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Food web analysis reveals effects of pH on mercury bioaccumulation at multiple trophic levels in streams

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ABSTRACT

Biomagnification processes and the factors that govern them, including those for mercury (Hg), are poorly understood in streams. Total and methyl Hg concentrations and relative trophic position (using $\delta^{15}N$) were analyzed in biofilm and invertebrates from 21 streams in New Brunswick, Canada to assess food web biomagnification leading to the common minnow blacknose dace (*Rhinichthys atratulus*), a species known to have Hg concentrations that are higher in low pH waters. Biomagnification slopes within stream food webs measured using Hg vs. $\delta^{15}N$ or corresponding trophic levels (TL) differed depending on the chemical species analyzed, with total Hg exhibiting increases of 1.3–2.5 per TL (mean slope of total Hg vs. $\delta^{15}N = 0.14 \pm 0.06$ S.D., range = 0.06–0.20) and methyl Hg showing a more pronounced increase of 2.8 to 6.0 per TL (mean slope of methyl Hg vs. $\delta^{15}N = 0.30 \pm 0.08$ S.D., range = 0.22–0.39). While Hg biomagnification slopes through the entire food web (Trophic Magnification Factors, TMFs) were not influenced by water chemistry (pH), dietary concentrations of methyl Hg strongly influenced biomagnification factors (BMFs) for consumer-diet pairs within the food web at lower trophic levels, and BMFs between dace and predatory invertebrates were significantly higher in low pH waters. These analyses, coupled with observations of higher Hg in primary producers in streams with low pH, suggest that pH influences both baseline concentrations and biomagnification of Hg in these systems. Because higher Hg concentrations in the diets of primary consumers and predatory insects in lower pH waters led to lower BMFs, these feeding groups showed insignificant relationships between Hg and pH; thus, altered BMFs associated with dietary concentrations can dampen the effects of environmental conditions on Hg concentrations.

1. Introduction

Contaminants such as mercury (Hg) pose worldwide threats to the health of fish-eating humans and wildlife because they can biomagnify to high concentrations in predators occupying the top of the food web. Much has been learned about Hg biomagnification (defined as an increase in concentration from food to consumer) in lentic and marine food webs through comparative studies, particularly those that use stable nitrogen isotopes ($\delta^{15}N$) as an indicator of trophic level (Kidd et al., 1995; Jardine et al., 2006; Al-Reasi et al., 2007). Far less is known about the dynamics of Hg biomagnification in streams (Chasar et al., 2009), and only recently have studies been conducted on Hg in small lotic systems (Peterson et al., 2007; Brigham et al., 2009; Chasar et al., 2009; Ward et al., 2010) despite the high and rising occurrence of fish consumption advisories in running waters associated with Hg contamination (USEPA 2011) and the potential toxic effects in wildlife that consume prey with high Hg concentrations (Burgess and Meyer, 2008).

Chemical and biological processes at the base of aquatic food webs can have considerable influence on Hg concentrations in higher order consumers. For example, strong correlations exist between Hg concentrations in biota and water chemistry variables such as pH and dissolved organic carbon (DOC) in lakes and streams (Watras et al., 1998; Greenfield et al., 2001; Jardine et al., 2012a) due to increased solubility and greater methylation and uptake of Hg at lower pH and higher DOC by lower trophic level biota (Mason et al., 1996; Pickhardt and Fisher, 2007). Low pH and high aqueous organic carbon content are also associated with wetlands that are known producers of methyl Hg (Rudd, 1995). However, while some authors have speculated that acidic water may directly affect Hg concentrations of higher order consumers such as fish because of reductions in growth efficiency (Watras et al., 1998; Greenfield et al., 2001), little is known about the influence of pH on the movement of Hg through the entire food web (Trophic Magnification Factors, TMFs; Borgå et al., 2012) or components thereof [Biomagnification Factors (BMFs) for specific predator–prey pairs], and whether certain taxa are more susceptible to the effects of reduced pH on Hg bioaccumulation (Jardine et al., 2012a).

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In addition to effects of water chemistry, dietary concentrations of metals can also affect the relative increase (BMF) between trophic levels (DeForest et al., 2007). Dietary concentrations are inversely related to BMFs in lab-based studies, a phenomenon believed to be related to the regulation of uptake/excretion that occurs even for non-essential metals due to similarities in their chemical structure to essential metals (Phillips and Rainbow, 1989). Accounting for these effects in the field is more challenging because of the difficulty in assessing dietary habits, but the more recent use of $\delta^{15}N$ to calculate trophic levels (TLs) in studies of contaminant biomagnification (Borgå et al., 2012) provides a means to assess the effects of water chemistry and dietary concentrations on the trophic transfer of mercury.

In this study, biofilm, aquatic invertebrates and the common minnow blacknose dace (*Rhinichthys atratulus*) were collected from 21 streams in New Brunswick (NB), Canada to assess Hg biomagnification through riverine food webs. The large number of sites sampled in this study, with wide ranging chemical conditions and Hg concentrations (Jardine et al., 2012a), presented a unique opportunity to examine the transfer of Hg through the food web. The relative influence of water chemistry (pH) and dietary concentrations on BMFs was assessed to determine where in the food web these drivers act to increase Hg concentrations in biota. Our working hypothesis was that food webs of streams with lower pH would have higher TMFs because of previously observed negative correlations between fish Hg concentrations and pH (Watras et al., 1998; Greenfield et al., 2001; Jardine et al., 2012a).

2. Materials and methods

2.1. Field sampling

Samples were collected from 21 forested streams of second to sixth order over a three year period (2005–2007) in August and September, under baseflow conditions (Table 1). Samples were collected once at each site, and included biofilm, primary consumers (Baetidae, Ephemerellidae, Heptageniidae, freshwater mussels, Hydropsychidae, Isonychiidae, Leptophlebiidae, Psephenidae, and Pteronarcyidae) and predatory invertebrates (Perlidae, Gomphidae, Gerridae, Aeshnidae, Cordulegastridae, and Megaloptera), and the fish blacknose dace (*R. atratulus*). Length and weight measurements were made on the fish samples. Previous studies with stable carbon isotopes ($\delta^{13}C$) have shown that these invertebrates rely 29–100% on in-stream production and thus are well-connected enough to the biofilm food source and blacknose dace (52% reliance on in-stream production) to warrant their inclusion in analyses of biomagnification (Jardine et al., 2008, 2012a). Baseline methyl Hg at the 21 sites, as indicated by concentrations in biofilm, ranges over more than one order of magnitude from <0.01 to 0.11 μg/g (Jardine et al., 2012a).

Biofilm was analyzed as composite samples scraped from at least three rocks (three composite samples per site) and invertebrates were analyzed as pooled samples of several individuals for each taxon; fish white muscle tissue was dissected from above the lateral line and analyzed individually. All samples were freeze-dried and ground to a homogenate prior to analysis. Water chemistry was measured at each site by collecting one surface sample in 250 mL high density polyethylene bottles and frozen for analysis at the NB Department of Environment Analytical laboratory (Fredericton, NB). A suite of measurements, including sulfate and total phosphorus was made on each sample but analyses herein focused on pH because it consistently affects fish Hg (Greenfield et al., 2001; Jardine et al., 2012a) and was strongly correlated with an indicator of wetland influence, total organic carbon ($r = -0.62$ for the 21 sites). Water was not analyzed for methyl or total Hg. Previous studies have shown links between the proportion of

Table 1
Mercury (Hg) concentrations (μg g^{-1} dry weight) in biofilm, primary consumers, predatory invertebrates, and blacknose dace, water chemistry ([pH, total organic carbon (TOC), sulfate (SO_4) and total phosphorus (TP)], mean body length (mm) of blacknose dace and biomagnification of total and methyl Hg through the food web in New Brunswick, Canada streams.

Site	Lat	Long	pH	logTOC	logSO4	logTP	Biofilm	Primary consumer	Predator	Dace	Dace	Log Total Hg vs. $d^{15}N$			Log Methyl Hg vs $d^{15}N$		
							methyl Hg	methyl Hg	methyl Hg	total Hg	length	Slope	r^2	n	Slope	r^2	n
Black River	45.33	65.78	7.21	0.45	0.57	−2.30	0.03	0.03	0.33	0.96	53	0.13	0.62	10	0.22	0.96	10
Burpee Millstream	45.98	66.38	7.26	0.40	0.60	−1.80	0.01	0.10	0.24	0.48	64	0.16	0.79	10	0.26	0.93	10
Cains River	46.43	66.02	7.01	0.57	1.14	−1.82	0.01	0.11	0.13	1.00	41	0.12	0.67	16	0.28	0.96	16
Cains River downstream	46.54	65.84	7.15	0.60	1.09	−1.85	0.02	0.13	0.16	1.20	58	0.16	0.79	13	0.26	0.93	11
Clark Brook	46.06	65.54	6.87	0.25	1.02	−1.60	0.11	0.99	–	3.33	53	0.15	0.92	9	0.24	0.82	9
Cumberland Stream	46.04	65.87	7.52	0.42	1.09	−1.89	0.01	0.13	0.24	1.15	61	0.19	0.95	9	0.35	0.92	11
English Brook	46.43	66.60	7.33	0.40	0.74	−2.15	0.02	–	0.14	0.91	47	0.08	0.90	6	0.34	0.91	8
Forks Stream	46.11	65.57	7.40	0.61	1.07	−1.92	0.01	0.11	0.22	0.73	61	0.20	0.88	9	0.33	0.88	9
Gounamitz River washout	47.53	67.65	8.11	0.52	−0.30	−2.60	0.01	–	–	0.22	42	0.12	0.96	11	0.22	0.92	9
Hutchinson Brook	46.19	65.83	7.44	0.57	0.83	−1.47	0.01	–	0.42	1.03	60	0.14	0.79	10	0.39	0.95	11
Kelly's Brook	45.94	65.78	7.34	1.03	1.11	−1.92	0.02	0.26	0.35	1.27	57	0.17	0.90	11	0.39	0.92	13
Little Southwest Miramichi	46.88	66.09	7.10	0.32	0.73	−2.30	0.01	–	0.13	0.74	56	0.18	0.89	13	0.33	0.98	10
Renous River @ Red Bridge Road	46.81	65.87	7.52	0.47	0.51	−2.30	0.01	0.04	0.26	0.70	50	0.13	0.79	9	0.31	0.96	10
South Branch Mill Brook	45.78	65.88	7.47	0.59	0.20	−2.15	0.05	0.47	0.76	3.19	65	0.13	0.81	8	0.29	0.82	11
Smith Forks	46.96	66.58	7.17	0.37	0.72	−2.00	0.01	0.09	0.15	1.01	57	0.14	0.63	15	0.32	0.96	13
Stickney Brook	46.38	67.57	8.29	0.65	0.60	−2.15	0.01	0.09	0.14	0.25	55	0.06	0.77	7	0.22	0.72	11
Starkey Brook	45.91	65.82	7.27	0.86	0.91	−1.96	0.04	0.67	0.40	3.77	61	0.10	0.89	7	0.24	0.92	9
Trout Brook Tobique	46.78	67.51	7.73	0.45	0.04	−2.22	0.01	0.20	0.32	0.76	53	0.16	0.87	9	0.33	0.86	11
Weldon Creek	45.89	64.72	7.82	1.29	0.18	−2.22	0.00	0.08	0.16	0.37	55	0.14	0.94	11	0.28	0.67	12
Waweig River	45.25	67.14	7.11	0.38	0.75	−1.92	0.03	0.11	0.26	0.76	64	0.17	0.69	10	0.34	0.95	11
Young's Cove Brook	46.00	65.94	6.08	0.67	1.09	−1.64	0.02	0.28	0.23	1.74	53	0.19	0.93	8	0.37	0.97	10

wetlands in a catchment and the availability of Hg in the water column of streams (e.g. Brigham et al., 2009); therefore it was assumed that any links observed between pH and Hg in lower trophic level organisms (biofilm and primary consumers) was mediated by these processes. The focus of this study was instead on Hg in the food web.

2.2. Laboratory analyses

All fish and most invertebrates were analyzed for total Hg, a subset of these samples was analysed for methyl Hg, and all biofilm samples were analysed for both total and methyl Hg. All reported units are μg g^{-1} dry weight. Fish and invertebrate samples were analysed for total Hg using a Direct Mercury Analyser (DMA-80, Milestone Microwave Laboratory Systems, Shelton, CT). Recoveries of certified reference materials (CRM; National Research Council, Ottawa, ON) analyzed alongside samples were 93.7 ± 2.9% and 104.0 ± 3.9% for DORM-2 (dogfish muscle, n = 47) and TORT-2 (lobster hepatopancreas, n = 27), respectively. Blanks were analyzed after every ten samples and were consistently less than 10% of sample concentrations; blank values were not used to adjust sample values. Sample repeats had a mean difference of 8.7 ± 8.2% S.D. (n = 16) and 10.1 ± 6.5% S.D. (n = 18) within and across analytical runs, respectively. For methyl Hg, these samples were digested first with potassium hydroxide followed by acidic copper sulfate, then extracted in dichloromethane using a procedure described in Al-Reasi et al. (2007) and analysed by GC–MS on a HP 6890 series with HP injector series 7863 (Cai et al., 1997). Recovery of methyl Hg in the CRM (DORM-2) that was extracted along with samples was 94.1 ± 11.5% (n = 21). As determined from a subset of samples analyzed for both methyl and total Hg, all of the Hg in dace muscle tissue was methyl Hg (mean % methyl Hg = 109.3 ± 17.1 of total Hg), so all remaining fish samples not analyzed for methyl Hg were assumed to have concentrations equal to total Hg (Bloom, 1992). Because % methyl Hg was variable in invertebrates, methyl Hg concentrations were measured directly. For the biofilm samples, methyl Hg was measured by GC-AFS on a Brooks Rand Model III following digestion in 25% KOH/MeOH and ethylation with $NaB(C_2H_5)_4$ This method also generates Hg(II) data and thus total Hg was calculated from the sum (Liang et al., 1994).

Biotic samples were analyzed for $\delta^{15}N$ as described previously (Jardine et al., 2008). $\delta^{15}N$ was converted to trophic level ($TL_{consumer}$) for invertebrates and dace using the formula $TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{biofilm})/\Delta^{15}N + 1$, where $\Delta^{15}N$ is 2.0‰, the estimated mean difference in $\delta^{15}N$ between an organism and its diet (McCutchan et al., 2003). This lower value of 2.0‰ was used because recent field-based fractionation estimates suggest that a lower $\Delta^{15}N$ value is appropriate for stream food webs (Bunn et al., in press).

2.3. Statistical analyses

All regressions were analyzed with SPSS software. Biomagnification is calculated and presented in different ways in the literature using $\delta^{15}N$ (Jardine et al., 2006), including the slope (m) of log- or ln-transformed Hg vs. $\delta^{15}N$ and as a trophic magnification factor [TMF, 10^m or e^m where m is the slope of log- or ln- Hg vs. trophic level (TL, calculated from $\delta^{15}N$), Borgå et al., 2012]. In this study, regression slopes of log total Hg or methyl Hg vs. TL were used to calculate TMFs across sites. Because TL calculations are sensitive to $\Delta^{15}N$ (Post, 2002), slopes of log total or methyl Hg vs. $\delta^{15}N$ are also reported here for comparison with other studies. In addition, because of differences in biomagnification between total and methyl Hg, the remaining statistical comparisons used only methyl Hg. TMFs were calculated from the log methyl Hg-TL slopes (m) using 10^m and compared to streamwater pH using regressions.

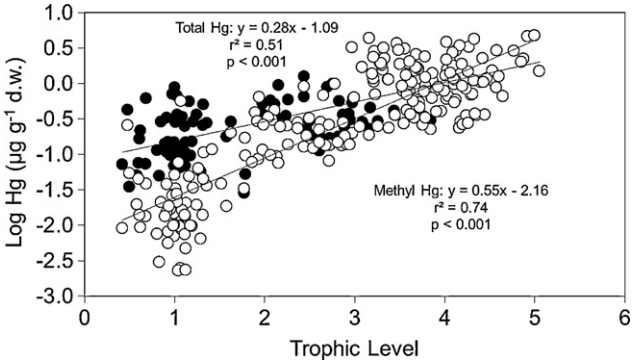

Fig. 1. Total (solid circles) and methyl (open circles) mercury (μg/g dry weight) in biota from New Brunswick, Canada streams (all sites combined) versus trophic level (TL) estimates derived from $\delta^{15}N$ data [$TL = (\delta^{15}N_{consumer} - \delta^{15}N_{biofilm})/2.0 + 1$].

The effect of dietary concentrations on consumer-diet BMFs was measured by regressing the site-specific methyl Hg concentration (Hg_{diet}) at a given trophic level against the relative increase to the next trophic level ($Hg_{consumer}/Hg_{diet}$) for the site. For simplicity, this calculation assumed that each integer trophic level fed 100% on the trophic level below it. Finally, to illustrate how methyl Hg-pH relationships might be influenced by BMFs at different levels of the food web, concentrations in a given trophic level were regressed against pH across sites.

3. Results

3.1. Food web biomagnification

Within each stream food web, mercury concentrations were strongly related to trophic level of biota as measured by $\delta^{15}N$. Slopes of the linear regressions of log total Hg vs. $\delta^{15}N$ within streams, as indicators of biomagnification, were all significant ($p < 0.05$) and ranged from 0.06 to 0.20 (mean slope = 0.14 ± 0.06 S.D., Table 1). These log total Hg-$\delta^{15}N$ slopes correspond to log total Hg-TL slopes of 0.12 to 0.40 and TMFs of 1.3–2.5 when $\Delta^{15}N$ = 2.0‰. When data were pooled across sites, the overall log total Hg-TL slope was 0.28 (TMF of 1.9) and significant ($r^2 = 0.51$, $p < 0.001$, Fig. 1). If a more conventional, higher value for $\Delta^{15}N$ (3.4‰) was used, log total Hg-TL slopes would have ranged from 0.20 to 0.68 and TMFs from 1.6 to 4.8.

Regressions of log methyl Hg vs. $\delta^{15}N$ were also significant at all sites ($p < 0.05$) and had steeper slopes than corresponding total Hg regressions (mean slope = 0.30 ± 0.08 S.D., range = 0.22–0.39, Table 1). These log methyl Hg-$\delta^{15}N$ slopes correspond to log methyl Hg-TL slopes of 0.44–0.78 when $\Delta^{15}N$ = 2.0‰ and TMFs of 2.8–6.0. When data were pooled across sites, the log methyl Hg-TL slope was 0.55 (TMF of 3.5) and significant ($r^2 = 0.74$, $p < 0.001$, Fig. 1). With a higher value for $\Delta^{15}N$ (3.4‰), log methyl Hg-TL slopes would range from 0.75 to 1.33 and TMFs from 5.6 to 21.2.

3.2. Trophic magnification between specific consumer-diet pairs

Changes in methyl Hg concentrations with trophic level depended on pH and dietary methyl Hg concentrations, but these effects were only observed at specific locations in the food web. Biomagnification of methyl Hg through the entire food web, as measured by TMFs, was not higher in low pH waters. Regression of TMFs on streamwater pH yielded $r^2 = 0.10$ and $p = 0.162$ (data not shown). Biomagnification factors, however, were affected by both dietary concentrations and pH, depending on the predator-prey pair. BMFs for biofilm to primary consumers averaged 11.0 ± 7.4

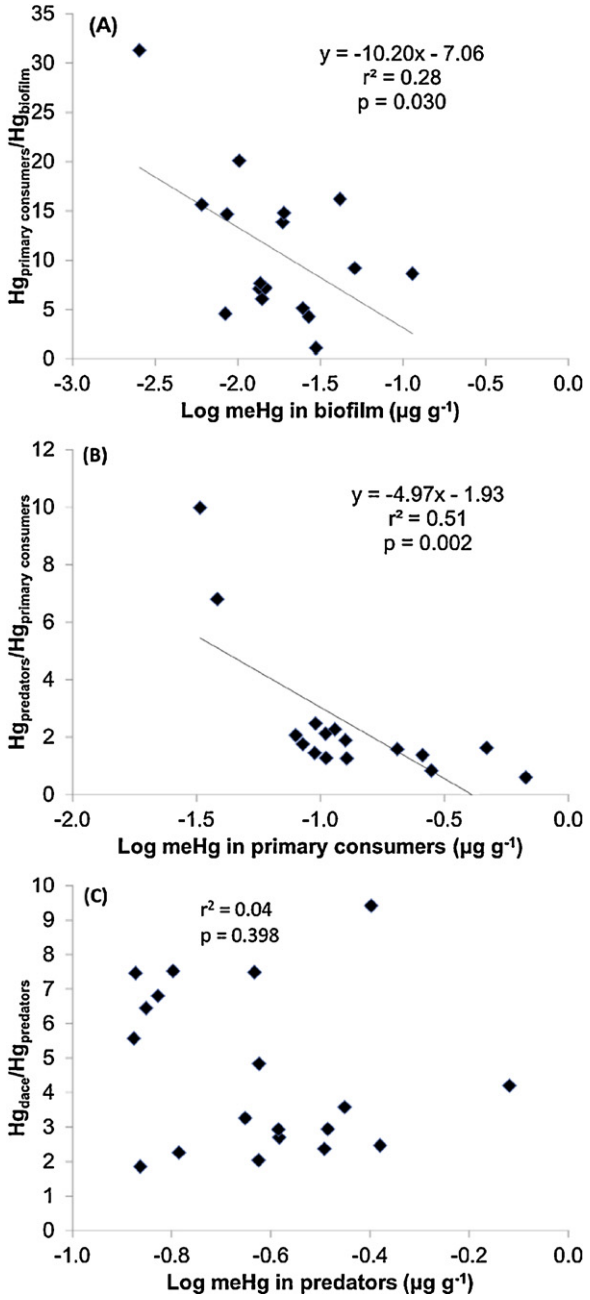

Fig. 2. Biomagnification of methyl mercury (μg/g dry weight) from a) biofilm to primary consumers as a function of biofilm methyl mercury concentrations, b) primary consumers to predatory insects as a function of primary consumer methyl mercury concentrations, and c) from predatory insects to blacknose dace as a function of predatory insect methyl mercury concentrations.

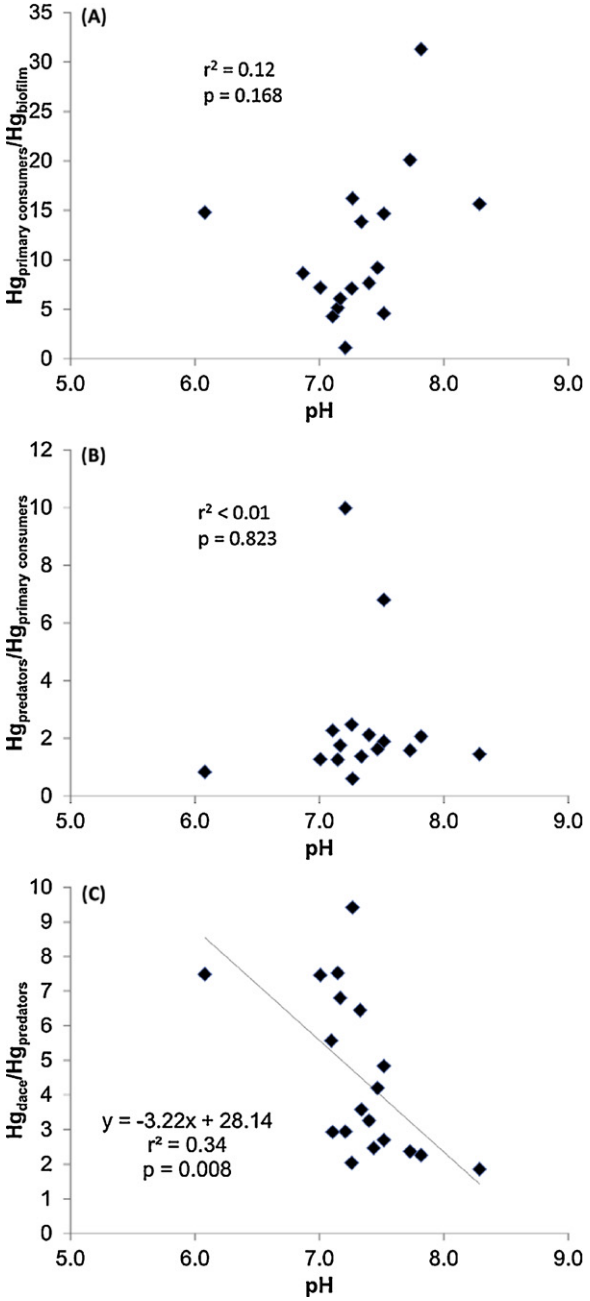

Fig. 3. Biomagnification of methyl mercury (μg/g dry weight) as a function of streamwater pH from a) biofilm to primary consumers, b) primary consumers to predatory insects, and c) from predatory insects to blacknose dace.

S.D. and ranged from 1.1 to 31.3 across sites. BMFs for primary consumers to predatory invertebrates averaged 2.5 ± 2.4 S.D. and ranged from 0.6 to 10.0 across sites. BMFs for both of these consumer-diet pairs were highest when concentrations in the diet were low (Fig. 2a,b), but for neither pair was there an effect of pH on BMFs (Fig. 3a,b). BMFs for predatory invertebrates to dace averaged 4.5 ± 2.3 S.D., ranged from 1.8 to 9.4, and showed the opposite pattern to the previous two consumer-diet pairs, with dietary concentrations (i.e. predator Hg) having no effect on the BMF (Fig. 2c), but pH had a significant negative effect, with lower BMFs in higher pH waters (Fig. 3c). Dace Hg concentrations significantly increased with increasing mean body size ($r^2 = 0.38$, $p = 0.003$, Table 1) but BMFs from predatory invertebrates to dace were unrelated to the average size of dace ($r^2 = 0.08$, $p = 0.230$, Table 1).

4. Discussion

4.1. Food web biomagnification of mercury

Measurements of stable nitrogen isotopes and Hg in biota from streams that ranged in baseline Hg concentrations and water chemistry yielded new insights into how Hg increases in concentration as it moves through food webs. Despite no apparent relationship between overall TMFs and pH, there was a significant increase in BMFs between predatory insects and blacknose dace associated with lower pH, while lower trophic level biota had BMFs that were

controlled instead by Hg concentrations in the diet. The findings support previous observations that high dietary concentrations can cause lower biomagnification (DeForest et al., 2007), and they also show that pH can act directly on higher-trophic level species such as fishes to increase biomagnification of Hg from the diet (Watras et al., 1998; Greenfield et al., 2001). The mechanism for this latter phenomenon is likely to involve effects on growth efficiency (Suns and Hitchin, 1990; Trudel and Rasmussen, 2006)

Biomagnification slopes for streams in this study, using $\delta^{15}N$, were similar to those reported previously for fresh and salt water food webs (Kidd et al., 2012). The mean slope for all streams was 0.14 for total Hg and 0.30 for methyl Hg. These compare favorably with studies from other lotic environments including streams in Oregon, Wisconsin and Florida, USA that had log Hg-$\delta^{15}N$ slopes ranging from 0.15 to 0.27 for invertebrates and fishes (Chasar et al., 2009). In that study, invertebrates were analysed for methyl Hg and fish were analysed for total Hg, while other riverine studies that reported lower Hg-$\delta^{15}N$ slopes analyzed only total Hg in both invertebrates and fishes. These include 0.10 for the food web of the Mekong Delta (Ikemoto et al., 2008) and an average slope of 0.08 for rivers and wetlands from the Mitchell River in tropical northern Australia (Jardine et al., 2012b). In the latter study, only three of 14 food webs had total Hg-$\delta^{15}N$ regressions that were significantly greater than zero. These comparisons highlight the need for increased focus on methyl Hg in food webs as this is the form that biomagnifies.

The large difference between total Hg and methyl Hg slopes in the current study stems, in part, from the inclusion of biofilm that had low % methyl Hg (3–27%, Jardine et al., 2012a). Many studies that use $\delta^{15}N$ to quantify Hg biomagnification analyse only animal samples. Because animals generally have higher % methyl Hg than plants (Kidd et al., 2012) and approach 100% methyl Hg in higher-trophic level organisms such as fish (Bloom, 1992), total Hg and methyl Hg slopes are often similar for food webs dominated by higher trophic level biota (Campbell et al., 2005; Al-Reasi et al., 2007; Chasar et al., 2009). However, some invertebrates also have low % methyl Hg, including mussels (22 ± 7%, Kidd et al., 2012), grazing insects ($\leq$50%, Tremblay et al., 1996; Edmonds et al., 2012) and zooplankton (as low as 11%, Watras et al., 1998). These observations and our results illustrate the importance of including plant to primary consumer and primary consumer to predator linkages to properly quantify Hg transfer and demonstrate the enhanced food web biomagnification of organic vs. inorganic Hg through food webs (Mason et al., 1996).

The slopes reported here correspond to a mean methyl Hg TMF of 4.1 ± 1.1 (range = 2.7 to 6.1), a four-fold increase in Hg with each TL when a $\Delta^{15}N$ of 2.0‰ is assumed (McCutchan et al., 2003; Bunn et al., in press). By adjusting $\Delta^{15}N$ only slightly to another mean value (2.5‰) reported from a summary of controlled dietary experiments (Vanderklift and Ponsard, 2003), the mean TMF increases to 5.9 ± 1.9 (range = 3.5–9.6), illustrating its sensitivity to this parameter ($\Delta^{15}N$) that has some uncertainty (Post, 2002; Jardine et al., 2006). This latter average TMF is similar to predator prey factors (PPFs) calculated from field and lab studies (USEPA 1997). PPFs in those studies were 6.3 ± 1.3 from phytoplankton to zooplankton (TL 1 to TL 2), 6.2 ± 2.1 for zooplankton to insectivorous fish (TL 2 to TL 3) and 5.0 ± 1.5 for insectivorous fish to piscivorous fish (TL 3 to TL 4) (USEPA 1997). Our highest and most variable TMF was for biofilm to primary consumers (11.0 ± 7.4) which may be a consequence of a broad range of assimilation efficiencies for the biofilm diet (Reinfelder et al., 1998) or inaccuracies in biofilm concentrations associated with impure samples (Hamilton et al., 2005). Further study of these biomagnification trends within and among food webs may reveal species- or ecosystem-specific patterns in the transfer of Hg, which would assist in risk assessment models for this metal.

4.2. Influence of water chemistry and dietary concentrations

Though the average regression slope in this study was similar to those from other studies, there was a broad range in slopes for individual stream food webs supporting blacknose dace, providing evidence that factors such as water chemistry and physiology may affect biomagnification. This supports the use of a range of biomagnification factors in risk assessments designed to set benchmarks for total maximum daily loads to watersheds (Hope, 2003). By measuring the concentrations of Hg in primary food sources at the base of the food web and applying the range of biomagnification slopes observed here and in other studies (Kidd et al., 1995; Al-Reasi et al., 2007; Chasar et al., 2009), concentrations in top predators and associated error can be predicted. Given that some species may accumulate less Hg than predicted based on their trophic level (Swanson et al., 2003), ideally food web-specific BMFs and TMFs that incorporate the Hg-sensitivity of the species involved will be used in these risk assessments rather than applying a fixed value to all food webs and predator-prey pairs.

Dace accumulated a large amount of Hg (Table 1) despite their small body size (30–76 cm, typical of one to three year old fish, Reed and Moulton, 1973), and BMFs for predatory invertebrates to dace were significantly higher in streams with lower pH. Though mean body size significantly influenced dace Hg concentrations, consistent with other studies (e.g. Watras et al., 1998 and references therein), the effect of size was in addition to the pH effect as indicated by both variables being significant in a multiple regression model ($p < 0.001$). Dace body size did not affect BMFs from predators to dace; these were instead controlled solely by pH. According to kinetic models of trace element accumulation (Reinfelder et al., 1998; Luoma and Rainbow, 2005), this suggests that dace effectively assimilate Hg from their diet, are inefficient at eliminating Hg, or accumulate high Hg loads due to slow growth in lower pH waters (Karimi et al., 2007). Acidic conditions have been shown to reduce growth rates of fishes (Mills et al., 2000) and high Hg concentrations in fishes are often associated with low pH waters and poorer growth conditions (e.g. Greenfield et al., 2001).

Dace are sensitive to acidic conditions in streams (Simonin et al., 1993). Higher plasma sodium concentrations have been observed in dace from low alkalinity waters (Dennis and Bulger, 1995), suggesting that dace have difficulty with ion regulation in low pH streams. Indeed, consistent with stream surveys in New York State (Schofield and Driscoll, 1987), no dace were captured in streams that had pH < 6 and where brook trout that are known to have a higher tolerance to lower pH were collected (Jardine et al., 2012a). The pH tolerance threshold for dace may be near a value of 6 and when living in waters with a pH near this threshold, Hg concentrations increase dramatically, possibly due to an enhanced stress response. An enhanced stress response could reduce overall growth efficiency (i.e. growth relative to food consumption) leading to higher Hg concentrations (Trudel and Rasmussen, 2006; Karimi et al., 2007). While only one of our study streams was acidic at the time of sampling (Youngs Cove Brook, pH 6.08), these single spot measurements of pH were made late in the summer under low flow conditions and therefore likely underestimated the potential for exposure to acidic water through acid pulses (Dennis and Bulger, 1995) that often occur during high flows in spring associated with snowmelt (Baker et al., 1996).

4.3. Implications for ecosystem monitoring of mercury

Earlier work on common loons (*Gavia immer*) showed strong links between blood Hg and lake water pH (Burgess and Meyer, 2008), suggesting that pH effects on Hg bioaccumulation are transferred to piscivorous birds. We should expect, therefore, that the high concentrations measured in blacknose dace (up to 4.8 μg/g

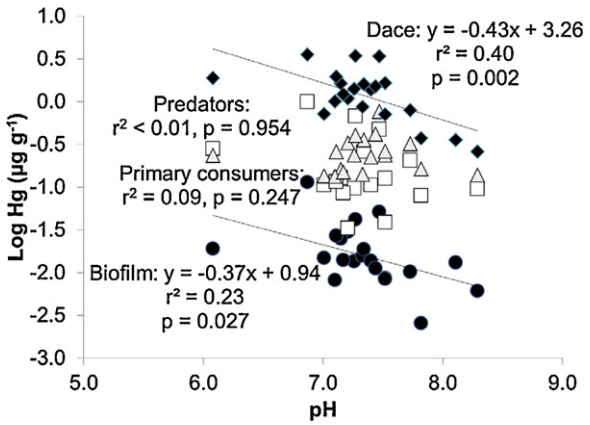

Fig. 4. Mercury concentrations (μg/g dry weight) as a function of streamwater pH for biofilm (solid circles, methyl Hg), primary consumers (open squares, methyl Hg), predatory insects (shaded triangles, methyl Hg) and blacknose dace (solid diamonds, total Hg).

d.w.) that are partly caused by enhanced biomagnification in low pH waters pose a threat to fish-eating birds (White, 1957). Low pH therefore has both direct (higher baseline concentrations, Jardine et al., 2012a) and indirect (higher BMFs in some species) effects on Hg bioaccumulation in food webs. This explains why this variable often emerges as an important covariate in explaining biotic Hg concentrations across a gradient of lakes or rivers (Watras et al., 1998; Scheuhammer and Graham, 1999; Greenfield et al., 2001; Edmonds et al., 2012). Dietary links to acid-sensitive species or internal sources of Hg within waterbodies will dictate whether an organism is susceptible to these effects or if its diet shields it from Hg exposure (Jardine et al., 2012a). In addition to blacknose dace and other minnows (family Cyprinidae), many species of river-dwelling fish including white sucker (*Catostomus commersoni*), smallmouth bass (*Micropterus dolomieu*) and pumpkinseed sunfish (*Lepomis gibbosus*) may be sensitive to the effects of reduced pH (Schofield and Driscoll, 1987). If reductions in pH are accompanied by corresponding increases in Hg BMFs, river reaches dominated by these species could be areas of high risk to fish-eating wildlife.

Results from this study indicate that Hg biomagnification slopes in streams are comparable to those found in other aquatic environments. However, differences in Hg biomagnification can occur due to the influence of abiotic factors (i.e. pH) and to concentrations of Hg in the diet (Tsui and Wang, 2004; DeForest et al., 2007). The reduced BMFs associated with higher dietary concentrations for both primary consumers and predatory invertebrates, coupled with higher biofilm Hg concentrations in low pH waters, leads to a dampening of the pH effect at these two higher trophic levels (Fig. 4). This has implications for the choice of taxa in research and monitoring studies because the effect of dietary concentrations on BMFs reduces concentration differences across the pH gradient at the next highest trophic level and also reduces the overall variability among sites. Significant effects of pH on biofilm Hg concentrations (Jardine et al., 2012a) were no longer apparent in primary consumers and predatory invertebrates (Fig. 4). Furthermore, variability among sites was lower in predatory invertebrates (coefficient of variation (CV) = 0.41) compared to primary consumers (CV = 1.18). These findings stress that, in addition to calculating TMFs for the entire food web, partitioning the web into distinct integer trophic levels (ideally with stable N isotopes) and calculating BMFs for predator-prey pairs can help disentangle the competing effects of water chemistry and dietary concentrations on Hg bioaccumulation. This approach may apply equally well to other trace elements (Jardine and Kidd, 2011) and organic contaminants (Borgå et al., 2012), yielding new insights into the uptake and transformation of toxic substances in food webs.

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