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Methylmercury Bioaccumulation in Stream Food Webs Declines with Increasing Primary Production

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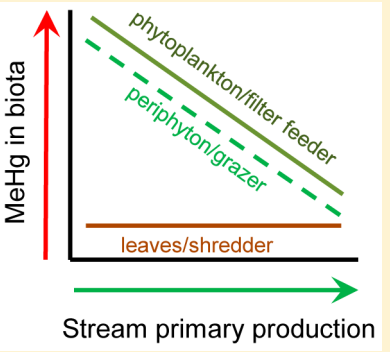
Supporting Information

ABSTRACT: Opposing hypotheses posit that increasing primary productivity should result in either greater or lesser contaminant accumulation in stream food webs. We conducted an experiment to evaluate primary productivity effects on MeHg accumulation in stream consumers. We varied light for 16 artificial streams creating a productivity gradient (oxygen production =0.048−0.71 mg O_2 L^{-1} d^{-1}) among streams. Two-level food webs were established consisting of phytoplankton/filter feeding clam, periphyton/grazing snail, and leaves/shredding amphipod (*Hyalella azteca*). Phytoplankton and periphyton biomass, along with MeHg removal from the water column, increased significantly with productivity, but MeHg concentrations in these primary producers declined. Methylmercury concentrations in clams and snails also declined with productivity, and consumer concentrations were strongly correlated with MeHg concentrations in primary producers. Heterotroph biomass on leaves, MeHg in leaves, and MeHg in *Hyalella* were unrelated to stream productivity. Our results support the hypothesis that contaminant bioaccumulation declines with stream primary production via the mechanism of bloom dilution (MeHg burden per cell decreases in algal blooms), extending patterns of contaminant accumulation documented in lakes to lotic systems.



INTRODUCTION

Aquatic ecosystems are prominent in the global mercury (Hg) cycle, but levels of understanding of Hg bioaccumulation pathways through different aquatic ecosystems are not equal. Mercury is commonly introduced to aquatic ecosystems by atmospheric deposition and watershed runoff.[1,2] Anaerobic microbes convert inorganic Hg to organic methylmercury (MeHg), which is bioaccumulated and biomagnified through food webs via dietary trophic transfers.[3,4] Food-web dynamics of MeHg in lentic systems have been examined extensively in the field (e.g., refs 5 and 6) and experimentally;[7−10] in contrast and until recently,[11−16] investigations of the cycling and accumulation of MeHg in streams have lagged behind those in lakes and wetlands.[12,14,17−19] Mercury contamination is pervasive in lotic systems, and is the leading cause of fish consumption advisories in the U.S. where 1.8 million river kilometers were under advisory in 2010.[20] However, uncertainty remains regarding how Hg contamination interacts with eutrophication (artificially elevated primary production resulting from nutrient pollution and other anthropogenic factors), another pervasive stressor of lotic systems.[21]

Differences in stream food-web assemblages and degrees of primary production may influence MeHg accumulation. Smaller, headwater streams often differ from lakes by having periphyton, a biofilm consisting of attached algae, bacteria, mucilage, and entrained particulate organic matter, rather than either phytoplankton or macrophytes as the dominant primary producer. Periphyton can both absorb MeHg from water[22] and methylate Hg,[23] and accumulated MeHg can be passed to benthic macroinvertebrate food webs when periphyton is consumed. Greater primary productivity in streams has been hypothesized to lead to increased accumulation in consumers through greater uptake of either Hg[22] or organic contaminants[24,25] by stream periphyton. Likewise, MeHg accumulation in stream food webs can increase with primary production in systems where dense mats of algae (*Cladophora glomerata*) form during summer months.[18,19] These mats and their associated epiphyte communities create zones of enhanced in situ production of MeHg that can be released to overlying water and accumulate in consumer tissues.

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Alternatively, increased primary productivity could reduce MeHg accumulation in consumers through either bloom or growth dilution phenomena.[17] Bloom dilution is a process whereby algal cell division distributes a fixed amount of contaminant among an increased number (and mass) of cells resulting in lower contaminant concentration.[9] Bloom dilution of MeHg in phytoplankton reduces bioaccumulation in lentic zooplankton, and could explain field observations of lower Hg concentrations in fish living in eutrophic lakes compared to those in oligotrophic lakes.[5,9] Greater productivity also can fuel faster growth of consumers that results in somatic growth dilution, where efficient growth of animals dilutes contaminant concentrations in tissues.[7] Bloom dilution of Hg, MeHg, and other metals has been documented experimentally for stream algal communities,[26] and Ward et al.[17] have argued that MeHg accumulation in stream food webs is amplified in less productive systems, in part due to the process of bloom dilution by primary producers. These opposing hypotheses regarding contaminant accumulation relative to primary production in lotic systems have not been tested experimentally.

We conducted a laboratory experiment with artificial streams to examine mechanisms of MeHg accumulation in lotic food webs. We established two-level invertebrate food webs consisting of leaves and a shredding amphipod, periphyton and a grazing snail, and phytoplankton and a filtering clam. This experiment differed from previous tests by directly examining benthic stream consumers as opposed to pelagic lake consumers, and by continually adding MeHg to the experimental units as opposed to a single loading at the start.

EXPERIMENTAL SECTION

Preparation Phase. Sixteen recirculating artificial streams (fiberglass raceways 105 cm long × 72 cm wide × 46 cm deep; JMS Composites, Springfield, OH) were constructed at the Miami University Aquatics Facility (Oxford, OH, USA) (Supporting Information Figure S1, Table S1). Including an extensive preparation phase, the experiment was conducted between December 18, 2008 and May 27, 2009 (Supporting Information Table S1). Water in each artificial stream was recirculated with a polycarbonate paddle powered by a gear motor (17 HP DC, Bison Gear & Engineering Corp., St. Charles, IL). Artificial stream interiors and paddles were cleaned before use by filling the artificial streams with culture water for 3 days, draining and refilling three additional times. Culture water was prepared by dechlorinating and UV-sterilizing water drawn from the City of Oxford, OH Water Treatment Plant, which obtains water from groundwater wells, and blending it with deionized water to a hardness of 120 mg $CaCO_3$/L. Culture water was stored in a 9500 L polyethylene tank, recirculated through a UV-sterilization system, and distributed to artificial streams via Schedule 80 PVC plumbing. During the experiment, artificial streams contained 30 cm depth of culture water that was maintained at this level by the addition of deionized water (140 L total water volume, 1.83 m^2 total submerged surface area). Surface velocities ranged from 0.012 to 0.37 m s^{-1} with the greatest flow near the paddle. Bottom velocities ranged from 0.015 to 0.17 m s^{-1} with the greatest flow on the side of the raceway opposite of the paddle.

A primary productivity gradient was established by varying exposure of the streams to light with a 16:8 h light:dark photoperiod. Four treatment groups of four replicates each included artificial streams that were: (a) unshaded and placed directly under lights (high-light treatment, 22 W visible light, 100% of maximum light level), (b) unshaded and placed near lights (midlight treatment, 14W, 63%), (c) shaded on top and sides by a single layer of black window screening (midshade treatment, 2.5W, 11%), and (d) shaded on top and sides by two layers of black window screening (high-shade treatment 1W, 0.05%; Supporting Information Figure S1). Unglazed ceramic tiles and leaf packs were placed in Elams Run, Oxford, OH (N34.521072, W84.736653) to allow colonization of naturally occurring microbial assemblages (Supporting Information Table S1, Figure S2A). Colonized tiles were transferred to the artificial streams to inoculate the streams with algae. Leaf packs were filled with similar amounts of air-dried and partially crushed tulip poplar (*Liriodendron tulipifera*) leaves in pouches made from 500 μm mesh Nitex (Supporting Information Figure S2B). Primary consumers consisted of the filtering Asian clam *Corbicula fluminea*, grazing snail *Elimia* sp., and shredding amphipod *Hyalella azteca* (here after referred to as clams, snails, and *Hyalella*). Clams and *Hyalella* were reared in separate holding tanks for three months, and snails for several weeks, prior to introduction to artificial streams (Supporting Information Figure S3).

Dissolved NH_4NO_3 and NaH_2PO_4 were added to water in the artificial streams in equal amounts after the colonized tiles were installed. Nutrient concentrations were spot-checked during the experiment with rapid assessment kits; subsequent nutrient additions were made to maintain nominal concentrations of 0.70 mg N L^{-1} and 0.077 mg P L^{-1}. Concentrations were adjusted downward later in the experiment to avoid algal blooms (Supporting Information). Separate water samples were collected every other day for analysis of NO_3^- and soluble reactive phosphorus (SRP). Measured nutrient concentrations ranged on average between 0.052−3.36 mg N L^{-1} and 0.005−1.380 mg P L^{-1} (Supporting Information Figure S4A, B). These are representative of concentrations measured in natural lotic systems receiving varying degrees of anthropogenic nutrient inputs.[27]

Autotrophic production was evaluated from in situ measurements of dissolved oxygen.[28] We calculated the total change in dissolved oxygen (mg O_2 L^{-1} d^{-1}) during the light period as a measure of total primary production among streams (here after referred to as "primary production"). This method accounts for changes in oxygen related to photosynthesis, but does not account for variance in either community respiration or gas exchange with the atmosphere,[28] the latter of which is assumed to be relatively constant among our experimental streams. Dissolved oxygen was recorded at 2 h intervals beginning before, and ending after, the light period on April 7 of the experiment following 67 days of nutrient additions.

Preconsumer Addition Phase. Detailed information on experimental conditions, sample collection, and analytical chemistry methods are provided in Supporting Information. Briefly, MeHg (as CH_3HgCl) was added to streams on April 21, 9 days prior to addition of consumers to allow MeHg uptake in periphyton, phytoplankton, and leaves. Methylmercury was subsequently added daily for the duration of the experiment in order to maintain a nominal concentration of 2 ng MeHg L^{-1} in filtered water (i.e., 280 ng MeHg per each 140 L stream). Total MeHg added to each stream was 10,360 ng over the course of the experiment. Water, periphyton, phytoplankton, and leaves were collected from each stream every 3 days prior to adding consumers (Supporting Information Table S1). Water was sampled for analysis of

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total suspended solids (TSS). Prior to addition of consumers, the only suspended solids present in the streams were phytoplankton. Afterward, suspended solids consisted of phytoplankton with the likely addition of feces and pseudofeces (packets of filtered particulates ejected from the inhalant siphon of clams in response to an overabundance of suspended solids) produced by the consumers, and hereafter we refer to this suspended organic matter as "seston". Seston was analyzed for chlorophyll *a* and MeHg concentrations. Leaves were sampled for MeHg and ergosterol content, a measure of fungal biomass.[29] Samples were prepared for MeHg analysis with trace-metal clean techniques.[30] Samples of leaves, periphyton, and consumers were stored frozen (−20 °C) in acid-cleaned vials until analysis. Subsamples of lyophilized material (±0.1 mg) were digested with dilute HNO_3 in a 60 °C water bath for 12−14 h,[31] whereas MeHg was extracted from filtered water after treatment with 1% H_2SO_4.[32] Sample MeHg was quantified with gas chromatographic cold-vapor atomic fluorescence spectrometry.[32−34] All MeHg concentrations for organic matter and animal tissues are reported on a dry-weight basis.

Consumer Addition Phase. *Hyalella*, snails, and clams were added to artificial streams on April 30, 2009, marking the beginning of the 27-d animal exposure to MeHg (Supporting Information Table S1, Figure S3). Clams were suspended in the water column in flow-through baskets, and *Hyalella* were placed in leaf packs (Supporting Information Figure S2B). Clams and snails were labeled individually with tags prior to deployment. Shell lengths and mass, including samples of amphipods, were measured prior to deployment and at the time of collection to assess growth, but no change in either body size or mass was observed. Sampling of consumers began the day they were added to streams; three composite samples of each consumer were collected randomly for determination of biomass, moisture, ash-free dry mass (AFDM), and MeHg concentration. The number of individual animals in composite samples varied based on animal size and survival in streams over time, and all sample masses were >4.0 mg dry weight. Periphyton, seston, leaves, and consumers were sampled 3, 7, 14, 21, and 27 days after the consumer additions. Periphyton, seston, and leaves were analyzed for MeHg, chlorophyll *a* (in seston and periphyton), and ergosterol (in leaves). Methylmercury concentrations in water (ng L^{-1}) were measured in samples passed through a 0.7 μm glass fiber filter. Methylmercury in seston was calculated volumetrically as the mass of MeHg in seston per liter of water (ng L^{-1}). Concentrations in phytoplankton were calculated by dividing seston MeHg concentration by the chlorophyll *a* concentration associated with that sample (ng g^{-1} chlorophyll *a*). Similarly, periphyton MeHg concentrations were calculated on a mass basis (ng g^{-1}) for a given sample and on a per mass of chlorophyll *a* basis (ng g^{-1} chlorophyll *a*) by dividing periphyton MeHg concentration by the mass of chlorophyll *a* (g m^{-2}) in the stream.

Statistical Analyses. Primary production (mg O_2 L^{-1} d^{-1}) varied within and among light treatments. We used the resulting oxygen gradient to test the hypothesis that MeHg concentrations vary as a function of stream primary production using linear and nonlinear regression analyses. This allowed for a more robust statistical analyses of the main effect of altered stream productivity than an analysis of variance on light treatments.[9,35,36] Snail mortality was high by the end of the experiment (particularly in midshade and high-shade treatments), so models of MeHg concentrations in leaves, primary producers, and consumers used data from May 14 (14 days after consumers were added to streams) to be consistent among organic matter types and consumers. These models were similar in all respects to those with data from the end of the experiment, although the magnitude of observed responses (i.e., total MeHg concentrations) was higher at the end of the experiment. Standing crop producer biomass (measured as chlorophyll *a*) and total MeHg removed from the water column were also modeled on the last day of the experiment (May 27) to demonstrate light treatment effects over the entire course of the experiment. Total MeHg removed from the water column (%) was calculated by mass balance; aqueous MeHg concentration on a given day (Supporting Information Figure S5A) was converted to total mass of MeHg in 140 L of streamwater and then compared with the cumulative amount of MeHg added to streams up to that sampling event. MeHg concentrations in producers, leaves, and consumers were $\log_{10}$ transformed prior to analyses to improve normality and because they typically varied over orders of magnitude among streams. Statistical outliers (studentized residuals > |2|) were excluded from regression models, and all analyses were performed using R.[37]

RESULTS

The light treatment strongly affected stream primary production, with oxygen production increasing over an order of magnitude among streams (0.048−0.71 mg O_2 L^{-1} d^{-1}; Figure 1). As expected, phytoplankton and periphyton biomass increased significantly and positively with oxygen production (Figure 1A), indicating that oxygen production effectively characterized total autochthonous primary production among streams. The increase in primary production (and producer biomass) corresponded with greater nutrient removal from the water column (Supporting Information Figure S4). Over 98% of MeHg, on average, was removed from the water column on the first day of additions, indicating rapid incorporation of MeHg into primary producer biomass, and removal remained high (>90%) throughout the experiment (Supporting Information Figure S5B). The percentage of aqueous MeHg removal increased significantly with primary production (Figure 1B).

Methylmercury concentrations in seston increased significantly with primary production while concentrations in phytoplankton declined with productivity (Figure 2A). The inverse response of MeHg in phytoplankton is consistent with bloom dilution. Greater uptake of MeHg from the water column led to an increase of MeHg in seston per liter of water, but increasing phytoplankton biomass led to lower MeHg concentrations per cell. As we observed for phytoplankton, MeHg concentrations in clams also declined significantly with primary production (Figure 2B). Similarly, MeHg concentration in periphyton (per unit mass or chlorophyll *a*) and snails declined significantly with primary production (Figure 2C, D). As expected, leaf fungal biomass (i.e., ergosterol content) was unrelated to primary production (Supporting Information Figure SI 6A), indicating that the light treatment had no effect on this heterotrophic community. MeHg concentrations in leaves and *Hyalella* were unrelated to primary production and were 1−2 orders of magnitude lower than those in primary producers and their consumers (Figure 2E, F).

Methylmercury concentrations in clams (34−625 ng g^{-1}) and snails (26−463 ng g^{-1}) declined by an order of magnitude with stream primary production. Concentrations of MeHg in clams declined significantly with MeHg concentrations in seston measured on a volumetric basis (Figure 3A), but

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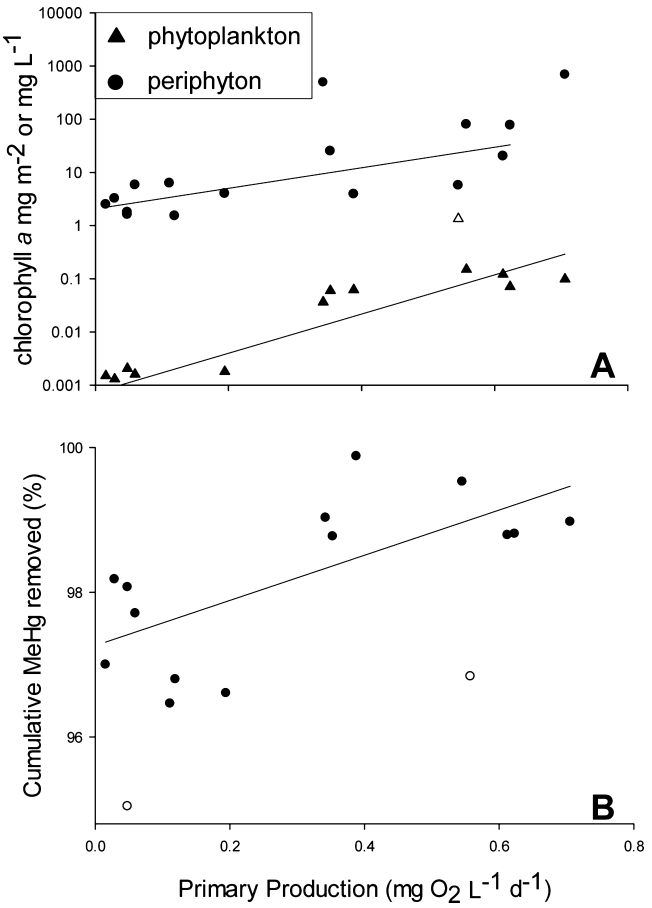

Figure 1. (A) Changes in phytoplankton (triangles, mg L^{-1}) and periphyton (circles, mg m^{-2}) chlorophyll *a* biomass relative to primary production among streams at the end of the experiment. Log_{10} phytoplankton chlorophyll *a* = 3.683(mg O_2 L^{-1} d^{-1}) − 3.138, R^2 = 0.84, $P < 0.0001$. Log_{10} periphyton chlorophyll *a* = 1.932(mg O_2 L^{-1} d^{-1}) + 0.313, R^2 = 0.63, $P < 0.0007$. (B) Effect of primary production on cumulative MeHg removed at the end of the experiment. MeHg removed (%) = 3.123(mg O_2 L^{-1} d^{-1}) + 97.26, R^2 = 0.48, $P < 0.001$. Open symbols are statistical outliers excluded from models.

increased with phytoplankton concentrations assessed on a chlorophyll-*a* basis (Figure 3B). Snail MeHg concentrations increased with periphyton concentrations on a mass basis (Figure 3C), but were unrelated to MeHg concentrations normalized to chlorophyll *a* (Figure 3D). Methylmercury concentrations in *Hyalella* were unrelated to concentrations in leaves (Supporting Information Figure S6B).

DISCUSSION

Testing Primary Production-Mercury Accumulation Hypotheses for Stream Food Webs. Eutrophication and contaminants are common stressors in stream ecosystems, but the ways in which these stressors interact to influence contaminant accumulation in stream food webs are unresolved. Some researchers have argued that productivity and contaminants act synergistically to increase accumulation of either organochlorines or MeHg,[22,24] while a competing model has posited that MeHg accumulation declines with increasing stream primary productivity,[17] similar to the behavior of MeHg accumulation in lake and marine systems.[5,9,38] Central to these hypotheses is the role periphyton plays in contaminant uptake and transfer to consumers. The argument for higher contaminant accumulation in more productive systems is that greater periphyton biomass increases uptake and retention of contaminants (e.g., MeHg and organochlorines) resulting in greater bioaccumulation by stream consumers.[22,24] Alternatively, bloom dilution by periphyton could result in lower MeHg accumulation by consumers, such that less productive streams have greater MeHg accumulation.[17]

Our results strongly support the latter hypothesis with bloom dilution as the mechanism. Productive streams removed more MeHg from the water and incorporated it into food webs to a greater extent than less productive streams. Yet phytoplankton and periphyton had lower concentrations of MeHg in more productive streams due to bloom dilution. This is clearly illustrated by the inverse response of seston and periphyton chlorophyll *a* MeHg concentrations to primary production. Productive streams had higher seston concentrations, which concentrated a greater mass of MeHg from the aqueous phase than less productive streams (seston MeHg increases with primary production). However, increasing algal biomass led to lesser MeHg per cell (phytoplankton chlorophyll *a* MeHg declines with primary production) as expected under bloom dilution. This resulted in lower dietary inputs of MeHg to consumers, which accumulated an order of magnitude less MeHg along the stream productivity gradient. These results are consistent with observations of bloom dilution and MeHg accumulation in pelagic food webs,[5,7,9,38] and thus extend MeHg uptake patterns from lake and marine systems to stream benthic and sestonic food webs. We enhanced primary production in our experiment by varying light intensity, and photochemical degradation of MeHg could have played a minor role in lowering MeHg accumulation in the most productive streams. Based on measured photon fluxes and published rate constants for photochemical decomposition of MeHg in natural waters,[39,40] photodecomposition is estimated to have removed ≤1% of MeHg added to artificial streams.

Primary producer biomass in our experiment was representative of natural variation in aquatic systems, although phytoplankton concentrations were relatively high in some streams. Phytoplankton chlorophyll *a* at the end of the experiment was 0.00025−0.150 mg L^{-1}, and the geometric mean among streams over time was 0.0005−0.184 mg L^{-1} (excluding two dates where algal blooms were observed), except for one stream that had a mean concentration of 0.516 mg L^{-1}. All of these values are within the range of phytoplankton chlorophyll *a* measured across 115 temperate streams,[41] except for the two highest mean concentrations which exceeded the maximum value of 0.170 mg L^{-1} reported in that study. These high values are more representative of phytoplankton chlorophyll *a* concentrations in larger rivers, which can be upward of 0.300−0.400 mg L^{-1} in highly eutrophic systems,[42,43] and eight of our streams would be classified as eutrophic (suspended chlorophyll a concentrations >0.03 mg L^{-1}) using the boundaries suggested by Dodds et al.[44] Periphyton chlorophyll *a* biomass in our streams followed a similar distribution to those in temperate streams, with 92% of our measurements falling below the mesotrophic-eutrophic boundary for streams.[44]

Our results may have run counter to the higher productivity/higher accumulation hypothesis for two reasons. First, empirical evidence for this hypothesis[24,25] as well as conceptual models of contaminant fate[22,24] were derived from small, headwater streams where food webs are dominated by periphyton/grazer and detritus/shredder pathways.[45] Our experimental streams

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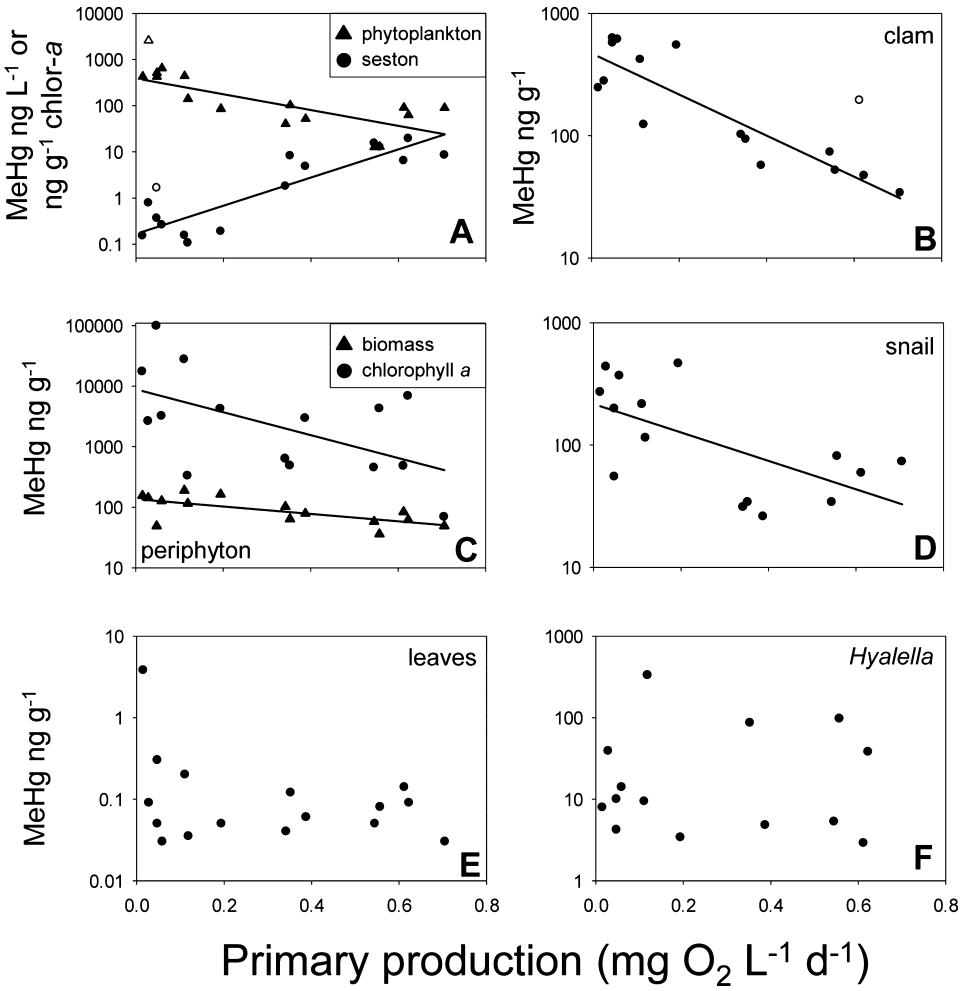

Figure 2. Effect of primary production on MeHg concentrations in (A) phytoplankton (triangles) and seston (circles). (A) Log_{10} phytoplankton chlorophyll *a* MeHg = −1.714 (mg O_2 L^{-1} d^{-1}) + 2.590, R^2 = 0.59, P < 0.0009; Log_{10} seston MeHg = 3.055(mg O_2 L^{-1} d^{-1}) − 0.780, R^2 = 0.80, P < 0.0001. (B) Log_{10} clam MeHg = −1.680 (mg O_2 L^{-1} d^{-1}) + 2.673, R^2 = 0.78, P < 0.0001; (C) Log_{10} periphyton biomass MeHg = −0.613(mg O_2 L^{-1} d^{-1}) + 2.134, R^2 = 0.47, P = 0.005; Log_{10} periphyton chlorophyll *a* MeHg = −1.890(mg O_2 L^{-1} d^{-1}) + 3.949, R^2 = 0.31, P = 0.03; (D) Log_{10} snail MeHg = −1.162(mg O_2 L^{-1} d^{-1}) + 2.336, R^2 = 0.40, P = 0.01. MeHg in leaves (E) and (F) *Hyalell*a were unrelated to primary production. Open symbols are statistical outliers excluded from models.

had substantial phytoplankton biomass and thus may be more representative of larger, turbid river systems where phytoplankton/filter feeder pathways have greater importance.[45] Our results suggest that contaminant accumulation in food webs of larger rivers is more similar to lentic systems, contradicting the higher productivity/higher accumulation conceptual model that Berglund[24] generalized to all rivers. While the Berglund[24] model was developed explicitly for organic contaminants, it is reasonable to extend it to MeHg because the mechanism for food web accumulation (i.e., uptake by primary consumers and trophic transfer to primary consumers) is similar for MeHg and organochlorines.

Second, the interactions among contaminant uptake, periphyton biomass, and consumer accumulation are central to the competing stream productivity/accumulation models. Our ability to characterize these important interactions was somewhat limited, owing to snail mortality toward the end of the experiment. However, our results still support the lower productivity/higher accumulation hypothesis.[17] By day 14 of the experiment, MeHg concentrations in stream periphyton was much lower in productive streams and corresponded with MeHg concentrations in snails that were an order of magnitude less than those in low-productivity streams. These results align with prior modeling and experimental findings that primary production significantly reduces concentrations of MeHg, Hg, and other metals in stream algal biofilms.[26] However, the relationship between primary production and MeHg accumulation in streams may vary depending on the primary producer community. Accumulation of MeHg can increase with primary production where dense algal mats foster methylation of inorganic Hg.[18,19] Our experiment was not designed to assess this potentially important in situ process as our benthic algae community was composed of periphyton rather than attached macroalgae and associated epiphytes. While not measured, it also is likely that Hg methylation by benthic algae was trivial as a result of low Hg availability: concentrations of total Hg, as Hg(II), in southwest Ohio groundwater (the source water used in this experiment) are <1 ng L^{-1}.

Dynamics of MeHg Accumulation in Stream Food Webs. Lower stream primary production was related to less phytoplankton biomass and higher MeHg concentrations in phytoplankton and clam tissues. These results are consistent with comparatively lower MeHg concentrations resulting from greater production as demonstrated by experiments manipulat-

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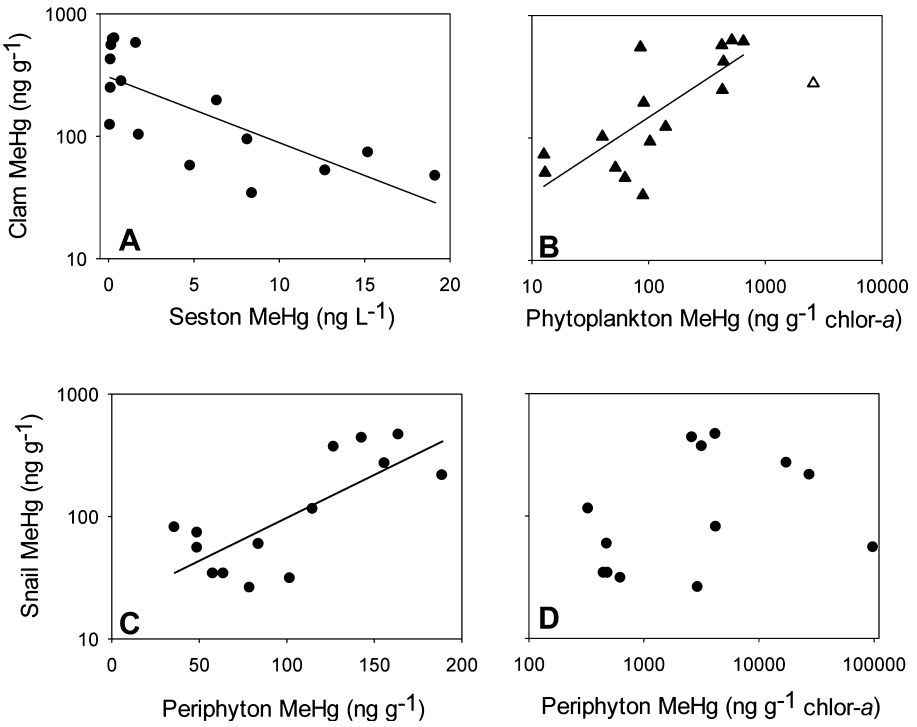

Figure 3. Effect of MeHg in food on MeHg concentrations in consumers. (A) Log_{10} clam MeHg = −0.054(seston MeHg) + 2.483, R^2 = 0.55, P < 0.01. (B) Log_{10} clam MeHg = 0.627(log_{10} phytoplankton chlorophyll *a* MeHg) + 0.913, R^2 = 0.58, P < 0.001. (C) Log_{10} snail MeHg = 0.007(periphyton MeHg) + 1.287, R^2 = 0.57, P = 0.002. (D) MeHg concentrations in snails and periphyton chlorophyll *a* were unrelated. Panels B and D are plotted on a log−log scale to better illustrate the distribution of MeHg concentrations. Open symbols are statistical outliers excluded from models.

ing lentic food webs with nutrient gradients (e.g., refs 9 and 10). However, clams produce pseudofeces whereas cladoceran zooplankton used in the previous experiments do not.[9,10] Pseudofeces could have altered MeHg cycling and accumulation in our experimental systems if pseudofeces preferentially scavenged MeHg and reduced its bioavailability. If this were the case, then we would not have expected the strong, significant relationship between MeHg concentrations in phytoplankton and clams. Likewise, primary production had similar effects on MeHg concentrations in phytoplankton and seston prior to addition of clams (results not shown), and these results are independent of pseudofeces effects. Collectively, the greater mass removal of MeHg from the water column and higher concentrations of MeHg in seston, along with lower concentrations in phytoplankton and clams, are all consistent with bloom dilution, and the potential addition of pseudofeces appears to have little influence on this main finding.

Terrestrial detritus is an important resource for stream food webs, particularly in forested headwater streams,[45] yet this pathway is relatively understudied in contaminant accumulation investigations.[13,46] Our results support a growing body of evidence that heterotrophic biofilms on leaves are less important for MeHg uptake into stream food webs than autotrophic pathways.[13,19,47] Leaf MeHg concentrations were unaffected by primary productivity, and ergosterol measurements indicated no effect of light levels on fungi growth, as would be expected for heterotrophic organisms. However, nutrient enrichment can greatly enhance flows of elemental nutrients (carbon, nitrogen, and phosphorus) to primary consumers in detritus-based stream food webs,[48] and it is conceivable that MeHg accumulation would respond similarly in streams where heterotroph biomass and leaf decomposition are enhanced by elevated nutrients. Our results do not support this process because nutrient availability was high in streams with low primary production and neither ergosterol content nor leaf MeHg concentrations responded to excess nutrients. Methylmercury concentrations in *Hyalella* were unrelated to, and much higher than, those in leaves, suggesting an alternate source of MeHg to *Hyalella*. However, *Hyalella* MeHg concentrations were also unrelated to MeHg concentrations in water, seston, periphyton, and phytoplankton (results not shown), so the dynamics leading to MeHg accumulation by *Hyalella* in this study are unresolved.

The relative contribution of periphyton and phytoplankton assemblages to primary production varies with stream size, becoming increasingly dominated by phytoplankton in larger rivers.[45] We observed that primary production appears to dilute concentrations of MeHg in both periphyton and phytoplankton assemblages, as observed for Hg and other metals in other fluvial systems.[17,26] Given that phytoplankton (large rivers) and periphyton (small streams) are an entry point of MeHg into many stream food webs, we hypothesize that MeHg accumulation relative to primary productivity should behave similarly in rivers regardless of size. However, other ecological, geochemical, and landscape factors (e.g., in-stream processes governing Hg methylation, predator size and trophic position, pH and sulfate concentrations, and Hg loadings) will likely interact with primary production to create spatial variation in MeHg accumulation across stream networks.[15,16,49−51]

The negative relationship between primary production and Hg accumulation in lentic systems has been well established through abundant field studies and experiments. In contrast, the relationship between primary production and Hg accumulation in lotic systems has remained unresolved, leading

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to diametrically opposed hypotheses (contaminant accumulation does/does not increase with primary production in streams) in the literature. These hypotheses were founded upon scant field data and had not been tested experimentally. Our mesocosm experiment not only resolved these opposing hypotheses but also identified the mechanism, bloom dilution, leading to lower MeHg accumulation with increasing primary production in streams. These hypotheses have remained untested in part because stream ecosystems are far more complicated to replicate experimentally than are lentic systems (i.e., easily replicated in beakers or aquaria), and there are few facilities equipped to replicate streams. Stream mesocosms provide an ideal platform for scaling ecotoxicology experiments from more traditional single-species tests to ecosystem-level investigations of stressor responses.[52] Our experimental design allowed us to assess multiple pathways of exposure (two autotrophic and one heterotrophic pathway), and this multitrophic approach illustrates the power of stream mesocosms to provide controlled studies of food web dynamics as they interact with multiple stressors, such as contaminant exposure and eutrophication, that are common across stream ecosystems.[52]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental set up and conditions; sample collection and chemical analyses; table summarizing time line of events; photographs of experimental facility and streams as well as stream resources and consumers; results for nutrients, ergosterol concentration and *Hyalella* MeHg concentrations; aqueous MeHg concentrations and % MeHg removed from the water during the experiment. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00911.

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Notes

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