Mercury in Tadpoles Collected from Remote Alpine Sites in the Southern Sierra Nevada Mountains, California, USA

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Abstract Amphibians in alpine wetlands of the Sierra Nevada mountains comprise key components of an aquatic-terrestrial food chain, and mercury contamination is a concern because concentrations in fish from this region exceed thresholds of risk to piscivorous wildlife. Total mercury concentrations were measured in whole tadpoles of the Sierra chorus frog, *Pseudacris sierra*, two times at 27 sites from high elevations (2786 – 3375 m) in the southern Sierra Nevada. Median mercury concentrations were 14 ng/g wet mass (154 ng/g dry mass), which were generally low in comparison to tadpoles of 15 other species/location combinations from studies that represented both highly contaminated and minimally contaminated sites. Mercury concentrations in *P. sierra* were below threshold concentrations for risk to predaceous wildlife. Concentrations in tadpoles were also lower than those observed in fish in the study region presumably because tadpoles in the present study were much younger (1-2 mo) than fish in the other study (3-10 years old), and tadpoles represent a lower trophic level than fish. Mercury concentrations were not related to distance from the adjacent San Joaquin Valley, a source of agricultural and industrial pollutants.

Keywords Pacific chorus frog, *Pseudacris sierra*, Amphibian, High elevation, Tadpole, Mercury

The Sierra Nevada mountains of California (hereafter, Sierra Nevada) lie downwind from major regional sources of airborne pollutants from agriculture, industry, and transportation, and the mountains may also receive pollutant inputs from trans-Pacific sources and the global atmospheric pool (Cahill et al. 1996; Landers et al. 2008). Atmospherically transported pollutants at high elevation (e.g., >2750 m) in the Sierra Nevada include both historic- and current-use pesticides, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and metals (e.g., Cahill et al. 1996; Landers et al. 2008; Bradford et al. 2010). Of particular concern, mercury (Hg) concentrations in brook trout (*Salvelinus fontinalis*) at high elevation have exceeded thresholds of risk to piscivorous wildlife (Schwindt et al. 2008). Moreover, mercury was associated with tissue damage in the kidneys and spleen of these fish, as indicated by increases in macrophage aggregates, suggesting that Hg or another pollutant has affected fish health (Schwindt et al. 2008).

Amphibians often serve as vital links for energy and nutrient flow between lower and higher trophic levels, and they may also be important in transferring contaminants from aquatic to terrestrial food webs (Bergeron et al. 2010). Amphibians have historically been nearly ubiquitous among the abundant water bodies at high elevation in the Sierra Nevada (Vredenburg et al. 2007). At least three amphibians, the Sierra chorus frog (*Pseudacris sierra)*, the southern mountain yellow-legged frog (*Rana muscosa*), and the Sierra Nevada yellow-legged frog (*Rana sierrae*), are important components of an aquatic-terrestrial food web with the garter snakes (*Thamnophis couchi* and *T. elegans*) as the primary top carnivore (e.g., Knapp 2005). Indeed, the occurrence of garter snakes in this area is highly dependent on the occurrence of these prey species, and there is no evidence that the snakes switch to other aquatic or terrestrial prey in the absence of aquatic amphibians (e.g., Knapp 2005). Unfortunately, populations of two of these species (*R. muscosa* and *R. sierrae*) have dramatically declined in recent decades throughout their range in the Sierra Nevada (Vredenburg et al. 2007).

The present study examines Hg concentrations in tadpoles of *P. sierra* because the abundant and widespread populations of this species allow us to address two objectives. First, we evaluate total Hg concentration in *P. sierra* relative to tadpoles of other species elsewhere and relative to thresholds of concern to predatory wildlife (Lazorchak et al. 2003). If Hg concentrations are high relative to other species/locations or concentrations exceed predator thresholds, concern would be raised that Hg may be affecting other wildlife and contributing to regional amphibian population declines. Second, we evaluate the spatial distribution of Hg in the study region because it is unknown whether certain areas tend to have higher Hg concentrations than others (i.e., “hotspots”). Specifically, we test the hypothesis that Hg at high elevation is related to distance from the adjacent San Joaquin Valley. During much of the year air reaching high elevations in the Sierra Nevada passes through the San Joaquin Valley and receives pollutant inputs from agricultural and industrial sources (including coal-fired power plants) in the Valley and the San Francisco Bay area (Hayes et al. 1984; Shair 1987; Ewell et al. 1989). Within the southern Sierra Nevada, the general geographic pattern for some atmospherically deposited organic pollutants is a decrease in concentration with distance from the San Joaquin Valley up to about 40 km, beyond which elevations are high (e.g., >2750 m) and concentrations remain static or decrease relatively little (Bradford et al. 2010).

Materials and Methods

In the Sierra Nevada at high elevation, *P. sierra* females oviposit in water bodies within days of ice-off, and tadpoles develop over a period of approximately 2-3 months before completing metamorphosis (unpublished data). Tadpoles feed primarily by removing material from benthic, rock, or plant surfaces, but will scavenge dead animal matter when available (unpublished data). We collected tadpoles of *P. sierra* from two water bodies > 200 m apart from each of 14 areas (with one exception, yielding total of 27 sites) dispersed throughout the high-elevation (>2750 m) portion of Sequoia and Kings Canyon National Parks, California (see map of site locations in Bradford et al. 2010). Sampling was conducted during two periods (Period 1, 30 July to 12 August 2005; Period 2, 29 August to 12 September 2005) to capture much of the developmental time of pre-metamorphic tadpoles. Elevation at sampling sites averaged 3219 m (range 2786 to 3375 m), water pH averaged 6.3 (range 5.0 – 7.4), and electrical conductivity averaged 12 µS/cm (range 1 – 127) (Bradford et al. 2010). We calculated two metrics to represent the distance for each sampling site to the San Joaquin Valley (Bradford et al. 2010). First, linear distance (measured using Arc Map 9.2; ESRI) is the distance to the closest point on the mountain-valley boundary, defined as the boundary between mountain slopes and the relatively flat valley, roughly following certain contour levels but smoothed to eliminate prominent lateral deviations (e.g., river valleys). Second, upslope distance was calculated using Arc Info (ESRI) as the path that runoff water would follow from the site to the mountain-valley boundary. Upslope distance was used as a surrogate for the flow path taken by daily upslope/downslope winds common in the southern Sierra Nevada during summer (Shair 1987; Ewell et al. 1989). Linear distance for the sampled sites ranged from 42.9 to 82.5 km and upslope distance ranged from 59.6 to 187.3 km) (Bradford et al. 2010).

Tadpoles were collected by hand or dip net using clean, powder-free latex gloves and placed in plastic bags filled with water from the collection site. A median of 11 (range 5-75) tadpoles were transferred to a 25-ml certified pre-cleaned glass vial with Teflon™ lined cap and placed on dry ice. Tadpoles at metamorphic stages (i.e., Gosner 1960 stages > 41) were excluded. Duplicate sampling frequency was 10%. Vials were stored on dry ice or in a freezer at -20^{o}C until analysis. Median tadpole stage (Gosner 1960) was determined from a sample of approximately 16 tadpoles collected simultaneously and used for other analyses

(Bradford et al. 2011). Details for site selection, site characteristics, and sampling methods are provided in Bradford et al. (2010).

In the laboratory tadpole samples were homogenized using a Kinematica® Polytron PT1200E (Lucerne, Switzerland) handheld homogenizer for approximately 2 min. A microwave oven, Anton-Parr Multiwave™ 3000 (Graz, Austria), with Teflon™ vessels was used in a microwave-assisted acid digestion of tissues. Approximately 4 g of ground tadpole tissue (equivalent to a median of 4 tadpoles; range 2 - 46) were combined with 4 mL HNO_3 and 4 mL dionized H_20 and microwaved with increasing power to approximately 1200 W and 160 ^oC.

Detailed methods used to analyze the tadpole tissue for Hg can be found in (Kramer and Gerstenberger 2010). In short, total mercury was analyzed in accordance with U.S. Environmental Protection Agency (EPA) Method 245.6 (One EPA 1991) using a PerkinElmer® Flow-Injection Mercury System 100 (FIMS 100) (Sheldon, Connecticut, USA). equipped with an AS-91 autosampler using the flow-injection mercury cold-vapor technique. The instrument detection limit is reported to be 0.2 parts per billion (ppb). The method detection limit (MDL) was calculated to be 0.010 µg/g (10 ppb)

.Three replicates were performed on each sample and an average of the three measurements was reported. To compare Hg concentrations with studies that reported concentrations on a dry mass basis, tadpole moisture content was determined by oven drying a subsample (approx. 0.5 g) of the homogenized tadpoles.

Quality assurance and quality control were ensured by performing a calibration blank each day prior to analysis. Calibration standard solutions were prepared from 1000 ug/mL Hg in 5% HNO_3 JT Baker® stock reference solution (Phillipsburg, New Jersey, USA) by serial dilution. A 0.995 or higher correlation coefficient was considered acceptable for the calibration curve. Each microwave digestion tray contained 16 samples including a reagent blank and two certified standard reference materials: National Research Council Canada DORM-3 dogfish muscle tissue (Ontario, Canada) and National Institute of Standards and Technology Standard Reference Material® (SRM) 1946 Lake Superior Fish Tissue (Gaithersburg, Maryland, USA). For further assurance, 10% of samples were randomly selected for duplicate analysis. A recovery between 80% and 120% of expected value was accepted for the SRMs, duplicate samples, and spiked samples. Samples were not recovery corrected.

For statistical analysis Hg concentration values below the estimated method detection limit were replaced with half this value (i.e., 0.005 µg/g). Values for concentration during Period 1 were not normally distributed (Shapiro-Wilks test) even if log-tranformed. Consequently, Spearman rank tests were used to test for correlations between concentration and distance from the San Joaquin Valley and tadpole stage Statistical analysis was conducted with SAS 9.3 (SAS Institute, Cary, North Carolina, USA).

Results and Discussion

Tadpoles increased in developmental stage from an average Gosner stage of 31.5 during Period 1 to 36.6 during Period 2, whole tadpole mass increased from an average of 0.58 g to 1.02 g between the two periods, and water content decreased from an average of 92.3% to 89.3% (Table 1, t-test $P < 0.0001$ in all cases). Tadpoles were absent during site visits approximately 30 d prior to the first sampling at each site when ponds were still snow covered or recently thawed; thus, tadpoles were ≤ 1 mo old during Period 1 and ≤ 2 mo old during Period 2.

Mercury concentrations were generally similar between the two sampling periods, although detection frequency was lower during Period 1 than Period 2 (Table 1). No geographic pattern for non-detects was apparent. Mercury concentrations in tadpoles were not significantly related to tadpole developmental stage or to whole tadpole mass during either sampling period (Spearman rank correlation tests, $P > 0.15$ in all cases). By contrast, results from some studies of other species showed both positive and negative associations (e.g., Bank et al. 2007; Weir et al. 2010).

Mercury concentrations in *P. sierra* tadpoles were generally low in comparison to tadpoles for 15 other species/location combinations in other studies (Table 2).

These studies collectively included highly contaminated sites and sites thought to have minimal contamination. Specifically, the overall mean concentration in the present study (16 ng/g wet mass) was low among eight species/location combinations reported on a wet-mass basis, whereas the overall mean (195 ng/g dry mass) in the present study was in mid range among eight species/location combinations reported on a dry-mass basis. Although these results are consistent with some degree of Hg contamination at the alpine sites in the present study, concentrations in tadpoles were well below maximum values observed at known contaminated sites. Specifically, concentrations in *P. sierra* averaged more than an order of magnitude less than the maximum site averages reported for *Bufo bufo* near a mercury mine in Slovenia (450 ng/g wet mass) and for *Anaxyrus americanus* among five sites along a contaminated river in Virginia (3930 ng/g dry mass; Table 2). To our knowledge, the body burden concentration that represents a harmful level is unknown for pre-metamorphic tadpoles of any species.

Average Hg concentrations in *P. sierra* tadpoles in the present study (16 ng/g wet mass) were below threshold concentrations in fish for risk to piscivorous wildlife (i.e., river otter, 100 ng/g wet weight; mink, 70 ng/g; and kingfisher, 30 ng/g; Lazorchak et al. 2003). However, four of the 54 tadpole samples in the present study exceeded the threshold for kingfishers. In contrast, whole fish (3–10 years old) sampled from two lakes 2-3 km from our nearest site in 2003 equaled or exceeded all consumption thresholds for these predatory wildlife (averaging approximately 100 and 110 ng/g wet mass in the two lakes; Landers et al. 2008; Schwindt et al. 2008). Greater Hg concentrations in fish compared to *P. sierra* tadpoles in alpine waters is not surprising given that Hg tends to bioaccumulate (Unrine et al. 2007), trout are higher in the food web (carnivores) than in *P. sierra* tadpoles (omnivores; unpublished data), and the fish sampled were 3 to 10 years old (Schwindt et al. 2008) whereas tadpoles were only 1-2 months old.

Although the dietary toxicity is unknown for the common snake predators of aquatic amphibians in the study region, our data suggest that consumption of *P. sierra* tadpoles poses low risk to snakes from mercury contamination. This might not be the case, however, for their consumption of other aquatic amphibians in the region because these amphibians likely accumulate mercury to a greater extent than *P. sierra* tadpoles. Specifically, adults of the frogs *Rana muscosa* and *R. sierrae* are carnivorous and can live for many years, and tadpoles of these species typically take two or more years to reach metamorphosis whereas *P. sierra* tadpoles metamorphose in a few months (Vredenburg et al. 2004; Matthews and Miaud 2007; DFB, unpublished data).

Mercury concentration in *P. sierra* tadpoles in the present study was not significantly related to either linear or upslope distance from the San Joaquin Valley during either sampling period (Spearman rank correlation, $p>0.25$ in all cases). Thus, there is no evidence for spatial structuring of Hg distribution at high elevation in the southern Sierra Nevada, i.e., > 43 km from the San Joaquin

Valley). This finding is generally consistent with that for pesticides, PCBs, and PAHs in *P. sierra* tadpoles, sediment, and air from the same sites (Bradford et al. 2010). For these compounds and media there was no general pattern of concentration as a function of distance metrics among these high elevation sites. Nevertheless, the possibility remains that Hg concentrations would be greater at lower elevations and closer to the San Joaquin Valley, as has been found for several pesticides (Bradford et al. 2010). In summary, there is no evidence that Hg contamination is a threat to *P. sierra* tadpoles or their predators, and there is no evidence for the existence of Hg "hot spots" at high elevation in the southern Sierra Nevada.

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Table 1 Characteristics and total mercury concentration in *Pseudacris sierra* tadpoles at high elevation during two sampling periods: 30 July to 12 August 2005 (Period 1) and 29 August to 12 September 2005 (Period 2). Multiple tadpoles comprised each sample (see text). Individual sample values for wet mass and water content are averages per tadpole, whereas sample values for stage represent the median.

	Period 1	**Period 2**
Wet Mass (g)	0.58 ± 0.08[a] (0.07-1.57)	1.02 ± 0.06 [a] (0.39-1.50)
Median Gosner Stage	31.5 ± 0.6[a] (26 - 37)	36.6 ± 0.4 [a] (30 – 39.5)
Water Content (%)	92.3 ± 0.5 [a] (84.4 - 96.9)	89.3 ± 0.3 [a] (86.6 - 94.1)
Total Hg (ng/g wet) [b]	13 [5 - 17] [c] (5 - 53)	16 [13 - 20] [c] (5 - 33)
Total Hg (ng/g dry) [b]	150 [70 - 247] [c] (61 - 1730)	151 [104 - 216] [c] (53 - 376)
Hg Detection Frequency (%)	74.1	96.3
Number of Sites (Samples)	27	27

[a] Values are mean ± SE (range)
[b] Hg concentration below the estimated method detection limit (MDL) were replaced with half of the MDL (i.e., 5 ng/g wet mass)
[c] Values are median [interquartile range] (range)

Table 2 Total mercury concentration in whole tadpoles.

Location	Context	Species	No. Sites	Mean Hg [a] (ng/g Wet)	Mean Hg [a] (ng/g Dry)	Study [c]
Acadia National Park, Maine, USA	Atmospheric Hg deposition from anthropogenic sources	*Lithobates catesbeianus*	3	19 (17-22)	----	(Bank et al. 2007)
"	"	*Lithobates clamitans*	6	25 (14-38)	----	"
Southern Illinois, USA	Upwind & downwind from coal-fired power plants; upwind/downwind Hg not significantly different	*Lithobates catesbeianus*	12	72	----	(Weir et al. 2010)
"	"	*Lithobates clamitans*	14	37	----	"
"	"	*L. catesbeianus* & *L. clamitans*	23	---- (16-75)	----	"
Cottage Grove Reservoir, Oregon, USA	Reservoir contaminated by mine drainage	*Lithobates catesbeianus*	1	< 20	----	(Curtis 2003)
Vicinity Idrija, Slovenia	Vicinity of mercury mine	*Bufo bufo*	1	450	----	(Bryne et al. 1975)
US Dept. of Energy Savannah River Site, South Carolina, USA	1 site undisturbed; 2 with agriculture before 1951; 1 metals-contaminated remediated	*Rana sphenocephalus*	4	< 0.2 (<0.2-<0.2)	----	(Burger and Snodgrass 2001)
Fox River, Wisconsin, USA	Tadpoles in enclosures along contamination gradient along river	*Rana clamitans*	3	16	93 (50-120)	(Karasov et al. 2005)

US Dept. of Energy Savannah River Site, South Carolina, USA	Wetland downstream from sluiced ash from coal-fired power plant	*Lithobates catesbeianus*	1	----	110 (median) [b]	(Unrine et al. 2007)
Axios Delta, Greece	Estuarine and delta complex; anthropogenic Hg input via rivers	*Hyla* sp.	----	----	840 (median)	(Goutner and Furness 1997)
"	"	*Rana ridibunda*	----	----	50	"
South River, Virginia, USA	Reference site upstream of contamination source	*Anaxyrus americanus*	1	----	540[b]	(Bergeron et al. 2010)
"	Contaminated sites along river	"	5	----	2130[b] (115-3830)	"
Lake Nkuruba, Uganda	Lake in conservation area	Unspecified species	1	----	40	(Campbell et al. 2006)
Southern Georgia, USA	Stork prey item from unknown specific location	Ranidae	1	----	<100	(Gariboldi et al. 1998)
Southern Sierra Nevada mountains, California, USA	High-elevation in national park	*Pseudacris sierra*	27	16 [d] (<10-37) [median=14]	195 [d] (67-975) [median=154]	Present study

[a] Hg concentrations are for mean among sites (range of site means)
[b] Tadpoles held 48 h to void gut contents
[c] Studies included had method detection limit < 20 ng/g wet mass or < 50 ng/g dry mass, if reported
[d] Hg concentrations differ slightly from values in Table 1 because values in present table are averaged by site.