

In Situ Production of Methylmercury within a Stream Channel in Northern California

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Natural stream ecosystems throughout the world are contaminated by methylmercury, a highly toxic compound that bioaccumulates and biomagnifies in aquatic food webs. Wetlands are widely recognized as hotspots for the production of methylmercury and are often assumed to be the main sources of this neurotoxin in downstream ecosystems. However, many streams lacking wetlands in their drainage basins (e.g., montane and semiarid regions in the western United States) have significant methylmercury contamination, and the sources of methylmercury in these streams remain largely unknown. In this study, we observed substantial production of methylmercury within a highly productive stream channel in northern California (South Fork Eel River) within a drainage basin lacking wetlands. We found that in situ methylmercury production is positively related to phosphorus removal and water temperature within the stream channel, supporting hypothesized biological mediation of in situ mercury transformation. Moreover, our data suggest that epiphytic microbial communities on a dominant filamentous alga (*Cladophora glomerata*) could play a role in in situ methylmercury production. Because peak in situ methylmercury production coincides with the period of the highest biological productivity during summer baseflow, methylmercury produced internally may be efficiently routed into local stream food webs. Our study provides strong evidence that stream channels, especially those associated with high primary productivity, can be important for regulating the bioavailability and toxicity of this global contaminant.

Introduction

Mercury (Hg) is distributed throughout the global environment through atmospheric transport and deposition (1, 2). Aquatic ecosystems are especially vulnerable to Hg contamination due to the production of bioaccumulative methylmercury (MeHg) within the watershed (3). In stream

ecosystems, the prevailing assumption is that most of the MeHg is derived from upstream wetlands (4, 5) where MeHg production is promoted by the highly reduced environment (6, 7). However, natural streams without hydrologically connected wetlands can often have high MeHg contamination (e.g., accumulated by fish) such as streams throughout the mountainous region in the western United States (8, 9), and their sources of MeHg are essentially unknown.

Within stream channels, biological activity can transform the speciation and bioavailability of essential chemicals such as organic carbon (10) and nitrogen (11). In situ transformation of Hg is not often examined but is assumed to have a negligible effect on the bioavailability of Hg to stream food webs (5, 12, 13). Nevertheless, since Hg cycling is strongly mediated by photochemically- and microbially mediated processes (3), sunlit and productive stream channels could provide prime conditions for transformations (methylation and/or demethylation) of Hg, leading to MeHg contamination even in streams without upstream sources of MeHg (8, 9). In fact, there is some evidence showing in situ Hg transformation within flowing waters. For example, Jackson (14) demonstrated in situ production of MeHg within a Hg-contaminated and eutrophic riverine lake system in Saskatchewan (Canada) and found that in situ MeHg production was mainly driven by the releases of organic nutrients from phytoplankton blooms and sewage during the low flow period (14). Furthermore, Balogh et al. (15) showed in situ production of MeHg in an agricultural stream in south-central Minnesota following an algal bloom in the summer as well as during extensive litter accumulation in the autumn. The results of both studies point out that the availability of organic matter and/or nutrients are key factors in promoting in situ MeHg production; Jackson (14) also found that the abundance of inorganic Hg (the substrate for MeHg) is of lesser importance. To date, there are no studies examining in situ production of MeHg in stream ecosystems lacking anthropogenic influences (e.g., enriched nutrients, suspended solids, and elevated Hg levels). Mercury cycling in natural ecosystems may be regulated by different factors compared to highly human-altered ecosystems.

In this work, we examined in situ MeHg production in a third-order and highly productive stream, the South Fork Eel River, in Branscomb, California. The watershed is largely undisturbed, with old growth forest and a long, sustained summer baseflow period (16, 17). We regard this stream as a model system to examine in situ MeHg production for three reasons: First, the sustained dry summer period precludes surface runoff to the stream channel that might complicate the identification of sources of MeHg (4, 5, 18). Second, the study site is representative of streams unaffected by point source discharges, and the aqueous Hg concentrations (typically below 1 ng L⁻¹ during baseflow (9)) are comparable to, or lower than, many other streams receiving primarily atmospheric Hg (5, 18). Third, since the drainage basin of this stream lacks wetlands, aqueous MeHg concentrations ([MeHg]) are low, and any in situ MeHg production can elevate [MeHg] considerably, which improves our ability to detect in situ MeHg production.

In summer 2009, we sampled surface water monthly along a 8.4-km productive stream reach of South Fork Eel River and at the mouth of four major tributaries draining to this reach. We filtered and analyzed water samples for total-Hg, MeHg, and a suite of cations and anions. Our previous work (9) showed that the tributaries often had very low dissolved MeHg concentrations (<20 pg L⁻¹ as our method detection limit), but the mainstem channel had considerably higher

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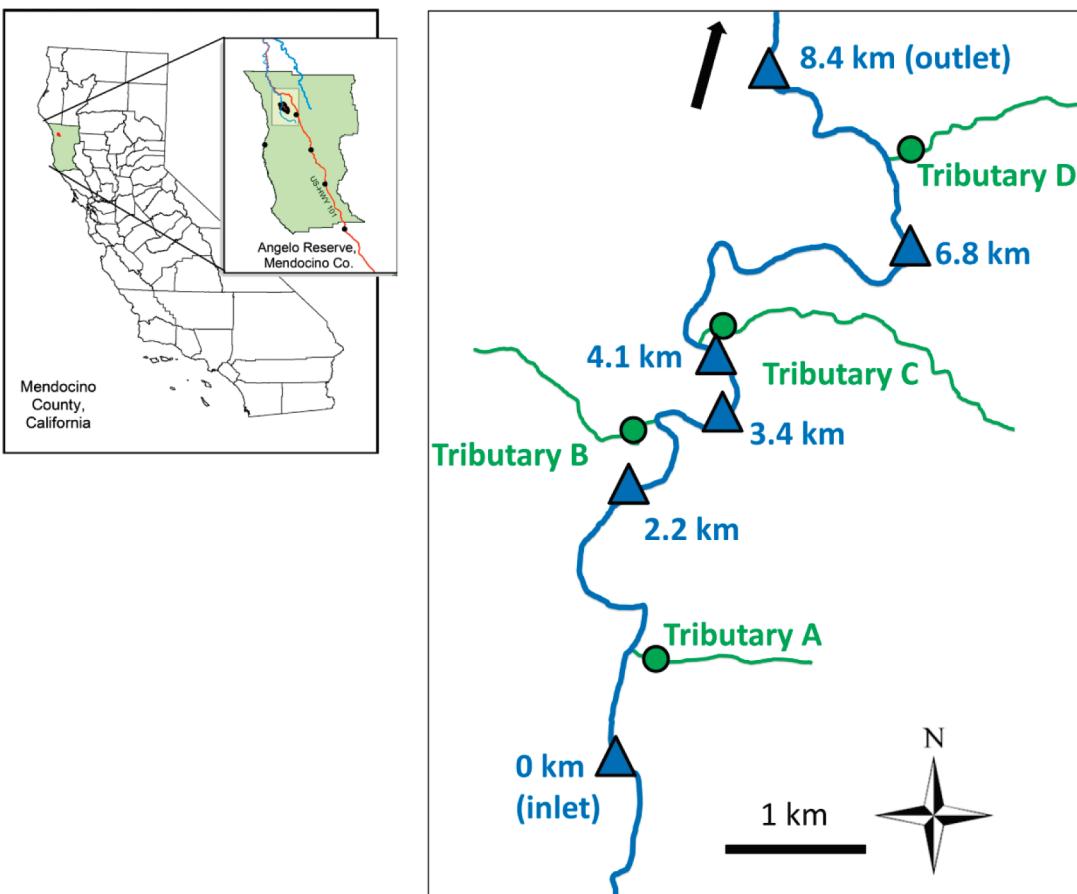


FIGURE 1. Locations of the sampling points on the mainstem stream channel (triangles) and at the mouths of the four major tributaries draining to this reach (circles).

dissolved MeHg concentration during summer baseflow ($>100 \text{ pg L}^{-1}$). Therefore, in the absence of rainfall during the baseflow period we expected that dissolved MeHg concentrations in the main channel would decrease downstream (or longitudinally) as MeHg in the mainstem was diluted by the water from the drained tributaries.

Experimental Section

Our study site is the South Fork Eel River, and sampling points were within or near the Angelo Coast Range Reserve of the University of California Natural Reserve System (39°44' N, 123°39' W) in Mendocino County, California (Figure 1, *left panel*). Most of the rainfall in the region occurs in the winter, and stream discharge declines steadily following winter rain, with summer baseflow typically maintained from early June through late September (16, 17). From May through October, 2009, we performed monthly sampling of surface water along the 8.4-km stream reach and the mouth of four major tributaries (Figure 1, *right panel*) during daytime (between 8 a.m. and 6 p.m., Pacific Standard Time) (Supporting Information (SI) part I). Discharge was measured monthly in the mainstem channel (points at 0 and 8.4 km) and at the mouth of four major tributaries, following Gore (19).

Since we intended to use daytime MeHg and phosphorus (P) concentration data to estimate daily mass balances of MeHg and P, it was necessary to ensure that diel variation of these constituents was not significant. To address this, we conducted diel sampling in the mainstem channel at a single site in our study reach (6.8 km in Figure 1) (SI part II). The results show expected diel cycles for both water temperature and pH (SI Figure S1) in response to daily variation in solar inputs. However, we found no significant diel cycles for either MeHg or P (SI Figure S1), so that our daytime measurements

of MeHg and P data could be used for daily mass balance estimates in this study.

Filtered samples were analyzed for total-Hg and MeHg by cold vapor atomic fluorescence spectrometry with appropriate standards and quality controls (SI part III), while filtered samples were submitted to outside laboratories for analyzing total-P, total-iron (Fe), and sulfate (SO_4^{2-}) (SI part III). Using discharge measurements at points 0 km and 8.4 km of the mainstem channel and at the mouths of the four major tributaries draining to the study reach (Figure 1), we performed a simple hydrologic mass balance to estimate filtered MeHg concentrations ($[\text{MeHg}]$) in the mainstem channel, assuming there was no in situ MeHg production and destruction within the stream reach (SI part IV).

Our previous study (9) suggested that the dominant primary producer in the stream (the filamentous alga *Cladophora*) may act as a hotspot of MeHg production and elevate aqueous MeHg concentrations in the study stream. In this study, we investigated whether *Cladophora* can be a net source of MeHg by incubating freshly collected algae in streamwater and then quantifying the release of MeHg to the water (SI part V). Moreover, an algal survey conducted by other researchers (Furey, P. C. and Power, M. E., University of California, Berkeley) at our study site from April through October 2009 recorded the colonization stages of *Cladophora* in the stream reach (SI part VI). We used this survey data to assess whether the composition of the algal community is related to changes in aqueous MeHg levels in the stream channel.

Results and Discussion

Environmental Characteristics. Through the dry summer period, we observed exponential declines in discharge in the

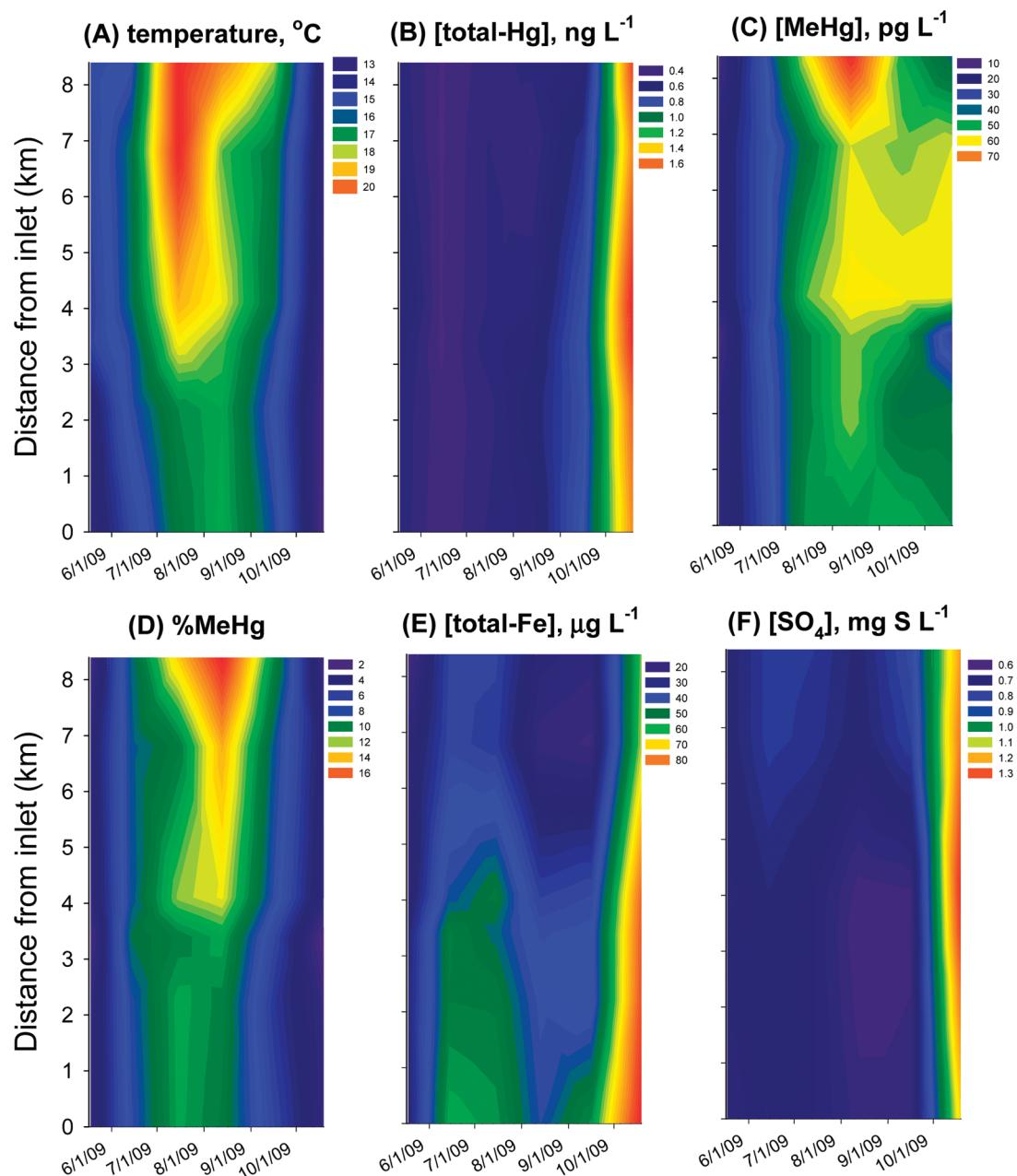


FIGURE 2. Spatiotemporal plots of (A) water temperature, (B) [total-Hg], (C) [MeHg], (D) fraction of total-Hg as MeHg (%MeHg), (E) [total-Fe] and (F) [SO₄²⁻] in the mainstem channel from May–October, 2009. Vertical axis indicates the distance along the sampling reach from inlet (bottom) to outlet (top).

mainstem and tributaries (SI Figure S2); discharge became stable in September and increased in October due to several rainfall events. Typically, winter rainfall results in discharge several orders of magnitude higher than those measured during summer (17). From May through August, water temperature in the mainstem channel increased due to the simultaneous increase of air temperature and decrease of water depth; water temperature also increased longitudinally in July and August owing to the reduction in canopy cover along the reach (Figure 2A); water temperature then decreased from August to October, 2009. Overall, water temperature in the mainstem was on average 1.0–7.3 °C higher than those in the shaded tributaries at the same time. Varying canopy cover in the stream network drives these spatial differences in solar inputs and water temperature, both of which influence primary productivity, leading to an increase in algal productivity with stream size (16).

Water Chemistry. In the stream network, dissolved total-Hg concentration ([total-Hg]) was consistently low, ranging from 0.24–0.58 ng L⁻¹ (median value = 0.43 ng L⁻¹, n = 106) for both the mainstem and tributaries from May through August (Figure 2B). However, [total-Hg] increased slightly in September (0.46–0.88 ng L⁻¹) and was further elevated in October (1.4–1.8 ng L⁻¹) in the mainstem (Figure 2B). Also, some tributaries showed elevated [total-Hg] in September (e.g., mean = 0.89 ng L⁻¹ in A and 0.78 ng L⁻¹ in B) and October (e.g., mean = 1.4 ng L⁻¹ in A and 1.5 ng L⁻¹ in B), which could be attributed to throughfall and/or terrestrial surface runoff which may desorb Hg from the leaf and/or surface soils (18). In fact, the October samples had higher dissolved organic matter content and were tinted yellow in comparison to very clear waters at all sites during the entire summer baseflow (9, 16).

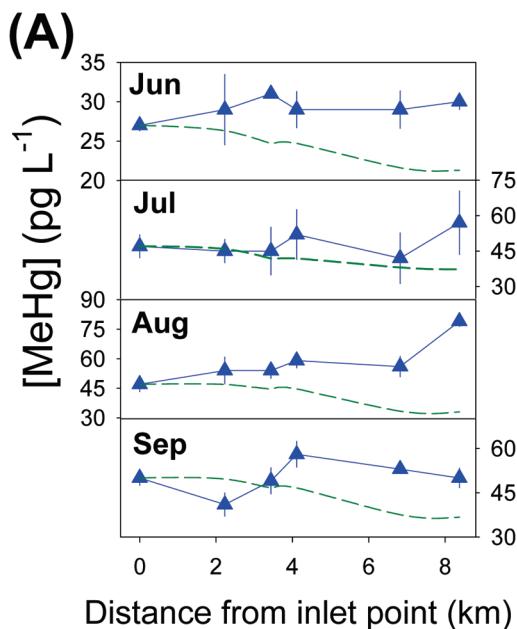
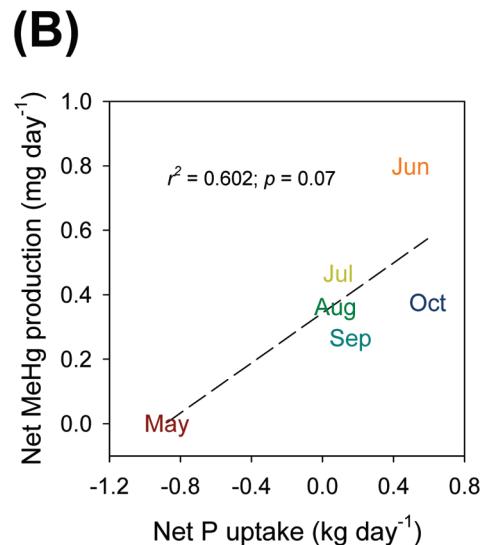


FIGURE 3. (A) [MeHg] (mean \pm s.e.m., $n = 3$) along the 8.4-km stream reach during baseflow period (June–September, 2009) plotted with the “dilution curve” (dotted gray line) that would result from inputs of very low [MeHg] water from the tributaries in the absence of in situ MeHg production and surface runoff within the study reach. Data for May and October were not included as they were regarded as not having baseflow. (B) Relationship between net MeHg production and net P uptake within the 8.4-km stream reach over the study period. The equation for the linear regression is as follows: Net MeHg production in $\text{mg day}^{-1} = 0.343 \times (\text{Net P uptake in } \text{kg day}^{-1}) + 0.388$.

All water samples in May had [MeHg] close to or below our method detection limit (MDL) of 20 pg L^{-1} . From June through October, [MeHg] in the mainstem channel was higher compared to all tributary streams (Figure 2C). Notably, [MeHg] increased significantly downstream ($p < 0.05$) during August (up to 84 pg L^{-1}). In October, the mainstem channel was influenced by surface runoff and [MeHg] was highly variable, ranging from relatively high [MeHg] to values below our MDL. Considering the fraction of total-Hg as MeHg in the water (or %MeHg), we found that %MeHg peaked during July and August and also increased longitudinally (Figure 2D), in accordance with the spatiotemporal pattern of water temperature (Figure 2A). In addition, the spatiotemporal pattern of [total-Fe] was somewhat opposite to either %MeHg or water temperature, suggesting that Fe cycling was related to MeHg production within the channels (Figure 2E), as was found in other ecosystems with in situ MeHg production (20). $[\text{SO}_4^{2-}]$ did not vary from May through September ($0.60\text{--}0.81 \text{ mg L}^{-1}$) but increased considerably in October ($1.15\text{--}1.34 \text{ mg L}^{-1}$) due largely to terrestrial inputs through surface runoff and/or throughfall (Figure 2F).

Methylmercury Mass Balance. In the tributaries, [MeHg] was consistently either close to or below the MDL (data not shown), and water from the tributaries should have diluted [MeHg] as it mixed into the mainstem channel. Using a hydrologic mass balance, we predicted that [MeHg] should decrease by 20–30% over the 8.4-km stream reach during the baseflow period. However, we found that longitudinal patterns of [MeHg] in the mainstem (except July due to a larger variation of [MeHg] at each site) deviated significantly ($p < 0.05$) from the “dilution curve” predicted based on the input of very low [MeHg] water from the tributaries (Figure 3A). [MeHg] actually increased by up to 60% over the 8.4 km stream reach during summer baseflow period. These results clearly indicate substantial input of MeHg within the stream channel, and this pool of MeHg could not be derived from an upland source as there was no rainfall and runoff from June through September. Gross production of MeHg would actually be much higher since extensive photodecomposition of MeHg in the water column is likely to occur due to the full



sunlight conditions and high transparency of the water column (due to the very low level of suspended solids) (21).

Mass-balance estimations for both MeHg and P in the entire 8.4-km stream reach showed a positive relationship between net production of MeHg and net uptake of P (Figure 3B). Phosphorus is widely regarded as an essential nutrient for biological activity including autotrophic and heterotrophic metabolism (22), and the positive relationship between MeHg production and P uptake in the stream reach may suggest biological mediation of MeHg production within the stream channels, such as microbial sulfate- and iron-reduction (23, 24).

Potential Sources of in Situ Methylmercury. Without rainfall and runoff during summer baseflow, upland sources of MeHg cannot explain the observed rise of MeHg within the study stream reach (Figure 3A), leaving in situ source(s) of MeHg as the most likely explanation. It is widely recognized that Hg methylation is regulated by organic matter availability in aquatic ecosystems (6, 14). In our study stream, extensive algal mats and filaments of *Cladophora* could represent potential in situ sources of MeHg. Previously, we found very high %MeHg in algal tissues (9) implying high net Hg methylation rate (7, 12). Our incubation of freshly collected *Cladophora* in streamwater showed substantial rates of MeHg release to the water ($0.35 \pm 0.06 \text{ pg g}^{-1} \text{ wet wt. h}^{-1}$) (mean \pm s.e.m., $n = 5$). This result indicates that *Cladophora* either released MeHg to the water directly or facilitated methylation of inorganic Hg in situ or both.

While it is possible that the *Cladophora* algal mats represent a source of MeHg to the stream, MeHg production is actually mediated by Hg methylating bacteria (6), and therefore the algal mats may facilitate MeHg production indirectly through accommodating these Hg methylating bacteria. We found that in situ MeHg production is related to the successional stages of the algal surfaces. Green (non-epiphytized) *Cladophora* started to build up throughout the study stream reach around April to May. As temperature increased and discharge declined (Figure 4A), microalgae (e.g., diatoms) and bacteria colonized algal filaments (25). Furthermore, the proportion of green, or non-epiphytized,

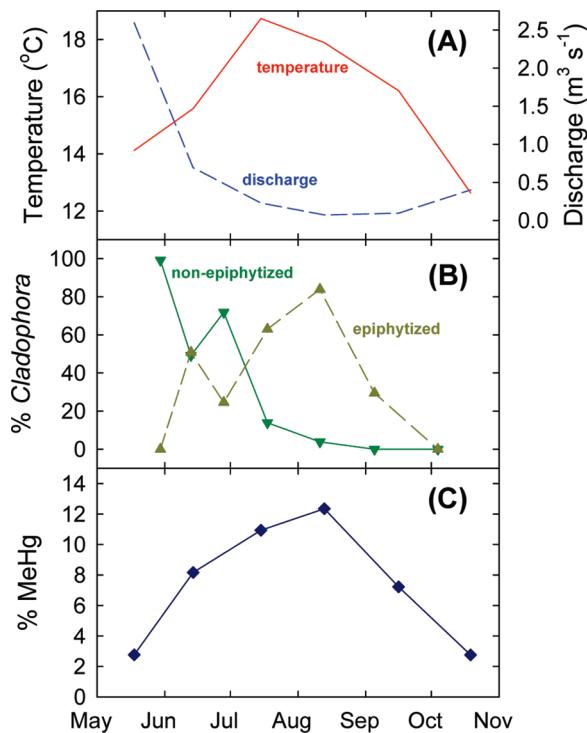


FIGURE 4. Temporal variability in (A) mean discharge and water temperature and (B) relative proportion of non-epiphytized and epiphytized *Cladophora* surveyed in two transects in the study stream in 2009 and (C) %MeHg in filtered streamwater.

Cladophora decreased from 100% in May to less than 10% in August in two surveyed transects (SI part VI), while the proportion of epiphytized (yellow and rusty) *Cladophora* rose (Figure 4B). After August, the proportion of epiphytized *Cladophora* decreased dramatically and decaying *Cladophora* subsequently dominated (data not shown). Interestingly, we found the temporal variation in the proportion of epiphytized alga (Figure 4B) was in parallel to that of %MeHg in water (Figure 4C) in the study reach. Since %MeHg can imply the net Hg methylation efficiency (12), therefore, these results suggest that microbial communities on the surface of *Cladophora* play a role in in situ MeHg production by accommodating Hg methylating bacteria on the algal surfaces.

Apart from algae, there are several other potential sources of in situ MeHg in the study stream. Surface sediment is widely regarded as a hotspot for MeHg production in standing waters (6, 26, 27), but in this bedrock-dominated stream, we previously found that %MeHg in sediment porewater was even lower than that in the surface water (9). Therefore, sediments do not appear to be active sites of MeHg production in this stream, consistent with the results of a recent large-scale study on streams across the United States (13). Hyporheic zone could be a possible source of in situ MeHg (28), but we do not have any direct evidence or measurement to confirm the existence of hyporheic zone in our study site, which clearly needs further study. Suspended solids (either organic or inorganic) have been shown to be associated with water column Hg methylation (14). However, our study stream does not have a high load of suspended particles ($<0.5 \text{ mg L}^{-1}$) during summer baseflow, and, therefore, it is very unlikely that suspended particulate matter is an important contributor to in situ Hg methylation during summer baseflow. Fish are known to contain a substantial amount of MeHg, and their carcasses can release MeHg during decay in water (29). However, during the summer baseflow period semelparous salmon species are not present (Finlay, J. C., personal observation) and the fish assemblage

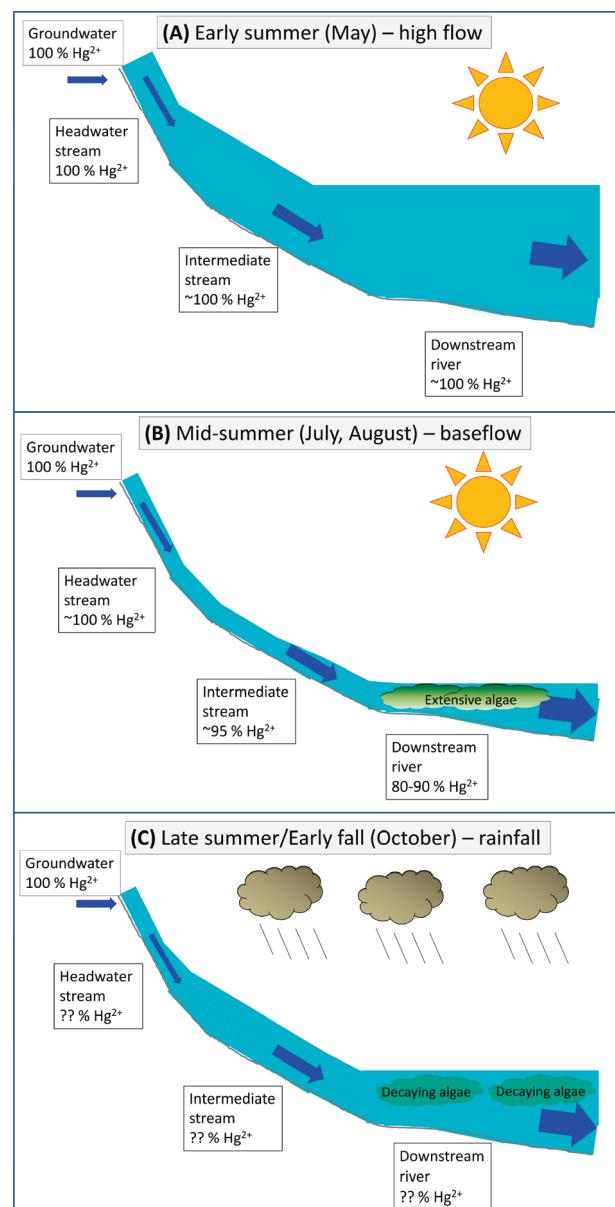


FIGURE 5. Schematic diagram summarizing our understanding of Hg dynamics in the study stream network from (A) early summer, to (B) middle summer, to (C) late summer or early fall, as influenced by weather, hydrology, and biological productivity. Note: total-Hg is assumed to consist of Hg^{2+} and MeHg in the water; “?? % Hg^{2+} ” in (C) means variable and unpredictable % Hg^{2+} or %MeHg in water due to the influences of episodic surface runoff.

is dominated by steelhead trout, which return to the ocean after spawning in the springtime.

Conceptual Framework of Mercury Transformation in the Stream Network. Based on the current and previous studies in this stream network (9), we summarize our current understanding of in situ Hg transformation in a schematic diagram (Figure 5). In early summer (May) when the streamflow is high, water temperature and algal productivity are relatively low (Figure 5A), and Hg speciation in the groundwater-dominated flow (almost exclusively inorganic Hg or Hg^{2+}) would remain largely unchanged as the water flows downstream. In midsummer, however, as the streamflow decreases and water temperature increases, biological (algal) productivity peaks in the downstream sites. These conditions (low flow and high biological activities) promote in situ methylation of Hg^{2+} and elevate the fraction of Hg^{2+}

that is methylated when water travels downstream (up to 20% (9)) (Figure 5B). In late summer and early fall, when the water temperature and primary productivity decrease and streamflow increases due to occasional rainfall events, in situ Hg methylation may decrease, while terrestrial runoff now represents a new source of MeHg to the streams (potentially through prior methylation in the upland). [MeHg] in the mainstem becomes more variable due to the episodic inputs of surface runoff which are accompanied by high $[Hg^{2+}]$ or [total-Hg], [Fe], and $[SO_4]$ (Figure 5C). Under baseflow conditions, in situ Hg cycling leads to increasing Hg bioaccumulation in the stream network as observed previously (9). Hydrographs in this stream network vary little year-to-year (17), and we can use this conceptual framework to predict when in situ MeHg production is substantial and becomes an important input to local food webs.

The present results should apply to many other ecosystems with similar hydrological regimes, such as the forested watersheds with Mediterranean climate located along the northern California and Oregon coast range (17), and with modest to high levels of primary productivity (either driven by high solar inputs or nutrient enrichment). Moreover, in situ MeHg production may not necessarily be restricted to ecosystems lacking extensive wetland coverage in the watershed. For example, it was previously observed that summer algal blooms and autumn litter fall accumulation can stimulate in situ MeHg production in nutrient-rich agricultural streams in Minnesota (15). In fact, it is likely that there are multiple sources of MeHg (both external and internal) to stream food webs, but their relative importance may change temporally and spatially with variation in temperature, hydrology, and biological productivity (5, 18).

Overall, our study provides new information on the ecosystem-scale mediation of Hg cycling in stream channels without substantial anthropogenic disturbance; in situ MeHg production appears to be more dynamic and sensitive to many external factors than the Hg cycling in standing waters (26, 27). Continued warming of the planet, widespread eutrophication and increasing global Hg emissions (30) will have important but complex effects on the in situ cycling of Hg in stream ecosystems and thus the contamination of stream food webs. More studies are clearly needed in this area to understand the mechanisms of Hg contamination in diverse stream ecosystems.

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Supporting Information Available

SI I: water sampling and preservation; SI II: diel water sampling; SI III: mercury and chemical analyses; SI IV: hydrologic and chemical mass balances; SIV: algal incubation experiment; SI VI: algal survey in South Fork Eel River; Figure S1: diel variation in the mainstem channel; Figure S2: discharges at the mainstem and tributaries in the study watershed. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information for

***In-Situ Production of Methylmercury within a Stream Channel in
Northern California***

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Supporting Information I – Water sampling and preservation

We sampled surface water at the mainstem channel and the mouth of four major tributaries from May through October, 2009. The actual sampling dates were May 17-19, 2009, June 13-15, 2009, July 14-16, 2009, August 12-14, 2009, September 15-17, 2009 and October 19-20, 2009. At each sampling site, surface water was collected into a vigorously acid-cleaned 500-mL Teflon bottle with personnel wearing non-powder cleanroom gloves; each site was sampled on 2 or 3 occasions (i.e. 2 or 3 days) each month. Within 6 h of collection, each sample was individually filtered through a 0.45- μm cellulose nitrate membrane housed in an acid-leached disposable filter unit (Nalgene). Filtered sample was immediately transferred into another acid-cleaned 500 mL Teflon bottle, acidified to 0.4 % (v/v) with trace-metal grade hydrochloric acid (Fisher Scientific) (Parker and Bloom, 2005), and stored in the dark at 4 °C. All samples were transported back to the University of Minnesota on ice for further processing.

Supporting Information II – Diel water sampling

At a single site in the stream reach (at 6.8 km point in Fig. 1), we collected surface water samples about every 2 h from 10 am on August 13, 2009 through 10 am on August 14, 2009. We filtered the water and analyzed for MeHg and P as for all other ambient water samples. We also measured *in-situ* water temperature and pH.

Supporting Information III – Mercury and chemical analyses

All MeHg analysis was performed in a cleanroom laboratory in Metropolitan Council Environmental Services (St. Paul, Minnesota). Filtered sample (90 mL) was first distilled to remove matrix interferences, buffered with sodium acetate at pH 4.9, and ethylated by sodium tetrathylborate; alkyl mercury (Hg) species were purged from solution with Hg-free N₂ and pre-concentrated onto Tenax traps. Methylmercury was quantified by atomic fluorescence detection following gas chromatographic separation and pyrolysis (Bloom, 1989; Liang et al., 1994). The method detection limit (MDL) was established as 20 pg L⁻¹. Samples with MeHg concentrations below the MDL were assumed to be half of the MDL, i.e. 10 pg L⁻¹ (Clarke, 1998) for all subsequent calculations. Analytical accuracy was checked by analyzing certified reference materials between sample runs. DORM-3 dogfish muscle and DOLT-4 dogfish liver (National

Research Council of Canada, Ottawa, Ontario, Canada) were digested in KOH/methanol. The mean MeHg concentrations were 0.347 mg kg^{-1} ($n=27$; CV=7.9%) for DORM-3 (certified value = $0.355 \pm 0.056 \text{ mg kg}^{-1}$) and 1.29 mg kg^{-1} ($n=16$; CV=9.6%) for DOLT-4 (certified value = $1.33 \pm 0.12 \text{ mg kg}^{-1}$). Matrix spikes resulted in a mean recovery of $89 \pm 3 \%$ ($n=16$; CV=14%). All bottle and filter blanks had MeHg below the MDL.

For total-Hg analysis, filtered samples were digested by 0.5 % bromine monochloride at 60°C overnight, and total-Hg was analyzed by a single gold-trap amalgamation technique (Liang et al., 1993). Analytical accuracy was checked by repeated analyses of NIST 1641d Mercury in Water certified reference material, with an average recovery of $97 \pm 5 \%$ ($n=35$; CV=5.3%).

Filtered P and Fe were determined by inductively coupled plasma optical emission spectrometry (Thermo Scientific iCAP 6500 dual view) at the Department of Geology and Geophysics, University of Minnesota. Filtered SO₄ was determined by ion chromatography (Dionex DX120) at the Research Analytical Laboratory, University of Minnesota.

Supporting Information IV – Hydrologic and chemical mass balances

We performed a simple hydrologic mass balance to estimate filtered MeHg concentrations ([MeHg]) in the mainstem channel, assuming there was no *in-situ* MeHg production and destruction within the stream reach. Since we did not have discharge data at points of 2.2 km, 3.4 km, 4.1 km and 6.8 km of the mainstem channel, and therefore we estimated the discharge at these points through a linear relationship between discharge and watershed area in the drainage network during summer baseflow period (from June through September only).

We estimated [MeHg] at each sampling site in the main channel using the measured and/or estimated discharge values and the measured [MeHg] at point 0 km, assuming simple mass mixing (i.e. assuming no net change in total amount of MeHg or mass of water). Within the 8.4-km stream reach, we constructed mass balances for both MeHg and P to estimate the net change of both chemical species in the study reach using the following equation:

$$\begin{aligned} \text{Net change (g day}^{-1}\text{)} &= \text{Flux at 8.4 km main channel (g day}^{-1}\text{)} - [\text{flux at 0 km main channel (g day}^{-1}\text{)} \\ &\quad + \text{flux at tributary A (g day}^{-1}\text{)} + \text{flux at tributary B (g day}^{-1}\text{)} + \text{flux at tributary C (g day}^{-1}\text{)} + \\ &\quad \text{flux at tributary D (g day}^{-1}\text{)} + \text{unaccounted flux (g day}^{-1}\text{)}] \end{aligned}$$

where flux is calculated by multiplying chemical concentration and daily discharge. The unaccounted flux includes any water draining into the study reach other than that from the four monitored tributaries. We assumed this water flux, primarily from very small channels, would have the same [MeHg] and [P] as the average [MeHg] and [P] of the four major tributaries in each month. This is a reasonable assumption because we had previously sampled smaller tributaries in the study site and found similar [MeHg] and [P] as in the four major tributaries we measured in this study (Tsui et al., 2009). To express the net change effectively, we used net production of MeHg and net uptake of P in the study reach.

Supporting Information V – Algal incubation experiment

To investigate potential source of MeHg within the stream channels, we performed a short-term algal incubation experiment on July 18, 2009. Briefly, we collected fresh *Cladophora* biomass from the mainstem channel (at 2.2 km point in Fig. 1). The *Cladophora* biomass had a light load of epiphytes such as diatoms (Power et al., 2009). We filtered stream water through 0.45- μm and transferred 100 mL into new and sterile Hg-free Nalgene polyethylene terephthalate glycol (PETG) bottles. About 0.5 g wet weight of fresh *Cladophora* was added to each bottle with filtered stream water; bottles were placed just below the water surface of the mainstem channel for 3.5-4 h under full sunlight conditions. Afterward, water sample was filtered through 0.45- μm and the filtered solution was acidified with 0.4 % hydrochloric acid and analyzed for MeHg as described above for ambient water samples.

Supporting Information VI – Algal survey in South Fork Eel River

Algal survey was conducted within our stream reach by Drs. Paula C. Furey and Mary E. Power (Department of Integrative Biology, University of California, Berkeley) from April to October, 2009. Transects were set up across two pools (at approximately 3.4 and 6.8 km points in Fig. 1) and were surveyed for different components on the streambed including *Cladophora* and assessed their colors or stages. Colonization of different groups of epiphytes or microbes change the visual appearance (i.e. color) of *Cladophora* as demonstrated by Power et al. (2009). For our convenience, we presented the average values of the relative proportions of non-

epiphytized (green) and epiphytized (yellow and rusty) *Cladophora* from their surveys in order to determine if the colonization may have effects on the transformation of Hg in the stream reach.

References for Supporting Information

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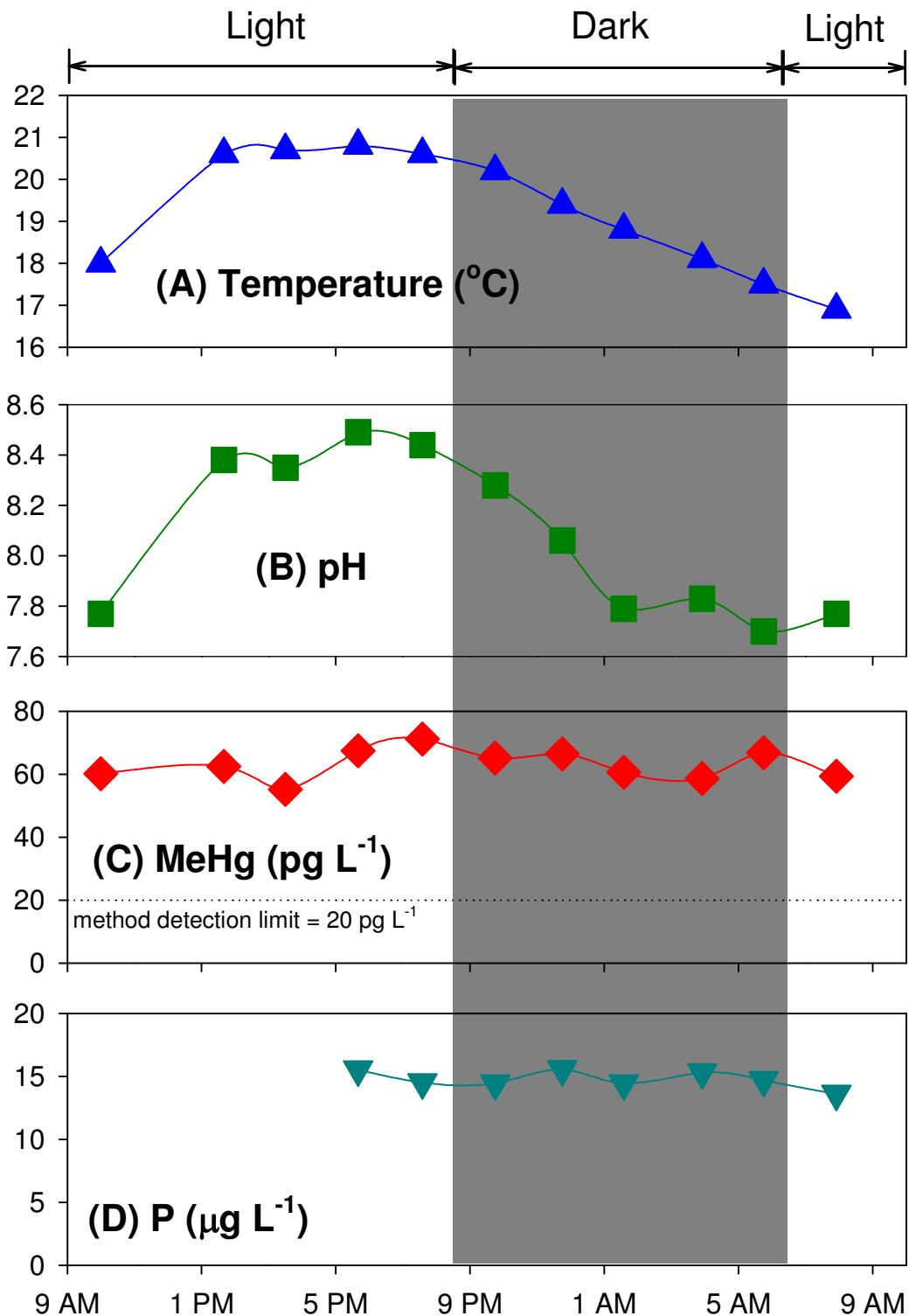


Fig. S1. Diel variation in (A) water temperature, (B) pH, and (C) filtered MeHg and (D) P concentrations at the mainstem channel during mid-August, 2009.

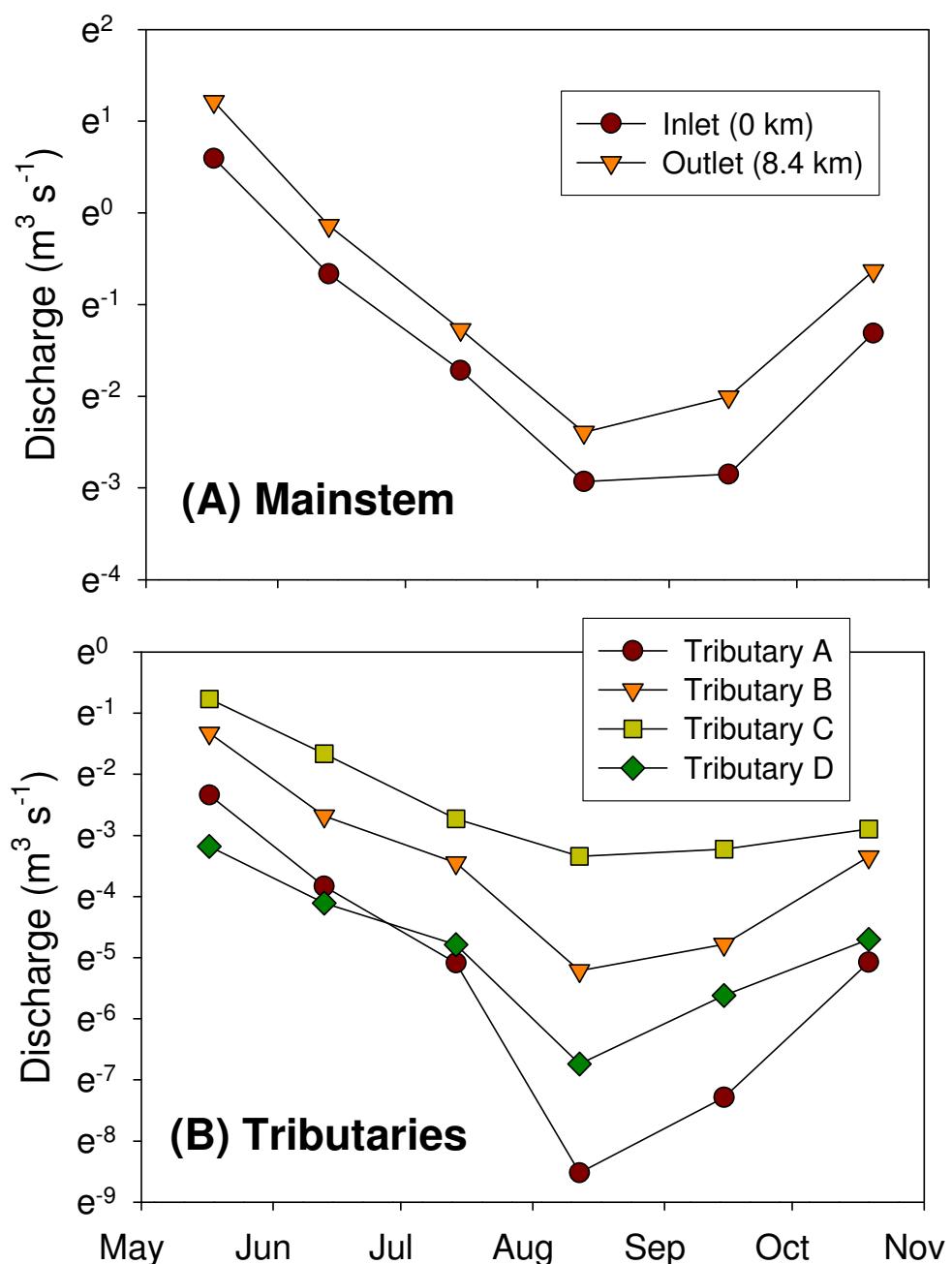


Fig. S2. Discharges measured at **(A)** mainstem and **(B)** tributaries in the study site in 2009. Vertical axis is ln-transformed to show the exponential decline from mid-May through mid-August, 2009.