Survey of mercury in boreal chorus frog (*Pseudacris maculata*) and wood frog (*Rana sylvatica*) tadpoles from wetland ponds in the Prairie Pothole Region of Canada

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Abstract

Tadpoles are important prey items for many aquatic organisms and often represent the largest vertebrate biomass in many fishless wetland ecosystems. Neurotoxic mercury (Hg) can, at elevated levels, decrease growth, lower survival, and cause developmental instability in amphibians. We compared total Hg (THg) body burden and concentration in boreal chorus frog (*Pseudacris maculata*) and wood frog (*Rana sylvatica*) tadpoles. Overall, body burden and concentration were lower in boreal chorus frog tadpoles than wood frog tadpoles, as expected, because boreal chorus frog tadpoles consume at lower trophic levels. The variables species, stage, and mass explained 21% of total variation for body burden in our models but had negligible predictive ability for THg concentration. The vast majority of the remaining variation in both body burden and THg concentration was attributable to differences among ponds; tadpoles from ponds in three areas had considerably higher THg body burden and concentration. The pond-to-pond differences were not related to any water chemistry or physical parameter measured, and we assumed that differences in wetland geomorphology likely played an important role in determining Hg levels in tadpoles. This is the first report of Hg in frog tadpoles in the Prairie Pothole Region of North America.

Key words: mercury, wetlands, tadpole, amphibians, Prairie Pothole Region, North American Great Plains, Saskatchewan

Introduction

The elevated transport and deposition of mercury (Hg) to remote pristine areas, such as the prairie region of North America, occurs via anthropogenic Hg emissions (Schuster et al. 2002). Hg in the atmosphere is deposited onto the landscape via wet or dry deposition (Hall et al. 2005; Graydon et al. 2008; Weiss-Penzias et al. 2016), and once HgII enters aquatic ecosystems it can be readily transformed into the neurotoxic organic methylmercury (MeHg; Gilmour and Henry 1991; Branfireun et al. 1999). Although MeHg can be produced abiotically, most is methylated via microbial activity in wetlands and lakes, where HgII is reduced and bound to a methyl group (Boening 2000; Hintelmann 2010). Once present in aquatic ecosystems, MeHg can be taken up and biomagnified with increasing trophic level (Morel et al. 1998; Rimmer et al. 2005; Scheuhammer et al. 2007; Hall et al. 2009). Effects of MeHg exposure in humans and wildlife include reduced reproductive rates,

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hormonal and immune system changes, altered brain chemistry, behavioral or visual abnormalities, and mortality (Scheuhammer et al. 2007; Hightower 2009).

The Prairie Pothole Region (PPR) of North America is an area of abundant isolated wetland ponds that are usually small, shallow, and productive water bodies surrounded by riparian zones that are usually dominated by cattails (*Typha latifolia*), bulrushes (*Schoenoplectus* spp.), sedges (*Carex* spp. and *Scirpus* spp.), and willows (*Salix* spp.) (Batzer and Sharitz 2006; Johnson et al. 2010; Hayashi et al. 2016). These wetlands provide many ecosystem services including groundwater recharge, flood control, water quality, and carbon and nutrient sequestration (van der Kamp et al. 2003; Hayashi et al. 2016). They are also important breeding and nesting habitats for avian wildlife in North America (Batt et al. 1989). Atmospherically deposited Hg may be readily methylated (Hoggarth et al. 2015) and accumulated (Sando et al. 2007; Hall et al. 2009) in these environments because prairie wetlands have potentially high dissolved organic carbon (DOC) concentration, a warm temperature in the summer months, and low dissolved oxygen; all variables which lead to anoxic conditions in which the main methylating organisms (sulfate and iron reducing bacteria and methanogens (Gilmour et al. 2013; Parks et al. 2013)) thrive (Paranjape and Hall 2017).

Amphibians can be highly sensitive to MeHg because it may reduce reproductive success, growth, and survival, especially in the early developmental stages (Zillioux et al. 1993; Unrine et al. 2004, 2007; Bank et al. 2007b; Bergeron et al. 2010a). Despite the sensitivity of amphibians to Hg, fish are the most studied organism in terms of MeHg toxicity. Within aquatic habitats, amphibians and other mid-level trophic consumers are prey for fish and other aquatic biota, which in turn may be consumed by humans or other top predators of the ecosystem. Thus, lower trophic levels can mediate the transfer of MeHg from aquatic to terrestrial ecosystems (Bank et al. 2007a). Although tadpoles are important prey for many fish and waterfowl (Bergeron et al. 2010b), there is a lack of studies focused on amphibians compared with other aquatic vertebrates (Bradford et al. 2012). Furthermore, tadpole biomass is often the largest of all vertebrates in many fishless wetland ecosystems, making them an important part of ecosystem functioning (Ugarte et al. 2005). Finally, Hg concentration and body burden in frog tadpoles from the PPR have not been reported in the literature. Our objective here was to compare total Hg (THg) concentration in herbivorous boreal chorus frog (*Pseudacris maculata* Agassiz, 1850) and omnivorous wood frog (*Rana sylvatica* LeConte, 1825) tadpoles from Saskatchewan wetlands. By studying lower trophic levels, inferences can be made about higher level consumers, and precautions can be implemented if a high concentration of Hg is found. Frog tadpoles are a good biomonitor of processes within wetland habitats as almost all of their food sources are aquatic, unlike other amphibians or adult frogs (Hothem et al. 2010). Global amphibian population declines have become a concern (Stuart et al. 2004) and Hg monitoring in wetland amphibians can be useful as some of these declines may be attributed to environmental Hg contamination (Corn 2000; Ficken and Byrne 2013).

Methods

Site selection

Twenty-two sampling sites were located across central Saskatchewan (Table 1 and Fig. 1). From 1981 to 2010 this region had daily mean temperatures ranging from a mean winter temperature of −14.7 °C in January to a mean summer temperature of 18.9 °C in July (Environment Canada 2016). Over the same period, mean annual precipitation was 389.7 mm, with approximately 26% as snowfall (Environment Canada 2016). The area of the wetland ponds ranged from 0.2 to 13 ha (data not shown), and the maximum depth at the time of sampling was between 0.45 and 3.15 m (Table 1). Ponds did not have surface water connectivity, and although we did not measure groundwater dynamics, prairie wetlands typically have limited surface and groundwater connectivity (Hayashi et al. 2016). Ponds were surrounded by conventional farmland, organic farmland, minimum tillage farmland, or wildlife areas

Table 1. Geographic coordinates, maximum depth, pH, temperature, conductivity, whole water total mercury concentration, and methylmercury concentration for sampled wetland ponds.

Area	Pond	Latitude			Longitude			Maximum depth (cm)	pH	Temperature (°C)	Conductivity (µS/cm)	Whole water total mercury (ng/g)	Whole water methylmercury (ng/g)
Amey	MinTill	52°	30′	14.04″	107°	18′	04.32″	90	7.12	19.38	978	8.18	1.445
	Org	52°	29′	15.00″	107°	21′	33.00″	50	6.01	18.38	202	4.63	0.214
	Conv	52°	31′	03.00″	107°	18′	23.00″	70	7.14	18.96	1026	12.06	5.396
Baumel	Wild	52°	10′	45.80″	105°	15′	42.20″	230	8.57	22.87	789	3.28	0.840
Bull	MinTill	52°	44′	19.68″	105°	01′	17.04″	95	8.19	27.22	162	5.45	0.091
Demong	Org	52°	33′	27.70″	105°	35′	24.80″	200	7.49	21.08	386749	3.63	0.624
	Conv	52°	33′	27.70″	105°	35′	24.80″	140	6.97	21.37	151	2.33	0.520
Friesen	MinTill	52°	42′	57.96″	106°	29′	08.16″	45	7.14	27.7	270	4.71	0.258
	Org	52°	40′	27.00″	106°	38′	57.00″	70	6.39	16.16	318	7.91	0.088
Gillis	MinTill	52°	40′	54.12″	104°	14′	27.96″	130	8.04	20.84	397	2.66	1.330
	Conv	52°	47′	39.84″	103°	52′	48.36″	99	8.04	20.898	1026	9.54	3.391
Leight	Wild	52°	28′	11.00″	104°	32′	11.00″	75	8.9	23.34	941	7.13	2.739
	MinTill	52°	25′	06.00″	104°	24′	41.00″	70	7.38	19.87	573	7.87	3.193
	Org	52°	18′	36.00″	104°	20′	21.00″	100	8.21	22.94	427	4.08	1.112
Loiselle	MinTill	52°	18′	05.40″	106°	13′	18.48″	315	6.68	16.13	268	6.83	2.598
	Conv	52°	15′	57.00″	106°	04′	21.00″	70	7.05	21.7	330	2.96	0.386
Mumm	Conv	53°	16′	48.00″	106°	10′	23.16″	100	7.34	19.1	944	No data	No data
Naharney	Wild	52°	45′	07.00″	107°	07′	59.88″	155	9	23.2	1633	3.33	0.561
	Conv	52°	43′	47.00″	107°	16′	04.00″	100	7.7	18.8	1240	5.61	2.084
Weiland	Wild	52°	14′	39.70″	104°	57′	58.10″	85	8.85	25.8	688	6.42	1.289
	MinTill	52°	27′	34.00″	105°	12′	52.56″	83	7.36	21.02	933	4.47	1.852
	Conv	52°	27′	34.00″	105°	07′	17.00″	140	7.97	21.12	1476	3.42	1.766

Note: Wild, wildlife area; MinTill, minimum tillage farms; Org, organic farms; Conv, conventional farms.
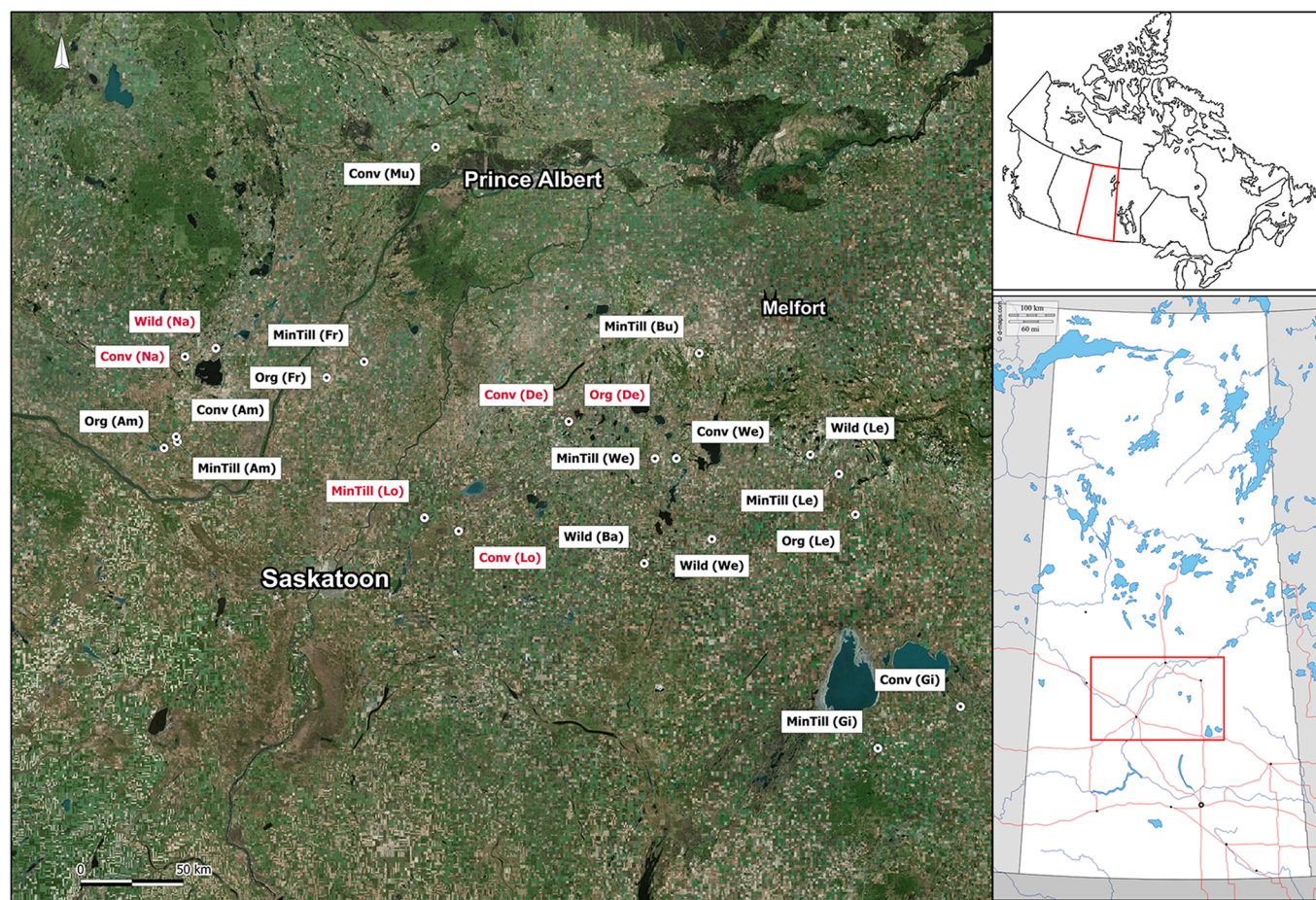
Fig. 1. Locations of sampled wetland ponds. Wetland pond sample sites are identified with a farming practice type (wildlife area (Wild), minimum tillage farms (MinTill), organic farms (Org), and conventional farms (Conv)) and family names of local residents are provided in parentheses. Sites with high Hg concentrations are indicated in red. See Table 1 for additional geographic information. Images attributed to: Microsoft Bing Maps (formally Virtual Earth) and Earthstar Geographics binged.it/241EGxJ (imagery), theblog.ca/wp-content/uploads/2006/11/bigmapofcanada.png (Canada), and d-maps.com/carte.php?num_car=23476&lang=en (Saskatchewan).

(Donald et al. 2001). Conventional and organic farms were generally tilled three times a year, whereas minimum tillage farms limited tilling to once a year or were not tilled at all (Donald et al. 2001). Wildlife areas were parcels of Saskatchewan grasslands that were not altered for agricultural use. These areas included native prairies, nature park reserves, and recreational park reserves. Ponds were selected in a previous study (Donald et al. 2001) examining the impact of adjacent farming practices on pesticide concentrations. Ponds were named according to family name of a resident of the area and adjacent farming practices. Although we do not consider the impact of farming practices here, we have maintained the names for consistency.

Sample collection

Tadpoles

Boreal chorus frogs and wood frogs are common across the entire geographic area. Tadpoles were collected opportunistically during the day within each pond from 14 to 18 June 2010. Dip nets were used to collect tadpoles in the shallow water nearest to the shore around the perimeter of each wetland

pond for 30 min. All tadpoles collected were separated into plastic containers where they were visually identified according to Preston (1982). A subsample of three boreal chorus frog and three wood frog tadpoles were randomly selected, euthanized using buffered tricaine methane/sulfonate (MS-222), rinsed in ultrapure water, placed in separate Ziploc® bags, flash frozen with dry ice, and stored in the field on dry ice. Size was not a criterion for tadpole selection. If three frogs could not be collected in 30 min, one or two frogs were taken instead. Euthanization of frogs was performed using procedures approved by the Canadian Council on Animal Care (2010) with appropriate permits. We obtained a total of 32 boreal chorus frog and 61 wood frog tadpoles from 20 different wetland sites. Developmental stage (using Gosner 1960) was recorded prior to sample preparation for THg analysis (see below). Tadpoles were stored in a laboratory freezer (−14 °C) until analysis.

Water samples

Water samples for both standard water quality parameters and THg and MeHg concentration were collected by wading into the center of the pond to chest height. A "clean-hands–dirty-hands" technique was used to collect unfiltered water samples into precleaned glass bottles for Hg analysis (St. Louis et al. 1994). Hg samples were preserved by acidification using trace metal grade HCl (at 0.2% and 0.4% by volume for THg and MeHg, respectively). Water chemistry samples were collected concurrently, filtered using 0.45 μm glass fiber filters, and stored at 4 °C until analysis. Wetland ponds were sampled for conductivity (mS/cm), pH, and water temperature (°C) using a YSI 556 multiprobe sonde. Maximum depth (cm) was estimated at the center of each wetland pond using the cord of the probe, which was marked every 25 cm.

Sample preparation and Hg analysis

We used THg as an indication of potential MeHg uptake of tadpoles in this study. A relationship between THg and MeHg concentration in both adult and larval amphibians has been previously demonstrated (%MeHg in tadpoles in other studies ranged from 7% to 61% (Bergeron et al. 2010a; Loftin et al. 2012)); however, the use of THg as a proxy for MeHg is generally not appropriate if %MeHg is less than 90%. As a result, we used THg as an indication of potential MeHg uptake, with caution, in the context of possible sources of Hg to higher trophic level organisms.

Samples were freeze-dried, and whole tadpoles (ranging from dry weights of 5.8–73.5 mg and 70–450 mg for boreal chorus frog and wood frog tadpoles, respectively) were individually digested in Teflon bombs with a metal grade nitric:sulfuric acid ratio of 7:3 at 125 °C for 2 h. Samples were then further digested in 9 mL of ultrapure water and 1 mL of bromine monochloride at 60 °C overnight. Aliquots (1–2 mL dependent on the estimated THg concentration of the sample) were then analyzed for THg using a Tekran® Series 2600 Cold Vapor Atomic Fluorescence Spectrophotometer (United States Environmental Protection Agency 2002). Recoveries of certified reference material (National Research Council DORM-3 for THg) were between 99.6% and 100% of the certified values. The concentration of THg in the water samples was similarly analyzed following EPA Method 1631 (United States Environmental Protection Agency 2002). MeHg concentration was quantified using a Tekran 2700 at the University of Western Ontario, following distillation on a Tekran 2750 at the University of Regina. Spike recoveries on acid blanks and spike samples were between 97% and 109% and 101% and 112%, respectively. Minimum detection limits were less than 0.3 ng/L for THg and 0.02 ng/L for MeHg. Approximately 10% of samples were analyzed in duplicate.

Water chemistry analysis

Water samples were analyzed for concentrations of DOC, total nitrogen, anion (Cl, F, and SO_4), cation (Na, Ca, Mg, and K), total dissolved solid and total phosphorus concentrations, and turbidity using standard methods (Stainton et al. 1977). Anion and cation data are presented as total concentrations of all anions and cations.

Statistical analysis

All data are presented in both concentration (ng THg/g dry body tissue ± standard error) and body burden (ng THg/dried frog ± standard error). We analyzed both THg concentration and body burden in whole tadpoles using linear mixed models (package lme4 in R version 3.1.1; R Core Team 2016). Both response variables were ln-transformed prior to analysis as the original data spanned 2–3 orders of magnitude and showed strong heteroskedasticity. We developed a set of eight models for each response variable that included all subsets of terms for species, metamorphic stage, and body mass as fixed effects. Each model also included a random effect for pond. We ranked models using the second-order Akaike information criterion (AIC), then calculated model weights (ω_i) as a measure of evidence for each model having the best predictive ability from among those considered. We assessed the relative importance of each predictor variable based on the sum of model weights ($\Sigma\omega_i$) for all models in which it was included: variables with $\Sigma\omega_i$ approaching one were very likely to improve model fit, those with $\Sigma\omega_i$ near zero were very unlikely to improve fit, and those with intermediate $\Sigma\omega_i$ had mixed support (Burnham and Anderson 2002).

We performed a principal components analysis (PCA) to explore how patterns of MeHg variation among ponds were related to water chemistry. All variables except for water temperature, depth, and pH were log-transformed prior to analysis. We substituted the mean values for missing observations for three cases in which ponds did not have data for total dissolved solids, MeHg, or THg; results using these substitutions were not noticeably different from those obtained by removing ponds with missing data from the PCA.

Results and discussion

Differences in THg in wood frog vs. boreal chorus frog tadpoles

Body burden and THg concentration in all tadpoles ranged from 0.5 to 2300.5 ng/frog dry weight (dw) and 25.9 to 7889.3 ng/g dw, respectively (Tables S1 and S2). Lower values were not outside the range of those measured in other studies; however, upper values exceeded those of any previous study, including in amphibians from some contaminated studies (see Bradford et al. 2012 for a summary). The variables species, stage, and mass explained 21% of total variation for body burden in our models but had negligible predictive ability for THg concentration (Table S3). Nearly all the remaining variation in both body burden and THg concentration was attributable to differences among ponds ($R^2 = 96\%$ when including the random pond effect in both cases; Table S3).

The THg body burden of boreal chorus frog tadpoles ($n = 32$, mean ± standard error: 33 ± 17 ng/frog) was lower than that of wood frog tadpoles ($n = 61$, mean = 329 ± 76 ng; Fig. 2a). THg concentration showed a similar trend (mean = 597.2 ± 132.1 ng/g and 1668.9 ± 351.6 ng/g for boreal chorus frogs and wood frogs, respectively; Fig. 2b). Bioaccumulation of MeHg through trophic levels has been well documented, as prey items are the primary source of MeHg for aquatic organisms (Hall et al. 1997; Rimmer et al. 2005; Ward et al. 2010). Boreal chorus frog tadpoles are herbivorous and feed on algae, whereas wood frog tadpoles are omnivorous, and likely supplement a primary diet of algae with invertebrates (e.g., Odonata and Dytiscidae) and eggs of amphibians including wood frog tadpoles (McDiarmid and Altig 1999; Wells 2007). Thus, the lower values observed in boreal chorus frogs were not surprising considering the trophic position of both species.

There was also high variation within species within ponds, especially for wood frog tadpoles. This may be due to life history parameters such as stage of development, because greater development leads to more opportunity for Hg biomagnification and a change in diet as the organism approaches metamorphosis (Bradford et al. 2012). To address differences in development timelines, we tested the effect of metamorphic stage on body burden and THg concentration. Models that included both
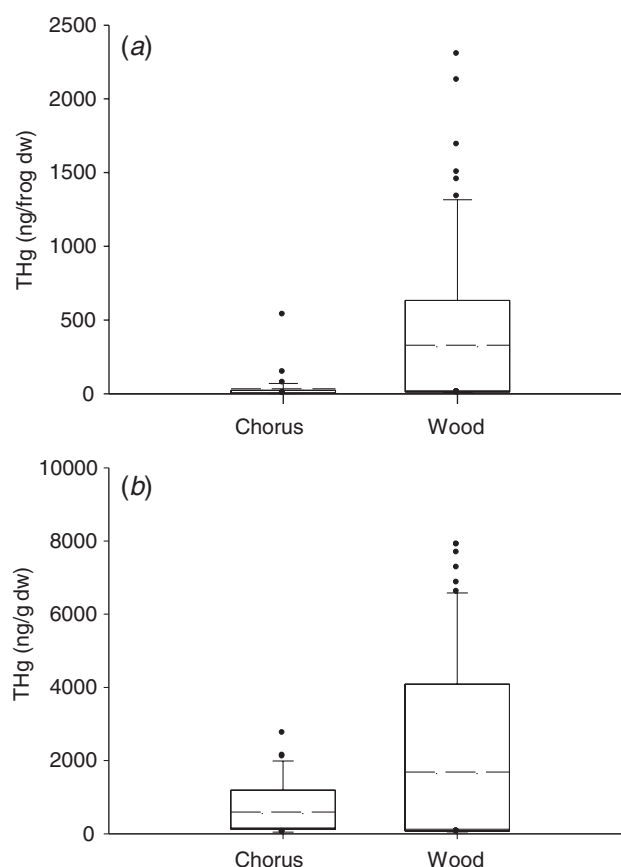
Fig. 2. Total mercury concentration and body burden of different species of frog tadpoles. Total mercury (THg) (*a*) body burden (ng/frog dry weight (dw)) and (*b*) concentration (ng/g dw) in boreal chorus frog and wood frog tadpoles. Sample size was 32 and 61 for boreal chorus frog and wood frog tadpoles, respectively. The boundary of the box closest to zero indicates the 25th percentile, a solid line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles. Means are represented by the dashed line.

species and stage were unambiguously supported over other models for body burden ($\Sigma\omega_i > 0.999$ for both variables; **Table 2**), providing further evidence of Hg bioaccumulation with metamorphic stage. There was also some evidence that tadpoles with greater mass had a higher body burden, after accounting for effects of both species and metamorphic stage ($\Sigma\omega_i > 0.77$; **Table 2**). We could not definitively conclude that any of the predictors had an effect on THg concentration ($\Sigma\omega_i = 0.661$, 0.727, and 0.407 for species, stages, and mass, respectively), but there was some evidence that more developed wood frogs had a higher THg concentration than less developed boreal chorus frogs (**Fig. S1**).

Given the lack of study of Hg in amphibians in prairie wetlands, we decided to compare our results with lower trophic organisms from other studies in similar systems. Gastropods and some Corixidae feed on algae and are therefore at a similar trophic level as boreal chorus frog tadpoles (Clarke 1981; Merritt and Cummins 1996). The THg concentration in pond snails (Gastropods and Lymnaeidae) and aquatic insects (Corixidae, Odonata, Dytiscidae, and Notonectidae) from wetlands in the PPR ranged from 11.7 to 161.2 ng/g (Bates 2010) and 9.7 and 507.1 ng/g (Bates and Hall 2012), respectively. These values were lower than our boreal chorus frog tadpoles (25.9–2736.4 ng/g). The potentially higher accumulation in tadpoles compared with other organisms at similar trophic levels suggests that tadpoles may be an important vector for Hg within aquatic and terrestrial food webs as the Hg accumulated by tadpoles is retained throughout metamorphosis (Bergeron et al. 2010a). Elevated THg levels may be problematic in areas where tadpoles are abundant prey sources for higher trophic levels, as tadpoles may increase the amount of MeHg accumulated through food webs.

Table 2. Ranking of top-performing models predicting total mercury concentration and body burden in 93 tadpoles from 22 ponds within the Prairie Pothole Region, based on the second-order Akaike information criterion (AIC).

Response	Predictors	ΔAIC_C	ω_i
Natural logarithm of total mercury concentration (ng/g)			
	Species, stage	0.00	0.40
	Stage, mass	0.95	0.25
	Species	1.66	0.17
	Mass	3.45	0.07
	Species, stage, mass	3.74	0.06
	Species, mass	5.39	0.03
	Stage	6.07	0.02
Natural logarithm of body burden (ng/frog)			
	Species, stage, mass	0.00	0.77
	Species, stage	2.43	0.23

Note: ω_i, model weight.

Differences in THg in tadpoles in different ponds

After accounting for species, stage, and weight, we observed significant pond-to-pond variability in both body burden and concentration (Table S3). Tadpoles from wetland ponds in three areas (labelled as Demong, Loiselle, and Naharney) had THg body burdens and concentrations that were an average of 60 and 38 times higher, respectively, than tadpoles from ponds in other areas (Figs. 3a, 3b). These very large differences dwarfed variation in THg among the remaining ponds.

Why were concentrations in these ponds so high?

Factors that influence HgII methylation and MeHg bioavailability are important controls in MeHg bioaccumulation in aquatic organisms (Wiener 2010; Li and Cai 2013; Hsu-Kim et al. 2013; Paranjape and Hall 2017). We explored water chemistry and physical parameters that are often useful in explaining the variation of MeHg and THg in whole water (such as DOC, pH, total anion concentrations; see Methods section for complete list), and thus Hg in aquatic animals, using PCA (Fig. 4). We did not identify any water chemistry parameters, including THg and MeHg concentrations in water, as being strongly associated with the concentration of Hg in tadpoles in our systems.

Differences in Hg concentration in the ponds were apparent for both species of tadpoles, suggesting that Hg concentration in algae and lower trophic organisms must also have been elevated because diet is the main vector of Hg in most vertebrates (Burger et al. 2014). Another explanation of high values in certain ponds is that the gastrointestinal tracts (GT) of tadpoles sampled from the high Hg ponds contained sediment with an elevated Hg concentration. Burger and Snodgrass (1998) showed that the concentration of Hg in tadpoles GTs was elevated compared with Hg concentration in the tail or rest of the body, suggesting that gut contents can contribute to overall body concentration. In addition, tadpoles have been shown to accumulate metals from sediment only (Snodgrass et al. 2004). We did not measure THg concentration in sediments in our ponds. However, our previous work has shown that Hg concentration in sediments can vary in these types of systems (Hoggarth et al. 2015), and it is therefore conceivable that the high Hg ponds had elevated Hg concentration in the sediments. If sediments were a significant source of Hg for tadpoles, we would expect to find a high inorganic Hg to MeHg ratio in the organisms. A further exploration of Hg speciation in tissues
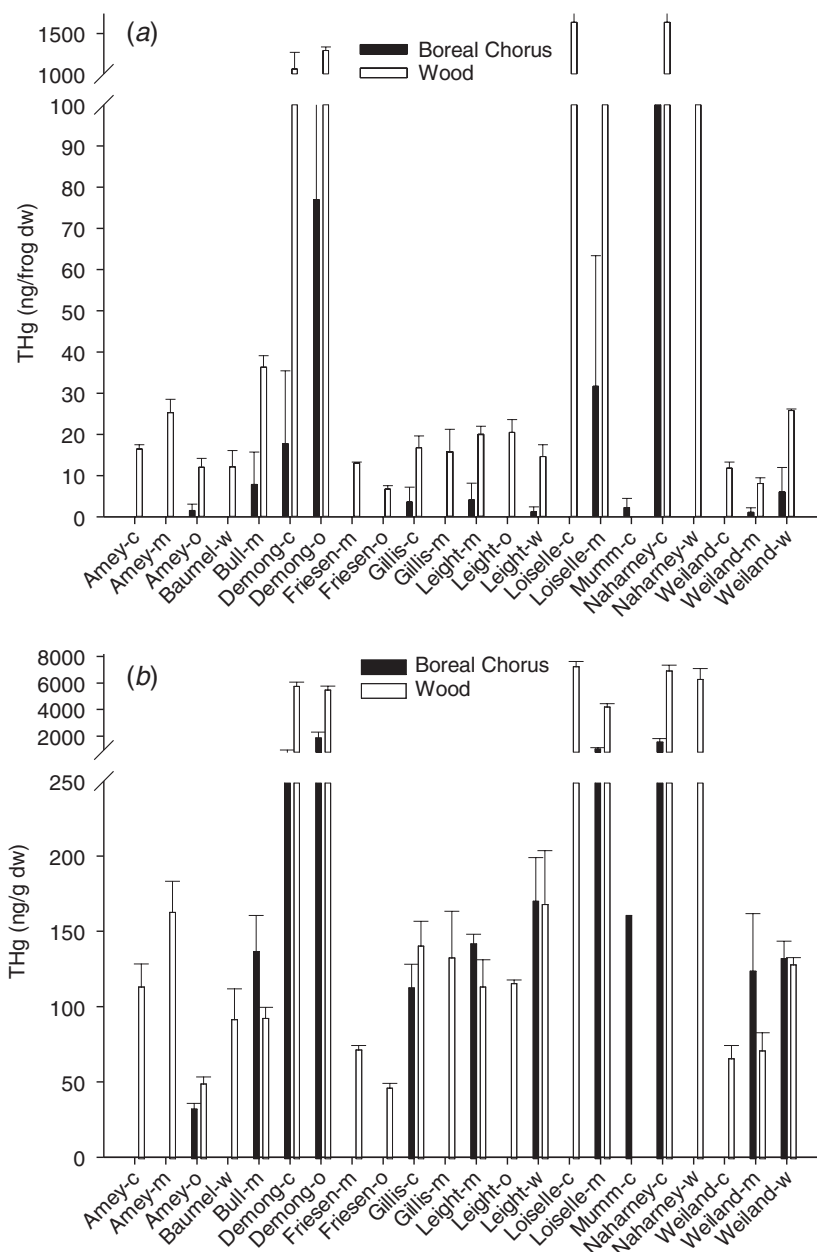
Fig. 3. Total mercury concentration and body burden of frog tadpoles from different sampling ponds. Mean total mercury (THg) (*a*) body burden (ng/frog dry weight (dw)) and (*b*) concentration (ng/g dw) of boreal chorus frog tadpoles (black bars) and wood frog tadpoles (white bars) sampled from all wetland ponds in each area. Ponds are identified with the area name and the first letter of the farming practices adjacent to each pond (see **Table 1** for full pond names). Frog tadpole species coexisted in ponds with two data bars. Error bars represent standard error of the mean.

is required, and clearly a more thorough study on the biogeochemical cycling of Hg, including concentration in the sediments, in these high Hg ponds is warranted.

Wetland geomorphology is likely important in the determination of Hg levels in aquatic organisms. For example, natural areas tend to retain more water in soils and thus input less water into wetlands compared with agricultural areas (van der Kamp et al. 2003), leading to a greater diversity in
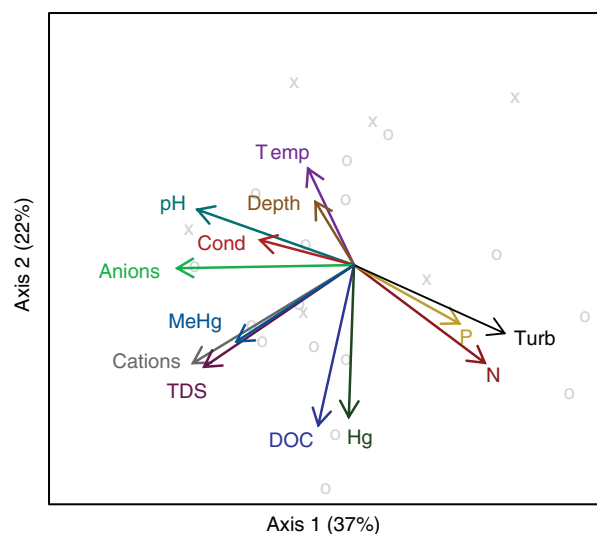
Fig. 4. Principal component analysis (PCA) of water chemistry across ponds. Biplot showing results from a PCA of water chemistry data across ponds. "o" and "x" designate ponds from low and high Hg CLUSTERs, respectively. MeHg, methylmercury concentration; Hg, total Hg concentration; Temp, temperature; Cond, conductivity; Turb, turbidity; TDS, total dissolved solids; DOC, dissolved organic carbon; Anions, total anion concentration; Cations, total cation concentration.

hydrological patterns, especially in prairie wetland ponds (Hayashi et al. 2016). Wet–dry cycling is important for the methylation process as it reoxidizes sulfur for metabolism by sulfate reducing bacteria, and therefore, methylation of HgII, and subsequent bioaccumulation of MeHg in biota, may be influenced by the frequency of drying and flooding in wetlands (Sando et al. 2007; Eckley et al. 2015; Singer et al. 2016). Consequently, intermittent wetlands will contain higher amounts of MeHg in the water and in residing fish (Ackerman and Eagles-Smith 2010; Coleman Wasik et al. 2015). Second, the vegetation composition of riparian zones may also be an important factor in determining MeHg levels in aquatic habitats because a higher amount of shading by riparian zones is associated with higher MeHg levels in aquatic organisms (Balogh et al. 2002; Ward et al. 2010). Riparian vegetation of our ponds was typical of PPR wetlands and could provide shading of sediments where methylation could be occurring. These two examples demonstrate that the processes controlling Hg accumulation in these tadpoles are much more complex than inorganic Hg deposition alone.

Conclusions

Hg cycling in the wetland ponds of the PPR is vastly understudied. We present the first data set on Hg in frog tadpoles from this region. Because wood frog tadpoles had a higher THg concentration and body burden than boreal chorus frog tadpoles, our results supported our hypothesis that biomagnification of Hg occurred with increasing trophic level in tadpoles in the wetland ponds of the PPR. Both boreal chorus frog and wood frog tadpoles contained relatively high THg levels compared with invertebrates at similar trophic levels in the PPR and tadpoles in other studies. After accounting for species, stage, and weight, we observed high pond-to-pond variability in both body burden and concentration (Table S3). Tadpoles from ponds in three areas had an ~40× higher THg body burden and concentration than those from other ponds, and these differences were not related to any water chemistry or physical parameter measured. Differences in wetland geomorphology likely played an important role in the determination of Hg in aquatic organisms. Elevated Hg concentration is potentially problematic for both the tadpole populations and tadpole predators due to the toxicological impacts of Hg bioaccumulation, and tadpoles may be important vectors of Hg from aquatic to terrestrial systems.

Acknowledgements

Author contributions

Conceived and designed the study: SAB, BDH. Performed the experiments/collected the data: SAB, MCV, BDH. Analyzed and interpreted the data: SAB, MCV, BDH. Contributed resources: BDH. Drafted or revised the manuscript: SAB, MCV, BDH.

Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

All relevant data are within the paper and in the Supplementary Material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2016-0041

Supplementary Material 1

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