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SONGBIRD FEATHERS AS BIOINDICATORS OF HYDRAULIC FRACTURING RELATED CONTAMINATION

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ABSTRACT

An increasing number of oil and gas wells that are directionally drilled and hydraulically fractured using high volumes of water (HVHF) could jeopardize local water supplies and aquatic/riparian ecosystems. HVHF produced water or flowback water contains elevated measurements of Total Dissolved Salts (TDS), such as strontium (Sr) and barium (Ba), and elevated activities of Naturally Occurring Radioactive Materials (NORM). Improper water treatment or accidental leaking/spills of flowback water can possibly contaminate surface water. Species living in/around polluted water can ingest the water and bioaccumulate HVHF related contaminants. Specifically, Louisiana Waterthrush are riparian birds that feed on aquatic macroinvertebrates. The macroinvertebrates live in areas of HVHF and could bioaccumulate Sr and Ba in their bloodstream. By analyzing and comparing the chemical makeup of riparian bird feathers that live near active and non-active HVHF sites, the effect of HVHF on surface water quality could potentially be quantified. A recent study shows that Sr and Ba concentrations in feathers (n = 285) collected near active HVHF sites are higher than those collected near non-active HVHF sites (Latta et al. 2015).

Louisiana Waterthrush feathers (n = 96) were collected in both active and non-active HVHF sites in the Marcellus Shale and Fayetteville Shale regions. The results are consistent with previous studies by demonstrating that Sr, Ba, and Ca concentrations correlate strongly (Ebel and Comar 1968). When Ca is needed from the bloodstream for incorporation into newly grown feathers, Sr and Ba can act as replacements and will bioaccumulate. Aluminum (Al) feather concentrations were measured to indicate improper feather cleaning/handling and the presence of dust/clay particles. Inconsistent with a previous study within the sample set, there was no statistical difference in this study between Sr, Sr/Ca, Ba, Ba/Ca, Ca, and Al measurements for active and non-active HVHF sites. In the Fayetteville Shale region, ^{87}Sr/^{86}Sr feather ratios are consistent with Fayetteville Shale flowback water (Warner et al. 2013). The ^{87}Sr/^{86}Sr data are inconclusive as to whether or not active HVHF sites are distinguishable from non-active HVHF sites. A continuation of this study with a larger sample size and greater statistical power would help draw critical conclusions in discovering the effects that HVHF has on surface water quality.

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Chapter 1

INTRODUCTION

Maintaining good water quality is important to both human and ecological health. Surface water contamination due to the extraction of fossil fuels is a rising concern. Specifically, studies have shown that water supplies and aquatic/riparian ecosystems local to oil and gas drilling operations are in jeopardy due to the increase in the number of oil and gas wells directionally drilled and hydraulically fractured using high volumes of water, which is referred to as High Volume Hydraulic Fracturing (HVHF) (Vengosh et al. 2013, Burton et al. 2014, Entrekin et al. 2011, Vidic et al. 2013, Gordalla et al. 2013, Warner et al. 2012). The increased need for energy diversity and the continued improvement of drilling and fracturing techniques have caused rapid and concentrated development of HVHF well pads across shale basins that did not previously hold any economically extractable resources (Burton et al. 2014). The industrial activity associated with HVHF increases the number of roadways and truck traffic. These increases lead to habitat loss. The damage inflicted on the habitats raises concern regarding water quality degradation. Other water quality concerns are derived from: 1) the possibility of gas migration into shallow groundwater and surface water, 2) wastewater discharge following inadequate treatment, and 3) unintentional spills of HVHF associated wastewater (Vidic et al. 2013, Myers 2012, Warner et al. 2012).

HVHF often requires construction of new roads to gain access to promising drill sites. Numerous trucks haul equipment, water, and chemicals to complete the well. First, roadways are built to access the drilling areas. Then, well pads are constructed at each HVHF site. At a well pad, the ground is leveled and stone is placed in an area that is roughly the size of three football fields. The well pads are often located near streams and waterways to minimize the distance that trucks need to travel in order to obtain water for the fracturing process. Large volumes of surface water are required for HVHF procedures. Each well pad can contain up to eight well laterals. Each well lateral uses up to 15 million liters, or 4 million gallons, of surface water that is infused with chemicals that inhibit bacterial growth, mineral buildup, sludge formation, polymerization, and pipe corrosion. Other chemicals added to the water increase viscosity, aid in transporting proppant, increase flow, and balance pH (Burton et al. 2014, Steliga et al. 2015, Ferrer and Thurman 2015). A detailed list of added chemicals and their corresponding purposes can be found in Burton et al. (2014). Over 75% of the added chemicals pose threats to human and ecological health (Burton et al. 2014). Continuing the HVHF procedure, the water/chemical mixture is pumped under high pressure into the well bore to fracture the low permeability rock layer several miles below the Earth's surface. The low-permeability rock layer contains the oil or gas. The fractures increase the permeability of the rock layer, often shale rock, and allow natural gas to flow more easily to the well at the Earth's surface. The water/chemical mixture returns to the surface and is often referred to as flowback water or produced water. The flowback water now contains high concentrations of salts. The flowback water salt concentrations are often greater than those of seawater. The salty flowback water is defined as brine, which has a Total Dissolved Salts (TDS) measurement greater than 35,000 parts per million (ppm). In addition to high concentrations of salts, the brine also contains elevated activities of Naturally Occurring Radioactive Materials (NORM). NORM is most often in the form of two long-lived isotopes of radium, 226-radim and 228-radium (Ferrer et al. 2013, Jackson et al. 2013, Yuhe et al. 2017).

The main components contributing to the high concentrations of TDS HVHF flowback water are sodium (Na) and Chloride (Cl). Other contaminants of concern include strontium (Sr) and barium (Ba). Sr and Ba are found naturally in high concentrations in the Marcellus Shale, which is a low-permeability shale layer in the Appalachian Basin. High Sr and Ba concentrations help to distinguish HVHF flowback water from the Marcellus Formation, when compared to other oil and gas formations in Pennsylvania, West Virginia, and Ohio (Steliga et al. 2015). Untreated flowback water is too salty to be reused, for example, as irrigation water. In HVHF procedures, the flowback water is often treated at municipal or industry-based WasteWater Treatment Plants (WWTPs). Unfortunately, the WWTPs are often not fully equipped to properly treat the flowback water (Ferrar et al. 2013, Warner et al. 2013). Post-treatment flowback water is released in WWTP effluent into local streams and surface water. WWTP effluent increases the salinity of the freshwater streams and the radioactivity of sediments (Warner et al. 2013). The WWTP effluent can affect water quality for creatures and animals that live in the aquatic/riparian ecosystems. The effluent can also have effects on local drinking water treatment plant intake facilities (VanBriesen and Good 2017, Wilson et al. 2014, VanBriesen and Wilson 2012).

Poor water quality can often lead to lower levels of biodiversity in aquatic organisms, including benthic macroinvertebrates. Local surface water quality is often assessed by studying the degradation of the biodiversity of aquatic organisms. Environments in and around rapid HVHF development areas have experienced a decrease in biodiversity in terms of population density (Kiviat 2013, Entrekin et al. 2011). Additionally, independent from sample size, local water quality has historically been studied through an examination of bioaccumulation in remaining animals that live in or near the aquatic/riparian biome. Contaminants of concern bioaccumulate in the bloodstream and tissues of the animals after they are exposed to contaminated water (Carson 1962, Furness and Camphuysen 1997). Studying the animals' bloodstream and tissue accumulation can be used to help quantify regional surface water contamination.

Bird feathers are commonly used as bioindicators to assess the quality of a bird's ecosystem and to measure possible environmental contaminants. Specifically, by studying contaminant bioaccumulation in feathers from riparian bird species living exclusively near waterways, such as the Louisiana Waterthrush bird species, researchers can assess local water quality. The Louisiana Waterthrush is a songbird that often nests near freshwater streams in linear territories that are roughly 300-1,200 meters, or 980-4,000 feet, long, in parallel with the streams (Robinson 1995, Mulvihill et al. 2008, Mattsson and Cooper 2006). The Louisiana Waterthrush has a substantial population density overlapping with many shale regions (Figure 1). A Waterthrush diet consists mainly of benthic macroinvertebrates that live exclusively in freshwater streams. Stream contaminants bioaccumulate in the bloodstream of the macroinvertebrates. Once the macroinvertebrates are consumed by the bird, stream contaminants will then accumulate in the bloodstream of the birds (Robinson 1995). Growing feathers are connected with blood vessels; therefore, the quality of the food and water that the bird intakes during a feather's growth period is represented in the makeup of the feather (Burger 1993, Furness 1993). While direct measurements of blood samples from riparian bird species best represent bloodstream contamination, the contaminants of interest are also incorporated into newly grown bird feathers. When compared to the process for obtaining blood samples, bird feathers are obtained through a less invasive process.

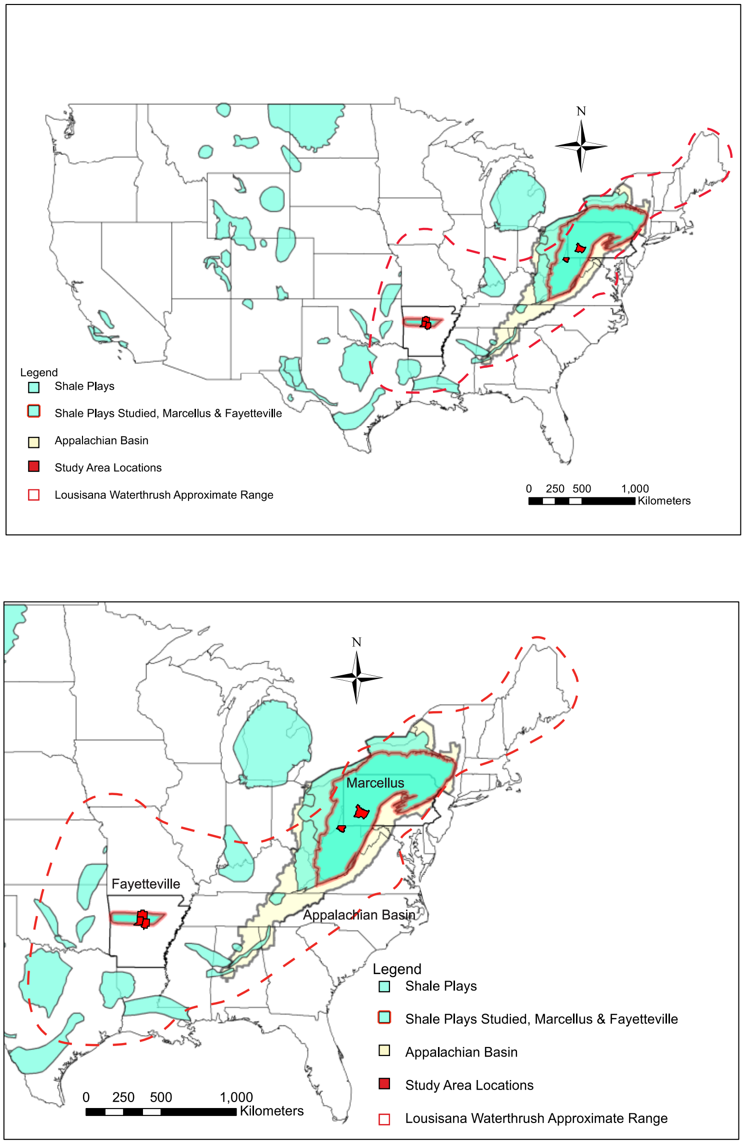

Figure 1. The Louisiana Waterthrush bird species territory overlaps with many shale regions. Specifically, the Marcellus Shale region and the Fayetteville Shale region are of main focus in this study. Similar methods of bioaccumulation analysis can be implemented in other shale regions with substantial Louisiana Waterthrush population density.

Bioaccumulation is measured using contaminant concentration per mass of feather (Macko et al 1997). However, the mass of contaminants can be normalized to other elements necessary for feather growth. For example, Sr, Ba, and calcium (Ca) have similar chemical properties and follow analogous biological pathways within the organism. Sr and Ba are capable of bioaccumulating in bird feathers, and are reliable markers for the determination of the impacts of HVHF on surface water quality. Sr and Ba enter the organism proportionally to Ca via bloodstream absorption through food and water intake (Ebel and Comar 1968). Sr and Ba concentrations can be normalized to the total concentrations of Ca that are present in the feather to account for greater incorporation of Ca (and therefore Sr and Ba) that may occur throughout the feather growth period. While there are no strong statistics supporting health risks due to high Sr and Ba exposure, it is likely that other harmful contaminants from HVHF and oil and gas development have impacted regional freshwater streams if other indicators of HVHF, such as Sr and Ba, are found in high ratios in the bird feather (Latta et al. 2015, Maurer et al. 2012).

While feathers are thought to be good indicators of bioaccumulation and water quality contamination, there are potential issues that could complicate their use. For example, the presence of small dust particles due to incomplete cleaning of the feathers could result in artificially high metal concentration results. Remaining dust particles can cause misleading measurements of apparent bloodstream incorporation of contaminants. Dust pollution can be caused from construction of roads and well pads, truck traffic, and many other industrial and human activities. Dust pollution is a means for dust particles to be incorporated onto the feathers, but not into the bloodstream or into the feather. Aluminum (Al) is a key indicator that dust particles are present on the bird's feather and not in the bloodstream. Studying Al concentrations relative to Ca, Sr, and Ba can determine if dust particles are incorporated onto the feather. If substantial Al concentrations are found, it would suggest that contaminant levels measured in the feathers might be due to dust pollution and surface contamination from inadequate cleaning of feathers prior to analyzation. However, correlation of Al with Ca, Sr, and Ba can indicate dust pollution is present in the bird's ecosystem (Borghesi et al. 2017, Simon et al. 2011).

Latta et al. (2015) reports that the amounts of Sr and Ba bioaccumulated in Louisiana Waterthrush that live near sites with HVHF activity is significantly higher compared to those without HVHF activity. The research group analyzed Sr and Ba concentrations in newly grown feathers. Ca concentration measurements were not reported in this specific study; therefore, a mass ratio approach was not investigated. The sample size of the study includes 285 feathers. Statistical results of the Latta et al. (2015) study show that HVHF is associated with increased Sr (p-value = 0.01) and Ba (p-value = 0.04) concentrations. In addition to increased Sr and Ba concentrations near HVHF sites, a significant difference in the concentration values was observed between the Marcellus Shale region and the Fayetteville Shale region. The Marcellus Shale regional data showed increased concentrations of both Sr (p-value < 0.01) and Ba (p-value < 0.01) compared to the Fayetteville Shale region. The interaction of regional differences and HVHF history was not statistically significant for either Sr (p-value = 0.62) or Ba (p-value = 0.78) concentrations. Historical testing did not observe natural increases in Sr and Ba concentrations in sites without HVHF. A ratio was used to compare Sr and Ba concentration data from 1997 – 2002 with Sr and Ba data from 2010 – 2013. Concentration levels remained relatively constant over the observed timeframe for both Sr (ratio = 0.89 – 1.67) and Ba (ratio = 0.64 – 1.40) concentrations.

The goal of this study is to demonstrate the use of Ba/Ca and Sr/Ca concentration ratios to track water contamination and bioaccumulation in a riparian bird species. Through collaboration with the authors of the Latta et al. (2015) study, this study will analyze Sr/Ca and Ba/Ca concentration ratios in feather duplicates (i.e, feathers collected in the same nest and at the same time) of feathers measured, analyzed and published in 2015. An objective of this study is to determine if high concentrations of Sr and Ba and $^{87}Sr/^{86}Sr$ ratios observed in Louisiana Waterthrush feathers correspond to oil and gas development. Another objective is to determine if observed bioaccumulation reflects water quality degradation or air quality and dust contamination of the bird's habitat.

Chapter 2

METHODS

2.1 SAMPLE COLLECTION

Louisiana Waterthrush feather samples were removed from birds that were nesting near streams in the Marcellus (PA & WV) and Fayetteville (AR) shale regions (Table 1). Feathers were collected from stream habitats that are considered to have “active” and “non-active” HVHF activity. “Active” is defined as one or more active shale gas well pads, access roads, or pipelines upstream from the collection site. A mist net method was used to temporarily capture the birds. The outer rectrix (R6) feathers were collected from adult birds. Nestling R6 feathers were collected 7-8 days from the start of the nesting period. Feathers are assured to be grown at the sample habitat stream because only newly grown adult, nestling, and color-banded adult bird R6 feathers were collected. A year before the feather collection stage begun, color-band tags were placed on birds to ensure feather samples were grown in the sample habitats and regions. (A detailed version of the feather sampling strategy can be found in Latta et al 2015.)

Table 1. Feathers from the Louisiana Waterthrush bird species were sampled in various streams in the Marcellus (PA and WV) and Fayetteville (AR) shale regions.

Shale Region	US State	State County	Freshwater Habitat		
Marcellus	Pennsylvania	Westmoreland	Loyalhanna Creek Powdermill Run Camp Run		
Marcellus	West Virginia	Wetzel	Buffalo Run Carpenter Run Hile’s Run Huss Penn Run	Megan’s Run Nettles Run Olive Run Owl Run	Sees Run Slabcamp Run Snake Run Wyatt’s Run
Fayetteville	Arkansas	Van Buren Conway Faulker	Little Red River Cedar Creek Sis Hollow	Point Remove Creek East Fork Point Remove Creek	Sunnyside Creek Black Fork South Fork

2.2 LABORATORY TESTING

To prepare feathers prior to contaminant analysis, they were washed with non-ionic surfactant Triton X-100 soap for 1 minute and rinsed a minimum of 6 times with deionized (DI) water. The feathers were freeze dried to remove all moisture. The dry mass of the feather was recorded, and is on average about 0.05 mg. The feathers were acid digested using 1.0 mL of 9:1 HNO_3:HCL Omni-trace solution in a pressurized microwave Teflon vessel. One feather, cut in half with ceramic scissors, was placed into each vessel. For every eleven feather samples digested, a twelfth vessel was filled with acid but contained no feather sample to collect method blank correction data. The vessels were heated to 105°C for 30 minutes in a CEM MARS 6 Express microwave digestion system. When the vessels cooled, 4 mL of DI water and 2 drops of peroxide (H_2O_2) were added to the vessels. The vessels were heated again to 90°C for 10 minutes. A 9:1 DI water:digestion dilution was prepared for inductively coupled plasma mass spectroscopy (ICP-MS) on a Thermo-Fischer Scientific X Series II-SBM and X Series II-MFM ICP-MS Quadrapole. The following isotopic elements were tested for: Sr, Ba, Al, and Ca. Samples were also analyzed for $^{87}Sr/^{86}Sr$ on a ThermoFisher Scientific Triton Plus thermal ionization mass spectrometer (TIMS). The precision of $^{87}Sr/^{86}Sr$ of the IAPSO standard used in the Triton Plus was $^{87}Sr/^{86}Sr = 0.70917 \pm 0.0003$. Sr was separated from the digested feathers using an Elemental Scientific prepFAST-MC. Yield checks confirmed that over 98% of the Sr was recovered.

2.3 STATISTICS

The statistical computing and graphics software, “R,” was used for the statistical analysis of the Sr, Ba, Ca, and Al feather concentrations and concentration ratios. Feathers samples were collected from Arkansas and Pennsylvania in both active and non-active drilling areas. A comparison of Sr and Ba concentrations was also completed for comparison to results from a previous study. For the statistical comparison, samples were standardized according to feather mass, not feather length. After standardization, it is assumed that juvenile and adult feathers can be included in the same category (Latta et al. 2015). Sample data were sorted into six categories that depend on drilling activity and location: 1) Active – Marcellus & Fayetteville, 2) Non-Active – Marcellus & Fayetteville, 3) Active – Marcellus, 4) Non-Active – Marcellus, 5) Active – Fayetteville, and 6) Non-Active – Fayetteville. Data in all categories were not normally distributed, according to a Kruskal-Wallis test. The values were log-transformed to normality. A Pearson’s test, Spearman’s test, and Wilcoxon Rank Sum were completed on the log-transformed data to compare the desired categories. Table 2 indicates the number of feather samples (n-value) for each category.

Table 2. All samples were placed into one of six categories based on drilling activity and location. The number of samples for each category is represented by the n-value.

Category			n-value
1	Active	Marcellus & Fayetteville	50
2	Non-Active	Marcellus & Fayetteville	46
		Marcellus & Fayetteville TOTAL	**96**
3	Active	Marcellus	25
4	Non-Active	Marcellus	25
		Marcellus TOTAL	**50**
5	Active	Fayetteville	25
6	Non-Active	Fayetteville	21
		Fayetteville TOTAL	**46**

Chapter 3

RESULTS & DISCUSSION

The concentration data (μg/g) of part per billion (ppb) for Ca, Sr, Ba, and Al do not fit a normal distribution. The distributions for Ca, Sr, Ba, and Al can be found in Figures 2a, 2b, 2c, and 2d, respectively. The log transformed concentration data for Ca, Sr, Ba, and Al fit normal distributions and can be found in Figures 2e, 2f, 2g, and 2h, respectively. The log-transformed Ca data fits a one-sided normal distribution curve. The log-transformed Sr, Ba, and Al concentration data fit a two-sided normal distribution curve.

The Pearson's and Spearman's Test are used to determine if data sets are correlated. P-value (p) and correlation factor (ρ) results for the Pearson's Test (p_p, ρ_p) and Spearman's Test (p_s, ρ_s) are shown in Table 3 and Table 4, respectively. A p-value less than 0.05 and a correlation factor greater than 0.50 indicates with a 95% confidence interval that there is correlation between the data sets. Sr vs. Ca ($p_p = 8.2e\text{-}10$, $\rho_p = 0.61$; $p_s = 2.3e\text{-}09$, $\rho_s = 0.60$), Ba vs. Ca ($p_p < 2.2e\text{-}16$, $\rho_p = 0.79$; $p_s < 2.2e\text{-}16$, $\rho_s = 0.66$), and Sr vs. Ba ($p_p = 3.5e\text{-}12$, $\rho_p = 0.67$; $p_s < 2.2e\text{-}16$, $\rho_s = 0.62$) all contain a p-value less than 0.05 and a correlation factor greater than 0.50. A correlation exists between Sr, Ba, and Ca concentrations in both the Marcellus and Fayetteville Shale regions. The statistical significance was expected to demonstrate Sr, Ba, and Ca undergo similar biological pathways within an organism. Changes in bioaccumulation of Sr, Ba, and Ca will occur in similar amounts since they contain comparable biological pathways. The method of using high Sr/Ca and Ba/Ca ratios as a key indicator for bioaccumulation at active HVHF sites relied on the study supporting similar biological pathways existed between Sr, Ba, and Ca (Ebel and Comar 1968). The statistical significance in the Pearson's and Spearman's Test that there is a correlation between Sr, Ba, and Ca concentrations is consistent with previous studies. Higher Sr/Ca and Ba/Ca ratios in surface water could potentially indicate HVHF contamination. Flowback fluids contain high Ca concentrations, but elevated ratios are thought to indicate flowback fluid, in particular. It is possible that both Sr, Ba, and Ca concentrations are elevated in flowback fluid contaminated surface water.

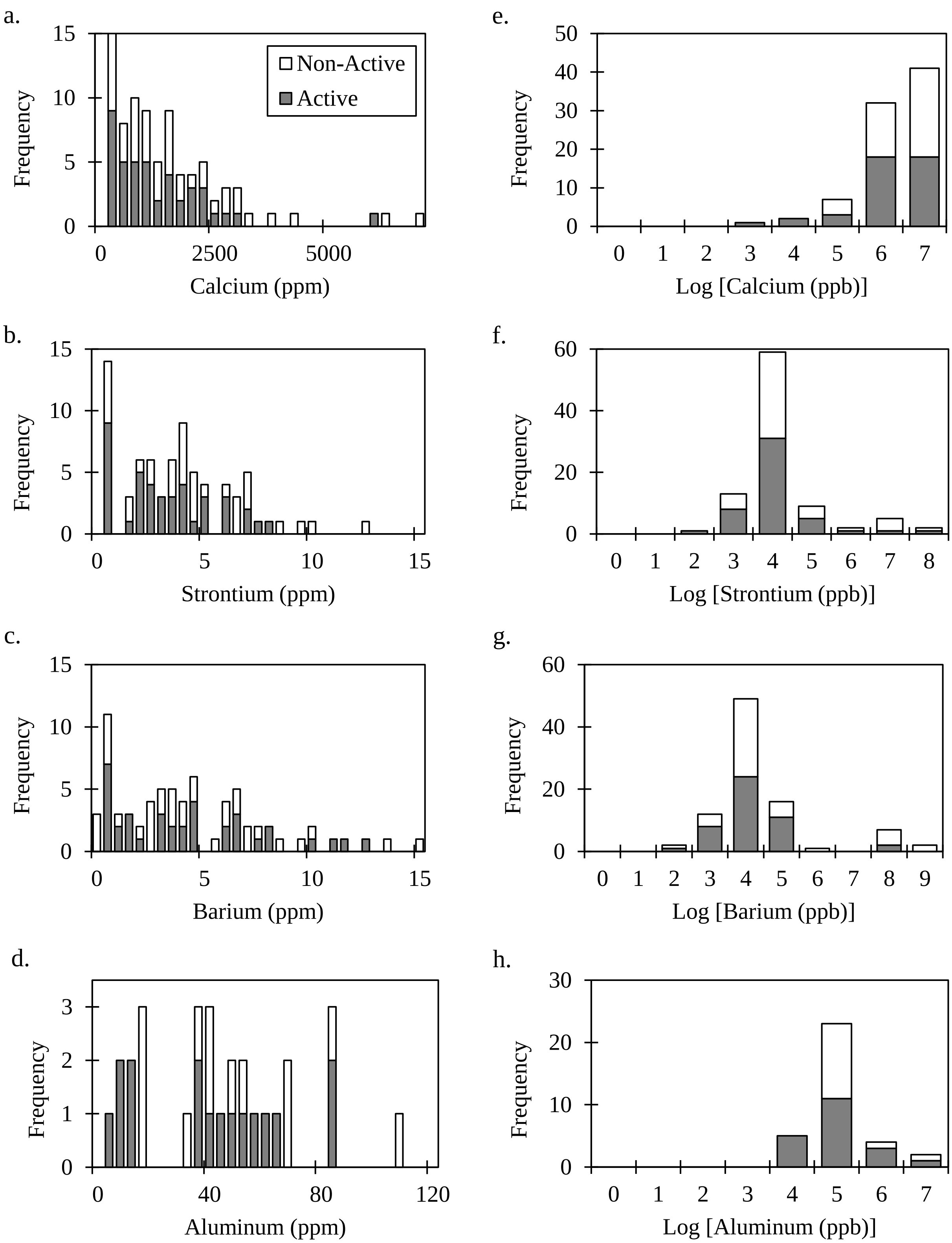

Figure 2. Non-normal data distribution sets for Ca, Sr, Ba, and Al concentrations can be found in Figures a, b, c, and d, respectively. Normal data distribution sets for log-transformed Ca, Sr, Ba, and Al concentrations can be found in Figures e, f, g, and h, respectively. The log-transformed Ca data fits a one-sided normal distribution curve. The log-transformed Sr, Ba, and Al concentration data fit a two-sided normal distribution curve.

Table 3. The Pearson's Test results in correlation between Ba, Sr, and Ca. No correlation exists between Al with Sr, Ba, and Ca. All statistical conclusions are drawn using a 95% confidence interval.

Location		Element	n-value	Pearson's Test	
				p-value	Correlation Factor
1	Marcellus & Fayetteville	Sr	92	8.2e-10	0.61
	Marcellus & Fayetteville	Ca	85		
2	Marcellus & Fayetteville	Ba	89	<2.2e-16	0.79
	Marcellus & Fayetteville	Ca	84		
3	Marcellus & Fayetteville	Sr	92	3.5e-12	0.67
	Marcellus & Fayetteville	Ba	89		
4	Marcellus & Fayetteville	Al	40	0.85	0.03
	Marcellus & Fayetteville	Sr	92		
5	Marcellus & Fayetteville	Al	40	0.17	0.22
	Marcellus & Fayetteville	Ba	89		
6	Marcellus & Fayetteville	Al	40	0.52	0.10
	Marcellus & Fayetteville	Ca	85		

Table 4. The Spearman's Test results in correlation between Ba, Sr, and Ca. No correlation exists between Al with Sr and Ca, while a correlation does exist between Al and Ba. All statistical conclusions are drawn using a 95% confidence interval.

Location		Element	n-value	Spearman's Test	
				p-value	Correlation Factor
1	Marcellus & Fayetteville	Sr	92	2.3×10^{-9}	0.60
	Marcellus & Fayetteville	Ca	85		
2	Marcellus & Fayetteville	Ba	89	$< 2.2 \times 10^{-16}$	0.66
	Marcellus & Fayetteville	Ca	84		
3	Marcellus & Fayetteville	Sr	92	$< 2.2 \times 10^{-16}$	0.62
	Marcellus & Fayetteville	Ba	89		
4	Marcellus & Fayetteville	Al	40	0.22	0.19
	Marcellus & Fayetteville	Sr	92		
5	Marcellus & Fayetteville	Al	40	1.7×10^{-3}	0.47
	Marcellus & Fayetteville	Ba	89		
6	Marcellus & Fayetteville	Al	40	0.10	0.26
	Marcellus & Fayetteville	Ca	85		

The Pearson's Test indicates no correlation exists between Al with Sr, Ba, and Ca in the Marcellus Shale region. No Al data was available from the Fayetteville Shale regional feather samples. A p-value less than 0.05 and a correlation factor greater than 0.50 indicates with a 95% confidence interval that there is correlation between the two data sets. Al vs. Sr ($p_p = 0.85$, $\rho_p = 0.03$), Al vs. Ba ($p_p = 0.17$, $\rho_p = 0.21$), and Al vs. Ca ($p_p = 0.52$, $\rho_p = 0.10$) all contain a p-value greater than 0.05 and a correlation factor less than 0.50. The Spearman's Test indicates no correlation exists between Al with Sr and Ca; however, there is a correlation between Al and Ba. Al vs. Sr ($p_s = 0.22$, $\rho_s = 0.19$) and Al vs. Ca ($p_s = 0.10$, $\rho_s = 0.26$) all contain a p-value greater than 0.05 and a correlation factor less than 0.50. Al vs. Ba ($p_s = 1.7e\text{-}3$, $\rho_s = 0.47$) contains a p-value less than 0.05 and a correlation factor slightly less than 0.50. With five out of the six statistical tests reporting no correlation between Al with Sr, Ba, and Ca, it can be concluded that majority of the feather samples were washed well and were likely not contaminated with dust/clay particles. This indicates that Sr, Ba, and Ca feather concentrations are not likely caused by dust contamination. Possible reasons for the correlation between Al and Ba using the Spearman's Test is that dust/clay particles contaminated certain feather samples due to improper washing, or that the laboratory surroundings during feather sample preparation could have been dusty. Another hypothesis is that the bird's habitat contains high levels of dust pollution due to nearby human development. In this case, dust/clay particles can become incorporated into the outer layer of a newly grown feather. In general, there is a slight positive relationship between Sr/Ca, Ba/Ca, Sr, Ba, and Ca versus Al. The active HVHF site samples dominate the positive trend. The log-transformed Sr/Ca, Ba/Ca, Sr, Ba, and Ca versus Al data plots are shown in Figure 3a, 3b, 3c, 3d, and 3e, respectively.

The Wilcoxon Rank Sum Test indicates there is no statistically significant difference between Sr/Ca and Ba/Ca feather concentration ratios collected from active versus non-active HVHF sites in neither the Marcellus Shale region nor the Fayetteville Shale region. Additionally, the Wilcoxon Rank Sum Test indicates that there is no statistically significant difference between Sr, Ba, Ca, or Al concentrations at active versus non-active HVHF sites in neither the Marcellus Shale region nor the Fayetteville Shale region

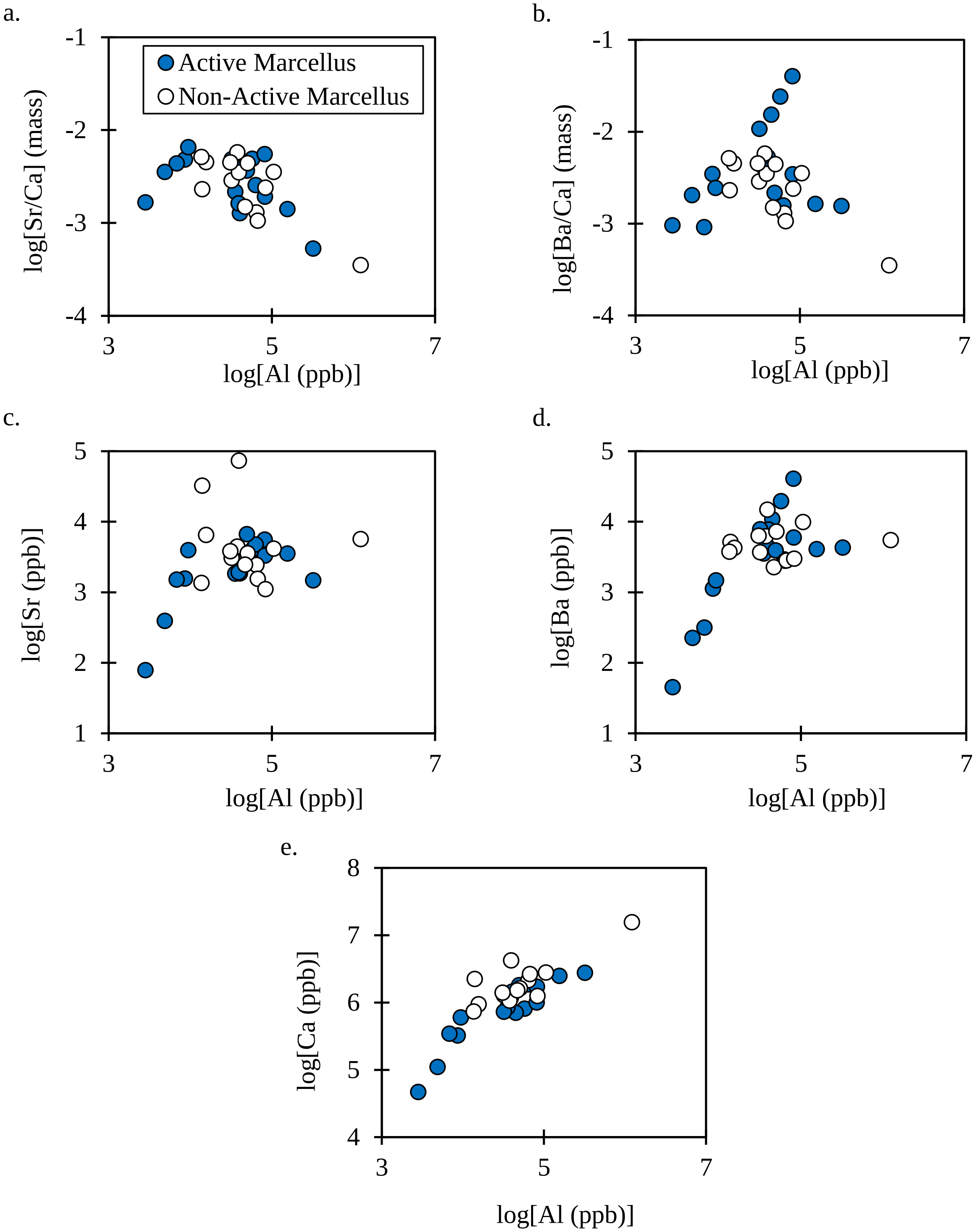

Figure 3. Figures a and b show the comparisons of log-transformed data for Sr/Ca and Ba/Ca concentration ratios versus Al concentration. Figures c, d, and e show log-transformed data for Sr, Ba, and Ca concentrations compared to Al concentration. In general, all data plots observe a positive relationship with Al concentration and is best observed in the active Marcellus Shale regional samples.

in this data set. A p-value less than 0.05 indicates with a 95% confidence interval that there is a statistically significant difference between the two data sets. The p-value results for the Wilcoxon Rank Sum Test (p_{wrs}) are shown in Table 5. Neither the concentration ratios nor the concentration data supports that there is a statistically significant difference in populations. That statistical significance of this data set indicates that Sr and Ba are likely not bioaccumulating in higher amounts in feathers obtained from HVHF regions than Sr and Ba naturally found in the bird feathers from regions without HVHF. Furthermore, if HVHF is contaminating surface waters, it is unlikely that it has an effect on the bioaccumulation of organisms relying on the aquatic ecosystem as its habitat.

Recent studies have shown that contamination of surface water near active HVHF sites has likely occurred, and that concentrations of Sr and Ba at active HVHF sites are significantly higher compared to non-active HVHF sites (Latta et al. 2015). The statistical results of this study suggest that there is no significant difference in the feathers measured (n = 96), which is a notably smaller sample size than that studied in Latta et al. (2015) (n = 285). Upon analysis of the plots in Figure 2, it can be found that the Fayetteville Shale regional data is more spread out compared to the Marcellus Shale regional data. More precisely, the active Fayetteville Shale regional samples have a more diverse concentration and concentration ratio than all other samples. The active and non-active Marcellus Shale regional samples are more concentrated around a similar value. It is also observed that the calculated ratios (Ba/Ca and Sr/Ca) are controlled by the Ca concentrations. The Sr/Ca and Ba/Ca concentration ratios have a negative relationship with Ca concentrations, while the Sr and Ba concentrations are positively correlated with Ca concentrations. As Ca concentrations increase, Sr and Ba concentrations also increase, while Sr/Ca and Ba/Ca concentration ratios decrease. This can possibly indicate less Sr and Ba is incorporated into the feather during growth periods when Ca is in great abundance and uptake. The log-transformed Sr/Ca, Sr, Ba/Ca, and Ba versus Ca data plots are shown in Figure 4a, 4b, 4c, and 4d, respectively.

Table 5. Wilcoxon Rank Sum Test results show that with a 95% confidence interval no correlation exists between Active and Non-Active HVHF sites at any of the tested locations.

Location		Drilling Activity	Element or Elemental Ratio	n-value	Wilcoxon Rank Sum Test
					p-value
1	Marcellus & Fayetteville	Non-Active	Sr/Ca	43	0.51
	Marcellus & Fayetteville	Active		44	
2	Marcellus & Fayetteville	Non-Active	Ba/Ca	42	0.28
	Marcellus & Fayetteville	Active		46	
3	Marcellus	Non-Active	Sr/Ca	24	0.36
	Marcellus	Active		25	
4	Marcellus	Non-Active	Ba/Ca	25	0.88
	Marcellus	Active		25	
5	Fayetteville	Non-Active	Sr/Ca	19	0.36
	Fayetteville	Active		19	
6	Fayetteville	Non-Active	Ba/Ca	17	0.06
	Fayetteville	Active		21	
7	Marcellus & Fayetteville	Non-Active	Ca	43	0.19
	Marcellus & Fayetteville	Active		42	
8	Marcellus & Fayetteville	Non-Active	Sr	44	0.21
	Marcellus & Fayetteville	Active		48	
9	Marcellus & Fayetteville	Non-Active	Ba	43	0.20
	Marcellus & Fayetteville	Active		46	
10	Marcellus & Fayetteville	Non-Active	Al	21	0.25
	Marcellus & Fayetteville	Active		19	
11	Marcellus	Non-Active	Ca	25	0.12
	Marcellus	Active		25	
12	Marcellus	Non-Active	Sr	24	0.63
	Marcellus	Active		25	
13	Marcellus	Non-Active	Ba	25	0.44
	Marcellus	Active		25	
14	Fayetteville	Non-Active	Ca	18	0.57
	Fayetteville	Active		17	
15	Fayetteville	Non-Active	Sr	20	0.57
	Fayetteville	Active		23	
16	Fayetteville	Non-Active	Ba	18	0.99
	Fayetteville	Active		21	

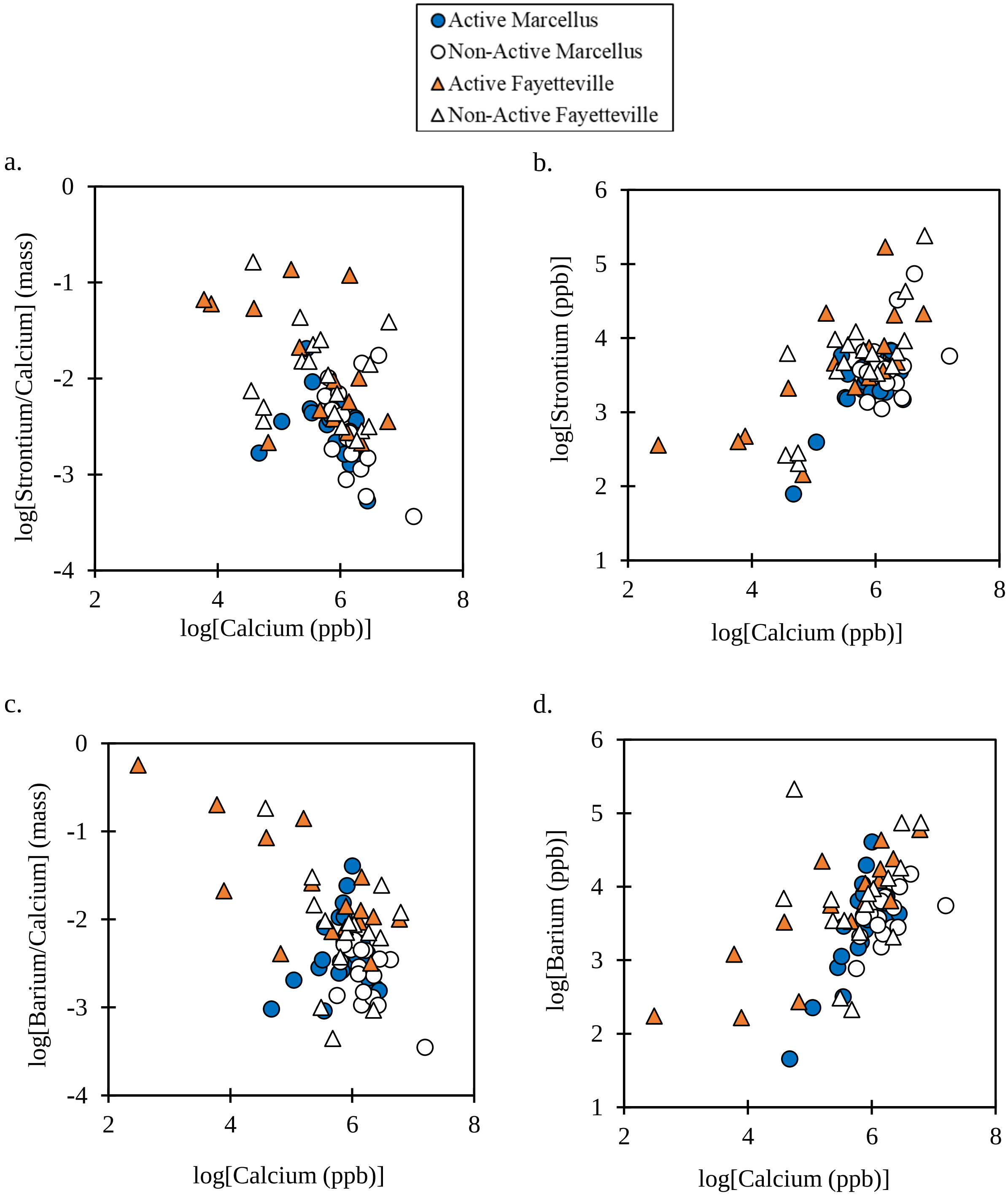

Figure 4. Figures a and b show the comparisons of log-transformed data for Sr and Ba concentrations versus Ca concentration. Figures c and d show log-transformed data for Sr/Ca and Ba/Ca concentration ratios compared to Ca concentration. In general, the data is driven by the Ca concentrations. A negative relationship exists between Sr/Ca and Ba/Ca concentration ratios versus Ca concentration. A positive relationship exists between Sr and Ba concentrations versus Ca concentration. In addition, the Fayetteville Shale regional samples are more diverse/spread out in all plots.

While Sr/Ca and Ba/Ca concentration ratios are thought to be good indicators of flowback fluid, $^{87}Sr/^{86}Sr$ ratios can also be a good indicator. $^{87}Sr/^{86}Sr$ ratios are generally lower in flowback fluids compared to average surface waters. In the Fayetteville Shale region, flowback fluids generally had a $^{87}Sr/^{86}Sr$ ratio ranging from 0.7095 – 0.7105. In contrast, shallow groundwater collected in areas of Fayetteville Shale HVHF development had a $^{87}Sr/^{86}Sr$ ratio ranging from 0.7100 to 0.7170. The majority of the water samples had a $^{87}Sr/^{86}Sr$ ratio ranging from 0.7130 – 0.716 (Warner et al. 2013). None of the shallow groundwater samples were thought to be contaminated with Fayetteville flowback water (Warner et al. 2013). The $^{87}Sr/^{86}Sr$ ratios measured in the bird feathers (n = 12) do not conclusively indicate $^{87}Sr/^{86}Sr$ ratios from the Fayetteville Shale formation flowback fluids and/or shallow groundwater (Warner et al. 2013). The majority of the $^{87}Sr/^{86}Sr$ ratios for bird feathers lie in the overlap region of $^{87}Sr/^{86}Sr$ ratio for Fayetteville formation flowback fluid and shallow groundwater (Figure 4). The bird feather sample size tested for a $^{87}Sr/^{86}Sr$ ratio is too small to conduct a full statistical analysis on the active versus non-active groups. High Sr bird feather concentrations do not directly correspond to lower $^{87}Sr/^{86}Sr$ ratios, which could indicate HVHF. A continued study of $^{87}Sr/^{86}Sr$ ratios for bird feathers collected in both active and non-active Fayetteville Shale regions should be performed, increasing the sample size for accurate statistical analysis.

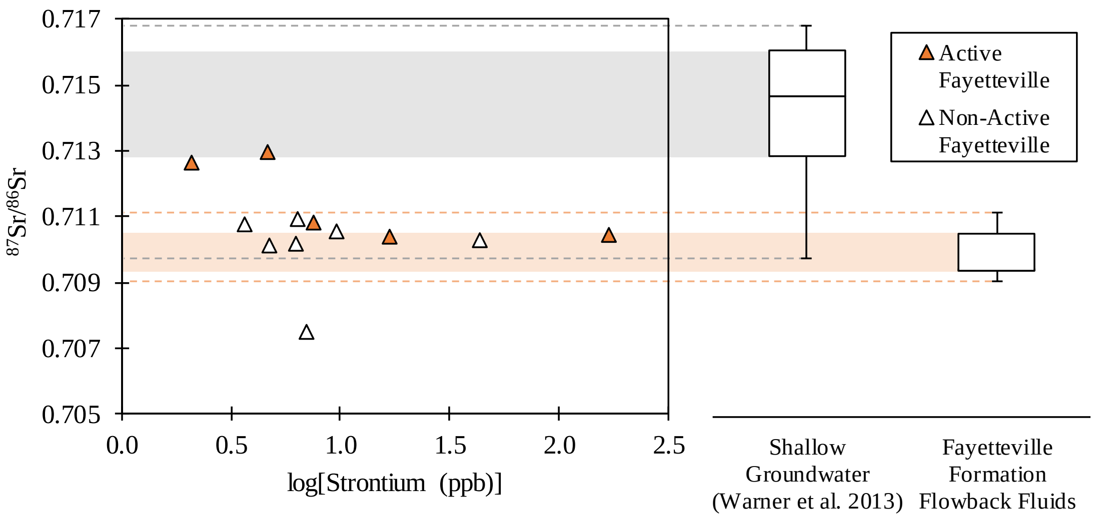

Figure 5. The $^{87}Sr/^{86}Sr$ results for feathers collected in the Fayetteville Shale region (n = 12), both at active and non-active sites, is comparable to $^{87}Sr/^{86}Sr$ data for Fayetteville formation flowback fluid and shallow ground water reported by Warner et. al (2013). Majority of the $^{87}Sr/^{86}Sr$ bird feather measurements lie in the overlap region for the flowback fluid and shallow groundwater. Further studies with a larger sample size should be performed.

Chapter 4

CONCLUSION

The study obtained consistent results with previous findings that Sr, Ba, and Ca contain similar biological pathways within an organism. Specifically, during the growth period of a bird feather, when Ca is needed from the bloodstream for incorporation into the newly grown feather, Sr and Ba can act as a replacement and therefore bioaccumulate in the feather. Dust/clay particulate contamination is indicated by high Al concentrations. Al contamination can occur on the surface of the bird feather, or through incorporation into the feather from high dust pollution in the surrounding air. Al surface contamination is possible, but unlikely in this study due to the thorough cleaning technique used.

There is no statistical difference in this study between Sr, Ba, Ca, Al, Sr/Ca, and Ba/Ca measurements in active versus non-active HVHF sites in neither the Marcellus Shale region nor the Fayetteville Shale region. Compared to the non-active Fayetteville Shale regional samples, the active Fayetteville Shale regional samples have a more diverse concentration and concentration ratio. The active and non-active Marcellus Shale regional samples are concentrated around a similar value. Upon evaluation of the Sr/Ca and Ba/Ca ratios versus Ca concentration, samples with high Ca concentrations have a lower ratio. The data is controlled by the Ca concentration, suggesting that less Sr and Ba is incorporated into the feather during growth periods when Ca is in great abundance. Further studies with a larger sample size can help conclude whether or not there is a statistical difference between feathers collected at active versus non-active HVHF sites.

The $^{87}Sr/^{86}Sr$ ratios for bird feather samples collected in the Fayetteville Shale region are inconclusive as to whether or not the active HVHF sites are distinguishable from the non-active HVHF sites. Previous data from Warner et al. (2013) show that $^{87}Sr/^{86}Sr$ for Fayetteville formation flowback fluids are lower than $^{87}Sr/^{86}Sr$ for shallow groundwater. This distinction in the $^{87}Sr/^{86}Sr$ ratios suggests that higher

^{86}Sr isotope concentrations are found in active HVHF produced fluids than ^{86}Sr isotope concentrations found naturally occurring in shallow groundwater and therefore surface water. Only 12 bird feather samples from the Fayetteville Shale region were tested for ^{87}Sr/^{86}Sr results. The majority of the bird feather samples resulted in the overlap region where the ^{87}Sr/^{86}Sr could resemble either Fayetteville formation flowback fluid or shallow groundwater. The sample size is too small to conduct proper statistical analysis. A continued study with a larger sample size can have the greater statistical power needed to draw significant conclusions.

Lastly, directly evaluating Sr, Ba, and Al bioaccumulation in the food source of the Waterthrush bird species eliminates a step in the food chain. Digestion of the macroinvertebrates that live in freshwater streams is conducted in a similar manner to that of the bird feather digestion. Analysis of macroinvertebrate samples for HVHF associated contaminants reaches closer to a goal of the study – discovering whether or not HVHF flowback water is contaminating surface water and imposing lasting threats on aquatic life and riparian habitats.

Appendix A

CONCENTRATION VALUES

The master table for all measured concentration values was made in an Excel Spreadsheet. The values below were used to prepare all figures. An empty cell signifies no data was recorded or the data was below the detection limit.

Ca ppb	log(Ca) log[ppb]	Sr ppb	log(Sr) log[ppb]	Sr ppb	log(Sr/Ca) log[ppb]	Ba ppb	log(Ba) log[ppb]	Ba/Ca ppb	log(Ba/Ca) log[ppb]	Al ppb	log(Al) log[ppb]	Active Non-Active	Marcellus Fayetteville
1756593	6	6831	4	0.0039	-2.4102	7308	4	0.0042	-2.3809			A	M
608213	6	2015	3	0.0033	-2.4798	6466	4	0.0106	-1.9734			A	M
797950	6	5746	4	0.0072	-2.1426	2596	3	0.0033	-2.4877			A	M
673622	6	2529	3	0.0038	-2.4254	1762	3	0.0026	-2.5825			A	M
353617	6	3267	4	0.0092	-2.0344	2907	3	0.0082	-2.0851			A	M
282700	5	5789	4	0.0205	-1.6887	800	3	0.0028	-2.5480			A	M
817483	6	4041	4	0.0049	-2.3060	19721	4	0.0241	-1.6175	57639	5	A	M
709614	6	2923	3	0.0041	-2.3852	10893	4	0.0153	-1.8139	45003	5	A	M
1456039	6	1864	3	0.0013	-2.8928	7777	4	0.0053	-2.2724	40702	5	A	M
850976	6	1851	3	0.0022	-2.6625	3567	4	0.0042	-2.3776	35539	5	A	M
1009619	6	5588	4	0.0055	-2.2569	40793	5	0.0404	-1.3936	81455	5	A	M
325160	6	1573	3	0.0048	-2.3155	1128	3	0.0035	-2.4599	8627	4	A	M
1178878	6	1917	3	0.0016	-2.7889	4494	4	0.0038	-2.4188	39123	5	A	M
2502733	6	3547	4	0.0014	-2.8485	4109	4	0.0016	-2.7847	155230	5	A	M
47184	5	79	2	0.0017	-2.7763	45	2	0.0010	-3.0167	2821	3	A	M
346208	6	1523	3	0.0044	-2.3566	317	3	0.0009	-3.0388	6825	4	A	M
603906	6	3979	4	0.0066	-2.1812	1480	3	0.0025	-2.6107	9426	4	A	M
1860756	6	4770	4	0.0026	-2.5912	2932	3	0.0016	-2.8025	63241	5	A	M
110538	5	393	3	0.0036	-2.4487	227	2	0.0021	-2.6880	4883	4	A	M
1812124	6	6672	4	0.0037	-2.4340	3926	4	0.0022	-2.6643	49353	5	A	M
733026	6	3576	4	0.0049	-2.3117	7883	4	0.0108	-1.9684	32097	5	A	M
1729926	6	3332	4	0.0019	-2.7153	5983	4	0.0035	-2.4611	81991	5	A	M
2769360	6	1476	3	0.0005	-3.2733	4308	4	0.0016	-2.8081	320380	6	A	M
2484195	6	6601452	7	2.6574	0.4245	78033846	8	31.4121	1.4971	475810	6	A	M
2169962	6	10740723	7	4.9497	0.6946	34771762	8	16.0241	1.2048	1177680	6	A	M
1658139	6	3237	4	0.0020	-2.7094	4055	4	0.0024	-2.6116			A	F
1.31E+06	6	3.59E+03	4	0.0027	-2.5630	1.22E+04	4	0.0093	-2.0308			A	F
2.23E+06	6	4.73E+03	4	0.0021	-2.6735	2.40E+04	4	0.0108	-1.9678			A	F
5.95E+06	7	2.13E+04	4	0.0036	-2.4470	6.01E+04	5	0.0101	-1.9959			A	F
1.38E+06	6	7.89E+03	4	0.0057	-2.2422	1.74E+04	4	0.0127	-1.8974			A	F
7.72E+05	6	2.94E+03	3	0.0038	-2.4191	6.32E+03	4	0.0082	-2.0867			A	F
-8.55E+05		1.67E+04	4	-0.0196		9.60E+03	4	-0.0112				A	F
-4.50E+04		3.06E+04	4	-0.6788		2.74E+04	4	-0.6094				A	F
2.17E+05	5	4.60E+03	4	0.0212	-1.6733	5.63E+03	4	0.0260	-1.5859			A	F
7.84E+05	6	7.43E+03	4	0.0095	-2.0235	1.11E+04	4	0.0142	-1.8485			A	F
-1.25E+05		2.06E+03	3	-0.0164		8.28E+02	3	-0.0066				A	F
4.66E+05	6	2.18E+03	3	0.0047	-2.3296	3.38E+03	4	0.0073	-2.1387			A	F
1.42E+06	6	1.69E+05	5	0.1190	-0.9244	4.31E+04	5	0.0302	-1.5195			A	F
2.02E+06	6	2.04E+04	4	0.0101	-1.9948	6.44E+03	4	0.0032	-2.4964			A	F
-2.16E+06		-3.34E+03		0.0015	-2.8113	-1.50E+04		0.0069	-2.1589			A	F
-1.87E+06		-2.88E+03		0.0015	-2.8113	-1.29E+04		0.0069	-2.1589			A	F
38914	5	2100	3	0.0540	-1.2679	3307	4	0.0850	-1.0706			A	F
66881	5	144	2	0.0022	-2.6673	272	2	0.0041	-2.3900			A	F
7852	4	473	3	0.0602	-1.2204	166	2	0.0211	-1.6752			A	F
-18186		217	2	-0.0119		-96		0.0053	-2.2762			A	F
-20330		296	2	-0.0146		-34		0.0017	-2.7764			A	F
5990	4	400	3	0.0668	-1.1754	1208	3	0.2016	-0.6954			A	F
307	2	359	3	1.1667	0.0669	174	2	0.5664	-0.2469			A	F
-3958		465	3	-0.1175		186	2	-0.0469				A	F
158611	5	21696	4	0.1368	-0.8640	22232	4	0.1402	-0.8533			A	F

Ca ppb	log(Ca) log[ppb]	Sr ppb	log(Sr) log[ppb]	Sr ppb	log(Sr/Ca) log[ppb]	Ba ppb	log(Ba) log[ppb]	Ba/Ca ppb	log(Ba/Ca) log[ppb]	Al ppb	log(Al) log[ppb]	Active Non-Active	Marcellus Fayetteville
565008	6	3712	4	0.0066	-2.1824	771	3	0.0014	-2.8652			NA	M
732446	6	3461	4	0.0047	-2.3255	4048	4	0.0055	-2.2576			NA	M
642079	6	6527	4	0.0102	-1.9929	2118	3	0.0033	-2.4816			NA	M
1423782	6	4019	4	0.0028	-2.5493	1523	3	0.0011	-2.9707			NA	M
1285874	6	3093	3	0.0024	-2.6188	3706	4	0.0029	-2.5403	32058	5	NA	M
2154206	6	2465	3	0.0011	-2.9415	2808	3	0.0013	-2.8849	64540	5	NA	M
4235774	7	74039	5	0.0175	-1.7575	14859	4	0.0035	-2.4550	39400	5	NA	M
2252973	6	32575	5	0.0145	-1.8399	5207	4	0.0023	-2.6362	14025	4	NA	M
15681311	7	5727	4	0.0004	-3.4374	5536	4	0.0004	-3.4522	1222004	6	NA	M
943136	6	6549	4	0.0069	-2.1584	4278	4	0.0045	-2.3433	15668	4	NA	M
2810319	6	4179	4	0.0015	-2.8277	9976	4	0.0035	-2.4498	105686	5	NA	M
1074667	6	4479	4	0.0042	-2.3801	6232	4	0.0058	-2.2366	37599	5	NA	M
1635910	6	3579	4	0.0022	-2.6601	7252	4	0.0044	-2.3533	50507	5	NA	M
736364	6	1358	3	0.0018	-2.7340	3790	4	0.0051	-2.2885	13661	4	NA	M
1521133	6	2474	3	0.0016	-2.7888	2276	3	0.0015	-2.8250	47063	5	NA	M
2655232	6	1563	3	0.0006	-3.2301	2825	3	0.0011	-2.9731	67140	5	NA	M
1253920	6	1110	3	0.0009	-3.0529	3015	3	0.0024	-2.6190	83274	5	NA	M
1412927	6	3839	4	0.0027	-2.5659	6381	4	0.0045	-2.3452	30881	4	NA	M
758652	6	5855207	7	7.7179	0.8875	97442817	8	128.4421	2.1087	2195945	6	NA	M
6775510	7	-987705		-0.1458		407886352	9	60.2001	1.7796	12743952	7	NA	M
17450386	7	126744569	8	7.2631	0.8611	691320891	9	39.6164	1.5979	10210369	7	NA	M
3732991	7	8315992	7	2.2277	0.3479	82133881	8	22.0022	1.3425	4053367	7	NA	M
2632467	6	4439146	7	1.6863	0.2269	43169892	8	16.3990	1.2148	2097818	6	NA	M
1055693	6	6045294	7	5.7264	0.7579	65815684	8	62.3436	1.7948	566845	6	NA	M
1311781	6	10633703	7	8.1063	0.9088	53218680	8	40.5698	1.6082	1195540	6	NA	M

Ca ppb	log(Ca) log[ppb]	Sr ppb	log(Sr) log[ppb]	Sr ppb	log(Sr/Ca) log[ppb]	Ba ppb	log(Ba) log[ppb]	Ba/Ca ppb	log(Ba/Ca) log[ppb]	Al ppb	log(Al) log[ppb]	Active Non-Active	Marcellus Fayetteville
2220482	6	6354	4	0.0029	-2.5434	2075	3	9.34E-04	-3.0295			NA	F
6209604	7	241434	5	0.0389	-1.4103	74731	5	1.20E-02	-1.9196			NA	F
2918033	6	9185	4	0.0031	-2.5020	18082	4	6.20E-03	-2.2078			NA	F
1851257	6	4189	4	0.0023	-2.6453	13076	4	7.06E-03	-2.1510			NA	F
1064874	6	3348	4	0.0031	-2.5026	9391	4	8.82E-03	-2.0546			NA	F
-101777		-157		0.0015	-2.8113	-706		6.94E-03	-2.1589			NA	F
236965	5	3612	4	0.0152	-1.8169	3485	4	1.47E-02	-1.8325			NA	F
37812	5	6197	4	0.1639	-0.7854	6948	4	1.84E-01	-0.7358			NA	F
634985	6	6892	4	0.0109	-1.9644	2388	3	3.76E-03	-2.4247			NA	F
807639	6	3529	4	0.0044	-2.3596	5764	4	7.14E-03	-2.1465			NA	F
308690	5	4677	4	0.0152	-1.8196	309	2	9.99E-04	-3.0003			NA	F
3034677	6	42830	5	0.0141	-1.8504	74419	5	2.45E-02	-1.6104			NA	F
881748	6	6141	4	0.0070	-2.1571	8086	4	9.17E-03	-2.0376			NA	F
359019	6	8129	4	0.0226	-1.6451	3462	4	9.64E-03	-2.0158			NA	F
221074	5	9594	4	0.0434	-1.3625	6724	4	3.04E-02	-1.5169			NA	F
478291	6	12137	4	0.0254	-1.5956	213	2	4.46E-04	-3.3511			NA	F
56437	5	204	2	0.0036	-2.4424	-105		-1.86E-03				NA	F
-26441		325	3	-0.0123		66	2	-2.50E-03				NA	F
35325	5	264	2	0.0075	-2.1266	-181		-5.12E-03				NA	F
-37357		463	3	-0.0124		200	2	-5.35E-03				NA	F
55961	5	282	2	0.0050	-2.2973	213123	5	3.81E+00	0.5807			NA	F

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ACADEMIC VITA

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EDUCATION

Bachelor of Science in Chemical Engineering May 2018
Minor in Environmental Engineering
Minor in Engineering Leadership Development
The Pennsylvania State University, University Park, PA
Schreyer Honors College

WORK EXPERIENCE

Engineering Intern May 2017 – Aug. 2017
Washington Penn Plastic Company, Inc., Washington, PA

- Applied problem solving methodologies to the plastic compounding and manufacturing industry
- Bridged the technical gap between the testing laboratories and the sales and marketing units
- Established informative sales tools that emphasize the company's engineering capabilities and goals

Engineering Leadership Development Program Teaching Assistant Aug. 2016 – Present
The Pennsylvania State University, University Park, PA

- Assist student teams with engineering projects to verify that appropriate leadership skills are being demonstrated
- Balance the class focus on interpersonal and technical communication within multidisciplinary engineering teams

Undergraduate Research Assistant June 2016 – Present
The Pennsylvania State University, University Park, PA

- Explore characteristics of bird feathers from ecosystems both near and far from hydraulic fracturing sites
- Investigate correlations in the feather compositions and compare results to elements in hydraulic fracturing flow back water

Undergraduate Research Assistant Jan. 2015 – May 2016
The Pennsylvania State University, University Park, PA

- Explored alternative methods for pneumonia vaccination techniques through membrane separation of proteins
- Utilized nanofiltration stir cells and HPLC systems to acquire test results

CERTIFICATION

Lean-Sigma Process Improvement Yellow Belt Spring 2016

LEADERSHIP

Leadership Team, PSU Women in Engineering Program Orientation 2015 – Present
Chair, Society of Women Engineers Executive Director Assistant 2015 – 2016