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The Effects of Pulsed Exposures of Suspended Clay on the Survival, Growth, and Reproduction of *Daphnia magna*

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THE EFFECTS OF PULSED EXPOSURES OF SUSPENDED CLAY ON THE SURVIVAL, GROWTH, AND REPRODUCTION OF *DAPHNIA MAGNA*

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by
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ABSTRACT

Suspended sediments are a natural component of aquatic ecosystems but anthropogenic activity such as land development can result in significant increases, especially following rain events due to surface erosion. Continuous exposures of suspended clay and silt have been shown to effect growth and reproduction of Cladocera, leading to a decrease in population growth rate. The mechanism of clay toxicity in these filter-feeding organisms is clogging of the gut tract resulting in decreased food uptake and assimilation. When placed in clean water, daphnids can purge clay from their gut and recover. In many surface waters, aquatic organisms experience episodic exposures of high concentrations of suspended solids driven by rain events. However, little is known about the consequences of pulsed exposures on individuals and populations. The objective of the present study was to characterize the effects of continuous and pulsed exposures of natural and defined clays on survival, growth, and reproduction of *Daphnia magna*. Two defined clays, montmorillonite and kaolinite, as well as clay isolated from the Piedmont Region of South Carolina, USA were used. Continuous exposures of clays elicited a dose dependent decrease in survival. Toxicity varied depending on clay source with montmorillonite > natural clay > kaolinite. Pulsed exposures caused a decrease in survival in a 24 h exposure of 734 mg/L kaolinite. Exposure to 73.9 mg/L also caused an increase in the time to gravidity although there was not a corresponding decrease in neonate production over 21 d. No significant effects

resulted from 12 h exposures even at 730 mg/L, almost 10 times the 24 h reproductive effects concentration. This suggests that exposure duration impacted toxicity more than exposure concentration in these pulsed exposures.

DEDICATION

I dedicate this work to my parents, Cathy and David Robinson, and my sisters, Elizabeth, Elaine, and Marianne. Without their love and support this would not have been possible.

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I would like to thank my advisor, Steve Klaine, for all he has taught me and all the guidance he has given throughout my time at Clemson. Everything you have done is greatly appreciated and has aided me in becoming a good scientist. Also, thanks to my committee members, Steve Klaine, Cindy Lee, Yuji Arai, and Aaron Roberts, for help with the writing process and supporting me through one tough decision. Thanks to Billy Bridges for his help with statistics and to the two anonymous reviewers at *Environmental Toxicology and Chemistry* for their helpful critiques on my manuscript. Also to John Smink for his help with all of the TSS data on Lost Creek and the Reedy and Saluda Rivers.

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INTRODUCTION

Suspended solids are natural components of aquatic ecosystems. They result from the resuspension of bottom sediments or the erosion of surrounding lands. Anthropogenic activity such as changing land use can increase exposed soil making it more vulnerable to erosive forces of air and water, especially during rain events. Due to their small size, clay- and silt-sized particles stay in suspension longer while larger particles, such as sand-sized particles, settle rapidly from the water column (EPA, 2006). Studies have shown that these suspended particles can affect aquatic organisms in several ways.

Suspended sediments can have both direct and indirect impacts on zooplankton. Zooplankton are an important link in aquatic ecosystems between primary producers and carnivorous invertebrates and fish. Suspended sediments reduce light attenuation thereby reducing phytoplankton productivity and reducing food for many zooplankton species (Kirk and Gilbert, 1990). Many filter feeding zooplankton such as *Daphnia* are generalists when it comes to feeding, and therefore, do not discriminate between food particles and suspended sediments. Ingestion of significant amounts of suspended particles can clog the intestinal tract and reduce the ability of an organism to take in and process food (Arruda et al., 1983; McCabe and O'brien, 1983; Kirk, 1991b; Levine et al., 2005). Suspended silts and clays decreased overall size, size at maturity, and fecundity in several species of Cladocera (Kirk and Gilbert, 1990; Kirk, 1992). Individual effects can have population level consequences such as reduced population growth rate (McCabe and O'brien, 1983; Kirk and Gilbert, 1990). Furthermore, these effects could possibly lead to a change in zooplankton community structure by allowing rotifers to dominate since they have a more selective feeding behavior, and can therefore avoid ingestion of clay (Kirk and Gilbert, 1990).

Imbalance of suspended and bedded sediments is a major source of impairment of water bodies. There are very few federal regulations on suspended sediments with much being left up to individual states. In 2006 the U.S. Environmental Protection Agency published a framework to aid states and territories when developing criteria for sediments. One of the necessary steps for developing suspended sediment criteria was finding a biological response indicator.

Most of the published research has examined effects of continuous exposures to natural, suspended sediments, silts, and clays. In many surface waters, organisms are subjected to episodic exposures of suspended sediments, usually driven by rain induced surface erosion. The objective of the present study was to characterize the toxicity of suspended clays to *Daphnia magna*, during episodic exposures. The hypotheses to be tested were: one, *D. magna* can recover from short duration exposures because the gut tract is cleared of clay particles relatively quickly; two, exposure duration and exposure concentration are equivalent, that is if duration is doubled and concentration is halved they should elicit the same response; and three, the response from episodic exposures depends on the type of clay as seen with longer, continuous exposures. This research used a novel bioassay system that kept the exposure scenario constant, facilitating more quantitative relationships between suspended solids and organism response.

LITERATURE REVIEW

Suspended solids in the environment

Suspended solids, which are made up of particulate organic matter including decaying plant matter and animal waste, as well as inorganic particles such as sand, silt and clay, are an important component of aquatic environments. Suspended particulate matter plays a role in nutrient cycling, provides habitat for microorganisms, and acts as a food source for many aquatic invertebrates and some fish (Whiles and Dodds, 2002). Suspended particulates can also affect the transport and bioavailability of toxicants including pesticides, aromatic hydrocarbons, heavy metals and other environmental contaminants (Simon and Makarewicz, 2009). However, an excess of even clean suspended sediment can put a stress on aquatic organisms.

Suspended solids can have adverse affects on aquatic organisms including fish, benthic invertebrates, zooplankton, phytoplankton and aquatic plants. Suspended solids reduce light attenuation that results in decreased primary productivity, and therefore, decreased food abundance for organisms that feed on algae and aquatic plants (Arruda et al., 1983; Wood and Armitage, 1997; Alin et al., 1999; Levine et al., 2005). This can lead to herbivores being restricted to living at shallower depths where primary production can still take place (Alin et al., 1999). Suspended solids can also reduce visibility for organisms that hunt for prey visually (Wood and Armitage, 1997) and affect benthic organisms by altering the benthic habitat by deposition and scouring (Wood and Armitage, 1997; Shaw and Richardson, 2001; Dodds and Whiles, 2004). Suspended solids can damage gills of fish and impact feeding of filter feeding organisms (Shaw and Richardson, 2001; Whiles and Dodds, 2002). Which can result in decreased growth and reproduction or even death of these organisms (McCabe and O'brien, 1983; Kirk and Gilbert, 1990; Newcombe and MacDonald, 1991; Wood and Armitage, 1997; Shaw and Richardson, 2001; Whiles and Dodds, 2002).

The amount of damage caused by suspended solids depends on properties of the solids. One important factor is the particle size. Sediment particle range from clays (<2 μm diameter) to silt (2 μm- 50 μm diameter) to sand (50μm-2 mm diameter) (Sparks, 2003). Larger, sand particles will tend to cause a greater effect on benthic habitats as sand-sized particles tend to settle out rapidly. Smaller particles, clays, will stay in suspension affecting light attenuation as well as pelagic organisms such as fish and zooplankton. Much of the research done on effects of suspended solids to these organisms uses suspended clays and silts.

Effect of suspended solids on aquatic organisms

Suspended solids affect different types of animals differently and can have both direct and indirect effects. Indirectly they reduce light penetration, and therefore, the photosynthetic activity of aquatic plants, reducing the growth of algal cells and other aquatic plants that act as a food source for many aquatic invertebrates and fish (Arruda et al., 1983; Wood and Armitage, 1997; Alin et al., 1999; Levine et al., 2005). Suspended solids can also lead to change in water quality parameters. For example suspended solids containing organic particulate matter can deplete dissolved oxygen by increasing the biological oxygen demand (Whiles and Dodds, 2002). Increased turbidity can reduce the hunting ability of fish which depend on vision to find prey items. Murkiness can be beneficial for the prey organism but disadvantageous for the predator. However, some fish hunt easier because suspended particles can create a contrast between prey items and their surroundings (Wood and Armitage, 1997; Berry et al., 2003).

Suspended sediments can also deposit and scour a benthic habitat, which modifies the habitat used by benthic invertebrates and for fish spawning. Large inputs of sediment can result in the burial of these organisms. Excess suspended sediments can also increase drift of benthic invertebrates (Wood and Armitage, 1997; Berry et al., 2003). In addition suspended solids can harm fish by causing abrasions to or clogging the gills thus decreasing their respiratory ability (Shaw and Richardson, 2001; Berry et al., 2003).

Another direct effect to aquatic animals, and one that is a focus of this research, is the effect on zooplankton. Zooplankton are an important part of aquatic ecosystems, acting as a food source for many larger invertebrates and fish. Zooplankton such as *Daphnia*, used in this study, are generalist filter feeders, and therefore, do not discriminate between food particles and suspended sediments (Porter et al., 1982; Kirk and Gilbert, 1990; Kirk, 1991b; Kirk, 1991a). Suspended particles can have undesirable consequences on the feeding of these organisms. Suspended silts and clays have been shown to decrease overall size, size at maturity, and fecundity in several species in the order Cladocera (Kirk and Gilbert, 1990; Kirk, 1992). As a consequence suspended sediments can lead to a reduced population growth rate in these species (McCabe and O'brien, 1983; Kirk and Gilbert, 1990; Kirk, 1992). The most probable cause for these effects is a reduction in food uptake rate and assimilation efficiency in the presence of suspended sediments (Arruda et al., 1983; McCabe and O'brien, 1983; Kirk, 1991b; Kirk, 1991a). This is thought to be caused by behavioral changes as a result of filling of the gut tract. These behaviors include an increase in rejection of food and clay particles using the postabdominal claw, a decreased thoracic limb beat frequency, and decreased chewing movements of the mandible (McCabe and O'brien, 1983; Kirk, 1991b; Kirk, 1992). The effects caused by suspended sediments and clays of reduced growth, reproduction, and survival are the same as that caused by poor food quality or quantity (McCabe and O'brien, 1983; Kirk, 1992).

Suspended clay can also have the potential to change the zooplankton community structure. Cladocera are not selective when it comes to feeding, ingesting particles ranging in diameter of 0.5-40 μm (Kirk, 1991a). Rotifers on the other hand capture food items in the 4-18 μm diameter range (Kirk, 1991a), which leads to rotifers ingesting fewer clay particles than Cladocerans, thus experiencing fewer ill effects when exposed to suspended clay. The difference between the two can allow rotifers to dominate over cladocerans, where without suspended sediments Cladocera have the advantage because they feed on smaller food particle that rotifers do not (Kirk and Gilbert, 1990; Kirk, 1991a). The increase in rotifers compared Cladocera will result in a change in higher community structure as different predator species may feed on different zooplankton based on size or other characteristics.

Land use and episodic exposures

Anthropogenic activities can cause a significant increase in suspended sediment. How land is being used greatly affects the amount of exposed soil that is likely to be affected by the erosive forces of wind and rain. Lenat and Crawford (1994) show that catchments that were made up of mostly agricultural or urban land had increased suspended sediment concentrations compared to a catchment that was mostly forest land. Furthermore, suspended solids is greatly increased after storm events when there is increased flow across land and through stream channels. Figure 1 compares the total suspended solids (TSS) that occurred during one rain event, for two streams in the upper Saluda watershed of South Carolina. Lost Creek is in a developed area while the Knight Creek drainage area is mostly forested land. In Lost Creek the TSS reached a peak of 2,579 mg/L with an event mean of 326 mg/L, while Knight Creek stayed below 300 mg/L with an event mean of 116 mg/L. With this it is evident that pelagic organism are likely to only be exposed to suspended sediments for a limited amount of time and are in clean water between storm events. This is why it is important to study organisms being exposed to sediments episodically rather than over a long duration.

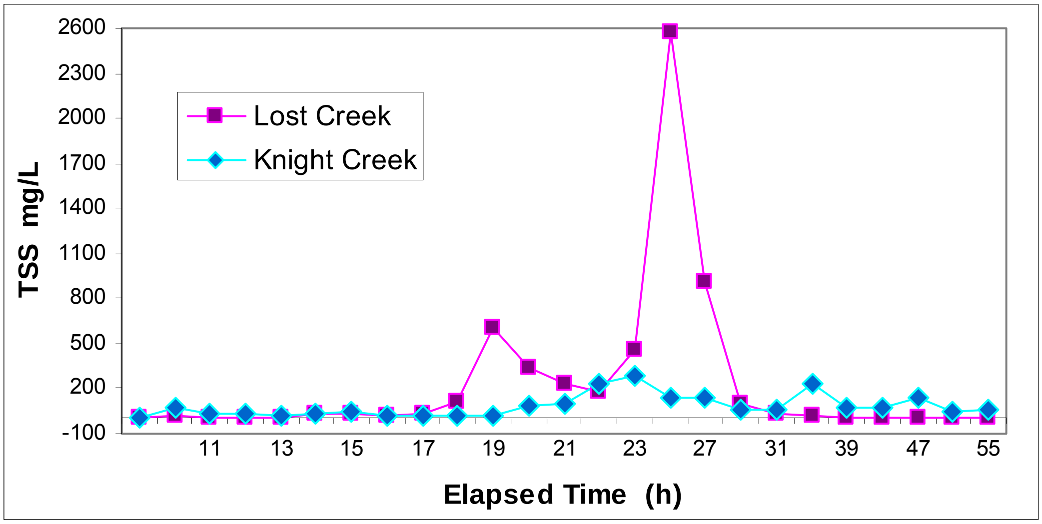

Figure 1. Total suspended solids during a storm event for two streams in the Saluda watershed in South Carolina. Lost Creek is in a highly developed area and Knight Creek is a reference site that has little development. Data from February 5, 2004.

Much of the research done on the response of organisms to suspended sediments does not take into account the duration of the exposure, only the concentration. Newcombe and MacDonald (1991) were one of the first researchers to realize that, like chemical contaminants, the duration of suspended sediment exposures determines the magnitude of response of exposed organisms. Prior to this, researchers discussed effects caused by suspended sediments and clays but they rarely discussed the length of the exposure that caused these effects. Even their methods are vague when discussing duration with them giving ranges (Kirk and Gilbert, 1990) or indicating that organisms were exposed longer but only a given period of time was used for analysis (Kirk, 1992).

Regulation on Total Suspended Solids

Imbalance of suspended and bedded sediments is a major source of impairment of water bodies. One of the major consequences is the harm done to aquatic biota but suspended sediment also affects preparation of water for drinking, and recreation and navigation in impaired water bodies. According to the U.S. Environmental Protection Agency (EPA) 40% of the U.S. river miles assessed in 1998 had some kind of impairment due do sediment (EPA, 2006).

There are few federal regulations on suspended sediments in the United States. How sediments are regulated is mainly left up to individual states. According to a U. S. EPA survey in 2001, only 32 of the 53 U.S. states and territories had numerical criteria for suspended sediment (EPA, 2006). Thirteen of the remaining states and territories reported narrative criteria, leaving eight states with no criteria for suspended sediments (EPA, 2006). Within the numerical criteria, there is no standard method in determining the amount of suspended sediments. Some states use turbidity while others use total suspended solids (TSS). These two measurements can not be compared between different systems because TSS is the weight of solid material per volume of water and turbidity is a measure of how light penetrates the water. Turbidity depends on the composition and size of the suspended particles and can be affected by dissolved organic matter, which can differ between systems. Turbidity, however, is easier to measure than TSS. Turbidity, measurements use a single instrument with a small water sample while TSS requires filtering, drying and weighing large sample volumes. In 2006 the U.S. EPA published a framework to aid states and territories when developing water quality criteria for sediments. One of the necessary steps for developing suspended sediment criteria was finding a biological response indicator (EPA, 2006). Research into the response of organisms to suspended sediments is important before criteria can be developed. Hence, this project will look at the effect of several types of suspended clay on the zooplankton, *Daphnia magna*, using episodic exposures of varying duration and concentration.

MATERIALS AND METHODS

Suspended solids

Three types of suspended solids were used in this research: kaolinite (KN), montmorillonite (MN), and natural clay isolated from a local source (LC). Kaolinite clay powder (CAS # 1332-58-7) was purchased from VWR International and MN clay powder was purchased from Ward's Natural Science. Stock suspensions of these defined clays (KN and MN) were prepared by mixing clay powder in deionized water on a stir plate overnight. This was followed by sonication and concentration determination as described below.

The natural sediment was collected in 5-gallon plastic buckets from the banks of Lost Creek (LC) in the Saluda River watershed within the Piedmont ecoregion of South Carolina, and transported to the Clemson University Institute of Environmental Toxicology (CU-ENTOX), Pendleton, South Carolina. The clay-sized fraction (<2μm) was separated using gravimetric techniques following Stoke's Law and assuming a particle density of 2.65 g/cm^3. The clay-sized fraction was concentrated by centrifugation at 3,000 *g* for 30 min, using an International Equipment Company Centra® GP8 centrifuge. The resulting pellets were resuspended in deionized water and sonicated for 1 h. The concentration of this LC and the clay suspensions was determined using Standard Method 2540 D (Eaton et al., 2005). Pall Metrigard® glass fiber filters, with retention of 0.5 μm, were used as the filtering media. The collected clay-sized particles (LC) were analyzed by X-ray diffraction (XRD) (The MineralLab, Lakewood, CO, USA) and were found to be 60% kaolinite (Table 1) (Capper, 2006).

Table 1. X-ray diffraction analysis of clay-sized fraction (<2 μm) of Lost Creek (SC,USA) sediment (Capper, 2006).

Mineral Name	Chemical Formula	Approximately Wt %
Kaolinite	$Al_2Si_2O_5(OH)_4$	60
Gibbsite	$Al(OH)_3$	10
Goethite	FeO(OH)	12
Quartz	SiO_2	<5
Mica/illite	$(K,Na,Ca)(Al,Mg,Fe)_2(Si,Al)_4O_{10}(OH,F)_2$	5
K-feldspar	$KAlSi_3O_8$	<3
Plagioclase feldspar	$(Na,Ca)Al(Si,Al)_3O_8$	<3
Smectite	$(Ca,Na)_x(Al,Mg,Fe)_4(Si,Al)_8O_{20}(OH,F)_4 \cdot nH_2O$	<5
"Unidentified"	NA	<5

Test Organism

Daphnia magna were obtained from cultures maintained at CU-ENTOX. Routine reference acute toxicity tests have been performed with this culture to ensure consistent culture sensitivity to sodium chloride. Organisms were cultured in 1 L glass beakers containing 600 ml reconstituted soft water (14.4 g $NaHCO_3$, 9.0 g $CaSO_4$, 9.0 g $MgSO_4$, 0.62 g KCl in 300 L Milli Q water (Millipore)) with a hardness of 45±5 mg/L as $CaCO_3$ and alkalinity of 35±5 mg/L as $CaCO_3$. Water with low hardness and alkalinity were used to minimize interaction between clay and dissolved ions, thus decreasing clay aggregates in test media. Culture media was renewed three times a week and cultures were fed daily. Food consisted of *Selenastrum capricornutum* at a final concentration of 300,000 cells/ml, and yeast-cerophyll-trout chow mixture that was fed at half the volume of algal stock added.

Bioassay design

Organisms were exposed to clay using 4-L plastic beakers on a stir plate with a 2-inch stir bar to keep clay suspended (Figure 2). Organisms were held in the center of the beaker in glass test chambers within a modified test tube rack. Each test chamber consisted of 4 cm of 2.5 cm outer diameter, fused quartz glass tubing (Technical Glass Products, OH) capped on both ends with 500 µm Teflon® mesh (Rickly Hydrological Company, OH). Mesh was secured to the bottom of the tube with aquarium safe silicon, and to the top with a 1 cm piece of 2.6 cm inner diameter fused quartz glass tubing (Martin, 1987). Eight test chambers containing one *D. magna* each were placed in each test tube rack. Two replicate test beakers, each containing one test tube rack holding eight organisms, were used for each treatment for all tests.

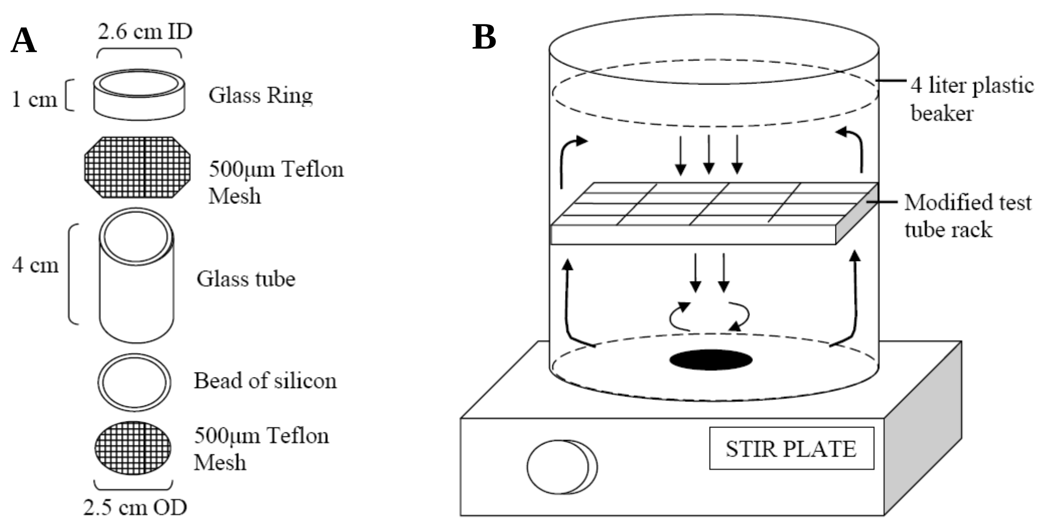

Figure 2. Bioassay design. (A) Diagram of glass test chamber used to contain one daphnid. (B) Diagram of test beaker containing modified test tube rack used to hold eight glass test chambers suspended in media.

Experimental design

For all bioassays, *Daphnia* were 72 h old at test initiation to keep them within the glass cylinders capped with 500 μm mesh. Test organisms were fed *Selenastrum capricornutum* every day at a final concentration of 150,000 cells/ml. The photoperiod for all tests was a 16:8 h light:dark cycle.

Initial bioassays were conducted for 7 d to determine mortality, growth, and reproduction endpoints with all three clay types: KN, MN, and LC clay. For mortality experiments, media were made from prepared stock suspensions diluted with soft water, to produce concentrations of 0, 25, 50, 100, and 200 mg/L for KN, concentrations of 0, 5, 15, 30, 50, and 75 mg/L for MN, and concentrations of 0, 25, 50, 75, 100, and 150 mg/L for LC. Test media was renewed everyday, with pH, dissolved oxygen, and temperature measured before and after each renewal. Also, TSS was measured at initiation, before and after each renewal, and at test termination. Due to the adhering of clay to surfaces, the concentration decreased over time; therefore, the average concentration was used in the analysis. During renewals the test tube rack containing the test chambers was placed in a dish of clean media while the test media was replaced (Capper, 2006).

For 7 d sublethal tests, medium was prepared from stock suspensions to produce a concentration of 50 mg/L of each clay type. This concentration is not uncommon in the environment. This lab has records, from 2004 to 2009, of the concentrations of total suspended solids in the Reedy and Saluda Rivers, South Carolina. The annual mean TSS concentration of an average rain event ranged from 10 to 100 mg/L in 2004 to 2006. Also, Jones and Knowlton (2005) saw average concentrations of up to 47 mg/L from 135 reservoirs in Missouri. During sublethal experiments, in addition to daily renewals of medium, the length of each organism was measured from its eye to the base of its tail using an ocular micrometer on a dissecting microscope, and the presence of eggs in the brood chamber of the organisms was also recorded daily. Organisms that survived to the end of the test but had yet to become gravid were assumed to become gravid the following day and were assigned a value of 11 for days to gravidity (Capper, 2006).

The 21-day, episodic exposure bioassays were conducted to examine the effect of sediment pulses on mortality, growth, and reproduction for the majority of the daphnid life span. Organisms underwent a 24 h or less, single or double exposure, and were kept in clean media for the remainder of the 21 d. For the single exposures, organisms were exposed for 24 h to each of the three types of clay or for 12 h to kaolinite only. The 24 h exposures were at concentrations of 50, 100, 200, and 400 mg/L KN, 5, 10, 25, and 50 mg/L MN, and 25, 50, 100, and 200 mg/L LC clay. The 12 h exposure was at concentrations of 100, 200, 400, and 800 mg/L KN. The double pulse exposures were for 12 h and only KN was used. Organisms were exposed to 800 mg/L KN for the first 12 h of the test period, then after a recovery period of 0, 48, 96, 192, or 384 h, were exposed to a second 12 h pulse of 800 mg/L KN. Concentrations chosen for 12 and 24 h exposures covered a range that is seen in the environment as a result of rain events. Table 2 shows data from Lost Creek, a first order stream in a developing area of South Carolina, for several rainfall events from 2004 to 2009. Also, total suspended solids measured by the lab, using an autosampler, in the Reedy and Saluda Rivers during a few selected rain events exhibited maximum concentration around 100 mg/L to more than 500 mg/L, with event mean concentrations ranging 10 to 100 mg/L. These sampling sites are just upstream of a reservoir, Lake Greenwood, and would be more reprehensive of a lake system were daphnids are more likely to be found.

During all episodic exposures, test beakers were only stirred on magnetic stir plates during the exposure; therefore, neonates that fit through the mesh would not be killed by the stir bar and could be counted. Medium was renewed daily, as with the 7-d tests. Each day organism length was measured, the presence of the first brood of eggs in the brood chamber was noted, and neonates were counted to obtain the average number of neonates produced by each organism over the period of the test. Due to some mortality during the test, the average number of neonates per live organism for the extent of 21 d was calculated by taking the number of neonates on a particular day and dividing this by the number of live organisms in that beaker for that day. The resultant values were then summed to obtain the average number of neonates produced per organism during the 21 d test period. During the second exposure of the double pulse neonates could not be counted due to poor visibility in the media. For this 12 h period of time, the number of neonates was estimated by photographing each organism before and after the exposure and counting the neonates in the brood chamber that should have been released during the exposure.

Table 2. Summary of rain event data for Lost Creek. For 21 select rain events that occurred from 2004 to 2009. Water samples taking using an autosampler. TSS is total suspended solids; EMC in event mean concentration.

Event	Storm duration (h:m)	rainfall amount (inches)	runoff volume (L)	TSS total load (kg)	EMC (mg/L)	TSS_{max} (mg/L)
2/5/2004	20:00	0.9	1.20E+06	1211.68	1013.5	2579
6/25/2004	5:00	0.43	1.52E+05	133.23	876.6	2007
1/13/2005	3:30	0.73	4.33E+06	7945.66	1834.8	7820
2/14/2005	6:15	0.27	1.15E+06	135.58	117.8	286
7/7/2005	4:15	0.35	1.03E+06	1352.74	1316.3	3338
7/12/2005	4:00	0.43	7.67E+05	159.68	208.3	2373
7/29/2005	3:00	0.48	7.07E+05	997.54	1411.8	3446
3/10/2006	4:00	0.15	2.53E+05	65.07	257.4	1280
3/20/2006	9:00	0.85	2.21E+06	1241.29	561.3	1196
6/23/2006	4:00	0.72	1.05E+06	740.80	707.8	1923
6/26/2006	3:00	0.49	1.17E+06	855.48	734.0	1915
8/10/2006	3:00	0.28	3.78E+05	71.51	189.1	283
5/5/2007	15:00	1.68	6.05E+06	2449.04	404.9	858
9/14/2007	7:30	1.68	4.74E+06	4028.10	849.2	915
11/26/2007	10:45	0.31	4.80E+05	17.56	36.6	54.1
2/13/2008	9:00	0.22	4.18E+05	72.33	173.2	270
2/21/2008	9:00	0.65	3.54E+06	449.22	126.9	209
8/17/2008	4:10	0.75	1.73E+06	1055.44	610.4	891
8/26/2008	11:00	1.71	6.96E+06	6980.00	1003.1	3309
2/27/2009	5:00	0.36	6.92E+05	103.60	149.7	285
5/6/2009	3:30	0.35	1.09E+06	90.34	83.1	197

Water quality analysis

Total suspended solids samples were analyzed using Standard Method 2540 D (Eaton et al., 2005) and Pall Metrigard® glass fiber filters with retention of 0.5 µm were used as filter medium. A Yellow Springs Instruments Model 85 handheld probe was used to measure temperature and dissolved oxygen. pH was measured using an Orion 710A+ pH meter.

Statistical analyses

Seven day median lethal concentration (LC50) values were calculated using Trimmed-Spearman-Karber with ToxStat (version 1.5, WEST) (Capper, 2006). Differences in days to gravidity and the number of neonates produced per organism over 21 d were analyzed by a one-way analysis of variance (ANOVA) and Tukey's multiple comparison test with the statistical analysis software SAS (SAS institute). Growth rates were compared by defining a nonlinear growth curve model for each treatment, combining them into one model and comparing growth coefficients using a pairwise comparison. Also, the length of organisms recorded each day were compared using one-way ANOVA, and using sequential Bonferroni to adjust for type I and type II error.

RESULTS

Concentrations of suspended clay decreased over the 24 h period between renewals, due to adhesion onto surfaces of the test chambers. Consequently, concentrations used to develop exposure response relationships were the average of the measured initial and final concentrations for each renewal.

Daphnia magna exhibited dose-dependent mortality for all three clay types in the 7-d bioassays. Montmorillonite was the most toxic clay, with 7-d LC50 values for MN, LC, and KN of 5.17 (95% confidence interval 2.81, 9.54), 51.02 (31.25, 83.31), and 74.51 (65.08, 85.3) mg/L, respectively. All three clay types decreased growth after 7 d ($p<0.05$) (Figure 3). After 24 h, organisms in all exposures showed decreased growth compared to control organisms ($p<0.05$), and by 72 h all treatments showed differences in growth from other treatments as well as the controls ($p<0.05$). The pattern of toxicity was consistent with that of mortality: MN > LC > KN (Capper, 2006).

Fecundity was characterized by noting the day organisms became gravid. Organisms exposed to LC clay experienced a significant increase in the number of days to gravidity ($p<0.05$) from both the controls and KN (Table 3). Kaolinite-exposed organisms did not show a significant increase from the control in days to gravidity ($p>0.05$) while MN-treated organisms died before they became gravid (Capper, 2006).

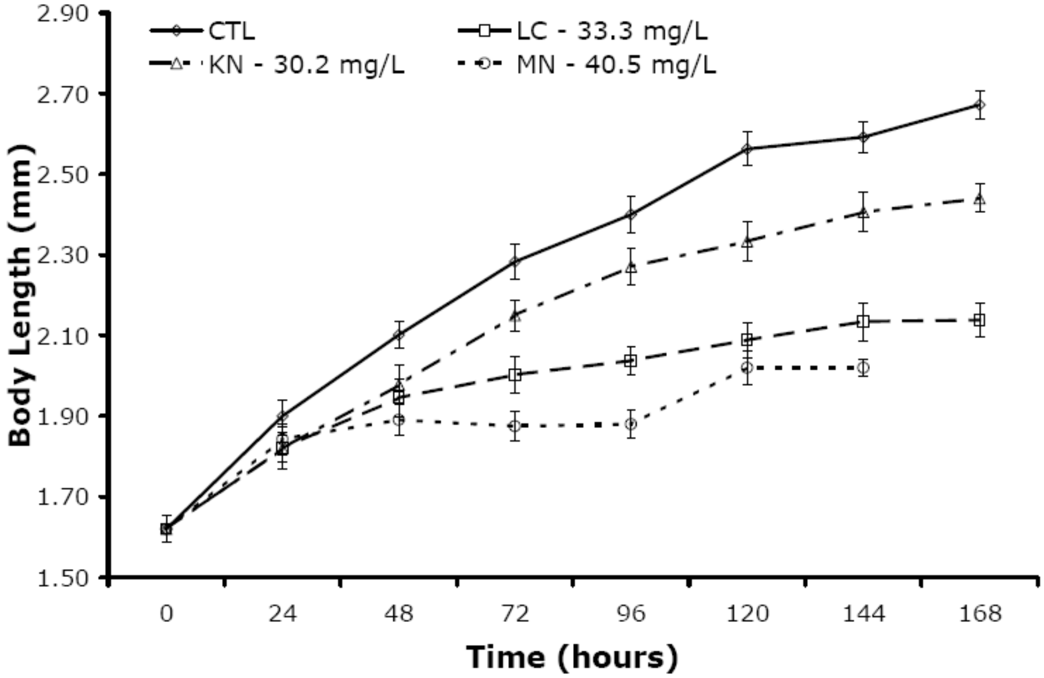

Figure 3. Body length of *Daphnia magna* over time (mean ± standard error) for the control (CTL), Lost Creek (LC), kaolinite (KN), and montmorillonite (MN) treatments. Actual exposure concentrations are shown in the legend. Exposures were statistically different form controls at all time points >24h (Capper, 2006).

Table 3. Average number of days since birth to become gravid for *Daphnia magna* control (CTL) and those exposed to suspended kaolinite (KN), Lost Creek (LC) clay, and montmorillonite (MN) for 7 d (SE=standard error). Only one organism survived MN exposure long enough to become gravid; therefore, days to gravidity was not available (NA) (Capper, 2006).

Sediment	mean	SE
CTL	5.4	0.2
KN	5.4	0.2
LC	8.6	0.9
MN	NA	NA

Organisms exposed to a single pulse of KN for 12 or 24 h, or MN or LC for 24 h, did not show any dose-dependent response for survival over 21 d. There were no exposure concentrations that led to a significant decrease in survival from the controls ($p>0.05$). For the double pulse consisting of two 12 h KN exposures of 800 mg/L (measured concentration 734.2 mg/L), the organisms with a 0 h recovery time (or 24 h exposure) showed a significant decrease in survival from both the control and organisms with a recovery time greater than or equal to 48 h ($p<0.05$) (Figure 4).

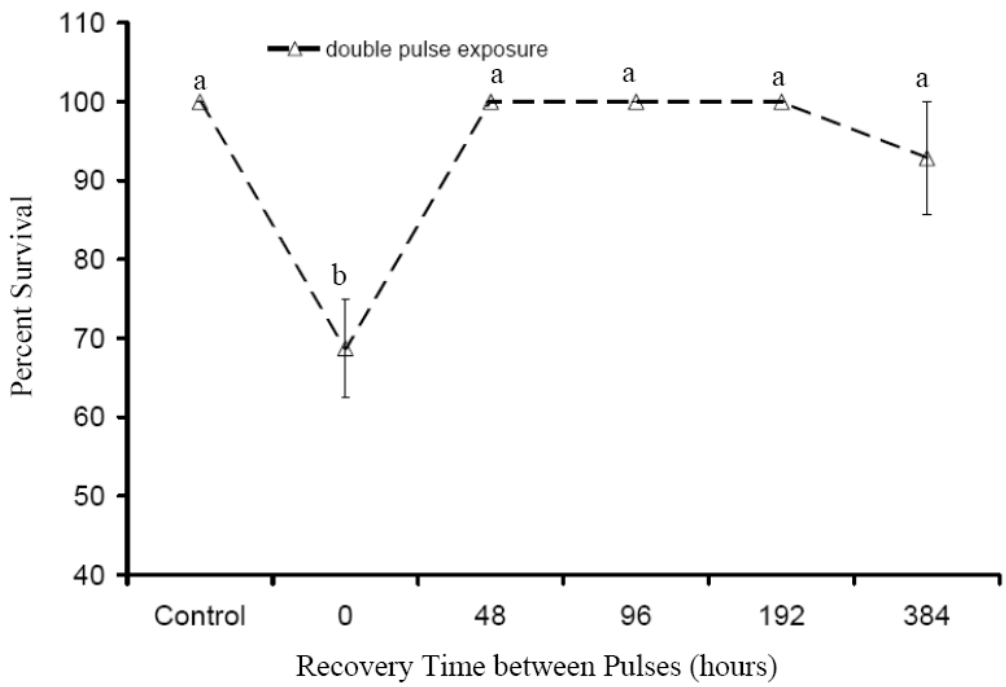

Figure 4. Percent survival of *Daphnia magna* for double pulse exposure of varying recovery time between pulses (mean± standard error). Only 0 h recovery time elicited a significant decrease in survival. Points with the same lowercase letter were not significantly different ($p>0.05$).

Organisms exposed for 24 h to concentrations of 73.9, 152.5, and 312.0 mg/L KN; 9.0, 21.7, 48.9 mg/L MN; and 193.1 mg/L LC showed a significant increase in days to gravidity compared to control ($p<0.05$). Days to gravidity for organisms exposed for 24 h at lower concentrations and all 12 h KN exposed organisms were not significantly different from the control ($p>0.05$) (Figure 5). Organisms exposed to all single pulse treatments showed no significant reduction in number of neonates produced over 21 d ($p>0.05$; data not shown).

Organisms exposed to two 12 h KN exposures with a 0 h recovery time showed a significant increase in time to gravidity compared to control and organisms with a recovery time of 96 h or more ($p<0.05$; Figure 5C). Days to gravidity for the 0 h recovery was not significantly greater than organisms with the 48 h recovery ($p>0.05$). There was no significant difference in the number of neonates produced for the double pulse exposures compared to controls ($p>0.05$; data not shown).

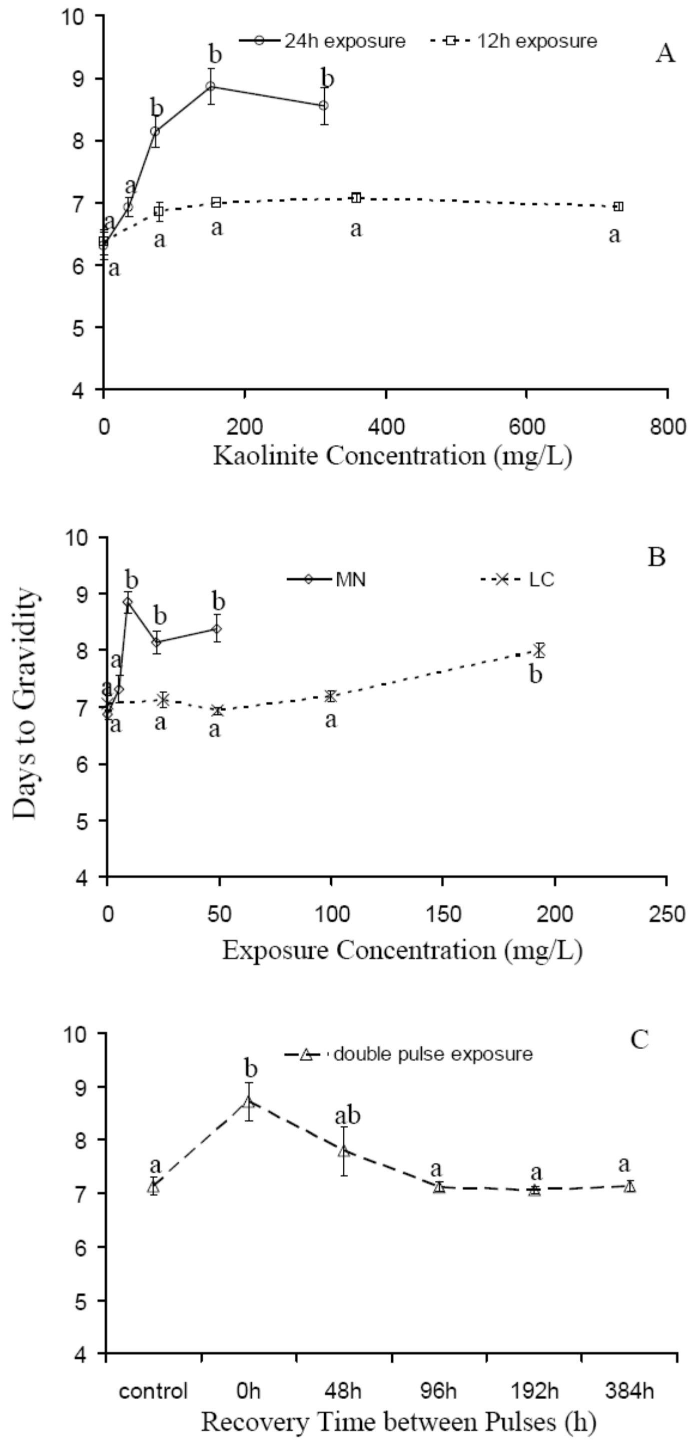

Figure 5. Number of days from birth for organisms to become gravid with first brood for (A) single pulse kaolinite (KN), (B) montmorillonite (MN) and natural clay-sized particles (LC), and (C) double pulse kaolinite (KN) exposures (mean± standard error). Note that experiments were started on day three of the organisms' lives. Points with the same lowercase letter were not significantly different ($p>0.05$).

Growth was characterized by measuring the length of the organisms each day. These results are shown in Figure 6. A significant difference in growth rate was observed over the 21-d bioassay; therefore, length was compared for each day to determine when differences occurred. Because comparing length required 21 ANOVAs, sequential Bonferroni was used to adjust for type I and type II error. For the 24 h exposures of all three clay types there was a significant decrease in length, compared to the control, after the exposure. The number of days in which the length was significantly smaller depended on the type of clay and the exposure concentration. For day 1 to 14 (KN) and day 1 to 9 (MN and LC), there was at least one concentration that had a significant difference in length (p<0.0056, 0.0031, and 0.033, respectively). For the 12 h KN exposure the results differed from the 24 h exposure in that there was no significant difference after the exposure, but by day 16 the controls were significantly smaller than exposed organisms for all concentrations (p<0.05). In the double pulse exposure only, organisms with a 0 or 48 h recovery time were significantly smaller than controls on days 1 through 13 and 5 through 19, respectively (p<0.05).

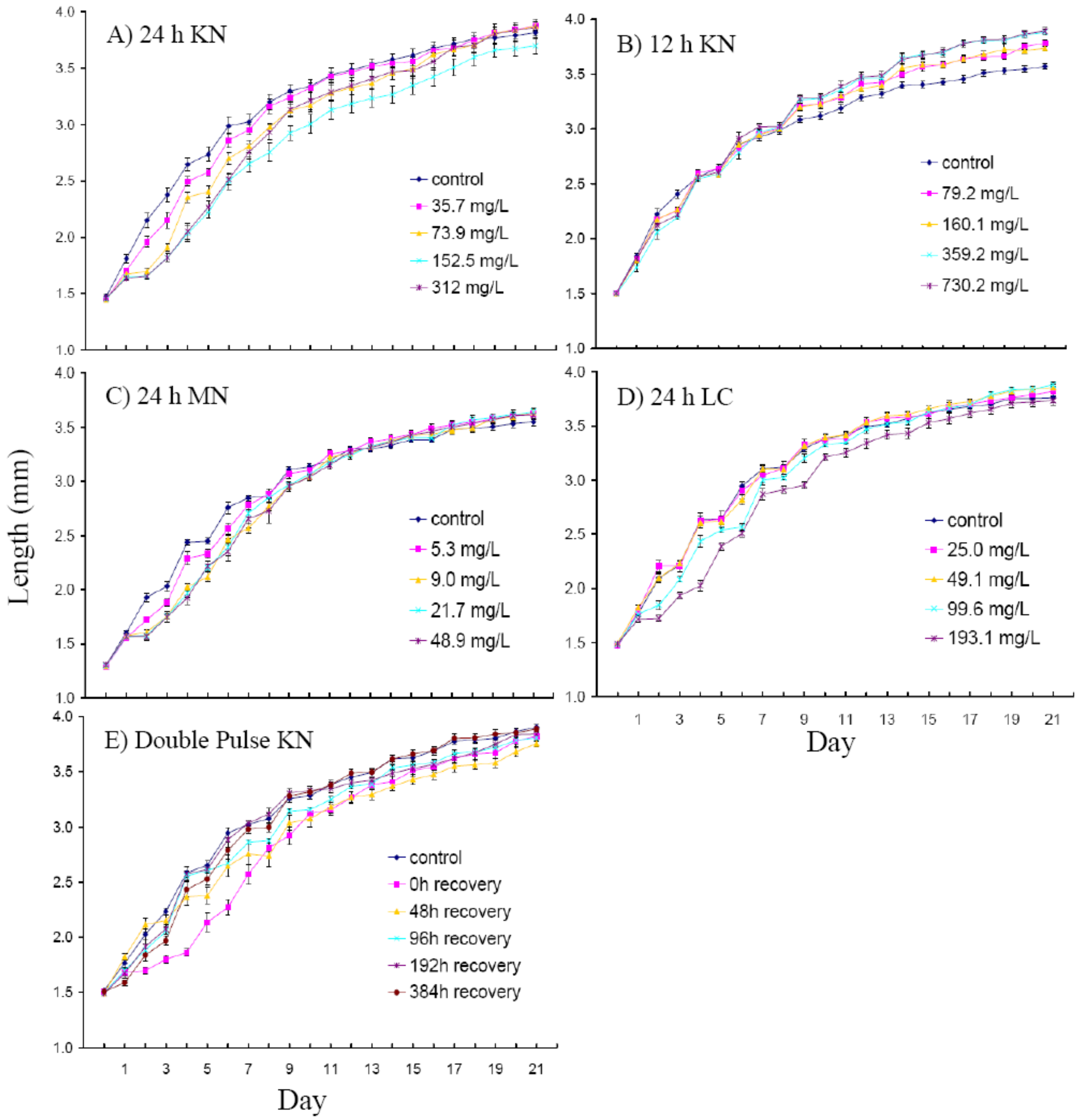

Figure 6. Length of organisms for each treatment over 21-d test period for (A) 24 h kaolinite (KN) single pulse, (B) 12 h kaolinite (KN) single pulse, (C) 24 h montmorillonite (MN) single pulse, (D) 24 h natural clay-sized particles (LC) single pulse, and (E) kaolinite (KN) double pulse exposure (mean± standard error).

DISCUSSION

Dose-dependent mortality was observed in *D. magna* for all three clay types at low concentrations compared to the concentrations observed in the environment during storm events. Table 2 gives data for several rain events on Lost Creek and TSS can reach up to 8,000 mg/L. In a larger system, the Saluda River upstream from Lake Greenwood, South Carolina, TSS has been measured at more than 100 mg/L. Also, Aruda et al (1983) state that concentrations of 100 mg/L are common in Tuttle Creek reservoir in Kansas, a source that contains *Daphnia* species. These mortality results support previous research with filter feeding invertebrates using similar concentrations of suspended clay (Kirk and Gilbert, 1990). The toxicity of the clays varied greatly among types of clay. Montmorillonite was the most toxic (7 d LC50 = 5.17 mg/L), KN the least toxic (7 d LC50 = 74.51 mg/L), and LC fell between these two but closer to KN (7 d LC50 = 51.02 mg/L). The toxicity of LC compared to the other clays becomes clear when the composition of the particles are considered. Lost Creek clay is 60% kaolinite (Table 1) and the response of *D. magna* to LC was nearer to the response to KN than to MN. However, the LC particles were not analyzed for organic carbon content or any adsorbed contaminants. The presence of either of these could play a part in the toxicity of these particles. These results imply that mineralogy, ultimately may have a significant influence on the toxicity of suspended clays.

The clays also reduced growth and reproduction in *D. magna*. These effects have been observed to occur as a result of decreased food quality or quantity (McCabe and O'brien, 1983; Kirk, 1992). The mechanism of impairment of *Daphnia* when exposed to suspended sediments and clays is mainly a reduction of feeding efficiency. Other researchers have shown decreased feeding rates result from decreased filtering appendage beat rate and an increased rejection rate when exposed to clays (Kirk, 1991b). The decreased appendage beat rate and increased rejection frequency in the presence of suspended clay observed by Kirk (1991b) were similar to those observed by Porter et al. (1982) when *Daphnia* were exposed to high food concentrations. Because the ratio of food to nonfood particles is lower when suspended clay is present, these mechanisms lead to a smaller amount of ingested food when suspended clay is present.

The concentration of algae used in the present study was 150,000 cells/ml, which is higher than typical environmental concentrations and those used by other researchers (5,000 cell/ml *Cryptomonas* sp. (Kirk and Gilbert, 1990); 31,000 cells/ml *Ankistrodsmus falcatus* (Kirk, 1992); 50,000 cells/ml *Chlorella vulgaris*, (Arruda et al., 1983)). Therefore, organisms in the current study consumed a greater ratio of algae to clay than they might experience in the environment suggesting that the environmental implications of the results might be conservative. Kirk and Gilbert (1990) observed relatively small effects when *Daphnia ambigua* were exposed to suspended clay at higher food concentrations (20,000 cells/ml) than at more environmentally relevant food concentrations

Continuous exposure to significant concentrations of suspended solids occur in some turbid waters, but it is important to know how organisms respond to and recover from episodic suspended sediment exposures that occur as a result of rain induced surface erosion. During storm events, suspended sediments can reach relatively high concentrations for short periods of time (Shaw and Richardson, 2001). It has been observed that surviving *D. magna* can purge their clay-clogged gut tract within approximately 30 min after transfer to clean medium (Capper, 2006) (Figure 7). Organisms exposed to a single 12- or 24 h pulse of clay exhibited no significant mortality (*p*<0.05; data not shown). Given that it required approximately 75 mg/L KN, 5 mg/L MN, or 51 mg/L LC over 7 d for 50% of the organisms to die, it is expected that much higher concentrations would be required for significant mortality to be experienced with exposures of only 12 or 24 h. During the double pulse exposures with a 0 h recovery time before the second pulse (essentially a 24 h exposure), there was a significant decrease in survival (*p*>0.05; Figure 4). This double pulse exposure was at a concentration two times that used for highest 24 h exposures. Recovery times of 48 h or greater allowed organisms enough time to feed normally before being exposed again and experiencing reduced food consumption for a second time.

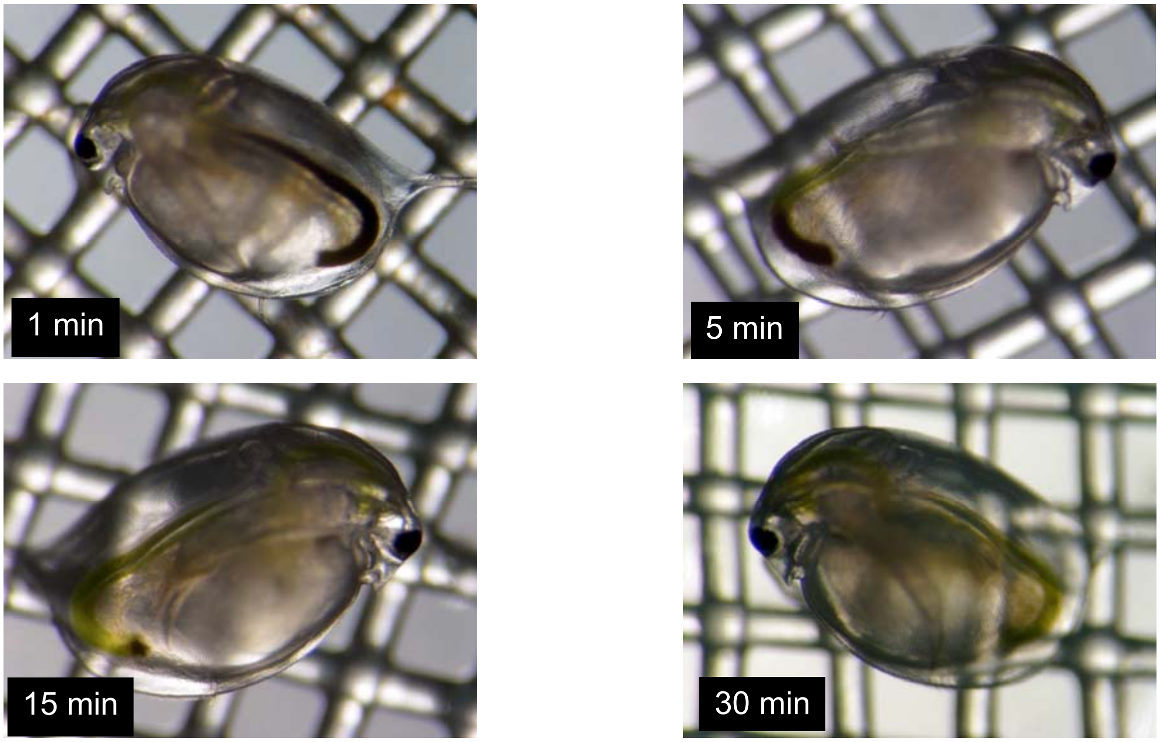

Figure 7. Photos showing clay movement out to the gut track after being exposed to natural clay-sized particles from Lost Creek (LC) clay and moved to clean media for 1, 5, 15, and 30 min. Photos are of the same organism (Capper, 2006).

Influences of exposure concentration and exposure duration were not equivalent. Doubling the exposure concentration for half of the duration did not yield the same result (Figure 5a). Single 12 h pulsed exposures did not significantly increase the number of days to gravidity; however, there was a significant increase in days to gravidity for single 24 h exposure at the three highest KN concentrations (73.9, 152.5, and 312 mg/L KN). There was also a significant increase in days to gravidity in the double pulse exposure when the recovery time was 0 h, which is essentially a 24 h exposure. The exposures that elicited a significant increase in days to gravidity (0 h recovery double pulse and the 24 h exposures of 312, 152.5, and 73.9 mg/L) were approximately equal, one half, one quarter, and one tenth, respectively, of the highest 12 h KN exposure that showed no effect. This result is one indication that duration of exposure is more important than concentration. The cause is likely due to the gut filling with clay particles for a range of concentrations, preventing food assimilation. During the 24 h exposure the organisms had decreased feeding for a longer time than the 12 h exposure, consequently causing a greater effect even at lower concentrations. Also, at the lowest concentrations the ratio of food to clay ingested should be greater, since all treatments received the same amount of food. Therefore, feeding would not be inhibited as much at this greater food-to-clay ratio. It may also take longer for the gut to fill with clay at the lowest concentration, decreasing the time during which the gut is filled.

Even with an increase in days to gravidity for organisms in the 24 h exposure, the number of neonates produced over the 21-d bioassay did not decrease (data not shown). If the organism lives to reproduce there should be little effect on the population. However, a delay in reproduction increases the risk of predation occurring before reproduction, which was not considered in this research. Also, results by Ratte (1996) suggested that increasing the time to first reproduction by one day could result in greater effects on the *Daphnia* population than decreasing the offspring produced. Hence, these results suggest that 24 h exposures could decrease the ecological fitness of *D. magna*. Previously, researchers reported a decrease in population growth rate during longer exposures to suspended sediments and clay, but the duration of the exposure was not discussed by these researchers (Kirk and Gilbert, 1990; Kirk, 1992).

Another indication that the longer exposures caused a greater effect even at lower concentrations is the decreased organism length observed for the 24 h exposures. In the days following these exposures there was a significant decrease in growth that was not observed in the days following the 12 h exposure at higher concentrations. By the end of the test period the length of the organisms exposed for 24 h were not significantly different from the controls, while those exposed for 12 h were significantly larger than the control organisms. This difference suggests a shift of more energy into growth after exposure. McCabe and O'Brien (1983) observed similar results with *D. pulex* that had been exposed to suspended silt, although they used a continuous exposure. They also used turbidity to measure the suspended solids making it difficult to compare to the results of the present study. The exposed organisms in their experiments were significantly longer than control organisms while reproduction was reduced. They explained this increased length as resulting from organisms funneling energy to growth rather than reproduction when exposed to suspended silt.

These results could aid in developing criteria for suspended sediments. Not only the amount of suspended particles is important but also the duration of exposure. It is possible to predict the occurrence and frequency of these kinds of exposures using runoff models such as WASP (Di Toro et al., 1983). Benthic as well as pelagic organisms should be considered as sediments can alter substrate composition, increase drift of the organisms, and even bury benthic invertebrates (Berry et al., 2003).

Another quality that must be considered is the type of clay. These results indicate that MN was much more toxic than KN. These differences may be due to differences in clay structures. Kaolinite is 1:1 clay meaning each sheet is composed of one to one layers of silicon tetrahedral and aluminum octahedral with the sheets held together by hydrogen bonds. Kaolinite has no interlayer for water molecules or ions to enter. It also has no permanent charge, only a pH dependent charge, due to protonation and deprotonation at the edge sites. On the other hand, MN is a 2:1 clay with an aluminum octahedral layer sandwiched between two silicon tetrahedral layers. Montmorillonite contains isomorphic substitution, or replacement of some of the Al^{+3} atoms by Fe^{+2} and Mg^{+2} atoms, resulting in a permanent negative charge of approximately 0.33 as well as pH dependent charges. The sheets are held together by weak electrostatic forces allowing an interlayer, and therefore, an expandable clay. Water molecules and cations can enter the interlayer. This expandability, as well as greater cation exchange capacity of MN compared to KN, is likely the reason for its greater toxicity. However, this study did not focus on exactly how it influences toxicity. Future research should focus on the mechanism for this difference so that more predictive relationships that include particle composition can be generated. The knowledge of the mechanism might lead to site-specific criteria based on local geology.

CONCLUSIONS

This study looked at the effect to *Daphnia magna* of exposure to suspended clay. Specifically, it looked at the effects of episodic exposures as this commonly occurs in the environment as a result of erosion of exposed soils during rain events. Three different types of clay were used and *Daphnia* were exposed to a single or double pulse of varying duration and concentration. The results suggest that the type of clay daphnids are exposed to is very important. The 7 d LC50 varied between clays with montmorillonite being the most toxic and kaolinite the least toxic. Episodic exposures demonstrated the same results. For 24 h exposures, kaolinite concentrations (74 mg/L) had to be much higher than montmorillonite concentrations (9 mg/L) before an increase in days to gravidity or decreased growth was observed. The difference between the clays indicates that the harm done to these organisms is dependent on the geology of the sediment in the system, and all systems are not the same.

The results of this study also suggest that the duration of the exposure is important when considering the effects of suspended sediments. Duration and concentration were not equivalent, that is, doubling the concentration for half the time did not elicit the same effect. Even at 10 times the lowest concentration of 24 h exposure that increased days to gravidity, there was no increase in days to gravidity for the 12 h exposure. Few researchers considered the duration of suspended sediment exposures prior to Newcombe and MacDonald (1991). Much of the time suspended sediment exposures are short as a result of storm events and then organisms have time to recover before the next exposure.

The results of this study could be important when developing suspended sediment criteria. The result that different clays elicit different effects means that criteria may need to differ depending on the geology of the area. Also the duration of exposure was more important than concentration. Therefore, suspended solids reaching high concentrations for a short duration may be better than lower concentrations for a longer duration. However, filter feeding zooplankton should not be the only biological indicator for suspended sediments criteria. High concentrations for short duration may be more harmful to other organisms, so this needs to be considered.

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