Ecotoxicology and Environmental Safety 114 (2015) 318–325

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Environmental influence on cyanobacteria abundance and microcystin toxin production in a shallow temperate lake

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ARTICLE INFO

Article history:
Received 7 May 2014
Accepted 8 May 2014
Available online 22 July 2014

Keywords:
qPCR
Cyanobacteria
ELISA
NMDS
MIC 16S
mcyE

ABSTRACT

The increasing frequency of harmful cyanobacterial blooms in freshwater systems is a commonly recognized problem due to detrimental effects on water quality. Vancouver Lake, a shallow, tidally influenced lake in the flood plain of the Columbia River within the city of Vancouver, WA, USA, has experienced numerous summertime cyanobacterial blooms, dominated by *Aphanizomenon* sp. and *Anabaena sp*. Cyanobacteria abundance and toxin (microcystin) levels have been monitored in this popular urban lake for several years; however, no previous studies have identified which cyanobacteria species produce toxins, nor analyzed how changes in environmental variables contribute to the fluctuations in toxic cyanobacteria populations. We used a suite of molecular techniques to analyze water samples from Vancouver Lake over two summer bloom cycles (2009 and 2010). Both intracellular and extracellular microcystin concentrations were measured using an ELISA kit. Intracellular microcystin concentrations exceeded WHO guidelines for recreational waters several times throughout the sampling period. PCR results demonstrated that *Microcystis* sp. was the sole microcystin-producing cyanobacteria species present in Vancouver Lake, although *Microcystis* sp. was rarely detected in microscopical counts. qPCR results indicated that the majority of the *Microcystis* sp. population contained the toxin-producing gene (*mcy*E), although *Microcystis* sp. abundance rarely exceeded 1 percent of overall cyanobacteria abundance. Non-metric multidimensional scaling (NMDS) revealed that PO_4–P was the main environmental variable influencing the abundance of toxic and non-toxic cyanobacteria, as well as intracellular microcystin concentrations. Our study underscores the importance of using molecular genetic techniques, in addition to traditional microscopy, to assess the importance of less conspicuous species in the dynamics of harmful algal blooms.

1. Introduction

Harmful cyanobacterial blooms are an increasingly common occurrence in eutrophied aquatic systems, and may result in a broad range of environmental, social, and economic consequences. For example, cyanobacterial blooms can increase oxygen demand, which may lead to localized incidents of hypoxic or anoxic conditions that lead to fish kills (Anderson et al., 2002; Paerl, 2008). Surface blooms can also block light from reaching benthic primary producers, which may then adversely affect food web dynamics dependent on lake-bottom habitat (Bricelj and Lonsdale, 1997; Gallegos and Bergstrom, 2005). Social and economic impacts of cyanobacterial blooms include negative effects on recreational opportunities due to closure of affected areas, reduced local fisheries, and increased water treatment costs (Hoagland et al., 2002; Paerl, 2008).

In addition to their broader ecological impacts, cyanobacteria are known to produce a suite of secondary metabolites that include hepatotoxins, neurotoxins, and dermatotoxic compounds. These toxins have been linked to decreased water quality and detrimental effects on higher trophic levels (Leonard and Paerl, 2005; Ferrão-Filho et al., 2009), as well as small animal mortality and illness (Boyer, 2007; Jacoby and Kann, 2007), and adverse health risks to humans (Paerl, 2008). A complex suite of interacting environmental factors influence cyanobacterial bloom dynamics and toxin production. These include abiotic factors such as nutrient inputs (Downing et al., 2001; Elliott, 2012; Heisler et al., 2008), hydrodynamics (i.e. wind-driven currents, turbulence, and stratification) (Fortin et al., 2010; Hotto et al., 2007), light availability (Cires et al., 2011; Renaud et al., 2011), and temperature (Cires et al., 2011; Davis et al., 2009), but also biotic interactions such as competition and grazing (Elser, 1999; Ger et al., 2010; Gobler et al., 2007).

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http://dx.doi.org/10.1016/j.ecoenv.2014.05.004
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Not all cyanobacteria species produce toxins, therefore being able to identify and quantify the abundance of toxin and non-toxin-producing cyanobacteria is essential to understanding toxic cyanobacterial bloom dynamics. In particular, toxin and non-toxin producing individuals of the same species often coexist (Davis et al., 2009; Otsuka et al., 1999; Rantala et al., 2006), and toxin producing individuals cannot be morphologically distinguished from non-toxin producing individuals (Dittmann et al., 1997). Moreover, cyanotoxins are not species specific: several different species are known to produce the same toxin and the regulation of toxin production varies considerably as environmental conditions change. Without identifying the toxin-producing species, managing for toxic cyanobacterial bloom effects on water resources may be ineffective.

Vancouver Lake is a large, temperate, shallow, non-stratifying lake located in the floodplain of the Columbia River, within the city limits of Vancouver, Washington, USA. Vancouver Lake is a local recreational destination serving residents throughout the Portland (OR)-Vancouver (WA) Metropolitan area. It also serves as critical habitat for wildlife and migratory waterfowl. Cyanobacterial blooms have been documented in Vancouver Lake since 1979, although anecdotal references suggest blooms also occurred prior to that (Bhagat and Orsborn, 1971). In recent years the lake has often been closed during late summer due to cyanobacterial blooms and the presence of toxins. Although the overall plankton community of Vancouver Lake has been studied and documented (Boyer et al., 2011; Rollwagen-Bollens et al., 2012), no studies have investigated the identity of the microcystin-producing cyanobacteria and the potential water quality variables associated with toxin production.

More broadly, we are interested in Vancouver Lake as a model system to elucidate cyanobacterial bloom dynamics in shallow, temperate lakes generally. Thus the objectives of this study were to use molecular genetic techniques to identify any microcystin-producing species in Vancouver Lake, and to investigate which environmental variables influence changes in microcystin concentration and the toxin-producing cyanobacteria population.

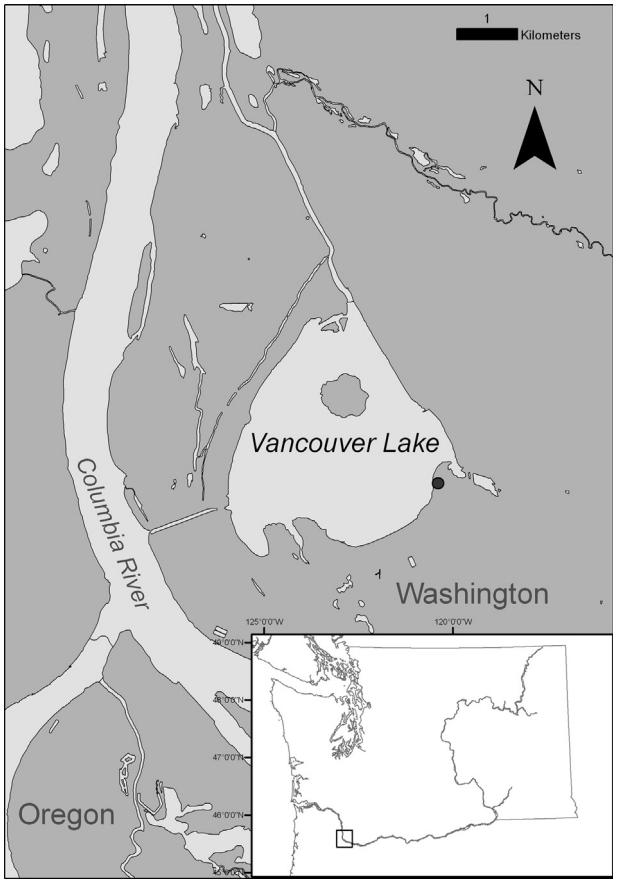

Fig. 1. Vancouver Lake, Washington, is located just north of Portland, Oregon. The filled circle represents the sampling station at Vancouver Lake Sailing Club.

2. Materials and methods

2.1. Field collection

Sampling for the current study was conducted from the Vancouver Lake Sailing Club dock (Fig. 1) on a weekly basis during the periods of May through October 2009, and June through September 2010. A prior study examining the spatial distribution of plankton communities in Vancouver Lake showed no significant differences in plankton abundance or taxonomic composition among eight study sites throughout the lake (Bollens and Rollwagen-Bollens, 2009). Briefly, eight different sampling sites representing both littoral and limnetic zones were sampled on a quarterly basis for one year to measure spatial and temporal variability in plankton. Although there were significant seasonal differences, there were no significant spatial differences among sites (Kendall's tau, $p > 0.5$), thus we consider the dock site to be representative of the lake as a whole. On each sampling date, vertical profiles of temperature, dissolved O_2, pH, and turbidity were measured every 0.2 m from the surface to the bottom using a YSI 91 probe. Total water column depth and Secchi depth were also recorded. Because the lake is very shallow and well-mixed during the summer, we collected surface water samples using a clean bucket, and triplicate subsamples were taken for later nutrient analyses. Subsamples for DNA extraction and toxin analysis were also collected and kept chilled in a cooler for transport and subsequent analysis in the laboratory. Triplicate 200 mL subsamples were also obtained and preserved in 5 percent acid Lugol's solution for microscopical analysis to determine abundance and taxonomic composition of phytoplankton.

2.2. Nutrient analyses

Nutrient samples were filtered through 0.45 μm Millipore disposable filter capsules, stored in plastic bottles and kept refrigerated until analysis. Dissolved nitrate (NO_3–N), nitrite (NO_2–N), ammonium (NH_4–N), phosphate (PO_4–P), and silicate (SiO_4–Si) concentrations were analyzed to assess the effects of nutrients on the phytoplankton community generally and the cyanobacteria community specifically. Samples were analyzed by the Marine Chemistry Lab at the University of Washington's School of Oceanography.

2.3. Microscopic analysis

Lugol's preserved phytoplankton samples were concentrated in settling chambers (≤ 10 mL) and at least 200 cells were enumerated using an Olympus CK-40 inverted microscope ($400\times$) and identified at least to genus, and to species whenever possible (Prescott et al., 1978; Wehr, 2002).

2.4. DNA extraction and purification

For DNA extraction, 250 mL aliquots of lake water were filtered onto 0.45 μm GF/F filters at the time of collection. Filters were kept frozen in polyethylene centrifuge tubes at −80 °C until analyzed. Filters were initially suspended in Buffer ATL (Qiagen, Valencia, CA) and disrupted by three cycles of freezing at −20 °C and thawing at 70 °C. Proteinase K was added to a final concentration of 50 μg/mL and incubated at 50 °C for 2 h. DNA was extracted by first adding phenol/chloroform/isoamyl alcohol (25:24:1) (v/v), and then a second chloroform/isoamyl alcohol (24:1) extraction (v/v). DNA was precipitated overnight at −20 °C after adding 95 percent ethanol (v/v) and 9 M sodium acetate (0.1 percent v/v). DNA was collected the following day by centrifugation (16,000 × *g*, 30 min). A second rinse step was performed using 75 percent ethanol (v/v) and then centrifuged (16,000 × *g*, 15 min). DNA pellets were air-dried and re-suspended in sterile water. DNA was further purified using GeneClean II (MP Biomedicals). Concentration and purity of extracted DNA was measured spectrophotometrically (SmartSpec Plus Spectrophotometer, BioRad).

2.5. PCR

Samples were initially examined to assess the presence of cyanobacteria by targeting 16S rDNA (Urbach et al., 1992), and microcystin-producing cyanobacteria by targeting genera-specific microcystin synthetase gene *mcyA* (Hisbergues et al., 2003) and genera-specific microcystin synthetase gene *mcyE* (Rantala et al., 2004). Each assay was carried out in 10 μL reactions and contained 400 μM of each primer,

400 μM dNTPs, 400 μM BSA, 10× buffer (10 percent v/v), and 1 unit Taq polymerase (Genscript). PCR protocol for cyanobacteria 16S rDNA was as follows: initial denaturation step of 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 60 °C for 45 s, 72 °C for 45 s, and a final extension step at 72 °C for 7 min, and then held at 4 °C. Amplification for *mcyA* and *mcyE* genes was similar, except that the annealing temperatures were set at 51 °C and 55 °C, respectively. PCR products were visualized on 1.5 percent agarose gels stained with 1 percent GelRed.

Cultures of microcystin-producing *Microcystis aeruginosa* (UTEX 2385) and non-microcystin-producing *M. aeruginosa* (UTEX 2386) were used as positive and negative controls for initial target screening of lake water samples (University of Texas Culture Collection). These cultures were grown and maintained using 3N Bold liquid medium on a 12:12 h light:dark regime. PCR products isolated from lake water samples from three different sampling dates, for each of the *mcyA* and *mcyE* procedures (for a total of six products), were sequenced on an ABI3130xl Sanger sequencer (according to the manufacturer's protocol) and compared among sequences available in GenBank. Sequences were uploaded to GenBank with accession numbers KC603867 and KC603858.

2.6. qPCR

Based on results from initial screening of lake water samples using conventional PCR, UTEX 2385 (microcystin-producing *M. aeruginosa*) and UTEX 2386 (non-microcystin-producing *M. aeruginosa*) were used as external standards to determine cyanobacteria 16S rDNA (Baxa et al., 2010) and *Microcystis* sp. 16S rDNA (Neilan et al., 1997) gene copy numbers. UTEX 2385 was used as the external standard for determining *Microcystis* sp.-specific *mcyE* gene copy numbers (Vaitomaa et al., 2003). Gene copy numbers were calculated according to Vaitomaa et al. (2003), however we did not assume two 16S rDNA gene copies per cell. All gene copy numbers for both *Microcystis* sp. and cyanobacteria genera are reported here as gene copy per ng DNA, due to extraction efficiencies and variable rRNA operon copy number. Throughout the extraction and subsequent clean-up process, we could not assume the final amount of DNA extracted from the filters represented the initial volume of lake water used. We also did not assume an average of four 16S rDNA copies per cell for quantifying the cyanobacteria population, nor did we assume an average of two 16S rDNA copies per cell for *Microcystis* sp. Environmental conditions influence 16S rRNA operon copy numbers in bacteria, unfavorable environmental conditions for some species exhibiting low growth rates will result in fewer rRNA operons, since expression from multiple rRNA operons incurs a metabolic expense of slower growing cells (Klappenbach et al., 2000). Also, using cultured strains derived from a different aquatic system has been shown to differ in operon copy number compared to the same species found in a different environment (Acinas et al., 2004). We assumed one *Microcystis* sp. 16S rDNA copy number per cell and *mcyE* copy per cell. Although this can lead to inaccurate estimates of cell abundances (Crosby and Criddle, 2003; Rinta-Kanto et al., 2005, 2009), relative changes in population abundance can still be determined, and the utility of qPCR in monitoring potentially toxin producing *Microcystis* sp. populations remains high (Martins and Vasconcelos, 2011).

A series of 10-fold dilutions was prepared for standard curve calculations. Standard curves used to calculate gene copy numbers in lake water samples met the minimum requirement of efficiencies ranging from 90 to 110 percent and $r^2 > 0.99$. Reactions (20 μL) were prepared using 1 μL of DNA from extracted standard strains or 2–20 ng of DNA from lake water samples, 400 μM BSA, 400 nM of each primer, and 10 μL of Power SYBRGreen (Invitrogen), and then run on an ABI 7500 instrument (Applied Biosystems, SDS software v.2.0.5). We used the same amplification protocol from our conventional PCR for our qPCR. To avoid errors caused by primer dimers we also added a dissociation step from 65 to 95 °C after the final extension step for each assay. All lake water samples were run in triplicate.

2.7. Microcystin toxin analysis

Lake water subsamples for toxin analysis were filtered in 50 mL aliquots through 0.22 μm polycarbonate filters. Filters were kept in glass scintillation vials and the filtrates were stored in polyethylene centrifuge tubes. Both filter and filtrate were then kept frozen (−80 °C) until analyzed. Intracellular and extracellular microcystin toxin concentrations were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Beacon Analytical Systems, Saco, ME). Although the ELISA kit does not distinguish between microcystin-LR (calibrator) and other variants of microcystin (i.e., cross reactivities among different microcysin variants are possible and noted by the manufacturer), this method is acceptable for monitoring microcystin concentrations and has been used in previous studies (Gurbuz et al., 2012). Extracellular toxin analysis was performed on the filtrate according to the manufacturer's protocol. Intracellular toxin analysis was conducted according to a modification of the procedures described in Sevilla et al. (2009). Briefly, intracellular toxins were extracted from phytoplankton cells by submerging the polycarbonate filters for 30 min in a buffer mixture of 80 percent methanol, 0.1 percent Tween, and 0.1 percent trifluoroacetic acid. Aliquots (50 μL) of the supernatant were then diluted 1:20 with 5 percent phosphate buffered saline and then analyzed with the ELISA kit according to the manufacturer's protocol.

2.8. Statistical analysis

Non-metric multidimensional scaling (NMDS) was used to examine associations among water quality variables, intra- and extra-cellular microcystin concentrations, and gene copy numbers of each assay. We used NMDS because it is an unconstrained ordination method that does not assume linear relationships and is robust in handling discontinuous and non-normal data (McCune and Grace, 2002). All ordinations were performed using PC-ORD version 5.3 software. A total of five "species" were included in the analysis: cyanobacteria 16S rDNA gene copy numbers (CYA 16S), *Microcystis* sp. 16S gene copy numbers (MIC 16S), *Microcystis* sp.-specific *mcyE* gene copy numbers (*mcyE*), intracellular microcystin concentration, and extracellular microcystin concentration. Environmental variables included in the analysis were total water depth, Secchi depth, temperature, dissolved O_2, NO_2–N, NO_3–N, NH_4–N, PO_4–P, SiO_4–Si, turbidity, and pH. Species abundances were log $(x+1)$ transformed. We used Euclidean distance because we had two different types of variables in representing "species" data: gene copy numbers and microcystin concentrations (Clarke and Warwick, 2001). Due to missing data points of various environmental variables, the NMDS was performed on a subset of data May–October 2009 and June–September 2010 ($n=26$).

3. Results

3.1. Physical and nutrient characteristics

Total water depth ranged from 0.91 to 3.1 m in 2009, and 0.97–3.2 m in 2010 (Fig. 2a). Secchi depth ranged from 0.10 to 0.99 m in 2009, and 0.2–0.66 m in 2010 (Fig. 2a). Summer temperatures in 2009 reached as high as 28 °C, but in 2010 only reached 23 °C (Fig. 2b). On average, dissolved O_2 was lower in 2009 (5.8 mg/L) than in 2010 (10.4 mg/L) (Fig. 2b). In 2009, pH ranged from 8.0 to 10, and ranged between 7.9 and 9.9 in 2010 (Fig. 2c). Turbidity ranged from 11 to 170 NTU in 2009, and in 2010 turbidity ranged from 23 to 130 NTU (Fig. 2c).

During the sampling period in 2009, PO_4–P concentrations showed greater variation (1.1–240 μg/L) than in 2010 (2.1–50 μg/L) (Fig. 2d). Similarly, the range of NH_4–N concentrations was greater in 2009, when it peaked at 460 μg/L, compared to 2010 when the highest concentration was 160 μg/L (Fig. 2d). NO_3–N concentrations were lower in 2009, with a maximum concentration of 20 μg/L, than in 2010, with a maximum concentration of 95 μg/L (Fig. 2e). NO_2–N levels remained relatively low in both 2009 and 2010 (Fig. 2e). SiO_4–Si availability was greater in 2009, with the maximum concentration of 1.1×10^4 μg/L, than in 2010, when the maximum was 6.3×10^3 μg/L (Fig. 2f).

3.2. Microscopic enumeration of cyanobacteria community

In a separate analysis Lee et al. (unpublished) showed that the summer phytoplankton communities in Vancouver Lake were significantly different between 2009 and 2010, largely due to the presence of a distinct cyanobacterial bloom in 2009 that did not occur in 2010. More specifically, in 2009 the cyanobacterial bloom was initially dominated by *Anabaena sp.*, but then progressed to an *Aphanizomenon sp.*-dominated cyanobacteria community for the rest of the bloom (Fig. 3a). In 2010, although there was a brief period with increased *Aphanizomenon sp.* abundance in July and August, the summertime phytoplankton community was dominated by chlorophytes and diatoms (Fig. 3a). In both 2009 and 2010 a wide range of cyanobacteria species were observed; however, *Anabaena sp.* and *Aphanizomenon sp.* together made up >80 percent of the relative abundance as determined through traditional light microscopy (Fig. 3b).

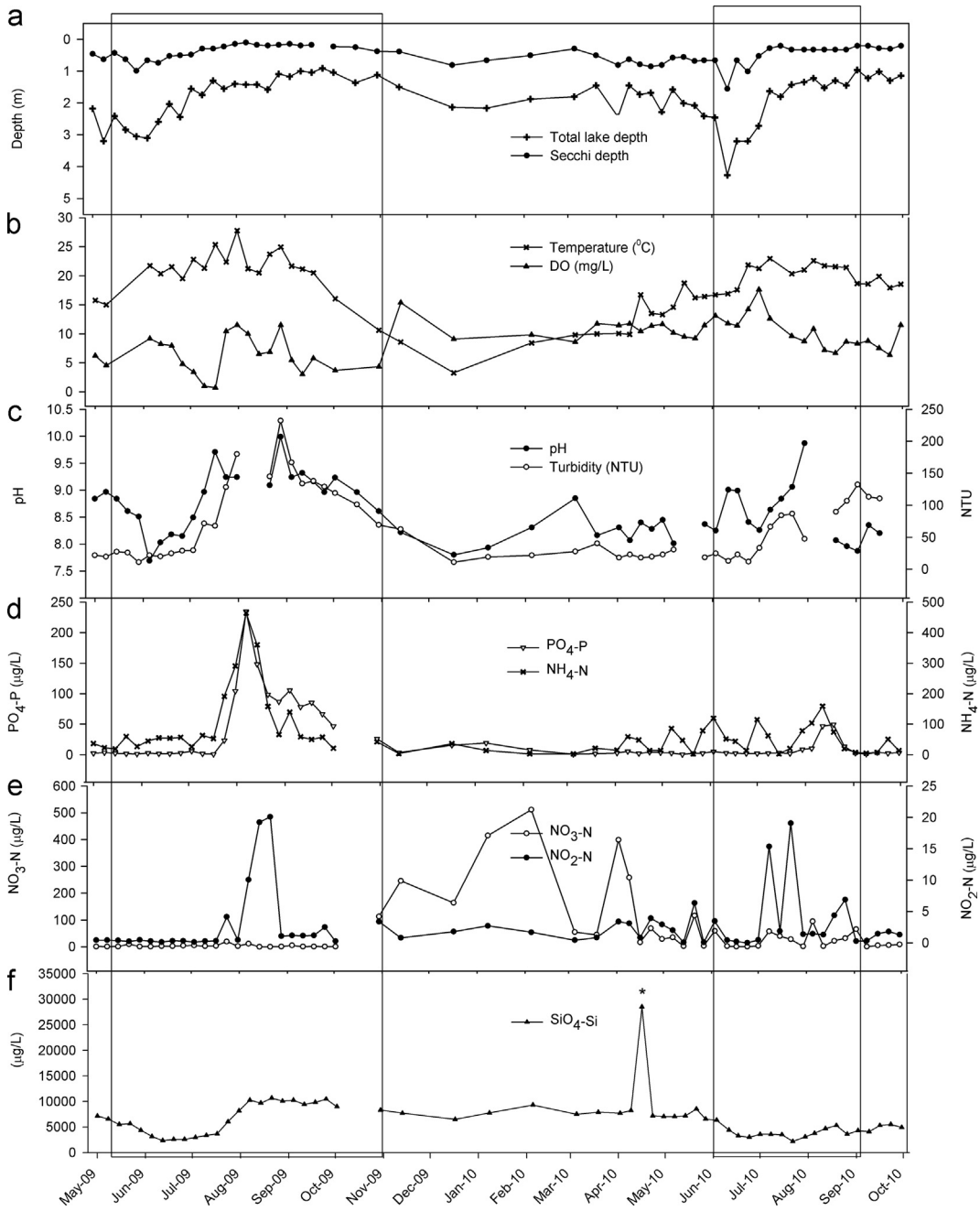

Fig. 2. Environmental variables for 2009 and 2010: (a) total water depth and Secchi depth, (b) temperature and dissolved O_2 (DO), (c) pH and turbidity, (d) PO_4–P and NH_4–N, (e) NO_3–N and NO_2–N, (f) SiO_4–Si. Boxed areas represent the sampling period and data used for 2009 and 2010 analyses. *Although this value falls outside the analyzed sampling period, it is recognized as a potentially erroneous value.

3.3. Identification and quantification of toxin and non-toxin producing cyanobacteria species

DNA sequence results from PCR amplicons using genera specific *mcyA* and *mcyE* primers indicated that a species of *Microcystis* was the only potential microcystin producer detected in Vancouver Lake. Yet, *Microcystis* sp. populations were rarely observed microscopically throughout the sampling period (Fig. 3b). Because we were unable to reliably detect or monitor *Microcystis* sp. using traditional microscopy, we were unable to correlate the qPCR results with cell counts. In addition, on several dates we observed *Anabaena sp.* to account for >50 percent of the cyanobacteria community; however, we did not detect any molecular signature of *Anabaena sp.* using either of the genera specific *mcyA* or *mcyE* primers. Although only one microcystin-producing cyanobacteria species was detected throughout our samples, we do not exclude the possibility of other cyanobacteria producing microcystin existing in Vancouver Lake.

Gene copy numbers for total cyanobacteria, *Microcystis* sp., and potentially microcystin-producing *Microcystis* sp. were variable throughout the sampling period in 2009 and 2010 (Fig. 3c). qPCR results comparing cyanobacteria 16S rDNA gene copy numbers and *Microcystis* sp. 16S rDNA gene copy numbers indicated that *Microcystis* sp. made up <1 percent of the total cyanobacteria community throughout the sampling period. Overall, *Microcystis* sp. gene copy numbers in 2010 were less than in 2009, as were total cyanobacteria gene copy numbers. Notably, potentially toxin-producing *Microcystis* sp. was present in every lake water sample except one during the 2009 and 2010 sampling periods, and in most cases made up >50 percent of the total *Microcystis* sp. population (Fig. 3d).

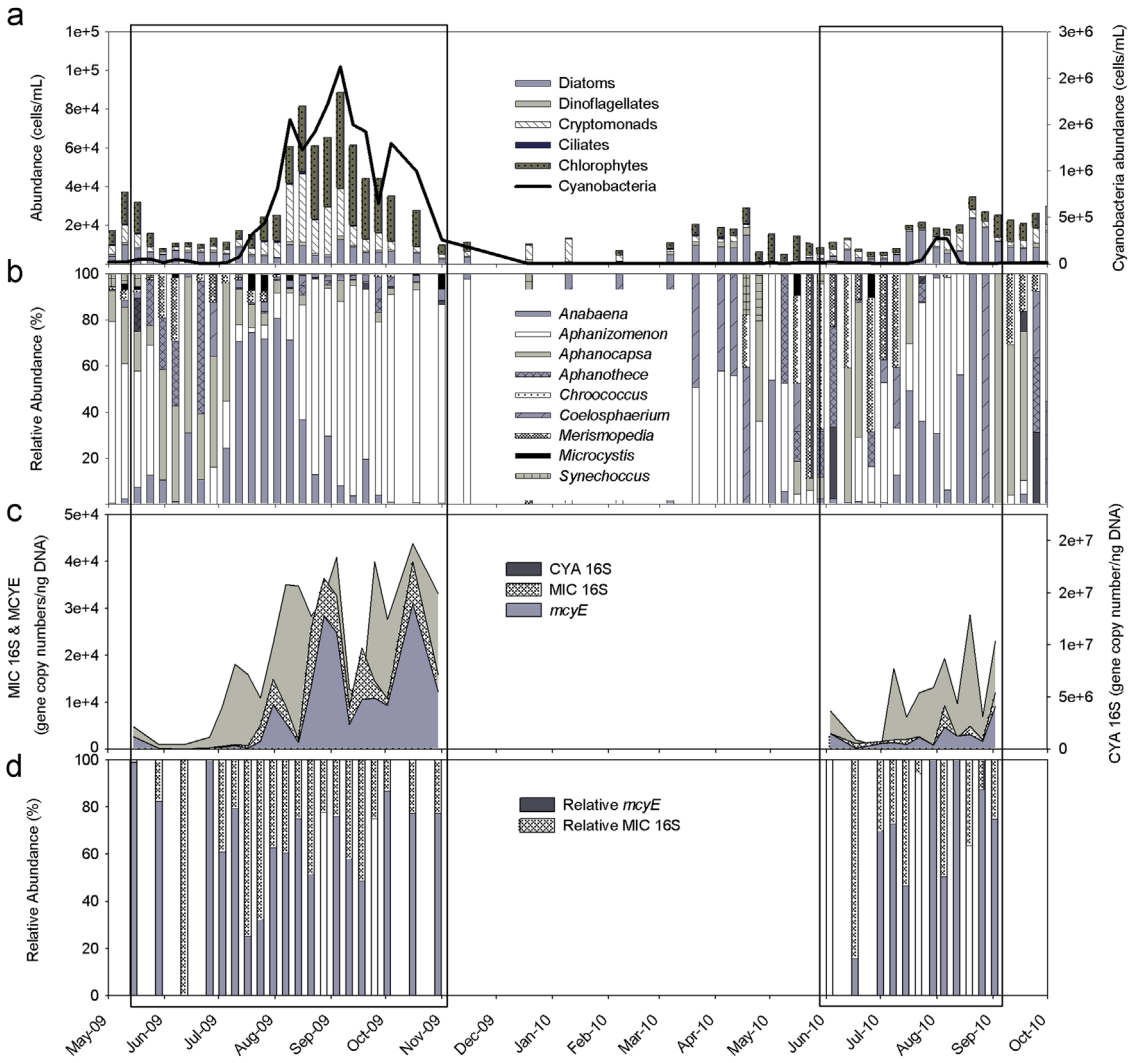

Fig. 3. Vancouver Lake water samples: (a) the absolute abundance of cyanobacteria in relation to other phytoplankton groups during the 2009 and 2010 sampling period based on microscopical counts, (b) relative abundance of cyanobacteria species found in Vancouver Lake, (c) gene copy numbers for MIC 16S, *mcyE*, and CYA 16S, (d) relative abundances of MIC 16S and *mcyE* gene copy numbers. Boxed areas represent the sampling period for 2009 and 2010.

3.4. Intracellular and extracellular microcystin concentrations

Intracellular microcystin concentrations ranged from undetectable to 15 μg/L in 2009 (Fig. 4). As *mcyE* abundance increased in 2009, intracellular microcystin concentration also increased. Further, intracellular microcystin decreased as *mcyE* abundance decreased at the beginning of October 2009, and then increased in mid-October when *mcyE* abundances also increased. In 2010, intracellular microcystin concentrations fluctuated from undetectable to 12 μg/L (Fig. 4). Similar to 2009, in 2010 intracellular microcystin increased as *mcyE* increased. However, overall intracellular microcystin concentration and *mcyE* abundances were less in 2010 than in 2009.

Extracellular microcystin concentrations ranged from undetectable to 0.3 μg/L in 2009 and from undetectable to 0.5 μg/L in 2010 (Fig. 4). No discernible or significant trends were observed between extracellular microcystin concentrations and intracellular microcystin concentrations or *mcyE* abundance.

3.5. NMDS results comparing species abundance and environmental variables

Results from NMDS analysis indicated that SiO_4–Si, PO_4–P, and turbidity were strongly associated with increased toxin and non-toxin producing cyanobacteria populations, and intracellular microcystin concentration (Fig. 5). In 2009, SiO_4–Si levels increased during the summer and plateaued for the duration of the bloom. In 2010, SiO_4–Si levels increased somewhat at the beginning of the period of elevated cyanobacteria abundance, but were variable over the duration of the sampling period. PO_4–P levels peaked in August 2009 and gradually decreased throughout that year's bloom, but in summer 2010 PO_4–P levels were lower in magnitude and duration.

In addition, total lake water depth and Secchi depth were inversely associated with toxin and non-toxin producing cyanobacteria populations. As total lake water depth and Secchi depth decreased, total cyanobacteria, *Microcystis* sp., and microcystin-producing *Microcystis* sp. gene copy numbers increased. Additionally, NMDS results showed total cyanobacteria gene copy numbers and extracellular microcystin concentrations were not strongly associated with any other variable; intracellular microcystin was most strongly associated with microcystin-producing *Microcystis* sp., and toxic and non-toxic *Microcystis* sp. were positively associated with each other.

4. Discussion

In Vancouver Lake, microcystin concentrations have been monitored for several years; however, the cyanobacteria species responsible for producing the toxin had not been positively identified

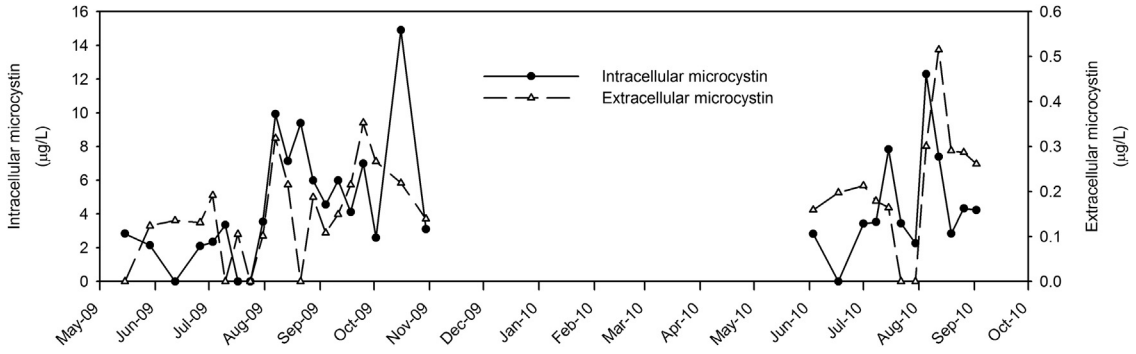

Fig. 4. Intracellular and extracellular microcystin concentrations for 2009 and 2010.

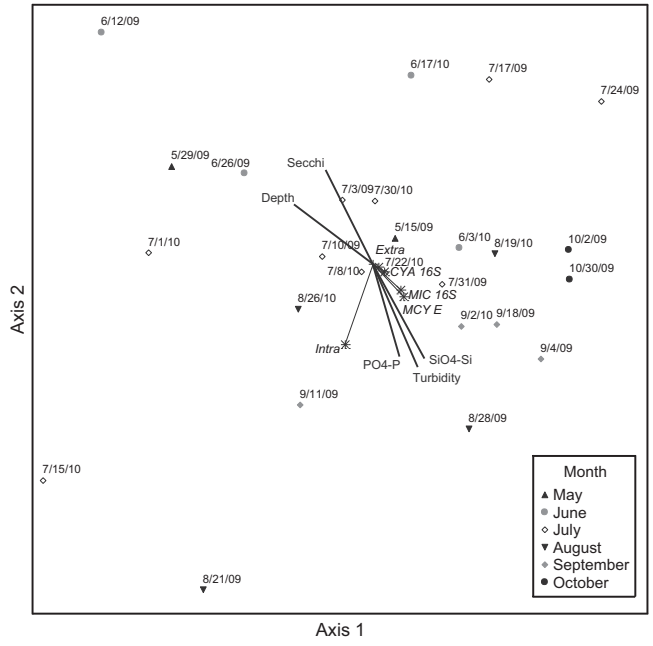

Fig. 5. NMDS ordination of gene copy numbers of toxic (*MCYE*) and non-toxic *Microcystis* sp. (*MIC 16S*), cyanobacteria (*CYA 16S*), and intra- (*Intra*) and extracellular (*Extra*) microcystin concentrations. Each point represents a sampling date. Axis 1 represented 56.5 percent of the observed variance and axis 2 represented 43.3 percent of the observed variance. Vectors are environmental variables associated with species points ($r^2 > 0.2$).

until now. Our previous microscopic examinations of Vancouver Lake plankton assemblages have shown that, since 2006, the annual cyanobacterial blooms have been dominated by *Aphanizomenon sp.* and *Anabaena sp.*, with rare observations of *Microcystis* sp. (Lee et al. unpublished). Surprisingly, in this two-year study using molecular methods, *Microcystis* sp. was the only microcystin-producing cyanobacteria species detected. Prior to our own studies, *Microcystis* sp. was visually observed to be dominant during one summer bloom in 2003, when microcystin levels ranged from < 0.5 µg/L to 18.5 µg/L (Jacoby and Kann, 2007), but despite microcystin levels having been detected and reported since then, *Microcystis* sp. has rarely been observed visually. During the summer of 2011, the Clark County Public Health Department measured microcystin levels periodically throughout the monitoring season, and in one instance levels were high enough to trigger a lake closure for recreational uses, despite the absence of *Microcystis* sp. in microscopic counts (Clark County, 2011). This suggests that there can be significant adverse effects on water quality regardless of the absolute or relative abundance of microcystin-producing *Microcystis* sp.

Therefore, our results demonstrate the utility of using molecular tools to detect and monitor microcystin-producing cyanobacteria. *Microcystis* sp. was rarely detected when quantified using light microscopy, yet our PCR results indicated that potentially toxin-producing *Microcystis* sp. was present during almost all sampling dates. The discrepancy between qPCR and microscopy results may be due to several factors, including preservation and counting methods, and the potential amplification of other species using *Microcystis* sp.-specific 16S rDNA primers. However, the total MIC 16S gene copy numbers represented < 1 percent of the total CYA 16S gene copy numbers, suggesting the discrepancy between qPCR and microscopy results may instead be due to the scarcity of the species and therefore a low probability of accurately enumerating the species through microscopy. Although we only monitored one microcystin gene, *mcyE*, qPCR results suggested the potential microcystin-producing *Microcystis* sp. population was highly variable.

Our results are similar to observations in Upper Klamath Lake, OR, where an *Aphanizomenon sp.*-dominated cyanobacterial bloom (> 90 percent by volume) was shown to contain microcystin-producing *Microcystis* sp., even though *Microcystis* sp. was rarely detected via microscopy (Eldridge et al., 2012; Saker et al., 2007). In addition, in *Microcystis* sp.-dominated cyanobacterial blooms, Rinta-Kanto et al. (2009) showed the proportion of toxin-producing *Microcystis* sp. to non-toxin producing *Microcystis* sp. to be highly variable in Lake Erie, ranging from 0 to 60 percent. Kardinaal et al. (2007) found microcystin-producing *Microcystis* sp. dominated total *Microcystis* sp. populations in two unstratified lakes located in the Netherlands. Finally, Baxa et al. (2010) showed that microcystin-producing *Microcystis* sp. makes up less than 20 percent of the total *Microcystis* sp. population in the San Francisco Estuary.

Our analysis showed PO_4–P was associated with increased populations of toxin- and non-toxin producing *Microcystis* sp., total cyanobacteria, and intracellular microcystin concentration in Vancouver Lake. Assimilation of phosphorus is critical for cellular function and influences toxin-producing cyanobacteria dynamics, including growth, life cycle stages, and toxin production (Dyhrman, 2008; Jacoby et al., 2000). Previous studies have shown that increased PO_4–P availability positively influences cyanobacteria populations (Rinta-Kanto et al., 2009; Xu et al., 2010) and more specifically toxin and non-toxin producing *Microcystis* sp. populations (Davis et al., 2009; Li et al., 2012; Xu et al., 2010). Increased PO_4–P availability has also been shown to influence microcystin concentrations in other lake systems (Jacoby et al., 2000; Rinta-Kanto et al., 2009), and in some cases higher inorganic phosphorous levels have been shown to yield higher microcystin content per cell (Rapala et al., 1997). To better understand the relationship between microcystin and non-microcystin producing cyanobacteria populations we recommend that future experiments be done to measure gene expression for microcystin producing cyanobacteria under varying PO_4–P availability.

Although our analysis also showed turbidity and SiO_4–Si concentrations to be associated with toxin and non-toxin producing *Microcystis* sp., total cyanobacteria, and intracellular microcystin concentration, we do not suggest that turbidity or SiO_4–Si directly influence these relationships. Instead, we suggest these two factors broadly describe the environmental conditions associated with the summer blooms. The lake is very well mixed during the summer months, when total lake depth is at a minimum (<1.5 m). This, along with increased cyanobacteria abundance during the summer, contributes to increased turbidity. With respect to silicate, Rinta-Kanto et al. (2009) showed that SiO_4–Si was positively correlated with microcystin concentrations in Lake Erie. Similarly, Aboal et al. (2005) found that increased intracellular microcystin levels were associated with increased silicate concentrations in a river in southeast Spain. However, neither study has shown nor even suggested that SiO_4–Si directly contributes to microcystin production. Instead, SiO_4–Si has been linked to the dissolution of diatoms (Spears et al., 2008), with increased SiO_4–Si availability signifying a shift in phytoplankton community composition away from a diatom-dominated community to a cyanobacteria-dominated community (Bennion and Smith, 2000; Aboal et al., 2005).

Total lake depth and Secchi depth were inversely related to SiO_4–Si and PO_4–P. Secchi depth and lake water depth were strongly associated with each other—as lake water depth decreased, Secchi depth also decreased. These two variables describe seasonal lake hydrology where lake water depth and Secchi depth decrease during the summer months and increase during the winter and spring months during the rainy season and spring snow melt.

Intracellular microcystin concentrations were strongly associated with the abundance of microcystin-producing *Microcystis* sp., and to a lesser extent associated with the total *Microcystis* sp. population (Fig. 5). As toxic *Microcystis* sp. populations increased, intracellular microcystin concentrations also increased. Our results are similar to those of Rinta-Kanto et al. (2009) in Lake Erie, who found intracellular microcystin concentrations to be correlated with both the total *Microcystis* sp. population and microcystin-producing *Microcystis* sp. Davis et al. (2009) also showed microcystin-producing *Microcystis* sp. were significantly correlated with intracellular microcystin in several lakes in New York state, USA.

Intracellular microcystin concentrations in Vancouver Lake were two to three orders of magnitude higher than extracellular concentrations. This difference may be due to how microcystin is released into the water column and the rate of microcystin degradation. Microcystin is primarily released through biotic means, including cell lysis, and to a lesser extent by active transport through the cell wall (Rapala et al., 1997). Once released into the water column, microcystin can rapidly degrade (average half-life of one day) due to heterotrophic bacteria (Christoffersen et al., 2002) and other chemical interactions (Tsuji et al., 1994), and is thus unlikely to accumulate in the water column (Christoffersen et al., 2002; Lahti et al., 1997). However, other research indicates that microcystin can persist in the water column for up to several weeks (Harada et al., 1996; Gągała and Mankiewicz-Boczek, 2012).

In conclusion, *Microcystis* sp. was the only microcystin-producing cyanobacteria species detected in Vancouver Lake, a species that had previously gone unnoticed due to the limitations of traditional microscopical analysis and the numerical dominance of other cyanobacteria genera, namely *Aphanizomenon sp.* and *Anabaena sp*. Cell counts and species diversity determined by microscopy may provide a general understanding of the phytoplankton community composition; however, without the use of molecular and genetic techniques it is effectively impossible to quantitatively monitor changes in toxic populations that are relatively low in overall abundance.

PO_4–P was the main environmental variable associated with increased toxin and non-toxin producing *Microcystis* sp. gene copy numbers, and intracellular microcystin concentrations. These results are consistent with other *Microcystis* sp. dominated systems, although studies of lakes of similar physical and phytoplankton community characteristics to that of Vancouver Lake remain underrepresented. While using 16S rDNA to monitor changes in *Microcystis* sp. and other cyanobacteria populations may not provide absolute abundance, qPCR still remains an important tool in assessing relative changes in potential toxic cyanobacteria populations. Quantifying *mcyE* gene copy number, rather than relying solely on visual cell counts, may be a better explanatory metric for overall toxin concentration. As natural resource and public health managers continue to monitor regional and local freshwater systems for toxic cyanobacterial blooms, a multifaceted approach is needed to understand cyanobacterial bloom dynamics on both species-specific and community levels.

Acknowledgments

We thank Drs. Jenefer DeKoning, Steve Sylvester, and Ruth Philips for their invaluable support and resources throughout this project, Julie Zimmerman for helping with the sampling and data collection, and the Vancouver Lake Sailing Club for lake access. This research was partially funded through Grant# 06HQGR0126 from the United States Geological Survey (USGS) to G.R.B and S.M.B, through the State of Washington Water Research Center (06HQCR0126). The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the USGS. The lead author received additional funding as an NSF GK-12 Fellow, as part of a STEM Fellows in K-12 Education (GK-12) Fellowship grant (DGE 07-42561) awarded to G.R.B and S.M.B, and a Robert Lane Fellowship in Environmental Science through Washington State University.

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