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Free Zinc Ion and Dissolved Orthophosphate Effects on Phytoplankton from Coeur d'Alene Lake, Idaho

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Coeur d'Alene Lake in northern Idaho is fed by two major rivers: the Coeur d'Alene River from the east and the St. Joe River from the south, with the Spokane River as its outlet to the north. This phosphorus-limited lake has been subjected to decades of mining (primarily for zinc and silver) and other anthropogenic inputs. A 3^2 full-factorial experimental design was used to examine the interactive effects of free (uncomplexed) zinc ion and dissolved-orthophosphate concentrations on phytoplankton that were isolated from two sites along a longitudinal zinc-concentration gradient in Coeur d'Alene Lake. The two sites displayed different dominant taxa. *Chlorella minutissima*, a dominant species near the southern St. Joe River inlet, exhibited greater sensitivity to free Zn ions than *Asterionella formosa,* collected nearer the Coeur d'Alene River mouth with elevated dissolved-zinc concentrations. Empirical phytoplankton-response models were generated to describe phytoplankton growth in response to remediation strategies in the surrounding watershed. If dissolved Zn can be reduced in the water column from >500 nM (i.e., current concentrations near and down stream of the Coeur d'Alene River plume) to <3 nM (i.e., concentrations near the southern St. Joe River inlet) such that the lake is truly phosphorus limited, management of phosphorus inputs by surrounding communities will ultimately determine the limnologic state of the lake.

Introduction

Varied land use in close proximity to Coeur d'Alene Lake is reflected in diverse point and non-point sources of nutrients, toxicants, and sediment from two major riverine inputs; the St. Joe River from the south and Coeur d'Alene River from the east. The Coeur d'Alene River, distinct from the St. Joe River, drains historic heavy-metal mining and smelting areas. The lake transitions longitudinally from mesotrophic in the south to oligotrophic north (down gradient) of the Coeur d'Alene River Plume. Primary productivity is phosphorus limited (dissolved orthophosphate consistently <50 nM; refs *1*, *2*). Yet the lake's location down gradient of the Bunker Hill Superfund Site, a "Mining Megasite" (*3*), has raised concerns about potential toxic response by phytoplankton and higher-trophic-level organisms due to elevated dissolved-metal concentrations, in particular zinc (Zn). Despite being an essential micronutrient, Zn toxicity to phytoplankton at submicromolar concentrations has been found to disrupt the metabolism of phosphorus (*4*, *5*), the limiting macronutrient. An excess of free Zn ions suppresses cell division, and consequently, phosphorus merely accumulates intracellularly, but that suppression can be mitigated by increasing dissolved orthophosphate. So, as Zn bioavailability increases, phosphorus utilization is inhibited, and conversely, as phosphate bioavailability increases, Zn toxicity is mitigated. Because of management concerns in Coeur d'Alene Lake and other lentic systems receiving trace-contaminant inputs to the water column, the interaction between elevated Zn (i.e., beyond nutritional requirements that vary among species from 0.8 to 30 000 nM, ref *6*) and limiting orthophosphate on phytoplankton from the lake is examined.

Previous studies on the regulation of primary productivity in Coeur d'Alene Lake have been equivocal. Wissmar (*7*) found no primary Zn suppression of carbon-14 uptake by natural phytoplankton assemblages at a site near the Coeur d'Alene River inlet. In constrast, subsequent bioassays using a test organism not isolated from the lake (*Selenastrum capricornutum*; ref *8*) indicated that the high dissolved-Zn concentrations typically observed in Coeur d'Alene Lake should suppress phytoplankton growth and, hence, affect biomass production and fisheries resources. Chemically defined media studies were performed on phytoplankton species isolated from the lake to consider speciation effects over concentration ranges representing the entire lake. Those studies resulted in a "binary effect" where all treatments aside from basal-Zn concentrations caused total growth suppression (*1*). Because previous results highlighted potential toxicological controls on primary production in the lake, the following two questions were posed: Are dissolved Zn and orthophosphate concentrations interactively associated with growth parameters of dominant phytoplankton species in Coeur d'Alene Lake? If so, can these interactions be quantitatively expressed to facilitate the development of process-interdependent models for the lake? Culturing experiments conducted between June 2004 and January 2006, examined phytoplankton-growth response to a range of dissolved orthophosphate and free Zn ion concentrations representative of the region within- and up-gradient of the Coeur d'Alene River inlet to the lake. Within the longitudinal Zn-concentration gradient in Coeur d'Alene Lake, two dominant phytoplankton species, *Chlorella minutissima* and *Asterionella formosa*, were used as test organisms for bioassays using chemically defined media. Ancillary chemical characterizations of the water column were also developed to facilitate formulation of culturing media, interpret algal-culturing results, and provide a comparison with similar characterizations performed a decade before (*1*). This work, coordinated with physical-transport studies by others, represented a concerted effort to develop an initial process-interdependent water-quality model for the lake. The approach used in this study may be applicable to other aquatic systems where primary productivity and subsequent trophic transfer are impacted by point or non-point inputs of biologically reactive chemicals.

Experimental Section

Triplicate samples from the lake water column were collected on June 28, 2004, and then processed for taxonomic analysis of phytoplankton communities. Dominant species from two locations within and up-gradient of the Coeur d'Alene River plume were isolated for batch-culturing experiments using chemically defined media prepared in polypropylene Class-100 laminar flow hoods. Culturing methods without addition

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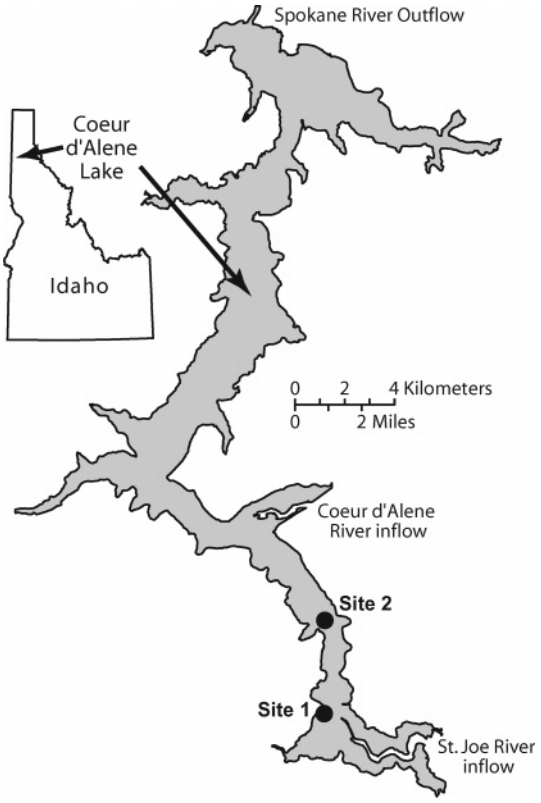

FIGURE 1. Map of Coeur d'Alene Lake and sampling locations: (Site 1) St. Joe River inlet (47° 23.391′ N 116° 45.256′ W), (Site 2) a water-column monitoring site between the St. Joe and Coeur d'Alene River inlets (47° 25.161′ N 116° 45.400′ W).

of defined mineral particulates (i.e., a mono-phasic medium; ref *5*) followed Kuwabara et al. (*9*). Toxicity of metals like Zn to phytoplankton is dependent on metal speciation (i.e., presence of bioavailable metal forms; refs *4*, *5*, *10*). For example, Zn toxicity to phytoplankton can be significantly reduced by increasing major cation (e. g, Ca, K, Mg, Na) concentrations (*11*). The culturing medium was, therefore, formulated to represent the dissolved major and minor constituents in the lake water column (Supporting Information Table S1; refs *1*, *12*). pH was set at 7.5 given an observed range of 6.6−8.2 for the lake. No pH-buffering solution was added, but the suspension was sparged with a fluoroethylene polymer (FEP) aerator. pH was typically within 0.2 pH units of 7.5 and adjusted daily if necessary within 0.1 pH units. After micromanipulator-controlled pipet isolations of dominant algal species, test organisms were maintained in media formulations representative of the sampling site from which they were collected. A chlorophyte, *Chlorella minutissima* from the southern St. Joe River inlet (hereafter referred to as Site 1; Figure 1), and a diatom, *Asterionella formosa* from a long-term monitoring site in the lake's main channel between the St. Joe River inlet and the Coeur d'Alene River plume (hereafter referred to as Site 2; Figure 1), were used in these experiments. During the culturing period, 400 mL suspensions of phytoplankton cells were maintained in acid-washed, media-conditioned 500 mL FEP vessels to minimize adsorption/desorption effects between wetted culturing surfaces and the bulk solution. Initial cell densities for *C. minutissima* and *A. formosa* cultures were 20×10^3 and 2.5×10^3 cells mL^{-1}, respectively, so as to begin the culturing period with similar biovolumes. To simulate spring surface water conditions in the lake, temperature was regulated by using culturing-room controls (7 ± 2 °C) and further refined by a circulating water bath with an immersion heater (10 ± 0.5 °C). Cool-white fluorescent bulbs were used to provide

TABLE 1. Experimental Design for Algal-Culturing Media[a]

treatments (9 total)	basal P (μM) N:P molar ratio = 40	mid P (μM) N:P molar ratio = 16	high P (μM) N:P molar ratio = 4
basal Zn (μM)	5×10^{-6}/0.1	5×10^{-6}/0.25	5×10^{-6}/1.0
mid Zn (μM)	1×10^{-2}/0.1	1×10^{-2}/0.25	1×10^{-2}/1.0
high Zn (μM)	3×10^{-2}/0.1	3×10^{-2}/0.25	3×10^{-2}/1.0

[a] Computed free zinc (Zn) ion concentrations and dissolved orthophosphate (P) concentrations in micromolar units (μM) are separated by a slash

illumination at approximately 64 μE-m^{-2}-s^{-1}. Nine media formulations were used for the bioassays (Table 1) with three replicate cultures monitored per formulation for each test organism (i.e., 54 monitored cultures), allowing second-order effects to be estimated. Zn treatment levels were selected for the cultures to represent the concentration gradient between Sites 1 and 2 (near-surface, euphotic-zone concentrations in Table S2). Dissolved-orthophosphate concentrations represented (1) phosphorus-limiting concentrations with a nitrogen to phosphorus molar ratio of 40, as is the case for the lake (*2*), (2) Redfield-ratio conditions ($N:P_{(molar)} = 16$; ref *13*), and (3) an excess of phosphorus ($N:P_{(molar)} = 4$). In ascending order, the three free Zn ion concentrations or three dissolved-orthophosphate concentrations selected for the cultures are hereafter referred to as "basal", "mid", and "high" treatments (Table 1). Chemical speciation of constituents added to the culturing media was computed using the program HYDRAQL (ref *14*; Table S1). On each culturing day, the cell concentration and mean-cell volume were determined by triplicate measurements per treatment using a particle counter (Coulter Multisizer IIe). Daily measurements continued for 6 days or until stationary-phase cell density was achieved. Elevated Zn concentrations in *A. formosa* cultures caused a clumping behavior that required sonication prior to counting (B. Braun LabSonicator with micro-tip probe at 30 W for 20 s). Once counted, sampled aliquots were discarded. Linear regression of culturing data was used to calculate mean and 95% confidence intervals for growth rate (doublings per day) and lag-phase duration (an estimate, in days, of the culturing time required to begin exponential growth), and cell yield (represented as the logarithm of maximum biovolume in μm^3-mL^{-1}). Although *C. minutissima* was reasonably estimated by a sphere, cellular volume of *A. formosa* was estimated as a cylinder where particle diameter from the counter was converted to cell length and the cross-sectional area was assumed constant at 12.6 μm^2 (i.e., a cross-sectional radius of 2 μm). Using the computer programs S-Plus (Insightful Corporation) and STATISTICA (StatSoft, Inc), these estimates were, in turn, used to develop empirical response-surface models to describe species-growth response to interactive dissolved-orthophosphate and free Zn ion effects.

Two field-sampling events in June 2004 and June 2005 were used to supplement the chemical characterization of the lake water column (*1*, *2*) and to determine trace-metal concentrations associated with phytoplankton cells. Dissolved organic carbon (DOC), representing organic ligands to complex free Zn ions, determined by high-temperature, noncatalytic combustion (*15*), remains fairly constant (120−140 μM) in lake surface waters. Trace-metal samples were collected in acid-washed high-density polyethylene bottles, filtered (0.2 μm polycarbonate membrane), acidified with double quartz-distilled hydrochloric acid to pH 2, and analyzed by inductively coupled plasma mass spectrometry (ICP−MS; ref *16*). Laboratory and field blanks as well as water samples from Site 1 were below method detection limits for dissolved Zn (<3 nM). On June 10, 2005, at two locations (a

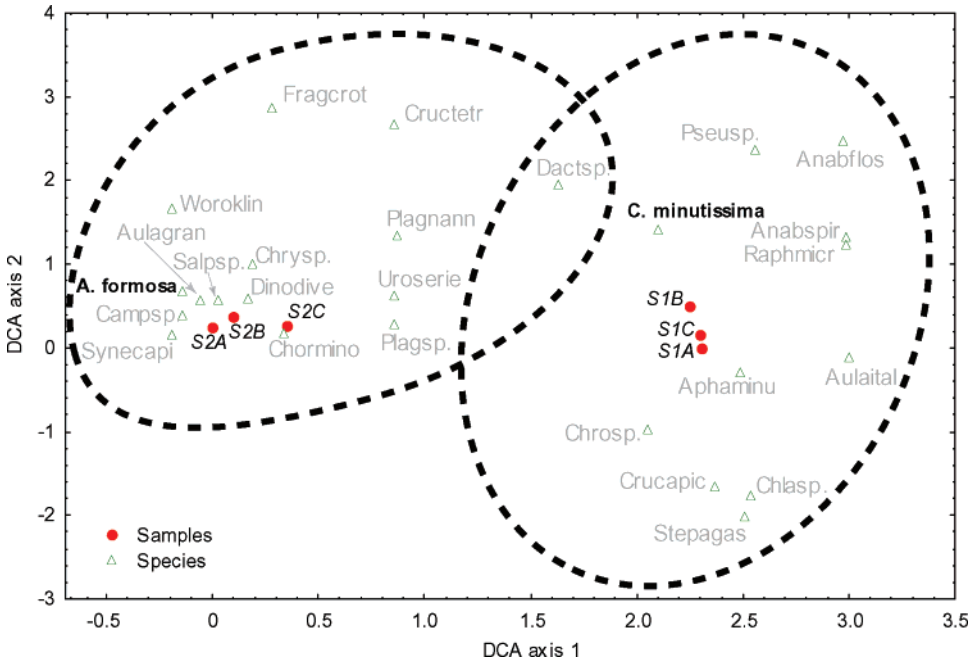

FIGURE 2. Ordinations of dominant phytoplankton taxa (species acronyms defined in Table S3) by detrended correspondence analysis (DCA; ref *34*). Samples that are similar in species composition appear closer to one another on a DCA plot than do sites with dissimilar species composition. Shown is a high intra-site similarity among samples compared to a low inter-site similarity in species composition. Axis values represent species turnover. On either axis, a difference of 4 between sites would indicate close to a 100% difference in species composition. The relative positions of the two test organisms (*C. minutissima* and *A. formosa*) reflect the two sampling-site groups.

main-channel site just downstream of the Coeur d'Alene River plume and Site 2, up gradient of that plume), and at the chlorophyll-maximum depth, phytoplankton cells were collected on preweighed, acid-washed, 0.2-μm polycarbonate filters to obtain initial measurements of trace-metals accumulated in and on these primary producers. Samples were freeze-dried and weighed for subsequent chemical digestion and trace-metal analysis (*16*, *17*). Dissolved-metal concentrations at the chlorophyll-maximum depth were used to calculate a dimensionless bioconcentration factor.

Results and Discussion

Phytoplankton Response. The depth and vertical extent of the subsurface chlorophyll-a maximum layer varied along a longitudinal transect of the lake (*18*). That variability extended to phytoplankton-community composition as a major shift in dominant species occurred between Sites 1 and 2 (Figure 2, Table S3). For test organisms selected to represent that compositional shift, cell numbers for *C. minutissima* decreased from 1505 ± 74 cells per milliliter ($n = 3$) at Site 1 to 258 ± 0 (approximately an 83% reduction) down gradient at Site 2. Conversely, the cell numbers for *A. formosa* increased from 8 ± 4 cells per milliliter ($n = 3$) at Site 1 to 877 ± 107 (approximately a two-order-of-magnitude increase) at Site 2. Regarding temporal variability, the current phytoplankton community displays differences from a decade ago. For example, of seven cyanophyte species identified in June 2004, only two matching genera were observed throughout 1991 and 1992 in monthly monitoring of the entire lake (*1*). These changes may reflect an ecosystem response to remedial activities implemented in the watershed because water-column dissolved-Zn concentrations have decreased in the lake during the past decade (*1*, *2*), although the point source input from the Coeur d'Alene River is still evident.

As a general observation, both test organisms displayed similar relative responses, but greater sensitivity to free Zn ions was exhibited by *C. minutissima*, compared to the attenuated response by *A. formosa* (Figure 3, Table S4). This observation is consistent with the predominance of *C. minutissima* at Site 1 (<3 nM dissolved Zn; Table S2) in comparison to the increased presence of *A. formosa* at Site 2 closer to the Coeur d'Alene River plume with elevated dissolved-Zn concentrations (254 ± 2 nM dissolved Zn). Both Figure 3 and Table S4 indicate that nonlinear effects are important in describing the observed response by both test organisms.

Lag-phase duration significantly increased with increased free Zn ion concentration, but much more so for *C. minutissima* (>6 days at the highest free Zn ion concentration, Figure 3A and B) than for *A. formosa* (consistently <2 days). Exponential growth was not observed by *C. minutissima* in the high-Zn treatments regardless of the dissolved-orthophosphate concentration (Table S4). The bulk residence time for the lake is approximately 6 months, but the stratum of maximum chlorophyll within the water column, at ~10 m depth, moves through the lake much more rapidly with transit times of days to weeks (*18*), similar to the duration of our culturing experiments. Based on biovolume measurements for *A. formosa*, lag-phase duration increased from zero (0.0 ± 0.1) days in basal-Zn medium to an average of 1.0 ± 0.5 days in mid and high Zn media. Unlike *C. minutissima*, the difference in lag-phase duration between mid and high-Zn media was not statistically significant for *A. formosa*.

Growth rates for both test organisms were maximized (Table S4) in basal-Zn media. This was expected for *C. minutissima* (0.84−1.27 d^{-1} in basal-Zn media) having been collected at Site 1 with <3 nM dissolved Zn, but less so for *A. formosa* (0.53−0.88 d^{-1} in basal-Zn media) that was maintained in culturing media of elevated free Zn ion concentration, representative of its collection at Site 2 (254 ± 2 nM dissolved Zn). An inverse relationship between growth rate and free Zn ion concentration was evident, ultimately exhibiting no discernible growth for *C. minutissima* at high-Zn levels (Figure 3, Table S4). Growth of *A. formosa* was also adversely affected by elevated Zn concentrations, but measurable growth was consistently observed. For *A. formosa*, growth rates ranged from 0.49 ± 0.14 d^{-1} at the highest free Zn ion concentration to 0.70 ± 0.14 d^{-1} at the lowest. The

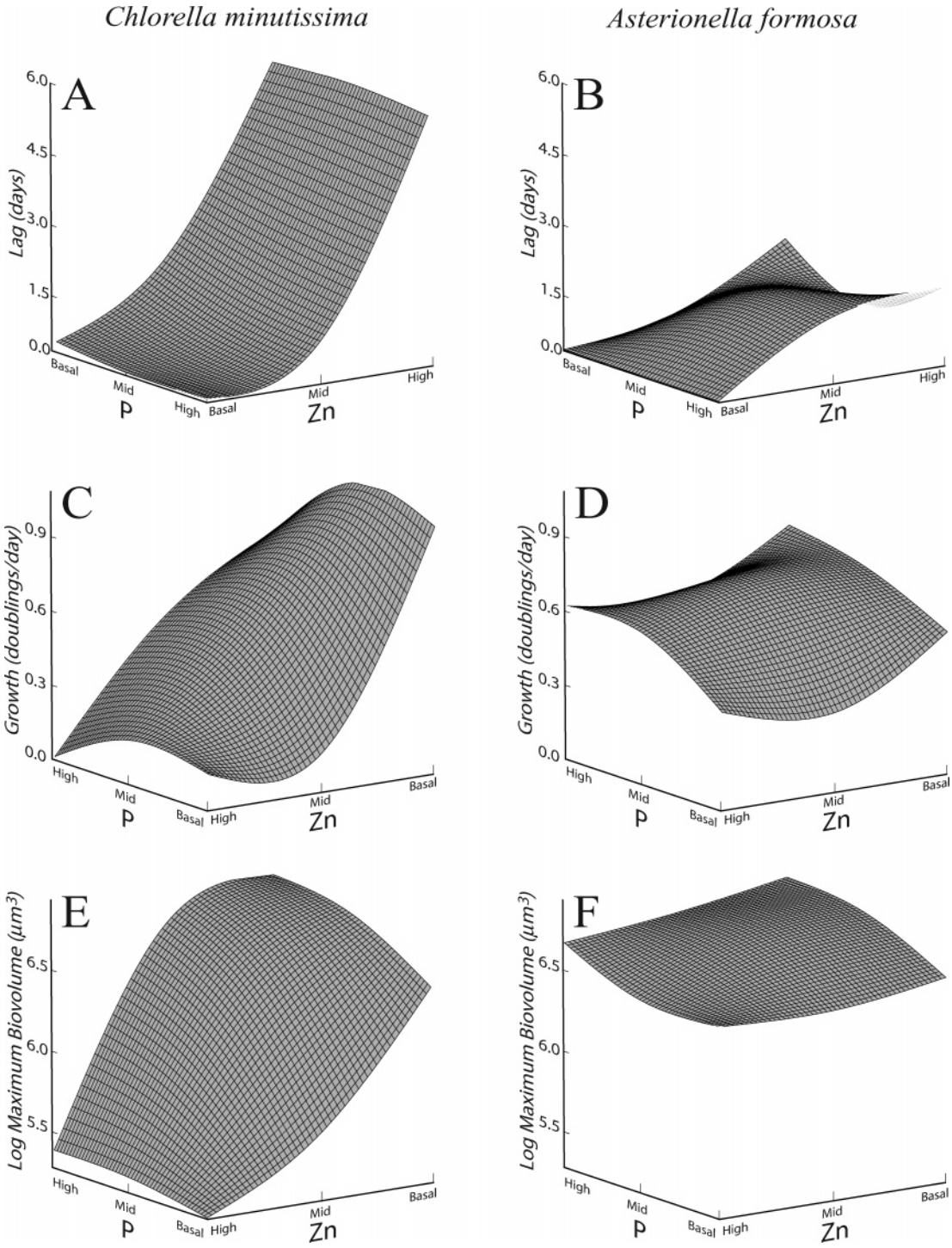

FIGURE 3. Modeled phytoplankton-response contours with the left and right columns depicting responses for *C. minutissima* and *A. formosa*, respectively: (Plates A and B) lag phase duration in days based on biovolumes, (Plates C and D) Growth rate in days^{-1} also based on biovolumes, and (Plates E and F) logarithm of cell yield in μm^3-mL^{-1}. Concentrations for bioassay treatment levels are provided in Table 1. In Plate A and B only, the ordination of the axes are reversed to show a response-surface view from above, consistent with the other 4 plates.

maximum growth rate for *A. formosa* in this study (0.88 ± 0.03 d^{-1}) is comparable to that reported by others (0.81 ± 0.08 d^{-1}; 19), despite the fact that our test organism was collected from Site 2. At each of the three free Zn ion concentrations, growth rates for *A. formosa* were lowest at the basal dissolved-orthophosphate concentration Empirical modeling of *C. minutissima* response consistently exhibited a positive primary (first-order) effect of dissolved-orthophosphate concentration and an adverse primary effect in response to free Zn ion on growth rate (Table S4). Although a positive effect of dissolved orthophosphate was also determined for *A. formosa*, the adverse effects of free Zn ion were not consistently depicted. For example, the first-order adverse effect of Zn on growth rate was statistically significant when based on changes in biovolume, but not when based on cell concentration.

Cell yield is another phytoplankton growth parameter that is typically constrained by limiting nutrients (phosphorus for Coeur d'Alene Lake; refs *1*, *2*), but may also be affected by a toxic response. At basal-Zn concentrations, both

phytoplankton species exhibited increased cell yields (log-transformed biovolume in μm^3-mL^{-1}) of up to 6.93 ± 0.19 and 6.95 ± 0.13 for *C. minutissima* and *A. formosa*, respectively, with increased orthophosphate concentrations. This increase was most evident between basal and mid levels of dissolved orthophosphate, as the high-P treatments provided an excess of phosphorus relative to nitrogen (i.e., N:P molar ratio of 4 << Redfield molar ratio of 16). As free Zn ion concentrations and, hence, bioavailability increased, the positive effect of dissolved orthophosphate on cell yield became less pronounced to the point where dissolved orthophosphate concentration had no effect on cell yield for *C. minutissima* at the highest free Zn ion concentrations. The cells simply did not grow and perhaps were not viable by the end of the culturing period. Among other responses to increased Zn concentrations, *A. formosa* cells presumably formed exudates (*20*), which led to cell aggregation and complicated cell counting. Aggregated cells had a decrease in exposed surface to volume ratios and hence a decrease in toxicant exposure from the bulk solution (*20*, *21*). Clumping (aggregation) of cells due to the release of mucilaginous compounds may have the indirect morphological effect of increasing particle size and, hence, settling rate to the lakebed. Replicate variability in bioassays of *A. formosa* was encountered as sonication of culture aliquots was required to break clumps into individual cells amenable to particle counting. In summary, although both test organisms exhibited a positive effect of dissolved orthophosphate concentration and an adverse reaction to free Zn ion as quantified by all three response parameters, species-specific differences were evident in their relative response to both culturing-media variables.

As part of a collaborative study to develop a process-interdependent water-quality model for the lake, qualifications about this work should be noted. First, our phytoplankton-growth models represent the response of species that were dominant in the phytoplankton community during the time of the study. Despite our efforts to use representative test organisms and media, these models must be applied with caution in the field, because the observed community structure, particularly in a chemically "fragile", oligotrophic system, may be altered by a variety of naturally and anthropogenically affected environmental conditions not considered in the experimental design. Those conditions include river discharge, water temperature, and macrophyte density in littoral zones of the lake that shift over multiple time scales as demonstrated by changes in the phytoplankton community over the past decade (ref *1*, Table S3). Second, extrapolation of the response models beyond the concentrations ranges used in the study is imprudent (*22*). For example, it was noted above in the introduction that free Zn ion concentrations representative of lake waters down gradient of the Coeur d'Alene River plume, and intentionally beyond the experimental design used here, have totally suppressed phytoplankton growth (*1*). Third, because the concentration intervals used in the culturing treatments represent lake conditions, the experimental design was not orthogonal (i.e., coefficient values depended on the units and intervals of the independent variables). The micromolar concentrations for dissolved orthophosphate spanned 1 order of magnitude, but the free Zn ion concentrations were 1−5 orders of magnitude smaller. Therefore, the significant modeling coefficients describing the effect of free Zn ion can be orders of magnitude greater than those describing the effects of dissolved orthophosphate on a dependent variable. Fourth, the model description of lag-phase duration for *C. minutissima* underestimated adverse Zn effects because the input lag phase for the model was set at 6 days when, in fact, that was the lower bound as growth was not measurable over the 6 day culturing period at the highest Zn concentration, regardless of orthophosphate additions. Finally, although composition of the media at the outset of the culturing period is chemically defined (Table S1; refs *9*, *10*), it was not monitored during the batch-culturing period. Despite the use of chelating agents, chemical composition and speciation of media can change in response to nutrient or toxicant uptake and exudate release, particularly with the initial cell concentrations for *C. minutissima* of 20×10^3 cells-mL^{-1} (*23*, *24*). Batch cultures were run until stationary-phase cell densities were achieved or as long as 6 days to monitor cultures that were adversely affected by media formulations (typically due to elevated free Zn ions). In such cases where exponential growth was not observed (i.e., lag-phase duration was greater than 6 days), calculations were made to determine the effect of cellular uptake on the medium formulation. Using maximum Zn concentrations from Site 2 (1400 μg-Zn/g-phytoplankton), the effect on the culturing medium due to Zn uptake by the *C. minutissima* innoculum (isolated and stored in basal-Zn medium) would be approximately 2 nM or approximately 1% of the total Zn added to the mid and high-Zn formulations. Inherent decreases in concentrations of some media constituents may have occurred during batch cultures, but (1) did not greatly affect dissolved-Zn concentrations when free Zn ions adversely affected the test organisms, and (2) is expected to underestimate rather than overestimate the adverse effects of free Zn ion on these taxa if exponential growth is achieved during transit times in the lake (*23*, *24*).

Management Implications. Under oxic, pH neutral conditions, typical of Coeur d'Alene Lake, orthophosphate has a high affinity to adsorb onto metal oxide surfaces (*25*, *26*). Repartitioning reactions are likely to be an important factor in the availability of orthophosphate considering (1) depleted dissolved-orthophosphate concentrations typical of oligotrophic lakes, as with Coeur d'Alene Lake north of Site 2 (tenths of micromolar; refs *27*, *28*), and (2) ubiquitous surficial distribution of iron oxides in Coeur d'Alene Lake sediments (*2*, *29*). Once available in the water column, results presented herein indicate that metabolism of the limiting nutrient by phytoplankton is further constrained by inhibitory free Zn ion effects that affect lag-phase duration and growth rates over time scales of days to a week. Based on measured 3-dimensional current velocities (*18*), the time scales of these growth parameters are environmentally significant because they are comparable to transit times associated with stratified flow within and through Coeur d'Alene Lake. That is, process-interdependent chemical-transport models may be a critical management tool in such lakes because biological response occurs over the same time scales as physical transport.

Knauer et al. (*30*) observed that Zn accumulated by phytoplankton from four Swiss lakes (3−30 μmoles-Zn-g^{-1} dry weight) was "tightly regulated" over a broad range of free Zn ion concentrations (10^{-4} to 10^{0} μM). Although Zn concentrations associated with phytoplankton from Site 2 were consistently within this regulated range (21.0 ± 0.8 μmoles-Zn-g^{-1}dry weight, $n = 3$), phytoplankton near the Coeur d'Alene River plume consistently exceeded that range (50.2 ± 15.9 μmoles-Zn-g^{-1} dry weight, $n = 3$) despite a computed free Zn ion concentration of <0.5 μM (lower than the maximum reported by Knauer et al., ref *30*). Using the taxonomic data from Site 2 (Table S3), a logarithmic conversion of phytoplankton biovolume to cellular carbon (*31*), and a carbon to dry weight ratio of 100:40 (*13*), the mass concentration of phytoplankton at Site 2 can be estimated at 370 ± 90 μg-L^{-1}. Multiplying the phytoplankton mass concentrations by the Zn associated with phytoplankton presented above provides an estimate for phytoplankton-Zn in the water column (8 ± 3 nM) that is 2 orders of magnitude lower than the dissolved-Zn concentrations at the chlorophyll-maximum depth at Site 2 (515 ± 1 and 379

± 4 nM in 2004 and 2005, respectively; Table S2). Although it has been hypothesized that trace-metal accumulation, settling, and decomposition of phytoplankton can represent a major internal source of dissolved trace metals to lake bottom waters (*32*), our analysis indicates that Zn concentrations associated with lake phytoplankton cannot provide that source, at least based on the temporal and spatial coverage of this study. Furthermore, there is a confined subsurface chlorophyll maximum in the lake that is longitudinally maintained between water column temperatures of 10−13 °C (∼5−15 m depths; ref *18*). If phytoplankton transport through the lake is constrained to this subsurface layer, then settling would be negligible and phytoplankton accumulation would represent a net water-column sink for dissolved Zn due to repartitioning and advection to the Spokane River. Although this extreme condition is probably not met, low benthic-chlorophyll concentrations measured in the lake (1.3−3.5 μg-Chl-cm^{-2}) relative to other oligotrophic/mesotrophic lakes (*2*) once again suggest a benthic source of dissolved trace-metals like Zn other than settled phytoplankton (*2*, *33*). However, concerns expressed about potential changes in metal cycling within the lake due to future increases in anthropogenic phosphorus inputs to the lake (*32*) are valid based on cell-yield results observed in this study (Table S3).

Although the concept of P−Zn interactive effects on phytoplankton growth have been previously documented (*4*, *5*, *10*), the response models developed here for Coeur d'Alene Lake have management implications that may be applicable to other P-limited lentic environments. If, through management constraints, dissolved Zn could be regulated down to Site 1 (St. Joe River inlet) concentrations, and phosphorus inputs were not regulated, the phytoplankton community would presumably shift toward an assemblage similar to the St. Joe River inlet. The response model indicates that (1) lag phases north of the St. Joe River inlet for Zn-sensitive species like *C. minutissima* would be virtually eliminated, (2) growth rates that are nearly absent for Zn-sensitive species would increase to approximately 1 d^{-1} and, hence, organic carbon deposition to the lakebed would increase, but (3) despite the accelerated growth of Zn-sensitive species, algal biomass would remain limited by phosphorus availability in the water column.

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Note Added after ASAP Publication. The assignment of copyright for the version published ASAP March 9, 2007 was in error; the corrected version published ASAP March 13, 2007.

Supporting Information Available

Additional information describing media formulations, water-column chemistry, phytoplankton community composition, and culturing results (Tables S1−S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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