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Relationships Between Landscape Factors and Crayfish Density and the Interacting Effects of Grazing and Increased Sedimentation on Algal Biomass

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Recommended Citation

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Relationships Between Landscape Factors and Crayfish Density and the Interacting Effects of
Grazing and Increased Sedimentation on Algal Biomass

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology

by

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University of Central Arkansas
Bachelor of Science in Biology 2008

December 2013
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

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ABSTRACT

In recent years in the United States there has been a rapid expansion in anthropogenic sources of sediment in streams including construction, agriculture, and drilling for natural gas. Potential effects land disturbance associated with activities from natural gas development on aquatic biota in surrounding streams have not yet been well documented. An increase in inorganic sediment in streams can be detrimental to organisms through a variety of mechanisms including alteration of dominant substrate type, higher turbidity resulting in lower visibility, and burial of food resources such as algae and detritus. Increasing sedimentation in stream environments through anthropogenic disturbance is a widespread problem, but few studies examine sediment effects on biological interactions and processes. I had two main objectives, which were 1) to investigate whether abundances of crayfish, an important stream organism, were correlated to natural gas well density or other natural gas related variables in stream catchments, and 2) to determine if sediment altered the grazer-periphyton interaction and if that effect was dependent upon grazer type (i.e., if sediment impacted the grazer-periphyton relationship differently between scrapers and collectors). I sampled crayfish in streams draining catchments with differing well activity to address the first research objective. A negative correlation existed between the predictor variables of the number of gas wells and the density of unpaved roads and the response variable of crayfish abundance suggesting further research examining potential natural gas activity impacts on crayfish populations may be important to avoid negative impacts of gas drilling on crayfish. Greenhouse experimental streams were employed to address the second objective and I found that increasing sediment affected the grazer-periphyton interaction differently between two grazers (crayfish and snails), where crayfish under high sediment levels provided a marginally significant net benefit to algal

biomass. In contrast, snails under high sediment conditions caused a statistically significant decline in algal biomass. This result suggests that sediment effects on grazer-periphyton interactions will depend on the grazer species and future studies could examine impacts on insect grazers that may be more sensitive to sedimentation.

ACKNOWLEDGEMENTS

<u>DEDICATION</u>

I dedicate this work to my grandfather, Joseph Cruse, who passed away before the

conclusion of this project, but whose constant encouragement throughout my life helped to

motivate me to finish my degree.

> Nature's first green is gold,
> her hardest hue to hold.
> Her early leaf's a flower;
> but only so an hour.
> Then leaf subsides to leaf,
> so Eden sank to grief,
> so dawn goes down to day,
> nothing gold can stay.
>
> Robert Frost
> (Frost, 1923)

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INTRODUCTION

This thesis explores the potential effects land disturbance associated with activities from natural gas development on grazing stream invertebrates, and the potential effects of natural gas drilling *via* increased sedimentation on grazer-periphyton interactions. Drilling for natural gas has rapidly expanded in the United States (US) over the last decade and has potential ecological consequences that may not be yet fully realized. The number of producing wells in the US has increased 1.5 times from 2000 to 2011 (US EIA 2013). Natural gas drilling in the Fayetteville Shale often takes place near streams, and the potential impacts to surface waters as outlined in Entrekin et al. (2011) include increased sedimentation (Williams et al. 2008) and turbidity, potential contamination by hydraulic fracturing fluids or produced waters, and alteration of stream flow due to local water withdrawals. Chapter one of this thesis looks at the relationships between the density of crayfish, an important stream grazer, and natural gas activity measures as well as other landscape- and local-scale variables. Chapter two investigates how a documented natural gas activity impact on streams, increasing sedimentation (Williams et al. 2008), may affect the grazer-periphyton interaction within streams.

Crayfish have been shown to account for nearly half the invertebrate production in streams (Momot 1995) and can function as "ecological engineers" by significantly redistributing stream substrates (Statzner et al. 2000, Statzner et al. 2003). Thus, crayfish play important roles in stream ecosystems and any alteration in the environment that negatively impact crayfish abundances will further impact stream food webs and substrate structures. Crayfish have been shown to change in relation to land use, but these changes are species and land use specific and sometimes counter-intuitive. In research conducted by Simon and Morris (2000) in Indiana, crayfish species from five different genera (*Cambarus*, *Orconectes*, *Procambarus*,

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Fallicambarus, and *Palaemonetes*) all declined significantly with an increase in commercial land use development. Crayfish from the *Cambarus* genera declined with increases in residential development, but tolerated increases in agricultural land use. One unexpected result was that one crayfish species, *Palaemonetes kadiakensis* actually increased in relative abundance as agricultural land use increased, though a potential explanation for this is not given. This study also documented tolerance levels of crayfish to many aromatic hydrocarbons (e.g. naphthalene and perylene) and metals (e.g. manganese and strontium). The effects of these chemicals on crayfish is of particular interest when considering the effects of natural gas drilling, as many of the chemicals in hydraulic fracturing fluid are aromatic hydrocarbons (US House of Representatives Committee on Energy and Commerce 2011), and metals are often found in the waters produced from drilling (Entrekin et al. 2011). Any of these could be mechanisms by which crayfish populations may be negatively impacted, which could alter stream properties *via* aforementioned means. Further, natural gas drilling has been shown to increase the amount of fine sediments coming off of gas pads and running into the stream (Williams et al. 2008), which can impact stream organisms in other detrimental ways.

Increasing sedimentation in streams has been cited as the number one threat to surface waters by the US EPA (2006). An increase in the amount of fine sediments that compose the substrate in gravel bed streams has been shown to negatively impact organisms at all trophic levels, including deleterious effects to algae *via* blocking sunlight (Waters 1995, Steinman 1996, Wood and Armitage 1997, Izagirre et al. 2009), burial creating anoxic conditions (Peterson 1996), and increased scour during high flow events (Newcombe and MacDonald 1991, Francoeur and Biggs 2006). Similarly, an increase in fine sediments negatively affects primary consumers such as macroinvertebrate and fish grazers through mechanisms of reduction in food

quality (Sloane-Richey et al. 1981, Cline and Short 1982), loss of interstitial spaces used for shelter (Richards and Bacon 1994, Gayraud and Philippe 2003, Bo et al. 2007) and egg laying surfaces (Berkman and Rabeni 1987). While the negative effects on these organisms have been studied independently, it is not as well understood how increasing sediment may interact with the process of grazers consuming algae. The process of grazing has been shown to have measured, predictable impacts on algal production and composition, generally reducing overall periphyton biomass, increasing biomass specific productivity, and shifting the community physiognomy towards smaller algal growth forms, as the taller forms are grazed directly or dislodged by grazers indirectly (Steinman 1996). While the impacts of sediment and grazing on algal biomass have been studied independent of one another, few studies have investigated how a variable like increasing sediment may affect the established grazer-periphyton interaction.

The goal of this thesis was to first establish if there were any observed relationships between the density of crayfish, a dominant stream grazer, and landscape-scale variables that might be associated with increased sediment loads to streams, such as agricultural land use or natural gas activity. Then, I wanted to determine how increased sedimentation may alter the grazer-periphyton interaction. Deleterious impacts of sediments in streams, can impact multiple trophic levels and their interactions at once, and therefore understanding these processes is essential to fully understand the way threats like increasing sedimentation will impact the whole stream. This paper seeks to add to that body of knowledge by linking how natural gas and other anthropogenic practices that contribute sediments to streams may be impacting the density of an important stream grazer and altering the interaction between grazers and their periphyton food source.

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CHAPTER 1

The Potential Influences of Land Use on Crayfish Density

ABSTRACT

Crayfish are an integral part of stream food webs, acting as carnivores, grazers, and detritivores. In addition, they often compose the majority of macroinvertebrate biomass and are major contributors to secondary production as prey for many species of fish. Crayfish abundance can be related to landscape and local habitat factors and anthropogenic land use change, such as increasing natural gas activity, in catchments may reduce abundance by increasing fine sediment in streams. This study, conducted spring 2012, examined the relationship between catchment natural gas activity and other landscape and local variables on stream crayfish densities. Crayfish densities were quantified in ten streams draining catchments with a range of natural gas wells (0.0-3.0 wells km^{-2}). I hypothesized that crayfish densities would be lower in streams with higher densities of natural gas wells in their catchments. Catchment and in-stream variables were examined using multiple linear regression analysis and the best fit model for each was selected using Akaike Information Criteria. While no in-stream variable models had significant effects on crayfish density, it was determined that crayfish density was significantly related to a combination of catchment variables. The best fit model of catchment variables included the average density of unpaved roads, the runoff distances from the wells to the stream (flow inverse path), and the percent of urban and pasture land (R^2=0.8327; p=0.0096). This study is the first to document a negative relationship between natural gas drilling activity and crayfish densities. While this study was limited to one sampling season, it suggests that continued monitoring of the effects of natural gas drilling on crayfish populations is warranted. Studies should also endeavor to determine what specific factors of gas drilling may be negatively impacting crayfish.

INTRODUCTION

Natural gas is an important transition fuel between traditional petroleum and cleaner energy sources, but until recently it has been locked in shale formations and was too difficult and expensive to access. However, a combination of recent advances in hydraulic fracturing, or "fracking", and favorable legislation like the "Halliburton Loophole" have made natural gas more accessible and less expensive to extract. Hydraulic fracturing involves the building of infrastructure to access the drilling sites, clearing off land to construct the well pad, pushing a mix of chemicals and water under high pressure into the shale layer to fracture the rock, and disposing of the produced waters that return from the well. Many of the environmental concerns raised by the increase in natural gas drilling have been outlined in Entrekin et al. (2011), and include potential threats to surface waters via contamination by the fracking fluids or produced waters, which contain a number of potentially harmful chemicals (US House of Representatives Committee on Energy and Commerce 2011) and heavy metals (Soeder and Kappel 2009), and increased sedimentation from the clearing of land for roads and well pads (Williams et al. 2008). Increased sedimentation is a problem that results from other anthropogenic disturbance such as agriculture (Costa 1975, Lenat 1984, Clark et al. 1985, Zaimes et al. 2004) and urbanization (Wolman 1967, Paul and Meyer 2001), and has been shown to negatively impact biota via reduction in suitable habitat through the filling of interstitial spaces (Richards and Bacon 1994, Gayraud and Philippe 2003, Bo et al. 2007).

Crayfish variables such as density and species diversity can be related to stream catchment as well as to local environmental variables. Relationships to catchment variables include positive relations with forested or suitable riparian buffer (Page and Mottesi 1995). Studies that have found relationships between crayfish and catchment-scale land use have

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suggested that one of the primary mechanisms for the land-use effect on crayfish abundance were associated changes in the substrate composition (Page and Mottesi 1995, Taylor et al. 1996, Butler et al. 2003, Ngulo and Grubbs 2010). Several crayfish species have been found to prefer or to have positive associations with habitat variables such as availability of pebble, cobble, and boulder substrates (Bovbjerg 1970, Bouchard and Robinson 1980, Mitchell and Smock 1991, Hill and Lodge 1994, Kershner and Lodge 1995, Riggert et al. 1999, Flinders and Magoulick 2005, Westhoff et al. 2006). Many studies associate this preference for larger substrates with predator avoidance (Stein and Magnuson 1976, Stein 1977, Hill and Lodge 1994, Kershner and Lodge 1995). If land-use change in a streams watershed leads to increasing fine sedimentation (Williams et al. 2008, Entrekin et al. 2011), then it has the potential to negatively impact crayfish abundances by filling interstitial spaces.

Negative impacts of natural gas activity in catchments on crayfish populations could alter many aspects of the stream ecosystem. Crayfish have been called both a keystone species, for their role in stream food webs, as well as ecosystem engineers for the way they impact the structure of the streambed. Studies examining the role of crayfish in food webs have determined that they serve many functional roles. Momot et al. (1978) explored the role of crayfish in lake food webs, and concluded that crayfish were key energy transformers between the trophic links and that their functional importance exceeds their biomass dominance, which is one definition of a keystone species (Paine 1995). His study found that in their systems, crayfish served roles as primary consumers, primary carnivores, and decomposers. Momot also found that in their system crayfish served roles as primary consumers, primary carnivores, and decomposers. These findings are similar to those in a study by Whiteledge and Rabeni (1997), which found that crayfishes in the Jacks Fork River (in the Ozarks) have myriad functional roles in the tropic

system and have the potential to significantly affect the production and biomass of lower tropic levels. They concluded that crayfish play important roles as predators, shredders, and algal grazers, often consuming more of these food resources (animal matter, CPOM, and benthic algae, respectively) annually than that estimated to be consumed by all other benthic invertebrates. In addition to the role of crayfish in the food web, crayfish can restructure their habitat in search for food, by means such as macrophyte reduction or removal and sediment disturbance (Momot 1995). This ability of crayfish to restructure their environment has led to other scientists deeming them ecosystem engineers (Statzner et al. 2000, Statzner et al. 2003, Helms and Creed 2005) for their ability to change the structure of the benthic environment by removal of filamentous algae (Creed 1994, Nystrom 1999) and macrophytes (Momot 1995, Nystrom 1999), as well as their ability to redistribute sediments on stream beds. Statzner et al. (2000) found that crayfish significantly affected the form of the substrate in artificial streams by causing increased bedform roughness in riffles, and decreased sand in gravel interstitial spaces in riffles.

Research on whether land use change associated with natural gas drilling and other human activities is affecting crayfish populations is especially important in Arkansas, as it has one of the highest crayfish diversities in the U.S. (Bouchard and Robinson 1980, Hobbs 1988) and natural gas drilling has been substantially increasing over the past few years (Entrekin et al 2011). The purpose of this study was to determine if crayfish abundance was related to land use measures, particularly natural gas activity metrics, in the streams of north central Arkansas. I hypothesized that if land use changes associated with increasing natural gas wells (NGWs) were negatively impacting streams, then streams with a higher density of NGWs in their catchment will have lower crayfish densities than streams with a low density of NGWs in their catchment.

METHODS

Study Design

Ten study stream reaches approximately 200 meters long were selected that had differing

NGW densities in their respective catchments ranging from 0.3 to 3.0 wells/km^2. These study

reaches were in low order, gravel bed streams located across north central Arkansas. The ten

streams are part of four different drainages (Figure 1). Data on well densities and locations

within each catchment were collected from the Arkansas Oil and Gas Commission. Inverse flow

path lengths were calculated by The Nature Conservancy and the process is detailed in Entrekin

et al. 2011. In summary, the inverse flow path lengths are defined as the flow distances from the

well pads to the stream channels. Because the area around many of these streams contains

elevated topography, an attempt was made to account for this and define the actual distance that

the water would travel, rather than assuming a straight path. Well pads that had a shorter flow

distance to the streams were given a higher impact value. The gas well point data used to

calculate the flow inverse path lengths were obtained from the Arkansas Oil and Gas

Commission and were based on data current as of March 28, 2012. Land use, classified as

forest, urban and pasture, in each catchment was quantified based on 2009 aerial photography

(Gorham and Tullis USGS).

Habitat Metrics

Habitat metrics were collected in each stream along a 200m delineated section. Every

10m of stream length, the substrate was categorized every 0.5m across the stream width into

bedrock, silt, sand, pebble, gravel, cobble, and boulder. Percent of the stream that was covered

by tree canopy was also estimated and core samples were taken from within the pools and riffles

of each delineated reach of the streams by collecting all the course benthic organic matter

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(CBOM) from within a cylinder of a known area and then disturbing the substrate within the cylinder and collecting a water sample from within to determine the average amount of fine benthic organic matter (FBOM). All of this work was completed by professors and students at the University of Central Arkansas and the University of Arkansas as a part of a large scale study on the effects of NGWs on streams.

Crayfish Collection

In May 2012, ten streams were sampled for crayfish in north-central Arkansas. In each stream, five riffles were sampled for crayfish. Each riffle was sampled in six different, haphazardly selected locations using a $0.485m^2$ pvc quadrat which was placed upstream of a $500\mu m$ mesh kicknet. The area in the quadrat was vigorously disturbed with a hand rake for 1 minute to dislodge any crayfish. After the area was disturbed, the net was brought forward through the disturbed area and the contents of the net were examined for crayfish.

Crayfish were removed from the net, sorted into similar groups and identified on site using Pflieger and Dryden (1996). Each individual was sexed, and its carapace length was measured. If available, one form I male from each group was preserved in 80% isopropyl alcohol and returned to the lab to confirm the identification.

Statistical analysis

In this study there were many potential predictor variables, including in-stream variables and landscape level variables that may have influenced the one response variable of crayfish density (crayfish/m^2). A principle component analysis (PCA) was used to rank in-stream and landscape level predictor variables in order to eliminate variables with lower explanatory power. The top three variables were chosen from both in-stream and landscape level predictor variables and examined against each other to check for and eliminate variables that were collinear. If two

variables were collinear, the variable with lower explanatory power was not included in the model selection process. The model selection was accomplished using the Akaike Information Criteria (AIC) method. All statistical analyses were conducted using SAS statistical software (version 9.2, SAS Institute, Cary, NC).

RESULTS

The resulting top three landscape level predictor variables from the PCA (Table 1) were the density of unpaved roads (r^2=0.445), total wells (r^2=0.358), and flow inverse path (r^2=0.281). While total wells (Figure 2A) had a marginally significant effect on crayfish density (p=0.068), it was not included in the AIC model selection because it was significantly correlated with the density of unpaved roads (Figure 2D). The best fit model, as determined using AIC (Table 2) included the independent variables density of unpaved roads (Figure 2C) and flow inverse path (Figure 2B).

The resulting top three in-stream predictor variables from the PCA (Table 1) were percent pebble substrate (r^2=0.191), average amount of fine benthic organic matter (r^2=0.105), and average amount of course benthic organic matter (r^2=0.077). None of the variables independently had a significant relationship with crayfish density (Figures 3A-3C), and there was no statistically significant best fit model resulting from the AIC selection process (Table 3).

DISCUSSION

This is the first study, to my knowledge, to examine potential relationships between land use activities related to natural gas drilling by hydraulic fracturing and crayfish, even though this process poses potential threats to surrounding stream biota (Entrekin et al. 2011). I hypothesized that streams that had a higher density of natural gas wells within their catchments would have lower crayfish densities. A negative relationship was found between a combination of some

natural gas and land use-related variables and crayfish density censused during one season in ten north central Arkansas streams.

Crayfish have been shown to be affected by changes in land use, primarily through changes in substrate composition (Page and Mottesi 1995, Ngulo and Grubbs 2010). Other studies have reinforced the notion that substrate composition impacts crayfish abundance and distribution, where crayfish often prefer or are correlated with increases in pebble, gravel, and cobble substrates (Bovbjerg 1970, Bouchard and Robinson 1980, Mitchell and Smock 1991, Hill and Lodge 1994, Kershner and Lodge 1995, Riggert et al. 1999, Flinders and Magoulick 2005, Westhoff et al. 2006). Due to these known effects, the crayfish density data in this study were examined against variations in in-stream habitat metrics to determine whether or not any of them significantly explained the observed variation in crayfish abundance. A principle component analysis (PCA) revealed the top three in-stream variables potentially influencing crayfish densities to be the percent pebble substrate, and the average amount of FBOM and CBOM, respectively. However, after using AIC model selection, no significant combinations of these predictor variables were significant (Table 3; Figures 3A-3C). It is important to note that the studies that have found relationships between substrate types and crayfish distribution have varied in the number of streams or lakes sampled, ranging from three sites with multiple sampling dates (Mitchell and Smock 1991) to 65 sites in 54 streams sampled two consecutive years (Flinders and Magoulick 2005), and that this study is at the low end of necessary samples and therefore explanatory power may be limited. In order for this result to be more thoroughly evaluated, more streams and sampling sites should be included. However for this study, once it had been determined that no in-stream habitat variables were affecting crayfish abundances, landscape level variables could then be examined for any potential impacts.

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The landscape level variables examined for their potential impacts on crayfish densities included natural gas related variables like the density of unpaved roads (which significantly increases with total wells; Figure 2D) and the flow inverse path (Figure 2B). Crayfish did have a significant negative relationship with the density of unpaved roads (Figure 2C), but no other relationships were statistically significant. When the variables were combined and a model was selected using AIC, it was determined that the combination of variables that had the most significant impact on crayfish density was density of unpaved roads*flow inverse path (Table 2). While it is clear that these natural gas related variables (density of unpaved roads and flow inverse path) have a negative relationship with crayfish densities, the exact mechanisms underlying this relationship cannot be determined with this study. Unlike the studies mentioned previously, this negative relationship between landscape factors and crayfish density to not appear to be due to changes in in-stream variables like substrate composition, but again the power of this study is low due to limited sample sites and a large number of explanatory variables. It is also important to note that a previous study in these streams (Entrekin et al. 2011) did observe an increase in suspended sediments with gas well activity at these same study sites, which could lead to substrate changes and in turn further affect crayfish abundances. It is tempting to speculate that there may be a release of some chemical or heavy metal in these streams with higher total wells (highly correlated with unpaved roads; Figure 2D) which may be negatively impacting crayfish densities, as crayfish have been shown to negatively respond to increases in mercury and other heavy metals (Ramo et al. 1987, Simon and Morris 2009), but further water quality testing and comparative analysis would be needed to draw such conclusions. Another possibility could be that the land use changes related to gas well activity is impairing or shifting the algal or macroinvertebrate densities, thereby causing a bottom –up

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effect on crayfish by negatively impacting their primary food resources. As stated previously, crayfish have a diverse diet and have been found to consume large amounts of detritus, animal matter, and algae (Momot 1978, Whitledge and Rabeni 1997). While there was no significant relationship between CBOM and crayfish densities (Figure 3C), there may have been undetected relationships between land use changes related to natural gas drilling and macroinvertebrate densities or algal biomass, which could be causing the decline in crayfish densities, but a more direct study would need to be conducted to determine if there is a causal relationship between these variables.

This study has been the first to document a negative relationship between variables related to natural gas drilling on crayfish, but the study was limited by having only one sampling season and few study streams. Further, correlation does not equal causation. However, this study highlights, the importance for further study and monitoring the effects of natural gas drilling on the surrounding surface waters and their biota. Natural gas may be a useful energy to reduce dependence on foreign oil and serve as a cleaner burning alternative to coal and oil, but it needs to be mined in a manner that is ecologically responsible.

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Table 1: Catchment and in-stream variables and their respective means and ranges. See Figure 1 for location of the streams used.

Catchment variable	Mean	Range	In-stream variable	Mean	Range
Density of Unpaved Roads (m/km^2)	839.1	567.7-1307.1	% Pebble Substrate	8.7	2.8-16.7
Total Well Density (#/km^2)	1.3	0.3-3.0	Fine Benthic Organic Matter (g)	645.1	214.1-1510.1
Flow Inverse Path	1.45	0.03-5.24	Coarse Benthic Organic Matter (g)	44.0	0.6-205.3

Table 2: Models of the effects of catchment variables on crayfish density from a multiple linear regression using Akaike Information Criteria.

Model	Adj. R^2	AIC
Unpaved Roads*Flow Inverse Path	0.6055	4.9449
Unpaved Roads	0.3757	8.8701
Flow Inverse Path	0.1907	11.4646

Table 3: Models of the effects of in-stream variables on crayfish density from a multiple linear regression using Akaike Information Criteria.

Model	Adj. R^2	AIC
% Pebble substrate	0.1907	12.6430
% Pebble substrate*CBOM	0.2392	14.0243
FBOM	0.1048	13.6516
% Pebble*FBOM	0.2139	14.3518
CBOM	0.0770	13.9570
FBOM*CBOM	0.1791	14.7844
% Pebble*FBOM*CBOM	0.2658	15.6685
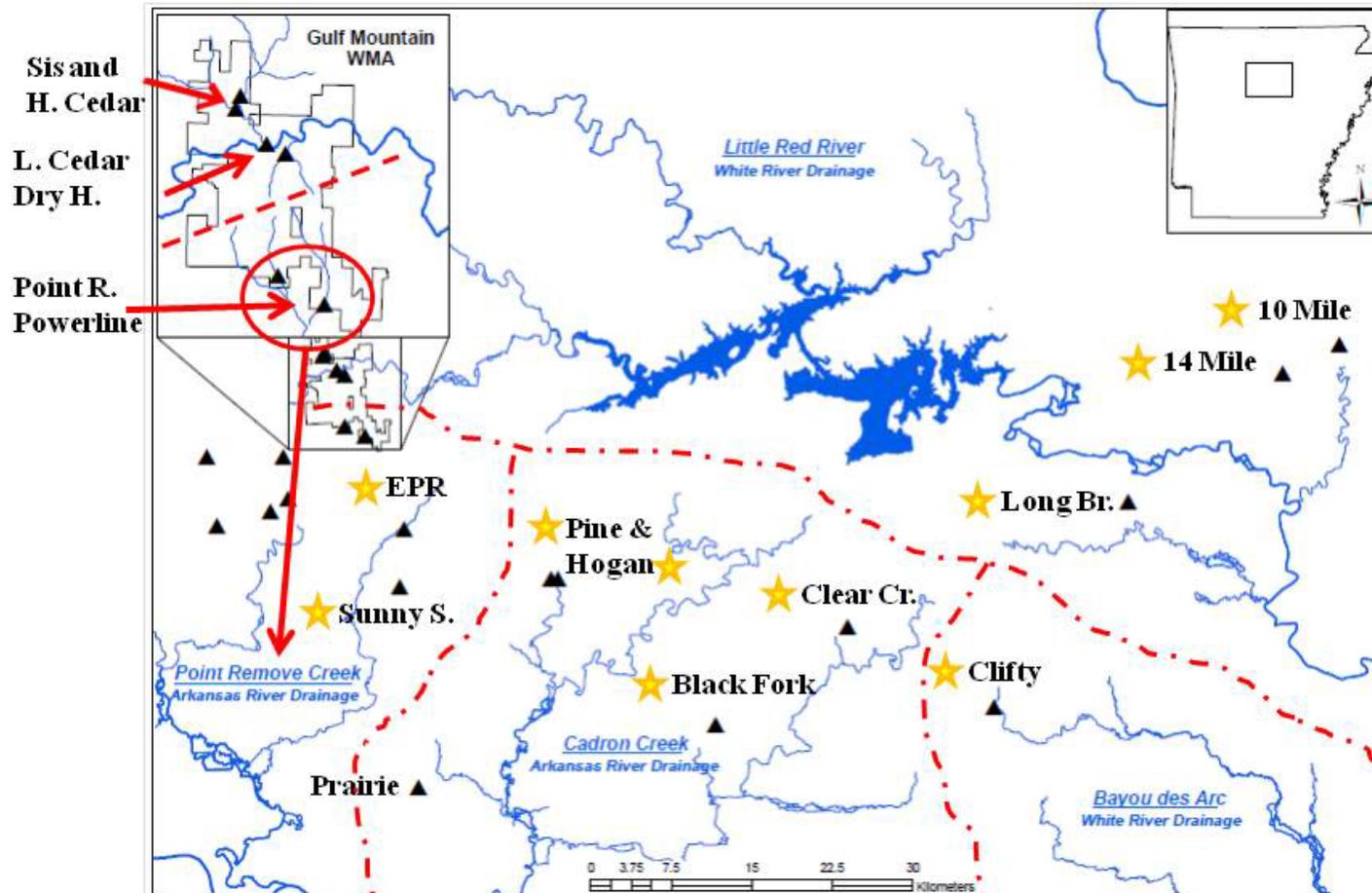
Figure 1: The streams sampled and their respective drainages. The streams that were sampled in this study are marked with a star. Map courtesy of Loren Stearman.
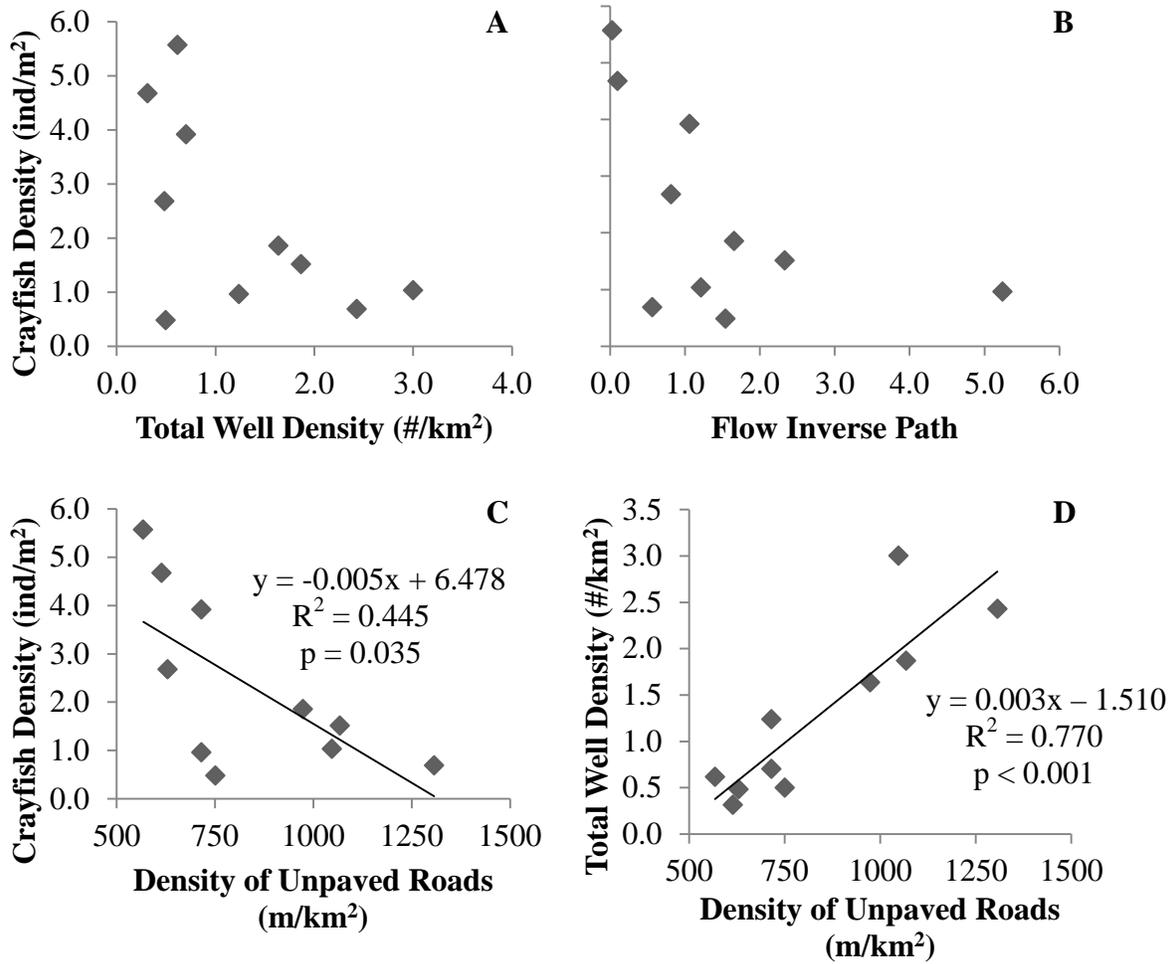
Figure 2: Relationships between the density of unpaved roads within the catchment and crayfish density (A), total well density and crayfish density (B), average inverse distance between the wells and the streams (Flow Inverse Path) and crayfish density (C), and density of unpaved roads and total well density. Linear regression equations, correlation coefficients (R^2), and probability (p) values are reported for relationships where p≤0.05.

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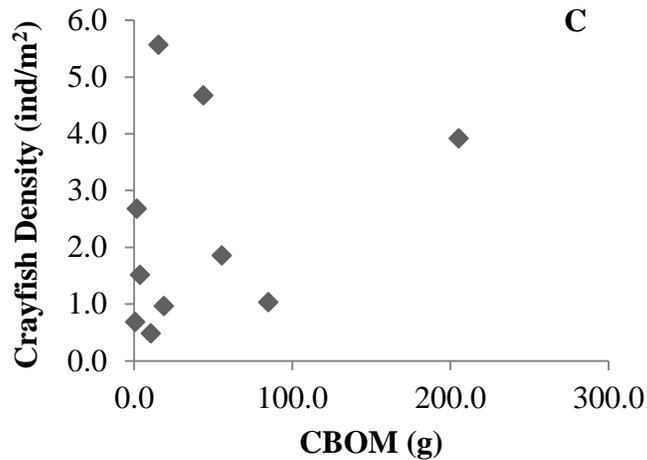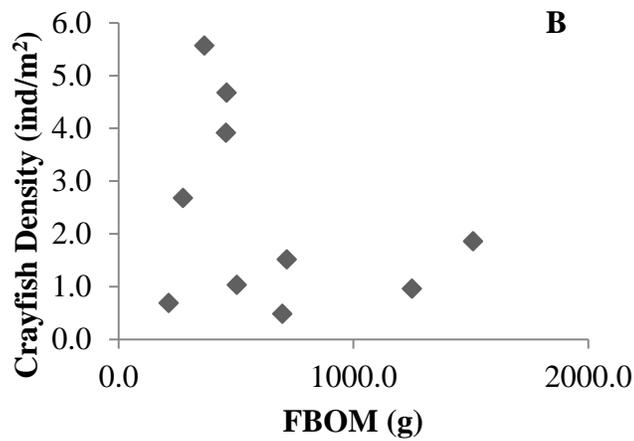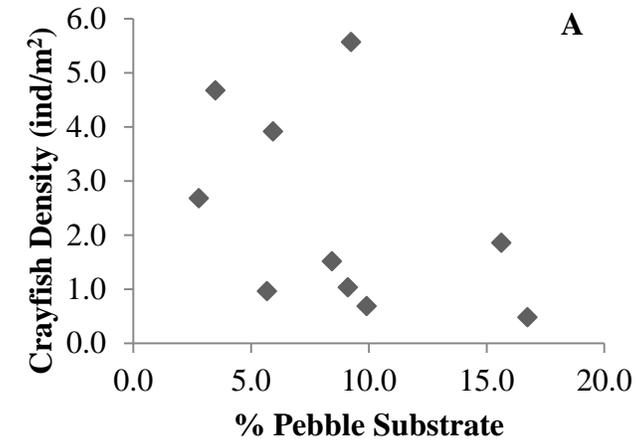
Figure 3: Relationships between average percent pebble substrate and crayfish density
(A), average mass of fine benthic organic matter (g) in the stream and crayfish density
(B), and average mass of course benthic organic matter (g) in stream and crayfish
density (C). Linear regression equations, correlation coefficients (R^2), and probability
(p) values are reported for relationships where $p \leq 0.05$.

CHAPTER 2

The Interacting Effects of Grazing and Increased Sedimentation on Algal Biomass

ABSTRACT

Increasing sedimentation is one of the main factors leading to reduced biological condition of US streams and can directly negatively impact stream periphyton via reduction of light and habitat availability. It can also indirectly affect periphyton by altering the interaction between grazers and periphytic algae, but few have examined this relationship. This study examined whether increasing sediment affects grazer-periphyton interactions using two common grazing species, the crayfish (*Orconectes palmeri*) and the snail (*Elimia* sp.), which have both been shown to reduce periphyton biomass in the absence of sedimentation. I hypothesized that sediment would have a negative effect on periphyton biomass and limit snail grazing more than crayfish grazing due to the snails smaller size and feeding mode (scraping) as opposed to the crayfishes larger body size and feeding mode (collecting). Therefore, crayfish grazing would increase chlorophyll *a* (chl *a*) by providing a net benefit to shorter algal growth forms via a reduction in cobble sediments. I expected snail grazing would have no effect on cobble sediments and periphyton biomass. To test this, I conducted two, 4-wk experimental stream sediment (low sediment [LS]=100g/m^2; intermediate sediment=1000g/m^2; and high sediment=5000g/m^2) and grazer (*Orconectes palmeri* present or absent, and *Elmia* sp. present or absent) manipulations. In both experiments, periphyton biomass was stimulated by benthic sediment addition and the presence of grazers reduced both the amount of sediment on the cobbles collected (p<0.0001 for both) and the amount of loosely-attached filamentous algal biomass (crayfish, p<0.0001; snails, p≤0.0126); however, the presence of crayfish increased the amount of periphytic chl *a* under all sediment conditions, whereas in the snail grazing experiments, the presence of snails decreased the amount of chl *a* under all sediment conditions. This result suggests that grazing by crayfish facilitated the growth of smaller growth forms of

algae, whereas snail grazing removed even these growth forms. This is the first experiment to my knowledge to indicate that sediment effects on stream periphyton may depend on the dominant benthic grazing species present.

INTRODUCTION

Streams are important freshwater ecosystems that are becoming increasingly imperiled by anthropogenic disturbances such as eutrophication (Smith 2003), changes in stream morphology due to channelization (Lau et al. 2006, Smiley and Dibble 2008), and increasing sedimentation (Waters 1995, USEPA 2006). Sedimentation can be defined as an increase in small size fraction particles, organic and inorganic, in aquatic ecosystems. Increased sedimentation was recently listed by the United States Environmental Protection Agency (USEPA) as the number one contaminant of wadeable United States (U.S.) streams (USEPA 2006). Traditionally, the main sources of sediment have been cited as row-crop agriculture (Costa 1975, Lenat 1984, Clark et al. 1985, Zaimes et al. 2004), livestock grazing (Platts 1979, Zaimes et al. 2004), forestry (Tebo 1955, Bormann et. al. 1974, Beschta 1978, Kreutzweiser and Capell 2001), mining (Wagener and LaPerriere 1985, James 1989) and urban development (Wolman and Schick 1967, Jones and Holmes 1985, Paul and Meyer 2001, Walters et al. 2003). As the human population continues to increase, land use modification will likely increase sediment contamination in streams. Manipulative studies examining the impacts of this increasing sediment on biota and their interactions are needed to fully understand the consequences of sedimentation in stream ecosystems. Sedimentation has been shown to have negative effects on algae and periphyton, which is a community of heterotrophic microbes and algae within a polysaccharide matrix growing on surfaces in aquatic ecosystems. This periphyton is an important basal food resource in freshwater ecosystems.

The main effect of sedimentation, both suspended (Ellis 1936, Lloyd et al. 1987, Newcombe and MacDonald 1991, Steinman 1996, Wood and Armitage 1997, Yamada and Nakamura 2002) and benthic sediment (Waters 1995, Steinman 1996, Wood and Armitage 1997,

Izagirre et al. 2009), on algae comes from the reduction of light for photosynthesis. Suspended

sediment can also scour algae from the substrate (Newcombe and MacDonald 1991, Francoeur

and Biggs 2006). Sedimentation can also cause decreases in overall periphyton biomass due to

scour by sediment during high flow events (Horner et al. 1990, Blenkinsopp and Lock 1994).

Further, increases in clay particles can reduce the organic portion of periphyton by adhering to

the sticky polysaccharide matrix (Sloane-Richey et al. 1981, Graham 1990). The incorporation

of inorganic sediment particles into the organic component of periphyton may reduce periphytic

food quality for grazing stream biota (Sloane-Richey et al. 1981, Graham 1990).

The negative impact of sedimentation on grazing organisms comes not only through a

potential reduction in food quality (Sloane-Richey et al. 1981, Cline et al. 1982, Graham 1990),

but also through a variety of other mechanisms. Increasing suspended and benthic sediment can

affect respiration through gill clogging in both macroinvertebrates (Lemly 1982) and fish (Berg

and Northcote 1985, Bruton 1985, Power 1990a, Servizi and Martens 1992, Wood and Armitage

1997). Macroinvertebrates are further affected by benthic sedimentation through the loss of

habitat by filling of interstitial spaces (Richards and Bacon 1994, Gayraud and Philippe 2003, Bo

et al. 2007). The reduction in "clean" substrate by increased siltation can also affect the

spawning habitat of lithophilous fishes like the central stoneroller (Berkman and Rabeni 1987).

A reduction in grazing organisms due to factors such as sediment could impact algal

communities, as grazing by organisms have been shown to effect algal communities in various

ways.

The effect of grazing on periphyton community structure is best observed when

examining changes in physiognomy (Steinman 1996). Steinman (1996) quotes Whittaker (1975)

as defining physiognomy as the study of form and structure in natural communities. The

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physiognomy of benthic algal communities yields fairly consistent responses to grazing, with grazing typically causing in a decrease in overstory forms of algae and an increase in understory forms (Steinman 1996). The decrease in percent overstory has been seen using myriad grazers including snails (Hunter 1980, Kesler 1981, Sumner and McIntire 1982, Cuker 1983, Lamberti et al. 1987a, Steinman et al. 1987a, Lowe and Hunter 1988, Swamikanu and Hoagland 1989, Tuchman and Stevenson 1991, Hill et al. 1992, Steinman et al. 1992, Rosemond et al. 1993), mayfly larvae (Colletti et al. 1987, Hill and Knight 1987, Hill and Knight 1988), caddisfly larvae (Lamberti and Resh 1983, Jacoby 1987, Steinman et al. 1987a, Hill and Knight 1988, Lamberti et al. 1989, Feminella and Resh 1991, Katano et al. 2007), crayfish (Vaughn et al. 1993, Creed 1994), and minnows (Power and Matthews 1983, Power et al. 1985, Gelwick and Matthews 1992). The declines in overstory forms come not only through direct grazing (Hill and Knight 1987, Leiss and Hillebrand 2004), but also through dislodgement as grazers move through the periphyton matrix (Hill and Knight 1987, Hill and Knight 1988, Lamberti et al. 1989). The increase in percent understory forms is typically relative, as the number of understory algal cells typically declines with grazing, but they decline at a far lower rate than the overstory forms, increasing their percent abundance (Steinman et al. 1987a, Mulholland et al. 1991, Hill et al. 1992). Understory forms may also increase with grazing as they may benefit from the removal of overstory forms through increased nutrient and light availability (Feminella and Resh 1991, Mulholland et al. 1991, Steinman 1996). Changes in the structural makeup of periphyton due to grazing can lead to, or be accompanied by changes in biomass and primary production.

Periphyton biomass, like periphyton physiognomy, responds to grazing with fairly consistent patterns (Steinman 1996). Periphyton biomass almost always declines in response to grazing (Steinman 1996), and that pattern has again been observed using multiple grazer types

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including chironomid larvae (Power 1990b), mayfly larvae (Colletti et al. 1987, Hill and Knight 1987, Hill and Knight 1988, Scrimgrour et al. 1991, Karouna and Fuller 1992), caddisfly larvae (Lamberti and Resh 1983, McAuliffe 1984, Jacoby 1987, Lamberti et al. 1987b, Steinman et al. 1987a, Hill and Knight 1988, Lamberti et al. 1992, Katano et al. 2007), snails (Hunter 1980, Mulholland et al. 1983, Jacoby 1985, Steinman et al. 1987a, Lowe and Hunter 1988, Osenberg 1989, Underwood and Thomas 1990, Bronmark et al. 1991, Tuchman and Stevenson 1991, Hill et al. 1992, Steinman 1992, Rosemond et al. 1993), shrimp (Pringle et al. 1993), tadpoles (Lamberti et al. 1992), and fish (Power and Matthews 1983, Power et al. 1988, Gelwick and Matthews 1992, Wootton and Oemke 1992, Huchette et al. 2000). However, overall periphyton biomass sometimes shows no response to grazing, which could result from different reasons including low grazer density (Colletti et al. 1987, Steinman et al. 1987b), a mismatch between the grazer and dominant alga type (Jacoby 1987, Karouna and Fuller 1992), the algae being resource limited (Feminella et al. 1989) or by the replacement of a large, slow-growing alga lost through herbivory by a small, fast-growing alga (Steinman 1996). Given that grazing produces these repeatable patterns, it is important to understand how outside factors, like increasing sediment, may impact the way algae responds to herbivory.

Little work has been done to examine the interactions that may exist between sediment and grazing and how those interactions may affect algal community properties and much of that work has been done in tropical stream ecosystems. In these tropical stream ecosystems, grazing organisms tended to remove epilithic sediment and stimulate periphyton biomass. Power (1990a) found that the armored catfish (family Loricariidae) could reduce the rate of sediment accumulation in moderately ($\sim$15-45mg/cm^2) and highly ($\sim$20-80mg/cm^2) sedimented stream enclosures. Further, armored catfish individuals that were stocked at levels 1/6 of their natural

density actually reduced epilithic sediment and positively affected periphyton standing crops and productivity. Similar results were found by Pringle et al. (1993) when studying omnivorous freshwater shrimp in Costa Rican streams. With their foraging activities, the shrimp also removed sediment from the rocky substrate resulting in significantly higher algal biomass relative to exclusions. Schofield et al. (2004) performed stream exclusions in temperate streams of macroconsumers (fishes and crayfish, specifically) in which the exclusions were subjected to experimentally increased sediment bedloads. They found that after ~30 days there was significantly more periphytic chlorophyll a (mg/m^2) in the macroconsumer exclusion treatments than in the macroconsumer access treatments under ambient sediment conditions. In contrast, they found that under increased sediment loads, there was no significant difference in the periphytic chlorophyll a between the macroconsumer access and exclusions treatments. They noted that there was not a significant difference in how often macroconsumers visited the ambient and sedimented treatments, which suggests that the difference in the effects of macroconsumer exclusion between these treatments was not a direct effect of macroconsumer avoidance. They hypothesized that the difference may instead be an indirect effect related to alterations in prey density caused by sedimentation, noting that the top-down effects on sediment sensitive taxa were lower in the sediment treatments, whereas top-down effects on sediment-tolerant taxa were unaffected. The results of temperate and tropical studies examining sediment effects on grazer-periphyton interactions differ, which may be partly due to differences in grazer species types, but this hypothesis has not been addressed. Further, the existing studies do not incorporate smaller grazing macroinvertebrates that can also have important effects on periphyton (Lamberti et al. 1987a, Steinman et al. 1987a) and may have different responses to increasing fine sediments.

My objective was to determine whether increasing benthic sedimentation altered grazer-periphyton interactions and whether that effect was dependent upon grazer type. I used two common grazing species in temperate freshwater stream ecosystems: crayfish and snails. Crayfish grazing has previously been shown to decrease filamentous algal cover (Hart 1992, Creed 1994) which can facilitate the growth of understory algae such as smaller diatoms (Creed 1994). Crayfish grazing has also been shown to produce an overall decline in algal biomass (Evans-White et al. 2001). The effect of different densities of crayfish on primary production has also been demonstrated by Flint and Goldman (1975) where they found that low intensity crayfish grazing stimulates periphyton primary productivity, whereas at high intensities of crayfish grazing, primary productivity decreases because production can no longer compensate for the increased removal of algal cells. During the process of crayfish foraging, crayfish also become effective sediment movers/removers. Crayfish can act as bioturbators by changing the distribution of sand, gravel, and benthic organic matter in streams (Parkyn et al 1997, Statzner et al. 2000, Angeler et al. 2001, Statzner et al. 2003, Creed and Reed 2004, Usio and Townsend 2004, Helms and Creed 2005). Crayfish often account for a significant proportion of the biomass of macroinvertebrates (Momot et al. 1978, Rabeni 1992) and are keystone species both as consumers and as ecosystem engineers.

Snails were chosen because they are not only abundant grazers in the south east U.S. (Newbold et al. 1983, Richardson et al. 1988, Hill and Harvey 1990), but throughout North America (Kehede and Wilhm 1972, Hunter 1980, Elwood et al. 1981, Mulholland et al. 1983, Lamberti et al. 1989). Given the abundance and importance of snails not only in the U.S., but worldwide, it is no surprise that they have been the subject of numerous grazing studies (Hunter 1980, Cuker 1983, Underwood and Thomas 1990, Tuchman and Stevenson 1991). However,

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even though snails have been the subject of myriad grazing studies, few of these studies have examined the association between increasing sediment and snails. One study has suggested that sedimentation increases snail (*Lavigeria grandis*) mortality (Donohue et al. 2003). Another study has examined the impact of sediment on snails, finding that snails in disturbed sites with increased sedimentation tend to ingest more material than snails (3 *Lavigeria* sp. and *Reymondia borei*) at reference sites with lower sediment as determined by fecal matter, but that the ingested material contains a high level of sediment suggesting a lower food quality (McIntyre et al. 2005). While this study suggested that snails can consume more periphyton under increased sediment conditions, it did not examine the periphyton to determine how it may respond to this increased consumption or clearing of sediment by the snails.

I hypothesized that at low sediment levels, crayfish would exhibit the same effects that they have previously been shown to have on algal communities, reducing both overall periphyton biomass and filamentous algal biomass when compared to non-grazed treatments. Crayfish at intermediate sediment levels may have effects similar to those observed from armored catfish (Power 1990a), where crayfish grazing has a net beneficial effect on overall periphyton biomass, but still reduces filamentous algal biomass when compared with non-grazed treatments. Crayfish at high sediment levels may no longer be able to provide a positive effect on overall periphyton biomass, but will still cause reductions in filamentous algal biomass when compared with non-grazed treatments. Snails at low sediment levels may reduce overall periphyton biomass and filamentous algal biomass when compared with non-grazed treatments. Snails at high sediment levels may consume a higher degree of organics to compensate for lower food quality and ingesting a high amount of sediments shown in a previous study (McIntyre et al.

2003), which may result in either no direct benefit, or even a negative impact on algal biomass in grazed treatments.

METHODS

Crayfish greenhouse experimental design

A greenhouse experimental stream study conducted from November 11 through December 26, 2011, was designed to test the interacting effects of crayfish grazing and increasing levels of sediment on periphyton. The experimental design employed a fully factorial combination of two levels of crayfish grazing (present and absent), and three levels of sediment (low=100 g/m^2, intermediate=1000 g/m^2, high=5000 g/m^2) and included 30 experimental units (recirculating streams; five replicate streams per treatment). Sediment data collected from study streams in north central Arkansas (located in the Little Red, Point Remove, and Cadron drainages) as part of a study to determine if natural gas wells impact stream ecosystems was used to parameterize my greenhouse stream experiment. Mean benthic sediment levels in the study streams ranged from 149 to 2210 g/m^2 (Entrekin 2011). The sediment levels in the high sediment treatment were chosen to represent a level higher than observed at field sites, but were within values observed in the literature (Power 1990a, Waters 1995).

In the summer of 2011 (June 13 through June 26), crayfish were sampled from a subset of the study streams to determine the dominant crayfish species of the area and to estimate crayfish densities in these streams. To accomplish this, crayfish were sampled from three riffles and three pools per stream by placing a 0.485m^2 quadrat in front of a 500µm kicknet facing upstream. The area in the quadrat was vigorously disturbed using a hand rake for one minute and dislodged crayfish were moved into the net by the current. Crayfish were sampled three

times per riffle and three times per pool. The crayfish were then preserved in 80% isopropyl alcohol and returned to the lab for identification.

The crayfish *Orconectes palmeri* was the dominant crayfish species in four out of the five streams sampled. The mean density of *O. palmeri* varied across streams (one to nine individuals (ind)/m^2), but averaged to five ind/m^2. Individual quadrat densities ranged from 0 to 20 ind/m^2. I chose to stock *O. palmeri* in my experimental streams at 15 ind/m^2 as densities were around this level in some areas. The total biomass stocked was 25.94 g/m^2. The area of the experimental streams was 0.068m^2 and the densities stocked were near levels observed in the study streams and those reported in the literature for *Orconectes* spp., which have been found at 8 individuals/m^2 (*O. punctimanus*; Flinders and Magoulick 2005), and ~13 individuals/m^2 for *O. neglectus chaenodactylus*; Rabalais and Magoulick 2006).

Snail greenhouse experimental design

A similar greenhouse experimental stream study was conducted from May 1 through June 7, 2012, which was designed to test the interacting effects of snail grazing and increasing levels of sediment on periphyton. The experimental design employed a fully factorial combination of two levels of snail grazing (present and absent), and two levels of sediment (low=100 g/m^2 and high=5000 g/m^2) and included 20 experimental units (five replicates per treatment). I decided to focus only the low and high sediment levels in this experiment because the previous crayfish experiment had shown resulted in no significant sediment by grazing interactions on the algal response variables.

In the summer of 2012 (May 8), snails were sampled from a stream in northwest Arkansas (Clear Creek) to determine the dominant snail species of the area and to estimate snail densities in a local stream. To accomplish this, snails were sampled from one stream by placing

a $0.45m^2$ quadrat on the substrate in a shallow part of the stream. The area in the quadrat was visually scanned for snails which were removed and placed in a container for counting. This was repeated 10 times in different areas of the stream and an average density was calculated. A subsample of the snails were placed in 70% ethanol and returned to the lab for identification. The snail *Elmia* sp. was the only snail species collected. *Elmia* sp. density ranged from 68 to 186 ind/m^2. The mean density of *Elmia* was 114 ind/m^2. The snails were stocked at 8 snails per experimental stream which was equivalent to 118 snails/m^2.

Experimental stream setup:

Each experimental stream was created from a 6.5 L circular pan (31 cm diameter) with a 9 cm diameter PVC pipe sealed in the middle. Streams were filled with 3.5 L of dechlorinated tap water and a 5 watt pump (Shkerry Aqua, HJ-531) was used to create unidirectional flow at an average velocity of 0.17 m/s. The bottom of each pan was covered with cobbles that had been scrubbed free of organic material and sterilized in an autoclave to avoid contamination from any previously colonized algae or bacteria. A periphyton slurry aliquot (10 mL) collected by scraping several cobbles from a local stream was evenly dispersed through each artificial stream. Two weeks were allowed for periphyton colonization and cobbles were collected to establish starting chl *a* levels prior to any experimental manipulation (Crayfish experiment mean±1SE=0.5513µg/cm^2 ± 0.0604; Snail experiment mean±1SE =0.3583µg/cm^2 ± 0.0571). The snail experiment did start with one treatment (low sediment/snails absent) having lower chl *a* values than the other treatments ($p<0.040$), but by week 2 the chl *a* values in this treatment had reached the starting values of the other treatments and then leveled off. Nutrient levels in the experimental streams were monitored on a weekly basis by taking filtered water samples (1 µm GF/F; Pall), which were analyzed for soluble reactive phosphorus (SRP) and nitrite+nitrate

($NO_2^- + NO_3^-$). SRP was measured using standard molybdate/ascorbic acid APHA benchtop methods and $NO_2^- + NO_3^-$ was measured on a Lachat QuickChem 8500 (Lachat Instruments, Milwaukee, WI). Nutrient levels were maintained at predetermined levels (SRP=20µg/l, NO_2^- +NO_3^-=70µg/L) based on prior research of the study streams in north central Arkansas (B. Austin unpublished data). The average light levels in the greenhouse were 1928 watts/m^2 in the daytime (7:00:00-19:00:00) and 1 watt/m^2 in the nighttime (19:00:01-6:59:59) for the crayfish experiment and 5328 watts/m^2 in the daytime and 90 watts/m^2 in the nighttime for the snail experiment. The average air temperatures were 19.5^{o}C in the daytime and 15.3^{o}C at night for the crayfish experiment (Range: 7.83-42.46^{o}C) and 26.1^{o}C in the daytime and 18.1^{o}C at night for the snail experiment (Range: 9.03-34.01^{o}C).

After the two week colonization period, sediment treatments were added to pre-determined, randomly selected experimental streams. The sediment (previously collected from a stream in the Little Red drainage) was sorted through a 500µm sieve, dried, and weighed into the appropriate treatments. After the sediment was added, it was given two days to settle before crayfish grazers (*O. palmeri*, previously collected from a stream in the Cadron drainage) or snail grazers (*Elmia* sp., previously collected from Clear Creek in northwest Arkansas) were added to the pre-determined, randomly selected streams. Prior to adding the crayfish, each individual's wet mass was recorded and its carapace length was measured to ensure that all crayfish were relatively the same size (3.52±1.32 g; 23.75±2.21 mm carapace length). Prior to the addition of snails, each snails shell length was measured to ensure each stream received an average shell length per stream relatively the same (average ranged from 14.36-15.45mm across experimental streams).

Response Variables

Once a week a cobble was collected from each experimental stream and frozen within 2 hours of collection until chl *a* analysis could be performed. Later, chl *a* was analyzed by scrubbing each cobble to create a periphton slurry, which was homogenized on a stir plate and subsampled onto a pre-ashed (500°C) Pall GF/F glass fiber filter. The filters were then submerged in 10 mL of 95% EtOH solution, boiled at 78°C for 5 minutes (Sartory and Grobbelaar 1984), removed, allowed to cool in the dark at 4°C for 24 hours, then measured for absorbency using a Genesys 10 VIS spectrophotometer (Thermo Fischer Scientific Inc., Waltham, MA) as described in APHA (2005). After chl *a* analysis, the filters and extracts were returned to pre-ashed (500°C), pre-weighed aluminum tins which were dried (50°C), weighed to determine dry mass, ashed at 500°C, desiccated, and reweighed to obtain the ash mass. The ash free dry mass (AFDM) is the difference between the dry mass and the ash mass. Chl *a* and AFDM were both calculated as a mass per unit area, the area being the surface of the cobble which was determined by the aluminum foil method (Lamberti et al. 1991). An autotrophic index (AI) equal to the chl *a* biomass (mg/cm^2) divided by the AFDM (mg/cm^2) was used to determine the photosynthetic content of the periphyton in each experimental stream. Other response variables were also measured on a weekly basis.

Inorganic sediment collected on the filter from each periphyton slurry subsample was measured by taking the difference between the ash weight and the pre-weight of each tin and calculated as mass per unit area as described earlier.

At the conclusion of the experiment, all of the remaining cobbles were scrubbed and the attached filamentous algae were collected in a 1 mm sieve. The filaments were placed in pre-

ashed (550°C) pre-weighed aluminum tins, dried (50°C), weighed, ashed at 550°C, desiccated, and reweighed to obtain filamentous algal biomass (g).

Data analysis

A repeated-measures two way analysis of variance (ANOVA) was used to test for all statistically significant effects and interactions, including a time*grazer*sediment interaction for the response variables of chl *a*, AFDM, AI, and cobble sediment mass. All response variables were logged transformed to meet the assumptions of normality. If there were no significant interactions, any significant main effects were examined. A two-way ANOVA was used to test for all statistically significant effects and interactions for the filamentous algal biomass response variable, which was not logged transformed. All significant differences were determined using a post-hoc Tukey analysis. All statistical analyses were conducted using SAS statistical software (version 9.2, SAS Institute, Cary, NC).

RESULTS

Crayfish Experiment

Sediment levels differed between high and intermediate, and intermediate and low treatments based cobble sediment mass (Figure 1). Cobble sediment mass (CSM) was dependent on the sediment and the grazing manipulation (Table 1) and showed lower sediment in the crayfish present (CP) treatments verses the crayfish absent (CA) treatments regardless of initial sediment levels (Figure 1).

Time, sediment, and grazing did not interact to affect any of the periphyton response variables (Table 1). Benthic chl *a*, however, had some main effects that were statistically significant (Table 1). There was a significant time effect on chl *a*, where there was a significant increase in chl *a* during the first week of the experiment (combined chl *a* values from all

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treatments increased from 0.51±0.06 to 1.05±0.14 μg/cm^2 [mean±1SE]), but then levels were stable throughout the remainder of the experiment (Figure 2A). There was also a significant sediment effect on chl *a* (Table 1) which increased as benthic sediment increased (Figure 3A), with combined chl *a* values (CA and CP) for the whole experiment being 1.03±0.07, 0.85±0.09, and 0.75±0.07 μg/cm^2 (mean±1SE) for the high, intermediate and low sediment treatments, respectively. While there was not a significant grazer effect on chl *a* (Table 1), there was a tendency for CP treatments to have lower chl *a* than CA treatments at all sediment levels (Figure 4A). Ash free dry mass had a significant time effect (Table 1), increasing from 0.20±0.01 to 0.24±0.02 mg/cm^2 (mean±1SE) in the third week of the experiment (Figure 2B). AFDM responded to different sediment levels (Table 1) and tended to increase as benthic sediment increased (Figure 3B), with combined AFDM values (CA and CP) for the whole experiment being 0.26±0.01, 0.20±0.01, and 0.18±0.01 mg/cm^2 (mean±1SE) for the high, intermediate, and low sediment treatments, respectively. There was a significant grazer effect on AFDM (Table 1) with CP treatments having lower AFDM than CA treatments for all sediment levels (Figure 4B). Time significantly affected the AI, with a the chl *a*/AFDM increasing significantly over the first week of the experiment, but returning to starting levels by the third week (Figure 2C). The AI responded differently to grazing absence and presence (Table 1), where the AI was 5.05±0.36 for the CP treatments (all sediment levels) for the entire experiment and was 3.35±0.18 for the CA treatments (Figure 4C). Filamentous algal biomass was significantly reduced by the presence of crayfish (Table 1; Figure 4D).

Snail Experiment

The sediment treatments added at the start of the experiment did achieve different sediment levels between high and low treatments when examining the cobble sediment mass per

treatment (Figure 5B). The CSM response variable had two statistically significant interactions, week by grazing and sediment by grazing (Table 2). At the end of the experiment, the difference between the snails absent (SA) and snails present (SP) treatments became greater with the SA treatments having higher CSM than the SP treatments (Figure 5A). The sediment by grazing interaction in the CSM response variable showed that when averaged over the whole experiment, the HS/SA treatments had higher sediment than all other treatments, followed by the HS/SP treatments. Further, the LS/SA treatments had higher sediment than the LS/SP treatments (Figure 5B).

Chlorophyll *a* did have two statistically significant interactions, a week by grazing interaction and a sediment by grazing interaction (Table 2). During the first two weeks of the experiment there was a divergence in chl *a* values with the SA treatments gaining chl *a* while the SP treatments lost chl *a* (Figure 6A). Towards the end of the experiment, chl *a* did not differ between SP and SA treatments. The HS/SA treatments had higher chl *a* values than all other treatments while the rest of the treatments did not differ significantly from each other (Figure 7A). The AFDM response variable also had statistically significant week by grazing and sediment by grazing interactions, but it also had a week by sediment interaction (Table 2). The week by sediment interaction showed that at the beginning of the experiment the HS treatments had higher AFDM than the LS treatments, but by week 2 that difference had disappeared (Figure 8). The week by grazing interaction suggested that at the start of the experiment the SA and SP treatments did not differ in AFDM, however by week 1 the SA treatments had higher AFDM than the SP treatments, and that trend continued throughout the experiment (Figure 6B). The sediment by grazing interaction suggested that the HS/SA treatments had the highest AFDM, followed by the LS/SA treatments, then the HS/SP treatments. All differences were statistically

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significant (Figure 7C); however the reduction in AFDM by snail grazing was greater in the HS treatments than the LS treatments. Finally, the AI response variable had two statistically significant interactions, a week by grazing interaction and a sediment by grazing interaction (Table 2). The week by grazing interaction showed that from the start of the experiment to week 1 the SP AI decreased, but recovered the next week with no other differences (Figure 6C). The AI sediment by grazing interaction was statistically significant (Table 2), however when the Tukey analysis was run, there were no differences between the treatments (Figure 7B). Filamentous algal biomass did not have any interactions; however the grazing main effect was statistically significant (Table 2). The SA treatments had higher filamentous algal biomass than the SP treatments when it was collected at the conclusion of the experiment (Figure 7D).

DISCUSSION

The interaction between crayfish and snail grazing and increasing sediment on algae has not, to my knowledge, been directly examined even though crayfish and snails are important grazers (Lamberti et al. 1989, Creed 1994, Evans-White et al. 2001) and increasing sediment in streams is a growing problem (Richter et al., 1997, Wood and Armitage 1997, Owens et al. 2005, USEPA 2006), with documented negative effects on algae (Biggs et al. 1999, Parkhill and Gulliver 2000, Izagirre et al. 2009). I expected that high sediment conditions would cause a reduction in algal biomass via shading and burial. I also expected that crayfish might cause a net increase of algae via sediment removal and due to their inability to graze smaller algal growth forms. Finally, I hypothesized that snails with their smaller body size and rasping feeding mode would not be as effective at removing sediment and their ability to graze smaller growth forms of algae would have a negative effect on algal biomass. I did find that crayfish presence reduced sediment cover (Figure 1) and that it tended to provide a net benefit to algal biomass (Figure

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4A). However, contrary to my expectations, I found that higher sediment levels actually resulted in increased algal biomass (Figures 3A & 7A), and that snails were effective at sediment removal (Figure 5A), but unlike in the crayfish grazing, snail grazing decreased algal biomass (Figure 6A).

Previous studies have reported only an initial negative impact of deposited sediments on benthic algal biomass (Yamada and Nakamura 2002, Izagirre et al. 2009), but I found that deposited sediment had a stimulatory effect on algal biomass in the crayfish and snail experiments (Figures 3A & 7A). Nutrients such as phosphorus and nitrogen have been found to bind to sediments (Vaze and Chiew 2004) and may have increased nutrient availability to algae. However, there were no noticeable differences in the water chemistry between the low, intermediate, and high sediment treatments in either grazing experiment. It may be possible that the sediments leached nutrients into the water quickly, which was not observed in the water chemistry analysis that was first collected a week after sediment was added in both experiments. Furthermore, loosely attached filamentous green algae were not affected by sediment, but were negatively affected by both grazers (Figures 4D & 7D).

While sediment had the unexpected effect of stimulating algal growth, it was effectively removed from cobbles by the presence of both crayfish and snails (Figures 1 & 5B). Not only were snails effective at sediment removal, they were more effective than crayfish (Table 3; Figure 9A), which was not anticipated, especially given that snails (*Lavigeria grandis*) have been shown to react negatively to increasing sediments (Donohue et al. 2003). It was also observed that both crayfish and snails were able to effectively reduce filamentous algae (Figures 4D & 7D). In this experiment, there was also no evidence that sediment negatively impacted the grazing abilities of either grazer. Both types of grazer were able to effectively graze on

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filamentous algae (Figures 4D &7D) and both effectively reduced overall AFDM (Figures 4C & 7C).

While both grazers reduced sediment cover and effectively grazed on the algae, the resulting effect on chl *a* concentration differed between grazers. Crayfish grazing had a stimulatory effect on chl *a* concentration (Figure 2A) whereas snail grazing had a negative effect on chl *a* concentration (Figure 7A). Crayfish grazing stimulated chl *a* (Figure 4A) and generally reduced overall periphyton biomass (Figure 4C). Increasing sediment levels had a similar stimulatory effect on chl *a*, however unlike crayfish grazing, increasing sediment also increased overall periphyton biomass (Figure 3B). Therefore, the trend was that crayfish presence resulted in a more autotrophic periphyton mat than did increasing sediment levels. Snails, by contrast, always had a negative impact on chl *a* concentration and overall periphyton biomass, and only made the periphyton mat more autotrophic under low sediment conditions (Figure 7B). The stimulation in chl *a* concentration leading to an increased AI (chl *a*/AFDM) has been observed previously when crayfish were used as grazers (Evans-White and Lamberti 2005). In the Evans-White and Lamberti (2005) experiment, crayfish grazing treatments had significantly higher chl *a* concentration than ungrazed and snail grazed treatments. The experimenters suspect that this was probably not due to an increase in algal biomass, because no increases were seen in other periphyton response variables such as AFDM and algal biovolume, but rather was probably due to an increase in the amount of chl *a* per cell. The difference observed between these two grazers under the same sediment conditions could be due to shifts in algal community structure due to the differences in the grazer's mode of feeding; however in a previous study, algal community composition was not shown to differ between snail and crayfish grazing (Evans-White and Lamberti 2005). The difference may also be due in part to what was seen in the

McIntyre (2003) study, where snails under high sediment conditions tended to consume more than their counterparts under normal sediment conditions. The snails in this experiment may have been consuming more periphyton than they normally would to make up for the loss in food quality from ingesting a higher proportion of sediment.

This study was the first to document that sediment levels equivalent to levels seen from anthropogenic disturbance affect the grazer-periphyton interaction using two common North American grazers, crayfish and snails. The way increasing sediment may affect grazer-periphyton interactions, particularly crayfish-periphyton interactions and snail-periphyton interactions, is of particular importance in Arkansas. Arkansas not only has a high density and diversity of crayfish (Hobbs 1988) and a high density of snails (north Arkansas; personal observation), but north central Arkansas has seen a growth in population by up to 25% since the year 2000 (US 2010 Census Data). Further, north central Arkansas has recently had a boom in natural gas drilling (Entrekin et al. 2011), which may lead to increases in sediment loads. The substrate of most streams in the northern part of Arkansas is composed primarily of gravel and cobble (Brown et al. 1998, personal observation). With the threat looming of increasing sediment loads in these streams, it is important to study how rising sediment levels may affect the grazer-periphyton interaction in these streams, particularly with key large-bodied abundant grazers like crayfish.

This study found that increasing sediment affects the grazer-periphyton interaction differently between the two grazers, where crayfish under high sediment levels provided a marginally significant net benefit to algal biomass. In contrast, snails under high sediment conditions caused a statistically significant decline in algal biomass. This difference is likely due to the differences in grazer feeding mode and body size. Further research could also be done in

this area by repeating the study using in-stream enclosures and different sediment levels, or different grazers. This may be important because the grazers used in this study are relatively large bodied and may be more tolerant to highly sedimented conditions than other smaller, soft bodied grazers such as mayflies, caddisflies, and other grazing macroinvertebrates. Under the same conditions, these grazers may be more highly impacted, and with these organisms, the grazer-periphyton may be disrupted by anthropogenic sedimentation. This would allow for further extrapolation of how these processes occur in real-world situations.

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Table 1: ANOVA table gives the F and p-values of every effect for all response variables in the **crayfish** experiment. Cobble Sed. Mass, Chl a, AFDM, and AI were all analyzed using a repeated measures two-way ANOVA and the degrees of freedom (DF) for the effects are: Week=4, Sed=2, Graz=1, Week*Sed=8, Week*Graz=4, Sed*Graz=2, and Week*Sed*Graz=8. Filamentous Algal Biomass was analyzed using a two-way ANOVA (DF in table).

Effect	Cobble Sed. Mass		Chl a		AFDM		AI		Filamentous Algal Biomass		
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	DF	p-value
Week	1.08	0.3698	6.86	<0.0001	2.48	0.0479	8.16	<0.0001	N/A		N/A
Sediment	249.34	<0.0001	5.03	0.008	23.96	<0.0001	0.38	0.6872	0.64	2	0.5362
Grazing	124.42	<0.0001	3.41	0.0674	23.12	<0.0001	18.37	<0.0001	83.24	1	<0.0001
Week*Sed	0.46	0.8802	0.81	0.5961	1.14	0.3412	0.86	0.5556	N/A		N/A
Week*Graz	1.52	0.2017	0.81	0.5222	0.45	0.7718	1.01	0.4038	N/A		N/A
Sed*Graz	6.15	0.0029	0.03	0.9724	2.03	0.1364	0.32	0.7271	0.72	2	0.498
Week*Sed*Graz	0.29	0.9674	1.09	0.3762	0.88	0.5384	0.85	0.5571	N/A		N/A

Table 2: ANOVA table gives the F and p-values of every effect for all response variables for the snail grazing experiment. Cobble Sed. Mass, Chl *a*, AFDM, and AI were all analyzed using a repeated measures two-way ANOVA, and the degrees of Freedom (DF) for the effects are: Week=4, Sed=1, Graz=1, Week*Sed=4, Week*Graz=4, Sed.*Graz=1, and Week*Sed*Graz=4. Filamentous Algal Biomass was analyzed using a two-way ANOVA (DF in table).

| Effect | Cobble Sed. Mass | | Chl *a* | | AFDM | | AI | | Filamentous Algal Biomass | | |
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	DF	p-value
Week	6.01	0.0003	5.90	0.0003	7.26	<0.0001	2.91	0.0266	N/A		N/A
Sediment	295.85	<0.0001	35.33	<0.0001	58.71	<0.0001	0.94	0.3359	2.96	1	0.1047
Grazing	176.62	<0.0001	51.41	<0.0001	194.81	<0.0001	0.08	0.7772	12.83	1	0.0025
Week*Sed	2.27	0.0692	1.60	0.1812	2.86	0.0287	0.23	0.9186	N/A		N/A
Week*Graz	14.69	<0.0001	7.49	<0.0001	15.90	<0.0001	2.61	0.0417	N/A		N/A
Sed*Graz	16.78	<0.0001	19.27	<0.0001	10.20	0.0020	6.68	0.0116	2.10	1	0.1670
Week*Sed*Graz	0.85	0.4948	1.57	0.1897	1.27	0.2895	0.16	0.9585	N/A		N/A
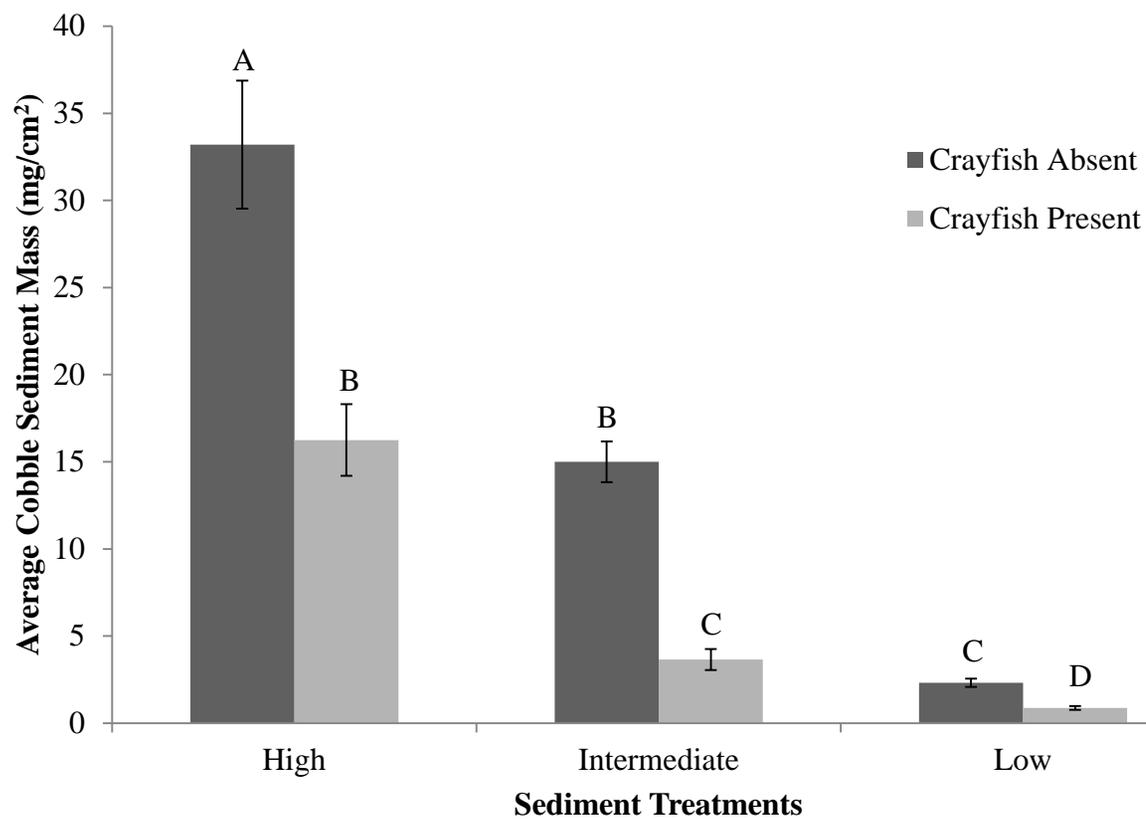
Figure 1: Inorganic sediment mass per unit area (non-transformed data) collected from the periphyton slurry subsamples averaged over the whole crayfish experiment. Bars not sharing a common letter are statistically significantly different, based on log transformed data ($p<0.0001$). Error bars=±1 Standard Error.
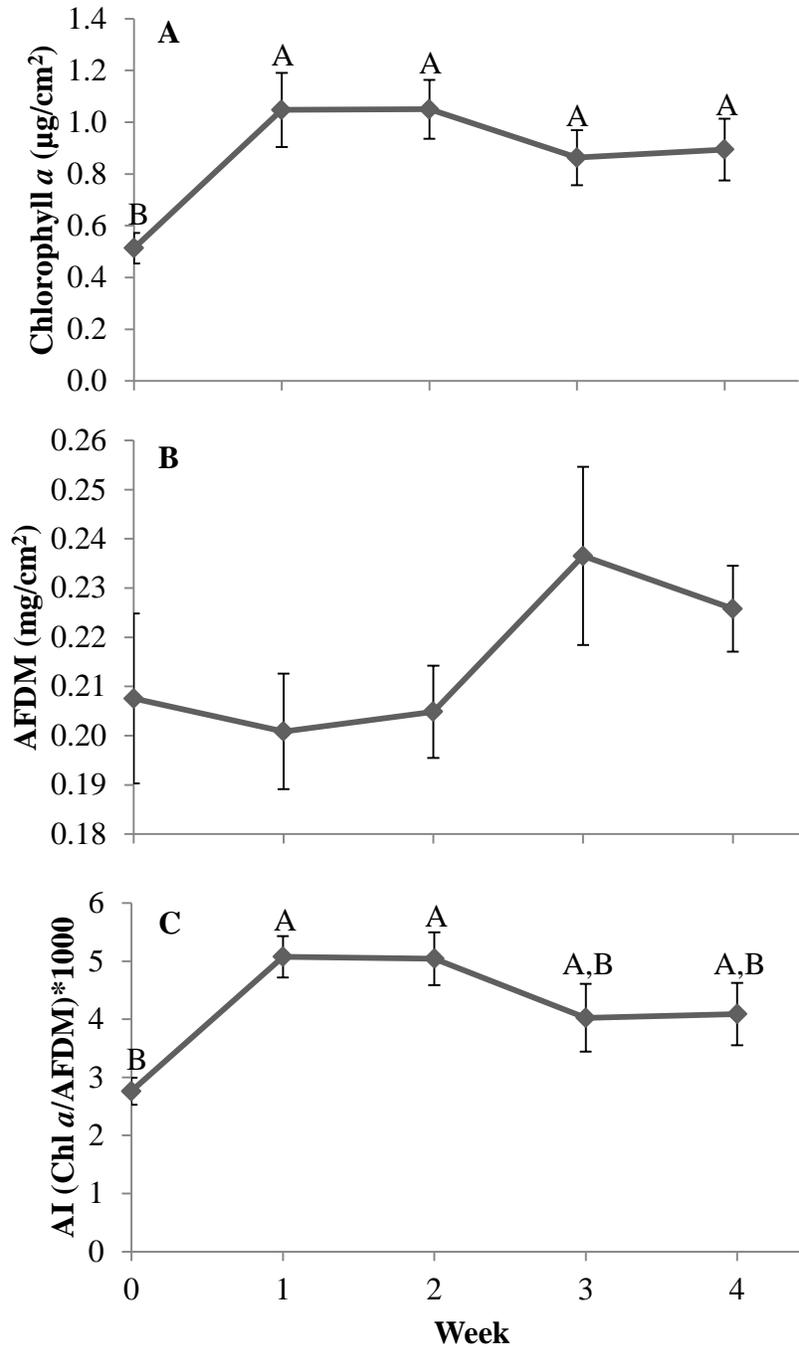
Figure 2: Graphs represent averages of all treatment combinations (all sediment and all grazing combinations) for each week of the crayfish grazing experiment. Crayfish were added immediately after the week 0 rocks were collected. **A.** Average chlorophyll *a* per week (p≤0.0171). **B.** Average ash free dry mass per week. **C.** Average autotrophic index per week (p<0.0001). Bars within each graph not sharing a common letter are significantly different based on the log transformed data. All error bars=±1 Standard Error.
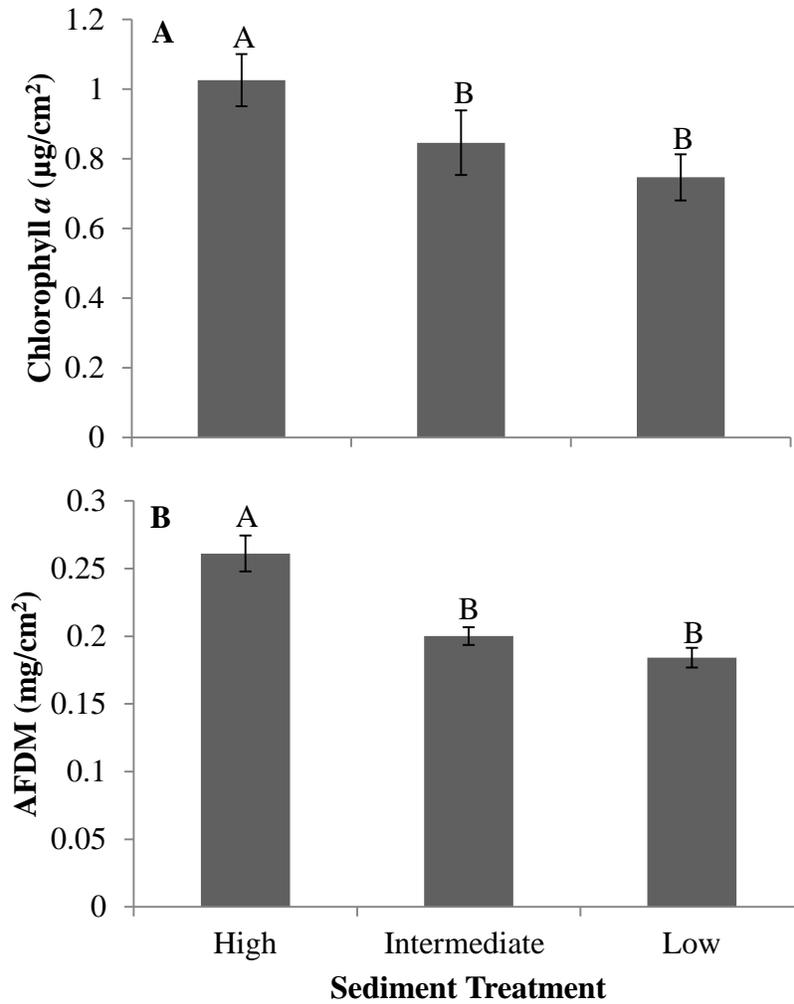
Figure 3: **A.** Chlorophyll *a* versus sediment treatments averaged for the whole crayfish experiment ($p \leq 0.0204$). **B.** Ash free dry mass by sediment treatment averaged for the whole crayfish experiment ($p < 0.0001$). Bars within each graph not sharing a common letter are significantly different based on the log transformed data. All error bars=±1 Standard error.
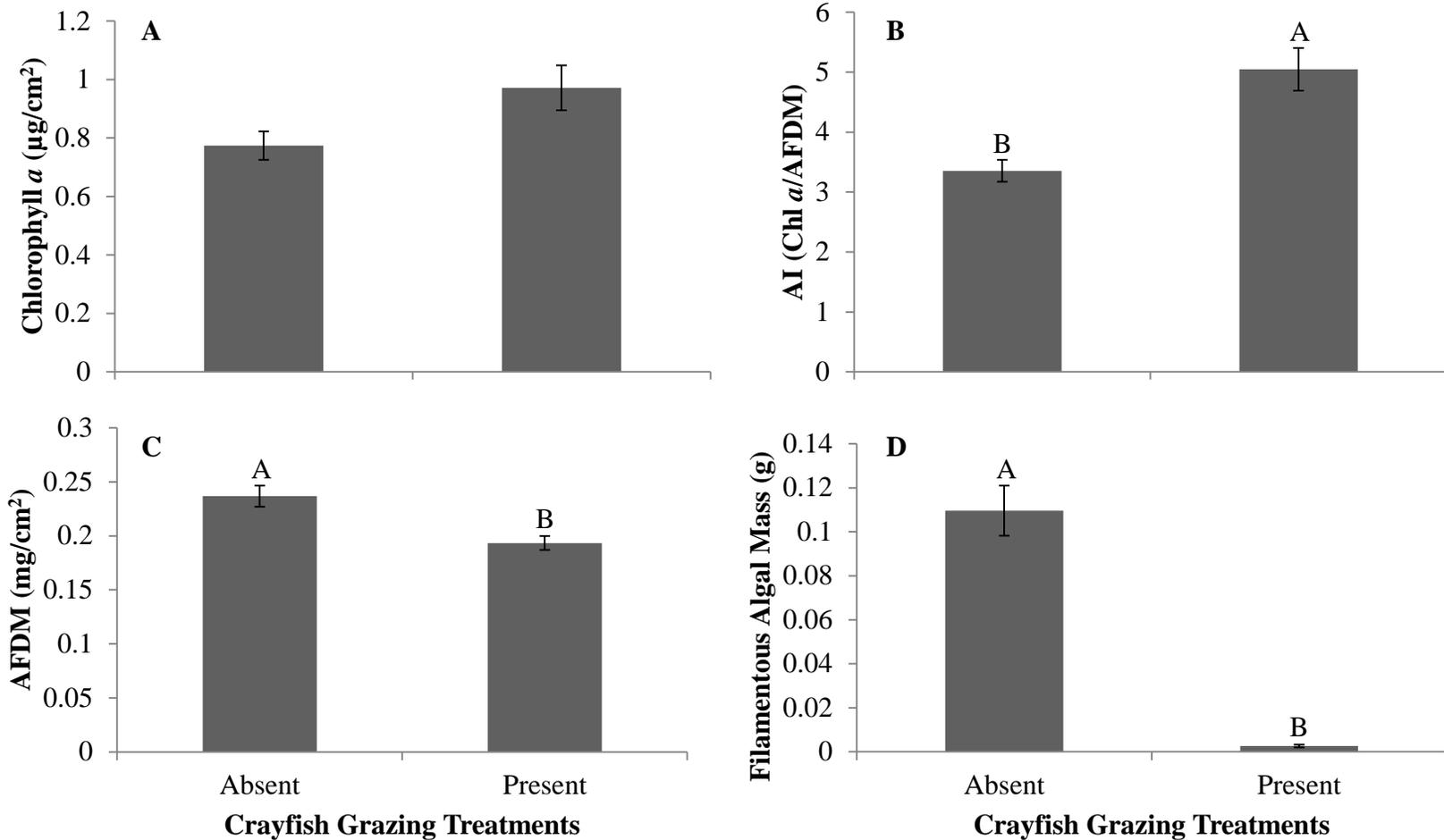
Figure 4: Graphs represent averages of all sediment treatments for the whole crayfish experiment. **A.** Chlorophyll *a* by crayfish grazing treatment (p=0.0647). **B.** Autotrophic index by crayfish grazing treatment (p<0.0001). **C.** Ash free dry mass by crayfish grazing treatment (p<0.0001). **D.** Filamentous algal biomass by crayfish grazing treatment (p<0.0001). Bars not connected by a common letter within each graph are statistically significantly different based on log transformed data. Error bars=±1SE.
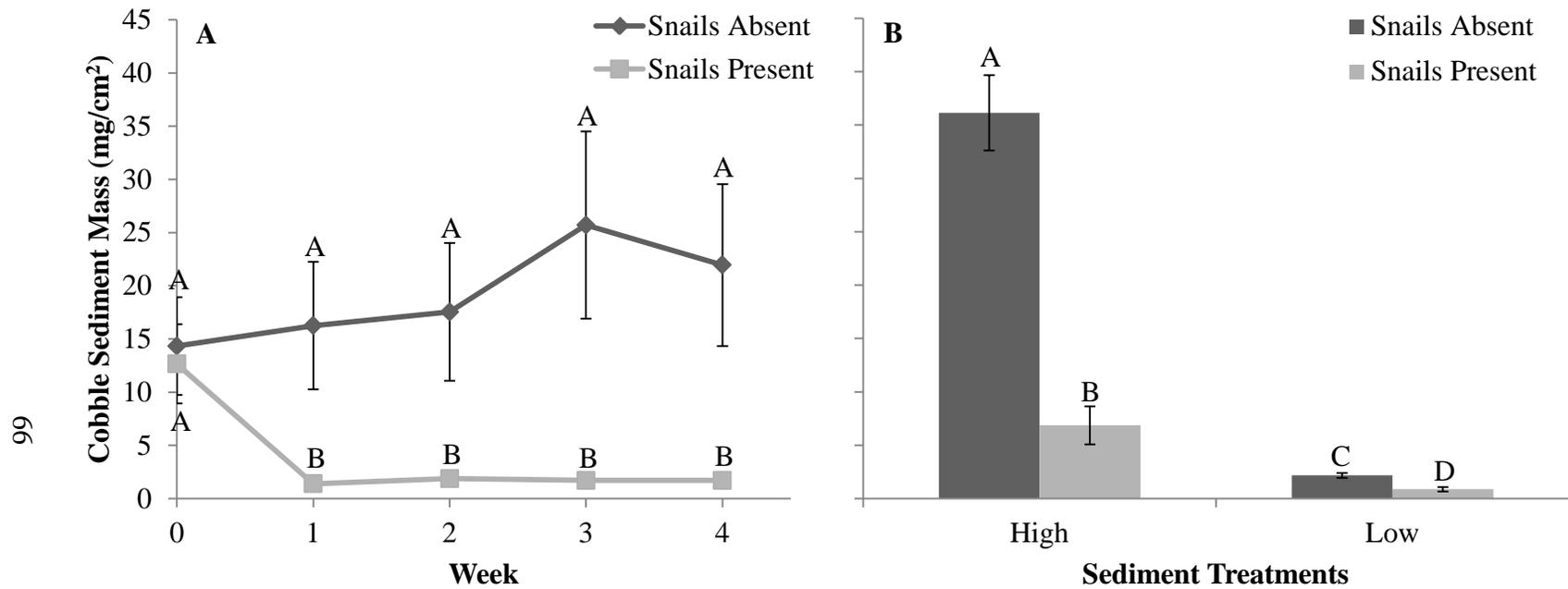
Figure 5: **A.** Average cobble sediment mass per week by snail grazing treatment ($p<0.0001$). **B.** Cobble sediment mass per snail grazing treatment averaged for the whole experiment ($p \leq 0.0349$). Bars within each graph not sharing a common letter are significantly different based on the log transformed data. All error bars=±1 Standard Error.
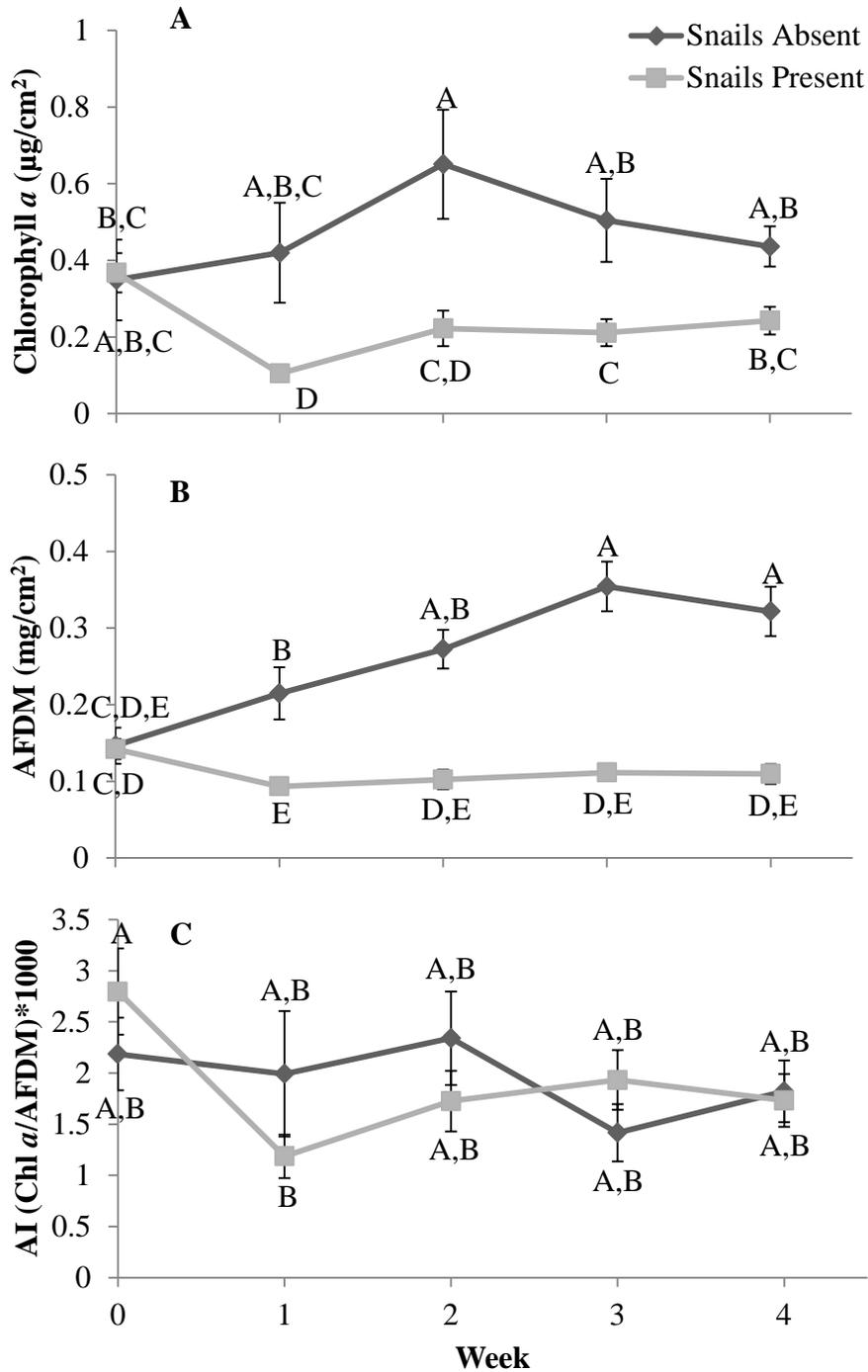
Figure 6: **A.** Average chlorophyll *a* per week by snail grazing treatment
(p≤0.0396). **B.** Average ash free dry mass per week by snail grazing treatment
(p≤0.0454). **C.** Autotrophic index (chl *a*/AFDM) per week by snail grazing
treatment (p=0.0169). Bars within each graph not sharing a common letter are
significantly different based on the log transformed data. All error bars=±1
Standard Error.
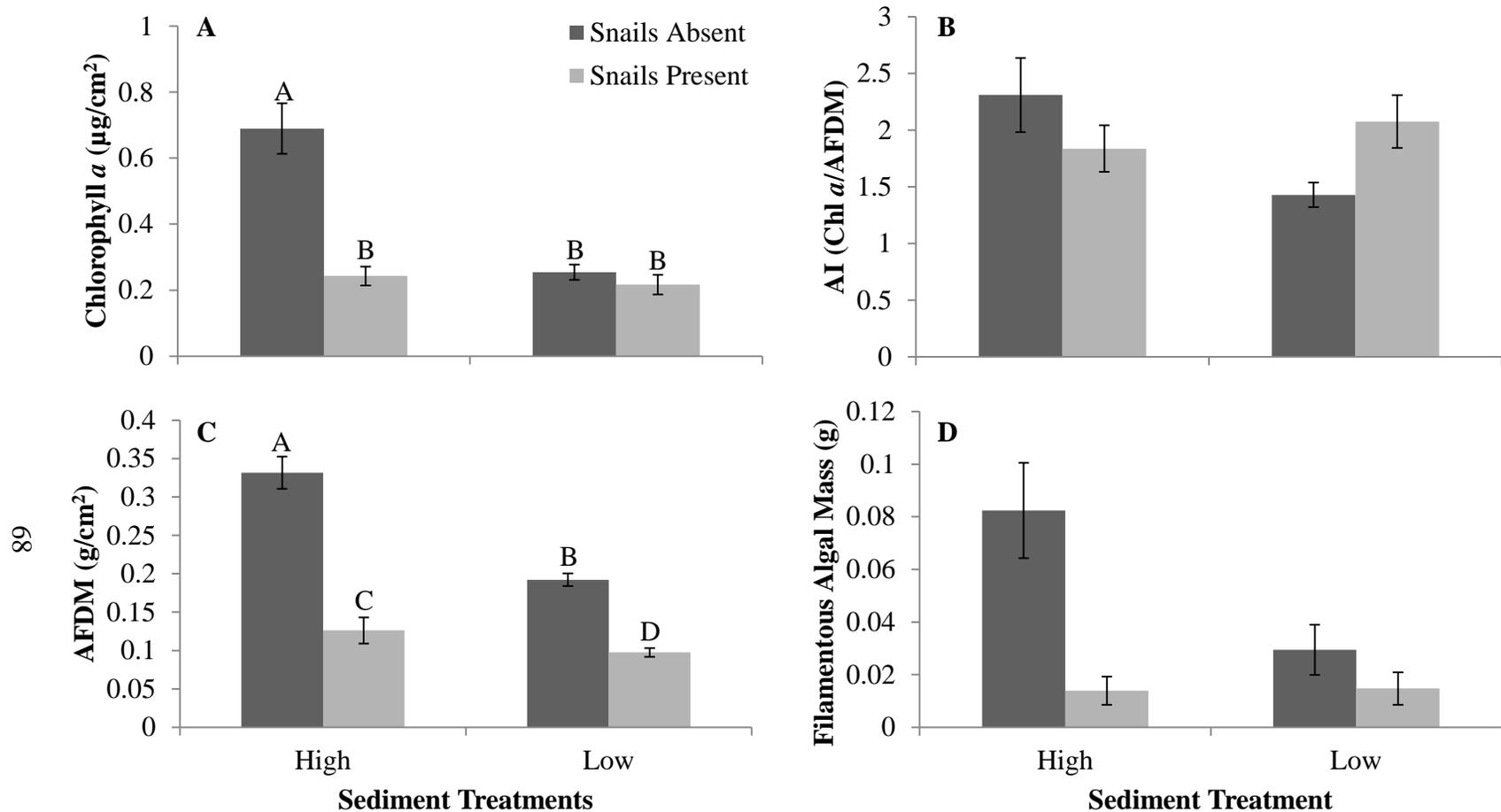
Figure 7: **A.** Chlorophyll *a* by snail grazing and sediment treatment averaged for the whole experiment (p<0.0001). **B.** Autotrophic index by snail grazing and sediment treatment averaged for the whole experiment. **C.** Ash free dry mass by snail grazing and sediment treatment averaged for the whole experiment (p≤0.0117). **D.** Mass of filamentous green algae collected at the end of the experiment by snail grazing and sediment treatment. Bars within each graph not sharing a common letter are significantly different based on the log transformed data. All error bars=±1 Standard Error.
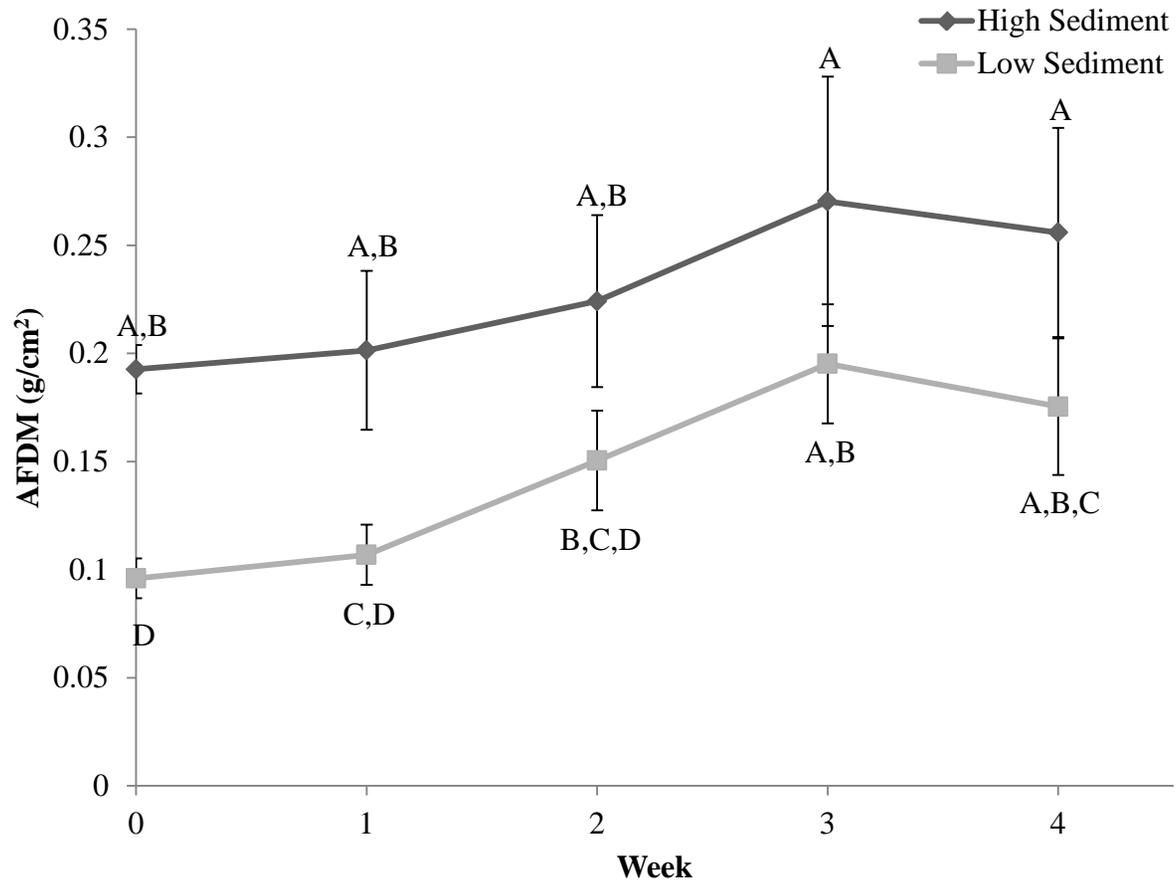
Figure 8: Graph shows the average ash free dry mass per sediment treatment for each week of the experiment (non-transformed data). Bars not sharing a common letter are significantly different based on the log transformed data ($p \leq 0.0164$). Error bars=±1 Standard Error.

CONCLUSIONS

Natural gas drilling has many potential environmental impacts, and the increase in drilling has expanded so rapidly that we may not be able to fully comprehend the impacts before the problems become too pervasive. Drilling for natural gas has already been linked to increases in sediment in surface flow near streams (Williams et al. 2008) and increases in stream turbidity (Entrekin et al. 2011), both of which have been shown to negatively impact aquatic biota (Wood and Armitage 1997) when they have been introduced into streams *via* other anthropogenic processes such as urbanization (Paul and Meyer 2001). This study's results indicate that natural gas variables were correlated to decreasing crayfish abundance, but the power of the study is low due to the small sample size and a short sampling timeframe. This result does however highlight the importance of continued monitoring of streams and crayfish populations near gas wells in order to document any negative impacts so that improvements may be made in the drilling process to avoid further degradation of our watersheds.

While it is not clear whether or not sedimentation was the mechanism influencing crayfish abundance in the streams with more natural gas wells in this study, it is known from previous studies that increased sedimentation can negatively influence both algae (Waters 1995, Steinman 1996, Wood and Armitage 1997, Izagirre et al. 2009) and grazers (Graham 1990, Richards and Bacon 1994, Gayraud and Philippe 2003, Bo et al. 2007). Fewer studies had been done to examine how sediment might impact how grazing affects algae (Power 1990, Pringle et al. 1993, Schofield et al. 2004) and no studies had examined this relationship at sediment levels equivalent to those seen from anthropogenic disturbance. This study found that the way anthropogenic levels of sediment affects the grazer-periphyton interaction can differ based on grazer type. This brings attention to the fact that there is still more that needs to be learned about

how increasing sedimentation can impact not only aquatic biota individually, but their

interactions and other stream processes as well.

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