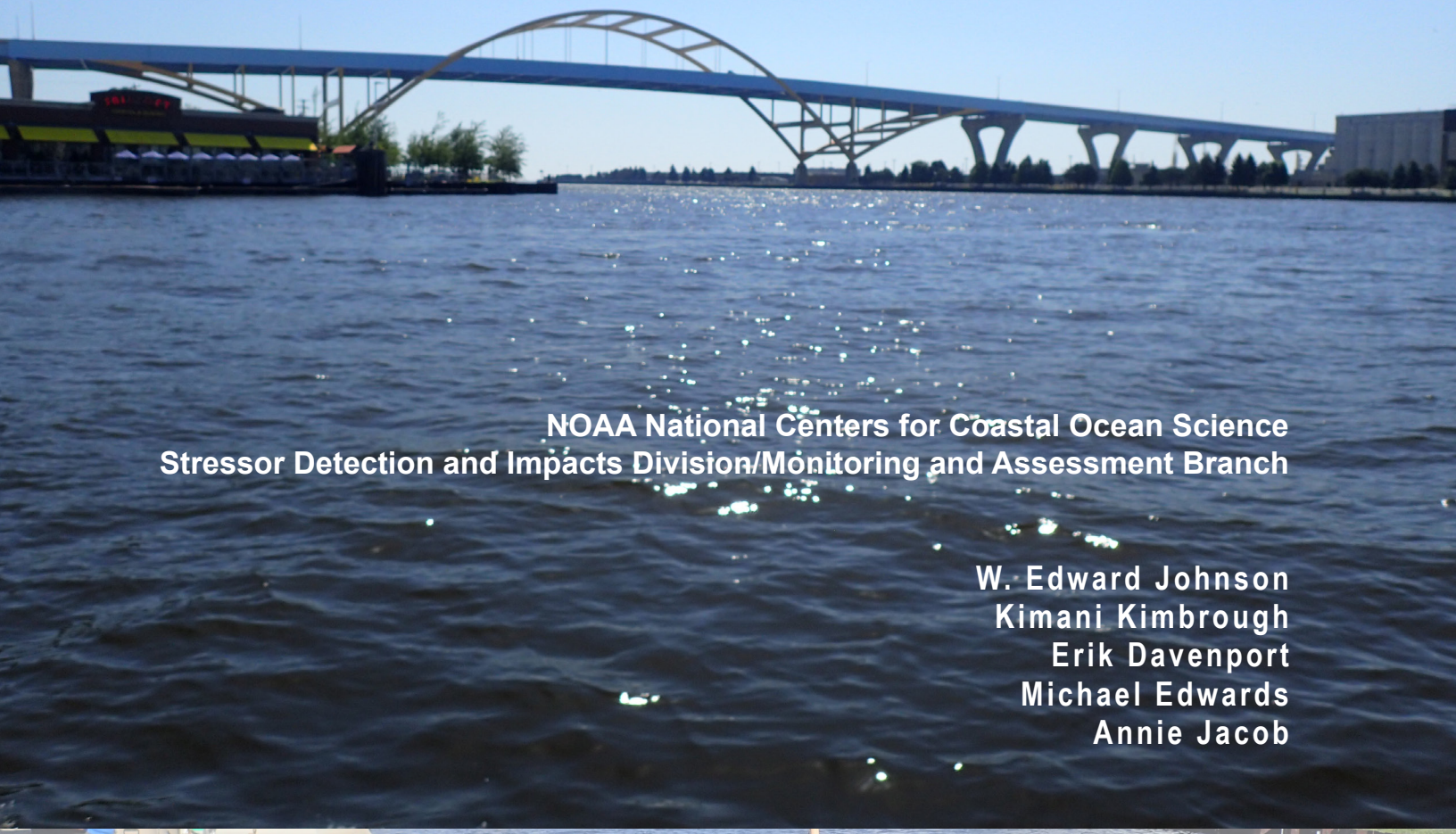
Great Lakes Restoration Initiative Interagency Agreement Final Report: NOAA Mussel Watch FY 18



NOAA National Centers for Coastal Ocean Science
Stressor Detection and Impacts Division/Monitoring and Assessment Branch

W. Edward Johnson
Kimani Kimbrough
Erik Davenport
Michael Edwards
Annie Jacob

June 2021

GOVERNMENT CONTRACT LABOR WAS PROVIDED BY CSS-DYNAMAC, FAIRFAX, VA UNDER NOAA CONTRACT NUMBER # EA-133C-14-NC-1384.

Cover photos.
Top: Milwaukee Bridge, credit NOAA; bottom: NOAA boat, credit NOAA

Table of Contents

Project Information

A. Title: (2018-666)Integrated Emerging Contaminant Monitoring in the Great Lakes by NCCOS Mussel Watch Program.

B. Principal Investigator: Dr. Ed Johnson, NOAA, Great Lakes Chief Scientist,

Ed.Johnson@noaa.gov, 240-533-0345

C. Project Period: 2018-2020

D. Funded Amount: $638,273

Project Approach for FY18 effort

- Participation in the Integrated Case Assessment Study at Milwaukee Bay Estuary along with several other federal partners.
- Mussel collection from riverine and nearshore sites along eastern and western Lake Michigan and Lake Ontario.
- Characterize the temporal variability of dressined mussel metabolome by sampling mussels from May- Nov from a site near NOAA Muskegon Field Station.
- Utilize a multi-indicator approach to characterize contaminant exposure and effects.
- Use of mussel bioeffects techniques such as untargeted metabolomics, targeted metabolomics and DNA damage assays.

The National Oceanic and Atmospheric Administration's (NOAA) Mussel Watch Program (MWP) administered by the National Centers for Coastal Ocean Science (NCCOS) has used dreissenid mussels to monitor contaminants in the nearshore zones of the Great Lakes since 1992. MWP has 25 long-term monitoring sites in the Great Lakes from Duluth, MN on Lake Superior at the mouth of the St. Louis River to Cape Vincent, NY where Lake Ontario flows into the St. Lawrence River. 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.

In FY12 and beyond, MWP initiated a more focused effort in select AOCs and incorporated newer techniques and approaches such as the use of caged mussels, bivalve health metrics, etc. in order to better address contamination and remediation issues of specific AOCs. While the main focus of Phase 1 efforts was on providing data on legacy contaminants, MWP did opportunistic monitoring of contaminants of emerging concern (CEC) in mussels, which paved the way for participation in the GLRI Action Plan II efforts.

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." Accordingly, a multi-agency team of federal scientists formulated a Strategic Plan, which sets forth two major goals:
(1) To characterize and evaluate the extent to which CECs threaten fish and wildlife populations relative to other chemical stressors present in the Great Lakes, and,
(2) To pilot and develop a short and long term state-of-the-art bioeffects surveillance program for the Great Lakes basin.

The major goals are to be achieved through three separate, but integrated study components including Surveillance Program (SP), Integrated Assessment Case Studies (IACS) and Priority Contaminant Mixtures (PCM). While the SP component is conducted at the Great Lakes basin-wide scale, the latter two are conducted at the individual watershed scale.

In FY18, MWP participated in the IACS study initiated by the federal team, the main objective of which is to identify associations between watershed attributes, such as land use and point sources, and CEC prevalence in watersheds. In 2018, the focus was on Pharmaceuticals and Personal Care Products (PPCPs) in the urban watershed of Milwaukee Estuary AOC to help identify CEC stress associated with urban land use. MWP also conducted surveillance sampling of mussels from western and eastern Lake Michigan and Lake Ontario. For MWP, the specific objectives for the 2018 field season were:
Objective 1: Support the GLRI CEC Team's "Year of the PPCPs" study of the Milwaukee River.
Objective 2: Nearshore and riverine sampling of mussels from western and eastern Lake Michigan & Lake Ontario.
Objective 3: Measure chemical body burden,

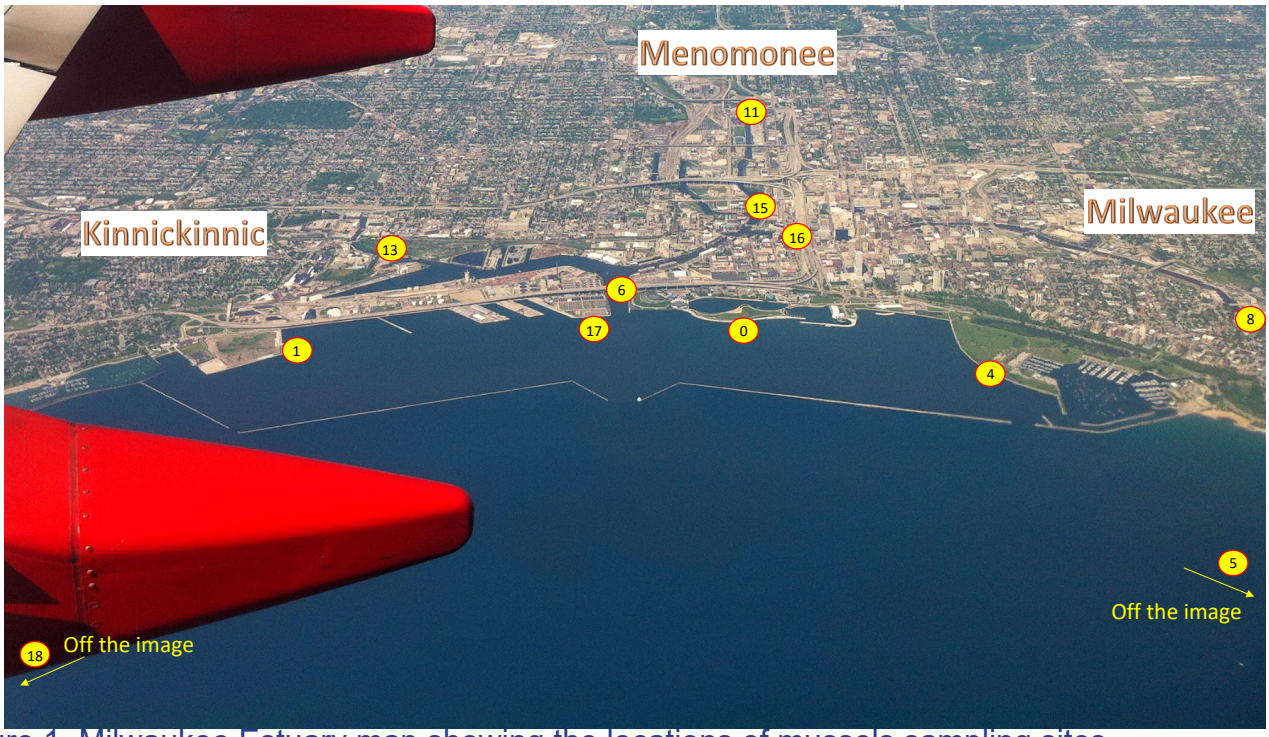


Figure 1. Milwaukee Estuary map showing the locations of mussels sampling sites.

metabolomics, cellular biomarker and DNA damage response in mussels
Objective 4: Study the change in mussel metabolome from May- Nov by repeated sampling of in situ mussels at Muskegon Field Station.

Milwaukee Estuary- IACS

The Milwaukee Estuary Area of Concern (AOC) was designed as one of the 31 US AOCs in 1987 by the International Joint Commission. The AOC includes the lower 3.1 miles of the Milwaukee River, the lower 3 miles of the Menomonee River down, the lower 2.5 miles of the Kinnickinnic River and the inner and outer Harbor and nearshore waters of Lake Michigan. In 2008, the boundary was expanded to include the following: Cedar Creek downstream from Bridge Road to confluence with Milwaukee River Milwaukee River and Lincoln Creek from confluence with Cedar Creek to North Avenue Dam; Little Menomonee River from Brown Deer Road to confluence with Menomonee River, and Menomonee River downstream from confluence with Little Menomonee River to 35th Street.

Past industrial activities coupled with urbanization in subsequent years have caused pollution and impairments to the beneficial use of the waters within Milwaukee estuary AOC boundaries. Out of a total of 14 possible beneficial use impairments (BUIs), eleven were designated within the original AOC and four within the expanded boundary. Legacy contamination from polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals are considered to be the predominant cause of the impairments. Further, water quality has been affected by pollution sources associated with land use from the entire drainage basins of the Milwaukee, Menomonee, and Kinnickinnic Rivers.

In situ mussels were collected from the outer harbor site (LMMB 0, 1, 4 & 6) in Milwaukee Estuary and from two sites (LMMB 5 & 18) in Lake Michigan, Wisconsin. Mussels from LMMB 4 were used to deploy at LMMB 4, 6, 8, 11,13 and 17. Mussels from LMMB 5 were caged at the same site to assess cage effect.

Surveillance and Temporal Sampling

In situ mussels were collected from 21 riverine and near shore sites distributed in Lake Michigan, Ontario and Lake Huron. Additionally, in situ mussels were collected from the same location in Muskegon Lake (near NOAA field station) 9 times from May- Nov to examine the temporal variability of bivalve health indicators and body burden in relation to biological and environmental factors.

Field Component

- Collections of in situ mussels from 2 outer harbor sites in Milwaukee Estuary and relocation in cages at 7 sites.
- Deployment of mooring with caged mussels, data loggers (temperature and dissolved oxygen) and passive samplers (POCIS or SPMDs or both).
- Retrieval of moorings after 4 weeks.
- Processing of mussels for bivalve health metrics.
- Sampling of mussels from riverine and nearshore sites along eastern and western Lake Michigan and Lake Ontario.
- Temporal sampling of mussels from May- Nov from a site near NOAA Muskegon Field Station to examine the changes in mussel health metrics.

Laboratory analyses

- Mussel tissue chemistry (legacy organics and contaminants of emerging concern)
- POCIS chemistry
- SPMD chemistry
- Targeted and untargeted metabolomics
- DNA damage analyses
- Cellular biomarker analyses

Site Descriptions

LMMB 0 is located in the outer harbor north of the harbor entrance ship channel. This site is adjacent to Lakeshore State Park, a 7-ha manmade island constructed mainly of dolomite limestone from a deep tunnel project of the Milwaukee Metropolitan Sewerage District in 1991.

The LMMB 1 site is located adjacent to the Milwaukee Harbor Confined Disposal Facility (dredge spoil) in the outer harbor south of the harbor entrance ship channel and approximately 4 km south of LMMB 4.

LMMB 4 is located in the outer harbor north of the harbor entrance slip channel adjacent to Juneau Park, whose land use has remained largely unchanged since the early 1900s.

LMMB 5 is approximately 0.4 km offshore of a public swimming beach and about 4 km north of the entrance to Milwaukee harbor.

LMMB 6 is in Jones Island N end. About 35m E of the USGS water quality station building.

LMMB 8 is in Upper Milwaukee River (MKE). E side of MKE river (N) about 20m S of Humboldt Ave bridge.

LMMB 11 is in Upper Menomonee (MM) River. N side of MM river near the intersection of Mt Vernon Ave and 21st Street. Mooring tied off near the second mooring bollard E of the first bollard.

LMMB 13 is in Upper Kinnickinnic (KK) River. W side of KK about halfway between the Becher St and Lincoln Ave bridges.

LMMB 14 is in Lower Kinnickinnic River. W side of KK about 25m downstream of the turning basin w/ in 3m of the concrete ship wall.

LMMB 15 is in Lower Menomonee River. N side of MM river about 40m E of the 6th Street bridge.

LMMB 16 is in Lower Milwaukee River. W side of MKE river about halfway between the Clybourn and St Paul St bridges. W side of river on bulkhead about 40 m N of the E St Paul Ave bridge and 325 m N of the Milwaukee River and Menomonee River confluence. This site only had a pressure sensor logger (no mooring and no mussels). The pressure sensor was tested to determine if it could identify flood events (increased pressure due to increased water depth).

Figure 2. Sites in Lake Michigan, Lake Huron and Lake Ontario sampled for mussels in 2018.

LMMB 17 is a new river site. Approximately 160m S of where the confluence of Milwaukee's three river discharge into the outer harbor. Primary outfall of the Jones Island WWTP (Milwaukee Metropolitan Sewerage District - MMSD). Sides scan sonar on the R2604 confirmed the position of the outfall on the concrete wall. Water depth at this location is 30' due to maintenance dredging. Two moorings were deployed on the same chain – total of 4 galvanized Frabill minnow traps. This was a microplastic site along with 6, 0, 4, and 5.

LMMB18- Western Lake Michigan nearshore site. 2.5km SE of the S Milwaukee harbor breakwater opening used by the Lake Express High Speed Ferry and 2km from Lake Michigan shoreline. Water depth 15 feet.

LMSJ- St Joseph's river. Site established in Southeast Lake Michigan at the St Joseph's River mouth approximately 100 m E of the end of the N pier on its south side (channel side, not lake side) between the inner and outer lights (closer to the inner light). St Joseph's River bisects the towns of Benton Harbor and St Joseph's.

LMBR- Black River- Site established in Southeast Lake Michigan at the Black River mouth (channel side) under the south pier head light. Town of South Haven, Michigan.

LMKZ- Kalamazoo River. Site established in Southeast Lake Michigan at the Kalamazoo River mouth approximately 150m east of the south pier head light and along the stone boulder inset where the south jetty meets the shoreline.

LMPS0- Port Sheldon. Site established in Southeast Lake Michigan at the Pigeon River mouth approximately 350 m E of the north pier head light (channel side).

TBID0- Thunder Bay Island Davidson. Site in Lake Huron approximately 200m south of Thunder Bay Island at the permanent boat mooring for the historic James Davidson ship wreck. Water depth 35 feet.

TBIW0- Thunder Bay Island Wilson- Site in Lake Huron approximately 2.5km NE of Thunder Bay Island at the permanent boat mooring for the historic D.W. Wilson ship wreck. Water depth 40 feet.

TBRD0- Thunder Bay River dock- Site in Thunder Bay River 1.25km upstream from the river mouth at the dock (river-east) behind the Great Lakes Maritime Heritage Center.

LMMW0- Manitowoc-Site established in Lake Michigan at the mouth of the Manitowoc River on the harbor side of the north breakwater about 150m NW of the Manitowoc Breakwater Lighthouse.

LMMW01- Manitowoc- Site established in Lake Michigan approximately 1.8km NE of the Manitowoc Breakwater Lighthouse and 1.2km from the nearest lake shoreline.

LMTR0- Two Rivers-Site established in Lake Michigan at the mouth of Two Rivers in the channel near the south pier head and approximately 125m from the end of the south pier head.

LMAG0- Algoma- Site established in Lake Michigan at the mouth of the Ahnapee River along the harbor side of the main north pier's east end and west of the foot bridge across the break in the wall to the detached outer north pier head light.

LMAG01-Algoma- Nearshore site in Lake Michigan 0.95km SSE of the Algoma north pier head light and 0.8km E of the shoreline. Water depth 26 feet.

LMKW0- Kewaunee River- Site in Lake Michigan at the mouth of the Kewaunee River in the channel and from the sheet pile wall, approximately 500m NW of the south pier head light.

LMKW01- Kewaunee River- Nearshore site in Lake Michigan at the Kewaunee Shoal Light, approximately 2.25km E of the Kewaunee River south pier head light. Water depth 14 feet.

LMSB0- Sturgeon Bay- Nearshore site near the entrance to Sawyer Bay about 0.5km south of Cabot Point and 0.45km NE of Quarry Point.

LOFC0- Fourmile Creek- new nearshore site. Lake Ontario 0.85km NW of the mouth of Fourmile Creek, near Fourmile Creek State Park, NY.

NRYT0- Niagara River- Youngstown- Site established in connecting channel in Lower Niagara River at Youngstown, approximately 4km upstream from the Niagara River and Lake Ontario and about 60m from E shoreline (US side of the Niagara R.).

LOGR0-Genesee R- Site established in Lake Ontario at the mouth of the Genesee River in the channel off of sheet pile approximately 0.5km from the end of the west pier head.

LOOR0- Oswego River- Site established in Lake Ontario at the mouth of the Oswego River on the east side of the dock at the H. Lee White Museum.

LOCV0- Cape Vincent- Site established in the connecting channel in Lake Ontario at the entrance to the St Lawrence River on shore side (U.S.A.) of the east end of the 425m breakwater. Water depth 20 feet.

LOFO- offshore Lake Ontario. Site is one of two in Lake Ontario that is part of EPA's Fish Monitoring and Surveillance Program.

MUS 1-9 (Muskegon site): The site is located at the mouth of the Muskegon River (Lake Michigan) about 40 feet west of the end of the south pier head in 20 feet of water. An abundant population of mussels (quagga) inhabit the stone boulders protecting the concrete and sheet pile wall of the pier head. A mooring was deployed on the sand bottom at the base of the stone boulders that slope upwards from the sand bottom to about 5 feet below the water surface against the sheet pile wall. Mussels were collected from the boulders within 20 feet of the mooring placement at a depth of about 20 feet.

Mussel Sampling for IACS

In situ mussels were sampled by divers from the riverine and nearshore sites by SCUBA divers. For the IACS study at Milwaukee Estuary, divers harvested mussels from LMMB 4 and LMMB 5 using a metal paint scraper from rocks and the sheet pile wall on the north side of the island. The mussels were placed into a separate nylon mesh dive bag; the bags were gently shaken underwater to remove debris. Upon surfacing, the harvested mussels in individual mesh bags were immediately placed in a 26 L cooler with aerated site water until deployment. A subsample comprising 200-400 harvested in situ mussels were taken to analyze for baseline tissue chemistry and metabolomics before caging. The cages were deployed for 4 weeks and retrieved between July 9-11, 2018. Approximately 300-500 mussels were placed inside each cage, which consisted of torpedo shaped metal minnow traps that were tightly secured with cable ties. The cages were deployed approximately 0.5 m above the river bottom and secured to shore with #2 zinc plated double loop steel chain. One composite sample comprising 200-400 harvested mussels was taken to analyze for tissue chemistry and bivalve health assays.

The Polar Organic Chemical Integrative Sampler (POCIS) is composed of two sheets of microporous

(0.1µm pore size) polyethersulfone membrane encasing a solid phase sorbent (Oasis HLB) which retains sampled chemicals. The Oasis HLB is a universal solid-phase extraction sorbent widely used for sampling a large range of hydrophilic to lipophilic organic chemicals from water.

The Semipermeable Membrane Device (SPMD) is composed of lay flat, low density polyethylene tubing containing a thin film of a pure, high-molecular weight lipid (triolein). Both POCIS and SPMD were purchased from EST-lab (St. Joseph, Missouri) and deployed at 6 sites in Milwaukee Estuary and at one site in Muskegon Lake.

Sample Processing

Tissue Chemistry
One composite sample comprising 200-400 mussels was taken from each cage to analyze for tissue chemistry. Mussel samples were rinsed with site water to remove debris, placed in freezer bags, packed on water ice and shipped to laboratories within two days.

Cellular biomarker and DNA damage
Two FEP bags each containing 15-20 mussels were prepared for DNA damage and cellular biomarker analyses. Each FEP-bag received a unique number. The four FEP bags from each site are placed into one labeled cloth bag, and flash-frozen by placing in a cryogenic dry vapor shipper (-196 degrees C).

Metabolomics
Each metabolomics sample consists of 24 individually FEP-bagged mussel plus two additional composite bags containing 15-20 extra mussels for archive. Prior to bagging the 24 individual mussels, digital calipers are used to measure their longest length and recorded on the data sheet. The composited bags of bivalves are not measured. Each FEP-bag received a unique number. The entire group of 26 FEP bags from each site are placed into one labeled cloth bag, and flash-frozen by placing in a cryogenic dry vapor shipper (-196 degrees C).

Analysis Methods

Tissue Chemistry
Tissue samples were analyzed by TDI-Brooks International, Inc., Texas for PAH and legacy organics, and by SGS AXYS Analytical Services Ltd. in British Columbia, Canada for CECs. Protocols for analytical methods for organic contaminants including PAHs in mussel tissue are detailed Kimbrough et al. (2006).
The SGX AXYS 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).

DNA damage
DNA damage analyses were conducted by scientists at National Institute for Standards and Technology and methods are described in detail in Jaruga et al., 2017. Briefly, mussels were thawed on ice, then washed with ice-cold deionized water. Mussel tissues (≈100 mg) separated from shells with a scalpel were processed according to the product manual of E.Z.N.A. Mollusc DNA Kit, Omega Bio-tek (Norcross, Georgia) with modification involving homogenization with Bullet Blender Storm 24 high-throughput bead-mill homogenizer (Next Advance, Averill Park, New York). Tissues were placed in the 1.5 mL Rhino Screw cap tubes (Next Advance) kept on ice, containing 350 mL of ML1 Buffer from the kit and three 2 mm zirconium oxide beads. Tubes were transferred into the Bullet Blender kept in the refrigerator at 4 0C and processed 2 x 30 s at speed 12 with 30 s break between runs. Subsequently 25 mL of Proteinase K from the kit was added and samples were incubated for 2 h at 60 0C. Then, subsequent steps of the Mollusc DNA Kit protocol were applied. For the final DNA elution, two portions of 100 mL of sterile high-performance liquid chromatography grade water (Sigma- Aldrich, St. Louis, Missouri) warmed to 70ºC were used. The UV absorbance spectrum of each DNA sample was recorded by absorption spectrophotometry between the wavelengths of 200 nm and 350 nm to ascertain the quality of DNA and to measure the DNA concentration at 260 nm (absorbance of 1 = 50 mg of DNA per mL).

Aliquots (50 mg) of DNA samples were dried in 1.5 mL deoxyribonuclease-free Eppendorf tubes in a SpeedVac under vacuum and then kept at –80 0C for further analysis. Gas chromatography-tandem mass spectrometry with isotope-dilution was used to identify and quantify modified DNA bases and 8,5' -cyclopurine-2'-deoxynucleosides. Six modified DNA bases (5 hydroxy-5-methylhydantoin (5-OH-5-MeHyd), thymine glycol (ThyGly), 5,6-dihydroyuracil (5,6-diOH-Ura), 4,6-diamino-5-formamidopyrimidine (FapyAde), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 8-hydroxyguanine (8-OH-Gua)) and three 8,5′-cyclopurine-2′-deoxynucleosides ((5'S)-8,5′-cyclo-2′-deoxyadenosine (S-cdA), (5'R)-8,5′cyclo-2′-deoxyguanosine (R-cdG) and (5'S)-cyclo-2′-deoxyguanosine (S-cdG)) were identified and quantified in the mussels' tissue samples.

Targeted Metabolomics

AXYS Analytical Services Ltd. provided the analytical services for targeted metabolomics on dreissenid mussels. The methods are proprietary and confidential. Mussels were extracted from their shells, exterior water removed and the whole body homogenized. Extraction with methanol and chloroform used a bead blender and a portion of the extract was subject to further workup and analysis for the six lists of metabolites:

· MABA: Amino acids and Biogenic amines (43 metabolites)
· MFAAHEX: Fatty acids (18 metabolites) and Hexose (1 metabolite)
· MLIP: Phospholipids and Acylcarnitines (144 metabolites),
· MNRG: Metabolites associated with energy pathways (17 metabolites).

Untargeted Metabolomics

Mussels samples were analyzed by scientists at National Institute of Standards and Technology using nuclear magnetic resonance spectroscopy. Methods are described in detail in Watanabe et al., 2015.

Cellular biomarkers

Acetylcholinesterase (AChE) activity was used as a biomarker of neurological toxicity, and is often a target of pesticides. A standard kinetic assay that measures the reaction rate of DTNB through coupling of acetylthiocholine iodide (ACTC) is used to measure AChE activity (Fulton and Key, 2001; Binelli et al., 2005). Mussel and clam tissues were weighed and homogenized in 20 mM sodium phosphate buffer (pH 7.6) with Triton-x-100 at 1:5 mass to volume ratio and centrifuged at 10,000 g for 20 minutes at 4°C. Subsamples of the supernatant were used for total protein analysis (Biorad protein assay) and AChE activity (40 µl of supernatant, 180 µl sodium phosphate buffer, 30 µl of 5 mM 5,5'-di-thio-bis-2-nitobenoic acid (DTNB), and 30 µl of 25 mM acetylthiocholine iodide (ACTC) to initiate the kinetic reaction). The AChE activities were measured using Thermo Scientific Multiskan GO 96-well plate spectrophotometer (3 replicates per sample) at 412 nm at 22°C for 7 minutes at 30 second intervals. The AChE activity was then calculated based on the following standard equation:

AChE activity µmol ACTC/min/mg protein $=(\Delta A_{412}\times Vol_t\times 1000)/(1.36\times 10^4\times lightpath\times Vol_s\times Protein)$

ΔA_{412} = Change in absorbance per min, corrected for spontaneous hydrolysis
Volt = total assay volume (ml)
1.36×10^4 = extinction coefficient of TNB (M/cm)
Lightpath = microplate well depth (1 cm)
Vol_s = sample volume (in ml)
Protein = concentration of protein (mg/ml)

It has been demonstrated that baseline AChE levels are temperature-dependent (Binelli et al., 2005; Ricciardi et al., 2006), changing in a parabolic pattern over temperatures ranging from 5 – 30oC. Therefore, the expected concentrations based on ambient water temperature (T) can be estimated based on the following equation: AChE activity = -0.0117T2 + 0.3946T - 0.5747.

Lipid peroxidation assays (based on malondialdehyde (MDA) concentrations were used as a biomarker of oxidative stress (Ringwood et al., 1999). Mussel and clam tissues were weighed and homogenized in 20 mM sodium phosphate buffer (pH 7.6) with Triton-x-100 at 1:5 mass to

volume ratio. The homogenates were centrifuge at 13,000 g for 5 minutes at 4°C. Supernatant samples (50 µl) were added to 700 µl of 0.375% thiobarbituric acid (TBA) and 7 µl of 2% butylated hydroxytoluene (BHT), boiled for 15 minutes at 100°C, and centrifuged for 5 minutes at room temperature at 13,000 g. The MDA levels of the supernatants (200 µl / well) were measured at 532 nm using a Thermo Scientific Multiskan GO 96-well plate spectrophotometer (3 replicates per sample). Malondialdehyde tetraethyl acetal (MDA) was used for standard curves (slopes and blanks were checked for consistency between batches), and the results are reported as MDA nmol/g wet weight.

Total glutathione (GSH) concentrations were used as an indicator of antioxidant status and detoxification capacity, and also as an indicator of toxicity. The assay is a kinetic assay that measures the change in DTNB as driven by glutathione reductase (Ringwood et al., 1999). Mussel and clam tissues were weighed and homogenized in 5% sulfosalicylic acid (SSA) at 1:5 mass to volume ratio, and centrifuged at 13,000 g for 5 minutes at 4°C. Supernantant samples (30 ul) were mixed with 150 µl of 280 mM NADPH and 40 µl of 10 mM DTNB (NADPH and DTNB were prepared in sodium phosphate buffer (143 mM, 6.33 mM Na4EDTA at pH 7.5)). Then 30 µl of 50 U/ml glutathione reductase was added to each well to start the reaction, measured at 412 nm absorbance for 2 minutes with a Thermo Scientific Multiskan GO 96-well plate spectrophotometer (3 replicates per sample). Reduced glutathione was used to generate standard curves (slopes and blanks were checked for consistency between batches), and the results are reported as GSH nmol/g wet weight.

Table 2. Chemical list A) PAHs

Acenaphthene	Acenaphthylene	Anthracene	Benz[a]anthracene
Benzo[a]pyrene	Benzo[e]pyrene	Benzo[b]fluoranthene	Benzo[k]fluoranthene
Benzo[g,h,i]perylene	Benzothiophene	C1-Benzothiophene	C1-Chrysenes
C1-Dibenzothiophenes	C1-Fluorenes	C1-Fluoranthenes_Pyrene	C1-Naphthobenzo-thiophene
C1-Naphthalenes	C1-Phenanthrenes_ Anthracenes	C2-Dibenzothiophenes	C2-Fluorenes
C2-Fluoranthenes_Pyrenes	C2-Naphthobenzo-thiophe	C2-Naphthobenzothiophene	C2-Naphthalenes
C2-Phenanthrenes_Anthracenes	C3-Benzothiophene	C3-Chrysenes	C3-Dibenzothio-phenes
C3-Fluorenes	C3-Fluoranthenes_ Pyrenes	C3-Naphthobenzothiophene	C3-Naphthalenes
C4-Chrysenes	C4-Naphthalenes	C4-Phenanthrenes_Anthra-cenes	Chrysene
Dibenzo[a,h]anthracene	Dibenzofuran	Dibenzothiophene	Fluoranthene
Fluorene	Indeno[1,2,3-c,d] pyrene	Naphthobenzothiophene	Naphthalene
Phenanthrene	Pyrene		

B) Pesticides by GC/HRMS (MLA-035.R07.02).

2,4'-DDD	Chlorothalonil	Fenitrothion	Nonachlor, cis-
2,4'-DDE	Chlorpyriphos	Flufenacet	Nonachlor, trans-
2,4'-DDT	Chlorpyriphos-Oxon	Flutriafol	Octachlorostyrene
4,4'-DDD	Chlorpyriphos-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	
Chlordane, oxy-	Ethion	Mirex	

C) Octylphenol, Nonylphenol & Nonylphenol Ethoxylates (MLA-080.R02.04).

4-NP	4n-OP
NP1EO	NP2EO

D) Pharmaceuticals and personal care products (MLA-075.R06.01).

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

FY18 study was undertaken with the support and collaboration with the NOAA office listed below:

- NOAA-GLERL- boat and diving support for SCUBA diving operations, and mooring deployment and recovery; GLERL ecologist provided scientific and logistical support.

Twelve missions were carried out between April - November, 2018 with 274 person field days, 72 mission days, 32 dive days and 30 boat days.

Event	Date
Annex 3 activities update EPA-NOAA brief	Q1
GLRI PFAS coordination meeting	Q2
GLRI Phase 1 report	Q2-Q3
Field planning and logistics meeting for Milwaukee IACS	Q2- Q3
GLRI CEC monthly call	Q1-Q4
GLRI CEC session at SETAC in Sacramento, CA	Q2
GLRI F2F Meeting in Athens	Q2
GLRI-CEC Phase 2 report	Q4
IACS coordination call	Q2

Table 3: List of significant events during the project period.

What data is there

1. Mussel chemistry
2. Targeted metabolomics for mussels
3. Non-targeted metabolomics for mussels
4. DNA damage assay
5. POCIS chemistry
6. SPMD chemistry
7. Dissolved oxygen and temperature data

Purpose of data

1. Contaminant characterization of Milwaukee Estuary using bivalves and passive sampler chemistry.

2. Monitoring of nearshore and riverine sites along eastern and western Lakem Michigan, and Lake Ontario to obtain baseline chemistry and bivalve health data.

2. Assessment of bivalve health indicators (metabolomics and DNA damage assays) to inform on bioeffects of contaminants.

3. Temporal study of targeted and untargeted metabolomics of mussels in relation to environmental and biological factors.

How and when it was collected

1. Mussels were collected by scuba divers.
2. Mussel collections, mooring deployment and recovery were conducted between April- November 2018.

Where it resides

1. Chemistry data resides on a NOAA web page as downloadable excel files.

2. Chemistry data will permanently reside as part of NOAA's Query Manager database and be accessible through ERMA and DIVER.

3. Bivalve health indicator data reside on local drives and will be made available to public eventually after the publication of journal articles.

Table 4. Compilation of data collected are listed and their associated filename found on the website http://www.coastalscience.noaa.gov/projects/detail?key=179

Description	Filename
Great Lakes Sampling Sites	GL_Sampling sites_MWP_2018
Tissue Chemistry- Contaminants of Emerging Concern	GL_CEC_AXYS_2018.xls
Tissue Chemistry- PAH	GL_PAH_TDI_2018.xls
Tissue Chemistry- Legacy Organics	GL_Organics_TDI_2018.xls
POCIS Chemistry	GL_POCIS_AXYS_2018.xls
SPMD Chemistry	GL_SPMD_AXYS_2018.xls
Water Quality Data	GL_Water quality_2018.xls

Analysis of the data

Mussel Data Analysis

Mussel and passive sampler chemistry data were manipulated using R-code for both data formatting and analysis. In this report, we present the data in bar charts and box plots. In-depth analysis of the data will be conducted in associated technical memorandums and manuscripts.

List of Project Outputs

Following manuscript/reports are in development:

- Characterization of Great Lakes Mussel Watch (2013 - 2018) Pharmaceuticals and Personal Care Products using land-use and machine learning.
- Temporal variability of mussel health metrics in relation to biological and environmental factors.
- A manuscript titled 'Characterization of Polycyclic Aromatic Hydrocarbons in the Great Lakes Basin using Dreissenid Mussels' that include 2018 data has been submitted to the Journal of Environmental Monitoring and Assessment.

Conclusions and Recommendations

Specific conclusions/recommendations will be made once the entire data is analyzed.

Jaruga, P., E. Coskun, Kimbrough, K.L, A. Jacob,W.E. Johnson and M. Dizdaroglu. 2017. Biomarkers of oxidatively induced DNA damage in dreissenid mussels: A genotoxicity assessment tool for the Laurentian Great Lakes. Environmental Toxicology32:2144–2153.

Kimbrough, K. L., and G. G. Lauenstein (eds.). 2006. Major and trace element analytical methods of the National Status and Trends Program 2000-2006. NOAA Technical Memorandum NOS NCCOS 29 Silver Spring, MD 19pp.

Kimbrough, K. L., G. G. Lauenstein, and W. E. Johnson (eds.). 2006. Organic contaminant analytical methods of the National Status and Trends Program: Update 2000-2006. NOAA Technical Memorandum NOS NCCOS 30 Silver Spring, MD 65pp.

Kimbrough, K.L., W. E. Johnson, A. Jacob, M. Edwards, E. Davenport, G. Lauenstein, T. Nalepa,M. Fulton and A. Pait. 2014. Mussel Watch Great Lakes Contaminant Monitoring and Assessment:Phase 1. Silver Spring, MD. NOAA Technical Memorandum NOS NCCOS 180, 113 pp.

U.S. Environmental Protection Agency, 2007, Method 1694: Pharmaceuticals and personal care products in water, soil,sediment, and biosolids by HPLC/MS/MS: USEPA, Washington, DC, EPA-821-R-08-008, 77 p.

U.S. Environmental Protection Agency, 2007b, Method 1699:Pesticides in water, soil, sediment, biosolids, and tissue by HRGC/HRMS: USEPA, Washington, DC, EPA-821-R-08-001,96 p.

Watanabe, M., K. A. Meyer, T. M. Jackson, T. B. Schock, W. E. Johnson, and D. W. Bearden. 2015. Application of NMR-Based Metabolomics for Environmental Assessment in the Great Lakes Using Zebra Mussel (*Dreissena Polymorpha*). Metabolomics. http://dx.doi.org/10.1007/s11306-015-0789-4. https://repository.library.noaa.gov/view/noaa/13686

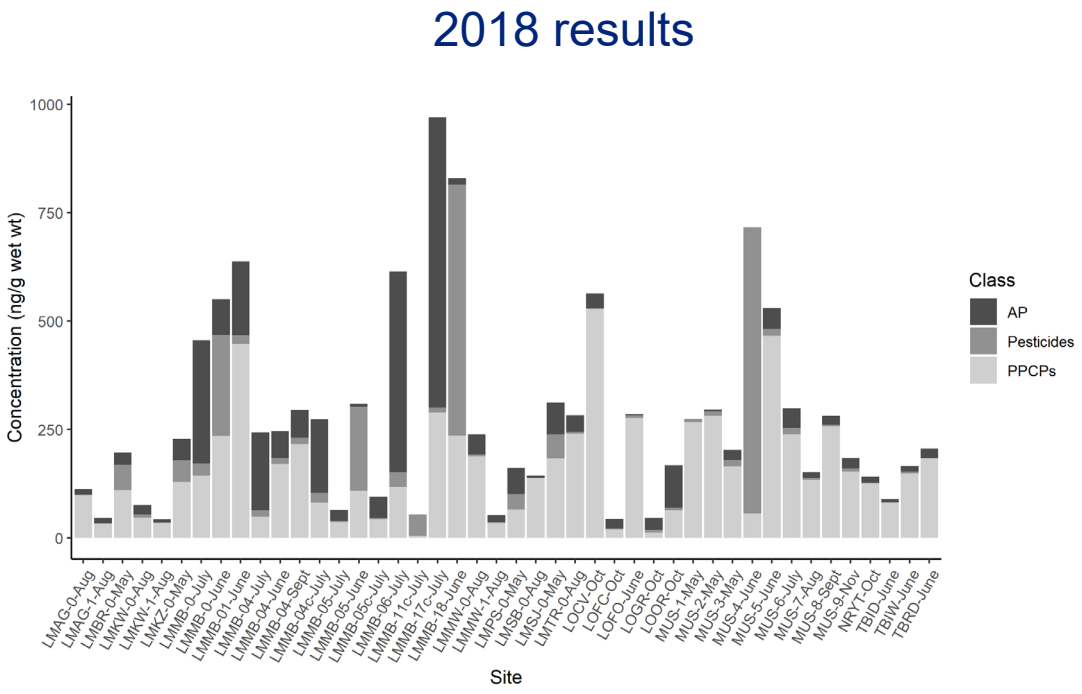


Figure 3: Total concentration of the three classes of contaminants of emerging concern (Alkylphenols (AP), Pesticides and Pharmaceuticals and Personal Care Products (PPCPs) at the sites sampled in 2018.

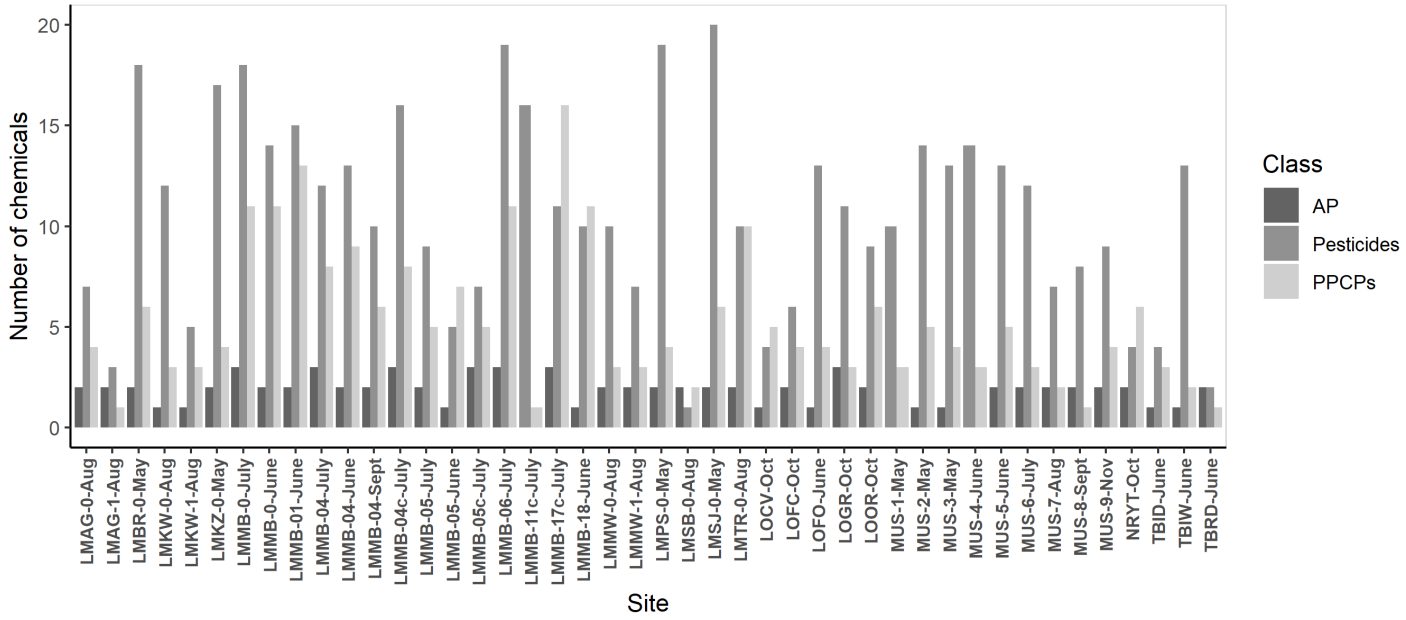


Figure 4: Total number of chemicals detected within the three classes of contaminants of emerging concern (Alkylphenols (AP), Pesticides and Pharmaceuticals and Personal Care Products (PPCPs) at the sites sampled in 2018.

Percent detection of CECs and legacy pesticides in mussel tissue

COMPOUND	PERCENT DETECTION
10-hydroxy-amitriptyline	2.3
4n-OP	2.3
beta-Endosulphan	2.3
Clotrimazole	2.3
Cocaine	2.3
Desmethyldiltiazem	2.3
Flutriafol	2.3
HCH, beta	2.3
Lomefloxacin	2.3
Metformin	2.3
Triamterene	2.3
2-Hydroxy-ibuprofen	4.5
Cypermethrin	4.5
Diltiazem	4.5
HCH, alpha	4.5
Sulfamethazine	4.5
Trimethoprim	4.5
Venlafaxine	4.5
Heptachlor	6.8
Norverapamil	6.8
Octachlorostyrene	6.8
Warfarin	6.8
Pendimethalin	9.1
Propranolol	9.1
Melphalan	11.4
Ranitidine	11.4
Mirex	13.6
NP1EO	13.6
Triclocarban	13.6
2,4'-DDT	18.2
Chlordane, oxy-	18.2
Etoposide	18.2
Fluoxetine	18.2
Miconazole	18.2
Azithromycin	20.5
Dacthal	20.5
Verapamil	22.7

COMPOUND	PERCENT DETECTION
Trifluralin	27.3
2,4'-DDE	29.5
Aldrin	29.5
Chlorpyriphos	31.8
Diphenhydramine	43.2
4,4'-DDT	52.3
Citalopram	52.3
Nonachlor, cis-	52.3
2,4'-DDD	54.5
Amitriptyline	65.9
Chlordane, gamma (trans)	72.7
Heptachlor Epoxide	72.7
Chlordane, alpha (cis)	79.5
NP2EO	79.5
4,4'-DDD	81.8
Sertraline	81.8
4-NP	84.1
Nonachlor, trans-	88.6
4,4'-DDE	93.2
Clonidine	95.5
Dieldrin	95.5
Hexachlorobenzene	97.7

Table 5: Percent detection of compounds detected above three times the detection limit in mussel tissue samples from 2018.

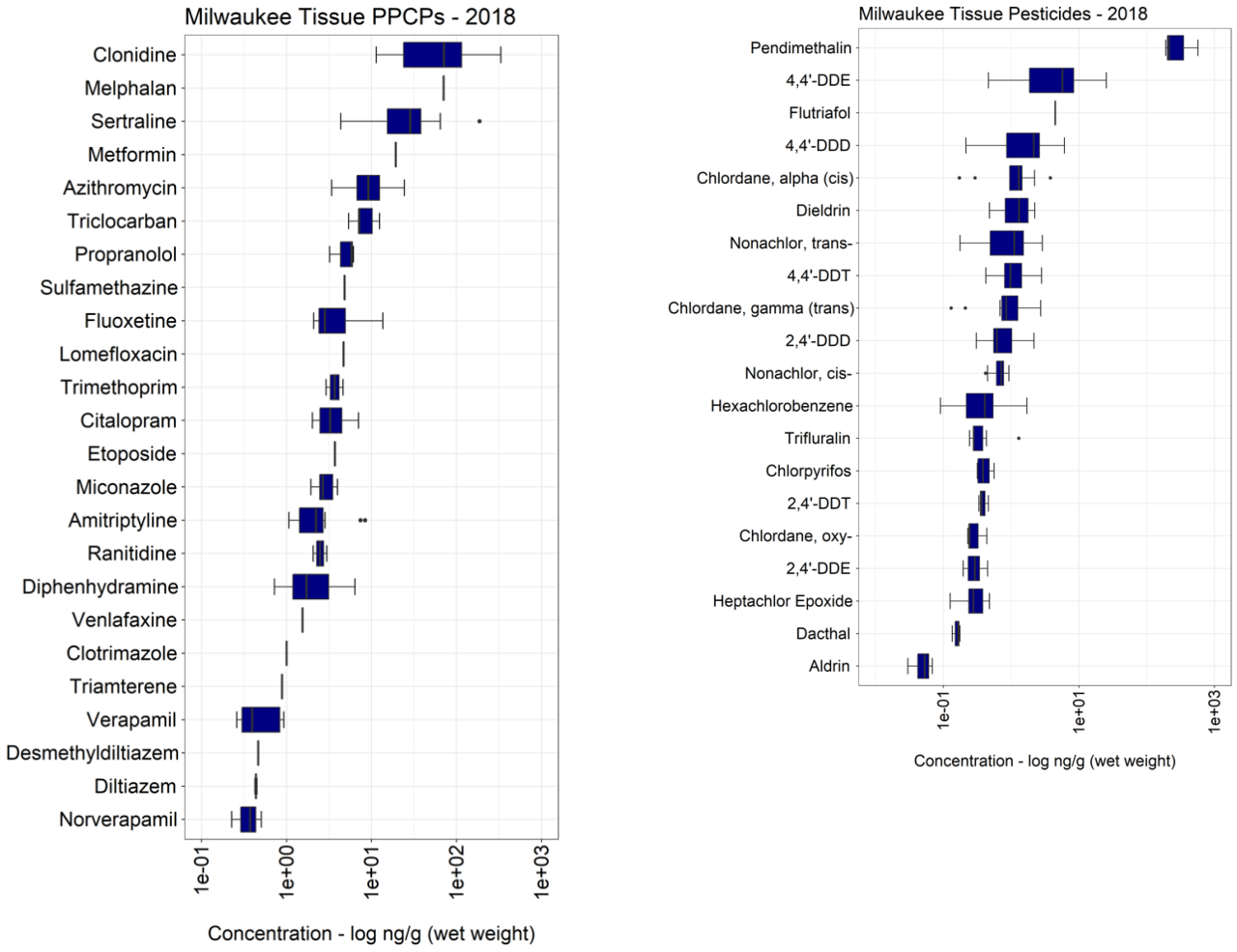


Figure 5: Horizontal boxplots of pharmaceuticals and personal care products (left panel) and pesticides (right panel) detected above three times the detection limit in mussel tissue samples from Milwaukee Estuary in 2018.

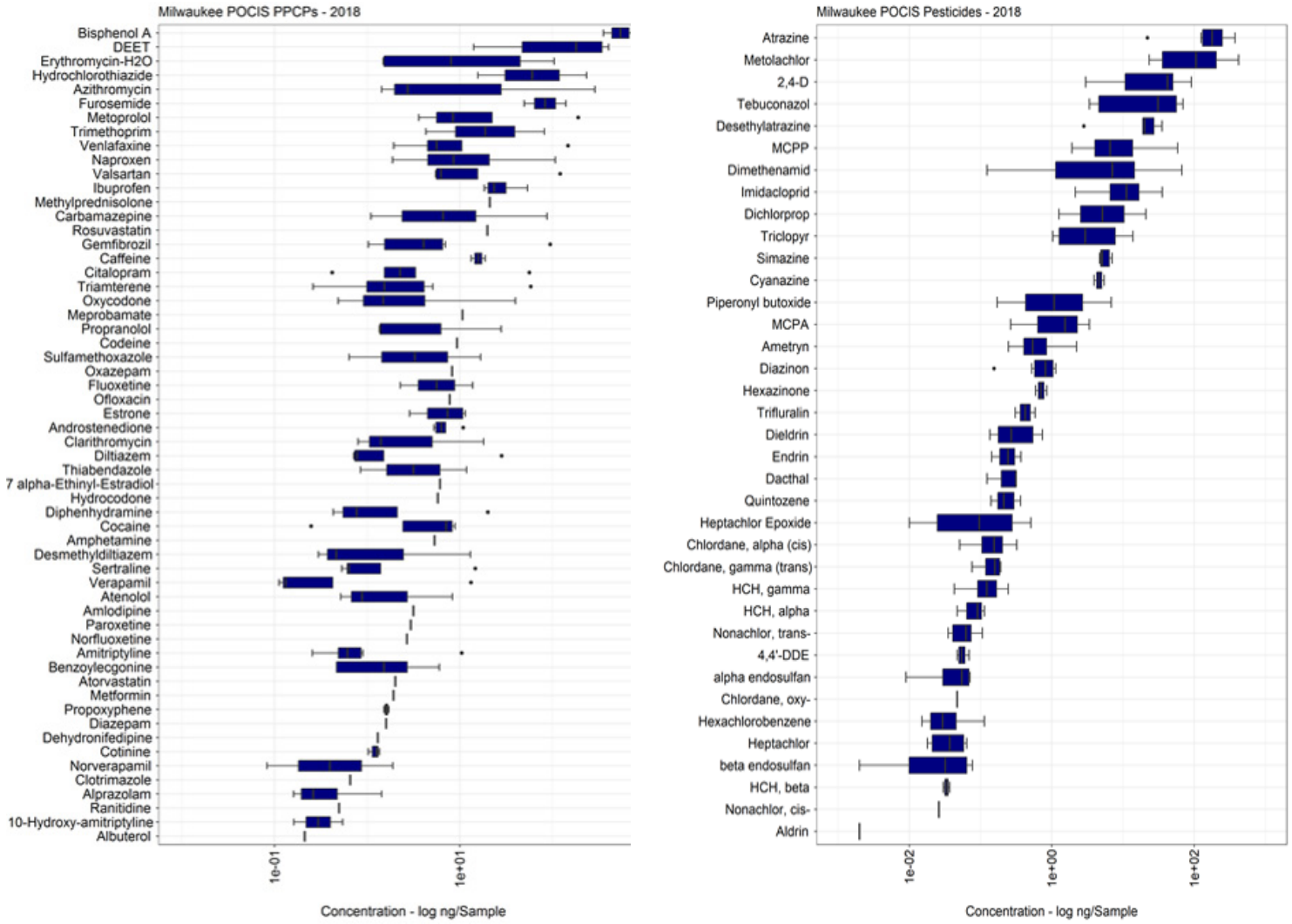


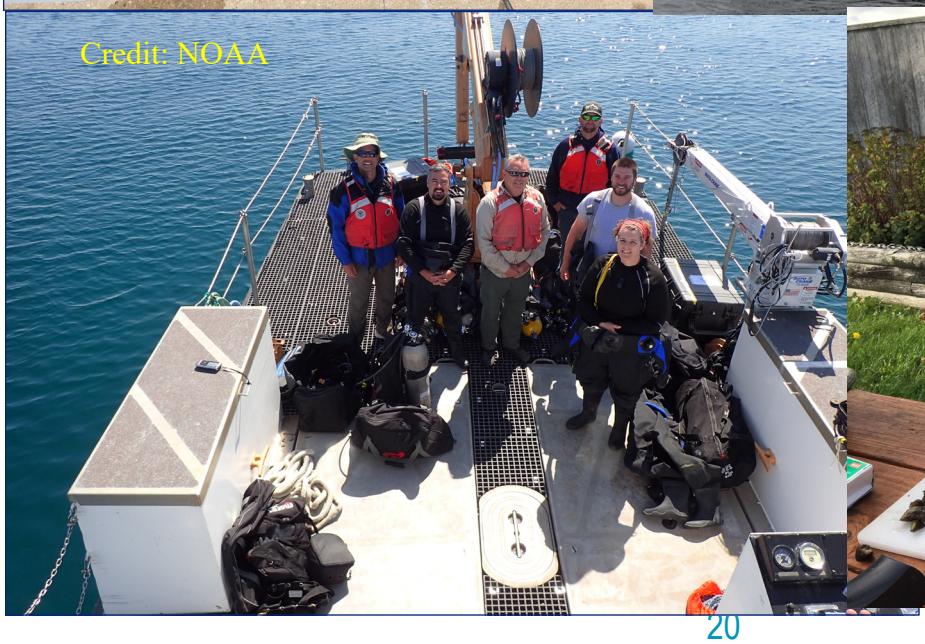
Figure 6: Horizontal boxplots of pharmaceuticals and personal care products (PPCPs;left panel) and pesticides (right panel)detected above detection limit in POCIS samples from Milwaukee Estuary in 2018.


Credit: NOAA


Credit: NOAA


Credit: NOAA


Credit: NOAA
R2604
YAMAHA
150
YAMAHA
150
Credit: NOAA


Credit: NOAA


Credit: NOAA