Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs

Paul C. Pickhardt*†, Carol L. Folt*, Celia Y. Chen*, Bjoern Klaue‡, and Joel D. Blum‡

*Department of Biological Sciences, Dartmouth College, Hanover, NH 03755; and ‡Department of Geological Sciences, University of Michigan, Ann Arbor, MI 48109

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Mercury accumulation in fish is a global public health concern, because fish are the primary source of toxic methylmercury to humans. Fish from all lakes do not pose the same level of risk to consumers. One of the most intriguing patterns is that potentially dangerous mercury concentrations can be found in fish from clear, oligotrophic lakes whereas fish from greener, eutrophic lakes often carry less mercury. In this study, we experimentally tested the hypothesis that increasing algal biomass reduces mercury accumulation at higher trophic levels through the dilution of mercury in consumed algal cells. Under bloom dilution, as algal biomass increases, the concentration of mercury per cell decreases, resulting in a lower dietary input to grazers and reduced bioaccumulation in algal-rich eutrophic systems. To test this hypothesis, we added enriched stable isotopes of Hg to experimental mesocosms and measured the uptake of toxic methylmercury ($CH_3{}^{200}Hg^+$) and inorganic $^{201}Hg^{2+}$ by biota at several algal concentrations. We reduced absolute spike detection limits by 50–100 times compared with previous techniques, which allowed us to conduct experiments at the extremely low aqueous Hg concentrations that are typical of natural systems. We found that increasing algae reduced CH_3Hg^+ concentrations in zooplankton 2–3-fold. Bloom dilution may provide a mechanistic explanation for lower CH_3Hg^+ accumulation by zooplankton and fish in algal-rich relative to algal-poor systems.

Nutrient enrichment with subsequent eutrophication is one of the most important problems impacting lakes worldwide (1, 2). Increased nutrient concentrations produce algal blooms, which in turn alter concentrations of nutrients, gases, pH, and metal ions in the water (3). It is our hypothesis that by increasing algal abundance, nutrient enrichment also alters Hg inputs to lake food webs. Mercury concentrations in fish have been related to metal burdens in their zooplankton prey (4–8), but the connection between Hg accumulation by zooplankton and increasing algal density under nutrient enrichment has not been established. It is critical to discern this association because algae can concentrate Hg from the aqueous phase (e.g., by 100–10,000+ times) and thus provide the greatest inputs of Hg to the food chain (9, 10). Here we report how an induced algal bloom affects the accumulation of methyl and inorganic Hg in the cladoceran *Daphnia* after 2 and 3 weeks of grazing on algae labeled with stable isotopes of Hg. *Daphnia* is a common zooplankton herbivore and known to be a major food for planktivorous fish (11), therefore factors affecting Hg burdens in this "keystone" (12, 13) prey taxon may have important ramifications for predicting CH_3Hg^+ burdens in fish across lakes of varying trophic status.

We experimentally tested the hypothesis that at equal initial concentrations of aqueous Hg, an increase in algae will result in a decrease in Hg uptake—by zooplankton grazers. Our rationale for this hypothesis was that the concentration of metal per cell would be lower in dense algal blooms (hereafter, bloom dilution) because the same amount of metal would be distributed among a greater number of algal cells. A related but different phenomenon, *growth biodilution* of trace metals, is observed in rapidly growing phytoplankton, whereby biomass-specific concentrations of metal diminish as cells divide (14). How either process of dilution with the phytoplankton affects the zooplankton, however, is not known. Possible bloom dilution has been observed for polychlorinated biphenyls (15, 16), As (17), Po, Cd, and Co (18) but has not been reported for Hg. To our knowledge, this is the first experimental manipulation to test bloom dilution in freshwater plankton.

Materials and Methods

Preparation of Algal Density Gradient. To test for effects of algal density on mercury accumulation in algae and on *Daphnia* subsequently grazing on those algae, 12 mesocosm stock tanks were used. The 550-liter resin tanks were scrubbed clean with a low detergent, low trace metal soap, rinsed, and then filled with approximately 450 liters of low ionic-strength water from a crystalline bedrock well. Samples of well water were first analyzed for trace metals by means of magnetic sector inductively coupled plasma-MS to ensure that the well water was low in metals and there were no significant differences between tanks (P.C.P., unpublished data). To buffer the systems from fluctuations in pH and to provide an adequate microbial community, 50 g (wet weight) of leaves (locally collected *Fagus grandifolia*, *Betula papyrifera*, *Acer saccharum*, and *Quercus rubra*) were added to each tank (Fig. 1*A*). Tanks were covered securely with fiberglass window screening to reduce unwanted colonization by invertebrates and to minimize airborne nutrient inputs. Water in the tanks was equilibrated with the atmosphere for 48 h before further additions. Each tank was then inoculated with phytoplankton and microzooplankton, by adding 3 liters of 48 μm of filtered Post Pond (Lyme, NH) water (Fig. 1*B*). Baseline nitrogen and phosphorus were measured after phytoplankton had been in the tanks for 48 h (Fig. 1*C*). Twenty-four hours after baseline nutrient measurements, tanks were randomly assigned to one of six nutrient levels with two tanks at each level. The lowest phosphorus level was 7.4 μg of P·liter^{-1} with inorganic nutrients doubling at each of the subsequent nutrient levels to a maximum of 44.6 μg of P·liter^{-1} at level six. Additions of nitrogen and phosphorus in the form of dissolved $NaNO_3$ and K_2HPO_4 (2.51 and 36.72 g·liter^{-1}, respectively) were made so as to achieve the desired atomic ratio of 30:1 (N:P) (Fig. 1*D*). Phosphorus concentrations added to the tanks corresponded to concentrations found routinely in lakes in the northeastern U.S. (19). Standing stocks of phytoplankton within the 12 tanks were left to develop for 9 days after the application of the 6 inorganic nutrient levels (Fig. 1 *D* and *E*).

Adding Hg Isotopes and Zooplankton. On day 14 (Fig. 1*E*), stable isotopes were added to the tanks. A stock solution of 50 mg·liter^{-1} enriched ^{201}Hg (Oakridge National Laboratory, 98.11% ^{201}Hg) was prepared in 0.01 M HCl. Enriched mono-

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†To whom reprint requests should be addressed. E-mail: pickhardt@dartmouth.edu.

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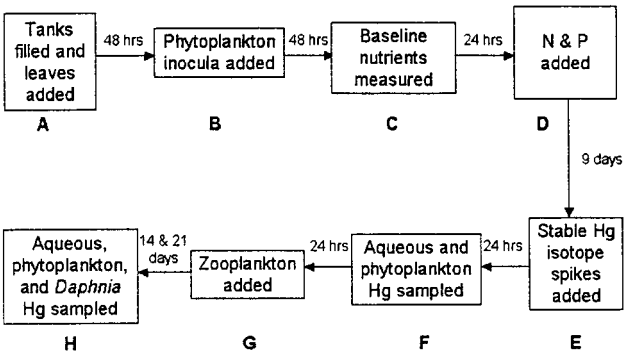

Fig. 1. Chronology of mesocosm tank experiments. Time intervals given between boxes indicate time elapsed between the respective procedures. Note that the sampling described in *H* was conducted at two separate periods after zooplankton addition (*G*).

methylmercury, $CH_3{}^{200}Hg^+$ (Oakridge National Laboratory, 96.41% ^{200}Hg), was synthesized by methylating $^{200}HgCl_2$ with methylcobalamin (20). After extraction with CH_2Cl_2 and back extraction into dilute HCl, a stock solution of 8 mg·liter^{-1} $CH_3{}^{200}HgCl$ in 0.01 M HCl was made. Of the $^{201}HgCl_2$ and $CH_3{}^{200}HgCl$ stock solutions, 1.00 and 1.25 ml, respectively, were added and thoroughly mixed with a wooden paddle to each of the 12 tanks to achieve an initial tank water concentration of 100 ng·liter^{-1} ^{201}Hg and 20 ng·liter^{-1} $CH_3{}^{200}Hg$ (Fig. 1*E*). Forty-eight hours after the stable isotope spikes, macrozooplankton collected from Post Pond with an 80-μm net were added at approximately 2 times the natural density to allow for mortality in transition.

Tank Monitoring. Physical conditions in all of the tanks were monitored throughout the experiments. Specific conductivity, dissolved oxygen, temperature, and tank water pH were measured every 48 h between 13:00 and 15:00. By the addition of small volumes of dilute (2.0 M) H_2SO_4, the pH was maintained between 7.8 and 8.2 for all tanks. Samples for phytoplankton biomass (by means of chlorophyll *a* samples) were collected 24 h after mercury spike additions (Fig. 1*F*) and at the two zooplankton sampling periods (Fig. 1*H*). Samples for zooplankton taxonomy, density, length, and biomass were also collected when zooplankton were sampled for Hg (Fig. 1*H*).

Collection and Inductively Coupled Plasma (ICP)-MS Analyses of Isotope Samples. The isotope spike analyses were performed by continuous-flow cold-vapor generation magnetic sector-ICP-MS (8, 21–23). Collection and digestion of samples for $CH_3{}^{200}Hg^+$ and $^{201}Hg^{2+}$ in water, particulates, and zooplankton were conducted as follows. Sampling equipment and sample vials were acid-cleaned in sequential 1 M nitric acid, 1:5 hydrochloric acid, and trace metal-grade (distilled) dilute nitric acid with ultra-pure water rinses before and after each acid bath (8). Aqueous mercury samples were collected in borosilicate glass vials with Teflon septa and preserved to ≈pH 1 with Seastar Baseline HNO_3 (Seastar Chemicals, Sidney, BC, Canada). Particulate samples (particles >0.45 μm and <45 μm) were collected by filtering 100 ml of tank water on to cellulose acetate filters that had been rinsed with dilute (≈0.33 M) distilled nitric acid and ultra-clean water. Cellulose acetate filters with sample were immediately transferred to Teflon vials. Aqueous and particulate samples were collected 24 h after metal spike additions (Fig. 1*F*) and again when live zooplankton were sampled (Fig. 1*H*). Live zooplankton were field-sorted into Teflon vials under a dissecting scope 2 and 3 weeks after metal spike additions (Fig. 1*H*). *Daphnia* mercury burdens were calculated for two tanks at each respective treatment level with two samples (10–20 *Daphnia mendotae*) from each tank. All samples were stored in the dark at ≈4°C before digestion and analysis. Particulate and zooplankton samples were digested for 10–12 h at 70°C with a mixture of HNO_3 and HCl (2:1; Seastar Baseline acids). Acidified water samples were not digested further (8).

The quantification of the enriched isotope spikes of ^{200}Hg and ^{201}Hg was performed by standard-sample-standard bracketing with certified external Hg standards of natural isotopic abundance. The natural background of ^{200}Hg and ^{201}Hg was subtracted based on the measured $^{198}Hg/^{200}Hg$ and $^{198}Hg/^{201}Hg$ ratio of the bracketing standards. The external calibration of the ^{200}Hg and ^{201}Hg spike concentrations was based on the atomic mass fraction of ^{200}Hg and ^{201}Hg in the natural abundance standards (46.24 g ^{200}Hg·mol^{-1} Hg and 26.54 g ^{201}Hg·mol^{-1} Hg). The procedural detection limits by isotope dilution were a function of the precision of the isotope ratio measurements (about 0.1%) and the background concentrations. Our method allows for the unambiguous tracking of picograms/femtomols of CH_3Hg^+ and Hg(II) from aqueous spikes into algae and zooplankton.

Detection Limits. Twenty-four hours after the stable isotope additions, aqueous Hg concentrations were close to our method detection limits for water samples (0.5 ng·liter^{-1} for ^{200}Hg and ^{201}Hg). These extremely low aqueous Hg concentrations met our goal of conducting experiments at dilute concentrations typical of most lakes (8). We achieved detection limits of the isotopically labeled Hg species for the particulate and zooplankton samples for ^{200}Hg and ^{201}Hg of 1 ng·liter^{-1} or 0.5 pg, respectively, which is a 50–100-fold improvement over traditional analytical techniques using additions of isotopically unlabeled Hg or radioactive Hg tracers (8, 24).

Statistical Analyses. We adopted a gradient approach with our mesocosm experiments wherein we traded off lower replication at each treatment level ($n = 2$) in favor of increasing the number of treatment levels ($n = 6$). This design is intended for regression analysis and allows for a more robust examination of trends and overall effects of a treatment in the face of high variation within treatments. This gradient approach was ideal for our goal to identify the general direction and magnitude of nutrient addition and increasing algal biomass effects on mercury uptake by grazers. The strength and generality afforded by this approach to ascertain the overall effect of treatments on specific dependent variables has made it a common approach for experiments involving ecological gradients (25, 26). Treatment effects were assessed by means of regression analysis [*F* test comparison of model mean squares divided by error mean squares, JMP (version 4.04, SAS Institute, Cary, NC)]. Least squares regression lines and 95% confidence intervals are plotted for variables only when the relationship is significant at the $P \leq 0.05$ level.

Results and Discussion

As expected, 9 days after the inorganic nutrient gradient was applied to the mesocosms there were significant differences in standing algal biomass measured as chlorophyll *a* (Fig. 2*A*) across tanks. Temperature, conductivity, and pH did not vary across treatments although there was a significant increase ($R^2 = 0.17$, $P < 0.0001$) in dissolved oxygen at higher nutrient concentrations as expected with increased algal density (P.C.P., unpublished data). The conditions in the tanks at the time of zooplankton addition (11 days after inorganic nutrient additions as per Fig. 1) were well within the range of conditions experienced in the pelagic water of oligotrophic to mesotrophic lakes in temperate North America (7, 19).

Our first important finding was that at the time of zooplankton addition there was considerable bloom dilution of the Hg spikes

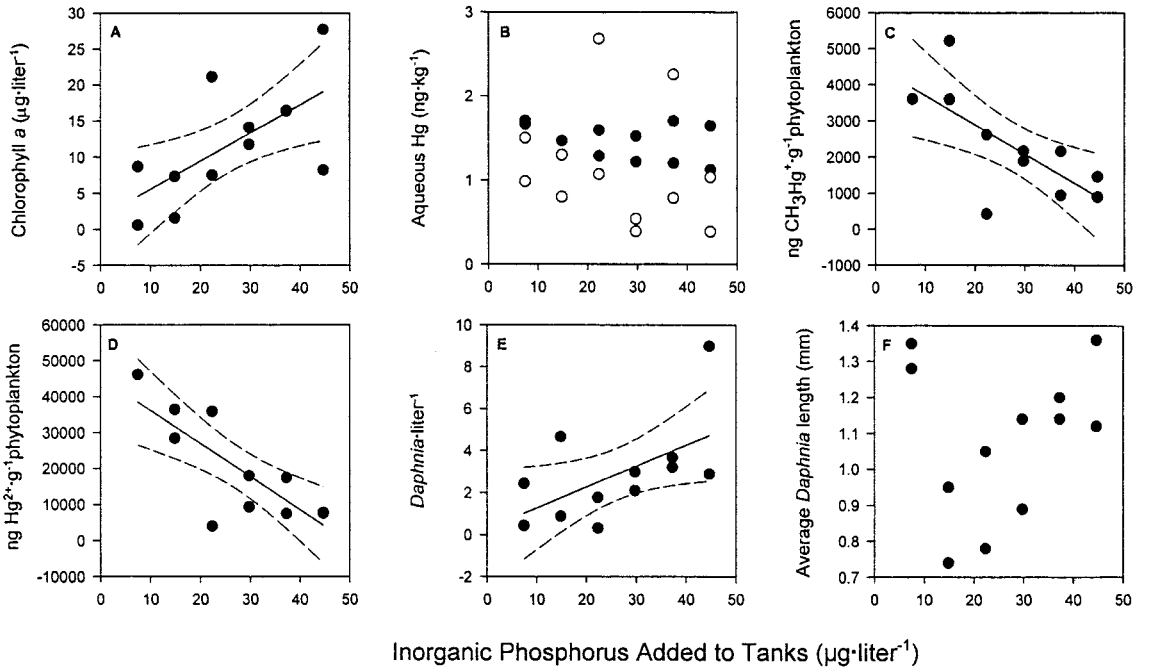

Fig. 2. Effect of added phosphorus on chlorophyll *a* concentrations at the time of the metal spike additions (*A*). $n = 12$, chlorophyll $a = 0.389(\mu g\ P\ added{\cdot}liter^{-1}) + 1.69$, $R^2 = 0.431$, $P < 0.021$. Aqueous concentrations of $CH_3{}^{200}Hg^+$ (●) and $^{201}Hg^{2+}$ (○) 24 h after additions to experimental tanks (*B*). For aqueous $CH_3{}^{200}Hg^+$: $n = 12$, $CH_3Hg^+ = -0.005(\mu g\ P\ added{\cdot}liter^{-1}) + 1.59$, $R^2 = 0.102$, $P > 0.311$. For $^{201}Hg^{2+}$: $n = 12$, $Hg^{2+} = -0.010(\mu g\ P\ added{\cdot}liter^{-1}) + 1.41$, $R^2 = 0.035$, $P > 0.558$. Effect of nutrient additions to CH_3Hg^+ (*C*) and Hg^{2+} (*D*) associated with algal biomass 24 h after metal spike additions. For CH_3Hg^+ (*C*): $n = 11$, $CH_3Hg^+ = -80.14(\mu g\ P\ added{\cdot}liter^{-1}) + 4502$, $R^2 = 0.499$, $P < 0.016$. For Hg^{2+} (*D*): $n = 11$, $Hg^{2+} = -917(\mu g\ P\ added{\cdot}liter^{-1}) + 45290$, $R^2 = 0.623$, $P < 0.004$. Algal biomass derived from chlorophyll *a* concentrations at time of metal spikes, assumes 1.25% of algal biomass is chlorophyll *a* (27). For both *C* and *D* an outlier from the lowest phosphorus addition level was excluded from regression analyses—in each case, the measured value exceeded 1 SD from a 0.99 confidence interval. Effect of nutrient additions on adult *Daphnia* density 3 weeks after metal spikes (*E*): $n = 12$, *Daphnia*·liter$^{-1} = 0.74(\mu g\ P\ added{\cdot}liter^{-1}) + 0.27$, $R^2 = 0.323$, $P < 0.054$. Effect of nutrient additions on the mean length of adult *Daphnia* 3 weeks after metal spikes (*F*): $n = 12$, mean *Daphnia* length $= 0.02(\mu g\ P\ added{\cdot}liter^{-1}) + 1.01$, $R^2 = 0.030$, $P > 0.59$. Total nitrogen and total phosphorus addition were kept at the atomic ratio of 30:1 as described in *Materials and Methods*. The 95% confidence intervals (---) are plotted for significant regressions.

under reasonable levels of nutrient enrichment. Twenty-four hours after the mercury spikes were added there was no detectable difference in aqueous Hg concentrations across tanks (Fig. 2*B*). Yet organic CH_3Hg^+ and inorganic Hg^{2+}spike concentrations in particulates were 10^3-10^4 times greater than in the water after 24 h of exposure to the isotope spikes (Fig. 2 *C* and *D*), demonstrating the rapid and successful incorporation of the isotope spikes into algal biomass. Moreover, there were significant differences in total algal Hg measured across the nutrient gradient after 24 h (Fig. 2 *C* and *D*). In general, tanks with greater nutrient enrichment had greater algal biomass and lower Hg per gram of algal material (Fig. 2 *A*, *C*, and *D*). This evidence is a sound demonstration of bloom dilution.

Our second major finding was that as hypothesized, bloom dilution of Hg in algae initiated different mercury uptake dynamics in the zooplankton under high- vs. low-nutrient enrichment. Specifically, methylmercury concentrations were consistently and significantly lower in *Daphnia* from the high nutrient, high initial algal biomass tanks compared with *Daphnia* from the low nutrient, and low initial algal biomass tanks at 2 and 3 weeks after zooplankton additions (Fig. 3 *A* and *C*). Correspondingly, low algal abundances resulted in a 2–3-fold increase in the accumulation of CH_3Hg^+ in *Daphnia* from low-nutrient tanks (Fig. 3 *A* and *C*). From these results, we infer that the concentration of CH_3Hg^+ in *Daphnia* across treatments was related to the concentration of CH_3Hg^+ (Fig. 2*C*) in the algal cells they ingested, which was in turn affected by algal biomass; e.g., that bloom dilution drives a diminution of metal in the zooplankton. We also observed similar results for effects of bloom dilution on calanoid and cyclopoid copepods (P.C.P., unpublished data). This result has important implications for trophic transfer of toxic CH_3Hg^+ to fish in oligotrophic lakes.

Despite the highly significant relationships measured in *Daphnia* CH_3Hg^+ burdens across the nutrient gradient, there is a substantial amount of unexplained variation in our data. Varying *Daphnia* ages, feeding rates, the number of developing embryos in *Daphnia* brood pouches, or possible genetic differences are possible factors contributing to this unaccounted variance. Moreover, there are other possible explanations for our finding. For example, as hypothesized for rapidly growing algae [e.g., growth biodilution (14)], a diminution of the mass-specific metal spike in animals could result whenever there are rapid increases in zooplankton density or biomass (i.e., when the production of new tissue outpaces the uptake of metal). Growth biodilution cannot explain our results at 2 weeks because there were no differences in zooplankton density across treatments even though marked differences in methylmercury levels of individuals were evident. Growth biodilution did not occur by means of increases in body size either, because there were no significant body-size differences in *Daphnia* with increasing nutrient addition 2 and 3 weeks after spike additions (see Fig. 2*F* for lengths at week 3). However, 3 weeks after the zooplankton additions there was a marginally significant trend for lower methylmercury concentrations in treatments with higher *Daphnia* densities (Fig. 2*E*). This pattern provides some support for the hypothesis that growth biodilution leads to lower mass-specific CH_3Hg^+ in *Daphnia* at high density over time.

Finally our third significant finding was that unlike CH_3Hg^+, bloom dilution of inorganic Hg^{2+} concentrations in the algae (Fig. 2*D*) had no measurable influence on the accumulation of

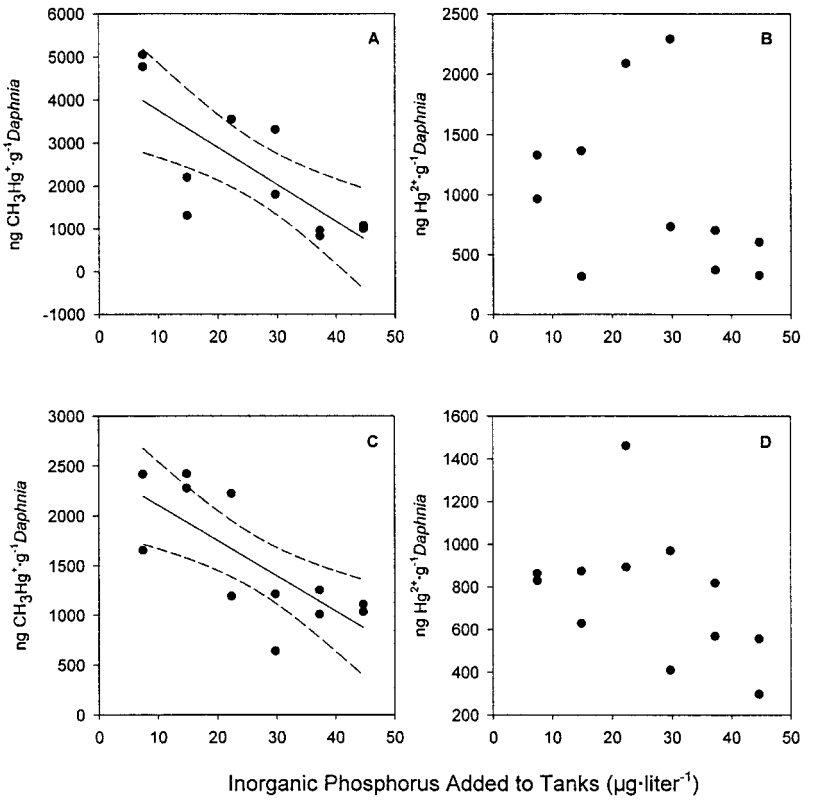

Fig. 3. Mean CH_3Hg^+ (*A*) and Hg^{2+} (*B*) concentration in *Daphnia* (g^{-1} dry weight *Daphnia*) against phosphorus addition (as per *Materials and Methods*) 2 weeks after zooplankton were added to the tanks. For CH_3Hg^+: $n = 12$, CH_3Hg^+ $g^{-1} = -643(\mu g$ P added·liter$^{-1}) + 4630$, $R^2 = 0.583$, $P < 0.0063$. For Hg^{2+}: $n = 12$, Hg^{2+} $g^{-1} = -125.4(\mu g$ P added·liter$^{-1}) + 1455$, $R^2 = 0.115$, $P > 0.306$. Mean CH_3Hg^+ (*C*) and Hg^{2+} (*D*) concentration in *Daphnia* with nutrient addition 3 weeks after zooplankton were added to the tanks. For CH_3Hg^+: $n = 12$, CH_3Hg^+ $g^{-1} = -265(\mu g$ P added·liter$^{-1}) + 2465$, $R^2 = 0.554$, $P < 0.0056$. For Hg^{2+}: $n = 12$, Hg^{2+} $g^{-1} = -78.7(\mu g$ P added·liter$^{-1}) + 1041$, $R^2 = 0.213$, $P > 0.130$. The 95% confidence intervals (---) are plotted for significant regressions.

inorganic Hg^{2+} in *Daphnia* (Fig. 3 *B* and *D*). To our knowledge, this is the first study to experimentally demonstrate the preferential accumulation of CH_3Hg^+ relative to inorganic Hg^{2+} in grazing invertebrates feeding on an intact phytoplankton assemblage. Preferential accumulation of CH_3Hg^+ in zooplankton is reasonable to expect because zooplankton show the greatest assimilation rates of Hg from algal cytoplasm (28), where CH_3Hg^+ is concentrated in algal cells (6, 9, 10). In contrast, inorganic mercury tends to remain surface-bound and thus is less likely to be assimilated (10).

Our study did not include data for mercury accumulation by nonalgal particulate matter, which is known to be a significant Hg source to nonselective grazers such as *Daphnia* in some natural systems (29). In these experiments, the tanks were low in nonalgal particulates. Another important determinant of mercury cycling in aquatic systems that we did not quantify was the scavenging of mercury compounds by suspended particulate matter and detritus (30).

We conclude that CH_3Hg^+ transferred to grazing zooplankton, and eventually to fish and other vertebrates, will be influenced by nutrient pulses and algal blooms. More specifically, algae effectively and rapidly concentrate both inorganic and organic Hg, but the metal burden per cell decreases in algal blooms. Bloom dilution of CH_3Hg^+ in algae results in a substantial reduction of CH_3Hg^+ uptake by cladocerans in high-nutrient, high-algae conditions. Conversely, cladocerans feeding within low-nutrient, low-algae treatments accumulate more CH_3Hg^+. Further, zooplankton that graze on algae preferentially accumulate CH_3Hg^+ relative to inorganic Hg^{2+}. This difference is instrumental in the efficient trophic transfer of CH_3Hg^+ relative to inorganic Hg to vertebrates. A final, unique feature of this research is demonstration of the value of using specific, stable isotope spikes of Hg to unambiguously track mercury through the food web near ambient concentrations. In particular, we tracked spikes of CH_3Hg^+ and inorganic Hg^{2+} and obtained exceptionally low absolute detection limits of those isotopic spikes (0.5–1 pg), which represents a significant improvement over traditional natural Hg or radioisotope methods.

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