

A Preliminary Study of the Selenium Levels Found in Fishes Collected Downstream from Active Coal Mining & Valley Fill Operations

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INTRODUCTION

During the collection of water chemistry, benthic macroinvertebrate, and fisheries data for the Mountaintop Mining Environmental Impact Study, low levels ($< 25\mu\text{g/l}$) of selenium were found in some of the streams. The current water quality standard for selenium is $5\mu\text{g/l}$, which is lower than the levels measured in some of the streams studied during the Mountaintop Mining Study. Streams in the areas west of the Mississippi River have selenium levels several times higher compared to the central Appalachian streams.

The primary purpose of this paper was to study the levels of selenium found in various fishes collected in a stream located below coal mining operations. Secondary goals of the study were to: 1) examine the levels of selenium concentrations among different fish species, 2) to examine the levels of selenium concentrations found in various organs, and 3) to compare the variances associated with two different methodologies of determining dry weights of selenium in fish tissues.

The study site is located in Wayne county on Kiah Creek near Harts, West Virginia. Kiah Creek and the East Fork of Twelvepole Creek join at East Lynn Lake also located in Wayne County. The station is at an elevation of approximately 800 feet above sea level, and is located just upstream from the Parker Branch confluence, and below the Rollem Fork confluence (FIGURE 1). This site on Kiah Creek has been sampled for water chemistry, habitat, and benthic macroinvertebrates bi-yearly since the Spring of 1995. Previous fisheries evaluations have been conducted at this Kiah Creek site in the Spring and Fall of 2000 and in Spring 2001, but these studies only examined the fisheries communities for abundance and taxa diversity, not tissue analysis.

METHODS

Physical & Chemical Water Quality

Water temperature, Dissolved Oxygen (DO), pH, and conductivity were measured with a Hydrolab™ Minisonde multi-parameter probe. Flow was measured with a Marsh-McBirney™ Model 2000 portable flow meter. Stream width, depth, and velocity was measured, and the resulting average discharge was reported for the station. Water chemistry samples were collected on April 23, 2004 and were returned to R.E.I. Consultants, Incorporated for processing. Parameters analyzed included acidity, alkalinity, total hardness, nitrate/nitrite, chloride, sulfate, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), fecal coliforms, total phosphorus, dissolved organic carbon, total aluminum, dissolved aluminum, antimony, arsenic, beryllium, cadmium, calcium, chromium, copper, total iron, dissolved iron, lead, total manganese, dissolved manganese, magnesium, mercury, nickel, potassium, selenium, silver, sodium, thallium, and zinc.

Fisheries Collection

Fisheries collections were conducted April 23, 2004 using the EPA's Rapid Bioassessment Protocols For Use in Streams and Wadeable Rivers (EPA 841-B-99-002) in accordance with the "Programmatic Environmental Impact Statement (A Survey of the Condition of Streams in the Primary Region of Mountain Top Removal/ Valley Fill Coal Mining - March 1999, U.S. EPA, Region III)". Block nets were placed at each end of a 100-meter sampling reach to prevent immigration or emigration within the sampling reach. A Smith-Root™ battery-powered backpack electro-fishing unit was used to conduct a triple pass depletion. Collected fishes were identified to species, numerated, measured to the nearest millimeter total length, and weighed to the nearest 1.0 or 0.1 gram total weight depending on fish size. Fishes to be used for selenium analysis were then individually wrapped and packed in ice and returned to the laboratory. Fishes selected for tissue analysis were then dissected and organs such as the heart and liver, as well as reproductive tissues (gonads or eggs) and muscle tissue were separated to be individually weighed. To test for differences in selenium tissue analysis, tissues were homogenized and then split between the two laboratories. The University of Missouri-Columbia Research Reactor (MURR) Center was utilized for tissue analysis, but not water chemistry. In addition, to test for variances among individual samples, six replicates were derived from some of the individual samples, and split between the two laboratories. Individual methodologies are described below.

Selenium Analyses for Tissue and Water (REI Consultants, Inc)

Tissue samples are prepared for analysis using SW-846 Method 3050B. This is an acid digestion involving nitric acid, hydrogen peroxide and the application of heat. After digestion, the resulting extract is then analyzed for selenium using method SW 7740. This method utilizes Graphite Furnace Atomic Absorption Spectroscopy. The minimum detection level for this

procedure was 0.5 mg/kg.

Waters are digested for total selenium using EPA method 200.2. This is an acid digestion which utilizes nitric and hydrochloric acids and the application of heat. The resulting extract can then be analyzed for selenium using any one of EPA methods 270.2 (Graphite Furnace Atomic Absorption Spectroscopy), 200.9 (Stabilized Temperature Graphite Furnace Atomic Absorption Spectroscopy) or 200.8 (Inductively Coupled Plasma-Mass Spectroscopy). For the determination of dissolved selenium, an unpreserved sample is first filtered through a 0.45 µm membrane filter. The resulting sample is then treated and analyzed following the same procedures as outlined for total selenium. The minimum detection level for these procedures was 2 µg/l

Selenium Analyses (University of Missouri-Columbia Research Reactor (MURR) Center)

The fish tissue samples were frozen, and shipped frozen on dry ice by Federal Express to the University of Missouri-Columbia Research Reactor (MURR) Center. The fresh (or wet) mass of each sample (as received) was measured and recorded. The samples were lyophilized at a trap temperature of -50 C and pressure of approximately 500 millitorr and the dry sample mass was measured and recorded. The percent dry matter was calculated for each sample. Aliquots of the dry samples were transferred by mass into HDPE irradiation vials. Client samples and quality control (QC) samples were analyzed by instrumental neutron activation analysis. Replicate QC samples (NIST SRM 1577 Bovine Liver) were analyzed for QA/QC.

RESULTS

Because of the expense of analyzing tissue samples for selenium, only seven fish were selected to be analyzed for selenium levels in various tissues and organs. These seven individuals were selected due to their size and habitat. Larger and older fish were selected in attempts to obtain a “worse-case” scenario for selenium concentrations found in fish from streams located below active mining operations. This included individuals from the following species: smallmouth bass *Micropterus dolomieu*, golden redhorse *Moxostoma erythrurum*, common carp *Cyprinus carpio*, and bluegill *Lepomis macrochirus*.

From the data presented in TABLES 1 and 2, one can see that selenium concentrations varied widely between the different fish species examined. Unfortunately, whole body selenium levels were not measured because all individuals were dissected for individual organ/tissue analysis. An ANOVA performed on the data revealed that there was a statistically significant ($p < 0.001$) difference between selenium levels found between carp eggs and redhorse eggs, and a statistically significant ($p < 0.001$) difference between selenium levels found between carp muscle and redhorse muscle. However, not enough individuals have been examined to date to make an accurate determination if selenium levels vary among fish species.

Also from the data presented in TABLES 1 and 2, one can see that selenium concentrations varied widely between the different tissues and organs for each of the specimens. For example, Smallmouth Bass #01 had noticeably higher levels of selenium in its heart and liver compared to eggs or muscle. This was not observed in Smallmouth Bass #02. Golden Redhorse #01 contained highest levels of selenium within its eggs; Golden Redhorse #02 contained highest levels within its heart, and Golden Redhorse #03 had highest levels in its muscle tissue. However, not enough individuals have been examined to date to make an accurate determination if selenium levels vary between individual tissues/organs, or if certain organs consistently contain higher levels than others.

From the data presented in TABLES 1 & 2, when comparing the oven drying method (REI Consultants, Inc) and the freeze drying method (MURR) of preparing tissue samples, there was a statistically significant ($p < 0.001$) difference only between golden redhorse eggs examined between the two laboratories. The oven drying and subsequent Graphite Furnace Atomic Absorption Spectroscopy method detected lower levels of selenium than the freeze drying and subsequent instrumental neutron activation analysis.

DISCUSSION

Water chemistry from the most recent sampling event (TABLE 3) revealed fair water quality with neutral pH, and low levels of acidity, alkalinity, hardness, nitrate/nitrite, sulfate, TDS, TSS, and almost all metals. Conductivity was relatively elevated during this sampling event with 366 μS , and there was an extremely high level of fecal coliform with 2000 cfu/100ml. Level of iron was well within recommended ranges; levels of total aluminum exceeded recommended ranges, but the dissolved level was within tolerance levels. Total selenium was $< 0.0010 \text{ mg/l}$, and has usually been less than this detectable limit. However, in Spring 2003, total selenium at this station was 0.002 mg/l , and in Fall 2003 was 0.0046 mg/l which was the highest recorded level.

Habitat has usually been the primary limiting factor at this Kiah Creek station with a total score of 138 out of a possible 200 (TABLE 4). Limiting scores for “embeddedness”, “velocity depth combinations”, and “frequency of riffles” have been the parameters most limiting. This site is comprised primarily of a single long pool habitat, with only a very small riffle area, which has proven to be conducive to fish, but not to benthic macroinvertebrates.

Because of the limiting habitat, in conjunction with some water quality limitations, the benthic macroinvertebrate community has been considered to be marginal to poor. Only 291 individuals and 8 taxa were collected during the Spring 2004 study, and the benthic community was comprised of over 95% pollution tolerant individuals (TABLE 5). The WV-SCI score during the Spring 2004 sampling event was 21.6 (TABLE 6), and the average WV-SCI score since Spring 1995 is calculated to be 52.1. Additionally, only one taxa each from the mayfly and stonefly groups was collected; caddisflies were absent.

Historically, this site on Kiah Creek has been comprised of an abundant fish community comprised of a wide variety of taxa. During an intensive fish population survey conducted in April 2001 at the same Kiah Creek site used for this study, 680 fish from 21 taxa were collected (TABLE 7). The majority were considered to be intermediate tolerant individuals (70%), and the community was considered to be fairly healthy.

As stated in the Results section of this paper, although there was selenium analysis performed on organs such as the heart, liver, and gonads, not enough individuals have been tested to date for statistical comparisons between species. However, there was a significant difference in mean selenium levels between common carp *Cyprinus carpio* eggs and golden redhorse *Moxostoma erythrurum* eggs (TABLE 1). Both taxa depicted (FIGURE 2) levels below recommended levels of 10 mg/kg for eggs and ovaries (Lemley 2002). A study performed by the United States Fish and Wildlife Service (USFWS) in the spring and summer of 2003, and was conducted in East Lynn Lake and Beech Fork Lake as well as streams in their watersheds. This study revealed levels of selenium in bluegill *Lepomis macrochirus* eggs to be 1.08 mg/kg; for gizzard shad *Dorosoma cepedianum* level of selenium in eggs was 3.54 mg/kg; and for largemouth bass *Micropterus salmoides* eggs ranged from 2.06 to 4.73 mg/kg (USFWS 2004).

Also from TABLE 1, there was a significant difference in mean selenium levels between common carp *Cyprinus carpio* muscle tissue and golden redhorse *Moxostoma erythrurum* muscle tissue. Mean selenium level in carp muscle tissue was 8.14 mg/kg which was slightly higher than the recommended level of 8.0 mg/kg as reported by Lemley (2002). However, mean selenium level in golden redhorse muscle tissue was well below the recommended range with a mean of 5.34 mg/kg (FIGURE 3). During the USFWS study, levels of selenium in largemouth bass *Micropterus salmoides* fillets ranged from 1.26 to 3.25 mg/kg (USFWS 2004).

Because enough replicate samples were performed on carp eggs, carp muscle, redhorse eggs, and redhorse muscle at both the REI Consultants, Incorporated (REIC) and the University of Missouri-Columbia Research Reactor (MURR) Center laboratories, the two different methodologies could be statistically analyzed between the two facilities. REIC utilized the oven drying method to dry the sample prior to acid digestion and selenium analysis by Graphite Furnace Atomic Absorption Spectroscopy. MURR utilized a freeze drying method of preparation and subsequent instrumental neutron activation analysis. Analysis between the two facilities revealed a significant ($p < 0.001$) difference in selenium levels in golden redhorse eggs between the two facilities (FIGURE 4). The high water content found in eggs compared to muscle tissue was thought to be the reason for the differences between the two facilities. It was thought that this high ratio of wet weight (water) to dry weight may make the accurate measurement of selenium more difficult. Nevertheless, levels of selenium found in golden redhorse muscle and carp eggs and muscle compared between the two facilities were not significantly different (FIGURES 5 - 7).

Considerably more research is needed to evaluate the differences between these two methods of analyzing tissues for selenium. In addition, this study did not attempt to compare

selenium levels of fishes from streams located below mining operations to “control”, or non-mining related streams. This is difficult to assess, however, as fish are mobile enough to potentially enter or leave streams of various water qualities, thereby possibly providing misleading information.

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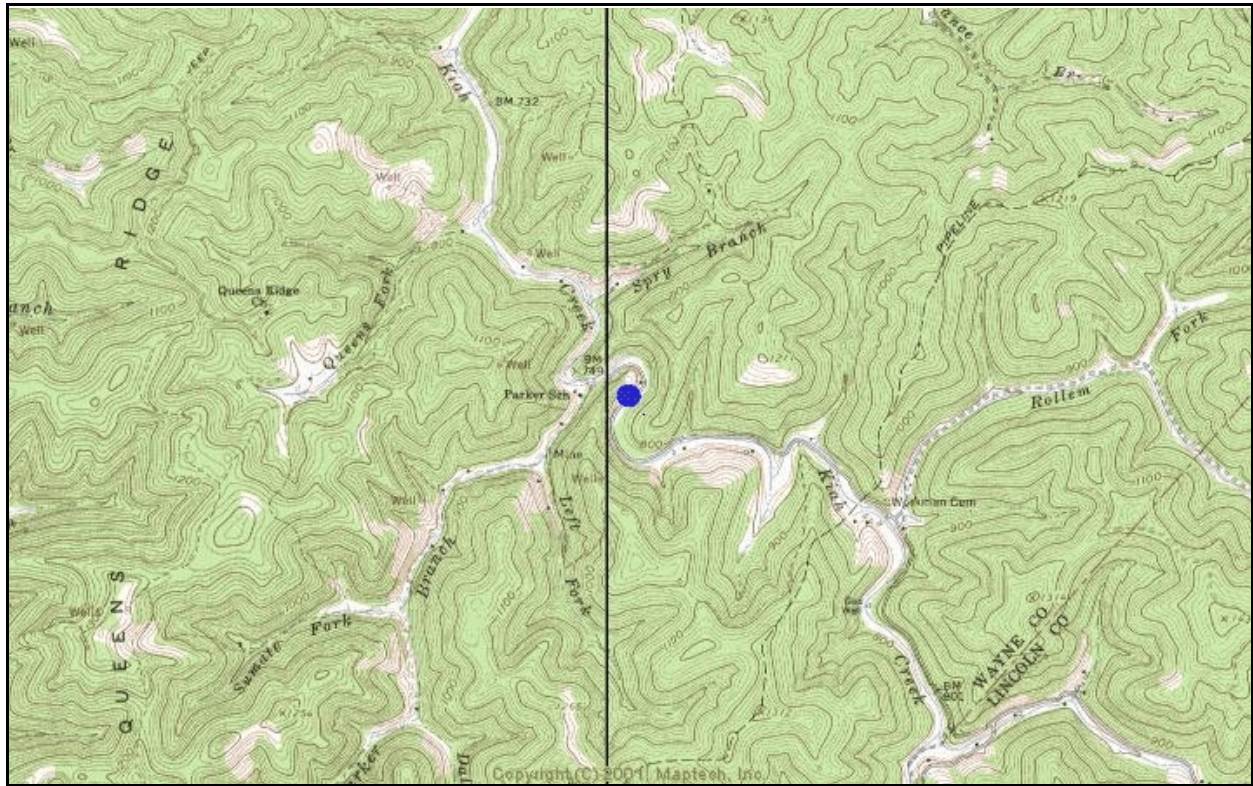


FIGURE 1. Fish sampling location for the fish station located on Kiah Creek. Argus Energy-WV, LLC, April 2004.

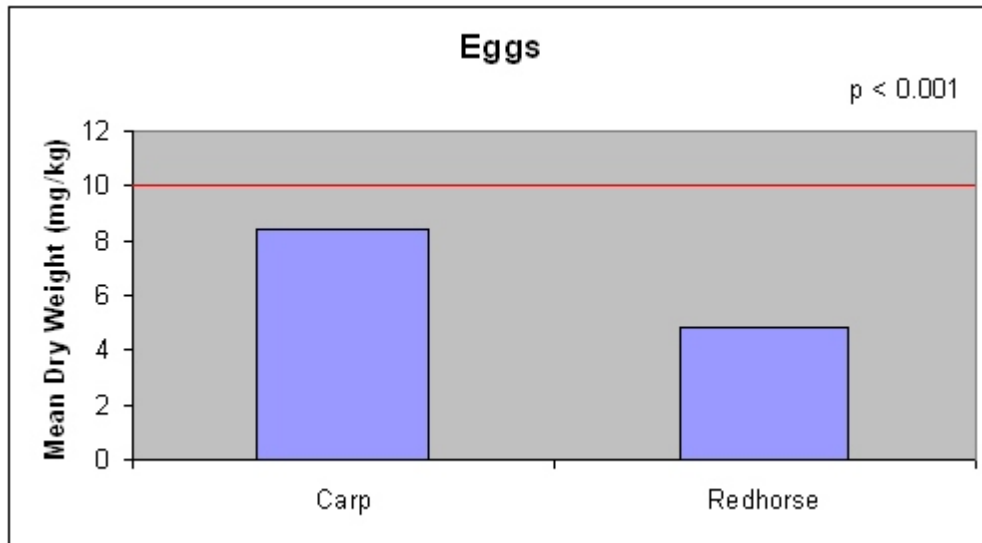


FIGURE 2. Mean dry weight (mg/kg) of selenium found in common carp *Cyprinus carpio* eggs versus golden redhorse *Moxostoma erythrurum* eggs. Argus Energy-WV, LLC, April 2004. Red line is Lemley's recommended selenium level for eggs and ovaries of 10 ug/g (mg/kg).

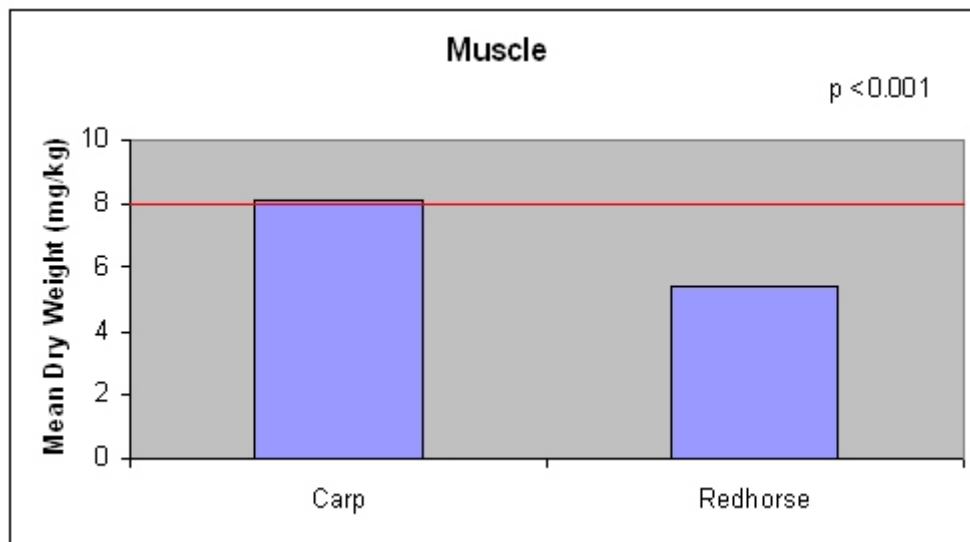


FIGURE 3. Mean dry weight (mg/kg) of selenium found in common carp *Cyprinus carpio* muscle tissue versus golden redhorse *Moxostoma erythrurum* muscle tissue. Argus Energy-WV, LLC, April 2004. Red line is Lemley's recommended selenium level for muscle tissue of 8 ug/g (mg/kg).

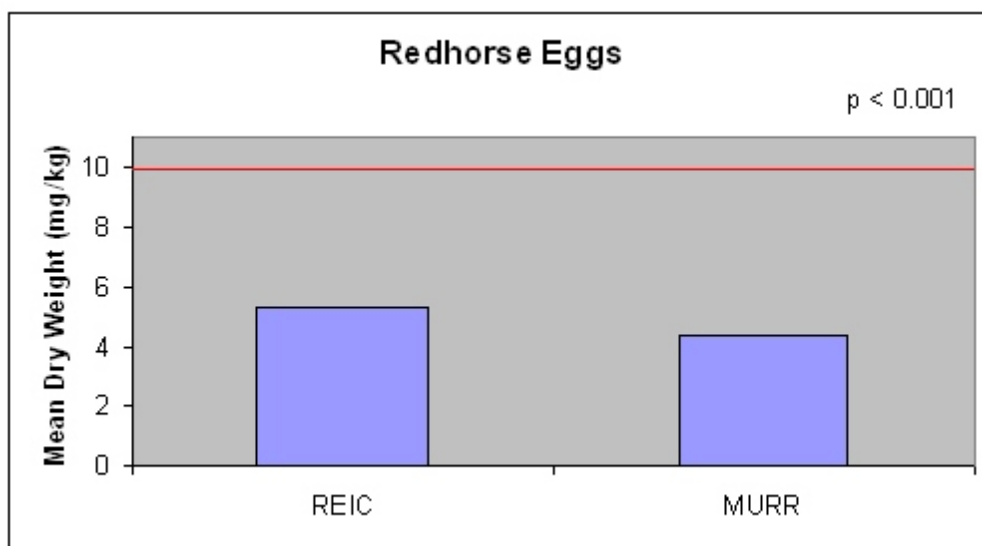


FIGURE 4. Mean dry weight (mg/kg) of selenium found in golden redhorse *Moxostoma erythrurum* eggs as analyzed by REI Consultants, Inc. and MURR, April 2004. Red line is Lemley's recommended selenium level for eggs and ovaries of 10 ug/g (mg/kg).

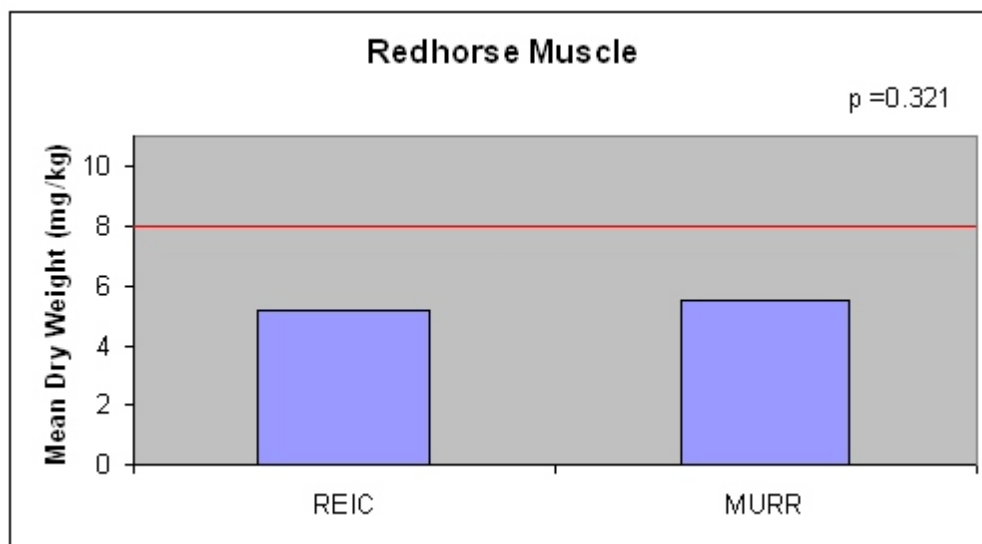


FIGURE 5. Mean dry weight (mg/kg) of selenium found in golden redhorse *Moxostoma erythrurum* muscle tissue as analyzed by REI Consultants, Inc. and MURR, April 2004. Red line is Lemley's recommended selenium level for muscle tissue of 8 ug/g (mg/kg).

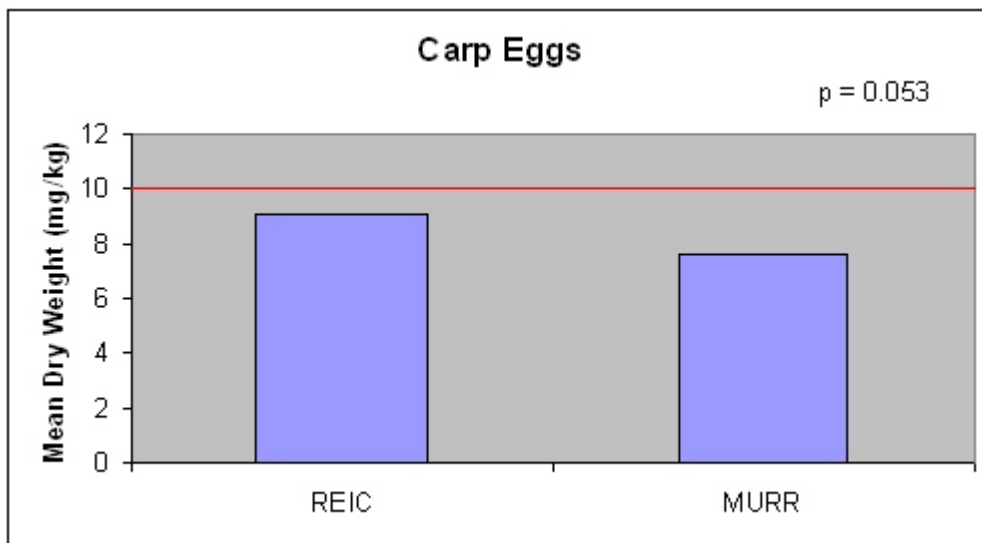


FIGURE 6. Mean dry weight (mg/kg) of selenium found in common carp *Cyprinus carpio* eggs as analyzed by REI Consultants, Inc. and MURR, April 2004. Red line is Lemley's recommended selenium level for eggs and ovaries of 10 ug/g (mg/kg).

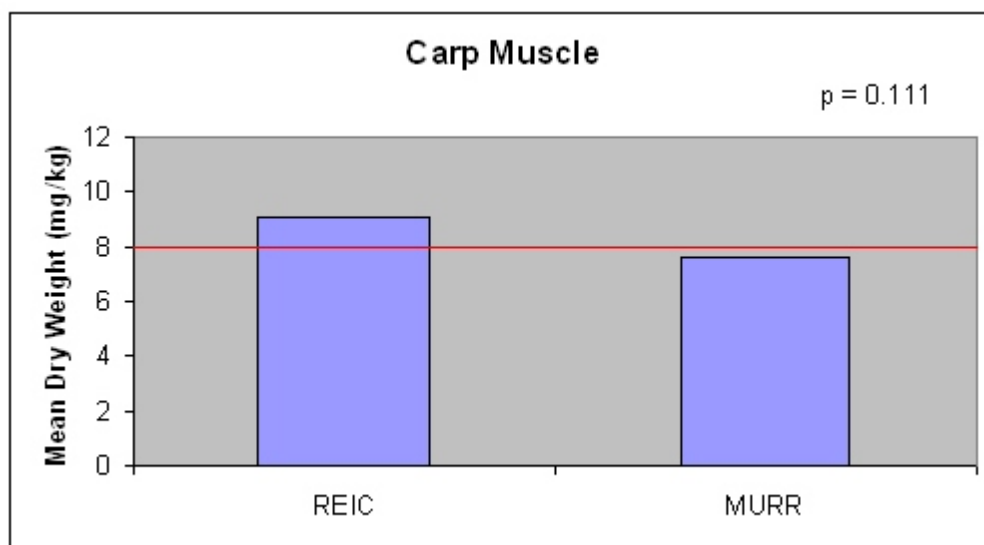


FIGURE 7. Mean dry weight (mg/kg) of selenium found in common carp *Cyprinus carpio* muscle tissue as analyzed by REI Consultants, Inc. and MURR, April 2004. Red line is Lemley's recommended selenium level for muscle tissue of 8 ug/g (mg/kg).

TABLE 1. Total selenium (dry weight analysis, mg/kg) values in tissues of fish collected in Kiah Creek. REI Consultants, Inc (REIC) and University of Missouri-Columbia Research Reactor (MURR) Center, July 2004.

Species	Specimen #	Tissue	Total Selenium (REIC; dry wt) mg/kg	Total Selenium (MURR; dry wt) mg/kg
Carp Female: 420 mm, 1074 g	01	Eggs (1)	6.65	9.45
		Eggs (2)	8.00	8.91
		Eggs (3)	8.35	9.07
		Muscle (4)	8.05	7.91
		Muscle (5)	7.82	8.69
		Muscle (6)	7.71	8.66
Golden Redhorse Female: 385 mm, 590 g	03	Eggs (7)	4.25	5.34
		Eggs (8)	4.40	5.20
		Eggs (9)	4.50	5.34
		Muscle (10)	5.91	5.29
		Muscle (11)	5.54	5.22
		Muscle (12)	5.05	5.13

Difference between Dry Oven Method (REIC) and Freeze Method (MURR)

<u>Sample</u>	<u>p-value</u>
Carp Eggs	0.053
Carp Muscle	0.111
Redhorse Eggs	<0.001*
Redhorse Muscle	0.321

Difference between Carp and Redhorse

<u>Sample</u>	<u>p-value</u>
Eggs	<0.001*
Muscle	<0.001*

* = p value < 0.050 indicating a significant difference between means.

TABLE 2. Total selenium (dry weight analysis, mg/kg) values in tissues of fish collected in Kiah Creek. REI Consultants, Inc (REIC) and University of Missouri-Columbia Research Reactor (MURR) Center, April 2004.

Species	Specimen #	Tissue	Total Selenium (REIC; dry wt) mg/kg	Total Selenium (MURR; dry wt) mg/kg
Smallmouth Bass	01	Heart	6.13	5.55
		Liver	3.92	7.15
		Eggs	1.45	4.79
		Muscle	1.92	4.58
Smallmouth Bass	02	Heart	1.12	6.13
		Liver	2.38	10.38
		Gonads	2.61	6.18
		Muscle	3.14	5.29
Golden Redhorse	01	Heart	2.95	4.74
		Eggs	7.32	8.13
		Muscle	4.05	5.70
Golden Redhorse	02	Heart	4.89	4.70
		Eggs	3.55	4.90
		Muscle	1.32	4.39
Bluegill	01	Heart	NA	5.67
		Liver*	22.3	17.84
		Muscle	3.81	5.67

NA = sample too small too be split

* = this sample had a relatively small mass, which was largely water, thus resulting in a substantially different % dry matter compared to other samples. Therefore, these results should be viewed with caution.

TABLE 3. Physical and chemical water quality for the fish station located on Kiah Creek. Argus Energy-WV, LLC, April 2004.

PARAMETER	Fish Station
Dissolved Oxygen (DO) mg/l	10.3
pH (SI)	7.03
Conductivity (μ S)	366.0
Acidity (mg/l)	<1.0
Alkalinity (mg/l)	22.4
Total Hardness (mg/l)	148.0
Fecal Coliform (cfu/100 ml)	2000
Nitrate/Nitrite (mg/l)	0.3
Chloride (mg/l)	3.3
Sulfate (mg/l)	133.0
TSS (mg/l)	4
TDS (mg/l)	237
Total Phosphorus (mg/l)	<0.05
Dissolved Organic Carbon (mg/l)	1.7
Dissolved Aluminum (mg/l)	0.066
Total Aluminum (mg/l)	0.188
Antimony (mg/l)	<0.0010
Arsenic (mg/l)	<0.0100
Beryllium (mg/l)	<0.0010
Cadmium (mg/l)	<0.0010
Calcium (mg/l)	29.7
Chromium (mg/l)	<0.0050
Copper (mg/l)	<0.0050
Dissolved Iron (mg/l)	0.030
Total Iron (mg/l)	0.273
Lead (mg/l)	<0.0010
Dissolved Manganese (mg/l)	0.181
Total Manganese (mg/l)	0.207
Magnesium (mg/l)	17.8
Mercury (mg/l)	<0.0002
Nickel (mg/l)	<0.0100
Potassium (mg/l)	2.57
Selenium (mg/l)	<0.0010
Silver (mg/l)	<0.0020
Sodium (mg/l)	4.18
Thallium (mg/l)	<0.0010
Zinc (mg/l)	0.007

TABLE 4. Habitat scores for the fish station located on Kiah Creek. Argus Energy-WV, LLC, April 2004.

Fish Station				
<u>Primary - Substrate and Instream Cover</u>				
1. Epifaunal Substrate and Available Cover (0-20)				17
2. Embeddedness (0-20) 6				6
3. Velocity Depth Combinations (0-20)				7
<u>Secondary - Channel Morphology</u>				
4. Sediment Deposition (0 - 20)				20
5. Channel Flow Status (0 - 20)				20
6. Channel Alteration (0 - 20)				20
7. Frequency of Riffles (0 - 20)				1
<u>Tertiary - Riparian and Bank Structure</u>				
8. Bank Stability (0 -10)				
Left Bank				10
Right Bank				8
9. Bank Vegetation Protection (0 -10)				
Left Bank				10
Right Bank				7
10. Riparian Zone Width (0 - 10)				
Left Bank				9
Right Bank				3
Total Score				138
Note: The scoring for each category	<u>Optimal</u>	<u>Sub - Optimal</u>	<u>Marginal</u>	<u>Poor</u>
Primary	16 - 20	11 - 15	6 - 10	0 - 5
Secondary	16 - 20	11 - 15	6 - 10	0 - 5
Tertiary	9 - 10	6 - 8	3 - 5	0 - 2

TABLE 5. Total abundances and sensitivities of benthic macroinvertebrates collected from the fish station located on Kiah Creek. Argus Energy-WV, LLC, April 2004.

TAXON	Fish Station
Insecta	
Ephemeroptera (Mayflies)	
Ephemerellidae	
Eurylophella (S)	3
Plecoptera (Stoneflies)	
Nemouridae	
Amphinemura (S)	4
Diptera (True Flies)	
Chironomidae (T)	262
Empididae (T)	7
Odonata (Dragonflies)	
Gomphidae	
Stylogomphus (S)	2
Coleoptera (Beetles)	
Elmidae (F)	2
Dubiraphnia (F)	3
Annelida (Aquatic Worms)	
Oligochaeta (T)	8
Total Individuals	291
Taxa	8
Sensitive Individuals (%)	3.1%
# Sensitive Taxa	3
Facultative Individuals (%)	1.7%
# Facultative Taxa	2
Tolerant Individuals (%)	95.2%
# Tolerant Taxa	3
() Classification of Pollution Indicator Organisms	
(S) = Sensitive (F) = Facultative (T) = Tolerant (U) = Unclassified	

TABLE 6. Selected benthic macroinvertebrate metrics for the fish station located on Kiah Creek.
Argus Energy-WV, LLC, April 2004.

	Fish Station
Metric 1. Taxa Richness	8
Metric 2. Modified Hilsenhoff Biotic Index	5.81
Metric 3. Ratio of Scrapers to Collector/Filterers	2:0
Metric 4. Ratio of Ephemeroptera, Plecoptera, Trichoptera (EPT) and Chironomidae Abundances	7:262
Metric 5. Percent Contribution of Mayflies	1.0%
Metric 6. Percent Contribution of Dominant Taxa	90.0% Chironomidae
Metric 7. EPT Index	2
Metric 8. Percent of Shredders to Total	1.4%
Metric 9. Simpson's Diversity Index	0.19
Metric 10. Shannon-Wiener Diversity Index	0.73
Metric 11. Shannon-Wiener Evenness	0.23
Metric 12. West Virginia Stream Condition Index (WV-SCI)	21.6

TABLE 7. Summary of fisheries catch for the fish station located on Kiah Creek. Argus Energy-WV, LLC, April 2001.

Common Name	Scientific Name	Total Abundance	Relative Abundance	Mean Length (mm)	Mean Weight (g)	Standing Stock (kg)	Sensitivity Index
Sand Shiner	Notropis ludibundus	10	0.015	59.8	2.5	0.025	M
Bluntnose Minnow	Pimephales notatus	84	0.124	68.8	4.4	0.373	T
Central Stoneroller	Campostoma anomalum	80	0.118	83.2	8.9	0.710	M
Creek Chub	Semotilus atromaculatus	65	0.096	101.0	17.3	1.126	T
Silverjaw Minnow	Ericymba buccata	28	0.041	59.8	2.4	0.068	M
Striped Shiner	Luxilus chrysocephalus	140	0.206	86.9	8.6	1.199	M
River Chub	Nocomis micropogon	5	0.007	114.2	20.2	0.101	I
Northern Hogsucker	Hypentelium nigricans	15	0.022	160.5	56.2	0.844	I
White Sucker	Catostomus commersonii	15	0.022	134.1	27.8	0.417	T
Smallmouth Bass	Micropterus dolomieu	1	0.001	119.0	12.0	0.012	M
Spotted Bass	Micropterus punctulatus	2	0.003	134.5	19.0	0.038	M
Rockbass	Ambloplites rupestris	1	0.001	58.0	4.3	0.004	M
Green Sunfish	Lepomis cyanellus	1	0.001	114.0	24.0	0.024	T
Longear Sunfish	Lepomis megalotis	3	0.004	74.0	8.0	0.024	I
Bluegill	Lepomis macrochirus	8	0.012	87.0	11.2	0.089	M
Lepomis spp.	Lepomis spp.	15	0.022	58.5	4.6	0.070	NC
Logperch	Percina caprodes	4	0.006	93.3	7.4	0.029	M
Johnny Darter	Etheostoma nigrum	104	0.153	46.1	1.0	0.107	M
Fantail Darter	Etheostoma flabellare	10	0.015	52.6	1.8	0.018	M
Rainbow Darter	Etheostoma caeruleum	78	0.115	43.0	1.2	0.094	M
Least Brook Lamprey	Lampetra aepyptera	11	0.016	132.7	5.0	0.055	M
Total		680				5.427	

Sensitivity Index:

(I) = Pollution Intolerant (M) = Intermediately Pollution Tolerant (T) = Pollution Tolerant (NC) = Not Classified