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Evidence from two shale regions that a riparian songbird accumulates metals associated with hydraulic fracturing

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Citation: Latta, S. C., L. C. Marshall, M. W. Frantz, and J. D. Toms. 2015. Evidence from two shale regions that a riparian songbird accumulates metals associated with hydraulic fracturing. Ecosphere 6(9):144. http://dx.doi.org/10.1890/ES14-00406.1

Abstract. The risk of contamination of surface waters from hydraulic fracturing activities (i.e., fracking) to extract gas from underground shale formations has been viewed primarily in the context of localized point-source events such as spills with no evidence of contaminants entering food chains. We showed that in watersheds where hydraulic fracturing occurs, an obligate riparian songbird and top predator in headwater systems, the Louisiana Waterthrush (*Parkesia motacilla*), accumulated metals associated with the fracking process. In both the Marcellus and Fayetteville shale regions, barium and strontium were found at significantly higher levels in feathers of birds in sites with fracking activity than at sites without fracking. The question of what pathway these metals followed from the shale layers to enter the food chain was not resolved by this study, but our data suggested a recent origin for these metals in the riparian systems we studied because levels of barium and strontium in feather samples from reference sites in the Marcellus Region without fracking activity did not differ from historical samples of waterthrush feathers gathered prior to any fracking in the region. Our finding of similarly elevated levels of metals associated with fracking in two geographically distant shale formations suggests hydraulic fracturing may be contaminating surface waters and underscores the need for additional monitoring and study to further assess ecological and human health risks posed by the increasingly widespread development of unconventional sources of natural gas around the world.

Key words: Arkansas; bioindicator; contamination; Fayetteville Shale; flowback; Louisiana Waterthrush; Marcellus Shale; natural gas; *Parkesia motacilla*; Pennsylvania; produced water; West Virginia.

Received 22 October 2014; revised 31 March 2015; accepted 21 April 2015; **published** 11 September 2015. Corresponding Editor: B. Maslo.

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Introduction

New technologies such as horizontal drilling and hydraulic fracturing (or fracking) have recently allowed the profitable extraction of gas from deep underground shale layers (Kerr 2010, Entrekin et al. 2011, Gregory et al. 2011), but critics have pointed towards a variety of potential environmental risks associated with fracking (Entrekin et al. 2011, Jackson et al. 2013, Burton et al. 2014). Much of the public debate around the environmental impacts of this unconventional natural gas development has focused on surface disturbances associated with land clearing and the development of infrastructure (Entrekin et al. 2011, Drohan et al. 2012), erosion and sedimentation of waterways (Entrekin et al. 2011, Drohan et al. 2012, Vidic et al. 2013), and the release of methane into the atmosphere (Howarth et al. 2011*b*). Fracking has also been controversial because it involves the high pressure injection of ~7–25 million liters of water and other additives into the shale layers each time the well is fracked (Kerr 2010, Howarth et al. 2011*a*). These additives promote the release of the natural gas from the shale layers. Products used in the hydraulic fracturing of natural gas-bearing shale vary among operators, but may often include highly toxic compounds or known carcinogens (Colburn et al. 2011, Entrekin et al. 2011).

Fracking compounds do not all remain underground; water that returns to the surface immediately following fracking is known as flowback, while fluids that return over longer time periods and at slower rates is known as produced water. Both flowback and produced waters may contain fracking compounds, as well as naturally occurring metals, formation brines, radionuclides, and other potential environmental contaminants from the shale layers (Arthur et al. 2008, Jackson et al. 2013). The amount of flowback and produced water from a well varies considerably, but in portions of the Marcellus Shale only 10–30% of introduced fluids may be recovered (Entrekin et al. 2011), and there is as yet no information on the fate of the remaining millions of gallons of introduced fluids.

Contamination of surface waters and terrestrial systems may occur during the drilling process or during the transport and disposal of the flowback or produced waters (Entrekin et al. 2011, Gregory et al. 2011, Jackson et al. 2013). However, the possibility that fracking itself can contaminate underground water supplies is often minimized because of the large vertical separation between shale gas formations and aquifers, and the relatively narrow zone fractured to release gas (Warner et al. 2012, Jackson et al. 2013). As a result, the primary risk of contamination of surface waters has been viewed as localized point-source pollution events (Kerr 2010). There have been few studies of the ecological effects of drilling contaminants entering surface waters (Entrekin et al. 2011, Burton et al. 2014, Souther et al. 2014), and a general lack of baseline environmental monitoring prior to unconventional natural gas development has frequently hindered the drawing of any robust conclusions from the few ecological studies (Burton et al. 2014, Souther et al. 2014). As a result, we have no knowledge of whether contaminants from fracking may be entering food chains, and we have inadequate data to predict the ecological risks associated with these operations (Entrekin et al. 2011, Jackson et al. 2013, Burton et al. 2014, Souther et al. 2014).

The use of birds as bioindicators of pollution has a long history as a catalyst for environmental protection (Carson 1962, Furness and Camphuysen 1997). The breeding habitat of the Louisiana Waterthrush (*Parkesia motacilla*) is forested, freshwater streams, and the species has been shown to be an excellent bioindicator of riparian habitat quality (Mattsson and Cooper 2006, Mulvihill et al. 2008). Waterthrushes feed largely on benthic macroinvertebrates taken directly from the stream, or on recently emerged adults of these same macroinvertebrate species (Robinson 1995); changes in water quality can affect the diversity and abundance of macroinvertebrates, and densities and productivity of waterthrushes (Mulvihill et al. 2008). As a top predator, the waterthrush may also be exposed to, and bioaccumulate through their diet, the chemical contaminants occurring in the macroinvertebrates and the riparian ecosystem. Contaminants occurring in the bird's food will pass to their blood and then be incorporated in growing feathers. Once feather growth ends, the feather structure is representative of the bird's blood physiology and toxicology during its period of growth (Burger 1993, Furness 1993).

In this multi-regional study from the Marcellus Shale of the Appalachian region and the Fayetteville Shale of Arkansas, we used the Louisiana Waterthrush as an indicator species to assess the accumulation of contaminants associated with the hydraulic fracturing process. Barium (Ba) and strontium (Sr) are two naturally occurring metals found in very high concentrations in the shale layers (Entrekin et al. 2011, Chapman et al. 2012), and the flowback and produced water is also known for its high Ba and Sr concentrations (Chapman et al. 2012). These metals would be expected in above-ground environments if leaks, accidental spills, illegal dumping, or migration were occurring (Chapman et al. 2012). While there is a shortage of data for evaluating the risk posed by exposure to Ba (WHO 1990) and Sr (WHO 2010) above natural levels, both metals can be bioaccumulated in organisms (WHO 1990, 2010). Regardless of the potential impact of Ba or Sr on aquatic or terrestrial organisms, we consider these metals to be markers for potentially numerous other toxic chemicals that could result from shale gas development. We hypothesized that waterthrushes foraging and nesting along streams with hydraulically fractured shale gas wells in the watersheds would accumulate Ba and Sr occurring in the environment and incorporate these markers in their feathers. Contaminants in feathers known to have been grown on target streams were compared between impacted, fracked sites and unfracked, reference sites, and to feathers collected prior to any fracking activities, to understand whether contaminants may be entering food chains in riparian areas in regions dominated by gas well development.

Methods

We sampled birds along first- and second-order streams in West Virginia and Pennsylvania where hydraulic fracturing occurs in the Marcellus Shale and in Arkansas in the Fayetteville Shale formation (Fig. 1). In the Marcellus Shale region, study streams were tributaries of Buffalo Run located in the Lewis Wetzel Wildlife Management Area in northwestern West Virginia (Buffalo Run, Carpenter Run, Hile's Run, Huss Pen Run, Megan's Run, Nettles Run, Olive Run, Owl Run, Sees Run, Slabcamp Run, Snake Run, Wyatt's Run). Additional reference streams were located in Westmoreland County, Pennsylvania (Loyalhanna Creek, Powdermill Run, Camp Run). In the Fayetteville Shale region, streams were located in the Gulf Mountain Wildlife Management Area of Van Buren County, and in Conway and Faulkner counties, Arkansas, and were tributaries of Point Remove, Cadron, and the South Fork of the Little Red River. These included Cedar Creek, Sis Hollow, Point Remove, East Fork Point Remove Creek, Sunnyside Creek, and Black Fork. Additional reference streams were tributaries of the Kings and Buffalo National River and included Piney Creek, Jimmy Creek and Adds Creek in Carroll and Newton counties, Arkansas.

Louisiana Waterthrushes maintain linear territories along streams, typically 300–1200 m in length (Robinson 1995, Mulvihill et al. 2008). Waterthrush territories were considered impacted if one or more shale gas well pads or other shale gas infrastructural elements (roads or pipelines) occurred in the watershed upstream from the waterthrush territory. In the Marcellus Shale region we used a sequence of aerial and satellite imagery, Google Maps (https://www.google.com/maps) and map data from Fracktracker.org and PAGasLease.com to determine areas of shale gas activity and development. In West Virginia gas well pads were mapped by downloading recent gas well production data from the WVDEP (WVDEP 2014) to determine when the well was constructed, if the well was currently active, and if it targeted the Marcellus formation. In the Fayetteville Shale region, the presence of unconventional gas extraction and location of stream catchments were based upon Arkansas oil and natural gas well maps along with online production and well information (AOGC 2012), the Arkansas Watershed Information System, and guidance from ongoing research in the area (Entrekin et al. 2011). In all cases and for each year of the study our site classifications were confirmed with on-site observations.

We began locating and monitoring Louisiana Waterthrushes soon after their arrival on their streamside territories. We used behavior to determine when pairs were nesting and searched for every nest attempt at each site. Nests were checked at 3- to 4-day intervals for survival of eggs and nestlings. Adult males and females were mist netted to obtain feather samples, while feathers from nestlings were collected near the end of the nestling period (generally day 7–8).

Contaminants were measured in nesting Louisiana Waterthrush by sampling the outer rectrix (R6) and testing this feather for Ba and Sr concentrations. To help insure that tested feathers were grown on the target stream we only used rectrices from focal individuals from which we could safely deduce that the feather was grown on that study site. These included: (1) adults with adventitiously molted feathers (i.e., feathers regrown following our early-season plucking of R6), (2) nestlings, and (3) uniquely color-banded

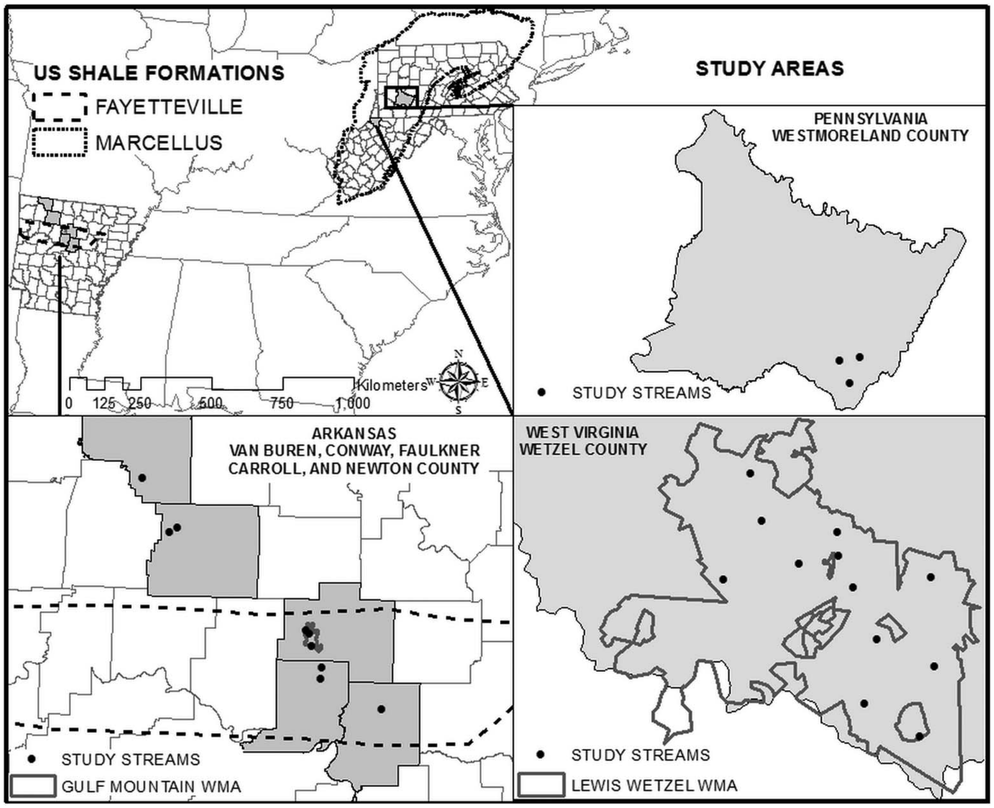

Fig. 1. Location of first- and second-order streams in the Marcellus Shale region of West Virginia and Pennsylvania and the Fayetteville Shale region of Arkansas, where Louisiana Waterthrush were sampled.

adults that were known to have nested on the same stream in the previous year. This latter group of samples are useful because this species molts and regrows feathers on the breeding stream, immediately following nesting (Robinson 1995, Mulvihill et al. 2008), so concentrations of contaminants in feathers will reflect concentrations of the metal encountered in that same stream during the previous post-breeding season.

Feather samples were processed for contaminants by the Dartmouth Toxic Metals Superfund Research Program at Dartmouth College and the Dartmouth Medical School in Hanover, New Hampshire. Feathers were washed with the nonionic surfactant Triton X-100 and rinsed 6 times with deionized water. The feathers were air dried in a clean drying box and then freeze dried to remove residual moisture. Each feather was then acid digested in an open vessel procedure using 0.5 mL of 9:1 HNO_3:HCl. The feather was left in the acid overnight and then heated at 105°C for 20 min in a MARS Express (CEM, Mathews, North Carolina, USA) microwave digestion unit. After digestion, 200 μL of H_2O_2 was added and the sample was heated again at 90°C for 10 min. The digested sample was then diluted to ~7 mL with deionized water. The digested sample was analyzed for trace metals by inductively coupled plasma mass spectrometry (Agilent 7700x, Santa Clara, California, USA) following EPA procedure 6020a (USEPA 2008). Quality control included analysis of duplicates, spikes and digestion reference materials, as well as blanks and fortified blanks.

Statistics

Contaminant levels were first standardized among feathers based on feather length and feather mass. In order to determine whether the two correction methods yielded similar results, for each individual contaminant we fit a calibration model (Type II regression) to compare estimated contaminant levels corrected for feather mass with estimated contaminant levels corrected for feather length. We used the lmodel2 package to fit a reduced major axis regression in R (version 2.15.1; R Development Core Team 2012), after first transforming the data to normality using Box-Cox transformations in package Car. We then looked at overall model fit as estimated by r^2 and the presence of non-linearity. Because the calibration models fit extremely well for both contaminants (Ba, $r^2 = 0.96$; Sr, $r^2 = 0.96$), and no non-linearity was present, we concluded that both methods of correction gave substantively the same results. Subsequent tests used feather mass as the correction factor.

We also determined in advance of other analyses whether siblings from the same nest constituted independent samples by comparing a null (intercept only) model with one including family as a random covariate. If siblings are truly independent, then including family in the model would not explain any variation. Therefore, a likelihood ratio test (LRT) comparing these models tests a null hypothesis of independence among siblings. The LRT is known to be conservative, so a significant p-value is a strong indication that siblings are not independent samples. Data for both contaminants was transformed so that the assumptions of normal distribution of residuals were met. Independence was rejected for both Ba (LRT = 9.85, $p < 0.01$) and Sr (LRT = 32.74, $p < 0.01$), therefore, we pooled samples from siblings for all subsequent analyses.

We then compared Ba and Sr concentrations between fracked and non-fracked sites. Our model to compare contaminant levels between fracked and non-fracked sites included the covariates of fracked versus non-fracked, and Fayetteville Shale region versus Marcellus Shale region. Contaminant level was our response variable and was log transformed to ensure that residuals followed a normal distribution.

We also added an additional control by establishing an historical baseline for observed contaminant levels using Louisiana Waterthrush feathers which were collected along our Pennsylvania study streams in 1997–2002 prior to the commercial implementation of hydraulic fracturing techniques. Within the Marcellus Shale region, reference samples (from sites that were not impacted by fracking) were available from two time periods: 1997–2002 and 2010–2013. We determined whether the levels of either contaminant had changed during that time period by fitting an ANOVA model with a single covariate, time period. Our response variable (contaminant level) was first adjusted with log transformations so that residuals approximated a normal distribution. As a result, once back-transformed the model estimated the ratio of contaminant levels in 2010–2013 to those in 1997–2002.

Results

From 2010 to 2013 we collected 285 feather samples from Louisiana Waterthrush for testing: 167 samples from the Marcellus Shale region and 118 samples from the Fayetteville Shale region. Samples were divided between impacted, fracked sites and unfracked, reference sites within each region (Marcellus impacted, $n = 50$, reference, $n = 117$; Fayetteville impacted, $n = 55$, reference, $n = 63$). Additional reference samples ($n = 40$) were obtained from 1997 to 2002 collections in the Marcellus Shale prior to any fracking operations.

Feather contaminant data from 2010 to 2013 were used to determine whether contaminant levels varied between sites with and without fracking activity. Contaminant levels were log-transformed, so tests examined ratios of contaminant levels rather than differences. We found that fracking was associated with significant increases in both Ba ($F = 4.37$, $p = 0.04$, $n = 276$) and Sr ($F = 10.08$, $p < 0.01$, $n = 284$; Table 1). Differences between regions were also significant, with higher levels of Ba ($F = 11.29$, $p < 0.01$) and Sr ($F = 39.49$, $p < 0.01$) in the Marcellus Shale region than in the Fayetteville Shale region (Table 1). The interaction of region $\times$ fracking history was not significant for either Ba ($F = 0.08$, $p = 0.78$) or Sr ($F = 0.25$, $p = 0.62$).

The levels of Ba and Sr at reference sites not directly impacted by fracking did not increase significantly between 1997–2002 and 2010–2013 (ratio for Ba, $n = 93$, estimate = 0.95, 95% CI = 0.64–1.40; Sr, $n = 93$, estimate = 1.22, 95% CI = 0.89–1.67), suggesting that observed increases in Ba and Sr concentrations in the feathers of

Table 1. Results of ANOVA model comparing contaminant levels for barium (Ba) and strontium (Sr) with the covariates of Fayetteville Shale region versus Marcellus Shale region and fracked versus non-fracked. The response variable of contaminant level was log-transformed to ensure that residuals followed a normal distribution. Fracking was associated with significant increases in both barium and strontium. Differences between regions were also significant with higher contaminant loads occurring in the Marcellus Shale region. The interaction of region × fracking history was not significant for either contaminant.

		Region		Fracked		Region × fracked	
Contaminant	*n*	*F*	*p*	*F*	*p*	*F*	*p*
Ba	276	11.29	< 0.01	4.37	0.04	0.08	0.78
SR	284	39.49	< 0.01	10.08	< 0.01	0.25	0.62

waterthrushes in fracked sites are of recent origin (Fig. 2).

Discussion

The detection of significantly elevated concentrations of Ba and Sr in feathers grown at fracked sites in both the Marcellus and Fayetteville Shale regions indicates that bioaccumulation of contaminants is occurring and that there is communication between natural gas activities and riparian systems. The presence of these markers further suggests that other toxic chemicals used in hydraulic fracturing of natural gas-bearing shale may also be contaminating surface waters. We did not resolve the pathway by which these metals move from the shale layers to enter the food chain. While Ba and Sr were chosen as indicators of fracking contamination based on the low likelihood that they would occur in surface waters due to widespread road salting, leakage of septic systems, or wastewater outflows as would Na, Cl, and other compounds which are also characteristic of produced waters (Chapman et al. 2012, Warner et al. 2012), our hypothesis that the observed elevated concentrations of Ba and Sr are the result of fracking activities is strongly reinforced by our findings from the Marcellus Shale region where data showed that levels of Ba and Sr have not changed significantly in reference sites since 1997–2002. Results from these control sites also help exclude the possibility that the Ba and Sr may have originated with drainage from abandoned coal mines or older shallow oil and gas wells which can be a source of Ba and Sr (Chapman et al. 2012). Our results support the hypothesis that the recent initiation of fracking has been responsible for the increase in Ba and Sr in sites with fracking, and we have no reason to think that other activities that could result in additions of these metals to these systems have changed in the few years since we initiated our study in fracked watersheds.

Widespread contamination of surface waters in areas where fracking occurs could be explained by migration of waters directly from the shale layers. Fracking may allow the migration of contaminants through natural faults and fractures, or fracking might compromise supposedly impermeable layers of rock thousands of feet below aquifers through induced fractures and thus contaminate underground and above-ground water supplies (Myers 2012, Warner et al. 2012, Jackson et al. 2013). Such a hypothesis is supported by a model of the migration of fracking fluids over time in the Marcellus Shale which suggests that fracking would exacerbate natural fractures in the shale, and that pressures exerted by the fracking can continue for up to a year, resulting in a high likelihood of chemicals reaching the surface in just a few years (Myers 2012). While this model has proven controversial (Saiers and Barth 2012, Cohen et al. 2013, Jackson et al. 2013), geochemical evidence from the Marcellus Shale region also shows that pathways exist between deep underlying formations and shallower aquifers used for drinking water (Warner et al. 2012), leading to the conclusion that water resources are at risk of contamination. In addition, case studies show that stray or fugitive gas from deep gas-rich formations has migrated from the subsurface into shallow aquifers where it has affected groundwater quality (Jackson et al. 2013, Souther et al. 2014).

Contamination of surface waters near fracked gas wells is most frequently associated not with

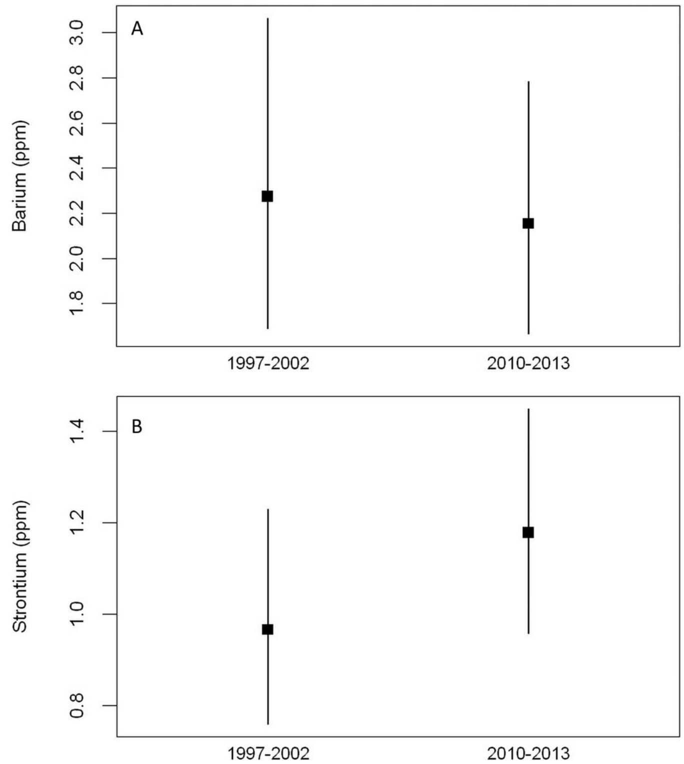

Fig. 2. Estimated mean contaminant load of birds (with 95% CI), based on fitted ANOVA models, indicate that contaminant loads in the Marcellus Shale region did not differ between 2010 and 2013 reference sites with no fracking and historical samples (1997–2002) that were gathered before fracking occurred for both (A) Ba ($n = 93$, estimate = 0.95, 95% CI = 0.64–1.40) and (B) Sr ($n = 93$, estimate = 1.22, 95% CI = 0.89–1.67). These results suggest that the significantly higher levels of Ba and Sr that we observed in sites with fracking activity are of recent origin.

hydraulic fracturing per se, but with spills or the improper or illegal storage or disposal of flowback and produced water (Osborn et al. 2011, Myers 2012, Warner et al. 2012, Jackson et al. 2013). The number of reported regulatory violations varies dramatically between states, attributable to the variable degree of regulation and regulatory resources at the state level (Entrekin et al. 2011, Burton et al. 2014). Nearly half of the violations in Pennsylvania involved the illegal direct discharge of pollutants, improper erosion control, or failure to contain wastes (PADEP 2010, Entrekin et al. 2011), while in Arkansas, 20% of surface water violations from fracking involved illegal discharges and spills (Burton et al. 2014). We question, however, whether our results could be accounted for only by reported violations which are spatially and temporally distinct point sources of pollution, because isolated spills might be expected to show up only as spikes in contaminant loads carried by individual birds along a given stream, and would not be statistically significant across our study sites.

Evidence suggests, however, that leaks and spills associated with hydraulic fracturing activities may be much more frequent than reported, and this may play a role in the results presented here. For example, reporting requirements from Pennsylvania have been described as "routinely violated" (Souther et al. 2014), with the Auditor General in Pennsylvania citing inaccuracy in the Department of Environmental Protection's (DEP) tracking of wastes from Marcellus production sites, and noting that clear violations in DEP inspections were not recorded as violations in summary reports (PADAG 2014). In addition, reported violations may overlook inferior well-casings as a source of contamination, as recent research has implicated faulty cement and well-casings that allow the escape of methane and other contaminants to the environment (Jackson et al. 2013, Darrah et al. 2014, Ingraffea et al. 2014). If correct, under-reported spills and discharges, rather than migration induced by hydraulic fracturing itself, may explain the widespread occurrence of elevated Ba and Sr levels necessary to find statistically significant patterns across two geographically distant shale layers as seen here.

Our findings of similar patterns from both the Marcellus and Fayetteville shale regions, suggests that whatever the pathway for contaminants, the contamination problem is larger than an isolated watershed or even a single shale formation. The regulatory efforts and industry standards for hydraulic fracturing and the development of unconventional natural gas wells in the regions where our study took place would appear to be inadequate for controlling contamination of surface waters with fracking byproducts. Our finding of significantly higher levels of Ba and Sr also suggest the possibility of surface water contamination by any of the hundreds of chemicals that may be used in hydraulic fracturing, including friction reducers, acids, biocides, corrosion and scale inhibitors, pH adjusting agents and surfactants (Colburn et at. 2011, Entrekin et al. 2011).

The occurrence in the Louisiana Waterthrush of contaminants associated with fracking has general implications for wildlife and human health, as many of the potential contaminants introduced in the fracking process should not be ingested at any concentration (Colburn et al. 2011, Kiviat 2013, Souther et al. 2014). A better understanding, however, of gas migration processes and potential pathways of contamination is required. Some success has been shown in the use of isotope ratios to confirm the deep origin of dissolved solids occurring in ground or surface waters (Chapman et al. 2012, Darrah et al. 2014), so an analysis of isotopes of Ba and Sr occurring in feathers of birds within and outside areas of fracking activity may be useful in determining their origin. Further studies using indicator species in a variety of taxa should also be pursued to improve our understanding of the possible effects of hydraulic fracturing for natural gas on water quality, wildlife fitness, ecosystem dynamics, and human health.

Acknowledgments

S. C. Latta conceived the study and coordinated its design, implementation, and analysis and manuscript preparation. L. C. Marshall and M. W. Frantz coordinated field teams and data entry and assisted with writing. J. D. Toms designed and conducted statistical analyses and assisted with writing. S. C. Latta, L. C. Marshall, and M. W. Frantz secured funding to complete the work. We thank the numerous field personnel involved with our study. Study design was improved through valuable discussions with Dan Volz (Center for Healthy Environments and Communities, University of Pittsburgh), Jerry Mead (Academy of Natural Sciences of Philadelphia), and Sally Entrekin (University of Central Arkansas). Early versions of the manuscript were improved with comments from Robert Ricklefs, John Wenzel, Walter Carson, and members of the Don Quixote de la Resaca Eminent Ecologists Forum, and by reviews from Erik Kiviat and an anonymous reviewer. Research was funded by The Heinz Endowments, M. Graham and Jane S. Netting Environmental Fund, the National Aviary Conservation Endowment, a Marion Orton Memorial Grant, WVDNR/USGS (to P. B. Wood), and private donors. Funding for some field personnel was also provided by the National Science Foundation (grant DEB-1349870 to Tim Nuttle). In-kind support was provided by West Virginia University, West Virginia Cooperative Fish and Wildlife Research Unit, West Virginia Department of Natural Resources, U.S. Geological Survey, and University of Arkansas. Permissions to complete the work were provided by the Pennsylvania Game Commission, Pennsylvania Department of Conservation and Natural Resources, West Virginia Department of Natural Resources, and Arkansas Department of Game and Fish. Study protocols were approved by the Institutional Animal Care and Use Committee of the National Aviary/Pittsburgh Zoo.

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