Blood Selenium Concentrations in Ravens, Bald Eagles, and Mallard Ducks

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Abstract

Elevated blood selenium concentrations have been observed in various species of invertebrates that were sampled in and around the Gros Ventre River drainage. This inspired the analysis of an incidental data set on blood selenium concentrations in ravens, bald eagles, and mallard ducks. Mean values for these species were 346 ng/ml,1424 ng/ml, and 6274 ng/ml respectively. A single factor ANOVA using these means yielded a p-value of 6.4099E-09 which supports the hypothesis that mean selenium levels in these species are not equal. To aid in the development of future study designs for investigating variation in selenium bioaccumulation in this ecosystem , a R script was developed that will allow researchers to estimate necessary sample sizes for discriminating between different mean values between species or sub-populations.

INTRODUCTION

Selenium is a paradoxical nutrient with complex and ecologically significant biogeochemistry. It has been labeled a paradox because in many organisms there is a narrow range in between essential and toxic levels. This is why it is an ecologically significant element and target of substantial biogeochemical research. Selenium research has focused on both the biogeochemical cycling (i.e. weathering of source rocks, atmospheric deposition, anthropogenic sources, speciation dynamics) as well as the effects of its bioavailability, and propensity for bioaccumulation, on flora and fauna. Research involving this later topic has exploited both experimental and empirical methodologies. Often this research is concerned with producing some manner of toxicity threshold or dose response curve for use in managing and predicting the ecological consequences of selenium in the environment. Typically the empirical studies have focused on systems with anthropogenically elevated selenium levels due to irrigation drainage waters or power plant waste disposal. Most cases of toxic bioaccumulation occurred in aqueous systems such as wetlands, lakes, or rivers (Frankenberger and Engberg, 1998, p. 1-8). Management may not be appropriate or feasible for selenium loads that are not anthropogenically derived but anytime selenium levels are elevated there may be ecologically significant effects. Based on the growing body of research involving toxicity thresholds and dose response curves for various species, one may make potentially insightful discoveries about an ecosystem's dynamics by investigating selenium concentration patterns, even in largely natural environments.

The Gros Ventre River drainage feeds into the Snake River just outside of the southern boundary of Teton National Park. Elevated selenium concentrations in fish and ospreys that utilize this drainage have been documented by Wyoming Game and Fish and Craighead Beringia South, unpublished data.

This could have demographic implications for any species that utilize fish or other biotic resources in which selenium bioaccumulation occurs. Though management for selenium loads in or around the park will not likely occur, it could be of benefit to wildlife managers to identify species who may be adversely affected by some form of selenosis. This study analyzes an incidental data set on blood selenium levels in birds that was collected as part of lead blood level research undertaken by the organization Craighead Beringia South. The analysis focuses on differences in blood selenium levels between ravens, bald eagles, and mallards. It also develops a methodology for determining appropriate sample sizes for doing a Tukey analysis as part of future research.

METHODS

Data acquisition

A data set consisting of blood selenium concentrations for ravens, bald eagles, and mallards was obtained from Craighead Beringia South. This small wildlife research non-profit oraganization obtained the data during exploratory research that was primarily concerned with blood lead levels in raptors. In all cases a net launcher was used to ensnare baited birds in a net propelled by a remotely triggered blank rifle round. All birds were trapped opportunistically within the study area which is largely comprised of the southeast corner of Teton National Park and includes private lands in between the National Elk Refuge and the Snake River. The ravens were captured from November 11th of 2006 thru March 5th of 2007. The eagles were captured in January and February of 2007 and all of the mallards were captured on March 3rd of that same year. Blood samples were aseptically collected using syringes on the brachial vein and blood was deposited into EDTA Vacutainer® tubes as per lab instructions. All blood samples were sent to the Diagnostic Center for Population and Animal Health at Michigan State University (Lansing) for the toxic elements in whole blood test.

ANOVA

In order to assess the differences in blood selenium levels between species, a single factor analysis of variance (ANOVA) was performed with species as the explanatory variable and selenium concentration (ng/ml) as the response variable.

This analysis is only appropriate if a number of assumptions are met. The first assumption is that groups (species) must be independent of one another and the data within each group must also be independent. The independence assumption is met by considering possible relationships between the groups as well as considering whether the data within each group was gathered with randomization from a homogenous population. In this case the independence assumption is made with caution due to uncertainty about the randomness of the sampling technique and homogeneity of the population.

A second major assumption is the equal variance assumption. There must be approximately equal variance between all of the groups. To assess this condition, side-by-side box plots were constructed in Excel to visually contrast the data spread between groups. Both a difference in spreads as well as a systematic change with center was observed. This systematic increase in variance with increasing means was confirmed by looking at summary statistics. In order to compensate for this violation the data were log transformed. Plotting the re-expressed data in side-by-side boxplots as well as a plot of residuals vs. predicted values (both in Excel) demonstrated that the re-expression made the variance between groups more nearly equal. The equal variance assumption was thereby met.

The third and final major assumption for an ANOVA is that each population from which the data were sampled must be normal. To check this condition a normal probability plot was made in R using the residuals from all three groups. The plot depicted a reasonably good correspondence between the

residuals and the theoretical (expected) quantiles, thereby confirming the approximate normality of the data.

After evaluating the degree to which the data met the assumptions for an ANOVA, the single factor ANOVA from the Excel data analysis extension was utilized. This output a p-value for interpreting whether or not the null hypothesis for the ANOVA was to be rejected. See chapter twenty eight of De Veaux, Velleman and Bock, *Intro Stats*, 2006, for a strong basic treatment of ANOVA.

Tukey critical difference

A script was written in R to model Tukey critical differences depending on a range of parameters. A Tukey critical difference is the difference between group means that will correspond to a rejection of the null hypothesis (all group means equal) for an ANOVA with a given type I error level (alpha level) (www.statsoft.com and Jon Graham, personal correspondence). The script was constructed with the intended purpose of estimating proper sample sizes for examining if mean selenium concentrations differed in eagles that utilized different drainages. Jon Graham, an applied statistician at the University of Montana, constructed the script in R such that graphs of Tukey critical differences would be output along with the input parameters: standard deviation, site number, and sample size. By examining the graphs one can discern an appropriate sample size for each group depending on how subtle a difference one wishes to detect.

RESULTS

The statistics for selenium concentrations among the data sets for ravens, mallards and eagles exhibit notable differences. This is especially true when contrasting the statistics for ravens and eagles with those of mallards. It is apparent that the variance of the three species is not equal. Of special concern is the trend of increasing variance with increasing means among the three species. This is a significant violation of the equal variance assumption for an ANOVA. See Table 4 and Figure 1. This trend motivated the log transformation of the selenium concentrations for ravens, eagles and mallards.

Figure 2 and figure 3 are box plots of residuals by group and residuals by predicted value respectively. These figures are plotted from data that has been re-expressed as the $\log_{10}$ of the original selenium concentrations. The figures demonstrate that this transformation has remedied the trend of increasing variance with increasing means. The transformation also made the intra-group variance more nearly equal and demonstrates that there are no outliers.

Figure 4 is a normal probability plot for the residuals of the $\log_{10}$ transformed data for all three groups. The degree to which the plotted data approximates a straight line with a slope of one is an indication of how normal the population that supplied the data is. Any deviance from this line is a potential violation of the nearly normal condition for an ANOVA. Figure 4 depicts a fairly straight plot with a slope near one. This indicates that an ANOVA can be performed but interpretations should be made with some reservation (De Veaux et al., 2006).

The p-value from the F-model with 2 and 38 degrees of freedom is 6.4099E-09. Based on this p-value and despite some potentially slight violations of the conditions for an ANOVA, I am confident in rejecting the null hypothesis that all of the group means are equal. See table 5. We can be extremely

confident that at least two of the mean blood selenium concentrations for ravens, eagles, and mallards (346 ug/ml, 1424 ug/ml, and 6274 ug/ml) are statistically different.

Table 1. Raven selenium concentrations.

Species	Selenium (ng/ml)	Selenium (ug/ml)	Selenium (ppm)
Raven	734	0.734	0.70
Raven	831	0.831	0.79
Raven	496	0.496	0.47
Raven	712	0.712	0.68
Raven	412	0.412	0.39
Raven	585	0.585	0.56
Raven	370	0.37	0.35
Raven	120	0.12	0.11
Raven	249	0.249	0.24
Raven	52	0.052	0.05
Raven	67	0.067	0.06
Raven	75	0.075	0.07
Raven	79	0.079	0.08
Raven	65	0.065	0.06

Table 2. Bald eagle blood selenium concentrations.

Species	Selenium (ng/ml)	Selenium (ug/ml)	Selenium (ppm)
BAEA	897	0.897	0.85
BAEA	2287	2.287	2.17
BAEA	1980	1.98	1.88
BAEA	857	0.857	0.81
BAEA	1074	1.074	1.02
BAEA	749	0.749	0.71
BAEA	2125	2.125	2.02

Table 3. Mallard selenium concentrations.

Species	Selenium (ng/ml)	Selenium (ug/ml)	Selenium (ppm)
Mallard	2893	2.893	2.75
Mallard	3253	3.253	3.09
Mallard	1434	1.434	1.36
Mallard	6662	6.662	6.33
Mallard	6445	6.445	6.12
Mallard	2601	2.601	2.47
Mallard	6129	6.129	5.82
Mallard	8730	8.73	8.29
Mallard	14270	14.27	13.55
Mallard	10320	10.32	9.80

Table 4. Summary statistics for raven, eagle, and mallard Selenium concentrations.

Se Concentration (ng/ml)	*Raven*	*Eagle*	*Mallard*
Mean	346.21	1424.14	6273.70
Standard Error	76.05	254.61	1264.97
Median	309.50	1074.00	6287.00
Standard Deviation	284.57	673.63	4000.18
Sample Variance	80979.26	453774.81	16001445.34
Range	779.00	1538.00	12836.00
Minimum	52.00	749.00	1434.00
Maximum	831.00	2287.00	14270.00
Sum	4847.00	9969.00	62737.00
Count	14.00	7.00	10.00
Largest(2)	734.00	2125.00	10320.00
Smallest(2)	65.00	857.00	2601.00
Confidence Level(95.0%)	164.30	623.00	2861.56

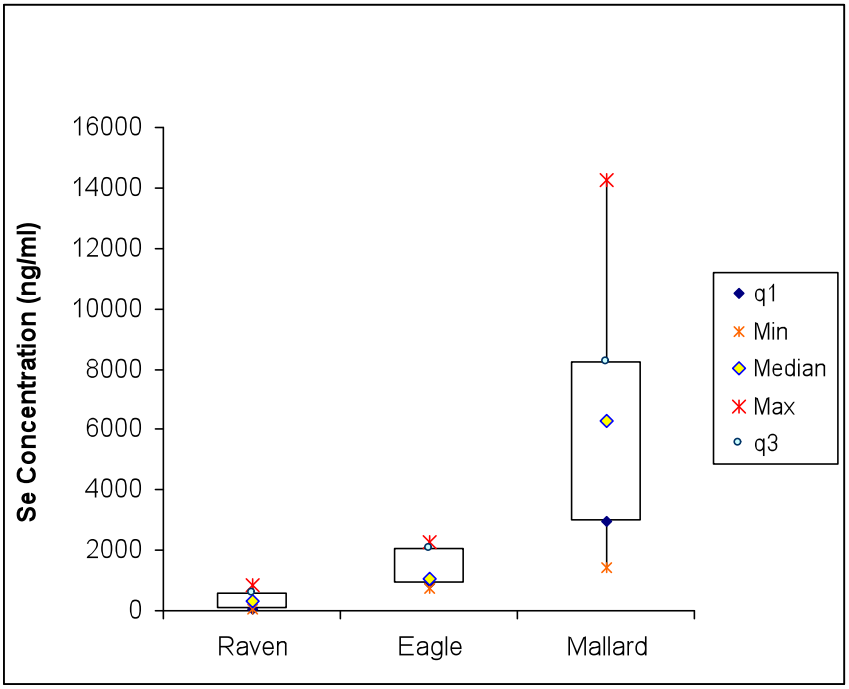

Figure 1. Side-by-side boxplot for raven, eagle, and mallard Selenium concentrations.

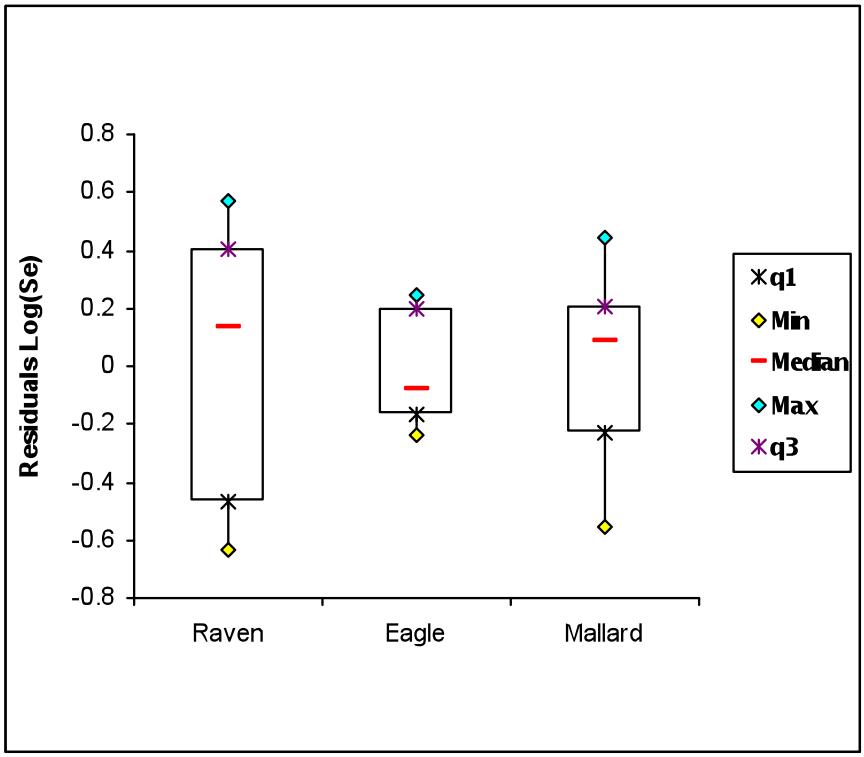

Figure 2. Side-by-side boxplot for raven, eagle, and mallard $\log_{10}$(Se concentrations).

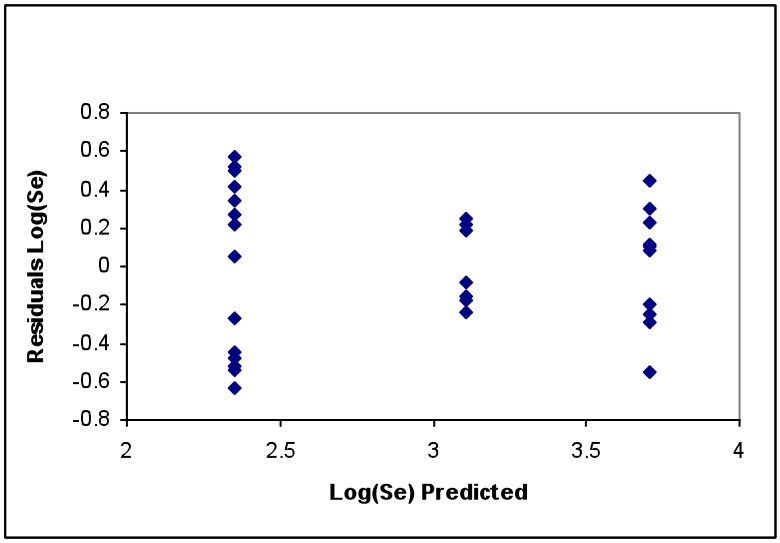

Figure 3. Plot of log predicted values vs. residuals log for raven, eagle, and mallard Se concentrations.

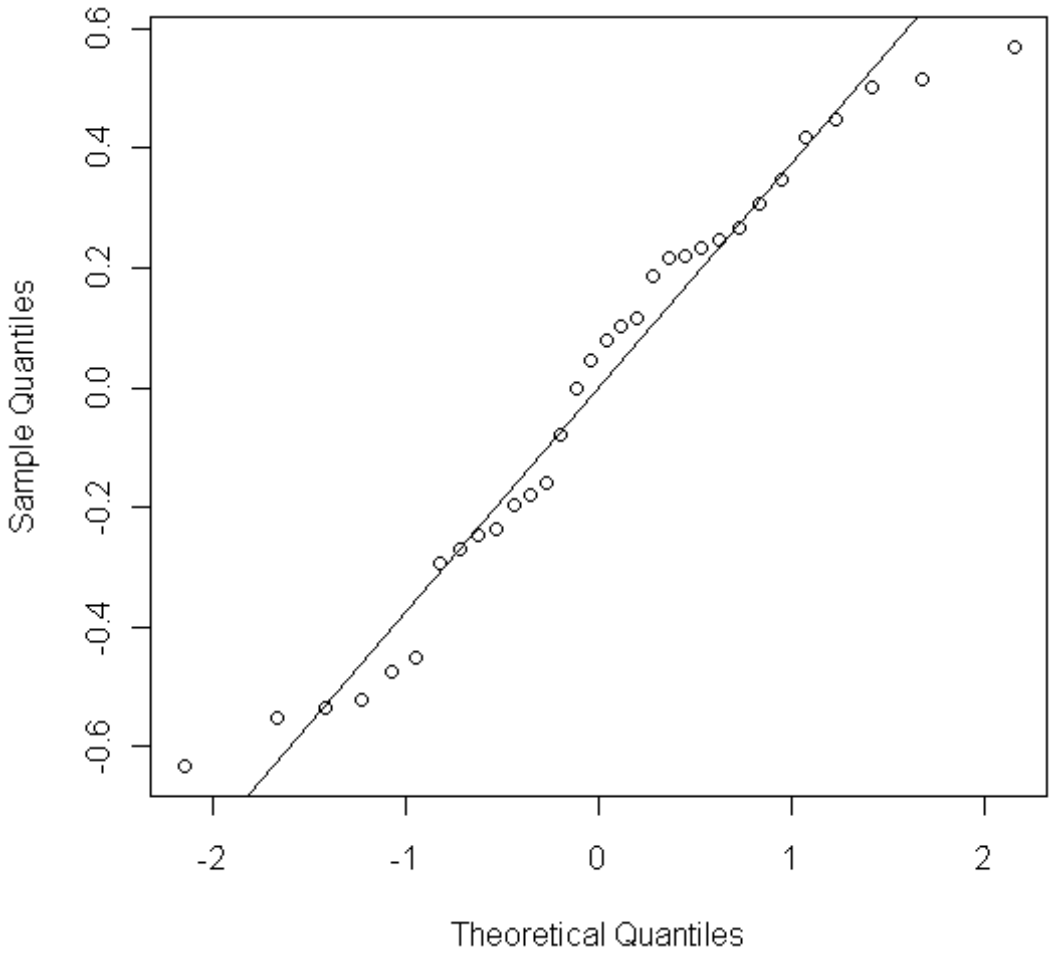

Figure 4. Normal q-q plot.

Table 5. Single factor ANOVA with species as the factor and $\log_e$(Se) as the response.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Log (Raven Se (ng/ml))	14	32.8865	2.349	0.2096
Log (Eagle Se (ng/ml))	7	21.7745	3.110	0.0437
Log (Mallard Se (ng/ml))	10	37.0746	3.707	0.0962

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	***P-value***	*F crit*
Between Groups	10.9717	2	5.485	39.871	**6.4099E-09**	3.340
Within Groups	3.8525	28	0.137			
Total	14.824	30				

DISCUSSION

Variation in blood selenium concentrations

A major result of the data analysis was that there is a statistically significant difference between the mean selenium concentrations for ravens, eagles, and mallards. This result from the ANOVA must be interpreted cautiously because there is no conclusive test for the independence assumption and mild violations occurred with respect to the equal variance and normality assumptions. The later two assumptions are of minimal concern because of the subtle manner in which these assumptions were violated. This is especially true of the equal variance assumption because unequal variances decrease the likelihood of a type I error (De Veaux et al., 2006). In other words, the minor violation of the equal variance assumption is of no practical importance in light of the fact that the null hypothesis was rejected. There is always the possibility that the independence assumption was violated and this would drastically restrict the applicability of the ANOVA. In this case there is no reason to doubt that the

species (groups) are independent, but the assumption of independence for individuals is more tenuous. For instance, the sampling technique could be biased toward capturing younger more inexperienced ravens that approach the bait first. In that case, a representative random sample would not have been taken from the overall population, and the results would be invalid or only applicable to the population of young ravens. Similar scenarios should be considered so as to limit the possibility of erroneously interpreting the results.

The variation in selenium concentrations both within and between the species could indicate that selenium exposure or bio-uptake is not uniform within this ecosystem. Based on what is known of selenium biogeochemistry, it is not surprising that mallards had the highest mean selenium concentration. The selenium cycle often involves a leaching of selenium from seleniferous soils and subsequent enrichment of water bodies through convergence and evaporation of contaminated source waters. When aqueous selenium is present, bioaccumulation is often observed in aquatic organisms and can increase in magnitude with trophic level. There have been a number of cases where selenium bioaccumulation was observed in terrestrial organisms that fed in wetland, riparian, or lucustrian environments where selenium concentrations were elevated (Engberg et al., 1998, p. 315-347). This could be the scenario responsible for the selenium levels seen in all three species and the variation may be explained by different feeding habits among the individuals and species. Rigorous further investigation would be necessary to test this hypothesis.

The mean selenium concentrations for ravens, eagles, and mallards were 346 ng/ml (.33 ppm), 1031 ng/ml (.98 ppm) and 6274 ng/ml (5.96 ppm) respectively. No published research addressing the background selenium levels or physiological effects of selenosis in ravens or eagles is known. For mallards there is an appreciable body of research on these topics but most often egg or tissue concentrations are used instead of blood concentartions. To my knowledge, no selenium toxicity

threshold exist for avian species based on blood selemium levels. This makes it difficult to postulate about the potential biological or demographic significance of the levels observed here. An estimate of baseline mean selenium concentrations for free-ranging freshwater birds is 0.4 ppm or less (Franson et al, 2002). The DCPA lab that performed the sample analysis and generated the data for the current study stated that 0.13 to 0.2 ppm is the range considered normal for birds in general. O'Toole D, and Raisbeck MF, 1997, did experimental research on mallards and found that a treatment group fed 25 ppm selenium enriched (dry weight) food stock obtained a maximum blood selenium level of 8.9 ug/ml (~8.9 ppm). Many individuals from this group exhibited a plethora of adverse physiological effects that were attributed to selenosis. This was not the case for a control group (0.4 ug/ml blood selenium) and a lower dose treatment group that obtained a maximum blood selenium level of 4.5 ug/ml (~4.5 ppm). Based on these published findings and the results of the current analysis on mean blood selenium concentrations for ravens (0.35 ppm), eagles (1.03 ppm), and mallards (6.27 ppm), the mallard population is suspect for exhibiting ecologically significant bioaccumulation of selenium.

Tukey critical difference and future research

The data analysis and interpretation has established that there are different mean blood selenium concentrations for ravens, eagles, and mallards, and previous research only supports potential adverse effects for mallards. To further investigate selenium concentrations in aquatic and terrestrial bird species a first step may be to survey other species. While blood samples are a more efficient and less invasive metric for selenium bioaccumulation in birds, egg concentrations are more relevant to previous research. Regardless of the metric, a great deal of time, money, and effort must be invested in collecting each sample. For this reason, it is crucial that an intelligent compromise be struck between sample sizes and statistical power.

The R script for Tukey critical differences will output a graph that will allow researchers to determine the ideal compromise between data volume and statistical power. This is accomplished by allowing researchers to visualize the important factors in a Tukey analysis and how they relate to one another. The easiest way to think about the script is that it models the difference in means between groups at which a given alpha value would support the rejection of the null hypothesis that all group means are statistically indistinguishable, for a Tukey analysis. In other words, based on possible standard deviations, sample sizes, group numbers, and an arbitrary alpha value one can model answers to the following question: how big of differences between group means can one expect to be able to detect with a Tukey analysis? The model user must determine the standard deviations, sample sizes, number of groups, and the alpha value, for which they would like to see theoretical Tukey critical differences. The ideal scenario would involve a pilot study that obtains an estimate of the within group standard deviation for a population, for instance, the average standard deviation for different species of birds. See figure 5 for an example of the model output. This example displays the modeled Tukey critical differences, corresponding sample sizes for six groups (treatments) at an alpha level of 0.05, and exploratory standard deviations of 60, 70, 81, 90, and 150; these standard deviations are arbitrary, but could come from the actual estimates of a pilot study. The figure is labeled with the critical difference between group means on the vertical axis and sample size for the groups on the horizontal axis. In the upper right hand corner is the legend that corresponds to the modeled relationship (curve) for a given intra-group standard deviation.

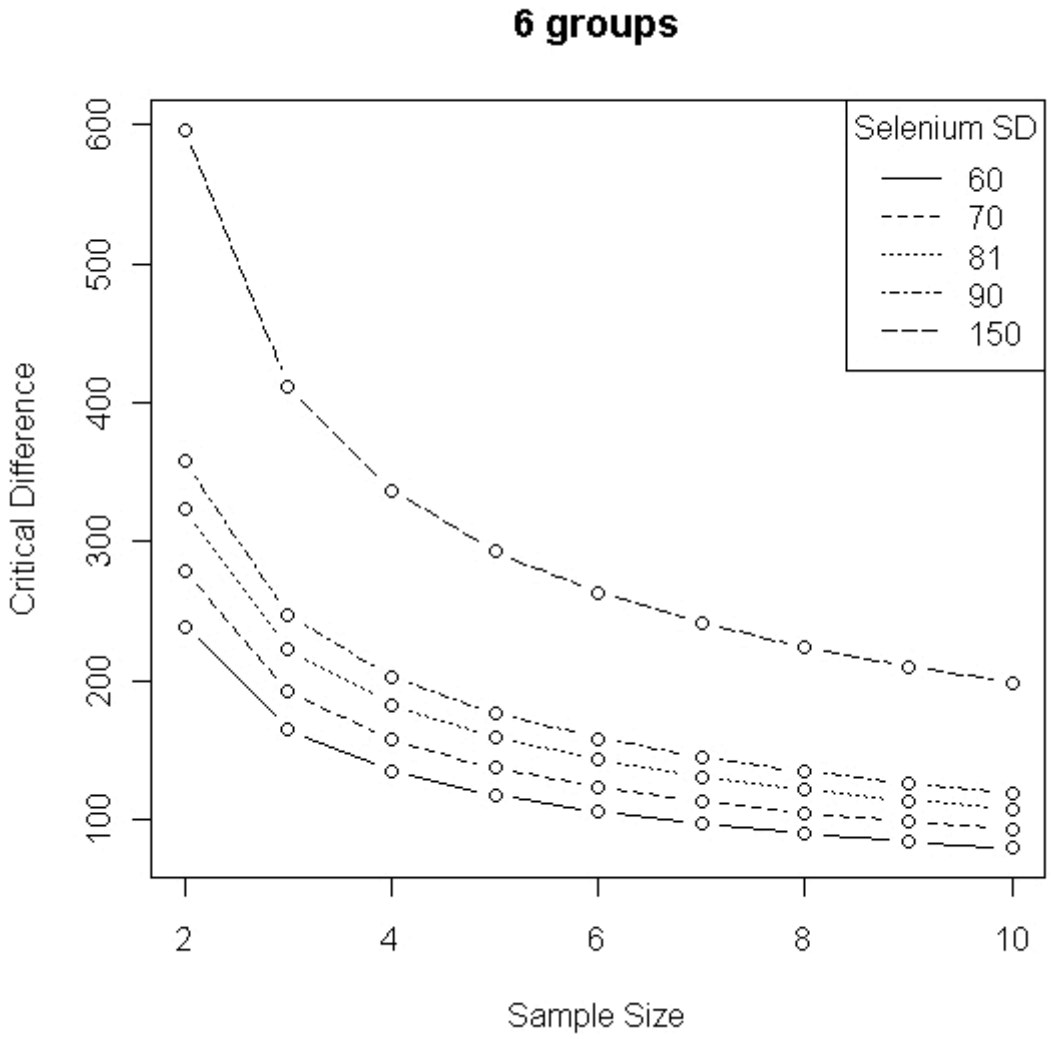

Figure 5. Critical difference vs. sample size (6 groups, alpha .05)

To use the R script an assortment of standard deviations and sample sizes must be entered along with a desired alpha value and number of treatments. A chart similar to figure 5 will be output when the script is run. Looking at the chart, an appropriate sample size can be estimated based upon desired

Tukey critical differences; in figure 5, for example, there is marginal gain in statistical power when moving from a sample size of four to six for all of the exploratory standard deviations. This methodology can be used to estimate a sample size necessary to distinguish blood selenium levels between avian species or between different sub-populations of the same species.

CONCLUSION

During the late winter and early spring of 2006-2007 in and around the Gros Ventre River drainage, there was considerable variance in mean blood selenium concentrations for ravens, eagles, and mallards, and the mean concentrations appeared to be significantly different. A single factor ANOVA confirmed that the mean concentrations were not all equal. This indicates that there is differential uptake and or bioaccumulation between these species as well for individuals within these species. The mean concentration for mallards was elevated to the point that it may produce adverse effects for the mallard population. This study serves as a pilot study to determine theoretical sample sizes necessary to detect differences between group means for different species or sub-populations in at-risk avian species (i.e. waterfowl and osprey); the R script for modeling Tukey critical differences can be used to estimate sample sizes that balance sampling effort and statistical power.

REFERENCES

De Veaux RD, Velleman PF, Bock DE. 2006. Intro Stats. 2nd ed. Pearson Addison-Wesly, New York, NY, USA. Chp 28.

Engberg RA, Westcot DW, Delmore M, Holz DD. 1998. Federal and State Perspectives on Regulation and Remediation of Irrigation-Induced Selenium Problems. In Frankenberger WT, Engberg RA, eds, *Environmental Chemistry of Selenium,* 98. Marcel Dekker Inc, New York, NY, USA.

Franson JC, Hoffman DJ, Schmutz JA. 2002. Blood selenium concentrations and enzyme activities related to glutathione metabolism in wild emperor geese. *Environ Toxicol Chem* 21:2179-2184

O'Toole D, Raisbeck MF. 1997. Experimentally induced selenosis of adult mallard ducks: Clinical signs, lesions, and toxicology. *Vet Pathol* 34:330–340

<http://www.statsoft.com/textbook/statistics-glossary/t/button/t/#Tukey%20HSD>