Introduction

OVERVIEW

The Pennsylvania Water Resources Research Center (PA-WRRC), founded in 1964, is authorized by Congress as one of the nation's 54 water resources research institutes comprising the National Institutes of Water Resources (NIWR). In keeping with the Land Grant mission of the host Universities, each institute serves a tripartite mission of University research, education, and outreach in advancing pressing problems in water quality and quantity. The program is administered by the U.S. Department of the Interior through the U.S. Geological Survey, in a unique Federal-State-University partnership. The PA-WRRC is located on the University Park campus at the Pennsylvania State University. There, PA-WRRC resides within the Penn State Institutes of Energy and the Environment (PSIEE). The PA-WRRC continues to receive support contributions from PSIEE, which funds the Director’s time spent in water center administration and provides additional staff support for administrative, accounting, communications, and research functions.

The PA-WRRC receives USGS 104B federal base funding that is distributed via a small grants competition to researchers at academic institutions across Pennsylvania. A request for proposals for this competition was broadly disseminated. Given the level of funding available in FY15, we were able make several awards as listed below; intended to be small grants allowing faculty to conduct research our outreach on problems important to Pennsylvania. None of the federal funding was used to pay overhead costs, and PA-WRRC matched every dollar of its base appropriation with at least two dollars from non-federal sources.

Research Projects.

Three projects supported during FY15 were research-oriented, addressing unanswered questions in water resources and seeking solutions to water challenges.

• Principal Investigator William Strosnider and colleagues from St. Francis University led a research project entitled “Passive Co-Treatment of Acid Mine Drainage and Municipal Wastewater: A Novel Solution to Protect and Restore Water Quality.” This work indicates that carbon substrate complexity controls microbial community structure but does not affect pollutant removal rates during the co-treatment of acid mine drainage and municipal wastewater

• Principal Investigator William Burgos and colleagues from Penn State University led a research project entitled “Assessment of Shale Gas Contaminants in Sediment Profiles of the Conemaugh River Lake.” This research found that peak concentrations of barium, radium, strontium, chloride, bromide and sodium in the sediments of the Conemaugh River Lake corresponded to the years (2007 – 2011) when the highest volumes of oil & gas wastewater were discharged into the watershed.

• Principal Investigator Victoria Braithwaite and colleagues from Penn State University led a research project entitled “Determining how fish populations cope with rapid environmental fluctuation: A case study in Pennsylvania streams.” They found high behavioral heterogeneity that could limit fish movement and adaptive potential.

Information Transfer Projects.

Two projects supported during FY15 were oriented toward outreach and information transfer, making research-based information available to user communities.
• PI Bryan Swistock from Penn State University and a group of extension educators led a project entitled “Monitoring and Education for Harmful Algal Blooms in Pennsylvania Inland Ponds and Lakes.” This project created a network of Penn State Extension water specialists who provide education and identification of harmful algae bloom species in inland ponds and lakes across the state to reduce potential risks to animals and humans associated with these algae.

• PI Beth Boyer from Penn State University and PA-WRRC graduate student interns worked to deliver educational and outreach programs. PA-WRRC co-sponsored numerous conferences and academic seminar series, and presented talks and poster presentations about water resources of Pennsylvania and beyond.

Educational Impact.

37 students (33 undergraduate students, 3 doctoral students, and 1 postdoctoral fellow) were supported or partially supported as part of the PA-WRRC 104B projects during FY15 from multiple academic institutions across Pennsylvania.

Further, PA-WRRC facilitated a summer internship that was offered by the USGS through the National Institutes for Water Resources Student Internship Program, via the Pennsylvania Water Resources Research Center. This work is part of a research project being done in partnership with Presque Isle State Park, Erie County Department of Health, and the Tom Ridge Environmental Center (TREC) Regional Science Consortium (RSC). The intern, undergraduate student Erika Levy from Allegheny College, studied water quality of Lake Erie. She worked with mentors Jeannette Schnars from the RSC and Tammy Zimmerman from USGS.
None.
Determining How Fish Populations Cope with Rapid Environmental Fluctuation: A Case Study in Pennsylvania Streams

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Publications

There are no publications.
PROJECT TITLE & PRINCIPAL INVESTIGATORS

Determining how fish populations cope with rapid environmental fluctuation: a case study in Pennsylvania streams

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PROBLEM and RESEARCH OBJECTIVES

Rates of population extirpation from habitat loss have reached unprecedented levels and climate change is predicted to be a leading cause of future species extinctions (Travis 2002, Mantyka-Pringle et al. 2012). Within Pennsylvania, processes such as Marcellus Shale gas drilling and variable climate patterns are already affecting aquatic environments in multiple ways (Weltman-Fahs and Taylor 2013). Accordingly, conservation of emergent properties that promote resistance and resilience to environmental perturbation will be vital to future population persistence. Though it has been demonstrated that phenotypic plasticity increases resilience to habitat loss (Greene et al. 2010), the ability for plasticity to promote population persistence under climate change and habitat degradation has not been explored (Chevin et al. 2010, Meier et al. 2014). If plasticity does increase survival, failure to conserve highly plastic genotypes could accelerate species extinction.

Phenotypic plasticity is a heritable trait that is only expressed after interactions with the environment. Thus, an individual could have a genotype capable of exhibiting high plasticity, but will maintain a uniform response unless exposed to a heterogeneous environment (Scheiner 1993, Nijhout 2003). In many species, lifetime plastic potential is determined during a short phase in juvenile development. During this time, exposure to diverse habitats and fluctuating environmental conditions triggers higher plasticity, whereas homogenous conditions promote fixation of phenotypes (Kihslinger and Nevitt 2006, Olvido and Mousseau 1995, Jones et al. 2013).

Studies have shown that plasticity increases behavioral flexibility, thus allowing organisms to exploit new habitats (Busch et al. 2012), increase prey consumption (Keathley and Potter 2011), and increase reproductive success (Betini and Norris 2012). Loss of genetic connectivity and environmental heterogeneity reduces plasticity which, in turn, reduces population resistance to environmental disturbance (Reed et al. 2011, Miner et al. 2005). As such, plasticity may determine the pace for population extirpation due to habitat loss.

Natural resource management often focuses on conservation of landscapes and populations. The significance of these efforts cannot be ignored; however, the certainty of future habitat loss necessitates management become more forward-thinking and shift focus to promoting resistance.
and resilience. To accomplish this goal, a better understanding of emergent properties of landscapes and populations is vital.

This study focused on an economically and socially important species, brook trout (*Salvelinus fontinalis*), to determine how the interactive effects of genetics and behavior may influence differential survival of fish populations under a changing climate. Warming stream temperatures are projected to extirpate brook trout populations from much of the species’ current range (Hudy et al. 2008). However, landscape habitat data used to generate models may not accurately reflect patterns of local habitat use. At small spatial scales, phenotypic plasticity may enable populations to continue occupying heterogeneous habitat mosaics despite loss of broad-scale habitat characteristics. These cross-scale interactions produce unanticipated, nonlinear patterns and dynamics that reduce the ability to predict future outcomes of climate change on species resiliency, adaptive potential, and persistence (Nogués-Bravo and Rahbek 2011, Angert et al. 2013). As such, understanding the factors that control plasticity, and the ability of plasticity to increase survival, will be critical for future natural resource management.

The objectives of this research were to (1) determine the degree of genetic connectivity among populations in Loyalsock Creek, (2) determine the degree of behavioral heterogeneity and plasticity in natural brook trout populations, and (3) hypothesize the effects of genetics and behavior on phenotypic plasticity.

**METHODOLOGY**

*Study site*

This research focused on brook trout populations in the Loyalsock Creek watershed in Pennsylvania. Loyalsock Creek is a 103-km tributary to the West Branch Susquehanna River that flows through Sullivan and Lycoming counties (Figure 1). The watershed is an important recreational area for fishing, hunting, rafting, and hiking and contains the Loyalsock State Forest, World’s End State Park, and several State Game Lands.

With future climate change scenarios and projected increase of gas and coal mining, there is concern about the long-term security of brook trout populations in the watershed. In the future, these processes will likely operate synergistically with the increase presence of invasive species to accelerate brook trout population extirpation. And, because most brook trout populations are
already isolated to higher headwaters where immigration may be limited, many populations may already susceptible to decline due to genetic drift and loss of diversity.

Genetic tissue collection

Tissue samples were collected at 27 sites in the Loyalsock Creek watershed in summer 2015 (Figure 2). Because surveys were completed in conjunction with other research activities, sampling effort was variable but ranged from single to triple-pass electrofishing. We attempted to collect 50 adult (i.e., >100mm total length; Whiteley et al. 2012) fish from each site; however, we were able to collect less than 30 adults at two locations. We included only adult fish in this analysis because juveniles rarely disperse outside of their natal streams and analyses conducted on sibling groups can provide inaccurate estimates of genetic diversity (Aunins et al. 2014).

For all adults, we excised a 5mm portion of the upper caudal fin clip and preserved the tissue in 95% non-denatured...
ethyl alcohol. To assist in age estimation, we recorded the total length and weight for each fish.

**Genotyping and Data Analysis**

Genetic laboratory work at the U.S. Fish and Wildlife Service Northeast Fishery Science Center in Lamar, PA is ongoing. Individuals are being genotyped using 12 microsatellite loci as described by Aunins et al. (2013). Loci include SfoC-113, SfoD-75, SfoC-88, SfoD-100, SfoC-129, SfoC-24, SfoB-52, SfoC-28, SfoC-38, SfoC-79, SfoC-86, and SfoD-91, all of which have been used in previous studies to successfully genotype brook trout populations (Hudy et al. 2010, Kanno et al. 2011, King et al. 2012). All alleles will be individually scored using GeneMapper software.

After all fish have been genotyped, within-population genetic diversity will be quantified using allelic frequency, number of alleles per locus, effective number of alleles, and observed heterozygosity. We will also test each population for deviation from Hardy-Weinberg equilibrium. Patterns of genetic differentiation among populations will be determined by calculating pairwise fixation indices (Fst). We will also determine whether there is a significant isolation-by-distance effect.

**Fish Collection for Behavior Trials**

In summer 2015 we sampled 16 sites to collect adult brook trout for behavior trials (Figure 3). At each site we attempted to collect at least 20 individuals in order to accurately estimate population phenotypic diversity. While larger samples sizes would have improved estimates of within-population heterogeneity, we were limited by the number of assessments that could be completed in one day. Further, for streams with low brook trout abundance, the spatial extent needed to exceed 20 individuals would have decreased the precision of estimates of environmental covariates. The length of
stream sampled at each site was variable, but ranged from approximately 100-750m. In total, data were collected on 377 fish.

Fish were captured via single-pass electrofishing using sub-optimal amperage to minimize effects of collection on behavior. After capture, fish were held in a net pen in groups of approximately 30. The net pen was large enough that fish were able to swim freely and maintain rheotaxis.

**Behavioral Assays**

Personality assessments were completed in a 76cm x 76cm square white plastic tank filled with stream water to a height of 45cm. A remote controlled camera was mounted approximately 1.5m from the water surface using PVC pipe. Tanks were placed next to the stream, and away from possible sources of disturbance.

Fish were transported from the net pen to the testing tank in a 19L bucket filled halfway with stream water. Fish were poured from the bucket to the center of the testing tank and, immediately after observers were hidden from view, a 30-min video recording was started. At the end of video recording, we measured total length and weight for each individual and released it back to the stream.

**Video Analysis**

A rapid assessment of videos indicated a dichotomy in behavior that describes differences in exploration tendency among individuals. This personality metric has been described by others by quantifying the amount of time a fish spends moving in the testing tank (Conrad et al. 2011). Accordingly, we are currently viewing videos to record the proportion of time an individual spends swimming. We will also attempt to estimate distance moved by overlaying a grid on the tank and determining how many times a fish crosses into a new cell.

Videos are being analyzed using Noldus EthoVision 11.5 software. For each video, the first 20 minutes are not analyzed, thus allowing the fish time to acclimate to the testing tank. The remaining 10 minutes are analyzed using EthoVision to automatically track the fish’s movements.
Behavior Data Analysis

With nearly 200 hours of video, we are still working to complete data collection. However, we will compare the proportion of exploratory individuals in each population across sample sites to determine the degree of phenotypic variance across populations. If there is significant variance, we will model the proportion of exploratory individuals as a function of environmental covariates. In particular, we will see if exploration can be predicted by stream gradient or the presence of brown trout. Both of these variables could limit the amount of movement a fish is allowed to experience during juvenile development.

**PRINCIPAL FINDINGS AND SIGNIFICANCE**

While analyses are ongoing, preliminary results suggest that the proportion of exploratory individuals is highly variable among streams. Whereas some streams contained upwards of 80% exploratory fish, other sites had 15% or fewer. The cause for this finding is still under investigation, but it could be genetic, abiotic, and/or biotic. Genetically, populations could have different behaviors because of local adaptation to abiotic stream conditions (e.g., high gradient, low pH) causing directional selection for certain genotypes in the populations. Also possible is that the presence of brown trout, a piscivorous, nonnative species that predates on juvenile brook trout, encourages the development of more sedentary behaviors as a means to decrease predation risk.

This research is the first large-scale study of fish behavior and the first to document behavioral heterogeneity across a watershed. Our findings suggest that local abiotic and biotic conditions may shape individual-level behavior and, as exploration may predict plasticity, these conditions could influence plasticity and resilience to habitat loss. Accordingly, our results indicate that accounting for individual-level processes and small-scale spatial patterns may improve trout conservation.

**STUDENTS & POSTDOCS SUPPORTED**

Shannon White, Intercollege Graduate Degree Program in Ecology, Pennsylvania State University Ph.D.; Nathan Newton, Susquehanna University, Bachelor of Science; Laurel Seemiller, Susquehanna University, Bachelor of Science
PUBLICATIONS

INFORMATION TRANSFER ACTIVITIES

AWARDS & ACHIEVEMENTS
N/A

PHOTOS OF PROJECT
Loyalsock Creek has a diverse range of trout habitat with many streams containing waterfalls. These waterfalls can be a limitation to fish movement and can decrease genetic variation which could also decrease behavioral plasticity.
Brook trout are state fish of Pennsylvania and the only native trout species to the state. A tissue sample was taken from every adult fish we collected and will be used to infer the degree of genetic diversity and population connectivity in the watershed.

Works Cited


Assessment of Shale Gas Contaminants in Sediment Profiles of the Conemaugh River Lake

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Publications

There are no publications.
PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)
Assessment of Shale Gas Contaminants in Sediment Profiles from the Conemaugh River Lake

William Burgos, Professor of Environmental Engineering, Department of Civil and Environmental Engineering, The Pennsylvania State University, 212 Sackett Building, University Park, PA 16802, wdb3@psu.edu, phone: (814)-863-0578

Patrick Drohan, Associate Professor of Pedology, Department of Ecosystem Science and Management, The Pennsylvania State University, 452 Agricultural Sciences and Industries Building, University Park, PA 16802, pjd7@psu.edu, phone: (814)-863-4246

PROBLEM and RESEARCH OBJECTIVES

In collaboration with the US Army Corps of Engineers Pittsburgh District, we collected intact sediments cores from the Conemaugh River Lake in western Pennsylvania. This lake was selected because it is located in a watershed with significant Marcellus shale gas activity, is downstream of several brine treatment facilities, and the sediments are well-structured allowing for temporal resolution of contaminant loads entering the lake. Our central hypothesis was that treated effluent from brine treatment facilities can increase concentrations of inorganic constituents in sediments and porewater at a watershed scale and lead to potential long-term risks to aquatic and human health. Results from this study will be used to develop a sediment management strategy for the Army Corps, to inform state and federal agencies about these conditions, and to propose future research activities in this area.

METHODOLOGY

A Vibracore sediment sampling package was operated on a floating platform to drive 6 m sections of 7.5 cm diameter Al agricultural drainpipe into the lake sediments. A rescue tripod mounted over a drill hole in the platform was used to pull the core out of the lake bottom. After extraction, caps were placed on the ends of the core and secured with duct tape. Nine cores were collected. Cores were immediately returned to the boat launch, ‘flash frozen’ on dry ice, and stored in a freezer trailer (-9 °C) for transport to Penn State.

Frozen cores were thawed and dried for core logging and 226Ra/228Ra isotope measurements, or halved or sectioned while frozen for porewater analyses. The intact core halves were scanned on a Geotek MSCL-S system using an Innov-X handheld X-ray fluorescence (XRF) spectrometer (elemental analyses) and a Bartington magnetic susceptibility loop sensor. Frozen cores used for porewater analyses were sectioned and material from each section was placed into 250 mL centrifuge tubes. Sediment sections were transferred to an anaerobic chamber, allowed to thaw, centrifuged at 10,000 rpm for 30 min, and then returned to the chamber. Porewater was
aspirated from the tubes and used to measure a suite of inorganic contaminants. Porewater samples analyzed for pH, ORP, conductivity, major anions (Cl, Br, SO4) by ion chromatography, cations (Na, Ca, Ba, Sr, Mg) by ICP-AES, and trace metals (e.g., Cd, Cr, As, Pb) by ICP-MS. 86/87Sr isotopes were measured in the porewater using a multi-collector ICP-MS (MC-ICP-MS). Sediment samples were extracted in aqua-regia and analyzed for major cations and trace metals, and for 226/228Ra by gamma spectroscopy.

**PRINCIPAL FINDINGS AND SIGNIFICANCE**

We found Conemaugh River Lake sediments and porewaters to contain elevated concentrations of radium, barium, strontium, and bromide. Oil & gas wastewater from two large brine treatment facilities has been discharged into the Blacklick Creek upstream of the Conemaugh River Lake. Using a preliminary sediment age model based on $^{228}$Ra and $^{210}$Pb we found that the peak concentration of many of these contaminants corresponded to 2007 – 2011 when the highest volumes of O&G wastewater were discharged into the watershed. In this case, surface water disposal of oil & gas wastewater impacted sediments 10 km downstream of the CWT plants.

**STUDENTS & POSTDOCS SUPPORTED**

Luis Castillo Meza, Civil and Environmental Engineering, Ph.D. candidate.

**PUBLICATIONS**

In progress.

**INFORMATION TRANSFER ACTIVITIES**

“Impact of shale gas wastewater disposal on Conemaugh River Lake sediments”

Invited seminar presented by W.D. Burgos to the Environmental Engineering program at University of Pittsburgh, March 2, 2016.

**PHOTOS OF PROJECT**

Please include 2 graphics or photos with captions, if possible. These may be used in our annual report, web page, and/or brochure, and may be used by the National Institutes of Water Resources.
Pat Drohan (PSU) and Carl Nim (USACE) collecting sediment cores on the Conemaugh River Lake, May 20, 2015.
Porewater and sediment concentrations versus depth from Core 3 collected from the Conemaugh River Lake. The highlighted region in both panels corresponds to 2007-2011, years of maximum discharge of oil & gas wastewater to Blacklick Creek,
Passive Co-Treatment of Acid Mine Drainage and Municipal Wastewater: A Novel Solution to Protect and Restore Water Quality

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Publications

There are no publications.
PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)
Passive Co-Treatment of Acid Mine Drainage and Municipal Wastewater: A Novel Solution to Protect and Restore Water Quality

Principal investigator: William Strosnider, Assistant Professor of Environmental Engineering, Saint Francis University, wstrosnider@francis.edu, 814-471-1144

Co-investigator: Rachel Wagner, Assistant Professor of Environmental Engineering, Saint Francis University, rwagner@francis.edu, 814-471-1155

Co-investigator: Joel Bandstra, Associate Professor of Environmental Engineering, Saint Francis University, jbandstra@francis.edu, 814-471-1147

PROBLEM and RESEARCH OBJECTIVES

Municipal wastewater (MWW) and acid mine drainage (AMD) are commonly co-occurring waste streams in Pennsylvania (PA). More than 2,500 miles streams and rivers in PA are impacted by untreated AMD which constitutes the greatest threat to water quality in our state. Untreated AMD discharges are often in impoverished rural settings where MWW is untreated or poorly treated. Even when treated, MWW and AMD are generally addressed with material- and energy-intensive technologies, consuming considerable financial and natural resources. Recent studies have indicated that these waste streams may be passively treated together to affect significant material and energy savings thus offering a more sustainable tool to restore and improve local water resources. However, a careful investigation of the factors that affect the efficiency of AMD and MWW passive co-treatment is required so that this approach can be optimized and implemented to achieve these substantial savings. Regional nonprofit watershed restoration organizations, watershed associations, conservation districts, and governmental agencies are interested to harness this developing technology because it offers a feasible and sustainable method to simultaneously address the two greatest detractors to water quality in their area. This approach is also consistent the increasing expansion of effective local implementation of passive AMD treatment systems by such groups throughout PA and broader Appalachia, because the problem of poor treatment of AMD and MWW persists throughout the Appalachian coal belt. The objectives of this study were to evaluate the role of the key components of the MWW in the co-treatment process and their effect on metal removal and sulfate reduction rates. In particular, co-treatment bioreactors containing MWW with complex and simple carbon substrates were evaluated to assess their pollutant removal rates and efficiencies. In addition, this work investigated the composition and temporal dynamics of the microbial communities involved in the co-treatment process in order to better understand aspects of the microbial ecology that drives this emerging remediation technology. The microbial diversity of AMD-MWW co-treatment bioreactors has not previously been well explored, and the role of particular groups of microbes during the co-treatment process has not been well defined.
**METHODOLOGY**

Clear polyvinyl chloride (PVC), flow-through columns (inner diameter 9.6 cm, height 100 cm) were used as the bioreactors for the mixing of synthetic AMD (sAMD) and real MWW or synthetic MWW (sMWW). These bioreactors were filled with Kaldnes K1 plastic media (porosity of 0.80; Liao and Ødegaard 2002), which is designed to promote microbial biofilm growth. Five sampling ports were evenly spaced along the bioreactor. Water flow was driven by peristaltic pumps, which introduced the MWW and sAMD through the bottom of the bioreactor, operating for 1 minute every hour. Flow rates averaged 29-33 ml/min, with a residence time of approximately 2.1, 3.6, 5.0, 6.4 and 7.9 days for the five sampling ports at a 3:1 - 4:1 mixing ratio of MWW to sAMD. In addition, a theoretical mixed concentration that represented the initial concentration of the constituents upon mixing of the sAMD and MWW within the bioreactor (a retention time of 0) was calculated based on the mixing ratio of these two waste streams, which was tracked using the concentration of chloride (Cl−), a conservative tracer ion.

The sAMD was prepared by dissolving 5.67 g of FeSO₄ in 19 liters of deionized water and adjusting the pH to approximately 2.5 by adding 10.1 ml of trace metal grade 3.49 M H₂SO₄, giving a concentration of approximately 60 mg L⁻¹ Fe²⁺. In addition, the SO₄²⁻ concentration was increased by adding, 2.24g, 8.99g and 22.48g of sodium sulfate after the second, third and fourth sampling dates, respectively to prevent the rate of SO₄²⁻ reduction due to be limited by SO₄²⁻ availability. The simple sMWW was prepared by dissolving 4.51 g of sucrose in 19 liters for a concentration of 100 mg C/L. Sodium carbonate (2.00 g – equivalent to ~100 mg L⁻¹ CaCO₃), potassium phosphate (at a concentration of 7.5 mg L⁻¹ phosphate) and ammonium chloride (at a concentration of 25 mg L⁻¹ ammonium) were also added as well as trace micronutrients (calcium chloride, sodium selenite, magnesium chloride and nickel carbonate) in the required concentrations for SRB (Tsukamoto et al. 2004). The second version of sMWW (named intermediate sMWW) contained the same constituents as above as well as soluble potato starch at a concentration of 50 mg C L⁻¹. The final type of sMWW (named complex sMWW) contained the same constituents as the second version and additionally lauric acid at a concentration of 20 mg C L⁻¹. In addition, primary wastewater obtained weekly from the Loretto Wastewater Treatment plant and sieved through a 150 micron sieve to remove large particles was used as a comparison for the final treatment. This real MWW was diluted with deionized water following measurement of its alkalinity and COD in order to approximate the concentrations of these key constituents in the sMWWs. The sMWWs and real MWW were placed in an ice bath to minimize microbial alteration of the nutrient and carbon content prior to mixing with the sAMD within the bioreactor.

The experiment was run in triplicate for the real MWW and the simple sMWW, and in duplicate for the intermediate and complex sMWW treatments due to leaking and related problems with a bioreactor column from each of these treatments. The bottom four sampling ports of each sMWW bioreactor were injected with 10 ml of a mud slurry from passive AMD treatment pond in order to inoculate these bioreactors with an appropriate microbial community. This represented the start or day 0 of the experiment. The bioreactors with the real MWW were started on day 40 due to logistical delays and were first sampled on day 55 of the experiment. Water samples (approximately 250 ml) were collected in high density polyethylene (HDPE) sample bottles from all 5 ports on each bioreactor on day 5, 27, 41, 55, 69, 82 and 96. Calibrated YSI probes were used to measure the pH, temperature, dissolved oxygen concentration and specific conductance of each of the samples.
Water samples were filtered through 0.22 micron glass fiber filters and collected for dissolved organic carbon (DOC) and were stored in brown amber glass vials at -20°C until analysis on a Shimadzu total organic carbon (TOC) analyzer. Filtered water samples were also collected for analysis of anions (Cl⁻ and SO₄²⁻) on a Dionex ICS-1500 ion chromatograph using standard methods (Rice et al. 2012), stored at 4°C and analyzed within 1-2 days of collection or frozen for later analysis. Filtered water samples were also collected for analysis of Fe and preserved with trace metal grade concentrated nitric acid. These samples were analyzed on a Thermo Scientific flame atomic absorption spectrometer using standard methods (Rice et al. 2012).

Microbial DNA samples were collected on 0.22 micron glass fiber filters from 100 ml of filtered water. The filters were stored in sterile 50 ml centrifuge tubes at -20°C until DNA extraction could be performed. DNA extractions were carried out on one-half of each filters using MOBIO Powerwater DNA extraction kits following the manufacturer instructions. The inoculum sample as well as bioreactor samples on day 29 (55 for the real MWW treatment), 69, 82 and 96 were analyzed for each of the treatments in duplicate or triplicate. The extracted DNA was stored at -20°C until the next-generation sequencing of the V3-V4 region of the microbial 16S rRNA gene was undertaken at the Research and Testing Laboratory (Lubbock, Texas). The details of the high-throughput sequencing process and analysis are described in the Research and Testing data methodology (2014). The Shannon-Weiner diversity index and the EVAR evenness index (Smith and Wilson 1996) were calculated from the operational taxonomic unit (OTU) data for each sample.

PRINCIPAL FINDINGS AND SIGNIFICANCE

Key pollutant removal rates and efficiency

The importance of biological SO₄²⁻ reduction to sulfide has been documented in the treatment of AMD (Sánchez-Andrea et al. 2014; Neculita et al. 2007). It is similarly important in co-treatment, resulting in alkalinity generation, DOC removal, and metal precipitation (Strosnider et al. 2011a). By the end of the experiment, all treatments had effectively equal sulfate removal rates, regardless of carbon substrate. By the last sampling date (day 96), there were no statistically significant differences in SO₄²⁻ removal rates among treatments (Figure 1; one-way ANOVA, as above, $F = 3.15, p = 0.108, df = 9$). All treatments, including the real MWW, were able to achieve rates of SO₄²⁻ removal between 60 mg/ L day⁻¹ and 73 mg/ L day⁻¹. Studies of AMD treatment reporting sulfate reduction at low pH were critically reviewed by Sanchez-Andrea et al. (2014), who found sulfate reduction rates ranging from 0.03 g/L day⁻¹ to 15 g/L day⁻¹. The SO₄²⁻ removal rates of the synthetic treatments are also similar to those documented by Zagury et al. (2006), who saw a maximum reduction rate of 95 mg L⁻¹ day⁻¹ in their experiment involving a variety of complex organic substrates.
Figure 1. Removal rates of sulfate ($\text{SO}_4^{2-}$), dissolved organic carbon (DOC) and iron (Fe) in the four municipal wastewater (MWW) treatments on day. Distinct letters between treatments indicate a statistically significant difference based on one-way ANOVAs and Tukey’s HSD tests at $\alpha < 0.05$.

The intermediate and complex sMWW exhibited greater $\text{SO}_4^{2-}$ removal efficiencies, 71.2% and 80.5% respectively, compared to the simple sMWW (55.5%) and the real MWW (66.5%) treatments on the final sampling date. For all treatments, $\text{SO}_4^{2-}$ removal efficiencies increased temporally. Chang et al. (2000) examined the use of complex organic materials in AMD bioremediation. A minimum $\text{SO}_4^{2-}$ concentration of 200 mg L$^{-1}$ was reached when using sludge from a wastewater recycling plant, corresponding to a removal efficiency of about 92% (Chang et al. 2000). The authors suggested that more easily degradable organic materials could be added to decrease the $\text{SO}_4^{2-}$ concentration further. Although this study did not directly assess MWW, the function of carbon as fuel for SRB is consistent with the current study. The carbon sources used by Chang et al. (2000) were much more cellulosic than the sugar, starch, and fatty acid used in the simplified sMWWs as well as in much greater initial concentrations, but the observed $\text{SO}_4^{2-}$ removal efficiencies were both greater than 50%.

The dissolved organic carbon (DOC) removal rate was not significantly different between the sMWW treatments, which varied between 21 mg/ L day$^{-1}$ and 26 mg/ L day$^{-1}$ on day 82. Overall the sMWW treatments had much higher initial DOC concentrations than the real MWW, where the initial DOC was almost five times lower, resulting in much higher removal rates and efficiencies in the sMWW treatments. DOC increases in the real MWW treatment may have been due to fermentative bacteria that degrade particulate or bulky organic materials, such as partially soluble lipids (Johnson and Hallberg 2005, Chang et al. 2000). The DOC removal rate observed in the complex sMWW treatment (37 mg/ L day$^{-1}$) on the final sampling date was significantly greater than all of the other treatments (Figure 1; one-way ANOVA, as above, $F = 71.62, p < 0.001, df = 9$). The simple and intermediate sMWW treatments had similar rates that were almost 10 times faster than the real MWW, which still had low initial DOC. The highest removal efficiencies also occurred on the last sampling date for the real, simple, intermediate, and complex treatments (36.3%, 79.0%, 68.1%, 69.2%) DOC removal can be linked with SRB...
activity, since organic carbon is necessary for bacterial sulfate removal (Lefticariu et al. 2015). Thus by the end of the experiment, the similar DOC removal efficiencies observed across the sMWW treatments may be attributable to the activity of the fermentative bacteria and the SRB.

Iron removal increased throughout the study, with the highest removal rates and efficiencies seen during the final two sampling events. On the last sampling date, the simple and intermediate sMWW treatments had a significantly higher Fe removal rate than the complex treatment at 4.5 mg L⁻¹ day⁻¹, 4.8 mg L⁻¹ day⁻¹, and 2.5 mg L⁻¹ day⁻¹, respectively (Figure 1; one-way ANOVA, as above, \( F = 5.43, p = 0.038, \text{df} = 9 \)). In the complex treatment, Fe removal accelerated more slowly than in other treatments, with equal Fe removal happening after a longer retention time. The real and intermediate sMWW treatments showed similar removal efficiencies of 97.1% and 97.0% respectively, followed closely by the complex treatment (95.8%) and the simple treatment (89.9%). The final concentrations and removal efficiencies were on par with Zagury et al. (2006), who saw between 93-100% of Fe removed from the treated water. Fe removal was furthermore comparable with Deng et al. (2016), who saw close to 97% Fe removal in their AMD-MWW co-treatment bioreactors. Fe can also be removed through flocculation with phosphate (PO₄²⁻), and it is possible that FePO₄ formed initially (Omoike and Vanloon 1999). However Fe reducing bacteria are capable of reducing this form of Fe under anaerobic conditions. Thus, while FePO₄ may have formed, it would have only been a temporary removal mechanism since both Fe and PO₄²⁻ would have been released into solution again. It is also possible that at a pH greater than 4, ferric Fe precipitated as oxy hydroxides (Younger et al. 2002). Sulfide generation is likely the key removal mechanism responsible for decreasing Fe in the bioreactors through the formation of Fe sulfides (Younger et al. 2002). This could have led to the similarly efficient Fe removal among all of the treatments.

**Microbial Composition and Diversity**

The pH and the type of organic substrate available are two of the most important factors in determining the complexity of microbial communities (Hiibel et al. 2011). A more diverse bacterial community was expected to accompany the increase in carbon source complexity among the sMWW treatments (Strosnider et al. 2011b). The real wastewater treatment had the greatest diversity in terms of number of phyla (14.5), families (105), Shannon-Weiner diversity index (4.11), and evenness (0.23) on day 96 of the experiment. The simple sMWW treatment had the second largest number of phyla and family, with a maximum of 10 phyla and 74 families detected on day 96. Both the intermediate and complex treatment had lower numbers for both of these taxonomic categories. The complex treatment showed an average of 7.5 phyla and 60.5 families on day 96, which was slightly more abundant in both categories compared to the intermediate sMWW. The phyla detected were: *Bacteroidetes, Firmicutes, Proteobacteria, Verrcomicrobia, Acidobacteria, Actinobacteria, Spirochaetes, Chloroflexi, Cynobacteria, Elusimicrobia, Ignavibacteria, Chlorobi, Lentisphaerae, Synergistetes, Deferrribacteres, Fibrobacteres, Fusobacteria, Tenericutes, and Planctomycetes*. With an exception for the intermediate treatment on day 69, the phyla *Bacteroidetes*, *Proteobacteria*, and *Firmicutes* composed greater than 97% of all bacterial OTUs for all treatments.

The *Firmicutes* phyla dominated the simple treatment with 58.3% of the bacterial OTUs coming from this phylum by the last sampling date, while all other synthetic treatments showed less than 8% *Firmicutes*. All synthetic treatments began with an average of 35.3% *Firmicutes*. The real MWW stayed relatively constant in its *Firmicutes* composition, averaging 13.6% between all sampling dates. *Firmicutes* are known to form endospores, possibly explaining why
this phyla would dominate in the treatment with the simplest carbon source, in the case of the simple sMWW (Margosch et al. 2006). The real MWW, which consistently had the lowest DOC concentration, also had a significant Firmicutes population by day 96 at 17.0%. The Clostridium spp. dominated this phyla in all treatments by day 96. Deng et al. (2016) also noted the appearance of clones most similar to the dehalogenating and fermentive Clostridium spp. in their AMD-MWW co-treatment bioreactors. The Clostridia class has been identified as containing avid sugar and protein fermenters, and is often found in bioreactors with simple organics (Kaksonen et al. 2004). Sucrose was the main carbon source for the simple treatment, further explaining the clear Clostridium spp. and Firmicutes phyla dominance in these bioreactors. The Clostridium spp. may have also played a role in supplying SRB with suitable organic substrate to oxidize, in the same manner as the Bacteroides spp. in the more complex treatments.

All treatments except for the complex sMWW showed an increase in the relative abundance of Proteobacteria throughout the experiment. The Proteobacteria have one of the highest number of OTUs per sequence recorded among all of the phyla. This may indicate that they are a key source of diversity to the microbial communities within the co-treatment systems. The real MWW had the highest proportion, with Proteobacteria composing nearly half of the OTUs. By the final sampling date, the Proteobacteria phyla composed 45.2%, 16.2%, 3.9%, and 16.0% respectively of the real, simple, intermediate, and complex treatments (Figure 2). The Epsilonproteobacteria class composed the majority of the Proteobacteria in the real treatment at 25.2%, followed by the Gammaproteobacteria class at 11.4% of the total OTUs by day 96 (Figure 2). Epsilonproteobacteria was less than 0.1% of OTUs in all other treatments on the same date (Figure 2). Gammaproteobacteria was also the most abundant class in the intermediate treatment, but overall this class composed the smallest proportion of the Proteobacteria. The Betaproteobacteria class dominated in the complex treatment at 9.5% of total OTUs on day 96 (Figure 2). Alphaproteobacteria and Betaproteobacteria have been documented as strongly adaptive to more acidic environments, possibly explaining their appearance in the low pH reactors in this study (Kuang et al. 2013). The simple treatment showed similar proportions of the Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria on day 96, ranging from 3.6% to 5.1% of total OTUs (Figure 2). Alphaproteobacteria are broadly distributed in environments affected by AMD and are capable of respiring ferric to ferrous iron by oxidizing reduced carbon compounds (Johnson and Bridge 2002). Deng et al. (2016) found that their co-treatment system was dominated by the Deltaproteobacteria class, with a high portion of genes associated with sulfate reduction. The Deltaproteobacteria class is categorized as a gram negative SRB (Castro et al. 2000). Although this class composed a variable percentage of the OTUs in each treatment, the rate of sulfate reduction was not significantly different among treatments by the end of the study. This may be because organisms from multiple phyla are able to perform similar functions of sulfate and iron reduction, compensating for one another’s absence. Alternatively, the absolute number of microbes from this class may have remained similar although the relative distribution changed due to the varying abundance of other types of bacteria such as the fermentative bacteria.

While the different MWW treatments showed differences in the relative abundance of the dominant phyla, their community structure and diversity provided multiple routes for effective pollutant removal. The simple treatment was dominated by the Firmicutes phyla and still showed rates of pollutant removal on par with other treatments such as the intermediate sMWW, which was strongly dominated by Bacteroidetes (Figure 2). Most notably, SO42− removal rates were not significantly different on day 96, when the real, simple, and final two treatment streams were
strongly dominated by Proteobacteria, Firmicutes, and Bacteroidetes respectively. Microbial community composition, diversity, and evenness appear to be heavily influenced by the carbon sources present in the co-treatment system. The synthetic waters used in this study allowed for better identification of the role of varying complex carbon resources in microbial community development. In the simple treatment, which had sucrose as a carbon source, the Clostridia class, noted sugar fermenters, dominated. In systems with more complex carbons such as the starch in the intermediate and the lipid in the complex treatment, the fermentative Bacteroides spp. dominated. These results seem to indicate that various microbial community structures are able to remove SO$_4^{2-}$ and Fe at effectively the same rate and efficiencies. The microbial ecosystem appears to adapt to take advantage of the available carbon source. Subsequent study of the microbial communities in AMD-MWW co-treatment can explore the use of other qualitative and quantitative sequencing methods to better characterize how microbial community structure in co-treatment bioreactors can change temporally and with variation in AMD and MWW composition.

Conclusions

· The co-treatment of synthetic AMD with synthetic MWW was found to be an effective bioremediation approach, with particularly efficient removal of Fe.
· By the end of the experiment no substantial differences in the removal rates of SO$_4^{2-}$ and Fe were observed among treatments, despite large differences in the complexity of the available carbon sources.
· The carbon substrate complexity appeared to affect the microbial community composition and diversity within the bioreactors.
· Three bacterial phyla (Bacteroidetes, Firmicutes, and Proteobacteria) dominated the relative abundance of bacteria in all treatments, and their distinct distributions between treatments could be explained in terms of the preference of key bacterial representatives within each phyla for particular types of carbon.
· The ability of distinct microbial communities to exhibit equivalent pollutant removal efficiencies suggests that the microbial ecosystem with the bioreactors readily adapts to take advantage of the available carbon resources.
· The flexibility of microbial community composition appears to impart robustness to AMD-MWW co-treatment, which may facilitate the application of this remediation technology in many diverse environments.
Figure 2: The dominant phyla (Bacteroidetes, Firmicutes, Proteobacteria and “other”; left side) and Proteobacteria classes (right side) in the real (A), simple (B), intermediate (C) and complex (D) municipal wastewater (MWW) treatments on day 96 of the experiment.
STUDENTS & POSTDOCS SUPPORTED (name, Department, University, degree) -- of all undergrads, grads, and post-docs who worked on the project

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Stefan Long, Department of Math, Engineering and Computer Science, St. Francis University, B.Sc. in Environmental Engineering
PUBLICATIONS

Marcillo, Cristina E.; Peter M. Smyntek; John E. Fortunato; Walber C. Andrade; Joshua Vinglish; Rachel C. Wagner; Joel Z. Bandstra; William H. Strosnider, In Review, The role of microbial community structure and diversity in the biological co-treatment of acid mine drainage with municipal wastewater, Water Research.


INFORMATION TRANSFER ACTIVITIES


Strosnider William H. J., Brandon K. Winfrey, Rebecca A. M. Peer, Jacob A. McCloskey, Jeff Chastel, Peter M. Smyntek, Robert W. Nairn, 2015, Passive co-treatment of mine drainage with sewage: An approach to save material, energy, and fiscal resources, Environmental Considerations in Energy Production Conference, Pittsburgh, USA.

Smyntek, Peter M., Joel Z. Bandstra, Cristina E. Marcillo, Rachel C. Wagner and William H. J. Strosnider, 2015, Removal and behavior of metal contaminants during passive co-treatment of synthetic acid mine drainage and synthetic municipal wastewater, American Chemical Society Meeting, Denver, USA.

AWARDS & ACHIEVEMENTS

Honor’s Thesis awarded to Cristina E. Marcillo for her research and writing on this project.

Undergraduate Research Grant awarded to Joshua Vinglish for microbial DNA analyses of samples obtained through this project.

Undergraduate Research Grant awarded to Cristina E. Marcillo to extend the microbial DNA analyses of samples generated during this project.
PHOTOS OF PROJECT
Please include 2 graphics or photos with captions, if possible. These may be used in our annual report, web page, and/or brochure, and may be used by the National Institutes of Water Resources.

Acid mine drainage – municipal wastewater co-treatment columns with black iron sulfide precipitates that indicate effective pollutant removal from these waste streams.
The setup and analyses in this co-treatment experiment involved over 30 environmental engineering undergraduate students, and the project was integrated into 3 courses as well as multiple summer research projects.
LITERATURE CITED


Lefticariu, L., E. R. Walters, C. W. Pugh, K. S. Bender, 2015, Sulfate reducing bioreactor dependence on organic substrates for remediation of coal-generated acid mine drainage: Field experiments, Applied Geochemistry, 63, 70–82. doi:10.1016/j.apgeochem.2015.08.002.


Information Transfer Program Introduction

None.
Building Capacity to Assess Harmful Algal Blooms in Surface Waters of Pennsylvania

Basic Information

<table>
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<tr>
<th>Title:</th>
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<td>3/1/2015</td>
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<td>End Date:</td>
<td>2/29/2016</td>
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<tr>
<td>Principal Investigators:</td>
<td>Bryan Reed Swistock</td>
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Publications

There are no publications.
FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)
Monitoring and Education for Harmful Algal Blooms in Pennsylvania Inland Ponds and Lakes

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PROBLEM and RESEARCH OBJECTIVES
Pennsylvania contains thousands of natural and man-made ponds and lakes. Past surveys of the owners of these waterbodies have found that 77% have management problems. A 2003 survey of over 500 pond and lake owners in Pennsylvania found that 52% complained of nuisance levels of aquatic plants and/or algae. While most forms of pond and lake algal growth are harmless, certain types of blue-green algae can produce toxins that can cause injury or death to animals or humans who interact with the water. These blue green algae, such as Microcystis and Anabaena, are actually a type of photosynthetic bacteria called cyanobacteria.
Cyanobacteria blooms can have important implications for the use of pond and lake water during mid to late summer. Over 80 different toxins produced by these bacteria can cause noxious odors, kill aquatic life, produce skin irritation, and cause various gastrointestinal illnesses. These toxins could limit the use of pond water for fishing, swimming, irrigation or animal watering and could also severely degrade the aesthetic appearance of the pond which are each important uses of ponds and lakes in Pennsylvania. While networks and expertise from Pennsylvania SeaGrant and other agencies are already in place to monitor for harmful algae blooms in the Great Lakes and other larger water bodies, inland pond and lake owners would benefit from a network of trained educators who can identify harmful algae and provide information on proper management of harmful algae blooms. Such a network would also provide some data on the occurrence of harmful algae blooms in these previously unstudied waterbodies and changes in algae distributions over time.

The overall goal of this project was to reduce human and animal risks associated with cyanobacteria blooms in inland ponds and lakes across Pennsylvania by providing assistance and education for pond and lake owners. The objectives of the project were:
1. Provide a network of trained educators to help pond and lake owners identify suspicious algae samples and provide data on the occurrence of these problems in Pennsylvania.
2. Create numerous educational methods to extend education about HAB’s to pond and lake owners through webinars, workshops, displays and publications.
METHODOLOGY
The project began with a training workshop for Penn State educators on March 12, 2015, at Penn State University. Presenters at the workshop were:

- Nathan Irwin, Aquatic Biologist, Pennsylvania Department of Environmental Protection. Nate has extensive experience with harmful algal blooms in the Lake Erie watershed.
- Dr. Lisa Murphy, VMD, University of Pennsylvania, Associate Professor of Toxicology, Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine. Dr. Murphy has worked with the effects of harmful algae blooms on animals.

The workshop agenda, shown below, provided four hours of content on harmful algae blooms.

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>10:00am</td>
<td>Introduction to Harmful Algal Blooms (HABS)</td>
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<tr>
<td>10:00-10:45</td>
<td>Speaker: Nate Irwin, HABs in the news, what are HABs, why cyanobacteria matter, bioaccumulation, field conditions that may fuel HABs, seasonal succession, exist everywhere (in general not invasive)</td>
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<tr>
<td>10:45-11:00</td>
<td>Break</td>
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<tr>
<td>11:00-11:30</td>
<td>HABs impacts on Livestock</td>
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<tr>
<td>11:00-11:30</td>
<td>Speaker: Dr. Lisa Murphy, HABs and livestock in the news, symptoms of cyanotoxin exposure, case studies</td>
</tr>
<tr>
<td>11:30-12:00</td>
<td>Livestock Water Supplies</td>
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<tr>
<td>11:30-12:00</td>
<td>Speaker: Nate Irwin, Examples of good and &quot;not so great&quot; water supplies, what makes a good water supply?, water supply maintenance, new water supplies</td>
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<tr>
<td>12:00-1:00pm</td>
<td>Lunch</td>
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<tr>
<td>1:00-1:30</td>
<td>P.A.D.L.S.</td>
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<tr>
<td>1:00-1:30</td>
<td>Speaker: Dr. Lisa Murphy, What is it? What does PADLS do? What samples do they process? In what format should samples be submitted?</td>
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<tr>
<td>1:30-2:00</td>
<td>Identifying Cyanobacteria</td>
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<tr>
<td>1:30-2:00</td>
<td>Speaker: Nate Irwin, Field Tests, look-a-likes, under the scope</td>
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<tr>
<td>2:00-2:15</td>
<td>Break</td>
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<tr>
<td>2:15-3:00</td>
<td>Identifying Cyanobacteria (continued)</td>
</tr>
<tr>
<td>3:00-3:30</td>
<td>HAB Education and Outreach</td>
</tr>
<tr>
<td>3:00-3:30</td>
<td>Speaker: Bryan Swistock, How to advertise our ID services, workshops, grant deadlines, etc.</td>
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Thirteen Penn State Extension educators and water specialists attended the full day workshop. A subset of nine of the attendees received microscopes and other materials to identify algae samples during the summer. The locations of these nine water specialists are shown in Figure 1.
Figure 1. Locations of Penn State Extension water specialists who attended the HAB training and received materials to assist in identification of potentially harmful cyanobacteria.

The nine specialists located in Figure 1 each received a OM139 Infinity Corrected Plan Optics Trinocular Microscope with a Summit SK2-5.2X 5.0MP Digital Microscope Camera for identification of algae species and sharing of sample photos. Attendees also received various identification keys and textbooks to facilitate algae identification including *Freshwater Algae: Identification, Enumeration and Use as Bioindicators* (2015) by Edward Bellinger and David Sigee.

After the training workshop, the nine Extension educators began advertising for regional HAB workshops and for the public and for pond owners to submit water samples for identification of algae. A short fact sheet on HAB’s was created by Pennsylvania SeaGrant in 2015 (available at: https://seagrant.psu.edu/topics/pa-coastal-ecosystems/harmful-algal-blooms/resources) and this fact sheet was utilized by project staff at workshops and exhibits to create awareness about HAB’s in inland ponds and lakes. A statewide webinar was also presented on April 29, 2015 to provide basic education and publicity about the HAB network for pond and lake owners and professionals.

Project personnel organized and presented a total of 17 workshops between June 2015 and February 2016. These workshops ranged from 1 to 3 hours and some included outdoor sessions at ponds and lakes. Attendees to workshops were permitted to bring samples of aquatic algae or plants for identification. A one page data sheet was created to document information about each algae sample submitted to the network. This data was compiled to include the size of each pond, species identified, and location of the waterbody.

All of the workshops were evaluated to determine actions taken by pond and lake owners to monitor and manage for HAB’s and other common pond/lake problems. Attendees received the
PA SeaGrant HAB fact sheet along with several other Penn State Extension publications on pond and lake management.

A set of two 24” tall pull-up displays were designed and created during summer 2015 for use at county fairs, Ag Progress Days, and various other events. The displays were drafted by project staff members and created by staff from the College of Agricultural Sciences publications office. Each of the nine water specialist in Figure 1 received copies of these displays.

![Pull-up displays](image-url)

Figure 2. The two pull-up banners created during this project for use by project staff in educating the public about harmful algae blooms.

Near the conclusion of the project, Penn State Extension collaborated with the Pennsylvania Lake Management Society (PALMS) to convene a half-day HAB session as part of the PALMS annual conference in State College, PA on February 24-25, 2016.

**PRINCIPAL FINDINGS AND SIGNIFICANCE**

As a result of the training of Extension water specialists and the workshops that they organized, a total of 92 algae or aquatic plants samples were submitted as part of the HAB project with 30 samples (33%) being dropped off or mailed to project personnel and 62 (67%) being brought to a workshop or event. The 92 samples came from 62 different waterbodies in 23 Pennsylvania counties and three other states totaling 730.2 acres. Overall, 17 of the 92 samples (18%) contained at least one HAB species capable of producing toxins (Figure 2). The commonly
observed species were *Aphanizomenon* (9% of samples), *Microcystis* (7%), and *Anabaena* (5%). Many samples contained more than one HAB species. The most common aquatic plant was watermeal (*Wolffia spp.*), a plant commonly confused with harmful algae that occurred in 5% of the samples submitted to project staff.

![Figure 2](image.png)

**Figure 2.** Percent of the 88 samples submitted to project staff that were identified as aquatic plants or various types of algae.

Additional findings and outcomes from the project are listed below under the Information Transfer Activities.

**STUDENTS & POSTDOCS SUPPORTED**

The project did not involve training of students or postdocs but 13 Penn State Extension water specialists and educators received training about proper identification and management of harmful algae blooms. These educators will be able to extend their knowledge to farmers and residents who own or live near the thousands of inland ponds and lakes located throughout Pennsylvania.

**PUBLICATIONS**

- A paper entitled *Monitoring and Education for Harmful Algal Blooms in Pennsylvania Inland Ponds and Lakes* has been accepted for oral presentation at the 2016 Association of Natural Resources Extension Professionals Conference in Burlington, Vermont in June 2016.
- We anticipate submitting a manuscript to the *Journal of Extension* in 2016.

**INFORMATION TRANSFER ACTIVITIES**

The project investigators presented results from this study at a variety of Penn State Extension water resources workshops and events across the state including pond&lake workshops and pesticide recertification courses. Details of educational efforts include:
• **Workshops** – Project staff organized and presented 17 workshops across the state which were attended by 432 pond and lake owners or managers representing 3,588 acres of inland waters. Onsite surveys at workshops were completed by 319 pond owners with 316 (99%) indicating that they learned new information and 230 (72%) planning on using information from the workshop to better manage their pond or lake. A follow-up evaluation of 120 attendees several months after the workshops had 46 responses (38% return) and found that 73% of attendees had actually taken action on their pond or lake after attending a workshop.

• **Exhibits** - Another 337 attendees visited the HAB educational display staffed by the Water Resources Extension team at Ag Progress Days in Centre County, PA in August 2015. The displays were also used a variety of other events such as county fairs but exact numbers of visitors to the displays could not be recorded.

• **HAB Conference** – Project staff worked with the Board of Directors of the Pennsylvania Lake Management Society (PALMS) to organize a half-day session and table-top display on HAB’s as part of the PALMS annual conference in State College, PA on February 24-25, 2016. Dr. Lewis Molot from York University in Toronto, Canada provided the keynote presentation followed by a panel discussion among three researchers working on HAB projects. The HAB workshop was attended by 140 pond and lake professionals and lake association members from across Pennsylvania and surrounding states. The program or speakers for the HAB session at the PALMS conference are shown in Figure 3. Past surveys have found that attendees at the PALMS conference each own or manage an average of 15.3 acres of pond and lakes for a total of 2,142 acres of waterbodies in 2016. A follow-up evaluation of attendees at the PALMS conference (n=50) found that 100% learned new information and 88% plan to use this information to take actions to better manage ponds and lakes.

• **Publicity** - A statewide news release was prepared and disseminated in the *Watershed Winds* online newsletter in Spring 2014 and again in Spring 2015. These resulted in numerous newspaper interviews about the project. An additional news release is planned for spring 2016 to summarize results from the project. A statewide webinar was presented in April 2015 on *Harmful Algal Blooms in Pennsylvania*. The live webinar had 84 attendees with another 217 viewing a recorded version of the webinar as of March 10, 2016. The recorded webinar and supporting materials can be viewed at: [http://extension.psu.edu/natural-resources/water/courses/water-resource-webinars/webinars/harmful-algae-blooms](http://extension.psu.edu/natural-resources/water/courses/water-resource-webinars/webinars/harmful-algae-blooms)

• **Overall Impact** – Cumulatively, project activities educated over 1,200 pond/lake owners or managers representing 5,730 acres of inland waterbodies across the state.
PA LAKE MANAGEMENT SOCIETY
2016 CONFERENCE AGENDA

Wednesday – February 24th

8:00 AM  Registration, Continental Breakfast & Visit Exhibits
9:00 AM  Welcome: PALMS President – Lance Bowes, District Manager, Venango Conservation District

Ponds, Lakes and Cyanobacteria Session

9:15 AM  Keynote Speaker – Dr. Lewis Molot, York University – Preventing Cyanobacteria Blooms: Getting Sediment Oxygen and Redox Levels Up (1 credit – NJ, Core)
10:15 AM  Break
10:45 AM  Cyanobacteria Panel Introductions – Jim Clark, Penn State Extension - McKean County
10:50 AM  Nate Irwin, Pennsylvania Department of Environmental Protection – An Introduction to Harmful Algal Blooms (1 credit – PA, NJ)
11:10 AM  Stephen Souza, Princeton Hydro – PARE: A Comprehensive Program for Tracking and Quantifying Harmful Algal Blooms (1 credit – NJ)
11:30 AM  Bryan Swistock, Penn State Extension – Penn State Extension Harmful Algal Bloom Project Results (1 credit – NJ)
11:50 AM  Panel Discussion Q&A
12:15 PM  Lunch (Hotel Atrium)
1:15 PM  Concurrent Sessions
(A) Dr. Lewis Molot, York University – The Impacts of Multiple Stressors (Invasive Mussels, Climate Change and Phosphorus Reductions) on Oxygen Profiles in a Large Lake (1 credit – NJ)
(B) Andrew Zadnick, Western Pennsylvania Conservancy – Management of a Glacial Lake (1 credit – PA, NJ)

1:55 PM  Break
2:15 PM  Concurrent Sessions
(A) Nate Irwin, Pennsylvania Department of Environmental Protection – Local Water Monitoring Response to Harmful Algal Blooms (1 credit – NJ)
(B) Rich Ruby, U.S. Army Corps of Engineers – Demonstration Project to Eradicate Hydrilla in Tonawanda Creek section of the Erie Canal, NY (1 credit – PA, NJ)

2:55 PM  Break
3:10 PM  Concurrent Sessions
(A) Mike Lovegreen, Bradford County Conservation District – Stephen Foster Lake: A Case Study in a Comprehensive Approach to Lake Restoration (1 credit – NJ)
(B) Keith Beamer & Michael Swartz, Pennsylvania Fish and Boat Commission – Channel Catfish Spawning Boxes (1 credit – NJ)

4:00 PM  Hospitality Hour (Conference Lobby)
5:00 PM  Adjourn for the day

THANK YOU TO OUR CYANOBACTERIA SESSION SPONSORS!

[Logos for Pennsylvania Water Resources Research Center and PennState Extension]

Note: PALMS reserves the right to make changes to the agenda.
AWARDS & ACHIEVEMENTS
Penn State Extension’s Water Resources Team received the 2015 Outreach and Education Award from the North American Lake Management Society (NALMS). Ongoing work on this project was included in the award nomination. More information can be found at:

PHOTOS OF PROJECT

Dr. Lewis Molot gives the keynote presentation during the Harmful Algae Bloom workshop at the Pennsylvania Lake Management Society Conference in State College, PA on February 24, 2016 (photo by Jim Clark).

Diane Oleson, a water quality educator with Penn State Extension in York County, teaches youth about harmful algae blooms at Ag Progress Days in August 2015 (photo by Jim Clark). The youth are viewing HAB species through one of the microscopes purchased through this project.
Bryan Swistock, a water resources specialist with Penn State Extension, identifies algae samples at a workshop in Jefferson County during July 2015 (photo by Jim Clark).

Various species of harmful cyanobacteria from a sample submitted during the project from a 40-acre recreational lake (photo by Bryan Swistock).
Amy Galford, a water resources educator with Penn State Extension in Cumberland County, presents a workshop on harmful algae blooms in Lebanon County, PA, on August 25, 2015 (photo by John Bray).

An example of the table-top HAB display at the Pennsylvania Lake Management Society Conference in State College, PA on February 24-25, 2016 (photo by Jim Clark).
# Pennsylvania Water Resources Research Center Education & Outreach Program

## Basic Information

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<th><strong>Title</strong></th>
<th>Pennsylvania Water Resources Research Center Education &amp; Outreach Program</th>
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<td><strong>Principal Investigators</strong></td>
<td>Elizabeth W. Boyer</td>
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## Publications

There are no publications.
FINAL PROJECT REPORT
Pennsylvania Water Resources Research Center

PROJECT TITLE & PRINCIPAL INVESTIGATOR(S)
Pennsylvania Water Resources Research Center Education & Outreach Program
Elizabeth W. Boyer, Department of Ecosystem Science and Management, Penn State University

PROBLEM and RESEARCH OBJECTIVES
Concerns over water resources have been growing in Pennsylvania in recent years, in response to severe droughts and floods, a growing population, increasing demands for water, and the need to understand how changes in land use, climate, and energy extraction (e.g., natural gas) affect water quantity and quality. The Pennsylvania Water Resources Research Center sponsored conferences, symposia, and outreach activities that promote dialog of important water resources issues in Pennsylvania and beyond. By supporting conferences and symposia, we facilitate dialog about water issues of importance in Pennsylvania; encourage synthesis of data and results; enable networking; stimulate research collaborations, and provide support to early career researchers. By offering educational outreach programs, we widely disseminate information about water issues, and teach graduate students how to communicate water science and research to a broad audience.

METHODOLOGY
The Pennsylvania Water Resources Research Center (PA-WRRC) will help to plan, co-sponsor, and participate in conferences, seminars, and other outreach activities related to water resources issues in Pennsylvania. Goals of these activities are to:

• Discuss water issues of importance to Pennsylvania. We aim to bring together diverse audiences to discuss water issues of regional importance. We will encourage dialog and discussion among stakeholders, involving academia, state and local agencies, utilities, non-governmental organizations, consultants, and more. This will foster collaboration and cooperation toward articulating water challenges, common goals, and potential solutions. Further, we aim to discuss the role of research in advancing understanding of important water resources issues in Pennsylvania. This will encourage synthesis of data, information, and results.

• Enable networking. We recognize that networking among researchers, educators, and stakeholders is one key to both professional growth and to impactful science. The conferences provide mechanisms for professional networking for all participants through formal activities (e.g., introductions, panel discussions and breakout groups, and the oral/poster presentation sessions), and informal activities (ample time for discussions to unfold during breaks, meals, and social activities).

• Mentor early career academic researchers. We aim to facilitate strong participation of early career researchers (including assistant professors, post-doctoral associates, and graduate students), inviting them to participate in the activities, and providing awards to offset their travel and registration costs.
PRINCIPAL FINDINGS AND SIGNIFICANCE

Conference Sponsorship. In FY2015, the Pennsylvania Water Resources Research Center co-sponsored the following conferences:

1. **2015 Water Symposium at Penn State.** In celebration of Earth Day, Penn State Institutes of Energy and the Environment along with the Pennsylvania Water Resources Research Center sponsored this symposium at Penn State on April 22, 2015. The symposium featured invited speakers, poster presentations, and artwork on all aspects of water resources research, education, and outreach. More information is available at: [http://news.psu.edu/story/348895/2015/03/23/research/water-symposium-make-splash-hub-earth-day](http://news.psu.edu/story/348895/2015/03/23/research/water-symposium-make-splash-hub-earth-day)

2. **2015 Pennsylvania Groundwater Symposium: An Unconventional Look at PA Groundwater.** In celebration of National Drinking Water Week, Penn State Extension and the Pennsylvania Department of Environmental Protection along with PA-WRRC and other sponsors offered this annual symposium in State College, PA on May 6, 2015. This provided a forum for researchers, students, professionals and educators to exchange information and promote protection of groundwater resources throughout the state. More information about the symposium is featured here: [http://extension.psu.edu/natural-resources/water/news/2015/groundwater-professionals-gather-for-the-2015-pennsylvania-groundwater-symposium](http://extension.psu.edu/natural-resources/water/news/2015/groundwater-professionals-gather-for-the-2015-pennsylvania-groundwater-symposium)

3. **2015 Annual Symposium of the American Water Resources Association, Pennsylvania State Section: Planning for Water Quality Improvements on a Watershed Basis.** PA-AWRA, PA-WRRC, and other sponsors offered this symposium on October 9, 2015. The agenda from the meeting is available at: [http://state.awra.org/pennsylvania/conference/annual_conf.htm](http://state.awra.org/pennsylvania/conference/annual_conf.htm)
• **2016 Pennsylvania Lake Management Society Conference.** PALMS along with PA-WRRC and other co-sponsors offered this event in State College, PA in February 2016. The research theme of the PA-WRRC co-sponsorship was Harmful Algae. There were 143 attendees. More information about the symposium can be found here: [http://www.palakes.org/news-and-events/conference-wrap-up/](http://www.palakes.org/news-and-events/conference-wrap-up/)


**Research Seminars**

**Series on Emerging Contaminants.** In FY15, PA-WRRC Director Boyer served as a coPI (along with PI Heather Gall and others) on a grant from Penn State’s College of Agricultural Sciences to support a campus wide seminar series on emerging contaminants. The series was sponsored by the Agriculture and Environment Center. Links to recordings of most of the seminars are available at: [http://agsci.psu.edu/aec/research-extension/aec-webinars-and-events/aec-webinars/science-for-solutions-seminar-series-1/emerging-contaminants-series](http://agsci.psu.edu/aec/research-extension/aec-webinars-and-events/aec-webinars/science-for-solutions-seminar-series-1/emerging-contaminants-series). Seminars during FY15 are listed below.

• **March 19, 2015**
  1:00pm-2:00pm, 244 Ag Engineering Building
  Dr. Linda Lee and Dr. Michael Mashtare, Purdue University: Source and Remediation Potential of Perfluoroalkyl Acids

• **March 20, 2015**
  12:20pm-1:10pm, 104 Forest Resources Building
  Dr. Maria S. Sepulveda, Purdue University: Environmental Hormones: Can they Elicit Changes in Sex Ratios in Fish Populations?

• **March 27, 2015**
  12:20pm-1:10pm, 106 Forest Resources Building
  Edward Topp, University of Western Ontario: Mitigating the Spread of Antibiotic Resistance: Managing Human Exposure to Antibiotic Resistance Genes Enriched in Food Production Systems

• **April 2, 2015**
  3:30-4:30pm, 217 Forest Resources Building
  Clinton Williams, United States Department of Agriculture, Agricultural Research Service: Reclaimed Water and the Fate of Pharmaceuticals in the Environment.
• April 3, 2015
  12:20-1:10pm, 106 Forest Resources Building
  Alan Kolok, University of Omaha: Endocrine Disrupting Compounds in Agriculturally
  Intensive Landscapes: Muddy Waters.

• April 16, 2015
  3:30-4:30pm, 217 Forest Resources Building
  Scott Yates, United States Department of Agriculture: Protecting Agro-Ecosystems by
  Controlling Emissions of Pesticides.

• April 17, 2015
  11:15am-12:05pm, 106 Forest Resources Building
  Dana Kolpin, Research Hydrologist, U.S. Geological Survey, Iowa City: Contaminants of
  Emerging Concern: New Environmental Challenges.

• April 24, 2015
  2:30-3:30pm, 244 Agricultural Engineering Building
  Edward P. Kolodziej, Associate Professor, University of Washington: Addressing the
  Challenges of Agricultural Pharmaceuticals and Bioactive Contaminants in Aquatic
  Systems.

Special Seminar

• September 4, 2015
  2:00-3:00pm, 215 Armsby Building
  Gregory W. Characklis, Professor, Dept. of Environmental Sciences & Engineering; and
  Director, Center for Watershed Science & Management, Institute for the Environment,
  University of North Carolina at Chapel Hill. Integrating engineering, economics and
  finance to manage the risks of hydrologic variability.

Outreach Activities.

• PA-WRRC provided a scientific briefing on Shale Gas Exploration Potential Impacts on
  Water Resources to Congressional Representative Glenn Thompson and one of his staff
  members (April 1, 2015; 431 Forest Resources Building, Penn State University). Topics
  included:
  • Elizabeth Boyer, Director, Pennsylvania Water Resources Research Center. Overview of
    shale gas research, education, and outreach at Penn State.
  • David Yoxtheimer, Penn State Marcellus Center for Outreach and Research. Water
    management, and stats on the latest use/reuse/disposal practices used by the industry to
    minimize risks to water quality.
  • Susan Brantley, Director, Earth & Environmental Systems Institute. Overview of the
    Shale network Research Coordination Network, and recent methane & violations data in
    Pennsylvania.
• Bryan Swistock: Penn State Extension shale gas activities. Water extension team’s research and outreach activities.
• Tom Murphy: Director, Marcellus Center for Outreach and Research. Efforts underway to convey related information constituents in Pennsylvania and beyond.

• PA-WRRC Director Elizabeth Boyer served as an invited Panelist in a session on Science and Policy, as part of the Cornell University, Biogeochemistry, Environmental Science, and Sustainability Fall Retreat, Ithaca, NY, September 2015.

• PA-WRRC Director Elizabeth Boyer along with several other PSIEE staff (Jenni Evans, Lara Fowler, and Patty Hickman, and Tom Richard) are involved in search committees for the following new water-related positions at Penn State: 1) Open Rank; Director of the Water Institute and Faculty Position. View the job announcement at: https://psu.jobs/job/57767; PSIEE interim Director Jenni Evans, Chair). 2) Assistant Professor, Hydrology Faculty Position, Department of Ecosystem Science and Management and Penn State Institutes of Energy & the Environment View the job announcement at: https://psu.jobs/job/58596; PA-WRRC Director Elizabeth Boyer, Chair.

• PSIEE was involved in aspects of the Nile Project visit to Penn State in April 2015. The Nile Project uses music to raise awareness of the cultural and environmental challenge along the Nile River of Africa. More information about this cultural event is found here: http://news.psu.edu/story/352368/2015/04/09/arts-and-entertainment/nile-project-bring-east-african-music-cooperation

• PSIEE sponsored a research mixer on February 4, 2016. This event brought together Penn State researchers interested in water and biogeochemical cycles, for informal discussion and networking.

Service to the Scientific Community by PA-WRRC Director Elizabeth Boyer
• Pennsylvania Sea Grant External Advisory Council
• American Water Resources Association, Pennsylvania State Chapter, Board of Directors
• Lead University representative to Universities Council on Water Resources.
• National Atmospheric Deposition Program Technical Committee
• Spring Creek Water Resources Monitoring Project Advisory Committee.
• U.S. Environmental Protection Agency, Hydraulic Fracturing Research Advisory Panel.
• U.S. Environmental Protection Agency, Clean Air Scientific Advisory Committee, Secondary National Ambient Air Quality Standards Review Panel for NOx and SOx.
• American Geophysical Union, Hydrology Section, Water & Society Technical Committee
• Hydrological Processes, an international journal, Associate Editor

STUDENTS SUPPORTED
Lidiia Iavorivska, PhD student, Department of Ecosystem Science and Management, Penn State University
PUBLICATIONS


PRESENTATIONS

- Buda AR, SS Tzilkowski, LC Kibet, RB Bryant, EW Boyer, AL Allen, PJ Kleinman, and EB May. Terrestrial sources of urea to water in a mixed land use watershed: exploring the roles of current and past nitrogen management. Joint Aquatic Sciences Meeting, Portland, OR, May 2014.


- Eklöf, K., Boyer, EW., Drohan, P. and Iavorivska, L. Will refilling a northern Appalachian impoundment cause extensive methylation? International Union of Forest Research Organizations World congress, 5-11 October 2014, Salt Lake City, UT, USA.


- (Invited) Boyer EW. Atmospheric Mercury Deposition and Accumulation in Mid-Appalachian Catchments. Cornell University, Biogeochemistry and Environmental Science and Sustainability Seminar Series, February 27, 2015.
USGS Summer Intern Program

Basic Information

| Start Date: | 3/1/2015 |
| End Date:   | 2/29/2016 |
| Sponsor:    | USGS     |
| Mentors:    | Students: Erika Levy |

Internship Evaluation

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<td>Utilization of your knowledge and experience</td>
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Additional Remarks

Erika worked as the USGS PA-WRRC intern at Regional Science Consortium at the Tom Ridge Environmental Center in Erie, PA during the summer of 2015. She was responsible for using the Virtual Beach software developed by the USGS to predict swimming advisories at six zones located on Presque Isle State Park. Each zone was based on a specific number of parameters including turbidity, wave height, wind speed and many more which were used within the model software. The software then gave threshold values which, if exceeded, predicted poor water quality in that zone. The threshold exceedance values were used to determine whether smaller amounts of water samples from each beach should be plated in order to achieve more accurate counts. (the high the number of coliforms, the more difficult it is to count them on the plate) Erika was also responsible for working directly with USGS team members working on the Lake Erie Time of Travel Study. During this study, she coordinated with members of the Pennsylvania Health Department to send teams to each of 25 waypoints around Lake Erie. Erika was then responsible for the plating of each waypoint water sample with help from the Regional Science Consortium interns. She reported the plate counts to USGS and cataloged all data taken at each waypoint Finally, Erika constructed a master spreadsheet of all summer 2015 data collected at each zone on Presque Isle. This master spreadsheet included ancillary data collected from the field, weather station data from a weather tower located on Presque Isle, E.coli plate counts, and much more. Erika also assisted in water collection and sample processing as part of the Regional Science Consortium intern team in 2015.