BRITISH COLUMBIA | Ministry of Environment

NON – LETHAL TISSUE SAMPLING OF MIDDLE FRASER RIVER WHITE STURGEON (ACIPENSER TRANSMONTANUS)

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Environmental Protection Division
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EXECUTIVE SUMMARY

Non-lethal tissue samples were collected from 31 Fraser River white sturgeon of varying sizes to determine baseline metal levels prior to the discharge of mine effluent. Tissue samples were analyzed for six metals: cadmium, copper, mercury, molybdenum, selenium, and zinc. Of the 31 sturgeon sampled 6 were juvenile mortalities that were used to compare the results from the non-lethal tissue plug to the results from the accepted standard of whole body analysis. The non-lethal tissue plugs showed no difference in copper and selenium results from that of the whole body results; however, mercury and zinc results exhibited a difference between the two sampling methods.

Copper, mercury and zinc concentration generally increased with increased sturgeon size and by inference, increased sturgeon age. Selenium concentrations decreased as sturgeon length increased. There appears to be a correlation between the decreasing selenium concentrations and the increasing mercury concentrations in white sturgeon. Cadmium was not detectable in any of the muscle tissue. Molybdenum was rarely detected and in the few cases, was close to the method detection limit.

Baseline metal levels in middle Fraser River white sturgeon, when detectable, appear to be consistent with findings in previous studies on Fraser River fish.

INTRODUCTION

Knowledge of middle Fraser River white sturgeon (*Acipenser transmontanus*) is limited and it is thought that this population remains localized within seasonally established areas to maximize the use of food resources in a harsh and limiting environment (RL&L, 2000). This sedentary approach to habitat use as well as its bottom-feeding behaviour puts the white sturgeon at an increased risk from nearby effluent sources. The approval of an effluent discharge to the middle Fraser River from a copper-molybdenum mine north of William Lake, British Columbia (BC) required corroboration that white sturgeon were not taking up additional metal. To determine if metal accumulation in resident white sturgeon was occurring, an understanding of pre-discharge metal levels in the fish was needed. A non-lethal tissue sampling program was initiated and operated in conjunction with the Ministry of Forests, Lands, and Natural Resource Operations (FLNRO) Williams Lake Fisheries Section white sturgeon radio-tagging program.

The tissue sampling project was developed to evaluate the effectiveness of non-lethal tissue sampling for determining metal concentrations and to obtain baseline tissue levels for the middle Fraser River white sturgeon population. Due to the small population, slow growth, late sexual maturity, and long lifespan of white sturgeon, the development of a non-lethal tissue sampling method was necessary. Tissue samples from white sturgeon were analyzed for metals and fin samples were collected and analyzed for dioxins, furans and PCBs. The fin sampling and analyses data will be addressed separately. This document examines the baseline metal results and the effectiveness of the non-lethal study method.

STUDY AREA

Sampling was conducted at different locations along the Fraser River section shown in Figure 1. The mine effluent discharge diffuser is within the Fraser River adjacent to Tingley Creek outlet.

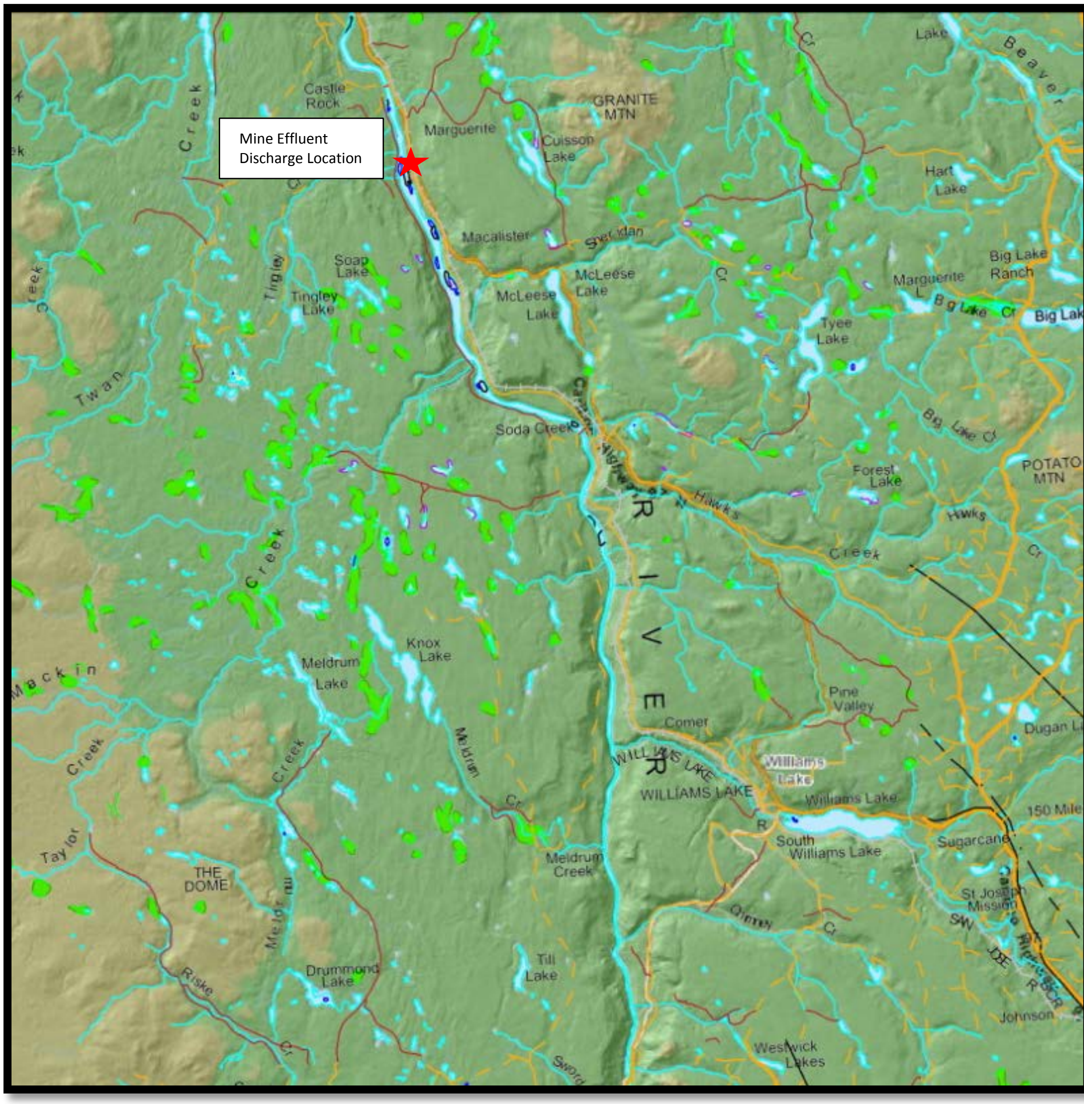

Figure 1. Middle Fraser River near Williams Lake, B.C.

METHODS

White sturgeon were caught on rod and reel with barbless baited hooks and brought to shore for non-lethal plug sampling. Sampling took place during September of 2007 and July, August, and September of 2008. Samples were collected from 31 white sturgeon.

The non-lethal methodology developed for this project includes procedures, in part, from the federal guidelines for non-lethal fish tissue sampling for mercury (Env. Canada, 2012). Other guidance and research projects were also reviewed to establish the protocol (Rolfhus et al., 2008; US EPA, 2003). Nitrile gloves were used for all sampling and handling of fish. Every tissue plug was created using a new sterile 12mm *Acuderm®* tissue biopsy punch (Fig. 2).

Figure 2. Tissue punches being collected

Tissue plugs from each fish were removed from the body of the sturgeon using a newly-bladed stainless steel autopsy knife and stainless steel tweezers that had been washed with deionized water (Fig. 3). Both the knife and tweezers were rinsed in laboratory-grade acetone just prior to use.

Figure 3. Extracting the plug

Two adjacent tissue plugs were combined to make up one tissue sample for each fish and together weighed approximately 0.5 grams. Tissue plugs were placed in laboratory-grade glass bottles, labelled and immediately stored on ice until they could be frozen at the MOE laboratory in Williams Lake, BC. Some veterinary-grade tissue adhesive *3MVetbond*™ was applied to the plug location and allowed to dry for approximately 30 seconds prior to the fish being released. Sampling was only done when the water temperature was above 8 degrees Celsius to ensure successful healing as shown in Figure 4. All samples were kept frozen and shipped with ice to Brooks Applied Labs Ltd. in Seattle, Washington for metal analyses.

Figure 4. Tissue plug extraction site a year later

Six elements were selected for analysis: cadmium (Cd), copper (Cu), mercury (Hg), molybdenum (Mo), selenium (Se) and zinc (Zn). Cadmium, copper, and molybdenum in the mine effluent were predicted to reach concentrations that would, at times, exceed BC water quality guidelines or the upstream concentrations in the Fraser River. Cadmium and copper at higher concentrations in the environment can adversely affect behavioural functions and the health of fish. Molybdenum was not known to affect fish but it was included due to its high concentrations in the mine effluent. Mercury, selenium, and zinc were not related to the mine effluent but are key elements associated with anthropological activities such as placer mining, non-point source runoff, and agriculture. At high concentrations, all three can have extremely adverse effects on fish health including the increased likelihood of mortality.

As white sturgeon in the middle Fraser River are consider endangered (COSEWIC, 2012), a non-lethal method was needed to determine if metal concentrations in the sturgeon. As this non-lethal procedure had only been established for mercury concentrations in fish, it needed to be validated against whole fish analysis to ensure it was representative of the other metals as well. To facilitate the verification, 6 juvenile sturgeon, less than 1 meter in length, were caught, killed and frozen. A few months later, each fish was left to thaw in the MOE laboratory for approximately 24 hours prior to sampling and dissection. The laboratory table was stainless steel, washed and then rinsed with deionized water, and finally rinsed with laboratory-grade acetone. Tissue plugs were removed following the same equipment and procedures used in the field (Fig. 5).

Figure 5. Plug removal on unfrozen juveniles

Replicate plugs were also collected to quantify the variability within the muscle (Fig. 5). The plugs from the juvenile sturgeon were refrozen and then shipped to Brooks Applied Labs Ltd. using the same process as the other samples. After the internal organs were removed, the sturgeon were filleted. Both muscle fillets, including skin, from each fish were considered one sample. The kidney, liver and fillet tissues were refrozen and sent to Brooks Applied Labs Ltd for metal analyses. The results of the metal concentrations from the organs are not discussed in this document.

Fishing effort occurred at locations of known and probable habitat use within the middle Fraser River shown in Figure 1. White sturgeon had to be greater than 100 cm in length to minimize any effect from non-lethal sampling on fish health. White sturgeon caught and sampled were also evaluated by three length categories to determine if the body burden metal concentrations varied across growth and life stages. The length distributions were based on established middle Fraser River population growth stages such as sexual maturity at 20 years (140 cm) and a slowed growth rate after 200 cm (RL&L, 2000). All fish lengths were based on fork length of the fish and measured by FLNRO Fisheries staff. All fish caught and brought to shore were sampled if greater than 100 cm in length.

Statistical analyses of tissue results for metals above detection limits were completed using SAS*jmp®* statistical software. The minimum baseline sample size was determined to be 25 through a power analysis that could detect an effect with a 95% confidence interval for ± 25% around the mean. From the 6 metals Hg was chosen for the power analysis as initial results had shown Hg concentrations were well above the method detection limits. Replicate and regular plugs from the 6 juvenile sturgeon were averaged to provide one result in all analyses except the within-muscle comparison. Outliers were determined through a combination of reviewing field notes and scientific literature as well as completing various statistical analyses such as residual analysis. Regression analyses were used to evaluate the relationship between metal concentration and fish length. All statistical analyses used a significance level (alpha) of 0.05. The mean metal concentrations were assessed against the length categories using one-way ANOVAs and the means of the length categories were also compared pair-wise using Tukey-Kramer HSD to determine statistical differences. The different sampling methods, fillet versus plug, were compared to determine if the results were a factor of the method or differences between the fish. Matched pairs analysis using a paired t-test and a Tukey mean-difference plot was performed for Hg, Cu, Se and Zn concentrations to compare fillet to plug values and to compare replicate and regular tissue plugs within the same fish. The one-way ANOVA was used to compare the mean concentrations of each metal from the 6 juvenile sturgeon for regular and replicate plugs as well as plugs and fillets.

RESULTS

Concentrations of Cd, Cu, and Mo were predicted to be higher in the mine effluent discharge than the BC water quality guidelines or the current levels in the Fraser River. The tissue analysis for baseline Cd concentrations revealed that all results were below detection limits. For baseline Mo concentrations, 28 results were below detection limit and 3 samples were above the detection limit but only 1 sample was high (0.289 µg/g wet weight) compared to the method detection limit of 0.006 µg/g. Copper was variable in tissue samples and there was no statistical evidence (p=0.37) that Cu tissue concentrations increased with fish length despite the slightly increasing slope of the fit line (Fig. 6).

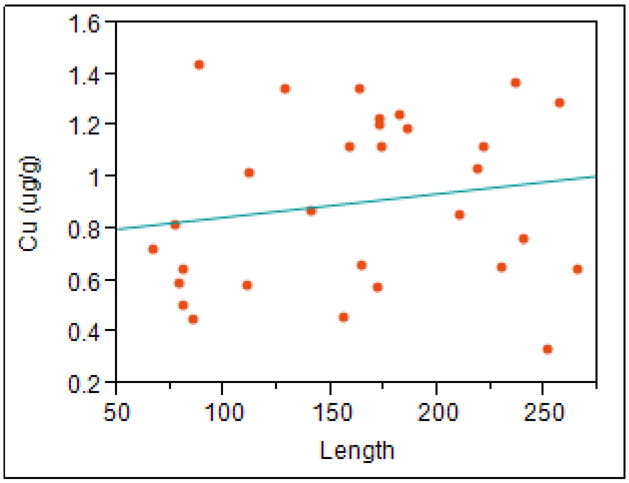

Figure 6. Fish length versus copper concentration in muscle plugs

In addition to the three metals sampled for baseline information related to the mine effluent, Se, Hg and Zn were also analyzed. These elements often have increased levels in water and sediments due to anthropological activities and can have very adverse effects on fish health at high concentrations. All results for these three metals were above the detection limit. Of the three metals, Se showed the strongest evidence (p=0.0002) of a relationship between concentration and fish length (Fig. 7).

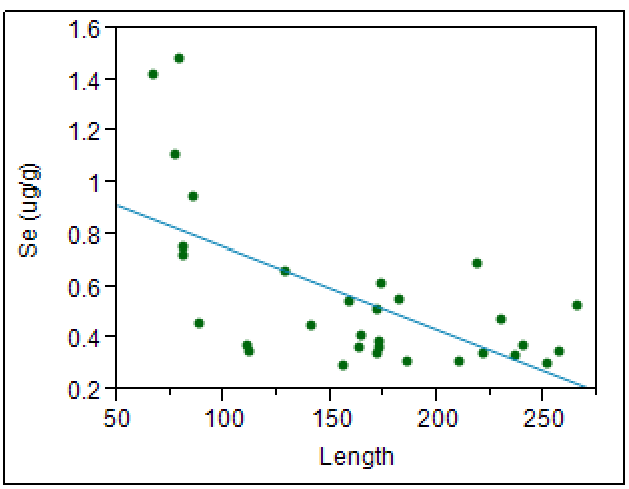

Figure 7. Fish length versus selenium concentrations in muscle plugs

The regression analysis of Hg concentrations had strong evidence of a relationship (p=0.0074) between increasing fish length and increasing Hg concentrations (Fig. 8). There was no statistical evidence of a relationship for Zn (p=0.1330) and fish length. But like Cu, the fit line for Zn indicates an increasing trend between fish length and metal concentration (Fig. 9).

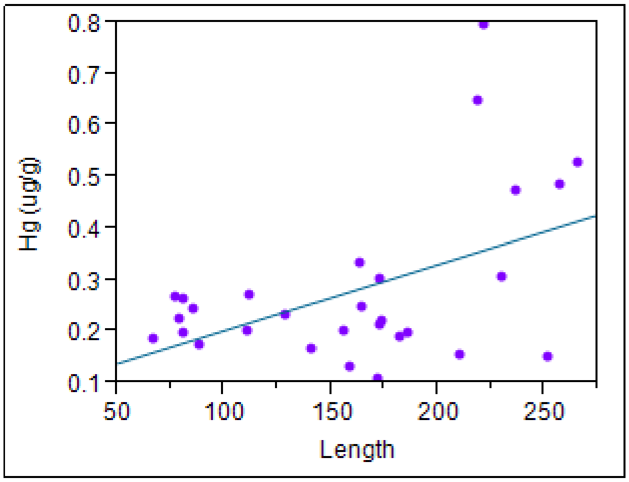

Figure 8. Fish length versus mercury concentration in muscle plugs

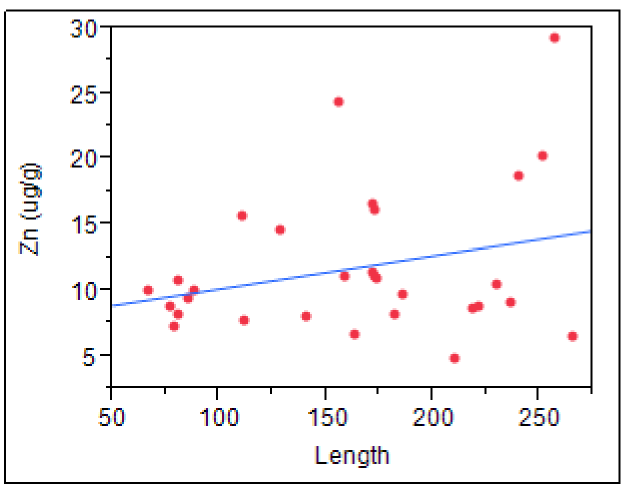

Figure 9. Fish length versus zinc concentrations in muscle plugs

Lengths categories were based on the white sturgeon study by RL&L, 2000, which determined growth stages such as sexual maturity at 20 years of age and approximately 140 cm for fish length, and slowing of growth, which happened after 200 cm as shown in Figure 10. Lengths of white sturgeon sampled had almost equal representation across three length categories (Fig. 11). Fish caught and sampled included 10 fish shorter than 140 cm in length, 11 fish between 140 and 200 cm in length, and 9 fish that were greater than 200 cm in length.

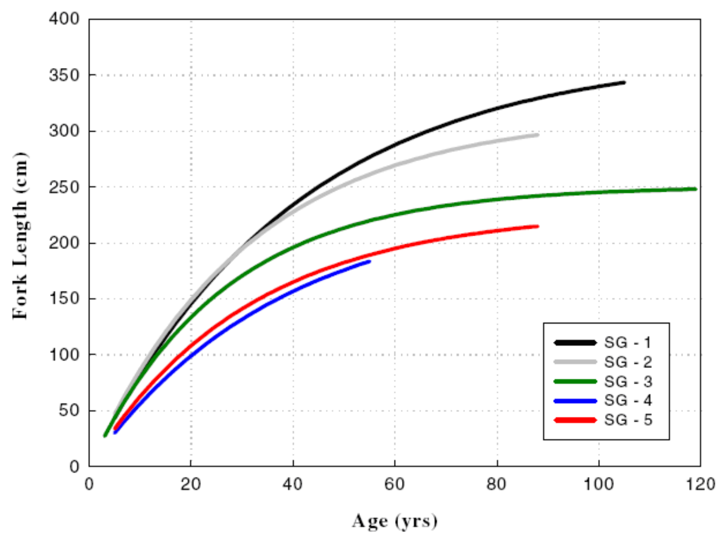

Figure 10. Age Growth Curves of White Sturgeon Stock Groups (SG) in the Fraser River Drainage (RL&L, 2000)

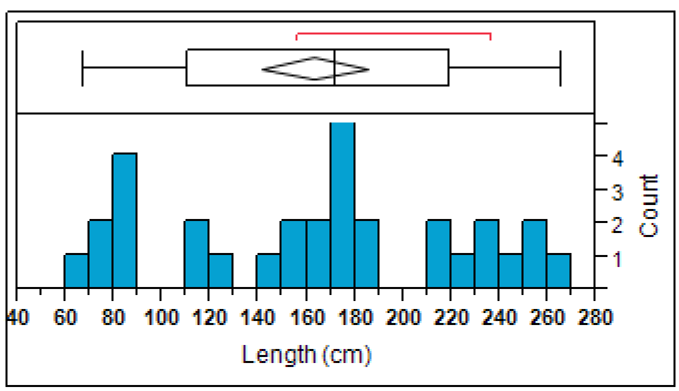

Figure 11. Distribution of sampled sturgeon length

Mean Hg concentrations in Figure 12 showed a strong similarity across the two shortest categories but the longest category (200 and over) did not overlap with either of the shorter fish lengths. The Tukey-Kramer all pairs analysis showed very strong evidence that the mean concentration of the shorter fish groups was different than the longest fish group (p=0.00136 and p=0.0036). Also of note is the greater variability in Hg concentrations at the >200 cm fish lengths (Fig. 12).

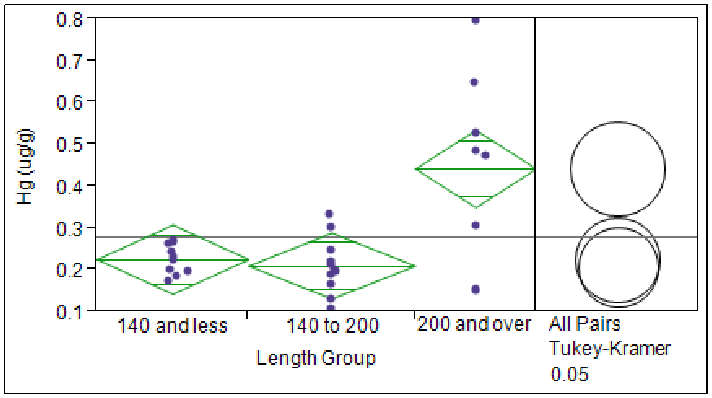

Figure 12. Comparison of mean mercury tissue concentrations across length categories

Green diamonds represent the mean, statistical overlap, and 95% confidence limit for each length group.
Circles on the right illustrate strength of relationship where more overlap means more statistical evidence for similarity between the means.

The Tukey-Kramer all pairs analysis of Se showed very strong evidence that the mean tissue concentration of the shortest category was statistically different (p=0.0031 and p=0.0023) than the means of the other length categories. As seen in the graph (Fig. 13), Se concentrations in the shortest category are variable when compared to the limited range of concentrations in the other two length categories.

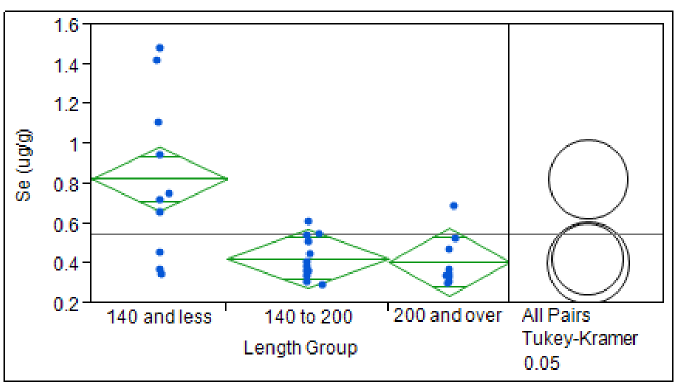

Figure 13. Comparison of mean selenium tissue concentrations across length categories

A comparison of mean concentrations for Cu (Fig. 14) and Zn (Fig. 15) across length categories showed no evidence of a difference between the categories which is consistent with the results of regression analyses for these metals.

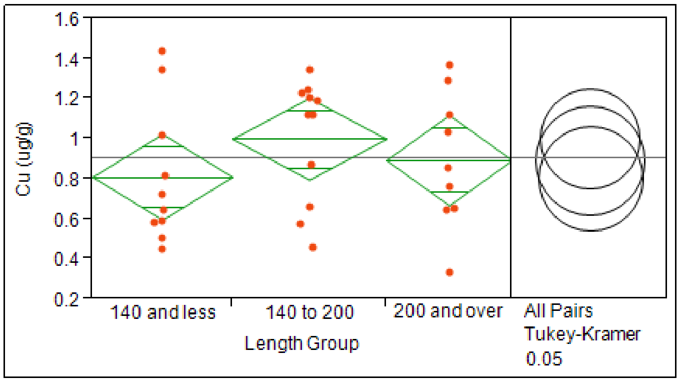

Figure 14. Comparison of mean copper tissue concentrations across length categories

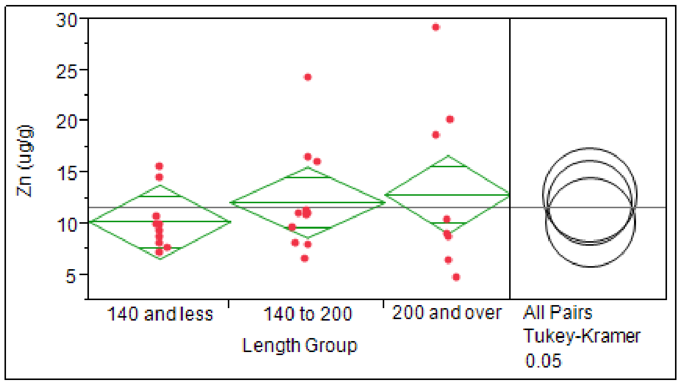

Figure 15. Comparison of mean zinc tissue concentrations across length categories

To verify, whether the non-lethal plug sample was representative of the whole sturgeon, comparisons were made between the concentrations found in the plugs and fillets of six juvenile white sturgeon.

Copper concentrations in the plugs of the juveniles ranged from 0.45 to 0.82 µg/g wet weight (ww). The fillets from the same six fish had a similar range of copper concentrations from 0.27 to 0.67 µg/g ww. Selenium concentrations in plugs (0.73 to 1.49 µg/g ww) had similar ranges to fillets (1.05 to 1.37 µg/g ww). Copper and selenium concentrations did not exhibit a statistical difference between the plug and fillet method but the copper results were slightly less definitive (Cu Prop > |t| = 0.1101, Se Prop > |t| = 0.3186).

Mercury concentrations in plugs ranged from 0.184 to 0.266 µg/g ww and in fillets from 0.180 to 0.374 µg/g ww for the six juveniles. Zinc concentrations ranged from 7.27 to 10.8 µg/g ww in the juvenile sturgeon plugs and from 5.77 to 8.02 µg/g ww in the fillets of the same six sturgeon. Mercury and zinc analysis reported some evidence that plug sampling may not be representative of concentrations for the whole fish (Hg Prob >|t| = 0.0193, Zn Prob >|t| = 0.0159). Fillets generally had higher Hg concentrations and lower Zn concentrations than the plugs taken from the same fish.

As the results for Cd and Mo for both the plugs and the fillets were below the method detection limits, no analysis was completed.

Matched pairs analyses was also used to compare concentrations of metals in regular and replicate plugs to look for variability within the muscle tissue but no evidence was found. The paired t-test results were Prob > |t|= 0.6609 for Hg, Prob >|t|= 0.3662 for Cu, Prob >|t|= 0.6648 for Se and Prob >|t| = 0.9737 for Zn. Lastly, the mean concentrations for each of the six metals from the regular plugs of the juvenile sturgeon were compared to the mean concentrations of the replicate plugs and no differences were identified.

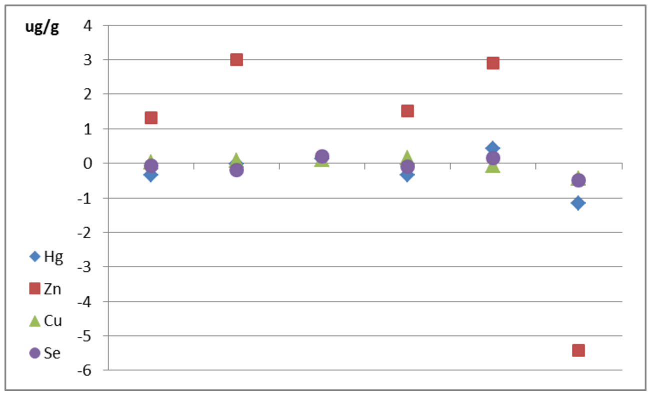

Figure 16. Metal concentration differences between regular and replicate plugs

DISCUSSION

Tissue sampling from middle Fraser River white sturgeon was dual purposed: to establish baseline metal values prior to the mine effluent discharge and to determine if non-lethal plug samples were representative of the more standardized lethal muscle sampling. The following section discusses how baseline tissue metal results compare to similar sampling of fish tissue in the Fraser River. Further, this section looks at other plug sampling and compares the information to that of this study.

Baseline Metal Concentrations

Cadmium

Cadmium is known to accumulate primarily in the kidneys and liver of aquatic organisms so muscle tissue will typically have the lowest concentrations of the different body tissues (BC Min. of Env. 2015; Chowdhury, 2005).

All the Cd results from the tissue plugs were below method detection limits. An earlier study (MacDonald et al., 1997) on three Fraser River white sturgeon of varying lengths found Cd concentrations in muscle were also below detection levels though these ranged from 0.05 to 0.44 µg/g wet weight (ww).

A study on Cd accumulation in the muscle tissue of common carp (Malekpouri et.al, 2011) found that Cd could accumulate to levels significantly different ($P<0.05$) than the control population. The study captured nine combinations of three Cd concentrations of 10 µg/L, 50 µg/L and 100 µg/L across three exposure timeframes of 30, 60 and 90 days. The lowest muscle concentration of 0.03 µg/g dry weight (dw) occurred in the lowest exposure (30 days) and the lowest concentration (10 µg/L) of waterborne Cd. The other experiments in this study resulted in more exposure and/or higher Cd concentrations with the maximum combination (100 µg/L for 90 days) resulting in a mean Cd concentration of 12 µg/g dw in carp muscle tissue. The increased Cd in muscle did not appear to have any consistent effect on fish growth as either measured by fork length or fish weight (Malekpouri et al., 2011). The method detection limit for Cd analysis in middle Fraser River white sturgeon ranged from 0.002 to 0.004 µg/g ww which is a magnitude lower than the common carp results and the method detection level used in the earlier McDonald et al. (1997) study but cadmium was still not detected.

Molydenum

Molybdenum is an essential element but like many metals is stored in the liver and kidneys (Regoli et.al, 2012).

Molybdenum results were below detection levels in all but 3 samples. Of the three samples, two had Mo concentrations near detection limits (0.004 to 0.01 µg/g ww) but one sample had a high Mo concentration (0.289 µg/L). A study examining accumulation of molybdenum in rainbow trout muscle tissue found that even when the initial Mo concentration in the water was 11,000 µg/L, the muscle concentrations peaked at day 28 in the range of 0.7 µg/g, but by day 120, had dropped back to below the detection limit (Regoli et al., 2012). The study concluded that Mo does not accumulate in fish muscle tissue despite high levels occurring in the aquatic environment. While it is not known if the three Mo concentrations above detection limits were due to procedural contamination, it can be concluded that the one high concentration was an outlier and generally Mo concentrations were near or below detection limits.

Copper

Copper at sub-lethal levels can affect many functions in fish including reproduction, behaviour, growth, blood chemistry, olfaction, and metabolism (Singleton, 1987). While copper can be found in muscle tissue it typically accumulates in the liver.

The baseline results for the middle Fraser River found that Cu concentrations were highly variable across all the white sturgeon lengths and ranged from 0.27 to 1.44 µg/g ww. Tissue sampling of 3 white sturgeon in the Prince George area in 1991 found an average copper concentration for muscle tissue of 0.29 ± 0.07 ug/g ww (MacDonald et al., 1997). A study on Fraser River fish caught near Marguerite in 1994 and 1995 ascertained that average Cu concentrations in peamouth chub muscle were 0.62 (0.5 – 0.7) µg/g ww and 0.36 (0.24 – 0.44) µg/g ww respectively (Raymond et al., 2001). The same study found the Cu concentration in mountain whitefish muscle were 1.26 (0.54 – 1.88) µg/g ww and 0.46 (0.43 – 0.50) µg/g ww. The study suggested that mountain whitefish, as a bottom feeder, had higher Cu concentrations on average due to their ingestion of sediment (Raymond et al., 2001). The average plug concentration for the 31 Fraser River sturgeon which includes the 6 juveniles was 0.91 ± 0.34 µg/g ww which was similar to the average concentrations in mountain whitefish near Marguerite in 1994.

In a study of uncontaminated lakes in BC, it was established that the mean Cu concentration in 112 rainbow trout from 24 lakes was 0.39 ± 0.29 µg/g ww (Rieberger, 1992). The 1992 study also collected 20 mountain whitefish from 4 lakes which had an average Cu concentration of 0.35 ± 0.20 µg/g ww. While this study is not directly relevant to sturgeon living in a river, it does provide further comparison of baseline metal concentrations in BC.

In 2008, a US Fish and Wildlife Service study found that 6 hatchery shortnose sturgeon (3.5 to 5.5 years old) of similar weight and with an average fork length of 62.8 cm, had an average Cu concentration in the fillet of 0.32 ± 0.01 µg/g ww (Mierzykowski, S. 2012). In the same study a combined sample from two 2.5 year old sturgeon which were half the weight of 3.5 to 5.5 year old fish identified above, had a Cu concentration of 0.24 µg/g ww (Mierzykowski, S. 2012). The whole body of a single 1.5 year old sturgeon was analyzed which resulted in a high Cu concentration of 1.40 µg/g (ww), probably due to the inclusion of organs which are typically higher in Cu. The 6 fillets from the Fraser River juvenile white sturgeon, with fork lengths ranging from 67 cm to 86 cm, had an average Cu concentration of 0.42 ± 0.14 µg/g ww. While slightly higher than the hatchery sturgeon in the US Fish and Wildlife Service study, the Fraser River sturgeon are assumed to be older as reflected in their larger length.

Copper concentrations in white sturgeon seem to be consistent with the concentrations found in other fish studies in the Fraser River and in white sturgeon juveniles found in other areas.

Zinc

The effects of zinc to fish mainly involve breathing such as gill damage, hypoxia, and impaired gas exchange (Eisler, 1993). As implied by the toxic effects, accumulation of zinc in fish is typically greatest in the gills and least in the body muscle although the exact pattern of accumulation may vary by species (Rajkowska and Protasowicki, 2013).

Zinc concentrations in the middle Fraser River white sturgeon had a similar pattern and trend to Cu concentrations. Like Cu, Zn displayed a high degree of variability at all fish lengths but with a slight increase in Zn concentrations with increasing fish length. Zinc concentrations ranged from 4.82 µg/g ww to 29.2 µg/g ww with an average concentration of 6.7 ± 0.87 µg/g ww.

The average Zn muscle concentration of the 3 white sturgeon in the Prince George area was 3.06 ± 0.29 µg/g ww which is much lower than the middle Fraser River concentrations (MacDonald et al., 1997). The difference in average Zn concentrations between the two sections of the river could be a factor of progressive zinc inputs over the years, additional zinc contributions from upstream or due to the small Prince George area sample size. Mountain whitefish from the Fraser River near Marguerite had average Zn concentrations of 5.52 (4.09 – 7.40) µg/g ww in 1994 and 5.36 (4.90 – 5.69) µg/g ww in 1995 (Raymond et al., 2001). Peamouth chub from the same study had average Zn concentrations of 12.8 (11.6 – 13.9) µg/g ww and 9.57 (8.63 – 10.70) µg/g ww over the two years (Raymond et al., 2001). Shortnose sturgeon in Mierzykowski's 2012 study of hatchery fish found the average Zn concentration in fillets was 4.1 µg/g ww with little variation. Furthermore, average Zn concentrations in rainbow trout and mountain whitefish from uncontaminated lakes in BC were 4.28 ± 1.35 µg/g ww and 4.16 ± 1.00 µg/g ww respectively (Rieberger, 1992).

Zinc concentrations in middle Fraser River white sturgeon where higher on average than the concentrations in the Prince George white sturgeon, the shortnose sturgeon in Mierzykowski's 2012 study, and the mountain whitefish in the Fraser River. Conversely, peamouth chub in the Fraser River had higher average Zn concentrations than fish in all other studies including middle Fraser River white sturgeon.

Selenium

Like Zn, selenium is an essential element but can be toxic to fish at high concentrations. Selenium is typically transferred through the food chain where it has the potential to magnify as it moves up the chain. As such, the top feeders, such as sturgeon, are at highest risk although accumulation rates depend greatly on site-specific conditions and how individuals use the habitat (Beatty and Russo, 2014).

White sturgeon in the middle Fraser River showed a very strong correlation between decreasing Se concentrations and increasing fish length. Selenium concentrations ranged from an average of 1.2 ± 0.12 µg/g ww in the 6 juvenile sturgeon to an average of 0.43 ± 0.13 µg/g ww in fish approximately 200 cm in length. Selenium was not measured in the Prince George area sturgeon or in fish from uncontaminated BC lakes; however, it was analysed in mountain whitefish and peamouth chub in the Fraser River during 1994 and 1995. Concentrations of Se in mountain whitefish ranged from 0.34 to 0.58 µg/g ww and peamouth chub had Se concentrations ranging from 0.43 to 0.61 µg/g ww (Raymond et al., 2001).

Large (>200 cm in length) white sturgeon from the middle Fraser River had Se concentrations comparable to the mountain whitefish and peamouth chub sampled in 1994 and 1995. Smaller white sturgeon and especially juveniles had high concentrations of Se comparatively.

Mercury

Mercury can bioaccumulate and biomagnify much like Se. Unlike Se, Hg does not have a biological function and is not an essential element (Nagpal, 1989). Methyl-mercury, the most biologically available form, adheres strongly to fish protein and is readily available in fish muscle.

Mercury concentrations in middle Fraser River white sturgeon ranged from 0.094 µg/g ww to 0.796 µg/g ww, increasing with increasing fish length with an average concentration of 0.286 ± 0.172 µg/g ww. There was strong statistical evidence that longer and presumably older white sturgeon had higher levels of mercury in their muscle tissue. Previous analysis of Fraser River white sturgeon found Hg concentrations ranging from 0.18 to 1.44 µg/g ww for the Prince George area and results of 0.27 µg/g ww and 0.83 µg/g ww for two white sturgeon caught in the Williams Lake area (MacDonald et al., 1997). The 1994 and 1995 sampling of Fraser River fish near Marguerite found average Hg concentrations of 0.17 and 0.09 ug/g ww in peamouth chub and 0.02 and 0.03 ug/g ww in mountain white fish (Raymond et al., 2001).

Mercury concentrations in the middle Fraser River white sturgeon were lower or equal to the concentrations reported in the 1997 MacDonald et al. Fraser River white sturgeon study. The middle Fraser River white sturgeon had higher concentrations than other Fraser River fish species previously sampled from the same area which could be expected as it is a top feeder.

Mercury and Selenium Interaction

While Hg and high concentrations of Se can be toxic, Se may also protect against Hg toxicity (Beatty and Russo, 2014). It has been suggested that an inverse relationship can be found in the tissue concentrations of Se and Hg within fish exposed to both Se and Hg (Nagpal, 1989).

Mercury concentrations in middle Fraser River white sturgeon muscle increased with increasing fish length while Se concentrations strongly decreased. The fish length category analysis for the Fraser River white sturgeon also highlighted the inverse relationship of Se and Hg and fish length. There was strong evidence that white sturgeon over 200 cm had greater average concentrations of Hg in the tissues than those in the shorter categories. There was also very strong evidence that higher average Se concentrations were found in sturgeon under 140 cm when compared to the longer fish.

Similar relationships between Se and Hg were seen in a number of other studies. An Ontario study of several lakes, found that the lake with the highest fish tissue Se concentrations also had the lowest tissue methyl-mercury concentrations (Yang et al., 2009). In a San Francisco Bay study on sport fish, the leopard shark, a top feeder, had the highest Hg concentrations and also the lowest Se concentrations (Davis et al., 2011). In the same study, white sturgeon had the highest Se concentrations of sport fish (Davis et al., 2011), but Hg concentrations were not collected for this species so it could not be determined if a correlation existed. A recent study on hammerhead sharks found a strong positive correlation between Hg in muscle and fish weight (Bergés-Tiznado et al., 2015). The same study did not find any statistical evidence of a relationship between fish size and Se concentrations in muscle tissue; however, the brain Se concentrations decreased with increasing shark length (Bergés-Tiznado et al., 2015).

The trends for Hg and Se tissue concentrations in Fraser River white sturgeon exhibit the probability of a relationship in accumulation between these metals. Similar trends were identified in the studies on hammerhead sharks, leopard sharks and walleye.

Plug Method Validation

The non-lethal plug method has been shown to work well for quantifying Hg concentrations in fish in other studies (Ackerson et al. 2014; Peterson et al., 2005; Baker et al., 2004; Cizdziel et al., 2002;) and is provided in various tissue sampling guidance documents (BC Min of Env., 2012; Env. Canada, 2005; US EPA, 2003). Non-lethal plug collection for selenium was tested on 12 white sturgeon in the San Francisco Bay 2009 study. This study found that the Se concentrations from plugs were on average 25% higher than fillets, though they correlated well with fillets and were determined to be a suitable alternative to lethal sampling (Davis et al., 2011). The study on fish in several US rivers by Peterson et al., 2005 evaluated the effectiveness of biopsies to accurately identify mercury concentrations. This study compared the mercury concentrations of 210 filet biopsies to the same whole bodies from 13 species of fish collected across 12 of the western USA states. The concentrations were log transformed and using the very strong correlation (r^2 = 0.96), an equation was developed for predicting whole body concentrations from biopsy results (Peterson et al., 2005). This predictive equation demonstrated that whole body concentrations were expected to be about 50 to 60 percent lower than biopsy concentrations. A similar study was conducted on smallmouth bass from rivers in Missouri; it found an almost identical relationship to that of the Peterson et al. study, between biopsied muscle plugs and fillets from the posterior half of the same fish (Schmitt & Brumbaugh, 2007). Both the Peterson et al. 2005 study and the Schmitt & Brumbaugh, 2007 study used regression analyses to establish the relationship and a predictive equation between the plugs and fillets. A similar statistical evaluation between plug and fillet results for the white sturgeon was not possible due to the limited sample size (n=6).

While a regression was not feasible for the six juvenile fish results, concentrations of metals in plugs were compared using a paired t-test to those of the fillets from the same fish. Plugs and fillet results for Cd and Mo were equivalent as all the results for both tissue collection methods were below detection limits. This confirms that Cd and Mo were not present in the muscle samples from the other fish and that the plug method was producing reliable results. Concentrations of copper and selenium were not statistically different between fillets and plugs. There was some evidence of a statistical difference in the concentrations between fillets and plugs for both Hg and Zn.

Muscle Variability

The use of non-lethal plug sampling was based on guidance documents and previous studies that were specific to sampling for Hg. In 2002, Cizdziel et al. reported on mercury concentrations of multiple locations of biopsied muscle on each fish in the study and determined that the dorsal area was best for accuracy of mercury measurements. The study also confirmed this by comparing single plugs to homogenized fillets of six fish and got a mean relative percent difference of 5.4% (Cizdiel et al., 2002). Peterson et al., 2005 reported that the study E. Pearson did in 2000 for the North Dakota Department of Health showed that dorsal biopsies were generally more accurate than anterior or posterior biopsies. The non-lethal plugs taken in the field were collected just below the dorsal fin (Fig. 3) though more posterior than identified in the Peterson et al., 2005 and Cizdiel et al., 2002 studies. The replicates were collected fairly close to the original plugs and may not truly represent muscle variability but possibly variations due to sampling technique. No evidence of statistical difference were identified between the regular and replicate plugs in the six fish juveniles.

CONCLUSIONS

The collection of non-lethal muscle plugs provided baseline levels for some metals in white sturgeon from the middle Fraser River. Cadmium was not detected in the muscle tissue and molybdenum was rarely detected. Copper and zinc concentrations were variable in the muscle tissue though showed a tendency for increased metal concentration with increased fish length. Both Hg and Se concentrations exhibited a very strong correlation with sturgeon length. While Hg concentrations increased with longer fish sizes, Se concentrations decreased across increasing lengths. The baseline results of all six metals were consistent with concentrations found in previous studies on BC mountain whitefish and peamouth chub and Fraser River white sturgeon.

The sampling of tissue with dermal punches and collecting plugs is a useful non-lethal method of measuring some metals in white sturgeon. The method was quick, appeared to not hurt the fish, and recaptures showed good healing of previous biopsies (Fig. 4). No statistical differences were identified between the plugs and fillets for Cu and Se concentrations; however, mercury and zinc results indicated differences. Molybdenum does not appear to accumulate in muscle tissues; consequently, plug sampling may not be needed. Collection of additional fillet and plug concentrations would be necessary to provide more certainty on the validity of the method and the relationship between plugs and fillets for the other five metals. While the validity of using non-lethal plug sampling for Hg and Zn requires further examination, it appears to be a valid method for Cd, Cu, and Se muscle tissue analysis for white sturgeon. Collection of replicates confirmed that the technique provided replicable results however, variation between dorsal, posterior and anterior muscle areas should be studied further.

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APPENDIX A. METAL CONCENTRATIONS IN WHITE STURGEON TISSUE PLUGS

Fish No.	Length (cm)	Hg (ug/g)	Cd (ug/g)	Cu (ug/g)	Mo (ug/g)	Se (ug/g)	Zn (ug/g)
1	252	0.150	<0.004	0.33	<0.01	0.30	20.20
2	186	0.198	<0.004	1.19	<0.01	0.31	9.61
3	156	0.203	<0.004	0.46	*0.089*	0.29	24.30
4	222	0.796	<0.004	1.12	<0.01	0.34	8.70
5	173	0.214	<0.004	1.20	<0.01	0.39	11.00
6	257	0.484	<0.004	1.29	<0.01	0.35	29.20
7	172	*0.664*	<0.004	*2.62*	<0.01	0.34	11.30
8	237	0.473	<0.004	1.37	<0.01	0.33	9.10
9	210	0.155	<0.004	0.85	<0.01	0.31	4.82
10	173	0.301	<0.004	1.23	<0.01	0.36	16.10
11	163	0.333	<0.004	1.34	<0.01	0.36	6.67
12	111	0.202	<0.004	0.58	<0.01	0.37	15.60
13	88	0.175	<0.004	1.44	<0.01	0.46	9.94
14	112	0.270	<0.004	1.02	<0.01	0.35	7.73
15	182	0.191	<0.003	1.24	<0.005	0.55	8.13
16	129	0.234	<0.003	1.34	<0.005	0.66	14.60
17	159	0.131	<0.003	1.12	<0.006	0.54	11.00
18	219	0.647	<0.003	1.03	<0.005	0.69	8.68
19	240	*0.094*	<0.003	0.76	<0.006	0.37	18.70
20	266	0.529	<0.003	0.64	<0.006	0.53	6.42
21	230	0.306	<0.002	0.65	<0.005	0.47	10.40
22	140.5	0.168	<0.003	0.87	<0.005	0.45	8.00
23	164	0.247	<0.002	0.66	*0.025*	0.41	*54.80*
24	174	0.221	<0.003	1.12	<0.005	0.61	10.90
25	172	0.109	<0.003	0.57	<0.005	0.51	16.50
A	79	0.224	<0.002	0.59	<0.004	1.49	7.27
B	81	0.263	<0.003	0.65	<0.006	0.73	10.80
C	77	0.266	<0.003	0.82	*0.289*	1.11	8.82
D	86	0.244	<0.002	0.45	<0.004	0.95	9.35
E	67	0.184	<0.003	0.72	<0.006	1.42	10.05
F	81	0.198	<0.002	0.50	<0.004	0.75	8.19

Note: Outliers are in italics.

APPENDIX B. METALS CONCENTRATIONS IN JUVENILE MUSCLE TISSUES

Fish ID	tissue	Hg (ug/g)	Cd (ug/g)	Cu (ug/g)	Mo (ug/g)	Se (ug/g)	Zn (ug/g)	Length
A	plug reg	0.207	<0.002	0.600	<0.004	1.450	7.92	79
	plug rep	0.242	<0.003	0.580	<0.006	1.520	6.61	
	Fillet	0.244	<0.003	0.430	<0.006	1.370	6.57	
B	plug reg	0.261	<0.003	0.680	<0.006	0.630	12.30	81
	plug rep	0.264	<0.003	0.610	<0.006	0.820	9.30	
	Fillet	0.374	<0.003	0.440	<0.005	1.050	6.48	
C	plug reg	0.272	<0.003	0.860	*0.289*	1.210	*132.00*	77
	plug rep	0.26	<0.003	0.770	<0.006	1.010	8.82	
	Fillet	0.36	<0.003	0.270	<0.005	1.200	5.96	
D	plug reg	0.227	<0.002	0.520	<0.004	0.900	10.10	86
	plug rep	0.261	<0.002	0.380	<0.004	0.990	8.59	
	Fillet	0.368	<0.003	0.670	<0.005	1.110	5.77	
E	plug reg	0.205	<0.003	0.680	<0.006	1.500	11.50	67
	plug rep	0.163	<0.002	0.760	<0.004	1.340	8.59	
	Fillet	0.18	<0.003	0.360	<0.005	1.220	7.45	
F	plug reg	0.139	<0.002	0.280	<0.004	0.510	5.47	81
	plug rep	0.256	<0.002	0.720	<0.005	0.990	10.90	
	Fillet	0.285	<0.003	0.340	<0.006	1.090	8.02	

Note: Outliers are in italics.

APPENDIX C. STATISTICAL ANALYSES RESULTS

Test	Comparison	Statistical Result	
Regression analysis		**Prob > F**	
	Hg vs Length	0.0074	
	Cu vs Length	0.37	
	Se vs Length	0.0002	
	Zn vs Length	0.133	
One-way analysis with Tukey-Kramer HSD all pairs comparison	Mean of Length Group for Hg	**p-Value**	
	140 and Less vs 140 to 200	0.9587	
	140 to 200 vs 200 and Over	0.0016	
	140 and Less vs 200 and Over	0.0036	
	Mean of Length Group for Cu	**p-Value**	
	140 and Less vs 140 to 200	0.3977	
	140 to 200 vs 200 and Over	0.7543	
	140 and Less vs 200 and Over	0.8475	
	Mean of Length Group for Se	**p-Value**	
	140 and Less vs 140 to 200	0.0023	
	140 to 200 vs 200 and Over	0.9875	
	140 and Less vs 200 and Over	0.0031	
	Mean of Length Group for Zn	**p-Value**	
	140 and Less vs 140 to 200	0.7189	
	140 to 200 vs 200 and Over	0.9488	
	140 and Less vs 200 and Over	0.5568	
Matched pairs analysis and one-way analysis with Tukey-Kramer HSD pairs comparison	Fillet vs Plug Comparison	**Prob > |t|**	
	Hg	0.0196	
	Cu	0.1101	
	Se	0.3186	
	Zn	0.0159	
	Regular and Replicate Plug Comparison	**Prob > |t|**	**p-value**
	Hg	0.6609	0.3920
	Cu	0.3662	0.7453
	Se	0.6648	0.7059
	Zn	0.9737	0.3271