Freshwater Institute (DFO Winnipeg) Stable Isotope Laboratory Quality Assurance/Quality Control and Inter-lab Comparison Report

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FRESHWATER INSTITUTE (DFO WINNIPEG) STABLE ISOTOPE LABORATORY QUALITY ASSURANCE/QUALITY CONTROL AND INTER-LAB COMPARISON REPORT

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ABSTRACT

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The use of stable isotopes as a food web biotracers has become accepted and commonly used globally in marine, freshwater and terrestrial ecosystems. This report was written to provide researchers interested in working with Fisheries and Oceans-Freshwater Institute's (FWI) stable isotope laboratory with quality control and quality assurance information. FWI's stable isotope analysis system was evaluated relative to three well established stable isotope analysis laboratories (University of Waterloo's Environmental Isotope Laboratory, University of Windsor's Chemical Tracers Lab and University of Ottawa's G.G Hatch Stable Isotope Laboratory). The internal precision and inter-laboratory reproducibility of $\delta^{13}C$ and $\delta^{15}N$ analysis was determined for a variety of fish, whale and invertebrate samples. Long term internal repeatability of an in-house fish standard is +/-0.08 ‰ for $\delta^{13}C$ and +/-0.1 ‰ for $\delta^{15}N$. Overall, agreement between FWI and reference laboratories was acceptable (average $\delta^{13}C$ +/-0.08 ‰, average $\delta^{15}N$ +/-0.16) demonstrating that $\delta^{13}C$ and $\delta^{15}N$ data produced by FWI can be readily compared with data produced by established SIA laboratories.

RESUME

Rosenberg, B., D. Neumann, and L. Loseto. 2015. Institut des Eaux Douces (MPO Winnipeg) Isotopes Stables Laboratoire AQ/CQ et Inter-laboratoire Rapport de Comparaison. Rapp. Stat. Can. Sci. Halieut. Aquat. 1260: vi + 7 p.

L'utilisation des isotopes stables comme biotraceurs alimentaires est désormais acceptée et couramment utilisée pour l'étude des écosystèmes marins, aquatique et terrestres. Ce rapport a été rédigé pour fournir aux chercheurs intéressés en travailler avec Pêches et Océans Canada-Institut des eaux douces (IED) et le laboratoire d'isotopes stables, avec des contrôles de qualité et l'information nécessaire à l'assurance de qualité. Le laboratoire d'isotopes stable de l'FWI a été évaluée par rapport à trois laboratoires d'analyse d'isotopes stables bien établies (Université de Waterloo, Laboratoire d'isotopes de l'environnement, Université de Windsor, Laboratoire de traceurs chimiques et l'Université d'Ottawa, Laboratoire GG Hatch des isotopes stables). La précision et la reproductibilité inter-laboratoire interne des analyses $\delta^{13}C$ et $\delta^{15}N$ a été déterminée pour une variété d'échantillons de poissons, baleines et d'invertébrés marins. Répétabilité interne à long terme d'un poisson type standard est de +/-0,08 ‰ pour $\delta^{13}C$ et +/-0,1 ‰ pour $\delta^{15}N$. Globalement, l'accord entre l'FWI et des laboratoires de référence était bon ($\delta^{13}C$ moyenne +/-0,08 ‰, $\delta^{15}N$ moyenne +/-0,16), démontrant que les données de $\delta^{13}C$ et $\delta^{15}N$ produites par FWI peut facilement être comparées avec les données produites par les laboratoires établis pour l'étude des isotopes stables.

INTRODUCTION

Stable isotope (SI) analysis is a tool used to study trophic ecology in both freshwater and marine ecosystems. Carbon and nitrogen stable isotopes can be used to reconstruct diet and understand trophic linkages within a biological system. Both carbon (C) and nitrogen (N) isotopes exist primarily as their lighter isotopes (^{12}C and ^{14}N) but a small percentage of each exists as a heavier component (1.11% ^{13}C and 0.36% ^{15}N). The ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes are determined relative to standard materials and expressed as differences between the heavy isotope in a sample from that in a standard material ($\delta^{13}C$ and $\delta^{15}N$) (Peterson and Fry 1987). These ratios can be used to elucidate energy sources/flows and food web structure (Boecklen et al. 2011). While SI analysis is widely used in ecological research, results can be affected by the type of equipment used, operating conditions, standards and normalization (Carter and Fry 2013). For this reason, SI analyzes performed at different laboratories may not result in comparable findings.

SI analysis is conducted at the Fisheries and Oceans Canada (DFO) Freshwater Institute (FWI) biotracers lab (Winnipeg, Manitoba) to support research on ecosystem structure and function. The objective of this paper is to perform a quality assurance/quality control (QA/QC) exercise and address uncertainties that might arise when comparing data produced by FWI with data produced at other labs. The results of this study will provide researchers who use FWI's biotracers lab with a sense of both its internal repeatability and reproducibility relative to three well established labs.

MATERIALS AND METHODS

The FWI-biotracers lab includes a Thermo Advantage V Plus continuous flow Isotope Ratio Mass Spectrometer (CF-IRMS), a Costech 4010 Elemental Analyser (EA), a Temperature Conversion Elemental Analyser (TC/EA) and a Gas Chromatograph-Combustion (GC/C) system. The EA and TC/EA allow us to analyze carbon, nitrogen, sulfur and oxygen isotopes in bulk organic and inorganic samples. The GC/C system will allow us to perform compound specific stable isotope analysis on compounds containing carbon and nitrogen. As analysis of bulk samples for $\delta^{13}C$ and $\delta^{15}N$ will be the primary type of analysis performed, this report will focus on those isotopes.

Samples analyzed and compared for this study were selected from samples previously analyzed for $\delta^{15}N$ and $\delta^{13}C$ at either the University of Ottawa-G.G Hatch Stable Isotope Laboratory, University of Waterloo-Environmental Isotope Laboratory or the University of Windsor-Chemical Tracers Lab. Samples selected for analysis were also representative of the research needs and interests of the staff at the DFO-FWI.

Samples were analyzed as received from the other labs (no additional sample treatment such as lipid extraction or carbonate removal was performed). Tissues from 11 species of fish (marine, freshwater and anadromous), 12 species of invertebrate, Beluga muscle and liver, Killer Whale muscle and skin, Narwhal skin, Ring Seal muscle and hair, Bowhead Whale and Bearded Seal muscle, Bowhead Whale baleen and sediments were included in this study (scientific names are listed in Appendix 1). Most samples were analyzed in duplicate or triplicate. Results of the analysis are provided in Tables 1-5.

Our sample analysis protocols are similar to those used in other laboratories. Tissue samples were homogenized, weighed into tin cups and analyzed with the CF-IRMS interfaced with the EA. The EA includes a chromium/cobalt combustion column (1020 $^{\circ}$C), a copper reduction column (650 ºC), a magnesium perchlorate column to remove residual water and a 3 meter packed GC column (50 ºC) which separates N_2, CO_2, and other gaseous products.

The methods used to operate the EA are based on methods used by the United States Geological Services (USGS) Reston lab (Révész et al. 2012). Normalization software (Light Isotope Management Systems -LIMS) was made available by Mr. Ty Coplen of the USGS Reston lab. Internationally certified normalization standards include USGS 40 and 41 (glutamic acid), IAEA-N-2 (ammonium sulfate) IAEA-CH-6 (sucrose), IAEA-CH-7 (polyethylene), IAEA-NO-3 (potassium nitrate), IAEA-S-3 (silver sulfide) and NBS123 (sphalerite).

USGS 40 and 41 are spaced throughout every EA run and used to normalize each sample run. An in-house fish sample is run several times with each sample set to monitor instrument performance and long-term drift. The long-term variance of the in-house fish sample is +/- 0.08 ‰ for δ^{13}C and +/- 0.1 ‰ for δ^{15}N. Duplicate samples are run at least every 10 samples.

As the IRMS' linear range is limited and C is normally abundant, sample weight is adjusted according to N content. Our target sample weight is 400 µg +/-10 µg and is intended to provide a sample containing 40-60 µg N. If there is no history on a species (i.e., C:N % is unknown), the sample must be run on the EA to determine appropriate sample weights for N and required dilution for C.6.

RESULTS

Tables 1 through 5 demonstrate that while both intra-laboratory precision and inter-laboratory repeatability are generally better for δ^{13}C than δ^{15}N they are acceptable for both isotopes. Variability, which is quite dependent on sample homogeneity (De Groot 2004), was generally lower for more homogeneous samples and higher for less homogeneous samples.

In a paper based on collaborative analysis of Bowhead baleen by their respective labs, Hobson and Schell (2012) considered inter-lab differences of ±0.2 ‰ for carbon and ±0.3 ‰ for nitrogen small enough that results from the labs could be directly compared. For Table 6, fish, marine mammals and invertebrates were grouped and the variability of δ^{13}C and δ^{15}N results between

FWI and the Universities of Ottawa, Waterloo and Windsor were determined. The average variability between the FWI and Universities of Windsor, Waterloo and Ottawa for fish, invertebrates and marine mammals falls within the range provided by Hobson and Schell (2012).

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Table 1. Means and standard deviations of repeat analyses of $\delta^{13}C$ in a variety of fish and marine mammal samples. Intra and inter-laboratory repeatability between the Freshwater Institute (FWI) and the University of Waterloo Environmental Isotope Laboratory are compared.

Sample ID	Sample Size	$\delta^{13}C$ FWI		$\delta^{13}C$ U Waterloo		FWI - U Waterloo
		Mean (‰)	Standard Deviation (‰)	Mean (‰)	Standard Deviation (‰)	Standard Deviation (‰)
Arctic Cisco	4	-23.39	0.05	-23.32	0.05	0.05
Lake Cisco	11	-25.23	0.05	-25.23	0.02	0.00
Broad Whitefish	10	-27.55	0.06	-27.46	0.07	0.06
Saffron Cod	9	-22.95	0.06	-22.99	0.07	0.03
Arctic Flounder	7	-24.90	0.04	-24.87	0.09	0.02
Pacific Herring	10	-24.99	0.10	-25.02	0.35	0.02
Atlantic Poacher	10	-21.66	0.03	-21.73	0.07	0.05
Longear Eelpout	5	-21.06	0.05	-21.13	0.10	0.05
Greenland Halibut	7	-22.31	0.04	-22.26	0.28	0.04
Arctic Cod	8	-23.46	0.12	-23.46	0.06	0.00
Arctic Alligatorfish	5	-21.57	0.14	-21.45	0.07	0.09
Beluga - muscle	10	-19.47	0.06	-19.40	0.03	0.05
Beluga - liver	10	-21.32	0.07	-21.25	0.05	0.05
Paraeuchaeta sp.	5	-27.12	0.14	-27.00	0.16	0.09
Themisto abyssorum	5	-27.63	0.10	-27.43	N/A	0.15
Themisto libellula	5	-27.37	0.18	-27.11	N/A	0.18
Calanus hyperboreus	5	-27.34	0.12	-27.11	0.41	0.16
Beroe cucumis	5	-25.47	0.13	-25.50	0.02	0.02
Thysanoessa raschii	5	-25.43	0.12	-25.34	0.06	0.06
Clione limacina	4	-26.93	0.10	-27.09	N/A	0.11
Limacina helicina	3	-26.59	0.17	-26.72	N/A	0.09
Aglantha digitale	5	-23.30	0.14	-23.53	0.06	0.16
Mysidae sp.	3	-25.81	0.20	-25.92	N/A	0.08
Mertensia ovum	3	-26.36	0.09	-26.33	0.13	0.02
Sagitta sp.	3	-25.38	0.34	-25.53	N/A	0.11
Sediment	5	-18.01	0.15	-17.89	0.19	0.08

Table 2. Means and standard deviations of repeat analyses of $\delta^{15}N$ in a variety of fish and marine mammal samples. Intra and inter-laboratory repeatability between the Freshwater Institute (FWI) and the University of Waterloo Environmental Isotope Laboratory are compared.

Sample ID	Sample Size	$\delta^{15}N$ FWI		$\delta^{15}N$ U Waterloo		FWI - U Waterloo
		Mean (‰)	Standard Deviation (‰)	Mean (‰)	Standard Deviation (‰)	Standard Deviation (‰)
Arctic Cisco	4	13.73	0.09	13.73	0.18	0.00
Lake Cisco	11	11.42	0.08	11.52	0.10	0.07
Broad Whitefish	10	9.50	0.08	9.50	0.09	0.00
Saffron Cod	9	15.48	0.10	15.09	0.15	0.27
Arctic Flounder	7	11.54	0.08	11.21	0.09	0.23
Pacific Herring	10	13.17	0.16	13.18	0.38	0.01
Atlantic Poacher	10	17.39	0.11	17.24	0.18	0.11
Longear Eelpout	5	17.91	0.12	17.70	0.12	0.15
Greenland Halibut	7	15.93	0.08	15.65	0.32	0.20
Arctic Cod	8	14.69	0.08	14.66	0.22	0.02
Arctic Alligatorfish	5	14.95	0.06	14.94	0.18	0.01
Beluga - muscle	10	17.64	0.05	17.55	0.17	0.07
Beluga - liver	10	18.15	0.06	17.85	0.14	0.22
Paraeuchaeta sp.	5	12.20	0.37	11.98	0.14	0.16
Themisto abyssorum	5	10.94	0.22	10.52	N/A	0.30
Themisto libellula	5	10.21	0.19	10.02	N/A	0.13
Calanus hyperboreus	5	8.90	0.03	8.60	0.28	0.21
Beroe cucumis	5	12.51	0.07	11.88	0.09	0.45
Thysanoessa raschii	5	10.86	0.14	11.18	0.42	0.23
Clione limacina	4	9.49	0.11	9.02	N/A	0.33
Limacina helicina	3	8.05	0.06	7.91	N/A	0.09
Aglantha digitale	5	11.50	0.15	11.33	0.07	0.12
Mysidae sp.	3	10.93	0.38	11.18	N/A	0.18
Mertensia ovum	3	10.54	0.16	10.50	0.19	0.03
Sagitta sp.	3	12.84	0.16	12.88	N/A	0.03
Sediment	5	3.95	0.07	3.60	0.38	0.25

Table 3. Means and standard deviations of repeat analyses of $\delta^{13}C$ in a variety of fish and marine mammal samples. Intra and inter-laboratory repeatability between the Freshwater Institute (FWI) and the University of Windsor Chemical Tracer Laboratory are compared.

Sample ID	Sample Size	$\delta^{13}C$ FWI		$\delta^{13}C$ U Windsor		FWI - U Windsor
		Mean (‰)	Standard Deviation (‰)	Mean (‰)	Standard Deviation (‰)	Standard Deviation (‰)
Greenland Halibut	4	-19.81	0.24	-19.81	0.31	0.01
Bowhead - muscle	5	-19.50	0.06	-19.67	N/A	0.12
Narwhal - skin	10	-19.58	0.11	-19.90	0.09	0.23
Ring Seal - muscle	5	-19.95	0.02	-20.07	0.14	0.08
Bearded Seal – muscle	5	-18.43	0.03	-18.66	0.05	0.16
Ring Seal - fur	5	-17.44	0.02	-17.32	0.09	0.09
Killer Whale - muscle	1	-14.52	0.31	-14.58	0.04	0.04
Killer Whale -skin	1	-13.92	0.11	-14.32	0.28	0.28

Table 4. Means and standard deviations of repeat analyses of $\delta^{15}N$ in a variety of fish and marine mammal samples. Intra and inter-laboratory repeatability between the Freshwater Institute (FWI) and the University of Windsor Chemical Tracer Laboratory are compared.

Sample ID	Sample Size	$\delta^{15}N$ FWI		$\delta^{15}N$ U Windsor		FWI - U Windsor
		Mean (‰)	Standard Deviation (‰)	Mean (‰)	Standard Deviation (‰)	Standard Deviation (‰)
Greenland Halibut	4	16.43	0.06	16.25	0.13	0.12
Bowhead - muscle	5	12.64	0.05	12.40	N/A	0.17
Narwhal – skin	10	16.50	0.09	16.20	0.18	0.21
Ring Seal - muscle	5	15.22	0.02	14.99	0.17	0.17
Bearded Seal – muscle	5	15.60	0.07	15.38	0.08	0.15
Ring Seal - fur	5	16.12	0.08	15.67	0.05	0.32
Killer Whale - muscle	1	18.28	0.13	17.93	0.11	0.25
Killer Whale -skin	1	18.38	0.13	17.90	0.00	0.34

Table 5. Mean and standard deviation of repeat analyses of $\delta^{13}C$ and $\delta^{15}N$ in Bowhead baleen analyzed at the Freshwater Institute (FWI) and the University of Ottawa GG Hatch Stable Isotope Laboratories. Intra and inter-laboratory repeatability standard deviations are also compared.

Stable Isotope	Sample Size	FWI		U Ottawa		FWI – U Ottawa
		Mean	Standard Deviation (‰)	Mean	Standard Deviation (‰)	Standard Deviation (‰)
δ13C	4	-18.69	0.02	-18.78	0.18	0.06
δ15N	4	13.12	0.10	13.31	0.15	0.14

Table 6. Average difference for $\delta^{13}C$ and $\delta^{15}N$ between fish, invertebrates and marine mammals analyzed at the Freshwater Institute (FWI), University of Waterloo, University of Windsor or University of Ottawa.

Sample Type	Average difference $\delta^{13}C$ (‰)	Average difference $\delta^{15}N$ (‰)
Fish	+/-0.09	+/-0.18
Invertebrates	+/-0.19	+/-0.27
Marine mammals	+/-0.14	+/-0.21

Appendix 1. Common and scientific names of fish and marine mammals analyzed for this study.

Common Name	Species Name
Arctic Cisco	*Coregonus autumnalis*
Lake Cisco	*Coregonus artedii*
Broad Whitefish	*Coregonus nasus*
Saffron Cod	*Eleginus gracilis*
Arctic Flounder	*Liopsetta glacialis*
Pacific Herring	*Clupea pallasii*
Atlantic Poacher	*Leptagonus decagonus*
Longear Eelpout	*Lycodes seminudus*
Greenland Halibut	*Reinhardtius hippoglossoides*
Arctic Cod	*Boreogadus saida*
Arctic Alligatorfish	*Ulcina olrikii*
Beluga Whale	*Delphinapterus leucas*
Bowhead	*Balaena mysticetus*
Narwhal	*Monodon monoceros*
Ring Seal	*Phoca hispida*
Bearded Seal	*Erignathus barbatus*
Killer Whale	*Orcinus orca*