



# Stable Carbon and Nitrogen Isotopes of A Sentinel Species, the Western Alaska Red Fox (*Vulpes Vulpes*)

Dainowski BH<sup>1,3</sup>, Duffy LK<sup>1,3\*</sup>, McIntyre J<sup>2</sup> and Jones P<sup>4</sup>

<sup>1</sup>Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775

<sup>2</sup>Department of Mathematics and Statistics, University of Alaska Fairbanks, Fairbanks, AK 99775

<sup>3</sup>Department of Chemistry and Biochemistry, University of Alaska Fairbanks, Fairbanks, AK 99775

<sup>4</sup>Alaska Department of Fish and Game, Bethel AK, 99559

\*Corresponding author: Lawrence K. Duffy, Department of Chemistry and Biochemistry, 900 Yukon Drive, Room 194, University of Alaska Fairbanks, Fairbanks, AK 99775-6160, USA, Tel: 907-474-7525; Email: lkduffy@alaska.edu

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## Abstract

Mercury (Hg) is a metal of both natural and anthropogenic origins that bioaccumulates in wildlife. Environmental risk assessments to arctic wildlife, such as the red fox (*Vulpes vulpes*), often use mercury analysis of hair due to its minimally invasive sampling method. The fate and transport of Hg provides refined risk assessment information. Stable carbon and nitrogen isotopes in hair and other tissues from 65 members of a wild, free-ranging population of red foxes in western Alaska were investigated to establish the trophic position for a northern red fox population in western Alaska. Hair stable isotopes  $\delta^{13}\text{C}$  correlated with stable isotope values from bone and muscle. Stable isotope ratios of the wild red fox are distinct from published farmed red fox data. Data demonstrated that hair  $\delta^{15}\text{N}$  positively correlated with hair total mercury levels. The western Alaska red fox consumes a diet of rodents and birds, which agrees with stable isotope data. The data establishes a baseline for monitoring changes in trophic level patterns as climate change impacts the red foxes' food web.

**Keywords:** Nitrogen Isotopes; *Vulpes Vulpes*; Western Alaska Red Fox; Sentinel Species

## Introduction

The red fox (*Vulpes vulpes*) is considered a generalist predator [1-3], whose movements are usually driven by local, seasonal food availability. The red fox expanded its range to the Arctic regions of the far north during the 20th century [4-6]. Found throughout Alaska, red fox are considered abundant on the Yukon Kuskokwim river delta [7]. Because of their abundance and value, red fox are ranked as the most important species to trappers in western Alaska and second most important overall statewide [8].

Red fox food sources consist of rodents, invertebrates, fruit, and even anthropogenic garbage in urban settings [9-11]. Red fox survive in a variety of ecosystems from semi-arid deserts to the [1] feeding on a wide range of prey species specific to their habitat [12-14]. In Alaska, the natural habitat provides red back voles (*Myodes rutilus*) and other microtine rodents, shrews (*Sorex cinereus*), wood frogs (*Rana*

*sylvatica*), black fish (*Dalia pectorallice*), and birds, such as willow ptarmigans (*Lagopus lagopus*) [13,15]. Historically, foxes were captured and farms established to produce fur for the retail clothing market. Over time, the farms also became associated with some behavioral and domestication studies [16]. Stable isotope analysis is a common tool used to trace pathways of organic matter through the food chain [17]. The stable isotope ratios reflect dietary sources from coastal, terrestrial, benthic and pelagic environments [18,19]. Differences in carbon stable isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) arise in the photosynthetic pathways of C3 and C4 plants [20,21]. In western Alaska, the native vegetation is exclusively composed of C3 plants [22]. The animals' tissue isotopes reflect naturally occurring stable isotope values in both local diet and seasonal patterns [15,23]. The difference in carbon isotopes [24,25] can distinguish wild foods from processed animal kibble or human food in their diet. The stable nitrogen isotope ( $^{15}\text{N}/^{14}\text{N}$ ) is used to identify food web structures [12,26-28].

Besides general isotopic incorporation, stable isotope ratio can also discriminate between an animal's diet and accumulation of nutrient values in various tissues [29-31]. Each tissue incorporates the nutrients from diet differently as each tissue is influenced by different turnover rates [32]. The  $\delta^{15}\text{N}$  signature is incorporated into tissues at a slower rate when herbivores consume a nitrogen-poor diet [33]. An animal's feeding ecology also determines the uptake of contaminants, such as mercury [3,34-36].

In this study, we used stable isotopes to test the hypothesis that hair could be used to monitor food chain changes within the red foxes' ecosystem. We additionally hypothesized that the stable isotopes would be correlated between hair, muscle and tissue. Thieszen, et al. [37] noted that muscle tissue infers diet one to two months prior to sampling and bone collagen infers past diet and increases in mercury. Since winter hair begins growing in August and is fully prime by late November or early December [12,38,39], contaminants like mercury, accumulated during late summer, would be observed. Analysis of seasonal impacts on fox tissues infer past diet, increases in mercury bioaccumulation help identify changes in the environment or feeding patterns. Roth and Hobson [12] reported stable isotope data for captive red foxes raised on a commercial pellet diet. A qualitative comparison, using wild foxes from western Alaska would add to Roth and Hobson's foundational concept of using stable isotopes to monitor food webs.

## Materials and Methods

### Sample Preparation

A total of 200 red fox carcasses were donated during the period of November 2010 through February 2011. Trappers anonymously and voluntarily provided carcasses to the Alaska Department of Fish and Game (ADF&G) in Bethel, Alaska. Carcasses remained frozen and were stored outside at the ADF&G office (temperature was continuously below 0°C).

Frozen carcasses were transported to the University of Alaska Fairbanks (UAF) where they were stored at -20 °C. Carcasses were partially thawed and sub-samples of liver, muscle, and one whole kidney, femur, and rear paw with fur were collected into Whirl-Pak™ bags and stored at -20 °C (liver, muscle, kidney, rear paw) and -80°C (femur). All samples were collected from the right side of the carcass using stainless steel scalpels or scissors. Only foxes with all five available tissues were analyzed for stable isotope analysis. Based on these criteria, 65 foxes were used in this study, 35 males and 30 females.

Approximately 14 g of frozen liver was cut into small

pieces (~1 cm<sup>3</sup>). The entire quadriceps muscle was removed from the femur bone while partially frozen, and cut into small pieces. For dissection of cortex and medulla, kidneys were kept partially frozen on a clean stainless steel tray placed on ice. All instruments used to separate renal cortex and medulla (scalpels and trays) were also kept at -20 °C before use. All tissues were placed into individual pre-weighed Whirl-Pak™ bags and lyophilized for 72 hours (Labconco FreeZone 4.5™ Freeze Dry System).

Hair was collected from the right rear paw using a Wahl stainless steel trimmer (carbon blades) and stainless steel scissors. Blades were thoroughly cleaned between samples. Hair was placed in a 50 ml metal-free conical bottom tube and filled with 1% Triton X, covered, and allowed to sit for 15-30 minutes, shaking occasionally. The Triton X was then poured off and the tube was filled with H<sub>2</sub>O, covered, sat for 10-15 minutes, with occasional shaking. This process was repeated at least four times until all soap was gone. The hair samples were placed into individual pre-weighed Whirl-Pak™ bags and lyophilized for 48 hours (Labconco FreeZone 4.5™ Freeze Dry System).

Bones were cored under a fume hood while wearing a 3MTM particulate respirator N95. The bones were cored using a Dremel™ glass diamond drilling bit, 1/4" (6.350mm), 663DR, drill speed #23. Each femur was drilled completely through the shaft in three locations to produce six core. Four cores were used in a similar study of THg [3], and the remaining 2 cores were used in this stable isotope study. The periosteum and any trabecular bone were removed using a Dremel™ glass diamond taper point sander, 3/32" (2.381mm), #7144. The cores were stored at -20 °C in acid-washed (5% HNO<sub>3</sub>) vials prior to degreasing. The degreasing method used is as follows: bone core samples were sonicated in deionized water in order to remove any debris. Next, they were soaked in chloroform for two 8-hour periods to remove any lipids, then finally rinsed with deionized water and air-dried under a fume hood for 3 days.

The bone collagen extraction is as follows: bone samples were demineralized by soaking in an HCl-H<sub>2</sub>O solution until they were translucent. After which they were rinsed with deionized water. In order to separate the collagen from other organic compounds and proteins, the bone samples were gelatinized by placing the collagen samples, with HCl-H<sub>2</sub>O solution (pH between 3.0 and 4.0) in a culture tube. The tube was heated to 70°C under a stream of nitrogen, to dissolve the collagen. The supernatant was lyophilized to retrieve the collagen after centrifugation.

### Stable Isotope Analysis

Isotope ratios are presented as stable isotope abundances

relative to the international standard (delta ( $\delta$ ) notation) in parts per thousand (permil (‰))

$$\delta X = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 103 \text{ ‰}$$

where, element X =  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the molar ratios of heavy to light isotope of the sample and the standard [24]. These isotope ratios are expressed in delta notation of permil relative to the Vienna PeeDee Belemnite international standards for carbon, and atmospheric N for nitrogen. These standard values are measured using a laboratory standard (peptone) calibrated against several certified reference materials [24].

Between 0.1 and 0.5 mg of hair, liver, muscle, renal cortex, renal medulla, and bone collagen samples were placed in tin capsules then loaded into an autosampler. Samples were analyzed for stable carbon and nitrogen isotopes at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks. A continuous-flow isotope ratio mass spectrometry with a Costech ECS4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced to a Finnigan Delta Plus XP isotope ratio mass spectrometer via the Conflo III interface (Thermo Fisher Scientific, Waltham, MA, USA) was used for analyzing. The laboratory standard was measured multiple times between samples to evaluate the accuracy and precision of analyses. Accuracy and precision was assessed using multiple peptone standards. Precision was within 0.3‰ and accuracy was within 0.1‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

### Tissue turnover, Growth Rates and Lipid Contamination Methods used for Analysis

Analysis of the pelage (hair) cycle of the red fox has shown that annual molt begins in early spring, approximately in April, and regrowth begins in late summer. The cycle is complete when the fur obtains its full length and is fully prime in early December. Therefore, the hair collected

in winter will be representative of a diet August through October [39]. Bone collagen, however, describes the lifetime diet of an animal [37], while muscle tissues will show a diet within the last two months prior to sampling [12]. Renal cortex and medulla, as well as the liver show the diet within the last week prior to sampling [12,40].

Lipids are typically depleted in  $^{13}\text{C}$  and have  $\delta^{13}\text{C}$  values more negative than those in carbohydrates and proteins within an individual organism [41]. Lipids for hair and bone were extracted and corrected, while muscle, liver and kidney were not [41,42]. The C:N ratios were analyzed to compare the effect of lipid extractions. A C:N ratio of around four (~10% lipids) is an acceptable range for terrestrial mammals.

### Statistical Analyses

Histograms and normal probability plots were used to assess normality of the distribution of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope measurements for each tissue. Individual isotope measurements greater than three standard deviations from the mean for each tissue were identified as potential outliers [43]. Means and standard deviations were used to summarize  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C:N values for all tissues for all foxes, as well as separately for male and female foxes. T-tests were used to compare mean isotope values of males and females for each tissue. Paired t-tests were used to test mean differences in isotope values between pairs of tissues, for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Pearson's product-moment correlation was used to measure and test the significance of correlations between isotope values in each pair of tissues, for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . We used the scale established by Andale [44] to classify correlations as follows: High correlation: 0.5 to 1.0 or -0.5 to 1.0, Medium correlation: 0.3 to 0.5 or -0.3 to 0.5, and Low correlation: 0.1 to 0.3 or -0.1 to -0.3. A significance level of  $\alpha = .05$  was used for all hypothesis tests. All analyses were performed using the statistical software R [45].

	Hair Mean (STD)	Bone	Liver	Muscle	Kidney Cortex	Kidney Medulla
$\delta^{13}\text{C}$ Carbon	-24.89 (1.08)	-22.56 (0.67)	-25.37 (1.34)	-25.69 (1.14)	-25.35 (1.21)	-25.31 (1.21)
$\delta^{15}\text{N}$ Nitrogen	5.57 (1.48)	4.64 (1.47)	5.72 (1.64)	5.20 (1.53)	5.63 (1.66)	5.85 (1.53)
C:N Ratio	2.62 (0.05)	2.51 (0.04)	4.01 (0.61)	3.38 (0.31)	4.02 (0.22)	3.83 (0.30)

**Table 1:** Mean and standard deviation for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and C:N ratios for all red foxes (n=65).

## Results

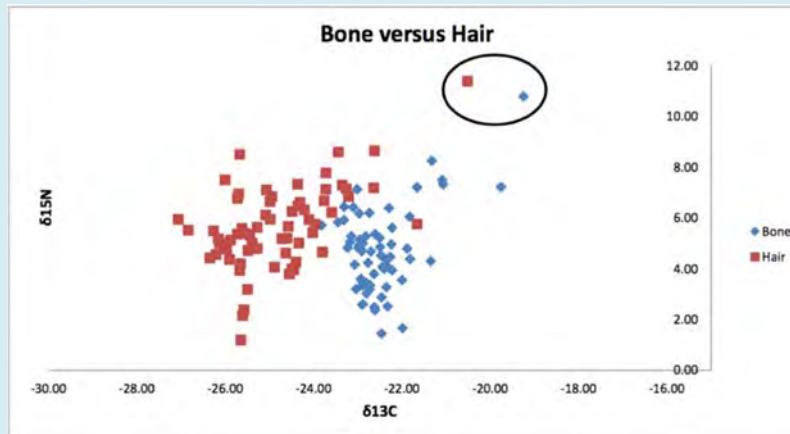
### Tissue Isotope values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and C:N ratios in wild foxes

The C:N ratios (Table 1) demonstrated a ratio that would be expected for terrestrial mammals [41]. Table 1

summarizes the means and standard deviations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and C:N ratios for all tissues from the sample of free-ranging red foxes from Bethel Alaska. There was no significant difference between male and female foxes. Histograms and normal probability plots of isotope values were consistent with normality for all tissues.

Figure 1 compares the relationship of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for bone and hair. The  $\delta^{15}\text{N}$  values of the two tissues had similar ranges. On the other hand, the  $\delta^{13}\text{C}$  values differed between hair and bone. An outlier was observed in one fox that had isotope values outside the three-standard deviation range for  $\delta^{13}\text{C}$  in hair and bone [43]. This same fox

was considerably larger and heavier (5.5 kilograms (kg) than the other foxes. Weight averages for all foxes were 3.85 kg, with males having averages of 4.06 kg, and females 3.62 kg. This fox was not included in the statistical analyses (Figure 1). The C:N ratio for male and female foxes demonstrated ratios that would be expected for terrestrial mammals.



**Figure 1:** Comparison  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  levels for hair and bone in western Alaska wild red foxes. One male fox outlier is circled.  $\delta^{13}\text{C}$  values illustrate differences in carbon isotope biochemistry.

Paired t-tests indicated significant differences in the means for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between pairs of tissues. Table 2 ( $\delta^{13}\text{C}$ ) reports the mean differences and p-values for all tissues. For  $\delta^{13}\text{C}$  pairwise mean of differences, hair, bone

and muscle were significantly different from kidney and liver. Kidney cortex and medulla were not significantly different from each other.

	Hair	Bone	Muscle	Liver	Kidney Cortex	Kidney Medulla
Hair	----	-2.33	0.79	0.483	0.46	0.42
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bone		----	3.12	2.81	2.79	2.75
			<0.0001	<0.0001	<0.0001	<0.0001
		Muscle	----	-0.311	-0.33	-0.37
				0.001	<0.0001	<0.0001
			Liver	----	-0.02	-0.05
					0.791	0.466
				Kidney Cortex	----	-0.03
						0.404

**Table 2:** Carbon stable isotope paired t-test among all tissues. Mean of differences (top number) / p-values (bottom number).

For  $\delta^{15}\text{N}$  pairwise mean of differences (Table 3), hair was significantly different from bone and muscle. Bone was significantly different from all tissues. Kidney cortex and

medulla were significantly different from all tissues except for hair and liver.

	Hair	Bone	Muscle	Liver	Kidney Cortex	Kidney Medulla
Hair	----	0.92	0.37	-0.15	-0.05	-0.27
		<0.0001	<0.0001	0.328	0.696	0.044
Bone	----	----	-0.55	-1.08	-0.98	-1.2
			0.002	<0.0001	<0.0001	<0.0001
		Muscle	----	-0.52	-0.42	-0.64
				<0.0001	<0.0001	<0.0001
			Liver	----	0.09	-0.12
					0.002	0.01
				Kidney Cortex	----	-0.21
						<0.0001

**Table 3:** Nitrogen stable isotope paired t-test among all tissues. Mean of differences (top number / p-values (bottom number)).

	Hair	Bone	Muscle	Liver	Kidney Cortex	Kidney Medulla
Hair	----	0.423	0.819	0.673	0.709	0.692
		0.0004	<0.0001	<0.0001	<0.0001	<0.0001
Bone	----	----	0.308	0.328	0.318	0.346
			0.013	0.007	0.01	0.005
		Muscle	----	0.838	0.913	0.904
				<0.0001	<0.0001	<0.0001
			Liver	----	0.891	0.875
					<0.0001	<0.0001
				Kidney Cortex	----	0.952
						<0.0001

**Table 4:** Carbon stable isotope correlations among all tissues. Pearson's product-moment correlation (top number) / p-values (bottom number).

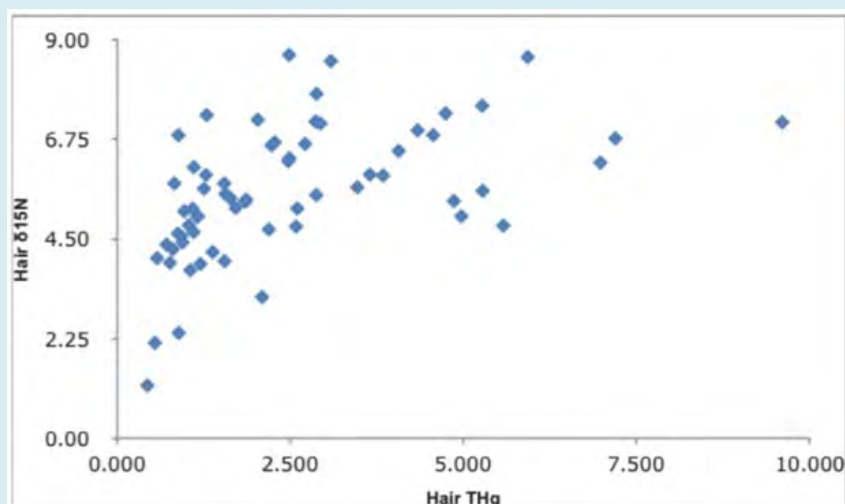
	Hair	Bone	Muscle	Liver	Kidney Cortex	Kidney Medulla
Hair	----	0.613	0.851	0.68	0.719	0.744
		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bone	----	----	0.55	0.405	0.433	0.48
			<0.0001	<0.0001	<0.0001	<0.0001
		Muscle	----	0.928	0.939	0.945
				<0.0001	<0.0001	<0.0001
			Liver	----	0.988	0.975
					<0.0001	<0.0001
				Kidney Cortex	----	0.985
						<0.0001

**Table 5:** Nitrogen stable isotope correlation among all tissues. Pearson's product-moment correlation (top number) / p-values (bottom number).

Pearson's product-moment correlation was used to estimate correlations in isotope measurements between pairs of tissues. Table 4 ( $\delta^{13}\text{C}$ ) and Table 5 ( $\delta^{15}\text{N}$ ) also show the p-values for testing significance. All p-values were significantly different from zero. Specifically, high correlations were observed for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope measurements between hair and muscle, liver, kidney cortex and medulla. Hair and muscle correlated with liver, kidney cortex and medulla. Bone showed a small correlation with all tissues for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope measurements. Bone is less likely to be useful in predicting stable isotope ratios in other tissues of the red fox.

### Relationship of Mercury Concentration to Hair

A correlation between  $\delta^{15}\text{N}$  values and THg concentrations of hair was observed for the western Alaska red foxes total mercury (THg) concentration in hair as reported by Dainowski, et al. [3]. When  $\delta^{15}\text{N}$  of hair increased, hair THg increased. Figure 2 shows an increasing pattern of hair  $\delta^{15}\text{N}$  ranging from 2 to about 7 per mil, with most foxes between 4.5 and 7 per mil. The Hg levels range from 0.5 ppm to 9 ppm with most foxes Hg concentrations between 1 and 5 ppm.



**Figure 2:**  $\delta^{15}\text{N}$  versus THg of hair in free-ranging red foxes from western Alaska. THg concentrations are reported in ppm (mg/kg) and  $\delta^{15}\text{N}$  values are reported in per mil.  $R^2 = 0.42$ . Mean (std dev) of hair 5.57 (1.48), and THg 2.58 (1.96). Stable isotope values for hair correlate with THg levels in hair.

### Farmed Foxes Versus Wild Foxes

Roth and Hobson [12] reported a study of captive red foxes in which the objective was to measure diet-tissue isotopic fractionation in foxes raised on commercial pellet

feed. A non-statistical, qualitative comparison is presented in Table 6. The red fox  $\delta^{13}\text{C}$  hair mean was -24.89 while the farmed fox hair was -16.10 per mil (Table 6). Muscle and bone tissues also differed between the two groups. Similarly, the  $\delta^{15}\text{N}$  means varied for both groups (5.57 vs. 8.10 per mil).

	$\delta^{13}\text{C}$ Alaska free-ranging foxes	$\delta^{13}\text{C}$ Ontario commercial fed farmed foxes	$\delta^{15}\text{N}$ Alaska free-ranging foxes	$\delta^{15}\text{N}$ Ontario commercial fed farmed foxes
Bone	-22.56 (0.67)	----	4.64 (1.47)	----
Kidney Medulla (KidneyM)	-25.31 (1.21)	----	5.85 (1.53)	----
Kidney Cortex (KidneyC)	-25.35 (1.21)	----	5.63 (1.66)	----
Liver	-25.37 (1.34)	-18.3	5.72 (1.64)	8.2
Muscle	-25.69 (1.14)	-17.6	5.20 (1.53)	8.2
Hair (fur)	-24.89 (1.08)	-16.1	5.57 (1.48)	8.1

**Table 6:** Stable Isotope mean values (std dev) for tissues of Alaskan free-ranging red foxes and Southern Ontario, commercially fed, farmed red foxes [12]. All values are reported in parts per mil (‰).

## Discussion

The small percentage of lipid content in the fox tissues did not influence the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope values. The red fox tissues had a 1% difference in their mean values between lipid extracted, e.g. bone, and non-lipid extracted tissues, e.g. hair, for  $\delta^{13}\text{C}$ .

Potential western Alaska red fox nutrient sources include terrestrial, avian, aquatic, and occasionally anthropogenic derived foods. Plants, both C3 and C4, are significant due to the  $\delta^{13}\text{C}$  ratios, which differ depending on the photosynthetic pathway [21,46]. The ratio of  $^{13}\text{C}/^{12}\text{C}$  isotopes in C3 plants range from approximately -24.00 to -34.00‰, while the ratio in C4 plants range from approximately -6.00 to -19.00‰ [47]. Alaska's native vegetation is exclusively C3, such as rice, shrubs and grasses [22,47]. The  $\delta^{13}\text{C}$  isotopes indicate that C3 plant eating terrestrial animals comprise the majority of the red fox's diet. The red fox hair mean value  $\delta^{13}\text{C}$  of -24.89‰ (male and female) results from a diet high in microtines (Table 6).

The farmed red fox diet is  $\delta^{13}\text{C}$  isotopically different from the wild Alaskan fox (Table 6). Roth and Hobson [12] reported the pellet diet of their farmed foxes contained up to fifty percent carbohydrate, resulting in an average  $\delta^{13}\text{C}$  isotope fractionation value lower than the Alaskan red fox whose diet is based on microtines eating C3 plants. For farmed fox  $\delta^{13}\text{C}$  isotope values ranged between -16.10 to 18.31 per mil in the tissues (Table 6). Corn, found in domestic animal feed, is a major component of the commercial rodent pellet diet and human diet [11,25].

Nitrogen stable isotope ratios vary as trophic levels increase [48], and between terrestrial and marine sources [13,24,26,27]. The increase in trophic levels can be 2.00‰ to 4.00‰ between levels; however, this depends on the tissue water content [49-51]. The environment and physiological factors of animals (e.g. hair growth) [24,49] and wetter and cooler ecosystems plants would have a lower  $\delta^{15}\text{N}$  ratio [24,51].

After comparing our muscle data with Roth and Hobson [12],  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope measurements illustrate that a wild diet of mainly microtines consumed by the free-ranging red foxes can easily be distinguished from the carbohydrate rich pellet diet that includes carbon from C4 plants. Our stable isotope demonstrates that free-ranging Western Alaska red fox diets vary greatly from the commercial fur farmed foxes from Ontario, Canada (Table 6). The isotope data suggests that the Alaska red foxes have a major diet of herbivores, such as microtines that feed on C3 plants. Our data validates Roth and Hobson's concept using stable isotope changes to monitor terrestrial mammalian

omnivores' food web [52].

The isotope values of the red fox's diet items versus the THg concentrations found in their hair tissue implies that the THg may be coming through a mixed lemming/aquatic/avian pathway (possibly fish or other fish-eating birds) for those foxes with the higher mercury contents. The free-ranging red foxes should retain a summer signal in their hair. A summer seasonal effect of THg in the diet may explain the variation in the Hg values (Figure 2) [53].

Since red foxes are the second most important species overall in the state of Alaska, as well as among the top most important species to trappers in western Alaska, the different statistical analysis provided in this research has shown to be important. The stable isotope statistical analyses, as seen in various tissues of the Alaskan red fox, have shown the red fox's dietary food source, the components of their food chain as well as seasons diet patterns in their changing Alaskan environment. In addition, the mercury analyses, as seen in various tissues of these same red fox's, helped to identify not only bioaccumulation of mercury through season changes but also the accumulation of mercury through the fox's food chain. Since the red fox's movements are driven by local and seasonal food availability, the implications of this research is important in order to monitor the health of the Western Alaska red fox. By monitoring the health of the Alaskan red fox and their food chain, we can also monitor the health of their ecosystem, through mercury analysis, for nutritional value. By monitoring the red fox's health and the health of their ecosystem, we can then address various health issues that may be indicated in future red fox tissue analysis. In this way, we can make every effort to insure a healthy ecosystem and population of red foxes in western Alaska [54].

## Conclusion

This is the first report on red fox population stable isotope ratios from western Alaska. The  $\delta^{15}\text{N}$  stable isotope ratio of hair are significantly different from bone and muscle tissues. The western Alaska red fox differs in trophic position with the farmed red fox. Stable isotope data is correlated with the mercury levels. Identifying the trophic position of red fox populations allows for ecological risk assessment of contaminants such as mercury as climate change and industrial development impacts the physical and biological composition of western Alaska ecosystems.

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