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# Accumulation of Marcellus Formation Oil and Gas Wastewater Metals in Freshwater Mussel Shells

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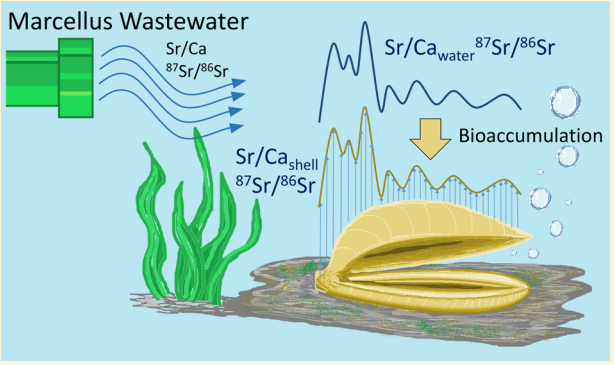
Supporting Information

**ABSTRACT:** For several decades, high-salinity water brought to the surface during oil and gas (O&G) production has been treated and discharged to waterways under National Pollutant Discharge Elimination System (NPDES) permits. In Pennsylvania, USA, a portion of the treated O&G wastewater discharged to streams from 2008 to 2011 originated from unconventional (Marcellus) wells. We collected freshwater mussels, *Elliptio dilatata* and *Elliptio complanata*, both upstream and downstream of a NPDES-permitted facility, and for comparison, we also collected mussels from the Juniata and Delaware Rivers that have no reported O&G discharge. We observed changes in both the $Sr/Ca_{shell}$ and $^{87}Sr/^{86}Sr_{shell}$ in shell samples collected downstream of the facility that corresponded to the time period of greatest Marcellus wastewater disposal (2009−2011). Importantly, the changes in $Sr/Ca_{shell}$ and $^{87}Sr/^{86}Sr_{shell}$ shifted toward values characteristic of O&G wastewater produced from the Marcellus Formation. Conversely, shells collected upstream of the discharge and from waterways without treatment facilities showed lower variability and no trend in either $Sr/Ca_{shell}$ or $^{87}Sr/^{86}Sr_{shell}$ with time (2008−2015). These findings suggest that (1) freshwater mussels may be used to monitor changes in water chemistry through time and help identify specific pollutant sources and (2) O&G contaminants likely bioaccumulated in areas of surface water disposal.




## INTRODUCTION

Advances in combining horizontal drilling and high volume hydraulic fracturing (HVHF) have greatly increased the ability to extract hydrocarbons from low-permeability formations, facilitating an increase in U.S. production of natural gas from "unconventional" wells in the U.S.[1] This technology has allowed a shift in electricity generation away from coal to cleaner-burning natural gas, which may prove an important intermediate in the search for clean energy independence.[2,3] Despite the benefits, concerns have been raised about the potential negative impact of HVHF on water quality.[3−7] In the Marcellus Shale Formation, the third largest shale play in the U.S., the median well requires 27.9 million liters (ML) of water injected for natural gas extraction. Approximately 4 ML of the injected water returns to the surface with high concentrations of total dissolved solids (TDS > 100 000 mg/L), metals, and radioactivity.[2,5,8−14] Since 2011, in Pennsylvania, the state with the largest oil and gas production from the Marcellus, between 70% and 90% of wastewater fluid that returns to the surface is reused in subsequent drilling and HVHF operations.[15,16] However, between 2008 and 2011, over 2.9 billion liters of oil and gas (O&G) wastewater was discharged to waterways following treatment at centralized waste treatment facilities under National Pollutant Discharge Elimination System (NPDES) permits[4,7,15,16] (Supporting Information Figure S1).

Despite treatment, researchers observed several changes to water and sediment quality downstream of the NPDES discharges, including increases in the (1) salinity of the receiving streams,[4] (2) radioactivity of sediments at the point of discharge[4,17] and kilometers downstream,[18] and (3) mortality of freshwater mussels.[19] In one study, the effects of discharges to the Allegheny River from an NPDES-permitted facility in Warren, PA were examined (Figure 1). The facility discharged to the Allegheny River and increased the specific conductance to values between 4000 and 12 000 $\mu$S/cm, as much as 120 times the background conductance (103−188 $\mu$S/cm).[19] The elevated conductivity was observed over 2 km downstream, in a temporal pattern that mimicked the discharges from the O&G effluent (i.e., during the work-day). A concern with the increased salinity is the potential negative impacts to freshwater biota, often utilized as indicators of water quality for ecological and

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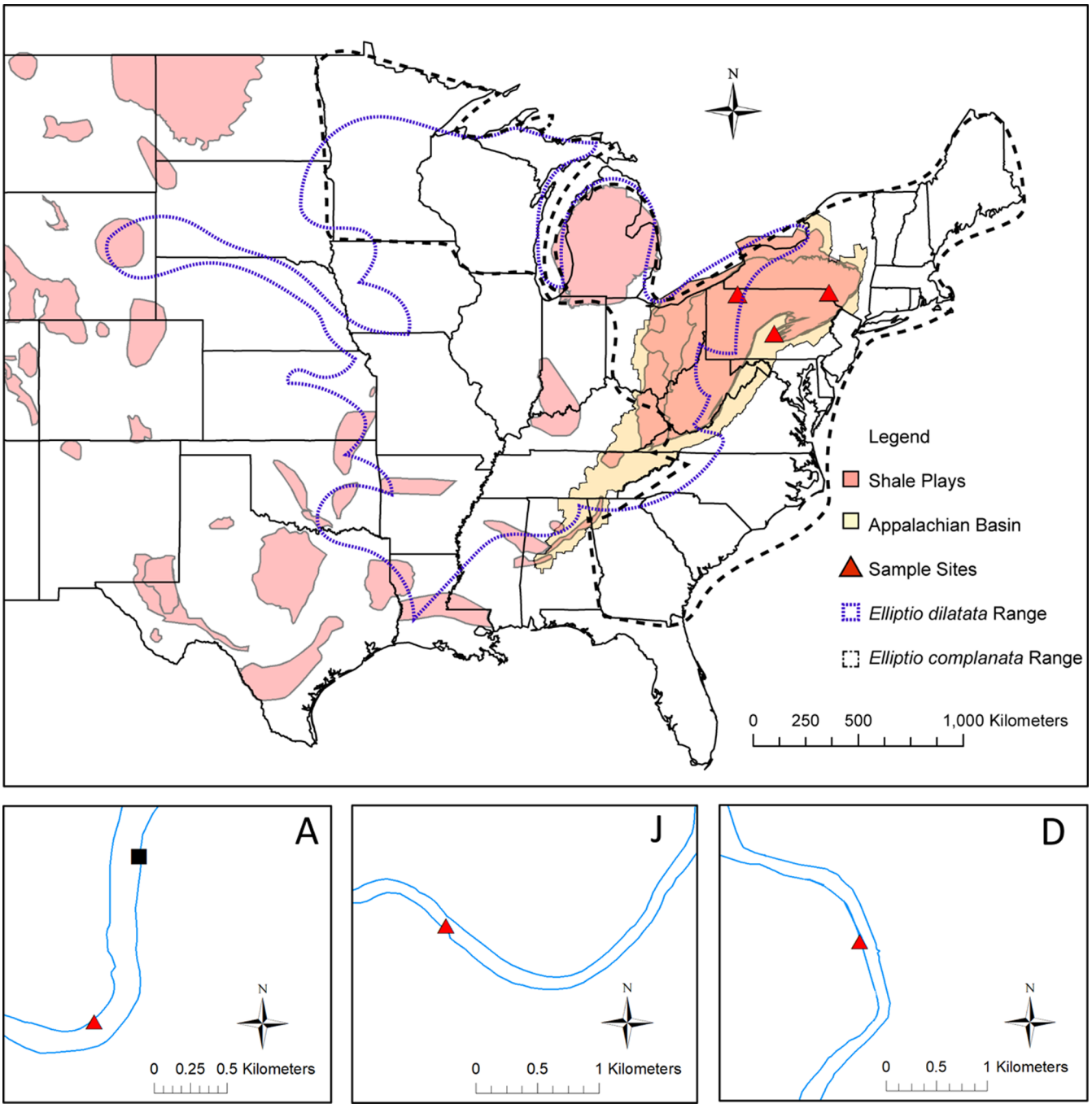


**Figure 1.** Ranges of *Elliptio dilatata* and *Elliptio complanata* outlined as shown. Note the overlap of the *Elliptio dilatata* range and several shale plays such as the Antrim and Fayetteville. Juniata and Delaware River samples are within the Applachian Basin but are not downstream of O&G wastewater disposal facilites (see Figure S1 in SI). The lower panels show the sample locations, indicated by red triangles, where samples were collected along the Allegheny (A), Juniata (J), and Delaware (D) Rivers. The black square in panel A represents the centralized waste treatment discharge to the Allegheny River.

human health. Indeed, population density, diversity, and mortality of mussels in the Allegheny River were all negatively affected downstream of the Warren facility discharge. Survival rates were between 80 and 100% 2 km downstream and 20−50% 0.5 km downstream of the discharge.[19] Mussels may be particularly sensitive to changes in salinity because glochidia, the larval stage of mussels, are sensitive to chloride concentrations and thus have the potential to be good indicators of high salinity O&G wastewater disposal impacts to streams. Freshwater mussels are currently the most endangered North American faunal group, experiencing a large scale and widespread extinction event, with 35 of the 297 species becoming extinct since 1900.[20,21] It is projected that 55% of the species that existed in 1900 will be extinct by 2100.[21]

Bivalves such as the freshwater mussels examined in this study precipitate a shell of biogenic carbonate (aragonite) that can be used as a proxy for a variety of water quality parameters such as temperature, pH, and salinity.[22−34] Carbon, oxygen, and strontium isotopes in carbonates can be used to reconstruct water conditions, trace environmental contaminants, and


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observe seasonal cycles using a variety of analytical techniques.[23,24,26,35−44] Changes in shell composition with subdaily levels of resolution are possible in some samples.[32,33,45−49]

In freshwater mussel species, metal (Mg, Mn, Ba, and Sr) incorporation into shells[23,28,50−52] is thought to be controlled by different processes, depending on the metal of interest. For example, $Mg/Ca_{shell}$ ratios are thought to be influenced by stress factors such as high TDS, changes in water quality,[53,54] the presence of organic material, or incorporation of noncarbonate minerals.[47,55] Changes in $Mn/Ca_{shell}$ ratios are thought to be controlled by phytoplankton productivity,[23] a mussel food source, while $Ba/Ca_{shell}$ ratios have been found to be relatively constant across the shell, with intermittent spikes[33,34,56,57] that may be the result of incorporation of Ba-rich particles into inclusions of the shell. Barium-rich particles, such as clays, are rejected by the mollusk to the extrapallial fluid (EPF) and may subsequently precipitate into new shell material.[33,34,52,56] Of primary interest to this study is strontium, which has been studied frequently in bivalves.[47,54,58−60] In freshwater systems, biological processes likely regulate the incorporation of Sr into shell material[22,61] and measurement of the $Sr/Ca_{shell}$ strongly correlates with the $Sr/Ca_{water}$.[52] Strontium is found in high concentrations (>1 g/L) in O&G wastewaters, and isotopic ratios of strontium ($^{87}Sr/^{86}Sr$) often distinguish the source of the Sr,[4,13,62] including the formation in which that wastewater originated (e.g., Marcellus Formation).

Here, we analyze shells of freshwater mussel species *Elliptio dilatata* and *Elliptio complanata* collected in the Allegheny River approximately 0.5−1 km downstream of an NPDES-permitted facility for evidence of accumulation of strontium from O&G wastewater. *Elliptio dilatata* and *Elliptio complanata* are present over a wide geographical area and may therefore prove to be useful biomonitors in other oil and gas basins (Figure 1). The Allegheny River is home to 49 species of freshwater mussel, many of which are endangered.[63] For comparison, we also collected shells ∼100 m upstream of the facility and from two Pennsylvania Rivers with no history of O&G wastewater disposal through NPDES permits, the Juniata and Delaware Rivers. We hypothesize that changes in water quality associated with the disposal of high volumes of Marcellus O&G wastewater in 2008−2011, observed in previous studies,[4,19] are discernible in the metal and isotopic composition recorded in the shells of freshwater mussels.

## MATERIALS AND METHODS

**Sample Site Location and Information.** An NPDES-permitted facility is located in Warren, PA along the left bank of the Allegheny River (Figure 1A). The Allegheny River in Warren has an average discharge of 192 $m^3$ per second ($m^3/s$) with flood control provided by the Kinzua Dam located upstream. Under typical flow conditions the facility discharges below the water surface. Between 1995 and 2001 the facility reported total annual discharges of treated O&G wastewater between 0.17 and 7.1 ML. Then, from 2002 to 2008 the discharges rose to between 23 and 85 ML. The discharge volume more than doubled again in the period between 2009 and 2014 with a range in discharge volumes between 160 and 194 ML. Changes in concentrations, ion ratios, and isotopic ratios in the Allegheny from the O&G discharges were calculated with mixing models presented in the Supporting Information (SI).

**Field Methods.** Multiple shells of the species *Elliptio dilatata* and *Elliptio complanata*, colloquially the Northern and Eastern Spike, respectively, were collected approximately 1 km downstream of the NPDES discharge (Allegheny Down 1 and 2) in November 2015 (Figure 1A) under the direction and guidance of a Pennsylvania Natural Heritage Program biologist. This distance allowed for complete mixing of the NPDES effluent and the Allegheny River (see SI). A single shell of *Elliptio complanata* was collected ∼100 m upstream of the discharge. These species were chosen because they are classified as of "least concern" in terms of population health, and their geographic range extends throughout the entire Marcellus Shale Region of the Appalachian Basin (Figure 1). Other shale plays such as Utica, Antrim, Chattanooga, Fayetteville, and New Albany Shales[64] are also located within the geographic range of *Elliptio dilatata* and *Elliptio complanata*. The shells were empty, and the time of death was estimated to be less than 6 months prior (i.e., May−Oct 2015) as evidenced by the integrity of the periostracum and the organic portion of the hinge. Individual shells were selected based on shell integrity (minimal post-mortem dissolution) and adequate length and cross-sectional thickness. Shells of the same two species were collected from the Delaware River (Delaware) and the Juniata River (Juniata) in an effort to establish natural fluctuations in background values and assess seasonal variability. Water samples were also collected from the same locations, field filtered, and preserved with nitric acid for major cation and $^{87}Sr/^{86}Sr$ analysis. Stream sediment was also collected at the same time and location as Allegheny Down 1 and 2 shell samples.

**Sediment Leaching.** To extract Sr from sediment where the mussels live and determine the $^{87}Sr/^{86}Sr$ of pore water in equilibrium with the sediment, a simple deionized water leach was performed. Sediment samples were homogenized and then mixed in a 1:9 ratio with ultrapure (>18.2 MΩ.cm) water. The mixture was allowed to react for 5 days. Liquid aliquots were then drawn and centrifuged, diluted with ultrapure water, and acidified with 7N Omni-pure nitric acid prior to analysis.

**Sample Preparation.** Shells were physically cleaned with a brush, sonication, and ultrapure (>18.2 MΩ.cm) water to remove any sediment or soft-tissue material. Shells were then thick-sectioned from the umbo to the outer edge of the shell using an abrasive cutting wheel. Within the same shell sample, three types of sample preparation methods were used: (1) Sr/Ca ratios were collected via direct ablation along the cross sectional surface with laser ablation inductively coupled plasma mass spectrometer (LA-ICP-MS). (2) Subsamples for analyses of oxygen isotopes and $^{87}Sr/^{86}Sr$ were collected by micromilling material along the cross section, and (3) on a subset of samples (Allegheny Down 1, Juniata, and Delaware), shells were segmented with an abrasive cutting wheel for subsequent analysis of $^{87}Sr/^{86}Sr$.

**Shell Sr/Ca Profiles.** For direct ablation, thick sections of shell were mounted to the stage of an ESI NWR193 laser coupled to a Thermo Xseries II ICP-MS located at the Penn State University Energy and Environmental Sustainability Laboratories (EESL) using a soft putty to ensure stability. Shells were ablated in a continuous profile along the outermost edge of the cross section with the laser set to 50% power, traveling at 40 $\mu$m/s with a pulse repetition rate of 40 Hz and a spot size of 100 $\mu$m. A 10 s delay followed by a 15 s drawdown time were added at the start and end of each ablation period. NIST SRM 612, used as an external calibration standard, and USGS MACS-3, a pressed-powder carbonate standard, were ablated at the start of each test to calibrate and correct for instrument drift. The coefficients of variation (CV), defined as the standard deviation divided by the mean, of the Sr/Ca ratio in


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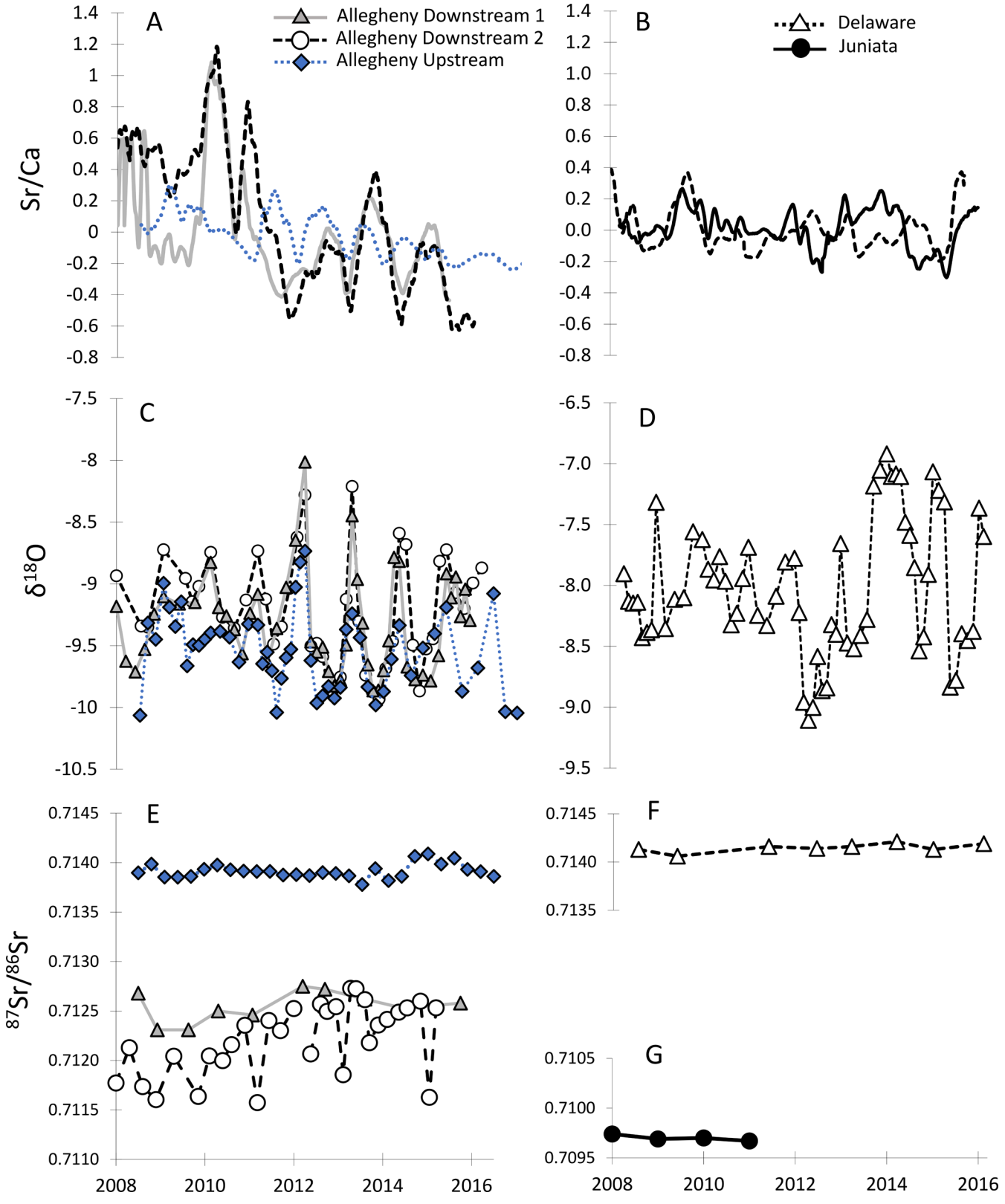


**Figure 2.** $Sr/Ca_{shell}$ plotted as the coefficient of variation from the mean for *Elliptio dilatata* and *Elliptio complanata* shells collected along the Allegheny River (A) and the Juniata and Delaware Rivers (B). Note both the larger variation in CV and trend with time (2008−2016) for shells collected downstream compared to those collected upstream or on the Juniata and Delaware Rivers. Time of shell precipitation was determined using the seasonal fluctuations in $\delta^{18}O_{shell}$ that are temperature dependent and peak to enriched values during the winter months for the three shells collected along the Allegheny River (C) and the single shell from the Delaware River (D). Note that the Juniata shell was completely consumed in analysis prior to obtaining $\delta^{18}O_{shell}$. $^{87}Sr/^{86}Sr_{shell}$ for shells collected in Allegheny River (E), Delaware River (F), and Juniata River (G). Two shells collected downstream of an NPDES-permitted facility on the Allegheny River (Allegheny Down 1 and 2) show higher $Sr/Ca_{shell}$ and lower $^{87}Sr/^{86}Sr_{shell}$ relative to the three shell samples collected from background areas either upstream of the facility (Allegheny Up) or from Rivers without any permitted



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**Figure** 2. continued

discharges of oil and gas wastewater (Juniata and Delaware). Combined, the data indicate that the NPDES discharges resulted in greater changes in $Sr/Ca_{water}$ and $^{87}Sr/^{86}Sr_{water}$ with time. Specifically, the period of greatest reported volumes of discharge (2009−2011) increased the $Sr/Ca_{shell}$ and decreased $^{87}Sr/^{86}Sr_{shell}$ in samples collected downstream. The tables of shell results are available in the SI.

the MACS-3 and NIST 612 were consistent (CV = 0.03 and 0.01, respectively) between days on which the instrument was run. The CV of standards was at least 10 times smaller than those observed in shell samples.

**Oxygen Isotope Ratios.** Shell thick sections were mounted to glass slides using epoxy so that the exposed face was directly adjacent to the ablated face sans the 0.65 mm cutting edge. The slide was mounted to the stage of an ESI New Wave Research micromill. A 300 $\mu$m diameter drill bit was used to mill sample locations spaced every 700 $\mu$m along the outer edge of the shell. Depth was adjusted to produce approximately 90 $\mu$g of shell powder from each milling. Carbonate powders were reacted with phosphoric acid at 70 °C for at least 3 h in a ThermoScientific Gas Bench II coupled to a ThermoScientific Delta Advantage at Union College, Schenectady, New York. LSVEC, NBS18, and NBS19 standards were used to perform a regression-based correction, achieving $\delta^{18}O$ precisions of <0.1‰ with precisions most commonly below 0.06‰.

**Strontium Isotopes.** Following ablation and milling, 2−4 mm subsections of the thick section were cut perpendicular to the radius of the shell. The shell subsections weighed approximately 12 mg and were digested in 700 $\mu$L of >18.2 MΩ·cm water, 200 $\mu$L of 70% Omni-pure $HNO_3$, 100 $\mu$L of 35% trace metal grade HCl, and 100 $\mu$L of 33% Omni-pure $H_2O_2$. When the reaction was no longer visibly bubbling, the vials were capped and placed on a hot plate at 40 °C for 24 h. After 24 h, the temperature was raised to 75 °C for 4 days until the shells were completely dissolved into solution. An aliquot containing approximately 400 ng of strontium was drawn, centrifuged, and separated manually using strontium spec EICHROM resin or an Elemental Scientific prepFAST-MC for isotopic analysis. Yield checks confirmed >98% Sr recovery.

$^{87}Sr/^{86}Sr$ ratios were collected on the ThermoFisher Neptune MC ICP-MS or ThermoFisher scientific Triton Plus thermal ionization mass spectrometer (TIMS) located at Penn State University EESL. NIST SRM 987 and IAPSO seawater standards, after passing through the prepFAST resin columns, were run concurrently with each set of samples. The precision of $^{87}Sr/^{86}Sr$ of the NIST SRM 987 standard during the course of this study on the Neptune was $^{87}Sr/^{86}Sr$ = 0.710260, SE = 0.000006 and on the Triton was $^{87}Sr/^{86}Sr$ = 0.710244, SE = 0.000012. The precision of $^{87}Sr/^{86}Sr$ of the IAPSO standard during the course of this study on the Neptune was $^{87}Sr/^{86}Sr$ = 0.709186, SE = 0.0000009 and on the Triton was $^{87}Sr/^{86}Sr$ = 0.709175, SE = 0.000016. Additionally, powdered carbonate standards USGS MACS-3 and USGS EN-1 were analyzed using the same Sr separation techniques as all samples (see full methods in SI) and measured on the Triton. During the course of this study, our mean $^{87}Sr/^{86}Sr$ values for both carbonate standards were within the accepted and published values. For MACS-3, it was mean = 0.707570, SE = 0.000008, SD = 0.0000045, $n$ = 6. MACS-3 has a reported value = 0.707553 ± 0.000037.[65] For EN-1, we measured mean = 0.709179, SE = 0.000008, $n$ = 2, which has an accepted value = 0.0709167 ± 0.000008.[66] Both the Triton and the Neptune were used to maximize sample throughput. Percent Relative Standard Deviation (%RSD) and 1$\sigma$ error bars for both instruments were smaller than the symbols in figures. Resin column extractions and concurrent running of standards concurrently with samples may help improve sample corrections, as the standards would show any fractionation or contamination introduced by a given set of columns but would simultaneously increase the distribution of measured values for a given standard, as each individual column extraction run may introduce unique amounts of uncertainty between runs.

## RESULTS AND DISCUSSION

**Sclerochronology.** Freshwater mussels precipitate carbonate in isotopic equilibrium with the surrounding water with a temperature dependent fractionation between the oxygen in the water molecule ($\delta^{18}O_{water}$) and the oxygen precipitated in the carbonate shell ($\delta^{18}O_{carb}$).[26,67,68] This fractionation factor can be expressed as

$$1000 \ln(\alpha) = 2.559(10^6 \times T^{-2}) + 0.715 \quad (1)$$

[24,69]

Mean oxygen isotope values $\delta^{18}O_{water}$ (reported in this paper relative to VSMOW) for surface waters in the eastern USA, including Pennsylvania, vary seasonally with average reported values ($\delta^{18}O_{water}$ = −8‰ to −10‰).[70] $\delta^{18}O_{water}$ values are lowest during the winter months and highest during warmer months,[70] but shells record the opposite trend in $\delta^{18}O_{shell}$ values.[24] Recent measurements of surface waters in portions of the Allegheny watershed in western PA ($\delta^{18}O_{water}$ = −6.4‰ to −9.4‰) and northern West Virginia ($\delta^{18}O_{water}$ = −7.2‰ to −10.6‰)[71] were within this range. Seasonal variations within the Allegheny River near our study area ranged from $\delta^{18}O_{water}$ = −6.7‰ to −10.27‰, with lower $\delta^{18}O_{water}$ values reported during the colder winter months (Jan−March) and higher $\delta^{18}O_{water}$ values reported in late summer and fall (Aug−Oct). Similar but smaller variations are observed in the Juniata River ($\delta^{18}O_{water}$ = −7.9‰ to −9.44‰).[70,72] The range in reported $\delta^{18}O_{water}$ values and the seasonal temperature variations allow for $\delta^{18}O_{shell}$ variations to be used as a proxy for changing seasons and therefore time using eq 1. Because of the temperature dependent fractionation (eq 1), the $\delta^{18}O$ value recorded in the $CaCO_3$ shell ($\delta^{18}O_{shell}$) decreases in warm summer months.[69] But in the winter, the cold temperatures result in rapidly increasing $\delta^{18}O_{shell}$ values during the winter months Dec−Feb.[67] Typically, the least negative $\delta^{18}O_{shell}$ value is measured in a narrow interval of the shell and can be used to separate yearly growth and infer age of each portion of the shell.

We used $\delta^{18}O_{shell}$ values as a proxy to assign seasonal dates to $^{87}Sr/^{86}Sr_{shell}$ and $Sr/Ca_{shell}$. On the basis of the date of shell collection, the age of the shell can be accurately determined by counting the punctuated, enriched $\delta^{18}O_{shell}$ values that represent winter months. Allegheny Down 1 and 2 were estimated to be 8 and 7 years old at the time of harvesting, respectively, covering the spring of 2008 to late fall of 2015 (Figure 2). The shell collected upstream of the discharge on the Allegheny was harvested approximately 1 year after Allegheny Down 1 and 2 but is also of the same species, general size, and shell thickness and covers the years of 2009−2016. The $\delta^{18}O_{shell}$ values from



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Table 1. Mean $Sr/Ca_{shell}$ and $^{87}Sr/^{86}Sr_{shell}$ for Samples Collected Compared to $Sr/Ca_{water}$ and $^{87}Sr/^{86}Sr_{water}$ Values of Surface Water Samples or Leachates of Sediments Collected at the Sample Locations

| sample | mean $Sr/Ca_{shell}$ (mmol/mol) | calculated $Sr/Ca_{water}$ (mmol/mol) | observed $Sr/Ca_{water}$ (mmol/mol) | measured $^{87}Sr/^{86}Sr_{shell}$ | measured $^{87}Sr/^{86}Sr_{water}$ |
|---|---|---|---|---|---|
| Allegheny Up | 0.25 | 1.6−0.9 | 2.13 | 0.713914 | 0.713983 |
| Allegheny Down 1 | 0.29 | 1.8−1.0 | 2.18 | 0.712546 | 0.713977 |
| Allegheny Down 2 | 0.25 | 1.6−0.9 | 2.18 | 0.712221 | 0.713977 |
| Delaware | 0.26 | 1.6−0.9 | 1.94[a] | 0.71415 | 0.714085 |
| Juniata | 0.52 | 3.3−1.9 | 3.70[b] | 0.70970 | 0.709716 |
| Allegheny Downstream sediment leachate | | | 2.38 | | 0.711804 |
| Allegheny Upstream sediment leachate | | | | | 0.714046 |

[a]Sample collected December 2015. [b]Sample collected November 2016.

mussels collected in the Delaware displayed roughly the same variation and estimated time period of growth (2008−2015) as observed in the shells collected in the Allegheny. The shell collected from the Juniata appears to be the youngest, only spanning 2010−2015. However, the digestion procedure for the Juniata shell consumed all of the carbonate sample, and therefore ages assigned to the Juniata are only estimates based on shell size and growth lines (Figure 2).

On the basis of the $\delta^{18}O_{shell}$ values calculated, growth rate in Allegheny Down 1 and 2 increased as the shell aged, ranging from 3.15 to 3.61 mm/year between 2008 and 2011 to 5.13−5.19 mm/year between 2012 and 2016. The slower growth recorded in the shells while the bivalves were younger is the opposite of what we would expect and may correlate to poor water quality (higher TDS and Cl) during the period of large discharge volumes of O&G wastewater (2008−2011). The lower shell growth rates corresponded to the periods of highest $Sr/Ca_{shell}$ and the period of highest discharge from the NPDES facility.

**Strontium Profiles in Shells.** The shells collected downstream of the NPDES discharge (Allegheny Down 1 and 2) exhibited two apparent differences in $Sr/Ca_{shell}$ relative to both the upstream shell (Allegheny Up) and the background samples (Juniata and Delaware). First, the downstream samples displayed higher $Sr/Ca_{shell}$ in the chronologically older portions of the shell near the umbo (i.e., when the mussel was ontogenically youngest) with a strong downward trend recorded in $Sr/Ca_{shell}$ as the mussel aged. Both of the background samples (Delaware and Juanita) and the sample collected upstream on the Allegheny (Allegheny Up) did not display any trends in $Sr/Ca_{shell}$ over the lifetime of the mussels (2008−2015/16). Second, the downstream shells display larger deviations from the mean $Sr/Ca_{shell}$ ratio compared to the shells collected from background locations (Delaware and Juanita) or upstream on the Allegheny (Allegheny Up). The coefficient of variation was used to describe intrashell variability in Sr/Ca ratios and is defined as the standard deviation normalized to the mean. The CV of Sr/Ca was 0.35 and 0.44 in the downstream Allegheny shells. The CVs of $Sr/Ca_{shell}$ were much lower in the shell collected upstream (CV = 0.14) and the shells from the Delaware and Juniata (CV = 0.13 and 0.14, respectively). The CV of $Sr/Ca_{shell}$ for the two shells downstream (CVs = 0.35−0.44) was approximately 3 times higher than the background samples (CVs = 0.13−0.14). Each of these three nonimpacted shells showed remarkably similar CVs, indicating that this is likely the natural variation in $Sr/Ca_{shell}$ through time for nonimpacted waterways. CVs of downstream shell samples were likely higher than upstream and background samples due to variable Sr/Ca ratios downstream of the effluent, rising during periods of discharge and returning toward background when the facility was not discharging. This variable behavior of the discharge was observed by Patnode et al. using salinity monitors placed downstream of the discharge.[19]

The variations in $Sr/Ca_{shell}$ observed in downstream shell samples could arise from greater changes in water temperature, shell growth rate, or $Sr/Ca_{water}$ relative to all other shell samples collected (Figure 2). A seasonal variation is apparent in all shells regardless of sample location, with higher $Sr/Ca_{shell}$ recorded during the summer and lower values during the winter. Indeed, $Sr/Ca_{shell}$ has been proposed as a temperature proxy in marine systems, but in freshwater systems seasonal fluctuations such as what we observed in $Sr/Ca_{shell}$ are more likely related to metabolic effects[52,61] and the $Sr/Ca_{water}$.[52] Additionally, fluctuations and ranges in water temperature in all three rivers are similar during a yearly cycle—likely ruling out variations in water temperature as the control on changes in $Sr/Ca_{shell}$.

The rate of shell precipitation is thought to exert significant controls on the $Sr/Ca_{shell}$, with the lowest values recorded when a mussel is young and growing rapidly and an increasing ratio recorded as the organism ages and its growth slows.[58,59] However, differences in $Sr/Ca_{shell}$ are likely not the result of differences in growth rates because all background shells exhibited relatively uniform $Sr/Ca_{shell}$ with no obvious trends over their lifetimes. Additionally, the samples collected downstream of the discharge showed the opposite trend, with the highest $Sr/Ca_{shell}$ in the ontogenically youngest portions of the shell.

Changes in the $Sr/Ca_{water}$ are likely controlling both the trend with age of the downstream mussels as well as the greater variability in $Sr/Ca_{shell}$ compared to background mussels. The $Sr/Ca_{shell}$ in freshwater mussels is generally controlled by the $Sr/Ca_{water}$ ratio of the surrounding water and a partition coefficient ($D_{Sr}$):

$$D_{Sr} = \frac{Sr_{shell}/Ca_{shell}}{Sr_{water}/Ca_{water}} \quad (2)$$

Mean $Sr/Ca_{shell}$ in samples collected downstream of the facility were 0.29 and 0.25 mmol/mol for Allegheny Down 1 and 2, respectively. While the $Sr/Ca_{shell}$ of the Delaware and Juniata shells were 0.26 mmol/mol and 0.52 mmol/mol, respectively. All shells recorded concentrations that are consistent with Sr/Ca reported in the literature for freshwater mussels.[23,52] If we assume that $D_{Sr}$ = 0.16−0.28,[52] we can calculate the range in mean $Sr/Ca_{water}$ (Table 1). We observed slightly higher $Sr/Ca_{water}$ for all rivers relative to those calculated using the $D_{Sr}$


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range of 0.16−0.28. This may indicate a slightly lower $D_{Sr}$ for *Elliptio dilatata* and *Elliptio complanata* than the freshwater species (e.g., *Lampsilis cardium*) used in previous studies.[52]

On the basis of the $Sr/Ca_{shell}$ ratio measured in the samples collected downstream of the discharge, it is most likely that O&G wastewaters with high Sr concentrations (see SI) changed the $Sr/Ca_{water}$ ratio of the Allegheny River and led to the elevated $Sr/Ca_{shell}$ in the shells collected downstream of the NPDES facility. Notably, the $Sr/Ca_{shell}$ in both samples collected downstream do not correlate with river flow rate or periods of extreme drought, conditions that could potentially increase $Sr/Ca_{water}$ and, subsequently, $Sr/Ca_{shell}$.

**Strontium Isotope Profiles.** While $Sr/Ca_{shell}$ may vary due to a wide variety of factors, changes in $^{87}Sr/^{86}Sr_{shell}$ are dependent only on the isotopic ratio of the water.[36,73,74] Strontium is a relatively high-mass element, so mass-dependent fractionation due to physical, chemical, or biological processes is very small compared to other, lighter isotopic systems.[36] Fractionation of $^{87}Sr/^{86}Sr$ occurs primarily due to ingrowth as its parent isotope $^{87}Rb$ decays, and with a half-life of 48.8 billion years, no change is detectable on the time scales considered herein. The $^{87}Sr/^{86}Sr_{shell}$ collected from the Juniata and Delaware Rivers both ranged less than 0.00009(±) over the lifetime of the organism with ratios that were consistent with the $^{87}Sr/^{86}Sr_{water}$ (Juniata = 0.70970, Delaware = 0.71415). The water and shell samples collected from the Allegheny River upstream of the discharge showed slightly greater variation of 0.00017(±), but the average $^{87}Sr/^{86}Sr_{shell}$ was consistent with the $^{87}Sr/^{86}Sr_{water}$ (Table 1). As with $Sr/Ca_{shell}$, the samples collected downstream of the NPDES discharge displayed a much larger variation in the $^{87}Sr/^{86}Sr_{shell}$ compared to all background shells. Most importantly, the $^{87}Sr/^{86}Sr_{shell}$ ratios in shells downstream of the discharge (range = 0.71150−0.71275) were lower than the upstream sample (range = 0.713779−0.714189). The CV of $^{87}Sr/^{86}Sr_{shell}$ in the downstream sample ($5.1 \times 10^{-4}$) was 5 times higher than the CV of $^{87}Sr/^{86}Sr_{shell}$ in the upstream sample ($9.9 \times 10^{-5}$).

The Marcellus Shale Formation has both a high Sr/Ca ratio (mean = 94 mmol/mol) and a relatively distinct $^{87}Sr/^{86}Sr$ isotopic signature, residing almost entirely between 0.70900 and 0.71100.[62] The reported $^{87}Sr/^{86}Sr$ ratios of NPDES effluents in 2010−2012 ranged from 0.710124 to 0.71110 (mean = 0.7106) and are consistent with the isotopic ratios of Marcellus Formation wastewaters.[62] These ratios are significantly different than values reported for local watersheds that were not impacted by NPDES permitted discharges ($^{87}Sr/^{86}Sr$, range 0.71220−0.71450, mean = 0.7132) or other sources of contamination such as acid mine drainage ($^{87}Sr/^{86}Sr$ = 0.714474−0.714549).[4] Importantly, the background $^{87}Sr/^{86}Sr_{water}$ ratios agree with our $^{87}Sr/^{86}Sr_{water}$ (0.713983) measured on the Allegheny upstream of the discharge. Additionally, based on the age and lithology of surface rock exposures in the majority of the Appalachian Basin, $^{87}Sr/^{86}Sr$ ratios should range from 0.713 to 0.720,[75] which is also the expected range of streams in the geologic region.[4,36,62,73,74,76−79]

The portions of the mussels with the highest $Sr/Ca_{shell}$ in Allegheny Down 1 and 2 were also the portions with the lowest $^{87}Sr/^{86}Sr_{shell}$. Combined, this provides further evidence that O&G waste effluent from the NPDES facility containing high strontium concentrations with lower $^{87}Sr/^{86}Sr$ (Table 1 SI)[62] altered the Allegheny $Sr/Ca_{water}$ and lowered the $^{87}Sr/^{86}Sr_{water}$ from background values of 0.713983 to values between 0.711500 and 0.712750. Additionally, the $^{87}Sr/^{86}Sr$ measured in the leachate of a sediment sample collected concurrently with Allegheny Down 1 was 0.711804, while the surface water collected at the same time measured 0.713977. That suggests that Sr discharged from the facility has precipitated out with sediment that has accumulated and persisted downstream of the facility. Previous studies indicated Marcellus Sr/Ca and $^{87}Sr/^{86}Sr$ signatures up to 19 km downstream of oil and gas treatment facilities, more than 4 years after Marcellus waste was reportedly discharged. *Elliptio dilatata* and *Elliptio complanata* are generally sessile and interact extensively with the sediment in which they burrow and anchor themselves.[80] The apparent discrepancy of $^{87}Sr/^{86}Sr_{shell}$ and $^{87}Sr/^{86}Sr_{water}$ in shells collected downstream of the facility is explained by sediment with a persistent signature of Marcellus oil and gas wastewater because a portion of the $^{87}Sr/^{86}Sr_{shell}$ is sourced from sediment porewater.

**Environmental Significance.** We observed elevated $Sr/Ca_{shell}$ and lower $^{87}Sr/^{86}Sr_{shell}$ of freshwater mussels *Elliptio dilatata* and *Elliptio complanata* collected from the Allegheny River downstream of a NPDES permitted O&G wastewater discharge compared to $Sr/Ca_{shell}$ and $^{87}Sr/^{86}Sr_{shell}$ values recorded upstream of the facility. The observed shift in ratios recorded in the shells collected downstream was most significant from 2009 to 2011, which corresponds to periods of elevated unconventional O&G discharge from the NPDES facility (2009−2011). No such variations in either the $Sr/Ca_{shell}$ or the $^{87}Sr/^{86}Sr_{shell}$ were observed upstream or in rivers with similar hydrologic systems (Juniata and Delaware) that do not have NPDES permitted O&G wastewater discharge. The elevated Sr in the *Elliptio dilatata* and *Elliptio complanata* shells likely indicates incorporation of Sr that is sourced in part from O&G wastewater that was discharged to the Allegheny River. To our knowledge, this represents the first indication of bioaccumulation of unconventional O&G wastewater downstream of surface water disposal facilities.

Following a decrease in Marcellus wastewater treated at the facility (post-2011), an overall trend of decreasing $Sr/Ca_{shell}$ and increasing $^{87}Sr/^{86}Sr_{shell}$ is recorded from 2011 to 2015 in shells collected downstream of the facility, while the shells collected upstream or from streams without O&G discharge facilities show no corresponding temporal trends. The observed changes in the shells likely reflect the decreased volume of Marcellus O&G wastewater released to the Allegheny River during this time period (2012−2015/16) compared to 2009−2011. Combined, our data indicate that changes in water quality from O&G discharges are recorded in the shells of freshwater mussels that live downstream.

$^{87}Sr/^{86}Sr_{water}$ in surface waters throughout the Appalachian Basin are significantly different than the measured ratios in effluent from facilities that accept and treat O&G wastes and reported values for waste fluids associated with O&G formations. The difference in $^{87}Sr/^{86}Sr$ indicates this tool could be utilized throughout the Appalachian Basin and perhaps over the entire range of *Elliptio dilatata* and *Elliptio complanata* (Figure 1) to trace O&G wastewater disposal in other areas. Future work will evaluate surface waters downstream of other O&G facilities for changes in $Sr/Ca_{shell}$ and $^{87}Sr/^{86}Sr_{shell}$ in freshwater mussel shells to confirm the spatial and temporal scale of O&G wastewater impacts on water quality.



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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b02727.

Figures, methods, and oil and gas signatures (PDF)
Allegheny SrCa, $\delta^{18}O$, and $^{87}Sr/^{86}Sr$ tables (XLSX)

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### Notes

The authors declare no competing financial interest.

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