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MERCURY BIOACCUMULATION IN GREEN FROG (*RANA CLAMITANS*) AND BULLFROG (*RANA CATESBEIANA*) TADPOLES FROM ACADIA NATIONAL PARK, MAINE, USA

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Abstract—Mercury contamination in the northeastern United States, including Acadia National Park (ANP; ME, USA), is well documented and continues to be a public health issue of concern. Mercury contamination of wild amphibians has received little attention, however, despite reports of worldwide population declines. Here, we report total Hg and methyl Hg (MeHg) concentrations for water, sediment, and green frog (*Rana clamitans*) and bullfrog (*Rana catesbeiana*) tadpoles (age, approximately one year) from ANP. Total Hg concentrations (mean ± standard error) in green frog and bullfrog tadpoles were 25.1 ± 1.5 and 19.1 ± 0.8 ng/g wet weight, respectively. Mean total Hg was highest for green frog tadpoles sampled from the Schooner Head site (ANP, Bar Harbor, ME, USA), a small, semipermanent beaver pond where Ranavirus was detected during the summer of 2003 sampling period. Methyl Hg comprised 7.6 to 40% of the total Hg in tadpole tissue (wet-wt basis), and mean total Hg levels in tadpoles were significantly different among pond sites ($n = 9$). Total Hg in pond water was a significant predictor of tadpole total Hg levels. Dissolved organic carbon was a significant predictor of both total Hg and MeHg in water, and total Hg in water also was strongly correlated with MeHg in water. Of the nine pond ecosystems sampled at ANP, 44% had a methylation efficiency (water MeHg to total Hg ratio) of greater than 10%, and 33% had total Hg levels in sediment that were approximately equal to or greater than the established threshold level effect concentration for freshwater sediments (0.174 mg/kg dry wt). Our data indicate that wetland food webs in ANP likely are susceptible to high levels of total Hg bioaccumulation and that methylation dynamics appear to be influenced by local abiotic and biotic factors, including disturbances by beavers and in situ water chemistry patterns. These findings may be important to National Park Service resource managers, especially considering the class I airshed status of ANP and the strong potential for negative effects to aquatic ecosystem structure and function from Hg pollution.

Keywords—Mercury Amphibian Bioaccumulation

INTRODUCTION

Mercury contamination in the northeastern United States, including Acadia National Park (ANP), is well documented [1] and continues to be a serious public health issue [2,3]. Fish consumption advisories as a result of elevated Hg levels have been issued in 48 states, because Hg contamination has been identified as a serious health risk for pregnant women, the developing fetus, and children [4]. Effects of this pollutant are not isolated to humans. Fish and amphibians from remote aquatic sites can bioaccumulate significant amounts of methyl Hg (MeHg) [5–7]. Mercury has no beneficial role in metabolic function and no known benefit to biota [8,9].

Atmospheric deposition is believed to be an important source of Hg in the northern United States, with deposition rates in the range of 5 to 10 μg/m²/year [10]. Wet deposition of Hg at ANP has occurred at a mean rate of 7.9 μg/m²/year since 1995 (Mercury Deposition Network [MDN], National Atmospheric Deposition Program [ANP, Hancock County, ME, USA; McFarland Hill, MDN site ME98; hhtp://nadp.sws.uiuc.edu/mdn/]). Accumulation rates of Hg to the sediment at two locations in Acadia were 100 to 200 μg/m²/year during the 1980s, suggesting that a large amount of dry Hg input is not measured by the wet-only MDN collector [11]. These rates are comparable to those reported from urban lakes and, presumably, reflect deposition of Hg from upwind sources (e.g., the metropolitan regions and solid waste incinerators in the northeastern United States [12]). These deposition rates at ANP are a major concern for the Park Service, considering the class I air-quality status of ANP and the ecological implications of Hg pollution [13]. This high deposition of Hg makes ANP especially well suited as a field laboratory for investigations of Hg bioaccumulation in palustrine biota.

Mercury contamination of wild amphibians has received little attention (see, however, [8,9,14–17]), despite reports of worldwide population declines [18–20]. Amphibians are widely distributed across watersheds, are a potential vector of Hg transport into the surrounding riparian zone and uplands, and comprise an energy base on which biota from higher trophic levels depend [21]. Methyl Hg can be toxic to aquatic biota, impairing productivity, growth, and development as well as eliciting aberrant behavior and, potentially, causing death [22]. Chronic exposure to MeHg also potentially may increase the susceptibility of individuals to disease [23,24]. Moreover, Parris and Baud [25] reported that the effects of chytridiomycosis (an emerging infectious disease that poses a significant risk to wild amphibian populations) may be affected by the presence of Cu, a common environmental contaminant known to occur in some aquatic ecosystems.

The specific objectives of the present investigation were to

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Table 1. Temperature and water chemistry values for the nine sample ponds in Acadia National Park (ME, USA) for June 2003[a]

Pond site	No. of samples	Species sampled	Temp (°C)	Specific conductance (μS/cm)	Dissolved oxygen (%)	pH	Acid-neutralizing capacity (μEq/L)	Dissolved organic carbon (mg/L)	Chlorophyll *a* (μg/L)[b]
Leech[c]	4	Bullfrog	23.5 ± 1.3	53.13 ± 14.6	72.8 ± 27.2	6.3 ± 0.29	144.5 ± 9.5	3.7 ± 0.29	23.0
Lower Hadlock[d]	4	Bullfrog	23.5 ± 0.8	48.9 ± 8.1	56.1 ± 15.5	6.5 ± 0.03	40.9 ± 0.6	2.9 ± 0.09	1.1
New Mill[d]	1	Bullfrog	22.9	45.5	75.9	6.5 ± 0.0	85.2 ± 3.1	3.1 ± 0.02	2.2
Duck	4	Green frog	21.6 ± 0.7	28.5 ± 0.42	36.9 ± 6.7	4.9 ± 0.0	2.6 ± 0.25	7.9 ± 0.7	1.2
Duck Brook[d]	1	Green frog	22.6	43.9	76.5	5.5 ± 0.1	37.1 ± 2.6	8.6 ± 0.6	9.7
Hodgdon	4	Green frog	22.1 ± 0.9	60.5 ± 11.8	31.9 ± 5.1	6.2 ± 0.13	120.0 ± 8.0	8.5 ± 0.3	3.7
Upper Hadlock	4	Green frog	22.2 ± 0.9	54.6 ± 6.4	55.1 ± 15.6	6.5 ± 0.1	46.6 ± 1.8	3.5 ± 0.17	1.4
Heath	4	Green frog	19.9 ± 1.2	46.1 ± 3.2	17.5 ± 5.8	5.5 ± 0.01	50.5 ± 4.6	16.8 ± 1.6	7.1
Schooner Head Beaver[e]	4	Green frog	24.9 ± 0.9	95.2 ± 3.2	58.1 ± 13.4	6.5 ± 0.13	231.5 ± 15.5	17.2 ± 1.5	13.0

[a] Values are presented as the mean ± standard error.
[b] Only one sample was taken. No standard error is available.
[c] Confirmed disease site (Icthyophonus, 2001; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).
[d] Confirmed disease site (Ribeiroia, 2001; Ranavirus, 2002; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).
[e] Confirmed disease site (Ranavirus, 2001, 2003; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).

evaluate the relationships between total Hg and MeHg concentrations for water, sediment, and bullfrog (*Rana catesbeiana*) and green frog (*Rana clamitans*) tadpoles; to characterize and compare Hg concentrations among the two sampled species and among pond sites, including three sites where amphibian die-offs and disease have been reported; to evaluate trends between tadpole length and weight measurements with total Hg concentrations; and to determine total Hg and MeHg bioconcentration factors (BCFs) from each pond site. The use of these species as indicators of Hg bioaccumulation in wetland ecosystems also is discussed.

MATERIALS AND METHODS

Study area

Bullfrog and green frog tadpoles were collected from ANP (44°21′N, 68°13′W). The park includes 12,260 ha on Mount Desert Island and 2,973 ha in surrounding parcels. It encompasses 26 mountains, and approximately 20% of the park is classified as wetland habitat, such as marshes, lakes, ponds, streams (elevation range, 50–250 m), vernal pools, swamps, and bogs [26]. The park also contains salt marshes, marine aquatic beds, and intertidal shellfish flats. Terrestrial habitats include peatlands, coniferous forest, and upland as well as riparian deciduous forests. The area is dominated by white spruce (*Picea glauca*), red spruce (*Picea rubens*), and balsam fir (*Abies balsamea*). Dominant deciduous tree species include birch (*Betula* spp.), aspen (*Populus* spp.), maple (*Acer* spp.), and red oak (*Quercus rubra*). In 1947, a human-caused fire burned 6,880 ha of northeastern Mount Desert Island, including watersheds sampled during the present investigation.

Field sampling design

We collected tadpoles (age, approximately one year; Gosner stages 27–37 [27]) from nine pond sites in ANP using standard minnow traps baited with dried cat food. Traps were baited during the afternoon hours (later than 1400 h) and checked by 0800 h the next day. We randomly selected 10 to 11 tadpoles to be analyzed individually for total Hg and 4 to 10 individuals for composite analysis of total Hg and MeHg. Selected tadpoles were measured (total length, snout–vent length, and tail length), rinsed with pond water, and killed using more than 500 mg/L of tricaine methane sulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA) buffered with equal amounts of sodium bicarbonate. Tadpoles were rinsed with pond water; placed in individual, sterile plastic bags (Whirl-Pak; M-Tech Diagnostics, Cheshire, UK); and frozen before analysis.

Water chemistry sampling and analyses

Water samples for chemical analyses (closed-cell pH, dissolved organic carbon [DOC], chlorophyll *a,* and acid-neutralizing capacity) also were collected (twice; one sample from each pond was collected on the same day, and sampling was repeated the following week). Low-level trace metal–clean techniques were used for field sampling and storage of water Hg and MeHg samples [28–30]. Samples were preserved with low-Hg, 6 M HCl (1% by volume) and double-bagged in the field. Teflon® acid (10% trace metal grade HCl) cleaned bottles were uncapped and capped under water to minimize contamination. Water temperature, percentage dissolved oxygen, and specific conductance were measured (Table 1) in the field with glass electrodes (model 85 and 63; YSI Incorporated, Yellow Springs, OH, USA).

Total Hg and MeHg water samples were collected from a shoreline location close to tadpole capture sites in 0.3 to 0.6 m of water. During June and July of 2003, one sediment composite (comprised of samples from three locations) was collected at the center of the tadpole trapping area using a clean, plastic scoop. Water and sediment chemistry samples were analyzed by staff at the University of Maine Environmental Water Chemistry Laboratory (Orono, ME, USA).

Mercury analyses

Total Hg in water was determined by cold-vapor atomic fluorescence spectroscopy (CVAAS) [30–32]. Methyl Hg in water was analyzed using distillation and aqueous ethylation followed by measurement with CVAAS according to U.S. Environmental Protection Agency (U.S. EPA) method 1630 [33]. All tadpole samples were processed for total Hg using acid digestion and CVAAS measurement based on U.S. EPA method 245.6 [30–32].

Tadpole composite samples were freeze-dried, freeze-fractured, homogenized, and analyzed for MeHg content with a modified version of U.S. EPA method 1630 [33] combined

with alkaline digestion and for total Hg using U.S. EPA method 245.6 [30–33]. Tadpole composite MeHg and total Hg concentrations were calculated on a dry-weight basis. Dry-weight MeHg and total Hg data were converted [34] to wet weight using the mean percentage moisture following the formula wet weight = dry weight·(1 − % moisture/100).

Accuracy of analytical techniques was evaluated by analyzing certified reference materials (International Atomic Energy Agency-356 and TORT-2 [lobster hepatopancreas; National Research Council Canada, Ottawa, ON] for MeHg, and DOLT-2 [dogfish live tissue; National Research Council Canada] for total Hg) with each sample batch and by determining the Hg recovery from spiked homogenates. Precision of Hg analytical techniques was evaluated using one duplicate sample for each composite analysis run. Reagent blanks were used to document any laboratory contamination. All water, biota, and sediment chemistry laboratory analyses were completed by staff at the University of Maine Environmental Water Chemistry Laboratory except for the MeHg water samples, which were analyzed at Brooks Rand Laboratories (Seattle, WA, USA).

Statistical analyses

Data normality was evaluated using Lillefor's test [35], and we used log transformations as necessary for Hg concentration data. Relationships between water chemistry variables and frog tissue Hg concentration data were examined with correlation matrices. We discarded uncorrelated independent variables ($r \leq 0.6$, $p > 0.05$). When independent variables were correlated, we used the variable with the higher *r*-value in linear-regression modeling [35]. We also used correlation matrices to test for significant trends between individual tadpole body measurements (total length, snout–vent length, and tail length) and total Hg concentrations (log transformed). The BCFs were calculated for individual ponds using the formula BCF = $\log(\text{biota}_{\text{concentration}}/\text{water}_{\text{concentration}})$. We used the tadpole total Hg composite and MeHg composite samples to determine the BCFs for each pond site. We also calculated the mean BCF based on total Hg data from individual tadpoles and made comparisons among ponds with one-way analysis of variance (ANOVA). Mean tadpole tissue total Hg concentrations among sites were compared with ANOVA. We used nested ANOVA to test for differences in total Hg between species with individual tadpoles, and Mann–Whitney *U* tests were used to evaluate total Hg and MeHg in sediment and tadpole composite samples. We accepted statistical significance at $p < 0.05$. All statistical tests were performed with Systat 10.2 [36].

RESULTS

Total Hg and MeHg bioaccumulation and BCFs

Total Hg concentrations (mean ± standard error) were not significantly different ($\text{ANOVA}_{\text{nested}}$: $F = 1.06$; $df = 1, 7$; $p = 0.34$) between green frog (25.1 ± 1.5 ng/g wet wt) and bullfrog (19.1 ± 0.8 ng/g wet wt) tadpoles (Fig. 1). Methyl Hg comprised 7.6 to 40% of the total Hg in tadpole tissue (wet-wt basis), and mean total Hg levels in tadpoles were significantly different among pond sites ($F = 10.84$; $df = 8, 83$; $p < 0.001$) (Fig. 1). Because these species were considered to be phylogenetically and functionally similar, and because Hg levels did not differ between species, we pooled species data for subsequent analyses.

Total Hg BCFs for individual tadpoles were significantly different among sampling ponds ($F = 45.05$; $df = 8, 83$; $p < 0.001$). The data indicate that MeHg generally was more readily bioconcentrated in tadpoles in comparison to the inorganic Hg fraction (Fig. 2).

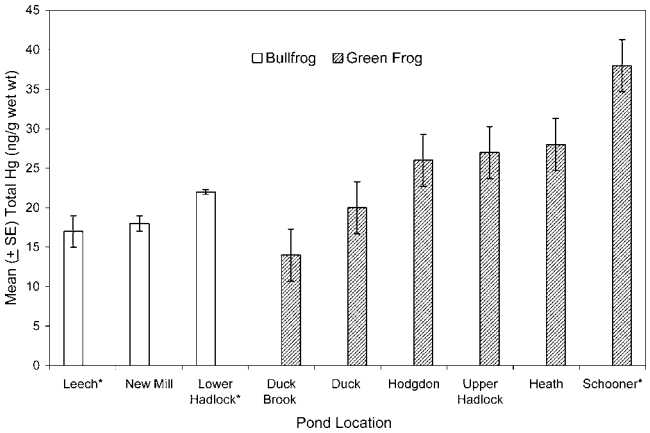

Fig. 1. Total Hg concentration (mean ± standard error [SE]) in green frog and bullfrog tadpoles sampled from nine ponds in Acadia National Park (ME, USA) during June 2003. An asterisk denotes a disease/die-off site (National Park Service Archive, Bar Harbor, ME, USA; unpublished data).

Levels of MeHg in green frog composite samples ranged from 0.003 to 0.023 mg/kg wet weight, and levels of MeHg in bullfrog composite samples ranged from 0.011 to 0.022 mg/kg wet weight. No statistical difference was detected between species ($U = 9.0$, $df = 1$, $p = 1.0$) (Table 2). Total Hg in green frog composite samples ranged 0.027 to 0.110 mg/kg wet weight, and total Hg in bullfrog composites ranged from

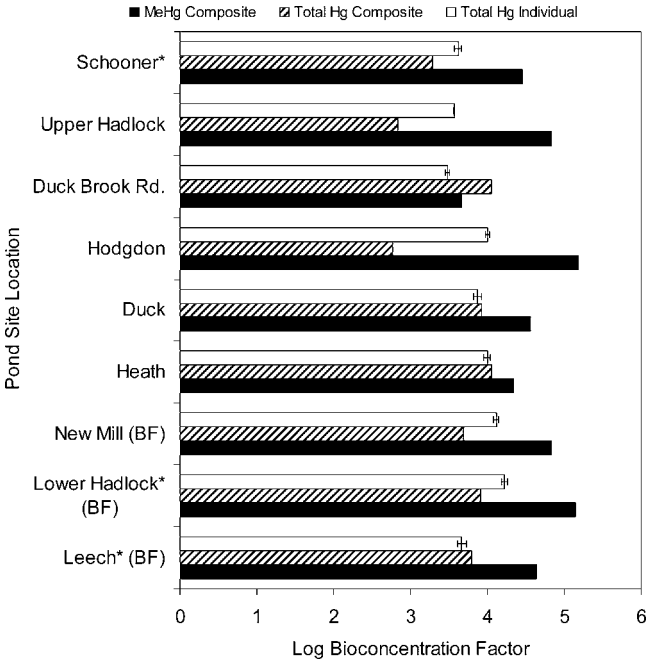

Fig. 2. Bioconcentration factors (log[biota concentration/water concentration]) for total Hg (composite and individual samples) and methyl Hg (MeHg) from nine ponds in Acadia National Park (ME, USA) during June 2003. Values (mean ± standard error) are presented for individual tadpole total Hg samples. An asterisk denotes a disease/die-off site (National Park Service Archive, Bar Harbor, ME, USA; unpublished data). Sites with (BF) on the *y* axis denote bullfrog sample ponds; all other sites represent green frog tadpole sample ponds.

Table 2. Total Hg and methyl Hg (MeHg) concentrations in water, sediment, and green frog and bullfrog tadpoles (age, approximately one year) from nine ponds sampled in Acadia National Park (ME, USA) during June 2003

Pond site	Species sampled	Water total Hg (ng/L)[a]	Water MeHg (ng/L)[a]	Sediment		Tadpole		
				Total Hg (mg/kg dry wt)	Methyl Hg (μg/kg dry wt)	Composite Methyl Hg (mg/kg wet wt)	Composite total Hg (mg/kg wet wt)	%MeHg
Leech[b]	Bullfrog	2.8 ± 0.4	0.51 ± 0.04	0.157	2.22	0.022	0.075	29.3
New Mill	Bullfrog	2.5 ± 0.2	0.23 ± 0.03	0.020	0.16	0.016	0.042	38.1
Lower Hadlock[c]	Bullfrog	1.3 ± 0.05	0.08 ± 0.003	0.038	0.14	0.011	0.075	14.7
Duck	Green frog	2.5 ± 0.2	0.34 ± 0.08	0.186	0.63	0.012	0.030	40.0
Duck Brook	Green frog	3.5 ± 0.6	0.57 ± 0.04	0.107	1.04	0.003	0.027	11.1
Hodgdon	Green frog	4.4 ± 0.1	0.19 ± 0.11	0.038	1.63	0.03	0.110	27.3
Upper Hadlock	Green frog	1.4 ± 0.05	0.07 ± 0.03	0.016	0.09	0.005	0.066	7.6
Heath	Green frog	7.0 ± 0.9	1.05 ± 0.17	0.172	0.88	0.023	0.103	22.3
Schooner Head Beaver[d]	Green frog	8.4 ± 1.1	0.56 ± 0.49	0.069	0.70	0.016	0.075	21.3

[a] Values are presented as the mean ± standard error ($n = 2$).
[b] Confirmed disease site (Icthyophonus, 2001; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).
[c] Confirmed disease site (Riberiroia, 2001; Ranavirus, 2002; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).
[d] Confirmed disease site (Ranavirus, 2001, 2003; National Park Service Archive, Bar Harbor, ME, USA; unpublished data).

0.042 to 0.075 mg/kg wet weight. No statistical difference between species was detected ($U = 8.0$, $df = 1$, $p = 0.8$) (Table 2). Composite samples consistently had higher levels of total Hg in comparison to the individual samples. The reason for this difference is unknown, although it may be a result of the different matrices used for analyses (i.e., freeze-dried composite material using multiple individuals vs individual wet samples) or simply of the uncertainty associated with each analytical technique. Total Hg and MeHg concentrations in water or sediment collected from ponds occupied only by green frog tadpoles compared to ponds occupied only by bullfrog tadpoles did not differ (Table 2).

Mercury bioaccumulation and tadpole morphometry

We detected a significant relationship between green frog tadpole body measurements and total Hg concentrations (green frog total length: $r = 0.28$, $p < 0.05$; snout–vent length: $r = 0.27$, $p < 0.05$; tail length: $r = 0.26$, $p < 0.05$). We also detected a significant relationship between total Hg concentrations and bullfrog tadpole total length ($r = 0.38$, $p < 0.05$) and tail length ($r = 0.42$, $p < 0.05$) but not snout–vent length ($r = 0.15$, $p > 0.05$).

Mercury and pond chemistry

A positive relationship was found between mean pond methylation efficiency (Table 3 and Fig. 3) and total Hg in sediment ($r^2 = 0.70$, $p = 0.005$) and between DOC levels and mean MeHg concentrations ($r^2 = 0.58$, $p = 0.02$) (Fig. 4). We also detected a significant relationship between mean total Hg in tadpole tissue and mean total Hg in water ($r^2 = 0.48$, $p = 0.038$) (Table 3). Additionally, pond sediment MeHg was best predicted by mean chlorophyll *a* concentrations ($r^2 = 0.57$, $p = 0.02$) (Table 3). Mean DOC concentrations also were a good predictor of mean total Hg in water ($r^2 = 0.91$, $p = 0.001$) (Fig. 4). We also detected a significant relationship between mean MeHg in water and mean total Hg in water ($r^2 = 0.52$, $p = 0.03$) (Fig. 4). Total Hg concentration in sediment was negatively correlated with pH ($r^2 = 0.61$, $p = 0.01$). We detected significant differences in mean total Hg in water ($F = 41.11$, $df = 8$, $p < 0.001$) among sampling ponds; however, the average concentration of MeHg in water was not statistically different among sites ($F = 3.13$, $df = 8$, $p = 0.054$) (Table 2).

Table 3. Correlation matrix of pond chemistry variables and total Hg and methyl Hg (MeHg) in water, sediment, and tadpoles sampled from nine ponds in Acadia National Park (ME, USA) during June 2003[a]

	Mean tadpole Hg	Chlorophyll *a*	Mean DOC	Mean ANC	Mean water total Hg	Mean water MeHg	Sediment MeHg	Sediment total Hg	Pond methylation capacity	Tadpole MeHg	pH
Mean tadpole Hg	1.000										
Chlorophyll *a*	−0.036	1.000									
Mean DOC	0.656*	0.204	1.000								
Mean ANC	0.575	0.610*	0.373	1.000							
Mean water Hg	0.694*	0.350	0.953*	0.611*	1.000						
Mean water MeHg	0.159	0.482	0.763*	0.132	0.718*	1.000					
Sediment MeHg	−0.178	0.755*	0.125	0.381	0.225	0.349	1.000				
Sediment total Hg	−0.207	0.418	0.381	−0.191	0.247	0.703*	0.461	1.000			
Pond methylation efficiency	−0.559	0.579	0.092	−0.212	0.034	0.654*	0.505	0.838*	1.000		
Tadpole MeHg	0.303	0.265	0.298	0.453	0.435	0.273	0.583	0.175	−0.002	1.000	
pH	0.276	0.133	−0.343	0.564	−0.102	−0.476	−0.096	−0.782*	−0.580	0.115	1.000

[a] ANC = acid-neutralizing capacity; DOC = dissolved organic carbon. *$p < 0.05$.

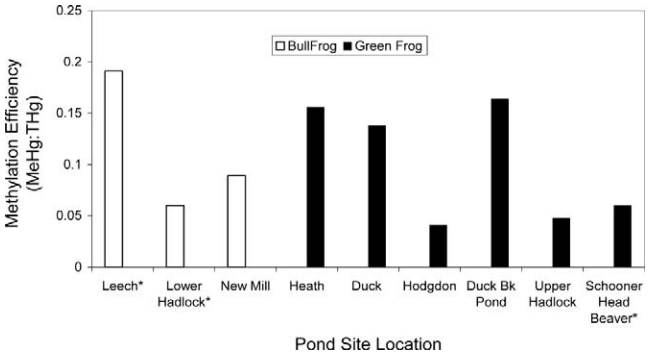

Fig. 3. Distribution of methylation efficiency (methyl Hg [MeHg]: total Hg [THg] in water) rates for nine ponds in Acadia National Park (ME, USA) during June 2003. An asterisk denotes a disease/die-off site (National Park Service Archive, Bar Harbor, ME, USA; unpublished data).

DISCUSSION

Total Hg and MeHg bioaccumulation and bioconcentration factors

Mercury bioaccumulation in tadpoles varied across ponds in ANP (Fig. 1), and total Hg concentrations in pond water were significantly correlated with mean tadpole total Hg concentrations (Table 3). The percentage MeHg present in tadpoles (7.6–40%) was relatively similar to the range reported for biota with similar food habits, such as benthic invertebrate detritivores (20–25%) and grazers (30–40%) sampled from two hydroelectric reservoirs in Quebec, Canada [37]. Mean total Hg was highest for green frog tadpoles sampled from the Schooner Head site, a small, semipermanent pond with extensive beaver activity and where Ranavirus was detected in green frog tadpoles during the summer of 2003 (National Park Service Archive, Bar Harbor, ME, USA; unpublished data). Currently, no data exist regarding the potential link between MeHg, total Hg, and disease in amphibians. Bennett et al. [24] reported that mean liver concentrations of Hg, Se, and Zn, as well as the Hg to Se molar ratio, were significantly higher in harbor porpoises (*Phocoena phocoena*) that died from infectious disease compared to healthy porpoises. Although Hg has no known benefit to biota [8,9], the mechanism between MeHg and amphibian disease is unknown, and further research is required to determine the possible effects of chronic exposure on susceptibility to disease in amphibians.

Total Hg concentration (mean ± standard error) of 66.1 ± 3.4 ng/g wet weight (n = 116) for one- to three-year-old larval northern two-lined salamanders (*Eurycea bislineata bislineata*) collected from 14 streams in ANP [7] exceeded the mean total Hg concentration in individual green frog and bullfrog tadpoles (Fig. 1). Individual larval two-lined salamanders likely had higher Hg concentrations because of their invertebrate diet [38] in comparison to both green frog and bullfrog tadpoles, which are grazers.

Gerstenberger and Pearson [16] reported Hg concentrations in adult bullfrogs (n = 28) from a wetland in Meadow Valley Wash (NV, USA). Mercury concentrations from the investigation by Gerstenberger and Pearson [16] ranged from 0.004 to 0.142 mg/kg for brain, 0.012 to 0.117 mg/kg for muscle, 0.036 to 0.252 mg/kg for kidney, and 0.046 to 0.458 mg/kg for liver. Burger and Snodgrass [15] reported no effect of depuration on Hg concentrations before laboratory analysis. Whole-body concentrations of total Hg in bullfrog tadpoles (n = 40) sampled from the Carolina Bay wetland (a site receiving wastewater and surface runoff as well as cooling water from a coal-fired plant and storm-water runoff from a vehicle maintenance parking lot) at the Savannah River (SC, USA) site were only slightly higher than those in ANP bullfrog tadpoles (mean ± standard error: 36.0 ± 1.7 μg/kg wet wt and 181.0 ± 9.9 μg/kg dry wt basis [16]).

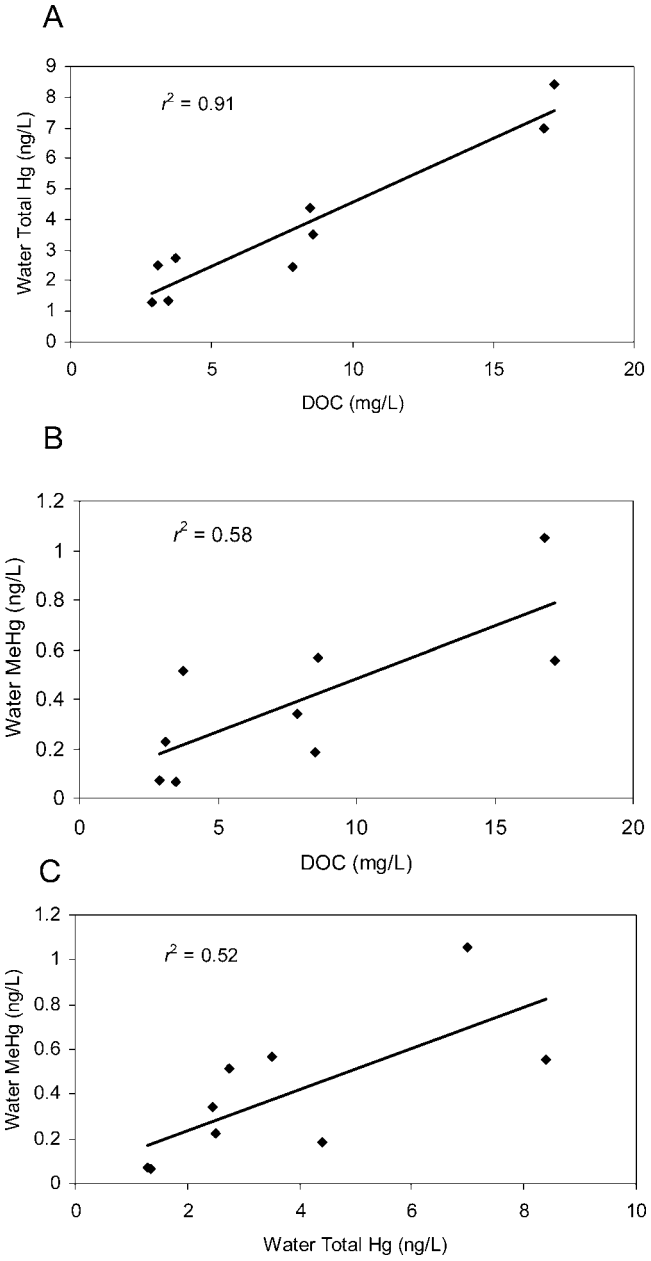

Fig. 4. (**A**) Relationship between dissolved organic carbon (DOC) and total Hg in water. (**B**) Relationship between DOC and methyl Hg (MeHg) in water. (**C**) Relationship between total Hg and methyl Hg in water. Data are from the nine sample ponds in Acadia National Park (ME, USA) during June 2003. An asterisk denotes a disease/die-off site (National Park Service Archive, Bar Harbor, ME, USA; unpublished data).

Bioconcentration factors for green frog and bullfrog tadpoles from the present study (Fig. 2) were comparable to those of biota found at similar trophic levels (amphipod BCF for total Hg, 4–4.5; amphipod BCF for MeHg, 5–5.5) collected from Seal Cove Pond and Hodgdon Ponds in ANP (J. Burgess, University of Maine, Orono, ME, USA; Master's thesis). Bio-

concentration factors for MeHg were higher than those for total Hg on all except the Duck Brook pond (Fig. 2). Boudou and Ribeyre [39], Burgess (Master's thesis), as well as Watras and Bloom [40] demonstrated that MeHg was transferred up the food chain more efficiently than inorganic Hg, primarily because of uptake at low trophic positions and differential Hg species compartmentalization [41]. Pickhardt et al. [42] reported that as the algal biomass increased, the concentration of MeHg per cell decreased. In turn, this resulted in lower rates of dietary intake and reduced bioaccumulation for grazers (*Daphnia* sp.) in oligotrophic aquatic ecosystems [42]. Future Hg research at ANP should evaluate the potential relationships between local algae population dynamics, community structure, and sediment S, C, and Hg biogeochemistry with patterns of MeHg uptake by local biota.

Mercury and tadpole morphometry

We detected statistically significant relationships between tadpole body measurements (total length, snout–vent length, and tail length) and total Hg concentrations for green frog and bullfrog tadpoles, although snout–vent length was not significant for bullfrogs. This relationship may reflect increased Hg bioaccumulation as a function of age. In contrast to the present investigation, Gerstenberger and Pearson [16] reported that length and weight measurements were poor predictors of total Hg concentrations in adult bullfrog tissue.

Mercury and pond chemistry

Acidified freshwater ecosystems with high temperatures, DOC, sulfate-reducing bacteria, sulfate, or inorganic Hg(II) levels facilitate MeHg bioaccumulation and biomagnification [5,43–45]. Concentrations of total Hg (1.2–9.4 ng/L) and MeHg (0.04–1.22 ng/L) in ANP pond water were comparable to those in lakes of eight western national parks (Lassen Volcanic, Yellowstone, Glacier, Grand Teton, Rocky Mountain, Sequoia, Kings Canyon, and Yosemite) investigated by Krabbenhoft et al. [46]. Dissolved organic carbon levels at ANP ponds (2.79–18.6 mg/L) overlapped those reported by Krabbenhoft et al. [46] for the western national parks mentioned above (range, 0.2–11.7 mg/L) except for the Schooner Head beaver and the Heath pond ANP sites, which were substantially higher (Table 1). Dissolved organic carbon was a significant predictor of both total Hg and MeHg in water, and total Hg in water was strongly correlated with MeHg in water (Table 3 and Fig. 4). Johnson et al. [47] similarly showed a strong association ($r^2 = 0.60$, $p < 0.05$) between DOC and total Hg for two streams in ANP. These findings were expected, because Hg has a strong binding affinity for dissolved organic matter [48]. The relationship between DOC and Hg bioaccumulation is complex, and regression data from the present investigation show that DOC was more strongly correlated with total Hg ($r^2 = 0.91$) than with MeHg ($r^2 = 0.58$) in ANP pond water (Fig. 4). Wallschlager et al. [48] reported that in ecosystems where Hg is transported primarily by organic matter and is derived from soils and wetlands, Hg and DOC would be expected to be positively correlated. Ravichandran [49] suggested that correlations between DOC and Hg may or may not exist for aquatic ecosystems that are being directly affected by atmospheric deposition. Dissolved organic carbon reactivity with Hg also likely is dependent on its structural and chemical composition and by the presence and activity of other ions in the water column (see [49] and references therein).

Percentage MeHg as total Hg is a good predictor of methylation efficiencies in aquatic ecosystems [50]. Surface waters with methylation efficiencies that exceed 10% often contain biota with high concentrations of total Hg [46,50,51]. Methylation efficiencies in ANP exceeded those reported for selected western national parks (range, 0.0–0.13) [46]. Four of the nine ponds (44%) had methylation efficiencies of greater than 10% (Fig. 3), suggesting that ANP wetland food webs likely are susceptible to bioaccumulating high levels of total Hg. Correlations between ANP pond methylation efficiency and total Hg in sediment and between chlorophyll *a* and MeHg in sediment were significant (Table 3). Sulfate-reducing bacteria may have been more active and abundant at ponds with greater chlorophyll *a* concentrations (i.e., sites with higher primary productivity). However, future research concerning the complex interactions between pond methylation efficiency, sediment dynamics, and pond productivity is required to further evaluate this hypothesis.

Conservation implications

Mercury pollution in aquatic ecosystems remains a daunting challenge for natural resource managers [13], especially considering the class I airshed status of ANP (i.e., requiring the highest level of protection under the Clean Air Act [http://www2.nature.nps.gov/air/regs/cleanAir.cfm]) and the potential long-term ecological ramifications of surface-water contamination. Where community-level studies are impractical, green frog and bullfrog tadpoles may be effective indicators of Hg bioaccumulation in pond ecosystems, given that these species are relatively abundant, are easily identified in the field, use local resources, are widely distributed, and can be reared for companion laboratory experiments or for in situ toxicology studies [52]. Future studies should evaluate the relationship between beaver activity, subsequent changes in water chemistry (including DOC mobilization) [53], and total Hg in water and biota to establish and test spatially explicit predictive models of bioaccumulation patterns and for risk assessment purposes.

Although to our knowledge the present investigation is the first to characterize Hg bioaccumulation in tadpole tissue in the northeastern United States, where amphibian disease has been confirmed [54] (http://www.mainenature.org/archive/6-11-02.html), the relationship between occurrence of amphibian disease in tadpoles and MeHg exposure is unknown. Future investigations should evaluate if MeHg, other environmental contaminants, or other abiotic conditions (i.e., drought and warm water temperatures) potentially increase the susceptibility of amphibian populations to disease. Toxicological studies with MeHg exposure regimes that reflect natural pond conditions and that examine the long-term and sublethal effects of this contaminant with the synergistic or cumulative effects of stress from the presence of predators and/or predator cues [55] may be necessary as well. Expanding amphibian monitoring programs to include heavy metal biomonitoring of amphibians, water, and sediments also should be considered for the effective management of surface waters.

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