



Mercury Bioaccumulation in Pacific Giant Salamanders from Lotic Ecosystems in the Pacific Northwestern United States

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BACKGROUND

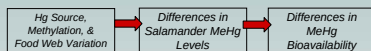
Why study mercury in the Pacific Northwest?

- Methylmercury (MeHg) contamination in the U.S. is well-documented (USEPA 2001) and continues to be a public-health issue of great concern (Schober et al 2003, Mahaffey et al. 2004). Mercury (Hg) has no beneficial role in metabolic function, and Hg accumulation has no known benefit to biota (Eisler 2000).
- Despite the documentation of worldwide amphibian declines Hg contamination of amphibians inhabiting stream ecosystems has received little attention, especially in lotic environments from the Pacific Northwest. These species, however, may be widely distributed across watersheds, are possible vectors of transport into the surrounding riparian zone and uplands, and comprise an energy base on which higher trophic levels in riparian ecosystems depend. Although fish are sentinels for monitoring levels of Hg contamination relative to human health in many states, salamanders are often less expensive to collect and may be a more efficient biomonitor of aquatic system health, since they use local resources and may be less transient (Bank et al. 2005).

OBJECTIVES & HYPOTHESES

The specific project objectives are to:

1. Examine and compare geographic patterns of Hg and MeHg in stream water, sediment, riparian soils, larval giant salamanders (Cope's Giant Salamander - *Dicamptodon copei*, WA, California giant salamander - *Dicamptodon ensatus*, CA and Pacific Giant Salamander - *Dicamptodon tenebrosus*, CA), and stream invertebrate prey items from the same streams.
2. Analyze potential relationships between Hg and MeHg levels in the collected surface water, sediment, salamanders, stream (i.e., water chemistry), and watershed-scale conditions. We also evaluate effects of current and legacy mining on Hg speciation and bioavailability.
3. Compare Hg and MeHg levels in salamanders with variations in trophic complexity ($\delta^{15}N$) and energy flow ($\delta^{13}C$).



STUDY AREA

Samples were collected in coastal forested watersheds distributed along a latitudinal gradient throughout the Pacific Northwest and included headwater stream sites in Olympic National Park, WA ($n=3$), Marin County, CA ($n=3$), and Redwood National and State Parks, CA ($n=4$). For comparative purposes we also collected samples from Whiskeytown National Recreation Area (NRA) and vicinity ($n=2$), including a watershed with a known historical gold mining site, on adjacent BLM land, where Hg contamination is substantial and widespread.

History of Mining at French Gulch

The French Gulch mining district resides along the Shasta-Trinity County line in the general vicinity of the town of French Gulch. It has been the largest gold-producing area in the Klamath Mountains province of southwestern Oregon and northwestern California. French Gulch was named for the French Canadians who mined gold here in 1849, from whom the town received its name in 1856. Clear Creek yielded large amounts of placer gold at this time. The Washington Mine, discovered in 1852, was the first quartz mine worked in Shasta County. From around 1900 to 1914, the output for the district averaged between \$300,000 and \$500,000 worth of gold per year. There was some activity during the 1920s and 1930s, and there has been minor prospecting until fairly recently with the reopening of the Washington Mine by the Bullion River Gold Corporation. Total production has been estimated to be over \$20,000,000. As a supply and stopping place, this town was located on the California-Oregon Trail and the it rivaled Shasta in its day. Sniping is common in the area.

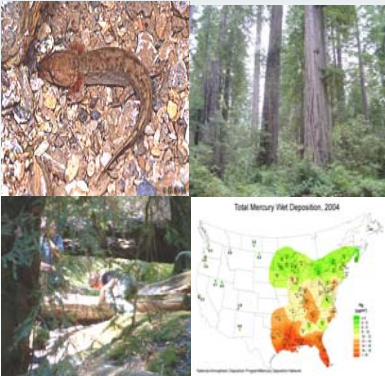


Figure 1. Clockwise - Pacific giant salamander (*Dicamptodon* spp.) larvae photo, Redwood National Park study area (photo), 2004 Total Hg deposition map (USGS-NADP-MDN, 2004), and stream field sampling (photo).

METHODS

Salamander Collection, Measurements & Storage

Using sterile, powder free, gloves, salamander larvae ($n = 60$) were collected opportunistically with dip nets or a standard aquatic d-ring net. Prior to sacrificing the salamander, larvae were retained in an individual clean plastic bag and the following measurements were recorded: a) total length (cm), b) snout-vent length (cm), and c) tail length (cm). We recorded the UTM coordinates of the location where the animal was captured and the elevation (meters) if possible. We used tricaine methanesulfonate (FINQUEL-MS-222) to kill salamander larvae in a humane way approved by the Harvard Medical Animal Use and Care Committee (IACUC). Larvae were killed using a lethal dose of MS-222 (650-850 mg/L buffered with sodium bicarbonate - at approximately equal levels), thoroughly rinsed with stream water, and then placed in an individual, labeled (date of collection, park name, stream name, and individual identification number) sterile plastic bag to be frozen prior to laboratory analysis.

General Stream Chemistry

Using a YSI-meter (Model 556, YSI, Inc., Yellow Springs, OH) we recorded the following water chemistry variables at 3 random locations (≥ 25 m apart) on the stream: a) pH, b) dissolved oxygen, c) temperature ($^{\circ}C$), d) redox potential (mV) and, e) specific conductance ($\mu S/cm$).

Total Hg and Stable Isotope and Determination

PGS samples were freeze-dried and analyzed following EPA Method 7473 for Hg analysis by thermal decomposition, amalgamation, and atomic absorption spectrophotometry using a Milestone DMA-80 direct Hg analyzer. Wet weights and dry weights (i.e., after freeze drying) of individual, sacrificed larvae were recorded in the laboratory (i.e., post-death). Stable isotope analyses were conducted at the Boston University Stable Isotope facility.

Statistical Modeling

We used linear regression to test for trends between snout-vent length and Hg concentration. Box-plots, Student's t -tests and one-way analysis of variance were used to compare Hg averages across sites and parks.

RESULTS

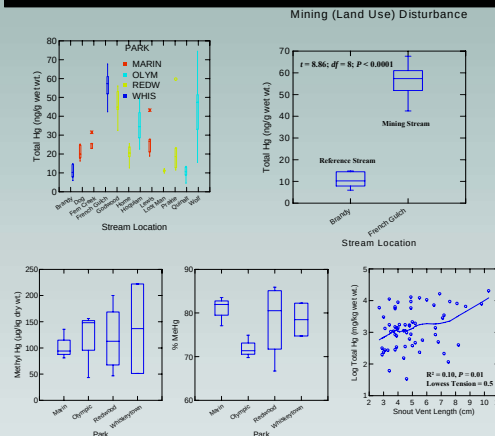


Figure 2-6. Figure 2 (upper left) Box-plot of Hg in PGS larvae from west coast DOI lands, September 2005. Figure 3 (upper right). Comparison of total Hg concentration in PGS larvae from reference (Whiskeytown NRA) and mining sites (adjacent BLM land), September 2005. Figure 4 (lower left). MeHg concentration (dry wt. basis) in PGS composites pooled by stream and plotted by park. Figure 5 (middle left) Percent MeHg in PGS composites pooled by stream and plotted by park, September 2005. Figure 6 (right left). Scatter-plot of Hg concentration in PGS individuals and snout-vent-length (SNV), September, 2005. Lowess smoother (0.5 tension) used to show fine scale changes in the overall SNV-Hg trend.

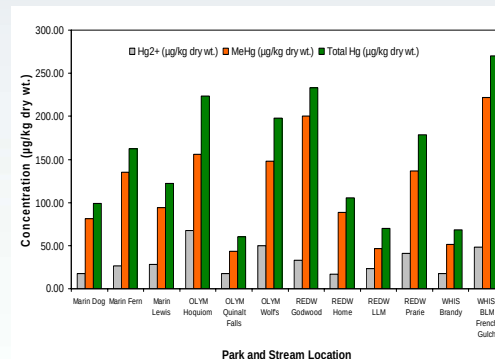


Figure 7. Broad-scale distribution of Hg^{2+} , MeHg and Hg in PGS larvae composites from the selected Pacific Northwest DOI lands, September, 2005. Data in graph presented on a dry wt. basis. French gulch is a known contaminated stream, on BLM land, with active and historic mining within the catchment.

RESULTS

Table 1. Water chemistry characteristics from the 12 selected study stream sites from DOI lands in the Pacific Northwest, September, 2005. Values represent mean ($\pm$ SE) from three replicate samples per stream.

PARK			Temperature (C°)	Dissolved Oxygen (mg/L)	Redox (ORP-mV)	Specific Conductance (µS/cm)
Stream Name	pH					
OLYMPIC NP						
Wolf's	7.28	10.90	12.72	191.23	52.00	
	(0.068)	(0.01)	(0.143)	(3.15)	(0.00)	
Hoquiam	7.09	11.72	11.06	201.03	51.00	
	(0.134)	(0.01)	(0.15)	(6.85)	(0.00)	
Quinalt Falls	7.12	12.06	9.88	194.50	33.00	
	(0.08)	(0.01)	(0.23)	(2.95)	(0.00)	
MARIN						
Fern	7.89	14.03	12.83	166.80	146.33	
	(0.03)	(0.11)	(0.69)	(0.99)	(0.667)	
Dog	7.96	13.63	10.93	156.53	250.00	
	(0.01)	(0.07)	(0.17)	(1.11)	(0.58)	
Lewis	7.72	14.77	9.11	157.63	240.33	
	(0.03)	(0.11)	(0.06)	(1.75)	(1.67)	
REDWOOD NP						
Little Lost Man	6.74	12.77	9.64	168.67	54.00	
	(0.01)	(0.00)	(0.06)	(0.76)	(0.00)	
Godwood	7.39	11.90	10.07	151.10	92.33	
	(0.00)	(0.01)	(0.12)	(0.25)	(0.33)	
Prairie	7.39	13.19	9.40	153.57	69.00	
	(0.10)	(0.05)	(0.19)	(3.17)	(0.00)	
Home	7.44	12.24	10.07	144.73	101.33	
	(0.06)	(0.00)	(0.17)	(3.09)	(0.33)	
WHISKEYTOWN NRA						
Brandy	6.75	13.84	9.73	181.97	47.00	
	(0.11)	(0.00)	(0.21)	(4.73)	(1.00)	
French Gulch (BLM Land)	7.95	16.52	10.15	131.87	127.00	
	(0.03)	(0.02)	(0.19)	(4.90)	(0.00)	

Table 2. Stream chemistry characteristics, PGS Hg speciation data, total length, and stable isotope from Brandy creek (Reference – WHIS, NRA) and French Gulch (Mining Land Use - BLM) in central California, September, 2005. Values represent mean ($\pm$ SE) when available. Composite samples represent value from one sample only.

Parameter	French Gulch (Mining Land-Use)	Brandy Creek (Reference)
Total Hg (ng/g wet wt. PGS Individual)	56.08 (4.27)	10.69 (1.76)
Total Hg (ng/g dry wt. PGS Composite)	270.19	68.64
Hg ²⁺ (ng/g dry wt. PGS Composite)	48.01	17.35
MeHg (ng/g dry wt. PGS Composite)	222.18	51.29
% MeHg (PGS Composite)	82.23	74.73
Total Hg Water (ng/L)	5.32	1.24
pH	7.95 (0.03)	6.75 (0.11)
Redox - ORP (mV)	131.87 (4.90)	181.97 (4.73)
Temperature ($^{\circ}C$)	16.52 (0.02)	13.84 (0.00)
Dissolved Oxygen (mg/L)	10.15 (0.19)	9.73 (0.21)
Specific Conductance ($\mu S/cm$)	127.00 (0.00)	47.00 (1.00)
PGS Total Length (cm)	8.72 (0.13)	12.12 (0.62)
PGS Larvae $\delta^{15}N$ ($n = 5$)	3.64 (0.01)	1.43 (1.22)
PGS Larvae $\delta^{13}C$ ($n = 5$)	-25.61 (0.09)	-22.39 (0.19)

CONCLUSIONS & FUTURE WORK

1. Overall, despite their large size, Hg levels were low to moderate in PGS larvae (Figure 2). No water chemistry variables (Table 1) were correlated ($r < 0.58$, $P > 0.05$) to the Hg or MeHg concentrations in PGS larvae. Hg in water samples increased with increasing stream temperature ($R^2 = 0.51$, $P < 0.05$). Hg in PGS larvae was significantly different when categorized by stream site ($F = 12.65$; $df = 11, 48$; $P < 0.001$; Figure 2). No observed difference in PGS larvae Hg concentration was detected among parks ($F = 0.12$; $df = 3, 56$; $P > 0.05$; Figure 2).
2. The west coast Hg and MeHg distribution survey data (Figure 2-5) and the snout-vent length-Hg scatter-plot (Figure 6) show that PGS Hg and MeHg concentrations were highest at the French Gulch (Figure 2-5, Figure 7) site, where mining has occurred. Mercury also increased with increasing snout-vent length (Figure 6) of PGS larvae. Therefore, total Hg bioaccumulation in PGS larvae appears to be influenced by both body size and local watershed conditions that exert controls on stream biogeochemical and food web processes likely created by the spatial and temporal patterns and effects of mining land use practices.
3. At French Gulch, PGS Hg and MeHg bioavailability were approximately 500% higher (Hg t -test on individuals, $P < 0.0001$) in comparison to the reference stream (Brandy), and this was in accord with a similar % increase in Hg in surface water (Table 2). We also detected significantly higher $\delta^{15}N$ values in PGS larvae from French Gulch potentially indicating that the sampled individuals fed at a higher trophic position, were older, or had higher $\delta^{15}N$ due to enrichment of nitrogen from the mining wastes in comparison to larvae from the reference stream (Brandy). However, evaluations of between site variation in $\delta^{15}N$ using baseline corrected nitrogen isotope data should be undertaken to confirm these findings.
4. Future work will analyze sediments, invertebrates, and water methylation efficiency (MeHg/Hg). Watershed land cover geomorphology and stream chemistry modeling is also planned. Additional trace metals analyses are planned for PGS tissue samples including copper and cadmium analyses. Stable isotope analyses will also be conducted using baseline corrected $\delta^{15}N$ data.

Literature Cited: Need to add.