Great Lakes Mussel Watch: Assessment of Contaminants of Emerging Concern

NOAA National Centers for Coastal Ocean Science
Stressor Detection and Impacts Division

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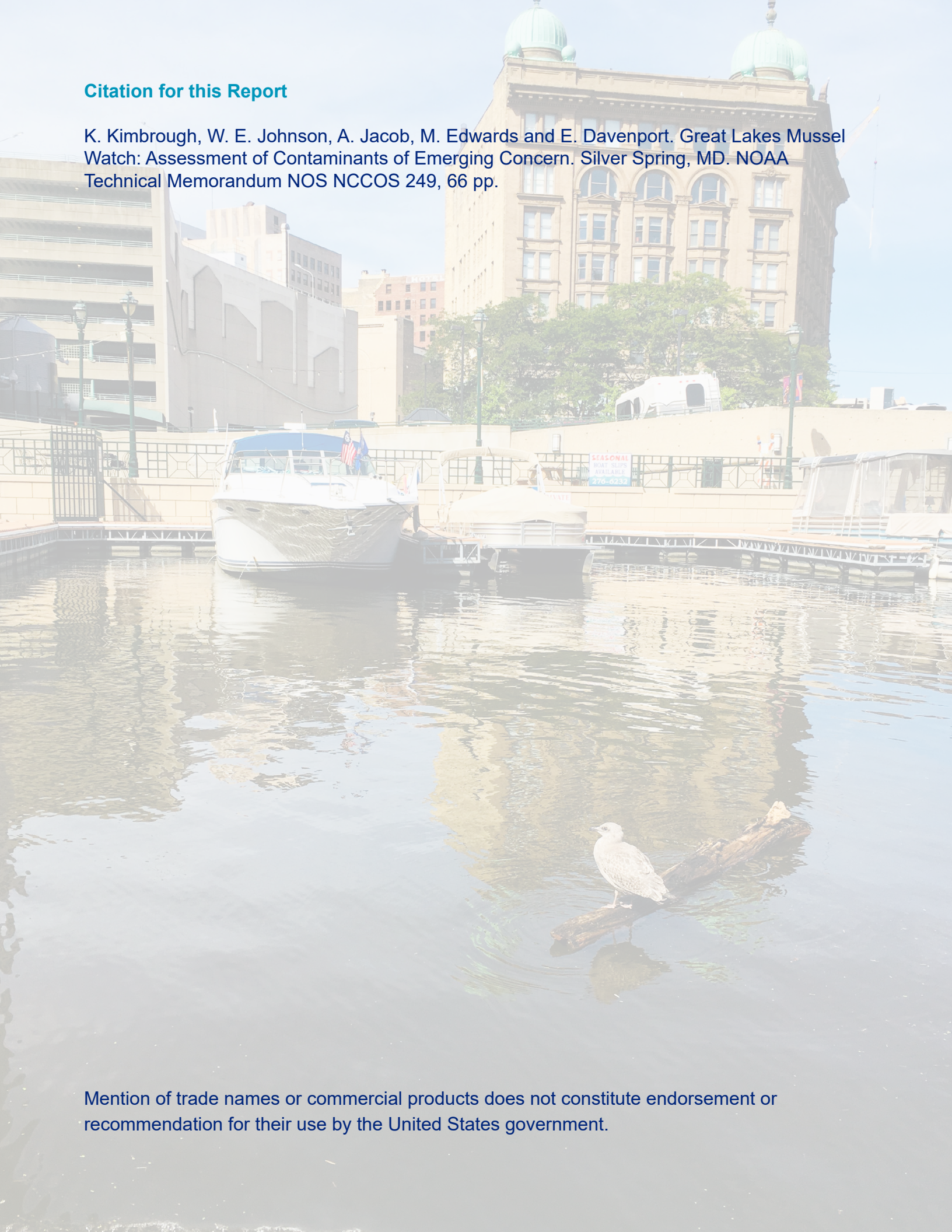
August 2018

NOAA TECHNICAL MEMORANDUM NOS NCCOS 249

NOAA NCCOS Stressor Detection and Impacts Division



Citation for this Report

K. Kimbrough, W. E. Johnson, A. Jacob, M. Edwards and E. Davenport. Great Lakes Mussel Watch: Assessment of Contaminants of Emerging Concern. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 249, 66 pp.

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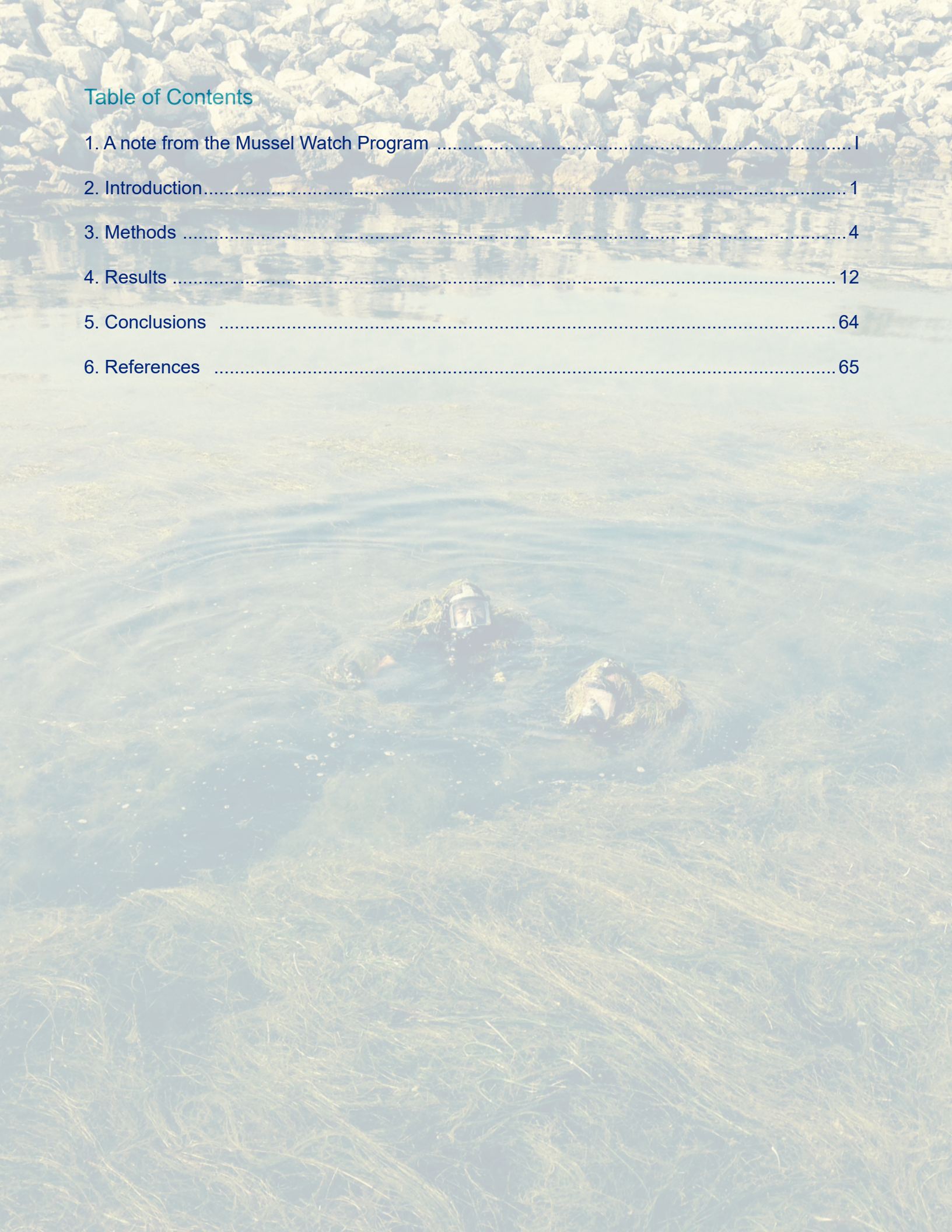


A note from the Mussel Watch Program

NOAA's National Status and Trends (NS&T), Mussel Watch Program has been in operation since 1986, when it was designed to monitor the status and trends of a broad suite of chemical contaminants at sites that represented large coastal areas in order to construct a nationwide assessment. Of the Program's analyte list, some compounds have had their use restricted and declined in concentration over time, while others persist. At the same time, there are huge numbers of newly measured chemicals entering the environment every year and there is often a lack of comprehensive information on the sources, distribution, persistence, and potential effects of these contaminants of emerging concern (CECs). Previous efforts by NS&T in accessing CECs have been limited to localized studies and few classes of emerging contaminants. In 2006, NS&T collaborated with local scientists and stakeholders to conduct a survey of the occurrence of residue of human use pharmaceuticals in the Chesapeake Bay, Biscayne Bay, and the Gulf of Farallones. Other efforts to date includes a nationwide Mussel Watch Program assessment of polybrominated diphyenyl ethers in 2009, and a regional CEC characterization led by the Southern California Coastal Water Research Project in 2011.

Mussel Watch is now conducting additional pilot work in other portions of the United States, in order to assess CEC classes for potential inclusion into the national monitoring program. These pilot studies are regional in nature, balancing short-term flexibility in study design against the cost of broad CEC surveys. We tested multiple bivalve species and sediment samples for accumulation of flame retardants, stain resistant compounds, pharmaceutical and personal care products, endocrine-disrupting chemicals, and current use pesticides. Our 2014-15 Great Lakes effort was a retrospective analysis of dreissenid (zebra/quagga) mussels collected for multiple other studies in our ongoing work under the Great Lakes Restoration Initiative.

In 2015, NOAA funded two case studies; a survey of natural oyster beds in a range of land use types around Charleston, SC and a Chesapeake Bay survey which utilized a combination of traditional Mussel Watch sites and caged oyster deployments to target land use and wastewater outfalls. We conducted a regional assessment of blue mussels in the Gulf of Maine in 2016, combining traditional Mussel Watch sites with those of the Gulf Watch program. Combined with the previous work in California, these pilot studies represent the range of bivalve species and land use types surveyed in the national Mussel Watch program.

Introduction

The Clean Water Act of 1972 calls for preserving and maintaining the chemical, physical and biological integrity of our nation's waters. Numerous water quality monitoring programs of varying scopes run by government agencies, universities and citizen volunteer groups operate at local, regional and national scales. Water quality data thus generated are used to characterize waters, describe status and temporal trends, identify emerging issues, and prioritize areas where resources need to be directed in restoring and rehabilitating water bodies.

NOAA's National Centers for Coastal Ocean Science (NCCOS) Mussel Watch Program (MWP) is a national contaminant monitoring program established in 1986 to monitor chemical contaminants in coastal waters. As the name suggests, the program uses bivalves as indicator organisms to monitor persistent, hydrophobic organic contaminants and trace metals at approximately 300 coastal sites nationwide. Given the unique attributes such as the limited ability of bivalves to metabolize the bioaccumulated chemical pollutants, limited mobility, their widespread distribution and abundance throughout the U.S. coast, these organisms serve as ideal indicators of chemical pollution on a national scale.

The summary of nearly two decades of MWP's national monitoring data was published in a report in 2008 (Kimbrough *et al*., 2008). Many of the monitored chemicals (restricted use or banned before the initiation of the program) had reached asymptotic decline in concentrations and further continuous monitoring of these chemicals was debated. Concurrently, due to great strides in ecotoxicological awareness and advances in analytical methods and instrumentation, a new class of chemicals termed contaminants of emerging concern (CEC) gained the attention of the scientific community. CECs are chemicals or other substances for which there are not regulatory standards, whose presence in the environment has only recently been documented, and have the potential to cause adverse biological effects at environmentally relevant concentrations. Often the CECs are not included in routine environmental monitoring programs, pending further evaluation of their toxicity, potential health effects, and environmental persistence. These CECs include but are not limited to complex mixtures of industrial chemicals (e.g., flame retardants), agrochemicals (e.g., pesticides), pharmaceuticals and personal care products (PPCP), alkylphenols, nanomaterials, etc. Wastewater treatment plants supply a nearly continuous low-level, complex mixture of CECs to the receiving water-body. Many of these CECs are inherently biologically active and though detected only at minute concentrations can still elicit biological impacts on biota (Schoenfuss *et al*., 2016). In response to the growing concern of the potential adverse effects posed by CECs, MWP began assessing how best to incorporate CECs in the national monitoring protocol in early 2000s.

To date, Mussel Watch efforts on CECs have included a nationwide assessment of polybrominated diphyenyl ethers in bivalve tissue (Kimbrough *et al*., 2009), and a regional CEC characterization led by the Southern California Coastal Water Research Project in 2011(Bricker *et al*., 2014). Mussel Watch is now conducting additional pilot work in other portions of the United States (Great Lakes, Charleston, SC and Chesapeake Bay, MD) to assess CEC classes that can be detected in mussels. These pilot studies are regional in nature, balancing short-term flexibility in study design, including targeted site selection at known or suspected contaminated areas, against the cost of broad CEC surveys.

In the Great Lakes, Mussel Watch initiated contaminant monitoring in 1992 after the invasion and proliferation of Ponto-Caspian mussels in the region. Beginning in 2010, MWP expanded its monitoring activities in the Great Lakes under the Great Lakes Restoration Initiative (GLRI), Action Plan I (2010-2014), Focus Area "Toxic Substances and Areas of Concern". MWP added sites in all the U.S. Areas of Concern (AOC) and data from the basin-wide assessment conducted in 2009-2010 is summarized in Kimbrough *et al*., 2014. AOCs were a target for work because of heir importance to the GLRI initiative. Under GLRI, MWP also initiated the use of caged mussels to conduct place-based contamination assessments in the Manistique River, Milwaukee Estuary, and Niagara River. Caged mussels were typically deployed for approximately four weeks and strategically located in areas with known or suspected pollution. Mussel reference

site(s) were established in the lake nearshore zones or connecting channels.

The GLRI Action Plan II (2015-2019) Focus Area "Toxic substances and Areas of Concern" calls for federal partners to "identify emerging contaminants and assess impacts on Great Lakes fish and wildlife." The multi-agency team of federal agencies uses a collaborative research, monitoring and surveillance program to achieve the goals outlined in the Action Plan II to effectively address the issue of CECs in the Great Lakes. MWP initiated its first Great Lakes CEC study in 2015 using caged mussels in the Maumee River, the largest Great Lakes watershed with predominant agricultural land use. In the following year, Great Lakes Mussel Watch repeated and expanded the Maumee study by increasing the spatial extent of caged mussel deployments. The primary objective of this two-year study was to determine the frequency and magnitude of current use pesticides in mussel tissue. However, other CECs were also monitored to provide a broad characterization of CECs in mussels.

Additionally, MWP collected mussel samples from several sites in Lake Erie for CEC characterization. Coupled with the field efforts under GLRI, NCCOS conducted a retrospective analysis of dreissenid mussels collected in 2013 from Milwaukee Estuary and 2014 from Niagara River as part of the ongoing work under GLRI. Both Milwaukee Estuary and Niagara River are polluted areas with urban land use and hence these samples maximized the potential for finding CECs in this exploratory effort. By leveraging various resources to achieve common goals of CEC characterization, MWP investigated the occurrence and magnitude of a broad list of CECs in mussels collected from several Great Lakes locations with various land-use characteristics. Furthermore, MWP initiated bivalve health assessments using various techniques (DNA damage, metabolomics, gene expression, and cellular biomarkers) in partnership with academia, other federal agencies and private sector to link CEC exposure to biological effects in mussels. These results will be summarized in a subsequent report.

The current report summarizes all the mussel tissue CEC data obtained between 2013-2015 in the aforementioned efforts grouped by analytical method in a user-friendly format. The study design as initially intended is to inform MWP management, stakeholders and general public about the frequency of occurrence and the magnitude of CECs in mussels. As the dataset grows spatially and temporally, we anticipate patterns will emerge with respect to land use and proximity to pollutant sources. These preliminary results from the Great Lakes along with pilot studies in other regions of the country will aid MWP in determining the best way forward in incorporating CECs in the national monitoring protocol.

Methods

Mussel samples from contamination assessment studies in the Great Lakes were chosen for CEC analysis. To increase the likelihood of finding CECs, samples from rivers and harbors were selected preferentially over samples collected from relatively less polluted nearshore lake sites. The data presented here are from multiple studies including the Maumee and Ottawa Rivers, Niagara River and select tributaries, Milwaukee Estuary, Ashtabula River, Cuyahoga River, Presque Isle Bay, and Black River. The location and number of samples are given in Figure 1. All samples included in this report were collected between June 2013 and June 2015. Because some sites were sampled multiple times, letters have been added to the site names to indicate temporal variations in sampling. Samples are a combination of *in situ* mussels collected from outer harbor stone breakwaters and caged mussels deployed in rivers. Site location is provided in Figures 2 A-H. The Maumee River sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in June and July of 2014 respectively.

All samples were measured for Pharmaceuticals and Personal Care Products (PPCPs). Pesticides were measured in samples from the Maumee and Ottawa Rivers, because they come from a watershed with a high percentage of planted cropland, and are of local interest. A subset of samples were measured for phenols (octylphenol, nonylphenol, nonylphenol ethoxylates), and hexabromocyclododecane (HBCDD) based on stakeholder interest. We have documented where each method was used along with a map and site location for each tissue measurement in Figures 2A-H.

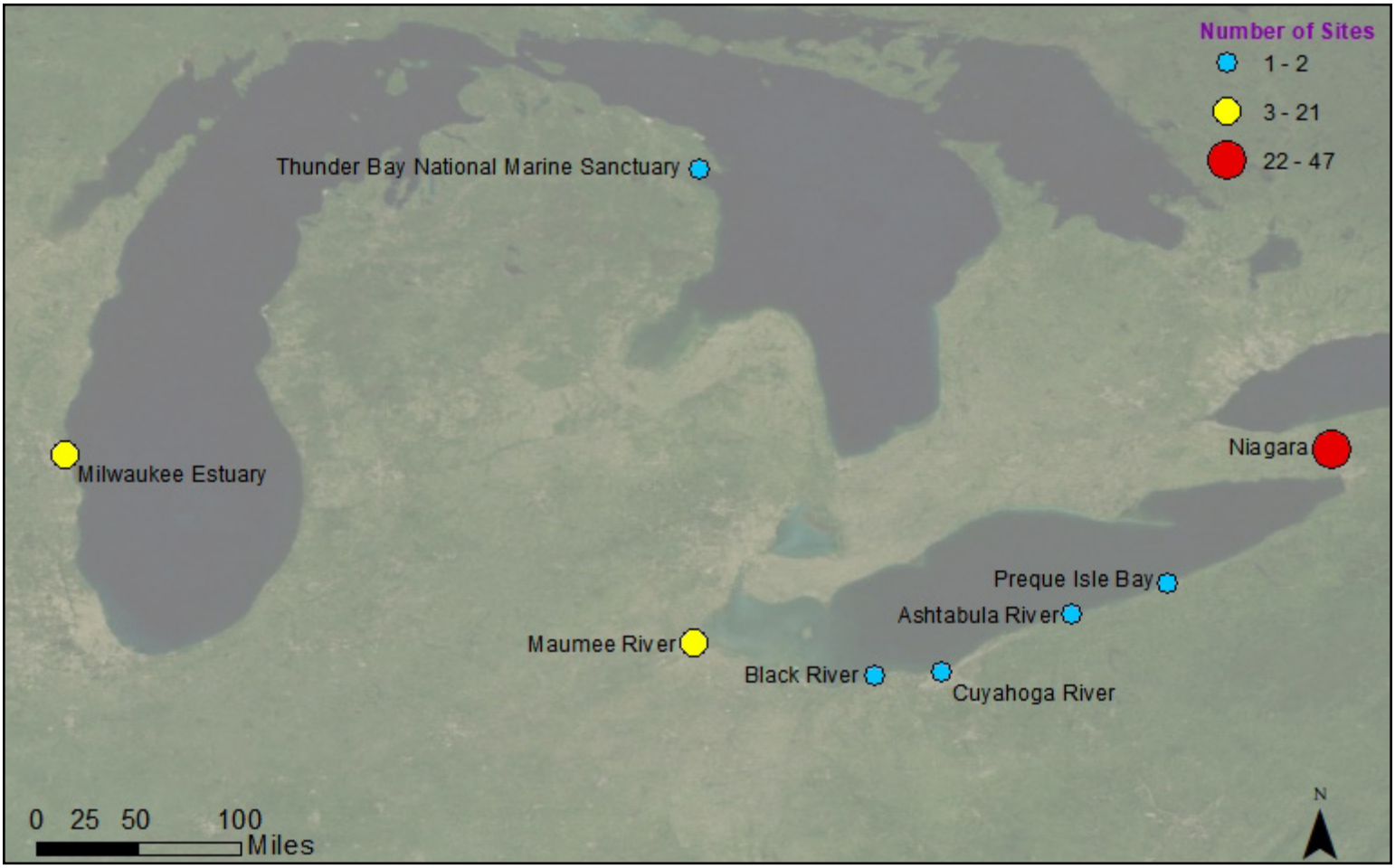
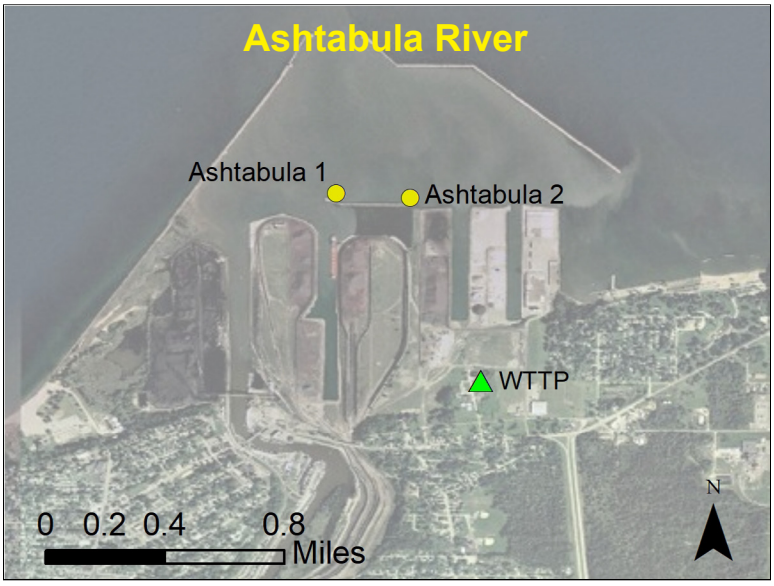


Figure 1. Great Lakes Mussel Watch CEC study locations. Size and color of circles designate the number of sites at each location. The Niagara, Maumee and Milwaukee Rivers have relatively more sites in addition to multiple time periods for some sites. Locations with 1-2 sites are a part of our screening effort and are not meant to characterize the entire location.



Method

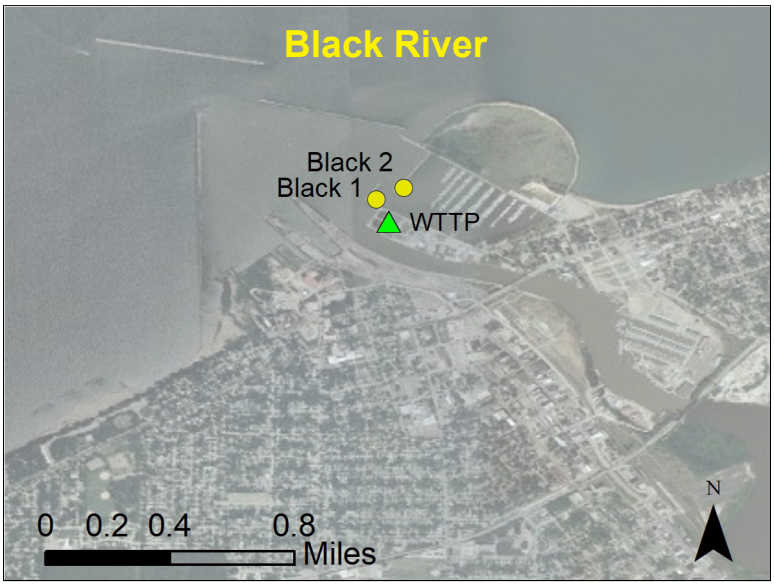
Pesticides (AXYS method MLA-035.R07.02)

Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)

Site	Latitude	Longitude
Ashtabula 1	41.9123	-80.7935
Ashtabula 2	41.9121	-80.7900



Method

Pesticides (AXYS method MLA-035.R07.02)

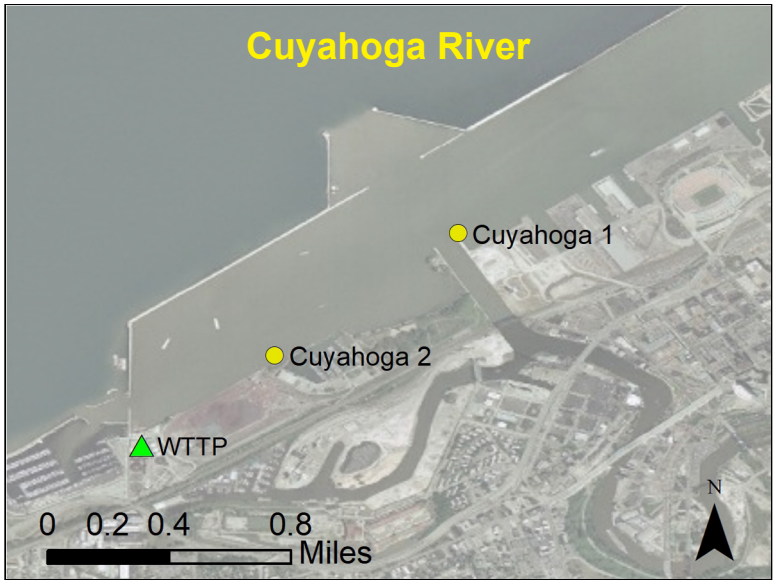
Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)

Site	Latitude	Longitude
Black 1	41.4736	-82.1822
Black 2	41.4742	-82.1810

Figures 2A-B. Great Lakes Mussel Watch CEC study site locations. Method names in bold red text were performed at the listed location. Site table provides approximate nominal latitude and longitude for each site. Where applicable, wastewater treatment plants (▲) and reference sites (*) are identified on the map.



Method

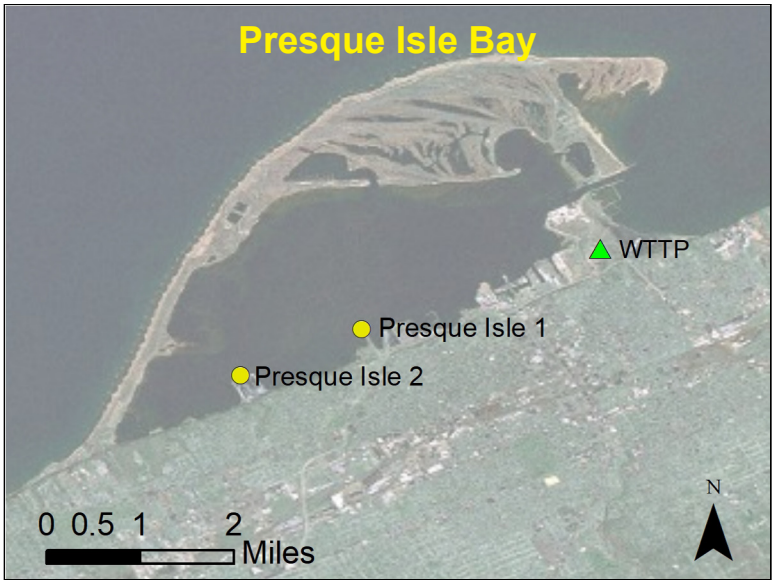
Pesticides (AXYS method MLA-035.R07.02)

Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)

Site	Latitude	Longitude
Cuyahoga 1	41.5042	-81.7114
Cuyahoga 2	41.4984	-81.7201



Method

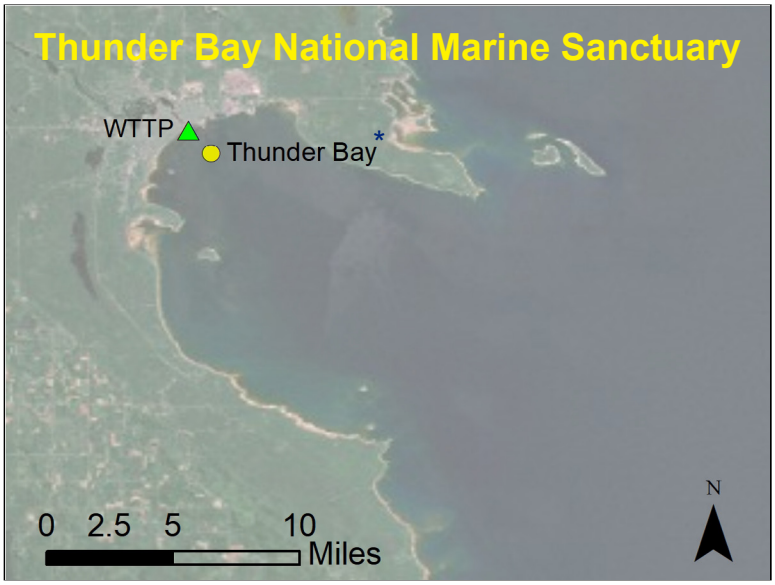
Pesticides (AXYS method MLA-035.R07.02)

Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)

Site	Latitude	Longitude
Presque Isle 1	42.1305	-80.1132
Presque Isle 2	42.1234	-80.1320



Method

Pesticides (AXYS method MLA-035.R07.02)

Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)

Site	Latitude	Longitude
Thunder Bay	45.0461	-83.4156

Figures 2C-E. Great Lakes Mussel Watch CEC study site locations. Method names in bold red text were performed at the listed location. Site table provides approximate nominal latitude and longitude for each site. Where applicable, wastewater treatment plants (▲) and reference sites (*) are identified on the map.

Site	Latitude	Longitude
Maumee 0	41.7006	-83.4601
Maumee 1	41.6886	-83.4750
Maumee 2	41.6554	-83.5251
Maumee 3	41.7618	-83.3290
Ottawa 1	41.7329	-83.4682
Ottawa 2	41.7248	-83.4798
Ottawa 3	41.7106	-83.4994

Method Tissue
Pesticides (AXYS method MLA-035.R07.02)
Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)
Alkylphenols (AXYS method MLA-080.R02.04)
Hexabromocyclododecane (AXYS method MLA-070. R02.03)

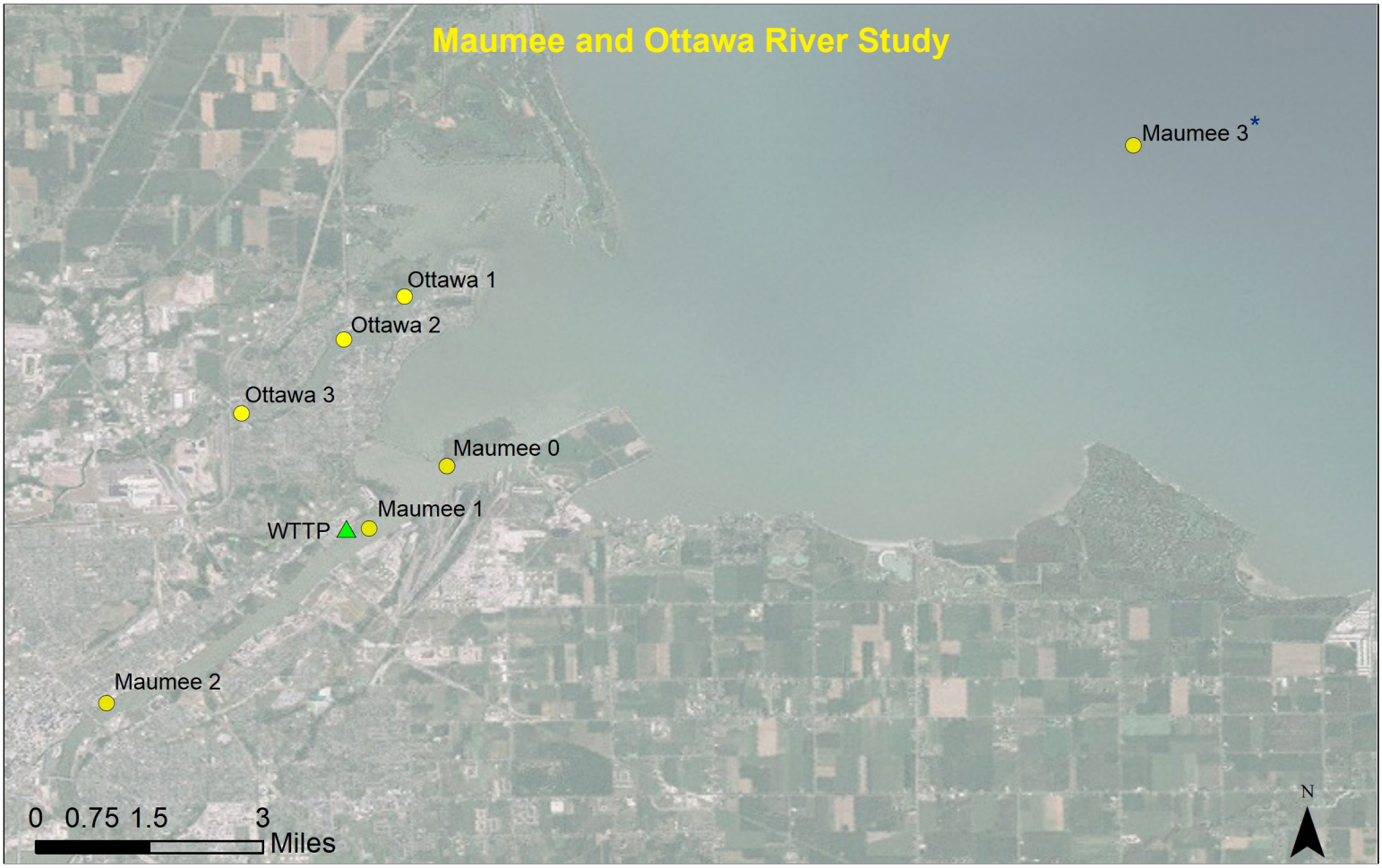


Figure 2F. Great Lakes Mussel Watch CEC study site locations. Method names in bold red text were performed at the listed location. Site table provides approximate nominal latitude and longitude for each site. Where applicable, wastewater treatment plants (▲) and reference sites (*) are identified on the map.

Site	Latitude	Longitude
Milwaukee 0	43.0340	-87.8948
Milwaukee 1	43.0078	-87.8872
Milwaukee 10	43.0334	-87.9176
Milwaukee 12	43.0318	-87.9469
Milwaukee 13	43.0095	-87.9067
Milwaukee 4	43.0432	-87.8878
Milwaukee 5	43.0597	-87.8670
Milwaukee 6	43.0245	-87.8987
Milwaukee 7	43.0440	-87.9129
Milwaukee 8	43.0570	-87.8998
Milwaukee 9	43.0284	-87.9256

Method
Pesticides (AXYS method MLA-035.R07.02)
Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)
Alkylphenols (AXYS method MLA-080.R02.04)
Hexabromocyclododecane (AXYS method MLA-070. R02.03)

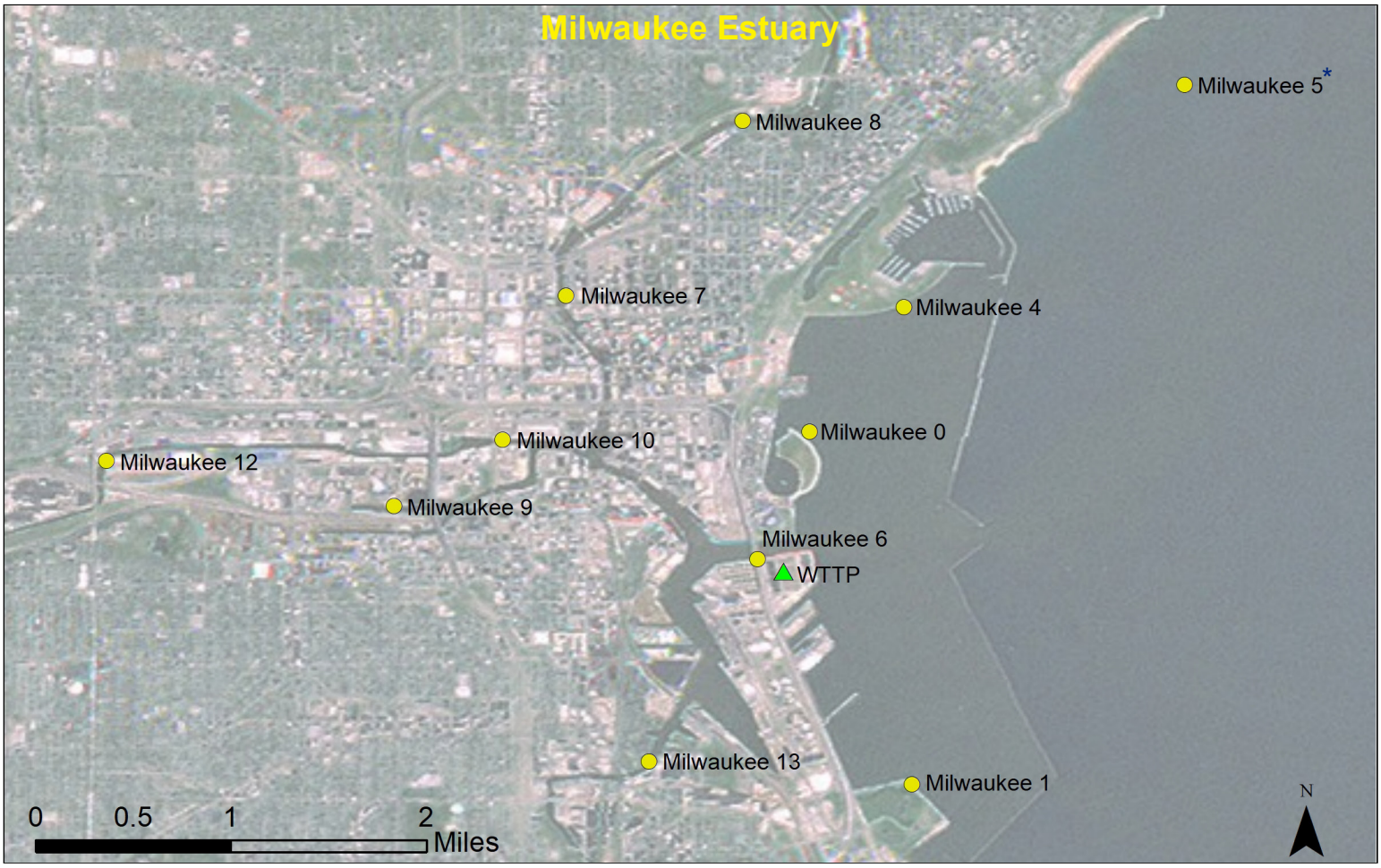


Figure 2G. Great Lakes Mussel Watch retrospective CEC study site locations. Method names in bold red text were performed at the listed location. Site table provides approximate nominal latitude and longitude for each site. Where applicable, wastewater treatment plants (▲) and reference sites (*) are identified on the map.

Site	Latitude	Longitude
Cayuga	43.0750	-78.9639
Ellicott	43.0204	-78.8754
Gill 1	43.0781	-79.0267
Gill 2	43.0783	-79.0259
Gill 3	43.0788	-79.0258
Niagara 0	42.8442	-78.8644
Niagara 1	42.8708	-78.9022
Niagara 4	42.8846	-78.8908
Niagara 9	43.0612	-79.0028
Scajaquada 0	42.9301	-78.8999
Scajaquada 1	42.9291	-78.8985
Smokes	42.8114	-78.8637
Niagara 17	42.8710	-78.8852
Two Mile 0	43.0113	-78.9062
Two Mile 1	43.0108	-78.9064
Tonawanda 0	43.0248	-78.8816
Tonawanda 1	43.0224	-78.8812
Tonawanda 2	43.0196	-78.8530

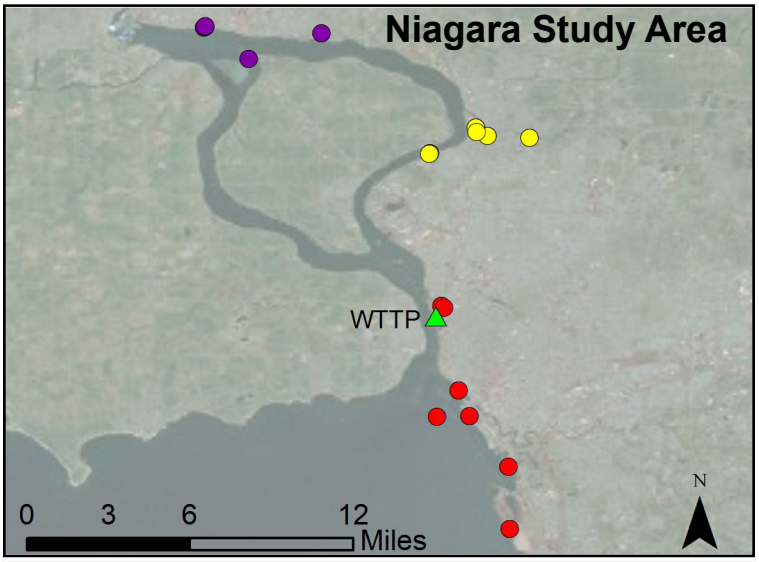
Method

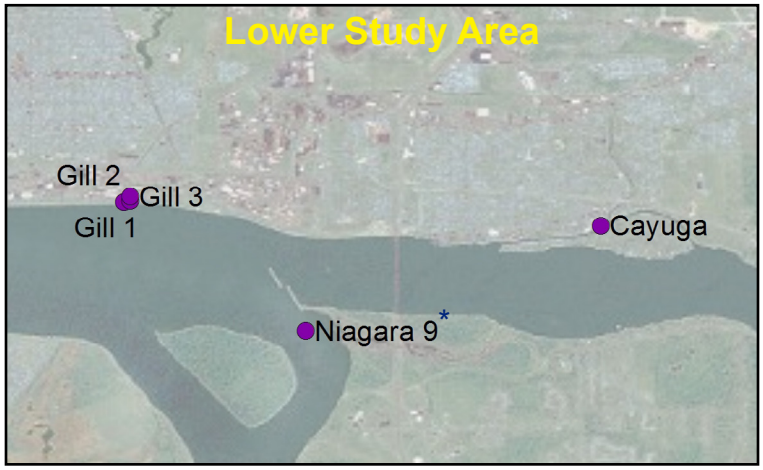
Pesticides (AXYS method MLA-035.R07.02)

Pharmaceuticals & Personal Care Products (AXYS method MLA-075.R06.01)

Alkylphenols (AXYS method MLA-080.R02.04)

Hexabromocyclododecane (AXYS method MLA-070. R02.03)





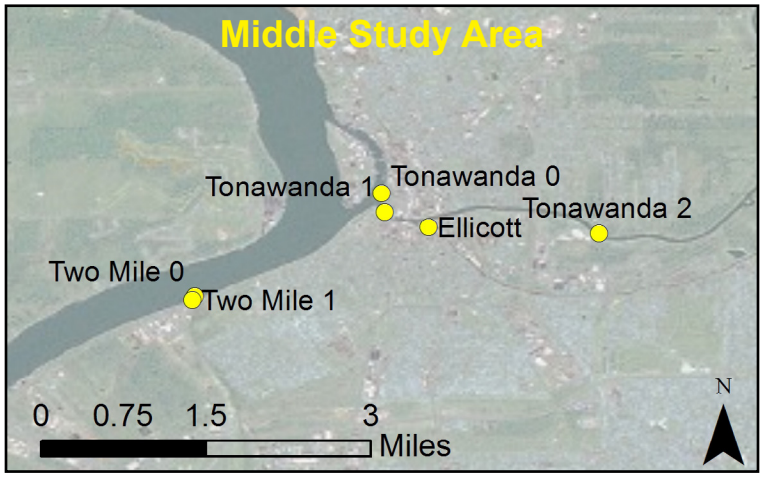


Figure 2H. Great Lakes Mussel Watch retrospective CEC study site locations. Method names in bold red text were performed at the listed location. The Niagara study Area figure displays relative location of all study sites. Site table provides approximate nominal latitude and longitude for each site.

Sampling Procedures

Divers harvested *in situ* mussels from established populations in the open lake, nearshore lake zone, or outer harbor breakwaters. Locations with 1-2 sites (Figure 1) and harvest/reference sites used *in situ* mussels for chemical analyses. At the Niagara, Maumee and Milwaukee locations *in situ* mussels from harvest sites were relocated in cages. All samples included in this report were collected between June 2013 and June 2015. Sample analysis occurred between June 2015 and March 2016.

Chemical analysis

All samples were analyzed by SGS AXYS Analytical Services Ltd. in British Columbia, Canada. The methods are proprietary and confidential. Hence, in this document, we will refer to the name of the method and revision number provided by SGS AXYS. Note that MLA-075 and MLA-035 are based on EPA methods 1694 and 1699 respectively (U.S. EPA 2007a, b).

To simplify the presentation of results, all analytes were grouped by analytical method. Not all methods were performed at all sites. As a result, the number and location of samples analyzed for each method may be different. Contaminant concentrations were blank corrected. A more liberal filter of data was used for recording presence/absence (heat map) compared to those results presented for concentration. Specifically, samples with concentrations above detection limit were recorded as detected, and are only used in summaries of presence/absence (heat maps and method tables). Where a numeric concentration is provided (bar charts, and chemical concentration table), values below 3 times detection limit were changed to zero. Handling of data with analytical flags is detailed in Table 1. Compounds that were not quantifiable (NQ flag) by the method were not part of the overall analysis. The results include parent compounds and metabolites/degradation products.

Table 1. Handling of data flags for reporting presence/absence and concentration.

Flag	Definition	Presence/ Absence	Concentration
U	Not detected	Yes	Yes
K	Peak detected, but did not meet quantification criteria, result reported represents the estimated maximum possible concentration	Yes	No
D	Dilution data	Yes	Yes
J	Concentration less than LMCL (Lower Method Calibration Limit)	Yes	3x
NQ	Data not quantifiable	No	No
T	Analyte recalculated against alternate labelled compound(s)or internal standard	Yes	Yes
H	Concentration is estimated (information only value)	Yes	No

Results

In this section, the contaminant concentration results from mussel tissue samples are presented by analytical method. Results are summarized and characterized using the format described in the following readers guide section. The analytes detected, found in the compound list table and heat map for each method, are measurements that are above the detection limit. Compound specific concentrations and summaries, provided in the compound summary table, bar charts, and site concentration tables include all measurements, but any measurement below 3 x the detection limit was changed to zero. The box and whisker plots only utilize measurements that are three times the detection limit.

These results serve as an initial assessment to characterize various suites of CECs that had the highest probability of being detected in tissue, and to identify what methods could be used in the future.

Readers Guide

The results section contains tables and figures organized by analytical method.

Site table. Presentation of all the locations where PPCP analysis was used

Pharmaceuticals & Personal Care Products	n
Ashtabula River	2
Black River	2
Cuyahoga River	6
Maumee and Ottawa River Study	15
Milwaukee Estuary	13
Niagara River	19

The site table lists all locations where the specified analytical method was applied. Details regarding site location can be found in Figures 1 and 2. "n" reflects the number of samples analyzed at each location.

Method table. Summary of compound detections for the PPCP analysis. Percentage categories are used to characterize how often a compound was found in a sample at 3 x DL.

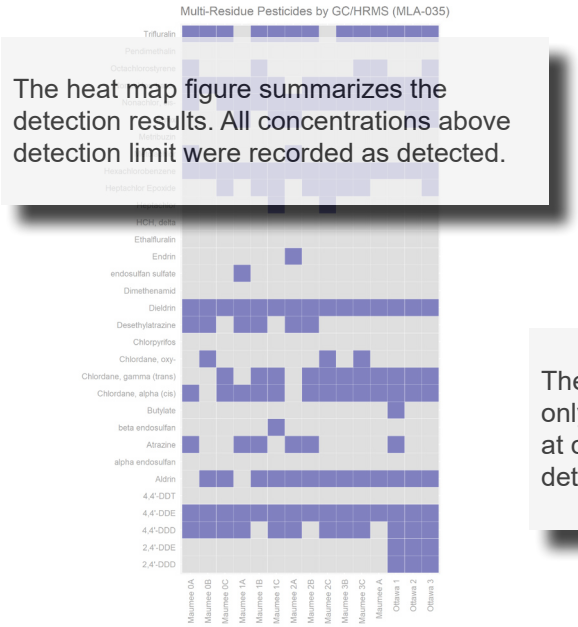
Compound percentage categories	Samples	
	n	%
Compound not found	96	68
Compound found in 1-24 % of samples	29	21
Compound found in 25-49 % of samples	7	5
Compound found in 50-74 % of samples	4	3
Compound found in 75-100 % of samples	4	3

The method table summarizes the percentage of compounds found above the detection limit (DL) with respect to number (n) and percentage (%) of sites.

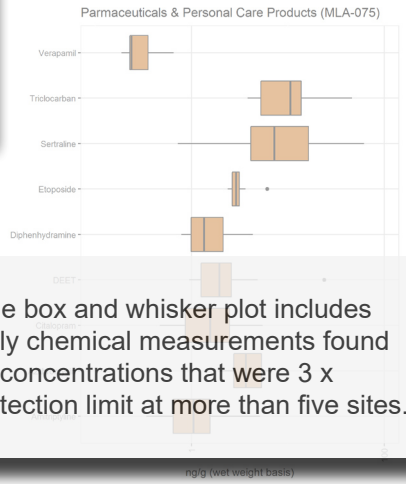
Chemical concentration table. Summary of compound concentrations in ng/g wet weight. Concentrations below 3 x detection limit were changed to zero.

COMPOUND	Usage	Class	Concentration (ng/g wet weight)		
			maximum	mean	median
2-Hydroxyibuprofen	Pharmaceutical	Anti-inflammatory	269000	12947	0
Iopamidol	Pharmaceutical	Contrast agent	261000	31824	0
10-Hydroxy-amitriptyline	Pharmaceutical	Antidepressant	401	44	0
Cocaine	Recreational drug	Stimulant	311	38	0

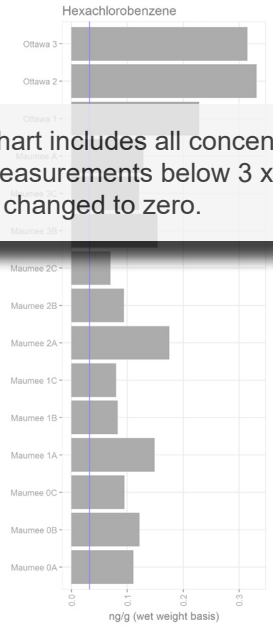
The chemical concentration table summarizes concentration results where measurements below 3 x detection limit were changed to zero. These results are visualized in the associated box and whisker and bar charts below.



The heat map figure summarizes the detection results. All concentrations above detection limit were recorded as detected.



The box and whisker plot includes only chemical measurements found at concentrations that were 3 x detection limit at more than five sites.



The bar chart includes all concentration results. Measurements below 3 x detection limit were changed to zero.

Site concentration table. Concentration (ng/g wet weight) results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero.

Label	Maumee 0A	Maumee 0B	Maumee 0C	Maumee 1A	Maumee 1B	Maumee 1C
2,4'-DDD	0	0	0	0	0	0
Octachlorostyrene	0.147	0	0	0	0.072	0
Trifluralin	0.159	0.32	0.237	0	0.319	0.388

The site concentration table summarizes results for dreissenid mussels by site and time. Concentration results below 3 x detection limit were changed to zero.

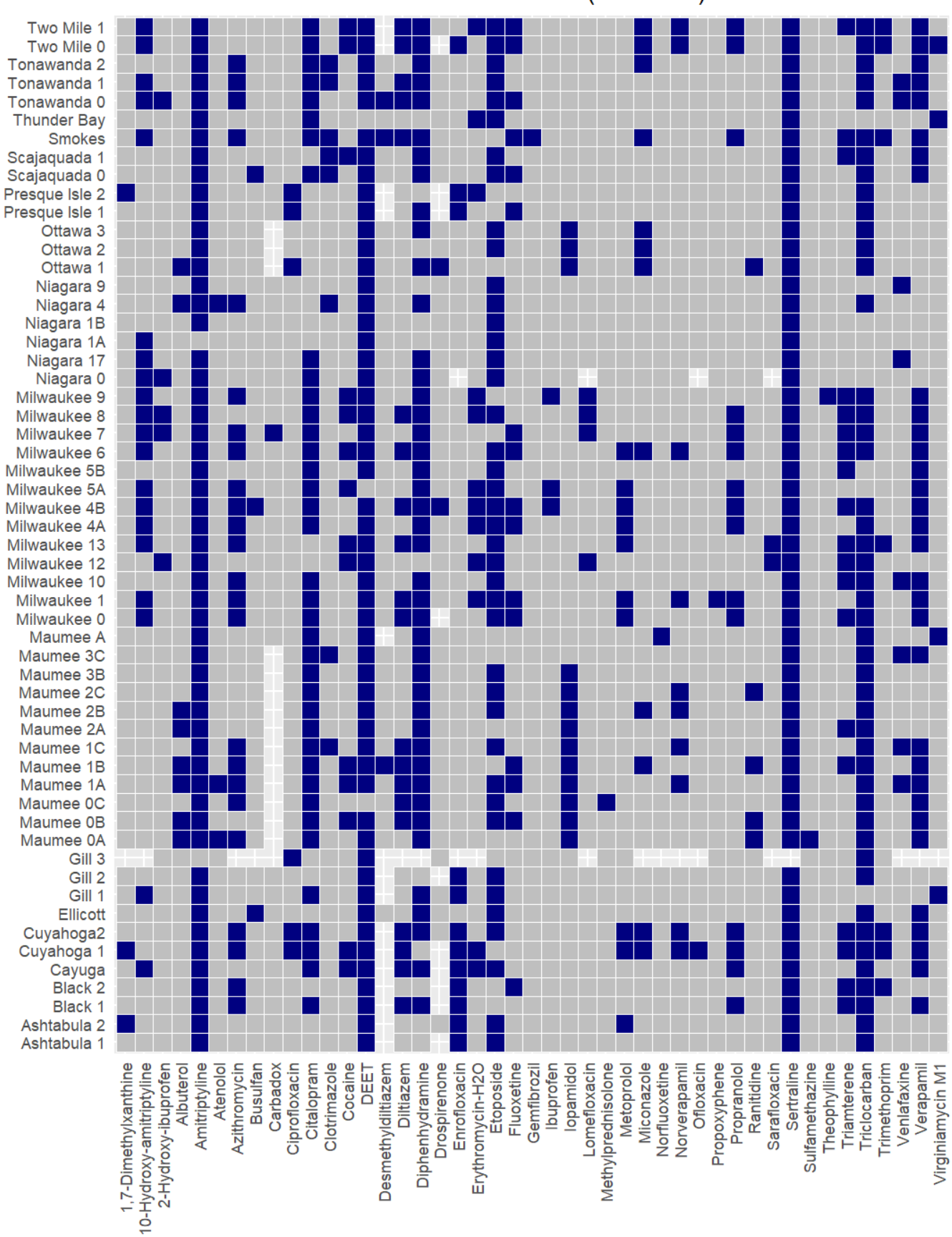
Pharmaceuticals and Personal Care Products

Pharmaceuticals and personal care products were analyzed at all sites. Pharmaceuticals are inherently bioactive and many are chronically or acutely toxic with some being identified as endocrine disruptors. The AXYS PPCP method MLA-075.R06.01 includes analyses of over-the-counter, elicit and prescription drugs; synthetic musks; antimicrobials; antibiotics; and insect repellents. The pharmaceuticals have human and veterinarian uses making both wastewater treatment plants and concentrated animal feeding lots potential sources.

Chemical list table. Pharmaceuticals and personal care products (MLA-075.R06.01). Concentrations above the detection limit, found in at least one sample, are identified in bold red type.

1,7-Dimethylxanthine	**Citalopram**	**Gemfibrozil**	Prednisone
10-Hydroxy-amitriptyline	Clarithromycin	Glipizide	Progesterone
17 alpha-Dihydroequilin	Clinafloxacin	Glyburide	Promethazine
17 alpha-Estradiol	Clonidine	Hydrochlorothiazide	**Propoxyphene**
17 alpha-Ethinyl-Estradiol	**Clotrimazole**	Hydrocodone	**Propranolol**
17 beta-Estradiol	Cloxacillin	Hydrocortisone	**Ranitidine**
2-Hydroxy-ibuprofen	**Cocaine**	**Ibuprofen**	Rosuvastatin
4-Epianhydrochlortetracycline [EACTC]	Codeine	**Iopamidol**	Roxithromycin
4-Epianhydrotetracycline [EATC]	Colchicine	Isochlortetracycline [ICTC]	**Sarafloxacin**
4-Epichlortetracycline [ECTC]	COMPOUND	Lincomycin	**Sertraline**
4-Epioxytetracycline [EOTC]	Cotinine	**Lomefloxacin**	Simvastatin
4-Epitetracycline [ETC]	Cyclophosphamide	Medroxyprogesterone Acetate	Sulfachloropyridazine
Acetaminophen	Daunorubicin	Melphalan	**Sulfadiazine**
Albuterol	**DEET**	Meprobamate	Sulfadimethoxine
Allyl Trenbolone	Dehydronifedipine	Mestranol	Sulfamerazine
Alprazolam	Demeclocycline	Metformin	Sulfamethazine
Amitriptyline	**Desmethyldiltiazem**	**Methylprednisolone**	Sulfamethizole
Amlodipine	Desogestrel	**Metoprolol**	Sulfamethoxazole
Amphetamine	Diatrizoic acid	Metronidazole	Sulfanilamide
Amsacrine	Diazepam	**Miconazole**	Sulfathiazole
Androstenedione	Digoxigenin	Minocycline	Tamoxifen
Androsterone	Digoxin	Moxifloxacin	Teniposide
Anhydrochlortetracycline [ACTC]	**Diltiazem**	Naproxen	Testosterone
Anhydrotetracycline [ATC]	**Diphenhydramine**	Norethindrone	Tetracycline [TC]
Atenolol	Doxorubicin	Norfloxacin	**Theophylline**
Atorvastatin	Doxycycline	**Norfluoxetine**	Thiabendazole
Azathioprine	**Drospirenone**	Norgestimate	Trenbolone
Azithromycin	Enalapril	Norgestrel	Trenbolone acetate
Benzoylecgonine	**Enrofloxacin**	**Norverapamil**	**Triamterene**
Benztropine	Equilenin	**Ofloxacin**	**Triclocarban**
Betamethasone	Equilin	Ormetoprim	Triclosan
Bisphenol A	**Erythromycin-H2O**	Oxacillin	**Trimethoprim**
Busulfan	Estriol	Oxazepam	Tylosin
Caffeine	Estrone	Oxolinic Acid	Valsartan
Carbadox	**Etoposide**	Oxycodone	**Venlafaxine**
Carbamazepine	Flumequine	Oxytetracycline [OTC]	**Verapamil**
Cefotaxime	Fluocinonide	Paroxetine	**Virginiamycin M1**
Chlortetracycline [CTC]	**Fluoxetine**	Penicillin G	Warfarin
Cimetidine	Fluticasone propionate	Penicillin V	Zidovudine
Ciprofloxacin	Furosemide	Prednisolone	

Pharmaceuticals and Personal Care Products



Heat map. Presence (■) and absence (□) of each compound found in mussel tissue at various locations. Maumee sites ending in A, B, and C represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in June and July of 2014 respectively. Analytes that were not quantifiable (NQ) are designated by white lines in the box.

Pharmaceuticals and Personal Care Products

Site table. Presentation of all the locations where the PPCP analysis was used to analyze tissue samples.

Pharmaceuticals & Personal Care Products	Sites (n)
Ashtabula River	
Black River	2
Cuyahoga River	
Maumee and Ottawa Rivers	15
Presque Isle Bay	
Thunder Bay National Marine Sanctuary	1
Milwaukee Estuary	
Niagara River	19

Method table. Summary of compound detections for the PPCP analysis. Percentage categories are used to characterize how often a compound was found in a sample at 3 x DL.

Compound percentage categories	Samples	
	n	%
Compound not found	96	68
Compound found in 1-24 % of samples	30	
Compound found in 25-49 % of samples	7	5
Compound found in 50-74 % of samples	4	
Compound found in 75-100 % of samples	4	3

Summary of detections (Heat map figure)

- Of the 141 compounds analyzed, 45 compounds (32%) were detected in dreissenid mussels.
- Sertraline was the only compound detected at all sites.
- Twenty two compounds were detected at one of the five reference sites (Maumee 3, Milwaukee 5, Thunder Bay National Marine Sanctuary, Niagara 9 and Niagara 1) including: alpha-HBCDD, 10-Hydroxy-amitriptyline, Amitriptyline, Azithromycin, Citalopram, Clotrimazole, Cocaine, DEET, Diphenhydramine, Erythromycin-H2O, Etoposide, Ibuprofen, Iopamidol, Metoprolol, Norfluoxetine, Propranolol, Sertraline, Triamterene, Triclocarban, Venlafaxine, Verapamil, and Virginiamycin M1.

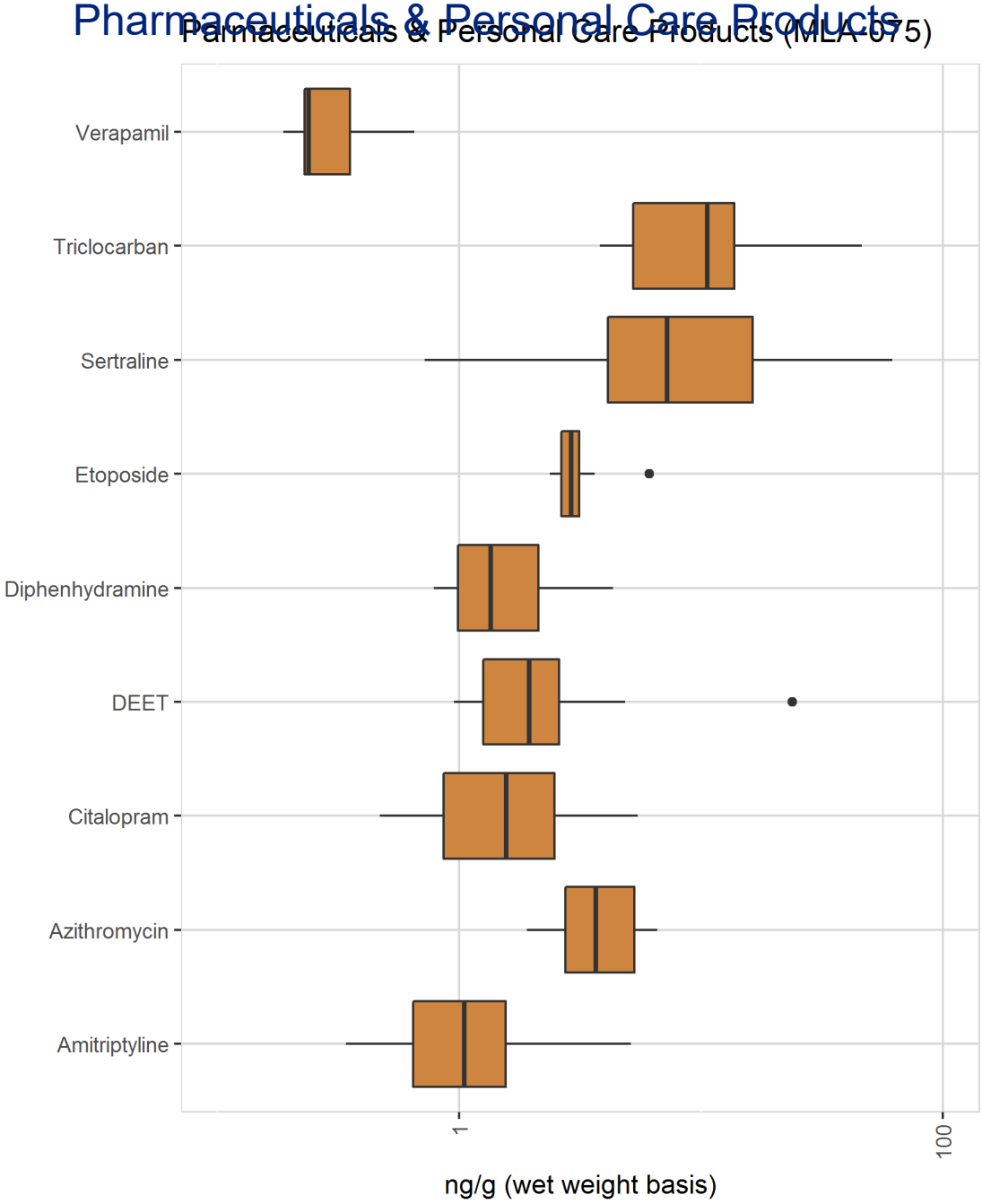
Summary of concentration data (3 x DL)

- The highest mean concentrations found were for Iopamidol, Sertraline and 2-Hydroxy-ibuprofen.
- Some of the PPCPs were in the same concentration range as compounds measured historically by the Mussel Watch Program such as aldrin and chlordane (see pesticide section).
- PPCP concentrations ranged four orders of magnitude for those compounds that were detected (0.06 - 269 ng/g wet).

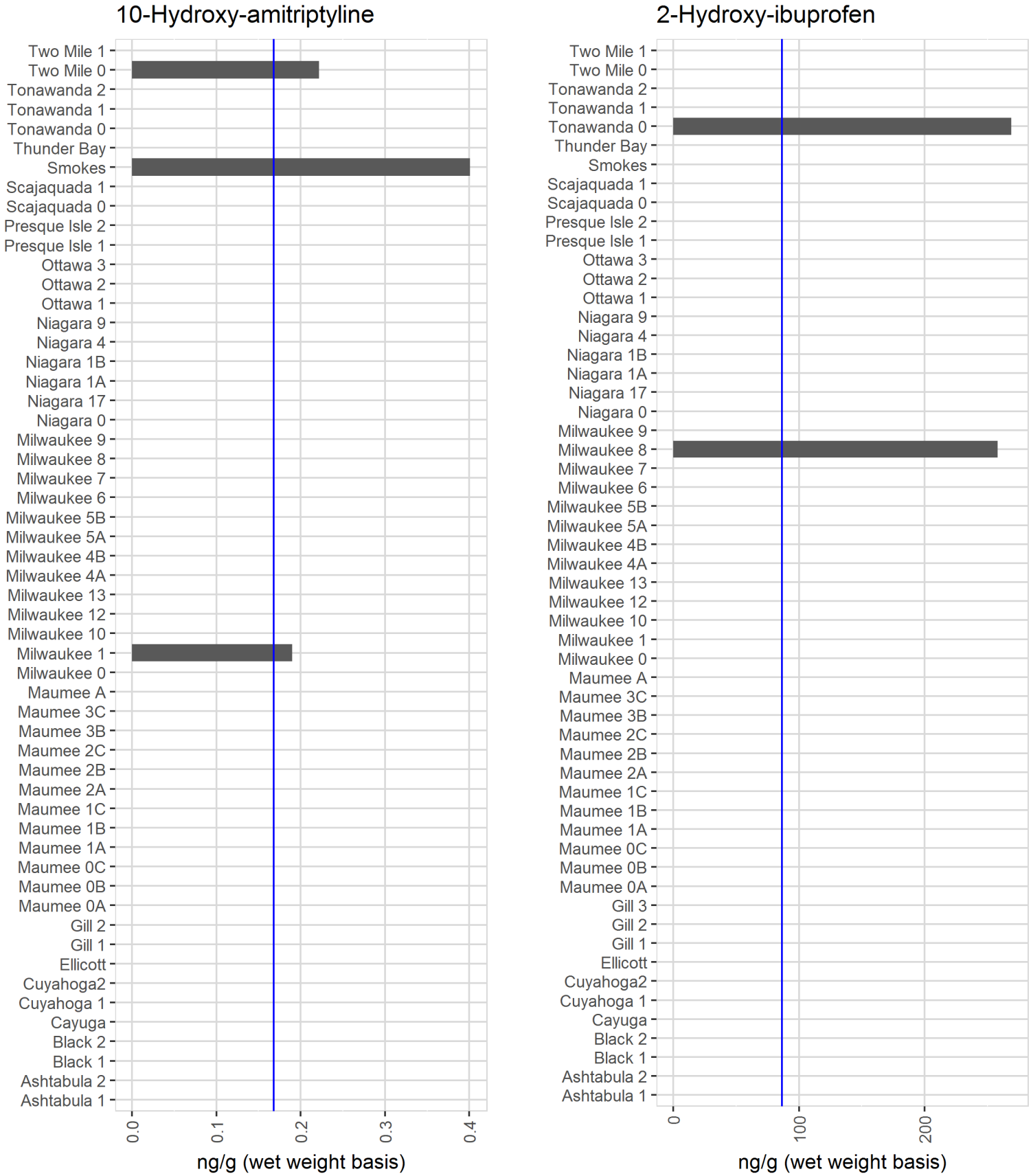
Chemical concentration table. Summary of compound concentrations in ng/g wet weight. Concentrations below 3 x detection limit were changed to zero. * List is not comprehensive and represents some of the most common usages of each compound.

			Concentration (ng/g wet weight)		
Compound	**Usage***		**maximum**	**mean**	**median**
2-Hydroxy-ibuprofen	Pharmaceutical	Anti-inflammatory	269	9.41	0.00
Iopamidol	Pharmaceutical	Contrast agent	261	19.27	0.00
Sertraline	Pharmaceutical	Antidepressant	61.7	12.42	7.25
Triclocarban	Pharmaceutical	Antibacterial	46.2	6.55	4.09
Ciprofloxacin	Pharmaceutical	Antibiotic	44.5	1.02	0.00
DEET	Personal Care	Insect repellent	23.75	1.18	0.00
Azithromycin	Pharmaceutical	Antibiotic	6.59	0.81	0.00
Etoposide	Pharmaceutical	Cancer	6.1	0.78	0.00
Citalopram	Pharmaceutical	Antidepressant	5.48	0.93	0.68
Fluoxetine	Pharmaceutical	Antidepressant	5.45	0.15	0.00
Amitriptyline	Pharmaceutical	Antidepressant	5.12	1.01	0.72
Diphenhydramine	Pharmaceutical	Antihistamine	4.33	0.58	0.00
Triamterene	Pharmaceutical	Blood pressure/Diuretic	3.62	0.09	0.00
Propranolol	Pharmaceutical	Blood pressure/Chest pain	3.11	0.16	0.00
Diltiazem	Pharmaceutical	Blood pressure/Chest pain	0.702	0.02	0.00
Venlafaxine	Pharmaceutical	Antidepressant	0.662	0.01	0.00
Verapamil	Pharmaceutical	Antihypertensive	0.654	0.06	0.00
10-Hydroxy-amitriptyline	Pharmaceutical	Metabolite of Amitriptyline	0.401	0.01	0.00
Cocaine	Recreational drug	Stimulant	0.311	0.01	0.00

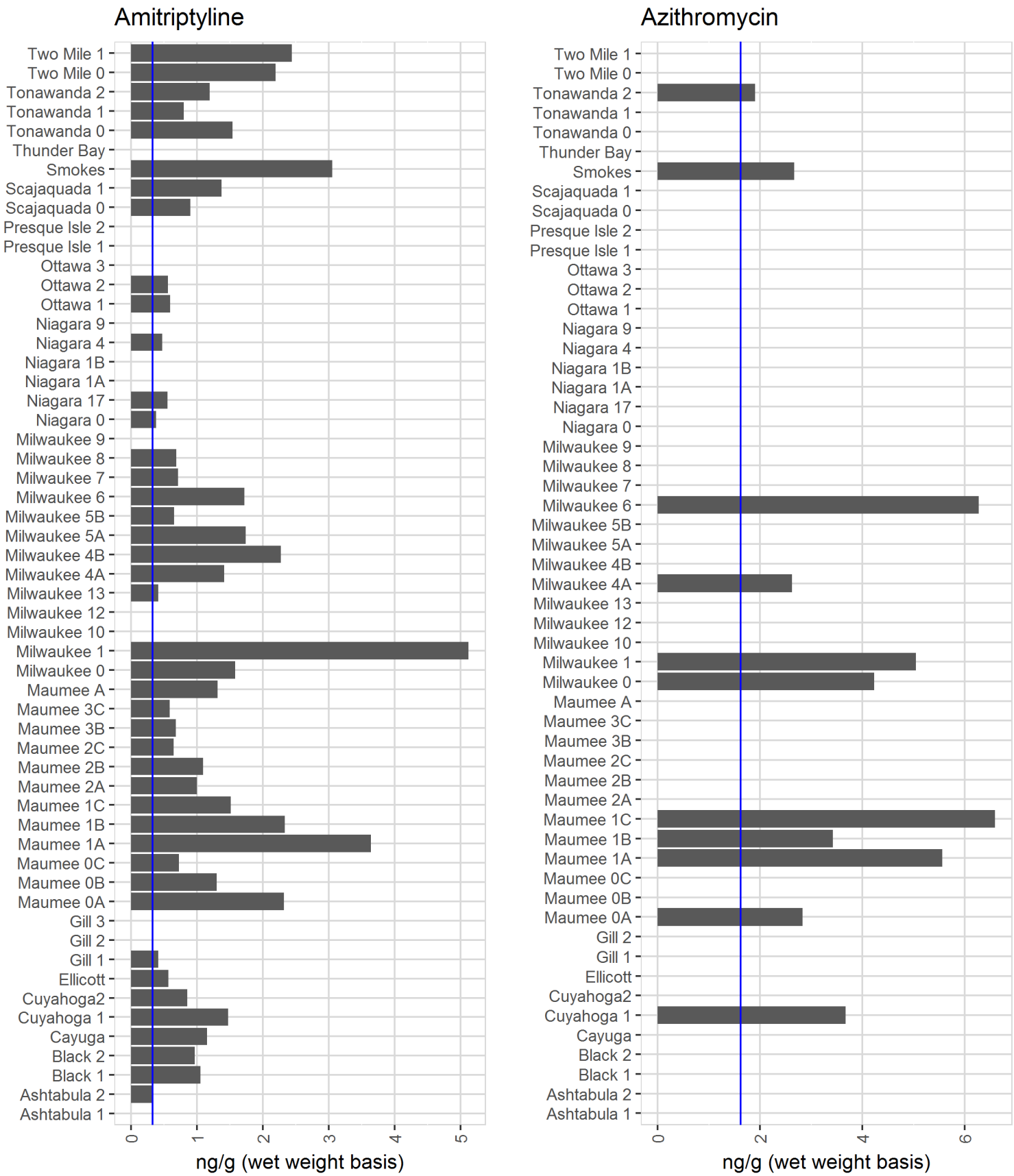
Pharmaceuticals & Personal Care Products



Box and whisker plot. Only chemical compounds found at more than five sites that had concentrations above 3 x detection limit are included. Measurements of zero were not included. This plot provides perspective to the relative concentrations of most commonly found PPCPs in dreissenid mussel tissue.



Bar plots. Concentration results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Blue line represents 3 x detection limit. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in June and July of 2014 respectively.



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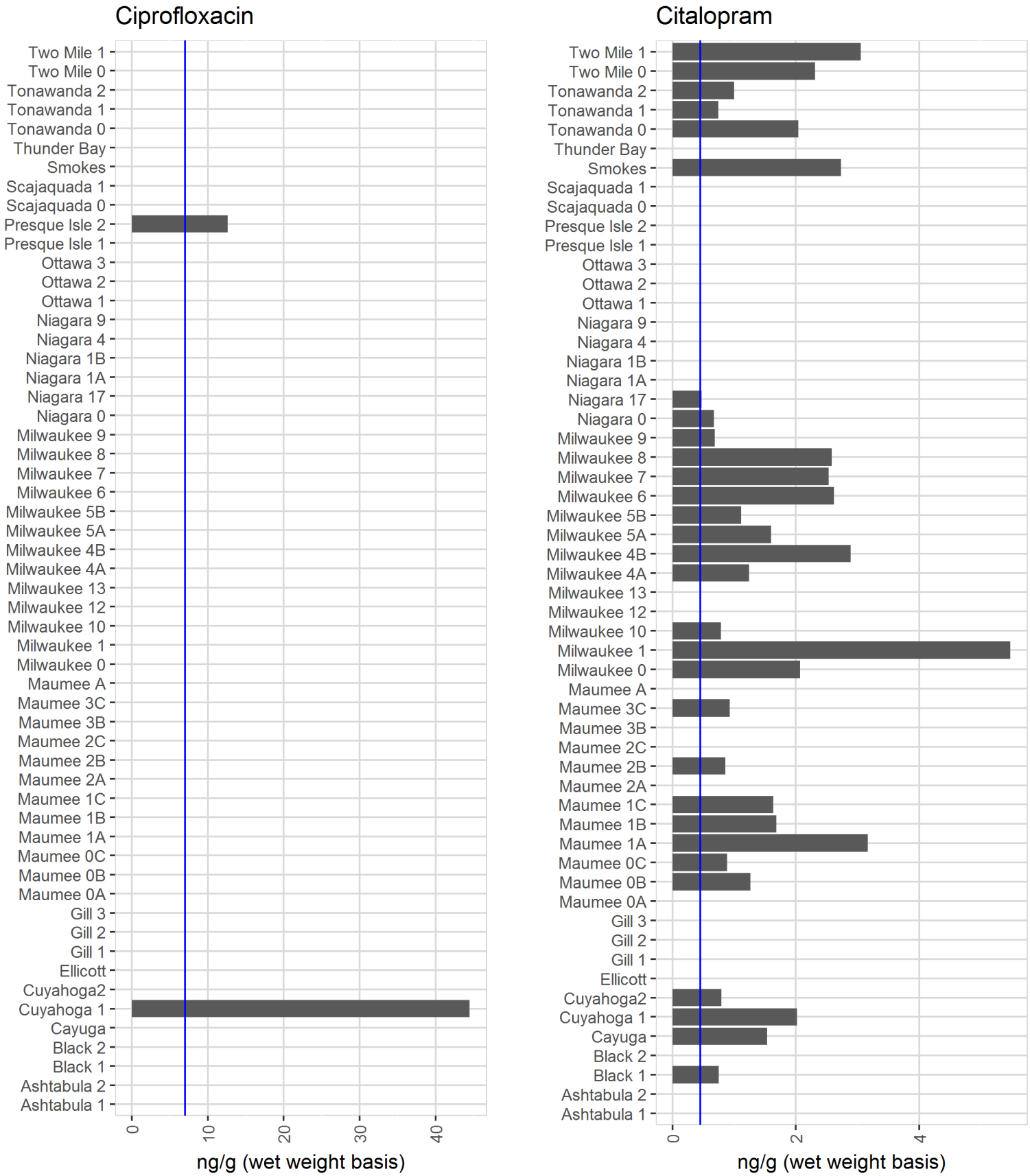

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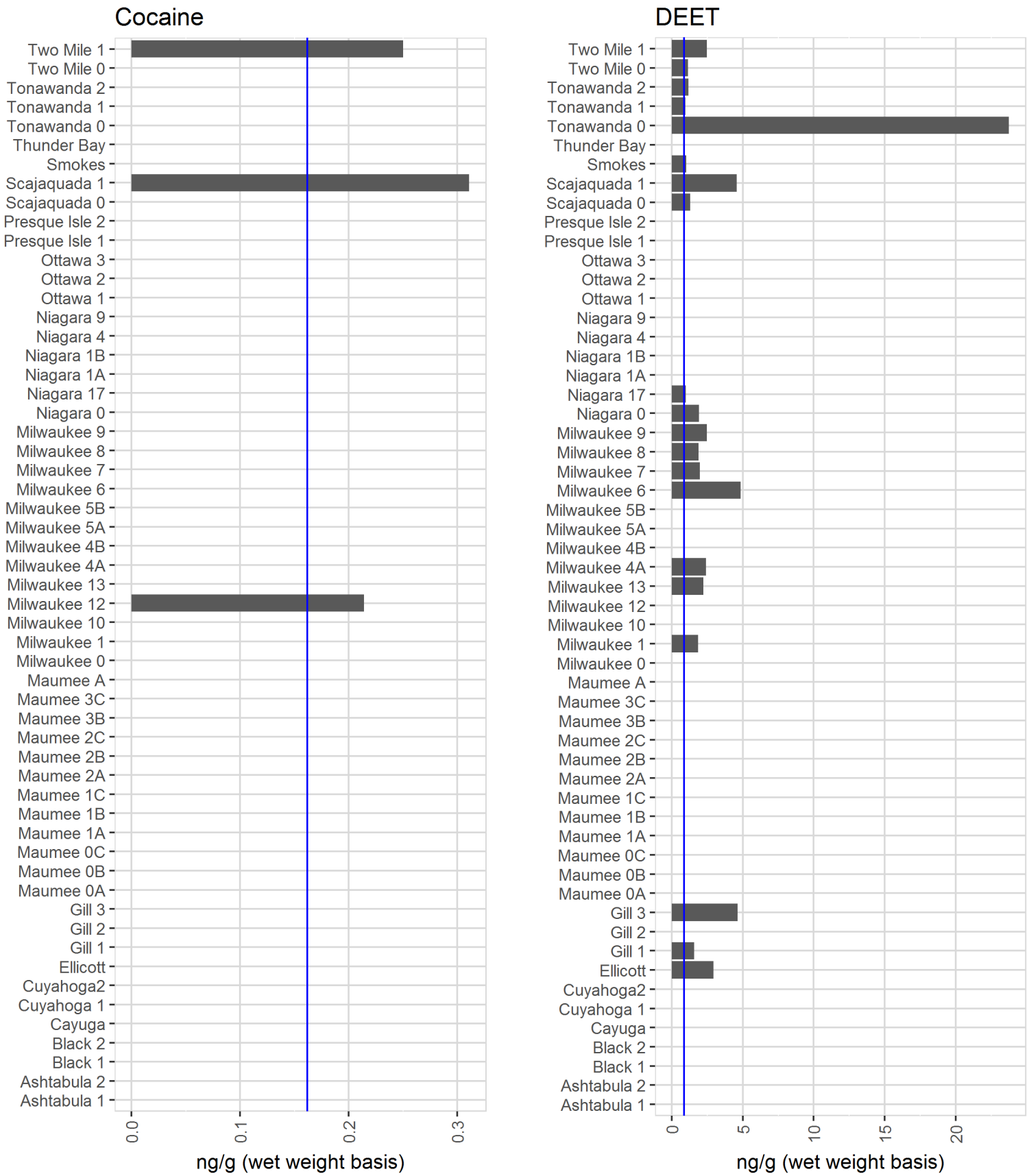

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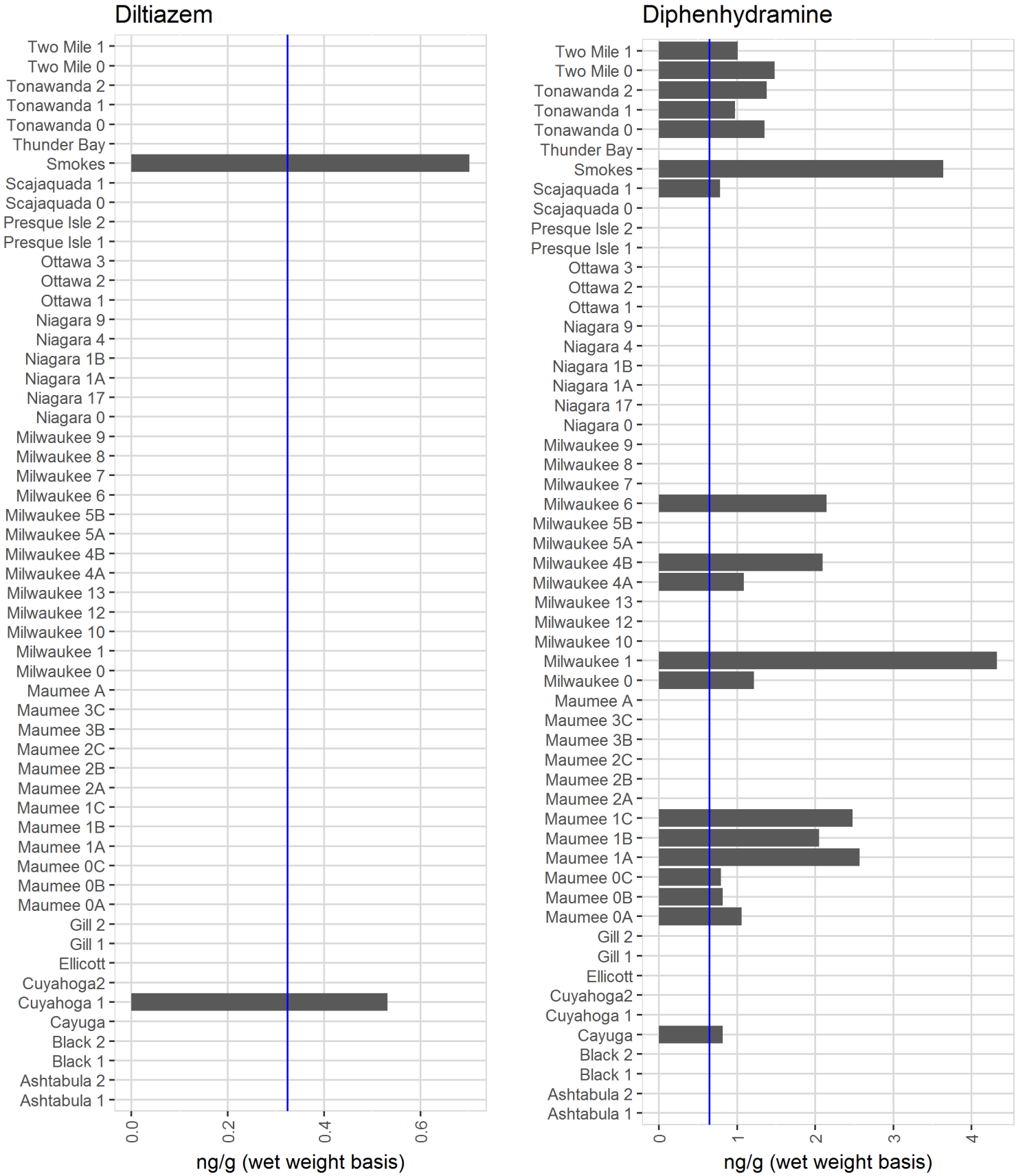

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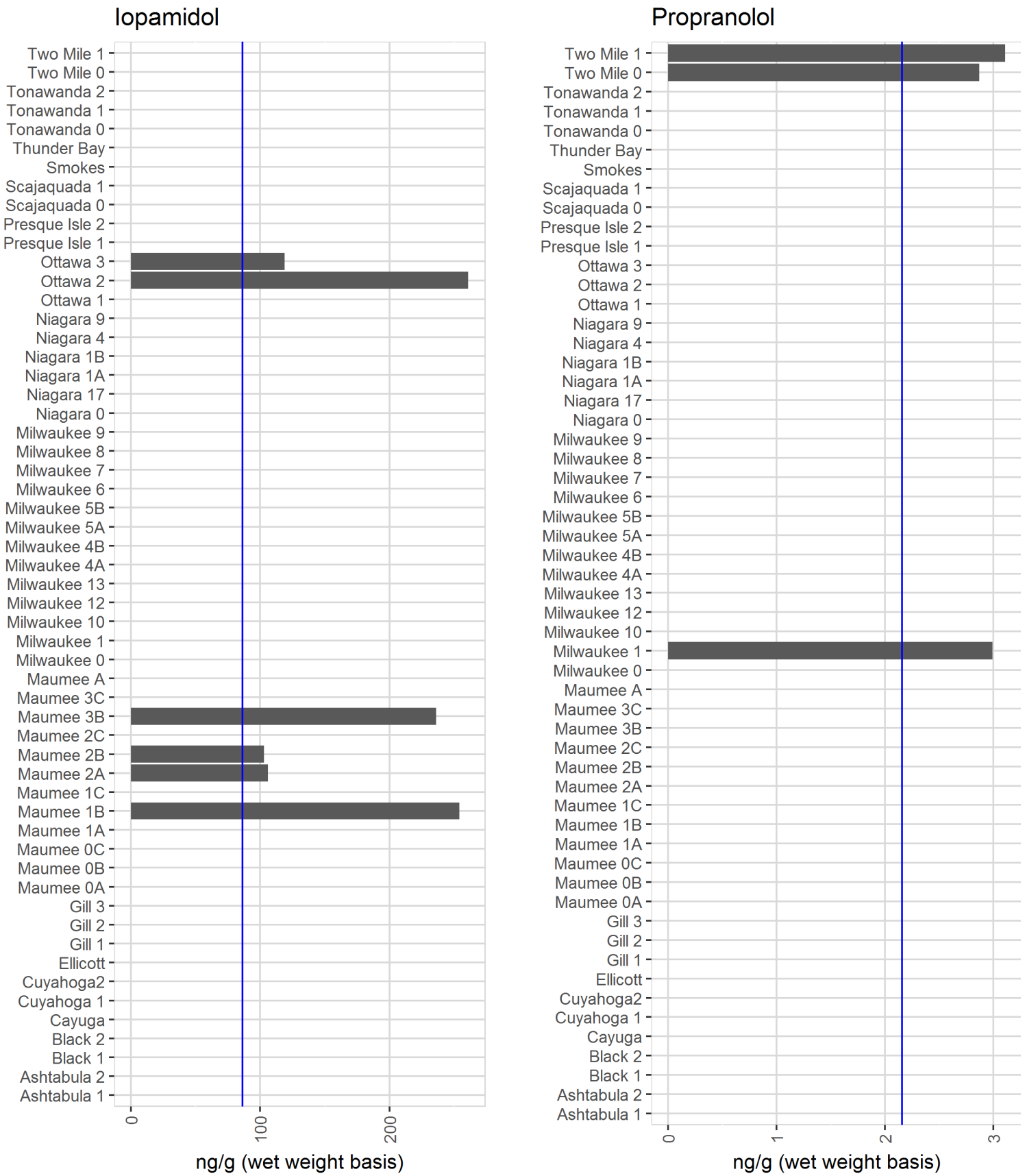

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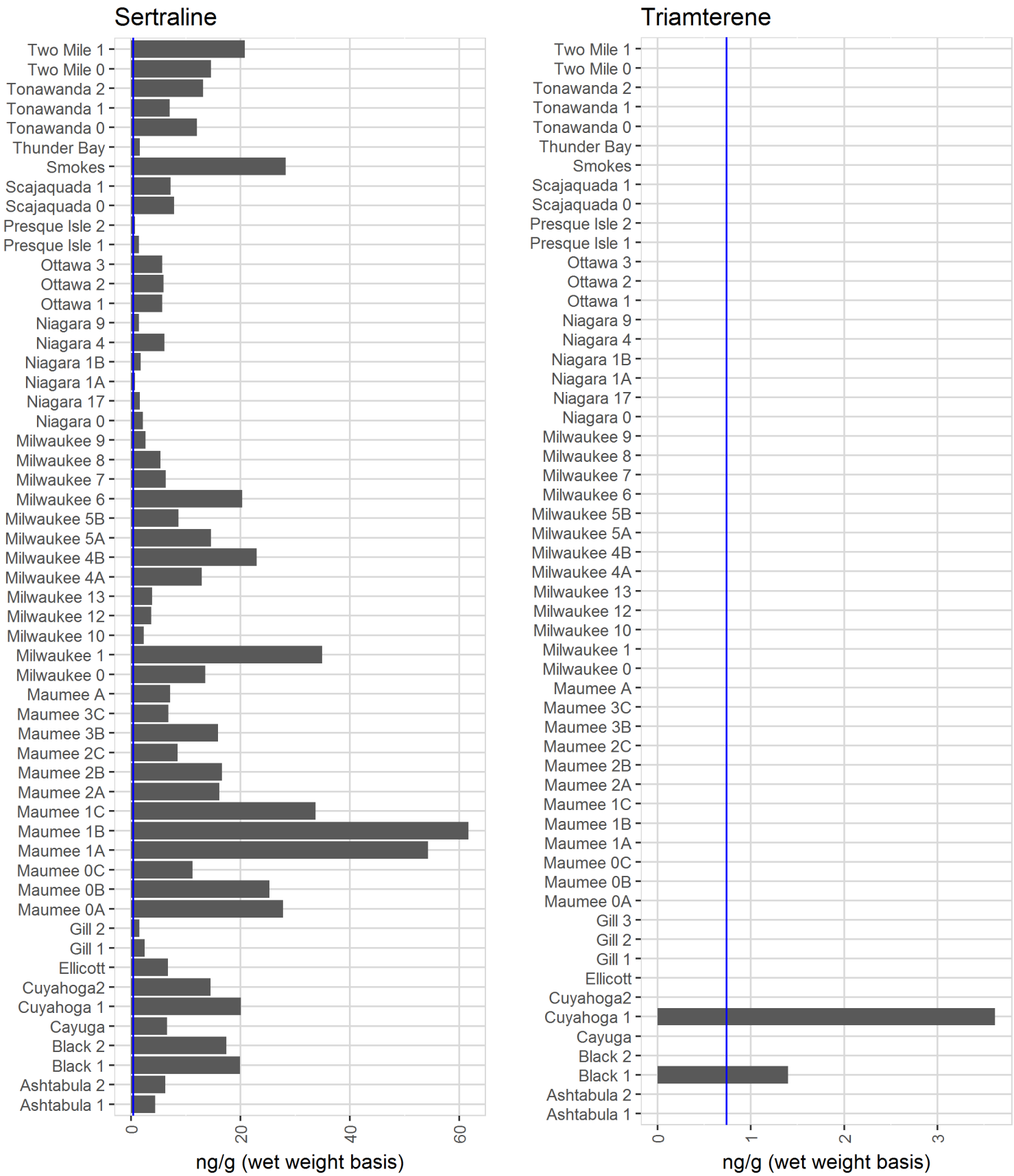

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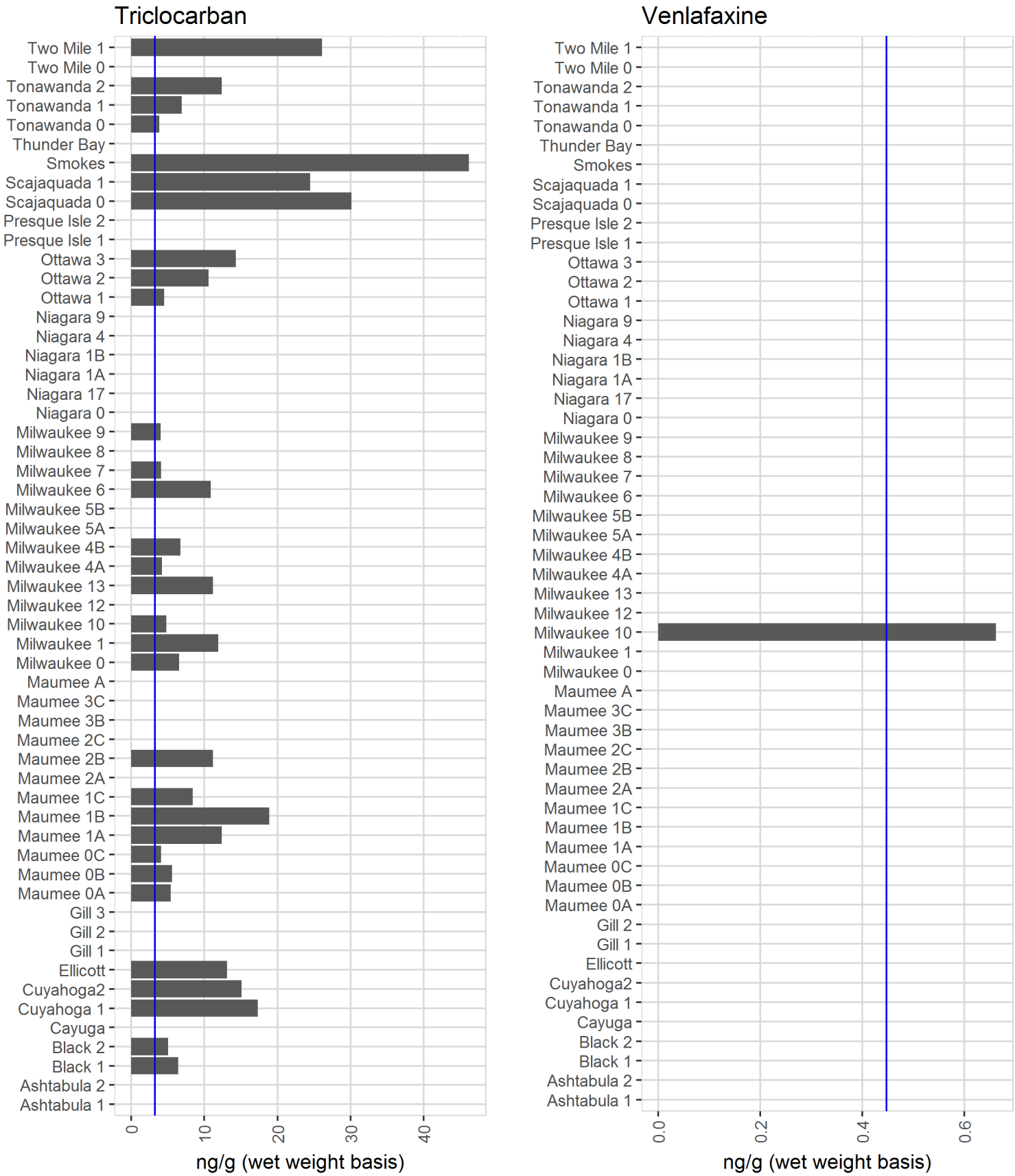

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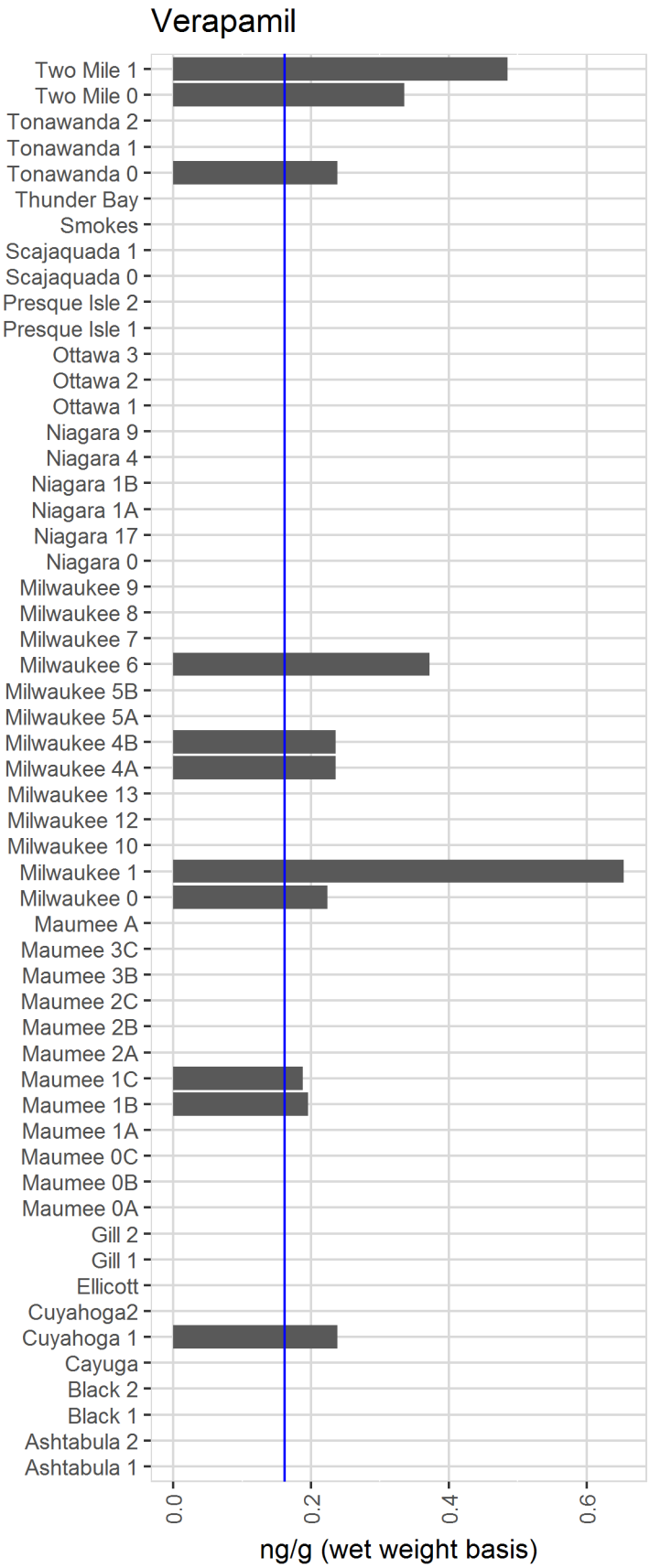

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Site concentration table. Concentration results (ng/g wet weight) for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site Name	10-Hydroxy-amitriptyline	2-Hydroxy-ibuprofen	Amitriptyline	Azithromycin	Ciprofloxacin	Citalopram	Cocaine	DEET	Diltiazem	Diphenhydramine
Ashtabula River 1	0	0	0	0	0	0	0	0	0	0
Ashtabula River 2	0	0	0.34	0	0	0	0	0	0	0
Black 1	0	0	1.05	0	0	0.746	0	0	0	0
Black 2	0	0	0.97	0	0	0	0	0	0	0
Cayuga	0	0	1.15	0	0	1.53	0	0	0	0.814
Cuyahoga 1	0	0	1.47	3.67	44.5	2.02	0	0	0.532	0
Cuyahoga 2	0	0	0.85	0	0	0.792	0	0	0	0
Ellicott	0	0	0.57	0	0	0	0	2.944	0	0
Gill 1	0	0	0.41	0	0	0	0	1.564	0	0
Gill 2	0	0	0	0	0	0	0	0	0	0
Gill 3		0	0		0	0	0	4.644		
Maumee 0A	0	0	2.32	2.83	0	0	0	0	0	1.06
Maumee 0B	0	0	1.3	0	0	1.26	0	0	0	0.817
Maumee 0C	0	0	0.73	0	0	0.884	0	0	0	0.791
Maumee 1A	0	0	3.64	5.56	0	3.17	0	0	0	2.57
Maumee 1B	0	0	2.33	3.42	0	1.68	0	0	0	2.05
Maumee 1C	0	0	1.51	6.59	0	1.63	0	0	0	2.48
Maumee 2A	0	0	1.00	0	0	0	0	0	0	0
Maumee 2B	0	0	1.09	0	0	0.858	0	0	0	0
Maumee 2C	0	0	0.64	0	0	0	0	0	0	0
Maumee 3A	0	0	0.68	0	0	0	0	0	0	0
Maumee 3B	0	0	0.59	0	0	0.925	0	0	0	0
Maumee 3C	0	0	1.31	0	0	0	0	0	0	0
Milwaukee 0	0	0	1.58	4.23	0	2.07	0	0	0	1.22
Milwaukee 1	0.19	0	5.12	5.04	0	5.48	0	1.84	0	4.33
Milwaukee 10	0	0	0	0	0	0.787	0	0	0	0
Milwaukee 12	0	0	0	0	0	0	0.214	0	0	0
Milwaukee 13	0	0	0.41	0	0	0	0	2.22	0	0
Milwaukee 4A	0	0	1.41	2.62	0	1.24	0	2.42	0	1.09
Milwaukee 4B	0	0	2.27	0	0	2.89	0	0	0	2.10
Milwaukee 5A	0	0	1.74	0	0	1.6	0	0	0	0
Milwaukee 5B	0	0	0.65	0	0	1.11	0	0	0	0
Milwaukee 6	0	0	1.72	6.27	0	2.62	0	4.84	0	2.15
Milwaukee 7	0	0	0.71	0	0	2.53	0	1.97	0	0
Milwaukee 8	0	258	0.69	0	0	2.58	0	1.89	0	0
Milwaukee 9	0	0	0	0	0	0.683	0	2.47	0	0

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Site Name	10-Hydroxy-amitriptyline	2-Hydroxy-ibuprofen	Amitriptyline	Azithromycin	Ciprofloxacin	Citalopram	Cocaine	DEET	Diltiazem	Diphenhydramine
Niagara 0	0	0	0.38	0	0	0.67	0	1.924	0	0
Niagara 17	0	0	0.55	0	0	0.47	0	0.994	0	0
Niagara 1A	0	0	0	0	0	0	0	0	0	0
Niagara 1B	0	0	0	0	0	0	0	0	0	0
Niagara 4	0	0	0.47	0	0	0	0	0	0	0
Niagara 9	0	0	0	0	0	0	0	0	0	0
Ottawa 1	0	0	0.59	0	0	0	0	0	0	0
Ottawa 2	0	0	0.56	0	0	0	0	0	0	0
Ottawa 3	0	0	0	0	0	0	0	0	0	0
Presque Isle 1	0	0	0	0	0	0	0	0	0	0
Presque Isle 2	0	0	0	0	12.6	0	0	0	0	0
Scajaquada 0	0	0	0.90	0	0	0	0	1.28	0	0
Scajaquada 1	0	0	1.37	0	0	0	0.311	4.58	0	0.784
Smokes	0.401	0	3.05	2.67	0	2.73	0	1.01	0.702	3.64
Thunder Bay National Marine Sanctuary	0	0	0	0	0	0	0	0	0	0
Tonawanda 0	0	269	1.54	0	0	2.04	0	23.8	0	1.35
Tonawanda 1	0	0	0.802	0	0	0.739	0	0.954	0	0.971
Tonawanda 2	0	0	1.19	1.9	0	1	0	1.184	0	1.38
Two Mile 0	0.222	0	2.19	0	0	2.31	0	1.15	0	1.48
Two Mile 1	0	0	2.44	0	0	3.05	0.25	2.48	0	1.01

Site concentration table. Concentration results (ng/g wet weight) for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site Name	Etoposide	Fluoxetine	Iopamidol	Propranolol	Sertraline	Triamterene	Triclocarban	Venlafaxine	Verapamil
Ashtabula River 1	0	0	0	0	4.44	0	0	0	0
Ashtabula River 2	3.09	0	0	0	6.27	0	0	0	0
Black 1	0	0	0	0	19.9	1.4	6.41	0	0
Black 2	0	0	0	0	17.4	0	5.07	0	0
Cayuga	0	0	0	0	6.59	0	0	0	0
Cuyahoga 1	0	0	0	0	20.1	3.62	17.3	0	0.238
Cuyahoga 2	2.52	0	0	0	14.5	0	15.1	0	0
Ellicott	0	0	0	0	6.77	0	13.1	0	0
Gill 1	0	0	0	0	2.47	0	0	0	0
Gill 2	0	0	0	0	1.52	0	0	0	0
Gill 3	0	0	0	0		0	0		
Maumee 0A	0	0	0	0	27.8	0	5.43	0	0
Maumee 0B	0	0	0	0	25.3	0	5.59	0	0
Maumee 0C	2.91	0	0	0	11.2	0	4.09	0	0
Maumee 1A	0	0	0	0	54.3	0	12.4	0	0
Maumee 1B	0	0	254	0	61.7	0	18.9	0	0.196
Maumee 1C	0	0	0	0	33.7	0	8.43	0	0.188
Maumee 2A	0	0	106	0	16.1	0	0	0	0
Maumee 2B	0	0	103	0	16.6	0	11.2	0	0
Maumee 2C	2.89	0	0	0	8.53	0	0	0	0
Maumee 3A	6.1	0	236	0	15.9	0	0	0	0
Maumee 3B	0	0	0	0	6.83	0	0	0	0
Maumee 3C	0	0	0	0	7.12	0	0	0	0
Milwaukee 0	0	0	0	0	13.6	0	6.53	0	0.224
Milwaukee 1	0	5.45	0	2.99	34.9	0	11.9	0	0.654
Milwaukee 10	0	0	0	0	2.35	0	4.81	0.662	0
Milwaukee 12	0	0	0	0	3.68	0	0	0	0
Milwaukee 13	2.48	0	0	0	3.83	0	11.2	0	0
Milwaukee 4A	0	0	0	0	12.9	0	4.21	0	0.236
Milwaukee 4B	2.76	0	0	0	23	0	6.73	0	0.236
Milwaukee 5A	0	0	0	0	14.6	0	0	0	0
Milwaukee 5B	3.51	0	0	0	8.64	0	0	0	0
Milwaukee 6	3.63	0	0	0	20.3	0	10.9	0	0.372
Milwaukee 7	0	0	0	0	6.33	0	4.08	0	0
Milwaukee 8	0	0	0	0	5.39	0	0	0	0
Milwaukee 9	0	0	0	0	2.64	0	4.01	0	0

Site concentration table. Concentration results (ng/g wet weight) for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site Name	Etoposide	Fluoxetine	Iopamidol	Propranolol	Sertraline	Triamterene	Triclocarban	Venlafaxine	Verapamil
Niagara 0	0	0	0	0	2.12	0	0	0	0
Niagara 17	0	0	0	0	1.59	0	0	0	0
Niagara 1A	2.37	0	0	0	0.727	0	0	0	0
Niagara 1B	0	0	0	0	1.72	0	0	0	0
Niagara 4	0	0	0	0	6.11	0	0	0	0
Niagara 9	0	0	0	0	1.45	0	0	0	0
Ottawa 1	0	0	0	0	5.7	0	4.49	0	0
Ottawa 2	2.9	0	261	0	5.93	0	10.6	0	0
Ottawa 3	0	0	119	0	5.71	0	14.3	0	0
Presque Isle 1	0	0	0	0	1.41	0	0	0	0
Presque Isle 2	0	0	0	0	0.72	0	0	0	0
Scajaquada 0	0	0	0	0	7.83	0	30.1	0	0
Scajaquada 1	0	0	0	0	7.25	0	24.5	0	0
Smokes	0	2.7	0	0	28.3	0	46.2	0	0
Thunder Bay National Marine Sanctuary	0	0	0	0	1.6	0	0	0	0
Tonawanda 0	2.9	0	0	0	12	0	3.82	0	0.238
Tonawanda 1	0	0	0	0	7.02	0	6.93	0	0
Tonawanda 2	3.14	0	0	0	13.2	0	12.4	0	0
Two Mile 0	0	0	0	2.87	14.6	0	0	0	0.335
Two Mile 1	2.6	0	0	3.11	20.8	0	26.1	0	0.485

Pesticides

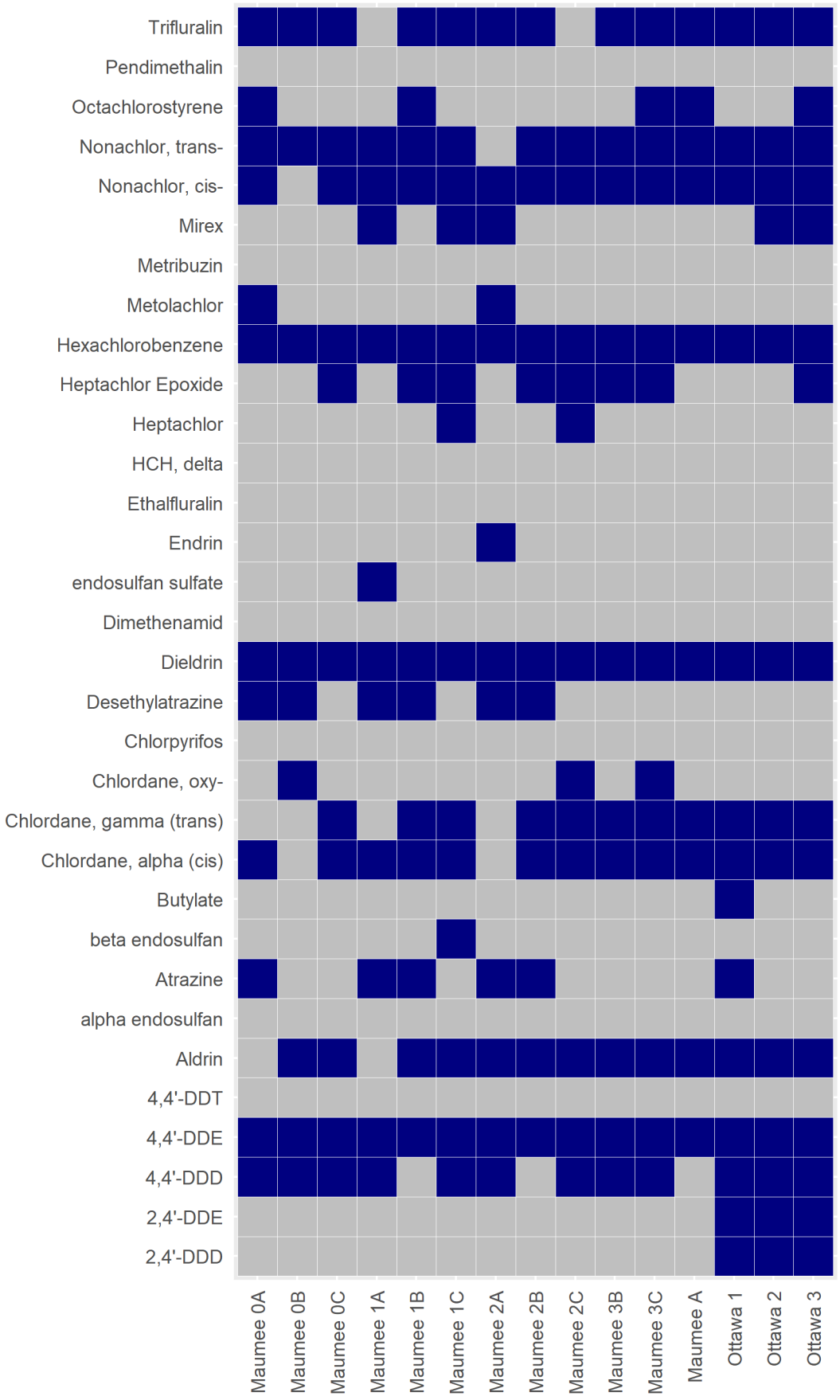
The pesticides method contains both current use pesticides (CUPs) and pesticides historically measured by the Mussel Watch Program. One of the benefits of utilizing this method is that it allows for CUPs to be compared to concentrations of the more persistent pesticides that have been historically measured by the program. This method analyzed a total of 74 pesticides and metabolites in samples from the Maumee and Ottawa River study exclusively. Adverse biological effects to non-target species such as fish, birds, amphibians including non-target insect and plant species is a concern. The Ottawa and the Maumee study sub-watersheds include the city of Toledo and are therefore primarily developed. The Maumee and Ottawa Rivers both have substantial cultivation taking place at the larger watershed level.

Chemical list table. Pesticides by GC/HRMS (MLA-035.R07.02). Concentrations above the detection limit, found in at least one sample, are identified in bold red type. * represents compounds not included in analyses because they were all NQ.

2,4'-DDD	Chlorothalonil	Fenitrothion	**Nonachlor, cis-**
2,4'-DDE	**Chlorpyrifos**	Flufenacet	**Nonachlor, trans-**
2,4'-DDT	Chlorpyrifos-Methyl	Flutriafol	**Octachlorostyrene**
4,4'-DDD	Chlorpyrifos-Oxon	Fonofos	Parathion-Ethyl
4,4'-DDE	Cyanazine	HCH, alpha	Parathion-Methyl
4,4'-DDT	Dacthal	HCH, beta	**Pendimethalin**
Alachlor	**Desethylatrazine**	**HCH, delta**	Perthane
Aldrin	Diazinon	HCH, gamma	Phorate
alpha-Endosulfan	Diazinon-Oxon	**Heptachlor**	Phosmet
Ametryn	**Dieldrin**	**Heptachlor Epoxide**	Pirimiphos-Methyl
Atrazine	**Dimethenamid**	**Hexachlorobenzene**	Quintozene
Azinphos-Methyl	Dimethoate	Hexazinone	Simazine
beta-Endosulfan	Disulfoton	Linuron	Tebuconazol
Butralin	Disulfoton Sulfone	Malathion	Tecnazene
Butylate	**Endosulfan Sulfate**	Methoprene	Terbufos
Captan*	**Endrin**	Methoxychlor	Triallate
Chlordane, alpha (cis)	Endrin Ketone	**Metolachlor**	**Trifluralin**
Chlordane, gamma (trans)	**Ethalfluralin**	**Metribuzin**	**Trifluralin**
Chlordane, oxy-	Ethion*	**Mirex**	

Pesticides

Multi-Residue Pesticides by GC/HRMS (MLA-035)



Heat map. Presence (■) and absence (□) of each compound found in mussel tissue from Maumee and Ottawa Rivers. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively.

Pesticides

Site table. Presentation of all the locations where the pesticide analysis was used to analyze tissue samples.

Multi-Residue Pesticides	Sites (n)
Maumee River	12
Ottawa River	3

Method table. Summary of compound detections for the pesticide analysis. Percentage categories are used to characterize how often a compound was found in a sample at 3 x DL.

Compound percentage categories	Samples	
	n	%
Compound not found	40	56
Compound found in 1-24 % of samples	11	15
Compound found in 25-49 % of samples	6	8
Compound found in 50-74 % of samples	3	4
Compound found in 75-100 % of samples	12	17

Summary of detections (Heat map figure)

- Of the 72 compounds analyzed, 32 (44%) were detected in dreissenid mussels.
- Trifluralin was the only CUP detected at all sites.
- Many CUPs -Atrazine, Butylate, Desethylatrazine, Metolachlor, Octachlorostyrene, and Trifluralin were detected at the Maumee 3 reference site.
- Most of the compounds found with this method were persistent compounds historically measured by the Mussel Watch Program.

Summary of concentration data (3 x DL)

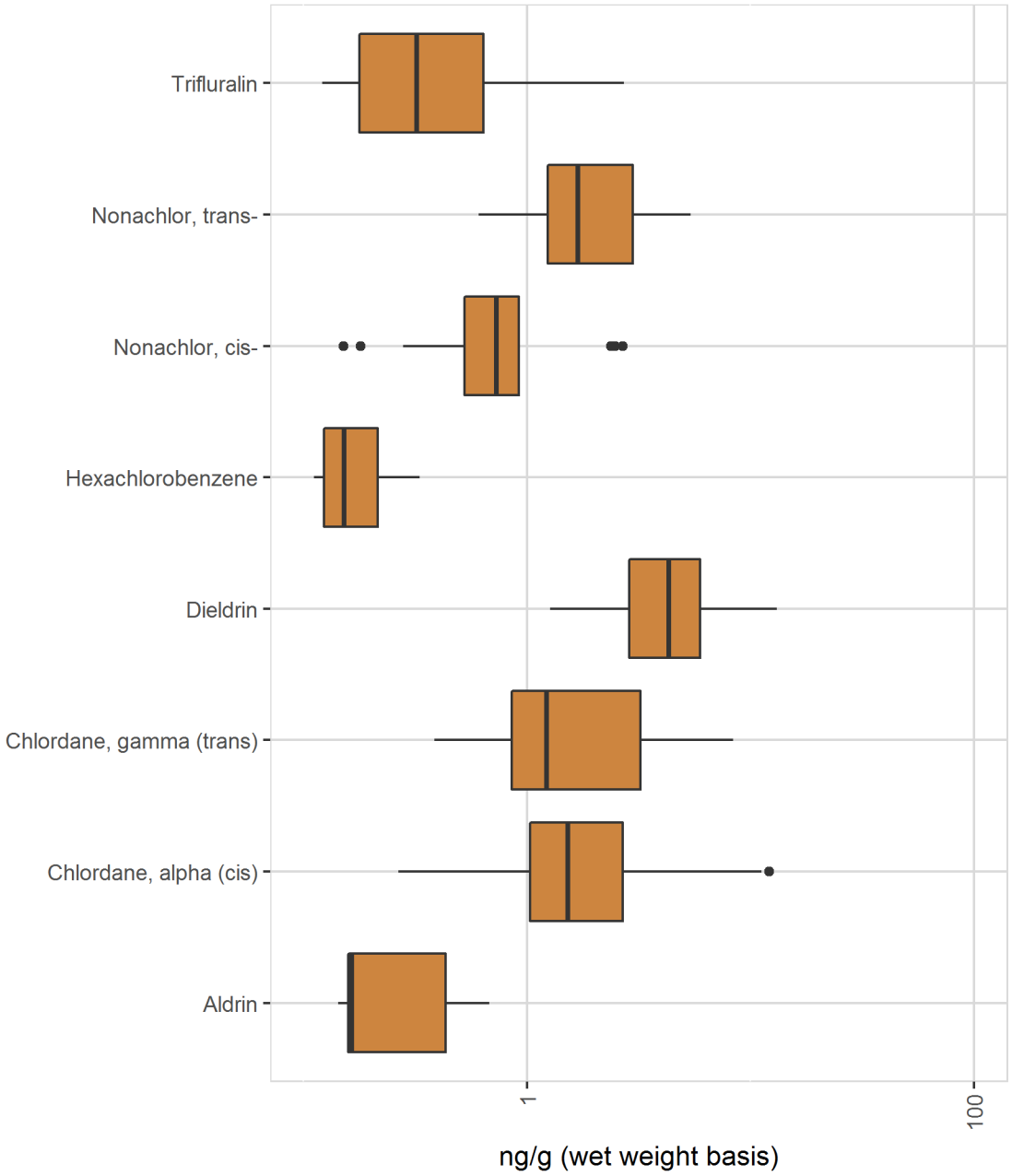
- Concentrations for CUPs ranged about three orders of magnitude for those compounds that were detected (0.02 - 48.3 ng/g wet).
- Atrazine was the CUP with the highest concentration (23.2 ng/g wet) in mussels.
- Fewer CUPs were detected relative to historically measured pesticides, however,concentration ranges were similar in tissue samples (pg/g wet to ng/g wet).

Chemical concentration table. Summary of compound concentrations in ng/g wet weight. Concentrations below 3 x detection limit were changed to zero.

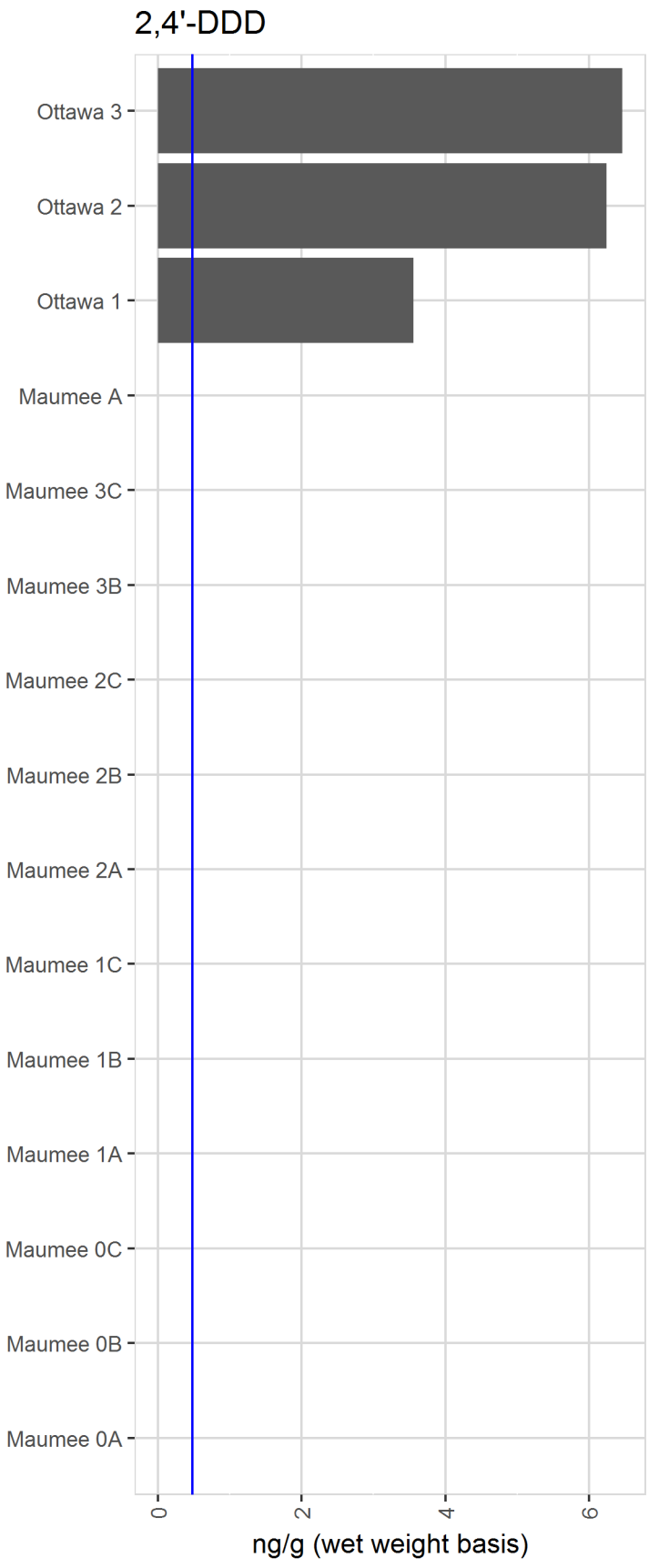
			Concentration (ng/g wet weight)		
Compound	**Usage**		**maximum**	**mean**	**median**
4,4'-DDE	Legacy	Insecticide	32.4	7.25	2.32
Atrazine	CUP	Herbicide	23.2	5.44	0.00
4,4'-DDD	Legacy	Insecticide	22.2	4.55	1.44
Metolachlor	CUP	Herbicide	13.7	1.37	0.00
Dieldrin	Legacy	Insecticide	13.1	5.10	4.32
Chlordane, alpha (cis)	Legacy	Insecticide	12.1	3.04	1.31
Chlordane, gamma (trans)	Legacy	Insecticide	8.34	1.94	0.88
2,4'-DDD	Legacy	Insecticide	6.46	1.08	0.00
Nonachlor, trans-	Legacy	Insecticide	5.4	2.06	1.56
Trifluralin	CUP	Herbicide	2.71	0.53	0.32
Nonachlor, cis-	Legacy	Insecticide	2.68	0.92	0.71
Butylate	CUP	Herbicide	1.65	0.11	0.00
2,4'-DDE	Legacy	Insecticide	1.34	0.23	0.00
Desethylatrazine	CUP	Herbicide	1.23	0.34	0.00
Heptachlor Epoxide	Legacy	Insecticide	0.758	0.27	0.40
Aldrin	Legacy	Insecticide	0.678	0.18	0.14
Endosulfan Sulfate	Legacy	Insecticide	0.499	0.03	0.00
beta-Endosulfan	Legacy	Insecticide	0.36	0.02	0.00
Chlordane, oxy-	Legacy	Insecticide	0.355	0.06	0.00
Hexachlorobenzene	Legacy	Insecticide	0.331	0.15	0.12
Heptachlor	Legacy	Insecticide	0.256	0.03	0.00
Octachlorostyrene	CUP	Byproduct	0.185	0.04	0.00
Mirex	Legacy	Insecticide	0.107	0.02	0.00
Endrin	Legacy	Insecticide	0.059	0.00	0.00

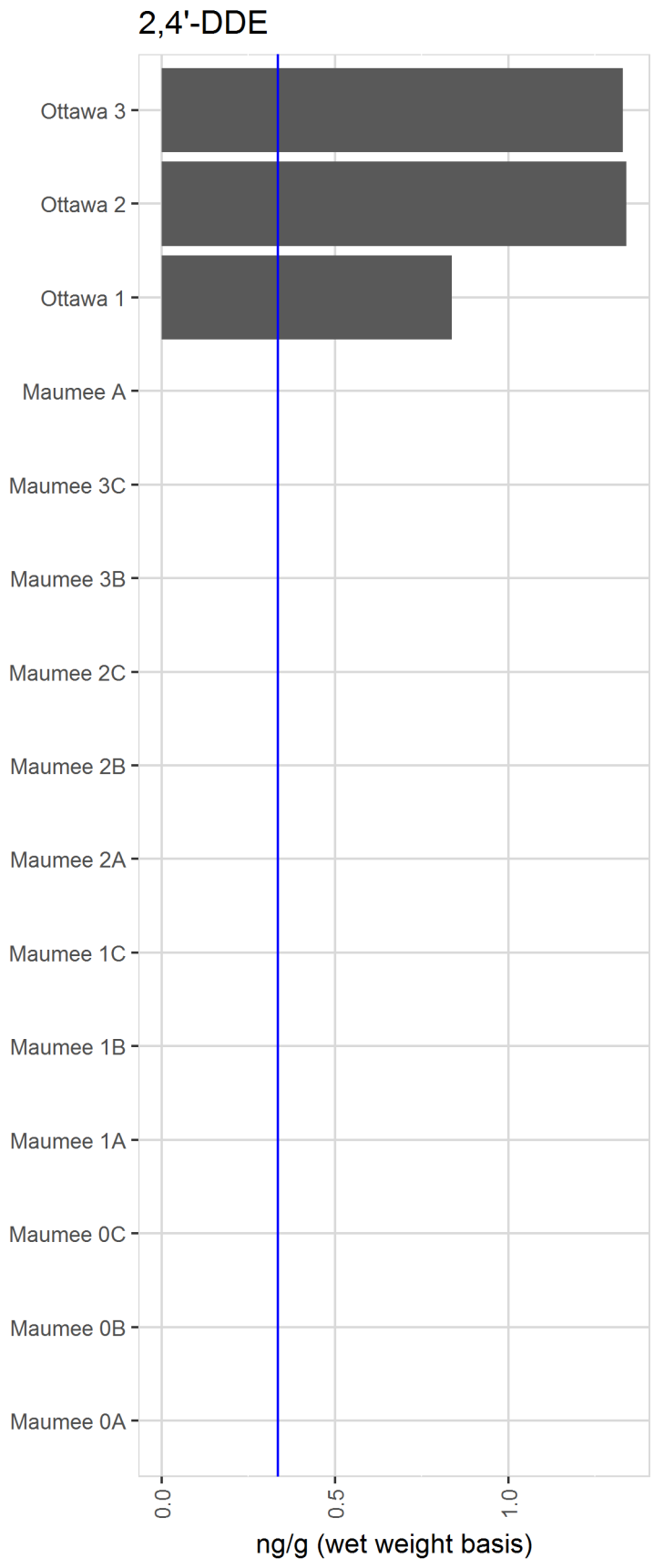
Pesticides

Multi-Residue Pesticides by GC/HRMS (MLA-035)

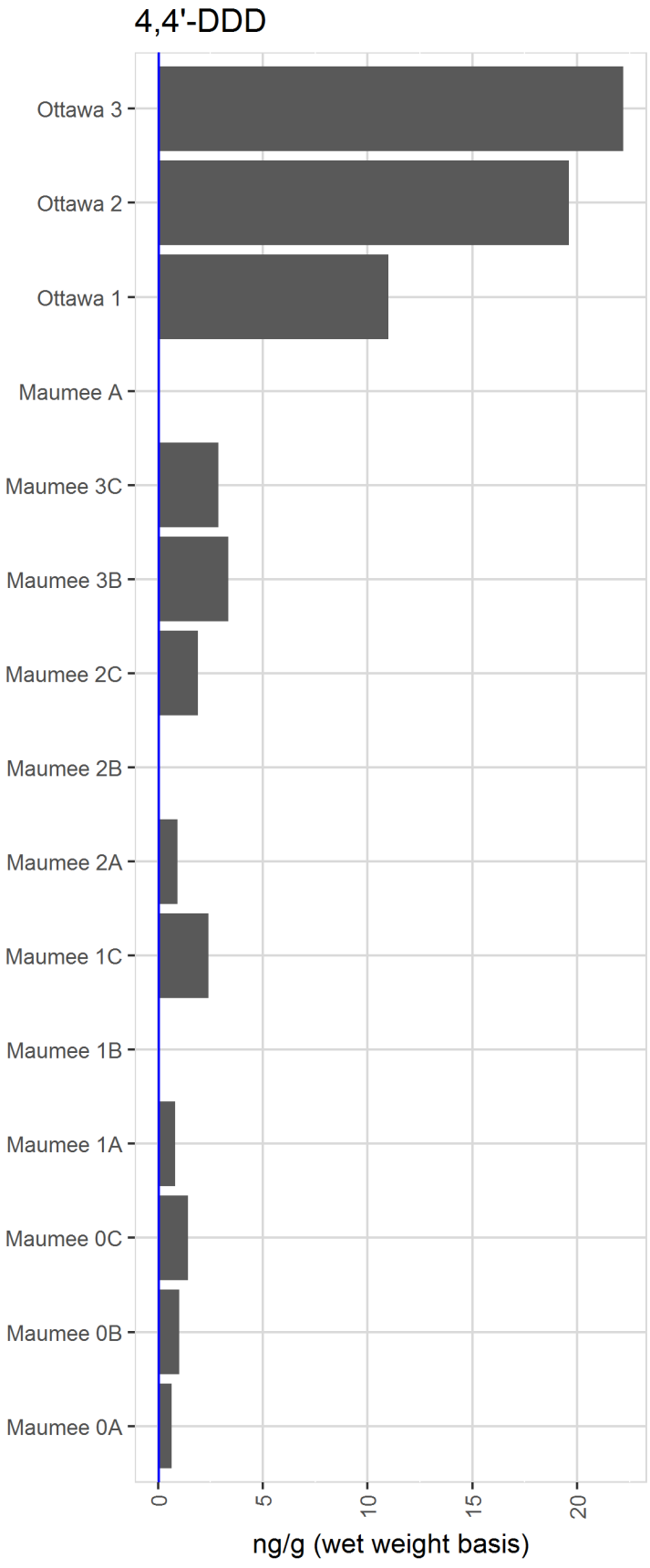


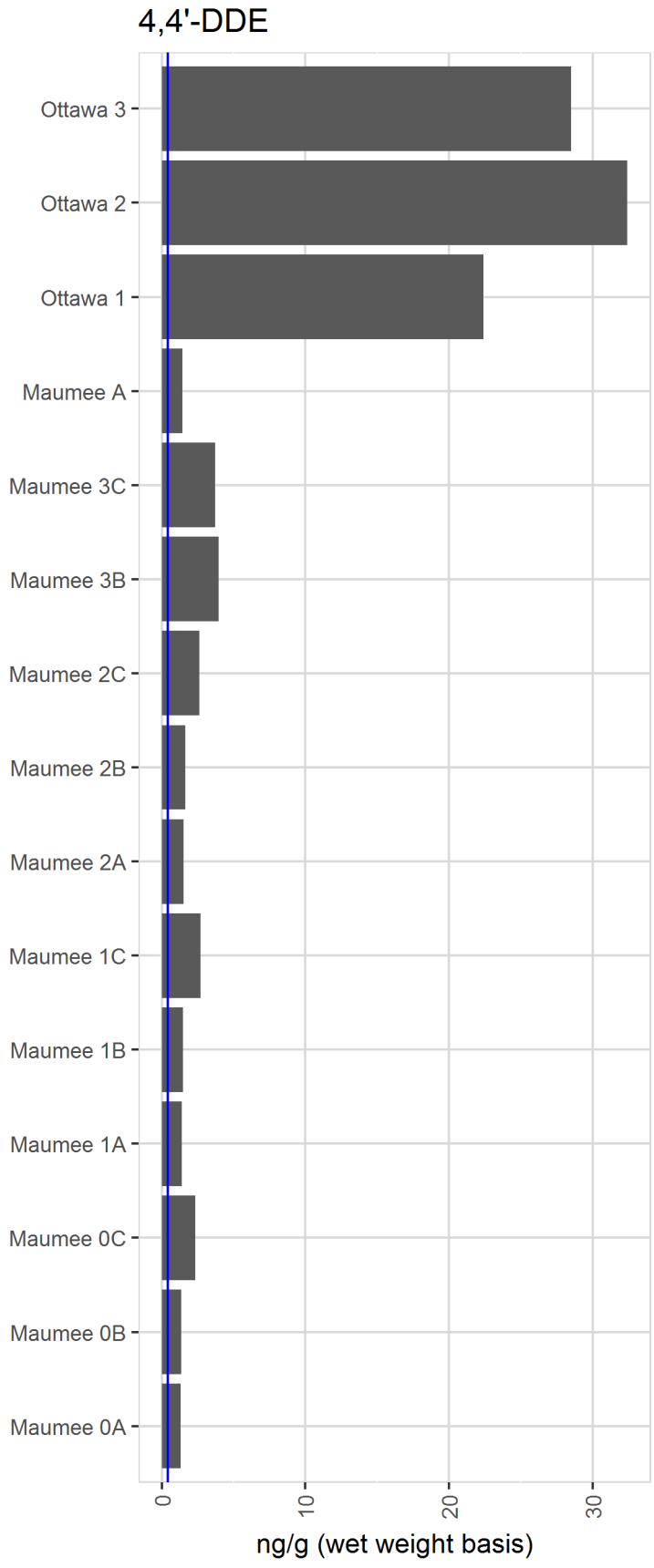
Box and whisker plot. Only chemical compounds found at more than five sites that had concentrations above 3 x DL were included. Measurements of zero were not included. This plot provides perspective to the relative concentrations of most commonly found pesticides in dreissenid mussel tissue.





Bar plots. Concentration results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Blue line represents 3 x detection limit. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively.





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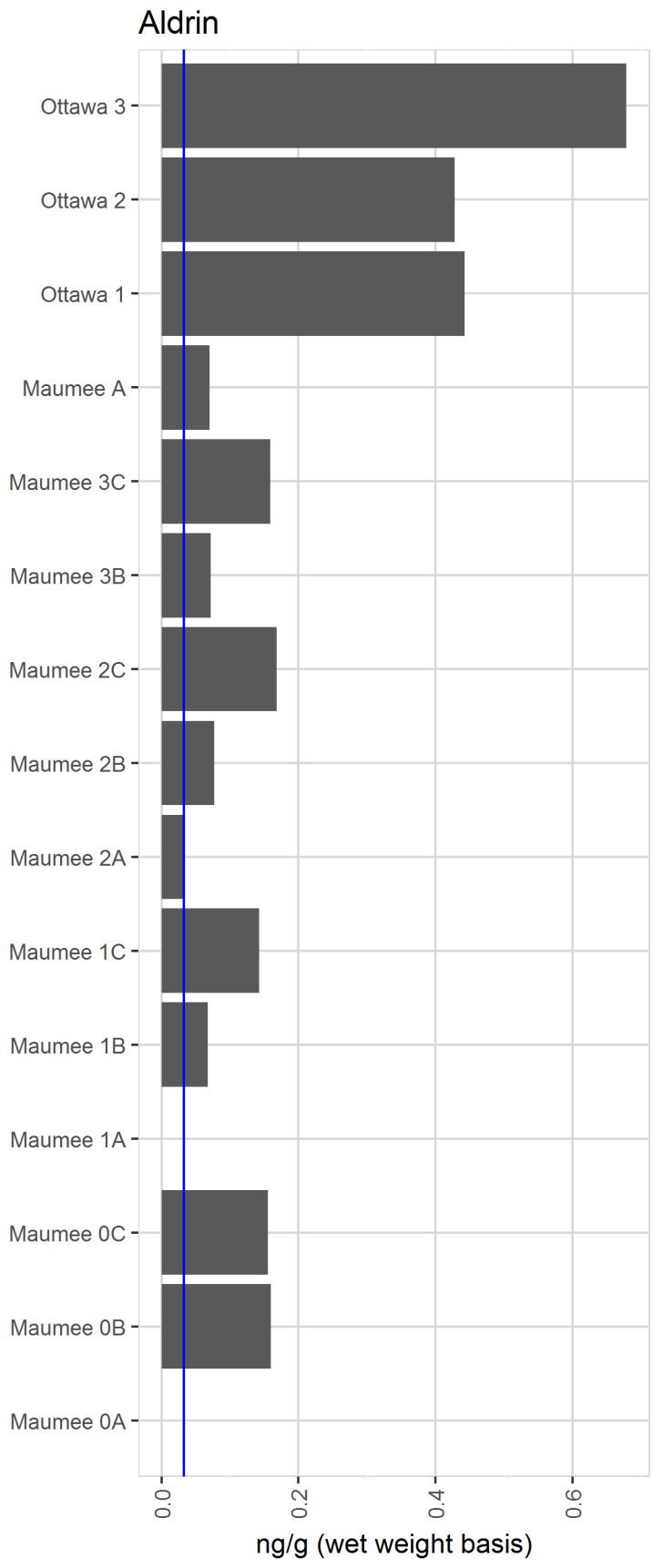

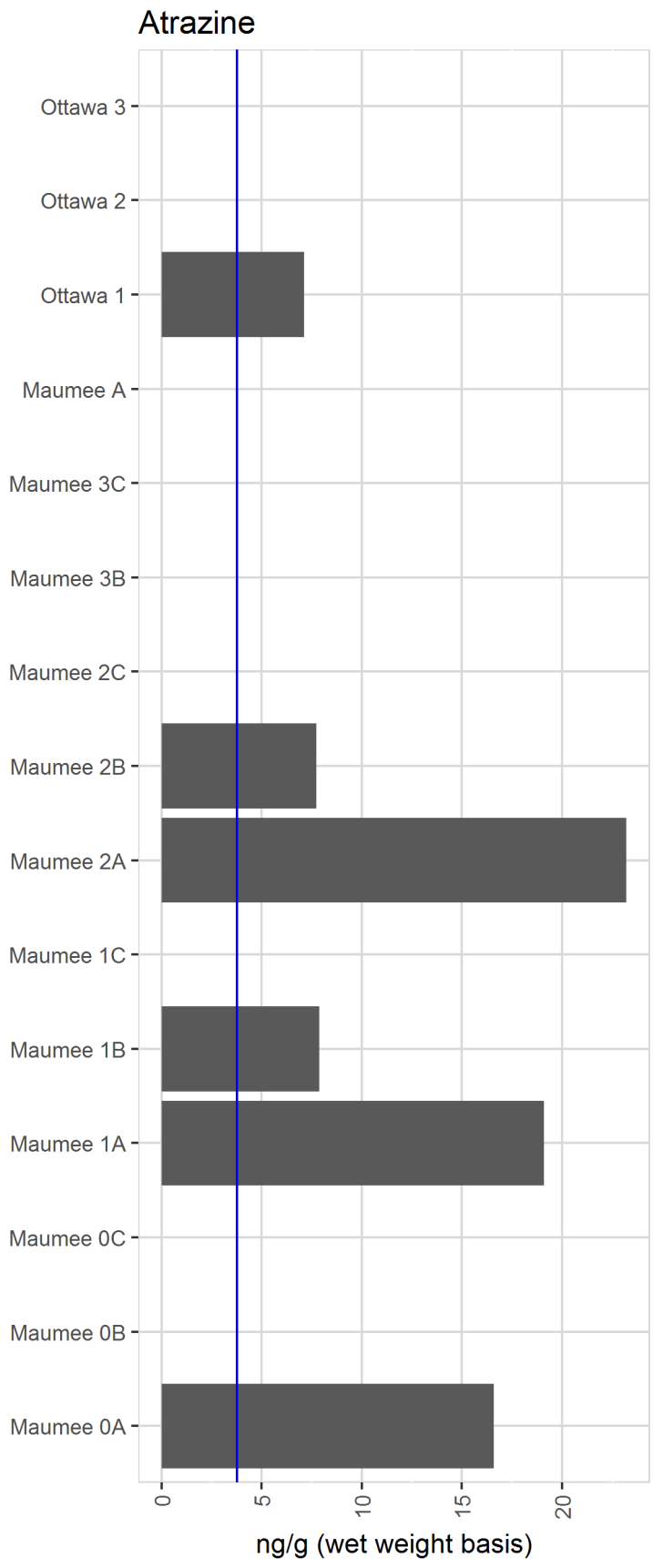

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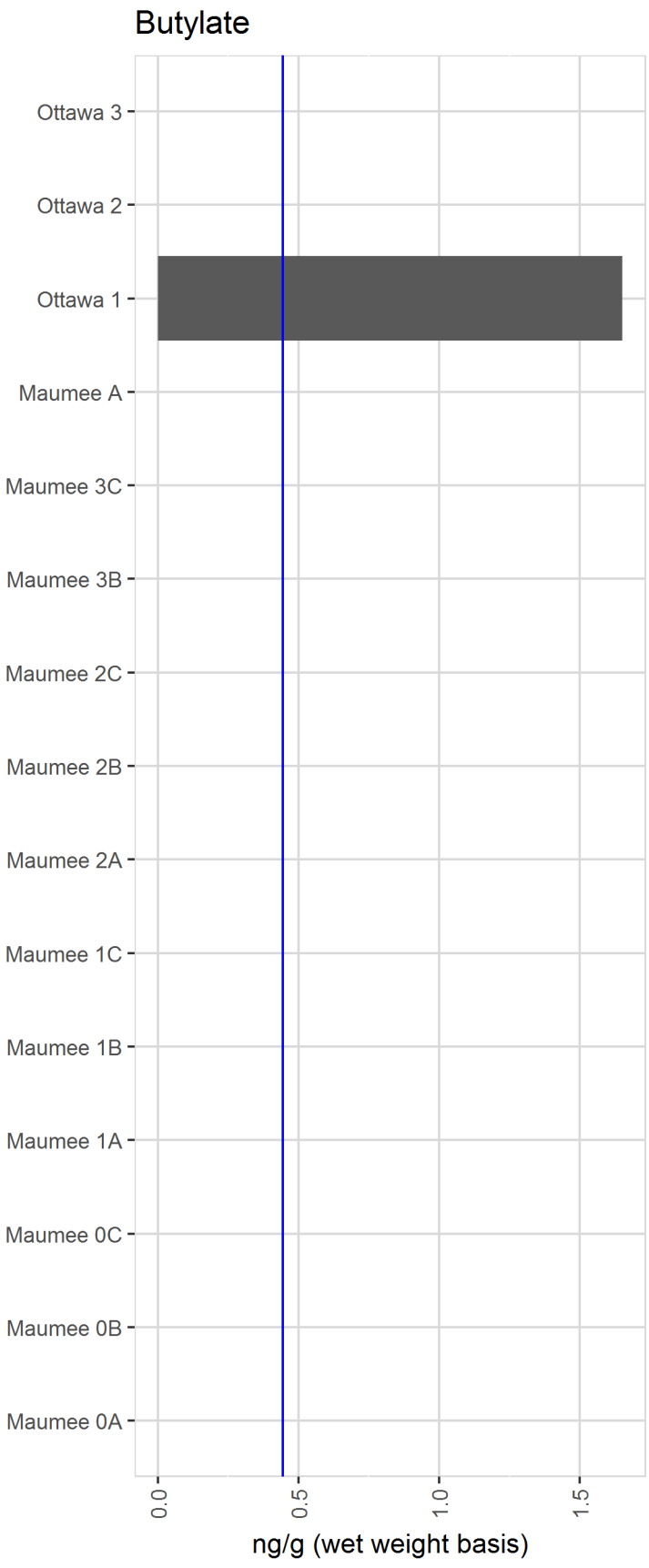

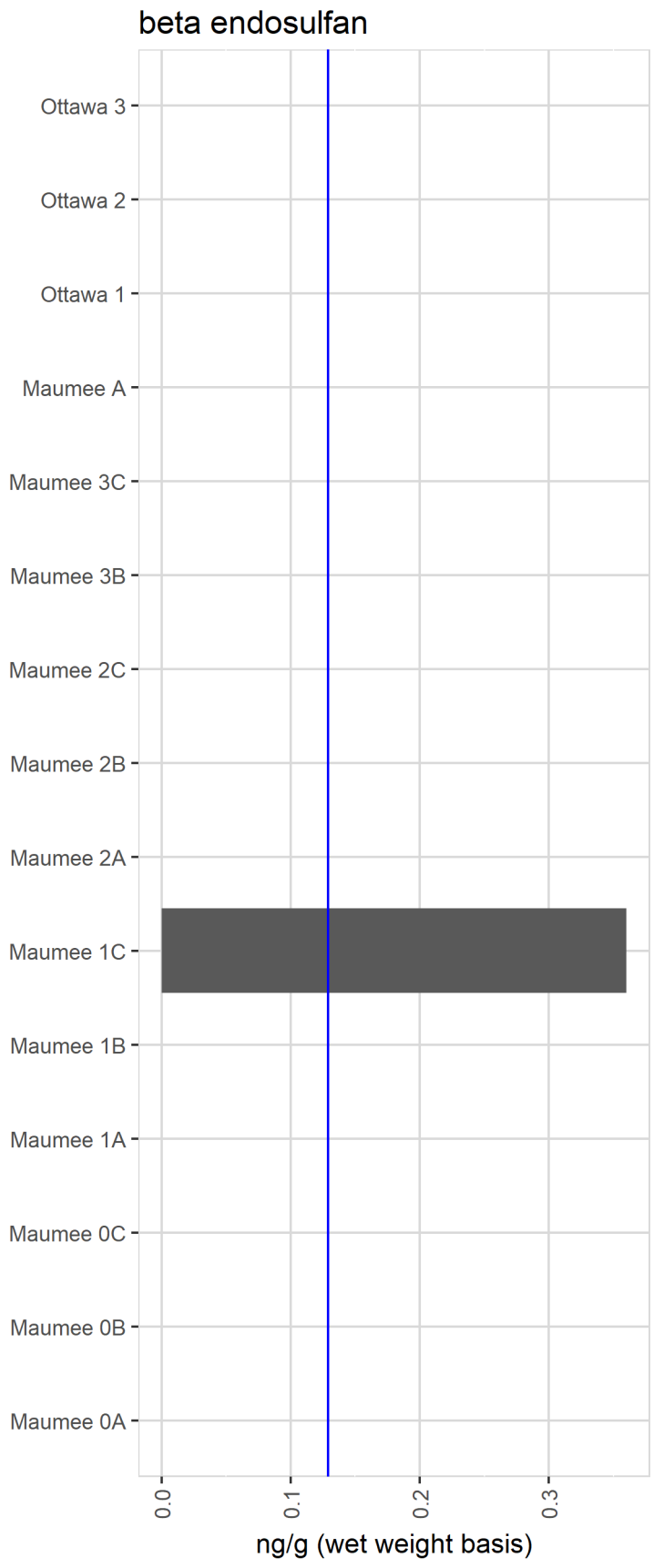

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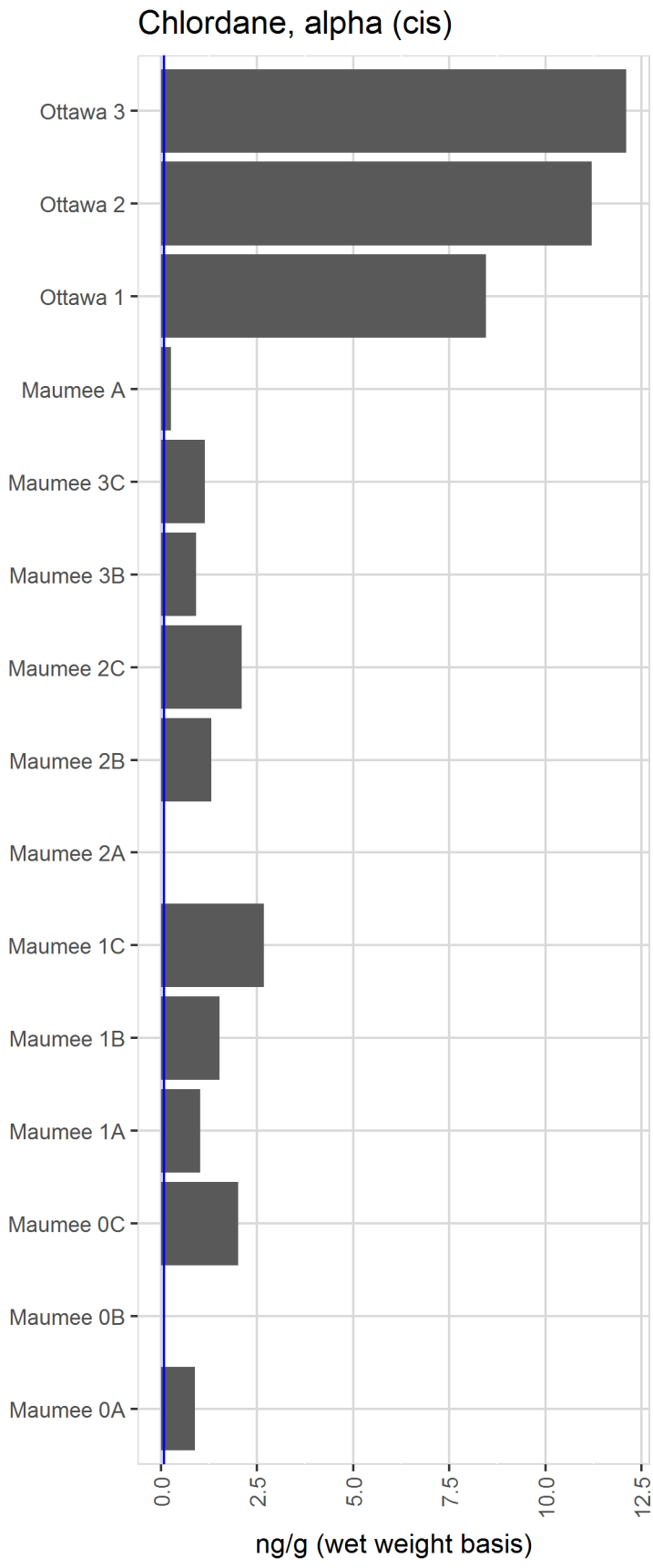

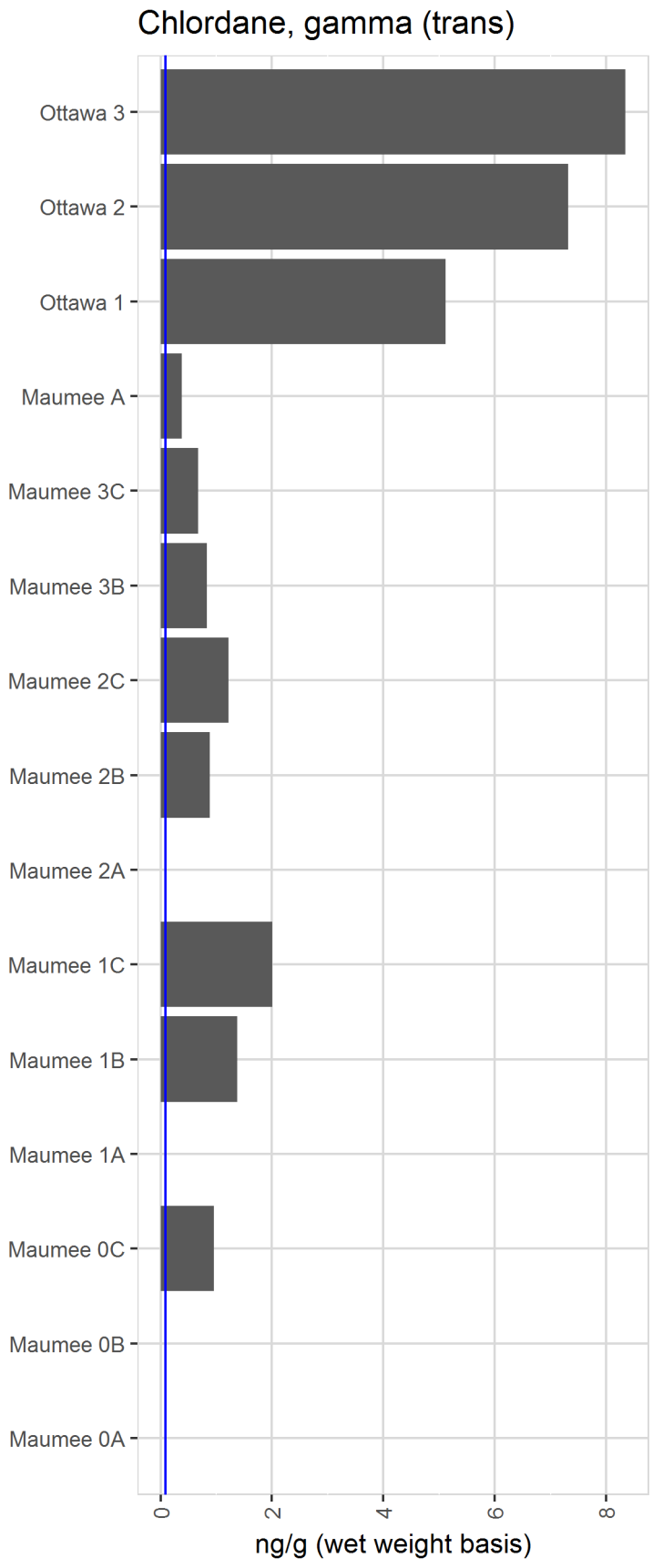

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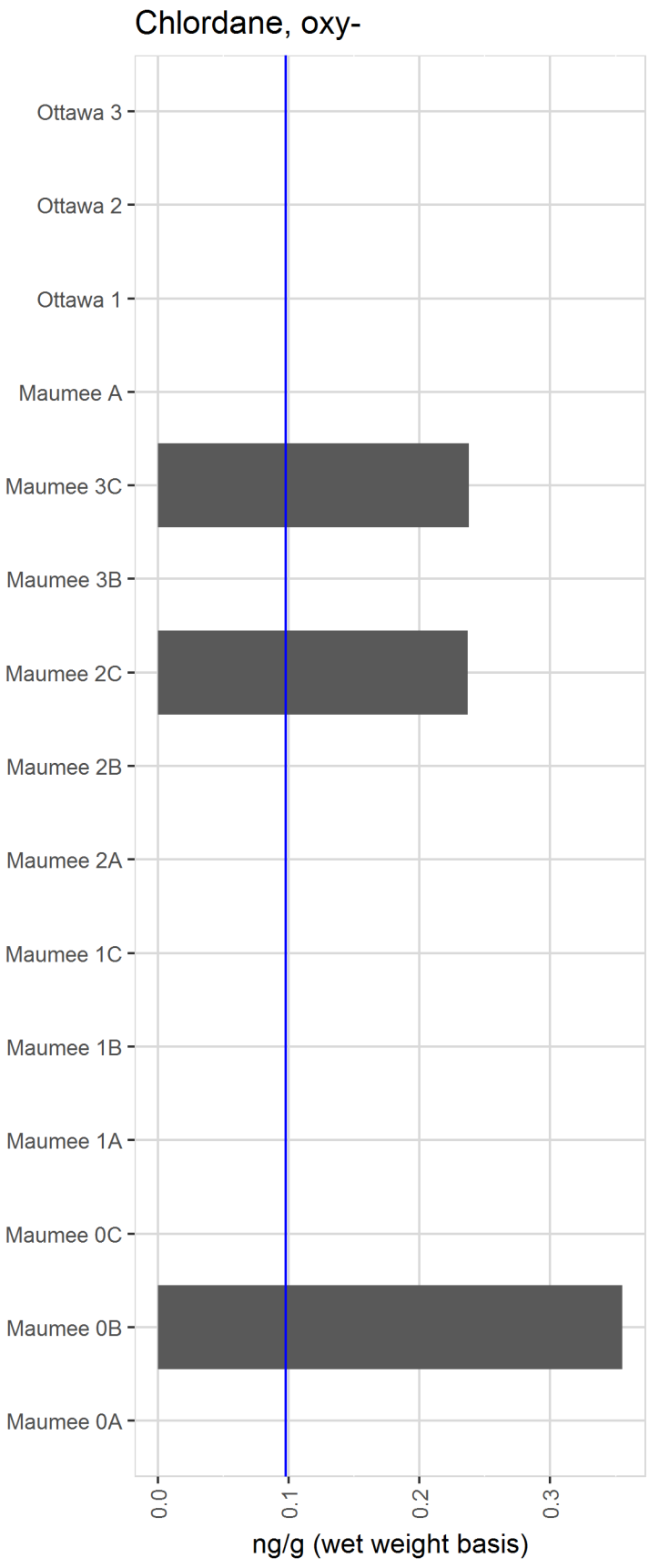

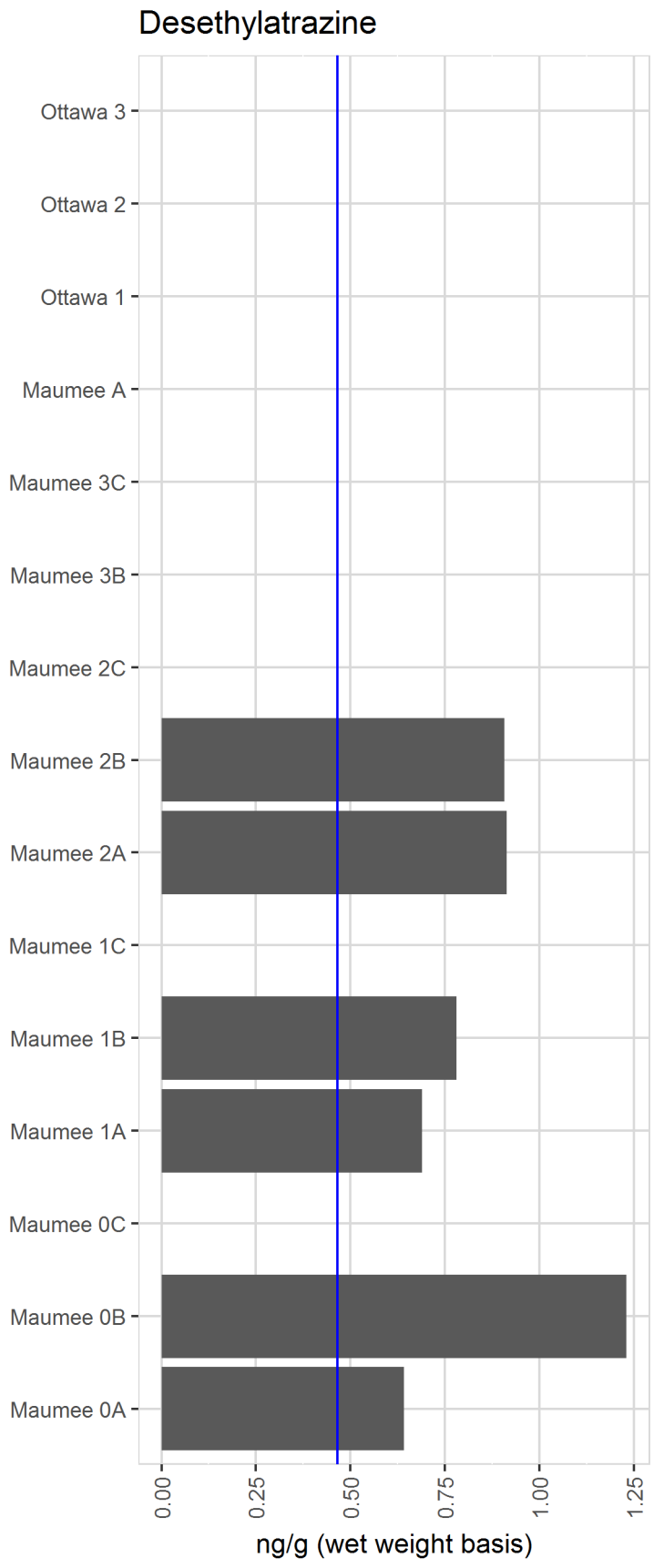

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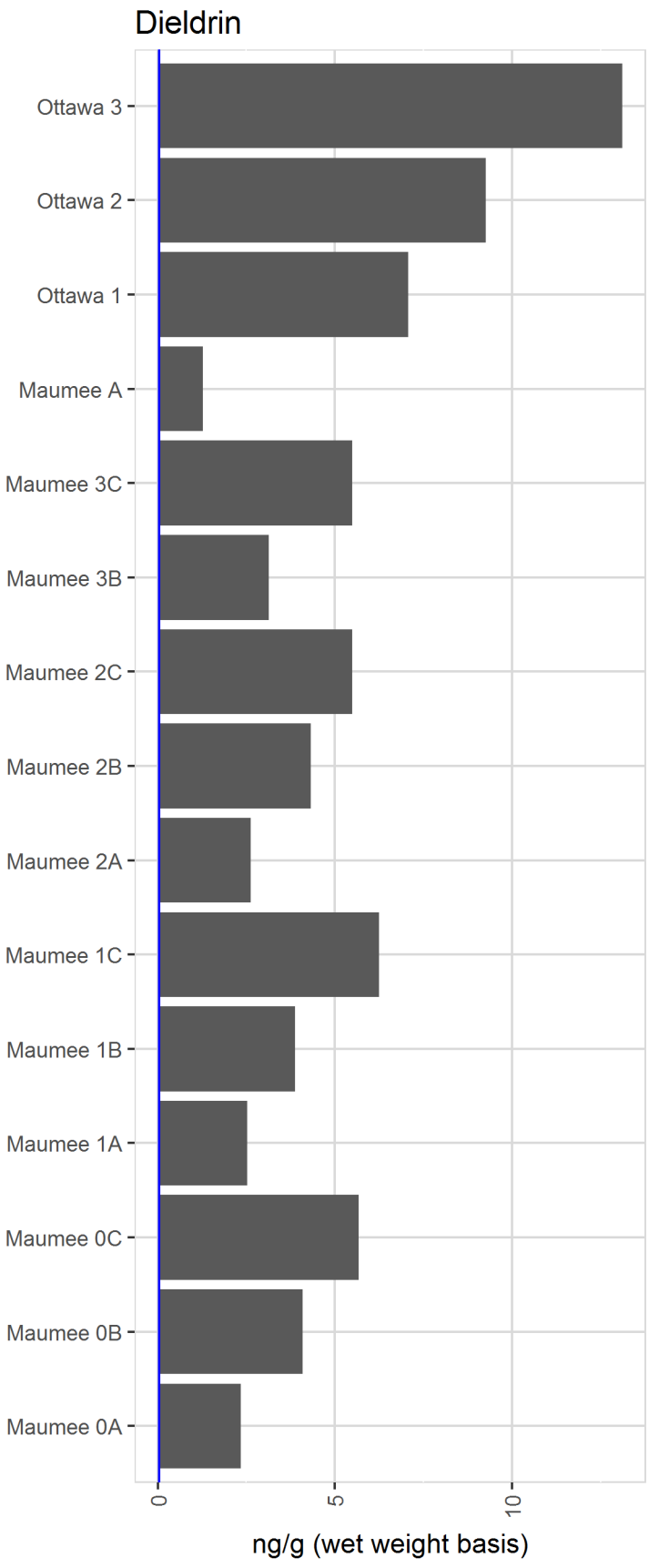

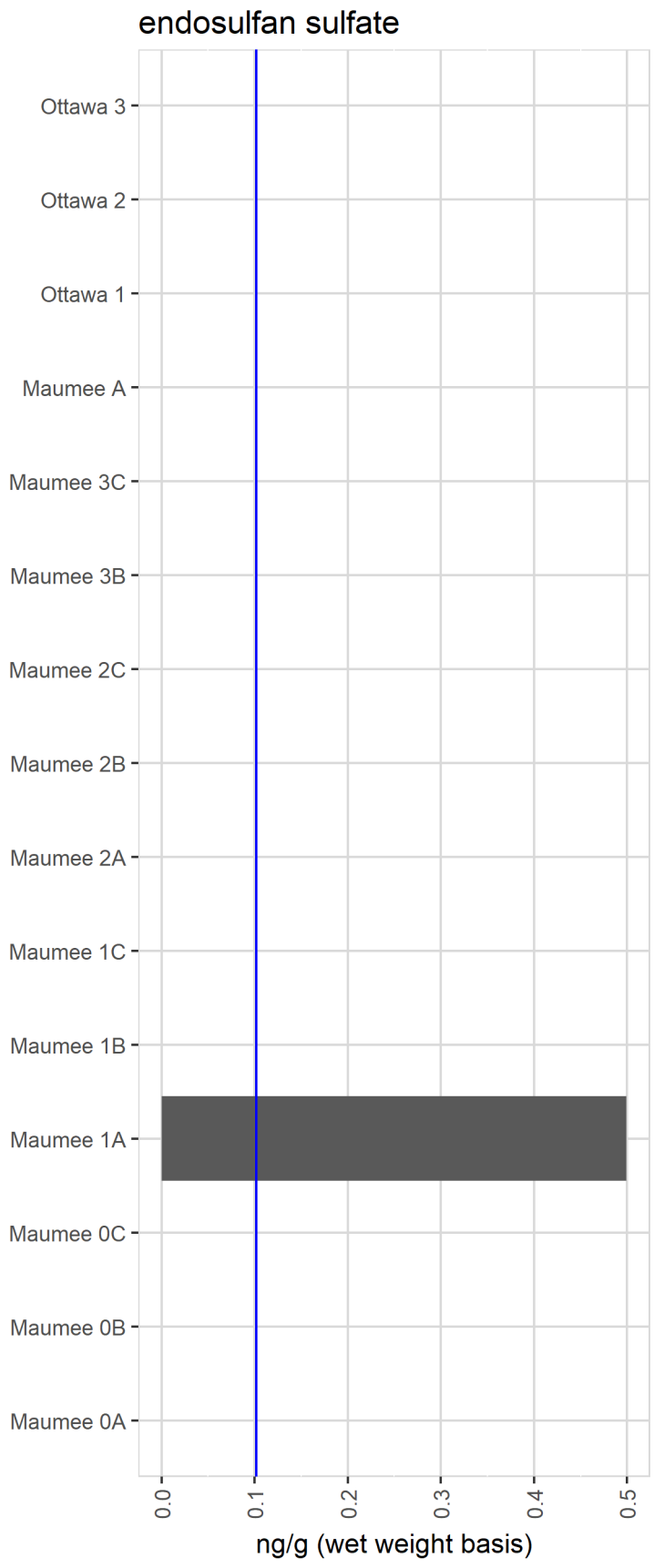

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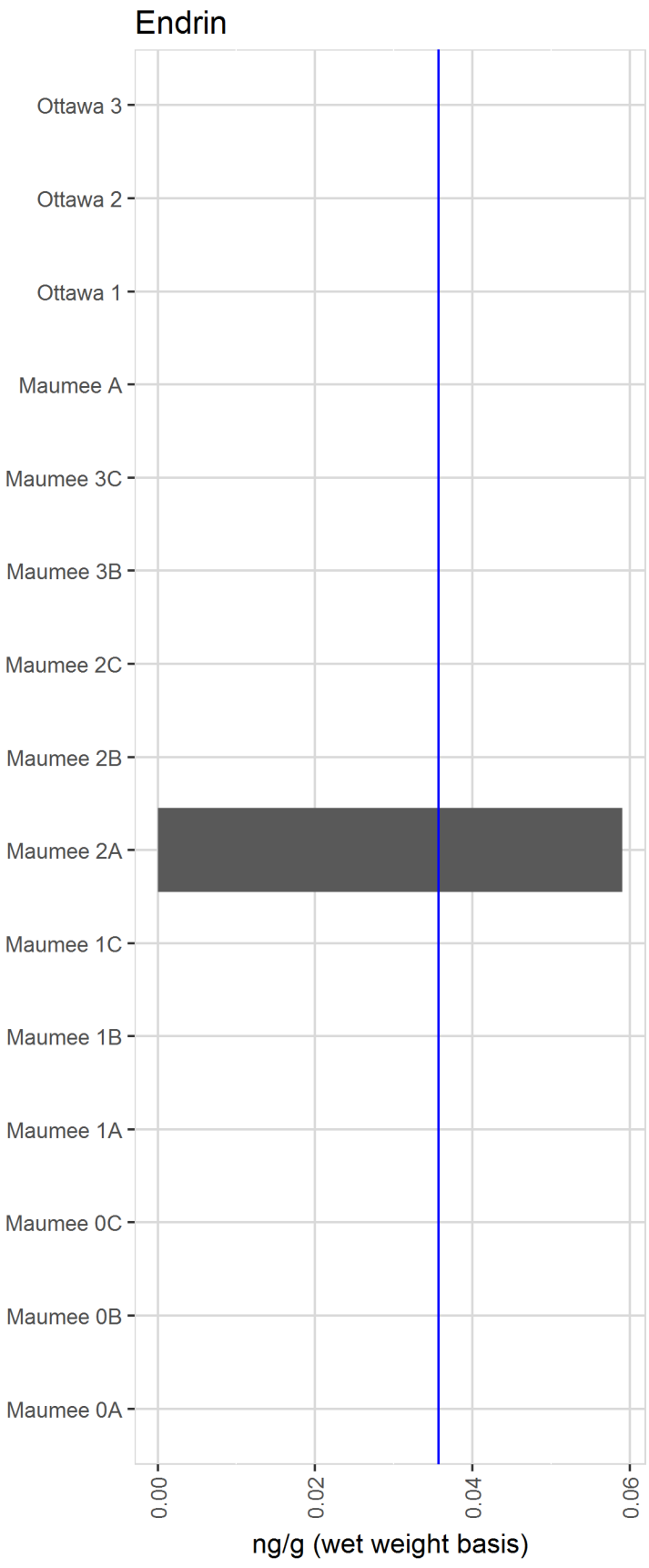

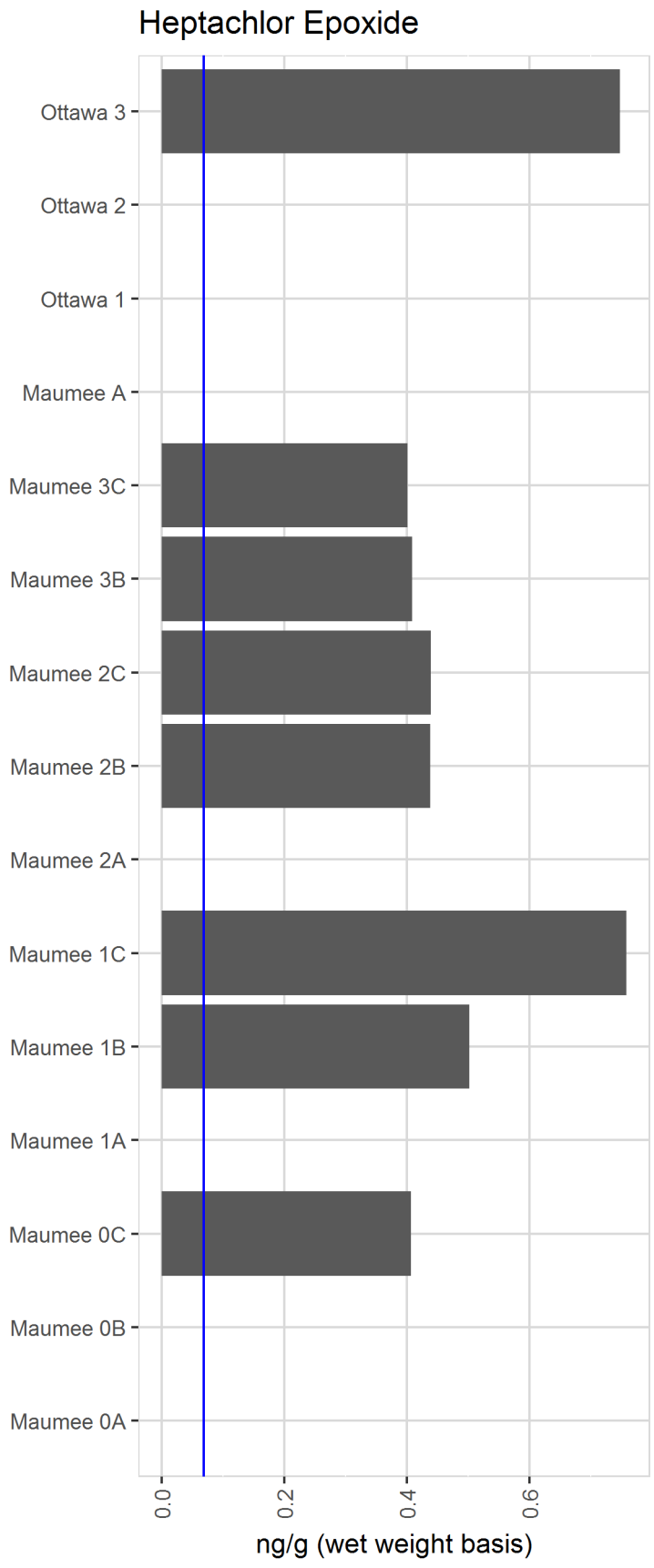

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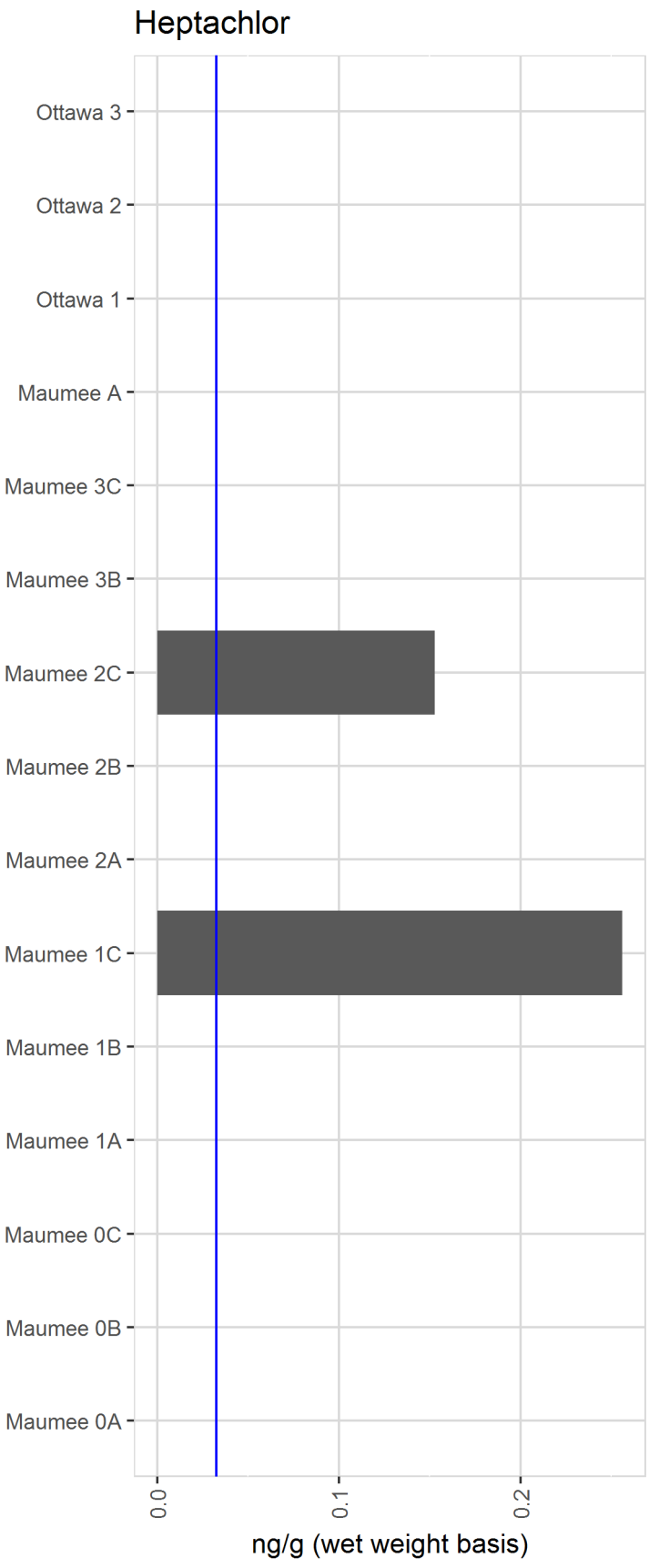

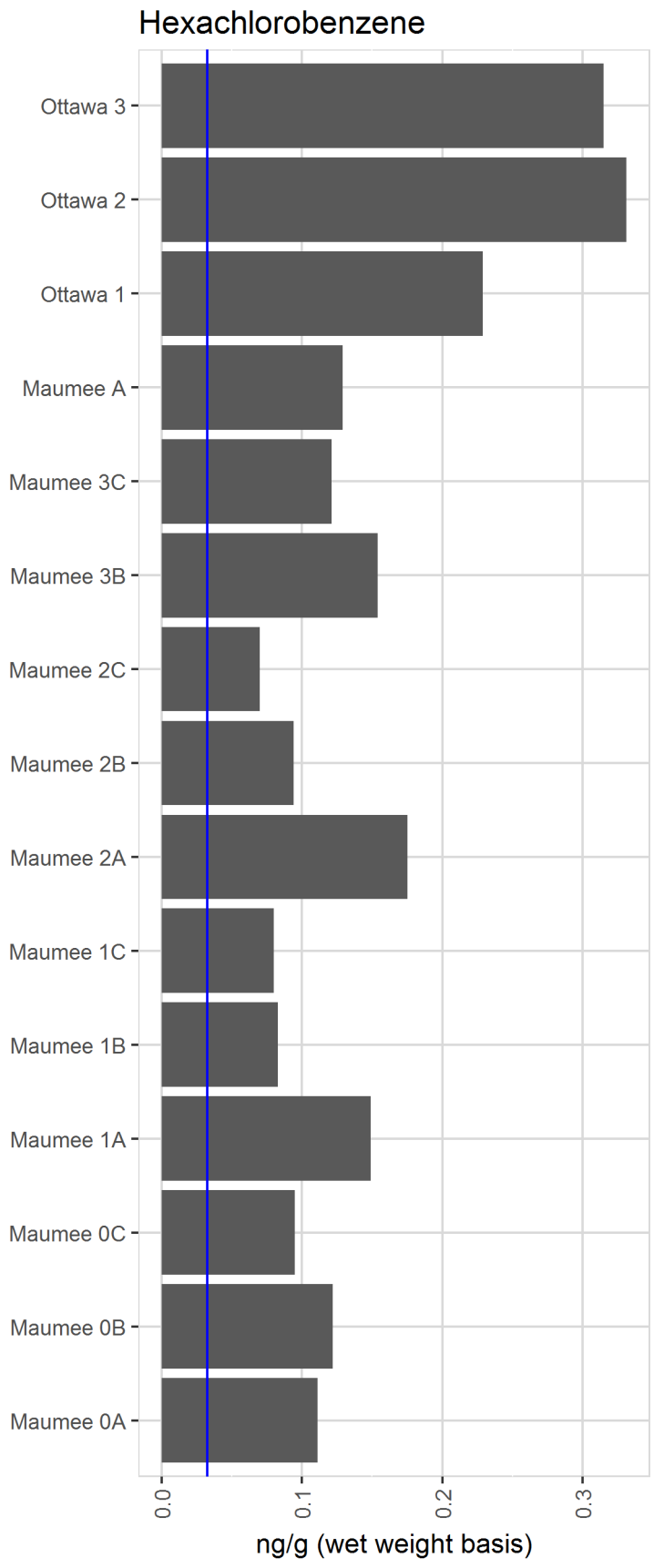

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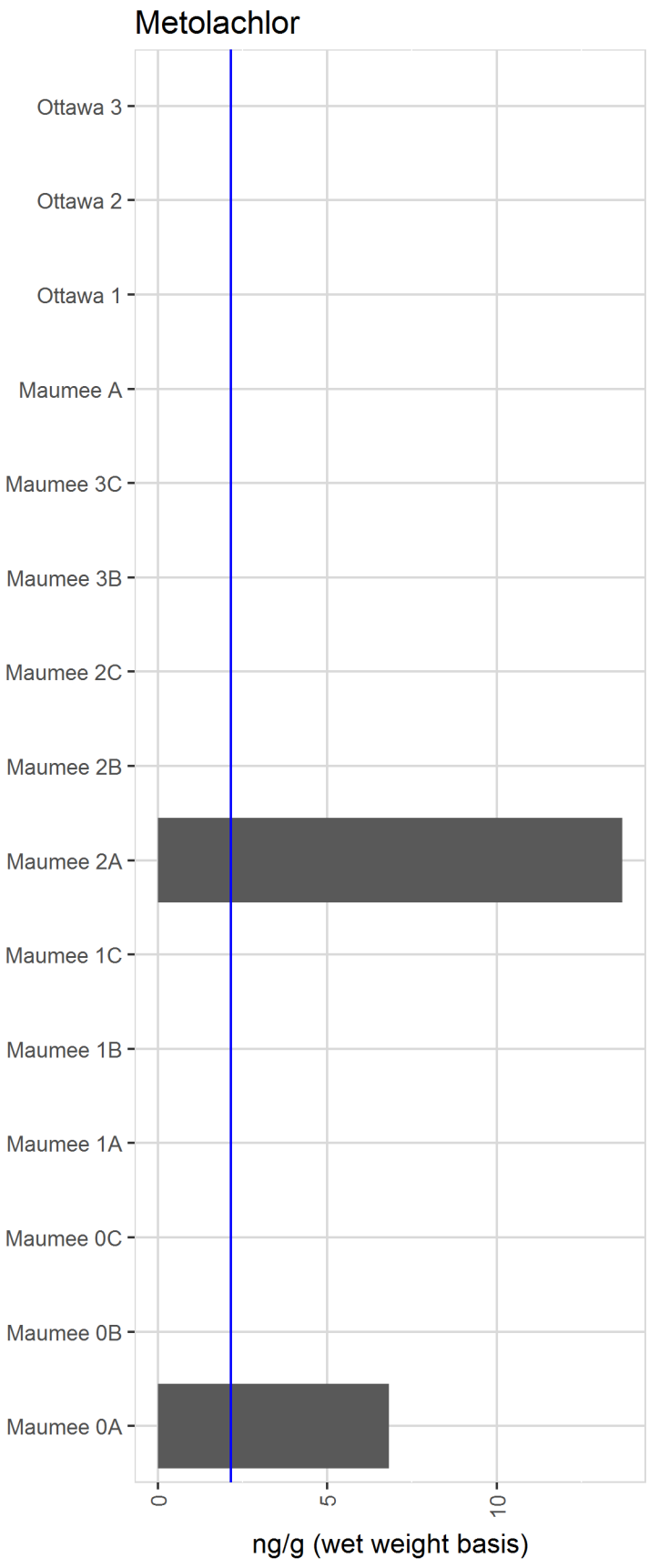

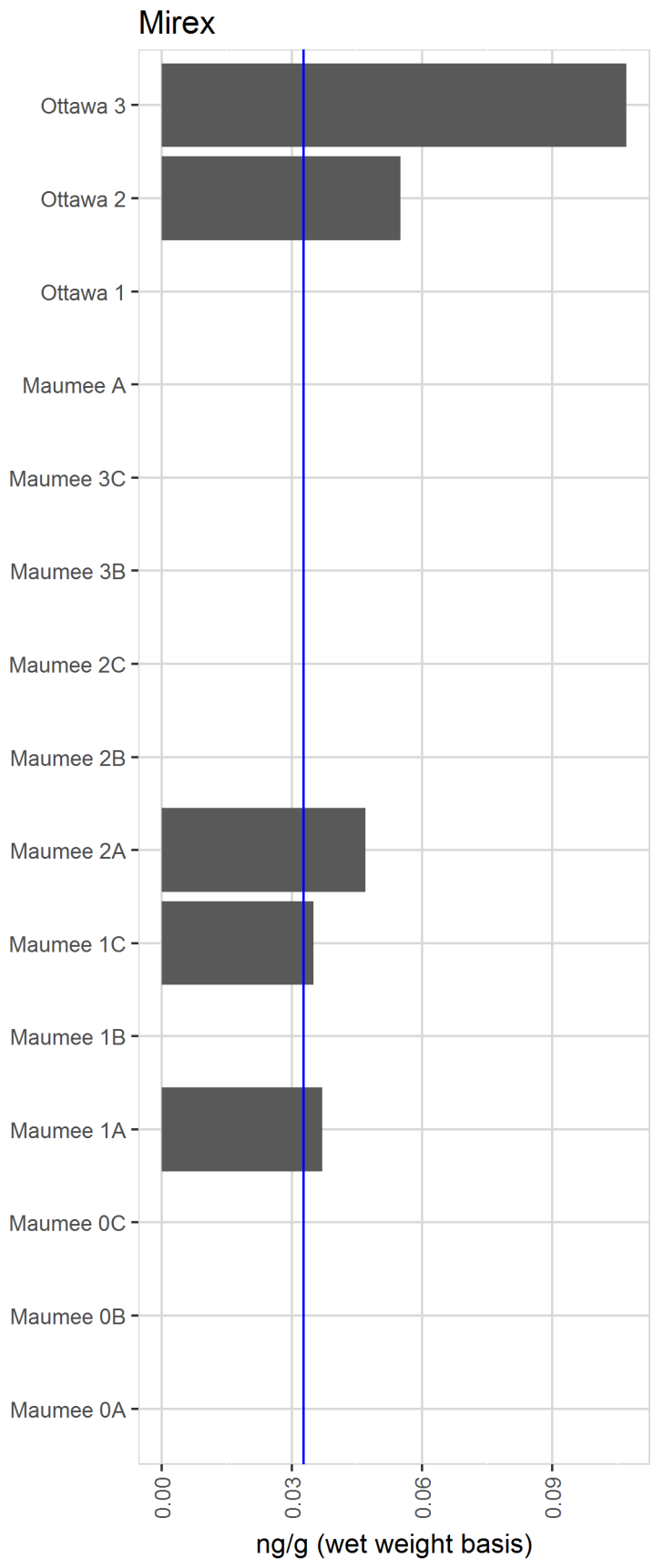

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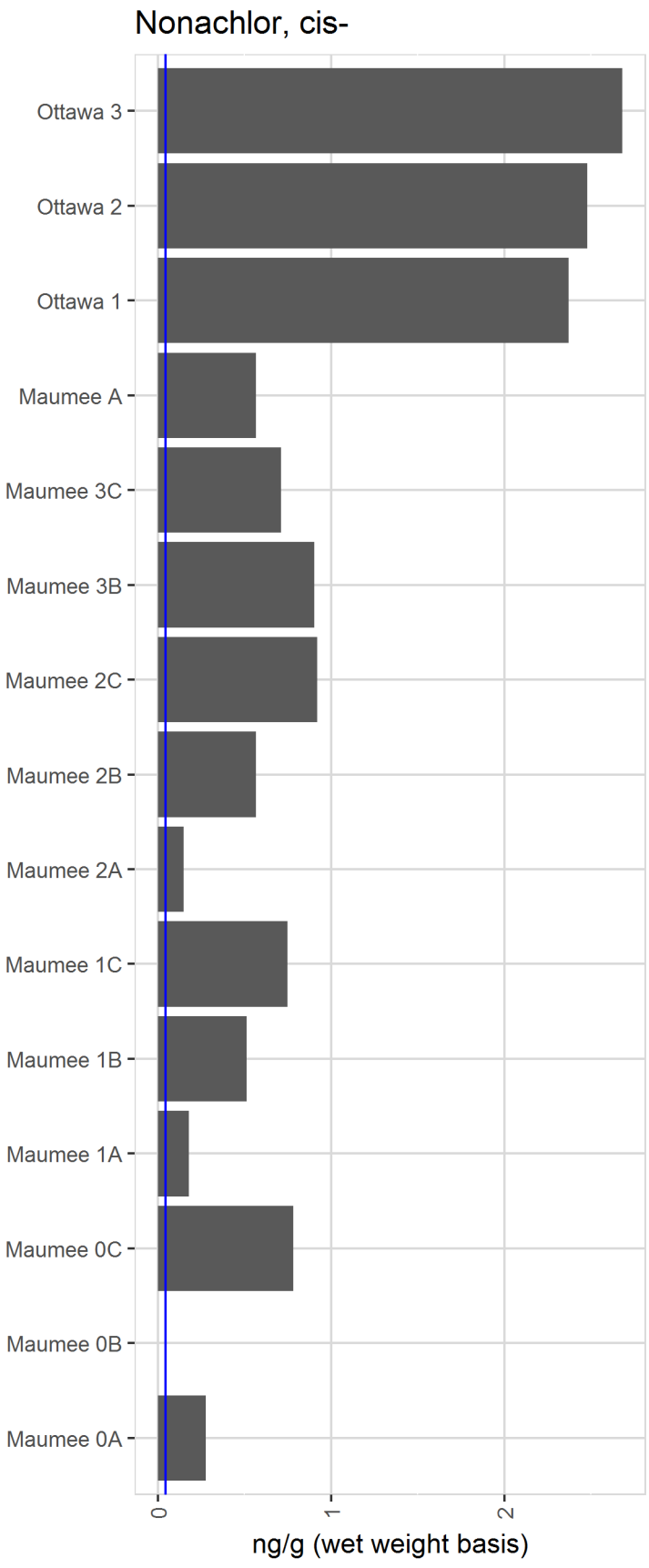

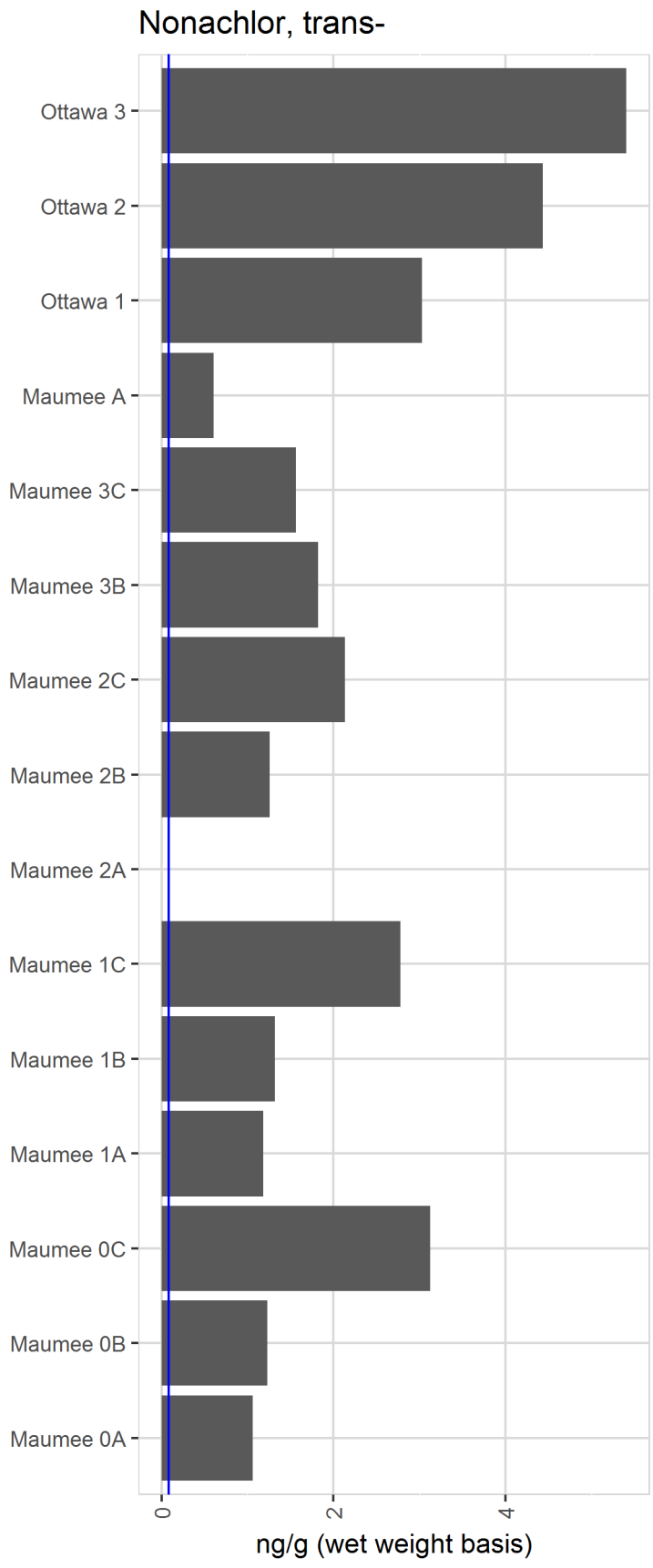

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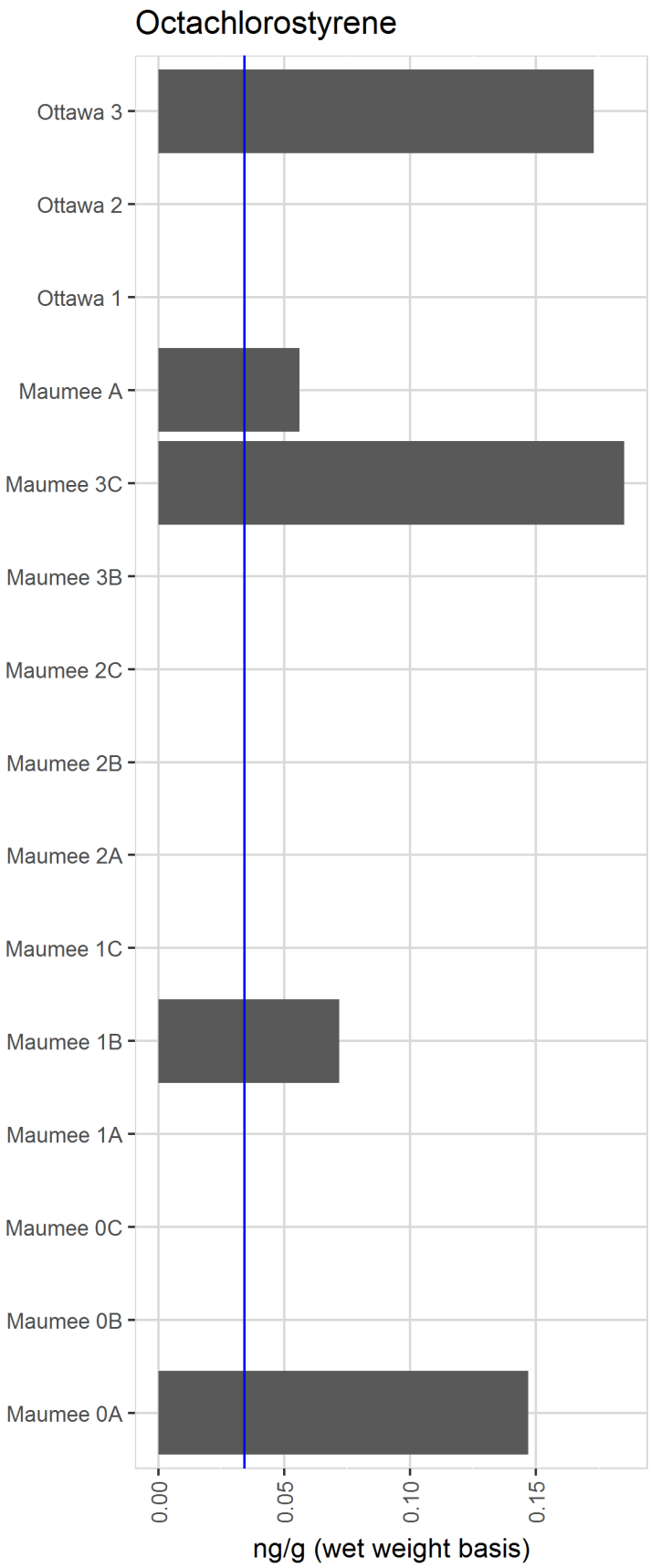

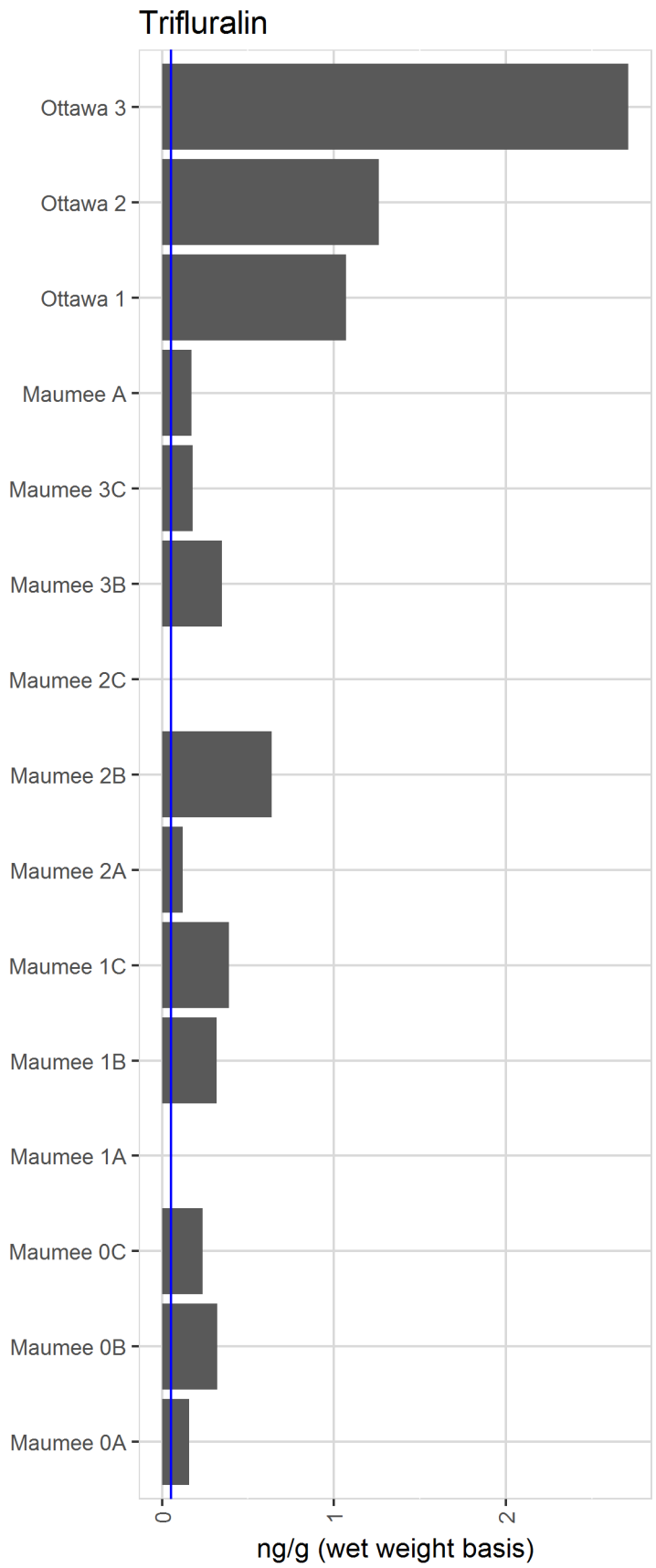

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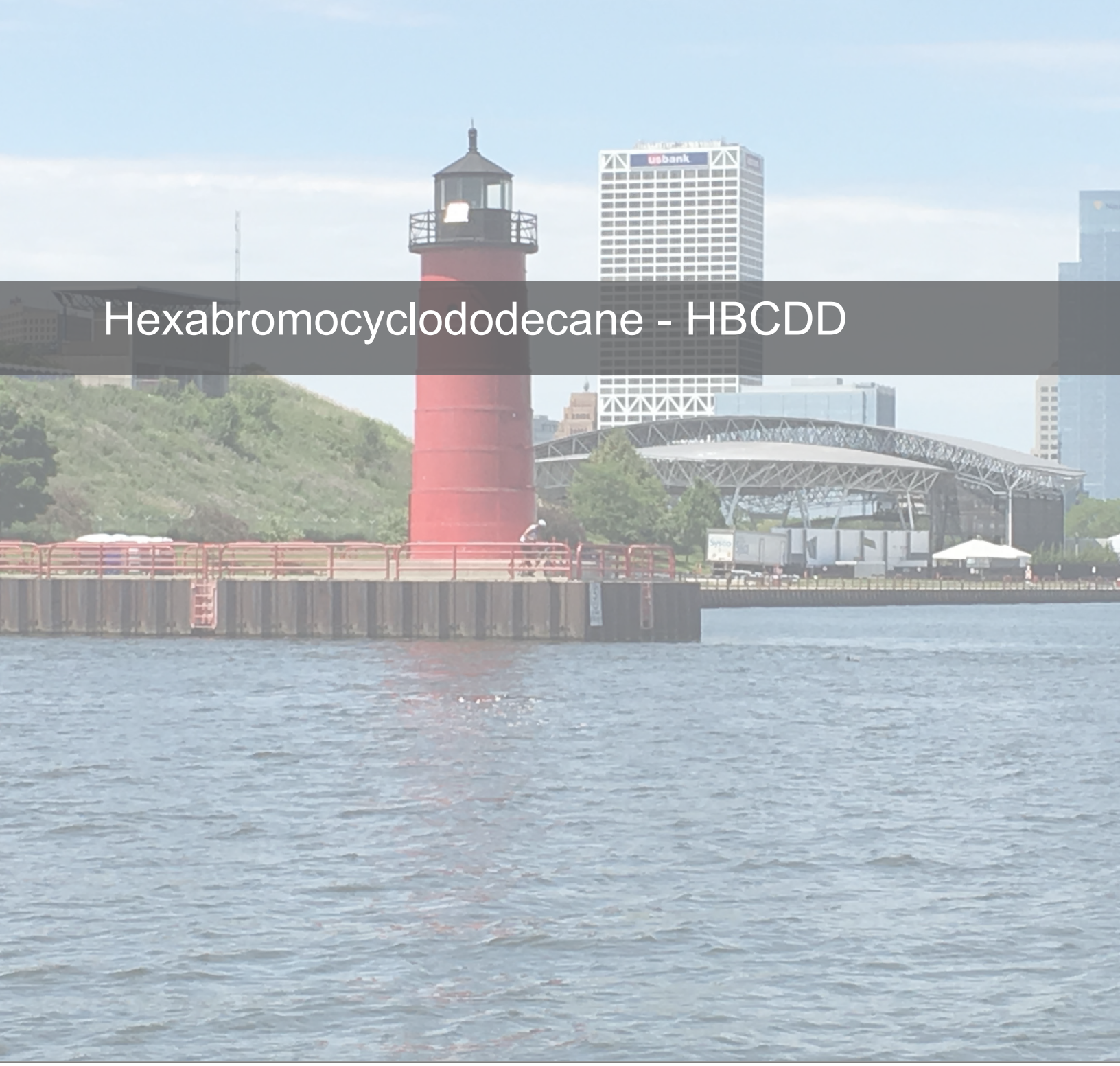
Site concentration table. Concentration (ng/g wet weight) results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively.

Label	Maumee 0A	Maumee 0B	Maumee 0C	Maumee 1A	Maumee 1B	Maumee 1C	Maumee 2A	Maumee 2B	Maumee 2C
2,4'-DDD	0	0	0	0	0	0	0	0	0
2,4'-DDE	0	0	0	0	0	0	0	0	0
4,4'-DDD	0.649	1.03	1.44	0.833	0	2.4	0.922	0	1.92
4,4'-DDE	1.33	1.37	2.32	1.39	1.49	2.69	1.51	1.64	2.63
Aldrin	0	0.16	0.155	0	0.068	0.143	0.033	0.077	0.168
Atrazine	16.6	0	0	19.1	7.88	0	23.2	7.72	0
beta-Endosulfan	0	0	0	0	0	0.36	0	0	0
Butylate	0	0	0	0	0	0	0	0	0
Chlordane, alpha (cis)	0.884	0	2.02	1.03	1.52	2.68	0	1.31	2.11
Chlordane, gamma (trans)	0	0	0.963	0	1.38	2.01	0	0.882	1.22
Chlordane, oxy-	0	0.355	0	0	0	0	0	0	0.237
Desethylatrazine	0.642	1.23	0	0.689	0.781	0	0.914	0.908	0
Dieldrin	2.35	4.08	5.67	2.52	3.88	6.24	2.63	4.32	5.49
Endosulfan Sulfate	0	0	0	0.499	0	0	0	0	0
Endrin	0	0	0	0	0	0	0.059	0	0
Heptachlor	0	0	0	0	0	0.256	0	0	0.153
Heptachlor Epoxide	0	0	0.407	0	0.502	0.758	0	0.438	0.439
Hexachlorobenzene	0.111	0.122	0.095	0.149	0.083	0.08	0.175	0.094	0.07
Metolachlor	6.82	0	0	0	0	0	13.7	0	0
Mirex	0	0	0	0.037	0	0.035	0.047	0	0
Nonachlor, cis-	0.279	0	0.782	0.18	0.512	0.748	0.151	0.567	0.921
Nonachlor, trans-	1.06	1.23	3.12	1.18	1.32	2.78	0	1.26	2.13
Octachlorostyrene	0.147	0	0	0	0.072	0	0	0	0
Trifluralin	0.159	0.32	0.237	0	0.319	0.388	0.121	0.638	0

Site concentration table. Concentration (ng/g wet weight) results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively.

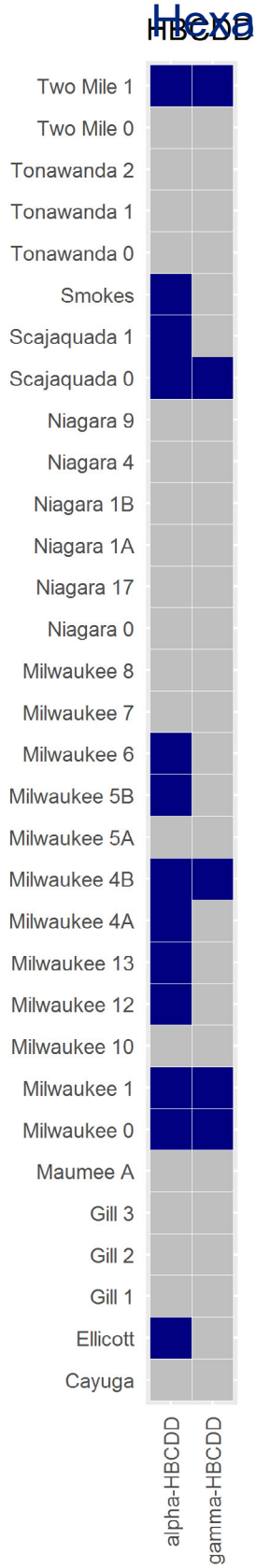
Label	Maumee 3A	Maumee 3B	Maumee 3C	Ottawa 1	Ottawa 2	Ottawa 3
2,4'-DDD	0	0	0	3.56	6.24	6.46
2,4'-DDE	0	0	0	0.837	1.34	1.33
4,4'-DDD	3.35	2.89	0	11	19.6	22.2
4,4'-DDE	3.96	3.72	1.46	22.4	32.4	28.5
Aldrin	0.072	0.159	0.07	0.442	0.428	0.678
Atrazine	0	0	0	7.12	0	0
beta-Endosulfan	0	0	0	0	0	0
Butylate	0	0	0	1.65	0	0
Chlordane, alpha (cis)	0.918	1.14	0.266	8.45	11.2	12.1
Chlordane, gamma (trans)	0.828	0.68	0.386	5.12	7.32	8.34
Chlordane, oxy-	0	0.238	0	0	0	0
Desethylatrazine	0	0	0	0	0	0
Dieldrin	3.13	5.49	1.27	7.06	9.26	13.1
Endosulfan Sulfate	0	0	0	0	0	0
Endrin	0	0	0	0	0	0
Heptachlor	0	0	0	0	0	0
Heptachlor Epoxide	0.409	0.401	0	0	0	0.748
Hexachlorobenzene	0.154	0.121	0.129	0.229	0.331	0.315
Metolachlor	0	0	0	0	0	0
Mirex	0	0	0	0	0.055	0.107
Nonachlor, cis-	0.903	0.711	0.567	2.37	2.48	2.68
Nonachlor, trans-	1.82	1.56	0.606	3.03	4.43	5.4
Octachlorostyrene	0	0.185	0.056	0	0	0.173
Trifluralin	0.348	0.178	0.173	1.07	1.26	2.71



Hexabromocyclododecane - HBCDD

Hexabromocyclododecane is mainly used as a flame retardant in polystyrene foam, textiles, and electronic equipment. Commercial HBCDD products are a mixture of mainly three isomers (alpha, beta, and gamma), which are measured by this method. This is a suite of persistent, bioaccumulative compounds that are subject to long range transport and have been found in remote areas and in biological samples (UNEP 2007). As a result of the potential threat, these compounds are found on the EPA's List of Chemicals of Concern. HBCDD is toxic to some aquatic organisms and may have recruitment consequences for some species (Desjardins *et al*., 2004; Deng *et al*., 2009, Drottar and Krueger 1998). HBCD was binationally designated as a Chemical of Mutual Concern by Annex 3 of the Great Lakes Water Quality Agreement.

Hexabromocyclododecane - HBCDD



Heat map. Presence (■) and absence (□) of each compound found at various locations in tissues. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Chemical list table. HBCDD by LC-MS/MS (MLA-070. R02.03). Concentrations above the detection limit, found in at least one sample, are identified in bold red type.

alpha-HBCDD
gamma-HBCDD
beta-HBCDD

Site table. Presentation of all the locations where the HBCDD analysis was used to analyze tissue samples.

HBCDD	n
Milwaukee Estuary	12
Maumee River	1
Niagara River	19

Method table. Summary of compound detections for the HBCDD analysis. Percentage categories are used to characterize how often a compound was found in a sample at 3 x DL.

Compound percentage categories	Samples	
	n	%
Compound not found	1	33
Compound found in 1-24 % of samples	1	33
Compound found in 25-49 % of samples	1	33
Compound found in 50-74 % of samples	0	0
Compound found in 75-100 % of samples	0	0

Summary of detections

- Of the 3 compounds analyzed, 2 were detected in dreissenid mussels.
- alpha-HBCDD was the dominant isomer found in mussel tissue followed by gamma. beta-HBCDD was not detected in mussel tissue.
- alpha-HBCDD was found at one or more reference sites (Milwaukee 5, Niagara 9 and Niagara 1).

Hexabromocyclododecane - HBCDD

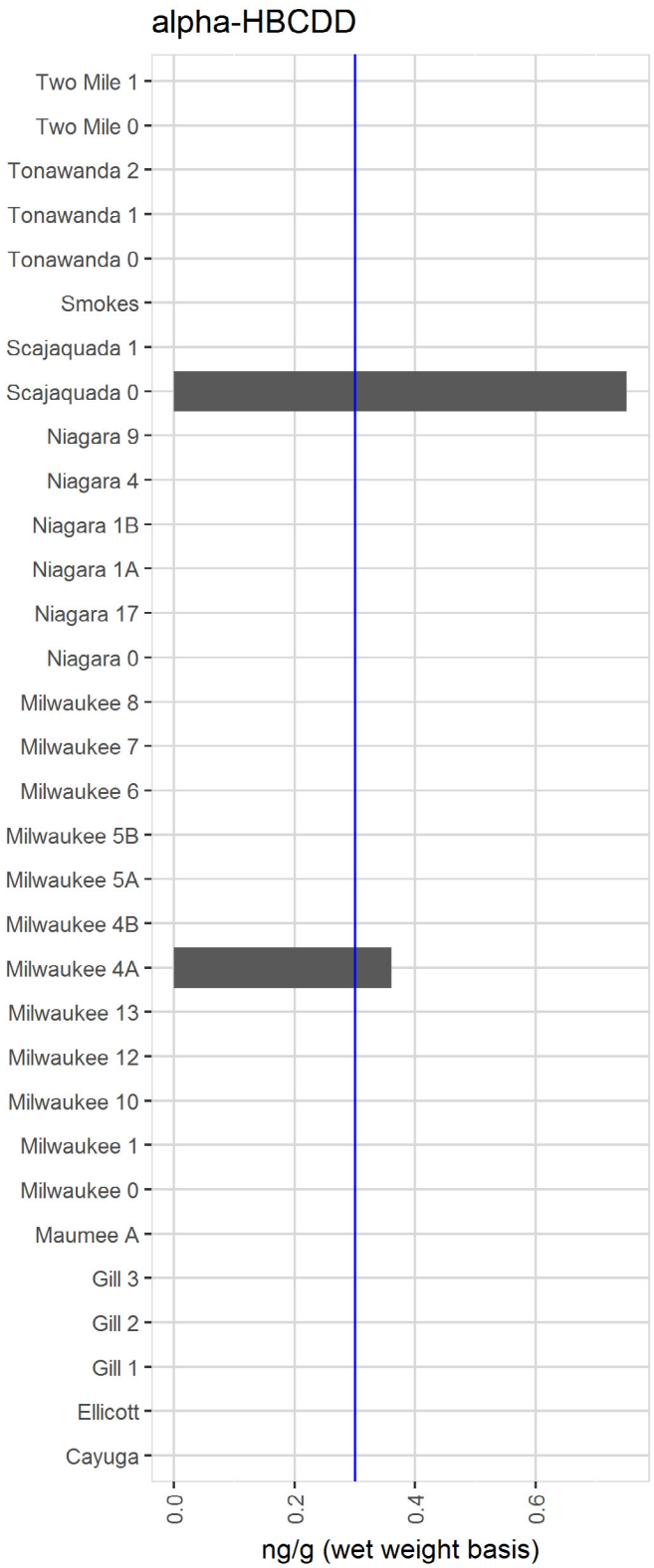
Summary of concentration data (3 x DL)

- Alpha-HBDD was the only compound found with concentrations above 3 x DL.

- Concentrations were in the low, relative compounds measured historically by the Mussel Watch Program such as aldrin and chlordane (see pesticide section).

Chemical concentration table. Summary of compound concentrations in ng/g wet weight. Concentrations below 3 x detection limit were changed to zero.

		Concentration (ng/wet weight)		
COMPOUND		**maximum**	**mean**	**median**
alpha-HBCDD	Brominated Flame Retardant	0.751	0.03	0.00



Bar plots. Concentration results for dreissenid mussels by site and time. Blue line represents 3 x detection limit. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site concentration table. Concentration (ng/g wet weight) results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Maumee sites ending in A, B, and C, represent samples collected in May, June and July of 2015 respectively. Milwaukee sites ending in A and B represent samples collected in June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site Name	alpha-HBCDD
Cayuga	0
Ellicott	0
Gill 1	0
Gill 2	0
Gill 3	0
Maumee 3C	0
Milwaukee 0	0
Milwaukee 1	0
Milwaukee 10	0
Milwaukee 12	0
Milwaukee 13	0
Milwaukee 4A	0.361
Milwaukee 4B	0
Milwaukee 5A	0
Milwaukee 5B	0
Milwaukee 6	0
Milwaukee 7	0
Milwaukee 8	0
Niagara 0	0
Niagara 17	0
Niagara 1A	0
Niagara 1B	0
Niagara 4	0
Niagara 9	0
Scajaquada 0	0.751
Scajaquada 1	0
Smokes	0
Tonawanda 0	0
Tonawanda 1	0
Tonawanda 2	0
Two Mile 0	0
Two Mile 1	0

Alkylphenols and Ethoxylates

The analysis of these compounds was performed on samples from the Milwaukee and Niagara River studies. The compounds in this group are derived from alkyphenols that are useful because of their surfactant behavior (both hydrophilic & hydrophobic properties). Alkylphenols have a wide variety of commercial and industrial applications ranging from additives in oil to laundry detergents. These compounds have been in use for decades, but continue to be assessed as they have environmental implications such as toxicity to some aquatic organisms. They also degrade slowly in aquatic systems making them persistent in addition to being bioaccumulative.

Alkylphenols and Ethoxylates

Chemical list table. Octylphenol, Nonylphenol & Nonylphenol Ethoxylates (MLA-080.R02.04). Concentrations above the detection limit, found in at least one sample, are identified in bold red type.

4n-OP
4-NP
NP1EO
NP2EO

Site table. Presentation of all the locations where the Alkylphenol analysis was used to analyze tissue samples.

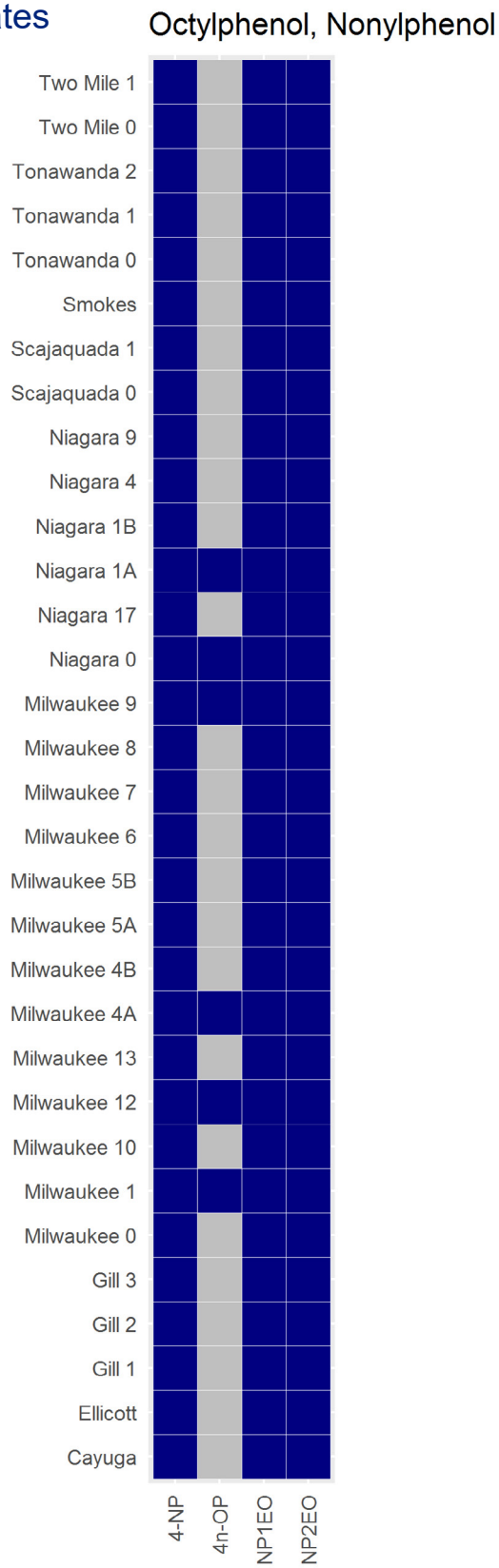
Octylphenol, Nonylphenol & Nonylphenol Ethoxylates	n
Milwaukee Estuary	13
Niagara River	19

Method table. Summary of compound detections for the Alkylphenol analysis. Percentage categories are used to characterize how often a compound was found in a sample at 3 x DL.

Compound percentage categories	Samples	
	n	%
Compound not found	0	0
Compound found in 1-24 % of samples	1	25
Compound found in 25-49 % of samples	0	0
Compound found in 50-74 % of samples	0	0
Compound found in 75-100 % of samples	3	75

Summary of detections (Heat map figure)

- All four compounds measured by this method were detected in dreissenid mussels
- Three of the four compounds measured were detected at all sites.
- One or more of all four compounds analyzed were detected at one or more of the reference sites (Milwaukee 5, Niagara 9 and Niagara 1).



Heat map. Presence (■) and absence (□) of each compound found at various locations in tissues. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively. NQ is designated by white lines in the box.

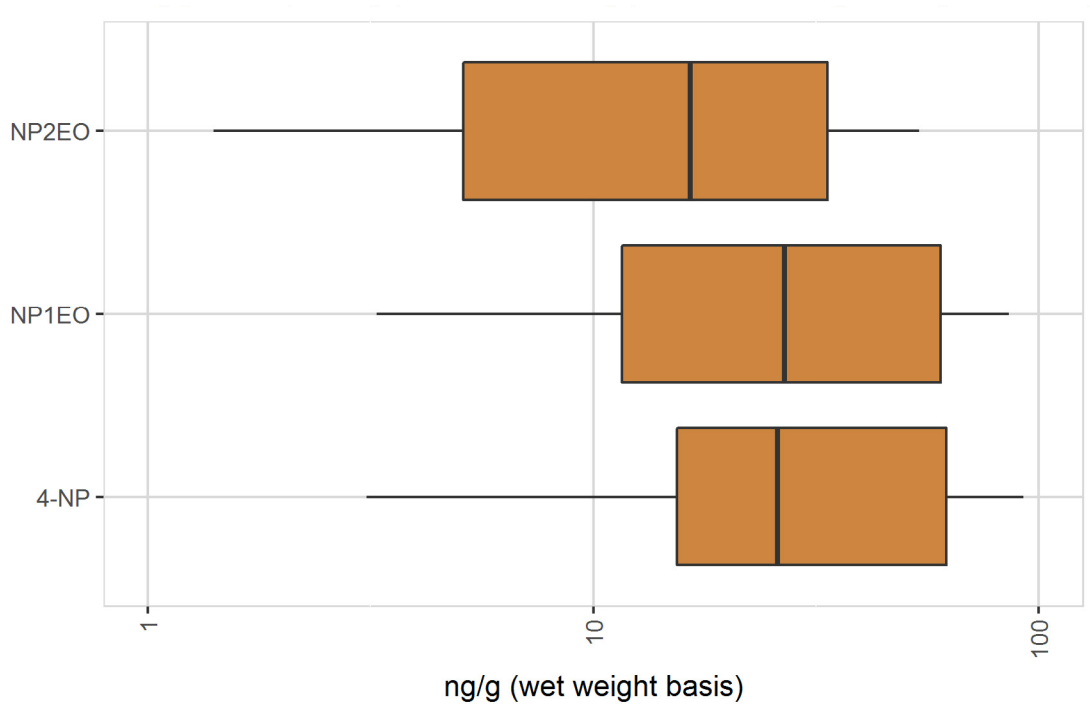
Alkylphenols and Ethoxylates

Summary of concentration data (3 x DL)

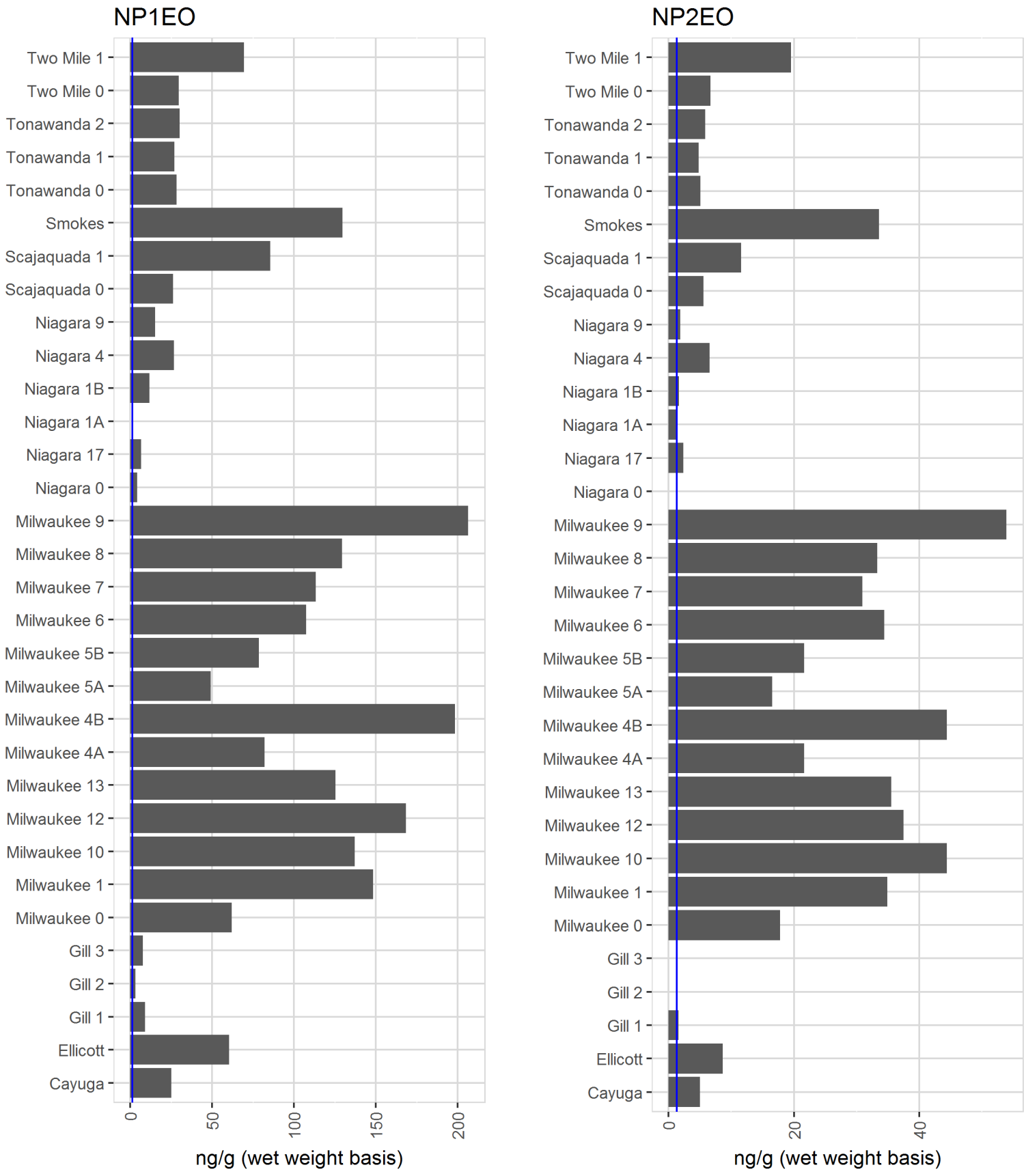
- NP1EO, NP2EO, and 4-NP are nearly ubiquitous in the environment and were found at the Milwaukee 5, Niagara 9, and Niagara 1 reference sites.
- Concentrations of these compounds (ng/g wet) were in general elevated relative to other CEC classes.
- 4n-OP was the only compound not found at the majority of sites.

Chemical concentration table. Summary of compound concentrations in ng/g wet weight. Concentrations below 3 x detection limit were changed to zero.

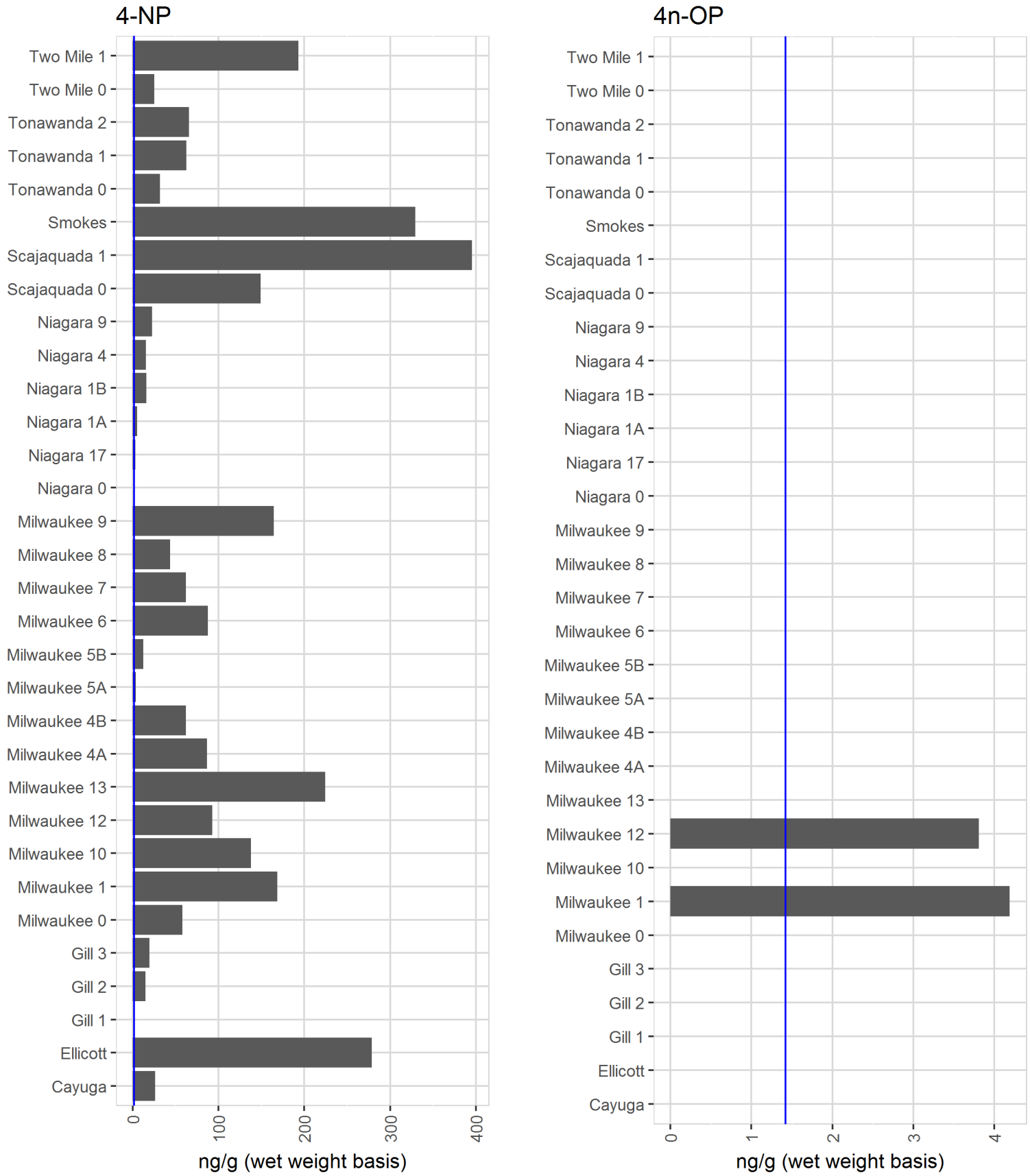
			Concentration (ng/g wet weight)		
COMPOUND	**Usage**		**maximum**	**mean**	**median**
NP1EO	Industrial	Alkylphenols	206	68.75	54.65
NP2EO	Industrial	Alkylphenols	53	17.14	10.12
4-NP	Industrial	Alkylphenols	395	89.19	59.82
4n-OP	Industrial	Alkylphenols	4.19	0.25	0.00



Box and whisker plot. Only chemical compounds found at more than five sites that had concentrations above 3 x DL were included. Measurements of zero were not included. This plot provides perspective to the relative concentrations of alklyphenols and ethoxylates in dreissenid mussel tissue.



Bar plots. Concentration results for dreissenid mussels by site and time. Blue line represents 3 x detection limit. Concentrations below 3 x detection limit were changed to zero. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

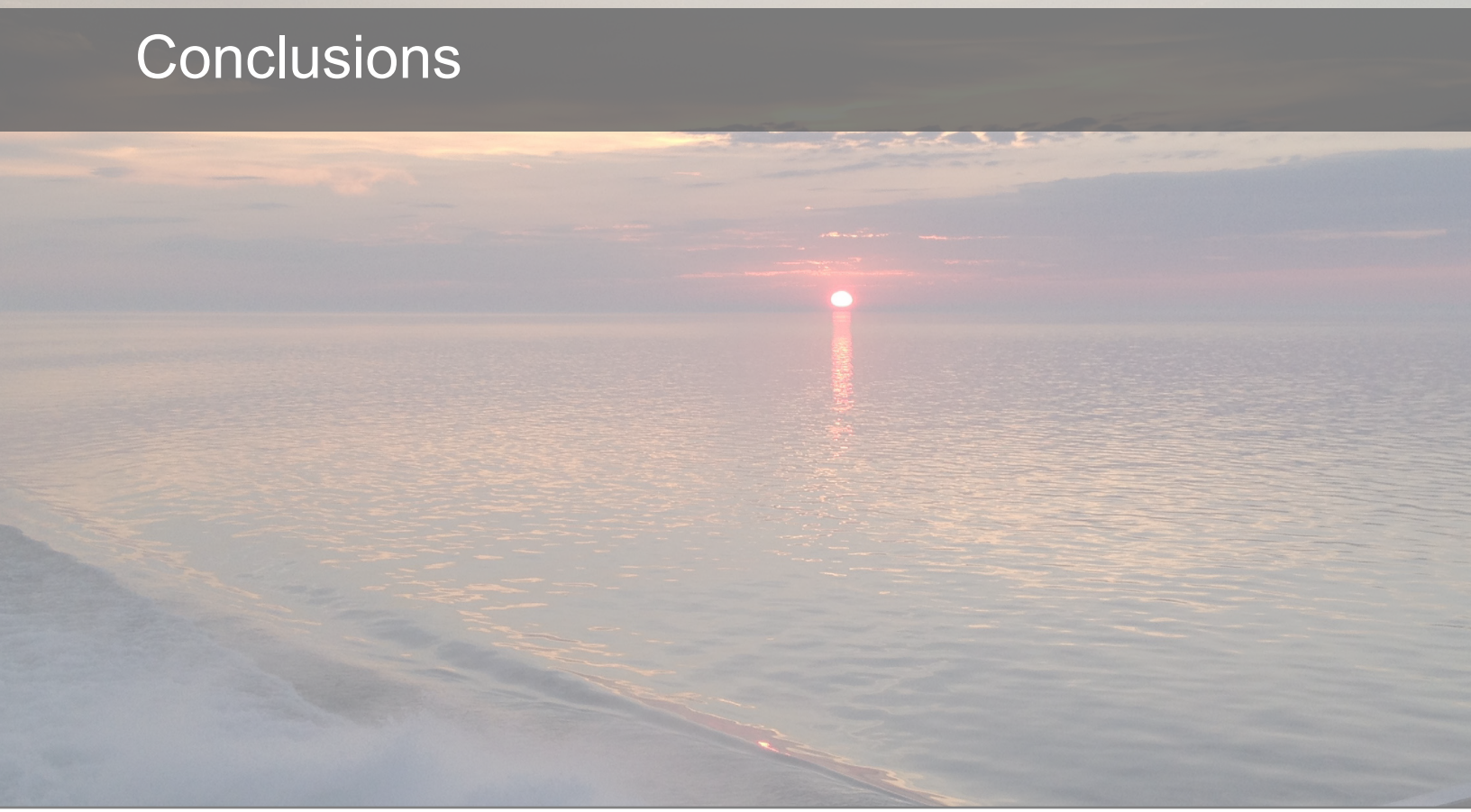


Bar plots. Concentration results for dreissenid mussels by site and time. Blue line represents 3 x detection limit. Concentrations below 3 x detection limit were changed to zero. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

Site concentration table. Concentration (ng/g wet weight) results for dreissenid mussels by site and time. Concentrations below 3 x detection limit were changed to zero. Milwaukee sites ending in A and B represent samples collected in May, June and July of 2013 respectively. Niagara sites ending in A and B represent samples collected in July and June of 2014 respectively.

SiteName	4n-OP	4-NP	NP1EO	NP2EO
Cayuga	0	25.9	24.94	5.032
Ellicott	0	278.6	60.28	8.63
Gill 1	0	0	9.04	1.592
Gill 2	0	14.8	3.26	0
Gill 3	0	19.4	7.54	0
Milwaukee 0	0	57.57	61.82	17.8
Milwaukee 1	4.19	168.47	148.32	34.9
Milwaukee 10	0	137.6	137.18	44.4
Milwaukee 12	3.81	92.47	168.32	37.5
Milwaukee 13	0	224.6	125.18	35.5
Milwaukee 4A	0	86.27	81.92	21.6
Milwaukee 4B	0	62.07	198.32	44.4
Milwaukee 5A	0	3.57	49.02	16.5
Milwaukee 5B	0	11.9	78.58	21.6
Milwaukee 6	0	87.47	107.32	34.4
Milwaukee 7	0	62.07	113.32	30.9
Milwaukee 8	0	43.47	129.32	33.3
Milwaukee 9	0	164.47	206.32	53.9
Niagara 0	0	0	4.14	0
Niagara 17	0	3.1	6.54	2.372
Niagara 1A	0	5	0	1.402
Niagara 1B	0	15.7	11.58	1.64
Niagara 4	0	15.4	26.68	6.51
Niagara 9	0	22.5	15.28	1.87
Scajaquada 0	0	149.2	26.04	5.552
Scajaquada 1	0	395.6	85.58	11.6
Smokes	0	329.2	129.64	33.552
Tonawanda 0	0	31.5	28.28	5.11
Tonawanda 1	0	62.3	26.88	4.79
Tonawanda 2	0	65.6	30.18	5.83
Two Mile 0	0	25	29.64	6.702
Two Mile 1	0	193.2	69.44	19.552

Conclusions



Of the 237 compounds analyzed by all analytical methods, 99 compounds (42%) were detected in dreissenid mussels. Trifluralin, Sertraline, NP1EO, NP2EO, and 4-NP were the CECs found at all sites. Our results indicate that some CECs are accumulated in mussel tissue at concentrations that can be measured by current analytical methods. CEC detections occurred at all sites and the frequency of detection was greater at sites located in river/harbor areas with known contaminant sources such as runoff of agrochemical and wastewater treatment outfalls. Some CECs were detected in references sites distant from known sources of pollution suggesting persistence and/or transport offshore.

These results support the use of biomonitoring of CECs. However, when chemicals are not detected in mussel tissue, it does not necessarily mean that they are not present in the water column. The next steps are to include water sampling along with mussel sampling in order to confirm waterborne exposure of CECs to biota. Concurrent with this effort of water and bivalve CEC monitoring should be the inclusion of bivalve health indicators, techniques utilizing omics and biomarker assays that measure biological response to environmental stressors that help link CEC exposure to biological effects.

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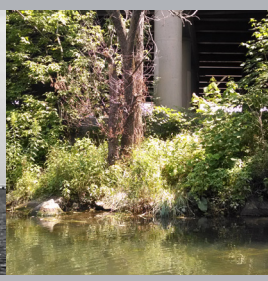

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