Forensic Attribution of CBRNE Materials: A Chemical Fingerprint Database

Final Status Report

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IMPORTANT INFORMATIVE STATEMENTS

Forensic Attribution of CBRNE Materials CRTI-08-0116 was supported by the Canadian Safety and Security Program which is led by Defence Research and Development Canada's Centre for Security Science, in partnership with Public Safety Canada. The project was led by Public Safety Canada in partnership with Royal Canadian Mounted Police, DRDC Ottawa, University of Ottawa, and the Department of National Defence.

Canadian Safety and Security Program is a federally-funded program to strengthen Canada's ability to anticipate, prevent/mitigate, prepare for, respond to, and recover from natural disasters, serious accidents, crime and terrorism through the convergence of science and technology with policy, operations and intelligence.

Table of Contents

Introduction: Scope of the Work

The initial project was to study hair and its relation to drinking water as a possible forensic tool to help in identifying unknown corpses in law enforcement investigations. This tool could also be used as an indicator of geographical movement for persons of interest. Hair is an ideal medium to obtain information about an individual [(1)]. Since hair grows at approximately 1 cm per month, it can provide a chronological snapshot of the habits of a person over a monthly or bi-monthly time frame that is roughly equal to the length of their hair (in cm).

The scope of the work increased when a DRDC – Centre for Security Science awarded a CRTI to PSC to also include modern pollens and soil analysis. The development of custom software, now known as "Signature", was tasked by PSC through a private company (CSDi) to aid in the data mining and probability plotting of the results on a geographical map. This has been brought to fruition and a copy of the database has been delivered to each partner at the end of the project in late spring of 2013.

The U of Ottawa was tasked with handling all of the Canadian field sampling including the distribution of specific samples to all parties. The U of Ottawa was further tasked with the in-house stable isotope analysis of water and hair samples for the various isotopes of interest including testing the limitations of the technology. The G.G. Hatch Stable Isotope Laboratory in the Earth Sciences department was used to analyse routine samples (e.g. water) but also to develop and execute various techniques for many of the samples (e.g. hair); for which no procedures existed at this facility.

As well, UO was tasked to coordinate the pollen analysis with the U of Montréal group to populate the database for PSC. However, the exploitation of the pollen data would need to be done as a future project.

The RCMPs "Forensic Science and Identification Services" were tasked with the analysis of the soils samples, as well as the quartz grains within, for trace elements as a forensic tracking tool.

All Canadian provinces are identified by their acronyms in the report: Newfoundland (NL), Nova Scotia (NS), New Brunswick (NB), Prince Edward Island (PE), Québec (QC), Ontario (ON), Manitoba (MB), Saskatchewan (SK), Alberta (AB) and British Columbia (BC).

Carbon and Nitrogen

Different isotope measurements can give different information about a person. In general, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) as well as sulphur ($\delta^{34}S$, discussed below) isotope measurements provide information about diet. The $\delta^{13}C$ values show the relative proportion of the type of foods consumed, which is often linked to the photosynthetic cycle of carbon in plants and are categorized as C3 (e.g. corn, sugar cane, maize), C4 (e.g. rice, wheat, maple sugar, beans, nuts) and to a lesser extent CAM (e.g. pineapple, cactus). C3 plants typically have a $\delta^{13}C$ range of -22 to -34 ‰, whereas the typical $\delta^{13}C$ range for C4 plants is -6 to -19 ‰ [(2-9)] and CAM plants cover both ranges, with values linked to water stress.

In addition, the $\delta^{13}C$ values of livestock can reflect the dietary source that it is fed. The tissue of livestock that is fed a maize (i.e. C4 plant) diet tends to have more positive $\delta^{13}C$ values compared

to livestock that is fed a grass (i.e. C3 plant) diet. Jahren et al [10] surveyed beef and chicken from three major fast food chains in the US, and found that the $\delta^{13}C$ value of 100% of the chicken and 93% of the beef was consistent with these animals being fed a corn (C4) based diet. Nakamura et al [7] found that the protein portion ($\delta^{15}N$) of beef, pork and chicken was related to geographical location (Japan vs. USA vs. Germany), but was not correlated to the diet of the animal.

The consumption of animals raised in different geographical regions can also influence the $\delta^{13}C$ range of hair. McCullagh et al [6] showed that the average $\delta^{13}C$ value of hair from the US was more positive than that for hair from UK residents (-17.6 vs. -20.5 ‰ for US and UK hair, respectively). The researchers attributed this difference to the various feeding practices of livestock: the UK livestock is fed primarily grasses (C3), while the US livestock is fed a mixture of grass (C3) and corn (C4).

The $\delta^{13}C$ value is useful in determining a person's dietary habits [2,8,9,11], if a person changed their diet [3,4,8], or if a person lives in a different region [2,3,6,8,9,12]. The time it takes for a change in the $\delta^{13}C$ value to be noted in hair can range from 1-3 months [4,8]. However, O'Connell noted that it took 7-12 months for the $\delta^{13}C$ value to reach equilibrium.

$\delta^{15}N$ measurements are an indicator of the amount of animal protein (both terrestrial and/or marine products) a person consumes [2,4,8,9,13]. The more terrestrial animal protein and/or marine products consumed, the more positive the $\delta^{15}N$ value. For example, the $\delta^{15}N$ values of omnivores, vegetarians and vegans were determined for a German population. The omnivores had the most positive $\delta^{15}N$ values (average of 9.9 ‰), followed by the vegetarians (average of 7.7 ‰), and then the vegans (average of 6.2 ‰) [9]. A similar study was undertaken by O'Connell et al [13] which again showed omnivores with the highest $\delta^{15}N$ values (average of 8.8 ‰) followed by vegetarians (average of 8.7 ‰) and vegans (average of 6.9 ‰). However, in this case, the ovo-lacto vegetarians were not distinguishable from the omnivores.

O'Connell et al [8] showed that it can take 7-12 months, after changing diet, for the $\delta^{15}N$ values to reach equilibrium, even though a change in the $\delta^{15}N$ values was recorded a few months after the change occurred. Huelsemann et al [4] performed a study with a controlled change in diet in both the types of plants and types of protein (shift form terrestrial to marine) consumed. The researchers found that there was an immediate rise in the $\delta^{15}N$ values with an increase in the consumption of marine products.

In addition, $\delta^{15}N$ analysis may also be used as an indicator of health [14-18]. For example, during a "nutritional stress" time where the person is suffering from an illness or not retaining proper nutrients (e.g. anorexic [15,17,18] and bulimic patients [16], or pregnant women who reported nausea and vomiting during pregnancy [14]), researchers have shown that the $\delta^{15}N$ value generally becomes more positive; this can be termed as catabolism where a person begins to consume their own reserves. Mekota et al [17] reports that it takes approximately 2 weeks for a change to be seen in the $\delta^{15}N$ values after a return was made to a healthy diet.

It is important to note that the shift due to "nutritional stress" is in the same direction as increasing protein intake or changing the type of protein intake from terrestrial to marine products (i.e. both

result in a more positive $\delta^{15}N$ value). As such, these two scenarios cannot be differentiated using $\delta^{15}N$ analysis.

Hydrogen and Oxygen

While dietary information and differences between populations are useful anthropological and forensic information, hydrogen (δ^2H, or deuterium (D)) and oxygen ($\delta^{18}O$) isotope analysis can be used to track movement of a person and to determine residency [1,12,19-24]. The reason for this is due to fact that the δ^2H and $\delta^{18}O$ values of water (H_2O) are different in different locations [25,26]. As people consume their local water, the δ^2H and $\delta^{18}O$ values of the water are reflected in the δ^2H and $\delta^{18}O$ values of the hair [19-21,23,24]. As such, if these two isotope values of a person's hair remain constant over the length the hair, then that person likely did not change geographical locations. Alternatively, if the values do change, this indicates that the person likely travelled outside their region.

There are relatively few publications of this nature in the literature. Ehleringer et al [19] analysed hair from individuals residing in 18 US states, and determined that the δ^2H and $\delta^{18}O$ values are dependent on where the individual resided. O'Brien et al [23] showed that persons residing in Alaska and New York had different isotopic values, and Fraser et al [21] showed that persons residing in different parts of the world also could be distinguished using δ^2H values. Additionally, the use of multiple isotope systems can further aid in gathering information about an individual. Fraser et al [20] showed $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δ^2H values of hair could be used to distinguish between persons residing in different countries around the world. Meier-Augenstein et al [22] used $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δ^2H to determine the recent geographical origin of an unknown person found in Dublin, Ireland. Mutzel et al [12] used $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and δ^2H analysis to determine the country (or area) of origin of several samples. Thompson et al [29] noted differences in $\delta^{13}C$, $\delta^{15}N$, δ^2H, $\delta^{18}O$ and $\delta^{34}S$ isotope values in hair from people residing in four different Asian countries.

Researchers have estimated a range of the amount of water δ^2H signal incorporated into the δ^2H hair signal: 40-49% [21], 42 % [29], 36 % [23], 31 % [24] and 27 % [19]. The analysis of ~ 60 pan-Canada samples collected from Phase I of the project (Previous Initiative) showed approximately 27 % of the water δ^2H signal is incorporated into the hair. Similarly, the estimated amount of water $\delta^{18}O$ signal incorporated into the $\delta^{18}O$ hair signal by different researchers was: 27 % [23], 35 % [19] and 40 % [29].

Conversely, between 51 and 73 % of the hydrogen signal and 60 to 73 % of the oxygen signal in hair comes from other sources, such as food. The link between food sources and geographical regions are significantly less understood, and are very difficult to measure, as supermarkets may consistently obtain food from non-local sources. This is discussed further under Limitations.

Sulphur

$\delta^{34}S$ measurements can be useful in determining dietary habits [11,27,28], and can be used for geo-location purposes [2,3,11,12,29]. Sulphur sources for humans include food, ocean deposition, local soil/rock geochemistry, and deposition from the atmosphere, which can be from both anthropogenic or natural (e.g. volcanoes) sources. In terms of diet, more positive $\delta^{34}S$ values in

hair have been linked to higher consumption of marine animals, which in turn have a higher $\delta^{34}S$ value than terrestrial animals. In terms of geo-location, persons residing in coastal habitats tend to have a higher hair $\delta^{34}S$ values than those residing more inland. This may be due to two factors, namely increased intake of marine-based protein due to the readily availability of seafood/fish in coastal areas, or the deposition of S which has a relatively positive $\delta^{34}S$ value often in the form of sea spray from salt water on coastal vegetation and soils. Further, atmospheric deposition and local geochemistry may also contribute to the use of $\delta^{34}S$ as a geo-location tool.

$\delta^{34}S$ values have been measured for several different populations, and have been used, in conjunction with other isotope values, to determine geo-location. Valenzuela et al [11] reported a range between -1.2 and 9.9 ‰ (average 3.4 ± 1.1 ‰) for $\delta^{34}S$ values of American hair, and noted that $\delta^{34}S$ values were the least positive in the Great Plains area, and became progressively more positive moving towards the south, east and west. Valenzuela et al [27] also analyzed hair from several European countries, and noted the average $\delta^{34}S$ values of hair from each country ranged between 5.9 ± 0.6 and 7.3 ± 0.7 ‰. Katzenberg and Krouse [28] showed hair from Canberra, Australia to have a $\delta^{34}S$ value around 14 ‰, while hair from Calgary, AB was closer to 0 ‰. Thompson et al [29] found $\delta^{34}S$ values ranging from 3.2 to 13.8 for persons residing in China, India, Mongolia and Pakistan.

By itself, $\delta^{34}S$ values have not been very successful in identifying origins of unknown hair samples. However, when analyzed in conjunction with other isotopes (i.e. $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ and $\delta^{18}O$), researchers have been able to better differentiate between hair samples with similar $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ and $\delta^{18}O$ values. Mutzel et al [12] found that the $\delta^{34}S$ values of hair for residents of Australia were different than those for residents of several European and Asian countries, Brazil and Costa Rica. However, when analyzed using multiple isotopes, residents from Brazil, Costa Rica and Australia were readily distinguished from each other, and from European residents. Thompson et al [29] measured $\delta^{34}S$ in hair from Pakistan, Mongolia, China and India. Of these countries, Pakistan had the least positive $\delta^{34}S$ values, while India had the most positive. Further, the researchers noted that the trend in $\delta^{34}S$ values was geographically related. When analyzed in conjunction with $\delta^{18}O$ and $\delta^{2}H$ values, the researchers were able to more fully distinguish the origin of hair samples between these countries. Bol et al [2,3] used $\delta^{34}S$ values, in conjunction with other isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ and $\delta^{18}O$) values to identify which hair samples belonged to visitors (i.e. non-locals), and which hair samples were from locals.

Limitations

Although isotopic analysis has the potential to provide much information about an unknown person, there are some limitations with this technique that should be noted.

There is the concept of the "supermarket diet". This means that people can buy food from virtually anywhere in the world at any given time. Although it has been shown that different plant groups (C3 vs C4) are more predominant in certain regions than others, it does not mean that a person is necessarily eating locally. As such, the $\delta^{13}C$ values may not necessarily be a reflection of the consumption of locally grown food. Despite this, continental differences between North America and Europe/UK have been noted [2,3,6,8,12]. *As political, environmental and local food*

production concerns become more prevalent, the food consumption tendency of people is moving back into the direction of eating locally available foods.

The impact that food has on the δ^2H and $\delta^{18}O$ values of hair is less known. O'Brien et al [23] measured various food products purchased in Alaska and New York. While variations in δ^2H and $\delta^{18}O$ values were found between similar foods, the average δ^2H and $\delta^{18}O$ values of all the food taken collectively was similar in both regions. Chesson et al [30] found that the δ^2H value of ground beef purchased from a supermarket was related to the δ^2H value of tap water from the same region. However, the δ^2H value of hamburger patties from fast food chains were not related to that of local tap water. A more systematic approach is required to determine the effect of food consumed on δ^2H and $\delta^{18}O$ values in hair.

The determination of the location of an individual based on δ^2H and $\delta^{18}O$ values is linked to those same isotope values of the local water consumed. Since the δ^2H and $\delta^{18}O$ values of water varies systematically with latitude and elevation [25,26], there should be systematic variations in the δ^2H and $\delta^{18}O$ measured in hair as you change latitudes. However, there can be local variability in the isotopic values of the water sources. Further, humans do not all process water identically, which can lead to variability in δ^2H and $\delta^{18}O$ values at local scales. It should be noted that this technique is not intended to pinpoint a city. Instead, it is to be used as a tool to predict the ***probability*** that an unknown person resided in a region, and to estimate the time that person spent in that region.

In order for this technique to be used to help identify unknown persons, the isotopic data collected must be compared against a database. While differences over the length of a hair can be measured, this information needs to be compared to a database in order to make any inferences as to where this person had been residing. To date, few, if any, systematic isotopic databases exist and most of the inferences of locality are based on precipitation (rainfall) data; which is not necessarily based on actual local water sources for consumption. In Canada, a project was recently undertaken to create an isotopic database of Canadian human hair as well as the local water consumed by various Canadian residents.

Previous Initiative

The preliminary stage of this collaborative Forensic Isotope Ratio Mass Spectrometry (FIRMS) project (RCMP, Phase I, 2007-2008) focused on the analysis of 70 to 120 pan-Canadian hair, water and protein samples for their stable isotopic signature (δ^2H, $\delta^{13}C$, $\delta^{15}N$). This large scale study was to assess the use of FIRMS for geo-location and to evaluate protocols of sampling, sample preparation, sample storage and method of sample analysis for hair, water and proteins. Based on those preliminary results the Ottawa-FIRMS group has decided to focus on two major center-east provinces in Canada that contain many significant ports of entry and urban centers for the next phase (i.e., RCMP, Phase II).

The information generated by Phase II (2008-2009) assisted in the development of Global Information System (GIS) maps of the δ^2H and $\delta^{18}O$ stable isotope ratios of waters of the most populated areas within Ontario and Quebec. This was used to "predict" what stable isotope ratios in human hair would look like if an individual had lived or traveled within those two provinces. Canadian expertise in FIRMS will continue to be developed, as well as participation in the FIRMS Network to access international research efforts involving this emerging forensic technique, which

can be applied to unidentified human remains, movement of persons of interest, country of origin for explosives, drugs, etc.

Current Project

In 2009, the Ottawa FIRMS group (PSC, RCMP, U of Ottawa) was awarded a four year grant from CRTI (Chemical, Biological, Radiological, Nuclear and Explosives Research and Technology Initiative) to pursue a project entitled: *Forensic Attribution of Chemical, Biological, Radiological, Nuclear and Explosives Material*s. This project combines isotope ratio mass spectrometry (IRMS), inductively coupled plasma-mass spectrometry (ICP-MS) of hair and water samples, in conjunction with pollen analysis and ICP-MS of bulk soil and quartz samples to produce a "regional fingerprint" of unique chemical signatures. This project builds on knowledge and expertise accumulated from the Previous Initiatives (Phases I and II).

Over 425 cities/towns were sampled across Canada for hair, water, soil and pollen during the summers of 2008 through 2011. The following table outlines the tasks performed for this project.

Table 1. Major partners performing tasks for this project.

Organization	Acronym	Task(s)
University of Ottawa	UO	Hair, water, soil and pollen sample collection and distribution* IRMS analysis of hair and water samples
Public Safety Canada	PSC	ICP-MS analysis of hair and water samples
Royal Canadian Mounted Police	RCMP	ICP-MS analysis of soil and quartz grain samples
University of Montréal	UM	Soil and pollen collection for ON and QC. Morphological and statistical analysis of pollen samples

*all sample collection except for soil and pollen samples from ON and QC; which were not part of the original mandate of Phase II.

Custom software (Signature©) was developed by the company Chaumont Systems Development Inc. (CSDi) of Ottawa, Ontario to manage the analytical results used to build a searchable database. This database is used to calculate the probability origin of unknown samples of hair, water, soil or pollen collected by law enforcement or other agencies and plot the results on a map.

Report

This report summarizes the work completed by UO on this project, (including sample distribution), and all results performed to date for the isotopic analysis of hair and water collected from all provinces: Newfoundland (NL), Prince Edward Island (PE), Nova Scotia (NS), New Brunswick (NB), Quebec (QC), Ontario (ON), Manitoba (MB), Saskatchewan (SK), Alberta (AB) and British Columbia (BC).

Sample Collection

All hair and water samples to be included in the database have been collected. Table 2 summarizes all samples collected across Canada.

Table 2. Total number of samples collected across Canada.

Location	Hair	Tap water	Other water	Soil	Pollen
ON, QC	143	141	18	243	243
NB, NS, PE, NL	131	136	34	246	253
MB, SK	100	114	26	150	150
AB, BC	216	188	42	234	234
TOTAL	**592***	**579^**	**120^**	**873**	**880**

*Includes one hair sample collected form Iqaluit, NT, and one hair sample from Weatherby, UK.
^Includes one sample that was analyzed for $\delta^{18}O$ only and one sample that was analyzed for $\delta^{2}H$ only

All recorded information for pollen and soil samples are summarized in the attached Excel file CRTI_project_08-0116RD_sample_info_import_template.xlsx. All results from the pollen analysis are summarized in the attached Excel file CRTI_project_08-0116RD_pollen.xlsx.

Water Samples

All water samples were collected in triplicate: one 50 mL vial was used for IRMS analysis, and two 50 mL vials were used for ICP-MS analysis. The vial used for IRMS analysis was used as received. The two 50 mL vials for ICP-MS analysis were prepared in the following manner.

1) Prior to sample collection, 1 mL of 20 % Ultrex II nitric acid was added.
2) After all samples had been collected, an additional 1 mL of Ultrex II nitric acid was added.

All water samples for ICP-MS analysis were sent to the RCMP. The RCMP added 1 mL of Ultrex II nitric acid within 1 month of receipt. All water samples were picked up by PSC.

Hair Samples

All volunteers who donated hair samples answered a questionnaire. This questionnaire varied from year to year. All questions asked are included in Table 3.

Hair samples were collected in two manners: haircuts (for those who have short hair), and cut from the scalp (for those who had longer hair). Based on the travel question, we are confident that the hair we collected is from local people who resided in their community during the timeframe that we collected it.

Each of the hair samples were divided into two aliquots – one aliquot for IRMS analysis, and one aliquot for ICP-MS analysis. If there was not enough hair to split into two, PSC did not receive an aliquot of the sample. All hair samples for ICP-MS analysis were picked up by PSC.

All hair for isotope analysis was prepared by first washing the hair in a 2:1 solution of chloroform:methanol (CHCl3:MeOH), then grinding the hair into a powder using a Retsch ball mill and stainless steel grinding jars. Hair was stored in 1 dram glass vials, lightly covered, on the bench, until analyzed.

Table 3. Questions answered by individuals who donated hair to populate the database.

Question	Year (s) and Areas Sampled
Sex: MALE or FEMALE Age: 18-29; 30-39; 40-49; 50-59; 60-69; 70+ Source of drinking water: - groundwater (deep or shallow well; how deep?) - surface water (name of lake or river)	all years
Are you a vegetarian or a vegan? If yes, what is your source of protein?	all years
Are you a smoker? If yes: how long have you smoked? If no: did you ever smoke? If yes, how long ago did you quit?	2008 (ON, QC) 2010 (MB, SK) 2011 (AB, BC)
Do you consume bottled water? If yes, how often and in what quantity?	2008 (ON, QC)
How often, and in what quantity, do you consume the following beverages: bottled water, alcohol, soft drinks?	2009 (NB, NS, PE, NL)
What types of beverages do you consume, how often, and in what quantity?	2010 (MB, SK) 2011 (AB, BC)
Is your hair dyed? YES or NO	2009 (NB, NS, PE, NL) 2010 (MB, SK) 2011 (AB, BC)
How often do you consume seafood and/or fish? Please specify types consumed.	all years
Have you travelled outside your local area for more than one day in the last year? If yes, please fill in the following details.	all years

Soil and Pollen Samples

While on sampling trips, all soil samples were shipped to the RCMP, and all pollen samples were shipped to UM, both on a weekly basis.

Sample Analysis

All hair and water samples collected from across Canada have been analyzed using IRMS. All water samples have been analyzed for δ^2H and $\delta^{18}O$. All hair samples have been analyzed for δ^2H, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and $\delta^{18}O$. However, in some cases, there was not enough hair for all isotopic analyses.

Water Report

This project is intended to tie together all data collected from across Canada. As such, this report includes all water δ^2H and $\delta^{18}O$ analyses for all locations. The results are reported relative to the international standard SMOW (Standard Mean Ocean Water); which is the internationally recognized "zero" for both isotopes

The locations of water collection and all isotopic results are summarized in the attached Excel file CRTI_project_08-0116RD_hair_and_water.xlsx.

All recorded information for water samples are summarized in the attached Excel file CRTI_project_08-0116RD_sample_info_import_template.xlsx.

Preparation of water and details of isotopic analyses of water are summarized in APPENDIX 1.

All water samples were divided into three categories:
1) Tap water whose source is groundwater (GW; e.g. shallow or deep wells).
2) Tap water whose source is surface water (SW; e.g. rivers & lakes).
3) Purchased water from a bottled water source (B) or from a water cooler (WC), and "eau de source" water, which is unfiltered, untreated water that people collect and consume.

Tap Water

In total, 579 tap water samples were collected. One tap water sample from Coaldale, AB was discarded due to a leaking vial, so a total of 578 water samples were analyzed. One sample from North Battleford, SK was not analyzed for δ^2H, so a total of 577 water samples with both δ^2H and $\delta^{18}O$ were analyzed. All tap water samples were identified as coming from ground water (TG) or surface water (TS) sources, and are plotted in Figure 1a. All tap water is plotted in Figure 1b. Figures 2 a-e are plots for different regions of Canada.

In Figure 1a, the regression lines are different between TG and TS: the regression line for TG has a larger slope and more positive intercept than that for TS (Tables 4a and 4b). The different lines result from various processes occurring on assorted waters sources. Specifically, surface water is subjected to evaporation, which affects the larger mass isotope more intensely, while ground water is significantly less affected by this physical process, which in part can lead to different regression lines and thus some different isotopic values for the same area. Major effects are linked to latitude (North-South temperature effects; winter/summer) and altitude (pressure & temperature) such as the Rocky Mountains areas.

In Figure 1b, all tap water shows an intermediate slope and intercept between those for TG and TS. The line is also very similar to the "Canadian Meteoric Water Line" (CMWL).

Also plotted on Figures 1a and 2a-e is the CMWL derived from rainfall measurements throughout Canada. It is the average δ^2H and $\delta^{18}O$ values of precipitations collected from across Canada (excluding the Arctic) over 7 years [26].

$$\delta^2H = 7.75 \times \delta^{18}O + 9.83$$

It is very similar to the planetary "Global Meteoric Water Line" (GMWL) derived from rainfall as measured on a worldwide basis and defined by Craig [31] as: $\delta^2H = 8 \times \delta^{18}O + 10$

and later refined by Rozanski [26]: $\delta^2H = 8.17\ (\pm 0.07) \times \delta^{18}O + 11.27\ (\pm 0.65)$

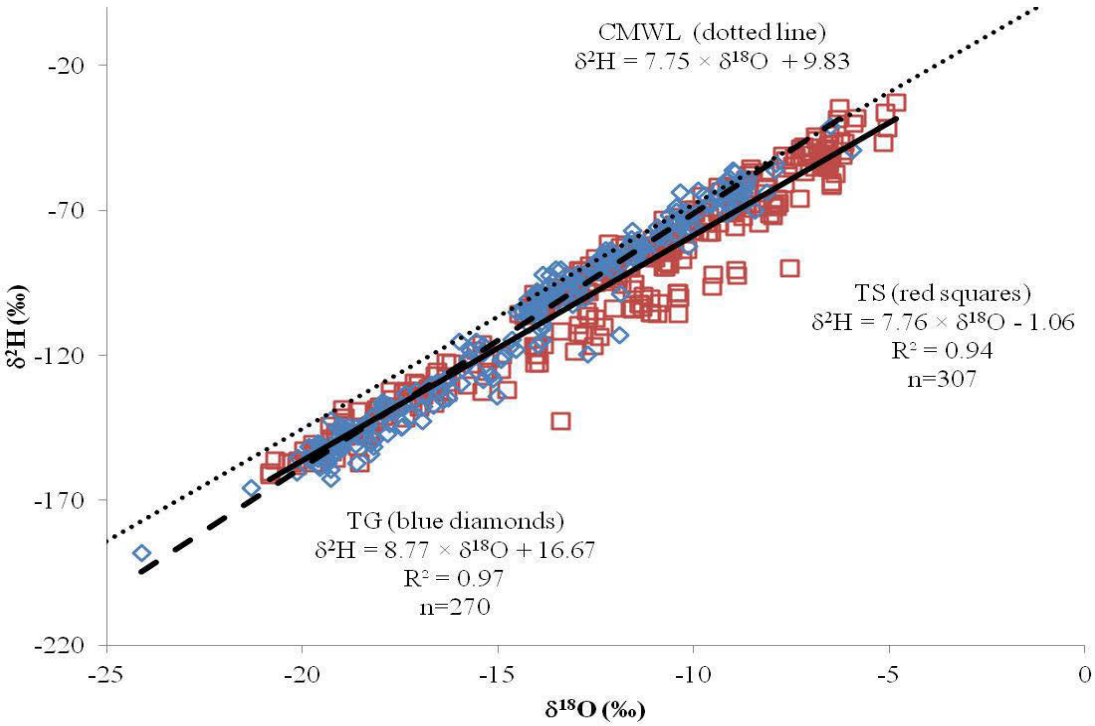

Figure 1a. All tap water collected from all provinces. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

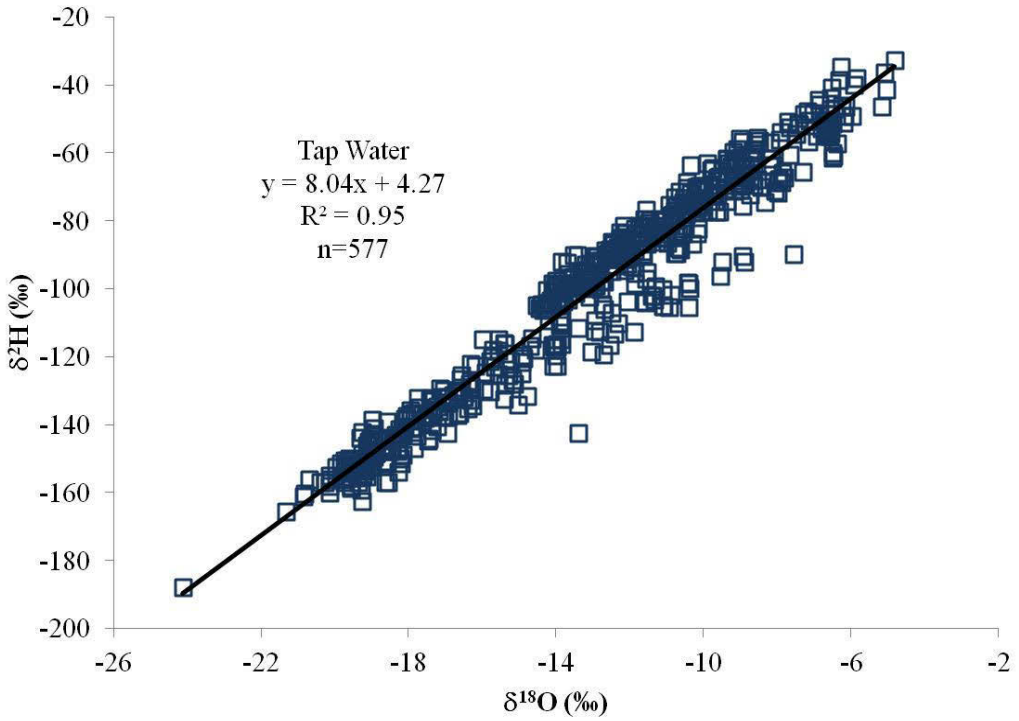

Figure 1b. All tap water collected from all provinces.

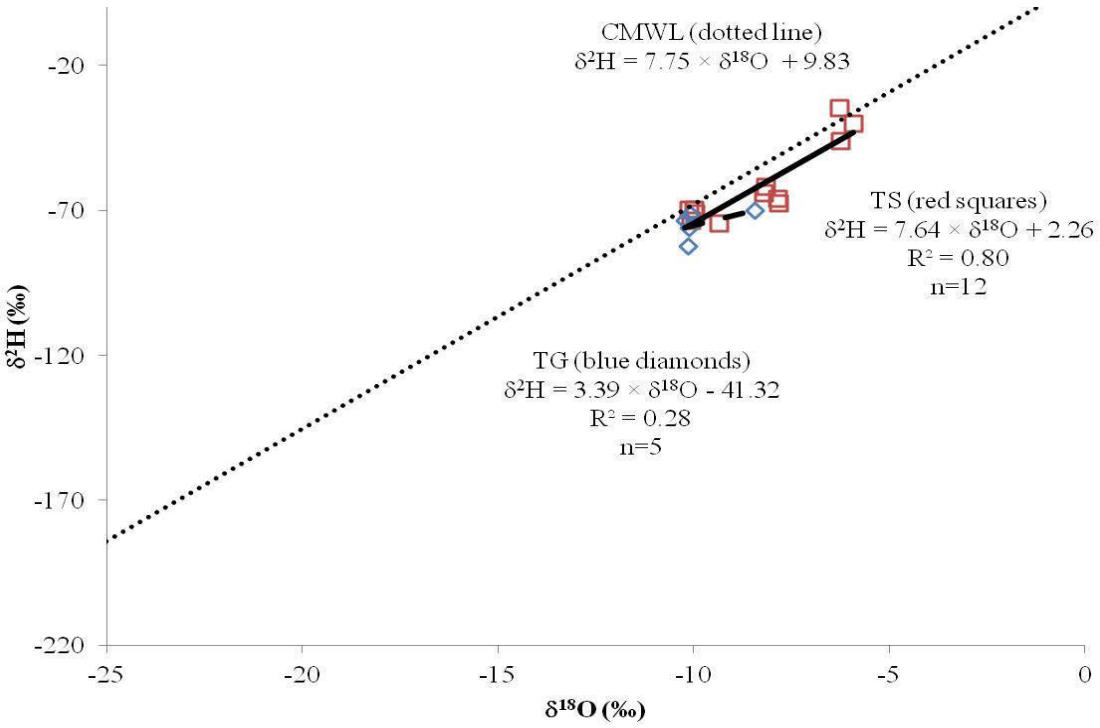

Figure 2a. Tap water collected from NL. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

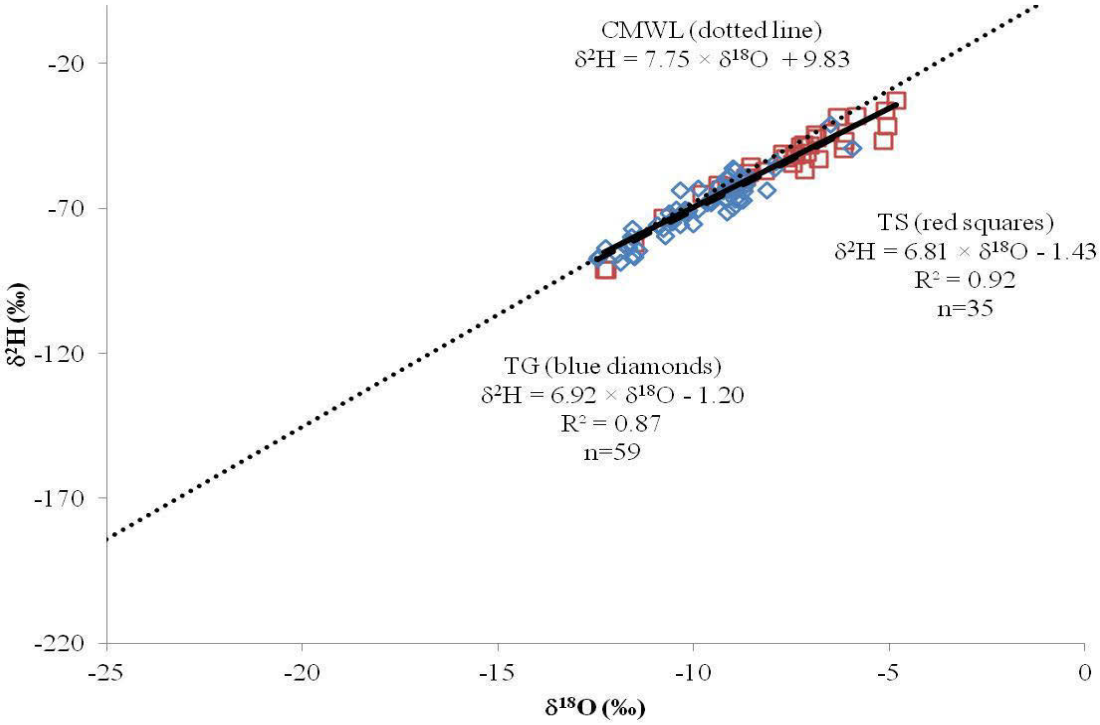

Figure 2b. Tap water collected from NB, NS and PE. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

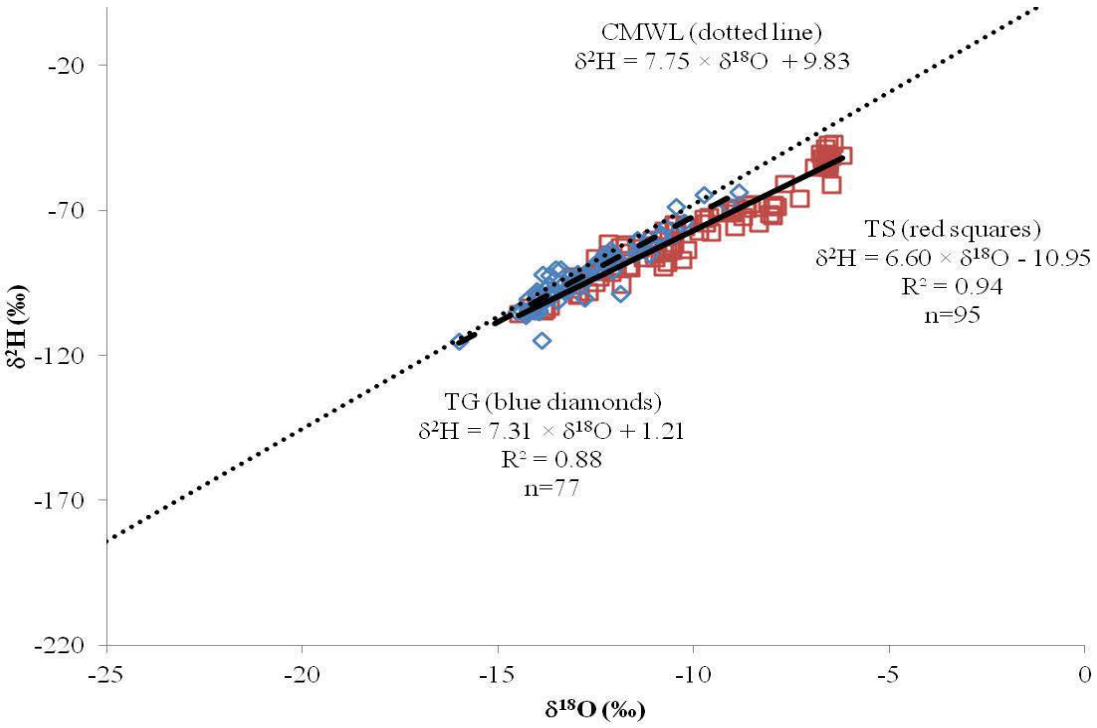

Figure 2c. Tap water collected from ON and QC. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

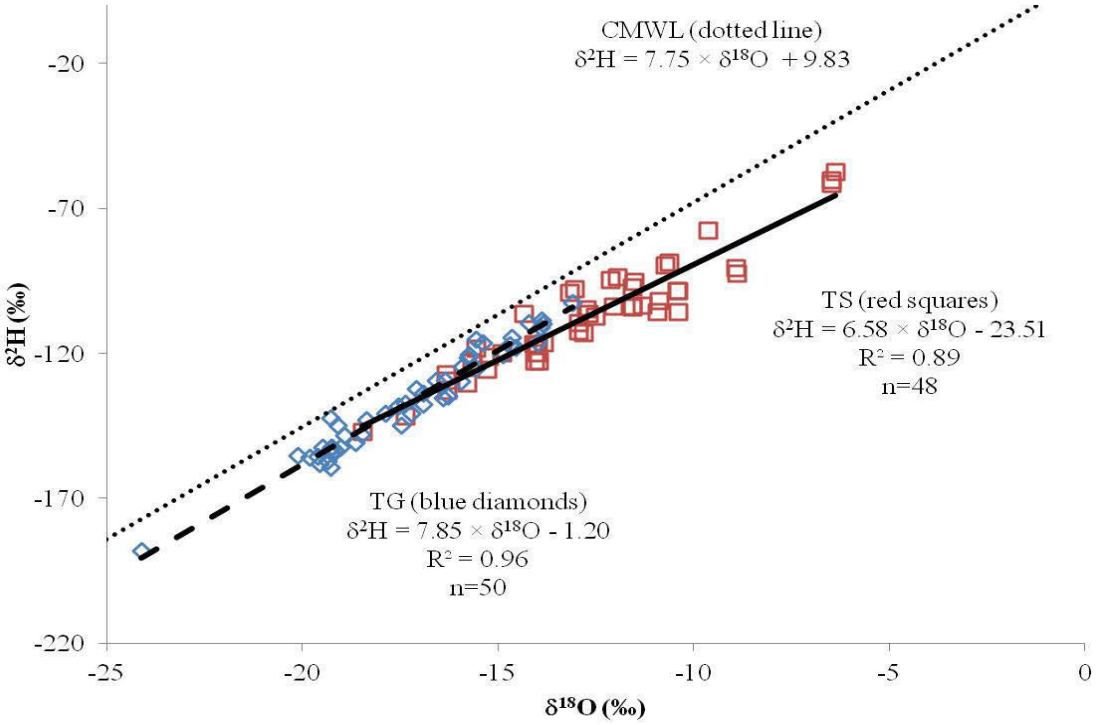

Figure 2d. Tap water collected from MB and SK. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

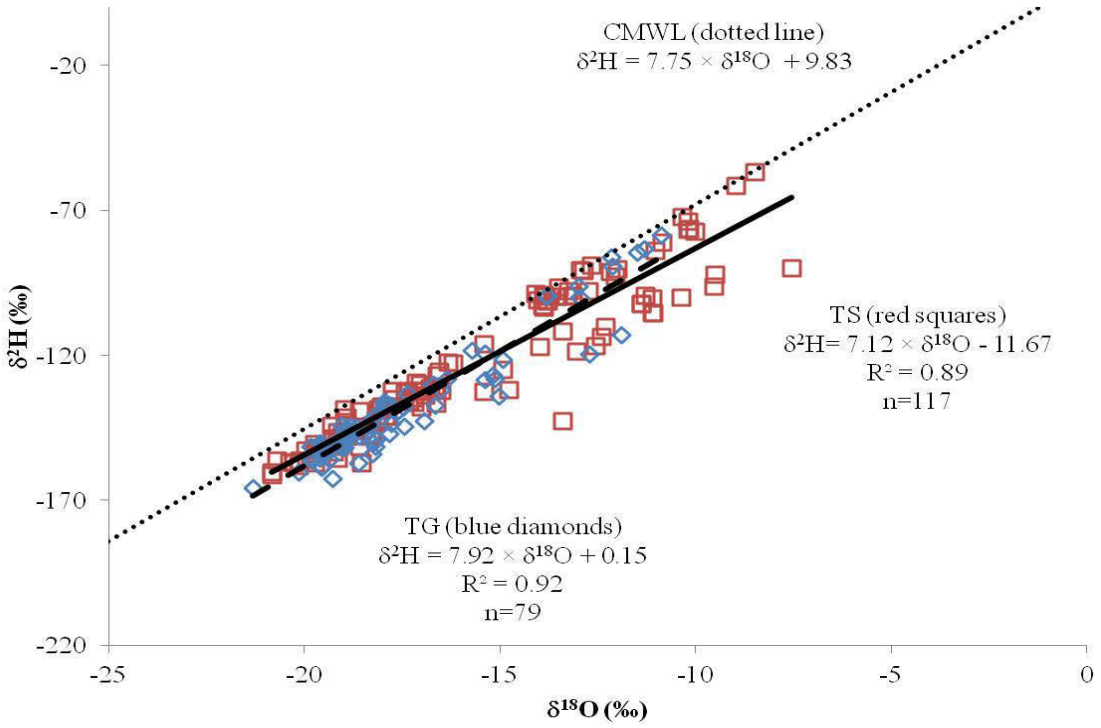

Figure 2e. Tap water collected from AB and BC. TS = tap water from a surface water source. TG = tap water from a ground water source. CMWL = Canadian meteoric water line [26].

The Meteoric Water Lines (MWL) result from isotopic fractionation effects (i.e. a change in the isotope value) on water. The main causes of isotopic fractionation are temperature and pressure (i.e. latitude, altitude, seasonal), which are directly related to the variables affecting the equilibrium constant (K) in a chemical reaction. For stable isotopes, the equilibrium constant is symbolized by "α" and represents the distribution, or exchange of isotopes, between two substances or two phases of the same substance. For example, the $^{18}O/^{16}O$ exchange between water vapour and liquid is represented by [26]:

$$H_2O_{water} \leftrightarrow H_2O_{vapour;} \text{ where}$$

$$\alpha^{18}O_{water\text{-}vapour} = \frac{(^{18}O/^{16}O)_{water}}{(^{18}O/^{16}O)_{vapour}}$$

These isotopic effects result in a shift in the **same** direction for both δ^2H and $\delta^{18}O$ data where the isotopic values of groundwater are generally more negative than those from surface water of the same (or nearby) location. Although the magnitude of the shift is larger for $\delta^{18}O$ compared to δ^2H, it follows predictable thermodynamic patterns resulting in Local, Canadian and Global MWL.

The CMWL has a similar slope to the regression line of the MWL for both TS and TG (Figure 1). However, the slope for TG is larger than that for TS. Since the main recharge for lakes and rivers is generally precipitation, it follows that TS and the CMWL would have similar slopes. On the other hand, once the water infiltrates the ground and remains there, in some cases for many years,

evaporation no longer significantly affects this water, resulting in a slightly different regression line.

It is important to keep in mind that the CMWL is essentially a combination of several "Local Meteoric Water Lines" (LMWL). These LMWL can vary significantly from the CMWL due to varying local climate conditions and geographic parameters. Figures 2a-e show "Tap Surface Water Lines" (TSWL) and "Tap Ground Water Lines" (TGWL) for several regions of Canada; their regression lines are summarized in Tables 4a and 4b.

Table 4a. Slope and intercept for TS from several regions across Canada.

Figure	Location	Slope	Intercept	N
2a	NL	7.64	2.26	12
2b	NB, NS, PE	6.81	-1.43	35
2c	ON, QC	6.60	-10.95	95
2d	MB, SK	6.58	-23.51	48
2e	AB, BC	7.12	-11.67	117
1	All	7.76	-1.06	307

Table 4b. Slope and intercept for TG from several regions across Canada.

Figure	Location	Slope	Intercept	N
2a	NL	3.39	-41.32	5
2b	NB, NS, PE	6.92	-1.20	59
2c	ON, QC	7.31	1.21	77
2d	MB, SK	7.85	-1.20	50
2e	AB, BC	7.92	0.15	79
1	All	8.77	16.67	270

With the exception of NL, which had limited samples, the slope of TS is always smaller than that of TG.

Comparing Figures 2a-e, there is a geographical dependence on the δ^2H and $\delta^{18}O$ values of the waters. Figure 2a represents the province furthest to the east (i.e. NL), and the locations progressively move towards the west with increasing letters. As you move west, there is a general progression in the water δ^2H and $\delta^{18}O$ values: the eastern locations are amongst the most positive δ^2H and $\delta^{18}O$ values, and the more western locations are amongst the most negative δ^2H and $\delta^{18}O$ values. This trend is consistent with the isotope values of water varying with latitude (northward) and altitude (near the Rocky Mountains).

With the exception of NL (Figure 2a; small sampling size), the slopes of the WLs were comparable to the CMWL (± 1.2). The shallower slope for all the TSWLs suggests that the water plotted along these lines has undergone significant evaporation, as compared to the TGWLs. The deviations in the TSWLs from the CMWL infer that the water collected in these areas have undergone different processes than water that plots along the CMWL. Other factors such as altitude (melt waters from mountains), humidity, local weather conditions and temperature can give rise to these deviations. This is discussed further under Deuterium Excess (below).

Figures 3a and 3b show the GIS maps of δ^2H and $\delta^{18}O$ values for all tap water (i.e. TS and TG combined). Both figures show the same pattern: the δ^2H and $\delta^{18}O$ values vary with latitude in the east (i.e. ON and east), and the isotopic values vary with longitude in the west (i.e. Manitoba and west). The longitudinal pattern is a result of the Canadian Rockies, which show an altitude effect. This pattern is similar to that seen for precipitation in Canada (Figure 4) [26].

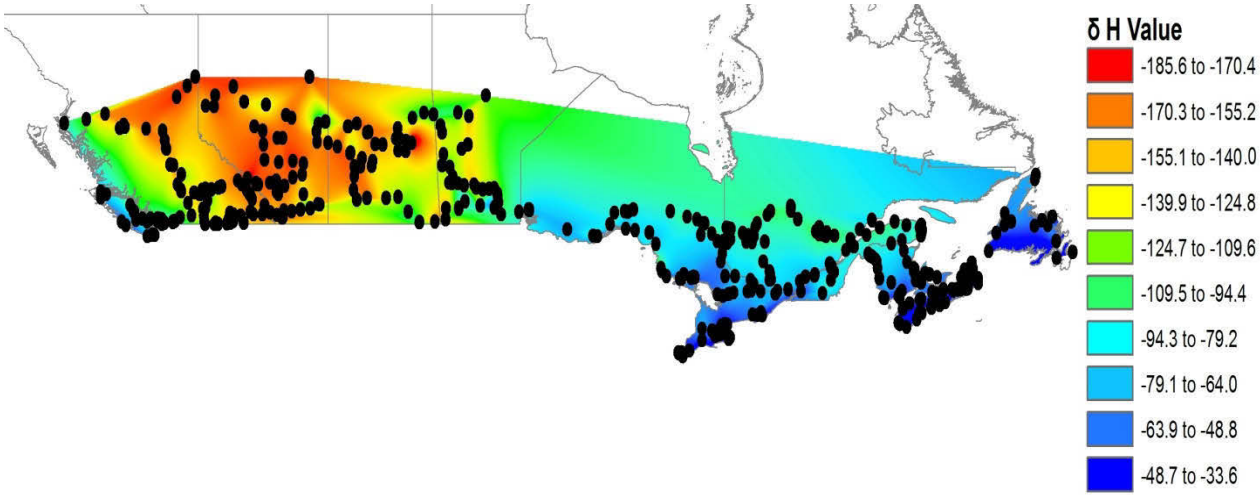

Figure 3a. δ^2H of all tap water collected from Canada. Black dots represent sampling locations.

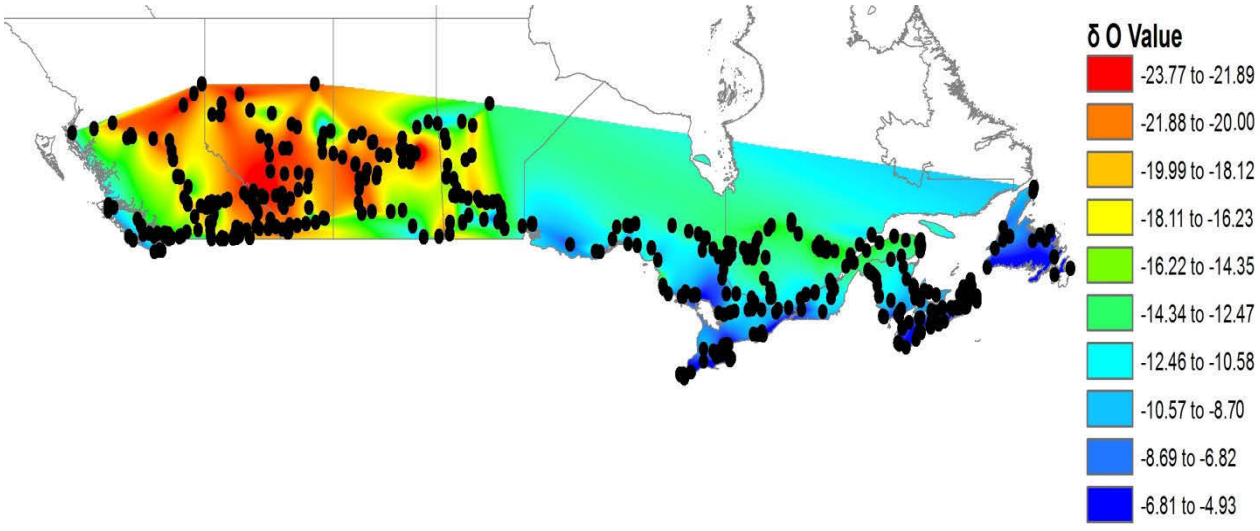

Figure 3b. $\delta^{18}O$ of all tap water collected from Canada. Black dots represent sampling locations.

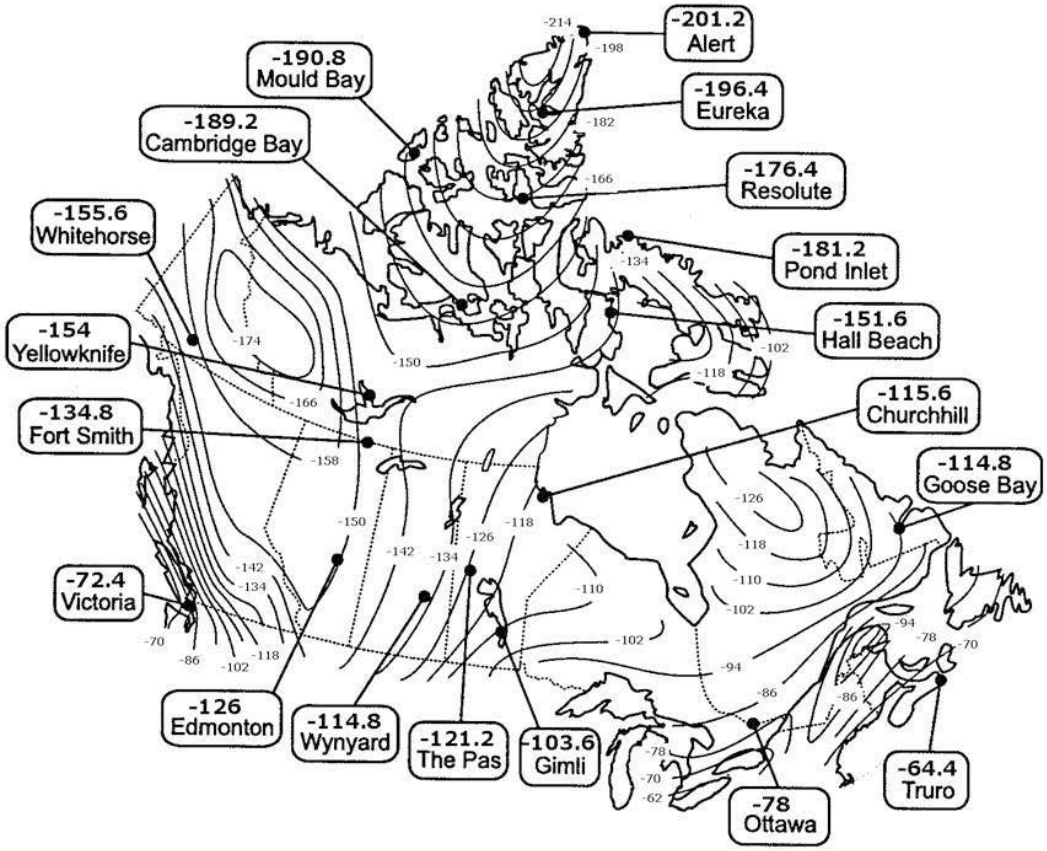

Figure 4. δ^2H of groundwater (lines) and precipitation (bubbles) for samples collected across Canada. Modified from Clark and Fritz [26].

Figures 5a and 5b show the δ^2H and $\delta^{18}O$ values for TS. As with Figures 3a and 3b, the δ^2H and $\delta^{18}O$ values vary with latitude in the east, and with longitude (altitude) in the west.

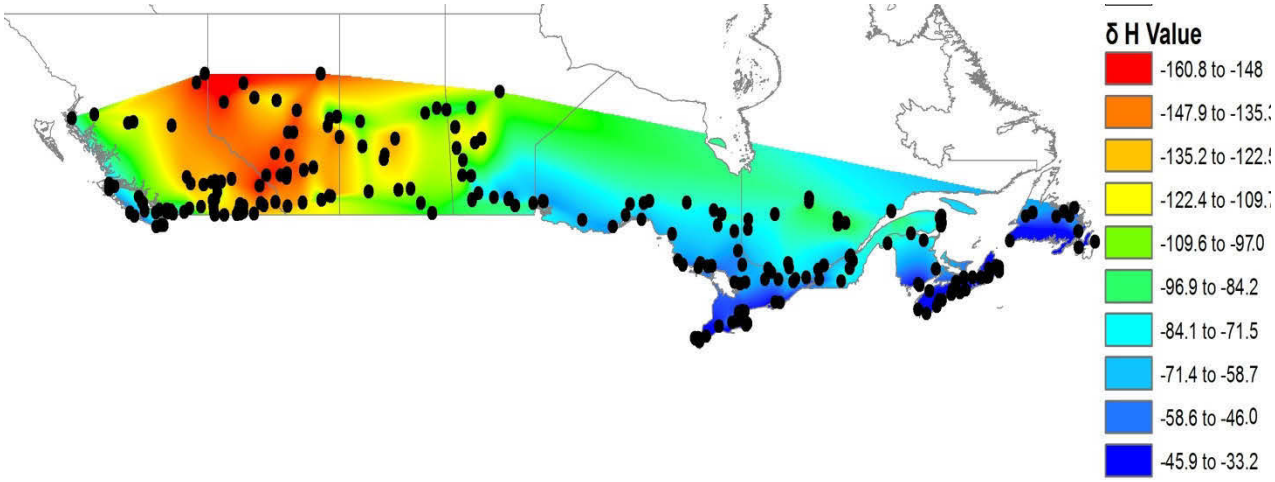

Figure 5a. δ^2H of TS water collected from Canada. Black dots represent sampling locations.

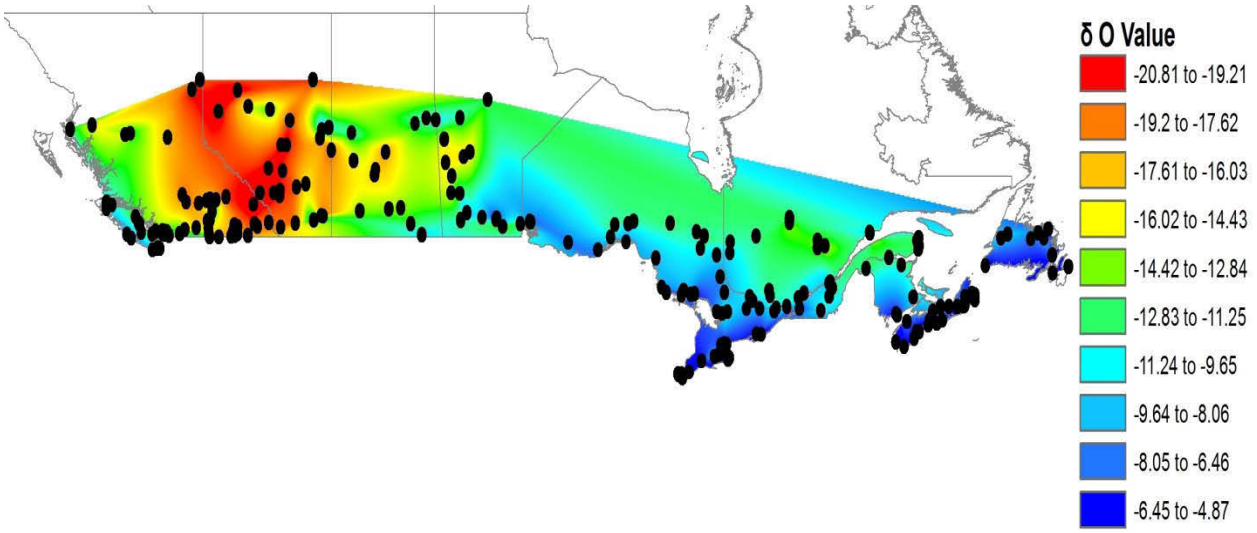

Figure 5b. $\delta^{18}O$ of TS water collected from Canada. Black dots represent sampling locations.

Figures 6a and 6b show the $\delta^{2}H$ and $\delta^{18}O$ values for TG. Again, the pattern of latitude variation is preserved in the east, and longitude (altitude) variation is preserved in the west.

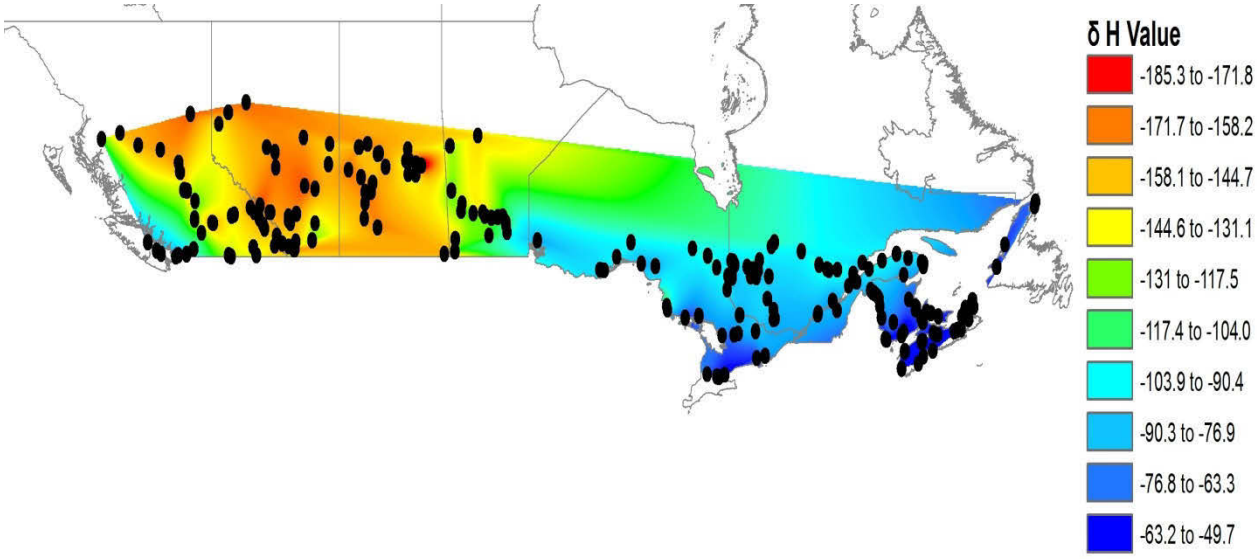

Figure 6a. $\delta^{2}H$ of TG water collected from Canada. Black dots represent sampling locations.

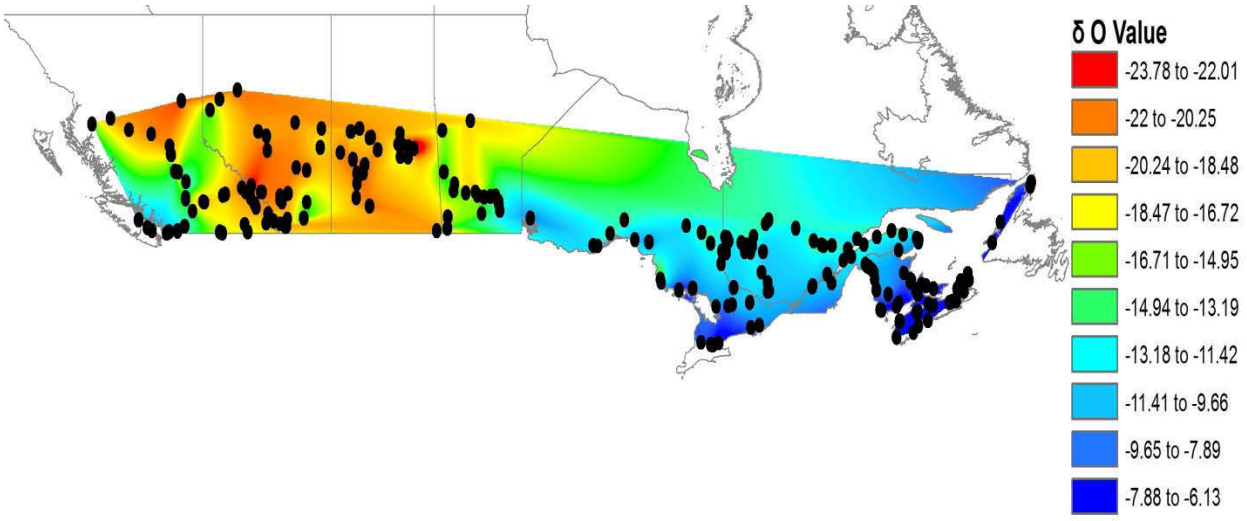

Figure 6b. $\delta^{18}O$ of TG water collected from Canada. Black dots represent sampling locations.

Deuterium Excess

All MWL result from the fractionation of water isotopes in predictable patterns. However, different local geographical conditions such as altitude (pressure), humidity, sea surface temperature and wind speed, result in different patterns of fractionation. These different fractionation patterns can be characterized from the GMWL using deuterium-excess (*d*) which distinguishes these local variations and is defined for a slope of 8 (from the GMWL) and calculated as [26]:

$$\text{GMWL is defined as } [31]: \ \delta^2H = 8 \times \delta^{18}O + 10$$
$$\text{and } d = \delta^2H - 8 \times \delta^{18}O$$

Calculating *d* for global precipitation, a value of 10 ‰ for an average global humidity of 85% for seawater (with a value of δ^2H= 0‰) is demonstrated in Figure 7 [26].

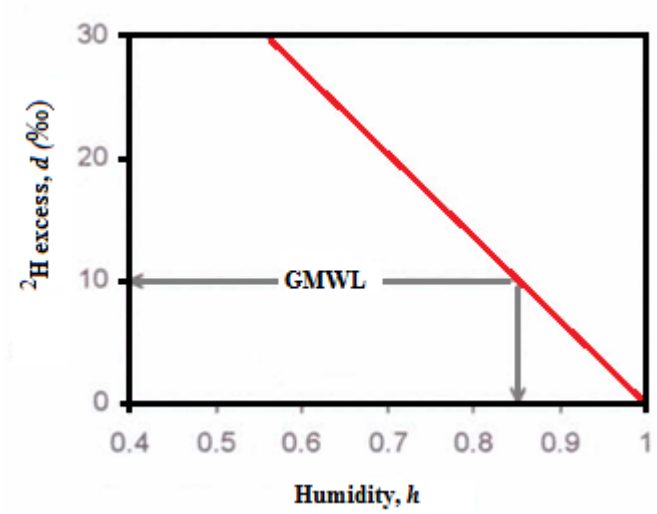

Figure 7. The deuterium excess parameter, *d,* as a function of humidity, *h*, during kinetic evaporation from the ocean surface [26].

As such, by calculating *d* for every water sample, the extent to which a water sample has deviated from the GMWL can be quantified. It is of importance to mention that all the samples, except for the re-sampling set, were collected during the early summer months which give a single seasonal value for our temperate climate in Canada. An example of this is shown in Table 5 [(26)] where seasonal sampling occurred showing the expected variations due to seasonal changes.

Table 5. Meteoric water lines for stations from varying climates across Canada [(26)].

Station	Climate	Season	$\delta^{18}O$ vs. $\delta^{2}H$		d
			Slope	Intercept	
Victoria	Pacific marine	Year	7.8	2.9	3.6
		Summer	8.3	3.9	1.6
		Winter	7.5	-1.6	3.9
Le Pas	Western interior	Year	7.6	0.6	5.6
		Summer	7.4	-3.0	3.5
		Winter	8.0	11.3	10.4
Ottawa	Eastern interior	Year	7.6	6.5	12.2
		Summer	7.5	4.8	11.0
		Winter	7.9	11.0	14.7
Truro	Atlantic marine	Year	7.4	5.6	10.6
		Summer	7.8	8.3	8.2
		Winter	7.4	5.2	12.8
Fort Smith	Arctic continental	Year	7.5	-4.9	0.3

Figures 8a and 8b show the map of *d* for TG and TS, respectively. In simple terms, the further the *d* value is from 10, the further away that water sample is from the GMWL. In general, the lower the *d*, the lower the humidity of the area, resulting in larger fractionation effects due to evaporation.

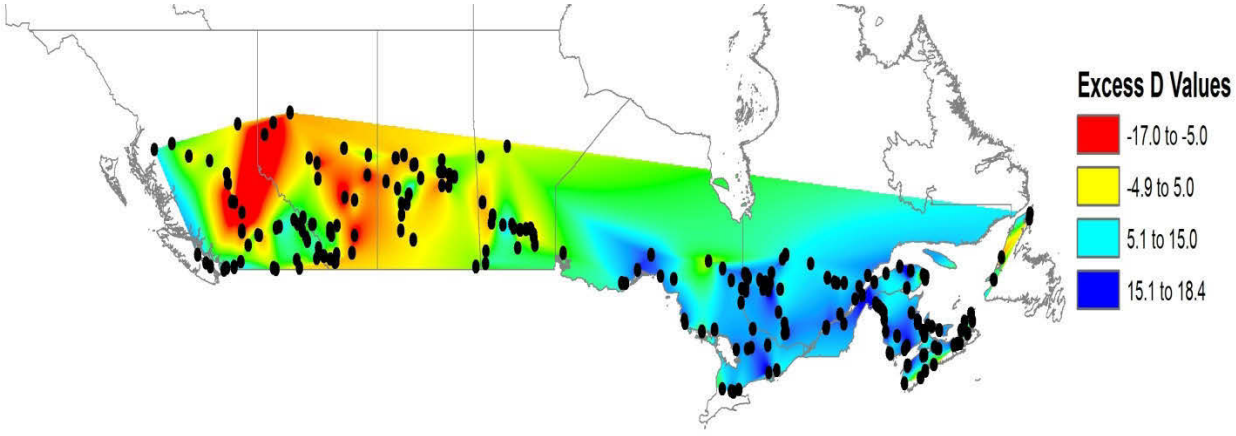

Figure 8a. GIS map showing *d* (deuterium excess) for ground water sources (TG).

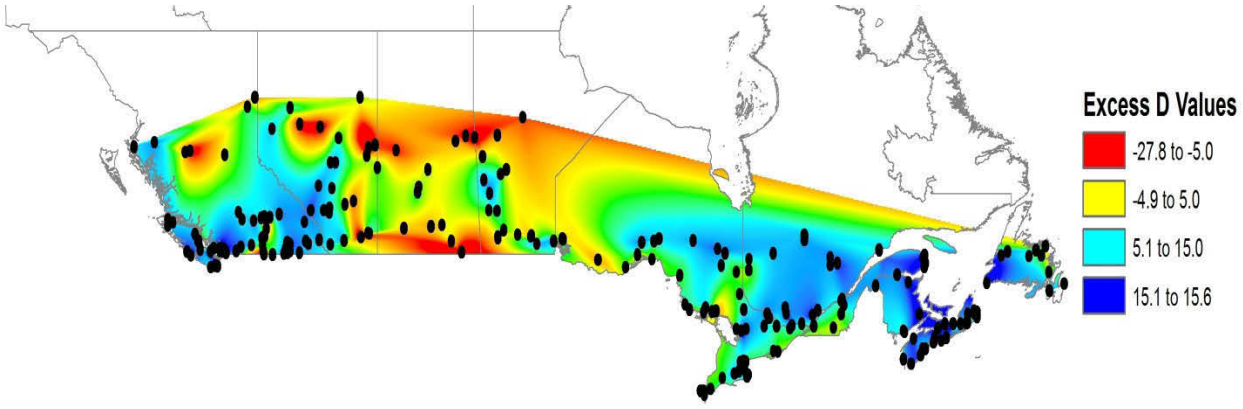

Figure 8b. GIS map showing *d* (deuterium excess) for surface water sources (TS).

The blue colour symbols represent water that had a *d* around 10 (i.e. 5 to 18.4 ‰), which is similar to the GMWL. Yellow symbols represent areas with lower *d* values (i.e. -5 to 5 ‰), which can represent moderate evaporation effects. From Figure 8a, yellow symbols are concentrated in the western provinces (MB and west) for TG. In contrast, TS sources showing moderate evaporation are found AB, SK, and MB, with pockets in northern BC and ON (Figure 15b). Red symbols, which show extreme lower *d* values (< -5‰) can represent areas where extreme evaporation has occurred. Extremely evaporated sources occurred in both TS and TG samples, and occurred in the western provinces. In general, there is lower humidity in BC east of the Rockies to MB, due to the inland location, which can result in more extreme evaporation of water sources.

Multiple Tap Water Samples from the Same Location

In several instances, more than one sample of water was obtained from the same city. In most cases, the tap water samples showed nearly identical δ^2H and $\delta^{18}O$ values (i.e. within ± 4 and ± 0.4 ‰ for δ^2H and $\delta^{18}O$, respectively). However, there were some occasions as outlined below, where this was not the case.

Baie Comeau, QC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
House 1	-103.5	-14.16
House 2	-86.4	-10.98
Hotel	-84.6	-10.79

House 1 is outside of the city, while House 2 and Hotel are within the city. After speaking to a person who designed the water distribution system, House 1 receives its water from a different river than House 2 or Hotel.

Liverpool, NS

Location	δ2H (‰)	δ18O (‰)
RCMP	-40.9	-6.52
House	-49.2	-5.96

The town water supply is a lake. However, these two samples are from two different wells, presumably from different aquifers.

Sault Ste. Marie, ON

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
House	-89.4	-12.56
Hotel	-74.3	-9.70

This city has two water sources: wells and Lake Superior. Both of these sources were sampled.

St. Anthony, NL

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-75.8	-10.12
House	-82.3	-10.15

This town uses two wells for their water supply. It is possible that samples were taken from the two different source wells, which may be supplied from different aquifers.

Summerside PE

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-62.9	-9.89
Restaurant	-74.5	-10.55

All tap water sources in PEI are from ground water. The RCMP detachment is outside of the city of Summerside, so perhaps the water from the RCMP is from a different aquifer than the city of Summerside.

Woodstock, NB

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-86.8	-11.53
Restaurant	-76.8	-11.57

There is not enough information available to explain this discrepancy.

Radisson, SK

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-142.2	-19.27
Radisson Tap	-152.2	-19.25
House 1	-153.7	-19.16

House 1 is located outside of Radisson. Occupants of this house used House 1 water from their personal well to cook with, and Radisson Tap to drink (no other details were given). These two water samples appear to be from the same water source. Water from the RCMP is town water, which is also groundwater. The RCMP tap water may be from a different aquifer than the other house, resulting in a different isotope value.

Burns Lake, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
House 1	-153.1	-19.38
House 2 – filtered water	-144.8	-18.64
House 2 – directly from tap	-147.9	-18.60
House 3	-144.3	-17.44
House 4	-110.1	-12.31
House 5	-113.4	-12.41

The source of drinking water in the Village of Burns Lake is groundwater; however, the source of drinking water for residents living outside the village is Burns Lake itself [32].

The same participant lived in House 1 for three years before moving to House 2 six months prior to sampling. House 1 and House 2 were across the road from each other. House 1 obtained their water from a different source as House 2: House 1 source is unknown (likely a personal well), but House 2 is on city water (groundwater). For House 2, it is evident that filtering the water does not result in a change in the isotopic value of the water.

House 3 also has city drinking water. The δ^2H value of the water is identical to House 2, but the $\delta^{18}O$ value is different. This may be due to the city drawing water from different aquifers, but no information is available at this time to support this hypothesis.

Houses 4 and 5 both claim their drinking water source is the Tchesikut River, which accounts for the different δ^2H and $\delta^{18}O$ values observed for their drinking water compared to those on city water.

Vancouver Area, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
Coquitlam – restaurant	-90.3	-12.94
Langley - home	-90.8	-12.88
Burnaby – house	-102.7	-13.90
Delta – house	-101.2	-13.80
Richmond – restaurant	-101.1	-13.79
Surrey – house	-103.4	-13.88
Surrey – hotel	-99.0	-13.88
Vancouver – restaurant	-99.7	-13.53
West Vancouver – gas station	-96.5	-13.51
North Vancouver – house	-97.8	-13.26
North Vancouver – house	-101.0	-14.04
North Vancouver – RCMP	-98.6	-14.09

The Vancouver area is supplied by three main reservoirs: the Capilano, the Seymore and the Coquitlam. Each reservoir is fed by the corresponding areas watershed, and each reservoir provides ~ 33 % of drinking water to the metro Vancouver area [33].

Water samples were taken from the main areas of metro Vancouver. The Coquitlam reservoir presumably supplies the suburb of Coquitlam with drinking water, and the city of Langley. Water from the Coquitlam reservoir is isotopically different from the remaining areas, suggesting that the Coquitlam reservoir is slightly different (isotopically-speaking) than the other two reservoirs.

Canmore, AB

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-150.5	-19.53
House – tap	-151.5	-19.54
House – filtered	-151.6	-19.54
House – reverse osmosis	-154.3	-19.54
Facility – other side of river	-144.3	-18.81

A resident from Canmore stated the drinking water source is a municipal well. The RCMP detachment and House are located on the same side of the river, and the isotope values of the water are the same. Filtering and reverse osmosis treatment of the water did not alter the isotope value. The water sample obtained from a facility located on the other side of the river had a different isotopic value, suggesting that this facility drew its water from a different source.

Chetwynd, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-154.2	-19.55
House	-160.1	-20.14

The source of tap water for Chetwynd is groundwater. The House uses a personal well for its drinking water. It may be that the water from the House is from a different aquifer than water from the RCMP (city water), but there is not enough information to determine if this is the case.

Glenwood, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
House 1	-158.6	-19.55
House 2	-155.6	-19.36
House 3	-144.2	-18.42
House 4	-139.0	-17.61
House 5	-137.9	-17.46

All 5 houses have personal wells for their drinking water supply. The different isotope results obtained may be due to more than one aquifer supplying the houses.

Invermere, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-156.2	-19.80
Hotel	-148.2	-18.88

The source of tap water for Invermere is groundwater. If the water from the RCMP is from a different aquifer than water from the hotel, this may explain the difference in isotope values.

Kelowna, BC

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
House 1	-99.2	-11.30
House 2	-102.1	-11.39
RCMP	-102.0	-11.40
Restaurant in Rutland	-138.5	-18.05
Gas Station in West Kelowna	-126.7	-16.60

Kelowna is divided into several areas, including Rutland (to the east) and West Kelowna. Houses 1 and 2 and the RCMP all have identical isotope values, and are all located in Kelowna. However, both Rutland and West Kelowna have different isotope values, suggesting that these areas are not on the same water source as Kelowna.

Pincher Creek, AB

Location	δ^2H (‰)	$\delta^{18}O$ (‰)
RCMP	-144.0	-19.31
Hotel	-142.1	-18.17

Pincher Creek uses surface water as their source of drinking water. The difference in isotope values for both locations within Pincher Creek suggests two different sources; where one is ground and the other surface water. This conclusion is suggested by the ^{2}H result being almost identical whereas the ^{18}O results are different enough. However, there is not enough information available to determine if this is the case.

Quesnel, BC

Location	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
Restaurant	-140.7	-18.15
House 2	-143.4	-17.78
House 3	-144.1	-18.31
House 4	-151.8	-18.97

The source of tap water in Quesnel is ground water. Both the restaurant and House 2 are on city water, which give identical isotopic results. House 3 has a personal well, which may be from the same aquifer as the town supply. House 4 also has a personal well, which may be from a different aquifer than the town, resulting in different isotope values.

Salmon Arm, BC

Location	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
RCMP	-135.7	-17.32
House	-131.9	-16.66
Hotel	-131.6	-16.63

The source of tap water in Salmon Arm, BC is surface water. Both the House and Hotel had the same isotopic results for the water. The RCMP water is slightly different, suggesting it may be a different source than the other two.

Williams Lake, BC

Location	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
RCMP	-119.5	-12.72
House	-147.0	-17.84

Five deep wells provide the city of Williams Lake with drinking water and the RCMP detachment is on city water. The water obtained from the House is described as coming from a "community well", which may explain the isotopic difference between these two water samples: the community well may be a different aquifer than the city.

Groundwater and Surface Water from the Same Location

During the collection of water samples, tap water from the same location (or very close locations) which had both surface and ground sources could be sampled. The results are shown in Table 6. In most cases, the ground water has more negative $\delta^{2}H$ and $\delta^{18}O$ values compared to surface water (shown in grey) which tracks well with evaporative effects occurring on the surface water compared to the ground water.

Table 6: Surface water vs. ground water from the same, or very close, locations. Locations showing different δ^2H and/or $\delta^{18}O$ values are in grey. Locations with similar δ^2H and/or $\delta^{18}O$ values have no highlighting.

Location	Province	Surface Water		Ground Water	
		δ^2H (‰)	$\delta^{18}O$ (‰)	δ^2H (‰)	$\delta^{18}O$ (‰)
Yarmouth (S) / Arcadia (G)	NS	-32.8	-4.82	-56.0	-7.94
Lunenburg (S) / Centre (G)	NS	-36.2	-5.10	-53.8	-7.90
Dartmouth (S) /East Hants County (G)	NS	-48.2	-7.03	-59.6	-9.19
Dartmouth (S) /Still Water Lake (G)	NS	-48.2	-7.03	-63.7	-8.13
Dartmouth (S) /Folly Mountain (G)	NS	-48.2	-7.03	-62.4	-9.11
Port Hawkesbury (S) /Port Hastings (G)	NS	-56.6	-7.16	-64.0	-8.71
Baddeck	NS	-54.4	-7.48	-66.8	-9.15
Cochrane	ON	-97.9	-12.70	-105.0	-14.52
Geraldton (S) /Outside Geraldton(G)	ON	-83.8	-10.55	-99.0	-13.96
Sault Ste. Marie	ON	-74.3	-9.70	-89.4	-12.56
Hearst (S) / Kapuskasing (G)	ON	-93.1	-12.40	-100.1	-12.79
Barry's Bay (S) / Killaloe (G)	ON	-70.9	-9.38	-89.9	-12.55
Toronto (GTA) (S) /Milton (G)	ON	-51.1	-6.57	-63.7	-8.86
Cobalt (S) / New Liskeard (G)	ON	-75.5	-8.95	-98.6	-13.52
Nipigon	ON	-86.8	-10.29	-98.8	-14.02
Parry Sound	ON	-67.7	-8.54	-79.9	-11.45
Napanee (S) / Plainfield (G)	ON	-52.0	-6.71	-68.6	-10.44
Thunder Bay	ON	-69.4	-8.64	-96.7	-13.25
Kingston (S) /Harrowsmith (G)	ON	-50.9	-6.49	-83.6	-11.35
Chapais	QC	-94.8	-12.50	-102.5	-14.55
Tadoussac (S) /Sacre Coeur (G)	QC	-81.8	-11.08	-93.5	-13.01
Brandon (S) / Virden (G)	MB	-97.2	-11.56	-138.0	-17.55
Portage La Prairie	MB	-88.5	-10.63	-120.0	-15.74
Prince Albert (S) /Shellbrook (G)	SK	-141.7	-17.37	-152.5	-19.26
Meadow Lake*	SK	-104.0	-11.58	-150.8 -125.1	-18.63 -15.56
Bonnyville	AB	-87.9	-7.56	-150.1	-18.23
Burns Lake*	BC	-110.1 -113.4	-12.31 -12.41	-144.3 -153.1 -144.8 -147.9	-17.44 -19.38 -18.64 -18.60
Kamloops	BC	-129.3	-17.14	-151.4 -148.8	-19.83 -18.15
Antigonish (S) /St. Andrews (G)	NS	-62.0	-9.16	-62.1	-9.36
Truro (S) / Bible Hill (G)	NS	-63.2	-9.31	-67.2	-9.62
Liverpool	NS	-38.1	-5.85	-49.2	-5.96
Nanaimo	BC	-88.8	-12.67	-89.1	-12.14
Terrace	BC	-115.9	-15.42	-118.1	-15.71
Peace River	AB	-158.0	-20.14	-158.3	-19.57
Ashcroft^	BC	-142.8^	-18.14^	-128.2^	-15.39^
Courtenay^	BC	-99.5^	-13.29^	-86.1^	-12.15^
Hope^	BC	-99.7^	-13.84^	-99.7^	-12.98^
Langley^	BC	-90.8^	-12.88^	-84.4^ -83.1^	-11.50^ -11.32^

* More than one water sample was obtained from the area.

^ See text below for explanation.

For Antigonish/St. Andrew, both the ground water and surface water δ^2H and $\delta^{18}O$ values were similar. The St. Andrew sample from is from a personal well. The water supply for the town of Antigonish is the James River Reservoir. It is hypothesized that the James River serves to recharge water from the personal well, resulting in very similar water isotope values.

Similarly, the ground water and surface water isotopic values are very close for Truro/Bible Hill. The Lepper Brook serves as the water source for Truro, which also likely recharges the personal well from Bible Hill.

In the case of Liverpool, the δ^2H value of the surface water is more positive than that of groundwater; however, the $\delta^{18}O$ values are very similar. The water source for Liverpool is Town Lake. It is unclear as to why the $\delta^{18}O$ values are very similar. It is possible that the well was a shallow well, which may be recharged mainly from precipitation.

For Nanaimo, Terrace and Peace River, both the δ^2H and $\delta^{18}O$ values are very similar, suggesting that the recharge of the well and surface water in these areas may be from the same source.

There are some cases where the surface water is more negative than the ground water, namely in Ashcroft, Courtenay, Hope and Langley. In these cases, it is plausible that the source of surface water is from snow or ice melt. The isotopic values of winter ice and snow are usually more negative whereas liquid water can evaporate and get more enriched. However, there is insufficient evidence to prove that this is the case.

Tap Water and Purchased Water from the Same Location

Purchased water was separated into two categories:

a. water cooler (WC) water, which is the typical blue 18.5 L (or 5 gallon) jugs (n=87)
b. bottled water (B), which is in 1 L or less containers (n=28)

The δ^2H and $\delta^{18}O$ values for all purchased water are plotted in Figure 16. Both the bottled water and WC water have similar slopes to the CMWL.

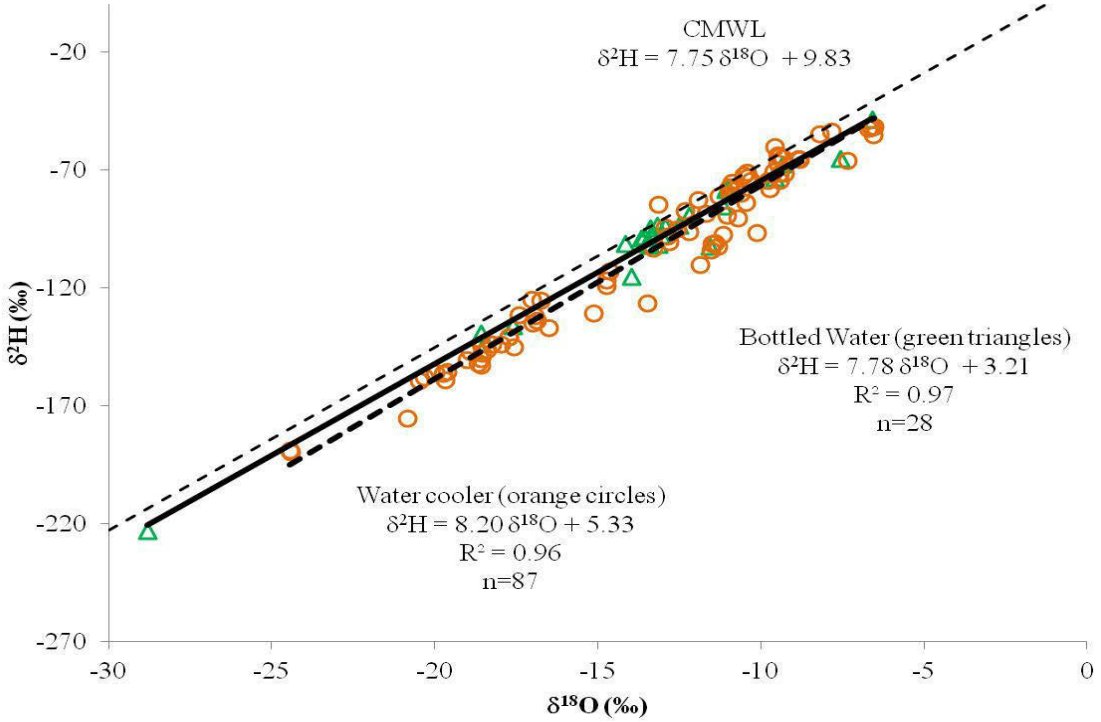

Figure 9. δ^2H and $\delta^{18}O$ of bottled (B) and water cooler (WC) water purchased from locations across Canada.

The calculated *d* (deuterium excess) for the purchased waters ranged from 20.5 to -18.4, and behaved similarly to tap water. Fifty of the 115 purchased water samples had a *d* close to the GMWL (i.e. between 5 and 15). Only 2 purchased water samples (both purchased in NB) had a *d* > 15, which is generally due to extreme dry conditions causing fractionation in the vapour phase along the air column. Fifty-one of the 115 purchased water samples have a lower *d* values (i.e. -5 to 5 ‰), which can be indicative of moderate evaporation effects. Extreme lower *d* values (< -5‰) can represent areas where extreme evaporation has occurred, and these were found in only 12 of the 115 purchased water samples. All water with extreme lower *d* values was purchased in western Canada.

Water Cooler Water

Water samples from 18 L water jugs for water coolers (WC; i.e. often known as spring water or filtered water) were collected from several locations. Table 7 shows the results of two samples from Grizzly and Arrowhead water companies.

Table 7. Comparison of isotope values from two samples of WC water from several cities. Those locations which showed different δ^2H and/or $\delta^{18}O$ values are in grey.

Water Company	Location Sampled	Province	δ^2H (‰)	$\delta^{18}O$ (‰)
Grizzly	Osoyoos	BC	-102.6	-11.33
	Osoyoos	BC	-101.2	-11.49
Arrowhead	Calgary	AB	-175.4	-20.84
	St. Albert (Edmonton)	AB	-153.0	-18.60

For Grizzly water, there was no variation between the two different water coolers sampled.

For Arrowhead water, it appears as though this company may use two different water sources for their bottled water, **or** these water samples may be from different times and may be exhibiting a seasonal fractionation effect. It is not unusual for various bottled water sources to be close to their target market to keep shipping and selling price cost down.

In many cases, the label of the WC water stated the location of the source of water, and tap water from the same city was sampled to compare to the source of the WC water. Table 8 shows the results of this comparison.

In several cases, the WC water had the same isotopic value as the town/city water from which it was bottled. However, this was not true for 6 of the WCs. This may mean that the WC company uses a different source of water than the city water system; such as a spring nearby).

Table 8: Comparison of WC water with city sources to that city/town water. Locations which showed different δ^2H and/or $\delta^{18}O$ values are in grey.

Water Company	Where Bottled	WC		Town/City Water	
		δ^2H (‰)	$\delta^{18}O$ (‰)	δ^2H (‰)	$\delta^{18}O$ (‰)
Water Pure and Simple	Drumheller, AB	-147.7	-18.56	-147.2 -151.4	-18.25 -18.59
Water Pure and Simple	Grand Forks, BC	-125.4	-16.75	-129.9	-16.70
Cariboo Clear	Cache Creek, AB	-130.9	-15.15	-126.9	-15.17
Mountain Manna Water & Ice Co.	Elkford, BC	-144.1	-18.59	-144.6	-18.93
Aqua Pure	Kamloops, BC	-124.9	-17.04	-129.3	-17.14
Ultra Pure	Prince Rupert	-88.6	-11.69	-92.8	-12.06
Northland Pure Water	Quesnel, BC	-144.0	-17.98	-140.7 -143.4	-18.15 -17.78
Purely H2O	Burns Lake, BC	-150.2	-18.58	-147.9 -153.1 -144.3	-18.60 -19.38 -17.44
Arrowhead	Calgary, AB	-175.4 -153.0	-20.84 -18.60	-151.9 -148.6 -148.1	-19.61 -19.25 -19.06
Grizzley	Osoyoos, BC or Pencticton, BC	-102.1 -102.6	-11.49 -11.33	-116.5 -111.6	-12.55 -13.39
Canadian Springs	Richmond, BC	-97.6	-11.18	-101.1	-13.79
Water Pure and Simple	Courtenay, BC	-78.0	-9.73	-99.5	-13.29
Pure Water	Red Deer, AB	-156.2	-19.75	-148.0 -149.9	-18.90 -19.14
Williams Lake Water Factory	Williams Lake, BC	-126.4	-13.50	-119.5	-12.72

WC water was compared to tap water from the same city from which it was purchased. Table 9 shows the locations and isotope values of the local tap water and the purchased water. Cities with different δ^2H and $\delta^{18}O$ values (i.e. outside of ± 4 and ± 0.4 ‰ for δ^2H and $\delta^{18}O$, respectively) are highlighted in grey.

Table 9: Comparison of tap water and WC water sampled from the same city. Locations which showed different δ^2H and/or $\delta^{18}O$ values are in grey.

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
Yarmouth	NS	TS	-32.8	-4.82
		President's Choice	-70.6	-9.62
Liverpool	NS	TS	-38.1	-5.85
		Canadian Springs	-74.4	-9.42
St. John	NB	TS	-52.9	-7.41
		Canadian Springs	-74.5	-9.79
Antigonish	NS	TS	-62.0	-9.16
		Canadian Springs	-71.3	-9.44
St. Peter's	NS	TS	-46.5	-5.17
		Highland Water Cooler	-54.6	-8.20
Fredericton	NB	TG	-84.4	-11.41
		Gilbert Mountain Springs	-72.3	-10.52
Baddeck	NS	TS	-54.4	-7.48
		Canadian Springs	-64.0	-9.46
Thetford Mines	QC	TS	-95.5	-11.85
		Everest Water	-82.6	-11.93
Woodstock	NB	TG	-86.8	-11.53
		Crystal Springs	-73.1	-10.41
Abrams Village	PE	TG (personal well)	-75.4	-10.36
		Canadian Springs	-64.2	-9.44
Perth	NB	TG	-82.0	-11.66
		Crystal Springs	-74.7	-10.30
Placentia	NL	TS	-40.0	-5.91
		True North	-89.4	-11.06
Edmundston	NB	TG	-87.1	-12.46
		Their'eau	-84.7	-13.14
St. John's	NL	TS	-46.0	-6.26
		Mount Pearl Springs	-65.6	-8.83
Port-Aux-Basques	NL	TS	-35.4	-6.28
		local grocery store, bottled in P-A-B	-53.7	-7.84
Campbellton	NB	TS	-91.1	-12.22
		Unknown water cooler	-80.4	-10.96
Cornerbrook	NL	TS	-69.7	-9.99
		Big 8 Springs	-80.1	-10.62
Baie Comeau	QC	TS	-86.4	-10.98
		Nutrinor	-112.9	-14.66
Battleford	SK	TG	-152.9	-19.28
		Pure water to go	-146.4	-18.39
Melfort	SK	TG	-103.2	-16.4
		from pharmacy	-136.8	-16.52
		Purely Natural Purified Water	-188.1	-24.45
Moose Jaw	SK	TS	-122.7	-13.97
		Prairie Spring Water	-135.1	-16.99
Outskirts of Portage La Prairie	MB	TG	-120.3	-15.74
		World of Water	-100.6	-12.82
Portage La Prairie	MB	TS	-88.5	-10.63
		Bluemoon Water	-90.5	-10.72
		World of Water	-98.1	-12.89
The Pas	MB	TS	-121.2	-15.24
		Can Aqua	-104.0	-11.52

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
Vermilion	AB	TG	-142.6	-16.94
		Glaysmore Springwater	-143.8	-18.26
Winnipeg	MB	TS	-61.3	-6.49
		World of Water	-66.1	-7.34
Ashcroft	BC	TS	-142.8	-18.14
		Cariboo Clear	-130.9	-15.15
Williams Lake	BC	TG	-119.5	-12.72
		Williams Lake Water Factory	-126.4	-13.50
Whitecourt	AB	TG	-149.5	-18.92
		WC – reverse osmosis tap from Whitecourt	-159.1	-19.68
Terrace	BC	TS	-115.9	-15.42
		WC – refill at Canadian Tire	-116.9	-14.73
St. Albert	AB	TS	-151.9	-19.58
		Arrowhead	-153.0	-18.60
Radium Hot Springs	BC	TS	-160.2	-20.82
		TS	-161.3	-20.84
		WC – Grocery Store	-131.7	-17.43
		WC – Sobey's	-155.8	-20.00
Port Alberni	BC	TS	-90.2	-11.99
		Water Pure and Simple	-78.0	-9.73
Osoyoos	BC	TS	-116.5	-12.55
		GrizzlyWC-1	-101.2	-11.49
		Grizzly WC-2	-102.6	-11.33
Fort McMurray	AB	TS	-153.4	-19.24
		TS	-155.4	-19.14
		Culligan	-159.5	-20.47
Fernie	BC	TG	-137.0	-17.95
		Mountain Manna Water & Ice	-144.1	-18.59
Chase	BC	TS	-136.2	-17.18
		Canadian Springs	-97.6	-11.18
Calgary	AB	TS	-151.9	19.61
		TS	-148.6	-19.25
		TS	-148.1	-19.06
		Arrowhead - filtered	-175.4	-20.84
Burns Lake	BC	TG	-144.3	-17.44
		TG	-147.9	-18.60
		Purely H2O	-150.2	-18.58
Atikokan	ON	TS	-74.4	-8.33
		WC	-87.4	-12.32
Moncton	NB	TS	-73.1	-10.78
		83 Natural Springs	-77.6	-10.97
		Big 8 Springs	-67.2	-9.49
		Unknown WC	-81.3	-11.29
Red Deer	AB	TS	-148.0	-18.90
		TS	-149.9	-19.14
		WC-Costco	-147.1	-18.93
		Pure Water	-156.2	-19.75
New Liskeard	ON	TG	-98.6	-13.52
		water cooler	-103.3	-13.30
St. Catherine's	ON	TS	-52.7	-6.63
		water cooler	-51.6	-6.52
Kingston	ON	TS	-55.1	-6.57

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
		water cooler	-50.9	-6.49
Geraldton	ON	TS	-83.8	-10.55
		water cooler	-84.0	-10.45
Napanee	ON	TS	-52.0	-6.71
		water cooler	-52.4	-6.72
Welland	ON	TS	-50.4	-6.75
		water cooler	-52.1	-6.61
Chapais	QC	TS	-94.8	-12.50
		water cooler	-95.1	-12.58
Chibougamau	QC	TS	-99.2	-12.98
		water cooler	-94.5	-12.95
Ste Foy	QC	TS	-70.5	-9.39
		water cooler	-71.4	-9.27
St. Anthony	NL	TG	-75.8	-10.12
		TG	-82.3	-10.15
		deionized water from co-op	-75.4	-10.89
Wolfville	NS	TG	-70.3	-9.88
		Bottled in Coldbrook, NS	-70.9	-10.43
St. Stephen	NB	TG	-66.8	-8.95
		Canadian Springs	-60.4	-9.59
Tracadie	NS	TG (personal well)	-66.9	-8.74
		Unknown water cooler	-59.3	-9.40
Cheticamp	NS	TG	-68.1	-9.57
		Co-op, Eau Du Source	-64.7	-9.33
Ingonish	NS	TG	-65.4	-9.49
		Unknown water cooler	-63.7	-9.48
Bathurst	NB	TS	-81.8	-11.53
		Vienneau	-77.3	-10.94
Clarenville	NL	TS	-65.9	-7.85
		Unknown water cooler	-65.1	-8.85
Carrot River	SK	TG	-188.0	-24.12
		DBN	-189.8	-24.43
Dauphin	MB	TS	-94.5	-12.13
		Ultrapure	-96.3	-12.21
Flin Flon	MB	TS	-98.3	-10.43
		Can Aqua	-96.5	-10.13
Prince Albert	SK	TS	-141.7	-17.37
		Iceberg Pure Water	-140.7	-17.73
Swan River	MB	TG	-114.6	-14.65
		Co-op filtered brand	-119.2	-14.74
Tisdale	SK	TG	-132.0	-17.09
		Watertech	-131.8	-16.93
Weyburn	SK	TS	-132.7	-16.31
		Natural Choice Prairie Springwater	-133.7	-16.89
100 Mile House	BC	TG	-112.8	-11.89
		High Tech Water Co.	-110.3	-11.87
Athabasca	AB	TS	-151.4	-19.13
		WC – distilled	-150.3	-19.02
		WC – from Home Hardware	-155.7	-19.62
Dawson Creek	BC	TS	-157.0	-18.55
		Northern Source	-152.2	-18.68
Drumheller	AB	TS	-147.2	-18.25
		TS	-151.4	-18.59

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
		Water Pure and Simple	-147.7	-18.56
Grand Forks	BC	TG	-129.9	-16.70
		Pure and Simple	-125.4	-16.75
Grande Prairie	AB	TS	-156.9	-20.38
		TS	-157.0	-20.27
		WC – unknown source	-158.0	-20.33
Kamloops	BC	TS	-129.3	-17.14
		Aqua Pure	-124.9	-17.04
Kelowna	BC	TS	-99.2	-11.30
		TS	-102.0	-11.40
		TS	-102.1	-11.39
		WC – filtered city water	-100.9	-11.41
Port Hardy	BC	TS	-81.1	-10.87
		WC – unknown source	-78.4	-10.76
Prince Rupert	BC	TS	-92.8	-12.06
		Ultra Pure	-88.6	-11.69
Quesnel	BC	TG	-140.7	-18.15
		TG	-143.4	-17.78
		Northland Pure Water	-144.0	-17.98
		WC - unknown	-140.9	-17.74

From the Summer 2008 sampling campaign in ON and QC, 9 of the 10 WC samples had similar δ^2H and $\delta^{18}O$ values as the tap water from the town from which it was sampled. With the exception of Atikokan, it appeared as though water sold in WCs has the same source as the town from which it was purchased.

However, for WC samples collected from the remainder of Canada, there was not as high a percentage of WC water bottled from the same water source as the city from which it was sampled from. Looking at Canada as a whole, 45 of the 85 WC samples had different δ^2H and $\delta^{18}O$ values compared to tap water from the same city (highlighted in grey in Table 9). In these cases, it is possible the WC water was NOT bottled in the same city in which it was sampled. However, there were still 40 of 85 cities with isotopically identical tap and WC water purchased from that city, suggesting that many companies do indeed bottle from city tap water and sell it as "filtered" WC water.

Bottled Water

In AB and BC, Nestlé Purelife bottled water was frequently seen in stores, which is bottled in Hope, BC. The following table compares the isotopic value of several purchased bottles of Nestle Purelife to Hope, BC tap water (Table 10).

Table 10: Comparison of Hope, BC city tap water to several purchased Nestle Purelife bottles.

Where Purchased	Nestle PureLife		Hope, BC City water	
	δ^2H (‰)	$\delta^{18}O$ (‰)	δ^2H (‰)	$\delta^{18}O$ (‰)
Chetwynd, BC	-98.3	-13.57	-99.7	-13.84
Edmonton, AB	-101.5	-13.46		
Fort St. John, BC	-101.6	-13.15		
Kamloops, BC	-98.8	-13.68		
Kelowna, BC	-102.3	-11.57		

With the exception of the bottle purchased in Kelowna, BC, all Nestle Purelife bottles had virtually the same isotopic values, which corresponded to Hope BC city tap water. This suggests that Nestle Purelife and the city of Hope BC share the same water source.

Bottled water was also purchased from several cities, and its isotopic values were compared to that of tap water from the same city. The results are shown in Table 11. Cities with different δ^2H and $\delta^{18}O$ values (i.e. outside of ± 4 and ± 0.4 ‰ for δ^2H and $\delta^{18}O$, respectively) are highlighted in grey.

In all but 5 cases, the δ^2H and $\delta^{18}O$ values of the bottled water were very different than the δ^2H and $\delta^{18}O$ values of the tap water from the city in which they were purchased. This is not very surprising, as the source of the bottled water is not necessarily close to the area of purchase. Also, a selling point for bottled water is the "exotic" aspect of the water source that brings a premium price and is often sold as a "clean" & "healthy" choice.

Table 11: Comparison of tap water and bottled water sampled from the same city. Locations which showed different δ^2H and/or $\delta^{18}O$ values are in grey.

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
St-Felicien	QC	TG	-106.1	-14.29
			-85.4	-11.18
Alma	QC	TG	-105.7	-14.46
			-79.0	-11.08
Montreal	QC	TS	-48.5	-6.64
			-77.4	-11.05
Meadow Lake	SK	TS	-103.7	-11.58
		Aquafina (from Toronto or Vancouver)	-93.7	-12.52
Rosetown	SK	TG	-153.4	-19.18
		Co-op (Kawkawa, BC)	-98.0	-13.34
Swift Current	SK	TS	-116.2	-13.84
		Nestle (Hope Spring BC)	-97.3	-13.36
Winnipeg	MB	TS	-61.3	-6.49
		World of Water	-65.4	-7.55
Aldouane	NB	TG (PW)	-79.5	-10.73
		Life	-94.7	-13.39
New Glasgow	NS	TS	-57.1	-8.19
		Big 8 Spring	-67.4	-9.34
St-Leonard	NB	TG	-85.7	-11.96
		Aquifina	-48.4	-6.59
North Sydney/ Sydney Mines	NS	TS	-38.4	-6.33
		Nestle	-73.4	-9.57

City/Area	Province	Source	δ^2H (‰)	$\delta^{18}O$ (‰)
Outskirts of Portage La Prairie	MB	TG	-120.3	-15.74
		Lifebrand (High River, AB)	-139.4	-18.58
Thompson	MB	TS	-112.3	-12.94
		Davren Springs (Middlebro, MB)	-93.4	-13.19
		BV Spring Water (Middlebro, MB)	-97.9	-13.06
		Aquafina	-89.2	-12.19
St. Anthony	NL	TG - RCMP	-75.8	-10.12
		80 Degrees North	-223.0	-28.80
Calgary	AB	TS	-151.9	-19.61
		TS	-148.6	-19.25
		TS	-148.1	-19.06
		Natural Springs Water	-146.8	-18.58
Chetwynd	BC	TG	-154.2	-19.55
		Nestle Purelife	-98.3	-13.57
Edmonton	AB	TS	-150.6	-19.73
		TS	-153.8	-19.72
		B – Kirkland-1	-101.5	-13.48
		B – Kirkland-2	-101.9	-13.39
		Nestle Purelife	-101.5	-13.46
Fort St. John	BC	TS	-157.0	-19.73
		Nestle Purelife	-101.6	-13.15
Kamloops	BC	TS	-129.3	-17.14
		Nestle Purelife	-98.8	-13.68
Quesnel	BC	TG	-140.7	-18.15
		TG	-143.4	-17.78
		Aquafina	-136.3	-17.98
Kelowna	BC	TS	-99.2	-11.30
		TS	-102.0	-11.40
		TS	-102.1	-11.39
		Nestle Purelife	-102.3	-11.57
New Liskeard	ON	TG	-98.6	-13.52
		Ice River Springs	-94.1	-12.95
Demaiarisville	QC	TG	-96.9	-13.82
		Eska	-101.4	-14.15
Gimli	MB	TG	-108.3	-13.89
		Sobey's	-115.2	-13.97

The most notable difference between tap water and bottled water is "80 Degrees North" water from St. Anthony, NL. This brand of bottled water was extremely popular in NL, and is seen sold in Montreal, QC. The company who sells it uses melted water from an iceberg (a selling point), which is why the δ^2H and $\delta^{18}O$ values of this water are extremely negative.

As evidenced from Tables 9 and 11, water purchased for a town or city is not necessarily isotopically similar to the tap water depending on its selling purpose and expected target market. Since the δ^2H and $\delta^{18}O$ value of hair are related to the water that a person consumes, this finding may be significant when evaluating the isotopic composition of hair, and relating this to a geographical location. It is important to keep in mind that cooking water is generally from the tap and not a pot filled with bottled water. The cooking process in turn replaces most of the water from the original food (e.g. potatoes, carrots, etc).

Hair Report

The locations of hair collection and all isotopic results are summarized in the attached Excel file CRTI_project_08-0116RD_hair_and_water.xlsx.

All recorded information for hair samples are summarized in the attached Excel file CRTI_project_08-0116RD_sample_info_import_template.xlsx.

Details on hair preparation and all isotopic analyses performed on hair can be found in APPENDIX 1.

In total, 592 hair samples were collected over 4 sampling trips. However, not all hair was used in the database. Samples that were collected but are not included in the database are:

#5: from Cochrane, ON: hair sample collected from a salon; no questionnaire
#7: from Cochrane, ON: hair sample collected from a salon; no questionnaire
#29: hair from unknown source
#44: from Parry Sound, ON: participant was nervous about us cutting her hair, only let us collect the very tip of her > 30 long hair, so excluded from database; also did not have a home water sample
#50: duplicate hair from a participant
#134: duplicate hair from a participant
#607: hair from Iqaluit, NT: large geographical distance between sampling points skewed models
#707: hair from Weatherby, UK

The following table shows the number of analysis of hair for each isotope.

Table 12. Number of hair samples included in the database for each isotope.

Hair analyzed for $\delta^{2}H$	Hair analyzed for $\delta^{18}O$	Hair analyzed for $\delta^{13}C$	Hair analyzed for $\delta^{15}N$	Hair analyzed for $\delta^{34}S$
577	563	577	577	529

Dyed Hair

A hair sample from Ottawa, ON that was frequently highlighted with several colours of blond was obtained. Each hair sample was prepared in the same manner as all other samples, and was ground to a powder for analysis (See APPENDIX 1). Care was taken to ensure that the dyed and non-dyed hair were taken for the same time frame, to minimize temporal effects, but it is not 100% certain that all the hair was from the same time frame. In addition, a dyed and un-dyed hair sample from two participants: one from Regina, SK, the other from Shirley, BC, were obtained. In both cases, their hair was naturally a brown colour, and each had blond highlights in their hair. All isotopic values are single measurements. The results of the $\delta^{13}C$ and $\delta^{15}N$ analysis are presented in Tables 13 a, b and c.

Table 13a. $\delta^{13}C$ and $\delta^{15}N$ values of undyed and dyed hair from ON. Analytical error is ± 0.20 ‰.

Hair Sample from ON	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Undyed	-18.06	8.38
Colour #1	-18.24	8.55
Colour #2	-18.20	8.73
Colour #3	-18.12	8.49
Colour #4	-18.10	8.08
Colour #5	-18.06	8.56

Table 13b. $\delta^{13}C$ and $\delta^{15}N$ values of undyed and dyed hair from SK. Analytical error is ± 0.20 ‰.

Hair Sample from SK	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Undyed	-18.14	10.21
Dyed	-18.13	9.92

Table 13c. $\delta^{13}C$ and $\delta^{15}N$ values of undyed and dyed hair from SK. Analytical error is ± 0.20 ‰.

Hair Sample from BC	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Undyed	-18.91	9.67
Dyed	-18.88	9.63

Since these are single measurements, no statistical analysis was performed. The analytical error for $\delta^{13}C$ and $\delta^{15}N$ analysis is ± 0.20 ‰. With the exception of the $\delta^{15}N$ value of Colour #4 for the ON hair sample (Table 13a), the dyed hair and non-dyed hair are all within analytical uncertainty, and there is no difference between dyed and non-dyed hair in terms of $\delta^{13}C$ and $\delta^{15}N$. The single outlier may be from a different time frame than the other hair, but there is insufficient information to determine if this is the case.

In order to systematically determine if dyeing hair results in a change in isotope values, hair was obtained from one individual who regularly shaved his head. Hair was approximately 1.5 to 2 cm long, and was taken from a single haircut to minimize heterogeneity. All dyes were purchased from chain stores and are commonly used dyes. Prior to dyeing, the hair was washed with a 2:1 chloroform:methanol solution and left to dry.

The haircut was divided into 5 aliquots:

1) no treatment (undyed)
2) dyed red using a common brand of dye purchased at a national drugstore (Red)
3) dyed blond using a common brand of dye purchased at a national drugstore (Blond)
4) dyed brown using a common brand of dye purchased at a national drugstore (Brown)
5) dyed using henna purchased at a natural products store (Henna)

All 4 aliquots were dyed on the same day. Three days after dyeing the hair, each aliquot was further divided into 2 parts. Part 1 was ground into a powder, and Part 2 remained as cut hair. Each hair was measured in triplicate. For Cut Brown hair, one of the replicate measurements was an outlier, and was discarded. The results are shown in Tables 14 a to e.

Table 14a. $\delta^{13}C$ values of undyed and dyed hair. Analytical uncertainty is ± 0.20 ‰.

	$\delta^{13}C$ CUT HAIR (‰)	$\delta^{13}C$ GROUND HAIR (‰)
Undyed	-17.25	-17.09
Red	-17.35	-17.15
Blond	-17.37	-17.21
Brown	-17.23	-17.27
Henna	-17.33	-17.20

Table 14b. $\delta^{15}N$ values of undyed and dyed hair. Analytical uncertainty is ± 0.20 ‰.

	$\delta^{15}N$ CUT HAIR (‰)	$\delta^{15}N$ GROUND HAIR (‰)
Undyed	9.00	9.32
Red	9.05	9.29
Blond	9.08	9.32
Brown	9.00	9.21
Henna	9.14	9.25

Table 14c. $\delta^{34}S$ values of undyed and dyed hair. Analytical uncertainty is ± 0.4 ‰.

	$\delta^{34}S$ CUT HAIR (‰)	$\delta^{34}S$ GROUND HAIR (‰)
Undyed	2.1	2.0
Red	1.9	2.0
Blond	2.0	2.0
Brown	2.0	1.9
Henna	1.9	1.8

Table 14d. $\delta^{2}H$ values of undyed and dyed hair. Analytical uncertainty is ± 2.0 ‰.

	$\delta^{2}H$ CUT HAIR (‰)	$\delta^{2}H$ GROUND HAIR (‰)
Undyed	-77.1	-78.9
Red	-77.4	-76.0
Blond	-76.9	-75.9
Brown	-76.6	-76.1
Henna	-77.0	-77.6

Table 14e. $\delta^{18}O$ values of undyed and dyed hair. Analytical uncertainty is ± 0.4 ‰.

	$\delta^{18}O$ CUT HAIR (‰)	$\delta^{18}O$ GROUND HAIR (‰)
Undyed	9.5	9.7
Red	7.7	8.1
Blond	7.6	8.1
Brown	7.5	8.0
Henna	9.2	9.8

In all cases, the isotopic values of the cut hair were within analytical uncertainty (see table titles for analytical uncertainty values and APPENDIX 1) of the corresponding ground hair, further confirming that grinding hair does not significantly change the isotope value. However, it should be noted that for $\delta^{15}N$ and $\delta^{18}O$, there appeared to be a small (but within analytical uncertainty) offset, with the ground hair always having more positive values than the corresponding cut hair.

For δ^2H, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, the isotopic values of the undyed hair were within analytical uncertainty of all 4 treated hair aliquots. This suggests that dying hair does not significantly affect these isotope values for hair.

However, differences were noted in the $\delta^{18}O$ value. The $\delta^{18}O$ values of the undyed and henna dyed hair were within analytical uncertainty, suggesting that the henna dye did not affect the $\delta^{18}O$ value. However, there was a significant difference in $\delta^{18}O$ values between the undyed hair and the Red, Blond and Brown hair. This suggests that dying hair with store-bought dyes can alter the $\delta^{18}O$ value of hair. Care must be taken when interpreting $\delta^{18}O$ values, and comparing $\delta^{18}O$ values to existing databases.

Since information concerning if a person's hair was dyed was collected, a statistical analysis of the effect of dyeing hair on isotope values was undertaken. Box plots were created to visually display differences between populations. Box plots display statistical data. For each variable, there is a solid black line through the middle of the box – this represents the **MEDIAN** of the data. The upper line of the box is the 75^{th} percentile, and the lower line of the box is the 25^{th} percentile. Thus, 50 % of the data is encompassed by this box. The bars that extend vertically from the box encompass ~ 95 % of the data, assuming the data is normally distributed. However, the bars may encompass all data points, assuming there are no outliers. The circles represent outliers from the data set.

Levene's test determines if the data sets had equal variance was also performed. Next, t-tests were performed to determine if there was a statistical difference between the means of the populations. T-tests give a give a p-value, which is dependent on if both data sets have equal variance. To determine the significance of the t-test, a confidence interval of 95 % was chosen. Thus, those t-tests with a p-value < 0.05 demonstrate that the means of the populations tested are statistically different. Conversely, a p-value > 0.05 demonstrates that the means of the populations tested are not statistically different.

The average isotope values of undyed and dyed hair from people across Canada, and p-values from the t-tests comparing undyed and dyed hair is presented in Table 15. The results from Levene's test determine if there is equal variance between the two populations. Boxplots to visually illustrate the distribution of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, δ^2H and $\delta^{18}O$ are presented in Figures 10 a-e.

Table 15. Average hair isotope values and p-values from t-tests comparing isotope values for dyed and undyed hair. A p-value less than 0.05 (highlighted in grey) shows the isotope value is significantly different between undyed and dyed hair.

Isotope	Average for Undyed Hair	Average for Dyed Hair	p-value
$\delta^{13}C$	-18.64	-18.69	0.35
$\delta^{15}N$	9.22	9.21	0.79
$\delta^{34}S$	1.7	1.6	0.85
δ^2H	-91.7	-94.4	0.017
$\delta^{18}O$	9.1	7.7	9.9e-12

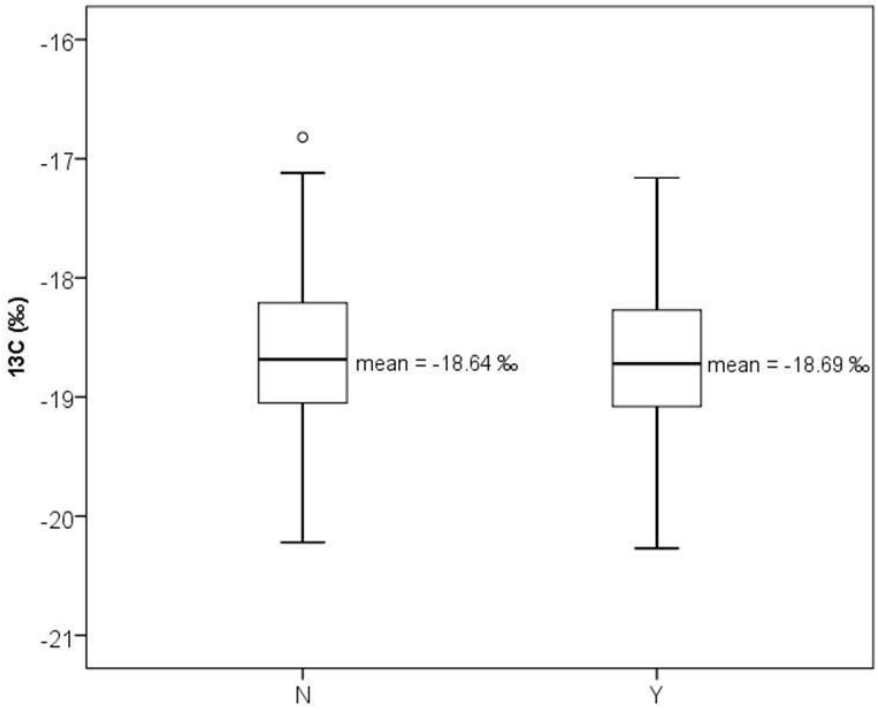

Figure 10a. Box plot of $\delta^{13}C$ values for undyed (N) and dyed (Y) hair. The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

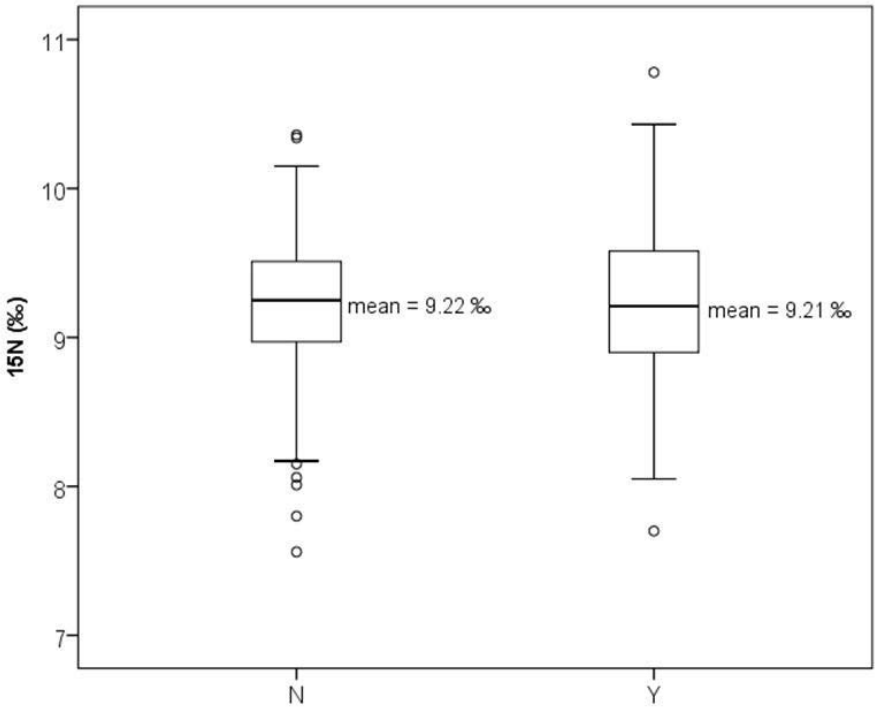

Figure 10b. Box plot of $\delta^{15}N$ values for undyed (N) and dyed (Y) hair. The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

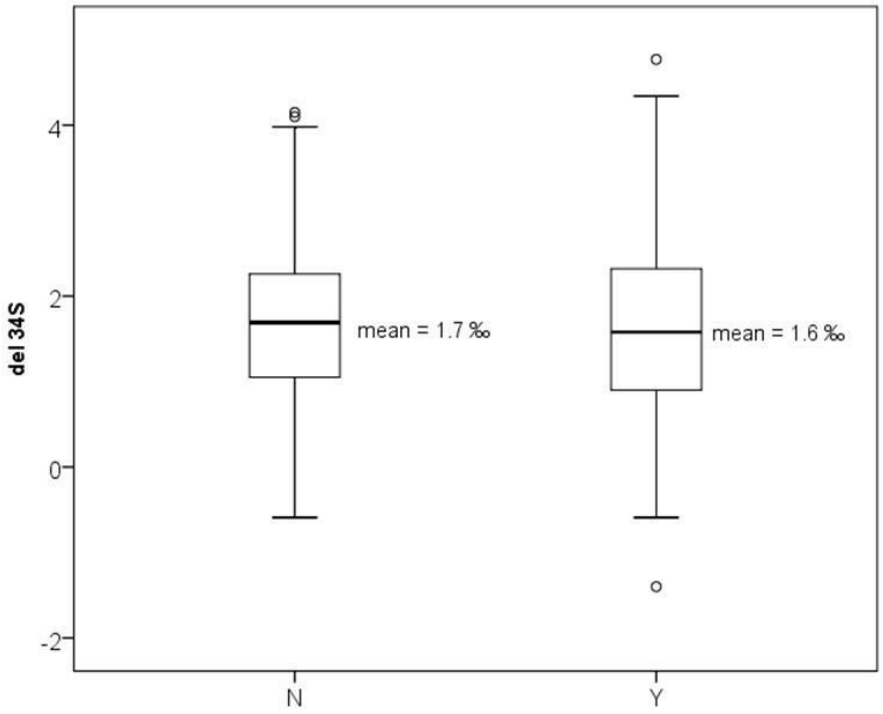

Figure 10c. Box plot of $\delta^{34}S$ values for undyed (N) and dyed (Y) hair. The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

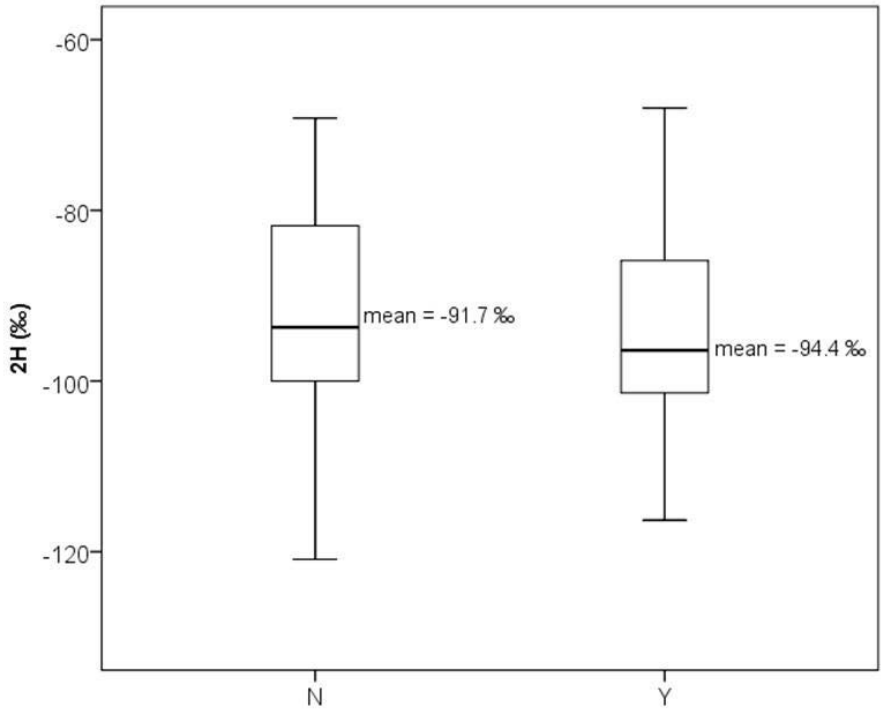

Figure 10d. Box plot of $\delta^{2}H$ values for undyed (N) and dyed (Y) hair. The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

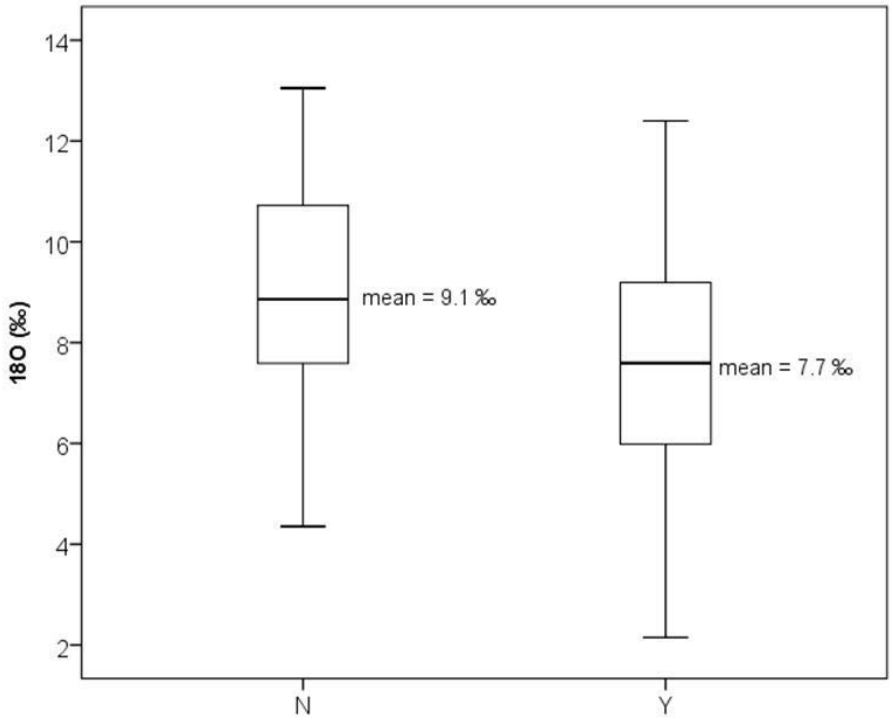

Figure 10e. Box plot of $\delta^{18}O$ values for undyed (N) and dyed (Y) hair. The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

For $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, there was no statistical difference between undyed and dyed hair from across Canada (p values > 0.05), and means of the undyed and dyed hair are very close to one another. This suggests that the hair from the database follows the same pattern observed for the systematic dyed hair experiment.

For $\delta^{2}H$, there was a statistical difference between the dyed and non-dyed hair from across Canada ($p<0.05$). However, the boxplots look very similar, and the means for each population are within analytical uncertainty (± 2 ‰). As will be discussed under the section Hydrogen and Oxygen below, the $\delta^{2}H$ values of hair varies across Canada. This variation by location may explain the statistical difference observed between $\delta^{2}H$ of undyed and dyed hair, as this analysis includes hair from many locations. This analysis, coupled with the fact that no difference outside of analytical uncertainty was observed in the systematic dyed hair experiment, makes us confident that the effect of dyeing hair will have minimal to no impact on the interpretation of $\delta^{2}H$ values for geolocation.

For $\delta^{18}O$, there was a large statistical difference between the dyed and non-dyed hair from across Canada. The boxplots do not look similar, and the means are not within analytical uncertainty (± 0.4 ‰). As is the case for $\delta^{2}H$, $\delta^{18}O$ values of hair are also expected to vary across Canada. However, the magnitude of the difference in $\delta^{18}O$ values between undyed and dyed hair is much larger, suggesting that geolocation cannot be the only source of this variation. Further, as was observed in the systematic dyed hair experiment, the undyed hair had a more positive $\delta^{18}O$ value compared to the dyed hair. Based on this evidence, $\delta^{18}O$ values may not be confidently used for geolocation purposes.

Temporal Hair Analysis

To determine the effect of sampling hair at different times of the year, hair samples from volunteers in three different cities were collected at various times and analyzed for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$. The results are shown in Tables 16a, 16b and 16c. Hair was collected in such a way that any travel would not have been included in the timeframe analysed.

Table 16a: $\delta^{13}C$ values of hair from participants in different cities over time.

City	Summer 08	Fall 08	Winter 08/09	Winter 09/10	Winter 10/11	Winter 11/12	Maximun Spread
Montreal 45	-18.58	-18.24	-18.16	-18.17	-18.12		0.46
Montreal 24	-18.52	-18.35	-18.37	-18.58	-18.21	-18.73	0.52
Montreal 10	-18.72	-18.61	-18.66	-18.65	-18.62	-18.86	0.25
Montreal 12	-18.43	-18.39	-18.36		-18.28		0.15
Ottawa 41	-18.15	-17.68	-17.68	-17.61	-17.63	-17.73	0.54
Ottawa 4	-19.12	-18.87	-18.91	-18.25	-18.40	-18.96	0.87
Ottawa 6	-17.99	-18.06	-17.74	-17.76	-17.59	-17.86	0.47
Ottawa 9	-16.92	-16.93	-17.30	-17.02	-17.46	-17.49	0.57
Ottawa 5	-17.15	-17.57	-17.68	-17.65	-17.58	-17.39	0.53
Ottawa 2	-17.78	-17.35	-17.62	-17.78	-17.79	-18.02	0.67
Sudbury 3	-18.07	-18.28	-18.28	-17.48	-18.60	-18.84	1.36
Sudbury 8	-18.37	-18.22	-17.85	-18.13	-18.58	-18.64	0.79
Sudbury 13	-16.73	-16.60	-16.45	-16.86	-16.45	-16.68	0.41
Sudbury 23	-17.92	-17.81	-17.77	-17.53	-17.63	-17.96	0.43
Sudbury 18	-18.04	-17.82	-17.98	-18.09	-17.87	-18.08	0.27
Sudbury 25	-17.46	-17.39	-17.55	-18.51	-17.54	-17.30	1.21
Sudbury 7	-17.37	-17.38	-17.18	-17.39	-17.64	-17.53	0.48
Sudbury 11	-17.79	-17.59	-17.88	-17.78	-17.61	-17.99	0.40
Sudbury 17	-17.89	-18.01	-17.95	-17.90	-17.15		0.86
Sudbury 49	-18.27	-18.46	-18.20	-17.70	-18.18	-17.90	0.76
Sudbury 29	-17.05	-17.17	-16.96	-16.53	-16.96	-16.78	0.64

With the exception of 4 samples (highlighted in grey), the maximum spread observed was less than 0.80 ‰ (2 x analytical error) over the four years analyzed. This suggests that the carbon portion of the participant's diet did not greatly change over time for most people. For the 4 participants with larger spreads, 3 had only one value that was substantially different from the others, suggesting that in general, the carbon portion of their did not significantly change. The fourth participants with a larger spread had two values that were substantially different from the others, which may be explained by some diet changes potentially due to seasonality changes in food availability and/or lifestyle choices.

Table 16b: $\delta^{15}N$ values of hair from participants in different cities over time.

City	Summer 08	Fall 08	Winter 08/09	Winter 09/10	Winter 10/11	Winter 11/12	Maximun Spread
Montreal 45	8.62	8.91	8.86	9.08	9.05		0.29
Montreal 24	9.76	9.40	9.33	9.47	9.53	9.52	0.43
Montreal 10	9.79	10.37	10.06	10.02	9.56	9.93	0.81
Montreal 12	9.80	9.32	8.89		9.76		0.91
Ottawa 41	9.75	9.58	9.79	9.60	9.62	9.49	0.30
Ottawa 4	7.52	8.04	8.34	8.78	8.23	8.52	1.26
Ottawa 6	8.69	9.57	9.62	9.01	9.34	9.47	0.93
Ottawa 9	8.77	8.89	7.87	9.19	9.19	9.06	1.32
Ottawa 5	8.58	9.19	9.89	8.77	8.71	8.95	0.61
Ottawa 2	8.42	9.02	8.90	8.61	8.90	9.06	0.54
Sudbury 3	9.56	9.42	9.46	9.46	9.23	9.07	0.49
Sudbury 8	8.58	8.64	8.68	8.51	8.42	8.62	0.26
Sudbury 13	9.46	9.77	9.60	9.59	9.45	9.70	0.32
Sudbury 23	9.52	9.91	9.51	9.41	9.43	9.54	0.50
Sudbury 18	9.18	9.24	9.02	9.20	9.05	9.30	0.28
Sudbury 25	9.22	9.59	9.53	9.11	9.44	9.78	0.67
Sudbury 7	10.10	9.78	9.80	9.87	9.86	10.28	0.50
Sudbury 11	9.46	10.04	9.29	9.73	9.42	9.52	0.75
Sudbury 17	8.86	8.84	9.13	9.24	9.14		0.38
Sudbury 49	9.17	9.19	8.68	9.38	8.95	9.26	0.70
Sudbury 29	9.55	9.43	9.36	9.51	9.27	9.84	0.57

With the exception of 5 samples (highlighted in grey), the maximum spread observed was less than 0.80 ‰ (2 x analytical error) over the four years analyzed. As was observed for carbon, this suggests that the nitrogen portion of the participant's diet did not greatly change over time for most people. For the 5 participants with larger spreads, 4 had only one value that was substantially different from the others, suggesting that in general, the nitrogen portion of their diet did not significantly change. The fifth participant with a larger spread had two values that were substantially different from the others and may be explained by some diet changes potentially due to seasonality changes in food availability and/or lifestyle choices.

Table 16C: δ^2H values of hair from participants in different cities over time.

City	Summer 08	Fall 08	Winter 08/09	Winter 09/10	Winter 10/11	Winter 11/12	Maximun Spread
Montreal 45	-75.5	-77.4	-76.0	-76.7	-77.3		1.9
Montreal 24	-73.4	-72.4	-74.0	-78.6	-78.8	-82.4	10.0
Montreal 10	-72.5	-75.1	-74.2	-75.4	-81.0	-78.5	8.5
Montreal 12	-77.1	-79.5	-78.7		-82.6	-82.0	5.5
Ottawa 41	-80.0	-79.6	-80.3	-80.3	-80.0	-83.5	3.9
Ottawa 4	-86.3	-80.4	-80.9	-84.9	-87.4	84.9	7.0
Ottawa 6	-74.8	-74.4	-74.4	-76.6	-78.0	-77.4	3.7
Ottawa 9	-83.8	-86.0	-82.9	-83.4	-79.2	-76.6	9.4
Ottawa 5	-81.0	-82.6	-83.0	-82.1	-85.3	-88.7	7.7
Ottawa 2	-83.1	-87.3	-77.4	-85.1	-86.0	-86.7	9.8
Sudbury 3	-88.8	-86.8	-89.5	-92.0	-94.3	-92.4	7.5
Sudbury 8	-83.6	-86.4	-81.8	-85.6	-89.4	-85.9	7.6
Sudbury 13	-81.8	-81.8	-80.8	-84.6	-85.6	-84.6	4.8
Sudbury 23	-82.8	-82.8	-78.0	-80.2	-81.5	-83.1	5.1
Sudbury 18	-82.8	-82.2	-82.9	-82.6	-88.3	-87.6	6.1
Sudbury 25	-84.7	-86.3	-86.5	-95.2	-88.2	-86.0	10.5
Sudbury 7	-74.1	-77.1	-76.9	-78.0	-85.5	-83.5	11.4
Sudbury 11	-83.8	-81.9	-84.9	-87.6	-88.6	-87.5	6.7
Sudbury 17	-78.3	-81.7	-78.0	-85.3	-85.8		7.8
Sudbury 49	-88.8	-89.6	-90.6	-84.8	-95.8	-83.6	12.2
Sudbury 29	-79.0	-84.8	-85.0	-85.6	-82.8	-87.1	8.1

With the exception of 8 samples (highlighted in grey), the maximum spread observed was less than 8.0 ‰ (2 x analytical error) over the four years analyzed. For the 8 participants with larger spreads, 3 had only one value that was substantially different from the others, and 5 had two values that were substantially different from the others. This may be explained by some diet changes potentially due to seasonality changes in food availability and/or lifestyle choices. In the case of hydrogen and oxygen isotopes, there may be some seasonality changes to the drinking water; especially if the water is from a small surface source such as a stream or lake. Further exploitation of this data will be done in the course of a published paper.

Carbon and Nitrogen

$\delta^{13}C$ and $\delta^{15}N$ measurements are indicators of a person's diet and general health [2-4,8,9,14,15,17,18]. The $\delta^{13}C$ signal in a person's hair is a reflection of the relative amounts of plant photosynthetic cycle (C3, C4 & CAM) based foods a person consumes, as well as the $\delta^{13}C$ signal of the protein a person consumes. On the other hand, the $\delta^{15}N$ signal reflects the amount and type of protein consumed, and it can serve as an indicator of general health. In order to determine changes in a person's general health, one must monitor the $\delta^{15}N$ value of a person's hair over time - a single $\delta^{15}N$ measurement is not sufficient to draw a picture of a person's general health, nor can the magnitude of the $\delta^{15}N$ value be used to assess general health. Since our database consists of single points (i.e. a chronological analysis is not performed), only $\delta^{15}N$ in terms of protein intake can be measured. A person's general health may play a role, but there are no means to measure this unless a time based analysis (e.g. time cuts along the length of the hair) is performed.

Carbon and Nitrogen Isotope Values Across Canada

In total, 577 hair samples are included in the $\delta^{13}C$ and $\delta^{15}N$ isotope database of Canadian hair. A plot of all hair analyzed for $\delta^{13}C$ and $\delta^{15}N$ is presented in Figure 11.

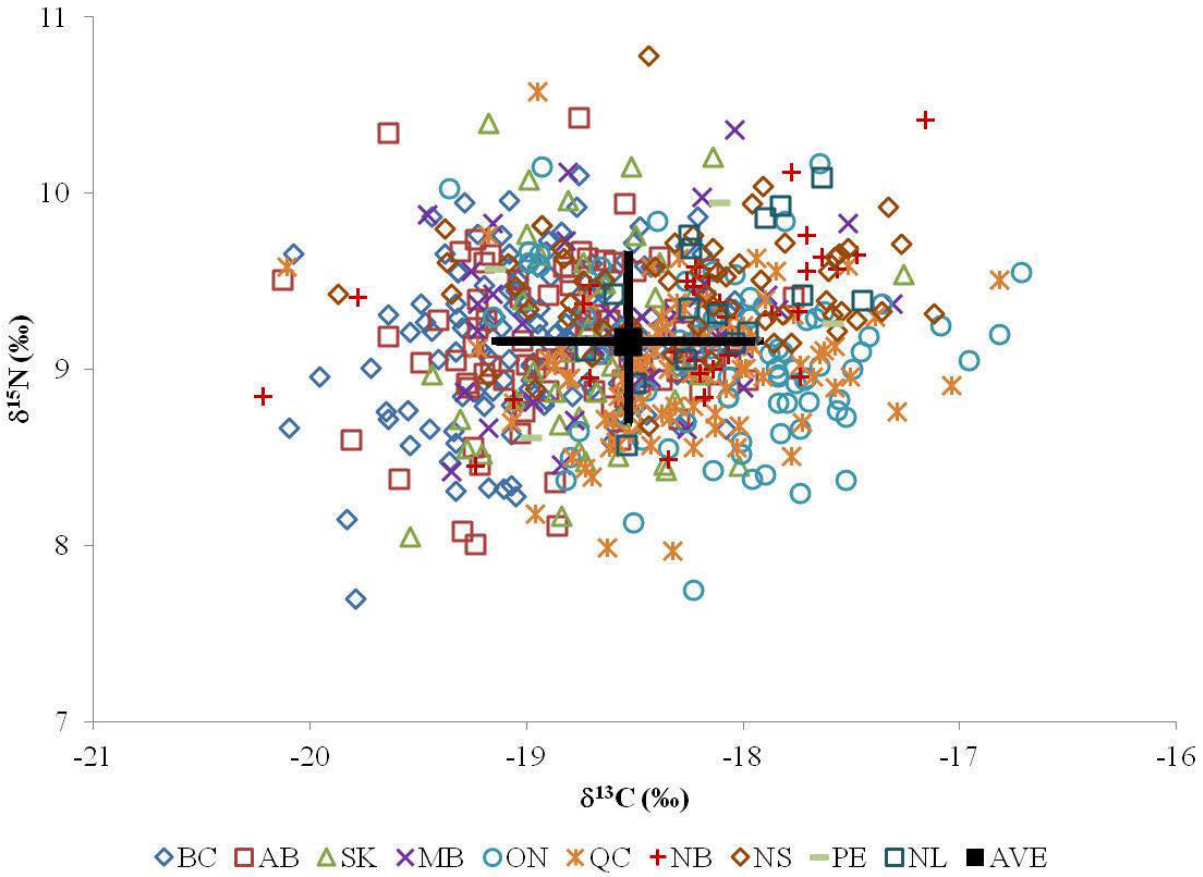

Figure 11. $\delta^{13}C$ and $\delta^{15}N$ values for all hair. The large square represents the average and standard deviation for all hair samples (-18.54 ± 0.61 ‰ and 9.16 ± 0.46 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively).

All points are separated by province collected. The $\delta^{13}C$ and $\delta^{15}N$ isotope values in Canadian hair range from -20.27 to -16.72 ‰ and 7.56 to 10.78 ‰, respectively. The average and standard deviation for $\delta^{13}C$ is -18.54 ± 0.61 ‰ and for $\delta^{15}N$ it is 9.16 ± 0.46 ‰.

The most negative $\delta^{13}C$ value measured for Canadian hair was -20.27 ‰, and was from a vegetarian from Tofino, BC. The most positive value is from an omnivore in Scarborough, ON (-16.72 ‰). The range of all $\delta^{13}C$ values measured, regardless of dietary choices, was completely encompassed by the range of $\delta^{13}C$ values of hair from the US (-14.7 to -21.6 ‰ [11]), suggesting that Canadians and Americans eat a similar diet. The US hair shows a larger range than Canada, which may be due to higher variations in their food sources.

However, compared to hair of volunteers from the UK/Europe, there is a difference between typical Canadian diets and typical European/UK diets. This is likely due to two differences:

1) Food products consumed in Canada are typically produced from sugar cane, corn syrup or corn products (higher proportion of C4 signal), while food products consumed in the UK/Europe are typically produced from beet sugar (higher proportion of C3 signal)
2) Livestock is primarily fed maize or a mixture of maize and grasses (higher proportion of C4 signal) in Canada vs. primarily grasses (C3 signal) in the UK/Europe

There is an overlap in the $\delta^{13}C$ values of hair from Canada with hair from UK/Europe, the latter which report $\delta^{13}C$ values ranging from -19.2 to -22.6 ‰ [2,6,8,13,20]. However, the averages for both Canada and the UK/Europe are clearly distinguished from each other. As such, $\delta^{13}C$ analysis of hair can be used as a tool to determine if the unknown person ate a typical Canadian diet, or a typical European/UK one.

Hair from someone residing in Weatherby, UK was also collected. With a $\delta^{13}C$ value of (-21.43 ‰), it was more than 1 ‰ less than the most negative Canadian hair sampled (-20.27 ‰). While this person frequently travelled, the $\delta^{13}C$ value of his hair clearly showed a strong European/UK signal, which is consistent with where this person resides. His $\delta^{15}N$ value was typical of an omnivore diet (9.32 ‰ see Section Omnivore vs Vegetarian below).

The following analysis was performed on omnivores only. For more details, see Section Omnivores and Vegetarians below. The average and range of $\delta^{13}C$ and $\delta^{15}N$ values for omnivores for each province are presented in Table 17 and Figure 12. Figures 13a and 13b show the boxplots of $\delta^{13}C$ and $\delta^{15}N$ values for omnivores for each province.

Table 17. $\delta^{13}C$ and $\delta^{15}N$ values of hair from persons residing in different provinces.

Province	n	Average $\delta^{13}C \pm SD$ (‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$ (‰)	Range $\delta^{15}N$ (‰)
BC	129	-18.96 ± 0.43	-18.04 to -20.10	9.17 ± 0.43	7.70 to 10.10
AB	83	-18.81 ± 0.46	-17.77 to -20.13	9.14 ± 0.43	8.01 to 10.43
SK	42	-18.69 ± 0.42	-17.26 to -19.54	9.11 ± 0.58	8.05 to 10.40
MB	43	-18.54 ± 0.51	-17.31 to -19.46	9.25 ± 0.42	8.42 to 10.36
ON	77	-18.04 ± 0.53	-16.72 to -19.36	9.06 ± 0.46	7.75 to 10.17
QC	83	-18.28 ± 0.52	-16.82 to -20.11	8.99 ± 0.40	7.97 to 10.58
NB	30	-18.25 ± 0.66	-17.16 to -20.22	9.30 ± 0.43	8.45 to 10.42
NS	58	-18.26 ± 0.62	-17.12 to -19.87	9.47 ± 0.32	8.68 to 10.78
PE	4	-18.46 ± 0.74	-17.51 to -19.15	9.35 ± 0.57	8.61 to 9.95
NL	16	-18.13 ± 0.36	-17.45 to -18.73	9.39 ± 0.40	8.57 to 10.09

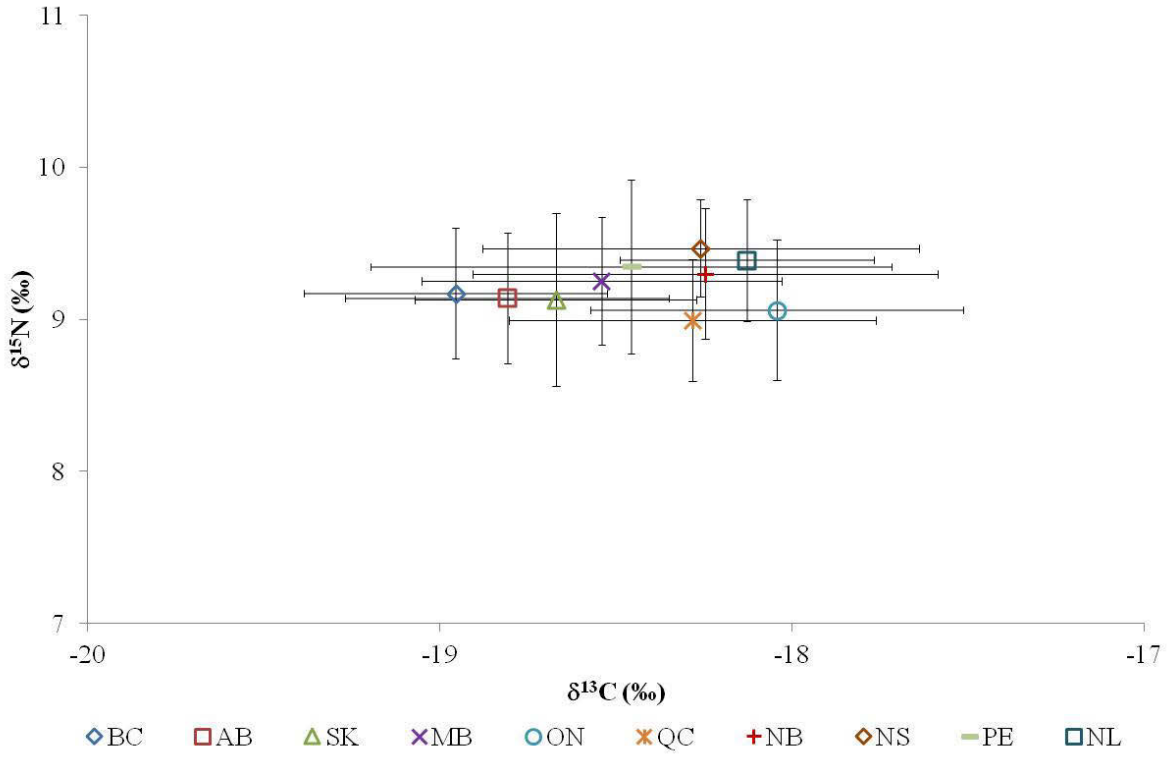

Figure 12. Average $\delta^{13}C$ and $\delta^{15}N$ values for each province.

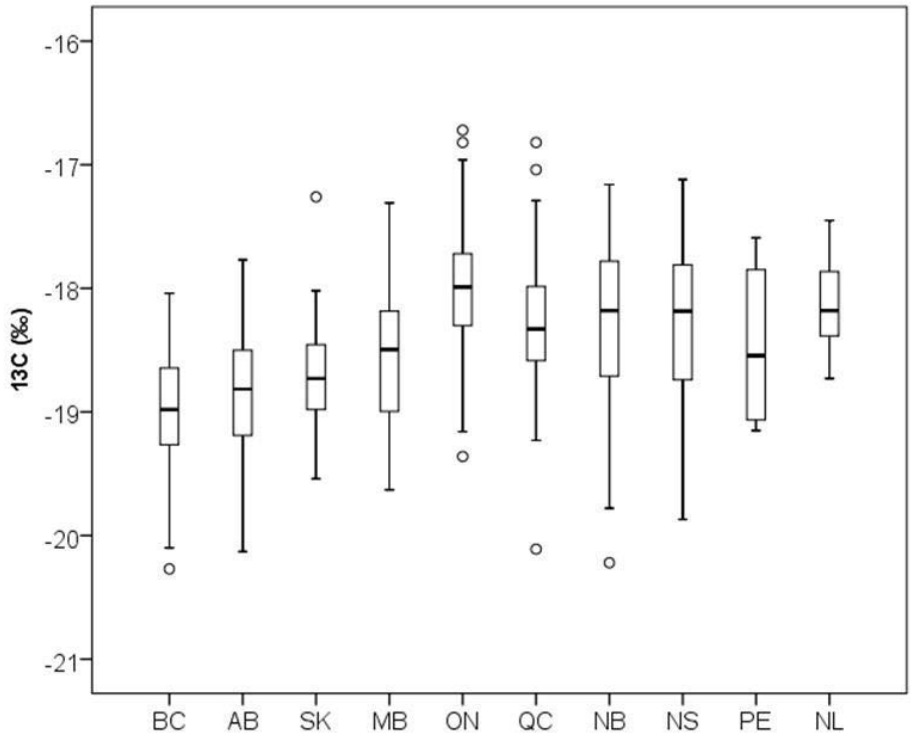

Figure 13a. Box plot of $\delta^{13}C$ values for all provinces. The solid black line inside the box is the MEDIAN.

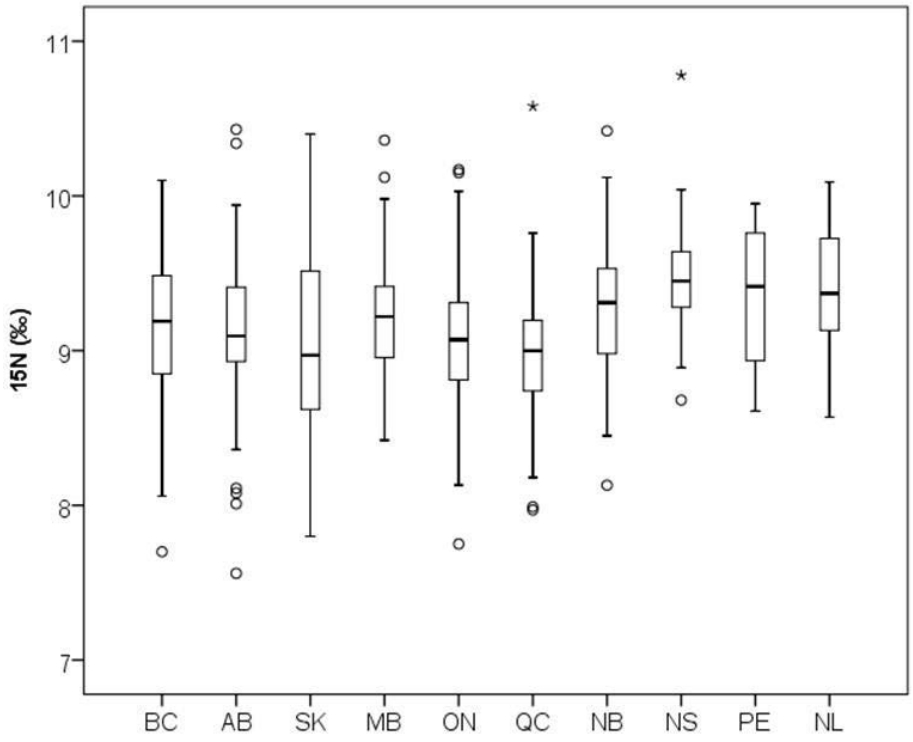

Figure 13b. Box plot of $\delta^{15}N$ values for all provinces. The solid black line inside the box is the MEDIAN.

While the range of measured $\delta^{13}C$ and $\delta^{15}N$ values were relatively small, there was a noted difference between provinces. Further, as evidenced from Figure 12, there is a larger spread in average $\delta^{13}C$ values compared to $\delta^{15}N$ values. Since PE had only 4 samples, this province was excluded from the statistical analysis.

Levene's test for equal variance and a t-test were performed on the $\delta^{13}C$ and $\delta^{15}N$ values from each province to determine if there is a statistical difference in these isotopes between the provinces. p-values (from the t-test) with *unequal variance* (as determined from Levene's test) are presented in *italics*. A p-value of less than 0.05 demonstrates that the two sets of data analyzed are statistically different at a 95% confidence interval. The p-values from the t-tests are presented in Table 18a and 18b.

Table 18a. p-values from t-tests comparing hair $\delta^{13}C$ values from different provinces. A p-value less than 0.05 (highlighted in grey) demonstrates the $\delta^{13}C$ values are significantly different between those provinces. Values in italics represent provinces with unequal variance (Levene's test).

Province	AB	SK	MB	ON	QC	NB	NS	NL
BC	0.014	0.00053	1.6E-06	1.0E-13	1.0E-13	*1.5E-06*	*1.1E-11*	1.7E-11
AB		0.16	0.0062	1.0E-13	3.9E-13	2.1E-06	*7.1E-08*	1.4E-07
SK			*0.20*	1.6E-10	1.4E-05	*0.0027*	*5.2E-05*	1.2E-05
MB				7.5E-07	0.0045	0.041	0.010	0.0037
ON					0.0044	0.046	0.031	0.55
QC						1.0	0.81	0.26
NB							0.87	0.39
NS								*0.28*

Table 18b. p-values from t-tests comparing hair $\delta^{15}N$ values from different provinces. A p-value less than 0.05 (highlighted in grey) demonstrates the $\delta^{15}N$ values are significantly different between those provinces. Values in italics represent provinces with unequal variance (Levene's test).

Province	AB	SK	MB	ON	QC	NB	NS	NL
BC	0.53	*0.43*	0.25	0.13	0.0076	0.29	*1.7E-07*	0.043
AB		*0.70*	0.13	0.43	0.069	0.17	*2.7E-07*	0.027
SK			*0.14*	*0.88*	*0.44*	0.18	*3.0E-04*	*0.026*
MB				0.030	0.0014	0.97	0.0028	0.23
ON					0.34	0.051	*9.6E-09*	0.0083
QC						0.0041	7.7E-12	4.6E-04
NB							*0.019*	0.29
NS								0.43

For $\delta^{13}C$, there were significant differences observed between many provinces. Each province showed a statistical difference to several other provinces, suggesting that the local diets may be different in different parts of Canada.

For $\delta^{15}N$, QC, NS and NL showed the highest rates of statistical differences to other provinces, which may reflect differences in dietary protein choices. BC, AB, SK, MB, ON and NB showed similar average $\delta^{15}N$ values, and in general were not statistically different from each other.

Despite the statistical differences, the range $\delta^{13}C$ and $\delta^{15}N$ values overlap for each province (Figures 13a and 13b). Thus, taken on an individual basis, $\delta^{13}C$ and $\delta^{15}N$ values alone are not useful as a geographical indicator but combined with other evidence may be useful.

Omnivore vs Vegetarian

Dietary choices in terms of protein intake can affect the $\delta^{15}N$ value of hair. In general, the more terrestrial animal protein and/or marine products consumed, the more positive the $\delta^{15}N$ value. This is often referred to as the trophic effect; or the higher up in the food chain the more positive the $\delta^{15}N$ value. Previous researchers [9,13] have shown that vegans have a lower average $\delta^{15}N$ value in hair than vegetarians and omnivores. In one study, vegetarians had a lower average $\delta^{15}N$ value in hair than omnivores [9], but in the other, these two groups were indistinguishable [13]. Further, the type of protein consumed can also affect the $\delta^{15}N$ value in hair. Huelsemann et al [4] reported an increase in the $\delta^{15}N$ values with an increase in the consumption of marine products.

Table 19a shows the range and average $\delta^{13}C$ and $\delta^{15}N$ values between omnivores (including those who ate little meat) and vegetarians. The vegetarians were further sub-divided into those who ate seafood/fish, and those who did not.

Table 19a. $\delta^{13}C$ and $\delta^{15}N$ hair values for omnivores and vegetarians.

Group	Average $\delta^{13}C \pm SD$ (‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$ (‰)	Range $\delta^{15}N$ (‰)
Omnivores (n=565)	-18.52 ± 0.60	-16.72 to -20.22	9.17 ± 0.45	7.75 to 10.78
Vegetarians who ate seafood/fish (n=5)	-18.87 ± 0.90	-17.95 to -20.27	8.94 ± 0.19	8.65 to 9.13
Vegetarians who ate no seafood/fish (n=7)	-19.16 ± 0.30	-18.99 to -19.63	8.24 ± 0.48	7.56 to 8.79

Trends were observed in both the average $\delta^{13}C$ and $\delta^{15}N$ values for these groups. One could think of these three groups in terms of their place in the food chain in terms of animal protein consumption: omnivores would be at the higher level than vegetarians who consume seafood/fish, who are, in turn, at a higher level than vegetarians who do not consume seafood/fish. This is reflected in the increasing $\delta^{15}N$ values as you go higher up the food chain. Further, there is a noticeable difference in the $\delta^{13}C$ values as well, which is likely due to an increased amount of C3 sources consumed by the vegetarians compared to omnivores from this study.

The vegetarians who ate no seafood/fish were amongst the lowest $\delta^{15}N$ values measured, which is consistent with other studies showing that on average, vegetarians have a lower $\delta^{15}N$ value than omnivores [9]. Further, there was one vegetarian who did not consume any seafood/fish with a $\delta^{15}N$ value more typical of an omnivore diet ($\delta^{15}N$ = 8.79 ‰). More samples of persons who are vegetarian are needed to better understand the effect of protein dietary choices on hair $\delta^{15}N$ values.

Because there were so few vegetarians in our study, both groups of vegetarians (i.e. those who ate seafood/fish, and those who did not) were combined for statistical analysis (see Table 19b for combined Vegetarian statistics).

Table 19b. $\delta^{13}C$ and $\delta^{15}N$ hair values for combined vegetarian groups.

Group	Average $\delta^{13}C \pm SD$ (‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$ (‰)	Range $\delta^{15}N$ (‰)
Vegetarians (n=12)	-19.04 ± 0.61	-17.95 to -20.27	8.53 ± 0.52	7.56 to 9.13

Levene's test and a t-test were performed on the $\delta^{13}C$ and $\delta^{15}N$ data sets from omnivores and vegetarians. A p-value (from the t-test) of less than 0.05 demonstrates that the two sets of data analyzed are statistically different at a 95% confidence interval. The p-values from the t-tests are presented in Table 20.

Table 20. p-values from t-tests comparing hair $\delta^{13}C$ and $\delta^{15}N$ values between omnivores and vegetarians. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{13}C$ or $\delta^{15}N$ values are significantly different between omnivores and vegetarians.

Isotope	p-value
$\delta^{13}C$	0.0035
$\delta^{15}N$	1.5e-6

Figures 14a and 14b show the box plots for vegetarians (n=12) and non-vegetarians (n=565) for $\delta^{13}C$ and $\delta^{15}N$, respectively. The mean of the data is indicated on the graph, but it is NOT represented by the solid middle line in the box (which is the median).

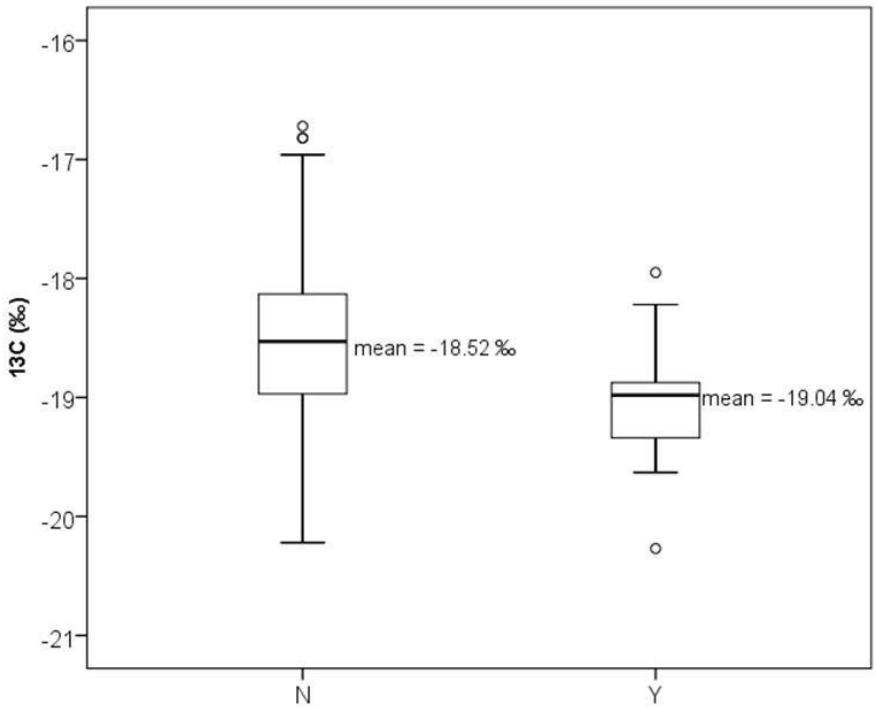

Figure 14a. Box plot of $\delta^{13}C$ values for omnivores (N) and vegetarians (Y). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

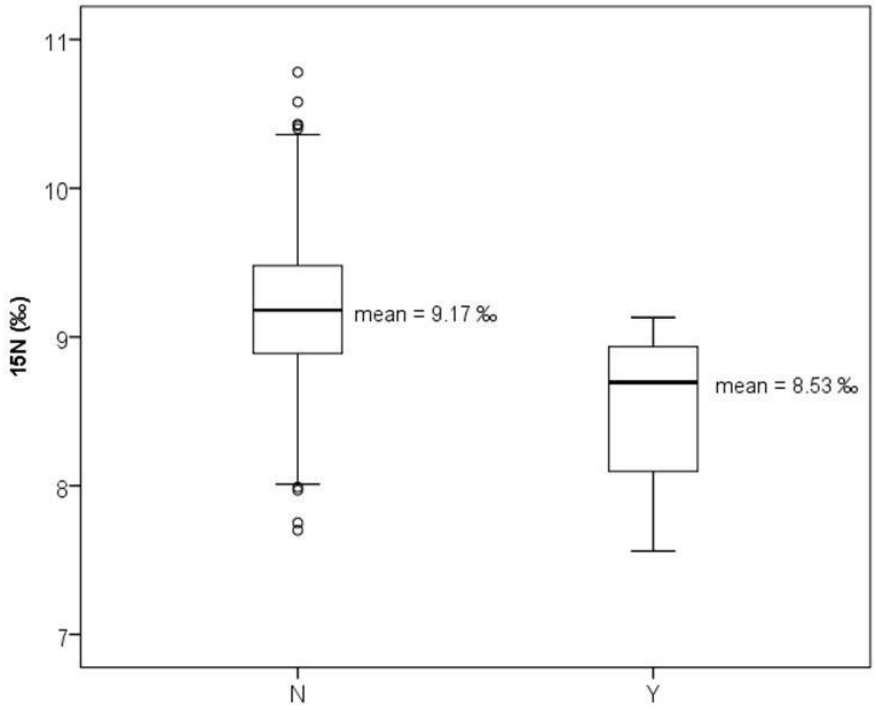

Figure 14b. Box plot of $\delta^{15}N$ values for omnivores (N) and vegetarians (Y). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

Levene's test shows equal variances for the two groups compared. The p-values for both $\delta^{13}C$ and $\delta^{15}N$ are less than 0.05, confirming that there is a statistical difference between the $\delta^{13}C$ and $\delta^{15}N$ values of omnivores and vegetarians. While trends are apparent, there is still considerable overlap in the $\delta^{13}C$ and $\delta^{15}N$ values of omnivores and vegetarians. As such, vegetarian diet to an unknown hair sample based on the $\delta^{13}C$ and $\delta^{15}N$ values of hair cannot be definitely assigned.

Marine Protein Consumption

In an effort to quantify the effect of consuming seafood/fish on hair $\delta^{15}N$ values, and to determine if there is an appreciable effect on hair $\delta^{15}C$ values, the omnivore group was further divided into groups based on their marine protein consumption. Of the people who consumed seafood/fish, the quantity consumed was rated based on their reported average intake of marine protein according to the following scale:

Table 21a: Rating system to quantify seafood/fish consumption, and $\delta^{13}C$ and $\delta^{15}N$ values for each rating group.

Rate	Quantity of Seafood/Fish consumed	n	Average $\delta^{13}C \pm SD$(‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$ (‰)	Range $\delta^{15}N$ (‰)
0	Never	82	-18.52 ± 0.59	-16.72 to -19.64	9.05 ± 0.49	7.80 to 10.36
1	0.1 to 3 portions per month	181	-18.55 ± 0.60	-16.82 to -20.27	9.11 ± 0.48	7.70 to 10.43
2	1 to 1.9 portions per week	174	-18.52 ± 0.64	-16.82 to -20.22	9.20 ± 0.41	8.15 to 10.78
3	2 to 3.9 portions per week	110	-18.52 ± 0.59	-16.96 to -20.11	9.20 ± 0.44	7.56 to 10.15
4	4 to 6.9 portions per week	20	-18.63 ± 0.58	-17.74 to -19.64	9.28 ± 0.48	8.30 to 9.76
5	7+ portions per week	10	-18.58 ± 0.48	-17.98 to -19.46	9.42 ± 0.70	7.97 to 10.58

The average $\delta^{15}N$ becomes more positive as the quantity of seafood/fish consumed increases. This is consistent with what was observed by Huselmann et al [4]. In contrast, no pattern was observed for $\delta^{13}C$ with increasing consumption of seafood/fish.

Because Rates 4 and 5 had few participants, they were combined for the following statistical analysis (see Table 21b for combined Rate 4/5 statistics).

Table 21b: Rating system to quantify seafood/fish consumption, and $\delta^{13}C$ and $\delta^{15}N$ values for each rating group.

Rate	Quantity of Seafood / Fish consumed	n	Average $\delta^{13}C \pm SD$(‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$(‰)	Range $\delta^{15}N$(‰)
Combined 4/5	4 + portions per week	30	-18.61 ± 0.54	-17.74 to -19.64	9.33 ± 0.55	7.97 to 10.58

Levene's test and a t-test were performed on the $\delta^{13}C$ and $\delta^{15}N$ data sets from omnivores and vegetarians. A p-value (from the t-test) of less than 0.05 demonstrates that the two sets of data analyzed are statistically different at a 95% confidence interval. The p-values from the t-tests are presented in Tables 22a and 22b. Figures 15a and 15b show the box plots for $\delta^{13}C$ and $\delta^{15}N$, respectively for the different rates of seafood/fish consumption.

Table 22a. p-values from t-tests comparing hair $\delta^{13}C$ values from participants consuming different amounts of seafood/fish. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{13}C$ values are significantly different between the groups.

Group	1	2	3	4/5
0	0.67	0.94	0.98	0.45
1		0.67	0.66	0.61
2			0.96	0.48
3				0.45

Table 22b. p-values from t-tests comparing hair $\delta^{15}N$ values from participants consuming different amounts of seafood/fish. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{15}N$ values are significantly different between the groups. Values in italics have unequal variances between the data sets.

Group	1	2	3	4/5
0	0.35	0.009	0.31	0.012
1		*0.049*	0.13	0.028
2			0.89	0.15
3				0.18

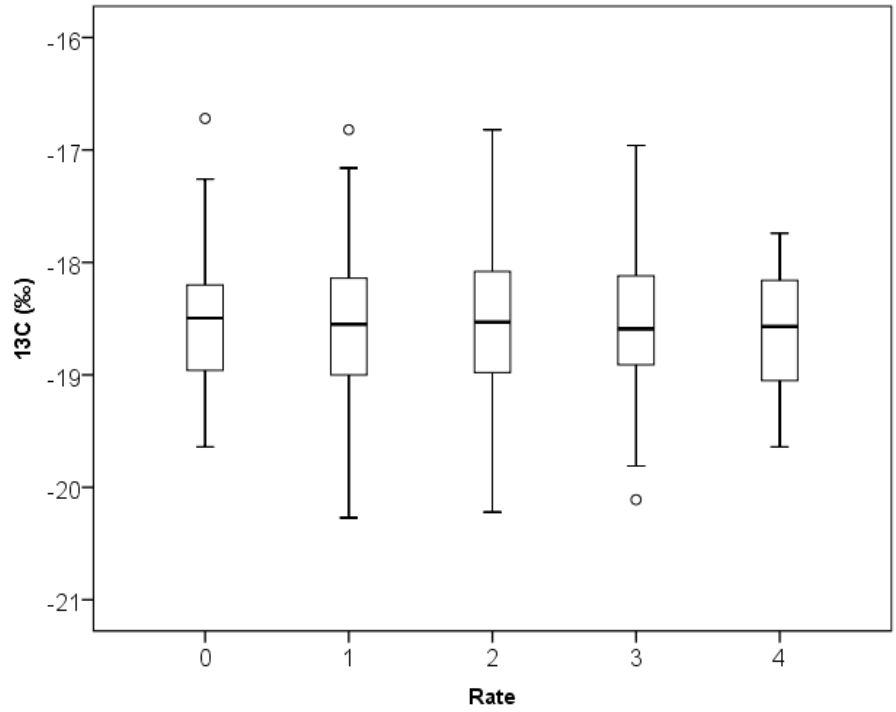

Figure 15a. Box plot of $\delta^{13}C$ values for different rates of marine protein consumption. See text for details. Rate 4 is the combined group of Rates 4/5. The solid black line inside the box is the MEDIAN

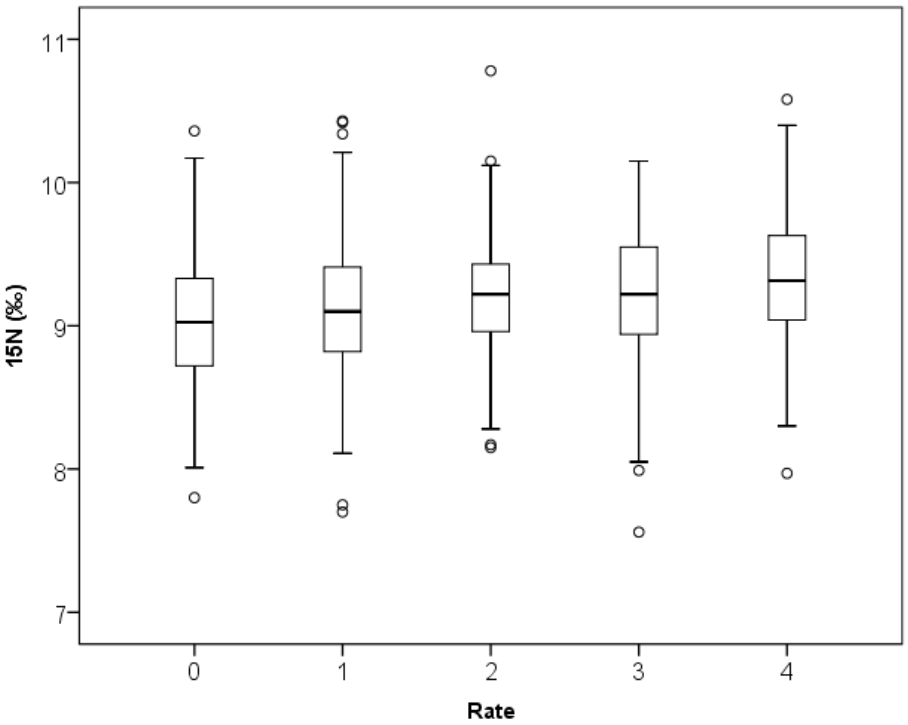

Figure 15b. Box plot of $\delta^{15}N$ values for different rates of marine protein consumption. See text for details. Rate 4 is the combined group of Rates 4/5. The solid black line inside the box is the MEDIAN.

For $\delta^{13}C$, there was no statistical difference between all rates of marine protein consumption, suggesting that seafood/fish intake does not significantly affect $\delta^{13}C$ hair values.

For $\delta^{15}N$, there was a statistical difference between several groups. Participants who never consumed marine products (Rate 0) did not have a statistically different average $\delta^{15}N$ value than those who consumed marine products less than once per week (Rate 1), suggesting that the consumption of marine protein less than one portion per week does not significantly affect the average $\delta^{15}N$ value of hair. In contrast, consuming more than 4 portions of marine protein per week (Rate 4/5) did significantly affect average $\delta^{15}N$ values compared to consuming none or less than one portion per week (Rates 0 and 1).

While trends are apparent, there is still considerable overlap in the $\delta^{13}C$ and $\delta^{15}N$ hair values of persons who consume varying amounts of marine protein. As such, the reason for the amount of protein intake based on $\delta^{13}C$ and $\delta^{15}N$ values of hair cannot be accurately determined.

Gender

$\delta^{13}C$ and $\delta^{15}N$ values for males and females were analyzed to determine if there is a difference in these average isotope values between these two groups. Levene's test and a t-test were performed on the $\delta^{13}C$ and $\delta^{15}N$ values from males and females. The p-values from the t-tests, along with the averages and standard deviations for each group are presented in Table 23, and the boxplots are shown in Figures 16a and 16b.

Table 23. Average and standard deviations, and p-values from t-tests comparing hair $\delta^{13}C$ and $\delta^{15}N$ values between males and females. A p-value less than 0.05 (highlighted in grey) indicates a significant difference between the two groups.

Isotope	Average ± SD (‰) for Male	Average ± SD (‰) for Female	p-value
$\delta^{13}C$	-18.42 ± 0.60	-18.61 ± 0.60	2.0e-4
$\delta^{15}N$	9.19 ± 0.43	9.14 ± 0.48	0.20

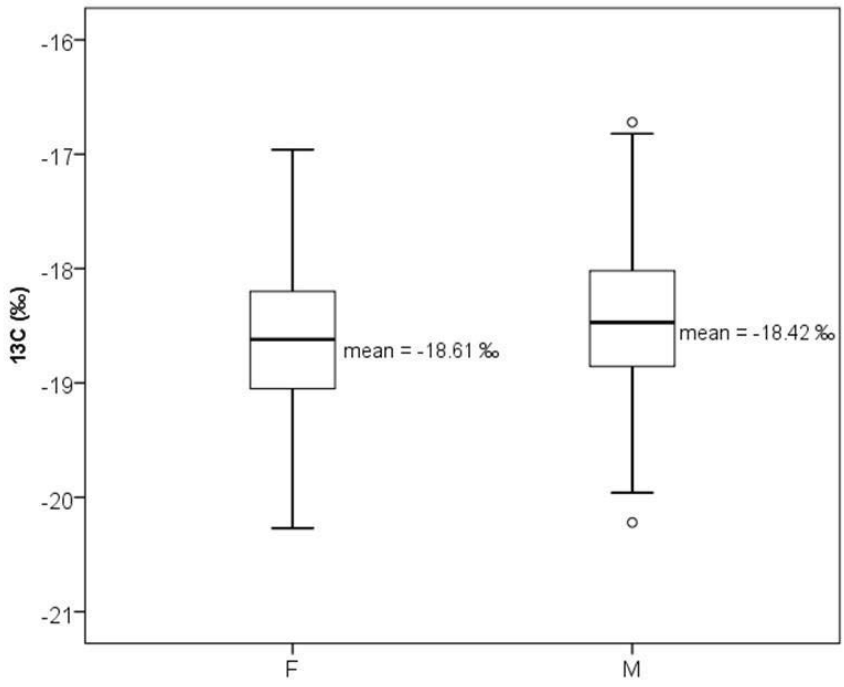

Figure 16a. Box plot of $\delta^{13}C$ values for females (F) and males (M). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

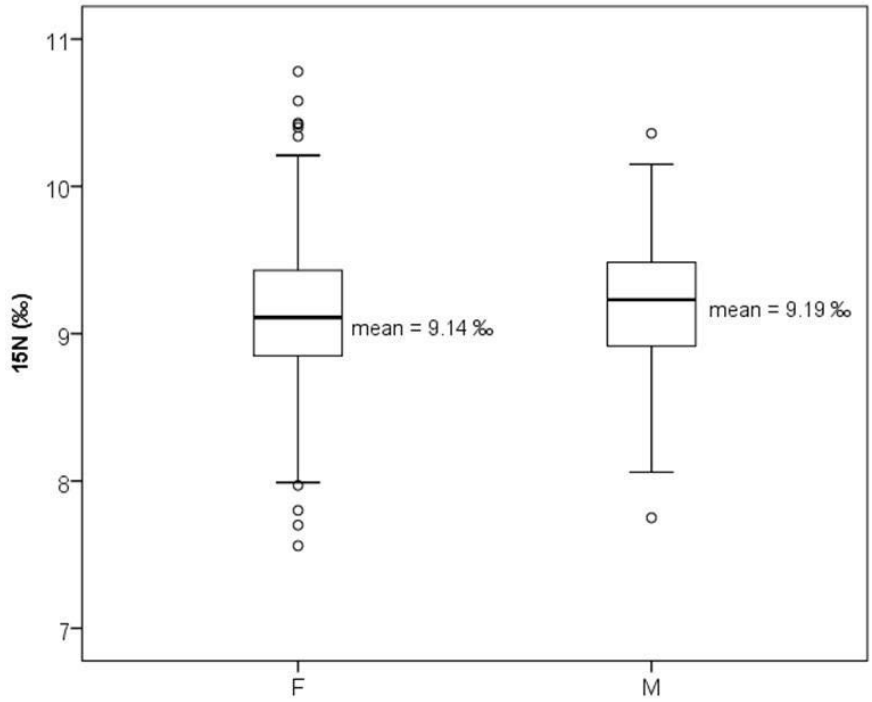

Figure 16b. Box plot of $\delta^{15}N$ values for females (F) and males (M). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

The average $\delta^{13}C$ and $\delta^{15}N$ hair values from male and female participants were similar, suggesting that gender does not affect these isotope values.

Levene's test shows equal variances for the two groups compared. The p-value for $\delta^{13}C$ was less than 0.05, showing that there is a statistical difference between the $\delta^{13}C$ values of hair from males and females. However, due to the overlapping range of $\delta^{13}C$ values for males and females, $\delta^{13}C$ values are not useful in determining the gender of an unknown hair donor.

The p-value for $\delta^{15}N$ was greater than 0.05, confirming that there is no statistical difference between $\delta^{15}N$ values of males and females, and that $\delta^{15}N$ are not useful in determining the gender of an unknown hair donor.

Smoker vs. Non-Smoker

$\delta^{13}C$ and $\delta^{15}N$ values for smokers and non-smokers were analyzed to determine if smoking could significantly affect these isotope values for hair. This question was not asked during the collection campaign in Atlantic Canada (2009), resulting in less data points.

Levene's test and a t-test were performed on the $\delta^{13}C$ and $\delta^{15}N$ values for smokers and non-smokers. The p-values from the t-tests, along with the averages and standard deviations for each group are presented in Table 24, and the boxplots are shown in Figures 17a and 17b.

Table 24. Average and standard deviations, and p-values from t-tests comparing hair $\delta^{13}C$ and $\delta^{15}N$ values between smokers and non-smokers. A p-value less than 0.05 (highlighted in grey) indicates a significant difference between the two groups.

Isotope	Average ± SD (‰) for Smoker	Average ± SD (‰) for Non-Smoker	p-value
$\delta^{13}C$	-18.52 ± 0.48	-18.63 ± 0.60	0.18
$\delta^{15}N$	9.15 ± 0.47	9.09 ± 0.46	0.28

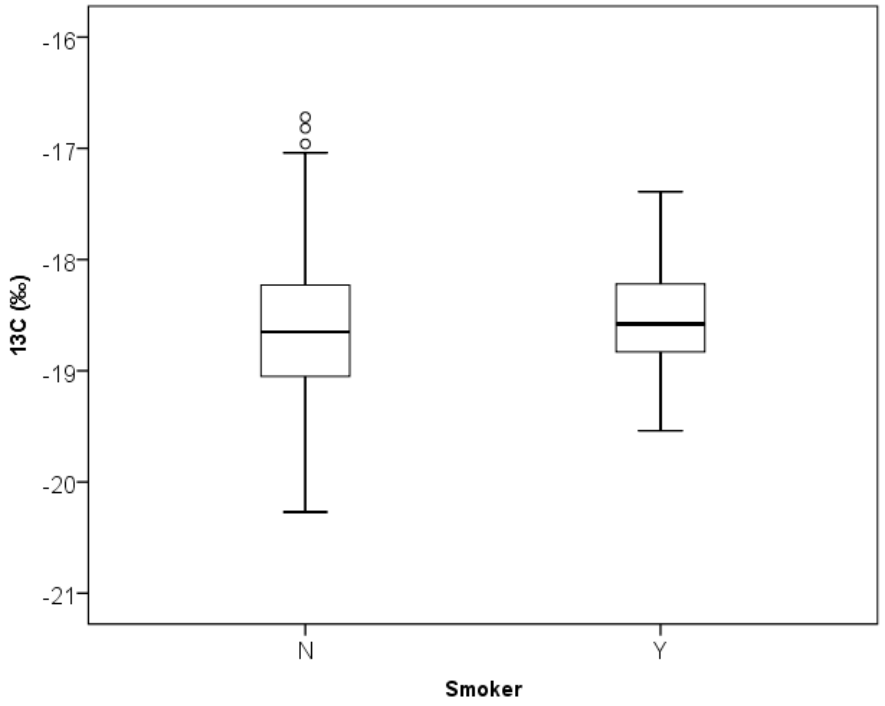

Figure 17a. Box plot of $\delta^{13}C$ values for smokers and non-smokers. The solid black line inside the box is the MEDIAN.

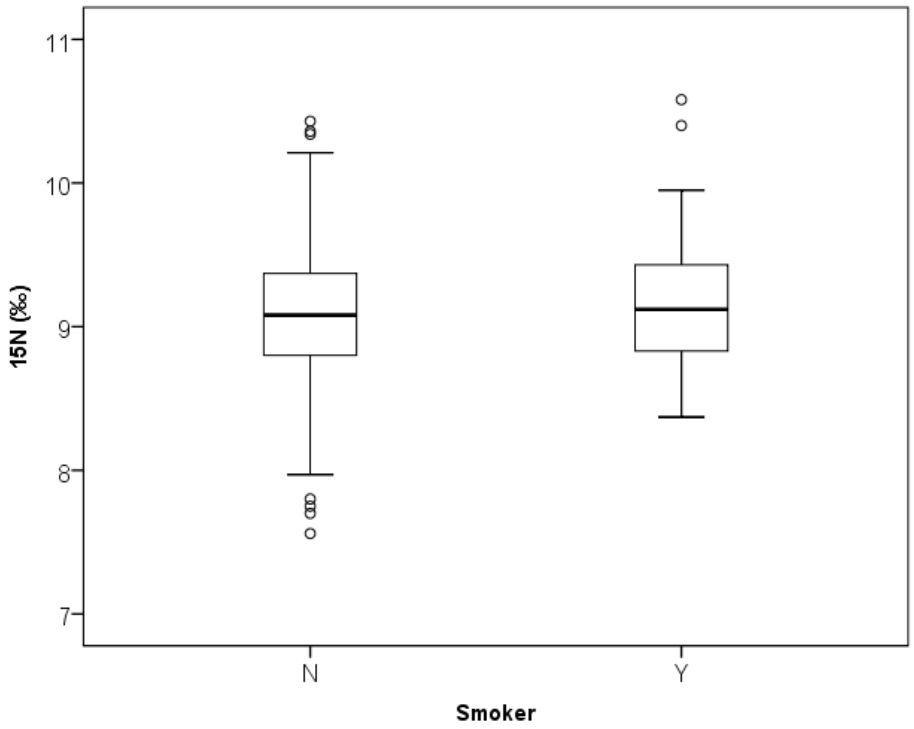

Figure 17b. Box plot of $\delta^{15}N$ values for smokers and non-smokers. The solid black line inside the box is the MEDIAN.

The average $\delta^{13}C$ and $\delta^{15}N$ hair values for smokers and non-smokers were similar, suggesting that smoking does not affect these isotope values.

Levene's test shows equal variances for all groups. The p-values for both $\delta^{13}C$ and $\delta^{15}N$ were greater than 0.05, confirming that there is no statistical difference in these isotopes between smokers and non-smokers. Further, due to the overlapping range of $\delta^{13}C$ and $\delta^{15}N$ values for smokers and non-smokers, these isotope values are not useful in determining if an unknown hair donor smoked.

Age

Statistical analysis was performed on $\delta^{13}C$ and $\delta^{15}N$ hair values from participants of different ages to determine if different age categories had different $\delta^{13}C$ and $\delta^{15}N$ hair values. The age of the participant was divided into several categories. The age ranges, average $\delta^{13}C$ and $\delta^{15}N$ values and ranges are shown in Table 25.

Table 25: Age ranges and $\delta^{13}C$ and $\delta^{15}N$ values for each age group.

Age Range	N	Average $\delta^{13}C \pm SD$ (‰)	Range $\delta^{13}C$ (‰)	Average $\delta^{15}N \pm SD$ (‰)	Range $\delta^{15}N$ (‰)
18-29	81	-18.39 ± 0.48	-17.53 to -19.37	9.20 ± 0.38	8.33 to 10.36
30-39	132	-18.49 ± 0.59	-16.72 to -20.10	9.12 ± 0.48	7.75 to 10.17
40-49	179	-18.65 ± 0.60	-17.16 to -20.13	9.20 ± 0.45	7.70 to 10.58
50-59	138	-18.48 ± 0.64	-16.82 to -20.11	9.16 ± 0.47	8.13 to 10.78
60-69	27	-18.63 ± 0.61	-17.33 to -19.78	9.19 ± 0.41	8.18 to 9.92
70+	20	-18.70 ± 0.79	-17.29 to -20.22	8.85 ± 0.56	7.97 to 10.34

For $\delta^{13}C$, all the age ranges showed similar average $\delta^{13}C$ hair values, suggesting that Canadians all ate similar diets.

For $\delta^{15}N$, all the age ranges showed similar average $\delta^{15}N$ hair values except for the 70+ category. This may suggest that people 70+ make different dietary choices than younger people.

Levene's test for equal variance and a t-test was performed on the $\delta^{13}C$ and $\delta^{15}N$ values from the different age categories. The p-values from the t-tests are presented in Tables 26a and 26b, and the boxplots are shown in Figures 18a and 18b.

Table 26a. p-values from t-tests comparing hair $\delta^{13}C$ values from people of different age groups. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{13}C$ values are significantly different between those age groups. Values in italics have unequal variances between the data sets.

Age	30-39	40-49	50-59	60-69	70+
18-29	*0.15*	*0.00025*	*0.22*	0.037	*0.10*
30-39		0.026	0.87	0.29	0.17
40-49			0.019	0.88	0.71
50-59				0.28	0.17
60-69					0.72

Table 26b. p-values from t-tests comparing hair $\delta^{15}N$ values from people of different age groups. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{15}N$ values are significantly different between those age groups. Values in italics have unequal variances between the data sets.

Age	30-39	40-49	50-59	60-69	70+
18-29	0.21	0.98	*0.56*	0.98	0.0015
30-39		0.13	0.44	0.44	0.025
40-49			0.50	0.97	0.0017
50-59				0.74	0.0080
60-69					0.019

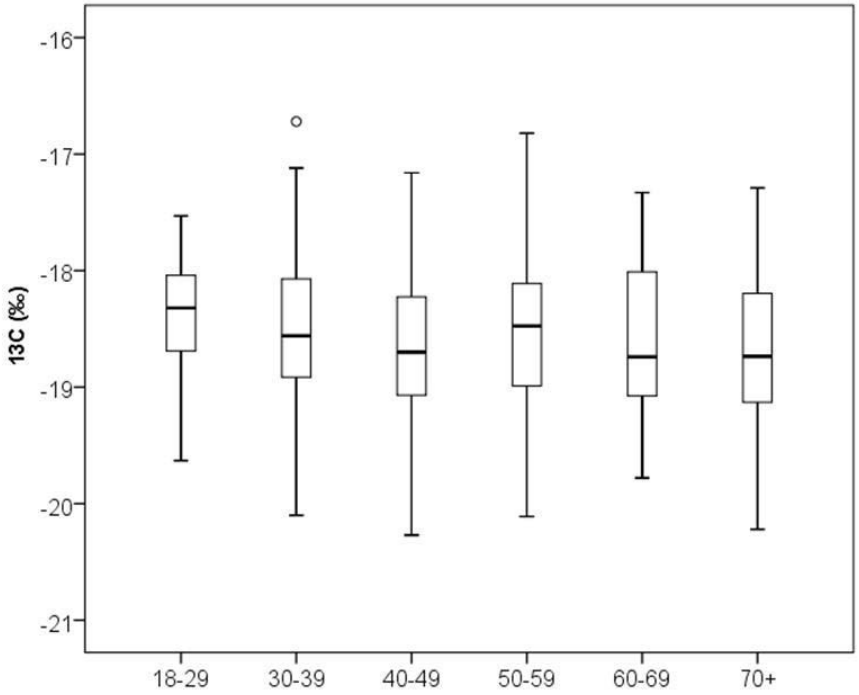

Figure 18a. Box plot of $\delta^{13}C$ values for all age ranges. The solid black line inside the box is the MEDIAN.

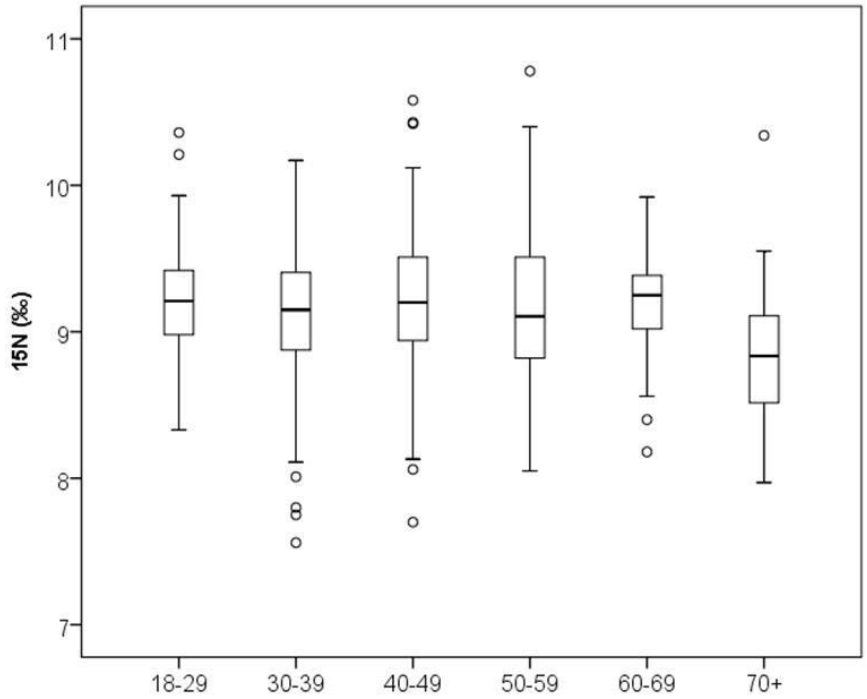

Figure 18b. Box plot of $\delta^{15}N$ values for all age ranges. The solid black line inside the box is the MEDIAN.

For $\delta^{13}C$, age group 40-49 was statistically different from all age groups above 60. The only age groups that were statistically different were 18-29 and 60-69. These differences suggest these age groups may choose different food options. However, since the range of $\delta^{13}C$ values for all groups overlap, individual measurements of hair $\delta^{13}C$ values are not useful in determining the age of an unknown hair donor.

Interestingly, the only group which was statistically different from any other with regards to $\delta^{15}N$ is the 70+ age range. This age group may make different dietary choices in regards to protein intake, or perhaps it is a reflection of overall health; though there is no basis for measuring this for this project. However, due to the small number of samples for people in this range, and due to the overlapping range of $\delta^{15}N$ values for all age groups, individual measurements of hair $\delta^{15}N$ values are not useful in determining the age of an unknown hair donor.

Sulphur

$\delta^{34}S$ measurements can be useful in determining dietary habits [11,27,28], and may have uses for geo-location purposes [2,3,11,12,29]. Food, ocean deposition, local soil/rock geochemistry, and deposition from the atmosphere can all be sources of sulphur for humans. More positive $\delta^{34}S$ values in hair have been linked to higher consumption of marine animals, and to persons residing in coastal habitats.

Sulphur Isotope Values across Canada

In total, 529 hair samples were analyzed for $\delta^{34}S$. The following table compares the range of $\delta^{34}S$ values measured in this study to other studies.

Table 27. Range of $\delta^{34}S$ values from hair from different locations.

Hair Sample Collection Location	Range $\delta^{34}S$ (‰)	Reference
Canada	-1.4 to 4.8	this study
Canada	~0 to ~ 3	28
United States	-1.2 to 9.9	11
UK	3.55 to 7.48	2
Western Europe	4.8 to 8.3	27
Canberra (AUS)	~ 14	28
China, India, Mongolia, Pakistan	3.2 to 13.8	29

The Canadian range measured for samples collected from this study was very similar to other Canadian hair measured [28]. The most negative $\delta^{34}S$ value measured for Canadian hair was -1.4 ‰ from a person residing in Dauphin, MB. The most positive value, 4.8 ‰, was from a person living in Edmunston, NB.

Similar to $\delta^{13}C$ measurements, the Canadian range of $\delta^{34}S$ values is almost completely encompassed by the range reported for the United States [11], with the US having a much more positive end member (9.9 ‰) for the range. Compared to other countries (Table 26), the $\delta^{34}S$ value of Canadian hair is more negative. This difference may be due to dietary or geological differences between Canada and other countries [2,3,11,12,27-29]. This difference in $\delta^{34}S$ values may be used as a tool in conjunction with other evidence to distinguish between Canadian (North American) hair and foreign hair. As an example, hair was collected from a person from Weatherby, UK. The $\delta^{34}S$ value of his hair was 5.2 ‰, which was within the range of $\delta^{34}S$ values in hair from the UK [2]. This value was more enriched than any Canadian hair measured, suggesting that $\delta^{34}S$ values may be used as a line of evidence to distinguish between Canadian and foreign hair.

All $\delta^{34}S$ values were plotted against $\delta^{13}C$ (Figure 19a), $\delta^{15}N$ (Figure 19b) and $\delta^{15}H$ (Figure 19c) values for the same hair sample. In the case of $\delta^{13}C$ and $\delta^{15}N$, very poor correlation was observed, suggesting that the S portion of the diet is not necessarily related to the C and N portion.

However, there is a weak correlation between $\delta^{34}S$ and $\delta^{2}H$ values ($R^2 = 0.31$). Since $\delta^{2}H$ values vary with geographic location [1,12, 19-24], the correlation of $\delta^{2}H$ values with $\delta^{34}S$ values may suggest that $\delta^{34}S$ values can also vary with geographic location.

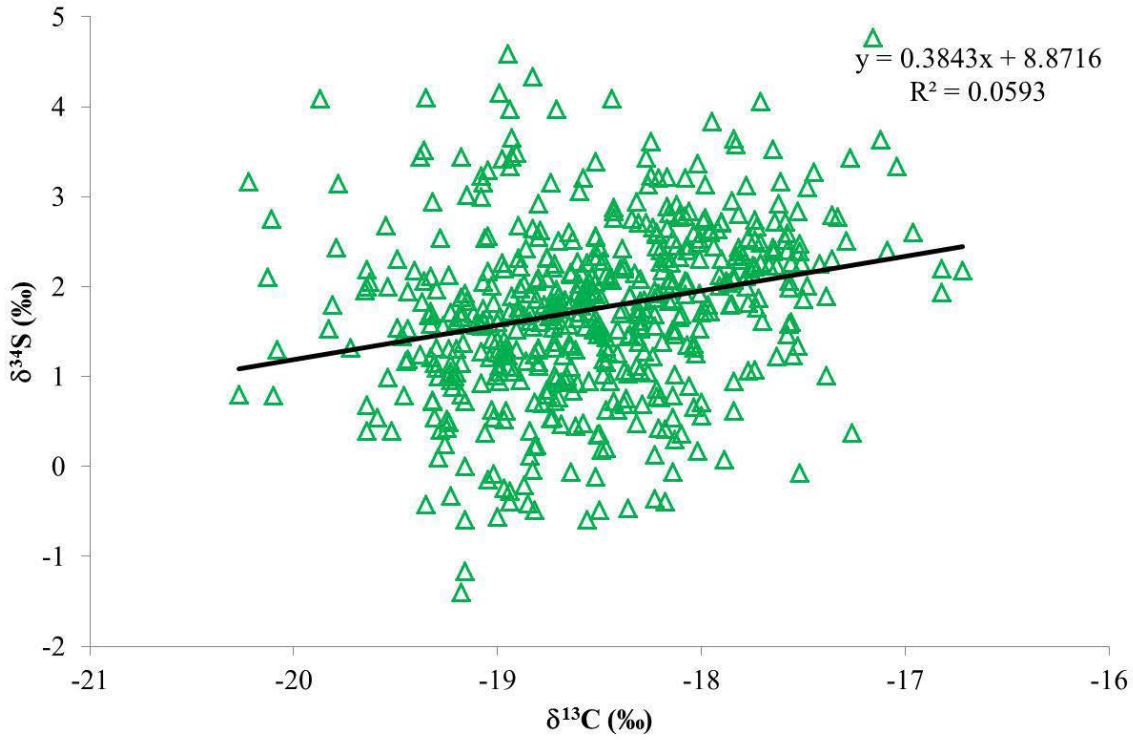

Figure 19a. $\delta^{34}S$ and $\delta^{13}C$ values in hair showing very poor correlation.

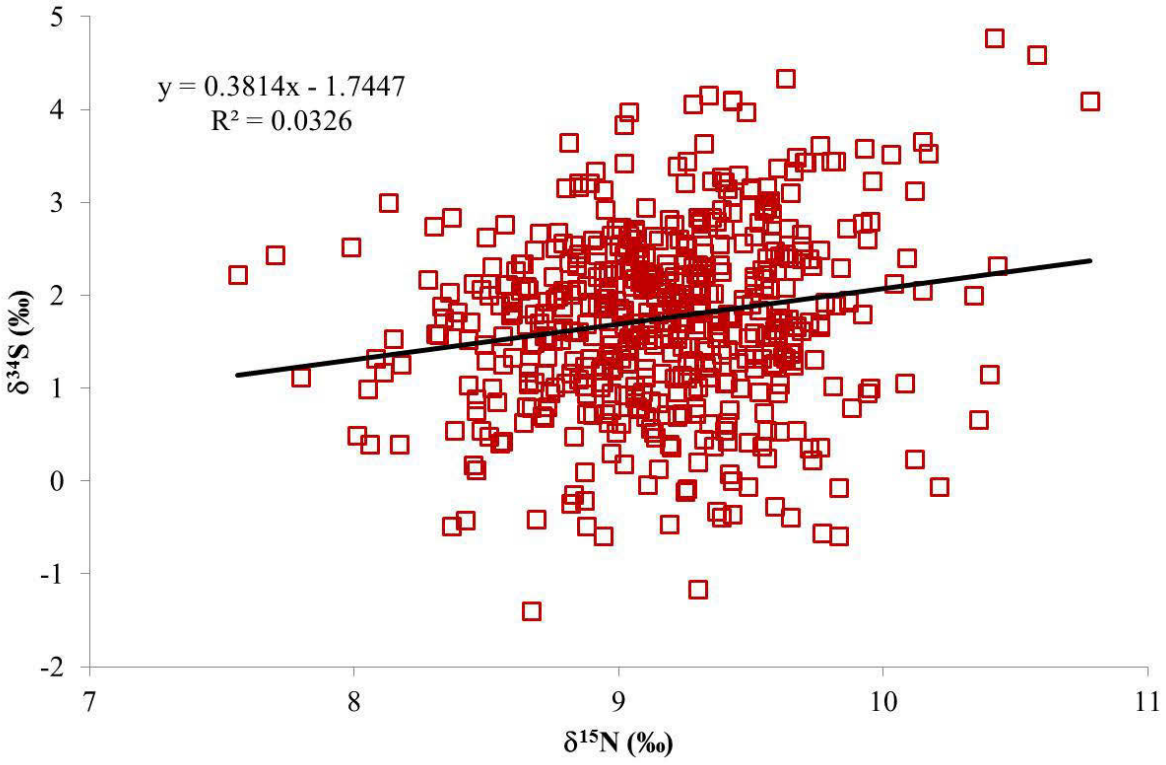

Figure 19b. $\delta^{34}S$ and $\delta^{15}N$ values in hair showing very poor correlation.

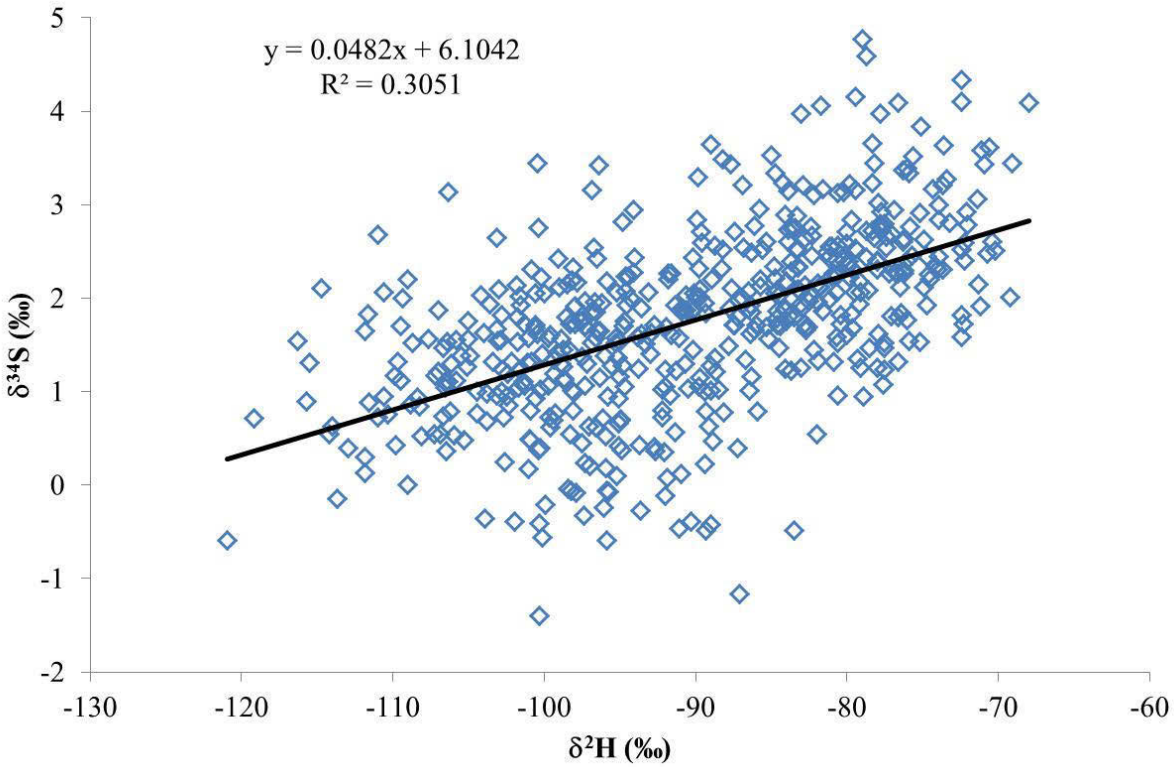

Figure 19c. $\delta^{34}S$ and $\delta^{2}H$ values in hair showing a weak correlation.

Figure 20 shows the GIS map of all $\delta^{34}S$ values for Canadian human hair.

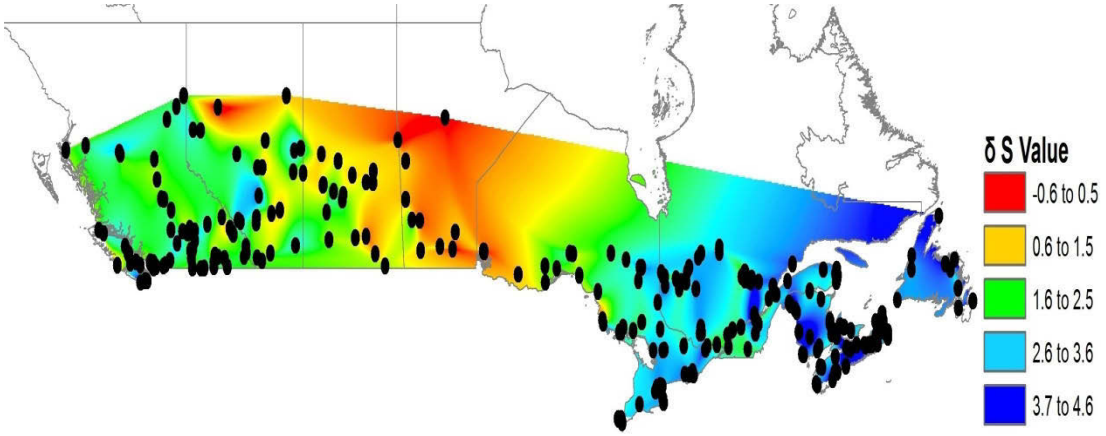

Figure 20. $\delta^{34}S$ values from hair collected across Canada.

The average and range of $\delta^{34}S$ values for each province are presented in Table 28. Figure 21 shows the box plot of $\delta^{34}S$ for each province.

Table 28. $\delta^{34}S$ values of hair from persons residing in different provinces.

Province	n	Average $\delta^{34}S \pm SD$ (‰)	Range $\delta^{34}S$ (‰)
BC	118	1.6 ± 0.7	0.0 to 3.5
AB	79	1.4 ± 0.7	-0.6 to 3.1
SK	42	0.7 ± 0.7	-0.6 to 2.4
MB	43	0.3 ± 0.7	-1.4 to 2.1
ON	65	2.0 ± 0.9	-1.2 to 4.1
QC	72	2.2 ± 0.6	1.0 to 4.6
NB	33	2.6 ± 0.7	1.5 to 4.8
NS	57	2.6 ± 0.8	1.2 to 4.3
PE	4	2.7 ± 0.3	2.3 to 3.0
NL	16	2.7 ± 0.6	1.8 to 3.6

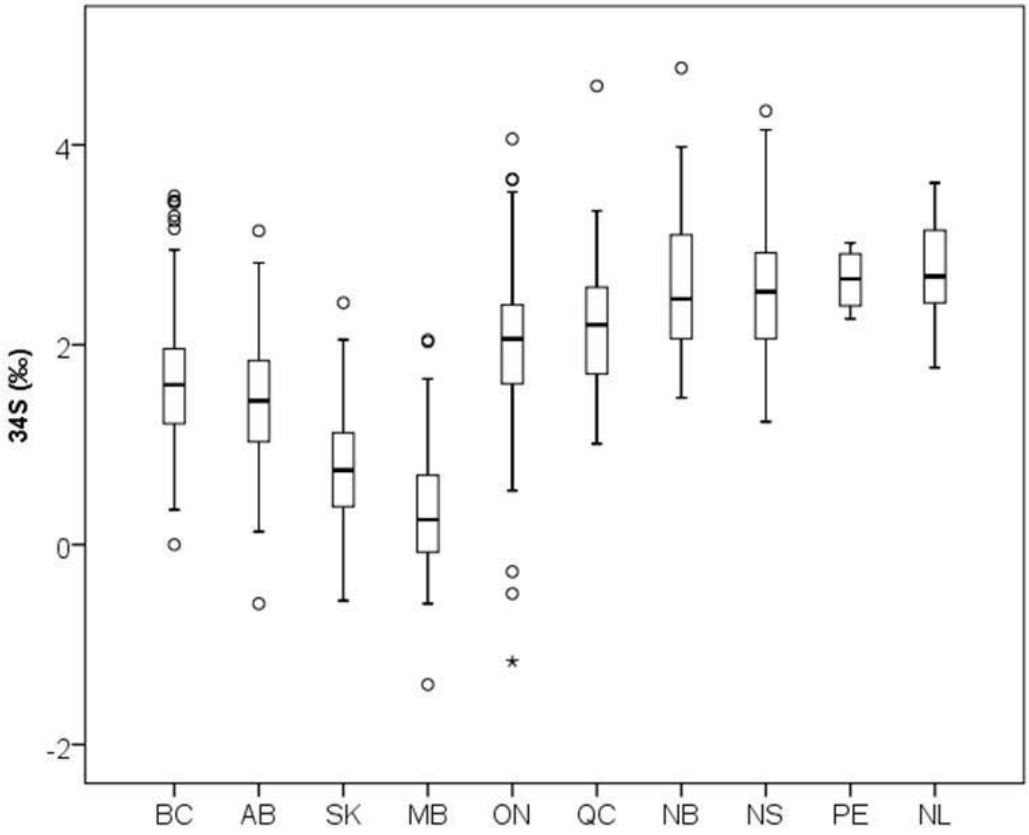

Figure 21. Box plot of $\delta^{34}S$ values for all provinces. The solid black line inside the box is the MEDIAN.

From the literature, more positive $\delta^{34}S$ values in hair have been observed in persons residing in coastal habitats [2,3,11,12,29]. The $\delta^{34}S$ values in Canadian hair show this same trend – the more positive $\delta^{34}S$ hair values are primarily found in the coastal areas (blue areas on Figure 20), and the less positive $\delta^{34}S$ hair values (yellow and red) are primarily found in inland regions. The same is true for the average $\delta^{34}S$ of hair. The observed geographic pattern may be due to two competing processes:

1) Geolocation – coastal regions receive sulphur deposition from the ocean which is enriched (more positive) in $\delta^{34}S$, and is thus reflected in the $\delta^{34}S$ value in hair.
2) Increased consumption of $\delta^{34}S$ enriched marine protein maybe due to higher availability and dietary habits to coastal areas.

Levene's test and a t-test were performed on the $\delta^{34}S$ values from each province to determine if there is a statistical difference between the provinces. The p-values from the t-tests are presented in Table 29.

Table 29. p-values from t-tests comparing hair $\delta^{34}S$ values from different provinces. A p-value less than 0.05 (highlighted in grey) demonstrates the $\delta^{34}S$ values are significantly different between those provinces. Values in italics represent provinces with unequal variance (Levene's test).

	AB	**SK**	**MB**	**ON**	**QC**	**NB**	**NS**	**PE**	**NL**
BC	0.052	1e-12	1e-13	0.0055	2e-7	3e-11	1e-13	0.0036	2e-8
AB		4e-8	1e-13	1e-4	1e-10	6e-13	1e-13	5e-4	2e-10
SK			0.015	5e-12	1e-13	1e-13	1e-13	6e-7	1e-13
MB				1e-13	1e-13	1e-13	1e-13	4e-8	1e-13
ON					0.13	7e-4	1e-4	0.14	0.0028
QC						0.0020	0.0011	0.13	0.0024
NB							0.83	0.92	0.68
NS								0.85	0.55
PE									0.86

Levene's test shows all comparison groups had equal variance. There were significant differences observed between many provinces. The coastal Maritime Provinces (NB, NS, PE and NL) were not statistically different from each other, and tthey had the most enriched average $\delta^{34}S$ values in Canada. This is in agreement with the literature, were more positive $\delta^{34}S$ values in hair have been observed in persons residing in coastal areas [2,3,11,12,29].

Omnivore vs Vegetarian

The effect of consuming animal protein on hair $\delta^{34}S$ has not been reported in the literature. From the collected Canadian hair, there are only 11 vegetarians, 6 of which do not eat fish/seafood. Table 30a shows the average and range of $\delta^{34}S$ values from Canadian hair.

Table 30a. $\delta^{34}S$ hair values for omnivores and vegetarians.

Group	**Average $\delta^{34}S \pm SD$ (‰)**	**Range $\delta^{34}S$ (‰)**
Omnivores (n=518)	1.7 ± 1.0	-1.4 to 4.8
Vegetarians who ate seafood/fish (n=5)	2.7 ± 1.3	0.8 to 4.0
Vegetarians who ate no seafood/fish (n=6)	1.8 ± 0.9	0.4 to 3.0

The vegetarians who did not eat seafood/fish had a similar average $\delta^{34}S$ value to omnivores. In contrast, vegetarians who ate fish had an enriched $\delta^{34}S$ value, which is consistent with the consumption of marine protein [11, 27, 28]. However, it must be noted that for this analysis, the amount of marine protein was not quantified for omnivores and vegetarians. In fact, there were

many omnivores who consumed seafood/fish, and many that did not. Further, this analysis did not take geolocation into account, which can also influence the $\delta^{34}S$ values. As such, it is difficult to draw any conclusions concerning the effect of diet choices on $\delta^{34}S$ values.

Due to the low number of vegetarians in our study, both groups of vegetarians (i.e. those who ate seafood/fish and those who did not) were combined for statistical analysis (see Table 30b for $\delta^{34}S$ average and range for the combined vegetarian group).

Table 30b. $\delta^{34}S$ hair values for the combined vegetarian group.

Group	Average $\delta^{34}S \pm SD$ (‰)	Range $\delta^{34}S$ (‰)
Vegetarians (n=11)	2.2 ± 1.1	0.4 to 4.0

Levene's test and a t-test were performed on the $\delta^{34}S$ values from omnivores and vegetarians. A p-value (from the t-test) of less than 0.05 demonstrates that the two sets of data analyzed are statistically different at a 95% confidence interval.

Figure 22 shows the box plots for vegetarians (n=11) and omnivores (n=518) for $\delta^{34}S$. The mean of the data is indicated on the graph, but it is NOT represented by the solid middle line in the box (which is the median).

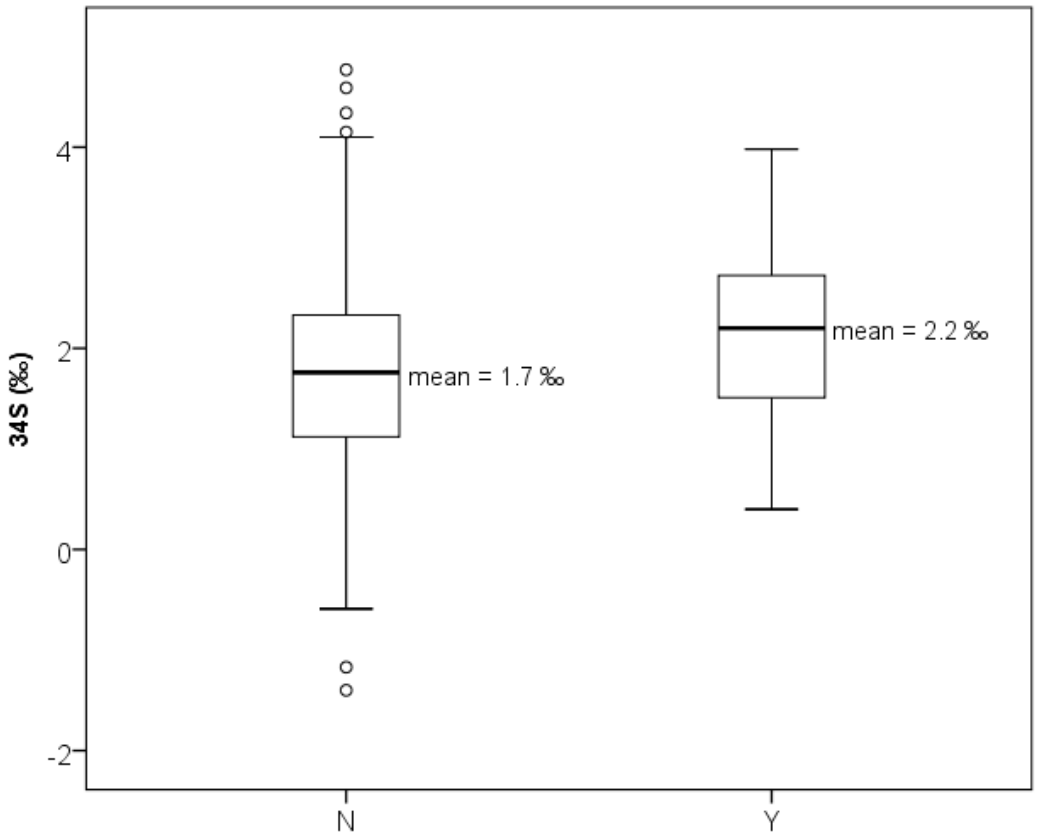

Figure 22. Box plot of $\delta^{34}S$ values for omnivores (N) and vegetarians (Y). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

Levene's test shows equal variances for the two groups compared. Interestingly, the p-value was 0.13, meaning the $\delta^{34}S$ values from omnivores are not statistically different than the $\delta^{34}S$ values from vegetarians, despite the difference in means.

As was observed for $\delta^{13}C$ and $\delta^{15}N$, trends in the average $\delta^{34}S$ values were apparent. However, the $\delta^{34}S$ values may also be influenced by geolocation, which is not necessarily taken into account with diet information (i.e. vegetarians or omnivore). Further, there is overlap in the $\delta^{34}S$ values of omnivores and vegetarians. Because of this, vegetarian diet to an unknown hair sample based on the $\delta^{34}S$ values of hair cannot be definitely ascertained.

Marine Protein Consumption

The same analysis concerning the effect of marine protein consumption on the isotope value of Canadian hair was performed for $\delta^{34}S$. Of the people who consumed seafood/fish, the quantity consumed was rated based on their reported average intake of seafood/fish according to the following scale:

Table 31a: Rating system to quantify seafood/fish consumption, and $\delta^{34}S$ values for each rating group.

Rate	Quantity of Seafood/Fish consumed	n	Average $\delta^{34}S \pm SD$ (‰)	Range $\delta^{34}S$ (‰)
0	Never	77	1.2 ± 0.9	-1.4 to 3.5
1	0.1 to 3 portions per month	163	1.5 ± 0.9	-1.2 to 4.8
2	1 to 1.9 portions per week	160	1.9 ± 1.0	-0.5 to 4.2
3	2 to 3.9 portions per week	103	2.2 ± 0.8	0.1 to 4.1
4	4 to 6.9 portions per week	18	2.0 ± 0.7	0.8 to 3.3
5	7+ portions per week	8	2.6 ± 1.4	0.8 to 4.6

In general, there is a trend of more positive $\delta^{34}S$ values with increasing consumption of marine protein. However, this is not true for Rate 4 (4-6.9 portions per week), which has a lower average $\delta^{34}S$ value than Rate 3 (2-3.9 portions per week). This may be due to the small number of participants in this group, or due to geographic variability, or a combination of both.

Since groups 4 and 5 had few participants, they were combined for statistical analysis (see Table 31b for $\delta^{34}S$ average and range for combined group).

Table 31b: Rating system to quantify seafood/fish consumption, and $\delta^{34}S$ values for combined Rates 4 and 5 group.

Rate	Quantity of Seafood/Fish consumed	n	Average $\delta^{34}S \pm SD$ (‰)	Range $\delta^{34}S$ (‰)
Combined group 4/5	4+ portions per week	26	2.2 ± 1.0	0.8 to 4.6

Combined Group Rate 4/5 (4+ portions per week) had the same $\delta^{34}S$ value as Rate 3 (2-4 portions per week). Not taking geolocation into account, this suggests that the consumption of 2 or more servings of seafood/fish per week will, on average, result in a more enriched $\delta^{34}S$ hair value.

Levene's test and a t-test were performed on the $\delta^{34}S$ values for each rate of seafood/fish consumption. The p-values from the t-tests are presented in Table 32. A p-value of less than 0.05 demonstrates that the two sets of data analyzed are statistically different at a 95% confidence interval. Figure 23 shows the boxplot for $\delta^{34}S$ for the different rates of seafood/fish consumption.

Table 32. p-values from t-tests comparing hair $\delta^{34}S$ values from participants consuming different amounts of seafood/fish. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{34}S$ values are significantly different between the different groups.

Group	1	2	3	4/5
0	0.005	7e-8	2e-13	3e-6
1		7e-4	3e-8	2e-3
2			0.019	0.19
3				0.98

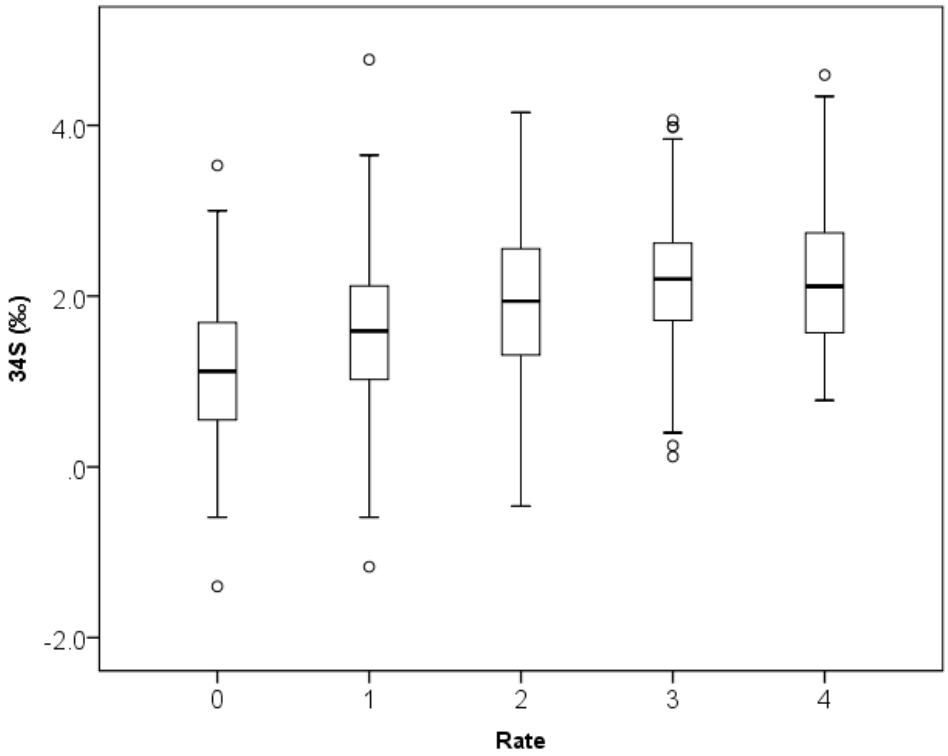

Figure 23. Box plot of $\delta^{34}S$ values for different rates of marine protein consumption. See text for details. Rate 4 is the combined group of Rates 4/5. The solid black line inside the box is the MEDIAN.

Levene's test shows equal variances for all compared groups. Rate groups 0, 1 and 2 were not significantly different from each other. Not taking geolocation into account, this suggests that the consumption of less than 2 portions of marine protein per week does not significantly affect the $\delta^{34}S$ value of hair. In contrast, Combined Group 4/5 was significantly different from Rate groups 2 and 3. Again, disregarding geolocation, this suggests that increasing marine protein consumption to 4 + times per week can significantly enrich $\delta^{34}S$ value of hair. However, the effect of geographic location cannot be ignored, and it cannot be conclusively determined that exclusively increasing the consumption of marine protein can result in a more enriched $\delta^{34}S$ hair value.

Gender

$\delta^{34}S$ values for males and females were analyzed to determine if there was a difference in the average isotope values between these two groups. Levene's test and a t-test were performed on the $\delta^{34}S$ values from males and females. The p-value from the t-test, along with the averages and standard deviations for each group are presented in Table 33, and the box plot is shown in Figure 24.

Table 33. Average and standard deviation, and p-value from t-test comparing hair $\delta^{34}S$ values between males and females. A p-value less than 0.05 (highlighted in grey) indicates a significant difference between the two groups.

Isotope	Average ± SD (‰) for Male	Average ± SD (‰) for Female	p-value
$\delta^{34}S$	1.7 ± 0.10	1.8 ± 1.0	0.66

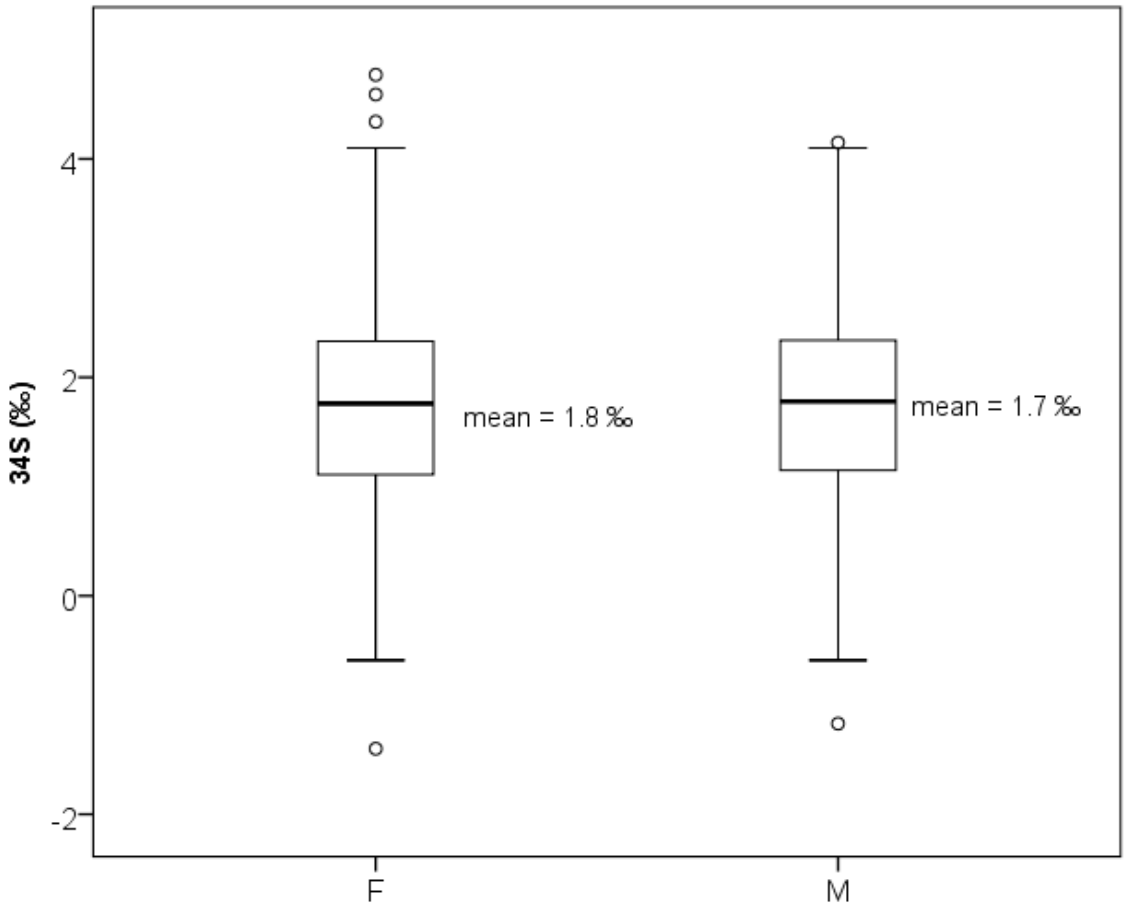

Figure 24. Box plot of $\delta^{34}S$ values for females (F) and males (M). The solid black line inside the box is the MEDIAN. MEANS are presented in text on the graphs.

The mean $\delta^{34}S$ values of males and females were similar to each other, suggesting that gender does not affect the $\delta^{34}S$ values of hair

Levene's test shows equal variances for the two groups compared. The p-value for $\delta^{34}S$ was greater than 0.05, demonstrating that there is no statistical difference between the $\delta^{34}S$ values of

hair from males and females. Further, due to the overlapping range of $\delta^{34}S$ values for males and females, $\delta^{34}S$ values are not useful to determine the gender of an unknown hair donor.

Smoker vs. Non-Smoker

$\delta^{34}S$ values for smokers and non-smokers were analyzed to determine if smoking could significantly affect the $\delta^{34}S$ values for hair. This question was not asked during the collection campaign in Atlantic Canada (2009), resulting in less data points.

Levene's test and a t-test were performed on the $\delta^{34}S$ values for smokers and non-smokers. The p-value from the t-test, along with the average and standard deviation for each group are presented in Table 34, and the boxplot is shown in Figure 25.

Table 34. Average and standard deviation, and p-value from t-test comparing hair $\delta^{34}S$ values between smokers and non-smokers. A p-value less than 0.05 (highlighted in grey) indicates a significant difference between the two groups.

Isotope	Average ± SD (‰) for Smoker	Average ± SD (‰) for Non-Smoker	p-value
$\delta^{34}S$	1.5 ± 0.9	1.3 ± 1.0	0.14

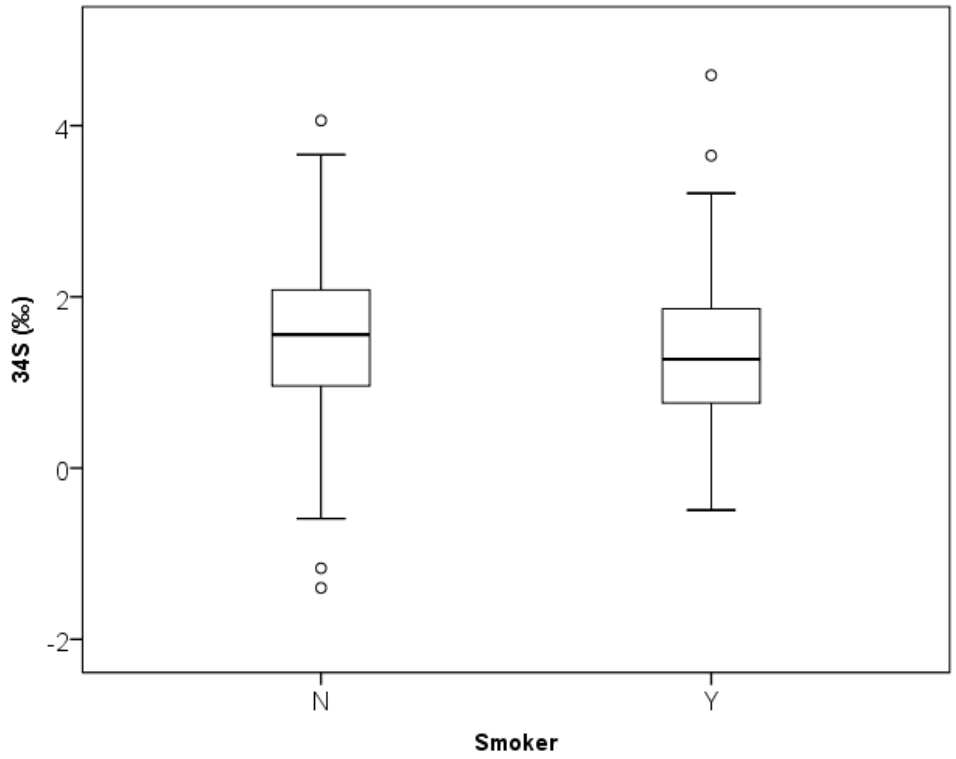

Figure 25. Box plot of $\delta^{34}S$ values for smokers (Y) and non-smokers (N). The solid black line inside the box is the MEDIAN.

The average $\delta^{34}S$ hair values for smokers and non-smokers were similar, suggesting that smoking does not affect the $\delta^{34}S$ value of hair.

Levene's test shows equal variances for the two groups compared. The p-value was greater than 0.05, confirming that there is no statistical difference between the $\delta^{34}S$ values of hair from

smokers and non-smokers. Further, due to the overlapping range of $\delta^{34}S$ values for smokers and non-smokers, $\delta^{34}S$ values are not useful to determine if an unknown hair donor smoked.

Age

Statistical analysis was performed on $\delta^{34}S$ hair values from participants of different ages to determine if different age categories showed different $\delta^{34}S$ hair values. The age of the participant was divided into several categories. The age ranges, average $\delta^{34}S$ values and range are shown in Table 35.

Table 35: Age ranges and $\delta^{34}S$ values for each age group.

Age Range	N	Average $\delta^{34}S \pm SD$ (‰)	Range $\delta^{34}S$ (‰)
18-29	77	1.7 ± 0.9	-0.4 to 4.3
30-39	118	1.7 ± 0.9	-1.2 to 3.7
40-49	163	1.6 ± 1.0	-0.6 to 4.8
50-59	131	1.9 ± 1.0	-1.4 to 4.1
60-69	22	1.7 ± 1.0	-0.4 to 3.4
70+	18	2.1 ± 1.0	0.0 to 4.2

All the age ranges showed similar average $\delta^{34}S$ hair values, except for the 70+ category. This may suggest that people 70+ make different dietary choices than younger people.

Levene's test for equal variance and a t-test were performed on the $\delta^{34}S$ values from the different age categories. The p-values from the t-tests are presented in Table 36, and the boxplot is shown in Figure 26.

Table 36. p-values from t-tests comparing hair $\delta^{34}S$ values from people of different age groups. A p-value less than 0.05 (highlighted in grey) shows the $\delta^{13}C$ values are significantly different between those age groups.

Age	30-39	40-49	50-59	60-69	70+
18-29	0.88	0.83	0.081	0.78	0.066
30-39		0.94	0.032	0.70	0.052
40-49			0.022	0.69	0.065
50-59				0.44	0.44
60-69					0.24

Levene's test shows equal variance for all compared groups. The only group which showed a statistically significant difference is age group 50-59, which was statistically different than the age groups 30-39 and 40-49. These differences may suggest these age groups may choose different food options, or perhaps live in different geographic regions. However, since the range of $\delta^{34}S$ values for all groups overlap; individual measurements of hair $\delta^{34}S$ values are not useful in determining the age of an unknown hair donor.

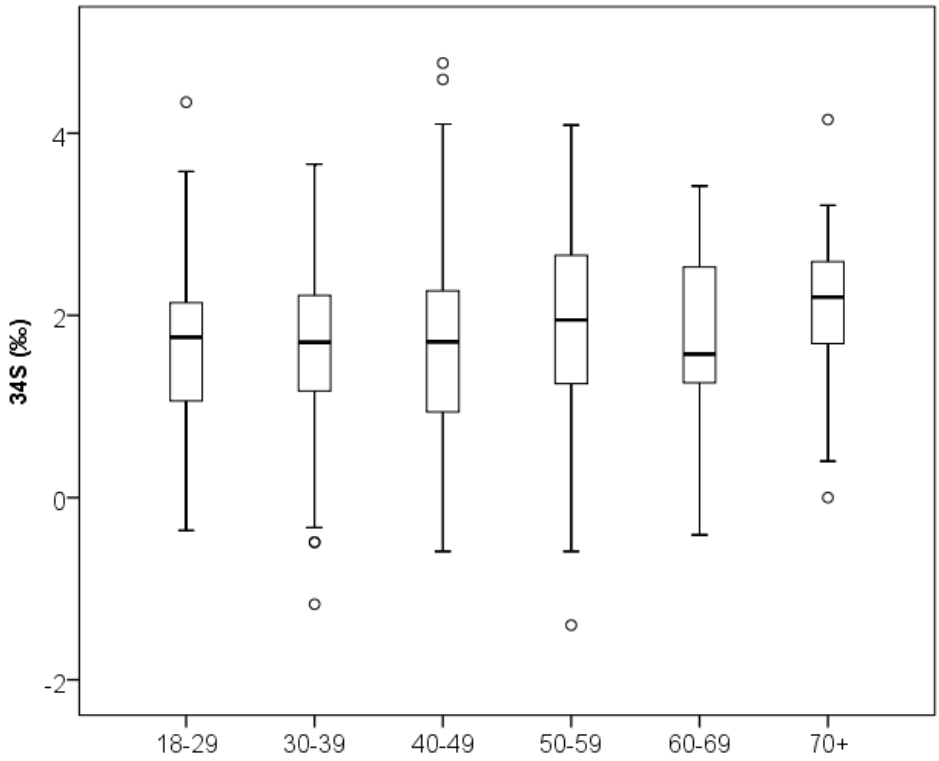

Figure 26. Box plot of $\delta^{34}S$ values for all age ranges. The solid black line inside the box is the MEDIAN.

Hydrogen and Oxygen

Verification of $\delta^{2}H$ and $\delta^{18}O$ Analysis

At the time, no international standards existed that were suitable to analyze $\delta^{2}H$ for hair. Four in-house hair standards were prepared for this project, specifically for $\delta^{2}H$ analysis. Details on the preparation are in APPENDIX 1.

In order to verify that the $\delta^{2}H$ values for the four in-house hair standards were accurate, aliquots were sent to two other laboratories for verification. These results were published in Rapid Communications in Mass Spectrometry in 2011 [34]. Table 37a shows the results of the comparison. Analytical error is ± 2 ‰.

Table 37a. Comparison of $\delta^{2}H$ values for the four in-house hair standards between three labs.

Isotope Standard	$\delta^{2}H$ (‰) U of Ottawa	$\delta^{2}H$ (‰) UK	$\delta^{2}H$ (‰) USGS
Hair- AND (G737)	-71.6	-72.9	-70.4
Hair- COL (G738)	-88.8	-88.8	-87.8
Hair- CAL-CAN (G739)	-106.8	-105.9	-109.8
Hair- CAL-SAL (G740)	-102.1	-101.0	-102.9

The results verified that the method used for $\delta^{2}H$ hair analysis was both accurate and precise.

Details of $\delta^{18}O$ analysis can be found in APPENDIX 1. In order to verify that our procedure for analyzing for $\delta^{18}O$ was accurate, aliquots of our 4 in house hair standards were sent to UC Davis for analysis. Table 37b shows the results of the comparison. Analytical error is ± 0.4 ‰.

Table 37b. Comparison of $\delta^{18}O$ values for the four in-house hair standards.

Isotope Standard	$\delta^{18}O$ (‰) U of Ottawa	$\delta^{18}O$ (‰) UC Davis
Hair- AND (G737)	12.2	11.9
Hair- COL (G738)	9.1	9.0
Hair- CAL-CAN (G739)	6.9	7.0
Hair- CAL-SAL (G740)	6.0	6.1

The results verified that the method used for $\delta^{18}O$ hair analysis was both accurate and precise.

Hydrogen Isotope Values Across Canada

$\delta^{2}H$ analysis of hair can be used as a geolocation tool and has been shown to track movement of a person as well as to determine where a person has been living [1,12,19-24,34]. $\delta^{2}H$ values of hair are related to the $\delta^{2}H$ of water from the location where they reside. Since the $\delta^{2}H$ value of water varies with latitude and altitude (See Water Report above), and people tend to cook and consume their local water, the isotopic signals found in water are reflected in the isotopic signals found in hair.

$\delta^{2}H$ analysis was performed on 569 hair samples. $\delta^{2}H$ values spanned 53 ‰, ranging from -68.0 to -120.9 ‰. Ehleringer et al [19] showed a similar span of 45 ‰ for $\delta^{2}H$ values from hair collected in the US. However, the range of hair cannot be compared, as this group (U. of Utah) sent us an e-mail on November 3 2008, stating that the accuracy of these analyses may be questionable. The e-mail is pasted below:

"Recently, we have started an in-depth recalibration effort for two of our laboratory reference materials used during H&O stable isotope analysis, Florida Horsehair (FH) and Utah Horsehair (UH). The original calibration effort for these materials was completed in 2005, which is partly documented in the Bowen et al. 2005 Rapid Communications in Mass Spectrometry publication.
Because we've previously analyzed samples for you, we feel the need to caution you when the interpreting absolute values of the data we've provided to you. Relative comparisons (i.e. the difference in δ^2 H values between unknowns analyzed at the University of Utah) among samples are correct, but these values may be off in an absolute sense, if we determine that the hydrogen and oxygen isotope ratios of FH and UH are different than we previously thought. The absolute values may not be and, therefore, should not be compared to hydrogen data produced by another lab until we conclude what values to assign to FH and UH.
As we progress in the recalibration effort, we will continue to send updates. If we find the values for FH and UH should be redefined, we will re-evaluate all data analyzed for you. If you have any questions on these issues, please do not hesitate to contact us."

NOTE: After repeated requests, this lab has never returned the results of their recalibration and thus forced us to move on with other groups for standardisations checks.

In addition, two hair samples were analyzed that were not from the 10 Canadian provinces: one from Iqaluit, and the other from Weatherby, UK. The sample from Iqaluit had a δ^2H value of -83.0 ‰, which is more positive than expected from the arctic. The hair sample from Weatherby, UK had a δ^2H value of -67.0 ‰, which is consistent with the other UK hair sample analyzed at -71.6 ‰; which is the in house hair standard AND (G737)) from Manchester, UK.

Figure 27 shows a plot of δ^2H of hair vs. δ^2H of ground water (TG) and δ^2H of surface water (TS).

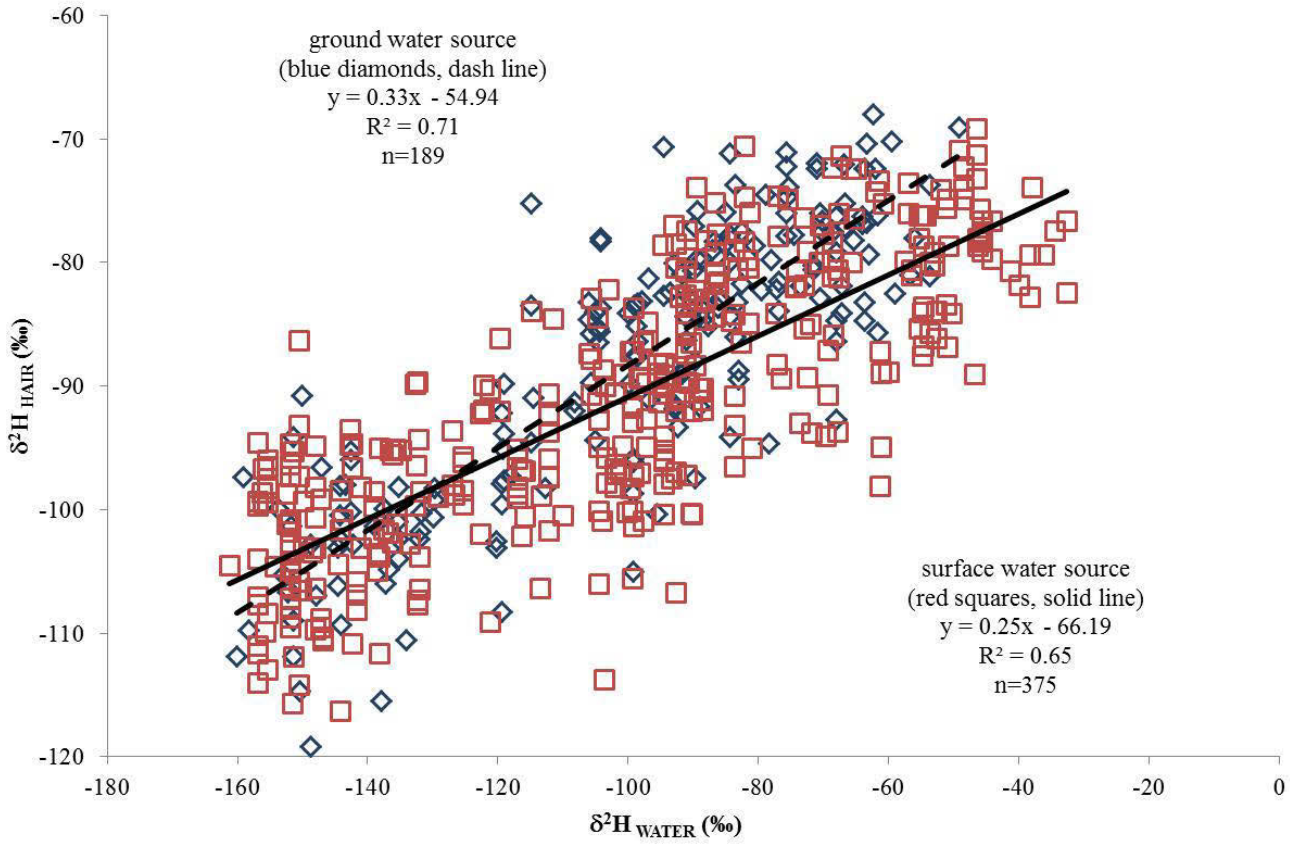

Figure 27. Correlation of δ^2H in hair to δ^2H of water (ground water (TG) = blue diamonds and surface water (TS) = red squares) from Canadian samples.

From a first order approximation, the slope can be used to estimate the percentage of the water δ^2H value that is expressed in the hair δ^2H value. From Figure 27 it is observed that 25% and 33% of the hydrogen water signal from surface and ground water, respectively, is reflected in hair. The remaining 67-75 % of the hair δ^2H signal is from other, "non-geographical" sources such as food or drinks.

Figure 28 shows a plot of δ^2H of hair vs. δ^2H of all water, regardless of water source. In total, there were 564 hair-water pairs.

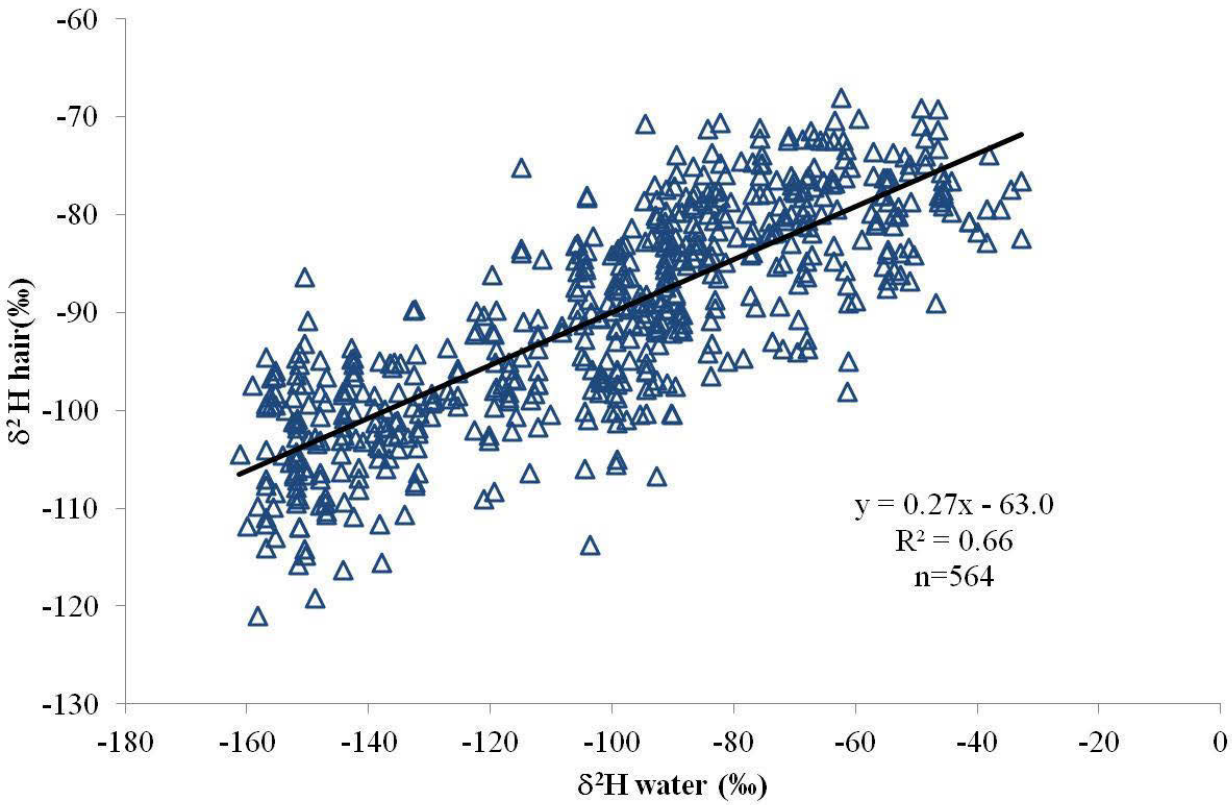

Figure 28. Correlation of δ^2H in hair to corresponding δ^2H in water

Taking all water together, regardless of source, there is ~27 % of the water δ^2H signal reflected in hair, with ~ 73% of the δ^2H signal in hair coming from non-geographical sources.

Although there are differences in the slopes, from the perspective of analyzing unknown samples, it is best to use the relationship between hair and all water, and not have it divided into ground and surface water.

The estimate of 27% is similar to what others have reported in the literature: 40-49% [21], 42% [29], 36% [23], 31% [24] and 27% [19], and it is identical to what was measured from Phase I of our project.

The correlation of δ^2H in water to that in hair is only 0.66. There is no doubt that there is a correlation between δ^2H in water and in hair. Human variability is the most likely reason for the calculated correlation. While there is good reproducibility of δ^2H in hair collected from most locations, there were some locations where a large scatter of δ^2H in hair was observed. Those locations are presented in Table 38. Only cases where the difference in δ^2H between all the hairs sampled exceeded 5 ‰ (1.25 times the analytical error of ± 2 ‰) are presented.

Table 38. Variability observed in δ^2H of hair sampled from the same location.

City	Pv.	δ^2H Hair (‰)	City	Pv.	δ^2H Hair (‰)
Cornerbrook	NL	-80.0, -94.0	Peace River	AB	-109.8, -120.9
Chéticamp	NS	-76.3, -92.7	Athabasca	AB	-95.3, -106.6, -111.9
Fredericton	NB	-71.2, -77.8, -80.0	Bonnyville	AB	-88.3, -97.5, -111.9
Centre	NS	-73.7, -81.1	Medicine Hat	AB	-94.6, -96.6, -110.8
Ingonish	NS	-72.0, -76.5, -78.2, -84.1	Grande Prairie	AB	-99.3, -104.0, -107.0, -107.7, -111.6, -114.0
Lebel-Sur-Quevillon	QC	-80.7, -82.6, -83.3, -83.3, -85.6, -85.1, -86.9, -86.9 -89.6	Fort McMurray	AB	-96.5, -96.0, -96.6, -98.3 -99.4, -108.4, -112.9
Montreal	QC	-74.9, -84.0, -87.5	Chase	BC	-95.6, -102.6
Roberval	QC	-84.5, -91.3	Hanna	AB	-89.6, -107.3, -107.6
Val D'or	QC	-70.7, -82.7	Port McNeill	BC	-84.2, -93.1
Thetford Mines	QC	-88.2, -100.4	Fort St. John	BC	-94.5, -111.0
Amos	QC	-78.0, -83.7	Campbell River	BC	-87.2, -97.2
Rouyn-Noranda	QC	-78.2, -83.0, -84.7	Red Deer	AB	-94.8, -98.2, -99.3, -100.6, -103.0, -103.1, -106.4, -109.7
Rawdon	QC	-76.2, -84.9	Burns Lake	BC	-98.1, -98.9, -100.4, -100.5, -107.0
Waswanipi	QC	-85.6, -94.4	Sardis	BC	-87.7, -95.9, -96.2, -98.7 -105.0
Alma	QC	-82.9, -87.8, -90.7	Vancouver and area	BC	-86.4, -94.8, -95.8, -96.9 -97.8, -98.3
Dupuy	QC	-80.1, -91.8	Tofino	BC	-87.2, -98.1
La Malbaie	QC	-80.9, -89.0	Comox & Courtenay	BC	-91.6, -91.8, -92.9, -100.1, -105.5
Kenora	ON	-78.5, -80.6, -85.9, -93.7	Kelowna	BC	-90.6, -96.7, -98.1, -101.3
Toronto and area	ON	-74.1, -75.6, -78.7, -79.2, -80.2, -83.4, -83.9, -86.1, -86.8, -89.0	Edmonton and area	AB	-86.3, -93.1, -94.7, -96.3 -97.3, -102.8, -104.7, -105.0, -105.1,-105.8, -107.8, -109.5, -108.7, -109.5
Windsor	ON	-76.2, -78.7, -86.7	Merritt	BC	-89.8, -93.8, -97.6
Goulais River	ON	-75.2, -83.5, -94.6	Port Alberni	BC	-83.0, -92.0, -100.4
North Bay	ON	-72.3, -78.3, -79.9, -81.1	Lethbridge	AB	-95.0, -98.1, -103.1, -103.8, -111.6
Elliot Lake	ON	-79.1, -89.3	Prince George	BC	-95.2, -106.4
Geraldton	ON	-78.9, -90.8, -96.5	Prince Rupert	BC	-88.4, -91.1, -92.0, -96.9 -106.7

City	Pv.	δ^2H Hair (‰)	City	Pv.	δ^2H Hair (‰)
Nipigon	ON	-79.3, -80.9, -84.4	Revelstoke	BC	-98.4, -101.6, -103.6, -116.3
Dauphin	MB	-88.2, -89.1, -90.1, -94.0, -95.2, -95.9, -96.1, -97.9, -100.3	Calgary	AB	-97.3, -100.8, -101.0, -101.7, -103.4, -106.2, -107.2
Thompson	MB	-90.6, -92.2, -92.4, -93.7, -95.1, -95.9, -97.3, -97.4, -97.5, -101.7	Williams Lake	BC	-92.2, -96.6, -97.9, -99.6 -108.2
Weyburn	SK	-92.7, -94.9, -100.1, -106.0	Vernon	BC	-82.8, -89.4, -90.9, -91.3
Meadow Lake	SK	-113.7, -100.9			

While a correlation of 0.66 is generally acceptable for biological samples, it may not be useful as a prediction tool for geographic location. The original hypothesis for this project was to use the water δ^2H value to predict what the δ^2H value of hair might be for a given location. To do this, a strong linear correlation is required between δ^2H of water and δ^2H of hair. Based on the correlation factor of 0.66, this would not provide sufficient resolution to accurately predict an area. Instead, the direct comparison of δ^2H of hair from the person of interest to δ^2H values of hair from the database is the more powerful geo-location tool.

It has been established that the δ^2H value of tap water collected from several provinces across Canada varies with location. Figure 29 shows how the δ^2H value of hair varies across Canada.

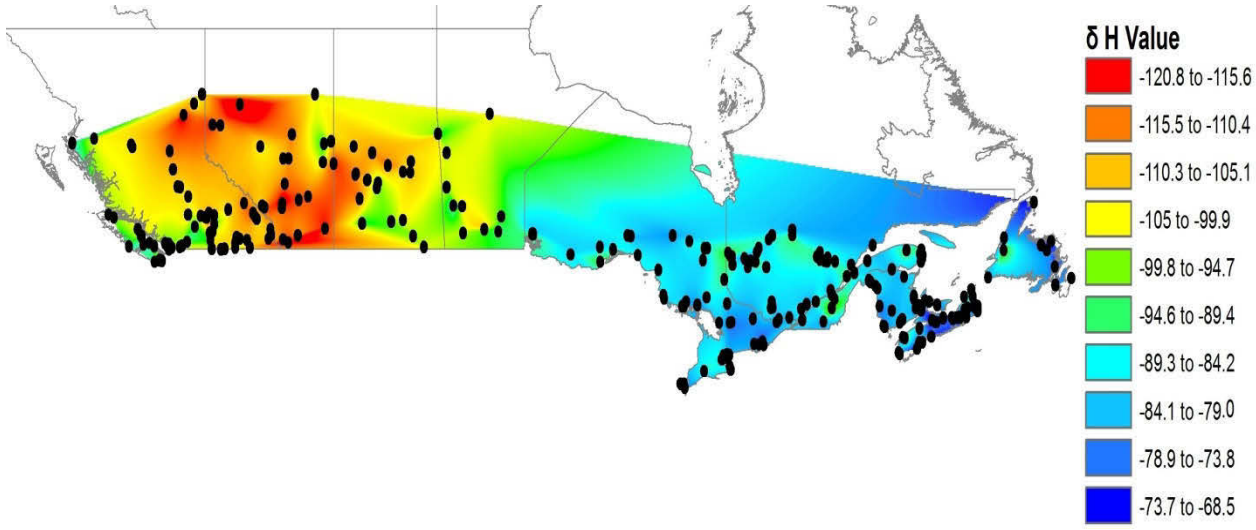

Figure 29. δ^2H values from hair collected across Canada.

Based on Figure 29, there is a pattern in the δ^2H values of hair that correlates with the distribution of δ^2H values for tap water across Canada. For ON, QC and Atlantic Provinces, the δ^2H values of hair varies with latitude; where the δ^2H values of hair become more negative with increasing latitude. The δ^2H values of hair for MB, SK, AB and BC are significantly more negative than that

for ON, QC or the Atlantic Provinces. In the west, the geographical variation is more longitudinal. This is due to an altitude effect from the Rockies, which creates an effect that mimics their North-South direction.

A statistical analysis of δ^2H values by province (as was performed for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) to determine if the δ^2H values of hair in different provinces were statistically different would not be meaningful. The δ^2H value of Canadian hair varies primarily with latitude in the east, and with longitude in the west. Because of the two different effects on δ^2H values, provincial borders are not ideal for dividing Canada into regions. Any other kind of divisions would be arbitrary, or would be based on δ^2H values, thus making a statistical analysis circular. In addition, because δ^2H values of hair are linked to geolocation, it is difficult to determine if other variables such as vegetarianism, marine protein consumption, gender, smoking and age will affect the δ^2H values of hair. In order to properly evaluate these parameters, a static sample of sufficient quantity must be taken from the same geolocation. As such, no further statistical analyses was performed on δ^2H values from hair in Canada.

Oxygen Isotope Values Across Canada

As has been shown for δ^2H, $\delta^{18}O$ values of hair are linked to the $\delta^{18}O$ value of water [19-21,23,24]. Since the $\delta^{18}O$ value of water varies with latitude and altitude (See Water Report above), and people tend to cook and consume their local water, the isotopic signals found in water are reflected in the isotopic signals found in hair.

$\delta^{18}O$ analysis was performed on 563 hair samples. $\delta^{18}O$ values spanned 11 ‰, ranging from 2.2 to 13.1 ‰. Ehleringer et al [19] showed a slightly smaller span of 8.5 ‰, with a range of 7.4 to 15.9 ‰ for $\delta^{18}O$ values from hair collected in the US. This is not unexpected for Canada as we are closer to the arctic circle, which would result in less positive $\delta^{18}O$ values.

The two samples collected which were not from the 10 Canadian provinces were also analyzed for $\delta^{18}O$. The Iqaluit sample had a $\delta^{18}O$ value of 10.0 ‰, which is in-line with what is expected for a sample from the Arctic. The Weatherby, UK sample had a $\delta^{18}O$ value of 13.2, which is more positive than all of the Canadian hair, but consistent with the other UK hair sample analyzed (12.2 ‰; in house hair standard AND (G737)), who was from Manchester, UK.

However, it was determined that the dyeing of hair significantly affects its $\delta^{18}O$ value. As such, only hair that is not dyed can be reliably used in the database. For the sampling campaign in ON and QC (2008), this question was not asked on the questionnaire. Therefore, any samples collected during that sampling campaign cannot be used to populate the $\delta^{18}O$ hair database. In total, 257 hair samples, ranging from 4.6 to 13.1 ‰, were used to populate the $\delta^{18}O$ hair database.

Figure 30 shows a plot of $\delta^{18}O$ of hair vs. that of all water. TS were not distinguished from TG. In total, there were 257 hair-water pairs.

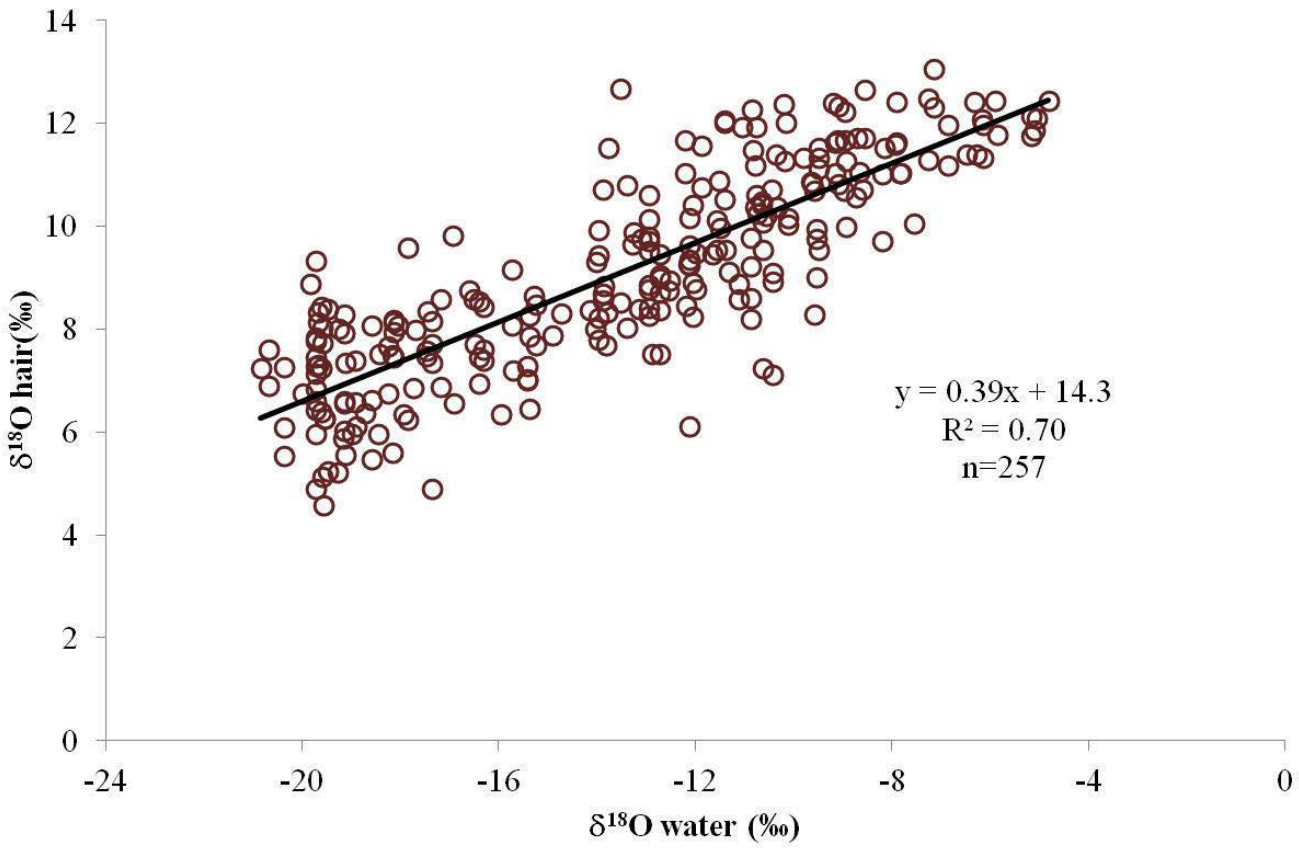

Figure 30. Correlation of $\delta^{18}O$ in hair to corresponding $\delta^{18}O$ in water.

Taking all water together, regardless of source, there is 39 % of the water $\delta^{18}O$ signal reflected in hair, with 61% of the $\delta^{18}O$ signal coming from non-geographical sources. The estimate of 39% is similar to what others have reported in the literature: 40% [29], 35% [19] and 27% [23].

The correlation of $\delta^{18}O$ in water to $\delta^{18}O$ in hair is 0.70, which is similar to that found for $\delta^{2}H$ (0.66). As is true for $\delta^{2}H$, there is no doubt that there is a correlation between $\delta^{18}O$ in water and $\delta^{18}O$ in hair. The scatter is likely due to human variability. The locations with variability exceeding 1 ‰ (1.25 times the analytical error of ± 0.4 ‰) are presented in Table 39.

Table 39. Variability observed in $\delta^{18}O$ of hair sampled from the same location.

City	Pv.	$\delta^{18}O$ Hair (‰)
Castlegar	BC	5.5, 6.6, 8.1
Chase	BC	6.9, 8.6
Comox and Courtenay	BC	8.0, 9.6
Vancouver area	BC	7.5, 8.0, 8.3, 8.5, 8.7, 9.1, 9.8, 9.9, 10.0
Fort St. John	BC	6.6, 7.8
Kamloops	BC	7.5, 7.9, 8.9
Merritt	BC	6.5, 7.9, 8.3
Nelson	BC	6.9, 8.0
Prince Rupert	BC	8.2, 8.9, 10.4
Vernon	BC	9.5, 9.8, 9.9, 11.1
Athabasca	AB	5.6, 7.3
Calgary	AB	6.4, 7.2, 8.0
Edmonton and area	AB	4.9, 5.1, 6.0, 6.4, 6.8, 7.1, 7.3, 7.5, 7.7, 7.8, 8.0, 9.3,
Fort McMurray	AB	6.0. 6.6, 7.9, 8.3
Grande Prairie	AB	5.5, 6.1, 7.3
Medicine Hat	AB	5.6, 7.5
Melfort	SK	6.9, 8.5
Moose Jaw	SK	7.8, 9.3
Prince Albert	SK	4.9, 7.3, 7.7
Regina	SK	8.2, 9.4, 9.9
Rosetown	SK	5.2, 8.4
Dauphin	MB	6.1, 9.3, 9.4, 9.6, 10.2
Flin Flon	MB	7.1, 9.1
Portage La Prairie	MB	7.2, 8.1, 9.2, 9.5, 10.1
Thompson	MB	8.3, 8.4, 8.8, 8.9, 9.5, 9.7, 10.1, 10.6
Sherbrooke	QC	9.8, 11.2
Fredericton	NB	10.5, 12.0, 12.1
Grand Falls	NB	9.5, 10.8
West Branch	NB	10.6, 11.9
Cheticamp	NS	8.3, 10.7
New Glasgow	NS	9.7, 11.0
Port Hawkesbury	NS	11.3, 12.3, 12.5, 13.1

As is true for $\delta^{2}H$, the $\delta^{18}O$ value of tap water collect from several provinces across Canada varies with location. However, because no data for ON and only limited values for QC are available, spatial analysis of $\delta^{18}O$ using GIS was not possible due to the large gap of data in the middle of Canada. Also, for the same reasons as outlined for $\delta^{2}H$, no further statistical analysis was performed.

Hydrogen and Oxygen in Hair

δ^2H and $\delta^{18}O$ values of tap water from across Canada were compared to the CMWL. Similarly, the δ^2H and $\delta^{18}O$ values of hair from across Canada were used to create a Canadian hair line which is shown in Figure 31. Note that only $\delta^{18}O$ values from non-dyed hair is shown.

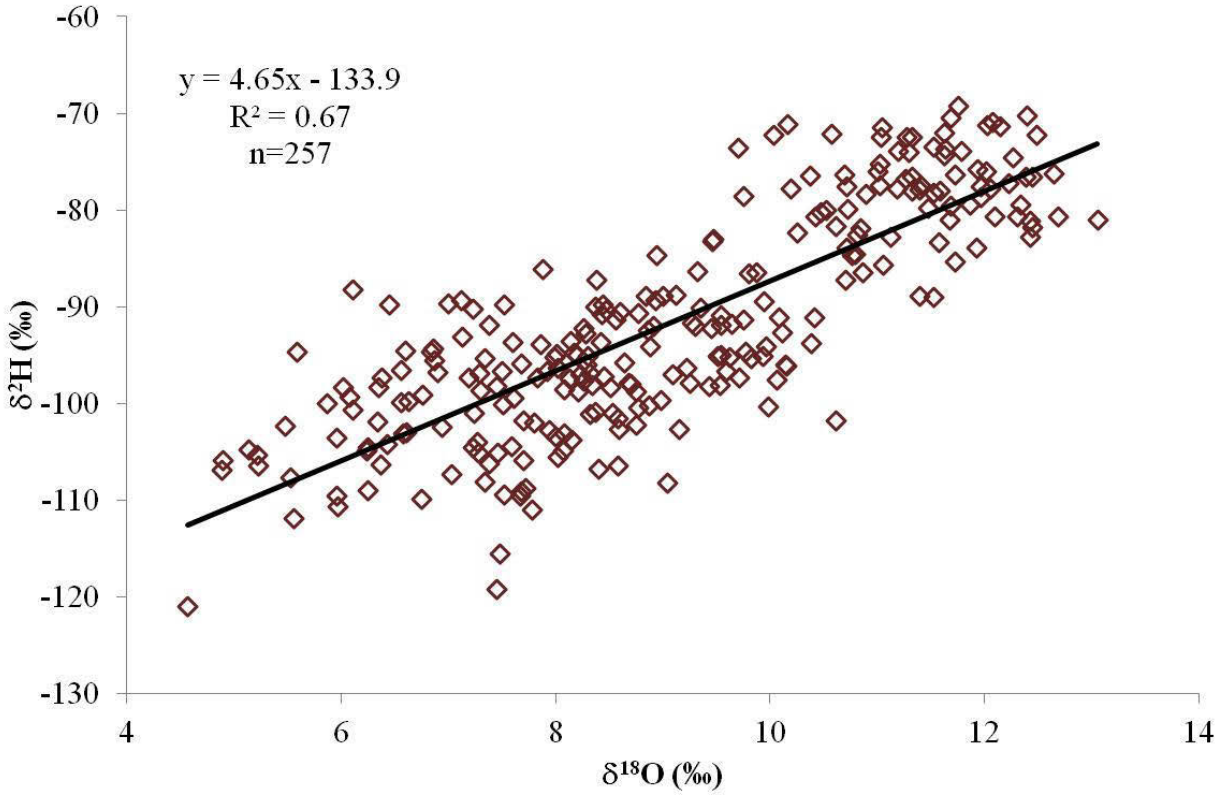

Figure 31. Correlation of δ^2H and $\delta^{18}O$ in hair.

The slope of the Canadian hair line (4.65) is similar to that found for hair from the USA (5.73) [19], and from international hair samples (6.85) [20].

The Canadian hair line was compared to the water line derived from Canadian Tap water in Figure 32. The slope of the tap water line (8.04) is very close to the CMWL slope (7.75) and the GMWL (8.17 ± 0.07) [26]. Figure 32 suggests that the isotopic composition of Canadian tap water is reflected in the isotopic composition of Canadian hair. The differences between the lines may be attributed to non-geographic sources of hydrogen and oxygen which are taken up by the body.

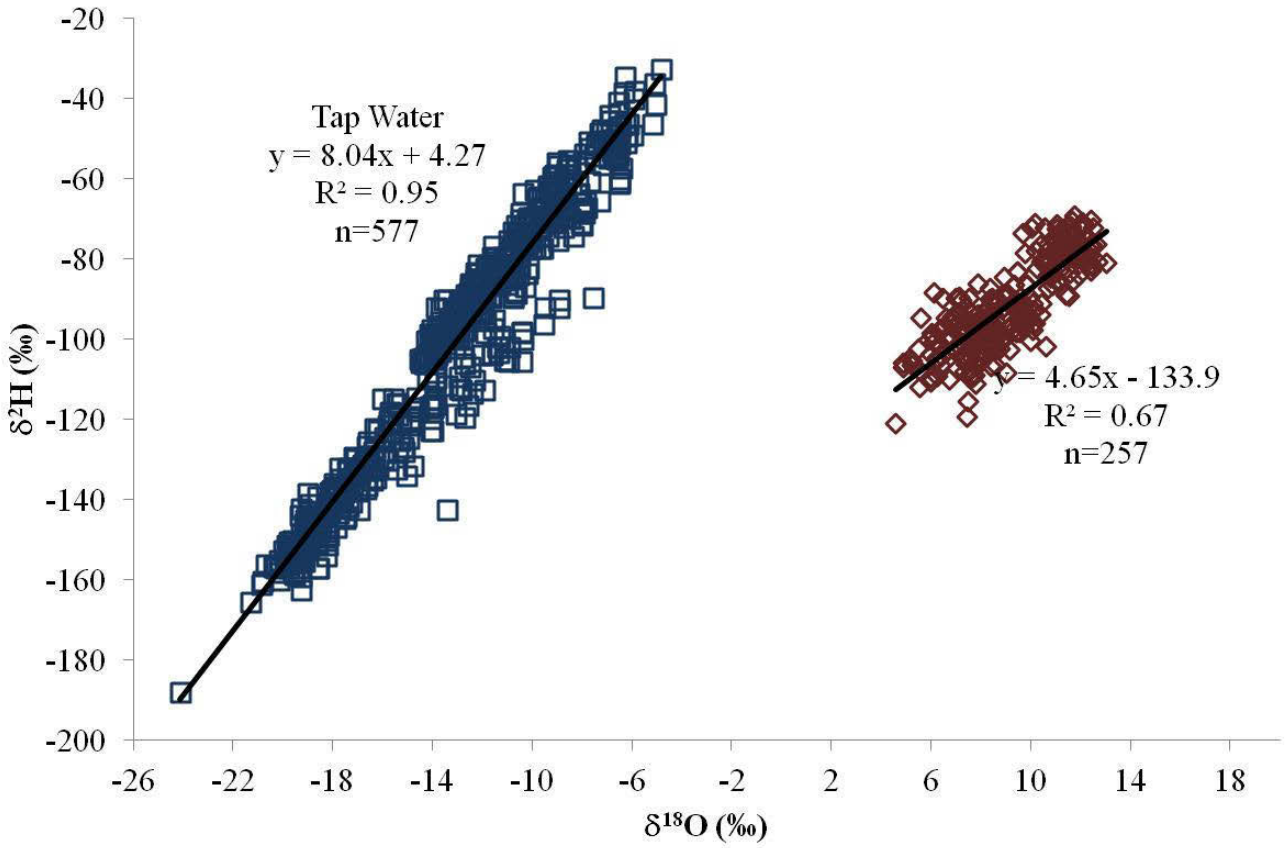

Figure 32. Comparison of Canadian hair line (red diamonds) to water line from Canadian tap water (blue triangles).

Application of Database to Unidentified Hair

Three police cold cases were processed where the database for isotopic values of Canadian hair was used to predict an unidentified person's geolocations in the months prior to their deaths. These cases were analysed during different stages in the creation of the database and thus the interpretation methods for geolocating have evolved. Moving forward, a method is established for interpretation where an extensive database for hair comparison is now available. These cases would benefit from the added analysis of trace elements in order to execute cross-linked comparisons with the isotope data.

The three completed cases are attached to this report in the following PDF files:

2010-11-16-Report – Mme Victoria Case.pdf
2011-10-14-Report – Halifax Case.pdf
2013-07-15 Final Report IHIT File 2012-2454.pdf

Conclusions

The following conclusions can be drawn from this study:

1) The δ^2H and $\delta^{18}O$ values in tap water varied in predictable patterns according to latitude and altitude.

2) Regionally, tap water isotope values deviated from the CMWL, particularly for surface water in areas with high evaporation.

3) The δ^2H and $\delta^{18}O$ values in hair varied across Canada in predictable patterns according to latitude and altitude. This variation makes these isotopes a powerful indication of geolocation.

4) With some exceptions, several tap water samples collected from the same city were reproducible to within ± 4.0 and ± 0.40 ‰ for δ^2H and $\delta^{18}O$, respectively.

5) With few exceptions, the δ^2H and $\delta^{18}O$ values of TS were more positive relative to TG from the same location.

6) The δ^2H and $\delta^{18}O$ values of purchased water (both WC and bottled water) from a city were not necessarily similar to the δ^2H and $\delta^{18}O$ values of tap water from that city.

7) The isotopic composition of tap water is reflected in the isotope composition of hair. From a first order approximation, 27 % of the δ^2H signal and 39 % of the $\delta^{18}O$ signal in water is reflected in the hair isotope value.

8) In some instances, significant differences in hair δ^2H and $\delta^{18}O$ values were observed from persons sampled from the same city. These differences are attributed to human variability.

9) Based on the findings in this report, it is recommended that the **direct comparison** of δ^2H of hair from the person of interest to δ^2H values of hair from the database is a more powerful geo-location tool, rather than predicting hair δ^2H values based on the δ^2H values of tap water.

10) The application of hair dye did not significantly affect the $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and δ^2H values of hair. However, significantly different $\delta^{18}O$ values were measured for dyed hair. Based on this evidence, $\delta^{18}O$ values may not be confidently used for geolocation purposes.

11) Temporal analysis of $\delta^{13}C$, $\delta^{15}N$ and δ^2H values of hair demonstrated that for most participants, these isotope values did not significantly change over the analysis time frame.

12) Some significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of hair samples were noted between provinces. However, the range of differences in these measurements is too small to be used as a geographical indicator.

13) A statistical difference in the $\delta^{13}C$ and $\delta^{15}N$ values of hair samples were demonstrated between omnivores and vegetarians.

14) For $\delta^{13}C$, there was no statistical difference noted for persons with different amounts of marine protein intake. For $\delta^{15}N$, there was a significant statistical difference between persons who consumed less than 4 portions of marine protein per week, and those who consumed less than 4 portions.

15) Some significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of hair samples were noted between gender, smokers and non-smokers, and age. However, the range of these isotope values for these parameters overlapped, and cannot be reliably used for geolocation purposes in Canada.

16) $\delta^{34}S$ values varied with geolocation: more positive $\delta^{34}S$ values were observed in persons residing in coastal habitats, and less positive values were seen in inland regions.

17) There was no significant difference in $\delta^{34}S$ values between omnivores and vegetarians, males and females, smokers and non-smokers. There was a statistical difference between some age ranges. However, the range of $\delta^{34}S$ values for these parameters overlapped, and cannot be used as a reliable geolocation tool.

18) The $\delta^{34}S$ values were significantly different for varying amounts of marine protein consumption. However, the effect of geolocation cannot be ignored, and it cannot be conclusively determined that the significant differences in the $\delta^{34}S$ values can be solely attributed to marine protein consumption.

Recommendations for Future Development

Several recommendations can be made for future developments:

1) Hair should be collected from areas which are lacking in $\delta^{18}O$ information due to the sampling of persons with dyed hair.

2) Hair can be continually collected from areas which are missing in the database such as the Northern Territories and Yukon as well as adding to the existing database from the provinces. This can be achieved by including existing projects with other groups such as biologists, anthropologist and medical professionals; all of which travel throughout Canada.

3) The trace element results can be linked to the isotope results to determine if these two different analyses provide complimentary information. It would be advisable to transfer the techniques developed at PSC to the U of Ottawa geochemistry labs to increase the capabilities of forensic hair analysis in one institution.

4) The pollen database can be exploited to potentially serve as a geolocation tool. This should be done through the creation of either a post-doc or of a post-graduate thesis in collaboration with the U of Montréal partner.

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APPENDIX 1. Sample Preparation for Isotopic Analysis

Water Sample preparation

Add a piece of Cu (to remove any S species which may be present) and a few grains of activated charcoal (to remove any organics) to the vial containing the water sample. Allow the water sample with the added Cu and activated charcoal to sit for 24 hours.

Add 200 μL of the water to an exetainer and cap it with a gas-tight cap.

Calibrated internal standards are prepared with every batch of samples for normalization of the data.

Water Isotopic Analysis

$\delta^{18}O$ analysis

Flush the headspace of the exetainer with 2% CO_2 in He for 4 minutes. Allow the CO_2 and H_2O to equilibrate for 24 hours.

The CO_2 gas is analyzed automatically at a constant temperature in continuous flow using a GasBench II with a Delta^{XP} IRMS. All $\delta^{18}O$ measurements are normalized to VSMOW. The routine precision (2 sigma) of the analysis is ± 0.15‰.

$\delta^{2}H$ analysis

Add a piece of Hoko platinum catalyst to the exetainer containing the water sample. Flush the headspace of the exetainer with 2% H_2 in He for 4 minutes. Allow the H_2 and H_2O to equilibrate for at least 2 hours.

The H_2 gas is analyzed automatically at a constant temperature in continuous flow using a GasBench II with a Delta^{XP} IRMS. All $\delta^{2}H$ measurements are normalized to VSMOW. The routine precision (2 σ) of the analysis is ± 2.0 ‰.

Hair Sample Preparation

Place the hair sample into a 50 ml beaker. Cover the hair with a 2:1 solution of chloroform:MeOH. Gently agitate the solution for 10 mins.

Place a coffee filter in a funnel and pour off the solution, allowing the filter to catch the cleaned hair. Allow the hair sample to dry on the filter. Once the hair sample is dry, store it in a newly labeled clean coin envelope.

Add an appropriate amount of hair to a grinding jar. Add the grinding ball to the grinding jar and close up the other end. Place the capsule on the Retch grinding machine.
NOTE: over-pulverizing resulted in the burning of the sample, and much can be lost.
Pulverize the sample at 30 Hz for 30 sec. and check the sample. If it is not completely pulverized, repeat at 30 Hz for 15 sec. and check the sample again. If it is not completely pulverized, repeat at 30 Hz for 10 sec.

Transfer the pulverized sample to a sheet of folder weighing paper, scraping out as much as possible out of the grinding jar. Store the ground sample in a clean 1 dram vial.

Hair Carbon and Nitrogen Isotopic Analysis

Samples and standards were weighed into tin capsules and loaded onto an Elemental Analyser interfaced to an isotope ratio mass spectrometer. Samples and isotope standards were combusted in an oxygen atmosphere at about 1800°C (Dumas combustion), and the resulting gas products were carried by helium through columns of oxidizing/reducing chemicals optimized for CO_2 ($\delta^{13}C$ analysis) and N_2 ($\delta^{15}N$ analysis). The gases were separated by a "purge and trap" adsorption column and each gas was sent to the IRMS for isotope analysis.

Isotope standards were used to normalise (correct) the sample data to the accepted international scales for each different isotope. These materials were chosen because they cover most of the natural range for carbon and nitrogen containing compounds. One isotope standard was used as a blind standard (i.e. the isotope value is known), as a check to ensure the normalisation was performed properly. Table 1 shows the internal isotope standards used in the normalisation of the samples and their $\delta^{13}C$ and $\delta^{15}N$ values relative to the international scales.

Table 1. Carbon and nitrogen isotope standards.

Isotope Standard	Use	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{15}N_{AIR}$ (‰)
Mix of ammonium sulphate and sucrose (C52)	Normalisation	-11.94	+16.58
Nicotinamide (C51)	Normalisation	-22.95	+0.07
Caffeine (C54)	Normalisation	-34.46	-16.61
Glutamic Acid (C55)	Blind standard	-28.53	-3.98

All $\delta^{15}N$ values are reported vs. AIR. These standards were calibrated to International standards IAEA-N1 (+0.4‰), IAEA-N2 (+20.3‰), USGS-40 (-4.52‰) and USGS-41 (47.57‰).

All $\delta^{13}C$ values are reported vs. V-PDB. These standards were calibrated to International standards IAEA-CH-6 (-10.4‰), NBS-22 (-29.91‰), USGS-40 (-26.24‰) and USGS-41 (+37.76‰).

Analytical precision was based on our Blind Standard (C55, which is not used for normalisation) and was usually better than ± 0.2 ‰ for both carbon and nitrogen.

Hair Hydrogen Isotopic Analysis

Four in house hair samples of hair, AND, COL, CAL-CAN and CAL-SAL were obtained and developed for this project. Three of the four stock samples were obtained from three different individuals who did not frequently travel outside of their local community for extended periods of time. All three individuals were omnivores.

The sample AND was from an individual who moved to Ottawa in January 2007 after previously residing in Manchester, United Kingdom, for the previous 7 years. The subject donated a haircut from July 2007, and the cut hair strands were approximately 2-6 cm in length. Since hair grows at

approximately 1 cm per month [1], and the remaining hair on the scalp of the subject was ~6-10 cm long, it was presumed that the majority of the sampled hair represented the signal from Manchester.

Sample COL was from an individual who moved to Ottawa, Ontario, Canada, in November 2004, after previously living in Los Angeles, California, USA, for the previous 5 years. The subject also donated a haircut in June 2007. This subject had quite long hair before it was cut (~25 to 30 cm long), where the hair strands cut were ~3-12 cm long and could therefore be presumed to represent an Ottawa signal.

Sample CAL-CAN was from an individual who had only ever resided in Calgary, Alberta, Canada. A haircut with cut strands ~ 10-15 cm in length was donated in June 2007. It is unknown how long the scalp hair remaining after the hair cut was.

The final hair collection was also from Calgary, and consisted of a large bag of hair from several individuals in a hair salon located in Calgary. A large amount of hair that by visual appraisal appeared to be from the same individual was separated from the bag and used to make the sample CAL-SAL.

For each of the four hair samples, several (>20) aliquots were pulverized and collected together to make a large volume. The collected pulverized aliquots were shaken for hours in large glass jars to thoroughly mix them together. The hair samples were not sieved, so a variety of "grain" sizes presumably exists. All four hair samples are stored in separate glass jars with a loose-fitting lid at the University of Ottawa.

δ^2H analysis is more complicated than $\delta^{13}C$ or $\delta^{15}N$ analysis due to exchangeable hydrogen. What this means is that hydrogen which is present in the chemical structure of the hair molecule in the form of O-H (hydroxyl) or C(O)N-H (amide) will exchange with the hydrogen of water molecules in the ambient air [2-4]. Since the isotopic composition of ambient air is different in various parts of the world [5,6], the analysis of compounds which have exchangeable hydrogen would give different results. Because the exact chemical structure of keratin (and hair) is unknown, the amount of exchangeable hydrogen cannot be calculated, and must be determined experimentally. Further, it has been shown that different compounds, or the same compound which is prepared in different ways, will have different amounts of exchangeable hydrogen [2,3].

In order to accurately determine the true δ^2H value of hair, and account for the exchangeable hydrogen, we performed an "exchange experiment" modeled after Bowen et al [2]. We weighed out two identical sample sets. Each sample set contained isotope standards with no exchangeable hydrogen, which were used for the normalisation of the sample data, and our four in-house ground hair isotope standards.

Table 2. Hydrogen isotope standards.

Isotope Standard	Use	δ^2H_{VSMOW} (‰)
Polyethylene Foil , International standard IAEA-CH-7 (C61)	No exchangeable hydrogen, normalisation	-100.3
Kaolinite (G731)	No exchangeable hydrogen, normalisation	-58.0

The two sample sets were each equilibrated in separate sealed chambers with 5 mL of water having different isotopic compositions (-198.5 and 11.3 ‰). The waters are referred to as "heavy" (11.3 ‰) and "light" (-198.5 ‰). After 4 days of equilibration, the sample sets were removed and placed in their own vacuum chambers for 7 days to remove any residual water from the surface of the samples. On the 7th day, the samples were analysed for δ^2H.

Samples and isotope standards were weighed into silver capsules and loaded onto a Pyrolysis Elemental Analyser interfaced to an isotope ratio mass spectrometer. Samples and isotope standards are pyrolyzed at about 1400°C. The resulting gases are separated on a gas chromatography (GC) column, and sent to the IRMS.

The calculation for the "true" hydrogen isotope value is outlined in Fraser et al [(4)]. Briefly, the fraction of exchangeable hydrogen is:

$$F_{ex} = (\delta^2H_{hair,\ heavy} - \delta^2H_{hair,\ light}) / (\delta^2H_{water,\ heavy} - \delta^2H_{water,\ light})$$

Where:

F_{ex} = fraction of exchangeable hydrogen
$\delta^2H_{hair,\ heavy}$ and $\delta^2H_{hair,\ light}$ = the normalised δ^2H values of the hair equilibrated in heavy and light water, respectively
$\delta^2H_{water,\ heavy}$ and $\delta^2H_{water,\ light}$ = the δ^2H values of the heavy and light water used for equilibration

The "true" δ^2H can be calculated using either the "light" or "heavy" measured isotope values, as they both yield the same "true" δ^2H. It is calculated as:

$$\delta^2H_{hair,\ true} = [\delta^2H_{hair,\ heavy} - (F_{ex} \times (\delta^2H_{water,\ heavy})] / (1-F_{ex})$$

Or

$$\delta^2H_{hair,\ true} = [\delta^2H_{hair,\ light} - (F_{ex} \times (\delta^2H_{water,\ light})] / (1-F_{ex})$$

All reported δ^2H values were the "true" δ^2H values corrected for exchangeable hydrogen, and were reported to the international scale V-SMOW. Analytical precision was based on the reproducibility of our internal hair standards identified as AND (G737), COL (G738), CAL-CAN (G739) and CAL-SAL (G740) which was usually better than ± 2.0 ‰.

Hair Oxygen Isotopic Analysis

Samples and standards were weighed into silver capsules and loaded onto a PyroCube Elemental Analyzer. The samples fell into a glassy carbon reactor held at 1450°C to produce CO, H_2 and/or

N_2. H2 and/or N2 were removed from the gas flow, and CO was trapped by a chemical trap. The CO was then released and entered the Delta Plus XP IRMS via the Conflo 4 interface.

Isotope standards were used to normalise (correct) the sample data to the accepted international scales. In addition, another isotope standard was used to correct for linearity. These materials were chosen because they cover most of the natural range for oxygen containing compounds. Table 2 shows the internal isotope standards used in the normalisation of the samples and their $\delta^{18}O$ values relative to the international scales.

Table2. Oxygen isotope standards.

Isotope Standard	Use	$\delta^{18}O_{SMOW}$ (‰)
IAEA 600: Caffeine	Normalisation	-3.48
IAEA601: Benzoic Acid	Normalisation	23.14
CAL-SAL (G740)	Linearity	

All reported $\delta^{18}O$ values were reported to the international scale V-SMOW. Analytical precision was based on the reproducibility of AND (G737), COL (G738), CAL-CAN (G739) and CAL-SAL (G740) which was usually better than ± 0.4 ‰.

Hair Sulphur Isotopic Analysis

Samples and standards were weighed into tin capsules, loaded onto a Vario EL III Elemental Analyzer, and flash combusted at 1800°C. Released gases were trapped, with the exception N2, which flowed though and was then purged. All gases were released, and SO_2 was trapped a second time (double purge and trap method) while all other gases were purged. The SO_2 was then released and entered the Delta Plus XP IRMS via the Conflo 4 interface.

Isotope standards were used to normalise (correct) the sample data to the accepted international scales for each different isotope. In addition, another isotope standard was used to correct for linearity. These materials were chosen because they cover most of the natural range for sulphur containing compounds. Table 3 shows the internal isotope standards used in the normalisation of the samples and their $\delta^{34}S$ values relative to the international scales.

Table 3. Sulphur isotope standards.

Isotope Standard	Use	$\delta^{34}S_{CDT}$ (‰)
Silver Sulphide (S-1)	Normalisation	-0.3
Silver Sulphide (S-2)	Normalisation	21.7
Silver Sulphide (S-6)	Linearity	-0.7

All reported $\delta^{34}S$ values were reported to the international scale CDT. Analytical precision is based on the reproducibility of AND (G737), COL (G738), CAL-CAN (G739) and CAL-SAL (G740) which was usually better than ± 0.4 ‰.

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