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ANALYSIS OF UNCONVENTIONAL OIL AND GAS IMPACTS ON DOWNSTREAM FISH ASSEMBLAGES AND PHYSIOLOGICAL STRESS

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ANALYSIS OF UNCONVENTIONAL OIL AND GAS IMPACTS ON DOWNSTREAM FISH ASSEMBLAGES AND PHYSIOLOGICAL STRESS

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ABSTRACT

Analysis of Unconventional Oil and Gas Impacts on Downstream Fish Assemblages and Physiological Stress

Joshua Ankeny

Unconventional Oil and Gas (UOG) production has been steadily expanding throughout the mid-Atlantic since 2008. Increased sedimentation, degraded water chemistry and an overall decrease in habitat quality due to UOG is anticipated to negatively impact aquatic inhabitants, a common observation in other stressed landscapes (i.e., mining, agriculture, development). We assessed stream health through both community analysis and physiological parameters (i.e., growth and hematocrit). A before-after-control-impact study uncovered three fish metrics that were significantly different following UOG disturbances. The invertivore-piscivore metric decreased following UOG disturbance ($p = 0.045$) whereas two benthic metrics saw a surprising increase ($p = 0.003$ and $p = 0.011$). Further analysis revealed that fish communities are becoming more uniform as tolerant taxa proliferate through the chronically degraded systems. An ANCOVA alongside a linear mixed effect model failed to find a significant difference between the weight and length of *Semotilus atromaculatus* residing in both treatment conditions. *S. atromaculatus* were tested for hematological responses within eleven UOG impacted sites and eight reference sites with a linear mixed effect model. Hematocrit levels were found to be significantly lower in *S. atromaculatus* residing within UOG impacted streams ($p = 0.029$). A weak negative correlation ($r = -0.397$) suggests that as UOG well density increases, resident fish health decreases. Our findings indicate that in systems chronically impaired by anthropogenic stressors, physiological health indicators may provide better insight than community analyses. Additionally, we predict that the steady expansion of horizontal wells could lead to an overall degradation of resident fish populations as they exhibit degraded health. Our findings have potential to shape management practices and establish UOG protocols that protect aquatic environment.

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CHAPTER 1: LITERATURE REVIEW OF THE ENVIRONMENTAL IMPACTS OF UOG

INTRODUCTION

Shale gas production through means of unconventional oil and gas (UOG) is a rapidly growing industry in the United States. Starting in the early 2000s, the oil and gas industry employed some 225,000 employees or more, and spiked to nearly 450,000 employees in 2011 (Brown et al. 2013). Previously, natural gas trapped in these underground shale reserves was both hard to reach and hard to extract. Now, with further technological advances and an increasing drive to find the next new energy source, the potential benefit outweighs the previously prohibitive costs of operation. It is projected that shale gas will account for 46% of all natural gas production in the US, up from the 14% in 2009 (Rahm and Riha 2012).

A large contributor to this increase in oil and gas extraction is the ability to extract from the Marcellus formation, which extends from southwestern New York through eastern Ohio, and includes much of Pennsylvania and portions of West Virginia (King n.d.). As of 2011, the Energy Information Administration calculated that the Marcellus formation would produce around 141 trillion cubic feet of natural gas. In 2015, the operating wells were averaging a total output of 14.4 billion cubic feet per day. This natural gas was an astonishing 36% of the total natural gas used in the United States for that year (King n.d.). In the state of West Virginia alone, there has been nearly a 40-fold increase in UOG wells since 2007.

Pathways of Impact:

With a rapid spike in a field that is so minimally explored, a major concern is how UOG impacts downstream freshwater ecosystems. A previous study by Weltman-Fahs (2013) described three primary pathways through which UOG can impact freshwater environments, including; (1) increased sedimentation linked to deforestation and runoff from impermeable surfaces including but not limited to well pads and roadways; (2) the leaching of chemicals into both surface and ground waters through spills and leaks that occur during the fracturing process; and (3) the rapid withdrawal or dispersal of water associated with the UOG causing a hydrological change in the waterway (Weltman-Fahs and Taylor 2013a).

The first of pathway of impact is sedimentation. An increase in sedimentation directly increases the nitrogen and phosphorus levels of the stream. With increased nitrogen and phosphorus levels, eutrophication occurs more readily (Entrekin et al. 2011). Williams et al. goes on to say that increased sedimentation can lead to decreased channel depths resulting in habitat change, and furthermore, a decrease in the recreational use of the waterway (Williams et al. 2008). This sedimentation caused by UOG can be a factor of two activities; deforestation and the development of impermeable surfaces. Drilling for UOG requires the construction of a well pad and, as with other construction jobs, deforestation is immanent. Essentially, through the deforestation of an area you are removing what keeps the soil immobilized. With this removed, any rain will easily wash the now mobilized sediment into the adjacent stream. A well-established mountain stream derives a large proportion of its energy from the leaf litter of the surrounding vegetation. If an area is deforested, this leaf litter no longer exists. Decreased canopy cover allows for more sunlight to reach the surface water resulting in an increase in temperature and an increased abundance of photosynthesizing plants and algae. With this change the stream now has an autochthonous energy base, something more similar to streams of larger width (Stone and Wallace 1998).

Access roads to the well pads are another UOG feature that are deleterious to surrounding ecosystems. This increase in impermeable surfaces leads to yet another source of sediment runoff, as well as a whole new problem; increased salinization of waterways. In a 2005 study, it was determined that the increase in roadways and the use of deicing methods are leading to the salinization of freshwaters (Kaushal et al. 2005). In their study, they explain the ecological implications of such a phenomenon. An increase the salinity levels of surface waters can lead to a change in mortality and reproduction of aquatic organisms (Kaushal et al. 2005). A change in seasons, correlating with a natural change in surface water levels, can lead to fluctuation in the concentration of chloride in a stream. This fluctuation of chloride concentration can amplify the effects of the salinity change on organisms unable to regulate their osmotic potential of their cells within a short time frame (Kaushal et al. 2005). In a 2014 study, Kassotis et al. explained that chloride is only one of over 750 chemicals used in the shale gas extraction process. In this study, Kassotis primarily focused on endocrine disrupting chemicals. They found that all of 39 unique samples contained some form of endocrine disrupting chemicals (Kassotis et al. 2014). These chemicals have tremendous effects on the reproductive organs of the animals inhabiting the impacted stream. Another study, performed in 2017, explained how the presence of flowback and produced water in streams has a negative effect on rainbow trout gill morphology via oxidative stress (Blewett et al. 2017).

These untreated fracturing fluids are finding their way into surface waters through multiple means. Accidental spills during the drilling process is the main pathway. Canada reported that 2,500 spills occurred over a seven year span. 113 of these spills were known to have entered freshwater bodies of water (Blewett et al. 2017). Another source of surface water contamination is the improper treatment of flowback. Flowback is the fluid slurry that “flows back” up the well after the initial fracturing process. This cocktail consisting of brine, heavy metals and nucleotides is then hauled to the treatment facilities where the intention is to treat it and disperse it into surface waters. A common problem is that flowback is high in chloride which proves hard to remove in treatment plants (Olmstead et al. 2013). Chloride is only one of the many hard to remove chemicals found in flowback that leads to the inadequate treatment of dispersed wastewater.

Defects in well casings is yet another pathway for these chemical rich liquids to contaminate surface waters. After a well is drilled and before the fracking fluid is injected, a cement well casing is constructed. This casing is supposed to stop any contamination of groundwater and support the well's infrastructure. Though the intentions are good, there are recorded events of well casing failures that allow fracking fluid to leak into the surrounding groundwater and eventually the surface waters (Entrekin et al. 2011).

The final of the three main impacts of UOG is the rapid withdrawal and dispersal of water used in the UOG fracturing process. Water is the primary carrier of the chemicals deep into the well. Many UOG sites operate on a multi-stage fracturing method resulting in an incredible need for easily accessible fresh water. According to Entrekin, each UOG well uses between 2-7 million gallons of water (Entrekin et al. 2011) .To accumulate this much water, one of two things must occur. The oil and gas company either dams a waterway, or directly extracts the water from a nearby stream. Both scenarios negatively impact the stream. Both methods change the natural hydrology of the river. The damming of a stream creates a sediment sink for particles that would otherwise flow downstream. These pools can be lacking in aquatic life as the substrate becomes uninhabitable and the flow decreases the amount of suspended food available to fishes. On the other hand, removing massive amounts of water from an active stream channel leads to inadequate dilution of chemicals, ultimately affecting the downstream biota (Wildi 2010).

The lack of regulations on an UOG operation is becoming an increasingly apparent problem. As far as water withdrawal and dispersal are concerned, there is a lack of mandatory protocol. According to Rahm and Riha, there is no regulation on water withdrawal that takes into consideration the size and discharge of the used stream. They go on to say that there is no law stating where the used water must be returned to the stream from which it is extracted (Rahm and Riha 2012). The lack of regulation allows those 2-7 million gallons of water to be dispersed in a different location, leaving both the dispersal and extraction sites with altered hydrological regimes. A stream not equipped to handle an increased discharge of this magnitude could experience major geomorphological changes. These changes could result in a less diverse and less stable biotic community structure.

Rahm and Riha studied these withdrawal and dispersal effects on streams of different sizes and concluded with a simple strategy to mitigate harm to the waterways. They determined that the smaller the stream, the more monitoring of water withdrawal and dispersal should be used. Larger streams (> 1000 cfs) have the ability to lose and gain larger amounts of water without an impact on habitat and flow. Whereas, smaller streams (< 100 cfs) are prone to worse consequences (Rahm and Riha 2012).

Consequential Effects on Communities:

The three leading environmental impacts of UOG, sedimentation, chemical leaching, and hydrological changes, all have a direct impact on the organisms living within the impacted environment. Though not exactly the same, other land uses have been shown to have similar deleterious effects. A stream's ecosystem consists of a multitude of flora and fauna, most of which are sensitive to the side effects of anthropogenic disturbances.

Deforestation of land surrounding a stream for the construction of well pad and roads can lead to increased sedimentation within the stream. This sedimentation and reduced canopy cover results in an energy source change from allochthonous to autochthonous (Stone and Wallace 1998). With a new energy source and a change in the composition of the stream bed habitat, the organismal populations of the stream changes. This leads to a decrease in fish and invertebrate diversity between headwater streams and their downstream constituents (Stone and Wallace 1998).

Impervious surfaces, such as those in residential developments, have been found to have deleterious effects on stream habitat, water quality and biotic structure (Walters et al. 2001; Morse et al. 2003; Roy et al. 2003). Upon construction of impermeable surfaces, the turbidity of adjacent surface waters systems increased. Studies have shown that increased stream turbidity decreases the index of biological integrity (IBI) score for both macroinvertebrates (Morse et al. 2003; Roy et al. 2003) and fish (Meyer et al. 1999; Walters et al. 2001). Morse et al. (2004) explained that increased development leads to degraded water chemistry through increased specific conductivity and TSS. Other studies have shown that residential development leads to degraded habitat conditions (Merriam et al. 2011). We anticipate that the impervious surfaces constructed during UOG operations will mimic the effects of residential development.

Johnson et al. (2017) explained that biofilms are subject to species change relatable to an anthropogenic change in land use (Johnson et al. 2017). Biofilms can contain a variance in species relative to the water quality. This feature allows for the identification of increased salts, increased acidity and even increased nitrates. Brittingham et al. proposed that this deforestation has an impact on the amphibians in the area as well (Brittingham et al. 2014). They determined that deforestation leads to habitat fragmentation and can change dispersal and breeding habits of frogs.

Fish Communities as a Proxy for Stream Condition:

Before 1981, fish communities were rarely used as a metric to determine stream condition. In 1981 this changed when James R. Karr wrote a methods paper outlining the use of fish communities as a simplistic and inexpensive way of analyzing a stream (Karr 1981). Since then, his method has been used on both a national (Barbour et al. 1999, USEPA 2006) and local (McCormick et al. 2001) scale. Biotic indicators are superior to chemical or physical monitoring as they give a better picture of what is happening over a span of time (Fausch et al. 1990). They explain that chemical or physical parameters are so complex that they rarely can predict the biological integrity of the system. Karr goes on to explain that fish are a better biological monitoring taxa than macroinvertebrates and diatoms due to their ease of sampling (i.e., less sorting, less identification training), our wealth of background knowledge and the ability to translate the results into a form easily understood by the general public.

Fish IBI analysis, involves analysis of community, population and the individual organism (Karr 1981; Fausch et al. 1990). IBIs are specific to their geographic location and must be created before analyzing a given area. Anderson (2015) used data collected from statewide sampling events to create a West Virginia IBI (Anderson et al. 2015). Each individual species found in the studies were classified based on their life history traits (i.e., spawning, distribution, tolerance, trophic guild and family). Fish community samples from our study will look at the difference of these metrics between UOG impacted and reference conditions. In the case of a significant change in community structure, there is potential to advise environmental legislation (Karr 1990).

Hematocrit as a Proxy for Fish Health:

Stress in an organism is can be caused by a variety events. Whatever the cause, the stress event leads to the creation of corticosteroids and catecholamines. These hormones then regulate secondary stress responses (i.e., compromised immune system, alteration of oxygen consumption [Schreck and Tort 2016]). Sopinka et al. (2016) outlines various methods that are used to measure these stress responses (Sopinka et al. 2016). They outline hematocrit as an indicator of stress response that requires a simple test using inexpensive equipment.

Hematocrit is the volume percentage of red blood cells found in the blood (Sopinka et al. 2016). Typically, a baseline hematocrit value is obtained for a target species in a control setting. After this baseline value is determined, the fish is subjected to a stressor and the change in hematocrit is recorded. Depending on the stressor, the hematocrit can either increase or decrease. In events that require the subject to consume more energy (i.e., avoiding predators), the red blood cell count increases and subsequently the hematocrit value increases (Marshall et al. 2012). In stress events that involve exposure to heavy metals, pesticides and disease it is common to see a decrease in hematocrit as red blood cells are being destroyed and white blood cell counts are increasing (Barnhorn 1996; Buthelezi et al. 2000; Nussey et al. 2006; Ghaffar et al. 2018). We expect to see a decrease in the hematocrit levels of fish residing in UOG impacted stream as they are potentially subject to decreased water quality including heavy metal exposure.

OBJECTIVES

The goal of this research project is to determine the effects of UOG on fisheries within West Virginia watersheds. Using both pre- and post- UOG development data, it is possible to directly test for UOG development impacts on fishes. To meet this goal, I addressed the following objectives:

1. Quantify the effect of UOG development on fish assemblage structure
2. Quantify the effect of UOG development on the physiological stress levels of resident *Semotilus atromaculatus* (creek chub)

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CHAPTER 2: ANALYSIS OF UNCONVENTIONAL OIL AND GAS IMPACTS ON DOWNSTREAM FISH COMMUNITIES USING A BEFORE AFTER CONTROL IMPACT STUDY DESIGN

Joshua N. Ankeny, J. Todd Petty, Eric R. Merriam, Quinton E. Phelps

ABSTRACT

Since 2008, Unconventional Oil and Gas (UOG) production has been expanding throughout West Virginia's portion of the Marcellus shale formation. An increase in UOG is expected to negatively impact headwater aquatic systems through increased sedimentation, degraded water chemistry, altered hydrologic regimes and an overall decrease in habitat quality. In this study, we utilized a before-after-control-impact study design in tandem with fish community metrics to quantify the effects of UOG on fish community metrics in 8 UOG impacted sites and 10 reference streams. A generalized linear mixed effect model revealed three metrics that had a significant interaction between treatment type (i.e., reference and UOG impacted) and sampling period (i.e., pre- or post-). Invertivores and Piscivores metrics were found to decrease following UOG events, whereas benthic metrics increased. Our findings suggest that community level analyses are not accurately assessing stream condition as fish communities are experiencing statewide degradation. An ANCOVA alongside a linear mixed effect model failed to find a significant difference between the weight and length of *Semotilus atromaculatus* residing in both treatment conditions. Our findings indicate that in systems chronically impaired by anthropogenic stressors, community analyses may not be capable of detecting a degraded ecosystems.

INTRODUCTION

The deleterious effects of anthropogenic land uses on both biotic and abiotic aquatic factors have been extensively studied. Researchers have explored the effects of mining (Freund and Petty 2007; Merriam et al. 2011; Merovich et al. 2013), agriculture (Cuffney et al. 2000; King et al. 2005), development (Morse et al. 2003; Merriam et al. 2011) and various combinations of land uses (Merovich and Petty 2007; Merriam et al. 2015). However extensive the research, there is a lurking concern for the potential impacts of new understudied land uses. This research focuses on the rapid expansion of Unconventional Oil and Gas (UOG) exploration in the Marcellus shale formation. The Marcellus shale play, spanning across much of the Appalachian Basin, has seen a rapid uptick in UOG activity following new technological advances in 2008 (Rahm and Riha 2012; Brown et al. 2013). In 2007 there were 62 drilled UOG wells throughout the state of West Virginia. As of 2017, this number has increased to 2382.

Unconventional Oil and Gas is aptly referred to as horizontal drilling or hydraulic fracturing. The hydrocarbons targeted with this approach lie deep within horizontal shale formations that are relatively thin but very wide. To extract hydrocarbons from these formations, wells are drilled between 1500 and 3000 meters deep. Once the shale formation is breached, the drill is turned parallel to the earth's surface and a hole is drilled horizontally through the shale. A slurry of fracking fluid consisting of water, chemicals, biocides and proppants are forced in the well under extreme pressure. This process fractures the shale formation and props it open as to stimulate the release of hydrocarbons (EPA, 2018).

Unconventional Oil and Gas, like other anthropogenic stressors (i.e., mining, agriculture, development), has deleterious effects on the surrounding watershed (Morse et al. 2003; Merriam et al. 2011). Deforestation for the purpose of well pad construction, road construction and pipeline corridor clearing lead to sedimentation of surrounding surface waters and habitat fragmentation (Stone and Wallace 1998; Weltman-Fahs and Taylor 2013b; Brittingham et al. 2014). The cracking of well casings, the spilling of brine solutions and the improper treatment of fracturing fluids have all lead to recorded contamination events in surface water systems (Brittingham et al. 2014; Shrestha et al. 2017). In the Marcellus shale formation, each UOG well requires 7.5 - 26.5 million liters of water during the fracturing process (Entrekin et al. 2011). Extracting this much water from headwater systems can be detrimental to the hydrologic regime (Rahm and Riha 2012).

In the early 1980s, two innovative stream assessment protocols were created: The Index of Biological Integrity (IBI) and the Index of Well Being (IWB) (Gammon 1980; Karr 1981). These protocols analyzed fish community metrics to determine the health of aquatic ecosystems, a method rarely used before their time (Gammon 1980; Karr 1981). Since their creation, the IBI and IWB have been extensively studied and used at both the national (Barbour et al. 1999) and local (McCormick et al. 2001) scales. Gagic et al. (Gagic et al. 2015) determined that trait-based community analyses provide better understanding of ecosystem functionality than taxonomic-based analyses. Gagic's approach was used to construct the West Virginia IBI which included metrics based on spawning characteristics, trophic levels, taxonomic families and tolerance levels (Anderson et al. 2015). Alterations in fish metrics are commonly used to indicate changes in habitat quality, while consistent results can indicate a stable environment. Many past studies have used fish community analyses to determine the effects of an anthropogenic land use (Meyer et al. 1999; Walters et al. 2001). In addition to community level analyses, physiological parameters (i.e., weight and length regression, condition factor) of individual species are also commonly used to determine fish health as it relates to the surrounding environment (Peters 1983; Reiss 1989; Riedel et al. 2009). Decreased weight length regressions and condition factors imply a degree of malnourishment and degraded individual health (Williams 2000).

In this study, we sampled 18 fish communities spread across the Monongahela River basin and the Ohio River basin of northwestern West Virginia (Figure 2). To our knowledge, this is the first study that utilizes a before-after-control-impact study design alongside fish community metrics to assess watersheds impacted by UOG disturbance. Our research objectives include: (1) quantify changes in fish community metrics as a response to UOG development within the watershed; (2) analyze variations in weight (g) and length (mm, standard length) of *Semotilus atromaculatus* (creek chub) within both treatment conditions.

METHODS

Study Area:

The Marcellus shale formation lies underneath six US states: West Virginia (21.33%), Pennsylvania (35.35%), New York (20.06%), Ohio (18.19%), Virginia (3.85%), Maryland (1.09%). Of the 245,771km^2 that the Marcellus shale covers, only 27,510km^2 are leased by oil and gas companies for UOG extraction (U.S. Energy Information Administration, 2011). Both the Monongahela River Basin and the Ohio River basin, analyzed in this study, fall completely within the range of the Marcellus shale play (Figure 2). The Monongahela River basin covers 6800km^2 of north central West Virginia and is predominately forested (75%) land. Agriculture (14%) development (5%) and mining (4%) are the primary land use stressors in the Monongahela River basin. The Ohio river basin covers 9600km^2 of northwestern West Virginia and is predominantly forested (82%) land. Agriculture (10%) is the primary land use stressor in the Ohio River basin.

Site Selection:

Data provided by the West Virginia Department of Environmental Protection (WVDEP) and West Virginia Division of Natural Resources (WVDNR) was used to select sampling events that predated UOG activity. Eighteen sites throughout both the Ohio River basin and the Monongahela River basin were selected for resampling based on presence or absence of UOG, watershed land use attributes, sampling methods, and sampling date. Pre-UOG sites were mapped and joined to a National Land Cover Database (U.S. Geological Survey, 2014) land use file. Sites were removed from selection if they contained any upstream mining disturbance, upstream agriculture disturbance greater than 20%, upstream development disturbance greater than 2.5% or did not use backpack electrofishing as the sampling method. Detailed polygons of UOG well pads were overlaid on the land use map. From the sites that did not contain UOG disturbance within their watersheds, ten sites of least disturbance (Stoddard et al. 2006), henceforth referred to as reference, were selected. Eight impacted sites were selected based on the presence of UOG disturbance within their watersheds and a sampling date prior to the spud date of all upstream wells. All mapping was performed in version 10.3 ArcGIS software (Environmental Systems Research Institute [ESRI] 2005).

Fish Community:

All collection methods followed the single pass backpack electrofishing protocol outlined in the WVDEP Fish Collection Protocol (WVDEP, 2011). Samples were collected between June and October in 2017 and 2018. Sampling events corresponded with stable flow conditions as to reduced turbidity and increase capture efficiency (Hense et al. 2010). Stream reaches were initiated at the coordinates provided from the agencies who performed the pre-UOG sampling. If reach lengths were not provided, it was calculated as 40 times the average wetted width for a minimum of 160 meters and a maximum of 300 meters, as to follow WVDEP standard operating procedures. Table 10 of the WVDEP Fish Collection Protocol (WVDEP, 2011) was used to determine the number of electrofishing units and netters to use based on stream width and depth. Smith-Root Model 24LR backpack electrofishing units (Vancouver, Washington) were used alongside ¼" mesh nets (Freund and Petty 2007). As sampling progressed through the reach, debilitated fish were netted and placed in live wells. Upon completion of the reach, fishes were identified to the species level, weighed (nearest 0.01g) and measured (nearest mm, standard length). Fish that were not able to be identified in the field were immediately euthanized in 95% ethanol and identified in the lab. Fish identified in the lab were not weighed as preservation in 95% ethanol significantly reduces fish weight (Shields and Carlson 1997).

Physical and Chemical Parameters:

To assess habitat quality, we performed U.S. Environmental Protection Agency (EPA) rapid visual habitat assessments (RVHA) at each site (Barbour et al. 1999). Using RVHA, various physiological parameters were assessed to give the site a score (maximum possible RVHA = 200).

In situ water parameters in tandem with lab analyses were used to assess water quality. A YSI 650 equipped with a 600XL sonde was used to measure dissolved O_2 (mg/L), pH, temperature (C), and specific conductivity (µS/cm) (Yellow Springs Instruments, Yellow Springs, OH, USA). A single filtered sample was obtained using a Nalgene ® filtration device with a 0.45 µm mixed cellulose-ester membrane filter. This sample was used to measure Al, Ca, Fe, Mg, Mn, K, Na, Sr, Zn (EPA method 200.7) as well as Ba, Cd, Cr, Ni and Se. (EPA method 200.8). Three unfiltered samples were obtained and used to measure Br^-, Cl^-, SO_4^{2-} (EPA method 300.0), NO_2^-, NO_3^- (EPA method SM4110B-2000), total P (EPA method SM4500-P BE-1999), total dissolved solids (EPA method SM2540 C-1997), total and bicarbonate alkalinity (EPA method SM2320 B-1997). The samples were stored at 4℃ until they were analyzed at Research Environmental and Industrial Consultants Inc. (Beaver, WV, USA).

Statistical Analysis:

Fish community response to UOG development:

The first objective set forward in this study was to assess changes in fish community metrics as a response to UOG development within the watershed. Initially, raw counts as well as relative abundances were calculated for multiple functional groups derived from Anderson's dissertation (Anderson et al. 2015). Of the 65 metrics outlined in Anderson's doctoral dissertation, 51 were selected for analysis based on expected response to UOG disturbance. Eleven of the 51 metrics were removed as more than ⅓ of the samples contained zero detections (Stoddard et al. 2008). The resulting 40 functional groups used for this study are outlined in Table 1 along with their expected response to UOG. Statistical analysis was performed in R statistical software version 3.4.1 (R Core Team, 2017). A significance level α = 0.05 was predetermined for this study. All functional groups were tested for normality using Q-Q plots.

Normally distributed data was analyzed with a generalized linear mixed effects model with a poisson distribution and sample site as a random effect. Total fish caught per sampling event was used as an offset when determining relative abundances. If the original poisson generalized linear mixed effects model showed overdispersion a negative binomial model was substituted. Homogeneity of variance was tested for using the Brown-Forsythe test. For each metric containing a significant interaction between treatment type (i.e., reference or impacted) and sampling period (i.e., pre- or post-) a post hoc Tukey test was ran using a Dunn-Sidak adjustment to correct for experiment-wide errors. Generalized linear mixed effects models were used to assess the abundances of individual fishes comprising each community metric that was found to be statistically significant.

The BACI model tested in this study was, $\gamma_{ijk} = \mu + \tau_i + \rho_j + \tau\rho_{ij} + \omega_{ik} + \varepsilon_{ijk}$, where γ is the community metric, τ is the treatment effect (i.e., impact or reference), ρ is the time aspect (i.e., pre- or post-), $\tau\rho$ is the interaction between treatment and time (i.e., pre-reference, post-reference, pre-impact, post-impact), ω is the random site error, and ε is the random experimental error (McDonald et al. 2000).

Weight and length response to UOG development:

The weight (g) and standard length (mm) of 987 *S. atromaculatus*, spread across all sites and treatment types (i.e., reference and impacted), were recorded. Weight and lengths were log transformed and an ANCOVA was used to compare the slopes of the weight-length regression between treatment types (Figure 3). A linear mixed effect model, with site as a random effect, was used to compare both the log weight and log length, independently of one another, between treatment types (Table 4; Figure 4).

Allometry within treatment groups was tested for using the following equation:

$$W = \alpha L^{\beta}$$ (Peters 1983; Reiss 1989; Riedel et al. 2009)

In this equation, W is the log weight (g) of each specimen, L is the log length (mm) of each specimen, α is the intercept and β is the allometric parameter (Riedel et al. 2009; Ogle 2013).

Fulton's condition factor was calculated for each individual specimen using the following equation:

$$K_{TL} = [(100{,}000)(W)] / L^3$$ (Ricker 1975)

In this equation, K is the coefficient of condition, W is the weight (g) and L is the length (mm). A two-sample t-test was used to compare the means of the condition factors of each treatment type (Table 3).

Water quality and habitat quality response to UOG development:

Water quality measurements were not available for the pre-Impacted and pre-Reference samples. Therefore, each water quality parameter as well as RVHA was compared between post- treatment conditions (i.e., post-Impacted and post-Reference) using two-sample t-tests (Table 6).

RESULTS

Habitat and Water Quality:

Though insignificant ($p = 0.377$), mean rapid visual habitat assessment scores appeared to decrease in the presence of UOG. Two sample t-tests revealed six water quality parameters that were significantly different with regard to treatment type: Calcium ($p = 0.003$), Magnesium ($p = 0.020$), Strontium ($p = 0.009$), Conductivity ($p = 0.003$), TDS ($p = 0.024$) and Alkalinity ($p = 0.018$). Remaining water quality parameters were not found to be significantly different between treatment conditions ($p > 0.05$ for all parameters [Table 6]).

Community Metrics:

Of the 40 community metrics analyzed, three had a significant interaction between treatment (i.e., reference and impacted) and sampling period (i.e., pre- and post- [Table 2]). The invertivore and piscivore metric (IP) was significantly different when looking at raw count data, while the benthic (Benthic) and benthic minus white sucker (Benthic_CACO) were significantly different when comparing relative abundances of the metrics. The mean abundance of IP species decreased from 324.9±79.5 in pre-UOG impacted sites to 200.4±139.6 in post-UOG impacted sites. The mean relative abundance of species within the Benthic metric increased in relative abundance from 0.551±0.175 in pre-UOG impacted sites to 0.678±0.164 in post-UOG impacted sites. The mean relative abundance of species within the Benthic_CACO metric increased in relative abundance from 0.537±0.172 in pre-UOG impacted sites to 0.662±0.167 in post-UOG impacted sites (Table 2).

Redside dace ($p < 0.001$), rainbow darters ($p = 0.026$), johnny darters ($p < 0.001$), longear sunfish ($p < 0.001$), golden redhorse ($p < 0.001$) and mimic shiner ($p < 0.001$) are all intolerant or moderately tolerant species within the invertivore or piscivore trophic levels. These fish species decreased in abundance following UOG disturbances. Central stoneroller ($p < 0.001$), mottled sculpin ($p = 0.020$), northern hogsucker ($p = 0.007$), logperch ($p < 0.001$) and eastern blacknose dace ($p < 0.001$) are all moderately tolerant or tolerant benthic species. These species were found to increase following UOG disturbance.

Size Metrics:

The ANCOVA for the weight-length regressions did not reveal any significant difference between treatment types ($p = 0.536$). Figure 3 the log transformed data weight and length data. The slopes are nearly indiscernible from one another implying that both populations are increasing in weight and length at a similar ratio. A linear mixed effect model comparing the length and weight independently failed to find a significant difference between treatment types ($p > 0.05$ for both metrics [Table 4; Figure 4]).

Treatment type was not found to be a significant predictor of allometric growth. Fish within both conditions were found to have negative allometric growth. Reference conditions had a β of 2.82 ± 0.037, while Impacted conditions had a β of 2.84 ± 0.044. Additionally, Fulton's condition factor was not found to be significantly different between treatment types ($p = 0.28$, Table 3).

DISCUSSION

Fish Assemblages:

Analysis of 18 fish communities revealed that three (Table 2) of our 40 metrics are statistically different with regard to the interaction between treatment type (i.e., impacted and reference) and sampling period (i.e., pre-UOG and post-UOG). The first of the three significant metrics is unique in that it represents two trophic levels, invertivores and piscivores (IP). Our study found that the IP metric is significantly lower in watersheds containing UOG (Table 2; Figure 5). Previous studies (McCormick et al. 2001) suggest that the IP metric is negatively correlated with increased turbidity, reduced canopy cover and degraded habitat quality, all of which are common in sites containing UOG disturbances (Entrekin et al. 2011; Weltman-Fahs and Taylor 2013a; Brittingham et al. 2014). Piscivores are typically visual predators and it has been shown that increased turbidity reduces their foraging efficiency (Mazur and Beauchamp 2003). One explanation for this decrease is optimal foraging theory which explains that as habitat quality decreases fishes move to a habitat patch that provides a better cost-benefit ratio (Aspey and Lustick 1983). Invertivores have been found to decrease in systems with increased sedimentation as macroinvertebrate composition shifts (Pirhalla 2004). A decrease in the abundances of intolerant and moderately tolerant invertivore-piscivore species (i.e., redside dace, rainbow darter, johnny darter, longear sunfish, golden redhorse, mimic shiner) is driving the decrease in the IP metric (Table 8).

Additionally, our analyses determined that fishes comprising the Benthic and Benthic minus white sucker (Benthic_CACO) metrics increased in relative abundance following UOG development (Table 2; Figure 5). These findings disagree with other trait based indexes (Barbour et al. 1999; McCormick et al. 2001; Anderson et al. 2015) which predict that anthropogenic stressors associated with a decrease in benthic habitat (i.e., sedimentation or substrate composition) negatively impact metrics consisting of benthic species as they require clean substrate for habitat and spawning. Since increased stream sedimentation is one of the leading impacts of UOG (Weltman-Fahs and Taylor 2013b; Brittingham et al. 2014) we initially hypothesized a decline in these metrics. Our findings suggest that UOG sites are not experiencing increased sedimentation load. Comparison of RVHAs did not reveal significant degradation in habitat quality of UOG impacted sites (Table 6), implying that the evaluated parameters (i.e., embeddedness, substrate characterization, sediment deposition, etc) are uniform (Barbour et al. 1999) throughout treatment conditions. An increase in the abundances of tolerant and moderately tolerant benthic species (i.e., central stoneroller, mottled scuplin, northern hogsucker, logperch, eastern blacknose dace) is driving the increase in these benthic metrics (Table 8). We failed to find a significant increase in the Benthic metrics when we removed the tolerant species (Benthic2.DEP).

Our metrics were analyzed using both raw count data as well as relative abundance data utilizing total fish as an offset. Though both pre- and post- sampling methods followed the WVDEP Fish Collection Protocol (WVDEP 2018), we expected relative abundance data to have stronger results as it reduces differences associated with different collection crews. Additionally, it has been determined that both mean stream width and stream gradient are important factors in single-pass electrofishing efficiency (Hense et al. 2010). As these factors do not change within a site following UOG development, we can assume that they are not reducing detection efficiency. The Benthic and Benthic_CACO metrics were found to be statistically different when analyzing relative abundances while the IP metric was found to be statistically different only when analyzing raw data.

Under ideal circumstance, both pre-impacted and pre-reference treatment conditions should be similar in habitat characteristics as all sites are of similar size, similar geographic location and have only UOG as the variable land use stressor. The remaining 37 metrics revealed no significant change following UOG development despite our initial hypotheses. This suggests that with a continual rise in anthropogenic disturbance throughout the state of West Virginia there is potential to reduce even the pristine headwater streams to degraded habitats through dispersal and mass effects (Leibold et al. 2004; Heino et al. 2015; Merriam and Petty 2016). A decreased habitat quality of neighboring systems has been shown to reduce the functionality of isolated headwater streams within West Virginia (Merriam and Petty 2016). Though our site selection methods controlled for upstream land uses, we disregarded downstream impacts. The fourth principle of the riverscape-concept explains that “unintended consequences of habitat degradation will occur in all directions, including upstream” (Fausch et al. 2002). This statewide degradation could result in communities that are uniform between treatment and reference conditions, rendering community level analyses useless.

It appears that reference and impacted sites are becoming more uniform which could result in an overall decrease in fish richness and an eventual extirpation of less tolerant species.

Similar patterns have been recorded in macroinvertebrate communities within the mountaintop removal-valley fill mining region of West Virginia (Merriam and Petty 2016). We believe we are seeing a proliferation of tolerant taxa throughout the headwater streams of our study area predating even our pre-UOG samples. Continued UOG disturbance, in conjunction with other anthropogenic stressors, could result in overall degradation of West Virginia headwater streams to lower biological integrity classes (Karr 1981, 1990).

Size Metrics:

S. atromaculatus are a widely distributed species with a range that extends across much of eastern United States and southeastern Canada. Though *S. atromaculatus* are omnivorous they mainly feed on macroinvertebrates and other fishes (Nico and Fuller 2019). Metric construction for various IBIs has labeled *S. atromaculatus* as a tolerant species (McCormick et al. 2001; Anderson et al. 2015). These characteristics lend *S. atromaculatus* to being a good candidate for field studies as they are ubiquitous among West Virginia stream conditions. *S. atromaculatus* chub were selected for size analysis as they were detected in all 18 of sites at an average of 55 individuals per site (N = 987).

An ANCOVA of the length-weight slope revealed that *S. atromaculatus* are increasing in size at similar ratios regardless of treatment condition (Figure 3). A linear mixed effects model failed to reveal a significant difference between weight and length (Table 4). Additionally, there is no difference in allometric growth or Fulton's condition factor between treatment condition (Table 3). Weight and length characteristics are highly variable between season (Moutopoulos and Stergiou 2002). Our sampling period ranged from June to October. A different sampling approach where weight and length measurements are taken within the same season may reveal different results.

Management Potential:

West Virginia has a long history of anthropogenic disturbance through mining, mountaintop removal, agriculture and development (Yarnell 1998). Moreover, legacy land uses that occurred prior to our pre- data could be determining community composition within these streams (Harding et al. 1998). These historic events make it nearly impossible to find a stream that is truly of the reference condition. For our study, we instead selected streams of the least disturbed condition (LDC [Stoddard et al. 2008]). Stoddard et al. (2008) labeled streams of least disturbed condition as those in the landscape that still have anthropogenic disturbance but on a lesser scale than the defined impacted sites. Both treatment conditions had agricultural disturbance (< 20%) and developmental disturbance (< 2.5%). Mining disturbances were controlled against as studies have shown that mining in West Virginia has deleterious effects on biological condition that can be long-lasting (Petty et al. 2010; Merriam et al. 2011 [Table 5]). Additionally, mining has been shown to affect surface waters to a greater extent in the presence of additional land use stressors (Merriam et al. 2015). The only significantly different anthropogenic land use stressor between treatment conditions was UOG ($p < 0.001$ [Table 5]). Management agencies should consider both the individual impact of UOG as well as the cumulative effects with other land use stressors.

Karr (1981; 1990) describes poor lotic habitats as those "dominated by omnivores, pollution-tolerant forms, and habitat generalists; growth rates and condition factors commonly depressed; hybrids and diseased fish often present." As fish communities in these watersheds become increasingly degraded and dominated by tolerant taxa we begin to question if community level analysis is sufficient. If the cumulative effects of anthropogenic stressors result in predominantly streams of poor condition, IBI cannot be used to make informed management decisions. From our findings, we suggest that in chronically impaired systems the headwaters streams are threatened by downstream anthropogenic stress (Fausch et al. 2002). Therefore, a significant change in community metrics will prove hard to detect as the communities within these ecosystems are at the lowest biotic integrity class.

Conclusion:

In summary, analysis of fish community metrics determined that IP abundance decreased following UOG disturbance, indicating a reduction in optimal foraging conditions (Aspey and Lustick 1983; Mazur and Beauchamp 2003). Contrary to expectations (Karr 1981; McCormick et al. 2001; Anderson et al. 2015), we saw an increase in the relative abundance of fishes within the Benthic and Benthic minus white sucker metrics. Fish assemblage results suggest that community level analyses in watersheds with extensive anthropogenic stress, both current and legacy, may not provide an adequate measurement of an aquatic ecosystem's health. We suggest that management agencies should conduct physiological studies as opposed to community studies to determine changes in resident fish health associated with landscape alterations (chapter 3 of this thesis).

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FIGURES AND TABLES

Figure 1:

A time series depicting the expansion of UOG across the state of West Virginia. Each dot (●) represents a drilled UOG well. In 2007 there were 62 wells. In 2008 there were 314 wells. In 2011 there were 1189 wells. In 2017 there were 2382 wells. Map created in ArcGIS version 10.3 (Environmental Systems Research Institute [ESRI] 2005).

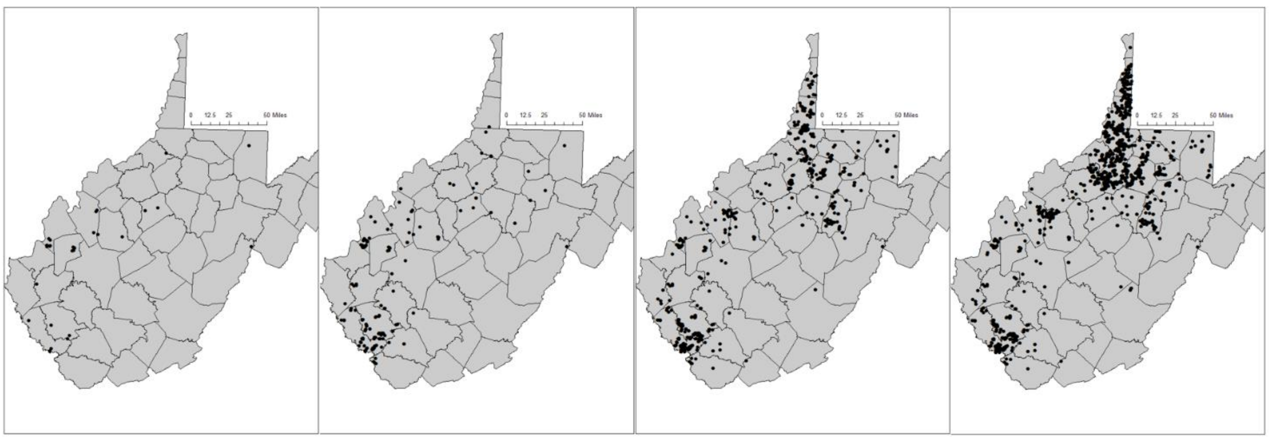

Table 1:

All fish metrics analyzed in this study along with their predicted response to UOG. Metrics expected to decrease in abundance after UOG disturbance (-). Metrics expected to increase in abundance after UOG disturbance (+). Metrics and expected results were selected from a larger table found in Anderson, 2015.

Metric Abbreviation	Description	Expected Response
Game	Classified game fish from WV DNR	–
RGS	Rock-gravel spawners	–
GSS	Gravel and sand spawners	–
NGL	Non-guarding lithophilic spawners	–
MO	Macro-omnivore	–
IN	Invertivore	–
IP	Invertivore-Piscivore	–
ISEAT	Invertivore-Piscivore minus *S. atromaculatus*	–
Benthic	Benthic species	–
Benthic_CACO	Benthic species minus *C. commersonii*	–
Cyprinid	Cyprinidae family	–
Cyprinid_BNDSEAT	Cyprinidae family minus *R. atratulus* and *S. atromaculatus*	–
BND_CACO_SEAT	Blacknose dace, white sucker and *S. atromaculatus*	+
OH	Omnivore-Herbivore	+
OH_CAAN	Omnivore-Herbivore minus *C. anomalum*	+
OH_CAAN_CACO	Omnivore-Herbivore minus *C. anomalum* and *C. commersonii*	+
OH_NG	Non-game omnivore-herbivore	+
IBenthicNG	Benthic and non-game invertivore-piscivore	–
INonGameNB	Non-game and non-benthic invertivore-piscivore	–
DMS	Darters, madtoms and sculpins	–
Percidae	Percidae family	–

Centrachidae	Centrarchidae family	–
Catostomidae	Catostomidae family	–
CGS_RGS	Clean gravel and rock gravel spawners	–
Cavity Spawn	Cavity spawners	–
Fish2.DEP	Total Fish minus tolerant	–
RGS2.DEP	Rock-gravel spawners minus tolerant species	–
NGL2.DEP	Non-guarding lithophilic spawners minus tolerant species	–
IP2.DEP	Invertivore-piscivore minus tolerant species	–
Benthic2.DEP	Benthic minus tolerant species	–
Cyprinid2.DEP	Cyprinidae family minus tolerant species	–
Game2.DEP	Game fish minus tolerant species	–
Tol.DEP	Tolerant species	+
Mod.DEP	Moderately tolerant species	–
Int.DEP	Intolerant species	–
Tol_Benthic.DEP	Tolerant benthic species	+
Tol_Cyprinid.DEP	Tolerant species in the Cyprinidae family	+
McC_CGS2.DEP	Clean gravel spawners minus tolerant species	–
CGS_RGS2.DEP	Clean gravel and rock-gravel spawners minus tolerant species	–
CavitySpawn2.DEP	Cavity spawners minus tolerant species	–

Figure 2:

8 impacted sites (★) and 10 reference sites (●) were sampled within both the Monongahela River drainage basin (light grey) and the Ohio River drainage basin (dark grey). Dashed polygon represents the Marcellus Shale formation. Map created in ArcGIS version 10.3 (ESRI, 2005).

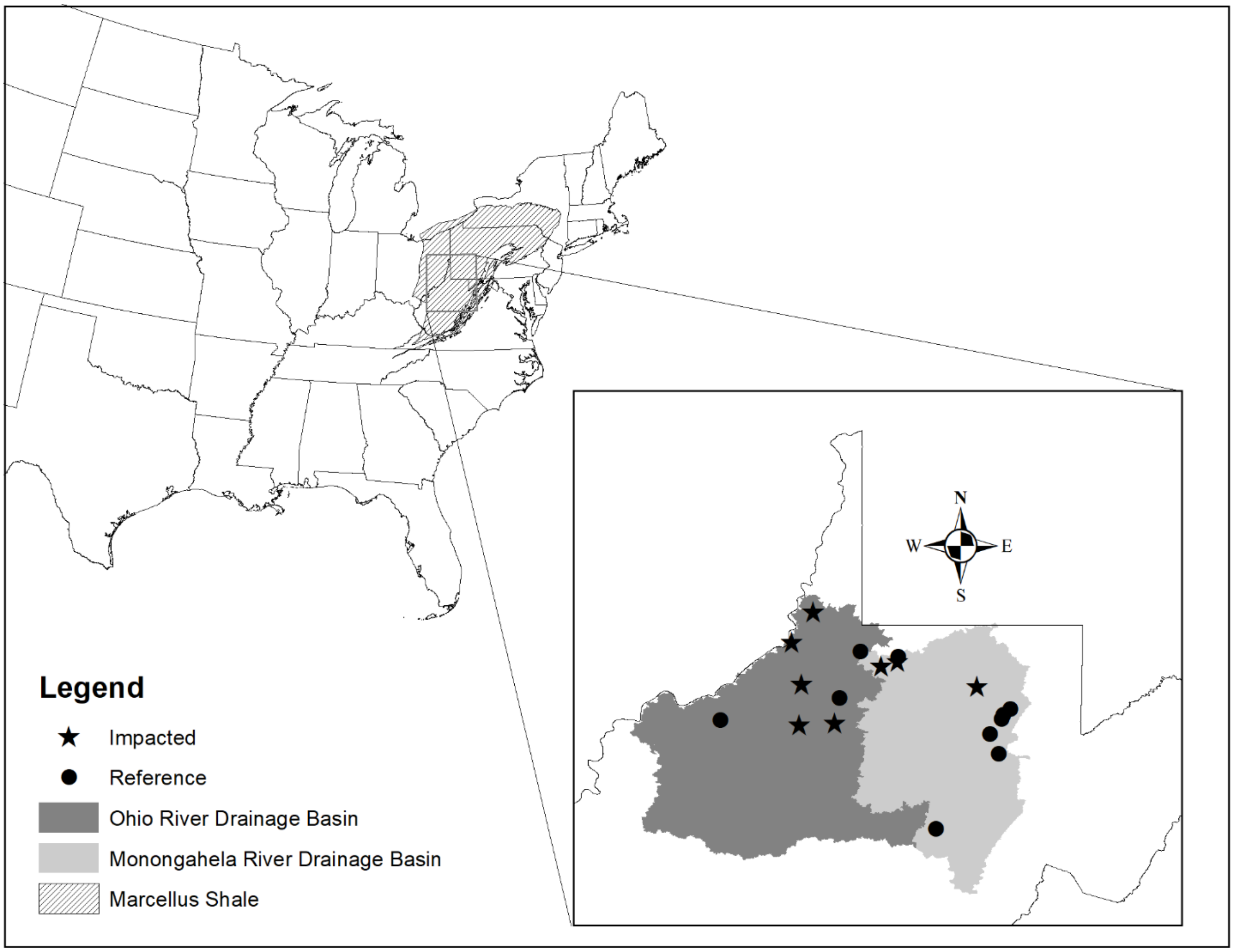

Table 2:

The results from generalized linear mixed effects models comparing fish community metrics between treatment conditions (i.e., reference and impacted) and sampling period (i.e., pre- and post- UOG).

Raw Counts							
Metrics	Treatment	Period	Mean	SD	SE	Grouping	*p* value
Invertivore - Piscivore	Reference	Pre	248.2	166.5	52.66	AB	0.045
		Post	300.9	110.1	34.81	AB	
	Impacted	Pre	324.9	79.50	28.11	B	
		Post	200.4	139.6	49.37	A	
Relative Abundances							
Metrics	Treatment	Period	Mean	SD	SE	Grouping	*p* value
Benthic Species	Reference	Pre	0.698	0.137	0.043	AB	0.003
		Post	0.677	0.146	0.046	AB	
	Impacted	Pre	0.551	0.175	0.062	A	
		Post	0.678	0.164	0.058	B	
Benthic Species minus White Suckers	Reference	Pre	0.679	0.145	0.046	AB	0.011
		Post	0.663	0.149	0.047	AB	
	Impacted	Pre	0.537	0.172	0.061	A	
		Post	0.662	0.167	0.059	B	

Figure 3:

Boxplots of significant metrics. p-value derived from generalized linear mixed effect models. White boxes represent reference conditions. Gray boxes represent impacted conditions.

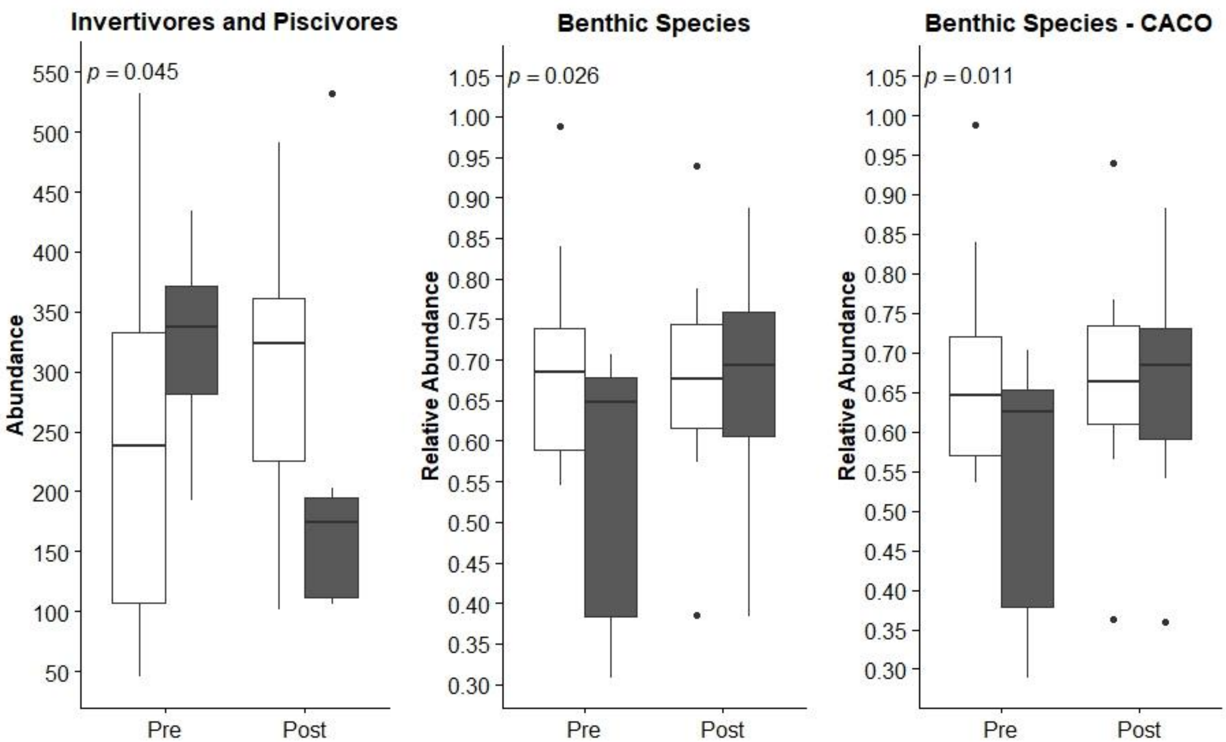

Figure 4:

Weight and length regression of *Semotilus atromaculatus* in both treatment conditions. Reference samples and fit are represented by hollow circles (o) and a dashed line respectively. Impacted samples and fit are represented by solid circles (●) and a solid line respectively.

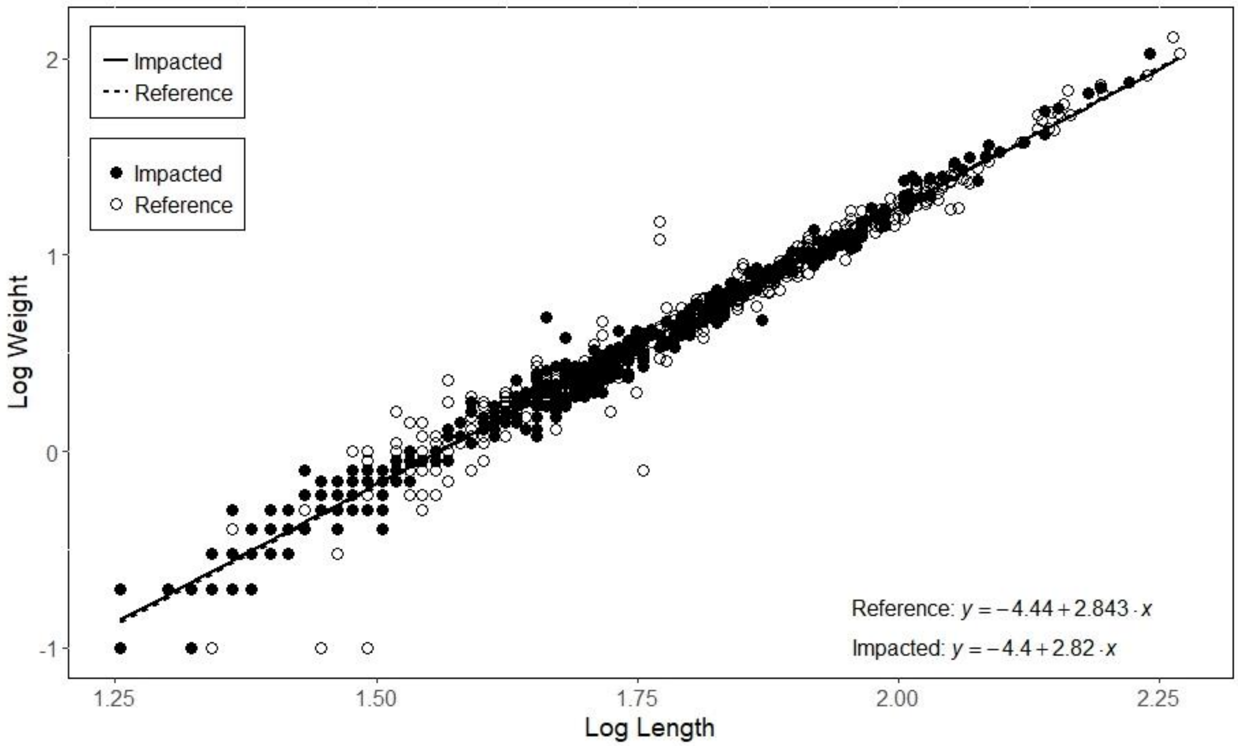

Table 3:

Fulton's condition factor for *Semotilus atromaculatus* in both treatment conditions.

	Mean	SD	SE	*p* value
Reference	1.97	0.503	0.022	0.28
Impacted	2.01	0.398	0.019	

Table 4:

The results of a linear mixed effect model for standard length (mm) and weight (g) of *Semotilus atromaculatus* in both treatment conditions.

	Treatment	N	Mean(±SD)	*p* value
Log Length (mm)	Reference	537	1.77±0.19	0.073
	Impacted	450	1.71±0.18	
Log Weight (g)	Reference	537	0.595±0.54	0.080
	Impacted	450	0.440±0.51	

Figure 5:

Boxplot of the log length and log weight of *Semotilus atromaculatus* in both treatment conditions.

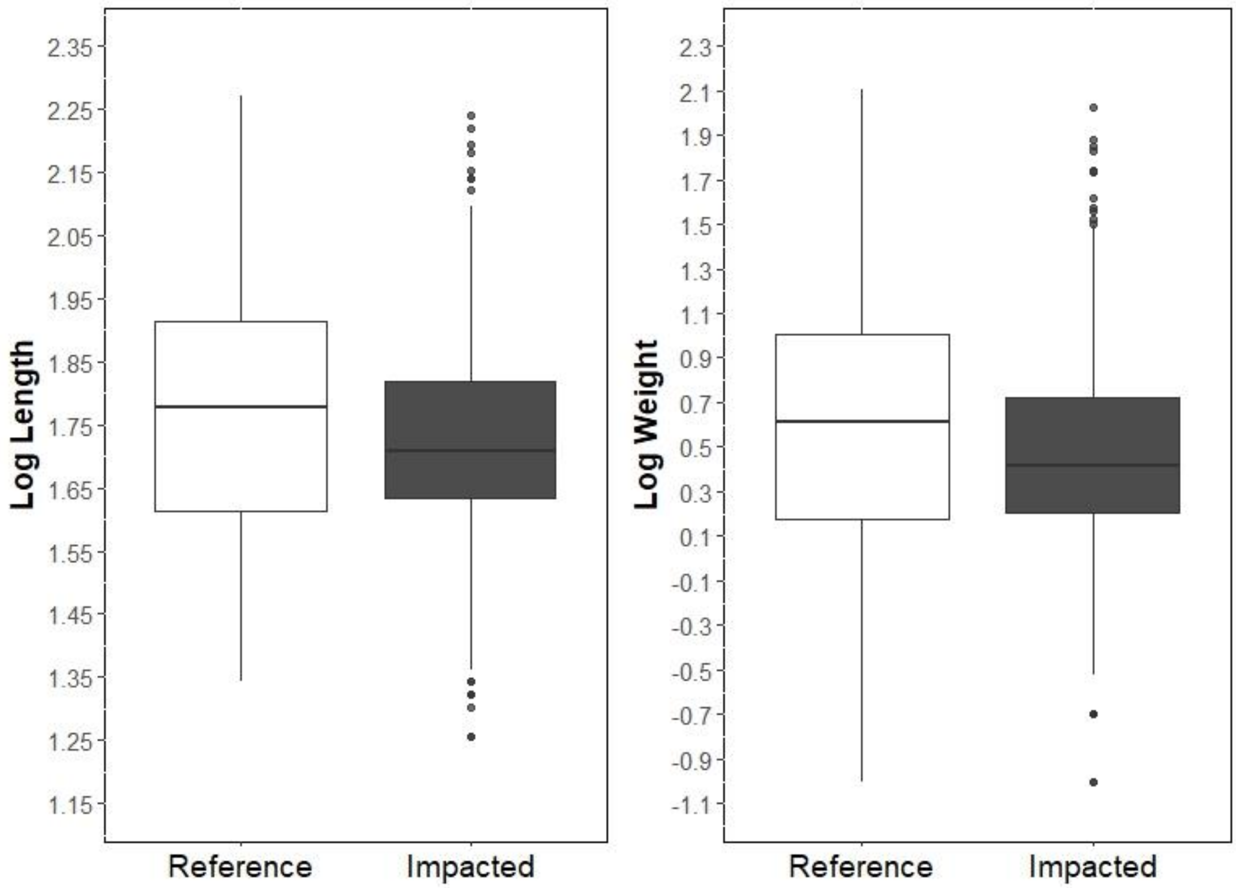

Table 5:

Comparison of watershed land uses between treatment types. Unconventional Oil and Gas was calculated as wells per upstream area. Other land uses were calculated as a percent of total upstream area. An asterisk (*) denotes statistically significant parameters.

Watershed Land Uses	Mean ± SD (%)		P-Value
	Reference	Impacted	
Agriculture (%)	8.60 ± 5.90	7.42 ± 2.65	0.583
Development (%)	0.65 ± 0.79	0.63 ± 0.46	0.955
Forest Cover (%)	88.5 ± 7.04	90.1 ± 3.03	0.530
Open Water (%)	0.058 ± 0.057	0.037 ± 0.032	0.356
Roads (%)	1.80 ± 0.49	1.66 ± 0.74	0.650
Herbaceous Wetlands (%)	0.030 ± 0.060	0.032 ± 0.045	0.907
Woody Wetlands (%)	0	0.012 ± 0.035	0.351
UOG (Wells/km^2)	0	1.07 ± 0.65	0.003*
Pre SMCRA Grass (%)	0.0032 ± 0.010	0	0.343
Pre SMCRA Barren (%)	0	0	NA
Pre SMCRA Forest (%)	0.0010 ± 0.0033	0	0.343
Post SMCRA Grass (%)	0.075 ± 0.185	0	0.233
Post SMCRA Barren (%)	0.014 ± 0.042	0	0.322
Post SMCRA Forest (%)	0.172 ± 0.340	0.0010 ± 0.0019	0.147

Table 6:

Comparison of water quality and habitat parameters between treatment types. An asterisk (*) denotes statistically significant parameters. All metrics are mg/L except for those specified. RVHA is a score ranging between 0 and 200.

Parameters	Mean ± SD		P-Value
	Reference	Impacted	
Aluminum	0.021 ± 0.027	0.035 ± 0.045	0.450
Calcium	17.82 ± 7.27	29.36 ± 6.73	0.003*
Iron	0.098 ± 0.086	0.124 ± 0.074	0.498
Magnesium	3.21 ± 1.67	5.02 ± 1.31	0.020*
Manganese	0.026 ± 0.037	0.043 ± 0.061	0.488
Potassium	1.77 ± 0.88	2.05 ± 0.56	0.437
Sodium	6.55 ± 6.98	18.57 ± 22.1	0.176
Strontium	0.071 ± 0.05	0.141 ± 0.05	0.009*
Zinc	0.0029 ± 0.005	0.0039 ± 0.005	0.681
Barium	0.056 ± 0.021	0.064 ± 0.017	0.344
Bromide	0.006 ± 0.19	0.041 ± 0.047	0.078
Chloride	4.63 ± 3.61	7.76 ± 4.13	0.113
Sulfate	17.81 ± 20.7	15.25 ± 8.26	0.727
Nitrate	0.382 ± 0.90	0.075±0.08	0.312
Phosphorous	7.85 ± 0.41	7.96 ± 0.45	0.717
pH	7.85 ± 0.41	7.99 ± 0.45	0.513
Conductivity (µS/cm)	99.3 ± 64.5	214.3 ± 72.3	0.003*
TDS	0.065 ± 0.042	0.139 ± 0.047	0.024*
Dissolved Oxygen	11.05 ± 1.85	10.36 ± 2.61	0.540
Alkalinity	51.4 ± 36.6	102.7 ± 42.8	0.018*
RVHA	136.3 ± 25.96	126.3 ± 19.37	0.377

Table 7:

All 50 species that occurred within samples were classified based on traits. Spawning habits are rock-gravel spawners (RG), gravel-sand spawners (GS), non-guarding lithophils (NGL), cavity spawners (CAV), Lithophilic spawners in sand or rock (LSR) and clean gravel spawners (CGS). Trophic levels are invertivore-piscivore (IP), invertivore (IN), macro-omnivore (MO), and omnivore-herbivore (OH). Tolerance levels are intolerant (I), moderately tolerant (M), and tolerant (T). Other classifications included benthic species (B) and game species (G).

Common	Scientific	Code	Family	Spawn	Trophic	Tol.	Other
Yellow Bullhead	*Ameiurus natalis*	AMNA	Ictaluridae		MO, OH	T	B, G
Brown Bullhead	*Ameiurus nebulosus*	AMNE	Ictaluridae		MO, OH	T	B, G
Rock bass	*Ambloplites rupestris*	AMRU	Centrachidae		IP	M	B, G
Central Stoneroller	*Campostoma anomalum*	CAAN	Cyprinidae	RG, CGS	MO, OH	T	B
River Carpsucker	*Carpiodes carpio*	CACA	Catostomidae		MO, OH	M	B
White Sucker	*Catostomus commersoni*	CACO	Catostomidae	GS, NGL	MO, OH	T	B
Southern Redbelly Dace	*Chrosomus erythrogaster*	PHER	Cyprinidae		MO, OH	M	
Redside Dace	*Clinostomus elongatus*	CLEL	Cyprinidae	RG	IN, IP	I	
Mottled Sculpin	*Cottus bairdii*	COBA	Cottidae	CAV	IN, IP	M	B
Spotfin Shiner	*Cyprinella spiloptera*	CYSP	Cyprinidae	CAV	IN, IP	T	
Muskellunge	*Esox masquinongy*	ESMA	Esocidae		IP	I	G

Greenside Darter	*Etheostoma blennioides*	ETBL	Percidae	RG, NGL	IN, IP	I	B
Rainbow Darter	*Etheostoma caeruleum*	ETCA	Percidae	RG, CGS	IN, IP	M	B
Fantail Darter	*Etheostoma flabellare*	ETFL	Percidae	RG, CAV	IN, IP	M	B
Johnny Darter	*Etheostoma nigrum*	ETNI	Percidae	RG, CAV	IN, IP	M	B
Banded Darter	*Etheostoma zonale*	ETZO	Percidae	NGL	IN, IP	I	B
Northern Hogsucker	*Hypentelium nigricans*	HYNI	Catostomidae	RG, CGS, NGL	IN, IP	M	B
Channel Catfish	*Ictalurus punctatus*	ICPU	Ictaluridae		MO, OH	T	B, G
Least Brook Lamprey	*Lampetra aepyptera*	LAAE	Petromyzontidae	GS, CGS	MO, OH	I	B
Redbreast Sunfish	*Lepomis auritus*	LEAU	Centrachidae	GS	IP	M	G
Green Sunfish	*Lepomis cyanellus*	LECY	Centrachidae		IP	T	G
Pumpkinseed	*Lepomis gibbosus*	LEGI	Centrachidae		IN, IP	M	
Bluegill	*Lepomis macrochirus*	LEMA	Centrachidae		IN, IP	T	G
Longear Sunfish	*Lepomis megalotis*	LEME	Centrachidae		IN, IP	M	G
Striped Shiner	*Luxilus chrysocephalus*	LUCH	Cyprinidae	RG	OH	T	
Redfin Shiner	*Lythrurus umbratilis*	LYUM	Cyprinidae		IN, IP	T	
Smallmouth Bass	*Micropterus dolomieu*	MIDO	Centrachidae		IP	M	G
Spotted Sucker	*Minytrema melanops*	MIME	Catostomidae	RG, NGL	OH	M	B
Spotted Bass	*Micropterus punctulatus*	MIPU	Centrachidae		IP	M	G

Largemouth Bass	*Micropterus salmoides*	MISA	Centrachidae		IP	M	G
Black Redhorse	*Moxostoma duquesni*	MODU	Catostomidae	RG, NGL	IN, IP	I	B
Golden Redhorse	*Moxostoma erythrurum*	MOER	Catostomidae	GS, CGS, NGL	IN, IP	I	B
Emerald Shiner	*Notropis atherinoides*	NOAT	Cyprinidae		MO, OH	M	
Silverjaw Minnow	*Notropis buccatus*	NOBU	Cyprinidae	GS, NGL	IN, IP	T	
Mountain Madtom	*Noturus eleutherus*	NOEL	Ictaluridae	CAV	IN, IP	I	B
Stonecat	*Noturus flavus*	NOFU	Ictaluridae	CAV	IN, IP	M	B
River Chub	*Nocomis micropogon*	NOMI	Cyprinidae	RG, CGS	IN, IP	M	
Brindled Madtom	*Naturus miurus*	NOMU	Ictaluridae	CAV	IN, IP	M	B
Silver Shiner	*Notropis photogenis*	NOPH	Cyprinidae		IN, IP	T	
Rosyface Shiner	*Notropis rubellus*	NORU	Cyprinidae	RG, NGL	IN, IP	I	
Sand Shiner	*Notropis stramineus*	NOST	Cyprinidae	LSR	OH	M	
Mimic Shiner	*Notropis volucellus*	NOVO	Cyprinidae		IN, IP	M	
Logperch	*Percina caprodes*	PECA	Percidae	GS, CGS	IN, IP	M	B
Blackside Darter	*Percina maculate*	PEMC	Percidae	GS, CGS	IN, IP	M	B
Trout-perch	*Percopsis omiscomaycus*	PEOM	Percopsidea		IN, IP	M	B
Bluntnose Minnow	*Pimephales notatus*	PINO	Cyprinidae	CAV	MO, OH	T	
Eastern Blacknose Dace	*Rhinichthys atratulus*	RHAT	Cyprinidae	GS, CGS	MO, OH	T	B

Longnose Dace	*Rhinichthys cataractae*	RHCA	Cyprinidae	CGS	IN, IP	M	B
Brook Trout	*Salvelinus fontinalis*	SAFO	Salmonidae	CGS	IP	I	G
Creek Chub	*Semotilus atromaculatus*	SEAT	Cyprinidae	GS	IP	T	

Table 8:

Results of a generalized linear effects model comparing species within the Invertivore-Piscivore and Benthic metrics. Raw counts were used to determine mean and standard deviation for the Invertivore-Piscivore metric. Relative abundances were used to determine mean and standard deviation for the benthic metric.

Invertivore–Piscivore Metric			
Species	Period	Mean (±SD)	*p value*
Redside Dace	Pre	7.5±21.2	<0.001
	Post	0.375±1.06	
Rainbow Darter	Pre	22.75±27.07	0.026
	Post	17.75±22.96	
Johnny Darter	Pre	22.5±20.42	<0.001
	Post	7.875±9.88	
Longear Sunfish	Pre	5.125±12.9	<0.001
	Post	0.625±1.41	
Golden Redhorse	Pre	5.75±13.9	<0.001
	Post	0.125±0.35	
Mimic Shiner	Pre	10.875±22.28	<0.001
	Post	2.5±5.24	
Benthic Metric			
Central Stoneroller	Pre	0.345±0.264	<0.001
	Post	0.40±0.234	
Mottled Sculpin	Pre	0.011±0.038	0.020
	Post	0.021±0.032	
Northern Hogsucker	Pre	0.054±0.060	0.007
	Post	0.062±0.060	
Logperch	Pre	0.0008±0.002	<0.001
	Post	0.0087±0.022	
Eastern Blacknose Dace	Pre	0.072±0.071	<0.001
	Post	0.123±0.172	

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CHAPTER 3: HEMATOCRIT AS A PREDICTOR OF *SEMOTILUS ATROMACULATUS* STRESS DERIVED FROM UNCONVENTIONAL OIL AND GAS DISTURBANCE

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ABSTRACT

Unconventional Oil and Gas (UOG) production has been steadily expanding throughout the mid-Atlantic since early 2008. Increased sedimentation, degraded water chemistry and an overall decrease in habitat quality due to UOG is anticipated to negatively impact aquatic inhabitants, a common observation in other stressed landscapes (i.e., mining, agriculture, development). We tested for differences in *Semotilus atromaculatus* hematological responses within eleven UOG impacted sites and eight reference sites with a linear mixed effect model. Treatment type was found to be a significant predictor of hematocrit levels in resident *S. atromaculatus* within UOG impacted streams ($p = 0.029$). With the findings outlined in this manuscript, we predict that the steady expansion of horizontal wells could lead to an overall degradation of resident fish populations as they become increasingly stressed.

INTRODUCTION

Headwater streams are an essential element of the holistic ecosystem as they provide resources through functions that cannot occur elsewhere. Headwater systems are the nexus between land and higher order streams. They transform allochthonous nutrients into fine particulate organic matter and dissolved organic matter that can be processed by downstream residents (Vannote et al. 1980; Cuffney et al. 2000). They provide habitat, thermal refugia and spawning grounds to higher order residents (Curry et al. 1997; Power et al. 1999; Petty et al. 2005; Meyer et al. 2007). These headwater streams are vital to the preservation of fish taxa and they are in jeopardy as they are highly susceptible to anthropogenic disturbances (Trexler et al. 2014).

Unconventional Oil and Gas (UOG), also known as hydraulic fracturing or horizontal drilling, has been rapidly increasing throughout North America since the early 2000s (Rahm and Riha 2012; Brown et al. 2013). UOG, like other land use stressors (i.e., mining, agriculture, development), has been shown to stress aquatic systems. UOG increases sediment load through the construction of impermeable surfaces (i.e., roadways, cement well pads) and the deforestation of pipeline corridors (Williams et al. 2008; Adams et al. 2011), leaches chemicals associated with the fracturing process into both surface and ground waters (Rahm and Riha 2012) and alters the natural hydrologic regimes by means of rapid withdrawal or dispersal of water used in the fracturing process (Entrekin et al. 2011; Weltman-Fahs and Taylor 2013a).

With a rapid spike in a field that is so minimally explored, a major concern is how UOG impacts receiving freshwater residents. Previous lab studies have analyzed alterations in various physiological functions of fishes after the introduction of UOG produced brines (Papoulias and Velasco 2013; Blewett et al. 2017). These studies have concluded that fishes exposed to UOG produced brines have damaged gill morphology and decreased organ function. Although these studies are integral, they do not take into account the flashy nature of produced water spills, nor do they account for the natural fluctuation in flows that alter chemical concentrations. The only way to ensure that these parameters are accounted for is to study streams *in situ*.

In this study, we sampled wild populations of *Semotilus atromaculatus* with regard to their exposure to upstream UOG. We used hematocrit levels to compare fishes residing in impacted conditions to those residing in reference conditions. Hematocrit is the volume ratio of packed red blood cells in a given blood sample and can be used as a stress indicator for fishes (Sopinka et al. 2016). Studies have shown that hematocrit levels have the potential to decrease in fish exposed to heavy metals as erythrocyte production is inhibited (Wepener et al. 1992; Buthelezi et al. 2000; Nussey et al. 2006). The same is true for fish exposed to synthetic chemicals commonly found in anthropogenically stressed landscapes (Ghaffar et al. 2016, 2018; Qureshi et al. 2016). For the first time, this study showed a decrease in the hematocrit levels of fishes residing in systems impacted by UOG.

METHODS

Site Selection:

Sites were selected with regard to their exposure to upstream UOG, watershed land use attributes (i.e., mining, development, agriculture), watershed size and sampling date. Using version 10.3 ArcGIS software (Environmental Systems Research Institute [ESRI] 2005), UOG well pad polygons were overlaid on an accumulation land use file. Eight reference sites were selected by the following criteria: there were no upstream mining impacts, sites had an upstream agricultural disturbance less than 17%, an upstream developmental disturbance less than 2.5%, and no UOG well pads within their watershed. Eleven impacted sites were selected if they had UOG well pads with one or more active wells within their watersheds, had no upstream mining impacts, an upstream agricultural disturbance less than 17%, an upstream developmental disturbance less than 2.5% and had a sampling date prior to the spud date of the UOG operation. Land cover values were accumulated from the 2011 edition of the National Land Cover Database (U.S. Geological Survey, 2014).

Fish and Blood Sampling:

Approximately thirty *S. atromaculatus* were sampled at each site using singlepass backpack electrofishing techniques outlined in the WVDEP Fish Collection Protocols (WVDEP, 2011). Smith-Root Model 24LR backpack electrofishers (Vancouver, WA, USA) were used alongside ¼" mesh nets. Table 10 of the WVDEP Fish and Collection Protocol was used to determine how many electrofishers and netters to use (WVDEP, 2011). Debilitated fish were immediately euthanized and a single blood sample from the caudal artery was collected in a 70μL heparinized glass microhematocrit capillary tube and sealed with Sigillum Wax (LW Scientific, Lawrenceville, Georgia). Upon collection of the blood sample, the specimen and corresponding blood sample were placed in a Whirl-Pak bag (Nasco, Fork Atkinson, Wisconsin) and placed on ice. Once in the lab, the blood samples were centrifuged using a CritSpin Microhematocrit Centrifuge Model M961 (IRIS International, Chatsworth, Los Angeles, California). The centrifuged samples were read using the Digital Reader (IRIS International, Chatsworth, Los Angeles, CA, USA). Samples too small to read in the Digital Reader were read with a microscope.

Physical and Chemical Parameters:

To assess habitat quality, we performed U.S. Environmental Protection Agency (EPA) rapid visual habitat assessments (RVHA) at each site (Barbour et al. 1999). Using RVHA, various physiological parameters were assessed to give the site a score (maximum possible RVHA = 200).

A YSI 650 equipped with a 600XL sonde was used to measure dissolved O_2, pH, temperature, total dissolved solids, and specific conductivity (Yellow Springs Instruments, Yellow Springs, OH, USA). A single filtered sample was obtained using a Nalgene ® filtration device with a 0.45 μm mixed cellulose-ester membrane filter. This sample was used to measure Al, Ca, Fe, Mg, Mn, K, Na, Sr, Zn (EPA method 200.7) as well as Ba, Cd, Cr, Ni and Se. (EPA method 200.8). Three unfiltered samples were obtained and used to measure Br^-, Cl^-, SO_4^{2-} (EPA method 300.0), NO_2^-, NO_3^- (EPA method SM4110B-2000), total P (EPA method SM4500-P BE-1999), total dissolved solids (EPA method SM2540 C-1997), total and bicarbonate alkalinity (EPA method SM2320 B-1997). The samples were stored at 4℃ until they were analyzed at Research Environmental and Industrial Consultants Inc. (Beaver, WV, USA).

Statistical Analysis:

The goal of this study was to determine if hematocrit levels of *Semotilus atromaculatus* inhabiting reference streams were significantly different than those of individuals inhabiting impacted streams. Hematocrit results were tested for normality using a Q-Q plot. We used a linear mixed effects model, with sampling site as a random effect, to analyze the raw hematocrit data. A two-sample Kolmogorov-Smirnov test was used to compare the cumulative frequency distributions of hematocrit for both reference and impacted treatments. A two-sample t-test was used to compare land use attributes between reference and impacted sites. Land use attributes were selected based on their likelihood to impact aquatic habitats. All statistical analyses were performed in R 3.4.1 (R Core Team). The significance level (α) used for this study was 0.05. Two-sample t-tests were used to compare water quality parameters and RVHA between treatment conditions (Table 2).

RESULTS

Hematocrit:

Blood samples were taken from 200 specimens within eight reference sites and 269 specimens within eleven UOG impacted sites. Hematocrit levels from these blood samples were recorded. A linear mixed effects model, with sampling site as a random effect, revealed that hematocrit levels are significantly lower in *S. atromaculatus* residing within UOG impacted systems ($p = 0.029$). Compared to reference conditions (51.95 ± 12.5), hematocrit levels decreased significantly in impacted sites (47.20 ± 14.3). A cumulative frequency plot of the hematocrit results supports this finding (Figure 1). A two-sample Kolmogorov-Smirnov test was used to compare frequency distributions within both treatment conditions ($p < 0.001$ [Figure 1]). Comparison of upstream well density to average hematocrit levels revealed a slightly negative correlation, though insignificant ($r = -0.397$, $p = 0.092$ [Figure 2]).

Water Chemistry:

Analysis of land uses as a percent of watershed area determined that only UOG was significantly different ($p < 0.001$) between impacted and reference sites. Upstream agriculture, development, forest cover, open water, roads, wetlands and mine lands were not significantly different between treatment types ($p > 0.05$ for all parameters [Table 1]). Water chemistry values differed significantly in calcium ($p = 0.005$), strontium ($p = 0.040$) and TDS ($p = 0.049$) between treatment types. All three parameters were higher in UOG impacted systems (Table 2).

DISCUSSION

As we advance and progress as a civilization, it is critical to ensure that our exploitation of oil and gas reserves does not result in diminished ecosystems. Our technological advances allow for the extraction of hydrocarbons that were previously inaccessible. Consequently, the environmental side effects of these extraction methods are understudied. In the present study, we explored the negative environmental impacts of UOG (i.e., sedimentation, chemical leaching, alterations of the hydrological regime [Weltman-Fahs and Taylor 2013]) and studied their hematological effect on resident *Semotilus atromaculatus*. Blood samples were taken from individuals residing in aquatic systems downstream of UOG operations as well as reference conditions. These blood samples were used to determine hematocrit levels, a common stress level indicator (Sopinka et al. 2016). Other studies have performed lab experiments that subject specimen to UOG produced waters and surrogates (Papoulias and Velasco 2013; Blewett et al. 2017; He et al. 2017). To our knowledge this is the first study that analyzes hematological responses to UOG disturbance *in situ*.

Land use stressors (i.e., agriculture, mining, development, UOG) have been known to degrade receiving aquatic systems (Cuffney et al. 2000; Fitzpatrick et al. 2004; Merriam et al. 2011; Weltman-Fahs and Taylor 2013a). Because of these underlying effects, our site selection methods controlled for UOG as the only significantly different land use attribute between treatment types (p, 0.001 [Table 2]). Other land uses of concern for this study were agriculture, development, forest cover, open water, roads, wetland, and both pre- and post- SMCRA mine lands.

When exploring the water chemistry data, we did observe significantly higher strontium between our sites. This could indicate a long-term impact of UOG development in the watershed as high strontium levels have been found in produced waters (Cozzarelli et al. 2017; Geeza et al. 2018). Although strontium appeared significant within the UOG impacted systems, other water parameters (i.e., magnesium, conductivity, bromide and barium) which have been found to be positively correlated with UOG activity did not stand out (Cozzarelli et al. 2017; Austin et al. 2018; Keller et al. 2018). The flashy nature of produced water spills and leaching suggests that the chemicals are readily diluted and transported downstream. From the water quality data we collected, we can argue that single sample water chemistry analysis at baseflow is not an effective way to measure UOG's impact on stream chemistry.

Compared to control sites, hematocrit levels significantly decreased in UOG impacted specimens ($p < 0.001$ [Table 1]). Hematocrit decrease after exposure to pollutants agrees with the finding of other aquatic studies. Wepener et al. (1992) determined that the presence of chromium in aquatic systems has the potential to decrease hematocrit levels (Wepener et al. 1992). A similar response was seen with copper exposure (Nussey et al. 2006), manganese exposure (Barnhorn 1996), arsenic exposure (Ghaffar et al. 2016) and exposure to pesticides (Qureshi et al. 2016; Ghaffar et al. 2018). In these studies, as well as the present study, it is assumed that exposure to chemical pollutants (i.e. strontium and calcium) and degraded water quality (i.e., TDS) result in either an inhibition of erythrocyte formation or hemolysis. Both physiological responses contribute to reduced red blood cell counts and decreased hematocrit levels.

In Figure 2, we compared the average hematocrit levels to the density of UOG wells within the watershed (wells/km^2) at the site level. A negative correlation ($r = -0.397$) revealed that as upstream well density increased the health of resident *Semotilus atromaculatus* decreased. This finding could aid the Department of Environmental Protection (DEP) in making better informed decisions regarding the permitting of UOG wells. With future studies to support our finding, there is potential to create permitting restrictions that take into account the density of UOG wells within a given watershed.

The methods outlined in this study provide a fast and cost-effective way to analyze the effects of land use stressors on aquatic systems. Previous study methods require either a large team of technicians (i.e., fish community analyses), an intricate knowledge of invertebrate species (i.e., aquatic macroinvertebrate studies), access to laboratory space or access to expensive equipment (i.e., water chemistry analyses). The present study requires the following: a small team of technicians, the ability to identify the target species and access to inexpensive microhematocrit sampling equipment.

CONCLUSION

In the Ohio and Monongahela River Basins of northern West Virginia, an area known for its anthropogenically stressed landscape, we discovered that UOG is negatively impacting the health of resident *Semotilus atromaculatus*. When compared to reference conditions, fish residing in UOG impacted systems had significantly lower hematocrit levels. Previous studies have attributed this decrease in hematocrit levels to hemolysis or hindered erythrocyte production. Consequently, as UOG is the only significantly different land use between treatment types, we are confident in suggesting that an increase in sedimentation and chemical leaching associated with UOG operations is responsible for the decreased health of the inhabitants.

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FIGURES AND TABLES

Figure 1:

A cumulative frequency plot of hematocrit data. The dark gray bars represent the frequency of hematocrit values for the impacted sites while the light gray bars represent the control sites. The solid line represents to the percentage frequency for the impacted sites while the dashed line represents the reference sites. p = significance factor, N_R = number of reference samples, N_I = number of impacted samples

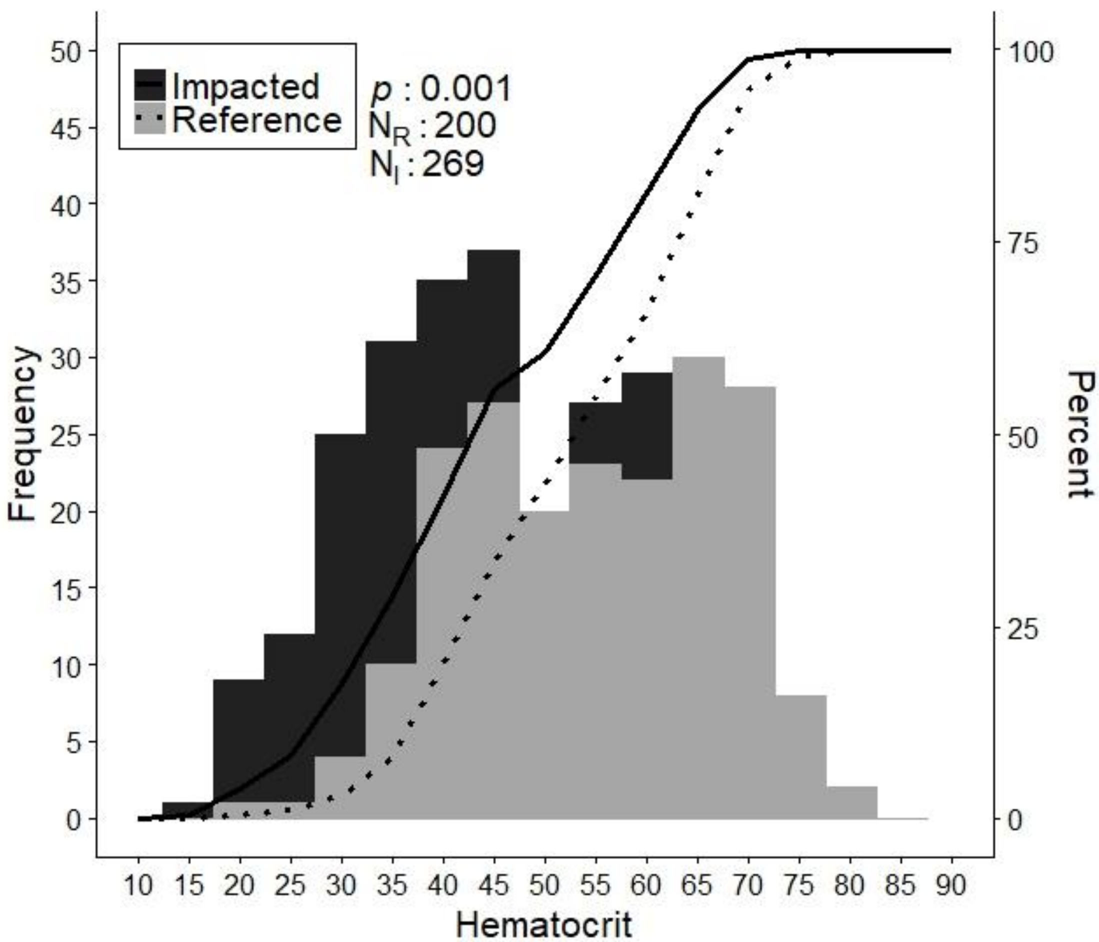

Figure 2:

The relationship between upstream UOG density (wells/km^2) and average hematocrit levels. The points represent individual sites. The dotted line represents the best fit line.

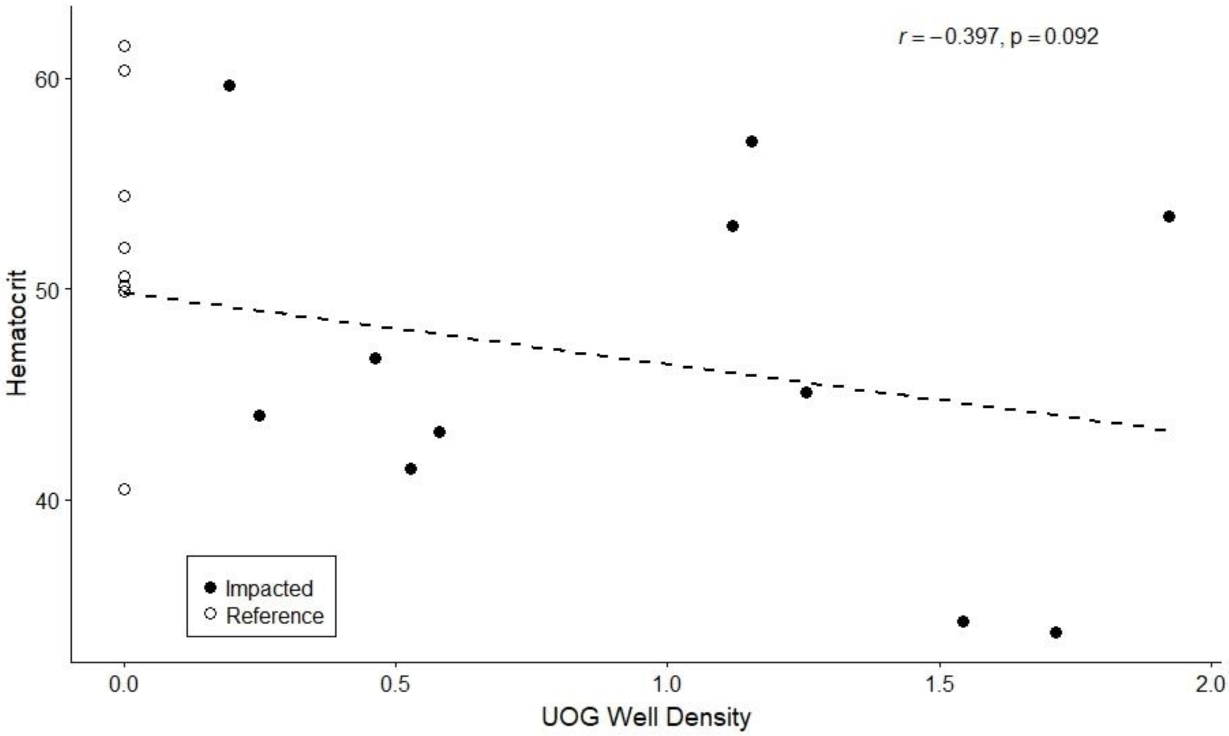

Table 1:

Comparison of watershed land uses between treatment types. Unconventional Oil and Gas was calculated as wells per upstream area. Other land uses were calculated as a percent of total upstream area. An asterisk (*) denotes statistically significant parameters.

Watershed Land Uses	Average Area ± SD (km^2)		P-Value
	Reference	Impacted	
Agriculture (%)	7.88 ± 5.04	8.72 ± 3.17	0.685
Development (%)	0.74 ± 0.86	0.57 ± 0.39	0.611
Forest Cover (%)	89.1 ± 6.55	88.8 ± 3.56	0.927
Open Water (%)	0.050 ± 0.059	0.060 ± 0.080	0.758
Roads (%)	1.86 ± 0.51	1.71 ± 0.66	0.605
Herbaceous Wetlands (%)	0.016 ± 0.040	0.028 ± 0.046	0.543
Woody Wetlands (%)	0	0.0008 ± 0.002	0.249
UOG (Wells/km^2)	0	1.03 ± 0.66	< 0.001*
Pre SMCRA Grass (%)	0	0	NA
Pre SMCRA Barren (%)	0	0	NA
Pre SMCRA Forest (%)	0	0	NA
Post SMCRA Grass (%)	0.094 ± 0.21	0	0.238
Post SMCRA Barren (%)	0.018 ± 0.047	0	0.329
Post SMCRA Forest (%)	0.21 ± 0.37	0.0003 ± 0.0009	0.148

Figure 3:

11 impacted sites (★) and 8 reference sites (●) were sampled within both the Monongahela River drainage basin (light grey) and the Ohio River drainage basin (dark grey). Dashed polygon represents the Marcellus shale formation. Map created in ArcGIS version 10.3 (ESRI, 2005).

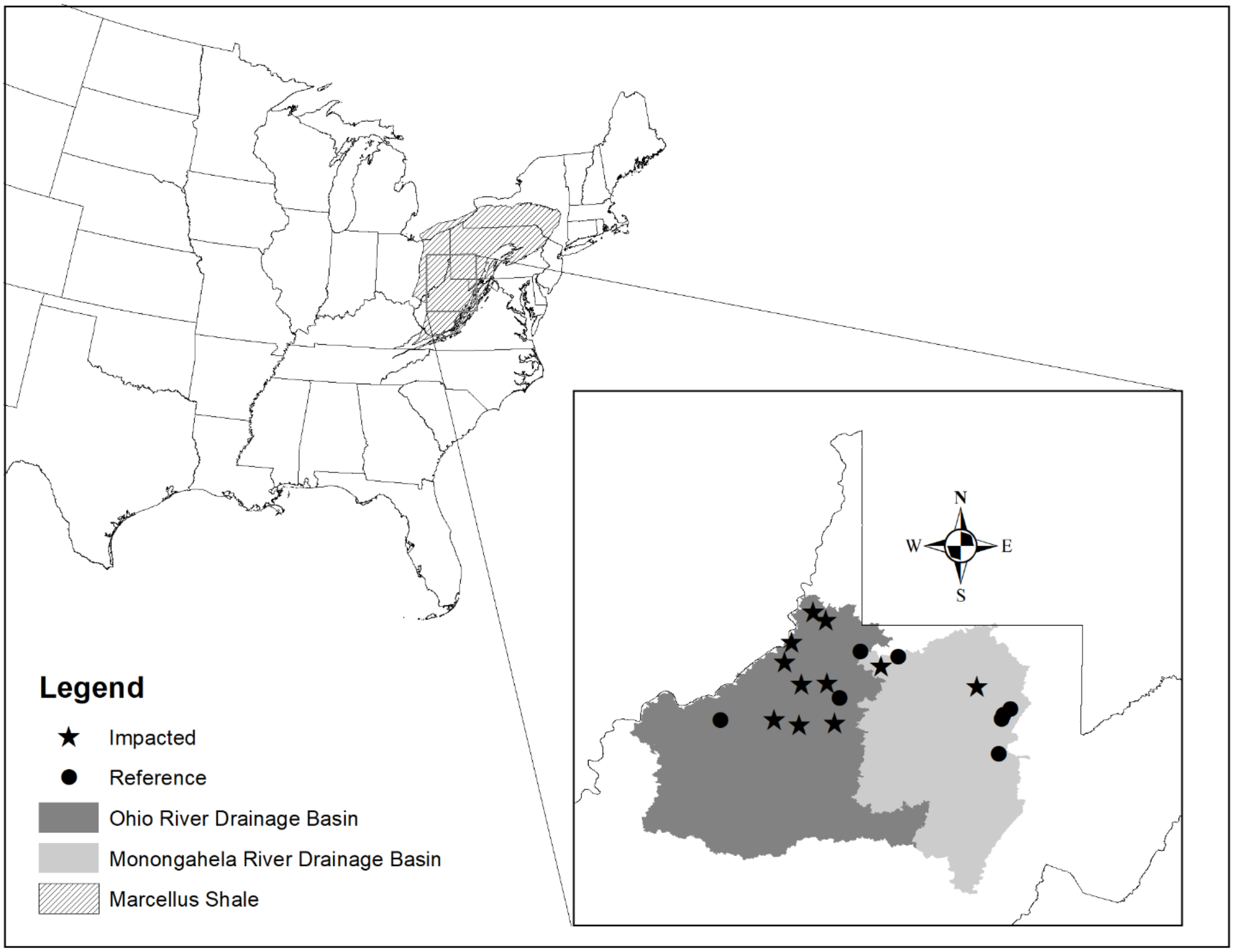

Table 2:

Comparison of water quality and habitat parameters between treatment types. An asterisk (*) denotes statistically significant parameters. All metrics are mg/L except for those specified. RVHA is a score ranging between 0 and 200.

Parameters	Mean ± SD		P-Value
	Reference	Impacted	
Aluminum	0.024 ± 0.030	0.045 ± 0.050	0.300
Calcium	19.55 ± 6.73	30.44 ± 7.11	0.005*
Iron	0.109 ± 0.092	0.120 ± 0.073	0.804
Magnesium	3.56 ± 1.66	5.06 ± 1.40	0.065
Manganese	0.030 ± 0.040	0.043 ± 0.059	0.616
Potassium	1.81 ± 0.88	1.92 ± 0.42	0.767
Sodium	7.44 ± 7.56	16.16 ± 20.10	0.253
Strontium	0.080 ± 0.047	0.148 ± 0.074	0.040*
Zinc	0.003 ± 0.005	0.006 ± 0.013	0.509
Barium	0.058 ± 0.022	0.062 ± 0.018	0.747
Bromide	0.008 ± 0.021	0.131 ± 0.299	0.251
Chloride	4.85 ± 3.82	15.53 ± 24.98	0.240
Sulfate	20.49 ± 22.53	13.15 ± 7.30	0.403
Nitrate	0.473 ± 1.00	0.078 ± 0.077	0.302
Phosphorous	0.01 ± 0.013	0.007 ± 0.007	0.535
pH	7.97 ± 1.12	8.03 ± 0.324	0.881
Conductivity (μS/cm)	157.88 ± 83.63	223.78 ± 107.12	0.176
TDS	99.5 ± 39.89	162.1 ± 74.45	0.049*
Dissolved Oxygen	7.22 ± 1.90	8.28 ± 2.39	0.356
Alkalinity	56.94 ± 38.76	90.59 ± 33.31	0.077
RVHA	127.38 ± 18.85	129.14 ± 21.07	0.868

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