Mercury Source Fingerprinting in Arid Lands Aquatic Ecosystems

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Problem and Research Objectives

Mercury (Hg) is well-known as a global pollutant; its main source is coal combustion, and deposition of Hg into aquatic systems and landscapes produces subtle neurotoxic effects in vertebrates at low concentrations. Identifying Hg sources and unraveling their relative contribution to the Hg inventories in water, soil, sediment, and biota remains an elusive problem despite decades of environmental studies of Hg. Recent work by several groups, however, indicates that Hg exhibits small variations in its stable isotope compositions. This variation is at least partially source-related and can potentially be used to understand sources, transport, and biogeochemical cycling of Hg. Research in Hg isotopes is not well developed, but is the subject of intense interest among several research groups.

The principal investigators have been working to build the capability at Northern Arizona University to track the movement of Hg through ecosystems in order to understand the sources of Hg and mechanisms by which some fisheries in arid lands ecosystems are impaired by Hg and others are not. This project supplements an award from the Technology and Research Initiative Fund (TRIF) at NAU, titled "Developing the Capacity to Trace Sources of Mercury in the Environment Using Hg Stable Isotopes". The TRIF award permits the investigators to address the instrumental challenges in perfecting the technique of detecting Hg isotope ratios. Funding from the Water Resources Research Institute, through WRRA enabled us to fund a graduate student to apply this technique for her master's thesis work.

The specific objectives of this project were to (1) acquire an instrument to analyze total Hg (T-Hg) and establish a laboratory and protocols to support this instrument; (2) develop the capability to track the movement of Hg through aquatic food webs using a variety of stable isotope techniques; and (3) collaborate with an external Hg isotope expert to develop the capability to measure Hg isotope ratios.

Methodology

Our first objective involved measurement of T-Hg in solid and semi-solid samples (as opposed to aqueous or other liquid samples). We acquired a Teledyne/Leeman Hydra-C analyzer. This

instrument is designed to analyze specifically for Hg by combusting samples, accumulating Hg by amalgamation onto gold-coated sand, releasing the Hg vapor by heating the gold sand, and detecting the Hg by cold-vapor atomic absorption spectrometry. This technique corresponds to EPA Method 245.5 (USEPA, 1993). This instrument can detect greater than about 2 ng Hg. For sediment samples of about 200 mg, this corresponds to a detection limit of about 0.4 ng/g Hg.

Our second objective involved tracing the movement of Hg through aquatic food webs. This was the subject of the master's student funded through this research. She selected three stream reaches in the upper Verde River of northern Arizona (Figure 1). The project consisted of sampling algae, biofilm, leaves, and fish at upstream and downstream locations in each stream. Samples were analyzed for T-Hg; nitrogen-15 isotope ratios ($\delta^{15}N$) to detect the trophic position in the food web; and carbon-13 ($\delta^{13}C$) and organic-matter deuterium ($\delta Dorg$) isotope ratios to detect carbon sources. Hermosillo (2010) provides a complete description of field and laboratory methods.

Our third objective was to make measurements of Hg isotope ratios for inorganic and biotic samples. We used a modification of the technique described by Foucher and Hintelmann (2006). Samples were digested in *aqua regia* (HNO_3/HCL 9:1 v/v) and diluted to about 5 ng/mL Hg. Samples were introduced to a multi-collector inductively coupled plasma mass spectrometer (MC ICPMS) using an apparatus that provided a continuous flow of stannous chloride ($SnCl_2$) for Hg reduction, injection of a thallium (Tl) solution of known isotopic composition an internal standard, and a moisture separation device which uses argon gas to separate moisture from the cold Hg vapor. The ICPMS was operated in multi-collector mode, with Faraday collectors tuned to detect the Mass 198, 199, 200, 201, and 202 isotopes of Hg and the Mass 203 and 205 isotopes of Tl.

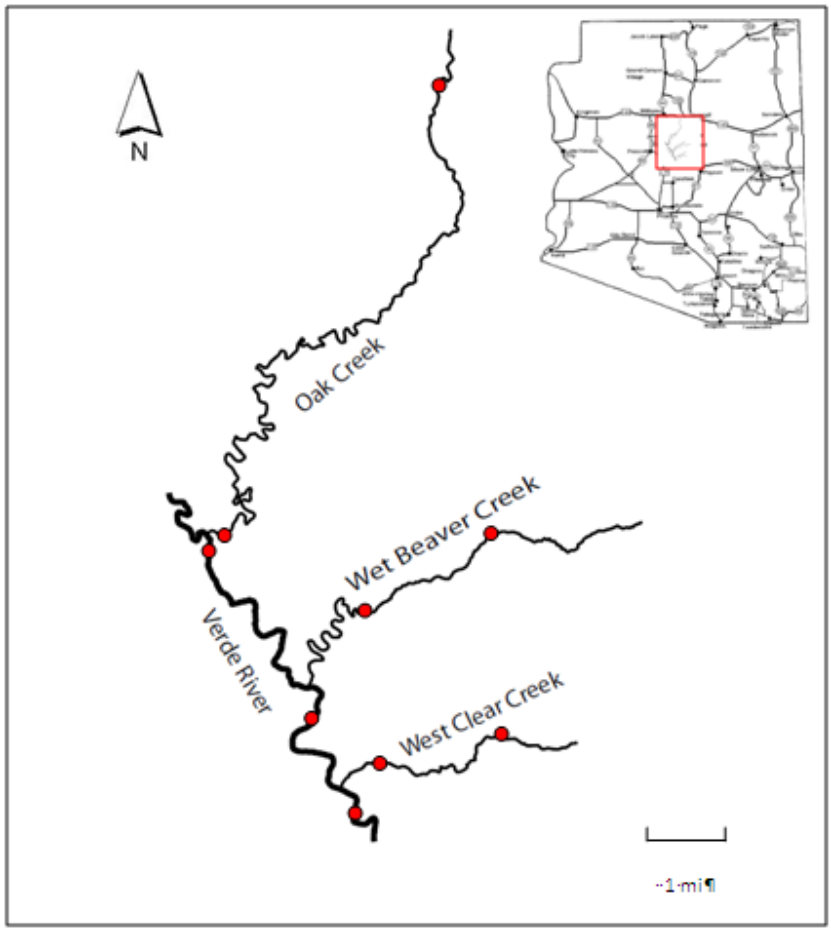

Figure 1. Study Location for Food Web Study in Arizona.

Principal Findings and Significance

We were able to complete our first two objectives, which were to establish a laboratory for environmental Hg analysis and developing the techniques to detect movement of Hg in aquatic food webs. We also made significant progress on the third objective, which was to make accurate measurements of Hg isotope ratios.

Objective 1. Establish a Laboratory for Environmental Mercury

Pooling a variety of funding sources, which included this project, enabled us to acquire and make operational a Teledyne/Leeman Hydra-C total Hg analyzer. Table 1 provides a summary of the performance of the instrument over an operational period of about a year. The materials analyzed were standard reference materials (SRMs) from certified sources and selected to reflect the

composition of the materials we analyze most commonly: sediments, plant material, and fish tissue. DORM-3 and MESS-3 are from the National Research Council Canada, Institute for National Measurement Standards; IAEA-336 is from the International Atomic Energy Agency in Seibersdorf, Austria; and SRM 1547 and SRM 2702 are from the US National Institute of Standards and Technology. Shown in the table are the standard deviation of the mean (Stdev); relative standard deviation (RSD), which is the standard deviation divided by the mean and reported as a percent; and error, which was the arithmetic difference between the certified and mean measured valued divided by the average of the two values and reported as a percent.

Table 1. Analyses of Standard Reference Materials for Total Hg.

SRM	Material	n	Mean (ng/g)	Stdev (ng/g)	RSD	Certified (ng/g)	Error
DORM-3	Fish Protein	5	410	18.8	4.3%	382	7.1%
IAEA-336	Lichen	6	187	17.7	8.5%	200	6.5%
MESS-3	Marine Sediment	22	91.6	5.9	5.4%	91.0	0.7%
SRM 1547	Peach Leaves	19	37.2	6.3	11.7%	31.0	18.2%
SRM 2702	Marine Sediments	40	422	83.5	18.4%	445	5.1%

Instrument performance was excellent or acceptable for all materials except SRM 1547. Additionally, 10 to 20 percent of samples were analyzed in duplicate and duplicate precision was generally better than five percent relative percent difference (calculated same as error in Table 1). This instrument was used for measuring T-Hg for all samples in Objectives 2 and 3. We consider the instrument and laboratory fully operational, but are concerned about the relatively high error in the analysis of SRM 1547. In response, we continue to analyze high numbers of duplicate samples and SRMs to verify accuracy in measurements.

Objective 2: Track Mercury in Aquatic Food Webs

This objective was pursued by a graduate student in the Environmental Sciences and Policy program at NAU. The student worked closely with the stream ecosystem research group in Jane Marks's laboratory at NAU. Her objective was to evaluate differences in bioaccumulation of Hg in terms of the River Continuum Concept (RCC). This phenomenon in stream ecology considers shifts in energy sources from upstream to downstream in river continuums. RCC theory predicts that upstream regions should be dominated by decomposing leaf matter and other allochthonous

material as primary carbon sources. Downstream reaches should be dominated by the autothonous carbon sources of primary producers. Traditional carbon isotope ($\delta^{13}C$) and more innovative deuteurium isotope (δDorg) measurements were used to determine energy source in food webs, along with more direct measurements of leaf litter and mass of algae and biofilm at sampling locations.

More relevant to this report however, is the capability provided by this project to use the combination of T-Hg and nitrogen stable isotopes ($\delta^{15}N$) to infer bioaccumulation. Nitrogen isotopes have become recognized as reliable indicators of trophic position, or the position in a food web occupied by individuals (e.g., Fry, 2006). Table 2 summarizes the Hg and isotopic data collected as part of the graduate thesis work supported by this project.

Table 2. Food Web Data Collected in Arizona Stream Ecosytems.

	Hg (ng/g)			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			δD (‰)		
	n	Mean	Stdev	n	Mean	Stdev	n	Mean	Stdev	n	Mean	Stdev
Algae	32	30.8	9.1	29	-29.1	5.0	29	4.2	3.5	29	-229.1	25.3
Biofilm	10	22.6	4.3	10	7.3	4.4	10	0.5	0.5	10	-137.1	25.2
Leaves	40	56.3	5.5	40	-29.0	0.9	40	-0.2	1.5	40	-137.6	7.9
Ambloplites rupestris – Rock bass	6	482.9	156.3	6	-24.0	1.0	6	14.7	1.2	6	-142.0	12.1
Pomoxis annularis -- Crappie	2	214.0	42.3	2	-25.2	0.7	2	11.4	0.2	2	-178.7	6.7
Ictalurus punctatus – Channel catfish	1	420.5	-	1	-23.3	-	1	12.7	-	1	-135.4	-
Lepomis cyanellus – Green sunfish	15	496.3	251.1	15	-22.6	0.5	15	9.5	0.9	15	-119.3	10.6
Lepomis macrochirus – Bluegill sunfish	18	536.9	259.7	18	-23.0	10.3	18	9.9	5.2	18	-141.0	39.2
Micropterus dolomieui – Smallmouth bass	69	384.0	266.1	67	-23.4	1.6	67	11.0	2.3	67	-143.5	15.9
Micropterus salmoides – Largemouth bass	5	737.3	281.9	5	-23.6	1.1	5	13.0	1.2	5	-127.9	24.6
Oncorhynchus mykiss – Rainbow trout	12	73.9	7.2	10	-17.9	1.2	10	11.1	0.4	10	-116.6	10.1
Pylodictis olivaris – Flathead catfish	19	606.3	415.3	19	-24.3	0.9	19	11.4	2.4	19	-160.5	23.5
Salmo trutta – Brown trout	12	138.0	138.0	12	-25.4	-25.4	12	10.9	10.9	12	-149.8	-149.8

A complete analysis of the data are provided in Hermosillo (2010), however an important result of this research was that differences in Hg bioaccumulation between nearby ecosystems were revealed. Figure 2 shows plots of T-Hg versus $\delta^{15}N$ for the downstream sampling locations in two of the streams sampled. The data show that in both systems Hg concentrations increase with trophic position, providing at least partial confirmation that the ultimate Hg sources are diffuse, rather than point sources. Point sources of Hg, such as mines or industrial waste, would likely result in high concentrations of Hg without systematic increases with trophic position.

More importantly though, the data show that the food web in Oak Creek concentrates less Hg at high trophic positions than in Wet Beaver Creek. With relevance to fish specifically, for a given trophic position, there are lower concentrations of Hg in fish from Oak Creek than from Wet

Beaver Creek. It is not yet clear whether this difference is due to differences in the supply of Hg to the streams, differences in the methylation of Hg between the streams, or differences in the trophic structures of the streams.

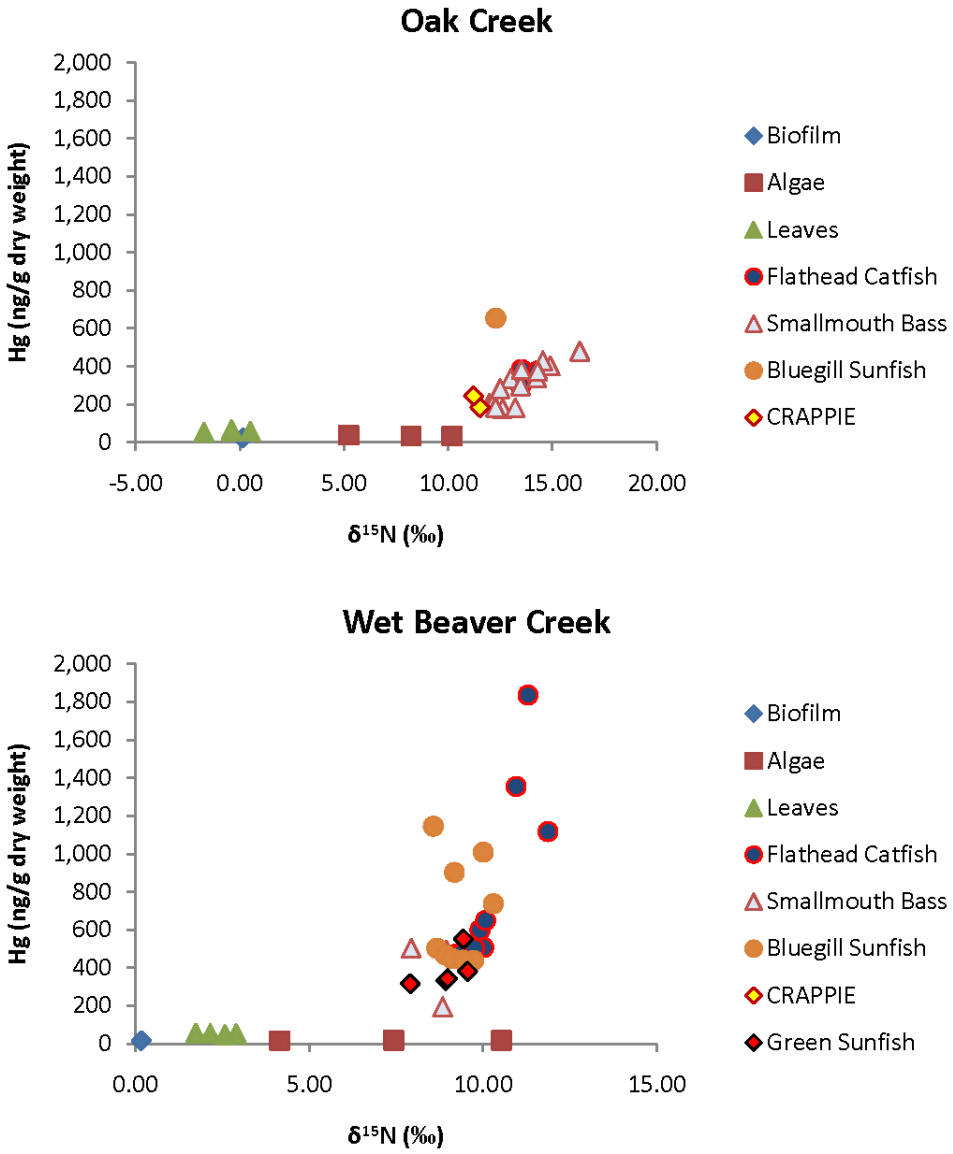

Figure 2. Mercury Concentration (T-Hg) Versus Nitrogen-15 ($\delta^{15}N$) for Food Webs in Two Arizona Streams (From Hermosillo, 2010).

Much work has yet to be done with these data, however this study does demonstrate that we have developed the techniques to detect relationships between trophic position and Hg

bioaccumulation in arid lands aquatic ecosystems. An important next step is to be able to address more directly the question of Hg sources.

Objective 3: Measure Mercury Stable Isotopes

Little debate remains that the major source of Hg in fish in most aquatic ecosystems is atmospheric (Grigal, 2003), yet the ability to detect the relative contributions of Hg from different sources has been elusive. Because Hg is volatile and readily forms Hg vapor in combustion sources, the potential exists for global transport of Hg. Indeed, coal-fired power plants in rapidly developing Asian countries have been blamed for Hg deposition in the western United States (e.g., Hope, 2006). Mercury stable isotopes may provide a direct indication of sources, if the various sources of Hg in the environment, for example the native geologic material, coal used by local sources, and coal used by more distant sources, have sufficiently distinctive isotopic ratios.

Mercury has seven stable isotopes: ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg. Instrumentation and methods now exist to measure all seven of these isotopes simultaneously for a given sample. Our objective was to adopt these techniques, acquire the apparatus necessary to introduce the samples into the MC ICPMS at NAU, and make isotope ratio measurements at NAU. To date we have worked with colleagues at Trent University in Onario, Canada. At their laboratory, the principal investigators were able to make measurements on samples collected from two Arizona ecosystems. Table 3 shows quality assurance data for the sample runs.

Table 3. Quality Assurance Data from Hg Isotope Analytical Runs.

Almaden	δ^{199}Hg (‰)	δ^{200}Hg (‰)	δ^{201}Hg (‰)	δ^{202}Hg (‰)
Sample 1	-0.08	-0.25	-0.42	-0.63
Sample 2	-0.20	-0.35	-0.56	-0.64
Mean	-0.14	-0.30	-0.49	-0.63
Blum and Bergquist (2007)	-0.14	-0.27	-0.44	-0.54
RPD	2.2%	9.5%	11.4%	16.1%

The reference material, Almadén, was an in-house reference material currently in use at the University of Michigan laboratory of Dr. Joel Blum. In the absence of certified reference materials, it is used as an uncertified reference material in the Hg isotope community. Precision was good or acceptable, except for the δ^{202}Hg observations.

The notation for Hg stable isotopes uses the customary *del* notation and in most current literature is expressed relative to the Mass-198 isotope (^{198}Hg). The standard being adopted for comparison in the del notation is NIST 3133 (Bergquist and Blum 2007) using the following formula:

$$\delta^{xxx}Hg = \left\{\left[\left(\frac{^{xxx}Hg}{^{198}HG}\right)_{unknown} / \left(\frac{^{xxx}Hg}{^{198}HG}\right)_{SRM\ 3133}\right] - 1\right\} x1000$$

Table 4 shows a summary of analytical results. Total Hg (T-Hg) was analyzed in our laboratory at NAU. Isotopic analyses were conducted in the Hintelmann laboratory at Trent University, Ontario, Canada.

Table 4. Hg Isotopic Analysis of Inorganic and Biotic Material from Arizona Ecosystems.

	T-Hg (ng/g)	δ^{199}Hg (‰)	δ^{200}Hg (‰)	δ^{201}Hg (‰)	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{200}Hg (‰)	Δ^{201}Hg (‰)
Upper Lake Mary - Upper Core	207	-0.31	-0.71	-1.12	-1.57	0.08	0.07	0.04
Upper Lake Mary - Mid Core	92	-0.30	-0.67	-1.05	-1.43	0.09	0.07	0.08
Parker Canyon Lake	101	-0.43	-0.48	-1.05	-1.18	-0.14	0.11	-0.17
Parker Canyon Soil 104-A	43	-0.12	-0.20	-0.50	-0.63	0.04	0.10	-0.01
Parker Canyon Soil 104-B	28	-0.36	-0.54	-0.97	-1.14	-0.06	0.03	-0.10
Parker Canyon Soil 104-D	17	-0.24	-0.42	-0.73	-0.85	-0.02	0.01	-0.08
Upper Lake Mary Fish 28	2,337	1.84	0.03	1.36	-0.07	1.87	0.07	1.43
Upper Lake Mary Fish 34	897	1.79	0.13	1.37	0.02	1.80	0.13	1.38
Upper Lake Mary Fish 63	747	1.61	0.17	1.34	0.16	1.57	0.09	1.22

An important aspect in the study of stable isotopes is the concept of *fractionation*. This is a change an isotopic ratio due to a physical or biological process. In heavy isotopes, such as strontium (Sr) and lead (Pb), as well as Hg, there is a fractionation process associated with the mass of the isotope itself. This is known as *mass-dependent fractionation* (MDF) and can be accounted for as essentially a data processing step. Researchers have discovered that for the Hg isotopic system, a second fractionation process occurs, but only in the odd numbered isotopes (^{199}Hg and ^{201}Hg). This has been termed mass-independent fractionation (MIF).

MIF can be calculated as the difference between the observed isotope ratio and the theoretical MDF (Blum and Bergquist, 2007):

$$\Delta^{xxx}Hg = \delta^{xxx}Hg - \left(\delta^{202}Hg\ x\ F\right)$$

Where xxx is the mass of the isotope and F is a fractionation factor (See Blum and Bergquist (2007) for a listing of these factors.

These separate phenomena have the potential to increase the power of Hg stable isotopic analysis in making inferences regarding sources and processes of transfer and transformation, but require levels of control on sampling and analytical procedures. The samples from Table 4 are consistent with values reported for similar materials in other studies. The phenomenon of mass-independent fractionation is particularly evident as Hg moves through food webs and can be seen in the fish samples. This can be seen in Figure 3, which plots δ^{199}Hg (a MIF isotope) versus δ^{202}Hg (a MDF isotope).

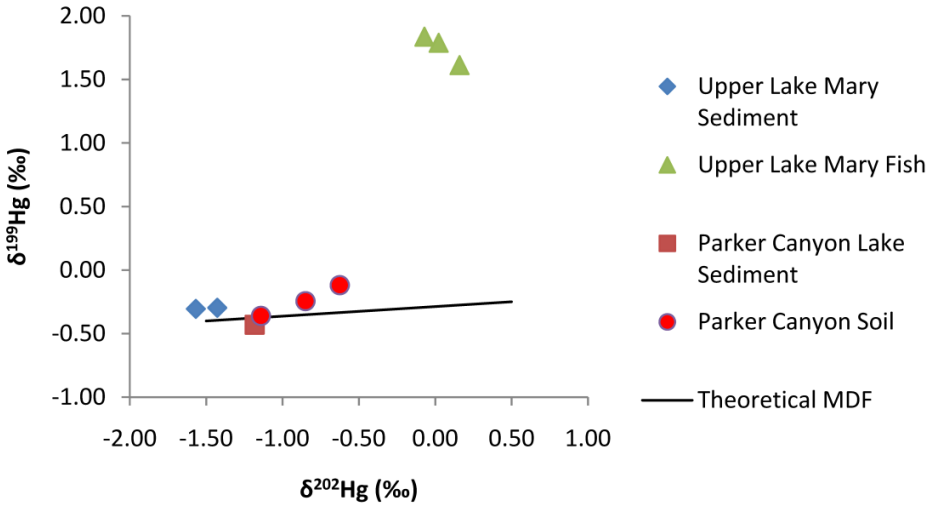

Figure 3. Mercury Stable Isotope Analysis of Soils, Sediments, and Fish from Arizona Ecosystems.

The solid line in Figure 3 shows the theoretical MDF. The fish show a distinct departure from the theoretical MDF value. The cause of this fractionation is not yet well understood, but in a study in the Arctic, Gantner and others (2009) found that the magnitude of the departure of the MDF from the theoretical value was consistent across a region and that an offset could be applied to estimate the isotope ratios of Hg in lower trophic level and the sediment. This has the potential value of being able to use Hg isotopic ratios from fish tissue alone to infer sources, rather than reconstructing isotopic ratio studies of entire food webs.

At this point we have successfully applied techniques for analyzing Hg stable isotopes and have reasonable, but limited, data from Arizona ecosystems. We have acquired a specialized apparatus for introducing samples into the MC ICPMS at NAU, but have not yet tested it.

In summary, this project, in conjunction with research funded by TRIF, and in close cooperation the stream ecosystem group led by Jane Marks at NAU, have enabled NAU to develop the capability to track Hg movement through aquatic ecosystems in arid lands. We now have all of the laboratory instrumentation necessary to make further investigations in total Hg and Hg stable isotopes and have developed strong research collaborations both with a leading Hg isotope laboratory as well as within NAU among the Chemistry, Environmental Engineering, Biology, Environmental Sciences faculty. Importantly, this project enabled funding for a master's student to develop skills in aquatic ecology, while providing crucial initial data for our research effort.

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