

# Taking the trophic bypass: aquatic-terrestrial linkage reduces methylmercury in a terrestrial food web

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**Abstract.** Ecosystems can be linked by the movement of matter and nutrients across habitat boundaries via aquatic insect emergence. Aquatic organisms tend to have higher concentrations of certain toxic contaminants such as methylmercury (MeHg) compared to their terrestrial counterparts. If aquatic organisms come to land, terrestrial organisms that consume them are expected to have elevated MeHg concentrations. But emergent aquatic insects could have other impacts as well, such as altering consumer trophic position or increasing ecosystem productivity as a result of nutrient inputs from insect carcasses. We measure MeHg in terrestrial arthropods at two lakes in northeastern Iceland and use carbon and nitrogen stable isotopes to quantify aquatic reliance and trophic position. Across all terrestrial focal arthropod taxa (Lycosidae, Linyphiidae, Acari, Opiliones), aquatic reliance had significant direct and indirect (via changes in trophic position) effects on terrestrial consumer MeHg. However, contrary to our expectations, terrestrial consumers that consumed aquatic prey had lower MeHg concentrations than consumers that ate mostly terrestrial prey. We hypothesize that this is due to the lower trophic position of consumers feeding directly on midges relative to those that fed mostly on terrestrial prey and that had, on average, higher trophic positions. Thus, direct consumption of aquatic inputs results in a trophic bypass that creates a shorter terrestrial food web and reduced biomagnification of MeHg across the food web. Our finding that MeHg was lower at terrestrial sites with aquatic inputs runs counter to the conventional wisdom that aquatic systems are a source of MeHg contamination to surrounding terrestrial ecosystems.

**Key words:** aquatic-terrestrial linkages; ecological tracer; food web; Iceland; methylmercury; spider; stable isotopes.

## INTRODUCTION

Matter, energy, and nutrients commonly cross ecosystem boundaries (Polis et al. 2004). While there has been significant focus on the ecological implications of such cross-habitat food web linkages or spatial subsidies (Polis et al. 1997), there is growing interest in understanding the potential for cross-habitat linkages to serve as vectors for the movement of harmful contaminants (Cristol et al. 2008, Raikow et al. 2011). Water to land linkages are of particular concern since aquatic systems have long accumulated harmful waste materials from anthropogenic sources (Carson 1962). In the case of the highly toxic heavy metal, mercury, aquatic systems are of additional concern because they provide the anoxic conditions necessary for the microbially mediated conversion of inorganic mercury ( $\text{Hg}^{2+}$ ) to the highly bioavailable form, methylmercury (MeHg; see Downs et al. [1998]).

Emergent aquatic insects can be important vectors creating aquatic to terrestrial linkages (Baxter et al.

2005, Gratton et al. 2008), and recent work has highlighted their potential role in transporting contaminants from aquatic to terrestrial systems. Raikow et al. (2011) found PCB concentrations in aquatic insects to be orders of magnitude higher than terrestrial insects, corresponding with elevated PCB concentrations in riparian consumers. Similarly, terrestrial-feeding birds adjacent to streams had elevated mercury concentrations, presumably a result of consuming aquatic insect-feeding spiders (Cristol et al. 2008).

Despite recent research, mechanisms for aquatic to terrestrial contaminant movement remain poorly resolved. If aquatic insects are more contaminated than their terrestrial counterparts, then the degree of contamination of terrestrial consumers should be a function of reliance on aquatic prey and should decrease with distance from the shoreline. Yet aquatic insects can have other consequences for terrestrial food webs and ecosystems. Consumption of aquatic insects may increase or decrease the trophic position of terrestrial consumers, depending on the specific situation. This can, in turn, affect levels of contaminants such as MeHg and PCBs, which are known to correlate positively with trophic position since they strongly biomagnify (Cabana

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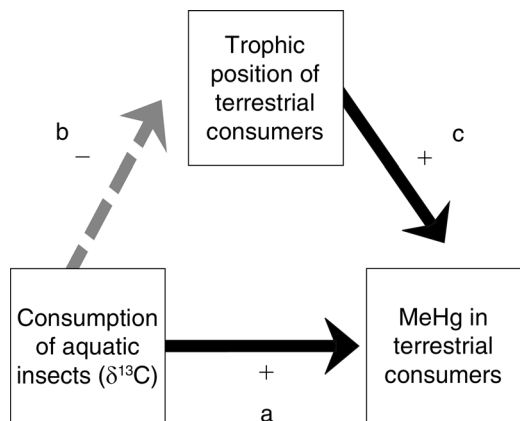


FIG. 1. Hypothesized roles of consumption of emergent aquatic insects and trophic position as drivers of methylmercury (MeHg) in terrestrial arthropods. Aquatic insects are generally more contaminated than their terrestrial counterparts, such that we predict that (arrow a) MeHg in terrestrial consumers will be positively correlated (+) with aquatic insect reliance. The trophic position of midges is lower than that of most terrestrial prey that would otherwise be consumed by terrestrial arthropods (Tachet 2010). Thus, (arrow b) consumption of aquatic insects should cause terrestrial consumers to have a lower (–) trophic position. (Arrow c) Trophic position and MeHg in terrestrial consumers should be positively correlated as a result of food chain biomagnification. Consumption of aquatic insects is expected to have direct (consumption of more contaminated aquatic prey) and indirect (reducing trophic position) effects on mercury in terrestrial arthropods that counteract each other.

and Rasmussen 1994, Vander Zanden and Rasmussen 1996, Wiener et al. 2003). In addition, deposition of aquatic insect carcasses on land may fertilize terrestrial ecosystems (Gratton et al. 2008, Gratton and Vander Zanden 2009), potentially altering basic ecosystem properties, such as primary productivity, that can subsequently affect the cycling of MeHg (Ward et al. 2010).

We quantify MeHg in terrestrial arthropods adjacent to two Icelandic lakes that differ in the magnitude of aquatic insect inputs. We use natural abundances of stable carbon and nitrogen isotopes to elucidate aquatic insect reliance and trophic position of terrestrial arthropods in an effort to reveal the potential factors determining MeHg concentrations in the biota. Since methylation of Hg is known to occur predominantly in aquatic habitats, aquatic insects are predicted to be more contaminated than their terrestrial counterparts. We therefore predicted that MeHg in terrestrial consumers would be positively correlated with aquatic insect reliance (see Fig. 1, arrow a). In addition, the trophic position of aquatic midges (~2; Tachet 2010) is lower than that of most terrestrial prey that would otherwise be consumed by terrestrial arthropods (Dreyer et al. 2012). Thus, we expected that consumption of aquatic insects would correspond with a decrease in the trophic position of terrestrial consumers (Fig. 1, arrow b). Finally, we expected that trophic position and MeHg

in terrestrial consumers would be positively correlated as a result of food chain biomagnification (Fig. 1, arrow c). From these arguments, we hypothesized that consuming aquatic insects would have both direct (consumption of more contaminated aquatic prey, see Fig. 1, arrow a) and indirect (via reductions in trophic position, see Fig. 1, arrows b and c) effects on MeHg in terrestrial arthropods, which are expected to counteract each other. We therefore used path analysis (Grace 2006) to examine the direct and indirect effects of consuming aquatic prey on MeHg in terrestrial consumers. Our work highlights the potential interactions among biogeochemical and ecological factors in determining the fate and movement of contaminants in the environment, and specifically, contaminant movement across habitat boundaries.

## METHODS

### Study sites

Our two terrestrial study sites in northeast Iceland (~65°4' N, 17°0' W) have similar terrestrial arthropod food webs, but differ in terms of aquatic insect (midge) inputs (Gratton et al. 2008, Dreyer et al. 2012). Aquatic midge larvae can reach extraordinarily high densities in the naturally eutrophic Mývatn and are consistently low in oligotrophic Helluvastjörn (Gratton et al. 2008). Midge deposition on land within the first 150 m of shore is approximately 50 times higher at Mývatn compared to Helluvastjörn (Dreyer et al. 2012). The midge community in Mývatn is dominated by two species, *Tanytarsus gracilentus* Holmgren and *Chironomus islandicus* Kieffer, which represent roughly 79% and 9% of total abundance, respectively (Lindegaard and Jónasson 1979). Helluvastjörn is dominated by *C. islandicus*.

### Sample collection

The terrestrial arthropod community was sampled along four transects at Mývatn (high midge lake) and along two transects at Helluvastjörn (low midge lake). At each transect, we sampled the arthropod community 5 m and 200 m from shore. Hereafter, we refer to these four lake distance combinations as habitat type. Furthermore, to account for the potential effect of soil moisture conditions on MeHg concentrations, we sampled arthropods at both wet and dry locations at each transect. Wet sites were wetlands generally dominated by *Sphagnum* spp. and *Carex* spp., while dry sites were dominated by heathland species, including ericaceous shrubs (*Betula nana* (L.), *Arctostaphylos uva-ursi* (L.) Spreng, *Calluna vulgaris* (L.) Hull, *Vaccinium uliginosum* (L.), *Empetrum nigrum* (L.), *Salix lanata* (L.), *Salix phylicifolia* (L.)), grasses (*Poa* spp. and *Deschampsia* spp.), and forbs (*Gallium verum* (L.) and yarrow *Achillea millefolium* (L.)). Arthropod sampling was performed by vacuum sampling with a modified leaf blower (SH 85 Shredder Vac/Blower, Stihl, Waiblingen, Germany). At each site, the vacuum sampler was pressed over the ground to remove arthropods from the

vegetation and leaf litter. The contents of each vacuum bag were emptied into white plastic trays in the field, and arthropods were removed using plastic ultracleaned forceps and/or aspirators. In July 2010, the dominant functional groups in the terrestrial food web were collected: Lycosidae (wolf spiders, *Pardosa sphagnicola* (Dahl, 1908) and *Pardosa palustris* (Linnaeus, 1758)), Linyphiidae (sheet-web spiders, *Erigone arctica* (White, 1852)), Gnaphosidae (ground spiders, *Gnaphosa lapponum* (L. Koch, 1866) and *Haplodrassus signifier* (C.L. Koch, 1839)), Opiliones (harvestmen, *Mitopus morio* (Fabricius, 1779)), Acari (mites, *Trombididae*), Collembola (springtails), Tingidae (*Acalypta nigrina* (Fallén, 1807)), and Coccoidea (scale insects, *Arctorthezia cataphracta* (Olafsen, 1772)). Since most plants and herbivorous and detritivorous arthropods sampled in July 2010 had MeHg concentrations below the detection limit, sampling in July 2011 was restricted to taxa with higher MeHg concentrations (Lycosidae, Linyphiidae, Opiliones, and Acari). Lycosidae and Linyphiidae are predatory and specialize on live prey, while Opiliones and Acari are also predators, but feed on dead and decaying arthropods. In 2010 and 2011, larva and adult chironomids (*Tanytarsus* sp. and *Chironomus* sp.) were collected from each lake.

#### Mercury analyses

All sampling, sample processing, and analysis for MeHg and total Hg (THg) followed established clean techniques to minimize contamination of samples (US EPA 1996b, Hurley et al. 1998). Samples were freeze-dried, ground with an acid-washed glass mortar and pestle, and weighed. Samples were analyzed at the Wisconsin State Lab of Hygiene (WSLH), Environmental Health Division, and Inorganic Chemistry Department.

For MeHg samples, a known amount of tissue was placed into a clean Teflon vial with 10 mL of a 25% KOH/methanol mixture. The contents of each vial were sonicated for 8 h and extracted in warm (60°C) KOH/MeOH overnight before analysis (Bloom 1992). Three blanks and three standard reference materials (SRMs) were also processed and analyzed along with each batch of samples. Methylmercury analysis followed ethylation, gas chromatograph separation, pyrolysis, and cold vapor atomic fluorescence spectroscopy (CVAFS) detection techniques (Olson et al. 1997, Hurley et al. 1998). The limit of detection (LOD; MeHg as Hg) was 2 ng/g in tissue. The instrumental LOD and limit of quantification (LOQ) were calculated as 0.074 and 0.185 ng/L.

For THg analysis, a known amount of sample was placed into a clean Teflon container along with 7 mL of a nitric and sulfuric trace metal grade acid mixture (5 HNO<sub>3</sub>:2 H<sub>2</sub>SO<sub>4</sub>), microwaved, cooled, and 23 mL of Milli-Q (Millipore, Bedford, Massachusetts, USA) water were added to each. Finally, 1 mL of BrCl was added to each sample and placed in an oven at 60°C overnight. Blanks and SRMs each constituted at least

10% of each processing batch. THg analysis used established protocols for dual gold amalgamation and CVAFS techniques (Gill and Fitzgerald 1987, US EPA 1996a, Olson et al. 1997, Hurley et al. 1998). The LOD was 1 ng/g in tissue.

#### Isotope analysis and trophic position estimation

Arthropod samples were freeze dried, ground to a fine powder, and analyzed for stable isotopes of C and N using a DELTA plus Advantage (Thermo Scientific, Waltham, Massachusetts, USA) configured through a CONFLO III (Thermo Finnigan, Mississauga, Ontario, Canada) using an NC2100 Elemental Analyzer (EA; Carlo Erba, Milan, Italy) at Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, Arizona, USA). Ratios of <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N are expressed relative to known standards (Vienna Pee Dee Belemnite [VPDB] and atmospheric N, respectively) in parts per million (‰) notation. Mean standard error on laboratory standards were 0.04 for δ<sup>15</sup>N and 0.13 for δ<sup>13</sup>C. Mean standard error of the estimate on the duplicate samples were 0.1 for δ<sup>15</sup>N and 0.07 for δ<sup>13</sup>C.

δ<sup>13</sup>C values provide an indicator of terrestrial consumer reliance on aquatic sources (midges) because midges are isotopically enriched (−14‰ and −23‰ in Mývatn and Helluvastjörn, respectively) relative to terrestrial plants (−29‰, −28‰, −28‰, and −29‰ in Mývatn at 5 m and 200 m and Helluvastjörn at 5 m and 200 m in plants, respectively). The mean habitat type-specific δ<sup>15</sup>N and δ<sup>13</sup>C of terrestrial plants (*Salix lanata*, *Salix phylicifolia*, *Betula nana*, *Arctostaphylos uva-ursi*, *Galium verum*, *Empetrum nigrum*, *Achillea millefolium*, and Poaceae: mean δ<sup>15</sup>N of −1.8‰, −4.5‰, −5.7‰, and −6.4‰ in Mývatn at 5 m and 200 m and Helluvastjörn at 5 m and 200 m, respectively) and the periphyton biofilm (mainly composed by benthic diatoms) were used as the isotopic end-members (mean δ<sup>15</sup>N of −0.6‰ and 1.1‰ in Mývatn and Helluvastjörn, respectively). We estimated trophic position of arthropod consumers (Lycosidae, Linyphiidae, Opiliones, and Acari) using stable nitrogen and carbon isotope as follows:

$$\text{Trophic position} = 1 + \left( \delta^{15}\text{N}_{\text{arthropod}} - [\delta^{15}\text{N}_{\text{aquatic}} \times \alpha + \delta^{15}\text{N}_{\text{terrestrial}} \times (1 - \alpha)] \right) / f_1 \quad (1)$$

$$\alpha = \frac{(\delta^{13}\text{C}_{\text{arthropod}} - f_2 - \delta^{13}\text{C}_{\text{terrestrial}})}{(\delta^{13}\text{C}_{\text{aquatic}} - \delta^{13}\text{C}_{\text{terrestrial}})} \quad (2)$$

where  $f_1$  and  $f_2$  are δ<sup>15</sup>N and δ<sup>13</sup>C trophic discrimination, respectively, and  $\alpha$  is the degree of aquatic reliance. Trophic discrimination factors,  $f_1$  and  $f_2$ , were assumed to be 3‰ per trophic level for δ<sup>15</sup>N and 0.8‰ for δ<sup>13</sup>C (total fractionation, assuming two trophic steps),

TABLE 1. MeHg concentrations (ng/g dry mass) in terrestrial arthropods sampled near (5 m) and far (200 m) from a high midge (Mývatn) and a low midge (Helluvastjörn) lake, in northeastern Iceland.

Taxon and species	Helluvastjörn		Mývatn	
	200 m	5 m	200 m	5 m
Midges	4.5 ± 1.9			
Lycosidae <i>Pardosa</i> spp.	127.3 <sup>a</sup> ± 24.2	116.4 <sup>a</sup> ± 20.3	102.1 <sup>a</sup> ± 40.9	40.5 <sup>b</sup> ± 20.8
Linyphiidae <i>Erigone arctica</i>	180.3 <sup>a</sup> ± 57.3	188.5 <sup>a</sup> ± 55.5	129.2 <sup>ab</sup> ± 58.9	64.2 <sup>b</sup> ± 52.7
Opiliones (Phalangiidae) <i>Mitopus morio</i>	33.0 <sup>a</sup> ± 16.9	45.3 <sup>a</sup> ± 5.1	26.7 <sup>ab</sup> ± 1.5	12.3 <sup>b</sup> ± 6.5
Acari (Trombiidae)	42.9 <sup>a</sup> ± 18.9	35.2 <sup>ab</sup> ± 2.9	46.1 <sup>a</sup> ± 11.6	15.7 <sup>b</sup> ± 3.5
Other trophic levels (detritivores, herbivores)	<2	<2	<2	<2
Plants, <i>Salix lanata</i>	<2	<2	<2	<2
Soils	0.28 <sup>a</sup> ± 0.03	0.18 <sup>a</sup> ± 0.07	0.40 <sup>a</sup> ± 0.37	0.10 <sup>a</sup> ± 0.04

Notes: Values are means ± SD. Letters indicate statistically significant differences ( $P < 0.05$ ) for multiple comparisons of MeHg values across habitat types within a taxon.

respectively (Oelbermann and Scheu 2002). We used primary producers as baseline for trophic position calculations instead of primary consumers because they are easier to sample and there is no uncertainty regarding their trophic position. Trophic positions of primary consumers from the four habitat types (Mývatn 200 m from shore, Mývatn 5 m from shore, Helluvastjörn 200 m from shore, Helluvastjörn 5 m from shore) were calculated and all were close to 2.0 (Appendix: Table A1).

#### Data analysis

We used linear models, general linear models, and linear mixed-effects models to estimate MeHg as a function of aquatic reliance ( $\delta^{13}\text{C}_{\text{arthropod}}$ ), consumer trophic position, and taxa (Lycosidae, Linyphiidae, Opiliones, and Acari). Interactions between taxa and  $\delta^{13}\text{C}_{\text{arthropod}}$ , and between taxa and trophic position were included in the model to account for heterogeneity of slopes (e.g., ANCOVA). Additionally, transect number and wet/dry locations were included in initial models as random effects. These two random effects were not significant and were subsequently dropped from the model. Data were ln-transformed as necessary to satisfy assumptions of normality. We checked for normality and homogeneity by visual inspections of residuals plotted against fitted values. Data were analyzed using SAS v9.3, R v3.0.1 (R Development Core Team 2013), and the R packages lme4 (Bates and Maechler 2009) and multcomp (Hothorn et al. 2008).

We used path analysis to examine the direct and indirect effects of  $\delta^{13}\text{C}$  and trophic position on terrestrial arthropod MeHg. We hypothesized that aquatic reliance (i.e., terrestrial arthropod  $\delta^{13}\text{C}$ ) can affect MeHg both directly (through the consumption of contaminated aquatic prey) and indirectly (by altering consumer trophic position, Fig. 1). Trophic position and MeHg concentration were ln-transformed prior to analysis. We used maximum likelihood to estimate path coefficients and used a bootstrapping process with 10 000 replicate bootstrapped samples to determine the significance of direct and indirect effects. The four key taxonomic groups were analyzed in four separate path

analyses and grouped together for a combined path analysis. For each taxonomic grouping, we considered alternative model structures by removing nonsignificant paths, one at a time, and then comparing alternative models using the Akaike information criterion (AIC). Lower AIC scores indicate better models, and a model is considered sufficiently better than a competing model if its AIC score is two or more points lower; models with AIC scores within two points are considered similar (Burnham and Anderson 2002). Path analysis, the bootstrapping procedure, and model selection were performed using Amos v5.0.1 (Arbuckle 2003).

#### RESULTS

MeHg concentrations were highly variable at our terrestrial study sites (Table 1). MeHg in terrestrial plants, herbivores (Tingidae and Ortheziidae), and detritivores (Collembola) was below the detection limit (<2 ng/g DM [dry mass]). MeHg for adult midges averaged  $4.5 \pm 1.9$  ng/g dw (mean ± SD;  $N = 6$ ) and was not significantly different between the two lakes ( $t$  test,  $P > 0.5$ ). Bulk soil MeHg concentrations were not significantly different among the four habitat types and averaged  $0.22 \pm 0.18$  ng/g (ANOVA,  $P > 0.5$ ). MeHg represented a small fraction ( $0.4\% \pm 0.3\%$ ) of the soil total mercury (THg) pool.

Across all taxa of terrestrial arthropod predators and predator/detritivores, MeHg concentrations were about three times lower for nearshore (5 m) Mývatn (high midge lake) compared to 200 m at Mývatn and 5 m and 200 m at Helluvastjörn (low midge lake; Table 1). The largest difference in MeHg was for nearshore predators (Lycosidae and Linyphiidae) at Mývatn, which had only about one-fourth and one-third the concentrations of MeHg observed at Helluvastjörn. A similar pattern of low MeHg concentrations for nearshore Mývatn was evident for predator/detritivores (Opiliones and Acari), although MeHg concentrations for these two taxa were consistently lower than Lycosidae and Linyphiidae across all habitat types (Table 1).

Contrary to our expectation (Fig. 1), MeHg in terrestrial arthropods decreased with aquatic insect reliance (as indexed by terrestrial arthropod  $\delta^{13}\text{C}$ ):



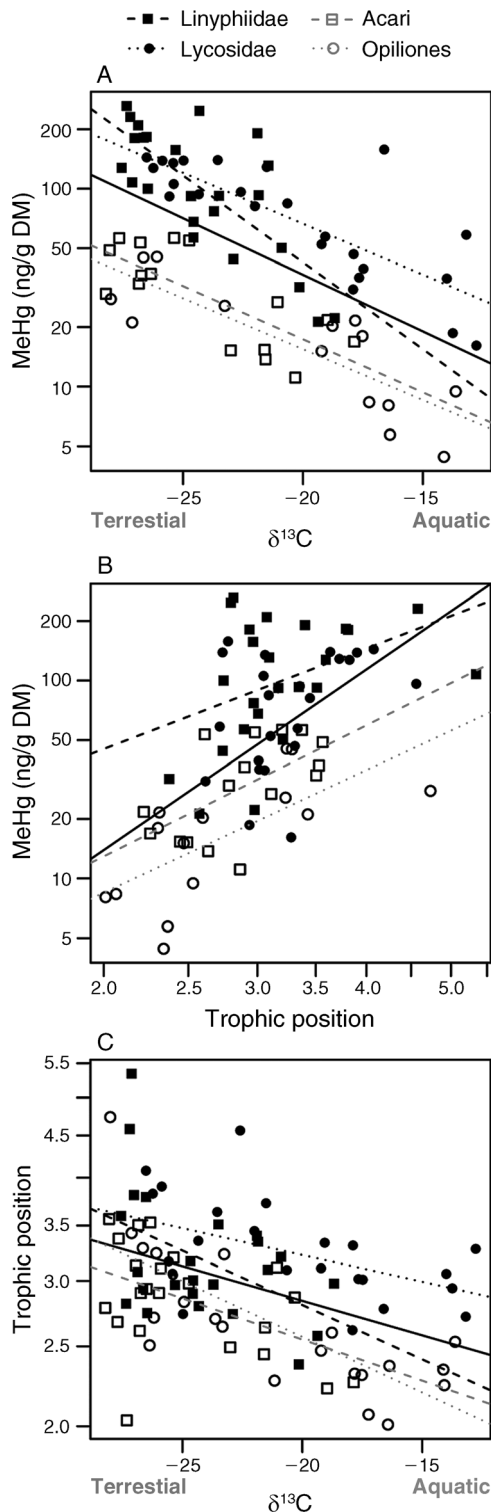


FIG. 2. Relationships among aquatic reliance ( $\delta^{13}\text{C}$ ), trophic position, and MeHg for terrestrial arthropods from two Iceland lakes. Relationships between (A)  $\delta^{13}\text{C}$  and MeHg (ng/g dry mass [DM]), (B)  $\delta^{13}\text{C}$  and trophic position, and (C) trophic position and MeHg (ng/g DM). Symbols represent different taxa. Solid lines indicate the relationship for all species

$\ln(\text{MeHg}) = -0.13 \times \delta^{13}\text{C} + 0.97$ ;  $F = 39.38$ ,  $df = 1, 76$ ;  $r^2 = 0.34$ ,  $P < 0.0001$  (Fig. 2A). There was no significant  $\delta^{13}\text{C} \times \text{taxa}$  interaction (ANCOVA,  $P = 0.16$ ), indicating no significant difference in slopes among taxa. Intercepts did differ among some taxa pairs and could be combined into two statistically different groups, predators (Lycosidae and Linyphiidae) and predator/detritivores (Opiliones and Acari):  $\ln(\text{MeHg}) = -0.13 \times \delta^{13}\text{C} [+ 1.54 \text{ predator} + 0.20 \text{ predator/detritivores}]$ ;  $F = 147.46$ ,  $df = 2, 75$ ;  $r^2 = 0.80$ ,  $P < 0.0001$ .

Consistent with our expectation (Fig. 1), terrestrial arthropod MeHg was positively correlated with trophic position (all taxa combined):  $\ln(\text{MeHg}) = 3.03 \times \ln(\text{TP}) + 0.53$ ;  $F = 39.15$ ,  $df = 1, 76$ ;  $r^2 = 0.34$ ,  $P < 0.0001$  (Fig. 2B). There was no significant difference in slopes among taxa (ANCOVA,  $P = 0.97$ ). Taxa had statistically distinct intercepts ( $\ln(\text{MeHg}) = 1.92 \times \ln(\text{TP}) [+ 1.32 \text{ Acari} + 0.84 \text{ Opiliones} + 2.37 \text{ Linyphiidae} + 1.99 \text{ Lycosidae}]$ ;  $F = 32.42$ ,  $df = 4, 73$ ;  $r^2 = 0.64$ ,  $P < 0.0001$ ).

Terrestrial arthropod trophic position and  $\delta^{13}\text{C}$  (index aquatic reliance), our two main hypothesized drivers of MeHg (Fig. 1), were themselves negatively correlated:  $\ln(\text{TP}) = -0.02 \times \delta^{13}\text{C} + 0.66$ ;  $F = 21.79$ ,  $df = 1, 89$ ;  $r^2 = 0.20$ ,  $P < 0.0001$  (Fig. 2C).

In light of this correlation and our explicit hypotheses regarding the potential direct (aquatic insects are more contaminated) and indirect (altered trophic position) effects of aquatic insect reliance on MeHg accumulation, we used path analysis to examine direct and indirect drivers of MeHg in terrestrial arthropods. The AIC-based model selection process was not used on the model with all taxa combined because all paths in this model were significant (Fig. 3; Appendix: Table A2). Model selection for each of the taxon-specific models indicated that removing the nonsignificant path from trophic position to MeHg did not significantly improve the model relative to the saturated (fully connected) model; that is the saturated and reduced models were similar as the difference between their AIC scores was not greater than 2 (Appendix: Table A3). We therefore retain this ecologically and biologically relevant path in each of the final taxon-specific models. Results of the saturated and reduced model analyses were qualitatively similar (e.g., path coefficients common to the two models were of the same sign and similar magnitude). Chi-square tests were not used to assess model fit because our saturated models have zero degrees of freedom. When all terrestrial arthropod taxa are combined (Fig. 3), trophic position had a significant positive effect on MeHg ( $P = 0.0005$ ; Appendix: Table A2). Aquatic reliance ( $\delta^{13}\text{C}$ ) had significant negative direct effects on trophic position ( $P = 0.0003$ ) and MeHg ( $P = 0.0007$ ) and a significant negative indirect effect on MeHg ( $P = 0.0003$ ) through

← combined, dashed lines are for each taxa separately where significant ( $P < 0.05$ ).

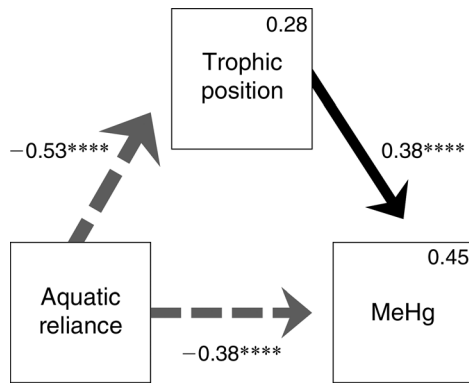


FIG. 3. Path diagram showing the direct and indirect effects of aquatic reliance ( $\delta^{13}\text{C}$ ) and trophic position on the concentration of MeHg. Arrow widths are scaled to standardized path coefficients. The solid black line indicates a positive effect and dashed gray lines indicate negative effects. The  $r^2$  values are given in the upper right corner of variable boxes where appropriate. Path coefficients and  $P$  values are given in Appendix: Table A2.

\*\*\*\*  $P < 0.0001$ .

trophic position. Accounting for both direct and indirect effects, aquatic reliance had a significant net negative effect on MeHg ( $P = 0.0002$ ). When examining taxonomic groups separately, aquatic reliance had significant negative effects on both trophic position and MeHg (Appendix: Fig. A1 and Table A2). However, trophic position had no significant effect on arthropod MeHg for any taxon individually (Appendix: Fig. A1 and Table A2), indicating that the taxa-specific correlations between trophic position and MeHg (Fig. 2B) are fully explained by the effect of aquatic reliance ( $\delta^{13}\text{C}$ ) on trophic position and MeHg. Therefore, for taxa individually, there is no indirect effect of aquatic reliance on MeHg through trophic position, and variation in MeHg is explained by the direct effect of aquatic reliance. Trophic position may not be an important factor explaining variation in MeHg because, within taxa, there is too little variation in trophic position. In contrast, inclusion of diverse taxa introduces variation in trophic position, thereby allowing trophic position to explain variation in MeHg.

#### DISCUSSION

The most striking finding of this study was that consumption of aquatic prey (midges) by terrestrial predators had a negative effect on these consumers' MeHg concentration (Figs. 2A and 3). Although in the path analysis one might expect a negative indirect effect of aquatic reliance on MeHg (i.e., by decreasing trophic position), the dominating negative direct effect of aquatic reliance on MeHg concentration is surprising. Moreover, terrestrial arthropod MeHg was lowest directly adjacent to Lake Mývatn, which has the highest midge inputs (Table 1). The concentration of MeHg in terrestrial predators was generally similar and notably

higher at the other sites (200 m from Mývatn, and both near and far from our low midge lake, Helluvastjörn; Tukey's HSD,  $P > 0.05$ ). This strongly suggests that MeHg from lake-derived midges was not a major MeHg input to terrestrial food webs. This finding contrasts with our initial hypothesis that lakes would be a source of mercury to terrestrial food webs (Fig. 1) and with nearly all previous literature reports (Walters et al. 2008, Walters et al. 2010, Raikow et al. 2011, Tsui et al. 2012, Tweedy et al. 2013). These previous studies highlight the importance of emergent aquatic insects as a vector of contaminant transfer (often PCBs or MeHg) from aquatic systems to terrestrial or riparian food webs. Thus, while previous literature indicates that high levels of contaminants can serve as a tracer for the consumption of aquatic resources by terrestrial consumers, for our study systems the opposite is true: a high degree of aquatic reliance results in low MeHg in terrestrial consumers.

How can we explain this pattern? One potential explanation is that emergent aquatic insect prey has lower concentrations of MeHg than terrestrial prey. However, this is not so. MeHg concentrations for adult midges (which are aquatic herbivores, i.e., trophic level 2) averaged 4.5 ng/g DM and did not differ between the two study lakes ( $P > 0.05$ ), which contrasts with other studies that show strong differences in macroinvertebrate MeHg from lakes with very different trophic status and acidity (Parkman and Meili 1993). MeHg concentrations in plants, terrestrial herbivores, and detritivores, on the other hand, were below the detection limit of 2.0 ng/g, and no comparisons among MeHg across sites could be performed for these taxa. We therefore cannot quantify the difference, but it is clear that MeHg in aquatic herbivores (midges) is higher than their terrestrial counterparts, and thus inconsistent with our finding that terrestrial arthropods that consume aquatic prey have lower MeHg concentrations. Moreover, the  $\delta^{13}\text{C}$  of some consumers was significantly enriched and close to that of the midges ( $> -15\text{‰}$ ) suggesting that a large fraction of the diet of the consumers was derived from aquatic resources (from 39% in Acari to 75% in Lycosidae, according to Eq. 2). Thus, the effect of aquatic reliance on MeHg concentration is not merely a significant statistical relationship but a biologically meaningful pattern, as well.

Evaluation of terrestrial arthropod trophic position (derived from  $\delta^{15}\text{N}$ ) provided some additional insights. Terrestrial arthropods that consume aquatic prey tended to have lower trophic position (Fig. 2C). When relying on terrestrial prey, terrestrial arthropod trophic position tends to be high, indicative of multiple trophic steps and a complex food web. In contrast, in the presence of substantial midge inputs, terrestrial arthropods (or possibly their prey) switch to feeding on midges, which directly reduces terrestrial arthropod trophic position. Studies have shown that contaminants such as MeHg are positively correlated with trophic position as a result

of food web biomagnification (Cabana and Rasmussen 1994). Thus, our results are consistent with the idea that consumption of aquatic insects, though perhaps slightly more contaminated (for a given trophic level), has the effect of reducing terrestrial consumer trophic position, which in turn, results in lower MeHg concentrations. In other words, the existence of an aquatic insect subsidy allows the longer (multitrophic level) terrestrial food web to be bypassed (Fig. 4). The occurrence of such a trophic bypass in coupled food webs could have substantial consequences for MeHg concentrations ultimately found at higher trophic levels. For example, studies generally find a three to six times increase in MeHg with each trophic level (Vander Zanden and Rasmussen 1996, Wiener et al. 2003), such that a trophic bypass equivalent of one trophic level could result in dramatic differences in MeHg concentrations, as reported here. Whether the decrease in trophic position of riparian consumers feeding on aquatic prey is a general phenomenon needs to be more fully evaluated. Reliance on aquatic resources by terrestrial consumers has been shown in several studies, including work at Mývatn (Gratton et al. 2008, Hoekman et al. 2012). In none of these studies, however, was trophic position of the consumers explicitly estimated. We are also unaware of other studies that have examined changes in trophic position of riparian consumers in the presence of aquatic prey. As a result, it remains to be seen whether feeding on aquatic prey, and the trophic bypass that it can create, would result in lower MeHg concentrations in terrestrial consumers in other situations.

One important factor is the relative concentration of MeHg in aquatic insects. Mean MeHg concentration in emergent aquatic insects from Lake Myvatn was 4.5 ng/g, which is lower than MeHg concentrations in aquatic insects reported in other studies. For example, concentrations of MeHg in chironomids from Canadian high arctic lakes ranged from 40 to 100 ng/g (Chételat et al. 2008), from 32 to 106 ng/g in Quebec lakes (Le Jeune et al. 2012), 23 ng/g in Alaska lakes (Hammerschmidt and Fitzgerald, 2005), or 7.9 ng/g in Lake Superior (Back et al. 2003). Indeed, if MeHg concentrations are high in aquatic insects, whether due to point source Hg contamination or aquatic chemical conditions that favor MeHg methylation, this could override the trophic bypass. It is also possible that inputs of aquatic insects affect the terrestrial ecosystem in some other way that reduces MeHg concentrations. For example, lake-derived midges change the abundance and community composition of shoreline terrestrial arthropods (Dreyer et al. 2012). Perhaps midge inputs shift the arthropod community towards greater dominance of lower trophic position consumers. This does appear to be the case: mean trophic position of consumers sampled at Mývatn at 5 m (mean trophic position, 2.7) was lower than the other three sites (Helluvastjörn at 200 m, mean trophic position, 3.2; Helluvastjörn at 5 m, mean trophic

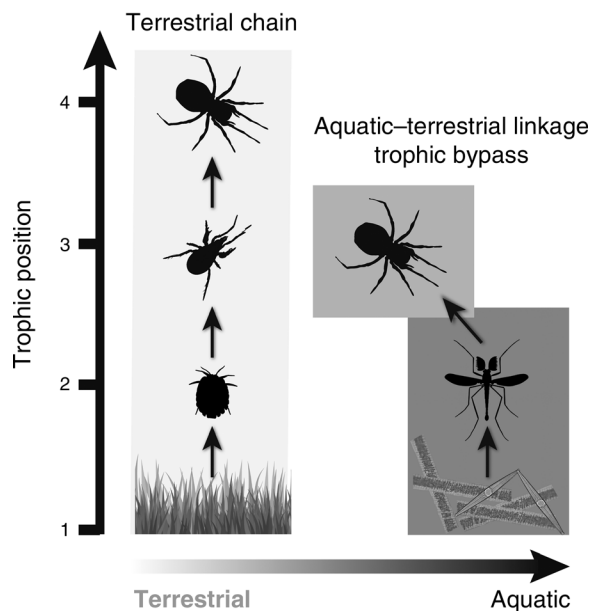


FIG. 4. Conceptual illustration of the trophic bypass concept. Consumption of aquatic resources by terrestrial consumers, such as spider, corresponds with lower MeHg concentrations, possibly due to shorter food chains or some other change in the food web or ecosystem.

position, 3.3; Mývatn at 200 m, mean trophic position, 3.2).

Deposition of lake-derived midges can also fertilize terrestrial systems (Gratton et al. 2008), thereby increasing ecosystem productivity and food quality (as estimated by tissue C:N or C:P). Consistent with this, among our study sites, plants nearshore at Mývatn had the lowest C:N (22) compared to locations either away from shore (Mývatn at 200 m, C:N = 41) or at the low midge Helluvastjörn (C:N = 42 and 43, at 5 m and 200 m in plants, respectively). Bultman et al. (2014) found similar results in *Salix phylicifolia* shrubs. Leaf N concentrations were 8–11% higher at Mývatn compared to Helluvastjörn. In freshwater systems, increased food quality has been found to reduce the trophic transfer efficiency of MeHg as a consequence of somatic growth dilution and, to a lesser extent, reduced consumption rates (Pickhardt et al. 2002, Ward et al. 2010). If this were a factor, the prediction is that Mývatn at 5 m would exhibit a reduced biomagnification factor (i.e., lower slope in the trophic position–MeHg relationship) compared to unfertilized sites. Interestingly, the opposite was true. Mývatn at 5 m was the only one of the four sites with a significant relationship between trophic position and MeHg, and the relationship was positive at this site:  $\ln(\text{MeHg}) = 4.07 \times \ln(\text{trophic position}) - 0.86$ ;  $P < 0.0001$ ,  $r^2 = .53$ ;  $F = 34.4$ ,  $df = 1, 31$ . Thus, nutrient enrichment caused by midge deposition does not appear to lead to reduced biomagnification, as predicted from the growth dilution hypothesis. We do note that trophic position values of consumers from Mývatn at 5 m tend

to be lower than the other three sites, indicating a contributing role of the trophic bypass idea.

Methylmercury concentrations in terrestrial arthropods from our Icelandic study sites averaged  $75 \pm 63$  ng/g dw, which is high relative to the few previous literature reports for sites lacking point source contamination. Mean MeHg in spiders from other uncontaminated sites did not exceed 25 ng/g dw. In remote sites from New Hampshire (White Mountain National Forest), MeHg concentrations in spiders were  $16 \pm 4$  ng/g (Wyman et al. 2011). Similar concentrations were reported in spiders from uncontaminated reference sites in Virginia ( $24 \pm 14$  ng/g). In contrast, MeHg concentrations in spiders near Hg-contaminated rivers were much higher ( $608 \pm 720$  ng/g (Cristol et al. 2008)). One possible reason for the high MeHg in terrestrial arthropods could relate to Hg emitted from volcanic eruptions or atmospheric chemical pollution from nearby geothermal power production (see Coderre and Steinhórsson 1977, Rühling and Steinnes 1998, Kristmannsdóttir and Ármannsson 2003). This phenomenon could explain why, despite the remoteness and low industrial activity in northeastern Iceland, MeHg concentrations in terrestrial arthropods are elevated, although it is unclear why MeHg concentrations for midges are not similarly elevated. Notably, studies have found high MeHg concentrations in Icelandic lamb (Reykjal and Thorlacius 2001) and mosses (Rühling and Steinnes 1998).

In summary, terrestrial arthropods showed high reliance on emergent aquatic insects adjacent to our high midge lake (Mývatn), as measured using stable carbon isotopes. High aquatic reliance corresponded with low trophic position and low MeHg concentrations. Our findings highlight the role of aquatic prey, in this case midges, in depressing MeHg in terrestrial consumers via both direct and indirect mechanisms. Also, our results contrast with previous studies that showed increased pollutant levels in terrestrial consumers adjacent to streams (Walters et al. 2008, Walters et al. 2010, Raikow et al. 2011, Mogren et al. 2013). Despite recent advances in the ecological understanding of aquatic–terrestrial linkages (Nakano and Murakami 2001, Baxter et al. 2005, Hoekman et al. 2011, Dreyer et al. 2012, Hoekman et al. 2012), our research highlights the fact that we still know little about the potential implications of such linkages for terrestrial food webs and ecosystems, particularly with regard to societally important applied issues such as contaminant bioaccumulation. Considering the widespread nature of aquatic to terrestrial linkages on many landscapes (Bartrons et al. 2013), understanding these linkages is important both for the use of contaminant such as MeHg as ecological tracers, as well as for understanding the cycling of contaminants in the environment.

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#### LITERATURE CITED

- Arbuckle, J. L. 2003. AMOS 5.0.1. AMOS Development Corporation, Spring House, Pennsylvania, USA.
- Back, R. C., P. R. Gorski, L. B. Cleckner, and J. P. Hurley. 2003. Mercury content and speciation in the plankton and benthos of Lake Superior. *Science of the Total Environment* 304:349–354.
- Bartrons, M., M. Papeş, M. Diebel, C. Gratton, and M. J. Zanden. 2013. Regional-level inputs of emergent aquatic insects from water to land. *Ecosystems* 16:1353–1363.
- Bates, D., and M. Maechler. 2009. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-32. R Foundation for Statistical Computing, Vienna, Austria. [www.r-project.org](http://www.r-project.org)
- Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology* 50:201–220.
- Bloom, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1010–1017.
- Bultman, H., D. Hoekman, J. Dreyer, and C. Gratton. 2014. Terrestrial deposition of aquatic insects increases plant quality for insect herbivores and herbivore density. *Ecological Entomology* 39:419–426.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York, New York, USA.
- Cabana, G., and J. B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable isotopes. *Nature* 372:255–257.
- Carson, R. 1962. Silent Spring. Houghton Mifflin, Boston, Massachusetts, USA.
- Chételat, J., M. Amyot, L. Cloutier, and A. Poulain. 2008. Metamorphosis in chironomids, more than mercury supply, controls methylmercury transfer to fish in high arctic lakes. *Environmental Science and Technology* 42(24):9110–9115.
- Coderre, J. A., and S. Steinhórsson. 1977. Natural concentrations of mercury in Iceland. *Geochimica et Cosmochimica Acta* 41:419–424.
- Cristol, D. A., R. L. Brasso, A. M. Condon, R. E. Fovargue, S. L. Friedman, K. K. Hallinger, A. P. Monroe, and A. E. White. 2008. The movement of aquatic mercury through terrestrial food webs. *Science* 320:335.
- Downs, S. G., C. L. Macleod, and J. N. Lester. 1998. Mercury in precipitation and its relation to bioaccumulation in fish: a literature review. *Water, Air, and Soil Pollution* 108:149–187.
- Dreyer, J., D. Hoekman, and C. Gratton. 2012. Lake-derived midges increase abundance of shoreline terrestrial arthropods. *Oikos* 121:252–258.
- Gill, G. A., and W. F. Fitzgerald. 1987. Picomolar mercury measurements in seawater and other materials using stannous chloride reduction and two-stage gold amalgamation with gas phase detection. *Marine Chemistry* 20:227–243.
- Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge, UK.
- Gratton, C., J. Donaldson, and M. J. Vander Zanden. 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in northeast Iceland. *Ecosystems* 11:764–774.
- Gratton, C., and M. J. Vander Zanden. 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. *Ecology* 90:2689–2699.



- Hammerschmidt, C. R., and W. F. Fitzgerald. 2005. Methylmercury in mosquitoes related to atmospheric mercury deposition and contamination. *Environmental Science and Technology* 39(9):3034–3039.
- Hoekman, D., M. Bartrons, and C. Gratton. 2012. Ecosystem linkages revealed by experimental lake-derived isotope signal in heathland food webs. *Oecologia* 170:735–743.
- Hoekman, D., J. Dreyer, R. D. Jackson, P. A. Townsend, and C. Gratton. 2011. Lake to land subsidies: experimental addition of aquatic insects increases terrestrial arthropod densities. *Ecology* 92:2063–2072.
- Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363.
- Hurley, J. P., S. E. Cowell, M. M. Shafer, and P. E. Hughes. 1998. Partitioning and transport of total and methyl mercury in the Lower Fox River, Wisconsin. *Environmental Science and Technology* 32:1424–1432.
- Kristmannsdóttir, H., and H. Ármannsson. 2003. Environmental aspects of geothermal energy utilization. *Geothermics* 32:451–461.
- Le Jeune, A.-H., F. Bourdiol, L. Aldamman, T. Perron, M. Amyot, and B. Pinel-Alloul. 2012. Factors affecting methylmercury biominification by a widespread aquatic invertebrate predator, the phantom midge larvae *Chaoborus*. *Environmental Pollution* 165:100–108.
- Lindegaard, C., and P. M. Jónasson. 1979. Abundance, population dynamics and production of zoobenthos in Lake Mývatn, Iceland. *Oikos* 32:202–227.
- Mogren, C. L., W. E. Walton, D. R. Parker, and J. T. Trumble. 2013. Trophic transfer of arsenic from an aquatic insect to terrestrial insect predators. *PLoS ONE* 8:e67817.
- Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences USA* 98:166–170.
- Oelbermann, K., and S. Scheu. 2002. Stable isotope enrichment ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia* 130:337–344.
- Olson, M. L., L. B. Cleckner, J. P. Hurley, D. P. Krabbenhoft, and T. W. Heelan. 1997. Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades. *Fresenius' Journal of Analytical Chemistry* 358:392–398.
- Parkman, H., and M. Meili. 1993. Mercury in macroinvertebrates from Swedish forest lakes: influence of lake type, habitat, life cycle, and food quality. *Canadian Journal of Fisheries and Aquatic Sciences* 50:521–534.
- Pickhardt, P. C., C. L. Folt, C. Y. Chen, B. Klaue, and J. D. Blum. 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proceedings of the National Academy of Sciences USA* 99:4419–4423.
- Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics* 28:289–316.
- Polis, G. A., M. E. Power, and G. R. Huxel. 2004. *Food webs at the landscape level*. University of Chicago Press, Chicago, Illinois, USA.
- R Development Core Team. 2013. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. [www.r-project.org](http://www.r-project.org)
- Raikow, D. F., D. M. Walters, K. M. Fritz, and M. A. Mills. 2011. The distance that contaminated aquatic subsidies extend into lake riparian zones. *Ecological Applications* 21:983–990.
- Reykaldal, O., and A. Thorlacius. 2001. Cadmium, mercury, iron, copper, manganese and zinc in the liver and kidney of the Icelandic lamb. *Food Additives and Contaminants* 18:960–969.
- Rühling, A., and E. Steinnes. 1998. Atmospheric heavy metal deposition in Europe 1995–96. *Rounborgs grafikse hus*, Copenhagen, Denmark.
- Tachet, H. 2010. *Invertébrés d'eau douce: systématique, biologie, écologie*. CNRS Editions, Paris, France.
- Tsui, M. T. K., J. D. Blum, S. Y. Kwon, J. C. Finlay, S. J. Balogh, and Y. H. Nollet. 2012. Sources and transfers of methylmercury in adjacent river and forest food webs. *Environmental Science and Technology* 46:10957–10964.
- Tweedy, B. N., R. W. Drenner, M. M. Chumchal, and J. H. Kennedy. 2013. Effects of fish on emergent insect-mediated flux of methyl mercury across a gradient of contamination. *Environmental Science and Technology* 47:1614–1619.
- US EPA. 1996a. Method 1631: mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. EPA 821-R-96-012. US EPA, Office of Water, Washington, D.C., USA.
- US EPA. 1996b. Method 1669: method for sampling ambient water for determination of metals at EPA ambient criteria levels. EPA 821-R-96-011. US EPA, Office of Water, Washington, D.C., USA.
- Vander Zanden, M. J., and J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecological Monographs* 66:451–477.
- Walters, D. M., K. M. Fritz, and R. R. Otter. 2008. The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. *Ecological Applications* 18:1835–1841.
- Walters, D. M., M. A. Mills, K. M. Fritz, and D. F. Raikow. 2010. Spider-mediated flux of PCBs from contaminated sediments to terrestrial ecosystems and potential risks to arachnivoracious birds. *Environmental Science and Technology* 44:2849–2856.
- Ward, D. M., K. H. Nislow, and C. L. Folt. 2010. Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. Pages 62–83 in R. S. Ostfeld and W. H. Schlesinger, editors. *Year in ecology and conservation biology 2010*. Wiley-Blackwell, Malden, Massachusetts, USA.
- Wiener, J. G., D. P. Krabbenhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury. Pages 409–464 in D. J. Hoffman, B. A. Rattner, G. A. Burton, and J. Cairns, editors. *Handbook of toxicology*. Second edition. CRC Press, Boca Raton, Florida, USA.
- Wyman, K. E., N. L. Rodenhouse, and M. S. Bank. 2011. Mercury bioaccumulation, speciation, and influence on web structure in orb-weaving spiders from a forested watershed. *Environmental Toxicology and Chemistry* 30:1873–1878.

## SUPPLEMENTAL MATERIAL

## Ecological Archives

The Appendix is available online: <http://dx.doi.org/10.1890/14-0038.1.sm>