. Most simply migrate from a lake or reservoir to a nearby river or stream, or from a larger downstream section of the river to a smaller upstream tributary. For example, some Walleye (*Sander vitreus*) spawn in lakes with suitable habitat, and some return to river systems or streams that connect with the lake. However, some Pikeminnows (genus *Ptychocheilus*) migrate over 100 miles to spawn. In riverine systems, some individuals migrate short distances to suitable habitat, while others migrate longer distances. The proximity of the selenium source sampling locations should also be considered; the nearest source of selenium may be located some distance upstream, or it may be located at or near a sampling site. If Pacific anadromous species are selected as target species to be used for sampling, EPA recommends that states and authorized tribes use the whole-body criterion element for juvenile (smolt) as the primary criterion element over the other elements. This recommendation is due to the unique life history of these species, specifically, the lack of exposure to adult salmonids from selenium in freshwater prior to reproduction (see Section 6.4.1.1 in USEPA 2016).

The use of a limited number of target species allows comparison of fish contaminant data among sites over a broad geographic area. It is difficult to compare contaminant monitoring results within a state or among states unless the data are from the same species because of differences in habitat, food preferences, and rate of contaminant uptake among various fish species. However, it is impracticable to sample the same species at every site. Limiting the number of species allows for collection and comparison of contaminant data from across a state, region, or nationally. Table 3 lists EPA's recommended target species for implementation of the selenium criteria (adapted from existing EPA guidance on fish tissue monitoring (USEPA 2000a). Common molluscivorous fish species are indicated in bold. Molluscivorous fish species have a higher potential to bioaccumulate selenium, since the available data indicate that mollusks generally have a higher trophic transfer factor than other invertebrate taxa (USEPA 2016a).

Leveraging Existing Fish Tissue Monitoring Programs and Sample Designs

Considerations for Augmenting Existing Fish Tissue Monitoring Programs

In 2010, forty-five states monitored chemical contaminants in fish tissue for assessing human health risks. The design of an agency's existing fish tissue monitoring program will likely drive its approach to

selenium monitoring. Twenty-eight states identify selenium as a contaminant in their monitoring program (USEPA 2010a). Many states already have monitoring programs and sample designs that can be leveraged for the new selenium criterion. Several case studies are provided in the following sections as examples of programs that might have the capacity and framework to augment their existing monitoring strategies to include fish tissue monitoring for the selenium criteria.

Consistency with Existing Programs

To the extent possible within a state or tribal program, EPA recommends that fish tissue monitoring for the assessment of the selenium aquatic life criterion should be consistent with state's current practices regarding spatial and temporal considerations of the program, species collected, and sample type collected. In this way, logistical modifications to a state's fish tissue monitoring program can be minimized. However, care should be taken when utilizing existing sampling programs that are designed for human health protection, as existing sampling designs and methods for human health may need to be amended before being used for selenium sampling. States should take into consideration the information presented in this document when amending their programs. Where deviation from existing state or tribal programs is necessary because of spatial or temporal considerations, or species/sample type due to concerns regarding specific waterbodies with selenium inputs, these can potentially be accommodated by leveraging expertise and logistical assistance from other agencies. Various state (e.g., Department of Natural Resources) or federal (i.e., National Oceanic and Atmospheric Administration - National Marine Fisheries Service, United States Fish and Wildlife Service [USFWS], United States Geological Survey [USGS]) agencies have the expertise to provide such assistance. Alternatively, in the absence of an existing program, additional monitoring may need to be planned for criteria implementation.

Temporal Considerations

Various temporal factors will influence fish tissue monitoring strategies for selenium. For example, as described earlier in this document, most fish species that are synchronous spawners do so in the spring, whereas fish tissue collection for advisories typically occurs in the late summer or early fall, when contaminant loads in the edible portion of the fish are highest. If an agency is limited to sampling outside of the pre-spawning period due to resource constraints, that will need to be considered when incorporating selenium fish tissue monitoring into the existing programs, or when developing a new program (e.g., sampling whole body or muscle tissue instead of egg-ovary tissue).

The only appropriate time to collect egg-ovary tissue from suitable species is when the female is gravid in the pre-spawn stage, just prior to mating and spawning. This is typically a very small window (see Appendix B) of time for most synchronous species, and may occur in the spring or early summer, or in the fall to early winter. In northern latitudes, spawning may occur slightly later than in southern latitudes. It is the selenium concentration in eggs that drives early life stage toxicity, so adult female fish must be collected during the late vitellogenic or pre-ovulatory periods of oogenesis for this criterion to be scientifically and toxicologically meaningful. Measuring selenium concentration in ovarian tissue during other periods of oogenesis will be much less informative. Summer and fall may be prime periods for whole-body and muscle tissue collection due to the engorgement of populations to replenish fat and energy reserves post-spawn.

For egg-ovary tissue sampling, agencies with fish tissue monitoring responsibilities should consult with a state fisheries biologist to determine the appropriate time for sampling specific species in their region in order to capture the specimens in their pre-spawning phase. These regional experts will be familiar with

the local species, and able to use their best professional judgment to determine which are appropriate for selenium sampling, and the appropriate sampling time frame based on spawning season. If agency resources limit fish tissue collection to times outside of these species-specific windows, then the only appropriate samples to collect are whole-body and muscle tissue. Target fish species collected in the fall may be common to selenium monitoring and human health risk assessment. In this case, muscle tissue can be composited and evaluated for selenium in addition to contaminants of interest for fish consumption advisories. Seasonal restrictions (e.g., due to spawning seasons, high flows) on fish sampling may also prevent sampling for egg-ovary tissue in specific areas.

Spatial Considerations

Spatial factors will need to be considered when augmenting existing programs, or when developing a new program. For example, as described earlier in this document, some fish species migrate to upstream areas to spawn; these areas may be harder to access than larger order downstream segments that are inhabited during non-spawning seasons. However it may still be possible to sample such species on their way up stream. It may be necessary to monitor smaller order stream segments of a larger stream network than is traditionally monitored (e.g., downstream river segment) to get closer to the selenium input. This may require some adjustment to monitoring plans that would consider the species of fish available in the small stream segment, temporal issues (e.g., spring flood/safety, low flow availability of fish), and the types of appropriate sampling gear. Agencies should 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.

States currently use a number of different methods for selecting sites for sampling fish tissue. Monitoring agencies generally will target high-use fishing areas, areas of special concern, and areas of suspected contamination, such as water bodies where fish advisories have been issued in the past (USEPA 2010a). States using this survey design should consider possible selenium prevalence and potential areas of contamination when targeting areas for sampling. If problem areas are identified through best professional judgment or through screening studies to determine the magnitude of chemical contamination in sensitive fish species, these areas can then continue to be targeted to monitor trends. Additional information regarding screening studies and intensive studies can be found in the "Existing Guidance" section of this document.

Geology may cause certain areas to be prone to selenium bioaccumulation, resulting in elevated concentrations. This should be kept in mind when selecting sites, and when analyzing data from these areas (Beatty and Russo 2014). In many areas, selenium sources have been well characterized; in these areas an intensive study designed to capture the magnitude and geographical extent of the selenium contamination in fish tissue (rather than following the results of a screening study) is recommended to ensure protection of aquatic life from reproductive impacts and aquatic community balance. Results of these intensive studies could be used to help identify the geographic extent of the selenium contamination, either downstream in a lotic environment, or by area in a lentic environment.

Forty agencies monitor fish sampling areas at regular intervals, and several conduct statewide, rotating basin sampling programs over a multi-year period (USEPA 2010a). Agencies can 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 the targeted basins as indicated (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 2000a). Probability sampling provides the basis for estimating 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). 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 tribe. Where selenium is already a primary parameter of interest, the state or tribe may have the data to support more intensive studies in certain water bodies.

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 50 or more fixed and rotating stations. The KDHE selects sites based on targeted, census, and probability based study designs. Specific sub-program objectives determine the numbers, species, and sizes of fish collected from a particular water body, and the tissues and parameters of interest.

Highlights (KDHE 2013):

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

The KDHE maintains a comprehensive fish tissue sampling program that routinely collects various tissue types.

http://www.kdheks.gov/environment/qmp/download/Fish_Tissue_Part_III.pdf

Selenium Differences in Lentic and Lotic Environments

Selenium concentrations and bioaccumulation patterns are different in lotic (flowing water) versus lentic (very slow moving or still water) environments. It is of greatest concern in lentic water bodies, where reducing conditions create an environment where selenium accumulates in sediment more readily. Benthic organisms are therefore 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). Several studies have concluded that fish feeding on benthic organisms are expected to have higher selenium concentrations than fish feeding from the water column (Schneider et al., 2015; Simmons and Wallschläger, 2005). This suggests that bottom feeding fish may have higher selenium levels, at least for the lifecycle that ties their energy needs to food webs with benthic insects. Other studies (Saiki et al. 1993; Saiki and Lowe 1987) have shown that detritivores may

be exposed to high levels of dietary selenium, as high concentrations of selenium were measured in detritus. Reducing conditions may also lead to higher bioavailability in the water column (Luoma and Rainbow 2008).

Hillwalker et al. (2006) found that the body burden concentrations of selenium in insects within similar taxa were up to 7 times greater in lentic systems than lotic systems within the same watershed. Additionally, they concluded that selenium bioaccumulation in insects gave a more accurate measurement of accumulation risk throughout the food chain than surface water selenium concentrations (Beatty and Russo 2014).

Mollusks such as mussels and clams accumulate selenium to a much greater extent than planktonic crustaceans and insects due to higher ingestion rates of both particulate-bound (algae) and dissolved selenium from the water column through filter feeding. These organisms also have a lower selenium elimination rate (Johns et al. 2008; Reinfelder et al. 1997). Certain ecosystems with mollusk-based food-webs may create a pathway for more selenium to bioaccumulate, particularly in molluscivorous fish, since the available data indicate that mollusks generally have a higher trophic transfer factor than other invertebrate taxa (USEPA 2016a). Common molluscivorous fish species are indicated in Table 3.

Existing Resources and Information

Available Expertise

The fish tissue sampling infrastructure (experience, equipment, etc.) for the purposes of implementing the selenium fish tissue criterion typically resides in the agency charged with protection of natural resources (e.g., a natural resources department or a fish and game department). EPA recommends that states or authorized tribes leverage the appropriate expertise and logistical knowledge for compiling the necessary information and data to implement sampling.

All states, in addition to most authorized tribes and interstate commissions, have established biological assessment programs. This means that there should be capacity to establish or modify existing fish tissue monitoring programs to facilitate implementation of the new fish tissue-based criteria elements in the new selenium water quality criterion. In addition to individual state and tribal agencies and local expertise, federal (e.g., USFWS) and state resource agency collaborations could be used as necessary to 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, water bodies, and watersheds for selenium sampling beyond the coverage of current monitoring program
- Design an appropriate monitoring strategy
- Select target species
- Identify pre-spawning periods
- 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 monitoring strategy. They could also use their extensive database to determine where to conduct more intensive studies in certain water bodies.

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 (DNR), Health (MDH), and Agriculture (MDA) 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):

- Approximately 130 lakes and river sites are sampled annually.
- The Fish Contaminant Monitoring Program database contains over 31,000 data records.
- As of 2008, the program has sampled 22% of the estimated 5,500 fishing lakes in the state (15% of the lakes <2,000 acres and 80% of the lakes >2000 acres).

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

https://www.pca.state.mn.us/sites/default/files/p-p2s4-05.pdf

Existing Guidance

Existing EPA guidance related to monitoring of contaminants in fish was published in *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis* (USEPA 2000a). This Guidance was developed specifically for assessing human health risks associated with consumption of fish and shellfish, so there are aspects of the aquatic life selenium fish tissue-based criterion that are not covered by the 2000 Guidance (e.g., fish egg-ovary sampling). The 2000 Guidance recommends selenium as a target analyte based on its relevance to human health and focuses on fish consumption advisories.

The monitoring strategy in the 2000 Guidance document discusses two tiers of studies with the goal of identifying locations where fish consumption advisories may be needed. Tier 1 studies are screening studies that cover a large number of sites for chemical contamination with few samples per site. These are most useful in water bodies, regions, or states where there are no known or expected selenium problems. Screening studies help states identify those sites where selenium concentrations are elevated relative to other water bodies in the state and prioritize water bodies for future monitoring, thus enabling resources to be used more efficiently. For example, water bodies with fish having low selenium may be monitored less frequently in the future, while water bodies with fish having elevated selenium at or near the tissue elements may be prioritized for more frequent or more intensive monitoring. Other information (e.g., location of sources), can also be used to prioritize sites for screening and prioritization.

Tier 2 studies are intensive studies of problem areas identified in screening studies to determine the magnitude of chemical contamination in sensitive fish species, and to assess the geographic extent of the

contamination. 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.

For the purposes of implementing the aquatic life selenium criterion recommendations, the process is different. In the waterbody assessment context, once a criterion element threshold is exceeded, the waterbody is considered impaired (and placed on the state's or tribe's CWA section 303(d) list), and the next step would be additional monitoring for a TMDL or site specific criterion. Data from intensive studies might help to support TMDL development for those waters where fish tissue criteria elements are exceeded by identifying the magnitude of selenium in fish tissue ("worst case scenario"). Monitoring at points downstream in a lotic water body may define the area of impact for an impairment based on selenium in tissues of sensitive resident fish species. In lentic systems, intensive monitoring in a large lake or reservoir, for example, might demonstrate that selenium contamination in fish is limited to a certain area such as an embayment or a tributary arm of a reservoir.

Although the focus of the 2000 Guidance document is different, it still provides information that is useful to state and tribal programs monitoring for implementation of the fish tissue components of EPA's aquatic life selenium criterion recommendations. In particular, the 2000 Guidance document discusses the importance of selecting target species for tissue samples, and provides lists of species for various feeding habits and habitats (bottom feeder, predators) that are recommended by EPA, USFWS, and USGS as targets for monitoring. The 2000 Guidance also discusses study design considerations and the major parameters that must be specified for field collection activities, such as site selection, analyte screening values, sampling times, sampling type, and quality assurance/quality control (QA/QC) samples such as replicate samples.

Additionally, numerous documents on bioassessment techniques have been produced by EPA and other stakeholders. Specific sections of these documents contain information that may be helpful for developing guidelines for sampling fish (particularly for species like cyprinids not typically targeted by state monitoring programs) for the purposes of selenium fish tissue analysis. 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 for fish collections and fish collection methodologies. 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	USEPA 2000a	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://nepis.epa.gov/Exe/ZyPDF.cgi/20004OQK.PDF?Dockey=20004OQK.PDF
Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue	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)	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-long-term-ecological-monitoring-studies/508A10FEE39E7E93EF07B005D06952F5
Spatially Balanced Sampling of Natural Resources	Stevens and Olsen, 2004	https://archive.epa.gov/nheerl/arm/web/pdf/grts_asa.pdf
Application of Global Grids in Environmental Sampling	Olsen et al. 1998	https://archive.epa.gov/nheerl/arm/web/html/abolsen98.html

Using Existing Data to Enhance Selenium Monitoring

All available existing data should be considered and utilized as necessary 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 monitoring program (USEPA 2010a). Several states have conducted extensive statewide assessments, and could have existing state selenium data. The Ohio River Valley Water Sanitation Commission (ORSANCO) collects 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 EPA's 2008-2009 National Rivers and Streams Assessment; the data are publicly available at http://www.epa.gov/fish-tech/fish-tissue-data-collected-epa. One hundred paired mercury and selenium fish fillet concentration data from samples collected in 2007 are available at http://www.epa.gov/sites/production/files/2015-07/mercury-finaldata2012.xlsx. Sample sites are randomly selected U.S. locations where existing mercury advisories were in place at the time of sampling. The USGS has also conducted numerous state surveys of selenium in fish tissue. The USGS National Water Quality Assessment (NAWQA) database (http://cida.usgs.gov/nawqa_www/nawqa_data_redirect.html) contains analytical results for fillet and whole-body fish tissue samples from across the country.

Sample Assessment: Analytical Chemistry

Fish tissue sampling for the selenium criterion will involve many of the same types of analytical concerns as with any tissue monitoring and assessment program. Various researchers have shown that analytical results on the same population of fish can differ between studies and even within studies. These inherent uncertainties are minimized through a rigorous study design, clear data quality objectives, meticulous QA/QC protocols, and careful execution of the monitoring and assessment program in the field. Standardized 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 procedures should be used across implementation programs, (e.g., ambient monitoring, NPDES compliance monitoring).

Quality assurance in the laboratory should be closely monitored, and laboratories should be selected carefully based on lab 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 EPA's 2000 Fish Advisory Guidance (USEPA 2000a) includes detailed direction for preparing muscle and whole body samples. Please refer to Appendix A of this document for egg and ovary sample preparation.) EPA does not have approved methods under 40 CFR Section 136 for measuring selenium in fish tissue. However, states and authorized tribes are not required to use EPA-approved methods for monitoring and assessment of criteria attainment or other activities not related to permit applications or permit compliance reports (USEPA 2016a). Several methods for selenium analysis in animal tissue are presented in Table 5. Four methods have a method detection limit (MDL) that is ten times lower than the range expected given the criteria limits for tissue (the exception is EPA Method 6010C).

Table 5: List of Test Procedures for Total Selenium in Tissue

Method	Digestion / Preparation in reference method?	Example MDL[1]	Links to Methods
EPA Method 6010C – Inductively Coupled Plasma - Atomic Emission Spectroscopy	No – Recommended: 3052 (total), or 3050B (total recoverable)	5 mg/kg	http://www.epa.gov/sites/production/files/2015-07/documents/epa-6010c.pdf 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 6020A – Inductively Coupled Plasma - Mass Spectrometry (ICP - MS)	No – Recommended: 3052 (total), or 3050B (total recoverable)	0.2 mg/kg	https://www.epa.gov/sites/production/files/2015-07/documents/epa-6020a.pdf 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 – Atomic Absorption, Borohydride Reduction	No – References 3010A for water (total) Recommended: 3052 (total), or 3050B (total recoverable)	0.05 mg/kg	https://www.epa.gov/sites/production/files/2015-12/documents/7742.pdf 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
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)	0.008 µg/g	https://pubs.usgs.gov/tm/2006/tm5b1/PDF/TM5-B1.pdf 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
NOAA 140.1 – Graphite Furnace-Atomic Absorption for the Analysis of Trace Metals in Marine Animal Tissues	Yes – Teflon Bomb	0.1 µg/g	https://www.nemi.gov/methods/method_summary/7185/

[1] **MDL -** Establish empirically; MDLs will be laboratory, and potentially instrument, or analyst-specific. To determine MDLs, commercial laboratories generally follow the procedures described in 40 CFR Part 136 Appendix B using analyte free reference material for spiking.

States can also use methods for analyzing selenium in water to measure selenium in fish tissue, as long as the samples are made soluble. Tissue samples are homogenized and digested prior to analysis using strong acid or dry-ashing digestion. The suitability for a given technique should be determined by the individual lab and its capabilities and preference. Care should be taken to use a process that will minimize the loss of volatile selenium. For example, fluorometric techniques require sample digestion and sample reduction; loss of volatile selenium compounds is possible because several steps are required (ATSDR 2003). Standard reference materials, analytical duplicates, and matrix spike samples are recommended to determine the applicability of a selected digestion procedure. EPA recommends three specific EPA-approved analytical methods for aqueous selenium; these methods are presented in Table 6 (USEPA

2016a). All three methods have an MDL that is ten times lower than the range expected given the criteria limits for tissue.

Table 6. List of Test Procedures for Total Selenium in Water

Method	Digestion / Preparation in reference method?	Example MDL[1]	Links to Methods
American Public Health Standard Method 3114 B – Arsenic and Selenium by Manual Hydride Generation/Atomic Absorption Spectrometry (2009) or 3114 C – Arsenic and Selenium by Continuous Hydride Generation/Atomic Absorption Spectrometry (2009)	Yes – 3114 B includes digestions (Section 4), but references SM 3030F for sample preparation	2 µg/L	https://www.nemi.gov/methods/method_summary/9703/ https://www.scribd.com/doc/17718890/Standard-Methods-21st-ed-Part-3000-Metals
EPA Method 200.8, Rev 5.4 – Determinations of Trace Elements in Waters by ICP- MS (1994a)	Yes – Section 11.2 (total recoverable) Alternative digestion 3010A (total)	7.9 µg/L	https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf https://www.epa.gov/sites/production/files/2015-12/documents/3010a.pdf
EPA Method 200.9, Rev.2.2– Determination of Trace Elements by Stabilized Temperature Graphite Furnace Atomic Absorption (1994b)	Yes – Section 11.2, (total recoverable) Alternative digestion 3010A (total)	0.6 µg/L	https://www.epa.gov/sites/production/files/2015-08/documents/method_200-9_rev_2-2_1994.pdf https://www.epa.gov/sites/production/files/2015-12/documents/3010a.pdf

[1] **MDL -** Establish empirically; laboratory- and potentially instrument- or analyst-specific. To determine MDLs, commercial laboratories generally follow the procedures described in 40 CFR Part 136 Appendix B using "analyte free" reference material for spiking.

The North American Metals Council (NAMC) has published a comprehensive discussion of analytical concerns relevant to selenium, contained in Ohlendorf et al. 2008 and 2011. An additional NAMC document (Ralston et al. 2008) presents guidance on analytical methods and considerations for selenium and its chemical species. 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. 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 water quality standard for selenium.

If a state or authorized tribe is using a data set that includes several values below the detection level, it must decide how it will evaluate these values. EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories (USEPA 2000a), recommends using one-half of the MDL for non-detects in calculating mean values (Section 9.1.2). Measurements between the MDL and the method quantitation limit are assigned a value of the detection limit plus one-half the difference between the detection limit

and the quantitation limit. Other statistical methods could also be used to calculate the average of data that includes values below the detection limit. States or authorized tribes could conduct a sensitivity analysis to determine how best to quantify samples below the detection limit (USEPA 2010b). For further discussion on handling non-detects, see USEPA 2000a and USEPA 2010b.

Additional information regarding analysis can be found in Appendix L of the Criteria Document (USEPA 2016a). 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.

Sample Assessment: Statistical Analysis

EPA guidance related to recommended statistical approaches for comparing contaminant measurements measured at different locations or over time is outlined in Appendix N of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Vol 1: Fish Sampling and Analysis* (USEPA 2000a). The guidance recommends using the t-test to statistically compare the mean of all fish tissue data for a single species to the criterion. States and authorized tribes can evaluate whether the t-test statistic of the mean exceeds the water quality standards. Intensive studies may include the collection of fish contaminant 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 (i.e., between stations) or temporal (i.e., over time) analyses. Spatial and temporal comparisons of contaminant data may yield important information about the variability of target analyte concentrations in specific populations of a particular target species. EPA recommends that states and authorized tribes consult a statistician to determine the specific statistical tests needed for a particular data set, and choose a method best suited to how they express their water quality standards.

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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 that includes gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish, and 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. 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. A composite homogenate sample of 20 grams of tissue should be collected for analysis of selenium (USEPA 1994a).

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 acetone-washed and baked aluminum foil. The left body wall should be removed by using fine dissecting instruments. 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 (FIN 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). A composite homogenate sample of 20 grams of tissue should be collected for analysis of selenium (USEPA 1994a).

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. It is recommended to transfer the samples collected from each individual female into sealed Ziploc® bags to prevent water (from ice melting) entering the sample. Storage time is 6 months to 2 years at –20°C for the majority of trace metals, including selenium (Janz and Muscatello, 2008).

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).

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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. Monitoring agencies should use all available locally relevant resources to determine the appropriate time to collect fish for the purpose of implementing the selenium criteria.

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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
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)

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
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)

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
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

Family	Scientific Name	Common Name	Spawning Season
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
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)

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, N.A. 1982, Page and Burr 1991)

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)

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
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

Family	Scientific Name	Common Name	Spawning Season
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 or wet weight concentrations. It is essential that exposure assessors be aware of this difference so that they may ensure consistency between units. If the contaminant concentration is measured in wet weight of fish, then the concentration must be converted to dry weight units to compare against the selenium criterion, which is expressed in dry weight (USEPA 2008). Wet weight may be converted to dry weight using the following equation:

$$WW = DW \times [1 - (\text{percent moisture}/100)]$$ (Lusk et al. 2005)

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 is unavailable for a fish species, percent moisture data for a similar species (i.e., same genus or, if unavailable, same family) should be used. Table C-1 lists percent moisture targeted species by tissue type (USEPA 2016). Percent moisture can vary within species; therefore, these data should generally be used when dealing with historical data. Field collected samples can be analyzed for % 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]		[a]USEPA 2014; [b]Chatakondi et al. 1995
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[a]			75.3[b]	USEPA 2014; [b]USEPA 2015
Pimephales notatus	Bluntnose Minnow	74.8				USEPA 2014
Nocomis micropogon	River Chub	75.2				USEPA 2014
Ictalurus punctatus	Channel Catfish			81.22[a] 78.43[b]		[a] Pinkney 2003; [b] May et al. 2009
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

Scientific Name	Common Name	Average % Moisture	% Moisture by Tissue			Reference
			Whole body	Muscle	Egg-ovary	
Oncorhynchus mykiss	Rainbow Trout			77.54	61.2	USEPA 2016
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]		[a] USEPA 2014; [b] Pinkney 2003, [c] May et al. 2009
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 2016
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

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