Importance of Ultraviolet Radiation in the Photodemethylation of Methylmercury in Freshwater Ecosystems

Igor Lehnherr* and Vincent L. St. Louis

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9

Received January 27, 2009. Revised manuscript received May 29, 2009. Accepted June 9, 2009.

Photodemethylation (PD) is thought to be the most important biogeochemical sink of methylmercury (MeHg) in freshwater lakes. However, we possess little mechanistic knowledge of this important biogeochemical process with regard to, for instance, the role of ultraviolet (UV) radiation versus visible light in mediating MeHg PD. This information is critical to correctly model MeHg PD at the whole-lake level, since wavelengths in the UV and visible regions of the solar spectrum are attenuated at very different rates in the water column of lakes. Furthermore, the established methodology for quantifying MeHg PD requires the addition of a MeHg spike, which often increases the concentration of ambient MeHg by 1 to 2 orders of magnitude; however, the assumption that the MeHg spike behaves like ambient MeHg has never been verified. We quantified MeHg PD rates using an isotopically enriched Me199Hg tracer added to lake waters already containing high concentrations of ambient MeHg, allowing us to simultaneously monitor the decomposition rate of the spike and ambient MeHg. Experiments were conducted at the Experimental Lakes Area to quantify the first-order rate constant ($k_{pd}$) of MeHg PD in samples exposed to (1) full solar radiation, (2) UV-A and visible light (i.e., with UV-B blocked), or (3) visible light only. We demonstrate for the first time that the use of a MeHg spike to quantify PD rates is appropriate since spike and ambient MeHg—both in filtered and unfiltered particulate and dissolved phases and binding of ligands, respectively. Finally, we show that the decomposition of MeHg in lake waters, including partitioning between the particulate and dissolved phases and binding of MeHg to water samples prior to incubation in the study lake because the low ambient MeHg concentrations naturally present in lake waters usually limit MeHg PD to rates that are not detectable. The addition of the MeHg spike often increases the concentration of aqueous MeHg by 1 to 2 orders of magnitude, potentially disrupting the chemical speciation of MeHg in lake waters, including partitioning between the particulate and dissolved phases and binding of ligands, which could in turn yield measured MeHg PD rates which are not representative of in situ rates.

To evaluate whether MeHg PD rates measured using the methodology described above are representative of true rates, we examined MeHg PD in a boreal lake at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada, by adding an isotopically enriched Me199Hg tracer to lake waters already high in ambient MeHg. This approach allowed us to simultaneously quantify PD rates of the added Me199Hg tracer and ambient MeHg to determine whether the Me199Hg tracer behaves like ambient MeHg or otherwise affects the PD of ambient MeHg. To examine the effect of UV radiation on MeHg PD rates, we quantified MeHg PD rates in samples exposed to (1) full solar radiation including UV-B (280–400 nm), (2) UV-A and visible light (i.e., with UV-B blocked), and (3) visible light only. Experiments were performed with filtered and unfiltered water to elucidate whether particulate matter has a direct effect on measured MeHg PD rates. Finally, we present a model which incorporates light-specific rate constants of MeHg PD with UV and visible light attenuation to calculate whole-lake MeHg PD fluxes.

Introduction

Methylmercury (MeHg) is a potent neurotoxin (1) that has the ability to bioaccumulate and biomagnify in aquatic food webs (2). As a result, fish in numerous lakes worldwide contain elevated MeHg concentrations and the consumption of fish is the main pathway for human exposure to MeHg (3). While many studies have focused on identifying sources of MeHg to aquatic systems and quantifying the biogeochemical processes responsible for the production of MeHg (see 4 for a review), decomposition pathways of MeHg have not been thoroughly examined, although these processes are likely to be equally important in controlling the availability of MeHg to biota.

MeHg can be microbially demethylated in both the sediments (5, 6) and the water column (7) of lakes, as well as photolytically decomposed in surface waters (8). In fact, photodemethylation (PD) is the most important sink of MeHg in clear, oligotrophic lakes in both boreal (9) and tundra (10) ecosystems. Laboratory experiments using both artificial and natural light sources have shown that MeHg is degraded by ultraviolet (UV; 100–400 nm) radiation (11–14). However, because of the strong positive correlation between photosynthetically active radiation (PAR, 400–700 nm) incident at different depths in the water column and MeHg PD rates measured at those depths, it was recently suggested that visible light, and not UV radiation, is primarily responsible for MeHg PD in lake waters (15), despite evidence to the contrary (9, 16). Previous research has also demonstrated that rates of MeHg PD are similar in filtered and unfiltered waters taken from the same lake (8, 15), suggesting that particulate matter does not play a role in this process beyond attenuating and scattering incident light.

PD is often one of the principal fluxes considered in building lake-scale mass balance budgets of MeHg (9, 10, 17), and MeHg PD rates are typically quantified using the experimental methodology originally described by Sellers et al. (8). However, this method requires the addition of a spike of MeHg to water samples prior to incubation in the study lake because the low ambient MeHg concentrations naturally present in lake waters usually limit MeHg PD to rates that are not detectable. The addition of the MeHg spike often increases the concentration of aqueous MeHg by 1 or 2 orders of magnitude, potentially disrupting the chemical speciation of MeHg in lake waters, including partitioning between the particulate and dissolved phases and binding of ligands, which could in turn yield measured MeHg PD rates which are not representative of in situ rates.

* Corresponding author phone: (780) 492-0900; fax: (780) 492-9234; e-mail: lehnherr@ualberta.ca.
Methods

Experimental Design and Sample Collection. The MeHg PD experiments described here were conducted July 15–22, 2006, at the ELA located in the temperate boreal ecoregion, using water collected from Lake 979. Lake 979 was formed by the experimental flooding of a wetland complex in 1993 (18) and therefore had high concentrations of both DOC (12.8 mg L−1) and MeHg (0.81 ng L−1) due to the decomposition of flooded organic peatland soils and the resulting stimulation of Hg methylation.

Unfiltered epilimnetic water samples were collected into a 1-L Teflon bottle using Teflon tubing connected to a battery-powered gear pump (Greylor, PQ-12DC). Filtered samples were pumped through an additional in-line Teflon cartridge equipped with a 2.2 µm quartz microfiber filter that was previously muffled overnight at 500 °C. Samples were handled using trace-metal sampling procedures such as the “clean hands, dirty hands” protocol (19), and all sampling equipment was acid-washed and rinsed with MilliPore water (resistivity >18.2 MQ cm) prior to use. Bulk 1 L water samples were amended with a 1000 µL spike of a ~1.2 µg L−1 solution of isotopically enriched Me199Hg tracer in MilliPore water. The Me199Hg tracer was synthesized from 199HgO (92% purity, Trace Sciences International) using methylcobalamin (20). Spiked bulk water (200 mL) was distributed into each of five separate 250-mL FEP-Teflon bottles, and bottles were randomly assigned to one of five incubation times (0, 1, 2, 4, or 7 days). A rate of MeHg PD was obtained for each five-point time-series (see Data Analysis below) and every time-series was performed in duplicate.

Samples were incubated on the surface of Lake 239, located near the main research facilities at the ELA, inside a raft with different compartments (Figure S1). Two of the raft compartments were covered with either Mylar or Lee (model 226) film to control the quality of light to which samples were exposed. Mylar film has a cutoff wavelength of 320 nm while Lee film does not transmit light of wavelengths shorter than 400 nm (Figure S2); furthermore, the UV-blocking properties of the Mylar and Lee films were assessed before and after the experiment using a UV–vis spectrophotometer and remained unchanged over the short 7-day duration of the incubations. Therefore, MeHg PD rates in filtered and unfiltered lake water were quantified under the following light conditions: (1) full solar radiation, including UV-B, UV-A, and visible light, (2) UV-A and visible light (i.e., with Mylar film blocking UV-B radiation), and (3) visible light only (i.e., with Lee film blocking all UV). Duplicate time-series were performed for each possible combination of the light quality and filtration factors, and within each time-series, PD rates of ambient MeHg and spike Me199Hg were simultaneously quantified (spike versus ambient MeHg factor). A number of treatments were also replicated in the absence of light, by wrapping aluminum foil around sample bottles, or without the addition of Me199Hg to verify that the spike did not interfere with the measurement in situ MeHg PD rates. Bottles collected at the end of the desired incubation period were immediately frozen and stored in the dark at −20 °C until sample analysis.

Sample Analysis. Water samples were distilled and analyzed for MeHg using isotope-dilution gas chromatography–inductively coupled plasma mass spectrometry (GC-ICP-MS) (21, 22). Additional analytical details and QA/QC are provided in the Supporting Information.

Data Analysis. A rate constant of MeHg PD was obtained for each incubation time-series by modeling the MeHg concentration data using first-order chemical kinetics:

$$\ln[\text{MeHg}]_t = \ln[\text{MeHg}]_0 - kt$$  \hspace{1cm} (1)

where (MeHg) and (MeHg) are the MeHg concentrations at time t and time 0, respectively, and k is the first-order rate constant. However, for a photochemical process, incident photon flux density is a better predictor of the progress of reaction than reaction-time itself. Therefore, ln(MeHg), was regressed against the cumulative solar radiation flux, measured using a LiCor PAR sensor (LI-190SA Quantum Sensor) over the duration of the incubation time, t.

The rate constant of MeHg PD, kpd, was then obtained from the slope of the characteristic first-order kinetic plot (Figures S3–S5):

$$\ln[\text{MeHg}] = \ln[\text{MeHg}]_0 - \left( k_{pd} \times \text{Cumulative PAR photon flux} \right)$$  \hspace{1cm} (2)

The value of kpd, obtained for each time-series, was input into a repeated-measures ANOVA model with light quality and filtration as the between-time-series factors and ambient versus spiked MeHg as the within-time-series factor. All statistical analyses were performed at α = 0.05, using Systat (v. 11).

Results and Discussion

Effect of Light Quality on MeHg PD. We found that MeHg in lake water was decomposed when exposed to solar radiation (Figure 1), as has been shown in previous studies (8, 15, 17), and that UV radiation is the principal driver of MeHg PD in surface waters. Measured rate constants of MeHg PD (kpd, µmol2E−1) varied significantly with incident light quality (p < 0.0001), and decreased noticeably in the absence of UV radiation (Figure 1). In samples exposed to visible light only, kpd was 7–12 times smaller than in samples exposed to the full solar spectrum (Table 1, Tukey’s posthoc multiple mean comparison p < 0.0001), and 5–8 times smaller than in samples exposed to UV-A and visible light radiation (Table 1, Tukey’s p = 0.0003). Furthermore, blocking UV-B radiation resulted in 22–35% decreases in kpd, suggesting that UV-B radiation enhances MeHg PD above and beyond the effect of UV-A (Table 1, Tukey’s p = 0.015 and 0.023 for ambient and spike MeHg, respectively). However, this effect could in part be due to the fact that the Mylar film used to block UV-B radiation also blocked ~25% of the UV-A radiation in the range of 320–375 nm. Finally, kpd measured in filtered water samples incubated in the dark were ~70% lower than in samples incubated under visible light only. The mean rate constant (±standard error) for the decomposition of MeHg in the absence of light—expressed with respect to time as opposed to cumulative photon flux—was (5.3 ± 0.99) × 10−3 d−1; however, under these experimental conditions, such low rates of MeHg decomposition were at the limit of detection and are associated with a high degree of uncertainty (four regressions of ln[MeHg] versus Time, p-values = 0.004–0.36).

Effect of Filtration and Particulate Matter on MeHg PD. Overall, filtering the water prior to incubation had no effect on the measured value of kpd (p = 0.105; see also Figure 2). However, kpd tended to be higher in unfiltered samples for the experimental treatments performed in the absence of UV-B and under visible light only (Table 1). This tendency is somewhat surprising because we expected that if particulate matter did have an effect on MeHg PD, it would be to lower kpd due to increased scattering of incident light and decreased photoreactivity of particulate-bound MeHg. However, particle content in Lake 979 water was low and particulate bound MeHg in Lake 979 comprised only ~10% of the total aqueous MeHg; therefore, any potential differences in either light-scattering or photoreactivity of MeHg between filtered and unfiltered samples were likely too small to affect kpd. Our results are consistent with previous reports (8, 15) that there is no difference between MeHg PD rates quantified in filtered and unfiltered water. Therefore, par-
ticulate matter does not appear to play a critical role in MeHg PD beyond increasing the scattering and absorption of incident solar radiation in the water column. However, because our results suggest that rates are slightly higher in unfiltered water, we recommend quantifying MeHg PD in unfiltered samples for the purpose of modeling this process at the whole-lake level.

Effect of Spike Addition on MeHg PD. There was no difference between \( k_{pd} \) obtained for ambient MeHg and spike Me\(^{199}\)Hg (\( p = 0.802 \)). However, the interaction term Spike versus Ambient MeHg * Filtration was almost significant (\( p = 0.081 \)) suggesting that in unfiltered samples, the spike Me\(^{199}\)Hg was photodemethylated at a slightly higher rate than the ambient MeHg, while the reverse was true in filtered samples. This could be due to the spike Me\(^{199}\)Hg not partitioning between the particulate and dissolved phases in exactly the same manner as the ambient MeHg such that in unfiltered samples, more of the spike Me\(^{199}\)Hg remained in the dissolved, and more reactive, phase. However, the difference in \( k_{pd} \) resulting from a potential Spike versus Ambient MeHg * Filtration interaction is quite small (Table 1), and is perhaps better described as a statistical effect that is not relevant on an ecological scale. Additional experiments performed using samples that did not receive a spike of Me\(^{199}\)Hg showed that rates of ambient MeHg PD were no different than in samples spiked with Me\(^{199}\)Hg (Figure 2) and that this holds true in both filtered and unfiltered water (\( t \) test for sample means with unequal variances, \( p = 0.46 \) and \( p = 0.38 \) for filtered and unfiltered samples, respectively, and \( p = 0.16 \) when filtered and unfiltered data are pooled; Table 1). Together, these results validate the assumption that the added isotopically enriched MeHg tracer is distributed in a manner similar to ambient MeHg.

| TABLE 1. Rate Constants, \( k_{pd} \), of MeHg PD (Mean ± Standard Error; × 10\(^{-3}\) m\(^2\) E\(^{-1}\)) Measured for the Various Experimental Treatments |
|-------------------------------------------------|----------|----------|----------|----------|
|                                                   | full spectrum | UV-A + visible | visible only | dark     |
| filtered water                                    |            |            |            |          |
| ambient MeHg in spiked samples                    | 3.87\(^a\) | 2.31 ± 0.19 | 0.294 ± 0.010 | 0.137 ± 0.008 |
| spiked Me\(^{199}\)Hg                             | 3.82\(^a\) | 2.26 ± 0.20 | 0.269 ± 0.047 | 0.101 ± 0.023 |
| ambient MeHg in unspiked samples                  | 3.93 ± 0.02 | N/A        | N/A        | N/A      |
| unfiltered water                                  |            |            |            |          |
| ambient MeHg in spiked samples                    | 3.59 ± 0.08 | 2.81 ± 0.005 | 0.527 ± 0.002 | N/A      |
| spiked Me\(^{199}\)Hg                             | 3.69 ± 0.19 | 2.85 ± 0.06 | 0.505 ± 0.019 | N/A      |
| ambient MeHg in unspiked samples                  | 3.84 ± 0.18 | N/A        | N/A        | N/A      |
| best estimate\(^b\)                              | 3.69 ± 0.07 | 2.44 ± 0.18 | 0.280 ± 0.069 | 0.119\(^c\) ± 0.020 |
| \( n = 7 \)                                       | \( n = 4 \) | \( n = 4 \) | \( n = 2 \) |          |

\(^{a}\) Due to the presence of one outlier value (Q-test \( p < 0.05 \)) in the duplicate five-point time-series performed with filtered water and incubated under full solar radiation, the \( k_{pd} \) value obtained from this time-series was not used in the computation of mean \( k_{pd} \). \(^{b}\) Best estimate values of \( k_{pd} \) were corrected for the dark-decomposition of MeHg by subtracting the \( k_{pd} \) measured in the absence of light from all other \( k_{pd} \). \(^{c}\) The rate constant of dark MeHg decomposition expressed with respect to time is (5.36 ± 0.99) × 10\(^{-3}\) d\(^{-1}\).
TABLE 2. Relative Contribution of UV-B, UV-A, and Visible Light to the Whole-Lake Areal MeHg PD Flux, and Depth of MeHg PD Activity

<table>
<thead>
<tr>
<th>Light Attenuation Coefficient (m⁻¹)</th>
<th>L979</th>
<th>L239</th>
<th>% of Overall Areal MeHg PD Flux</th>
<th>L979</th>
<th>L239</th>
<th>Areal PD Flux (ng MeHg m⁻² d⁻¹)</th>
<th>10% Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV-B</td>
<td>0.871 (1.25)</td>
<td>35</td>
<td>14</td>
<td>0.18</td>
<td>7</td>
<td>2.0</td>
<td>0.30</td>
</tr>
<tr>
<td>UV-A</td>
<td>3.22 (2.16)</td>
<td>11</td>
<td>5.4</td>
<td>69</td>
<td>51</td>
<td>1.21</td>
<td>0.30</td>
</tr>
<tr>
<td>All UV</td>
<td>0.313 (0.280)</td>
<td>2.9</td>
<td>0.57</td>
<td>21</td>
<td>42</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Visible</td>
<td>0.313 (0.280)</td>
<td>2.9</td>
<td>0.57</td>
<td>21</td>
<td>42</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Notes:
- Rates were calculated using an average July PAR photon density flux at the ELA of 46.1 E m⁻² d⁻¹ and MeHg concentrations of 0.81 (this study) and 0.02 ng L⁻¹ (9) for the colored-water Lake 979 and clear-water Lake 239, respectively.
- All values were corrected for the attenuation of solar radiation by Teflon bottles (25) and Mylar/Lee film (Figure S2) where applicable, and uncorrected values are shown in parentheses.
- The areal MeHg PD flux was calculated assuming an equal volume of water in each 5 cm section of the water column within the zone of MeHg PD activity; this assumption is valid where lake depth is always greater than depth of MeHg PD activity, such as in the pelagic zone. The 10% depth is defined as the depth below which MeHg PD activity is less than 10% of the overall areal MeHg PD flux.

FIGURE 2. Photodemethylation of ambient MeHg in samples either spiked with 1.2 ng L⁻¹ of Me¹⁹⁹Hg or not amended with spike. Data for both filtered and unfiltered water samples are shown and error bars represent the difference between duplicate samples. Samples were collected following incubation periods of 0, 1, 2, 4, and 7 days under full solar radiation.
underestimates of the true $k_{pd}$. The transmission of visible and UV radiation through a lengthwise cross-section of a FEP-Teflon bottle was measured using a Shimadzu UV-2401PC spectrophotometer (Figure S2). Similar to recently published results (28), we found that 79% of visible, 51% of UV-A and 30% of UV-B energy was transmitted by the Teflon bottle, which is less than previously reported (99%, 82%, and 66% transmittance for visible, UV-A, and UV-B radiation, respectively) by measuring solar irradiance at the surface of a lake with the sensor placed inside a Teflon bottle (25). Because the emission spectrum of the radiation source in the spectrophotometer (deuterium and halogen lamps) is different from solar radiation at the earth’s surface, and because having the sensor inside of the Teflon bottle floating at the surface of a lake more closely replicates the conditions to which samples were exposed, the results reported in ref 25 are probably more relevant in this case.

To constrain the upper value of $k_{pd}$-overall, we corrected for the attenuation of UV and visible radiation by Teflon (and Mylar/Lee films) using the spectra shown in Figure S2, resulting in a $k_{pd}$-overall Value of $5.57 \times 10^{-3}$ m$^2$ E$^{-1}$ compared to $3.69 \times 10^{-3}$ m$^2$ E$^{-1}$ without any correction. However, using the transmittance of solar radiation through Teflon bottles reported in ref 25, a likely more accurate $k_{pd}$-overall value of $4.41 \times 10^{-3}$ m$^2$ E$^{-1}$ is obtained. To improve the accuracy of future measurements of $k_{pd}$, it would be helpful to measure UV and visible light irradiance inside a Teflon bottle throughout the experiment, using either a spectroradiometer or a solar actinometer. Alternatively, reaction vessels with better transmittance of solar radiation, such as PFA-Teflon bags (29) could also be used instead of FEP-Teflon bottles.

Modeling MeHg PD at the Whole-Lake Level. To scale up experimental results to the whole-lake level, we calculated a rate of MeHg PD at 5-cm depth-intervals according to eq 3, for both a colored-water lake (Lake 979) and a clear, oligotrophic lake (Lake 239) at the ELA.

MeHg PD Rate (ng L$^{-1}$ d$^{-1}$) =

\[
k_{pd} \text{ (m}^2 \text{ E}^{-1}) \times \text{MeHg (ng L}^{-1}) \times \text{PFD (E m}^{-2} \text{d}^{-1}) \quad (3)
\]

MeHg PD resulting from UV radiation and visible light was evaluated separately, such that three rate values were obtained for each depth (i.e., a separate rate value for UV-B, UV-A, and visible light mediated processes). $k_{pd,UVB}$ for UV-B mediated MeHg PD ($1.25 \times 10^{-3}$ m$^2$ E$^{-1}$) was calculated as the difference between the “full spectrum” $k_{pd}$ (UV-B + UV-A + visible) and $k_{pd}$ obtained with UV-B blocked (UV-A + visible) (Tables 1 and 2). Similarly, $k_{pd,UVA}$ for UV-A mediated MeHg PD ($2.16 \times 10^{-3}$ m$^2$ E$^{-1}$) was calculated as the difference between the “UV-A + visible” and “visible only” $k_{pd}$ (Tables 1 and 2).

Photon flux density (PFD) at each depth (z) was calculated according to eq 4, with $PFD_0 = 46.1$ E m$^{-2}$ d$^{-1}$, corresponding to the mean daily PAR photon flux density measured over the duration of our experiments.

\[
PFD_z = PFD_0 \times e^{-kz} \quad (4)
\]

Light attenuation coefficients ($k$) for UV-B and UV-A were estimated from DOC concentrations measured in July 2006 in each lake (Susan Kasián, Fisheries and Ocean Canada, Winnipeg, personal communication) using the equations provided in refs 26 and 27. Attenuation coefficients for PAR were calculated from duplicate light profiles conducted in July 2006 (Susan Kasián, personal communication). Finally, MeHg concentrations used to model MeHg PD rates for Lake
979 and Lake 239 were 0.81 ng L\(^{-1}\) (this study) and 0.02 ng L\(^{-1}\) (9), respectively.

The depth-specific rates calculated according to eq 3 can then be multiplied by the appropriate volume of water in each 5-cm “slice” of the water column, which depends on lake bathymetry, and summed to obtain depth-integrated whole-lake fluxes of MeHg PD (ng d\(^{-1}\)). For the purpose of our modeling exercise, however, we calculated areal fluxes of MeHg PD (ng m\(^{-2}\) d\(^{-1}\)) assuming an equal volume of water in each 5-cm section of the water column within the zone of MeHg PD activity. This assumption is valid for the pelagic zone of a lake, where lake depth is always greater than the depth of MeHg PD activity. Areal fluxes were then obtained by summing depth-specific rates from the surface down to the depth at which the modeled MeHg PD rate was 1% of the surface rate. This was done to exclude contributions to the areal MeHg PD flux from depth-specific rates which are not significantly greater than zero but which could contribute significantly to the sum value in a deep and/or colored lake, thus artificially inflating the modeled areal MeHg PD flux.

Modeled depth-specific and depth-integrated rates of MeHg PD (Figure 3) clearly demonstrate that for a colored, high DOC lake such as Lake 979, MeHg PD is primarily driven by UV radiation and is therefore largely restricted to the top 30 cm of the water column. Indeed, UV mediated MeHg PD accounts for over three-quarters of the overall areal flux, whereas the single-most important contributor accounts for only 30 cm of the water column. Indeed, UV mediated MeHg PD activity encompassed the top 2.5 m of the water column. However, the rates measured at depths of 0.75 and 1.5 m, which were used to build the relationship between incident PAR and MeHg PD rate, may have been influenced by non-negligible levels of UV radiation. We estimate that in Toolik Lake, UV mediated MeHg PD still accounts for 67% and 40% of the overall rate at depths of 0.75 and 1.5 m, respectively. We therefore propose that further measurements of MeHg PD rates throughout the water column, coupled with measurements of incident UV radiation and PAR at each depth, are warranted to clarify this matter.

Acknowledgments
We are grateful to Linnea Mowat for valuable assistance in the field and Po Yee Chan for assistance during sample analysis. We thank Jennifer Graydon for providing PAR data. Finally, we thank Rolf Vinebrooke and Rich Moses for contributing their knowledge of statistics to the experimental design and data analysis. This research was funded through a Natural Science and Engineering Research Council (NSERC) Discovery Grant to V.S.L., and a NSERC Post-Graduate Scholarship and Alberta Ingenuity Studentship to I.L.

Supporting Information Available
Analytical details and five additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited


