

THESIS

DEVELOPMENT OF PREDICTIVE GEOSMIN MODELS IN NORTHERN COLORADO  
LAKES, RESERVOIRS, AND RIVERS

Submitted by

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## ABSTRACT

### DEVELOPMENT OF PREDICTIVE GEOSMIN MODELS IN NORTHERN COLORADO LAKES, RESERVOIRS, AND RIVERS

Geosmin is a taste and odor (T&O) compound that is naturally occurring, produced by bacteria, and released into drinking water source waters. Geosmin in many parts of the country is a seasonal issue, so drinking water providers often look for temporary solutions to the T&O caused by geosmin. Being able to predict when geosmin will be an issue is vital if drinking water providers are going to succeed in using temporary mitigation methods. Therefore research is being performed to develop predictive models. This study is a broad sampling of Northern Colorado water bodies investigating the role of watershed and elevation, as well as biotic and abiotic water quality parameters. Water quality and zooplankton samples were collected from 20 different lakes and reservoirs as well as 20 sites on 4 rivers in Northern Colorado. Statistical models were developed using Multiple Linear Regression and Principal Component Analysis. Models show significant correlations between geosmin and zooplankton, particularly the species Nauplii and Daphnia in the lakes and reservoirs data. Modeling of the river data revealed geosmin relationships with elevation and dissolved oxygen, but did not show a significant correlation with stream flow. As expected from previous studies month of the year was also shown to be a significant factor.

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## INTRODUCTION

This thesis is composed of two main chapters, the first being a literature review and the second being the materials, methods, results, and discussion of this research. This study was performed over a two year period, 2011 and 2012, the majority of the sampling took place in the latter. The purpose of this research is to provide insight, and develop models, for predicting geosmin outbreaks in natural water bodies. Geosmin in Northern Colorado is a seasonal occurrence but has not necessarily occurred every year, or even at the same time every year. This creates just enough of a geosmin issue to be a problem for drinking water providers, but not enough of a problem to justify investing large amounts of capital to address it. If greater understanding of what causes geosmin episodes can be gained perhaps better watershed and source water management practices may be a viable option, or be able to predict when such events will occur so the water providers can be prepared with temporary treatment options or an alternative source. This study collected samples from 40 different sites including lakes, reservoirs, and rivers in Northern Colorado. Watersheds for these sites varied from agriculture influences in the eastern plains, to high mountain direct snowmelt runoff. Along with geosmin 13 other parameters were sampled in this study. Multiple linear regression, principal component analysis, and analysis of variance were used to develop models and identify relationships between geosmin and other parameters. Of particular interest for this study are the influence of zooplankton populations, and the role of elevation.

## CHAPTER 1: LITERATURE REVIEW

### *1.1 Background*

Public safety has historically been the primary concern of drinking water providers, however current consumers require not only safe water, but water that is aesthetically pleasant (e.g., odorless, colorless, tasteless) . The number of complaints to drinking water providers regarding the taste and/or odor of their water has been increasing every year in recent history (Turgeon, 2004). Utilities across the nation are spending billions of dollars to produce safe drinking water, yet a large number of consumers prefer to buy bottled water due to the taste and odor issues and the perceived lack of quality of their tap water (McGuire, 1995; Parinet et al., 2013). The reason for this divide is the presence of taste and odor compounds. Taste and odor compounds are a significant concern now because consumers have higher standards for their water, especially since their facet water can be compared to name brand bottled water. Therefore, citizens are getting a poor deal because they are essentially paying twice for the same product, one payment to the utility which provides safe drinking water whither the consumer drinks it or not, and another payment to the water bottlers, and to make things worse the Sierra Club estimates bottled water to be over 1,000 times more expensive than tap water (Sierra Club, 2008). Many examples of taste and odor problems exist along the front range of the Colorado Rocky Mountains including; the City of Greeley, the Fort Collins-Loveland District, the City of Loveland, the Town of Johnstown, and the City of Fort Morgan to name a few, have reported taste and odor issues to their constituents via the internet, newspaper, or fliers to address the public's concern regarding poor tasting and odorous tap water (Greeley Utilities, 2013; Fort Collins-Loveland Water District, 2013; City of Loveland, 2010; Town of Johnstown, 2011; Grubbs, 2012).

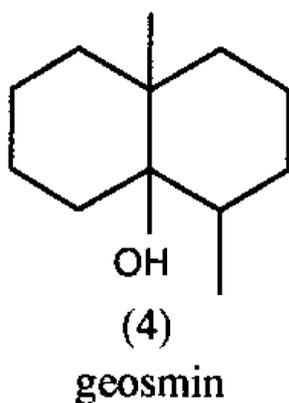
The two compounds known to be associated with taste and odor issues are the algal metabolites geosmin and 2-Methylisoborneol (MIB), which create a musty/soil flavor in the water (Parinet et al., 2013). Geosmin is a growing concern in America, Europe, South Africa and Australia (Mackey, 2012). In Northern Colorado, The City of Fort Collins has needed to switch water sources numerous times in the past decade due to geosmin breakouts in both Horsetooth Reservoir and the Poudre River (Billica and Oropeza, 2010; Billica and Loftis, 2008). Geosmin is a growing concern because more municipalities are relying on reservoirs to maintain a reliable source of water, and as these reservoirs age and experience eutrophication geosmin episodes get worse and last longer (Randtke et al., 2010). Environmental trends such as climate change, increased fertilization in the agriculture industry, treated sewage discharge, low river flows and droughts are all triggers associated with the proliferation of cyanobacteria (major geosmin producer) in natural and manmade waters (Paerl and Paul, 2009).

Geosmin is not removed using conventional water treatment methods. Effective Geosmin removal requires advanced treatment methods, such as; ozonation, activated carbon, biofilm filtration, reverse osmosis or nano-filtration (Ventura et al., 1995; Davies et al., 2004; Joe et al., 2007; Ho et al., 2012). Geosmin is a stable compound, and is not easily oxidized, volatilized, or affected by chlorination (Saito et al., 1999; Ng et al., 2002). Increasing the challenge to water providers is the low human detection limit of Geosmin by the average person. The average human can detect Geosmin in concentrations as low as 10ng/l (Ito et al., 1988; Lloyd et al., 1998). This means that drinking water providers need to remove nearly all the geosmin from the water which is very difficult and expensive.

Managing watersheds and source waters to prevent algal blooms and their associated T&O issues has a history of difficulty (Walve and Larson, 2007). Predicting geosmin episodes may provide a viable option for drinking water providers to creatively address T&O issues as they arise (Mackey, 2012). Effective and reliable geosmin predicting models do not exist to date.

### ***1.2 Geosmin the Most Common Taste and Odor Compound***

Geosmin is a metabolite produced by bacteria. Actinomycetes and cyanobacteria are both known to produce geosmin. In the case of bodies of water like lakes and reservoirs cyanobacteria is the most common culprit, whereas actinomycetes is soil bearing and therefore is often implicated with geosmin production in rivers (Wnorowski, 1992; Izaguirre and Taylor, 2004; Schollar et al., 2002).



**Figure 1- Molecular Structure of Geosmin (Pollak and Berger, 1996)**

Reasons for the appearance of geosmin episodes remain largely unexplained, which has hindered efforts to model geosmin concentrations (Juttner and Watson, 2007). Two articles from (Watson and Ridal, 2004) and (Zaitlin and Watson, 2006) reveal many possible causes for geosmin production, including; actinomycetes, cyanobacteria, and other complex interactions in periphyton, but do not clearly identify any direct relationships (Watson and Ridal 2004; Zaitlin and Watson, 2006).

### *1.2.1 Geosmin Producers*

Cyanobacteria all over the world are considered to be the major sources of geosmin in aquatic environments where photosynthesis is possible (Slater and Blok, 1983; Tsuchiya and Matsumoto, 1981; Izaguirre et al., 1982; Tabachek and Yurokowski, 1976). To complicate matters not all cyanobacteria taxa are equal, different taxa will produce geosmin and thrive under different conditions, so a broad analysis of total cyanobacteria concentrations will not likely produce a good correlation (Juttner and Watson, 2007). Other groups of algae have also been implicated with musty taste and odor episodes (Juttner, 1983). Other known producers of geosmin are protozoa and fungi, but have never been shown to cause taste and odor issues in drinking water (Hayes et al., 1991; Larsen and Frisvad, 1995).

Rivers and streams that are used for drinking water often experience geosmin outbreaks without the presence, or very small presence, of cyanobacteria, in which case another source must be considered. Actinomycetes are known to produce geosmin as a metabolite in soils (Zaitlin et al., 2003). Since actinomycetes exist in soil, rivers and streams are more susceptible to geosmin episodes due to actinomycetes (especially during low flows), whereas lakes and reservoirs have a smaller volume to bed/floor ratio and are likely not as affected by actinomycetes (Jensen et al., 1994).

Geosmin concentrations in algal cells are known to be higher than the concentration outside the cell (Peterson et al., 1995; Pan, 2002). Therefore, when the cell walls are broken down geosmin within the algal cells is released to the open water (Rashash et al., 1995). Certain zooplankton species, like *Daphnia*, feed on cyanobacteria which bursts the algal cells and releases the geosmin

inside the cell (Durrer et al., 1999). It has been shown that the filament structure of cyanobacteria is easily consumed by Daphnia because of the mechanical structure of the Daphnia filtering comb. However, this mechanical interference is limited when temperatures are increased and thus decreased viscosity (Abrusan, 2004). Increased cyanobacteria populations are not necessarily good for Daphnia because some taxa release toxins which will cause Daphnia deaths (Haney, 1987; Brett and Muller-Navara, 1997; Wilson and Sarnelle, 2006; Webster and Peters, 1978; Porter and McDonough, 1984).

### ***1.2.2 Conditions that Promote Producers***

A major trigger for the production of geosmin is the proliferation of nutrient sources such as, modern agricultural practices and sewage disposal. Drought conditions that reduce flows and reservoir volumes also increase cyanobacteria growth (Paerl and Paul, 2009). Correlations between bacteria interactions (Aoyama et al., 1995) and chlorophyll-a (Rosen et al., 1992; Bowmer et al., 1992) and geosmin concentrations have been reported. Trophic state alone has not been a good indicator of geosmin concentrations, but growth limitation due to inorganic phosphorus has had greater correlation with geosmin episodes. (Dzialowski et al., 2009) This is in large part because cyanobacteria are nitrogen fixing, and are therefore limited primarily by phosphorus (Mitsui et al., 1986). However, clear links have been shown between geosmin concentrations and nutrients (Rashash and Hoehn, 1996), light (Tsuchiya and Matsumoto, 1999), temperature (Blevins et al., 1995; Dionigi and Ingram, 1994), and metal concentrations (Schrader and Blevins, 2001).

The stratification regime in water bodies also plays in an important role in the fate and transport of contaminants and the interaction between phytoplankton and higher trophic levels. For instance,

zooplankton form aggregations during periods of increased stratification which may impact grazing on algae species that produce geosmin (Marcogliese and Esch, 1992; Thackeray et al., 2006). Temperature has been shown to influence geosmin production, but has not been a consistent indicator (Saadoun et al., 2001; Zhang et al., 2009). Temperature and season however are certainly factors, because it is common of geosmin episodes to occur in summer and fall months (Johnk et al., 2008). Evidence also exists suggesting that geosmin production is promoted during low-light conditions, because too much light can lead to the degradation of algal cells (Rashash et al., 1995; Zhang et al., 2009; Taylor, 2006).

### ***1.3 Geosmin Mitigation and Treatment Methods***

#### ***1.3.1 Source Water Management***

Managing watersheds to reduce algal growth by limiting nutrients is very difficult because cyanobacteria (blue-green algae) are nitrogen fixing, and are therefore only limited by phosphorus in concentrations as low as 0.005mg/l (Sawyer et al., 2003). Source water management is somewhat of an art, is less effective when the limiting a constituent to less than a tenth of ppm. Copper sulfate treatment or other algae bloom treatment chemicals and de-stratification techniques using solar bees or the Australian owned WEARS RESmix system disrupt algae habitat inactivating or destroying them (Elliot, 2005). One such municipality in Colorado is the Pagosa Springs Sanitation District, which installed two SolarBees into their source water reservoir and immediately reported improvements in drinking water quality, including lower total organic carbon (TOC) levels (Medora Inc., 2013). Other Colorado municipalities have had recent success with SolarBees as well including most recently the City of Englewood (Medora Inc., 2014). SolarBees have been shown to effectively prevent algal blooms when one solar bee is placed for

every 44 acre-feet. SolarBees and RESmix systems help prevent geosmin production by disrupting Cyanobacteria's ability to regulate its buoyancy, which enables other types of algae (blue, green, brown) to compete with the Blue-green algae. These other types of algae are easier to consume for most zooplankton, thus creating a healthy environment in which no species is dominant (Coetzee, 2013; Richards, 2013). An exploratory study of geosmin and MIB in catfish produced from a fish farm compared static ponds to ponds continually recirculated water through a wetland and found that ponds with a constant flow even using the same water reduced taste and odor compounds in the fish to levels below human detection (Zhong et al., 2011).

The use of copper-sulfate is effective but has many severe negative side-effects such as fish kills and copper accumulation in sediment. This method is therefore generally used as a last chance effort, and is discouraged by most utilities (Illinois State Water Survey, 1989). Managing the watershed itself to reduce nutrients will also reduce algal blooms and associated T&O episodes, however effective watershed management is difficult. The geosmin producing cyanobacteria are nitrogen fixing which means phosphorus must be the limiting reagent which is nearly impossible given that phosphorus levels must be reduced significantly to be effective (Walve and Larsson, 2007). Being able to predict geosmin spikes would enable water users to prepare for T&O episodes with temporary advanced treatment methods or source changes periodically (Mackey, 2012). However, no such reliable model exists and to date the best early detection method is to directly measure geosmin at key locations within a water supply (Taylor, 2006).

### ***1.3.2 WTP Treatment***

Geosmin is not easily removed once in a water supply, specifically traditional treatment methods are entirely inadequate to remove geosmin and often destroy algal cells increasing the dissolved geosmin concentration (Nowack et al., 2004; Chowdhury et al., 1988; Mackey, 2012).

#### ***1.3.2.1 Activated Carbon***

Activated carbon is an effective treatment method to reduce and remove geosmin concentrations (Nowack et al., 2004; Chowdhury et al., 1988; Mackey, 2012). Activated carbon has severe limitations when NOM (Natural Organic Matter) is present because the organic carbon blocks the activated carbon pores, more than three times as much activated carbon may be necessary to treat raw water with 1.8mg/l DOC (Dissolved Organic Matter) than pure water with the same concentration of geosmin (Zoschke et al., 2011; Matsui et al., 2012). The study also suggests that up to 10mg/l of PAC (Powder Activated Carbon) is necessary to completely remove 100ng/l geosmin. This number will vary depending on water quality. PAC costs about \$1 per pound, which adds up to tens of thousands of dollars a year to effectively remove geosmin (Carbon Activated Corp., 2013). Depending on a treatment facilities' current size, tanks and reservoirs may need increased capacity so that a longer contact time can be added. Up to an entire week may be necessary for activated carbon and NOM to equilibrate (Matsui et al., 2012). The addition of PAC will inevitably increase the amount of solids to be handled as well driving up costs.

#### ***1.3.2.2 Ozonation/Acidification***

Ozonation has been shown to reliably oxidize geosmin with high efficiency, but also with a high cost (Liang et al., 2007; Bruce et al., 2002). A less popular but still effective treatment option is

UV-Hydrogen-Peroxide (Peter and von Gunten, 2007; Rosenfeldt et al., 2005). Ozonation effectiveness has also been shown to be dependent on the water chemistry, mainly pH, and the presence of organics (humic acids, DOCs, NOM) (Brown, 2009). However, ozonation and UV-Hydrogen-Peroxide require as little as a few minutes contact time (Brown, 2009).

### ***1.3.2.3 Biofilm Filter***

Biofilms have had some success degrading and breaking down geosmin and other taste and odor compounds, but many shortcomings still exist that need to be addressed before biofilms become a reliable geosmin treatment option (Hoefel et al., 2006). Biological response time has been shown to be slow, which may not respond quickly enough to geosmin spikes which are known to rapidly vary in concentration (Senogles and Smith, 2002; Smith et al., 2008).

## ***1.4 Predictive Models for Geosmin***

Predictive models are important because geosmin is generally a seasonal occurrence, and given the cost associated with treating geosmin, preparation is key. Directly measuring geosmin is difficult, and there is a short period between being able to measure geosmin with instrumentation (1ng/l detection limit) and detecting it with the human tongue (5-10ng/l), which accounts for the benefit of a predictive model (Taylor, 2006).

### ***1.4.1 Regression***

Regression analysis is the basic and easiest model to develop. In 2009 Dzialowski and his team used two regression methods to predict geosmin in five Kansas reservoirs (Dzialowski et al., 2009). One method was the “stepwise” regression method which adds candidate explanatory variables to

the regression model one at a time, if they significantly increase the model's predictive power; the additional new variables are included until no significant increase in  $R^2$  occurs. The other method was the "best subsets regression" which modeled geosmin based on one or more parameters. (Sokal and Rohlf, 1995). These models and their significance are discussed further in Section 1.5 Recent Geosmin Study Results.

#### ***1.4.2 Canonical Correlation Analysis (CCA)***

A study of a catfish pond in China revealed a significant correlation between geosmin and specific cyanobacteria species using Canonical Correlation Analysis (CCA) (Zhong et al., 2011). CCA is considered a last resort statistical method by much of the science community when only one dependent variable is being considered. When multiple dependent variables are simultaneously being sought CCA is most appropriate (Hair and Anderson, 1998).

#### ***1.4.3 Principal Component Analysis (PCA)***

Recent previous studies have developed geosmin predictive models using Principal Component Analysis (PCA), most notably a City of Quebec study in 2010 (Parinet et al., 2010; Parinet et al., 2013). PCA turns original variables (abiotic, chemical, biological and microbiological parameters) into principal components (PCs), which are orthogonal and non-inter-correlated (Shrestha and Kazama, 2007; Barbieri, 1999). Correlations between the parameters can be discussions both visually using a loading plot, or in tabular format using eigenvalues.

#### ***1.4.4 Analysis of Variance (ANOVA)***

Analysis of Variance (ANOVA) is used to find variances within and between groups (or levels) of data (McIntyre, 2005). ANOVA defines similarities and differences between groups of data, called factors, which is always an important consideration in large scale environmental studies.

#### ***1.5 Recent Geosmin Study Results***

Many studies have been performed investigating sources of geosmin, particularly in the western regions of the United States and eastern municipalities in Canada. The most notable being, a 2009 study in Kansas by Dzialowski (Dzialowski et al., 2009), a 2010 study of the Colorado-Big Thompson Trans-basin Project in Colorado by the City of Fort Collins (Billica, 2011), a 2010 study in Quebec by Parinet regarding geosmin sources in streams (Parinet et al., 2010) (Parinet et al., 2013), a 2007 study performed by Juttner and Watson of the St. Lawrence river (Juttner and Watson, 2007), and a 2009 study by Peter in Sweden (Peter et al., 2009).

The Dzialowski study in 2009 was a follow up study of a similar geosmin study of Cheney Reservoir, near Wichita, KS. In the 1999 and 2000 water years the University of Kansas in conjunction with the City of Wichita conducted an in depth study of taste and odor in Cheney Reservoir which is characterized as a shallow eutrophic drinking water supply (Smith et al., 2002). The Smith study revealed only two peaks in geosmin concentrations between May 1999 and January 2001. This study highlights a common trend in geosmin episodes which is tremendous variability. Maximum geosmin concentrations went from less than 10ng/l in the summer of 1999 to almost 40ng/l in the following summer. Despite consistently low geosmin concentrations a

correlation factor of  $r^2 = 0.72$  was found using the model in Equation 1 where geosmin concentrations were modeled as dependent on chlorophyll -a (CHL) concentrations.

**Equation 1 - 2002 Smith Model of Geosmin in Cheney Reservoir Kansas**

$$Geosmin = 0.412 * (CHL) - 1.08 \quad R^2 = 0.72$$

The Dzialowski study included Cheney Reservoir as well as four other nearby Kansas lakes. The study took monthly samples between June and October in 2006 and two winter samples in December and January. The purpose of the study was to develop predictive models for each reservoir and attempt to develop broader geosmin predicting models both between reservoirs and within reservoir ecoregions. The study was very in depth and considered a variety of parameters and developed a number of regression models summarized in Table 1. Prior to the Dzialowski studies USGS conducted a study of taste of odor compounds in Cheney Reservoir and its main feeder stream the Ninnescah River. Christensen and fellows found using discrete sampling in conjunction with continuous monitoring, and developed no relationship between geosmin and any other common water quality parameters including; Chlorophyll-a, temperature, algal species, and nutrients (Christensen et al., 2006).

**Table 1 - Summary of the 2009 Dzialowski Models**

SITE	MODEL	CORRELATION	P VALUE	N
CROSS-SECTIONAL	LOG(GEOS)=1.1+2.67LOG(NO3)-0.19LOG(PO4)	0.34	0.001	57
CROSS-SECTIONAL	LOG(GEOS)=2.03-0.18LOG(PO4)-0.163LOG(ANA)	0.35	0.001	57
CROSS-SECTIONAL	LOG(GEOS)=0.2-0.42LOG(SD)	0.24	0.001	57
CROSS-SECTIONAL	LOG(GEOS)=1.19-0.22LOG(PO4)	0.25	0.001	57
BIG HILL	GEOS=69.48-8.32(CHL)+0.276(CHL)2	0.87	0.001	12
BIG HILL	GEOS=34.29-0.00002(TOTALG)	0.89	0.001	12
BIG HILL	39-0.609(TP)-0.00002(TOTCYANO)	0.94	0.001	12
CLINTON	LOG(GEOS)=2.48-0.276LOG(TOTCYANO)	0.36	0.009	18
CLINTON	LOG(GEOS)=0.97-0.004(SD)+0.000054(SD)2	0.61	0.001	18
CLINTON	LOG(GEOS)=1.24+0.37(PH)+0.005(SD)-.215LOG(TOTALG)	0.85	0.001	18
MARION	GEOS=-8.76+2.11(DO)	0.47	0.04	9
MARION	LOG(GEOS)=-9.93+3.02(DO)+9.33LOG(CHL)-3.58LOG(TOTALG)	0.93	0.002	9
CHENEY	NO SIGNIFICANT CORRELATION FOUND	-	-	-

A similar study of Lake Olathe, KS published by USGS found a 0.70 correlation between geosmin and three water quality parameters; secchi depth, specific conductance, and turbidity as shown below in Equation 2. The Olathe study had geosmin concentrations between 2.5 and 12 ng/l and consisted of 16 samples (Mau et al., 2004).

### Equation 2

$$Geosmin = 1.08Secchi - 0.064SpC + 0.24Turb + 31.04 \quad R^2 = 0.70$$

A similar and extensive study of taste and odor compounds in a Japanese lake developed predictive models for both MIB and geosmin (Sugiura et al., 2004). That study revealed a relationship between geosmin and cyanobacteria, diatom, and blue-green algae counts, as well as the abiotic

parameters; phosphorus, chemical oxygen demand, and dissolved oxygen. Using discriminate analysis Sugiura and fellows were able to explain 72.9% of geosmin concentrations from phytoplankton genera. Equation 3 developed in the Sugiura and fellows paper had a .670 correlation.

### **Equation 3**

$$\log(Geos) = -0.624 - 1.092TP + 0.153COD + 0.149DO \quad R^2 = 0.670$$

The second study performed by the City of Fort Collins presents a summary of geosmin concentrations at the city's drinking water plant from the Poudre River, Table 2 summary of this data. Geosmin results from the study during the period of 2010 and 2011 are summarized in Table 3 (Oropeza and Billica, 2011).

The significant findings of the upper Cache la Poudre river study reveal that the highest concentrations of geosmin occur in the winter months, unlike lentic water bodies which generally experience higher concentrations of geosmin in the summer months, or fall during lake turnover events. Geosmin concentrations may be related to discharge also because winter experiences lower flows, and very low concentrations of geosmin were reported during the spring runoff season. Concentrations near the Rustic reach of the river had significantly different concentrations than the Fort Collins WTP 25 miles away, suggesting that geosmin is not an inert compound. Low counts of geosmin producing cyanobacteria were recorded in the river periphyton.

**Table 2 - Geosmin concentrations near Rustic, CO in the Cache la Poudre R. (Oropeza and Billica, 2011)**

Geosmin (ng/l)	River Flow (cfs)	Date
20-40	< 50	Jan.-Feb. 2010
5	375	07/2010
3	200	08/2010
5	50	09/2010
3	75	10/2010
9	50	11/2010
11	< 50	11/2010
13	< 50	01/2011
12	< 50	02/2011
5	75	04/2011
5	100	05/2011
< 1	2500	06/2011
3	1000	07/2011

**Table 3 - Geosmin concentrations at key locations on the Poudre River from a 2010-2011 Fort Collins Utilities Study (Oropeza and Billica, 2011)**

Site	Geosmin	
Above Rustic	Mean	7.5
	(min-max)	(<0-28)
	Std. Dev.	7.1
Poudre Canyon Fire Stat.	Mean	9.2
	(min-max)	(3-25)
	Std. Dev.	6.5
Below Rustic	Mean	9.6
	(min-max)	(3-27)
	Std. Dev.	7.1
At Steven's Gulch	Mean	6.0
	(min-max)	(<0-24)
	Std. Dev.	6.2

That same City of Fort Collins study reviewed seven years' worth of geosmin samples taken from the Poudre River at the city water treatment plant and found a maximum concentration of geosmin at 18ng/l in 2003, and an average of less than 4ng/l between 2003 and 2010 (Oropeza and Billica, 2011). Another important study of a river water body was performed by Parinet and fellows, in 2010 of three Canadian rivers, Table 3 (Parinet et al., 2010). The 2010 study found found geosmin

concentration episodes during winter months as in the Oropeza and Billica study, the study also found interesting correlations between T&O and chlorophyl-a. A similar study by Parinet and fellows in 2013 followed up with farther research of geosmin episodes in the same area, and developed a simple model using stepwise regression modeling and using easily measured parameters for drinking water providers in the area to measure as a follow up to their study. The parameters used in the stepwise regression model were pheophytin, sum of green algae, CHL-a, and redox potential, resulting in an R<sup>2</sup> correlation of 0.657 (Parinet et al., 2013).

**Table 4 - Statistical Summary of Water Quality Results from Recent Geosmin Study (Parinet et al., 2010)**

	Site	Geosmin (ng/l)
	Mean	3.75
Levis	(min-max)	(<1-13.74)
	Std. Dev.	3.36
	Mean	1.68
Ste Foy	(min-max)	(<1-3.43)
	Std. Dev.	1.12
	Mean	0.6
Beauport	(min-max)	(<1-3.1)
	Std. Dev.	0.9

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## CHAPTER 2: DEVELOPMENT OF PREDICTIVE GEOSMIN MODELS IN NORTHERN COLORADO LAKES, RESERVOIRS, AND RIVERS

### 1.0 INTRODUCTION

Utilities across the nation spend billions of dollars annually to produce safe reliable drinking water, yet consumers are turning to bottled water in increasing numbers every year complaining that their tap water does not taste good (Turgeon, 2004; McGuire, 1995; Parinet et al., 2013). The source of these complaints is Geosmin, an organic compound which creates a musty/soil flavor in water (Parinet et al., 2013). Geosmin is a problem for water providers because no one wants their water to taste like dirt. Geosmin is a growing concern as more municipalities rely on aging, eutrophying reservoirs to maintain a reliable source of water (Randtke et al., 2010). Environmental trends such as climate change, increased fertilization in the agriculture industry, treated sewage discharge, low river flows and droughts are all triggers associated with the proliferation of cyanobacteria (a major geosmin producer) in natural and manmade waters (Paerl and Huisman, 2009). The compound structure of geosmin is shown in Figure 1, in Chapter 1.

Geosmin is a stable compound, not easily oxidized, volatilized, or affected by chlorination (Saito et al., 1999; Ng et al., 2002). Effective Geosmin removal requires advanced treatment methods, such as, ozonation, activated carbon, biofilm filtration, reverse osmosis or nano-filtration (Ventura et al., 1995; Davies et al., 2004; Joe et al., 2007; Ho et al., 2012). Increasing the challenge to water providers is the low human detection limit of Geosmin as low as 10ng/l (Ito et al., 1988; Lloyd et al., 1998). This means that drinking water providers need to remove nearly all the geosmin from

their finished water to prevent consumer complaints. Another alternative is source water management techniques such as, nutrient limiting, destratification, and copper sulfate.

Due to the difficulty and expense of treating and managing geosmin, another viable alternative is source water monitoring. Being able to predict geosmin episodes would enable water users to prepare for T&O events with temporary advanced treatment methods or source water changes (Mackey, 2012). However, no such reliable model exists and to date the best early detection method is to directly measure geosmin at key locations within a water supply, this method is still too slow however (Taylor et al., 2006). Reasons for the appearance of geosmin episodes remain largely unexplained, which has hindered modeling efforts (Juttner and Watson, 2007). Two articles from Watson and Ridal, 2004 and Zaitlin and Watson, 2006 reveal many possible causes for geosmin production, but do not clearly identify any direct relationships between geosmin concentrations and other water quality parameters (Watson and Ridal, 2004; Zaitlin and Watson, 2006).

To complicate matters not all geosmin producers are the same, different producers will peak production under different environmental conditions, therefore a broad range of bacteria must be sampled to detect a correlation (Juttner and Watson, 2007). Geosmin modeling is further complicated by the fact that geosmin concentrations within producing cells is always significantly higher than ambient concentrations, therefore factors affecting the health of the producers may also be correlated to outbreaks (Rashash et al., 1995; Peterson et al., 1995; Pan, 2002). Certain zooplankton species, like *Daphnia*, feed on cyanobacteria causing the algal cells to release the geosmin (Durrer et al., 1999). Studies have found correlations between geosmin and both bacteria

and zooplankton, however, trophic state alone has not been a good indicator (Bowmer et al., 1992; Rosen et al., 1992; Aoyama et al., 1995; Dzialowski et al., 2009). However, clear links have been shown between geosmin concentrations and nutrients (Rashash et al., 1996; Mitsui et al., 1986), light (Tsuchiya and Matsumo, 1999), temperature (Blevins et al., 1995); Saadoun et al., 2001); Zhang et al., 2009); Dionigi and Engram, 1994), and metal concentrations (Schrader and Blevins, 2001). Each of those papers listed have shown correlations for their specific body of water studied, but no successful model has been developed between water bodies. Larger municipalities in northern Colorado have begun geosmin monitoring programs, particularly the City of Fort Collins which is home to numerous breweries requiring high quality water. Studies on the Big Thompson Project, and Poudre River have shown unpredictable and intermittent geosmin episodes (Billica et al., 2010; Billica et al., 2008). Other northern Colorado lakes, such as Barr Lake, have had a much longer history of taste and odor issues (Sylvester, 1965).

The purpose of this study is to seek correlations between geosmin concentrations in natural water bodies with nutrients, temperature, zooplankton counts, and other basic water quality parameters for the purpose of developing a predictive model for northern Colorado. The project consists of sampling 24 lakes and reservoirs, as well as multiple locations on the Big Thompson and Cache la Poudre rivers, and using this data to develop statistical models that describe geosmin episode events in lakes as a group, rivers as a group, and as a whole. Using these statistical models drinking water providers shall be better prepared to handle taste and odor issues in Northern Colorado.

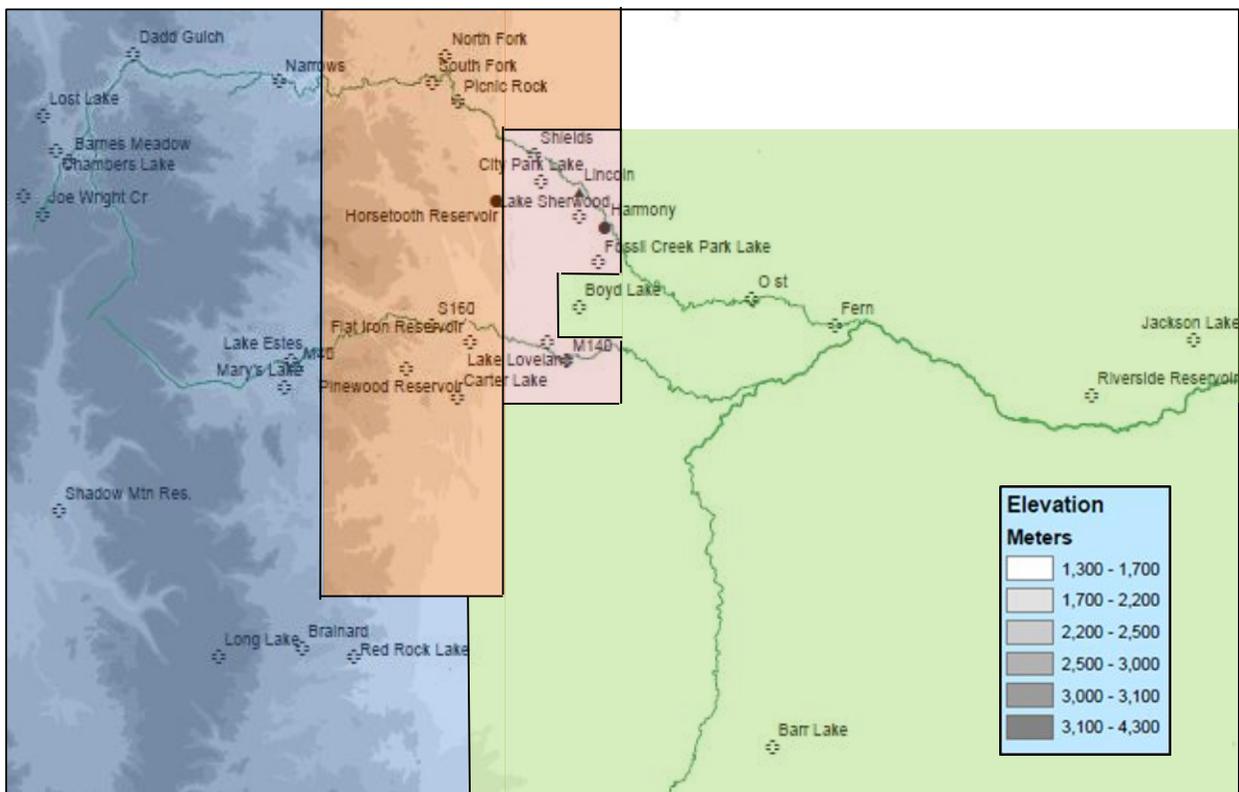
## 2.0 MATERIALS AND METHODS

### *2.1 Study Area*

This study covered a broad geographic region in Northern Colorado, as far west as the great divide, as far east as the City of Fort Morgan, and as far south as the City of Brighton. The study area has been intentionally designated to cover a large variety of watersheds, including agricultural, urban, forest, and high mountains. The study area therefore also covers a large range of elevations, from as low as 4,200' at Jackson Lake, to as high as 11,000' at Brainard Lake. Each lake and reservoir site was therefore sub-grouped into an eco-region based on elevation and watershed. Table 5 lists the lakes in each group, and Figure 2 shows the geographic delineation of the eco-regions. The high mountain region consisted of sites that are feed primarily by snowmelt runoff and had a watershed of alpine forest. The foothills region contains lakes and reservoirs lower in elevation than the high mountains lakes between 5,500' and 7,000'. These watersheds consist of forest, and mountain streams runoff, often containing the same water that passed through a high mountain lake. Lakes and reservoirs in the plains region are influenced by agricultural practices, higher temperatures, and low elevations, all between 4,000' and 5,500'. Urban lakes were located within the city limits of either Fort Collins or Loveland, and had sources from canal waters from local streams (Big Thompson, Poudre, or Fossil Creek) and storm water runoff. These lakes were also much smaller on average than lakes in the other regions.

**Table 5 - Eco-Region Delineation of Lakes and Reservoirs**

HIGH MOUNTAIN	FOOTHILLS	URBAN	PLAINS
ESTES LAKE	CARTER LAKE	CITY PARK LAKE	BOYD LAKE
MARY'S LAKE	HORSETOOTH RES.	FOSSIL CR. PARK LAKE	BARR LAKE
CHAMBER'S LAKE	PINEWOOD RES.	LAKE LOVELAND	JACKSON LAKE
JOE WRIGHT RES.	FLATIRON RES.	LAKE SHERWOOD	RIVERSIDE RES.
LOST LAKE			
BRAINARD LAKE			
LONG LAKE			
RED ROCK LAKE			



**Figure 2- Sample sites in Northern Colorado for this geosmin study. The eco-region was delineated by elevation and the blue color highlights the High Mountain region, the orange area highlights the Foothill region, the pink shades the Urban eco-region and the green highlights the Plains region.**

## ***2.2 Field Techniques***

Bottle (grab) samples of geosmin, TP, TN, TOC, and zooplankton were taken at approximately six week intervals from each river, lake, and reservoir site starting in May 2012. A number of less regular grab sample were taken in 2011 as well between May and November. Grab samples were taken in the same location and time of day for each site whenever possible and sampling locations varied site to site depending on accessibility, and ease. For instance, water bodies like Mary's Lake were only accessible by foot whereas other water bodies (e.g. Carter Lake, Horsetooth reservoir) were sampled off shore but far from center due to size and nature of the watercraft available. River sites were consistently taken from shore in a location 2 foot deep, or the deepest location nearby if all depths were under 2 foot. Geosmin samples taken in deep water (off shore) were always sampled using a Wildlife Supply Co Van-Dorn Horizontal Sampler at a depth of five feet or a foot and a half below the thermocline. Temperature, pH, DO, conductance, and CHL-a were measured in-situ with a Hach Hydromet MS5 sonde fitted with pH, Conductivity, LDO, Chlorophyll a, and Temperature attachments. Chlorophyll-a was assumed to correlate with phytoplankton concentrations in this study (Boyer et al., 2009). The sonde probe was rinsed in the water body and a period of 5 to 10 minutes was allowed for the probe to settle before recording any measurements. Secchi Depth measurements were recorded during sonde wait periods, using a Wildlife Supply Co. Secchi Disk, depths were recorded in feet.



**Figure 3 - Cache la Poudre River at Barnes Meadow showing Hach Hydrolab in use, a Nalgene bottle used for nutrient sampling, zooplankton net, and notebook with bag of extra supplies.**

Geosmin samples were collected in 40 mL Fisher Scientific amber glass EPA approved volatile organics analysis vials with Teflon caps with special consideration taken to ensure no air was trapped in the bottle. Duplicate samples were also taken at every site, but the duplicate sample was only laboratory tested if geosmin concentrations were discovered to be higher than 2ng/l in the first sample. Duplicates came from the same slug of water in the Van-Dorn Horizontal Bottle sampler. All samples were kept in a cooler and refrigerated between collection and laboratory analysis.

Zooplankton samples were collected using a Reliance 86um plankton net, and stored in 120ml Nalgene PP Jars. The net was submerged to a depth of 5ft, and quickly pulled up and poured into a plastic Nalgene bottle. At river sites, the net was pulled through five feet of water horizontally due to limitations of water depth. The zooplankton river measurements were less methodical because of this. Also zooplankton counts were very low and sampling of zooplankton in rivers eventually seized due to the low counts, and difficulty getting the net in the stream. However, it was not expected to find many zooplanktons at the river sites. Zooplankton samples were also stored in a cooler during the day, but each zooplankton sample was analyzed at the end of the day, so no storage preservation was used.

Nutrient samples (i.e., TN, TP, TOC) were taken using one Thermo Scientific Nalgene 2100-0032 Wide Mouth Teflon sample bottle at each sites that was rinsed once with the water from the water body prior to sample retrieval. All nutrient samples were taken at a depth of 2 feet, except in river conditions where only smaller depths were possible. In the case of rivers, samples were taken at 2 foot depths or half way between the bed and water surface, which ever was smaller. Nutrient grab samples were also stored in a cooler during field days and later stored in a refrigerator between sampling and laboratory analysis.

### ***2.3 Laboratory Techniques***

Sampling sets were taken on a four to six week basis, so to be sure the results were accurate duplicates were taken for Geosmin, TN and TP. However, Geosmin duplicates were only analyzed if a detectable concentration of Geosmin was discovered in the first sample. This was done to save

on cost. TOC and zooplankton samples were not duplicated either because of the high cost per sample.

### ***2.3.1 Geosmin***

An Agilent 5890 gas chromatograph (GC) equipped with an Agilent DB-5 MS (30 m, 0.25 mm i.d., 0.25  $\mu$ m) column connected to an Agilent 5973 mass spectrometer (MS) were used for geosmin sample analyses (Santa Clara, CA). Geosmin samples required preparation, which included 20ml of the raw grab sample, 3g of NaCl, and 20 $\mu$ L of 0.04mg/l TCA (Trichloroacetic Acid). Four standard solutions were also made for each sample set. The standard solutions are required to calibrate the gas chromatograph (GC), which does not have a calibration curve for Geosmin. For this project the standard solutions had concentrations of 1, 5, 10 and 50ng/l, providing the process a wide range of accuracy. Doing this every time also ensures that any differences in the GC are accounted for. A detailed procedure for this method can be found in Appendix I.

### ***2.3.2 Zooplankton***

Recording type and count of zooplankton present was performed using a microscope and manually counting and identifying species. A volume of 10ml of zooplankton sample was poured into a square microscope tray divided into 36 smaller squares etched into it. An average number of each organism per square was recorded using a Barska Monocular Compound Microscope and then multiplied by 119. The multiplier accounts for the volume of the sample, including the volume of water in the tray being analyzed in the lab, and the volume of the water the net passed through when sampled.

### ***2.3.3 Nutrients***

Total Nitrogen samples were analyzed using Hach Method 10071 (Hach Company, 2003), using a Hach DR/4000U spectrometer. Total Phosphorus samples were analyzed using Hach method 8190 (Hach Company, 2003), using a Hach DR4000 spectrometer. Total organic carbon samples were analyzed using Hach method 10129 (Hach Company, 2003), using a Hach DR2500 spectrometer and a Fisher Scientific Stirring Hotplate. All nutrient samples were analyzed within 10 days of being collected.

### ***2.4 Predictive Models***

Predictive models were developed for both measured geosmin concentrations and log of geosmin concentrations. Many of the geosmin samples were reported below the detection limit and these data were removed in some of the analyses. For this study zero values were excluded in model development in models that incorporated the log values. This method has been used in other geosmin studies as well, (Dzialowski et al., 2009), when a dataset has a large volume of non-detects. The non-detect limit for GC is between 1ng/l, but a value is still given. This study used the value recorded from the GC, but models using log datasets did not use some of the very low non-detect values because they were recorded as zero. However, to date no standard method for handling non-detects exists as described in the USGS produced “Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste and Odor Studies in Lakes and Reservoirs” (Graham et al, 2008). In contrast to this study, others have had datasets with geosmin concentrations above detect limits in the majority of dataset. In that case non-detects were not removed and were estimated to be at the detection limit or half the detection limit (Parinet et al, 2013; Parinet et al, 2010; Sugiura et al, 2004).

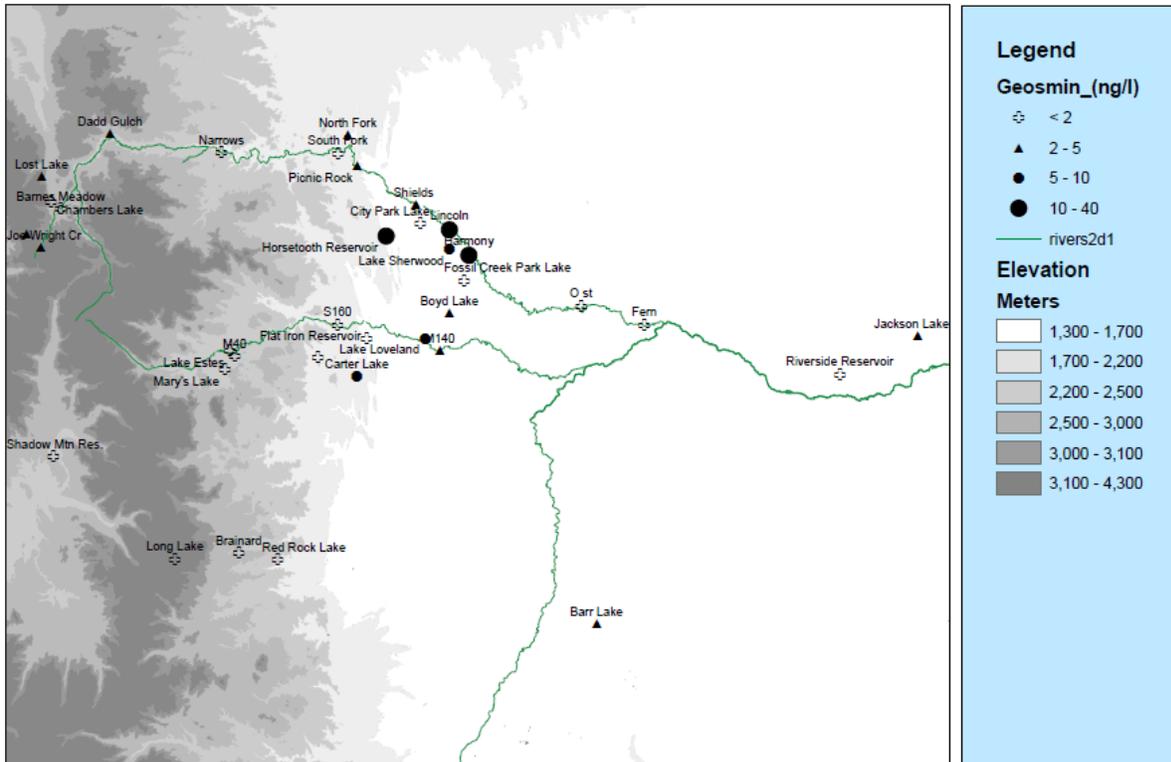
Statistical models were developed using Minitab16 Statistical Software functions; Regression, Stepwise Regression, One-way ANOVA, Probability Plot, Normality Test, and Principal Components Analysis. W test for Log Normality shows the null hypothesis that the geosmin data is log normal is not rejected, thus the data is assumed to be log normal. This does not include geosmin concentrations that were zero or non-detectable, since a value of zero cannot be converted to log. Geosmin data has been reported to have log normal characteristics in previous studies as well, but as mentioned previously this is not necessarily always the case (Dzialowski et al, 2009). The null hypothesis is that the geosmin data is normally distributed for the Shapiro-Wilk (W) test (Shapiro and Wilk, 1965). The result of the test shows that the geosmin data (with non-detect values removed) is not normally distributed since the p-value is less than the alpha value of 0.05, thus the null hypothesis is rejected. Previous studies' geosmin datasets were found to be normally distributed, for example studies by Parinet on the St Lawrence River in Canada had geosmin data with a normal distribution (Parinet et al, 2013; Parinet 2010).

An initial statistical review of the entire dataset was performed to begin the analysis phase of the study; separate models for lakes, rivers and eco-regions were developed because of the distinctly different characteristics between a flowing body of water, a relatively static body of water and the different ecological and elevation groupings. As mentioned earlier, studies have even suggested that the biological source of geosmin differs between lakes and rivers (Jensen et al., 1994). Significant correlations were found between reservoirs delineated in similar altitude and eco-regions. Lakes and reservoirs were grouped into four eco-regions: high mountain, foothill, urban, and agricultural/plains.

## 3.0 RESULTS AND DISCUSSION

### *3.1 Geosmin Concentrations in Colorado Water Bodies*

Geosmin concentrations recorded in reservoirs and lakes during this study varied from as high as 37ng/l to less than 1ng/l. Geosmin concentrations greater than 10 ng/L occurred in Horsetooth Reservoir, Fossil Creek Reservoir, and the Cache la Poudre River at Lincoln st. (**Error! Reference source not found.**). The distribution of recorded concentrations is heavily skewed toward the non-detectable range of less than 1ng/l. Nutrient concentrations for TN and TP in the lakes and reservoirs were also distributed primarily in low concentrations, TOC however had a much broader range of concentrations in lake and reservoir sites. River sites tended to have a distribution of nutrient concentrations that were lower and tighter. Table 6 shows water quality data for surface depth samples taken for this study.



**Figure 4 - Maximum Geosmin concentrations recorded during the study period for all Lakes, Reservoirs, and Rivers sampled.**

**Table 6 - Summary of Collected Data from Samples taken at Depths less than 2ft.**

Parameter	Minimum	Maximum	Average
Elev. (ft)	4460	10560	7316
Daphnia	0	5320	255
Calanoid	0	8640	463
Nauplii	0	14400	597
Keratella	0	53280	1349
Water Temp (F)	37.7	85.8	61
D.O. (mg/l)	3.98	15.5	8.1
pH	6.5	10.12	8.01
CHL (mg/l)	0	57.65	4.37
Sp. Cond. (uS/cm)	0	3748	420
Secchi Depth (ft)	0.25	13	4.22
TOC (mg/l)	0	20.1	5.83
Geosmin (ng/l)	0	37.10	1.35
TN (mg/l)	0	16.15	1.35
TP (mg/l)	0.02	4	0.66
Surface Area (Ac)	3	2500	587

### ***3.2 Regression Models***

Linear regression model results from the geosmin dataset shows that no statistically significant correlation ( $R^2 > 0.9$ ) exists between geosmin concentrations and any other measured parameter. The linear regression correlations are surprisingly very low, all  $R^2$  values were less than 0.1. Linear regression models developed for censored geosmin data produced equally poor correlation results. However, models of greater significance were developed using the log transformed geosmin dataset (Table 7). Although significance of linear regression relationships increased using log transformed geosmin datasets, the correlations were far from significant study wide, the most significant being with Nauplii and temperature with  $R^2$  values of just over 0.1. Significance for both these parameters and geosmin continued to increase as the dataset was further divided between static (lake/reservoir) and dynamic (river/stream) sites (Table 8). No correlations, however, are near the 0.9  $R^2$  value required to be considered statistically significant. Similar studies have suggested that causes for geosmin episodes have different sources depending on the

type of water body. Juttner and Watson in 2007 reported findings that suggest geosmin in rivers maybe more closely attributed to actinomycetes bacteria, whereas lakes and reservoirs maybe more susceptible to lentic organisms like cyanobacteria (Juttner and Watson, 2007).

Table 7 and Table 8 summarize the linear regression and ANOVA results respectively. The analysis included linear regression analysis between geosmin and the following parameters; Daphnia, Calanoids, Nauplii, Keratella, temperature, dissolved oxygen, pH, CHL, specific conductivity, Secchi depth, TOC, TN, TP, change in reservoir stage/stream discharge, and surface area. One-Way ANOVA used the following factors; Date, TOC, Total Phosphorous, Total Nitrogen, Body of water size and eco-region, and elevation, significance being a P value equal to or less than alpha of 0.05.

**Table 7 - Summary of R<sup>2</sup> values from linear regression models, using the raw geosmin data, the data with non-detects removed, and the log transformed geosmin data.**

VARIABLE	GEOSMIN, NO ZEROS			GEOSMIN, NON LOG			LOG(GEOSMIN)				
	ALL DATA	LAKE/RES. DATA	RIVERS DATA	ALL DATA	LAKE/RES. DATA	RIVERS DATA	ALL DATA	LAKE/RES. DATA	RIVERS DATA	POUDRE DATA	BIG THOMPSON DATA
DAPHNIA	0.001	0.000	0.010	0.000	0.000	0.006	0.034	0.032	0.004	0.048	-
CALANOID	0.002	0.001	0.229	0.000	0.001	0.025	0.005	0.003	0.120	0.154	0.000
NAUPLII	0.036	0.031	0.042	0.024	0.027	0.042	0.109	0.119	0.016	0.001	-
KERATELLA	0.003	0.002	0.072	0.004	0.002	0.072	0.041	0.045	0.032	0.024	0.000
TEMP.	0.040	0.048	0.007	0.040	0.045	0.009	0.110	0.190	0.016	0.018	0.009
DO	0.003	0.002	0.068	0.001	0.001	0.003	0.043	0.040	0.151	0.204	0.006
PH	0.001	0.000	0.012	0.013	0.003	0.008	0.005	0.016	0.015	0.103	0.044
CHL-A	0.003	0.007	0.025	0.002	0.004	0.048	0.048	0.089	0.005	0.010	0.008
SPEC. COND.	0.000	0.000	0.002	0.020	0.000	0.012	0.005	0.007	0.000	0.009	0.003
SECCHI D.	0.000	0.000	-	0.000	-	-	0.013	0.015	-	-	-
TOC	0.001	0.009	0.027	0.001	0.007	0.019	0.000	0.014	0.066	0.081	0.066
TN	0.002	0.007	0.005	0.002	0.007	0.002	0.008	0.031	0.007	0.019	0.013
TP	0.003	0.008	0.000	0.002	0.004	0.000	0.000	0.046	0.000	0.000	0.033
STAGE / Q.	-	0.075	0.010	0.055	-	-	0.048	-	0.008	-	-
SURF. AREA	-	0.006	-	-	0.006	-	0.000	-	-	-	-

**Table 8 - Summary of ANOVA results, using the raw geosmin data, the data with non-detects removed, and the log transformed geosmin data.**

VARIABLE	GEOSMIN, NO ZEROS			GEOSMIN, NOT LOG			LOG(GEOSMIN)				
	ALL DATA	LAKE/RES. DATA	RIVER DATA	ALL DATA	LAKE/RES. DATA	RIVER DATA	ALL DATA	LAKE/RES. DATA	RIVER DATA	POUDRE DATA	BIG THOMPSON DATA
LAKE SIZE	-	0.698	-	0.806	-	-	0.623	0.529	-	-	-
DRAINAGE TYPE	-	0.971	-	0.445	-	-	0.798	-	-	-	-
ELEVATION	0.397	0.250	0.314	0.464	0.475	0.249	0.015	0.235	0.034	0.047	0.732
TN	0.506	0.505	0.781	0.329	0.344	0.597	0.289	0.194	0.895	0.634	0.954
TP	0.294	0.354	0.675	0.247	0.282	0.618	0.237	0.133	0.869	0.232	0.471
TOC	0.451	0.442	0.249	0.517	0.502	0.270	0.109	0.515	0.21	0.541	0.742
DATE	0.283	0.651	0.832	0.403	0.382	0.441	0.001	0.017	0.459	0.602	0.807

Linear regression models were also developed for the following site categories: Lakes/Reservoirs, Rivers, The Big Thompson River, and the Cache la Poudre River. Of these models the most statistically significant model was with DO from the Poudre River dataset with a  $R^2$  value of 0.204. ANOVA tests reveal two significant factors, elevation and month. Many studies have previously suggested that season affects geosmin episodes (Johnk et al., 2009; Parinet et al., 2010; Oropeza et al., 2011). Interestingly, the studies performed by Parinet and Oropeza found that high geosmin concentrations occurred during winter months, this study however did not sample between November and April. Elevation appears to be a significant factor for river bodies, and month seems to be a common factor in lakes and reservoirs. To date no known study has investigated the significance of elevation in geosmin production. ANOVA analysis of log transformed geosmin data also shows that month of the year is significant factor effecting geosmin concentrations over the entire dataset, and the lentic only dataset.

### ***3.2.1 Eco-Region Regression Models***

As stated in the methods portion of the paper all the lake and reservoir sites were grouped into one of four categories, based on eco-region, watershed, and elevation. Models for each category, or eco-region, were developed using PCA, stepwise regression, and linear regression. The eco-region defined models produced the best correlations of the study. Table 9 summarizes all the linear regression  $R^2$  values, which contains correlations coefficients as high as 0.806.

These models were developed using the log transformed geosmin data, sampled only in the lakes and reservoirs. The linear regression models suggest that Nauplii is very likely to be correlated to geosmin concentration, particularly in the mountain and foothills regions. From the ANOVA tests,

Table 10, month of the year is a significant factor for geosmin production in foothills lakes but no other region. The same linear regression models developed using non log transformed geosmin data resulted in lower correlation values. However, Nauplii and Daphnia had the greatest correlation with geosmin of the parameters measured.

**Table 9 - R<sup>2</sup> Regression values correlated with Log(Geosmin) lakes and reservoirs data separated by region.**

VARIABLE	HIGH MOUNTAIN	FOOTHILLS	URBAN	PLAINS
DAPHNIA	0.126	0.185	0.002	0.107
CALANOID	0.001	0.369	0.002	0.003
NAUPLII	0.73	0.806	0.214	0.047
KERATELLA	0.003	0.014	0.037	0.15
TEMPERATURE	0.04	0.453	0.064	0.195
DO	0.14	0.13	0.007	0.367
PH	0.031	0.031	0.127	0.0
CHL-A	0.092	0.108	0.067	0.435
CONDUCTIVITY	0.006	0.157	0.017	0.08
SECCHI DEPTH	0.191	0.153	0.007	0.322
TOC	0.003	0.001	0.038	0.102
TN	0.022	0.036	0.049	0.089
TP	0.102	0.043	0.156	0.08

**Table 10 - P-Values from ANOVA Analysis with Log(Geosmin) from lakes and reservoirs data separated by region.**

FACTOR	HIGH MOUNTAIN	FOOTHILLS	URBAN	PLAINS
LAKE SIZE	0.808	0.304	0.412	-
TN	0.126	0.557	0.725	0.737
TP	0.161	0.104	0.363	0.802
TOC	0.262	0.397	0.773	0.341
MONTH	0.556	0.021	0.475	0.455

### 3.2.2 Stepwise Regression Models

Multivariate analysis produced models with greater significance. Stepwise regression was used, using an alpha to enter and remove of 0.05. The most significant models developed in each eco-region using linear regression and stepwise regression are shown below in Table 11. Stepwise regression was performed using both the log transformed and non-transformed geosmin datasets.

Other notable studies that used log transformed geosmin datasets include; the 2006 study by Christensen which developed a log transformed geosmin model using turbidity and specific conductivity, with a R<sup>2</sup> value of 0.709 (Christensen et al., 2006), Dzialowski in 2009 discussed further below (Dzialowski et al., 2009), and the Sugiura study of 2004 which reported a multiple linear regression model between the log(geosmin) and TP, COD, and DO, with a R<sup>2</sup> value of 0.5337 (Sugiura et al., 2004).

Transforming the geosmin data only increased correlation values some of the time. Particularly in the case of the foothills, the most correlated linear regression model used non-transformed data, but the most correlated stepwise regression model was with the log transformed dataset. Stepwise regression did not increase the Urban Lakes model correlation because the correlation between geosmin and the other parameters was so poor.

**Table 11 - Summary of Regression and Stepwise Regression Models with Highest Significance.**

DATASET	EQUATION	R2	N	P
ENTIRE DATASET	LOG(GEOSMIN) = -0.00047*[KERA]-0.101*[SECCHI]+1.36*[TP]+0.00226*[NAUPLII]-0.2044	0.922	145	0.005
ENTIRE DATASET	GEOSMIN = -0.60*[TOC]-0.0058*[DAPHNIA]+0.0382*[NAUPLII]+1.7846	0.88	145	0.024
ENTIRE DATASET	GEOSMIN = 0.00149*[NAUPLII]+0.6798	0.038	213	0.053
URBAN LAKES	LOG(GEOSMIN) = 0.00006*[NAUPLII]-0.214	0.214		
PLAINS LAKES	LOG(GEOSMIN) = -0.0321*[SECCHI]-0.100	0.322		
PLAINS LAKES	LOG(GEOSMIN) = -0.0279*[CHL]+0.120*[DO]+1.12885	0.688		
HIGH MOUNTAIN LAKES	LOG(GEOSMIN) = -0.048*[CHL]-0.045*[TOC]+0.00302*[DAPHNIA]-0.4740	0.432		
HIGH MOUNTAIN LAKES	GEOSMIN = 0.0028*[DAPHNIA]+0.233	0.246		
FOOTHILLS LAKES	LOG(GEOSMIN) = 0.00204*[NAUPLII]+0.0175*[SPC]-0.111*[SECCHI]+0.28*[TP]+0.45*[TN]-1.3166	0.9794		
FOOTHILLS LAKES	GEOSMIN = 0.00264*[NAUPLII]-0.697	0.806		
RIVERS DATASET	LOG(GEOSMIN) = 0.129*[DO]+0.037*[TOC]-1.589	0.224		
RIVERS DATASET	LOG(GEOSMIN) = 0.127*[DO]-1.42	0.151		

The following model was developed using the stepwise regression procedure using an alpha to enter and remove of 0.05.

**Equation 4**

$$\log(GEOS) = -0.00047 * [keratella] - 0.101 * [Secchi D.] + 1.36 * [TP] + 0.00226 \\ * [Nauplii] - 0.2044 \quad N = 145 \quad R^2 = 0.922 \quad P = 0.005$$

The null hypothesis that geosmin is not correlated with other water quality parameters is rejected based on,  $P < 0.05$ . Therefore, there appears to be significant correlation between log-geosmin and 4 other parameters (Total Phosphorous, Keratella, Nauplii, and Secchi Depth), with an R-squared value of 0.922. Given that the linear regression models showed the greatest significance with Nauplii, it would appear that geosmin concentrations in these water bodies are producing through means of grazing. It has been mentioned in previous studies that Geosmin concentrations in algal cells are known to be higher than concentrations outside the cell. (Peterson et al., 1995; Pan, 2002) Therefore, when zooplankton graze on geosmin producing algae, like cyanobacteria, the algal cell wall becomes compromised and releases the geosmin contained within the cell (Durrer et al., 1999; Rashash et al., 1995). TP is often the limiting nutrient for algae/bacteria growth, so the finding that TP is a significant parameter is not surprising (Mitsui et al., 1986).

Another model was developed using the stepwise regression procedure using alpha to enter and remove of 0.05. This model used the geosmin concentration without log transform, the correlation is less significant than the log transformed model, and interestingly only one of the same parameters.

### Equation 5

$$GEOSMIN = -0.60 * [TOC] - 0.0058 * [Daphnia] + 0.0382 * [Nauplii] + 1.7846$$

$$N = 145 \quad R^2 = 0.880 \quad P = 0.024$$

Just as in the case of the log transform model, the p-value is below our alpha value of 0.05, so the null hypothesis is rejected. Values recorded as zero were excluded from the previous model, just as the zero values were removed from the log transformed data. USGS have reported that removed non-detects, or in this case values of zero results in a loss of information (Gilliom et al, 1984). However, statically significant regression models could not be developed from the data if zero values are included in the geosmin dataset, so for the sake of this study they are removed. Shown below are the results from the stepwise regression model including geosmin results of zero. Clearly the model poorly explains geosmin episodes, but does highlight Nauplii as the only parameter that entered the stepwise regression model.

### Equation 6

$$GEOSMIN = 0.00149 * [Nauplii] + 0.6798 \quad N = 213 \quad R^2 = 0.038 \quad P = 0.053$$

Many studies have developed models successfully using multivariate linear regression models, using both geosmin concentration and the log transform (Christensen et al, 2006; Smith et al, 2002; Mau et al, 2004; Suguira et al, 2004; Dzialowski et al, 2009). Both the log transformed models and concentration models from previous studies have reported R-squared values over 0.70. The Dzialowski study of Cheney Reservoir developed models with R-squared values above 0.80 using both log transformed geosmin data and untransformed data. It is interesting to note as well that

these previous studies have all used different water quality parameters to develop their models. For example, Christensen and Mau used turbidity as the primary indicator of geosmin concentration, Smith used chlorophyll-a as the primary indicator, Suguira correlated log transformed geosmin with total phosphorous, chemical oxygen demand, and dissolved oxygen (Christensen et al, 2006; Smith et al 2002; Mau et al, 2004; Suguira et al, 2004). This study shows that zooplankton have a role to play in geosmin episodes, which should be expected given the algae and bacteria produce geosmin, algal grazers will influence these populations.

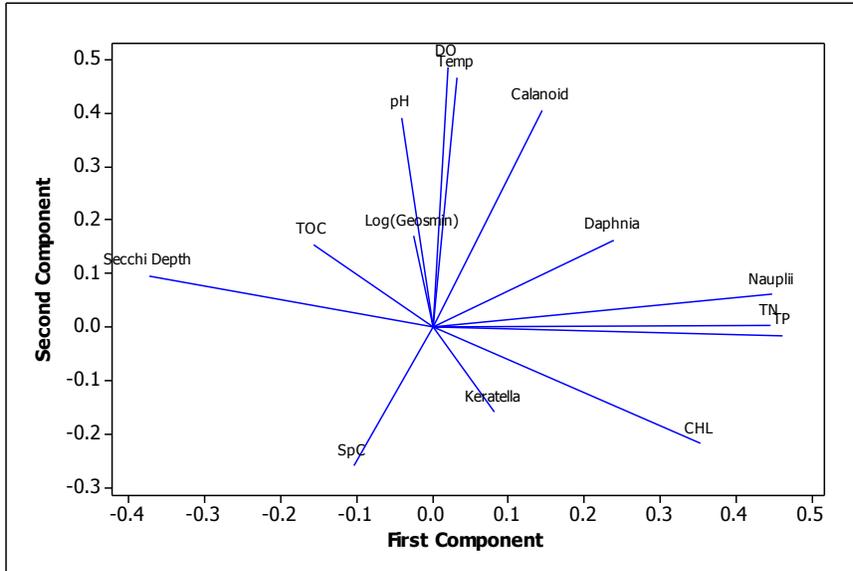
### ***3.3 PCA Results and Discussion***

Principal component analysis was performed using each grouping of sites as was used for the linear regression model and ANOVA (All data, lakes/reservoirs, rivers, Poudre River, Big Thompson River, High Mountain, Foothills, Urban, and Plains lakes). PCA also included analyzing both transformed and non-transformed geosmin data, however the component eigenvalues were the same for either input.

#### ***3.3.1 High Mountain Lakes PCA***

PCA results for the high mountain lakes region do not show any significant relationships between geosmin and other parameters. However, the loading plot, Figure 5 **Error! Reference source not found.**, shows relative grouping with geosmin and the following parameters; pH, DO, Temperature, and Calanoids. This is interesting given that linear regression modelling found that daphnia had greater correlation with geosmin than calanoids, and stepwise regression found chlorophyll-a and TOC to be the most significant abiotic parameters. Both regression models

have low  $R^2$  values and, as shown in Table 12, the component analysis does not reveal any obvious correlations either.



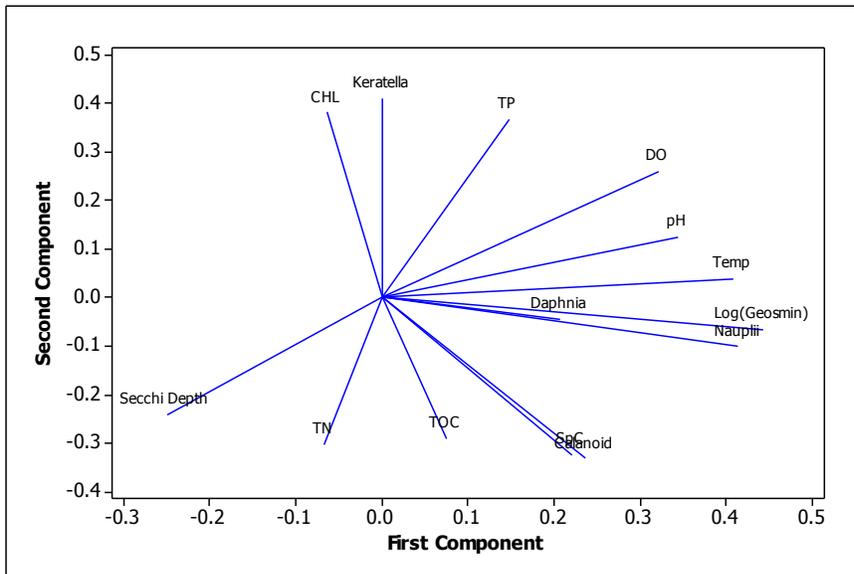
**Figure 5 - PCA of Log(Geosmin) and other sampled parameters from lakes and reservoirs considered to be “High Mountain Lakes” (Estes Lake, Mary’s Lake, Chambers Lake, Joe Wright Res., Lost Lake, Brainard Lake, Long Lake, and Red Rock Lake).**

**Table 12 - Log(Geosmin) PCA Eigenvalues for first and second components, High Mountain Lakes and Reservoirs.**

VARIABLE	PC1	PC2
DAPHNIA	0.239	0.162
CALANOID	0.145	0.404
NAUPLII	0.448	0.061
KERATELLA	0.080	-0.159
TEMP	0.032	0.465
DO	0.021	0.485
PH	-0.041	0.39
CHL	0.354	-0.219
SPC	-0.104	-0.259
SECCHI DEPTH	-0.373	0.095
TOC	-0.157	0.154
TN	0.445	0.003
TP	0.462	-0.016
LOG(GEOSMIN)	-0.025	0.170

### 3.3.2 Foothills Lakes PCA

The foothills eco-region produced the most significant models of this study, of which PCA confirms. Both Figure 6 and Table 13 show high correlation between geosmin and Nauplii. Nauplii and geosmin have first component eigenvalues of 0.414 and 0.443, respectively. This compares well to the regression models shown in Table 11 of the regression models section where Nauplii and geosmin had a high  $R^2$  value, and Nauplii with TN, TP, SpC, and Secchi Depth produced a very significant ( $R^2 > 0.95$ ) relationship in the stepwise regression model.



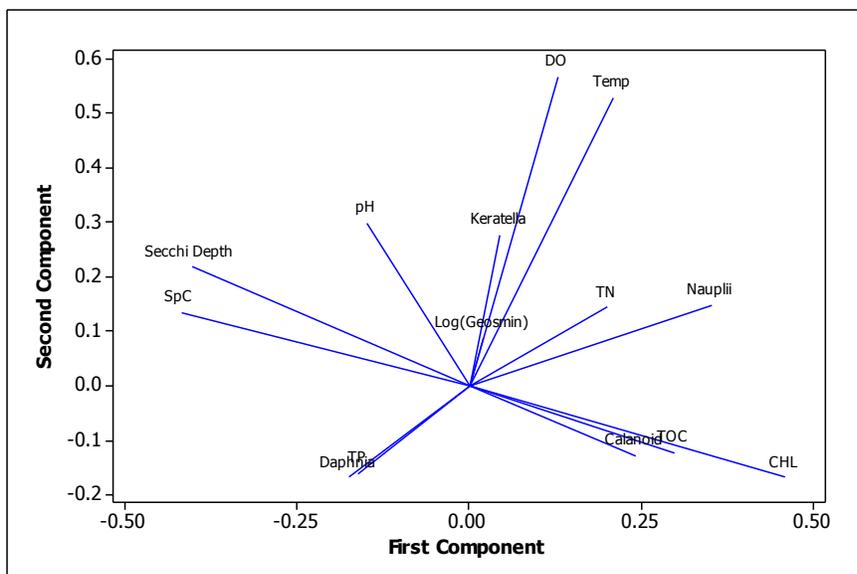
**Figure 6 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “Foothill Lakes” (Carter Lake, Pinewood Res., Flatiron Res., and Horsetooth Res.).**

**Table 13 - Log(Geosmin) PCA Eigenvalues for first and second components, Foothills Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	0.206	-0.045
CALANOID	0.236	-0.333
NAUPLII	0.414	-0.102
KERATELLA	0.001	0.41
TEMP	0.407	0.038
DO	0.321	0.259
PH	0.344	0.125
CHL	-0.064	0.381
SPC	0.22	-0.325
SECCHI DEPTH	-0.25	-0.242
TOC	0.074	-0.29
TN	-0.068	-0.303
TP	0.147	0.367
LOG(GEOSMIN)	0.443	-0.06

### **3.3.3 Urban Lakes PCA**

As found in the regression models, only small correlations were found between geosmin and other parameters in the urban lakes eco-region. Figure 7 shows geosmin, keratella, DO, and temperature clustered together, but the eigenvalues, shown in Table 14, do not support any significant trends. Perhaps with more data and a greater variety of geosmin concentrations a trend could be determined. Only one geosmin sample in this region produced a concentration over 10ng/l.



**Figure 7 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “Urban Lakes” (City Park Lake, Fossil Creek Park Lake, Lake Loveland, and Sherwood Lake).**

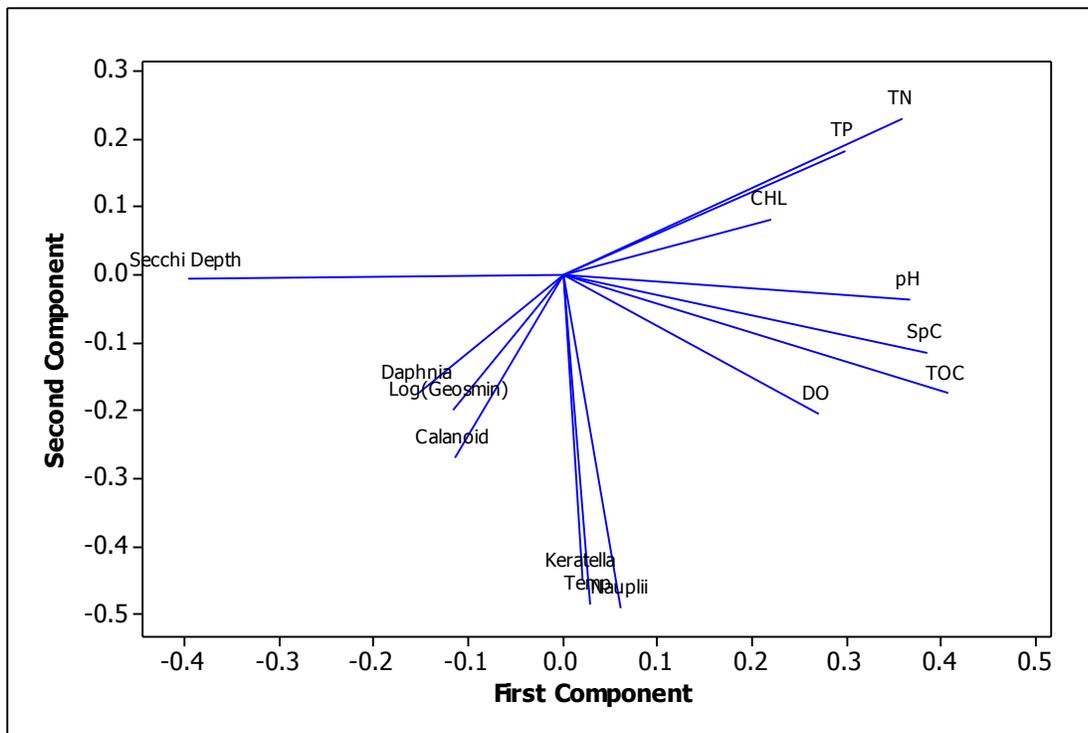
**Table 14 - Log(Geosmin) PCA Eigenvalues for first and second components, Urban Lakes and Reservoirs.**

VARIABLE	PC1	PC2
DAPHNIA	-0.174	-0.169
CALANOID	0.241	-0.129
NAUPLII	0.352	0.148
KERATELLA	0.044	0.276
TEMP	0.209	0.529
DO	0.129	0.568
pH	-0.148	0.3
CHL	0.458	-0.169
SPC	-0.419	0.135
SECCHI DEPTH	-0.404	0.218
TOC	0.297	-0.124
TN	0.201	0.144
TP	-0.161	-0.162
LOG(GEOSMIN)	0.02	0.087

### 3.3.4 Agricultural Lakes PCA

PCA results from the agricultural region shows a clear grouping of geosmin with zooplankton, particularly Daphnia and Calanoids. Although the grouping looks tight in Figure 8, Table 15 shows

that relationships between these parameters are not strong. It is also interesting that the parameters grouped with geosmin in the loading plot are not the same as the parameters found to have the highest linear correlation with. In fact, the results of the PCA show greater relationship between geosmin and biotic parameters, but the most significant regression models had abiotic parameters. Although, the abiotic parameters are related (TOC, CHL-a, and Secchi Depth).



**Figure 8 - PCA of Log(Geosmin) and other sampled parameters from lakes and reservoirs considered to be “Agriculture influenced Lakes” (Boyd Lake, Riverside Res., Jackson Lake, and Barr Lake).**

**Table 15 - Log(Geosmin) PCA Eigenvalues for first and second components, Plains Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	-0.152	-0.174
CALANOID	-0.115	-0.269
NAUPLII	0.061	-0.49
KERATELLA	0.021	-0.45
TEMP	0.028	-0.485
DO	0.271	-0.204
pH	0.367	-0.037
CHL	0.22	0.082
SPC	0.385	-0.116
SECCHI DEPTH	-0.397	-0.007
TOC	0.407	-0.175
TN	0.358	0.23
TP	0.298	0.183
LOG(GEOSMIN)	-0.117	-0.198

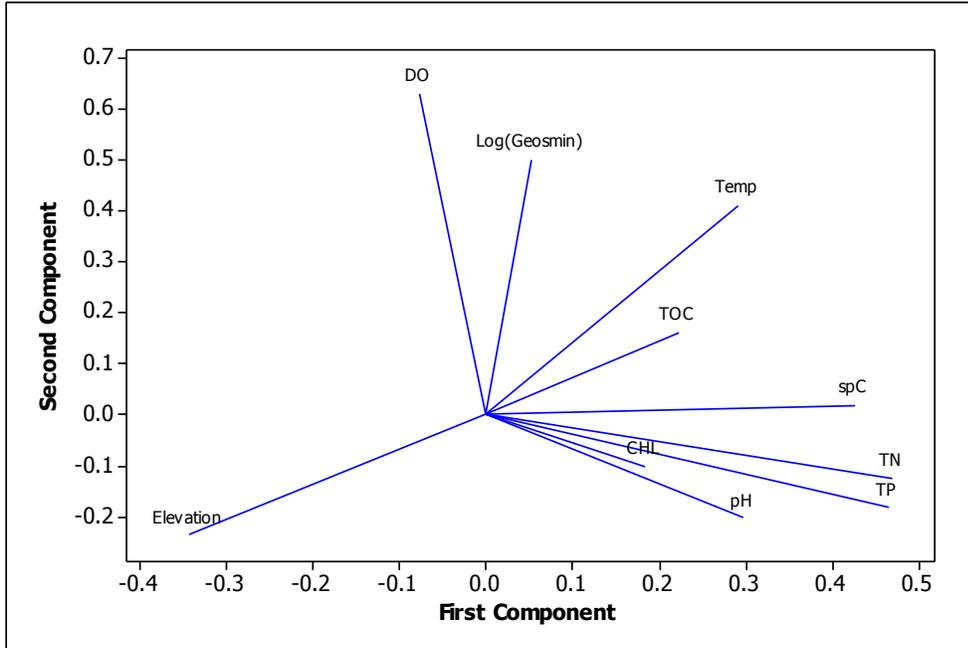
### ***3.3.5 River and Streams PCA***

Regression models showed small correlations between geosmin and DO, ANOVA revealed a significant relationship between geosmin and elevation. PCA shows a significant relationship between geosmin and both DO and temperature. The loading plot, Figure 9, shows Log(Geosmin), temperature and DO grouped together, but Table 16 clearly shows the significance of the relationship between geosmin, DO and temperature. Eigenvalues of 0.500 or higher are considered to be significantly related, Table 16 shows geosmin to be at 0.5 and DO and temperature are greater than 0.5. A study by Sugiura in 2004 reported a multiple linear regression model between the log(geosmin) and TP, COD, and DO, with a  $R^2$  value of 0.5337 (Sugiura et al., 2004).

A similar study by Parinet and fellows in 2010 and 2013 did not show a correlation between DO and Geosmin (Parinet et al., 2010; Parinet et al., 2013). The 2013 study by Parinet and fellows proposed a model for predicting Geosmin concentrations based on samples from phaeophytin, green algae counts, chlorophyll-a, and redox potential based on results from PCA. The rivers in the Parinet study vary from the rivers in this study in both climate, and flow. The rivers in the Parinet study are large slow moving rivers in Canada, colder climate, whereas the rivers in our study are much smaller, and are primarily snowmelt feed.

A similar study performed by Oropeza and fellows in 2011 of geosmin in the Cache la Poudre River found that geosmin concentrations were highest during low flow periods (Oropeza et al., 2011). The geosmin episodes recorded in the Oropeza study did occur during the winter which may have been the cause of the episodes as has been recorded by other studies (Dzialowski et al., 2009; Zaitlin and Watson 2006; Parinet et al., 2010; Christensen et al., 2006). The Oropeza study

took samples from a site near Rustic, CO located near our site designated Narrows Campground. Geosmin concentrations in our study from this site were very low, the highest being only 1.6ng/l so no correlation to discharge was recorded in this study unlike the Oropeza study.



**Figure 9 - PCA of Log(Geosmin) and other sampled parameters all river and stream data.**

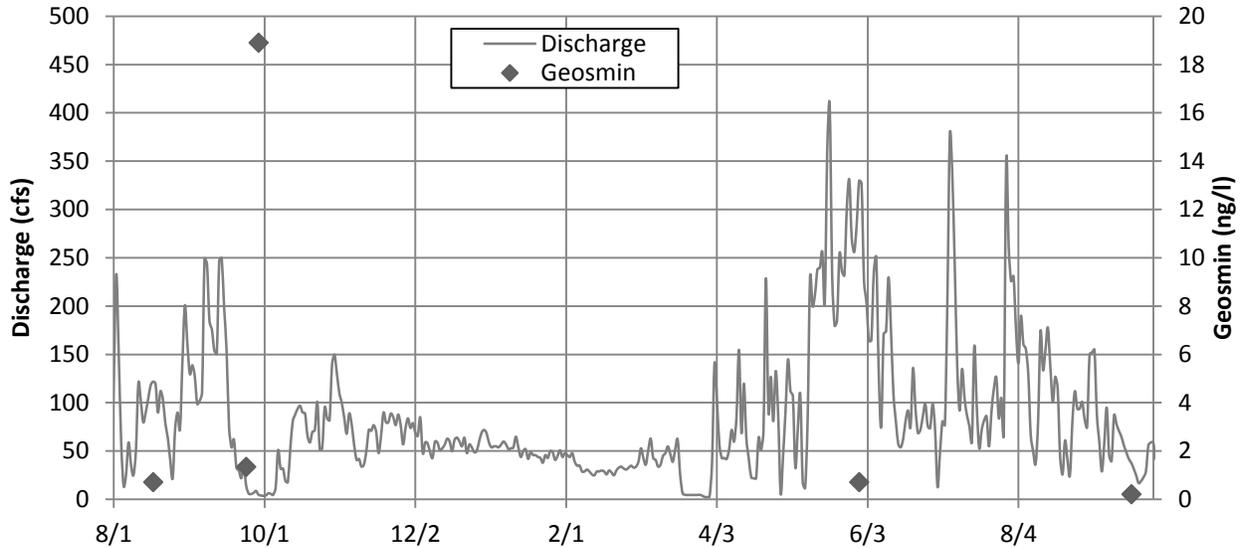
**Table 16 - Log(Geosmin) PCA Eigenvalues for first and second components, Rivers**

VARIABLE	PC1	PC2
TEMP	0.292	0.412
DO	-0.077	0.630
pH	0.297	-0.203
CHL	0.183	-0.102
SPC	0.425	0.019
TOC	0.222	0.162
TN	0.470	-0.125
TP	0.465	0.269
ELEVATION	-0.343	-0.237
LOG(GEOSMIN)	0.052	0.500

### 3.4 Times Series Results and Discussion

The highest geosmin concentration recorded on the Poudre River in this study did occur during a low flow period, which interestingly followed a period of higher flow in Fort Collins. As shown

in Figure 10, this high geosmin concentration occurred days after a low geosmin level was recorded. Many studies have found geosmin concentrations to rapidly increase as in this case.

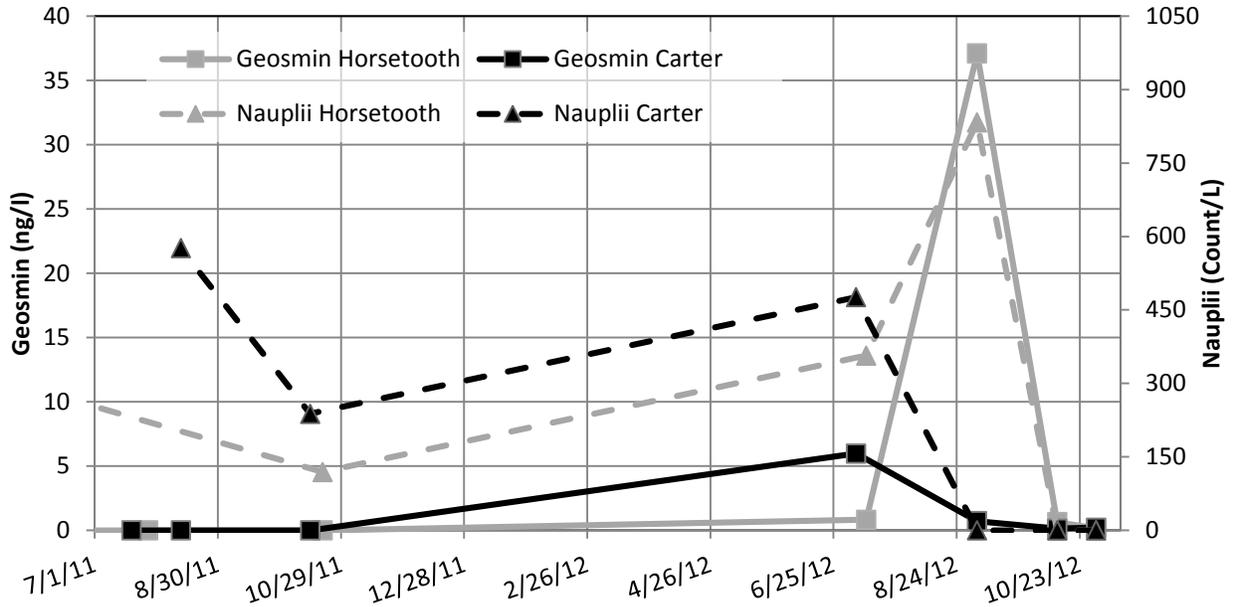


**Figure 10 - Time Series chart for Geosmin and Discharge on the Cache la Poudre River at Lincoln St.**

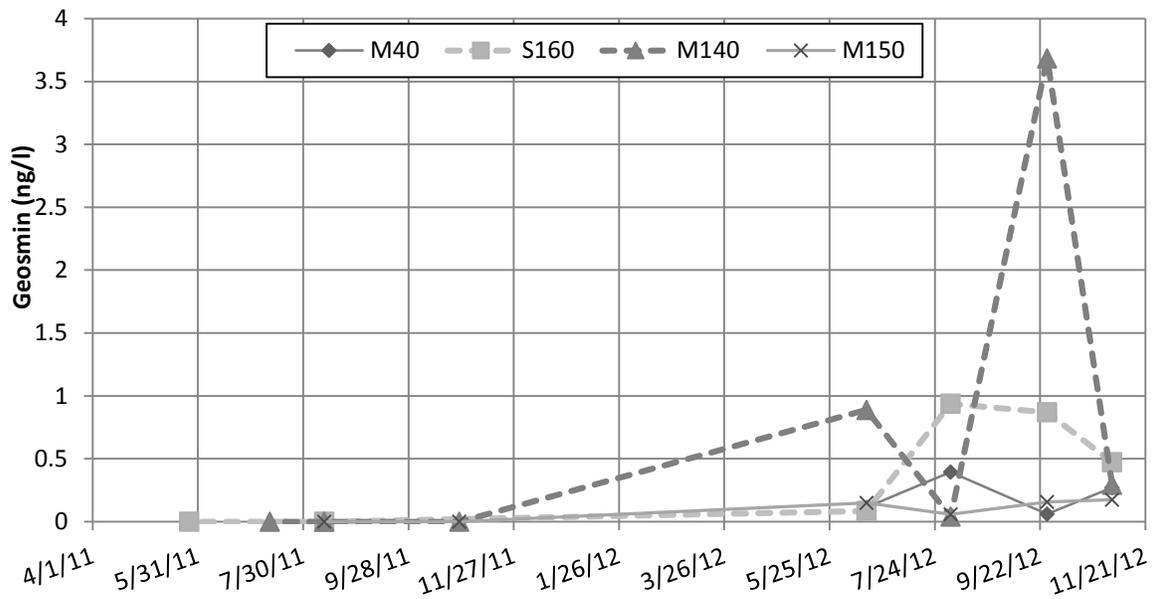
Time series data of geosmin concentrations have been shown to maintain low concentrations for extended periods of time with periodic and seemingly random large, but short peaks in concentration, this has been shown in previous geosmin studies of the Cache la Poudre River (Oropeza et al, 2011). Geosmin concentrations appear to remain far below human detection limit most of the year with periodic order of magnitude spikes in summer or early fall. Consider Horsetooth Reservoir, which has a known history of severe geosmin episodes most notably in 2011, Horsetooth had a peak recorded geosmin concentration of 37ng/l. The peak concentration however did not last more than two months, since no other measurements contained geosmin concentrations above 1ng/l. Clearly to detect a correlation between geosmin concentration and any other parameter in Horsetooth Reservoir a higher frequency of samples must be taken.

Carter Lake consistently had below human detection limit concentrations of geosmin as well. The highest concentration occurred in June, whereas the peak concentration in Horsetooth Reservoir a similar size and type of body of water was in September. Horsetooth and Carter Lake both have the same source of water (coming from a series of trans-basin diversions from the Colorado River watershed), and are very large reservoirs located at similar elevations, approximately only 40 miles apart. The fact that geosmin concentrations vary in both scale and time between these two reservoirs is a significant finding.

The foothills region which includes Horsetooth Res. and Carter Lake recorded the greatest significant regression models. Using linear regression geosmin and Nauplii had a  $R^2$  value of 0.81, and using stepwise regression the model improved to 0.98. Figure 11 shows the time series for Horsetooth and Carter lake geosmin concentrations and Nauplii. Both reservoirs appear to have a geosmin episode in conjunction with an increase in Nauplii counts.

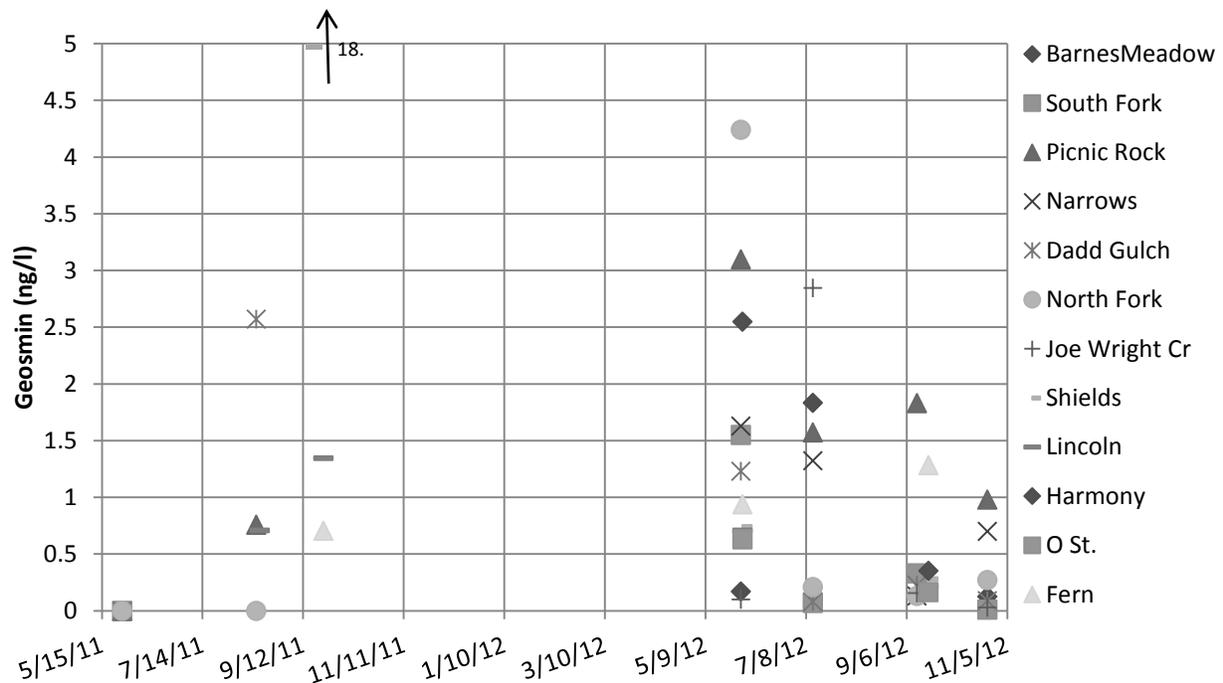


**Figure 11 - Geosmin and Nauplii time series data taken from Horsetooth Res. and Carter Lake between August 12th, 2011 and October 31st, 2012, excluding winter and spring months November through June.**



**Figure 12 - Geosmin time series data from four Big Thompson River sampling sites. M40 is located about a mile south of Estes Lake, S160 is located at Viestenz-Smith Mountain Park, M140 is located in the City of Loveland, and M150 is located downstream of Loveland at the I-25 bridge.**

Figure 12 displays the geosmin concentrations of the Big Thompson River at various locations along the stream. Notice that concentrations in 2012 are noticeably higher than in 2011, and even among same day samples the different locations along the stream have different concentrations of geosmin. Concentrations do not necessarily increase or decrease downstream either. Samples taken on September 26<sup>th</sup> 2012 show a drastic difference in Geosmin levels between sites M140 and M150. This is surprising because these locations are located just downstream of the City of Loveland only about a mile and a half apart. This is especially interesting because the concentration decreases rapidly downstream, rather than increasing. Either a source water is diluting the river, or the geosmin itself is being broken down or absorbed. This is similar to the findings by Oropeza and fellows in 2011 on the Poudre River where high geosmin concentrations in the Rustic, CO area were not seen downstream at the Fort Collins Water Treatment Plant diversion (Oropeza et al., 2011). However, the distance between the Oropeza sites could be measured in miles, not thousands of feet.



**Figure 13 - Times Series display of Geosmin results collected from sites along the Cache la Poudre River.**

The Poudre River was recorded to have non-human detectable geosmin concentrations throughout the majority of the sampling period. This is surprising because the Poudre has a history of recent geosmin episodes, particularly near and around the town of Rustic (Billica et al., 20007; Oropeza et al., 2011). One possible factor could be the presence of the High Park fire in June of 2012, however geosmin concentrations were low even at the Narrows Campground near Rustic, CO which is located upstream of the burn area. The summer of 2012 was a dry year especially relative to the previous year, so geosmin episodes were expected to be worse in 2012, but the data does not support this, see

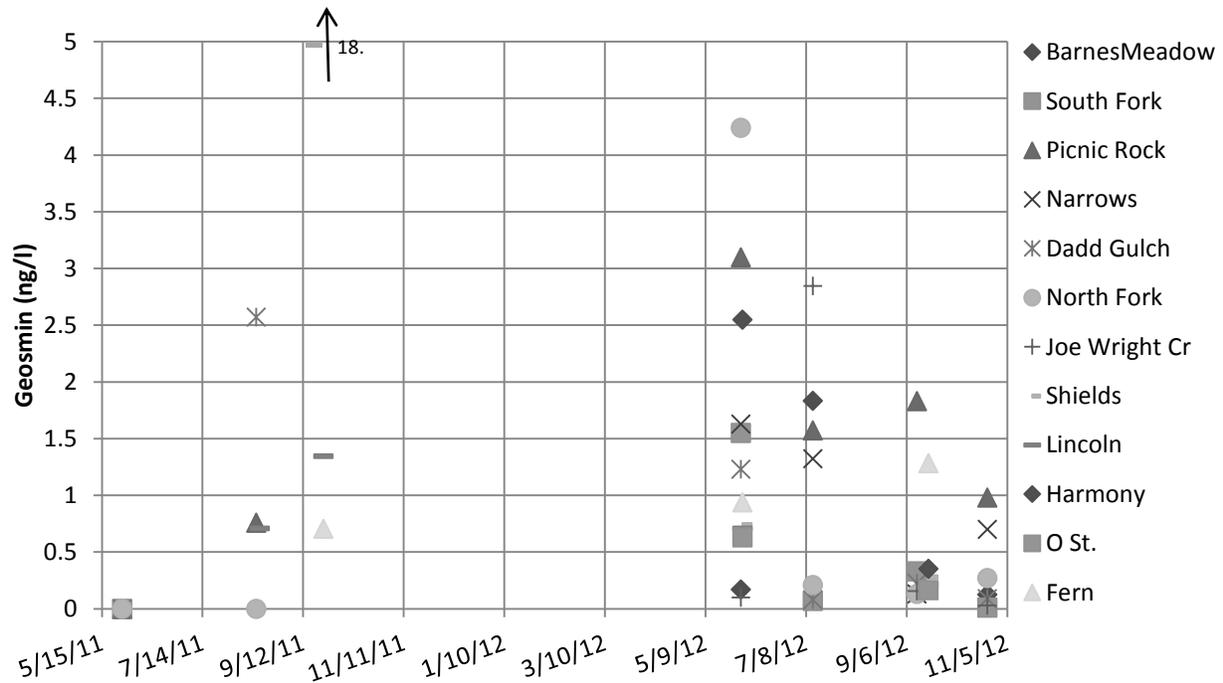


Figure 13. Because of the difference between the wet water year of 2011 and the dry water year of 2012 it was expected to find higher geosmin concentrations in 2012. The highest geosmin level recorded on the Poudre though occurred in 2011, and the overall average of geosmin concentrations in 2012 did not seem any difference than the year before. Of course we had many more samples from 2012 than 2011 so the comparison only tells so much.

Parinet in both 2010 and 2013 found that river sites with “Urban” contributing drainages had higher geosmin levels compared to a river with no anthropogenic influence (Parinet et al., 2010; Parinet et al., 2013). The fact that the highest recorded geosmin level on the Poudre River was sampled from downtown Fort Collins certainly supports this possibility. The two studies by Parinet also found that agricultural influenced increased geosmin level compared to areas with no anthropogenic influences. This study did not sample as many agricultural stream sites relative to others, but of the sites sampled agriculture did not significantly influence geosmin levels.

## 4.0 CONCLUSIONS

Geosmin concentrations were primarily correlated with differing elevations and regions. Geosmin concentrations needed to be transformed to log scale for these correlations to be seen. Previous studies have also transformed geosmin datasets to log scale to develop increased significance (Dzialowski et al., 2009; Christensen et al., 2006; Sugiura et al., 2004). The results of this study show no significant correlations between non-log transformed geosmin and any other measured parameter using linear regression modelling, which is in contrast to many previous studies including the Dzialowski paper which found significant relationships using both non and transformed datasets (Dzialowski et al., 2009; Mau et al., 2004; Smith et al., 2002; Parinet et al., 2010; Parinet et al., 2013). Studies from Mau and fellows 2004, Smith and fellows 2002, Parinet and fellows 2010 and Parinet and fellows 2013 found higher degrees of significance using non-log transformed datasets. ANOVA results showed a significant relationship between elevation, month of the year and log transformed geosmin concentrations, this was not the case with the original dataset or the censored dataset.

The most significant finding of this study is the relationship between zooplankton and geosmin, particularly *Daphnia* and *Nauplii*. The significance of these relationships varies depending on whether log transformed data is used or not. For example, the foothills eco region's most significant linear regression model is between *Nauplii* and the non-transformed geosmin data. But the most significant stepwise regression model for the foothill eco region is between the log transformed geosmin data and *Nauplii*, specific conductivity, Secchi depth, TP and TN.

- River data shows that elevation is a significant factor of geosmin concentrations. This may help account for unexplained geosmin episodes in the Poudre River near Rustic, CO found in other studies.
- Stream discharge did not appear to play a significant role given that the 2012 water year was much drier than the 2011 water year but higher geosmin concentrations were recorded in 2011.
- Geosmin levels in rivers appear to be influenced most by DO, and elevation. But neither parameter showed statistically significant correlations.
- Zooplankton, particularly Nauplii and Daphnia are shown to have a significant impact on geosmin concentrations in lentic water bodies.
- Those same water bodies display significant trends with month of the year, supporting the view that geosmin episodes are dependent on seasonal parameters.

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## APPENDIX I

### Geosmin Analysis Procedure

#### Sample Preparation:

##### Materials:

- Clean, screw-top amber vials with caps
  - Need enough for duplicate samples and 1 for each standard (4-5)
- Pipettors: 10-100 $\mu$ L, 100-1000 $\mu$ L, and 1-5mL
- Pipet tips for each of the above pipettors
- NaCl
- Scale

##### Procedure:

- Label each vial with the sample name and replicate edition, and each standard vial accordingly (usually 1ng/L, 5ng/L, 10ng/L, 25ng/L, and 50ng/L (optional depending on concentrations expected in samples))
- Weigh out 3g of NaCl into each of the sample and standard vials
- Using the 1-5mL pipettor & corresponding pipet tips, aliquot 20mL of sample into corresponding, salted, pre-labeled vials
  - Use the same pipet tip for the sample replicate
- Cap labeled vial containing sample/salt solution
- Cap sample, and place in refrigerator
- Change pipet tip, and repeat for the rest of samples
- Once all samples have been aliquoted, "spike" each sample with 20 $\mu$ L of .04mg/L TCA stock solution using the 10-100 $\mu$ L pipettor & corresponding pipet tips, upon adding TCA to sample, backwash tip 3-5 times to ensure all of the TCA has made it into the sample, discard pipet tip after each sample/replicate spiked, cap vial tightly
  - DO NOT use a dirty tip in the TCA solution as this will contaminate the stock
- After spiking all samples, cap TCA stock solution, place samples in refrigerator

#### Standard Preparation:

##### Materials:

- Pipettors: 10-100 $\mu$ L, 100-1000 $\mu$ L, and 1-5mL
- Pipet tips for each of the above pipettors
- Stock solutions
  - .04mg/L Geosmin
  - .04mg/L TCA
- 100mL volumetric flask
- 600mL beaker
- DI Water
- Parafilm

##### Procedure:

- Rinse the volumetric flask and beaker 3 times with DI water
- Measure 100mL DI water into rinsed volumetric flask
- Measure ~200mL DI water into rinsed beaker

- Using the 100-1000 $\mu$ L pipettor and tips
  - Remove and discard 125 $\mu$ L DI water from volumetric flask
  - Discard pipet tip and replace with new tip
  - Equilibrate pipet tip in .04mg/L Geosmin solution by backwashing (pipetting Geosmin solution in and out of pipet tip) 3-5 times
  - Add 125 $\mu$ L of .04mg/L Geosmin to the volumetric flask
  - Backwash tip 3-5 times in the DI water in the volumetric, discard pipet tip, cap stock geosmin solution.
  - Cut a piece of Parafilm large enough to cover opening of volumetric flask, stretch Parafilm over volumetric flask opening to cover tightly, invert and shake (with thumb over flask opening and Parafilm) several times to ensure complete mixing of Geosmin and DI water
  - This is a 50ng/L solution
  - Using a clean pipet tip, add 400 $\mu$ L of the 50ng/L solution to the salted vial labeled 1ng/L, cap tightly and set aside
- Using the 1-5mL pipettor and tips
  - Using the same tip, aliquot:
    - 2mL into prepared and labeled 5ng/L vial and cap tightly
    - 4mL into prepared and labeled 10ng/L vial and cap tightly
    - 10mL into prepared and labeled 25ng/L vial and cap tightly
    - 20mL into prepared and labeled 50ng/L vial and cap tightly (Optional)
  - Discard pipet tip and replace with a new one for the following:
    - From the 600mL beaker containing DI water add:
      - 19.6mL DI water to 1ng/L standard vial, cap tightly
      - 18mL DI water to 5ng/L standard vial, cap tightly
      - 16mL DI water to 10ng/L standard vial, cap tightly
      - 10mL DI water to 25ng/L standard vial
- Spike the standards with TCA, following the spiking procedure in the Sample Preparation Procedures, and place in refrigerator with prepared samples.
- Cut a 2 square X 2 square of Parafilm and wrap tightly around cap of vial containing stock Geosmin solution, place stock solutions in refrigerator
- Empty 600mL beaker, rinse 3 times with DI water and let air dry

Empty volumetric flask, rinse 3 times with tap water, followed by 3 rinses with DI water, let air dry

High purity sterile sodium chloride was acquired from Fisher Scientific (Pittsburgh, PA), and weighed using an Ohaus Adventurer balance. A 5.5 liter isotemperature Thermo Scientific Precision water bath, model number 2831, with  $\pm 0.2$  °C uniformity and  $\pm 0.1$  °C control resolution was purchased from Fisher Scientific (Pittsburgh, PA). Solid-phase microextraction (SPME) fibers coated with Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) at 65  $\mu$ m thickness, the SPME holder and the SPME GC-inlet liner were purchased from Sigma-Aldrich (Pittsburgh, PA). Agilent 5890 gas chromatograph (GC) equipped with an Agilent DB-5 MS (30 m, 0.25 mm i.d., 0.25  $\mu$ m) column connected to an Agilent 5973 mass spectrometer (MS) were used for sample analyses (Santa Clara, CA).

APPENDIX II

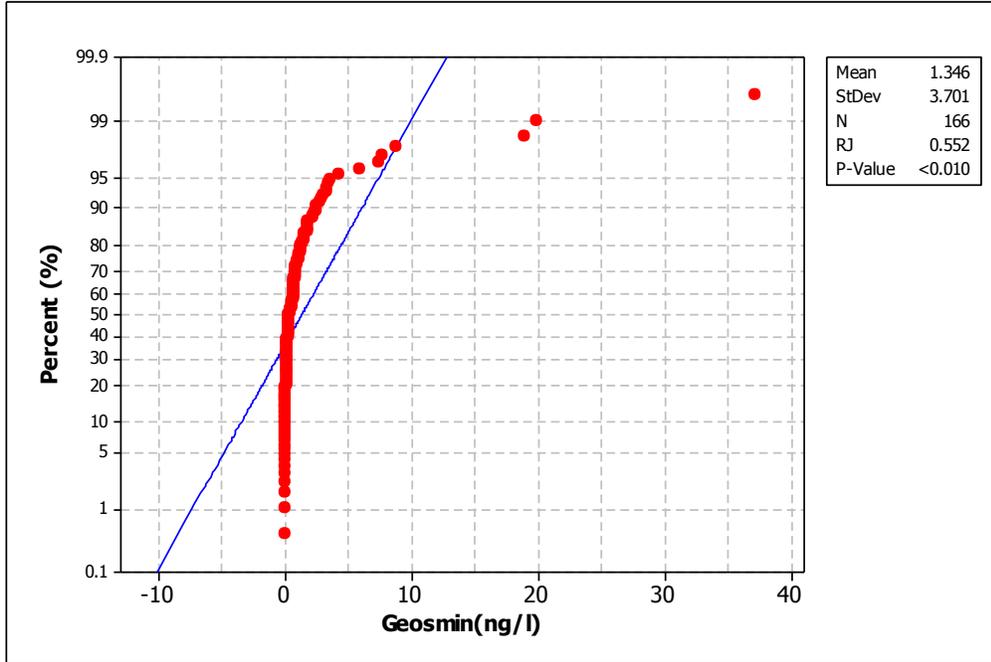


Figure 14 - W-Test for Normality including geosmin concentrations recorded below the detection limit.

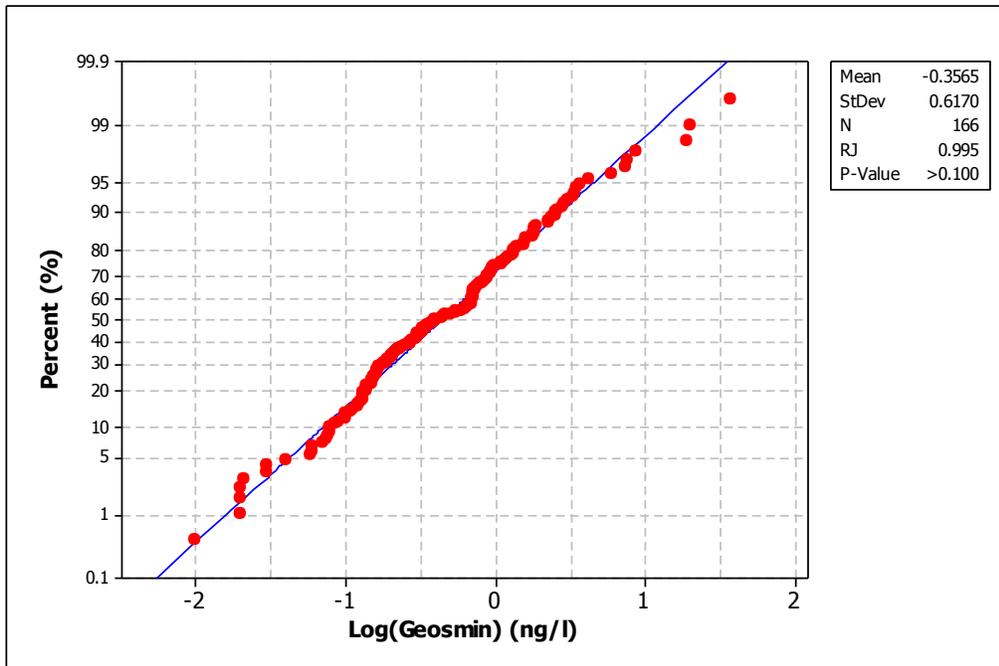


Figure 15 - MiniTab results from W-Test for the log of Geosmin data Normality; data does not include zeros/non-detect values from the entire dataset from all river and lake sampling locations.

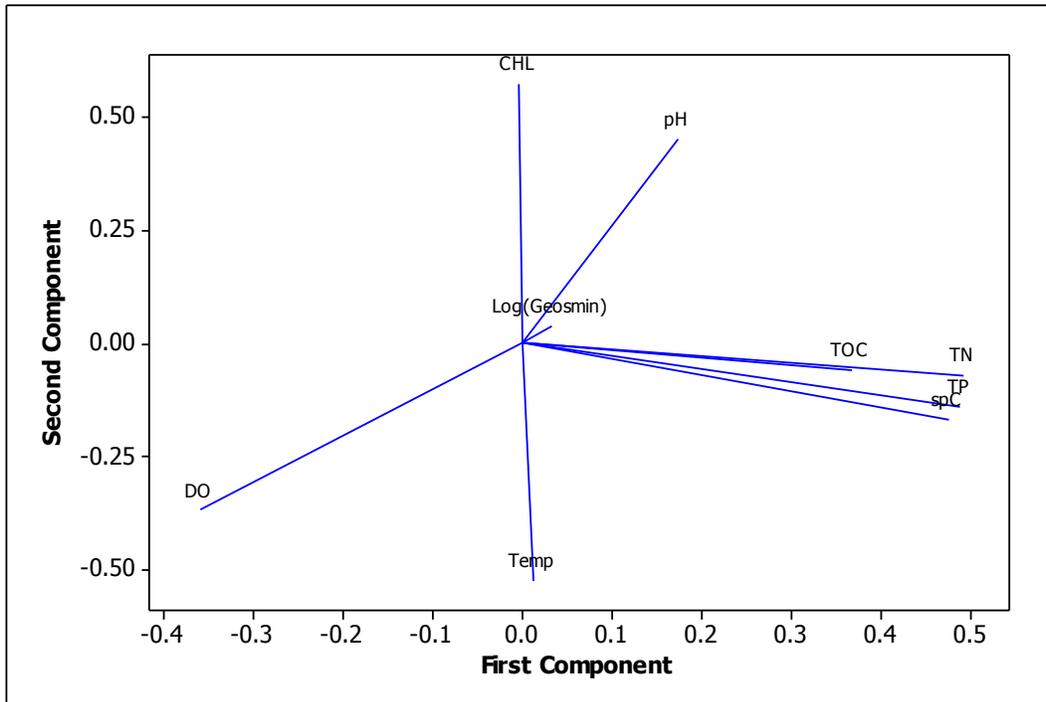
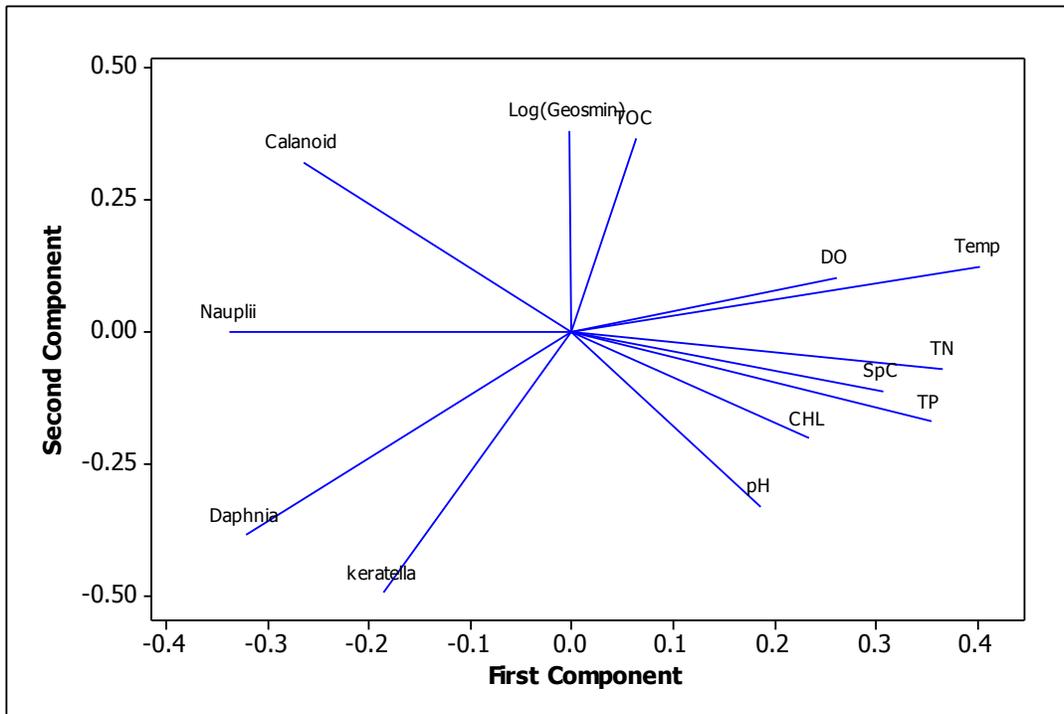
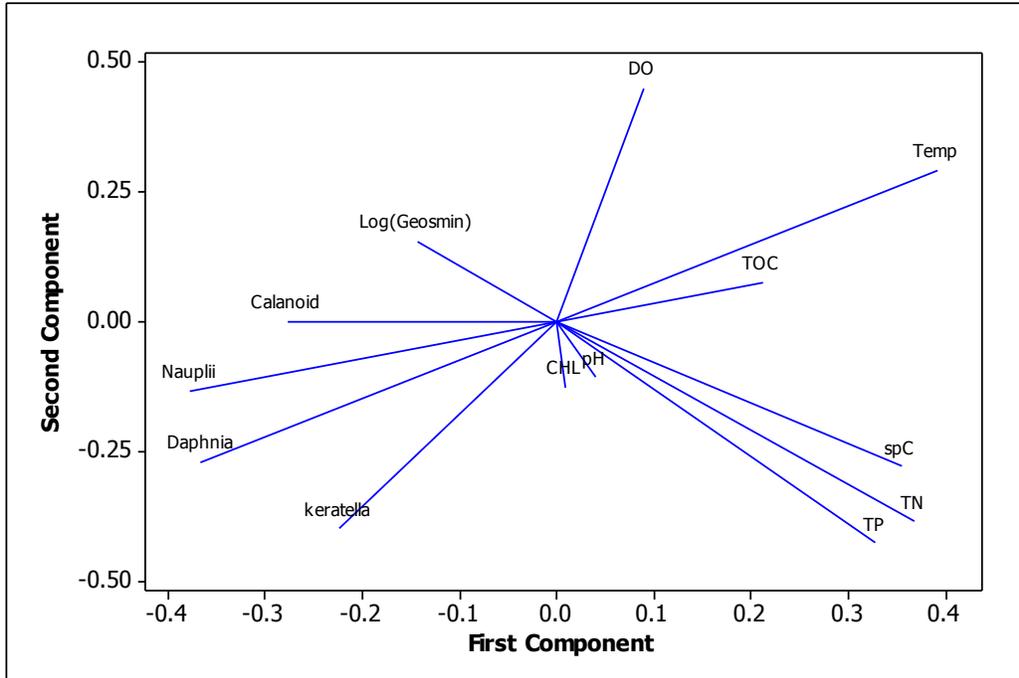


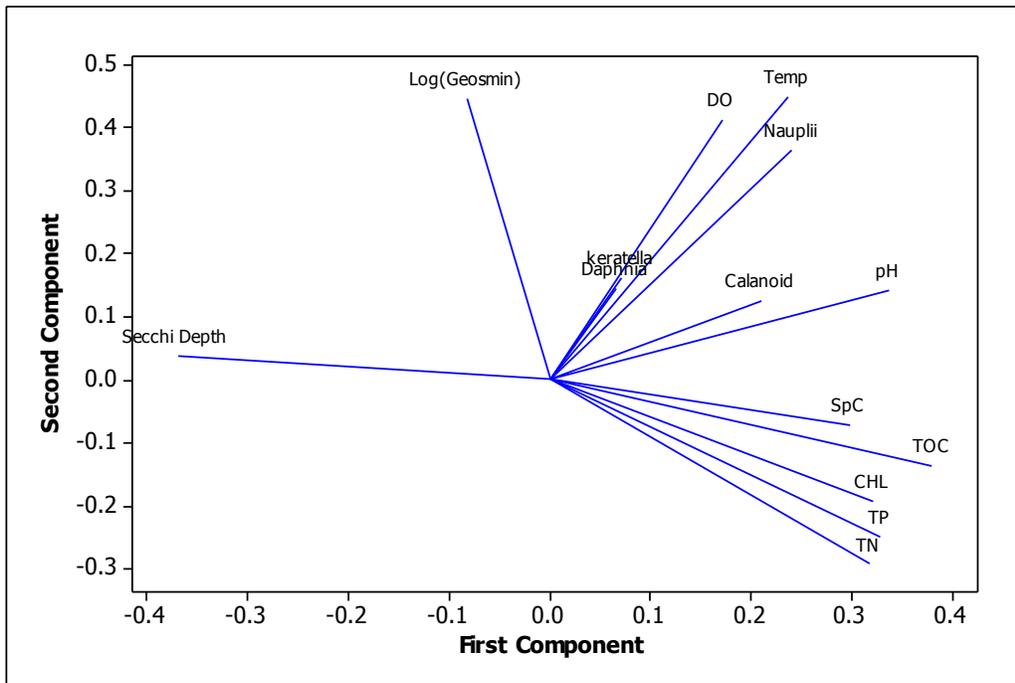
Figure 16 - PCA Loading Plot of Log(Geos), dissolved oxygen, pH, CHL-a, TOC, TN, TP specific conductivity, and temperature data taken from the Big Thompson River.



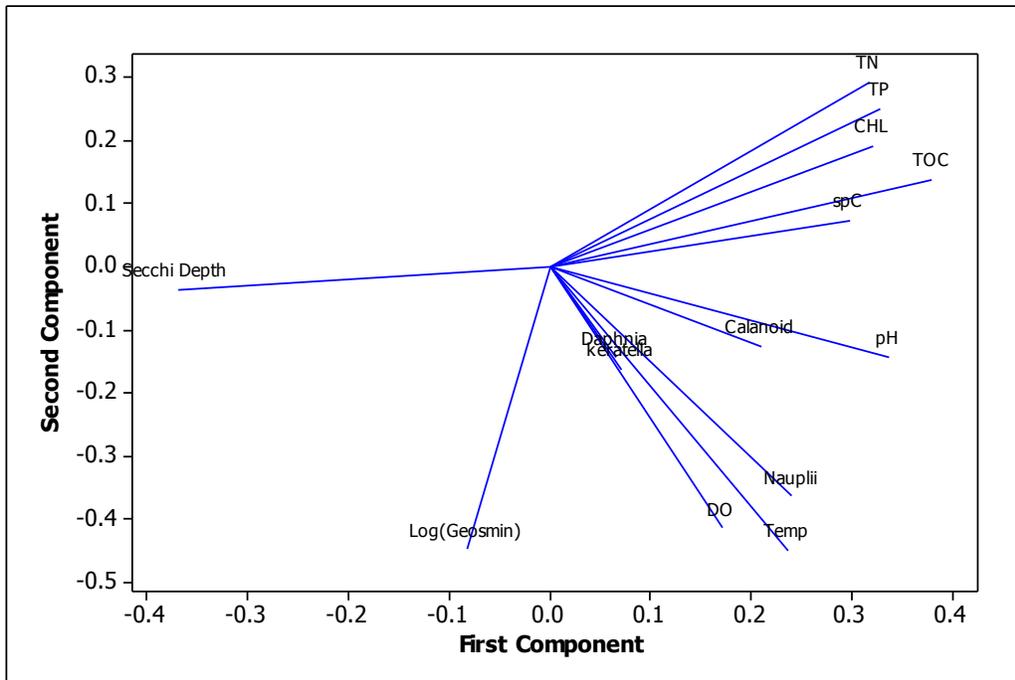
**Figure 17 - PCA Loading Plot of Log(Geosmin), dissolved oxygen, pH, CHL-a, TOC, TN, TP, specific conductivity, temperature, daphnia, keratella, Nauplii, and calanoid data taken from the Poudre River.**



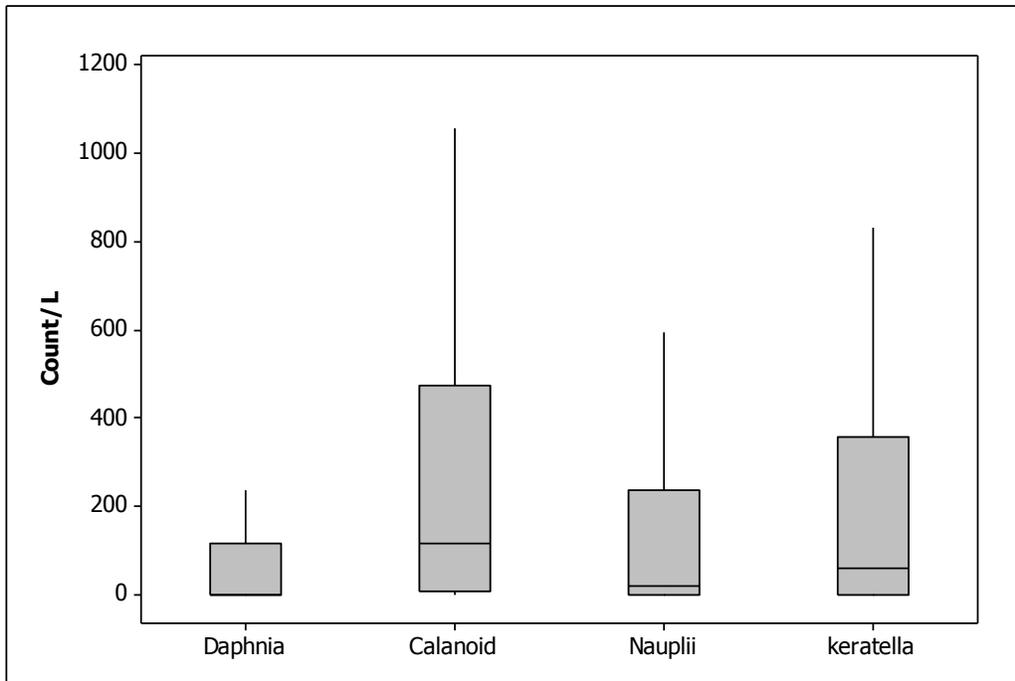
**Figure 18 - PCA Loading Plot - PCA Loading Plot of Log(Geosmin), dissolved oxygen, pH, CHL-a, TOC, TN, TP, specific conductivity, temperature, daphnia, keratella, Nauplii, and calanoid data from rivers sites only.**



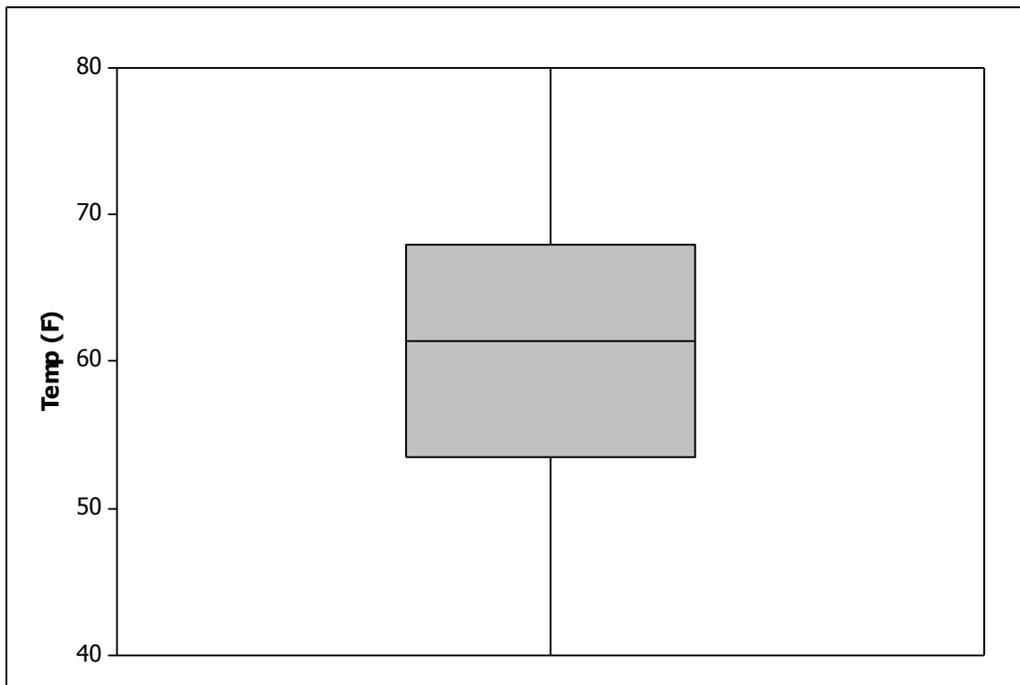
**Figure 19 - PCA Loading Plot of Log(Geos), dissolved oxygen, pH, CHL-a, TOC, TN, TP, specific conductivity, temperature, daphnia, keratella, Nauplii, and calanoid data from only the study lakes and reservoirs.**



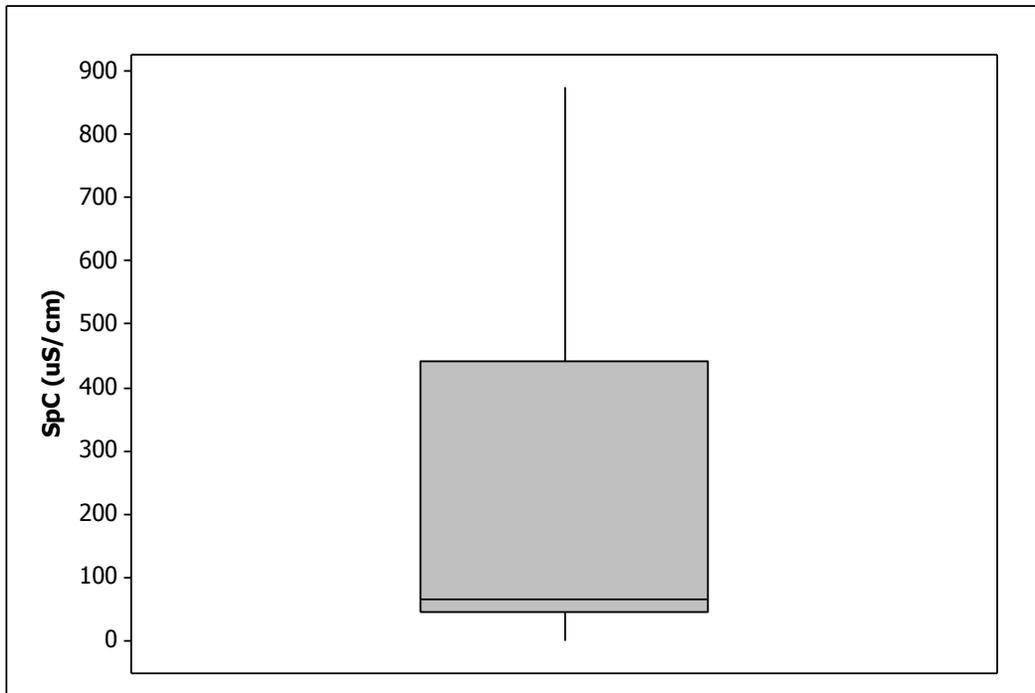
**Figure 20 - PCA Loading Plot of Log(Geos), dissolved oxygen, pH, CHL-a, TOC, TN, TP, specific conductivity, temperature, daphnia, keratella, Nauplii, and calanoid data from all the study including lake, reservoir and river locations.**



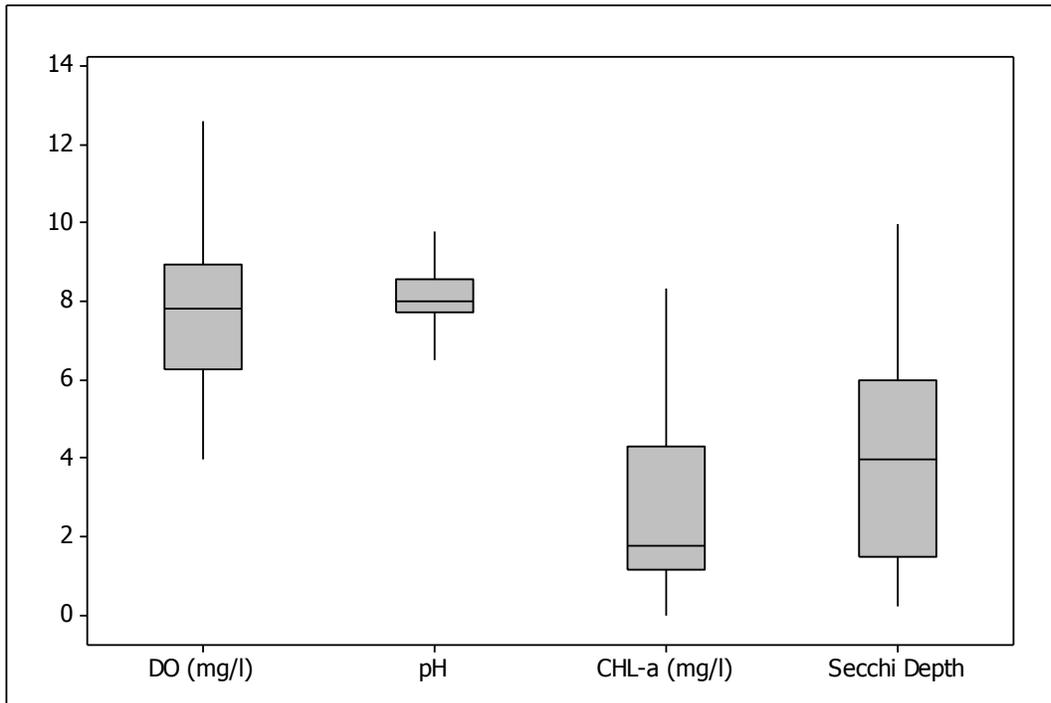
**Figure 21 - Box and Whisker plot of Biotic data from all sampling locations including lakes, reservoirs, and rivers.**



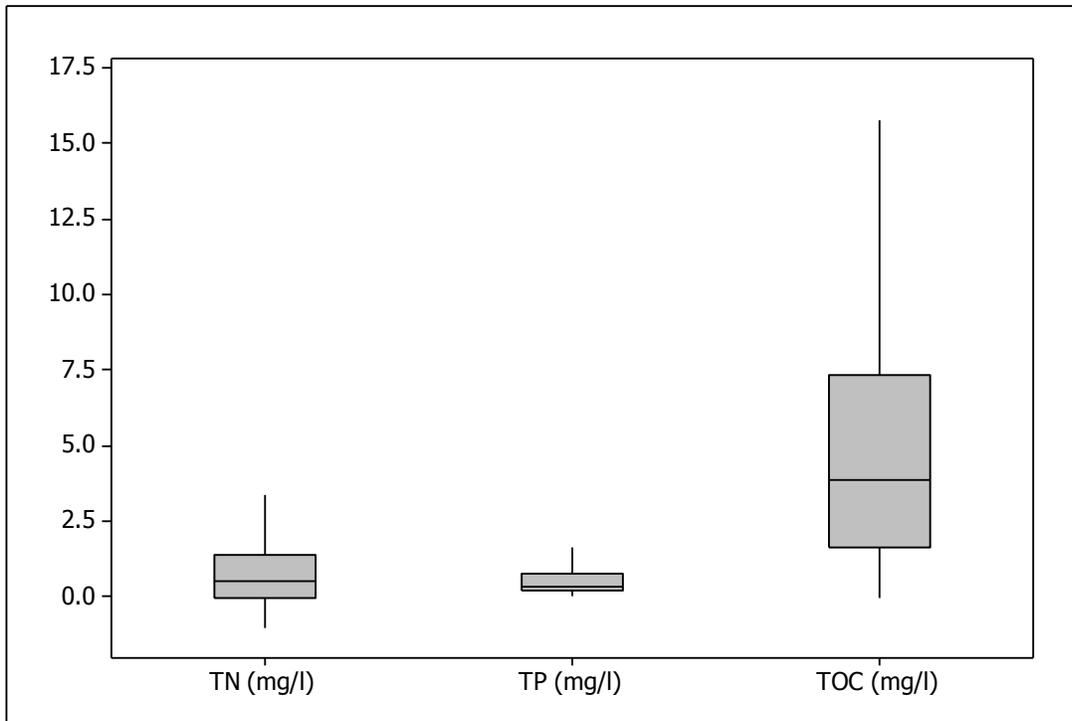
**Figure 22 - Box and Whisker plot of Temperature data from all sampling locations including lakes, reservoirs, and rivers.**



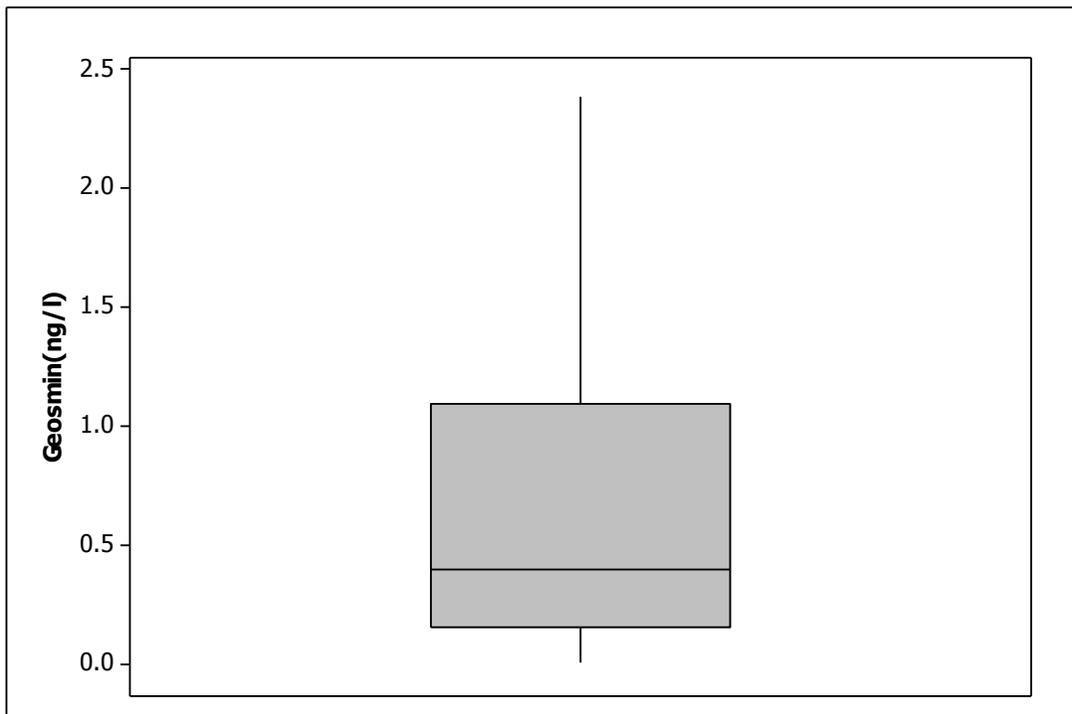
**Figure 23 - Box and Whisker plot of Specific Conductivity data from all sampling locations including lakes, reservoirs, and rivers.**



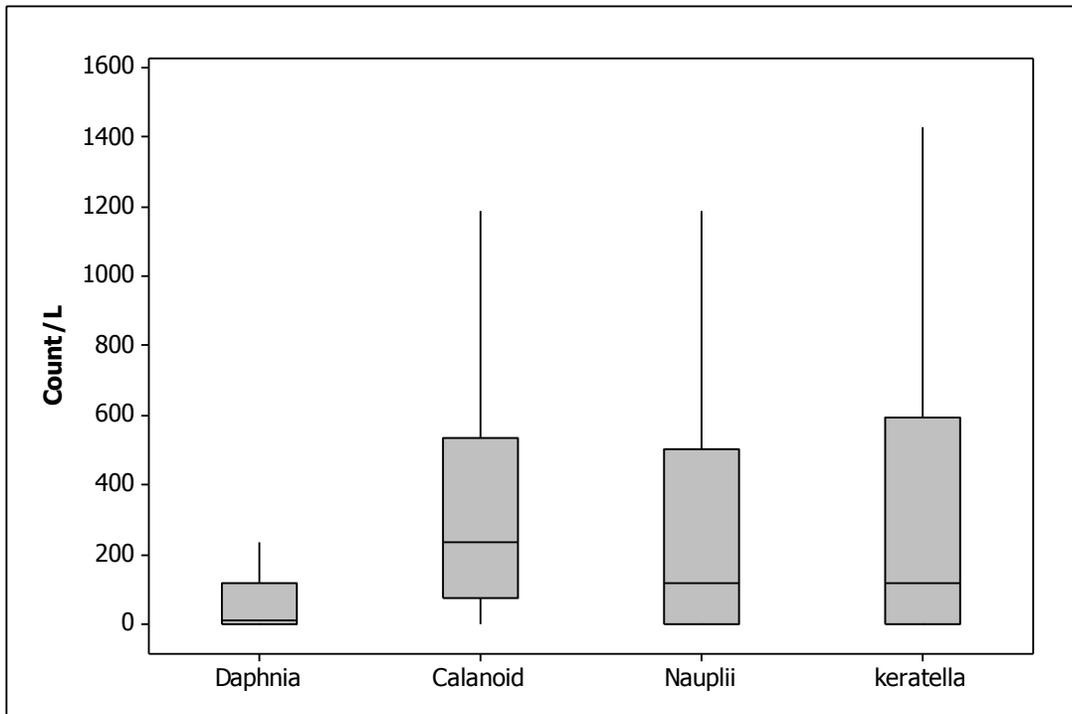
**Figure 24 - Box and Whisker plot of dissolved oxygen, pH, CHL-a, and Secchi depth measurements from all sampling locations including lakes, reservoirs, and rivers.**



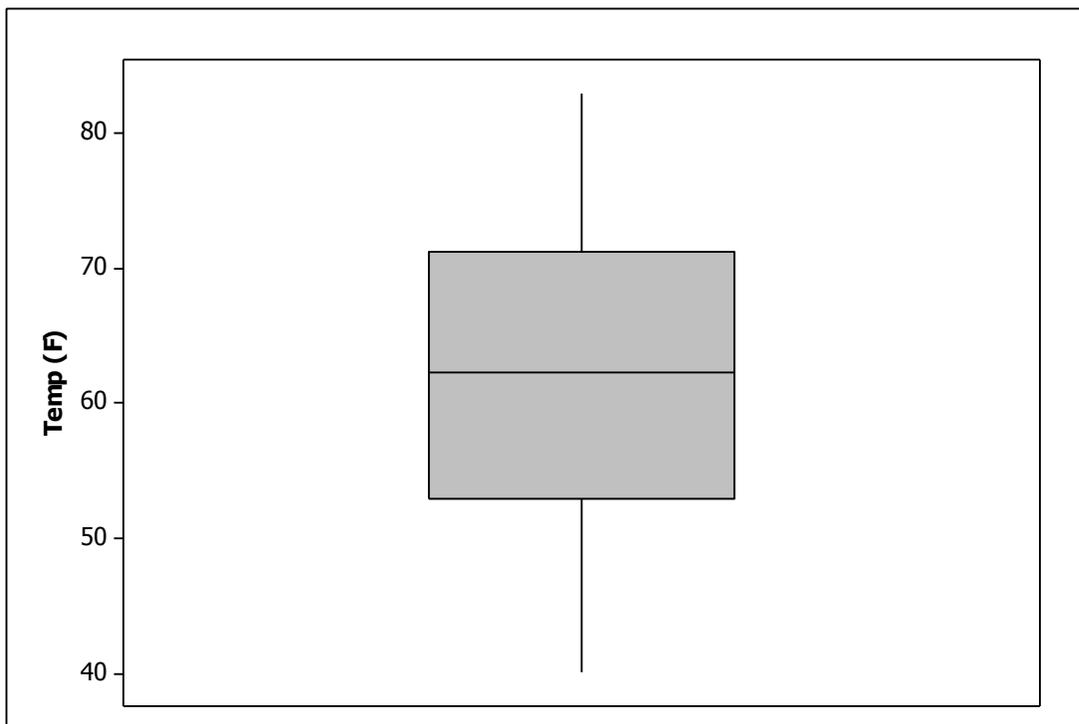
**Figure 25 - Box and Whisker plot of TN, TP, TOC data from all sampling locations including lakes, reservoirs, and rivers.**



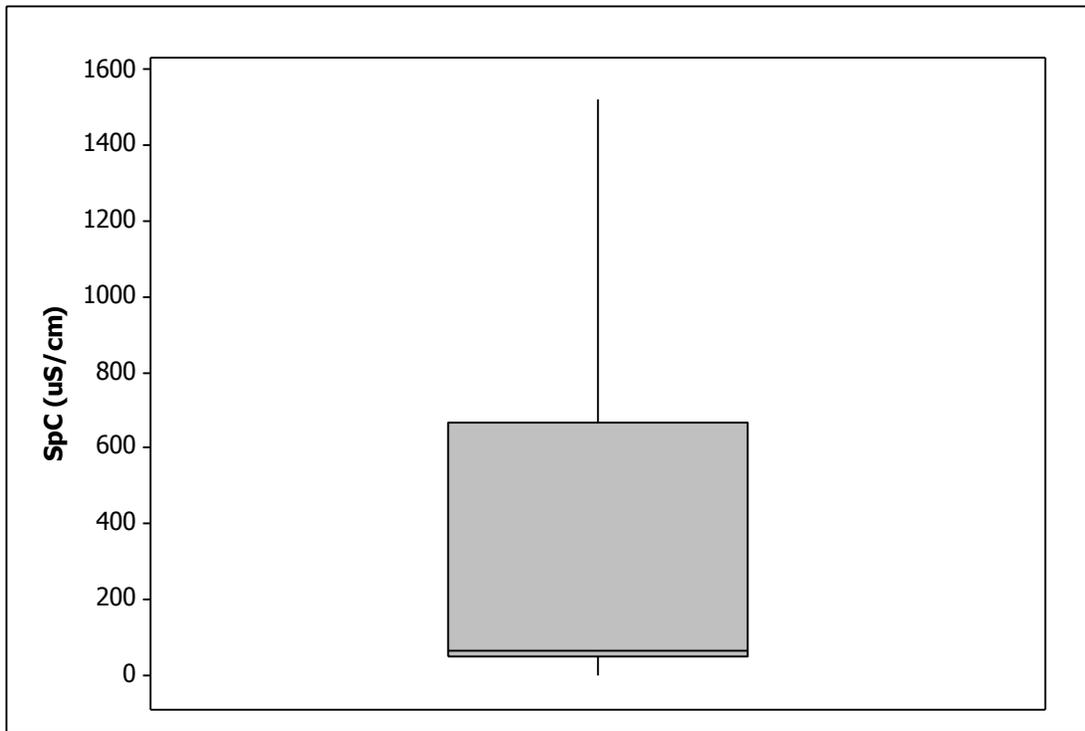
**Figure 26 - Box and whisker plot of Geosmin data from all sampling locations including lakes, reservoirs, and rivers.**



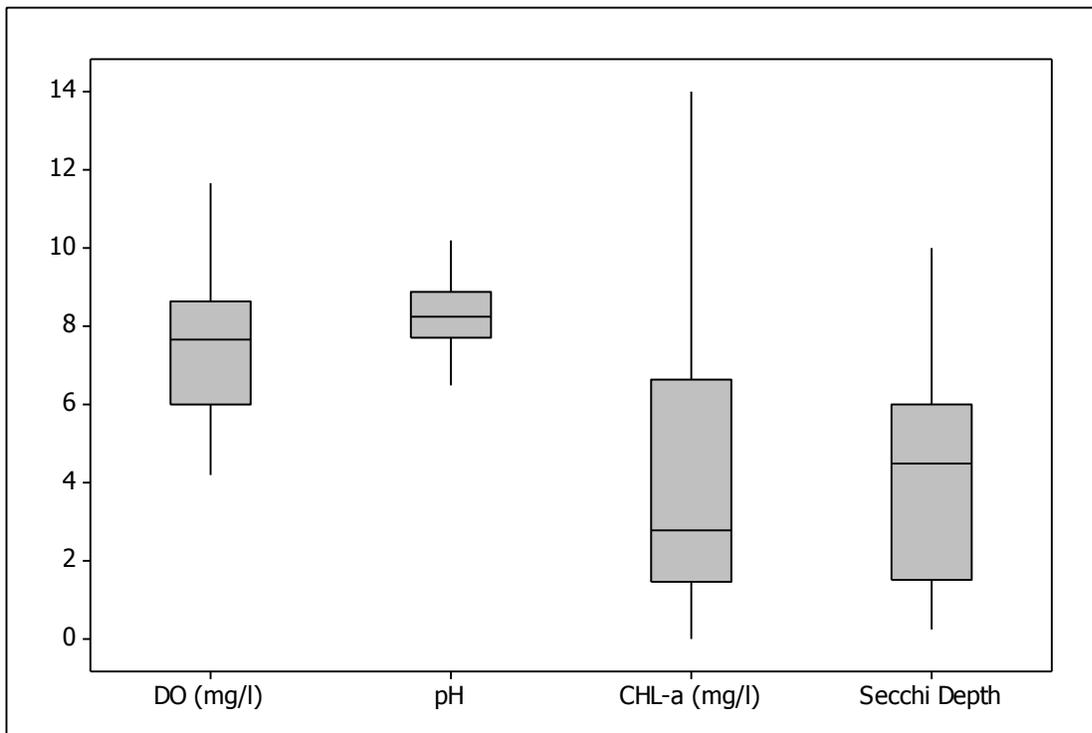
**Figure 27 - Box and Whisker plot of biotic parameters including daphnia, Calanoid, Nauplii, and Keratella from lake and reservoir samples.**



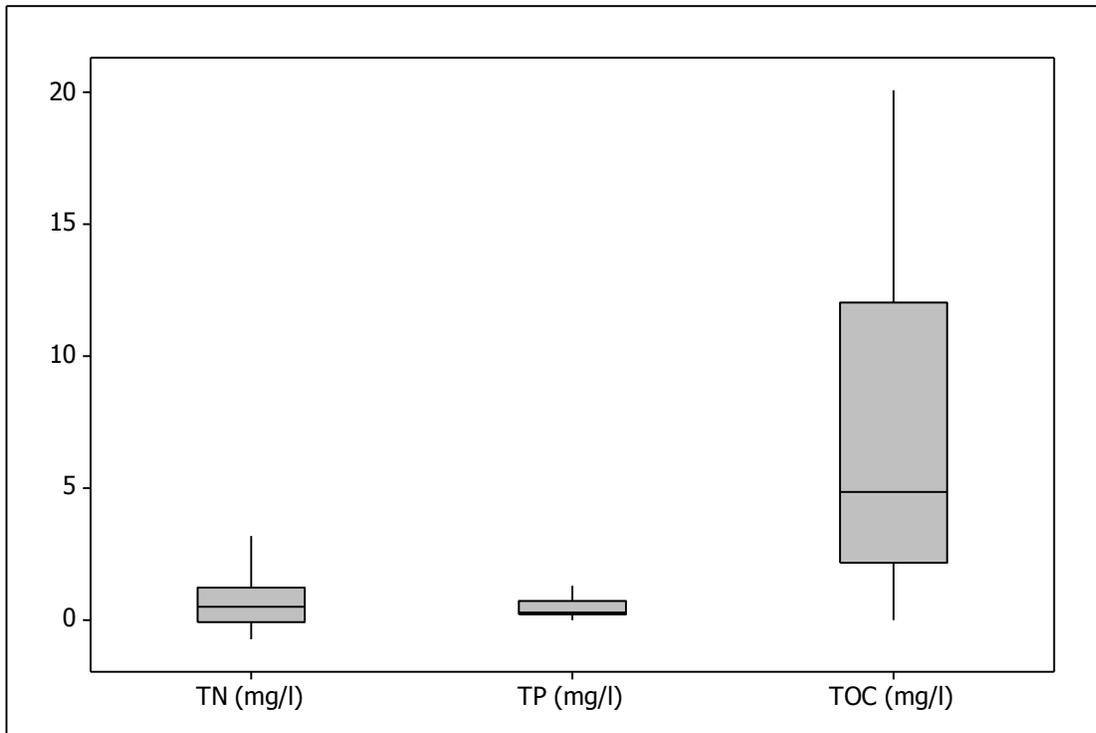
**Figure 28 - Box and Whisker plot of Temperature data from lakes and reservoirs only.**



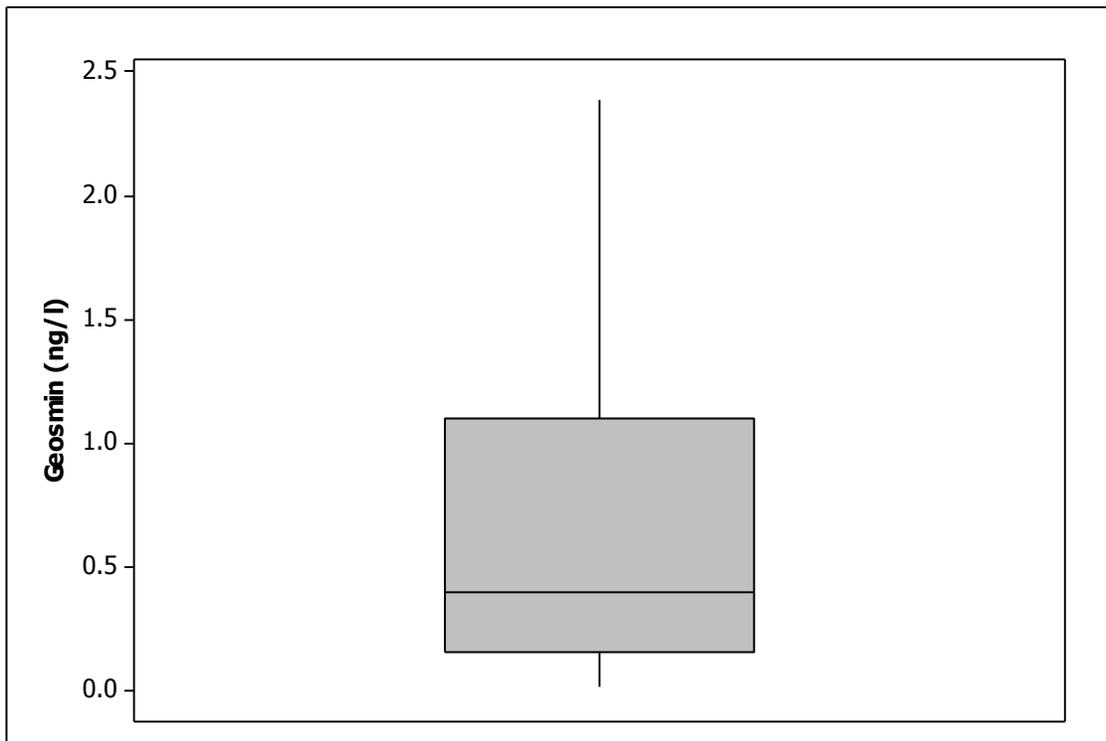
**Figure 29 - Box and Whisker plot of specific conductivity data from lakes and reservoirs only.**



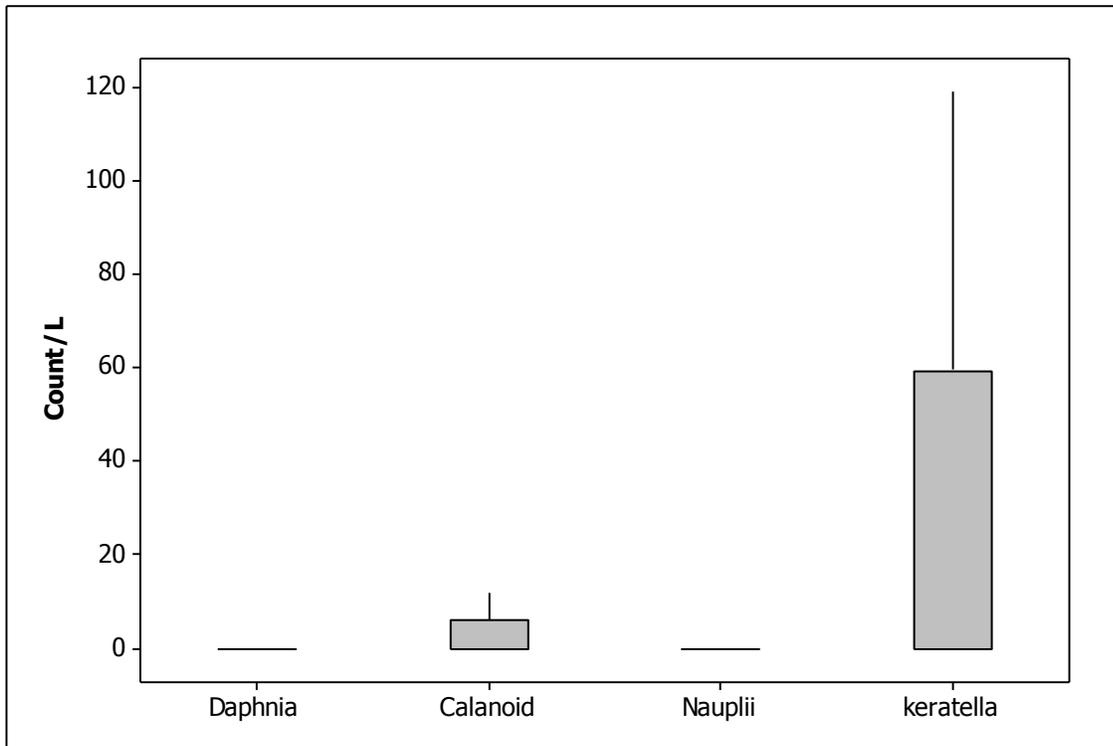
**Figure 30 - Box and Whisker plot of dissolved oxygen, pH, CHL-a, and Secchi depth measurements from lakes and reservoirs.**



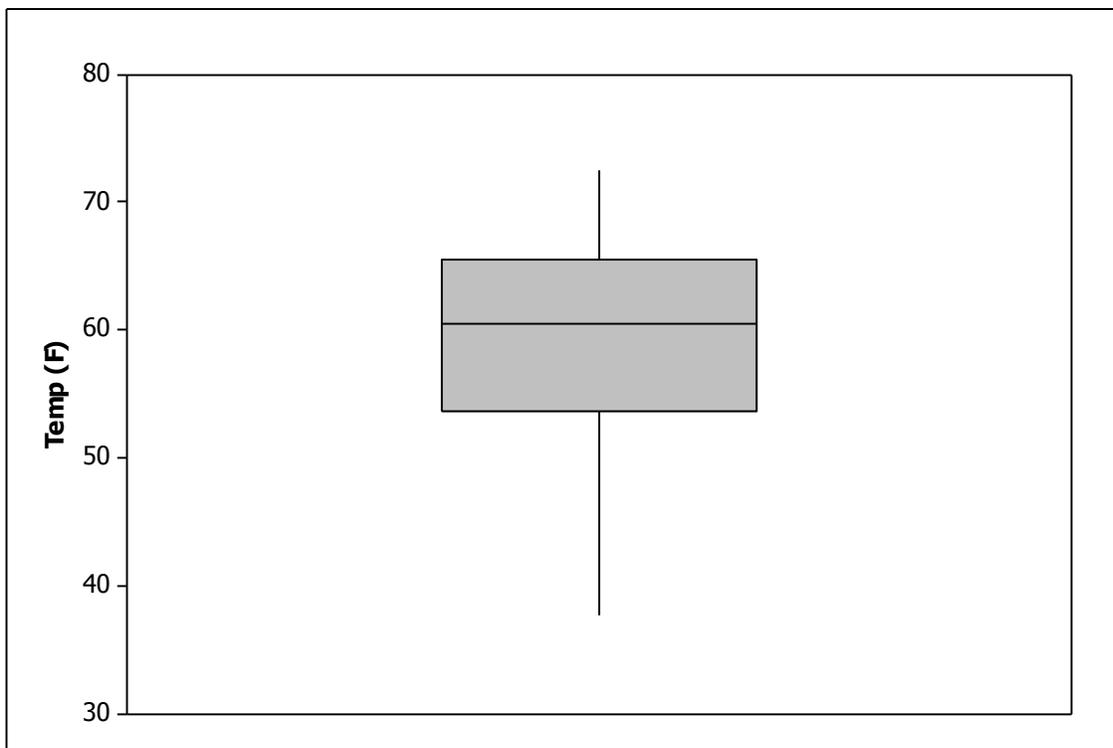
**Figure 31 - Box and Whisker plot of TN, TP, TOC data from lake and reservoirs sites only.**



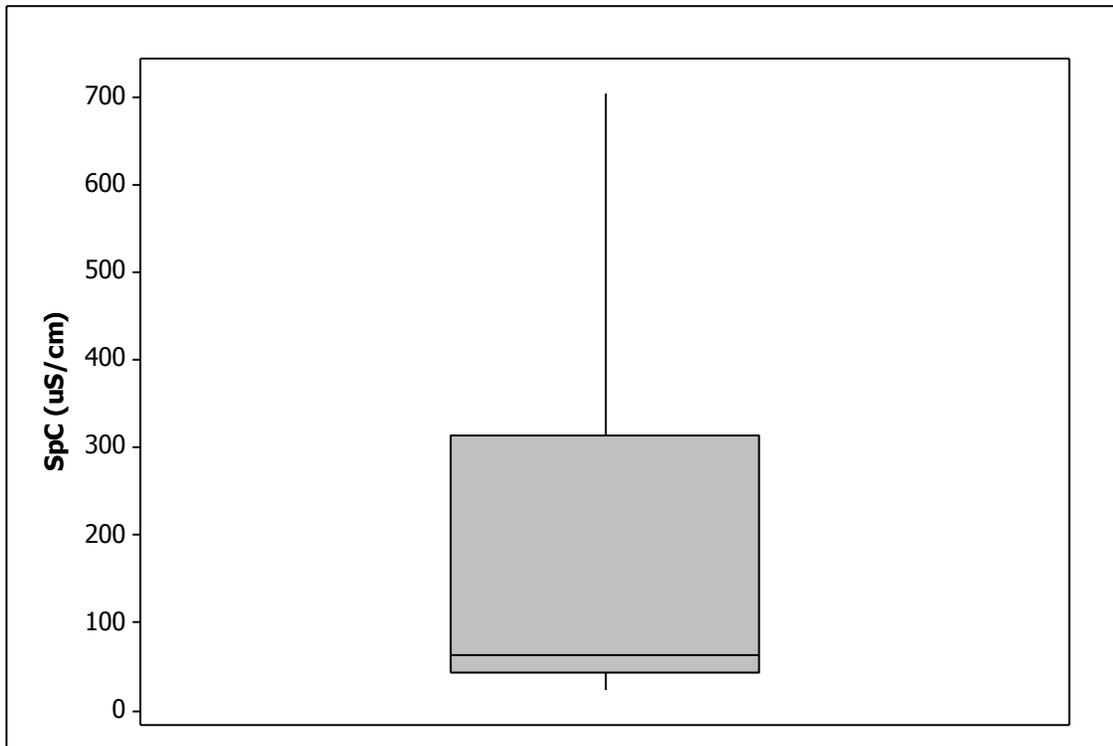
**Figure 32 - Box and whisker plot of Geosmin data from lake and reservoirs sites only.**



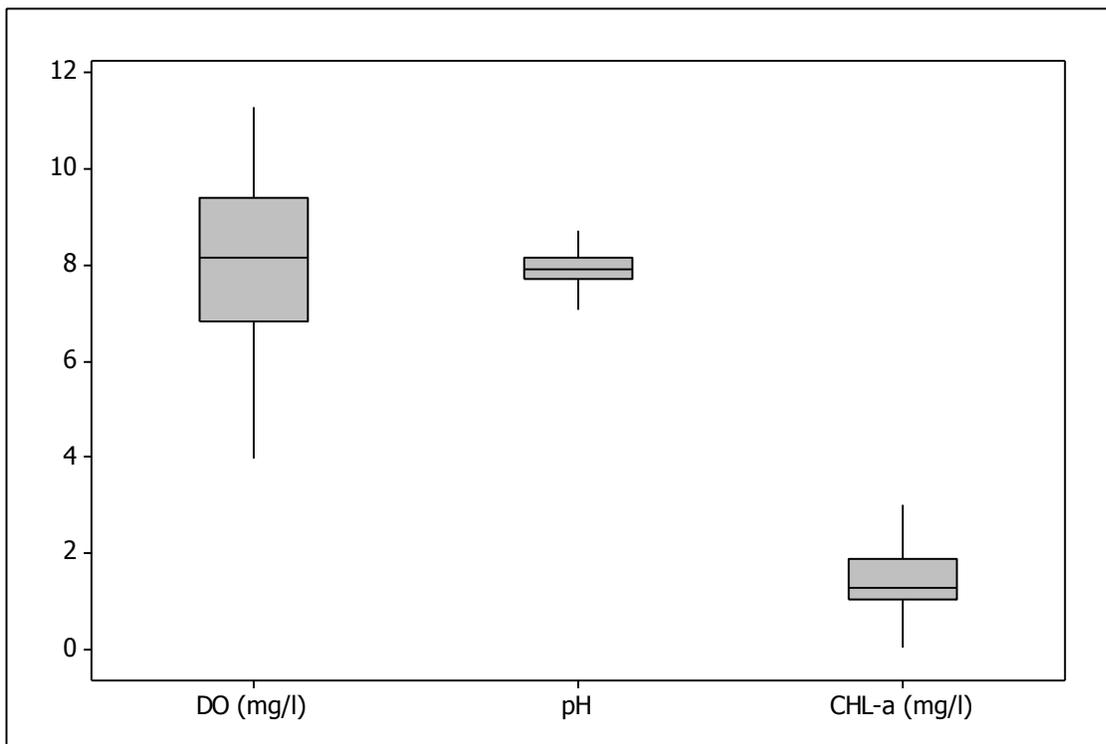
**Figure 33 - Box and Whisker plot of Biotic parameters; daphnia, Calanoid, Nauplii, and Keratella sampled from rivers sites.**



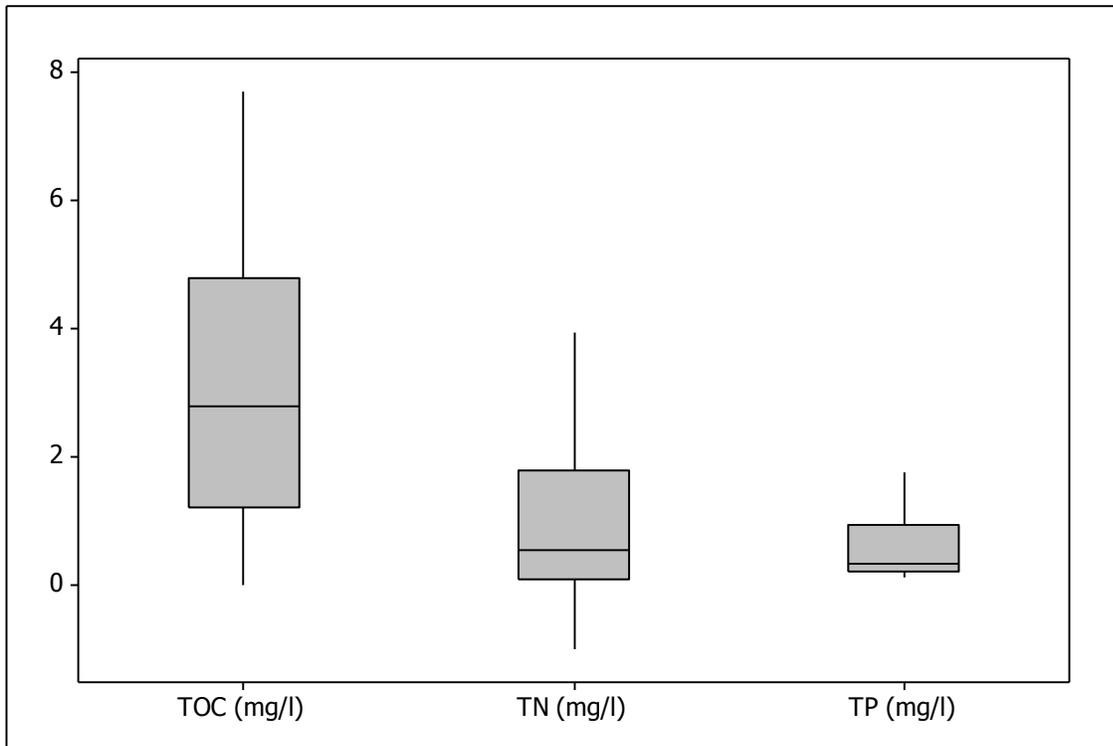
**Figure 34 - Box and Whisker plot of temperature data sampled from rivers sites.**



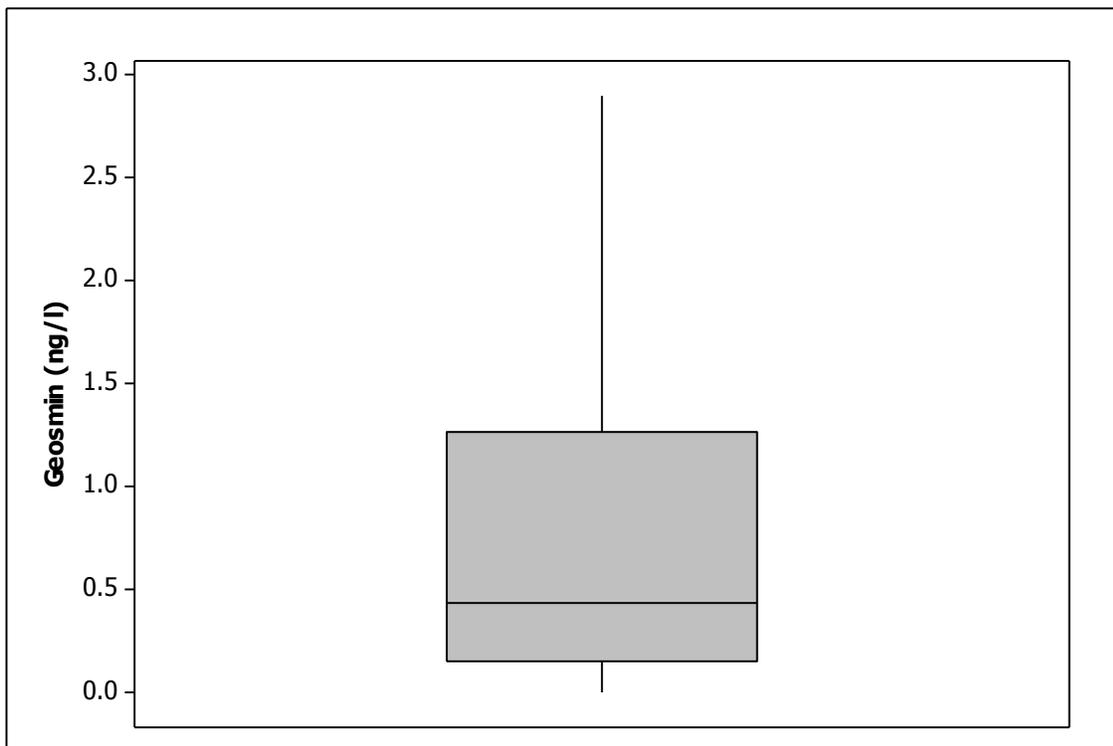
**Figure 35 - Box and Whisker plot of specific conductivity data from river sites.**



**Figure 36 - Box and Whisker plot of dissolved oxygen, pH, and CHL-a data from rivers sites only.**



**Figure 37 - Box and Whisker plot of TN, TP, TOC data from river sites only.**



**Figure 38 - Box and whisker plot of Geosmin data from river sites only.**

**Table 17 - Maximum, Minimum, and Average values for each parameter sampled from each of the Big Thompson River sites.**

LAKE/RESEVOIR SITES	RIVER SITES
ESTES LAKE	BIG THOMPSON @ M40
MARY'S LAKE	BIG THOMPSON @ M90
CHAMBER'S LAKE	BIG THOMPSON @ M130
JOE WRIGHT RES.	BIG THOMPSON @ M140
LOST LAKE	BIG THOMPSON @ M150
BRAINARD LAKE	BIG THOMPSON @ S160
LONG LAKE	POUDRE @ FERN AVE.
RED ROCK LAKE	POUDRE @ O ST.
CARTER LAKE	POUDRE @ HARMONY RD
HORSETOOTH RES.	POUDRE @ DADD GULCH
PINEWOOD RES.	POUDRE @ NARROWS CAMPGROUND
FLATIRON RES.	NF POUUDRE @ GATEWAY
CITY PARK LAKE	SF POUUDRE @ GATEWAY
FOSSIL CR. PARK LAKE	POUDRE @ PICNIC ROCK
LAKE LOVELAND	POUDRE @ BARNES MEADOW
LAKE SHERWOOD	POUDRE @ LINCOLN ST.
BOYD LAKE	POUDRE @ PROSPECT RD.
BARR LAKE	POUDRE @ SHIELDS RD.
JACKSON LAKE	JOE WRIGHT CR
RIVERSIDE RES.	S PLATTE @ POUUDRE RIVER

**Table 18 - Maximum, Minimum, and Average values for each parameter sampled from each of the Big Thompson River sites.**

	M40	S160	M140	M150	
DAPHNIA	0	0	0	0	MIN
	0	0	0	0	MAX
	0	0	0	0	AVE
CALANOIDS	0	0	0	0	MIN
	12	0	0	39	MAX
	1	0	0	5	AVE
NAUPLII	0	0	0	0	MIN
	0	0	0	0	MAX
	0	0	0	0	AVE
KERATELLA	0	0	0	0	MIN
	179	60	0	119	MAX
	30	7	0	20	AVE
TEMP (F)	45.4	42.0	47.9	0.0	MIN
	64.3	67.2	70.9	72.4	MAX
	55.7	55.1	62.8	50.9	AVE
DISSOLVED OXYGEN (MG/L)	5.26	4.68	3.98	0.00	MIN
	9.13	9.78	11.40	12.10	MAX
	7.37	7.98	8.05	6.52	AVE
PH	7.16	7.33	7.50	0.00	MIN
	9.06	8.27	8.34	8.59	MAX
	8.06	7.70	7.86	6.80	AVE
CHL-A (MG/L)	1.22	0.86	1.00	0.00	MIN
	11.17	2.00	7.20	7.60	MAX
	3.94	1.22	2.32	2.51	AVE
SPECIFIC CONDUCTIVITY (US/CM)	25	25	290	0	MIN
	50	58	1032	1065	MAX
	39	45	552	595	AVE
TOTAL ORGANIC CARBON (MG/L)	1.70	0.00	1.20	2.00	MIN
	3.20	2.30	13.20	9.00	MAX
	2.55	0.87	6.16	5.20	AVE
GEOSMIN (UG/L)	0.06	0.00	0.00	0.00	MIN
	0.40	0.94	3.68	0.18	MAX
	0.21	0.39	0.70	0.09	AVE
TOTAL NITROGEN (MG/L)	0.00	0.00	0.45	0.00	MIN
	0.85	0.75	12.15	11.45	MAX
	0.34	0.37	3.91	3.73	AVE
TOTAL PHOSPHORUS (MG/L)	0.16	0.10	0.92	0.73	MIN
	0.32	0.73	4.00	4.00	MAX
	0.25	0.34	2.21	2.04	AVE

**Table 19 - Maximum, Minimum, and Average values for each parameter sampled from each of the Cache la Poudre River sites.**

	Shields	Lincoln	Harmony	O St.	Fern	Barnes Meadow	South Fork	Picnic Rock	Narrows	Dadd Gulch	North Fork	Joe Wright Cr	
Daphnia	0	0	0	0	0	0	0	0	0	0	0	0	Min
	0	0	0	0	0	39	0	12	0	0	12	0	Max
	0	0	0	0	0	10	0	2	0	0	2	0	Ave
Calanoids	0	0	0	0	0	0	0	0	0	0	0	0	Min
	0	0	0	0	0	238	0	60	0	0	119	0	Max
	0	0	0	0	0	69	0	10	0	0	20	0	Ave
Nauplii	0	0	0	0	0	0	0	0	0	0	0	0	Min
	0	0	0	0	1	119	0	0	12	0	119	0	Max
	0	0	0	0	1	60	0	0	3	0	20	0	Ave
Keratella	0	60	60	40	0	0	0	0	0	0	0	0	Min
	0	60	60	40	0	238	0	0	0	0	0	0	Max
	0	60	60	40	0	60	0	0	0	0	0	0	Ave
Temp (F)	55.5	55.7	60.7	70.0	60.7	41.9	44.5	45.6	42.6	42.8	49.2	37.7	Min
	62.7	64.7	65.7	72.6	71.2	70.4	65.7	66.7	62.7	62.1	65.4	63.2	Max
	59.1	60.6	63.2	71.3	66.2	55.0	53.8	56.5	52.9	51.8	58.5	49.0	Ave
Dissolved Oxygen (mg/l)	5.99	9.39	6.95	7.82	9.40	5.41	5.75	5.90	5.85	5.84	4.82	5.70	Min
	9.49	11.29	10.02	11.22	11.00	8.98	10.00	10.45	9.76	9.90	9.40	9.15	Max
	7.74	10.08	8.49	9.52	10.13	7.85	8.26	8.17	8.07	8.63	7.34	7.60	Ave
pH	7.47	7.66	8.08	8.27	7.80	7.31	7.25	7.13	7.31	7.15	7.05	7.02	Min
	7.86	8.64	8.16	8.48	8.20	7.97	8.36	8.20	8.08	8.74	8.10	7.78	Max
	7.67	7.99	8.12	8.38	8.06	7.66	7.71	7.69	7.77	7.92	7.76	7.56	Ave

**Table 20 - Continued**

	Shields	Lincoln	Harmony	O St.	Fern	Barnes Meadow	South Fork	Picnic Rock	Narrows	Dadd Gulch	North Fork	Joe Wright Cr	
CHL-a (mg/l)	1.53	1.00	1.32	3.22	2.23	1.29	0.72	1.11	0.46	0.56	0.94	0.44	Min
	2.00	1.20	1.66	9.97	6.00	2.56	2.03	3.00	1.06	2.00	3.02	1.04	Max
	1.77	1.12	1.49	6.60	4.25	1.77	1.44	1.64	0.81	1.20	1.99	0.67	Ave
Specific Conductivity (uS/cm)	61	62	188	455	1200	38	33	33	31	31	29	35	Min
	226	233	758	826	1264	46	78	105	60	72	289	68	Max
	143	152	473	640	1226	41	57	71	49	47	171	56	Ave
Total Organic Carbon (mg/l)	0.00	0.70	2.10	4.80	2.80	0.40	0.00	0.00	0.00	0.00	0.00	0.80	Min
	4.60	3.90	2.40	5.60	3.90	5.60	18.50	19.70	5.80	16.80	10.80	4.80	Max
	2.30	2.15	2.25	5.20	3.33	2.93	5.80	6.43	2.20	4.35	5.18	2.35	Ave
Geosmin (ug/l)	0.28	0.22	0.35	0.16	0.71	0.13	0.00	0.00	0.13	0.00	0.00	0.03	Min
	2.89	18.90	19.95	0.63	1.28	1.83	1.55	3.10	1.63	2.57	4.24	2.85	Max
	1.30	4.38	7.62	0.40	0.98	0.61	0.39	1.37	0.95	0.70	0.81	0.78	Ave
Total Nitrogen (mg/l)	0.30	0.00	0.35	1.80	5.40	0.00	0.00	0.00	0.00	0.00	0.35	0.00	Min
	1.45	1.05	3.10	4.75	6.65	0.85	3.95	16.15	0.90	1.19	2.60	1.00	Max
	0.88	0.61	1.73	3.28	5.87	0.00	1.28	3.46	0.30	0.27	1.27	0.40	Ave
Total Phosphorus (mg/l)	0.20	0.22	0.32	0.81	0.87	0.15	0.14	0.11	0.20	0.15	0.31	0.14	Min
	0.72	0.48	0.96	1.06	1.65	0.50	2.92	4.00	0.48	0.81	2.16	0.31	Max
	0.46	0.35	0.64	0.94	1.21	0.25	0.92	0.96	0.30	0.30	0.76	0.22	Ave

**Table 21 - Maximum, Minimum, and Average values for each parameter sampled from each of the Lake and Reservoir sites.**

	Brainard	Barr Lake	Boyd Lake	Carter Lake	Chambers Lake	City Park Lake	Lake Estes	Flat Iron Reservoir	Fossil Creek Park Lake	Horseshoe Reservoir	
Daphnia	0	476	0	0	0	0	0	0	0	0	Min
	32	3510	198	1785	0	5320	238	952	238	1190	Max
	8	1839	79	449	0	914	42	248	79	329	Ave
Calanoids	39	540	39	119	60	0	0	0	119	40	Min
	238	8470	1386	833	595	3600	238	119	714	833	Max
	132	2251	502	446	417	1199	99	45	381	389	Ave
Nauplii	0	0	30	0	0	0	0	0	119	0	Min
	238	2496	4158	476	900	8640	119	119	9792	833	Max
	68	842	1095	119	240	1736	20	48	3470	268	Ave
Keratella	0	0	119	0	0	0	0	119	0	0	Min
	3213	963	5544	595	2880	51840	1190	952	14400	357	Max
	959	599	1466	152	790	7635	260	357	3094	126	Ave
Temp (F)	47.7	51.0	51.2	55.4	42.1	50.9	0.0	44.9	49.7	51.3	Min
	58.4	79.0	79.1	72.9	64.3	80.9	64.8	65.5	77.6	75.6	Max
	52.7	66.0	65.7	63.9	53.6	66.1	44.7	55.4	68.2	61.3	Ave
Dissolved Oxygen (mg/l)	6.86	4.75	4.68	4.20	5.22	4.77	0.00	5.18	5.05	4.20	Min
	9.38	15.50	9.34	7.71	10.60	8.75	10.50	8.24	15.04	9.60	Max
	8.00	8.93	7.73	6.02	8.24	7.35	6.23	6.41	9.80	7.11	Ave
pH	7.49	8.30	8.20	7.50	7.30	8.02	0.00	7.55	8.50	7.20	Min
	8.96	9.30	8.63	7.81	8.26	9.32	8.42	8.08	10.12	8.02	Max
	7.90	8.84	8.41	7.70	7.77	8.60	6.35	7.76	9.29	7.61	Ave
CHL-a (mg/l)	0.59	0.60	0.56	0.49	1.06	5.00	0.00	1.46	1.80	0.72	Min
	1.30	38.50	4.15	1.62	2.78	18.62	3.65	5.40	6.21	1.74	Max
	0.98	9.93	2.09	1.08	1.76	11.54	1.64	4.12	3.40	1.25	Ave

**Table 22 - Continued**

	Jackson Lake	Joe Wright Reservoir	Loveland Lake	Long Lake	Lost Lake	Mary's Lake	Pinewood Reservoir	Red Rock Lake	Riverside Reservoir	Shadow Min Res.	Sherwood Lake	
	0	60	0	0	0	0	0	12	0	0	0	Min
Daphnia	960	119	2142	0	476	12	1666	238	3094	2160	119	Max
	181	99	427	0	198	3	268	107	1031	580	20	Ave
	60	238	0	0	0	12	0	12	119	0	0	Min
Calanoids	952	714	357	119	238	476	357	238	4760	119	476	Max
	391	476	115	45	99	196	96	107	2340	33	238	Ave
	0	238	0	0	0	0	0	0	0	0	60	Min
Nauplii	1428	1428	14400	238	238	119	119	238	952	1296	3600	Max
	443	754	2060	89	79	20	37	119	337	324	1252	Ave
	0	0	0	0	0	12	0	0	0	0	0	Min
Keratella	34200	357	20160	119	595	1309	357	1071	0	833	8280	Max
	6521	198	2877	60	278	479	134	536	0	208	1420	Ave
	47.0	42.1	51.2	47.4	40.2	44.6	44.7	48.1	59.5	50.9	52.9	Min
Temp (F)	81.6	61.8	82.9	57.8	68.5	61.1	64.8	65.5	77.1	63.4	81.6	Max
	70.0	53.5	68.8	53.0	58.4	54.2	55.4	56.2	70.8	56.7	74.3	Ave
	6.42	5.18	5.14	5.97	5.33	4.78	4.71	5.62	4.33	7.31	6.16	Min
Dissolved Oxygen (mg/l)	12.60	6.76	10.71	8.95	8.60	8.94	13.00	7.32	6.79	9.00	10.60	Max
	8.77	6.14	8.68	7.73	6.44	7.39	8.74	6.47	5.89	7.98	8.23	Ave
	8.48	7.81	8.39	7.07	7.10	6.50	6.60	6.73	8.47	6.90	8.21	Min
pH	9.78	8.19	9.75	7.72	8.26	8.15	8.46	7.34	8.90	7.84	9.39	Max
	8.91	7.94	9.11	7.37	7.72	7.56	7.65	7.05	8.63	7.50	8.80	Ave
	5.80	1.20	1.36	0.58	2.75	0.00	1.10	1.56	2.11	1.14	3.00	Min
CHL-a (mg/l)	20.44	1.75	7.75	2.36	7.52	2.00	3.73	17.11	26.45	4.34	16.24	Max
	13.80	1.41	4.35	1.44	5.16	1.34	2.54	8.91	13.52	2.42	7.67	Ave

**Table 23 - Continued**

	Brainard	Barr Lake	Boyd Lake	Carter Lake	Chambers Lake	City Park Lake	Lake Estes	Flat Iron Reservoir	Fossil Creek Park Lake	Horseshoe Reservoir	
Specific Conductivity (uS/cm)	12	800	557	57	28	200	0	30	1861	55	Min
	27	1309	674	67	46	366	52	50	3748	71	Max
	19	974	627	60	38	281	35	45	2701	65	Ave
Total Organic Carbon (mg/l)	1.00	10.60	4.80	0.90	0.30	0.00	0.00	0.60	2.40	1.30	Min
	13.60	19.60	10.70	4.80	16.70	9.20	6.90	3.60	12.60	9.00	Max
	4.63	14.88	6.02	2.75	5.46	3.80	2.15	2.35	6.15	3.32	Ave
Geosmin (ug/l)	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.08	0.33	0.00	Min
	0.92	2.70	3.50	5.98	0.18	1.86	0.34	0.72	1.78	37.10	Max
	0.26	0.87	1.08	1.76	0.10	0.94	0.14	0.30	1.02	5.53	Ave
Total Nitrogen (mg/l)	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Min
	1.20	2.90	1.25	0.95	1.30	1.40	0.00	1.50	1.90	0.40	Max
	0.57	1.71	0.22	0.46	0.43	0.78	0.00	0.40	0.55	0.10	Ave
Total Phosphorus (mg/l)	0.07	0.05	0.02	0.11	0.03	0.31	0.00	0.10	0.19	0.04	Min
	0.70	1.33	1.49	0.57	0.74	0.65	0.31	0.49	2.16	0.41	Max
	0.36	0.93	0.52	0.27	0.31	0.44	0.18	0.33	0.75	0.19	Ave
Secchi Depth (ft)	0.0	0.5	3.0	5.0	9.0	1.0	0.0	4.5	4.0	5.0	Min
	0.0	10.0	6.0	10.0	9.0	2.0	7.0	6.0	6.0	6.0	Max
	0.0	4.4	4.8	7.3	9.0	1.5	5.0	5.5	5.1	5.6	Ave

**Table 24 - Continued**

	Jackson Lake	Joe Wright Reservoir	Lake Loveland	Long Lake	Lost Lake	Mary's Lake	Pinewood Reservoir	Red Rock Lake	Riverside Reservoir	Shadow Mtn Res.	Sherwood Lake	
Specific Conductivity (uS/cm)	1480	41	156	18	51	31	16	35	1375	31	344	Min
	2632	45	285	25	69	53	59	48	1407	55	433	Max
	1885	43	220	20	60	45	39	41	1388	43	385	Ave
Total Organic Carbon (mg/l)	11.60	0.00	1.50	0.00	7.90	0.80	0.40	0.80	11.70	0.00	7.50	Min
	20.10	5.80	20.00	14.80	15.10	3.20	9.00	14.20	14.50	10.90	18.20	Max
	16.62	2.37	7.59	4.35	12.30	1.85	3.18	10.18	12.73	4.30	12.38	Ave
Geosmin (ug/l)	0.10	0.16	0.00	0.00	0.03	0.13	0.00	0.00	0.10	0.00	0.00	Min
	3.40	2.59	7.40	0.40	3.30	1.60	1.10	0.44	0.30	0.90	7.66	Max
	1.07	1.05	1.14	0.23	1.63	0.68	0.22	0.16	0.18	0.34	1.58	Ave
Total Nitrogen (mg/l)	1.00	0.00	0.00	0.00	0.40	0.00	0.00	0.95	1.95	0.00	0.00	Min
	7.30	1.70	1.45	0.70	1.18	0.35	1.91	2.67	6.55	1.00	1.42	Max
	3.47	0.60	0.67	0.34	0.82	0.09	0.46	1.81	3.50	0.26	0.68	Ave
Total Phosphorus (mg/l)	0.09	0.15	0.02	0.09	0.04	0.15	0.22	0.06	1.12	0.02	0.06	Min
	2.71	1.18	1.19	0.37	0.41	0.52	0.39	0.84	2.36	0.87	0.60	Max
	1.21	0.71	0.38	0.23	0.24	0.30	0.26	0.44	1.84	0.28	0.39	Ave
Secchi Depth (ft)	0.5	4.0	0.5	0.0	6.0	4.0	4.0	1.0	0.3	4.0	0.5	Min
	3.0	4.0	4.0	0.0	6.0	7.0	9.0	2.0	2.5	9.0	3.0	Max
	1.3	4.0	2.3	0.0	6.0	5.7	6.6	1.5	1.2	6.0	1.6	Ave

**Table 25- Sample Dates for All River Sites**

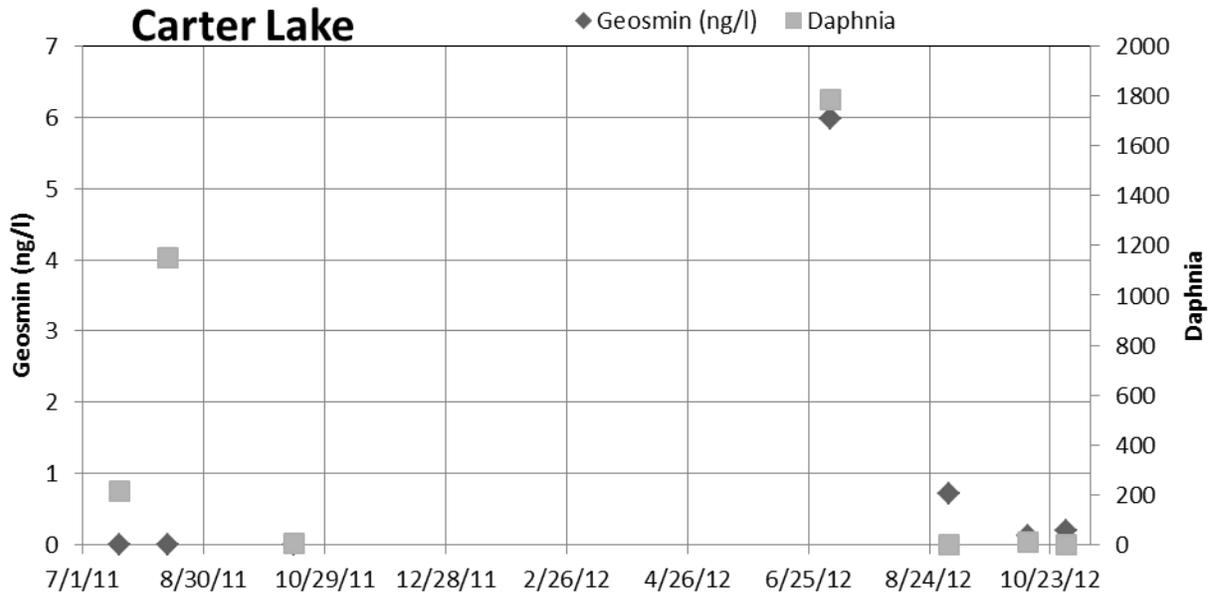
M40	S160	M140	M150	BARNES MEADOW	SOUTH FORK	PICNIC ROCK	NARROWS
11/2/12	11/2/12	11/2/12	11/2/12	10/24/12	10/24/12	10/24/12	10/24/12
9/26/12	9/26/12	9/26/12	9/26/12	9/12/12	9/12/12	9/12/12	9/12/12
8/2/12	8/2/12	8/2/12	8/2/12	7/12/12	7/12/12	7/12/12	7/12/12
6/15/12	6/15/12	6/15/12	6/15/12	5/30/12	5/30/12	5/30/12	5/30/12
	8/11/11	10/27/11	10/27/11		5/27/11	8/15/11	
	5/26/11	8/11/11	8/11/11			5/27/11	
		7/11/11					
DADD GULCH	NORTH FORK	JOE WRIGHT CR.	SHIELDS	LINCOLN	HARMONY	O ST.	FERN
10/24/12	10/24/12	10/24/12	9/19/12	9/19/12	9/19/12	9/19/12	9/19/12
9/12/12	9/12/12	9/12/12	5/31/12	5/31/12	5/31/12	5/31/12	5/31/12
7/12/12	7/12/12	7/12/12	9/29/11	9/29/11	9/29/11		9/24/11
5/30/12	5/30/12	5/30/12		9/24/11			
8/15/11	8/15/11			8/17/11			
5/27/11	5/27/11						

**Table 26 - Sample Dates for All Lakes and Reservoirs**

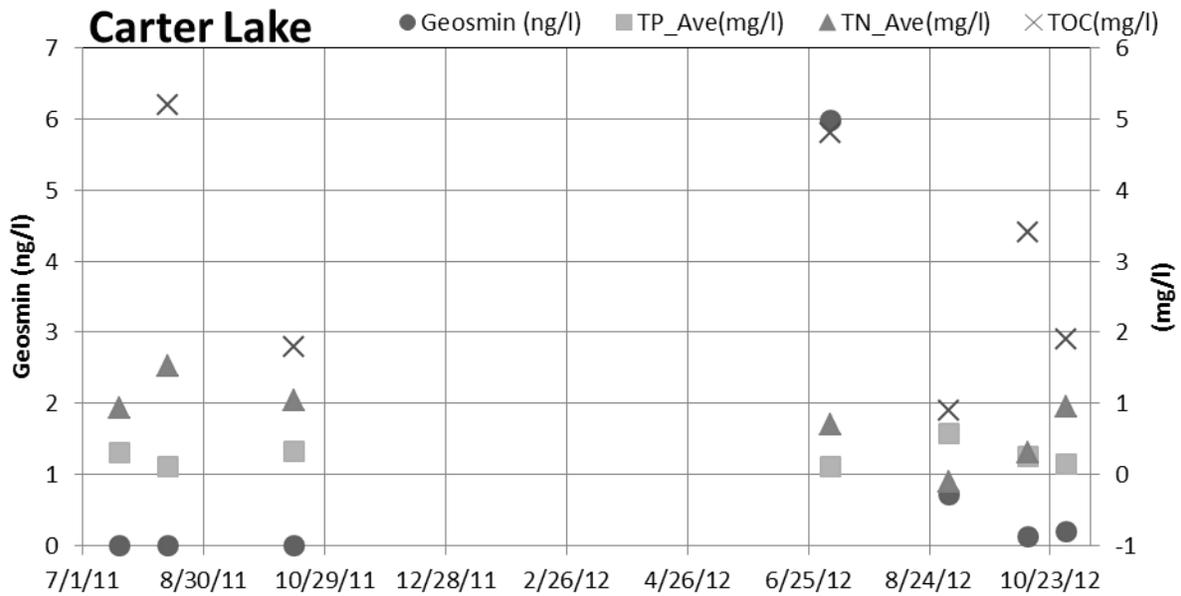
BRAINARD	BARR LAKE	BOYD LAKE	CARTER LAKE	CHAMBERS LAKE	CITY PARK LAKE	LAKE ESTES	FLAT IRON RESERVOIR	FOSSIL CREEK PARK LAKE	HORSETOOTH RESERVOIR	JACKSON LAKE
10/1/12	10/23/12	11/1/12	10/31/12	10/24/12	10/19/12	11/2/12	10/31/12	10/19/12	10/31/12	10/23/12
8/15/12	10/13/11	9/21/12	10/12/12	9/12/12	9/20/12	10/12/12	10/17/12	9/20/12	10/12/12	9/21/12
6/21/12	8/9/11	8/30/12	9/3/12	7/12/12	8/4/12	8/31/12	9/3/12	8/4/12	9/3/12	8/1/12
7/15/11	7/18/11	5/26/12	7/6/12	5/31/12	5/26/12	7/6/12	6/21/12	5/26/12	7/11/12	7/4/12
	6/17/11	10/19/11	10/14/11	8/15/11	10/12/11	6/27/11		8/5/11	10/20/11	10/29/11
		7/18/11	8/12/11		7/22/11	5/26/11		7/22/11	7/27/11	8/16/11
			7/19/11		6/24/11				5/25/11	7/26/11

**Table 27 - Continued**

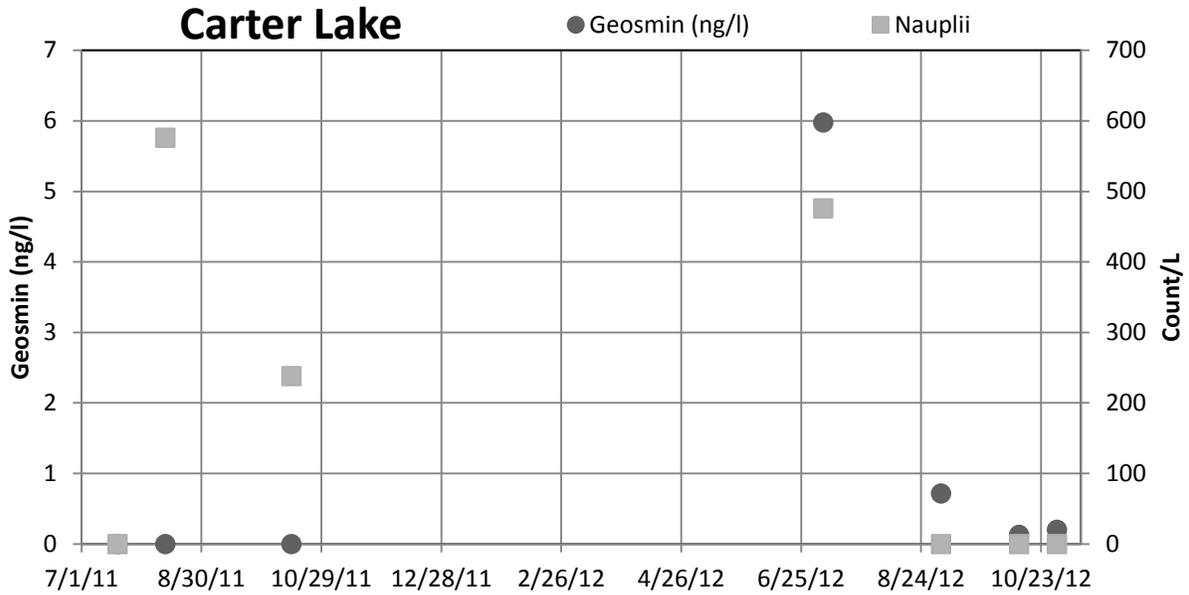
JOE WRIGHT RESERVOIR	LAKE LOVELAND	LONG LAKE	LOST LAKE	MARY'S LAKE	PINEWOOD RESERVOIR	RED ROCK LAKE	RIVERSIDE RESERVOIR	SHADOW MTN RES.	LAKE SHERWOOD
10/24/12	10/19/12	10/1/12	10/24/12	11/2/12	10/31/12	10/1/12	10/23/12	10/4/12	10/17/12
9/12/12	9/20/12	8/15/12	9/12/12	10/12/12	10/12/12	8/15/12	8/1/12	8/31/12	9/11/12
7/12/12	8/16/12	6/21/12	7/12/12	8/31/12	9/3/12	6/21/12	7/4/12	8/10/11	8/11/12
	7/11/12	10/5/11	5/30/12	6/21/12	6/21/12	10/5/11		6/29/11	7/14/12
	10/19/11		8/15/11	6/27/11	10/14/11				8/17/11
	8/9/11			5/26/11	8/12/11				7/25/11
	7/18/11				7/19/11				7/5/11
	5/24/11				5/25/11				6/30/11



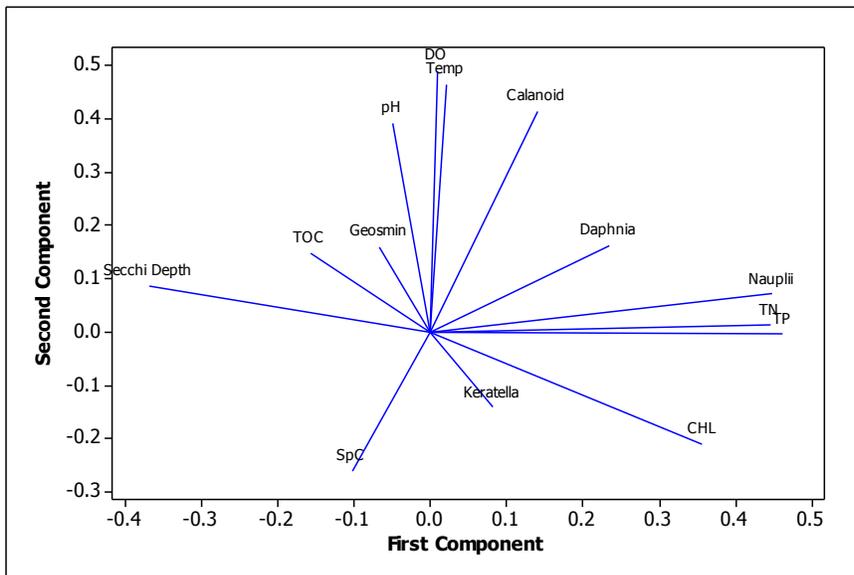
**Figure 39 – Times Series Graph displaying all geosmin and daphnia data points taken from Carter Lake. Units for Daphnia are number of organisms/liter.**



**Figure 40 - Time Series Graph displaying geosmin and nutrient results taken from Carter Lake during the study.**



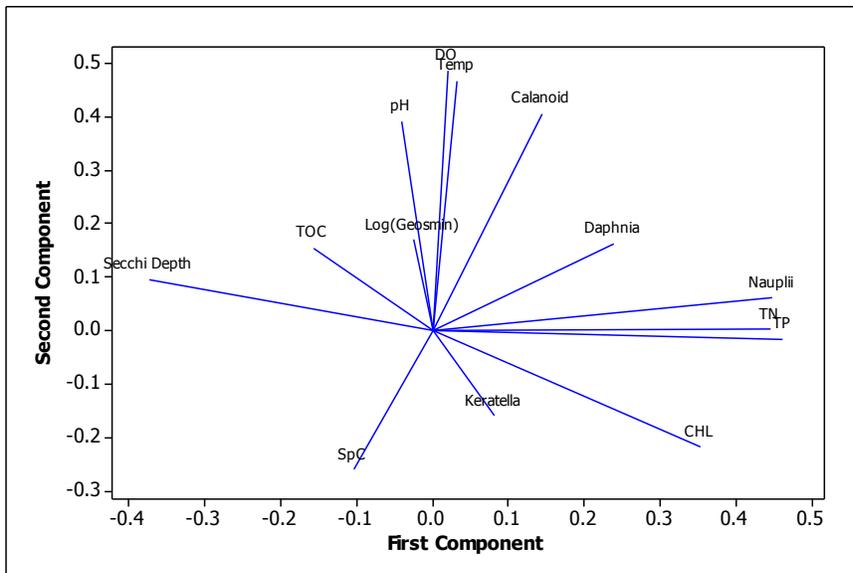
**Figure 41 - Times Series Graph displaying geosmin and Nauplii data taken from Carter Lake.**



**Figure 42 - PCA of geosmin and other sampled parameters from lakes and reservoirs considered to be “High Mountain Lakes” (Estes Lake, Mary’s Lake, Chambers Lake, Joe Wright Res., Lost Lake, Brainard Lake, Long Lake, and Red Rock Lake).**

**Table 28 - Geosmin PCA Eigenvalues for first and second components, High Mountain Lakes and Reservoirs**

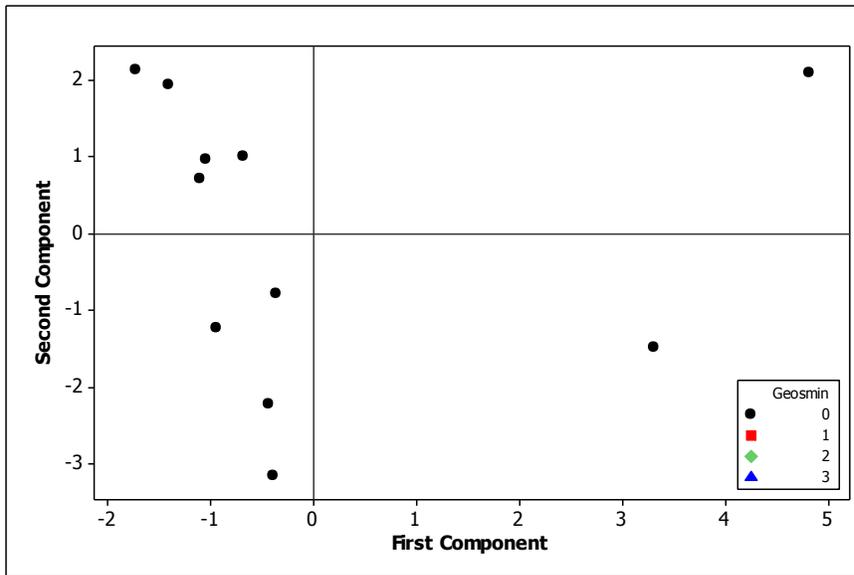
VARIABLE	PC1	PC2
DAPHNIA	0.235	0.162
CALANOID	0.14	0.414
NAUPLII	0.448	0.071
KERATELLA	0.082	-0.142
TEMP	0.021	0.466
DO	0.01	0.489
PH	-0.05	0.392
CHL	0.357	-0.211
SPC	-0.102	-0.261
SECCHI DEPTH	-0.369	0.086
TOC	-0.157	0.148
TN	0.445	0.012
TP	0.462	-0.005
GEOSMIN	-0.067	0.159



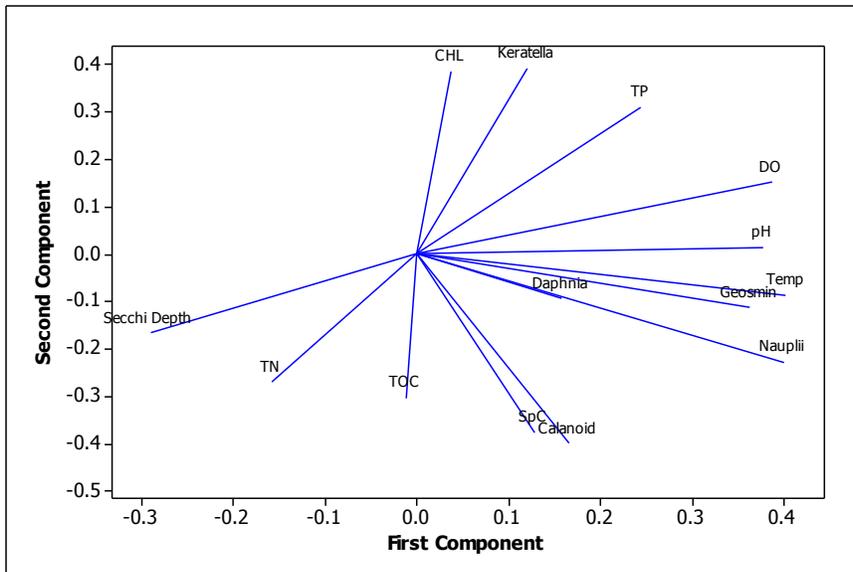
**Figure 43 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “High Mountain Lakes” (Estes Lake, Mary’s Lake, Chambers Lake, Joe Wright Res., Lost Lake, Brainard Lake, Long Lake, and Red Rock Lake).**

**Table 29 - Log(Geosmin) PCA Eigenvalues for first and second components, High Mountain Lakes and Reservoirs.**

VARIABLE	PC1	PC2
DAPHNIA	0.239	0.162
CALANOID	0.145	0.404
NAUPLII	0.448	0.061
KERATELLA	0.08	-0.159
TEMP	0.032	0.465
DO	0.021	0.485
PH	-0.041	0.39
CHL	0.354	-0.219
SPC	-0.104	-0.259
SECCHI DEPTH	-0.373	0.095
TOC	-0.157	0.154
TN	0.445	0.003
TP	0.462	-0.016
LOG(GEOSMIN)	-0.025	0.17



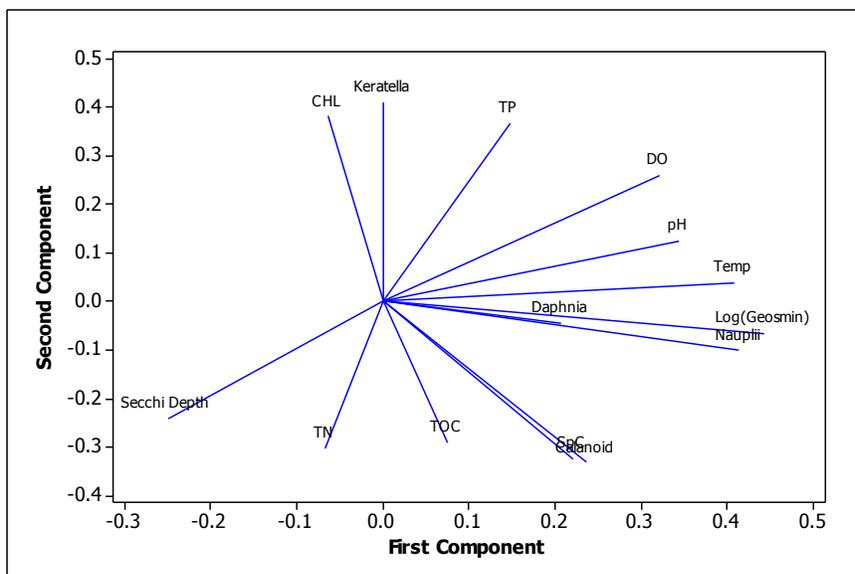
**Figure 44 - High Mountain Region PCA Score Plot**



**Figure 45 - PCA of geosmin and other sampled parameters from lakes and reservoirs considered to be “Foothill Lakes” (Carter Lake, Pinewood Res., Flatiron Res., and Horsetooth Res.).**

**Table 30 - Geosmin PCA Eigenvalues for first and second components, Foothills Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	0.158	-0.094
CALANOID	0.166	-0.4
NAUPLII	0.399	-0.228
KERATELLA	0.12	0.392
TEMP	0.403	-0.085
DO	0.387	0.151
pH	0.377	0.014
CHL	0.038	0.387
SPC	0.129	-0.377
SECCHI DEPTH	-0.291	-0.166
TOC	-0.012	-0.304
GEOSMIN	0.363	-0.114
TN	-0.158	-0.271
TP	0.245	0.312



**Figure 46 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “Foothill Lakes” (Carter Lake, Pinewood Res., Flatiron Res., and Horsetooth Res.).**

**Table 31 - Log(Geosmin) PCA Eigenvalues for first and second components, Foothills Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	0.206	-0.045
CALANOID	0.236	-0.333
NAUPLII	0.414	-0.102
KERATELLA	0.001	0.41
TEMP	0.407	0.038
DO	0.321	0.259
pH	0.344	0.125
CHL	-0.064	0.381
SPC	0.22	-0.325
SECCHI DEPTH	-0.25	-0.242
TOC	0.074	-0.29
TN	-0.068	-0.303
TP	0.147	0.367
LOG(GEOSMIN)	0.443	-0.06

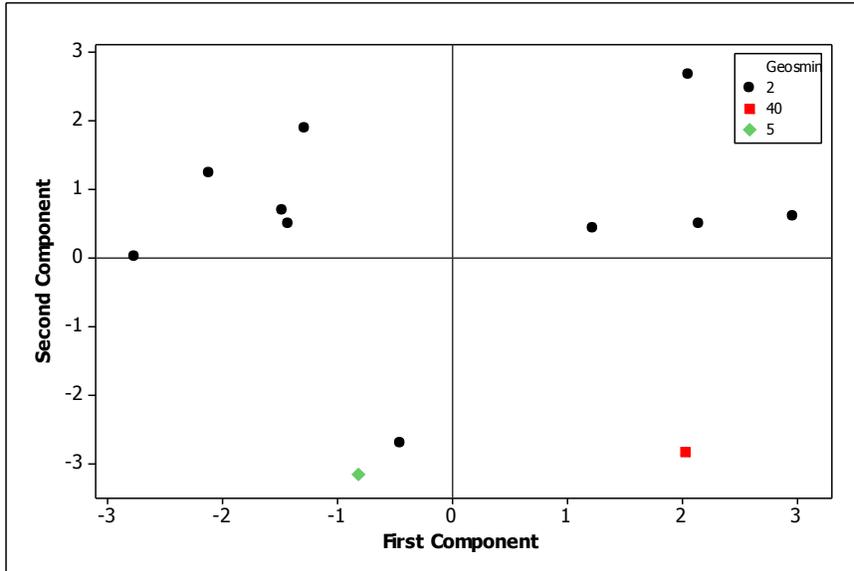


Figure 47 - Foothills Region PCA Score Plot

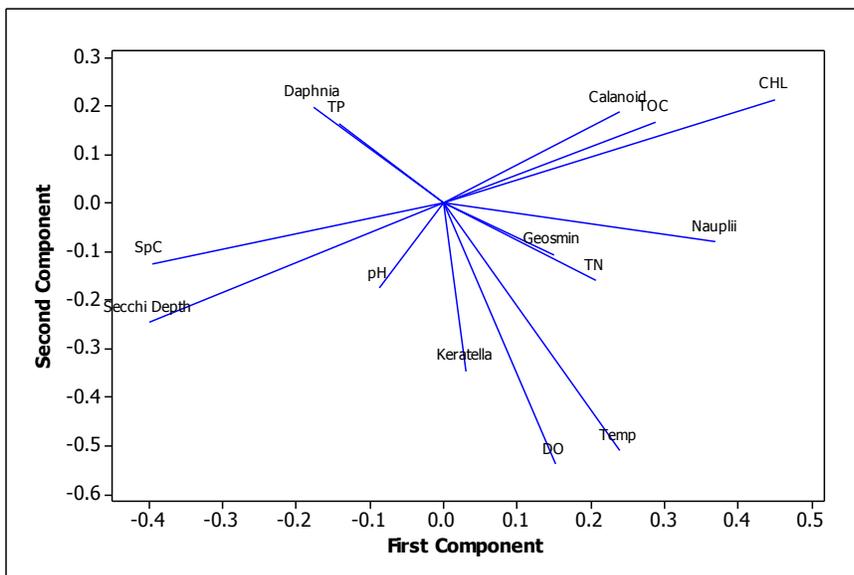
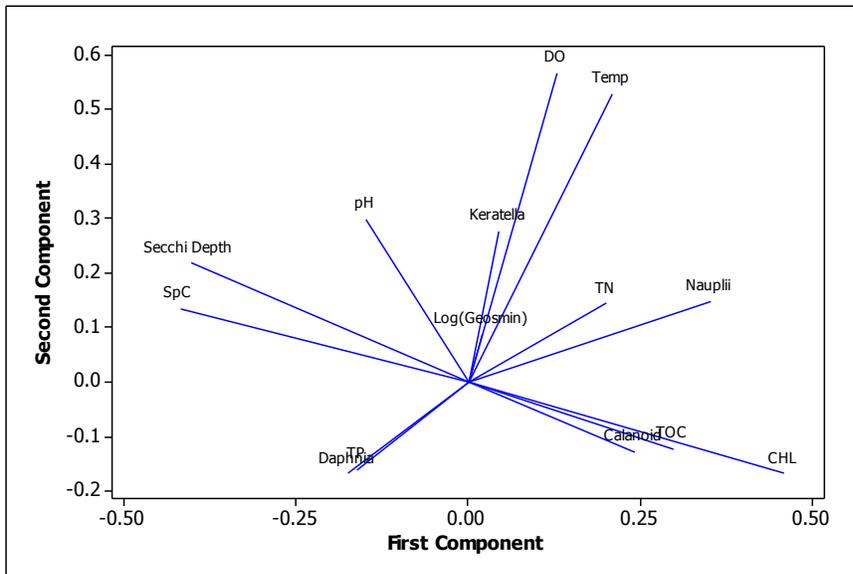


Figure 48 - PCA of geosmin and other sampled parameters from lakes and reservoirs considered to be “Urban Lakes” (City Park Lake, Fossil Creek Park Lake, Lake Loveland, and Sherwood Lake).

**Table 32 - Geosmin PCA Eigenvalues for first and second components, Urbans Lakes and Reservoirs**

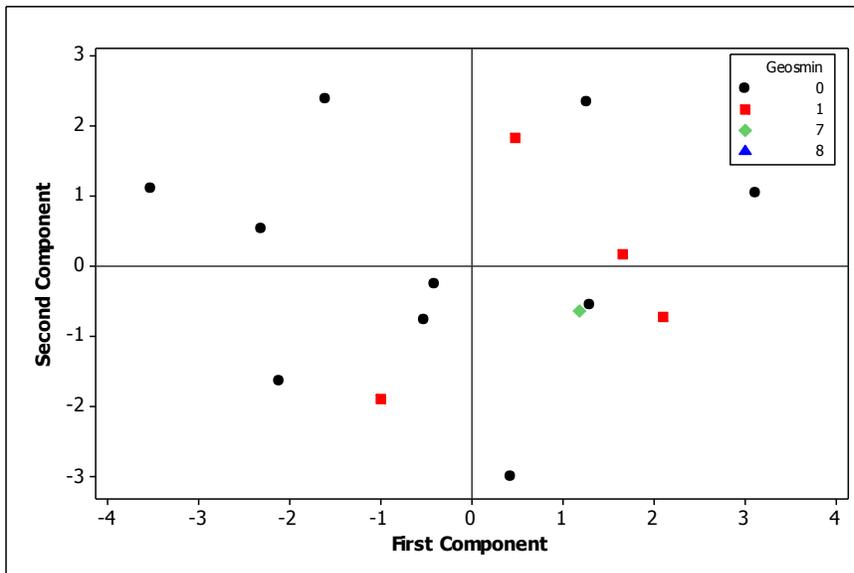
VARIABLE	PC1	PC2
DAPHNIA	-0.176	0.199
CALANOID	0.239	0.188
NAUPLII	0.369	-0.08
KERATELLA	0.031	-0.347
TEMP	0.239	-0.512
DO	0.152	-0.54
PH	-0.087	-0.176
CHL	0.45	0.215
SPC	-0.396	-0.124
SECCHI DEPTH	-0.399	-0.247
TOC	0.287	0.167
GEOSMIN	0.149	-0.107
TN	0.208	-0.16
TP	-0.143	0.164



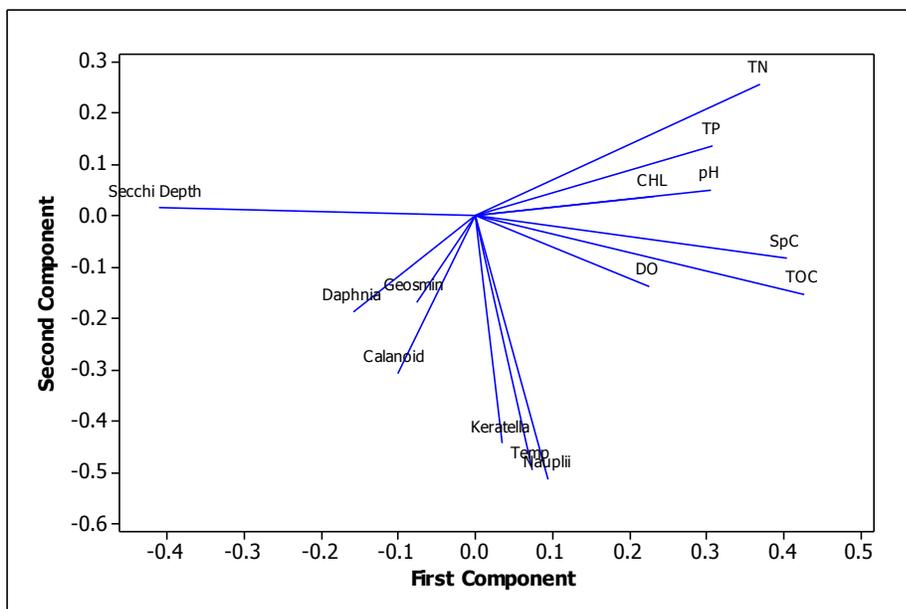
**Figure 49 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “Urban Lakes” (City Park Lake, Fossil Creek Park Lake, Lake Loveland, and Sherwood Lake).**

**Table 33 - Log(Geosmin) PCA Eigenvalues for first and second components, Urban Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	-0.174	-0.169
CALANOID	0.241	-0.129
NAUPLII	0.352	0.148
KERATELLA	0.044	0.276
TEMP	0.209	0.529
DO	0.129	0.568
PH	-0.148	0.3
CHL	0.458	-0.169
SPC	-0.419	0.135
SECCHI DEPTH	-0.404	0.218
TOC	0.297	-0.124
TN	0.201	0.144
TP	-0.161	-0.162
LOG(GEOSMIN)	0.02	0.087



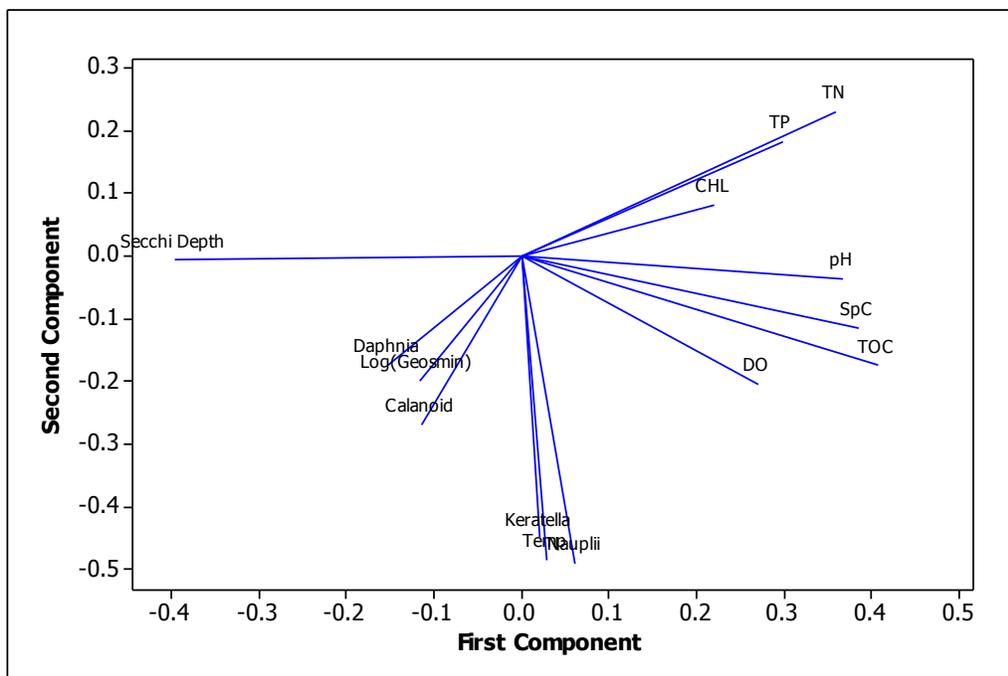
**Figure 50 - Urban Region PCA Score Plot**



**Figure 51 - PCA of Geosmin and other sampled parameters from lakes and reservoirs considered to be “Agriculture influenced Lakes” (Boyd Lake, Riverside Res., Jackson Lake, and Barr Lake).**

**Table 34 - Geosmin PCA Eigenvalues for first and second components, Plains Lakes and Reservoirs**

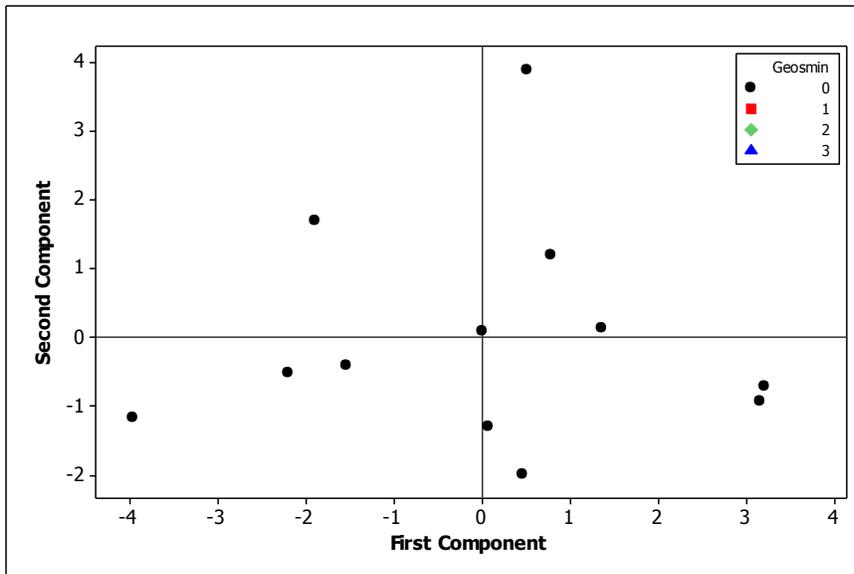
VARIABLE	PC1	PC2
DAPHNIA	-0.159	-0.186
CALANOID	-0.102	-0.307
NAUPLII	0.094	-0.513
KERATELLA	0.034	-0.444
TEMP	0.074	-0.494
DO	0.226	-0.137
PH	0.306	0.051
CHL	0.231	0.037
SPC	0.404	-0.082
SECCHI DEPTH	-0.412	0.015
TOC	0.426	-0.152
GEOSMIN	-0.077	-0.169
TN	0.368	0.257
TP	0.308	0.137



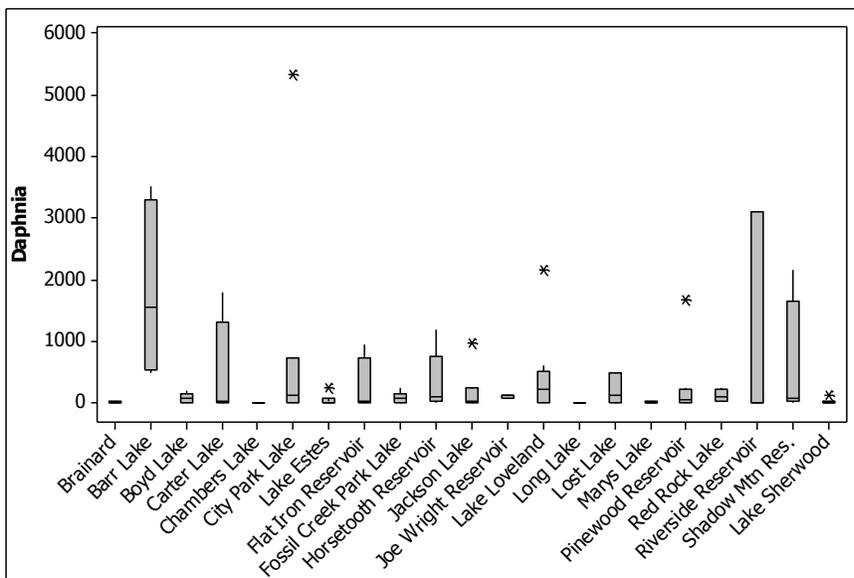
**Figure 52 - PCA of Log(geosmin) and other sampled parameters from lakes and reservoirs considered to be “Agriculture influenced Lakes” (Boyd Lake, Riverside Res., Jackson Lake, and Barr Lake).**

**Table 35 - Log(Geosmin) PCA Eigenvalues for first and second components, Plains Lakes and Reservoirs**

VARIABLE	PC1	PC2
DAPHNIA	-0.152	-0.174
CALANOID	-0.115	-0.269
NAUPLII	0.061	-0.49
KERATELLA	0.021	-0.45
TEMP	0.028	-0.485
DO	0.271	-0.204
pH	0.367	-0.037
CHL	0.22	0.082
SPC	0.385	-0.116
SECCHI DEPTH	-0.397	-0.007
TOC	0.407	-0.175
TN	0.358	0.23
TP	0.298	0.183
LOG(GEOSMIN)	-0.117	-0.198



**Figure 53 - Plains Region PCA Score Plot**



**Figure 54 - Box and Whisker Plot of Daphnia results for all Reservoirs.**

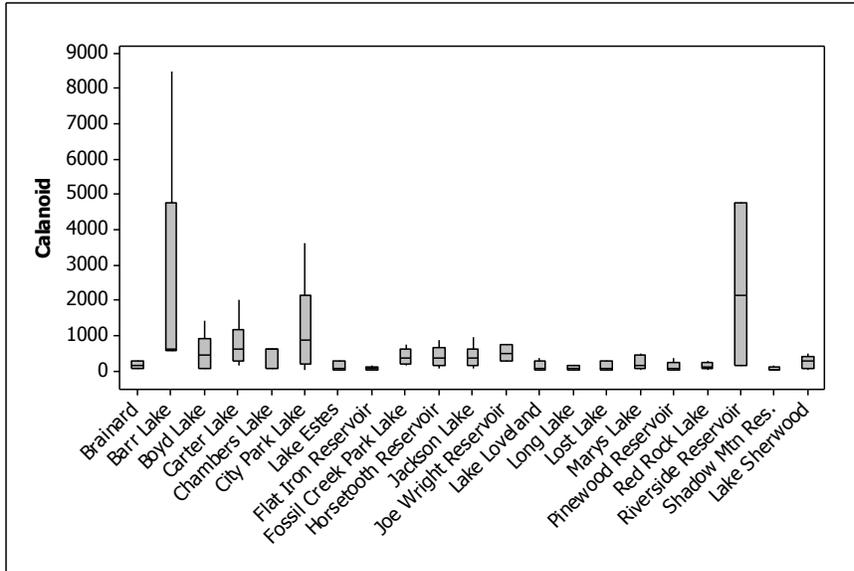


Figure 55 - Box and Whisker Plot of Daphnia results for all Reservoirs.

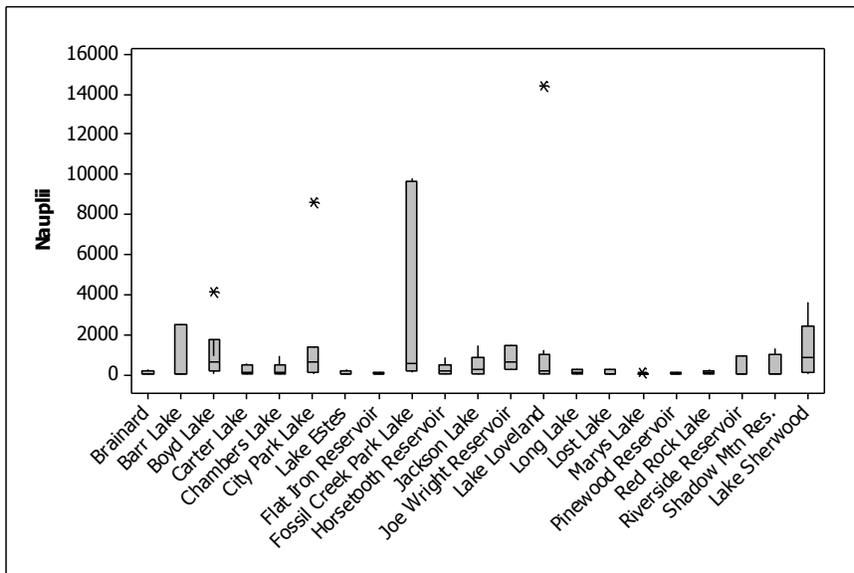
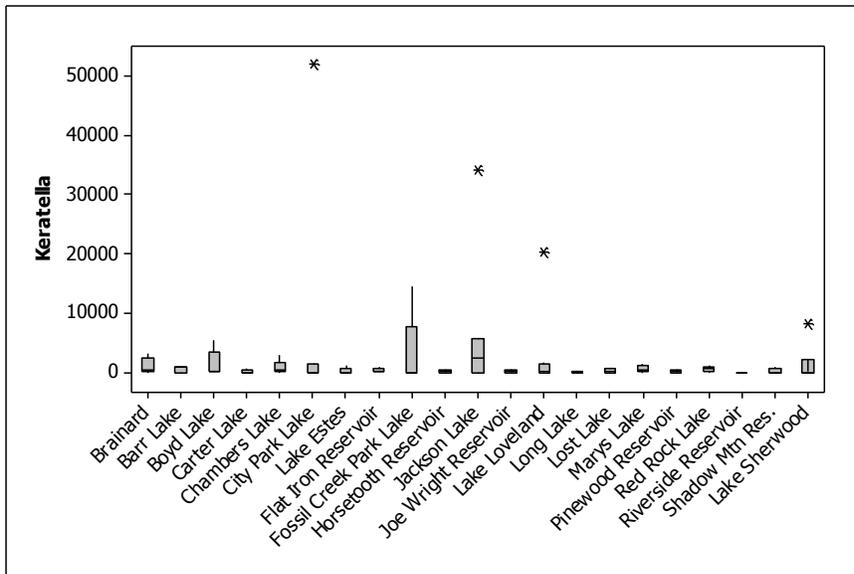
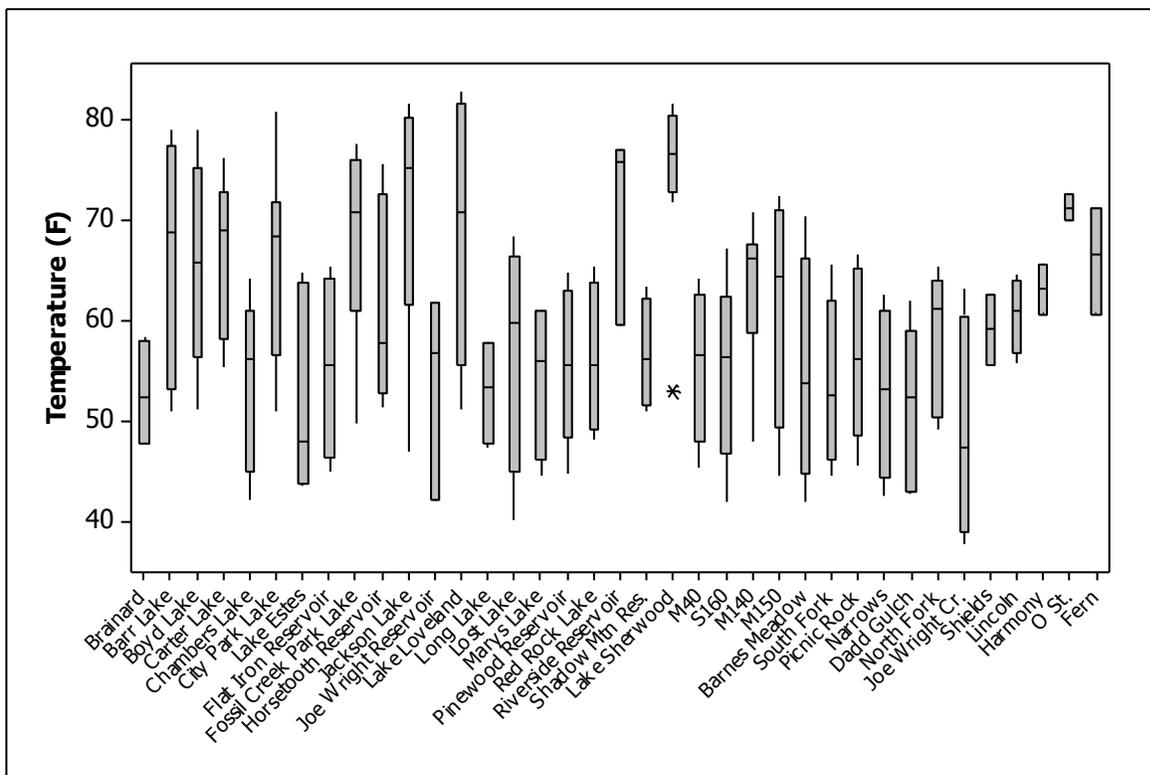


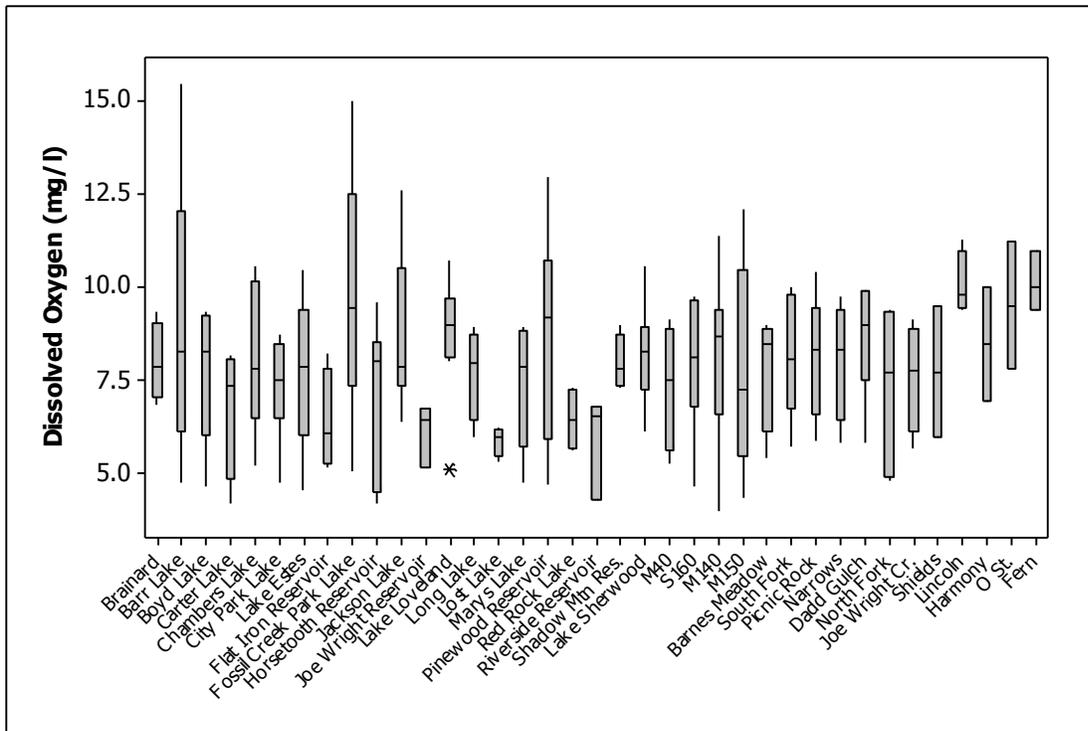
Figure 56 - Box and Whisker Plot of Nauplii results for all Reservoirs.



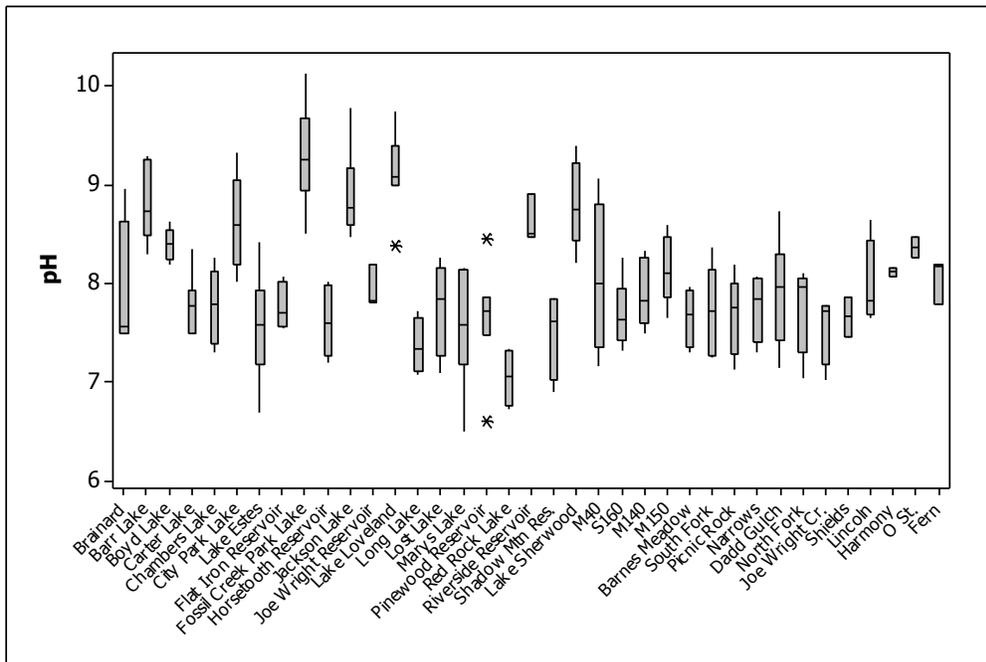
**Figure 57 - Box and Whisker Plot of Keratella results for all Reservoirs.**



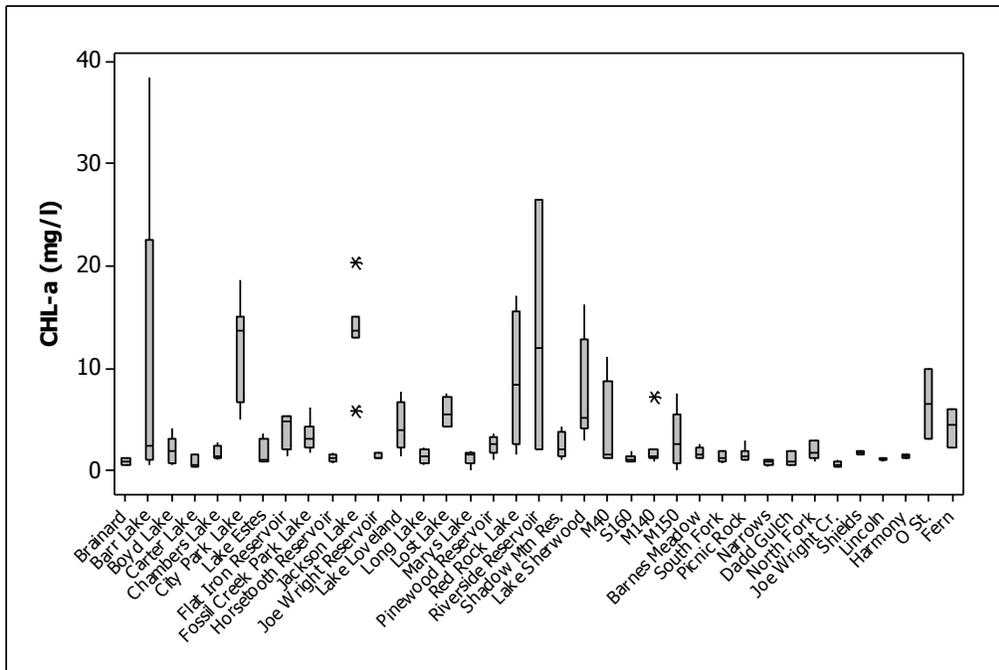
**Figure 58 - Box and Whisker Plot of Temperature Data for Each site including Lakes, Reservoirs and Rivers.**



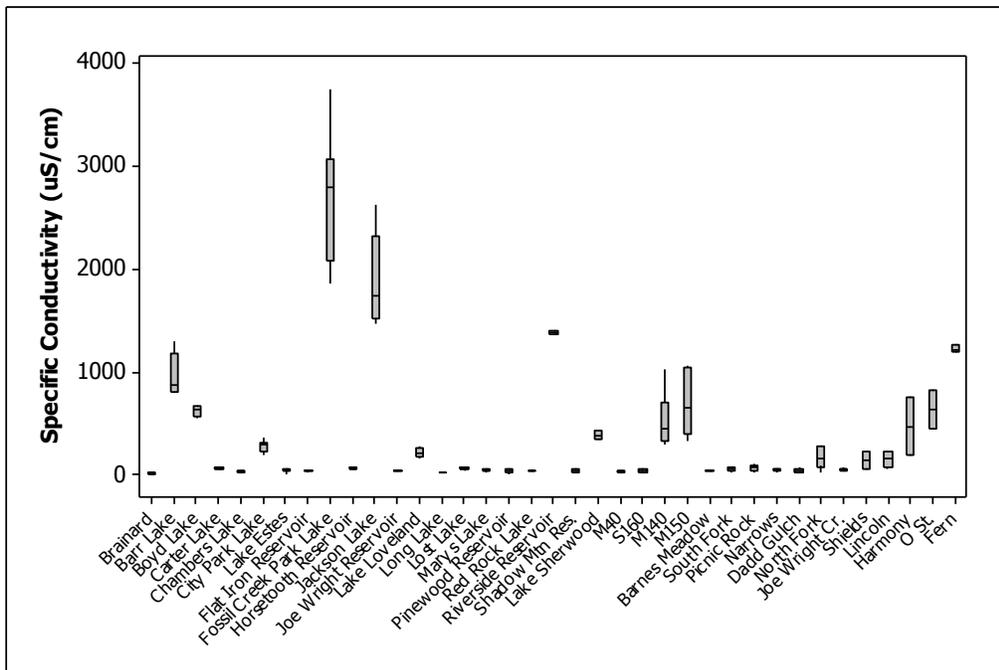
**Figure 59 - Box and Whisker Plot of Dissolved Oxygen Data for Each site including Lakes, Reservoirs and Rivers.**



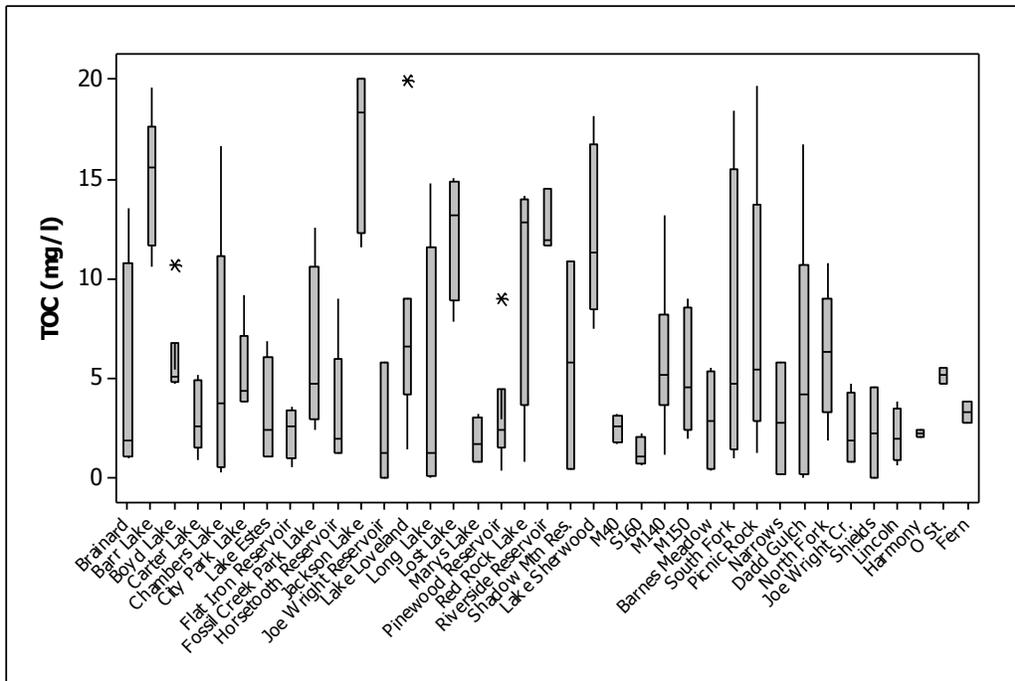
**Figure 60 - Box and Whisker Plot of pH Data for Each site including Lakes, Reservoirs and Rivers.**



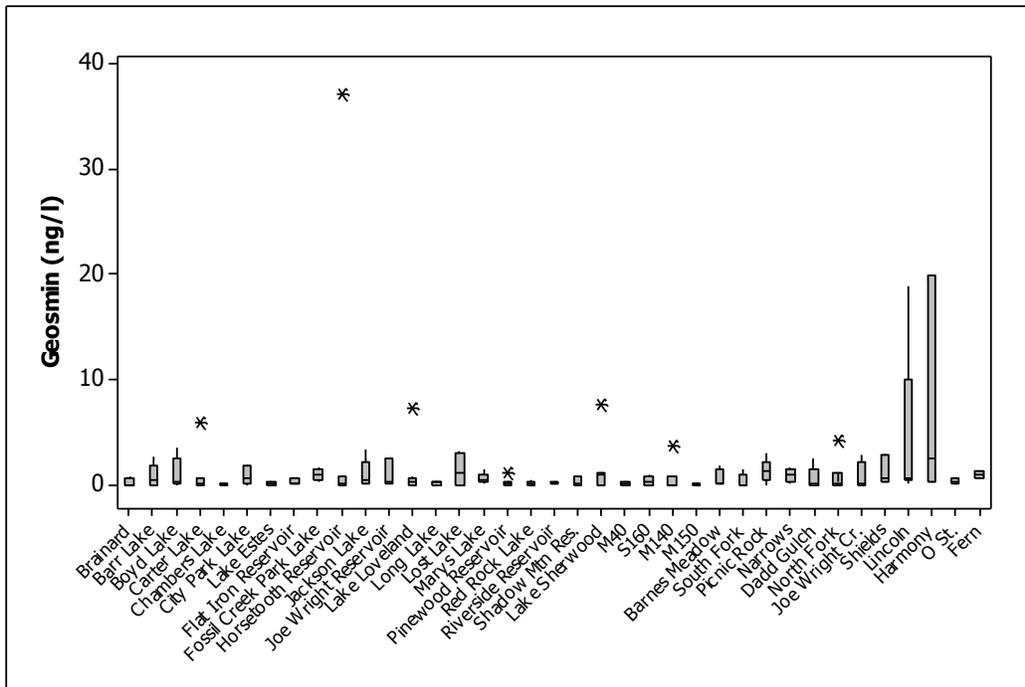
**Figure 61 - Box and Whisker Plot of Chlorophyll -a Data for Each site including Lakes, Reservoirs and Rivers.**



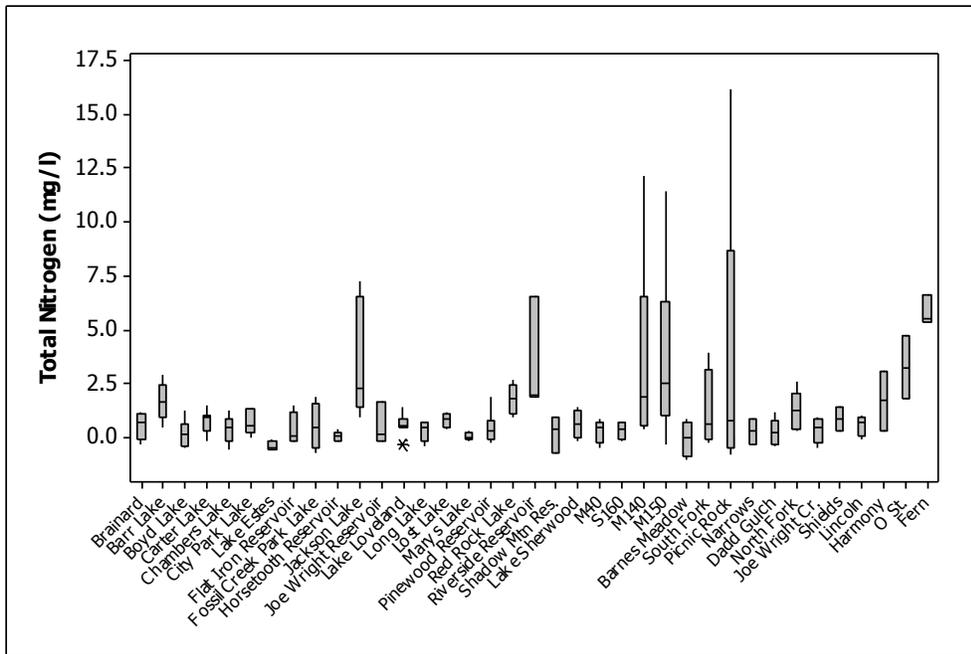
**Figure 62 - Box and Whisker Plot of Specific Conductivity Data for Each site including Lakes, Reservoirs and Rivers.**



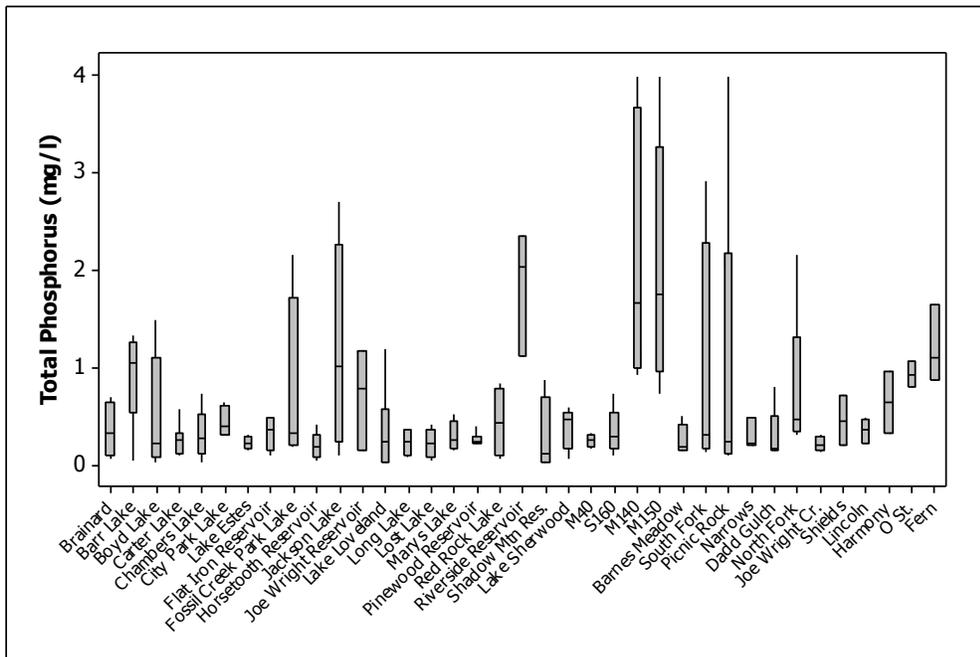
**Figure 63 - Box and Whisker Plot of Total Organic Carbon Data for Each site including Lakes, Reservoirs and Rivers.**



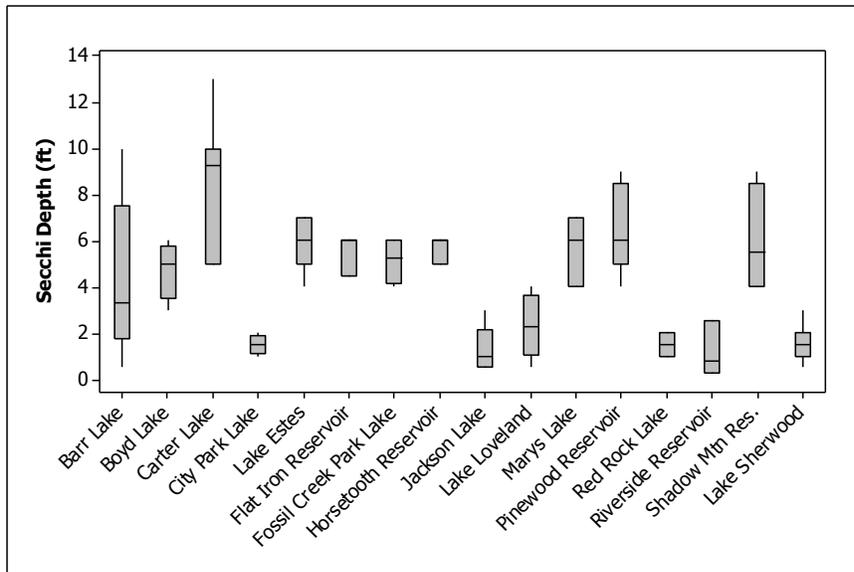
**Figure 64 - Box and Whisker Plot of Geosmin Data for Each site including Lakes, Reservoirs and Rivers.**



**Figure 65 - Box and Whisker Plot of Total Nitrogen Data for Each site including Lakes, Reservoirs and Rivers.**



**Figure 66 - Box and Whisker Plot of Total Phosphorus Data for Each site including Lakes, Reservoirs and Rivers.**



**Figure 67 - Box and Whisker Plot of Secchi Depth Data from Lakes and Reservoirs.**

**Table 36 - R<sup>2</sup> Regression values correlated with Log(Geosmin) lakes and reservoirs data separated by region.**

VARIABLE	HIGH MOUNTAIN	FOOTHILLS	URBAN	PLAINS
DAPHNIA	0.126	0.185	0.002	0.107
CALANOID	0.001	0.369	0.002	0.003
NAUPLII	0.73	0.806	0.214	0.047
KERATELLA	0.003	0.014	0.037	0.15
TEMPERATURE	0.04	0.453	0.064	0.195
DO	0.14	0.13	0.007	0.367
pH	0.031	0.031	0.127	0.0
CHL-A	0.092	0.108	0.067	0.435
CONDUCTIVITY	0.006	0.157	0.017	0.08
SECCHI DEPTH	0.191	0.153	0.007	0.322
TOC	0.003	0.001	0.038	0.102
TN	0.022	0.036	0.049	0.089
TP	0.102	0.043	0.156	0.08

**Table 37 - P-Values from ANOVA Analysis with Log(Geosmin) from lakes and reservoirs data separated by region.**

FACTOR	HIGH MOUNTAIN	FOOTHILLS	URBAN	PLAINS
LAKE SIZE	0.808	0.304	0.412	-
TN	0.126	0.557	0.725	0.737
TP	0.161	0.104	0.363	0.802
TOC	0.262	0.397	0.773	0.341
DATE	0.556	0.021	0.475	0.455

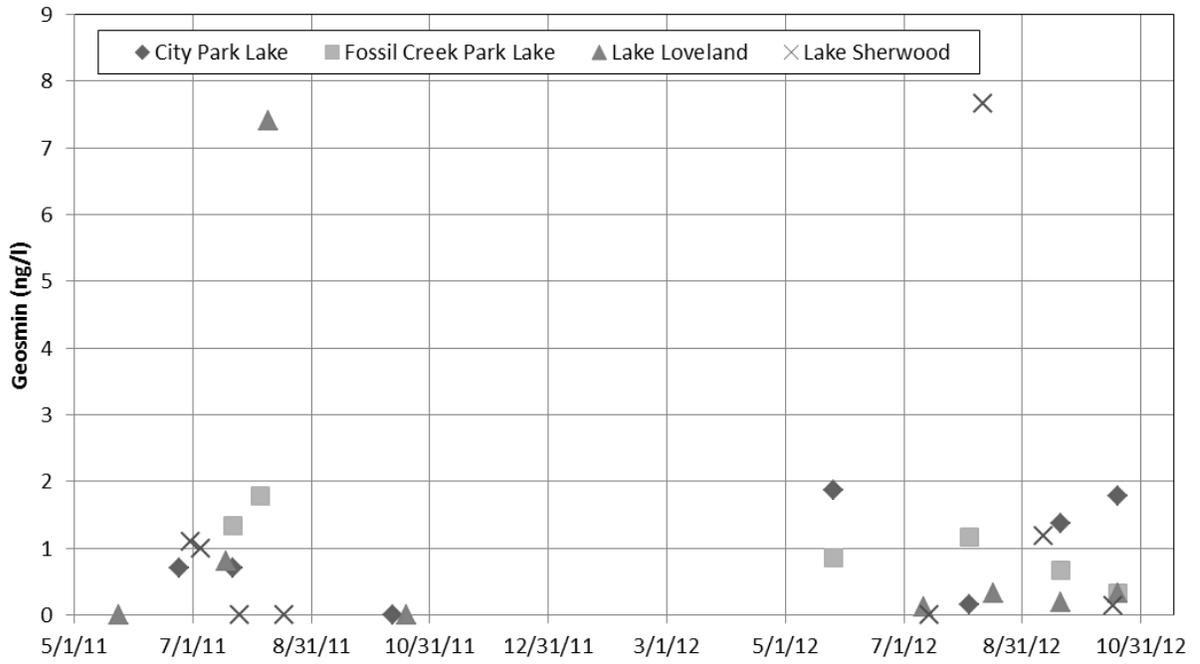
**Table 38 - R<sup>2</sup> Regression values correlated with Geosmin lakes and reservoirs data separated by region.**

VARIABLE	HIGH			
	MOUNTAIN	FOOTHILLS	URBAN	PLAINS
DAPHNIA	0.247	0	0.015	0.077
CALANOID	0.003	0.046	0.003	0.004
NAUPLII	0.002	0.378	0.17	0
KERATELLA	0.042	0.01	0.028	0.106
TEMPERATURE	0.072	0.085	0.057	0.209
DO	0.02	0.004	0	0.32
pH	0	0.036	0	0
CHL-A	0.001	0.004	0.022	0.076
CONDUCTIVITY	0.063	0.057	0.002	0.036
SECCHI DEPTH	0.008	0.064	0.001	0.094
TOC	0.066	0.026	0.004	0.048
TN	0.007	0.053	0.01	0.045
TP	0.041	0	0.03	0.049

**Table 39 - P-Values from ANOVA Analysis with Geosmin from lakes and reservoirs data separated by region.**

FACTOR	HIGH			
	MOUNTAIN	FOOTHILLS	URBAN	PLAINS
LAKE SIZE	0.128	0.648	0.862	-
TN	0.222	0.335	0.446	0.528
TP	0.553	0.446	0.756	0.935
TOC	0.297	0.919	0.501	0.495
DATE	0.903	0.357	0.398	0.292





**Figure 70 - Geosmin time series graph for Lakes and Reservoirs in the urban region.**

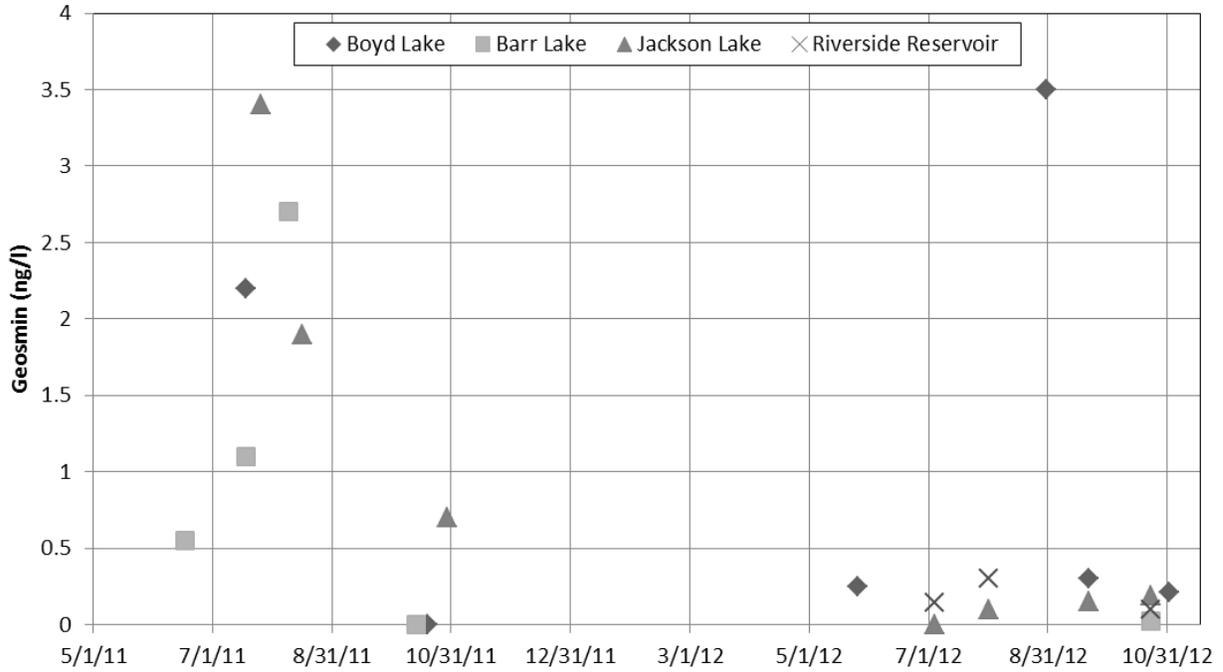


Figure 71 - Geosmin time series graph for Lakes and Reservoirs in the plains region.

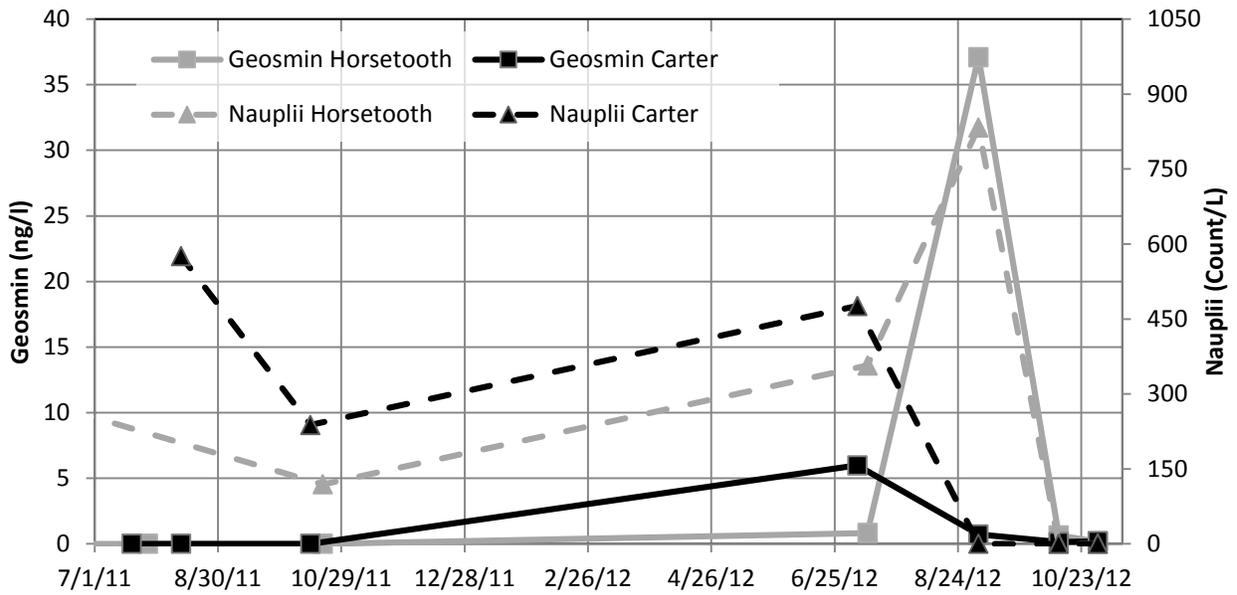


Figure 72 - Time Series graph of geosmin and Nauplii data for both Horsetooth and Carter Lake.

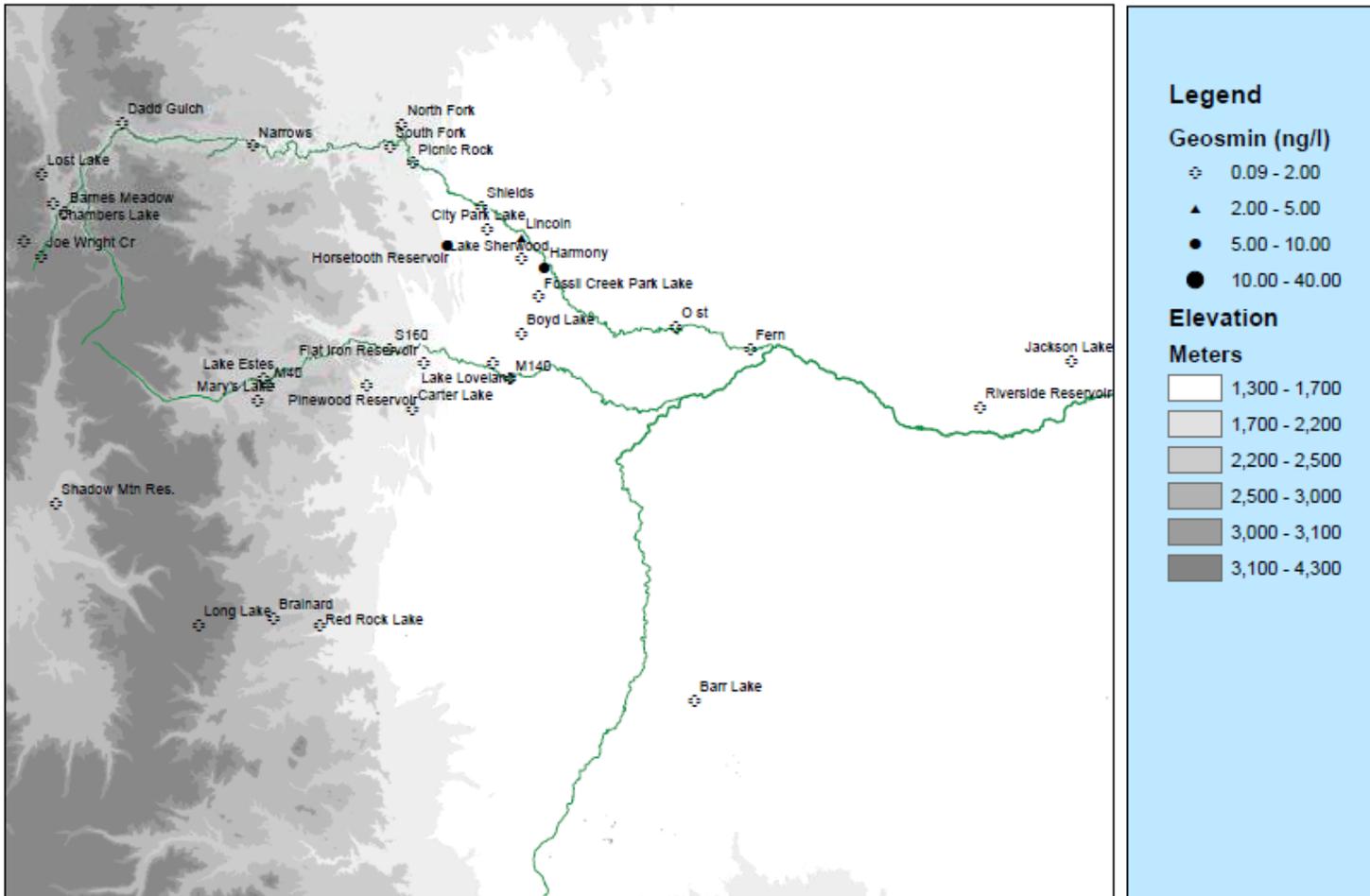
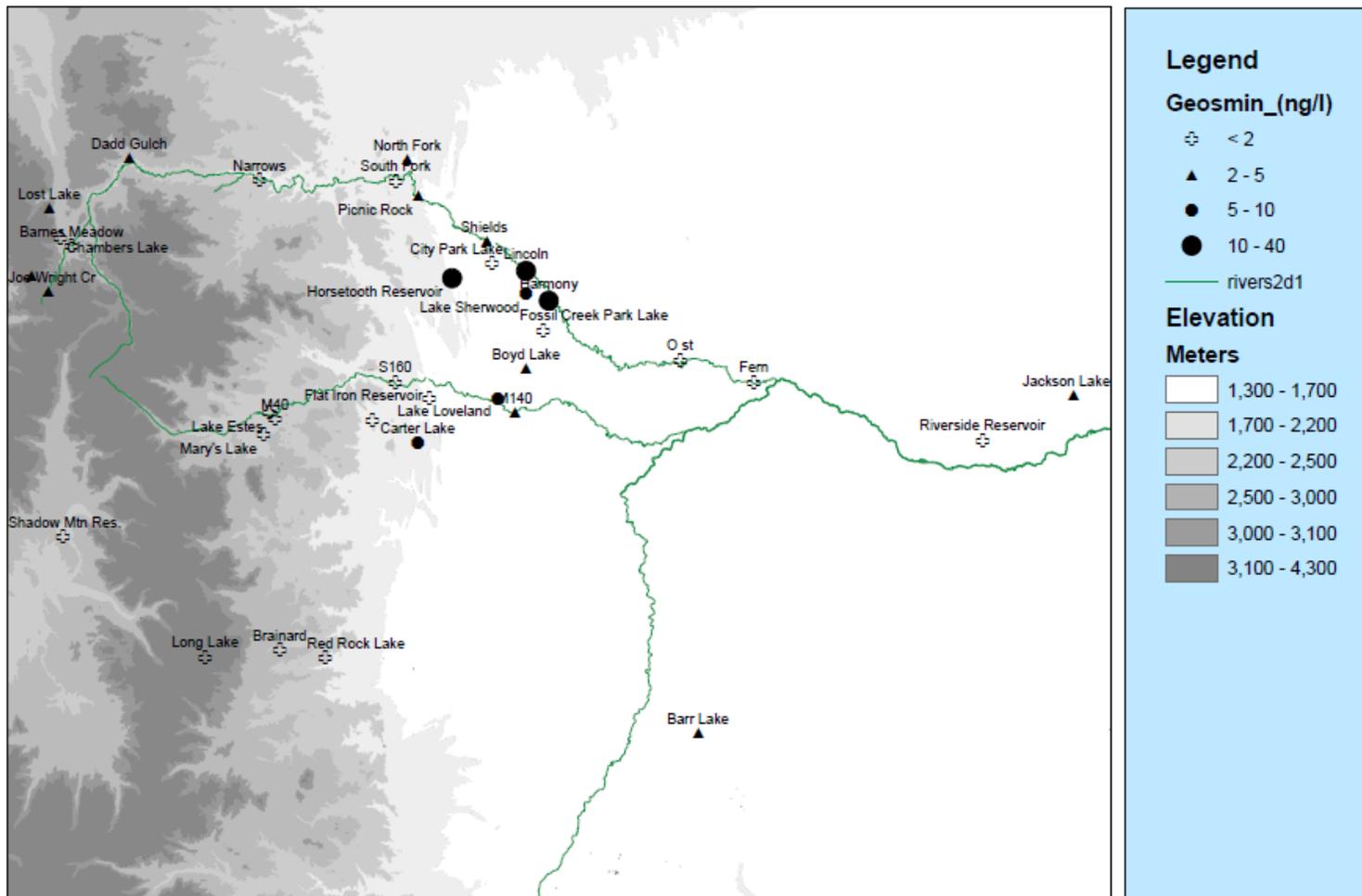


Figure 73 – Average Geosmin concentrations recorded during the study period for all Lakes, Reservoirs, and Rivers sampled.



**Figure 74 - Maximum Geosmin concentrations recorded during the study period for all Lakes, Reservoirs, and Rivers sampled.**

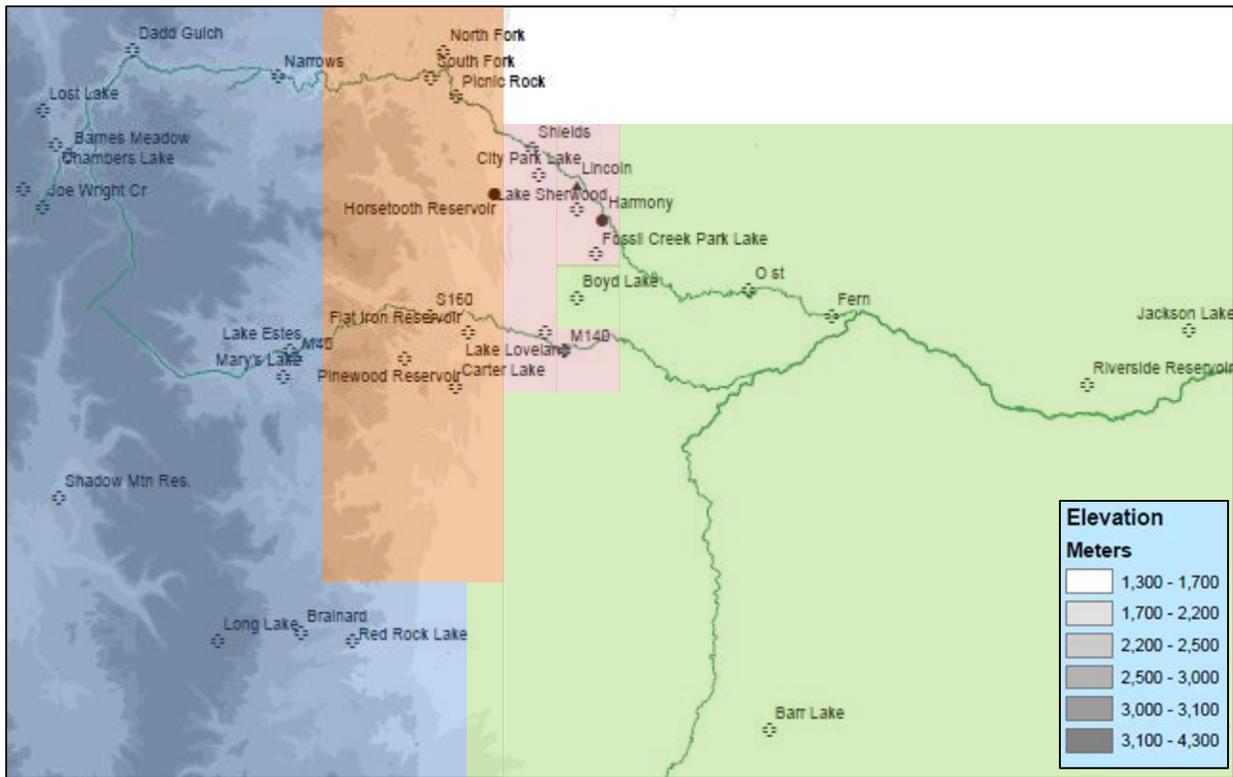


Figure 75 - Eco-Region Delineation by Elevation and Ecology

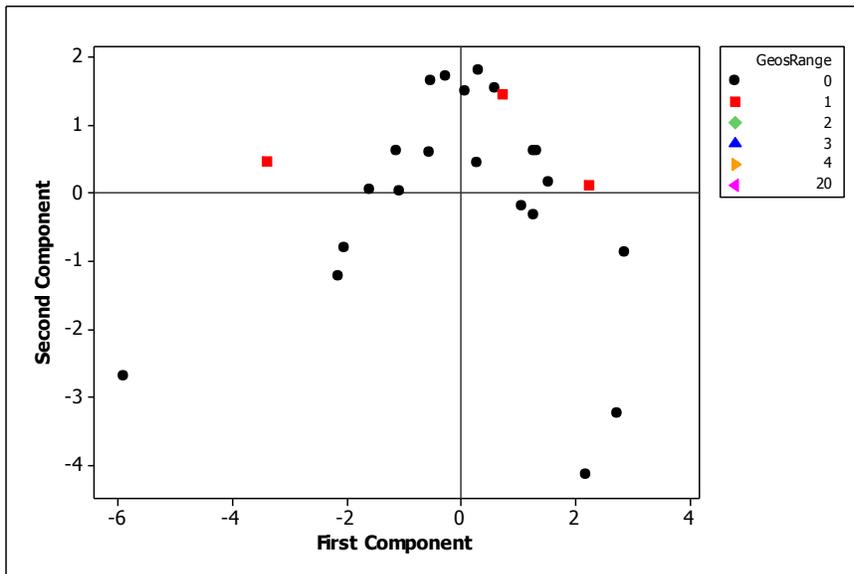


Figure 76 - Rivers PCA Score Plot

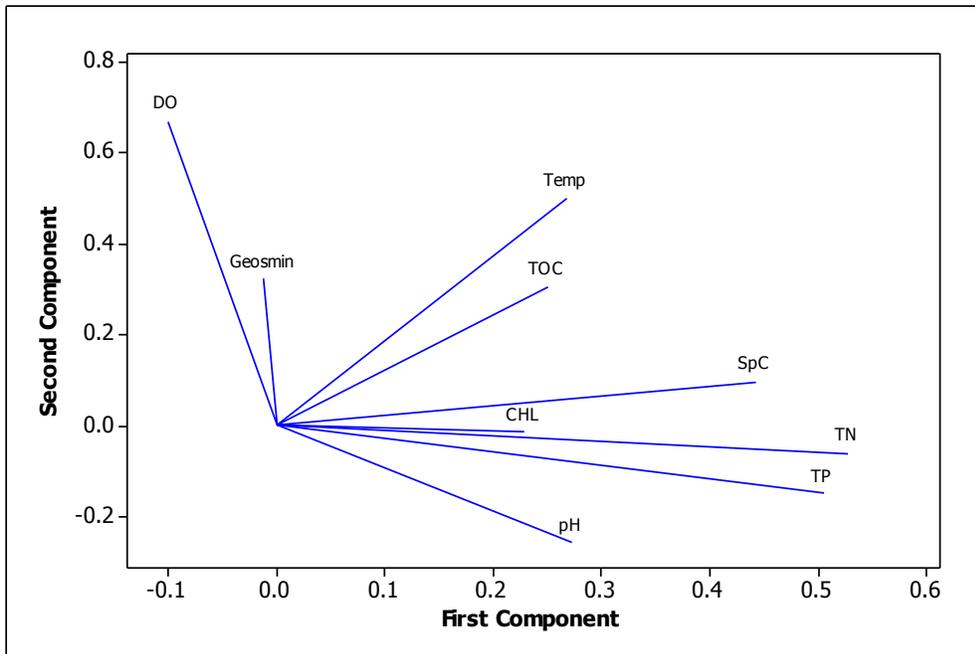


Figure 77 - PCA of Geosmin and other sampled parameters all river and stream data.

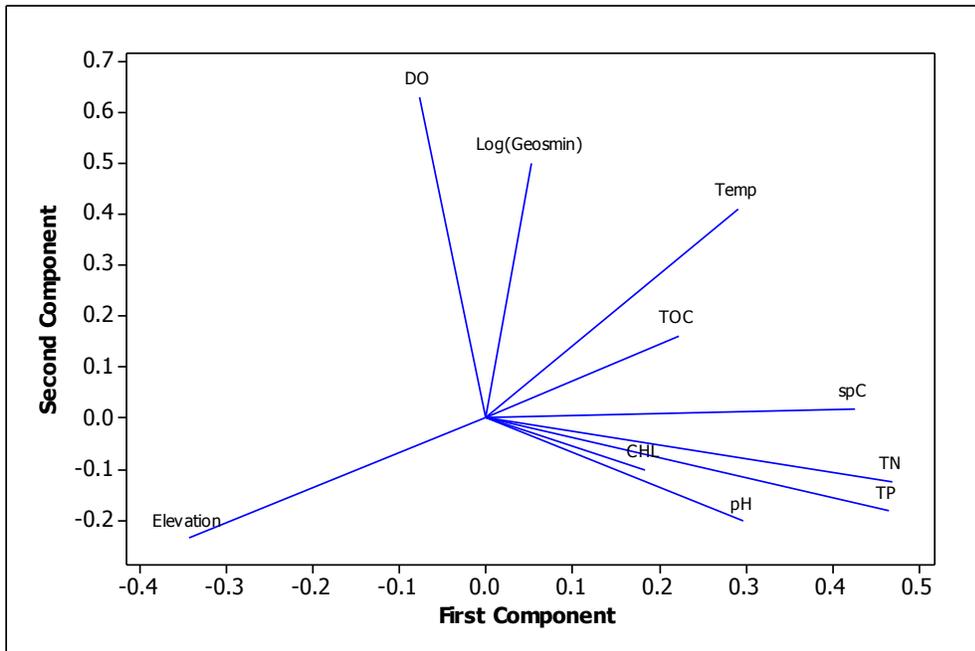


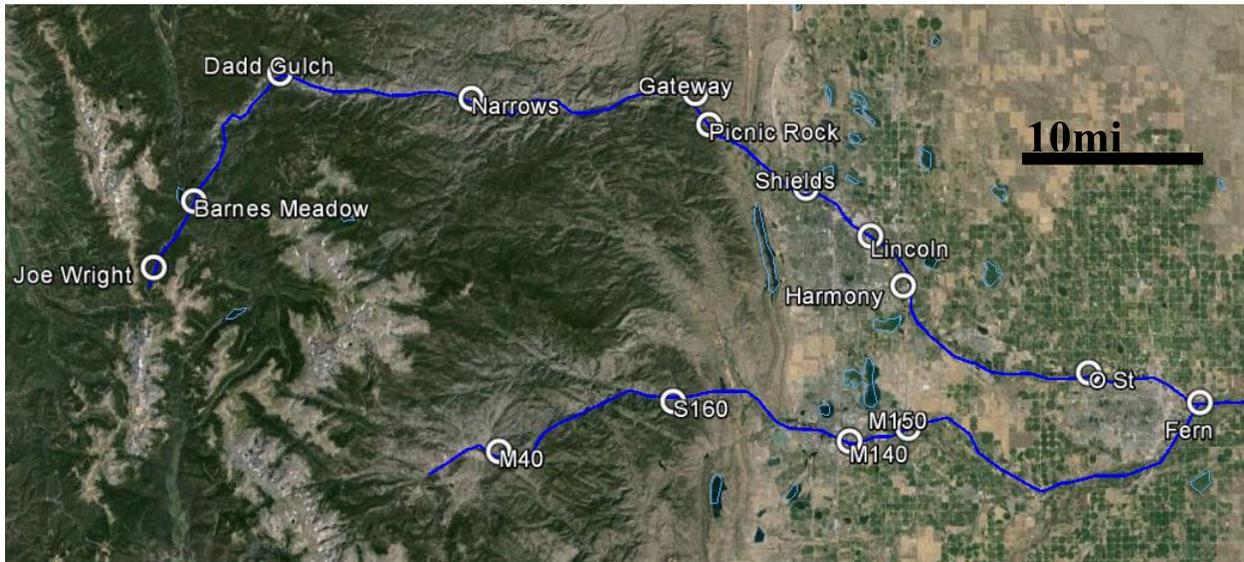
Figure 78 - PCA of Log(Geosmin) and other sampled parameters all river and stream data.

**Table 40 - Log(Geosmin) PCA Eigenvalues for first and second components, Rivers**

VARIABLE	PC1	PC2
TEMP	0.292	0.412
DO	-0.077	0.630
pH	0.297	-0.203
CHL	0.183	-0.102
SpC	0.425	0.019
TOC	0.222	0.162
TN	0.470	-0.125
TP	0.465	0.269
ELEVATION	-0.343	-0.237
LOG(GEOSMIN)	0.0520	0.500

**Table 41 - Geosmin PCA Eigenvalues for first and second components, Rivers**

Variable	PC1	PC2
Temp	0.268	0.501
DO	-0.101	0.67
pH	0.273	-0.258
CHL	0.228	-0.015
SpC	0.443	0.096
TOC	0.25	0.306
Geosmin	-0.012	0.323
TN	0.527	-0.061
TP	0.505	-0.15



**Figure 79 - Satellite imagery for displaying sampling locations on the Cache la Poudre and Big Thompson Rivers. (Courtesy Google Earth)**