

Assessment of the Great Bend Station (Sunflower Electric Power Corporation) Selenium Discharge on a Tributary of Dry Walnut Creek (Barton Co., KS)

Revised Final Report

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INTRODUCTION

Sunflower Electric's Great Bend Station (GBS) located near Heizer, Kansas in Barton County discharges spent cooling water to an unnamed tributary of Dry Walnut Creek, a tributary of the Walnut River that flows into the Arkansas River east of Great Bend Kansas (Figure 1). Currently, the State of Kansas's discharge limitations for selenium are based on 7µg/L daily and 23µg/L maximum concentrations of total recoverable selenium from water. In the past, selenium concentrations in the discharge water from GBS routinely exceeded State permitted levels. In addition, sporadic permit exceedances were observed within the receiving waterbody itself (AEC 2009). However, the U.S. Environmental Protection Agency (USEPA) has published an alternative draft that would establish criteria limits based upon biotoxicity/body burden levels rather than ambient water concentrations. The State is ready to adopt these new limits when USEPA finalizes their criterion which was projected to occur in 2010.

The Central Plains Center for BioAssessment (CPCB) designed and completed a study to evaluate the potential effects of the Great Bend Station discharge on the selenium levels associated with the sediment, water, fish, and fish eggs within the receiving tributary. The study would be similar to those done by CPCB on the Arkansas and Solomon Rivers (<http://www.cpcb.ku.edu/research.htm>).

Selenium (Se) is an essential trace nutrient but it can be toxic to aquatic life at excessive levels. High levels of selenium can also be toxic to humans. The drinking water Maximum Contaminant Level (MCL) for selenium is 50µg/L. Being a natural nonmetallic element, selenium can be found throughout the environment. Selenium has five oxidation states (-2, 0, 2, 4, 6) and the two major inorganic forms of selenium normally observed in aquatic environment are selenate ion (SeO_4^{2-}) and selenite ion (SeO_3^{2-}). High levels of selenium in waterbodies have mostly been related to irrigation of western soils that are naturally high in selenium, disposal of ash produced by coal-fired power plants, petroleum refinery effluents, and runoff or discharges from certain mining activities.

Selenium is a bioaccumulative pollutant. Aquatic life is exposed to selenium primarily through food consumption rather than from direct exposure to selenium in the water column. Although selenium bioaccumulates, it does not significantly biomagnify. Unlike mercury or PCBs, selenium concentrations do not increase significantly in successive levels of a food chain. Concentrations equal to or greater than 4.0 mg/kg in sediment are a concern because there is a potential for bioaccumulation in fish and wildlife (Lemly and Smith 1987).

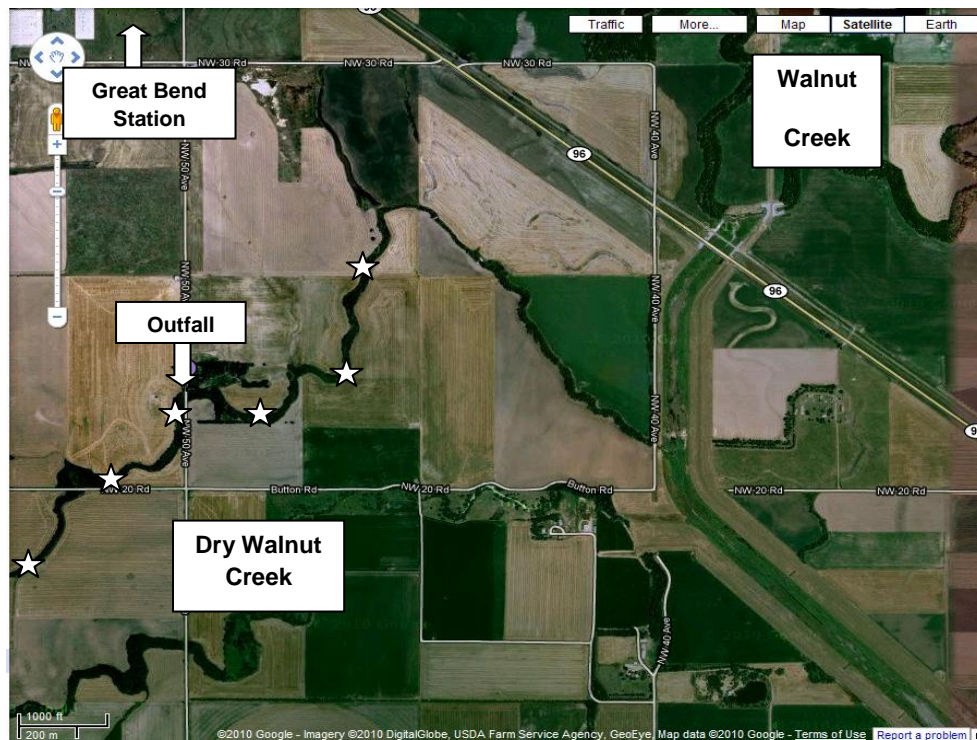


Figure 1. Aerial photo of location of Sunflower Electric's Great Bend Station outfall (38.3940667, -98.8669) on an unnamed tributary to Dry Walnut Creek. Stars indicate sampling sites.

USEPA is currently revising the aquatic life criterion for selenium and has been soliciting scientific information, data, and views pertaining to the criterion since December 2004. Due to the bioaccumulative property of selenium, fish-tissue based criterion has been proposed because fish tissue samples provide a better indicator of the presence of selenium in a particular waterbody. EPA's National Criterion states (USEPA 2004):

Freshwater aquatic life should be protected if the following conditions are satisfied.

- A. *The concentration of selenium in whole-body fish tissue does not exceed 7.91 µg/g dw (dry weight). This is the chronic exposure criterion. In addition, if whole-body fish tissue concentrations exceed 5.85 µg/g dw during summer or fall, fish tissue should be monitored during the winter to determine whether the selenium concentration exceeds 7.91 µg/g dw.*

- B. *The 24-hour average concentration of total recoverable selenium in water seldom (e.g., not more than once in three years) exceeds 258 µg/L for selenite, and likewise seldom exceeds the numerical value given by $\exp(0.5812[\ln(\text{sulfate})]+3.357)$ for selenate. These are the acute exposure criteria. At an example sulfate concentration of 100 mg/L, the 24-hour average selenate concentration should not exceed 417 µg/L.*

The goal of this project was to determine the degree of potential impairment of selenium in the unnamed tributary to the Dry Walnut River. The objectives were:

1. Monitor the selenium levels in the stream flow, bed sediments (reported in dry weight), aquatic life tissues (i.e. fishes) (reported in dry weight), and fish egg masses along the unnamed tributary of Dry Walnut River in Barton Co. KS during discharging and non-discharging periods
2. Compare levels of selenium in water, sediment, fish tissue, and eggs found in the tributary above and below the point of discharge.
3. Assess the selenium levels between the four media to determine the role of water-column selenium concentrations on impacting aquatic life.
4. Assess influence of period of discharge (June – September) concentrations on tributary concentrations during non-discharging periods (September - April).

METHODS

Site selection

The study area is along the unnamed tributary of Dry Walnut Creek in Barton County in central Kansas and includes one site on the Arkansas River (Figure 1). The outfall is located at 38.3940667 N, -98.8669 W near Button Rd. and SW 50 Ave. Six sites were chosen *a priori* to sample twice prior to and once after the power plant came on-line for the summer. Three sites were upstream of the Sunflower Power plant outfall and three sites downstream of the outfall, one of which was in proximity

to the discharge pipe. Sampling was conducted in March, May, and August of 2011. Sites 2 and 6 were dry in August, and thus no samples were collected at these sites.

Table 1. Sites sampled for this project. Coordinates are in NAD 83.

Site	Waterbody	Type	Latitude	Longitude	Location
1	Arkansas River	above	38.29319	98.89142	South of Dundee at dam. Dry in August, sampled pools below dam.
2	unnamed tributary	above	38.38639	98.88505	At NW 60 Ave. bridge crossing. Dry in August
3	unnamed tributary	above	38.39074	98.87086	At Button Rd. bridge crossing.
4	unnamed tributary	below	38.39380	98.86672	Upstream from NW 50 Ave., at effluent pipe.
5	unnamed tributary	below	38.39860	98.85532	Southwest of NW 30 Rd and NW 40 Ave. at pool flowing into diversion to Cheyenne Bottoms.
6	unnamed tributary	below	38.39226	98.84840	At NW 40 Ave. bridge crossing. Dry in August

Field methods

Water and sediment

During each of the field collections, DO, temperature, conductivity, pH, and turbidity were measured *in situ* using a Horiba U-10 Water Quality Checker. Concurrent with the field measurements, a one-liter grab water sample was collected and transported on ice in a cooler to the Kansas Biological Survey (KBS) Ecotoxicology Laboratory for water chemistry analysis. At each site, three sediment samples were collected (2.5 – 3 cm depth) using a hand-operated sediment corer, composited into one 500-ml wide-mouth HDPE bottle, and returned to the lab with the water samples for later analysis. Water and sediment samples were analyzed for selenium. A summary of water-quality parameters, analysis methods, and detection limits associated with parameters measured in this project is provided in Table 2.

Table 2. Water-quality parameters measured in this project.

Parameter	Container	Instrument	Method Citation	Detection Limit	Holding Time	Preservation
Laboratory Analyses						
Selenium in Water	1L Amber Glass	Perkin-Elmer Atomic Absorption (AA) Spectrophotometer Model 5100	EPA Method 7740	2 µg/L	180 days	pH < 2 with HNO ₃ , 4°C
Selenium in Soil	500ml. HDPE Jar	Perkin-Elmer Atomic Absorption (AA) Spectrophotometer Model 5100	EPA Method 3050B	-	180 days	4°C
Selenium in Fish	Aluminum Foil	Perkin-Elmer Atomic Absorption (AA) Spectrophotometer Model 5100	EPA Method 200.3	-	-	≤-20°C
<i>In situ</i> Measurements						
pH	none	Horiba U-10 Water Quality Checker	21 st Ed. Standard Methods 4500-H ⁺	0.1	-	-
Specific Conductance	none	Horiba U-10 Water Quality Checker	21 st Ed. Standard Methods 2510 A-B	0.001 mS/cm	-	-
DO	none	Horiba U-10 Water Quality Checker	21 st Ed. Standard Methods 4500-O G	0.1 mg/L	-	-
Turbidity	none	Horiba U-10 Water Quality Checker	21 st Ed. Standard Methods 2130-B	1 NTU	-	-
Water Temperature	none	Horiba U-10 Water Quality Checker	21 st Ed. Standard Methods 2550-B	0.1°C	-	-

* automated flow injection analyzer

Fish and Eggs

A representative sample of the fish community at each site was collected using a backpack electrofishing unit during most sampling events, or a seine at sites 4 and 5 in August 2011 when conductivity was too high for shocking. Species targeted for selenium analysis were black bullhead (*Ameiurus melas*), red shiner (*Cyprinella lutrensis*), and green sunfish (*Lepomis cyanellus*). One to three individuals of each of these three species were retained from each sampling event to determine whole body burdens or selenium. A total of 123 specimens were examined for selenium whole body burdens. In addition, eggs were extracted from gravid females of two of the three selected species (i.e. green sunfish and black bullhead) for selenium analysis. No gravid red shiner females were collected during the course of this study.

Laboratory Methods: Analytical DeterminationsWater quality parameters

Water total Se concentrations were evaluated using dissolution methods outlined in EPA Method 7740 and Graphite Furnace Atomic Absorption (GFAA). Water samples were digested using heat (hot plates at 95°C) and acid addition (H₂O₂, and HNO₃) to result in an acid concentration of 1% and a final volume of less than 50mL. Samples were brought back to 50mL with the addition of Type II water. 5mL of this solution was diluted to 10mL with a 1mL addition of 1% nickel nitrate. Samples were injected into the graphite furnace for analysis.

Sediment quality parameters

Sediment total Se concentrations were evaluated using dissolution methods outlined in EPA Method 3050B and GFAA. For the digestion of sediment samples, 1-2 grams (wet weight) of each sample was digested using heat (hot plates at 95°C) and with repeated additions of HNO₃ and H₂O₂. The resulting digestate was diluted to a final volume of 100 mL and was allowed to settle for particulate removal. Solution was then injected into the graphite furnace to analyze the concentrations in the sediment samples.

Fish quality parameters

Fish total Se concentrations were evaluated using digestion methods outlined in EPA Method 200.3 and GFAA. Up to 4-6 grams of frozen fish tissue or whole fish were solubilized using heat (hot plates at 120°C) with additions of HNO₃, H₂O₂, and HCl. Sample volumes were reduced to less than 5mL and diluted to 100-mL with Type II water. Any insoluble material in the samples was allowed to settle at the bottom of the vial prior to analysis using the graphite furnace.

Fish Eggs quality parameters

Fish eggs total Se concentrations were evaluated using digestion methods outlined in EPA Method 200.3 and GFAA. Whole egg masses were solubilized using heat (hot plates at 120°C) and with additions of HNO₃, H₂O₂, and HCl. Sample volumes were reduced to less than 5mL and diluted to 100-mL with Type II water. Any insoluble material in the samples was allowed to settle at the bottom of the vial prior to analysis using the graphite furnace.

Background: Climate

Precipitation and Temperature

As of November 21, 2011, Barton County, KS had been declared to be experiencing drought emergency according to the drought map provided by the Kansas Water Office (Figure 2). Kansas experienced severe drought conditions in 2011 due to record-breaking low precipitation values and temperatures that further exacerbated drought conditions throughout the state. Rainfall data for the years 2000-2011 (Table 3) for Barton County, KS, shows that the months of April, June and July experienced much lower rainfall on average as compared to the normal and new normal values, which are determined according to 30-year averages. Furthermore, the month of December experienced a high departure from normal precipitation values, which could be attributed to the fact that December was warmer than average throughout the state (1.3 degrees warmer than normal), according to the Kansas Water Office (2011).

RESULTS

General Water Chemistry

The chemistry of the March and May water samples collected at each site prior to the power plant operating for the summer was relatively typical of waterbodies located in this region (see Table 4

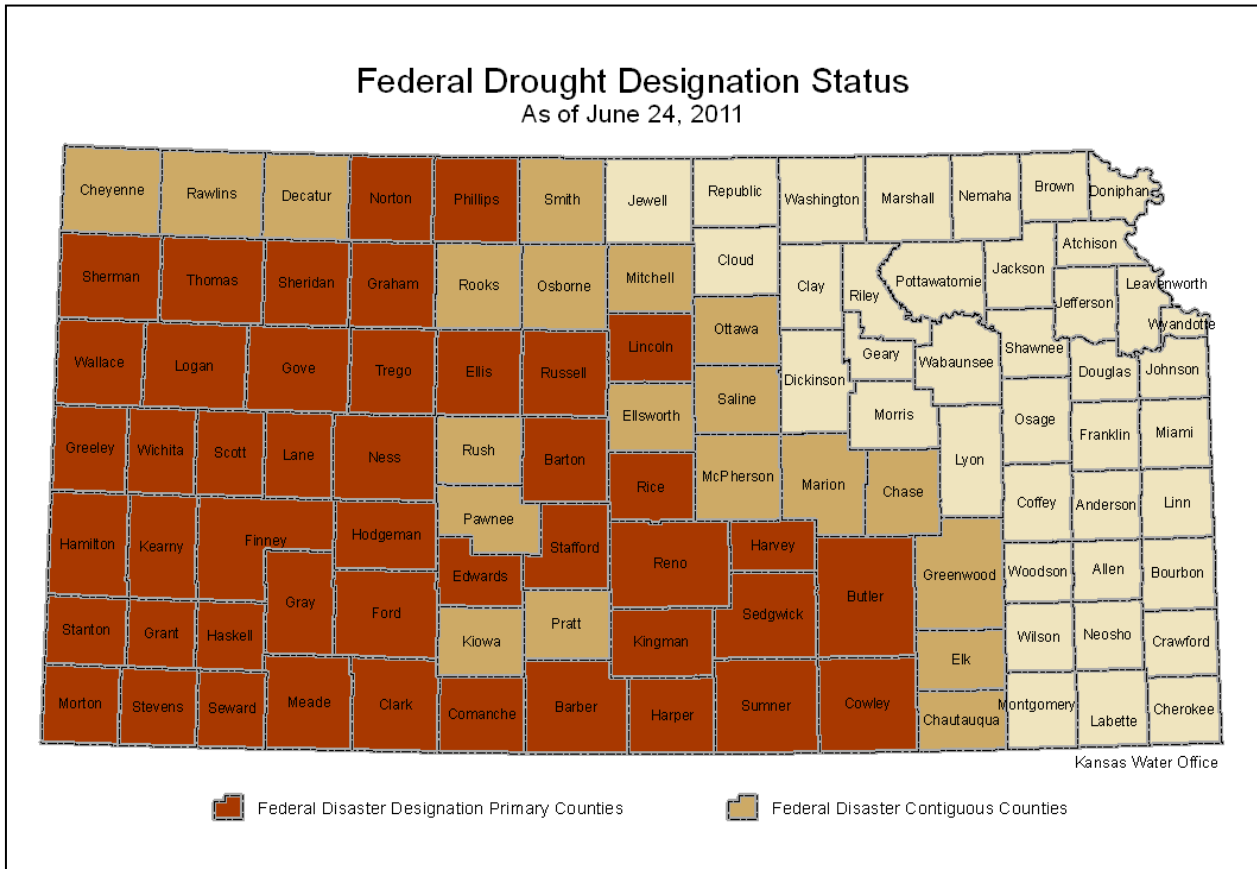


Figure 2. Federal drought designation status map released by the Kansas Water Office.

Table 3. Rainfall data for Barton County, KS for the years 2000-2011, in inches.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
New Normal	0.66	0.85	2.29	2.32	4.12	3.62	3.45	3.11	1.98	2.15	1.23	0.85	26.62
Normal	0.50	0.75	1.96	2.25	3.49	4.16	3.31	3.09	2.21	2.09	1.13	0.85	25.77
2000	1.50	2.02	5.53	1.43	4.73	2.71	5.91	0.10	0.00	4.26	0.69	0.47	29.32
2001	1.94	3.03	0.73	2.81	5.35	3.32	4.02	2.10	5.72	0.52	0.04	0.10	29.66
2002	0.73	0.41	0.85	1.50	2.24	2.54	1.21	3.24	0.70	5.49	0.10	0.02	19.01
2003	0.01	1.32	4.12	2.89	3.12	2.04	0.00	3.23	2.32	0.56	0.63	1.09	21.32
2004	0.37	0.93	3.42	1.31	1.96	5.17	5.08	2.69	1.40	1.75	1.21	0.12	25.38
2005	0.82	2.09	1.07	2.69	0.97	4.78	2.65	5.08	0.37	1.32	1.90	0.76	24.47
2006	0.07	0.00	1.41	3.36	3.06	3.29	2.61	8.63	1.50	1.07	0.22	2.84	28.04
2007	0.50	1.58	3.00	2.32	18.25	5.81	2.32	2.86	1.71	0.53	0.21	3.95	43.01
2008	0.42	0.97	0.55	3.68	5.21	4.17	3.42	4.14	3.13	6.23	0.89	0.28	33.06
2009	0.12	0.17	2.01	3.37	2.02	3.96	3.25	3.25	2.28	2.95	0.85	0.77	24.96
2010	0.51	0.65	1.74	3.08	5.43	5.63	3.28	3.13	1.87	0.29	2.30	0.20	28.09
2011	0.49	0.86	1.17	0.46	4.14	1.33	0.58	3.65	0.73	1.57	1.86	2.54	19.36

and Figure 3), which has relatively high selenium and conductivity. During sample collection in August, after the power plant had come on-line and operated during the summer, all sites were found pooled or dry, except for Sites 4 and 5 which were flowing due to the power plant discharge. The conductivity and selenium at this time in Sites 4 and 5 were notably higher than in all the other samples collected in 2011, even those collecting concurrently in August from Sites 1 and 3, which had only pooled water that would be concentrated due to evaporation. The selenium at the Site 4 effluent pipe and Site 5 directly downstream was twice as high as that from the other sites, so using sites 1, 2, 3, and 6 as control and subtracting their selenium values (August average = 21.9 μ g/L) from Sites 4 and 5 (August average = 68.1 μ g/L), the selenium (46.2 μ g/L) would still be over the maximum allowable 23 μ g/L total recoverable selenium from water.

Table 4. Water chemistry at the six study sites in 2011. Site 1 is the Arkansas River, the remaining are on the unnamed tributary of Dry Walnut Creek, Barton Co., KS.

Site	Event	Date 2011	Selenium μ g/L	pH	Water temp C	DO mg/L	Turbidity NTU	Conductivity mS/cm	Salinity %
1	1.1	9-Mar	14.11	8.62	11.8	---	2	1.26	0.05
1	1.2	11-May	30.68	7.35	20.9	6.83	18	1.4	0.06
1	1.3	16-Aug	13.64	7.79	18.9	4.38	9	1.26	0.05
2	2.1	10-Mar	23.68	8.76	13.4	6.4	6	1.25	0.05
2	2.2	11-May	34.80	7.88	21.6	7.33	45	1.39	0.06
2	2.3	16-Aug	dry	dry	dry	dry	dry	dry	dry
3	3.1	10-Mar	15.61	8.77	9.3	8.4	4	1.26	0.05
3	3.2	11-May	9.34	8.37	22.2	11.3	199	1.43	0.06
3	3.3	16-Aug	30.11	7.8	24	3.42	448	0.606	0.02
4	4.1	10-Mar	28.86	8.66	6.6	5.4	4	1.27	0.05
4	4.2	11-May	31.53	8.19	22.8	7.28	167	1.44	0.06
4	4.3	16-Aug	66.30	7.86	28.6	8.06	5	4.2	0.21
5	5.1	10-Mar	26.11	8.59	5.8	15.2	7	1.27	0.05
5	5.2	11-May	21.61	8.19	21.6	9.19	118	1.4	0.06
5	5.3	16-Aug	69.93	8.26	28.7	11.95	275	3.76	0.18
6	6.1	10-Mar	28.03	8.37	3.7	4.7	39	1.17	0.04
6	6.2	12-May	22.21	8.01	15.4	1.74	103	1.46	0.06
6	6.3	16-Aug	dry	dry	dry	dry	dry	dry	dry

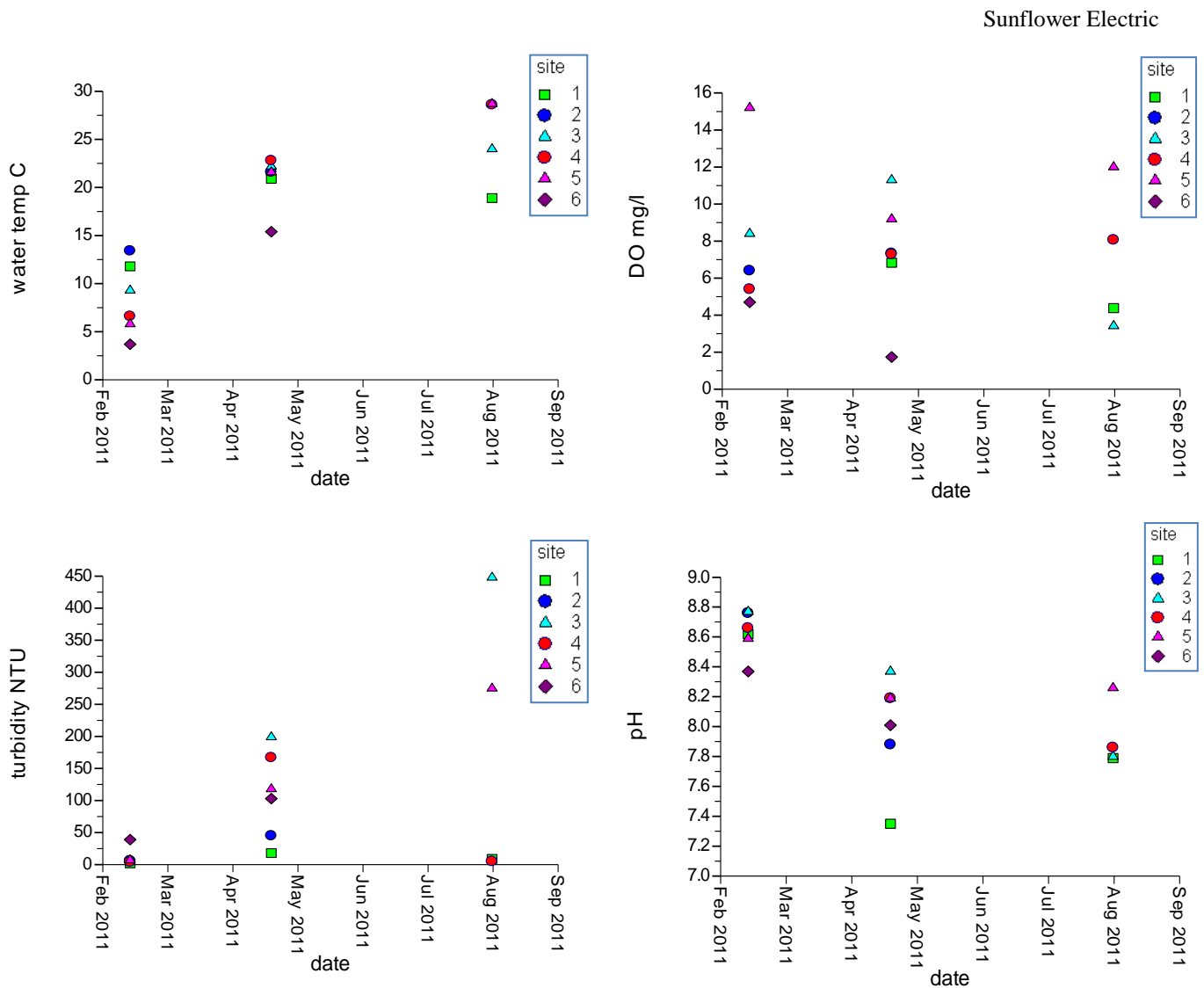


Figure 3. *In situ* water chemistry of each sampling event. Sites 1, 2, and 3 receive no water from the power plant; Sites 4, 5 and 6 receive the power plant discharge. Power plant came on-line in June.

Water: Selenium Concentrations

Detectable selenium concentrations were found in water samples taken at all sampling sites during each of the three sampling periods (See Appendix 1). For the most part, water concentrations of selenium were typically higher below the Sunflower Power discharge point at sampling Site 4 (Figure 4). While there was a visible difference in the mean concentration of selenium in stream water above and below, no statistically significant differences were noted when data from all dates were used in ANOVA testing (see below).

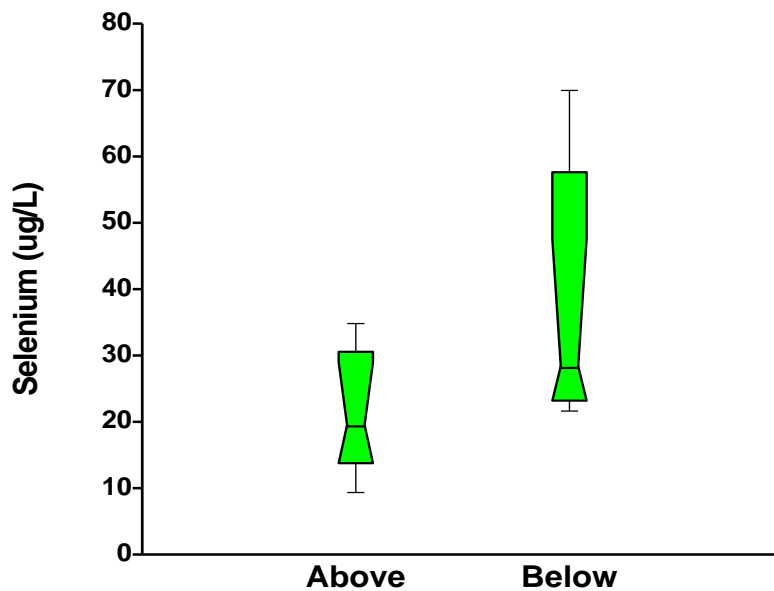


Figure 4. Boxplot of selenium water concentrations ($\mu\text{g/L}$) above and below treatment discharge for all months. Sites located above the Sunflower Power discharge point were Sites 1, 2 and 3 while Site 4 was immediately below the point to discharge and sites 5 and 6 were downstream of site 4.

A one-way ANOVA analysis of selenium water concentrations ($\mu\text{g/L}$) using water samples from all dates (i.e. March, May, and August) showed no significant difference ($p=0.0668$) between the two treatment groups (i.e. Above and Below groups). The one-way ANOVA results indicated the mean selenium water concentrations for the Above (21.49 ± 5.45) and Below (36.82 ± 5.45) groups did not overlap suggesting that while the p-value was slightly higher than the alpha value of 0.05, requiring the acceptance of the hypothesis of “no mean differences” these groups appear to differ if only slightly. This result may have been influenced by the inclusion of all sample dates – two of which were taken before the plant came on-line (i.e. pre-operational) and the last sampling date (August) represented a post-operation time period. Error bar plots were constructed to examine selenium concentrations in the water associated with each site (Figure 5) and post- and pre-operational time periods (August and March/May samples, respectively) above and below discharge point (Figures 6 and 7) because the limited number of water samples associated with each of the groupings precluded the use of boxplots.

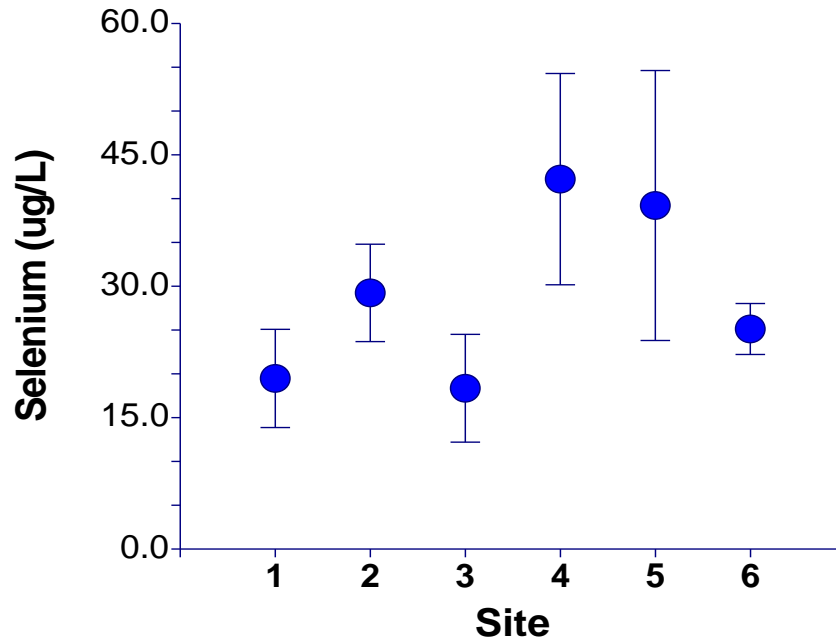


Figure 5. Error bar plots of mean selenium water concentrations ($\mu\text{g/L}$) and standard error in all six sites for all months.

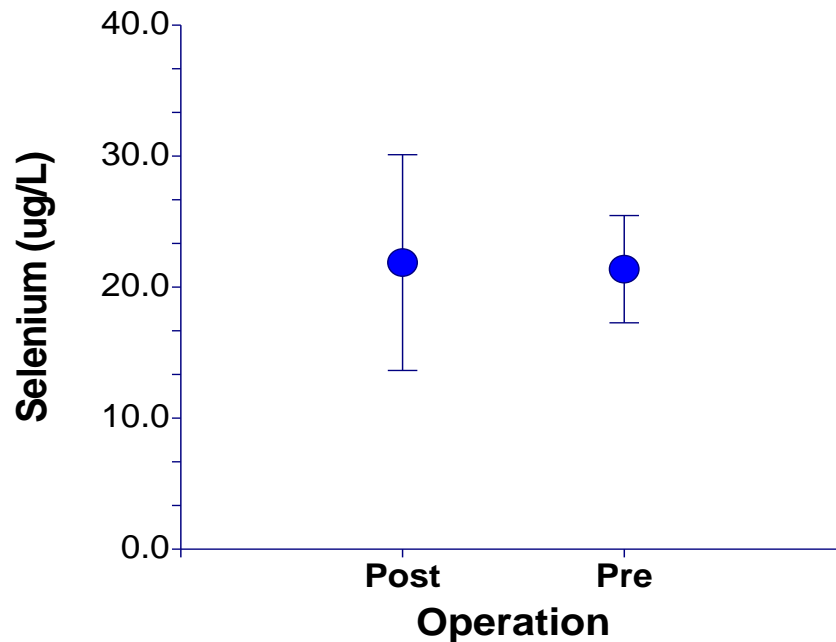


Figure 6. Error bar plots of mean selenium water concentrations ($\mu\text{g/L}$) pre and post operation *above* point of discharge.

Figure 5 clearly shows that all sites at or below the plant discharge point had elevated mean selenium concentrations but the standard error associated with nearly all site values suggests considerable overlap in values. Using all data for all months, the resulting error bar plots suggested

that there were no clear post- or pre-operational differences in selenium concentrations between these time periods above the discharge point (Figure 6). However, Sites 1-3 are never influenced by the Sunflower Power discharges regardless of whether the plant is in operation or not. Clearly both site location and sampling period have to be taken into account in order to identify possible impacts associate with plant discharges during operational time periods. In order to tease apart possible joint effects of location (above vs. below) and time (pre- vs. post-operational), an error bar plot (Figure 7) was constructed using only the data from the Below group of sites (i.e. Sites 4-6). This plot indicates that post operational values for selenium in the waters of the Below sites were greatly elevated during plant operation.

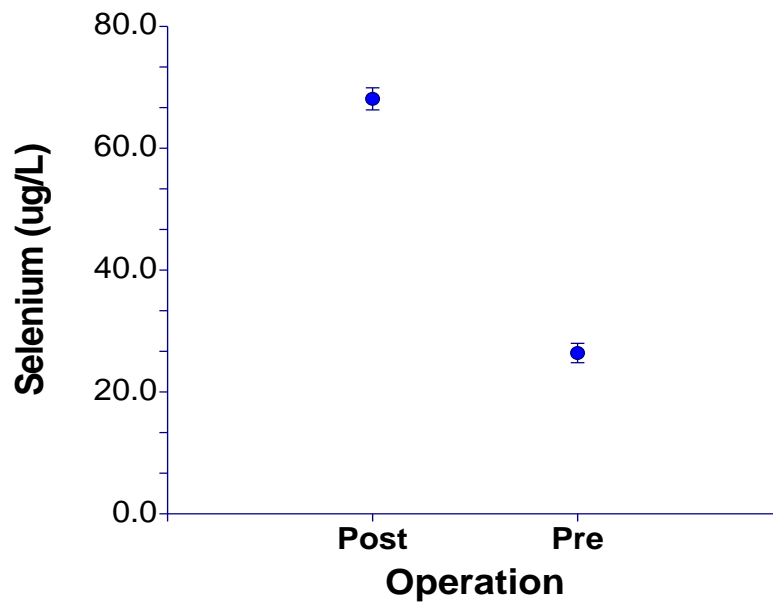


Figure 7. Error bar plots of mean selenium water concentrations ($\mu\text{g/L}$) pre and post operation *below* point of discharge.

This observation was supported by the results of one-way ANOVA analyses for selenium water concentrations ($\mu\text{g/L}$) for both the Above site group and the Below site group. The ANOVA using only the above sites (i.e. Above treatment) to test for pre-operational and post-operational differences was not significantly different ($p=0.9541$). However, the one-way ANOVA analysis for selenium water concentrations ($\mu\text{g/L}$) for the sites below the discharge point (i.e. Below treatment) indicated that

there was a significant difference in pre- and post-operational values different ($p \leq 0.0000$). Thus, it appears that selenium concentrations in stream water below the plant discharge point are much higher than waters above discharge but only during the period when the plant is in operation.

Sediment: Selenium Concentrations

Detectable selenium concentrations were found in sediment samples taken at all sampling sites during each of the three sampling periods (Appendix 1). For the most part, sediment concentrations of selenium were typically higher below the Sunflower Power discharge point at sampling Site 4 (Figure 8).

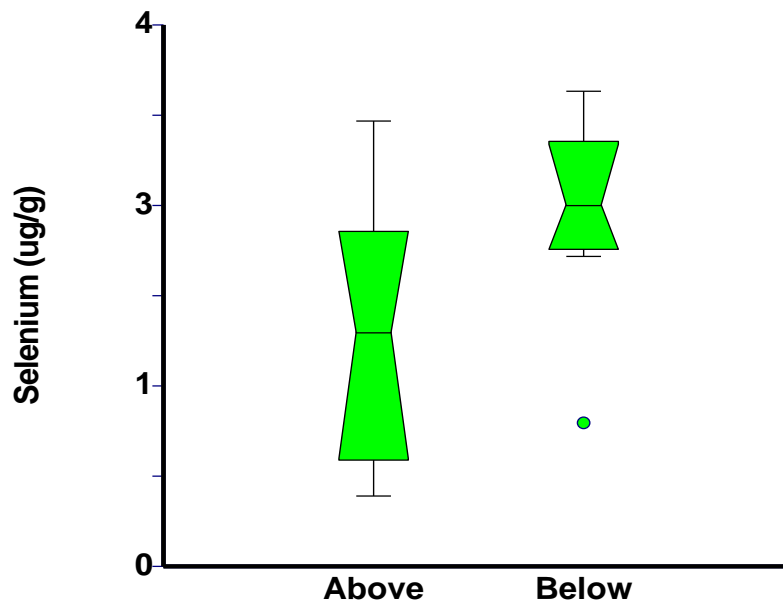


Figure 8. Boxplot of sediment selenium concentrations ($\mu\text{g/g}$) above and below the plant discharge point for all months.

This observation was confirmed by the results of a one-way ANOVA using all selenium sediment concentrations ($\mu\text{g/g}$) regardless of date collected when testing for Above or Below treatment differences ($p=0.0462$). Examination of site concentrations of selenium in stream bed sediments also suggested that most sites at or below discharge point were elevated regardless of the sampling period

(Figure 9). Sediment, unlike water, has a tendency to both retain selenium and to migrate downstream at a much slower time frame, thus allowing the selenium to remain elevated throughout the year.

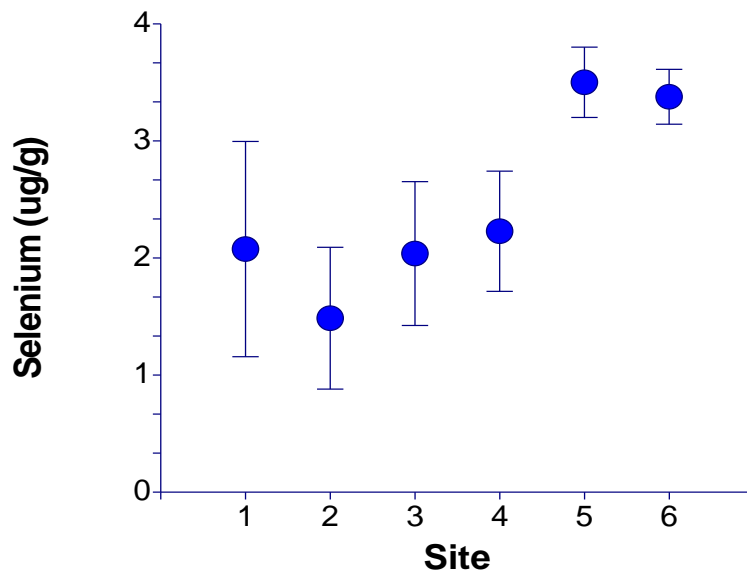


Figure 9. Error bar plots of mean selenium sediment concentrations ($\mu\text{g/g}$) and standard error in all six sites for all months.

Examination of selenium levels in sediments during post- and pre-operational periods for both Above and Below treatment groups showed similar relationships (Figures 10 and 11). It appears that post-operational sediment concentrations are always lower than pre-operational concentrations although a simple explanation for this seems to be lacking. One-way ANOVA results testing for post- and pre-operational effects on sediment concentrations for both the Above treatment group and Below treatment group were both non-significant yielding p-values of 0.0888 and 0.7333, respectively. While this is clearly the case for the Below treatment group, the differences in the Above treatment group appear to be distinctly different despite the slightly high p value associated with the ANOVA test.

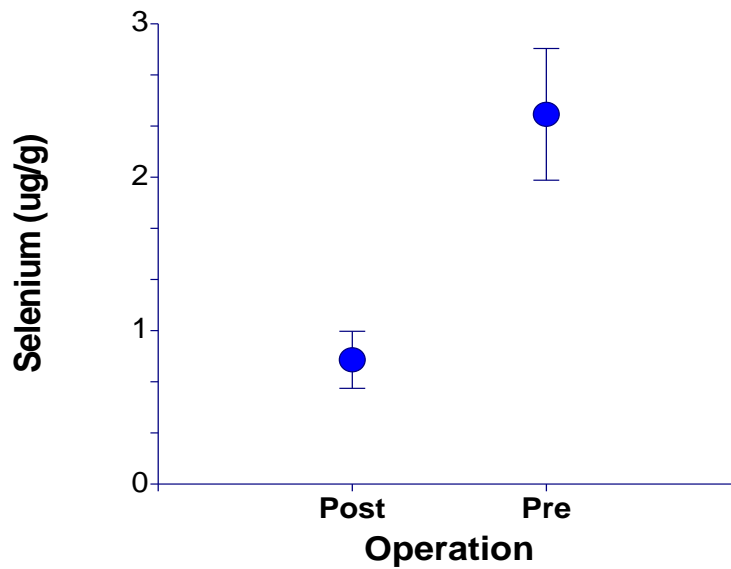


Figure 10. Error bar plots of mean selenium sediment concentrations ($\mu\text{g/g}$) pre and post operation *above* point of discharge.

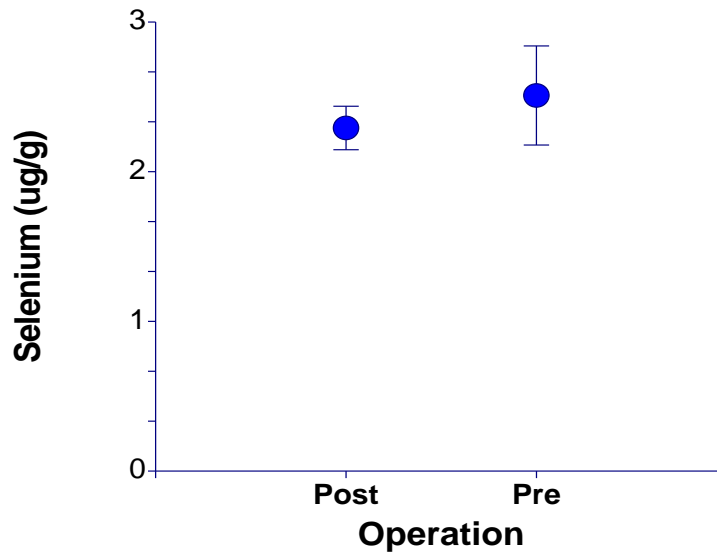


Figure 11. Error bar plots of mean selenium sediment concentrations ($\mu\text{g/g}$) pre and post operation *below* point of discharge.

Fish Tissue: Selenium Concentrations

Detectable selenium concentrations were found in most fish collected at all sampling sites during each of the three sampling periods (Appendix 2). For the most part fish body burdens of

selenium were not much different in fish taken below and above the Sunflower Power discharge point at sampling Site 4 regardless of time period (i.e. post- and pre-operational). Boxplots illustrating potential treatment and temporal effects (Figure 12) suggest minimal overall differences in the median values of treatment and temporal groups when all fish species were used in the construction of the boxplots.

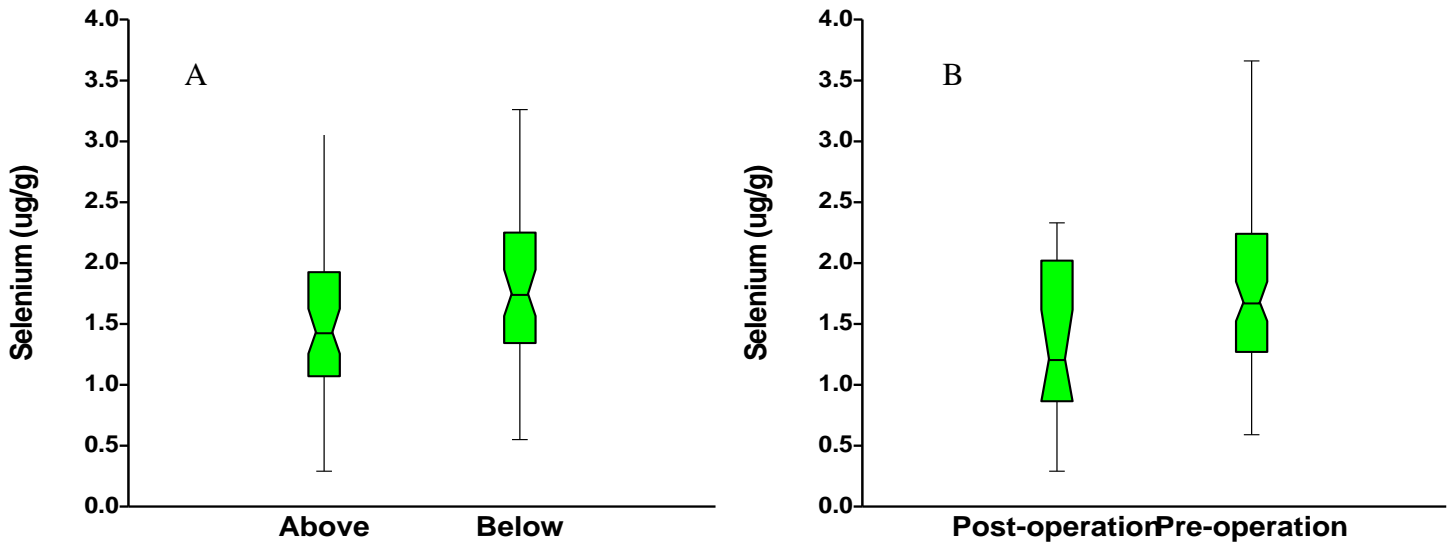


Figure 12. Whole fish body burden concentration for all fish associated with above and below sites (A) and pre- and post-operational (B) dates.

Black bullhead body burden concentrations of selenium showed a similar relationship as did all fish results when examining both treatment and operational period relationships (Figure 13). That is, fish concentrations tend to be higher below the plant discharge than above, but less during the post operational period. However, the limited number of black bullhead specimens taken during the post-operational period prevented the construction of a meaningful boxplot for this group.

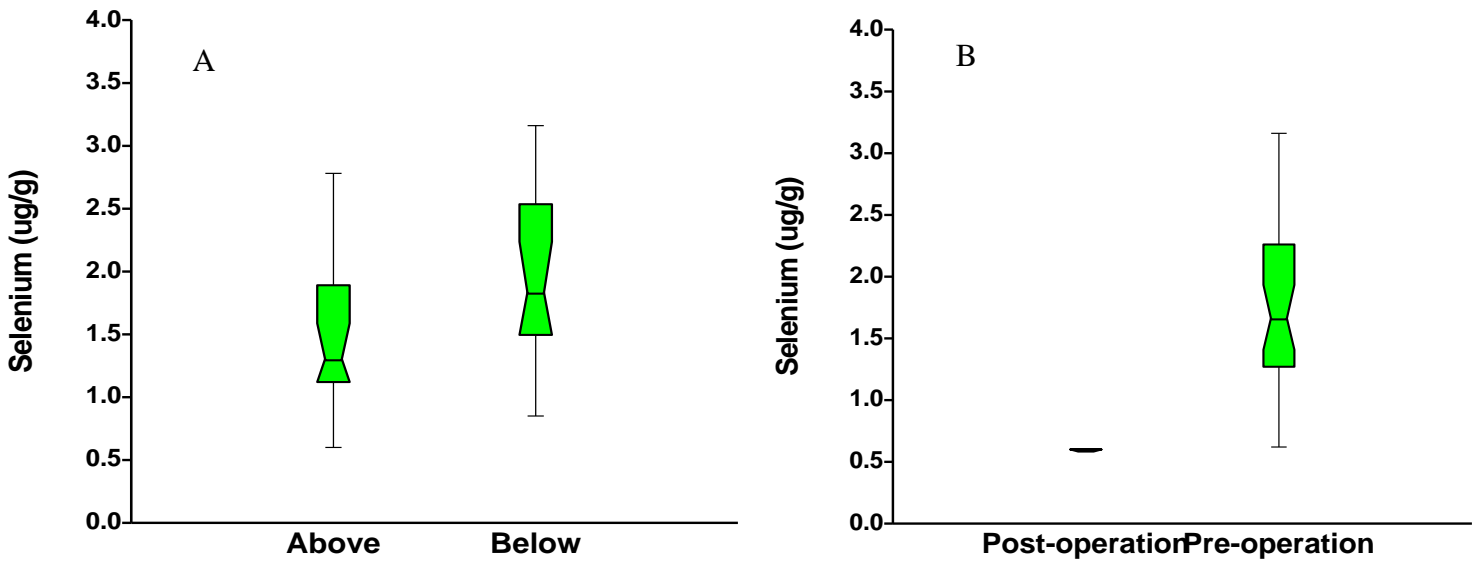


Figure 13. Whole fish body burden concentration for black bullhead associated with above and below sites (A) and pre- and post-operational (B) dates.

Whole body burdens of selenium found in green sunfish were very similar in both above and below discharge groups (i.e. treatments) while post-and pre-operational difference appeared to differ when all dates were used in constructing boxplots (Figure 14).

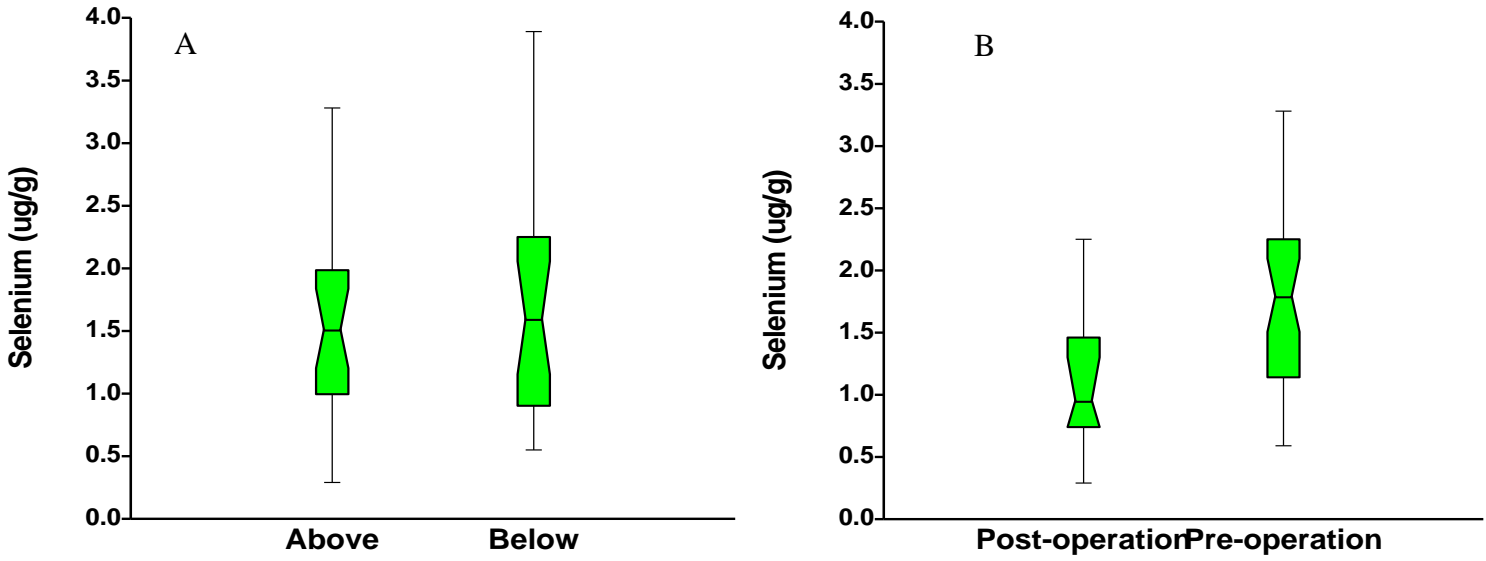


Figure 14. Whole fish body burden concentration for green sunfish associated with above and below sites (A) and pre- and post-operational (B) dates.

The last species examined for whole body burdens was the red shiner, a small minnow species also found throughout Kansas. This species did not seem to display any treatment or temporal differences when all data were again used in developing the boxplots of their relationships (Figure 15).

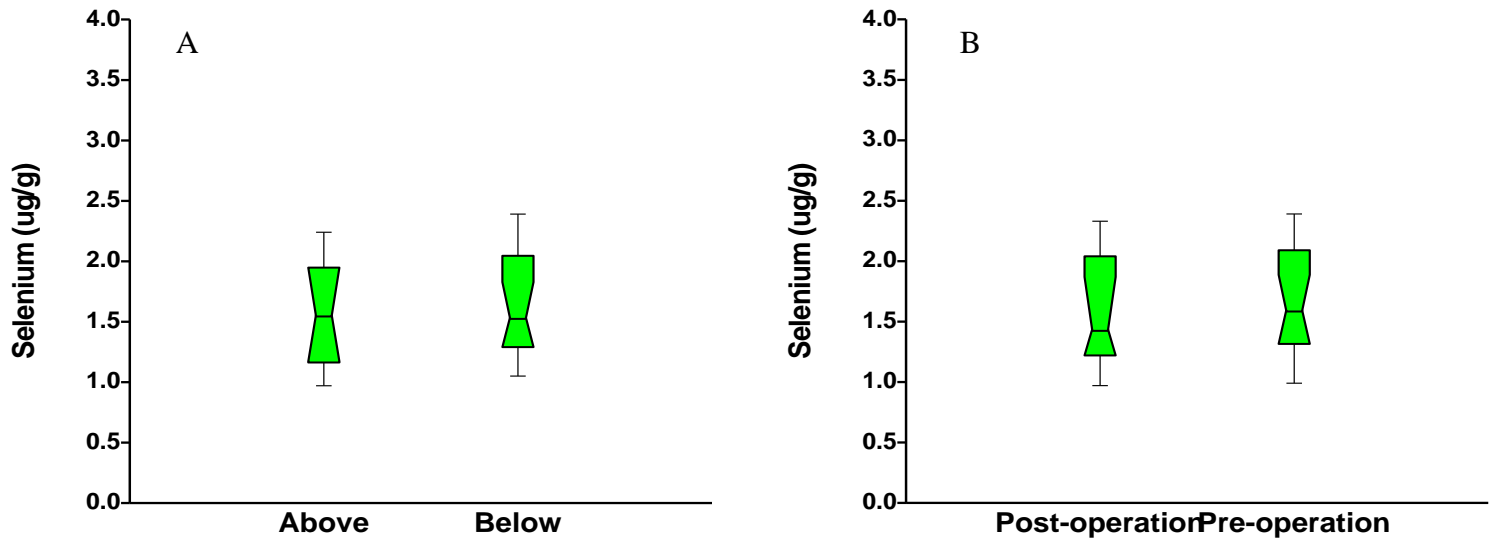


Figure 15. Whole fish body burden concentration for red shiners associated with above and below sites (A) and pre- and post-operational (B) dates.

In order to identify potential fish body burden differences associated with the Sunflower Power plant discharge, post- and pre- treatment concentrations were examined separately: first, for Above treatment sites and second, for sites located below the discharge point (i.e. Below treatment group). Then, the pre-operational data (i.e. March and May data) for all fish species was plotted by Above and Below groups; there appeared to be no distinct differences in selenium body burden values (Figure 16). However, when the post-operational data (i.e. August) data for all fish were graphically examined, fish body burden values for the Below group appeared to be much higher (Figure 16).

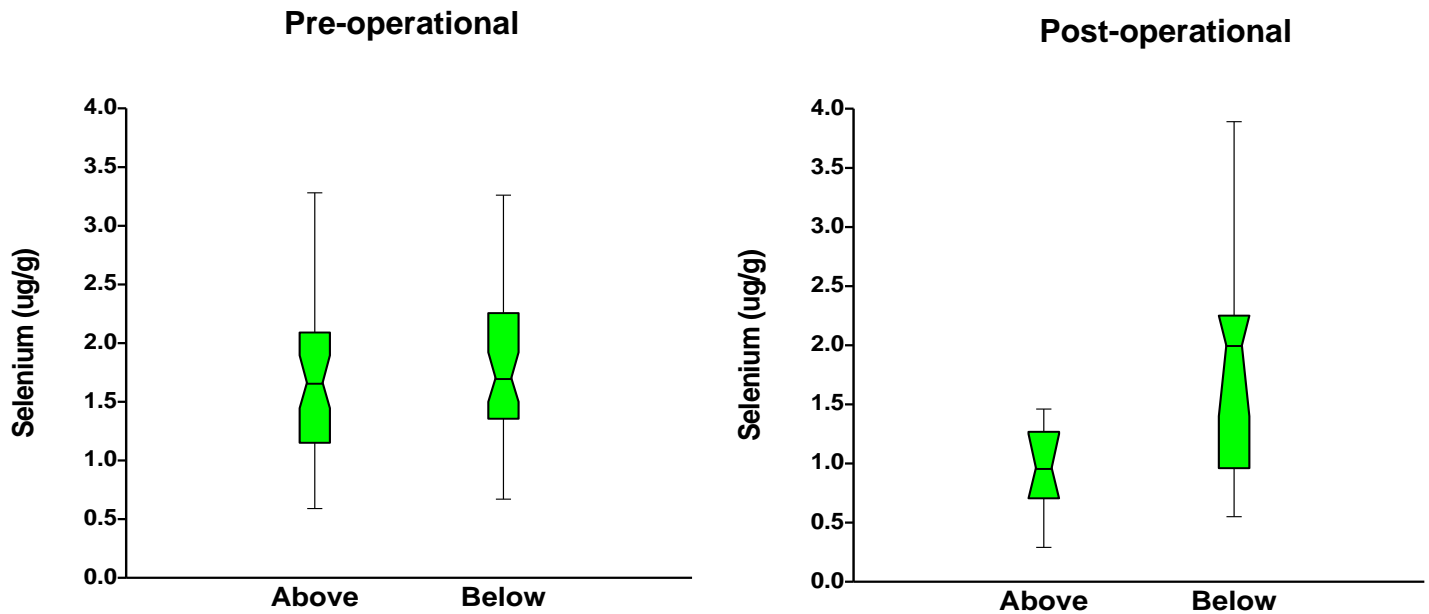


Figure 16. Whole fish body burden concentration for all fish associated with above and below sites for pre- and post-operational dates.

These observations were then examined by one-way ANOVA tests using the same two operational time periods to examine the selenium concentrations for all fishes in the Above and Below treatment groups. As noted, in the pre-operational period there was no significant differences between Above and Below group means ($p = 0.4522$), while significant body burden levels were noted in fish tissues in the Below group during the post-operational time period ($p = 0.0195$). Because so few black bullheads were taken during the post-operational time period only a pre-operational boxplot of selenium values could be produced (Figure 17). This boxplot for pre-operational values also showed slightly elevated levels in the Below group, but these differences could not be statistically tested due to the small sample size for bullheads in the Below group.

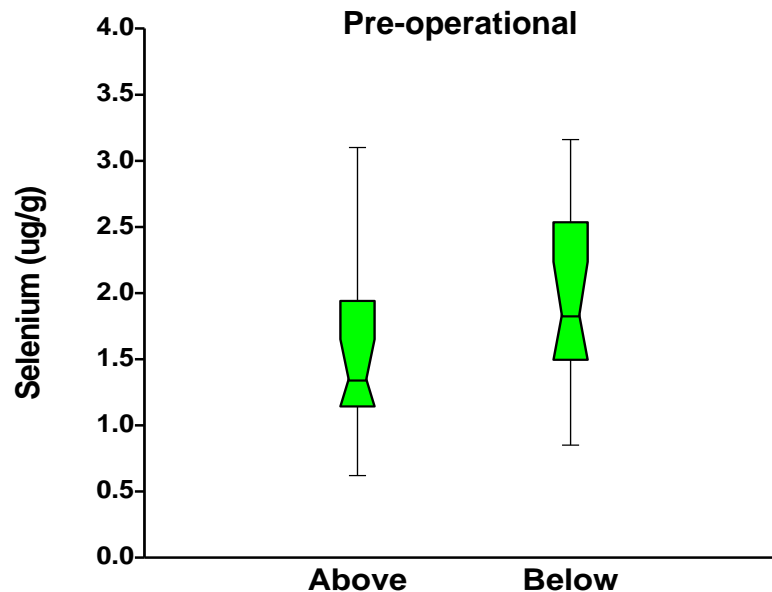


Figure 17. Whole fish body burden concentration for black bullheads associated with above and below sites for pre-operational dates. Only one bullhead was collected in each of the above/below site groups during post-operational sampling thus no box plot could be constructed for the post operational period.

Red shiner body burdens were also plotted separately for both pre- and post-operational time periods to assess possible treatment differences within these different time frames. The pre-operational boxplot showed like difference in body burden for red shiners during this time period (Figure 18) while post-operational body burdens were clearly higher in the Below group compared to the Above group of red shiners (Figure 18). These visual interpretations were verified when ANOVA tests were run on both the pre-operational time period which was non-significant for treatment differences ($p = 0.6735$) and the post-operational period which had significant treatment differences ($p = .0455$). The median body burden concentrations for red shiners in the Below group of fish was nearly double the median valued for the Above group during this operational time frame.

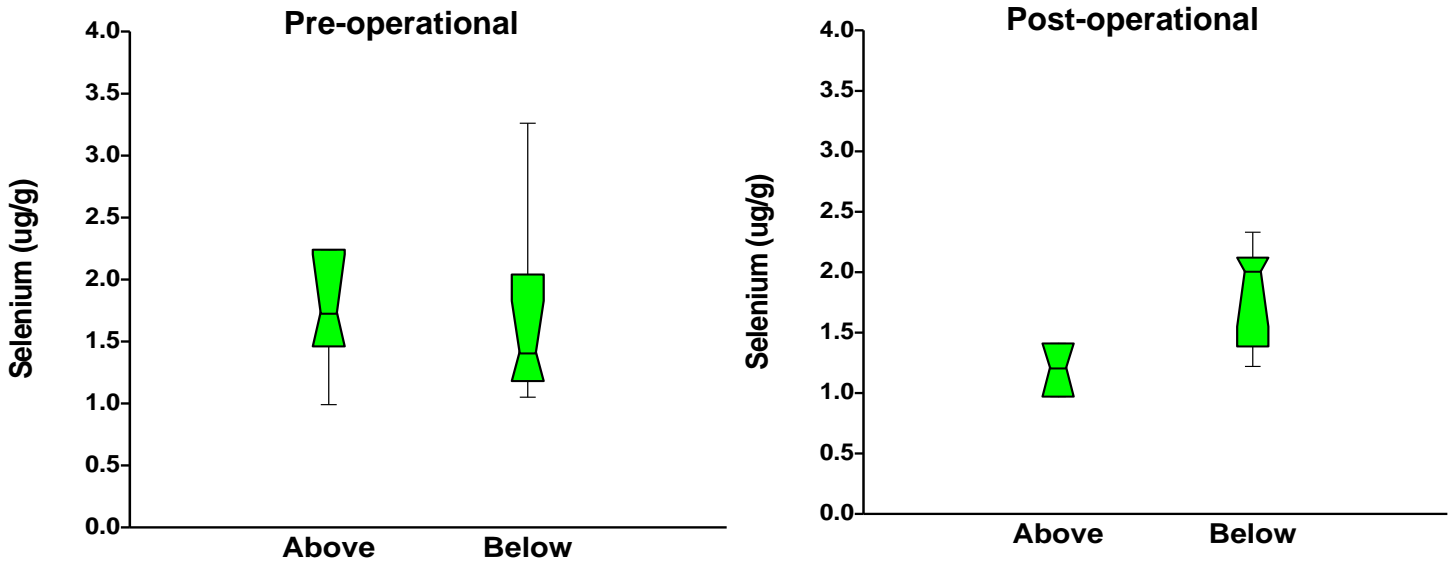


Figure 18. Whole fish body burden concentration for red shiners associated with above and below sites for pre- and post-operational dates.

Green sunfish body burden values were neither visually different (Figure 19) nor statistically different when ANOVA test were performed on treatments during the pre-treatment and post-treatment time periods ($p = 0.6777$ and 0.2291 , respectively).

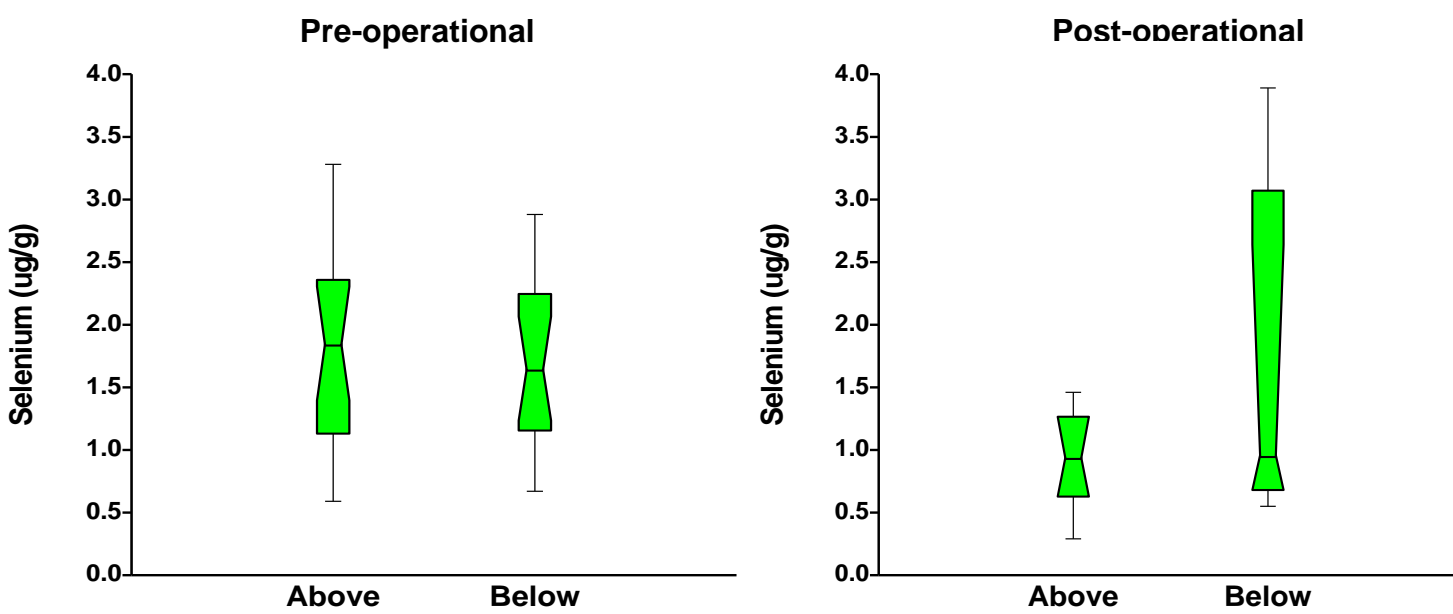


Figure 19. Whole fish body burden concentration for green sunfish associated with above and below sites for pre- and post-operational dates.

Fish Eggs: Selenium Concentrations

Few green sunfish and black bullhead females with egg masses were found during this study and no egg masses were found in red shiner females (Appendix 3). Thus, comparisons of egg masses concentrations were limited to examination of all species (i.e. black bullheads and green sunfish) and just green sunfish since too few egg masses were collected from black bullheads in the Below group. Boxplots of egg masses concentrations for each treatment group (i.e. Above and Below) using all data showed that egg mass concentrations of selenium was elevated in both the ‘all fish’ category and in green sunfish (Figure 20).

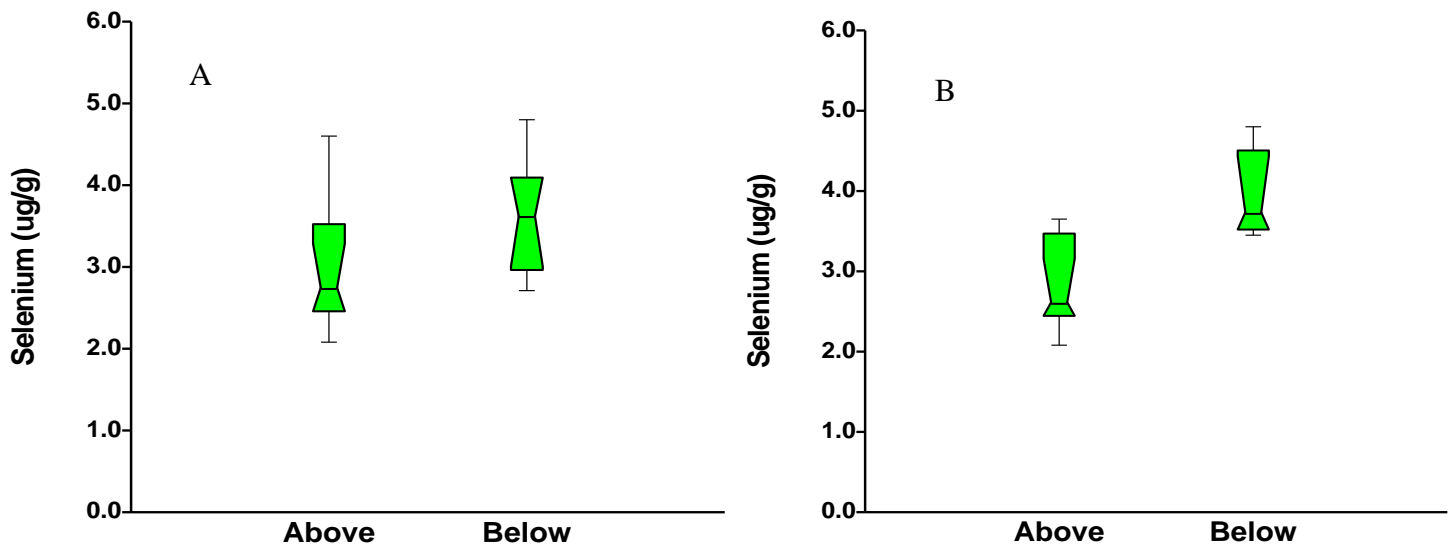


Figure 20. Egg mass concentration of selenium for all fish (A) and green sunfish (B) associated with above and below sites. Egg mass data for all dates were used in construction of these box plots.

One-way ANOVA results for the all fish egg mass concentrations indicated that there was no significant differences between Above and Below treatment groups ($p = 0.0631$). This p-value was just slightly greater than the alpha 0.05 level used in this study to determine significance. Then, green sunfish egg mass values were tested using the same ANOVA procedure significant differences in selenium concentrations were noted between Above and Below egg masses ($p = .0081$). That is green sunfish egg masses below the plant discharge had elevated levels of selenium when compared to egg

masses from green sunfish collected in sites above the plant discharge. The following discussion is based on these specific findings. In general, results indicated that selenium in water, sediment and fish tissues were elevated in Below sites mostly during the post-operational time frame. In addition, fish egg mass concentrations of selenium were higher in green sunfish found below the plant discharge. Overall, elevated selenium levels in the receiving water body (and fish) that can be attributed to plant discharges seems to be limited to post-operational time periods.

DISCUSSION

Water

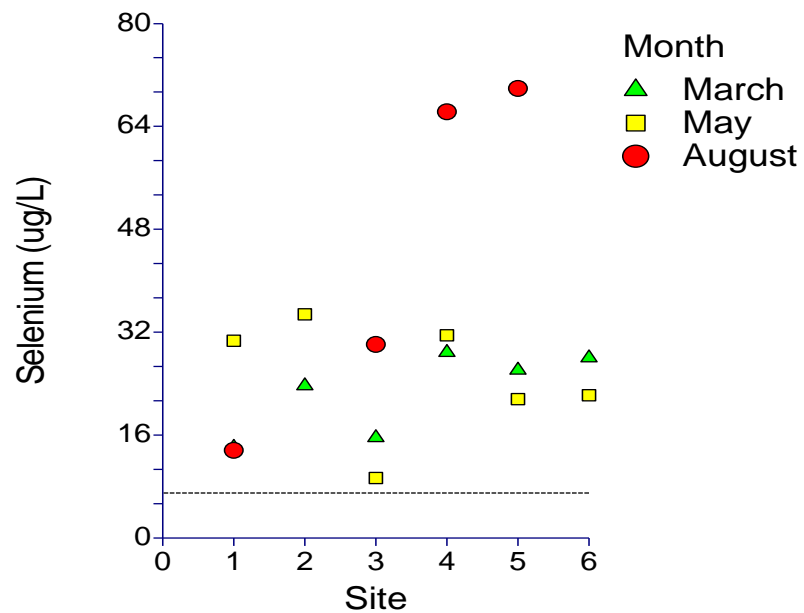


Figure 21. Scatter plot of selenium water concentrations ($\mu\text{g/L}$) and site numbers for all months. The dashed line represents the State of Kansas's daily discharge limitation of $7\mu\text{g/L}$.

Freshwater usually has background selenium concentration of $0.1 - 0.4 \mu\text{g/L}$ (DOI 1998).

Nevertheless, selenium levels in the water column at sites upstream and downstream of Great Bend Station discharge point all exceed the daily discharge limitation of $7\mu\text{g/L}$ set by the State of Kansas (Figure 21). The high selenium water levels at Sites 1, 2, and 3, which are above the discharge point, are not related to the Great Bend Station discharge. However, Figure 21 illustrates that after the plant

went online in June, selenium water concentrations during August were much higher at Sites 4 and 5, which are below the point of discharge.

Sediment

Selenium concentration in sediment equal to or greater than 4 mg/kg dw is a concern because there is a potential for bioaccumulation in fish and wildlife (Lemly and Smith 1987). The baseline selenium concentration in soil of the western United States is 0.23 mg/kg (Whitmer 2000). All sediment samples fell below 4 mg/kg and thus selenium levels in sediment within the study area are not likely to impose potential risk to aquatic life (Figure 22). It is important to note, however, that sediment samples collected in August display higher selenium concentrations below the discharge point, specifically at Sites 4 and 5. The plant went online in June, and thus the increase in selenium concentrations in sediment could possibly be a result of the discharge from the Great Bend Station.

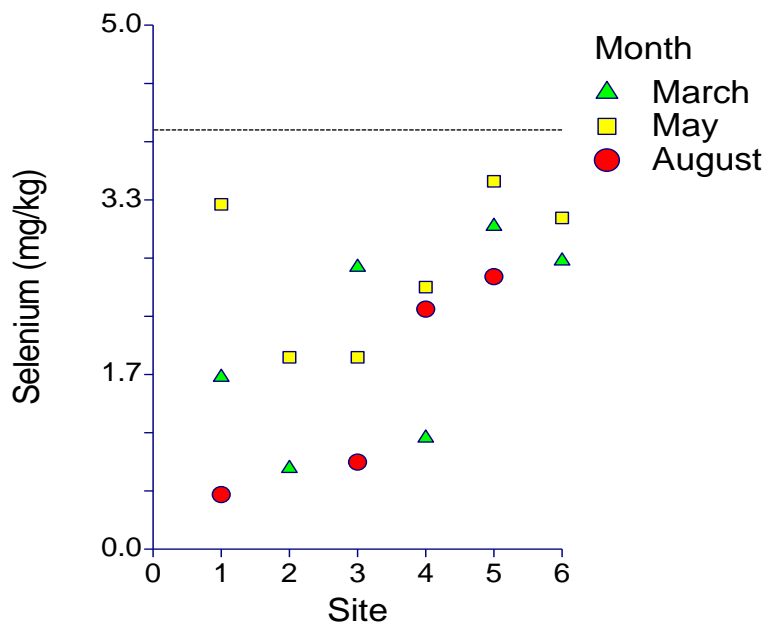


Figure 22. Scatter plot of selenium sediment concentrations (mg/kg) and site numbers for all months. The dashed line represents sediment concentration of 4mg/kg above which the potential for selenium bioaccumulation is a concern.

Fish

Due to the bioaccumulative properties of selenium, the proposed chronic criterion is expressed as selenium concentration in fish tissue rather than concentration in water because fish tissue samples provide a better indicator of the presence of selenium in a particular waterbody. Fish move throughout a waterbody and contaminants such as selenium can be absorbed into their tissue. Thus, fish tissue effectively reflects the level and duration of a particular contaminant in a waterbody over time. Most fish species have whole-body selenium concentrations of less than $4\mu\text{g/g}$ (DOI 1998). The EPA fish tissue-based criterion proposes that if selenium in whole-body fish tissue samples exceeds $5.85\mu\text{g/g}$ dry weight (dw) during summer or fall, fish should be monitored during winter to determine if selenium exceeds $7.91\mu\text{g/g}$ dw.

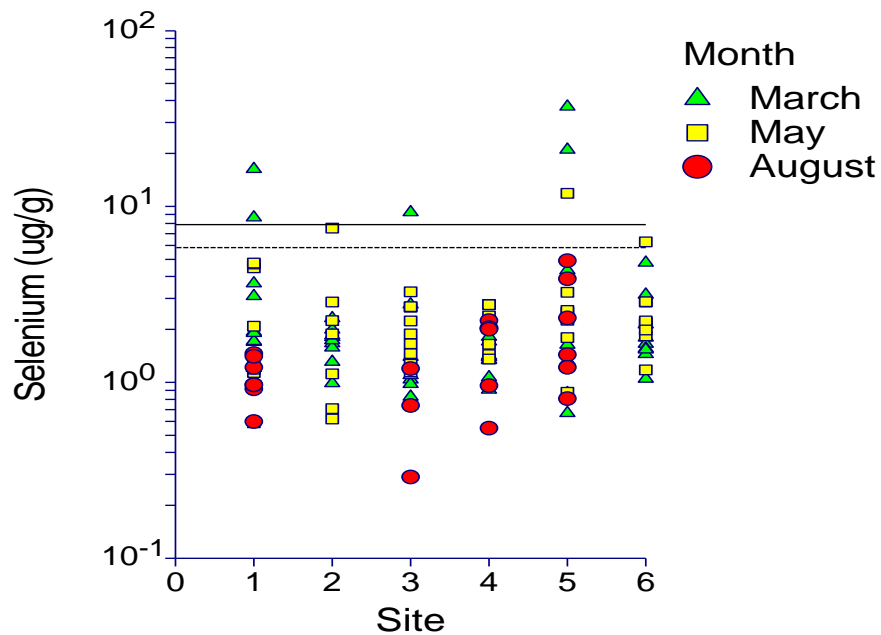


Figure 23. Scatter plot of selenium fish concentrations ($\mu\text{g/g}$) at each study site for all months. The dashed line represents selenium concentration of $5.85\mu\text{g/g}$ dry weight (dw) and the solid line represents selenium concentration of $7.91\mu\text{g/g}$ dw.

Based on Figure 23, only 8 out of 124 fish samples exceed the proposed selenium concentrations of $5.85\mu\text{g/g}$ dw and $7.91\mu\text{g/g}$ dw. None of the fish samples collected in August exceed the set concentrations, but March and May experienced higher selenium concentrations at Site 5 than

any other site even though the plant was offline during those two months. These results indicate that bioaccumulation of selenium is not occurring in fish at the six sites when all fish species are considered regardless of plant operation and downstream or upstream of discharge site.

However, when the data are analyzed based on fish species, red shiner shows possible bioaccumulation (Figure 24). Of the 39 samples of red shiner, eight contained selenium levels above the EPA criterion $5.86\mu\text{g/g dw}$. None of the eight samples with selenium concentrations above the criterion were from the month of August, but rather from March and May. Figure 15 displays that the body burden levels for red shiners almost doubled in the Below sites once the plant went online, even though the selenium concentrations for red shiners in August were all below the criterion levels. The size of the red shiner samples could be a factor for the lower selenium concentrations observed in August.

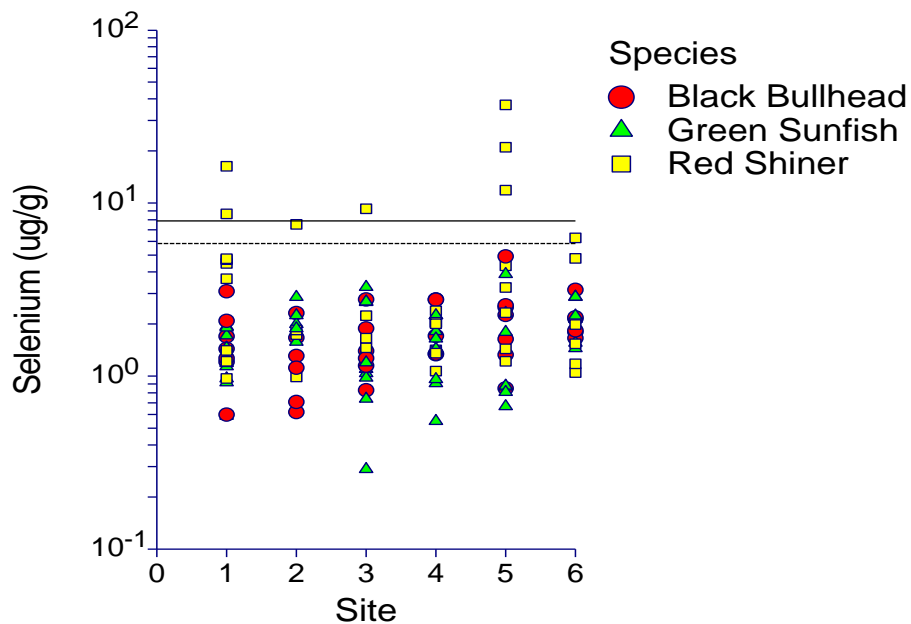


Figure 24. Scatter plot of selenium fish concentrations ($\mu\text{g/g}$) and site numbers for the three fish species. The dashed line represents selenium concentration of $5.85\mu\text{g/g dw}$ and the solid line represents selenium concentration of $7.91\mu\text{g/g dw}$.

Of the three fish species analyzed in this study, red shiner samples collected in March and May were the smallest in length and weight; August red shiner samples were larger and weighed more. A study conducted by USGS suggests that selenium concentrations were observed to be higher in smaller

fish samples (May *et al.* 2008). Specifically, the study concluded that selenium concentrations decrease at larger sizes in channel catfish. Figures 25 and 26 display a possible relationship between decreasing weight and length and increasing selenium concentrations in fish tissue. Of the 8 samples of red shiners that were above the $5.85\mu\text{g/g}$ criterion, 7 samples weighed less than any of the samples collected in August, and also contained the highest body burden levels. Such a pattern also exists for length, where 6 of the 8 samples are above the criterion and are also relatively smaller in length than any of the August red shiner samples. The findings from the USGS publication support other literature in regards to the presence of higher selenium concentrations in fish species that feed on aquatic insects or invertebrates. Red shiners are known to feed on aquatic and terrestrial invertebrates (Herrington 2008).

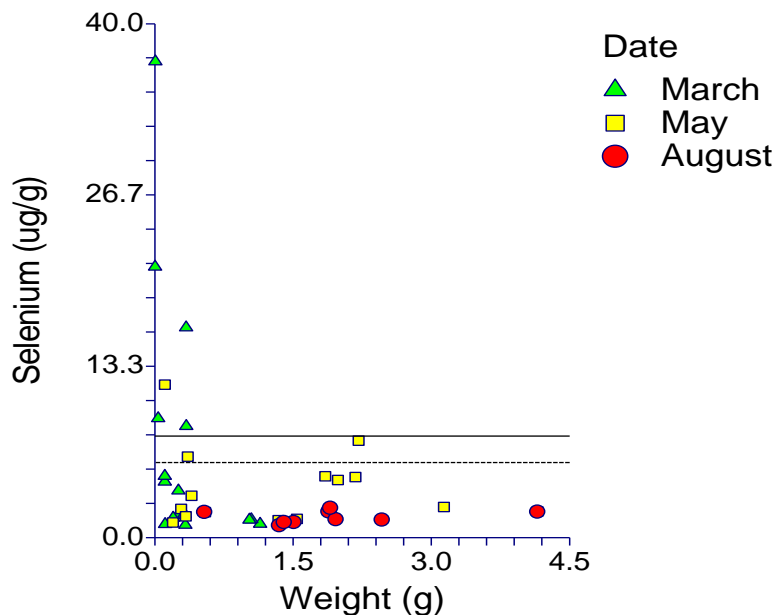


Figure 25. Scatter plots of selenium fish concentrations for red shiners and weight (g). The dashed line represents selenium concentration of $5.85\mu\text{g/g}$ dry weight (dw) and the solid line represents selenium concentration of $7.91\mu\text{g/g}$ dw.

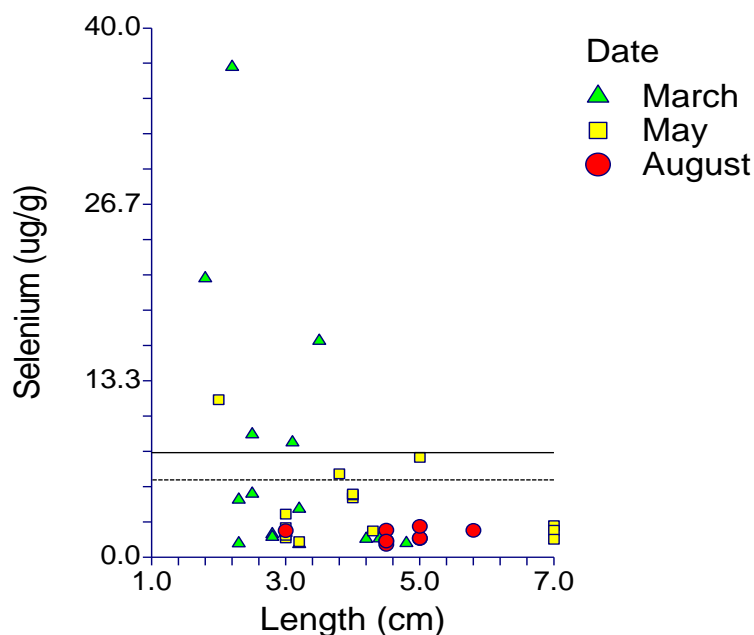


Figure 26. Scatter plots of selenium fish concentrations for red shiners and length (cm). The dashed line represents selenium concentration of $5.85\mu\text{g/g}$ dry weight (dw) and the solid line represents selenium concentration of $7.91\mu\text{g/g}$ dw.

Length and weight correlations with selenium concentrations were analyzed by using data reported in a Arkansas River study conducted by Kansas Biological Survey in 2005 (Huggins and Lim 2005). Scatter plots (Figures 27 and 28) indicate possible relationships between both weight and length and selenium body burden values. The highest selenium concentrations for all three months occur at the lower spectrum of the scale for both instances (weight and length). The same observations can be made for the data analyzed from the Solomon River study conducted by KBS in 2009 (Koontz *et al.* 2009). Again scatter plots (Figures 29 and 30) suggest that higher selenium values occurred in small fish specimens (e.g. lower weight and smaller length). It is not conclusive whether fish weight and length seem to influence the selenium concentrations in the whole-body samples for all three studies. However, the graphs clearly indicate that some of the fish samples with the highest selenium values were composed of the smaller fish, in regards to both weight and length.

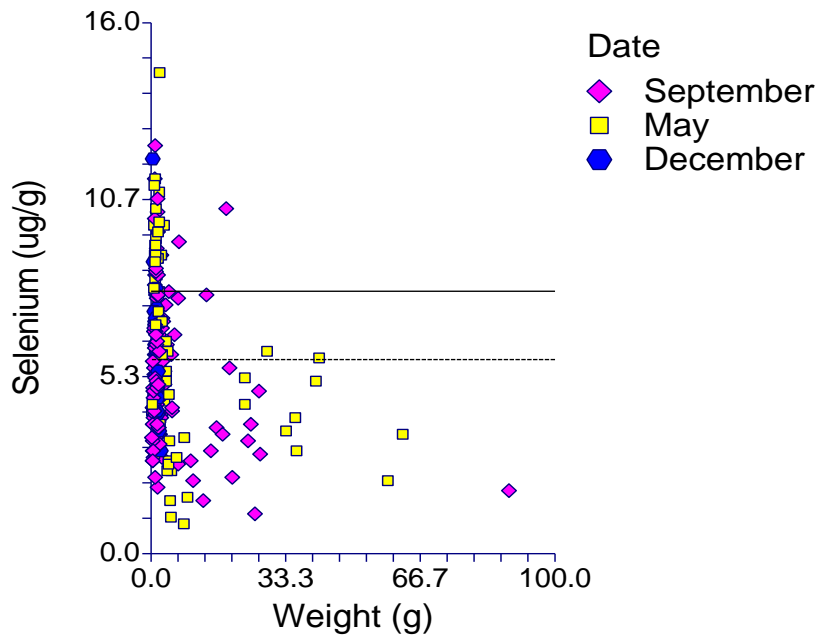


Figure 27. Scatter plots of selenium fish concentrations for all fish species and weight (g) from the Arkansas River study conducted by KBS. The dashed line represents selenium concentration of 5.85 μ g/g dry weight (dw) and the solid line represents selenium concentration of 7.91 μ g/g dw.

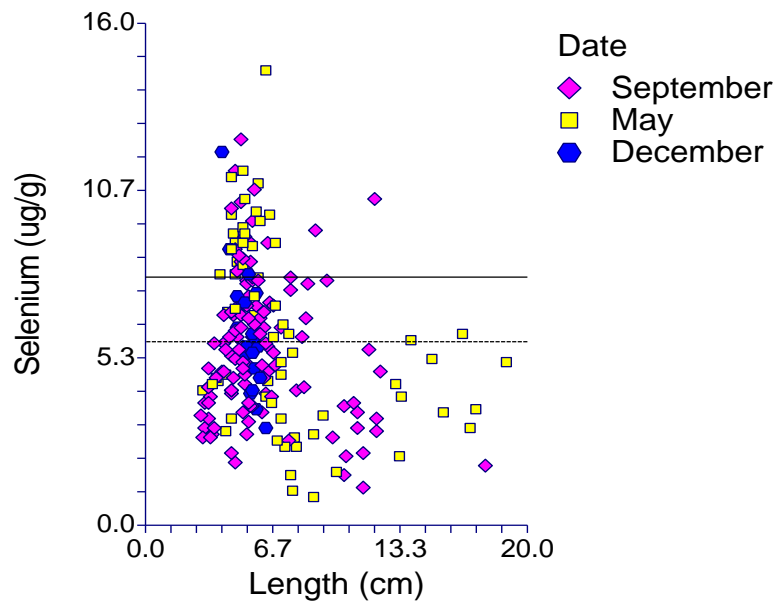


Figure 28. Scatter plots of selenium fish concentrations for all fish species and length (cm) from the Arkansas River study conducted by KBS. The dashed line represents selenium concentration of 5.85 μ g/g dry weight (dw) and the solid line represents selenium concentration of 7.91 μ g/g dw.

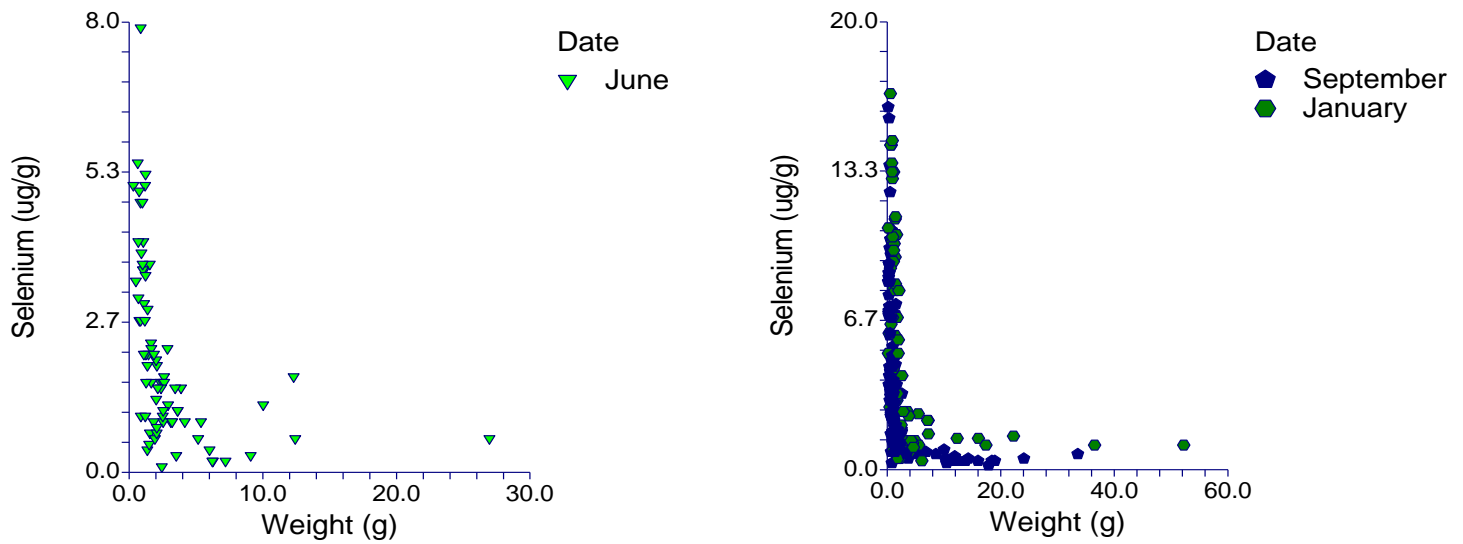


Figure 29. Scatter plots of selenium fish concentrations for all fish species and weight (g) from the Solomon River study conducted by KBS.

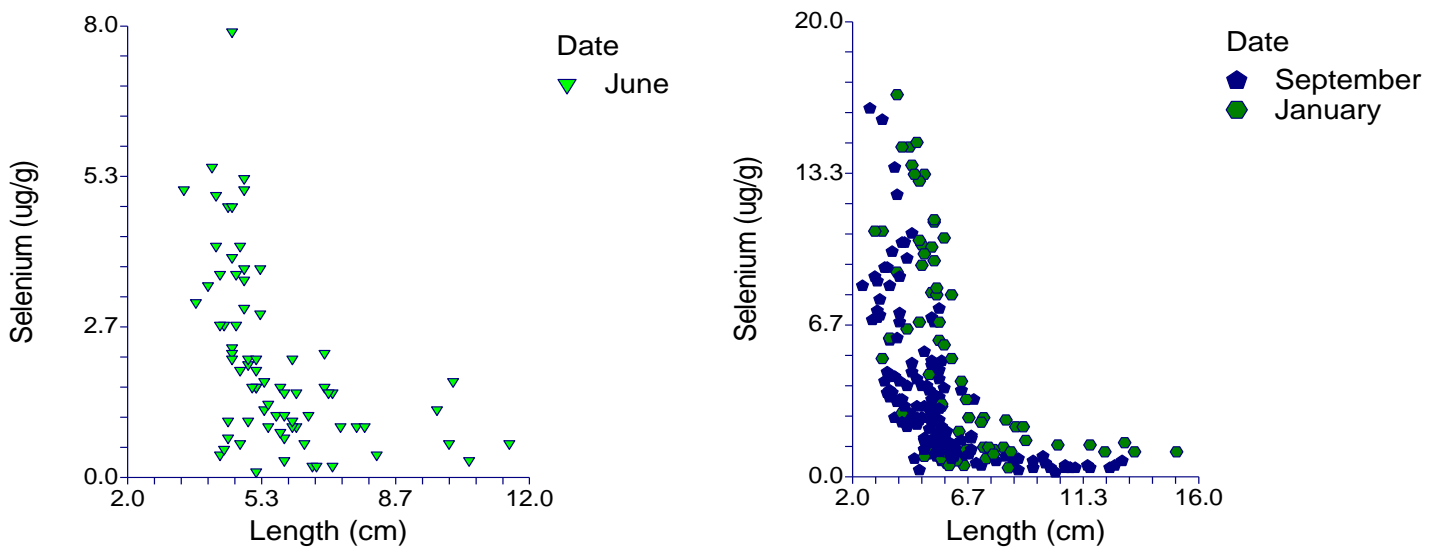


Figure 30. Scatter plots of selenium fish concentrations for all fish species and length (cm) from the Solomon River study conducted by KBS.

Data from the three studies (Arkansas, Solomon and Great Bend) were combined to statistically test for possible relationships between selenium concentrations in fish and fish size. Robust linear regression was performed using selenium as the dependant and weight as the independent variable and resulted in a significant linear regression model ($R^2 = 0.22$, $P = 0.00$, $DF = 643$) (Figure 31). Weight

explained approximately 22% of the variance in the selenium fish tissue concentrations among all fish species, regardless of study, site, and date. A similar significant model was found for length vs. selenium with ($R^2 = 0.18$, $P = 0.00$, $DF = 646$) (Figure 32). Length explained approximately 18% of the variance in the selenium fish tissue concentrations among all fish species, regardless of study, site, and date. In this study, red shiners displayed significant differences between sites. Therefore, red shiner selenium concentrations from Arkansas and Solomon studies were analyzed along with Great Bend data to increase sample size. Both, weight ($R^2 = 0.016$, $P = 0.20$, $DF = 101$) and length red shiner models ($R^2 = 0.072$, $P = 0.005$, $DF = 104$) were non-significant when sampling date, site, and study are excluded.

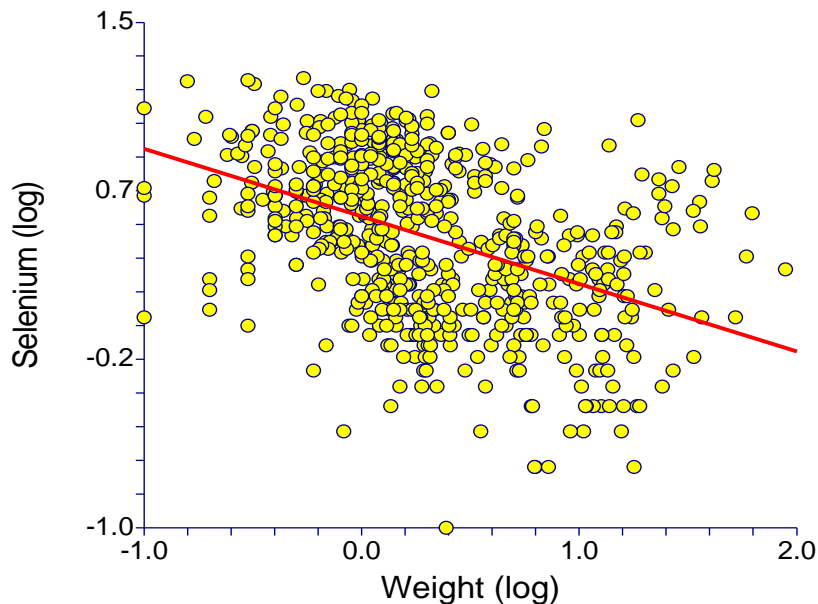


Figure 31. Scatter plot of fish tissue selenium concentration over weight with robust linear regression, NCSS.

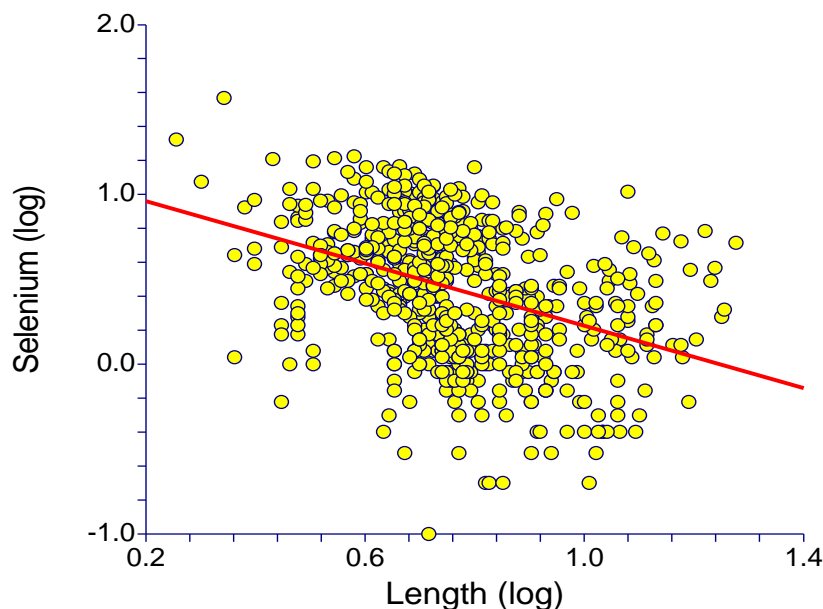


Figure 32. Scatter plot of fish tissue selenium concentration over length with robust linear regression, NCSS.

Fish Eggs

Selenium concentrations in eggs is related to larval fish deformities and thus may be a better indicator of potential negative impacts of selenium exposure than whole body burden concentrations (DeForest *et al.* 2011, Ohlendorf *et al.* 2011, WVDEP 2010). Selenium bioaccumulation can lead to reproductive failure, mortalities of eggs and embryos, and embryonic deformities and malformations in various species of fish. Teratogenic deformities can occur due to excessive selenium levels in fish eggs and can cause the deposition of selenium in developing eggs (Lemly 1997). These deformities can occur in fish eggs consisting of selenium concentrations exceeding $10\mu\text{g/g}$ (Lemly 1997). Figure 33 reveals that neither green sunfish eggs nor black bullhead eggs contain selenium levels above $10\mu\text{g/g}$. Green sunfish eggs from Site 5 did contain the highest selenium concentrations when compared to Site 1 and 2, but these are representative of months March and May and not of August due to the absence of eggs in the collected samples.

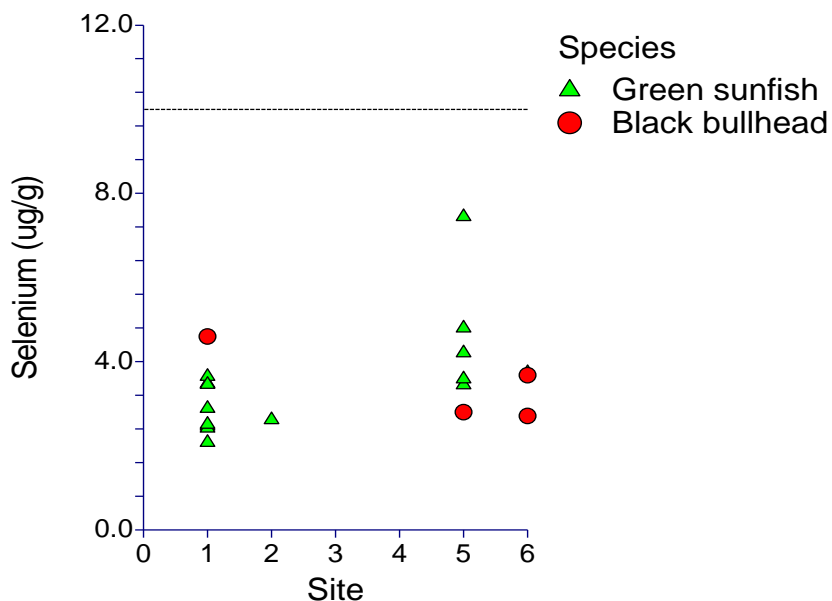


Figure 33. Scatter plot of selenium egg concentrations for green sunfish and black bullhead species. The dashed line represents selenium concentration of $10\mu\text{g/g}$, above which prevalence of teratogenic deformities increase.

In order to account for the absence of enough egg mass samples for proper selenium analysis, whole body fish selenium levels were used to calculate approximate selenium levels in eggs. Lemly's "Protocol for Aquatic Hazard Assessment" provides an estimation protocol which allows for the conversion of whole body fish selenium residues to calculate egg concentrations using the formula: fish egg selenium = fish whole body selenium \times 3.3 (Lemly and Smith 1987; Skorupa *et al.* 1996). This protocol can predict the aquatic hazard for reproductive impairment based on fish eggs. Therefore, all the whole body fish selenium values were converted to approximate fish egg concentrations and presented in Table 5. Fish egg concentrations range from 5.9 to $15.5\mu\text{g/g}$ selenium. The maximum fish egg concentrations found in all three species range from 16.2 to $122.3\mu\text{g/g}$ selenium. The hazard profile for selenium induced reproductive impairment in fish, based on fish egg concentrations ($\mu\text{g/g}$ dry wgt), is as follows (Lemly 1995, 2002): $>20\mu\text{g/g}$, high; $10\text{--}20\mu\text{g/g}$, moderate; $<5\text{--}10\mu\text{g/g}$, low; $3\text{--}5\mu\text{g/g}$, minimal; and $<3\mu\text{g/g}$, none.

Table 5. Concentrations of selenium ($\mu\text{g/g}$ dry weight) in fish and fish eggs: Categorization by fish species

Species	Number Collected	Se Conc. Range	Mean Se conc.	SD	Mean Se Conc fish egg basis	Maximum Se conc. Fish egg basis
Black bullhead	37	0.6-4.9	1.8	0.9	5.9	16.2
Green sunfish	46	0.3-3.9	1.6	0.8	5.3	12.8
Red shiner	40	0.9-37.1	4.7	6.8	15.5	122.3

^a fish mean Se egg concentration = fish wholebody mean Se concentration x 3.3 (Lemly and Smith 1987; Skorupa et al. 1996).

Only red shiners exhibited computed mean selenium concentration in fish eggs 10-20 $\mu\text{g/g}$, and therefore poses a moderate hazard rating. Of the 37 samples of black bullhead only one egg sample had a selenium concentration of 16.2 $\mu\text{g/g}$ that falls in the range of 10-20 $\mu\text{g/g}$ and two samples of green sunfish with selenium levels of 10.8 and 12.8 $\mu\text{g/g}$ as well. Of the 40 red shiner samples, eight samples had levels >20 $\mu\text{g/g}$, and seven samples had levels ranging from 10-20 $\mu\text{g/g}$. The hazard profile states that any fish egg concentration exceeding 20 $\mu\text{g/g}$ ranks this ecosystem component as a high hazard for selenium-induced reproductive impairment in freshwater fish. Thus, red shiner could potentially face reproductive failures or egg deformities, considering that the mean selenium concentration is 15.5 $\mu\text{g/g}$. Unfortunately, no actual egg masses were collected for red shiner in order to quantify and assess the potential risk.

CONCLUSIONS

Water column selenium concentrations ($\mu\text{g/L}$) did exceed the standard of 7 $\mu\text{g/L}$ set by the State of Kansas for selenium levels in water. Examination of site concentrations of selenium in stream bed sediments also suggested that most sites at or below discharge point were elevated regardless of the sampling period. However, none of the sediment samples in this study exceeded the criterion of 4 mg/kg. In order to identify selenium persistence in sediment, a more thorough analysis is recommended with a more representative sample collection throughout an entire year. Mean selenium

concentrations in fish tissue (whole body analysis) were found to be significantly higher at Sites 4, 5 and 6 after the plant went on-line (i.e. post-operational). In addition, red shiners displayed significant differences between sites above and below the discharge point once the plant went on-line. Green sunfish and black bullheads did not display significant differences when temporal or treatment effects were taken into consideration because of the limitations imposed by stochastic seasonal variability (i.e. drought).

Absence of fish and water at some sites in the month of August is undoubtedly a significant factor for the lack of identifying definitive relationships between fish tissue and sediment concentrations. Furthermore, though only eight out of 123 fish tissue samples exceeded the $5.85\mu\text{g/g}$ criterion, post-operational increases in selenium concentrations in fish tissue below the plant discharge point was revealed. Analysis of selenium samples collected throughout an entire year will give a better representation of fish tissue selenium levels because selenium levels vary in fish tissue based on warm or cold climates.

Green sunfish fish eggs displayed significant differences in selenium concentrations between Above and Below sites. When the protocol for aquatic hazard is taken into account, whole body fish concentrations can be converted to egg mass concentrations. This conversion revealed that red shiners warranted a moderate hazard rating, while black bullhead and green sunfish had a low hazard rating. In summary, all media: water, sediment, fish tissue, and fish eggs, displayed higher selenium concentrations at sites below the point of discharge after the plant was operational, but did not always exceed suggested criteria. In order to better understand the selenium trends in these media at the Great Bend Station discharge site, a more thorough investigation is required with a sampling period longer than three months to sample more fish and accurately describe the seasonal fluctuations of sediment in these four media.

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ACKNOWLEDGEMENTS

We would like to thank the landowners who granted access to the sampling sites. Thanks to Adam Blackwood for field assistance and fish identification and dissection. Thanks to the Kansas Wetlands Education Center in Great Bend for use of their fish seines.

APPENDICES

Appendix 1. Selenium concentrations for all sites and months for water and sediment.

Treatment	Operation	Site	Month	Month No.	Water Selenium (µg/L)	Sediment Selenium (µg/g)
Above	Pre	1	March	3	14.1	1.6
Above	Pre	2	March	3	23.7	0.8
Above	Pre	3	March	3	15.6	2.7
Below	Pre	4	March	3	28.9	1.1
Below	Pre	5	March	3	26.1	3.1
Below	Pre	6	March	3	28.0	2.8
Above	Pre	1	May	5	30.7	3.3
Above	Pre	2	May	5	34.8	1.8
Above	Pre	3	May	5	9.3	1.8
Below	Pre	4	May	5	31.5	2.5
Below	Pre	5	May	5	21.6	3.5
Below	Pre	6	May	5	22.2	3.2
Above	Post	1	August	8	13.6	0.5
Above	Post	2	August	8	n/a	n/a
Above	Post	3	August	8	30.1	0.8
Below	Post	4	August	8	66.3	2.3
Below	Post	5	August	8	69.9	2.6
Below	Post	6	August	8	n/a	n/a

*Values labeled n/a are not available due to the drought conditions that prevented sample collection

Appendix 2. Selenium concentrations for all sites and months for fish.

Site	Treatment	Month	Operation	Species	Fish Selenium (µg/g)	Length (cm)	Weight (g)
1	Above	March	Pre	Black Bullhead	1.7	6.5	4.0
1	Above	March	Pre	Black Bullhead	1.2	7.0	5.1
1	Above	March	Pre	Black Bullhead	3.1	13.5	6.4
1	Above	March	Pre	Green Sunfish	1.9	8.5	13.5
1	Above	March	Pre	Green Sunfish	1.7	8.2	13.6
1	Above	March	Pre	Green Sunfish	1.9	8.0	11.5
1	Above	March	Pre	Red Shiner	3.7	3.2	0.3
1	Above	March	Pre	Red Shiner	16.4	3.5	0.3
1	Above	March	Pre	Red Shiner	8.7	3.1	0.3
2	Above	March	Pre	Black Bullhead	2.3	13.5	4.9
2	Above	March	Pre	Black Bullhead	1.7	13.5	4.6
2	Above	March	Pre	Black Bullhead	1.3	14.5	7.6
2	Above	March	Pre	Green Sunfish	1.6	7.0	9.7
2	Above	March	Pre	Green Sunfish	1.8	6.8	8.3
2	Above	March	Pre	Green Sunfish	2.0	7.2	9.9
2	Above	March	Pre	Red Shiner	1.7	2.8	0.2
2	Above	March	Pre	Red Shiner	1.9	3.0	0.3
2	Above	March	Pre	Red Shiner	1.0	3.2	0.3
3	Above	March	Pre	Black Bullhead	1.4	11.2	5.4
3	Above	March	Pre	Black Bullhead	2.8	11.0	4.6
3	Above	March	Pre	Black Bullhead	0.8	11.5	5.0
3	Above	March	Pre	Green Sunfish	1.0	6.5	9.4
3	Above	March	Pre	Green Sunfish	1.0	7.0	8.7
3	Above	March	Pre	Green Sunfish	1.1	6.5	8.3
3	Above	March	Pre	Red Shiner	9.3	2.5	0.0
4	Below	March	Pre	Black Bullhead	1.3	13.0	5.6
4	Below	March	Pre	Black Bullhead	1.7	13.5	4.5
4	Below	March	Pre	Black Bullhead	1.4	13.0	5.1
4	Below	March	Pre	Green Sunfish	1.8	6.5	5.4
4	Below	March	Pre	Green Sunfish	1.4	6.3	5.2
4	Below	March	Pre	Green Sunfish	0.9	6.0	5.7
4	Below	March	Pre	Red Shiner	1.1	4.8	1.1
4	Below	March	Pre	Red Shiner	1.4	4.4	1.0
4	Below	March	Pre	Red Shiner	1.4	4.2	1.0
5	Below	March	Pre	Black Bullhead	0.9	8.2	12.7
5	Below	March	Pre	Black Bullhead	1.6	7.0	6.6
5	Below	March	Pre	Black Bullhead	1.3	8.0	8.2
5	Below	March	Pre	Green Sunfish	n/a	10.0	17.5
5	Below	March	Pre	Green Sunfish	0.9	9.5	14.5
5	Below	March	Pre	Green Sunfish	0.7	8.5	17.8
5	Below	March	Pre	Red Shiner	37.1	2.2	0.0
5	Below	March	Pre	Red Shiner	21.1	1.8	0.0
5	Below	March	Pre	Red Shiner	4.4	2.3	0.1
6	Below	March	Pre	Black Bullhead	1.8	12.0	4.1
6	Below	March	Pre	Black Bullhead	3.2	11.5	4.8
6	Below	March	Pre	Black Bullhead	1.7	11.5	4.9
6	Below	March	Pre	Green Sunfish	1.6	8.2	14.9
6	Below	March	Pre	Green Sunfish	1.5	8.0	12.4

6	Below	March	Pre	Green Sunfish	2.1	8.3	15.5
6	Below	March	Pre	Red Shiner	4.8	2.5	0.1
6	Below	March	Pre	Red Shiner	1.5	2.8	0.2
6	Below	March	Pre	Red Shiner	1.1	2.3	0.1
1	Above	May	Pre	Black Bullhead	2.1	18.0	5.7
1	Above	May	Pre	Black Bullhead	1.3	15.0	5.2
1	Above	May	Pre	Black Bullhead	1.4	16.0	5.9
1	Above	May	Pre	Green Sunfish	1.1	8.0	16.2
1	Above	May	Pre	Green Sunfish	1.2	8.5	17.5
1	Above	May	Pre	Green Sunfish	0.6	8.0	13.5
1	Above	May	Pre	Red Shiner	4.5	4.0	2.0
1	Above	May	Pre	Red Shiner	4.7	4.0	2.2
1	Above	May	Pre	Red Shiner	4.8	4.0	1.8
2	Above	May	Pre	Black Bullhead	1.1	8.0	8.6
2	Above	May	Pre	Black Bullhead	0.6	8.0	9.7
2	Above	May	Pre	Black Bullhead	0.7	8.5	12.8
2	Above	May	Pre	Green Sunfish	2.9	9.0	14.7
2	Above	May	Pre	Green Sunfish	2.3	10.0	19.0
2	Above	May	Pre	Green Sunfish	1.9	10.1	4.6
2	Above	May	Pre	Red Shiner	7.6	5.0	2.2
2	Above	May	Pre	Red Shiner	n/a	5.0	4.9
2	Above	May	Pre	Red Shiner	n/a	5.0	1.2
3	Above	May	Pre	Black Bullhead	1.9	12.0	5.2
3	Above	May	Pre	Black Bullhead	1.2	12.0	5.0
3	Above	May	Pre	Black Bullhead	1.3	11.0	6.0
3	Above	May	Pre	Green Sunfish	2.7	7.0	5.1
3	Above	May	Pre	Green Sunfish	3.3	7.0	5.0
3	Above	May	Pre	Green Sunfish	2.7	7.5	5.0
3	Above	May	Pre	Red Shiner	1.5	3.0	1.5
3	Above	May	Pre	Red Shiner	2.2	3.0	0.3
3	Above	May	Pre	Red Shiner	1.7	3.0	0.3
4	Below	May	Pre	Black Bullhead	2.8	9.0	11.5
4	Below	May	Pre	Black Bullhead	2.8	8.0	9.0
4	Below	May	Pre	Green Sunfish	1.7	8.0	10.1
4	Below	May	Pre	Green Sunfish	1.4	9.0	16.6
4	Below	May	Pre	Green Sunfish	2.3	8.0	13.1
4	Below	May	Pre	Red Shiner	2.4	7.0	3.1
4	Below	May	Pre	Red Shiner	2.0	7.0	4.2
4	Below	May	Pre	Red Shiner	1.4	7.0	1.3
5	Below	May	Pre	Black Bullhead	2.5	7.5	8.5
5	Below	May	Pre	Black Bullhead	2.3	10.5	13.6
5	Below	May	Pre	Black Bullhead	2.6	12.5	5.0
5	Below	May	Pre	Green Sunfish	0.9	7.5	12.7
5	Below	May	Pre	Green Sunfish	1.8	8.0	11.9
5	Below	May	Pre	Green Sunfish	2.3	7.5	11.1
5	Below	May	Pre	Red Shiner	11.9	2.0	0.1
5	Below	May	Pre	Red Shiner	3.3	3.0	0.4
6	Below	May	Pre	Black Bullhead	2.2	12.0	5.6
6	Below	May	Pre	Black Bullhead	1.8	10.2	16.0
6	Below	May	Pre	Black Bullhead	2.2	11.5	5.8
6	Below	May	Pre	Green Sunfish	2.9	7.0	9.8
6	Below	May	Pre	Green Sunfish	2.9	8.0	14.1
6	Below	May	Pre	Green Sunfish	2.2	8.5	16.1

6	Below	May	Pre	Red Shiner	1.2	3.2	0.2
6	Below	May	Pre	Red Shiner	2.0	4.3	0.5
6	Below	May	Post	Red Shiner	6.3	3.8	0.4
1	Above	August	Post	Black Bullhead	0.6	15.5	5.3
1	Above	August	Post	Green Sunfish	1.5	8.5	5.0
1	Above	August	Post	Green Sunfish	0.9	8.8	5.7
1	Above	August	Post	Green Sunfish	1.0	8.5	4.4
1	Above	August	Post	Red Shiner	1.2	4.5	1.5
1	Above	August	Post	Red Shiner	1.0	4.5	1.3
1	Above	August	Post	Red Shiner	1.4	5.0	2.5
3	Above	August	Post	Green Sunfish	0.3	8.0	15.6
3	Above	August	Post	Green Sunfish	0.7	7.0	4.8
3	Above	August	Post	Green Sunfish	1.2	6.5	11.7
4	Below	August	Post	Green Sunfish	0.6	2.8	0.6
4	Below	August	Post	Green Sunfish	1.0	2.9	0.9
4	Below	August	Post	Green Sunfish	2.3	2.8	0.6
4	Below	August	Post	Red Shiner	2.0	5.8	4.1
4	Below	August	Post	Red Shiner	2.1	4.5	1.9
4	Below	August	Post	Red Shiner	2.0	3.0	0.5
5	Below	August	Post	Black Bullhead	4.9	2.8	0.4
5	Below	August	Post	Green Sunfish	0.8	7.5	16.5
5	Below	August	Post	Green Sunfish	3.9	2.5	0.3
5	Below	August	Post	Red Shiner	1.4	5.0	2.0
5	Below	August	Post	Red Shiner	1.2	4.5	1.4
5	Below	August	Post	Red Shiner	2.3	5.0	1.9

Appendix 3. Selenium concentrations for all sites and months for fish eggs.

Site	Month	Treatment	Species	Fish Egg Selenium (µg/g)	Weight (g)
1	May	Above	Green sunfish	3.5	0.4
1	May	Above	Green sunfish	3.7	0.7
1	May	Above	Green sunfish	3.5	3.2
1	May	Above	Green sunfish	2.5	0.9
1	May	Above	Green sunfish	2.4	0.8
1	May	Above	Green sunfish	2.5	2.7
1	May	Above	Green sunfish	2.1	5.6
1	May	Above	Green sunfish	2.9	0.8
1	May	Above	Black bullhead	4.6	1.9
2	May	Above	Green sunfish	2.6	1.2
5	March	Below	Black bullhead	2.8	0.8
5	March	Below	Green sunfish	7.5	1.3
5	March	Below	Green sunfish	4.8	0.3
5	March	Below	Green sunfish	4.2	0.2
5	March	Below	Green sunfish	3.5	0.2
5	March	Below	Green sunfish	3.6	0.3
6	May	Below	Green sunfish	3.7	2.8
6	May	Below	Black bullhead	3.7	14.5
6	May	Below	Black bullhead	2.7	1.1