1   **Short Communication**
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3   **Low human health risks of algal toxins from consuming fish caught in Lake St. Clair**
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27 sampling. R.S.S., S.M.R., and X.O. analyzed the data. R.S.S. wrote the paper. All authors read,
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29
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34 **This file includes:** Main Text, Figure 2 to 2, Table 1 to 1
35

Abstract

Consuming fish exposed to harmful algal blooms (HABs) may be a major route of microcystin toxin exposure to humans. However, it remains unknown whether fish can accumulate and retain microcystins during pre- and post-HAB periods when fishing is active, raising uncertainties about fish catch in waterbodies with recurring seasonal HABs. We conducted a field study on Largemouth Bass (*Micropterus salmoides*), Northern Pike (*Esox lucius*), Smallmouth Bass (*Micropterus dolomieu*), Rock Bass (*Ambloplites rupestris*), Walleye (*Sander vitreus*), White Bass (*Morone chrysops*), and Yellow Perch (*Perca flavescens*) to assess the true human health risks to microcystin toxicity via fish consumption. We collected 124 total fish in 2016 and 2018 from Lake St. Clair, a large freshwater ecosystem in the North American Great Lakes that is actively fished and regularly experiences HABs. Muscles were analyzed using the MMPB Lemieux Oxidation method for total microcystins, which was used to perform a human health risk assessment for comparison against fish consumption advisory benchmarks available for Lake St. Clair. From this collection 35 fish livers were additionally extracted to confirm the presence of microcystins. Although microcystins were detected in all livers at widely varying concentrations (1-1,500 ng g^{-1} wet weight (ww)), muscle levels were consistently low (0-15 ng g^{-1} ww). Following the fish consumption advisories for microcystins in Lake St. Clair, our results suggest that fish filets may be safely consumed during pre- and post-HAB periods.

Main Text

Introduction

The global expansion of harmful algal blooms (HABs) is a pressing challenge for freshwater ecosystems. Their spread is due, in part, to climatic changes and an expansion in human activities, which render waterbodies increasingly vulnerable to biodiversity loss and threatened ecosystem services (Hellweger et al., 2022; Ho et al., 2019; Huisman et al., 2018; Reid et al., 2019; Smith et al., 2019). Frequently HABs produce microcystins, potent hepatotoxins that primarily inhibit protein phosphatases and can cause liver necrosis, tumor formation, and organ failure in humans and wildlife at sufficient levels (Chorus and Welker, 2021; Shahmohamadloo et al., 2022a, 2021).

The World Health Organization's (WHO) guidelines (WHO, 2020) are implemented in many countries to protect humans from microcystin toxicity in drinking water (12 µg L^{-1} short-term, 1 µg L^{-1} lifetime); however, longstanding concerns remain that fish consumption may be a viable route of algal toxin exposure (FAO, 2020; IPCC, 2022). These concerns stem from the possibility that microcystins may be one of several factors involved in fish kills during HAB events (Ibelings et al., 2005) or that microcystins can accumulate up the food chain (Gene et al., 2019; Shahmohamadloo et al., 2020a, 2020b). Moreover, previous studies that measured microcystins in fish tissues at levels exceeding the WHO's tolerable daily intake (TDI) value (0.04 µg kg^{-1} body weight per day) used enzyme linked immunosorbent assay (ELISA) (Dyble et al., 2011; Poste et al., 2011; Wituszynski et al., 2017). ELISA is prone to false positives in fish tissues because it only measures free fractions of microcystins, whereas bound fractions can make up to 90% of the total amount of toxins present in a sample (Birbeck et al., 2019; Flores et al., 2018; Schmidt et al., 2014). This has raised skepticism about the accuracy of such data in assessing risks to humans via consumption of fish. Intertwined in the debate about methodology are projections stating that climate change will increase the mean number of days of a HAB event from 16-23 days in 2050 to 18-39 days in 2090 (Chapra et al., 2017). Lake St. Clair, a large waterbody that is important for recreational fishers (OMNRF, 2016), regularly experiences microcystin-HABs (Davis et al., 2014) and may expose resident fish populations in the future to blooms with extended residency, severity, and toxicity.

We collected 124 recreationally important sport fish, namely Largemouth Bass, Northern Pike,
Smallmouth Bass, Rock Bass, Walleye, White Bass, and Yellow Perch from Lake St. Clair in 2016
and 2018 throughout pre- and post-HAB periods when recreational fishing is more active (i.e., the
duration of a HAB is a small fraction of the total fishing season). We measured microcystins in fish
muscles using the MMPB Lemieux Oxidation method (Anaraki et al., 2020). In addition, we
dissected 35 livers from these samples to confirm the presence of microcystins in fish tissues.
Results from muscles were then compared against fish consumption advisory benchmarks for
microcystins available to Lake St. Clair and performed a human health risk assessment to
determine whether fish tissues exceeded the WHO's TDI. Here, we hypothesize that microcystin
levels in fish muscles will vary among fish species but, overall, will pose low human health risks.
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98 **Methods**
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100 **Study location**
101
Eight locations in Lake St. Clair (42° 25' 20" N, 82° 39' 36" W) were sampled in 2016 and 2018
during pre- (June to July) and post- (October to November) HAB periods. *Microcystis*, *Planktothrix*,
and *Dolichospermum* are typically present in Lake St. Clair, with toxigenic strains of *Microcystis*
predominating (Davis et al., 2014). The Ontario Ministry of the Environment, Conservation and
Parks (MECP) additionally collected water samples during pre- and post-HAB periods from the
southern shoreline of Lake St Clair near Belle River (42° 18' 11" N, 82° 42' 46.7" W) and Puce
River (42° 18' 4" N, 82° 42' 19" W) to measure total phosphorus, chlorophyll *a*, and total
microcystins. Total phosphorus (28.7 and 13.4 µg L^{-1}), chlorophyll *a* (9.7 and 4.6 µg L^{-1}) and total
microcystins (0.2 and 1.0 µg L^{-1}) were highest in 2018. Microcystin concentrations in the present
study are similar to previous measurements from Lake St. Clair (Davis et al., 2014; Taranu et al.,
2019).
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114 **Fish sampling and tissue analysis**
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A total of 124 samples from Largemouth Bass, Northern Pike, Smallmouth Bass, Rock Bass,
Walleye, White Bass, and Yellow Perch (Table 1) were collected in 2016 and 2018 for microcystin
screening as a part of the MECP's Fish Contaminant Monitoring Program (Etobicoke, Ontario,
Canada) in partnership with the Ontario Ministry of Northern Development, Mining, Natural
Resources and Forestry (Wheatley, Ontario, Canada). Fish length and weight were recorded for all
samples, after which the dorso-lateral muscle tissues (i.e., skinless, boneless fillets) were
dissected for microcystin analysis. In 2018, a subset of 35 samples of Walleye, White Bass, and
Yellow Perch were additionally dissected for livers to confirm their exposure to microcystins. All
muscles and livers were frozen at -80 °C until homogenization and microcystin analysis. Fish
tissues were analyzed for total microcystins (free and bound) using an optimized MMPB Lemieux
Oxidation method (Anaraki et al., 2020).
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128 **Human health risk assessment**
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Total microcystins measured in muscles were compared against Ontario's fish consumption
advisory benchmarks for microcystin-LR developed by the MECP and Ohio's fish consumption
advisory benchmarks for total microcystins developed by the Ohio Environmental Protection
Agency. These benchmarks are based on the World Health Organization's recommended tolerable
daily intake (TDI) of 0.04 µg kg^{-1} bodyweight per day (WHO, 2020). The margin of exposure and
hazard quotient were additionally calculated to estimate the potential for adverse effects to develop
in humans from exposure to microcystins. The fish consumption advisory benchmarks and full
details on the human health risk assessment are fully described in Shahmohamadloo *et al.*,
(2022b).

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140 **Statistical analysis**
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142 For statistical analysis any values below the limit of detection of the LC-MS of 2.18 ng g^{-1} ww were
143 treated as zero concentrations. Linear mixed effect models with species treated as a random effect
144 were used to test whether concentrations of microcystins in the muscle and liver were greater than
145 the limit of detection.
146
147 **Results and Discussion**
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Fish muscles contained microcystins ($F_{1,117}$=20.42, p <0.0001), with a mean concentration of 1.29
ng g^{-1} ww across species (Figure 1). Only 24/124 samples had concentrations above the limit of
detection. 2/124 of samples had concentrations exceeding the WHO's TDI and the human health
risk assessment suggests microcystins are a low risk (Table 1). Applying these results against the
fish consumption advisory benchmarks in Ontario (Shahmohamadloo *et al.*, 2022b), 95% of
muscles were <6 ng g^{-1} ww microcystins, which would result in an 'unrestricted' fish consumption
advisory, and all muscles are in the 'unrestricted' meal category (<25 ng g^{-1} ww) in Ohio.
Considering 98% of muscles had microcystins below the WHO's TDI, our results indicate that
humans can safely consume Lake St. Clair Largemouth Bass, Northern Pike, Smallmouth Bass,
Rock Bass, Walleye, White Bass, and Yellow Perch fillets ranging from three times a week to every
day (12 to 32 meals per month), depending on the species and its size.
160
Microcystin concentrations in livers were higher with 34/35 samples exceeding the limits of
detection. Fish livers contained a significant amount of microcystins ($F_{1,32}$=21.33, p <0.0001) with
an average value of 224.81 ng g^{-1} ww across Walleye, White Bass, and Yellow Perch (Figure 2;
Table 1). The wide variability and presence of microcystin levels in all livers (1-1,500 ng g^{-1} ww)
supports our previous work in Lake Erie demonstrating that microcystins are present in fish livers
well beyond the cessation of HABs and may be a persistent contaminant in aquatic ecosystems
(Shahmohamadloo *et al.*, 2022b). Key microcystin-producing HAB species such as *Microcystis*, a
common algal species in Lake St. Clair (Davis et al., 2014) and in the majority of countries
worldwide (Harke et al., 2016), also possess genetic and ecophysiological diversity that allows
them to effectively overwinter on the sediments and rapidly form blooms under favorable conditions
(Dick et al., 2021). Evidence also shows microcystin-producing strains of *Microcystis* found in Lake
St. Clair are genetically similar to those found in Lake Erie (Davis et al., 2014). Advanced
evolutionary mechanisms in species such as *Microcystis* (e.g., key genes involved in biosynthesis
of secondary metabolites, carbon concentrating mechanisms, detoxification of reactive oxygen
species, and acquisition of nitrogen and phosphorus; see (Dick et al., 2021)) can help to explain
the microcystin burden evident in fish livers pre-HAB and offers further insight into the decades-
long postulation that microcystins are one among several stress factors involved in fish kills during
HAB events (Ibelings et al., 2005).
179
To alleviate concerns of microcystin exposure via human consumption, general and sensitive
populations (women of child-bearing age and children) should avoid eating fish organs from Lake
St. Clair and other waterbodies that experience regular HAB events. Our advice is consistent with
current advisories for many other contaminants which could be elevated in fish organs. While it is
convention to gut fish and strictly consume fillets, whole fish consumption is practiced in various
cultures and encouraged for those seeking additional nutrients in their diet (Beveridge et al., 2013;
He, 2009). However, microcystins do not break down during cooking and boiling (Harada et al.,
1996) and may therefore pose some level of risk to consumers eating fish whole. Considering 90%
of anglers consume 8 or less fish meals per month (Awad, 2006) and following the above
recommended practices, our results suggest the risk of microcystin toxicity from fish consumption
is low for Lake St. Clair.

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321 **Figures**
322
323 **Figure 1.** Total microcystins (ng g⁻¹ ww) in muscles across seven fish species (Largemouth Bass,
324 Northern Pike, Smallmouth Bass, Rock Bass, Walleye, White Bass, Yellow Perch) collected pre-
325 and post-harmful algal bloom in Lake St. Clair. The method detection limit (2.18 ng g⁻¹ ww) is
326 denoted by a dashed line. Refer to Table 1 for the number of fish analyzed per species.
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329
330 **Figure 2.** Total microcystins (ng g⁻¹ ww) in livers across three fish species (Walleye, White Bass,
331 Yellow Perch) collected pre- and post-harmful algal bloom in Lake St. Clair. The method detection
332 limit (2.18 ng g⁻¹ ww) is denoted by a dashed line. Refer to Table 1 for the number of fish analyzed
333 per species.
334

336 **Table**
337
338 **Table 1.** Fish collected from Lake St. Clair pre- and post-HAB for microcystin analysis in livers and
339 muscles. Data is reported in mean±SD.
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Species	n	Length (cm)	Weight (g)	Microcystins in liver (ng g^{-1} ww)	Microcystins in muscle (ng g^{-1} ww)	EDI (µg kg^{-1} body weight day^{-1}) [a]
Largemouth Bass	3	38±13	1133±833	-	1.9±1.4	0±0.005
Northern Pike	12	74±10	2500±1055	-	0.4±0.9	0±0.003
Rock Bass	16	23±3	250±82	-	1.1±2.1	0±0.007
Smallmouth Bass	25	43±5	1334±559	-	1.0±3.1	0±0.01
Walleye	42	48±10	1059±690	217.2±220.5	1.8±2.5	0±0.008
White Bass	12	36±3	566±161	120.4±17.8	0.6±1.3	0±0.004
Yellow Perch	16	25±2	161±59	233.9±359.8	1.9±2.4	0±0.008

341 [a] Estimated daily intake (EDI) is measured as ng g^{-1} d^{-1}, assuming one fish meal is 227 g and applying the mean tissue concentration as
342 the daily intake value. Values are compared against the World Health Organization's tolerable daily intake value (0.04 µg kg^{-1} body
343 weight day^{-1}).