Final Report

Arsenic Sources and the Feasibility of Using Nitrogen Isotopes to Determine Nitrogen Sources to Crowley Lake

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Section 1

Arsenic Sources in the Upper Owens River Watershed

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Introduction

Arsenic contamination of drinking water is now recognized as a significant world health problem (Sharpe 2003). While a number of arsenic contamination incidents have resulted from industrial sources, the far greater exposure and health risks have resulted from the increasing use and dependence on wells for drinking water (Kumar and Suzuki 2002). In light of accumulated evidence for chronic toxicological effects of arsenic, regulatory limits for drinking water have been lowered. The World Health Organization guideline was reduced from 50 $\mu g\ l^{-1}$ to 10 $\mu g\ l^{-1}$ in 1993 (WHO 1993) and the US-EPA guideline was also recently reduced to 10 $\mu g\ l^{-1}$ (Federal Register 2001).

Arsenic (As) concentrations in riverine waters are usually low (0.1 – 0.8 $\mu g\ l^{-1}$; Smedley and Kinniburgh 2002) but may be elevated in areas with large spring inputs from geothermal sources or high-arsenic ground waters. Hot Creek geothermal springs in Long Valley, California have high arsenic concentrations (85-153 $\mu g\ l^{-1}$, Wilkie and Hering 1998) and constitute a significant fraction of the inflows to Crowley Lake (Long Valley Reservoir). Here, we measure naturally occurring arsenic concentrations along the major tributaries to Crowley Lake and conclude that the only major sources are Hot Creek geothermal springs and lesser inputs from Big Springs and springs in the alkali lakes area. These inputs result in As concentrations in Crowley Lake of more than twice the newly adopted EPA standard.

Methods

Speciation and analysis

Arsenic may exist in any of several oxidation states (-3, 0, +3, +5) in the natural environment (As) but is mostly found as arsenite (III) or arsenate (V) (Smedley and Kinniburgh 2002). Arsenate is readily measured by a modification of the widely used phospho-molybdate for measuring phosphorous (Strickland and Parsons 1972) in which the arsenate is determined as the differences in the spectrophotometric absorption of the molybdate-blue complex before and after reduction of arsenate to arsenite (Johnson 1971). This modification is recommended to avoid over-estimation of phosphate concentrations whenever arsenate concentrations may be significant and was routinely employed during a 2-yr study of nutrient loading to Crowley Lake (Jellison and Dawson 2003) and during a subsequent assessment of internal nutrient loading to the lake (Jellison et al. 2003).

Oxidation of As(III) proceeds via both abiotic and biotic processes. As(III) is thermodynamically unstable in the presence of oxygen, but abiotic oxidation proceeds slowly with a half-life on the order of 100 days (Hering 1997). However, microbially-mediated oxidation may be much more rapid. Hering (1997) estimated a half-life of only 20 minutes with samples collected along Hot Creek geothermal area with nearly all the As(III) oxidized with 1.2 km of the source. Despite these findings, initial comparisons between total As derived from graphite furnace atomic absorption measurement and the modified molybdate blue method on samples collected at Benton Crossing Bridge approximately 9.5 km downstream of geothermal inputs suggested significant quantities

of non-As(V) species. Given this finding, all arsenic concentrations presented in this report are total arsenic as determined by the graphite furnace atomic absorption method. Determinations were made at the Marine Science Analytical Laboratory at University of California, Santa Barbara (www.msi.ucsb.edu).

Field sampling

All apparatus and bottles used in surface water sampling were soaked in de-ionized water (DIW) and then rinsed 3 times with DIW. For the purposes of this project, DIW is used to refer to filtered, de-ionized, reverse osmosis treated water. This is our primary washing and rinse water with a specific conductance of approximately 5 $\mu S\ cm^{-1}$. For reagent and standard preparation this water is further polished by ion exchange to a specific conductance of approximately 0.5 $\mu S\ cm^{-1}$. Sample collection bottles were rinsed with 10% HCl before DIW soaking and rinsing.

During October, 2000 as part of our sampling on our 319 project, a sample for arsenic analysis was taken at every one of our sampling sites in the watershed. Samples were filtered in the field with plastic syringes fitted with Gelman A/E filters (1 micron) which were rinsed with at least 150 ml of DIW or sample water. All stream samples were "grab" samples taken at a well-mixed location in the stream such as the outlet of a culvert. Samples were kept cool and in the dark during transport to the Sierra Nevada Aquatic Research Laboratory (SNARL). Samples were preserved with ultrapure nitric acid and transported cold to UCSB for analysis using graphite furnace atomic absorption.

During July, 2001 samples were collected at our monitoring stations on Owens River, Mammoth Creek, Crowley Lake and the outlet. Additional stations were added in order to determine exact sources from high input areas such as springs located at the fish hatchery and the Hot Creek Thermal area. Outlet samples were collected between August, 2001 and April, 2002. All samples were collected, preserved and analyzed in the same manner as the October, 2000 samples.

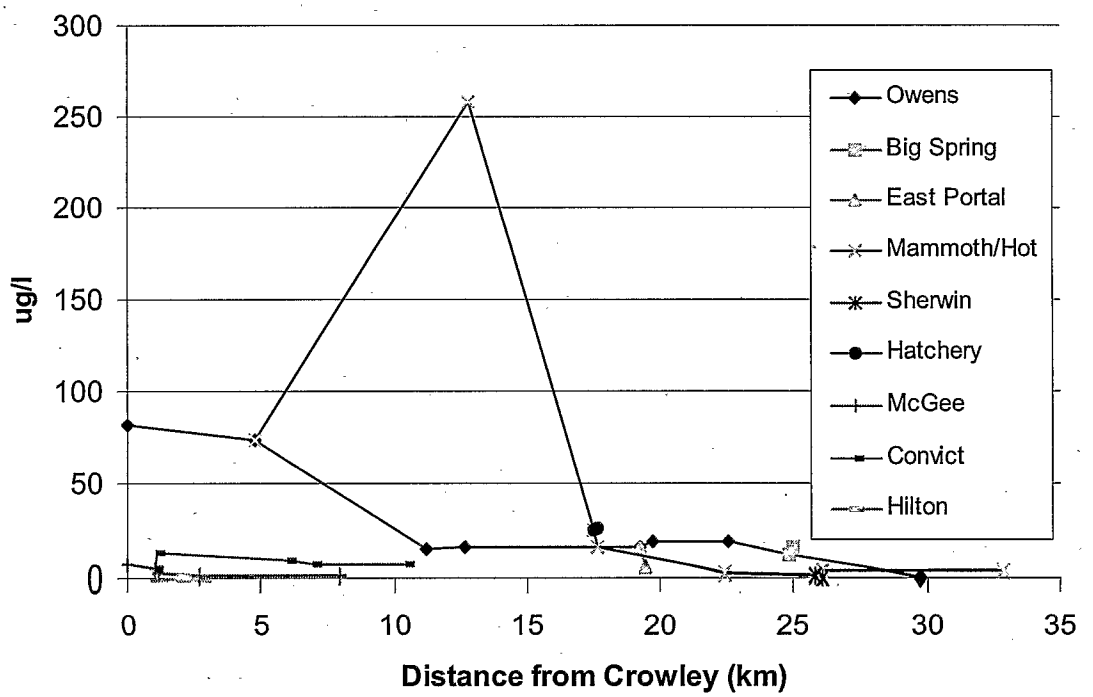
Samples from the outflow of Crowley Lake taken from the outflow pipe within the dam from August 2001 to April 2002 were also collected and analyzed for total As.

Results

The October 2000 survey (Fig. 1 and Table 1) clearly shows that the Hot Creek geothermal area is the predominant source of As loading to Crowley Lake with secondary inputs from Big Springs and the fish hatchery springs. Concentrations in Glass, Sherwin, and Hilton Creeks and at the Twin Lakes outflow were below the detection limit (<2 $\mu g\ l^{-1}$). Big Spring inputs on the Upper Owens River were 15 $\mu g\ l^{-1}$, while East Portal concentrations were ~5 μg l-1. Owens River concentrations remained fairly constant (14-19 $\mu g\ l^{-1}$) from below East Portel until mixing with Hot Creek waters above Benton Crossing. At Benton Crossing the As concentration of the combined flows was 74 $\mu g\ l^{-1}$ which increased slightly to 81 $\mu g\ l^{-1}$ before entering the lake.

Arsenic concentration in Mammoth Creek increased from <2 $\mu g\ l^{-1}$ at the LADWP gaging station just below US395 to 15 $\mu g\ l^{-1}$ at the confluence with Hot Creek Hatchery water. Hatchery water contained 25 $\mu g\ l^{-1}$ As. Arsenic increased to 257 $\mu g\ l^{-1}$ at the lower end of Hot Creek gorge. In contrast to upper McGee Creek, arsenic concentration in the outflow of Convict Lake was 6 $\mu g\ l^{-1}$ and increased slightly to 12 $\mu g\ l^{-1}$ prior to merging with McGee Creek before entering Crowley Lake. This latter increase is presumably due to spring inputs in wetland areas just above the confluence with McGee.

Figure 1 Total As concentration ($\mu g\ l^{-1}$) of samples collected along Crowley Lake tributaries during October 2000.



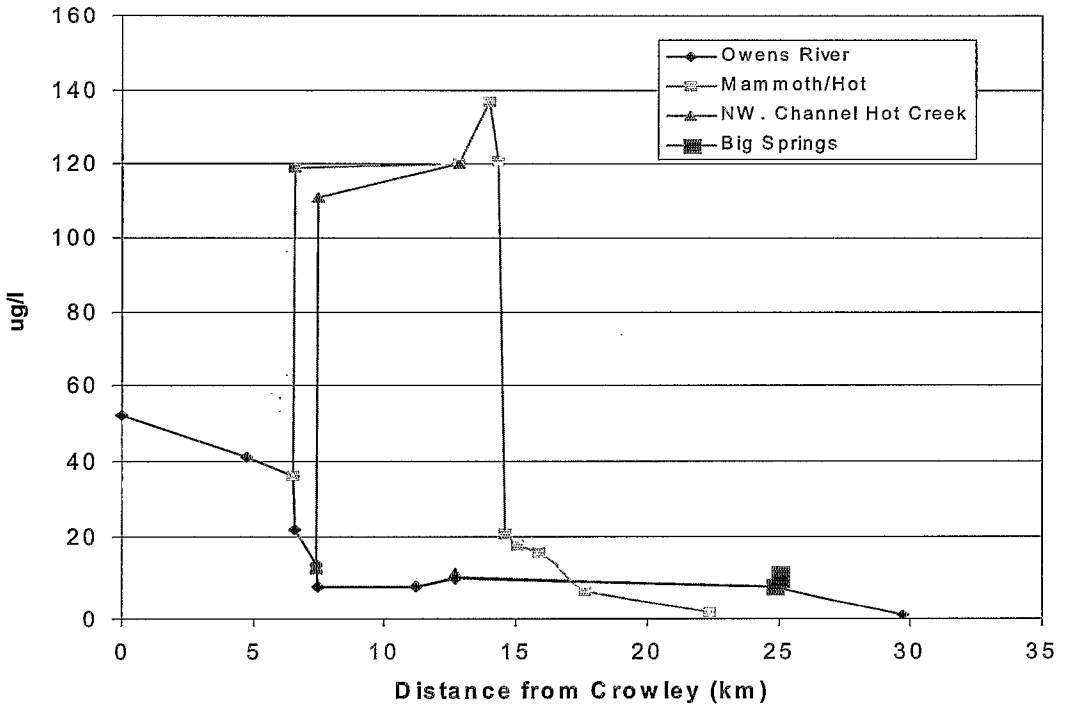
During July, 2001 samples were collected from Owens River, Mammoth/Hot Creek, Crowley Lake and the outlet. Additional stations were added along Mammoth/Hot Creek to determine the exact sources from high input areas such as springs located at the fish hatchery and the Hot Creek thermal area.

The July 2001 survey results (Table 2 and Fig. 2) were similar to those of October 2000 except that the concentrations measured at four stations along Hot Creek from just above the boiling pool area to the confluence with the Owens River were much lower (111 – 137 $\mu g\ l^{-1}$) than the single measurement (258 $\mu g\ l^{-1}$) at the lower end of Hot Creek gorge (USGS flume) sampled in October 2000. The sample taken directly from the boiling pool in Hot Creek gorge was 189 $\mu g\ l^{-1}$.

The Owens River series of samples indicate the only detectable inputs of As between the headwaters of Glass Creek and its confluence with Hot Creek to be the Big

Springs area. Riverine As concentrations increase from <2 μg l^{-1} above Big Springs to 9-11 μg l^{-1} below Big Springs. Concentration in a sample taken directly from one of the largest springs was 11 μg l^{-1}. Along Mammoth/Hot Creek concentrations were 2 μg l^{-1} at US395 and increased slightly to 8 μg l^{-1} between the highway and the Hot Creek fish hatchery. Hatchery spring waters had arsenic concentrations of ~20 μg l^{-1} resulting in a further increase to ~20 μg l^{-1} in the river below hatchery. Just above the thermal pools used for soaking by the public, the concentration was still only 21 μg l^{-1}. Just below the two largest thermal pools used for soaking, the concentrations had increased to 121 μg l^{-1} and then further to 137 μg l^{-1} at 200 m below the geothermal area fence. Concentrations decreased slightly through the rest of the gorge to 120 μg l^{-1} at the USGS flume gage site at the lower end of the gorge.

Figure 2 Total As concentration (μg l^{-1}) of samples collected from upper Owens River and Mammoth/Hot Creek during July 2001.

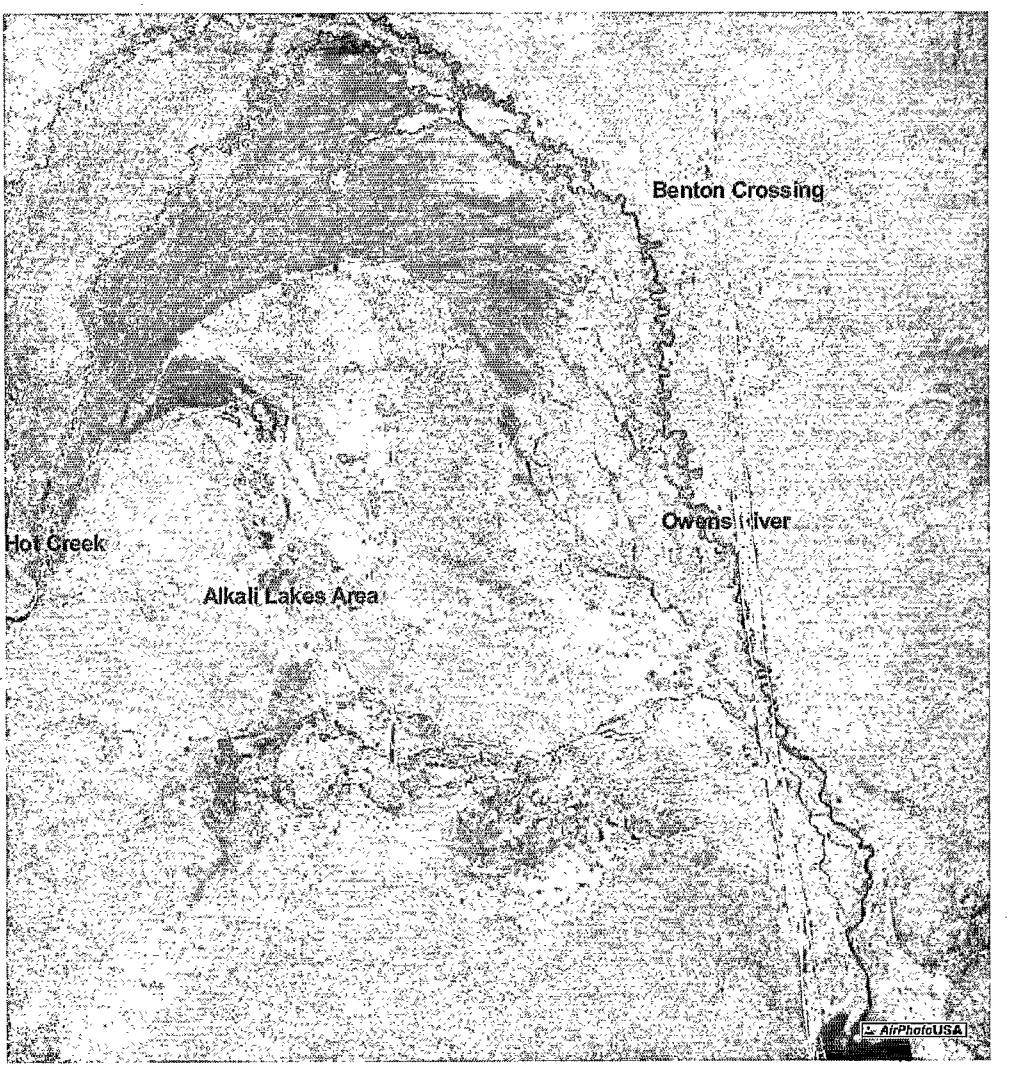


Below the gorge, irrigation practices result in braiding of the stream due to multiple irrigation diversions and spreading. Two of the streams were sampled at their confluence with the Owens River. Arsenic concentrations were similar in the two streams (111-119 μg l^{-1}) and resulted in increasing the concentration of the Owens River to 36 μg l^{-1} below the main Hot Creek tributary. Arsenic concentrations in the Owens River continued to increase prior to entering Crowley Lake, with concentrations of 41 and 52 at Benton Crossing Bridge and at the lake, respectively.

The observed increase between Benton Crossing and the lake indicates significant inputs below Benton Crossing Bridge. This increase was also observed in October 2000

and represents an increase of 11 and 29% of the loading for October 2000 and July 2001, respectively. Inputs below Benton Crossing Bridge include flows from the alkali lakes, springs, irrigation returns and possibly groundwater (Fig. 3).

Figure 3 Irrigated Pasture and Alkali Lakes inflowing to Owens River below Benton Crossing Bridge (2000 aerial photo, by AirPhoto USA)



Arsenic was also analyzed on a subset of samples collected monthly from August 2001 to April 2002 as part of a study of the nutrient budget for the lake, (Table 3). Arsenic concentration in these outflow samples ranged from 23 to 36 $\mu g\ l^{-1}$ with an overall mean of 28 $\mu g\ l^{-1}$.

Discussion

The arsenic surveys conducted in this study clearly indicate the natural sources of arsenic loading to Crowley Lake. The dominate source is the Hot Creek geothermal area, followed by Big Springs and other natural inputs below Benton Crossing. Glass Creek, upper Mammoth and McGee Creeks, and Hilton Creek had undetectable levels of arsenic (<2 $\mu g\ l^{-1}$). Outside of geothermal and Big Spring inputs, only Convict Creek had detectable concentrations ranging from 6-10 $\mu g\ l^{-1}$. However, we did not directly sample any springs other than Big Spring and many wells in the area have significant arsenic concentrations (see CWR 1967 and Mammoth City Water District data). Thus, unmeasured springs and groundwater may also contribute to arsenic loading of Crowley Lake.

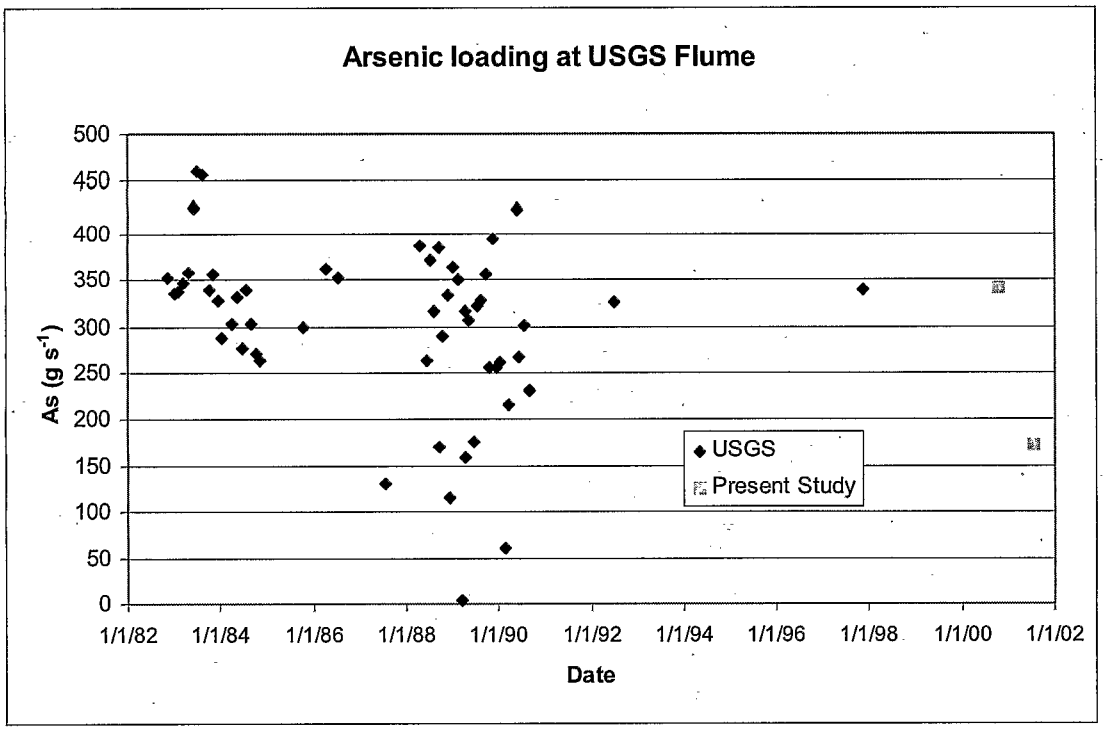
This distribution and the magnitude of the arsenic concentrations measured in this study were generally consistent with previous studies. DWR (1967) presents the most extensive previous study of arsenic in the Long Valley area. The mean arsenic concentration of 35 samples collected at the Benton Crossing Bridge from 1941 to 1947 was 70 $\mu g\ l^{-1}$, while the mean concentration of 58 samples collected on lower Hot Creek from 1953 to 1966 was 163 $\mu g\ l^{-1}$. DWR (1967) estimated that Hot Creek accounted for 68.9% of the arsenic loading to Crowley Lake, while Big Springs contributed 13.5 and other minor streams 33.8%. Although at least a yearly time series would be required to calculate an accurate loading budget, the October 2000 sampling from a single date in this study suggests Hot Creek accounts for ~80% of the loading, Big Springs ~11%, unidentified inputs from the alkali lake area ~8% and Convict Creek <1% of the arsenic loading.

The lower Hot Creek values observed in this study in July 2001, while within the range of previously reported values, are unusually low. A comparison can be made to USGS arsenic measurements at the flume at the lower end of Hot Creek gorge. Fifty determinations by the USGS between 1982 and 1997 range from 3 to 350 $\mu g\ l^{-1}$ with an overall mean of 191 $\mu g\ l^{-1}$. As the concentrations are due to spring inputs, they vary considerably due to dilution by the highly variable stream runoff. Comparing our values of As loading (concentration times flow) for the lower gorge station to USGS data shows the October 2000 value (340 $g\ s^{-1}$) lies just above the overall mean of 301 $g\ s^{-1}$, while the July 2001 loading value of 172 $g\ s^{-1}$ is substantially below the mean and only above the lowest 10% of USGS values (Fig. 4). Thus, while it is not possible to discount the July 2001 reading, it is suspiciously low. As all five measurements downstream are consistent with each other and we have no indication of suspect results from review of the raw data (standards, QA, internal recovery, etc.) provided by the analytical laboratory, we cannot reject this data. While potentially suspect, the primary purpose of delineating the sources of arsenic input is unaffected.

The increase in arsenic concentrations below Benton Crossing Bridge indicates significant inputs along the river reach between the bridge and the lake. This area includes flows from alkali lakes, springs, and return flows from diversions off of Hot Creek. Although not sampled in this study, CWR (1967) reports high arsenic concentrations in flows out of Alkali Lakes (600 $\mu g\ l^{-1}$). Hering (1997) suggests

microbially-mediated oxidation of As(III) occurs fairly rapidly in Hot Creek waters. Comparison of total As to As(V) derived from the phospho-molybdate method suggest 30-50% of the total arsenic in the Owens River at Benton Crossing Bridge and at its inflow to Crowley Lake are in non-As(V) forms. This may indicate significant inputs of As(III) from the alkali lakes and spring area.

Figure 4 Arsenic loading (g As / s) at the USGS flume (USGS data from http://nwis.waterdata.usgs.gov/usa/nwis/qwdata, station 10265150)



In general our findings are consistent with previous studies and confirm that arsenic loading to Owens River and Crowley Lake derives almost entirely from natural spring sources. The very minor contribution of Convict Creek present at the Convict Lake outflow likely arises from natural weathering of metamorphic rocks in the Convict Lake watershed. There was no evidence of arsenic loading from human activities aside from the remote possibility that irrigation return waters flowing through the alkali pond area could result in an increase in the amount of these arsenic-rich waters flowing into Owens River between Benton Crossing Bridge and the lake. This latter possibility cannot be appropriately addressed by the present study.

TABLES

Table 1 Arsenic concentrations in Crowley Lake Tributaries, 17-18 October 2000

River	Location	As (ppb)	As (µm)
Owens	At inflow to Crowley Lake	82	1.09
Owens	Benton Crossing Bridge	74	0.98
Owens	Downstream end of H. Arcularius ranch	14	0.19
Owens	Upstream end of H. Arcularius ranch	15	0.20
Owens	LADWP gauge below East Portal	16	0.21
Owens	Above East Portal on Gottwald ranch	19	0.25
Owens	Dwnstream end of Alpers ranch	18	0.24
Owens	At culvert below Big Springs	11	0.15
Owens	Glass Creek above US395	0	0.00
Big Springs	Big Springs (main spring)	15	0.20
East Portal	East Portal	5	0.07
Mammoth/Hot	At USGS flume below thermal area	258	3.44
Mammoth/Hot	Below Mammoth/Hot confluence	25	0.33
Mammoth/Hot	Mammoth Creek below just below Chance	15	0.20
Mammoth/Hot	At old 395 gaging station	2	0.02
Mammoth/Hot	Below confluence with Sherwin	2	0.02
Mammoth/Hot	Above confluence with Sherwin	3	0.04
Mammoth/Hot	Outllet of Twin Lakes	3	0.04
Sherwin	Sherwin Creek	0	0.00
Hatchery	Hatchery outflow	26	0.34
McGee	At inflow to Crowley Lake	6	0.08
McGee	Just below Convict confluence	4	0.05
McGee	Just above Convict confluence.	2	0.02
McGee	At US395	2	0.02
McGee	Above pack station	2	0.02
Convict	Just above McGee confluence	12	0.16
Convict	Just below SNARL	8	0.10
Convict	Just above SNARL	7	0.09
Convict	Outlet of Convict Lake	6	0.08
Hilton	At inflow to Crowley Lake	0	0.00
Hilton	At US 395	-1	-0.01
Hilton	At old US 395	-1	-0.01
Hilton	Above community at LADWP gauge	-1	-0.01

TABLE 2

Arsenic Concentrations As Determined By Graphic Furnace Atomic Absorption Spectroscopy, 7/17/2001 Sampling

Table 2 Arsenic concentrations in Crowley Lake Tributaries, 17 July 2001

River	Location	As (ppb)	As (µM)
Owens	At inflow to Crowley Lake	52	0.70
Owens	Benton Crossing	41	0.54
Owens	Just upstream of main Hot Creek. input	22	0.29
Owens	Just downstream of main Hot Creek input	36	0.48
Owens	Just upstream of westernmost Hot Creek input	9	0.12
Owens	Just downstream of westernmost Hot Creek input	13	0.18
Owens	Downstream end of Howard Arcularius prop.	9	0.11
Owens	Upstream end of Alpers Ranch	11	0.15
Owens	Culvert below Big Springs	9	0.11
Owens	Western primary spring at Big Springs	11	0.14
Glass	Just above confluence with Deadman Ck.	1	0.01
Mammoth/Hot	Westernmost Hot Creek input to Owens	111	1.49
Mammoth/Hot	Main Hot Creek input to Owens	119	1.58
Mammoth/Hot	USGS flume below thermal area	120	1.60
Mammoth/Hot	Above confluence of Hot Creek and hatchery inputs	8	0.11
Mammoth/Hot	Hatchery inputs above confluence with Hot Creek	17	0.23
Mammoth/Hot	Below confluence of Hot Creek and hatchery inputs	19	0.25
Mammoth/Hot	Hatchery AB springs	19	0.26
Mammoth/Hot	Hatchery CD springs	20	0.27
Mammoth/Hot	LADWP gauging station at US395	2	0.02
Mammoth/Hot	Downstream fence, Hot Creek Ranch	16	0.21
Mammoth/Hot	Beginning of visible geothermal area	18	0.24
Mammoth/Hot	Above upper swimming hole at Hot Creek public area	21	0.28
Mammoth/Hot	Below lower swimming hole at Hot Creek public area	121	1.62
Mammoth/Hot	200 m below geothermal area fence	137	1.83
Mammoth/Hot	Directly out of boiling pool	189	2.52
Crowley Lake	South station	58	0.78
Crowley Lake	Sest station	58	0.77
Crowley Lake	Outlet	49	0.65

Table 3 Arsenic concentrations in Crowley Lake Outflow

Sampling Date	AS (ppb)	AS (µM)
8/29/01	30	.40
9/12/01	26	.34
9/26/01	25	.33
10/10/01	23	.30
11/7/01	33	.44
12/5/01	25	.33
1/16/02	36	.48
2/13/02	29	.38
3/13/02	29	.38
4/10/02	26	.34

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Section 2

Tracing External Sources of Nitrate to Crowley Lake: An Isotope Feasibility Study

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1.0 Introduction

Nitrate has become a nearly ubiquitous pollutant in freshwater environments due to human alteration of the global nitrogen cycle. Emissions of nitrogen gases (principally NO_x) through burning of fossil fuels increased at a geometric rate through most of the 20th century (Schlesinger 1997). NO_x compounds quickly react with water in the atmosphere and are removed by precipitation or deposited on land as dry deposition. Through industrial fixation of atmospheric N_2 for fertilizers, humanity has increased nitrogen loading to the terrestrial biosphere and near-shore ocean ecosystems (Schlesinger 1997). Anthropogenic N in urban and agricultural runoff has contaminated groundwater with nitrate, and is a principal cause of eutrophication in aquatic ecosystems (both freshwater and near-shore ocean ecosystems e.g., estuaries) (Fenn et al. 1997, Fenn et al. 2003 a&b).

Crowley Lake, (Mono County, California) is the premier trout fishery in the eastern Sierra Nevada and the largest reservoir in the Los Angeles aqueduct system. In summer, large cyanophyte blooms impair recreational uses and water quality. The upper Owens River and Crowley Lake are listed under Section 303(d) of the federal Clean Water Act as impaired due to nutrients. Potential nitrate sources within the watershed include urban runoff from the Town of Mammoth Lakes, cattle ranching, and agricultural practices, runoff from high-elevation regions with little nitrogen uptake capacity and the Hot Creek Fish Hatchery.

Stable isotopes of nitrate may be useful to determine the sources of nutrients to the Owens River and Crowley Lake. In this study we evaluated the utility of $\delta^{15}N$ measurements of nitrate to determine sources of nitrate to Crowley Lake. In our analyses we isotopically characterized nitrate and samples of aquatic biomass in four landuse types within the Upper Owens River watershed (Tables 1 and 2): 1) groundwater nitrate sources in the largely undisturbed northern areas of the catchment (Glass Creek, Big Springs, and Mono Craters Portal), 2) urban runoff from the town of Mammoth (Mammoth Creek above the confluence with Hot Creek), 3) waters within and below the Hot Creek fish hatchery, and 4) runoff from undisturbed, granitic, high-elevation catchments (McGee Creek) with and without potential influence from livestock operations (above and below the McGee Creek horse pack station and in an area of intense cattle grazing bisected by McGee Creek).

Our major objectives were to: 1) evaluate current technologies for characterizing isotopes of nitrate in the Upper Owens watersheds, 2) look for correspondence between nitrate isotopic composition and aquatic biomass isotopic composition and 3) determine whether nitrate or biomass isotope composition can identify the major upland sources of nitrate to Crowley Lake. Section 2 of this chapter includes background information on environmental isotopes and a review of recent work on collection methods used for nitrate isotopes.

Table 1. Summary data for locations sampled during June 2000 in the Upper Owens River watershed.

Sub-Basin	Sampling Site	Landuse Classification	Date	Cl- µEq L^{-1}	NO_3^- µEq L^{-1}	SO_4^{2-} µEq L^{-1}	Sum Acid Anions µEq L^{-1}	DON µmol L^{-1}	Volume Processed liters	Anion Column Loading % of Capacity	$\delta^{15}N$-NO_3 o/oo	NO_3^- Recovery %
Owens River	Glass Creek	Groundwater	6/26/2000	5.7	0.8	18	25	2.9	20	7	11.3	69
Owens River	Big Springs	Groundwater	6/26/2000	188	8.9	131	329	1.2	30	141	8.7	175
Owens River	Alpers Ranch	Groundwater	6/26/2000	25	3.7	101	131	5.5	20	37	20.6	88
Owens River	East Portal	Groundwater	6/26/2000	164	15.4	227	407	8.6	10	58	3.0	99
Mammoth Creek	Twin Lakes Outlet	Urban	6/25/2000	4.7	0.1	42	4.8	5.0	18	12	8.5	306
Mammoth Creek	Above confluence with Sherwin Creek	Urban	6/25/2000	0.1	0.1	55	56	6.8	25	20	8.2	43
Mammoth Creek	Above confluence with Hot Creek	Urban	6/27/2000	19	0.5	49	69	7.8	20	20	12.0	15
Hot Creek	Above confluence with Mammoth Creek	Hatchery	6/26/2000	91	11.3	225	328	12.6	14	66	6.7	7
Hot Creek	Thermal Area	Hatchery	6/26/2000	130	7.4	361	500	9.8	10	71	15.4	87
McGee Creek	Above pack station	Granite+Livestock	6/25/2000	5.0	3.9	106	116		20	33	6.7	116
McGee Creek	Below US 395	Granite+Livestock	6/25/2000	6.2	2.6	119	128		20	37	4.9	118
	Crowley Reservoir - at inlet	Reservoir	6/27/2000	13	1.1	241	256	19.3	16.5	60	11.0	67
	Crowley Reservoir - Mid Basin 5m	Reservoir	6/27/2000	731	0.5	304	1036		30	444	8.7	35
	Crowley Reservoir - Mid Basin 15m	Reservoir	6/27/2000	218	15.9	139	374		25	134	10.6	1

Table 2. Summary data for locations sampled during December 2000 in the Upper Owens River watershed.

Sub-Basin	Sampling Site	Landuse Classification	Date	Cl- µEq L^{-1}	NO_3^- µEq L^{-1}	SO_4^{2-} µEq L^{-1}	Sum Acid Anions µEq L^{-1}	DON µmol L^{-1}	Volume Processed liters	Anion Column Loading % of Capacity	$\delta^{15}N\text{-}NO_3$ o/oo	NO_3^- Recovery %
Owens River	East Portal	Groundwater	12/18/2000	83	4.2	64	151		16.5	36	12.2	102
Owens River	Big Springs	Groundwater	12/1/2000	254	9.1	114	377		18	97	15.1	194
Mammoth Creek	Twin Lakes Outlet	Urban	12/3/2000	15	0.24	60.8	76		18	20	0.1	424
Mammoth Creek	Above confluence with Hot Creek	Urban	12/18/2000	159	1.3	171	331		18	85	0.8	91
Hot Creek	Above confluence with Mammoth Creek	Hatchery	12/18/2000	142	17.9	217	377		18	97	11.9	244
Hot Creek	Thermal Area	Hatchery	12/18/2000	155	13.5	510	678		15.8	153	18.1	79
McGee Creek	Above pack station	Granite+Livestock	12/11/2000	21	1.7	324	347		16.5	82	0.5	115
McGee Creek	Below US 395	Granite+Livestock	12/8/2000	20	0.08	362	382		16.5	90	3.6	1268
McGee Creek	At inlet to Crowley Reservoir	Granite+Livestock	12/8/2000	53	1.2	394	448		18	115	2.0	178
	Crowley Reservoir - East Basin	Reservoir	12/8/2000	787	0.71	301	1089		17.5	272	12.5	297
	Crowley Reservoir - West Basin	Reservoir	12/8/2000	801	1.4	312	1115		17	271	13.7	129

2.0 Isotope Fundamentals and Literature Review

2.1 Isotope Basics

The number of protons in the nucleus of an atom (atomic number) defines each element. The nuclei of an element may have variable numbers of neutrons owing to a variety of high-energy chemical reactions and radioactive decay. Because neutrons have weight (about the same as that of protons), atoms with the same number of protons, but varying numbers of neutrons differ in the atomic weight and are called isotopes. Radioactive (unstable) isotopes decay over time to form other radioactive or stable isotopes (for example radioactive carbon-14 decays to stable nitrogen-14 over thousands of years). Stable isotopes do not decay over time. Atomic weights of elements are expressed in terms of a standard atom: the isotope of nitrogen that has 7 protons and 7 neutrons in its nucleus. This atom is designated nitrogen 14 or ^{14}N and has atomic weight of 14 Daltons. Both protons and neutrons have weights very close to 1 Dalton each. Nitrogen-14 is the commonest isotope of nitrogen and its abundance in nature is 99.634%. Nitrogen-15 (^{15}N) with 7 protons and 8 neutrons is the second most abundant nitrogen isotope and has a natural abundance of 0.366%.

Isotopic ratios of an element are compared to those of a standard, and expressed in "delta" notation. For example, in the case of nitrogen isotope $\delta^{15}N$ is given by the relation:

$$\delta^{15}N = 1000[(^{15}N/^{14}N)_{Sample} - (^{15}N/^{14}N)^{Air}]/(\ ^{15}N/^{14}N)_{Air}\ ‰$$

where Air stands for standard mean atmospheric N_2, the standard to which nitrogen isotope ratios are compared. Note that the value of $\delta^{15}N$ is expressed in "per mil" (‰) rather than %, since the ratio of $^{15}N/^{14}N$ in nature is so small.

Element and compound isotope ratios are measured on mass spectrometers while radioactive isotopes are analyzed on gamma or beta counters or on accelerator mass spectrometers. Low mass, stable isotopes need to be quantitatively converted to pure gas from original compounds (e.g., CO_2 for carbon compounds and N_2 for nitrogenous compounds). Gases can be produced off-line using a variety of combustion and purification steps or in-line using a combination chromatography system and mass spectrometer (i.e., continuous flow). Gases are introduced into the mass spectrometer in their ionized state where they are dispersed in a strong magnetic field. The dispersion pattern in the field is controlled by the elements' atomic mass, causing atoms of different mass to impact on different detector surfaces (called collector cups). The rate at which atoms impact the cups effectively determines the abundance of the isotopes.

In the most accurate instruments, called dual inlet systems, both sample gases and reference standards (for example standard air for N isotopes) are introduced nearly simultaneously into the mass spectrometer. Parallel measurement of standard and sample materials compensates for subtle noise and error in the instrument and results in highly

accurate isotope measurements. In single inlet systems (like the one employed in the present study), standards and samples are introduced as a series so that several minutes might transpire between reference measurements and unknowns, leading to greater error.

Stable isotope can act as a "label" or "tracer" for a variety of chemical reactions in biotic and abiotic systems. The basis of this technique is that the weight of the nucleus of an atom has a slight effect on the chemical properties of that atom. For low mass elements, differences in mass are large enough that chemical, biological and physical processes fractionate or change the relative proportions of the different isotopes of the same elements in molecules. For nitrogen, mass-dependant fractionation processes include N-fixation (both biotic and by human production of fertilizers), assimilation (uptake of ammonium and nitrate by plants or microbes), mineralization and nitrification (microbial production of ammonium followed by oxidation to NO_x) and denitrification (microbial reduction of nitrate to NO, N_2O, and N_2 gases). As the delta value of a compound increases it becomes isotopically "heavier". Likewise if a sample has a lower delta value it said to be "lighter". Fractionation processes that result in isotopically heavier products than reactants are said to isotopically "enrich" compounds while reactions that result in lighter products are said to isotopically "deplete" the reactants.

2.2 Nitrate Collection and Source-Tracing Methods

Routine collection of nitrate in natural samples for $\delta^{15}N$ determination dates from the 1960s. The earliest methods used chemical reactions, such as the Kjeldahl digestion or Devarda alloy, to convert nitrate into ammonium (Bremner and Edwards 1965, Stark and Hart 1996). Ammonium is then removed from the sample and oxidized to N_2 gas using steam distillation/combustion methods (Velinsky et al. 1989, Kendall and Grim 1990) or by diffusion onto acidified filter paper to produce $(NH_4)_2SO_4$ which is combusted to produce N_2 gas (MacKown et al. 1987, Sigman et al. 1997). These techniques are applicable to both fresh and saline waters and to soil solutions.

For freshwaters the development of ion exchange resins has largely replaced earlier methods for collection of nitrate for $\delta^{15}N$ analysis owing to their ease of use, greater sample throughput and applicability to samples with low nitrate concentrations (Kendall et al. 1995, Wassenaar 1995, Harrington et al. 1998, Downs et al. 1999). In this method samples are passed through a column of chloride-form anion exchange resin. These resins are composed of positively charged functional groups covalently bounded to a solid support matrix. When a negatively charged compound is applied with greater affinity for the column than chloride, it is adsorbed, while compounds that are neutral or have the same or less affinity as chloride pass through the column. Adsorption of the negatively charged compounds is reversible with a salt solution or acid such as HCl.

One drawback to the use of anion exchange resins is their affinity for dissolved organic carbon (DOC). DOC is found in all natural waters and is composed primarily of humic substances derived from soils. Humic compounds possess a weak negative charge so they may adsorb to anion exchange resins (Croue et al. 1999). Fouling of the resin with DOC reduces the anion exchange capacity of the resin. Depending on the

compound, these substances may be desorbed from the column using salts or acids, possibly altering subsequent isotopic measurements since DOC contains nitrogen.

Various methods have been tried to remove DOC from natural waters prior to nitrate collection using resins. Silva et al. (2000) used activated charcoal to adsorb DOC. Inorganic forms of N can also be separated from DOC using ultra-filtration (Feuerstien et al. 1997). In a technique designed especially for dilute waters with low nitrate and DOC, Chang et al. (1999) used anion exchange resins that are less prone to DOC loading and incorporated pre-treatment of samples with cation exchange resins. These resins impart a positive charge to DOC thereby lowering its affinity for exchange sites on the resin. Still, none of these techniques completely solves the problem of DOC interference.

In complex systems the utility of using $\delta^{15}N\text{-}NO_3$ to trace sources of nitrate is often limited due to isotopic fractionation along hydrologic flowpaths (Kohl et al. 1971, Hauck et al. 1972). The original isotopic signature of nitrogen sources can quickly be altered by biologically mediated reactions that produce a mass-dependant fractionation (e.g. denitrification). Moreover, the range of $\delta^{15}N\text{-}NO_3$ typically encountered in surface waters is about half the range of $\delta^{18}O$ values measured for nitrate (Kendall 1998). It has been hypothesized that $\delta^{18}O\text{-}NO_3$ is a more conservative isotopic tracer and studies have shown that it provides an alternate method to trace nitrate sources to surface waters (Kendall et al. 1995, Campbell et al. 2002, Sickman et al. 2003, Chang et al. 2002). Use of both isotopes of nitrate greatly increases ones ability to de-convolute nitrate sources in watersheds.

Two recent methods have been published that may soon become the preferred method for collection and isolation of nitrate for isotopic analysis. Both techniques are based on the conversion of nitrate to N_2O gas by denitrifying bacteria. Sigman et al. (2001) and Casciotti et al. (2002), report that $\delta^{15}N\text{-}NO_3$ can be measured at natural abundance levels at ambient concentrations down to 1 $\mu mol\ L^{-1}$. The techniques are appropriate for both fresh and saline waters and applicable to samples as small as 5 milliliters which is < 1% of the size required for the exchange resin methods discussed earlier. Additionally, interference from DOC is low and explicitly dealt with in these methods.

The basic process involved in the Sigman and Casciotti methods is the quantitative conversion of nitrate to N_2O gas by *Pseudomonas sp.* – species of genetically modified bacteria that lack an active N_2O reductase:

$$2NO_3^- \overset{O_2}{\uparrow} \rightarrow 2NO_2^- \overset{O_2}{\uparrow} \rightarrow 2NO \overset{O}{\uparrow} \rightarrow N_2O \overset{O}{\uparrow} \rightarrow N_2$$

By stopping the reaction before reduction to di-nitrogen gas, both ^{15}N and ^{18}O isotopes of nitrate can be determined. Under controlled conditions the conversion of nitrate to nitrous oxide gas is nearly 100% so there is little nitrogen isotopic fractionation imparted on the products of this reaction. Oxygen isotopic fractionation does occur, however, due to preferential loss of ^{16}O, but by incorporating standard reference materials into every run

this error can be corrected. Interference from DOC is avoided because no exchange resin is used to collect the sample and the small amount of DOC-nitrogen in the water samples is overwhelmed by the organic nitrogen in the bacterial growth media in which the bacterial reduction takes place. The nitrogen isotopic composition of the growth media is known and corrected for in the computation of $\delta^{15}N\text{-}NO_3$ for the samples.

3.0 Methods

3.1 Stream and Lake Chemical Sampling

Nitrate and biomass samples for isotopic analysis were collected during spring runoff (June 2000) (Table 1) and in late autumn/early winter (December 2000) (Table 2). Filtered samples for major anions (chloride, nitrate and sulfate), ammonium and total dissolved nitrogen (TDN) were collected concurrently. Ammonium and nitrate samples were held in a coldroom at 5 °C. Ammonium was determined on filtered samples generally within 72 hours by the indophenol blue method (Strickland and Parsons 1972). The detection limit for the ammonium assay was 0.5 μmol L^{-1}. Nitrate was measured on a DIONEX ion chromatograph, employing an AS4A or AS14 separation column and conductivity detection. The nitrate detection limit was 0.05 μmol L^{-1}. For long-term storage, TDN samples were stored frozen at –20°C. Total dissolved nitrogen (TDN) was determined by Kjeldahl or Valderrama (1981) digestion methods. Dissolved organic nitrogen (DON) was computed as the difference between TDN and DIN (ammonium + nitrate). The detection limit for DON was 1.0 μmol L^{-1}.

3.2 Isotopic Measurements of Nitrate and Water

The silver nitrate technique of Chang et al. (1999) was used during the study. Only $\delta^{15}N\text{-}NO_3$ values were determined due to logistical constraints. Nitrate isotope samples were collected in collapsible polyethylene containers. Samples were filtered immediately with Gelman Groundwater Cartridges (0.45 μm), weighed to determine volume and a subsample collected for DIN and anion determination. Next, each sample was gravity-fed (ca. 0.5 L h^{-1}) through a cation exchange column containing 6 ml of hydrogen-form resin (AG50-WX8, 100-200 mesh, Biorad) and then through an anion exchange column containing 6 ml of chloride-form resin (AG2X 100-200 mesh, Biorad). The cation column was used to minimize clogging of the anion column by DOC and minimize transfer of unwanted nitrogen atoms from DOC to the ^{15}N portion of the nitrate that was retained by the anion column. The columns were stored at 5°C.

Nitrate was eluted from the anion columns with 30 ml of 3 N HCl and the acidic solution was neutralized with 16 g of silver oxide. The silver oxide was rinsed at least 30 times with deionized water to remove traces of nitrate prior to use; the background level of nitrate in these rinses was monitored until they fell below 0.1 μmol L^{-1}. The sample-silver oxide slurry was filtered and 4.5 ml of 1 M $BaCl_2$ was added to precipitate phosphate or sulfate in the sample. The sample was refrigerated overnight, filtered the following day to remove barium sulfate and phosphate precipitates and then passed through a 9 ml cation exchange column to remove excess barium and silver. To

neutralize and convert nitrate to silver nitrate, about 5 g of silver oxide was added to the sample after which it was filtered to remove silver chloride precipitate. After freeze-drying, the samples were prepared for introduction into the mass spectrometer using the procedures of Kendall and Grim (1990) and Chang et al. (1999).

Isotopic analyses of silver nitrate and biological materials were performed on a Europa Scientific Tracermass/Roboprep stable isotope mass spectrometer. Nitrogen isotope values ($\delta^{15}N$) are reported in per mil (‰) relative to atmospheric air, which is defined as 0 ‰. The precision for laboratory standards for $\delta^{15}N$ ranged from +/- 0.1 to 0.5 ‰ (SD). For all isotopic analyses, NIST-traceable standards were used to calibrate the mass spectrometer.

4.0 Results and Discussion

4.1 Isolation of Nitrate from Surface Water in the Upper Owens River Watershed

To evaluate whether the Chang method for isolation of nitrate was appropriate for surface waters in the Upper Owens watershed, we estimated the amount of nitrate recovered as silver nitrate following all isolation steps and compared these values to the theoretical recovery computed from sample nitrate concentrations and volumes of water processed. The moles of N in silver nitrate should equal the moles of nitrate loaded on the column (nitrate concentration x volume of water processed), however, in practice 100% recovery is rarely achieved. In our study, nitrate recoveries ranged from 1% to 306% for the June 2000 samples (Table 1) and from 79% to 1268% for the December 2000 samples (Table 2).

Under-recovery of nitrate shows that nitrate was lost by some mechanism during the isolation process and it is important to determine whether this process is conservative with respect to isotopic values or instead induces a change, or fractionation, in isotopic composition. In previous studies, recoveries were typically between 60-90% (Sickman et al. 2003, Chang et al. 1999). In these earlier studies nitrate was lost, without any alteration of the isotopic signature, during several steps of the chemical isolation of silver nitrate, most importantly during the neutralization of the samples with silver oxide. The dual inlet mass spectrometer used in Sickman et al. 2003 and Chang et al. 1999, could more accurately measure the mass of silver nitrate produced than the +/- 20% accuracy inherent to the Tracermass model used in the present study. For the feasibility study we believe that isotopic values from samples with nitrate recoveries from 50-120% are valid and unlikely to have much, if any, fractionation error. For the June 2000 samples, about one-third of the samples fall within the 60-90% range and 7 out of 15 between 50-120%. For the December samples less than 20% had recoveries between 60-90%, but almost half of the sample recoveries fell between 50-120%. In the remaining samples, under-recoveries may have been caused by fractionating processes, but the magnitude of the error is unknowable.

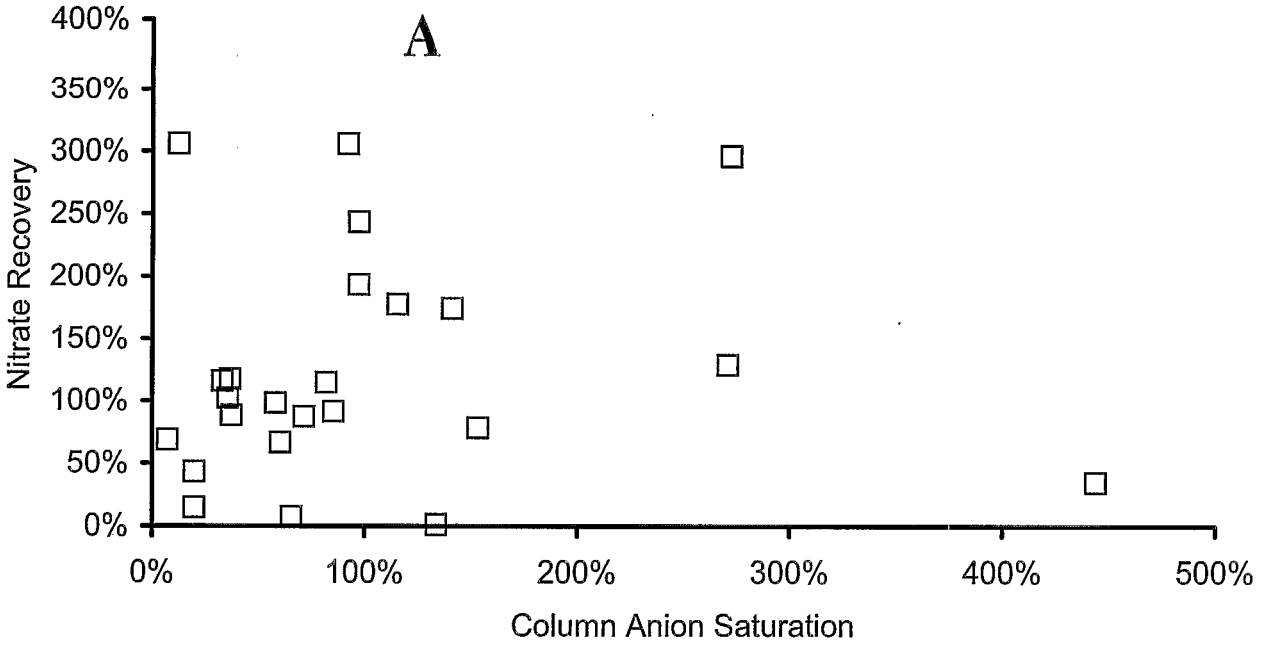
Many of the streams in the Upper Owens River watershed have high sulfate concentrations relative to nitrate (Tables 1 and 2). Sulfate concentrations in Hot Creek

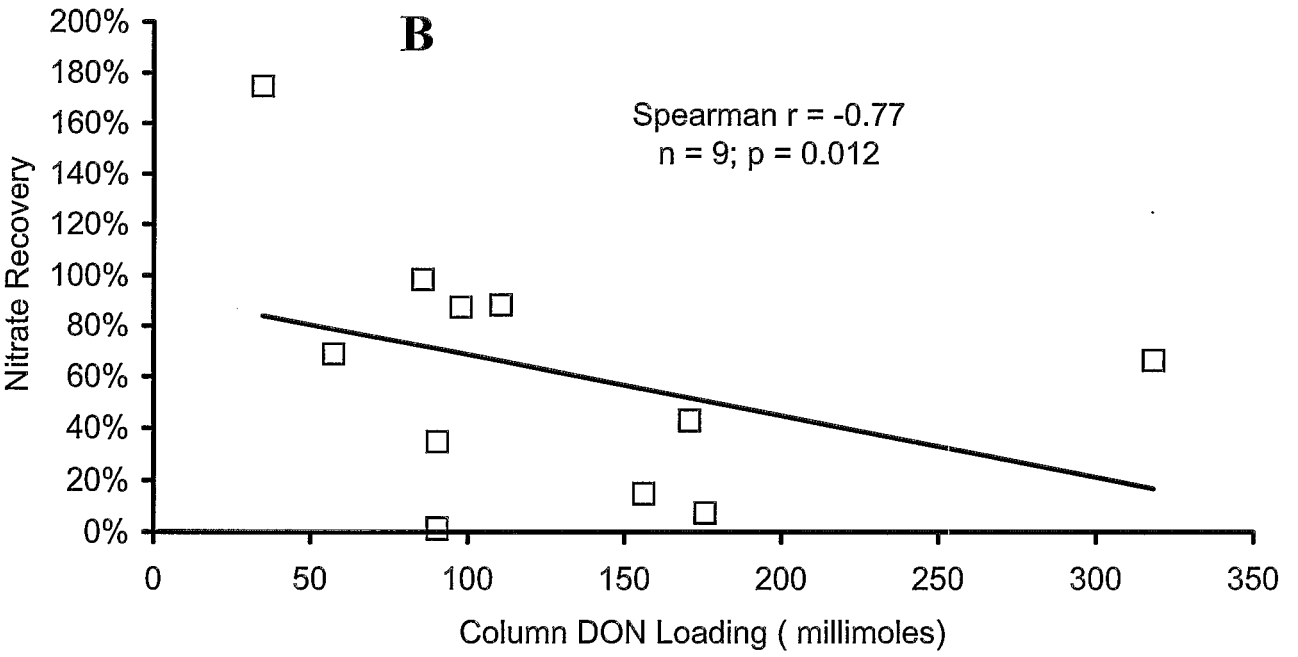
and McGee Creek ranged from 200-500 μEq L^{-1} and are much higher than any site where the Chang isolation method has been employed previously. High sulfate concentrations are problematic since the minimum sample size needed for isotopic analysis is approximately 75 μmoles of nitrate. The total exchange capacity of an anion column was 7000 μEq, so nitrate must comprise at least ~1% of the anion solutes for an adequate sample to be collected before the column is saturated. We used historical anion concentrations to plan how much water to load on the columns, but we did not know what the nitrate levels were until after the samples were processed. In some cases the amount of nitrate on the columns was less than required for replicate isotopic determinations; replicate measurements provide more accurate isotopic composition. Nineteen samples had nitrate concentrations greater than 0.5, but in nearly one-third of these samples, nitrate comprised less than 1% of the anions. To overcome this problem in the future, the size of the columns would need to be increased by 50-100% so that more water can be processed and more nitrate adsorbed.

High sulfate levels can cause low nitrate recoveries and may induce isotopic fractionation error. Even before the ion exchange capacity of the columns is reached, ions will compete for exchange sites on the resin (Lehmann et al. 2001). Anions with higher electronegativity (which is a function of the mass and charge of the element or compound), such as sulfate, have a greater affinity for the resin than nitrate and can strip-off adsorbed nitrate when the resin is saturated or if nitrate makes up only a small fraction of the anions in solution. Since, ion-exchange is a mass-dependent process, fractionation of nitrate isotopes may result from over-loading the columns or if the ratio of nitrate to total anions is very low. In this situation, nitrate composed of the heavier isotopes, ^{15}N and ^{18}O, will be preferentially lost from the resin and ^{14}N and ^{16}O will be preferentially retained. Low nitrate recoveries for the Crowley Lake samples in June 2000 may have resulted from column-saturation and the isotopic fractionation error is probably most severe in the 5 meter sample which had a lower $\delta^{15}N$ value. Overall, however, there was no relationship between column saturation and nitrate recovery or isotopic composition (Figure 1a), suggesting that: 1) there was not consistent isotopic fractionation owing to nitrate recoveries < 100% and 2) other factors, such as levels of dissolved organic matter played a role in low nitrate recoveries (discussed below).

Large over-recoveries occurred in samples with low nitrate levels. The over recoveries for Twin Lake outlet (6/25/2000 and 12/3/2000) and McGee Creek (12/8/2000) are likely an artifact of nitrate concentrations at or near the detection limit and the inaccuracy of the mass spectrometer to measure silver nitrate amounts below 10-20 μmoles. In other cases, excess nitrate could have resulted from nitrate contamination of the samples prior to column loading or in one of the steps required for silver nitrate isolation. However, great care was taken during all phases of the study and the same batches of resin, silver oxide and other reagents were used throughout, so we believe nitrate contamination is unlikely.

Figure 1. A) Relationship between column saturation and nitrate recovery.
B) Relationship between DON loading and nitrate recovery.





Because of its slight negative charge, DOC can adsorb to anion exchange resin. To minimize this effect, samples were run through a cation exchange column, to protonate the DOC and make it less likely to contaminate the anion resin. However, in nearly all of the samples where greater than 20 liters of water was processed, accumulation of colored organic compounds on the resin beds was noticed. Accumulation of organic carbon on the resin likely reduced the total exchange capacity of the column resulting in lower nitrate recovery from the samples. For the June 2000 sample we observed a significant, inverse relationship (Spearman rank correlation coefficient = -0.77, p=0.012) between DON loading and nitrate recovery suggesting that much of the under-recovery of nitrate in the study was due to DOC interference (Figure 1b; no DON data are available for December 2000). In addition, hydrophilic components of the adsorbed organic carbon can be removed from the columns during the HCl stripping procedure, potentially adding non-nitrate N atoms to the silver nitrate thereby contributing to over-recovery of nitrate in some samples.

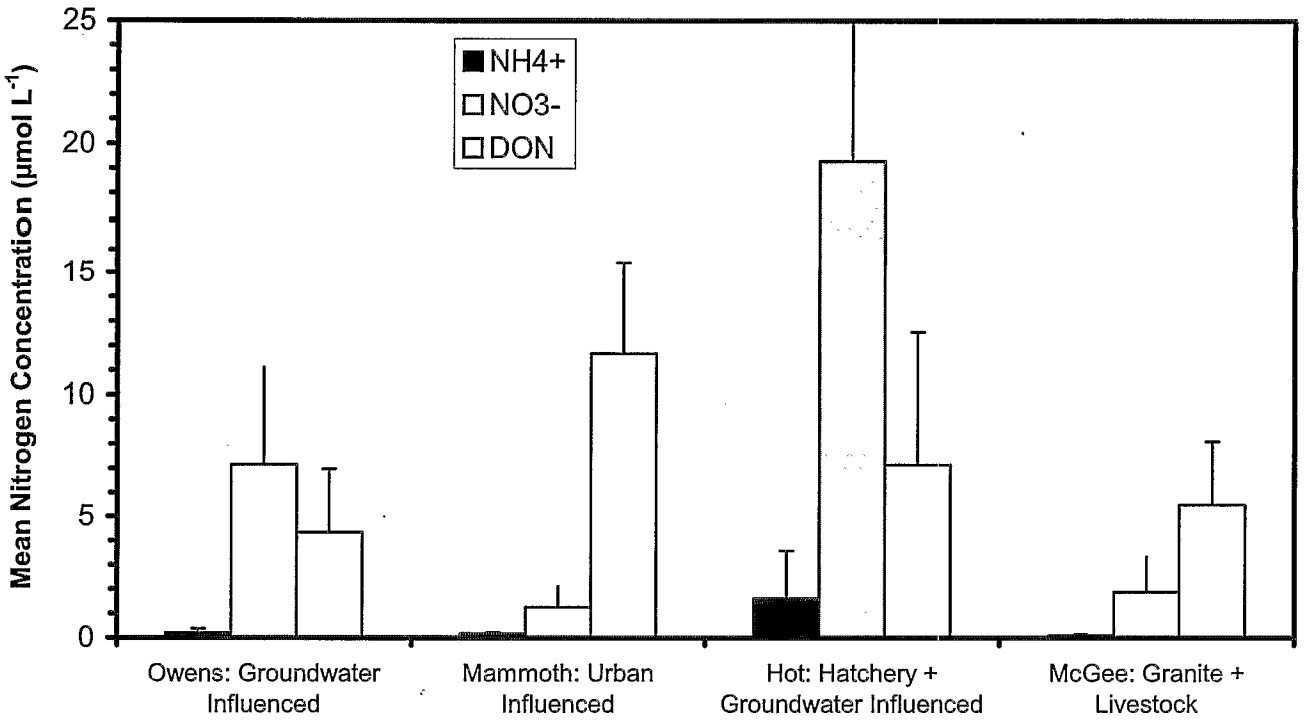
Contamination of the resin columns with DOC is a serious shortcoming of the Chang isolation method in waters with low ratios of nitrate to total anion. To overcome the low proportion of nitrate, more water must be passed through the column, however any advantage conferred by the larger column is mostly negated by increased DOC loading. Bacterial reduction procedures (Sigman et al. 2001, Casciotti et al. 2002) avoid interferences caused by DOC and are suitable for nitrate levels of < 1 mol L^{-1} and may be the most suitable method for nitrate isotope determination in the Upper Owens River watershed.

4.2 Nitrogen Concentrations vs. Landuse Type

Nitrate sources considered in this study were groundwater, urban runoff, the Hot Creek fish hatchery, and runoff from granitic, high-elevation catchments with and without livestock. In Tables 1 and 2 we have listed the landuse classification assigned to each of the sites sampled during the June and December 2000 field campaigns. Since some sampling locations are influenced by more than one landuse (for example streamflow from the Hot Creek Hatchery is influenced by fish production and groundwater nitrate), we classified them as to what we considered the greatest influence to be. In this case, Hot Creek was classified as hatchery influenced rather than groundwater influenced for data analyses.

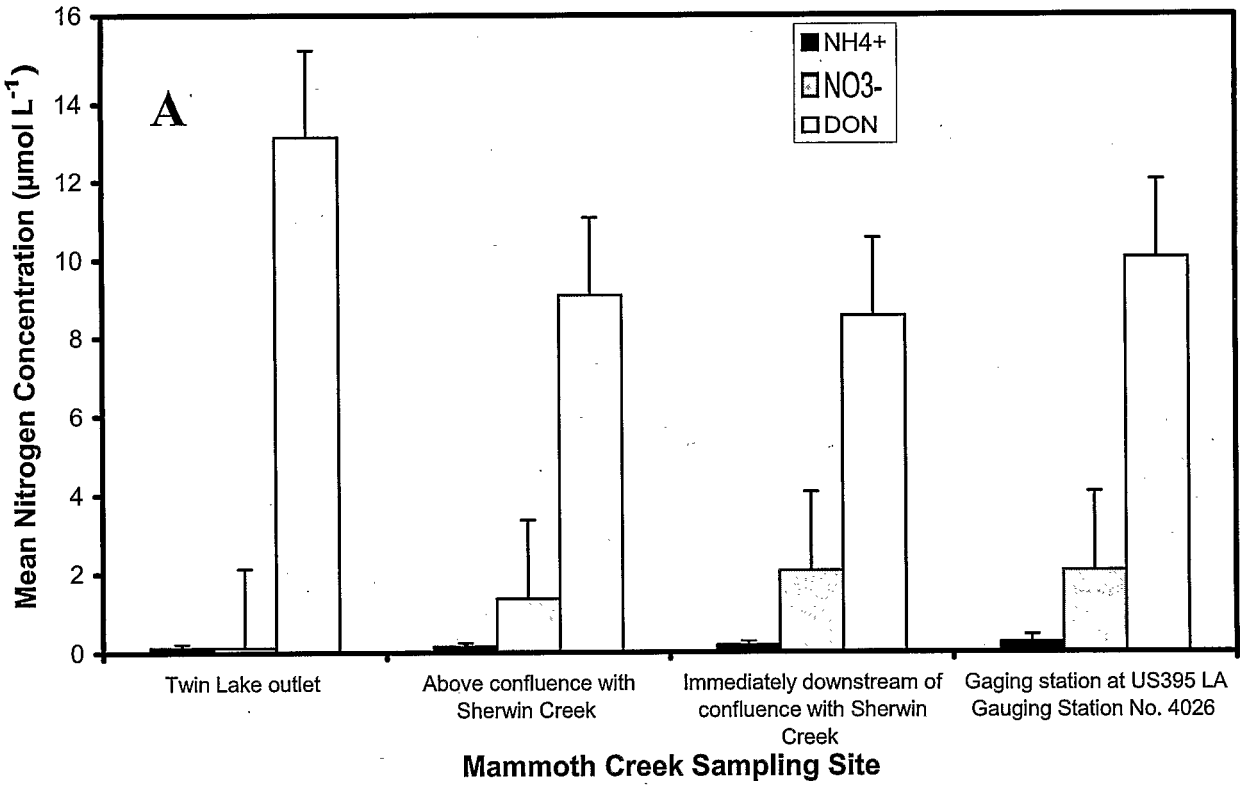
Using routine monitoring data for 2000 and 2001, we computed average nitrogen concentrations in the four landuse classifications. In general, the predominant form of nitrogen in groundwater-influenced waters in the upper Owens River watershed and in Hot Creek was nitrate (Figure 2). Organic nitrogen was the major form of nitrogen in Mammoth Creek and McGee Creek. Ammonium concentrations were near the detection limit at all but hatchery-influenced sampling sites. Mean total nitrogen concentration was 28 μmol L^{-1} in Hot Creek, 13 μmol L^{-1} in Mammoth Creek, 12 μmol L^{-1} in the Upper Owens Rivers sites and 7.5 μmol L^{-1} in McGee Creek.

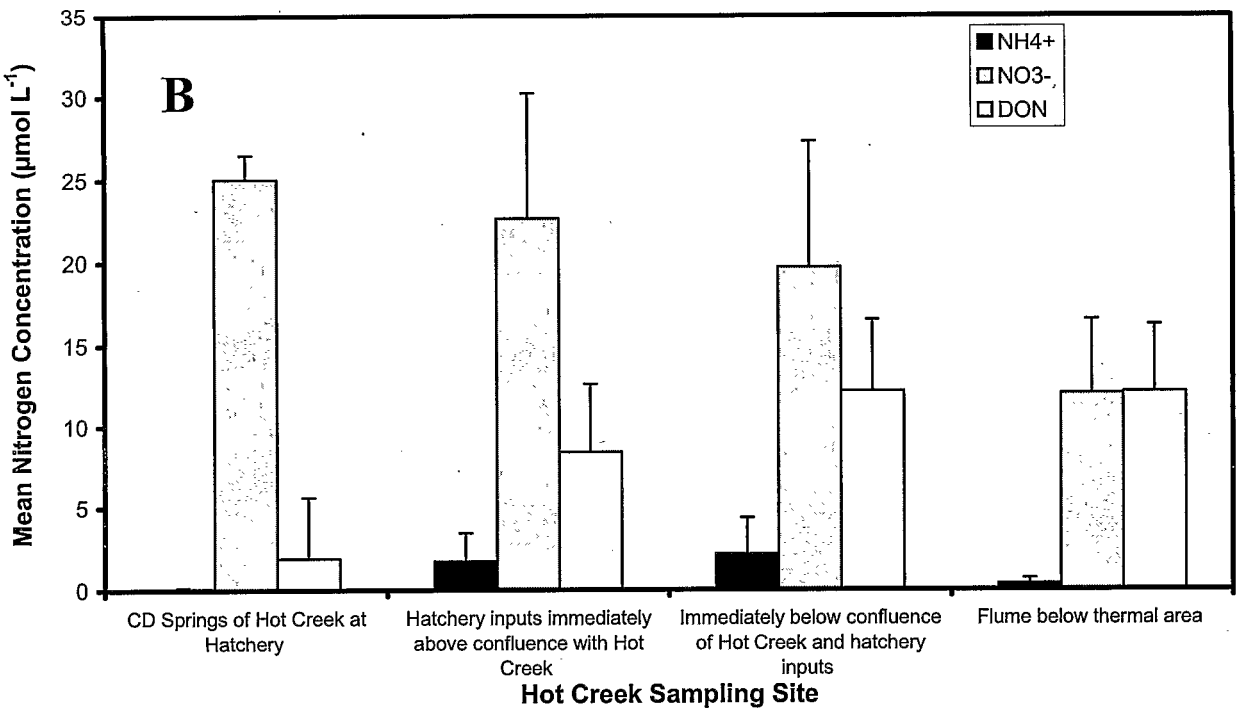
Figure 2. Mean nitrogen concentrations for four landuse classifications.



We observed changes in nitrogen concentrations and forms along the stream transects sampled which may be indicative of landuse influence (Figure 3). In Mammoth Creek nitrate concentrations increased downstream of the Twin Lake outlet while there was a slight tendency for DON to decline both in absolute terms and as a percentage of TN (Figure 3a). In Hot Creek the hatchery increased ammonium levels by 1-2 µmol L^{-1} and DON levels rose by 50-100% compared to the spring source waters (Figure 3b). Nitrate concentrations in inflowing waters to the hatchery were, on average, lower than below the hatchery. Fish excretion probably increased ammonium and DON levels in spring waters flowing through the hatchery. Nitrate losses may be explained by denitrification in sediments within and below the hatchery. In both June and December the $\delta^{15}N\text{-}NO_3$ value was higher in the thermal area below the hatchery; denitrification would result in isotopically heavier nitrate in downstream reaches of the creek.

Figure 3. Mean nitrogen concentrations at sites along A) Mammoth Creek and B) Hot Creek for calendar years 2000 – 2001.



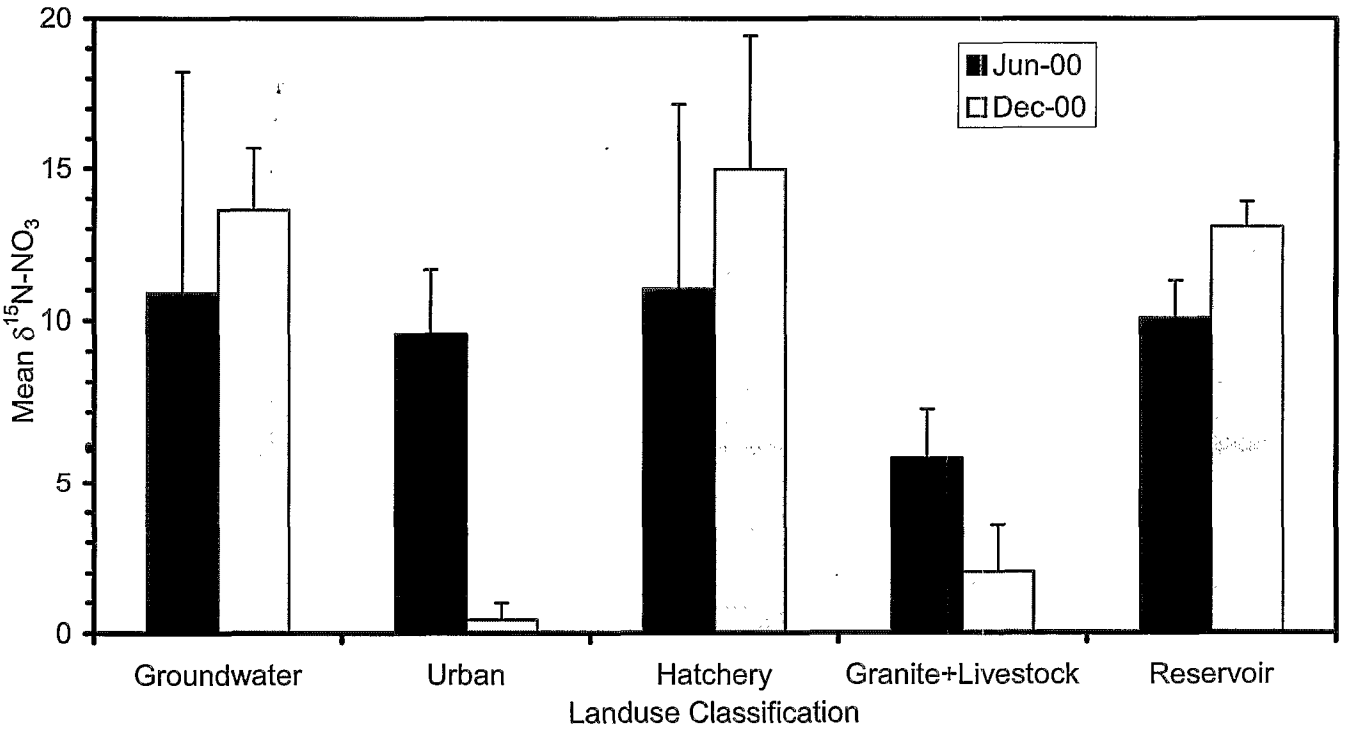


4.3 Nitrate and Biomass $\delta^{15}N$

As discussed in Section 4.1, the nitrate isotopic data collected during the study may be inaccurate due to interferences caused by sulfate and DON. Using nitrate recovery as a way to screen the data is problematic since the silver nitrate masses measured by the mass spectrometer are relatively inaccurate and it is uncertain whether under-recovery of nitrate was caused by mass-dependant processes affecting isotopic values. In addition, even when nitrate recoveries were reasonable, the isotopic composition was based on one rather than several replicate isotope measurements. Given the limited scope and resources for this project it was not possible to re-collect samples using more appropriate methods. Thus we were forced to decide whether analysis of the data is warranted. Given that this is a pilot study we decided to use all the isotopic data in the following analysis and discussion.

During both sampling dates, $\delta^{15}N\text{-}NO_3$ values ranged from ca. 0 to 20 ‰ (Tables 1 and 2). In June there was a large overlap in the $\delta^{15}N\text{-}NO_3$ values for the four landuse classifications and in Crowley Lake with no statistical differences among the means (Figure 4). Relative to June, December $\delta^{15}N\text{-}NO_3$ values in the lake and groundwater/hatchery influenced waters increased, but declined in the urban/granite/livestock influenced sources. In December the $\delta^{15}N\text{-}NO_3$ values in the urban and granite+livestock classification were statistically different than the remaining categories. There was no statistical difference between lake $\delta^{15}N\text{-}NO_3$ and the groundwater or hatchery values (Figure 4).

Figure 4. Mean isotopic composition of nitrate in four landuse types and Crowley Lake in June and December 2000.



A variety of aquatic plant and soil samples were collected from within and adjacent to the Upper Owens Rivers and in Hot Creek during June 2000. Additional phytoplankton samples were collected from Crowley Lake on December 18, 2000. The $\delta^{15}N$ value of the algae and phytoplankton at creek sites ranged from about 2 to 8 ‰ with generally higher values in Hot Creek compared with the Upper Owens River streams (Table 3). The isotopic composition of the algae in both streams is similar to typical values reported in the literature (Kendall 1998). Soil $\delta^{15}N$ values from the lakeshore around Crowley were relatively enriched (i.e., heavier), but dissimilar to values measured in cattle and horse droppings. Again, the values measured are within typically reported ranges.

The $\delta^{15}N$ value of phytoplankton within Crowley Lake was largely constant with both time and depth (Table 3). Values generally ranged between 2-3 ‰ and were quite similar to algal values found in inflowing creeks although they are heavier than those typically thought to be indicative of N_2-fixing blue-green alga (i.e., slightly less than 0 ‰; Kendall 1998).

4.4 Sources of Nitrate to Crowley Lake

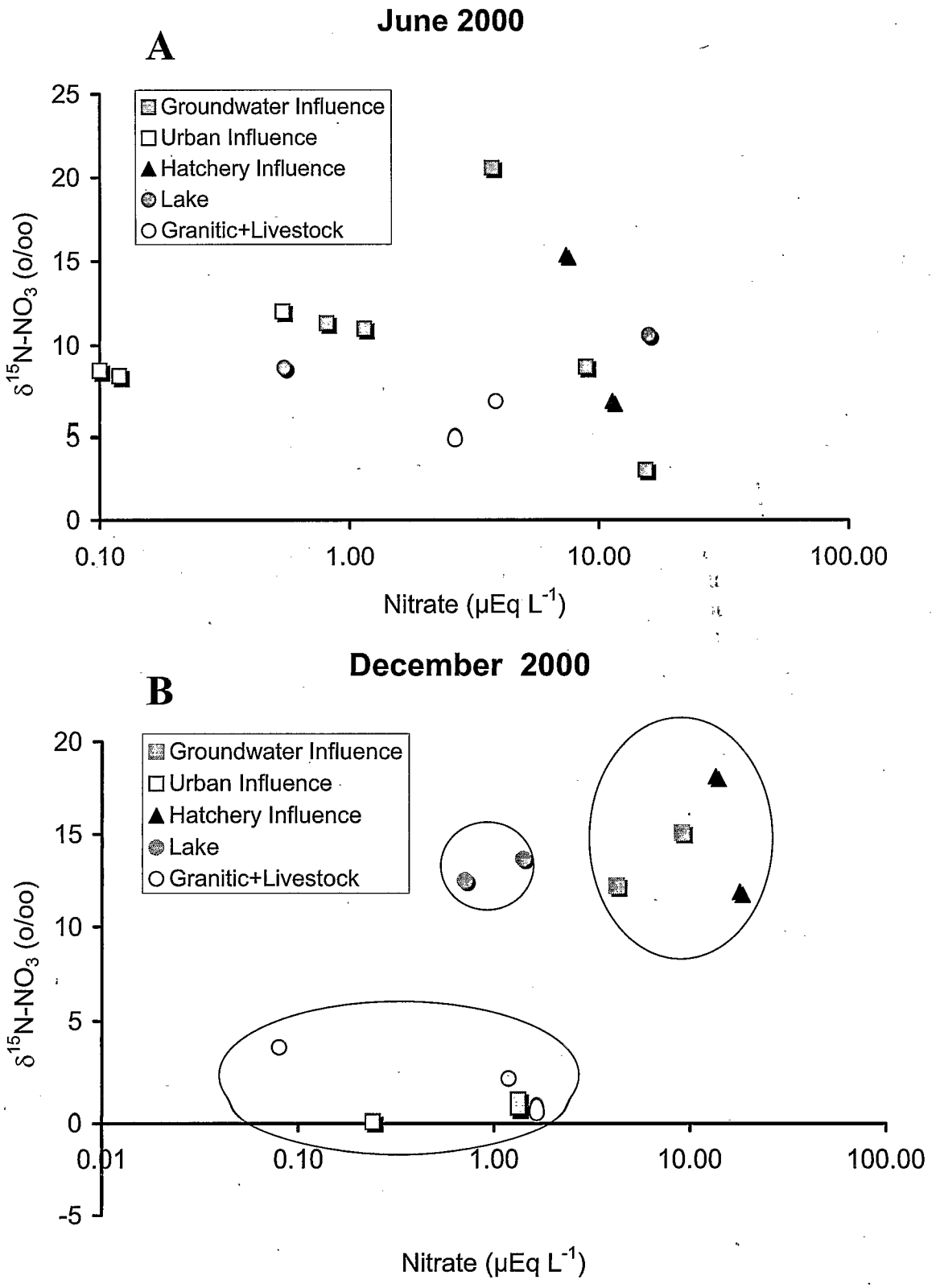
If the nitrate found in Crowley Lake was derived from streamwater inputs then both the concentration of nitrate and its isotopic composition should be explained by a mixture of these sources. Plots were drawn using the mean nitrate concentration and isotopic composition for the four landuse classifications and the lake (Figure 5). In June 2000 Crowley Lake nitrate could be a mixture of the identified external sources, although the widely overlapping signatures of the source end-members precludes us from determining their relative contributions (Figure 5a).

A seemingly more coherent story emerged from the December 2000 data (Figure 5b). Three clusters of samples were found: 1) relatively high concentration and isotopically enriched nitrate from the groundwater springs in the upper Owens River watershed and in Hot Creek, 2) low concentration and isotopically lighter nitrate in Mammoth and McGee creeks and 3) intermediate concentrations and isotopic composition in lake nitrate. From this bivariate plot it appears that nitrate concentrations and isotopic composition in Crowley Lake during December 2000 could be explained as a mixture of the four landuse types identified in the study.

Table 3. Summary data for biological materials sampled during June and December 2000 in the Upper Owens River watershed.

Sub Basin	Sampling Site	Landuse Classification	Date	Material	$\delta^{15}N$ ‰
Owens	Big Springs @pipe spring	Groundwater	6/26/00	Fern-like algae	3.2
Owens	Big Springs Main flow at campground	Groundwater	6/26/00	Green algae	2.5
Owens	Mono Portal	Groundwater	6/26/00	Fern-like algae	2.3
	Crowley Lake - mid station	Reservoir	6/27/00	Green surface algae	0.2
	Crowley Lake near dock	Reservoir	6/27/00	Black surface algae	1.7
	Crowley Lake mid station depth=15 m	Reservoir	6/27/00	Phytoplankton	2.8
	Crowley Lake mid station depth=5 m	Reservoir	6/27/00	Phytoplankton	2.4
	Crowley Lakeshore near McGee Inlet	Reservoir	6/27/00	Surface soil	8.9
	Crowley Lake inlet from McGee Creek	Reservoir	6/27/00	Bird droppings and soil	13.1
	Crowley Lakeshore near McGee Inlet	Reservoir	6/27/00	Cattle droppings	3.8
Hot Creek	Broodstock pond	Hatchery	6/27/00	Green Algae	8.2
Hot Creek	End of fingerling tank	Hatchery	6/27/00	Green Algae	3.1
Hot Creek	Headwaters of fingerling tank	Hatchery	6/27/00	Green Algae	8.9
Hot Creek	Hot Creek hatchery	Hatchery	6/27/00	Fish food from feeder	6.7
Hot Creek	Hot Creek below hatchery	Hatchery	6/26/00	Macrophyte	3.0
Hot Creek	Hot Creek below hatchery	Hatchery	6/26/00	Macrophyte	8.3
Hot Creek	Hot Crk @flume below thermal area	Hatchery	6/26/00	Macrophyte	7.4
Hot Creek	Hot Crk @flume below thermal area	Hatchery	6/26/00	Green Algae	7.6
McGee Creek	Below pack station	Granite+Livestock	6/25/00	Horse droppings	2.0
	Crowley south basin	Reservoir	12/18/00	Phytoplankton	2.5
	Crowley south basin	Reservoir	12/18/00	Phytoplankton	2.8
	Crowley east basin	Reservoir	12/18/00	Phytoplankton	3.2
	Crowley east basin	Reservoir	12/18/00	Phytoplankton	2.9

Figure 5. Bivariate plot of isotopic composition of nitrate and nitrate concentration in four landuse types and Crowley Lake. A) June B) December.



In a two-component separation computed for the mean isotopic composition of the three clusters shown in Figure 5b, groundwater+hatchery influenced sources contributed about 91% of the nitrate to Crowley Lake while the remaining 9% came from urban+granite+livestock influenced creeks (Table 4). In contrast, using nitrate concentration as the basis for the component separation would require that nearly all of the nitrate came from Mammoth and McGee creeks. Since assimilation of nitrate by phytoplankton induces isotopic fractionation at lower concentrations (Kendall 1998), it is unlikely that phytoplankton-uptake of nitrate from high-nitrate source waters (for example Hot Creek) could have reduced input concentration without appreciably affecting the isotopic composition. Thus, while the isotope analysis suggests groundwater+hatchery influenced sources, the concentration data contradict this conclusion. The apparent contradiction of the isotopic and chemical separations illustrates the limitations of using only a single isotope or line of inquiry to trace N sources. A multiple isotopic approach incorporating both the $\delta^{15}N$ and $\delta^{18}O$ composition of nitrate would greatly increase our ability to resolve the system and apportion the relative contribution of the nitrate sources.

Interestingly the $\delta^{15}N\text{-}NO_3$ value of nitrate in Crowley Lake, 5-15 ‰, was higher than what might be expected to result from the mineralization and nitrification of blue-green algae, which are thought to be a major source of N to the lake. Nitrogen fixation usually results in organic matter with a $\delta^{15}N\text{-}NO_3$ value near 0 ‰. Decomposition of blue-green biomass is unlikely to produce more than a 1-2 ‰ shift in the $\delta^{15}N$ value of ammonium and nitrification of ammonium typically produces isotopically lighter nitrate. Thus the net affect of these processes should produce a $\delta^{15}N\text{-}NO_3$ value lighter than is found in the lake. This finding suggests that either there was some other external or internal nitrate source to the lake (for example underground springs or sediment regeneration) or that the $\delta^{15}N\text{-}NO_3$ values we measured were erroneous.

Since N assimilation by algae typically induces a small isotopic fractionation, algae may take on the isotopic signature of their nutrient sources (Fry 1991). To investigate whether this is true in the Crowley Lake watershed we compared the mean isotopic composition of algae to the mean isotopic composition of nitrate in three of our four landuse types and in the Lake (Figure 6). For the inflowing creeks there was a general correspondence between the $\delta^{15}N$ of biomass and nitrate: sites with enriched $\delta^{15}N\text{-}NO_3$ had higher $\delta^{15}N$ values in biomass and the site with more depleted $\delta^{15}N\text{-}NO_3$ value had lower $\delta^{15}N$ values in biomass. At these sites the isotopic differences between dissolved and particulate N range from -0.4 to -3.8 ‰ (products → reactants) which is consistent with the expected direction and magnitude of the fractionation cause by N uptake in algae (Kendall 1998). Phytoplankton in Crowley Lake were, on average, 8 ‰ lighter than the nitrate in the lake, again suggesting that they utilized a different N source or that the nitrate isotope values are biased.

Table 4. Isotopic and chemical analysis of nitrate sources to Crowley Lake during December 2000. Presented are mean values of $^{15}N\text{-}NO_3^-$ and nitrate concentration for groundwater+hatchery influenced sources (Upper Owens River and Hot Creek), urban+granitic+livestock influenced sources (Mammoth and McGee creeks) and for nitrate in Crowley Lake. Nitrate-source percentages were computed using a two-compartment separation based on: 1) isotopic composition of nitrate and 2) nitrate concentration.

Isotopic Separation	$\delta^{15}N\text{-}NO_3$ Value ‰	% Owens+ Hot Creek NO_3	% Mammoth +McGee Creek NO_3
Owens+Hot Creek	14.3	-	-
Mammoth+McGee	1.4	-	-
Crowley Lake	13.1	91	9

Chemical Separation	Nitrate Value $\mu mol\ L^{-1}$	% Owens+ Hot Creek NO_3	% Mammoth +McGee Creek NO_3
Owens+Hot Creek	11.2	-	-
Mammoth+McGee	0.9	-	-
Crowley Lake	1.1	2	98

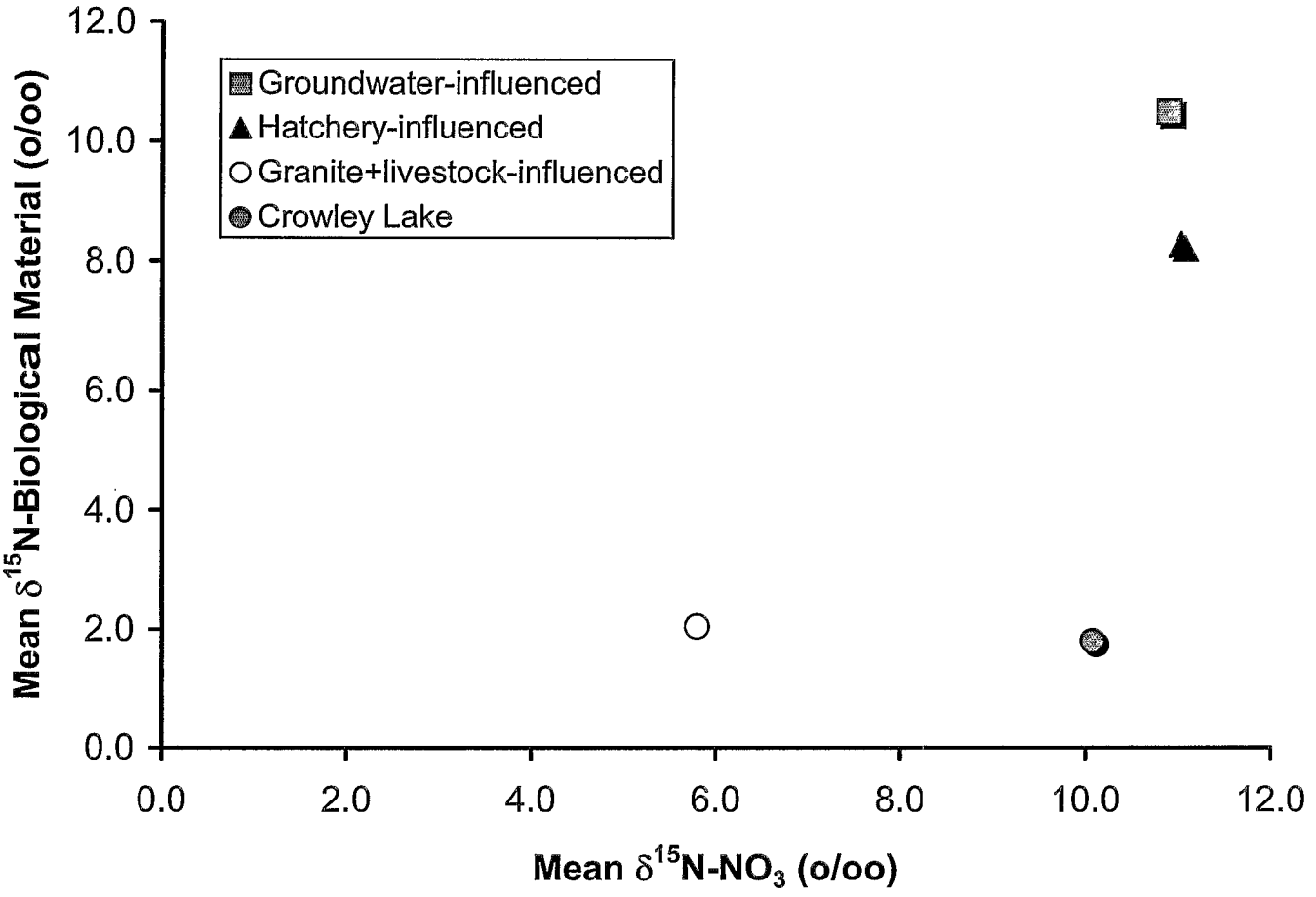
5.0 Conclusions

Owing to several factors inherent to surface waters within the upper Owens River watershed, $\delta^{15}N\text{-}NO_3$ values collected during the pilot study were not useful in resolving sources of nitrogen to Crowley Lake. While nitrate is the predominant form of N in groundwater-influenced source-waters, DON loading from the upland watersheds of Crowley Lake is probably the major input of allochthonous N to the lake. Furthermore, data presented in other portions of this report suggest that internal sources of N (N-fixation and internal cycling) are the main supplier of N to primary producers in the lake.

We have drawn the following conclusions regarding the feasibility of tracing nitrogen sources to Crowley Lake:

1. Nitrate levels from some landuse types, such as the Mammoth Village area, were near the detection limit (~0.1 $\mu mol\ L^{-1}$) and too low for stable isotope characterization by any method currently available.

Figure 6. Comparison of mean isotopic composition of algae and nitrate in four landuse types.



2. Even when nitrate concentrations were measurable, the vast majority of the anions in solution were chloride and sulfate. Low nitrate: total anion ratios make it impossible to use exchange resins to concentrate samples. The bacterial methods of Sigman et al. (2001) and Casciotti et al. (2002) are unaffected by anion concentrations and would be appropriate methods for a future study.

3. Dissolved organic carbon fouling of the anion columns used in this study interfered with the adsorption of nitrate on the anion resin. In addition it is possible that some of this DOC was released from the columns during the HCl stripping procedure and its nitrogen content affected the subsequent $\delta^{15}N\text{-}NO_3$ determinations. These finding suggest that using larger columns and larger samples to overcome the low proportion of nitrate to total anions in will not be effective. The bacterial methods of Sigman et al. (2001) and Casciotti et al. (2002) are not affected by DOC levels in samples.

4. Assuming the data we collected was accurate, there was a wide overlap of $\delta^{15}N$-NO_3 values in the watershed nitrate sources considered in this study. This overlap prevented us from resolving nitrate sources to Crowley Lake during June 2000. In December 2000 $\delta^{15}N$-NO_3 values in the reservoir were very similar to values from inputs from groundwater-and hatchery-influenced waters, but concentrations in source and receiving waters varied by a factor of 10 contradicting this inference.

5. There was correspondence between $\delta^{15}N$-NO_3 and aquatic biomass $\delta^{15}N$ in inflowing creeks, but not in Crowley Lake.

6. In Hot Creek the isotopic composition of nitrate and downstream declines in nitrate concentrations suggest that denitrification rates are high in creek sediments below the hatchery.

6.0 Recommendations

The chemistry of surface waters in the Upper Owens River watershed generally precludes the use of the Chang et al. (1999) method for collection and determination of $\delta^{15}N$-NO_3. In addition the $\delta^{15}N$-NO_3 values alone were not useful in resolving nitrate sources. If additional isotope study of nitrate sources is undertaken we recommend that both $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 be measured and that alternate methods such as those of Sigman et al. (2001) and Casciotti et al. (2002) be used. The bacterial techniques offer several advantages over older methods, including low sample requirements and no interference from DON. In addition, frozen archived samples already collected could be analyzed for nitrate isotopes with this method, saving the labor and expense of collecting new samples.

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