

**CARBON AND NITROGEN STABLE ISOTOPES ON THE TRUCKEE RIVER:
RESULTS OF MARCH 2004 SAMPLING**

Final Report

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Introduction

Dr. Laurel Saito and her students at the University of Nevada Reno (UNR) have been collaborating with the United States Geological Survey (USGS), the Pyramid Lake Paiute Tribe (PLPT), the Desert Research Institute (DRI), and the Nevada Division of Environmental Protection (NDEP) to investigate the use of stable carbon and nitrogen isotopes to understand anthropogenic impacts on the aquatic ecosystem in the Truckee River. Previous work included stable isotope sampling and analysis of the Truckee River aquatic food web (i.e., fish and macroinvertebrates, and periphyton) in the summers of 2002 and 2003 during relatively low flows, and in the spring of 2003 during higher flows. The scope of the current study involved collecting another set of aquatic food web samples in March 2004 on the Truckee River for carbon and nitrogen stable isotope analysis. This report presents the methods and results of this sampling.

The Truckee River is a vital resource to Nevadans in the northwest region of the state. It provides public water supplies to the cities of Reno and Sparks, and while little irrigated agriculture occurs directly adjacent to the river, about one-third of its flow is diverted to the Lahontan Valley for irrigation purposes. The river terminates in Pyramid Lake, which has experienced severe declines in water level because of the heavy water diversions along its length. In addition, there are numerous resort and recreational activities throughout the basin, and the river and Pyramid Lake provide valuable water and habitat for endangered Lahontan cutthroat trout and cui ui species. In 1998, the USGS's Nevada Basin and Range (NVBR) National Water-Quality Assessment (NAWQA) Program reported that while stream habitat at all sites (based on degradation indices related to riparian vegetation, stream modification, bank stability, and bank erosion) on the Truckee River system was better than the national median, fish communities in the lower reaches of the Truckee River were more degraded than the national median (Bevans et al. 1998). Furthermore, nutrients in the river and trace elements in its sediments increased 3 to 10 times downstream of the discharge from sewage treatment plants and the entrance of Steamboat Creek to the river. Thus, it appears that downstream influences on water quality and associated biological activity are detrimentally affecting the food web in the Truckee River.

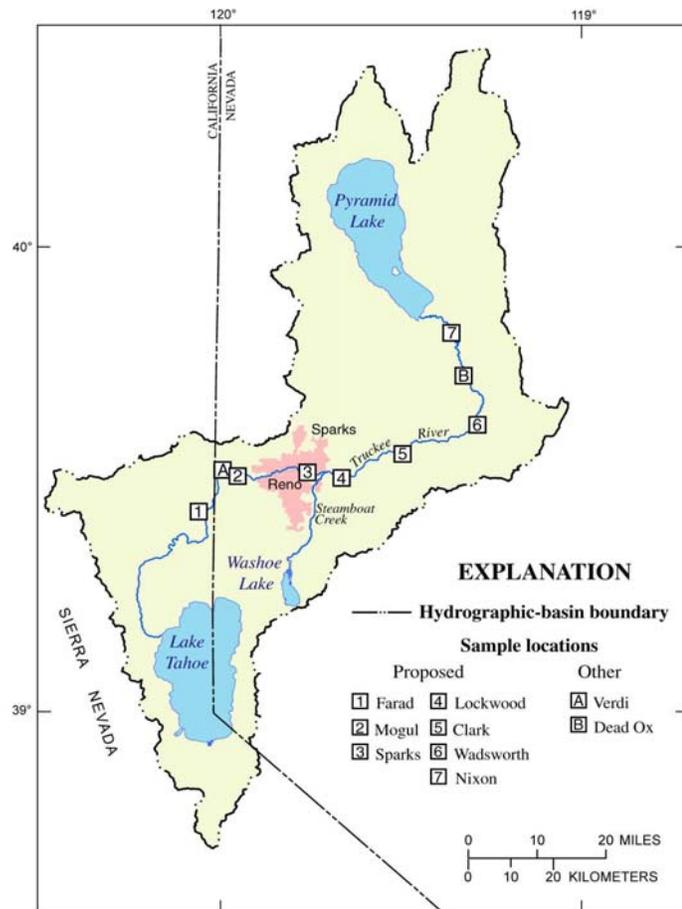


Figure 1. Truckee River Basin, Nevada and California

The current work involves the use of stable carbon and nitrogen isotopes to gain insight into the aquatic food web. The use of stable isotopes in trophic studies employs the fundamental concept that ‘you are what you eat.’ Stable isotopes incorporate two kinds of information: origin and fractionation. The isotopic signature of an individual will reflect the signature of the sources of the isotopes (i.e., where the isotopes first entered the food web) and the change in the isotopic signature due to isotopic fractionation by consumption and metabolism in the food web (Peterson and Fry 1987). Because isotopes accumulate in body tissues over time, a one-time analysis of stable isotopes provides a time-integrated measure of the diet (Fry and Sherr 1984; Hesslein et al. 1993; Vander Zanden et al. 1998). Stable isotope analysis can even be used in food webs with omnivory because isotope values can be measured in all levels of the food web, including phytoplankton, zooplankton, and aquatic insects (Michener and Schell 1994; Vander Zanden and Rasmussen 1996; France 1997). Carbon and nitrogen ratios are the most commonly used stable isotope ratios in food web studies. Carbon ratios ($\delta^{13}\text{C}$) are used because the slight ($0.2 - 1.1\text{‰}$) increase of $\delta^{13}\text{C}$ in animals relative to their diet means that the $\delta^{13}\text{C}$ signature of the primary producer (first organic food source) is likely to be preserved through several trophic levels (Peterson and Fry 1987; Michener and Schell 1994; Yoshioka et al. 1994; France and Peters 1997). Thus, carbon isotope analysis can be used to identify and distinguish the influence of different primary food sources if the isotopic signatures of those food sources are distinctive enough (Forsberg et al. 1993; Michener and Schell 1994). The nitrogen ratio ($\delta^{15}\text{N}$) is often used as an indicator of trophic position of a consumer (Fry 1988; Kling et al. 1992; Yoshioka et al. 1994) because the increase of $\delta^{15}\text{N}$ with trophic level is much greater than with carbon ($\sim 3\text{-}4\text{‰}$ per trophic level) (Michener and Schell 1994).

Stable carbon and nitrogen isotopes have value in potentially detecting anthropogenic influences on aquatic food webs. Human- and animal-derived wastewater should have higher $\delta^{15}\text{N}$ values because of the volatilization of ^{15}N depleted ammonia which occurs during the hydrolysis of urea, and because humans tend to eat higher in the food chain, which elevates their waste nitrogen signatures (Heaton 1986; Silva et al. 2002; Wayland and Hobson 2001). On the other hand, synthetic fertilizers are typically derived by industrial fixation of atmospheric nitrogen (which has a reference signature of 0‰), so waters draining fields using these fertilizers tend to have lower nitrogen signatures (Heaton 1986; Silva et al. 2002). Distinctive carbon signatures may be detected when aquatic-terrestrial interactions are altered (e.g. due to alteration of the stream channel and/or flooding regime) because terrestrial plants may have significantly different $\delta^{13}\text{C}$ signatures than their aquatic counterparts. Such approaches have been used to detect the importance of autochthonous versus allochthonous material in streams (Rounick and Winterbourn 1986; Finlay et al. 1999). In addition, shifts in food web dynamics such as shifts in diets or elimination of species may be detectable with stable isotopes; if the food chain shortens, we should see shifts in nitrogen signatures in the top predators, and if a food source is eliminated at the base of the food web, we may see shifts in the carbon signature.

The remainder of this report details the methods and results of stable isotope sampling of the Truckee River aquatic food web performed in March 2004.

Approach

During the week of March 15 – 17, 2004, we collected samples of fish, macroinvertebrates, periphyton, and water at Farad, Mogul, Sparks (i.e., Rock Park), Lockwood, Clark (i.e., Tracy), Wadsworth, and Nixon (i.e., below Marble Bluff Dam; Figure 1) as part of the scope of work for this study.

Macroinvertebrates were collected by using a 250-mm by 450-mm rectangular kick net with 500-micrometer (μm) mesh. The net was placed firmly in the streambed with the opening facing perpendicular to the river flow. The substrate immediately upstream of the net was disturbed by using a

stiff bristle brush, a garden claw, and by kicking the rocks. This released macrofauna residing in the substrate and on upstream rocks and allowed them to wash into the net. The macroinvertebrates were then hand-sorted by order (i.e., ephemeroptera, plecoptera, coleoptera, odonata, etc.) and in some instances by family. The sorted organisms were stored in labeled plastic whirlpacks, and frozen on dry ice.

Fish samples were collected using a backpack electroshocker provided by the USGS. At each location, we attempted to collect three samples of each size class and species of fish present. Total length and wet weight of each fish were recorded. For large fish, a biopsy punch was taken in the tissue behind the dorsal fin while in the field and the fish was then released back into the river. This process was especially important when dealing with the Lahontan cutthroat trout and the cui ui, which are threatened or endangered and must be returned to the river. Fish were stored in labeled plastic whirlpacks and placed on dry ice.

We also collected bullfrog tadpoles and crayfish when doing the macroinvertebrate and fish sampling. We generally took up to three samples of each size class present of these organisms, stored them in labeled plastic whirlpacks, and placed them on dry ice.

Macroinvertebrate, fish, and other samples were transported to the freezer at UNR until processing. To process the samples, the samples were defrosted and placed in labeled tin boats in an oven at 60 °C for at least 24 hours. Fish that were too big for processing were filleted and tissue from below the dorsal fin was taken and placed in the drying tins. Smaller fish were dried whole. Tissue from the tails of bullfrog tadpoles and crayfish were taken from large samples. The dried samples were ground in a mortar and pestle to form a homogenous powder. Approximately 2 milligrams (mg) of the powder was weighed into Costech 5 x 9 millimeter (mm) pressed tins that then were rolled into balls.

We also collected periphyton samples at each sampling location from three or four habitats within the river (i.e., pool, riffle, glide, and/or run). Because flows were relatively high at most of the sampling locations, the sites of the collections were limited to areas that could be reached via wading and therefore were not always as diverse. Three cobbles were collected at each of the different habitats within the river. These cobbles were placed in clean containers, rinsed with filtered local river water, and scrubbed with a wire brush. Any macroinvertebrates that were residing on the rocks were discarded and the water from the rinsing and scrubbing of the cobbles was placed in labeled plastic bottles and put on ice.

The periphyton sample bottles were transported to DRI, where each sample was placed in a blender to form a homogenized sample, and then poured back into the original container using filtered stream water from the appropriate site to rinse all of the contents back into the container. Approximately 3 milliliters (ml) of each solution was vacuum-filtered onto separate 25-mm Whatman GF/F filters. The filters were then placed in aluminum weigh boats in a 105 °C oven for 24 hours, after which one quarter of the dried filter was cut and placed on a 28-mm diameter piece of foil, which was rolled into a ball with the foil on the outside.

Once all samples were processed, the trays containing the completed samples were sent to the UNR stable isotope lab for analysis. The balls containing the homogenous powder were placed in a mass spectrometer and the carbon and nitrogen signatures were measured. The results were then sent via electronic mail to Dr. Laurel Saito and Ms. Christa Fay.

Water quality samples were collected in 2-0.5 liter (L) bottles after rinsing three times with river water at each site. One 0.5 L of the water was immediately filtered through a Whatman GF/F filter into another rinsed bottle, and both bottles were placed in a cooler for transport to the UNR lab. Mr. Peter Szameitat

and Ms. Christa Fay analyzed the filtered water for ammonium, nitrate, and orthophosphorus by using the colorimetric methods from Wetzel & Likens (2000) and Shimadzu UV 1201 spectrophotometer.

Results and discussion

We collected samples from the seven sites on the Truckee River during March 2004 (Table 1). Overall, we collected a total of 220 samples in the aquatic food web, of which 211 were analyzed for stable carbon and nitrogen isotope values. Statistical analysis was then completed on 197 samples. Some samples were discarded because there was not enough biomass within the sample or the sample was contaminated during preparation; see Table 2 for a description of these samples. All data is included in Appendix A and file *NDEPMarch 2004 Log.xls*.

Table 1. Sampling characteristics and results at seven sampling locations on the Truckee River in March 2004

	Sampling location						
	Farad	Mogul	Sparks	Lockwood	Clark	Wadsworth	Nixon
Distance from Tahoe (km)	55	76	97	107	125	150	187
Date	3/16/04	3/16/04	3/17/04	3/16/04	3/15/04	3/15/04	3/15/04
Time	8:30	10:00	9:00	12:00	14:00	12:00	9:00
Flow (cfs) ^a	721.67	797.67	719.33		372	84.33	87.33
Orthophosphorus ($\mu\text{g L}^{-1}$)	74.8	83.0	69.1	79.9	86.1	112.2	93.7
NH ₄ ($\mu\text{g L}^{-1}$)	121.0	121.0	108.1	64.0	100.9	116.7	136.9
NO ₃ ($\mu\text{g L}^{-1}$)	323.7	241.0	234.8	199.9	182.1	259.4	231.1
Number of isotope samples ^b	18/17	36/35	25/24	34/33	30/29	35/34	26/25
Average $\delta^{13}\text{C}$ ($^{\circ}/_{\text{oo}}$)	-22.53	-19.33	-18.01	-23.10	-23.175	-24.78	-24.16
Average $\delta^{15}\text{N}$ ($^{\circ}/_{\text{oo}}$)	9.88	10.03	9.96	15.92	15.14	15.63	10.21

^a Data provided by USGS web page: <http://water.usgs.gov/>. Accessed in June 2004

^b First number is number of reliable carbon isotope samples; second number is number of reliable nitrogen isotope samples

Table 2. Samples that were not included in the statistical analysis on the Truckee River in March 2004

Sample Number (mdddLL###)	Species	Reason for removing from analysis
0315CLA005	PER	Not enough nitrogen ^a
0315CLA025	AMP	Contaminated during processing
0315CLA026	TRI	Not enough nitrogen, too much carbon ^b
0315NIX005	PER	Not enough nitrogen ^a
0315NIX009	THS	Contaminated during processing
0315NIX026	TRI	Sample too big ^c
0315NIX027	TRI	Sample too big ^c
0315NIX028	TRI	Sample too big ^c
0315WAD005	PER	Not enough nitrogen ^a
0315WAD023	TRI	Sample too big ^c
0316FAR005	PER	Not enough nitrogen ^a
0316FAR015	COL	Sample too big ^c
0316LOC005	PER	Not enough nitrogen ^a
0316LOC030	AMP	Contaminated during processing
0316LOC036	PLA	Contaminated during processing
0316MOG003	PER	Missing sample
0316MOG006	PER	Not enough nitrogen ^a
0316MOG026	COL	Contaminated during processing
0316MOG034	EPH	Contaminated during processing
0316MOG037	TRI	Sample too big ^c
0316MOG041	PEL	Contaminated during processing
0316MOG042	SIM	Contaminated during processing
0317SPA005	PER	Not enough nitrogen ^a

^a Sample analyzed did not have enough nitrogen content; however $\delta^{13}\text{C}$ values were reliable

^b Sample analyzed did not have enough nitrogen content and too much carbon content

^c The sample was too big and therefore analysis did not result in reliable $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values

For quality control purposes, we analyzed five replicates of four samples to verify that the measured stable carbon and nitrogen isotope values for replicates of the same sample were not significantly different. To do this, we used the *proc means* and *proc glm* procedures in SAS, which confirmed that the replicates were not significantly different. The r-squared values for both the carbon and nitrogen signatures were 0.998, with p-values of less than 0.0001. Appendix B provides the input (*0304 C-Reps.sas* and *0304 NReps.sas*) and output (*0304 CReps.lst* and *0304 NReps.lst*) files for these analyses.

Average carbon and nitrogen stable isotope values with ± 1 standard deviation error bars for each of the sites sampled are shown in Figure 2 for the March 2004 sampling. Figure 2 shows that in March 2004, average nitrogen signatures were similar between Farad, Mogul, Sparks and Nixon. These locations have lower nitrogen signatures as compared to the remaining three sites, Clark, Lockwood and Wadsworth. Carbon signatures are higher at Sparks and Mogul and are lower at the remaining five sampling locations.

Figure 3 shows these average carbon and nitrogen signatures with distance from Lake Tahoe without the periphyton signatures, while Figure 4 shows the average carbon and nitrogen signatures with distance from Lake Tahoe for periphyton only for the March 2004 sampling. Average carbon and nitrogen signatures measured in August 2002 (summer 2002), March 2003 (spring 2003), and August 2003 (summer 2003) are also shown for comparison (note that periphyton signatures were not measured in August 2002). Plots showing actual carbon and nitrogen signatures of all species collected at each site in March 2004 are included in Appendix C (*appendix C.xls*). In general, average periphyton carbon and nitrogen stable isotope values are similar to, but slightly lower than, average signatures found in the rest of the food web (Figures 3 and 4). All sampling dates shown higher average nitrogen signatures and lower average carbon signatures between Sparks and Lockwood.

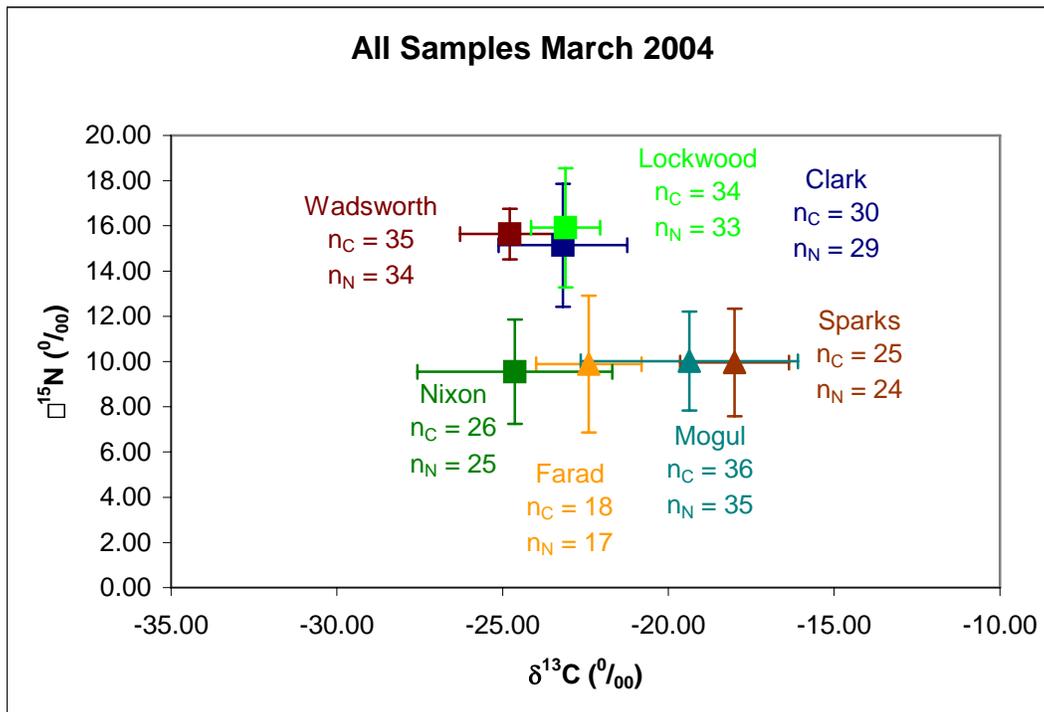


Figure 2. Average carbon and nitrogen stable isotope signatures measured at seven sampling locations on the Truckee River in March 2004; n_C and n_N are the numbers of samples with reliable signatures for carbon and nitrogen, respectively, at a particular location.

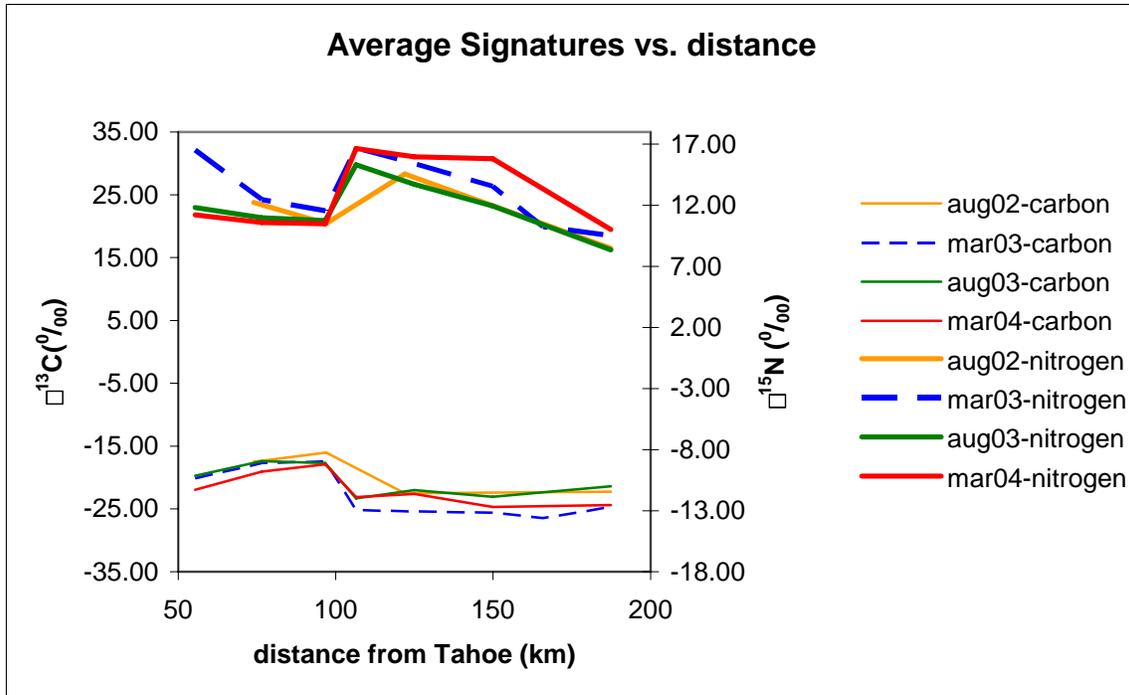


Figure 3. Average carbon and nitrogen stable isotope signatures without periphyton on the Truckee River

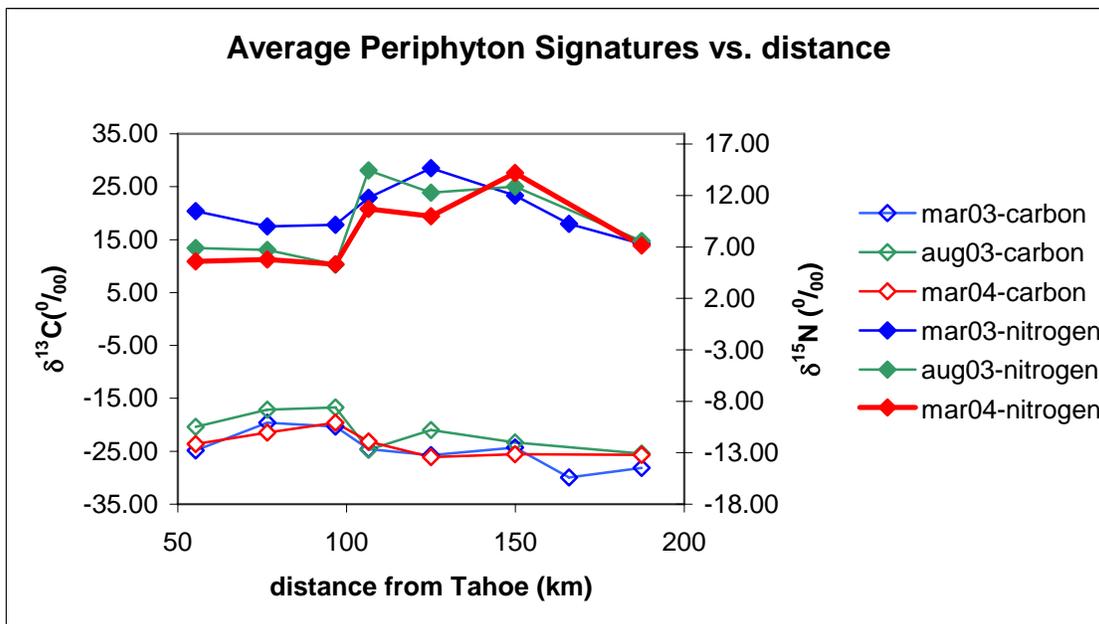


Figure 4. Average carbon and nitrogen stable isotope signatures for periphyton only on the Truckee River

We performed a two-way ANOVA test using SAS statistical software to determine if carbon and/or nitrogen signatures were significantly different between the seven sites sampled in March 2004. In

particular, we were interested to see if adjacent sites have significantly different signatures. Input and output files from our SAS runs are included in Appendix D and are summarized in Table 3. The null hypothesis for this analysis is that all sites have similar carbon and nitrogen signatures. α was adjusted for a small sample size (i.e., seven sites), where $\alpha = 0.05/7 = 0.00714$. Therefore, the null hypothesis was rejected wherever the p-value was less than α , meaning that there was a significant difference in signatures between the sites. The analysis confirmed our observations in Figure 2 that there were significant differences in the carbon and nitrogen signatures between the Sparks and Lockwood sites. For nitrogen signatures only, significant differences were observed between Lockwood and Clark, Clark and Wadsworth, and Wadsworth and Nixon. Bratberg (1980) and McKenna (1990) noted that the flows near Wadsworth are heavily influenced by groundwater inflows that primarily consist of irrigation return flow, and the significant difference in nitrogen signatures at the Wadsworth site from its adjacent site at Nixon may be indicating that influence. For carbon signatures only, significant differences were observed between Farad and Mogul and Mogul and Sparks. It is possible that changes in the carbon signatures may be reflecting influences of urbanization since the sites with significant differences represent a gradient of increasing urban impacts.

Table 3. p-values from comparison of adjacent sites for March 2003 sampling

Sites compared	p-values	
	$\delta^{13}\text{C}$ comparison	$\delta^{15}\text{N}$ comparison
Farad-Mogul	0.0017	0.3674
Mogul-Sparks	0.0001	0.4916
Sparks-Lockwood	<0.0001	<0.0001
Lockwood-Clark	0.4919	0.0011
Clark-Wadsworth	0.0170	0.0005
Wadsworth-Nixon	0.4292	<0.0001

Figures 3 and 4 also imply that the periphyton carbon and nitrogen signatures may be a good indicator of aquatic food web signatures. The linear regression r^2 statistic between average periphyton signatures at each site and the average signatures of all other food web components at each site were 0.82 and 0.75 for carbon and nitrogen signatures, respectively, indicating strong correlation.

We performed a two-way ANOVA test in SAS to determine if the periphyton signatures are correlated with any of the food web species in particular. The input and output files for this comparison are included in Appendix E, and the results are shown in Table 4. The analysis indicated that both carbon signatures between periphyton and amphipoda, annelida, chironomidae, and hemiptera were at least moderately correlated based on comparisons of samples at sites. Nitrogen signatures did not have any significant correlations. While some other p-values are high, they were based on one sample at one location, which was not enough to determine if a strong correlation exists or not.

Table 5. p-values from comparisons of periphyton stable isotope signatures with other food web species isotope signatures; n = number of observations compared; bolded values indicate strong correlations

Species description	Code	n	p-values from comparisons	
			Carbon signatures	Nitrogen signatures
amphipoda (scuds)	AMP	6	0.7765	<0.0001
annelida (worms/leeches)	ANN	7	0.3114	<0.0001
blue gill	BLG	1	0.0936	<0.0001
brown trout	BRT	17	<0.0001	<0.0001
chironomidae	CHI	11	0.9703	<0.0001
coleoptera (beetles)	COL	1	0.9981	0.8879
decapoda (crayfish/shrimp)	CRA	23	0.0006	<0.0001
ephemeroptera (mayflies)	EPH	14	0.0620	<0.0001
flathead minnow	FLM	1	0.7642	0.0036
gastropoda (snails)	GAS	6	<0.0001	<0.0001
hemiptera (water striders)	HEM	3	0.3139	0.0218
lahontan cutthroat trout	LCT	1	0.1553	<0.0001
lepidoptera (aquatic moths)	LEP	3	<0.0001	0.0148
lahontan redbreast shiner	LRS	26	0.0019	<0.0001
odonata (dragonfly/damselfly)	ODO	4	0.1181	0.0001
pelecypoda (clams)	PEL	3	0.0709	0.1123
planaria (flatworms)	PLA	2	0.6528	0.0003
plecoptera (stonefly)	PLE	7	0.0510	<0.0001
rainbow trout	RBT	8	0.0195	<0.0001
tahoe sucker	THS	39	<0.0001	<0.0001
trichoptera (caddisfly)	TRI	8	0.1376	<0.0001

Conclusions

The results of the stable carbon and nitrogen isotope analysis of aquatic food web samples collected along the Truckee River in March 2004 appear to confirm results obtained during similar sampling in August 2002, March 2003, and August 2003. General nitrogen signatures decrease with distance along the river, with the exception of a significant increase in the nitrogen signatures between the Sparks and Lockwood sampling locations. The March 2004 results also indicate a decrease in the carbon signatures at this location. Comparison of the periphyton signatures with signatures measured in the rest of the food web indicate that the periphyton signatures exhibit a correlation with food web signatures in general, but may be more strongly correlated with specific species, particularly chironomidae in terms of carbon signatures. Further analysis will be completed for all four sampling periods (August 2002, March and August 2003, and August 2004). Analysis will include determining if there are significant differences between in food web signatures between sites, species, and/or sampling dates. Once this has been completed, a food web model, or multiple food web models, will be constructed using the statistical information from the analyses and information about species' diets based on literature research.

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APPENDIX A

Sample data (*NDEP March 2004 Log.xls*)

APPENDIX B

SAS input and output files for sample replicates – carbon and nitrogen
(*0304 C-Reps.sas*, *0304 CReps.lst*, *0304 NReps.sas*, and *0304 NReps.lst*)

APPENDIX C

Plots of carbon and nitrogen signatures by location (*appendix C.xls*)

APPENDIX D

SAS input and output files for site comparisons
(*0304 location.sas and 0304location.lst*)

APPENDIX E

SAS input and output files for site comparisons
(*0304 species.sas and 0304 species.lst*)

APPENDIX F

Selected photographs from March 2004 sampling



Sorting macroinvertebrates at Nixon March 15, 2004



Collecting periphyton at Wadsworth March 15, 2004



Collecting periphyton and filtering water at Wadsworth March 15, 2004



Collecting macroinvertebrates at Sparks March 17, 2004



Electroshocking fish at Sparks March 17, 2004



Collecting periphyton at Sparks March 17, 2004