

# Applications médico-légales des isotopes stables

## (La science avec quelques histoires)

Gilles St-Jean

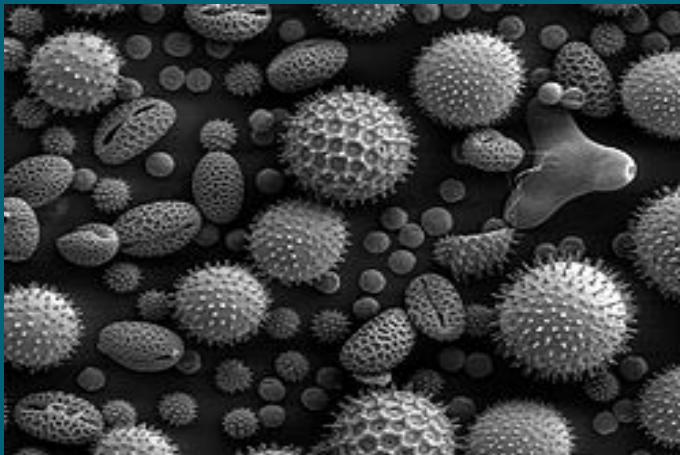
Université d'Ottawa / Sciences de la Terre et de l'Environnement  
Laboratoire André E. Lalonde de Spectrométrie de Masse par Accélérateur

MISTRAMO, 27-30 mars 2017; Sèvres, France

# Médico - juridique



Base de données?!

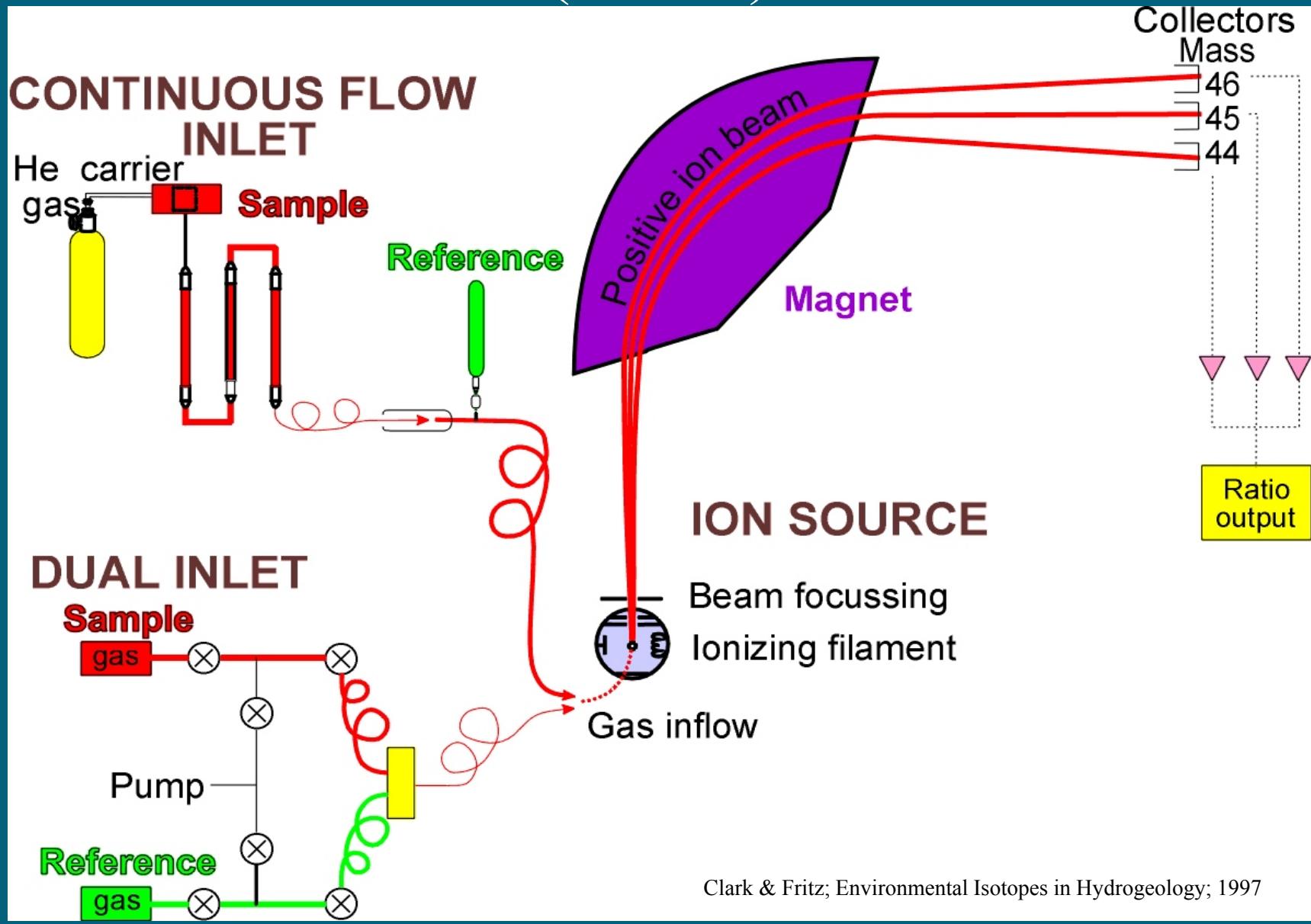




# Isotopes stables légers

Elements	Isotopes	Abondance Relative	Précision			
			ppm	%o		
Hydrogène	$^1\text{H}$	99.984	0.16	1		
	$^2\text{H}$ (D)	0.0156				
Carbone	$^{12}\text{C}$	98.892	0.56	0.05		
	$^{13}\text{C}$	1.108				
Azote	$^{14}\text{N}$	99.635	0.37	0.1		
	$^{15}\text{N}$	0.365				
Oxygène	$^{16}\text{O}$	99.759	0.20	0.1		
	$^{17}\text{O}$	0.204				
	$^{18}\text{O}$					
Soufre	$^{32}\text{S}$	95.02	9.16	0.2		
	$^{33}\text{S}$	4.22				
	$^{34}\text{S}$					
	$^{36}\text{S}$					

# Spectrométrie de masse à rapport isotopiques (SMRI)



# Spectrométrie de masse à rapport isotopiques (SMRI)

$$\delta^{13}\text{C} = \left\{ \frac{R_{\text{échantillon}} - R_{\text{référence}}}{R_{\text{référence}}} \right\} * 1000 \quad \text{Où } R = \frac{{}^{13}\text{C}}{{}^{12}\text{C}}$$

$$\delta^{13}\text{C} = \left\{ \frac{R_{\text{échantillon}}}{R_{\text{référence}}} - 1 \right\} * 1000$$

Exprimé en Delta pour mil ou ‰

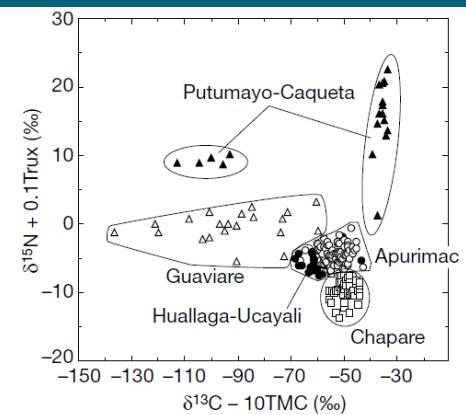
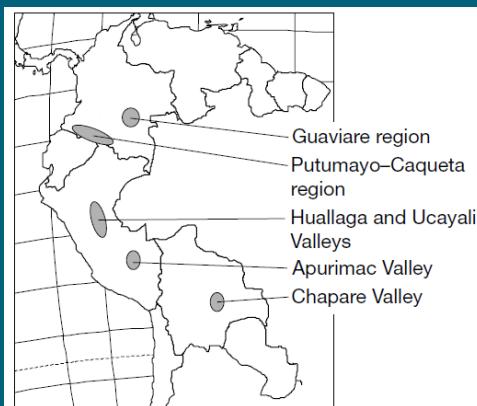
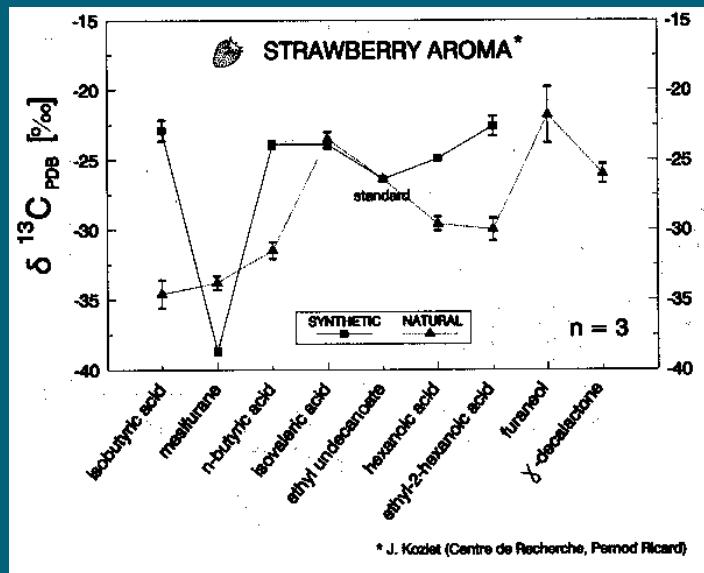
# Instrumentation CG-C/Py-IRMS ( $^2\text{H}$ ( $\text{D}$ )), $^{13}\text{C}$ , $^{15}\text{N}$ , $^{18}\text{O}$ )

- Techniques d'échantillonnage:  
Volume d'expansion (Headspace)-CG-SMRI, MEPS-CG-SMRI, etc.
- Produit d'adultération
  - Vin, bière, Vodka etc.
  - Saveurs (ex.. Vanilline)
  - Tabac/nicotine (ex.. cigares cubain)
- Tricherie athlétiques (Stéroïdes: Naturel vs. Synthétique; provenances)
- Médicale / Kinanthropologie / kinésiologie, Pharmaceutic: (ex: Cholestérol, H. Pilorie, dépenses d'énergie, traceurs isotopiques, etc.)
- Produit de biodégradation (ex.. acides gras, etc.)
- « Isotopic fingerprinting » (ex.. Produit pétroliers, saveurs, drogue, pharmaceutiques, etc.)
- Acides Aminées ( $^{13}\text{C}$ ,  $^{15}\text{N}$ )
- ETC...



# Instrumentation

## CG-C/Py-IRMS ( $^2\text{H}$ (D), $^{13}\text{C}$ , $^{15}\text{N}$ , $^{18}\text{O}$ )



**Figure 1** Identification of geographic regions in South America where coca is commonly grown. Left, regions producing illicit cocaine; right, identification of cocaine-growing regions based on a combined model derived from carbon- and nitrogen-isotope ratios as well as abundance of minor alkaloid components. Squares, Bolivia; triangles, Colombia; and circles, Peru. Regions within a country are distinguished by black and white symbols. Trux, truxilline; TMC, trimethoxycocaine. Isotope ratios are expressed as  $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000\text{‰}$ , where  $R$  is the molar ratio of heavy-to-light stable isotope; standards for carbon and nitrogen are PDB and air, respectively.

J. R. Ehleringer et al., NATURE | VOL 408 | 16 NOVEMBER 2000

# Instrumentation LC-IRMS ( $^{13}\text{C}$ )

- Produit d'adultération
  - - Sirop d'érable
  - Miel
- Source ( ex.. ASA)
- Détermination du carbone isotopique de l'ARN bactérien
- Analyse du  $\delta^{13}\text{C}$  des acides aminées non dérivés
- Analyse des acides organiques des plantes
- ETC...



# Instrumentation

## LC-IRMS (13C)

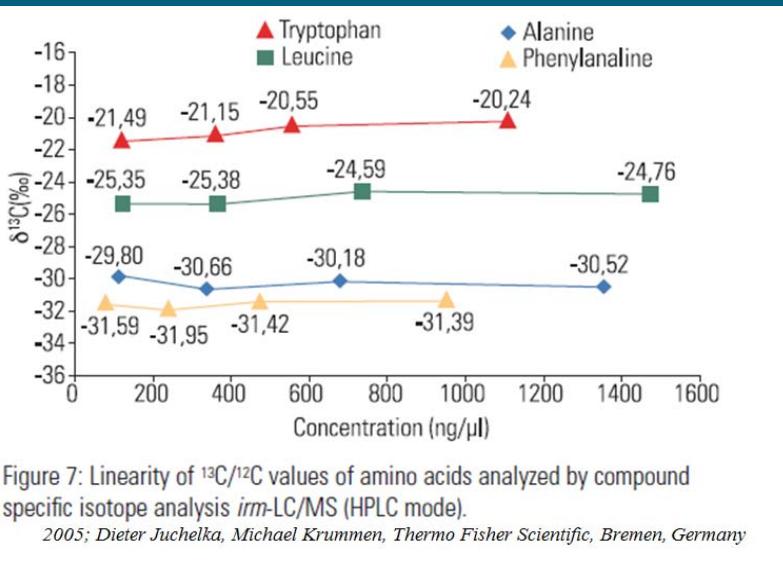


Figure 7: Linearity of  $^{13}\text{C}/^{12}\text{C}$  values of amino acids analyzed by compound specific isotope analysis *irm*-LC/MS (HPLC mode).

2005; Dieter Juchelka, Michael Krummen, Thermo Fisher Scientific, Bremen, Germany

D. Juchelka et al, Application Note 30065, Thermo Bremen

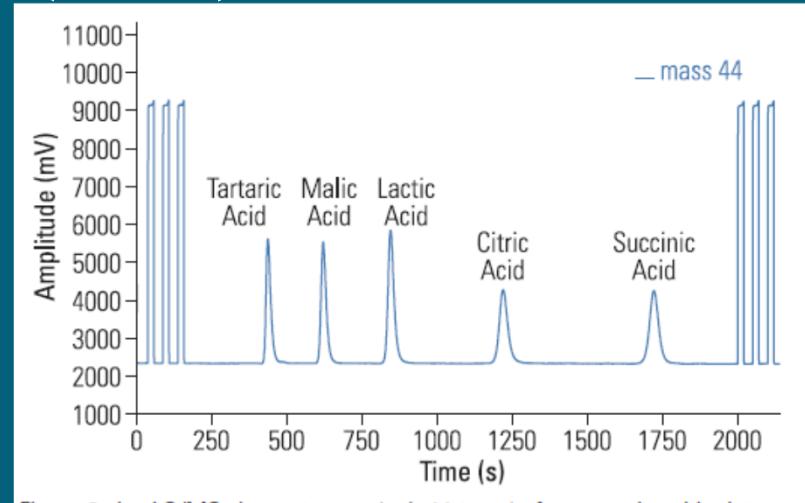


Figure 2: *irm*-LC/MS chromatogram ( $m/z$  44 trace) of an organic acid mixture.

2005; Elena Hettmann, Gerd Gleixner, Max Planck Institute for Biogeochemistry, Jena, Germany, Dieter Juchelka, Thermo Fisher Scientific, Bremen, Germany, dieter.juchelka@thermofisher.com

E. Hettmann et al, Application Note 30075, Thermo Bremen

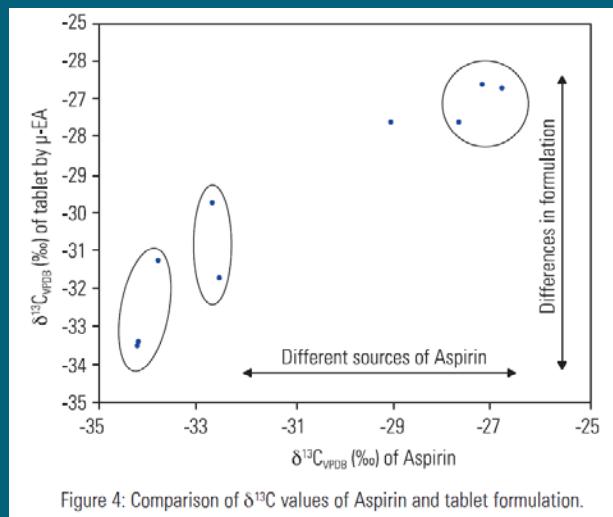


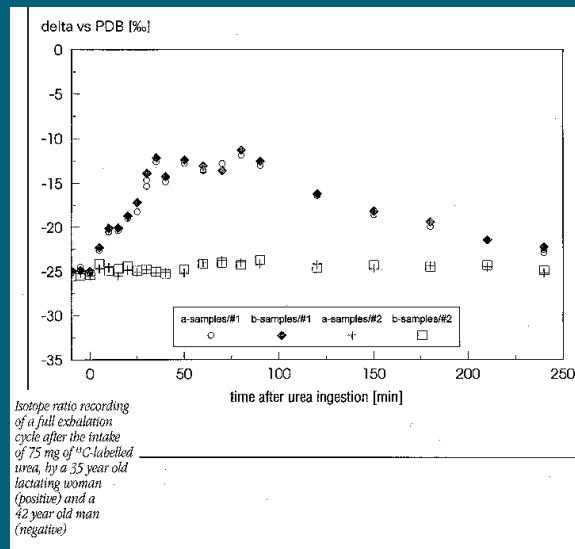
Figure 4: Comparison of  $\delta^{13}\text{C}$  values of Aspirin and tablet formulation.

A. Hilkert et al, Application Note 30025, Thermo Bremen

# Instrumentation

## Volume d'expansion (Gaz) CG-SMRI (C, N, O, S, Ar)

- Eau (D,  $^{18}\text{O}$ )
- Carbonates ( $^{13}\text{C}$ ,  $^{18}\text{O}$ )
- Analyse des gaz expirés (Breath Analysis)
  - Helicobacter pylori (Ulcère et autres)
  - Fibrose cystique (Malabsorption des gras)
- Dépenses énergétiques
  - Eau doublement marquée (D,  $^{18}\text{O}$ )
  - Leucine ( $^{13}\text{C}$ )
- ETC...



# Instrumentation

## Analyseur élémentaire-SMRI (D, C, N, O, S)



Analyses en bloc (Bulk analysis)

- Combustion (C & N)
- Conversion haute température (D, O)

Ex: Analyse élémentaires de cheveux

H = 5.8%

C = 50.1%

N = 17.0%

O = 21.5%

S = 5.0%



# Instrumentation

## Analyseur COD/CID-SMRI



Analyses en bloc (Bulk analysis)

- Combustion du DOC eau saline
- Conversion par acidification ( $H_3PO_4$ ) du CID
- Conversion par oxydation au persulfate du COD

# Instrumentation

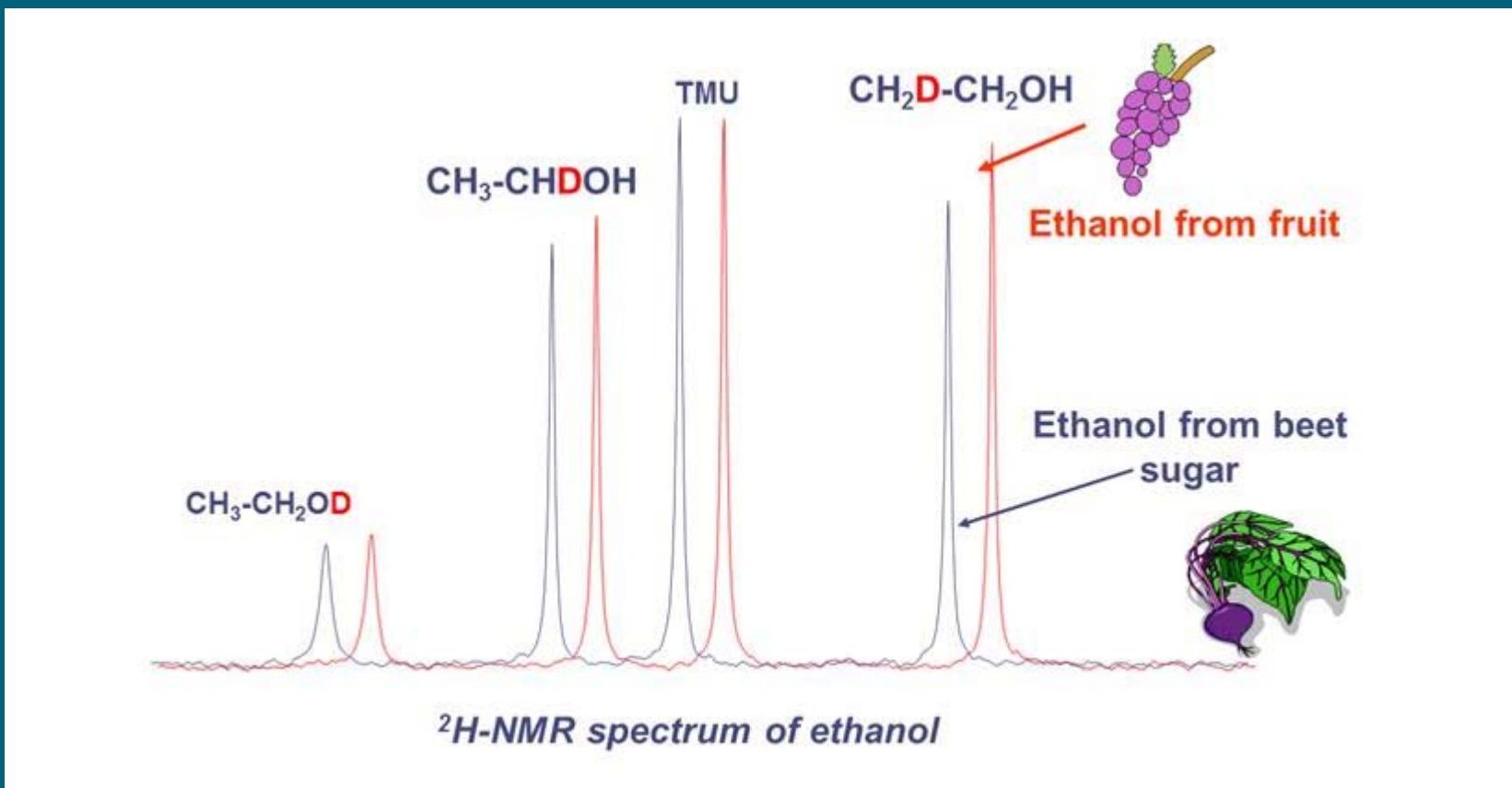
## SNIF-RMN (D, C, O)

Précision approximative

$$\delta D < 2\text{‰}$$

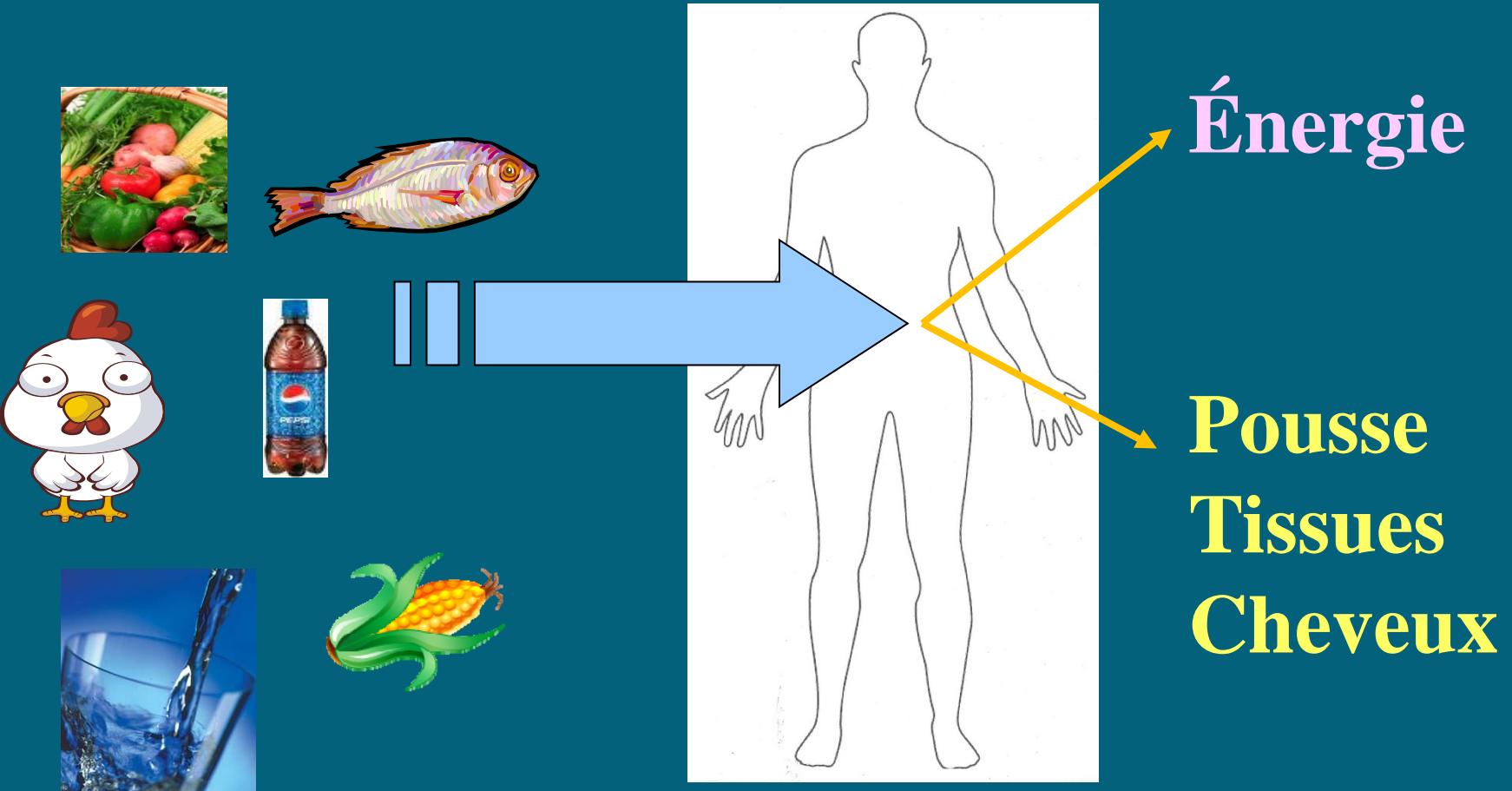
$$\delta^{13}\text{C} \pm 1.1\text{‰}$$

$$\delta^{18}\text{O} \pm 1.5\text{‰}$$



# On est ce qu'on mange (et bois)

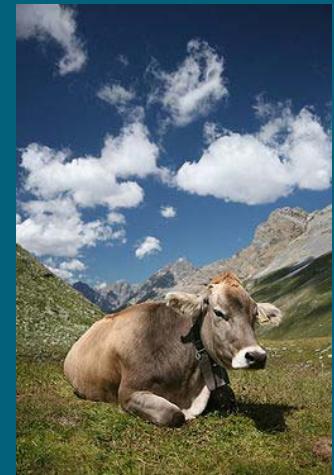
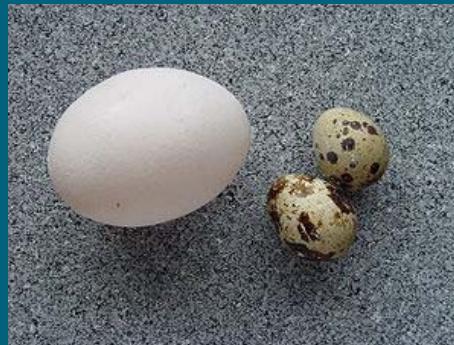
## Les plantes: ce qu'elles absorbent



# Azote

Le  $^{15}\text{N}$  reflètent la part protéinique de l'alimentation

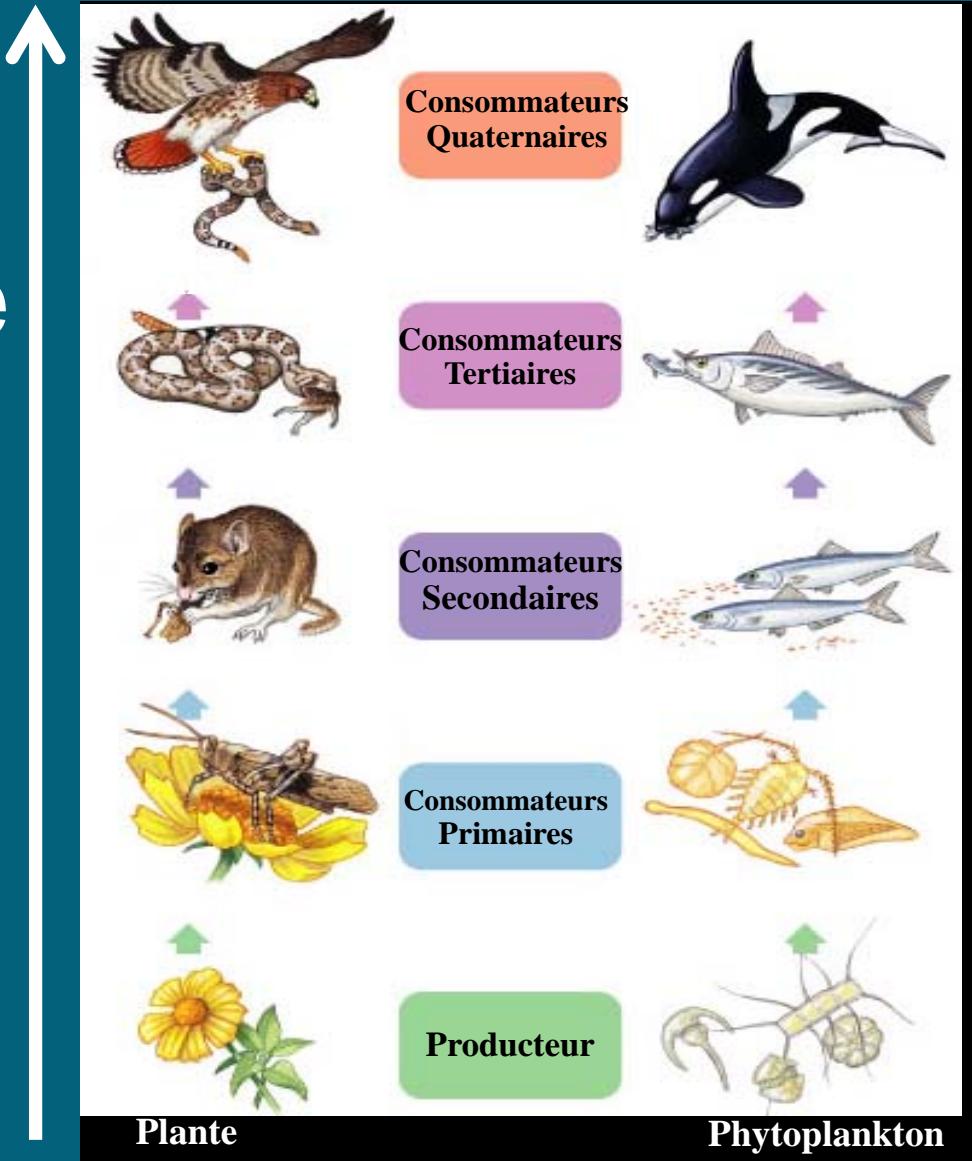
- Œufs, produits laitiers et substitut
- Viandes
- Poisson
- Légumes (Soyas, arrachide)
- Protéine de soya
- (Lactosérum "Whey ")
- Levure



# Azote

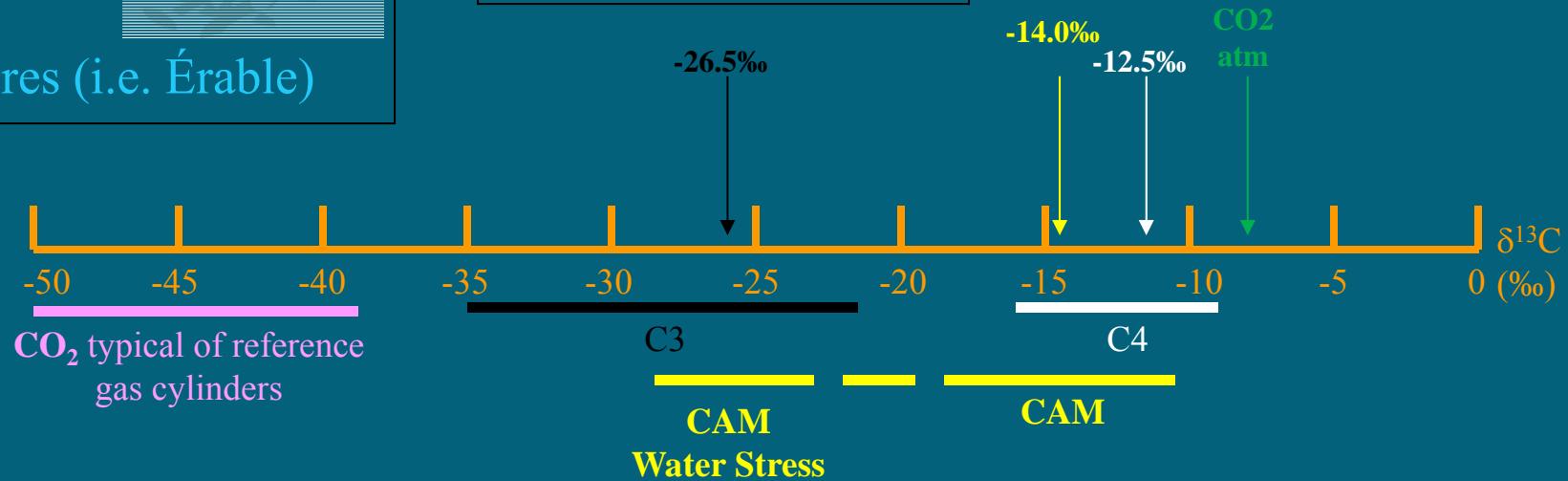
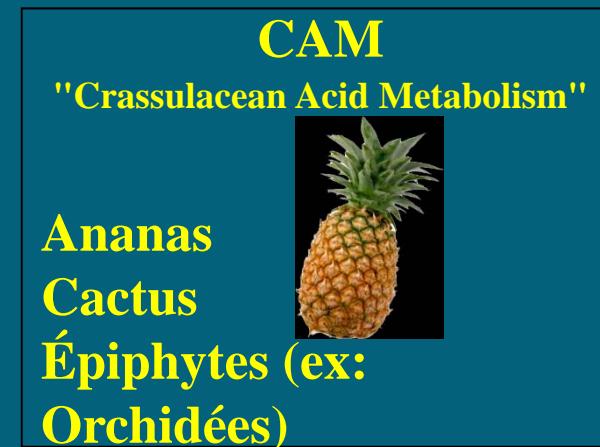
Niveau Trophique Augmente  
 $\delta^{15}\text{N}$   
(~ 3‰ / Niveau)

Catabolisme  
Résultat du recyclage des protéine corporel.  
(ex.. anorexie, boulimie, vomir de femme enceinte)  
↓  
Effet de niveau trophique  
ex.. Stress nutritionnel



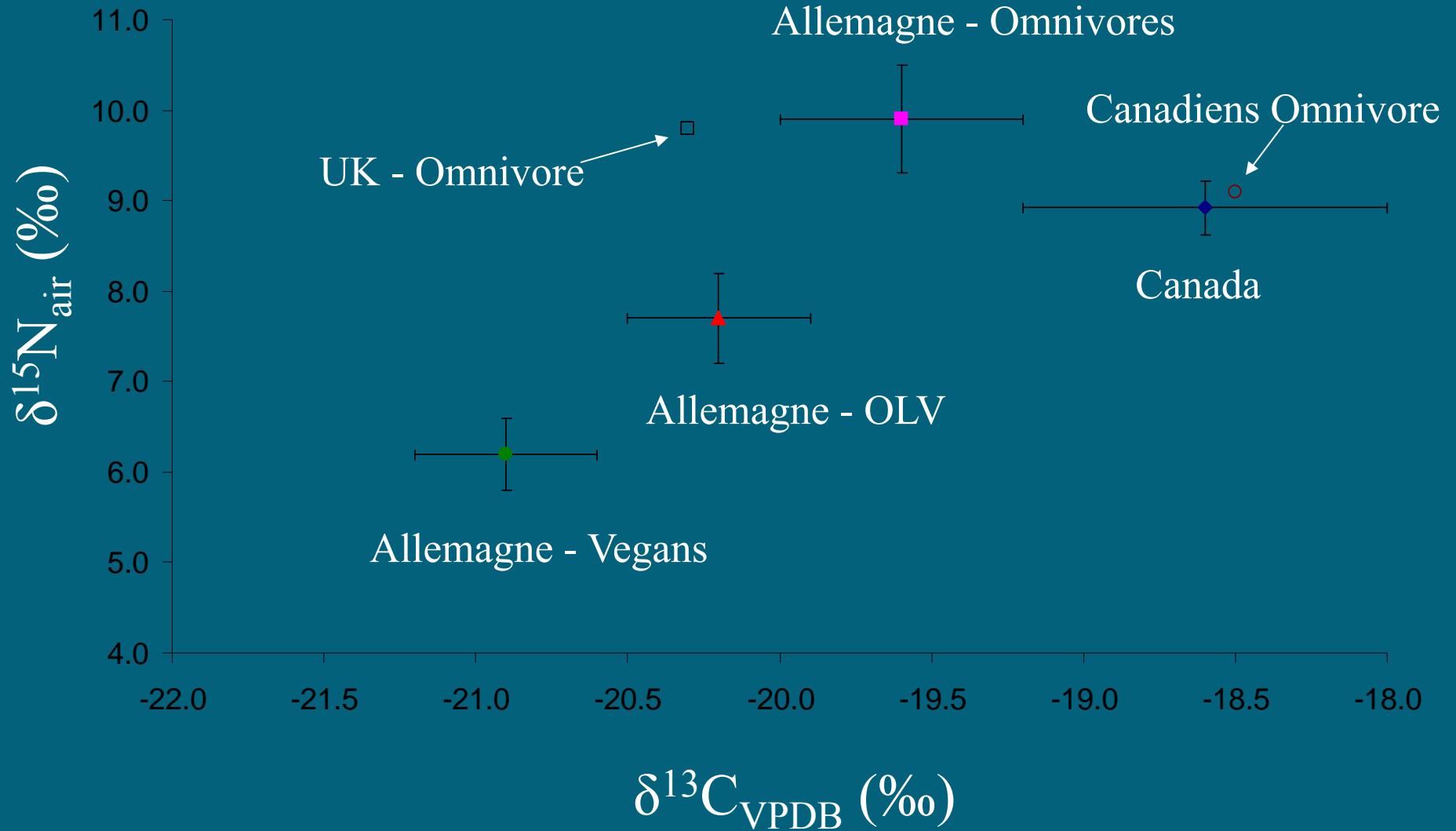
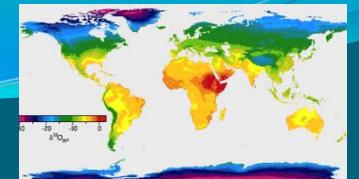
# Carbone (Fractionnement biogénique )

Le  $\delta^{13}\text{C}$  reflètent tous les composantes diététique, y compris les glucides et les graisses (c'est-à-dire la partie énergétique de l'aliment). Les valeurs proviennent de plantes appartenant à l'un des trois cycles photosynthétiques



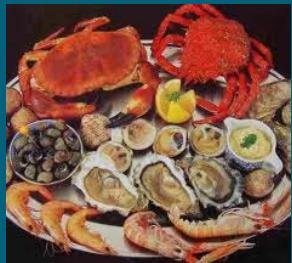


# Locale ou étranger?



Petzke, Boeing and Metges. RCMS 2005;19:1392-1400

# $\delta^{34}\text{S}$ – Diet and Geo-location



Marine protein – more positive  $\delta^{34}\text{S}$



More positive  
 $\delta^{34}\text{S}$   
from ocean  
SOURCES



More positive  
 $\delta^{34}\text{S}$

More negative  
 $\delta^{34}\text{S}$



# Nourriture et boissons

# Nourriture

## Origine et authenticité

- **Viandes** (ex. poulet, agneau, bœuf, porc, sanglier, cerf, etc.)
- **Poisson** (ex. saumon d'élevage vs sauvage, élevage méditerranéen de bars et de daurades , etc.)
- **Huiles** (ex. végétal, olive, palm, etc.)
- **Saveurs** (ex. Vanille)
- **Miel**
- **Sirop d'érable**
- **Jus de fruit** (ex: orange, canneberge, pomme)
- **Alcool et dérivé** (ex. Vin, vodka, whiskey, etc.)

# Miel



# Miel

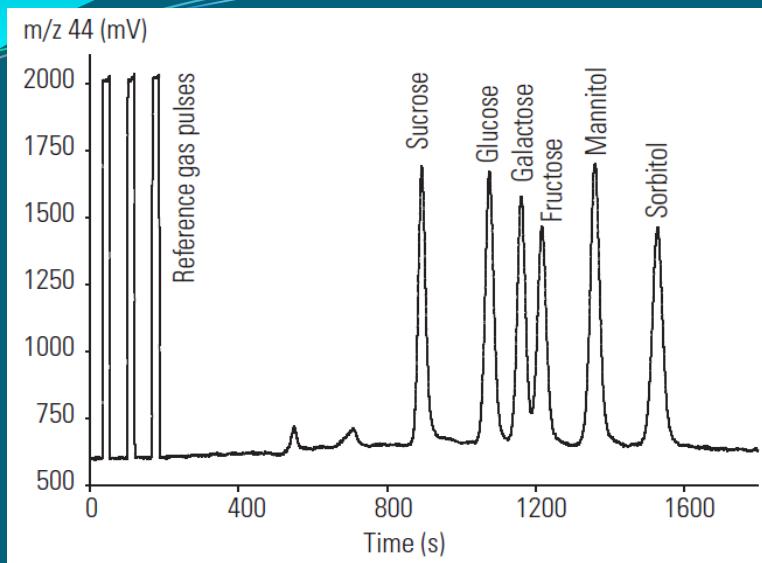


Figure 1: *irm*-LC/MS chromatogram of a sugar mixture.

A. Hilkert et al, Application Note 30024, Thermo Bremen

	$\delta^{13}\text{C}$ (‰)	Mean	S.D. ( $1\sigma$ )
Sucrose	-10.75	0.15	
Glucose	-10.20	0.12	
Galactose	-24.48	0.16	
Fructose	-11.50	0.05	
Mannitol	-11.59	0.32	
Sorbitol	-09.70	0.24	

Table 1:  $\delta^{13}\text{C}$  values and reproducibilities of a sugar mixture measured by *irm*-LC/MS.

A. Hilkert et al, Application Note 30024, Thermo Bremen

HONEY	SUCROSE ‰	GLUCOSE ‰	FRUCTOSE ‰	FRU/GLU RATIO OF AREAS	EA HONEY(4) ‰	EA PROT.(4) ‰	ADULT.(4) %	
1	-23.3	<b>-23.2</b>	<b>-22.9</b>	1.07	<b>-21.8</b>	-24.2	<b>16.7</b>	<b>adulterated</b>
2	-11.3	<b>-11.2</b>	<b>-13.9</b>	<b>0.65</b>	<b>-11.9</b>	n.a.	n.a.	<b>adulterated</b>
3	-25.3	-24.9	-24.9	1.42	-24.8	-24.8	0.0	
4	-26.4	-26.5	-26.4	0.97	-25.4	-21.6	0.0	
5	n.d.	-26.1	-26.0	<b>4.53</b>	-25.8	-26.1	1.9	<b>adulterated</b>
6	-26.1	-25.0	-25.3	1.62	-24.3	-24.3	0.0	
7	-25.0	-25.2	-25.1	1.16	-24.2	-24.7	3.4	
8	n.d.	<b>-25.1</b>	<b>-26.4</b>	2.17	-24.8	-25.1	1.5	<b>adulterated</b>

Table 3:  $\delta^{13}\text{C}$  values of eight honey samples analyzed by *irm*-LC/MS and by EA.

A. Hilkert et al, Application Note 30024, Thermo Bremen

# Sirop d'érable



# Authenticité des boissons alcoolisées

Composantes qui peuvent être analysées

Éthanol:  $\text{C}_2\text{H}_5\text{OH}$  ( $^{13}\text{C}/^{12}\text{C}$ , D/H,  $^{18}\text{O}/^{16}\text{O}$ )

Congénères: Sous-produits toxiques de distillation et de fermentation  
qui donne à chaque boisson son goût, couleur et arôme.

Isotopes des acétaldéhyde, éthyle acétate, n-propanol, isobutanol, alcool amylique

Eau: D/H,  $^{18}\text{O}/^{16}\text{O}$ ,  $^{17}\text{O}/^{16}\text{O}$

$\text{CO}_2$  (si pétillant):  $^{13}\text{C}/^{12}\text{C}$ ,  $^{18}\text{O}/^{16}\text{O}$

# Analyses isotopiques multiéléments d'une molécule

Éthanol



CG - combustion  
T = 1050°



Éch . du vol. d' expansion  
100 µL

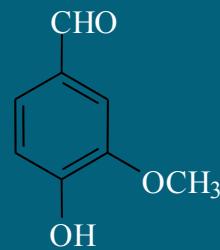
CG - pyrolyse  
T = 1400°



Benzaldéhyde

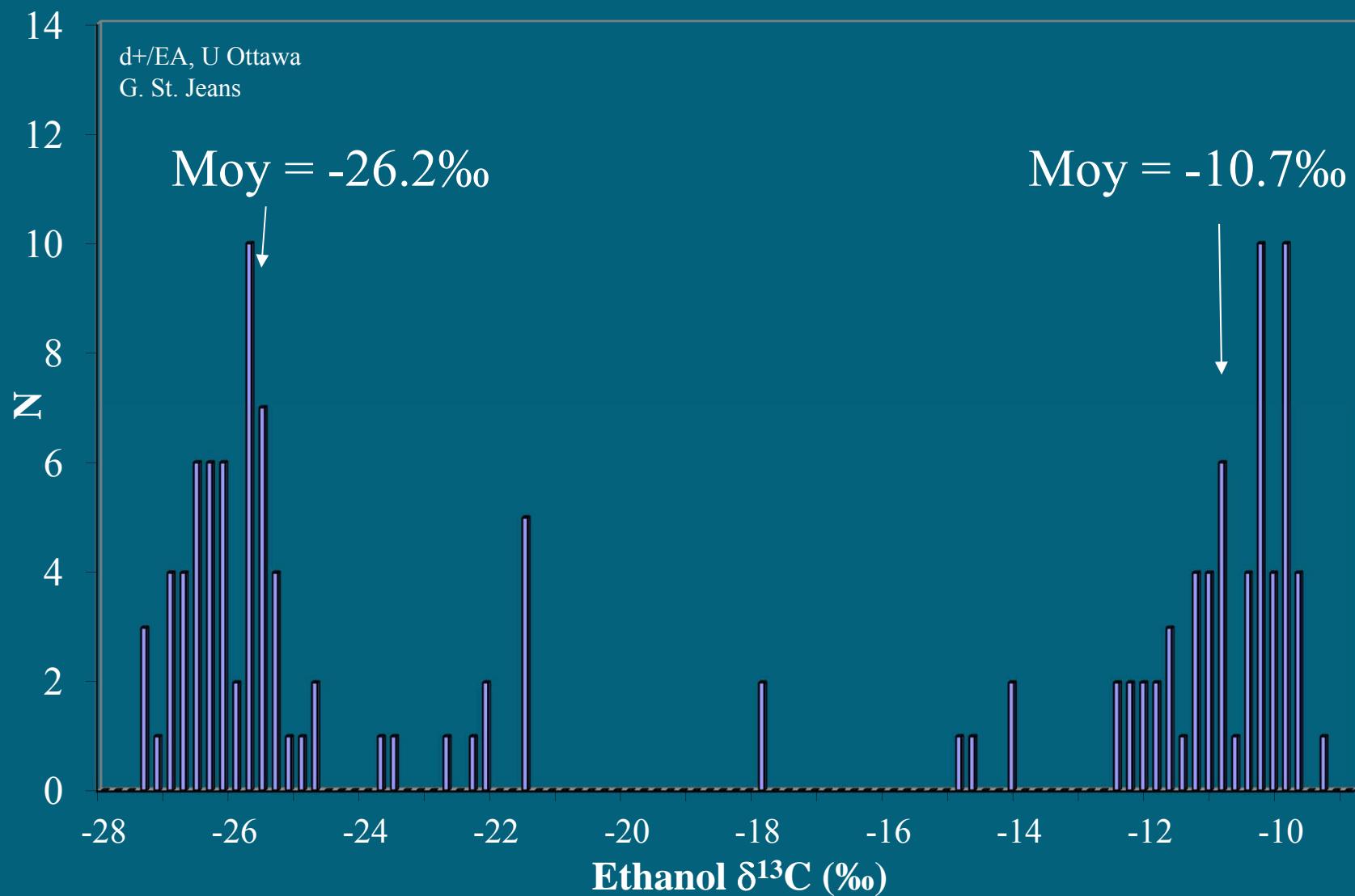


Vanilline



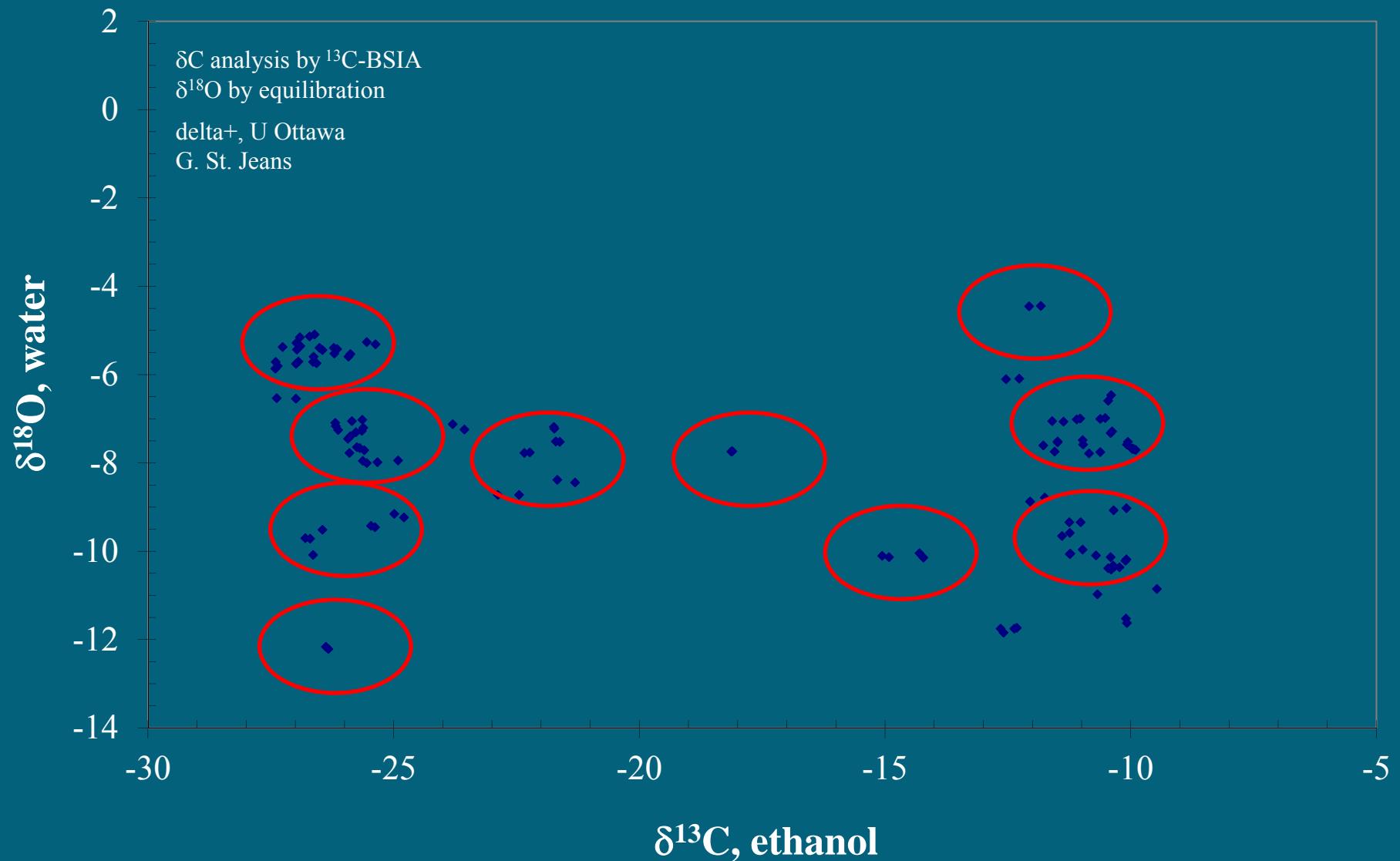
# Isotopic Analysis of Commercial Vodka

## Histogram of $\delta^{13}\text{C}$ of ethanol

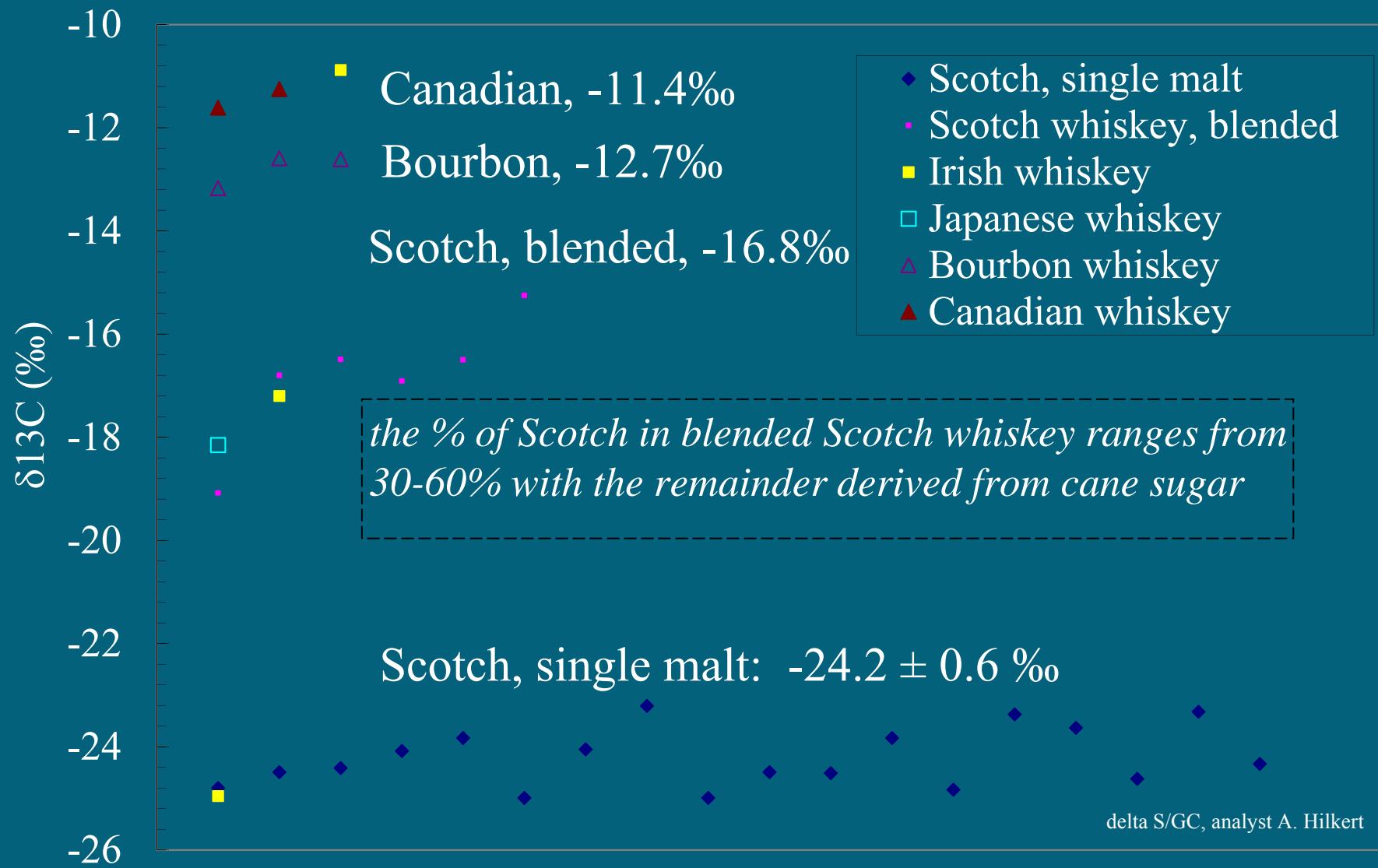


# Isotopic Analysis of Commercial Vodka

## $\delta^{13}\text{C}$ of ethanol vs. $\delta^{18}\text{O}$ of water

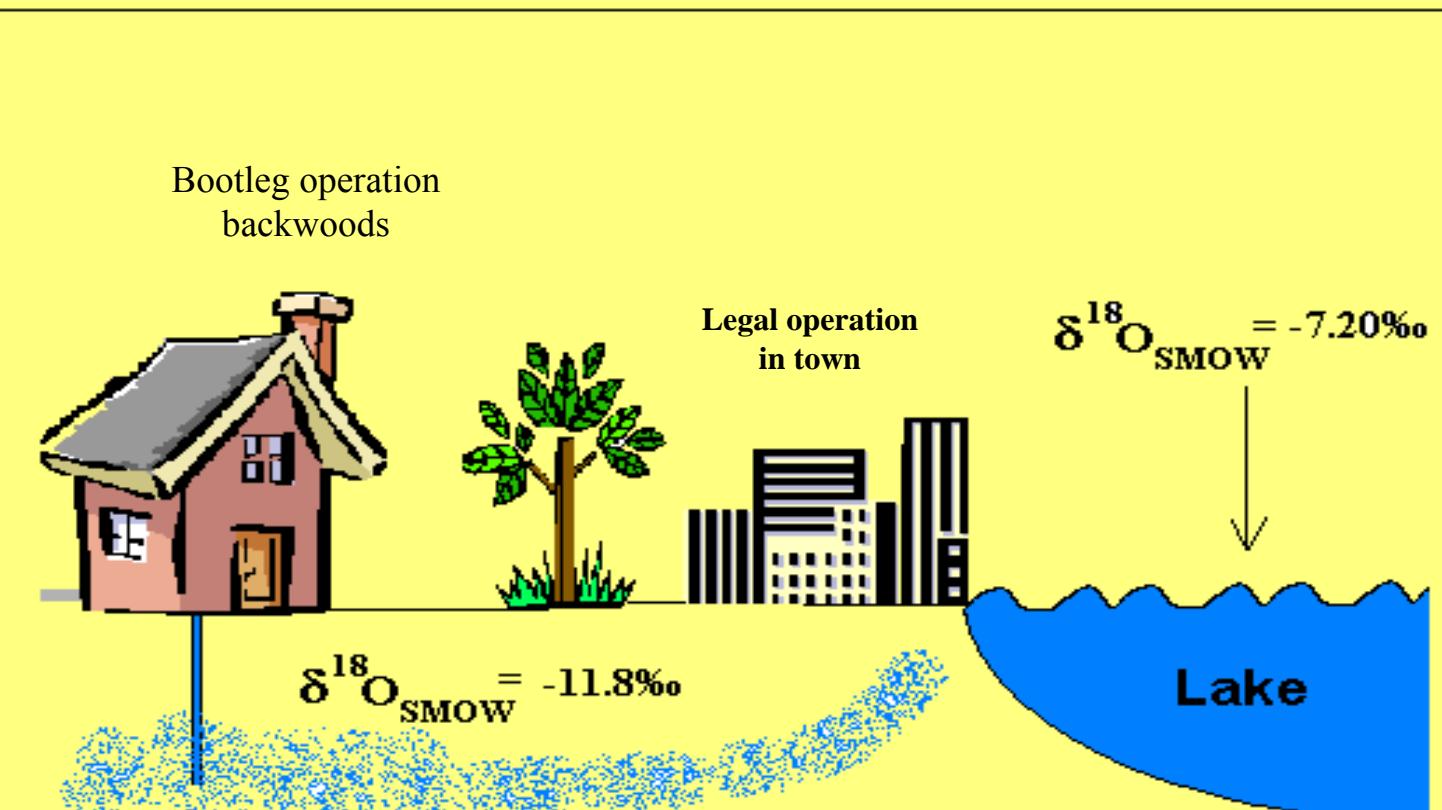


## $^{13}\text{C}$ -CSIA of ethanol by headspace sampling using $\delta^{13}\text{C}$ of ethanol in whiskey to calculate mixing



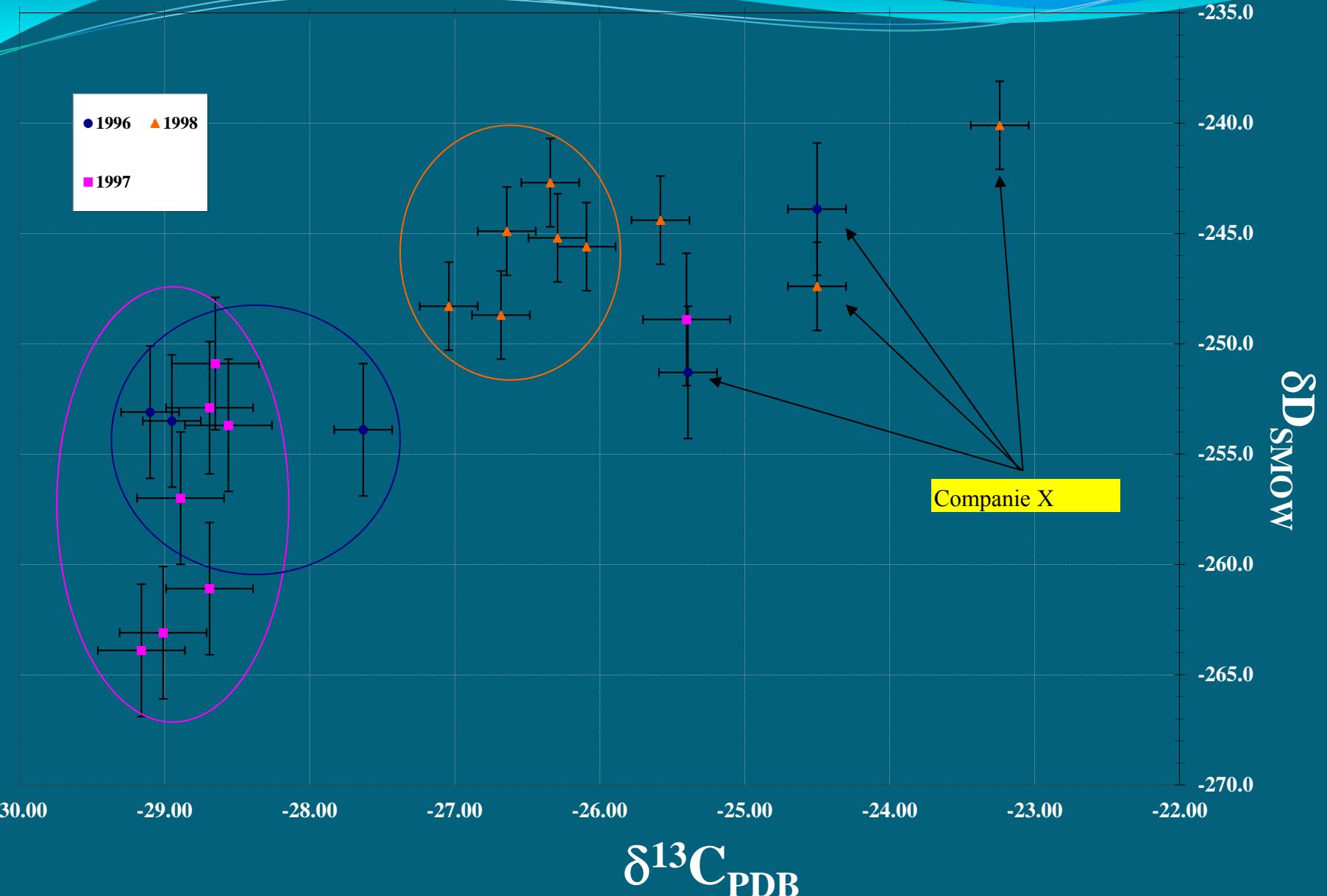
# $\delta^{18}\text{O}$ of dilution water: Whiskey, a case study

Gilles St-Jean, U Ottawa  
Daniel Perreault, Douane et Assise Canada



**Groundwater = local value**

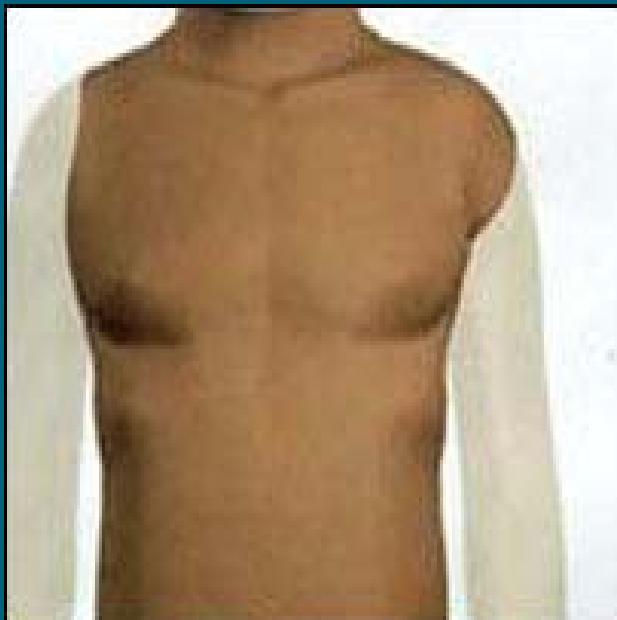
# Vin de glace : Données par année, $^{13}\text{C}$ vs D





# Médico-légale

# “Adam” Sept. 21, 2001

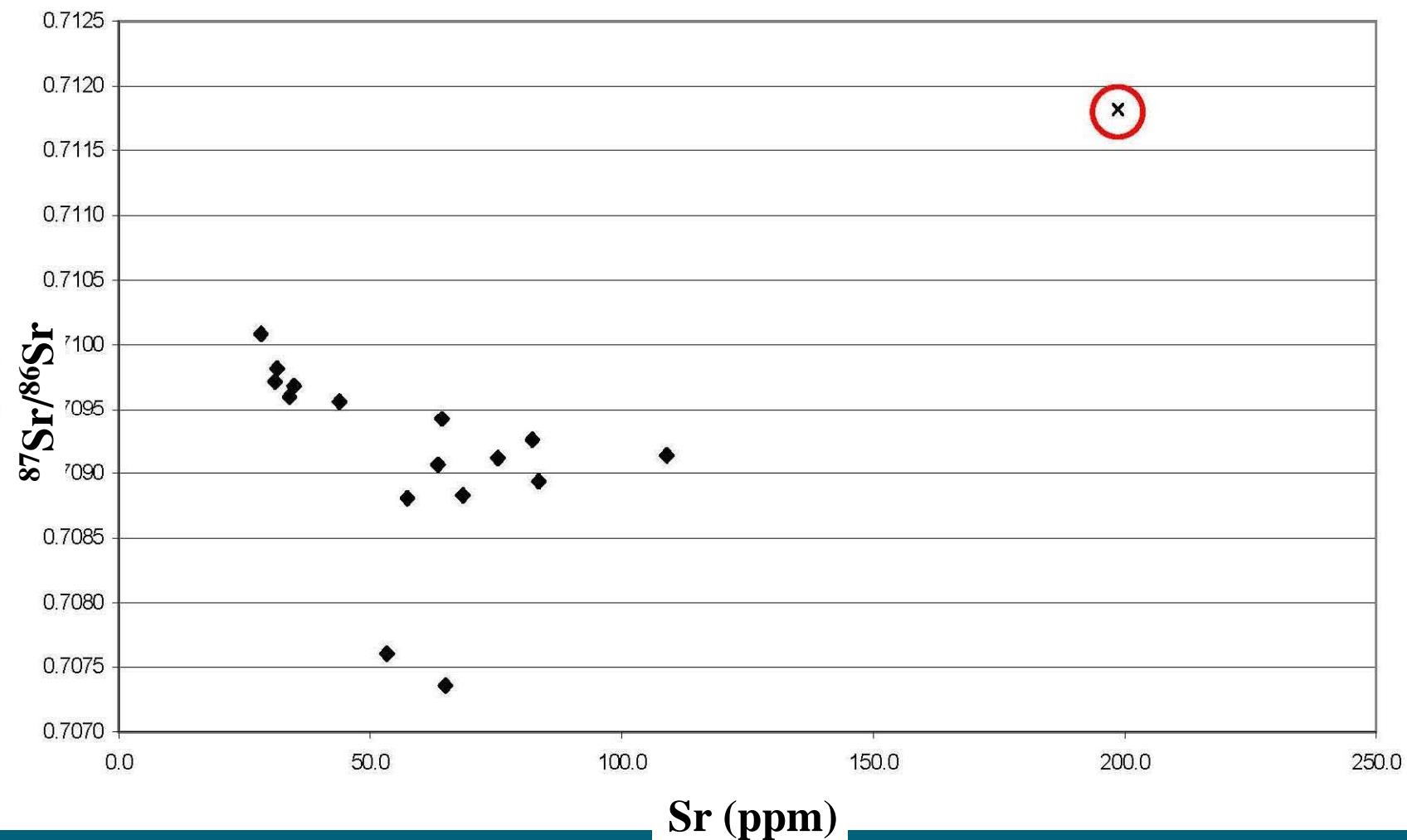


- Torso of child found in Thames River
- No head, arms, legs, exsanguination
- Evidence of ritualistic killing
- Standard forensics not useful

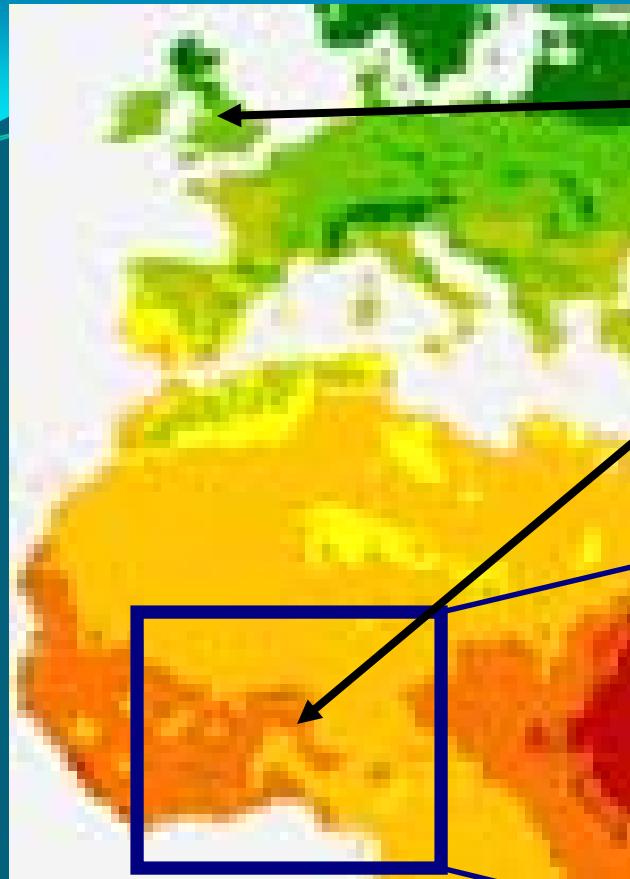
• [www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf](http://www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf)

# Strontium Isotopes

$^{84}\text{Sr}$	$^{86}\text{Sr}$	$^{87}\text{Sr}$	$^{88}\text{Sr}$
83.913426 0.56%	85.909203 8.88%	86.935532 7.00%	87.905517 32.88%



•[www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf](http://www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf)

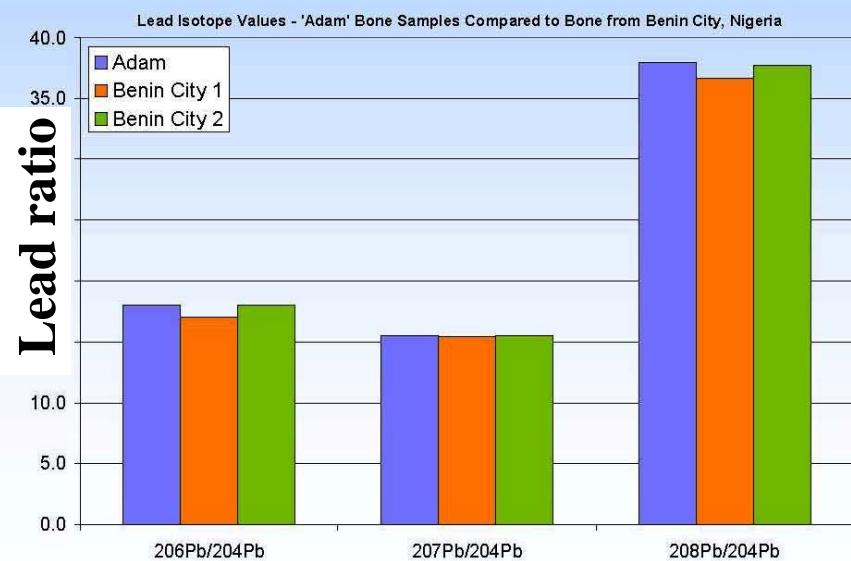


Torso found here

Sr data indicates from Africa



•[www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf](http://www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf)



# Benin

Likely from ~ 100 km  
Around Benin City

# “Adam” Outcomes

- Multivariate isotope analysis provided signature of the location where the child grew up

- Identity of child was identified



•[www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf](http://www.rsnz.org/events/sciencesecurity/presentations/RusselFrew.pdf)

# Preparation for analysis



First hair cutter: 1 cm resolution = 1 month  
Current hair cutter: 0.5 cm resolution = 0.5 months

# Exchange Experiment



**LIGHT WATER**



**HEAVY WATER**



4 days to equilibrate in water



Sealable  
container



7 days under vacuum

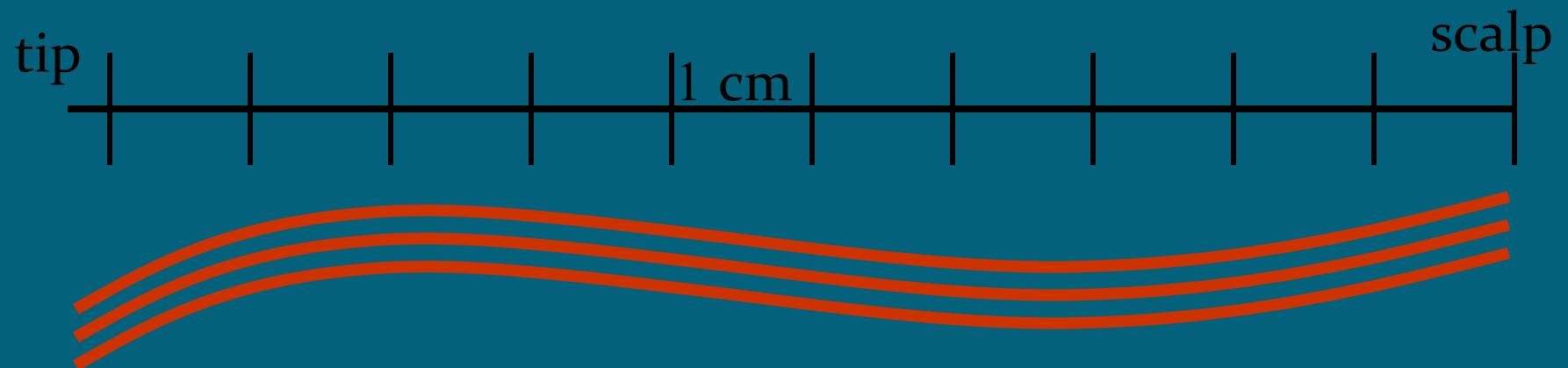
# Case: Hair Analysis

- Need between 50 and 200 hair for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^2\text{H}$  analysis
  - Depends on thickness of hair
- MUCH EASIER if hair is oriented AND clean
  - Know which end is the root and which end is the tip
  - Otherwise, spend \$\$ to clean hair and determine orientation



Since hair grows  $\sim$  1 cm each month...

### Chronological “map”

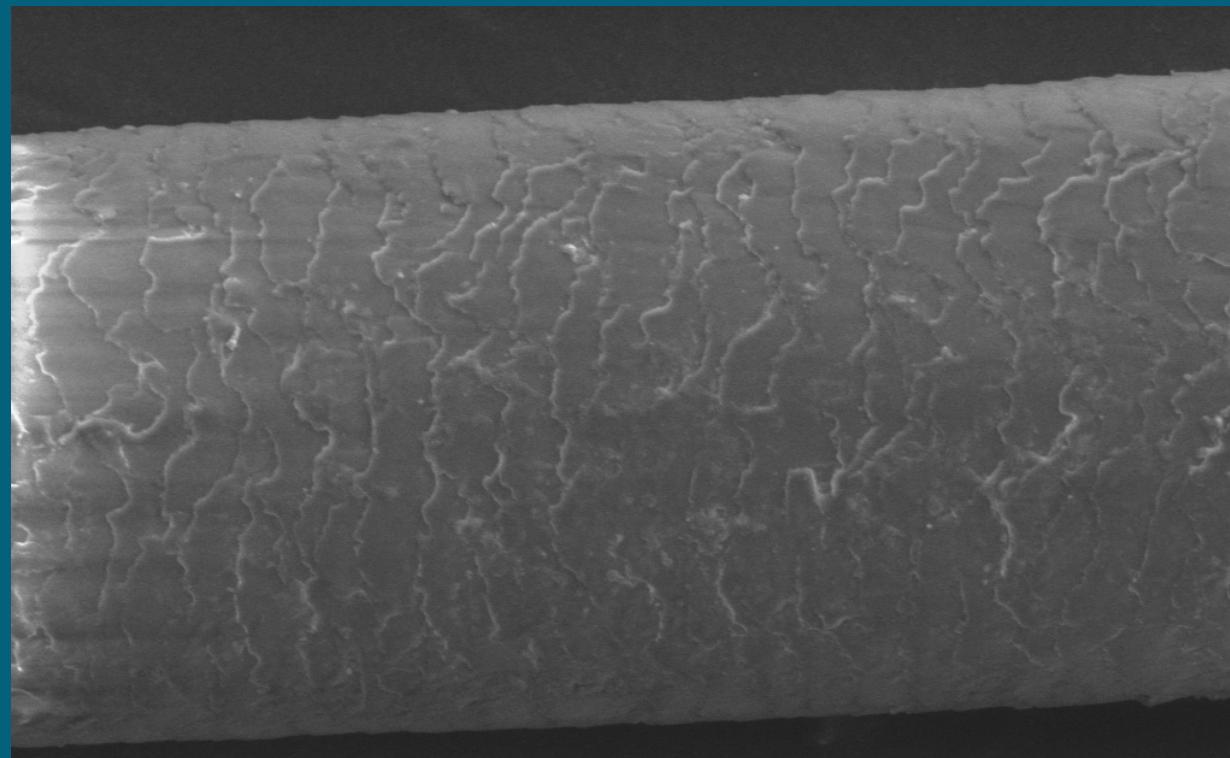


1 cm per month is AVERAGE  
MUST treat the time scale as “elastic”

# Hair Orientation

“Shingles” point towards direction of growth

TIP



ROOT

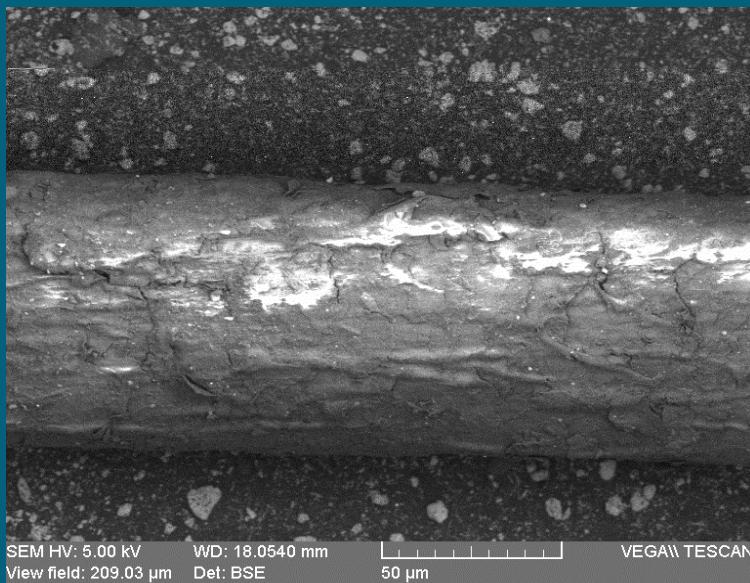
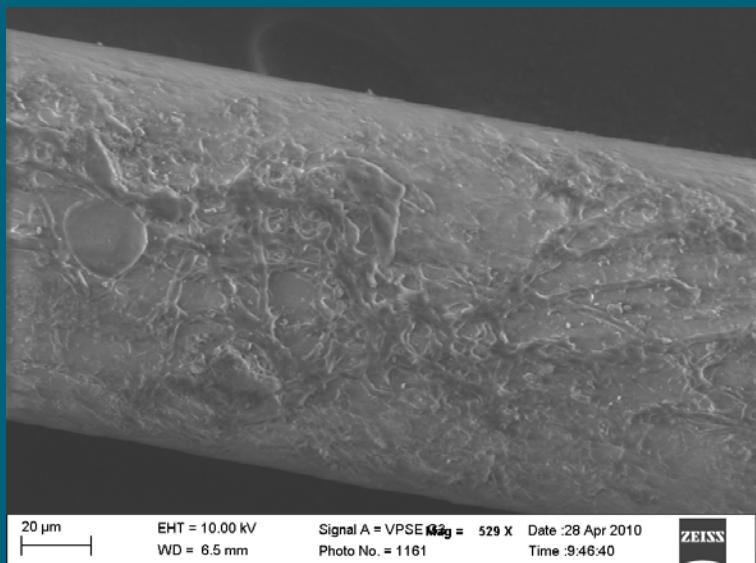
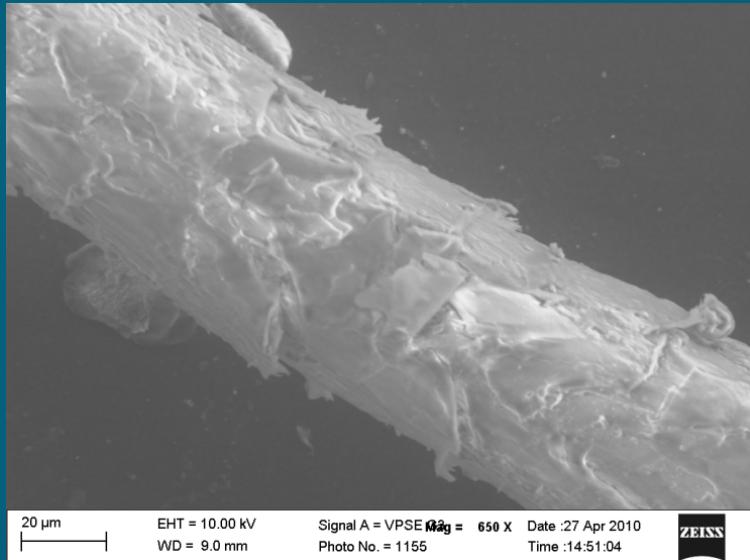
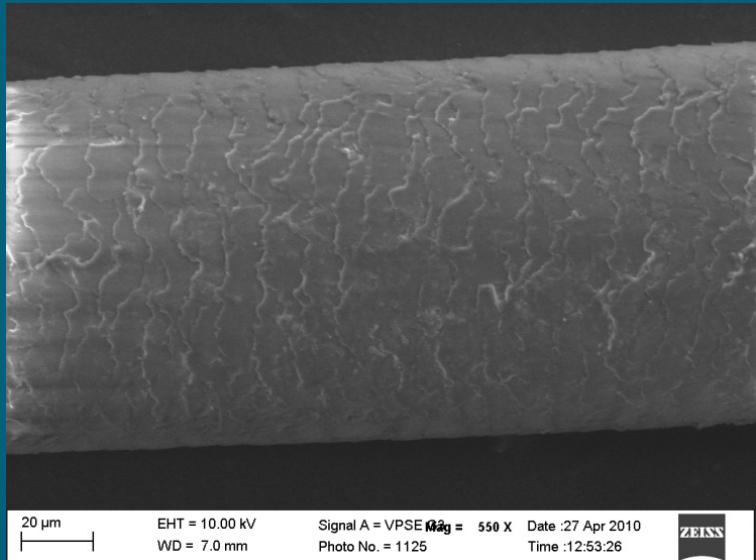
20  $\mu\text{m}$

EHT = 10.00 kV  
WD = 7.0 mm

Signal A = VPSE Mag = 550 X Date :27 Apr 2010  
Photo No. = 1125 Time :12:53:26



# Not all hair looks that good!



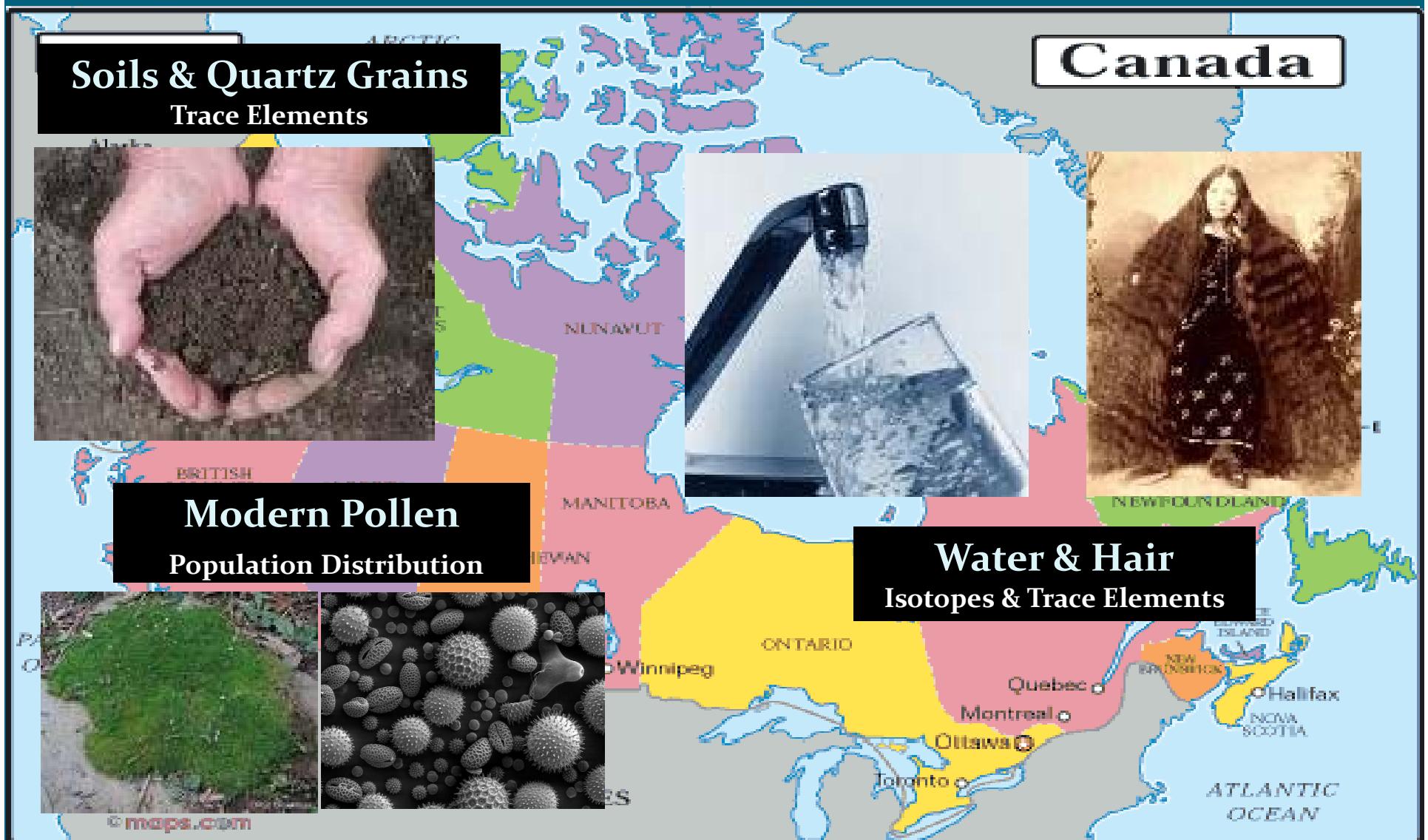
# Hair Reference Material

## Inter-laboratory Comparison ( $\delta D$ )

RM	$\delta^2H$ (‰) Ottawa U	$\delta^2H$ (‰) UK	$\delta^2H$ (‰) USGS	AVE $\pm$ SD
AND	-71.6	-72.9	-70.4	<b>-71.6 <math>\pm</math> 1.3</b>
COL	-87.9	-88.8	-87.8	<b>-88.2 <math>\pm</math> 0.6</b>
CAL-CAN	-106.4	-105.9	-109.8	<b>-107.4 <math>\pm</math> 2.1</b>
CAL-SAL	-101.2	-101.0	-102.9	<b>-101.7 <math>\pm</math> 1.0</b>

# Canadian Forensic Project

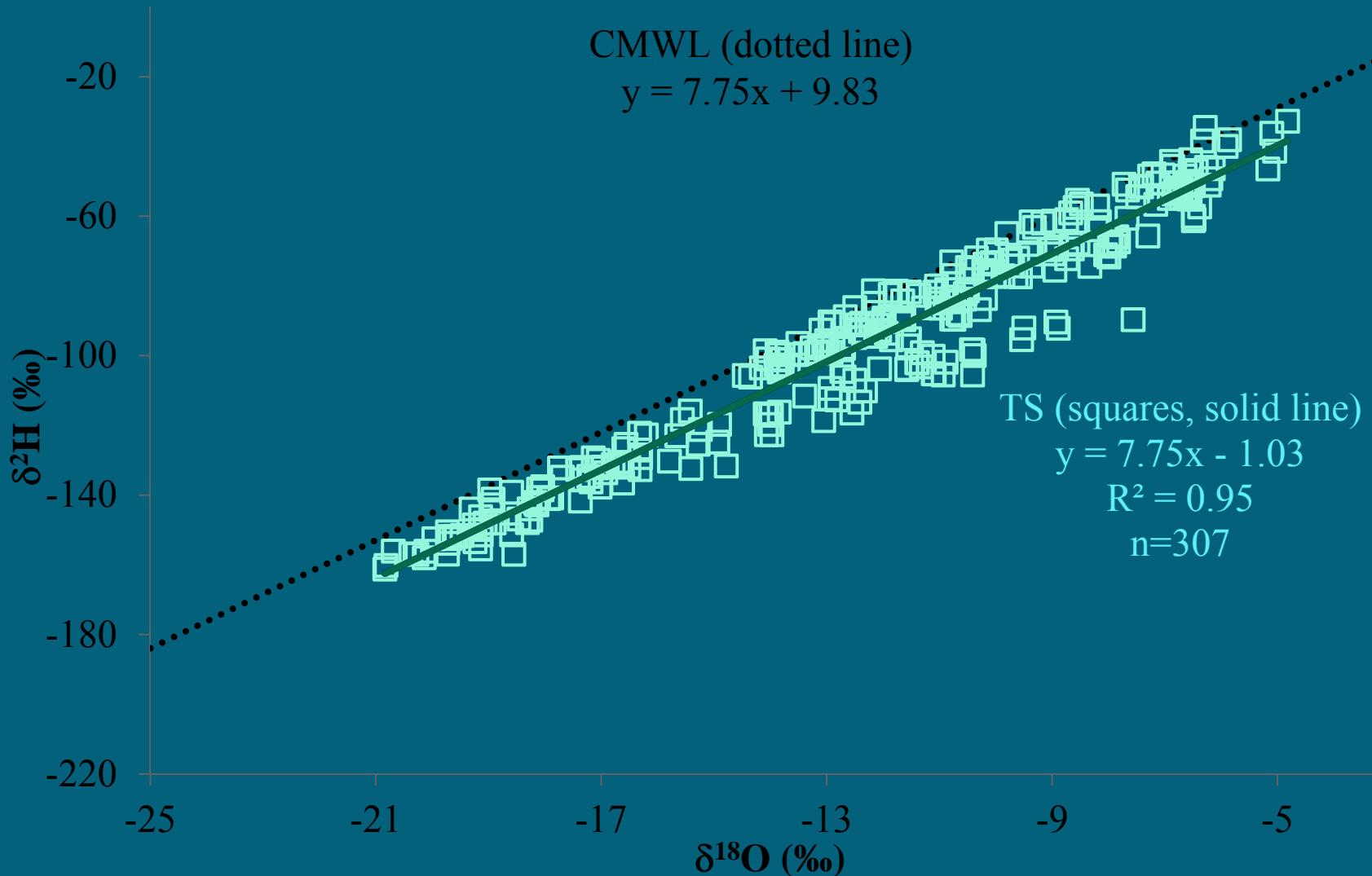
## 2009 - 2013



# Samples Collected

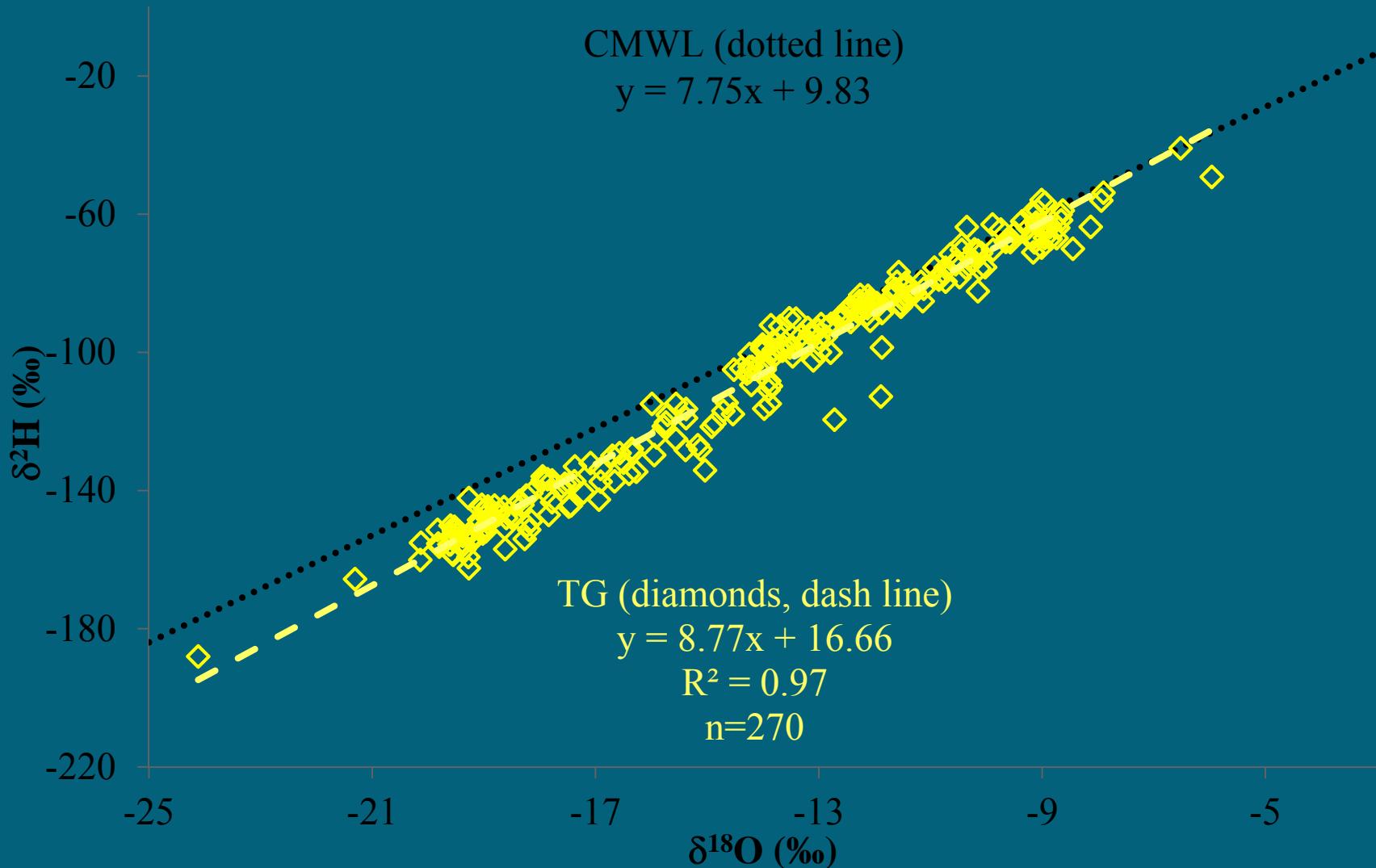
Where	Hair	Tap water	Other water	Soil	Pollen
ON, QC	140	140	15	243*	243*
NB, NS, PE, NL	131	136	39	246	253
MB, SK	100	114	27	150	150
AB, BC	216	188	41	234	234
<b>TOTAL</b>	<b>587</b>	<b>578</b>	<b>122</b>	<b>873</b>	<b>880</b>

# Canadian Tap Water Groundwater

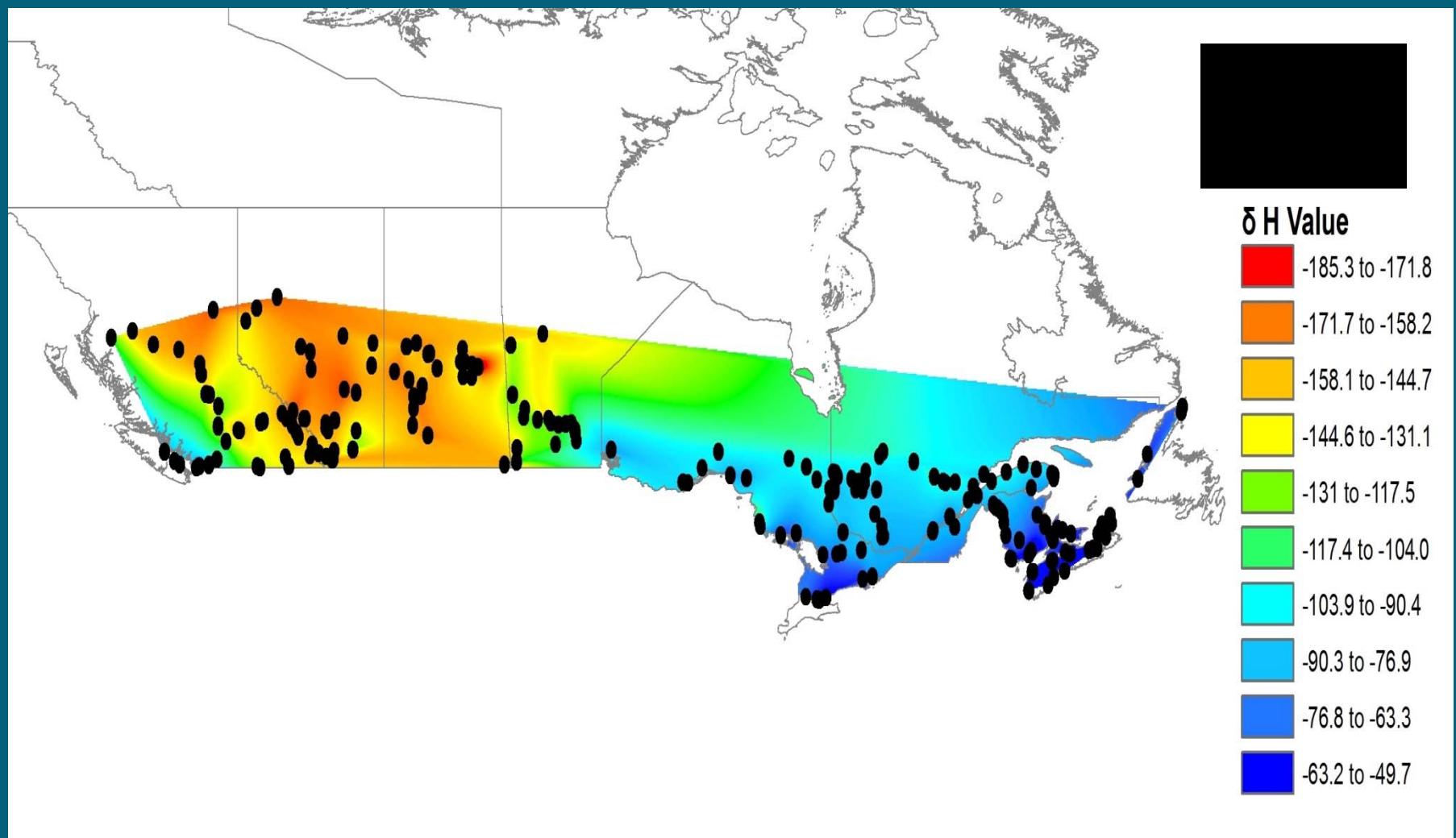


# Canadian Tap Water

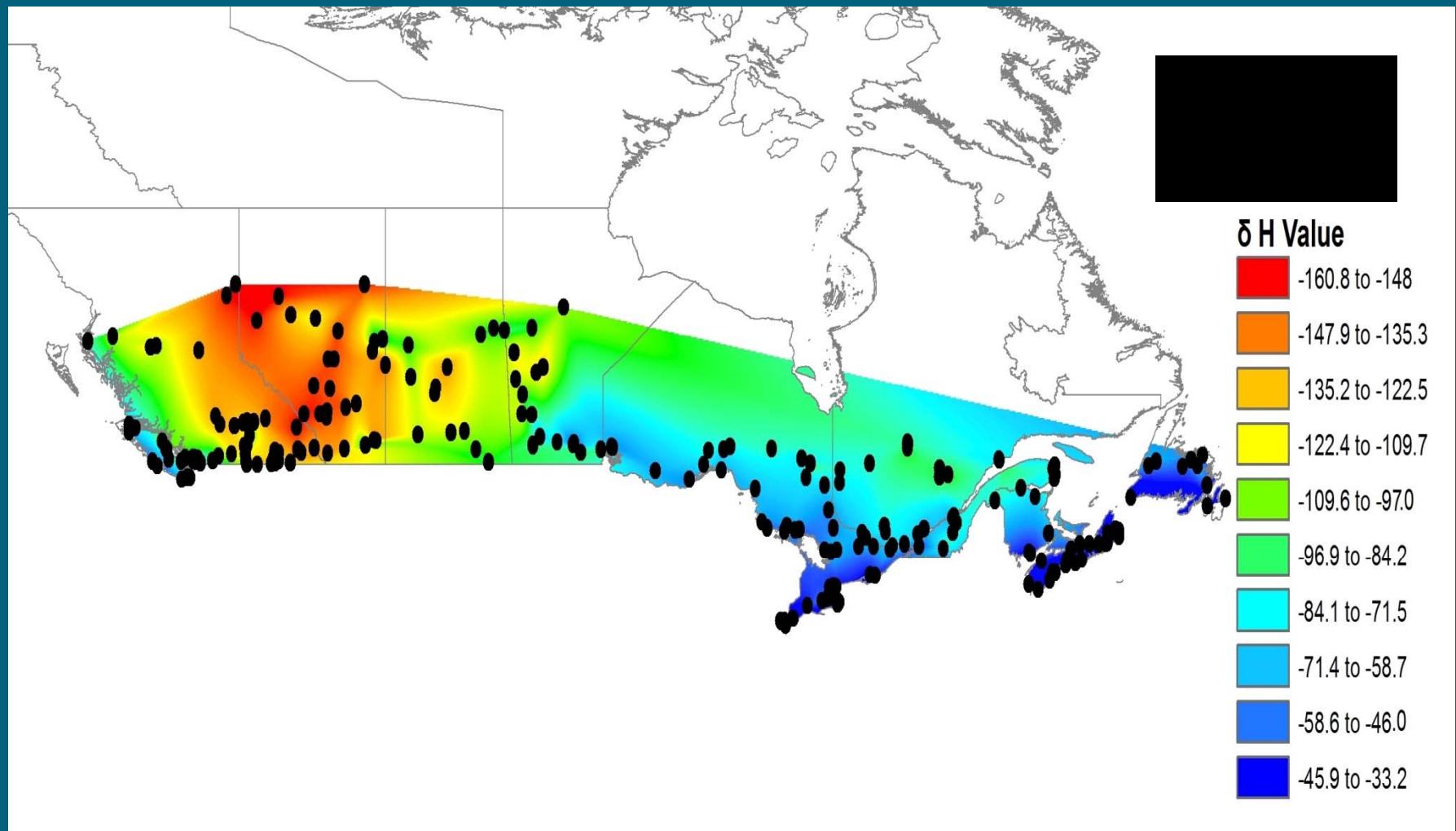
## Surface water



# Canadian Tap $\delta^2\text{H}$ from Ground Source

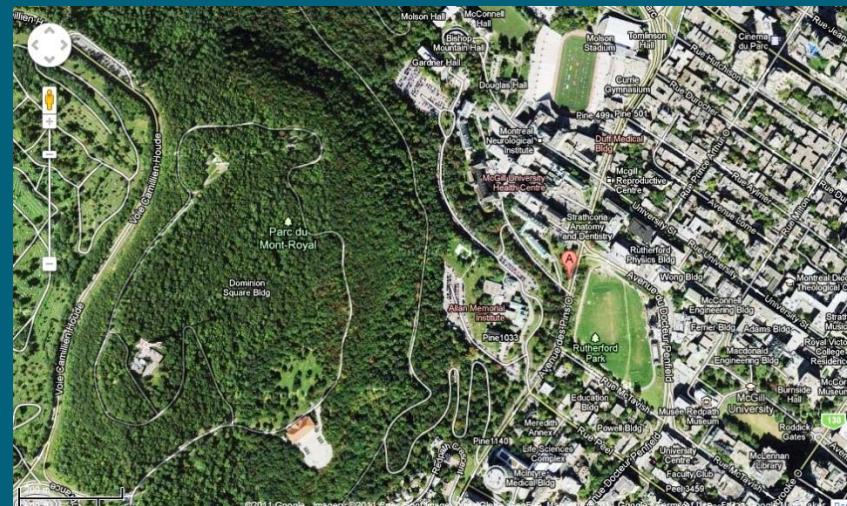


# Canadian Tap $\delta^2\text{H}$ from Surface Source

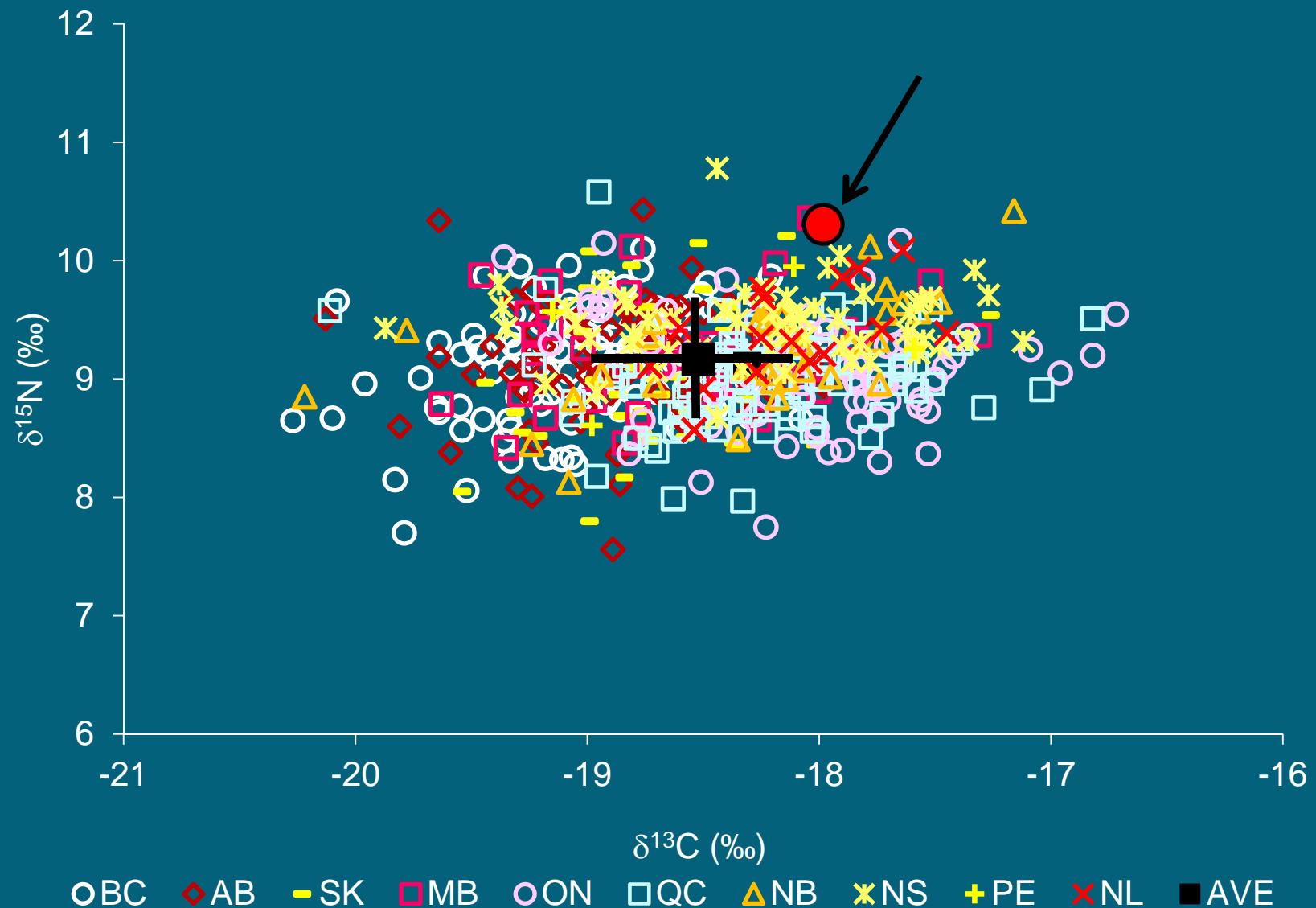


# Case 1: Madame Victoria

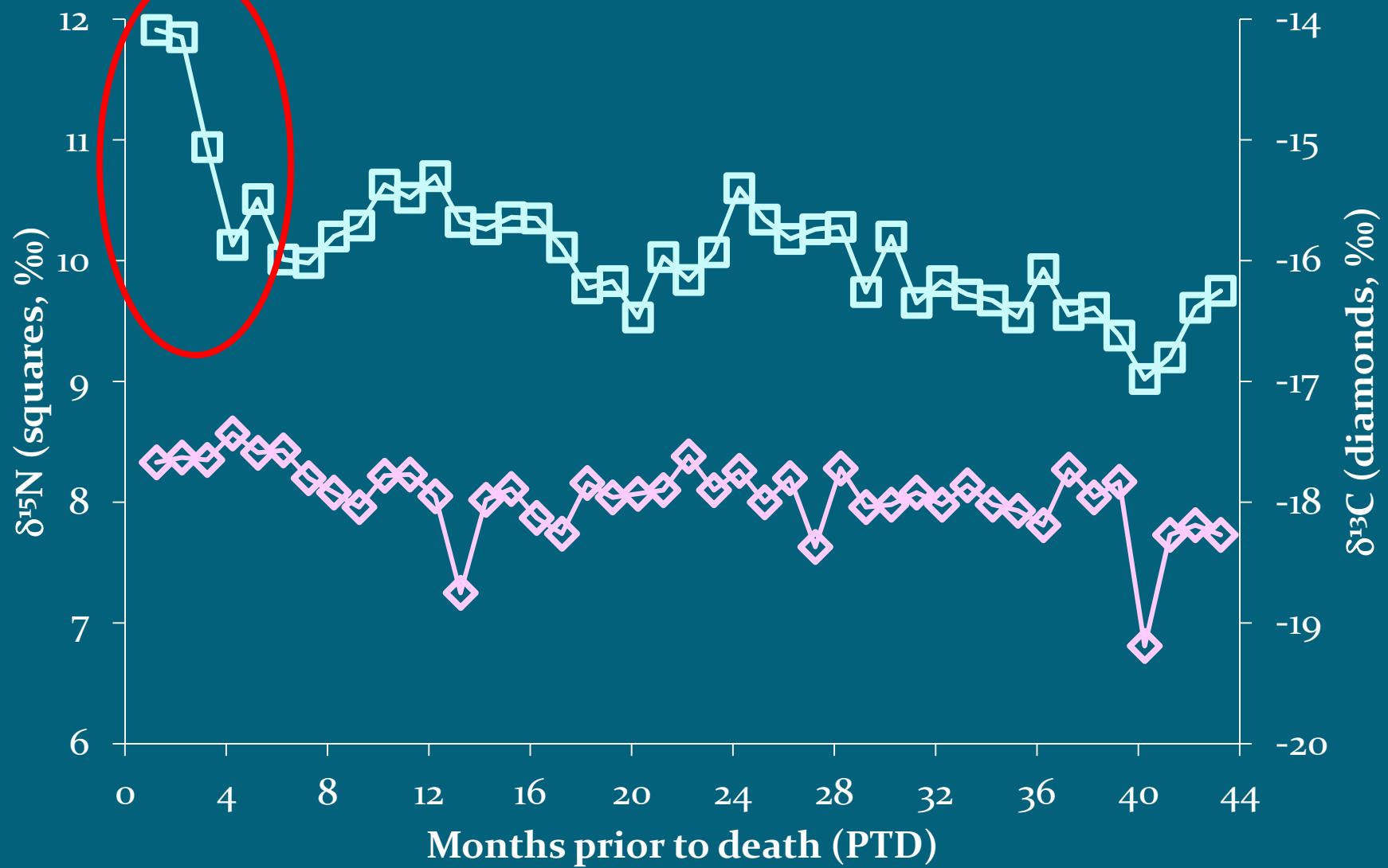
- Found near Royal Victoria Hospital in 2001
- Estimate died 2 years previous
- Received ~ 80 hair
  - Not-oriented
  - 43 cm long



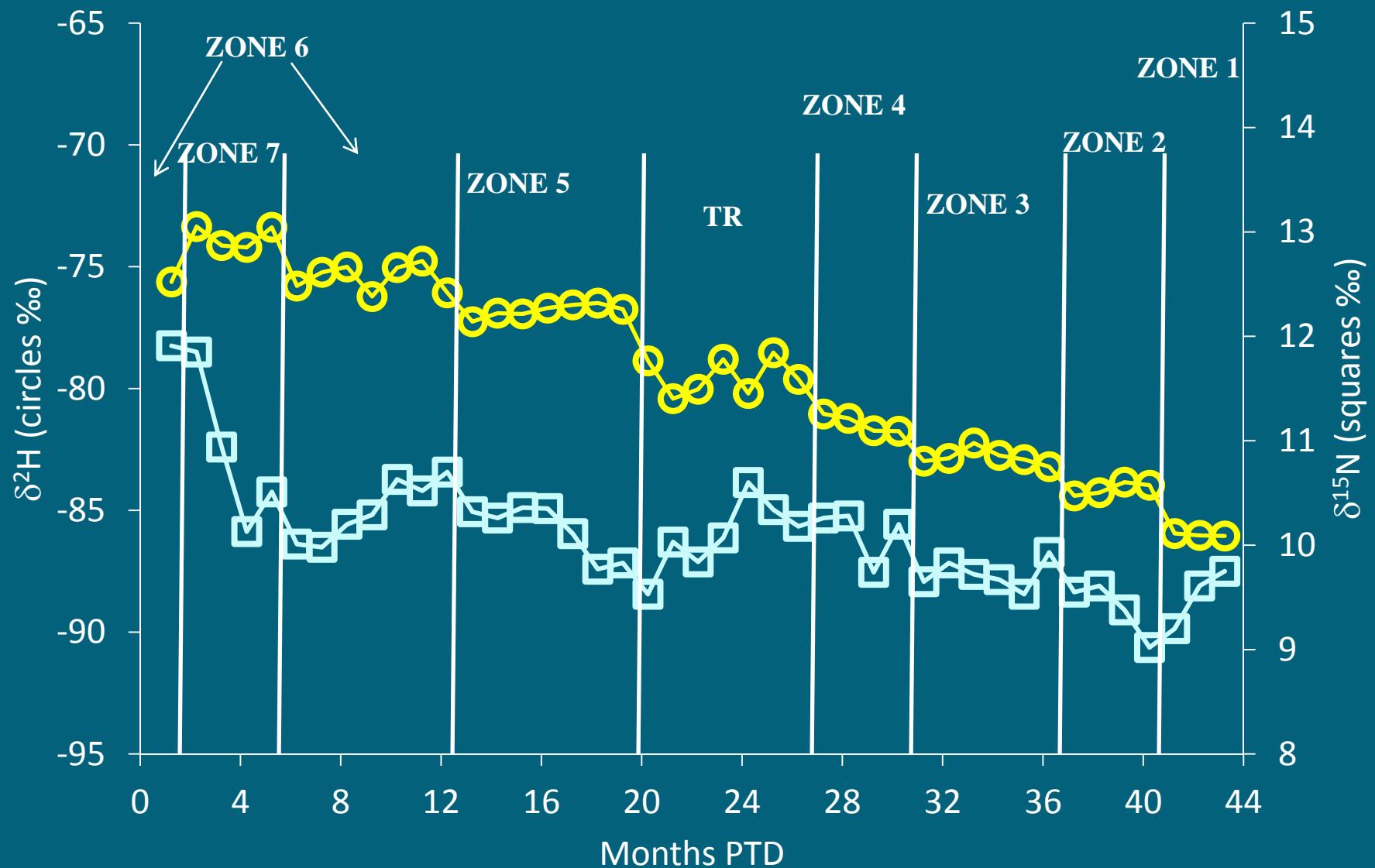
# Madame Victoria- CN



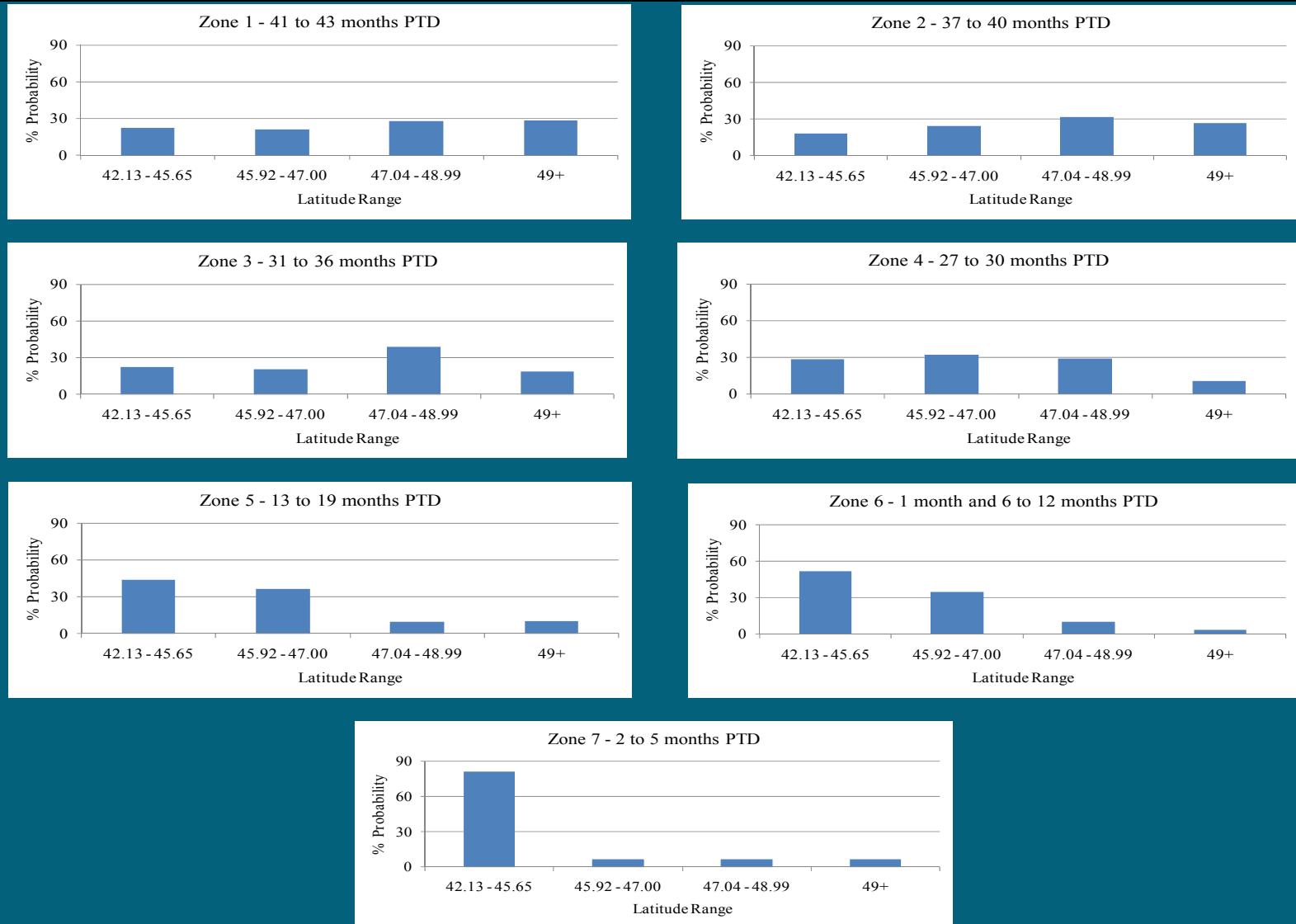
# Madame Victoria- CN



# H and N Isotopes- Zones



# Latitude Range Probability



**Figure 7.** Graphs depicting the probability that Mme Victoria resided in certain latitude ranges during the last 43 months PTD. For an explanation, see text.

# Custom Software for Analysis

- Signature<sup>tm</sup> database engine software developed by CSDi
- Used for all types of samples and analyses
  - Water and hair— isotope and trace element profile
  - Soil trace element profile
  - Pollen speciation profile
- Searches database and visually displays data
- Database mining to predict locations of unknown samples based on analysis profiles

# Zone Ranges

Variable Ranges Display

Select a Variable:

- del2H
- del34S
- del13C
- del15N

Values info:  
Min: -120.914  
Max: -68

Set Ranges Automatically

Clear All Current Ranges

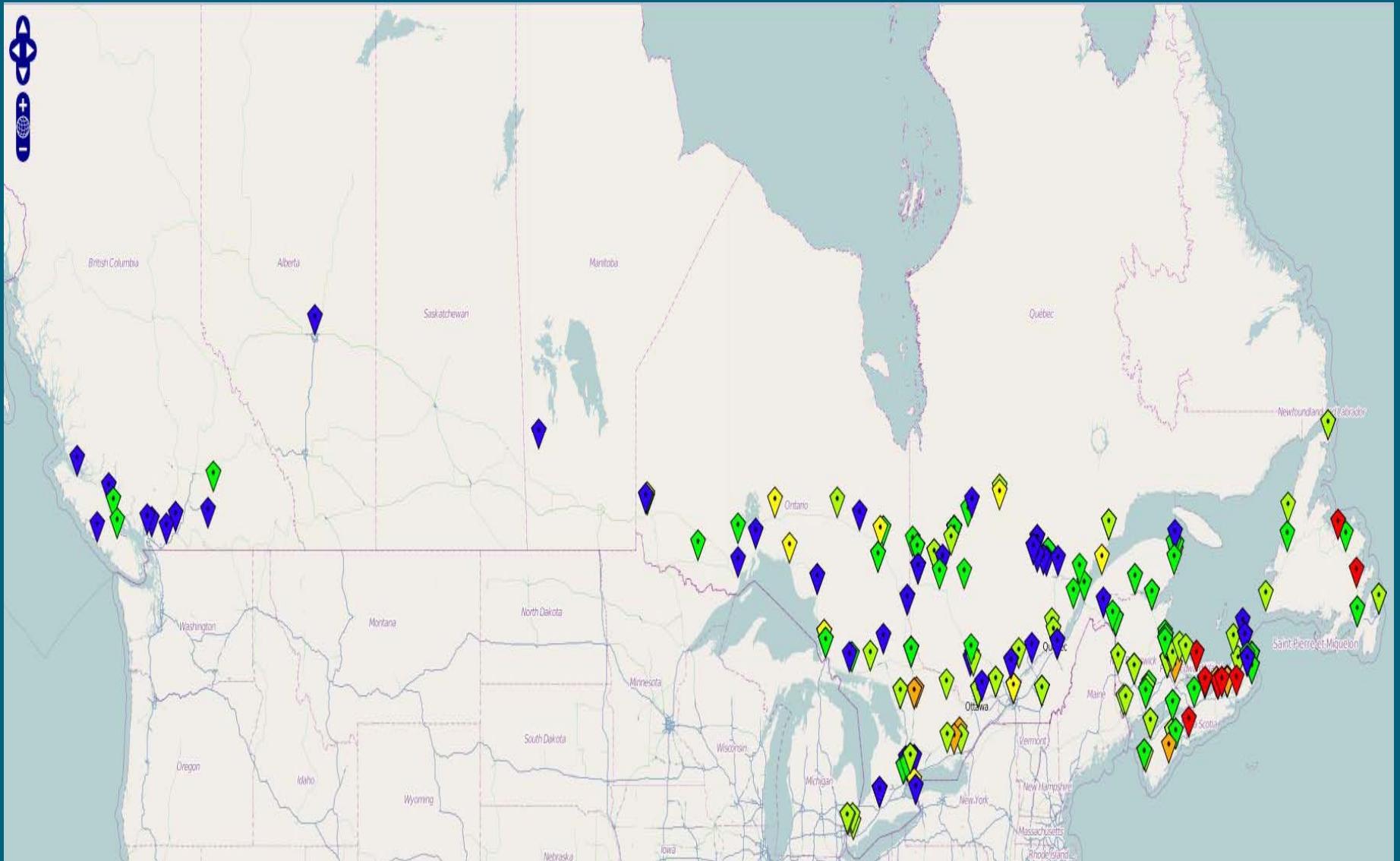
Set Ranges for GPS:

-75.8	to	-71.8
-77.5	to	-73.5
-78.8	to	-74.8
-83.4	to	-79.4
	to	
-88.0	to	-84.0

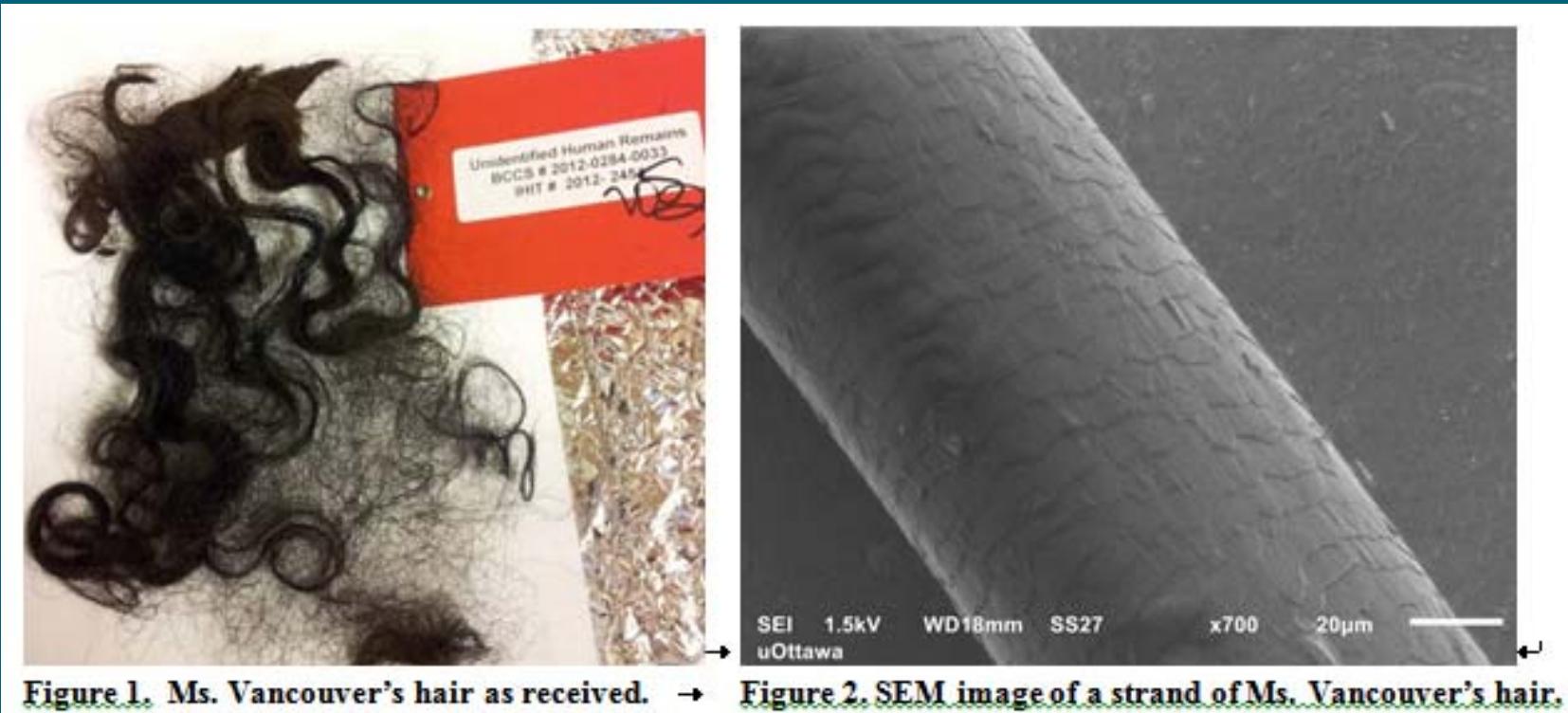
Ignore Out of Range Values

Generate Close

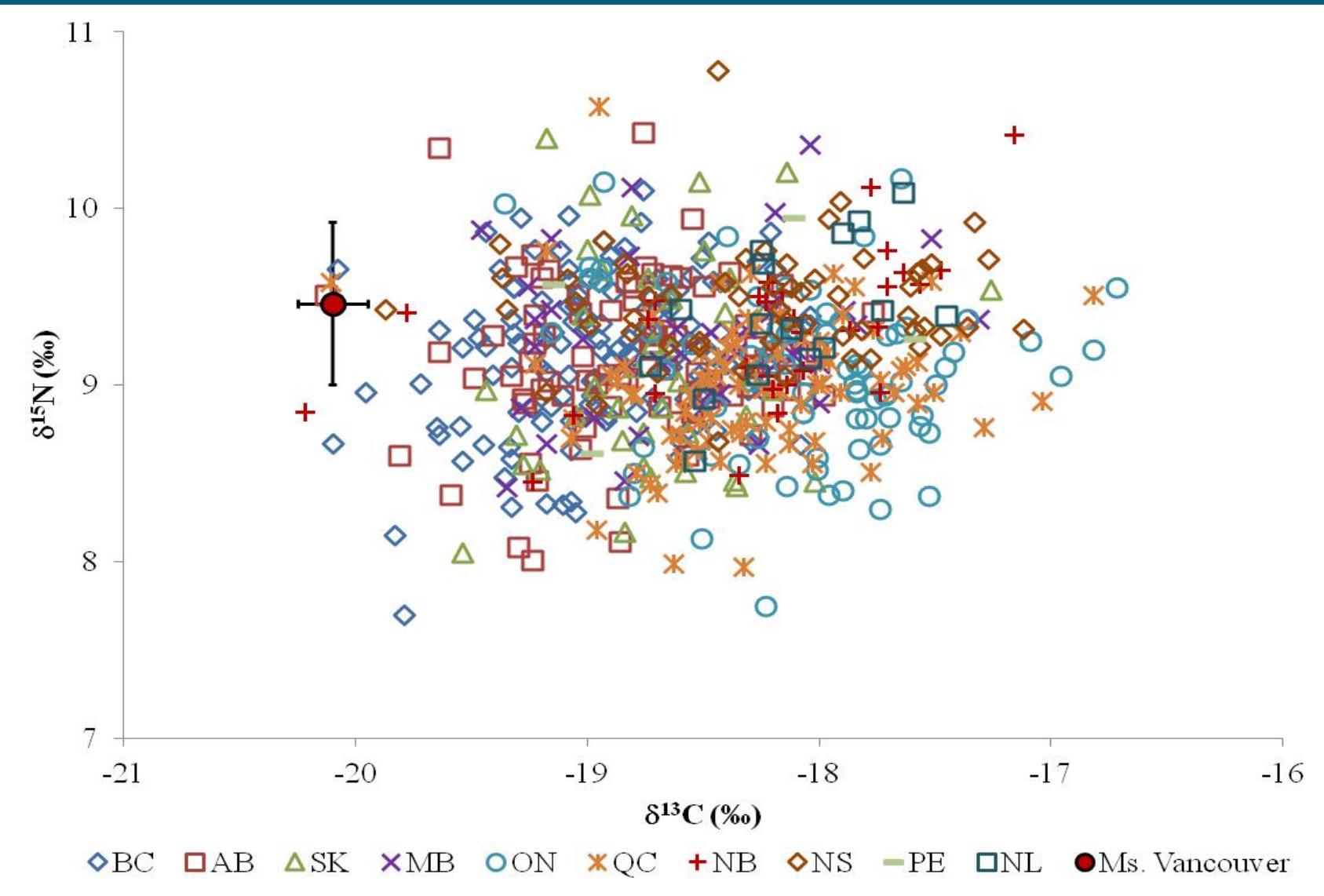
# Zone Ranges (All)



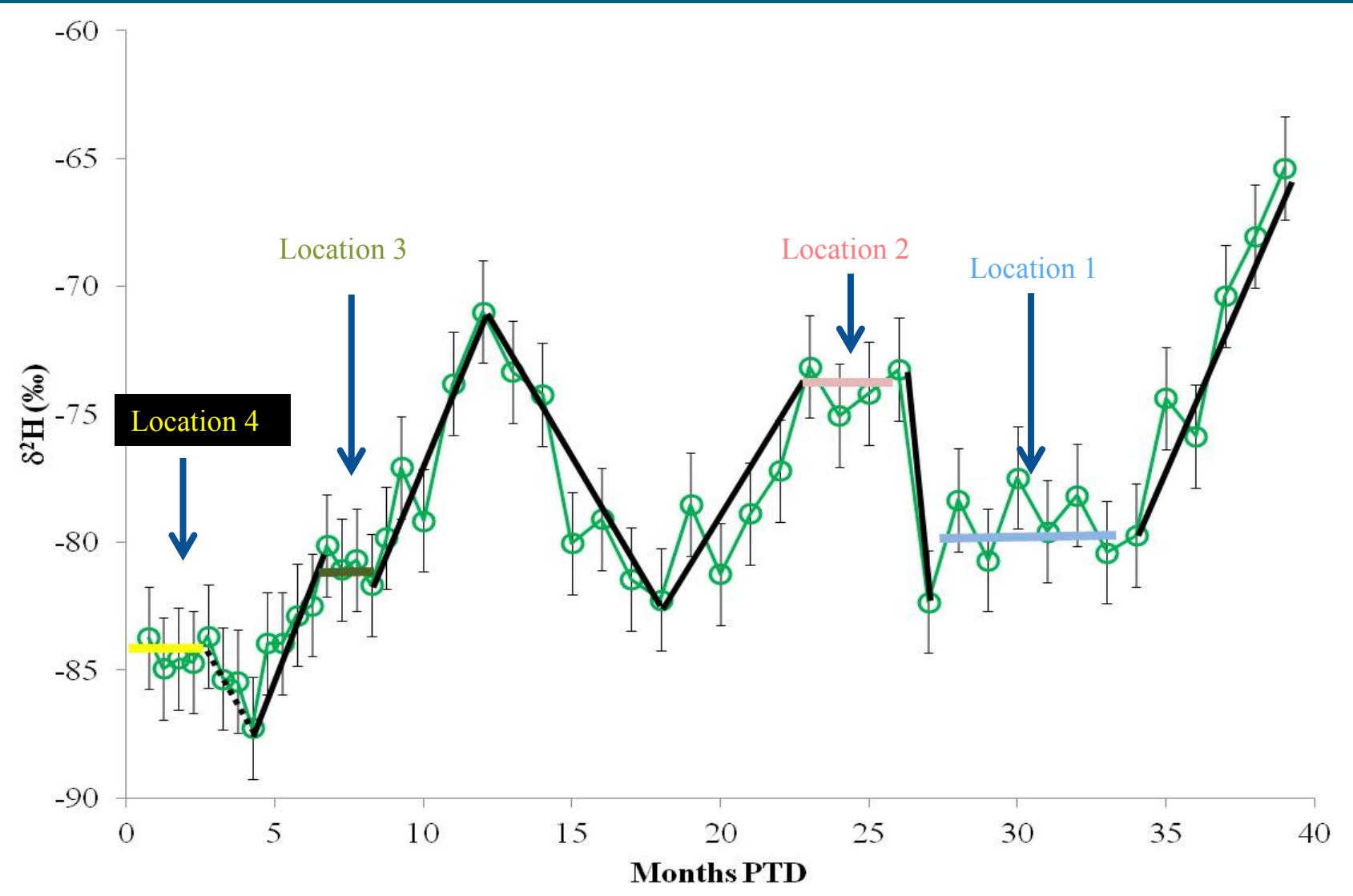
# Case 3: Madame Vancouver



# Madame Vancouver



# Madame Vancouver



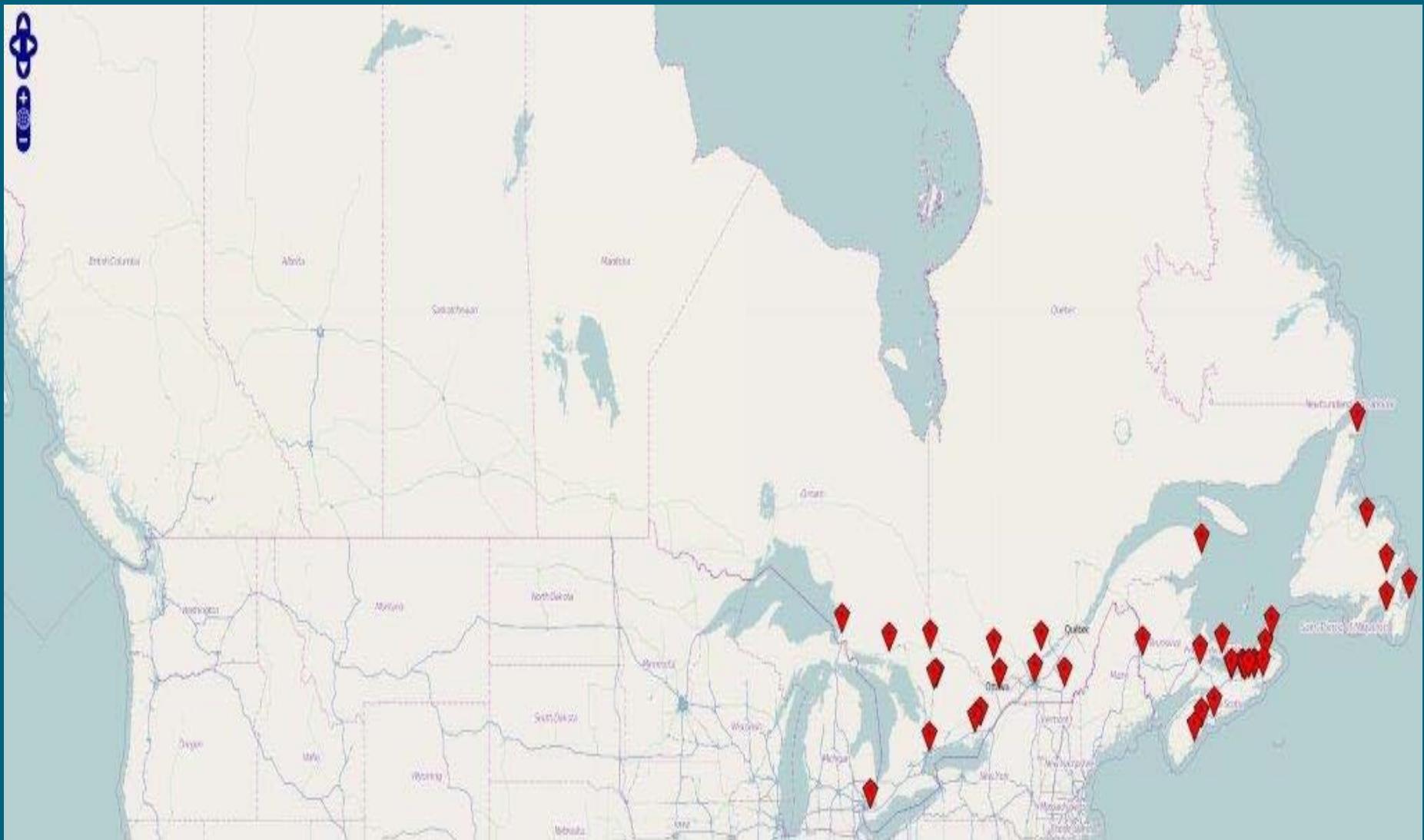
# Ms.Vancouver: Location 1

34 to 28 months PTD



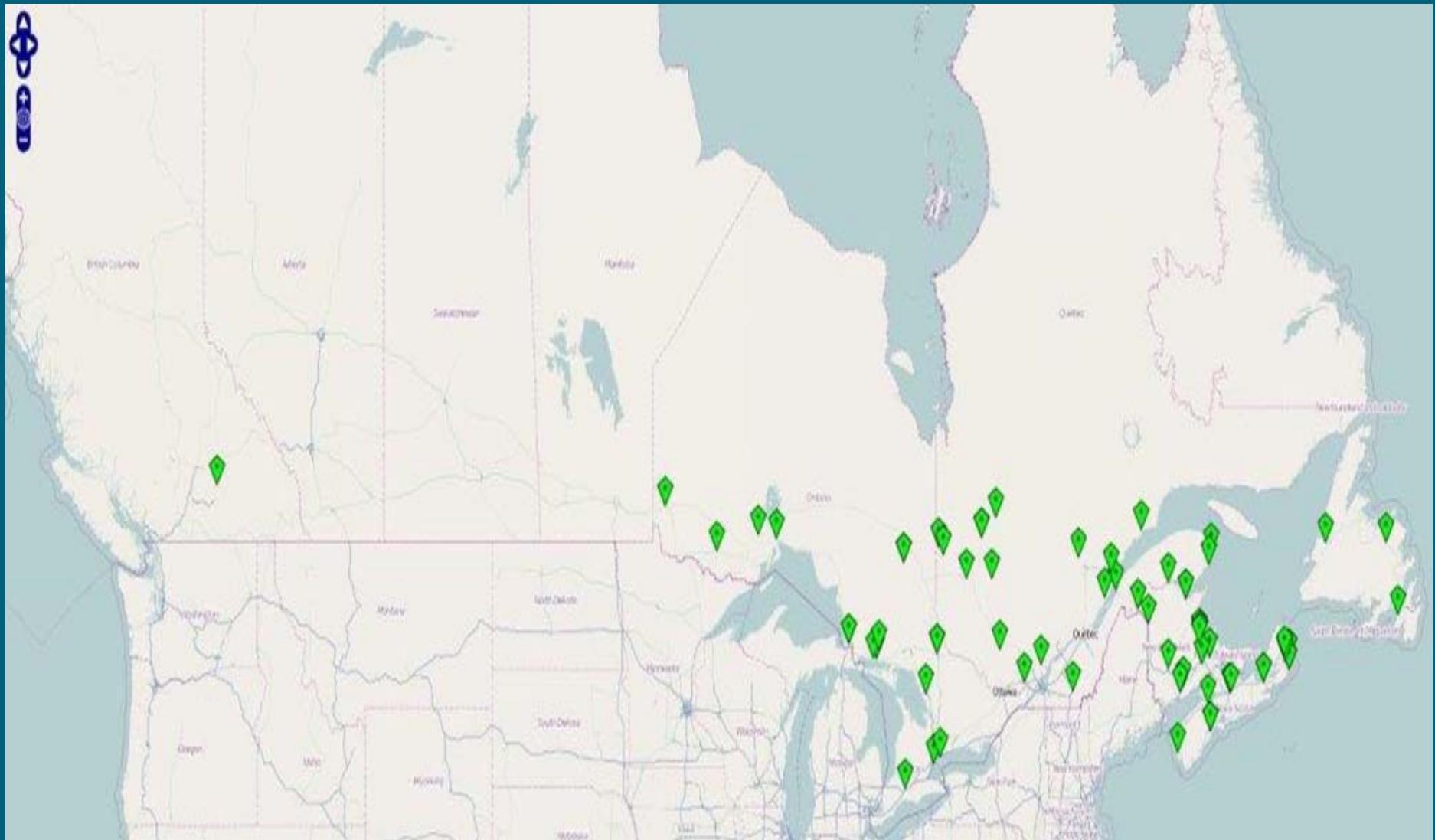
# Ms.Vancouver: Location 2

26 to 23 months PTD



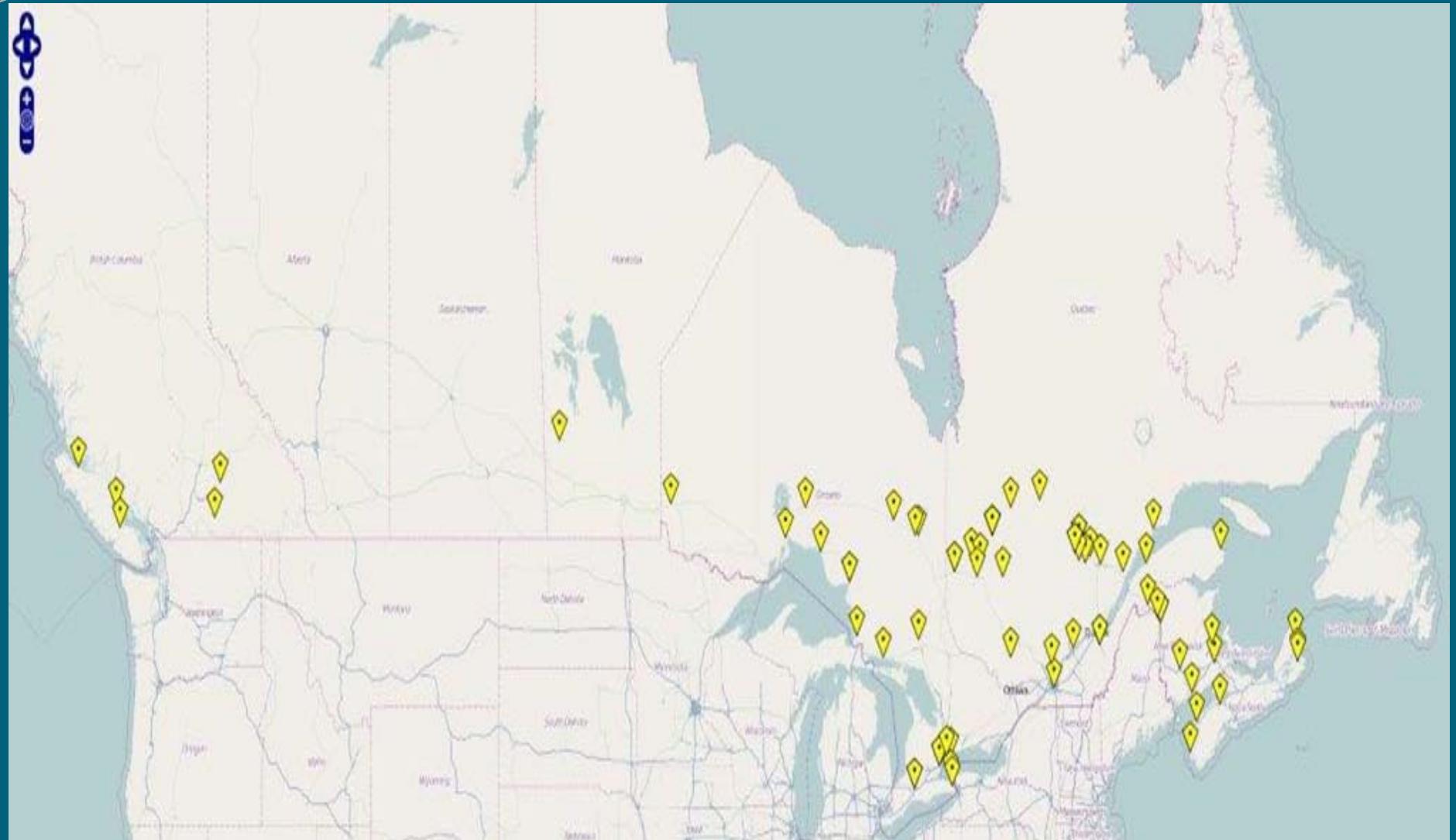
# Ms.Vancouver: Location 3

8 to 6.5 months PTD

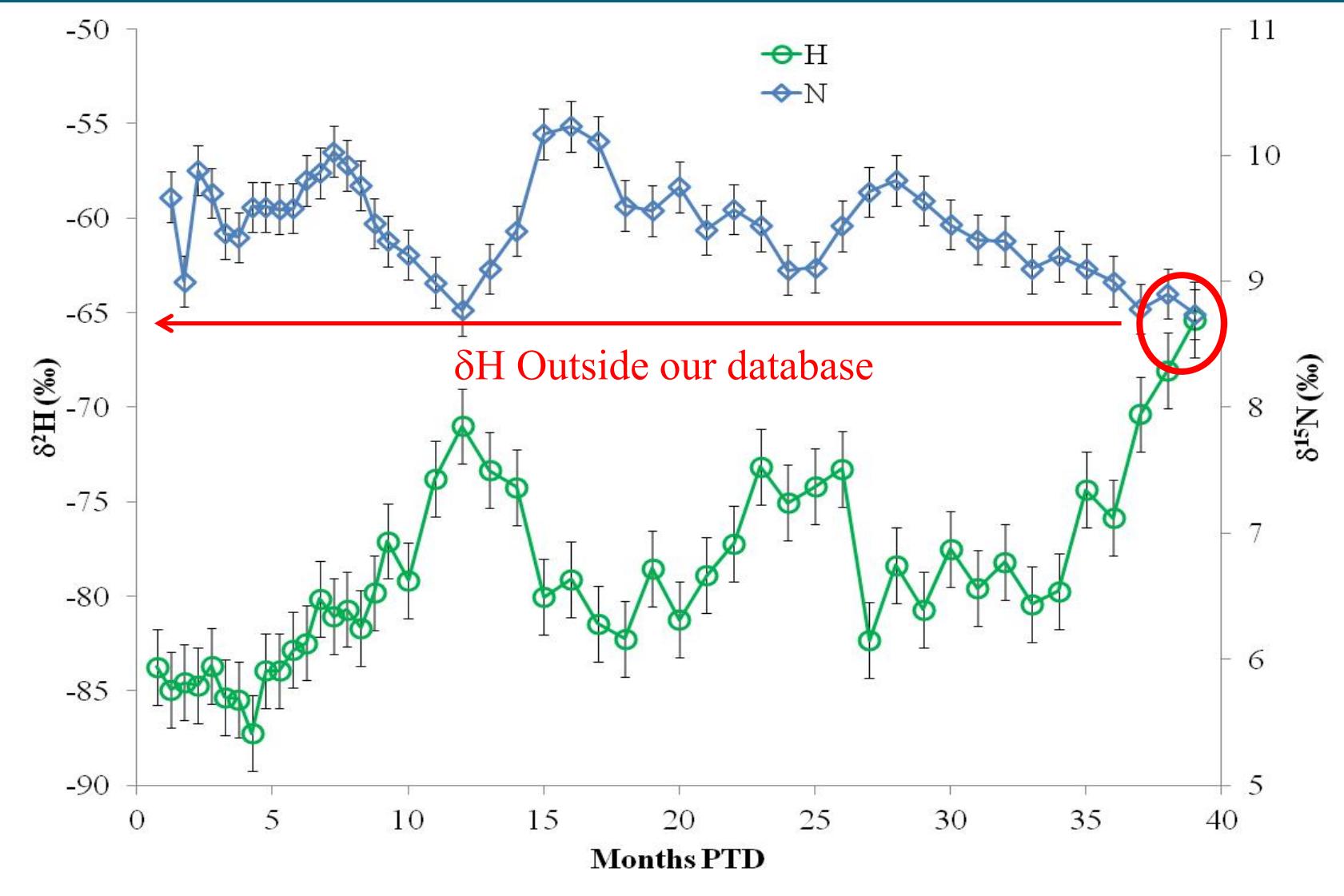


# Ms.Vancouver: Location 4

2.5 to 0.5 months PTD



# Madame Vancouver



# Overall Summary

- Techniques have been developed that can aid in the geo-location of forensically-relevant samples (e.g. hair, soil, pollen)
- Hair, water, soil and pollen databases have been populated in a Canadian database
- Isotopic analysis of hair can be used to track movements in unidentified persons

## Case 2: Mr. Halifax

- Decomposing body found Oct 8, 2004 near Halifax airport
- Hair was partially in dreadlocks, ~ 13 cm



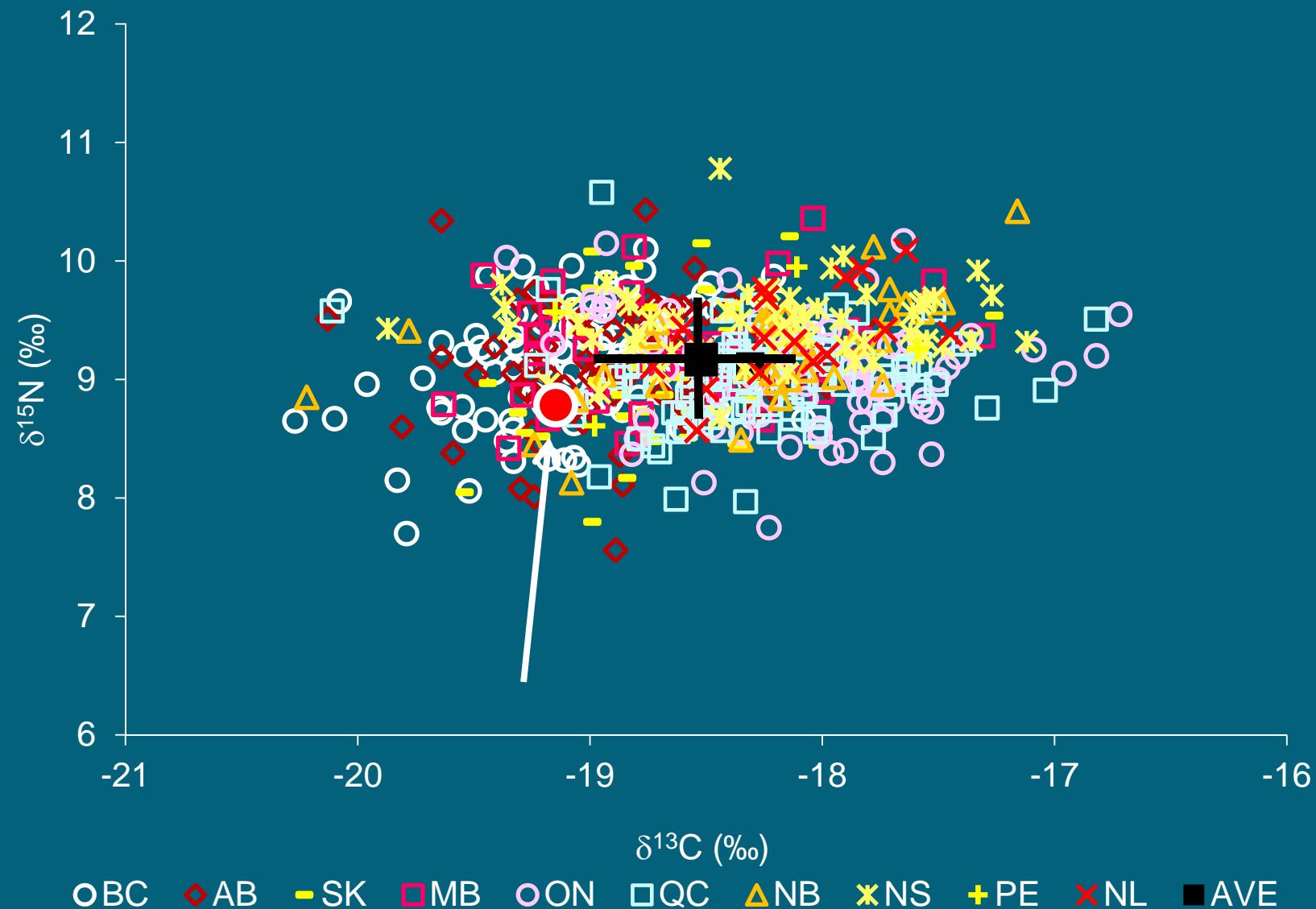
# Hair for Mr. Halifax

- When choosing hair for analysis, tried to pick hair that was ~ 0.5 cm  
NOT SO EASY!!

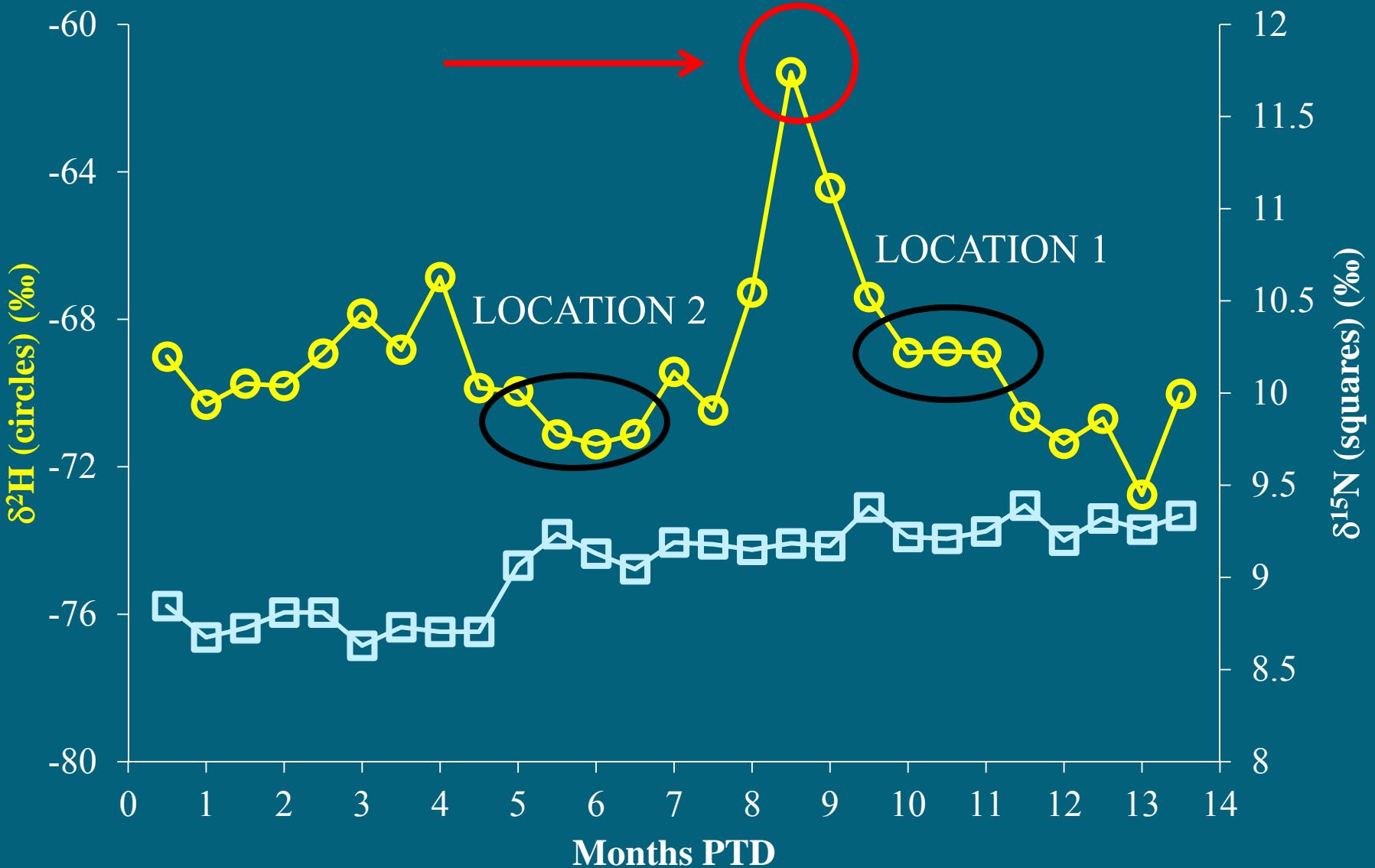
Time axis is ELASTIC



# Mr Halifax- CN

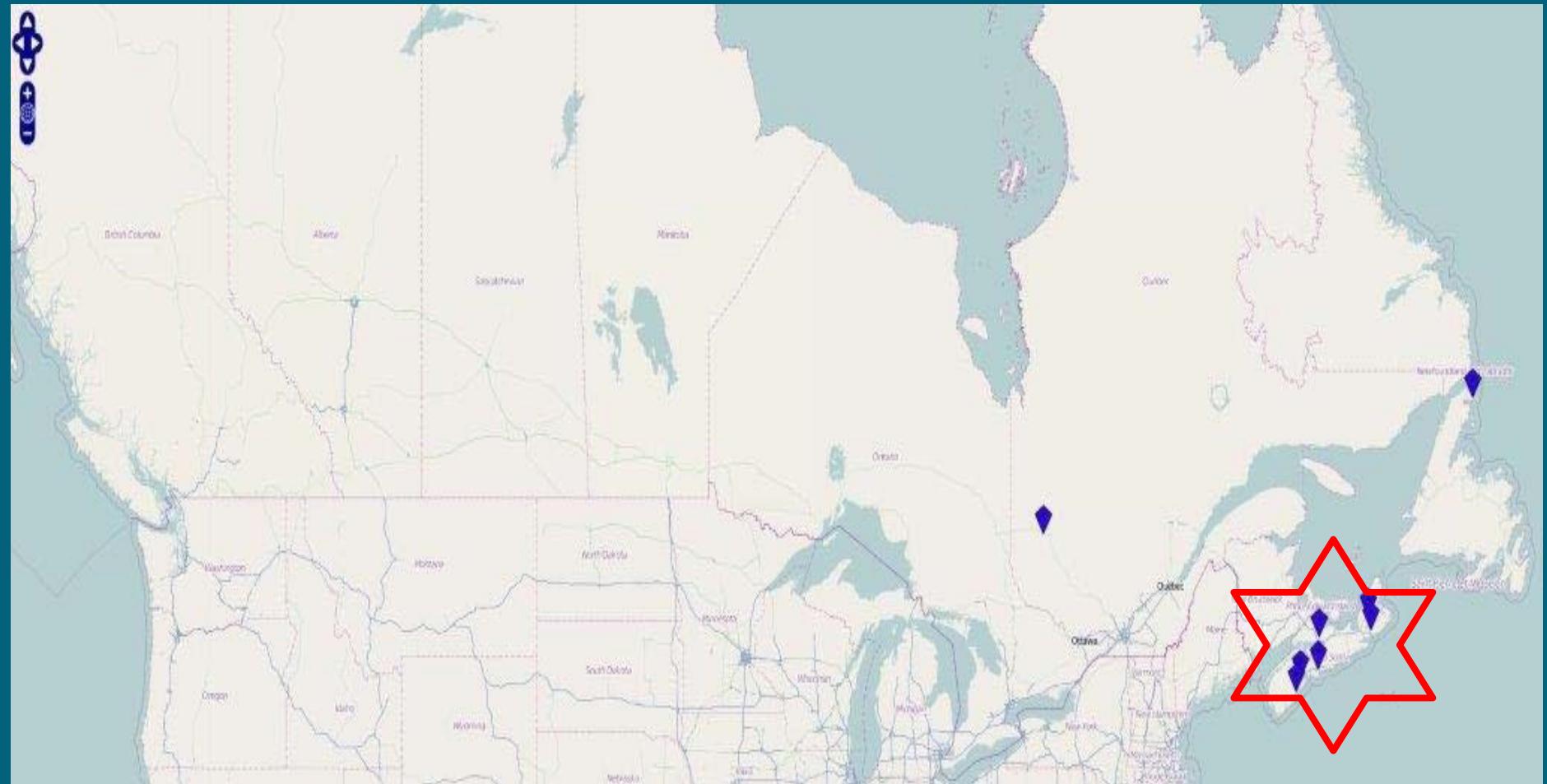


# Mr. Halifax – H & N



# Location 1

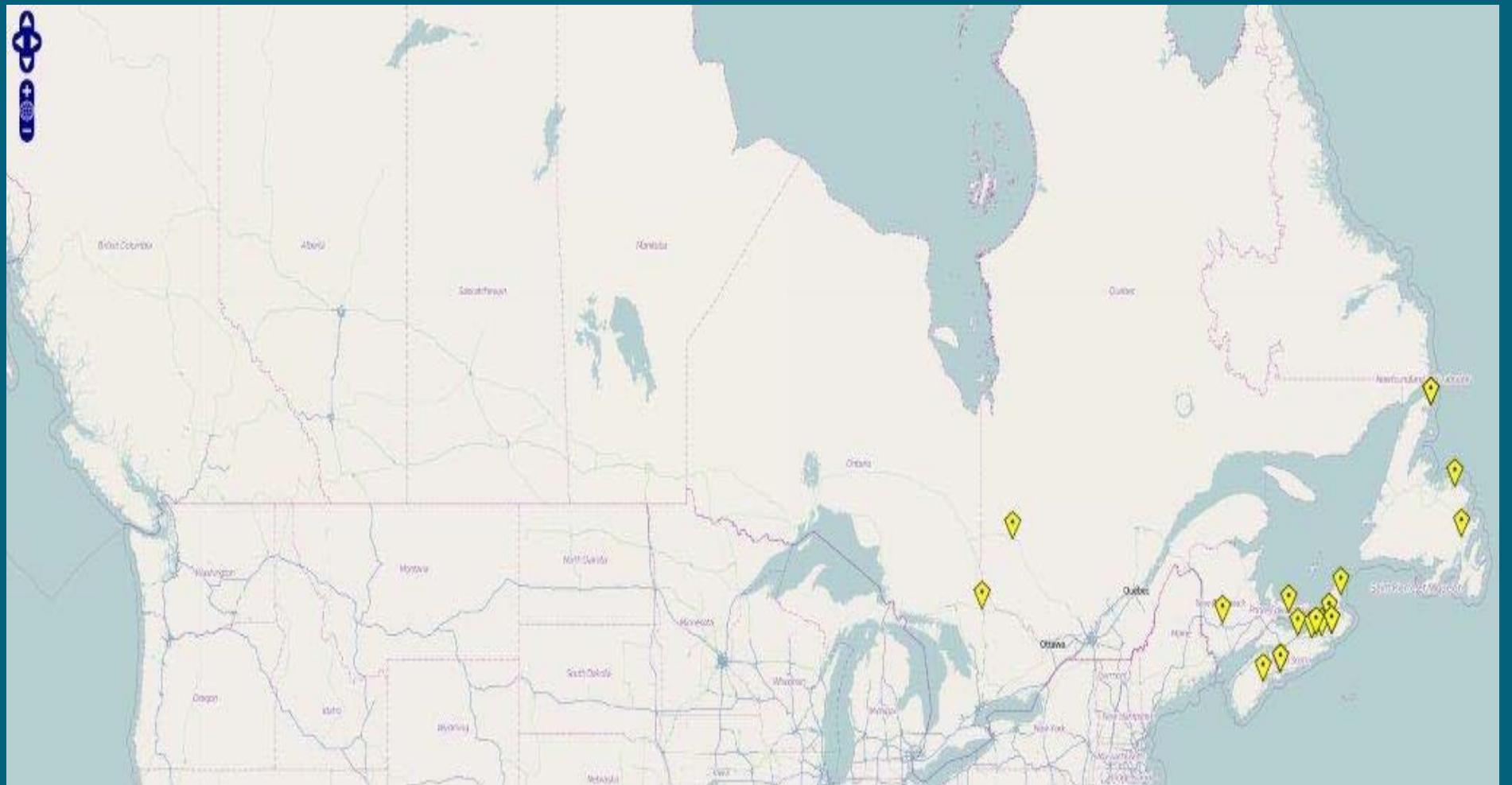
(10-11 months PTD)



$-68.9 \pm 2 \text{ \%o}$

# Location 2

(5.5-6.5 months PTD)



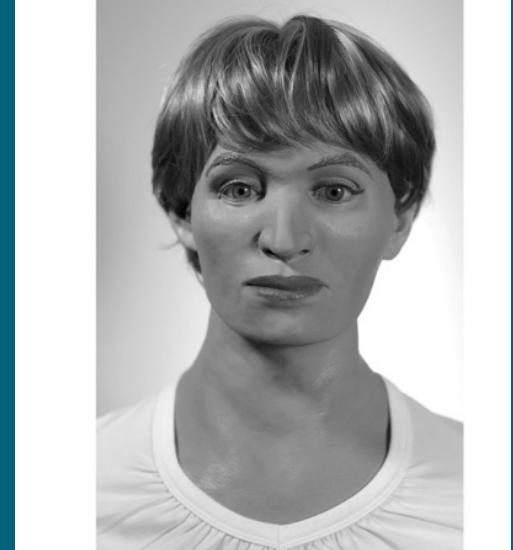
$-71.2 \pm 2\%$

# Summary – Mr. Halifax

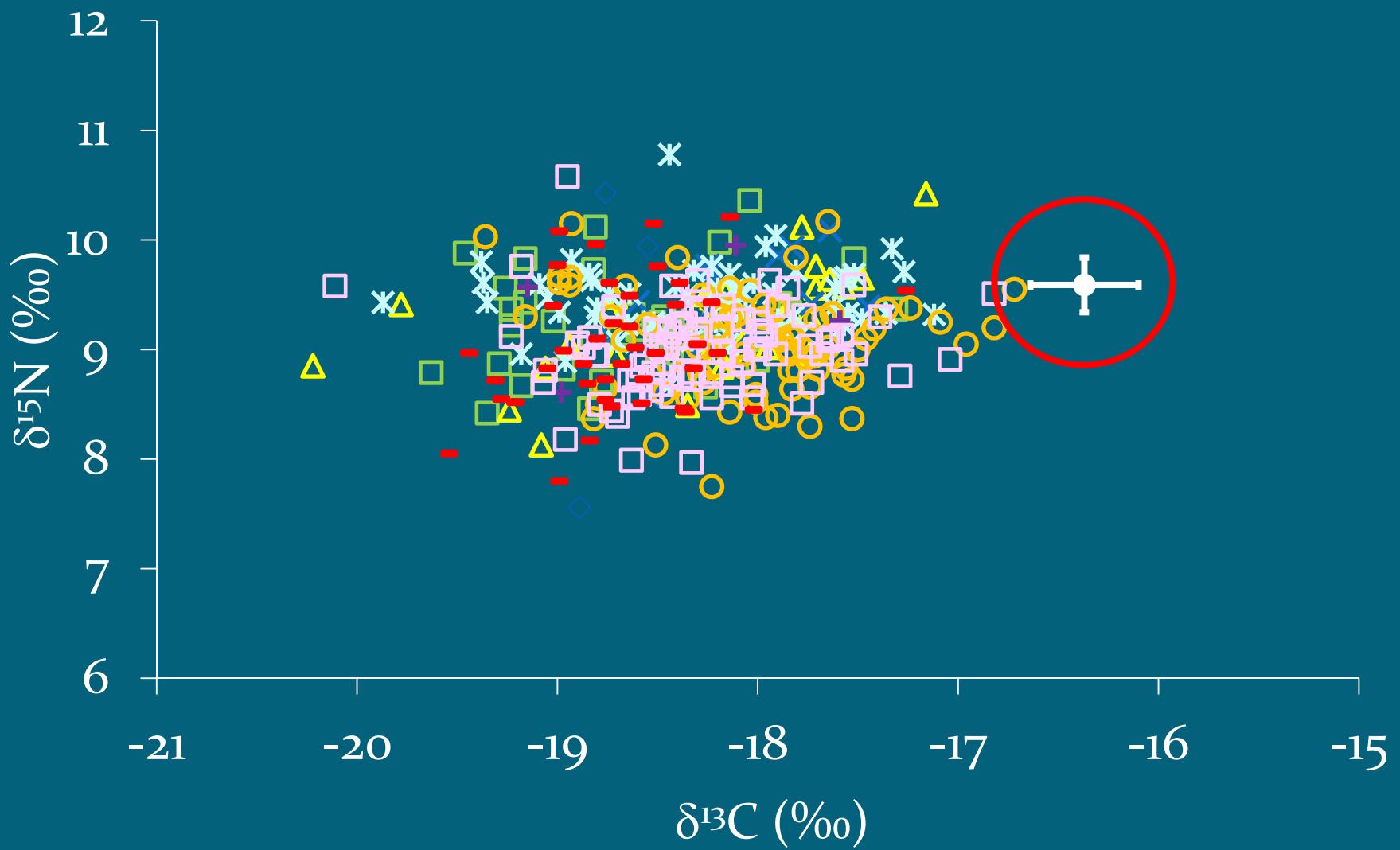
- $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were consistent with a North American omnivore
- Between 8 and 10 months PTD he likely travelled south for a short period of time
- Two locations where he may have resided for 1.5 to 2 months was the East Coast of Canada, or the North/Central east coast of US  
*(IDENTIFIED... actually travelled to Florida)*

# Ms. Napanee

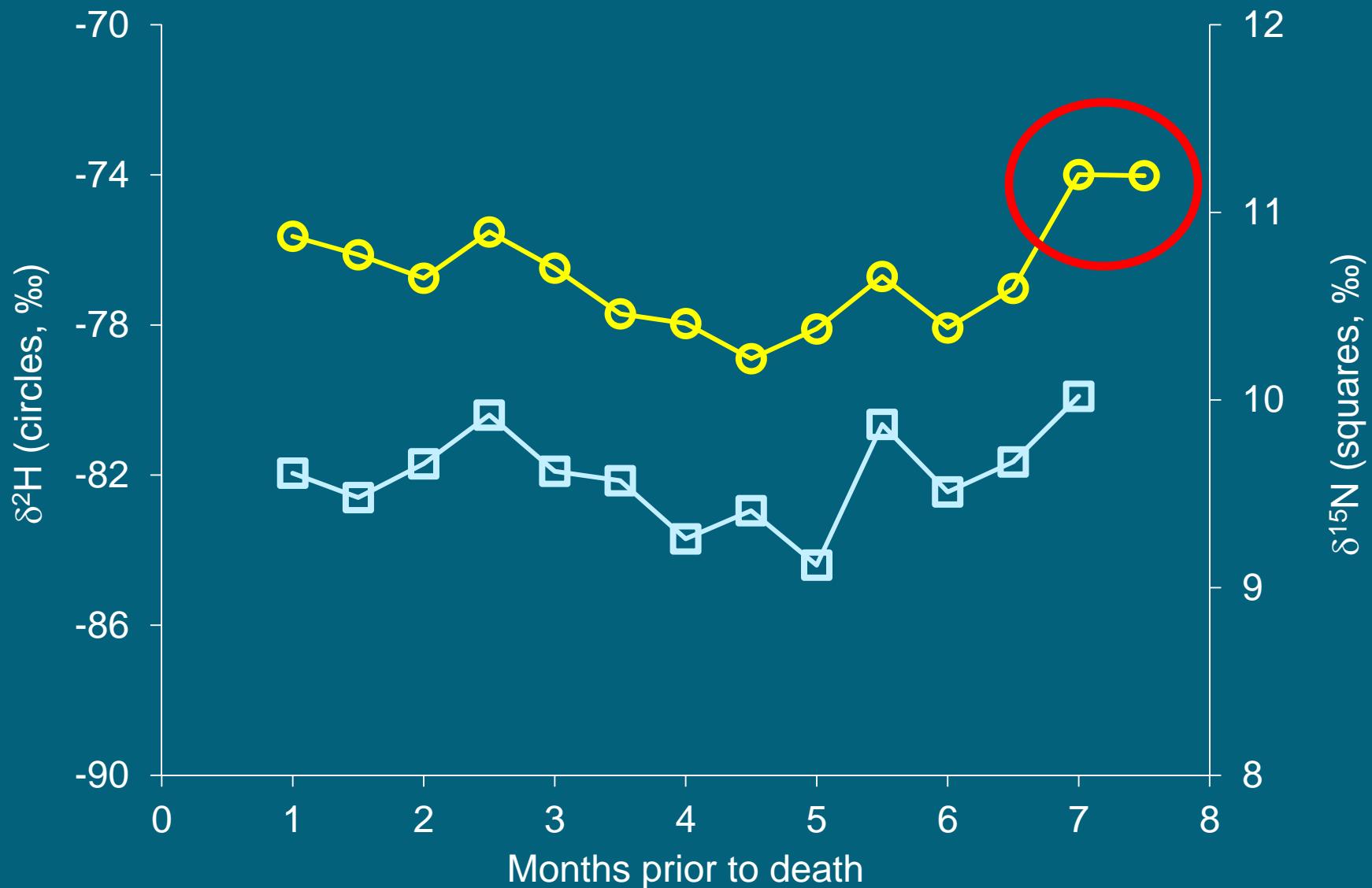
- Skeletal remains found Dec 30, 1984 near Napanee, ON
- Blond hair ~ 8 cm long, non-oriented
- Collected what looked like locks of hair from the evidence bag



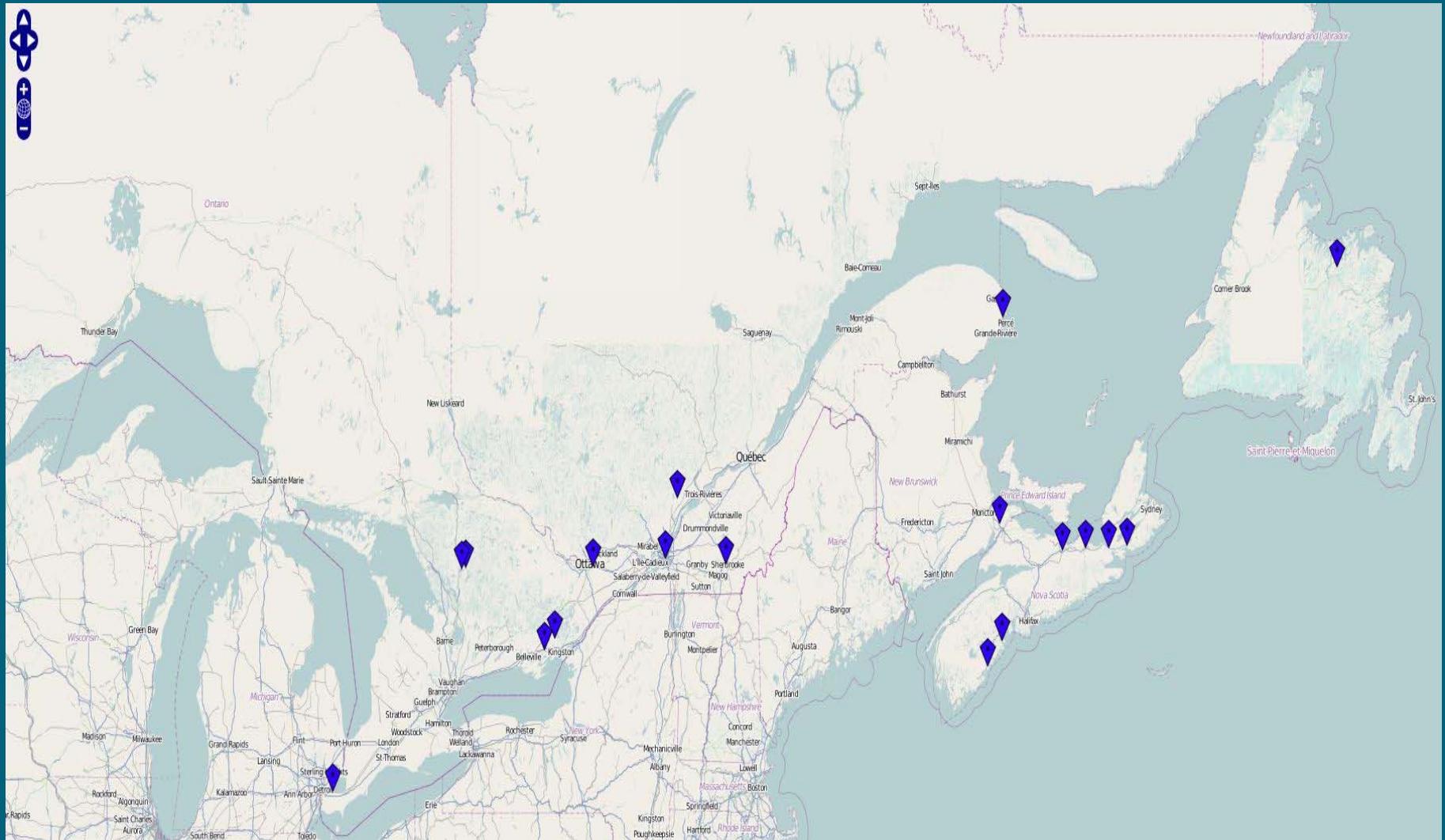
# Ms. Napanee- Average CN



# Ms. Napanee – H N



# Ms. Napanee – H N



# La géologie varie d'un endroit à l'autre...



# Ms. Napanee – Trace element result

Math Sub-Module

Source Compare

unknown bulk napanee

Compare Results

YOUR COMPARE RESULTS

Sample Name: Killaloe+Kingston  
SumDiff: 574650  
Probability: 8.48%

Sample Name: Calgary  
SumDiff: 621233  
Probability: 7.84%

Sample Name: AREF08H0000121 - Kingston - ON - Canada  
SumDiff: 661780  
Probability: 7.36%

Sample Name: Castlegar  
SumDiff: 857217  
Probability: 5.68%

Sample Name: Edmonton  
SumDiff: 898707  
Probability: 5.42%

Sample Name: Gracefield  
SumDiff: 911794  
Probability: 5.34%

View Excel View Graphic View GIS

Ok

Canada  
anada  
Canada

▼ Calgary

Sample Name	SumDiff	Probability
Killaloe+Kingston	574650	8.48%
Calgary	621233	7.84%
AREF08H0000121 - Kingston - ON - Canada	661780	7.36%
Castlegar	857217	5.68%
Edmonton	898707	5.42%
Gracefield	911794	5.34%

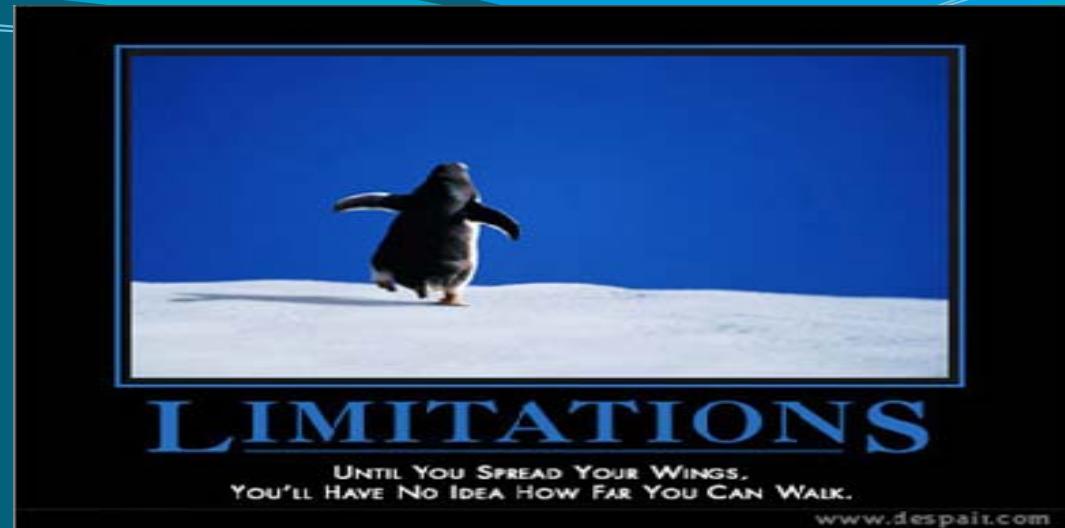
# Ms. Napanee – Trace element result



# Summary – Ms. Napanee

- More positive than average  $\delta^{13}\text{C}$  – possibly have resided in US or had a higher intake of C4 based foods
- Fluctuating  $\delta^2\text{H}$  values suggest she frequently changed locations
- $\delta^{15}\text{N}$  and in general  $\delta^{13}\text{C}$  values changed with movement
- From months 7.5 to 7 PTD,  $\delta^2\text{H}$  value of her hair was most consistent with her having resided in either southern Ontario, southern Quebec, the southern parts of New Brunswick and Nova Scotia or the north eastern states of the US.

# Limitations



- Specificity – broad (and possibly) multiple regions with similar isotope values
- Supermarket diet
- Non-existent databases
- Inter- and intra-individual attribution of data variations
- Not a “stand alone” technique

# Remerciement

- Center for Security Science / CRTI grant(Chemical, Biological, Radiological, Nuclear and Explosive Research and Technology Initiative) 2009-2013
- Royal Canadian Mounted Police
- PSC
- U Ottawa, Dept. Of Earth Sciences, G.G. Hatch Stable Isotope Lab- Paul, Wendy & Patricia
- Jonathan Mayo – Summer student extraordinaire