



## IDAHO DEPARTMENT OF FISH AND GAME

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Southeast Region  
1345 Barton Rd  
Pocatello, ID 83204

C.L. "Butch" Otter / Governor  
Virgil Moore / Director

September 27, 2017

Paula J. Wilson  
Hearing Coordinator  
Idaho Department of Environmental Quality  
1410 N. Hilton  
Boise, ID 83706

Dear Paula:

The Idaho Department of Fish and Game (IDFG) appreciates the opportunity to comment on the proposed rule for statewide and site specific selenium criteria. The proposed rule includes fish size and fish sample size criteria. Based on our analysis of whole body fish tissue concentrations collected in southeast Idaho, IDFG recommends increasing the proposed sample size from 5 to 10 fish and to modify the fish size criteria for trout and char to minimize biases associated with spawning migrations. A summary of each recommendation is described in more detail below.

### **Sample Size Analysis**

Idaho Department of Fish and Game used empirical data from previous sampling efforts to evaluate the effect of sample size (i.e. the number of individual fish from which tissue samples are taken) on estimates of mean selenium tissue concentrations in Yellowstone Cutthroat Trout and Brown Trout. To assess various sampling rates, we simulated a fish population with selenium levels representing the distribution (mean and standard deviation) observed in four streams for Yellowstone Cutthroat Trout and three streams for Brown Trout (Table 1).

Simulations were completed by sampling a hypothetical population with mean and standard deviations from Table 1. The number of fish sampled varied from 3 to 29 in increments of 2 fish. This process was repeated 10,000 times, which resulted in a distribution of 10,000 estimates of mean selenium level. Precision of mean selenium estimates was characterized by calculating the relative size (in percent) of the 95% confidence interval based on the distribution of the 10,000 estimates. Under this scenario, the standard deviation of the 10,000 estimates represents the empirical standard error. This number was multiplied by 1.96 to estimate the empirical 95%

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confidence interval and was divided by the mean selenium level and multiplied by 100 to equal the relative size of the confidence interval.

Table 1. Mean and standard deviation of selenium concentrations (whole body; mg/kg dw)) for Yellowstone Cutthroat Trout and Brown Trout. We pooled samples from all years (generally 2006 – 2016) and only fish under 200 mm in length were included.

Waterbody	Mean	Standard deviation
<b>Yellowstone Cutthroat Trout</b>		
Angus Creek	7.90	3.30
Blackfoot River	11.65	3.21
Crow Creek	7.53	3.48
Sage Creek	11.74	10.66
All	9.09	6.10
<b>Brown Trout</b>		
Crow Creek	10.84	6.18
Hoopes Spring	23.56	7.04
Sage Creek	20.76	6.63
All	14.98	8.32

The proposed fish tissue sample size of 5 fish generated 95% confidence intervals that average about 70% of the observed mean tissue concentration. For Sage Creek, a sample of 5 Yellowstone Cutthroat Trout resulted in simulated 95% confidence interval with a range of 2.3 to 21.1 mg/kg dw (Figure 1). The narrowest 95% confidence interval came from the Blackfoot River ( $\pm 24\%$  of the mean). If the proposed sample size was increased to 10 fish, the 95% confidence intervals would be reduced by about 50% for both Yellowstone Cutthroat Trout and Brown Trout (Table 2 and 3). Our simulations show that gains in confidence with increased sample size moderate after a 10 fish sample (Figure 2). Increasing the proposed sample size from a minimum of 5 to a minimum of 10 fish will significantly and reasonably increase the precision and reliability of fish tissue Se concentration estimates.

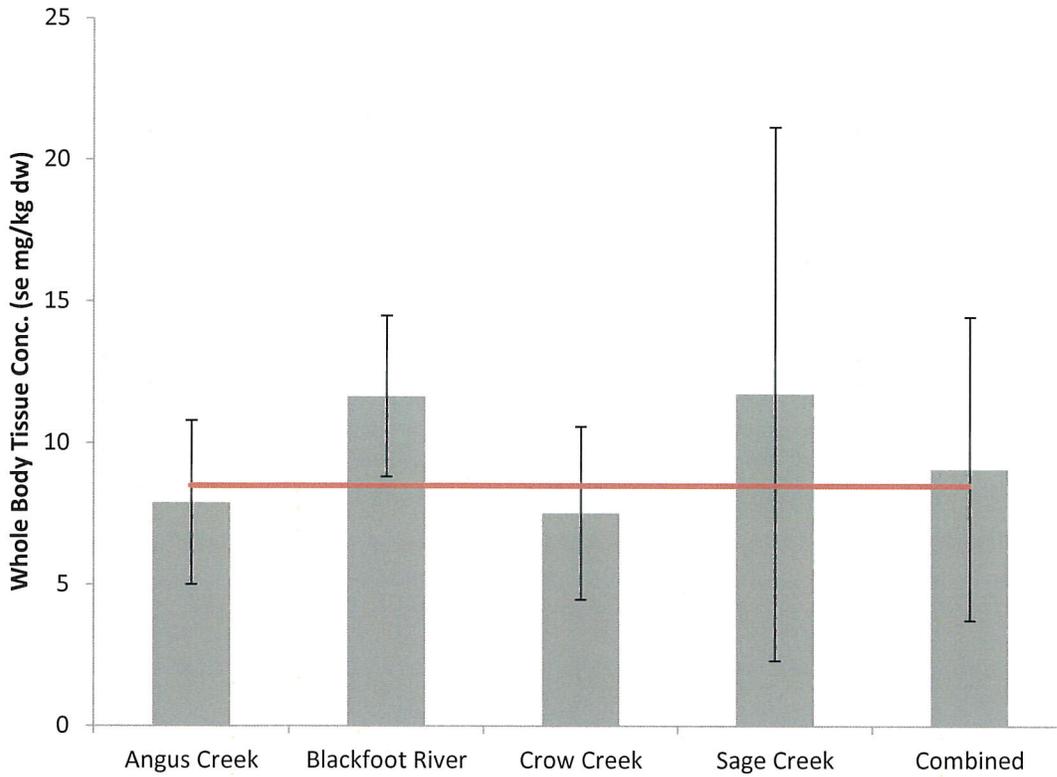


Figure 1. Mean fish tissue concentration plotted with the simulated 95% confidence intervals using the proposed sample size of 5 Yellowstone Cutthroat Trout. The mean values represent pooled data for all years and include only fish less than 200 mm in length. The horizontal line is the proposed whole body tissue criterion of 8.5 mg/kg dw.

Table 2. Simulated 95% confidence intervals for Yellowstone Cutthroat Trout. The values represent percentages of the mean selenium concentration. For example, a sample size of 3 fish generates a 95% confidence interval for Sage Creek that is  $\pm 101.6\%$  of the mean concentration for that stream. If 29 Yellowstone Cutthroat Trout were sampled from Sage Creek, the confidence interval drops to  $\pm 33.2\%$  of the mean.

Sample size	Stream				
	Angus Creek	Blackfoot River	Crow Creek	Sage Creek	Combined
3	47.2	31.6	52.3	101.6	76.3
Proposed 5	36.6	24.4	40.5	80.2	58.9
7	30.8	20.3	33.7	67.2	50.2
9	27.4	18.0	30.3	59.8	43.5
11	24.7	16.3	27.4	53.8	39.1
13	22.9	15.1	24.8	49.4	36.7
15	21.3	13.9	23.2	45.9	33.9
17	19.8	13.2	22.0	43.2	32.0
19	18.9	12.4	20.9	40.8	30.3
21	17.9	11.9	19.8	38.5	28.6
23	17.0	11.3	18.8	37.1	27.4
25	16.5	10.8	18.0	35.9	26.1
27	15.7	10.4	17.4	34.0	25.4
29	14.9	10.0	17.0	33.2	24.4

Table 3. Simulated 95% confidence intervals for Brown Trout. See description above for table interpretation.

Sample size	Stream			
	Crow Creek	Hoopes Spring	Sage Creek	Combined
3	65.4	33.7	36.2	63.8
Proposed 5	50.1	26.3	28.4	48.6
7	41.9	21.8	23.6	41.6
9	37.4	19.3	21.0	36.4
11	33.9	17.4	18.9	32.9
13	30.9	16.1	17.5	29.9
15	28.9	14.9	16.3	28.3
17	27.1	14.2	15.1	26.7
19	25.4	13.5	14.5	25.0
21	24.6	12.7	13.8	23.9
23	23.4	12.4	13.0	22.7
25	22.4	11.6	12.5	21.8
27	21.6	11.4	12.0	21.0
29	20.7	11.0	11.6	20.4

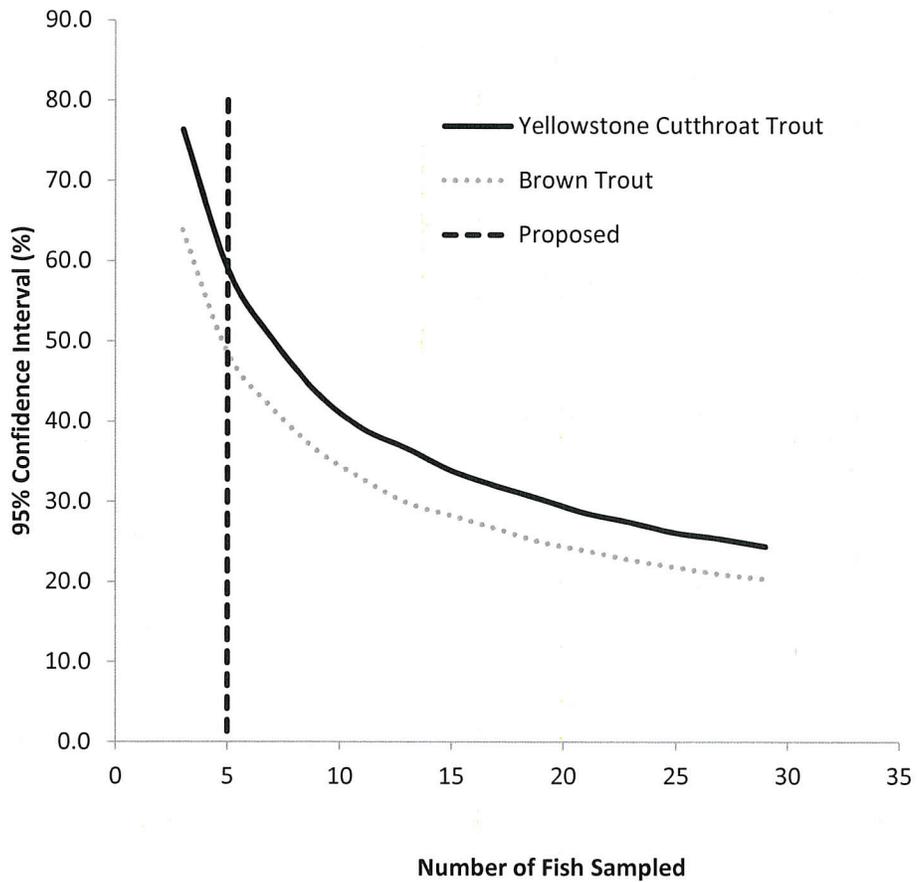


Figure 2. Simulated 95% confidence intervals plotted against the number of fish sampled. The vertical line represents the proposed rule for sample size of 5 fish. For Yellowstone Cutthroat Trout, a sample of 5 fish generates a confidence interval that is  $\pm 60\%$  of the mean selenium concentration. The benefits of increasing the number of fish sampled are marginal after 10 fish.

## **Fish Size Recommendation**

The proposed rule identifies that fish taken for whole body fish tissue analysis should be similar in size. Adult fish or juvenile fish can be used as long as the smallest individual is no less than seventy-five percent (75%) of the total length (size) of the largest individual. That size criteria allows for spawning adult trout and char to be used to describe a system's selenium condition. However, including adult trout/char in selenium tissue analysis does not account for significant migrations that can occur for spawning and foraging. Adult tissue concentrations may not represent the selenium levels present at the location where the fish were sampled. The following example from the Blackfoot Drainage illustrates the impact of fish migration on fish tissue results.

In 2009, fish tissue samples were collected from adult and juvenile Yellowstone Cutthroat Trout from the Blackfoot River. The adult trout were sampled during their spring spawning migration about 1 mile above the river's confluence with Blackfoot Reservoir. Prior to capture, those adults spent between 1 and 3 years maturing in the reservoir and had just begun their migration upriver to spawning tributaries. Once the adults reach the headwaters, they mix with juvenile fish produced in prior years. Sampling adult Yellowstone Cutthroat Trout in the headwater tributaries is consistent with the proposed methods. However, the associated selenium concentrations from adult fish reflect the reservoir not the headwater spawning tributaries where they were sampled.

Figure 3 shows whole body fish tissue concentrations from mature and juvenile Yellowstone Cutthroat Trout. The adult fish migrating out of the reservoir have a mean selenium concentration of 4.8 mg/kg dry weight. Conversely, fish tissue samples from juvenile fish, collected at the headwaters of the Blackfoot River, have a mean concentration of 10.5 mg/kg dry weight. The Blackfoot River example shows how samples from migratory adult trout may not accurately reflect conditions near where they were sampled.

A primary goal of collecting whole body fish tissue samples is to describe the stream or site from where the samples were collected. Juvenile fish are more likely to be living near a location where they have been foraging and should more accurately reflect local water quality conditions. For trout and char, we recommend setting a maximum fish size target for whole body tissue sampling of less than 200 mm total length. This will help minimize the potential impact of unknown past locations of adult trout and char.

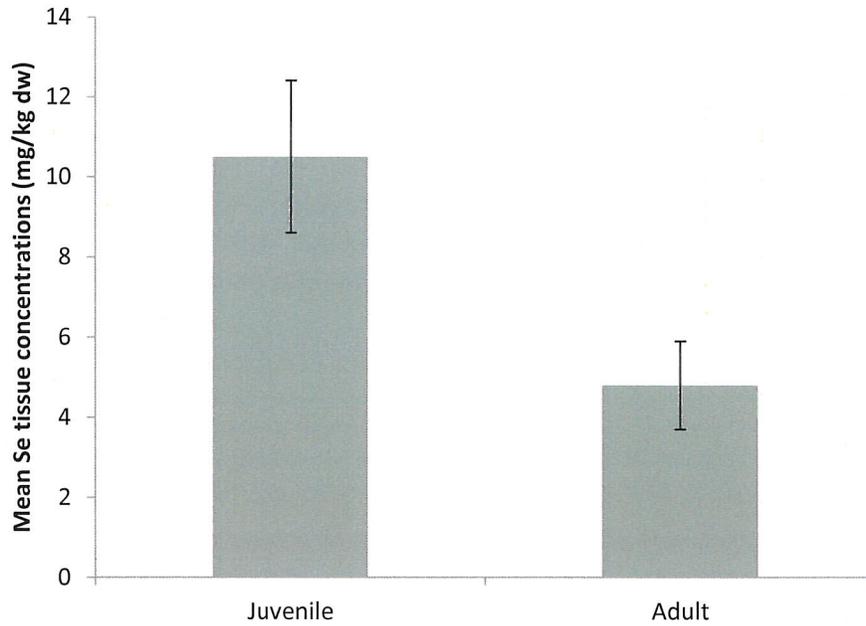


Figure 3. Comparison of mean whole body selenium tissue concentrations collected from the Blackfoot River in 2009 for juvenile (<200 mm TL) and adult (>350 mm TL) YCT. Error bars are standard deviations.

Sincerely,

Mark Gamblin

C: Sharon Kiefer, IDFG  
Bruce Olenick, DEQ