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Dissolved organic carbon modulates mercury concentrations in insect subsidies from streams to terrestrial consumers

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Abstract

Mercury (Hg) concentrations in aquatic environments have increased globally, exposing consumers of aquatic organisms to high Hg levels. For both aquatic and terrestrial consumers, exposure to Hg depends on their food sources as well as environmental factors influencing Hg bioavailability. The majority of the research on the transfer of methylmercury (MeHg), a toxic and bioaccumulating form of Hg, between aquatic and terrestrial food webs has focused on terrestrial piscivores. However, a gap exists in our understanding of the factors regulating MeHg bioaccumulation by non-piscivorous terrestrial predators, specifically consumers of adult aquatic insects. Because dissolved organic carbon (DOC) binds tightly to MeHg, affecting its transport and availability in aquatic food webs, we hypothesized that DOC affects MeHg transfer from stream food webs to terrestrial predators feeding on emerging adult insects. We tested this hypothesis by collecting data over two years from 10 low-order streams spanning a broad DOC gradient in the Lake Sunapee watershed in New Hampshire. We found that streamwater MeHg concentration increased linearly with DOC concentration. However, streams with the highest DOC concentrations had emerging stream prey and spiders with lower MeHg concentrations than streams with intermediate DOC concentrations; a pattern that is similar to fish and larval aquatic insects. Furthermore, high MeHg concentrations found in spiders show that MeHg transfer in adult aquatic insects is an overlooked but potentially significant pathway of MeHg bioaccumulation in terrestrial food webs. Our results suggest that although MeHg in water increases with DOC, MeHg concentrations in stream and terrestrial consumers did not consistently increase with increases in

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streamwater MeHg concentrations. In fact, there was a change from a positive to a negative relationship between aqueous exposure and bioaccumulation at streamwater MeHg concentrations associated with DOC above around 5 mg/L. Thus, our study highlights the importance of stream DOC for MeHg dynamics beyond stream boundaries, and shows that factors modulating MeHg bioavailability in aquatic systems can affect the transfer of MeHg to terrestrial predators via aquatic subsidies.

Keywords

aquatic insects; aquatic-terrestrial linkages; bioaccumulation; dissolved organic carbon; emergence; food web; mercury; non-linearity; resource subsidy; spiders; streams; threshold

INTRODUCTION

In addition to providing energy and nutrients, materials moving across ecosystem boundaries can be environmental stressors for the recipient food web (Rasmussen and Vander Zanden 2004, Paetzold et al. 2011, Kraus et al. 2014a). Aquatic contaminants such as polychlorinated biphenyls (PCBs) and heavy metals are examples of such potential environmental stressors, and their movement and propagation through aquatic to terrestrial food webs via consumption of aquatic prey by terrestrial predators has been well documented (Tremblay et al. 1998, Christensen et al. 2005, Walters et al. 2008, Raikow et al. 2011). However, little is known about the mechanisms controlling the movement of these contaminants. The ubiquity of aquatic contaminants and their potential to have negative impacts on wildlife (Scheuhammer et al. 2007, Brar et al. 2010, USEPA 2010), as well as the importance of aquatic prey for terrestrial consumers (Willson and Halupka 1995, Baxter et al. 2005), highlight the need for understanding what factors affect the movement of aquatic contaminants into terrestrial food webs.

Mercury (Hg) concentrations in aquatic environments have increased globally, exposing terrestrial consumers and ecosystems to elevated Hg levels (AMAP/UNEP 2013, Rowse et al. 2014). Hg is a toxic and naturally occurring element, but human activities such as the burning of coal for power production and artisanal small-scale gold mining have increased its presence in the environment by two-to-three fold since the Industrial Revolution (AMAP/UNEP 2013). The anthropogenic, less-toxic inorganic forms of Hg are transformed by microbes –mainly methanogens, and sulfate- and iron-reducing bacteria – into the more toxic methylmercury (hereafter: MeHg), which is prone to bioaccumulation, primarily in anaerobic aquatic environments (Fitzgerald and Clarkson 1991, Gilmour et al. 1992, Fleming et al. 2006, Hamelin et al. 2011, Kidd et al. 2012). Because aquatic food webs are disproportionately burdened by MeHg bioaccumulation (Driscoll et al. 2007, Chasar et al. 2009), studying the movement of Hg from those habitats onto land via linked aquatic and terrestrial (hereafter aquatic-terrestrial) processes is particularly relevant.

Emerging aquatic insects are potentially important vectors for MeHg transport from aquatic ecosystems to the diverse array of terrestrial consumers that feed on them (Power et al. 2004, Ballinger and Lake 2006, Speir et al. 2014). Researchers have reported high MeHg concentrations in non-piscivorous (e.g., insectivorous) terrestrial consumers that may be

forms strong bonds with Hg species (Cai et al. 1999), potentially transporting both inorganic Hg (Hg available for methylation) and MeHg from methylating sites, we hypothesized that streamwater inorganic Hg and MeHg concentration would increase as DOC increases. We also predicted that DOC concentration would modulate MeHg bioaccumulation in both emerging stream insects and their riparian predators, with MeHg concentration in emerging stream insects and spiders increasing with streamwater MeHg concentration up to a DOC concentration above which the relationship between streamwater MeHg and MeHg bioaccumulation would decrease.

METHODS

To investigate the effects of DOC and streamwater Hg and MeHg on the concentration of MeHg in emerging aquatic insects and terrestrial predators, we studied 10 streams in the Lake Sunapee watershed (Sullivan County, New Hampshire, USA, Appendix S1). This region has little industrial or commercial development, and only low-density residential development. Streams in this watershed were ideal because the region receives atmospheric Hg deposition (NADP 2015b, a), and the streams exhibit mean DOC concentrations between 2.09 and 12.52 mg C/L.

DOC

To explore the degree to which the concentration of DOC was associated with MeHg in emerging aquatic and riparian consumers, we collected one DOC sample per stream on 10 total sampling dates between May and July of 2011 and 2012. To ensure comparability of results, all streams were sampled the same day, in each sampling date. Stream water samples were collected in flowing water using a 60 mL syringe and filtered in the field using pre-combusted Whatman GF/F filters rinsed three times with stream water prior to collection. Samples were kept on ice and then frozen until they were analyzed at the Analytical Lab, Cary Institute of Ecosystem Studies, NY. Samples were analyzed on a Shimadzu® 5050 TC Analyzer as described by Findlay et al. (2010). For each stream we calculated a mean DOC concentration (mg C/L) for each year.

Stream temperature, pH and nutrient

We measured temperature, pH and nutrients in each study stream to account for potential effects of these stream variables on the MeHg concentration in stream water (Ullrich et al. 2001), as well as in emerging aquatic insects and riparian consumers. Temperature and pH were measured *in situ* using a pH/Conductivity/TDS Hanna meter (model number HI98129) at the same time samples were collected for DOC analysis. Due to logistical constraints temperature and pH were not sampled for two streams in 2011 (Appendix S2). We collected one sample for total phosphorus (TP) and total nitrogen (TN) in April, June and July 2012. Samples were kept on ice and then frozen until analysis. For TN, we used a basic persulfate digestion and measured nitrate using the second-derivative method (Crumpton et al. 1992). For TP, we used the persulfate digestion and the molybdate colorimetric method (Method 4500-P, American Public Health Association 1980).

Streamwater Hg and MeHg

To determine the Hg available for uptake by bacteria and algae and subsequent incorporation by stream consumers, we measured MeHg and total Hg (sum of MeHg and inorganic Hg) concentration in streamwater collected in June 2011, and April and June 2012, following methods in Chen et al. (2012). We collected water in certified clean polyethylene terephthalate bottles (500 mL) using the USEPA “clean hands/dirty hands” method (EPA Method 1669) by submergence into free-flowing surface water, following three rinses with stream water. We took duplicate samples and stored them double-bagged in 1% trace metal grade nitric acid-rinsed plastic bags on ice in the dark until filtering. The samples were filtered within 6 hrs of collection through pre-combusted (4 hrs at 550 °C) quartz fiber filters (particle retention of 0.3 µm). Blanks were 125 mL of distilled de-ionized (DI) water filtered before each duplicate set. Equipment was rinsed with DI water and 1% Optima grade HCl acid between samples from different streams. The filtrate was stored in certified clean 250 mL amber glass bottles in the dark at 4 °C before analyzing the actual form of the Hg molecules or ions present in the samples (i.e., Hg speciation analysis).

Terrestrial predator

We collected adult females of *Tetragnatha elongata* (Araneae: Tetragnathidae), a long-jawed orb weaver spider, from their webs using acid cleaned, non-metallic sampling gear. *T. elongata*'s low mobility and high dependence on aquatic insects (Gillespie 1987, Aiken and Coyle 2000) made this species ideal for our study because we could be confident that most of the Hg in the spiders came from consuming emerging stream insects rather than terrestrial insects. We collected spiders in June 2011, and June and July 2012. Spiders were found on their webs overhanging the rocks and riparian vegetation within 1 m from the stream at a maximum height of 2 m. Since *T. elongata* in the north temperate zone has one generation per year and overwinters in the antepenultimate instar (Aiken and Coyle 2000) performing collections in these months allowed us to specifically sample adult females of *T. elongata*. Thus we could control for spider gender and instar, which may confound Hg accumulation among individuals (Driscoll et al. 1994). Additionally, working only with females allowed us to avoid confounding potential diet differences between sexes (Sanzone et al. 2003). Females were freeze-dried in a trace metal-clean laboratory prior to Hg speciation analysis.

Quantifying spider diet

To characterize *T. elongata*'s diet we collected webs from all 10 streams during the last week of June 2011, the second and third weeks of May 2012, and the third weeks of June and July 2012. To identify the peak time of day for spider predation we performed collections at each stream as early as 0500 hrs and as late as 0100 hrs, and at multiple times in between, in June 2011 and May 2012. Once the time of peak predation was established, collections were made during the peak, which was between 2100 hrs and 0100 hrs, in June and July 2012. These months coincide with the bulk of aquatic insect emergence in these streams (Baer et al., unpublished data). Collections were performed from webs with active spiders to ensure that the insects reflected prey items rather than bycatch from uninhabited webs. We preserved the webs along with prey in 70% ethanol for identification in the lab. Similar to other web-building spiders, tetragnathids are non-selective predators (Lesar and Unzicker

1978, Culin and Yeargan 1982), making prey collection from webs a robust way to characterize their diet. Webs collected in May 2012 were occupied by juvenile spiders that had not developed the sexual characters that allow gender identification in the field. June and July collections were from webs occupied by adult females. Prey were identified down to family when possible using McCafferty (1983) and Merritt et al. (2008), otherwise taxa were identified to order. We also counted and measured individual prey insects to obtain number and biomass per web using published length-weight relationships (Sabo et al. 2002). We measured invertebrate body lengths (head to abdomen) under a dissecting scope with a 0.1 mm graduated handheld miniscale. Insects with an incomplete body (15% of total insects collected) were assigned the average body mass of the corresponding taxa in the same stream. To characterize the diet of spiders, and to estimate the dominant prey (by biomass) for Hg analysis in each stream, we took an average of the number and biomass of emerging aquatic insects in each web, and weighted this average by the number of webs collected each month, which allowed us to standardize diet for differences in collection effort across months in 2012.

Emerging stream insects

To measure the Hg concentration of the spider's prey we collected emerging stream insects in 2012 with constructed metal-free emergence traps (Appendix S3). Spider webs collect heavy metals from the environment (Hose et al. 2002, Xiao-li et al. 2006), thus using emerging stream insects rather than those insects collected directly from spider webs allowed us to avoid insect contamination with web material. Additionally, collecting emerging insects rather than those in webs allowed us to obtain sufficient biomass (>20 mg) for Hg speciation analyses of most of *T. elongata*'s potential prey taxa. Although terrestrial prey can alter MeHg concentrations in riparian predators (Bartrons et al. 2015), our focal riparian predator relies heavily on stream insect subsidies for its diet, with >97% of the potential prey biomass collected from the webs being aquatic origin (see Results). Thus we only analyzed MeHg concentrations in emerging stream prey. Emergence trap collection canisters (1 L Nalgene bottles) were acid cleaned sequentially in Citranox soap and DI water (10 mL soap in 2 L water), 1 N nitric acid (280 mL trace-metal-grade acid to 4 L DI water), and 1.4 N hydrochloric acid (1 L trace-metal-grade acid to 4 L DI water). Emergence traps had a capture surface area of 0.50 m². We deployed five traps per stream from May through July and collected insects from the traps twice a week. We removed the collecting canisters and placed them on ice until sorting. Insects were sorted and identified to family using acid-cleaned, non-metallic sampling gear in a laboratory set up for trace metal sample processing.

We selected a subset of the emerging insect taxa to analyze for Hg speciation based on their biomass dominance in *T. elongata*'s webs (Table 1). This was necessary due to the low biomass of emerging stream insects collected and the biomass required for accurate Hg speciation analyses (>20 mg). Thus we could not test the relationship between MeHg concentration in spiders and the MeHg concentrations of all the spiders' insect prey. For Chironomidae, the only biomass-dominant group present across all the focal streams, we had sufficient biomass to pool individuals by month for May, June and July. For the rest of

the prey taxa, we analyzed MeHg in aquatic prey that, combined, made up at least 70 % of the prey biomass in the spiders' webs in 2012.

We freeze-dried the insects and pooled individuals collected during the three months of sampling to obtain sufficient biomass for Hg speciation analyses. If the minimum biomass for Hg speciation analyses was not obtained from the emergence traps for one of these prey taxa, we analyzed the next-most-abundant taxon by biomass in the spider's webs until 80% of total prey biomass in webs was reached (Table 1, Appendix S4). However, this minimum percent biomass was not attained for five sites where we were unable to obtain 20 mg of sample for some of the taxa (Appendix S4). For those sites we analyzed taxa that comprised between 34% and 69% of the total biomass in the spider's web (Table 1, Appendix S4).

Hg speciation analysis

All Hg speciation analyses were performed by the Dartmouth Trace Element Analysis Core Facility using isotope dilution gas chromatography-inductively coupled plasma mass spectrometry (ICP-MS). Filtrates from water samples were spiked with labeled Hg and MeHg to give a ~1:1 ratio of spike to natural ^{202}Hg concentration of enriched inorganic ^{199}Hg (Hg_i) and enriched methyl ^{201}Hg (MeHg), and then extracted in 10 ml of 4M HNO_3 overnight at 60°C. Ionic Hg and MeHg were determined in the water sample filtrate following the ultra-low level methods described in Jackson et al. (2009). We calculated mean streamwater inorganic Hg and MeHg concentration for samples collected in each year. For determination of Hg speciation in biota, samples were freeze-dried, spiked with an appropriate amount of enriched inorganic ^{199}Hg and enriched Me^{201}Hg (Taylor et al. 2008) and then extracted in 2–3 ml of tetramethyl ammonium hydroxide, 25% w/v. MeHg and inorganic Hg were determined by species-specific isotope dilution purge and trap ICP-MS. Total Hg was calculated as the sum of MeHg and inorganic Hg (Point et al. 2007, Taylor et al. 2008). The method detection limit (MDL) for streamwater averaged 0.01 ng/L \pm 0.01 (mean \pm 1 SE) for MeHg and 0.1 ng/L \pm 0.01 for inorganic Hg. Percent recovery for streamwater samples spiked in the range of sample concentrations averaged 107.5% \pm 6.7 for MeHg and 96.2% \pm 2.3 for inorganic Hg. The MDL for the spider samples averaged 0.4 ng \pm 0.1 for both MeHg and inorganic Hg. Spiders were large enough (>20 mg) that we analyzed three individuals from each stream in each year for MeHg and inorganic Hg, then calculated the mean MeHg concentration in the spiders for each stream in each year. For the emerging aquatic insects, the MDL was 0.7 ng \pm 0.4 for MeHg and 1.5 ng \pm 0.7 for inorganic Hg. Quality control for biological samples was further evaluated by analyzing a standard reference material (SRM) for every batch of 20 samples. Recovery in SRMs averaged 101.6% \pm 2.5 for MeHg (DORM 4, NRC, Ottawa, 354 ng/g MeHg). For THg, recovery was 100.3% \pm 3.0 (DORM 4, NRC, Ottawa, 410 ng/g THg).

Data analysis

To test the hypothesis that streamwater inorganic Hg and MeHg concentrations increase with DOC, we used a general linear model relating average summer DOC concentration and average summer streamwater MeHg concentration, including an indicator variable for the year (2011 or 2012). Associations among streamwater MeHg, streamwater inorganic Hg, and stream parameters (temperature, pH, TN, and TP) were quantified using Spearman rank

correlation coefficients. We used Spearman rank correlation instead of Pearson's correlation due to the presence of outliers and non-linearities in the relationships.

Additionally, we tested our prediction that DOC concentration would modulate MeHg bioaccumulation in chironomid and non-chironomid prey and spiders using general linear models. Specifically, we tested whether DOC concentration and streamwater MeHg concentration interacted to determine the MeHg concentrations of these focal organisms. Explanatory variables were centered to minimize multicollinearity when interactions were included in the model (Chatterjee and Hadi 2012). We examined the degree of multicollinearity using the variance inflation factor (VIF). Although DOC concentration and streamwater MeHg concentrations were linearly related, all VIFs were lower than 3.3, indicating that effects of multicollinearity were minimal (Chatterjee and Hadi 2012). Because spiders were collected in 2011 and 2012, we included year of collection in that model. We analyzed chironomids separately from other stream prey to obtain information on the temporal variability in MeHg concentration in emerging stream insects, as well as the variability in prey MeHg concentration within a family. Thus, we included month of collection (May, June or July) as an indicator variable in that model. We removed one outlier (563.6 ng/g) from the chironomid data set after several outlier analyses (Mahalanobis distances, Jackknife distances, and Cook's D influence). Following a marginally significant outcome for the spiders, we performed a posthoc power analysis in JMP using an α of 0.05.

Data were checked to confirm that they met the assumptions for each statistical test. We used a Breusch-Pagan test (Breusch and Pagan 1979) to confirm that the residual variance did not depend on month or year ($P > 0.05$), and a Durbin-Watson test to confirm that there was no temporal autocorrelation ($P > 0.05$). The Breusch-Pagan test was performed using R statistical software (R development core team 2013), while all other statistical analyses were performed using JMP statistical software (SAS institute Inc. 2013).

RESULTS

Mercury concentrations in streamwater samples were dominated by inorganic Hg ($79.3\% \pm 2.5$; mean ± 1 SE). Streamwater inorganic Hg and MeHg were positively correlated ($\rho = 0.64$, $P = 0.002$). Streamwater MeHg concentrations across the studied streams increased with DOC concentration, with streamwater MeHg concentrations being marginally higher in 2011 compared to 2012 (Table 2a; Fig. 1). Stream temperature ranged from 12.5 to 21.2°C (median 15.2°C, interquartile range 14.1-17.5°C), all streams had circumneutral pH (median 6.7, interquartile range 6.3-6.9), TN ranged from 222.7 to 1125.9 $\mu\text{g/L}$ (median 319.0 $\mu\text{g/L}$, interquartile range 276.4-487.6 $\mu\text{g/L}$), and total phosphorus ranged from 6.4 to 40.5 $\mu\text{g/L}$ (median 13.6 $\mu\text{g/L}$, interquartile range 10.5-20.6 $\mu\text{g/L}$). TP was associated with streamwater MeHg ($\rho = 0.62$, $p = 0.05$), but the rest of the variables were not related to streamwater MeHg concentration ($|\rho| < 0.39$, $P > 0.23$; Appendices S2 and S5).

Aquatic insects were the dominant potential prey of *T. elongata* with a weighted mean percent ± 1 SE of $96.7\% \pm 0.3$ individuals and $97.9\% \pm 1.1$ of the insect biomass in their webs. However, we found considerable variation in the taxonomic composition of the

aquatic insects in *T. elongata*'s diet among the study sites, with Chironomidae being the only taxon that was common in webs at all streams (Table 1).

Most of the total Hg in the emerging insects and the riparian spiders was in the form of MeHg, with $90.8\% \pm 1.0$ MeHg in chironomids, $70.5\% \pm 4.0$ in non-chironomid emerging stream insects, and $78.5\% \pm 1.4$ MeHg in spiders. Non-chironomid and chironomid prey had MeHg concentrations that were much higher than the ambient water (ranging from 180 to 4500 and 480 to 4100 times higher, respectively). Spiders also had MeHg concentrations that were much higher than the stream water, with MeHg concentrations ranging from 690 to 11000 times higher than water in 2011, and from 1400 to 32000 times higher in 2012.

Streamwater MeHg concentration and DOC concentration interacted to explain MeHg concentration in emerging stream insects and riparian spiders, although the effect size and statistical significance varied among responses (Table 2b-d). In both cases, the association between streamwater MeHg concentration and both chironomid and spider MeHg switched from positive at low DOC concentrations to negative as DOC increased (Fig. 3a, c). The interaction was statistically significant for emerging stream insects and marginally significant for spiders, whose MeHg concentrations were lower in 2011 than in 2012 (Table 2b-d; Fig. 2a-f). This marginally significant outcome was associated with low statistical power (0.40). Temperature, pH, and nutrients were not associated with MeHg concentrations in stream emerging insects or spiders ($|p| < 0.52$, $P > 0.15$; Appendices S2 and S5).

To facilitate interpretation of the interactions between aqueous MeHg, DOC, and MeHg bioaccumulation, we used the parameter estimates from the regression models to generate response surfaces for the relationship between streamwater MeHg concentration and biota MeHg concentration as a function of DOC (Fig. 3). The response surface showed similar responses for chironomids and spiders. In streams with DOC concentrations below 5.03 and 4.65 mg C/L (respectively), there was a positive relationship between streamwater and either chironomid or spider MeHg concentrations (Fig. 3a,c). In contrast, there was a negative relationship between streamwater and either chironomid or spider MeHg concentrations in streams with more than 5.03 and 4.65 mg C/L (respectively). For the non-chironomid emerging stream insects, the response surface showed a negative relationship between the insect and streamwater MeHg concentrations regardless of the DOC concentration in the stream, although the slope became increasingly negative as DOC concentration increased (Fig. 3b).

DISCUSSION

To date, most of our knowledge about the factors affecting the movement of Hg through food webs comes from aquatic systems (Driscoll et al. 2007, Chasar et al. 2009). This is likely because MeHg production generally occurs under reducing conditions, such as in wetlands and the anoxic sediment bed of streams and lakes. Furthermore, human health concerns over the neurotoxic effects of eating contaminated fish has intensified research on MeHg bioaccumulation on aquatic food webs (Fitzgerald and Clarkson 1991, Hamelin et al. 2011, Driscoll et al. 2013). However, the strong connection between aquatic and certain terrestrial food webs caused by emerging aquatic insects (Baxter et al. 2005) suggests that

factors affecting Hg methylation and bioaccumulation in aquatic systems may impact a wider array of terrestrial consumers than previously thought. Recent research has focused on the movement of MeHg from freshwater to terrestrial food webs via aquatic insects (Wyman et al. 2011, Kraus et al. 2014b). However, as in aquatic food webs, the factors governing the movement of Hg between aquatic and terrestrial food webs are complex and not clearly understood. Our study shows that higher DOC concentrations may alter the bioavailability of MeHg to aquatic and terrestrial consumers. Taken together, this work extends our understanding of the ‘dark side’ of resource subsidies (*sensu* Walters et al. 2008), by revealing that streamwater chemistry (DOC) may alter the quality of subsidies to terrestrial consumers.

Our data suggest that DOC plays a dual role in MeHg dynamics. On the one hand, DOC concentration may affect MeHg bioaccumulation in emerging stream insects and their spider predators by increasing MeHg transport to streams from methylating sites. In contrast, DOC concentration may reduce the availability of MeHg for bioaccumulation. Streamwater MeHg concentration increased with DOC concentration, reinforcing the idea that DOC concentration is an important factor explaining streamwater MeHg concentrations (Brigham et al. 2009, Tsui et al. 2009). However, the increase in streamwater MeHg concentration did not translate into higher body MeHg concentrations in emerging stream insects and riparian spiders. Although streamwater MeHg concentrations increased with DOC (Fig. 1), our results suggest the MeHg bound to the DOC at higher DOC concentrations may be less readily available for uptake by the microorganisms that are the food source and pathway for MeHg uptake for many stream insects. This threshold effect of DOC on MeHg is supported by the decreasing slope with increasing DOC in all three panels of Fig. 3. Thus as DOC concentration increases there is an attenuation of MeHg bioaccumulation in chironomids and spiders. The negative relationship between streamwater MeHg concentrations and MeHg concentration in non-chironomid emerging stream insects along the entire DOC concentration range, depicted in the response surface, was unexpected, and may be related to the small sample size (n=3) for this group from streams below around 5 mg C/L DOC. Although the interaction between streamwater MeHg concentration and DOC concentration had low power and was marginally significant for the terrestrial consumer (P=0.09), the high reliance of *T. elongata* on emerging stream insects (here and by others, Gillespie 1987, Aiken and Coyle 2000), along with previous evidence of DOC attenuation of MeHg bioaccumulation, support our results.

The dual role of DOC in both transport of MeHg from methylating sites, and uptake attenuation has been hypothesized for years (Driscoll et al. 1994, Driscoll et al. 1995, Ravichandran 2004, Gorski et al. 2008, Tsui et al. 2009, Gerbig et al. 2011). Driscoll et al. (1994, 1995) suggested that MeHg concentrations in yellow perch from Adirondack lakes increased as DOC concentration increased up to 8 mg C/L, after which MeHg in perch was lower. Similarly, recent studies in Arctic lakes have quantitatively shown a threshold-type relationship between DOC and MeHg uptake by amphipods (French et al. 2014). Although the absolute concentration of DOC at which MeHg becomes attenuated has been highlighted, the absolute concentration may be context dependent (i.e., variable due to differences in consumer trophic level or system-specific DOC quality). Nonetheless, the attenuation of MeHg bioaccumulation and a threshold-type effect of DOC are common and

have application to understanding MeHg flow through food webs. The present study extends the observation of the threshold effects of DOC to emerging aquatic insects and their riparian predators along these streams. To our knowledge, this is the first study showing that attenuation of MeHg bioaccumulation at high DOC concentrations extends from aquatic to terrestrial food webs via predation on aquatic insects.

Obtaining data on MeHg concentrations in multiple food web compartments is typically limited by the costs of sample analyses and the need for a minimum biomass of prey, which is difficult to obtain for small organisms such as plankton and emerging aquatic invertebrates. Although stream characteristics such as cold temperatures, low pH, and low nutrient concentrations have been associated with high MeHg bioaccumulation in other systems (Ullrich et al. 2001, Chen and Folt 2005, Jardine et al. 2013, Lavoie et al. 2013), these characteristics did not explain the variability in MeHg concentrations in our focal organisms across the studied streams, perhaps in part because of the limited range in these variables across streams. Other biotic factors not investigated in this study have also been shown to impact MeHg bioaccumulation including microbial species composition and sediment or periphyton methylation potential in lakes and streams (Macalady et al. 2000, Hamelin et al. 2011, Buckman et al. 2015). These factors were beyond the scope of this study. However, the wide range of DOC concentrations among the focal streams in the Lake Sunapee system likely enabled us to detect effects of DOC on MeHg bioaccumulation.

Although it was beyond the scope of this study to speciate DOC, we hypothesize that heterogeneity in the functional groups of DOC might explain its attenuating effect on MeHg bioaccumulation observed at high DOC concentrations. Landscape variables, such as the presence of wetlands and differences in soil types, may be influencing the variation in DOC concentrations, and these factors may also affect the chemical structure of the DOC in the study streams (Nguyen and Hur 2011). Increases in DOC concentrations have been associated with increases in less bioavailable humic acids and aromatic DOC (Wu et al. 2007, Tsui and Finlay 2011, French et al. 2014). Thus, high DOC streams may have high MeHg concentrations but low bioavailability of it because the DOC-MeHg complexes are too large for microbial uptake or have strong binding sites that restrict ligand exchange (Kerner et al. 2003, Gorski et al. 2006). Another potential mechanism decreasing MeHg bioavailability is a decrease in Hg bioavailability to methylating bacteria (Chiasson-Gould et al. 2014). However, the positive, linear relationship between streamwater MeHg concentration and DOC suggests that the rate of methylation is not a limiting factor in our high DOC streams.

Differences in dietary sources, physiologies of the prey, and assimilation efficiency of taxa by predators may be among the factors influencing how DOC concentration affects organismal MeHg concentration within and across ecosystems. These factors can alter the effects of water chemistry on MeHg bioaccumulation (Jardine et al. 2013). For example, researchers found a decrease in MeHg bioaccumulation above a DOC concentration of 5 mg C/L in phytoplankton, seston, and hydropsychid larvae (Gorski et al. 2008, Tsui and Finlay 2011), while this concentration was reported at 8 mg C/L for amphipods and fish (Driscoll et al. 1994, French et al. 2014). Thus, the effect of DOC on MeHg concentration is inherently variable, and this variability is compounded with each additional trophic level, potentially

explaining the variability in spider MeHg concentration. Because percent MeHg typically increases with trophic level (Mason et al. 2000, Driscoll et al. 2007, Chen et al. 2009), and spider percent MeHg was less than that of chironomids but greater than that of non-chironomid emerging stream insects, non-chironomids are likely a more important source of assimilated food, and therefore MeHg, than chironomids. This suggests that the relative abundance of diet items found in webs may not be an accurate reflection of what was actually consumed and assimilated. Further research is needed to understand the factors affecting the role that DOC plays in MeHg's movement through different parts of the food web and in different food webs.

Furthermore, temporal changes in water chemistry and environmental factors affect MeHg availability and bioaccumulation in food webs (Chen et al. 2012, Eklof et al. 2015). In this study, there was little temporal variation of MeHg in chironomids during summer, however there may be large interannual variation. Although streamwater MeHg and DOC concentrations were higher in 2011, spiders collected in 2011 had lower MeHg concentrations than those collected in 2012. These interannual differences in spider MeHg are likely the result of spiders collected in 2012 having fed as juveniles on stream insects emerging in 2011; hence, MeHg bioaccumulated by the juveniles may have impacted MeHg loads in adult spiders. Further research is warranted to understand how temporal variation of DOC and streamwater MeHg concentrations affect MeHg bioaccumulation in stream-terrestrial food webs. Thus there is inherent variability in bioaccumulation of MeHg in terrestrial consumers due to myriad factors. The interaction between MeHg and DOC concentration is decipherable nonetheless, and merits further study.

Spiders connect aquatic and terrestrial food webs by preying on emerging aquatic insects and serving as prey for terrestrial wildlife such as birds and lizards (Ballinger and Lake 2006, Richardson et al. 2010). Furthermore, some studies report higher Hg concentrations in terrestrial invertivores than omnivores or piscivores, especially birds living near water (Cristol et al. 2008, Keller et al. 2014). Gann et al. (2015) found that *Tetragnatha* sp. near Caddo Lake and its associated wetlands exceeded the minimum tissue MeHg concentrations (19.4 -256 ng/g) that would cause physiological impairment in arachnivoracious birds (USEPA 1995) – and the mean MeHg concentration found in *Tetragnatha* spiders in this study was 3.9 times higher than the highest MeHg concentration reported by Gann et al. (2015). Moreover, MeHg concentrations in spiders in our study sites were also higher than in fish collected from the same streams (Broadley et al. unpublished manuscript), emphasizing the importance of MeHg bioaccumulation across aquatic-terrestrial boundaries in non-piscivorous terrestrial consumers. Because spiders are easy to collect, have enough biomass for accurate Hg speciation analyses, and reflect the effects of DOC on streamwater MeHg concentrations, we propose that they are good indicator species for monitoring Hg risk.

Because MeHg is transmitted through food webs (Hall et al. 1997), increased MeHg in prey is an important risk factor for MeHg exposure in both aquatic and terrestrial consumers. Our study shows that higher DOC concentrations may alter the relationship between MeHg in streamwater and its bioavailability to aquatic and terrestrial consumers. Thus our results highlight the complexity of the relationship between DOC, streamwater MeHg and MeHg concentration in biota, and suggest caution when using DOC as an indicator of biotic

sensitivity to Hg hotspots under the assumption of a linear relationship between DOC and MeHg bioaccumulation in biota (Driscoll et al. 2007, Evers et al. 2007). Moreover, DOC concentrations and chemical composition of freshwaters are changing globally (Findlay 2005, Evans et al. 2006, Dawson et al. 2009, Ritson et al. 2014); how these changes interact with MeHg risk to aquatic and terrestrial organisms warrants further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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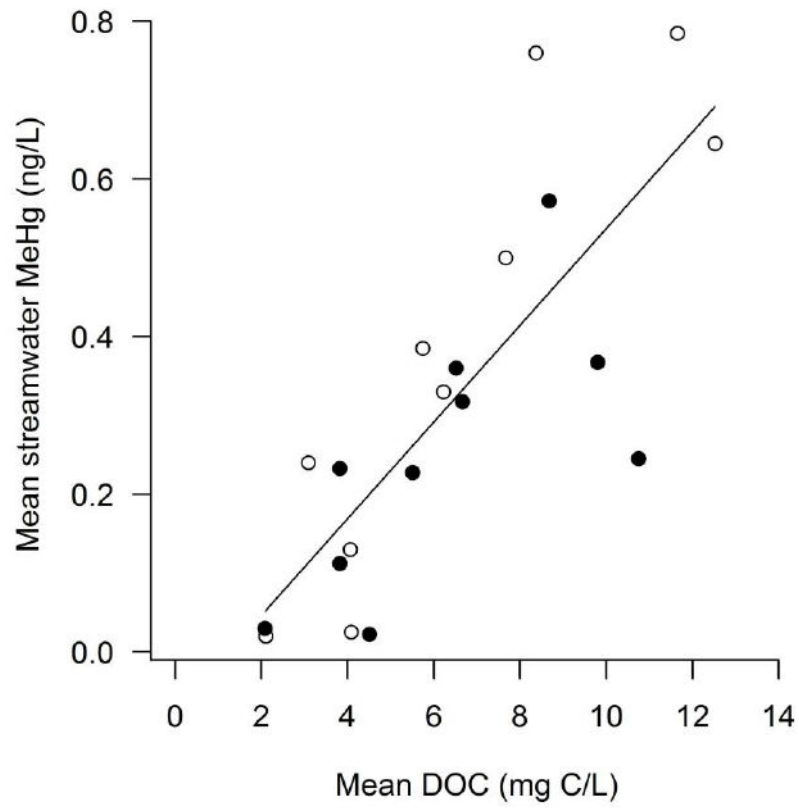


Figure 1. Relationship between streamwater MeHg concentration and DOC concentration in the Lake Sunapee, NH, watershed. Open symbols show data collected in 2011 and closed symbols show data from 2012.

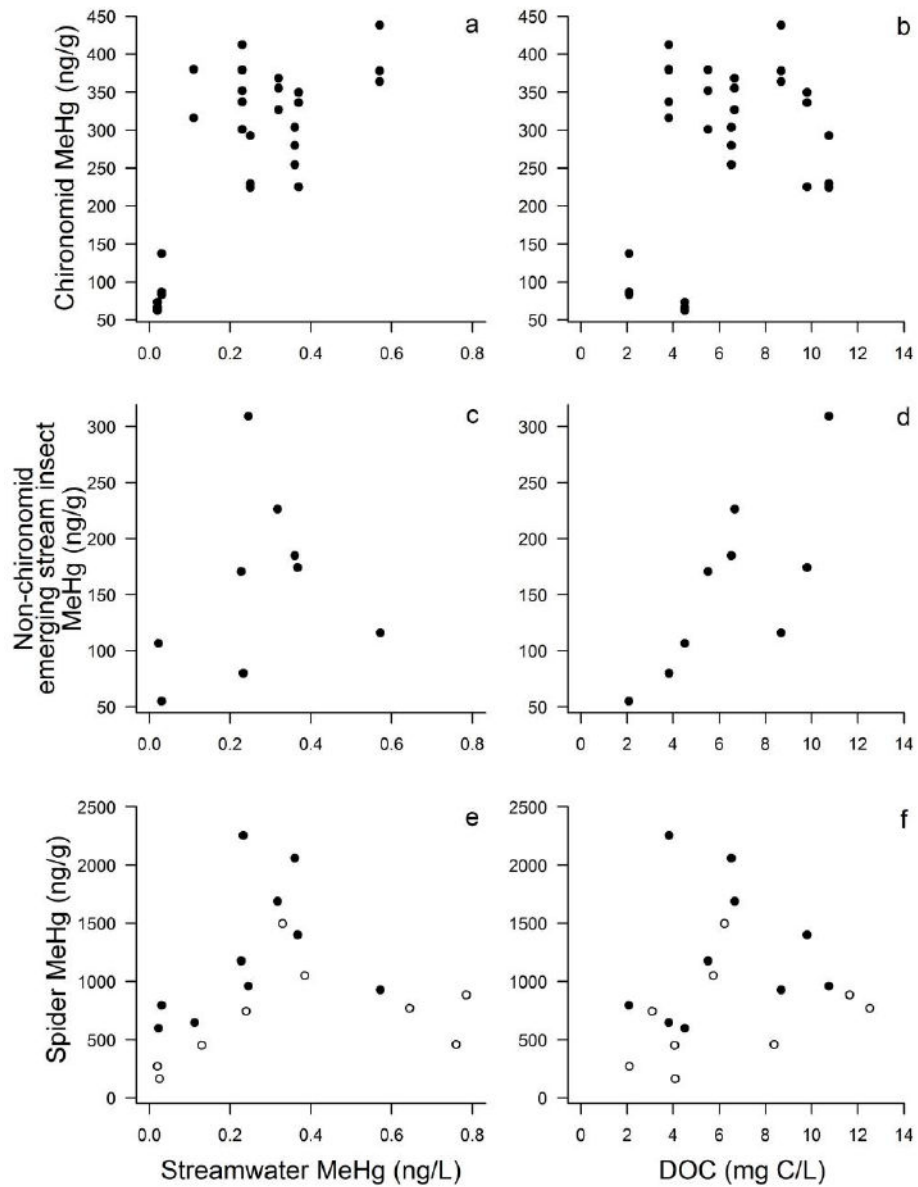


Figure 2. Relationship between mean streamwater MeHg concentration and mean DOC concentration, and mean MeHg concentration in emerging stream insects and riparian spiders for streams in the Lake Sunapee Watershed, NH. Open symbols show data collected in 2011 and closed symbols show data from 2012.

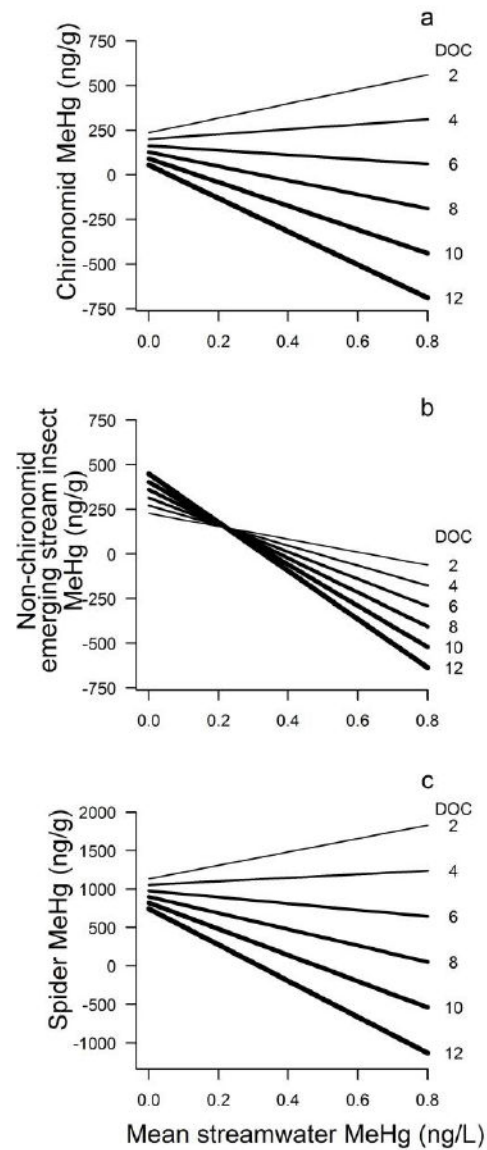


Figure 3. Response surfaces for the interactive effects of DOC concentration and streamwater MeHg concentration on the body MeHg concentration of the focal organisms associated with stream in the Lake Sunapee watershed, NH. Each line represents the relationship between body MeHg concentration and streamwater MeHg concentration at a particular DOC concentration, in mg C/L, based on the statistical models reported in Table 2.

Main elements of *T. elongata*'s diet across the focal streams. Streams are presented from low to high DOC concentrations. Dominant prey taxa are those that made up >70% biomass in the web. Taxa in italics were not analyzed for MeHg concentration due to low (<20 mg) biomass.

Table 1

| Stream | Year | # spider webs | # insects from webs | Mean \pm 1 SE # insect/web | Mean \pm 1 SE biomass/web | Dominant prey taxa by mass |
|-----------------|------|---------------|---------------------|------------------------------|-----------------------------|---|
| Beck Brook | 2011 | 11 | 149 | 13.1 \pm 9.9 | 3.0 \pm 1.5 | Chironomidae (58%) |
| | 2012 | 32 | 92 | 2.7 \pm 0.4 | 1.4 \pm 0.4 | Tipulidae (28%) <i>Tipulidae</i> (42%) |
| Bartlett Brook | 2011 | 6 | 19 | 3.0 \pm 0.7 | 2.4 \pm 1.0 | Chironomidae (34%) |
| | 2012 | 40 | 133 | 3.3 \pm 0.5 | 1.1 \pm 0.3 | Ephemerelellidae (79%) Chironomidae (63%) |
| Bloodgett South | 2011 | 7 | 21 | 3.0 \pm 1.0 | 1.0 \pm 0.6 | Tipulidae (19%) Leuctridae (52%) |
| | 2012 | 27 | 114 | 4.0 \pm 0.9 | 1.5 \pm 0.3 | Chironomidae (44%) Chironomidae (44%) |
| Otter Pond | 2011 | 3 | 11 | 3.0 \pm 2.0 | 0.9 \pm 0.4 | <i>Tipulidae</i> (34%) |
| | 2012 | 29 | 222 | 7.6 \pm 2.3 | 2.0 \pm 0.6 | Chironomidae (100%) Chironomidae (65%) |
| Pike Brook | 2011 | 6 | 18 | 2.7 \pm 0.6 | 2.8 \pm 1.7 | Hydropsychidae (29%) Tipulidae (63%) |
| | 2012 | 42 | 258 | 6.0 \pm 1.5 | 3.2 \pm 0.9 | Heptageniidae (46%) Chironomidae (36%) |
| Kidder Brook | 2011 | 13 | 200 | 15.1 \pm 8.2 | 3.3 \pm 1.4 | Chironomidae (70%) |
| | 2012 | 42 | 193 | 4.5 \pm 0.9 | 2.5 \pm 1.1 | <i>Ephemerelellidae</i> (41%) Chironomidae (25%) |
| King Hill | 2011 | 8 | 10 | 1.25 \pm 0.2 | 0.6 \pm 0.17 | Leuctridae (18%) Leptohyphidae (35%) |
| | 2012 | 35 | 132 | 3.5 \pm 0.6 | 2.0 \pm 0.5 | Tipulidae (28%) Chironomidae (19%) Heptageniidae (18%) Chironomidae (17%) <i>Leuctridae</i> (15%) |

| Stream | Year | # spider webs | # insects from webs | Mean \pm 1 SE # insect/web | Mean \pm 1 SE biomass/web | Dominant prey taxa by mass |
|-----------------|------|---------------|---------------------|------------------------------|-----------------------------|----------------------------|
| Blodgett North | 2011 | 6 | 19 | 3.2 \pm 1.4 | 0.9 \pm 0.5 | Tipulidae (10%) |
| | 2012 | 35 | 170 | 4.7 \pm 1.2 | 1.8 \pm 0.7 | Perlotidae (9%) |
| | | | | | | Chironomidae (97%) |
| | | | | | | Chironomidae (41%) |
| Red Water Creek | 2011 | 10 | 44 | 4.4 \pm 0.8 | 5.0 \pm 2.2 | Perlotidae (39%) |
| | | | | | | Zygoptera (45%) |
| | | | | | | Ephemeroptera (27%) |
| Herrick Cove | 2012 | 30 | 102 | 3.3 \pm 0.5 | 1.9 \pm 0.5 | Chironomidae (32%) |
| | | | | | | Leptophlebiidae (17%) |
| | | | | | | Heptageniidae (17%) |
| | | | | | | <i>Tipulidae</i> (16%) |
| Herrick Cove | 2011 | 13 | 57 | 4.3 \pm 1.1 | 1.4 \pm 0.4 | Chironomidae (96%) |
| | 2012 | 35 | 102 | 2.8 \pm 0.3 | 1.2 \pm 0.2 | Chironomidae (60%) |
| | | | | | | Hydropsychidae (18%) |

Numbers inside parenthesis represent contribution to spider diet. Collections were performed during the last week of June 2011, the second and third weeks of May 2012, and the third weeks of June and July 2012.

Table 2

General linear model results, for streams in the Lake Sunapee Watershed, NH. Explanatory variables were centered for the analyses that included interaction terms.

| Model | Estimate | Parameter Estimates | Std. Error | P |
|--------------------------------------|--|---------------------|------------|---------|
| a. Streamwater [MeHg] | R ² =0.66, F _{2,17} =19.72, p<0.0001 | | | |
| Intercept | -0.07 | 0.07 | | 0.35 |
| [DOC] | 0.06 | 0.01 | | <0.0001 |
| Year | 0.06 | 0.03 | | 0.09 |
| b. Chironomid prey [MeHg] | R ² =0.61, F _{5,23} =9.91, p<0.0001 | | | |
| Intercept | 317.38 | 18.22 | | <0.0001 |
| Streamwater [MeHg] | 671.75 | 113.82 | | <0.0001 |
| [DOC] | -18.18 | 7.02 | | 0.02 |
| Streamwater [MeHg]*[DOC] | -133.42 | 42.07 | | 0.004 |
| Month [May] | -8.82 | 18.48 | | 0.64 |
| Month [June] | 10.26 | 18.48 | | 0.58 |
| c. Non-chironomid prey [MeHg] | R ² =0.76, F _{3,5} =9.52, p=0.02 | | | |
| Intercept | 183.03 | 17.52 | | <0.0001 |
| Streamwater [MeHg] | -161.27 | 106.42 | | 0.19 |
| [DOC] | 22.14 | 6.77 | | 0.02 |
| Streamwater [MeHg]*[DOC] | -99.57 | 39.47 | | 0.05 |
| d. Spider [MeHg] | R ² =0.33, F _{4,14} =3.26, p=0.04 | | | |
| Intercept | 1180.02 | 150.63 | | <0.0001 |
| Streamwater [MeHg] | 1514.89 | 833.74 | | 0.09 |
| [DOC] | -38.94 | 60.55 | | 0.53 |
| Streamwater [MeHg]*[DOC] | -321.67 | 174.83 | | 0.09 |
| Year [2012] | -268.19 | 123.84 | | 0.05 |