Project Title: What role does nutrient cycling by zooplankton play in supporting HAB production in western Lake Erie?

Statement of Regional Water Problem:

Harmful algal blooms (HABs) have increased recently in the western basin of Lake Erie, resulting in serious economic and public health consequences (Raymond 2012, Carmichael and Boyer 2016). Binational HAB mitigation policies, requiring a 40% reduction in phosphorus (P) loading to Lake Erie, are largely premised on relationships between spring and early summer (March-July) P-loading from the Maumee River and summer bloom extent (Ho and Michalak 2017, International Joint Commission 2014, Obenour et al. 2014, Scavia et al. 2014, Stumpf et al. 2016). Underlying this relationship is a 30-150 day gap between P loading and cyanobacterial blooms in the western basin, which has a relatively short water residence time of about 50 days (Michalak et al. 2013). Thus, while much of the water from spring loading events has left the western basin by the time cyanobacteria blooms initiate, the P presumably stays in the basin to fuel the blooms (Fig. 1). This raises the question: *Where does the P 'go' before fueling HABs?* This is a **critical knowledge gap** because a lack of understanding of the mechanistic underpinnings of Lake Erie cyanobacteria predictive models contribute uncertainty to HAB forecasts and management efforts to reduce HABs and P loading to the lake. These uncertainties will likely compound in the face of future warming, more intense precipitation events, and new invasive species (Michalak et al. 2013, Paerl et al. 2016).

There are four primary sources of internal P cycling: entrainment of P from the sediments, organic matter decomposition, nutrient recycling by benthic macroinvertebrates (primarily dreissenid mussels), and nutrient cycling by zooplankton (ZNR; Fig. 1). Of these four, nutrient cycling by zooplankton is the most poorly constrained and understood component. Lake Erie models (Zhang et al. 2016) estimate that ZNR supports on average 15% of P demand by primary producers during the summer; yet, this estimate is limited in (1) not considering nutrient recycling by microzooplankton (2 – 200 μm; e.g., flagellates, ciliates, rotifers, nauplii, dreissenid veligers), which are often a substantial portion of total zooplankton biomass, (2) not considering N recycling by zooplankton, (3) being based on years (1997 and 1998) prior to the onset of HABs, and (4) lacking empirical validation. Consequently, we lack understanding of how seasonal patterns in micro- and mesozooplankton (200 μm – 20,000 μm) biomass and community structure combine with water temperature and zooplankton diets (Elser and Urabe 1999, Vanni 2002) to influence ZNR. A **lack of quantitative information** on rates and ratios of zooplankton nutrient recycling and the drivers of this component of internal nutrient cycling hinders our ability to understand the factors shaping the magnitude, timing, and duration of cyanobacteria blooms in the western basin of Lake Erie. Thus, our **overarching goal** is to evaluate one potentially important mechanism for maintaining P in western basin of Lake Erie between spring loading and summer cyanobacteria blooms – nutrient recycling by zooplankton (ZNR). This is a **research need** because an improved mechanistic understanding of the links between spring P loading and summer cyanobacteria blooms will benefit lake and HAB forecast models as well as managers and policy makers seeking to improve water quality, fisheries, and ecosystem resilience in a constantly changing environment. To address this research need, we have developed two research objectives and hypotheses:

Objective 1: Determine the relative importance of zooplankton-mediated N and P recycling in western basin of Lake Erie and how it contributes to HAB development, timing, and magnitude.

Hypothesis 1: *The relative importance of nutrient recycling by zooplankton is determined by the availability of dissolved nutrients within the lake, phytoplankton production, and seasonal patterns in zooplankton biomass and community composition.*

Objective 2: Identify the physicochemical and biological controls of zooplankton nutrient recycling rates and ratios.

Hypothesis 2: *Zooplankton N and P excretion rates and ratios are determined by seasonal shifts in water temperature, zooplankton community composition, biomass, and diet C:N:P.*

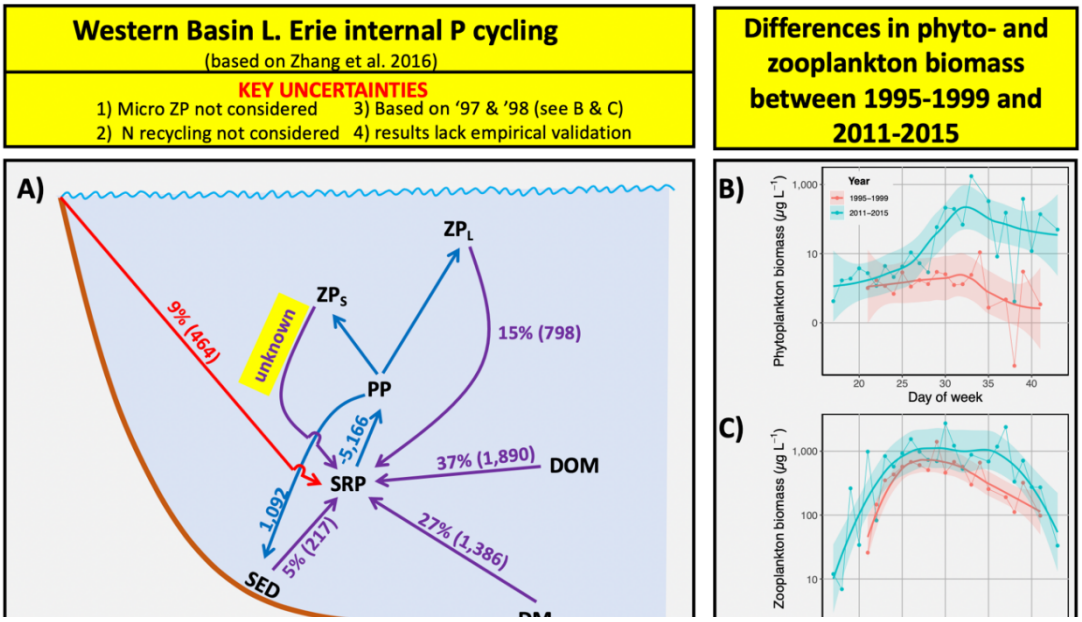

Figure 1. Our research would fill a key knowledge gap in our understanding of western Lake Erie internal P cycling (A) – the role of nutrient cycling by zooplankton in supporting phytoplankton and HAB production. Lake models (Zhang et al. 2016) indicate that internal P cycling largely supports the P demand of primary production through organic matter decomposition (DOM), entrainment of sediment P (SED), nutrient recycling by dreissenid mussels (DM), mesozooplankton (ZP_L), and potentially microzooplankton (ZP_S) although no data for these taxa exist for western Lake Erie. Yet, this model has critical limitations (listed at top of figure) with respect to our question including that it focused on 1997 and 1998 when phytoplankton (B) and zooplankton (C) biomass was considerably lower (note log scale on axis) than in recent years. Purple arrows represent fluxes from particulate P to SRP, while blue arrows represent fluxes from SRP to particulate P or between particulate P pools. The red arrow is external SRP loading from all sources. Numbers represent the size of the flux in metric tons of P between 1 March and 30 September based on averages presented in Zhang et al. (2016). Percentiles represent the percent of phytoplankton P demand supported by that flux. Phytoplankton and zooplankton data are from the Lake Erie Plankton Abundance Survey (LEPAS; supported by OH Division of Wildlife), which sampled eight sites in WBLE roughly biweekly between approximately May 1 and October 1 (Briland 2018a).

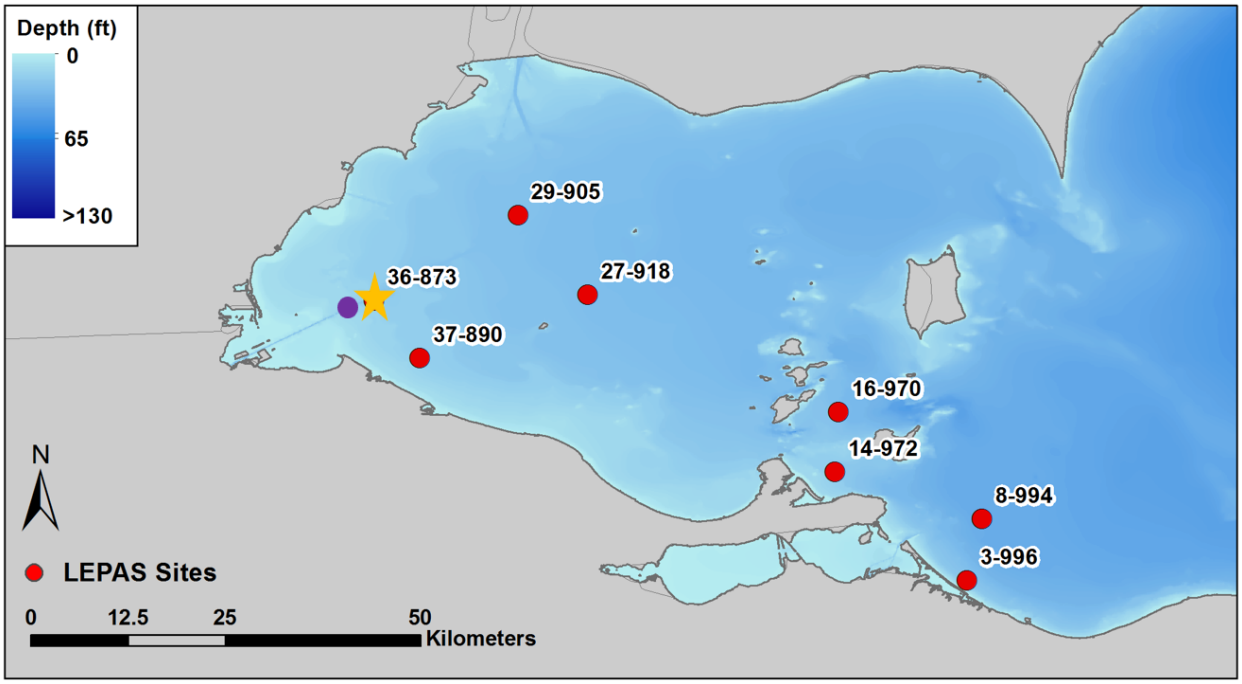

Figure 2. Map of western basin Lake Erie (WBLE) sites used for the proposed research. The yellow star indicates the focal site (36-873) for field-based nutrient recycling and grazing measurements, which is adjacent to NOAA-GLERL cHAB monitoring and buoy site WE2 (purple dot). Red points indicate the Lake Erie Plankton Abundance Study (LEPAS) monitoring sites.

Methods

Overview of Approach

To determine the relative importance of ZNR (**Obj. 1**) as well as the controls of ZNR rates and ratios (**Obj. 2**), we quantified N and P excretion rates by micro- and mesozooplankton regularly (once per month between April-June and September-October, biweekly between July and August), at one site in western basin of Lake Erie near the Maumee River mouth (Fig. 2). This site is located at a long-term OSU/ODNR monitoring station that is part of the Lake Erie Plankton Abundance Study (LEPAS). We evaluated the relative importance of ZNR by determining the proportion of phytoplankton P demand that is supported by ZNR. Here, we estimated phytoplankton P demand as the product of phytoplankton P:C and net primary production, which we will calculate using two approaches that leverage public data and ongoing work by collaborators. We will also evaluate ZNR's importance by comparing ZNR to publicly available estimates of other external and internal N and P sources (e.g., external P loading, nutrient recycling by dreissenid mussels, sediment P release as estimated by Matisof et al. 2016). To address **Obj. 2**, we will use statistical modeling to predict ZNR rates and ratios using water temperature as well as phytoplankton and zooplankton biomass and composition (both taxonomic and stoichiometric) as predictor variables. Additionally, to understand another important control on HABs—zooplankton feeding—we simultaneously measured zooplankton grazing on HABs and other phytoplankton groups. To maximize grant dollars our research heavily utilized publicly datasets (e.g., Heidelberg loading dataset) and collaborations with the ODNR, NOAA-GLERL, and Ohio EPA.

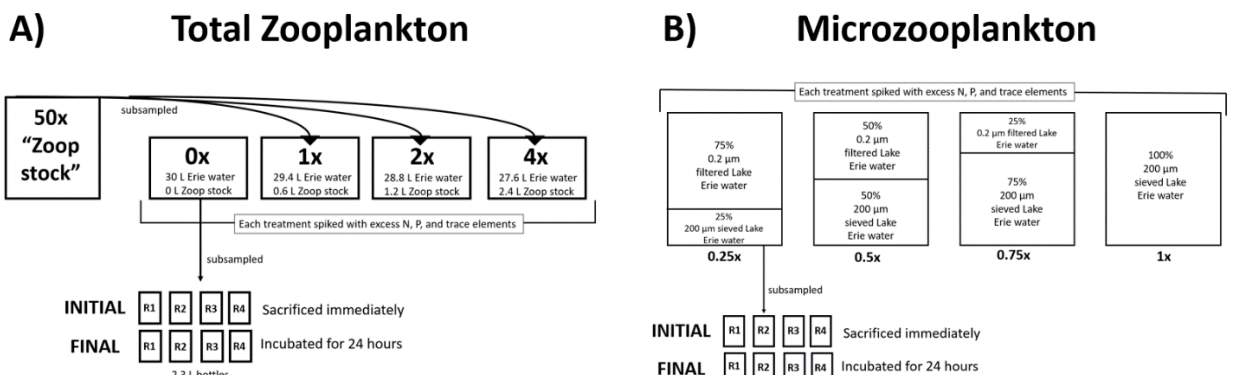

Figure 3. Experimental design for measuring total zooplankton (A) and microzooplankton (B) nutrient excretion and grazing rates. Mesozooplankton nutrient excretion and grazing rates are the difference between total zooplankton and microzooplankton measured rates.

Relative importance of ZNR (Obj. 1)

Measure zooplankton excretion (ZNR) and grazing (Task 1A)

Overview and field sampling: The first task required to evaluate the relative importance of ZNR (Obj. 1) was to measure community micro- and mesozooplankton N and P excretion rates and ratios (i.e., µg P L^{-1} d^{-1}). These measurements required two separate approaches. Total nutrient excretion by zooplankton (i.e., microzooplankton + mesozooplankton) was quantified via gradient-grazer assays (Lehman and Sandgren 1985, Hambright et al. 2007a) while microzooplankton excretion was measured using dilution assays (Landry and Hassett 1982). Mesozooplankton excretion is the difference between total zooplankton excretion and microzooplankton excretion. Combining these approaches also allowed for simultaneous estimation of total, micro-, and mesozooplankton grazing rates (Hambright et al. 2007a) which we will couple with measures of ZNR to better understand how zooplankton shape the development of HABs. We ran these experiments using surface water (1 m) and zooplankton collected from site 36-873 (Fig. 2) and immediately transported the samples back to OSU laboratory facilities for the experiments. Due to COVID-19 restrictions, zooplankton and water samples were collected by Ohio Department of Natural Resources personnel during routine LEPAS sampling. Mesozooplankton were collected from the whole water column with a vertical 200 µm mesh net tow (diameter = 0.5 m; length = 1.5 m) with a non-filtering cod end and flowmeter (Hydro-Bios model 438115, Altenholz, Germany). Water and microzooplankton was collected with a peristaltic pump (Geotech, Denver, CO, USA) from 1 m and immediately sieved through a 200 µm sieve. Zooplankton and lake water were stored in coolers and immediately transported to our laboratory at OSU for excretion and grazing measurements.

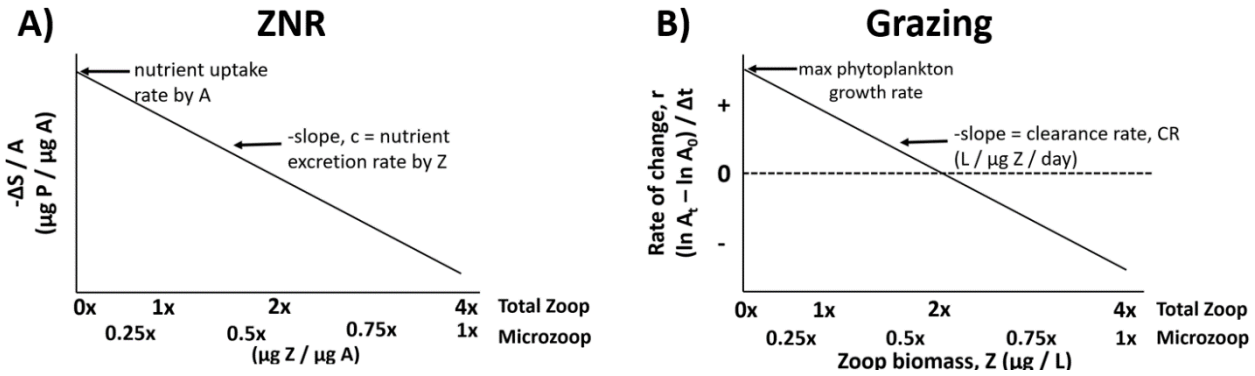

Figure 5. A) Mass-specific ZNR rates are calculated as the relationship between the change in nutrient concentration (SRP, DOP, NH_4^+) over 24 hours (*ΔS*; e.g., µg P L^{-1}) as a function of zooplankton density (*Z*; individuals L^{-1}), standardized by phytoplankton biomass (*A*; µg chl *a* L^{-1}). B) Zooplankton clearance rates (CR) are estimated as the phytoplankton rate of change over the experiment, r, as a function of zooplankton biomass (*Z*). Grazing rates are calculated as the product of the clearance rate and mean

ɛtion
)07a)

in which mesozooplankton were incubated at increasing densities in 200 µm filtered lake water with ambient phytoplankton and microzooplankton densities (Fig. 3A). To achieve this, we sieved surface water through a 200 µm mesh to remove mesozooplankton but retain phytoplankton and microzooplankton. Next, we split the water into four containers to create four mesozooplankton density treatments. These treatments consisted of: zero-controls lacking mesozooplankton (0x), ambient mesozooplankton densities (1x), 2x ambient density, and 4x ambient density. We sub-sampled mesozooplankton from our net tow samples to create the four mesozooplankton density treatments, and then enriched each container with dissolved inorganic nitrogen (DIN; ammonium (NH_4^+) + nitrate (NO_3^-)), soluble reactive phosphorus (SRP), trace elements, and vitamins to saturate biological uptake (Calbert and Saiz 2018). We then sub-sampled water from each treatment into eight 2.3 L borosilicate bottles, half of which were sacrificed and sampled immediately as "initial" samples, while the other half were incubated on a plankton wheel (Fig. 4) for 24 hours under natural temperature and light conditions (4 replicates per treatment). At the beginning and the end of the 24-hour incubation, samples of chemical (SRP, dissolved organic phosphorus (DOP), NH_4^+-N) and biological (chlorophyll *a*, phytoplankton major groups, meso-, micro-, nano-, and picoplankton) parameters were measured.

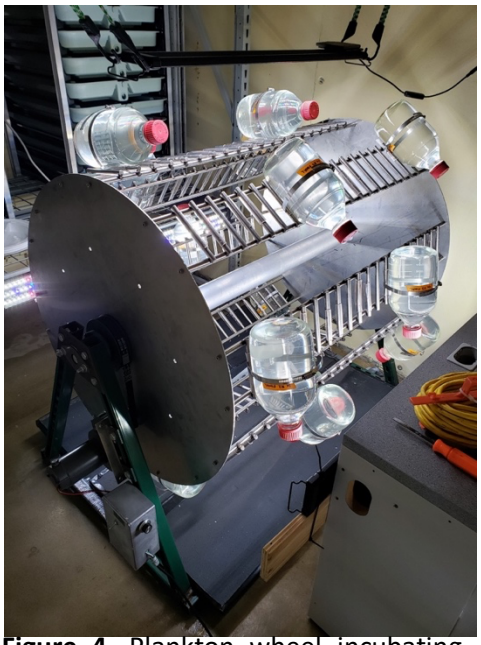

Figure 4. Plankton wheel incubating total zooplankton bottles (2.3 L) in an environmental chamber set at ambient western basin Lake Erie water temperature.

Following Hambright et al. (2007b), mean indivi
excretion rates (µg N or P ind^{-1} d^{-1}) for the

zooplankton community can be estimated from the relationship between the change in nutrient concentration (ΔS) and zooplankton density (Z; Fig. 5A):

$$-\frac{\Delta S}{A} = \mu + -c\frac{Z}{A} \qquad (1)$$

where A is the mean of the initial and final chlorophyll a concentration (µg chl a L^{-1}), μ is the maximum nutrient uptake rate of phytoplankton (µg N or P chl a^{-1} d^{-1}), and -c is the individual nutrient excretion rate (µg N or P ind^{-1} d^{-1}). The individual nutrient excretion rate (-c) and maximum nutrient uptake rate (μ) are the negative slope (i.e., slope × -1) and intercept, respectively, of the linear relationship between $-\frac{\Delta S}{A}$ and $\frac{Z}{A}$. Community excretion rates (µg N or P L^{-1} d^{-1}) under ambient conditions are the product of individual excretion rates (-c) and the total (micro- and meso) zooplankton density (individuals L^{-1}) at the time of collection.

Individual grazing rates (G_I; µg chl a $individual^{-1}$ d^{-1}) are (Fig. 5B):

$$G_I = A\,\frac{\Delta A}{Z} \qquad (2)$$

where A is the mean chlorophyll a concentration (µg chl a L^{-1}) and $\frac{\Delta A}{Z}$ is the slope of the relationship between the change in chlorophyll a concentration during the incubation (ΔA) and zooplankton density (Z; Hambright et al. 2007b). Community zooplankton excretion or grazing rates are the product of individual excretion or grazing rates and ambient zooplankton density (mesozooplankton + microzooplankton) in the lake.

This experimental design generated four final and initial bottles for each treatment which did not have a clear pairing for estimation of change in chlorophyll a or nutrient concentration (e.g., $R1_{final}$ v. $R1_{initial}$ or $R1_{final}$ v. $R3_{initial}$ are equally reasonable pairs of replicates to estimate ΔA or ΔS). To address this limitation, we used a bootstrapping approach to evaluate all potential pairings. We took the nutrient and chlorophyll a concentrations from the four final replicates and paired them with four randomly selected initial replicates, without replacement, calculated the individual excretion and grazing rates using least squared regression, and then repeated that process 100 times. The final individual excretion and grazing rates (± 90% confidence intervals) were expressed as the median and 10^{th} and 90^{th} percentiles of the distribution of bootstrap estimates of the least squared regression slopes. Since the expectation for nutrient recycling and grazing is a negative relationship between $-\frac{\Delta S}{A}$ and $\frac{Z}{A}$ or ΔA and Z, respectively, we evaluated whether recycling or grazing were measurable (i.e., above our detection limit) by determining whether the upper 90^{th} percentile for these terms exceeded zero (i.e., $\alpha = 0.05$ for a one-tailed test).

Dilution assays (microzooplankton): Microzooplankton excretion and grazing was measured with a dilution technique, in which microzooplankton and phytoplankton were incubated along a gradient of concentrations (0-100% lake water; Landry and Hassett 1982; Fig. 3B). We filled containers with different volumes of 200 µm sieved surface water (to remove mesozooplankton but retain the natural microzooplankton and phytoplankton assemblage) and 0.2 µm filtered surface water (to remove all plankton) to achieve the following treatment ratios (four replicates each): 25% (i.e., 25% 200 µm sieved water, 75% 0.2 µm filtered water), 50%, 75%, and 100%.

All containers were supplemented with DIN, SRP, trace elements, and vitamins to saturate biological uptake (Calbert and Saiz 2018). We sub-sampled treatments into eight 0.6 L borosilicate bottles. Half of the bottles were sacrificed immediately, while the other half were incubated for 24 hours under natural temperature and light conditions on a plankton wheel. Samples were collected at 0 and 24 hours to determine initial and final concentrations of the same parameters sampled for total zooplankton excretion and grazing. Microzooplankton individual nutrient excretion rates are estimated following equation 1 (Fig. 5A; Landry and Hassett 1982) while individual grazing rates are estimated following equation 2 (Fig. 5B; Hambright et al. 2007a). Community microzooplankton nutrient excretion (e.g., µg P L^{-1} d^{-1}) and grazing rates (µg chl *a* L^{-1} d^{-1}) are the product of individual rates and ambient microzooplankton density in the lake. Mesozooplankton excretion and grazing rates are the difference between total zooplankton rates and microzooplankton rates.

Sample analysis: We analyzed samples for SRP via the molybdenum blue reaction (Shimadzu UV-1800, Kyoto, Japan), and will analyze NH_4^+-N samples via the indophenol blue reaction using an autoanalyzer (SEAL Analytical, Mequon, Wisconsin, USA). Our old Latchet autoanalyzer had to be replaced and we are waiting on the delivery of a new SEAL autoanalyzer. Chlorophyll-*a* samples were measured via the fluorometric method after 90% acetone extraction (Trilogy, Turner Designs, San Jose, CA, USA). Phytoplankton composition (blue greens, diatoms, green algae, cryptophytes) was determined via fluoroprobe analysis (BBE Moldaenke, Schwentinental, Germany).

Due to the time-intensive nature of sample processing, all zooplankton and phytoplankton samples from the experiments have not yet been enumerated. Ambient mesozooplankton samples were enumerated and identified via light microscopy according to established LEPAS counting protocols (Conroy et al. 2005, Briland 2018b). Microplankton biomass (rotifers, protists, nauplii, phytoplankton 20-200 µm) will be estimated via light microscopy (Zeiss Axio Vert.A1; Lund et al. 1958, Hadas and Berman 1998, EPA 2016). Pico- and nanoplankton biomass (bacteria, protists, phytoplankton; 0.2-2 µm and 2-20 µm, respectively) will be determined via epifluorescence microscopy (Hobbie et al. 1977, Hadas and Berman 1998). Plankton biomass will be converted to carbon (C) units using established equations for phytoplankton (Hillebrand et al. 1999), microzooplankton (Dumont et al. 1975, Simon and Azam 1989, Putt and Stockner 1989, Hart et al. 2000) and mesozooplankton (Dumont et al. 1975, Culver et al. 1985).

Ambient phytoplankton composition, abundance, and biomass, as well as chlorophyll *a*, was determined from samples of the pumped surface water. Ambient nutrient concentrations (seston C, N, and P, NH_4^+-N, NO_3^--N , SRP, DOP) were determined from field collections at LEPAS site 36-873, as well as from weekly monitoring and continuous buoy measurements at NOAA-GLERL site WE2 (Fig. 2). Seston C and N content will be measured with an elemental analyzer (CE440 CHN, Exeter Analytical, Coventry, UK) and seston P content will be measured via the molybdate blue reaction using a spectrophotometer following sulfuric acid digestion (Shimadzu UV-1800, Kyoto, Japan).

Estimate relative importance of N and P release by zooplankton (Task 1B)
Overview: To assess the contribution of ZNR toward HABs and phytoplankton production, we will compare total excretion by zooplankton to phytoplankton N and P demand as well as other external and internal P sources within the western basin of Lake Erie.

Primary production (Task 1B.1): Net primary production on each sampling date was estimated using established relationships between GPP, chl-a, and water temperature (Morin et al. 1999). Estimates of chl-*a* and temperature were collected in-situ during each ZNR sampling event and converted to GPP. We calculated NPP as the difference between GPP and autotrophic respiration, which is assumed to be 50% of GPP (Hall and Tank, 2003, Hood et al. 2018).

Once data becomes publicly available, we will determine NPP using the diel oxygen technique to measure lake metabolism (Staehr et al. 2010). This approach requires high-frequency dissolved oxygen data as well as information on light availability, gas exchange, and mixed layer depth. We will use high-frequency dissolved oxygen data from NOAA-GLERL monitoring buoy WE2 located near our study site and the R package *LakeMetabolizer* (Winslow et al. 2016) to estimate metabolism. We will evaluate how these differing NPP estimates (chl-a vs. diel oxygen technique) influence our N and P demand estimates, and thus the contribution of ZNR toward phytoplankton nutrient demand.

Primary producer N and P demand (Task 1B.2): Phytoplankton N and P demand is the product of net primary production (NPP) and phytoplankton N:C and P:C, respectively. To determine phytoplankton C, N, and P content for our nutrient demand calculations, we collected seston samples during field sampling for ZNR measurements by pumping surface (1 m) water and filtering the water through a 0.7 μm filter in the laboratory. We will measure C and N content with an elemental analyzer (CE440 CHN, Exeter Analytical, Coventry, UK) and seston P content will be measured via the acid-hydrolysis method (Demott et al. 1998) using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) following the recommendations of Boros and Mozsár (2015). We will estimate phytoplankton P demand (μg P L^{-1} day^{-1}) for each sampling date as the product of seston P:C and NPP. Because the NH_4^+-N samples from our ZNR experiments have not yet been processed, our results will focus on P excretion by zooplankton.

Relative importance of nutrient release by zooplankton (Task 1B.3): For each sampling date, we will estimate the relative importance of ZNR toward HAB and phytoplankton primary production. To do so, we will calculate the ratio of P released by zooplankton and phytoplankton P demand. Once all samples have been processed, we will use generalized additive models (GAMs; Wood 2006, 2017) to evaluate seasonal changes in ZNR, and test for differences in splines to determine if patterns in ZNR importance differs between meso- and microzooplankton. To estimate confidence intervals for our measurements of ZNR importance, we will use a bootstrapping approach (Manly 2007, Benke and Huryn 2017) to propagate uncertainty associated with zooplankton N and P supply and phytoplankton N and P demand.

Compare ZNR to external P loads (Task 1C): As an additional approach to understand the importance of ZNR toward phytoplankton and cHAB production (Vanni et al. 2002, 2006), we will compare the magnitude of meso- and microzooplankton total ZNR to that of other publicly available estimates of other external and internal nutrient sources including external P loading,

nutrient recycling by dreissenid mussels, sediment P release (as estimated by Matisof et al. 2016). We will estimate P loading (Baker et al. 2014; National Center for Water Quality Research at Heidelberg University) during the two weeks prior to each ZNR measurement, and compare this to our estimates of ZNR, in order to understand how external nutrient availability influences the relative importance of ZNR.

Identifying controls on ZNR (Obj 2)

Identify determinants of zooplankton excretion rates and ratios (Task 2A): We will use statistical modeling approaches to identify the potential controls on nutrient recycling rates and ratios of micro- and mesozooplankton in WBLE. An information theoretic approach (Burnham and Anderson 2002) will be used to select among multiple alternative models incorporating combinations of surface water dissolved and total nutrient concentrations, zooplankton grazing rates, zooplankton biomass and composition, phytoplankton biomass and composition, as well as physiochemical variables. Temperature, nutrient concentration (standard dissolved and particulate constituents for N, P, and silica), cyanobacteria toxins, phytoplankton biomass and composition data will come from weekly NOAA-GLERL sampling at site WE2 nearby our sampling site (Fig. 2). The statistical models will take the form of mixed effect linear or generalized additive models (depending on preliminary evaluation of the data), which will account for temporal autocorrelation among response variables.

Principal Findings and Results:

Overview

Our planned sampling during the 2020 field season was postponed due to COVID-19; therefore, we conducted our ZNR and grazing measurements during the 2021 field season. We successfully completed measurements of ZNR and grazing on nine sampling events (Table 1). The plankton samples (nano-, pico-, micro-, and mesoplankton) collected for this project are multifaceted and time-consuming to enumerate, therefore, we project that all samples will be analyzed by summer 2023. These samples will likely contribute to two chapters of Lyndsie Collis' (Ph.D. student, Dept. of Evolution, Ecology, and Organismal Biology, OSU) dissertation. Below, we focus on communicating the days on which micro- and total zooplankton assays successfully measured P excretion and grazing.

Table 1. Table of sampling dates for zooplankton excretion and grazing experiments, with associated information on surface temperature, chlorophyll-*a*, percent cyanobacteria of the phytoplankton community, and mesozooplankton density at sampling site 36-873.

Sample Date	Temperature (°C)	Chlorophyll-a (µg / L)	% Cyanobacteria	Mesozooplankton density (# / L)
19 Apr. 2021	11.0	6.2	0	1
17 May 2021	15.2	26.8	0	18
29 Jun. 2021	25.2	10.5	<1%	11
14 Jul. 2021	23.9	5.5	11	31
26 Jul. 2021	25.7	6.7	24	23
9 Aug. 2021	25.7	72.8	43	50
24 Aug. 2021	27.7	15.3	70	122
10 Sep. 2021	20.9	32.9	46	152
4 Oct. 2021	19.1	29.8	55	NA*

*Sample still being processed

Objective 1: ***Determine the relative importance of zooplankton-mediated N and P recycling in the western basin of Lake Erie and how it contributes to HAB development, timing, and magnitude.***

Our preliminary results indicate that we obtained measurable individual zooplankton P excretion estimates (i.e., upper 90% confidence intervals was less than zero) on four of nine dates for total zooplankton (micro- plus mesozooplankton) excretion and on two of five dates for microzooplankton excretion (Figs. 5 and 6). Not all microzooplankton excretion SRP samples have been analyzed; the remaining SRP samples have low sample volume, so we are waiting to analyze those samples until the new autoanalyzer is installed. Estimates of community microzooplankton and total zooplankton excretion rates require density estimates of both zooplankton groups and thus will not be available until those samples are enumerated. Therefore, at this time, we cannot evaluate this objective, however, we are well positioned to do so once our samples are processed.

The total zooplankton and microzooplankton assays we conducted on nine dates generated numerous samples, many of which are time-consuming to process and still require analysis. We

have already processed the chlorophyll *a*, fluorometer, the ambient mesozooplankton samples from site 36-873 (excluding one date), the chlorophyll *a* and fluorometer samples from the experiments, most of the SRP samples, as well as pico- (2-20 µm) and nanoplankton (0.2-2 µm) samples from two dates. We still need to analyze the remaining SRP and NH_4^+-N samples, seston carbon, nitrogen, and phosphorus samples, as well as the remaining nano-, pico-, and microzooplankton samples. Since each microzooplankton community sample requires 5-10 hours to enumerate, we anticipate completing those samples by summer 2023.

Objective 2: ***Identify the physicochemical and biological controls of zooplankton nutrient recycling rates and ratios.***

We will not be able to address this objective until we finish analyzing the microzooplankton community samples. Sample processing should be completed by summer 2023 and we anticipate completing this analysis by the end of 2023.

Associated Additional Research: Zooplankton grazing rates and patterns

We obtained measurable individual grazing rates for total zooplankton and microzooplankton on six and five sampling dates, respectively (Figs. 7 and 8). During cyanobacteria blooms (9 August and 10 September), mesozooplankton grazed primarily on cyanobacteria, but also selected for diatoms or cryptophytes (Fig. 9). In contrast, microzooplankton did not have measurable grazing rates for any phytoplankton group during August and grazed only on green algae during September (Fig. 9). We will continue to process samples from these grazing experiments to obtain grazing rates, which will increase our understanding of zooplankton-HAB interactions in the western basin of Lake Erie.

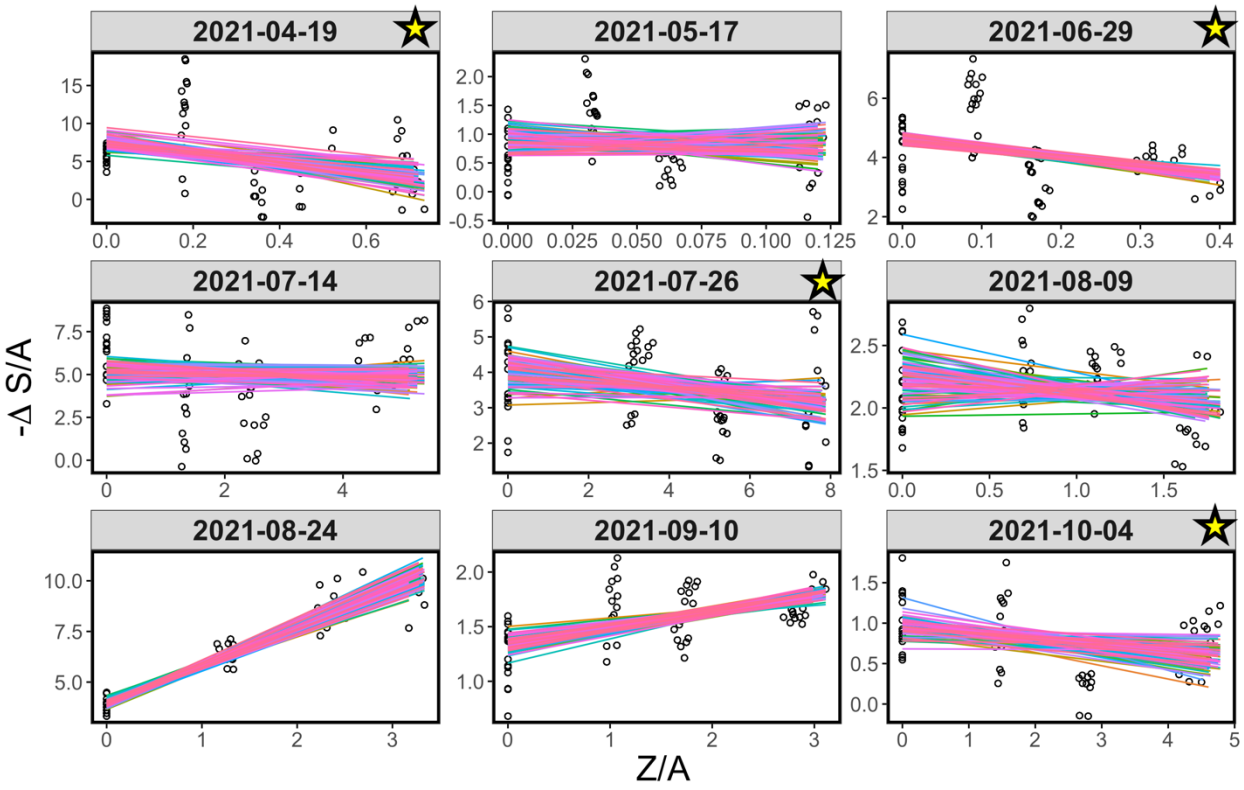

Figure 5. Results from total zooplankton phosphorus (P) excretion assays (gradient-grazer). The relationship between the quotient of the negative change in P concentration and algal biomass (-Δ S/A) and the quotient of zooplankton density and algal biomass (Z/A) were used to estimate individual excretion rates (-1 x slope; µg P ind^{-1} d^{-1}) for the total zooplankton community (micro- + mesozooplankton) on the nine sampling dates. For the purpose of this report, zooplankton density (Z) was estimated from the mesozooplankton density only, does not include microzooplankton densities and, therefore, cannot be used to estimate individual nutrient excretion rates. To estimate -Δ S/A and Z/A, we used a bootstrapping approach which paired each final SRP and chlorophyll *a* concentration with a randomly selected initial SRP or chlorophyll *a* concentration (n = 100), respectively, and then used linear regression to estimate the relationship between -Δ S/A and Z/A for each bootstrap replicate. Points are all bootstrapped estimates of -Δ S/A and Z/A and lines are the linear regression fits of the relationship between -Δ S/A and Z/A for each bootstrap replicate. Gold stars indicate slopes which were less than zero (i.e., the upper 90% CI of the slope estimate was less than zero) and indicate that phosphorus excretion was measureable. The x- and y-axis scales differ among each plot to improve visualization of the relationships between -Δ S/A and Z/A.

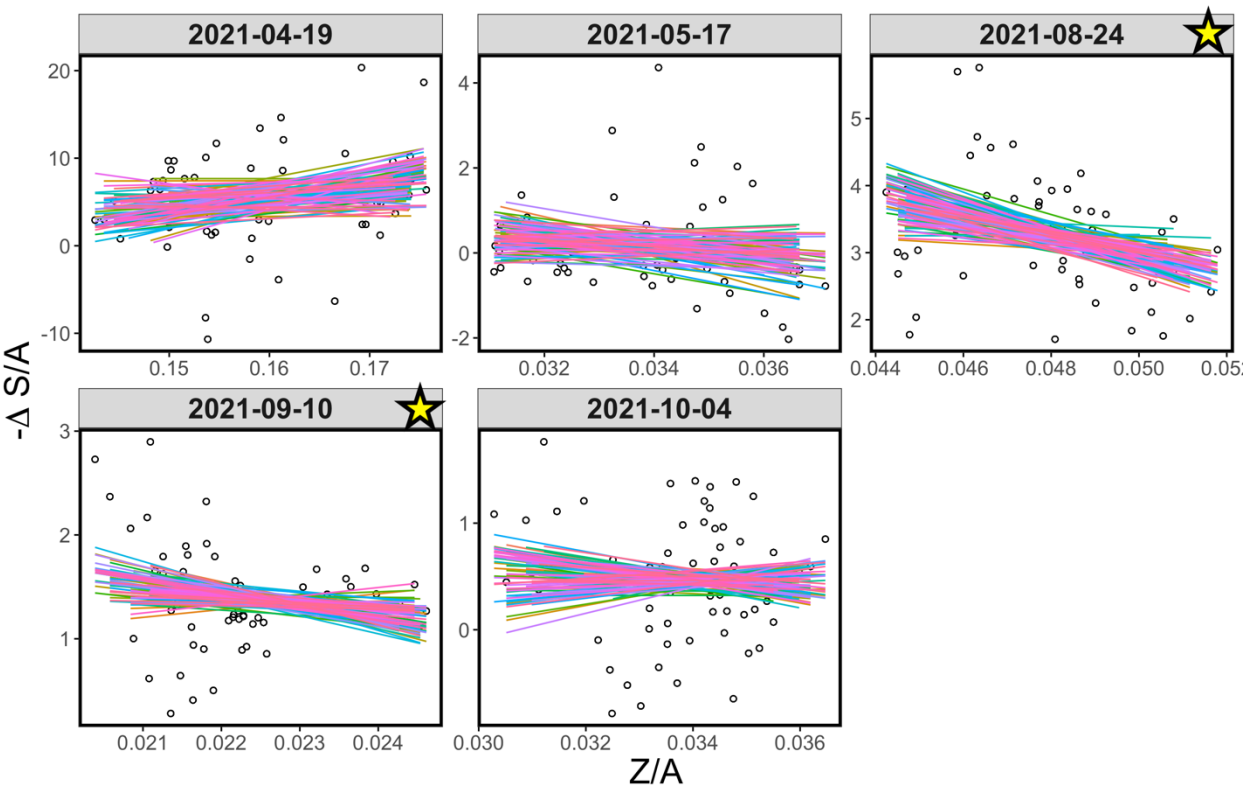

Figure 6. Results from microzooplankton phosphorus (P) excretion assays (dilution assays). The relationship between the quotient of the negative change in P concentration and algal biomass (-Δ S/A) and the quotient of zooplankton density and algal biomass (Z/A) were used to estimate individual excretion rates (-1 x slope; µg P ind^{-1} d^{-1}) for the microzooplankton community on the five sampling dates. Soluble reactive P samples for the other four dates will be proccessed at a later date. For the purpose of this report, microzooplankton density (Z) was represented by the dilution factors (25% ambient water, 50%, 75%, and 100%) and, therefore, cannot be used to estimate individual nutrient excretion rates. To estimate -Δ S/A and Z/A, we used a bootstrapping approach which paired each final SRP and chlorophyll *a* concentration with a randomly selected initial SRP or chlorophyll *a* concentration (n = 100), respectively, and then used linear regression to estimate the relationship between -Δ S/A and Z/A for each bootstrap replicate. Points are all bootstrapped estimates of -Δ S/A and Z/A and lines are the linear regression fits of the relationship between -Δ S/A and Z/A for each bootstrap replicate. Gold stars indicate slopes which were less than zero (i.e., the upper 90% CI of the slope estimate was less than zero) and indicate that phosphorus excretion was measureable. The x- and y-axis scales differ among each plot to improve visualization of the relationships between -Δ S/A and Z/A.

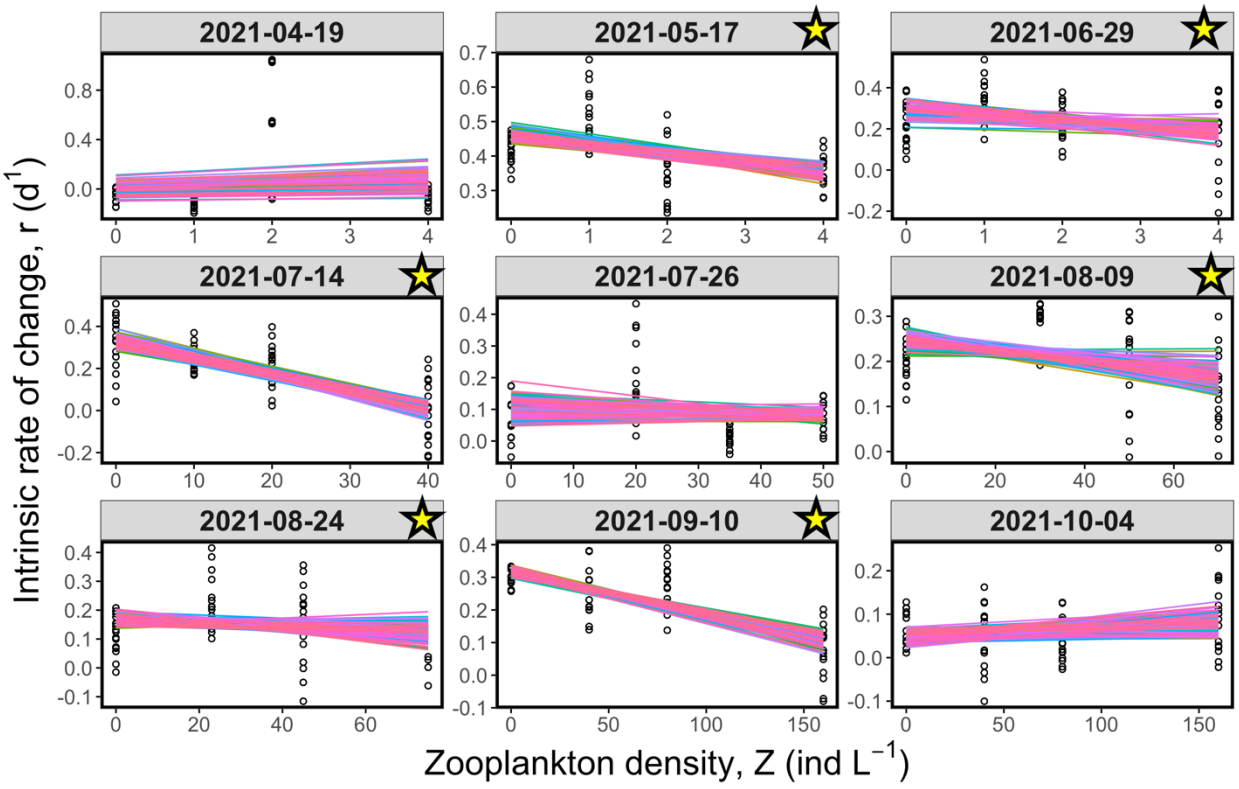

Figure 7. Results from total zooplankton grazing assays (gradient-grazer). The relationship between the intrinsic rate of change (*r*; [ln A_{final} – ln $A_{initial}$]/1 day) and the zooplankton density (*Z*) was used to estimate individual clearance rates (-1 x slope; L ind^{-1} d^{-1}) for the total zooplankton community (micro- + mesozooplankton) on the nine sampling dates. For the purpose of this report, zooplankton density was estimated from mesozooplankton density only, does not include microzooplankton densities and, therefore, cannot be used to estimate individual clearance rates. To estimate the intrinsic rate of change, we used a bootstrapping approach which paired each final chlorophyll *a* concentration with a randomly selected initial chlorophyll *a* concentration (n = 100) and then used linear regression to estimate the relationship between *r* and *Z* for each bootstrap replicate. Points are all bootstrapped estimates of *r* and lines are the linear regression fits of the relationship between *r* and *Z* for each bootstrap replicate. Gold stars indicate slopes which were less than zero (i.e., the upper 90% CI of the slope estimate was less than zero) and indicate that total zooplankton clearance rates were measureable. The x- and y-axis scales differ among each plot to improve visualization of the relationships between *r* and *Z*.

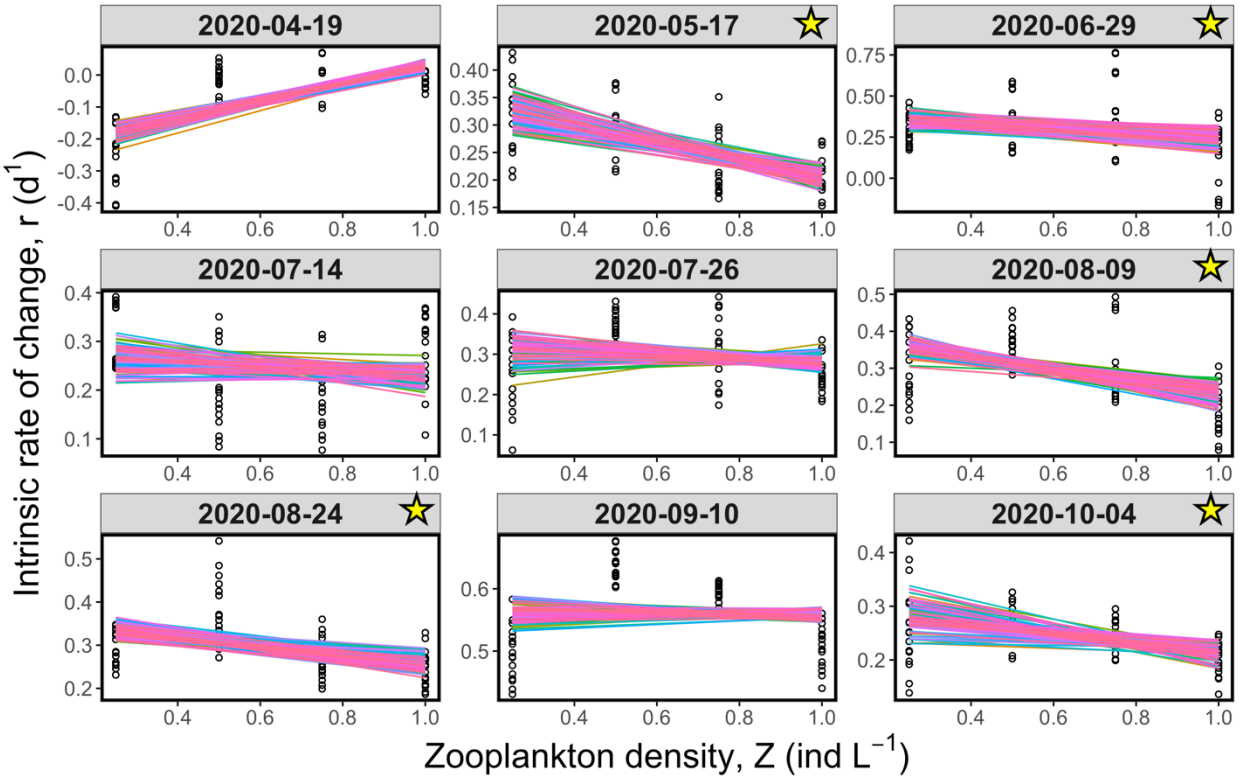

Figure 8. Results from microzooplankton grazing assays (dilution assays). The relationship between the intrinsic rate of change (*r*; [ln A_{final} – ln $A_{initial}$]/1 day) and the microzooplankton density (*Z*) was used to estimate individual clearance rates (-1 x slope; L ind^{-1} d^{-1}) for the microzooplankton community on the nine sampling dates. For the purpose of this report, microzooplankton density (Z) was represented by the dilution factors (25% ambient water, 50%, 75%, and 100%) and, therefore, cannot be used to estimate individual clearance rates. To estimate the intrinsic rate of change, we used a bootstrapping approach which paired each final chlorophyll *a* concentration with a randomly selected initial chlorophyll *a* concentration (n = 100) and then used linear regression to estimate the relationship between *r* and *Z* for each bootstrap replicate. Points are all bootstrapped estimates of *r* and lines are the linear regression fits of the relationship between *r* and *Z* for each bootstrap replicate. Gold stars indicate slopes which were less than zero (i.e., the upper 90% CI of the slope estimate was less than zero) and indicate that microzooplankton clearance rates were measureable. The x- and y-axis scales differ among each plot to improve visualization of the relationships between *r* and *Z*.

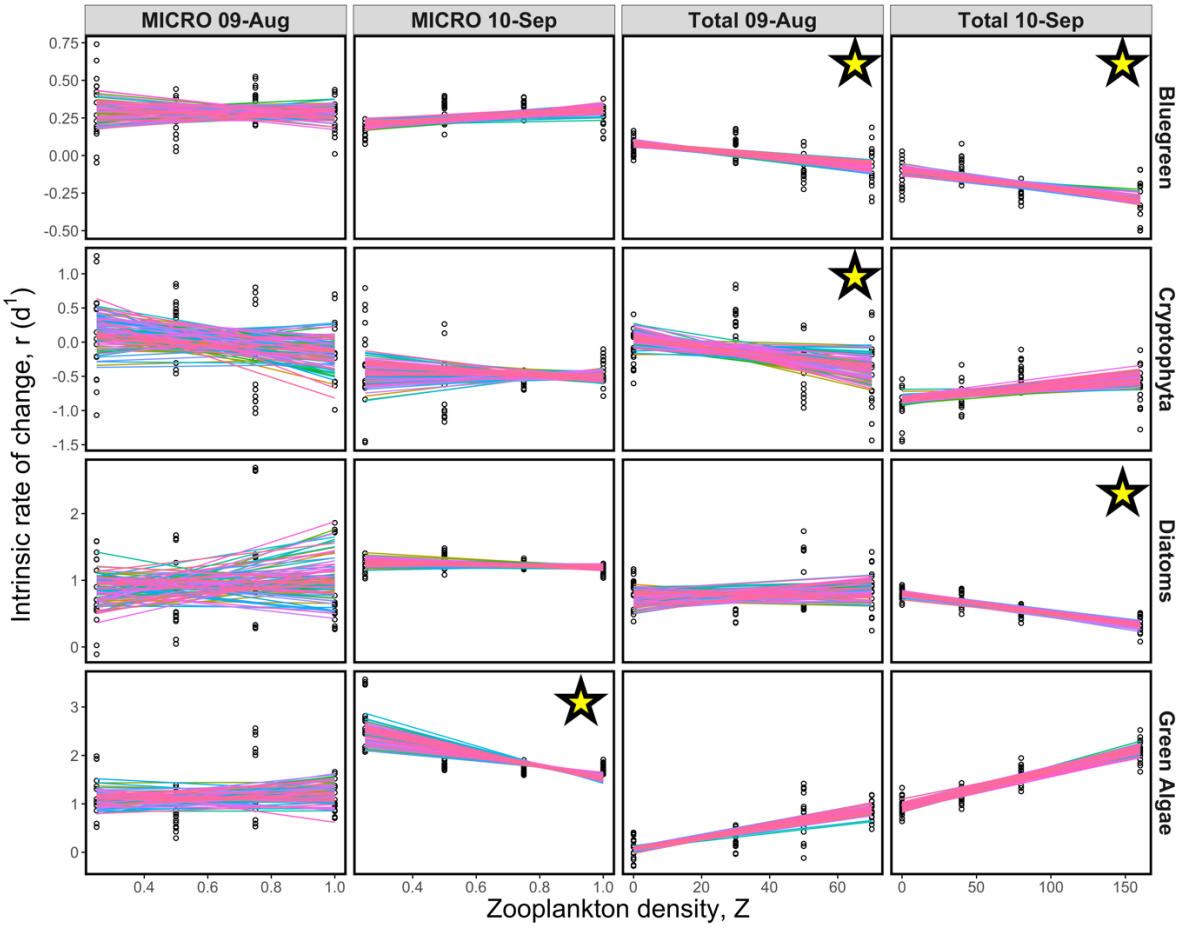

Figure 9. Results from micro- and total zooplankton grazing assays on two sampling dates (9 Aug 2021 and 10 Sept 2021), showing grazing patterns on major phytoplankton groups (blue greens, cryptophytes, diatoms, and green algae). The relationship between the intrinsic rate of change (*r*; [ln A_{final} – ln $A_{initial}$]/1 day) and the meso- or microzooplankton density (*Z*) was used to estimate individual clearance rates (-1 x slope; L ind^{-1} d^{-1}) for the total zooplankton and microzooplankton community on each sampling date. For the purpose of this report, zooplankton density (Z) for the total zooplankton assays was estimated from mesozooplankton density only, does not include microzooplankton densities and, therefore, cannot be used to estimate individual clearance rates. Furthermore, microzooplankton density (Z) for the microzooplankton assays was represented by the dilution factors (25% ambient water, 50%, 75%, and 100%) and, therefore, cannot be used to estimate individual clearance rates. To estimate the intrinsic rate of change, we used a bootstrapping approach which paired each final taxa-specific chlorophyll *a* concentration with a randomly selected initial taxa-specific chlorophyll *a* concentration (n = 100) and then used linear regression to estimate the relationship between *r* and *Z* for each bootstrap replicate. Points are all bootstrapped estimates of *r* and lines are the linear regression fits of the relationship between *r* and *Z* for each bootstrap replicate. Gold stars indicate slopes which were less than zero (i.e., the upper 90% CI of the slope estimate was less than zero) and indicate that total or microzooplankton clearance rates were measureable. The x- and y-axis scales differ among plots to improve visualization of the relationships between *r* and *Z*.

Significance:

Our research will increase understanding of interactions among nutrient cycles, phytoplankton, and zooplankton –an important nexus of HAB management– and will address mechanistic uncertainty associated with Lake Erie HABs forecasting models (e.g., Ho and Michalak 2017, Obenour et al. 2014, Stumpf et al. 2016). The fate of P between spring loading and HAB events is clearly mediated by internal nutrient cycling; however, the relative importance of different internal nutrient sources is poorly understood, particularly zooplankton-mediated nutrient cycling (Fig. 1). Once our samples our analyzed, we will be able to estimate nutrient recycling and grazing by zooplankton for our site near Toledo, OH and assess the relative importance of nutrient recycling by zooplankton relative to phytoplankton nutrient demand and other sources. This research will provide basic and applied science benefits including an improved understanding of the role of zooplankton in shaping nutrient recycling rates and ratios, knowledge about zooplankton-cyanobacteria feedbacks, as well as information that could be used to help parameterize and validate lake models.

This project has contributed to the training of an undergraduate (Dan Peters, OSU Biology Dept.), a technician (Morgan Shaw, recently accepted to graduate school at OSU), and a Ph.D. student (Lyndsie Collis) who co-wrote the proposal. This research will contribute data to at least two chapters of Lyndsie Collis' dissertation and to Dan Peters' undergraduate thesis which focuses on nano- and picoplankton grazing during HAB events. To date, this research has resulted in four abstract acceptances for posters and/or talks at two conferences in spring 2022 (State of Lake Erie and Joint Aquatic Science Meeting). We have developed collaborations and relationships with researchers and managers at NOAA-GLERL, Ohio Environmental Protection Agency, and Ohio Department of Natural Resources and will communicate our findings with those groups and others as this research progresses.

REFERENCES

Baker, D. B., R. Confesor, D. E. Ewing, L. T. Johnson, J. W. Kramer, and B. J. Merryfield. 2014. Phosphorus loading to Lake Erie from the Maumee, Sandusky and Cuyahoga rivers: The importance of bioavailability. Journal of Great Lakes Research **40**:502-517.

Benke, A.C. and A. D. Huryn. 2017. Secondary Production and Quantitative Food Webs. In: Lamberti, G.A. and F.R. Hauer (Eds.), Methods in Stream Ecology (Third Edition), Academic Press, Cambridge, MA, USA, pp. 235-254.

Boros, G. and A. Mozár. 2015. Comparison of different methods used for phosphorus determination in aquatic organisms. Hydrobiologia **758:** 235-242.

Briland, R. 2018a. Evaluating the causes and consequences of ecosystem change in Lake Erie: from plankton to fish. The Ohio State University, Columbus, OH.

Briland, R. 2018b. Evaluating the causes and consequences of ecosystem change in Lake Erie: from plankton to fish. PhD dissertation. The Ohio State University.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach. 2nd Edition. Springer, New York.

Calbert, A., and E. Saiz. 2018. How much is enough for nutrients in microzooplankton dilutioin grazing experiments? Journal of Plankton Research **40**:109-117.

Carmichael, W. W., and G. L. Boyer. 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. Harmful Algae **54**:194-212.

Conroy, J. D., D. D. Kane, D. M. Dolan, W. J. Edwards, M. N. Charlton, D. A. Culver. 2005. Temporal trends in Lake Erie phytoplankton biomass: roles of external phosphorus loading and dreissenid mussels. Journal of Great Lakes Research. **31(Suppl. 2)**:89-110

Culver, D.A., Boucherle, M.M., Bean, D.J., and J.W. Fletcher. 1985. Biomass of freshwater crustacean zooplankton from length-weight regressions. Canadian Journal of Fisheries and Aquatic Sciences. **42**:1380-1390.

DeMott, W. R., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. Limnology and Oceanography **43:**1147-1161.

Dumont, H. J., I. Van de Velde, and S. Dumont. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda, and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia **19**: 75-97.

Elser, J. J. and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology **80**: 735-751.

Hadas, O., and T. Berman. 1998. Seasonal abundance and vertical distribution of Protozoa (flagellates, ciliates) and bacteria in Lake Kinneret, Israel. Aquatic Microbial Biology. **14**: 161–170.

Hall, R. O., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography **48**:1120-1128.

Hambright, K. D., T. Zohary, and H. Güde. 2007a. Microzooplankton dominate carbon flow and nutrient cycling in a warm subtropical freshwater lake. Limnology and Oceanography **52**:1018-1025.

Hambright, K. D., N. G. H. Jr., W. R. Schaffner, and R. W. Howarth. 2007b. Grazer control of nitrogen fixation: synergisms in the feeding ecology of two freshwater crustaceans. Fundamental and Applied Limnology **170/2**:89-101.

Hart, D. R., L. Stone, and T. Berman. 2000. Seasonal dynamics of the Lake Kinneret food web: the importance of the microbial loop. Limnology and Oceanography **45**:350–361.

Hillebrand, H., C. Dürselen, D. Kirschtel, U. Pollingher, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology **35**:403-424.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology **33**:1225-1228.

Ho, J. C., A. M. Michalak. 2017. Phytoplankton blooms in Lake Erie impacted by both long-term and springtime phosphorus loading. Journal of Great Lakes Research **43**:221-228.

Hood, J. M., J. P. Benstead, W. F. Cross, A. D. Huryn, P. W. Johnson, G. M. Gislaon, J. R. Junker, D. Nelson, J. S. Olafsson, and C. Tran. 2018. Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming. Global Change Biology **24**:1069-1084.

International Joint Commission. 2014. A balanced diet for Lake Erie: Redcucing phosphorus loading and harmful algal blooms. Lake Erie Ecosystem Priority.

Landry, M. R., and R. P. Hassett. 1982. Estimating the grazing impact of marine microzooplankton. Marine Biology **67**:283-288.

Lehman, J. T., and C. D. Sandgren. 1985. Species-specific rates of growth and grazing loss among freshwater algae. Limnology & Oceanography **30**:34-36.

Lund, J.W.G., Kipling, C. and Le Cren, E.D. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia **11**:143-170.

Manly, B. F. J. 2007. Randomization, Bootstrap and Monte Carlo Methods in Biology. Chapman & Hall, CRC Press, Boca Raton, FL, USA.

Matisoff, G., E. M. Kaltenberg, R. L. Steely, S. K. Hummel, J. Seo, K. J. Gibbons, T. B. Bridgeman, Y. Seo, M. Behbahani, W. F. James, L. T. Johnson, P. Doan, M. Dittrich, M. A. Evans, and J. D. Chaffin. 2016. Internal loading of phosphorus in western Lake Erie. Journal of Great Lakes Research **42**:775-788.

Michalak, A. M., E. J. Anderson, D. Beletsky, S. Boland, N. S. Bosch, T. B. Bridgeman, J. D. Chaffin, K. Cho, R. Confesor, I. Daloglu, J. V. Depinto, M. A. Evans, G. L. Fahnenstiel, L. He, J. C. Ho, L. Jenkins, T. H. Johengen, K. C. Kuo, E. Laporte, X. Liu, M. R. McWilliams, M. R. Moore, D. J. Posselt, R. P. Richards, D. Scavia, A. L. Steiner, E. Verhamme, D. M. Wright, and M. A. Zagorski. 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proceedings of the National Academy of Sciences U S A **110**:6448-6452.

Morin, A., W. Lamoureux, and J. Busnarda. 1999. Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. Journal of the North American Benthological Society **18**:299-307.

Obenour, D. R., A. D. Gronewold, C. A. Stow, and D. Scavia. 2014. Using a Bayesian hierarchical model to improve Lake Erie cyanobacteria bloom forecasts, Water Resources Research **50**:7847-7860.

Paerl, H. W., W. S. Gardner, K. E. Havens, A. R. Joyner, M. J. McCarthy, S. E. Newell, B. Qin, and J. T. Scott. 2016. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae **54**:213-222.

Putt, M., and D. K. Stoecker. 1989. An experimentally determined carbon:volume ratio for marine ‘‘oligotrichous’’ ciliates from estuarine and coastal waters. Limnology and Oceanography **34**:1097–1103.

Raymond, H. A. 2012. Microcystin concentrations increasing at select Ohio drinking water

sources, positive correlation with pH and water temperature, and implications of surface scums. Ohio Journal of Science **112**:A7.

Scavia, D., J. David Allan, K. K. Arend, S. Bartell, D. Beletsky, N. S. Bosch, S. B. Brandt, R. D. Briland, I. Daloğlu, J. V. DePinto, D. M. Dolan, M. A. Evans, T. M. Farmer, D. Goto, H. Han, T. O. Höök, R. Knight, S. A. Ludsin, D. Mason, A. M. Michalak, R. Peter Richards, J. J. Roberts, D. K. Rucinski, E. Rutherford, D. J. Schwab, T. M. Sesterhenn, H. Zhang, and Y. Zhou. 2014. Assessing and addressing the re-eutrophication of Lake Erie: Central basin hypoxia. Journal of Great Lakes Research **40**:226-246.

Simon M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Marine Ecology-Progress Series **51**:201–213.

Staehr, P. A., D. Bade, M. C. V. D. Bogert, G. R. Koch, C. Williamson, P. Hanson, J. J. Cole, and T. Kratz. 2010. Lake metabolism and the diel oxygen technique: State of the science. Limnology and Oceanography: Methods **8**:628-644.

Stumpf, R. P., L. T. Johnson, T. T. Wynne, and D. B. Baker. 2016. Forecasting annual cyanobacterial bloom biomass to inform management decisions in Lake Erie. Journal of Great Lakes Research **42**:1174-1183.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual review of ecology and systematics **33**:341–70.

Vanni, M. J., A. M. Bowling, E. M. Dickman, R. S. Hale, K. A. Higgins, M. J. Horgan, L. B. Knoll, W. H. Renwick, and R. Stein. 2006. Nutrient cycling by fish supports relatively more primary production as lake productivity increases. Ecology **87**:1696-1709.

Winslow, L. A., J. A. Zwart, R. D. Batt, H. A. Dugan, I. Woolway, J. R. Corman, P. C. Hanson, and J. S. Read. 2016. LakeMetabolizer: an R package for estimating lake metabolism from free-water oxygen using diverse statistical models. Inland Waters **6**:622-636.

Wood, S. N. 2006. Generalized additive models: An introduction with R. Taylor & Francis, Boca Raton, FL, USA.

Wood, S. N. 2017. Generalized Additive Models: An Introduction with R. CRC Press, Boca Raton, FL, USA.

Zhang, H., L. Boegman, D. Scavia, and D. A. Culver. 2016. Spatial distributions of external and internal phosphorus loads in Lake Erie and their impacts on phytoplankton and water quality. Journal of Great Lakes Research **42**:1212-1227.