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Northern River Basins Study















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STABLE ISOTOPES OF SULFUR, CARBON AND NITROGEN IN BIOTA, UPPER ATHABASCA RIVER, 1992

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PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

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NORTHERN RIVER BASINS STUDY
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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,
IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;
this publication be subjected to proper and responsible review and be considered for release to the public.

F J Wrona — 19 Nov 1993
(Dr. F.J. Wrona, Ph.D., Science Director) (Date)

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;
this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.
SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No

P A Larkin — 21 Nov 1993
(Dr. P. A. Larkin, Ph.D., Chair) (Date)

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

Bev Burns — 21/12/93
(Bev Burns, Co-chair) (Date)

Lucille Partington — 04/01/93
(Lucille Partington, Co-Chair) (Date)

STABLE ISOTOPES OF SULFUR, CARBON AND NITROGEN IN BIOTA, UPPER ATHABASCA RIVER, 1992

STUDY PERSPECTIVE

A principal goal of the Northern River Basins Study is to understand the manner in which fish and wildlife become contaminated. Identification of their food is central to the interpretation of the sources and observed levels of contaminants in their tissues. Stable isotope analyses have been used successfully in other river systems to improve this understanding. This project involved the analysis of benthic organisms, fish tissue, and sediment for stable isotopes of sulphur, carbon and nitrogen. The differences in stable isotope concentration among the various analyzed medium assists scientists in interpreting data with respect to food sources, food chain relationships, contaminant bioaccumulation and biomagnification, and population movement.

Related Study Questions

6) What is the distribution and movement of fish species in the watersheds of the Peace, Athabasca and Slave river? Where and when are they most likely to be exposed to changes in water quality and where are their important habitats?

14) What long term monitoring programs and predictive models are required to provide an ongoing assessment of the sate of the aquatic ecosystems. These programs must ensure that all stakeholders have the opportunity for input.

Results of this project have identified the existence of unique stable isotope levels amongst the sampled media indicating that the technique is useful in discerning food chain structure. Some interesting differences in food chain pathways and seasonal differences in food availability and source were noted. Differences are significant enough to cause researchers to recommend additional analyses to determine the extent to which the dissimilarities are attributed to seasonal use and/or movement. In turn, this has the potential of helping researchers explain the variability of contaminant concentration in various media as well as possible influences on health indicators. Subsequent investigations will likely cover a more extensive geographic area within the basins, since preliminary results indicate there are significant shifts in food chain relationships amongst downstream trophic levels.

The technique also offers the potential to differentiate intra-species differences in contaminant body burdens and explain their probable movement patterns.

REPORT SUMMARY

A study was carried out of the stable isotope composition of sulfur, carbon, and nitrogen in the tissues of fish and invertebrates and sediment material from the Hinton Reach Specific Study Area. Samples taken in both the Spring and Fall were analyzed. Fish species analyzed consisted of mountain whitefish, northern pike, longnose sucker, and white sucker.

The purpose of the study was to determine whether the information on feeding and movement of the fish could be derived from the sulfur and carbon isotope data, and to use the nitrogen isotope data to define the trophic positions of the organisms. This kind of information was considered to be highly useful toward the interpretation of contaminant data and assessment of exposure of fish to effluents.

Based on the sulfur isotope data 12 of the 85 mountain whitefish could be easily assigned to a separate feeding group. Of these 12, 10 were from the fall selection of 40. The most of the remaining group of mountain whitefish had sulfur isotope compositions in a relatively small range which also contained the northern pike and the longnose sucker. A few mountain whitefish were intermediate to the two ranges, suggesting mixed feeding. Four of five white suckers caught above the confluence of the Berland River had carbon isotope compositions suggesting a different food source than other species at those sites. Benthic algal carbon is the most likely influence to that diet. Below the confluence of the Berland R. carbon isotopes of all white suckers were similar to other species.

Nitrogen isotopes defined four trophic levels. The single mayfly sample was in the lowest level. The stoneflies were in the second level, most longnose suckers and some white suckers in the third level, and mountain whitefish, northern pike, and some white suckers in the fourth trophic level. This suggests that trophic biomagnificaton should not be an explanation for potential differences in the contaminant levels which might be observed between the whitefish and pike. These preliminary studies have shown that stable isotope analyses have good potential to discriminate between food sources and to define trophic structures. These analyses should prove useful in adding ecological interpretive value to the very costly contaminant analyses.

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INTRODUCTION

The purpose of analyzing fish tissues and other components of the food chains for the stable isotopes of sulfur, carbon, and nitrogen is twofold: 1. to determine the trophic position of the organism in the food chain and 2. to determine the food sources, ie. allocthanous vs. autochthonous, and feeding locations ie. tributary or mainstem. For instance, if feeding in tributaries can be established, some inferences can also be made about fish movements.

The principle in using sulfur and carbon isotopes to determine food sources is "you are what you eat" or at least the aquatic organism is in this case. Sulfur and carbon isotopes are essentially unaffected by trophic transfers and therefore indicate the signal of the base the food chain in which they are found. Nitrogen isotopes are fractionated by a consistent amount at each trophic step and therefore can be used to indicate trophic level. Peterson and Fry (1986) have reviewed the general applications of these stable isotopes to food chain research. An application closer to that being used for the Northern River Basins Study (NRBS) has been published by Hesslein et al. (1991). It describes aquatic food chains in the lower Mackenzie River Basin.

METHODS

Samples from the Hinton Reach Specific Study Area (RSS) were supplied frozen by the NRBS either as homogenized muscle tissue or whole muscle tissue form. Samples for analyses reported on in this report were chosen with the intention of getting a representative look at the variety of species and locations. Toward this purpose, samples were chosen with the widest range of parameters of sex and size. This was a best guess at maximizing the variance. It was expected that if the preliminary investigation was successful, future studies would intensify the level of detail within species, different tissues, or over time etc.

The methods used for stable isotope analyses have been described in detail by Hesslein et al. (1989) and will be briefly described here. For carbon and nitrogen analyses a sample of approximately 20 mg is oven dried in air, placed in a Vycor glass tube (9mm OD x 25mm long) with 1 g copper wire and 1 g copper oxide (both high purity) and a small piece of silver foil (2 mm x 2 mm). The tube is then evacuated overnight to a pressure of < 10 um Hg and sealed with a torch using natural gas and oxygen. The sealed tube is then combusted at 800 °C for 2 hours and cooled slowly. The tube is attached to an evacuated glass extraction system and broken to release the H_20, CO_2, and N_2 to which the sample has been converted. The gases are then cryogenically purified. Water is removed at -55 °C, CO_2 at -293 °C, and N_2 at -293 °C on 5Å molecular sieve. The purified gases are transferred to the mass spectrometer in sealed glass vessels.

A high precision dual inlet isotope ratio mass spectrometer was used (VG Micromass 602E) for the analyses. The principle of operation is that the ratio of the isotopes in the sample gas is compared to a reference gas in the same system by switching the analyses back and forth from reference to sample many times. We have use

the same reference gases in our system over many years. The working reference gases have been calibrated to the internationally accepted standards for the stable isotopes of sulfur, carbon, and nitrogen.

The long term repeatability of analyses in our lab has had a standard deviation of 0.2 ‰ for $\delta^{34}S$, 0.1 ‰ for $\delta^{13}C$, and 0.3 ‰ for $\delta^{15}N$. For a single set of similar samples, as in this study, the precision is better than these values.

In January 1993 we began using a new method for the analyses of carbon isotopes. In this method the tissue sample is burned at high temperature in an automated elemental analyzer (Carlo Erba NA1500). The resultant CO_2 is cryogenically cleaned of water at -75 °C and frozen at -293 °C. The purified gas is the automatically introduced to a dual inlet isotope ratio mass spectrometer (VG Optima) and analyzed as above. The precision of this method is better than the manual one (<0.1 ‰) but we don't have enough experience to say how much better in the long term. A comparison of analyses of the same 30 tissue samples by both methods gave a standard deviation of the difference of 0.07 ‰, better than our long term manual results would have predicted.

In February 1993 we began using the automated elemental analyzer system for nitrogen isotope analyses. As with carbon, the sample is oxidized at high temperature. The oxides of nitrogen are reduced to N_2 over copper at 600 °C and trapped on silica gel at -293 °C. The gas is then introduced to the mass spectrometer as above for isotope analyses. The precision of the automated method is <0.1 ‰, and was standardised to the results of our manual methods.

RESULTS

The stable isotope data for the fish tissue, invertebrates, and biofilm samples analyzed are presented in Appendix A.

The mean at values for the three isotope analyses for each species at each sampling location and time are given in Table 1.

The standard deviations of most sets data for all three isotopes are near 1 pm. Instances of higher variance indicate inhomogeneous groups. These will be discussed below.

Sediment data is given in Appendix B. Nitrogen contents were too small to allow nitrogen isotope analyses.

DISCUSSION

Figure 1. shows that the food source indicators, $\delta^{34}S$ and $\delta^{13}C$, allow the definition of a major grouping of mainstem feeders. All four species are represented in this group. $\delta^{34}S$ allows definition of a group of mountain whitefish which we believe to be tributary feeders. This group contains two fish caught in the spring at the two most downstream stations. The remaining ten fish were all caught in the fall and came from all stations. We suspect the fish are from a tributary nearer the downstream locations. A few are in the mainstem in the spring, but more move up the mainstem with the fall upstream migration of mountain whitefish. A number of other fish have values intermediate between the "tributary" values and the mainstem values and indicate mixed feeding. These are found mostly at the downstream locations. A third distinct group, four white suckers, is discriminated by $\delta^{13}C$. Their $\delta^{34}S$ is slightly outside the main body of mainstem

feeders. This group could indicate tributary feeding or a different carbon source within the mainstem such as one supported by benthic algae. We favor the latter explanation.

Fig. 2 shows the three levels in the trophic structure in the mainstem. The lowest containing stoneflies, the middle, containing suckers, and the highest containing mountain whitefish and northern pike. The whitefish showing the tributary $\delta^{34}S$ have somewhat higher $\delta^{15}N$ but must be excluded from the mainstem trophic definition. The distinction between the $\delta^{15}N$ in the mainstem and tributary feeders is shown more clearly in Fig. 3. The mixture of fish of different feeding areas is the cause of the high variances of $\delta^{34}S$ and $\delta^{15}N$ found in the fall catches of mountain whitefish (Table 1). There is no change in the variances of $\delta^{13}C$ from spring to fall in the mountain whitefish even with the tributary fish included. There is, however, a small positive shift in the mean $\delta^{13}C$ from spring to fall at each sampling location. This could indicate a seasonal trend in the whole food chain due to physical conditions like temperature and light or a shift in the dietary mix of the mountain whitefish. Differences in the conditon of the fish (fat content in the muscle) can result in different $\delta^{13}C$, however, the shift observed would suggest more lipid in the spring (lipid has been shown to have lower $\delta^{13}C$ than associated muscle, R.H. Hesslein, unpublished) which seems unlikely.

Fig. 4 is a blowup of the $\delta^{34}S$ range of 7.5 ‰ to 11.5 ‰ from Fig. 1 which probably represents mainstem feeding. This range is applicable to the invertebrates as well as the fish. A range of 4 ‰ is not unusual for population feeding on material derived from a single sulfur source (Hesslein et al. 1991). The $\delta^{15}N$ shows the general trophic structure for mainstem feeders; a single herbivore (mayfly), predatory insects (stoneflies), suckers (longnose and white), and mountain whitefish and northern pike.

Of the four fish species only the northern pike show any trend with size. Fig. shows a trend of increasing $\delta^{15}N$ with increasing weight. This suggests a somewhat higher trophic position for the larger pike, but the range spans only one trophic step as predicted by $\delta^{15}N$ (3 ‰).

The sediments (Appendix B) had $\delta^{13}C$ in the range of the fish identified as mainstem feeders. The $\delta^{34}S$ in the sediments was not in the range of the "mainstem" fish (7.5 to 11.5 ‰). We are not certain that the sulfur analyzed came from organic sources as the organic contents of the sediments was very low. The sediment sulfur isotopes were not consistent with the $\delta^{34}S$ of the biofilm or invertebrates. Future sampling should concentrate on sediments with higher organic content or a method alteration will have to be imposed to extract only organic sulfur.

CONCLUSIONS

The $\delta^{15}N$ isotopic data has shown that it will certainly be useful in determining the trophic structure of the food chain. The $\delta^{13}C$ data suggest some interesting differences in food chain pathways and seasonal differences in food production or source which will require further examination. The $\delta^{34}S$ data suggest that a number of mountain whitefish which have fed primarily on a different food source than the bulk of the catch are present in the Hinton RSS, reach especially in the fall. It will be very interesting to compare in these groups of fish the contaminant burdens and health indicators

determined by other investigators. It would also be of value to extend the geographical coverage of the stable isotope data as the indications of alternate food sources are strongest at the most downstream locations. A much more complete understanding of the food chains and trophic structure could be determined by analyzing more species.

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Peterson, B.J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293-320.

Table 1. Means and standard deviations of stable isotopes in fish in Hinton RSS.

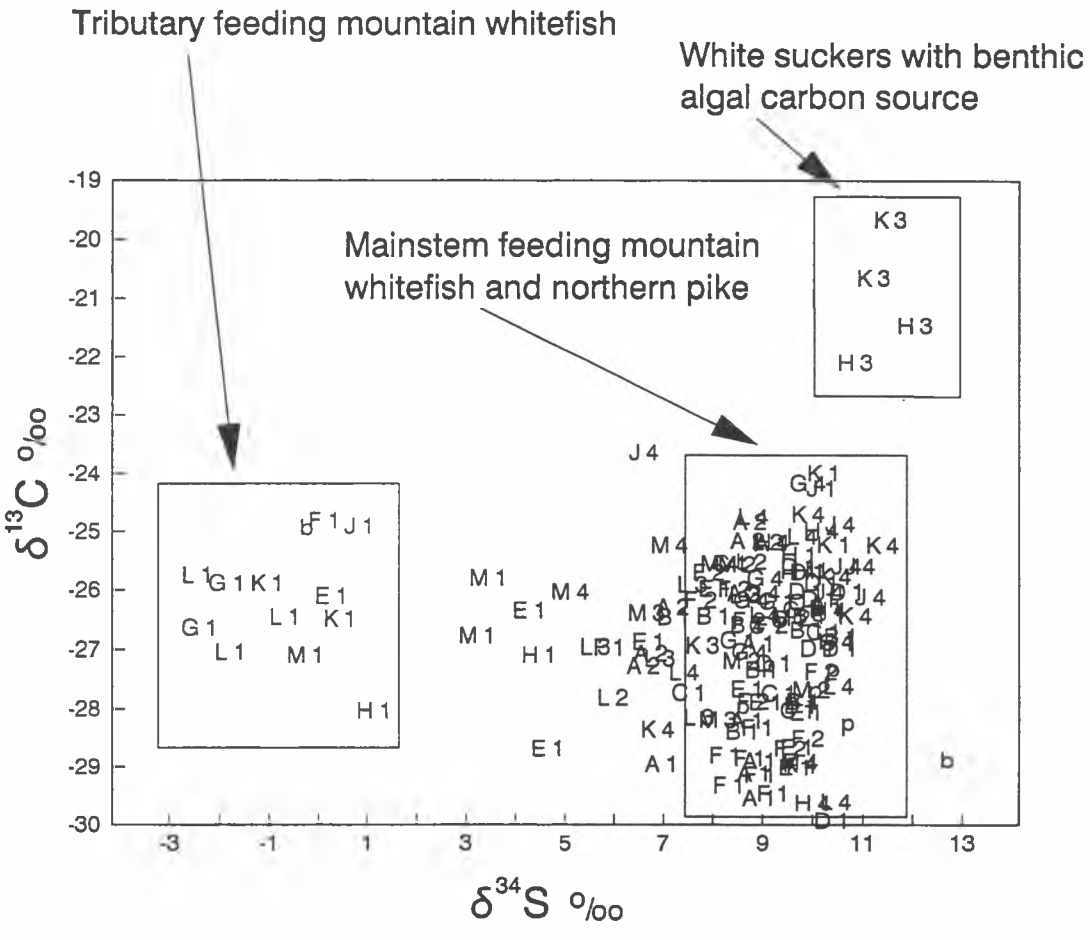
Site	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$
Mountain whitefish			
A	8.66	-28.69	8.15
	1.14	1.54	0.56
B	9.55	-26.98	6.38
	0.99	0.69	0.74
C	9.61	-26.84	6.92
	0.84	0.74	0.55
D	10.30	-26.66	7.56
	0.40	1.22	0.43
E	7.30	-27.53	8.18
	2.98	1.07	0.89
F	8.00	-28.48	8.18
	2.76	1.44	0.68
G	3.62	-26.40	8.41
	5.52	0.38	1.03
H	6.56	-26.56	7.55
	3.71	1.06	1.76
J	7.29	-26.65	7.30
	4.44	2.76	1.48
K	5.22	-25.41	8.81
	5.33	0.92	1.11
L	-1.45	-26.12	9.14
	0.73	0.68	1.16
M	2.39	-26.77	9.16
	1.70	0.59	0.62

Table 1 continued.

Site	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	
Northern Pike				
A	7.98	-26.13	8.03	
	0.94	0.89	0.78	
F	9.13	-27.15	7.54	
	0.79	1.02	0.72	
H		-25.55	7.48	n=1
K		-24.90	7.09	n=2
L	8.13	-26.15	7.40	
	1.40	0.83	0.52	
M	9.40	-26.78	7.94	
	0.64	1.14	0.67	
White sucker				
H	11.69	-21.79	5.04	n=1
K	10.41	-22.43	5.42	
	1.71	3.22	1.54	
L	7.48	-26.99	6.47	
	1.00	0.79	1.05	
M	7.64	-27.28	6.13	
	0.73	1.48	0.71	
Longnose sucker				
G	9.33	-25.63	6.44	
	0.42	1.00	0.65	
H	10.21	-26.46	5.11	
	0.42	1.56	0.24	

Table 1 continued.

Site	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$
J	10.22	-25.32	5.44
	1.56	0.85	0.63
K	10.08	-26.59	5.51
	1.45	1.56	0.35
L	9.53	-26.82	5.27
	1.07	1.64	0.21
M	7.78	-26.44	5.71
	1.68	1.45	0.48



Spring Fall

- A G = Hwy 40 Bridge
- B H = Weldwood Haul Bridge
- C J = Obed Mountain Coal Bridge
- D K = Emerson Lakes Bridge
- E L = Berland River confl. Bridge
- F M = Windfall Bridge

1 = Mountain whitefish
2 = Northern pike
3 = White Sucker
4 = Longnose Sucker
p = Stonefly (Plecoptera)
b = Biofilm
e = Ephemeroptera

Figure 1. Sulfur and carbon isotopes in all samples in the Hinton Reach Specific Study, spring and fall 1992. Highlighted areas are described in the text.

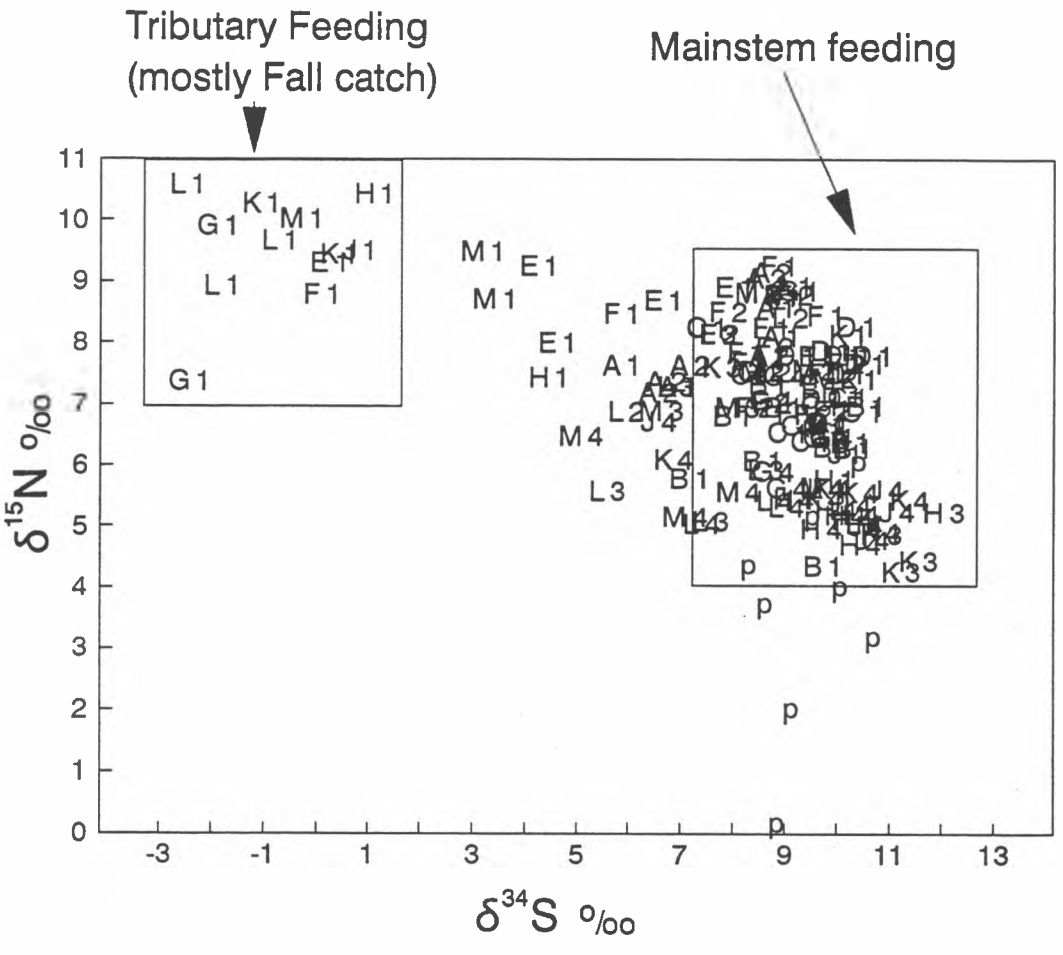


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Figure 2. Carbon and nitrogen isotopes in all samples in the Hinton Reach Specific Study, spring and fall 1992. Highlighted areas are described in the text.

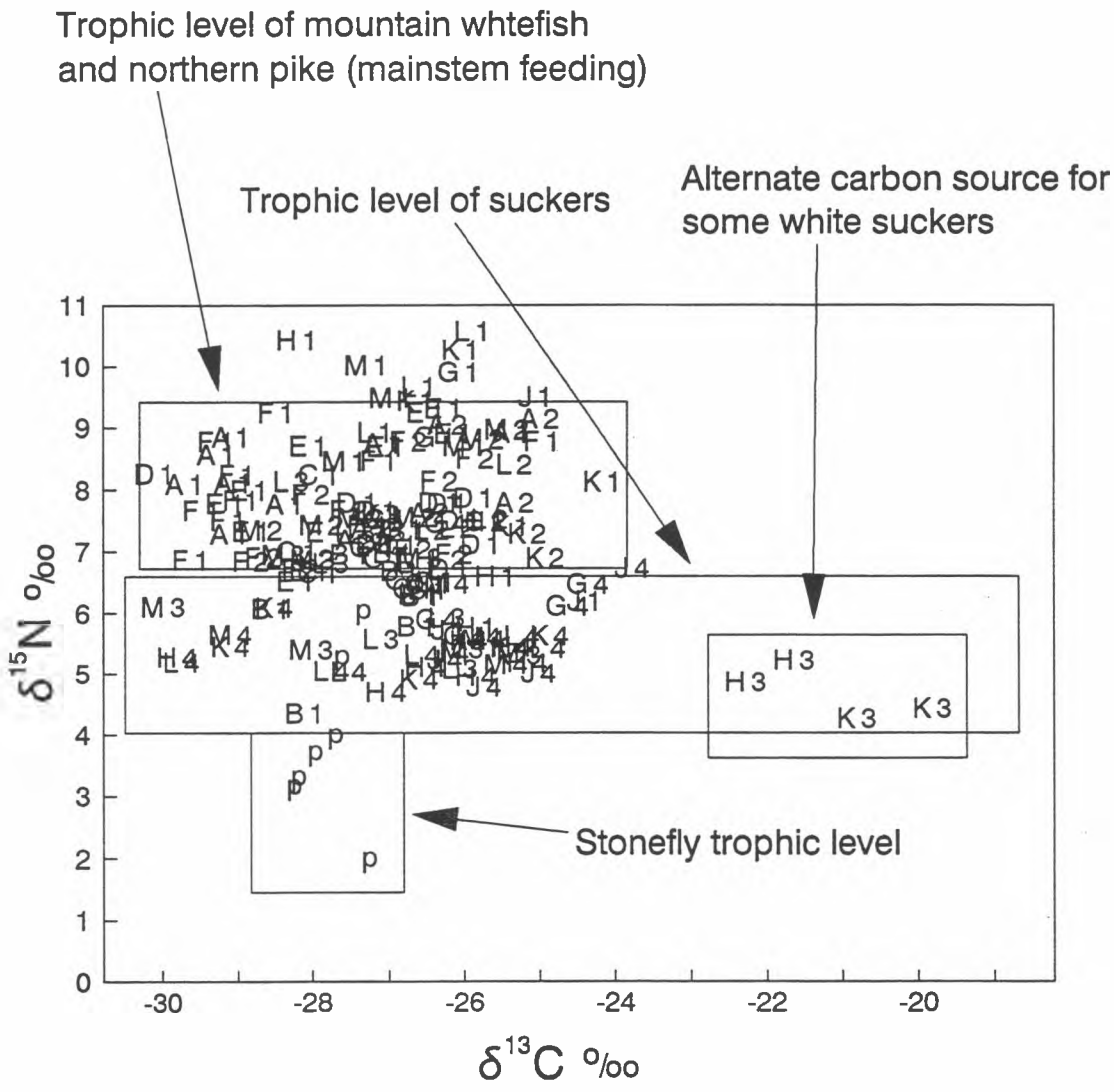

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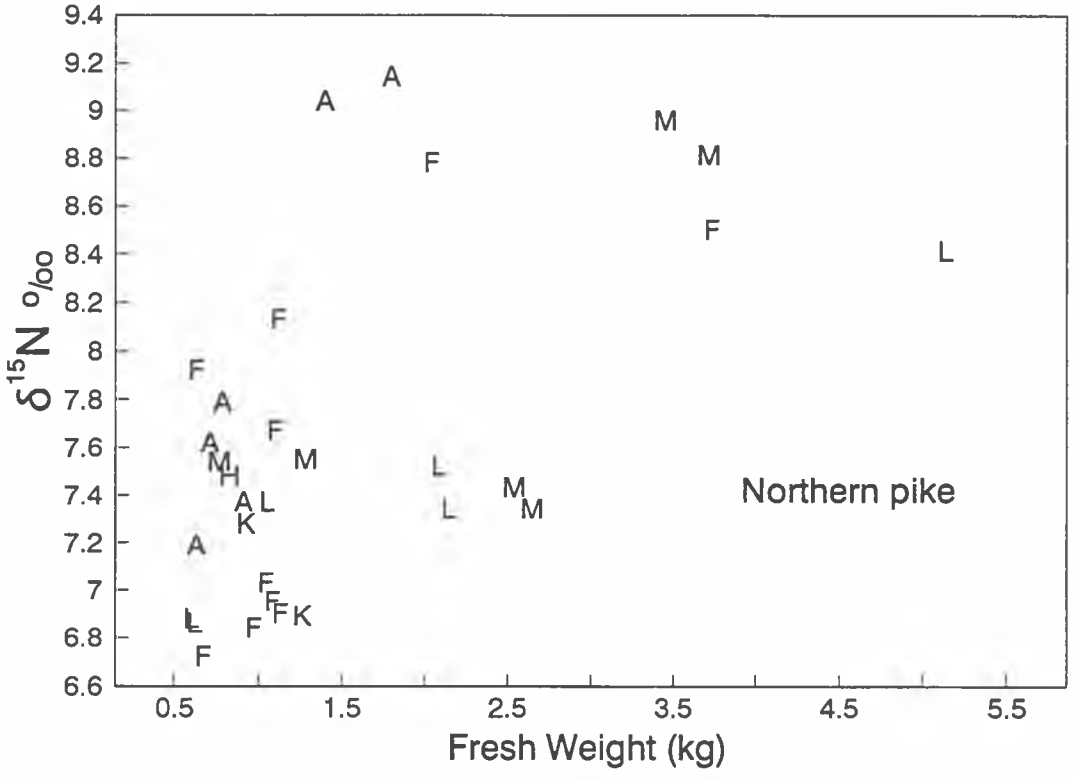
Figure 3. Sulfur and nitrogen isotopes in all samples in the Hinton Reach Specific Study, spring and fall 1992. Highlighted areas are described in the text.



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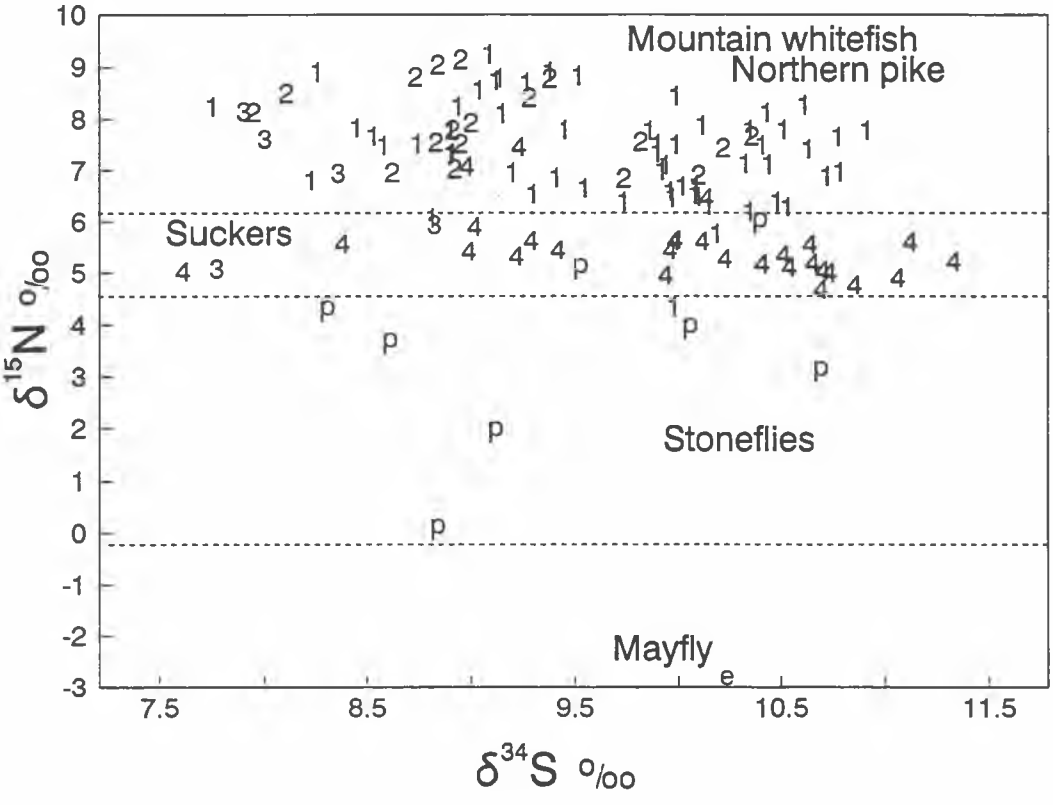
D	K	= Emerson Lakes Bridge
E	L	= Berland River confl. Bridge
F	M	= Windfall Bridge

Figure 4. Sulfur and nitrogen isotopes in "mainstem" range of sulfur isotopes in the Hinton Reach Specific Study, spring and fall 1992. Highlighted areas are described in the text.



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p = Stonefly (Plecoptera)
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e = Ephemeroptera

Figure 5. Nitrogen isotopes and fresh weight in northern pike in the Hinton Reach Specific Study, spring and fall 1992.

Appendix A. Northern River Basins Study stable isotope data.

NRBSID	LOCATION	SPECIES	SEX	WEIGHT	LENGTH	PART	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	ISOLAB
na	OBED COAL	PLEC RP2	U	na	na		9.12	-27.25	2.03	4731.0
na	EMERSON L.	PLEC RP1	U	na	na		10.06	-27.69	4.02	4732.0
na	OBED COAL	PLEC RP1	U	na	na		8.61	-27.96	3.74	4730.0
na	OBED COAL	PLEC RP1 H	U	na	na			-28.18	3.33	4730.1
na	EMERSON L.	PLEC RP2	U	na	na		10.40	-27.33	6.07	4733.0
na	WINDFALL BR.	BLANK	U	na	na		9.19	-25.93	6.11	4735.0
na	WINDFALL BR.	PLEC	U	na	na		9.53	-30.79	5.18	4736.0
na	EMERSON L.	PLEC RP2 H	U	na	na			-27.61	5.32	4733.1
na	KNIGHT BR.	PLEC	U	na	na		10.69	-28.24	3.19	4734.0
na	OBED COAL	BIOFILM	U	na	na		6.97	-10.88		4723.0
na	EMERSON L.	BIOFILM	U	na	na		-0.24	-24.94	14.80	4724.0
na	U/S HINTON	BIOFILM	U	na	na		2.62	-3.62		4721.0
na	WELDWOOD	BIOFILM	U	na	na		9.54	-16.62		4722.0
na	KNIGHT BR.	BIOFILM	U	na	na		12.70	-28.88		4725.0
na	WELDWOOD	PLEC	U	na	na		8.84	-30.66	0.18	4728.0
na	WELDWOOD	EPHEM	U	na	na		10.24	-31.24	-2.78	4729.0
na	WINDFALL BR.	BIOFILM	U	na	na		5.78	-12.56		4726.0
na	U/S HINTON	PLEC	U	na	na		8.31	-33.57	4.37	4727.0
A-I-1	U/S HINTON	MNWF	F	325.0	31.4	MUS	9.04	-29.12	8.57	4737.0
A-I-2	U/S HINTON	MNWF	M	381.0	33.0	MUS	8.90	-28.21	7.77	4843.0
A-I-3	U/S HINTON	MNWF	F	841.0	44.6	MUS	9.38	-25.22	8.91	4844.0
A-I-4	U/S HINTON	MNWF	F	308.0	29.2	MUS	9.15	-28.90	8.11	4738.0
A-I-5	U/S HINTON	MNWF	F	290.0	29.8	MUS	7.18	-28.95	7.28	4845.0
A-I-6	U/S HINTON	MNWF	F	531.0	36.6	MUS	9.12	-26.91	8.74	4846.0
A-I-7	U/S HINTON	MNWF	M	492.0	36.3	MUS	6.10	-30.22	7.62	4739.0
A-I-8	U/S HINTON	MNWF	F	795.0	41.3	MUS		-28.93	8.85	4847.0
A-I-9	U/S HINTON	MNWF	M	409.0	33.3	MUS	9.90	-30.87	7.51	4848.0
A-I-10	U/S HINTON	MNWF	F	360.0	31.4	MUS	9.15	-29.54	8.09	4740.0
A-II-1	U/S HINTON	NRPK	M	785.0	47.0	MUS	8.91	-25.18	7.79	4749.0
A-II-2	U/S HINTON	NRPK	U	712.0	46.7	MUS	7.40	-26.31	7.62	4881.0
A-II-3	U/S HINTON	NRPK	F	1792.0	65.9	MUS	8.95	-24.85	9.14	4750.0
A-II-4	U/S HINTON	NRPK	M	1399.0	59.0	MUS	8.84	-26.07	9.04	4751.0
A-II-5	U/S HINTON	NRPK	F	632.0	44.0	MUS	6.81	-27.28	7.19	4882.0
A-II-6	U/S HINTON	NRPK	F	910.0	48.3	MUS	6.95	-27.08	7.37	4752.0
B-I-1	HAUL BR.	MNWF	F	309.0	29.2	MUS	10.02	-27.88	6.68	4757.0
B-I-2	HAUL BR.	MNWF	F	310.0	28.5	MUS	8.81	-28.39	6.07	4849.0
B-I-3	HAUL BR.	MNWF	F	1199.0	45.9	MUS	10.78	-26.79	6.97	4850.0
B-I-4	HAUL BR.	MNWF	F	819.0	40.2	MUS	10.09	-26.69	6.68	4758.0
B-I-5	HAUL BR.	MNWF	F	291.0	28.3	MUS	10.48	-26.32	6.38	4851.0
B-I-6	HAUL BR.	MNWF	F	674.0	37.2	MUS	10.53	-26.41	6.28	4852.0
B-I-7	HAUL BR.	MNWF	F	472.0	32.8	MUS	10.15	-26.45	6.30	4759.0
B-I-8	HAUL BR.	MNWF	M	357.0	31.4	MUS	7.42	-26.47	5.78	4853.0
B-I-9	HAUL BR.	MNWF	F	400.0	32.0	MUS	9.98	-27.94	4.34	4854.0
B-I-10	HAUL BR.	MNWF	F	560.0	37.5	MUS	9.20	-27.35	6.95	4760.0
B-I-11	HAUL BR.	MNWF	F	526.0	35.7	MUS	8.23	-26.46	6.80	4855.0
B-I-12	HAUL BR.	MNWF	F	961.0	41.8	MUS	8.91	-26.60	7.33	4856.0
C-I-1	OBED COAL	MNWF	F	1029.0	40.1	MUS	9.92	-28.03	6.99	4741.0
C-I-2	OBED COAL	MNWF	F	1030.0	42.0	MUS	9.74	-26.51	6.39	4857.0
C-I-3	OBED COAL	MNWF	F	1030.0	42.1	MUS	9.97	-26.35	6.47	4858.0
C-I-4	OBED COAL	MNWF	M	742.0	39.3	MUS	10.44	-26.72	7.11	4742.0
C-I-5	OBED COAL	MNWF	F	550.0	38.5	MUS	7.75	-27.74	8.24	4859.0
C-I-6	OBED COAL	MNWF	F	435.0	31.3	MUS	10.10	-26.23	6.49	4860.0
C-I-7	OBED COAL	MNWF	F	341.0	29.7	MUS	9.30	-26.61	6.53	4743.0
C-I-8	OBED COAL	MNWF	F	599.0	38.5	MUS	8.58	-25.56	7.48	4861.0
C-I-9	OBED COAL	MNWF	M	692.0	40.6	MUS	9.55	-27.75	6.65	4862.0
C-I-10	OBED COAL	MNWF	F	812.0	41.4	MUS	10.72	-26.89	6.88	4744.0
D-I-1	EMERSON L.	MNWF	M	631.0	37.3	MUS	10.35	-26.16	7.79	4761.0
D-I-2	EMERSON L.	MNWF	F	361.0	30.5	MUS	9.94	-25.61	7.10	4863.0
D-I-3	EMERSON L.	MNWF	F	403.0	32.9	MUS	10.91	-26.05	7.78	4864.0
D-I-4	EMERSON L.	MNWF	M	422.0	32.6	MUS	10.61	-29.93	8.26	4762.0
D-I-5	EMERSON L.	MNWF	F	920.0	43.3	MUS	10.41	-25.89	7.50	4865.0
D-I-6	EMERSON L.	MNWF	F	1038.0	43.3	MUS	10.77	-26.99	7.65	4866.0
D-I-7	EMERSON L.	MNWF	F	415.0	32.0	MUS	10.12	-25.71	7.87	4763.0
D-I-8	EMERSON L.	MNWF	F	812.0	39.5	MUS	10.33	-26.99	7.12	4867.0
D-I-9	EMERSON L.	MNWF	F	602.0	35.0	MUS	10.09	-26.03	6.71	4868.0
D-I-10	EMERSON L.	MNWF	F	570.0	35.6	MUS	9.45	-27.24	7.79	4764.0
E-I-1	KNIGHT BR.	MNWF	M	412.0	33.5	MUS	4.86	-28.68	7.99	4765.0
E-I-2	KNIGHT BR.	MNWF	F	1173.0	45.3	MUS	4.49	-26.35	9.24	4869.0
E-I-3	KNIGHT BR.	MNWF	F	468.0	34.2	MUS	8.90	-27.67	7.31	4870.0
E-I-4	KNIGHT BR.	MNWF	F	715.0	39.2	MUS	10.08	-28.06	6.52	4766.0
E-I-5	KNIGHT BR.	MNWF	F	491.0	35.4	MUS	0.48	-26.11	9.30	4871.0

Appendix A. cont.

NRBSID	LOCATION	SPECIES	SEX	WEIGHT	LENGTH	PART	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	ISOLAB
E-I-6	KNIGHT BR.	MNWF	M	648.0	36.9	MUS	9.90	-28.66	7.33	4872.0
E-I-7	KNIGHT BR.	MNWF	M	748.0	38.9	MUS	9.86	-29.01	7.78	4767.0
E-I-8	KNIGHT BR.	MNWF	F	932.0	41.2	MUS	6.91	-26.88	8.69	4873.0
E-I-9	KNIGHT BR.	MNWF	F	952.0	45.9	MUS	8.26	-25.99	8.90	4874.0
E-I-10	KNIGHT BR.	MNWF	M	611.0	37.0	MUS	9.27	-27.89	8.70	4768.0
F-I-1	WINDFALL BR.	MNWF	M	891.0	41.8	MUS	9.14	-29.14	8.78	4745.0
F-I-2	WINDFALL BR.	MNWF	F	428.0	31.6	MUS	6.09	-26.98	8.47	4875.0
F-I-3	WINDFALL BR.	MNWF	M	502.0	34.3	MUS	9.41	-29.47	6.86	4876.0
F-I-4	WINDFALL BR.	MNWF	M	395.0	32.7	MUS	8.94	-28.86	8.24	4746.0
F-I-5	WINDFALL BR.	MNWF	F	472.0	33.3	MUS	9.99	-28.98	7.50	4877.0
F-I-6	WINDFALL BR.	MNWF	F	465.0	34.7	MUS	0.32	-24.82	8.78	4878.0
F-I-7	WINDFALL BR.	MNWF	F	497.0	33.8	MUS	9.99	-30.06	8.44	4747.0
F-I-8	WINDFALL BR.	MNWF	F	371.0	30.2	MUS	8.53	-29.33	7.67	4879.0
F-I-9	WINDFALL BR.	MNWF	M	914.0	41.4	MUS	8.45	-28.81	7.83	4880.0
F-I-10	WINDFALL BR.	MNWF	F	722.0	38.4	MUS	9.09	-28.33	9.24	4748.0
F-II-1	WINDFALL BR.	NRPK	F	632.0	45.5	MUS	9.00	-27.88	7.92	4753.0
F-II-2	WINDFALL BR.	NRPK	M	674.0	46.5	MUS		-28.02	6.72	4883.0
F-II-3	WINDFALL BR.	NRPK	M	1129.0	54.0	MUS	10.10	-28.51	6.90	4884.0
F-II-4	WINDFALL BR.	NRPK	M	1100.0	53.7	MUS	10.36	-27.38	7.67	4754.0
F-II-5	WINDFALL BR.	NRPK	M	1084.0	53.8	MUS	8.62	-26.00	6.95	4885.0
F-II-6	WINDFALL BR.	NRPK	M	970.0	53.0	MUS	9.74	-28.68	6.84	4886.0
F-II-7	WINDFALL BR.	NRPK	M	1118.0	54.6	MUS	7.95	-26.19	8.13	4755.0
F-II-8	WINDFALL BR.	NRPK	F	3735.0	79.2	MUS	8.11	-25.72	8.50	4887.0
F-II-9	WINDFALL BR.	NRPK	F	1045.0	52.1	MUS	8.92	-26.54	7.03	4888.0
F-II-10	WINDFALL BR.	NRPK	F	2045.0	63.7	MUS	9.38	-26.60	8.78	4756.0
G-I-1	NR ENTRANCE	MNWF	u	380.0	32.3	MUS	-2.16	-26.64	7.37	4889.0
G-I-3	NR ENTRANCE	MNWF	u	740.0	38.3	MUS	-1.61	-25.89	9.89	4890.0
G-I-8	NR ENTRANCE	MNWF	u	390.0	32.5	MUS	8.74	-26.86	7.52	4891.0
G-I-10	NR ENTRANCE	MNWF	u	575.0	37.0	MUS	9.52	-26.21	8.84	4892.0
G-IV-2	NR ENTRANCE	LNSK	F	820.0	41.6	MUS	9.02	-26.19	5.89	4945.0
G-IV-3	NR ENTRANCE	LNSK	M	570.0	36.2	MUS		-24.46	6.11	4946.0
G-IV-4	NR ENTRANCE	LNSK	M	470.0	37.5	MUS	9.23	-26.07	7.46	4947.0
G-IV-6	NR ENTRANCE	LNSK	F	940.0	42.6	MUS	8.98	-27.06	7.07	4948.0
G-IV-7	NR ENTRANCE	LNSK	M	676.0	37.8	MUS	10.14	-24.19	6.47	4949.0
G-IV-10	NR ENTRANCE	LNSK	F	630.0	37.8	MUS	9.29	-25.82	5.63	4950.0
H-I-7	HAUL BR.	MNWF	u	375.0	32.1	MUS	10.19	-25.68	5.76	4893.0
H-I-9	HAUL BR.	MNWF	u	485.0	34.1	MUS	1.37	-28.03	10.42	4894.0
H-I-10	HAUL BR.	MNWF	u	1220.0	46.9	MUS	9.96	-25.42	6.59	4895.0
H-I-11	HAUL BR.	MNWF	u	420.0	33.4	MUS	4.71	-27.10	7.43	4896.0
H-II-1	HAUL BR.	NRPK	F	825.0	48.5	MUS		25.55	7.48	4912.0
H-III-1	HAUL BR.	WHSK	M	730.0	37.0	MUS	11.09	-22.11	4.86	4927.0
H-III-2	HAUL BR.	WHSK	M	960.0	38.8	MUS	12.29	-21.47	5.22	4928.0
H-IV-2	HAUL BR.	LNSK	M	760.0	39.3	MUS	10.23	-29.63	5.27	4951.0
H-IV-4	HAUL BR.	LNSK	M	670.0	37.4	MUS	10.41	-25.00	5.18	4952.0
H-IV-5	HAUL BR.	LNSK	F	960.0	41.2	MUS	9.42	-25.20	5.44	4953.0
H-IV-7	HAUL BR.	LNSK	F	710.0	40.0	MUS	9.94	-25.68	4.95	4954.0
H-IV-8	HAUL BR.	LNSK	F	830.0	40.0	MUS	10.69	-26.87	4.69	4955.0
H-IV-9	HAUL BR.	LNSK	M	850.0	40.2	MUS	10.54	-26.35	5.11	4956.0
J-I-1	OBED COAL	MNWF	u	385.0	31.6	MUS	10.51	-31.30	7.79	4897.0
J-I-3	OBED COAL	MNWF	u	345.0	30.6	MUS		-26.08	5.72	4898.0
J-I-8	OBED COAL	MNWF	u	900.0	41.6	MUS	1.02	-24.92	9.49	4899.0
J-I-9	OBED COAL	MNWF	u	365.0	31.5	MUS	10.35	-24.28	6.19	4900.0
J-IV-1	OBED COAL	LNSK	F	680.0	37.6	MUS	10.51	-26.04	5.34	4957.0
J-IV-3	OBED COAL	LNSK	M	580.0	35.2	MUS	11.12	-25.61	5.60	4958.0
J-IV-6	OBED COAL	LNSK	F	750.0	38.6	MUS	10.85	-25.61	4.77	4959.0
J-IV-7	OBED COAL	LNSK	F	870.0	39.8	MUS	6.78	-23.65	6.72	4960.0
J-IV-8	OBED COAL	LNSK	M	715.0	39.7	MUS	11.33	-26.13	5.23	4961.0
J-IV-10	OBED COAL	LNSK	M	810.0	40.8	MUS	10.73	-24.89	5.00	4962.0
K-I-3	EMERSON L.	MNWF	u	380.0	32.4	MUS	0.65	-26.49	9.43	4901.0
K-I-6	EMERSON L.	MNWF	u	1160.0	44.4	MUS	-0.82	-25.89	10.26	4902.0
K-I-8	EMERSON L.	MNWF	u	425.0	33.7	MUS	10.63	-25.24	7.41	4903.0
K-I-10	EMERSON L.	MNWF	u	345.0	33.2	MUS	10.43	-24.02	8.12	4904.0
K-II-1	EMERSON L.	NRPK	M	925.0	49.1	MUS		-25.02	7.28	4913.0
K-II-2	EMERSON L.	NRPK	F	1260.0	56.5	MUS		-24.78	6.89	4914.0
K-III-1	EMERSON L.	WHSK	F	825.0	39.3	MUS	11.44	-20.66	4.25	4929.0
K-III-2	EMERSON L.	WHSK	F	550.0	34.0	MUS	11.78	-19.67	4.42	4930.0
K-III-4	EMERSON L.	WHSK	F	650.0	37.0	MUS	8.00	-26.95	7.60	4931.0
K-IV-1	EMERSON L.	LNSK	F	600.0	37.7	MUS	7.09	-28.36	6.09	4963.0
K-IV-2	EMERSON L.	LNSK	M	560.0	34.8	MUS	11.61	-25.25	5.43	4964.0
K-IV-3	EMERSON L.	LNSK	M	825.0	41.1	MUS	11.06	-26.45	4.90	4965.0
K-IV-7	EMERSON L.	LNSK	F	650.0	37.2	MUS	9.96	-28.95	5.45	4966.0

Appendix A. cont.

NRBSID	LOCATION	SPECIES	SEX	WEIGHT	LENGTH	PART	$\delta^{34}S$	$\delta^{13}C$	$\delta^{15}N$	ISOLAB
K-IV-8	EMERSON L.	LNSK	F	640.0	37.4	MUS	10.64	-25.79	5.55	4967.0
K-IV-10	EMERSON L.	LNSK	M	590.0	35.6	MUS	10.12	-24.72	5.62	4968.0
L-I-1	BELOW BERL.	MNWF	u	390.0	33.2	MUS	-0.49	-26.46	9.66	4905.0
L-I-2	BELOW BERL.	MNWF	u	450.0	31.9	MUS	-1.59	-27.04	8.92	4906.0
L-I-7	BELOW BERL.	MNWF	u	1010.0	42.6	MUS	-2.26	-25.75	10.57	4907.0
L-II-1	BELOW BERL.	NRPK	F	620.0	43.8	MUS		-26.08	6.86	4915.0
L-II-2	BELOW BERL.	NRPK	u	600.0	44.1	MUS	6.15	-27.82	6.88	4916.0
L-II-3	BELOW BERL.	NRPK	u	5135.0	87.6	MUS	9.28	-25.20	8.41	4917.0
L-II-4	BELOW BERL.	NRPK	M	1050.0	53.8	MUS		-25.96	7.37	4918.0
L-II-5	BELOW BERL.	NRPK	M	2150.0	64.3	MUS		-26.30	7.34	4919.0
L-II-7	BELOW BERL.	NRPK	u	2090.0	63.6	MUS	8.95	-25.53	7.52	4920.0
L-III-1	BELOW BERL.	WHSK	M	636.0	35.2	MUS	7.90	-28.15	8.14	4932.0
L-III-4	BELOW BERL.	WHSK	F	1105.0	42.5	MUS	5.79	-26.97	5.57	4933.0
L-III-5	BELOW BERL.	WHSK	M	570.0	36.0	MUS	7.77	-25.93	5.08	4934.0
L-III-6	BELOW BERL.	WHSK	F	950.0	41.0	MUS	7.11	-27.17	7.28	4935.0
L-III-7	BELOW BERL.	WHSK	M	720.0	37.7	MUS		-27.63	6.81	4936.0
L-III-8	BELOW BERL.	WHSK	F	800.0	39.5	MUS	8.82	-26.10	5.93	4937.0
L-IV-2	BELOW BERL.	LNSK	F	1060.0	44.2	MUS	10.70	-27.62	5.04	4969.0
L-IV-3	BELOW BERL.	LNSK	F	620.0	38.0	MUS	9.22	-26.43	5.32	4970.0
L-IV-5	BELOW BERL.	LNSK	M	540.0	35.8	MUS	10.65	-29.61	5.19	4971.0
L-IV-6	BELOW BERL.	LNSK	M	765.0	39.2	MUS	9.98	-25.10	5.62	4972.0
L-IV-9	BELOW BERL.	LNSK	F	800.0	41.2	MUS	7.61	-27.39	5.03	4973.0
L-IV-10	BELOW BERL.	LNSK	F	810.0	41.4	MUS	8.99	-24.76	5.42	4974.0
M-I-1	WINDFALL BR.	MNWF	u	960.0	43.1	MUS		-27.39	8.45	4908.0
M-I-2	WINDFALL BR.	MNWF	u	560.0	37.8	MUS	3.47	-26.78	9.48	4909.0
M-I-3	WINDFALL BR.	MNWF	u	435.0	35.9	MUS	-0.01	-27.10	10.01	4910.0
M-I-8	WINDFALL BR.	MNWF	u	1280.0	45.0	MUS	3.70	-25.81	8.70	4911.0
M-II-1	WINDFALL BR.	NRPK	u	1280.0	55.8	MUS	9.82	-26.48	7.55	4921.0
M-II-2	WINDFALL BR.	NRPK	F	3450.0	76.6	MUS		-25.25	8.96	4922.0
M-II-3	WINDFALL BR.	NRPK	u	765.0	47.1	MUS	8.83	-27.20	7.54	4923.0
M-II-5	WINDFALL BR.	NRPK	u	2540.0	67.7	MUS	10.22	-27.69	7.43	4924.0
M-II-6	WINDFALL BR.	NRPK	F	2650.0	70.0	MUS		-28.50	7.34	4925.0
M-II-10	WINDFALL BR.	NRPK	u	3715.0	77.0	MUS	8.73	-25.58	8.81	4926.0
M-III-4	WINDFALL BR.	WHSK	F	710.0	38.0	MUS	8.36	-28.20	6.94	4938.0
M-III-5	WINDFALL BR.	WHSK	F	980.0	40.6	MUS		-25.85	5.41	4939.0
M-III-6	WINDFALL BR.	WHSK	M	570.0	35.0	MUS	6.91	-26.40	6.90	4940.0
M-III-7	WINDFALL BR.	WHSK	M	750.0	38.9	MUS		-25.09	5.31	4941.0
M-III-8	WINDFALL BR.	WHSK	F	1020.0	43.1	MUS		-27.81	6.86	4942.0
M-III-9	WINDFALL BR.	WHSK	F	960.0	40.6	MUS		-29.80	6.10	4943.0
M-III-10	WINDFALL BR.	WHSK	F	1170.0	44.6	MUS		-27.83	5.40	4944.0
M-IV-2	WINDFALL BR.	LNSK	M	690.0	38.3	MUS	7.37	-25.26	5.15	4975.0
M-IV-3	WINDFALL BR.	LNSK	F	880.0	40.9	MUS	8.38	-25.56	5.56	4976.0
M-IV-4	WINDFALL BR.	LNSK	F	940.0	40.3	MUS	5.36	-26.04	6.48	4977.0
M-IV-6	WINDFALL BR.	LNSK	F	730.0	37.6	MUS	9.99	-28.91	5.64	4978.0

Appendix B. Northern River Basins Study sediment stable isotope data.

ISOLAB	IDENTITY	$\delta^{13}C$	$\delta^{34}S$
7536.0	HINTON	-26.16	5.30
7537.0	HINTON	-26.27	2.67
7538.0	HAUL BR.	-25.86	2.08
7539.0	HAUL BR.	-26.43	3.88
7540.0	OBED COAL	-26.80	0.15
7541.0	OBED COAL	-25.91	-0.08
7542.0	EMERSON L.	-25.59	1.63
7543.0	EMERSON L.	-25.91	3.29
7544.0	KNIGHT BR.	-26.33	7.18
7545.0	KNIGHT BR.	-24.79	2.85
7546.0	WINDFALL BR.	-24.64	4.96
7547.0	WINDFALL BR.	-24.41	3.46

NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

LABORATORY ANALYSES OF STABLE ISOTOPES IN FISH TISSUES AND STOMACH CONTENTS, AND STREAM BIOTA

PROJECT 3131-B1: Food Web and Stable Isotope Analyses

I. PROJECT DESCRIPTION

The contractor will analyze fish tissue and stream biota for the stable isotopes of carbon, sulphur and nitrogen. These analyses will assist in the interpretation of fish food sources and movement, contaminant concentrations in tissues and food web pathways.

II. TERMS OF REFERENCE

1. Tasks

   The contractor will analyze up to 516 samples of fish tissue (including liver), gut contents and stream biota (including bioslime) for stable isotopic composition of sulphur, carbon, and nitrogen. These results are to be interpreted with respect to fish food sources, fish movements and trophic status. The analysis is to follow the protocols outlined in Appendix A.

2. Frozen samples will be provided to the laboratory from the Northern River Basins Study collections.

III. REPORTING REQUIREMENTS

1. The values of $\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$ for each sample are to be reported.

2. Difficulties encountered with any samples are to be noted.

3. The contractor will prepare interim and final reports. To be included with the progress and final reports:

   a. a detailed description of the methodology used;

   b. a brief description of the mass spectrometer;

   c. a detailed description of the internal QA/QC procedures and estimation of precision;

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d. an interpretation of the meaning of the values with respect to the implications for food sources, fish migration, trophic levels (food web pathways); and

e. an assessment of the utility of the method in achieving the goals of improving the understanding of fish trophic structure, feeding sources, and fish movements between feeding areas. Recommendation of areas requiring further study.

4. In referring to specific samples, the contractor will use the same reference numbers as those attached to each sample.

5. The contractor will provide two copies of the computerized record of the analytical data on a 3.5" or 5.25" IBM-compatible disk.

IV. SCHEDULING

1. Interim report no later than October 31, 1992.

2. Final report by March 31, 1993.

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PROJECT 3131-B1: Food Web and Stable Isotope Analyses

TASK:

1. The contractor will analyze each of the samples for the following:

   a. The contract laboratory is required to analyze each fish tissue, gut content and biota sample for $\delta^{34}S$.

      i) The values shall be given relative to Canyon Diablo sulphur.

      ii) Precision of the analyses must be ±0.3% or better.

      iii) The influence of oxygen isotopic variation is to be eliminated through laboratory methods or corrected for using the mass 48/50 peak.

      iv) Digestion of fish and biota to yield sulphate must not produce any isotopic fractionation.

   b. The contract laboratory is required to analyze each fish tissue, gut content and biota sample for $\delta^{13}C$.

      i) The values shall be given relative to PDB carbon.

      ii) Precision of the analyses must be ±0.2% or better.

      iii) Acid treatment should be used to remove carbonate materials in the analyses of gut content and biota.

   c. The contract laboratory is required to analyze each fish tissue, gut content and biota sample for $\delta^{15}N$.

      i) The values shall be given relative to nitrogen in air.

      ii) Precision of the analyses must be ±0.3% or better.

      iii) Argon measurement on the mass spectrometer is to be used for the assessment of air contamination.

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2. Based on the distribution of the sample collections and the composition of the catches, the Project Manager will, in consultation with the Alberta Fish and Wildlife and Alberta Environment fisheries and contamination experts, stratify the sample in order of priority for analyses.

3. The samples will be analyzed in order of priority for the stable isotopes of sulphur, carbon, and nitrogen. The priority will be periodically reviewed in light of results to date.

4. The methodology and quality assurance procedures will be approved by the Northern River Basins Study Office prior to the commencement of the analytical work.

5. The contractor will also store the samples supplied by the Northern River Basins Study, at -20 Celsius while in possession of them and ship remaining materials back to the Northern River Basins Study storage facility within 6 months of the end of the contract.

6. The contractor will also supply all required equipment, chemicals, standard materials, etc., required to complete the work.