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DEMOGRAPHIC, SPATIAL, AND EPIGENETIC RESPONSE OF THE LOUISIANA WATERTHRUSH (PARKESIA MOTACILLA) TO SHALE GAS DEVELOPMENT

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DEMOGRAPHIC, SPATIAL, AND EPIGENETIC RESPONSE OF THE LOUISIANA WATERTHRUSH (*PARKESIA MOTACILLA*) TO SHALE GAS DEVELOPMENT

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ABSTRACT

DEMOGRAPHIC, SPATIAL, AND EPIGENETIC RESPONSE OF THE LOUISIANA WATERTHRUSH (*PARKESIA MOTACILLA*) TO SHALE GAS DEVELOPMENT

Mack W. Frantz

My study centered on a bioindicator songbird, the Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, an organism that co-occurs in both forested and aquatic habitat across the aquatic-terrestrial interface. This enabled the opportunity to quantify demographic, spatial, and epigenetic (i.e., DNA methylation) responses in a highly forested watershed of the Central Appalachians, the areas that have undergone the most rapid transformations over the last decade from unconventional shale gas development and activities. I organized my dissertation into 4 parts (Part 1: Introduction, Part 2: Louisiana Waterthrush Demography, Part 3: Spatial Assessment of Louisiana Waterthrush Foraging, Part 4: Louisiana Waterthrush Molecular Ecology) including 6 chapters that indicate multiple biotic and abiotic factors interacted with or were altered by shale gas development resulting in atypical, negative disturbances that drove a steep decline in a waterthrush population in West Virginia.

Part 1 includes Chapter 1 and is an introduction to my dissertation. I introduce the reader to the rationale for my study, the focal species, research objectives, and the study area. I also mention some limitations to my study that can be considered in any future research endeavors.

Part 2 comprises Chapters 2–3 which are a comprehensive examination of demographic parameters over a six-year period (2009–2011, 2013–2015). In Chapter 2, I examined demographic response to shale gas development for nest abandonment, nest survival, nest productivity, a source-sink threshold, riparian habitat quality, and territory density and length. Nest productivity was lower in areas disturbed by shale gas where a source–sink threshold suggested these areas were more at risk of being sink habitat. Overall results suggest a decline in waterthrush site quality as shale gas development increased. In Chapter 3, I focused on first-year return rates (site fidelity), site fidelity factors, and apparent survival. I related natal fidelity and pairing rates to territory density, and also compared # of breeding attempts between return and non-returning females with and without territory shale gas disturbance. The study identified

potential conflicts between factors that influence adult survival and site fidelity that may affect long-term population persistence.

Part 3 includes Chapters 4–5 and focuses on utilizing and accounting for spatial properties intrinsic to stream ecosystems to make informed decisions regarding waterthrush foraging. Chapter 4 was a follow-up to a waterthrush aquatic prey study at our site in 2011 that suggested shale gas development negatively affected waterthrush demography from alterations in their aquatic prey at a watershed scale. During 2013–2014, I quantified waterthrush demographic response and nest survival in relation to potential changes in its aquatic prey due to shale gas development. I utilized spatial generalized linear mixed models that accounted for both spatial and non-spatial sources of variability. I found waterthrush aquatic prey was negatively affected by shale gas development at the nest and territory level, and that there may be a disturbance threshold at which waterthrush can no longer adapt and respond negatively to changes in its aquatic prey. In Chapter 5, I used spatial stream network models (SSNMs) to explore relationships among the waterthrush, stream channel and monitoring data, and the aquatic prey of the waterthrush. I compared the spatial models to traditional regression models to see which ones performed best. We sampled aquatic prey in waterthrush territories and collected wetted perimeter stream channel and water chemistry data along a 50m fixed point stream grid that mapped the foraging substrate or stream channel where waterthrush forage. By relating foraging observations and data collected to the stream grid, I was able to develop a foraging probability index that determined what conditions or variables create or affect ideal foraging locations. Spatial models outperformed traditional regression models and made a statistical difference in whether stream covariates of interest were considered relatable to waterthrush foraging. My study also indicated waterthrush forage in areas of higher biotic stream integrity.

Lastly, Part 4 includes Chapter 6 where I examined epigenetic modifications. These are alterations to genes without changing the gene sequence and can be thought of as an evolutionary "soft" inheritance of gene expression that can either be adaptive or maladaptive for the individual. DNA methylation is one type of epigenetic modification that may vary in response to environmental stressors. We examined the association between DNA methylation and demographic characteristics in addition to potential differential methylation from shale gas development. There was differential methylation for demographic characteristics as well as for adult males between shale gas undisturbed and disturbed areas. Barium (Ba) and strontium (Sr)

data were collected in 2013 feather samples where adult males had fewer methylated sites at higher concentrations of Ba and Sr, while nestlings displayed no correlation of methylation to Ba and Sr concentrations. Females displayed increased methylation with increased Ba and Sr, a trend reflected in adult female recaptures. Overall, results of our study suggest sex-specific influences of shale gas development on gene expression that may affect long-term population survival and fitness.

DEDICATION

To my father, William C. Frantz (1949–2013).

Semper fidelis.

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PART 1. INTRODUCTION TO THE STUDY

CHAPTER 1. INTRODUCTION

Rationale

The rapid development of hydraulic fracturing techniques in the last decade have allowed the expansion of unconventional drilling activity and development, hereafter shale gas development, that overcome the low permeability of shale rock formations for hydrocarbon extraction (Arthur et al. 2008). The United States has twenty shale plays currently being developed (DOE 2009, EIA 2011), the Marcellus-Utica shale basin as one of the largest natural gas plays with substantial growth in gas production (EIA 2017a, EIA 2017b). While these techniques are being used globally (Boyer et al. 2011), the United States leads natural gas production and is becoming a net exporter (EIA 2015). The central Appalachian region is experiencing the quickest growth in shale gas development (MCOR 2016) since the underlying Marcellus-Utica shale is the most expansive basin and has the most potentially recoverable gas (DOE 2009).

Three-quarters of the Appalachian region categorized at highest potential risk from energy development, primarily shale gas, is forested (Dunscomb et al. 2014). As of 2015, over 140,000 ha of land had already been developed, with deciduous forest one of the major habitat types affected with high ecosystem service costs (Moran et al. 2017). West Virginia is one of two states (the other state Pennsylvania) with the highest probability for development (21%; Dunscomb et al. 2014). From 2009–2012, the majority (73.3%) of forest removed in West Virginia was the result of shale gas development (Widmann 2013), with about 1 ha of forest disturbed in addition to land cover disturbances associated with well and pipeline placement (Zinkhan 2016). Overall land cover disturbance due to Marcellus shale gas may be 3.6 ha per well pad (Zinkhan 2016), higher than shale gas footprints elsewhere (Entrekin et al. 2011, Drohan et al. 2012). By 2015, West Virginia had 20% of >15,000 producing Marcellus wells (WVGES 2015) with 40,000 new wells projected by 2030 (DOE 2010).

Shale gas development tends to outpace the ability to create adequate management practices that avoid risks to aquatic and terrestrial wildlife communities and their habitat (Brittingham et al. 2014). Since West Virginia is highly forested (80% of land cover; Gillespie 2012), forest loss and fragmentation from shale gas development has the potential to threaten Appalachian biodiversity (Kiviat 2013). In particular, species with specialized habitat needs that overlap these areas will be the most vulnerable (Brittingham et al. 2014). Only recently have we learned how shale gas development may positively and negatively influence Appalachian songbird communities (Barton et al. 2016, Farwell et al. 2016), predator-aquatic prey response (Wood et al. 2016), and how shale gas development may be associated with heavy metal bioaccumulation in songbirds (Latta et al. 2015). Even with recent insights, there has not been a mechanistic assessment of the interplay between shale gas disturbance and songbird demographic response (Northrup and Wittemyer 2013). Baseline data, such as population demography, are needed in both terrestrial and aquatic habitats of the Appalachian shale gas basin to detect and understand changes as they begin to occur (Brittingham et al. 2014). If multiple studies start to collect long-term baseline data, perhaps we can move beyond broad generalities in wildlife response to shale gas development and learn if a disturbance threshold exists that if exceeded has population-level consequences (Northrup and Wittemyer 2013; Becker et al. 2015).

There is a tendency for core forest disturbance from shale gas development to occur near forested headwater streams (Drohan et al. 2012). Proximity of shale gas development to water resources is of particular concern due to the potential for sedimentation runoff, reduced streamflow, contamination of surface waters (Entrekin et al. 2011), and alteration to the base of trophic food webs (Grant et al. 2016). Shale gas wells in the Marcellus shale region are commonly within 100–300 m of stream channels, and even closer for headwater drainage areas (Entrekin et al. 2011). Headwater streams are critical sources of water, sediment, organic matter, and nutrients for the rest of the stream system (Gomi et al. 2002), and therefore vital for ecological integrity (Freeman et al. 2007). Headwater streams, despite predominance of drainage area and total stream length, are largely overlooked for protection or regulation despite their potential effect on downstream reaches and aquatic life (MacDonald & Coe 2007). Consequently, biological communities and organisms that use resources downstream of shale gas development are at increased risk (Latta et al. 2015) and research of the highest priority (Souther et al. 2014), as indirect land use alterations to forest and water resources that may damage ecosystems represent the largest and most critical knowledge gap in scientific research (Costa et al. 2017). Using an organism that co-occurs in both forested and aquatic habitat across the aquatic-terrestrial interface (Gregory et al. 1991) may enable the most ideal scenario to quantify demographic, spatial, and epigenetic (i.e., DNA methylation) responses to shale gas development.

Focal species

The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is a forested headwater stream specialist known for its ability to respond to changes in ecological conditions (Mattsson and Cooper 2006). The waterthrush is a species of conservation concern on the U.S. Fish and Wildlife Service National List (USFWS 2008) due to its specialized habitat. As biological indicators of biotic stream integrity (O'Connell et al. 2000, Mulvihill et al. 2008), waterthrush feed primarily on benthic macroinvertebrate aquatic prey (Mattsson et al., 2009) in well-developed riffle and pool areas (Prosser and Brooks 1998), forming linear territories along the stream reach (Mulvihill et al. 2008). Many of the forested stream ecosystems in which the waterthrush primarily breed have rapidly undergone unconventional shale gas development, particularly in the Marcellus-Utica shale region (Evans and Kiesecker 2014), where almost all of its core breeding range also overlaps this region (Sauer et al. 2014). Species with specialized terrestrial or aquatic habitat needs that overlap forested areas undergoing shale gas development may be the most vulnerable to disturbance (Brittingham et al. 2014). As such, the increased intensity of core forest disturbance where headwater streams (Drohan et al. 2012, Farwell et al. 2016) and this species co-occur make waterthrush an ideal organism to assess potential demographic, spatial, and epigenetic consequences from shale gas development.

Objectives

My overall objective was to determine the degree to which shale gas development on our study area may or may not affect waterthrush and its aquatic prey by demographical, spatial, and epigenetic mechanisms. Specific objectives and hypotheses are below.

1) Examine how shale gas development influenced demographic response of waterthrush during 2009–2011 and 2013–2015 by quantifying waterthrush nest survival, productivity, and nest abandonment, an overall source-sink threshold, riparian habitat quality, and territory density and length.

> 1a) I hypothesized that we would detect an inverse relationship between the amount of shale gas disturbance and demographic metrics despite the species' ability to compensate for resource loss (Mulvihill et al. 2008, Wood et al. 2016).

1b) I hypothesized that productivity would differ between areas disturbed and undisturbed by shale gas development if source-sink dynamics (Pulliam 1988) exist in our local population.

2) Examine how shale gas development influenced first-year return rates (site fidelity), site fidelity factors, and annual survival of waterthrush during 2009–2011 and 2013–2015.

2a) I hypothesized apparent survival would decrease in concert with decreasing site fidelity.

3) After accounting for spatial and non-spatial sources of variability, examine how shale gas development influenced demographic response of waterthrush to aquatic prey changes in 2013–2014 as a follow-up to a 2011 aquatic prey study.

3a) I hypothesized that clutch size, number of fledglings, and territory density would have a positive association with aquatic prey metrics.

3b) Annual territory length increased as territory densities decreased (Frantz et al. 2018, Chapter 2), so we expected smaller territories to be indicative of higher quality aquatic prey and stream quality (e.g., Mulvihill et al. 2008).

3c) Nest survival was minimally affected by aquatic prey in 2011 (Wood et al. 2016) but I hypothesized that any stream impairment effects on the aquatic prey would affect nest survival.

3d) In evaluation of riparian quality indices we use to gauge waterthrush habitat (i.e., US EPA Rapid Bioassessment Protocol, EPA; Habitat Suitability Index, HSI), I hypothesized that aquatic prey would be positively linked with riparian quality habitat scores where higher scores indicate areas of higher aquatic stream health.

3e) In the same manner as waterthrush demographic response to shale gas development, I hypothesized an inverse relationship between aquatic prey metrics and the amount of shale gas disturbance or potential runoff in a territory or at a nest.

4) Using spatial stream network models, explore relationships between observed areas of waterthrush foraging and a) water chemistry, b) a waterthrush foraging score based on stream channel data, c) shale gas land use based on reach contributing area (i.e. catchment area), and d) multi-metric indices of biotic stream integrity at the family and genus level.

5) a) Examine how shale gas development may influence DNA methylation variation, hereafter differential methylation, of waterthrush between shale gas undisturbed and disturbed areas at the

territory scale, b) determine if differential methylation of DNA fragments or loci, hereafter restriction sites, varied by sex and age, c) identify differentially methylated restriction sites that were potentially under selection, d) correlate methylated restriction sites to barium (Ba) and strontium (Sr) heavy metal concentrations bioaccumulated in waterthrush feathers, and e) evaluate the degree to which an individual's methylated state was subject to change across years in individuals that returned over more than one breeding season (i.e., recaptures).

5a) Females can have a higher body condition index (BCI) than males even when there are no differences in territory quality (Latta et al. 2016). As such, I hypothesized differential methylation between males and females, as well as for males to have a stronger response to shale gas development since females tolerate a wider range of territory quality (Latta et al. 2016).

5b) I hypothesized older adults to have fewer methylated restriction sites than younger adults because decreased methylation is correlated with age in birds (De Paoli-Iseppi et al. 2019). Similarly, I expected nestlings to be differentially methylated from adults.

5c) Presuming nestlings are less exposed to stress during the hatching to fledging stage than adults during the breeding season, I hypothesized a weak response to shale gas development between shale gas disturbed and undisturbed areas if differential methylation can correlate to a gradient of stress (Sun et al. 2018).

5d). Finally, I hypothesized that Ba and Sr heavy metal concentrations are inversely correlated to the number of methylated restriction sites since contaminants interfere with methyl transfer (Hala et al. 2014).

Study Area

We studied waterthrush along 58.1 km of 1st- and 2nd-order forested headwater stream tributaries (*n* = 14) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia (39.490216°N, -80.650713°W). The study area lies within the Permian Hills subdivision of the Western Allegheny Plateau Ecoregion, an area of deeply dissected topography and relatively continuous Appalachian Oak and Mixed-Mesophytic Forest (Woods et al. 1999) with elevations of 221–480 m. The study area overlays the Marcellus-Utica shale region, covers 6521 ha (Farwell et al. 2016), and occurs where waterthrush reach their highest densities within the central Appalachians (Sauer et al., 2014). In 2008, LWWMA was 95.3% forested with only 0.4% shale gas land cover, the first shale gas well development starting in 2007 (Farwell et al. 2016). Shale gas at our study area and within the surrounding region since then has rapidly increased (WVGES 2015). By the end of the study in 2015, LWWMA was 90.8% forested with 2.4% shale gas land cover, where 83.1% of shale gas development resulted in direct forest loss (Farwell et al. 2016).

Limitations of the study

Since the first shale gas development began at LWWMA in 2007 (Farwell et al. 2016), ideally we would have collected "baseline" waterthrush demographic data before any shale gas development began. However, because shale gas development was only 0.4% land cover in 2008, we started our study early in development and presume the high territory densities in the beginning of the study (2009–2010) were typical of the study area before development activities. West Virginia University researchers were scouting LWWMA ridgetops and valleys in 2008 as part of Cerulean Warbler (*Setophaga cerulea*), raptor ecology, and avian community research endeavors, and also were exploring streams with the idea of starting a pilot waterthrush study the following year. Anecdotal evidence from graduate students and field technicians I spoke with who were there in both 2008 and 2009 suggest very similar stream occupancy by waterthrush.

No waterthrush data were collected in 2012. The peak of shale gas development occurred in 2011, and by 2013 shale gas development had abated and was no longer site-wide but concentrated in certain areas. We saw some of the most apparent differences in demography from 2011 to 2013. Ideally if we had collected data in 2012, we would have seen a transitional stage between the high and low demographic values as the trends suggest. Having data for the year 2012 would have improved our survival estimates, as well as if adult females were banded sooner in the project to avoid having dissimilar encounter histories.

Finally, our study occurred on one wildlife management area (WMA) or technically only one watershed consisting of many subwatersheds, which may limit broad inferences beyond our study area boundaries. Regardless of the debatable semantics of what constitutes a genuine replicate vs. pseudoreplicate in ecology (Davies and Gray 2015), I am confident I have taken careful consideration of all aspects of our study design and data collected to provide credible science. Fortunately, since we had multiple undisturbed and disturbed streams at LWWMA at varying degrees of disturbance, I was able to account for and separate out any site-level (stream) effects from shale gas disturbance effects in my analyses, among any other sources of variability, when necessary.

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PART 2. LOUISIANA WATERTHRUSH ECOLOGY

CHAPTER 2. DEMOGRAPHIC RESPONSE OF LOUISIANA WATERTHRUSH, A STREAM OBLIGATE SONGBIRD OF CONSERVATION CONCERN, TO SHALE GAS DEVELOPMENT

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ABSTRACT

Shale gas development continues to outpace implementation of best management practices for wildlife affected by development. We examined how shale gas development influenced demographic response of Louisiana Waterthrush (*Parkesia motacilla*) during 2009–2011 and 2013–2015 in a predominately forested landscape in West Virginia. Forest cover across the study area decreased from 95.3% in 2008 to 90.8% in 2015 while area affected by shale gas development increased from 0.4% to 2.4%. We quantified nest survival, abandonment, and productivity, a source-sink threshold, riparian habitat quality, and territory density and length by monitoring 58.1 km of forested headwater streams (*n* = 14 streams). Across years, we saw annual variability in nest survival with a general declining trend over time. Of 11 a priori models to explain nest survival (*n* = 280 nests), four models that had temporal, habitat, and shale gas covariates were supported and two of these models accounted for most of the variation in daily survival rate. Once accounting for temporal effects (rainfall, nest age, time within season), shale gas development had negative effects on nest survival. There was a weak, non-significant association between nest abandonment or percent failed attempts and shale gas disturbance. Population-level nest productivity declined, and individual-level productivity was lower in areas disturbed by shale gas, and a source-sink threshold suggested these areas are more at risk for being sink habitat. Riparian habitat quality scores, as measured by an US Environmental Protection Agency index and a waterthrush Habitat Suitability Index, differed by year and were negatively related to the amount of each territory disturbed by shale gas development. Territory density was not related to the amount of shale gas disturbance on streams, but decreased over time as territory lengths increased. Overall, our results suggest a decline in waterthrush site quality as shale gas development increased, despite relatively small site-wide forest loss.

Keywords: unconventional shale gas, hydraulic fracturing, energy impacts, Marcellus-Utica, bioindicator species, headwater stream, *Parkesia motacilla*, waterthrush

INTRODUCTION

The United States has twenty shale formations containing unconventional natural gas deposits that are being developed using new horizontal drilling and hydraulic fracturing techniques (DOE 2009, EIA 2011). The central Appalachian region is experiencing the most rapid growth in unconventional drilling activity and development, hereafter shale gas development, (MCOR 2016) since the underlying Marcellus-Utica shale is the most expansive basin with the most potentially recoverable gas (DOE 2009). The state of West Virginia alone had 20% of >15,000 producing Marcellus wells by 2015 (WVGES 2015) with 40,000 new wells projected by 2030 (DOE 2010). Within the Appalachian region, nearly 75% of close to 3.1 million forested hectares are at highest potential risk from energy development, primarily shale gas (Dunscomb et al. 2014). West Virginia is one of two states with the highest probability for development (21%; Dunscomb et al. 2014), and species with specialized habitat needs that overlap these forested areas will be the most vulnerable (Brittingham et al. 2014).

Recent studies have examined how shale gas development may positively and negatively influence Appalachian songbird communities (Barton et al. 2016, Farwell et al. 2016), how predators and their aquatic prey respond (Wood et al. 2016), and how shale gas development may be associated with bioaccumulated contaminants (Latta et al. 2015). However, there has not been a mechanistic assessment of the interplay between shale gas disturbance and songbird demographic response (Northrup and Wittemyer 2013). Baseline demographic data are needed in both terrestrial and aquatic habitats of the Appalachian shale gas basin to detect and understand changes as they begin to occur (Brittingham et al. 2014).

The proximity of shale gas development to water resources is of particular concern due to the potential for sedimentation runoff, reduced streamflow, and contamination of surface waters (Entrekin et al. 2011). Therefore biological communities and organisms that use water resources downstream are at increased risk from shale gas activities near surface waters (Latta et al. 2015). The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is a habitat specialist and species of conservation concern (USFWS 2008), that breeds along forested headwater streams and feeds primarily on benthic macroinvertebrates (Mattsson et al. 2009). Waterthrushes are well-established biological indicators of aquatic stream integrity (O'Connell et al. 2000, Mulvihill et al. 2008) and reach some of their highest abundances in the Marcellus shale region (Sauer et al. 2017). As such, the increased intensity of core forest disturbance where headwater streams and this species co-occur make waterthrush an ideal organism to assess potential demographic consequences from shale gas development (Drohan et al. 2012, Farwell et al. 2016). As a bioindicator species, we hypothesized that we would detect an inverse relationship between the amount of shale gas disturbance and demographic metrics despite the species' ability to compensate for resource loss (Mulvihill et al. 2008, Wood et al. 2016). Identifying how variability in habitat quality contributes to population surpluses (source habitat; Pulliam 1988) or deficits (sink habitat) is key to long-term conservation planning in landscapes undergoing development (Kirol et al. 2015). If source-sink dynamics exist in our local population, we also hypothesized that productivity would differ between areas disturbed and undisturbed by shale gas development.

We examined how increased shale gas development influenced waterthrush demography during 2009–2011 and 2013–2015. We quantified waterthrush nest abandonment, survival, and productivity, an overall source-sink threshold, riparian habitat quality, and territory density and length. In addition to identifying demographic responses, our results should inform well siting guidelines for shale gas development to minimize risk to ecological resources.

METHODS

Study Area

We studied waterthrush demography along 58.1 km of 1st- and 2nd-order forested headwater stream tributaries (*n* = 14) in the Lewis Wetzel Wildlife Management Area (LWWMA) in northwestern West Virginia (39.490216°N, -80.650713°W, Figure 1), an area that supports the highest waterthrush densities within the central Appalachians (Sauer et al., 2017). The study area lies within the Permian Hills subdivision of the Western Allegheny Plateau Ecoregion, an area of deeply dissected topography and relatively continuous Appalachian Oak and Mixed-Mesophytic Forest (Woods et al. 1999) with elevations of 221–480 m that overlays the Marcellus-Utica shale region. In 2008, LWWMA was 95.3% forested and had 0.4% shale gas land cover; the first shale gas well development began in 2007 (Farwell et al. 2016). Shale gas development in our study area and within the surrounding region since then has rapidly increased (WVGES 2015). In 2015, LWWMA was 90.8% forested and 2.4% in shale gas development land cover, with 83.1% of shale gas development resulting in direct forest loss (Farwell et al. 2016).

During our study, gas well development activities included building of conventional (shallower formations) and Marcellus well pads, forest clearing for yet unbuilt well pads, the expansion of existing road and pipeline infrastructure, and the construction of new infrastructure. Early in the study (2009–2010), the majority of Marcellus wells and their water holding ponds were located along the main stem of Buffalo Run, where the majority of our headwater study streams empty. Thus, although a few Marcellus well pads were located along our study streams, they tended to primarily impact the lower portions of the study streams. Between the 2010 and 2011 breeding seasons, shale gas development activities accelerated across the study area and began to increase especially on ridgetops. As a result of ridgetop activity, the whole downstream network of some streams became disturbed by sedimentation and surface runoff for the remainder of the study. LWWA experienced a 1.5% increase in area affected by timber harvests in 2010–2011 (Farwell et al. 2016), but these and pre-existing harvests did not result in complete forest canopy loss (Sheehan et al. 2014) and typically were not intersecting or influencing streams where we monitored waterthrushes. Shale gas development peaked in 2011, but starting in 2013, shale gas development abated site-wide and in general became concentrated on specific streams and ridgetops. Clearing for additional new well pads occurred late (June–July) in the 2013 breeding season with well pad completion in 2014, in addition to re-drilling of an existing well pad. There was no new shale gas development or activity in the 2015 breeding season. There were no “control” streams given that the majority of forest loss and fragmentation in the surrounding landscape resulted from recent shale gas activities (Farwell et al. 2016), but shale gas disturbance was concentrated on some streams more than others as indicated by the large annual range in percent of stream disturbed (range 0–66.9%; Table 1).

Mapping and Quantifying Disturbances

Within a Geographic Information System (GIS), we used a sequence of leaf-on and leaf-off aerial photographs from the National Agriculture Imagery Program (NAIP) for 2011 and 2014, satellite Quickbird imagery for 2009, and extensive annual ground-truthing to manually digitize areas of forest canopy disturbance within the study area for each year of the study. All forest canopy disturbances were classified as shale gas development related (i.e., well pads and associated road and pipeline infrastructure) or unrelated or pre-existing (i.e., forest roads, recent even-aged timber harvests, and various types of existing clearings) using FRAGSTATS 4 (McGarigal et al. 2012). We determined the percentage of forest canopy disturbance from shale gas development (GasFCD) and non-shale gas development (OtherFCD) sources within a 100 m radius of each nest for use as habitat covariates in nest survival models (Table 2). We used 100 m radius because forest edges may negatively affect the reproductive success of ground-nesting species at this scale (Flaspohler et al. 2001). We classified a few conventional impacts (i.e., stream-side vertical pump jacks) as related to shale gas development because their pads were managed in conjunction with nearby shale gas infrastructure and because their targeted formation, even though they remained shallow after development, was listed as Marcellus (WVGES 2015). Gas well records (WVDEP 2015) were used to verify target shale formations, drilling status, and start dates for all well disturbances.

Surface (i.e., 3D) lengths of each study stream (average length 4.1 ± 0.54 km, range 0.95–7.4 km) were calculated in GIS using a 3 m resolution digital elevation model and defined to have a drainage basin of 9.0 hectares (24 k scale or higher resolution) to delineate the uppermost headwater reaches. To describe and model waterthrush demography and riparian habitat quality as a function of shale gas disturbance, we created four continuous and one binary variable based on disturbance categories at the stream, territory, and nest scale. The first (StreamGas) described mostly localized streamside disturbance indicative of the presence of any shale gas infrastructure or activity (Figure 2A, Table 2). A section of stream was considered disturbed when well pads, infrastructure, or frequent vehicular activity (Figure 2E) were within 60 m of the stream centerline, the typical extent of waterthrush streamside use (Mattsson and Cooper 2009). When a stream had visually observable sedimentation from shale gas development (Figure 2F), we classified the entire stream network downstream of the sedimentation beginning point as disturbed. Streams were frequently and extensively ground-truthed each season, so there were no stream reaches where sedimentation events were likely to be missed.

We created a second shale gas disturbance category (StreamRunoff) that focused solely on potential run-off into streams from shale gas contaminants (Figure 2B, Table 2). A stream was considered disturbed at and below a well pad or retaining pond (similar to Latta et al. 2015), resulting in the whole downstream network classified as at risk for surface pollution based on elevational maps and ground truthing. The StreamRunoff category did not include pipeline or road disturbance and was a broader, distance-independent, disturbance category describing potential water pollution. For each year of the study, we then calculated the proportion of each stream disturbed for both the StreamGas and the StreamRunoff disturbance categories.

We calculated the proportion of each territory (a 60-m buffer around each territory vector; Mattsson and Cooper 2009) that was disturbed by StreamGas and called this metric TerrGas (Figure 2C, Table 2). The proportion of each territory disturbed by StreamRunoff was termed TerrRunoff (Figure 2D, Table 2). We classified each waterthrush nest location as undisturbed or disturbed by StreamGas within 60-m around the nest and called this variable NestGas.

Territory Density and Length

We mapped waterthrush territories in 2009–2011 and 2013–2015 along 14 streams with varying amounts of StreamGas and StreamRunoff (Table 1) to determine annual territory density and length. Territories were mapped for 11 streams in all years, Hiles Run and Huss Pen Run were mapped in 2014–2015, and Carpenters Run was mapped in 2009 and 2014–2015 due to restricted access. Territorial waterthrushes were target-netted and banded with an aluminum U.S. Geological Survey leg band and a unique combination of plastic color bands to allow identification of individuals. Waterthrush territories are typically described as a length (m) instead of area given that they form linear territories along a stream reach (Mulvihill et al. 2008), therefore all analyses were based on territory length. Waterthrush territory density is the number of individual territories per stream km monitored (Hallworth et al. 2011). Waterthrush territories were delineated typically from April 1–June 29 each year. Standardized territory mapping (Robbins 1970, Bibby et al. 1992) was conducted in 2009 (8 visits per stream) and 2010 (5 visits per stream). In 2011, we switched to a more opportunistic approach that allowed for mapping waterthrush locations and behaviors during nest searching of each stream (≥5 visits per stream). During 2013–2015, standardized territory mapping included ≥6 (average 11.5 ± 0.6) visits along each stream reach, with visits preceding peak incubation initiation, and visits within 4 hours after sunrise to ensure high rates of detection (Mattsson and Cooper 2006). Observations were recorded using in-field spot-mapping on topographic maps during 2009–2011 and with a WAAS-enabled Garmin 60CSX GPS unit with accuracy ≤5 m in 2013–2015. Given frequent and similar site visits each year and some of the same observers monitoring waterthrush in multiple years of the study, we can reasonably assume any changes in annual territory length would not be due to using in-field spot-mapping vs. GPS territory mapping methods.

Nest Abandonment, Survival, and Productivity

Nest searching and monitoring occurred concurrently with territory mapping to determine nest abandonment rate, survival and productivity. Waterthrush have the occasional behavior of creating "nonbreeding nests" (Mattsson et al. 2009) that are abandoned before nest construction is completed (Frantz personal observation). Shale gas disturbed areas may have higher nest abandonment rates (Davis 2014) where waterthrush possibly could increase the number of nonbreeding nests in these areas. In 2013–2015, any partially built or fully built nest of unknown, post-hoc fate was tallied on each stream as a potentially abandoned nest, which we called a failed attempt. Rarely a nest can survive more than one season partially intact and its older age evident (Frantz personal observation), but any questionable nest was not counted.

We monitored nests typically every 3–4 days initially and more frequently as fledging approached (Martin and Geupel 1993). We used nestling morphology to determine hatch date (Mattsson and Cooper 2009). We assumed an undamaged empty nest had fledged if the nest was active the day before and had approached the predicted fledge date. We attempted to verify fledging by looking for fledglings or adults carrying food if a nest was believed to have fledged. We counted number of eggs to determine clutch size of nests with complete clutches. The number of fledglings for each successful nest was the count of nestlings in the visit prior to fledging. Nests were considered successful if they produced at least one waterthrush fledgling, including nests parasitized by Brown-headed Cowbirds (*Molothrus ater*).

Riparian Habitat Quality Assessment

Riparian habitat quality was assessed using the Habitat Suitability Index specifically designed for waterthrushes (HSI; Prosser and Brooks 1998) and the US Environmental Protection Agency (EPA) Rapid Bioassessment for high gradient streams (Barbour et al. 1999). The HSI is a broad-scale evaluation of waterthrush instream foraging and upland habitat suitability that ranges from 0–1 (Prosser and Brooks 1998). The EPA index (range of 0–200) assesses stream quality based primarily on instream characteristics that relate to the abundance and composition of waterthrush aquatic macroinvertebrate prey, and therefore may indicate relative quality of instream foraging habitat (Wood et al. 2016). The HSI and EPA index were quantified in a 100 m stream segment centered on each nest in 2009. In later years we used a 50 m segment centered on each nest to make the indices more sensitive to habitat immediately surrounding waterthrush nests. Mattsson and Cooper (2006) conducted EPA assessments on stream reaches that were 20 X channel width in length. Our average channel width of 3.7 m X 20 approximates our 50 m segment. We did not collect EPA or HSI data in 2015 due to time constraints.

Analysis

Territory density and length. To model the effect of gas well development on waterthrush territory density, we used a generalized linear mixed model (GLMM) with study stream as a random effect and year and StreamGas as fixed effects. For these mixed models and all hereafter, we did not test more than one gas disturbance variable per analysis to avoid multicollinearity. The response variable was the number of territories for each stream in each year sampled with the length of each stream included as an offset. We specified a Poisson distribution based on the absence of overdispersion in the fixed-effects version of this model (Zuur et al. 2009). Modeling was performed using the *glmer* function within the lme4 package (Bates et al. 2015) in R (R Development Core Team 2014). For this model as well as the other mixed models mentioned below, model residuals were evaluated graphically and we used various data exploration diagnostic tools detailed in Zuur et al. (2010) to ensure model assumptions were met. Statistical significance ($\alpha = 0.05$) was assessed via a likelihood ratio test (Zuur et al. 2009). If year was significant, a post-hoc contrast Kruskal-Wallis sum rank test was completed with Bonferroni correction using the dunn.test R package (Dinno 2016) to determine between which years territory density differed.

We used a gamma family GLMM to test whether territory length with stream as a random effect differed among years (glmmADMB R package; Bolker et al. 2012). Overall statistical significance and post-hoc testing for year was done in the same manner as territory density. To test the hypothesis that territory length would increase with a decrease in territory density (Lack 1954), we related territory length to territory density with an Asymptotic Spearman Rho Correlation Test with R packages coin (Hothorn et al. 2015a) and psych (Revelle 2017).

Nest abandonment, survival, productivity, and source-sink threshold. To examine nest abandonment on streams from 2013–2015, we calculated percent failed attempts, hereafter nest abandonment, which was divided by the total of all nests on a stream, including fledged nests that could not be found (*n* = 18). We also calculated annual nest abandonment ± standard error (SE) to evaluate trends over time. We correlated nest abandonment per stream to StreamGas using an Asymptotic Spearman Rho Correlation Test with R packages "coin" (Hothorn et al. 2015a) and "psych" (Revelle 2017).

We used Program MARK 7.1 (White and Burnham 1999) to estimate daily survival rate (DSR) of waterthrush nests in each year of the study. Of 364 total nests across all years, we removed 84 nests that did not reach the egg-laying stage, that were discovered post-fate, or had unknown fates and thus did not meet the assumptions of MARK. We assumed a 29-day nesting period (egg-laying 5, incubation 14, nestling 10 days) based on the chronology of nests monitored on our study area to calculate annual nest survival using DSR. We plotted annual nest survival ± SE to graphically evaluate trends over time.

We developed a set of 11 a priori candidate models (Buckland et al. 1997) containing temporal, shale gas disturbance, and habitat covariates that we hypothesized might influence DSR of waterthrush nests. We did not include random effects (i.e., stream) in any model due to the difficulty of modeling such effects in nest survival analyses, but recognize that the random effect of stream could have accounted for variability among study streams if present. All a priori models included temporal covariates to account for their influence on nest survival based on previous literature: nest age, quadratic effect of time within-season (TT), and average daily rainfall. We included nest age because nests may be more vulnerable as they age (Dinsmore et al. 2002, Grant et al. 2005, Burhans et al. 2010), and because a similar covariate called nest stage was the most supported nest survival model in a 2011 waterthrush benthic aquatic prey study (Wood et al. 2016). We included TT because it was most parsimonious in a post-hoc waterthrush nest survival model (Mattsson and Cooper 2009). We included mean daily rainfall (Rain, mm) because headwater riparian systems are subject to seasonality and annual changes in rainfall (Richardson and Danehy 2006) that can affect waterthrush nest survival rates (Mattsson and Cooper 2009). For each nest, we averaged daily rainfall estimates across the period in which an active nest was under observation (Mattsson and Cooper 2009). Precipitation estimates were pooled from four Weather Underground, Inc. network stations closest (avg. 36 km) to the study area (three weather stations in 2009). We included an additional fixed year effect as a variable of interest in some models because shale gas development increased over the study period (Farwell et al. 2016) and to account for annual variation in DSR associated with biotic and abiotic factors not included in our models. We did not include a model with year only because we a priori evaluated nest survival graphically to review trends and found some overlap in annual estimates.

The primary variables of interest included three shale gas disturbance covariates (TerrGas, TerrRunoff, NestGas) and two habitat covariates (GasFCD, OtherFCD; Table 2). Gas disturbance covariates were not combined in an additive fashion in a single model because they are related metrics, and the habitat covariates were not combined in a single model as we wanted to distinguish if the source of forest canopy disturbance was important. We chose GasFCD and OtherFCD as habitat covariates as we hypothesized that shale gas development through the removal and fragmentation of riparian forest cover could negatively influence waterthrush reproduction through modified predator assemblages and activity as well as altered stream hydrology and water quality (Petit and Petit 1996, Mulvihill et al. 2008, Mattsson and Cooper 2009) and because waterthrushes are known to be sensitive to removal of forest canopy cover (O’Connell et al. 2003).

We used Akaike’s Information Criterion for small sample sizes (AICc) to evaluate support for candidate models (Burnham and Anderson 2002) in program MARK. We modeled the binomially distributed data with the user-defined, logit-link function while simultaneously considering associations with temporal, shale gas disturbance, and habitat covariates. We assessed the relative plausibility of each model in each model set by comparing Akaike weights (w_i). We considered the model with the lowest AICc value to be the best-supported model given the data, and any models with ΔAICc <2 were considered plausible (Burnham and Anderson 2002). We used model-averaged regression coefficients (Burnham and Anderson 2002) and 85% confidence intervals (hereafter CIs) to infer biological importance of covariates in plausible AICc models as 95% CIs with the information-theoretic approach can lead to variable selection uncertainty (Arnold 2010).

We quantified average overall individual and average annual population level nest productivity using an approach similar to Boves et al. (2015). Mean number of fledglings per successful nest per male (the capita value) was multiplied by nest survival (DSR^{29}) separately for areas undisturbed and disturbed by shale gas development. Areas undisturbed by shale gas development (*n* = 78) were categorized as territories with TerrGas = 0%, and areas disturbed by shale gas development (*n* = 55) as territories with any amount of TerrGas (range 2.7–100%). For population productivity, the individual productivity capita value was calculated per year, multiplied by annual nest survival, and then multiplied by average annual territory density to determine if average annual population productivity changed over time. Significance of individual productivity between areas undisturbed and disturbed by shale gas development and for population productivity across years was evaluated graphically by examining overlap of 95% CI error bars for simple biological inference (Payton et al. 2003, MacGregor-Fors and Payton 2013). Productivity SEs used to construct the CIs were unadjusted mean number of fledglings SE values to reflect the full range of variability for each metric (T. Boves, personal communication).

Additionally, we assessed whether productivity can compensate for adult mortality (e.g., Robinson and Morse 2007) by calculating a source-sink threshold (Pulliam 1988). Since the threshold is the minimum number of fledglings needed to compensate for adult mortality, productivity above or below the threshold allowed us to evaluate if habitat quality is sufficient for local populations to be maintained. In the manner of Robinson and Morse (2000), the source-sink threshold was the annual productivity per pair necessary to compensate for adult mortality modeled as $2(1-\phi)$ divided by ϕ_0 where ϕ is adult survival and ϕ_0 is juvenile survival. We calculated overall adult mortality ($2(1-\phi)$) using the average of our separate estimates of male and female apparent survival (ϕ). Male survival (ϕ) was 0.56 ± 0.04 and female ϕ was 0.44 ± 0.08 (Frantz et al. 2019). We assumed juvenile survival to be half the adult value (Nolan 1978) since low estimates (≤ 0.30) are likely more accurate than previously thought (McKim-Louder et al. 2013). The source-sink threshold value was multiplied by nest survival (DSR^{29}) to convert to the same scale as individual productivity values. We then graphically evaluated if average individual productivity in areas undisturbed and disturbed by shale gas development fell above or below the threshold value and based significance on overlapping 95% CI error bars for simple biological inference where CI proportion overlap of ≤ 0.5 is considered significant (Cumming and Finch 2005).

Riparian habitat quality assessment. We used a beta family GLMM to compare HSI scores for nest-centered segments located in shale gas disturbed and undisturbed territories using the glmmADMB R package (Bolker et al. 2012). Models included TerrGas and year as a fixed effect and stream as a random effect. Because the 2009 HSI index was collected at a 100 m stream length segment and following years were at 50 m segments, we used t-tests to compare HSI scores from 2009 to 2010, two years when percent of stream disturbed was the same. We found no differences (Z_{78} = 1.45, P = 0.15), so we did not account for stream segment length differences in our models. Our model had marginal overdispersion (χ_{240}^2 = 1.15, P = 0.051), so we added an observer-level random effect (OLRE) where each observation receives a unique random effect level that can absorb extra-parametric variability (Harrison 2015). Statistical significance (α = 0.05) was assessed via a likelihood ratio test (Zuur et al. 2009). If year was significant, we completed a post-hoc contrast Kruskal-Wallis rank sum test to indicate between which years HSI scores differed.

We used linear mixed effect modeling (LMM) in R package lme4 (Bates et al. 2015) to assess nest-centered EPA index scores in disturbed and undisturbed territories. Models included percent shale gas territory disturbance and year as a fixed effect and stream as a random effect. We did not test other gas disturbance variables within the same model to avoid multicollinearity. T-tests indicated a significant difference between EPA index scores from years 2009 (100 m assessment) and 2010 (50 m assessment; t_{78} = 6.12, P < 0.001. Therefore we dropped 2009 data from our model to avoid variability from stream segment length, and assumed 2010 was representative of initial gas disturbances to streams because % disturbance on streams was the same in 2009 and 2010. We used R package afex (Singmann et al. 2015) to retrieve p-values for the F test assessment of fixed effects. We used a post-hoc Tukey HSD test using R package multcomp (Hothorn et al. 2015b) to determine between which years EPA scores differed. We set significance as α = 0.05 for all tests.

RESULTS

Territory Density and Length

In six years we monitored 400 waterthrush territories. Waterthrush territory density was not related to StreamGas (χ_1^2 = 0.002, P = 0.97), but was significantly different by year and generally declined over time (χ_5^2 = 13.424, P = 0.02, Table 3). A post-hoc contrast Kruskal-Wallis sum rank test for year indicated territory density was significantly higher in 2010 than 2015 (χ_5^2 = 3.05, P = 0.02). Across years, study streams had a mean of 23.4% ± 0.03 of their length disturbed by gas development (range 0.0–66.9%, Table 1).

Territory length was significantly different by year and generally increased over time (χ_5^2 = 59.44, P = < 0.001, Table 3). A post-hoc test for year indicated territory length was greater in 2009 than in 2010 (χ_5^2 = 3.10, P = 0.01), but was less in 2009 than 2014 (χ_5^2 = -3.82, P = 0.001) and 2015 (χ_5^2 = -3.84, P = < 0.001). Territory length was less in 2010 than 2011 (χ_5^2 = -2.79, P =

0.02), 2013 ($\chi_5^2 = -4.90$, $P = < 0.001$), 2014 ($\chi_5^2 = -6.95$, $P = < 0.001$), and 2015 ($\chi_5^2 = -6.83$, $P = < 0.001$). Territory length also was less in 2011 than 2014 ($\chi_5^2 = -4.00$, $P = < 0.001$) and 2015 ($\chi_5^2 = -4.01$, $P = < 0.001$). Territory length significantly increased as territory density decreased (Rho = -0.49, $Z_{394} = -9.66$, $P < 0.001$).

Nest Abandonment, Survival, Productivity, and Source-sink Threshold

During 2013–2015, 59 nests of unknown fate were potentially abandoned or a failed nesting attempt (average 1.5 ± 0.3 failed attempts per stream). There was not a statistically significant relationship between nest abandonment on streams and StreamGas (Rho = 0.22, $Z_{38} = 1.40$, $P = 0.16$). Nest abandonment rates on streams were 16.9 ± 0.1%, 37.3 ± 0.1%, and 9.4 ± 0.04% annually. Annual daily and seasonal nest survival peaked in 2010–2011 and generally declined over time (Table 3). Overall mean DSR was 96.4 ± 0.3% and yielded average nest survival of 34.3 ± 3.1%. Across all years, eight nests were parasitized by Brown-headed Cowbirds, primarily in later years of the study (Table 3).

Of 11 a priori models (Table 4), four models that had habitat (GasFCD) and gas (TerrRunoff, TerrGas, NestGas) covariates were supported (ΔAICc <2). The two models that included TerrRunoff and TerrGas accounted for most of the variation in DSR ($w_i = 0.28$ and 0.27). Model-averaged regression coefficient 85% CIs did not overlap zero for Rain and GasFCD with positive influence on DSR, while TerrGas, TerrRunoff, and NestGas did not overlap zero and had a negative influence on DSR (Table 5, Figure 3). About a third (29.6%) of nests monitored had NestGas, and nest survival dropped from 37.3 ± 4.3% in undisturbed areas to 30.7 ± 4.5% in areas disturbed by shale gas. Territories containing nests had on average 24.7 ± 2.1% of TerrGas and 21.8 ± 2.2% of TerrRun. Nests across years were predominately forested (94.0 ± 0.5%) with 2.3 ± 0.3% GasFCD. NestAge and TT had regression coefficients that overlapped zero, indicating little or highly variable influence on DSR.

Overall population productivity was 2.3 ± 0.5 fledglings/km and generally declined from early years of the study (2009–2011) to later years (2013–2015, Table 3) based on 95% CIs. Overall individual productivity was 1.5 ± 0.1 (SE) fledglings per adult male. Individual productivity was higher in areas undisturbed (1.6 ± 0.2 fledglings) than disturbed (1.4 ± 0.2) by shale gas based on 95% CIs (Figure 4). The completed source-sink equation was 2(1-0.50) / 0.25, with 0.50 the average of male and female adult survival and juvenile survival assumed to be half that value at 0.25, resulting in 1.4 fledglings per pair. The source-sink threshold of 1.4

fledglings per pair was below the individual annual productivity in areas undisturbed by shale gas (Figure 4) suggesting these are source habitats. However, the threshold overlapped the productivity for areas disturbed by shale gas (average 56.7 ± 4.5% TerrGas in disturbed areas) suggesting these are borderline sink habitats.

Riparian Habitat Quality

HSI scores were negatively related to TerrGas ($\chi_1^2 = 65.34$, $P < 0.001$, Figure 5), and differed by year ($\chi_4^2 = 34.84$, $P < 0.001$, Table 3). A post-hoc contrast Kruskal-Wallis sum rank test for year indicated HSI scores were significantly higher in 2009 than in 2013 ($\chi_4^2 = 4.03$, $P < 0.001$) and in 2009 than in 2014 ($\chi_4^2 = 3.14$, $P = 0.01$).

EPA index scores were negatively related to TerrGas ($F_{1,158} = 14.54$, $P < 0.001$, Figure 5), and differed by year ($F_{3,196} = 14.07$, $P < 0.001$, Table 3). A post-hoc Tukey HSD test for year indicated EPA index scores were significantly higher in 2010 than in 2014 ($Z_{394} = 3.29$, $P = 0.005$), higher in 2011 than in 2013 ($Z_{394} = -4.26$, $P < 0.001$), and lower in 2013 than in 2014 ($Z_{394} = 6.18$, $P < 0.001$).

DISCUSSION

Over our six year study at LWWMA, we saw general declines in waterthrush territory density, nest survival, nest productivity, and riparian habitat quality concurrent with a site-wide increase in shale gas-related disturbance (Farwell et al. 2016). Our source-sink threshold suggests that individuals breeding in areas disturbed by shale gas development are potentially in sink habitat and are more at risk of population decline than individuals in areas undisturbed by shale gas development. Declines in waterthrush demography occurred despite <5% forest cover loss at our predominately forested study site (Farwell et al. 2016) which suggests that factors other than loss of forest cover also influenced demography (Wood et al. 2016). In general, all demographic parameters for waterthrushes appeared to be affected negatively by shale gas disturbances occurring in headwater stream ecosystems (Table 3). To our knowledge, our study is the first to establish the potential for Marcellus-Utica shale gas development to affect reproductive success and productivity of forest birds.

Waterthrush territory density declined across years, but was not explained by StreamGas. Streams on average had less than a quarter (22.5% ± 2.6) of their length disturbed by StreamGas (Table 1), and no stream was ever completely disturbed (maximum 66.9%). Consequently, undisturbed areas occurred on every stream, so waterthrush could shift their territories to forage and place nests in undisturbed sections of streams. Waterthrush on acidified streams in Pennsylvania used a similar strategy (Mulvihill et al. 2008). Despite waterthrush exhibiting high site fidelity (O'Connell et al., 2003), we noted that by the end of our study our initial high territory densities of 1.5 km^{-1} had dropped to 1 territory km^{-1}, lower than typical densities across the breeding range (Mattsson et al. 2009). A Headwater Stream Assessment based on waterthrushes in PA found that 0–1 territories km^{-1} indicated degradation and 1–2 territories km^{-1} indicated possible degradation (O'Connell et al., 2003). This suggests increased degradation of our study streams across our study period, as was also suggested by our declining HSI scores. The decline in riparian stream quality over time likely influenced the decrease in territory density and increase in territory lengths in our study. Increasing length of territories in disturbed areas may be a mechanism that allows waterthrushes to compensate for poor habitat quality (Mulvihill et al. 2008). Waterthrushes increasing their territory lengths may need additional foraging resources to meet minimal breeding requirements, as suggested by greater territory densities in 2011 in areas where macroinvertebrate density, biomass, and stream quality were higher (Wood et al. 2016).

Nest survival was positively influenced by average daily rainfall, similar to Mattsson and Cooper's (2009) finding of maximum daily survival rate at intermediate (3–10 mm) rainfall levels. This intermediate rainfall range is similar to what waterthrushes encountered during the active nesting period at our study site (range 0–11.2 mm, average 3.6 ± 0.1 mm). Rainfall in this range likely leads to increased prey availability, foraging efficiency, and therefore nest vigilance (Mattsson and Cooper 2009). Lack of sufficient water flow was likely more of a factor than flooding in our headwater system as only four nests were confirmed to have failed from high water events. At the beginning of the breeding period our streams were typically flowing, but by late summer when young were fledging, streams had intermittent or little flow. While not a documented threat to waterthrushes on our headwater study streams, shale gas operations withdraw large amounts of surface and groundwater from small streams (Entreken et al. 2011). As such, waterthrushes breeding downstream of water withdrawal operations have the potential to be negatively affected by altered hydrology in the same manner that water withdrawals affect other species, such as brook trout (*Salvelinus fontinalis*) (Weltman-Fahs and Taylor 2013).

After accounting for the positive influence of rainfall, waterthrush DSR also had a significant negative relationship with three shale gas development covariates. TerrRunoff was a measure of potential surface water contamination, while TerrGas and NestGas assessed the physical presence of shale gas infrastructure and included human activity and sedimentation (Table 2, Table 5). All previous bird community studies of Marcellus shale drilling in the Appalachians have focused primarily on presence of gas infrastructure, with less attention to noise and light levels (Davis 2014, Barton et al. 2016, Farwell et al. 2016). Waterthrushes in our study could have been directly affected by the presence of infrastructure given the similar findings of negative effects from oil and gas development on bird species from other regions (Van Wilgenburg et al. 2013, Thompson et al. 2015, Hethcoat and Chalfoun 2015), although we must also consider indirect effects on stream and terrestrial food webs from possible contamination (Entrekin et al. 2011). For example, waterthrushes in areas disturbed by shale gas development had higher levels of barium and strontium in their feathers than waterthrush in areas undisturbed by shale gas (Latta et al. 2015). Because barium and strontium are two heavy metals associated with the drilling process (Chapman et al. 2012), and LWWMA was a sampled region in Latta et al. (2015), this finding could be related to our modeling result that TerrRunoff negatively influenced DSR. Heavy metals can interfere with DNA methyl transfer (Hala et al. 2014), so one potential mechanism by which DSR can be affected is by differential methylation via epigenetics (see Chapter 6).

Previous studies have shown potential waterthrush vulnerability to forest habitat fragmentation (Robbins 1979, McIntyre 1995, Adams 2007) and declines in abundance after loss of ~16% forest from the landscape (Becker et al. 2015). In contrast, waterthrush in our study showed a slight positive relationship between GasFCD and DSR. Clearing of land for shale gas development in some instances may increase net primary production in streams (Johnson et al. 2015) and increase certain types of aquatic prey, such as shredders (Barton 2016), thus GasFCD potentially increased some aquatic prey taxa for waterthrushes. In addition, Davis (2014) found that while nest survival was lower for Field Sparrows (*Spizella pusilla*) in the presence of gas wells, gas pipelines and access roads had a lower index of predation risk, possibly from increased noise (Francis et al. 2012) or light (de Molenaar et al. 2006) levels, even though predation typically increases near forest edges (Paton 1994). However, these potential benefits from GasFCD could be offset by higher abandonment rates (Davis 2014) or cowbird nest parasitism in areas disturbed by shale gas development. Although few nests were parasitized (2.9% of nests) on our study area compared to other waterthrush studies (range 0–81%; Mattson et al. 2009), we observed an apparent increase in parasitism rates of waterthrush nests across years as forest cover declined and the majority of parasitized nests (75%) failed. Concurrently, cowbird detections increased from 2.1% of sample points in 2008 to 27.5% in 2015 (Farwell et al. 2016). Parasitized nests had double the amount of GasFCD (5.7% ± 2.2) than non-parasitized nests (2.7% ± 0.3). Given that average forest cover of nests was 94.0% ± 0.5 and waterthrush will not occupy areas of <40% forest cover (O'Connell et al. 2003), GasFCD may play only a minor role, at least initially, among several factors (i.e., rainfall and shale gas disturbance) in nest survival.

Waterthrush are persistent renesters with only 5–6 days between attempts (Mulvihill et al. 2002), in addition to the behavior of creating "nonbreeding nests" (Mattsson et al. 2009, Frantz personal observation). Average nesting attempts for our study site (including successful and unsuccessful individuals, and nest building attempts) was 1.04 ± 0.04 but up to five attempts were documented. We did not detect a statistically significant association between nest abandonment and amount of stream disturbance. A weak, negative relationship can be suggested, but there is no way to determine whether a nest of unknown fate (i.e., nests that cannot be used in typical nest survival analyses) were truly abandoned, failed, or left unfinished for other reasons. Negative discrimination of Brown-headed Cowbird eggs by waterthrush has been documented (Robinson 1990), so parasitism could be under-represented by nests that failed before clutch completion or before active observation, as we can only document apparent acceptance of parasitized nests. Further behavioral monitoring will be needed to discriminate nonbreeding nests from actual failed nests found post-hoc, and whether waterthrush will increase the number of nonbreeding nests or relocate a nest due to shale gas disturbance.

Population productivity generally declined over time, and areas disturbed by shale gas development had lower individual productivity, broadly overlapping with the source-sink threshold (Figure 3). Corresponding with lower individual productivity, lower nest survival, and decreased riparian habitat quality with increasing disturbance, areas disturbed by shale gas development may be at greater risk for being sink habitat. Our source-sink values match other studies showing productivity in optimal (or presumably undisturbed) source habitat to be barely above estimated source-sink thresholds (Morse 1996, Holmes et al. 1996). Headwater streams may need to be buffered from potential disturbances if they are only marginally source habitats even under ideal conditions (Morse and Robinson 2000).

Conclusion

Our study is one of the first to demonstrate that shale gas development can affect reproductive success and productivity in a wildlife population, likely by the presence of shale gas infrastructure and by indirect negative effects to stream health and aquatic prey (Wood et al. 2016). Increasing overall aquatic ecosystem health necessitates measures to protect water quality from upstream sediment load and pollutant sources (Cook et al. 2015) which would require watershed scale (Merovich et al. 2013) habitat conservation efforts. Spills and erosion are the most commonly reported environmental violations (Rahm et al. 2015), which could be avoided with setbacks from streams and avoidance of building in steep grades (Evans and Kiesecker 2014). Development is outpacing implementation of best management practices (Brittingham et al. 2014), so placing well pads further away from water than currently permissible may be the most effective way to avoid multiple disturbances from shale gas (Milt et al. 2016).

For effective mitigation strategies at a regional level, additional species and area-specific studies are needed (Northrup and Wittemyer 2013) as well as clarification of the specific mechanisms involved in species' responses (Hethcoat and Chalfoun 2015) to shale gas disturbance. Our study results combined with post-fledgling survival data (Streby and Anderson 2011), and a cross-ecosystem evaluation of food web interactions (Soininen et al. 2015) with potential contaminants would fill important knowledge gaps. Lack of information regarding the full range and interdependence of waterthrush demographic responses to shale gas development should not negate immediate risk management activities (Loss 2016), especially if multiple lines of evidence suggest decline and negative demographic response of a known important bioindicator of headwater stream ecosystems.

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TABLES

Table 1. Annual percent of each study stream’s length that was disturbed by shale gas development or activity (StreamGas, SG; see Table 2) or potentially affected by runoff (StreamRunoff, SR) from shale gas well pads (not including pipeline or road disturbance). The last new well pad construction occurred near study streams in 2014.

	2009		**2010**		**2011**		**2013**		**2014**		**2015**	
Study Streams	**SG**	**SR**	**SG**	**SR**	**SG**	**SR**	**SG**	**SR**	**SG**	**SR**	**SG**	**SR**
Buffalo East Run	14.9	7.6	14.9	7.6	14.9	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Buffalo West Run	53.5	2.6	47.4	2.3	58.7	2.9	2.9	2.9	2.8	2.8	2.7	2.7
Carpenter Run	0.0	3.3	*	*	*	*	0.0	3.3	0.0	3.3	0.0	3.3
Hiles Run	*	*	*	*	*	*	0.0	19.7	0.0	19.7	0.0	19.7
Huss Pen Run	*	*	*	*	*	*	0.0	18.4	0.0	18.4	0.0	18.4
Megans Run	0.0	4.3	0.0	4.0	13.7	4.2	0.0	40.5	0.0	40.5	0.0	40.5
Nettles Run	12.8	22.2	12.8	22.2	12.8	22.2	13.5	20.7	13.5	20.7	13.5	20.7
Olive Run	1.9	23.5	2.0	24.6	2.0	24.6	16.7	50.7	16.7	50.7	16.7	50.7
Owl Run	27.1	10.0	27.1	10.0	27.1	10.0	10.0	9.7	10.0	9.7	10.0	9.7
Sees Run	7.2	7.4	7.2	7.4	21.1	8.0	28.8	27.4	32.9	27.4	32.9	27.4
Slabcamp Run	54.6	49.0	57.4	51.5	56.8	51.0	25.8	53.1	45.2	53.1	41.4	53.1
Snake North Run	53.9	13.5	53.7	13.5	51.5	12.9	66.9	9.2	66.9	9.2	66.9	9.2
Snake South Run	51.9	9.7	50.5	9.5	50.5	9.5	58.7	8.6	58.7	8.6	58.7	8.6
Wyatt Run	0.0	3.8	0.0	3.8	46.7	59.2	10.9	66.7	47.0	66.7	10.9	66.7
Avg. Shale Gas Disturbance (%): StreamGas (SG)												
All study streams (n = 14)	23.1 ± 6.8		24.8 ± 7.0		32.3 ± 6.3		17.3 ± 5.7		21.5 ± 6.4		18.7 ± 6.0	
Consistently monitored (n = 9)	25.2 ± 7.1		24.8 ± 7.0		32.3 ± 6.3		22.0 ± 6.6		27.4 ± 7.2		23.7 ± 7.0	
Avg. Potential Runoff (%): StreamRunoff (SR)												
All study streams (n = 14)	13.1 ± 3.8		14.2 ± 4.3		19.3 ± 5.7		24.2 ± 5.5		24.2 ± 5.5		24.2 ± 5.5	
Consistently monitored (n = 9)	14.6 ± 4.5		14.9 ± 4.7		20.5 ± 6.2		28.9 ± 7.1		28.9 ± 7.1		28.9 ± 7.1	

*Not monitored

Table 2. Variables used in analyses evaluating the demographic response of Louisiana Waterthrush to shale gas development. Nest survival is daily survival rate (DSR) over a 29-day nesting period (DSR^{29}).

Variable of interest	**Notation**	**Analysis**
Intercept only	CONSTANT (C)	Nest Survival
Year of Study	Year	Nest Survival, Productivity, Territory Density, Territory Length, Riparian Habitat Quality
Nest Age	NestAge	Nest Survival
Time within-season (quadratic time trend)	TT	Nest Survival
Average Daily Rainfall	Rain	Nest Survival
Shale Gas Nest Disturbance (undisturbed=0, disturbed=1)	NestGas	Nest Survival
Percent of Stream Disturbed by Shale Gas	StreamGas	Territory Density, Nest Abandonment
Percent of Stream with Potential Contaminant Runoff	StreamRunoff	Description only
Percent of Territory Disturbed by Shale Gas	TerrGas	Nest Survival, Productivity, Source-sink Threshold, Riparian Habitat Quality
Percent of Territory with Potential Contaminant Runoff	TerrRunoff	Nest Survival
Shale Gas Forest Canopy Disturbance (%)	GasFCD	Nest Survival
Non-gas Forest Canopy Disturbance (%)	OtherFCD	Nest Survival
Habitat Suitability Index (HSI) score	HSI	Riparian Habitat Quality
Environmental Protection Agency (EPA) Rapid Bioassessment index	EPA	Riparian Habitat Quality

Table 3. Louisiana Waterthrush demography across six years in response to shale gas development at Lewis Wetzel Wildlife Management Area, WV. Population productivity is mean number of fledglings/successful nest/year multiplied by annual nest survival and average annual territory density/km of stream. Also shown are average annual EPA and habitat suitability index (HSI) scores ± SE. A larger EPA or HSI score indicates better riparian habitat quality.

Year	**Territory Density (territories /km)**	**Territory Length (m)**	**Daily Survival Rate (DSR)**	**Nest Survival (DSR^{29})**	**# of Nests**	**% Nests Parasitized**	**Population Productivity (per km) ± 95% CIs**	**EPA Index (range 0–200)**	**HSI (range 0–1)**
2009	1.5 ± 0.1	573.7 ± 23.9	96.4 ± 0.9	34.3 ± 8.9	41	0.0	2.32 ± 0.59	171.8 ± 2.0	0.82 ± 0.02
2010	1.8 ± 0.1	443.3 ± 23.1	98.2 ± 0.5	59.8 ± 9.3	39	2.6	4.63 ± 0.33	154.8 ± 1.9	0.79 ± 0.02
2011	1.5 ± 0.1	556.4 ± 31.2	96.7 ± 0.7	38.0 ± 8.0	47	0.0	2.56 ± 0.25	158.6 ± 1.8	0.78 ± 0.02
2013	1.2 ± 0.1	659.0 ± 34.3	95.8 ± 0.7	28.5 ± 6.1	65	4.6	1.61 ± 0.25	148.9 ± 2.1	0.76 ± 0.02
2014	1.1 ± 0.1	772.1 ± 41.9	95.4 ± 0.7	25.7 ± 5.8	54	5.6	1.16 ± 0.35	165.6 ± 2.2	0.77 ± 0.02
2015	1.0 ± 0.1	815.9 ± 49.3	96.1 ± 0.9	31.9 ± 8.4	34	3.0	1.31 ± 0.42	*	*

*not sampled in 2015

Table 4. AICc model results of 11 a priori nest survival models using Program MARK. See Table 2 for model notation. ΔAICc = difference from the top model, w_i = Akaike weight, and K = the number of parameters in each model.

Model	**ΔAICc**	***w_i***	***K***
C + Rain + NestAge + TT + TerrRunoff	0.00[a]	0.28	6
C + Rain + NestAge + TT + TerrGas	0.04	0.27	6
C + Rain + NestAge + TT + GasFCD	1.87	0.10	6
C + Rain + NestAge + TT + NestGas	1.97	0.10	6
C + Rain + NestAge + TT + OtherFCD	2.23	0.09	6
C + Rain + NestAge + TT + Year + TerrGas	2.97	0.06	11
C + Rain + NestAge + TT + Year + TerrRunoff	4.79	0.02	11
C + Rain + NestAge + TT + Year + GasFCD	5.59	0.01	11
C + Rain + NestAge + TT + Year + NestGas	5.59	0.01	11
C + Rain + NestAge + TT + Year + OtherFCD	5.92	0.01	11
CONSTANT (C)	9.72	<0.00	1

[a] The AICc value of the top model was 765.17

Table 5. Summary results for nest survival covariates (n = 7) from the top supported AICc models (n = 4) based on model-averaged regression coefficients, with unconditional standard error (SE) and 85% confidence intervals. Significant covariates with non-overlapping confidence intervals are bolded.

Parameter	**Estimate**	**Unconditional SE**	**Confidence Interval**
Rain	**0.207**	**0.064**	**0.114, 0.299**
TerrGas	**-0.004**	**0.001**	**-0.005, -0.003**
TerrRunoff	**-0.003**	**0.001**	**-0.005, -0.002**
NestGas	**-0.109**	**0.023**	**-0.142, -0.075**
GasFCD	**0.012**	**0.002**	**0.008, 0.015**
NestAge	-0.024	0.017	-0.049, 0.002
TT	0.010	0.042	-0.051, 0.070

FIGURES

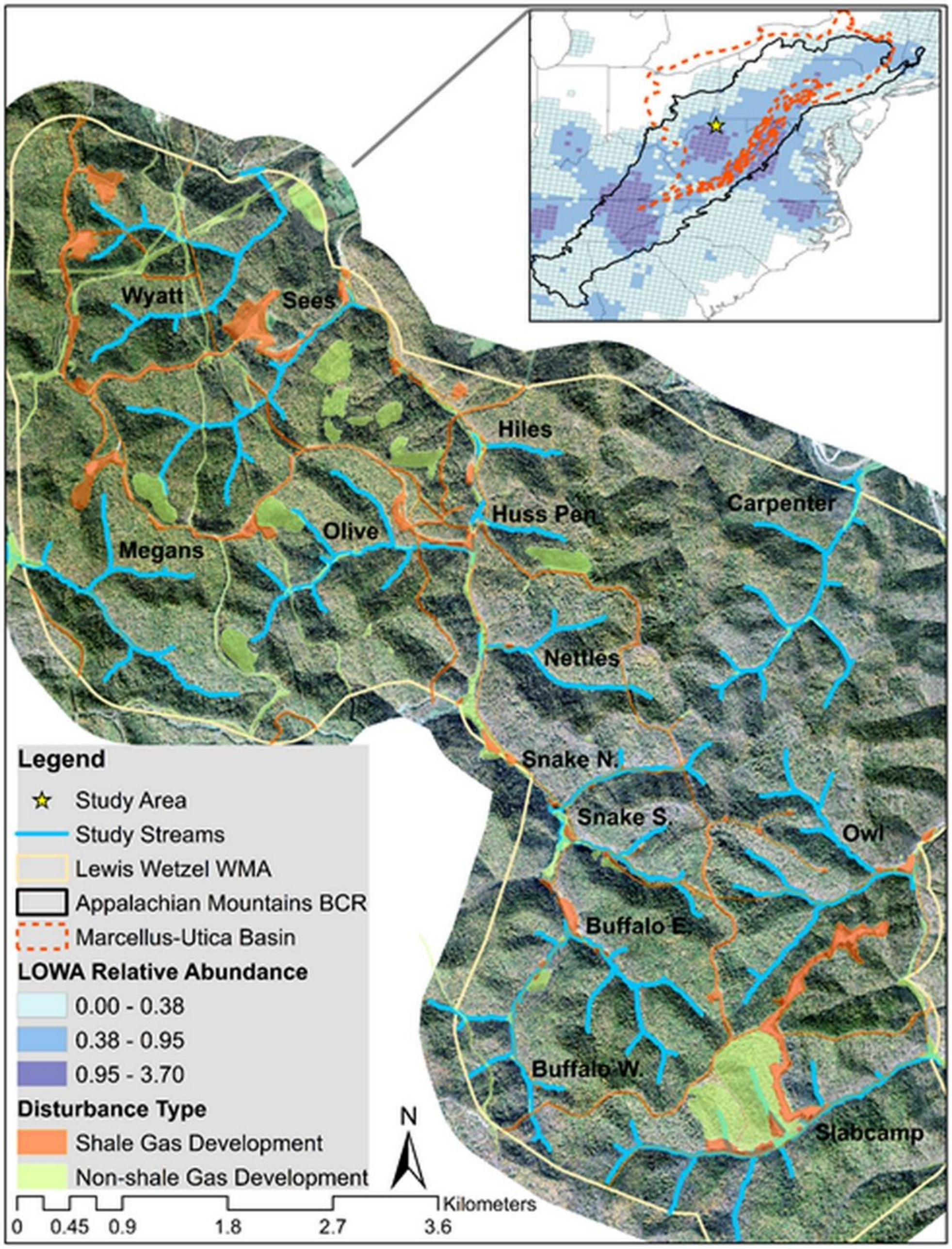

Figure 1. Our study area includes fourteen forested 1st and 2nd order headwater streams in the Lewis Wetzel Wildlife Management Area (WMA). It lies within the Marcellus-Utica shale basin, is within the core Louisiana Waterthrush (LOWA) range based on Breeding Bird Survey (BBS) data, and falls in the Appalachian Mountains Bird Conservation Region (BCR) in the eastern United States. LOWA Relative Abundance represents average waterthrush counts from nearby survey routes.

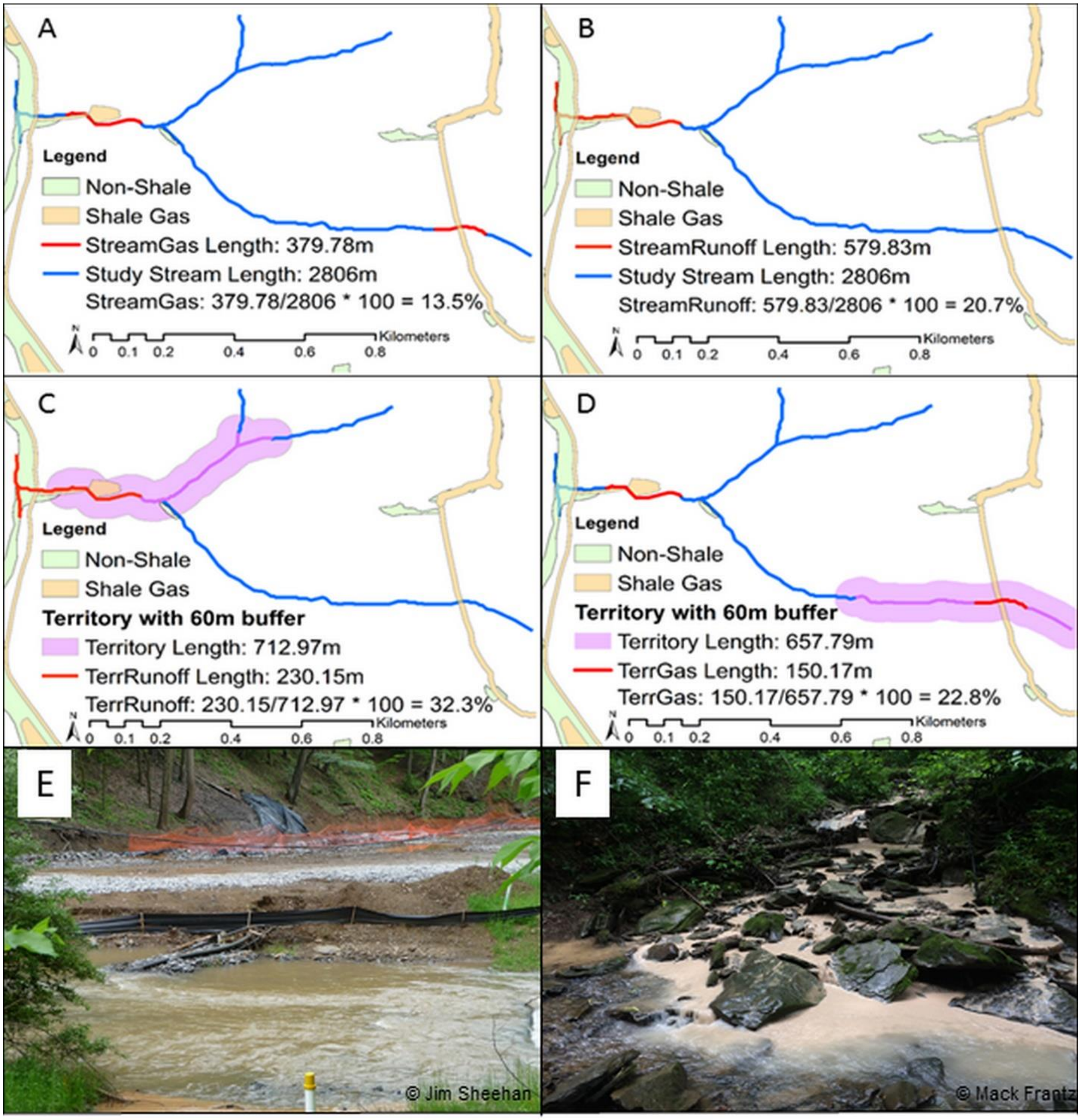

Figure 2. Examples of quantifying shale gas disturbances at the stream (A, B) and territory (C, D) scale and types of disturbance (E, F). A section of stream considered disturbed from frequent vehicular activity along a modified road (E). Sedimentation we traced upstream to its point of origin at an active shale gas well pad (F).

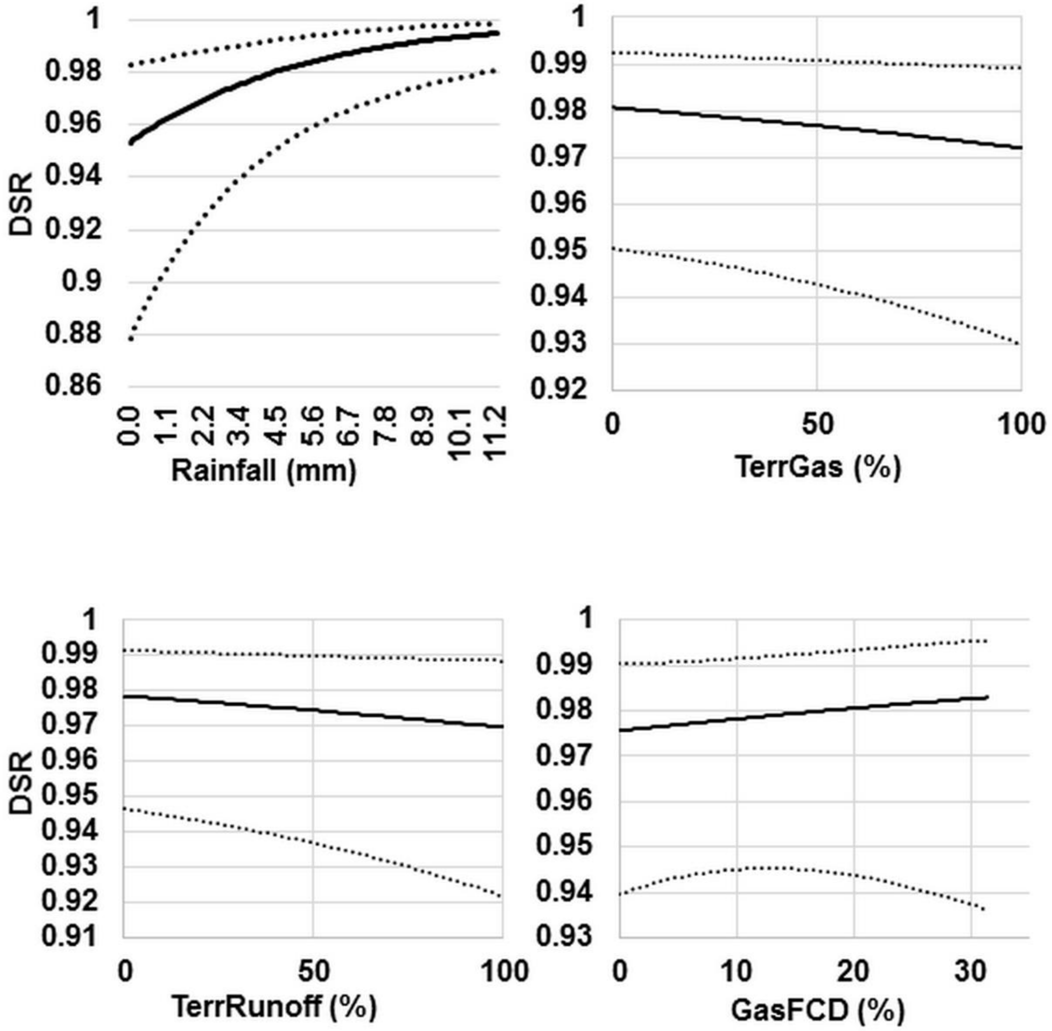

Figure 3. Four nest survival covariates that significantly influenced waterthrush daily survival rate (DSR). The binary response covariate NestGas also significantly influenced DSR (not depicted). See table 5 for model results and table 2 for covariate notation

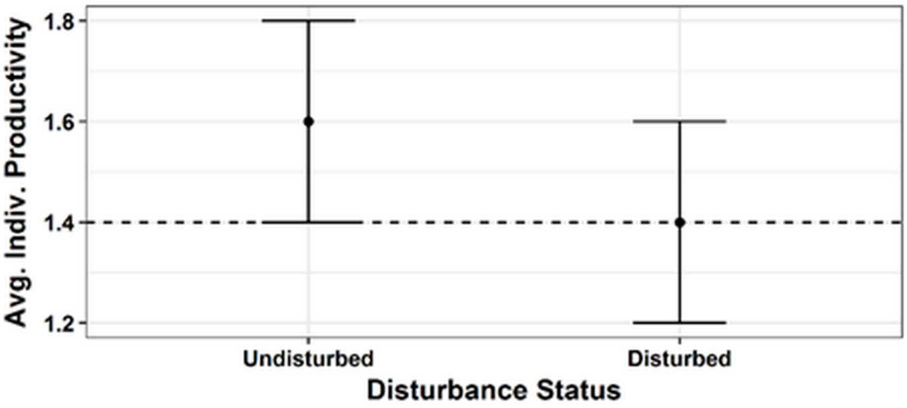

Figure 4. Average individual productivity (avg. # fledglings/successful nest/male * nest survival) ± 95% CIs in areas undisturbed and disturbed by shale gas to a source-sink threshold (gray bracketed line) of 1.4 fledglings.

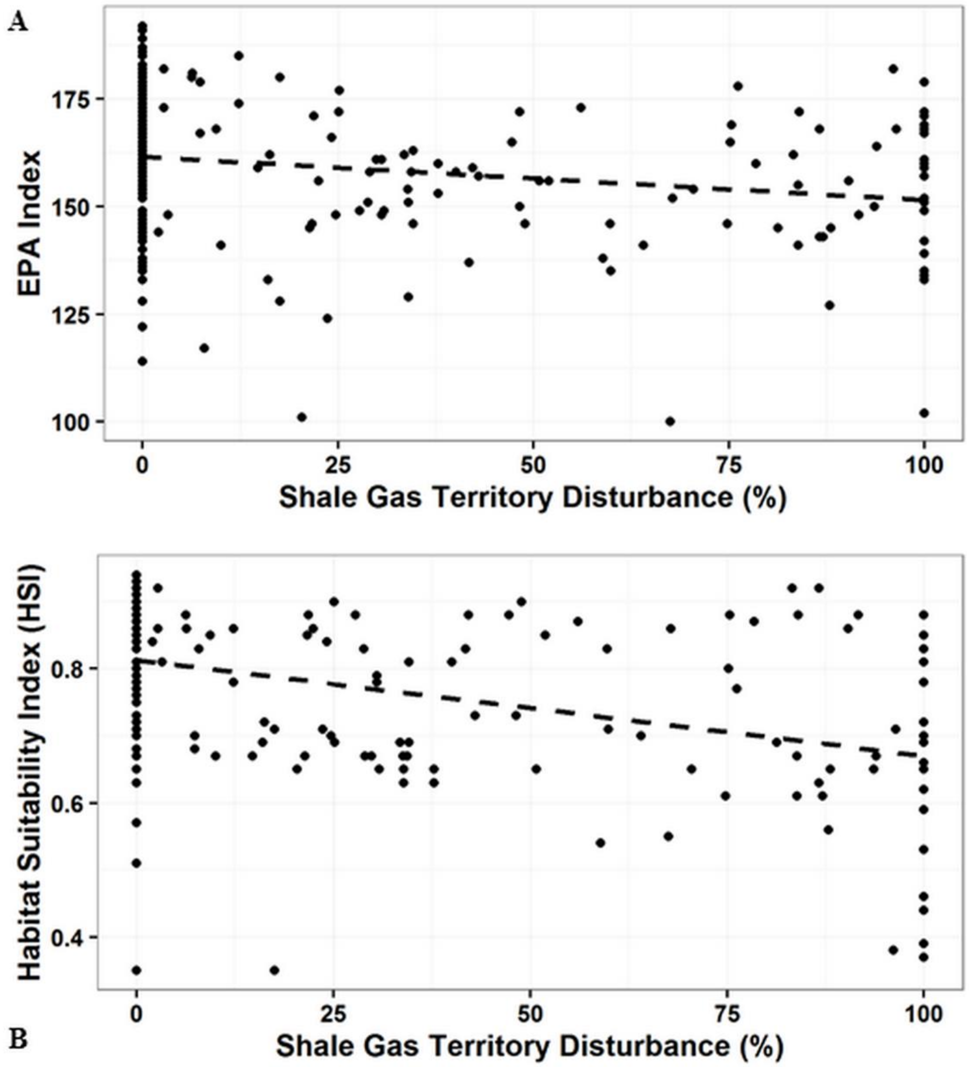

Figure 5. EPA Index and Habitat Suitability Index (HSI) scores in relation to TerrGas (A, B, respectively).

CHAPTER. 3. LOUISIANA WATERTHRUSH (*PARKESIA MOTACILLA*) SURVIVAL AND SITE FIDELITY IN AN AREA UNDERGOING SHALE GAS DEVELOPMENT.

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ABSTRACT

We quantified Louisiana Waterthrush (*Parkesia motacilla*) site fidelity and apparent survival across a 6 year period in an area undergoing shale gas development. Waterthrush initially exhibited high site fidelity that declined over time. At the same time, the number of unpaired males defending territories increased as did natal fidelity. We identified site fidelity factors that influenced if adult males and females returned. More males returned either due to or regardless of amount of shale gas disturbance and lower riparian habitat quality. Females were less likely to return with increased number of breeding attempts. Females in shale gas disturbed areas had a higher number of breeding attempts and lower individual productivity. We saw a general nonsignificant trend in declining apparent survival over time. Overall apparent survival estimates for adult males (0.56) and females (0.44) were similar to those reported for other populations. Apparent survival candidate models suggested weak, positive relationships of increased survival with shale gas territory disturbance, disturbance with year, and year for adult males, and a positive relationship of increased survival with hydraulic fracturing runoff for adult females although regression coefficients overlapped zero for all model-supported covariates implying no statistical significance. Since waterthrush can maintain pair bonds from the previous year and females must pick a nest site within the defended male's territory, there are potential conflicts between factors that influence adult survival and site fidelity that may affect long-term population persistence. Our study adds to previous evidence that shale gas disturbed areas may serve as sink habitats.

Key words: bioindicator, ecological trap, headwater stream, Marcellus-Utica, site fidelity, source-sink, survival

INTRODUCTION

The Louisiana Waterthrush (*Parkesia motacilla*; hereafter waterthrush) is a headwater stream specialist known for its ability to respond to changes in ecological conditions (Mattsson and Cooper 2006). Many of the forested stream ecosystems in which the waterthrush primarily breed have rapidly undergone unconventional shale gas development, particularly in the Marcellus-Utica shale region (Evans and Kiesecker 2014). The majority (71%) of the Appalachian region at greatest potential risk of energy development is forested, with shale gas overlapping 66% of this region (Dunscomb et al. 2014). The waterthrush is a species of conservation concern on the US Fish and Wildlife Service National List (USFWS 2008) due to its specialized habitat, and almost all of its core breeding range also overlaps the Marcellus-Utica shale region (Sauer et al. 2014).

Shale gas development can negatively affect waterthrush reproductive success and productivity (Frantz et al. 2018a) and their benthic macroinvertebrate prey (Wood et al. 2016, Frantz et al 2018b). However, it is not known if shale gas development can be a source of mortality, something that focal species-based modeling of potentially vulnerable taxa can answer (Brittingham et al. 2014). Stream disturbances such as acidification can affect waterthrush site fidelity (Mulvihill et al. 2008), so similar risks presented from shale gas are of particular interest (Souther et al. 2014), especially because waterthrush typically exhibit high site fidelity (Mulvihill et al. 2002, O'Connell et al. 2003) and forage on pollution-sensitive aquatic prey like Ephemeroptera (Trevelline et al. 2016). Across a 6 year study (2009–2011, 2013–2015), we quantified waterthrush annual first-year return rates (i.e., site fidelity), factors that might affect annual site fidelity, and apparent annual survival across 14 headwater streams with varying amounts of shale gas disturbance. Given that we observed annual declines in waterthrush habitat quality, nest survival, and productivity at our study site (Frantz et al. 2018a), we hypothesized that shale gas development would negatively affect site fidelity and apparent survival because site fidelity can directly influence fecundity and survival of individuals (Hoover 2003).

METHODS

Study area

We studied waterthrush along 14 1st- and 2nd-order headwater stream tributaries that totaled 58.1 km at Lewis Wetzel Wildlife Management Area (LWWMA) located in Wetzel County, West Virginia, USA (Frantz et al. 2018a, 2018b). The study area overlays the

Marcellus-Utica shale region, covers 6,521 ha (Farwell et al. 2016), and occurs where waterthrush reach their highest densities within the central Appalachians (Sauer et al. 2014). In 2009–2010, the majority of Marcellus wells and their water-holding ponds were located along the main stem of Buffalo Run where the majority of our 14 headwater study streams empty. Thus, although a few Marcellus well pads were located along our study streams, they tended to primarily impact the lower portions. Between the 2010 and 2011 breeding seasons, shale gas development activities began to increase on the ridgetops. Clearing for additional new well pads occurred late in the 2013 breeding season with well pad completion in 2014, in addition to redrilling of an existing well pad. There was no new shale gas development or activity in the 2015 breeding season. Shale gas disturbance on streams was highly variable within each year (Fig. 1), but on average across all years streams had 22.5 ± 2.6% of their length disturbed by shale gas development (range 0–67%). There were no "control" streams given that the majority of forest loss and fragmentation in the surrounding landscape resulted from recent shale gas activities (Farwell et al. 2016), but shale gas disturbance was concentrated on some streams more than others (Fig. 1; Frantz et al. 2018a).

Stream and territory disturbance mapping

Within a Geographic Information System (GIS), we used a sequence of leaf-on and leaf-off aerial imagery and extensive ground-truthing to manually digitize areas of disturbance within the study area. All disturbances were classified as shale gas related (i.e., well pads and associated road and pipeline infrastructure) or as being unrelated or pre-existing (e.g., powerline right-of-way). Gas well records (WVDEP 2015) were used to verify target shale formations, drilling status, and start dates for all well disturbances. Surface (i.e., 3D) lengths for each study stream vector were calculated in the GIS using a 3 m resolution digital elevation model and defined to have a drainage basin of 22.25 acres to delineate the uppermost headwater reaches.

To model site fidelity and apparent survival as a function of shale gas disturbance, we created 2 continuous variables based on our disturbance classifications. For each year of the study, we calculated the proportion of each study stream that was disturbed by shale gas (stream length disturbed divided by total stream length monitored). Any portion of a stream was considered disturbed where well pads or infrastructure were within 60 m of the stream centerline, which is the typical extent of waterthrush streamside use (Mattsson and Cooper 2009). Frequent vehicular activity on roads resulting from shale gas within 60 m of the streams additionally counted as disturbance, which was commonplace in 2009–2011. Stream reaches also were considered disturbed when sedimentation within a reach resulted from shale gas development (e.g., well pad construction) at any distance from the stream, resulting in the entire downstream network classified as disturbed. All of these stream disturbances collectively describe mostly localized streamside disturbance indicative of the presence of any shale gas infrastructure or activity, and we calculated a variable called TerrGas that was the proportion of each territory affected by these shale gas related disturbances. We created a second disturbance variable, TerrRunoff, focused solely on potential shale gas contaminant erosional runoff. For this variable, a stream reach was considered disturbed if it was directly at or below a well pad or retaining pond (similar to Latta et al. 2015), resulting in the whole downstream network classified as being at risk for surface pollution based on elevational maps and ground truthing. Thus, TerrRunoff was a broader, distance-independent, disturbance category related strictly to water quality.

We annually mapped waterthrush territories along each stream (see Frantz et al. 2018a for detailed methods). We then placed the 60 m buffer over individual waterthrush territory vectors, mapped on the same stream reach centerlines, to calculate the proportion (0–100%) of each territory disturbed by shale gas (territory length disturbed divided by total territory length); this created a TerrGas and TerrRunoff variable for each territory.

Louisiana Waterthrush banding and resighting

Territorial adult waterthrush were target-netted and banded during 2009–2011 and 2013–2015 with an aluminum US Geological Survey leg band and a unique combination of colored plastic bands to allow identification of individuals. Starting in 2010, nestlings were banded ~7–8 days after hatching. All study streams (n = 14) were searched intensively (≥5 stream visits) throughout each breeding season for banded individuals during territory mapping and nest monitoring (see Frantz et al. 2018 for full description). We used resightings of banded individuals to calculate first-year return rates as a measure of site fidelity, to calculate adult apparent survival, to aid in territory delineation, and to calculate male pairing rate (% males paired/year). We calculated pairing rate only for 2013–2015 since only records of banded females were kept prior to 2013.

First-year return rates were the proportion of newly color-banded individuals that were resighted the following year (e.g., Iverson 1988), in contrast to return rates including individuals banded in any given year (e.g., Hoover 2003), so that annual return rates reflected the amount of shale gas disturbance that occurred the year an individual was banded. Apparent survival was the probability an adult waterthrush remained alive and available for resighting, and therefore is not true survival probability of marked waterthrush in our population since mortality and permanent emigration cannot be distinguished (White and Burnham 1999, Lindberg and Rexstad 2002).

Nest and riparian habitat monitoring

Nest searching and monitoring occurred concurrently with territory mapping in the same manner as Wood et al. (2016) and Frantz et al. (2018a). We monitored most nests every 3–4 days initially and more frequently as fledging approached (Martin and Geupel 1993). We assumed an undamaged empty nest had fledged if the nest was active the day before and had approached the predicted fledge date. Waterthrush will have multiple breeding attempts if their nest fails (average 1.4 ± 0.05 attempts; MWF, unpubl. data). A pair was considered successful (i.e., nest success) if they produced at least one fledgling from their attempts.

Riparian habitat quality at each nest monitored was assessed as a factor influencing site fidelity using the Habitat Suitability Index specifically designed for waterthrush (hereafter HSI; Prosser and Brooks 1998) and the US EPA Rapid Bioassessment Protocol for high gradient streams (hereafter EPA; Barbour et al. 1999) in the same manner as Wood et al. (2016) and Frantz et al. (2018). The HSI is a broad-scale evaluation of waterthrush instream foraging and upland habitat suitability (Prosser and Brooks 1998) while EPA assesses stream quality based primarily on instream characteristics that relate to the abundance and composition of waterthrush aquatic macroinvertebrate prey (Wood et al. 2016, Frantz et al. 2018b).

Analysis

Return rates and site fidelity—We calculated apparent first-year return rates, hereafter site fidelity, as the number of newly banded individuals divided by the number that were resighted the following year for all adults, adult males, adult females, and nestlings to evaluate general trends in annual site fidelity for all years of study except 2013. Since the study did not occur in 2012, the 2013 return rate was based on individuals banded in 2011. Although we recognize this return rate is likely a low estimate, we felt it provided valuable information for examining the overall trend across years.

Site fidelity is high and persistent in waterthrush, thus annual return has been used as a proxy for survivorship (Latta et al. 2016) and also can be used to determine factors that may influence site fidelity (e.g., “decision rules” in Stracey and Robinson 2012). A conditional inference tree (ctree) analysis was performed separately for adult male and female waterthrush to determine what variables might affect the decision to return to a site. Return was modeled as a binomial response (returned or did not return the following year) in relation to TerrGas, TerrRunoff, nest success (yes/no), number of breeding attempts, HSI score, and EPA index in individually fitted models. The ctree analysis involved binary recursive partitioning and permutational testing on the site fidelity dataset structures with all returns for individuals included (n = 149 male and 35 female returns) using partykit (Hothorn and Zeileis 2016), with test statistics and P values obtained from a structural change test using strucchange (Zeileis et al. 2015) in program R (R Core Team 2014). Ctree is a nonparametric method that avoids overfitting, is less susceptible than traditional regression techniques to multicollinearity, and stops splitting the data once the null hypothesis of independence cannot be rejected ($\alpha = 0.05$; Hothorn and Zeileis 2016).

If any site fidelity factors were significant for males or females, we compared the average site fidelity factor value ± standard error (SE) between return and no return groups of the sex in question. If nest success or number of breeding attempts was significant for males or females, we also compared individual nest productivity between return and no return groups of the sex in question since productivity was lower in shale gas disturbed areas in our long-term study (Frantz et al. 2018a). We quantified individual productivity using an approach similar to Boves et al. (2015) where mean number of fledglings per successful nest per male was multiplied by nest survival (e.g., Frantz et al. 2018a). Additionally if TerrGas or TerrRunoff was a significant site fidelity factor for either sex, we compared these variables between areas with and without shale gas disturbance for each sex.

Apparent survival—We modeled apparent adult survival (ϕ) using the Cormack–Jolly–Seber (Cormack 1964, Jolly 1965, Seber 1965) model using RMark (Laake 2013) in R. We removed any individuals from analysis that were not confirmed as part of a breeding territory to avoid modeling possible transients (n = 12 males, n = 2 females). We modeled banded male adults (n = 94) separately from females since fewer females (n = 26) were banded and thus had a dissimilar encounter history. Encounter probability (p) was kept constant in all models because we assumed color bands were not lost and equal resight efforts occurred across years. Overall apparent survival from the constant model (covariate-independent null model) was estimated for males and females separately for relative comparison with survival values reported in other studies (e.g., Boulton et al. 2009). We also report survival estimates from the top supported male and female models to be inclusive of any differences.

We evaluated 3 covariates in models for males to determine if there was a disturbance level or annual influence to apparent survival: avgTerrGas, avgTerrRunoff, and year. The covariate avgTerrGas was the average of shale gas territory disturbance (i.e., TerrGas) across all years that an individual returned. The covariate avgTerrRunoff was the average potential hydraulic fracturing runoff (i.e., TerrRunoff) across all years an individual returned. Given high site fidelity in waterthrush (O'Connell et al. 2003), we felt avgTerrGas or avgTerrRunoff would indicate if there was an overall disturbance or runoff (e.g., sedimentation or surface water pollution) level that affected survival rather than modeling only initial disturbance levels. For males, we included all additive and interactive models for avgTerrGas and avgTerrRunoff with year. The 2 gas disturbance covariates were not combined in a single model since they are related metrics ($R^2 = 0.53$ males, 0.63 females).

We modeled year as we hypothesized that apparent survival would decrease in concert with decreasing return rates. Time intervals between occasions (year) were specified in the model, which corrects for inconsistent lengths of time (i.e., no data collected in 2012). For females, however, we could only model avgTerrGas and avgTerrRunoff because the sample size was too small to include a time-dependence parameter.

We used Akaike's Information Criterion for small sample sizes (AICc, as opposed to QAICc), to evaluate support for apparent survival candidate models (Burnham and Anderson 2002) based on lack of overdispersion using a bootstrapped goodness-of-fit test (Cooch and White 2016). We considered the model with the lowest AICc value to be the best-supported model given the data, and any models with ΔAICc<2 were considered plausible (Burnham and Anderson 2002). We assessed the relative plausibility of each model in each model set by comparing Akaike weights (w_i). We used model-averaged regression coefficients across all models that contained the covariate (Burnham and Anderson 2002) and 85% confidence intervals (hereafter CIs) to infer biological importance of covariates in plausible AICc models because 95% CIs with the information-theoretic approach can lead to variable selection uncertainty (Arnold 2010).

RESULTS

In our 6 year study, we banded 187 adult and 222 nestling waterthrush. Adult waterthrush initially exhibited high site fidelity (>63% during 2009–2010) that declined annually to 32% by 2015 (Table 1). Males and females also had declining trends. Resighting effort was ≥5 visits per stream in 2009–2011 and ≥6 visits per stream in 2013–2015, which suggests that the decline in site fidelity was not a result of decreased effort. Natal site fidelity was consistently low across years (Table 1) with a high of 5% of banded nestlings resighted in 2015.

Of the 6 factors we tested, 3 influenced site fidelity of adult males and one influenced that of adult females (Table 2, Fig. 2). Males that returned had higher TerrGas (37.0 ± 4.6%) values than those that did not return (25.1 ± 4.1%; $S_{147} = 3.75$, $P = 0.053$). Males that returned had lower EPA scores (154.8 ± 2.2) than those that did not return (160.8 ± 1.9 score; $S_{104} = 4.17$, $P = 0.041$). Males that returned also had lower HSI scores (0.73 ± 0.02) than those that did not return (0.78 ± 0.01 score; $S_{104} = 5.30$, $P = 0.021$). For females, individuals that did not return had more breeding attempts (2.1 ± 0.3) than females that did return (1.3 ± 0.1 attempts; Fig. 3, Table 2) ($S_{30} = 3.79$, $P = 0.051$).

Male apparent survival (ϕ) using the constant model was 0.56 ± 0.04 and 0.55 ± 0.04 from the top supported model (avgTerrGas). For males, 3 models were supported (ΔAICc<2), which included avgTerrGas, avgTerrGas plus year, and year (Table 3) with model weights of 0.33, 0.26, and 0.21, respectively. Model-averaged regression coefficient estimates for avgTerrGas and year were positive but 85% CIs overlapped zero for avgTerrGas and year indicating little or highly variable effect on male survival (Table 4). Yearly survival peaked in 2011 but in general decreased from the beginning to the end of the study (Table 1).

Female survival using the constant model was 0.44 ± 0.08 and 0.43 ± 0.08 from the top supported model (avgTerrRunoff). The constant model had the most support with weight of 0.54 but the avgTerrRunoff model competed with weight of 0.24 (Table 3). The regression coefficient estimate for avgTerrRunoff was positive, and while having model support, CIs overlapped zero indicating little or high variable effect on females (Table 4).

DISCUSSION

Over our 6 year study at LWWMA, we saw general declines in waterthrush first-year return rates concurrent with a site-wide increase in shale gas-related disturbance (Farwell et al. 2016). However, our study could not distinguish whether more males returned either due to or regardless of amount of shale gas territory disturbance. Waterthrush are known to exhibit high site fidelity (~40%; O'Connell et al. 2003), with males returning to the same territory annually and up to 50% of returning females occupying the same territories, frequently reuniting with the same male (Mulvihill et al. 2002). Observed return rates are the product of resighting probability, annual survival, and site fidelity (Schlossberg 2009). Given that we had thorough resight effort on our streams, return rates reflected some combination of annual survival and site fidelity. Apparent survival of adults in our study (males 0.56, females 0.44) was similar to the waterthrush survival rate of 0.47 reported for the northeastern United States (Mattsson et al. 2009). While year was not a significant covariate in apparent survival analysis, in general yearly survival peaked early in the study then declined toward the end of the study parallel to declining return rates (Table 1).

Natal fidelity is typically low in migratory passerines (Weatherhead and Forbes 1994, Schlossberg 2009) including waterthrush (O'Connell et al. 2003) as we also found in our study area (1.7 ± 1.2% resighted). Territory densities were high (>1.5 territories/km) in 2009–2011 compared to when the study ended (1.0 ± 0.1 territories/km; Frantz et al. 2018a); while there was a general increase in the number of nestlings banded over the study period, we did not document natal resights until 2014 and 2015 (Table 1) when territory densities were lowest. Increased natal fidelity was not enough to counter adult loss where the percent of unpaired males went from 0% in 2013, when the decline in adult site fidelity was first noticeable, to >10% in 2014 and 2015. Natal dispersal of waterthrush at our study area could be density-dependent (Greenwood and Harvey 1982) because when areas are saturated, dispersing may enhance individual survival or reproductive prospects of finding unoccupied, suitable breeding habitat (Förschler et al. 2010). Information on natal dispersal is currently lacking (Mattsson et al. 2009), and vital when shale gas development has the potential to heavily develop forest core ecosystems where headwater streams (Drohan et al. 2012) and waterthrush co-occur.

Covariates we tested to explain survival were inconclusive, but avgTerrGas and avgTerrRunoff may have positive relationships with male and female survival (Tables 3 and 4). This is in line with site fidelity factors for males where more males returned either due to or regardless of higher shale gas territory disturbance and lower riparian habitat quality (Table 2). Waterthrush frequently retain pair bonds (Mattsson et al. 2009), which may put factors that influence male and female site fidelity or nesting decisions at odds; in almost all cases where the banded male and female both returned from the previous year (71%, 5 of 7 pairs), they retained that bond in our study (MWF, unpubl. data). The number of breeding attempts can affect female return, and females with no shale gas territory disturbance had a lower number of breeding attempts than did females in disturbed areas (Table 2). Shale gas development had negative effects on nest survival and productivity in our long-term study (Frantz et al. 2018a). If we evaluate individual productivity adjusted for nest survival (i.e., Boves et al. 2015, Frantz et al. 2018a), females in shale gas disturbed areas also had lower productivity than did those in shale gas undisturbed areas (Table 2).

In the same manner that choice of nest site can create opposing selection pressures on nest success and fledgling survival (Streby et al. 2014), waterthrush may face perceived costs and benefits from TerrGas or TerrRunoff in a territory with its own survival and nest survival in conflict. If factors that influence site fidelity via annual return can also be a measure of survivorship (Latta et al. 2016), factors that affect reproductive success may also influence female return. This is particularly a concern if males who arrive first to set up a territory (Mattsson et al. 2009), and consequently females who must select a nest site within the defended male's territory, are initially "drawn" to shale gas disturbed areas. Shale gas disturbed areas may provide lower predation risk but lead to higher nest abandonment and parasitism (Davis 2014). Shale gas disturbed areas also can alter aquatic food webs (Grant et al. 2016) and benthic communities (Wood et al. 2016, Frantz et al. 2018b), the primary food of waterthrush. For instance, it may alter leaf pack breakdown creating patch-specific communities with more macroinvertebrates and shredders than sites without shale gas (Barton 2016). Waterthrush will leaf-pull at dead leaves submerged in water (Mattsson et al. 2009), but a benthic study in 2011 (Wood et al. 2016) and 2013–2014 (G.T. Merovich, Jr., West Virginia University, unpubl. data) at our study site suggest shale gas development negatively altered benthic macroinvertebrate communities. Waterthrush at our study site were likely able to meet all their foraging needs (Frantz et al. 2018b), so it is unclear otherwise why waterthrush would be "attracted" to these areas. For example, waterthrush can forage off-stream on terrestrial invertebrates or in undisturbed areas to compensate for loss of preferred aquatic prey (Mulvihill et al. 2008) and as observed on our study area (MWF and PBW, unpubl. data). Since other types of forest anthropogenic disturbances can serve as ecological traps (Weldon and Haddad 2005, Boves et al.

2013), concern is warranted when shale gas disturbed areas may be borderline “sink” (Pulliam 1988) habitats (Frantz et al. 2018a).

We cannot firmly establish if shale gas development was the causal mechanism for declining return rates. In the same manner that habitat specialists can exhibit delay to extinction until a new equilibrium is reached following habitat loss (i.e., extinction debt; Kuussaari et al. 2009), there may be a time lag in potential negative influences of shale gas development on waterthrush site fidelity and survival. Initial collective evidence from this and our long-term demographic study suggests shale gas development may have conflicting effects on waterthrush site fidelity and apparent survival that in the long term may negatively affect population persistence by impairing reproductive effort and productivity. However, if population growth rates are governed more through adult survival probabilities than reproductive rates (Sæther and Bakke 2000), there is the possibility population growth rates could be higher in shale gas disturbed areas. Low sample size (<100) can affect accuracy of typical survivorship analysis (Naef-Daenzer and Grüebler 2014), and there are carryover effects for this species as a result of body condition (Latta et al. 2016). As such, continued and full annual cycle monitoring will be required for more support and to understand all contributions to adult survival. Strong evidence is required that a habitat serves as an ecological trap (Robertson and Hutto 2006). Waterthrush need to be studied on a regional scale (Brawn and Robinson 1996) to know the extent to which shale gas disturbed areas could serve as potential sinks or ecological traps (Gates and Gysel 1978), and whether these are temporal, dynamic relationships (Fauth 2000).

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TABLES

Table 1. Annual percent apparent site fidelity (# banded in previous year divided by # resighted) for adult and nestling waterthrush. Annual adult male apparent survival (ϕ) and standard error (SE) from the year-only covariate model. Annual adult apparent survival was modeled for males only since female sample size was too small for a time-dependence parameter. Year interval represents a year +1 time interval for reporting apparent site fidelity and survival from the previous year. The study did not occur in 2012, so the 2011–2013 return rates are for individuals banded in 2011. Note only one female was banded in 2011 for the 0% female return rate from 2011–2013. Years where fidelity or survival could not be calculated are indicated with "–" either due to no newly banded waterthrush the previous year or because the study ended in 2015.

	Number Banded				Percent apparent site fidelity				
Year	Male	Female	Nestling	Year interval	Male	Female	All adult	Nestling	Male ϕ (SE)
2009	11	0	0	2009–2010	63.6	–	63.6	–	0.56 (0.17)
2010	17	3	10	2010–2011	64.7	67.0	65.0	0.0	0.63 (0.11)
2011	10	1	22	2011–2013	60.0	0.0	54.5	0.0	0.74 (0.07)
2013	47	14	112	2013–2014	42.5	57.1	45.9	1.8	0.53 (0.07)
2014	21	10	78	2014–2015	33.3	30.0	32.3	5.1	0.39 (0.07)
2015	33	20	0	–	–	–	–	–	–

Table 2. A descriptive summary of site fidelity factors (± standard error, SE) for adult male and female waterthrush that differed between returned vs. did not return birds (noted with *) and compared between shale gas disturbed and undisturbed territories. Productivity is the number of fledglings produced adjusted for nest survival in shale gas disturbed/undisturbed territories in the manner of Boves et al. (2015) and Frantz et al. (2018a). Higher EPA (range: 0–200) and HSI (range: 0–1) scores are indicative of better riparian habitat quality for waterthrush and their aquatic prey.

Return/Disturbance status	**Sample total**	**% TerrGas***	**EPA***	**HSI***
Males (*n* = 149)				
No return, TerrGas disturbance	52% (39 of 75)	48.3 (5.7)	159.9 (2.4)	0.78 (0.02)
No return, No TerrGas disturbance	48% (36 of 75)	0	161.7 (2.98)	0.79 (0.02)
Return, TerrGas disturbance	62% (46 of 74)	59.5 (4.9)	152.5 (3.1)	0.68 (0.02)
Return, No TerrGas disturbance	38% (28 of 74)	0	158.2 (2.9)	0.82 (0.01)

Return/Disturbance status	**Sample total**	**% TerrGas**	**# Breeding attempts***	**Productivity**
Females (*n* = 35)				
No return, TerrGas disturbance	48% (10 of 21)	40.9 (11.5)	2.2 (0.3)	1.44 (0.01)
No return, No TerrGas disturbance	52% (11 of 21)	0	1.9 (0.4)	1.49 (0.02)
Return, TerrGas disturbance	57% (8 of 14)	54.1 (9.6)	1.4 (0.2)	1.50 (0.01)
Return, No TerrGas disturbance	43% (6 of 14)	0	1.3 (0.3)	1.61 (0.01)

Table 3. Cormack–Jolly–Seber candidate models used to estimate male and female apparent survival (ϕ). AvgTerrGas was the average of shale gas territory disturbance across all years that an individual returned. AvgTerrRunoff was the average of potential hydraulic fracturing runoff in a territory across all years that an individual returned. The covariate year modeled time dependency (4 parameters). ΔAICc = distance from the top model, w_i = Akaike weight, K = the number of parameters in each model. The model term ϕ (~1) indicates apparent survival was held constant. Model term *p* (~1) for encounter probability was held constant in every model. The constant model is ϕ (~1) *p* (~1).

Model	**AICc**	**ΔAICc**	w_i	***K***
Males (*n* = 94)				
ϕ (~avgTerrGas) *p* (~1)	225.41	0.00	0.33	3
ϕ (~avgTerrGas + year) *p* (~1)	225.89	0.48	0.26	7
ϕ (~year) *p* (~1)	226.29	0.88	0.21	6
ϕ (~avgTerrRunoff + year) *p* (~1)	228.18	2.76	0.08	7
ϕ (~1) *p* (~1)	228.25	2.84	0.08	2
ϕ (~avgTerrRunoff) *p* (~1)	229.92	4.51	0.03	3
ϕ (~avgTerrGas * year) *p* (~1)	232.66	7.25	0.01	10
ϕ (~avgTerrRunoff * year) *p* (~1)	247.40	22.00	<0.001	10
Females (*n* = 26)				
ϕ (~1) *p* (~1)	50.74	0.00	0.58	2
ϕ (~avgTerrRunoff) *p* (~1)	52.56	1.82	0.24	3
ϕ (~avgTerrGas) *p* (~1)	53.08	2.34	0.18	3

Table 4. Summary results for male and female apparent survival (ϕ) in the top supported models (i.e., models where ΔAICc<2). Male results are model-averaged regression coefficients while female results are unstandardized regression coefficients ± standard error (SE). AvgTerrGas was the average of shale gas territory disturbance across all years that an individual returned. AvgTerrRunoff was the average potential hydraulic fracturing runoff in a territory across all years an individual returned. The covariate year modeled time dependency. Model supported covariates (*n* = 3) were not significant and had overlapping confidence intervals.

Parameter	**Estimate**	**SE**	**85% confidence interval**
Males			
avgTerrGas	0.011	0.072	−0.092, 0.115
year	0.327	2.282	−2.958, 3.613
Females			
avgTerrRunoff	0.006	0.008	−0.005, 0.018

FIGURES

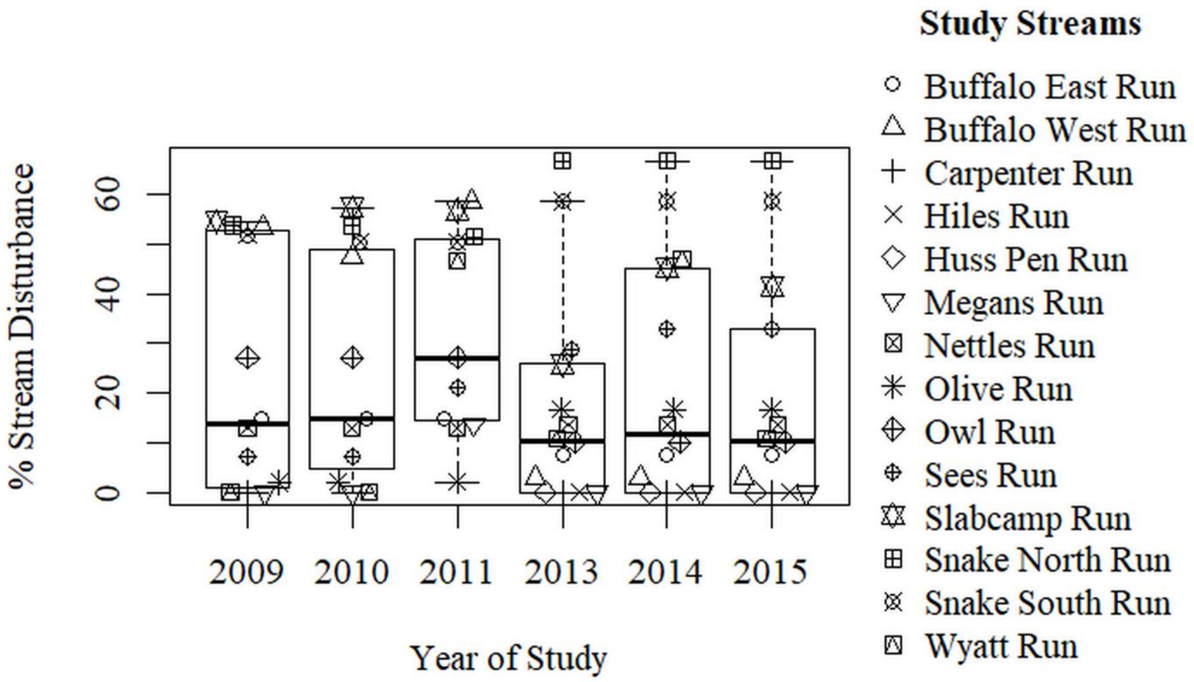

Figure 1. A box and whisker plot of disturbance on headwater streams (*n* = 14) resulting from shale gas development over a 6 year period (2009–2011, 2013–2015) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia.

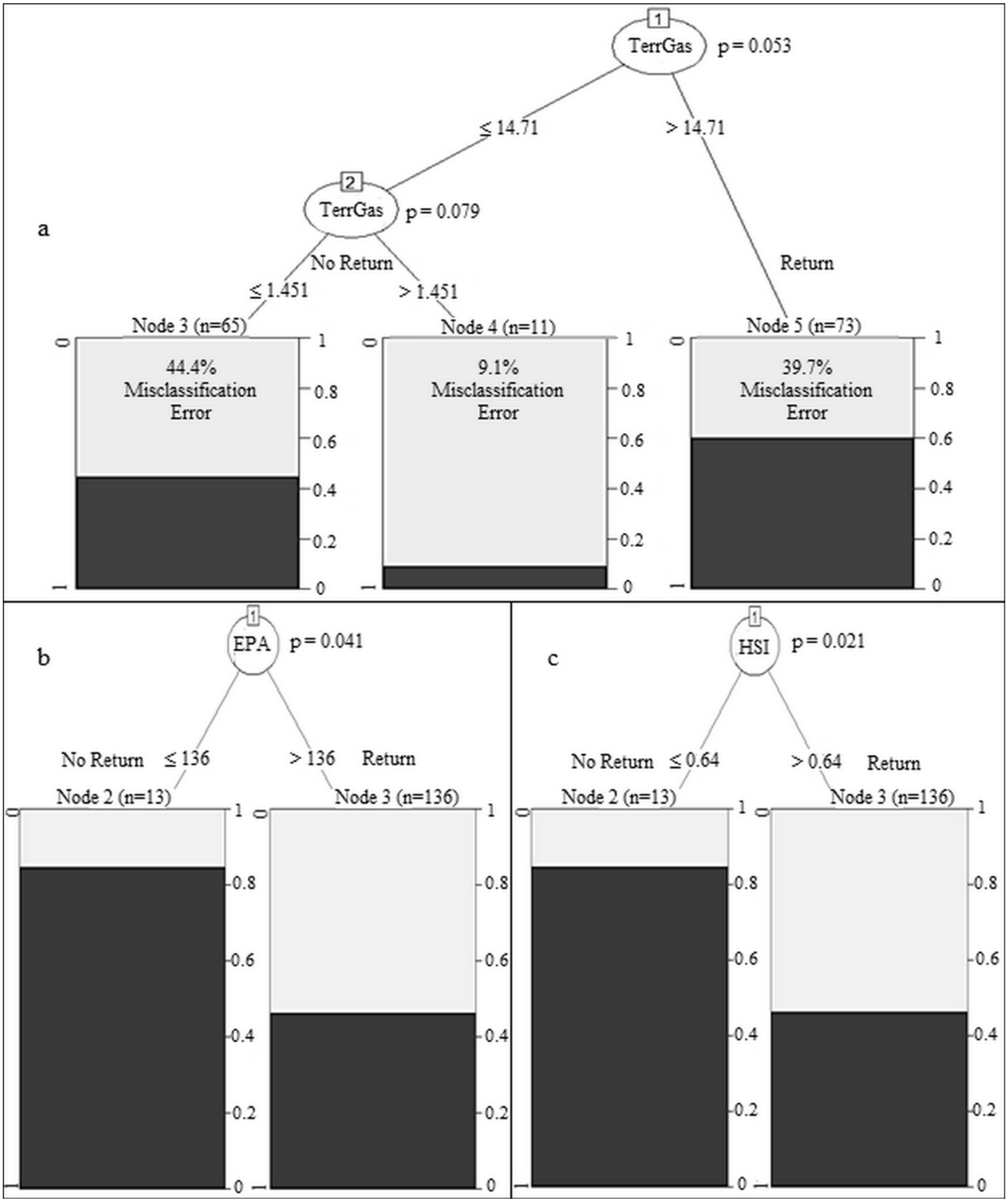

Figure 2. Conditional inference trees showing significant splits in data structure for TerrGas (a), EPA (b), and HSI (c) in explaining adult male waterthrush site fidelity, and percent misclassification error rate represented in assigning individuals to that group.

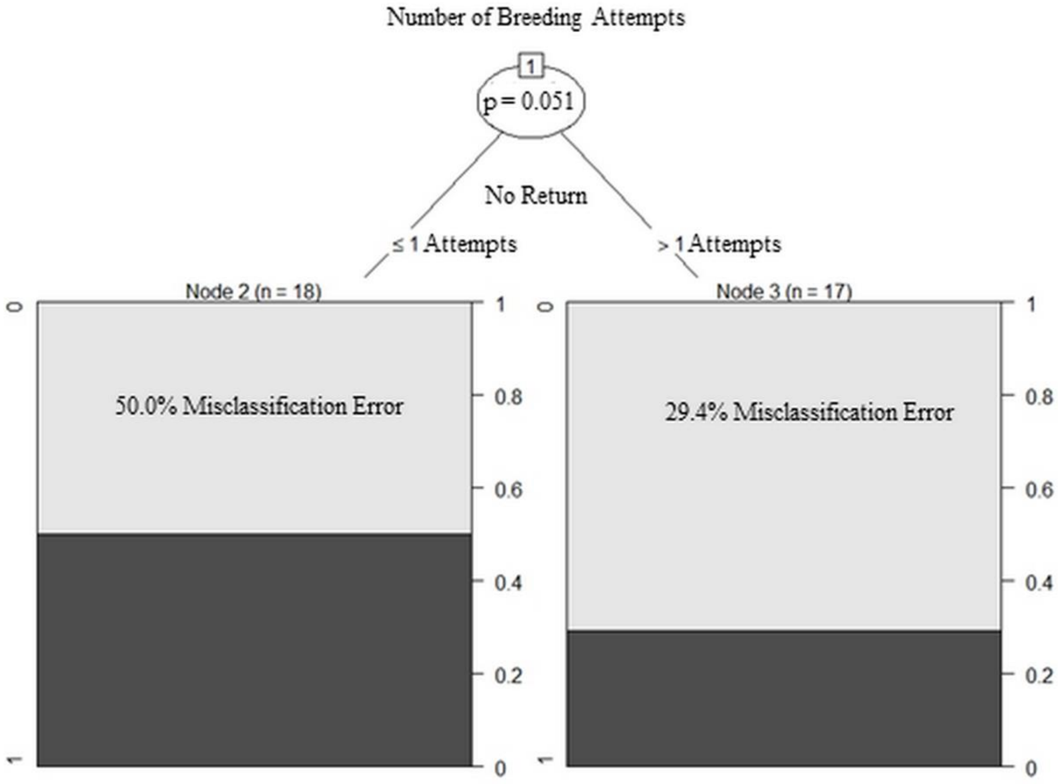

Figure 3. A conditional inference tree showing a significant split in data structure for the number of breeding attempts in explaining adult female waterthrush site fidelity, and percent misclassification error rate represented in assigning individuals to that group.

PART 3. SPATIAL ASSESSMENT OF LOUISIANA WATERTHRUSH FORAGING

CHAPTER 4. DEMOGRAPHIC CHARACTERISTICS OF AN AVIAN PREDATOR, LOUISIANA WATERTHRUSH (*PARKESIA MOTACILLA*), IN RESPONSE TO ITS AQUATIC PREY IN A CENTRAL APPALACHIAN USA WATERSHED IMPACTED BY SHALE GAS DEVELOPMENT.

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ABSTRACT

We related Louisiana Waterthrush (*Parkesia motacilla*) demographic response and nest survival to benthic macroinvertebrate aquatic prey and to shale gas development parameters using models that accounted for both spatial and non-spatial sources of variability in a Central Appalachian USA watershed. In 2013, aquatic prey density and pollution intolerant genera (i.e., pollution tolerance value <4) decreased statistically with increased waterthrush territory length but not in 2014 when territory densities were lower. In general, most demographic responses to aquatic prey were variable and negatively related to aquatic prey in 2013 but positively related in 2014. Competing aquatic prey covariate models to explain nest survival were not statistically significant but differed annually and in general reversed from negative to positive influence on daily survival rate. Potential hydraulic fracturing runoff decreased nest survival both years and was statistically significant in 2014. The EPA Rapid Bioassessment protocol (EPA) and Habitat Suitability Index (HSI) designed for assessing suitability requirements for waterthrush were positively linked to aquatic prey where higher scores increased aquatic prey metrics, but EPA was more strongly linked than HSI and varied annually. While potential hydraulic fracturing runoff in 2013 may have increased Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, in 2014 shale gas territory disturbance decreased EPT richness. In 2014, intolerant genera decreased at the territory and nest level with increased shale gas disturbance suggesting the potential for localized negative effects on waterthrush. Loss of food resources does not seem directly or solely responsible for demographic declines where waterthrush likely were able to meet their foraging needs. However collective evidence suggests there may be a shale gas disturbance threshold at which waterthrush respond negatively to aquatic prey community

changes. Density-dependent regulation of their ability to adapt to environmental change through acquisition of additional resources may also alter demographic response.

INTRODUCTION

The rapid development of hydraulic fracturing techniques in the last decade has allowed the expansion of development for unconventional drilling activity, hereafter shale gas development [1]. The Marcellus-Utica shale basin is one of the largest natural gas plays underlying part of the northeastern United States with substantial growth in gas production [2−3]. As of 2015, over 140,000 ha of land have been developed, with deciduous forest one of the major habitat types affected with high ecosystem service costs [4]. Shale gas development has outpaced the ability to create adequate management practices that protect against harm to aquatic and terrestrial wildlife communities and their habitat [5]. The trend for core forest disturbance from shale gas development where headwater streams occur [6] stresses the need for regional monitoring and research in these ecosystems.

Although there is local and regional variability in risks to water resources from shale gas development [7], shale gas development commonly occurs <300m from streams, increasing the threat of surface water degradation from sedimentation, altered stream flow, and the introduction of contaminants [8]. Johnson et al. [9] found that differences in benthic macroinvertebrate communities were dependent on the level of gas activity, and Grant et al. [10] found that stream pH, fish biodiversity, and taxa richness were negatively correlated with the number of gas wells. Additionally, Lutz and Grant [11] found that shale gas disturbed streams were more acidic and had lower index of biotic integrity (IBI) scores. However, other studies found shale gas development did not have any noticeable impact on water quality [12], or in least intrusive scenarios no evidence of impacts on fish, salamander, and crayfish assemblages [13]. Shale gas development has the potential to alter the base of aquatic food webs [14] and may be associated with bioaccumulated contaminants in an apex predator [15], but no study has yet followed potential effects from shale gas development across trophic levels of the aquatic-terrestrial interface.

Terrestrial and aquatic ecosystems are closely linked through cross-habitat physical mechanisms and energy fluxes, leaving research focusing only on land or water ecologically incomplete [16]. In particular, dynamics of forested headwater stream ecosystems occur at the aquatic-terrestrial interface [17]. Headwater streams are the critical sources of water, sediment, organic matter, and nutrients for the rest of the system [18], and are therefore vital for ecological integrity [19]. Furthermore, headwater streams, despite their predominance of drainage area and total stream length, are largely overlooked for protection or regulation despite their strong influence on downstream reaches [20].

Species with specialized terrestrial or aquatic habitat needs that overlap forested freshwater ecosystems [21−22] undergoing shale gas development may be the most vulnerable to disturbance [5]. The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is a habitat specialist and species of conservation concern [23] that breeds in contiguous riparian forests [24] and forages on benthic macroinvertebrates, hereafter aquatic prey, in well-developed riffle and pool areas [25]. Waterthrushes are considered bioindicators of riparian ecosystem integrity [25] due to their stream dependency [26–27].

Over a six-year waterthrush demography study (2009–2011, 2013–2015) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia, we observed general annual declines in territory density, reproductive success, and riparian habitat quality with increases in shale gas development [28], as well as declines in site fidelity and apparent survival (M. Frantz, *pers. comm.*). In 2011 and 2013–2014, two benthic studies on the same study area linked shale gas development to both strong (2011) and weak (2013–2014) negative influences on benthic community structure ([29]; G. Merovich, *pers. comm.*). The 2011 study also evaluated waterthrush demographic response to aquatic prey and found territory density and clutch size were greater in higher quality stream corridors during a year when shale gas activity was high [29]. However, Wood et al. [29] spanned only one breeding season with a limited sample size (n = 12 watershed samples) at the watershed-scale, meriting further evaluation with increased sampling efforts at other spatial scales (territory and nest).

As a follow-up to these previous studies, in 2013–2014 we: 1) evaluated the congruence between aquatic prey and riparian quality indices used to gauge waterthrush habitat (i.e., US EPA Rapid Bioassessment Protocol, EPA; Habitat Suitability Index, HSI), 2) evaluated if the amount of shale gas disturbance or potential hydraulic fracturing runoff in a territory or at a nest influence aquatic prey, and 3) quantified waterthrush demographic response to aquatic prey changes. We hypothesized that aquatic prey should be positively linked with riparian quality habitat scores. EPA and HSI scores were negatively affected by shale gas development [28]. As a consequence of habitat degradation, we expected a negative relationship between aquatic prey metrics and the amount of shale gas disturbance or potential hydraulic fracturing runoff in a territory or at a nest. We also hypothesized that clutch size, number of fledglings, and territory density would have a positive association with aquatic prey metrics. Annual territory length increased as territory densities decreased [28], so we expected smaller territories to be indicative of higher quality aquatic prey and stream quality (e.g., [30]). Nest survival was minimally affected by aquatic prey in 2011 [29] but we hypothesized that any stream impairment effects on the aquatic prey would affect nest survival.

METHODS

Study area

We studied waterthrush along 58.1 km of 1st- and 2nd-order headwater stream tributaries (n = 14) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia (Fig 1). Our waterthrush aquatic prey study in 2013–2014 was part of a waterthrush demography study over a six year period (2009–2011, 2013–2015; [28]). The study area overlays the Marcellus-Utica shale region and occurs where waterthrush reach their highest densities within the central Appalachians [31]. The LWWMA is part of a regional core designated as a priority conservation planning area for both aquatic and terrestrial targets [32].

During our study, shale gas development activities included building of conventional (shallower formations) and Marcellus well pads, timbering for yet unbuilt well pads, the expansion of existing road and pipeline infrastructure, and the construction of new infrastructure. In 2008, the LWWMA was 95.3% forested and had 0.4% shale gas land cover; the first shale gas well development began in 2007 [33]. Between the 2010 and 2011 breeding seasons, shale gas development activities that occurred since 2007 accelerated across the study area and began to increase especially on ridgetops. In 2011, study area-wide shale gas land cover was 1.3% and increased to 2.7% in 2013–2014 ([33]; Table 1). Starting in 2013, shale gas development abated study area-wide and instead became concentrated to specific streams and ridgetops. Clearing for additional new well pads occurred late (June–July) in the 2013 breeding season with well pad completion in 2014, in addition to re-drilling of an existing well pad. There was no new shale gas development or activity in the 2015 breeding season. In 2015, the LWWMA was 90.8% forested and 3.9% in shale gas development land cover, with 83.1% of shale gas development resulting in direct forest loss [33]. In summary, 2013 disturbances slowed and affected streams more noticeably late in the breeding season, while in 2014 shale gas activity peaked again, particularly at Slabcamp Run, but did not achieve 2011 levels of activity (Appendix Table 1).

Mapping of streams and shale gas disturbance

Within a Geographic Information System (GIS), we used a sequence of leaf-on and leaf-off aerial photographs from the National Agriculture Imagery Program (NAIP) for 2011 and 2014, satellite Quickbird imagery for 2009, and extensive annual ground-truthing to manually digitize areas of disturbance within the study area for each year of the long-term study, including years 2013–2014 of the aquatic prey study (see Frantz et al. [28] for full description). All forest canopy disturbances were classified as shale gas related (i.e., well pads and associated road and pipeline infrastructure, frequent truck traffic, and visual stream sedimentation) or as being unrelated or pre-existing (i.e., forest roads, recent even-aged timber harvests, and various types of existing clearings). We classified a few conventional impacts (i.e., stream-side vertical pump jacks) as related to shale gas development because their pads were managed in conjunction with nearby shale gas infrastructure and because their targeted formation, even though they remained shallow after development, was listed as Marcellus [34]. Gas well records [35] were used to verify target shale formations, drilling status, and start dates for all well disturbances.

Lengths of each study stream (average length 4.1 ± 0.54 km, range 0.95–7.4 km) were calculated in GIS using a 3D functional surface length tool and a 3 m resolution digital elevation model to account for topography, and study streams were defined to have a drainage basin of 9.0 hectares (i.e., <100 ha; [36]) to delineate the uppermost headwater reaches (24 k scale or higher resolution; e.g., [37]). To describe and model waterthrush demography and riparian habitat quality as a function of shale gas disturbance, we created four continuous and one binary variable based on disturbance categories at the stream, territory, and nest scale. The first (termed StreamGas) described mostly localized streamside disturbance indicative of the presence of any shale gas infrastructure or activity. A section of stream was considered disturbed when well pads, infrastructure, or frequent vehicular activity were within 60 m of the stream centerline, which is the typical extent of waterthrush streamside use (i.e., 60 m; [38]). When a stream had visually observable sedimentation that resulted from shale gas development at any distance from the stream, we classified the entire stream network downstream of the sedimentation beginning point as disturbed. Streams were frequently and extensively ground-truthed each season, so there were no stream reaches where sedimentation events were likely to be missed.

We created a second shale gas disturbance category (termed StreamRunoff) that focused solely on potential run-off into streams from shale gas contaminants. A stream was considered disturbed from at and below a well pad or retaining pond (similar to Latta et al. [15]), resulting in the whole downstream network classified as at risk for surface pollution based on elevational maps and ground truthing. This category did not include pipeline or road disturbance and was a broader, distance-independent, disturbance category describing potential water pollution. For each year of the study, we then calculated the proportion of each stream disturbed for each of these two disturbance categories.

We calculated the proportion of each waterthrush territory (a 60-m buffer around each territory vector) that was disturbed by StreamGas and called this metric TerrGas. The proportion of each territory disturbed by StreamRunoff was termed TerrRunoff. We classified each waterthrush nest location as undisturbed or disturbed by StreamGas within 60-m around the nest and called this variable NestGas. Hereafter we use StreamGas, StreamRunoff, TerrGas, TerrRunoff, and NestGas to describe shale gas disturbance metrics (Table 1, Appendix Table 2).

Waterthrush riparian habitat quality

Riparian habitat quality was assessed using the Habitat Suitability Index specifically designed for waterthrush (hereafter HSI; [25]) and the US EPA Rapid Bioassessment Protocol for high gradient streams (hereafter EPA [39]) in the same manner as Wood et al. [29] and Frantz et al. [28]. The HSI is a broad-scale evaluation of waterthrush instream foraging habitat, nesting habitat, and upland habitat suitability [25]. The EPA assesses stream quality based primarily on instream characteristics that relate to the abundance and composition of aquatic organisms, and therefore may indicate relative quality of instream foraging habitat for waterthrush [29]. The HSI and EPA indices were quantified in a 50-m stream reach centered on each nest location monitored to make the indices sensitive to habitat immediately surrounding waterthrush nests.

Waterthrush demographic monitoring

We quantified annual waterthrush territory length (m), territory density (# territories/km), and nest survival for our 14 study streams as described in Frantz et al. [28]. Waterthrush territories were delineated as linear vectors along each stream during April 1–June 29 using standardized territory mapping (≥6 stream visits [40–41]). Nest searching and monitoring occurred concurrently with territory mapping. Locations of waterthrush observations and nests were recorded with a WAAS-enabled Garmin 60CSX GPS unit with accuracy ≤5 m in 2013–2015.

To calculate daily survival rate (DSR) for nest survival, we monitored nests typically every three-four days initially and more frequently as fledging approached [42]. We assumed an undamaged empty nest had fledged if the nest was active the day before and had approached the predicted fledge date. Nest sites were revisited at least one more time to verify either no activity or renesting if the nest was not active prior to the expected fledge date. We counted number of eggs to determine clutch size of nests with complete clutches, and the number of fledglings for each successful nest was the count of nestlings in the visit prior to fledging. Nests were considered successful if they produced at least one waterthrush fledgling, including nests parasitized by Brown-headed Cowbirds (*Molothrus ater*).

Aquatic prey sampling

Aquatic prey occurring in riffle habitat adjacent to nest site locations were sampled once per nest using a Surber sampler. Nest site samples (n = 178) were collected shortly after the nest fledged, failed, or had been abandoned (May 22 – July 28, 2013; June 16 – July 6, 2014) to assess relative prey availability near the time a nest contained fledglings. During sample collection, we scrubbed rocks (>8 cm in diameter) and disturbed sediment 3-cm below the stream bed within the Surber frame for a total of 3 minutes [27]. We separated aquatic prey from detritus for each sample in the field and stored all organic matter in 70% or 95% ethanol.

Post-field season, aquatic prey in benthic samples were sorted, counted, and identified to genus level. Body lengths were also measured to estimate biomass (crayfish excluded). To summarize the aquatic prey composition for each sample, we calculated a family level multimetric IBI called the West Virginia Stream Condition Index (WVSCI [43]), and a genus level multimetric IBI called the Genus Level Index of Most Probable Stream Status (GLIMPSS), version CF, which does not require the genus-level identification of Chironomidae or Oligochaeta [44]. The values we calculated for both indices are based on sampling methods that are slightly modified [45] from the standard methods (i.e., Surber samples and all individuals used in calculations). Thus, they are not strictly interpretable as indicators of stream ecosystem health as originally intended. Nevertheless, they still quantify the composition and integrity of the aquatic prey resource available to waterthrush. We additionally calculated overall aquatic prey density and biomass using length-mass regressions [46]. In total, we selected six aquatic prey metrics to relate to waterthrush demography: WVSCI, GLIMPSS, biomass, density, EPT richness (component of WVSCI), and number of intolerant genera (component of GLIMPSS where pollution tolerance value is <4); Appendix Table 2).

Analysis

We used spatial generalized linear mixed models (hereafter SGLMMs) to assess relationships between waterthrush demography and aquatic prey as well as between riparian habitat quality and aquatic prey for each year (i.e., 2013, 2014) separately. SGLMMs accounted for possible effects of spatial autocorrelation and were modeled using *corrHLfit* within the spaMM package [47–48] in R [49]. Model residuals were evaluated graphically, extreme or influential data outliers identified graphically and with packages car [50] and stats [49] for potential removal, and other data exploration diagnostic tools were used [51] to ensure model assumptions were met. We used x-y coordinates as a spatial random effect in a Matern correlation model and included a stream random effect. For all SGLMMs, we determined statistical significance of fixed effects using a likelihood ratio test and set significance at $\alpha = 0.10$ to be cautiously moderate in our assessment of biological significance [52].

We evaluated the degree to which the six aquatic prey metrics were related to riparian habitat quality (i.e., EPA and HSI scores) in individual SGLMM models. We also tested the relationship between the six aquatic prey metrics and the shale gas disturbance metrics for the nest and territory scales (TerrGas, TerrRunoff, and NestGas) as G. Merovich (*pers. comm.*) found differences in benthic communities up and downstream of shale gas development. We modeled WVSCI, GLIMPSS, biomass, and density using a Gaussian distribution with biomass and density receiving a log10 transformation to approximate normality. We removed an outlier from our benthic density dataset because it was identified as a strong influential outlier not representative of other samples (6422.2 m^2 vs. 354.7 ± 31.3 per m^2 average density) by using the outlierTest and influence.measures functions with packages car and stat. EPT richness and number of intolerant genera were modeled using the Conway-Maxwell-Poisson (COM-Poisson [53–54]) distribution that generalizes the Poisson distribution to handle a wide range of under and over-dispersion typically found in ecological count data [55]. If a COM-Poisson model could not converge, we substituted with a Poisson distributed model (n = 12 models). Because Wood et al. [29] did not assess EPA and HSI in relation to aquatic prey metrics for their 2011 data, we completed a retrospective analysis of their 2011 data. We used Pearson (i.e., EPA) and

Spearman (i.e., HSI) correlation coefficients to relate 2011 EPA and HSI to aquatic prey metrics with package psych [56] for comparison to our 2013–2014 results.

We additionally assessed the relationship between waterthrush demography (number of fledglings, clutch size, territory length, and territory density) and the six aquatic prey variables in individual SGLMM models as above. Number of fledglings, clutch size, and territory density were modeled using the COM-Poisson distribution. Territory length was modeled using a gamma distribution. We removed an outlier sample from the fledging dataset where only one fledgling was represented due to Brown-headed Cowbird (*Molothrus ater*) parasitism. Because the Wood et al. [29] aquatic prey study did not assess territory length, we used a Pearson correlation on data collected in 2011 in the same manner as riparian habitat quality above to relate the six aquatic prey variables to territory length for comparison to our 2013–2014 results.

We used program MARK 7.1 (Colorado State University, Ft. Collins, Colorado, USA, [57]) to estimate daily survival rate (DSR) of monitored waterthrush nests in 2013 and 2014. We removed 63 nests that did not meet the assumption requirements of program MARK and 11 nests that had no benthic information, leaving 107 nests for analysis. We assumed a 29-day nesting period (egg-laying 5, incubation 14, nestling 10 days) based on the chronology of nests monitored on our study area [28].

We developed a set of 7 *a priori* candidate models [58] that we hypothesized might influence DSR of waterthrush nests based on the results of Wood et al. [29] and Frantz et al. [28]. All covariates are defined in Appendix Table 2. All *a priori* models included 3 temporal covariates and a shale gas covariate that influenced nest survival in our study area [28]; they included nest age (NestAge), quadratic effect of time of nesting within the breeding season (TT), average daily rainfall (Rain), and percent potential hydraulic fracturing runoff within a territory (TerrRunoff). Instead of an intercept model with no covariates, these 4 covariates formed our base null model given their known importance [28], allowing us to assess whether aquatic prey also influenced nest survival by accounting for them. Nest age indicates vulnerability as the nest ages [59] and within-season trends in DSR reflect dynamic activity patterns of nest predators (e.g., [60]). Mean daily rainfall (in mm) by influencing prey availability affects waterthrush nest survival [28, 38] as headwater riparian systems are subject to seasonality and annual changes in rainfall [61]. For each nest, we averaged daily rainfall estimates across the period in which an active nest was under observation [38]. Precipitation estimates were pooled from four Weather

Underground, Inc. network stations closest (avg. 36 km) to the study area. Six additive models included the null model plus each of our aquatic prey covariates of interest.

We used Akaike's Information Criterion for small sample sizes (AICc) to evaluate support for candidate models [62] in program MARK. We modeled the binomially distributed data with the user-defined, logit-link function while simultaneously considering associations with the covariates of interest. We considered the model with the lowest AICc value to be the best-supported model given the data, and any models with ΔAICc <2 were considered plausible. We used regression coefficients and 85% confidence intervals (hereafter CIs) to infer biological importance of covariates in plausible AICc models [63]. We model-averaged NestAge, TT, Rain, and TerrRunoff across all models [62].

RESULTS

Stream disturbance due to shale gas (i.e., StreamGas) was 32.3% in 2011, dropped to 17.3% in 2013, and elevated to 21.5% in 2014, reflecting different levels of shale gas activity despite study area-wide shale gas land cover not changing between 2013 and 2014 (Table 1; Appendix Table 1; Fig 2). The potential for hydraulic fracturing runoff within streams (i.e., StreamRunoff) increased from 19.3% in 2011 to 24.2% in 2013 and 2014 (Table 1). The percent of each waterthrush territory disturbed by shale gas (i.e., TerrGas) had the same patterns as StreamGas while TerrRunoff increased each year (Table 1). Only 20.3% of territories (39 of 192 total from 2011, 2013–2014) had their full territory length (100%) disturbed by TerrGas or TerrRunoff.

In 2013, aquatic prey biomass and density increased with increasing EPA score, while in 2014 intolerant genera increased with increasing EPA score (Table 2; Fig 2). No relationships were statistically significant in 2013 between HSI and aquatic prey, but in 2014 intolerant genera and WVSCI (approaching significance) increased with increasing HSI score (Table 2; Fig 2). For 2011 data, aquatic prey biomass had a statistically significant, positive correlation with EPA ($R^2 = 0.67$, $P = 0.02$) and HSI (Rho = 0.51, $P = 0.09$). In 2013, EPT richness increased with increasing TerrRunoff, but in 2014 EPT richness decreased with increasing TerrGas (Table 3; Fig 2). In 2014, intolerant genera decreased with increasing TerrRunoff, TerrGas, and NestGas (Table 3; Fig 2).

All tests for the relationships between clutch size, number of fledglings, and territory density with aquatic prey metrics were statistically non-significant (Table 4). Territory length decreased with increasing aquatic prey density and number of intolerant genera in 2013 (Table 4; Fig 2). For 2011 data, territory length had a statistically significant, negative correlation with GLIMPSS, EPT richness, and number of intolerant genera (R^2 = -0.65, -0.68, -0.67, P = 0.02; Fig 2), respectively.

Of 7 *a priori* nest survival models (Table 5), 6 different models were supported (ΔAICc <2) in 2013 and 2014. The null base model had the most weight in both years (*w*i = 0.25, 0.28). The model with EPT richness had the most weight of the 5 supported aquatic prey models in 2013 (*w*i = 0.17) and GLIMPSS the most in 2014 (*w*i = 0.18). Regression coefficient 85% CIs overlapped zero for all aquatic prey covariates indicating little, no, or highly variable influence on DSR, but the direction of the relationship between nest survival and aquatic prey switched from negative to positive for 5 of the 6 aquatic prey covariates from 2013 to 2014 (Table 6). In the null base model Rain had positive influence on DSR in 2013 and 2014, while TerrRunoff had negative influence on nest survival in 2014 (Table 6). MARK-formatted files (.inp file extension) used to analyze the relationship between waterthrush nest survival and aquatic prey are S1 Datasets 5 and 6.

DISCUSSION

Shale gas disturbances on our headwater stream ecosystem varied with the intensity of shale gas development that year ([28]; Table 1; Appendix Table 1). Our follow-up study was able to establish how shale gas alterations to riparian habitat quality and the food web can lead to potential effects at a higher trophic level in an apex predator. By also documenting waterthrush demography decline (Table 1, [28]) and shifts in aquatic prey community structure ([29]; G. Merovich *pers. comm.*) due to shale gas development, our study establishes the extent of dependency of waterthrush demographic response and adaptation due to the integrity of ecosystem conditions at the aquatic-terrestrial interface.

Waterthrush foraging resources

Our study builds a connection for decreasing riparian habitat quality due to shale gas altering, at least in part, waterthrush foraging resources. The EPA riparian habitat assessment has been successfully used in other studies in conjunction with waterthrush occupancy to explain biotic integrity [27]. Higher EPA index and HSI scores were indicative of a larger and healthier aquatic prey community in our system although not with all metrics and statistical significance was dependent on year (Table 2). Additionally, EPT richness and intolerant genera were negatively related to shale gas disturbance, mainly in 2014 (Table 3); this was important to establish since waterthrush riparian habitat quality was negatively affected by shale gas [28]. Overall, HSI was less reliable than EPA for describing aquatic prey, which may be due to HSI consisting of not just food (i.e., stream habitat and quality) scores, but also scores tabulated for waterthrush habitat cover, nesting, and a surrounding landscape classifier [25].

Waterthrush demographic responses

Most demographic responses to aquatic prey were variable or statistically non-significant. Even so, general demographic responses were negatively related to aquatic prey in 2013 then shifted to a positive response in 2014 when shale gas disturbance had a stronger negative influence on aquatic prey and nest survival (Table 4, Table 5). On streams acidified by mine drainage, waterthrush establish larger territories and forage on peripheral and novel prey items (e.g., terrestrial salamanders) to acquire sufficient prey resources [31]. We saw a similar effect where territory length increased with measures indicating poorer aquatic prey base (e.g., low EPT genus richness). However, territory length in 2014 tended to increase with increasing aquatic prey metrics, opposite of previous years (Table 4). Waterthrush likely had the ability to compensate for loss of food resources by foraging in undisturbed parts of their territory, in addition to increasing territory length, as only 20.3% of territories had their full territory length disturbed by TerrGas or TerrRunoff. The waterthrush’s compensation ability in combination with the decline in annual territory density likely contributed to the disassociation between territory length and aquatic prey in 2014. In contrast, given the stronger response and higher territory densities in 2011, under normal territory density conditions (≥1.5 territories/km) the hypothesis of smaller territories indicate higher quality habitat and foraging resources [30] likely still holds true.

Shale gas disturbance influences on nest survival and aquatic prey

Models used to explain nest survival were also dependent on year (Table 5) with TerrRunoff significantly decreasing daily survival rate in 2014 (Table 6). Our study aligns with Wood et al. [29] in that aquatic prey likely is less influential on nest survival than temporal effects like rain or shale gas disturbance [28]. While our waterthrush-related shale gas disturbance metrics (i.e., TerrGas, TerrRunoff, and NestGas) suggest conflicting yet overall weak negative effects on aquatic prey (Table 3), aquatic prey community structure at our study area quantified upstream and downstream of shale gas at a subwatershed scale also mirrored shale gas activity: community changes differed the most in 2011 [29], were weaker in 2013, and then slightly stronger in 2014 but not as much as 2011 when shale gas activity was highest (G. Merovich, *pers. comm.*; Appendix Table 1).

Implications

The year-to-year waterthrush demographic responses to aquatic prey in our study were not strongly proportional but instead followed relatively weaker patterning. Timing of benthic sampling in 2013 in relation to shale gas activity levels likely in part explain the lack of a clear signal between waterthrush demography and its aquatic prey. New shale gas activity in 2013 was not as evident until near or after sampling late in the breeding season (S1 Table 1), and shale gas well pad construction and drilling typical of our study site and elsewhere occur in “pulses” [5]. With our sampling design, we were able to detect benthic community responses as stronger in 2014 than 2013 (similar to G. Merovich, *pers. comm.*), but increased sampling efforts during appropriate sampling periods may be even more critical for higher food web organisms in overcoming variability of demographic response to shale gas.

Our study, through collective evidence, suggests the potential for localized negative effects to aquatic prey from shale gas development, in particular EPT and intolerant taxa that are believed to be the waterthrush’s preferred prey [24]. Additionally, the shift in demographic response in 2014 when shale gas disturbance had stronger negative effects on aquatic prey and nest survival may suggest a shale gas disturbance threshold ([64]; Fig 2) at which waterthrush respond and adapt to aquatic prey in the same manner aquatic prey community structure concurrently reflected levels of annual disturbance ([29]; G. Merovich, *pers. comm.*). Waterthrush are most likely to forage in locations that have higher EPT and intolerant genera [29], making it important to maintain or improve riparian habitat quality linked directly with their aquatic prey. In consideration of population regulatory mechanisms (e.g., [65]) that may influence annual demographic response and adaptability, continued long-term monitoring will be required to discern if a threshold of shale gas disturbance exists that alters aquatic prey communities and, in turn, affects demography of higher-level trophic linkages [66−67]. To some degree, waterthrush in our system appear to have the ability to adapt and meet their foraging needs. However, based on the response in 2011 and 2014 when aquatic prey was the most affected by shale gas, mechanisms used to compensate when stream disturbance is conservatively $\geq 25\%$ (Fig 2) may be less dependable, altering demography. The fact that benthic communities even within pristine streams may be at risk when isolated within heavily impacted regions [68], and the tendency for upper reaches of Appalachian headwater streams to have resource extraction activities [69], warrants more attention to multi-dimensional wildlife community responses within aquatic-terrestrial linkages associated to shale gas development.

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TABLES

Table 1. Louisiana Waterthrush annual demographic, riparian habitat quality, and shale gas disturbance metrics (mean ± SE) at Lewis Wetzel Wildlife Management Area, WV at peak (2011) and later stages (2013–2014) of shale gas development. Our study associated waterthrush response to aquatic prey community changes in relation to shale gas disturbance. All metrics are a subset of those originally reported in Frantz et al. [28] excepting % shale gas land cover which is cited from Farwell et al. [33]. Variable names are defined in Appendix Table 2.

Variable	**2011**	**2013**	**2014**
Riparian Habitat Quality			
EPA Index (range 0–200)	158.6 ± 1.8	148.9 ± 2.1	165.6 ± 2.2
HSI (range 0–1)	0.78 ± 0.02	0.76 ± 0.02	0.77 ± 0.02
Demography			
Territory Density	1.5 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
Territory Length (m)	556.4 ± 31.2	659.0 ± 34.3	772.1 ± 41.9
Nest Survival	38.0 ± 8.0	28.5 ± 6.1	25.7 ± 5.8
Clutch Size	4.8 ± 0.1	4.6 ± 0.1	4.4 ± 0.1
Fledglings	4.5 ± 0.1	4.7 ± 0.1	4.1 ± 0.2
Disturbance			
% TerrGas	38.0 ± 5.2	18.0 ± 3.4	27.2 ± 4.5
% TerrRunoff	20.0 ± 4.5	32.9 ± 5.2	36.0 ± 5.0
% StreamGas	32.3 ± 6.3	17.3 ± 5.7	21.5 ± 6.4
% StreamRunoff	19.3 ± 5.7	24.2 ± 5.5	24.2 ± 5.5
% Shale Gas Land Cover	1.3	2.7	2.7

Table 2. Association between waterthrush riparian habitat quality indices (i.e., EPA and HSI) and aquatic prey metrics in spatial generalized linear mixed models. In 2013, aquatic prey biomass and density increased with increasing EPA score, while in 2014 intolerant genera increased with increasing EPA score. No relationships were statistically significant in 2013 between HSI and aquatic prey, but in 2014 intolerant genera and WVSCI (approaching significance) increased with increasing HSI score. Results with ^{P} are from a Poisson model. P values of variables that are statistically significant are bolded. Variable names are defined in S1 Table 2. LRT = likelihood ratio test χ2 statistic. β = beta estimate of fixed effect.

Independent Variable	**β ± SE**	**LRT χ^2**	**p value**	**β ± SE**	**LRT χ^2**	**p value**
	GLIMPSS			**WVSCI**		
Year 2013						
EPA	0.120 ± 0.092	1.670	0.196	0.006 ± 0.081	0.010	0.922
HSI	13.700 ± 14.480	0.938	0.333	1.991 ± 12.515	0.030	0.864
Year 2014						
EPA	0.069 ± 0.066	1.128	0.288	0.014 ± 0.045	0.464	0.496
HSI	10.890 ± 11.221	0.961	0.327	11.540 ± 7.582	2.594	**0.107**
	Density			**Biomass**		
Year 2013						
EPA	0.005 ± 0.002	5.000	**0.025**	0.010 ± 0.004	2.862	**0.091**
HSI	-0.307 ± 0.399	0.601	0.438	0.219 ± 0.752	0.106	0.744
Year 2014						
EPA	0.0003 ± 0.002	0.017	0.896	0.002 ± 0.003	0.771	0.380
HSI	0.337 ± 0.351	0.645	0.422	0.148 ± 0.494	0.171	0.679
	EPT Richness			**Intolerant Genera**		
Year 2013						
EPA	0.010 ± 0.015	0.599	0.439	0.006 ± 0.005 ^{P}	1.665 ^{P}	0.197 ^{P}
HSI	-1.026 ± 2.517	0.036	0.850	-0.679 ± 0.718 ^{P}	0.869 ^{P}	0.351 ^{P}
Year 2014						
EPA	-0.005 ± 0.007	0.327	0.567	0.005 ± 0.002	3.160	**0.075**
HSI	1.581 ± 1.266	2.109	0.146	0.828 ± 0.399	4.573	**0.032**

Table 3. Association between waterthrush aquatic prey and shale gas disturbance metrics in spatial generalized linear mixed models. Results with ^{P} are from a Poisson model. In 2013, EPT richness increased with increasing TerrRunoff, but in 2014 EPT richness decreased with increasing TerrGas. In 2014, intolerant genera decreased with increasing TerrRunoff, TerrGas, and NestGas. P values of variables that are statistically significant are bolded. Variable names are defined in S1 Table 2. LRT = likelihood ratio test χ2 statistic. β = beta estimate of fixed effect.

Independent Variable	**β ± SE**	**LRT χ^2**	**p value**	**β ± SE**	**LRT χ^2**	**p value**
	GLIMPSS			**WVSCI**		
Year 2013						
TerrGas	-0.008 ± 0.062	0.020	0.888	0.012 ± 0.055	0.053	0.818
TerrRunoff	0.024 ± 0.044	0.253	0.615	0.046 ± 0.039	1.372	0.241
NestGas	0.303 ± 3.745	0.003	0.958	-1.028 ± 3.048	0.112	0.738
Year 2014						
TerrGas	-0.054 ± 0.046	1.398	0.237	-0.022 ± 0.033	0.391	0.532
TerrRunoff	-0.029 ± 0.035	0.622	0.430	-0.026 ± 0.025	1.640	0.200
NestGas	-1.989 ± 3.270	0.367	0.545	-0.748 ± 2.277	0.100	0.752
	Density			**Biomass**		
Year 2013						
TerrGas	0.002 ± 0.002	2.388	0.122	0.005 ± 0.003	2.338	0.126
TerrRunoff	0.002 ± 0.001	2.162	0.141	0.003 ± 0.002	0.469	0.493
NestGas	0.044 ± 0.095	0.219	0.640	0.215 ± 0.179	1.495	0.221
Year 2014						
TerrGas	-0.0004 ± 0.001	0.040	0.842	-0.00004 ± 0.002	0.003	0.960
TerrRunoff	-0.0002 ± 0.001	0.006	0.939	0.0004 ± 0.002	0.085	0.771
NestGas	-0.061 ± 0.098	0.280	0.597	0.003 ± 0.144	0.006	0.940
	EPT Richness			**Intolerant Genera**		
Year 2013						
TerrGas	0.003 ± 0.003 ^{P}	0.576 ^{P}	0.448 ^{P}	0.012 ± 0.012	1.071	0.301
TerrRunoff	0.017 ± 0.008	4.381	**0.036**	0.007 ± 0.008	0.789	0.375
NestGas	-0.034 ± 0.175	0.068	0.794	0.215 ± 0.672	0.114	0.736
Year 2014						
TerrGas	-0.010 ± 0.006	2.572	**0.109**	-0.004 ± 0.002	4.934	**0.026**
TerrRunoff	-0.003 ± 0.004	0.681	0.409	-0.003 ± 0.001	4.136	**0.042**
NestGas	-0.424 ± 0.399	1.056	0.304	-0.180 ± 0.112	2.756	**0.097**

Table 4. Association between waterthrush demographic response (i.e., clutch size, number of fledglings, territory length and territory density) and its aquatic prey in spatial generalized linear mixed models. All tests for the relationships between clutch size, number of fledglings, and territory density with aquatic prey metrics were statistically non-significant. Territory length decreased with increasing aquatic prey density and number of intolerant genera in 2013. Results with ^{P} are from a Poisson model. P values of variables that are statistically significant are bolded. Variable names are defined in S1 Table 2. LRT = likelihood ratio test χ2 statistic. β = beta estimate of fixed effect.

Dependent Variable	**β ± SE**	**LRT χ^2**	**p value**	**β ± SE**	**LRT χ2**	**p value**
	GLIMPSS			**WVSCI**		
Year 2013						
Clutch size	-0.009 ± 0.012	0.535	0.464	-0.004 ± 0.013	0.100	0.751
Fledglings	-0.004 ± 0.017	0.056	0.812	0.003 ± 0.019	0.831	0.362
Territory length	0.001 ± 0.001	0.143	0.705	-0.001 ± 0.003	-0.790	1.000
Territory density	-0.0003 ± 0.009	0.001	0.970	-0.002 ± 0.004 ^{P}	0.445 ^{P}	0.505 ^{P}
Year 2014						
Clutch size	0.002 ± 0.014	0.016	0.900	0.017 ± 0.020	0.734	0.392
Fledglings	-0.019 ± 0.026	0.523	0.469	-0.007 ± 0.041	0.033	0.859
Territory length	0.001 ± 0.001	0.341	0.559	0.002 ± 0.003	0.745	0.388
Territory density	0.001 ± 0.004 ^{P}	0.037 ^{P}	0.847 ^{P}	0.001 ± 0.012	0.007	0.934
	Density			**Biomass**		
Year 2013						
Clutch size	-0.00002 ± 0.001^{P}	0.001^{P}	0.975^{P}	0.00004 ± 0.0002^{P}	0.047^{P}	0.828^{P}
Fledglings	0.0001 ± 0.001^{P}	0.009^{P}	0.924^{P}	0.0001 ± 0.001	0.009	0.924
Territory length	-0.001 ± 0.0003	8.535	**0.003**	-0.0003 ± 0.0002	2.338	0.126
Territory density	-0.0001 ± 0.001	0.009	0.925	-0.0001 ± 0.001	0.086	0.769
Year 2014						
Clutch size	0.0003 ± 0.0005	0.465	0.495	0.00001 ± 0.0001^{P}	0.012^{P}	0.912^{P}
Fledglings	0.0005 ± 0.001	0.811	0.368	0.0004 ± 0.0003	2.125	0.145
Territory length	0.00002 ± 0.0001	0.098	0.754	0.000002 ± 0.00004	0.001	0.979
Territory density	-0.00002 ± 0.0003	0.014	0.907	-0.00004 ± 0.0002	0.048	0.826
	EPT Richness			**Intolerant Genera**		
Year 2013						
Clutch size	-0.005 ± 0.031 ^{P}	0.027 ^{P}	0.868 ^{P}	-0.079 ± 0.067	1.380	0.240
Fledglings	0.008 ± 0.047	0.027	0.870	-0.007 ± 0.041^{P}	0.031^{P}	0.860^{P}
Territory length	-0.014 ± 0.017	-0.460	1.000	-0.040 ± 0.018	4.62	**0.032**
Territory density	-0.008 ± 0.023 ^{P}	0.162 ^{P}	0.687 ^{P}	-0.001 ± 0.049	0.001	0.981
Year 2014						
Clutch size	0.019 ± 0.041 ^{P}	0.213 ^{P}	0.645^{P}	0.020 ± 0.074	0.072	0.788
Fledglings	0.076 ± 0.158	0.233	0.629	-0.054 ± 0.115	0.218	0.641
Territory length	0.023 ± 0.014	2.486	0.115	0.010 ± 0.007	1.864	0.172
Territory density	0.003 ± 0.051	0.004	0.947	-0.001 ± 0.038	0.0003	0.985

Table 5. Year 2013 and 2014 AICc model results of 7 *a priori* nest survival models with aquatic prey covariates using Program MARK. Of 7 a priori nest survival models, 6 different models were supported (ΔAICc <2) in 2013 and 2014 with the null base model having the most weight in both years (wi = 0.25, 0.28). ΔAICc = distance from the top model, and w_i = Akaike weight. Variable names are defined in Appendix Table 2.

Model	**AICc**	**ΔAICc**	***wi***
Year 2013			
Rain + NestAge + TT + TerrRunoff	152.33	0	0.25
Rain + NestAge + TT + TerrRunoff + EPT Richness	153.12	0.79	0.17
Rain + NestAge + TT + TerrRunoff + WVSCI	153.36	1.04	0.15
Rain + NestAge + TT + TerrRunoff + Density	153.51	1.18	0.14
Rain + NestAge + TT + TerrRunoff + GLIMPSS	154.00	1.67	0.11
Rain + NestAge + TT + TerrRunoff + Biomass	154.30	1.97	0.09
Rain + NestAge + TT + TerrRunoff + Intolerant Genera	154.35	2.02	0.09
Year 2014			
Rain + NestAge + TT + TerrRunoff	164.56	0	0.28
Rain + NestAge + TT + TerrRunoff + GLIMPSS	165.39	0.83	0.18
Rain + NestAge + TT + TerrRunoff + EPT Richness	166.35	1.79	0.11
Rain + NestAge + TT + TerrRunoff + WVSCI	166.36	1.80	0.11
Rain + NestAge + TT + TerrRunoff + Intolerant Genera	166.47	1.92	0.11
Rain + NestAge + TT + TerrRunoff + Density	166.48	1.92	0.11
Rain + NestAge + TT + TerrRunoff + Biomass	166.59	2.03	0.10

Table 6. Annual waterthrush nest survival covariates found in the top supported (ΔAICc <2, n = 6) and unsupported (n = 1) AICc models based on regression coefficients, standard error (SE), and 85% confidence intervals. In the null base model Rain had positive influence on daily survival rate (DSR) in 2013 and 2014, while TerrRunoff had negative influence on nest survival in 2014. Significant covariates with non-overlapping confidence intervals are bolded. Covariates included in every model to account for their influence (i.e., Rain, NestAge, TT, and TerrRunoff; [28]) have model-averaged regression coefficients and unconditional SEs. Variable names are defined in S1 Table 2.

Parameter	Estimate	SE	Confidence Interval
Year 2013			
Rain	**0.415**	**0.191**	**0.140, 0.690**
TerrRunoff	-0.001	0.002	-0.005, 0.002
NestAge	-0.052	0.043	-0.113, 0.009
TT	0.077	0.155	-0.147, 0.300
EPT Richness	-0.116	0.103	-0.317, 0.085
Density	-0.002	0.002	-0.005, 0.002
Biomass	-0.0002	0.001	-0.002, 0.001
WVSCI	-0.018	0.018	-0.054, 0.018
GLIMPSS	-0.009	0.015	-0.037, 0.020
Not in top supported:			
Intolerant Genera	-0.014	0.099	-0.208, 0.180
Year 2014			
Rain	**0.380**	**0.183**	**0.118, 0.643**
TerrRunoff	**-0.005**	**0.002**	**-0.008, -0.002**
NestAge	0.016	0.047	-0.052, 0.084
TT	-0.022	0.080	-0.137, 0.094
EPT Richness	-0.052	0.104	-0.255, 0.151
Density	0.0001	0.0004	-0.001, 0.001
WVSCI	0.012	0.023	-0.034, 0.057
GLIMPSS	0.016	0.015	-0.013, 0.045
Intolerant Genera	0.027	0.076	-0.121, 0.175
Not in top supported:			
Biomass	0.00004	0.0003	-0.001, 0.001

FIGURES

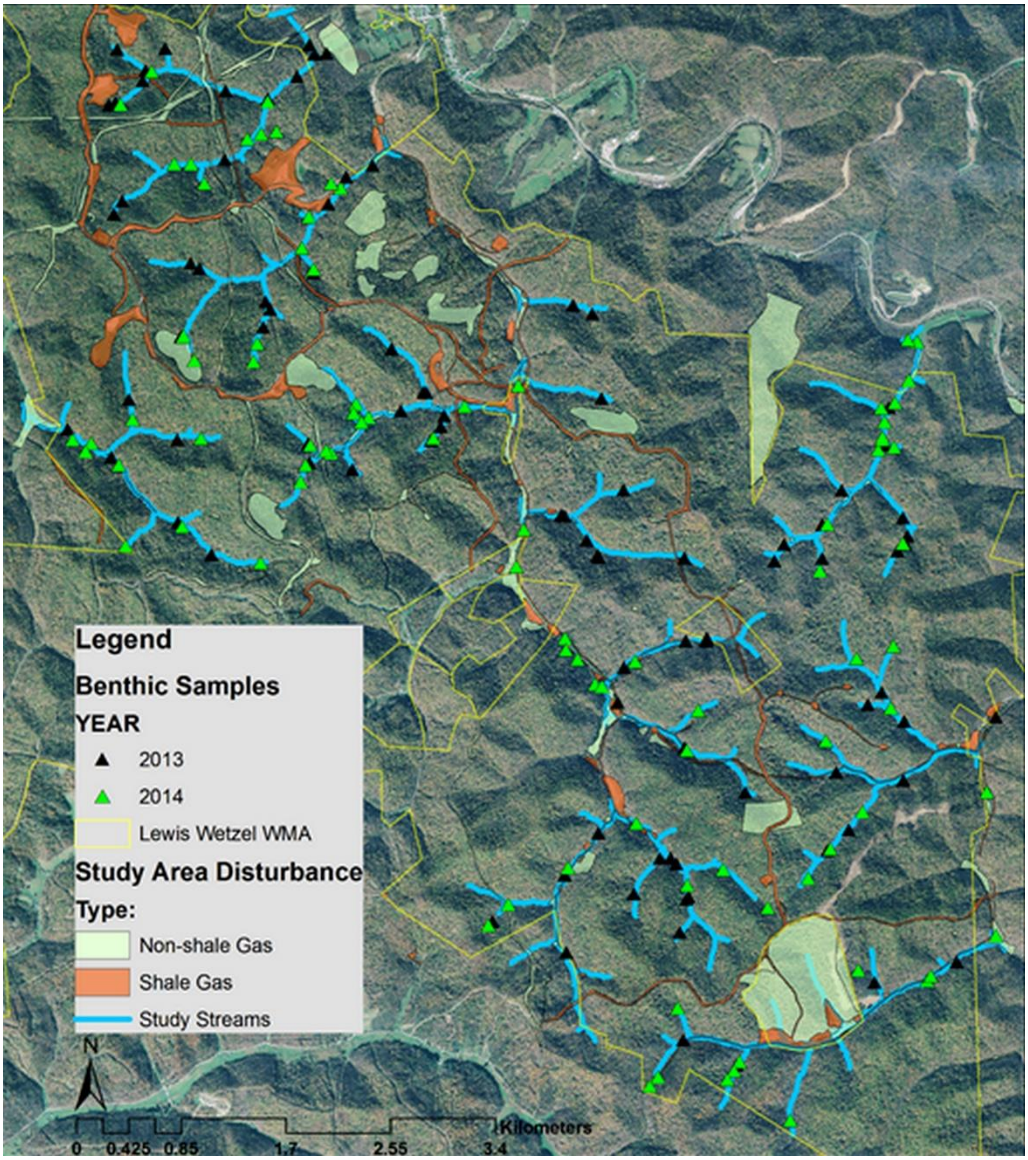

Fig 1. Location of study streams, benthic sampling locations, and stream reaches disturbed by shale gas development during 2013–2014 on the Lewis Wetzel Wildlife Management Area in northwestern West Virginia. The larger light green patches of non-shale gas disturbance are primarily timber harvests with partial canopy removal.

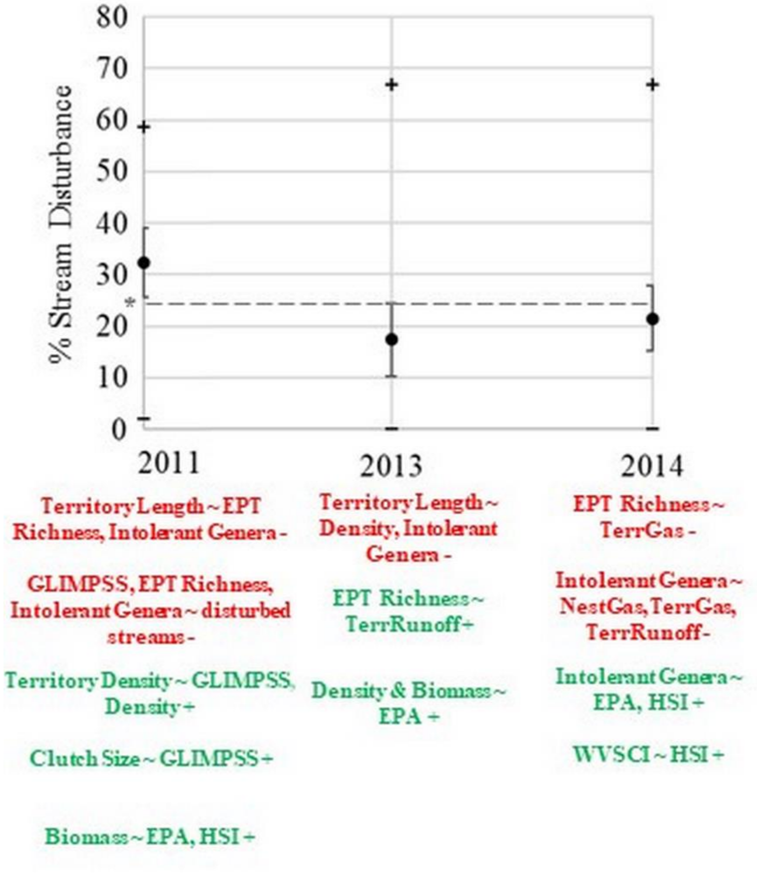

Fig 2. The average amount of shale gas related disturbance ± standard error (SE) and range (black + and -) on headwater streams (n = 14), in addition to statistically significant positive (green) and negative (red) demographic vs. aquatic prey responses over a six year period (2009–2011, 2013–2015) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia. Nest survival results are not displayed. The bracketed line represents a hypothetical, conservative disturbance threshold (≥25%) at which waterthrush demography may be more negatively affected based on the strongest and second strongest demographic responses to aquatic prey in 2011 and 2014. Variable names are defined in S1 Table 2.

APPENDIX

Appendix Table 1. Annual shale gas disturbance activity at Lewis Wetzel Wildlife Management Area study streams in 2011, 2013, and 2014. Our research season for several ridgetop and stream research projects occurred relatively from April–July of each year, with time periods referring to this research season range. R = new ridgetop activity, S = new stream activity (streamside road activity or stream sedimentation), W = new well pad activity, P = parts considered disturbed from activity in previous years, N = no new activity. Superscripts B = Brief or intermittent activity period(s), E= Early in waterthrush breeding season, L=Late in waterthrush breeding season, and C =continuous activity.

As a result of previous and newly started ridgetop activity in 2010–2011 the whole downstream network of some streams became at risk for sedimentation and surface runoff for the remainder of the study. In 2013, construction started on a new compressor station located before reaching Snake North, meaning all headwater stream bases emptying into Buffalo Run north of this site was near heavy truck traffic from 2013–2014. In late 2013 (June–July), construction started on a new well pad at the base of Owl Run which was previously a homestead with all drilling completed by July–August 2014. Well pad construction started on the ridgetops above Olive Run in 2013 with wells not becoming active until 2014; otherwise the stream remained mostly undisturbed except for maintenance of a forested track for pump jack accessibility. Activity at Buffalo East and West Run was mainly concentrated at their confluence where an active shale gas pad was located, especially in 2013–2014 where disturbance was otherwise minimal. In 2013–2014, Wyatt and especially Sees Run were subject to sedimentation slips into the stream from compromised erosional control below well pads or access roads, especially during any rainfall event; Wyatt Run had a new unsodded pipeline connector that caused brief sedimentation early in the research season.

In 2014, activity at Slabcamp Run increased from the previous year from a well pad being re-drilled but was commonly subject to streamside disturbance. Sees Run had increased sedimentation in 2014 from an active access road hillside partially collapsing and filling a stream valley. In summary, 2013 disturbances were just starting to occur but did not necessarily directly affect the streams during the time waterthrush were sampled in 2013, and in 2014 shale gas activity peaked again, particularly at Slabcamp Run, but did not achieve 2011 levels of activity.

Study Streams	**2011**	**2013**	**2014**
Buffalo East Run	P, S^{B}	P, W^{EB}	P, W^{B}
Buffalo West Run	P, S^{B}	P, W^{EB}	P, W^{B}
Carpenter Run	N	N	N
Hiles Run	N	N	N
Huss Pen Run	N	N	N
Megans Run	S^{B}, R^{C}	P, R^{C}	P, R^{C}, W
Nettles Run	P, R^{L}	P, S^{B}	P
Olive Run	P, W, S	P, S^{B}, R	P, S^{B}, R, W
Owl Run	P, S	P, W^{L}	P, W^{C}
Sees Run	P, R^{C}, W, S	P, R^{C}, S^{C}, W	P, R^{C}, S^{C}
Slabcamp Run	P, W, S	P, W, S	P, R^{L}, W^{C}, S^{C}
Snake North Run	P, S	P	P
Snake South Run	P, S	P	P
Wyatt Run	R^{C}, W	P, R^{C}, S^{EB}, W	P, R^{C}, S^{B}, W
Nest/Stream Sampling Dates	**May 6 – 9**	**May 22 – July 28**	**June 16 – July 6**

Appendix Table 2. Variables used to evaluate the demographic response and nest survival of Louisiana Waterthrush to aquatic prey and shale gas development. Nest survival is daily survival rate (DSR) over a 29-day nesting period. We evaluated Louisiana Waterthrush demographic response to aquatic prey and shale gas development using spatial generalized linear mixed models (SGLMMs).

Variable of interest	**Notation**	**Analysis**
Nest Age	NestAge	Nest Survival
Time within-season (quadratic time trend)	TT	Nest Survival
Average Daily Rainfall	Rain	Nest Survival
Percent of Stream Disturbed by Shale Gas	StreamGas	Description only
Percent of Stream with Potential Contaminant Runoff	StreamRunoff	Description only
Shale Gas Nest Disturbance (undisturbed=0, disturbed=1)	NestGas	SGLMM
Percent of Territory Disturbed by Shale Gas	TerrGas	SGLMM
Percent of Territory with Potential Contaminant Runoff	TerrRunoff	Nest Survival, SGLMM
Clutch Size	Clutch Size	SGLMM
Number of Fledglings	Fledglings	SGLMM
Territory Length	Territory Length	SGLMM
Territory Density	Territory Density	SGLMM
West Virginia Stream Condition Index	WVSCI	Nest Survival, SGLMM
Genus Level Index of Most Probable Stream Status	GLIMPSS	Nest Survival, SGLMM
Aquatic Prey Biomass	Biomass	Nest Survival, SGLMM
Aquatic Prey Density	Density	Nest Survival, SGLMM
Number of Intolerant Genera	Intolerant Genera	Nest Survival, SGLMM
Ephemeroptera, Plecoptera, Trichoptera (EPT) Richness	EPT Richness	Nest Survival, SGLMM
Habitat Suitability Index (HSI) score	HSI	SGLMM
Environmental Protection Agency (EPA) Rapid Bioassessment index	EPA	SGLMM

CHAPTER 5. SPATIAL STREAM MODELING OF LOUISIANA WATERTHRUSH (*PARKESIA MOTACILLA*) FORAGING SUBSTRATE AND AQUATIC PREY IN A WATERSHED UNDERGOING SHALE GAS DEVELOPMENT.

Chapter 5 is written in style of the peer-reviewed journal *Food Webs*.

ABSTRACT

We demonstrate use of spatial stream network models (SSNMs) for the first time on an aquatic-terrestrial organism, the Louisiana Waterthrush (*Parkesia motacilla*), to explore relationships between this bioindicator songbird and stream monitoring data in an area undergoing shale gas development. SSNMs allowed us to account for spatial autocorrelation inherent to stream monitoring data and properties that traditional modeling approaches cannot capture to elucidate factors that affect waterthrush foraging locations. We monitored waterthrush along 58.1 km of 1st- and 2nd-order headwater stream tributaries (n = 14) in northwestern West Virginia over a two year period (2013–2014), sampled benthic macroinvertebrates in waterthrush territories, and collected wetted perimeter stream channel and water chemistry data. Spatial models outperformed traditional regression models and made a statistical difference in whether stream covariates of interest were considered relatable to waterthrush foraging. Waterthrush foraging probability index (FPI) was greater in areas where family (West Virginia Stream Condition Index, WVSCI) and genus-level (Genus Level Index of Most Probable Stream Status, GLIMPSS) multi-metric indices of biotic stream integrity were higher. Waterthrush were found foraging both where stream locations were hydrologically connected and unconnected by stream flow. These stream foraging locations were relatively closer to undisturbed headwaters where WVSCI and GLIMPSS were predicted to be highest. While there was no significant relationship between FPI and shale gas land use on a catchment area scale, further information on bioaccumulation of contaminants in benthic macroinvertebrates of shale gas disturbed areas is needed before establishing the extent to which waterthrush foraging may be affected by shale gas development.

INTRODUCTION

A natural property of ecological data is autocorrelation where nearby objects are more likely to exhibit the same patterns for reasons not due to chance (Legendre, 1993). Since the advent of classical statistics (e.g. Fisher, 1935), many spatial models were developed that can account for the non-independence of ecological observations. However, spatial models are not created equally in handling violations of statistical assumptions (Dormann, 2007) and are designed mainly for terrestrial ecology (e.g. Fortin and Dale, 2005). Large, long-term datasets are being collected globally on streams as part of biomonitoring efforts to determine environmental conditions and change (Buss et al., 2015), making it increasingly important to choose appropriate statistical methods for valid assessment of stream network data (Rushworth et al., 2015). Spatial models that incorporate the unique properties of streams as dendritic networks with restricted, directed movement of resources through the landscape would be more ideal than the current trend of adopting terrestrial modeling techniques to streams (Issak et al., 2014). Recently, a series of spatial stream network models (SSNMs) were created for benthic organisms that account for stream properties (e.g. branching, flow direction and connectivity, confluences) and allow analysis of typical environmental monitoring data via stream-based spatial-weighting and autocovariance structures (Cressie et al., 2006; Ver Hoef et al., 2006; Peterson and Ver Hoef, 2010). Spatial autocorrelation is a confounding source of variability for covariates of interest on a stream network, where dismissing or ignoring it can lead to important information being discarded or lack of statistical inference (Legendre, 1993).

Wildlife communities in the Appalachian region, particularly in forested freshwater ecosystems (Dunscomb et al., 2014; Evans and Kiesecker, 2014), are threatened by unconventional shale gas development (Farwell et al. 2016; Frantz et al. 2018a). Shale gas wells in the Marcellus shale region are commonly within 100–300 m of stream channels, and often even closer to headwater drainage areas (Entrekin et al., 2011). Headwater streams are the critical sources of water, sediment, organic matter, and nutrients for the rest of the system (Gomi et al., 2002), and are therefore vital for ecological integrity (Freeman et al., 2007). Furthermore, headwater streams, despite their predominance of drainage area and total stream length, are largely overlooked for protection or regulation contrary to their potential effect on downstream reaches and aquatic life (MacDonald and Coe, 2007).

The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is an established biological indicator of aquatic stream integrity (O’Connell et al., 2000; Mulvihill et al., 2008) and species of conservation concern (USFWS, 2008). Waterthrush feed primarily on benthic macroinvertebrates (Mattsson et al., 2009) and breed along forested headwater streams, reaching some of their highest abundances in the Marcellus shale region (Sauer et al., 2014). Over a six-year period, shale gas disturbance negatively affected waterthrush riparian habitat quality, nest productivity, and nest survival suggesting potential long-term population consequences (Frantz et al., 2018a). Given the propensity for shale gas in the Appalachian region to be developed on ridgetops near headwater streams (Cook et al., 2015), there is a need to evaluate how down-stream communities, both aquatic and terrestrial, can be affected by potential surface water pollution (Entrekin et al., 2011). In particular, the food webs along the aquatic-terrestrial interface may be indirectly influenced by surface water contamination depending on where the organisms reside or forage along the stream network. Waterthrush are known to compensate for the loss of food resources by increasing their territory sizes and foraging in nearby undisturbed areas (Mulvihill et al., 2008; Frantz et al., 2018a). As such, a more detailed study of headwater streams and foraging of a stream-dependent organism would shed light on whether shale gas development is influencing food resources, especially if we can account for the spatial influence of stream properties.

In this study, we tested SSNMs on an aquatic-terrestrial organism to evaluate their utility in quantifying characteristics of waterthrush foraging areas based on 1) water chemistry, 2) a waterthrush foraging score based on stream channel data, 3) shale gas land use based on reach contributing area (i.e. catchment area), and 4) multi-metric indices of biotic stream integrity at the family and genus level. We hypothesized that waterthrush foraging would more likely occur in areas with higher biotic stream integrity and with higher abundance of pollution sensitive aquatic prey such as Ephemeroptera, Plecoptera, and Trichoptera (EPT) believed to be the waterthrush's preferred prey items (Mattsson et al., 2009). We also hypothesized that waterthrush foraging activity would be negatively related to areas of higher shale gas land use and water chemistry (i.e. higher conductivity, total dissolved solids, pH, and water temperature) as surface water pollution (e.g. Latta et al., 2015) and decreased riparian habitat quality (e.g. Wood et al., 2016; Frantz et al. 2018b) from shale gas development may negatively alter aquatic prey communities (Johnson et al., 2015).

METHODS

Study Area

We studied waterthrush along 58.1 km of 1st- and 2nd-order headwater stream tributaries (n = 14) at Lewis Wetzel Wildlife Management Area (LWWMA) located in northwestern West Virginia (Fig. 1). Our waterthrush foraging study occurred in 2013 and 2014 as part of a larger waterthrush demography study over a six year period (Frantz et al. 2018a, 2018b). The study area lies within the Permian Hills subdivision of the Western Allegheny Plateau Ecoregion, an area of deeply dissected topography and relatively continuous Appalachian Oak and Mixed-Mesophytic Forest (Woods et al., 1999) with elevations of 221–480 m. It overlays the Marcellus-Utica shale region and occurs where waterthrush reach their highest densities within the central Appalachians (Sauer et al., 2014).

Prior to our study, LWWMA was 95% forested with the first unconventional gas well development and activity, hereafter shale gas, starting in 2007 (Farwell et al., 2016). Shale gas at our study area and within the surrounding region since then has rapidly increased (WVGES, 2015). By 2015, LWWMA was 91% forested with forest loss primarily due to shale gas development (Farwell et al., 2016). Over the six year study period, gas well development activities included building of conventional and Marcellus well pads, timbering for yet unbuilt well pads, the expansion of existing road and pipeline infrastructure, and the construction of new infrastructure. Between the 2010 and 2011 breeding seasons, shale gas development activities began to increase on the ridgetops (Frantz et al., 2018a, 2018b). Therefore during the waterthrush foraging study in 2013–2014 the whole downstream network of some streams became disturbed by sedimentation and surface runoff from ridgetop activity (Frantz et al., 2018a, 2018b).

Mapping of Streams and Disturbance

Within a Geographic Information System (GIS), we used a sequence of leaf-on and leaf-off aerial (e.g. NAIP) imagery and extensive ground-truthing to manually digitize areas of disturbance within the study area (see Frantz et al., 2018a for full description). All disturbances were classified as shale gas related (e.g. well pads and associated road and pipeline infrastructure) or as being unrelated or pre-existing (e.g. forest roads, recent even-aged timber harvests, and various types of existing clearings). We classified a few conventional impacts (i.e., stream-side vertical pump jacks) as related to shale gas development because their pads were managed in conjunction with nearby shale gas infrastructure and because their targeted formation, even though they remained shallow after development, was listed as Marcellus (WVGES, 2015). Gas well records (WVDEP, 2015) were used to verify target shale formations, drilling status, and start dates for all well disturbances. Lengths of each study stream (average length 4.1 ± 0.54 km, range 0.95–7.4 km) were calculated in GIS using a 3D functional surface length tool and a 3 m resolution digital elevation model to account for topography, and study streams were defined to have a drainage basin of 9 hectares (i.e. <100 ha, Swanson et al., 1998) to delineate the uppermost headwater reaches (24 k scale or higher resolution; e.g. Strager et al., 2009).

Waterthrush Foraging Observations

We mapped waterthrush territories along 14 streams with varying degrees of shale gas disturbance in 2013–2014 from early April to late June each year. Standardized territory mapping (Robbins, 1970; Bibby et al., 1992) included ≥6 (average 11.5 ± 0.6) visits along each stream reach, with visits preceding peak incubation initiation, and visits within 4 hours after sunrise to ensure high rates of detection (Mattsson and Cooper, 2006). While delineating territories, observations were made whether waterthrush were foraging or not (e.g. singing, territorial dispute, flying) and mapped with a WAAS-enabled Garmin 60CSX GPS unit with accuracy ≤5 m. We recorded observations of both male and female waterthrush since neither foraging rate nor microhabitat use differs between the sexes (Robinson, 1990). When a waterthrush was detected, we only approached close enough for observation without perceptibly influencing behavior (Ritz and Rodewald, 2010). Waterthrush are just as likely to be "loafing" as they are foraging in a given location (Robinson, 1990). Therefore any observation where a waterthrush was observed to flush when first encountered was categorized as non-foraging, although we recognize the possibility that waterthrush may have flushed mid-forage. We varied the order and time of day we monitored study streams to prevent any time of day effects (Shield, 1977), so waterthrush observations overall should not be influenced by our presence nor time of day. We concurrently searched for and monitored waterthrush nests during these visits.

Wetted Perimeter Data

To evaluate in-stream riparian habitat quality for foraging waterthrush, we collected several stream channel metrics every 50m along each stream using a wetted perimeter protocol designed to determine optimal waterthrush foraging substrate locations (Master et al., 2005). Sampling locations were assigned in GIS prior to entering the field using Linear Referencing, which created routes along the stream that accounted for stream bend. Points were not sampled that fell on private property (n = 58 points), on completely dry sections of streams (n = 30 points), or stream sections with water flow too high for waterthrush (n = 5 points). This resulted in sampling of 1121 points among the fourteen headwater streams. Wetted perimeter data were collected in 2013 for Olive Run and Wyatt Run, and for the remainder of the streams in 2015. The data were collected in late June–July to be representative of year-round flow conditions.

At each 50m sampling point, a small-link metal chain was draped across the stream at points where water during a high flow event at each edge of the stream meets the bank. This resulted with the ends of the chain curving up each side of the stream bank and stopping at the uppermost portion at which water could flow, creating a “U-shape” with the chain. The chain conformed to all irregularities in the stream channel (e.g. rocks, logs) comprising the bottom substrate, including those sticking up above water. Using a meter tape, the lengths of chain that were above water and stretched over rocks or logs were measured (in meters) as an exposed point measurement with the number of exposed points tallied. The chain was then removed from the water and stretched to full length, with the distance between the two points that marked the edges of the stream measured. The full chain length was a wetted perimeter measurement, where a wet distance could be calculated from subtracting the exposed distance. We measured stream depth (in cm) at five regularly spaced intervals across the same start and end points of the chain: water’s edge on both sides, a quarter of the way in from middle on both sides, and middle of stream. A waterthrush foraging substrate score was calculated by taking the wetted perimeter value and dividing it by the average stream depth at that point (Mulvihill and Latta, Unpublished results). A higher wetted perimeter to mean depth ratio presumably indicated relatively better foraging substrate, and smaller values poorer foraging substrate (Mulvihill and Latta, Personal communication). At every 50m sampling point, we also collected water chemistry data in the form of pH, total dissolved solids (TDS, g/L), conductivity (μS/cm), and water temperature (°C) with Hannah Instruments and an Oakton PC 10 Series multi-parameter probes.

Benthic Macroinvertebrate Sampling

We sampled macroinvertebrates in riffle habitat adjacent to nest site locations using a Surber sampler in 2013 and 2014. Nest site samples (n = 178) were collected shortly after the nest fledged, failed, or had been abandoned (from mid-June to late July) to assess relative prey availability at the time the site was used by waterthrush. Additional macroinvertebrate samples were collected from waterthrush foraging locations (n = 65; average 165 ± 12.6 m from nest site locations) during two timed bouts, one each in May and June. The two bouts were later pooled into one sample after we found no taxa differences between the time periods. During sample collection, we scrubbed rock substrates and disturbed sediment 3-cm below the stream bed within the Surber frame for a total of 3 minutes (Mattsson and Cooper, 2006). We separated macroinvertebrates from detritus for each sample in the field and stored them in 95% or 70% ethanol.

Macroinvertebrates in benthic samples were sorted, counted, identified to genus level, and body lengths measured by an environmental scientist certified by the Society of Freshwater Scientists in macroinvertebrate identification. For each sample, we calculated several commonly used community metrics both at the family (e.g. family taxa richness) and genus (e.g. genus taxa richness) levels of resolution. These metrics were used to calculate multimetric indices of biotic integrity, one at the family level (West Virginia Stream Condition Index, WVSCI; Gerritsen et al., 2000) and one at the genus level (Genus Level Index of Most Probable Stream Status; GLIMPSS, version CF), which does not require the genus-level identification of Chironomidae (Pond et al., 2013).

GIS Data Preparation and Stream Formatting

In ArcMap GIS 10.2.2 (ESRI, Redlands, CA), foraging observations and macroinvertebrate sampling points were mapped along with the wetted perimeter point grid plotted along the headwater streams. Waterthrush typically travel no further than 60 m away from their linear territories (Mattsson and Cooper, 2009) and will forage off stream more often as the breeding season progresses (Robinson, 1990). Therefore we placed a 60 m buffer around each wetted perimeter sampling point. Using a spatial join, all foraging and non-foraging observations within those buffers were assigned to the wetted perimeter grid. Metrics from macroinvertebrate sampling points were merged into a single new output if they fell within a 60m buffer, and then averaged if more than one point fell within a buffer. Any wetted perimeter points that did not have any foraging or non-foraging observations nor macroinvertebrate data were removed from our response variable. A foraging probability index (FPI, 0–100%), our response variable, was derived from the number of foraging observations divided by the total observations (foraging and non-foraging) for the 60 m area. Calculating FPI in this manner gave a mostly continuous index since a 60m buffer overlapped the 50m wetted perimeter points, meaning observations and benthic samples could be assigned to more than one nearby wetted perimeter sample point. We defined FPI as a relative index that gauged where waterthrush were most likely to be found foraging. Rather than assume any areas with no waterthrush observations (i.e. points that we did not use for FPI) were non-optimal foraging areas, we reserved these wetted perimeter points for testing model prediction.

Gas variables (% Marcellus Pad, % Gas Pad (unconventional and conventional), % Gas Infrastructure) were created first as reach contributing area (RCA; i.e. catchment area scale) attributes using the STARS (Spatial Tools for the Analysis of River Systems) toolbox (Peterson and Ver Hoef 2014) in ArcMap GIS as a means of calculating land use. The Accumulate Values Downstream and Watershed Attributes tools were used to create and assign the gas RCA values to sampled points on the stream. To get a percentage contribution of each gas variable, we divided the value assigned to each stream sample by the total watershed area representative of all segment watersheds encompassing the study streams (33.4 km^2 total). Percent (%) Marcellus Pad included three retention ponds that may pose the same concerns to surface water contamination. While we included a metric that had conventional gas well pads (% Gas Pad), all major landscape alterations, development, and activity seen during the duration of our study would not have occurred without shale gas at our study site (Farwell et al., 2016). Percent (%) Gas Infrastructure included all well pads, pipelines, retention ponds, and access roads leading to well sites.

Stream segment vectors were simplified to avoid converging streams and have minimum pseudonodes (Peterson and Ver Hoef, 2014). In the original study design, parts of Buffalo Run that the headwater streams emptied into were included as part of the boundaries of each study stream since many times a waterthrush territory would border or include part of Buffalo Run (Frantz et al., 2018a). These sections of Buffalo Run were removed from each study stream since each stream can only have one outlet in SSNMs. Stream segments were also extensively preprocessed to ensure they were digitized in a downward flow direction and any network topology errors removed that may interfere with spatial weighting calculations.

Model Spatial Distance and Weight Preprocessing

All model analyses were done using the Spatial Stream Network (SSN) package (Ver Hoef et al., 2014) in R (R Core Team, 2014) and SSN object preprocessing for import in ArcGIS using the STARS toolbox (Peterson and Ver Hoef, 2014). In order to fit spatial models using spatial weights, we first determined stream segment proportional influence and additive function values using STARS. Stream segment proportional influence was based on RCA for each line segment watershed area (Peterson and Ver Hoef, 2010). Spatial weight was based on RCA since it serves as a surrogate for flow volume (Friedan et al., 2014). These values were contributed in R to create the spatial weights (Ver Hoef et al., 2014). Upstream distance between a stream outlet and each stream segment and sample point were calculated with STARS (Peterson and Ver Hoef, 2014) to be used in R for calculating hydrologic flow-connected and -unconnected distances (see Ver Hoef and Peterson, 2010) in R. The processed dataset was stored and displayed as a Landscape Network (LSN) that included all spatial and geographic relationships for the streams and stream dataset (Theobald et al., 2006; Peterson and Ver Hoef, 2014).

Model Variables, Covariance, Selection, and Evaluation

We initially reviewed all data graphically and through diagnostic tools to test assumptions of normality and applied data transformations if it improved approximation to normality (Zuur et al., 2010). Torgegrams (i.e. semivariograms for streams; see Zimmerman and Ver Hoef, 2016) were used to assess spatial autocorrelation which breaks up the semivariance into flow-connected and -unconnected structures (Ver Hoef et al., 2014). Based on diagnostic evaluation we added a log10 data transformation for temperature, total dissolved solids (TDS, g/L), conductivity (µS/cm), and foraging score.

Our SSNMs used a mixed-model autocovariance structure consisting of exponential tail-up (TU), tail-down (TD), and exponential Euclidean. TU and TD autocovariance models represent water flow-connected (i.e. TU and TD) and –unconnected relationships (i.e. TD) along the stream and is based on hydrologic (rather than "traditional" straight-line Euclidean) distance (extensive explanation can be found in Ver Hoef and Peterson, 2010). Euclidean distance was included for comparison since it is a traditionally used distance. Autocovariance models were not determined *a priori* (Friedan et al., 2014) as a partial sill, range parameter, and overall nugget effect estimated for each model helps determine relative influence of the components in individual models for either model improvement or removal (Ver Hoef and Peterson, 2010).

We used an exploratory multi-stage model selection process for model evaluation that allowed us to determine autocovariance structure and what covariates to keep for further evaluation (Friedan et al., 2014). We modeled covariates individually since we were interested in which ones had the most predictive power and were statistically significant before comparing the individual covariates in a final model set. As such, there was no need to examine correlations of covariates to avoid multicollinearity. We set $\alpha = 0.10$ to avoid missing any variables that may be of ecological relevance. We first ran a non-spatial linear regression model equivalent for each variable for comparison and evaluation of spatially-influenced properties in spatial models, and because non-spatial linear regression models are traditionally how the variables would have been modeled. Only significant variables ($P < 0.10$) from the non-spatial models were placed into spatial models. Initial covariance structures of spatial models were mixed and fixed to exponential TU, exponential TD, and exponential Euclidean since we expected variability in how spatial weights may affect each covariate.

Maximum likelihood (ML) estimation was used to estimate parameters of Gaussian models (response variable FPI). Akaike's Information Criterion (AIC; Akaike, 1974; Burnham and Anderson, 2002) was used to compare models which penalized for additional spatial autocovariance structures (Ver Hoef and Peterson, 2010). R^2 and root mean square prediction error (RMSPE) based on the observed response variable and leave one out cross validation (LOOCV) predictions were also calculated. Variance decomposition was used to determine the total amount of variation associated with a response variable (Ver Hoef et al., 2014). Predictions from wetted perimeter locations with no waterthrush observations were generated using universal kriging (Cressie, 1993).

The next stage of model selection involved selecting the best autocovariance structure (Friedan et al., 2014). If exponential TU/TD models had a higher partial sill than Euclidean autocovariance, we added Mariah, Spherical, and Linear-with-sill to test before final selection of autocovariance. Final models were evaluated by AIC, RMSPE, and by examining the influence of each variance component. We mapped and visually examined prediction values ± standard error (SE) as one means of determining overall model performance (Bennett et al., 2013) along with plotting of LOOCV predictions and SEs against the observed data. *Post-hoc* Spearman's Rho correlation index tests in R were used to determine what components of WVSCI, GLIMPSS, or foraging score were associated to FPI if those covariates were found important during model selection.

RESULTS

During 2013–2014, we collected 948 foraging and non-foraging observations of waterthrush. Each stream had an overall average of 30.6 ± 7.2 foraging and 37.1 ± 6.2 non-foraging observations (average 67.7 ± 11.1 total observations per stream, range 4–214) collected. We had 318 60-m buffered wetted perimeter sampling points for analysis that included both waterthrush observations and benthic samples, and an additional 103 saved for prediction modeling that had benthic samples but no waterthrush observations. Each stream had an average of 22.7 ± 3.4 sampling points (range 3–42). Each sampling point contained on average 4.1 ± 0.2

waterthrush observations (range 1–30). Average foraging observations at each sampling point were 2.0 ± 0.2 (range 0–30) and average non-foraging observations were 2.0 ± 0.1 (range 0–14). Overall average foraging probability index (FPI) on each stream based on the sampling points was 47.9 ± 4.5 % (range 20.3–80.4 %, Fig. 2).

Stream temperature, benthic biomass, benthic density, GLIMPSS, and WVSCI were significant in explaining foraging probability index (FPI) according to the non-spatial linear regression models ($P < 0.10$, Table 1). A torgegram for FPI suggested there may be higher spatial autocorrelation between flow-connected sample points at short distances, but both flow-connected and unconnected samples have high autocorrelation (Fig. 3). The torgegram also suggested using both tail up (TU) and tail down (TD) autocovariance structures in initial spatial models to obtain the full range of autocorrelation.

The five significant variables were placed into individual spatial models where only GLIMPSS and WVSCI remained significant ($P < 0.05$, Table 2). Given support that tail down (TD) models performed better than tail up (TU) models (Table 3), we added two more tail down variance components for AIC model comparison (Table 4). In final AIC model comparison between WVSCI and GLIMPSS, there was more support for WVSCI explaining FPI than GLIMPSS (Table 5) with competing indication of TD and Euclidean variance structure (Table 6). Final models for WVSCI and GLIMPSS had low predictive power with almost all model variance explained by the autocovariance component rather than the covariate (Table 6, Fig. 4 and 5). *Post-hoc* spearman rank correlation tests between FPI and WVSCI/GLIMPSS metrics (no. Ephemeroptera genera, no. Plecoptera genera, no. intolerant taxa tolerance value <4, and EPT richness) were all significant (Rho=0.24, 0.20, 0.23, and 0.22 respectively, $P < 0.001$, Fig. 6).

DISCUSSION

Our study is the first to apply SSNMs to relate trophic levels across the aquatic-terrestrial interface using a semi-aquatic organism that is not necessarily as restricted in movement as benthic organisms. Overall, spatial models outperformed traditional regression models, and made a statistical difference in whether stream covariates of interest were considered relatable to waterthrush foraging areas. While the spatial models had poor predictive power, SSNMs allowed us to assign variability due to spatial autocorrelation and evaluate potential trends involved in foraging on headwater streams. Stream temperature, biomass, and density were significant using standard linear regression, but were no longer significant once we considered spatial autocorrelation. Therefore using standard statistical approaches could have led to making a type I error for these covariates (Dormann et al., 2007).

We did not find a relationship between foraging substrate score based on wetted perimeter data and FPI (Table 1). The protocol was designed on 1st and 2nd order waterthrush study streams in Pennsylvania (PA) that are relatively wider, less bank, deeper water depth, and more perennial (Latta, 2009) than our narrow streams with steeper topography. Consequently, the wetted perimeter protocol may be region and stream-type specific, and water depth in relation to exposed or wetted areas that create available foraging microhabitat less important for perennial streams with many ephemeral and intermittent tributaries. For example, less availability of bank nest substrate in PA meant nests were commonly found off-stream in root balls of fallen trees (S. Latta, Unpublished results), whereas nests in root balls were rare at our study site (1 of 184 nests in 2013–2014; M. Frantz, Unpublished results). Master et al. (2005) found waterthrush densities on wintering grounds were higher on streams with higher wetted perimeter values, suggesting the need to test these protocols elsewhere in the waterthrush breeding range.

Waterthrush benthic studies were completed in 2011 and 2013–2014 during peak (2011), abated (2013), and elevated (2014) shale gas development at our study site. We found that waterthrush territory densities were greater on streams with higher GLIMPSS scores (Wood et al., 2016), which supports our significant spatial models with GLIMPSS and WVSCI. Higher GLIMPSS and WVSCI values indicate better riparian habitat quality and therefore more foraging resources for waterthrush (Frantz et al. 2018b). Our spatial model suggested at minimum a weak relationship between FPI and these multi-metric indices, at least relative to nesting locations where the majority of our benthic samples were collected. Friedan et al. (2014) used SSNMs to determine drivers of family and genus-level macroinvertebrate indices and wondered whether using coarser (mainly family-level) macroinvertebrate identification could have masked spatial patterns or reduced predictive power. In our case, using family vs. genus-level indices did not make a difference in residual variance of autocovariance, with WVSCI only having minor model selection support over GLIMPSS in explaining FPI (Tables 5 and 6). Our results suggest family-level taxonomic resolution may be good enough to indicate most likely waterthrush foraging areas.

Territory densities in 2011 were greater where Ephemeroptera, Plecoptera, and Trichoptera densities were higher, along with higher biomass (Wood et al., 2016). Territory density declines in 2013–2014 in part lead to the disassociation between aquatic prey biomass, density, and waterthrush demographic response at a nest and territory level (Frantz et al., 2018b). While biomass and density were no longer significant in our spatial models, there is still a positive association between richness of these sensitive taxa orders and FPI (Figure 6). We did not assess biomass or density by size class, by which waterthrush may have shown a stronger, significant response (Wood et al., 2016) in the spatial models. Overall riparian habitat site quality may be more important to waterthrush site assessment (Frantz et al., 2018a) than benthic metrics or in-stream characteristics alone for FPI. Indeed, waterthrush appear to be able to adapt to shale gas disturbance and meet all their foraging needs until potentially a certain disturbance threshold is reached (Frantz et al. 2018b). Nest survival in 2011 was best explained by Habitat Suitability Index (HSI), which describes both nesting and foraging components important to waterthrush (Wood et al., 2016).

The three % gas land use covariates were not significant in the initial non-spatial models (Table 1). These three variables were non-normal and could not approximate normality with traditional transformations, so technically were not appropriate for the linear models. Waterthrush have a negative demographic response to the physical presence of shale gas at localized levels of the nest or territory (Frantz et al., 2018a, 2018b), as well as their aquatic prey (Frantz et al. 2018b), so it is unclear the extent to which waterthrush foraging may change due to catchment-level shale gas disturbance. There are undisturbed stream segments and ephemeral tributaries on every stream giving opportunities to forage elsewhere (Wood et al., 2016), and waterthrush can compensate for food loss (Mulvihill et al., 2008). While this suggests to some extent adaptability in selecting foraging locations, shale gas disturbed areas have the potential to serve as sink habitats (Frantz et al., 2018a), so waterthrush may be breeding or foraging in an ecological trap (Gates and Gysel, 1978; Robertson and Hutto, 2006; Frantz et al. 2019). Additionally, waterthrush in shale gas disturbed areas bioaccumulate more heavy metals associated with the drilling process than those in undisturbed shale gas areas at our study site and elsewhere (Latta et al., 2015; Chapter 6). Foraging on macroinvertebrates is likely one way the heavy metals bioaccumulate, and we do not know how that factors into FPI. Shale gas well pad construction and drilling typical of our study site and others occur in “pulses” (Brittingham et al., 2014), making ephemeral disturbances such as sedimentation or potential runoff entering a stream system where waterthrush hold breeding territories difficult to quantify. SSNMs that can treat both spatial and temporal effects and allow non-linear data structures may be better suited to model these relationships (O'Donnell et al., 2014; Rushworth, 2014; Rushworth et al., 2015).

While our spatial models performed better than non-spatial models, they still had poor predictive power (Table 6, Fig. 4). Aquatic prey community responses were weaker in 2013–2014 at a nest and territory level than in 2011 in relation to shale gas activity levels (Frantz et al. 2018b) which may also translate to weaker predictive power at the catchment-level for these years of the study. Additionally, Friedan et al. (2014) found that spatial-weighting schemes made a substantial difference in model performance and affected variables differently. Our only spatial weighting scheme consisted of reach contributing area (RCA) (i.e. catchment area, Horizon Systems Corporation, 2007) and represented the aerial extent that contributes overland flow to a stream line segment. Some other spatial-weighting options to consider are Shreve's stream order (1967) or consideration of slope at stream segments. While catchment area may have been an appropriate scale for gas land use variables, a spatial weighting scheme such as slope that reflects local scale variability may have been more appropriate for headwater streams (Friedan et al., 2014). Our headwater streams have steep topography and many ephemeral tributaries, and headwaters are known to have high between stream variability of habitat and high macroinvertebrate beta diversity within and among catchments (Clarke et al., 2008).

Waterthrush have linear territories on the stream (Mulvihill et al., 2008) and typically fly up and down the stream corridor rather than around it (M. Frantz, *Personal observation*). As such it makes sense that tail-down (TD) autocovariance structure explained the most model variability as it allows correlation of samples between flow-connected and -unconnected stream segments. Euclidean distance having almost equal explanation of model variability likely reflects some combination of terrestrial components to waterthrush ecology and territory-scale or higher attributes. However predictive power depends not only on autocovariance structure but the covariates themselves (Friedan et al., 2014). For instance, FPI was likely not representative on Carpenter Run which could have introduced some unnecessary variability in the data (Fig. 2). Priority during our six year (2009–2011, 2013–2015) waterthrush demography project was to accurately delineate waterthrush territories, with emphasis on collecting new location points that reflected all boundaries of a waterthrush territory. While we monitored Carpenter Run for nests in 2013, we did not start collecting territory and foraging observations until 2014. Introducing stream (n = 14) as a random effect *post-hoc* in the WVSCI and GLIMPSS models may have explained between 4.6–6.5% of model variance (e.g. AIC = 41.81, RMSPE = 0.232 for WVSCI with Exponential TD autovariance + Stream). This emphasizes the importance of not only thorough observation collection but accounting for headwater stream heterogeneity.

Collecting large data sets due to stream monitoring programs is becoming commonplace (Rushworth et al., 2015), stressing the need to use the proper statistical tools that will provide optimal performance and prediction power. While our spatial models had poor performance power, we can still produce predictive maps that can direct us to potentially important waterthrush foraging areas to evaluate further such as upper reaches of headwater tributaries (Fig. 5). The utility of SSNMs have been used previously to predict fish densities (Issak et al., 2016), and now for an apex avian predator that habits the aquatic-terrestrial interface, and thus has the potential for land managers with waterthrush occurrence data to prioritize management or conservation areas given the waterthrush's role as a bioindicator of aquatic stream integrity (O'Connell et al., 2000; Mulvihill et al., 2008). Our exploratory SSNM analyses are a starting point to inquire further into food-web interactions between waterthrush, macroinvertebrates, and potential surface water contamination, and serves as an example of how spatial autocorrelation coming from multiple sources and scales may influence study implications.

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TABLES

Table 1. Initial non-spatial linear models (with nugget) to test the relationship between foraging score, water chemistry, macroinvertebrate metrics, shale gas land use and foraging probability index (FPI). Covariates with bolded *P* values were significant at $\alpha = 0.10$. R^2 is a generalized value of model fit and the partial sill (sill minus nugget) was included to assess variance of a covariate without the nugget effect.

Variable	Estimate	SE	*t* value	P value	R^2	Partial sill
Foraging Score	0.046	0.054	0.855	0.393	0.0023	0.122
Temperature (°C)	-1.073	0.619	-1.732	**0.084**	0.01	0.121
pH	0.048	0.077	0.627	0.531	0.0012	0.122
TDS, g/L	-0.052	0.106	-0.494	0.622	0.0001	0.122
µS/cm	-0.055	0.098	-0.554	0.580	0.001	0.122
GLIMPSS	0.004	0.001	3.008	**0.003**	0.03	0.119
WVSCI	0.005	0.002	2.948	**0.003**	0.03	0.119
Biomass	0.101	0.037	2.724	**0.007**	0.02	0.120
Density	0.115	0.056	2.053	**0.041**	0.01	0.121
% Marcellus Pad	-0.054	0.183	-0.294	0.769	0.0003	0.122
% All Pad	-0.062	0.175	-0.353	0.725	0.0004	0.122
% Gas Infrastructure	-0.018	0.086	-0.213	0.832	0.0001	0.122

Table 2. Initial spatial generalized linear models to test the relationship between foraging score, water chemistry, macroinvertebrate metrics, shale gas land use and foraging probability index (FPI). Covariates with bolded *P* values were significant at $\alpha = 0.10$. R^2 is a generalized value of model fit and the partial sill (sill minus nugget) was included to assess variance of a covariate without the nugget effect.

Variable	Estimate	SE	*t* value	P value	R^2	Partial sill
Temperature	-0.747	0.842	-0.887	0.376	0.002	0.0003
GLIMPSS	0.003	0.001	2.114	**0.035**	0.01	0.0003
WVSCI	0.004	0.002	2.493	**0.013**	0.02	0.0003
Biomass	0.051	0.039	1.281	0.201	0.01	0.0003
Density	0.074	0.054	1.360	0.174	0.01	0.0003

Table 3. Initial mixed autocovariance components (VAC) of the WVSCI and GLIMPSS spatial models. The nugget captures variability due to measurement error and/or spatial variability at less than the sampling distance. The range represents the distance at which the covariate is no longer spatially autocorrelated. The partial sill (sill minus nugget) assesses variance of a covariate without the nugget effect. Percent VAC is the percentage of residual variance accounted for by each autocovariance component. Based on higher partial sill values for Exponential TD, we added Mariah, Spherical, and Linear-with-sill TD to test before final selection of autocovariance components.

Variance component		**WVSCI**	**GLIMPSS**
Tail down (TD)	Autocovariance function	Exponential	Exponential
	Range	561.606	588.130
	Partial sill	0.073	0.067
	VAC_{TD} (%)	0.56	0.52
Tail up (TU)	Autocovariance function	Exponential	Exponential
	Range	433.213	734.33
	Partial sill	0.00000145	0.0000003
	VAC_{TU} (%)	0.00001	0.000002
Euclidean (Euc)	Autocovariance function	Exponential	Exponential
	Range	409.006	400.174
	Partial sill	0.055	0.061
	VAC_{Euc} (%)	0.42	0.47
Nugget	Nugget	0.0003	0.0003
	VAC_{Nugget} (%)	0.002	0.002

Table 4. AIC model comparison for WVSCI and GLIMPSS spatial models with Exponential Euclidean, Exponential tail down (TD), Spherical TD, and Linear plus sill TD autocovariance components in comparison to the non-spatial model with less parameters. Lowest leave-one-out cross-validation root-mean-square-prediction error (RMSPE) and AIC value was used to assess which models to select for final model comparison.

Variance component	AIC	RMSPE
WVSCI		
Nugget (non-spatial)	243.057	0.346
Exponential Euclidean + Nugget	41.250	0.233
Exponential TD + Nugget	40.469	0.233
Spherical TD + Nugget	41.971	0.236
Linear plus sill TD + Nugget	42.898	0.236
GLIMPSS		
Nugget (non-spatial)	243.403	0.346
Exponential Euclidean + Nugget	43.691	0.235
Exponential TD + Nugget	42.981	0.234
Spherical TD + Nugget	45.036	0.238
Linear plus sill TD + Nugget	45.969	0.238

Table 5. Final AIC model comparison for WVSCI & GLIMPSS. Lowest leave-one-out cross-validation root-mean-square-prediction error (RMSPE) and AIC value was used to select what model best explains foraging probability index (FPI).

Model	Variance component	AIC	RMSPE
FPI ~ GLIMPSS	Exponential Euclidean + Nugget	43.691	0.235
FPI ~ GLIMPSS	Exponential TD + Nugget	42.981	0.234
FPI ~ WVSCI	Exponential Euclidean + Nugget	41.250	0.233
FPI ~ WVSCI	Exponential TD + Nugget	40.469	0.233
FPI ~ WVSCI	Spherical TD + Nugget	41.971	0.236

Table 6. Final autocovariance components (VAC) of the WVSCI and GLIMPSS spatial models that best explain foraging probability index (FPI). The nugget captures variability due to measurement error and/or spatial variability at less than the sampling distance. The range represents the distance at which the covariate is no longer spatially autocorrelated. The partial sill (sill minus nugget) assesses variance of a covariate without the nugget effect. Percent VAC is the percentage of residual variance accounted for by each autocovariance component.

Variance component		**WVSCI**	**GLIMPSS**
Tail down	Autocovariance function	Exponential	Exponential
	Range	494.821	494.117
	Partial sill	0.128	0.128
	VAC_{TD} (%)	0.98	0.98
Tail down	Autocovariance function	Spherical	Spherical
	Range	282.836	NA
	Partial sill	0.129	NA
	VAC_{TD} (%)	0.97	NA
Euclidean (EUC)	Autocovariance function	Exponential	Exponential
	Range	462.148	459.309
	Partial sill	0.128	0.128
	VAC_{EUC} (%)	0.98	0.98
Nugget	Nugget	0.0003	0.0003
	VAC_{Nugget} (%)	0.002	0.002

FIGURES

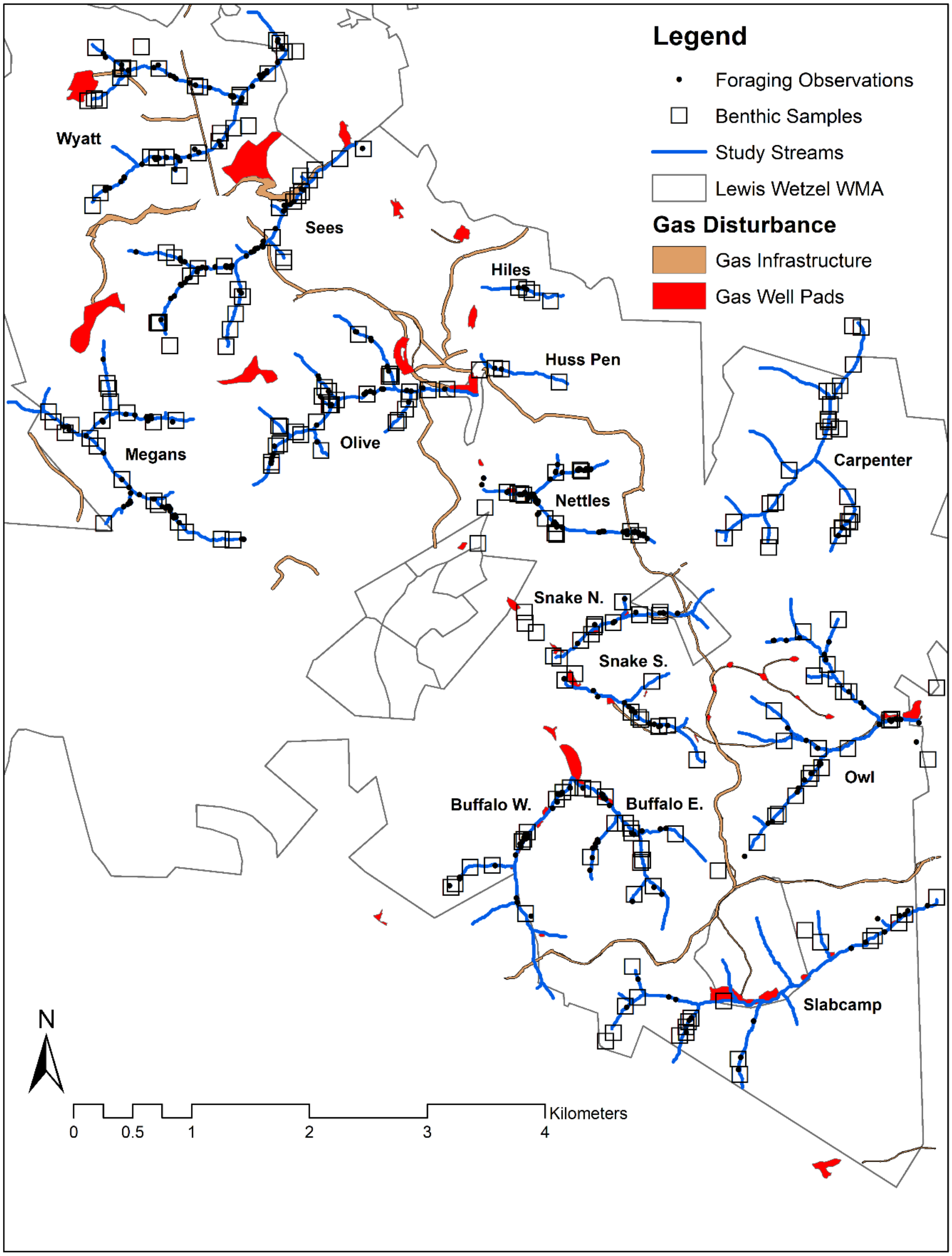

Fig. 1. **Study Area Map.** Our study area, Lewis Wetzel Wildlife Management Area (LWWMA), lies within the Marcellus-Utica shale basin. We observed Louisiana Waterthrush foraging on fourteen 1st and 2nd order headwater streams and collected benthic macroinvertebrate samples during 2013–2014.

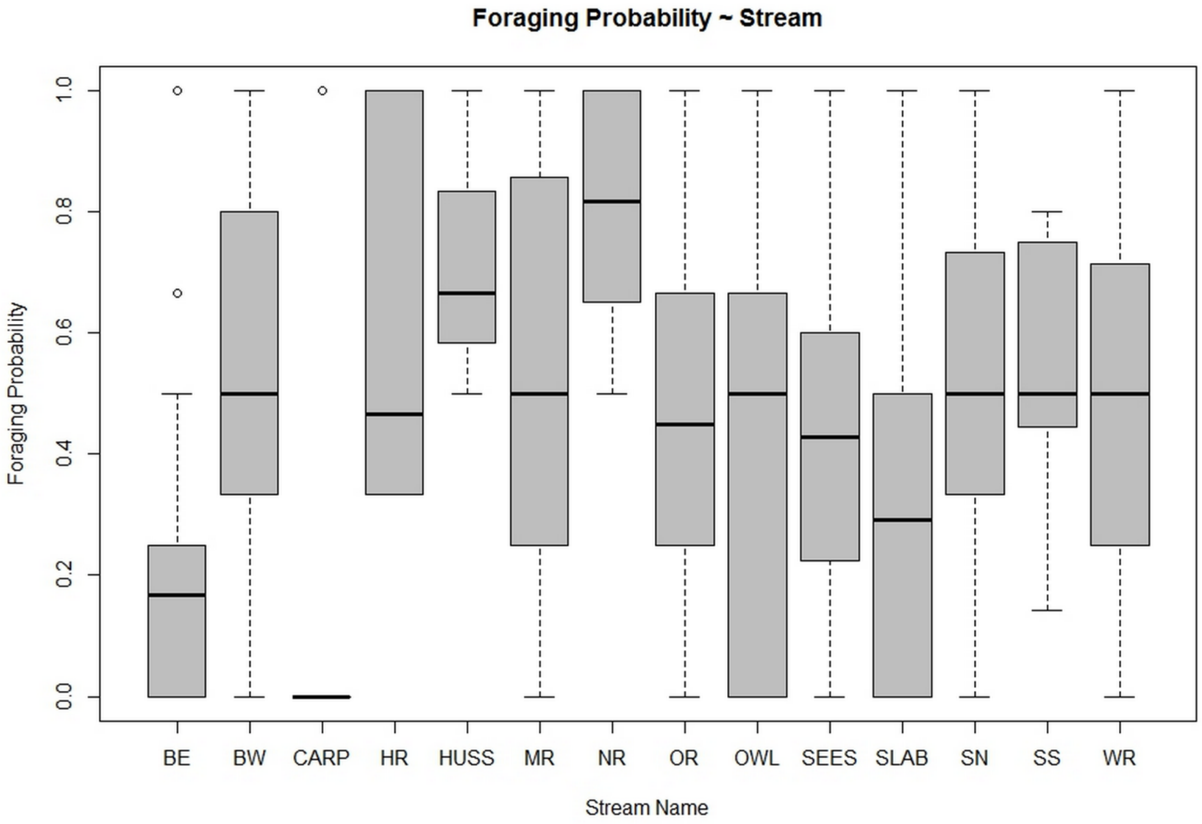

Fig. 2. **Foraging Probability Index by Stream.** Overall foraging probability index (FPI, 0–100%) on each stream during 2013–2014. FPI was derived from the number of foraging observations/total observations for a 60m area surrounding each wetted perimeter grid point on the stream. FPI was a relative index that gauged where waterthrush were most likely to be found foraging. Note Carpenter Run (CARP) only had two foraging observations, limiting the ability to make inferences about FPI on that stream.

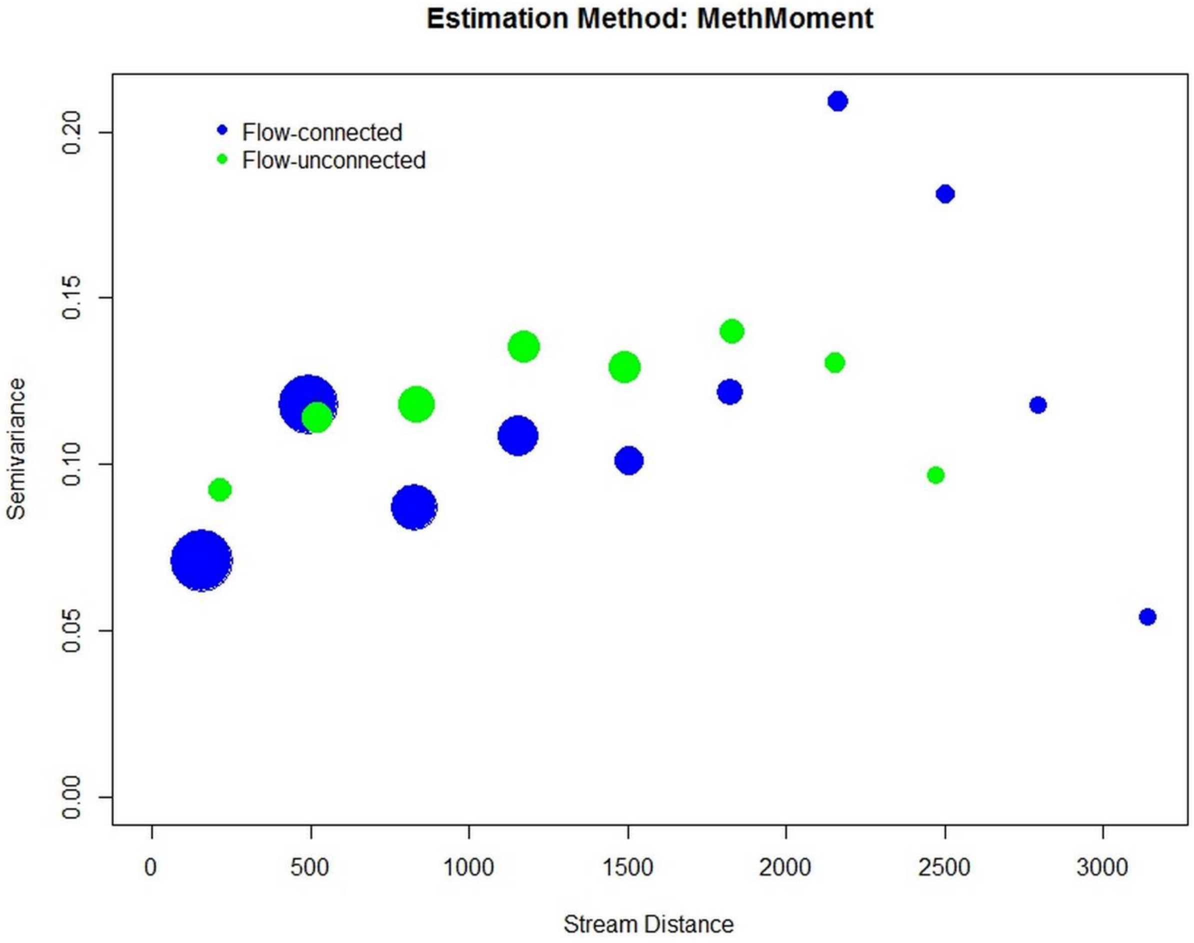

Fig. 3. **Foraging Probability Index Torgegram.** An example of a torgegram for foraging probability index (FPI) which is a modified type of semivariogram. A torgegram displays semivariance (spatial autocorrelation) for samples on streams into flow-connected and -unconnected structures to assist with model fitting. Diameters of circles are proportional to the number of pairs of points in each bin.

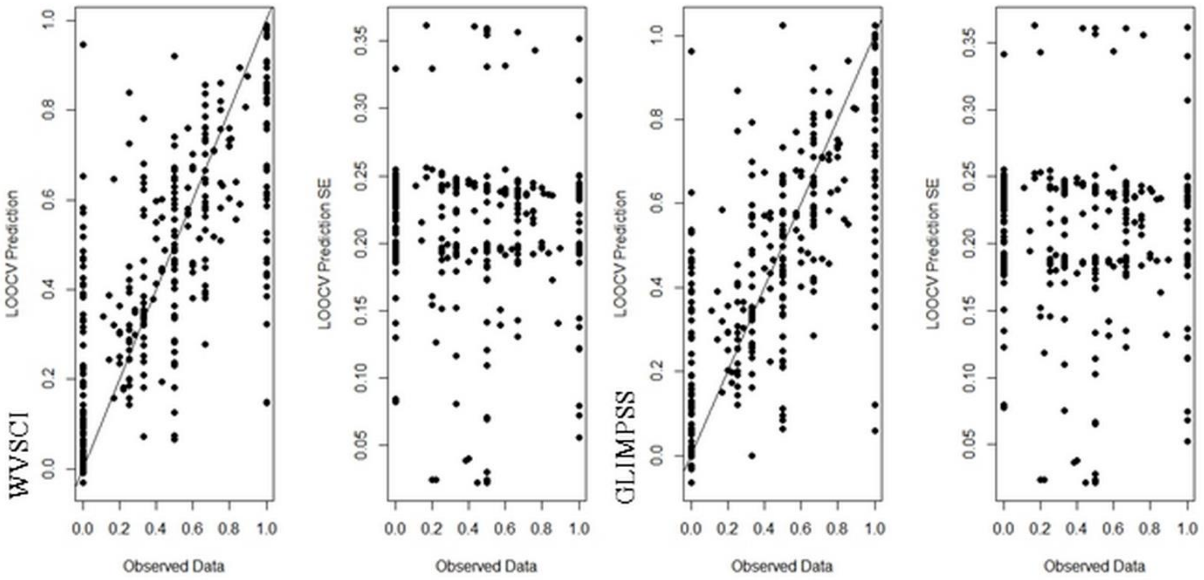

Fig. 4. **Model Performance.** Leave one out cross validation predictions (LOOCV) and standard error (SE) against the observed data for the top WVSCI and GLIMPSS spatial models as one means of assessing model performance.

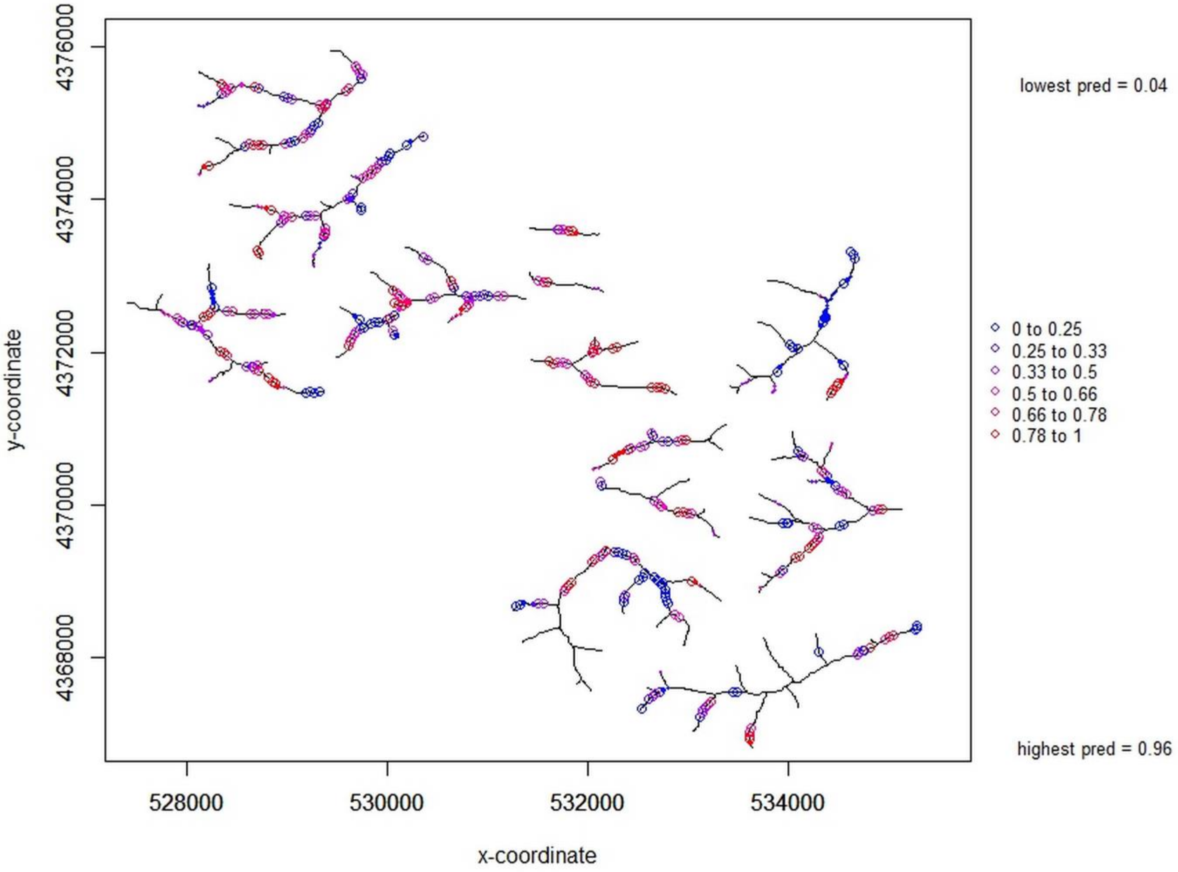

Fig. 5. **Prediction Map.** An example of prediction values mapped for WVSCI (solid circles) in relation to collected WVSCI data (open circles). The larger the solid circle, the more confidence in the prediction value (note most circles are small). Red values have a higher foraging probability index (FPI) than blue values.

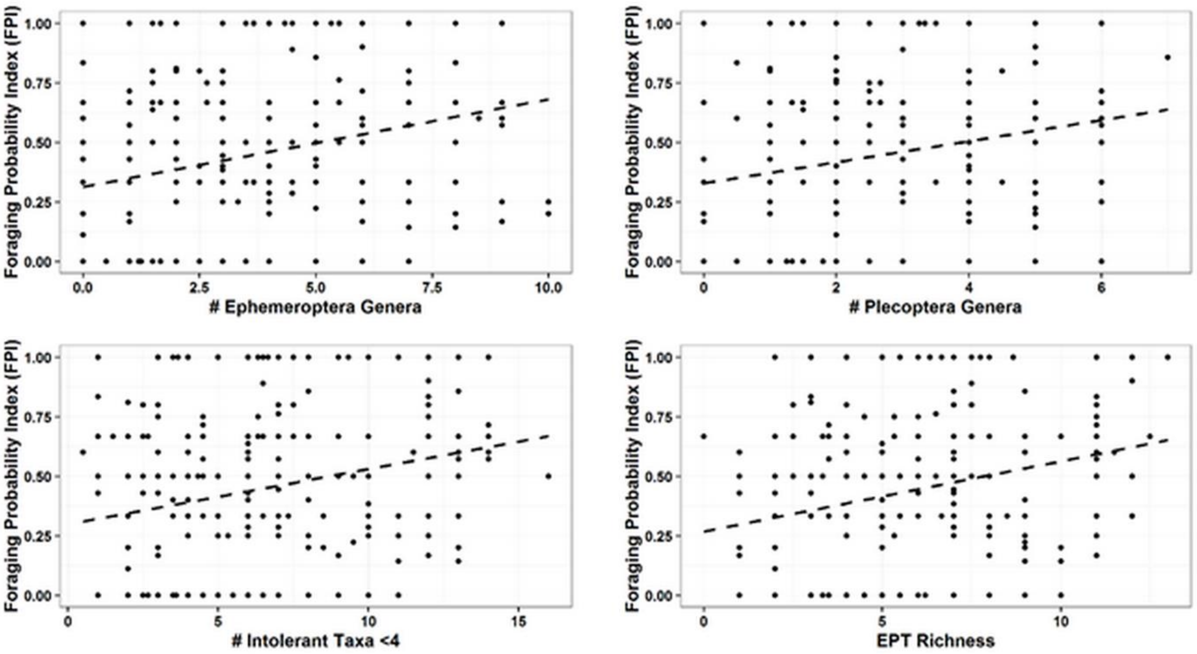

Fig. 6. **Foraging Probability Index Correlations.** *Post-hoc* spearman rank correlation tests between foraging probability index (FPI) and WVSCI/GLIMPSS metrics (no. Ephemeroptera genera, no. Plecoptera genera, no. intolerant taxa tolerance value <4, and EPT richness). All tests were significant (Rho=0.24, 0.20, 0.23, & 0.22 respectively, $P < 0.001$).

PART 4. LOUISIANA WATERTHRUSH MOLECULAR ECOLOGY

CHAPTER 6. EPIGENETIC RESPONSE OF LOUISIANA WATERTHRUSH (PARKESIA MOTACILLA) RELATED TO SHALE GAS DEVELOPMENT.

Chapter 6 is written in style of the peer-reviewed journal *Ibis*.

ABSTRACT

Epigenetic mechanisms such as DNA methylation may vary in response to environmental stressors and introduce adaptive or maladaptive gene expression within and among wild bird populations. We examined the association between DNA methylation and demographic characteristics of the Louisiana Waterthrush (*Parkesia motacilla*) in addition to potential differential methylation from shale gas development in a Central Appalachian watershed during 2013–2015. We also evaluated the degree to which an individual's methylated state was subject to change across years in individuals that returned over the course of more than one breeding season (i.e., recaptures). Overall population methylation differed between adult male and female waterthrush where adult males generally had fewer methylated restriction sites. Differential methylation also occurred between adult females and nestlings. Age influenced differential methylation in both adult males and females where there was generally decreased methylation with age. While adult male recaptures had decreased methylation with age, adult female recaptures had increased methylation with age. Adult males were differentially methylated between shale gas undisturbed and disturbed areas at a population and restriction site (i.e., loci) level, where restriction sites were predominately less methylated in shale gas disturbed areas, a trend consistently seen year to year in adult male recaptures. Barium (Ba) and strontium (Sr) data were collected in 2013 feather samples where adult males had fewer methylated sites at higher concentrations of Ba and Sr, while nestlings displayed no correlation of methylation to Ba and Sr concentrations. Adult females displayed increased methylation with increased Sr, a trend also seen year to year in adult female recaptures. Overall, results of our study suggest sex-specific influences of shale gas development on gene expression that may affect long-term population survival and fitness.

Keywords: bioindicator, contaminants, DNA methylation, Marcellus-Utica, shale gas.

INTRODUCTION

Natural gas production in the United States is expected to increase under all economic and demographic projections through 2050 primarily from continued unconventional drilling activity (hereafter shale gas development) of the Marcellus-Utica shale basin (EIA 2018). The central Appalachian region experienced some of the quickest growth over the last decade in shale gas development (MCOR 2016) since the underlying Marcellus-Utica shale is the most expansive basin and has the most potentially recoverable gas (DOE 2009). Between 2008 and 2018, 15,939 shale gas wells were developed at 5,674 sites across the Marcellus-Utica shale basin (Jacquet et al. 2018), with 2,528 wells developed in West Virginia (WVDEP 2018). The hydraulic fracturing process used to extract natural gas (Mongelli 2018) presents environmental challenges to wildlife (Brittingham et al. 2014) and watersheds (Latta et al. 2015), especially as it outpaces the ability to implement best management practices that avoid risks to aquatic and terrestrial wildlife communities and their habitat (Brittingham et al. 2014).

The Louisiana Waterthrush (*Parkesia motacilla*), hereafter waterthrush, is a forested headwater stream specialist known for its ability to respond to changes in ecological conditions (Mattsson and Cooper 2006) and is a species of conservation concern (USFWS 2008) due to its specialized habitat. As a biological indicator of biotic stream integrity (O'Connell et al. 2000, Mulvihill et al. 2008), waterthrush feed primarily on benthic macroinvertebrate aquatic prey (Mattsson et al., 2009) in well-developed riffle and pool areas (Prosser and Brooks 1998). In a long-term study, shale gas development negatively affected waterthrush demography (Frantz et al. 2018a, Frantz et al. 2019) and its aquatic prey (Wood et al. 2016, Frantz et al. 2018b), but we do not know if shale gas development may also influence waterthrush at a physiological (e.g., Kleist et al. 2018) or molecular level.

Little is known about epigenetic mechanisms in birds (Fresard et al. 2013), with only a small number of epigenetic studies conducted on wild bird populations (Schrey et al. 2012; Sheldon et al. 2018). Epigenetic modifications, such as DNA methylation, are an evolutionary "soft" inheritance of gene expression that can be either adaptive or maladaptive for the individual (Burdge and Lillycrop 2010). Epigenetic variation is dictated mostly by genetic control (Bossdorf et al. 2008), but it is the times when epigenetic variation is partly or completely independent of genetic control (Richards 2006) that is of the most interest to ecologists (Bossdorf et al. 2008). DNA methylation may vary in response to environmental stressors (Richards et al. 2010) and induce epigenetic changes that are inherited by future generations (Richards 2006; Herrera and Bazaga 2011). Chemical pollutants are one form of environmental stressor that may affect DNA methylation (Reyna-López et al. 1997; Pilsner et al. 2010). For instance, heavy metals may induce changes by affecting synthesis of substrate S-adenosylmethione (SAM) and its ability to donate methyl groups to methyltransferase enzymes (Hala et al. 2014). Epigenetic modifications therefore may facilitate a quick response to a changing environment (Rando and Verstrepen 2007; Bossdorf et al. 2008) by potentially introducing ecologically-relevant phenotypic variation within and among populations (Schrey et al. 2013).

As an apex predator, the waterthrush may be exposed to contaminants occurring in their aquatic prey or present in surface water of their riparian habitat. Previous work at our study sites found barium (Ba) and strontium (Sr), two heavy metals associated with the hydraulic fracturing process (Entrekin et al. 2011, Chapman et al. 2012), were bioaccumulated in waterthrush feathers at higher levels in shale gas disturbed areas than in undisturbed areas (Latta et al. 2015). Since waterthrush reach some of their highest abundances in the Marcellus shale region (Sauer et al. 2017), it is important to investigate the role of environmental stressors (especially anthropogenic ones) at the molecular level if stressors can increase DNA methylation variance (Dowen et al. 2012) or result in differential methylation by habitat type (Foust et al. 2016; McNew et al. 2017). Adult females can have a higher body condition index (BCI) than adult males in similar quality territories and can tolerate a wider range of territory quality (Latta et al. 2016). Thus, we expected differential methylation between adult males and females and for adult males to have a stronger response to shale gas development. We expected older adults to have fewer methylated restriction sites than younger adults and for nestlings to be differentially methylated from adults because decreased methylation is correlated with age in birds (De Paoli-Iseppi et al. 2018). Presuming nestlings are less exposed to stress during the hatching to fledging stage than adults during the breeding season, we hypothesized a weak response to shale gas development between shale gas disturbed and undisturbed areas if differential methylation can correlate to a gradient of stress (Sun et al. 2018). Finally, we hypothesized that heavy metal concentrations are inversely correlated to the number of methylated restriction sites since contaminants interfere with methyl transfer (Hala et al. 2014).

From 2013–2015, we 1) examined how shale gas development may influence variation in DNA methylation (hereafter differential methylation) of waterthrush occurring on territories identified as shale gas undisturbed and disturbed; 2) determined if differential methylation of DNA fragments or loci, hereafter restriction sites, varied by sex and age; 3) identified if differentially methylated restriction sites were potentially under selection; 4) correlated methylated restriction sites to Ba and Sr heavy metal concentrations bioaccumulated in waterthrush feathers; and 5) evaluated the degree to which an individual's methylated state was subject to change across years.

METHODS

Study Area

We studied waterthrush epigenetic response (2013–2015) as part of a six-year study along 14 1st- and 2nd-order forested headwater stream tributaries that totaled 58.1 km at the Lewis Wetzel Wildlife Management Area (LWWMA; 39° 29.654′ N, 80° 38.491′ E) located in Wetzel County, West Virginia, USA (Frantz et al. 2018a, 2018b, 2019). In 2008, LWWMA was 95.3% forested with the first shale gas development starting in 2007 (Farwell et al. 2016). By 2015, LWWMA was 90.8% forested with forest loss (4.5%) primarily due to shale gas (Farwell et al. 2016), but with no new shale gas development or activity during the 2015 breeding season. Shale gas disturbance on streams was highly variable within each year, but on average across 2013–2015 streams had 19.2 ± 3.4% of their length disturbed by shale gas development (range 0–67%; Frantz et al. 2018a).

Louisiana Waterthrush banding and monitoring

Annually, territorial adult waterthrush were target-netted and banded with an aluminum US Geological Survey leg band and a unique combination of colored plastic bands to allow identification of individuals while nestlings were banded ~7–8 days after hatching (Frantz et al. 2019). Age (nestlings = hatching year [HY]; adults = second year [SY], after second year [ASY], or after hatching year [AHY]) was determined from plumage characteristics (Pyle 1997)). Blood (5–10 µL) was collected from 146 individual adults and 159 nestlings by venipuncture from the ulnar vein of the wing for epigenetic analysis. Ten additional blood samples were collected from nine adult recaptures in following years with one individual recaptured in two subsequent years. Blood samples were preserved in RNA*later* Stabilizing Solution (Invitrogen Corporation, Grand Island NY) and were refrigerated until DNA extraction. Feather samples were collected from adults and nestlings in 2013 to measure Barium (Ba) and Strontium (Sr) bioaccumulation as part of a contaminant study (Latta et al. 2015).

Waterthrush territories were delineated along each stream during April 1–June 29 using standardized territory mapping (>6 stream visits) with nest searching, nest monitoring, and resightings of banded individuals occurring concurrently with territory mapping (Frantz et al. 2019). We digitized annual shale gas disturbance on the study area and classed each territory as undisturbed or disturbed by shale gas (see Frantz et al. 2018a for details), creating a binary (undisturbed or disturbed) TerrGas and TerrRunoff variable for each territory. TerrGas described presence or absence of localized streamside disturbance from any shale gas infrastructure or activity. TerrRunoff focused solely on potential shale gas contaminant erosional run-off from direct and mostly upstream sources (similar to Latta et al. 2015).

Differential Methylation

DNA was extracted with a DNeasy Kit (Qiagen, Valencia CA) and quantified using a Nanodrop (ThermoScientific) for analysis of differential methylation. Methylation-sensitive amplified fragment length polymorphism (MS-AFLP), a DNA fingerprinting method for non-model organisms (Schrey et al. 2013), was performed as outlined in Schrey et al. (2012) with the modification that digestion and ligation were performed in one step and digestion-ligation incubation for three hours. Selective PCR products were evaluated using a Beckman Coulter CEQ 8000 Genetic Analysis System. The protocol was performed with >10% random duplication of samples (n = 40) to choose consistent restriction sites for scoring and validate individual results; any restriction sites that could not be validated were removed. Each restriction site was scored as either methylated or unmethylated for individuals, creating an epigenotype per individual (Schrey et al. 2012).

Statistical Analysis

We completed Analysis of Molecular Variance (AMOVA) tests to calculate Φ_{st} (PhiPT, a genetic distance estimate) and characterize overall population differential methylation by sex, age, and shale gas disturbance using GenAlEx (Peakall and Smouse 2006, 2012). We tested if there was overall population differential methylation between adult males, adult females, and nestlings. For adult males and females, we further examined influence of age by comparing SY to ASY; AHYs (n = 7) were removed from analysis due to age uncertainty. We also tested overall population differential methylation between shale gas undisturbed and disturbed areas for TerrGas and TerrRunoff in adult males, adult females, and nestlings. When any overall population comparison was statistically significant, restriction sites were also analyzed individually to determine which restriction sites were driving the overall differential methylation. Statistical significance was determined by 9999 permutations with $\alpha = 0.10$ to not miss variation that may be of biological significance (Askins et al. 1990). BAYESCAN (Foll and Gaggiotti 2008) was used to identify any outlier restriction sites as potentially under selection from those previously determined to be statistically significant. Log10 values of posterior odds (PO) were interpreted as a type of Bayes factor for evidence of selection using Jeffreys' scale for Bayes factors (Jeffreys 1961; Foll and Gaggiotti 2008).

If TerrGas or TerrRunoff was statistically significant for adult males, adult females, or nestlings, the restriction sites driving the overall differential methylation were used to correlate the total amount of methylation across restriction sites to Ba and Sr heavy concentrations bioaccumulated in waterthrush feathers. Otherwise, all restriction sites were used. The test was limited to waterthrush sampled in 2013 that had both blood samples and Ba and Sr feather data from Latta et al. (2015). A Box-Cox transformation was performed on Ba and Sr data (in ppm) to approach normality using R package car (Fox et al. 2015) in R (R Core Team 2014). We used the transformed Ba and Sr data to conduct a Pearson correlation of the total amount of methylation across restriction sites to Ba and Sr concentrations using R package psych (Revelle 2017).

RESULTS

We confirmed 100 restriction sites ranging from 135–591 DNA base pairs in length that we could score for differential methylation. There was differential methylation in all individuals scored (n = 315), where all individuals except for two had a unique epigenotype across all restriction sites. Across all statistically significant population level comparisons, within-population variation ranged 97–99% and among population variation ranged 1–3% in the differentially methylated populations (Table 1).

Adult males (n = 107) were differentially methylated from adult females (n = 49; Table 1), with over 39 restriction sites differentially methylated by sex (Appendix Table 1). Across all restriction sites and only those that were statistically significant for adult males, adult males had fewer methylated restriction sites than adult females (Appendix Table 2). Adult females were differentially methylated from nestlings (Table 1) at 35 restriction sites (Appendix Table 1), but adult males were not (Table 2). Adult females had more methylated restriction sites than nestlings across all restriction sites and those that were statistically significant (Appendix Table 3).

Adult males and adult females were both differentially methylated by age (Table 1). Adult males had 21 restriction sites differentially methylated between SY and ASY individuals (Appendix Table 1), while adult females had 18 restriction sites (Appendix Table 1). All 21 restriction sites in adult males were less methylated as an ASY (Appendix Table 4), whereas in adult females 16 of 18 restriction sites were less methylated (Appendix Table 5). Out of the 21 and 18 restriction sites differentially methylated by age in adult males and females, only about 10% (four restriction sites: 340, 419, 532, 541) were the same between the sexes.

Adult males were not differentially methylated by TerrRunoff (Table 2). However, adult males from territories with presence of TerrGas were differentially methylated from those with absence of TerrGas (Table 1). Adult males had 10 restriction sites that were differentially methylated between shale gas undisturbed and disturbed territories (Appendix Table 1). Nine of the 10 restriction sites were less methylated in shale gas disturbed territories (Appendix Table 6); only one of these restriction sites (i.e., 541) was also a significant restriction site for adult male age. Adult females and nestlings were not differentially methylated by TerrGas or TerrRunoff, although adult females had 1% among population variance for TerrRunoff (Table 2). No evidence for restriction sites potentially under selection was found for any of the statistically significant differentially methylated restriction sites by sex, adult females vs. nestlings, adult male and female age, nor TerrGas (Appendix Table 7).

For adult males (n = 46), the number of methylated restriction sites decreased with increasing Sr ($R^2 = -32$, $P = 0.03$; Figure 1). The number of methylated restriction sites also tended to decrease with increasing Ba, but it was not statistically significant ($R^2 = -19$, $P = 0.20$). Adult female (n = 11) methylated restriction sites increased with increasing Sr ($R^2 = 0.55$, $P = 0.08$; Figure 1). The number of methylated restriction sites for adult females tended to increase with increasing Ba, but it was not statistically significant ($R^2 = 0.46$, $P = 0.16$). Nestlings (n = 29) did not have any statistically significant correlations for the number of methylated restriction sites with Sr ($R^2 = -11$, $P = 0.58$) or Ba ($R^2 = 0.02$, $P = 0.92$).

Three adult male and five adult female recaptures were evaluated for temporal trends in methylation, as well as one nestling recaptured as an adult female (Table 3). Two of the three adult male recaptures that had TerrGas presence in their territories had decreased methylation in the second year (Table 3). Adult male recaptures displayed decreased methylation in the second year, but adult female recaptures had increased methylation in subsequent years. The only nestling recaptured as an adult displayed the same trends as other adult female recaptures.

DISCUSSION

Our study detected at a molecular level differential methylation in a wild bird population relative to demographic characteristics and environmental influence of shale gas development despite a high amount of within-population variation (Table 1). Adult males and females were differentially methylated (Appendix Table 2), and nestlings were differentially methylated from females with methylation patterns similar to that of males (Appendix Table 3). Our study is the second for a wild bird population that suggests methylation varies with age. Humans have an epigenetic clock where methylation increases with age (Horvath 2013), but waterthrush generally displayed decreased methylation with age, similar to that found in a seabird at most age-related restriction sites (De Paoli-Iseppi et al. 2018). In addition to methylation varying with pollutant concentrations as previously seen in a wild bird population (Romano et al. 2016), our study is the first for a wild bird population to suggest a potential sex-specific epigenetic response to contaminants.

Adjacent, local wild bird populations can be differentially methylated due to living under different environmental conditions (McNew et al. 2017). Males in shale gas disturbed territories had fewer methylated restriction sites than males in undisturbed territories (Appendix Table 6) with unknown consequences on gene expression. Only one adult male recapture had no TerrGas disturbance each of the two years and had the same number of methylated restriction sites of the 10 identified as significant to TerrGas. The other two adult male recaptures had TerrGas disturbance each year and exhibited decreased methylation the following year among both TerrGas and age-related restriction sites (Table 3), consistent with overall population level results for adult males differentially methylated by TerrGas (Appendix Table 6). Sr and Ba were present in waterthrush feathers of both shale gas disturbed and undisturbed territories but was significantly higher in disturbed territories (Latta et al. 2015). Since heavy metals interfere with methyl transfer (Hala et al. 2014), evidence from our study suggests a strong possible link of males in shale gas disturbed areas having fewer methylated sites in response to direct exposure to contaminant sources. Only one age-related restriction site (i.e., 541) was shared with differentially methylated TerrGas restriction sites where there may be an interacting effect. Although Latta et al. (2015) did not trace the origin of the contaminants, the metals were hypothesized to have originated in the Marcellus Shale layer where they are known to be abundant (Entrekin et al. 2011; Chapman et al. 2012), and both surface waters and benthic aquatic prey were negatively affected by shale gas disturbance at our study site (Frantz et al. 2018a, 2018b).

Females have a higher body condition index than males and can cope with a wider range of territory quality conditions (Latta et al. 2016). In our long-term study, males exhibited very high site fidelity returning to the same shale gas disturbed territories despite lower riparian habitat quality, but females in disturbed territories had a higher number of breeding attempts and were less likely to return (Frantz et al. 2019). Brood size alone can affect nestling DNA methylation (Sheldon et al. 2018), so prenatal stress from the increased number of breeding attempts could potentially affect differential methylation in waterthrush nestlings. Males arrive first on the breeding grounds to set up a territory (Mattsson et al. 2009) where females must select a nest site within the defended male's territory, so female capacity to handle environmental perturbation does not necessarily mean the ability to avoid disturbance with potential consequences to long-term population persistence. The contribution that differential methylation has on survivorship and fitness, at least for males, needs to be determined if shale gas disturbed areas are borderline "sink" habitats (Frantz et al. 2018a).

Our study adds to existing evidence that methylation varies with pollutant concentrations (Romano et al. 2016) but is the first to indicate a potential sex-specific epigenetic response to contaminants in a wild bird population. Adult females had an increased number of methylated sites with increasing Sr whereas adult males had fewer methylated sites with increasing Sr (Figure 1). Epigenetic responses to contaminants can be sex-specific such as in polar bears (*Ursus maritimus*) where males had mercury-related brain hypomethylation, but females did not (Pilsner et al. 2010). Interestingly, adult female recaptures had *increased* methylation the following year across all restriction sites and at age-related restriction sites when methylation decreased with age for the adult male recaptures (Table 3) and for adult males overall (Appendix Table 4); the trend for adult female recaptures was also opposite that seen when analyzing adult females overall (Appendix Table 5). As such, the differing methylation patterns seen in adult female recaptures compared to overall adult female methylation patterns may be a typical response to contaminants but masked by small sample size (5 of 49 adult females). Levels of oxidative stress are affected by heavy metals (Stauffer 2017), where oxidative stress is a component of aging in birds (Xia and Møller 2018). Contaminants can create an "epigenetic trap" where epigenetic machinery is hijacked and may produce a phenotype that is maladaptive or mismatched for the environment (O'Dea et al. 2016), a possibility at not just our study area but other shale play regions (Latta et al. 2015).

DNA methylation is chiefly eliminated from germlines during gametogenesis and post fertilization but is not always complete (Wang et al. 2017). We did not detect differential methylation in nestlings between undisturbed and disturbed areas despite an expected weak signal if variability in methylation can establish over relatively short evolutionary time scales (Verhoeven et al. 2010). Adult waterthrush can provision nestlings with food items (i.e., terrestrial prey) that differ from what adults consume (Trevelline et al. 2016, 2018), which may buffer nestlings from any water-borne disturbances related to shale gas development. Because waterthrush nestlings typically disperse from their natal territory (Mattsson et al. 2009), our only return nestling was female and exhibited the same trend as the return adult females where methylation increased the following year (Table 3). Early environmental effects on a nestling's phenotype can be sex specific, and the environment experienced during early development may have a broader impact on the adult phenotype than that experienced later in life (Fresard et al. 2013). We should therefore caution drawing any conclusion that shale gas disturbance does not affect nestlings nor females from a lack of statistically significant differential methylation; nestlings that are males may be more susceptible, or females may be just as susceptible but exhibit a different methylation response to stressors (Figure 1).

No outlier restriction sites (i.e., loci) were identified as potentially under selection. While none were detected, our study identifies candidate restriction sites worthy of further attention. Given that we did see differentially methylated populations, these relationships may be complicated where only a few of these variable restriction sites may be ecologically important (Schrey et al. 2012). Methylation patterns are typically responsible for gene silencing (Li et al. 2011) but also can cause active transcription (Jones 2012). Epigenetic modifications are known to be involved in immune response (Gou et al. 2012), disease modulation (Luo et al. 2012a, 2012b), and may alter gene transcription from stress (Xu et al. 2012). Non-colonial, distributed bird species typically exhibit high gene flow (Barrowclough 1980; Avise 1994) which can both prevent and allow selection to occur. Factors that may explain a lack of selection include selection on restriction sites too weak to cause a "selective sweep" in the population without more time being involved, or too rare for selection to drive restriction sites to fixation without a certain number of generations since the environmental influence (e.g. Pritchard et al. 2010). Given the amount of within-population variability, higher statistical power may be necessary for detection (e.g., Schrey et al. 2012), which may also apply to the lack of population level differential methylation in females (15.6% of sample size) in relation to shale gas development.

Our study is the first to our knowledge to relate shale gas development to a molecular level, epigenetic response in a wildlife population. We provide a genome-wide snapshot of differential methylation in response to demographic and environmental factors, despite the limitations of MS-AFLP (see Schrey et al. 2013), where additional techniques can further be incorporated to identify the function of the methylated restriction sites in question. Differential methylation could provide a short-lived means to adapt in a rapidly changing environment, but its effects on genetic variation could have lasting impacts (O'Dea et al. 2016). Methylation levels are tissue-specific (Li et al. 2011), so the scope of epigenetic effects on waterthrush populations may be much broader. Most of the core breeding range of the waterthrush overlaps the Marcellus-Shale region (Sauer et al. 2017), meriting concern if there are potential long-term fitness and survival consequences from differential methylation between shale gas undisturbed and disturbed areas, especially when contaminants may further alter methylation (Nilsen et al. 2016).

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Competing Interests

The authors do not have any conflicts of interest related to this work.

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TABLES

Table 1. Summary results of Analysis of Molecular Variance (AMOVA) performed on 100 methylation-sensitive amplified fragment-length polymorphism (MS-AFLP) restriction sites grouped by sex, adult female vs. nestling, age (second year [SY] vs. after second year [ASY]) and shale gas disturbance status (TerrGas), showing differential methylation (*P* < 0.10) on a population level.

	df	**Sum of squares**	**Mean squares**	**% Variance**	**PhiPT**	**p value**
Male vs. Female						
Among Populations	1	46.810	46.810	2%	0.022	0.000
Within Populations	154	2841.652	18.452	98%		
Total	155	2888.462		100%		
Female vs. Nestling						
Among Populations	1	50.090	50.090	2%	0.023	0.000
Within Populations	206	3691.679	17.921	98%		
Total	207	3741.769		100%		
Male SY vs ASY						
Among Populations	1	26.645	26.645	2%	0.023	0.013
Within Populations	99	1694.266	17.114	98%		
Total	100	1720.911		100%		
Female SY vs ASY						
Among Populations	1	34.066	34.066	3%	0.032	0.017
Within Populations	46	961.288	20.898	97%		
Total	47	995.354		100%		
Male TerrGas						
Among Populations	1	21.469	21.469	1%	0.005	0.088
Within Populations	93	1592.594	17.125	99%		
Total	94	1614.063		100%		

Table 2. Summary results of Analysis of Molecular Variance (AMOVA) performed on 100 methylation-sensitive amplified fragment-length polymorphism (MS-AFLP) restriction sites grouped by adult male vs. nestling and shale gas disturbance status (TerrGas and TerrRunoff), indicating no differential methylation ($P > 0.10$) on a population level.

	df	**Sum of squares**	**Mean squares**	**% Variance**	**PhiPT**	**p value**
Male vs. Nestling						
Among Populations	1	19.073	19.073	0%	0.001	0.218
Within Populations	264	4477.168	16.959	100%		
Total	265	4496.241		100%		
Male TerrRunoff						
Among Populations	1	16.559	16.559	0%	-0.001	0.547
Within Populations	93	1597.504	17.177	100%		
Total	94	1614.063		100%		
Female TerrGas						
Among Populations	1	19.153	19.153	0%	-0.006	0.701
Within Populations	44	965.217	21.937	100%		
Total	45	984.370		100%		
Female TerrRunoff						
Among Populations	1	26.335	26.335	1%	0.009	0.147
Within Populations	44	958.034	21.774	99%		
Total	45	984.370		100%		
Nestling TerrGas						
Among Populations	1	18.719	18.719	0%	0.001	0.240
Within Populations	151	2556.223	16.929	100%		
Total	152	2574.941		100%		
Nestling TerrRunoff						
Among Populations	1	17.771	17.771	0%	0.001	0.343
Within Populations	151	2557.170	16.935	100%		
Total	152	2574.941		100%		

Table 3. A summary of recaptures (n = 9) that were sampled from year to year to evaluate the degree to which an individual's methylated state was subject to change. All Sites indicates the total number of methylated sites across all restriction sites (n = 100), while Male vs. Female (n = 39), Female vs. Nestling (n = 35), Male Age (n = 21), Female Age (n= 18), and TerrGas (n = 10) indicates the total number of methylated sites across restriction sites determined to be differentially methylated. TerrGas Presence and TerrRunoff Presence are the presence (1) or absence (0) of shale gas territory disturbance. The only nestling recapture was an adult female. Individuals with Strontium (Sr) and Barium (Ba) data from 2013 have Box-Cox transformed ppm values displayed as used in the Pearson correlations. NA = the data is not available. Data that is not applicable is indicated with "–" because differential methylation was not tested.

ID	**Year**	**Age**	**TerrGas Presence**	**TerrRunoff Presence**	**All Sites**	**Male vs. Female**	**Female vs. Nestling**	**Male Age**	**Female Age**	**Terr Gas**	**Sr**	**Ba**
Males												
12642	2013	3	0	0	24	5	–	3	–	3	1.22	1.07
12642	2014	4	0	0	21	2	–	2	–	3	NA	NA
12773	2014	3	1	0	28	6	–	2	–	1	NA	NA
12773	2015	4	1	0	6	0	–	0	–	0	NA	NA
12781	2014	3	1	0	19	5	–	4	–	2	NA	NA
12781	2015	4	1	0	15	7	–	0	–	0	NA	NA
Females												
12634	2013	3	1	0	15	3	2	–	1	–	1.26	1.05
12634	2014	4	1	0	48	16	17	–	8	–	NA	NA
12634	2015	5	1	1	55	21	20	–	10	–	NA	NA
12635	2013	3	1	1	16	3	4	–	2	–	1.38	1.07
12635	2014	4	1	1	29	12	7	–	5	–	NA	NA
12638	2013	3	0	1	20	5	3	–	3	–	1.36	1.08
12638	2014	4	0	1	29	10	8	–	7	–	NA	NA
12727	2013	3	NA	NA	13	3	1	–	0	–	1.35	1.09
12727	2014	4	0	0	48	16	14	–	4	–	NA	NA
12799	2013	3	NA	NA	12	2	1	–	1	–	1.44	1.16
12799	2014	4	0	1	45	21	18	–	6	–	NA	NA
Nestlings												
12839	2014	1	0	1	14	–	–	–	–	–	NA	NA
12839	2015	2	0	0	64	–	–	–	–	–	NA	NA

FIGURES

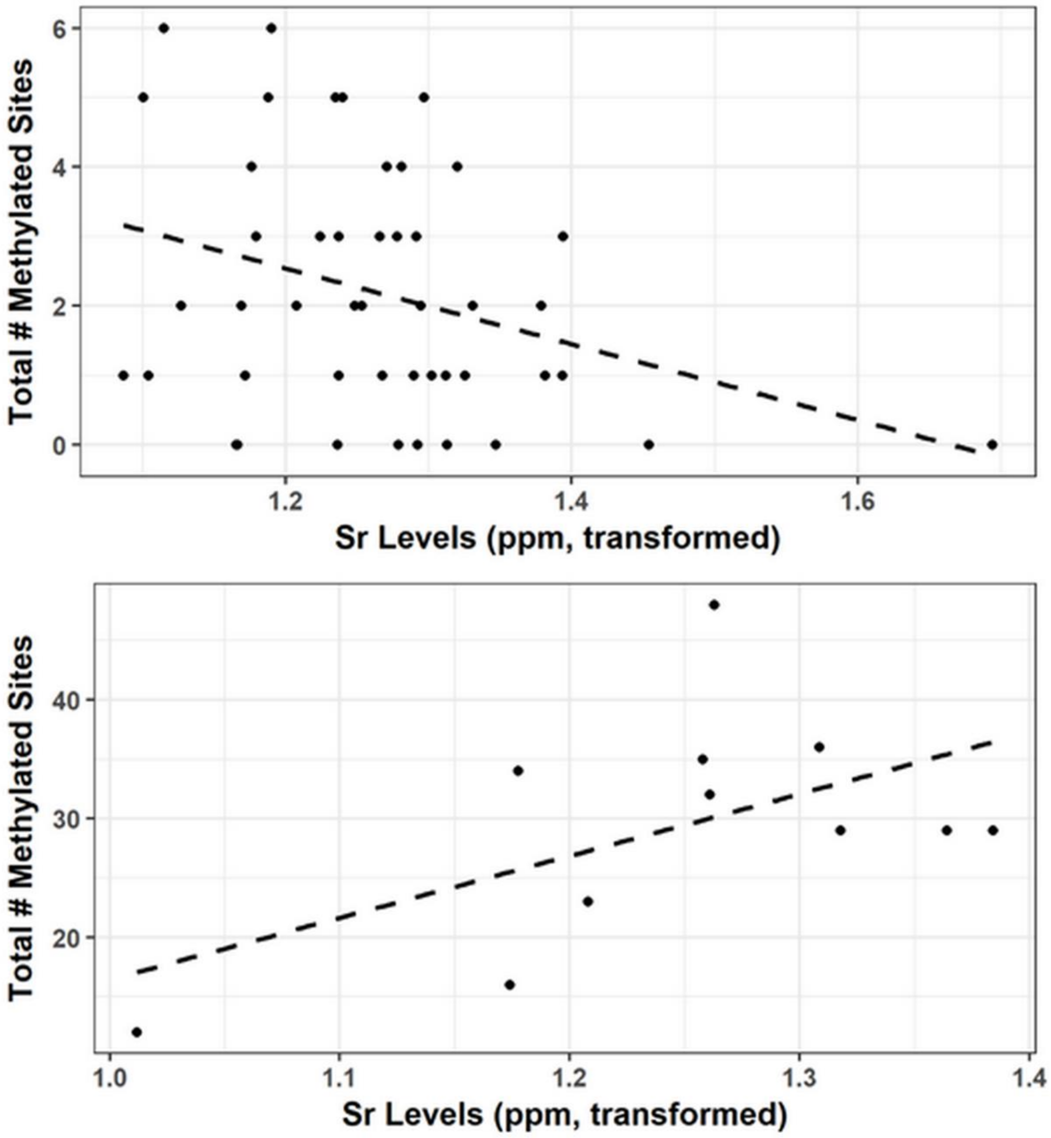

Figure 1. The number of methylated restriction sites identified as differentially methylated for TerrGas (n = 10) vs. strontium (Sr; R^2 = -32, *P* = 0.03) for adult males (top panel) and the number of methylated restriction sites across all restriction sites (n = 100) vs. strontium (Sr; R^2 = 0.55, *P* = 0.08) for adult females (bottom panel).

APPENDICES

Appendix Table 1. Summary results indicating differential methylation (*P* < 0.10) on a restriction site (i.e., locus) level after performing Analysis of Molecular Variance (AMOVA) grouped by sex (male, n = 107 vs. female, n = 49), adult females (n = 49) vs. nestlings (n = 159), male age (second year [SY], n = 14 vs. after second year [ASY], n = 87), female age (SY, n = 13 vs. ASY, n = 35), and TerrGas (undisturbed n = 46, disturbed n = 49) for adult males (n = 95).

Restriction Site	**PhiPT**	**P value**	**Restriction Site**	**PhiPT**	**P value**
Male vs. Female			Female vs. Nestling		
155	0.069	0.022	229	0.008	0.006
222	0.055	0.031	290	0.066	0.016
229	0.131	0.002	305	0.118	0.002
305	0.042	0.061	322	0.060	0.020
322	0.035	0.089	325	0.049	0.039
324	0.047	0.039	350	0.139	0.001
325	0.060	0.029	353	0.011	0.011
328	0.050	0.052	355	0.045	0.054
339	0.057	0.042	362	0.053	0.031
350	0.089	0.009	367	0.139	0.001
353	0.033	0.096	371	0.074	0.012
366	0.067	0.018	375	0.120	0.002
367	0.050	0.054	411	0.128	0.001
371	0.202	0.000	417	0.029	0.097
375	0.109	0.003	419	0.043	0.049
400	0.039	0.075	425	0.062	0.024
411	0.050	0.053	432	0.037	0.054
419	0.069	0.020	437	0.084	0.009
425	0.070	0.021	448	0.247	0.000
437	0.058	0.039	451	0.053	0.031
448	0.167	0.001	453	0.057	0.026
453	0.047	0.041	455	0.117	0.001
454	0.035	0.082	468	0.108	0.004
455	0.124	0.002	483	0.060	0.020
458	0.045	0.057	510	0.053	0.030
468	0.057	0.034	521	0.081	0.010
483	0.035	0.083	526	0.066	0.016
510	0.064	0.024	532	0.112	0.003
521	0.182	0.000	535	0.105	0.004
526	0.102	0.006	536	0.094	0.005
532	0.144	0.002	569	0.069	0.018
535	0.047	0.048	575	0.084	0.010
536	0.082	0.014	587	0.045	0.056
541	0.051	0.058	589	0.053	0.033
569	0.096	0.008	591	0.071	0.014
575	0.171	0.000			

582	0.07	0.022
587	0.035	0.090
591	0.041	0.059

Restriction Site	**PhiPT**	**P value**	**Restriction Site**	**PhiPT**	**P value**
Male Age			Female Age		
244	0.094	0.083	135	0.116	0.095
319	0.165	0.027	144	0.189	0.050
339	0.190	0.032	209	0.161	0.045
340	0.088	0.093	290	0.154	0.081
343	0.104	0.078	297	0.143	0.076
347	0.141	0.040	305	0.126	0.097
353	0.118	0.055	325	0.338	0.005
362	0.098	0.072	340	0.263	0.015
367	0.141	0.040	350	0.288	0.012
376	0.208	0.015	368	0.167	0.048
419	0.124	0.061	419	0.155	0.067
425	0.230	0.020	453	0.155	0.063
437	0.124	0.060	458	0.329	0.008
451	0.072	0.100	468	0.288	0.013
483	0.356	0.002	509	0.167	0.050
484	0.226	0.012	532	0.200	0.042
508	0.128	0.059	541	0.205	0.027
532	0.225	0.017	569	0.116	0.092
541	0.124	0.061			
589	0.165	0.024			
591	0.208	0.017			

Restriction Site	**PhiPT**	**P value**
Male TerrGas		
155	0.158	0.003
289	0.061	0.068
292	0.109	0.015
333	0.067	0.054
368	0.051	0.082
371	0.115	0.010
446	0.115	0.016
448	0.057	0.074
455	0.132	0.007
541	0.072	0.054

Appendix Table 2. Proportion (%) of differential methylation between male and female individual restriction sites (n = 39) that were statistically significant by sex (male, n = 107 vs. female, n = 49), and the collective proportion of differential methylation among all (n = 100) and just the statistically significant restriction sites.

Sex	All Sites		Sig. Sites		Site 155		Site 222		Site 229		Site 305		Site 322	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	68.08	31.92	68.66	31.34	44.90	55.10	53.06	46.94	36.73	63.27	59.18	40.82	71.43	28.57
Male	75.98	24.02	85.31	14.69	65.42	34.58	71.03	28.97	64.49	35.51	74.77	25.23	84.11	15.89

Sex	Site 324		Site 325		Site 328		Site 339		Site 350		Site 353		Site 366	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	67.35	32.65	69.39	30.61	69.39	30.61	77.55	22.45	67.35	32.65	69.39	30.61	67.35	32.65
Male	82.24	17.76	85.05	14.95	84.11	15.89	90.65	9.35	85.98	14.02	82.24	17.76	84.11	15.89

Sex	Site 367		Site 371		Site 375		Site 400		Site 411		Site 419		Site 425	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	69.39	30.61	67.35	32.65	65.31	34.69	79.59	20.41	69.39	30.61	73.47	26.53	77.55	22.45
Male	84.11	15.89	92.52	7.48	85.98	14.02	90.65	9.35	84.11	15.89	88.79	11.21	91.59	8.41

Sex	Site 437		Site 448		Site 453		Site 454		Site 455		Site 458		Site 468	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	73.47	26.53	53.06	46.94	73.47	26.53	77.55	22.45	69.39	30.61	81.63	18.37	67.35	32.65
Male	87.85	12.15	81.31	18.69	86.92	13.08	88.79	11.21	89.72	10.28	92.52	7.48	83.18	16.82

Sex	Site 483		Site 510		Site 521		Site 526		Site 532		Site 535		Site 536	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	71.43	28.57	71.43	28.57	67.35	32.65	67.35	32.65	63.27	36.73	73.47	26.53	73.47	26.53
Male	84.11	15.89	86.92	13.08	91.59	8.41	86.92	13.08	86.92	13.08	86.92	13.08	89.72	10.28

Sex	Site 541		Site 569		Site 575		Site 582		Site 587		Site 591	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	75.51	24.49	65.31	34.69	73.47	26.53	77.55	22.45	77.55	22.45	69.39	30.61
Male	88.79	11.21	85.05	14.95	94.39	5.61	91.59	8.41	88.79	11.21	83.18	16.82

Appendix Table 3. Proportion (%) of differential methylation between adult females vs. nestlings individual restriction sites (n = 35) that were statistically significant by adult females (n = 49) vs. nestlings (n = 159), and the collective proportion of differential methylation among all (n = 100) and just the statistically significant sites.

Group	**All Sites**		**Sig. Sites**		**Site 229**		**Site 290**		**Site 305**		**Site 322**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	68.08	31.92	68.86	31.14	36.73	63.27	67.35	32.65	59.18	40.82	71.43	28.57
Nestling	76.50	23.50	85.71	14.29	59.75	40.25	83.65	16.35	81.76	18.24	86.16	13.84
Group	**Site 325**		**Site 350**		**Site 353**		**Site 355**		**Site 362**		**Site 367**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	69.39	30.61	67.35	32.65	69.39	30.61	77.55	22.45	71.43	28.57	69.39	30.61
Nestling	83.65	16.35	88.68	11.32	86.16	13.84	89.31	10.69	85.53	14.47	89.94	10.06
Group	**Site 371**		**Site 375**		**Site 411**		**Site 417**		**Site 419**		**Site 425**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	67.35	32.65	65.31	34.69	69.39	30.61	71.43	28.57	73.47	26.53	77.55	22.45
Nestling	84.28	15.72	86.16	13.84	89.31	10.69	83.02	16.98	86.16	13.84	90.57	9.43
Group	**Site 432**		**Site 437**		**Site 448**		**Site 451**		**Site 453**		**Site 455**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	73.47	26.53	73.47	26.53	53.06	46.94	71.43	28.57	73.47	26.53	69.39	30.61
Nestling	85.53	14.47	89.31	10.69	85.53	14.47	85.53	14.47	87.42	12.58	88.68	11.32
Group	**Site 468**		**Site 483**		**Site 510**		**Site 521**		**Site 526**		**Site 532**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	67.35	32.65	71.43	28.57	71.43	28.57	67.35	32.65	67.35	32.65	63.27	36.73
Nestling	86.79	13.21	86.16	13.84	85.53	14.47	84.91	15.09	83.65	16.35	84.28	15.72
Group	**Site 535**		**Site 536**		**Site 569**		**Site 575**		**Site 587**		**Site 589**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Female	73.47	26.53	73.47	26.53	65.31	34.69	73.47	26.53	77.55	22.45	71.43	28.57
Nestling	90.57	9.43	89.94	10.06	82.39	17.61	89.31	10.69	89.31	10.69	85.53	14.47
Group	**Site 591**											
	Unmeth	**Meth**										
Female	69.39	30.61										
Nestling	85.53	14.47										

Appendix Table 4. Proportion (%) of differential methylation between male age (second year [SY], n = 14 vs. after second year [ASY], n = 87) individual restriction sites (n = 21) that were statistically significant by male age (n = 101), and the collective proportion of differential methylation among all (n = 100) and just the statistically significant sites.

Age	**All Sites**		**Sig. Sites**		**Site 244**		**Site 319**		**Site 339**		**Site 340**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	69.57	30.43	58.84	41.16	35.71	64.29	50.00	50.00	71.43	28.57	57.14	42.86
ASY	76.95	23.05	84.23	15.77	63.44	36.56	80.65	19.35	93.55	6.45	79.57	20.43

Age	**Site 343**		**Site 347**		**Site 353**		**Site 362**		**Site 367**		**Site 376**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	57.14	42.86	57.14	42.86	64.29	35.71	64.29	35.71	64.29	35.71	57.14	42.86
ASY	79.57	20.43	81.72	18.28	84.95	15.05	86.02	13.98	87.10	12.90	86.02	13.98

Age	**Site 419**		**Site 425**		**Site 437**		**Site 451**		**Site 483**		**Site 484**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	71.43	28.57	71.43	28.57	71.43	28.57	57.14	42.86	50.00	50.00	50.00	50.00
ASY	91.40	8.60	94.62	5.38	90.32	9.68	78.49	21.51	89.25	10.75	81.72	18.28

Age	**Site 508**		**Site 532**		**Site 541**		**Site 589**		**Site 591**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	42.86	57.14	64.29	35.71	71.43	28.57	50.00	50.00	57.14	42.86
ASY	72.04	27.96	90.32	9.68	91.40	8.60	79.57	20.43	87.10	12.90

Appendix Table 5. Proportion (%) of differential methylation between female age (second year [SY], n = 13 vs. after second year [ASY], n = 35) individual restriction sites (n = 18) that were statistically significant by female age (n = 48), and the collective proportion of differential methylation among all (n =100) and just the statistically significant sites.

Age	**All Sites**		**Sig. Sites**		**Site 135**		**Site 144**		**Site 209**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	63.15	36.85	52.14	47.86	46.15	53.85	84.62	15.38	38.46	61.54
ASY	70.80	29.20	77.94	22.06	74.29	25.71	48.57	51.43	71.43	28.57

Age	**Site 290**		**Site 297**		**Site 305**		**Site 325**		**Site 340**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	46.15	53.85	92.31	7.69	38.46	61.54	38.46	61.54	53.85	46.15
ASY	77.14	22.86	62.86	37.14	68.57	31.43	82.86	17.14	88.57	11.43

Age	**Site 350**		**Site 368**		**Site 419**		**Site 453**		**Site 458**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	38.46	61.54	61.54	38.46	53.85	46.15	53.85	46.15	53.85	46.15
ASY	80.00	20.00	88.57	11.43	82.86	17.14	82.86	17.14	91.43	8.57

Age	**Site 468**		**Site 509**		**Site 532**		**Site 541**		**Site 569**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
SY	38.46	61.54	61.54	38.46	38.46	61.54	53.85	46.15	46.15	53.85
ASY	80.00	20.00	88.57	11.43	74.29	25.71	85.71	14.29	74.29	25.71

Appendix Table 6. Proportion (%) of differential methylation between undisturbed (n = 46) and disturbed (n = 49) shale gas territory (TerrGas) individual restriction sites (n = 18) that were statistically significant for adult males (n = 95), and the collective proportion of differential methylation among all (n=100) and just the statistically significant restriction sites.

Status	**All Sites**		**Sig. Sites**		**Site 155**		**Site 289**		**Site 292**		**Site 333**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Undisturbed	74.33	25.67	71.74	28.26	50.00	50.00	60.87	39.13	63.04	36.96	65.22	34.78
Disturbed	77.02	22.98	87.55	12.45	79.59	20.41	79.59	20.41	85.71	14.29	83.67	16.33

Status	**Site 368**		**Site 371**		**Site 446**		**Site 448**		**Site 455**		**Site 541**	
	Unmeth	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**	**Unmeth**	**Meth**
Undisturbed	78.26	21.74	86.96	13.04	65.22	34.78	71.74	28.26	80.43	19.57	95.65	4.35
Disturbed	91.84	8.16	100.00	0.00	87.76	12.24	87.76	12.24	97.96	2.04	81.63	18.37

Appendix Table 7. Results of BAYESCAN analysis on a restriction site (i.e., locus) level grouped by demographic parameter and shale gas disturbance status that was differentially methylated at a population level. p is the posterior probability for the selection model. Log10(PO) is the logarithm of Posterior Odds to base 10 for the selection model. The estimated alpha coefficient indicates the strength and direction of selection. The averaged F_{st} coefficient is calculated as the posterior mean.

Restriction Site	**p**	**Log10(PO)**	**alpha**	**F_{st}**
Male vs. Female				
155	0.077	-1.076	-0.005	0.076
222	0.079	-1.065	-0.012	0.076
229	0.077	-1.080	0.014	0.078
305	0.090	-1.007	-0.019	0.076
322	0.092	-0.993	-0.023	0.075
324	0.086	-1.024	-0.015	0.076
325	0.083	-1.042	-0.012	0.076
328	0.078	-1.070	-0.009	0.076
339	0.084	-1.040	-0.010	0.076
350	0.073	-1.105	-0.003	0.076
353	0.080	-1.063	-0.023	0.075
366	0.089	-1.011	-0.006	0.076
367	0.082	-1.048	-0.013	0.076
371	0.085	-1.031	0.025	0.078
375	0.082	-1.047	-0.004	0.077
400	0.079	-1.065	-0.018	0.076
411	0.081	-1.057	-0.014	0.076
419	0.077	-1.076	-0.007	0.076
425	0.083	-1.043	-0.015	0.076
437	0.080	-1.062	-0.019	0.075
448	0.076	-1.082	0.013	0.077
453	0.083	-1.044	-0.018	0.076
454	0.080	-1.058	-0.022	0.075
455	0.080	-1.059	0.005	0.077
458	0.079	-1.067	-0.018	0.075
468	0.083	-1.041	-0.021	0.075
483	0.079	-1.064	-0.016	0.076
510	0.080	-1.059	-0.010	0.076
521	0.073	-1.105	0.017	0.078
526	0.085	-1.030	-0.001	0.077
532	0.081	-1.054	0.011	0.077
535	0.080	-1.061	-0.011	0.076
536	0.089	-1.012	-0.003	0.077
541	0.084	-1.036	-0.008	0.076
569	0.074	-1.095	0.004	0.077
575	0.072	-1.108	0.017	0.078

582	0.080	-1.058	-0.016	0.076
587	0.084	-1.035	-0.010	0.076
591	0.081	-1.055	-0.017	0.076

Restriction Site	**p**	**Log10(PO)**	**alpha**	**F_{st}**
Female vs. Nestling				
229	0.081	-1.057	-0.003	0.077
290	0.082	-1.047	-0.012	0.076
305	0.073	-1.102	0.003	0.077
322	0.083	-1.041	-0.016	0.076
325	0.088	-1.017	-0.015	0.076
350	0.080	-1.063	0.007	0.077
353	0.079	-1.069	-0.004	0.077
355	0.084	-1.036	-0.017	0.076
362	0.084	-1.035	-0.014	0.076
367	0.080	-1.059	0.003	0.077
371	0.080	-1.061	-0.004	0.077
375	0.073	-1.102	0.000	0.076
411	0.081	-1.052	0.000	0.077
417	0.086	-1.028	-0.023	0.075
419	0.081	-1.055	-0.026	0.075
425	0.086	-1.028	-0.014	0.076
432	0.090	-1.004	-0.023	0.076
437	0.082	-1.050	-0.009	0.076
448	0.079	-1.064	0.024	0.078
451	0.079	-1.064	-0.015	0.076
453	0.086	-1.024	-0.014	0.076
455	0.080	-1.058	0.001	0.077
468	0.076	-1.082	-0.001	0.077
483	0.083	-1.041	-0.013	0.076
510	0.079	-1.065	-0.013	0.076
521	0.081	-1.052	-0.006	0.076
526	0.078	-1.070	-0.009	0.076
532	0.077	-1.077	0.002	0.077
535	0.074	-1.095	-0.001	0.077
536	0.079	-1.068	-0.003	0.076
569	0.081	-1.054	-0.011	0.076
575	0.079	-1.069	-0.005	0.076
587	0.080	-1.063	-0.011	0.076
589	0.088	-1.013	-0.017	0.076
591	0.085	-1.034	-0.009	0.076

Restriction Site	**p**	**Log10(PO)**	**alpha**	**F_{st}**
Male Age				
244	0.079	-1.069	-0.015	0.129
319	0.079	-1.067	-0.004	0.130
339	0.089	-1.010	-0.002	0.131

340	0.087	-1.023	-0.016	0.129
343	0.081	-1.056	-0.012	0.130
347	0.072	-1.111	-0.011	0.129
353	0.082	-1.047	-0.018	0.129
362	0.085	-1.033	-0.014	0.129
367	0.078	-1.071	-0.010	0.130
376	0.074	-1.097	0.003	0.131
419	0.084	-1.040	-0.013	0.130
425	0.071	-1.118	-0.001	0.130
437	0.085	-1.032	-0.012	0.130
451	0.085	-1.031	-0.024	0.129
483	0.077	-1.081	0.012	0.132
484	0.083	-1.041	-0.006	0.130
508	0.083	-1.042	-0.014	0.129
532	0.078	-1.070	0.003	0.131
541	0.078	-1.073	-0.010	0.130
589	0.073	-1.101	-0.013	0.129
591	0.072	-1.110	-0.001	0.130

Restriction Site	**p**	**Log10(PO)**	**alpha**	**F_{st}**
Female Age				
135	0.080	-1.063	-0.021	0.150
144	0.080	-1.059	-0.014	0.151
209	0.082	-1.050	-0.010	0.151
290	0.081	-1.056	-0.013	0.151
297	0.088	-1.015	-0.021	0.151
305	0.078	-1.070	-0.016	0.151
325	0.074	-1.097	0.006	0.153
340	0.076	-1.084	-0.001	0.152
350	0.081	-1.057	0.003	0.152
368	0.078	-1.075	-0.011	0.151
419	0.081	-1.055	-0.012	0.151
543	0.081	-1.055	-0.018	0.150
458	0.082	-1.049	0.004	0.153
468	0.075	-1.089	-0.001	0.152
509	0.079	-1.064	-0.008	0.152
532	0.079	-1.068	-0.003	0.152
541	0.081	-1.054	-0.005	0.152
569	0.078	-1.070	-0.022	0.150

Restriction Site	**p**	**Log10(PO)**	**alpha**	**F_{st}**
Male TerrGas				
155	0.083	-1.044	-0.009	0.147
289	0.081	-1.052	-0.023	0.146
292	0.085	-1.030	-0.017	0.146
333	0.088	-1.018	-0.027	0.145
368	0.081	-1.054	-0.019	0.146
371	0.090	-1.003	0.020	0.151

446	0.078	-1.075	-0.010	0.147
448	0.081	-1.052	-0.028	0.145
455	0.076	-1.087	0.003	0.148
541	0.087	-1.020	-0.016	0.147