Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (*Perca flavescens*)

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Abstract: During the summer of 2006, the western basin of Lake Erie experienced a bloom of the toxigenic cyanobacterium *Microcystis aeruginosa*. Across 11 sites, intracellular, particulate-bound microcystin levels in the seston increased to levels that exceeded World Health Organization guidelines for drinking water exposure (1 μg toxin·L^{-1}). In contrast, toxin concentrations in yellow perch (*Perca flavescens*) muscle tissue (n = 68) declined from June to August, were negatively related to algal toxin levels, and never exceeded a conservative chronic exposure concentration estimated using proposed United States Environmental Protection Agency (US EPA) guidelines. Microcystin concentrations in yellow perch liver exceeded US EPA chronic exposure guidelines, were on average 125 times higher than muscle toxin concentrations per unit dry weight, and varied little throughout the summer. With current guidelines, humans do not appear to be at risk when consuming the muscle tissue of Lake Erie yellow perch collected during large-scale cyanobacterial blooms. However, this study highlights the need for a better understanding of the trophic transfer of cyanobacterial toxins through aquatic food webs in diverse ecosystems with an emphasis on understanding if these compounds could accumulate sufficiently to affect human health.

Résumé : Durant l'été 2006, le bassin occidental du lac Érié a connu une floraison de la cyanobactérie toxinogène, *Microcystis aeruginosa*. Dans 11 sites, les concentrations de microcystine intracellulaire liée aux particules dans le seston ont atteint des niveaux supérieurs aux normes de l'Organisation mondiale de la santé pour l'eau potable (1 μg toxine·L^{-1}). En revanche, les concentrations de toxines dans le tissu musculaire de la perchaude (*Perca flavescens*) (n = 68) ont diminué de juin à août et étaient en corrélation négative avec les concentrations de toxines dans les algues; elles n'ont jamais dépassé une concentration conservatrice d'exposition chronique estimée à partir des recommandations de l'Agence de protection de l'environnement des États-Unis (US EPA). Les concentrations de microcystine dans le foie des perchaudes dépassaient les concentrations mentionnées dans les recommandations sur l'exposition chronique de US EPA; elles étaient en moyenne 125 fois plus élevées que les concentrations de toxines dans le tissu musculaire par unité de masse sèche et elles ont peu varié au cours de l'été. D'après les recommandations actuelles, les humains ne semblent pas courir de risque en consommant du tissu musculaire de perchaudes du lac Érié capturées durant les floraisons à grande échelle de cyanobactéries. Cependant, notre étude met en évidence la nécessité de mieux comprendre le transfert trophique des toxines des cyanobactéries dans les réseaux alimentaires aquatiques dans les divers écosystèmes et, particulièrement, de savoir si ces composés peuvent s'accumuler suffisamment pour affecter la santé humaine.

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Introduction

Noxious cyanobacterial blooms are global phenomena that occur in freshwater and estuarine habitats in response to increased nutrient loading, alterations in nutrient stoichiometry, food-web manipulations, and biotic invasions (Schindler 1974; Paerl 1997; Sarnelle et al. 2005). Common cyanobacterial genera associated with harmful freshwater algal blooms include *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Microcystis*, and *Oscillatoria* (Carmichael 1992; Chorus and Bartram 1999; Zurawell et al. 2005). A significant consequence of cyanobacterial blooms is that intracellular toxins, such as the hepatotoxin microcystin or the neurotoxin anatoxin-*a*, released from these events can adversely impact aquatic systems used for recreation and drinking water (Francis 1878; Carmichael et al. 2001; Wiegand and Pflugmacher 2005). Although the ecological function of cyanotoxins is unresolved (Rantala et al. 2004; Wilson et al. 2006*a*; Schatz et al. 2007), it is clear that microcystins, a class of cyclic heptapeptides that are produced by several bloom-forming, cyanobacterial genera (e.g., *Microcystis*), reduce population growth in some zooplankton when consumed (Wilson and Hay 2007), inhibit protein phosphatases (An and Carmichael 1994), and promote liver tumors in mammals (Carmichael 1994; Watanabe et al. 1996; Codd et al. 2005). Consequently, to protect human health, the World Health Organization (WHO) has recommended that the maximum threshold of total dissolved and particulate microcystins in drinking water be set at 1 μg toxin·L^{-1} (Chorus and Bartram 1999). Microcystin concentrations in drinking water supplies that exceed this level may negatively impact human health and should concern water quality managers.

Cyanotoxin exposure for humans includes ingestion or inhalation of water containing toxin-producing cyanobacteria or dissolved compounds recently released by senescent cells (Christoffersen 1996; Sivonen 1996; Chorus and Bartram 1999). Consumption of fish containing cyanobacterial toxins represents a poorly studied, but potentially important, mechanism for the ingestion of harmful cyanotoxins by humans. Fresh- and brackish-water fishes are known to accumulate cyanotoxins in tissues, including muscle, viscera, and liver (Kotak et al. 1996; Ibelings et al. 2005; Gkelis et al. 2006). A recent study showed that microcystin accumulation rates in muscle and liver tissues are directly proportional to ingestion rates for at least one species of fish (i.e., Nile tilapia (*Oreochromis niloticus*); Zhao et al. 2006). Concentrations of microcystin are routinely shown to be much higher in fish liver than in other tissues (Magalhães et al. 2001; Xie et al. 2005; Chen et al. 2007), which is not surprising given the hepatotoxic properties of microcystins. In addition, although rarely reported in equivalent units, microcystin concentrations in fish liver tend to be orders of magnitude lower than concentrations in seston when collected from the same habitat (Ibelings et al. 2005). Possible explanations for this difference in tissue toxin concentrations include detoxification by higher organisms, microbial degradation, low biomagnifications, complex food-web transfer dynamics, and incomplete extraction procedures of cyanobacterial toxins (Christoffersen 1996; Sivonen 1996; Ibelings et al. 2005). Fewer studies have quantified microcystin levels in fish muscle, i.e., the tissue more generally consumed by humans (but see Sipiä et al. 2001; Magalhães et al. 2003; Xie et al. 2005). For the Great Lakes, we are unaware of any published study reporting microcystin levels in the muscle tissue of a sportfish, although toxin data for muscle tissues do exist for round goby (*Neogobius melanostomus*) collected in Hamilton Harbor, Lake Ontario (Murphy et al. 2003). Further, Babcock-Jackson (2000) reported mean gut (46 ng toxin·(g wet weight)$^{-1}$) and liver (127 ng toxin·(g wet weight)$^{-1}$) microcystin concentrations for fishes collected from western Lake Erie in August 1998.

The study presented herein focuses on microcystin accumulation in muscle and liver tissues of commercially and recreationally important yellow perch (*Perca flavescens*) from western Lake Erie. In Lake Erie, yellow perch are omnivorous, with diet patterns varying seasonally and ontogenetically (Tyson and Knight 2001). Similar to most fish species, as yellow perch grow in size, they shift from consuming mostly small-bodied prey (e.g., small zooplankton) to consuming larger prey (e.g., large zooplankton, benthic invertebrates, and fish; Hayes and Taylor 1990; Wu and Culver 1992). In western Lake Erie, benthic invertebrates consistently constitute the primary diet category (by weight) for age-1 and older yellow perch, whereas zooplankton (June–July) and fish (August–September) appear to be seasonally important (Tyson and Knight 2001). Thus it is feasible that seasonal microcystin accumulation by yellow perch will mirror such seasonal shifts in diet.

Our study represents the first, large-scale effort to document concentrations of microcystin in a Great Lakes fish species widely consumed by humans. Specifically, we aimed to elucidate potential health risks for humans who consume yellow perch containing this toxic intracellular compound. To this end, microcystin concentrations were determined for lake seston and the liver and muscle tissues of yellow perch, collected monthly (June to August 2006) across 11 sites throughout the western basin of Lake Erie. This sampling period coincided with the development of a massive cyanobacterial bloom (chlorophyll *a* range = 1–47 μg chlorophyll·L^{-1}) that was dominated by the toxigenic cyanobacterium *Microcystis aeruginosa*. We related yellow perch tissue microcystin concentrations to toxin levels in their environment to identify possible mechanisms of microcystin uptake (i.e., through food-web interactions) with an ultimate goal of understanding the threat that these fish pose to human health.

Materials and methods

Seston and yellow perch were collected from 11 sites (maximum depth range = 5.5–10.4 m; Table 1) throughout the western basin of Lake Erie (Fig. 1) on 22 June 2006, 18 July 2006, and 22 August 2006. Coincident with these collections, temperature, dissolved oxygen concentration (DO), conductivity, and pH were measured throughout the water column with an YSI sonde (model 6600EDS-M, YSI Incorporated, Yellow Springs, Ohio). Depth-integrated seston samples were collected with a tube sampler (3 cm diameter opening) from the surface to near the lake bottom and were then stored in a sealed, 10 L plastic cubitainer on ice. Yellow perch were collected with 10 min bottom trawls at each site and stored in plastic bags on ice until returning to the lab, where they were stored in a freezer (–20 °C).

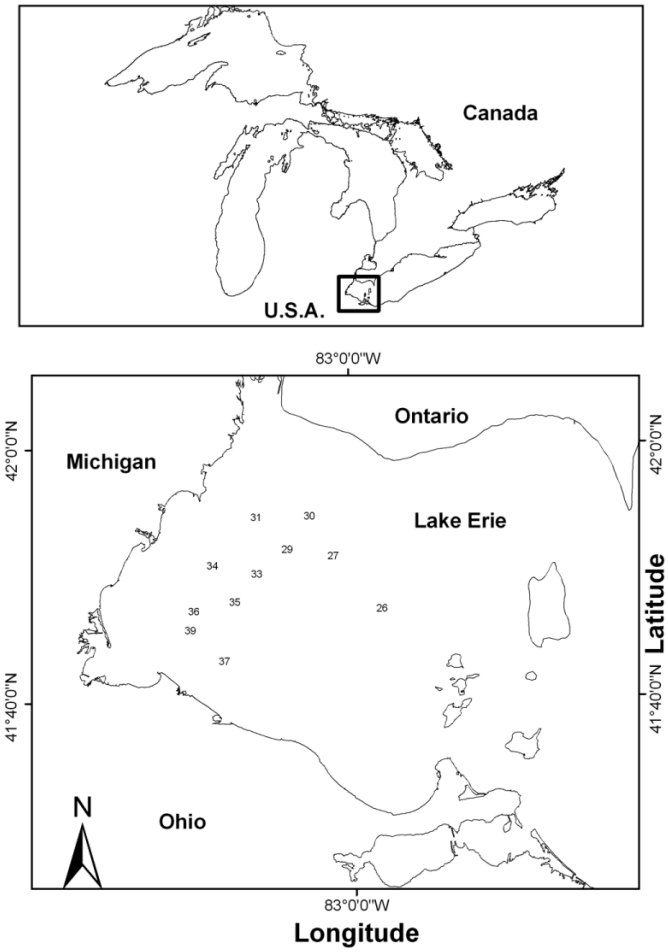
Table 1. Sample site locations and maximum depths (m) located in the western basin of Lake Erie.

Site	Latitude (N)	Longitude (W)	Depth (m)
26	41°46.1214′	82°58.2574′	10.4
27	41°50.2954′	83°03.3095′	10.1
29	41°50.8489′	83°08.1351′	9.8
30	41°53.4776′	83°05.7531′	9.5
31	41°53.4135′	83°11.3645′	8.8
33	41°48.9377′	83°11.3832′	8.2
34	41°49.6421′	83°16.0615′	7.9
35	41°46.7397′	83°13.7556′	8.2
36	41°46.0468′	83°18.0830′	6.7
37	41°42.0962′	83°14.9052′	7.3
39	41°44.5367′	83°18.4618′	5.5

In the laboratory, well-mixed 1000 mL seston samples were collected on filters (Whatman GF/F) for seston dry weight, chlorophyll, and microcystin content. Seston dry weight was determined after weighing tared and dried filters with collected seston (model AT250, Mettler-Toledo Inc., Columbus, Ohio). Chlorophyll was measured after a 24 h dark extraction at 4 °C in 95% ethanol via fluorometry (model 10AU, Turner Designs, Inc., Sunnyvale, Calif.). Intracellular microcystins in the seston were extracted twice (60 min·extraction^{-1}) from each seston sample using 15 mL 75% aqueous methanol. Concentrated acetic acid (60 μL) was added to the second extraction to lower solvent pH and to enhance extraction of microcystin. The extracts from each sample were collected via centrifugation, pooled, filtered through a glass-fiber prefilter (Nalgene, Nalge Nunc International Corp., Rochester, N.Y.), dried using vacuum evaporation, redissolved in water using sonication, and analyzed using enzyme-linked immunsorbant assay (ELISA; model EP-022, EnviroLogix Inc., Portland, Maine; An and Carmichael 1994). Using this protocol, seston microcystin concentrations can be quantified as microcystins per volume (ng toxin·L^{-1}), per dry weight (ng toxin·(g dry weight)$^{-1}$), and per unit chlorophyll (ng toxin·(μg chlorophyll)$^{-1}$). The volume metric provides a way to compare toxin abundances across water samples relative to the same volume of water filtered. The dry weight metric allows for cross-sample comparisons related to the amount of organic and inorganic material collected on the filter. The chlorophyll metric removes variance associated with inorganic, non-autotrophic material on filters and provides a technique to standardize toxin concentrations relative to the abundance of phytoplankton in the water sample. Because algal communities are comprised of mixtures of phytoplankton species that are able (i.e., cyanobacteria) or unable (e.g., green algae, diatoms, dinoflagellates) to produce microcystins, toxin concentrations relative to chlorophyll abundance are conservative and may be higher if only toxigenic cyanobacterial chlorophyll concentrations were known.

In the laboratory, whole fish were thawed and measured (total length and wet weight), and a sample of dorsal muscle tissue and the entire liver were collected, dried, and weighed. Microcystins were extracted twice from each tissue sample using 75% methanol. The first extraction (20 mL solvent) lasted for 2 h and included mechanical homogenization. The second extraction (30 mL solvent) included an addition of acetic acid (60 μL) and lasted for 24 h while the samples were stored at 4 °C in a dark environment. Extracts were collected via centrifugation, pooled, filtered through a glass-fiber prefilter (Nalge Nunc International Corp.) to remove particles, dried using vacuum evaporation, redissolved in water using sonication, and analyzed using ELISA.

Fig. 1. The Laurentian Great Lakes (upper map) and sampling sites in western basin Lake Erie (lower map). Sampling dates: 22 June 2006, 18 July 2006, and 22 August 2006. Latitude, longitude, and maximum depths for the sites can be found in Table 1.



The detection limit for microcystins present in extracts was 0.16 ng·mL^{-1}, and no microcystins were detected in filter and tissue blank samples. Microcystin recoveries of spiked samples were 92% for seston filter samples and 64% for liver and muscle tissue samples. Our recovery rates are consistent with recoveries from several past studies that extracted microcystins in lake water (91%–106% (Cong et al. 2006), 92%–111% (Wang et al. 2007)) and fish tissues (68% (Ernst et al. 2005), 68% (Ibelings et al. 2005), 63%–71% (Chen et al. 2007)). All reported toxin data, uncorrected for recovery rates, are for freely available toxins and do not include covalently bound microcystins. The concentrations are expressed as microcystin-LR equivalents based on the ELISA protocol (EnviroLogix Inc.); however, it is important to note that quantification is not consistent among microcystin variants. The ELISA kit used in this study (model EP-022, EnviroLogix Inc.) was developed to quan-

Table 2. Mean chemical and physical characteristics of surface and bottom waters across 11 sites (see Fig. 1 and Table 1) in the western basin of Lake Erie sampled in the summer of 2006.

Date	Sample location	Statistic	Temperature (°C)	Conductivity ($\mu S \cdot cm^{-1}$)	DO ($mg \cdot L^{-1}$)	pH
22 June	Surface	Mean	22.45	257.09	8.49	7.99
		SE	0.19	7.44	0.03	0.02
		Minimum	21.35	219.00	8.32	7.89
		Maximum	23.66	301.00	8.69	8.12
22 June	Bottom	Mean	21.80	251.00	8.29	7.93
		SE	0.08	6.92	0.03	0.03
		Minimum	21.28	219.00	8.18	7.81
		Maximum	22.13	296.00	8.57	8.11
18 July	Surface	Mean	25.58	259.27	7.82	8.39
		SE	0.20	10.61	0.10	0.06
		Minimum	24.71	220.00	7.10	8.14
		Maximum	26.55	330.00	8.22	8.76
18 July	Bottom	Mean	25.34	276.27	8.76	8.26
		SE	0.11	17.15	0.17	0.05
		Minimum	24.84	224.00	8.31	7.80
		Maximum	25.85	360.00	9.93	8.43
22 August	Surface	Mean	25.34	236.00	8.65	8.46
		SE	0.48	25.60	0.04	0.06
		Minimum	23.63	3.00	8.33	8.32
		Maximum	27.95	330.00	8.86	9.03
22 August	Bottom	Mean	23.96	255.09	9.30	8.31
		SE	0.07	11.64	0.16	0.04
		Minimum	23.57	217.00	8.78	8.09
		Maximum	24.35	317.00	10.31	8.56

Note: Sample size for all measurements is 11. DO, dissolved oxygen; SE, standard error.

tify microcystin-LR and thus was most sensitive for this variant. Microcystin-LR is the most toxic variant (Carmichael 1992) and the most common form found in the western basin of Lake Erie (Dyble et al. 2008). Thus, although our measurments may be slightly conservative, our results should be indicative of the relative human health threat that microcystins pose to humans consuming yellow perch tissue. Regarding toxicity due to protein-phosphatase inhibition (the primary mode of toxicity for microcystins towards mammals; An and Carmichael 1994), ELISA does not measure phosphatase inhibition directly, but does provide correlative data associated with real cyanobacterial toxicity as toxin abundance and phosphatase inhibition are highly correlated (Fastner et al. 2002).

Gut contents were analyzed for a subset of the yellow perch collected in June 2006 (n = 17), July 2006 (n = 20), and August 2006 (n = 16) that was also analyzed for tissue microcystins. Stomachs were removed from thawed fish and preserved in 95% ethanol. Gut contents were determined via microscopy and grouped into the following categories: zooplankton, benthic invertebrates, fish, and unidentified. Gut samples were then dried at 70 °C and weighed to determine dry mass.

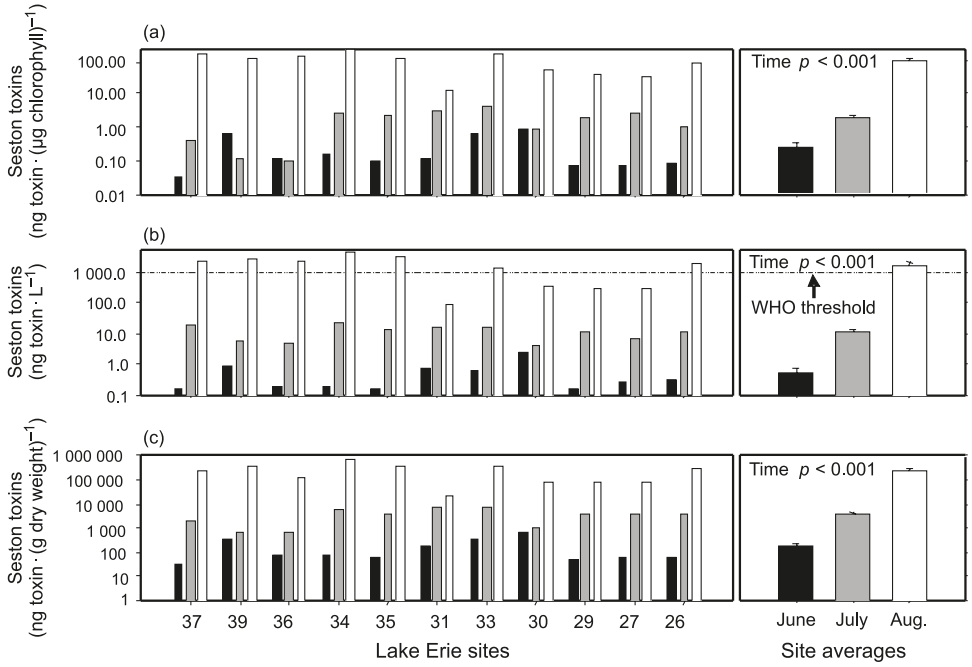
Differences in seston and tissue toxin levels were compared among sites and collection months via one-way analysis of variance (ANOVA). Unequal sample sizes precluded the use of two-way ANOVA for toxin abundance in yellow perch muscle and liver tissues across sites and time. Pearson's product moment correlations were used to evaluate associations among algal abundance, perch diet composition, and algal and tissue microcystin concentrations. Data were log- or arcsin-transformed as needed to conform to assumptions of parametric statistics. Nonparametric Kruskal–Wallis analyses were conducted when simple transformations failed to parameterize data. All analyses were performed with Systat 11 (Systat Software, Inc. 2004).

Results

Chemical and physical measures suggested that the western basin of Lake Erie was moderately well mixed from June to August 2006 (Table 2). Mean temperatures across all 11 sites ranged from 22.5 to 25.6 °C at the surface and from 21.8 to 25.3 °C at the lake bottom (Table 2). Conductivity and pH varied little over time (ranges 236–277 and 7.93–8.46 $\mu S \cdot cm^{-1}$, respectively), and the surface and bottom waters throughout the western basin of Lake Erie were well oxygenated (range 7.82–9.30 $mg \cdot L^{-1}$; Table 2).

Algal abundance (measured as chlorophyll *a*) ranged from 1 to 47 μg chlorophyll·L^{-1} among 11 sites throughout the western basin of Lake Erie during the summer of 2006. Chlorophyll concentration increased throughout the summer (June range 1.03–5.93 μg chlorophyll·L^{-1}; July range 2.91–46.95 μg chlorophyll·L^{-1}; August range 6.01–28.85 μg chlorophyll·L^{-1}) and varied significantly over time (ANOVA, p = 0.016). Microscopic observations confirmed that the dominant phytoplankter of the summer algal community was *M. aeruginosa*. Seston microcystin concentra-

Fig. 2. Mean seston microcystin concentrations measured as microcystin-LR equivalents for integrated water samples collected from the surface to near the lake bottom at 11 sampling stations during the summer of 2006: (*a*) ng toxin·(μg chlorophyll)$^{-1}$; (*b*) ng toxin·L^{-1}; (*c*) ng toxin·(g dry weight)$^{-1}$. Left panels contain monthly site concentrations (n = 1 per site). Right panels contain monthly averages (± standard error, SE) across sites (n = 11 per date). Sampling dates: solid bars, 22 June 2006; shaded bars, 18 July 2006; open bars, 22 August 2006. Note the $\log_{10}$ scale of the y axes.



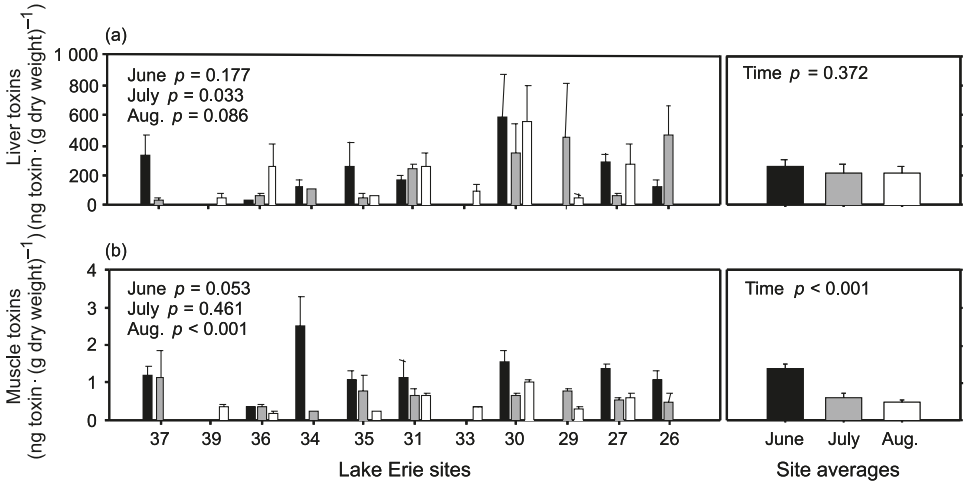
tions (measured as ng toxin·(μg chlorophyll)$^{-1}$, ng toxin·L^{-1}, and ng toxin·(g dry weight)$^{-1}$) showed similar patterns to algal abundance (ANOVA, time $p < 0.001$; Figs. 2*a*, 2*b*, 2*c*), and consequently both variables were positively correlated ($r \geq 0.440$, $p \leq 0.010$, $n = 33$). For all sampling sites and dates, microcystin concentrations in the seston were in the range of 0.04–189 ng toxin·(μg chlorophyll)$^{-1}$, 0.16–4284 ng toxin·L^{-1}, and 34 – 767 760 ng toxin·(g dry weight)$^{-1}$. In August, concentrations of microcystins exceeded the level (1 μg toxin·L^{-1}; Chorus and Bartram 1999) set by the WHO for safe drinking water standards (mean, 1.68 μg toxin·L^{-1}; range, 0.08–4.28 μg toxin·L^{-1}), but Lake Erie microcystin concentrations were below this recommended limit in June and July (range, 0.0002–0.02 μg toxin·L^{-1}; Fig. 2*b*).

Although mean (± standard error, SE) length and weight of analyzed yellow perch increased during the year (June, 166 ± 6 mm, 63 ± 5 g, n = 22; July, 174 ± 5 mm, 70 ± 5 g, n = 24; August, 184 ± 5 mm, 81 ± 6 g, n = 22), these seasonal trends were not statistically significant (ANOVA, $p \geq 0.069$). Further, total gut contents (grams dry mass of gut contents per gram dry mass of fish) did not vary over time (ANOVA, $p = 0.076$) for yellow perch collected in June (mean ± 1 SE = 0.127 ± 0.024 g), July (0.318 ± 0.108 g), and August (0.126 ± 0.048 g). Of the prey that could be identified, relative abundances (by dry weight) of the three prey types varied over time but were not correlated to yellow perch muscle or liver toxin concentrations ($p \geq 0.054$). Benthic invertebrates were relatively more abundant in yellow perch guts during June (mean ± 1 SE = 76% ± 11%, n = 16) than in July (19% ± 9%, n = 19) and August (44% ± 14%, n = 12; ANOVA, $p = 0.001$). The abundance of fish in yellow perch guts increased over time (mean ± 1 SE; June, 18% ± 10%, n = 16; July, 46% ± 11%, n = 19; August, 48% ± 14%, n = 12; Kruskal–Wallis, $p = 0.045$). Although no identifiable zooplankton were observed in yellow perch guts collected during August (mean ± 1 SE = 0% ± 0%, n = 12), zooplankton were significantly less abundant in yellow perch collected in June (mean ± 1 SE = 7% ± 6%, n = 16) than in July (mean ± 1 SE = 36% ± 10%, n = 19; ANOVA, $p = 0.010$).

Liver microcystin concentrations in individual yellow perch ranged from 17 to 1182 ng toxin·(g dry weight)$^{-1}$ and varied significantly across sites in July (ANOVA, $p = 0.033$; Fig. 3*a*), but not in June or August (ANOVA, $p \geq 0.086$; Fig. 3*a*). In general, fish collected closer to Maumee Bay appeared to have lower liver microcystin concentrations than those from sites in the central area of the western basin of Lake Erie (Figs. 1 and 3). Moreover, we found no differences in yellow perch liver toxin concentrations over time (ANOVA, $p = 0.372$; Fig. 3*a*). Liver microcystin concentrations were unrelated to fish length or wet weight (all fish data: length, $r = 0.215$, $p = 0.078$, n = 68; weight, $r = 0.203$, $p = 0.097$, n = 68). Similarly, mean monthly yellow perch liver toxin concentrations averaged within each site were not significantly correlated with seston microcystin concentrations, measured as microcystins per volume, dry weight, or chlorophyll content, throughout the summer of 2006 (site- and time-averaged data: $r \leq -0.107$, $p \geq 0.609$, n = 25).

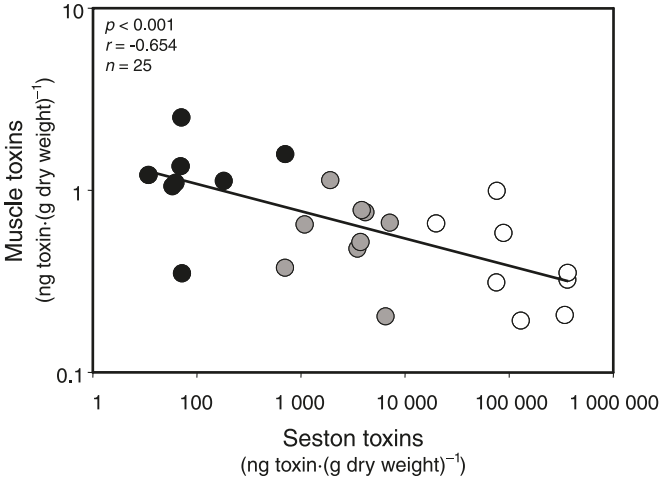
Microcystins concentrations in individual yellow perch muscle tissues ranged from 0.12 to 4.02 ng toxin·(g dry weight)$^{-1}$ and significantly varied across sites in August (ANOVA, $p < 0.001$; Fig. 3*b*), but not in June or July

Fig. 3. Mean (± standard error, SE) (*a*) liver and (*b*) muscle microcystin concentrations measured as microcystin-LR equivalents (ng toxin·(g dry weight)$^{-1}$) for yellow perch collected at 11 sampling stations during the summer of 2006. Left panels contain monthly site averages (n = 0–3 per site). Right panels contain monthly averages across sites (n = 22 (for June and August) or 24 (for July) per date). Sampling dates: solid bars, 22 June 2006; shaded bars, 18 July 2006; open bars, 22 August 2006.



(ANOVA, $p \geq 0.053$; Fig. 3*b*). In general, muscle microcystin concentrations of yellow perch were relatively low, and muscle toxin concentrations, measured as ng toxin·(g dry weight)$^{-1}$, represented only 0.8% (SE = 0.1%, n = 68) of the toxin found in liver tissue. However, we found large differences in yellow perch muscle toxin concentrations over time across all sites (ANOVA, $p < 0.001$; Fig. 3*b*). In contrast to the pattern observed for seston toxin concentration, which increased over time, muscle microcystin concentrations decreased over time (Figs. 3 and 4). Individual yellow perch muscle toxin concentrations were negatively related to total lengths, wet weights, and liver toxin concentrations (all fish data: length, r = –0.282, p = 0.020, n = 68; weight, r = –0.302, p = 0.012, n = 68; liver concentrations, r = 0.361, p = 0.003, n = 68). Interestingly, across sites from June to August 2006, mean yellow perch muscle microcystin concentrations were negatively related to seston microcystin concentrations regardless of whether seston toxins were measured relative to chlorophyll abundance, weight of material extracted, or volume of water filtered (site- and time-averaged data: $r \geq 0.609$, $p \leq 0.001$, n = 25; Fig. 4).

Fig. 4. Correlation between seston microcystin concentration (ng toxin·(g dry weight)$^{-1}$) and mean yellow perch (*Perca flavescens*) muscle microcystin concentration (ng toxin·(g dry weight)$^{-1}$) for all 11 sampling stations and three sampling dates (solid circles, 22 June 2006; shaded circles, 18 July 2006; open circles: 22 August 2006). Note that all data are represented as microcystin-LR equivalents. Each data point represents an n = 1 for seston toxins and an n = 1–3 for muscle toxins. Note the $\log_{10}$ scale of both axes.



Discussion

During July and August of 2006, the western basin of Lake Erie experienced a high biomass of phytoplankton (maximum site-specific algal abundance of 47 µg chlorophyll·L^{-1}) containing the toxigenic cyanobacterium *Microcystis aeruginosa*. Seston microcystin levels increased throughout the summer and exceeded the threshold established by the World Health Organization for safe drinking water (1 µg microcystin·L^{-1}; Chorus and Bartram 1999) at 7 of the 11 sites sampled in August 2006. Although orders of magnitude lower than seston toxin levels (maximum seston concentration of 768 µg toxin·(g dry weight)$^{-1}$), yellow perch liver samples contained relatively high amounts of microcystin (maximum liver concentration of 1.2 µg toxin·(g dry weight)$^{-1}$). In contrast, yellow perch muscle toxin concentrations remained relatively low throughout the summer (maximum muscle concentration of 0.004 µg toxin·(g dry weight)$^{-1}$). Further, although there was no clear relationship between yellow perch liver toxin levels and seston toxin concentrations over time, yellow perch muscle microcystin levels were negatively related to seston toxin concentrations.

Given the significance of protecting freshwater drinking and recreational water supplies, it is important to explore, across aquatic systems, toxic intracellular cyanobacterial compounds, such as microcystin (Chorus and Bartram 1999; Zurawell et al. 2005). Surprisingly, few data exist for measured seston toxin levels in the Laurentian Great Lakes (Babcock-Jackson 2000; Murphy et al. 2003; Dyble et al. 2008) and its watershed (Watzin et al. 2006; Lehman 2007; Knoll et al. 2008). Instead, research has been directed at

quantifying microbial diversity and the potential for cyanobacterial toxin production (e.g., *mcy* gene analysis) in waterbodies within the region (Wilson et al. 2005; Ouellette et al. 2006; Rinta-Kanto and Wilhelm 2006). Available toxin data for the Great Lakes show large spatial (western basin of Lake Erie: range = 0.1–15.4 μg toxin·L^{-1}; Rinta-Kanto et al. 2005) and temporal (western basin of Lake Erie: range = 0.002–0.085 μg toxin·L^{-1} (Babcock-Jackson 2000); Hamilton Harbor, Lake Ontario: range = 0–239 μg toxin·L^{-1}; eastern basin of Lake Erie: range = 0–0.4 μg toxin·L^{-1} (Murphy et al. 2003)) variation in summer seston microcystin concentrations, which is consistent with our observations in western Lake Erie (range = 0.0002–4.3 μg toxin·L^{-1}). Further, we found expected patterns in seston toxins within and across sites, with higher seston toxin concentrations later in the summer and at sites near mesotrophic Maumee Bay, respectively.

Field sampling techniques can significantly influence toxin estimates (Tillmanns et al. 2007), and our seston toxin data should be viewed as conservative, as the samples were integrated water samples (i.e., surface to near bottom). In contrast, other studies have measured seston toxin levels collected in the top 1 m of the water column (Murphy et al. 2003; Rinta-Kanto et al. 2005). If we had sampled the surface water, our seston toxin data likely would have been much higher on calm days when thick scums of buoyant cyanobacteria coated the surface of Lake Erie. Although surface samples are useful for estimating toxin concentrations that pose a threat to livestock and human health through tainted drinking water (Chorus and Bartram 1999), integrated samples were collected to estimate toxin concentrations throughout the water column (in part because yellow perch are rarely found near the lake surface). Moreover, few studies provide toxin data in units (e.g., toxin per biomass) that can be easily compared across trophic levels (but see Murphy et al. (2003) and Ibelings et al. (2005)). Instead, most studies present seston toxin data as μg toxin·L^{-1}, which is the unit typically considered by the World Health Organization (Chorus and Bartram 1999). Toxin per biomass data are easily collected by drying samples prior to weighing and are useful for direct comparisons among trophic levels both within and across studies. Given that water content of phytoplankton, zooplankton, fishes, bivalves, and other aquatic biota will vary, we suggest that future similar studies provide toxin data on a per dry biomass basis (ng toxin·(g dry weight)$^{-1}$).

Besides posing serious threats to global freshwater supplies, cyanobacterial toxins contained within fish tissues may present an alternative route of exposure to humans. However, this mechanism is poorly understood given that relatively few studies have determined the concentration of cyanobacterial toxins in the muscle tissue of fishes typically consumed by humans (Magalhães et al. 2001; Xie et al. 2004; Wood et al. 2006). Instead, many studies have documented toxin levels in those tissues where cyanobacterial toxins aggregate, e.g., liver tissue for the hepatotoxic microcystin (Kotak et al. 1996; Zimba et al. 2001; Ibelings et al. 2005). Moreover, in contrast to some past studies that analyzed the tissues of one or a few individuals for multiple fish species (Murphy et al. 2003; Gkelis et al. 2006), our focused study on the muscle and liver tissues from 68 yellow perch collected in the western basin of Lake Erie allowed us to draw more significant conclusions for this commercially important fish.

For the Great Lakes region, few data exist for microcystin levels in fish muscle or liver tissues (Babcock-Jackson 2000; Murphy et al. 2003). To our knowledge, the only other available toxin data for Lake Erie yellow perch are presented by Babcock-Jackson (2000), who showed liver microcystin concentrations of young-of-the-year (range of 94–230 ng toxin·(g wet weight)$^{-1}$, n = 10–15) and adult (127 ng toxin·(g wet weight)$^{-1}$, n = 7) yellow perch collected during 1998. Murphy et al. (2003) reported low toxin concentrations in the muscle (1 ng toxin·(g wet weight)$^{-1}$) and liver (1 μg toxin·(g wet weight)$^{-1}$) tissues of round gobies collected in Hamilton Harbor, Lake Ontario, during the summer of 2001. If the dry weight of fish tissue is 20% of the wet weight, data from these two studies for yellow perch and round gobies correspond to toxin levels of 5 ng toxin·(g dry weight)$^{-1}$ for muscle tissue and 5–1150 ng toxin·(g dry weight)$^{-1}$ (range) for liver tissue, which is consistent with our findings of toxin concentrations in yellow perch muscle (range of 0.12–4.02 ng toxin·(g dry weight)$^{-1}$) and liver tissue (range of 17–1182 ng toxin·(g dry weight)$^{-1}$).

Intriguingly, we found a significant, negative correlation between average seston and yellow perch muscle microcystin concentrations ($p < 0.001$), i.e., when seston toxins were lowest, muscle toxins were highest. Few studies have simultaneously measured microcystin abundances in the seston and fish muscle tissue collected from a lake over time. Of those, Magalhães et al. (2001) provided a similar negative relationship between seston and muscle microcystin concentrations for at least one year of their study. The other studies (Magalhães et al. 2003; Chen et al. 2006) showed a positive relationship between these variables. We consider finding these types of relationships between seston and fish muscle tissue microcystin concentrations within a lake to be remarkable given that the water mass where the fishes were found and that where they were exposed to microcystins are likely different because of the movement of the fishes, zooplankton prey, or the water through circulation within a lake. There is no single obvious explanation for this pattern as it is assumed that perch acquire cyanobacterial toxins through their diet, including zooplankton that prey on phytoplankton, such as toxigenic cyanobacteria. One potential explanation is that early in the summer when cyanobacteria have not become dominant, zooplankton populations are growing well while grazing on mixed diets containing various types of bacteria, cyanobacteria, and phytoplankton. When large-bodied zooplankton are abundant, yellow perch may preferentially consume such prey (which may have accumulated microcystins through their diet; Thostrup and Christoffersen 1999; Smith and Haney 2006). However, because of lower food quality (i.e., increasing dominance by cyanobacteria) and an increase in fish predation, large zooplankton are typically progressively replaced by smaller zooplankton (Brooks and Dodson 1965) during the seasonal succession of the plankton community towards cyanobacterial dominance, making zooplankton less important for yellow perch sustenance and thus reducing yellow perch exposure to microcystins. Yellow perch diet data support this idea, given that we found variation in the relative abun-

dances of zooplankton in the perch diets for fishes collected in June, July, and August (ANOVA, $p = 0.010$) and, in fact, found no identifiable zooplankton in the guts of yellow perch collected in August. Further, this seasonal pattern is consistent with that of previous studies (Tyson and Knight 2001).

Another explanation for the negative relationship between seston and perch muscle toxins could be related to the sizes of perch analyzed. Although fish size (measured as total length and wet weight) did not show significant trends, we did analyze slightly (insignificantly) smaller fish in June compared with in July and August. As fish grow, they tend to shift their diets from primarily small-bodied prey (e.g., zooplankton) to primarily large-bodied prey (e.g., benthic macroinvertebrates and fish). However, a multiple regression analysis describing mean muscle toxin levels as a function of mean seston toxins and perch wet weight estimates for each site per month ($n = 25$) reconfirmed that yellow perch size does not account for a significant amount of variation in muscle microcystin concentrations (dependent variable = log muscle toxins; independent variables = log seston toxins, $p < 0.001$, log wet weight, $p = 0.331$). Finally, because yellow perch feed on benthic organisms and many cyanobacteria overwinter on the sediments (Reynolds et al. 1981), it is possible that the counterintuitive pattern between seston and muscle toxins is related to toxin accumulation or biomagnification through the benthic food web. However, this mechanism for trophic transfer of cyanobacterial toxins is poorly understood. Thus, we support future observational and experimental studies intended to more fully understand the trophic transfer of cyanobacterial toxins.

Another motivation of this study, besides determining if microcystins are present in yellow perch tissues, was to determine if this class of hepatotoxins found within the edible muscle tissue of yellow perch could be harmful to human consumers. To determine this risk, we calculated a conservative microcystin total daily intake (TDI) threshold, which is the acceptable amount of a toxic compound that an individual can consume per day for a lifetime, based on established WHO (0.04 μg toxin·(kg of body weight)$^{-1}$·day^{-1}; Chorus and Bartram 1999) and proposed US Environmental Protection Agency (0.003 μg toxin·(kg of body weight)$^{-1}$·day^{-1}; US EPA 2006) guidelines. For a human weighing 80 kg and consuming 25.4 g dry weight (wet to dry weight conversion of 5:1) of fish tissue per day (Keill and Kissinger 1999, based on a consumption estimate from 90% of a Native American population that relies on a fish diet), the maximum concentration of muscle toxins we observed in yellow perch (maximum of 4.02 ng toxin·(g dry weight)$^{-1}$) was well below thresholds estimated using established WHO (117 ng toxin·(g dry weight)$^{-1}$) and proposed EPA (9.5 ng toxin·(g dry weight)$^{-1}$) guidelines.

Our findings show that even during an intense, large-scale bloom of microcystin-producing *Microcystis aeruginosa* in which seston toxin levels exceeded the threshold established by the WHO for microcystin in drinking water (Chorus and Bartram 1999), muscle tissue from the sportfish yellow perch contained concentrations of microcystin well below conservative thresholds established for human consumption. However, it is important to note that humans consuming fish tissue can potentially be exposed to both free and bound forms of toxins. Within organisms, microcystin may exist primarily in bound form owing to biotransformation. In fact, the bound form was found to represent 76% of the microcystin observed in salmon (*Salmo salar*) liver (Williams et al. 1997). Thus, total microcystin concentrations in yellow perch tissues may be higher than reported herein, given our inability to quantify covalently bound microcystins because of the limitations of our extraction technique. However, it is well recognized in toxicology that bound or conjugated forms of compounds are not readily absorbed, as these forms are created through biotransformation for detoxification and elimination of toxic compounds. Despite this general understanding, no studies adequately elucidate the actual potential for absorption of such forms of microcystin by humans. The toxicity of some metabolites of microcystin have been examined in mice using intravenous injection, and the glutathione and cysteine conjugates were found to be about 10 times less toxic than the parent microcystin-LR (Kondo et al. 1992). Thus, evaluation of the potential human exposure to cyanobacterial secondary metabolites would be best served by monitoring the extractable parent microcystin (because of limited bioaccumulation potential and toxicity of conjugates).

Moreover, understanding whether the low microcystin levels that we observed in the edible muscle tissues of Lake Erie yellow perch during a cyanobacterial bloom are typical will depend on many abiotic and biotic parameters. For example, populations of cyanobacteria exhibit high genetic and physiological diversity (Janse et al. 2004; Via-Ordorika et al. 2004; Wilson et al. 2006*b*), which could influence toxin dynamics. Moreover, individual strains of cyanobacteria have been shown to significantly alter their growth rates and toxin production in response to changes in temperature, light level, and availability of nutrients (Lee et al. 2000; Oh et al. 2000; Wiedner et al. 2003). In addition, some zooplankton may adapt to graze on toxic cyanobacteria (Hairston et al. 2001; Sarnelle and Wilson 2005), whereas some benthic grazers can actively avoid consuming cyanobacteria (Vanderploeg et al. 2001). Such ecological interactions may strongly influence the flow of cyanotoxins through food webs, although this has yet to be determined empirically. Thus, it is unclear if the trophic transfer of cyanobacterial toxins will always culminate in low fish muscle toxin concentrations. Additionally, although our results suggest that humans are not in danger of cyanotoxin poisoning when consuming muscle tissues from yellow perch harvested during cyanobacterial blooms, toxin concentrations in yellow perch livers exceeded conservative consumption thresholds for microcystin. Piscivorous fishes that feed on yellow perch could accumulate potentially dangerous levels of microcystins and could pose a health risk to humans who consume these fishes, but this possibility has not been well studied. Thus, there may be a health threat to humans consuming whole fish or fish livers during cyanobacterial blooms, and this threat should be communicated to public health officials. Consequently, we encourage future studies to quantify the type and abundance of toxic intracellular cyanobacterial compounds in important commercially and recreationally harvested fish species.

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