ACUTE TOXICITY OF POLYACRYLAMIDE FLOCCULANTS TO EARLY LIFE STAGES OF FRESHWATER MUSSELS

SEAN B. BUCZEK, W. GREGORY COPE, RICHARD A. MCLAUGHLIN, and THOMAS J. KWAK

Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina, USA
Department of Soil Science, North Carolina State University, Raleigh, North Carolina, USA
North Carolina Cooperative Fish and Wildlife Research Unit, US Geological Survey, Raleigh, North Carolina, USA

(Submitted 25 May 2016; Returned for Revision 15 November 2016; Accepted 10 April 2017)

Abstract: Polyacrylamide 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 polyacrylamide on many freshwater organisms, and prior to the present study, no information existed on the toxicity of polyacrylamide compounds to native freshwater mussels (family Unionidae), one of the most imperiled faunal groups globally. Following standard test guidelines, we exposed juvenile mussels (test duration 96 h) and glochidia larvae (test duration 24 h) to 5 different anionic polyacrylamide compounds and 1 non-ionic compound. Species tested included the yellow lampprussel (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 (LC50s) of polyacrylamide ranged from 411.7 to >1000 mg/L for glochidia and from 126.8 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. The results demonstrate that the polyacrylamide compounds tested were not acutely toxic to the mussel species and life stages tested, indicating minimal risk of short-term exposure from polyacrylamide applications in the environment. However, other potential uses of polyacrylamide in the environment (e.g., wastewater treatment, paper processing, mining, algae removal) and their chronic or sublethal effects remain uncertain and warrant additional investigation. Environ Toxicol Chem 2017:36:2715–2721. Published 2017 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

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 have caused, and continue to cause, increased 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, a process that alters land cover and leads 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. Intertidal spaces within the substrate become inundated with fine particulate matter, thereby reducing the structural heterogeneity, availability of habitat, and oxygen for benthic organisms [16].

The US Environmental Protection Agency (USEPA) determined sediment to be the greatest pollutant of rivers in the United States, and estimates of sediment release to US surface waters as a result of anthropogenic erosion are as high as 75 billion tons annually [17,18]. Among multiple sources, 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 those of natural areas, and these areas are responsible for >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 such as silt fences, check dams, erosion blankets, and sediment basins. However, effectively removing suspended sediment particles <20 μm requires the use of a chemical flocculant such as polyacrylamide [21], which 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 polyacrylamide that is used in turbidity control because it has a much lower aquatic toxicity than 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, NC, USA). The molecular weight is dependent on the length of the linear chains, which range from 12 to 17 Mg/mol [25]. When used for the reduction of turbidity, polyacrylamide is typically applied on-site...
at a rate of 1 to 5 mg/L of water prior to effluent release. However, the turbidity level, soil composition, and other physical parameters dictate the most effective compound for a given application [26,27]. It has been shown that polyacrylamide reduces the turbidity of runoff as much as 91% before reaching receiving waters [28]. When used in conjunction with other best management practices, polyacrylamide is especially effective at controlling turbidity [29–31]. Given their demonstrated efficacy, use of chemical flocculants such as anionic polyacrylamide is quickly becoming an essential best management practice 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 polyacrylamide 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 >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.

Toxicity concerns for anionic polyacrylamide to aquatic organisms center around the possible physical effect of its flocculation of invertebrate larval life stages and primary producers [24], as well as the polyacrylamide monomer acrylamide, which has been recognized as a neurotoxin and probable carcinogen [43]. Acrylamide exposure may occur because of incomplete polymerization during the manufacturing process, resulting in residual unbound acrylamide and, to a lesser degree, the possibility of acrylamide release during chemical, biological, and/or photodegradation [44,45]. However, laboratory studies have shown minimal release of acrylamide from polyacrylamide by intense UV irradiation and high thermal stress (95 °C for 10 d) [44,46]. 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 [45]. To date, there is no evidence of environmental concentrations of acrylamide above those allowed by the Safe Drinking Water Act (0.5 μg/L) during field applications to reduce turbidity [45,47]. Therefore, the main unresolved toxicity concerns of anionic polyacrylamide are related to its physical and chemical attributes. The objective of the present study was to develop toxicological information on 5 representative anionic polyacrylamide compounds and 1 nonionic 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 toxicity, or lack thereof, for each of the 6 polyacrylamide compounds provides important information for identifying potential environmental impacts of their use in water quality and erosion control.

**MATERIALS AND METHODS**

**Test chemicals**

Six compounds of polyacrylamide were selected for toxicity testing in the present study, to provide a range of charge density, molecular weight, and net charge (Table 2), all characteristics that may influence potential toxicity. All polyacrylamide compounds were obtained in granular form, and homogeneous stock solutions of polyacrylamide (1000 mg/L) were prepared by slowly adding (approximately 1000 mg/min) granular polyacrylamide 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: FLOPAM FA 920, AN 923, AN 923 SH, AN 923 VHM, and AN 913 VHM. Also, APS 705 was purchased from Applied

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Duration</th>
<th>LC50 (mg/L)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Raphidocelis subcapitata</em></td>
<td>Green algae</td>
<td>96 h</td>
<td>&gt;100</td>
<td>[24]</td>
</tr>
<tr>
<td><em>Chironomus dilutus</em></td>
<td>Midge</td>
<td>96 h</td>
<td>&gt;100</td>
<td>[24]</td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>Minnow</td>
<td>7 d</td>
<td>&gt;100</td>
<td>[24]</td>
</tr>
<tr>
<td><em>Phoxinus phoxinus</em></td>
<td>Minnow</td>
<td>96 h</td>
<td>&gt;1000</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Hyalella azteca</em></td>
<td>Amphipod</td>
<td>96 h</td>
<td>&gt;100</td>
<td>[24]</td>
</tr>
<tr>
<td><em>Eulimnogammarus verrucosus</em></td>
<td>Flatworm</td>
<td>96 h</td>
<td>&gt;100</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Baicalobilia guttata</em></td>
<td>Water flea</td>
<td>6–8 d</td>
<td>28.7</td>
<td>[24]</td>
</tr>
<tr>
<td><em>Ceriodaphnia dubia</em></td>
<td>Water flea</td>
<td>96 h</td>
<td>14.1</td>
<td>[49]</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Water flea</td>
<td>48 h</td>
<td>345</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Water flea</td>
<td>96 h</td>
<td>17</td>
<td>[50]</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Water flea</td>
<td>48 h</td>
<td>152</td>
<td>[51]</td>
</tr>
</tbody>
</table>

LC50 = 50% lethal concentration.

\[ \text{Test chemicals} \]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Net charge</th>
<th>Charge density (%)</th>
<th>Molecular weight classification</th>
<th>Molecular weight (Mg/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLOPAM AN 913 VHM</td>
<td>Anionic</td>
<td>13</td>
<td>Ultra high</td>
<td>13–16</td>
</tr>
<tr>
<td>FLOPAM FA 920</td>
<td>Nonionic</td>
<td>23</td>
<td>Very high</td>
<td>12–14</td>
</tr>
<tr>
<td>FLOPAM AN 923 SH</td>
<td>Anionic</td>
<td>23</td>
<td>Standard</td>
<td>9–12</td>
</tr>
<tr>
<td>FLOPAM AN 923</td>
<td>Anionic</td>
<td>23</td>
<td>Ultra high</td>
<td>14–17</td>
</tr>
<tr>
<td>FLOPAM AN 923 VHM</td>
<td>Anionic</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Polyacrylamide toxicity to freshwater mussels

Polymer Systems. The chemical property and compound information for SNF compounds tested was provided by the manufacturer (Table 2); but APS 705 is a proprietary mixture of anionic polyacrylamides, and it was included in testing because it is commonly used in environmental applications. The concentration range of polyacrylamide used during toxicity testing (0–1000 mg/L) was selected to guide applied management decisions regarding turbidity control in the presence of common and imperiled freshwater mussels. The highest test concentration of 1000 mg/L exceeded the current recommended concentration for turbidity control by a factor of 200 and was also based on previous aquatic organism toxicity data (Table 1) [24,49–51] and preliminary tests in our laboratory. Our pilot studies indicated that 1000 mg/L was the greatest homogeneous stock concentration of polyacrylamide that could be confidently achieved for use in toxicity testing because of desired dilution in water and the extreme viscosity of this concentration for accurate pipetting and recovering mussels. A greater test concentration could have likely been achieved through more aggressive mechanical agitation but could have risked polyacrylamide bond scissions and cleavage [44] because of shear stress, potentially adversely influencing the present results. Moreover, a greater maximum test concentration would have been even more environmentally unrealistic. Test exposure concentrations were verified using benzethonium chloride (Hyamine 1622; Acros Organics) and measurement of the resulting turbidity in a turbidity meter (LaMott 2020c; LaMott) [52]. Measured concentrations of polyacrylamide ranged from 84 to 109% of the calculated nominal concentrations.

Test organisms

We tested 3 species of native freshwater mussels, chosen based on geographical distribution, phylogenetic tribe, and conservation status: Lampsis cariosa (tribe Lampsilini), Alasmidonta raveneliana (tribe Anodontini), Megalonaias nervosa (tribe Quadrulini). The Appalachian elktoe (A. raveneliana) was propagated on the mottled sculpin (Cottus bairdii) as the host fish, whereas the yellow lampmussel (L. cariosa) was reared on largemouth bass (Micropterus salmoides), and the washboard (M. nervosa) was produced on blue catfish (Ictalurus furcatus). Lampsis cariosa is an Atlantic Slope species in various classifications of conservation status across its range from stable to critically imperiled (state endangered, North Carolina) [53]. Alasmidonta 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 [53,54]. Megalonaias nervosa, a common Interior Basin species, is widely distributed and stable in the Mississippi and Gulf of Mexico drainages [55].

Lampsis cariosa and A. raveneliana were provided by the Aquatic Epidemiology and Conservation Laboratory, College of Veterinary Medicine, North Carolina State University; and M. nervosa was supplied by the mussel culture laboratory at Missouri State University. 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 [56]. At the time of juvenile test initiation, L. cariosa ranged in age from 1 to 21 d, with an average (± standard deviation) shell length of 587.1 μm (± 125.2 μm); A. raveneliana ranged in age from 1 to 21 d, with an average shell length of 501.1 μm (± 50.1 μm); and M. nervosa ranged in age from 1 to 3 d, with an average shell length of 370.2 μm (± 22.6 μm).

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 [57]. Mean temperature (range in parentheses) of glochidia in culture water upon arrival to the laboratory was 17.4°C (13–22°C). Glochidia were acclimated to the reconstituted hard water [48] and the test temperature of 20°C by being placed into a 1:1 mixture of culture and reconstituted water for 2 h, allowing for a 2°C/h maximum rate of change. Glochidia were used in tests when initial viability was assessed at ≥90% using an Olympus SZ61 microscope and QCapture Pro 5.1 digital photographic software (Quantitative Imaging). 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 3 replicates for a given treatment. Test acceptability is specified to be >90% viability in the control treatment at test termination [57]; control viability in the present tests averaged 93% at 48 h. The number of glochidia that we used in each test replicate specified above was less than that recommended by the ASTM International guideline [57] (500–1000 glochidia in each treatment and a subsample of 100–150 glochidia from each replicate) because we were testing a federally endangered species (A. raveneliana) and a state endangered species (L. cariosa) and, therefore, had to be cognizant of the proper and efficient use of the glochidia of these species. Thus, fewer glochidia were used per test replicate to adhere to the measures put in place with our federal and state agency collaborators to minimize mussel use so as to not adversely impact the propagation, conservation, and population augmentation goals for these species. The reduced numbers of glochidia per replicate had no influence on the robustness of the median lethal concentration (LC50) calculation. Moreover, the adherence of the test validity threshold to the ASTM International guideline of >90% control survival at the end of the test ensured appropriateness and rigor of the test response. All tests were conducted in light- and temperature-controlled incubators (Precision Model 818 Thermo Fisher) held at 20°C, with 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°C (17–23°C). Juveniles were acclimated to the reconstituted hard water [48] and the test temperature of 20°C by being placed into a 1:1 mixture of culture and reconstituted water for 2 h, allowing for a 2°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 [57]. 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 (×3) contained 10 juveniles each, whereas all other treatment replicates (×3) contained 7 individuals. Test acceptability is specified to be >80% survival in the control treatment at 96 h [57]; control survival in the 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.
Table 3. Median lethal concentrations (LC50s) for acute toxicity of anionic polyacrylamide to native freshwater musselsa

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage</th>
<th>Time point (h)</th>
<th>FLOPAM AN 923 LC50 (mg/L)b</th>
<th>FLOPAM AN 923SH LC50 (mg/L)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alasmidonta raveneliana</td>
<td>Glochidia</td>
<td>24</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>48</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>329.8 (289.2–376.1)</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Lampsis cariosa</td>
<td>Glochidia</td>
<td>24</td>
<td>833.4 (769.7–902.4)</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>411.7 (373.4–454.0)</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>48</td>
<td>183.2 (139.8–240.2)</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>126.8 (99.9–161.0)</td>
<td>563.2 (414.2–765.8)</td>
</tr>
<tr>
<td>Megalonaia nervosa</td>
<td>Glochidia</td>
<td>24</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>Juvenile</td>
<td>48</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>705.5 (575.5–864.8)</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

aAcute exposures to the following polyacrylamide compounds resulted in insufficient mortality to calculate LC50s: APS 705, FLOPAM FA 920, FLOPAN AN 923 VHM, and FLOPAM AN 913 VHM.
bValues in parentheses represent 95% confidence intervals.

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 CaCO₃/L alkalinity (116–166 mg/L), 160.7 mg CaCO₃/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 [58], and all other water quality parameters were conducted using a calibrated multiprobe system (model 556 MPS; Yellow Springs Instruments).

Statistical analysis

The effect of each of the 6 polyacrylamide compounds on the survival of glochidia and juvenile mussels was used to determine the LC50 analyzed via the trimmed Spearman-Karber method (Comprehensive Environmental Toxicity Information Software [CETIS], V1.8.0.12; Tidepool Scientific). 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 [59]. Water chemistry variables from all tests were analyzed by one-way analysis of variance in SAS (SAS Institute) to assess any mortality attributable to variation in water chemistry during polyacrylamide 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 the polyacrylamide compound.

RESULTS

After exposing both glochidia and juvenile mussels to each of the 6 polyacrylamide compounds at concentrations up to 1000 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 point (Table 3). The 24-h LC50 was 833.4 mg/L (95% confidence interval [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 polyacrylamide compounds tested varied with mussel life stage (juveniles more sensitive than glochidia), species (L. cariosa most sensitive), and chemical properties of the compound (molecular weight, charge density, and net charge); but the compounds exhibited relatively low toxicity overall compared with the concentrations commonly applied for aquatic turbidity control. Of the 36 tests conducted with the early life stages of freshwater mussels and the 6 polyacrylamide compounds, 7 yielded calculable LC50 concentrations. Much of the previous toxicological research conducted on polyacrylamide and aquatic organisms had not generated LC50s but, instead, provided a NOEC (LC50 was greater than the highest concentration tested; Table 1). Even with testing a maximum polyacrylamide concentration of 1000 mg/L, many trials resulted in a NOEC at that highest concentration. This finding demonstrates that the risk of environmental polyacrylamide exposure to freshwater mussels seems minimal, especially at concentrations of 1 to 5 mg/L, where it is most effective for turbidity control [29,31]. Furthermore, the bioavailability of polyacrylamide is greatly reduced when it is added on-site and allowed to bind to suspended sediment, causing the formed floc to precipitate (commonly in a retention basin) before the effluent is released to receiving waters [60]. Thus, chronic environmental exposure to polyacrylamide resulting from turbidity control practices in construction effluent is unlikely to occur, and any polyacrylamide remaining in retention basins or transported to receiving waters [60].
waters degrades at a rate of 10 to 30% yr\(^{-1}\) [61,62]. For even the most toxic polyacrylamide tested (AN 923), there was a 24- to 126-fold margin of safety from common treatment concentrations.

The relative lack of acute toxicity in the present tests with anionic polyacrylamide and early life stages of native freshwater mussels compares similarly to previous acute toxicological studies of anionic polyacrylamide with other aquatic organisms (Table 1). For example, Weston et al. [24] found no significant mortality for the green alga Seleniastrum capricornutum, the 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 [49] reported a 96-h LC50 for Daphnia magna of 14.1 mg/L, and Biesinger et al. [50] 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 on the chemical properties (molecular weight, charge density, and net charge) of each polyacrylamide compound. According to Bolto and Gregory [23], anionic polyacrylamide toxicity is positively correlated with molecular weight. However, the present results indicate that it may actually be the inverse for freshwater mussels because 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 polyacrylamides (AN 913 VHM and AN 923 VHM) at 1000 mg/L experienced >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 polyacrylamide, 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 the present study, we tested 3 polyacrylamides with a charge density of 23% and varying molecular weights: AN 923, AN 923 SH, and AN 923 VHM. The first, AN 923, elicited the greatest level of toxicity and is distinguished from this group by having the lowest molecular weight (9–12 Mg/mol). Further evidence of the trend was the resulting serial toxicity of AN 923 SH (12–14 Mg/mol) to the most sensitive mussel species, L. cariosa. Thus, toxicity may be a result of reduced molecular weight allowing for greater accessibility of polyacrylamide to freshwater mussels. Shorter polyacrylamide chains may more readily access the internal organs of mussels via the incurrent siphon disrupting the biological processes. The polyacrylamide molecules, even at lower molecular weights, are too large to effectively pass through cellular membranes [63]. However, lower–molecular weight polyacrylamides have the potential to create increased interaction between polyacrylamide and surface membranes, eliciting behavioral alterations associated with labored respiration [64]. Upon histological examination of gill tissues acquired from lake trout fry (Salvelinus namaycush) exposed to 300 mg/L of anionic polyacrylamide, Liber et al. [64] reported increased red blood cells, hypertrophy, and hyperplasia in gill lamellae, all suggestive of stress and hypoxia. In fact, Goodrich et al. [65] found that a reduction of molecular weight increased toxicity of cationic polyacrylamide to rainbow trout (Oncorhynchus mykiss) juveniles, a trend that may also hold true for anionic polyacrylamide based on the present results. Beim and Beim [49] attributed mortality in aquatic invertebrates to the sorption of polyacrylamide on surface membranes resulting in decreased efficiency in biological functions, such as respiration, feeding, and reproduction. Further toxicity research with anionic polyacrylamides 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 polyacrylamide 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 [66]. In fact, of the 7 tests that resulted in the calculation of an LC50, 5 were for juveniles and just 2 were for glochidia.

We found varied responses among freshwater mussel species to the exposure of polyacrylamide. Lampsis cariosa was the most sensitive to polyacrylamide exposures, which resulted in the lowest LC50 (126.8 mg/L). Lampsis cariosa was the only species to have sufficient toxicity 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). Alasmidonta raveneliana was not as sensitive, with just one test eliciting sufficient toxicity to estimate an LC50 (329.8 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 polyacrylamide to freshwater mussels observed during the present study, the benefits of polyacrylamide 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 [67,68]. Also, polyacrylamide has the potential to decrease certain chemical toxicants and reduce nutrient loading [69,70]. Aldridge et al. [14] experimentally exposed 3 unionid species, the pimpleback (Quadruma putostos), gulf pigtoe (Fusconaia cerina), and Mississippi pigtoe (Pleurobema beadlearam) 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 as a result of starvation. The use of polyacrylamide may effectively reduce the amount of sediment entering surface waters to decrease the stress of excess sediment on this ecologically important group of imperiled organisms [28,71].

Strayer et al. [72] 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 because of their functional role in primary production, nutrient cycling, and other biological and chemical activities [73–75]. Freshwater mussels perform essential ecological processes including filtration, nutrient cycling, biodeposition, and bioturbation [37,75–77]. Unfortunately, the decline and extinction of many species in this group have occurred almost unnoticed, and identifying a single cause for the decline has been difficult because of the multiple stressors impacting water quality and habitat [37,78].

The aim of the present study was to determine the acute toxicity of anionic polyacrylamide to native freshwater mussels because it is used for the reduction of turbidity in construction effluents. However, anionic polyacrylamide is also used in
many different industries such as water and wastewater treatment, paper processing, and mining. Recent research has shown the efficacy of polyacrylamide used for other aquatic applications that apply the compound directly to water systems, such as infiltration barriers in water delivery systems and as an algal flocculant to remove unwanted algae ([45], K.J. Iwinski, 2013, Master’s thesis, Northern Michigan University, Marquette, MI, USA). These applications potentially require the addition of a polyacrylamide concentration (defined as the actual concentration of a known stock being added to achieve the theoretical environmental concentration) or an environmental polyacrylamide concentration (defined as a theoretical concentration based on complete mixing, volume, and flow; 698 and 386 mg/L, respectively) greater than several of the LC50 values calculated during the present study, to be effective for their desired use. The complete hazard (and risk) characterization for polyacrylamide and all of its possible uses were beyond the limited scope of the present turbidity control-focused study, and thus, the potential environmental applications of polyacrylamide that result in greater concentrations should be investigated for potential adverse effects to freshwater organisms.

CONCLUSION
In an effort to understand the environmental safety of some of the chemical tools being applied for turbidity control in aquatic systems, the present research focused on assessing the toxicity of selected anionic polyacrylamide compounds to early life stages of native freshwater mussels. Our findings indicate that anionic polyacrylamide poses minimal risk to freshwater mussels at optimal turbidity control concentrations of 1 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 because of the irreversible binding of polyacrylamide 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 polyacrylamides without additional toxicity testing or identification of the mode(s) of action. In fact, the present results highlight the differences in mussel species sensitivity and the toxicity of anionic polyacrylamide compounds, with generated LC50s ranging from 126.8 to >1000 mg/L. The present findings advance current knowledge of polyacrylamide toxicity to aquatic organisms and can be used to inform management decisions regarding turbidity control in the presence of common or imperiled freshwater mussels.

Acknowledgment—We thank C. Eads, C. Barnhart, and E. Glidewell for freshwater mussel propagation. We also recognize J. Archambault, B. Cope, J. Kang, J. Luther, J. McIver, A. Popp, M. Silliman, T. Sowers, and M. Walter for technical assistance in the laboratory and T. Pandolfo for a constructive manuscript review. This research was supported by a grant (RP-2014-20) from the North Carolina Department of Transportation. The North Carolina Cooperative Fish and Wildlife Research Unit is jointly supported by North Carolina State University, the North Carolina Wildlife Resources Commission, the US Geological Survey, the US Fish and Wildlife Service, and the Wildlife Management Institute.

Disclaimer—Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US government.

Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (ssbczce@ncsu.edu).

REFERENCES
Polyacrylamide toxicity to freshwater mussels


