How Small Stream Monitoring and a Benthic Algae DNA Metabarcoding Study has Informed **TMDL Development in a SW Ohio Watershed**

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Background

Establishing the the concentration of nutrients (namely nitrogen and phosphorus) where impacts to aquatic life begin to occur is difficult. This article describes how historical small stream monitoring and a study using DNA metabarcoding of algae growing attached to stream bottoms informed this issue in a watershed in Southwestern Ohio. USEPA Office of Research and Development (ORD) worked with USEPA Office of Water (OW) to advance the application of DNA metabarcoding methods in establishing nutrient reduction goals. ORD partnered with Ohio EPA's Total Maximum Daily Load (TMDL) development expert (co-author Gledhill) to help write a Loading Analysis Plan (LAP) that was published by the Ohio EPA in 2021. The LAP is a critical component of the state's required TMDL development process. The TMDL is a pollution budgeting tool that serves as a restoration plan for addressing a waterbody impairment.

Central science question and goals

Studying the nutrient target setting and implementation issue has been a focus of ORD's research in the East Fork of the Little Miami River (EFLMR) watershed. The EFLMR is a case study system established in 2006 with the goal of studying watershed nutrient management and the linkages to harmful algae blooms (HABs) and other impacts to aquatic life (Figure 1). The case study conducts routine monitoring (weekly) and watershed modeling that supports nutrient reduction planning and implementation efforts. This work has benefited from an established partnership that includes local and state professionals working in the watershed. One of the major goals of the partnership was to support Ohio's statewide TMDL efforts by using the EFLMR as a demonstration watershed.

The EFLMR is a 1300 km^2 mixed use watershed consisting of 19 HUC12 subwatersheds (HUC stands for "hydrologic unit code;" HUCs are geographic referencing units for the nation's watersheds). The upper portion, the UEFW, is dominated by row-crop agriculture while the lower watershed, the LEFW, is largely mixed urban and forested area (Figure 1). Harsha Lake, which separates the upper and lower watersheds, is a U.S. Army Corps of

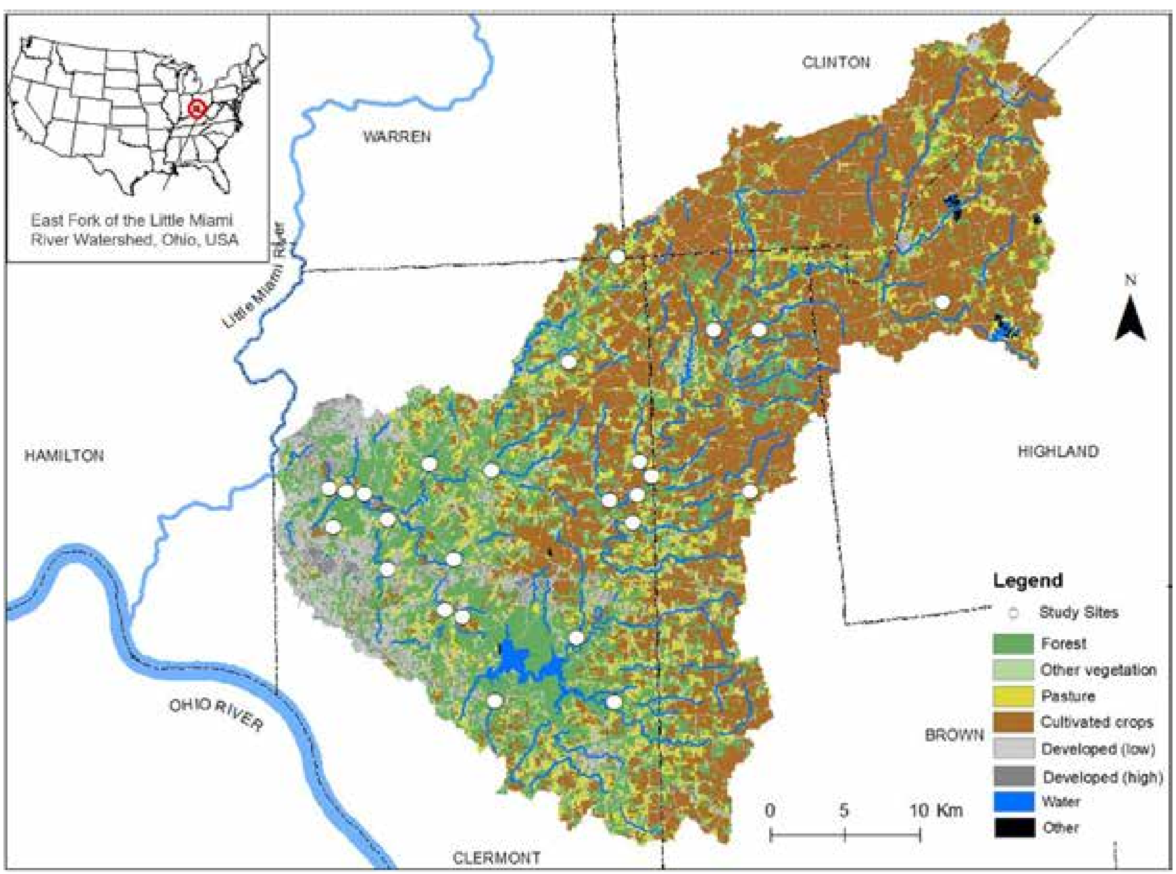

Figure 1. Land use, drainage network, stream sites map of the EFLMR.

Engineers reservoir built in 1978, serves as a major source for drinking water, and provides recreation for the surrounding region, but harmful algae blooms (HABs) have been occuring in this in the lake since 2009 (Smucker et al. 2021).

The Ohio EPA surveys large watersheds like the EFLMR on a rotational basis to comply with requirements for water quality assessment under sections 303(d) and 305(b) of the Clean Water Act (USEPA 2013). Assessments based on fish and macroinvertebrate communities are used in Ohio to determine whether the designated use for aquatic life is supported. If found not to be supported, the cause of the water quality impairment needs to be determined and a restoration plan, in this case a TMDL, developed. Before the reduction requirements can be determined for the TMDL, numeric targets must be set, which here are concentrations of nitrogen and phosphorus.

Ohio established a new framework for its TMDL program in 2017 (LSC 2017). It lists five steps leading up to the official TMDL (OhioEPA 2020). In 2012, the EFLMR was surveyed for CWA section 303(d) reporting and it was established that 52 percent of the 88 stream and river sites assessed did not support aquatic life designated uses (OhioEPA 2014). This survey's report constitutes Step 2 of Ohio's TMDL process. Since the state endeavors to write its TMDLs on a HUC12 subwatershed basis, if a HUC12 contains an impaired stream it requires a TMDL. Fifteen of the 19 HUC12s in the EFLMR contained at least one stream assessment site that did not support its aquatic life use.

Ohio has also listed under CWA section 303(d) the assessment unit containing Harsha Lake as impaired with regards to its public drinking water supply use. This impairment is due to HABs and is based on Ohio's algal toxin thresholds. An ORD analysis of reservoirs, including Harsha Lake, helped establish that excess nutrients were a key contributor to the harmful algae problem (Smucker et al. 2021). All the waters that feed the lake must be considered when addressing the nutrients causing these HABs. Therefore, 12 HUC12s (11 full ones and one partial one) require TMDLs.

The state considers TMDLs needed for stream aquatic life use impairments as "near-field" TMDLs, because these systems have smaller drainage areas, the would-be source are nearer to the impairment. TMDLs associated with systems like Harsha Lake address the impairment of the public drinking water use and receive loads from a larger drainage area. These types of TMDLs are referred to as "far-field" TMDLs. Ten of the HUC12 subwatersheds overlap in that they require a nutrient TMDL to address both near-field and far-field issues.

A major component of Step 2 of the state's TMDL process is establishing the sources of the impairment and causal mechanisms. Bob Miltner, a biologist with Ohio EPA, conducted an analysis of the 2012 survey data and proposed a "cautionary" model that linked fish index of biotic integrity and macroinvertebrate assemblage scores to a feedback mechanism linking low flow, low dissolved oxygen, and nutrient enrichment, with the statistical results pointing to organic nitrogen (N) as a potentially more important driver of impairment than phosphorus (P) (OhioEPA 2014). The finding that organic N was the most likely stressor raised questions relating to the exact mechanism of the impact from an ecological perspective. Enter the USEPA's monitoring and modeling research program in the EFLMR.

Supporting research

A major research goal of the EFLMR case study is to conduct stream monitoring to obtain data for performance evaluation of the watershed model and tracking the effectiveness of nutrient reduction practices through time. To meet this goal, stream monitoring sites needed to be located to capture nutrient conditions specific to dominant land uses and soil types in the system. This forced a focus on low-order, small-sized streams, which are not typically included in routine state monitoring programs (Figure 2). In larger streams, with larger drainage areas, land use types and soils can't be isolated. This monitoring design was important to identify "background" nutrient concentrations for streams needed for the TMDL development process.

ORD had established nutrient background conditions, operationally defined as the median of the distribution of nutrient concentrations in streams draining relatively undisturbed, forested sites, whose soils were reflective of the dominant classes in the system. ORD proposed using the 75th percentile of these streams' distributions as the targets to help move forward on specific research objectives – 77 µg/l for total phosphorus

Figure 2. Picture of one of ORD's small stream monitoring sites in the EFLMR.

(TP) and 707 µg/l for total nitrogen (TN). These target concentrations provided initial information to support the development of the state's LAP, Step 3 of Ohio's TMDL process. After careful consideration of this reference site approach, the state decided to adopt these targets for the far-field, drinking water use TMDL for the lake. However, further investigation into the effects of nutrients on stream organisms was desired to develop the targets for the near-field TMDLs to address the impact to aquatic life.

Owing to the difficulty linking mechanisms of nutrient stress to traditional measures of aquatic life use in streams, ORD designed a novel DNA metabarcoding study focused on characterizing the stream benthic algal community. Algae are of particular interest because they are highly responsive to nutrients and they are critical components of stream ecosystems that affect other organisms through changes in the food web and habitat. Important aspects of the study design included analyzing the existing nutrient data that were available for EFLMR streams to select sites that captured the broad range of nutrient concentrations in the watershed (25 sites shown in white in Figure 1).

Streams were visited weekly over one growing season. Benthic algae were collected from rocks during each sampling event, DNA was extracted, and primers targeting the diatom rbcL chloroplast gene were amplified with polymerase chain reaction (PCR) to characterize the diatom communities across both space and time (Smucker et al. 2020). Diatoms are a type of unicellular algae found in nearly all aquatic environments and they have a long history of research supporting their possible use as indicators of environmental conditions. Metabarcoding and bioinformatics identify the unique gene sequence reads in a sample and these were treated as "operational taxonomic units" (OTUs), which is a concept similar to species but is not a formal taxonomic grouping. Collectively, these OTUs comprise the diatom assemblage in a sample collected from a stream site.

Next, an approach was developed for analyzing the metabarcoding data. Three statistical methods were used to identify possible concentrations along the nutrient gradient at which large changes in the diatom assemblage occurred (Figure 3). The concentrations demarking large shifts in the structure of the diatom assemblage are referred to as change points, and they corresponded well with those established from the small-stream reference condition approach. These change points corresponded closely to the reference condition approach targets and Ohio EPA decided to use them in combination as one set of targets for phosphorus and nitrogen concentrations. This was the second critical piece of information from ORD Research that supported the LAP development.

The change point analysis aggregated the diatom assemblage information to site level means, but two additional exploratory data analyses focused on identifying how changes in nutrient

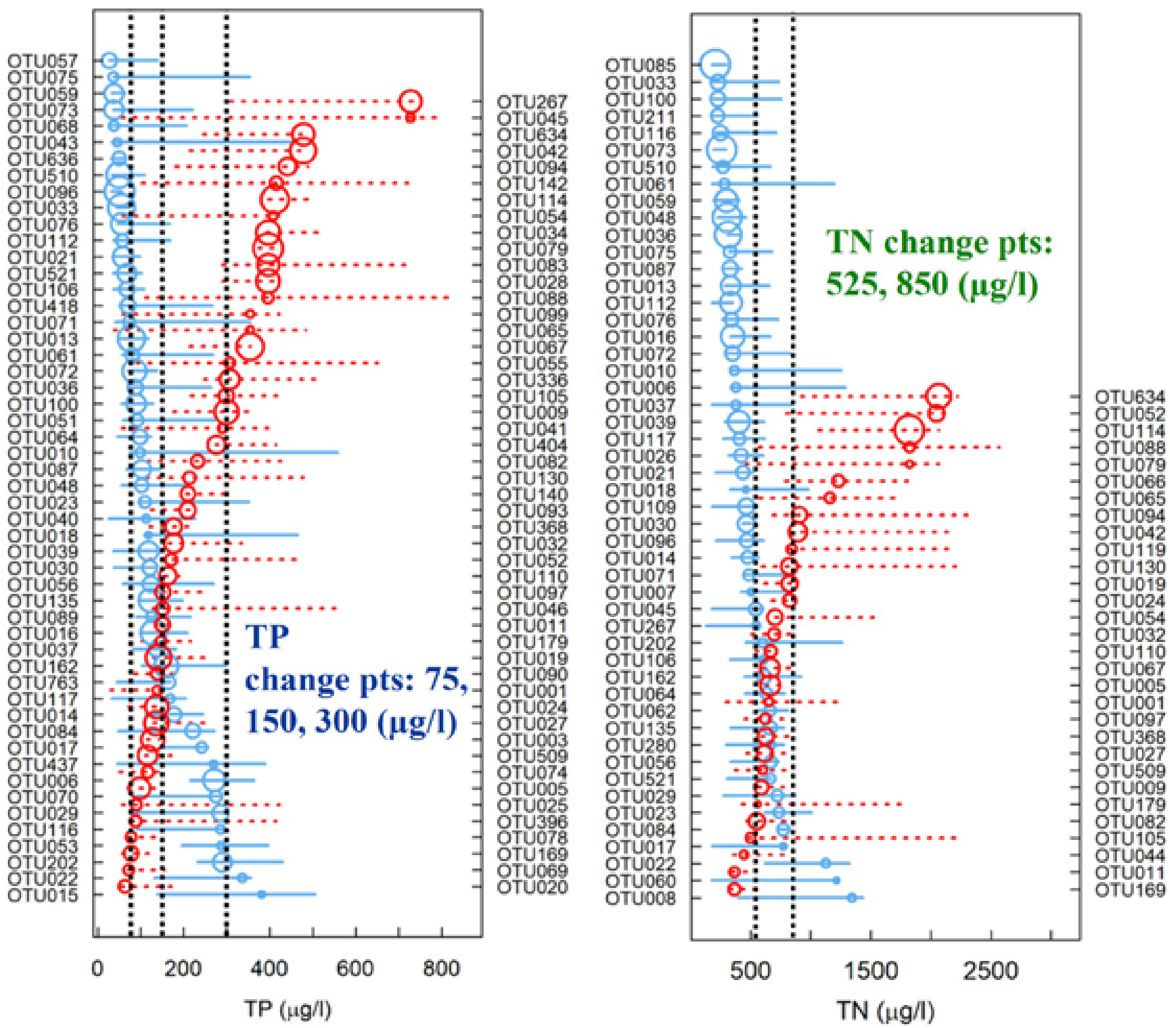

Figure 3. Graph adapted from Smucker et al. (2020). Possible nutrient targets (vertical dotted lines) based on considering all responses from Threshold Indicator Taxa Analysis (TITAN), boosted regression trees, and gradient forest analyses. Data are taxa change points those indicating low (blue) and high (red) TP or TN concentrations from TITAN.

concentrations and diatom assemblages over time might affect results and interpretations. Taken together these follow-on analyses supported the relative importance of phosphorus as a community driver and suggested that high frequency benthic algae sampling may not be necessary to capture significant relationships between nutrients and diatoms (Yuan et al. 2022 and Smucker et al. 2022, respectively). These conclusions proved to be a third critical piece supporting the subsequent TMDL development after the LAP was published.

Impact and future research

ORD and Ohio EPA partnered to write the sections of the LAP entitled "Linking impairment to TMDL pollutants," which is a critical component of the "Proposed Actions" to address the near- and far-field impairments of designated uses (OhioEPA 2021; https://epa.ohio.gov/static/Portals/35/tmdl/LAPs/Little percent20Miami/EFLMR_LAP.pdf. The diatom nutrient change points provided in Smucker et al. (2020) were used to bolster the rationale for setting specific targets for both P and N for the streams needing near-field TMDLs in the system by providing a direct link between nutrient concentrations and support for aquatic life use. The state justified the targets proposed by ORD as necessary for preserving low nutrient diatoms. Because diatoms are primary representatives of the base of stream food webs, loss of low-nutrient diatoms can cascade to higher trophic levels. The follow-on analyses helped the state decide to focus on P for setting proposed point source effluent limits. The LAP formally sets the nutrient targets for the subsequent TMDL calculations in the next step of the process.

Now the plan for the partnership is to use ORD's application of the Soil Water Assessment Tool (SWAT) model in the EFLMR (Karcher et al. 2013) to describe nutrient export for the TMDL calculations and develop nutrient reduction scenarios to meet the near and far-field targets. The SWAT output will be configured in terms of load duration curves using the exiting, background, and targeted nutrient concentrations. The model validated daily loads aggregated to an annual scale will be distributed among source-specific allocations and nutrient reduction requirements. This modeling analysis will be conducted for all the HUC12s and partial ones in the system needing a TMDL, and may serve as a valuable approach for use in other watersheds requiring TMDLs in the state.

Finally, the lessons learned from ORD's monitoring, modeling, and metabarcoding work in the EFLMR are beginning to be applied in other systems, with the intent of further demonstrating their utility toward establishing direct linkages between nutrient concentrations and aquatic life in streams. To this end, ORD is continuing to partner with Ohio and now is collaborating with Indiana on the processing of samples through the benthic algae metabarcoding workflow collected during the state's 303(d) surveys in East Fork of the White River Watershed, IN, and the Wabash, OH, in 2022. Stay tuned.

Acknowledgments

The authors would like to acknowledge Bob Miltner and Ruth Briland of the Ohio EPA for helpful suggestions and USEPA reviewers Michael Elovitz, Mark Bagley, and Dana Thomas for constructive edits and comments. *Agency Disclaimer:* The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency or the Ohio Environmental Protection Agency.

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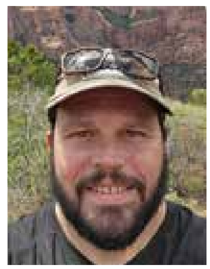

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