

Effect of ultraviolet radiation on alkaline phosphatase activity and planktonic phosphorus acquisition in Canadian boreal shield lakes

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Abstract

We examined how ultraviolet radiation (UVR) affects the activity of alkaline phosphatase (APase), a common extracellular enzyme. APase activity declined up to 57% under UVR exposure and decreased more often under ultraviolet A than ultraviolet B exposure, indicating that most of the observed decrease did not occur through direct enzyme inactivation. Enzyme activity in the particulate fraction ($>0.22 \mu\text{m}$) was less susceptible to UVR than activity in the total or dissolved fractions, suggesting that attachment to the cell surface may convey some protection against UVR-induced inactivation. Samples that were $0.22\text{-}\mu\text{m}$ filtered before being subjected to radiation treatments often showed increased enzyme activity, especially in the photosynthetically active radiation-only treatment, indicating reactivation of APase in the absence of de novo production of the enzyme. Decreases in APase can be severe near the lake surface, suggesting that UVR-induced variations in APase activity might contribute to microscale variations in nutrient availability and community composition.

Phosphorus (P) is the principal nutrient limiting primary producers in boreal lakes of the Northern Hemisphere (Schindler 1977). Thus, the ability of aquatic primary producers to acquire P both is fundamental to their survival and affects the interactions within and structure of planktonic communities. Because of this, a better understanding of the drivers and mechanisms that affect the phytoplankton's capacity to acquire P from its environment is crucial.

One driver that can affect phytoplankton P uptake is ultraviolet radiation (UVR; Hessen et al. 1995; Frost and Xenopoulos 2002).

Although the mechanism for UVR-induced reductions in P uptake is unclear, it certainly may compound other stresses faced by phytoplankton exposed to UVR and further affect structuring of planktonic communities. For example, in addition to the intense P limitation that can occur under UVR exposure, phytoplankton exposed to UVR also undergo decreases in photosynthesis and growth rates (e.g., Helbling et al. 1992; Xenopoulos and Frost 2003).

In this study, we undertook an investigation of the effects of ambient levels of ultraviolet A (UVA) and ultraviolet B (UVB) radiation on alkaline phosphatase (APase) activity. APase is a prevalent enzyme in aquatic environments, commonly found attached to cell surfaces or in the dissolved fraction. APase is produced by bacteria, algae, and zooplankton and acts by catalyzing the hydrolysis of phosphate esters to release orthophosphate. Its synthesis is repressed in the presence of P and promoted in low-P environments (reviewed in Jansson et al. 1988). Because of this attribute, the activity level of this enzyme is commonly measured to assess the degree of P limitation in aquatic systems (Healey and Hendzel 1980).

UVR has been shown to decrease the activity of several important phytoplankton enzymes in laboratory experiments, including the photosystem II enzyme Rubisco (Bischof et al. 2000) and the nitrogen fixing enzyme nitrogenase (Kumar et al. 2003). Several bacterial extracellular enzymes, which

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commonly act to cleave nutrients and carbon from dissolved organic matter, have also shown decreased activity with UVR exposure (Herndl et al. 1993). Ex situ studies have shown decreased APase activity with UVR exposure (e.g., Wetzel et al. 1995), which is proposed to occur through an indirect mechanism whereby photoreduced Fe binds with and inactivates the enzyme upon oxidization of Fe by H_2O_2 (Scully et al. 2003). This reversible process may allow for a source pool of inactivated APase in aquatic systems (Scully et al. 2003).

To date, the effect of UVR on APase activity in situ in freshwater environments is largely unstudied, and both the impact and magnitude of any UVR-induced changes in APase function on the structure of freshwater systems is poorly understood. We aimed to address this by undertaking measurements of UVR-induced variations in APase activity over several days, allowing us to obtain an integrated UVR-specific response and explore variations in phytoplankton P uptake and biomass that may occur concomitantly with changes in enzyme activity. We demonstrate that UVR exposure is an important factor regulating APase activity in situ and discuss the ecological implications of this effect in the freshwater environment.

Materials and methods

Study lakes—Lake 224 (L224) and Lake 302 South (L302S) are located in the Experimental Lakes Area (ELA) of northwestern Ontario, Canada (49°N, 93°W). The ELA lies within the Canadian Shield, and the catchments of both study lakes are composed of undisturbed boreal forest. L224 has a surface area of 0.259 km² and a maximum and mean depth of 27 and 11.6 m, respectively. L302S is smaller than L224, with a surface area of 0.109 km² and a maximum and mean depth of 10.6 and 5.1 m, respectively. A prolonged drought throughout the ELA and artificial acidification in L302S have caused clear, low dissolved organic carbon (DOC) conditions in both lakes (Schindler et al. 1996; Donahue et al. 1998). Additional information about L224 and L302S is presented in Xenopoulos et al. (2002).

Experimental design—Lake water was collected from directly below the water surface using a peristaltic pump and was incubated in polyethylene Whirlpak bags (Nasco Plastics), which were contained inside wire baskets (depth of 20 cm) suspended by floats at the lake surface. This allowed us to simulate the effects of near-surface thermoclines, which occur on the order of once every 2–3 d in these lakes (Xenopoulos and Schindler 2001). Ultraviolet filters (Cadillac Plastics) that allow the penetration of all ambient light (transmit >280 nm; Acrylite OP-4), block the penetration of UVB radiation (transmit >320; Mylar-D), and block the penetration of both UVA and UVB radiation (transmit >400 nm; Acrylite OP-3) were used to create the three light treatments. The plastic and mylar filters were attached to all sides of the baskets to ensure that all incoming radiation was screened.

The experiment was replicated three times in L302S (Table 1; experiments A, B, and C), and twice in L224 (Table 1; experiments D and E). Incubations commenced at 1000

Table 1. Experimental dates and average flux of UVA and UVB radiation during daylight hours of our experimental manipulations in experiments A through E in two boreal shield lakes in northwestern Ontario, Canada.

Lake	Experiment	Date	UVA (KJ m ⁻² d ⁻¹)	UVB (KJ m ⁻² d ⁻¹)
302S	A	17–19 Jun 1999	1,293.8	58.5
	B	14–16 Jul 1999	932.4	48.2
	C	23–25 Jul 1999	940.4	48.2
224	D	29 Jun–1 Jul 1999	1,157.3	56.1
	E	6–9 Jul 1999	1,088.9	58.2

h and were removed for analyses after 4 h (for experiment A only), 24 h (for all other incubations), and 48 h. Two replicate bags were removed from each treatment at each time interval. All collected samples (0 h lake water and incubated) were analyzed for chlorophyll *a* (Chl *a*), particulate carbon (C), particulate P, and APase activity. In three of the five experiments (twice in L302S and once in L224), lake water that had been filtered through a 0.22- μ m polycarbonate filter was incubated in addition to the routine lake water experiments. This prefiltered water allowed us to assess the effect of UVR on dissolved APase activity in the absence of any potential new APase production.

Determination of incident UVR—Downwelling UVA and UVB radiation were measured throughout the experiments at 10-min intervals using wideband UVA and UVB sensors (UVA = 320–400 nm, UVB = 280–320 nm; BW20 Vital Technologies) attached to a Li-Cor data logger, and cross-calibrated with a Li-Cor Spectroradiometer (model LI1800UM). Radiation measurements were made at the ELA meteorological station, which is located approximately 2.5 km from the study lakes. Levels of downwelling UVA and UVB during our incubations are shown in Table 1.

Determination of APase activity—APase activity was determined following the methods of Healey and Hendzel (1979). A portion of each sample was filtered through a 0.22- μ m polycarbonate filter; the following analysis was performed both on the filtrate and unfiltered samples. When available, samples filtered prior to the experiment were analyzed in an identical fashion. To distinguish these fractions from one another, unfiltered samples will be referred to as the “total” fraction, dissolved samples that were filtered after being exposed to light treatments as the “postfiltered” fraction, and dissolved samples that were filtered before being exposed to light treatments as the “prefiltered” fraction.

An aliquot of 4.5 ml of each sample was added to sterile fluorometer tubes; a 4.5-ml aliquot of sterile algal culture medium (autoclaved, P free) was also prepared as a standard. All fluorometer tubes were held in a 35°C water bath for 5 min, at which time 0.5 ml of 10 μ mol L⁻¹ 3-ortho-methylfluorescein phosphate (OMF-P; Sigma-Aldrich) adjusted to a pH of 8.5 was added to each tube. The hydrolysis of OMF-P to ortho-methylfluorescein (OMF) was measured fluorometrically (Turner Associates, model 111, equipped with a 47B primary filter and 2A12 secondary filter) by measuring

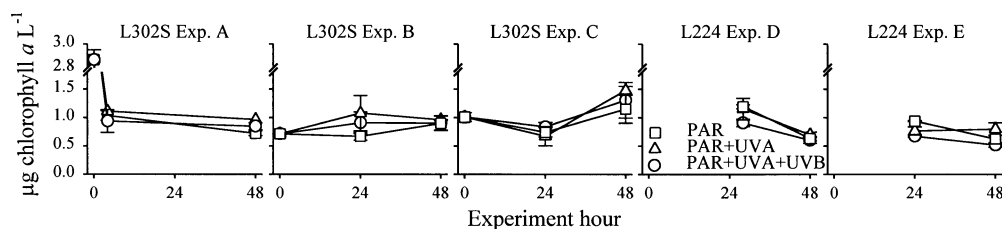


Fig. 1. Chl *a* concentrations in three UVR treatments, during five experimental periods at the Experimental Lakes Area, northwestern Ontario, Canada. Shown are mean values \pm 1 standard error for $n = 2$ replicates. Experimental periods A through E are as given in Table 1. Differences between UVR treatments were not significant at $p = 0.05$ in all experiments. Values for $t = 0$ in experiments D and E were not determined.

the genesis of fluorescent OMF at regular time intervals. Readings were zeroed at the start of each time interval using the standard. APase activity was calculated as $\mu\text{mol P L}^{-1} \text{h}^{-1}$.

Determination of particulate Chl *a*, C, and P concentrations—Chl *a* samples were collected by filtration onto Whatman GF-C filters within 2 h of lake water collection, frozen directly following filtration, and kept frozen until the time of extraction. Samples were extracted in the dark at 78–80°C in a solution of 90% ethanol, 10% water, for 5 min, and shielded from light upon removal. Extraction continued at 4°C for 24 h. Chl *a* concentration was determined spectrophotometrically (Shimadzu Scientific, model RF-1501) following Welschmeyer (1994).

Samples for particulate C and P determination were filtered onto precombusted (2 h at 475°C) Whatman GF/C filters. Particulate C was estimated after combustion at 975°C using a CHN analyzer (Control Equipment Corporation, model 440). Particulate P was estimated after digestion in potassium persulfate using the molybdate-ascorbic acid method (APHA 1992).

Data analysis—Two-way analyses of variance (ANOVAs) (JMP version 3.2, SAS Institute) were used to test for the effects of UVR treatment and time of sampling on Chl *a* and APase activity for each of the prefiltered, postfiltered, and total fraction samples. The $t = 0$ lake water sample was not included as part of the analysis because it was not subjected to our experimental manipulations. However, the $t = 0$ point is displayed on figures for comparison purposes. Data and residuals were inspected to ensure that they satisfied the assumptions of ANOVA. The Tukey–Kramer test was used for post hoc comparisons where differences were significant (Systat version 8.0, SPSS). Where interaction effects were highly significant, post hoc tests were not performed.

Results and discussion

Effect of UVR on Chl *a* concentrations—Exposure to UVR did not significantly affect Chl *a* concentrations in any of the five experiments (Fig. 1, $p_{\text{UVR}} > 0.05$ in all comparisons). Regardless of UVR treatment, Chl *a* concentrations significantly increased over time in experiment C but decreased with time in experiments D and E. Measured levels

of Chl *a* were similar throughout the summer and between the two study lakes. In experiment A, Chl *a* concentrations decreased significantly upon incubation, a trend that is also mirrored in the APase activity results (described following).

Although our result contrasts with several other studies that have shown decreased Chl *a* concentrations in the presence of UVR (e.g., Helbling et al. 1992), this lack of Chl *a* response to UVR exposure has been shown elsewhere for these lakes (Xenopoulos and Schindler 2003). In L302S, an artificially acidified lake (until 1990; pH \approx 6.0 in 1999), planktonic resistance to UVR may in part be a reflection of cotolerance in this community to the stresses of the clear water conditions that occur during acidification and to UVR exposure (Vinebrooke et al. 2004). Similarly, communities from clear lakes such as these are often better adapted, and thus more resistant, to UVR-induced stressors (e.g., Tank and Schindler 2004). Finally, nutrient limitation in our experimental communities (as discussed following) may have overwhelmed the effects of UVR exposure on algal biomass, as has been shown in other field studies (Frost and Xenopoulos 2002; Xenopoulos et al. 2002; Tank and Schindler 2004).

Seasonal and interlake variations in APase activity—Overall, APase activity was slightly higher in L302S than L224 (Fig. 2), although when expressed per unit of Chl *a*, total APase activity was similar between the two lakes, implying similar degrees of P limitation in these two systems. Chl *a*-corrected values of total APase activity between 0.14 and 0.80 $\mu\text{mol P } \mu\text{g Chl } a^{-1} \text{h}^{-1}$ (data not shown) indicate severe P stress in our experimental communities (Healey and Hendzel 1979). APase activity did not change significantly during the season in either of the study lakes. Sestonic C:P ratios were high (175–360 in L302S, 260–600 in L224; molar ratio from lake water samples), reinforcing the finding of severe P stress, as expected for lakes at the ELA (Healey and Hendzel 1980).

Effect of UVR on APase activity—Exposure to UVR significantly decreased total, postfiltered, and prefiltered APase activity (Table 2; Fig. 2). Activity declined significantly in each of the three fractions measured, with exposure to full-spectrum radiation decreasing activity by up to 41% in the total fraction and 57% in the postfiltered and prefiltered fractions, when compared with photosynthetically active radiation (PAR) alone. Of our five experimental replicates, one

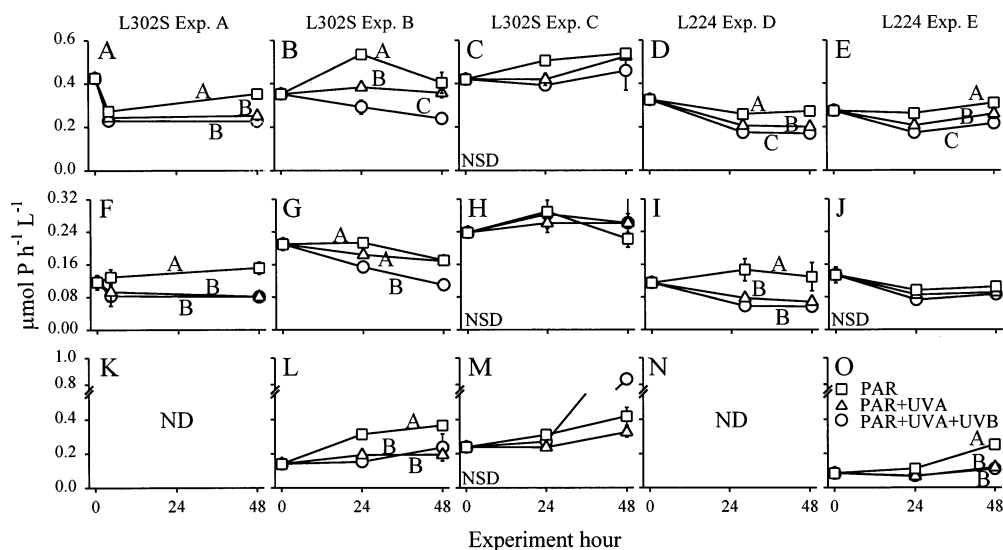


Fig. 2. Total APase activity in three UVR treatments, during five experimental periods at the Experimental Lakes Area, northwestern Ontario, Canada. Shown are mean values \pm 1 standard error for $n = 2$ replicates in the (A–E) total fraction, (F–J) postfiltered fraction, and (K–O) prefiltered fraction. Columns show the experimental periods as in Table 1. Significant differences between treatments are labeled using different letters. NSD = not significantly different. ND = not determined.

(experiment C) did not show significantly reduced APase activity when exposed to UVR. In experiment E, the post-filtered fraction only did not show a significant result.

In the total fraction, both UVA and UVB exposure decreased APase activity, with three experiments (B, D, and E) showing separate UVA and UVB effects, and one experiment (A) showing decreases from UVA exposure only (Table 2; Fig. 2). In contrast, in the postfiltered and prefiltered fractions, UVA was more effective than UVB at decreasing APase activity (Table 2; Fig. 2). Generally, UVB effects were greater in L224 than L302S.

It is unclear why a UVB effect occurred on some, but not other, experimental dates, although variable responses to UVB exposure are common in freshwater in situ studies (e.g., Xenopoulos et al. 2002). Ozone levels and fluxes of UVB to the lake surface were not greater during periods where significant UVB effects were observed (Table 1). Exposure history, which varies with changing water column stability, has been shown to affect sestonic susceptibility to UVR-induced damage (Xenopoulos and Schindler 2003). Similarly, shifts in community composition over time could affect sestonic susceptibility to UVR because different taxa vary significantly in their ability to synthesize APase (Rengefours et al. 2003). Both of these explanations could also account for the lack of significant response in experiment C.

The widespread efficacy of UVA radiation for decreasing APase activity indicates that direct inactivation of the enzyme was not the only cause of UVR-induced decreases in activity. As with other proteins, APase shows peak absorption of solar radiation at wavelengths between 270 and 290 nm and displays little to no absorption in the UVA range (Scully et al. 2003). Instead, the observed decrease in APase activity may follow a previously described indirect mechanism (Scully et al. 2003), whereby free Fe(II) is oxidized by

photochemically produced H_2O_2 and binds to and inactivates the enzyme as Fe(III). This process is reversible through photolysis of the Fe(III)-enzyme complex. Our study clearly demonstrates that inactivation of APase can occur in situ in freshwater environments and that UVR is an important factor controlling APase activity, even at current, ambient radiation levels.

The effect of UVR exposure on APase activity varied between the experimental fractions. The particulate component of the total fraction (total – postfiltered APase activity) was less affected by UVR exposure than either total or postfiltered treatments (Table 3), which may indicate that attachment to the cell surface partially protects these enzymes from being bound and inactivated by free Fe. In the prefiltered fraction, significant differences between treatments occurred because of increased APase activity under PAR-only exposure (i.e., in experiments B and E). For example, in experiment E, APase activity increased significantly in the PAR-only treatment, despite no significant result in the postfiltered fraction (Table 2; Fig. 2). De novo APase production is not possible in the prefiltered samples. Thus, this result suggests a reactivation of existing APase upon exposure to PAR. Although poorly studied, there is some evidence to suggest that complexed Fe can be photoreduced by visible band radiation (Emmenegger et al. 2001), allowing Fe-bound APase to be reactivated in the absence of UVR. The fact that increased enzyme activity was observed predominantly in PAR-only treatments suggests that a comparable amount of APase was recomplexed in PAR + UVR treatments. Across all UVR treatments, prefiltered samples were more likely to display increased APase activity over time than total or postfiltered samples. This difference may have occurred because our prefilter treatment removed particulate and some colloiddally bound Fe (Martin et al. 1995), thus

Table 2. Two-way ANOVA results for the effects of UVR on total, 0.22- μm postfiltered, and 0.22- μm prefiltered Apase activity under three different UVR treatments. Degrees of freedom, *F* statistics, and *p* values are reported. Significant differences are in bold. ND, not determined.

Lake	Date	Source of variation	Total fraction			Postfiltered			Prefiltered		
			df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
302S	17–19 Jun	UVR	2	41.574	0.0003	2	7.988	0.0204			
		time	1	12.486	0.0123	1	0.044	0.8406			
		UVR×time	2	10.720	0.0105	2	0.567	0.5950		ND	
	14–16 Jul	UVR	2	32.346	0.0006	2	54.541	0.0001	2	12.553	0.0072
		time	1	11.347	0.0151	1	49.857	0.0004	1	2.803	0.1451
		UVR×time	2	2.299	0.1814	2	3.406	0.1027	2	0.753	0.5107
23–25 Jul	UVR	2	3.156	0.1158	2	0.136	0.8759	2	62.162	<0.0001	
	time	1	4.712	0.0730	1	1.319	0.2945	1	54.673	<0.0001	
	UVR×time	2	0.438	0.6645	2	0.584	0.5863	2	58.340	0.0001	
224	30 Jun–1 Jul	UVR	2	04.297	<0.0001	2	11.408	0.0090			
		time	1	0.012	0.9181	1	0.401	0.5501		ND	
		UVR×time	2	1.412	0.3145	2	0.092	0.9133			
	6–9 Jul	UVR	2	34.955	0.0005	2	3.594	0.0942	2	18.507	0.0027
		time	1	28.638	0.0017	1	1.973	0.2097	1	29.966	0.0016
		UVR×time	2	0.105	0.9022	2	0.156	0.8587	2	4.962	0.0535

reducing the availability of Fe as a binding substrate and increasing competition for Fe binding sites.

The ecological implications of UVR-mediated decreases in APase activity—In laboratory experiments, algal P uptake has been shown to decrease in cells exposed to UVR (Hessen et al. 1995). To our knowledge, the mechanism for this has not been investigated. Our study indicates that decreases in P uptake may in part be caused by decreased labile P availability because of lower APase activity.

In our study, increased APase activity in the absence of UVR was expected to increase ortho-P availability and uptake through the hydrolysis of dissolved organic P (DOP). However, concentrations of particulate P and P normalized per unit Chl *a* and C were not significantly different across UVR treatments (Table 4). This result has been shown else-

where for these lakes under ambient P concentrations (Frost and Xenopoulos 2002).

Several explanations may account for the lack of change in particulate P concentrations despite significant differences in the activity of APase. First, measures of particulate C and Chl *a* often do not fully reflect changes in growth and biomass (Berman-Frank and Dubinsky 1999), which may have affected our normalized measures of particulate P. Several previous field experiments have shown decreases in molar C:P under UVR exposure (Xenopoulos et al. 2002; Tank et al. 2003), since UVR decreases C acquisition more strongly than any decrease in P uptake (Frost and Xenopoulos 2002). Second, as has been proposed elsewhere, UVR-induced decreases in bacterial biomass may have lessened competition for P in our UVR treatments (Xenopoulos and Bird 1997), thus compensating for decreased APase activity. Although

Table 3. Two-way ANOVA results for the effects of UVR on particulate APase activity under three different UVR treatments. Degrees of freedom, *F* statistics, and *p* values are reported. Significant differences are in bold. Where UVR treatment is significant, underlining indicates significant differences between treatments at *p* ≤ 0.05. P, photosynthetically active radiation (PAR)-only treatment; PA, PAR + UVA treatment; PAB, PAR + UVA + UVB treatment.

Lake	Source of variation	df	<i>F</i>	<i>p</i>	Post hoc comparisons	
302S	17–19 Jun	UVR	2	1.329	0.3329	
		time	1	3.656	0.1044	
		UVR×time	2	1.721	0.2565	
	14–16 Jul	UVR	2	13.965	0.0055	<u>P PA PAB</u>
		time	1	2.524	0.1632	
		UVR×time	2	1.357	0.3264	
224	23–25 Jul	UVR	2	1.590	0.2792	
		time	1	3.571	0.1077	
		UVR×time	2	0.009	0.9914	
	30 Jun–1 Jul	UVR	2	0.746	0.5135	
		time	1	0.764	0.4158	
		UVR×time	2	0.869	0.4660	
224	6–9 Jul	UVR	2	22.564	0.0016	<u>P PA PAB</u>
		time	1	20.537	0.0040	
		UVR×time	2	0.392	0.6916	

Table 4. Two-way ANOVA results for the effects of UVR on particulate P, molar C:P, and P per unit Chl α under three different UVR treatments. Analyses were conducted across three experiments in L302S and two experiments in L224. Degrees of freedom, F statistics, and p values are reported.

Lake	Source of variation	Particulate P			Molar C:P			P Chl α^{-1}		
		df	F	p	df	F	p	df	F	p
302S	UVR	2	0.240	0.792	2	0.173	0.844	2	1.107	0.371
	time	2	1.181	0.351	2	0.018	0.982	2	3.746	0.066
	UVR \times time	4	0.511	0.730	4	0.037	0.997	4	0.950	0.479
224	UVR	2	0.509	0.645	2	0.680	0.571	2	0.293	0.765
	time	2	1.128	0.431	2	4.159	0.137	2	0.378	0.714
	UVR \times time	4	0.479	0.756	4	1.963	0.303	4	0.301	0.862

bacteria are highly sensitive to UVR, they were likely poorly retained in our particulate P analyses. Finally, direct photolysis of DOP (Wetzel et al. 1995) may also have compensated for decreases in APase activity under UVR exposure.

Despite this, the observed decrease in activity and production of APase under UVR exposure has important ecological implications since P is a limiting nutrient in these lakes. Our laboratory measurement of APase-mediated P release occurred under ideal conditions and cannot be directly translated to field conditions. However, exposure to UVR resulted in decreases of up to 6 $\mu\text{g P cleaved L}^{-1} \text{ h}^{-1}$, a significant amount for P-starved cells. UVR-induced decreases in APase-mediated P release will be particularly acute near the surface and in small, tree-lined lakes, which often display secondary stratification that holds the sestonic community near the lake surface (Xenopoulos and Schindler 2001). Conversely, APase activity and production should increase at depths with no or low UVR relative to PAR. Such variation with depth might in turn contribute to microscale alterations in nutrient availability and community composition. In addition to specifically investigating the variations in this effect with depth, future studies should address UVR-induced variations in APase activity in lakes of differing humic content, given the increase in Fe photoreduction and H_2O_2 photoproduction in stained lakes.

Sestonic exposure to UVR can be expected to continue to change in coming years, not only because of changing ozone levels, but also because of the effects of acidification and drought on water clarity (e.g., Schindler et al. 1996). Despite this, our study shows that current, ambient fluxes of UVR decrease the activity of an important cell surface enzyme and therefore potentially affect nutrient cycling within aquatic systems.

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