Erosion, Sediment, and Turbidity Control and Monitoring Research to Meet Water Quality Goals

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Principal Investigators

Richard A. McLaughlin, Ph.D.
Professor
Department of Crop and Soil Sciences
And
W. Gregory Cope, Ph.D.
William Neal Reynolds Professor
Department of Applied Ecology

North Carolina State University

Raleigh, North Carolina

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16. Abstract

Construction sites usually create large areas of exposed soil which can produce runoff containing high sediment concentrations and turbidity. While standard practices can improve water quality, the use of surface outlets and chemical treatment can further reduce the discharged water turbidity by an order of magnitude or more. This project explored a variety of approaches to both measuring water quality and improving it, as well as the potential impacts to freshwater mussels from of construction site runoff. Four different surface skimmer outlet devices were tested under controlled, full-scale conditions to determine discharge rates as affected by orifice size and water depth. Water quality determination by up to three different sampling methods and two recording meters were also compared for turbidity values. A portable rainfall simulator capable of producing 2-3" hr^{-1} rainfall over a large (10' x 20') area was constructed for erosion testing. Two different dissolved flocculant dosers were constructed to have dosing controlled by either rainfall or runoff flow rates, and these were tested on three construction projects. Finally, a range of polyacrylamides (PAM) with different properties were tested for toxicity to three freshwater mussel species. Further testing of the toxicity of settled sediment, PAM-flocculated sediment, and suspended sediment was performed over two time periods for juvenile mussels. Three of the four skimmers had relatively linear discharges as water levels dropped from 5' to 1' in the test basin, with a fourth having steadily declining discharge rates. The discharge rates determined in this study were often different than those provided by the manufacturer, but test conditions were not necessarily the same. Turbidity determination by sampling or by recording probe often produced different values but usually similar trends, and the two recording probes provided similar values. The rainfall simulator produced droplets similar to those reported for other simulators and the rainfall distribution was highly influenced by wind speed, since no shielding was used. However, 2-3" h^{-1} occurred in most of the plot area. The two different dissolved flocculant dosers had success in reducing turbidity primarily during moderate events, but during high-flow and –sediment events there was turbidity reduction but turbidity remained high. These would have to be scaled up to treat those events. The mussels appeared to tolerate the PAM at concentrations >10X the targeted treatment level, and suspended sediment stressed the juvenile mussels more than settled or PAM-flocculated sediment. This suggests reducing turbidity with PAM would also reduce impacts on mussels in receiving waters.

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Executive Summary

Since surface outlets have been required by the United States Environmental Protection Agency, a number of devices have come on the market to “skim” the water from near the surface of sediment basins. Four of these were tested for discharge rate in a full-scale test basin, with three having a fairly constant discharge but a fourth having steadily declining discharge as the water depth decreased. The measured discharge rate was often different than the manufacturer stated, but testing conditions may have a large influence on discharge rates. Measurements of turbidity were compared between various types of sampling and continuous recording sensors (sondes) and were found to be different at times and similar at others. Sondes have the advantages of collecting more time points and eliminating the need to analyze a sample after collection, but there is also no way to explain data which might appear to be in error (e.g. outliers). A portable rainfall simulator was devised which can be used in the field for evaluating erosion control on large

areas (10’ x 20’). The droplet size distribution is similar to published values for simulators and it can produce 2” – 3” hr^{-1}.

Polyacrylamide (PAM) has become an effective tool for reducing construction related suspended sediment and turbidity, which are considered to have significant adverse impacts on aquatic ecosystems and are a leading cause of the degradation of North American streams and rivers. The most common approach to using PAM to reduce turbidity is to place the dry, granular form on check dams and in water conveyances upstream of an area for settling the resulting flocs. An alternative using dissolved PAM dispensed proportional to rainfall or flow, using only gravity, was demonstrated to work well within a certain range of conditions. High flows and turbidity can overwhelm this system, however. Prior to this research, no information existed on the toxicity of PAM compounds to native freshwater mussels (Family Unionidae), one of the most imperiled faunal groups globally. Following standard test guidelines, we exposed juveniles mussels (test duration 96-h) and glochidia larvae (test duration 24-h) to 5 different anionic PAM compounds and 1 non-ionic compound. Species tested included the Yellow Lampmussel (*Lampsilis cariosa*), an Atlantic Slope species that is listed as endangered in North Carolina, the Appalachian Elktoe (*Alasmidonta raveneliana*), a federally endangered Interior Basin species, and the Washboard (*Megalonaias nervosa*), a common Interior Basin species. We found that median lethal concentrations (LC50) of PAM ranged from 411.7 to > 1000 mg/L for glochidia and from 128.7 to > 1000 mg/L for juveniles. All LC50s were orders of magnitude greater (2–3) than concentrations typically recommended for turbidity control (1–5 mg/L), regardless of their molecular weight or charge density. Our results demonstrate that the PAM compounds tested were not acutely toxic to the mussel species and life stages tested, indicating minimal risk of short-term exposure from PAM applications in the environment. We also conducted acute (96 h) and chronic (20 d) laboratory tests with juvenile fatmucket (*Lampsilis siliquoidea*) and three exposure conditions (non-flocculated settled sediment, suspended sediment, and PAM-flocculated settled sediment), over a range of environmentally relevant turbidity treatments (50, 250, 1,250, and 3,500 nephelometric turbidity units; NTU). We found no effect of turbidity treatment or exposure condition on mussel survival in either the acute or chronic tests, suggesting a high level of tolerance for *L. siliquoidea* in short-term exposures. In contrast, we found significant reductions in protein concentration, ATP production, and oxidized proteins in mussels acutely exposed to suspended sediment, indicating physiological protective responses that limit energy production and reactive oxygen species accumulation under unfavorable environmental conditions. Our results suggest that anionic PAM applied to reduce suspended sediment may be effective at minimizing the adverse effects of short-term turbidity exposure on juvenile freshwater mussels without eliciting additional lethal or sub-lethal toxicity. Altogether, our findings should facilitate improved management and regulatory decision making for turbidity control best management practices in waters where freshwater mussels reside.

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Introduction and Literature Review

A proposed regulation issued by USEPA (2009), entitled the Effluent Limit Guidelines (ELG), mandated that the turbidity of water released from construction sites not to exceed 280 nephelometric turbidity units (NTU). This proposal was challenged in court and was rescinded, but it suggested that regulatory agencies were considering the impact of construction site stormwater on receiving waters. The current standard for North Carolina ranges from 10 – 50 NTU, depending on the receiving water, but this is generally ignored and instead evidence of increasing turbidity in receiving waters is the enforcement trigger. Sediment deposited beyond construction site boundaries are also considered a violation.

Our recent research has shown that improved sediment control methods can provide an economically viable strategy to reduce sediment discharges by increasing the retention capacity of the sediment traps to over 90% (McCaleb and McLaughlin, 2008; Thaxton et al., 2004; Thaxton and McLaughlin, 2005). This can be greatly improved through the addition of polyacrylamide to flocculate small particles, greatly reducing the settling time required for the deposition of the silt to clay size fraction of soils (McLaughlin and Bartholomew, 2007; Bhardwaj and McLaughlin, 2008; Bhardwaj et al., 2008; McLaughlin et al., 2009a,b). In addition, PAM can reduce soil erosion when applied with straw to levels similar to more expensive erosion control blankets (Babcock and McLaughlin, 2011). The great potential for PAM to reduce erosion at a relatively low cost suggests that it will be part of an integrated approach to meet the ELG.

Freshwater mussels (family Unionidae) are suspension- and deposit-feeding, aquatic organisms that live burrowed in sediments of streams and rivers. Unfortunately, they are one of the most rapidly declining faunal groups in the North America. About 70% of the nearly 300 freshwater mussel species found in the North America are considered vulnerable to extinction or already extinct (Bogan 1993; Williams et al. 1993). The decline of mussel populations in North America has occurred steadily since the mid 1800s and has been attributed to an array of factors associated with pollution and water quality degradation and habitat destruction and alteration (Strayer et al. 2004; Cope et al. 2008).

Native freshwater mussels have a unique life history and reproductive strategy (McMahon and Bogan 2001) that makes them susceptible to physical and chemical stressors in the water column and in aquatic sediments. Specifically, male mussels release sperm directly into the water that is then taken up through the siphoning action of the females. The eggs are fertilized inside the female and the embryos develop into larvae called glochidia in specialized pouches (marsupia) of the gills. Once fully developed, the female expels the glochidia into the water where they must attach to the fins or gills of an appropriate fish species to complete their metamorphosis into juvenile mussels. The glochidia develop on the fish for about 14 to 40 days and then fall to the bottom sediment where they burrow and reside as adults.

Recent research conducted in our laboratory and in several other laboratories from around the United States has led to the development and publication of a standard guideline for conducting toxicity tests with early life stages of freshwater mussels (ASTM 2006). This guideline has provided the foundation for the generation of consistent and robust toxicity information for early life stages of freshwater mussels that is now being used by the U.S. Environmental Protection Agency in the development or revision of National Water Quality Criteria. Specifically, our research with the method has contributed to much needed information on the acute and chronic toxicity of pesticides, metals, ions, ammonia, and temperature (Pandolfo et al. 2012, 2010a,b; Mosher et al. 2012; Bringolf et al. 2007a,b,c; Augspurger et al. 2003); however, data gaps for other important environmental stressors such as turbidty, suspended sediment, and the chemical compounds used to flocculate and remove sediment during erosion control practices such as polyacrylamide (PAM) remain unaddressed. This research will seek to fill those data gaps and aid in improved management and regulatory decision making.

IMPROVING WATER QUALITY FOR CONSTRUCTION SITE DISCHARGES

Task 1. Identifying cost-effective monitoring procedures and equipment to characterize turbidity levels in basin discharges and receiving waters.

The traditional method of monitoring storm water is to install an automatic sampler at an outlet of interest. This is typically programmed to obtain samples periodically based on flow (flow-paced or –weighted sampling), with the samples collected after each storm and analyzed in a laboratory. Another approach to sampling is to use stage samplers, which obtain a single sample once the water in the stream or pond has risen to a certain level. These are often called “bottles on a stick” because the sampling bottles are placed at different levels on a post in the water body to obtain samples on the rising limb of an event. An alternative is to use a sensor placed on a recording probe, often called a sonde when multiple parameters are measured, to directly measure the parameters in the water body. One of the problems with this for turbidity in sediment-rich waters is that the sensor can become clogged and either provide erroneous data or stop working altogether. This would not be known until the data is downloaded, so no data would be available for that event. In addition, there are no sensor for suspended sediment, so a relationship between turbidity and suspended sediment has to be established. It should be noted that automatic samplers often malfunction and do not obtain samples, as well. The water sampling equipment tested in this project are listed in Table 1 and the two sondes are shown in Figure 1.

Table 1. Sampling equipment tested for comparison of water quality values.

Sampler Type	Brand	Parameters	Approximate Cost
Automatic sampler	Isco	Flow-paced sampling	$5,000
Stage Sampler		Rising stage single samples	$50
Sonde	Manta 2 (Eureka)	Turbidity, temperature, level	$5,000
Sonde	Hydrolab MS5 Water Quality Multiprobe (Hach Environmental)	Turbidity, temperature, level	$5,000

Figure 1. The two sondes tested during this project: Eureka Manta 2 (top) and Hach Hydrolab MS 5 (bottom).

Task 2. Evaluating methods to achieve surface dewatering requirements.

Sediment basins are required to dewater from the surface and there are a number of commercial devices which are available to achieve this goal while also dewatering the basin after flow to it has stopped. These are listed in Table 2 and are shown at various stages of testing in Figures 2-5.

Table 2. Skimmers tested in our model sediment basin.

Skimmer	Outlet Type	Cost
Faircloth (Figure 1.2)	Screened opening suspended 10-12 cm below surface	2”: $545 4”: $1,290
Erosion Supply (Figure 1.3)	4 screened openings suspended below surface	3”: $625
Prodrain 70 (Figure 1.4)	Slots along pipe with an adjustable cover	
Marlee Model 1 (Figure 1.5)	Weighted ring with water entering from below	1 – 2.5”: $695

Figure 2. Faircloth skimmer at the beginning of a test.

Figure 3**.** Erosion Supply skimmer.

Figure 4. ProDrain-70 skimmer.

Figure 5. Marlee Model 1 skimmer.

Task 3. Developing a portable rainfall simulator capability and evaluate ground covers for erosion and vegetation establishment on construction sites.

Natural rainfall distribution has been shown to be the key element in establishing vegetation and the success or failure of erosion control products. We demonstrated that in several previous projects, where the success in establishing vegetation and preventing erosion was highly correlated with the timing, amount, and intensity of rainfall events. When evaluating erosion control products in the field, depending on natural events therefore introduces uncertainty about how they perform under heavy rainfall, since that may not occur during the evaluation period. Therefore, we wanted to develop a portable rainfall simulator with which we can test erosion control products in the field under a known rainfall amount and intensity.

There are a number of rainfall simulator designs and an ASTM standard design. In order to make a portable simulator which could test a relatively large area, we modified the ASTM design to include single nozzles mounted on 4 m PVC pipe and directed upward (Figures 6-7). In order to maintain the appropriate pressure for the desired droplet size distribution, pressure gauges were included at the bottom of each riser (Figure 8) and adjusted for the height of the water in the riser pipe. Each riser was supported by a steel pipe installed adjacent to the PVC pipe (Figure 9). To make the system portable, a 250 gallon tank was the reservoir and the water was delivered by a 2” gasoline pump (Figure 10). The reservoir capacity could be increased using larger tanks or water trucks using the same pump.

Figure 6. Constructed rainfall simulator in operation at SECREF.

Figure 7. Closeup view of droplet distribution from one nozzle.

Figure 8. View of the cutoff valve and pressure gauge for one of the nozzles.

Figure 9. Support pipes inserted flush with the ground so when the simulator nozzle system is removed the grass can be mowed. Metal pipe serves as addition support for the PVC pipe.

Figure 10. View of the rainfall distribution for the simulator. Pump and reservoir tank are shown beyond the simulator.

Task 4. Testing new products and approaches for reducing turbidity on active construction sites.

The main focus of this task was to try different systems for introducing PAM into stormwater flows on construction sites. Our system was based on the rain-driven, liquid-doser developed in New Zealand (Figure 11). We reduced the volume of the flocculant tank to approximately 110 L (30 gal) and used steel tubing for the roof support system to make it more portable (Figure 12). Two people can carry an assembled unit, or it can be broken down so that one person can install it. A second dosing system was devised using a simple float valve system behind a square-notch weir (Figure 13). This allows for the solution to be dispensed fairly proportionately to flow.

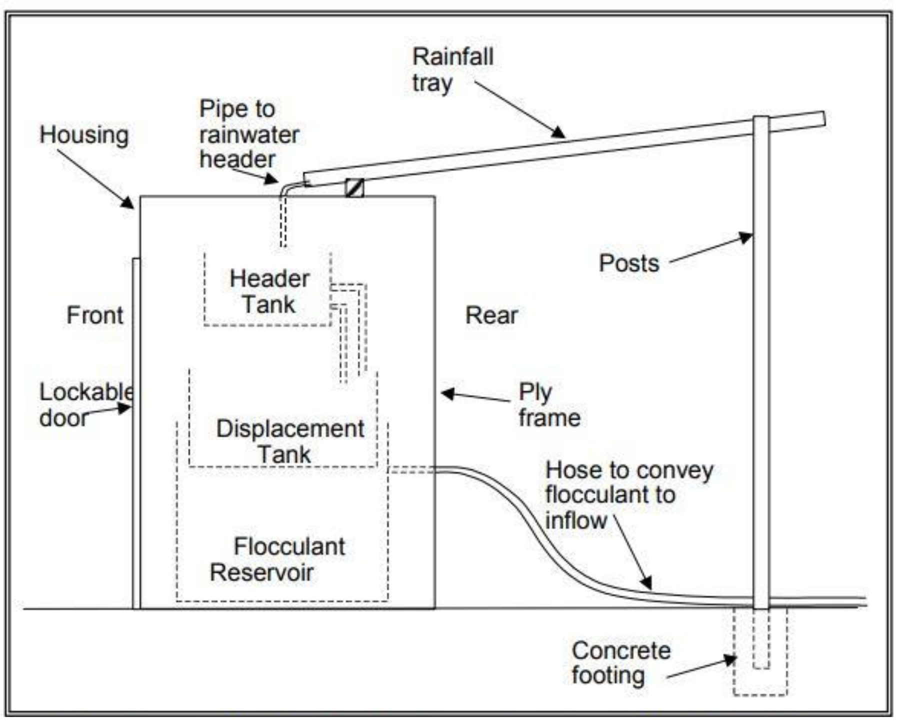

Figure 11. Picture of the New Zealand doser (top) and diagram of the dosing system (bottom).

Figure 12. Modified New Zealand dosing system deployed in the field. The system is dosing a slope drain prior to a small sediment basin.

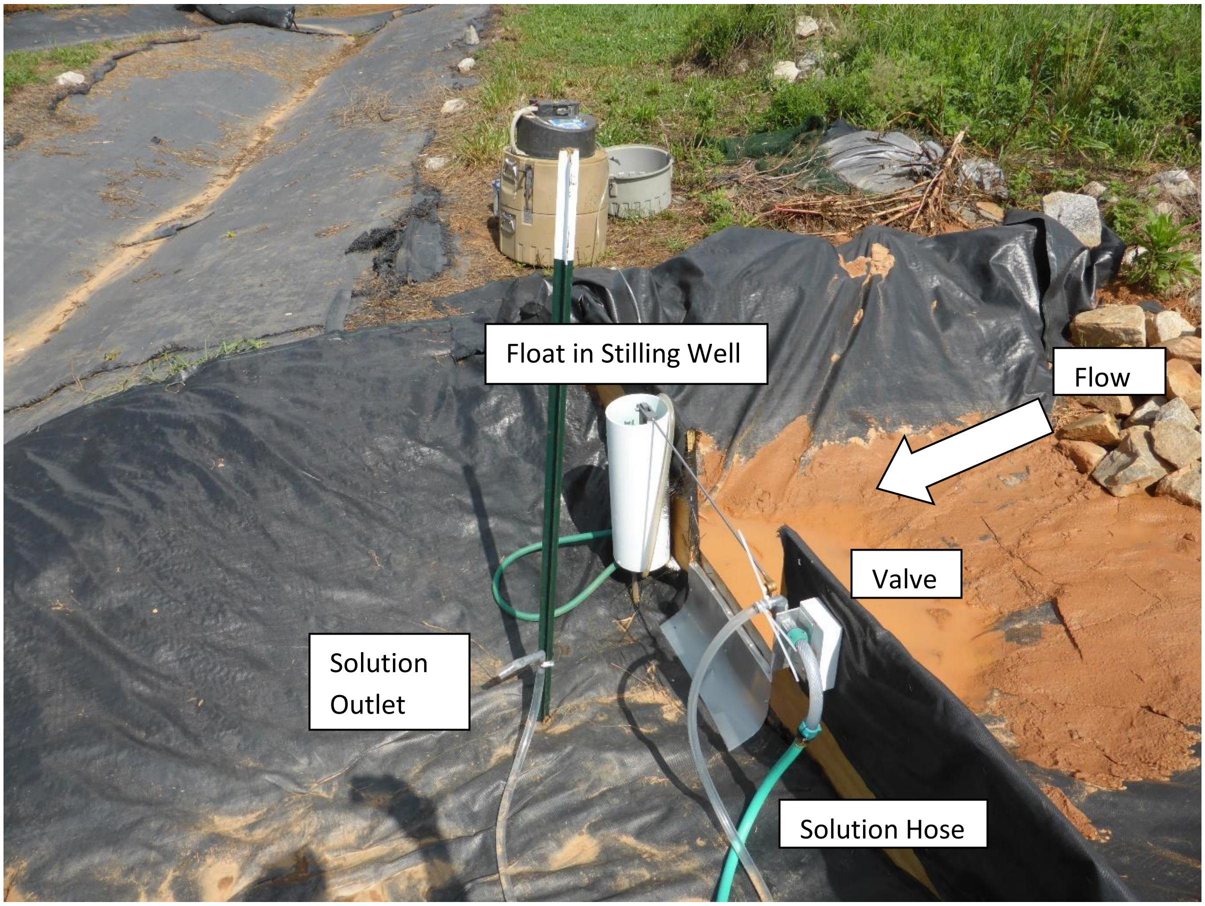

Figure 13. Float valve system for dispensing PAM solutions. Float is in PVC stilling well on left, valve on right with green hose coming from the source tank. PAM is released into clear hose on fence post in the middle of the channel below the weir.

RESULTS AND DISCUSSION

Task 1. Cost-Effective Monitoring of Turbidity

There were three water sampling methods tested including the automatic sampler (ISCO), which samples based on time or flow, the stage sampler, which simply takes a sample as the water level rises, and the grab sample. In all three cases the samples need to be analyzed for turbidity either in the field or in the laboratory. We also tested two water quality sondes, the Eureka Manta 2 and the Hach Hydrolab 5. These have the capability of logging turbidity, along with many other variables, in the water based on time intervals set by the user.

Data collected under controlled conditions indicated that the Eureka sonde produced somewhat lower turbidity levels compared to the three sampling methods (Figure 14). Testing at much lower turbidity levels, however, with the Eureka sonde and lab analysis of sampled water mostly similar, and the Hach sonde consistently lower (Figure 15). Recent testing in an actual skimmer basin (Figure 16) also had the Eureka readings mostly higher than the Hach unit (Figure 17). Both units had some anomalies during that testing. The Hach sonde responded to a rain event with higher turbidity, as expected, but the Eureka did not. Both units had some data points above the curve even when no rain event occurred, which could have been instrument error or something like a tadpole kicking up some sediment. This suggests that data would need to be scrutinized for anomalies and those points removed in order to get a true assessment of turbidity.

The change in turbidity with a rising water level in a skimmer basin at the Durham Connector site is demonstrated in Figure 18. The watershed draining to this basin is relatively undisturbed and vegetated, and the diversion ditches are lined to prevent erosion, so the turbidity levels are very low compared to others at this site (see below). The highest temperature recorded was 41^{o}C, or 105^{o}F, which is likely higher than mosquitoes can tolerate (Marinho et al., 2016) and this may partially explain why they are not a problem around sediment basins.

Marinho, R. A., E. B. Beserra, M. A. Bezerra-Gusmão, V. de S. Porto, R. A. Olinda, and C. A. C. dos Santos. 2016. Effects of temperature on the life cycle, expansion, and dispersion of Aedes aegypti (Diptera: Culicidae) in three cities in Paraiba, Brazil. J. Vector Ecology 41(1): 1-10.

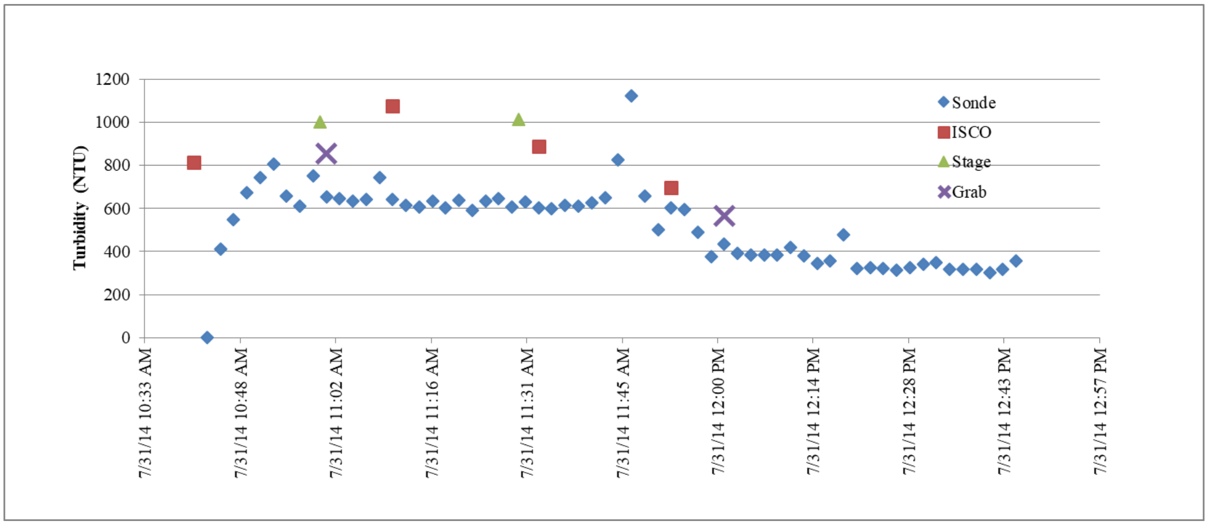

Figure 14. Turbidity measurements in a basin at SECREF using four different methods of obtaining data.

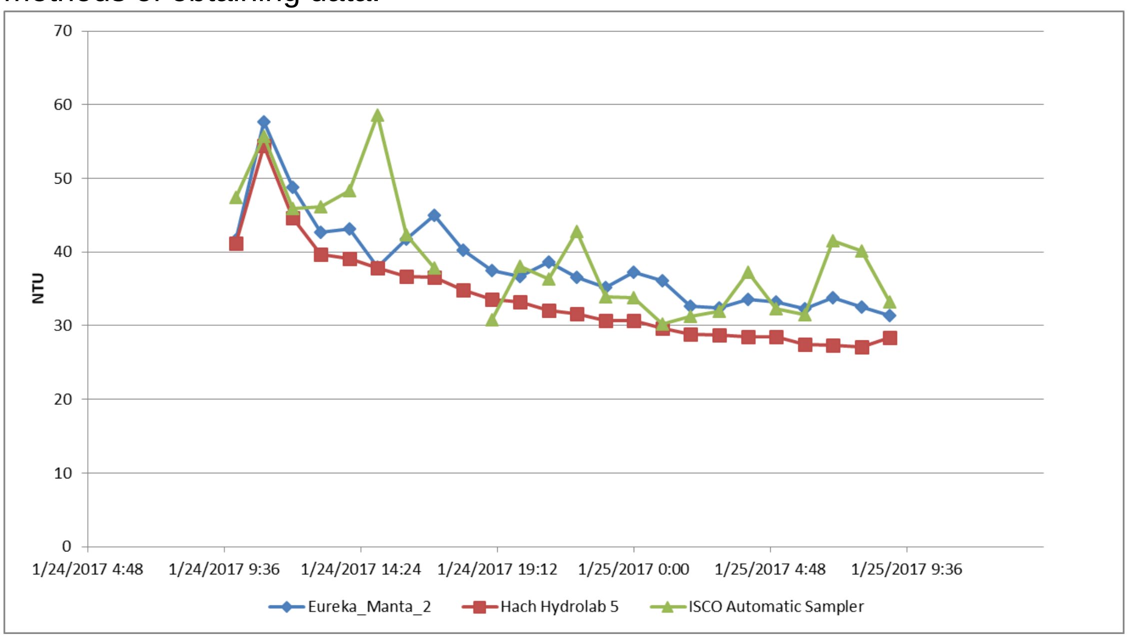

Figure 15. Turbidity in a skimmer basin at SECREF during a test run as measured by two sondes and a sampler.

Figure 16. Turbidity measurement testing site in Durham showing the two sondes, stage samplers, and automatic sampler intake tubing.

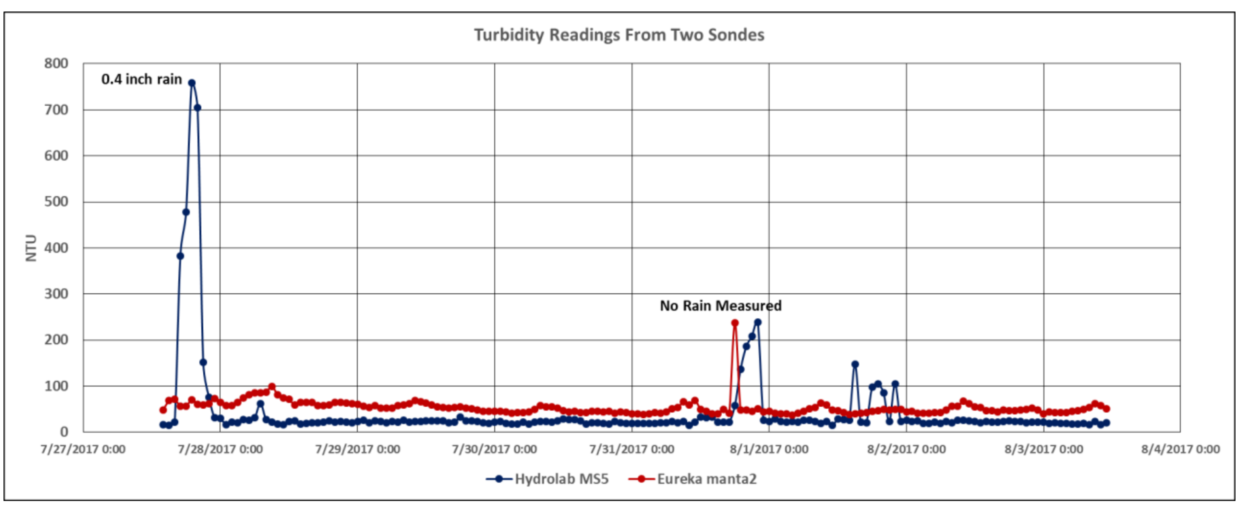

Figure 17. Turbidity measured by two sondes in a skimmer basin on the Durham Connector project.

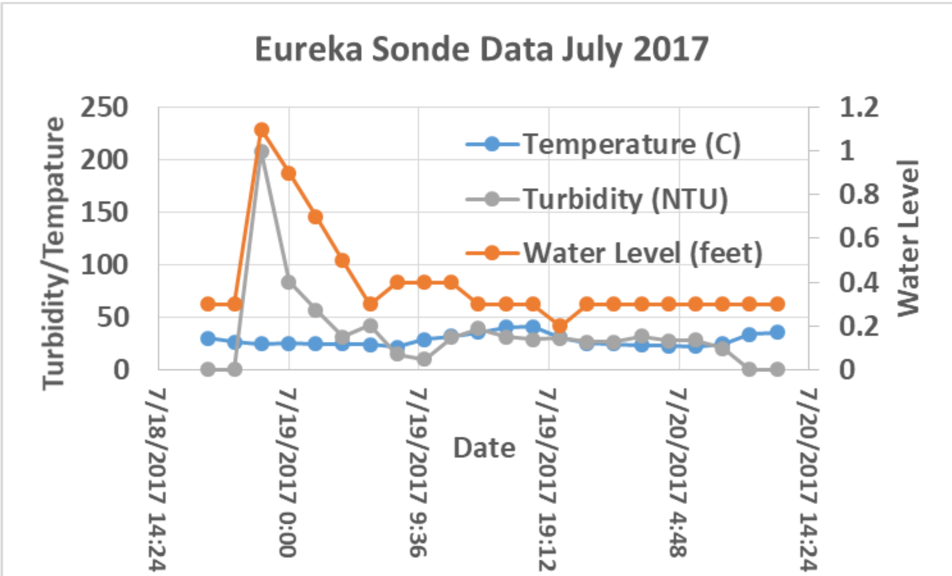

Figure 18. Temperature and turbidity in a skimmer basin after a storm event.

Task 2. Surface Dewatering Device Comparison

Four surface dewatering devices were tested for flow characteristics at different depths of water. Each one had tests conducted with at least three orifices or settings. The Faircloth, Erosion Supply, and Marlee skimmers had relatively steady discharges from depths of 5' down to around 1', with discharge rates dropping sharply after that (Figures 19-22). The Prodrain 70 skimmer had a steady decline in discharge rates as the water level dropped, but had the highest flow rate at fully open among the skimmers tested (Figure 23).

The average flow rate was calculated from the data and compared to the flow information from the manufacturers in Table 3. Deviations from the stated flows ranged from -60% for Marlee skimmer to +32% for the 2" Faircloth skimmer. The method of calculation can affect the flow estimate. We measured discharge rate continuously in a V-notch weir, but if you divide the total volume discharged by the time the discharge rate can vary from that substantially. This is particularly true for the skimmers with substantially different discharge rates at different water levels. The Marlee skimmer discharge rate was about half of what is listed by the manufacturer, possible due to testing conditions. We used a 10' length of cellular foam core (DWV) pipe to attach the skimmer to the outlet pipe, which might be longer than their test system. Because the Marlee attaches to the outlet pipe with a relatively stiff rubber hose, a longer pipe would result in a lower angle on the hose and so less downward pressure from it. The pipe is also lighter than schedule 40 or other pipe which might have been used in the manufaturer's tests. Both of these conditions would allow the Marlee skimmer to float higher in the water column and reduce flow. We also tested from about five feet of water, likely a greater depth than the manufacturer testing.

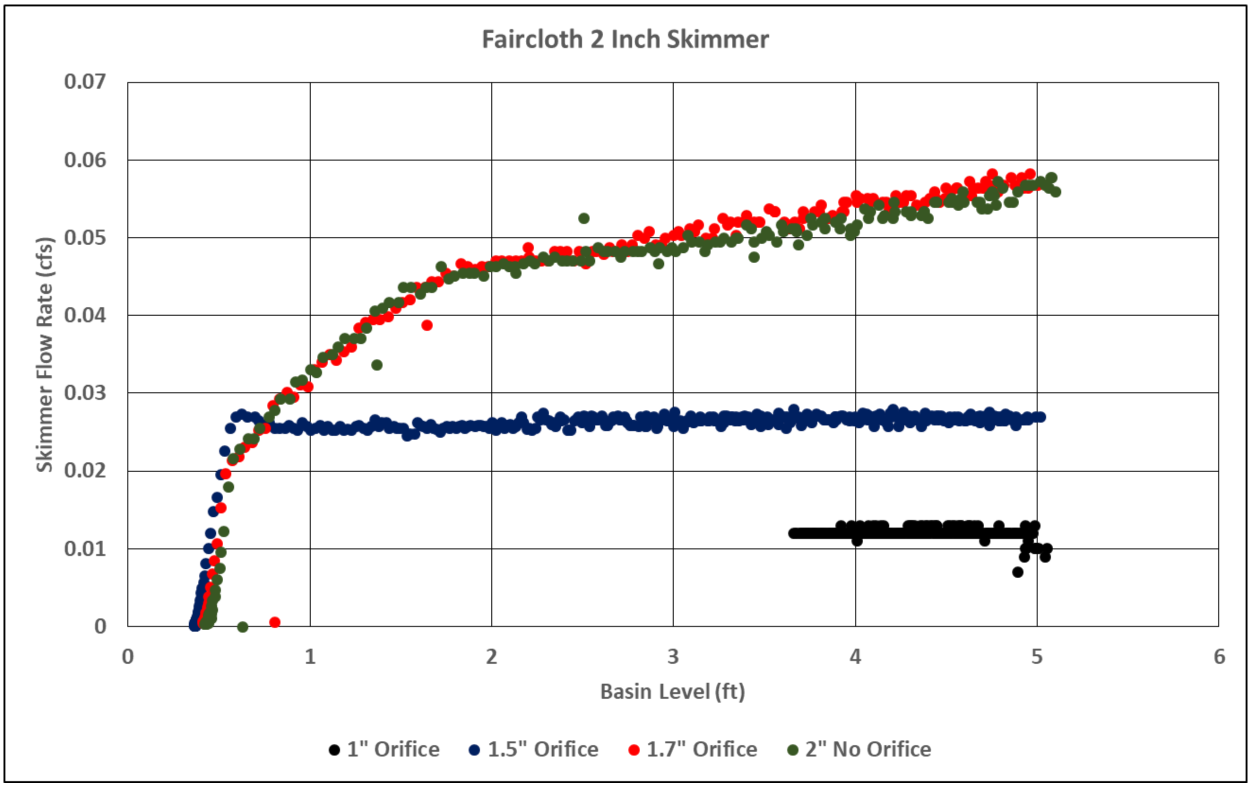

Figure 19. Discharge curves for the 2" Faircloth Skimmer with four different orifices.

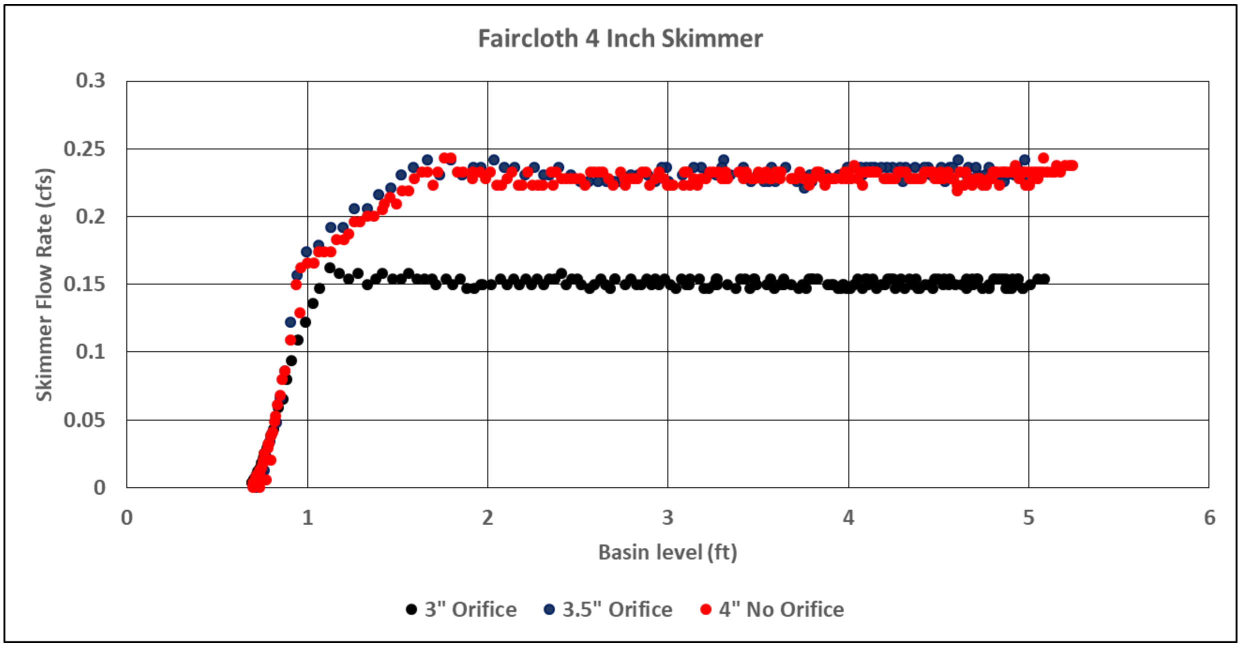

Figure 20. Discharge curves for the 4” Faircloth Skimmer with three different orifices.

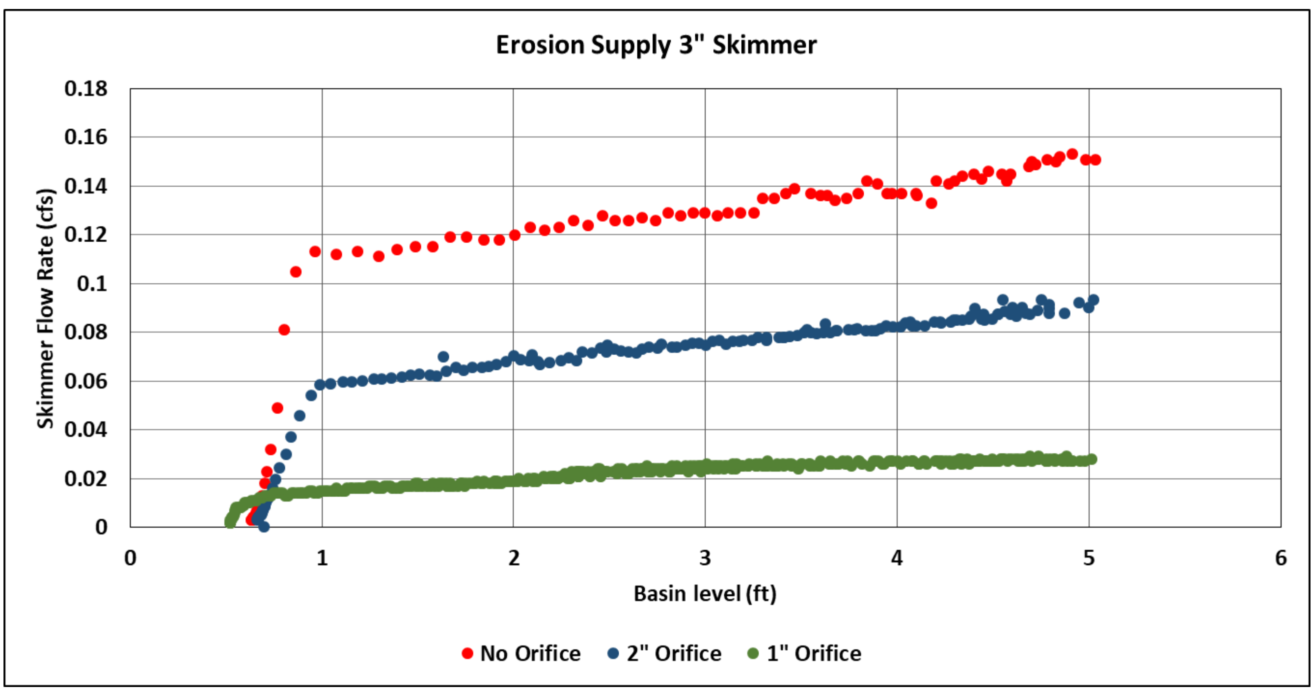

Figure 21. Discharge curves for the 3” Erosion Supply skimmer with three orifices.

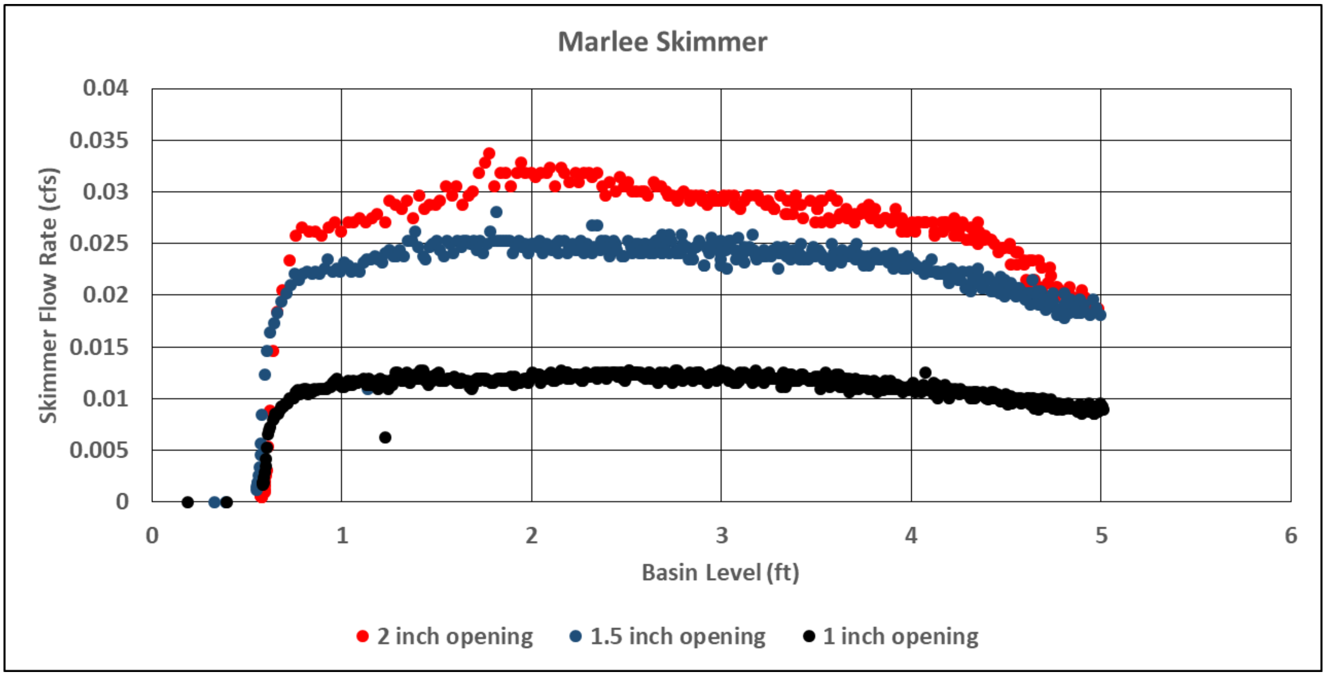

Figure 22. Discharge curves for the Marlee Model 1 Float Skimmer with three different orifices.

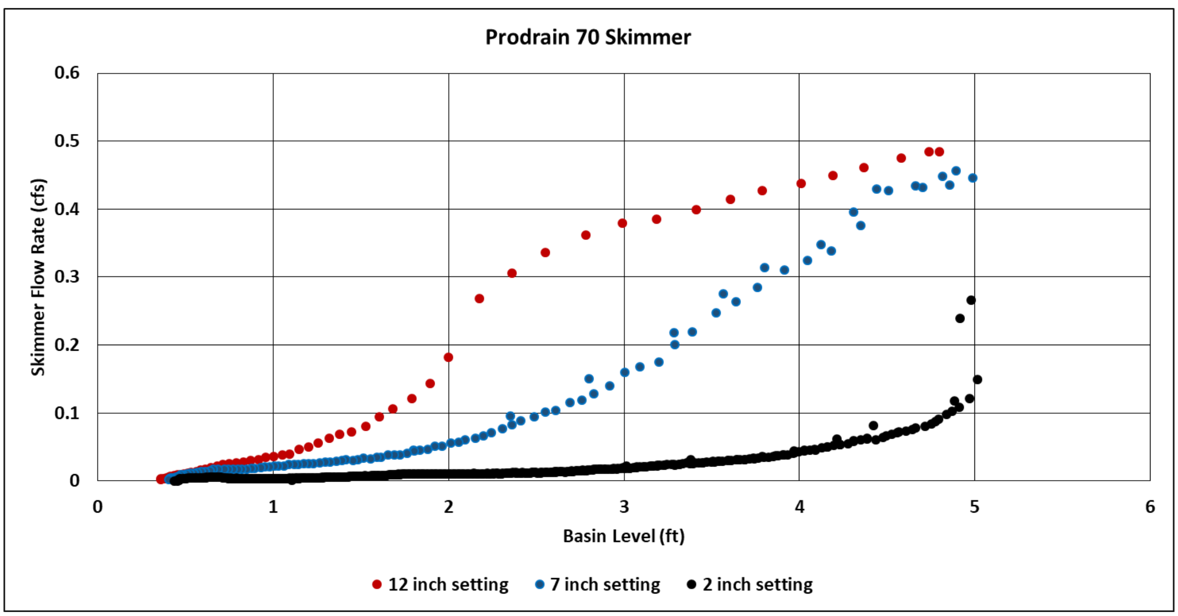

Figure 23. Discharge curves for the Prodrain 70 Skimmer at three different settings for slot openings.

Table 3. Discharge rates from our testing compared to the manufacturer data.

Skimmer	24-h Flow Rate (cubic feet)			
	Manufacturer	Average This Study (flow rate 5' – 1' depth)	Average This Study (volume/time 5' – 1' depth)	Difference (%) By flow/by volume
Faircloth 2" no plug	3,283	4,275	4,343	+30/+32
Faircloth 4" no plug	20,108	19,574	19,793	-3/-2
Erosion Supply 3" no plug	6,738-10,511 (1'-4' depths)	6,636	9,117	-2 to -37/+35 to -13
Marlee Model 1 2" orifice	5,652	2,362	2,281	-58/-60
ProDrain-70 12" (fully exposed slots)	17,952	20,940	24,600	+16/+37

Task 3. Develop a Portable Rainfall Simulator.

The portable rainfall simulator (PRS) was constructed as described in Methods using readily available materials. The key element in the system is the inclusion of pressure gauges for each riser pipe to the nozzles. The pressure needs to be within a small range in order to maintain droplet sizes which are somewhat similar to natural rainfall. Most of the droplets (92%) generated at the operating pressure (12 pounds per square inch (PSI) at the base of the riser pipe) fell within a range of 1-4 mm (Figure 24), similar to other rainfall simulators (Abudi et al., 2012). Because the simulator is run in an open environment, the effect of wind was also determine. There were two heavy rain areas when there was no wind, with around 5 in h^{-1} in those areas (Figure 25). Most of the area had 2-3 in h^{-1} with one corner receiving less than 1 in h^{-1}. At a high wind of 6-14 miles per hour (mph), most of the area received 1-2 in h^{-1} with heavier amount on the leeward side. With a more moderate wind of 3-10 mph, the distribution was actually the best over the entire area. This is reflected in the fact that the average rainfall rate was similar with the moderate wind compared to no wind but with somewhat less variation (Table 4).

Abudi, I., G. Carmi, and P. Berliner. 2012. Rainfall simulator for field runoff studies. J. Hydrology 454-455: 76-81. http://dx.doi.org/10.1016/j.jhydrol.2012.05.056.

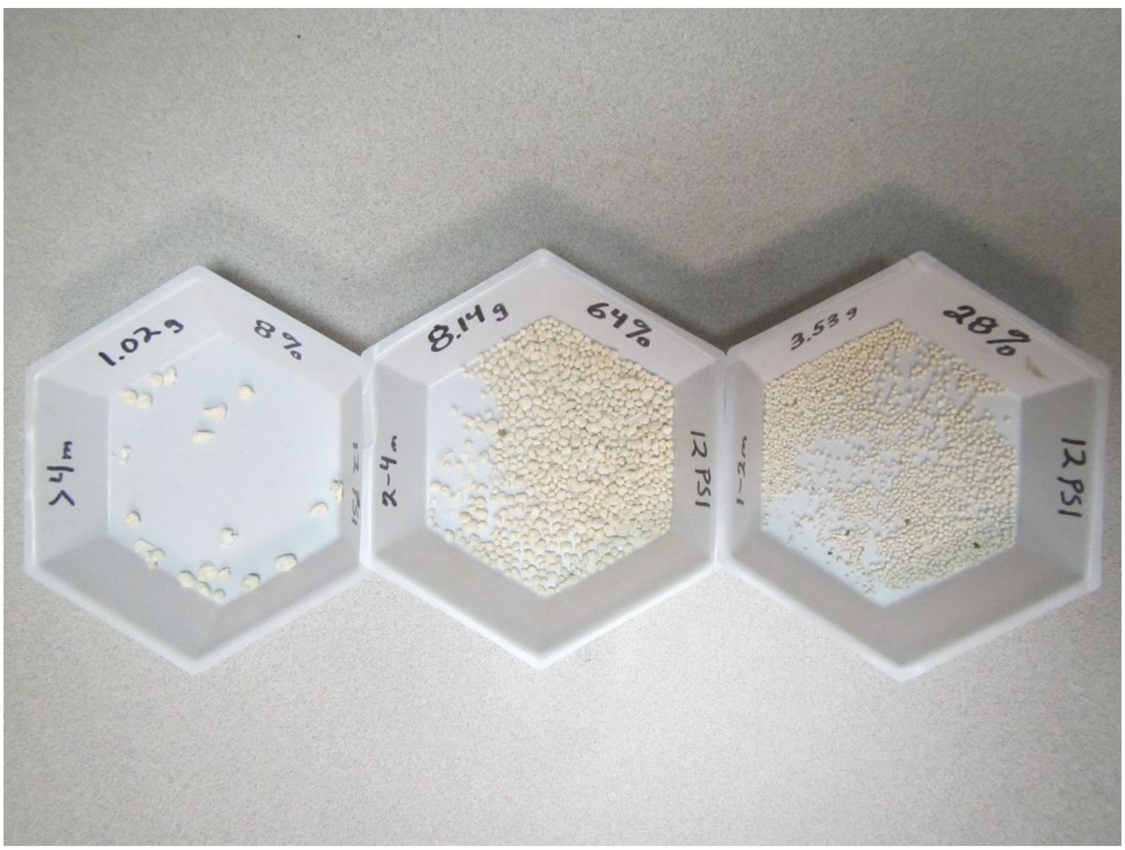

Figure 24. Raindrop size distribution for the rainfall simulator using the flour method.

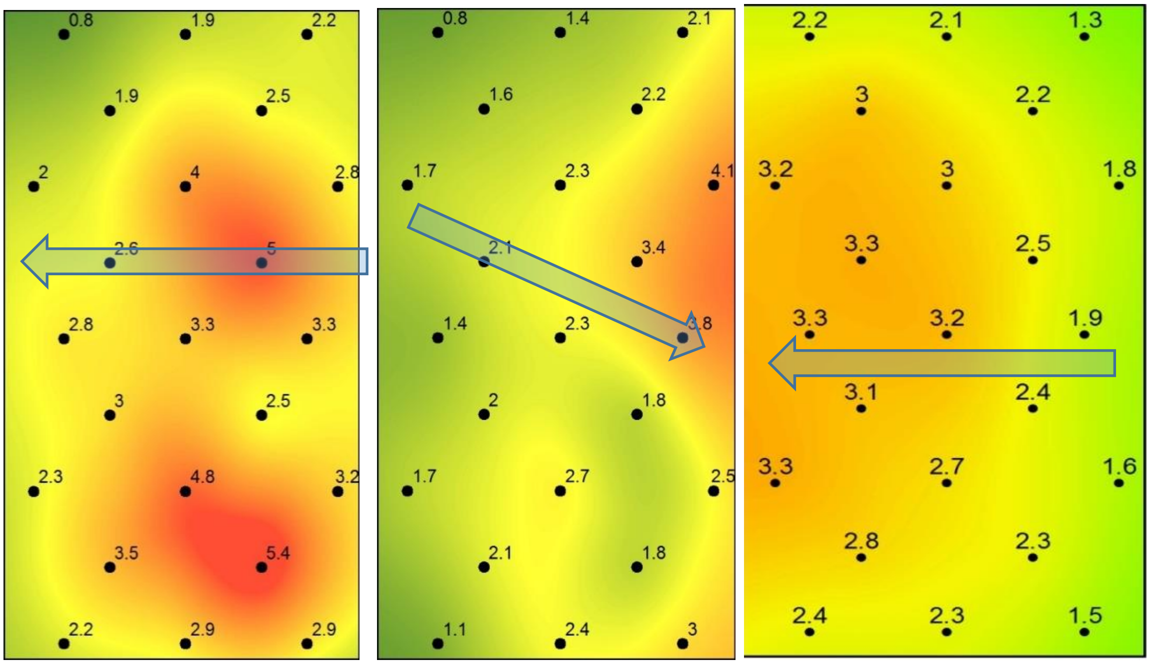

Figure 25. Wind effects on rainfall distribution. No wind on left, 6 -14 mph center, 3 – 10 mph on right, numbers represent rainfall rate (in h^{-1}), arrow shows wind direction.

Table 4. Effect of wind on the rainfall rate and variation in the rainfall simulator.

Test #	Average Rainfall Rate (inches/hr)	Standard Deviation (inches/hr)	Wind Speed (Avg. to Gust)	Wind Direction
1	2.21	0.78	6 to 11	NW
2	1.94	0.92	10 to 16	NE
3	2.94	0.79	3 to 7	S
4	2.20	0.81	6 to 14	NW
5	2.80	0.99	4 to 9	SE
6	2.65	0.76	3 to 9	SW
7	2.92	1.07	0	N/A

Task 4. Test New Products and Approaches for Reducing Turbidity.

The “standard” approach to reducing turbidity is to apply granular PAM to check dams upstream of a skimmer basin, with the flowing water dissolving the PAM as it passes through/over the dam. This requires two steps in attaining flocculation: first dissolving the PAM into the water and second, the PAM molecules unfolding (activating) in order to bind the suspended particles. A faster approach would be to dispense the PAM already dissolved in water, so the reaction can occur more quickly. However, this requires a system to dispense the dissolved PAM into the flowing water relatively proportional to the flow.

We constructed two systems for dispensing dissolved PAM. The first, referred to as the New Zealand dispenser, was adapted from the design introduced in New Zealand. This is essentially a displacement system, in which rainwater is collected into a container floating on the PAM solution. As the rainwater accumulates, the container sinks into the PAM solution and displaces it into a hose and out into the stormwater flow. In this way, the displacement is proportional to the rainfall amount and rate, which in turn should be relatively proportional to ditch flow. An example of an installation is shown in Figure 26, which was our first installation at the Rolesville bypass project.

The second was a simple float valve attached to a reservoir of dissolved PAM. This was installed on a square-notch weir, which allowed the valve to open in a linear proportion to flow as the water level rose behind the weir. This was demonstrated in testing at SECREF as shown in Figure 13. The solution release rate was found to be fairly linear in proportion to the water level in the weir (Figure 27). The calculated concentration of PAM in the runoff varies, but is mostly in the 0.5-1 mg L^{-1} range (Figure 28), usually enough to reduce turbidity depending on turbidity levels. In both types of dosers, the PAM concentration dictates the dosing concentration, but concentrations much above 1 g L^{-1} may become too viscous to flow easily in the tubing. The solution is also prone to freezing in cold weather unless the source tanks are buried.

An example of a successful treatment using the NZ system occurred on April 7, 2014 during an approximately 2.5 cm (1”) rain event. The rainfall and flow dynamics are shown in Figure 28, with peak flow occurring around the middle of the event. This is reflected in turbidity entering the 30 cm (12”) slope drain, with a peak of 1400

NTU about in the middle of the sample set, then tapering off (Figure 29). There were too few samples from the pipe inlet to note much of trend, but the discharge water leaving the basin was maintained at about 100 NTU throughout the event. We tested the PAM concentration in the basin exit and it was not detectable (< 5 mg L^{-1}) It is unlikely this low of a turbidity level could have been achieved by the skimmer alone.

In late 2014, the NZ Doser (Figure 31)and float valve doser (Figure 32) were also installed on the the I-840 Urban Loop extension project around Greensboro, NC, between US 70 and US 29 (STIP # U-2525B). The dosers were installed on two different ditches that contributed to a large two-tier sediment basin. Weirs were installed within each ditch to monitor flow and to take upstream water quality samples, using automated samplers, before dosing of PAM occurred. A third automated sampler was located at the exit of the upper tier of the basin to assess water quality as runoff exited the basin.

The turbidity reductions were not always substantial for various reasons. With higher flow, higher turbidity levels can be expected (Figure 33) and there might not be sufficient PAM dosing to achieve treatment. In other cases, the flow was so low that there was little change in turbidity (Figure 34). Figure 35 again shows the collected turbidity samples and that turbidity exiting the basin was lower than samples collected before PAM dosing occurred. Over a number of events, however, the turbidity in the basin discharge was reduced to 100-200 NTU, which is much lower than expected in skimmer basin discharges. This was demonstrated when we stopped dosing for several storms, with the resulting turbidity and TSS rising by 2X or more (Table 5).

The dosers were also installed on a tiered skimmer basin on the Durham Connector project. Both dosers we deployed at the basin, with the NZ doser dispensing inside the inlet pipe and the float valve doser dispensing inside one of the two outlet pipes from the upper basin (Figure 36). The area draining into the basin consisted of the roadbed, a parking and staging area, and several unvegetated slopes. As a result, sediment loads were very heavy and turbidities very high – sometimes >40,000 NTU (the upper limit of our meter). An example of the reduction in turbidity is shown in Figure 37, but in spite of substantial reduction the outlet turbidities were often in the 5,000 – 10,000 NTU. In most of the storms the float valve doser did not dispense PAM due to a mechanical problem. Laboratory tests suggested that the dosing level would need to be at least 10 mg L^{-1} to achieve substantial turbidity reduction, which is a much higher concentration than normally required. One example of the potential treatment occurred on May 10, when both dosers were functioning. The inlet turbidity was 9,000 – 15,000 NTU but grab samples of the outlet turbidity were 19-37 NTU. This also illustrates how mechanical systems, even when quite simple, can be unreliable unless carefully managed and protected from heavy sediment loads.

Figure 26. NZ Doser installed at the Rolesville 401 Bypass site. Slope drain brings water from the road bed to a small skimmer basin.

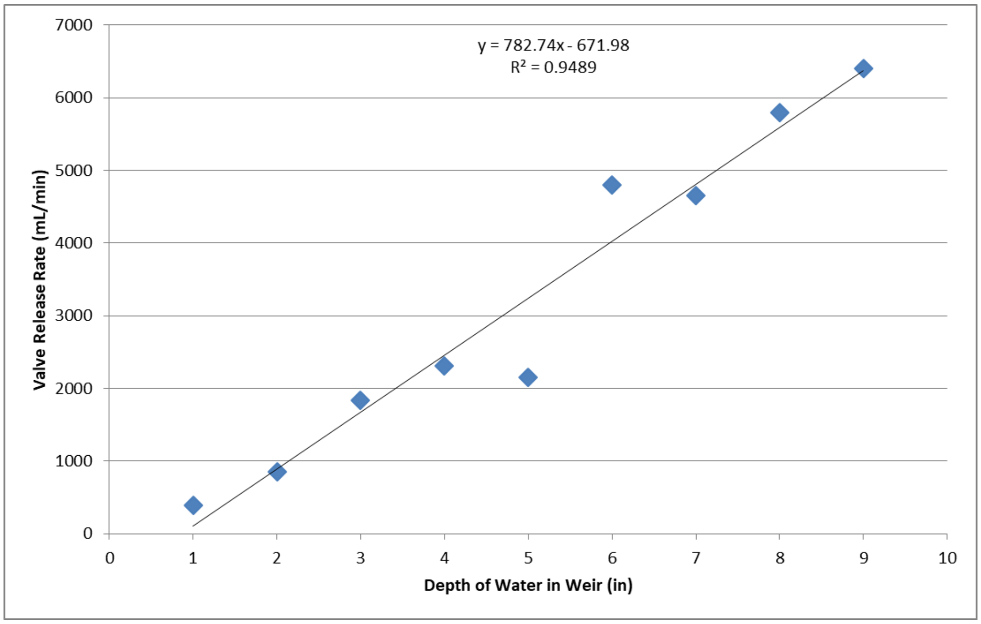

Figure 27. Float valve doser calibration curve as tested at SECREF.

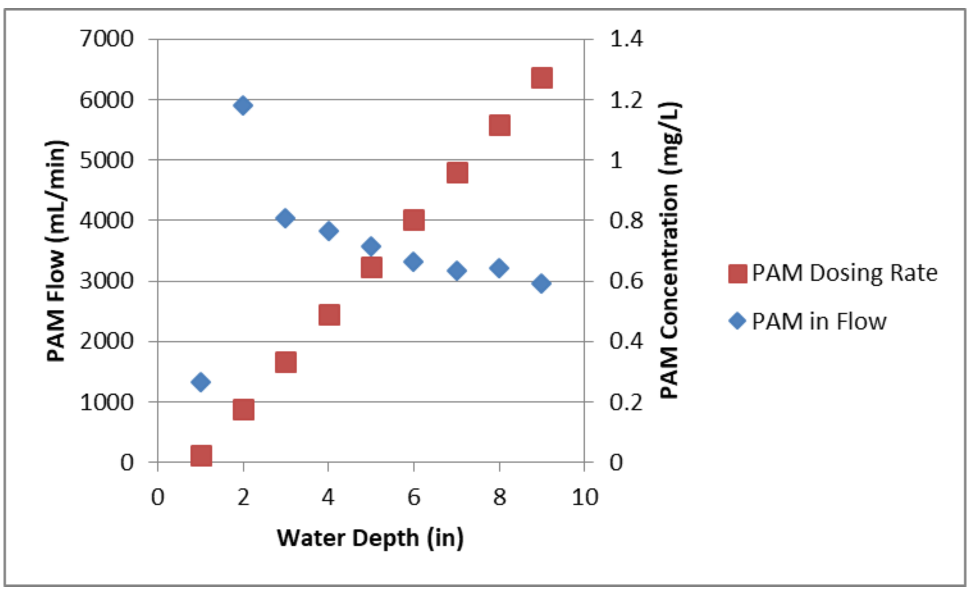

Figure 28. Calculated concentration of PAM in flow at different water depths behind weir.

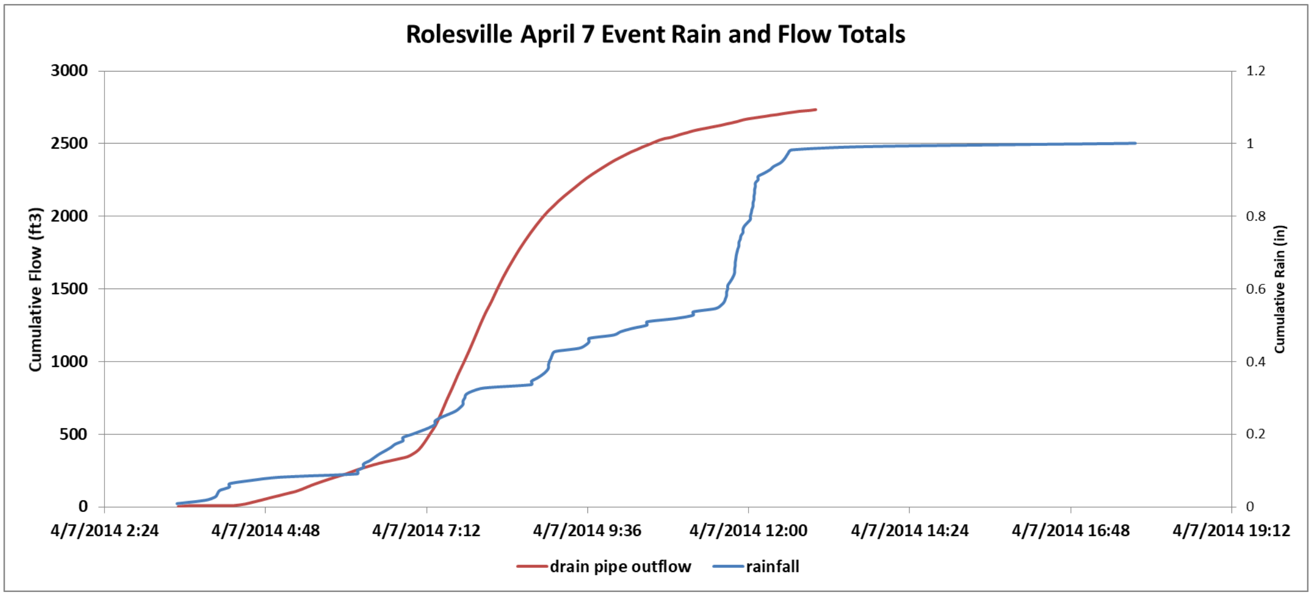

Figure 29. Cumulative rain and basin exit flow at the Rolesville NZ doser installation.

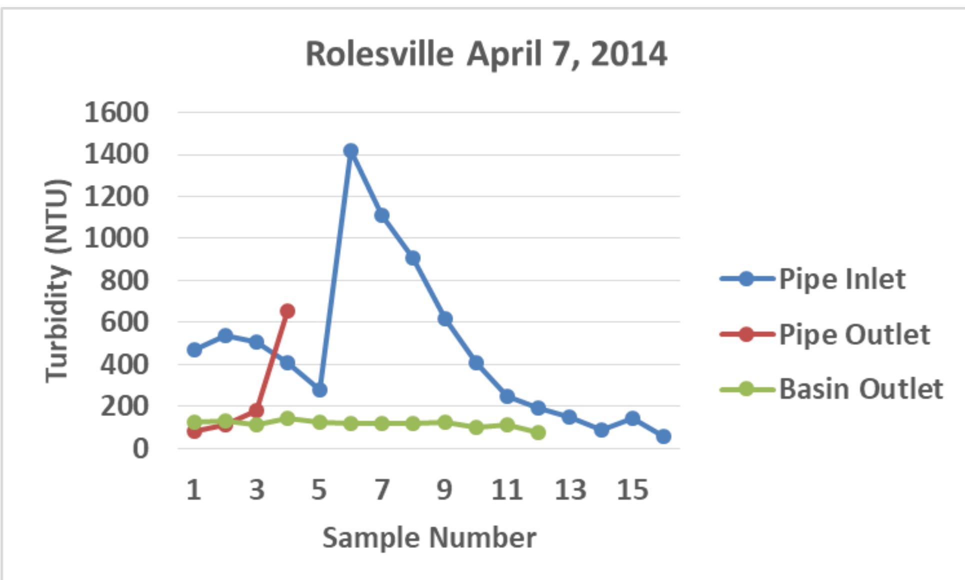

Figure 30. Turbidity in storm water flows into slope drain, out of slope drain, and out of skimmer basin at the Rolesville NZ Doser installation, April 7, 2014.

Figure 31. Rainfall driven liquid PAM dosing device (NZ Doser) installation in the foreground with skimmer basin (background right) and float valve doser (background left) at the Greensboro test site.

Figure 32. Closeup of the float valve dissolved PAM dosing device installation in Greensboro.

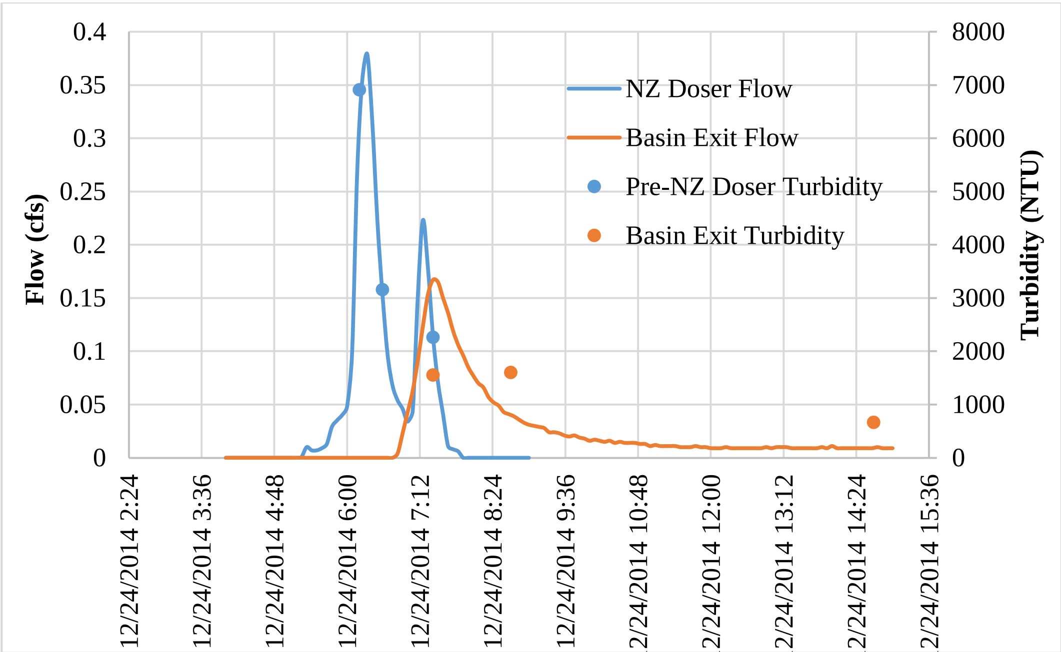

Figure 33. Rainfall driven doser (NZ Doser) flow and turbidity for a storm on 12/24/2014

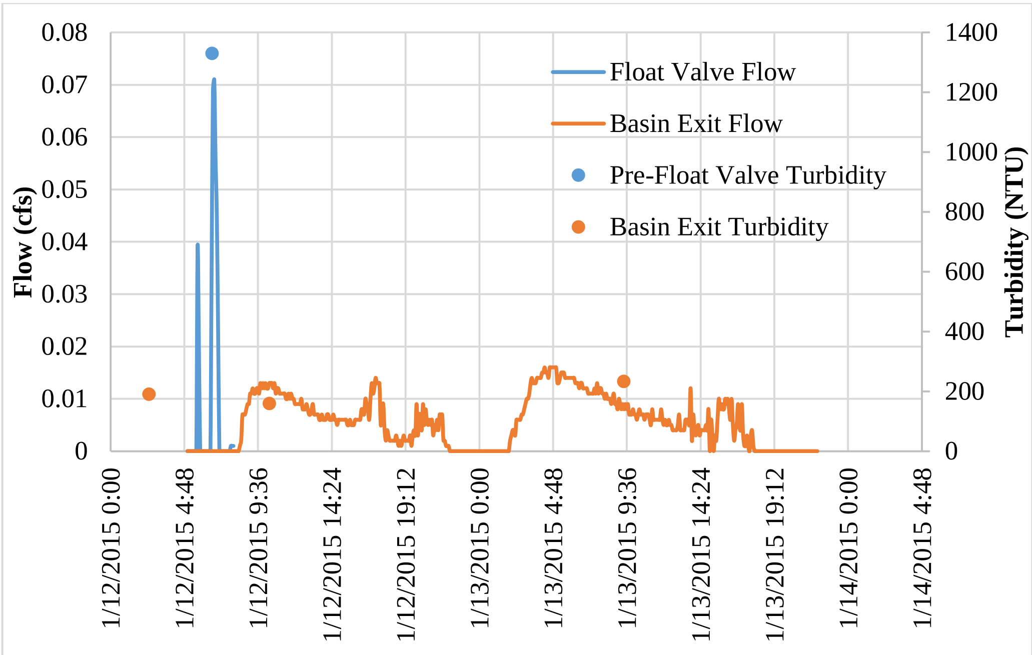

Figure 34. Float valve doser flow and turbidity for a storm on 1/12/2015

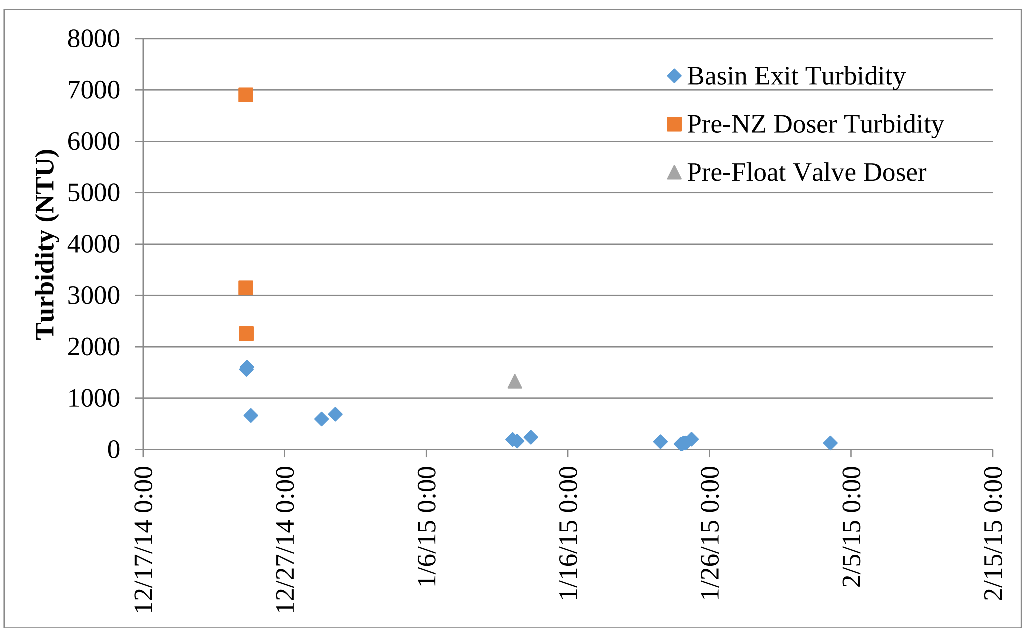

Figure 35. Turbidity samples by date at each of the three monitoring locations

Table 5. Average turbidity before the dosers and at the basin exit for the Greensboro installation. Values represent 2 events each for the "No PAM" averages and 9 events for the "With PAM Solution" averages.

Sample Locations	**With PAM Solution**		**No PAM**	
	Average Turbidity (NTU)	Average TSS (mg/L)	Average Turbidity (NTU)	Average TSS (mg/L)
Before New Zealand Doser	2969	1596	3243	2206
Before Float-Valve Doser	725	615	1324	1262
Basin Exit	163	57	372	286

Figure 36. Durham Connector site installation. NZ doser installed at upper basin inlet, float valve doser installed in one of the upper basin outlet pipes (source tank on dam).

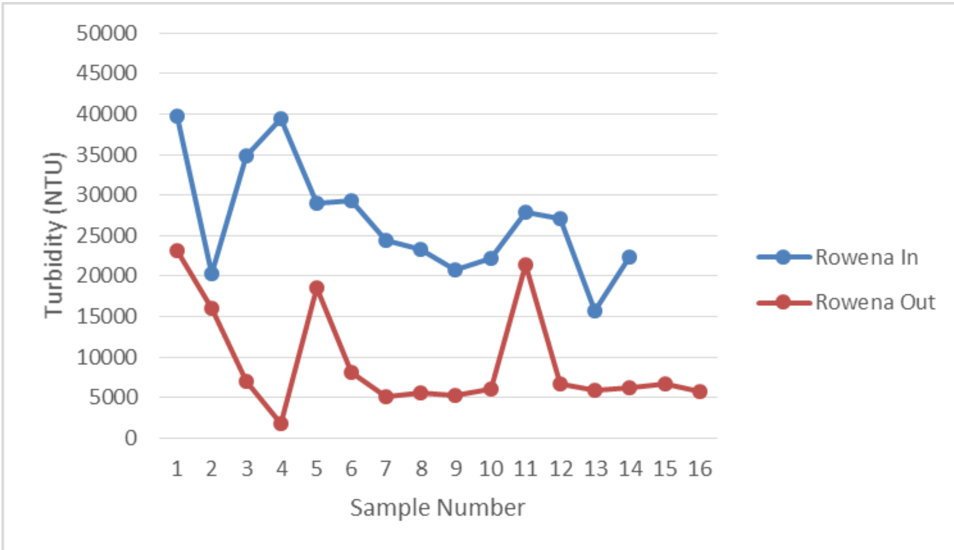

Figure 37. Turbidity at the inlet and outlet of the Durham Connector basin for the April 27, 2017 runoff event. Only the NZ doser was working during this storm. Values over 40,000 NTU were plotted as 40,000 NTU.

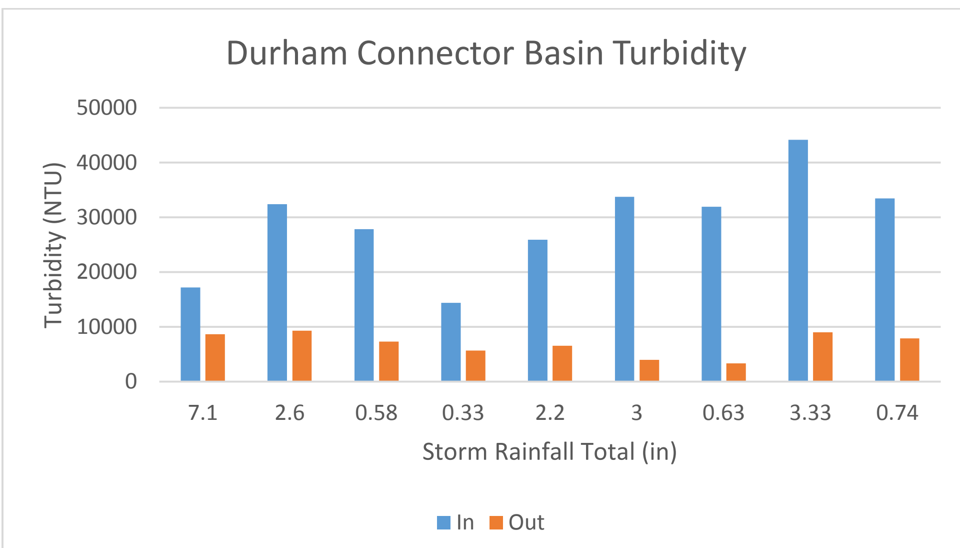

Figure 38. Turbidity reduction in the Durham Connector skimmer basin over 9 storm events with different rainfall totals.

Task 5: ASSESSMENT OF POLYACRYLAMIDE FLOCCULANTS, TURBIDITY, AND SEDIMENT ON NATIVE FRESHWATER MUSSELS

INTRODUCTION

The negative effects of soil erosion and suspended sediment on aquatic habitat and freshwater fauna are well-documented. Deleterious effects on aquatic ecosystems are often the result of the physical and chemical alterations that include sedimentation, light attenuation, and associated adsorbed contaminants. Urbanization, mining, road construction, and intensive agricultural practices can all lead to increased soil erosion and influxes of sediment to surface waters. These activities can result in landscape alterations permuting the natural hydrology, increasing runoff velocity, and sediment loading. The United States Environmental Protection Agency has concluded that nearly half of the waterways in the U.S. are significantly impaired by sediment and has designated sediment the primary pollutant of aquatic environments.

Increased turbidity from the suspended sediment fraction of inorganic sediments, which is the prevailing component contributing to turbidity during episodes of excessive runoff from disturbed soils, has been associated with adverse abiotic factors, such as decreased dissolved oxygen, light penetration, and increased water temperature. These changes have resulted in reduced diversity and biomass of primary producers (macrophytes, periphyton, and phytoplankton) in aquatic systems causing a cascade of deleterious effects on freshwater communities. Research has also identified a multitude of negative impacts and responses in fish as a result of suspended sediment exposure including avoidance, reduced hatching success, altered predator-prey interactions, damaged gill tissue, and direct mortality. However, the effects of turbidity on native freshwater mussels (family Unionidae), the most imperiled faunal group in North America have yet to be fully investigated, especially during early life stages.

Efforts to reduce suspended sediment released from construction sites to meet regulatory requirements have advanced through the implementation of a variety of Best Management Practices (BMPs). Many of these techniques are designed to reduce erosion by decreasing the velocity of runoff, thereby reducing the energy potential required to erode and suspend sediment. However, to remove the smallest fraction of suspended sediment < 20 μm from runoff effluent, chemical flocculants such as polyacrylamide (PAM) are used. PAM is a commercially available water-soluble polymer used in many different industries as a flocculating agent. PAM has been shown to reduce the turbidity of runoff by as much as 91% before reaching receiving waters, especially when used in conjunction with other

BMPs. Given the relatively high efficacy and putative low toxicity to aquatic organisms, chemical flocculants such as anionic PAM are quickly becoming an essential chemical tool to mitigate the well-studied impacts of increased suspended sediment on aquatic biota. However, more information is needed about the possible chemical and physical interactions of PAM within the environment to understand its risks and to determine its efficacy for mitigating the effects of suspended sediment on other organisms, such as native freshwater mussels, in a safe manner.

The overall goal of this research was to assess the practice of applying PAM to aquatic ecosystems in relation to potential impacts on freshwater mussels. The specific objectives were to develop toxicological information on 5 representative anionic PAM compounds and 1 non-ionic compound commonly used for the reduction of turbidity in stormwater runoff on the early life stages of 3 species of native freshwater mussels and to determine the relative sensitivity of freshwater mussels to a range of sediment and PAM-treated sediment conditions.

MATERIALS AND METHODS

Test chemicals

Six compounds of PAM were selected for toxicity testing in this study to provide a range of charge density, molecular weight, and net charge (Table 1), all characteristics that may influence potential toxicity. All PAM compounds were obtained in granular form, and homogeneous stock solutions of PAM (1 g/L) were prepared by slowly adding (approximately 1 g/min) granular PAM to reconstituted hard water and mixing on a stir plate for 24 h at room temperature. The stock solution was used in tests directly following mixing. The following polyacrylamide compounds were obtained from SNF Holding Company (Riceboro, Georgia, USA) FLOPAM: FA 920, AN 923, AN 923 SH, AN 923 VHM, and AN 913 VHM. APS 705 was purchased from Applied Polymer Systems (Woodstock, Georgia, USA). The chemical property and compound information for SNF compounds tested were provided by the manufacturer (Table 1), but APS 705 is a proprietary mixture of anionic PAMs, and it was included in testing because it is commonly used in environmental applications. In an effort to encompass both the typical effective range for turbidity reduction and to reach concentrations great enough to develop a median lethal concentration (LC50), each PAM compound had six treatment concentrations ranging from 5 to 1000 mg/L. Test exposure concentrations were verified and the using published methods and the measured concentrations of PAM in our tests ranged from 84 to 109% of the calculated nominal concentrations.

Test organisms

We tested four species of native freshwater mussels, chosen based on geographical distribution, phylogenetic tribe, and conservation status: *Lampsilis* *cariosa*, *Lampsilis siliquoidea* (tribe-Lampsilini), *Alasmidonta raveneliana* (tribe-Anodontini), and *Megalonaias nervosa* (tribe-Quadrulini). *L. cariosa* is an Atlantic Slope species in various classifications of conservation status across its range from stable to critically imperiled (state endangered, North Carolina). *A. raveneliana*, an Interior Basin species, endemic to the headwaters of the Tennessee River in western North Carolina and eastern Tennessee, is state (North Carolina and Tennessee) and federally endangered. *M. nervosa*, a common Interior Basin species, is widely-distributed and stable in the Mississippi and Gulf of Mexico drainages. *L. siliquoidea* is a common Interior Basin species widely-distributed and considered stable in the Mississippi and Gulf drainages of the U.S. and has been used extensively in toxicological testing.

L. cariosa and *A. raveneliana* were provided by the Aquatic Epidemiology and Conservation Laboratory, North Carolina State University, College of Veterinary Medicine (Raleigh, North Carolina, USA), and *L. siliquoidea* and *M. nervosa* were supplied by the mussel culture laboratory at Missouri State University (Springfield, Missouri, USA). With all species, glochidia were harvested from multiple (>3) gravid females <24 h before the initiation of each acute toxicity test. Juveniles were propagated by infecting host-fish with glochidia using standard propagation and culture methods. At the time of juvenile test initiation, *L. cariosa* ranged in age from 1 to 21 d, with an average ($\pm$SD) shell length of 587 µm ($\pm$ 125), *A. raveneliana* ranged in age from 1 to 21 d, with an average shell length of 501 µm ($\pm$ 50), and *M. nervosa* ranged in age from 1 to 3 d, with an average shell length of 370 µm ($\pm$ 23). Juvenile *L. siliquoidea* used for the turbidity and sediment experiments were approximately 17 months old, with an average ($\pm$ SD) shell length of 5.34 $\pm$ 0.80 mm.

RESULTS

After exposing both glochidia (mussel larvae) and juvenile mussels to each of the 6 PAM compounds at concentrations up to 1,000 mg/L only the AN 923 compound elicited mortality sufficient to calculate an LC50 for *L. cariosa* glochidia at the 24 or 48 h time points (Table 6). The 24 h LC50 was 833 mg/L (95% CI, 770–902 mg/L) and decreased to 412 mg/L (373–454 mg/L) at 48 h. For juvenile *L. cariosa*, AN 923 and AN 923 SH had 96 h LC50s of 130 mg/L (100–161 mg/L) and 563 mg/L (414–766 mg/L), respectively (Table 7). All other compounds showed no evidence of acute toxicity to either life stage at the highest concentration tested (no observed effect concentration [NOEC] = 1000 mg/L). The only test resulting in the calculation of an LC50 for *A. raveneliana*, the federally endangered species, was the 96 h juvenile exposure to AN 923 (330 mg/L: 95% CI 289–376 mg/L). Similarly, the only test that resulted in the calculation of an LC50 for *M. nervosa* was the 96 h juvenile exposure to AN 923 (706 mg/L: 95% CI 576–865 mg/L; Table 6).

When juvenile *L. siliquoidea* was exposed to three sediment test conditions (non-flocculated settled sediment, suspended sediment, and PAM-flocculated settled sediment) for a duration of 96 h, there was 100% survival in all treatments. Likewise, mussel survival at the 20-d chronic assessment was not significantly different among conditions or turbidity level ($p>0.05$). Mean percent survival (range in parenthesis) in the settled sediment, suspended sediment, and PAM-flocculated sediment conditions was: 89% (80 – 98%), 84% (81 – 87%), and 89% (87 – 93%), respectively (Figure 38).

SUMMARY

We found that the acute toxicity of the 6 PAM compounds tested varied with mussel life stage (juveniles more sensitive than glochidia), species (*Lampsilis cariosa* most sensitive), and chemical properties of the compound (molecular weight, charge density, and net charge), but exhibited relatively low toxicity overall, compared to the concentrations commonly applied for aquatic turbidity control. Of the 36 tests conducted with the early life stages of freshwater mussels and the 6 PAM compounds, 7 yielded calculable LC50 concentrations. For even the most toxic PAM tested (AN 923), there was still a 24- to 126-fold margin of safety from common treatment concentrations. The relative lack of acute toxicity in our tests with anionic PAM and early life stages of native freshwater mussels compares similarly to previous acute toxicological studies of anionic PAM with other aquatic organisms. Given the relatively low toxicity of PAM to freshwater mussels observed during this study, the benefits of PAM use for turbidity control may supersede the risk of toxic effects because PAM may effectively reduce the amount of sediment entering receiving waters and will decrease the stress of excess sediment on this ecologically important group of imperiled organisms.

CONCLUSIONS

Our findings indicate that anionic PAM poses a minimal risk to freshwater mussels at optimal turbidity control concentrations of 1 mg/L to 5 mg/L (a 24- to 126-fold margin of safety), as 127 mg/L was the lowest 96 h LC50 calculated (juvenile *L. cariosa*). Likewise, when we evaluated the effects of PAM-flocculated sediment on freshwater mussels by applying a 5 mg/ml concentration of anionic PAM (AN 923) to a range of turbidity treatments, the mussel results showed a protective quality of PAM-flocculated sediment during acute exposures; however, the chronic results were not as clear (refer to Appendix 1 and Appendix 2 for additional details). Anionic PAM appears to effectively mitigate the negative effects of acute turbidity exposure without creating additional risks due to flocculated sediment. Our overall findings advance the current knowledge of PAM toxicity to aquatic organisms and can be used to inform management decisions regarding turbidity control in the presence of common or imperiled freshwater mussels.

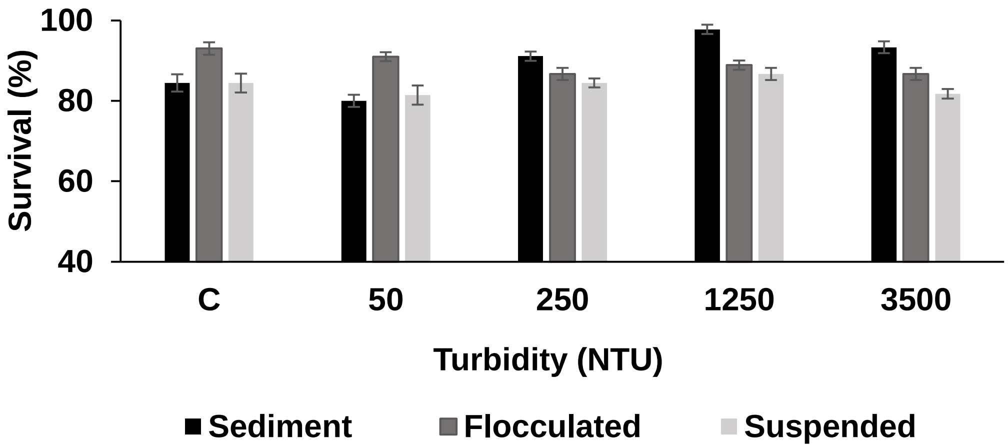

Figure 39. Mean (+SE) juvenile mussel survival at 20 d post exposure to sediment test conditions. No significant differences were found between any of test conditions (settled sediment, suspended sediment, PAM-flocculated sediment) or turbidity treatment levels (α = 0.05).

Table 6. Properties of selected anionic and non-ionic polyacrylamide (PAM) compounds used in acute toxicity tests with larval (glochidia) and juvenile freshwater mussels. Information derived via SNF online product brochure at http://www.snfgroup.com/images/pdf/Brochures_in_English/Catalogue%20Poudres.pdf.

Compound	Net charge	Charge density %	Molecular weight classification	Molecular weight (Mg/mole)
FLOPAM™ AN 913 VHM	Anionic	13	Ultra High	13–16
FLOPAM™ FA 920	Non-ionic	Non-ionic	High	5–6
FLOPAM™ AN 923 SH	Anionic	23	Very High	12–14
FLOPAM™ AN 923	Anionic	23	Standard	9–12
FLOPAM™ AN 923 VHM	Anionic	23	Ultra High	14–17
APS 705	Anionic	NA	NA	NA

NA=Information not available for product.

Table 7. Median lethal concentrations (LC50s) for acute toxicity of anionic polyacrylamide (PAM) to native freshwater mussels (95% CI). Acute exposures to the following PAM compounds resulted in insufficient mortality to calculate an LC50s: APS 705, FLOPAM™ FA 920, FLOPAM™ AN 923 VHM, and FLOPAM™ AN 913 VHM.

Species	Life stage	Time point (h)	FLOPAM™ AN 923 LC50 (mg/L)	FLOPAM™ AN 923SH LC50 (mg/L)
Alasmidonta raveneliana	Glochidia	24	>1000	>1000
		48	>1000	>1000
	Juvenile	48	>1000	>1000
		96	330 (289–376)	>1000
Lampsilis cariosa	Glochidia	24	833 (770–902)	>1000
		48	412 (373–454)	>1000
	Juvenile	48	183 (140–240)	>1000
		96	127 (100–161)	563 (414–766)
Megalonaias nervosa	Glochidia	24	>1000	>1000
		48	>1000	>1000
	Juvenile	48	>1000	>1000
		96	705 (576–865)	>1000

Recommendations

- Programmable samplers and water quality sondes usually provided similar turbidity readings. Samplers have the advantage of being able to run samples a second time to check the results, but they also require someone to retrieve and then analyze samples. Sondes can provide more continuous data, but the data needs to be scrutinized for unexplained readings. Overall, monitoring turbidity with sondes may be more cost-effective when other water quality parameters (e.g. Total Suspended Solids, nutrients, heavy metals) are not needed.
- The skimmers all performed reasonably well but some of the discharge rates provided by the manufacturers were considerably different from our test results. These differences would likely be less important than the installation and maintenance of the skimmers in the field.
- The plot-scale rainfall simulator produced rain droplet distributions similar to those reported for other simulators. The rainfall distribution was highly dependent on wind speed and direction, but generally ranged between 2" – 3" hr^{-1}, which is sufficient for testing erosion control products. It can be deployed to field sites on construction projects for "real conditions" testing of products.
- Alternative systems for controlling turbidity using dissolved PAM, deployed on three different projects, had highly variable impacts depending on site conditions. With moderate flows and turbidities, substantial reductions in turbidity could be achieved. The effects were less evident when flows and turbidities were high, such as >40,000 NTU. This is likely a function of the scale of the dosers relative to the volumes of runoff, as these dosers were only designed to handle <3,000 cubic feet of runoff at approximately 1 mg L^{-1}. Increasing the number or storage volume of the dosers would provide additional capacity. Close monitoring and maintenance of these systems is imperative.
- There was no evidence of PAM toxicity to freshwater mussels at both life stages at concentrations expected for turbidity treatment. In fact, there was a >10X safety margin even for the most sensitive species and life stage. This suggests the use of PAM for turbidity control should not have adverse effects on mussels in receiving waters.

Implementation and Technology Transfer Plan

1. Stormwater discharge quality on NCDOT construction sites can be maintained and improved using the skimmers currently on the market and dissolved PAM dosing systems in critical areas. There is little cause for concern about using PAM in areas where mussels are found. Monitoring of water quality using sondes may be cost effective when this data is needed.

2. The Roadside Environmental Unit can use this information in areas where turbidity concerns suggest additional measures should be used. The two dosing systems we developed represent two conceptual approaches to dispensing the solutions, but many others could be developed where no power is needed. Sondes could be deployed in sensitive areas where the turbidity needed to be closely monitored, such as borrow pits.

3. The low toxicity of PAM to freshwater mussels is information that should be widely disseminated to local, state, and federal agencies involved in water quality regulation. This, combined with information on PAM safety for a wide variety of aquatic organisms available from manufacturers and published studies, should ease concerns about its use for turbidity control.

   If NCDOT plans to implement turbidity monitoring using sondes or to deploy dissolved PAM dosing systems, additional training can be developed at NCSU. This could be incorporated into current certification training, at least as general information, or as part of an in-depth turbidity control workshop.

APPENDICES

APPENDIX 1: ACUTE TOXICITY OF POLYACRYLAMIDE FLOCCULANTS TO EARLY LIFE STAGES OF FRESHWATER MUSSELS

APPENDIX 2: SUBLETHAL EFFECTS OF TURBIDITY, SEDIMENT, AND POLYACRYLAMIDE ON NATIVE FRESHWATER MUSSELS

APPENDIX 1

Acute Toxicity of Polyacrylamide Flocculants to Early Life Stages of Freshwater Mussels (Accepted by Environmental Toxicology and Chemistry)

Abstract
Polyacrylamide (PAM) has become an effective tool for reducing construction related suspended sediment and turbidity, which are considered to have significant adverse impacts on aquatic ecosystems and are a leading cause of the degradation of North American streams and rivers. However, little is known about the effects of PAM on many freshwater organisms, and prior to this study, no information existed on the toxicity of PAM compounds to native freshwater mussels (Family Unionidae), one of the most imperiled faunal groups globally. Following standard test guidelines, we exposed juveniles mussels (test duration 96-h) and glochidia larvae (test duration 24-h) to 5 different anionic PAM compounds and 1 non-ionic compound. Species tested included the Yellow Lampmussel (*Lampsilis cariosa*), an Atlantic Slope species that is listed as endangered in North Carolina, the Appalachian Elktoe (*Alasmidonta raveneliana*), a federally endangered Interior Basin species, and the Washboard (*Megalonaias nervosa*), a common Interior Basin species. We found that median lethal concentrations (LC50) of PAM ranged from 411.7 to > 1000 mg/L for glochidia and from 128.7 to > 1000 mg/L for juveniles. All LC50s were orders of magnitude greater (2–3) than concentrations typically recommended for turbidity control (1–5 mg/L), regardless of their molecular weight or charge density. Our results demonstrate that the PAM compounds tested were not acutely toxic to the mussel species and life stages tested, indicating minimal risk of short-term exposure from PAM applications in the environment. However, potential chronic or sublethal effects remain uncertain and warrant additional investigation.

Keywords—Polyacrylamide, Freshwater Mussel, Toxicity, Unionidae, Turbidity Control

INTRODUCTION

Human activities influence ecosystem structure and function, as well as the organisms that occupy the ecosystems. Anthropogenic effects continue to accelerate extinction rates of many of the world's species [1]. Stress on the environment has intensified as global human populations have increased and shifted toward urban growth centers, which radically alter land-cover and lead to habitat destruction and alteration [2,3]. The change to urban and suburban land use alters local environments, leading to increased stormwater runoff, soil erosion, and reduced biodiversity, thereby creating regional disturbances that inherently alter lotic aquatic systems [4–8]. Increased turbidity and sediment load can alter the physical environment, reduce light penetration [9], decrease dissolved oxygen concentrations [7], and reduce habitat complexity [10]. These factors, among others, can result in deleterious effects on freshwater organisms, such as reduced food availability, increased water temperatures, altered feeding behavior, reduced respiration rate [11], decreased reproduction, decreased feeding rates [12], and direct mortality [6,7,13–15]. As suspended sediments settle from the water column, the complexity of the benthic substrate is reduced and the structure is altered. Interstitial spaces within the substrate become inundated with fine particulate matter thereby reducing the structural heterogeneity, availability of habitat, and oxygen for benthic dwelling organisms [16].

The United States Environmental Protection Agency (USEPA) determined sediment to be the greatest pollutant of rivers in the United States, and estimates of sediment release to U.S. surface waters due to anthropogenic erosion are as high as 75 billion tons annually [17,18]. Construction site runoff has been implicated as a major contributor of sediment and impairment of water quality [19]. Erosion rates of disturbed soil on construction sites are 7 to 500 times that of natural areas, and these areas are responsible for more than 90% of soil erosion in urban environments [19,20]. Efforts to reduce sediment release from construction sites have advanced through the implementation of a variety of Best Management Practices (BMPs) such as silt fences, check dams, erosion blankets, and sediment basins. However, to effectively remove suspended sediment particles < 20 μm requires the use of a chemical flocculant such as polyacrylamide (PAM) [21].

PAM is a water-soluble polymer commercially produced through the polymerization of acrylamide and available in various compounds of differing charge density and molecular weight. Both cationic and anionic polyacrylamide can be produced during the commercial manufacturing process through the addition of co-monomers such as trimethyl ammonium or sodium acrylate [22, 23], but it is the anionic form of PAM that is used in turbidity control because of toxicity concerns from the cationic form [24]. The amount of these substituents determines the degree of charge density, typically ranging from 7 to 50% (N. Bartholomew, 2003, Master's thesis, North Carolina State University, Raleigh, North Carolina, USA). The molecular weight is dependent on the length of the linear chains ranging from 12 to 17 Mg/mol [25]. When used for the reduction of turbidity, PAM is typically applied at a rate of 1–5 mg/L of water. However, the turbidity concentration, soil composition, and other physical parameters dictate the most effective compound for a given application [26,27].

PAM has been shown to effectively reduce the turbidity of runoff as much as 91% before reaching receiving waters [28]. When used in conjunction with other BMPs, PAM is especially effective at controlling turbidity [29–31]. Given their demonstrated efficacy, chemical flocculants like anionic PAM are quickly becoming an essential BMP on construction sites, as the industry strives to meet regulatory demands designed to mitigate the well-studied impacts of increased suspended sediment on aquatic biota [6,31–33].

Although acute toxicity studies of PAM have been conducted with standard aquatic test organisms (Table 1), toxicity data representing the highly imperiled native freshwater mussel fauna (Family Unionidae) have not been generated. Unionid mussels are experiencing significant declines across North America and throughout the world [34]. In fact, unionids are the most imperiled faunal group in North America with greater than 70% of the nearly 300 species considered endangered, threatened, of special concern, or already extinct [35]. Freshwater mussels are disproportionally sensitive to certain environmental contaminants and to other anthropogenic activities that impact aquatic habitat, facilitating the precipitous decline [5,35–39]. Previous toxicological research with freshwater mussels and environmental contaminants, such as chloride, ammonia and copper, have found freshwater mussels to be among the most sensitive aquatic organisms tested, especially when exposed during early life stages (glochidia and juveniles) [38,40–42]. Thus, utilizing exposure-response data from freshwater mussel tests to derive water quality criteria or environmentally acceptable levels may also be protective of other aquatic organisms.

The primary concerns for the toxicity of anionic PAM to aquatic organisms center around the monomer, acrylamide, which is recognized as a neurotoxin and probable carcinogen [43] and the physical effects of flocculation on larval life stages of invertebrates and the algal populations used as biological food sources [24]. The environmental risk for acrylamide exposure includes the possibility of incomplete polymerization during the manufacturing process, resulting in residual unbound acrylamide, as well as the possibility of acrylamide release during physical, chemical, biological, or photochemical degradation. Laboratory studies have shown minimal release of acrylamide from PAM by intense UV irradiation and high thermal stress (95^{0}C for 10 d) [44,45]. Moreover, field tests have shown no appreciable acrylamide release through environmental degradation, and any detected acrylamide is the result of free acrylamide from incomplete polymerization [46]. To date, there is no evidence of environmental concentrations of acrylamide above allowable levels (0.05%) during field applications when being applied to reduce turbidity [46,47]. Therefore, the main unresolved toxicity concerns of anionic PAM are related to its physical and chemical attributes.

The objective of this study was to develop toxicological information on 5 representative anionic PAM compounds and 1 non-ionic compound commonly used for the reduction of turbidity in stormwater runoff on the early life stages of 3 species of native freshwater mussels. Determining the LC50 for each of the 6 PAM compounds provides important data for identifying potential environmental impacts of their use in water quality and erosion control, while also, presenting possible implications for other environmental PAM applications that require greater concentrations (e.g., erosion control, canal lining, algae control).

MATERIALS AND METHODS

Test chemicals

Six compounds of PAM were selected for toxicity testing in this study to provide a range of charge density, molecular weight, and net charge (Table 2), all characteristics that may influence potential toxicity. All PAM compounds were obtained in granular form, and homogeneous stock solutions of PAM (1 g/L) were prepared by slowly adding (approximately 1 g/min) granular PAM to reconstituted hard water [48] and mixing on a stir plate for 24 h at room temperature. The stock solution was used in tests directly following mixing. The following polyacrylamide compounds were obtained from SNF Holding Company (Riceboro, Georgia, USA) FLOPAM: FA 920, AN 923, AN 923 SH, AN 923 VHM, and AN 913 VHM. APS 705 was purchased from Applied Polymer Systems (Woodstock, Georgia, USA). The chemical property and compound information for SNF compounds tested were provided by the manufacturer (Table 1), but APS 705 is a proprietary mixture of anionic PAMs, and it was included in testing because it is commonly used in environmental applications. In an effort to encompass both the typical effective range for turbidity reduction and to reach concentrations great enough to develop a median lethal concentration (LC50), each PAM compound had six treatment concentrations ranging from 5 to 1000 mg/L. Test exposure concentrations were verified using the turbidimetric reagent, benzethonium chloride (Hyamine 1622, Acros Organics, Geel, Antwerp, Belgium) and methods described by Kang et al. [49]. Measured concentrations of PAM in our tests ranged from 84 to 109% of the calculated nominal concentrations.

Test organisms

We tested three species of native freshwater mussels, chosen based on geographical distribution, phylogenetic tribe, and conservation status: *Lampsilis cariosa* (tribe-Lampsilini), *Alasmidonta raveneliana* (tribe-Anodontini), and *Megalonaias nervosa* (tribe-Quadrulini). *L. cariosa* is an Atlantic Slope species in various classifications of conservation status across its range from stable to critically imperiled (state endangered, North Carolina) [50]. *A. raveneliana*, an Interior Basin species, endemic to the headwaters of the Tennessee River in western North Carolina and eastern Tennessee, is state (North Carolina and Tennessee) and federally endangered [50,51]. *M. nervosa*, a common Interior Basin species, is widely-distributed and stable in the Mississippi and Gulf of Mexico drainages [52].

L. cariosa and *A. raveneliana* were provided by the Aquatic Epidemiology and Conservation Laboratory, North Carolina State University, College of Veterinary Medicine (Raleigh, North Carolina, USA), and *M. nervosa* was supplied by the mussel culture laboratory at Missouri State University (Springfield, Missouri, USA). With all species, glochidia were harvested from multiple (>3) gravid females <24 h before the initiation of each acute toxicity test. Juveniles were propagated by infecting host-fish with glochidia using standard propagation and culture methods [53]. At the time of juvenile test initiation, *L. cariosa* ranged in age from 1 to 21 d, with an average (±SD) shell length of 587.1 µm (± 125.2), *A. raveneliana* ranged in age from 1 to 21 d, with an average shell length of 501.1 µm (± 50.1), and *M. nervosa* ranged in age from 1 to 3 d, with an average shell length of 370.2 µm (± 22.6).

Glochidia test assessment

All toxicity tests (glochidia and juveniles) were conducted according to the standard guide for conducting toxicity tests with the early life stages of freshwater mussels [54]. Mean temperature (range in parentheses) of glochidia in culture water upon arrival to the laboratory was 17.4 ^{0}C (13-22 ^{0}C). Glochidia were acclimated to the reconstituted hard water [48] and the test temperature of 20 ^{0}C by being placed into a 1:1 mixture of culture and reconstituted water for 2 h, allowing for a 2 ^{0}C/h maximum rate of change. Glochidia were used in tests when initial viability was assessed at ≥90% using an Olympus SZ61 microscope (Olympus America, Center Valley, Pennsylvania, USA) and QCapture Pro 5.1 digital photographic software (Quantitative Imaging, Surrey, British Columbia, Canada). Viability (survival) was assessed by exposing glochidia to a saturated sodium chloride solution and individuals exhibiting a shell-closure response were considered viable. Static, water-only acute toxicity tests were conducted for 48 h, with viability assessed at 24 h and 48 h on subsamples of approximately 50 of the 150 glochidia in each of three replicates for a given treatment. Test acceptability is specified to be >90% viability in the control treatment at 48 h [54]; control viability in our tests averaged 93% at 48 h. All tests were conducted in light and temperature controlled incubators (Precision Model 818 Thermo Fisher, Marietta, Ohio, USA) held at 20 ^{0}C and a light:dark cycle of 16:8 h.

Juvenile test assessment

Mean temperature (range in parentheses) of juvenile mussels in culture water upon arrival to the laboratory was 19.8 ^{0}C (17–23 ^{0}C). Juveniles were acclimated to the reconstituted hard water [48] and the test temperature of 20 ^{0}C by being placed into a 1:1 mixture of culture and reconstituted water for 2 h, allowing for a 2 ^{0}C/h maximum rate of change, followed by a 1:3 ratio for an additional 2 h and then 100% reconstituted water for 72 h prior to test initiation. Static, water-only renewal tests were conducted for 96 h with a ≥90% water and chemical renewal at 48 h [54]. Survival was assessed at 48 and 96 h exposure time points by observing for foot movement outside or inside the shell or a heartbeat within a 5-min period. For each test, control replicates (x3) contained 10 juveniles each, whereas all other treatment replicates (x3) contained 7 individuals. Test acceptability is specified to be >80% survival in the control treatment at 96 h [54]; control survival in our tests averaged 99% at 96 h. All juvenile acute toxicity tests were conducted under the same temperature and light cycle conditions as for glochidia tests.

Water chemistry

Water chemistry analyses were performed at the 48 h time point for both glochidia and juvenile toxicity tests. Mean (range in parentheses) water quality conditions during the experiments were as follows: 134.5 mg $CaCO_3$ /L alkalinity (116–166 mg/L), 160.7 mg $CaCO_3$/L hardness (150–186 mg/L), 579.4 µS/cm conductivity (527–750 µS/cm), 8.4 pH (7.32–8.64), and 8.8 mg/L dissolved oxygen (6.66–9.47 mg/L) ; n=36 determinations for alkalinity and hardness, n=144 determinations for all other variables). Alkalinity and hardness were measured by titration following standard methods [55] and all other water quality parameters were conducted using a calibrated multi-probe system (YSI model 556 MPS, Yellow Springs Instruments, Yellow Springs, Ohio, USA).

Statistical analysis

The effect of each of the 6 PAM compounds on the survival of glochidia and juvenile mussels was used to determine the median lethal concentration (LC50) analyzed via the Trimmed Spearman-Karber method (Comprehensive Environmental Toxicity Information Software [CETIS], V1.8.0.12, Tidepool Scientific, LLC, McKinleyville, California, USA). The LC50 is the measure of toxicity and defined as the toxicant concentration resulting in the mortality of 50% of individuals exposed in the specified time period. Mortality was determined for glochidia by failure to respond via shell-closure to NaCl. Juvenile mortality was determined by observing no foot movement or heartbeat for individual mussels during the 5-min assessment period. In tests where sufficient mortality occurred to allow calculation of LC50s, values were considered significantly different when 95% confidence intervals did not overlap [56]. Water chemistry variables from all tests were analyzed by one-way analysis of variance (ANOVA) in SAS (SAS Institute, Cary, North Carolina, USA) to assess any attributable mortality due to variation in water chemistry during PAM exposures. All measured variables (alkalinity, hardness, pH, dissolved oxygen, conductivity and temperature) were not significantly different among tests, indicating no appreciable change in chemistry resulting from PAM compound.

RESULTS

After exposing both glochidia and juvenile mussels to each of the 6 PAM compounds at concentrations up to 1,000 mg/L only the AN 923 compound elicited mortality sufficient to calculate an LC50 for *L. cariosa* glochidia at the 24 or 48 h time points (Table 3). The 24 h LC50 was 833.4 mg/L (95% CI, 769.7–902.4 mg/L) and decreased to 411.7 mg/L (373.4–454.0 mg/L) at 48 h. For juvenile *L. cariosa*, AN 923 and AN 923 SH had 96 h LC50s of 126.8 mg/L (99.9–161.0 mg/L) and 563.2 mg/L (414.2–765.8 mg/L), respectively (Table 3). All other compounds showed no evidence of acute toxicity to either life stage at the highest concentration tested (no observed effect concentration [NOEC] = 1000 mg/L). The only test resulting in the calculation of an LC50 for *A. raveneliana*, the federally endangered species, was the 96 h juvenile exposure to AN 923 (329.8 mg/L: 95% CI 289.2–376.1 mg/L). Similarly, the only test that resulted in the calculation of an LC50 for *M. nervosa* was the 96 h juvenile exposure to AN 923 (705.5 mg/L: 95% CI 575.5–864.8 mg/L; Table 3).

DISCUSSION

We found that the acute toxicity of the 6 PAM compounds tested varied with mussel life stage (juveniles more sensitive than glochidia), species (*Lampsilis cariosa* most sensitive), and chemical properties of the compound (molecular weight, charge density, and net charge), but exhibited relatively low toxicity overall, compared to the concentrations commonly applied for aquatic turbidity control. Of the 36 tests conducted with the early life stages of freshwater mussels and the 6 PAM compounds, 7 yielded calculable LC50 concentrations. Much of the previous toxicological research conducted on PAM and aquatic organisms had not generated median lethal concentrations, instead, provided a NOEC (LC50 was greater than the highest concentration tested; Table 3). Even with testing a maximum PAM concentration of 1000 mg/L, many trials resulted in a NOEC at that highest concentration and demonstrates that the risk of environmental PAM exposure to freshwater mussels seems minimal, especially at the 1 to 5 mg/L concentrations where it is most effective for turbidity control [29,31]. For even the most toxic PAM tested (AN 923), there was a 24- to 126-fold margin of safety from common treatment concentrations.

The relative lack of acute toxicity in our tests with anionic PAM and early life stages of native freshwater mussels compare similarly to previous acute toxicological studies of anionic PAM with other aquatic organisms (Table 3). For example, Weston et al. [24] found no significant mortality for the green alga *Selenastrum capricornutum*, aquatic invertebrates *Hyalella azteca*, *Chironomus dilutus*, and *Ceriodaphnia dubia* and the fish *Pimephales promelas*, during exposures at the greatest concentration tested, 100 mg/L. However, Beim and Beim [57] reported a 96 h LC50 for *Daphnia magna* of 14.1 mg/L and Biesinger et al. [58] reported a 96 h LC50 for the same species of 17.0 mg/L, indicating a greater degree of toxicity and sensitivity than what we found for freshwater mussels. Toxicity appeared to be dependent upon the chemical properties (molecular weight, charge density and net charge) of each PAM compound. According to Bolto [23], anionic PAM toxicity is positively correlated with molecular weight. However, our results indicate that it may actually be the inverse for freshwater mussels, as we saw increasing toxicity with decreasing molecular weight when accompanied by an increase in charge density. In fact, juvenile mussels exposed to the highest molecular weight PAMs (AN 913 VHM and AN 923 VHM) at 1000 mg/L experienced greater than 90% survival among all species with the exception of *L. cariosa* (AN 913 VHM 52%), and juvenile survival was the least when exposed to the lowest molecular weight anionic PAM, AN 923 (< 9.5% survival). The aforementioned toxicity indicates a possible trend that appears to be the result of increased exposure to the co-monomer present in the compound. In this study, we tested three PAMs with a charge density of 23% and varying molecular weights: AN 923, AN 923 SH, and AN 923 VHM. AN 923 elicited the greatest level of toxicity and is distinguished from this group by the lowest molecular weight (9 to 12 Mg/mole). Further evidence of the trend was the resulting serial toxicity of AN 923 SH (12 to 14 Mg/mole) to the most sensitive mussel species, *L. cariosa*. Thus, toxicity may be a result of reduced molecular weight allowing for greater accessibility of PAM to freshwater mussels. Shorter PAM chains may more readily access the internal organs of mussels via the incurrent siphon disrupting the biological processes. Beim and Beim [57] attributed mortality in aquatic invertebrates to the sorption of PAM on surface membranes resulting in decreased efficiency in biological functions, such as respiration, feeding, and reproduction. Further toxicity research with anionic PAMs at greater charge densities may provide more clarity as to which components are eliciting toxicity and aid in identifying trends in compound toxicity.

Previous environmental contaminant research on the sensitivity of freshwater mussel early life stages has found glochidia to typically be more sensitive than juveniles [38]. However, because PAM may elicit mortality via membrane sorption and inhibition of essential biological functions, it was not surprising that we detected a higher degree of sensitivity in the more anatomically advanced juveniles. Glochidia lack many of the structures that are found in more advanced life stages, including gills, which are responsible for gas exchange and a likely site of sorption resulting in mortality [59]. In fact, of the seven tests that resulted in the calculation of an LC50, six were for juveniles and just one for glochidia.

We found varied responses among freshwater mussel species to the exposure of PAM. *L. cariosa* was the most sensitive to PAM exposures, which resulted in the lowest LC50 (126.8 mg/L). *L. cariosa* was the only species to have sufficient mortality to calculate an LC50 (833.4 mg/L) for glochidia, and the only species with a calculated LC50 for a compound other than AN 923 (AN 923 SH). *A. raveneliana* was not as sensitive, with just one test eliciting sufficient toxicity to estimate an LC50 (229.8 mg/L). The most tolerant of the species tested was *M. nervosa,* also with just one test resulting in an LC50 estimate (705.5 mg/L).

Given the relatively low toxicity of PAM to freshwater mussels observed during this study, the benefits of PAM use for turbidity control may supersede the risk of toxic effects. Freshwater mussels can be greatly affected by suspended sediments and the contaminants associated with them [60,61]. PAM also has the potential to decrease certain chemical toxicants and reduce nutrient loading [62,63]. Aldridge et al. [14], experimentally exposed three unionid species, the Pimpleback (*Quadrula pustulosa*), Gulf Pigtoe (*Fusconaia cerina*), and Mississippi Pigtoe (*Pleurobema beadleanum*) to frequent high levels of suspended sediment (600 mg/L every 0.5 h) and observed a reduction in filtering clearance rate. Such a reduction could lead to growth retardation, reproduction failure, or ultimately mortality due to starvation. The use of PAM may effectively reduce the amount of sediment entering receiving waters to decrease the stress of excess sediment on this ecologically important group of imperiled organisms [28,64].

Strayer [65] found that freshwater mussel declines caused by anthropogenic activity could lead to measurable changes in ecosystem structure and function. Other studies have described bivalves as keystone species due to their functional role in primary production, nutrient cycling, and other biological and chemical activities [66–68]. Freshwater mussels perform essential ecological processes including filtration, nutrient cycling, biodeposition and bioturbation [69,37,68,70]. Unfortunately, the decline and extinction of many species in this group has occurred almost unnoticed, and identifying a single cause for the decline has been difficult due to the multiple stressors impacting water quality and habitat [37,71].

The aim of this study was to determine the acute toxicity of anionic PAM to native freshwater mussels, as it is used for the reduction of turbidity in construction effluents. However, anionic PAM is also used in many different industries such as water and wastewater treatment, paper processing, mining, and others. Recent research has shown the efficacy of PAM used for other aquatic applications that apply directly to water systems such as infiltration barriers in water delivery systems and as an algal flocculant to remove unwanted algae [46, (K.J Iwinski, 2013, Master's thesis, Northern Michigan University, Marquette, Michigan)]. These applications potentially require the addition of a PAM concentration or an environmental PAM concentration (698 and 386 mg/L, respectively) greater than several of the LC50 values calculated during this study, to be effective for their desired use.

CONCLUSION

In an effort to understand the environmental safety of some of the chemical tools being applied for turbidity control in aquatic systems, this research focused on assessing the toxicity of selected anionic PAM compounds to early life stages of native freshwater mussels. Our findings indicate that that anionic PAM poses a minimal risk to freshwater mussels at optimal turbidity control concentrations of 1 mg/L to 5 mg/L (a 24- to 126-fold margin of safety), as 126.8 mg/L was the lowest 96 h LC50 calculated (juvenile *L. cariosa*). Furthermore, the hazard is reduced by the minimal likelihood of exposure due to the irreversible binding of PAM and sediment during environmental applications [23]. It is, however, not possible to ascribe a level of toxicity to a class of compounds as large and varying in chemical properties as anionic PAMs without additional toxicity testing or identification of the mode(s) of action. In fact, our results highlight the differences in mussel species sensitivity and the toxicity of anionic PAM compounds, with generated LC50s ranging from 126.8 to greater than 1000 mg/L. Our findings advance current knowledge of PAM toxicity to aquatic organisms and can be used to inform management decisions regarding turbidity control in the presence of common or imperiled freshwater mussels.

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TABLES

Table 1. Comparative acute toxicity of anionic polyacrylamide (PAM) to aquatic organisms.

Species	Common name	Duration	LC50 (mg/L)	Source
Raphidocelis subcapitata	Green algae	96 h	>100	[24]
Chironomus dilutes	Midge	96 h	>100	[24]
Pimephales promelas	Minnow	7 days	>100	[24]
Phoxinus phoxinus	Minnow	96 h	>1000	[57]
Hyalella azteca	Amphipod	96 h	>100	[24]
Eulimnogammarus verrucosus	Amphipod	96 h	2100	[57]
Baicalobia guttata	Flatworm	96 h	>100	[57]
Ceriodaphnia dubia	Water flea	6-8 days	28.7	[24]
Daphnia magna	Water flea	96 h	14.1	[57]
Daphnia magna	Water flea	48 h	345	[58]
Daphnia magna	Water flea	96 h	17	[58]
Daphnia magna	Water flea	48 h	218.1	[72]

Table 2. Properties of selected anionic and non-ionic polyacrylamide (PAM) compounds used in acute toxicity tests with larval (glochidia) and juvenile freshwater mussels. Information derived via SNF online product brochure at http://www.snf-group.com/images/pdf/Brochures_in_English/Catalogue%20Poudres.pdf.

Compound	Net charge	Charge density %	Molecular weight classification	Molecular weight (Mg/mole)
FLOPAM™ AN 913 VHM	Anionic	13	Ultra High	13–16
FLOPAM™ FA 920	Non-ionic	Non-ionic	High	5–6
FLOPAM™ AN 923 SH	Anionic	23	Very High	12–14
FLOPAM™ AN 923	Anionic	23	Standard	9–12
FLOPAM™ AN 923 VHM	Anionic	23	Ultra High	14–17
APS 705	Anionic	NA	NA	NA

NA=Information not available for product.

Table 3. Median lethal concentrations (LC50s) for acute toxicity of anionic polyacrylamide (PAM) to native freshwater mussels (95% CI). Acute exposures to the following PAM compounds resulted in insufficient mortality to calculate an LC50s: APS 705, FLOPAM™ FA 920, FLOPAM™ AN 923 VHM, and FLOPAM™ AN 913 VHM.

Species	Life stage	Time point (h)	FLOPAM™ AN 923 LC50 (mg/L)	FLOPAM™ AN 923SH LC50 (mg/L)
Alasmidonta raveneliana	Glochidia	24	>1000	>1000
		48	>1000	>1000
	Juvenile	48	>1000	>1000
		96	329.8 (289.2–376.1)	>1000
Lampsilis cariosa	Glochidia	24	833.4 (769.7–902.4)	>1000
		48	411.7 (373.4–454.0)	>1000
	Juvenile	48	183.2 (139.8–240.2)	>1000
		96	126.8 (99.9–161.0)	563.2 (414.2–765.8)
Megalonaias nervosa	Glochidia	24	>1000	>1000
		48	>1000	>1000
	Juvenile	48	>1000	>1000
		96	705.5 (575.5–864.8)	>1000

APPENDIX 2

Sublethal effects of turbidity, sediment, and polyacrylamide on native freshwater mussels (Accepted by Journal of American Water Resources Association)

ABSTRACT

Turbidity generated from sediment erosional processes is a ubiquitous pollutant adversely affecting water quality and aquatic life in waterways across the United States and elsewhere. Anionic polyacrylamide (PAM) is widely used as a chemical flocculent and has become an effective tool for reducing the impacts of suspended sediment and turbidity on aquatic ecosystems. However, no information exists on the toxicity of PAM-flocculated sediments to the imperiled, but ecologically important freshwater mussels of the family Unionidae. We conducted acute (96 h) and chronic (24 d) laboratory tests with juvenile fatmucket (*Lampsilis siliquoidea*) and three exposure conditions (non-flocculated settled sediment, suspended sediment, and PAM-flocculated settled sediment), over a range of environmentally relevant turbidity treatments (50, 250, 1,250, and 3,500 nephelometric turbidity units; NTU). We found no effect of turbidity treatment or exposure condition on mussel survival in either the acute or chronic tests, suggesting a high level of tolerance for *L. siliquoidea* in short-term exposures. In contrast, we found significant reductions in protein concentration, ATP production, and oxidized proteins in mussels acutely exposed to suspended sediment, indicating physiological protective responses that limit energy production and reactive oxygen species accumulation under unfavorable environmental conditions. Our results suggest that anionic PAM applied to reduce suspended sediment may be effective at minimizing the adverse effects of short-term turbidity exposure on juvenile freshwater mussels without eliciting additional lethal or sub-lethal toxicity. These findings will facilitate improved management and regulatory decision making for turbidity control best management practices in waters where freshwater mussels reside.

(KEY TERMS: turbidity, invertebrates, toxicology, erosion, best management practices [BMP’s], environmental impacts)

INTRODUCTION

The negative effects of erosion and suspended sediment on aquatic habitat and freshwater fauna have been well described (Ellis, 1936; Cordone and Kelley, 1961; Berkman and Rabeni, 1987; Newcombe and MacDonald, 1991; Wood and Armitage, 1997; Henley *et al.*, 2000; Gray *et al.*, 2014). Deleterious effects on aquatic ecosystems are often the result of the physical and chemical alterations that include sedimentation, light attenuation, and associated adsorbed contaminants (Thoms and Thiel, 1995; Henley *et al.*, 2000; Davies-Colley and Smith, 2001; Bilotta and Brazier, 2008). Urbanization, mining, road construction, and intensive agricultural practices can all lead to increased erosion and influxes of sediment to surface waters (Wolman and Schick, 1967; Lenat and Crawford, 1994; Henley *et al*., 2000; Wilkinson and McElroy, 2007). These activities can result in landscape alterations permuting the natural hydrology, increasing runoff velocity, and sediment loading (Henley *et al.*, 2000). In fact, Wilkinson and McElroy (2007) estimated the global anthropogenic erosion rate to be 75 Gigatons annually, which far surpasses the estimated 21 Gigatons per year from natural erosional processes. The United States Environmental Protection Agency has concluded that nearly half of the waterways in the U.S. are significantly impaired by sediment and has designated sediment the primary pollutant of aquatic environments (U.S. Environmental Protection Agency, 1990).

The term turbidity is often used to describe the visual clarity of water when assessing the ecological relevance of suspended sediment, but it also encompasses dissolved organic matter, exogenous pollutants, plankton, and microorganisms (Kirk, 1985; Davies-Colley and Smith, 2001). Turbidity can be quantified with a variety of methods, one of which measures the refraction of light through water using a turbidimeter (Kirk, 1985; Lloyd, 1987). This method generates a standard metric of nephelometric turbidity units (NTUs) that is used by many regulatory agencies to monitor suspended particles (O'Dell, 1993; Standard Methods, 1995; Davies-Cooley and Smith, 2001). In this laboratory study, turbidity refers only to the suspended sediment fraction of inorganic sediments, which is the prevailing component contributing to turbidity during episodes of excessive runoff from disturbed soils (Lloyd, 1987; U.S. Environmental Protection Agency, 2005). Understanding and monitoring this standard measure for suspended sediment is critical for the conservation and restoration efforts of both aquatic habitat and dependent biota.

Increased turbidity has been associated with adverse abiotic factors, such as decreased dissolved oxygen, light penetration, and increased water temperature. These changes have resulted in reduced diversity and biomass of primary producers (macrophytes, periphyton, and phytoplankton) in aquatic systems causing a cascade of deleterious effects on freshwater communities (Ellis, 1936; Chandler, 1942; Kirk, 1985; Van Nieuwenhuyse and LaPerriere, 1986; Lloyd *et al*., 1987; Davies-Colley *et al*., 1992; Wood and Armitage, 1997; Bilotta and Brazier, 2008). The adverse effects of turbidity on fish have also been well studied, from highly sensitive salmonid species to more tolerant warmwater species (Muncy 1979; Lloyd *et al*. 1987; Sigler *et al*. 1984). Research has identified a multitude of negative impacts and responses in fish as a result of suspended sediment exposure including avoidance, reduced hatching success, altered predator-prey interactions, damaged gill tissue, and direct mortality (Bisson and Bilby, 1982; Lake and Hinch, 1999; Sweka and Hartman, 2003; Sutherland and Meyer, 2007; Gray *et al.*, 2012). However, the effects of turbidity on native freshwater mussels (family Unionidae), the most imperiled faunal group in North America (Williams *et al*., 1993) have yet to be fully investigated, especially during early life stages.

Efforts to reduce suspended sediment released from construction sites to meet regulatory requirements have advanced through the implementation of a variety of Best Management Practices (BMPs). Many of these techniques are designed to reduce erosion by decreasing the velocity of runoff, thereby reducing the energy potential required to erode and suspend sediment. However, to remove the smallest fraction of suspended sediment < 20 μm from runoff effluent, chemical flocculants such as polyacrylamide (PAM) are used (Ward *et al.*, 1980). PAM is a commercially available water-soluble polymer used in many different industries as a flocculating agent (Sojka *et al*., 2007). PAM has been shown to reduce the turbidity of runoff by as much as 91% before reaching receiving waters (Soupir *et al*., 2004), especially when used in conjunction with other BMPs (McLaughlin and Bartholomew, 2007; McLaughlin and McCaleb, 2010; Kang *et al*., 2013a). Given the relatively high efficacy and putative low toxicity to aquatic organisms, chemical flocculants such as anionic PAM are quickly becoming an essential chemical tool to mitigate the well-studied impacts of increased suspended sediment on aquatic biota (Matson *et al*., 1997; Wood and Armitage, 1997; Sojka *et al.*, 2007; Weston *et al*., 2009; Kang *et al.*, 2013a; Buczek, in press). However, more information is needed about the possible chemical and physical interactions of PAM within the environment to understand its risks and to determine its efficacy for mitigating the effects of suspended sediment on freshwater organisms in a safe manner.

Although previous studies have been conducted on the effects of suspended sediment and PAM toxicity to a variety of freshwater organisms (Bisson and Bilby, 1982; Sigler *et al*., 1984; Beim and Beim, 1993; Lake and Hinch, 1999; Capper, 2006; Sutherland and Meyer, 2007; Weston *et al*., 2009; Acharya *et al.*, 2010; Robinson *et al.*, 2010; Gray *et al.*, 2012), none has attempted to elucidate the potential toxicity of the compounds when flocculated, especially to benthic dwelling freshwater mussels. The early life stages (glochidia and juveniles) of freshwater mussels are disproportionally sensitive to certain environmental contaminants and to other anthropogenic stressors that impact aquatic habitat, facilitating precipitous declines in their diversity and abundance (Keller and Zam, 1991; Williams *et al.*, 1993, Williams and Neves, 1995; Richter *et al.*, 1997; Augspurger *et al.*, 2003; Mummert *et al.*, 2003; Strayer *et. al*., 2004; Cope *et al.*, 2008; Archambault *et al.*, 2014). Thus, utilizing the sensitivity and exposure-response data from freshwater mussel toxicity tests to derive water quality criteria or environmentally acceptable levels may also be protective of other aquatic organisms. For these reasons, freshwater mussels have been used extensively over the past two decades as bioindicators of water quality (Gagné, 2002; Gundacker, 2000; Labieniec and Gabryelak, 2007), and there is growing interest in physiological, chemical, and molecular biomarkers to quantify sublethal responses of mussels to environmental toxicants (Gillis *et al.*, 2014; Machado *et al.*, 2014; Ridgway *et al.*, 2014).

Oxidative stress is one such biomarker, widely used in aquatic organisms for detection of the molecular response to environmental pollutants (Valavanidis *et al.*, 2006; Lushchak, 2011). Oxidative stress is the result of an imbalance of the steady-state reactive oxygen species (ROS) concentration and an organism's antioxidant defense system. When ROS generation exceeds steady-state, oxidative damage can occur to macromolecules such as DNA, proteins, and lipids (Lushchak, 2011). This damage can manifest as tissue damage, inflammation, disease, and aging (Goto *et al.*, 1999; Sohal, 2002). Indicators of general health and metabolism, such as adenosine triphosphate (ATP) and protein concentrations are also useful in the identification of induced biochemical processes and stress.

The specific objective of this study was to determine the relative sensitivity of juvenile freshwater mussels to a range of sediment and PAM-treated sediment conditions using survival and the sublethal endpoints of protein oxidation, ATP production, and protein concentration as measures of toxicity. The overall goal of this research was to assess the practice of applying PAM to aquatic ecosystems in relation to potential impacts on freshwater mussels.

MATERIALS AND METHODS

Experimental design and conditions

Two separate tests were conducted to evaluate the acute (96 h) and chronic (24 d) responses to three different sediment exposure conditions: suspended sediment, PAM-flocculated settled sediment, and non-flocculated settled sediment. Identical design and protocols were followed during both the acute and chronic tests. Target treatment concentrations of turbidity during the experiment were 0, 50, 250, 1,250, and 3,500 NTU. There were three replicates per treatment with 15 juvenile mussels in each replicate, including the control. Water chemistry analysis was performed at every 96-h time point, and the sediment was retained for particle size and total suspended solids analysis at the conclusion of each test. These three test conditions represented the three main possible scenarios of sediment exposure for freshwater mussels in the environment. Following total suspended solids determination protocols outlined by Clesceri *et al.*, (1998), each treatment replicate was vacuum filtered to a pre-weighed 1.5 µm Whatman glass fiber filter, baked at 120^{o}C until completely dry, and weighed [Figure A1]. Surviving juvenile mussels at the end of the tests were placed into 1.5 ml microcentrifuge tubes and submerged in midRIPA lysis buffer (25 mM Tris [pH 7.4], 1% NP-40, 0.5% sodium deoxycholate, 15 mM NaCl) then stored in an ultracold (-80 °C) freezer. For comparison, three representative baseline samples of *L. siliquoidea* were stored in an identical fashion and placed in an ultracold (-80 °C) freezer directly upon arrival for later biomarker analysis.

Test sediment

Sediment was obtained from the Sediment and Erosion Control Research and Education Facility (SECREF) at North Carolina State University (Raleigh, North Carolina, USA). The sediment originated from a nearby road construction site and had been previously characterized and used in several research projects; the sediment characteristics were as follows: 42.5% sand, 17.2% silt, 40.3% clay and USDA classification of clay. A working stock sediment was prepared by first passing the dried soil through a No. 10 standard testing sieve (VWR Scientific, Radnor, Pennsylvania, USA), and the resulting soil ≤ 2 mm was baked at 120ºC for > 24 h in a Fisher Scientific 600 series Isotemp standard oven to eliminate any indigenous organisms. To ensure the stock sediment sample would remain suspended under minimal agitation during laboratory exposures, dry sediment was eluted in

ASTM hard water (ASTM 2006a) and processed through a sequential series of mixing, settling, and decanting steps. The final supernatant particle size distribution was 12.2 μm (standard deviation: 11.8 μm) as determined by a Beckman Coulter LS particle size analyzer (Pasadena, California, USA) by the Department of Marine, Earth, and Atmospheric Sciences (MEAS) at North Carolina State University (Raleigh, North Carolina, USA) according to their standard methods.

To ensure that the test sediment was relatively uncontaminated and that any observed effects were not attributable to the presence of any potential toxicants, samples of sediment were analyzed for a suite of 22 metal and 146 organic compounds, including polycyclic aromatic hydrocarbons, polychlorinated biphenyls, legacy organochlorine pesticides and current use pesticides. The metal analysis was performed at RTI International (Research Triangle Park, North Carolina, USA) using a Thermo iCAP6500 ICP-OES (inductively coupled plasma optical emission spectrometer) following U.S. Environmental Protection Agency Method 200.7 and U.S. Environmental Protection Agency Method 3050B and their approved protocols. Triplicate readings of each sample were taken, the average of the three readings was reported as the final concentration. A rigorous quality assurance protocol was followed for the metals analyses that included reagent blanks, reagent blank spikes, duplicates, matrix spikes, and surrogate internal standards. Average recovery in surrogate standards was 95% (range 76 – 113%), relative percent difference of duplicates averaged 10% (range 0 – 20%), recovery of matrix spikes averaged 81% (range 29 – 105%), and reagent blanks were uncontaminated. None of the measured metals was of toxicological concern to mussels or other aquatic life (U.S. Environmental Protection Agency, 2009).

Organic contaminants in test sediment were analyzed in the Analytical Toxicology Laboratory at North Carolina State University, Raleigh, North Carolina, USA, with gas chromatography-mass spectrometry following standard approved procedures. A rigorous quality assurance protocol was followed for organics analyses and included procedural blanks, and surrogate internal standards. Quality assurance controls were all within acceptable levels and the surrogate recoveries for organic analysis were all between 67 and 97%. None of the measured organics were present at levels of toxicological concern to mussels or other aquatic life (U.S. Environmental Protection Agency, 2009).

Suspended sediment exposure

In an effort to maintain a constant exposure turbidity, the processed and highly concentrated sediment stock was added to each test beaker to achieve the target turbidity level. Turbidity was measured daily using a turbidimeter (Analite NEP260, Observator Instruments, formerly, McVan Instruments, Scoresby, Australia) and concentrated stock was added as needed to maintain the treatment turbidity at the desired treatment target. Suspended sediment treatments were maintained through the use of a Lab Companion® Multiposition magnetic stirrer (Billerica, Massachusetts, USA) and a 3.8 cm stir bar rotated at 70 rpm to provide the minimal agitation needed to maintain a suspension. Mussels were held in open top cylindrical cages constructed of 1 mm Nitex® mesh (dimensions: H: 11.5 mm, D: 39.0 mm) bound together with 0.4 mm stainless steel wire. All cages were suspended ~3 cm from the bottom of the 400 ml glass test beaker using 3 stainless steel wire support arms affixed over the lip of the beaker. The mean (range in parenthesis) measured turbidity during the 96-h assessment time points for each treatment was 56 NTU (7–113), 236 NTU (125–398), 1120 NTU (850–1488), and 3562 NTU (3325–3850).

Settled sediment exposure

Similar to the suspended sediment exposure, stock sediment was added to reach the equivalent target treatment turbidity of 50, 250, 1,250, and 3,500 NTU using a multiposition magnetic stirrer and a 3.8 cm stir bar rotated at 70 rpm to provide the minimal agitation needed to maintain a suspension and quantified using a turbidimeter (Analite NEP260). However, mussels exposed under this condition were added directly to the 400 ml beaker (no cages) after the stir bar was removed and sediment was allowed to settle.

Flocculated sediment exposure

Stock sediment was added as described for the previous exposures to reach the equivalent target treatment turbidity of 50, 250, 1,250, and 3,500 NTU. However, after the stir bar was removed anionic polyacrylamide FLOPAM AN 923 was added to all replicates and briefly stirred manually for a final PAM concentration of 5 mg/L. Mussels were then added directly to the 400 ml beaker, and flocculated sediment was allowed to settle. Directly prior to the 96-h assessment, a 10 ml sample of water was removed from each replicate and analyzed using the turbidimetric reagent, benzethonium chloride (Hyamine 1622, Acros Organics, Geel, Antwerp, Belgium) and methods described by Kang *et al.*, (2013b) to detect unbound or residual PAM concentrations. A standard curve was developed for the reactivity of PAM and benzethonium chloride using a stock solution of 1000 mg/L and turbidity readings for a serial dilution of PAM (0, 0.5, 1, 5, 10, 25, 50 mg/L). The flocculated sediment treatment concentrations were then compared to the standard curve values to determine the residual exposure concentration.

Test chemicals

FLOPAM AN 923 is a granular anionic polyacrylamide compound obtained from SNF Holding Company (Riceboro, Georgia, USA) and chosen for this experiment due to the relatively high degree of toxicity to freshwater mussels relative to other previously tested PAM compounds (Buczek, in press). A homogeneous stock solution of PAM (1000 mg/L) was prepared by slowly adding (approximately 1000 mg/min) PAM granules to ASTM hard water and mixing on a stir plate for 24 h at room temperature. The stock solution was used immediately following mixing and never stored for later use.

Test organisms

All sediment toxicity tests were performed with juvenile fatmucket (*Lampsilis siliquoidea*) provided by the mussel culture laboratory at Missouri State University (Springfield, Missouri, USA). *L. siliquoidea* juveniles were propagated by infecting host-fish (Largemouth bass; *Micropterus salmoides*) with glochidia using standard propagation and culture methods (Barnhart, 2006). *L. siliquoidea* is a common Interior Basin species widely-distributed and considered stable in the Mississippi and Gulf drainages of the U.S. (NatureServe, 2015) and have been used extensively in toxicological testing. Juvenile *L. siliquoidea* used for these experiments were approximately 17 months old, with an average ($\pm$ SD) shell length of 5.34 $\pm$ 0.80 mm.

Mussel assessment

Upon arrival at the laboratory, juvenile mussels were acclimated to the reconstituted hard water (ASTM, 2006a) and the test temperature of 20 ^{0}C by placement into a 50:50 mixture of culture and reconstituted water for 2 h, allowing for a 2 ^{0}C/h maximum rate of change, followed by a 25:75 mixture for an additional 2 h, and then 100% reconstituted water for 72 h prior to test initiation. Survival was assessed at 96 h by observing the occurrence of foot movement outside or inside the shell or a heartbeat within a 5-min period. Test acceptability in the acute (96 h) test was specified at > 90% control survival and the chronic (24 d) test acceptability was specified to be > 80% survival in the control treatment (ASTM, 2006b). All tests were conducted in light and temperature controlled incubators (Precision Model 818 Thermo Fisher, Marietta, Ohio, USA) and held at 20 ^{0}C and light:dark cycle of 16:8.

Water chemistry

Water chemistry analysis was performed at each 96-h time point for all toxicity tests. Mean (range in parentheses) water quality conditions during the experiments were as follows: 111 mg $CaCO_3$/L alkalinity (106–118), 163 mg $CaCO_3$/L hardness (150–170), 547 µS/cm conductivity (524–562), 8.21 pH (7.82–8.47), and 8.3 mg/L dissolved oxygen (7.5–8.8); n=6 for alkalinity and hardness, n=150 for all other variables. Alkalinity and hardness were measured by titration following standard methods (APHA, 1995) and all other water quality parameters were measured using a calibrated multi-probe system (YSI model 556 MPS, Yellow Springs Instruments, Yellow Springs, Ohio, USA).

Protein concentration/ATP

Whole mussels were homogenized in midRIPA lysis buffer (25 mM Tris (pH 7.4), 1% NP-40, 0.5% sodium deoxycholate, 15 mM NaCl) using a pestle inside of a 1.5 ml microcentrifuge tube. The samples centrifuged for 10 min at 16,000 rpm and the supernatant was saved for further analysis. We measured protein concentration, which has been previously used by Gillis *et al.* (2014) as an indicator of general health, to determine if subcellular stress corresponded to greater tissue level effects. Protein concentration was measured on a Bio-Rad SmartSpec3000 spectrophotometer following the manufacturer protocol for a protein assay kit (Bio-Rad, Life Science Research, Hercules, California, USA). Sample dilutions were made to uniform protein concentrations before analyzing for ATP concentrations by ATP dependent luciferin oxidation reactions. ATP production was quantified by the Enliten ATP assay system for bioluminescence detection kit and GLOMAX 20/20 Luminometer (Promega Corporation, Madison, Wisconsin, USA).

Oxidative stress-protein oxidation

Protein carbonyls can be used to determine protein oxidation and oxidative stress. Formed as a result of oxidative damage, protein carbonyls can be quantified by a series of derivatizing reactions following manufacturer Enzyme-Linked Immunosorbent Assay (ELISA) protocols (ENZO life sciences, Farmingdale, New York, USA). The sample was first reacted with dinitrophenylhydrazine (DNP) causing proteins to adsorb to the assay plate. The adhered proteins were then reacted with anti-DNP-biotin-antibody, streptavidin-linked horseradish peroxidase and chromatin. After the addition of an acid to stop the reactions, the absorbance was read at 450 nm using a plate reader (Multiscan FC, Thermo Scientific, Waltham, Massachusetts, USA). Each replicate sample was analyzed in duplicate, and samples were quantified by comparison with oxidized standards.

Statistical analysis

Comparisons of turbidity treatment concentrations (50, 250, 1,250, 3,500 NTU) and test conditions (non-flocculated settled sediment, suspended sediment, and PAM-flocculated settled sediment) were analyzed for normality (Shapiro-Wilk) followed by an analysis of variance (ANOVA) using SAS (SAS Institute, Cary, North Carolina, USA). To elucidate significance and rank between and within test conditions, results were further analyzed by Tukey's HSD post-hoc analysis ($\alpha = 0.05$).

RESULTS

Acute exposures

Juvenile *L. siliquoidea* exposed to the three sediment test conditions (non-flocculated settled sediment, suspended sediment, and PAM-flocculated settled sediment) for a duration of 96 h had 100% survival in all treatments. However, control survival during the chronic test dropped below the required 80% (ASTM, 2006b) in all test conditions at the 24-d assessment time point. Therefore, survival results presented are for the last acceptable assessment time point (20 d). Mussel survival at the 20-d assessment was not significantly different among conditions or turbidity level ($p>0.05$). Mean percent survival (range in parenthesis) in the settled sediment, suspended sediment, and PAM-flocculated sediment conditions was: 89% (80 – 98%), 84% (81 – 87%), and 89% (87 – 93%), respectively [Figure 1].

For the sublethal endpoints, analysis of protein concentration indicated no significant difference for mussels exposed to the range of treatment turbidity in the acute 96 h exposure [ANOVA: settled sediment ($p=0.167$), PAM-flocculated ($p=0.796$), suspended ($p=0.788$)]. However, there were significant differences with the effect of treatment condition on mussels. Using Tukey's HSD to test pairwise differences, we found that at lower turbidity concentrations [ANOVA: control ($p=0.018$), 50 NTU ($p=0.0004$), and 250 NTU ($p=0.006$)], mussels in the settled sediment and PAM-flocculated sediment conditions had higher protein concentrations than those in the suspended sediment conditions [Figure 2A]. When turbidity reached 1,250 NTU (ANOVA p=0.007) and 3,500 NTU (ANOVA p=0.018), mussels in the settled sediment had greater mean protein concentration than those exposed to the suspended sediment, with those in the flocculated sediment falling in between, and not different than either.

The acute (96 h) effect of turbidity on the production of ATP was different among the conditions [ANOVA: settled sediment (p=0.003), PAM-flocculated (p=0.021), suspended (p=0.191)]. ATP concentrations for mussels exposed to the settled sediment and PAM-flocculated sediment conditions generally declined with an increase in turbidity. Mussels in the lowest turbidity levels (control and 50 NTU) exposed to settled sediment had significantly greater concentrations of ATP than the two highest turbidity levels (1250 NTU and 3500 NTU), with the ATP concentration at 250 NTU falling in between [Figure 2B]. Similarly, ATP concentrations from mussels exposed to the PAM-flocculated sediment condition reached a significant low at the 1250 NTU level when compared to the control. We also found no effect of turbidity on ATP concentration for juvenile mussels exposed to the suspended sediment condition. When we analyzed for the effect of condition using Tukey's HSD, the suspended sediment condition had statistically lower ATP concentrations for all turbidity treatments but 1,250 NTU [control (p=0.001), 50 NTU (p=0.002), 250 NTU (p=0.01), 3,500 NTU (p=0.001)], where there was no significant difference between test conditions (p=0.219), [Figure 2B]. When we compared ATP concentrations from the acutely exposed mussels to the baseline mussel sample, those mussels stored in midRIPA lysis buffer and held in an ultracold (-80 ^{o}C) freezer upon arrival, we found that the baseline results were greater than 1.5 times those of the exposed mussels.

The analysis of protein carbonyl concentrations to detect protein oxidation during the acute exposure showed no effects of turbidity within the settled sediment (ANOVA p=0.104) and PAM-flocculated sediment (ANOVA p=0.542) conditions [Figure 2C]. However, within the suspended sediment condition, the concentration was lower at the 250 NTU level than the control or 3,500 NTU level (ANOVA p=0.001). This suggests no clear pattern of protein oxidation as a result of turbidity. The suspended condition had lower carbonyl concentrations than the settled or flocculated conditions for the 50, 250, and 3,500 NTU turbidities, again not showing a clear concentration-response pattern. The baseline mussels contained less than half the carbonyl than the exposed mussels, suggesting that there was increased protein oxidation under all test conditions.

Chronic exposures

Chronic exposure results for protein concentration revealed a similar trend. We found no significant differences based on treatment turbidity [ANOVA: settled sediment (p=0.49), PAM-flocculated (p=0.945), suspended (p=0.057)] and mussels exposed to the settled sediment condition had significantly greater protein concentration when compared to mussels exposed to the suspended sediment condition across all turbidity levels [control (p=0.03), 50 NTU (p=0.003), 250 NTU (p=0.0007), 1,250 NTU (p=0.001, and 3,500 NTU (p=0.04)]. In addition, mussels in the settled sediment condition also had significantly greater protein concentrations than those in the PAM-flocculated condition at turbidity levels 250 NTU and above [Figure 3A]. However, in all but one turbidity level (1,250 NTU), protein concentration was not significantly different between mussels in the PAM-flocculated condition and the suspended sediment condition.

When we analyzed the effect of turbidity on the production of ATP for chronically exposed (24 d) mussels, we found the inverse of our above acute (96 h) results in both the settled and PAM-flocculated sediment exposure conditions [ANOVA settled (p=0.001), PAM-flocculated (p=0.031)]. The greatest tissue concentrations of ATP found in the settled sediment condition occurred in the 1,250 NTU and 3,500 NTU turbidity levels, while the lowest concentration of ATP was found in the 50 NTU turbidity level [Figure 3B]. However, the only significant difference in ATP concentration found in the PAM-flocculated sediment condition was between the 50 NTU and 1250 NTU levels. Again, we found no effect of turbidity on ATP concentration for mussels exposed to the suspended sediment (ANOVA p=0.33). When we analyzed for the effect of condition using Tukey's HSD, we again found the chronic results to be the inverse of the acute, with mussels in the suspended sediment condition having statistically greater ATP concentrations for all turbidity levels except 1,250 NTU [control (p=0.014), 50 NTU (p=0.0003), 250 NTU (p=0.011), 3,500 NTU (p=0.025)], where there was no significant difference between test conditions (p=0.656), [Figure 3B]. The baseline ATP levels were 4 times greater than those of the chronically exposed mussels.

Chronic exposure results for protein oxidation revealed no significant differences across turbidity levels regardless of sediment conditions [ANOVA: settled sediment (p=0.711), PAM-flocculated (p=0.231), suspended (p=0.472)] and the same was true for condition effects, with the exception of the 50 NTU treatment level (ANOVA p=0.0001), [Figure 3C]. As in the acute tests, the baseline carbonyl concentrations were roughly 50% of the levels in the test mussels, regardless of treatment.

DISCUSSION

The results of this study illustrate the well-adapted behavior and physiology of juvenile *L. siliquoidea* to influxes in sediment (settled, suspended, and PAM-flocculated) during acute (96 h) and chronic (24 d) exposures. Notably, no mortality occurred during the acute exposures, even at the highest turbidity (3500 NTU) and there were no differences in mortality among the conditions and turbidity levels in the chronic test. The maximum turbidity level used was similar to, or higher than that reported in construction site runoff (Przepiora *et al*., 1998; Line and White, 2001; McCaleb and McLaughlin, 2008; McLaughlin *et al*., 2009), although spikes over 30,000 NTU can occur (McCaleb and McLaughlin, 2008; McLaughlin *et al*., 2009). There was little evidence for turbidity concentration related biochemical effects, but rather we observed a much greater difference when comparing the three experimental conditions.

The sublethal effects that we observed during the acute (96 h) and to a slightly lesser degree chronic (24 d) exposures, revealed that the suspended sediment exposures to juvenile *L. siliquoidea* resulted in significant decreases in protein concentration when compared to the settled sediment and PAM-flocculated sediment test conditions. This difference was maintained even when comparing control treatments, indicating strong condition related differences. One possible explanation for the observed decrease in protein concentration may be due to the condition design, as mussels in the suspended sediment treatments were subjected to a circular flow pattern generated to maintain a homogenous suspension of sediment. This increased flow may have initially elicited an energetically expensive elevation in clearance rate (Ackerman, 1999; Riisgård and Larsen, 2000) of nutrient poor inorganic sediment, contributing to possible injury and a greater energy deficit inducing shell closure and anaerobic metabolism (Ortmann and Grieshaber, 2003), which has been shown to further reduce the conversion of glucose to proteins (De Zwaan and Wijsman, 1976). Similarly, results from a study of environmental contaminant exposure to freshwater mussels found that protein concentration of exposed *Lasmigona costata* was significantly reduced with proximity to contamination (Gillis *et al*., 2014). Gillis *et al.* (2014) proposed that this reduction could be the result of altered resource partitioning as a strategy for survival. The primary mechanism for mussels to reduce subcellular energy demands is to convert to anaerobic metabolism, which could also explain the lack of significance along the turbidity gradient by excluding outside influence (De Zwaan and Wijsman, 1976).

During our acute test, we found an overall decreasing trend in ATP concentrations with increasing turbidity for both the settled sediment and PAM-flocculated sediment conditions, indicating cellular exposure related effects. However, no significant treatment differences in ATP were found in mussels exposed to suspended sediment, again possibly indicating shell closure avoidance behavior and physiochemical alterations to compensate for internal oxygen availability (McMahon, 1988). ATP is a coenzyme generated by organisms during aerobic respiration and used as energy currency within cells to perform essential biological functions. According to Conners (2004), the shift from aerobic to anaerobic metabolism reduces energy production by nearly 90%, explaining the significantly reduced

ATP concentrations in the suspended sediment condition. A reduction in ATP may also result in an inability of an organism to support regular cell processes, including ion regulation (O'Donnell *et al.*, 1996). Interestingly, chronic ATP results revealed an inverse condition rank with extended exposure, suggesting that early metabolic conversion may be an advantageous shift limiting further impairment. While anaerobic metabolism may reduce total energy production, it also greatly reduces energy demand as mussels perform fewer biological functions (De Zwaan and Wijsman, 1976).

Oxidative stress has become widely recognized as a mechanism of many disease processes, and protein oxidation is an important biomarker indicating oxidative damage by reactive oxygen species (Dalle-Donne *et al.*, 2003). In fact, Gillis *et al.* (2014) found that elevated levels of oxidative stress resulted in tissue damage that affected gill function in mussels. To quantify protein oxidation, we identified the production of protein carbonyls in homogenized whole body tissue samples of juvenile *L. siliquoidea*. Acute exposure results of this analysis revealed no significant treatment difference for the settled sediment and PAM-flocculated sediment conditions and a decreasing trend in protein oxidation for mussels exposed to the suspended sediment condition to a low of 0.422 nmol/mg at the 250 NTU treatment level before again increasing with increased turbidity. Interestingly, mussels in the suspended sediment condition that had previously demonstrated negative impacts to both protein concentration and ATP production appeared to show significantly less oxidative stress compared to their counterparts in the settled sediment and PAM-flocculated sediment conditions. These results lend more evidence to the conjecture that poor nutrition and increased flow elicited a protective shell closure avoidance response of the mussels that minimized damage and energy demand by sequestering them from unfavorable external conditions. However, anaerobic respiration is not feasible for prolonged periods, as it too leads to the accumulation of deleterious end products (De Zwaan and Wijsman, 1976; McMahon, 1988). Analysis of protein carbonyl concentration from our chronic exposure revealed a homogeneity among all treatments and conditions, with the exception of the 250 NTU treatment level, where the suspended sediment condition showed significantly higher protein carbonyl concentration. These results suggest a balance achieved over time through behavioral, physiological, and molecular alterations.

Bilotta *et al.* (2012) highlighted the ecological importance of understanding the natural suspended sediment regime and not only the amount of suspended sediment entering the system. Measuring the rate of erosion, as well as the influx and duration of sediment is essential to understanding the impacts to the system. These findings are in alignment with the literature review by Strayer *et al.* (2004), who found that erosion due to habitat alteration is among the most cited causes for mussel declines. Turbidity has been implicated in deleterious effects on all life stages of freshwater mussels, from reduced glochidia attachment to host fish and transformation success into juveniles (Beussink, 2007), juvenile recruitment failure (Osterling *et al.*, 2010), to reductions in adult filtration clearance rate, with consequences of growth retardation and mortality due to starvation (Aldridge *et al.*, 1987). Given these findings and the ubiquitous nature of turbidity and its stochastic temporal pattern, practices to limit its influx to aquatic systems are critical.

According to Weston *et al.* (2009), anionic PAM is recognized as safe for applications that may discharge to aquatic systems, and PAM has been shown to effectively reduce the turbidity of runoff before reaching receiving waters (Soupir *et al.*, 2004; McLaughlin and Bartholomew, 2007; McLaughlin and McCaleb, 2010; Kang *et al.*, 2013a). However, the toxicity of anionic PAM in the presence of suspended sediment was heretofore completely unknown. Furthermore, little was known about the influence of sediment when bound by PAM. Cheung and Shin (2005) previously illustrated the negative physical effects of suspended sediments to the gill tissues of mussels, however, when PAM binds with suspended sediment through electrostatic forces (Young *et al.*, 2007) the particle size and geometry are altered, forming flocs. These alterations may have increased potential to cause damage to the gill tissues of mussels. One of the primary objectives of this research was to evaluate the effects of PAM-flocculated sediment to freshwater mussels by applying a 5 mg/ml concentration of anionic PAM (AN 923) to a range of turbidity treatments. Comparing the biomarker endpoints of our three experimental conditions indicated a protective quality of PAM-flocculated sediment during acute exposures; however, the chronic results were not as clear.

These findings provide valuable information with direct implications for management practices and the conservation of freshwater mussels. Combined with our previous research on the toxicity of anionic PAM that found a median lethal concentration (LC50) with a 24- to 126-fold margin of safety to freshwater mussels (Buczek, in press), anionic PAM appears to effectively mitigate the negative effects of acute turbidity exposure without creating additional risks due to flocculated sediment. This research provides natural resource managers and decision makers with much needed information for future application of PAM for turbidity control. However, testing with additional mussel species and life stages may more clearly elucidate any adverse concentration-response impacts along a turbidity gradient, as turbidity tolerance appears to be species specific (Aldridge *et al.*, 1987).

APPENDIX

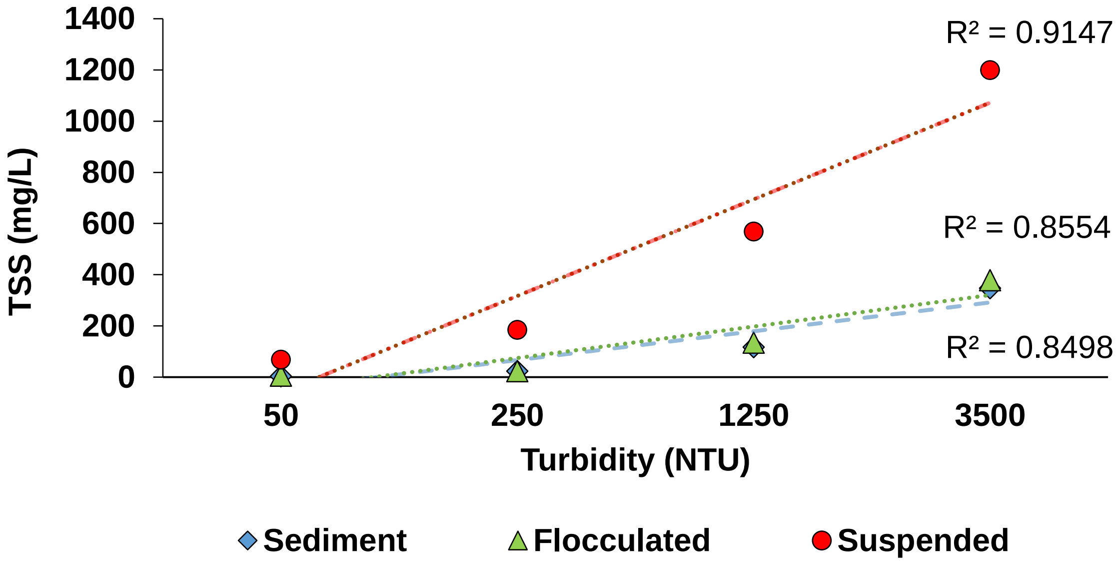

FIGURE A1. Linear relationship between total suspended solids (TSS) and target turbidity concentrations (NTU) for the three experimental conditions.

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FIGURES

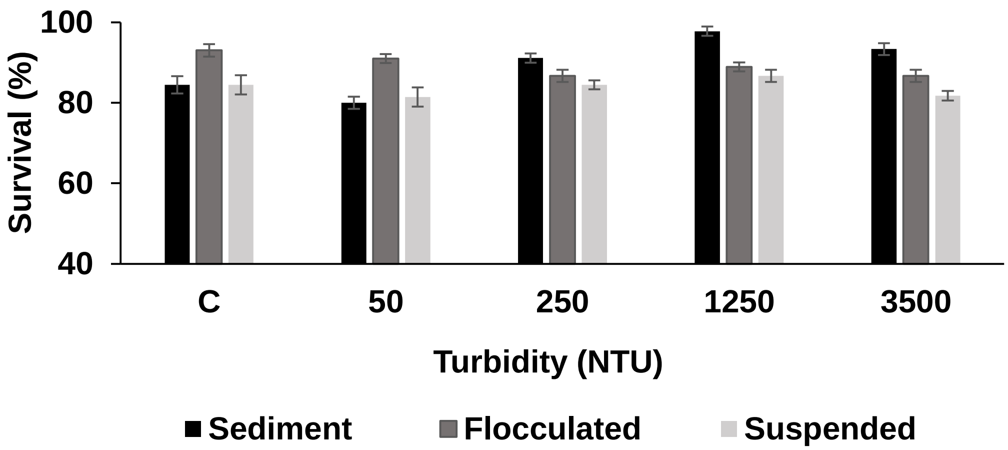

FIGURE 1. Mean (+SE) chronic survival at 20 d post exposure. No significant differences were found between test conditions (settled sediment, suspended sediment, PAM-flocculated sediment) or turbidity treatment levels ($\alpha = 0.05$).

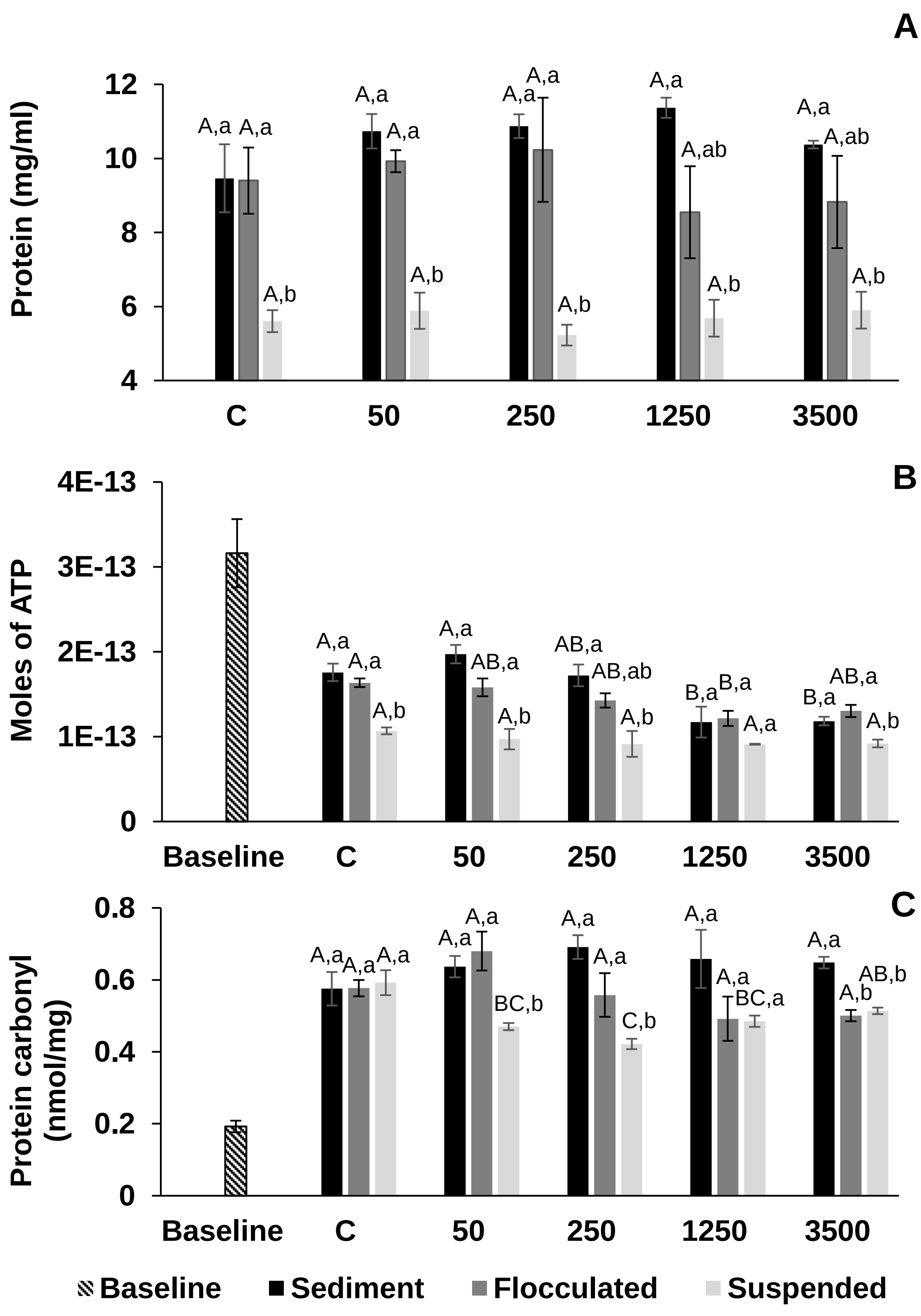

FIGURE 2. Acute (96 h) sublethal biomarker results to a range of turbidity and experimental conditions derived from whole body tissues of juvenile mussels. A) Mean (± SE) protein

concentration (mg/ml). B) Mean (± SE) adenosine triphosphate concentration. C) Mean (± SE) oxidized protein quantified by detection of protein carbonyls. Capital letters indicate significance ($\alpha = 0.05$) across treatment levels within a sediment condition and lower case indicates significance of test conditions within given treatment level.

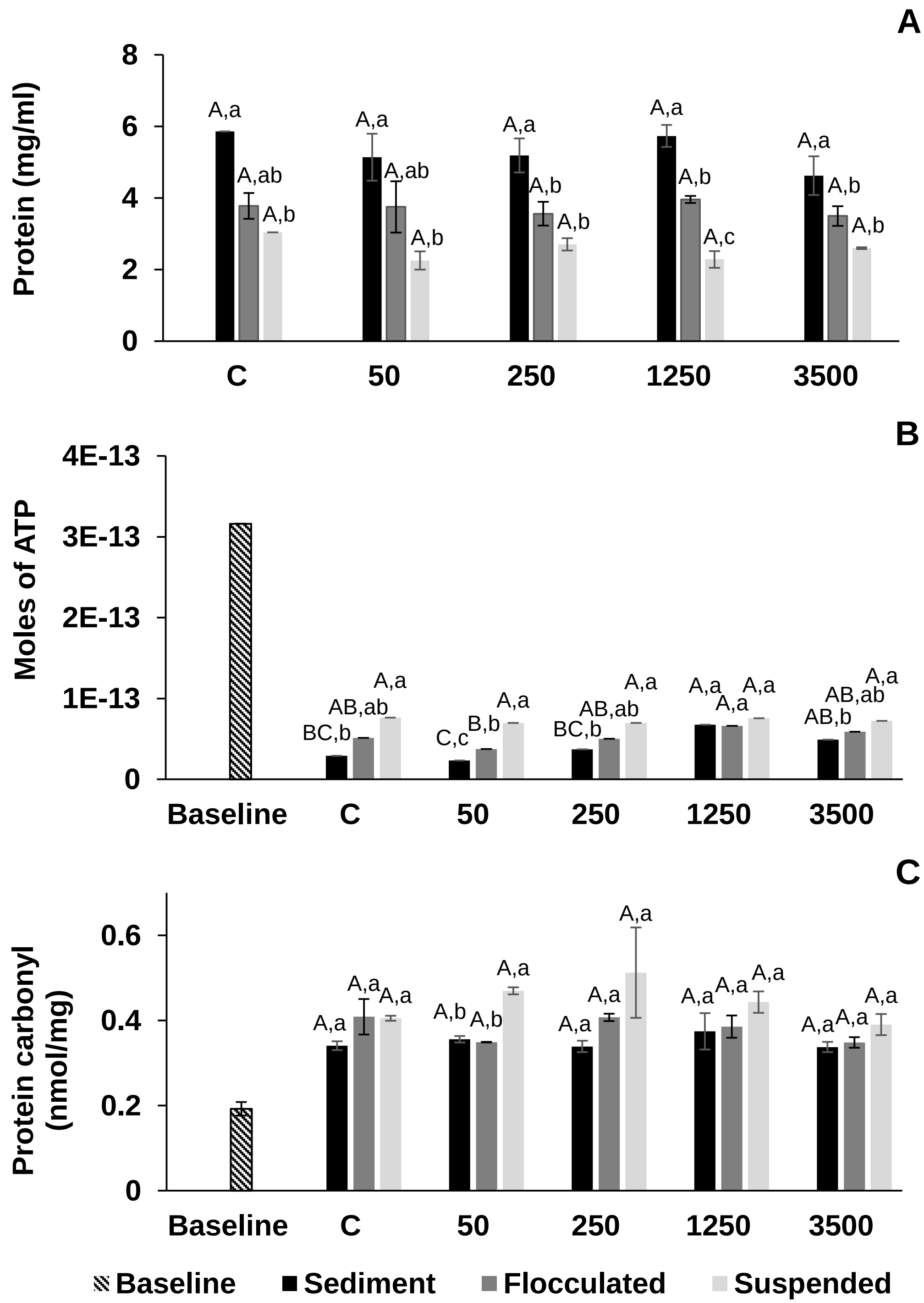

FIGURE 3.Chronic (24 d) sublethal biomarker result to a range of turbidity and experimental conditions. Whole body tissues of juvenile mussels. Whole body tissues. A) Mean (± SE) protein concentration (mg/ml). B) Mean (± SE) adenosine triphosphate concentration. C) Mean (± SE) oxidized protein quantified by detection of protein carbonyls. Capital letters indicate significance ($\alpha = 0.05$) across treatment levels within a sediment condition and lower case indicates significance of test conditions within given treatm