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Technical Report Overview

Report: Evaluation of Selenium Concentrations in Ovary of Northern Pikeminnow

Overview: This report presents the results of ovary selenium concentrations from northern pikeminnow in the Koocanusa Reservoir. This report also includes results from a study to characterize the influence of gonadal maturation stage, fish size, and fish sampling location on ovary selenium concentrations in northern pikeminnow in the Koocanusa Reservoir.

This report was prepared for Teck by Ecotox LLC, the University of Saskatchewan and Minnow Environmental Inc.

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FINAL REPORT

EVALUATION OF SELENIUM CONCENTRATIONS IN OVARY OF NORTHERN PIKEMINNOW (*Ptychocheilus oregonensis*)

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Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Elk River, BC

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Appendix C. Northern Pikeminnow Metal Tissue Data: 2019

1. INTRODUCTION

Ongoing monitoring of Koocanusa Reservoir indicates ovary Se concentrations from some northern pikeminnow (NPM; *Ptychocheilus oregonensis*) collected from the Canadian side of the Reservoir were above both the 11 mg kg^{-1} dry weight (dw) egg Se guideline established by the British Columbia Ministry of the Environment and Climate Change Strategy (BC ENV, 2014) and the 15.1 mg kg^{-1} dw egg Se criteria established by the U.S. Environmental Protection Agency (USEPA, 2016). However, the sensitivity of NPM to Se is currently unknown and so the ecological risk posed by observed egg Se concentrations is uncertain. Further, historical ovary Se concentrations were collected from unripe fish (i.e., not in spawning condition) and the influence of gonadal maturation stage on egg Se concentrations is uncertain. The following presents results from a study to characterize the influence of gonadal maturation stage, fish size, and fish sampling location on ovary Se concentrations in NPM collected from Koocanusa Reservoir.

Efforts to also conduct a toxicity test evaluating the effects of maternally transferred Se on NPM embryo-larval development were unsuccessful in 2019 due to the inability to collect a sufficient number of female fish in spawning condition. As such, this test is not discussed further in this report.

2. BACKGROUND

Northern pikeminnow are distributed throughout the Pacific drainages as far north as the Nass River drainage in BC, Canada to the Columbia River drainage in the U.S. They are most common along sandy, cobble, gravel, boulder or bedrock shorelines during summer and deeper waters during winter (Scott and Crossman 1973, Coker et al. 2001). Northern pikeminnow are late spring-summer spawners, typically spawning when water reaches 14-18 °C with males generally present in larger congregations on breeding grounds over gravel and cobble shallows (Gadomski et al., 2001). Females may have multiple spawning bouts with more than one male throughout the season. Eggs hatch after 8-10 days at 15-17 °C (Coker et al., 2001; Gadomski et al., 2001; Scott and Crossman, 1973).

Koocanusa Reservoir was formed by Libby Dam, located 30 km northeast of Libby, Montana at river mile 221.9 of the Kootenai River[1]. The reservoir is 145 km long, of which 68 km are in BC, Canada. The predominant source of water to the reservoir is the Kootenay River, of which the Elk River is a tributary. Northern pikeminnow are resident to Koocanusa Reservoir and have been sampled for ovary and muscle Se in BC, Canada and Montana (MT), U.S. over the last 11 years.

1. The Kootenay River is referred to as the 'Kootenai River' in the U.S.

Monitoring data indicate NPM ovary Se concentrations on the MT side of the reservoir have low variability within and across sampling years compared to fish collected from the BC side of the reservoir (Figure 1). Some fish collected on the BC side of the reservoir are above both the BC ENV guideline and the USEPA fish egg Se criteria of 11 and 15.1 mg kg^{-1} dw, respectively. These data also indicate ovary Se concentrations in fish collected from the BC side of Koocanusa Reservoir appear to be significantly ($p<0.05$) higher than those collected from the U.S. side.

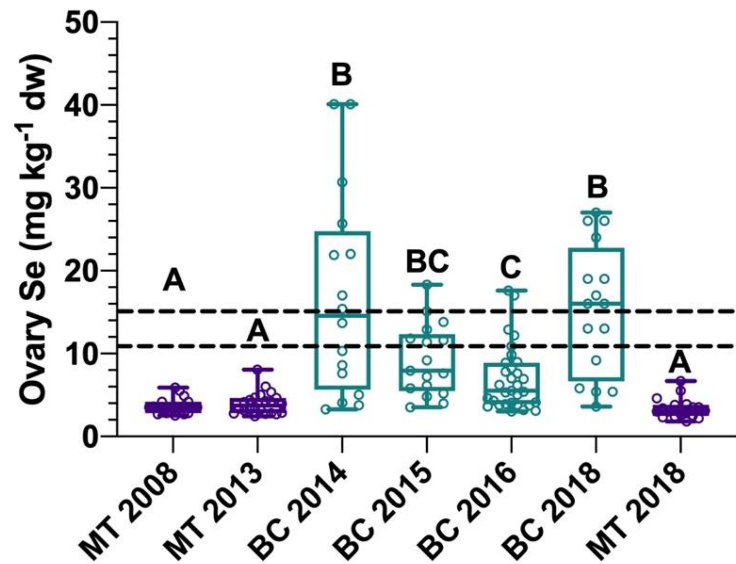

Figure 1. Ovary Se concentrations on the Montana (MT) and British Columbia (BC) sides of Koocanusa Reservoir (2008-2018). Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines. Different letters indicate significant differences ($p<0.05$).

There are several potential biases in the data collected to date that complicate the interpretation of differences in NPM ovary Se data. First, NPM typically reach spawning condition when they have a gonado-somatic index (GSI) of 8-12% (Gray and Dauble, 2001; Petersen and Ward, 1999). While GSI data are not available for fish caught in MT, only a single female on the BC side of the reservoir has been collected with a GSI in this range. The impact of collecting unripe ovaries on observed ovary Se concentrations is unknown, but much of the variability in ovary Se concentrations in the existing BC data is associated with a GSI <2%. Further 55% of ovaries collected from fish with a GSI <2% are above the BC ENV egg Se guideline of 11 mg kg^{-1} dw, while only 7% of ovaries collected from fish with a GSI >2% are above this guideline (Figure 2).

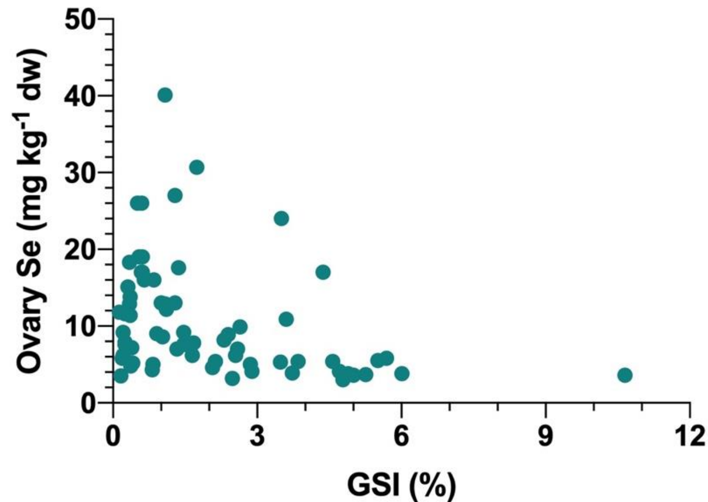

Figure 2. Relationship between ovary Se and GSI for northern pikeminnow collected on the BC side of Koocanusa Reservoir.

Second, there is a significant relationship between fish size and ovary Se concentrations (Figure 3A), and fish collected on the BC side of the reservoir tend to be smaller than those collected in MT (see Figure 3B). Collection of smaller fish on the Canadian side of the reservoir may be the result of sampling bias as fish collection has been restricted to angling, while on the MT side of the reservoir fish are collected using gill nets.

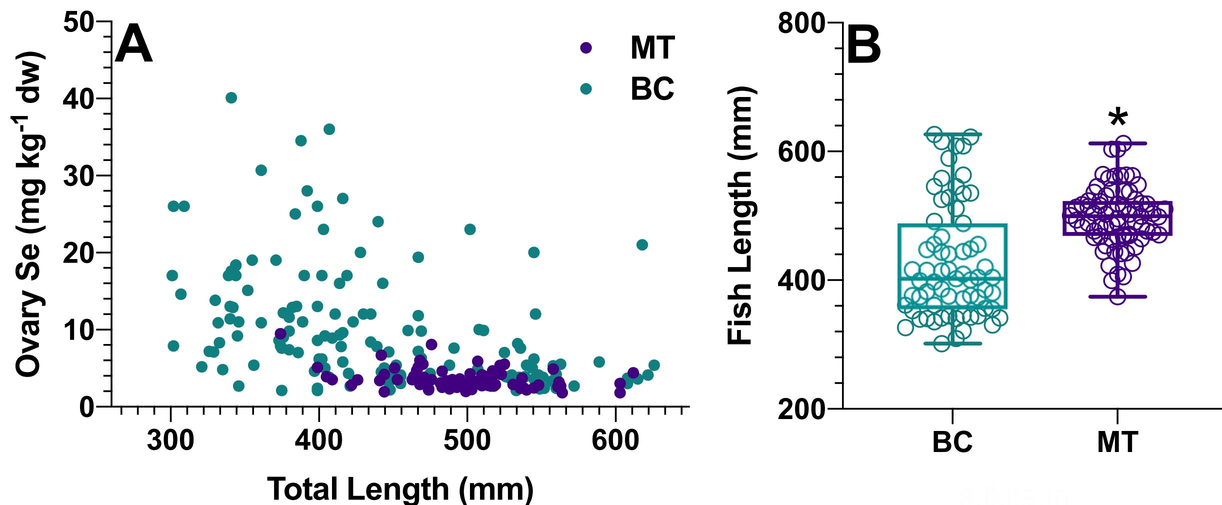

Figure 3. Relationship between ovary Se and fish length (A) and fish length distributions (B) for northern pikeminnow collected from Koocanusa Reservoir. Box plots represent mean, quartiles, maximum and minimum values. * = significant difference in the MT fish length compared to the BC fish lengths (p<0.001).

Overall, these observations suggest data collected to date on NPM ovary Se concentrations may be biased. However, this conclusion is uncertain due to the lack of ovary Se data in ripe fish, along with associated size and GSI data. Regardless of potential biases in historical ovary

Se sampling, the sensitivity of NPM to maternally transferred Se is not known. The original objectives of this study were to address both of these uncertainties.

3. OBJECTIVES AND STUDY DESIGN OVERVIEW

This study originally had two objectives:

1) To determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations; and
2) To evaluate the relationship between ovary Se concentrations and ovary development, fish size, and sampling location.

As discussed above, the inability to collect a sufficient number of female fish in spawning condition resulted in the first objective not being achieved. However, the extended effort to collect female fish in spawning condition lead to the collection of a large number (n=79 on the BC side of the reservoir) of samples for ovary Se analysis in support of the second objective. To achieve the second objective, the study had the following key elements:

1) Prior to NPM reaching spawning condition, unripe ovaries/eggs and muscle were collected and GSI measured in sexually mature females (30-60+ cm) to provide information on the relationships between ovary Se, GSI, fish size, and sampling location. As described earlier, historical data indicated fish size and sampling might influence egg Se concentrations, though these potential relationships may be confounded by other variables. The home range of NPM within the reservoir is unknown and so the extent to which ovary Se may reflect exposure to local Se sources (e.g., the Elk River) is also unknown. The developmental stage of a subset of ovaries were also characterized using histological techniques.
2) Attempts were made to collect a gradient of egg Se concentrations from ripe fish by collecting adult NPM of varying size (30-60+ cm) from several locations in Koocanusa Reservoir. This was ultimately unsuccessful but led to the collection of an increased number of ovary samples for Se analysis.

4. FIELD SAMPLING

Details of the field sampling strategy and methods employed are provided in the NPM Study Plan (EcoTox et al., 2019) and summarized here.

4.1 Sampling Strategy

There were originally two phases to the NPM field sampling program. In Phase 1 (beginning June 14, 2019), female NPM were collected from the BC side of the reservoir prior to reaching spawning condition to further characterize the effects of GSI, fish size, and sampling location on ovary Se concentrations as well as monitor spawning condition of the fish. Phase 2 sampling was

intended for once NPM reached spawning condition, and would involve collecting both male and female fish for the Se toxicity study. As only a few ripe fish were collected, Phase 2 sampling was never realized.

Although not explicitly part of this study, there was also an additional NPM sampling effort on the Montana side of the reservoir. In this effort (May 15, 2019), 15 female NPM were collected from Rexford in collaboration with personnel from Montana DEQ. This effort was made to ensure GSI data were collected and they represent the only fish from the Montana side of the reservoir for which GSI data are available.

Mature NPM were collected from various locations in Koocanusa Reservoir (BC side) using multiple sampling methods, consistent with scientific fish collection permit conditions and detailed in the NPM Study Plan (EcoTox et al., 2019). Six locations in Koocanusa Reservoir were initially identified in the study plan, but ultimately 10 locations were sampled in an attempt to collect additional females in spawning condition for the Se toxicity study (Figure 4). Sampling in these areas focused on inlets based on the assumption that NPM would congregate in these areas prior to moving upstream to spawn.

4.2 Sampling Methods

Northern pikeminnow were captured using multiple methods subject to and consistent with fish collection permit conditions. Short-set gill nets (starting with a maximum set time of 20 minutes) were used to reduce fish mortalities (Buchanan et al., 2002). Gill netting was anticipated to be the most efficient capture method and both cotton and monofilament 3-5” mesh nets were deployed. Short set times were used to avoid stress to both NPM and by-catch, particularly as species of concern, bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Onchorhynchus clarki lewisi*) are present in the reservoir. Three foot-diameter hoop nets were deployed and left to fish overnight (i.e., approximately 24 h). Cod pot traps were an additional capture method used, but not originally considered in the study plan. They function similarly to a minnow trap but on a larger scale (65” long x 40” wide, with 4” opening). These traps sit on the bottom substrate similar to the hoop net but sample a smaller area. Cod traps are quicker to deploy and pull; but are more difficult to transport as the metal frame cannot be collapsed. Similar to hoop nets, these traps were deployed and left to fish overnight (approximately 24 h). Angling was conducted from sampling boats. Angling was mainly employed between gill net sets as it has much lower catch per unit effort (CPUE) and often targets smaller individuals. Angling was also employed to scout the lower Elk River below the Elk River bridge at Kootenay Hwy 93. All fish captured were identified to species, enumerated, and all non-target individuals were released alive at the point of capture.

Northern pikeminnow sampled during Phase 1 were sacrificed by a decisive blow to the head. Fish processing and handling for tissue sampling was consistent with provincial guidelines (BC ENV, 2016).

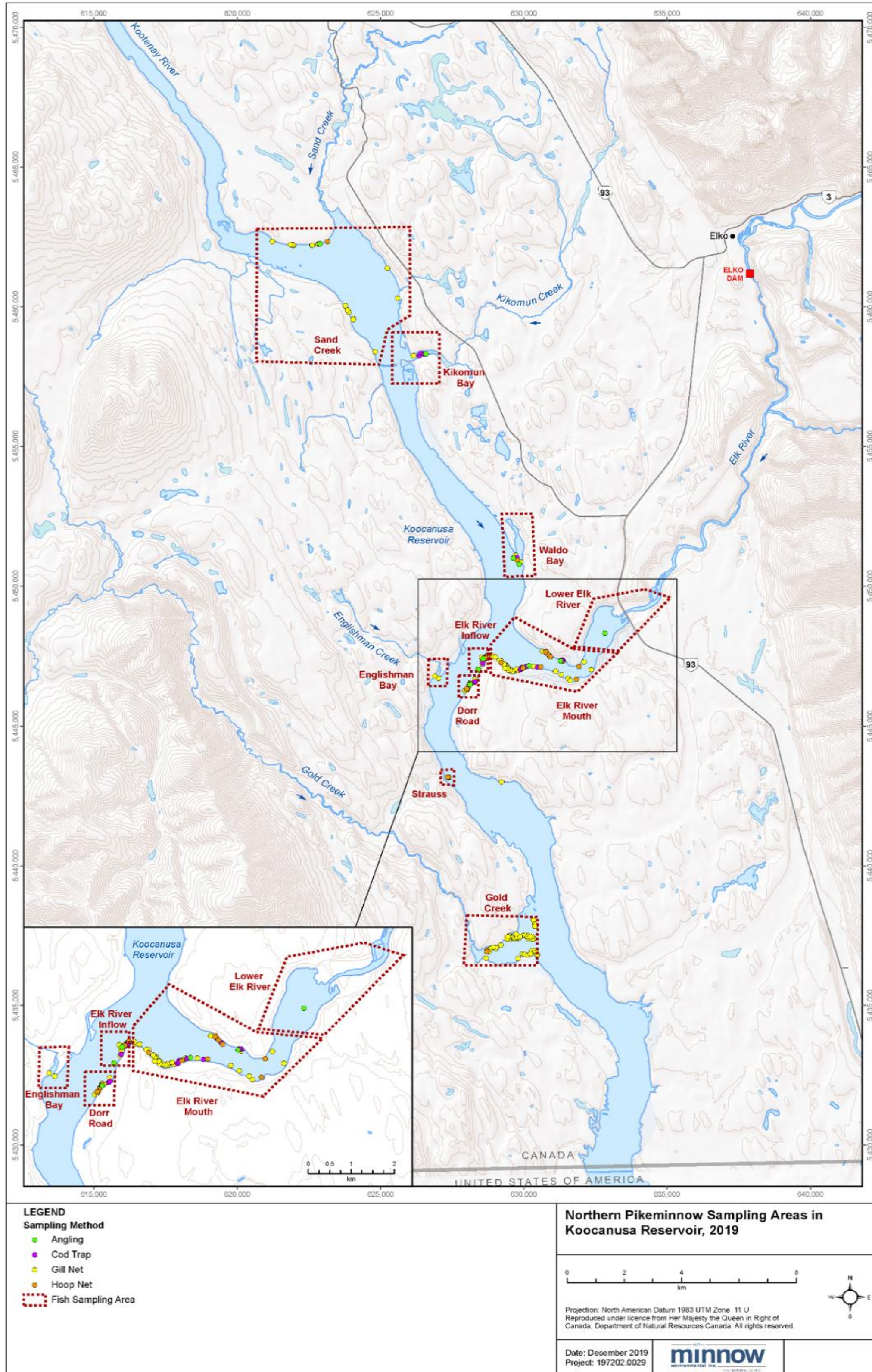

Figure 4. Northern Pikeminnow Sampling Areas on the Canadian Side of Koocanusa Reservoir.

Fish were kept on ice in coolers and transported to a dedicated field laboratory for processing as soon as possible following capture (i.e., within hours). Fork and total lengths and body weights were measured. Each fish was opened and the sex and/or sexual maturity recorded. Whole gonads and livers were removed from each fish and weighed to the nearest milligram using an analytical balance to allow for calculation of gonado-somatic indices. Whole ovaries were collected from each female and placed in separately labelled, polyethylene (Whirl-Pak®) bags. A skinless, boneless muscle fillet sample was also collected from each fish to provide supplemental data on muscle Se concentrations. Following these measures, age structures (i.e., otoliths) were removed from each fish. Each age structure was wrapped separately in waxed paper and placed inside a labelled envelope and archived for analysis. Internal or external deformities, erosions (fin and gill), lesions, or tumours (DELT) observed during processing (Sanders et al., 1999) and parasites were recorded. Tissue samples (ovaries, muscle, and age structures) were stored frozen pending shipment to the respective laboratory for analysis.

Mature female NPM retained for gonad and muscle collection were weighed and measured for total and fork length. Obvious external deformities, erosions (fin and gill), lesions, and tumours (i.e., DELT survey) and parasites observed during processing were recorded.

Ovary and muscle samples were all sent to Saskatchewan Research Council (SRC) in Saskatoon, SK for chemical analysis.

4.3 Permits

A permit for fish collection was obtained from the BC Ministry of the Environment and Climate Change Strategy (BC ENV *Application to Collect Fish for a Scientific Purpose*) and an additional permit was obtained for transport of eggs to the University of Saskatchewan (UoS) facility in Saskatoon, SK from Fisheries and Oceans Canada (License #119412), BC ENV (License #119412) and the Government of Saskatchewan (SK Import #2019-16).

5. LABORATORY METHODS

5.1 Ovarian Histology

All methods for histology preparation followed the UoS Toxicology Centre's draft standard operating procedure for histology (Appendix A). Field-collected NPM were dissected at the field laboratory and gonads excised, weighed and then immediately preserved in 10% buffered formalin. After 24 hours samples were transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming

table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

As, to the best of our knowledge, no previous studies have characterized NPM gonads histologically, oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish (OECD, 2009). Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

5.2 Analytical Chemistry

Ovary and muscle samples collected in the field were submitted for chemical analysis at SRC in Saskatoon, SK. In addition to Se, ovaries, eggs, and muscle were analyzed for 24 other elements (listed in Table 5 of the Study Plan). Results for these other elements are provided in Appendix C but are not discussed further in this report. Samples were analyzed using high resolution inductively coupled plasma mass spectrometry (HR-ICPMS). The detection limit for Se was 0.01 $\mu g\ g^{-1}$ dw. Moisture content was measured by freeze drying and results were reported on a dry weight basis along with moisture content to allow conversion to wet-weight values.

Standard quality control procedures for sample analysis were included as detailed in the Study Plan (EcoTox et al., 2019).

6. RESULTS

6.1 Fish Sampling

Four different methods were employed to capture NPM during the study: hoop nets, cod pot traps, gill nets and angling. The fish sampling was separated into 2 phases. The goal of Phase 1 was to sample approximately 36 sexually mature females of varying sizes and ranges of gonadosomatic index (GSI) values, with half being from sites directly influenced by the Elk River. This phase also allowed tracking of spawning condition within the population. When ripe fish were initially collected, Phase 2 sampling was initiated to focus on collecting fish for fertilization and assessment of larval deformities as a function of egg Se concentrations.

Different sampling methods had varying degrees of success in catching mature females and CPUE changed through the sampling period (Table 1). Monofilament gill nets were more successful than cotton mesh gill nets so after approximately two weeks remaining gill net sampling only used 3” and 4” monofilament nets. Overall, gill nets were the most successful capture method for mature females over the longest sampling period. Though gill nets had high

incidence of bycatch, survival rates were high (4 mortalities over 92 hours of effort in 9 sample areas) due to short set times. Elk Mouth, Elk Inflow and Gold Creek sites were sampled with greatest effort over the longest periods of time in response to capture success rates. Gold Creek and Elk Mouth, both at locations of tributary inflow, had the highest CPUE through the last weeks of June and tended to decrease through July. Elk Inflow area, where the Elk tributary inflow opens into the reservoir, had peak CPUE through mid-July then drastically declined moving into the last two weeks of July (Table 2).

Table 1. Female northern pikeminnow CPUE and total effort (hours) by gear type and sampling area.

CPUE	Dorr Rd.	Elk Inflow	Elk Mouth	Lower Elk	Sand Cr.	Gold Cr.	Englishman Bay	Strauss	Waldo Bay	Kikomun Bay	Total Gear CPUE	Total Gear Effort
Gill Net	5.24	60.00	55.34	-	7.78	136.61	0.00	8.11	0.00	-	273.08	
Gill Effort	7.68	18.00	19.47	-	10.85	31.45	1.70	1.50	1.20	-		91.85
Hoop Net	20.20	0.04	1.47	-	0.17	0.04	-	0.04	0.34	-	22.30	
Hoop Effort	596.25	67.68	492.08	-	46.43	93.50	-	95.18	117.37	-		1508.50
Cod Trap	0.43	0.19	0.25	-	-	-	-	-	0.00	0.04	0.91	
Cod Effort	238.43	237.76	420.62	-	-	-	-	-	24.00	99.42		1020.23
Angling	0.25	4.00	0.00	0.00	0.67	-	-	-	0.00	0.00	4.92	
Angling Effort	4.00	9.83	0.07	6.00	1.50	-	-	-	0.66	1.00		23.06
Total CPUE	26.12	64.23	57.06	0.00	8.62	136.65	0.00	8.15	0.34	0.04	**301.21**	
Total Effort	846.36	333.27	932.24	6.00	58.78	124.95	1.70	96.86	143.23	100.42		**2643.64**

Table 2. Gill net CPUE at most successful sample areas through the sampling period.

CPUE	Elk Inflow	Elk Mouth	Gold Creek
June	18.00	35.20	62.71
July 1-7	Not sampled	15.00	57.40
July 8-14	128.96	5.14	Not sampled
July 15-21	9.50	0.00	8.05
July 22-26	0.00	0.00	8.44

When sampling commenced many mature females had higher GSI than anticipated and there was difficulty capturing low GSI/large individuals and high GSI/small individuals. More fish were processed in an effort to capture the desired range of GSI and size. As the field season progressed and few ripe fish were captured, more fish were processed than originally anticipated with a total of 79 fish processed by end of the study (Table 3). This allowed inclusion of a greater GSI and size range as well as a range of egg development for histology analysis (15 fish). The high GSI/small size categories were eventually captured at Elk influenced sites but not at other sites (Table 3 and Figure 5).

Table 3. Fish GSI and total length for Rexford, Elk River and all other sites combined.

GSI/SIZE	300-400 mm	401-500 mm	501-600+ mm
Rexford, MT (n=15)			
<2%	2	6	0
2-4%	0	0	7
4-7%	0	0	0
7+%	0	0	0
Elk River, BC (n=49)			
<2%	3	6	1
2-4%	3	3	2
4-7%	2	3	3
7+%	3	11	9
Other Sites, BC (n=30)			
<2%	9	6	1
2-4%	0	2	1
4-7%	1	2	1
7+%	0	3	4

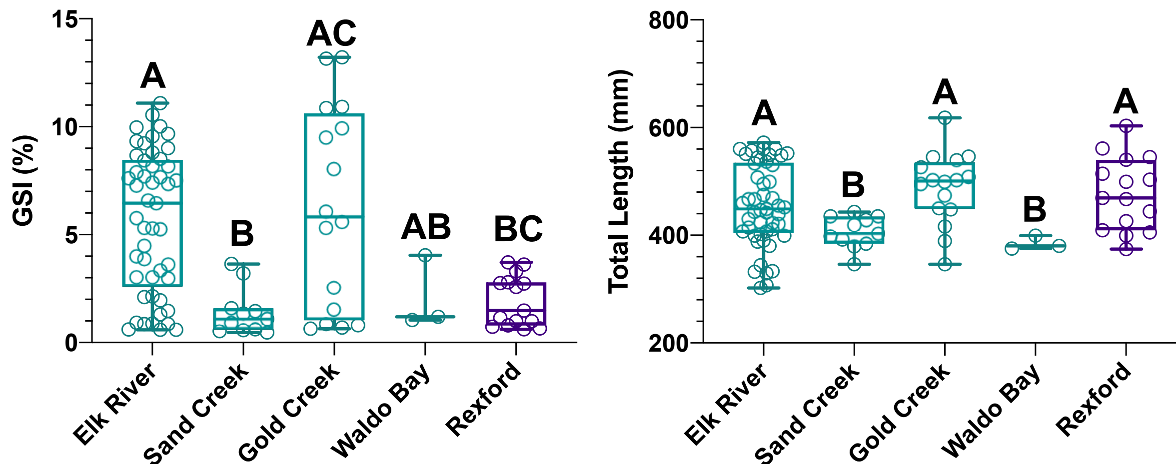

Figure 5. Northern pikeminnow GSI and total length by location for samples collected in 2019. Different letters indicate statistically significant differences ($p<0.05$) as determined by ANOVA.

6.2 Ovarian Histology

Between July 8 and 19, 2019, ovaries from 15 NPM were collected for histological analysis of maturation stages of oocytes across fish of different sizes and reproductive development (weight: 250 – 1800 g; fork length: 33.2 – 61.8 cm) and GSI (range: 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (Figure 6 and Table 4-1 in Appendix A). While there was no obvious relationship between the size of fish and GSI, there was a clear correlation between GSI and ovarian maturation stage (Figure 7) with fish having GSI >5% all being at oocyte maturation stage 3, with one exception. Similarly, there was a significant linear relationship between late stage vitellogenic oocytes (LVO) and GSI ($r^2 = 0.81$), revealing that ovaries of mature fish with a GSI > 5% consisted of over 50% LVOs (Figure 6).

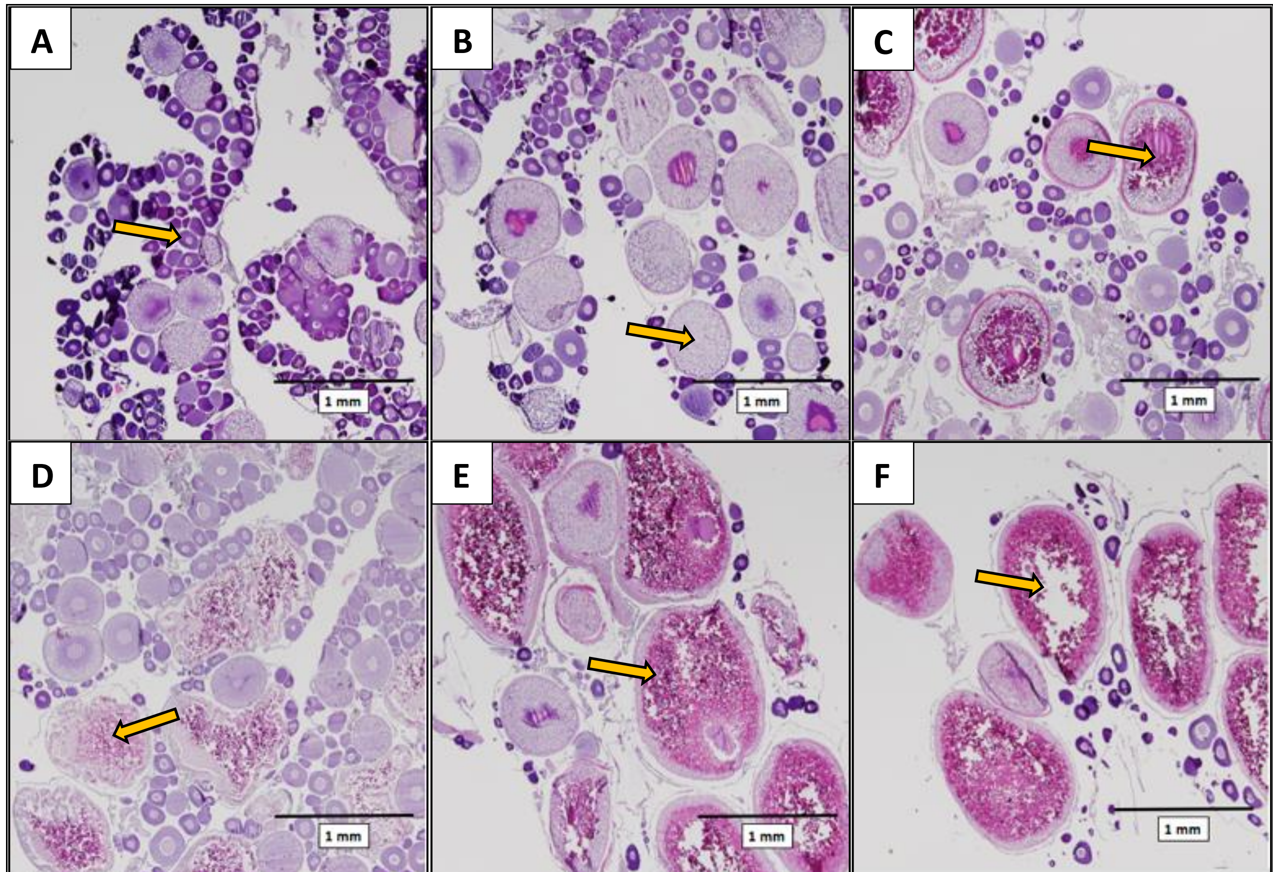

Figure 6. Histomicrographs of ovaries of northern pikeminnow representing early development stages. (Stage 1) consisting mainly of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E and F; Arrows).

Multivariate Analysis of Ovary Se Data

Analysis of historical ovary Se data for NPM suggests there are significant relationships with sampling location, fish size, and GSI (Figures 1-3). However, the historical data set lacks information on GSI for fish from the Montana side of the reservoir, may be confounded by correlations between fish size and GSI, and is limited for fish with a relatively high GSI (>5%). The sampling program for this study was designed to address these limitations and provide a robust dataset for evaluating the influence of multiple factors on ovary Se concentrations in NPM.

Historical sampling data (collected 2013-2018) were collated with data collected from 2019 (Appendix B). An initial exploratory analysis of natural log (ln)-transformed data was conducted by Principal Component Analysis (PCA) (prcomp, R) using z-scores of independent variables (total length, GSI, body weight, and gonad weight) to identify correlations among these variables and select the most appropriate variables for linear

modeling. The first two axes of the PCA with four input variables explained 99% of the variance in the four variables. Bivariate relationships among independent variables, and bivariate relationships between ovary Se and independent variables were plotted by area and year to help visualize effects of area and year on relationships. Natural log (ln) transformations of total length and body weight were highly correlated (R = 0.98), and a biplot from the first PCA with all four variables showed very similar relationship between body weight and total length and final PC scores. Because GSI includes body weight in its denominator, and body weight and total length were highly correlated, body weight was removed from the independent variables used in the MLR and total length was used as a measure of fish size in the model to reduce collinearity (Figure 8).

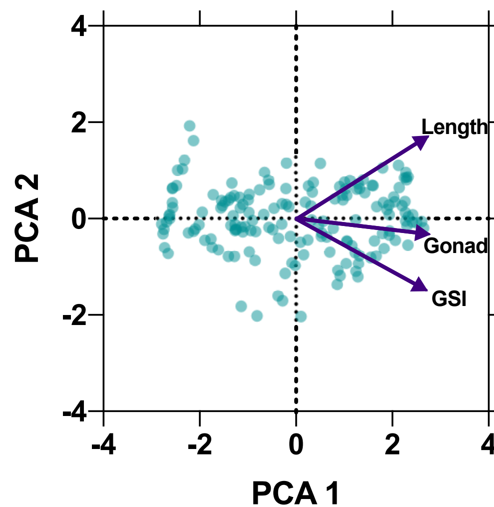

Figure 8. Biplot from PCA using z-scores of ln-transformed total length, gonad weight, and GSI.

After selecting initial independent variables (length, gonad weight, and GSI), exploratory linear and multiple linear regressions (MLR) were conducted to predict ln ovary Se for various subsets of the data. For example, models using one or more independent variables were developed for data sets for individual years, different combinations of years, individual locations, and different combinations of locations. These exploratory analyses were intended to gain a better understanding of how the data were distributed as a function of the independent variables, location, and sampling year. Based on these exploratory analyses, we concluded that the initial models should be developed using only the 2019 data because these data had been collected with a more balanced design of GSI and fish size classes than earlier data. Developing an initial model based on data from multiple years could introduce biases due to the incomplete sampling design with respect to the independent variables being evaluated.

The first model was developed to test for differences between area-specific slopes and intercepts with stepwise analysis using Bayesian Information Criterion (BIC) and to identify final models (Eq. 1). The contrasts used to test for area-specific intercepts tested for differences between Elk influenced sites and other sites.

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) +

Ln(GW) + area* Ln(GW) (Eq. 1)

where, OvSe = ovary Se, TL = total length (cm), GSI = gonadosomatic index, and GW = gonad weight. Variance inflation factors (VIFs) were relatively high for this model (>7) (Zuur et al., 2010) and gonad weight was not retained in the BIC version of the model, so gonad weight was removed and a second model was developed (Eq. 2).

Ln(OvSe) = area + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) (Eq. 2)

Area-specific slopes were not retained in the BIC model, resulting in a final model with area-specific intercepts and pooled slopes. Exclusion of area-specific slopes means that relationships between independent variables (total length and GSI) and ovary Se are statistically similar between sites. Retention of area-specific intercepts indicates that while differences in fish size and GSI between sites explains some of the observed differences in ovary Se concentrations, there are also statistically significant differences in ovary Se concentrations between some sites independent of the influence of fish size and GSI. This model performed reasonably well in terms of predicting ovary Se concentrations for the data on which it was based (Adj. $R^2 = 0.72$; Figure 9). Further, the predicted R^2 of 0.69 is just slightly lower than the adjusted R^2 of 0.72, indicating the model is not over-parameterized or unduly influenced by individual data points.

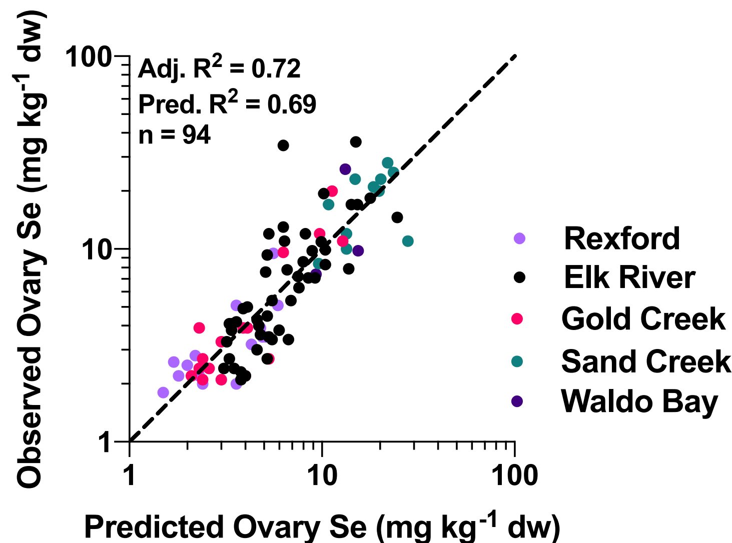

Figure 9. Ovary Se MLR model based on 2019 data only (see Eq. 2).

Once the MLR model based on data from 2019 only was developed, data from years prior to 2019 were then evaluated using the 2019 MLR model. Exploratory analyses indicated that data from 2014 and 2015 did not fit the model well. The majority of samples for 2014 were collected in February with the remaining 2014 samples and all 2015 samples collected in April. As would be expected given the sampling times, GSI was low in both data sets. The 2015 data set in particular consisted of fish with GSI <0.5%, which appears to introduce non-linearities into the overall relationship between GSI and ovary Se (Figure 10). Consequently, we opted to exclude the 2015 data from further analysis. It may have been possible to include the 2014 data set in the model, but given the limited amount of data (n=5) and limited ranged of GSI, we opted to exclude it from the analysis as well. Consequently, a model was fit using data from 2016-2019. The full model included "year" as a term to test for differences in ovary Se concentrations between years and as before was evaluated using BIC to select the most parsimonious variables for inclusion (Eq. 3).

$$Ln(OvSe) = area + year + Ln(TL) + area * Ln(TL) + Ln(GSI) + area * Ln(GSI) \quad \text{(Eq. 3)}$$

The final model selected by BIC using all data from 2016-2019 (n=141) retained the same variables as the model using only 2019 data with only slight differences in the model coefficients (Table 4). Adjusted and predicted R^2 for the BIC model were 0.67 and 0.65, respectively (Figure 11). The model tested for effects of year and area as well as area-specific slopes. Again, area-specific slopes were not retained in the BIC model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (ovary Se) across sites. Similarly, year was not retained as a factor in the model indicating there were no significant differences across the three sampling years (2016, 2018, and 2019) included in the analysis. Significant differences in area-specific intercepts were observed and retained in the model. The intercepts for both Gold Creek and Rexford were significantly lower than the Elk intercept ($p = 0.01$ and $p < 0.01$, respectively) indicating that after accounting for the influence of fish length and GSI, ovary Se concentrations in fish collected from Gold Creek and Rexford were significantly lower than those observed for fish collected near the Elk River (Table 4).

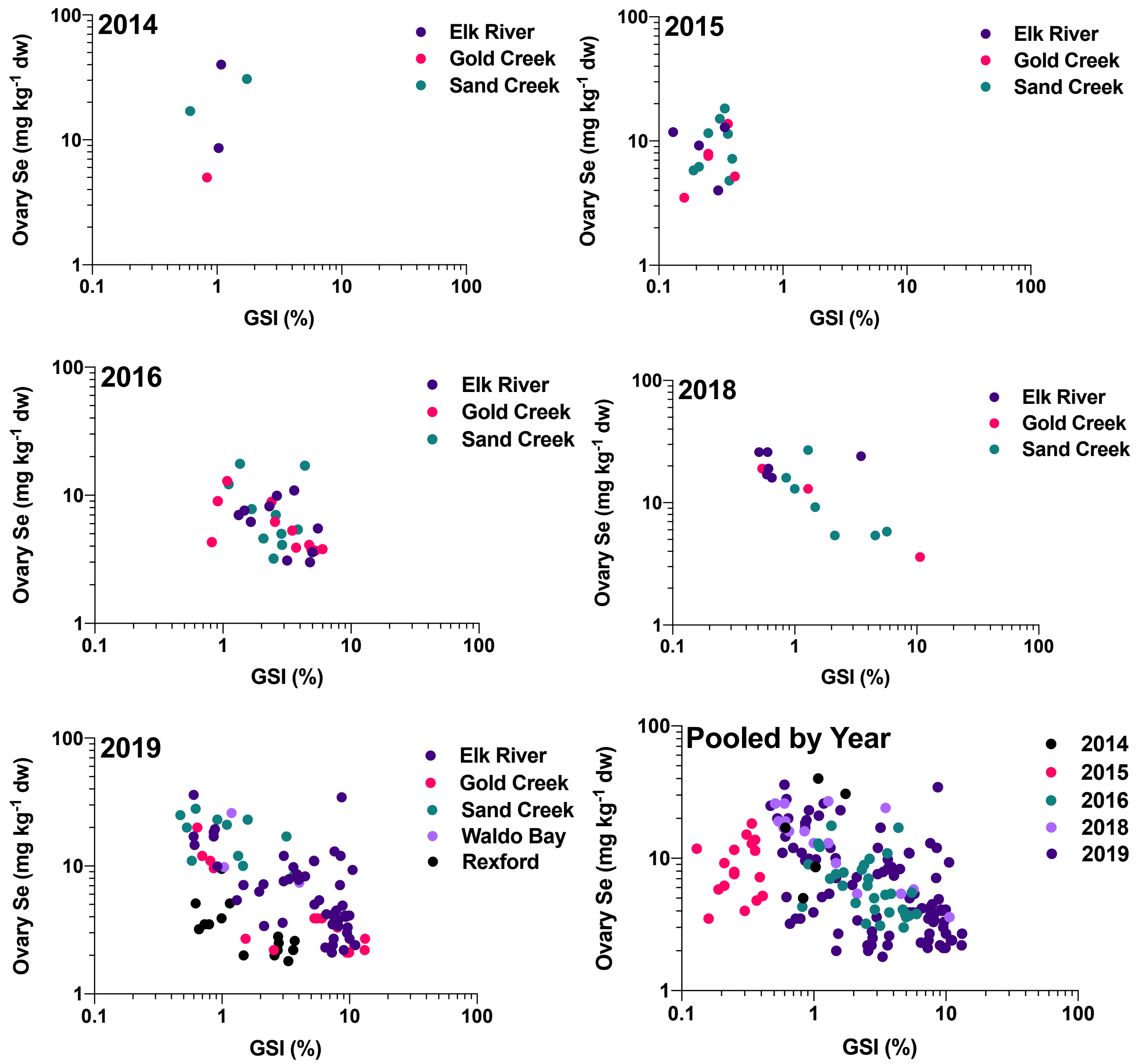

Figure 10. Relationship between GSI and Ovary Se by Year

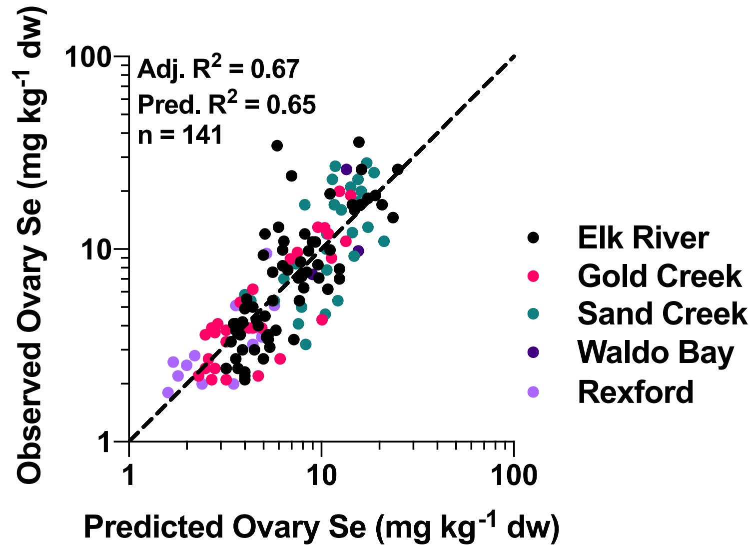

Figure 11. Final Ovary Se MLR model based on 2016-2019 data.

Table 4. Final ovary Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	7.94	0.96	8.31	-	
	Gold	7.66	0.10	-2.81	0.01	
	Sand	7.98	0.10	0.45	0.65	
	Waldo	8.02	0.26	0.33	0.75	
	Rexford	6.91	0.13	-7.62	<0.01	
Slopes	Ln Total Length	-1.45	0.26	-5.62	<0.01	-0.289
	Ln GSI	-0.39	0.05	-8.08	<0.01	-0.493

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that GSI (standardized slope = -0.49) has a stronger effect on ovary Se concentrations in the model than total length (standardized slope = -0.33) over the range sampled for these variables (Table 4). Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model appear to have equal variance (p = 0.145) but may not be normal (p = 0.031).

One potential caveat to this model is that the PCA analysis indicates a level of correlation between total length and GSI, as both variables have positive associations for PC1, though opposite associations for PC2 (Figure 8). A simple correlation analysis indicates these two variables are somewhat correlated (r=0.41; Figure 12). This correlation is primarily caused by the lack of data for fish with a total length >54 cm and GSI <3%. This observation is supported by the lack of a significant correlation (p >0.05) between total length and GSI for fish with a GSI >3%. It is unclear whether this data gap is due to sampling bias or some

mechanistic reason why fish in this category are not observed, though the former seems more likely. Regardless, this correlation introduces some uncertainty into the ovary Se model. While VIFs for the model were low (1.4 for both total length and GSI) suggesting the correlation is not unduly influencing the model, the full influence of this correlation can be difficult to characterize.

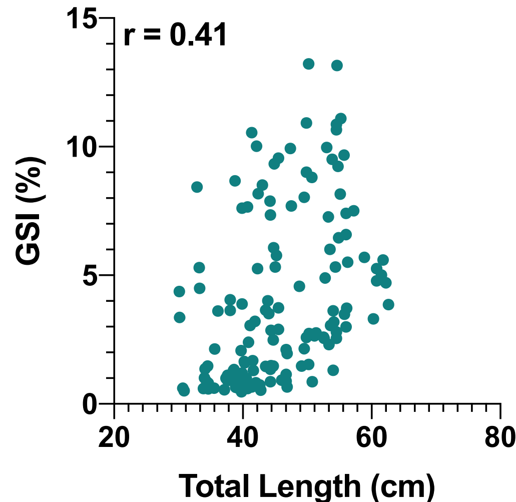

Figure 12. Correlation between total length and GSI in northern pikeminnow (2016-2019).

We further evaluated this issue by constraining the data set to only those data with GSI >3% (n = 69) where there is no correlation between fish length and GSI and re-parameterized the model. The resulting MLR model still retained both GSI and total length as variables (Adj. $R^2 = 0.55$), again supporting the premise that both variables are important predictors of ovary Se. However, the standardized model coefficients changed with total length (-0.49) now more important than GSI (-0.35). This reversal in relative importance of standardized model coefficients may simply be the result of constraining the original data set by ~50% or it could be an indication that the correlation between total length and GSI in the full data set is influencing the way variance is partitioned in the model.

Ultimately, the uncertainties associated with the correlation between total length and GSI appear to have relatively modest impacts on model predictions of ovary Se. Based on the final model using the full data set, differences in area-specific intercepts between sites would translate to predictions of ovary Se concentrations being, on average, 2.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold Creek and Elk River ovary Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.3 times higher than those from Gold Creek for a given fish length and GSI. Estimated mean ovary Se concentrations for small (30 cm) and large (60 cm) females with a

GSI of 6% (a conservatively low GSI for female fish ready to spawn) are below the BC ENV egg Se guideline (11 mg^{-1} kg dw) at all sampling locations using the MLR model based on the full data set. In comparison, the MLR model based on the data set constrained to a GSI >3% (i.e., the data set with no correlation between total length and GSI) provides higher estimates of mean ovary Se for small (30 cm) fish (Table 5), but estimates are generally within 30% of those using the MLR model based on the full data set. The somewhat larger increase in estimated mean ovary Se for Sand is the result of a higher intercept using the constrained data set.

Table 5. MLR model estimated mean ovary Se concentrations in female northern pikeminnow collected from different locations in Koocanusa Reservoir as a function of fish size and GSI. Estimated provided for the MLR model based on all data and the model based only on data where GSI was >3%.

Site	Fish Length (cm)	GSI (%)	Estimated Mean Ovary Se ($mg\ kg^{-1}$ dw) All Data	Estimated Mean Ovary Se ($mg\ kg^{-1}$ dw) Data with GSI >3%
Elk River	30	6	10.1	11.6
	60	6	3.7	3.5
Gold Creek	30	6	7.6	9.3
	60	6	2.8	2.8
Sand Creek	30	6	10.5	15.0
	60	6	3.8	4.6
Waldo Bay	30	6	10.9	9.0
	60	6	4.0	2.7
Rexford	30	6	3.6	5.0
	60	6	1.3	1.5

6.4 Multivariate Analysis of Muscle Se Data

Concurrent with ovary sampling, muscle samples have also been collected and analyzed for Se concentrations. The muscle Se data is a potential second line of evidence to support the observations and conclusions from the ovary Se analysis. As has been demonstrated in other species (USEPA, 2016), ovary Se and muscle Se concentrations in NPM are correlated (Figure 13). Consequently, observations based on ovary Se concentrations regarding the effects of fish size and sampling location are expected to also be observed for muscle Se data. While there is no identified mechanistic link between GSI and muscle Se concentrations, it is possible a correlation between GSI and muscle Se might be observed given the correlations between ovary Se and muscle Se, as well as total length and GSI in the data set.

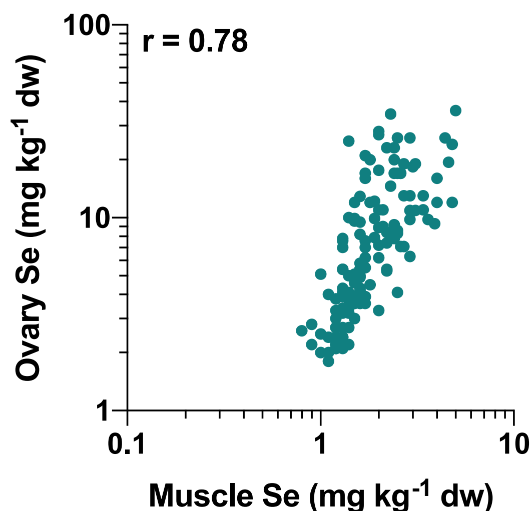

Figure 13. Correlation between muscle Se and ovary Se concentrations in NPM collected from Koocanusa Reservoir (2016-2019).

The multivariate analysis of muscle Se data used the same general multivariate approach described above for ovary Se data. The same data used in the ovary Se analysis was used in the muscle Se analysis for comparability except for a single fish collected from the mouth of the Elk River in 2016 for which no muscle data were collected (n = 140). Given the results of the ovary Se analysis, an initial model using only the 2019 data was not developed for muscle. Instead, the full data set (2016-2019) was used to evaluate the same general full model:

Ln(muscle Se) = area + year + Ln(TL) + area* Ln(TL) + Ln(GSI) + area* Ln(GSI) (Eq. 4)

The model selected by BIC retained both total fish length and GSI as variables. Adjusted and predicted R^2 for the BIC muscle Se model were lower than for the ovary Se model at 0.47 and 0.45, respectively (Figure 14). The lower performance of the muscle Se model appears to be driven by underprediction of the relatively high muscle Se data for fish collected from the mouth of Elk River, although area-specific slopes were not retained in the model indicating there were no significant differences in the relationship between the independent variables (total length and GSI) and dependent variable (muscle Se) across sites (Table 6). Significant differences in area-specific intercepts were identified in the model. The intercepts for Gold Creek, Sand Creek and Rexford were all significantly lower than the Elk intercept ($p < 0.01$) indicating that after accounting for the influence of fish length and GSI, muscle Se concentrations in fish collected from all three locations were significantly lower than for fish collected near the Elk River (Table 6). Based on the final model, differences in area-specific intercepts between sites would translate to predictions of muscle Se concentrations being, on average, 1.8 times higher for fish collected from the mouth of the Elk River compared to fish collected from Rexford for any given fish length and GSI. The differences between Gold and

Sand Creeks versus Elk River muscle Se concentrations are smaller, with Elk River ovary Se concentrations predicted to be, on average, 1.2-1.3 times higher than those from Gold and Sand Creeks for a given fish length and GSI.

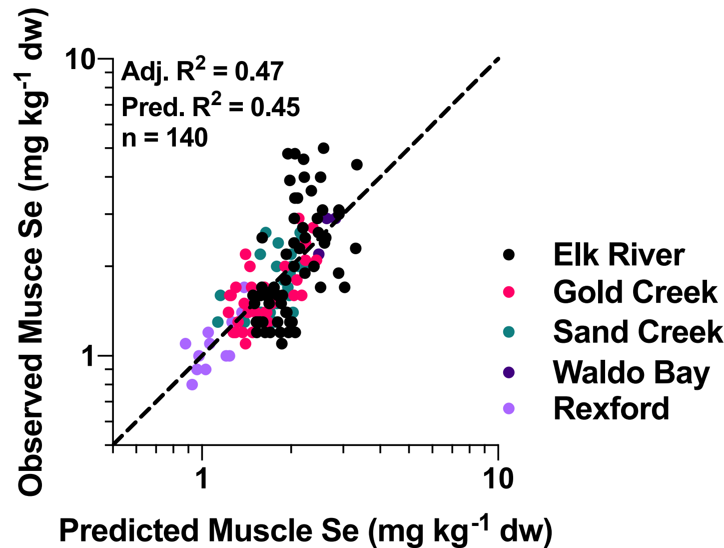

Figure 14. Final Muscle Se MLR model based on 2016-2019 data.

Table 6. Final muscle Se model coefficients and significance. Note: The t value and *p* value relate to testing for significant differences in intercepts relative to Elk.

		Estimate	Std. Error	t Value	*p* Value	Standardized Regression Slope
Intercepts	Elk	4.197	0.607	6.92	-	
	Gold	4.022	0.063	-2.77	<0.01	
	Sand	3.919	0.066	-4.21	<0.01	
	Waldo	4.263	0.167	0.40	0.69	
	Rexford	3.619	0.085	-6.76	<0.01	
Slopes	Ln Total Length	-0.889	0.164	-5.41	<0.01	-0.406
	Ln GSI	-0.086	0.030	-2.81	<0.01	-0.215

Standardized slope coefficients provide a relative measure of the slope of multiple independent variables. Standardized slope coefficients indicate that total fish length (standardized slope = -0.41) has a stronger effect on muscle Se concentrations in the model than GSI (standardized slope = -0.21) over the range sampled for these variables (Table 5). This is the opposite of what was observed for ovary Se, but again, should be treated with caution given the correlation between total length and GSI. Normality and homoscedasity of residuals were tested using the Shapiro Wilks test for normality (shapiro.test, R) and the Nonconstant Variance test (ncv, R). Residuals of the final model have unequal variance (p = 0.001) and are not normally distributed (p = 0.001) again demonstrating the model has some systematic biases.

7. DISCUSSION AND CONCLUSIONS

The objectives of this study were to: 1.) determine the effects of Se on early life stages of NPM across a range of maternally-derived egg Se concentrations, and 2.) to evaluate the effects of GSI, fish size, and sampling location on ovary and muscle Se concentrations. The first objective was not achieved due to the inability to collect a sufficient number of ripe female NPM. In the remainder of this report, the success in achieving the second objective and implications of study findings are discussed.

7.1 Effects of GSI, Fish Size, and Sampling Location on Ovary Se Concentrations

Historical monitoring data suggest GSI, fish size, and sampling location may influence ovary Se concentrations, but the data are confounded by relatively small sample sizes, are unevenly distributed for some variables (e.g., GSI), and potentially auto-correlated. To address these limitations, a total of 94 additional ovary Se samples were collected in 2019 that were relatively evenly distributed across size classes and to a lesser extent GSIs.

Using 2019 data and incorporating most of the data from historical monitoring (total n = 141), an MLR model that characterizes ovary Se concentrations as a function of fish size (total length) and GSI was developed. While the model has some uncertainties related to the correlation between total fish length and GSI in the data set used for model development, the conclusion that total length and GSI are important predictors of ovary Se concentrations in NPM appears robust and predictions using a constrained data set with no correlation between independent variables are generally within 30% of the model based on the full data set.

There were several key findings from this model. First, the model indicates that fish with lower GSI have higher ovary Se concentrations independent of any other variables. This indicates that fish collected early in the year (e.g., February-May) have ovary Se concentrations that overestimate the egg Se concentrations that the fish will have at the time of spawning. The mechanism underlying this reduction in egg Se with development is currently unclear. Transfer of Se into the eggs is known to be associated with vitellogenesis (Janz et al., 2010) and the ovarian histology component of this study demonstrates vitellogenesis coincides with egg development and an increase in GSI, as is typical of most teleost fish. Consequently, an increase in egg Se rather than a decrease in egg Se would be expected with increasing GSI. However, there are many species-specific complexities to the process of vitellogenesis including variations in the use of multiple vitellogenin isoforms, variations in timing of primary and secondary vitellogenesis, and the level of processing of vitellogenin in the egg and associated level of water absorption (Hara et al., 2016). These processes could all influence how Se concentrations in eggs change during the course of egg development and to the best our knowledge, have not been studied in any detail in NPM or closely related species. However, regardless of the mechanism, the reduction in ovary Se concentrations with increasing GSI is important for assessing potential Se risks to NPM in the

reservoir as it is the ovary/egg Se concentration at the time of spawning that should be compared to an egg Se effect concentration.

Given this finding, ovary Se data from fish with low GSI (i.e., <5%) should be excluded from the data set when assessing potential risk to NPM. The oocyte maturation study demonstrated a strong positive relationship ($R^2 = 0.81$) between GSI and oocyte development. Fish where the majority of oocytes in an ovary were stage 3 (late vitellogenic) oocytes were associated with a GSI >5% (Figure 6). Consequently, only ovaries collected from fish with a GSI ≥5% should be used in assessing Se risks to NPM as these ovaries are likely to provide a relatively unbiased estimate of egg Se concentrations for comparison to egg Se toxicity thresholds. Using this data usability qualifier (GSI ≥5%) restricts the ovary Se data set. All data prior to 2016 are eliminated from assessment due to either low GSI or GSI not being reported and the distribution of ovary Se concentrations are significantly lower (Figure 15). Of all the samples collected from fish with a GSI >5% (n=45), only a single fish has exceeded the USEPA egg Se criteria of 15.1 mg kg^{-1} dw and only 4 fish have exceeded the BC ENV guideline of 11 mg kg^{-1} dw.

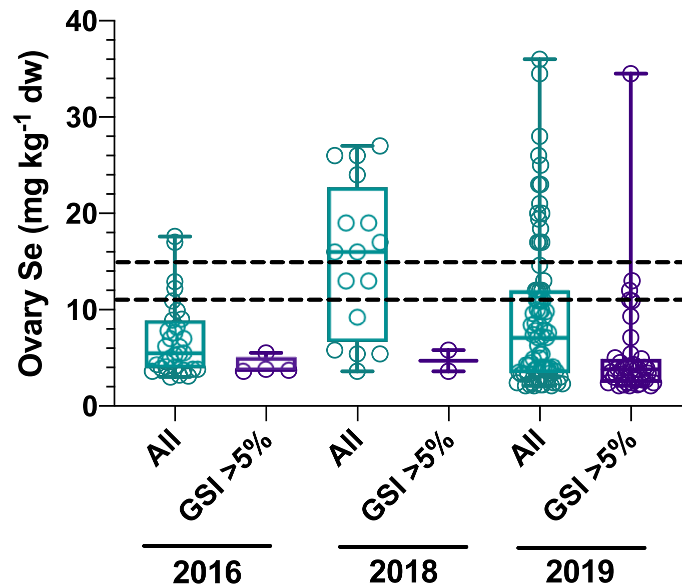

Figure 15. Comparison of ovary Se concentrations for all fish versus only fish with GSI ≥5%. Box plots represent mean, quartiles, maximum and minimum values. Dashed lines indicate BC ENV (11 mg kg^{-1} dw) and USEPA (15.1 mg kg^{-1} dw) egg Se guidelines.

The second significant finding from development of the ovary MLR model was that fish size has a significant effect on ovary Se concentrations in NPM, with smaller fish having higher ovary Se concentrations. This is likely the result of differences in dietary preferences between small and large adult NPM. Small adult NPM (<30 cm) typically have a primarily insectivorous diet, but become increasingly piscivorous with increasing size, feeding

primarily on juvenile salmonids (Clarke et al., 2005; Petersen, 2001; Zimmerman, 1999). Whole body trophic transfer factors (TTFs) for fish (i.e., invertebrate to fish or fish to fish) are typically <1 except at very low (<1 mg kg^{-1} dw) dietary Se concentrations (DeForest et al., 2007). A consequence of TTFs <1 is that consumers at progressively higher trophic levels will have progressively lower whole body Se concentrations (i.e., biodilution). This may explain the size effect observed in the current analysis as small NPM feeding on insects would be expected to have higher Se exposure than large NPM which have a higher trophic level and are feeding on juvenile salmonids.

The third, and final, significant finding resulting from the ovary Se MLR model was identification of effects of sampling location on ovary Se concentrations. By accounting for the influence of fish size and GSI on ovary Se, the model was able to test for differences in ovary Se concentrations between sampling locations. Results from this analysis indicate that fish collected from Gold Creek and Rexford have significantly lower ovary Se concentrations than locations sampled higher in the reservoir. Locations higher in the reservoir are generally closer to the Se input from the Elk River although the Sand Creek sampling location is further from the Elk River than the Gold Creek sampling location (Figure 4).

7.2 Effects of GSI, Fish Size, and Sampling Location on Muscle Se Concentrations

The muscle Se MLR model was not as robust as the ovary Se MLR model (Figures 11 and 13). There are likely several reasons for this outcome. First, the range in muscle Se concentrations (0.8-5.0 mg kg^{-1} dw) is much less than observed for ovary Se concentrations (1.8-36 mg kg^{-1} dw). Consequently, small errors in analytical precision will introduce significantly more variance in the muscle Se MLR model. Second, although apparently not significant enough to be detected by the BIC analysis, the muscle Se data collected from the mouth of the Elk River qualitatively appear to have a systematic bias (i.e., different slope) with respect to the MLR model, over-predicting low muscle Se concentrations and under-predicting high muscle Se concentrations.

Despite the muscle Se model being less robust, it did generally support the observations of the ovary Se model. Specifically, the muscle model supports that fish size is an important variable in determining NPM Se concentrations in Koocanusa Reservoir (Table 5). It also supports observations that fish collected from the mouth of the Elk River have higher Se tissue concentrations than fish from most other locations sampled in the reservoir (Table 5).

The finding that GSI is a significant variable in the muscle Se model was somewhat unexpected. Mechanistically, there is no obvious reason why GSI would be an important determinant of muscle Se concentrations. It is possible that retention of GSI in the model is simply an artifact of GSI being an important variable in predicting ovary Se and ovary Se being generally strongly correlated to muscle Se, or that total length and GSI are somewhat correlated. Supporting this hypothesis is the observation that the standardized slope for GSI is

only half of the slope for fish size (Table 6), indicating it has proportionally less influence on muscle Se concentrations while whereas the opposite is true for ovary Se where the standardized slope for GSI is twice as steep as for fish size (Table 4).

7.3 Conclusions and Recommendations

A primary objective of this study was to determine the sensitivity of embryo-larval NPM to maternally transferred egg Se, which was not achieved due to the limited number of ripe female fish collected. However, the second objective of this study to evaluate the effects of fish size, GSI and sampling location on NPM ovary Se was successfully accomplished. Results from this effort indicates that all three variables influence ovary Se concentrations. Importantly, the study concludes that ovary Se data collected from fish with a GSI <5% should not be used to assess Se risks to NPM as these data over-estimate egg Se concentrations. However, the study also demonstrates that small adult NPM have higher egg Se concentrations than large NPM likely due to a predominantly insectivorous diet and that NPM near the Elk River and further upstream (i.e., Sand Creek) have higher egg Se concentrations than those collected further down the reservoir.

Based on these results, this sub-population (small adult fish that reside in the upper reservoir) of NPM likely exhibit higher egg Se concentrations than the overall NPM population in the reservoir, although the mean ovary Se concentration is still predicted to be below the BC ENV egg Se guideline. The relative size of this sub-population and distribution of egg Se concentrations within it is not well characterized, but current results suggests understanding the sensitivity of NPM to maternally transferred egg Se concentrations may still be important. Consequently, additional sampling to characterize the distribution of ovary Se concentrations in NPM in the upper reservoir and to conduct a toxicity study to determine their sensitivity to egg Se concentrations is recommended.

The main limitation of the 2019 Se toxicity study was an inability to capture a sufficient number of ripe female NPM. It is currently unclear why there was so much difficulty collecting a greater number of ripe females. Relatively large numbers of females were collected in the second half of June with GSIs in the range expected for ripe females (Table 2). This continued into early July, but despite relatively high GSIs, only a few fish manually expressed eggs. By mid-July, the CPUE began to drop rapidly and fish that had already spawned began to be captured. As the field season progressed, it was apparent that NPM were not continually congregating in the same areas during the presumed spawning period. Where abundant ripe males were found one day, no ripe males were present only two days later. It was expected that ripe females would be present in these congregations of males or join them days after they were located. This was not the case. Considerable effort (2,644 fishing hours) was invested using four different capture methods across a large spatial scale (~30 km reach of the reservoir). Although an abundance of mature females with high GSIs were captured in

the first four weeks of sampling, CPUE dropped drastically through the last two weeks without locating the desired numbers of ripe females.

A clearer understanding of where Koocanusa NPM populations are spawning is needed, including whether it occurs in congregations and whether tributaries are possible spawning habitat areas. Some changes in gear use, particularly setting gill nets during dusk and dawn, may increase capture rates, but this introduces new safety issues for crews, which will need to be addressed in planning. Greater capture success may result from investing efforts in tracking NPM movements within Koocanusa Reservoir. Understanding NPM movements provides possibilities for improving understanding of habitat use during spawning and allows more focused fishing efforts in suspected spawning sites.

8. CLOSURE

We trust this report provides sufficient information for your present needs. Should you have any questions, please do not hesitate to contact Kevin Brix at (305) 773-8347.

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APPENDIX A

UNIVERSITY OF SASKATCHEWAN SELENIUM TOXICITY REPORT

APPENDIX B

NORTHERN PIKEMINNOW OVARY SELENIUM, MUSCLE SELENIUM, AND GSI DATA FOR KOOCANUSA RESERVOIR: 2008-2019

APPENDIX C

NORTHERN PIKEMINNOW METAL TISSUE DATA: 2019

Appendix A. Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

Report

Assessment of Early Development of Northern Pikeminnow (*Ptychocheilus oregonensis*) Collected from the Koocanusa Reservoir and the Elk River, BC

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March 2020

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1 Study Rationale

Ongoing monitoring in the transboundary Koocanusa Reservoir located in British Columbia (BC) indicated a range of selenium (Se) concentrations in wild northern pikeminnow (NPM; *Ptychocheilus oregonensis*) that in some cases exceeded the U.S. Environmental Protection Agency (USEPA) criterion for fish egg/ovary of 15.1 mg/Kg dry weight [dw] and the British Columbia Ministry of the Environment and Climate Change Strategy (ENV) guideline of 11 mg/Kg dw (Brix et al. 2019). Embryonic life-stages of fishes are particularly susceptible to Se exposure via maternal transfer (Janz et al. 2010). However, to the best of our knowledge no studies have investigated the sensitivity of NPM to Se. Also, recent data suggest that there is a negative correlation between relative gonad size of females (as represented by gonadosomatic index [GSI]) and ovary Se concentrations. While this trend could indicate lower exposures of embryos under the assumption that GSI is directly related to maturation stage (later maturation stages are assumed to have greater GSIs, which were reported to have lower Se concentrations), little is known about gonadal phenotypes and their correlation with GSI in this species.

Therefore, this study aimed to investigate 1) the potential effects of maternal transfer of Se to embryos of NPM collected from several sites on the BC side of Koocanusa Reservoir, representing a gradient of Se concentrations, and 2) to characterize gonadal maturation phenotypes prior to and during the reproductive season of NPM in Koocanusa Reservoir. Unfortunately, despite extensive efforts, an insufficient number of female NPM in spawning condition were collected to properly characterize the relationship between egg Se concentrations and NPM embryo-larval development. Consequently, this report only presents the methods and results of the gonadal maturation characterization.

2 Objective

The main objective of this study was to determine whether egg Se concentrations found in NPM from different locations in Koocanusa Reservoir as well as the Elk River, BC may have effects on developing embryos and larvae of NPM. The secondary objective of this study was to characterize ovarian phenotypes of NPMs prior to and during their reproductive season using histology. To accomplish this,

Specific objectives to be addressed during the 2019 NPM early life stage (ELS) studies were:

- Characterize concentrations of Se in parent fish and embryos collected from the BC portion of Koocanusa Reservoir.
- Collect gonadal tissues (representing a range of GSIs) from NPM of different sizes prior to and during the reproductive season to characterize ovarian maturation and oocyte developmental stages.
- Establish a field-fertilization, and an on-site and laboratory culture protocol for NPM embryos and fry.

- Characterize survival, growth, and development of ELS of NPM related to tissue Se concentrations in ovaries of parent fish and eggs/embryos.
- Describe (if detectable) the toxicity threshold concentration (LC_{10} [mortality] and/or EC_{10} [time to hatch, time to swim-up, teratogenicity, growth]) of maternally transferred Se in NPM embryos.

Unfortunately, an insufficient number of female NPM in spawning condition were collected during the study to allow for full development of a protocol and characterization of the effect of maternally transferred Se on developing NPM embryos and larvae. Consequently, only the methods and results for the ovarian histology assessment are provided in this report.

3 Methods

3.1 Ovarian Histology to Assess Gonadal Maturation Stages

All methods for histology preparation followed the UofS Toxicology Centre's draft standard operating procedure (Appendix A). Field-collected NPM were dissected on site and gonads were excised, weighed and then immediately preserved in 10% buffered formalin for 24 hours, and then transferred to 70% ethanol. Subsamples were excised and transferred to histology cassettes in 70% ethanol. Tissues were processed with an automated unit by the UofS Health Sciences Histology Core Facility, to dehydrate excess water, clear the alcohol for replacement with xylene, and infiltrate the tissues with molten paraffin. Processed tissues were embedded in molten paraffin in individual embedding rings, and cooled for 20 minutes to allow sufficient hardening. Because the ovary samples were fragile, blocks were pre-sectioned to expose the tissues and soaked in a glycerin-ethanol solution for 24 hours before section collection. Samples were sectioned with a microtome at a thickness of 5 µm. Sections were divided every 50 µm or as near as possible to the most intact section, and transferred to a glass microscope slide flooded with distilled water containing Mayer's Albumin Mounting Medium, on a warming table. Slides were dried in an oven set at 40°C for 24 hours before staining. Slides were immersed in a series of solvents, rinsing stages, and stained with hematoxylin and eosin, for section de-waxing and differential uptake of the two stains in cellular components. When staining was complete, sections were covered with cytoseal and coverglass.

Oocyte developmental stages were analyzed following the OECD Guidance Document for the Diagnosis of Endocrine-Related Histopathology of Fish Gonads (2009) - Criteria for Staging Ovaries in Fathead Minnow, Japanese Medaka and Zebrafish. Oocyte developmental stages were identified, counted, and the diameter of a subsample of each type was measured to calculate area.

Gonadosomatic indices (GSIs) were calculated for all fish from which gonads were collected for histological assessment as follows (Eq. 1):

$$GSI = \text{gonad weight [g]} / \text{body weight [g]} * 100 \qquad (1)$$

4 Results & Discussion

4.1 Ovarian Histology to Assess Gonadal Maturation Stages

Between July 8, 2019 and July 19, 2019, ovaries from a total of 15 NPM were collected for histological analyses of maturation stages of oocytes across fish of different sizes (weight range: 250 – 1800 g; fork length range: 33.2 – 61.8 cm), and GSIs (range 0.60 – 10.5 %). Fish represented all three stages of oocyte maturation ranging from immature (Stage 1) to preovulation (Stage 3) (*Figure 4-1*; ***Table 4-1***). While there was no obvious relationship between the size of fish and GSIs, there was a clear positive correlation between GSI and ovarian maturation stage (*Figure 4-1*) with fish having GSIs greater than or equal to 5% all grouping in the final maturation stage (3) with one exception. Similarly, there was a significant and linear relationship between late stage vitellogenic oocytes (LVO) and GSI (R^2 = 0.81), revealing that ovaries of mature fish with a GSI greater than 5% consisted of over 50% LVOs (*Figure 4-2B*). Finally, there was a negative relationship between ovarian Se concentrations and GSI as well as oocyte development stages (*Figure 4-3*).

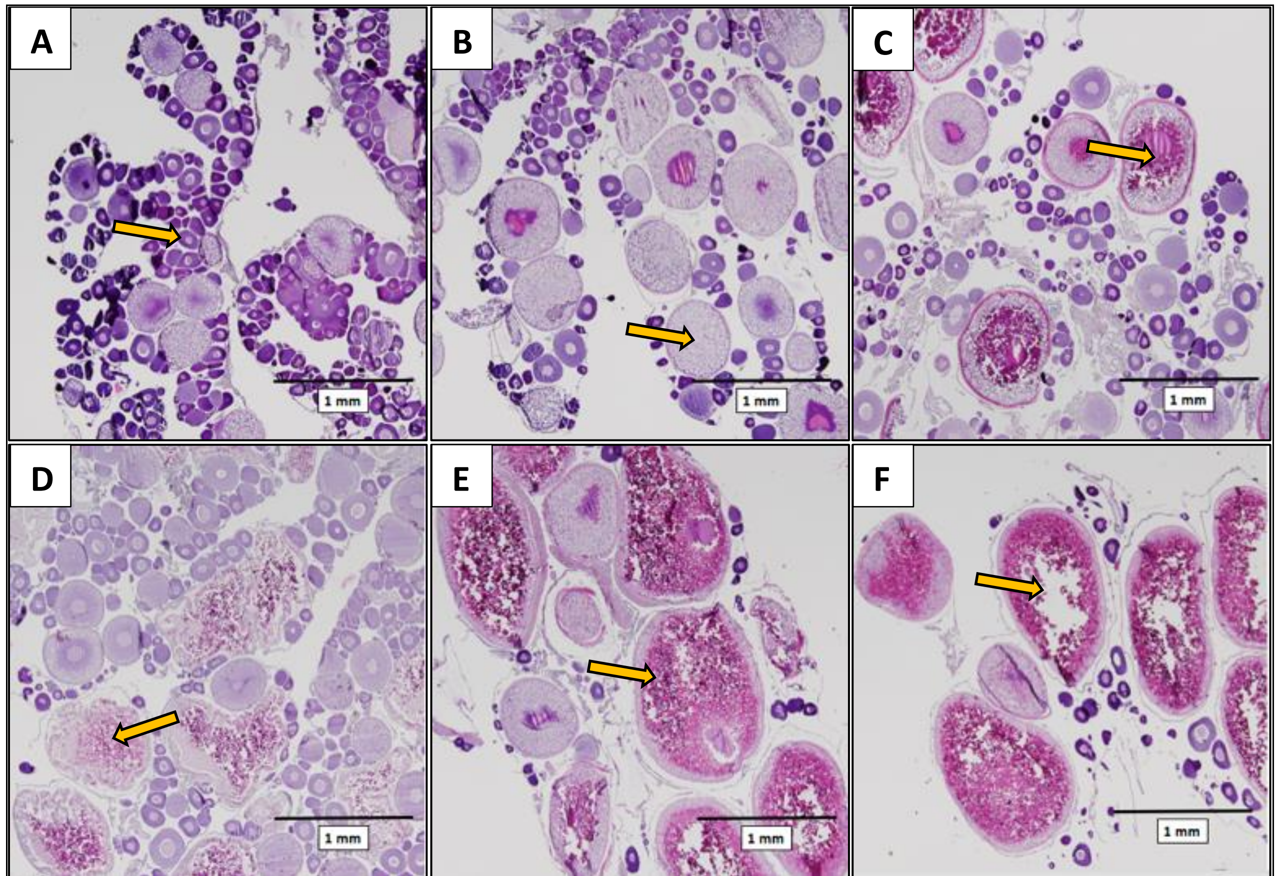

Figure 4-1. Histomicrographs of ovaries of northern pikeminnow representing early development stages (Stage 1) predominantly consisting of perinucleolar oocytes (A; Arrows) and cortical alveolar oocytes (B; Arrows), mid development stages (Stage 2) with increasing proportions of early (C; Arrows), and mid-vitellogenic oocytes (D; Arrows), and late pre-ovulatory stages (Stage 3) with the majority of oocytes representing late vitellogenic cells (E&F; Arrows).

Table 4-1. Summary of northern pikeminnow oocyte histology analysis detailing maternal morphometric characteristics, oocyte development counts, percent area covered by cell development categories, assessed gonadal developmental stage, and notes. Abbreviations are defined as follows: GSI – gonadosomatic index; PO – perinucleolar oocytes; CAO – cortical alveolar oocytes; EVO – early vitellogenic oocytes; LVO – late vitellogenic oocytes.

Sample ID	Maternal Assessment				Histological Assesment									
	Total Length (cm)	Total Weight (g)	GSI (%)	Ovary Se (µg/g)	Cell Type Count				Percent Area of Cell Type				Developmental Stage	Notes
					PO	CAO	EVO	LVO	PO	CAO	EVO	LVO		
SC-06	43.5	495	1.46	10	341	40	25	10	61.8	16.9	12.7	8.5	2	Atresia Present
GC-14	50.8	1030	0.86	9.6	364	38	4	0	67.7	28.9	3.4	0.0	1	
GC-15	61.8	1800	5.60	3.9	34	12	4	10	6.3	8.6	4.8	80.3	3	
ER-31	33.2	300	5.30	10.9	62	9	8	20	9.0	4.6	10.7	75.7	3	
ER-34	54.0	1470	1.31	5.4	156	46	10	8	37.4	27.6	13.1	21.9	2	
ER-35	40.7	530	7.65	3.8	47	9	4	18	11.2	3.7	5.3	79.7	3	
ER-36	41.4	650	10.54	9.3	19	8	5	14	3.9	6.9	9.8	79.4	3	
ER-37	45.2	750	5.77	5.4	67	8	3	3	32.5	14.2	17.6	35.7	2	
ER-38	41.1	560	3.05	12	54	11	12	9	11.5	11.6	38.5	38.4	2	
ER-39	42.1	580	10.02	2.7	42	10	4	12	10.6	7.4	7.5	74.4	3	Atresia Present
ER-40	34.4	250	0.86	18.4	367	37	0	0	73.5	26.5	0.0	0.0	1	
ER-41	42.4	530	8.17	3.4	48	6	3	10	18.3	5.2	6.8	69.7	3	
ER-42	42.3	620	5.25	11	35	7	4	5	12.0	10.9	22.8	54.3	3	
ER-44	40.7	610	0.60	36	255	64	0	0	41.7	58.3	0.0	0.0	1	
ER-45	49.9	1200	9.01	2.2	11	3	1	6	7.1	8.8	5.2	79.0	3	

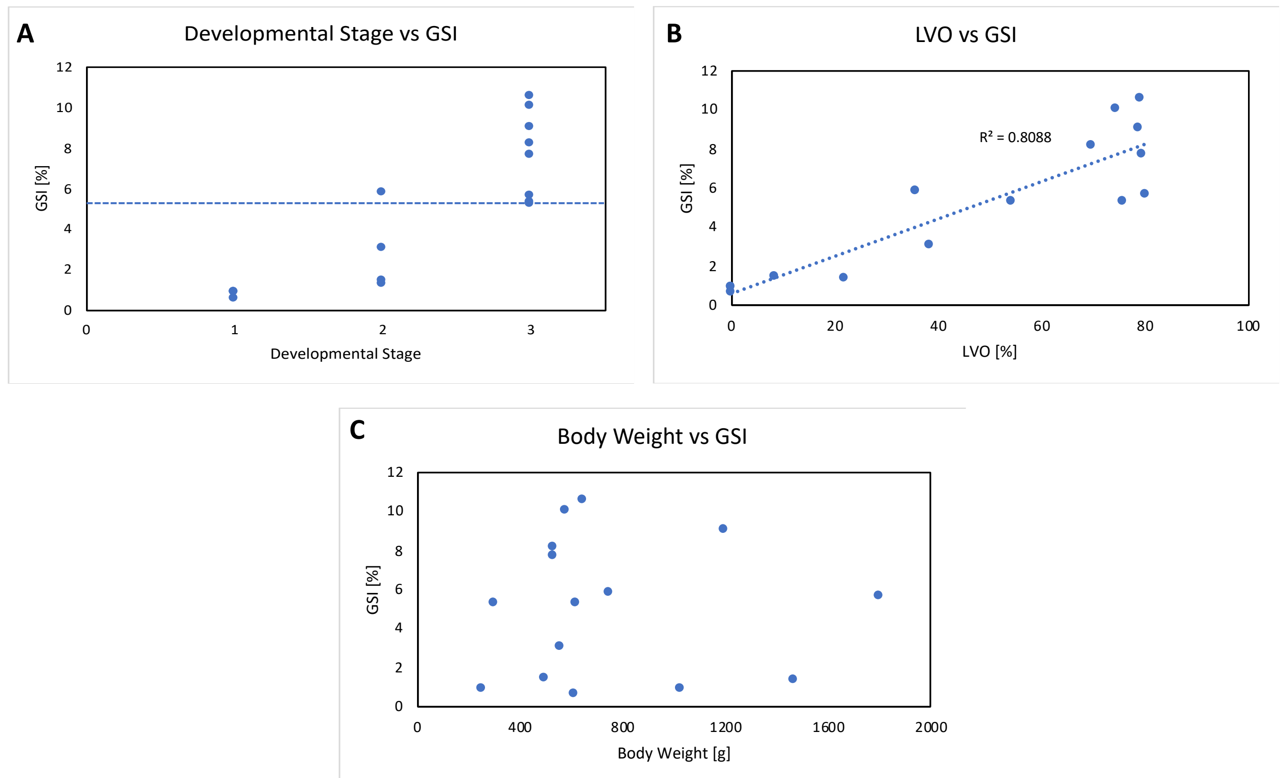

Figure 4-2. Relationships between gonadosomatic indices (GSIs; %) and A) body weight, B) LVO, and C) gonadal development stage in northern pikeminnow. Dotted line represents the 5% GSI level.

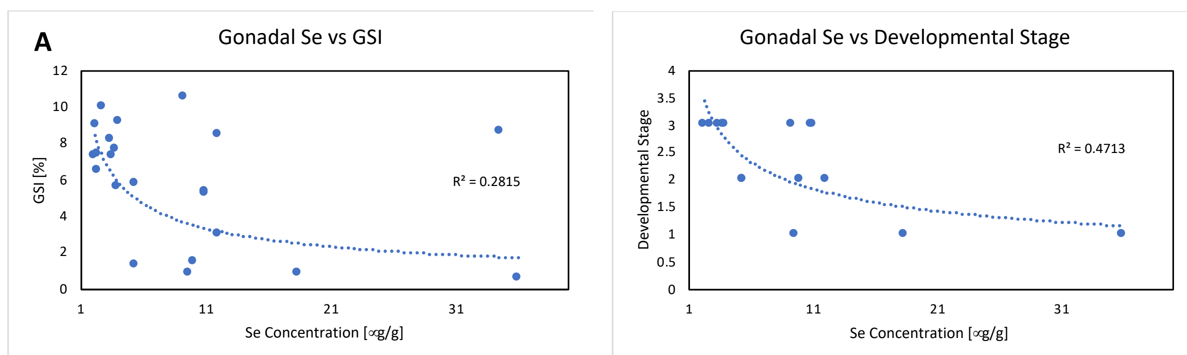

Figure 4-3. Relationship between ovarian Se concentration (µg/g d.w.) and A) gonadosomatic index (GSI), or B) Developmental Stage of oocytes in northern pikeminnow collected from the Koocanusa Reservoir. **Note:** Panel A includes data from 6 additional fish for which no histological evaluation was conducted.

5 Conclusions

This study successfully characterized, for the first time, the phenotypes of different ovarian maturation stage prior to spawning. Clear correlations between histologically determined proportion of follicular stages and GSI were described, demonstrating that fish in the final maturation stage (3) all had GSIs greater than or equal to 5%. Finally, there was significant, albeit weak, negative correlation between ovarian Se concentrations and maturation stage and GSI, indicating greater exposure of immature females. However, sample size and variability were such that future studies are required to confirm this relationship.

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Appendix A – Histological Procedures

University of Saskatchewan

Toxicology Centre

STANDARD OPERATING PROCEDURE

Histological Procedures

Draft

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DEFINITIONS AND ACRONYMS

PLEASE NOTE:

This protocol was produced for and by highly-qualified personnel of the Toxicology Centre of the University of Saskatchewan. It is therefore an in-house document and it should not be distributed without previous consent.

1.0 TISSUE SAMPLING & FIXATION

Morphometric measurements must be recorded as quickly as possible after the experimental animal is euthanized, because rapid degradation of tissues interferes with subsequent histological analyses. Record the following as applicable: individual ID#, sex, length (total length, fork length, standard length, snout-to-vent length, etc.), total weight, gonad weight, liver weight, appearance of secondary sex characteristics, and deformities or other external abnormalities.

There are several options for collecting histology samples, depending on the size of the organism:

1.1 Wole body/intact:

Fix the animal whole, and leave it intact for subsequent processing. In this case, the whole organism must be small enough to fit in a histocasette/standard paraffin block/microscope slide (e.g. fathead minnow at 30 days post-hatch);

1.2 Whole body/dissect

Fix the animal whole, and excise the tissues of interest at a later date prior to processing. For example, an adult fathead minnow or *Xenopus* metamorph can be fixed whole, transferred to 70% ethanol for storage, and then dissected to remove tissues of interest such as liver, gonad, thyroid etc. In this case, during sampling it is necessary to make an incision to expose the internal organs and allow rapid penetration of the fixative.

- Make a shallow mid-ventral incision through the body wall the entire length of the body cavity, being careful to not damage any of the internal organs;
- For adult fathead minnows, make a lateral incision up one side of the body wall to allow the fixative to penetrate the viscera. If possible, using fine forceps, gently move the viscera aside, grasp the swim bladder and discard;
- Attach an individual paper tag with ID# to the body using a needle, fishing line and a waterproof 'rite-in-the-rain' paper tag. Label with pencil only, because ethanol removes ink. Attach the tag by passing the needle and line through the body and tying it off.

1.3 Tissue necropsy:

Excise tissue samples from the freshly euthanized animal prior to fixation. This is done for large-bodied specimens that can't be fixed whole;

- In some cases an entire organ can be excised completely intact and fixed whole for histological analysis. Alternately, it may be necessary to remove only a portion of the tissue of interest, which should be done in a standardised manner, e.g.:

    - middle portion of the left or right gonad;
    - a particular lobe of the liver;
    - right 2nd gill arch;
    - middle portion of the posterior kidney;
- When possible, tissue samples should not exceed 1 cm in any direction, although there are exceptions.

When collecting samples for histology, it is preferable to use chemical overdose, because physical methods of euthanasia can sometimes damage histological samples. It is possible to collect different types of samples from a given individual, e.g. remove the fresh liver for biochemical analysis, and then fix the remaining tissues for histology.

1.4 Fixation:

Proper fixation of tissues is one of the most crucial steps in routine histology, and should be kept consistent across samples. The histology samples (i.e. tissue samples in histocassettes, or whole body samples) should be placed in fixative within 2-3 minutes of euthanasia. Ensure that samples are fully submerged, using a minimum of 10 volumes of fixative to 1 volume of tissue. Use Nalgene wide-mouth leak-proof polyethylene containers.

Samples should remain submerged in fixative for 48 hours, and are then transferred to 70% ethanol for storage. Fixative cannot be re-used, and should be disposed appropriately. The 70% ethanol should be poured off and replenished two more times (minimum 1-2 days each) to remove excess fixative prior to tissue processing.

Commonly used histological fixatives include: 10% Neutral Buffered Formalin, Cal-Ex™ II (Fisher), Davidson's Fixative, and Bouin's fluid. Those containing acids have superior tissue penetration with the added advantage of de-calcifying bone, which can improve tissue sectioning. Cal-Ex is therefore preferred for whole body fixation. Davidson's Fixative is also popular; it can be prepared in advance using stock chemicals, and has a reasonable shelf life for longer-term storage.

Davidson's Fixative	
Formalin	200 ml
100% Ethanol	300 ml
Glycerin	100 ml
Glacial Acetic Acid	100 ml
Distilled Water	300 ml

2.0 SPECIMEN GROSSING

Once fixed and stored in 70% ethanol, the specimens can be further trimmed if necessary prior to tissue processing. Whole body samples can be dissected to remove tissues of interest. In some cases, an entire organ can be excised (e.g. the gonad, liver), or alternately, a representative portion of the tissue of interest can be removed. Note: fixed weights and lengths can be used to generate condition factor, gonado- and hepatosomatic indices.

3.0 TISSUE PROCESSING and EMBEDDING

Fixed tissue specimens (stored in 70% ethanol) are loaded into a Vacuum Infiltration Processor (aka "**Tissue Processor**"). This programmable, automated unit contains reservoirs of various solvents as well as molten paraffin wax. The tissue processor can be programmed to control temperature, stir the solutions, and create pressure/vacuum cycles during sample processing, all of which can enhance the penetration of solutions through the tissues. The purpose of the process is: (1) **dehydration** - the tissues are bathed in a series of progressively stronger alcohols (70% up to 100%) to remove excess water from the cells, (2) **clearing** - the alcohol is flushed from the tissues and replaced with xylene or toluene (which are capable of dissolving paraffin) (3) **infiltration** - the tissues are infiltrated with molten paraffin. The final result is an intact tissue sample perfused with paraffin, which is immediately placed in a paraffin-filled mould and allowed to cool.

<u>4.1 Tissue Processing:</u>

It takes 14 hours to run a batch of samples through the tissues processor; this is typically done overnight, with sample embedding happening the following morning. Ensure that the samples are stored in a third rinse of 70% ethanol prior to loading them in the processor.

<u>4.2 Embedding</u>

1) Arrive at the Lab 20-30 minutes before the end of the processing run to prepare for embedding.

- Ensure that the Cryo station is turned on;
- Label an embedding ring for each sample to be embedded;
- Coat the embedding moulds with a thin layer of Mould Release, and place them on the warming console;

2) When the processing run is complete, remove the samples from the tissue processor and place them in the 'holding basin' full of molten paraffin wax in the embedding

console. Ensure that the samples do not cool down and solidify at this point, i.e. get the cassettes into the melted wax as quickly as possible, and keep the lid closed;

3) Using the heated paraffin dispenser, place a small amount of paraffin in the bottom of the mould. (Note that the paraffin dispenser flow rate can be adjusted);

4) Open a histocassette and spill the tissue sample out into the holding wax. Set the cassette aside.

5) Using heated forceps, gently grab the tissue and place in the bottom of the mould to attain the appropriate ***orientation for sectioning***. Place it on the cooling pad for 5-10 seconds, to ensure that the wax gels, and the tissue is held in place.

6) Place the labelled embedding ring on the mould with and fill with paraffin. The wax level should be above the rim of the embedding ring to account for shrinkage during cooling. Set it on the Cryo console to cool.

7) Repeat until all samples are embedded, working as quickly as possible.

8) Leave the blocks on the Cryo console for ~20 minutes. Gently pull the mould off and set the block on the benchtop to cool. Transport solidified blocks back to the Toxicology Centre, and let sit overnight prior to attempting any trimming or sectioning.

Table 1. Tissue processing program used at the University of Saskatchewan Histology Core Facility. Tissues are dehydrated in graded alcohols (Station 1 to 7), cleared in xylene (Station 8 to 10), and infiltrated with molten paraffin (Station 11 to 14).

Station	**Reagent**	**Time**	**Temp (°C)**	**Pres/Vac Cycle**	**Mix**
1	Ethanol 70%	1 hr	ambient	V	On
2	Ethanol 80%	1 hr	ambient	V	On
3	Ethanol 95%	1 hr	ambient	V	On
4	Ethanol 95%	1 hr	ambient	V	On
5	100% ethanol	1 hr	ambient	V	On
6	100% ethanol	1 hr	ambient	V	On
7	100% ethanol	1 hr	ambient	V	On
8	Ethanol/Xylene	1 hr	ambient	V	On
9	Xylene	1 hr	ambient	V	On
10	Xylene	1 hr	ambient	V	On
11	Paraffin	1 hr	60	V	On
12	Paraffin	1 hr	60	V	On
13	Paraffin	1 hr	60	V	On
14	Paraffin	1 hr	60	V	On

5.0 MICROTOMY (aka SECTIONING)

The embedding ring of the paraffin block is mounted on a rotary microtome. Ribbons of thin sections are created, and these are placed on glass microscope slides. The user can control the thickness of the sections (usually 5-7 μm, thinner is generally better), as well as the number and spacing of the sections retained on the microscope slide. There are several options for sectioning:

- **Single representative section** – one section is retained from each block, this is considered to be representative of the entire tissue;
- **Serial sectioning** - the user cuts through the entire tissue, and all sections are retained (labour-intensive);
- **Step sectioning** - the user cuts through the tissue and retains representative sections at pre-defined intervals;

5.1 General Methods for Sectioning:

- Turn on slide warming table, let it warm up to 40°C (temperature is generally pre-set, and shouldn't require adjustments);
- In a small beaker, prepare ~40mL of distilled water containing ~4 drops of Mayer's Albumin mounting medium, stored in fridge. This should be sufficient for 1 day of sectioning; fresh solution should be made up daily (1 drop Mayer's per 10 mL dH20);
- Use a razor blade to trim excess wax from the tissue blocks to within 2mm of the tissue edge. Maintain square sides on trimmed portion;
- Wipe down a fresh microtome blade with xylene to remove the oil coating, and mount it in the knife holder;
- Ensure microtome is clean and lubricated (see user manual);
- Pre-label a slide for the first paraffin block using solvent resistant marker (slides will be dipped in solvents during staining).
- Place the block firmly in the microtome chuck. Section the block according to the specific protocol (i.e. a single 'representative' section per block, step sections, or serial secions). The sections should come off the blade in continuous ribbons. Note that if the blocks are trimmed small, numerous sections and multiple rows of sections can be placed on a single slide;
- Place the labelled slide on the warming table and flood with the mounting medium. Float the sections of interest on the slide until they appear smooth and free of wrinkles;

- Once the sections are smooth, wipe away excess mounting medium from the slide (Kimwipe), and place it in a slide holder. Full racks of slides are stored in the 40C oven (minimum overnight) prior to staining;
- If scratches or nicks appear in the ribbons during sectioning, move the blade to an unused area, or replace entirely;

6.0 SLIDE STAINING - HEMATOXYLIN and EOSIN

Once the tissue sections have been allowed to dry overnight in a 40°C oven, they can be stained for light microscopy. Myriad staining techniques exist; Hematoxylin and Eosin ("**H&E**") is a common 2-part staining technique routinely used for basic paraffin sections. A rack full of slides is immersed in a series of solvents and stains, resulting in de-waxing of the sections and differential uptake of the 2 stains in various cellular components.

6.1 Staining:

- The stains and solvents can be used to stain app. 10 – 12 racks of slides, and then must be replaced. Check with other lab users regarding the status of the stain series, or if necessary check the quality of the most recently stained slides for fading or loss of contrast. Solvents can be topped up if they have evaporated down;
- Do not stain paraffin sections unless the slides have dried in 40°C oven for minimum 24 hours;
- Multiple racks can be stained at once. When the first boat is in the hematoxylin, a second rack can be started;
- It takes ~45 minutes to stain and coverglass one rack of slides;
- Staining and coverglassing are done in the fume hood;
- Before starting, check supplies of cytoseal and coverglass (use #1 thickness).

Table 2: Step-by-step staining process.

Station	Solution	Time	Notes
1	Xylene 1	2 min	
2	Xylene 2	2 min	
3	Xylene / 100% Ethanol	2 min	1:1 Ratio
4	100% Ethanol	2 min	
5	95% Ethanol	2 min	
6	70% Ethanol	2 min	
7	Tap Water	2 min	Replace often
8	Distilled Water	2 min	Replace often
9	Hematoxylin	5 min	
10	Tap water	rinse 4x	Water should run clear
11	Acid Alcohol (0.1%)	15 sec	0.1ml HCl/100ml 70% EtOH
12	Water	rinse 2x	
13	Phosphotungstic Acid (0.33%)	30 sec	(0.33 g/100ml Water)
14	Citric Acid (0.33%)	30 sec	(0.33 g/100ml Water)
15	Running Tap Water	5 min	
16	Eosin Y	2.5 min	
17	Tap Water	rinse 4x	Water should run clear
	70% Ethanol		
18	95% Ethanol	1 min	
19	100% Ethanol	2 min	
20	100% Ethanol	2 min	
21	Xylene / 100% Ethanol	2 min	1:1 Ratio
22	Xylene	2 min	
23	Xylene	Holding	

6.2 Coverglassing:

- Slides should be coverglassed as soon as possible after staining is completed. The slide rack is held in the last Xylene station until coverglassing is completed;
- Place a coverglass on a cork, add thin line of cytoseal full length;
- Remove slide from Xylene, blot slide edge on paper towel, do not allow Xylene to evaporate completely;
- Turn slide upside down, slowly lower it onto the coverglass at a slight angle, avoid trapping air bubbles in the cytoseal;
- Wipe off the back of the slide, and place flat on trays to dry, ensuring that the slide edges are not touching each other. Slides should air dry minimum 1 week before placing in slide boxes.

Appendix B. Northern Pikeminnow Ovary Selenium, Muscle Selenium, and GSI data for Koocanusa Reservoir: 2008-2019

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
MT	2008	May	14	-	Rexford	2.8	1.0	54.8	-	1973.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.5	1.0	48.3	-	1340.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.1	51.3	-	1347.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.1	51.8	-	1740.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.7	1.2	53.7	-	1708.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.2	1.2	56.2	-	1705.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.8	1.2	50.2	-	1592.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.7	1.2	50.0	-	1226.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.9	1.2	50.7	-	1306.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	2.9	1.3	53.1	-	1720.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.9	1.3	55.8	-	1789.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.3	49.5	-	1303.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.5	1.3	47.6	-	1183.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.6	1.4	50.9	-	1557.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.6	52.3	-	1586.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	4.2	1.7	51.8	-	1728.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	3.0	1.9	60.3	-	2259.0	-	-	-	-	-
MT	2008	May	14	-	Rexford	5.5	1.9	47.0	-	1140.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.7	1.5	56.3	-	1851.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.4	1.5	48.6	-	1134.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.7	1.7	51.7	-	1297.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	1.7	47.0	-	1039.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.3	1.8	52.2	-	1465.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	2.4	1.8	53.8	-	1506.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.3	1.8	50.2	-	1190.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.4	1.9	61.2	-	2696.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	6.0	2.0	46.8	-	953.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	8.1	2.3	47.6	-	1043.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	4.1	2.3	51.0	-	1361.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	5.0	2.3	45.1	-	925.0	-	-	-	-	-
MT	2013	May	14	-	Rexford	3.2	2.4	48.8	-	1052.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.5	56.2	-	1860.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.7	1.5	51.0	-	1343.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.3	1.6	50.4	-	1148.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.5	1.7	46.3	-	898.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.7	42.2	-	662.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	2.8	1.9	51.9	-	1134.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.2	1.9	44.4	-	776.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	3.9	1.9	46.4	-	1116.0	-	-	-	-	-
MT	2013	May	15	-	Tenmile	4.7	2.3	46.6	-	762.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	40.1	5.0	41.0	37.2	505.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	25.7	1.6	38.2	34.9	465.0	-	-	-	-	-
BC	2014	February	-	-	Elk River	3.3	2.4	29.5	27.2	316.0	-	-	-	-	-
BC	2014	April	-	-	Elk River	21.9	4.6	39.3	35.2	440.0	-	-	-	-	-
BC	2014	April	-	ER-PM-14G-Apr-14	Elk River	40.1	6.2	34.1	30.8	312.0	-	3.4	-	-	1.08
BC	2014	April	-	ER-PM-11G-Apr-14	Elk River	8.6	2.5	37.3	33.5	438.0	-	4.5	-	-	1.03
BC	2014	February	-	-	Gold Creek	7.6	2.8	39.3	35.7	495.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	15.4	2.2	35.5	32.3	360.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	10.3	2.5	38.6	34.9	500.0	-	-	-	-	-
BC	2014	February	-	-	Gold Creek	4.1	2.4	38.0	-	580.0	-	-	-	-	-
BC	2014	April	-	GC-PM-10G-Apr-14	Gold Creek	5.0	2.3	40.4	36.5	522.0	-	4.3	-	-	0.83

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2014	February	-	-	Sand Creek	13.7	1.6	35.5	32.1	320.0	-	-	-	-	-
BC	2014	February	-	-	Sand Creek	3.8	1.2	49.9	45.4	1200.0	-	-	-	-	-
BC	2014	April	-	SC-PM-10G-Apr-14	Sand Creek	17.0	1.6	34.4	31.2	300.0	-	1.8	-	-	0.61
BC	2014	April	-	SC-PM-01G-Apr-14	Sand Creek	30.7	2.9	36.1	32.8	355.0	-	6.2	-	-	1.74
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	4.0	-	35.8	32.3	380.0	-	1.1	-	-	0.30
BC	2015	April	-	ER-NSC-43-Apr-15	Elk River	12.9	4.1	34.2	30.8	320.0	13.0	1.1	4.01	315	0.34
BC	2015	April	-	ER-NSC-25-Apr-15	Elk River	9.2	3.1	40.4	36.5	560.0	13.0	1.2	7.81	551	0.21
BC	2015	April	-	ER-NSC-13-Apr-15	Elk River	11.8	6.0	46.7	42.7	885.0	13.0	1.1	12.97	871	0.13
BC	2015	April	-	GC-NSC-13-Apr-15	Gold Creek	5.2	1.9	32.1	28.9	252.0	9.0	1.0	5.50	245	0.41
BC	2015	April	-	GC-NSC-31-Apr-15	Gold Creek	13.8	2.0	33.0	30.2	261.0	12.0	0.9	2.30	258	0.36
BC	2015	April	-	GC-NSC-33-Apr-15	Gold Creek	7.9	2.3	37.4	34.0	390.0	10.0	1.0	2.41	387	0.25
BC	2015	April	-	GC-NSC-34-Apr-15	Gold Creek	7.6	1.6	37.5	34.0	390.0	9.0	1.0	2.10	387	0.25
BC	2015	April	-	GC-NSC-49-Apr-15	Gold Creek	3.5	1.3	44.8	40.1	880.0	12.0	1.4	17.22	861	0.16
BC	2015	April	-	SC-NSC-37-Apr-15	Sand Creek	7.2	1.7	32.6	29.4	261.0	9.0	1.0	3.22	257	0.39
BC	2015	April	-	SC-NSC-36-Apr-15	Sand Creek	4.8	1.9	33.5	30.0	275.0	10.0	1.0	2.51	271	0.37
BC	2015	April	-	SC-NSC-33-Apr-15	Sand Creek	11.4	1.4	34.0	30.2	300.0	8.0	1.1	3.30	296	0.36
BC	2015	April	-	SC-NSC-47-Apr-15	Sand Creek	18.3	2.4	34.4	30.8	325.0	13.0	1.1	3.66	320	0.34
BC	2015	April	-	SC-NSC-44-Apr-15	Sand Creek	15.1	2.4	35.2	31.7	370.0	13.0	1.2	5.49	363	0.31
BC	2015	April	-	SC-NSC-39-Apr-15	Sand Creek	11.6	2.0	38.0	34.0	445.0	12.0	1.1	3.34	441	0.25
BC	2015	April	-	SC-NSC-13-Apr-15	Sand Creek	6.2	1.5	40.0	36.3	492.0	14.0	1.0	8.33	483	0.21
BC	2015	April	-	SC-NSC-46-Apr-15	Sand Creek	5.8	1.7	41.6	37.5	630.0	13.0	1.2	14.58	614	0.19
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	3.1	-	54.1	49.5	1500.0		47.7			3.18
BC	2016	April	-	ER-NSC-21 O-Apr-16	Elk River	10.9	2.9	36.1	32.9	455.0	16.0	16.4	5.00	434	3.60
BC	2016	April	-	ER-NSC-19 O-Apr-16	Elk River	7.0	1.7	38.6	35.2	555.0	14.0	7.4	25.12	522	1.33
BC	2016	April	-	ER-NSC-17 O-Apr-16	Elk River	6.2	2.0	40.2	36.2	615.0	14.0	10.2	9.28	596	1.65
BC	2016	April	-	ER-NSC-27 O -Apr-16	Elk River	7.6	1.3	49.1	45.0	1200.0	15.0	17.6	24.42	1,158	1.47
BC	2016	April	-	ER-NSC-38 O-Apr-16	Elk River	9.9	1.5	51.1	47.0	1400.0	15.0	36.9	24.67	1,338	2.64
BC	2016	April	-	ER-NSC-16 O-Apr-16	Elk River	8.2	1.6	53.4	48.0	1540.0	14.0	35.5	17.35	1,487	2.31
BC	2016	April	-	ER-NSC-28 O-Apr-16	Elk River	5.5	1.7	56.3	50.8	1900.0	22.0	104.8	31.83	1,763	5.51
BC	2016	April	-	ER-NSC-15 O-Apr-16	Elk River	3.0	1.5	60.8	55.9	2600.0	20.0	124.3	46.89	2,429	4.78
BC	2016	April	-	ER-NSC-29 O-Apr-16	Elk River	3.6	1.6	61.5	56.7	2640.0	21.0	132.0	55.87	2,452	5.00
BC	2016	April	-	GC-NSC-17 O-Apr-16	Gold Creek	9.0	2.1	37.6	33.9	435.0	12.0	4.0	7.86	423	0.91
BC	2016	April	-	GC-NSC-01 O-Apr-16	Gold Creek	12.9	1.6	38.2	34.9	450.0	13.0	4.9	8.31	437	1.08
BC	2016	April	-	GC-NSC-14 O-Apr-16	Gold Creek	8.9	2.0	40.9	36.9	585.0	13.0	14.1	5.36	566	2.40
BC	2016	April	-	GC-NSC-16 O-Apr-16	Gold Creek	4.3	1.6	42.0	38.3	610.0	12.0	5.0	6.28	599	0.82
BC	2016	April	-	GC-NSC-12 O-Apr-16	Gold Creek	3.9	1.3	45.5	41.6	940.0	14.0	35.1	15.21	890	3.73
BC	2016	April	-	GC-NSC-26 O-Apr-16	Gold Creek	3.8	1.5	53.5	48.0	1600.0	15.0	96.2	42.73	1,461	6.01
BC	2016	April	-	GC-NSC-24 O-Apr-16	Gold Creek	3.8	1.4	52.8	48.2	1640.0	14.0	80.3	25.92	1,534	4.89
BC	2016	April	-	GC-NSC-06 O-Apr-16	Gold Creek	6.2	1.7	54.5	48.8	1400.0	15.0	35.7	22.15	1,342	2.55
BC	2016	April	-	GC-NSC-08 O-Apr-16	Gold Creek	5.3	2.2	55.8	50.6	1870.0	17.0	65.1	30.61	1,774	3.48
BC	2016	April	-	GC-NSC-13 O -Apr-16	Gold Creek	3.7	1.6	60.8	55.6	2360.0	17.0	124.2	62.56	2,173	5.26
BC	2016	April	-	GC-NSC-05 O-Apr-16	Gold Creek	4.1	1.6	62.2	57.0	2500.0	15.0	117.8	58.55	2,324	4.71
BC	2016	April	-	SC-NSC-25 O-Apr-16	Sand Creek	17.0	2.6	30.1	27.2	257.0	12.0	11.2	3.32	242	4.37
BC	2016	April	-	SC-NSC-09 O-Apr-16	Sand Creek	17.6	2.0	34.1	30.5	315.0	13.0	4.3	3.97	307	1.36
BC	2016	April	-	SC-NSC-21 O-Apr-16	Sand Creek	12.2	1.9	37.6	33.8	520.0	13.0	5.8	9.69	505	1.11
BC	2016	April	-	SC-NSC-29 O-Apr-16	Sand Creek	4.6	1.5	39.7	35.9	550.0	10.0	11.4	13.65	525	2.07
BC	2016	April	-	SC-NSC-03 O-Apr-16	Sand Creek	7.8	1.3	41.5	37.5	760.0	14.0	12.7	9.50	738	1.68
BC	2016	April	-	SC-NSC-35 O-Apr-16	Sand Creek	5.0	1.4	44.4	39.8	935.0	14.0	26.8	20.35	888	2.86
BC	2016	April	-	SC-NSC-36 O-Apr-16	Sand Creek	3.2	1.4	44.7	40.4	875.0	14.0	21.7	21.84	831	2.48
BC	2016	April	-	SC-NSC-32 O-Apr-16	Sand Creek	4.1	1.4	45.5	41.2	996.0	15.0	28.8	20.28	947	2.89
BC	2016	April	-	SC-NSC-34 O-Apr-16	Sand Creek	7.0	1.3	52.5	47.8	1420.0	15.0	36.8	19.82	1,363	2.59

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2016	April	-	SC-NSC-33 O-Apr-16	Sand Creek	5.4	1.3	62.6	57.4	2530.0	21.0	97.4	48.72	2,384	3.85
BC	2018	June	7	RG_ER_NSC06O_20180607	Elk River	26.0	4.4	30.9	27.7	205.0	6.0	1.1	2.93	201	0.51
BC	2018	June	7	RG_ER_NSC03O_20180607	Elk River	17.0	1.7	33.9	30.2	275.0	10.0	1.6	2.06	271	0.59
BC	2018	June	6	RG_ER_NSC02O_20180606	Elk River	19.0	3.1	35.5	31.8	315.0	9.0	1.9	2.70	310	0.61
BC	2018	June	7	RG_ER_NSC05O_20180607	Elk River	26.0	2.5	39.9	35.7	445.0	11.0	2.7	5.16	437	0.60
BC	2018	June	6	RG_ER_NSC01O_20180606	Elk River	16.0	4.0	41.4	37.0	545.0	10.0	3.6	6.42	535	0.65
BC	2018	June	7	RG_ER_NSC04O_20180607	Elk River	24.0	4.8	44.0	39.8	755.0	12.0	26.4	4.40	724	3.50
BC	2018	June	7	RG_GC_NSC02O_20180607	Gold Creek	19.0	2.7	37.1	33.5	350.0	9.0	1.9	4.32	344	0.54
BC	2018	June	7	RG_GC_NSC01O_20180607	Gold Creek	13.0	2.9	38.5	34.7	475.0	9.0	6.1	8.25	461	1.29
BC	2018	June	7	RG_GC_NSC03O_20180607	Gold Creek	3.6	1.7	54.5	50.1	1800.0	15.0	191.6	47.82	1,561	10.65
BC	2018	June	5	RG_SC_NSC05O_20180605	Sand Creek	13.0	2.7	34.0	31.0	280.0	8.0	2.8	2.76	274	1.00
BC	2018	June	5	RG_SC_NSC03O_20180605	Sand Creek	9.2	2.4	34.5	31.2	330.0	10.0	4.9	2.93	322	1.47
BC	2018	June	5	RG_SC_NSC04O_20180605	Sand Creek	5.4	1.6	35.6	32.4	340.0	9.0	7.2	3.65	329	2.13
BC	2018	June	10	RG_SC_NSC06O_20180610	Sand Creek	27.0	2.0	41.6	37.7	530.0	11.0	6.8	4.65	519	1.29
BC	2018	June	5	RG_SC_NSC01O_20180605	Sand Creek	16.0	1.7	44.3	40.4	685.0	12.0	5.8	10.55	669	0.85
BC	2018	June	5	RG_SC_NSC02O_20180605	Sand Creek	5.4	1.3	48.8	44.8	1140.0	13.0	52.1	24.00	1,064	4.57
BC	2018	June	10	RG_SC_NSC07O_20180610	Sand Creek	5.8	1.6	58.9	53.9	1690.0	17.0	96.2	29.16	1,565	5.69
MT	2018	May	8	-	Rexford	3.5	1.1	49.0	-	1215.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.2	1.1	47.4	-	1090.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	5.5	1.3	52.5	-	1575.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	4.6	1.4	48.3	-	1155.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.5	1.4	45.3	-	1110.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.7	1.4	51.6	-	1290.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.4	1.6	53.6	-	1360.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	6.7	1.6	44.2	-	760.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	3.9	1.9	51.3	-	1395.0	-	-	-	-	-
MT	2018	May	8	-	Rexford	2.3	1.4	48.8	-	1220.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.5	1.1	49.3	-	1190.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	2.9	1.1	46.8	-	915.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.4	1.1	44.1	-	960.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.1	1.2	48.2	-	1070.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	1.8	1.2	56.4	-	1575.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.3	49.8	-	1145.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.0	1.6	48.2	-	1150.0	-	-	-	-	-
MT	2018	May	9	-	Tenmile	3.8	1.6	47.1	-	955.0	-	-	-	-	-
BC	2019	June	14	6/14/2019 RG_ER-NPM-01_20190614	Elk River	3.8	1.6	55.1	50.2	1680.0	-	137.0	-	-	8.15
BC	2019	June	14	6/14/2019 RG_ER-NPM-02_20190614	Elk River	2.7	1.3	57.2	53.0	1520.0	-	114.0	-	-	7.50
BC	2019	June	17	6/17/2019 RG_ER-NPM-03_20190617	Elk River	3.3	1.2	55.7	50.5	1550.0	-	149.9	-	-	9.67
BC	2019	June	17	6/17/2019 RG_ER-NPM-04_20190617	Elk River	3.0	1.2	45.5	41.6	1050.0	-	100.3	-	-	9.55
BC	2019	June	18	6/18/2019 RG_ER-NPM-05_20190618	Elk River	4.9	1.6	50.7	46.6	1140.0	-	100.3	-	-	8.80
BC	2019	June	18	6/18/2019 RG_ER-NPM-06_20190618	Elk River	5.0	1.6	54.4	49.6	1540.0	-	81.9	-	-	5.32
BC	2019	June	18	6/18/2019 RG_ER-NPM-07_20190618	Elk River	4.3	1.3	47.5	43.0	1140.0	-	87.7	-	-	7.69
BC	2019	June	18	6/18/2019 RG_ER-NPM-08_20190618	Elk River	4.2	1.6	56.0	51.2	1400.0	-	92.1	-	-	6.58
BC	2019	June	19	6/19/2019 RG_ER-NPM-09_20190619	Elk River	7.2	2.0	46.7	42.4	880.0	-	18.6	-	-	2.11
BC	2019	June	19	6/19/2019 RG_ER-NPM-10_20190619	Elk River	9.9	1.9	46.0	42.2	720.0	-	6.6	-	-	0.92
BC	2019	June	19	6/19/2019 RG_ER-NPM-11_20190619	Elk River	17.0	2.5	40.2	36.5	620.0	-	3.7	-	-	0.60
BC	2019	June	20	6/20/2019 RG_ER-NPM-12_20190620	Elk River	2.4	1.3	55.2	50.3	1950.0	-	216.3	-	-	11.09
BC	2019	June	20	6/20/2019 RG_ER-NPM-13_20190620	Elk River	3.6	1.5	56.0	51.5	1590.0	-	47.6	-	-	2.99
BC	2019	June	20	6/20/2019 RG_ER-NPM-14_20190620	Elk River	7.6	1.7	53.6	48.8	1520.0	-	46.2	-	-	3.04
BC	2019	June	20	6/20/2019 RG_ER-NPM-15_20190620	Elk River	8.6	2.5	39.9	36.2	480.0	-	18.6	-	-	3.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-16_20190620	Elk River	17.0	2.4	39.0	34.9	430.0	-	3.7	-	-	0.86

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_ER-NPM-17_20190620	Elk River	4.5	1.8	44.2	40.0	840.0	-	66.2	-	-	7.88
BC	2019	June	20	6/20/2019 RG_ER-NPM-18_20190620	Elk River	4.1	2.5	53.0	48.0	1540.0	-	153.5	-	-	9.97
BC	2019	June	25	6/25/2019 RG_ER-NPM-19_20190625	Elk River	7.9	1.9	30.2	28.3	200.0	-	6.7	-	-	3.35
BC	2019	June	25	6/25/2019 RG_ER-NPM-20_20190625	Elk River	6.3	2.9	46.9	42.4	740.0	-	14.5	-	-	1.96
BC	2019	June	27	6/27/2019 RG_ER-NPM-21_20190627	Elk River	13.0	3.4	39.9	36.9	710.0	-	54.0	-	-	7.61
BC	2019	June	28	6/28/2019 RG_ER-NPM-22_20190628	Elk River	7.1	2.6	32.9	29.5	295.0	-	24.9	-	-	8.43
BC	2019	June	28	6/28/2019 RG_ER-NPM-23_20190628	Elk River	9.8	3.6	38.0	34.2	440.0	-	16.0	-	-	3.63
BC	2019	June	28	6/28/2019 RG_ER-NPM-24_20190628	Elk River	7.8	2.4	43.9	39.6	740.0	-	29.6	-	-	4.00
BC	2019	June	28	6/28/2019 RG_ER-NPM-25_20190628	Elk River	8.3	2.5	33.3	30.4	340.0	-	15.3	-	-	4.49
BC	2019	June	28	6/28/2019 RG_ER-NPM-26_20190628	Elk River	4.0	1.1	44.9	41.5	915.0	-	85.3	-	-	9.33
BC	2019	June	28	6/28/2019 RG_ER-NPM-27_20190628	Elk River	14.6	2.3	30.7	27.6	180.0	-	1.1	-	-	0.61
BC	2019	July	3	7/3/2019 RG_ER-NPM-28_20190703	Elk River	34.5	2.3	38.8	35.7	550.0	-	47.7	-	-	8.67
BC	2019	July	4	7/4/2019 RG_ER-NPM-29_20190704	Elk River	19.4	4.6	46.7	42.4	700.0	-	6.1	-	-	0.88
BC	2019	July	4	7/4/2019 RG_ER-NPM-30_20190704	Elk River	7.1	2.7	44.7	40.5	760.0	-	11.1	-	-	1.47
BC	2019	July	8	7/8/2019 RG_ER-NPM-31_20190708	Elk River	10.9	3.1	33.2	29.9	300.0	-	15.9	-	-	5.30
BC	2019	July	9	7/9/2019 RG_ER-NPM-32_20190709	Elk River	4.1	1.3	54.8	50.0	1550.0	-	143.1	-	20.09	9.23
BC	2019	July	9	7/9/2019 RG_ER-NPM-33_20190709	Elk River	3.5	1.4	44.3	41.6	780.0	-	57.3	-	-	7.35
BC	2019	July	9	7/9/2019 RG_ER-NPM-34_20190709	Elk River	5.4	1.6	54.0	49.0	1470.0	-	19.3	-	-	1.31
BC	2019	July	9	7/9/2019 RG_ER-NPM-35_20190709	Elk River	3.8	1.2	40.7	36.0	530.0	-	40.6	-	-	7.65
BC	2019	July	9	7/9/2019 RG_ER-NPM-36_20190709	Elk River	9.3	3.9	41.4	37.9	650.0	-	68.5	-	-	10.54
BC	2019	July	9	7/9/2019 RG_ER-NPM-37_20190709	Elk River	5.4	2.2	45.2	40.5	750.0	-	43.3	-	-	5.77
BC	2019	July	10	7/10/2019 RG_ER-NPM-38_20190710	Elk River	12.0	4.0	41.1	37.1	560.0	-	17.1	-	-	3.05
BC	2019	July	10	7/10/2019 RG_ER-NPM-39_20190710	Elk River	2.7	1.2	42.1	38.3	580.0	-	58.1	-	-	10.02
BC	2019	July	10	7/10/2019 RG_ER-NPM-40_20190710	Elk River	18.4	3.0	34.4	30.4	250.0	-	2.1	-	-	0.86
BC	2019	July	10	7/10/2019 RG_ER-NPM-41_20190710	Elk River	3.4	1.3	42.4	38.3	530.0	-	43.3	-	-	8.17
BC	2019	July	10	7/10/2019 RG_ER-NPM-42_20190710	Elk River	11.0	3.4	42.3	38.8	620.0	-	32.6	-	-	5.25
BC	2019	July	11	7/11/2019 RG_ER-NPM-43_20190711	Elk River	12.0	4.8	43.0	39.3	825.0	-	70.1	-	-	8.50
BC	2019	July	12	7/12/2019 RG_ER-NPM-44_20190712	Elk River	36.0	5.0	40.7	36.6	610.0	-	3.7	-	-	0.60
BC	2019	July	12	7/12/2019 RG_ER-NPM-45_20190712	Elk River	2.2	1.2	49.9	45.3	1200.0	-	108.1	-	-	9.01
BC	2019	July	13	7/13/2019 RG_ER-NPM-46_20190713	Elk River	2.4	1.3	56.0	50.6	1575.0	-	116.5	-	-	7.40
BC	2019	July	15	7/15/2019 RG_ER-NPM-47_20190715	Elk River	2.1	1.2	53.3	48.2	1200.0	-	87.2	-	-	7.27
BC	2019	July	16	7/16/2019 RG_ER-NPM-48_20190716	Elk River	2.3	1.3	54.9	50.5	1240.0	-	80.1	-	-	6.46
BC	2019	July	26	7/26/2019 RG_ER-NPM-49_20190726	Elk River	3.4	1.4	49.5	45.0	590.0	-	12.6	-	-	2.14
BC	2019	June	26	6/26/2019 RG_GC-NPM-01_20190626	Gold Creek	2.4	1.2	54.5	49.0	1375.0	-	149.5	-	-	10.87
BC	2019	June	26	6/26/2019 RG_GC-NPM-02_20190626	Gold Creek	2.1	1.3	53.9	49.3	1425.0	-	135.5	-	-	9.51
BC	2019	June	26	6/26/2019 RG_GC-NPM-03_20190626	Gold Creek	2.1	1.2	47.4	43.1	1075.0	-	106.7	-	-	9.93
BC	2019	June	26	6/26/2019 RG_GC-NPM-04_20190626	Gold Creek	20.0	2.4	38.9	34.9	460.0	-	2.9	-	-	0.64
BC	2019	June	26	6/26/2019 RG_GC-NPM-05_20190626	Gold Creek	3.9	1.7	44.8	40.4	915.0	-	55.5	-	-	6.07
BC	2019	June	26	6/26/2019 RG_GC-NPM-06_20190626	Gold Creek	2.4	1.1	49.9	44.9	1060.0	-	115.8	-	-	10.92
BC	2019	June	26	6/26/2019 RG_GC-NPM-07_20190626	Gold Creek	11.0	2.1	34.6	31.3	375.0	-	3.0	-	-	0.81
BC	2019	June	27	6/27/2019 RG_GC-NPM-08_20190627	Gold Creek	2.2	1.2	54.6	49.8	1600.0	-	210.4	-	-	13.15
BC	2019	June	27	6/27/2019 RG_GC-NPM-09_20190627	Gold Creek	2.2	1.4	52.6	47.8	1060.0	-	27.0	-	-	2.55
BC	2019	June	27	6/27/2019 RG_GC-NPM-10_20190627	Gold Creek	2.7	1.2	50.2	45.4	1280.0	-	169.2	-	-	13.22
BC	2019	June	27	6/27/2019 RG_GC-NPM-11_20190627	Gold Creek	12.0	1.8	41.6	37.4	600.0	-	4.2	-	-	0.70
BC	2019	June	27	6/27/2019 RG_GC-NPM-12_20190627	Gold Creek	3.9	1.4	45.0	40.5	920.0	-	48.9	-	-	5.32
BC	2019	June	27	6/27/2019 RG_GC-NPM-13_20190627	Gold Creek	3.3	2.0	49.5	44.8	1150.0	-	92.4	-	-	8.03
BC	2019	July	18	7/18/2019 RG_GC-NPM-14_20190718	Gold Creek	9.6	1.5	50.8	46.2	1030.0	-	8.8	-	-	0.86
BC	2019	July	19	7/19/2019 RG_GC-NPM-15_20190719	Gold Creek	3.9	1.4	61.8	57.0	1800.0	-	100.9	-	-	5.60
BC	2019	July	25	7/25/2019 RG_GC-NPM-16_20190725	Gold Creek	2.7	1.4	50.2	46.0	1200.0	-	18.3	-	-	1.53
BC	2019	June	20	6/20/2019 RG_SC-NPM-01_20190620	Sand Creek	8.4	2.2	43.5	39.3	740.0	-	26.9	-	-	3.64
BC	2019	June	20	6/20/2019 RG_SC-NPM-02_20190620	Sand Creek	20.0	1.8	42.8	38.0	600.0	-	3.2	-	-	0.53

Province/ State	Year	Month	Day	Sample ID	Area	Ovary Se (µg/g dw)	Muscle Se (µg/g dw)	Total Length (cm)	Fork Length (cm)	Body Weight (g)	Age	Gonad Weight (g)	Liver Weight (g)	Adjusted Body Weight (g)b	GSI (%)
BC	2019	June	20	6/20/2019 RG_SC-NPM-03_20190620	Sand Creek	11.0	2.0	34.6	31.6	340.0	-	2.0	-	-	0.58
BC	2019	June	20	6/20/2019 RG_SC-NPM-04_20190620	Sand Creek	17.0	2.6	41.9	38.9	640.0	-	20.5	-	-	3.20
BC	2019	June	20	6/20/2019 RG_SC-NPM-05_20190620	Sand Creek	28.0	2.0	39.2	35.5	490.0	-	3.1	-	-	0.62
BC	2019	July	24	7/24/2019 RG_SC-NPM-06_20190724	Sand Creek	10.0	1.4	43.5	34.2	495.0	-	7.2	-	-	1.46
BC	2019	July	25	7/25/2019 RG_SC-NPM-07_20190725	Sand Creek	21.0	1.7	38.4	35.0	525.0	-	5.7	-	-	1.09
BC	2019	July	25	7/25/2019 RG_SC-NPM-08_20190725	Sand Creek	23.0	2.4	40.3	37.3	540.0	-	8.6	-	-	1.59
BC	2019	July	25	7/25/2019 RG_SC-NPM-09_20190725	Sand Creek	12.0	1.5	44.3	39.9	790.0	-	10.6	-	-	1.34
BC	2019	July	26	7/26/2019 RG_SC-NPM-10_20190726	Sand Creek	25.0	1.4	39.8	35.7	510.0	-	2.4	-	-	0.47
BC	2019	July	26	7/26/2019 RG_SC-NPM-11_20190726	Sand Creek	23.0	2.2	37.9	34.2	495.0	-	4.6	-	-	0.92
BC	2019	June	21	6/21/2019 RG_WB-NPM-01_20190621	Waldo Bay	7.4	2.2	38.0	34.5	406.0	-	16.4	-	-	4.04
BC	2019	June	26	6/26/2019 RG-WB-NPM-02_20190626	Waldo Bay	26.0	2.9	39.9	35.9	480.0	-	5.7	-	-	1.19
BC	2019	June	26	6/26/2019 RG-WB-NPM-03_20190626	Waldo Bay	9.8	2.9	37.5	33.7	440.0	-	4.6	-	-	1.04
MT	2019	May	15	Rexford_NSC_01	Rexford	3.9	1.7	40.5	37.0	540.0	-	5.3	7.4	527.28	0.99
MT	2019	May	15	Rexford_NSC_02	Rexford	2.5	1.0	54.5	50.3	1785.0	-	49.7	32.04	1703.24	2.79
MT	2019	May	15	Rexford_NSC_03	Rexford	5.1	1.5	39.9	36.2	495.0	-	3.1	7.74	484.17	0.62
MT	2019	May	15	Rexford_NSC_04	Rexford	2.2	1.2	50.3	46.0	1500.0	-	41.0	29.007	1430.033	2.73
MT	2019	May	15	Rexford_NSC_05	Rexford	1.8	1.1	60.3	54.8	2060.0	-	68.2	29.69	1962.14	3.31
MT	2019	May	15	Rexford_NSC_06	Rexford	3.5	1.4	42.6	38.8	760.0	-	5.5	27.71	726.76	0.73
MT	2019	May	15	Rexford_NSC_07	Rexford	3.2	1.3	46.9	42.5	1070.0	-	7.1	20.16	1042.76	0.66
MT	2019	May	15	Rexford_NSC_08	Rexford	3.5	1.4	40.9	36.4	610.0	-	4.8	13.94	591.22	0.79
MT	2019	May	15	Rexford_NSC_09	Rexford	2.6	0.8	56.1	51.3	1620.0	-	60.3	28.73	1530.992	3.72
MT	2019	May	15	Rexford_NSC_10	Rexford	2.0	1.1	49.9	44.9	1200.0	-	30.9	14.03	1155.03	2.58
MT	2019	May	15	Rexford_NSC_11	Rexford	9.5	1.6	37.4	33.5	475.0	-	4.7	7.27	463.01	0.99
MT	2019	May	15	Rexford_NSC_12	Rexford	5.1	1.0	46.7	42.0	940.0	-	10.8	16.07	913.11	1.15
MT	2019	May	15	Rexford_NSC_13	Rexford	2.0	1.0	44.4	40.1	890.0	-	13.1	22.17	854.689	1.48
MT	2019	May	15	Rexford_NSC_14	Rexford	2.8	0.9	51.4	47.2	1540.0	-	42.4	20.822	1476.813	2.75
MT	2019	May	15	Rexford_NSC_15	Rexford	2.2	0.9	54.0	49.3	1490.0	-	53.9	21.269	1414.834	3.62

APPENDIX C. NORTHEN PIKEMINNOW TISSUE DATA: 2019

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG-ER-NPM-32-BF-20190709	Eggs	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	0.01	2.2	21	<0.01	0.6	0.037	0.03	<0.05	2.8	<0.01	0.10	<0.005	<0.05	<0.2	<0.005	<0.1	66	64.02
7/9/2019 RG-ER-NPM-33-BF-20190709	Eggs	<5	<0.02	<0.05	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.6	56	<0.05	0.9	0.04	<0.05	<0.5	3.0	<0.02	0.2	<0.01	<0.2	<0.5	<0.02	<0.2	80	65.11
7/12/2019 RG-ER-NPM-43-BF-20190712	Eggs	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.02	2.9	45	<0.01	2.4	0.046	0.04	0.07	11.7	<0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	69	64.87
7/13/2019 RG-ER-NPM-46-BF-20190713	Eggs	<2	<0.01	0.11	0.13	<0.01	<1	<0.01	<0.05	0.02	2.1	30	<0.01	0.3	0.042	0.03	<0.05	2.7	<0.01	0.10	0.010	<0.05	<0.2	<0.005	<0.1	98	63.44
7/16/2019 RG-ER-NPM-48-BF-20190716	Eggs	<2	<0.01	0.10	0.36	<0.01	<1	<0.01	0.06	0.02	2.6	22	<0.01	0.8	0.051	0.04	<0.05	2.3	<0.01	0.15	0.009	<0.05	<0.2	<0.005	<0.1	77	69.18
7/3/2019 RG-ER-NPM-28-AF-20190703	Eggs	120	<0.01	0.04	1.2	<0.01	<1	<0.01	0.06	0.04	2.4	74	0.07	2.9	0.019	0.04	0.10	8.4	<0.01	2.9	<0.005	<0.05	1.0	0.031	<0.1	77	95.04
7/9/2019 RG-ER-NPM-32-AF-20190709	Eggs	260	<0.02	0.17	2.6	<0.02	<2	<0.02	<0.1	0.04	2.2	140	0.51	7.4	0.03	<0.05	0.2	2.9	<0.02	3.0	<0.01	<0.1	4.3	0.05	<0.2	68	96.25
7/13/2019 RG-ER-NPM-46-AF-20190713	Eggs	100	<0.01	0.08	3.3	<0.01	<1	<0.01	0.07	0.04	2.2	130	0.20	12	0.041	0.05	0.10	2.3	<0.01	2.6	0.011	<0.05	3.8	0.028	<0.1	88	94.15
7/15/2019 RG-ER-NPM-47-AF-20190715	Eggs	60	<0.1	<0.5	<5	<0.02	<50	<0.02	<5	<5	<5	<50	<0.5	<5	0.07	<0.5	<5	1.8	<0.02	1	<0.1	<2	<5	<0.1	<1	70	94.79
7/16/2019 RG-ER-NPM-48-AF-20190716	Eggs	560	0.01	0.31	15	0.02	<1	0.01	0.09	0.11	2.6	760	1.1	85	0.048	0.12	0.21	2.7	0.01	6.7	0.015	<0.05	24	0.14	0.4	86	91.84
7/3/2019 RG-ER-NPM-28-PF_20190703	Eggs	<5	<0.02	0.25	<0.5	<0.02	<5	<0.02	<0.5	<0.5	2.1	34	<0.05	2.3	0.02	<0.05	<0.5	9.6	<0.02	0.1	<0.01	<0.2	<0.5	<0.02	<0.2	62	66.93
6/14/2019 RG_ER-NPM-01-M_20190614	Muscle	2	<0.01	0.15	0.25	<0.01	<1	<0.01	0.24	0.01	2.0	25	<0.01	0.3	1.3	<0.02	0.05	1.6	<0.01	3.8	0.016	<0.05	<0.2	<0.005	<0.1	19	72.64
6/14/2019 RG_ER-NPM-02-M_20190614	Muscle	<2	<0.01	0.21	0.95	<0.01	<1	<0.01	<0.05	0.02	2.6	24	0.02	0.8	2.2	<0.02	<0.05	1.3	<0.01	6.2	0.014	<0.05	<0.2	<0.005	<0.1	33	72.76
6/17/2019 RG_ER-NPM-03-M_20190617	Muscle	<2	<0.01	0.22	0.92	<0.01	<1	<0.01	0.15	0.02	3.5	33	0.06	0.6	1.2	<0.02	<0.05	1.2	<0.01	3.6	0.013	<0.05	<0.2	<0.005	<0.1	28	71.78
6/17/2019 RG_ER-NPM-04-M_20190617	Muscle	<2	<0.01	0.16	0.90	<0.01	<1	<0.01	<0.05	0.02	2.6	26	<0.01	0.6	0.66	<0.02	<0.05	1.2	<0.01	3.0	0.021	<0.05	0.2	<0.005	<0.1	22	71.16
6/18/2019 RG_ER-NPM-05-M_20190618	Muscle	<2	<0.01	0.04	0.72	<0.01	<1	<0.01	0.20	0.01	0.85	9	<0.01	0.6	1.7	<0.02	<0.05	1.6	<0.01	3.6	0.010	<0.05	<0.2	<0.005	<0.1	19	77.76
6/18/2019 RG_ER-NPM-06-M_20190618	Muscle	<2	<0.01	0.07	0.58	<0.01	<1	<0.01	<0.05	0.03	0.79	8	<0.01	0.8	2.1	<0.02	<0.05	1.6	<0.01	3.0	0.008	<0.05	<0.2	<0.005	<0.1	17	78.52
6/18/2019 RG_ER-NPM-07-M_20190618	Muscle	<2	<0.01	0.17	1.2	<0.01	<1	<0.01	<0.05	0.02	2.4	23	<0.01	1.0	0.90	<0.02	0.08	1.3	<0.01	5.7	0.013	<0.05	<0.2	<0.005	<0.1	24	72.59
6/18/2019 RG_ER-NPM-08-M_20190618	Muscle	<2	<0.01	0.03	0.75	<0.01	<1	<0.01	<0.05	0.01	0.95	13	0.01	0.4	1.9	<0.02	<0.05	1.6	<0.01	3.1	0.009	<0.05	<0.2	<0.005	<0.1	22	78.06
6/19/2019 RG_ER-NPM-09-M_20190619	Muscle	3	<0.01	0.06	1.7	<0.01	<1	<0.01	<0.05	0.03	2.5	27	0.09	1.2	0.94	<0.02	0.07	2.0	<0.01	8.5	0.017	<0.05	<0.2	<0.005	<0.1	38	76.79
6/19/2019 RG_ER-NPM-10-M_20190619	Muscle	<2	<0.01	0.03	0.95	<0.01	<1	<0.01	<0.05	0.02	1.7	16	0.02	0.7	0.98	<0.02	<0.05	1.9	<0.01	3.0	0.009	<0.05	<0.2	<0.005	<0.1	29	76.48
6/19/2019 RG_ER-NPM-11-M_20190619	Muscle	<2	<0.01	0.07	0.36	<0.01	<1	<0.01	<0.05	0.01	1.5	16	0.03	0.6	0.87	<0.02	<0.05	2.5	<0.01	1.4	0.012	<0.05	<0.2	<0.005	<0.1	18	78.43
6/20/2019 RG_ER-NPM-12-M_20190620	Muscle	<2	<0.01	0.17	0.20	<0.01	<1	<0.01	<0.05	<0.01	0.96	9	0.01	0.2	1.5	<0.02	<0.05	1.3	<0.01	0.61	0.010	<0.05	<0.2	<0.005	<0.1	15	72.41
6/20/2019 RG_ER-NPM-13-M_20190620	Muscle	<2	<0.01	0.08	0.78	<0.01	<1	<0.01	<0.05	0.02	1.1	11	0.01	0.7	1.5	<0.02	<0.05	1.5	<0.01	4.5	0.006	<0.05	<0.2	<0.005	<0.1	19	75.51
6/20/2019 RG_ER-NPM-14-M_20190620	Muscle	<2	<0.01	0.14	0.65	<0.01	<1	<0.01	<0.05	0.01	1.1	11	<0.01	0.6	1.3	<0.02	<0.05	1.7	<0.01	2.6	0.010	<0.05	<0.2	<0.005	<0.1	16	74.20
6/20/2019 RG_ER-NPM-15-M_20190620	Muscle	<2	<0.01	0.02	0.85	<0.01	<1	<0.01	<0.05	0.01	0.92	14	0.02	0.9	1.1	<0.02	<0.05	2.5	<0.01	2.0	0.008	<0.05	<0.2	<0.005	<0.1	24	79.46
6/20/2019 RG_ER-NPM-16-M_20190620	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	0.09	0.03	1.9	29	0.01	1.3	1.0	<0.02	<0.05	2.4	<0.01	4.8	0.011	<0.05	<0.2	<0.005	<0.1	34	80.14
6/20/2019 RG_ER-NPM-17-M_20190620	Muscle	<2	<0.01	0.08	1.0	<0.01	<1	<0.01	<0.05	0.02	2.2	22	<0.01	1.0	1.1	<0.02	<0.05	1.8	<0.01	3.7	0.013	<0.05	<0.2	<0.005	<0.1	26	78.24
6/20/2019 RG_ER-NPM-18-M_20190620	Muscle	<2	<0.01	0.07	0.46	<0.01	<1	<0.01	<0.05	<0.01	0.83	7	<0.01	0.4	1.7	<0.02	<0.05	2.5	<0.01	1.7	<0.005	<0.05	<0.2	<0.005	<0.1	20	74.74
6/25/2019 RG_ER-NPM-19-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.02	1.8	17	<0.01	1.0	0.41	<0.02	<0.05	1.9	<0.01	2.2	0.021	<0.05	<0.2	<0.005	<0.1	38	78.49
6/25/2019 RG_ER-NPM-20-M_20190625	Muscle	<2	<0.01	0.08	1.6	<0.01	<1	<0.01	<0.05	0.03	2.7	27	0.01	1.4	0.92	<0.02	<0.05	2.9	<0.01	7.1	0.018	<0.05	<0.2	<0.005	<0.1	29	76.00
6/27/2019 RG_ER-NPM-21-M_20190627	Muscle	<2	<0.01	0.04	1.3	<0.01	<1	<0.01	<0.05	0.02	2.6	35	<0.01	1.9	0.78	<0.02	<0.05	3.4	<0.01	7.1	0.011	<0.05	<0.2	<0.005	<0.1	28	76.67
6/28/2019 RG_ER-NPM-22-M_20190628	Muscle	<2	<0.01	0.02	0.84	<0.01	<1	<0.01	<0.05	0.02	0.92	9	<0.01	1.2	0.61	<0.02	0.05	2.6	<0.01	3.3	0.016	<0.05	<0.2	<0.005	<0.1	18	76.61
6/28/2019 RG_ER-NPM-23-M_20190628	Muscle	<2	<0.01	0.01	2.4	<0.01	<1	<0.01	0.08	0.02	2.3	21	<0.01	1.3	1.9	<0.02	<0.05	3.6	<0.01	3.4	0.012	<0.05	<0.2	<0.005	<0.1	25	74.79
6/28/2019 RG_ER-NPM-24-M_20190628	Muscle	<2	<0.01	0.06	2.0	<0.01	<1	<0.01	0.22	0.02	1.5	20	<0.01	1.4	0.88	<0.02	<0.05	2.4	<0.01	8.4	0.008	<0.05	<0.2	<0.005	<0.1	28	78.90
6/28/2019 RG_ER-NPM-25-M_20190628	Muscle	<2	<0.01	0.02	0.50	<0.01	<1	<0.01	0.06	<0.01	1.1	10	<0.01	0.7	1.0	<0.02	<0.05	2.5	<0.01	1.5	0.012	<0.05	<0.2	<0.005	<0.1	17	75.15
6/28/2019 RG_ER-NPM-26-M_20190628	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	0.07	0.02	1.7	21	<0.01	1.5	1.1	<0.02	<0.05	1.1	<0.01	7.1	0.010	<0.05	<0.2	<0.005	<0.1	24	77.37
6/28/2019 RG_ER-NPM-27-M_20190628	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	0.02	0.83	11	<0.01	1.2	0.35	<0.02	<0.05	2.3	<0.01	4.7	0.013	<0.05	<0.2	<0.005	<0.1	22	77.97
7/3/2019 RG_ER-NPM-28-M_20190703	Muscle	<2	<0.01	0.03	1.1	<0.01	<1	<0.01	<0.05	0.01	1.2	12	0.02	1.4	0.86	<0.02	<0.05	2.3	<0.01	4.7	0.010	<0.05	<0.2	<0.005	<0.1	19	76.85
7/4/2019 RG_ER-NPM-29-M_20190704	Muscle	<2	<0.01	0.03	0.37	<0.01	<1	<0.01	<0.05	0.01	1.7	21	<0.01	0.6	0.82	<0.02	<0.05	4.6	<0.01	0.75	0.011	<0.05	<0.2	<0.005	<0.1	21	78.32

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/4/2019 RG_ER-NPM-30-M_20190704	Muscle	<2	<0.01	0.03	0.58	<0.01	<1	<0.01	<0.05	<0.01	0.73	9	0.02	0.8	1.6	<0.02	<0.05	2.7	<0.01	2.3	0.006	<0.05	<0.2	<0.005	<0.1	15	77.17
7/8/2019 RG_ER-NPM-31-M_20190708	Muscle	3	<0.01	0.04	1.4	<0.01	<1	<0.01	13	0.05	2.3	170	0.02	1.6	0.72	<0.02	0.40	3.1	<0.01	2.7	0.019	<0.05	<0.2	<0.005	<0.1	33	78.99
7/9/2019 RG_ER-NPM-32-M_20190709	Muscle	<2	<0.01	0.12	0.77	<0.01	<1	<0.01	0.77	0.02	1.8	26	<0.01	0.5	1.2	<0.02	<0.05	1.3	<0.01	3.2	0.010	<0.05	<0.2	<0.005	<0.1	25	72.74
7/9/2019 RG_ER-NPM-33-M_20190709	Muscle	<2	<0.01	0.11	0.92	<0.01	<1	<0.01	0.11	0.02	2.2	21	<0.01	0.8	0.76	<0.02	<0.05	1.4	<0.01	3.9	0.021	<0.05	<0.2	<0.005	<0.1	26	75.45
7/9/2019 RG_ER-NPM-34-M_20190709	Muscle	<2	<0.01	0.12	1.5	<0.01	<1	<0.01	0.17	0.02	2.0	20	<0.01	1.1	1.4	<0.02	<0.05	1.6	<0.01	5.4	0.011	<0.05	<0.2	<0.005	<0.1	28	74.77
7/9/2019 RG_ER-NPM-35-M_20190709	Muscle	<2	<0.01	0.32	1.8	<0.01	<1	<0.01	0.14	0.02	2.2	24	<0.01	1.3	1.0	<0.02	<0.05	1.2	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	31	77.08
7/9/2019 RG_ER-NPM-36-M_20190709	Muscle	<2	<0.01	0.06	1.4	<0.01	<1	<0.01	0.17	0.03	2.7	25	<0.01	1.4	2.0	<0.02	<0.05	3.9	<0.01	4.3	0.020	<0.05	<0.2	<0.005	<0.1	34	78.53
7/9/2019 RG_ER-NPM-37-M_20190709	Muscle	4	<0.01	0.11	1.8	<0.01	<1	<0.01	0.07	0.03	2.2	26	0.02	2.0	0.87	<0.02	<0.05	2.2	<0.01	6.6	0.009	<0.05	<0.2	<0.005	<0.1	33	79.86
7/10/2019 RG_ER-NPM-38-M_20190710	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	0.10	0.02	2.4	25	0.02	1.4	0.80	<0.02	0.05	4.0	<0.01	4.0	0.011	<0.05	<0.2	<0.005	<0.1	29	78.21
7/10/2019 RG_ER-NPM-39-M_20190710	Muscle	<2	<0.01	0.11	0.46	<0.01	<1	<0.01	0.06	0.01	1.6	15	<0.01	0.5	0.92	<0.02	<0.05	1.2	<0.01	1.7	0.027	<0.05	<0.2	<0.005	<0.1	20	76.75
7/10/2019 RG_ER-NPM-40-M_20190710	Muscle	5	<0.01	0.09	0.74	<0.01	<1	<0.01	0.11	0.02	1.6	27	0.04	0.9	0.66	<0.02	0.09	3.0	<0.01	1.2	0.010	<0.05	<0.2	<0.005	<0.1	32	79.51
7/10/2019 RG_ER-NPM-41-M_20190710	Muscle	<2	<0.01	0.12	0.91	<0.01	<1	<0.01	0.18	0.01	1.0	11	<0.01	0.8	0.87	<0.02	<0.05	1.3	<0.01	3.1	0.024	<0.05	<0.2	<0.005	<0.1	21	76.75
7/10/2019 RG_ER-NPM-42-M_20190710	Muscle	<2	<0.01	0.03	1.5	<0.01	<1	<0.01	0.08	0.02	1.7	15	<0.01	1.4	0.92	<0.02	<0.05	3.4	<0.01	5.2	0.009	<0.05	<0.2	<0.005	<0.1	29	78.08
7/11/2019 RG_ER-NPM-43-M_20190711	Muscle	<2	<0.01	0.05	1.1	<0.01	<1	<0.01	0.13	0.02	2.6	17	0.02	1.3	0.69	<0.02	<0.05	4.8	<0.01	4.0	0.022	<0.05	<0.2	<0.005	<0.1	26	79.26
7/12/2019 RG_ER-NPM-44-M_20190712	Muscle	<2	<0.01	0.05	0.14	<0.01	<1	<0.01	<0.05	<0.01	0.73	10	<0.01	0.5	1.1	<0.02	<0.05	5.0	<0.01	0.10	0.008	<0.05	<0.2	<0.005	<0.1	17	78.91
7/12/2019 RG_ER-NPM-45-M_20190712	Muscle	<2	<0.01	0.10	0.19	<0.01	<1	<0.01	0.05	<0.01	0.77	6	<0.01	0.2	1.0	<0.02	<0.05	1.2	<0.01	0.80	0.015	<0.05	<0.2	<0.005	<0.1	14	76.31
7/13/2019 RG_ER-NPM-46-M_20190713	Muscle	2	<0.01	0.19	0.60	<0.01	<1	<0.01	0.06	0.02	2.2	23	0.01	0.4	1.3	<0.02	<0.05	1.3	<0.01	2.8	0.022	<0.05	<0.2	<0.005	<0.1	28	75.43
7/15/2019 RG_ER-NPM-47-M_20190715	Muscle	<2	<0.01	0.17	1.0	<0.01	<1	<0.01	0.07	0.02	1.6	16	<0.01	0.7	1.1	<0.02	0.12	1.2	<0.01	5.2	0.014	<0.05	<0.2	<0.005	<0.1	26	76.36
7/16/2019 RG_ER-NPM-48-M_20190716	Muscle	<2	<0.01	0.14	1.2	<0.01	<1	<0.01	<0.05	0.02	1.4	12	<0.01	0.8	1.7	<0.02	<0.05	1.3	<0.01	4.9	0.021	<0.05	<0.2	<0.005	<0.1	27	78.26
7/26/2019 RG_ER-NPM-49-M_20190726	Muscle	<2	<0.01	0.09	0.71	<0.01	<1	<0.01	0.06	0.02	1.5	15	<0.01	0.6	1.6	<0.02	<0.05	1.4	<0.01	3.6	0.014	<0.05	<0.2	<0.005	<0.1	24	77.86
6/26/2019 RG_GC-NPM-01-M_20190626	Muscle	<2	<0.01	0.18	0.17	<0.01	<1	<0.01	0.05	<0.01	0.84	8	<0.01	0.1	2.6	<0.02	<0.05	1.2	<0.01	0.51	0.007	<0.05	<0.2	<0.005	<0.1	12	75.39
6/26/2019 RG_GC-NPM-02-M_20190626	Muscle	<2	<0.01	0.07	0.07	<0.01	<1	<0.01	<0.05	<0.01	0.75	7	<0.01	0.1	1.5	<0.02	<0.05	1.3	<0.01	0.12	0.015	<0.05	<0.2	<0.005	<0.1	15	77.80
6/26/2019 RG_GC-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	0.54	<0.01	<1	<0.01	<0.05	<0.01	0.68	7	<0.01	0.5	1.1	<0.02	<0.05	1.2	<0.01	2.3	0.010	<0.05	<0.2	<0.005	<0.1	18	77.49
6/26/2019 RG_GC-NPM-04-M_20190626	Muscle	<2	<0.01	0.06	0.60	<0.01	<1	<0.01	<0.05	<0.01	0.82	12	<0.01	0.8	1.1	<0.02	<0.05	2.4	<0.01	2.3	0.011	<0.05	<0.2	<0.005	<0.1	22	78.87
6/26/2019 RG_GC-NPM-05-M_20190626	Muscle	<2	<0.01	0.04	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.8	20	0.01	1.4	1.1	<0.02	<0.05	1.7	<0.01	3.4	0.009	<0.05	<0.2	<0.005	<0.1	25	77.49
6/26/2019 RG_GC-NPM-06-M_20190626	Muscle	<2	<0.01	0.12	1.0	<0.01	<1	<0.01	<0.05	<0.01	1.7	17	<0.01	0.7	0.92	<0.02	<0.05	1.1	<0.01	3.8	0.021	<0.05	<0.2	<0.005	<0.1	22	75.90
6/26/2019 RG_GC-NPM-07-M_20190626	Muscle	<2	<0.01	0.02	1.1	<0.01	<1	<0.01	0.06	<0.01	1.3	20	0.01	1.2	1.1	<0.02	<0.05	2.1	<0.01	4.6	0.015	<0.05	<0.2	<0.005	<0.1	26	79.07
6/27/2019 RG_GC-NPM-08-M_20190627	Muscle	2	<0.01	0.10	1.4	<0.01	<1	<0.01	<0.05	<0.01	1.4	12	0.23	0.7	1.2	<0.02	<0.05	1.2	<0.01	6.6	0.022	<0.05	0.4	<0.005	<0.1	31	75.59
6/27/2019 RG_GC-NPM-09-M_20190627	Muscle	2	<0.01	0.04	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.91	12	<0.01	0.2	1.5	<0.02	<0.05	1.4	<0.01	0.78	0.017	<0.05	<0.2	<0.005	<0.1	14	78.43
6/27/2019 RG_GC-NPM-10-M_20190627	Muscle	<2	<0.01	0.09	0.92	<0.01	<1	<0.01	<0.05	<0.01	1.6	13	<0.01	0.7	1.2	<0.02	<0.05	1.2	<0.01	4.8	0.022	<0.05	<0.2	<0.005	<0.1	20	75.35
6/27/2019 RG_GC-NPM-11-M_20190627	Muscle	<2	<0.01	0.09	0.16	<0.01	<1	<0.01	<0.05	<0.01	0.63	10	<0.01	0.3	1.1	<0.02	<0.05	1.8	<0.01	0.60	0.005	<0.05	<0.2	<0.005	<0.1	14	77.35
6/27/2019 RG_GC-NPM-12-M_20190627	Muscle	<2	<0.01	0.10	1.3	<0.01	<1	<0.01	<0.05	<0.01	2.2	21	<0.01	1.4	1.0	<0.02	<0.05	1.4	<0.01	4.6	0.010	<0.05	<0.2	<0.005	<0.1	26	78.38
6/27/2019 RG_GC-NPM-13-M_20190627	Muscle	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	<0.01	0.82	11	<0.01	0.3	1.6	<0.02	<0.05	2.0	<0.01	0.57	<0.005	<0.05	<0.2	<0.005	<0.1	14	78.91
7/18/2019 RG_GC-NPM-14-M_20190718	Muscle	<2	<0.01	0.11	0.78	<0.01	<1	<0.01	<0.05	<0.01	2.9	34	<0.01	0.4	1.7	<0.02	<0.05	1.5	<0.01	1.3	0.014	<0.05	<0.2	<0.005	<0.1	27	76.81
7/19/2019 RG_GC-NPM-15-M_20190719	Muscle	2	<0.01	0.04	0.44	<0.01	<1	<0.01	<0.05	<0.01	0.84	6	<0.01	0.3	2.6	<0.02	<0.05	1.4	<0.01	1.8	0.011	<0.05	<0.2	<0.005	<0.1	20	80.92
7/25/2019 RG_GC-NPM-16-M_20190725	Muscle	<2	<0.01	0.04	1.2	<0.01	<1	<0.01	<0.05	<0.01	1.2	9	<0.01	0.8	1.3	<0.02	<0.05	1.4	<0.01	5.6	0.016	<0.05	<0.2	<0.005	<0.1	27	77.46
6/20/2019 RG_SC-NPM-01-M_20190620	Muscle	<2	<0.01	0.05	0.70	<0.01	<1	<0.01	<0.05	<0.01	2.5	31	<0.01	0.9	1.0	<0.02	<0.05	2.2	<0.01	2.7	0.013	<0.05	<0.2	<0.005	<0.1	27	78.73
6/20/2019 RG_SC-NPM-02-M_20190620	Muscle	<2	<0.01	0.03	0.69	<0.01	<1	<0.01	1.2	<0.01	1.2	24	<0.01	0.9	0.99	0.04	0.12	1.8	<0.01	3.8	0.010	<0.05	<0.2	<0.005	<0.1	23	80.68
6/20/2019 RG_SC-NPM-03-M_20190620	Muscle	<2	<0.01	0.04	1.1	<0.01	<1	<0.01	<0.05	<0.01	2.1	21	0.02	1.6	0.55	<0.02	<0.05	2.0	<0.01	5.1	0.012	<0.05	<0.2	<0.005	<0.1	34	79.58
6/20/2019 RG_SC-NPM-04-M_20190620	Muscle	<2	<0.01	0.07	0.77	<0.01	<1	<0.01	<0.05	<0.01	2.1	25	<0.01	0.8	1.1	<0.02	<0.05	2.6	<0.01	2.9	0.016	<0.05	<0.2	<0.005	<0.1	34	79.13
6/20/2019 RG_SC-NPM-05-M_20190620	Muscle	<2	<0.01	0.04	0.93	<0.01	<1	<0.01	<0.05	0.01	1.1	13	<0.01	0.9	0.81	<0.02	<0.05	2.0	<0.01	3.4	0.011	<0.05	<0.2	<0.005	<0.1	24	79.40

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/24/2019 RG_SC-NPM-06-M_20190724	Muscle	<2	<0.01	0.01	0.79	<0.01	<1	<0.01	0.32	0.01	0.85	17	<0.01	1.3	1.4	<0.02	<0.05	1.4	<0.01	4.8	<0.005	<0.05	<0.2	<0.005	<0.1	18	79.65
7/25/2019 RG_SC-NPM-07-M_20190725	Muscle	3	<0.01	0.06	1.1	<0.01	<1	<0.01	0.06	0.01	0.95	15	0.02	1.6	1.3	<0.02	<0.05	1.7	<0.01	4.7	0.007	<0.05	<0.2	<0.005	<0.1	44	80.43
7/25/2019 RG_SC-NPM-08-M_20190725	Muscle	<2	<0.01	0.10	1.0	<0.01	<1	<0.01	0.05	0.02	1.0	15	<0.01	1.8	1.3	<0.02	0.05	2.4	<0.01	6.6	0.014	<0.05	<0.2	<0.005	<0.1	24	78.64
7/25/2019 RG_SC-NPM-09-M_20190725	Muscle	<2	<0.01	0.02	0.24	<0.01	<1	<0.01	<0.05	0.01	1.8	19	<0.01	0.5	1.2	<0.02	<0.05	1.5	<0.01	1.1	0.006	<0.05	<0.2	<0.005	<0.1	19	76.51
7/26/2019 RG_SC-NPM-10-M_20190726	Muscle	<2	<0.01	0.07	0.39	<0.01	<1	<0.01	<0.05	0.01	0.76	10	<0.01	0.6	1.0	<0.02	0.06	1.4	<0.01	1.4	0.006	<0.05	<0.2	<0.005	<0.1	17	79.18
7/26/2019 RG_SC-NPM-11-M_20190726	Muscle	<2	<0.01	0.06	0.28	<0.01	<1	<0.01	<0.05	<0.01	1.1	14	<0.01	0.4	0.90	<0.02	<0.05	2.2	<0.01	1.1	0.013	<0.05	<0.2	<0.005	<0.1	21	79.53
6/21/2019 RG_WB-NPM-01-M_20190621	Muscle	<2	<0.01	0.02	0.97	<0.01	<1	<0.01	<0.05	0.02	1.7	23	0.04	0.9	1.1	<0.02	<0.05	2.2	<0.01	2.8	0.010	<0.05	<0.2	<0.005	<0.1	28	77.69
6/26/2019 RG-WB-NPM-02-M_20190626	Muscle	<2	<0.01	0.09	2.4	<0.01	<1	<0.01	<0.05	0.02	2.6	28	0.01	1.7	0.99	<0.02	<0.05	2.9	<0.01	9.4	0.015	<0.05	<0.2	<0.005	<0.1	44	79.29
6/26/2019 RG-WB-NPM-03-M_20190626	Muscle	<2	<0.01	0.08	1.5	<0.01	<1	<0.01	<0.05	0.02	2.5	26	<0.01	1.5	1.0	<0.02	<0.05	2.9	<0.01	3.9	0.006	<0.05	<0.2	<0.005	<0.1	32	79.01
6/14/2019 RG_ER-NPM-01-O_20190614	Ovary	<2	<0.01	0.07	0.12	<0.01	<1	<0.01	<0.05	0.02	3.2	43	<0.01	0.6	0.10	0.05	<0.05	3.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	97	64.38
6/14/2019 RG_ER-NPM-02-O_20190614	Ovary	<2	<0.01	0.07	0.14	<0.01	<1	<0.01	<0.05	0.03	3.1	38	<0.01	3.0	0.16	0.06	<0.05	2.7	0.01	0.19	0.007	<0.05	<0.2	<0.005	<0.1	110	65.57
6/17/2019 RG_ER-NPM-03-O_20190617	Ovary	<2	<0.01	0.06	0.22	<0.01	<1	<0.01	<0.05	0.03	2.8	45	0.01	1.3	0.10	0.05	<0.05	3.3	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	94	65.63
6/17/2019 RG_ER-NPM-04-O_20190617	Ovary	<2	<0.01	0.09	0.13	<0.01	<1	<0.01	<0.05	0.02	2.8	41	<0.01	0.7	0.051	0.04	<0.05	3.0	<0.01	0.11	0.009	<0.05	<0.2	<0.005	<0.1	87	61.36
6/18/2019 RG_ER-NPM-05-O_20190618	Ovary	<2	<0.01	0.04	0.21	<0.01	<1	<0.01	<0.05	0.03	3.0	37	0.01	2.2	0.12	0.04	<0.05	4.9	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	78	62.62
6/18/2019 RG_ER-NPM-06-O_20190618	Ovary	<2	<0.01	0.26	0.14	<0.01	<1	<0.01	<0.05	0.03	2.6	52	<0.01	2.2	0.14	0.07	<0.05	5.0	<0.01	0.20	0.006	<0.05	<0.2	<0.005	<0.1	120	67.92
6/18/2019 RG_ER-NPM-07-O_20190618	Ovary	<2	<0.01	0.06	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	41	<0.01	1.3	0.11	0.04	<0.05	4.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	87	63.03
6/18/2019 RG_ER-NPM-08-O_20190618	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.03	2.3	91	0.01	2.6	0.13	0.08	<0.05	4.2	<0.01	0.27	0.009	<0.05	<0.2	<0.005	<0.1	120	67.65
6/19/2019 RG_ER-NPM-09-O_20190619	Ovary	3	<0.01	0.06	0.16	<0.01	<1	<0.01	<0.05	0.03	2.8	60	0.18	2.2	0.054	0.07	<0.05	7.2	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	68.24
6/19/2019 RG_ER-NPM-10-O_20190619	Ovary	2	<0.01	0.04	0.26	<0.01	<1	<0.01	0.21	0.05	2.8	96	0.13	3.4	0.17	0.14	<0.05	9.9	<0.01	0.32	0.023	<0.05	<0.2	<0.005	<0.1	260	76.39
6/19/2019 RG_ER-NPM-11-O_20190619	Ovary	4	<0.01	0.06	0.32	<0.01	<1	0.01	0.11	0.06	4.1	130	0.18	2.7	0.096	0.12	0.06	17	<0.01	0.41	0.049	<0.05	<0.2	<0.005	<0.1	500	79.40
6/20/2019 RG_ER-NPM-12-O_20190620	Ovary	<2	<0.01	0.07	0.15	<0.01	<1	<0.01	<0.05	0.02	3.0	33	<0.01	0.5	0.081	0.04	<0.05	2.4	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	84	63.59
6/20/2019 RG_ER-NPM-13-O_20190620	Ovary	<2	<0.01	0.06	0.14	<0.01	<1	<0.01	<0.05	0.03	2.4	54	<0.01	1.9	0.23	0.06	<0.05	3.6	<0.01	0.22	0.008	<0.05	<0.2	<0.005	<0.1	120	71.25
6/20/2019 RG_ER-NPM-14-O_20190620	Ovary	<2	<0.01	0.06	0.12	<0.01	<1	<0.01	<0.05	0.04	2.6	60	<0.01	1.8	0.21	0.08	<0.05	7.6	<0.01	0.23	0.015	<0.05	<0.2	<0.005	<0.1	140	72.31
6/20/2019 RG_ER-NPM-15-O_20190620	Ovary	<2	<0.01	0.04	0.36	<0.01	<1	<0.01	<0.05	0.05	2.9	95	<0.01	5.5	0.078	0.10	<0.05	8.6	<0.01	0.24	0.009	<0.05	<0.2	<0.005	<0.1	150	70.05
6/20/2019 RG_ER-NPM-16-O_20190620	Ovary	2	<0.01	0.08	0.35	<0.01	<1	0.01	0.09	0.07	3.6	150	0.02	2.5	0.13	0.12	0.06	17	<0.01	0.26	0.033	<0.05	<0.2	<0.005	<0.1	520	83.66
6/20/2019 RG_ER-NPM-17-O_20190620	Ovary	<2	<0.01	0.07	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	2.3	0.085	0.04	<0.05	4.5	<0.01	0.16	0.005	<0.05	<0.2	<0.005	<0.1	74	65.19
6/20/2019 RG_ER-NPM-18-O_20190620	Ovary	<2	<0.01	0.05	0.22	<0.01	<1	<0.01	<0.05	0.02	1.8	32	<0.01	2.5	0.14	0.04	<0.05	4.1	<0.01	0.17	<0.005	<0.05	<0.2	<0.005	<0.1	80	65.88
6/25/2019 RG_ER-NPM-19-O_20190625	Ovary	<2	<0.01	0.08	0.38	<0.01	<1	<0.01	<0.05	0.04	2.6	66	<0.01	8.3	0.024	0.07	<0.05	7.9	<0.01	0.17	0.018	<0.05	<0.2	<0.005	<0.1	120	66.61
6/25/2019 RG_ER-NPM-20-O_20190625	Ovary	<2	<0.01	0.04	0.08	<0.01	<1	<0.01	<0.05	0.06	3.2	110	<0.01	2.6	0.12	0.08	<0.05	6.3	<0.01	0.21	0.030	<0.05	<0.2	<0.005	<0.1	180	75.96
6/27/2019 RG_ER-NPM-21-O_20190627	Ovary	<2	<0.01	0.03	0.11	<0.01	<1	<0.01	<0.05	0.02	2.7	49	<0.01	2.3	0.039	0.03	<0.05	13	<0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	80	60.36
6/28/2019 RG_ER-NPM-22-O_20190628	Ovary	<2	<0.01	0.05	0.17	<0.01	<1	<0.01	<0.05	0.02	2.2	37	<0.01	2.5	0.021	0.03	<0.05	7.1	<0.01	0.09	0.008	<0.05	<0.2	<0.005	<0.1	79	61.41
6/28/2019 RG_ER-NPM-23-O_20190628	Ovary	<2	<0.01	0.01	0.32	<0.01	<1	<0.01	<0.05	0.03	2.7	44	<0.01	4.1	0.12	0.04	<0.05	9.8	0.02	0.14	0.006	<0.05	<0.2	<0.005	<0.1	100	65.42
6/28/2019 RG_ER-NPM-24-O_20190628	Ovary	<2	<0.01	0.05	0.19	<0.01	<1	<0.01	<0.05	0.04	3.1	55	<0.01	1.5	0.12	0.05	<0.05	7.8	<0.01	0.15	0.006	<0.05	<0.2	<0.005	<0.1	120	67.86
6/28/2019 RG_ER-NPM-25-O_20190628	Ovary	<2	<0.01	0.02	0.22	<0.01	<1	<0.01	<0.05	0.03	3.3	51	<0.01	4.7	0.050	0.06	<0.05	8.3	<0.01	0.14	0.006	<0.05	<0.2	<0.005	<0.1	97	63.20
6/28/2019 RG_ER-NPM-26-O_20190628	Ovary	<2	<0.01	0.02	0.13	<0.01	<1	<0.01	<0.05	0.02	3.0	54	<0.01	2.1	0.037	0.04	<0.05	4.0	<0.01	0.15	<0.005	<0.05	<0.2	<0.005	<0.1	77	62.47
6/28/2019 RG_ER-NPM-27-O_20190628	Ovary	<2	<0.01	0.86	1.5	<0.01	<1	0.10	0.06	0.06	1.5	47	0.07	0.4	0.023	<0.02	0.06	14.6	<0.01	0.10	0.021	<0.05	<0.2	<0.005	<0.1	300	56.47
7/3/2019 RG_ER-NPM-28-O_20190703	Ovary	4	<0.01	0.03	0.20	<0.01	<1	0.01	<0.05	0.04	2.8	170	0.06	2.8	0.088	0.08	<0.05	34.5	<0.01	0.27	0.018	<0.05	<0.2	<0.005	<0.1	180	77.60
7/4/2019 RG_ER-NPM-29-O_20190704	Ovary	6	<0.01	0.05	0.39	<0.01	<1	0.04	0.22	0.07	3.9	150	0.06	2.8	0.097	0.15	0.10	19.4	<0.01	0.57	0.034	<0.05	0.3	<0.005	<0.1	330	77.92
7/4/2019 RG_ER-NPM-30-O_20190704	Ovary	14	<0.01	0.04	0.44	<0.01	<1	0.02	0.05	0.05	4.2	110	0.15	3.7	0.11	0.07	<0.05	7.1	<0.01	0.36	0.014	<0.05	0.3	<0.005	0.1	200	76.76
7/8/2019 RG_ER-NPM-31-O_20190708	Ovary	<2	<0.01	0.04	0.20	<0.01	<1	<0.01	0.09	0.03	3.3	64	<0.01	3.5	0.068	0.05	<0.05	10.9	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	100	65.74
7/9/2019 RG_ER-NPM-32-O_20190709	Ovary	<2	<0.01	0.08	0.13	<0.01	<1	<0.01	0.10	0.04	3.3	81	0.01	1.2	0.13	0.08	<0.05	4.1	<0.01	0.36	0.016	<0.05	<0.2	<0.005	<0.1	160	74.41

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/9/2019 RG_ER-NPM-33-O_20190709	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.6	47	<0.01	0.9	0.030	<0.02	<0.05	3.5	<0.01	0.18	0.010	<0.05	<0.2	<0.005	<0.1	97	63.66
7/9/2019 RG_ER-NPM-34-O_20190709	Ovary	3	<0.01	0.07	0.13	<0.01	<1	0.01	<0.05	0.05	3.2	77	<0.01	3.1	0.21	0.08	<0.05	5.4	<0.01	0.24	0.029	<0.05	<0.2	<0.005	0.1	260	76.24
7/9/2019 RG_ER-NPM-35-O_20190709	Ovary	<2	<0.01	0.06	0.24	<0.01	<1	<0.01	<0.05	0.02	2.4	57	<0.01	1.1	0.056	0.04	<0.05	3.8	<0.01	0.17	0.006	<0.05	<0.2	<0.005	<0.1	82	65.42
7/9/2019 RG_ER-NPM-36-O_20190709	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	0.02	3.0	43	<0.01	2.4	0.12	0.04	<0.05	9.3	0.01	0.10	0.006	<0.05	<0.2	<0.005	<0.1	78	62.62
7/9/2019 RG_ER-NPM-37-O_20190709	Ovary	<2	<0.01	0.06	0.23	<0.01	<1	<0.01	0.21	0.03	3.1	48	<0.01	1.5	0.050	0.05	<0.05	5.4	<0.01	0.24	<0.005	<0.05	<0.2	<0.005	<0.1	92	63.31
7/10/2019 RG_ER-NPM-38-O_20190710	Ovary	3	<0.01	0.04	0.19	<0.01	<1	<0.01	<0.05	0.04	3.6	57	<0.01	5.9	0.071	0.08	<0.05	12	0.01	0.28	0.007	<0.05	<0.2	<0.005	<0.1	130	69.92
7/10/2019 RG_ER-NPM-39-O_20190710	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.02	3.3	48	<0.01	2.0	0.038	0.04	<0.05	2.7	0.01	0.14	0.010	<0.05	<0.2	<0.005	<0.1	110	62.30
7/10/2019 RG_ER-NPM-40-O_20190710	Ovary	5	<0.01	0.06	0.35	<0.01	<1	0.02	0.12	0.04	4.0	110	0.07	1.4	0.060	0.07	0.06	18.4	<0.01	0.24	0.036	<0.05	0.2	<0.005	<0.1	540	79.88
7/10/2019 RG_ER-NPM-41-O_20190710	Ovary	<2	<0.01	0.07	0.17	<0.01	<1	<0.01	<0.05	0.02	2.6	52	<0.01	1.3	0.030	0.05	<0.05	3.4	<0.01	0.13	0.015	<0.05	<0.2	<0.005	<0.1	110	64.12
7/10/2019 RG_ER-NPM-42-O_20190710	Ovary	<2	<0.01	0.03	0.18	<0.01	<1	<0.01	<0.05	0.03	3.2	55	<0.01	5.1	0.079	0.07	<0.05	11	0.01	0.18	<0.005	<0.05	<0.2	<0.005	<0.1	100	65.47
7/11/2019 RG_ER-NPM-43-O_20190711	Ovary	<2	<0.01	0.06	0.17	<0.01	<1	<0.01	<0.05	0.03	3.0	42	<0.01	2.5	0.049	0.04	<0.05	12	0.01	0.12	0.007	<0.05	<0.2	<0.005	<0.1	78	64.30
7/12/2019 RG_ER-NPM-44-O_20190712	Ovary	3	<0.01	0.05	0.15	<0.01	<1	0.01	0.11	0.06	3.6	110	0.02	2.9	0.068	0.14	<0.05	36	<0.01	0.34	0.032	<0.05	<0.2	<0.005	<0.1	410	78.06
7/12/2019 RG_ER-NPM-45-O_20190712	Ovary	<2	<0.01	0.09	0.22	<0.01	<1	<0.01	<0.05	0.02	2.7	33	<0.01	0.5	0.049	0.04	<0.05	2.2	<0.01	0.13	0.010	<0.05	<0.2	<0.005	<0.1	91	62.46
7/13/2019 RG_ER-NPM-46-O_20190713	Ovary	<2	<0.01	0.12	0.07	<0.01	<1	<0.01	<0.05	0.04	3.3	91	<0.01	0.6	0.17	0.09	<0.05	2.4	<0.01	0.22	0.046	<0.05	<0.2	<0.005	<0.1	210	75.90
7/15/2019 RG_ER-NPM-47-O_20190715	Ovary	<2	<0.01	0.12	0.14	<0.01	<1	<0.01	<0.05	0.02	2.5	31	<0.01	0.7	0.091	0.03	<0.05	2.1	<0.01	0.12	0.011	<0.05	<0.2	<0.005	<0.1	99	66.82
7/16/2019 RG_ER-NPM-48-O_20190716	Ovary	<2	<0.01	0.11	0.06	<0.01	<1	0.01	<0.05	0.05	3.7	69	<0.01	1.4	0.37	0.11	<0.05	2.3	<0.01	0.33	0.059	<0.05	<0.2	<0.005	<0.1	230	81.71
7/26/2019 RG_ER-NPM-49-O_20190726	Ovary	2	<0.01	0.15	0.32	<0.01	<1	0.02	0.06	0.05	3.7	240	<0.01	1.2	0.23	0.07	<0.05	3.4	<0.01	0.43	0.051	<0.05	<0.2	0.008	0.1	380	82.16
6/26/2019 RG_GC-NPM-01-O_20190626	Ovary	<2	<0.01	0.08	0.20	<0.01	<1	<0.01	<0.05	0.02	2.5	41	<0.01	0.6	0.14	0.05	<0.05	2.4	<0.01	0.17	0.005	<0.05	<0.2	<0.005	<0.1	100	64.51
6/26/2019 RG_GC-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.02	2.5	38	<0.01	0.6	0.096	0.06	<0.05	2.1	<0.01	0.14	0.012	<0.05	<0.2	<0.005	<0.1	110	65.08
6/26/2019 RG_GC-NPM-03-O_20190626	Ovary	2	<0.01	0.05	0.18	<0.01	<1	<0.01	0.40	0.04	3.1	47	<0.01	0.6	0.049	0.04	<0.05	2.1	<0.01	0.12	0.006	<0.05	<0.2	<0.005	<0.1	77	63.05
6/26/2019 RG_GC-NPM-04-O_20190626	Ovary	<2	<0.01	0.17	0.19	<0.01	<1	<0.01	0.07	<0.01	2.5	75	0.01	1.1	0.096	0.04	<0.05	20	<0.01	0.21	0.032	<0.05	<0.2	<0.005	<0.1	360	72.82
6/26/2019 RG_GC-NPM-05-O_20190626	Ovary	<2	<0.01	0.03	0.26	<0.01	<1	<0.01	<0.05	<0.01	2.2	46	<0.01	1.9	0.076	0.03	<0.05	3.9	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	83	63.49
6/26/2019 RG_GC-NPM-06-O_20190626	Ovary	<2	<0.01	0.06	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.7	40	<0.01	1.9	0.048	0.04	<0.05	2.4	<0.01	0.12	0.012	<0.05	<0.2	<0.005	<0.1	100	63.02
6/26/2019 RG_GC-NPM-07-O_20190626	Ovary	<2	<0.01	0.11	0.13	<0.01	<1	0.01	0.12	<0.01	3.2	120	0.01	1.4	0.082	0.08	<0.05	11	<0.01	0.19	0.047	<0.05	<0.2	<0.005	<0.1	360	77.70
6/27/2019 RG_GC-NPM-08-O_20190627	Ovary	<2	<0.01	0.15	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	30	<0.01	0.4	0.078	0.03	<0.05	2.2	<0.01	0.11	0.012	<0.05	<0.2	<0.005	<0.1	100	62.90
6/27/2019 RG_GC-NPM-09-O_20190627	Ovary	<2	<0.01	0.10	0.06	<0.01	<1	<0.01	<0.05	<0.01	3.8	68	<0.01	1.5	0.25	0.10	<0.05	2.2	<0.01	0.30	0.036	<0.05	<0.2	<0.005	<0.1	180	77.62
6/27/2019 RG_GC-NPM-10-O_20190627	Ovary	<2	<0.01	0.10	0.14	<0.01	<1	<0.01	<0.05	<0.01	2.4	40	<0.01	1.1	0.093	0.04	<0.05	2.7	<0.01	0.11	0.010	<0.05	<0.2	<0.005	<0.1	90	61.14
6/27/2019 RG_GC-NPM-11-O_20190627	Ovary	<2	<0.01	0.04	0.12	<0.01	<1	<0.01	0.11	0.01	2.8	92	<0.01	2.4	0.13	0.10	<0.05	12	<0.01	0.29	0.018	<0.05	<0.2	<0.005	<0.1	340	78.10
6/27/2019 RG_GC-NPM-12-O_20190627	Ovary	<2	<0.01	0.04	0.25	<0.01	<1	<0.01	<0.05	<0.01	2.3	47	<0.01	3.3	0.070	0.03	<0.05	3.9	<0.01	0.12	<0.005	<0.05	<0.2	<0.005	<0.1	95	64.74
6/27/2019 RG_GC-NPM-13-O_20190627	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.1	33	<0.01	1.3	0.078	0.03	<0.05	3.3	<0.01	0.16	<0.005	<0.05	<0.2	<0.005	<0.1	65	61.68
7/18/2019 RG_GC-NPM-14-O_20190718	Ovary	7	<0.01	0.09	0.18	<0.01	<1	0.02	<0.05	<0.01	3.3	140	0.02	1.5	0.28	0.08	<0.05	9.6	<0.01	0.27	0.045	<0.05	0.2	<0.005	0.1	430	79.54
7/19/2019 RG_GC-NPM-15-O_20190719	Ovary	2	<0.01	0.10	0.21	<0.01	<1	<0.01	<0.05	<0.01	2.2	37	<0.01	0.5	0.27	0.04	<0.05	3.9	<0.01	0.11	0.008	<0.05	<0.2	<0.005	<0.1	120	67.63
7/25/2019 RG_GC-NPM-16-O_20190725	Ovary	<2	<0.01	0.12	0.18	<0.01	<1	<0.01	<0.05	<0.01	2.8	39	<0.01	1.1	0.083	0.03	<0.05	2.7	<0.01	0.11	0.011	<0.05	0.4	<0.005	<0.1	91	64.09
6/20/2019 RG_SC-NPM-01-O_20190620	Ovary	<2	<0.01	0.04	0.13	<0.01	<1	<0.01	<0.05	<0.01	3.1	56	<0.01	3.3	0.071	0.06	<0.05	8.4	<0.01	0.20	0.008	<0.05	<0.2	<0.005	<0.1	120	68.02
6/20/2019 RG_SC-NPM-02-O_20190620	Ovary	7	<0.01	0.06	0.26	<0.01	<1	<0.01	0.05	<0.01	2.6	130	0.03	1.2	0.12	0.06	<0.05	20	<0.01	0.84	0.041	<0.05	<0.2	<0.005	<0.1	490	80.46
6/20/2019 RG_SC-NPM-03-O_20190620	Ovary	<2	<0.01	0.09	0.07	<0.01	<1	<0.01	<0.05	<0.01	3.0	93	0.02	2.1	0.041	0.08	<0.05	11	<0.01	0.20	0.025	<0.05	<0.2	<0.005	<0.1	320	73.78
6/20/2019 RG_SC-NPM-04-O_20190620	Ovary	<2	<0.01	0.03	0.24	<0.01	<1	<0.01	<0.05	<0.01	3.6	110	0.02	3.0	0.098	0.09	<0.05	17	<0.01	0.72	0.012	<0.05	<0.2	<0.005	<0.1	140	69.48
6/20/2019 RG_SC-NPM-05-O_20190620	Ovary	<2	<0.01	0.10	0.33	<0.01	<1	<0.01	<0.05	0.05	3.0	98	<0.01	1.8	0.088	0.10	0.05	28	<0.01	0.23	0.034	<0.05	<0.2	<0.005	<0.1	360	75.91
7/24/2019 RG_SC-NPM-06-O_20190724	Ovary	<2	<0.01	0.08	0.18	<0.01	<1	<0.01	<0.05	0.05	2.5	120	<0.01	1.9	0.11	0.04	0.09	10	<0.01	0.78	0.008	<0.05	<0.2	<0.005	<0.1	250	77.04
7/25/2019 RG_SC-NPM-07-O_20190725	Ovary	<2	<0.01	0.07	0.09	<0.01	<1	<0.01	<0.05	0.04	3.0	100	<0.01	8.9	0.14	0.16	<0.05	21	<0.01	0.38	0.018	<0.05	<0.2	<0.005	<0.1	280	77.16
7/25/2019 RG_SC-NPM-08-O_20190725	Ovary	<2	<0.01	0.15	0.15	<0.01	<1	<0.01	<0.05	0.05	2.8	86	<0.01	5.1	0.092	0.10	<0.05	23	<0.01	0.57	0.018	<0.05	<0.2	<0.005	<0.1	200	74.33

Sample ID	Sample Type	Aluminum (µg/g dw)	Antimony (µg/g dw)	Arsenic (µg/g dw)	Barium (µg/g dw)	Beryllium (µg/g dw)	Boron (µg/g dw)	Cadmium (µg/g dw)	Chromium (µg/g dw)	Cobalt (µg/g dw)	Copper (µg/g dw)	Iron (µg/g dw)	Lead (µg/g dw)	Manganese (µg/g dw)	Mercury (µg/g dw)	Molybdenum (µg/g dw)	Nickel (µg/g dw)	Selenium (µg/g dw)	Silver (µg/g dw)	Strontium (µg/g dw)	Thallium (µg/g dw)	Tin (µg/g dw)	Titanium (µg/g dw)	Uranium (µg/g dw)	Vanadium (µg/g dw)	Zinc (µg/g dw)	Moisture (%)
7/25/2019 RG_SC-NPM-09-O_20190725	Ovary	<2	<0.01	0.08	0.07	<0.01	<1	<0.01	<0.05	0.05	2.7	75	<0.01	3.5	0.18	0.12	0.08	12	<0.01	0.33	0.007	<0.05	<0.2	<0.005	<0.1	220	75.90
7/26/2019 RG_SC-NPM-10-O_20190726	Ovary	3	<0.01	0.13	0.62	<0.01	<1	<0.01	0.09	0.05	3.2	100	<0.01	1.6	0.072	0.07	0.07	25	<0.01	0.28	0.023	<0.05	<0.2	<0.005	<0.1	440	75.75
7/26/2019 RG_SC-NPM-11-O_20190726	Ovary	<2	<0.01	0.12	0.15	<0.01	<1	<0.01	0.08	0.07	4.2	160	<0.01	2.8	0.12	0.05	<0.05	23	<0.01	0.62	0.028	<0.05	<0.2	<0.005	<0.1	320	80.08
6/21/2019 RG_WB-NPM-01-O_20190621	Ovary	5	<0.01	0.02	0.23	<0.01	<1	<0.01	<0.05	0.04	3.6	77	0.06	4.5	0.069	0.09	0.06	7.4	<0.01	0.40	0.006	<0.05	<0.2	<0.005	<0.1	120	67.47
7/13/2019 RG_ER-NPM-47-O_20190713	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.03	2.7	45	<0.01	0.9	0.045	0.03	<0.05	3.5	<0.01	0.10	0.009	<0.05	<0.2	<0.005	<0.1	94	64.10
6/26/2019 RG-WB-NPM-02-O_20190626	Ovary	<2	<0.01	0.08	0.15	<0.01	<1	<0.01	<0.05	0.05	3.0	83	<0.01	4.4	0.12	0.13	<0.05	26	<0.01	0.28	0.030	<0.05	<0.2	<0.005	<0.1	260	76.76
6/26/2019 RG-WB-NPM-03-O_20190626	Ovary	<2	<0.01	0.14	0.12	<0.01	<1	<0.01	<0.05	0.03	2.0	65	<0.01	1.6	0.18	0.06	<0.05	9.8	<0.01	0.14	0.007	<0.05	<0.2	<0.005	<0.1	240	70.13