Application of Bioactivity-Based Monitoring to Evaluate Remediation Effectiveness
A Case Study at Erie Pier Ponds, Duluth, MN.

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Background

Several small ponds neighboring the Erie Pier, in the St Louis River, Duluth, MN have been scheduled for remediation of sediments with elevated concentrations of mercury, dioxins, furans and PCBs. This remediation will involve bank to bank removal of the sediment, which will disrupt the habitat and basic ecosystem structure of the ponds.

Objectives

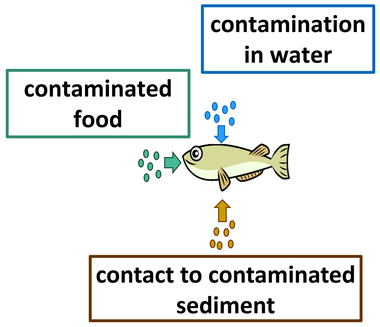
Examine whether molecular and physiological bioactivity profiles associated with samples and/or biota from the site could be used as a near-term indicator of remedy effectiveness while the ecosystem recovers from the physical disturbance associated with the dredging.

2018 Pilot Study:

- Establish a baseline bioactivity profile of sites.

2019 Follow-up Study:

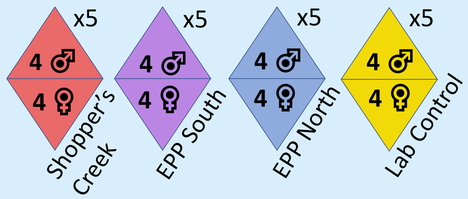
- Include additional endpoints based on the 2018 bioassay results and capture seasonal variability in the biological endpoints.



Methods

2018 Pilot Study

- Five cages with male and female fathead minnow fish were placed on each site for two separate exposure periods: 48 hours & 21 days.

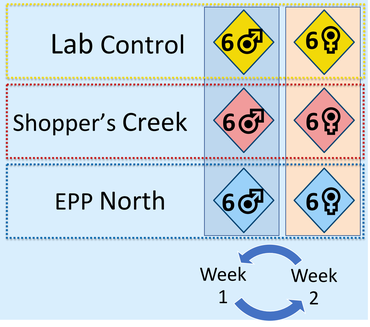


= Fish Cage

- Composite water samples were collected (one for the 48 h exposure and weekly for the 21 d exposure).

2019 Follow-up Study

- One cage with same sex fathead minnow fish was placed on each station for an exposure period of 7 days.
- Same sex cages were deployed weekly at each station from Aug 13 – Oct 9 of 2019 to capture seasonal variability of endpoints.
- Composite water samples were collected weekly.



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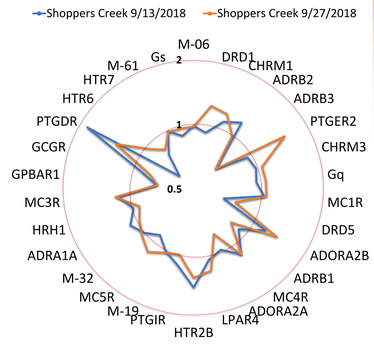
2018 Bioassay Results

- Composite water samples from the sites were evaluated for bioactivity using multifactorial in vitro screening assays, which activated several nuclear receptors and G-protein coupled receptors (Table 1 bellow)
- Based on these results, **PPAR-regulated genes** coding for lipoprotein lipase (lpl) and of acyl-CoA-oxidase (acox1) were selected as additional endpoints for the 2019 follow up experiment.



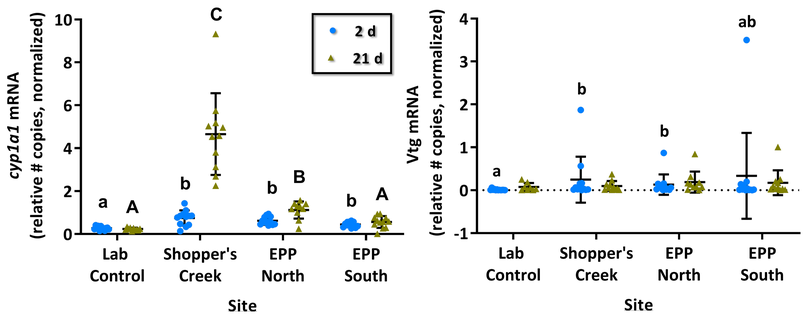
Assay	Receptors	Shopper's Creek	EPP North	EPP South
Attagene Trans Factorial Assay	Pregnane X Receptor (PXR)	X	X	X
	Peroxisome proliferator-activated receptor gamma (**PPARg**)	X	X	X
	Retinoid X receptor beta (RXRb)	X	X	
	Peroxisome proliferator-activated receptor alpha (**PPARa**)		X	X
G protein-coupled receptors (GPCRs) Factorial Assay	Prostaglandin D2 receptor	X	X	
	Prostaglandin E2 receptor	X		
	Dopamine receptor D1 (DRD1)		X	
	Adenosine A2b receptor (ADORA2B)		X	
	Melanocortin receptor (MC4R)		X	
	Prostaglandin I receptor		X	

X = Active hit on samples from site (>1.5 fold)

2018 Caged Fish Exposures

Male Liver qPCR Results:

- Cytochrome P450 1a1 (cyp1a1) was upregulated in the liver of male fathead minnows caged at the site, but there was no significant induction of vitellogenin (vtg) mRNA (an indicator of estrogenicity) at 21 days.

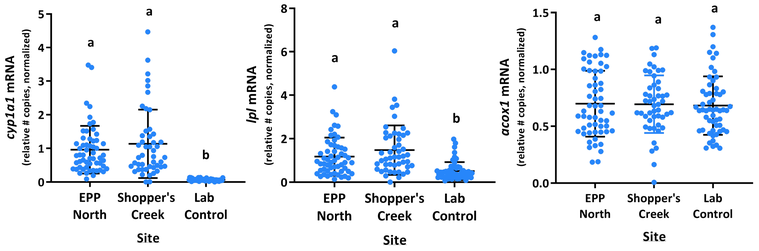


Lower case letters = Significant differences among sites at 2 d
Upper case letters = Significant differences among sites at 21 d
*No significant differences among sites at 21 d for vtg

2019 Caged Fish Exposures

Male Liver qPCR Results:

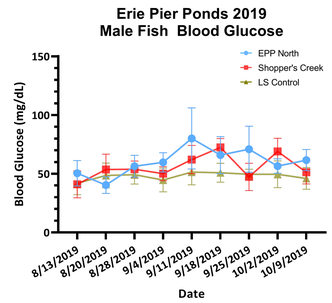
- Up regulation of cyp1a1 and lpl was detected in the liver of male fish .
- There was no significant induction of acox1 nor vitellogenin mRNA (consistent with 2018 results, vtg results graph not shown).

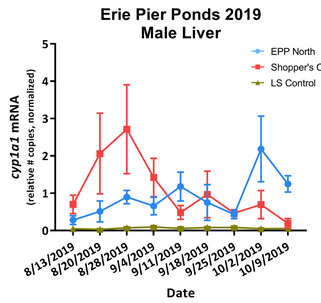


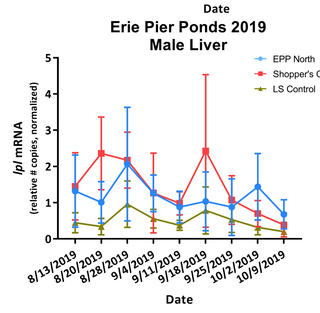
Lower case letters = Significant differences among sites

Seasonal Variation:

- To capture seasonal variability in the biological endpoints, weekly caged exposures were made from August to October of 2019.
- Seasonal variation was established for liver cyp1a1 and lpl, and the blood glucose of male fish when data were pooled across sample dates.







Conclusions

Cyp1a1 is a useful sensor of xenobiotic chemicals as it binds ligands such as polycyclic aromatic carbons and dioxin like compounds. Alterations in lpl gene product suggest activation of PPAR(s) which are involved in key pathways related to lipid metabolism and energy utilization. Therefore, based on monitoring conducted in 2018 and 2019, cyp1a1 and lpl were the endpoints identified as potentially useful for monitoring of remedy effectiveness.

Next Steps

Because caged fish responses were primarily influenced by water column contaminants, additional characterization of 2020 was focused on testing of extracts from sediment and food-base to identify additional endpoints suitable for monitoring remedy effectiveness.

Acknowledgements

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