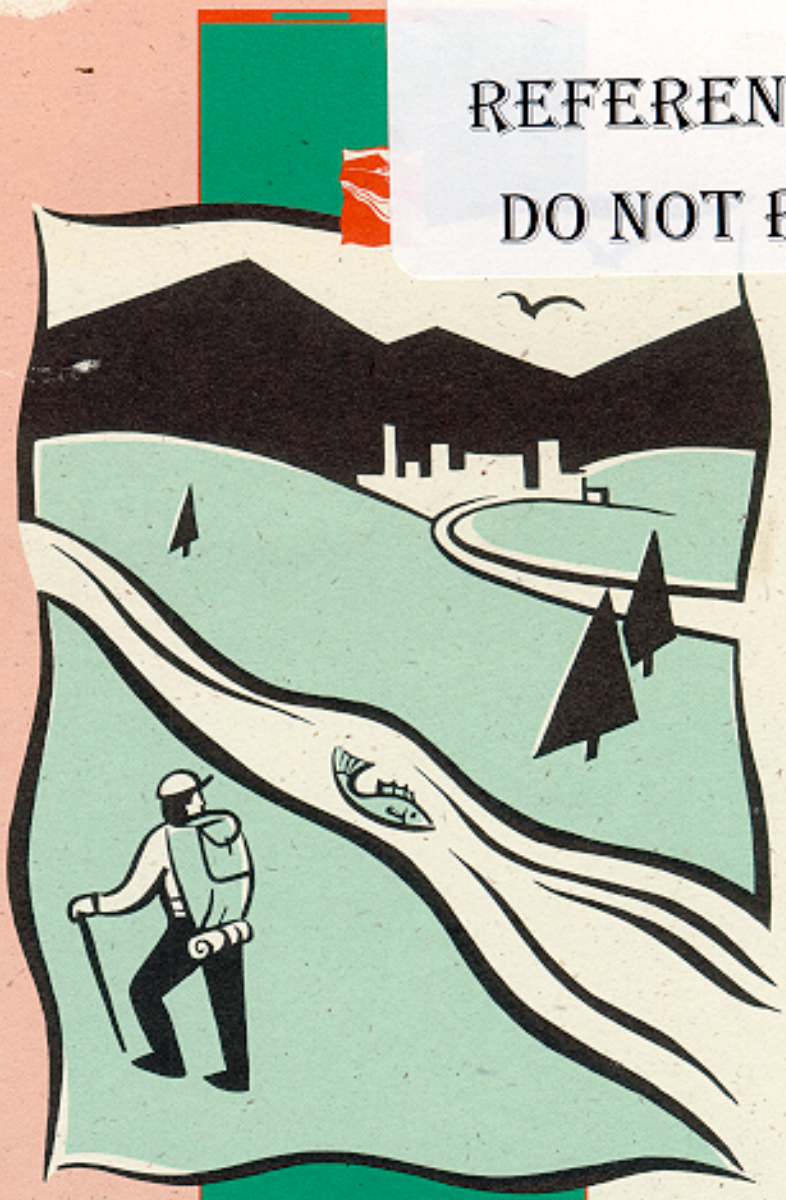
Fraser River Action Plan



Identification of Pulp Mill Effluent "Signals" in Riverine Food Webs by Stable Isotopic Analyses

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IDENTIFICATION OF PULP MILL EFFLUENT "SIGNALS" IN RIVERINE FOOD WEBS BY STABLE ISOTOPE ANALYSES

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Executive Summary

Although biological responses to pulp mill effluents (PME) can be measured, often it is difficult to quantify exposure of the biota to PME because of significant dilution in the receiving waters. Following the widely used stressor-exposure-response model, the quantitative establishment of exposure of biota to PME stressors is critical to developing cause-and-effect relationships among PME stressors and biological responses. A promising technique for establishing such exposure is the use of stable isotopic analyses, since stable isotopes of carbon, nitrogen and sulfur incorporated through food sources into biological tissues may serve as markers, or "signals", of the spatial extent of pulp mill effluent exposure (ingestion) in downstream riverine food webs.

The goal of this pilot project was 1) to assess whether stable isotopes of carbon and sulfur could be used to characterize and trace the fate of PME carbon and sulfur into the waters of the Thompson River, and 2) to determine the extent to which stable isotopes could be used to trace PME derived carbon and sulfur into the Thompson riverine food web. The objectives of the study were to be met by 1) chemically and isotopically characterizing PME carbon (dissolved inorganic carbon, DIC) and sulfur (SO_4) discharging into the Thompson River, 2) establishing natural background levels and the isotopic composition of DIC, sulfate and nitrate in the North, South Thompson and Thompson rivers, and 3) measuring the carbon, nitrogen and sulfur isotopic compositions aquatic organisms in the form of biofilm, invertebrates and fish.

Background concentrations of NO_3 along the Thompson River ranged between 0.3 and 0.6 ppm, and were below detection in both the sewage effluent and PME. Background sulfate concentrations ranged between 9.9 and 13 ppm in the Thompson Rivers, however, sulfate concentrations 8-30 times higher than background in the sewage effluent (89.5 ppm) and the PME (307 ppm) indicated an anthropogenic source of sulfate in these effluents. The $\delta^{34}S$ isotopic composition of the sewage effluent and the PME (2.1 and 1.3 o/oo, respectively) was isotopically distinct from both the North and South Thompson Rivers (3.2 and 0.0 o/oo). Unfortunately, the 36/64 mixing of the North and

South Thompson Rivers yielded a background $\delta^{34}S\text{-}SO_4$ that was virtually identical to both PME and the sewage effluent. As a result, $\delta^{34}S$ could not be reliably used to trace the fate PME or sewage sulfur into the Thompson River food web.

Background $\delta^{13}C$ data for dissolved inorganic carbon (DIC) in the Thompson River ranged between -7.1 and -8.1 o/oo, typical values for a well-mixed, bicarbonate dominated river in equilibrium with atmospheric CO_2. The sewage effluent, however, was significantly isotopically depleted in ^{13}C (-13.9 o/oo) relative to the background in the river, indicating the influence of terrestrial derived organic carbon. The negative shift in DIC was also observed in the Thompson River DIC just below the sewage plant diffusers. Although we were unable to measure the $\delta^{13}C$-DIC of the PME, a strong carbon isotopic depletion in the Thompson River DIC just below the PME diffusers indicated that PME contributed a significant terrestrial carbon input to the Thompson riverine DIC pool. We calculated that for the sample near the left bank below the PME diffusers, 23 % to 34 % of the riverine DIC at this site was derived from PME. Thus, the $\delta^{13}C$ data suggest that carbon isotopes may be a good tracer for following the fate of PME derived carbon into a riverine food chain. Unfortunately, during the single sampling trip, biofilm or aquatic organisms could not be obtained from sites near the pulp mill or sewage treatment plant.

Contrary to the single carbon-source model which has been assumed to be appropriate for food web of Thompson River, stable isotope analyses suggest the food web of this river is supported by two distinct carbon sources: (i) the algal biofilm which utilizes dissolved inorganic carbon from the river; and (ii) an unmeasured source with an isotopic signature similar to that of terrestrial plants. Our simple, two component, isotope mass balance models for carbon (and nitrogen) supports these conclusions and suggests that terrestrially derived organic carbon plays a much larger role in supporting the Thompson River food web than previously suspected.

Based upon nitrogen composition, the river has at least three trophic levels including algal biofilm, insect grazers, and insectivorous fish. However, this trend in trophic structure was partially obscured by the apparent input of an unmeasured terrestrial

source. Future dual-isotope research on this riverine food web must isolate the unmeasured source of carbon and nitrogen, which we hypothesize to be terrestrial plant input from upstream headwaters and PME.

Using stable isotopes to effectively trace the fate of PME carbon and sulfur was not completely successful in this pilot project due to time and sampling limitations, and to the complicated nature of the site - incomplete mixing of two rivers, and the presence of a sewage treatment plant near the PME diffusers. Nevertheless, the data clearly indicated that $\delta^{13}C$ of DIC derived from PME was isotopically distinct from riverine background values, and that carbon isotopes may be a useful PME tracer, particularly at a less complicated site. Similarly for $\delta^{34}S$, PME contributed significant anthropogenic sulfate inputs to the river, although unfortunately, its sulfur isotopic signal was indistinguishable from a mixture of the North and South Thompson rivers.

Despite the limitations encountered in the pilot study, we are convinced that stable isotopes of carbon and sulfur have excellent potential to trace PME derived carbon and sulfur from effluent source into riverine aquatic food webs. We recommend that a similar study be undertaken at a site that does not suffer from the complications of multiple effluent inputs and the mixing of two rivers. Finally, our study suggests that terrestrially derived carbon (perhaps from PME) plays an important role in supporting the food web of the Thompson River downstream of Kamloops Lake.

1.0 Introduction

Treated effluents from bleached kraft pulp mills have the potential to modify aquatic food webs by affecting the biota of primary and secondary producer trophic levels. These environmental impacts can be attributed to the effects of organic and nutrient enrichment, or to chemical toxicity caused by contaminants contained within effluents. For example, nutrients added by these effluents can produce major changes in the level of algal productivity (Bothwell 1992) and species diversity (Amblard et al. 1990). In severe cases organic enrichment from pulp mills can decrease species diversity of benthic invertebrates (Poole et al. 1978), but under moderate enrichment effluent addition may lead to increased invertebrate productivity (Swanson et al 1992). Impacts on fish include reduced recruitment, inhibited growth of gonads and induction of hepatic mixed function oxygenase (Servos et al. 1992; Munkittrick et al 1992).

Although biological responses to pulp mill effluents (PME) can be measured, often it is difficult to quantify exposure of the biota to PME because of significant dilution in the receiving waters. Following the widely used stressor-exposure-response model of Hunsaker and Carpenter (1990), the quantitative establishment of exposure of biota to PME stressors is critical to developing cause-and-effect relationships among PME stressors and biological responses. A promising technique for establishing such exposure is the use of stable isotopic analyses, since stable isotopes of carbon, nitrogen and sulfur incorporated through food sources into biological tissues may serve as markers, or "signals", of the spatial extent of pulp mill effluent exposure (ingestion) in downstream riverine food webs.

Naturally occurring stable isotopes of carbon, nitrogen or sulfur have been used in a variety of terrestrial studies to follow the fate of anthropogenic pollutants in natural systems (e.g., sewage, nitrate contamination, sour gas), but to a much lesser extent in aquatic environments and food chain studies (Van Dover et al., 1992; Gearing et al., 1991). The basis of the natural abundance stable isotope approach is that pollutants

entering an ecosystem may have isotopic signatures of ^{15}N, ^{13}C, or ^{34}S that are distinct from the unpolluted environment (Van Dover et al., 1992). Organisms that ingest these pollutants will assimilate, along with their other food sources, the isotopic signals of the contaminant into their body parts. In addition, for food chain studies, biological fractionation (or discrimination) against a particular isotope with increasing trophic status must also be accounted for (*cf* Fry and Sherr, 1984; Minagawa and Wada, 1984). Fortunately, significant isotopic discrimination appears to be limited to nitrogen (e.g. about 3 $^{o}/_{oo}$ isotopic enrichment per trophic level), and to a much lesser extent for carbon or for sulfur (about 0-1 $^{o}/_{oo}$; Minagawa and Wada, 1984; Fry and Sherr, 1984; Rundel et al., 1989; Fry, 1991). As a result, carbon and sulfur isotopes may be used to track the fate of pollutants into the food chain, and nitrogen isotopes can further be used to establish trophic status. To our knowledge no studies have attempted to trace the fate of pollutants into the food chain in terrestrial aquatic systems using stable isotope techniques.

The working hypothesis was that PME would contribute higher than background levels of dissolved or particulate organic carbon to the river, which could subsequently oxidize and contribute isotopically "light" CO_2 ($\delta^{13}C$ -25 to -30 $^{o}/_{oo}$ terrestrial organic matter) to the dissolved inorganic carbon (DIC) pool of the Thompson River. Further, sodium sulfate (saltcake) used in the pulp treatment process could add higher than background sulfate levels to the river, possibly with a unique $\delta^{34}S$ signature. The stable isotopes of carbon, nitrogen and sulfur in aquatic organisms could be used to establish food web patterns and trace the flow of PME derived carbon and sulfur into the aquatic food web of the Thompson River.

The goal of this pilot project was 1) to assess whether stable isotopes of carbon and sulfur could be used to characterize and trace the fate of PME carbon and sulfur into the waters of the Thompson River, and 2) to determine the extent to which stable isotopes could be used to trace PME derived carbon and sulfur into the Thompson riverine food web. The objectives of the study were to be met by 1) chemically and isotopically characterizing PME carbon and sulfur (DIC, SO_4) discharging into the Thompson River,

2) establishing natural background levels and the isotopic composition of DIC, sulfate and nitrate in the North, South Thompson and Thompson rivers, and 3) measuring the carbon, nitrogen and sulfur isotopic compositions aquatic organisms in the form of biofilm, invertebrates and fish.

It was anticipated *a priori* that the results of the study could be compromised by mixing of waters of different chemistries and isotopic composition from the North and South Thompson rivers, and further complicated by the presence of sewage discharge from the Kamloops sewage treatment plant just upstream from the pulp mill. The two month year-end time-frame of the pilot study limited the study to one sampling trip in March of 1993.

2.0 Sampling and Analytical Methods

A single field trip for the purpose of collecting river water, epilithic biofilm, invertebrates, and fish from the Thompson River was conducted between March 1-5, 1993. Sampling stations included sites on the North Thompson, South Thompson and Thompson rivers, as well as effluent samples from the Kamloops sewage treatment plant and the Weyerhaeuser pulp mill (Figure 1). Due to the extensive presence of ice cover on the North and South Thompson rivers, only water samples could be collected from all of the stations. A total of 52 water samples were collected, four from each of the 13 sampling stations. Twenty one samples of epilithic biofilm were collected at five sites along the Thompson River, and samples of invertebrates and fish were collected at only two sites along the Thompson River.

2.1 Water Samples

River water samples were collected from 11 sites along the Thompson River during the week of March 1-5, 1993 (Figure 1). River water samples were collected in

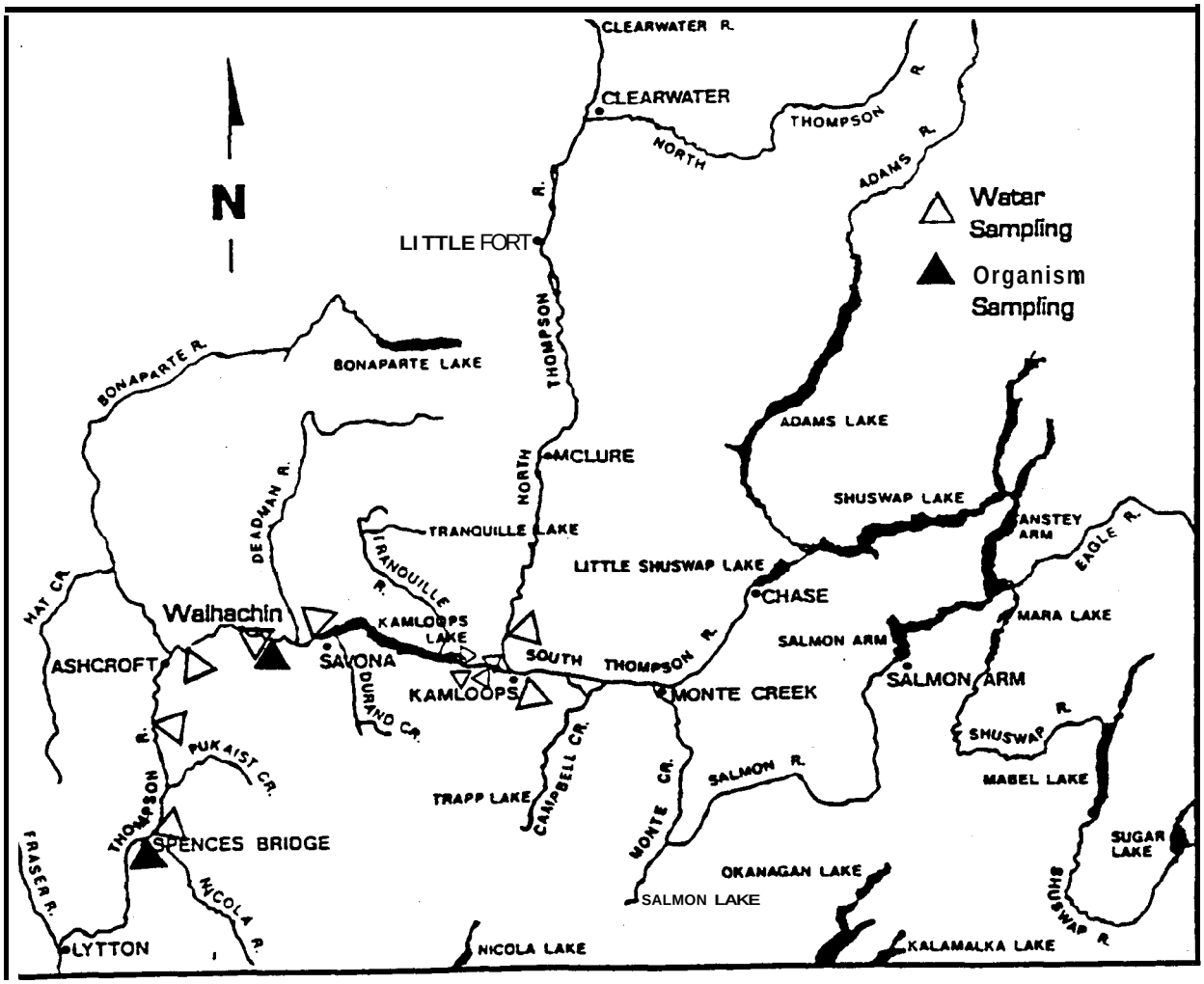

Figure 1. Location of the water and organism sampling sites on the South Thompson, North Thompson, and Thompson Rivers, B.C.

two 1 L plastic bottles by wading into the main flow of the river to a maximum depth of 0.5 m, rinsing the bottles with river water three times, filling the containers and placing the filled container on ice. In addition, two 1 L samples of PME were taken from the sewage treatment plant.

Water samples for oxygen and hydrogen isotope analyses were collected in tightly capped 50 ml plastic bottles. These samples for water isotopes were collected to aid in distinguishing mixing proportions of waters from the North and South Thompson River, the sewage treatment plant and the pulp mill. Water samples for selected nutrient ion concentration analyses (SO_4, NO_3) were collected in 50 ml plastic bottles, stored at 5ºC, and analyzed within one week using standard ion chromatography techniques. Dissolved sulfate for $\delta^{34}S$ analyses were precipitated as $BaSO_4$ from 1 L samples as by the addition of excess Barium chloride. River water DIC samples were collected in tightly sealed 1 L plastic bottles, and stored at 5ºC until further processing.

Stable carbon isotopic analyses of DIC from river water and sewage effluent were performed on 50 ml aliquots, injected via septum into an evacuated 500 ml flask containing 100 ml 85% H_3PO_4. Carbon isotope analyses of DIC could not be done on PME because of its lower viscosity. The CO_2 produced from the DIC extraction was cryogenically purified for $\delta^{13}C$ analyses. Sulfate isotopic analyses ($\delta^{34}S$) were conducted at the Department of Physics, University of Calgary. Water samples (2 µl) for hydrogen isotopic analyses were reduced to H_2 gas by reaction with zinc in 6 mm sealed Pyrex tubes at 500°C (Coleman et al., 1982). Water samples for oxygen isotope analyses were prepared using a VG Isoprep18™ water-CO_2 equilibrator. Stable isotope measurements for H, C, O, and N were performed using a VG Optima™ stable isotope ratio mass spectrometer at NHRI. All stable isotope data are reported relative to the pertinent international standard (carbon - PDB; hydrogen - SMOW; oxygen - SMOW; nitrogen - air; sulfur - CDT) in the typical delta ($^{O}/oo$) notation (Fritz and Fontes, 1980). Soluble nutrient ion (NO_3, SO_4) concentrations were conducted on water samples using standard liquid ion chromatography techniques at NHRI.

2.2 Aquatic Organisms

Samples of epilithic biofilm were collected from five sites along the Thompson River by using a metal scalpel blade to scrape the epilithic mat into a collecting jar. Each sample represented a composite of material from 2-3 large stones (64-256 cm^2). Stones with a thick biofilm mat were selected for sampling to facilitate collection of large amounts of biofilm.

Invertebrates and fish could only be collected from two of the biofilm sampling sites (Walhachin and Spences Bridge) due to the large investment of time required to collect sufficient biomass for the analyses. Invertebrates were sampled by placing a U-net (Scrimgeour et al. 1993) with 250 μm mesh downstream of an area of substratum and vigorously agitating the streambed to a depth of 5 cm. All animals collected in the net were placed in sorting trays on the streambank and the study animals live-sorted by taxa. Fish were collected by electrofishing and catching the stunned fish in a minnow net held downstream of the shocking area. In order to clear their guts, each taxonomic group of invertebrates or fish were held separately in aerated aquaria at 10ºC for 18-24 h before being placed into vials and frozen. Because organic debris, biofilm and mucus was found to adhere to some animals, considerable time was spent removing this material from individual specimens to reduce the possibility of sample contamination.

In the laboratory, freeze-dried epilithic biofilm, invertebrates and fish were analyzed for their stable carbon and nitrogen isotopic compositions. Fish samples were freeze dried and ground. For ^{13}C, 2 mg samples of whole biofilm and invertebrates were placed in 20 cm Vycor™ breakseal tubes along with 2 g CuO and 1 g Ag wire (Boutton et al., 1983). Samples were evacuated to $< 10^{-3}$ torr and the tubes sealed using a flame torch. Samples were combusted at 850ºC for 2 hours, followed by slow cooling at 0.8ºC per hour. The CO_2 gas produced was cryogenically purified and analyzed for $\delta^{13}C$ as described above. For ^{15}N, 10 to 40 mg samples of biofilm, insects and fish were prepared

using the CaO combustion method described by Kendall and Grim (1990). Purified dinitrogen was analyzed for $\delta^{15}N$ on a VG Optima™ as described above.

3.0 Results and Discussion

Results of all isotopic analyses of waters, dissolved inorganic carbon and sulfate are reported in Table 1. The measurements taken reflect a single snapshot in time, and it should be noted that the data presented cannot be over-interpreted, as the effect of seasonal variations in water chemistry, isotopes, effluent, and water volumes was not within the scope of the pilot project.

3.1 Effluent Inputs to the Thompson River

The waters of the North and South Thompson River were isotopically distinct in their oxygen isotopic composition, -18.3 and -17.2 ^{o}/oo, respectively (Table 1). These isotopic end-members could be used to calculate the downstream mixing proportion of the rivers using a simple 2 component isotope mass balance;

$$\delta^{18}O_{downstream} = X\,(\delta^{18}O_{N\ Thompson}) + Y\,(\delta^{18}O_{S\ Thompson}) \qquad (1)$$

where x+y =1. Using a downstream (Savona and below) average of -17.6 o/oo (Table 1), the mixing proportion of the North and South Thompson rivers was about 36% and 64% respectively. Hydrogen isotope mass balance calculations yielded a similar value.

Both the sewage effluent and pulp mill effluent (Table 1) were slightly isotopically heavier than either the North or South Thompson Rivers for $\delta^{18}O$ and $\delta^{2}H$. This suggested minor addition of isotopically heavier waters, possibly from reservoir storage or additional water from sources other than the Thompson river.

Table 1. Isotopic and chemical composition of water, DIC and dissolved nutrients at sampling sites on the South Thompson, North Thompson, and Thompson Rivers, B.C. March 1-5, 1993.

Sampling Station	$\delta^{18}O$ water	$\delta^{2}H$ water	$\delta^{13}C$ (DIC)	$\delta 34_S$ ($SO_4^{=}$)	NO_3^- ppm	$SO_4^{=}$ ppm
N. Thompson	**-18.3**	-140.0	-7.5	3.2	0.7	12.8
S. Thompson	**-17.2**	-131.9	-8.1	-0.0	0.3	10.0
Sewage Effluent	-16.9	-131.8	**-13.9**	2.1	<.16	**89.5**
Right Bank Below Sewage	-17.3	-133.0	-9.0	3.3	0.4	17.9
Left Bank Below Sewage	-17.6	-138.3	-9.3	2.2	0.5	15.4
Pulp Effluent	-16.3	-130.7	-	**1.3**	<0.8	**307.0**
Left Bank Below Pulp Mill	-17.9	-138.3	**-12.9**	2.6	0.6	40.3
Right Bank Below Pulp Mill	-17.3	-133.5	-8.6	2.3	0.4	13.2
Thompson River						
Savona	-17.6	-135.5	-7.1	3.7	0.5	10.0
Walhachin	-17.6	-134.8	-7.8	4.2	0.5	9.9
Ashcroft	-17.8	-134.3	-7.4	3.3	0.5	10.9
Highland Valley	-16.9	-134.9	-7.6	2.2	0.6	11.6
Spences Bridge	-17.6	-135.3	-7.5	0.2	0.6	13.0

The $\delta^{18}O$ data from the Thompson River below the confluence of the North and South (Figure 1) and just below the sewage treatment plant and the Pulp Mill indicated incomplete mixing of the North and South Thompson Rivers. The water sample collected from the left bank yielded $\delta^{18}O$ values closer to that of the North Thompson (-17.6 to -17.9 o/oo), whereas the water samples collected near the right bank more closely resemble the isotopic signature of the South Thompson river. The Savona site and other downstream occur below Kamloops Lake (Figure 1) and are presumably well mixed.

Nutrients and Isotopes of Carbon and Sulfur

Background concentrations of NO_3 along the Thompson River ranged between 0.3 and 0.6 ppm, and were below detection in both the sewage effluent and PME (Table 1). Background sulfate concentrations ranged between 9.9 and 13 ppm in the Thompson Rivers, however, sulfate concentrations 8-30 times higher than background in the sewage effluent (89.5 ppm) and the PME (307 ppm) indicated an anthropogenic source of sulfate in these effluents. The $\delta^{34}S$ isotopic composition of the sewage effluent and the PME (2.1 and 1.3 ^{o}/oo, respectively) was isotopically distinct from both the North and South Thompson Rivers (3.2 and 0.0 ^{o}/oo) (Table 1). Unfortunately, the 36/64 mixing of the North and South Thompson Rivers yielded a background $\delta^{34}S$-SO_4 that was virtually identical to both PME and the sewage effluent. As a result, $\delta^{34}S$ could not be reliably used to trace the fate PME or sewage sulfur into the Thompson River food web.

Background $\delta^{13}C$ data for dissolved inorganic carbon (DIC) in the Thompson River ranged between -7.1 and -8.1 o/oo (Table 1). These values are typical for a well mixed bicarbonate dominated river in equilibrium with atmospheric CO_2 (LaZerte and Szalados, 1982). The sewage effluent, however, was significantly isotopically depleted in ^{13}C relative to the background in the river. Values of -13.9 for the sewage effluent indicated the influence of terrestrial derived organic carbon (-25 to -30 o/oo) to the DIC pool. The negative shift in DIC was also observed in the Thompson River DIC just below

the sewage plant diffusers. Although we were unable to measure the δ^{13}C-DIC of the PME, a strong carbon isotopic depletion in the Thompson River DIC just below the PME diffusers indicated that PME contributed a significant terrestrial carbon input to the Thompson riverine DIC pool. The more depleted carbon isotope values resulted from the oxidation of terrestrial organic matter (in sewage and PME) to DIC;

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \rightarrow H_2CO_3 \qquad (2)$$

The DIC produced from the oxidation of terrestrial organic matter would have a similar carbon isotopic signature as its source (-25 to -30 ^{o}/oo, Deines, 1980). The isotopically depleted DIC produced, however, is diluted with background DIC in the PME, sewage effluent and the Thompson river. For example, if we assumed a PME DIC carbon isotopic value of -20 to -25 ^{o}/oo, an upstream DIC isotopic value of -9.3 (below sewage plant), then using equation 1 and substituting for carbon, we calculated that for the sample near the left bank below the PME diffusers, 23 % to 34 % of the riverine DIC at this site would be derived from PME. Thus, the δ^{13}C data suggested that carbon isotopes may be a good tracer for following the fate of PME derived carbon into a riverine food chain. Unfortunately, during the single sampling trip, biofilm or aquatic organisms could not be obtained from sites near the pulp mill or sewage treatment plant (see below).

3.2 Isotopes and Trophic Structure of Aquatic Organisms

The food web of the lower Thompson River is thought to be supported by autochthonous (i.e., within the ecosystem) primary production and consist of three trophic levels, namely algae, grazing insects and insectivorous fish (Bothwell et. al. 1992, Bothwell and Culp 1993). Filter-feeding insects are omnivorous, consuming algal and animal material trapped in their feeding nets. Of the potential autochthonous carbon

sources, production by diatoms has been identified as the major component of plant productivity. Conventional stream ecology theory suggests that in an open canopy river such as the Thompson, allochthonous carbon from terrestrial sources, including particulate (POM) and dissolved (DOM) organic matter, should be a relatively unimportant component of energy flow through the ecosystem (Vannote et. al. 1980).

A dual-isotope tracer study is a useful approach for checking conventional ideas about food web structure (Fry 1991). Carbon composition can be used to indicate the source of plant material for higher trophic levels because metabolism of the biota does not alter the $^{13}C/^{12}C$ ratio significantly (Rosenfeld and Roff 1992). Nitrogen is fractionated during metabolism such that the $^{15}N/^{14}N$ ratio is shifted by 2.5-4.0$^{o}/_{oo}$ with each successive trophic level (Minagawa and Wada 1984, Fry 1991). In addition, $\delta^{34}S$ can be used to trace food sources.

d*13C of Carbon Sources and Consumers*

Algal biofilms in the Thompson River had a carbon composition of -18 to -14$^{o}/_{oo}$ (Table 2; Fig. 2). The dominant carbon source for algae in this river appeared to be dissolved inorganic carbon. Isotopic fractionation of carbon on the order of 8-12 ^{o}/oo (LaZerte and Szalados, 1982) from DIC (-7 o/oo), indicated that algal carbon source was indeed from riverine DIC. In this pilot study we did not attempt to measure other sources of carbon, such as organic detritus (e.g., leaves, grasses, DOM from PME, etc.), that might be available to animal consumers.

In the Thompson River all benthic insects were depleted by about 4$^{o}/_{oo}$ relative to the algal biofilm (Table 2; Fig. 2), a shift which could not be attributed to fractionation alone. Because gut analyses of insects such as mayflies and chironomids demonstrate that these animals consume large quantities of the biofilm (Culp and Glozier unpubl. data), it is likely that in addition to algae, these insects consume a more isotopically depleted carbon source. This unknown food source is likely terrestrial in origin and may include leaf and

Table 2. Carbon and nitrogen isotopic composition of biofilm, invertebrates and fish at Thompson River stations, March 1-5, 1993.

Station/Sample Type		$\delta^{13}C$ (PDB)	$\delta^{15}N$ (AIR)
Epilithic Biofilm			
Savona		-14.8	3.8
Walhachin		-15.1	2.7
Ashcroft		-13.7	3.8
Highland Valley		-16.6	0.8
Spences Bridge		-14.3	-
Fish	***Species/Family***		
Walhachin	Sculpin	-22.9	9.1
	Sculpin	-22.8	8.6
	Rainbow Trout	-24.1	9.7
	Longnose Dace (large)	-25.8	8.8
	Longnose Dace (small)	-21.8	8.5
Spences Bridge	Longnose Dace (large)	-23.7	7.8
	Longnose Dace (small)	-19.1	6.8
	Sculpin	-20.1	8.5
Insects			
Walhachin			
	Ephemerella (small)	-21.9	3.9
	Ephemerella	-22.5	4.2
	Hydropsche	-26.5	6.8
	Chironomid	-18.2	2.2
	Baetis	-21.7	4.7
	Perlodidae	-22.8	4.8
	Ephemerella (small)	-21.5	3.2
Spences Bridge			
	Ephemerella	-22.0	3.5
	Chironomid	-18.3	1.8
	Baetis	-22.0	3.7
	Perlodidae	-21.9	4.8
	Ameletus	-21.3	4.8
	Heptagenidae	-23.0	3.7

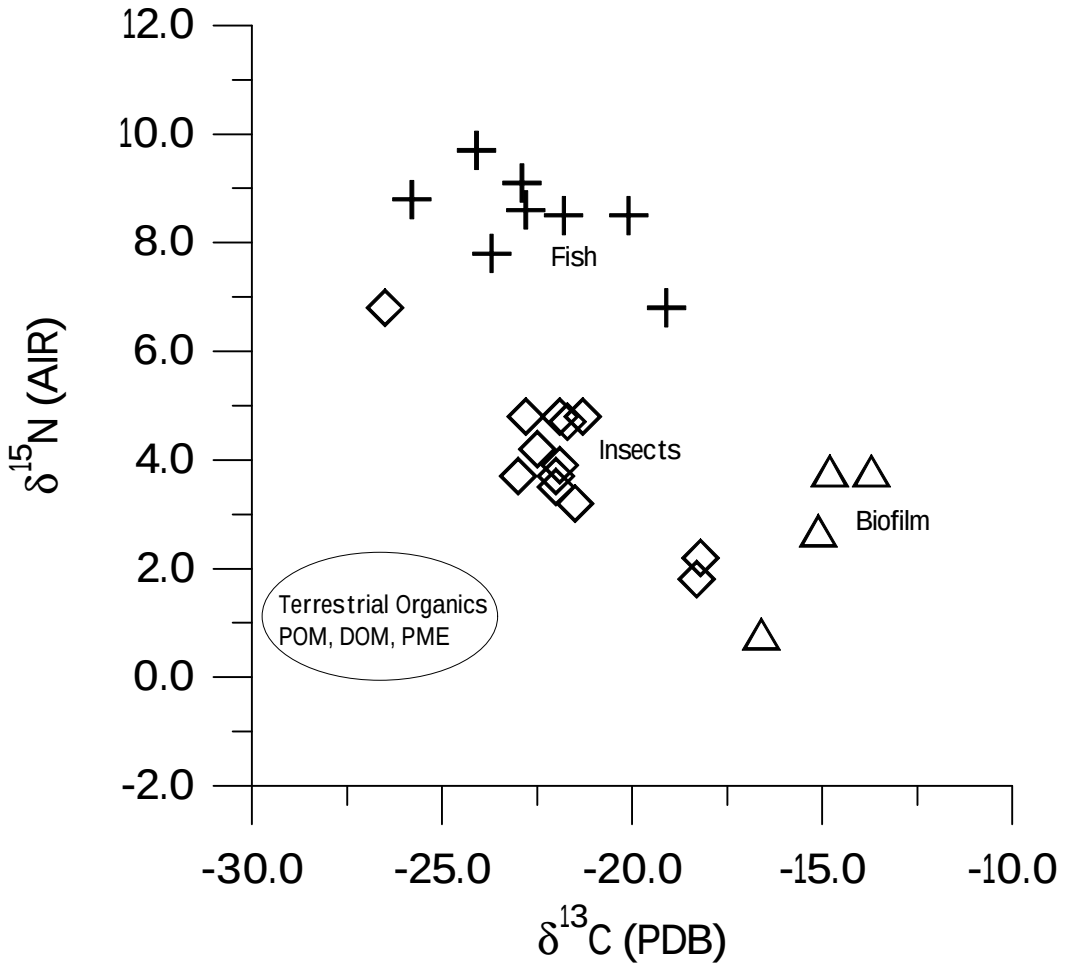

Figure 2. Carbon and nitrogen isotopic composition of aquatic organisms in the Thompson river. Triangles denote bofilm samples, diamonds denote insect samples and crosses denote fish samples. Range for terrestrial organics is from Deines (1980).

wood detritus, POM from upstream headwaters, or DOM from PME ($\delta^{13}C$ of -25 to -30 o/oo). Assuming that the unmeasured terrestrial food source had a $\delta^{13}C$ of -25 to -30 o/oo, biofilm an average $\delta^{13}C$ of -14.5 o/oo, the insects an average $\delta^{13}C$ of -22.5 o/oo, no trophic level carbon isotopic fractionation, and substituting into a simple two component isotope mass balance;

$$\delta^{13}C_{insects} = X(\delta^{13}C_{biofilm}) + Y(\delta^{13}C_{terrestrial\ organic}) \quad (3)$$

it would appear that most of the insects consumed between 24-48 % biofilm carbon and 51-76 % terrestrially derived organic carbon. Nitrogen isotopes were used to further support this estimate (see below).

Of the insects examined, chironomids had $\delta^{13}C$ values closest to that of their presumed carbon source, the algal biofilm. The mayflies, *Baetis tricaudatus*, *Ameletus*, and *Ephemerella* had more depleted $\delta^{13}C$ values indicating a greater dependency on the unmeasured terrestrial carbon source. Finally, the $\delta^{13}C$ of the filter-feeder, *Hydropsyche* (-26.5$^{o}/_{oo}$), was within the -30 to -25$^{o}/_{oo}$ range often measured for C_3 terrestrial plants (Fry 1991).

All of the fish sampled are thought to be predominantly insectivorous (Scott and Crossman 1973, Culp 1989, Nelson and Paetz 1992). In fact the $\delta^{13}C$ values corroborate this conventional view since the carbon composition of fish overlapped completely with that of aquatic insects (Table 2; Fig. 2). Small longnose dace (< 50 mm) and sculpin had less depleted $\delta^{13}C$ values (-22.9 to -19.1$^{o}/_{oo}$) than rainbow trout and large longnose dace (-25.8 to -23.7$^{o}/_{oo}$). The wide isotopic variability in dace (> 6$^{o}/_{oo}$) likely reflected size-dependent variability in feeding preferences.

d^{15}N of Nitrogen Sources and Consumers

Riverine food webs are supported by energy flow from allochthonous detritus and autochthonous primary production (Vannote et. al 1980), and consumers of this plant tissue should have an isotopic signature approximately 2.5-3.5$^{o}/_{oo}$ higher than the primary producer trophic level (Minagawa and Wada 1984, Fry 1991). In the Thompson River, the $\delta^{15}N$ values of the algal biofilm (0.8 to 3.8$^{o}/_{oo}$), chironomidae (1.8 to 2.2$^{o}/_{oo}$), and mayflies (3.2 to 4.8$^{o}/_{oo}$) were similar, suggesting that none of the insect consumers feed solely on algal biofilm (Table 2; Fig. 2). At the Walhachin site, the only location where biofilm and insect consumers where sampled simultaneously, mayfly $\delta^{15}N$ values were 0.5 to 2.0$^{o}/_{oo}$ higher than the biofilm. In contrast, chironomid $\delta^{15}N$ values (2.2$^{o}/_{oo}$) were depleted relative to algal biofilm (2.7$^{o}/_{oo}$). Thus, both mayflies and chironomids appear to consume algal biofilm and an unmeasured, isotopically depleted nitrogen source. This unknown source could be POM ($\delta^{15}N$ of 0 to 1 $^{o}/oo$) imported from upstream headwaters, or DOM in the PME. As with carbon, a simple two component nitrogen isotope mass balance was constructed to estimate relative proportions. The $\delta^{15}N$ values of terrestrial organic matter commonly range between 0-1 $^{o}/oo$. Assuming an average insect $\delta^{15}N$ of +4 $^{o}/oo$, an average biofilm $\delta^{15}N$ of 3.5 $^{o}/oo$, and a single trophic level fractionation of 3.5 $^{o}/oo$;

$$\delta^{15}N_{insects} = [\, X\,(\delta^{15}N_{biofilm}) + Y\,(\delta^{15}N_{terrestrial\ organic})\,] \quad + 3.5_{trophic\ fractionation} \qquad (3)$$

it would appear that the insects consumed about 20-40 % biofilm and 60-80 % terrestrial food sources. These estimates were supported by the carbon isotope data presented earlier.

In the higher trophic levels, nitrogen isotopic composition of the filter-feeding insect, *Hydropysche*, and all of the fish species indicate that aquatic insects are their dominant food source. *Hydropysche* may also consume algal biofilm.

In summary, contrary to the single, carbon-source model which has been assumed to be appropriate for food web of Thompson River (Bothwell et. al. 1992, Bothwell and Culp 1993), stable isotope analyses suggest the food web of this river is supported by two distinct carbon sources: (i) the algal biofilm which utilizes dissolved inorganic carbon from the river; and (ii) an unmeasured source with an isotopic signature similar to that of terrestrial plants. Based upon nitrogen composition, the river has at least three trophic levels including algal biofilm, insect grazers, and insectivorous fish. However, this trend in trophic structure was partially obscured by the apparent input of an unmeasured terrestrial source. Future dual-isotope research on this riverine food web must isolate the unmeasured source of carbon and nitrogen, which we hypothesize to be terrestrial plant input from upstream headwaters and PME.

3.3 Effects of Effluent on Food Web

Using stable isotopes to effectively trace the fate of PME carbon and sulfur was not completely successful in this pilot project due to time and sampling limitations, and to the complicated nature of the site - incomplete mixing of two rivers and the presence of a sewage treatment plant near the PME diffusers. Nevertheless, the data clearly indicated that $\delta^{13}C$ of DIC derived from PME was isotopically distinct from riverine background values, and that carbon isotopes may be a useful PME tracer, particularly at a less complicated site. Similarly for $\delta^{34}S$, PME contributed significant anthropogenic sulfate inputs to the river, although unfortunately, its sulfur isotopic signal was indistinguishable from a mixture of the two Thompson Rivers.

4.0 Recommendations

Despite the limitations encountered in the pilot study, we are convinced the stable isotopes of carbon and sulfur have excellent potential to trace PME derived carbon and sulfur from effluent source into riverine aquatic food webs. We recommend that a similar study be undertaken at a site that does not suffer from the complications of multiple effluent inputs and the mixing of two rivers. Finally, our study suggests that terrestrially derived carbon (perhaps from PME) plays an important role in supporting the food web of the Thompson River downstream of Kamloops Lake.

5.0 References

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