Title:

Changes to nitrate isotopic composition of wastewater treatment effluent and rivers after

upgrades to tertiary treatment in the Narragansett Bay watershed, RI

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ABSTRACT

Due to nitrogen load reduction policies, wastewater treatment facilities (WWTFs) have upgraded to tertiary treatment - where denitrification reduces and removes nitrogen. Changes to the stable isotopic composition of nitrate inputs after upgrades or how it transfers to the estuary have not been assessed in Rhode Island. We investigate whether these upgrades impact the isotopic signature of nitrate inputs to Narragansett Bay. Samples from rivers and WWTFs discharging to Narragansett Bay characterize the anthropogenic source nitrate (NO_3^-) isotopic composition ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) and temporal variability. At one WWTF, tertiary treatment increased effluent nitrate $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values by ~16‰. Riverine values increased by ~4‰, likely due to the combination of decreases in N and upgrades. Combined river and WWTF flux-weighted isotopic compositions showed enriched values and an amplitude reduction in monthly variability. When seasonal isotopic means are significantly different from other sources, $\delta^{15}N$-NO_3^- may be a useful tracer of inputs.

GRAPHICAL ABSTRACT & HIGHLIGHTS

Included as separate files

INTRODUCTION

To reduce the negative effects of anthropogenic nitrogen (N) loads, namely eutrophication and hypoxia, some wastewater treatment facilities (WWTFs) include nitrogen removal in their treatment schemes (USEPA 2004; RIDEM 2005). During processing, wastewater is initially strained to remove solids and grease/oil from water to be treated. Next, secondary treatment converts dissolved organic matter to inorganic N and then oxidizes ammonium (NH_4^+) to nitrate (NO_3^-), a microbial process called nitrification. The newest step, tertiary treatment, reduces NO_3^-, through microbially-mediated denitrification, to the largely biologically unavailable N_2 gas. Finally, wastewater is disinfected and released. The entire process takes about 1 day (USEPA 2004).

Addition of tertiary treatment has a dramatic effect on the final composition of wastewater. The Narragansett Bay Commission (NBC), the company that owns two of the largest WWTF that discharge into Narragansett Bay, RI, upgraded their facilities to tertiary treatment, starting in 2006. As of 2012, NBC reported total nitrogen in wastewater inflow to one of their treatment facilities to be composed of 61 % NH_4^+ and 1 % NO_3^-, with the remainder organic N. The final effluent nitrogen composition is 77 % NO_3^-, 6 % NH_4^+, and 17 % organic N. Moreover, total N is reduced by 75 % (NBC 2012). During our study period, NBC targeted a monthly average N load of 607 µM N (8.5 ppm) (NBC 2012). At the beginning of our study, in 2009, NBC used tertiary treatment at one of their plants, and by August 2012, the other plant had come online (achieving their post-upgrade targeted permit level of 5 ppm from May to October). In

this study, we focus on the NO_3^- component of WWTF effluent because it is the single largest pool entering the Narragansett Bay ecosystem.

Most of the freshwater input to Narragansett Bay, including both WWTF effluent and river water, enters in the northern reaches through the Providence River and Mount Hope Bay (Fig 1). The major N inputs to Narragansett Bay have been identified, and ~60% of total N input is anthropogenic in origin with rivers and wastewater treatment facilities contributing the majority of the anthropogenic N (Nixon et al. 1995; Nixon et al. 2008; Krumholz 2012). As of 2014, tertiary treatment upgrades were in place at many facilities in the Narragansett Bay watershed, including the three largest facilities (Field's Point, Worcester, and Bucklin), with more planned in the near future (Fig. S1). Decreases in N sources to Narragansett Bay were observed for the period of 2006-2010, where wastewater treatment facility N contributions fell by approximately 20 % (Krumholz 2012) due to implementation of tertiary treatment.

To assess the impact of nitrogen to an estuarine system, stable N isotopes ($^{15}N:^{14}N$, $\delta^{15}N$) are often combined with concentration and flux measurements to aid in distinguishing sources (Jordan et al. 1997; Tucker et al. 1999; Costanzo et al. 2001; Cole et al. 2004; Savage 2005). The $\delta^{15}N$ value of NO_3^- reflects the $\delta^{15}N$ value of its source and any transformations to which it was subject. Published values of $\delta^{15}N$-NO_3^- in anthropogenic N from secondary sewage treatment plants, septic system leachate, and rivers range from -3 to +40 ‰ (average >10 ‰) while marine (near shore) water values range from +3 to +6 ‰ (average 5 ‰) (Table S1) (Heaton 1986; Jordan et al. 1997; Costanzo et al. 2001; Cole et al. 2004; Chaves 2004; Pardo et al. 2004; Schlacher et al. 2005; DiMilla 2006; Deutsch et al. 2006; Dahnke et al. 2008; Deutsch et

al. 2009). The overlap in $\delta^{15}N$ decreases the certainty with which stable N isotopes can be used to uniquely identify anthropogenic discharges. Measurement of the oxygen isotopic composition ($\delta^{18}O$, ‰) of NO_3^- is typically coupled to the $\delta^{15}N$ measurement, enhancing the ability to distinguish nitrogen sources and nutrient processing pathways (Wassenaar 1995; Sigman et al. 2001; Mayer et al. 2002; Casciotti et al. 2002; Deutsch et al. 2005; Anisfeld et al. 2007; Saccon et al. 2013; Wexler et al. 2014).

Treatment of wastewater likely imparts variation in $\delta^{15}N$ and $\delta^{18}O$ values due to fractionation during nitrification (secondary treatment) and denitrification (tertiary treatment). Field work in fresh and salt water demonstrates that fractionation during consumption of nitrate through denitrification (and assimilation) leads to an increase in both $\delta^{18}O$ and $\delta^{15}N$ at a ratio of 0.5-1 depending on the study (Kendall 1998; Lehmann et al. 2003; Sigman et al. 2005; Anisfeld et al. 2007; and Wexler et al. 2014). Experimental work with laboratory cultures leads to an isotopic increase of equal magnitude within nitrate (1:1 $\delta^{18}O:\delta^{15}N$ change) across a range of measured isotope effects (+5 to +25 ‰) (Granger et al. 2004; Granger et al. 2008). Since tertiary treatment nitrogen removal is due to denitrification, both the $\delta^{15}N$ and $\delta^{18}O$ values of the NO_3^- in final WWTF effluent are expected to increase. However, nitrification, occurring in the second stage of treatment, the production of NO_3^- from NH_4^+, has isotope effect ranging from +15 to +30 ‰ (Casciotti et al. 2010a) and is likely to also contribute to the isotopic variation in WWTF effluent. Given a large, persistent NH_4^+ pool, the NO_3^- produced is likely to be isotopically depleted relative to the original DIN. During nitrification, the addition of oxygen atoms to the NO_2^- and NO_3^- molecules tends to produce a $\delta^{18}O$ of nitrate value resembling that of the water in which the nitrate was

created, with only a small fractionation (Casciotti et al. 2010b). During the second step of nitrification (nitrosification), the conversion of NO_2^- to NO_3^- is reversible, and has an inverse isotope effect, where the resulting NO_3^- is isotopically heavier than the NO_2^- (Casciotti 2009; Buchwald & Casciotti 2010). The inverse isotope effect imparts a signal when and where there is a significant accumulation of nitrite, as is the case in WWTFs, or during warm months at the sediment-water interface in rivers. Anammox bacteria may also be present in the treatment tanks (Sumino et al. 2006). It is difficult to predict the isotopic end result of these processes given their various impacts on the N and O isotopic composition of nitrate, although the balance of the processes should tend toward increasing the isotope values. Most local WWTFs discharge to the rivers, so riverine isotopic compositions are, at least in part, controlled by processes within WWTFs.

We measured the N and O isotopic compositions of dissolved NO_3^- inputs from WWTFs (which discharge directly to the bay) and rivers monthly during 2009-2010 and 2012-2013 to assess the impact of anthropogenic N source reductions on the nitrate isotopic contributions to Narragansett Bay. During the study period, the largest two WWTFs (Worcester and Field's Point; Fig. S1) in the watershed began using tertiary treatment. Our focus was to document temporal variability in the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate, both with and without tertiary treatment at the WWTFs, and to quantify the net impact of the change in treatment schemes. Finally, we evaluate the potential for using stable isotopes as a tracer of nitrogen source in highly dynamic, urban estuaries.

METHODS

Narragansett Bay and Watershed

Narragansett Bay, including Mount Hope and Greenwich Bays and the Providence River Estuary, is 328 km^2 and has a mean depth of 8.3 m (Fig. 1) (Pilson 1985). River input is relatively low (around 100 m^{3}s^{-1}, or 7.56×10^6 m^{3}d^{-1}) and most of the input occurs in the urbanized northern reaches (Fig. 1). Other freshwater sources, such as storm water and groundwater, are relatively small portions of the Narragansett Bay water budget, but are important to water budgets of smaller bays and coves, like Greenwich Bay (Spaulding 1987; Nixon et al. 1995).

The Blackstone, Pawtuxet, Ten Mile and Taunton Rivers (the rivers sampled for this study) are the largest rivers that discharge to the bay, in terms of flow, contributing a total of 5.79×10^6 m^3 d^{-1} (2009-2010) and 3.31×10^6 m^3 d^{-1} (2012-2013) with peak flow in the spring and winter (Table S2). Major known or potential nitrate sources to the rivers include WWTFs, stormwater runoff, rainfall, ground water and soil-based nitrate, and fertilizer run-off (Table S1). Less than 5 % of the land use for the Narragansett Bay watershed is considered agricultural, suggesting fertilizer runoff is only a small source of nitrate to the rivers (NBEP 2009). Additionally, a model of nitrogen loading to Narragansett Bay showed that fertilizer was a small source of N to the bay from the rivers (Vadeboncoeur et al. 2010). The majority of the land is either forested or residential, therefore, soil-based nitrate and WWTF discharge are probably the largest sources of nitrate (NBEP 2009; Krumholz 2012).

Within the watershed, a total of twenty-nine WWTFs discharge to the bay and its tributaries. Nineteen of these WWTFs discharge directly into the sampled rivers (Table S3). Three of the largest discharge directly to Narragansett Bay in the northern reaches

(Field's Point, East Providence and Bucklin), and one discharges directly to Mount Hope Bay (Fall River) (Fig. 1; Table S2) (Nixon et al. 2008; Krumholz 2012). These four treatment facilities do not discharge into the rivers we sampled.

Sample Collection

Water samples were collected from the riverine sources and four wastewater treatment facilities, 5-7 times from March 2009 to January 2010 (referred to as 2009), and 10-12 times from February 2012 to January 2013 (referred to as 2012). The river samples were collected from the last gauged point or upstream of the last dam using a bucket lowered from a bridge into the rivers. Final, 24-hour composite effluent samples from the WWTF were collected at the outflow pipe. The Narragansett Bay Commission (NBC, in 2009-2010 and 2012-2013), the City of East Providence through United Water, (2009-2010), and the City of Fall River, through Veolia Water (2012-2013) cooperated in the sampling. All samples were filtered using glass fiber filters, and either acidified with hydrochloric acid to a pH 2 or frozen and stored at -20°C until analysis.

Laboratory Analysis

Samples were analyzed on a Lachat QuickChem 2000 flow injection autoanalyzer using EPA method 353.4 (Grasshoff 1976; USEPA 1997) for NO_{3+2}, and NO_2^- at the University of Rhode Island (URI) or at NBC and Veolia Water (through subcontractor Premier Laboratory), and have a minimum detection limit of 0.05 µM for NO_3^- and a precision of 0.02 µM.

N and O isotope compositions were determined using the denitrifier method (Sigman et al. 2001; Casciotti et al. 2002) by gas chromatography-isotope ratio mass spectrometry. Stable isotope ratios are reported as the ratio of $^{15}N/^{14}N$ and $^{18}O/^{16}O$

between the sample and a standard, and are expressed as $\delta^{15}N$ or $\delta^{18}O$ where $\delta =$ [($R_{sample}/R_{standard}$) − 1] x 1000 and R = $^{15}N/^{14}N$ or $^{18}O/^{16}O$. Samples and working standards (IAEA N3, $\delta^{15}N$ = +4.7 ‰, $\delta^{18}O$ = +25.6 ‰; USGS 32, $\delta^{15}N$ = +180 ‰, $\delta^{18}O$ = +25.7 ‰; USGS 34, $\delta^{15}N$ = -1.8 ‰, $\delta^{18}O$ = -27.9 ‰) were analyzed in the same runs to normalize delta values to accepted standards (N_2 in air and VSMOW for $\delta^{15}N$ and $\delta^{18}O$ respectively). Precision of the method is < 0.3 ‰ for $\delta^{15}N$ and < 0.5 ‰ for $\delta^{18}O$ based on the standard deviation of all standards measured during study. Average precision for field duplicates is 0.4 ‰ for $\delta^{15}N$ and 0.6 ‰ for $\delta^{18}O$.

Flux-weighting Procedure

Isotopic measurements were flux-weighted with discharge flow measurements and NO_3^- concentrations from both freshwater sources (rivers or WWTFs) to quantify the $\delta^{15}N$ and $\delta^{18}O$ of anthropogenic inputs to Narragansett Bay. The resultant flux-weighted isotopic values are presented as yearly inputs from individual sources or monthly inputs from all sources. Data were presented in this fashion to show both changes to monthly/yearly trends from inclusion of tertiary treatment, and changes to monthly or seasonal isotopic compositions for the anthropogenic sources to the estuary.

To obtain annual average flow for rivers, we used Beale's unbiased estimator which compares the days we sampled to the average for the year and corrects for bias associated with individual events by assuming that the ratio of load to flow for the days when samples were taken is equal to the average annual ratio of load to flow (Beale 1962; Dolan et al. 1981; Fulweiler 2003) (Fig. 2). We chose Beale's based on extensive use in the Narragansett Bay watershed (e.g. Nixon et al. 1995; Fulweiler and Nixon 2005, 2005a; Nixon et al. 2008; Krumholz 2012). Additionally, Beale's is the best

estimator technique for our data (Fulweiler 2003, Krumholz 2012) – flow and concentration are weakly correlated, flow data are positively skewed and not normally distributed, and sample size in any given year is small (<12 times for both years). NO_3^- fluxes and flux weighted $\delta^{15}N$ and $\delta^{18}O$ values (units are flux-per mil) were then determined using the Beale's corrected flow. These results were then summed by year for individual collection sites and divided by the total flux for the year (flux-per mil / flux = per mil). The final result was a flux-weighted $\delta^{15}N$ and $\delta^{18}O$ value.

RESULTS AND DISCUSSION

WWTF Process and Tertiary Treatment

Nitrate concentrations in sewage effluent ranged from 9-584 µM for all WWTFs. Discharge from Fall River (2012) had the lowest observed concentrations and Bucklin discharged the highest, or among the highest, concentrations overall (Fig. 3). Average nitrate concentrations decreased by about 130 µM between 2009 and 2012, and effluent nitrate flux between years decreased by about 40 % (ANCOVA, $F_{(7,46)} = 7.41$, $p = 0.03$) (Fig. 2). We expected this reduction due to the addition of tertiary treatment at Field's Point, the largest WWTF in the watershed, where we observed significant reductions in nitrate flux.

For all treatment facilities, $\delta^{15}N\text{-}NO_3^-$ values ranged from -4 to +28 ‰ and $\delta^{18}O\text{-}NO_3^-$ values ranged from -16 to +30 ‰ (Fig. 3). Generally, Field's Point had the lowest $\delta^{15}N$ and $\delta^{18}O$ values in 2009, while in 2012 Fall River had the lowest $\delta^{15}N$ values and Bucklin had the lowest $\delta^{18}O$ values. During 2012, Field's Point generally had the

highest $\delta^{15}N$ and $\delta^{18}O$ values. No temporal pattern was evident in WWTF nutrient concentrations or isotopic compositions (Fig. 3).

Riverine nitrate concentrations ranged from 45-200 µM, with lower values occurring during warmer months (Fig. 4). In 2009, the Taunton River had the lowest concentrations, while, generally, the Blackstone River had the lowest concentrations in 2012. Average riverine nitrate flux significantly decreased by 35 % between years (ANCOVA, $F_{(7,67)}$ = 7.44, p = 0.01) (Fig. 2). Nitrate flux also significantly decreased between cooler months and warmer months (ANCOVA, $F_{(7,67)}$ = 5.06, p = 0.003) (Fig. 2).

The reduction in flux could be from a decrease in discharge from the treatment facilities along the rivers, or a decrease in river discharge. Comparing the Discharge Monitoring Reports (http://cfpub.epa.gov/dmr/) from the facilities discharging to the individual rivers, nitrogen discharge from the facilities was lower; however flow was not reported on all Discharge Monitoring Reports (Table S3; Krumholz 2012). Therefore, it is not clear if a decrease in WWTF flux affected the rivers. River discharge between 2009 and 2012 decreased significantly. The average river flow in 2009 was 1.45 10^6 m^3 d^{-1} and in 2012 was 0.83 10^6 m^3 d^{-1} (Table S2), representing a 42 % drop in discharge. Additionally, NOAA recorded ~13 inches less of rain in 2012 than 2009 (accessed through NOAA's "Climate at a Glance" database, www.ncdc.noaa.gov/cag). The change in nitrate loading to Narragansett Bay is most likely due to the change in river discharge resulting from lower precipitation despite lower contributions from WWTFs.

Riverine isotopic compositions ranged from +4 to +20 ‰ and -5 to +12 ‰ for N and O, respectively (Fig. 4). The $\delta^{15}N$ values increased through the warm months and

decrease through the cool months for both years, while the $\delta^{18}O$ values stayed roughly the same throughout both years (Fig. 4). When comparing between the two sampling periods, the Blackstone and Ten Mile Rivers also showed an increase in $\delta^{15}N$ and $\delta^{18}O$ values of ~ 4 ‰ in 2012 relative to 2009 (Table 1; flux-weighted, p = 0.01). The Pawtuxet and Taunton Rivers showed no statistically significant change (Fig. 4).

Between 2009 and 2012, the flux-weighted average of all WWTF final effluent N and O isotopes increased by ~7-8 ‰ (Table 1; t-test, p = 0.01). Given that only two of the treatment facilities were sampled in both years (Bucklin and Field's Point), we compared their flux-weighted averages, and found that the final effluent N and O isotopes increased by ~7-8 ‰ as well (Table 1; t-test, p = 0.01). At Field's Point, on average, WWTF effluent increased flux-weighted nitrate $\delta^{15}N$ and $\delta^{18}O$ by ~16-17 ‰ (Table 1; t-test, p < 0.005). The 1:1 increase in $\delta^{18}O$:$\delta^{15}N$ is consistent with the fractionation during denitrification and tertiary treatment (Granger et al. 2008).

Field's Point WWTF has the largest impact on the isotopic composition of WWTF effluent on the estuary. If Field's Point was removed from the annual average flux-weighed $\delta^{15}N$ and $\delta^{18}O$ for nitrate discharging from all WWTFs, the difference between 2009 and 2012 in $\delta^{15}N$ is minimal (~0.4 ‰) and in $\delta^{18}O$ is slight (~2 ‰). Even looking at just Bucklin WWTF (the only treatment facility to be monitored both years if we remove Field's Point), the changes in $\delta^{15}N$ and $\delta^{18}O$ for nitrate are similar (~0.7 ‰ and ~1.8 ‰, respectively). This indicates that tertiary treatment added at Field's Point significantly changed the N and O isotopic composition of the overall WWTF inputs to Narragansett Bay. The small but significant shift in $\delta^{18}O$ values likely stem from a change in the isotopic composition of the source water, rather than a processing effect. The ultimate

source of $\delta^{18}O$-NO_3^- is $\delta^{18}O$ of water ($\delta^{18}O$-H_2O) which changes with evaporation and precipitation patterns (Gat 1996; Sharp 2007), and is likely to be different between years. Bowen (2014) shows that the range of $\delta^{18}O$-H_2O for the region is over 7 ‰, so the difference of 2 ‰ between years is well within that that range. Moreover, given the large reduction in overall river flow and precipitation, this shift is likely caused by weather pattern changes between the years.

In the rivers, the inter-annual increase in $\delta^{15}N$ and $\delta^{18}O$ (0-4‰ depending upon the river) is likely due to a combination of factors. Nitrogen discharge from WWTFs discharging to the rivers was lower in 2012 (Discharge Monitoring Reports, see above), either the result of improved treatment reductions at the plants or a decrease of N coming into the plants prior to treatment. If N decreased because of treatment, then the isotopic composition of N and O may have increased due to increased removal through denitrification or assimilation within the tanks. Given the reduction in precipitation between 2009 and 2012, the input to the treatment facilities may have decreased, decreasing the amount of N being treated. The reduction of precipitation would most likely lead to an increase in $\delta^{18}O$-NO_3^- (Gat 1996; Sharp 2007), while the $\delta^{15}N$-NO_3^- would be more affected by processing. It's quite possible that these changes worked together to account for some of the 4 ‰ increase noted between 2009 and 2012.

Also of note is the timing of upgrades to WWTFs which discharge to the rivers. Individually, the Pawtuxet and Taunton Rivers showed no statistically significant change in isotopic composition during the study period, while the Blackstone and Ten Mile Rivers did. All plants discharging to the Pawtuxet River upgraded well before our study (2004-2006), while no plants have upgraded on the Taunton River (Krumholz 2012).

The lack of change during the study period may account for consistency of the isotopes, even with the reductions in N noted above. On the Blackstone River, most treatment facilities required to upgrade did so by 2006 (Krumholz 2012). However, the second largest facility in the watershed and largest to discharge to a river (Worcester), upgraded during our study at the end of 2009 (Krumholz 2012). On the Ten Mile River, one plant upgraded in 2008 (Krumholz 2012). These plants may have still been adjusting their methodology, achieving better N reduction techniques during our study period. These upgrades may have contributed to the changes in the N and O isotopic compositions of the rivers during our study period.

Individually, the majority of the upgraded plants are small, and add very little nitrate to the overall riverine inventory. However, the cumulative WWTF loading is a large source of nitrate to the river (Nixon et al. 2008; Krumholz 2012). Therefore, upgrades to tertiary treatment across the watershed could decrease the nitrate flux and impact the isotopes of nitrate. Additionally, non-point sources (such as runoff, groundwater, precipitation, and atmospheric deposition) may, post-upgrades, be considered larger pieces of the nitrate budget. Since the majority of the WWTF upgraded before our study, the number of plants which could have affected the isotopes is limited, however upgrades to tertiary treatment impart a large increase in the isotopic composition of the effluent, and, so we assert that the upgrades to the Worcester plant likely played a role in the observed isotopic changes in the rivers.

The slopes of $\delta^{18}O:\delta^{15}N$ for Bucklin and Field's Point WWTF and river data ranged from 0.5 to 1.7, suggesting that multiple processes – assimilation, nitrification, and denitrification – occur within the tanks, both pre- and post-upgrades, and in the

rivers (Fig. 5). During our study period, Field's Point upgraded to tertiary treatment. Dividing their data to pre- and post-upgrades (after Aug. 2012), the slope of the regression decreases substantially – from 1.7 to 0.5 (Fig. 5a). The slope of the pre-upgrade Field's Point data (1.7; Fig. 5a) is quite large. It implies that for every 1 ‰ increase in $\delta^{18}O$, the increase in $\delta^{15}N$ is less than 1 ‰. During the pre-upgrade portion of our study, Field's Point was using secondary treatment (nitrification). During nitrification, NO_3^- is produced from NH_4^+, and given the large, persistent pool of NH_4^+, the resultant NO_3^- is most likely going to be isotopically depleted. This would reduce the $\delta^{15}N$ of the NO_3^- while not affecting the $\delta^{18}O$ as significantly. The oxygen atoms in NO_3^- come from the water in which the nitrate was created, imparting a $\delta^{18}O$-NO_3^- similar to $\delta^{18}O$-H_2O (Casciotti et al. 2010b). This cycling could be present during the treatment process, particularly during the upgrades when the methodology was not set. The alteration of the method would have likely changed the ratio of O and N isotopic variation. Interestingly, when the Bucklin data are separated by year, the slope of the regression varies from 1 in 2009 to 0.5 in 2012 (not shown). This demonstrates a significant amount of variability in the $\delta^{18}O$:$\delta^{15}N$ between years, post upgrades (i.e. under similar operating conditions), and implies that the relative contributions from the range of processes within the plants is highly variable.

Rivers host nitrate assimilation and the uptake and incorporation of nitrate also results in a 1:1 $\delta^{18}O$:$\delta^{15}N$ increase (Mayer et al. 2002; Granger et al. 2004; Granger et al. 2008). This is supported by the slight seasonal trend in the $\delta^{15}N$-NO_3^- data. In both 2009 and 2012, a decrease in nitrate concentrations accompanied an increase in $\delta^{15}N$-NO_3^- during the warm months (May-October) (Fig. 4). In the rivers, it is harder to

attribute the observed 1:1 increases in $\delta^{18}O:\delta^{15}N$ to denitrification. Denitrification typically occurs in the sediments, which does not impart a significant isotope effect on the overlying water column due to the relatively complete use of the nitrate within sediment (Mariotti et al 1988; Brandes and Devol 2002). Therefore, seasonal increases in assimilation in the rivers may also play a role in setting the 1:1 of N and O isotopic (Fig. 5).

Changes to $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-

Diel changes within the rivers may add to the monthly variability observed (Gammons et al. 2011; Nimich et al. 2001). The variations should be small because the isotopes integrate all processes that affect a water parcel and do not reflect just the current process. While diel changes were not measured in the study, and samples were taken at approximately the same time each sample day, we cannot rule out diel changes in river properties, such as availability of nitrogen species and dissolved oxygen, to be part of the cause of wide variation in both $\delta^{15}N$ and $\delta^{18}O$ noted in this study.

Seasonally, nitrogen cycle processes, and discharge from WWTFs and rivers can change. Wastewater treatment facilities in Rhode Island are required to treat wastewater to the "maximum feasible extent", which includes the use of tertiary treatment when applicable and available (RIDEM 2005). The seasonal changes in N flux from the discharge are noted (Fig. 2); however, no seasonal trends are apparent in the isotopic time series data for Bucklin or Field's Point WWTF (Fig. 6).

Riverine $\delta^{15}N$ values displayed a temporally variable pattern with peaks in the late spring/early summer of both years (Fig. 4). Discharge rates decrease from cooler

to warmer months, and coincide with a decrease in NO_3^- concentration (Figs. 2 and 4). The increase in $\delta^{15}N$ and decrease in NO_3^- suggest that the rivers witness greater nitrate assimilation in warmer months compared with cooler months, as denitrification is unlikely to be a major contributor (see above; Mariotti et al 1988; Brandes and Devol 2002; Fig. 6). Assimilation should cause $\delta^{18}O$ values to increase as well (Granger et al. 2004; Granger et al. 2008), but seasonal trends in $\delta^{18}O$ data show no change or even a slight decrease from cooler months to warmer months, and maxima in the late spring (Figs. 4 and 6). Seasonal changes in precipitation can change $\delta^{18}O$ (Gat 1996; Sharp 2007; Bowen 2014), and the data presented here may be indicative of seasonal precipitation changes rather than processing.

Combined WWTF and Riverine Flux to Narragansett Bay

Narragansett Bay receives freshwater from both the WWTFs and rivers. The yearly pattern of N flux to the bay largely resembles those of the rivers, which was expected since the volume of WWTF discharge to Narragansett Bay is an order of magnitude less than the river flow (Fig. 2). However, the majority of the N within the rivers is sewage-derived, making the WWTFs that discharge into the Narragansett Bay watershed the largest N source (Nixon et al. 2008; Krumholz 2012). The WWTF and riverine data were flux-weighted and combined to analyze the isotopic compositions of anthropogenic NO_3^- (Fig. 2). This step was necessary to fully understand the impact of anthropogenic NO_3^- on the biogeochemistry of N in the estuary. By combining the freshwater sources, we are now able to address if and how the contributions to Narragansett Bay are different from the literature values, if they change over time, and how they might be used in mixing models.

A common assertion is that anthropogenic waste sources have high δ^{15}N values, and oceanic sources of nitrate have relatively low δ^{15}N values (Heaton 1986; Costanzo et al. 2001; Savage 2005; McClelland & Valiela 1998). On monthly timescales in 2009 this was not always true for NO_3^-. However, the combined WWTF and river flux-weighted average for 2009 was +8 ‰ (Table 1, analyzing both Field's Point and Bucklin together, as well as all the WWTFs sampled), similar to the canonical anthropogenic δ^{15}N signal of values >8 ‰ (Heaton 1986; Kendall 1998; Mayer et al. 2002). In 2012, on monthly timescales, the combined anthropogenic flux-weighted data ranges from +11 to +15 ‰ (Fig. 2), with an average of +13-14 ‰ (Table 1, analyzing both Field's Point and Bucklin together, as well as all the WWTFs sampled). These data suggest that mixing models that rely solely on flux-weighted yearly averages for seasonal outputs could overestimate (in the winter, when isotopic compositions may be lower) or underestimate (in the summer, when isotopic compositions are higher) the relative importance of WWTFs and rivers as a source of nitrate. This is especially true of the 2009 data (Table 2). During this time period, tertiary treatment had not been added to the largest treatment facilities, and seasonal changes in the isotopic compositions of WWTFs and rivers are evident with a standard deviation of 2-3 ‰ (Table 2). In 2012 riverine δ^{15}N values remained steady throughout the year, while WWTFs (all, and Bucklin and Field's Point isolated) varied about 3-4 ‰. The steady values of the rivers meant that the combined isotopic composition of the sources remained steady as well (Table 2). This may be due to lower river flows rather than a nitrogen cycle change, and, in this case, the yearly flux-weighted average and seasonal flux-weighted average yield the same result. Seasonality and overall flow rates (from rivers or WWTFs) need

to be considered when using anthropogenic isotopic values in mixing models. For example, if these values are being used to trace the ultimate source of nutrients to organic matter in sediment, essentially a time-integrated signal, then the annual means are likely appropriate. On the other hand, determining the source of nutrients to specific phytoplankton bloom would have required a more temporally limited evaluation of the nitrate sources, at least in 2009. Finally, mixing models using $\delta^{15}N$ as a tracer of anthropogenic influence require not only that NO_3^- sources to a system must be isotopically distinct but that the stable N isotope compositions must remain conserved. The increase in the flux-weighted average between years suggests the addition of tertiary treatment may make $\delta^{15}N$ a more robust tracer of nitrogen source, however, the conservation of the source $\delta^{15}N$ has yet to be rigorously tested in Narragansett Bay. Because there is actually less nitrogen carrying the anthropogenic isotopic signature as a result of tertiary treatment and nitrogen is still limiting in much of Narragansett Bay, it seems likely that the signal may not be conserved at significant distances from the sources.

CONCLUSION

In conclusion, our data show a wide range in both $\delta^{15}N$ and $\delta^{18}O$ values for riverine and WWTF final effluent nitrate discharging to the Providence River-Narragansett Bay system, consistent with work in other locations (Table S1). The large range of $\delta^{15}N$ and $\delta^{18}O$ values is the result of the multiple processes occurring in WWTFs and rivers.

An increase in $\delta^{15}N$ and $\delta^{18}O$ values of the nitrate inputs is associated with the overall decrease in nitrate flux resulting from the stimulation of denitrification as part of tertiary treatment in the facilities draining into the Narragansett Bay watershed. Results from a single plant initiating tertiary treatment suggest that the impact on WWTF effluent $\delta^{15}N$ and $\delta^{18}O$ is quite large (~16-17 ‰) (Table 1) and imply that WWTF upgrades are likely responsible for the $\delta^{15}N$ and $\delta^{18}O$ increases observed in the rivers as well. The 2012 flux-weighted averages suggest that overall anthropogenic N discharges contribute nitrate with high $\delta^{15}N$ values (~13 ‰; Table 1), with little seasonal variation (Table 2), supporting the use of anthropogenic N discharges in mixing models.

Tertiary treatment is an important step to reducing point source N pollution. It also increases the stable isotopic signature of NO_3^- to levels where $\delta^{15}N$-NO_3^- may be a better tracer of N source. The results of this study can be compared to other estuaries experiencing the same changes to wastewater treatment. These changes may improve N source distinction within estuarine N budgets, and reduce the isotopic range of anthropogenic N discharges in mixing models. Additionally, as point source N is reduced, non-point source N (stormwater runoff, atmospheric deposition, and agricultural runoff) may become a larger piece of estuarine N budgets, and may be able to be traced through changes to stable isotopes within receiving waters.

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FIGURE CAPTIONS

Figure 1. Narragansett Bay riverine and wastewater treatment facility (WWTF) collection map. Rivers are marked by circles, and WWTFs are marked by triangles. Sampled WWTFS do not discharge into the sampled rivers.

Figure 2. Top Panel: Flux (thousands moles N per day) from all WWTFs (open triangles), only Bucklin and Field's Point (filled triangles), all rivers (open circles), and all WWTFs and rivers combined (filled diamonds). Removal of East Providence and Fall River WWTFs did not change the combined flux. Bottom Panel: Average flux-weighted $\delta^{15}N$-NO_3^- (black) and $\delta^{18}O$-NO_3^- (gray) for all WWTFs (open triangles), Bucklin and Field's Point only (filled triangles), all rivers (circles), and all WWTFs and rivers combined (diamonds). Removal of East Providence and Fall River WWTFs did not change the combined flux-weight isotopes. March 2012 data for WWTFs were removed during the quality control process, and therefore not included in combined flux calculations.

Figure 3. Wastewater effluent [NO_3^-] (top), $\delta^{15}N$-NO_3^- (middle) and $\delta^{18}O$-NO_3^- (bottom) versus collection day. Bucklin (filled diamond), Field's Point (filled square), and East Providence (open circle) were sampled in 2009, while Bucklin, Field's Point, and Fall River (open diamond) were sampled in 2012. March 2012 data from Field's Point and Fall River were removed during the quality control process, as was May 2012 data from Fall River, resulting in breaks in the dataset.

Figure 4. Riverine [NO_3^-] (top), $\delta^{15}N$-NO_3^- (middle), and $\delta^{18}O$-NO_3^- (bottom) plotted against collection day (month/day/year). River samples are from the Blackstone (open diamond), Pawtuxet (filled triangle), Taunton Rivers (filled square), and Ten Mile (open circles).
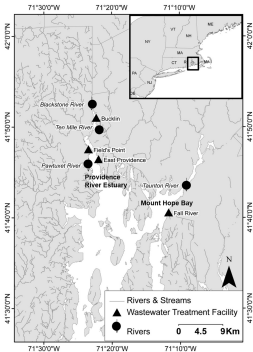
Figure 5. A: Bucklin and Field's Point WWTF $\delta^{18}O$-NO_3^- plotted against $\delta^{15}N$-NO_3^-. Bucklin WWTF 2009 and 2012 data are plotted together (filled diamonds). Field's Point data are divided by pre-upgrade (open squares), and post-upgrades (filled squares). B: Riverine data plotted by river name and year (2009: open symbols; 2012: filled symbols). C: Both riverine and Bucklin and Field's Point WWTF data plotted for 2009 (open symbols) and 2012 (filled symbols). For A and C reduced major axis (model II) linear regressions were performed (A: Bucklin: thin solid line; Field's Point pre-upgrade: dotted line; Field's Point post-upgrade: thick solid line, Field's Point all data: dashed line; C: WWTF: solid line, River: dashed line).
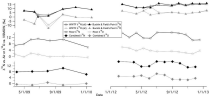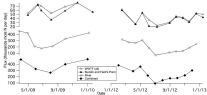
Figure 6. Bucklin and Field's Point WWTF (top) and Riverine (bottom) $\delta^{18}O$-NO_3^- plotted against $\delta^{15}N$-NO_3^- as a function of month of the year.
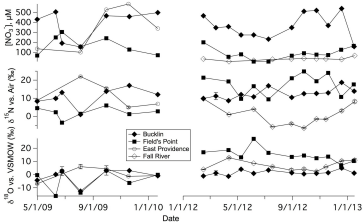

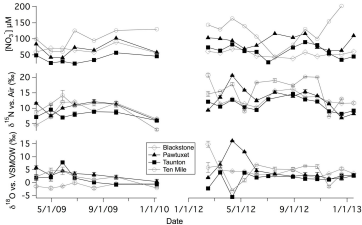SUPPLEMENTARY INFORMATION

Major NO_3^- sources to rivers and estuaries with their ranges in isotopic composition;

river and wastewater treatment facility yearly flow data; and a timeline of upgrades to

tertiary treatment by local wastewater treatment facilities and when sampling for this

manuscript occurred. This material is available free of charge via Marine Pollution

Bulletin's website.

Blackstone River

Bucklin

Ten Mile River

Field's Point

Pawtuxet River

East Providence

Providence River Estuary

Taunton River

Mount Hope Bay

Fall River

Rivers & Streams
▲ Wastewater Treatment Facility
● Rivers

0   4.5   9 Km

N

Figure axis labels: [NO₃⁻], μM; δ¹⁵O vs. VSMOW (‰); δ¹⁵N vs. Air (‰); Date.

Legend: Bucklin, Field's Point, East Providence, Fall River.

Figure showing time series of [NO₃⁻], δ¹⁵N, and δ¹⁸O measurements versus date for four sites.

Y-axis labels (top to bottom):
- $[NO_3^-]$ (μM)
- $\delta^{15}N$ vs. Air (‰)
- $\delta^{18}O$ vs. VSMOW (‰)

X-axis label: Date

Legend:
- Blackstone (○)
- Pawtuxet (●)
- Taunton (■)
- Ten Mile (+)

X-axis tick labels: 5/1/09, 9/1/09, 1/1/10, 1/1/12, 5/1/12, 9/1/12, 1/1/13

A

$\delta^{15}N$ AIR (‰)

$\delta^{13}C$ PDB (‰)

B

$\delta^{15}N$ AIR (‰)

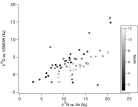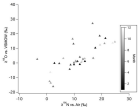$\delta^{13}C$ PDB (‰)

C

$\delta^{15}N$ AIR (‰)

$\delta^{13}C$ PDB (‰)

Table 1. Annual average flux-weighted $\delta^{15}N$ and $\delta^{18}O$ values for nitrate discharging from all WWTFs, rivers, and combined WWTF and river sources. "Average" includes Bucklin and Field's Point data, while "Average (all)" includes all WWTF data presented in Figure 3 for either 2009 or 2012. "Combined" includes Bucklin and Field's Point WWTF data combined with river data, while "Combined (all)" includes all data presented in Figures 3 and 4 for either 2009 or 2012. "--" means no data available.

	2009		2012		Difference	
	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
WWTFs						
Bucklin	+12.0	-0.2	+12.6	+1.6	+0.6	+1.8
Field's Point	+1.5	-4.6	+17.8	+13.1	+16.3	+17.7
East Providence	+9.4	+0.1	--	--	--	--
Fall River	--	--	+2.5	+7.4	--	--
Average	*+6.9*	*-2.3*	*+14.4*	*+5.5*	*+7.5*	*+7.8*
Average (all)	*+7.1*	*-2.1*	*+13.8*	*-5.6*	*+6.7*	*+7.7*
Rivers	+8.5	+0.5	+12.7	+4.0	+4.2	+3.5
Combined	+8.1	-0.5	+13.3	+4.2	+5.1	+4.7
Combined (all)	+8.2	-0.5	+13.1	+3.9	+4.9	+4.4

Table 2. Seasonal average flux-weighted δ^{15}N for nitrate discharging from Bucklin and Field's Point WWTFs, rivers, and combined WWTF (Bucklin and Field's Point only) and river sources. "WWTFs (all)" refers to seasonal average flux-weight δ^{15}N for all data included in Figure 3. "Combined (all)" refers to seasonal average flux-weight δ^{15}N for all data included in Figure 3s and 4. Riverine flux was calculated using Beale's unbiased estimator (see "Flux-weighting Procedure"). St. Dev. is the standard deviation for all seasons for that source and year.

Season	2009 δ^{15}N (‰)	2012 δ^{15}N (‰)	2009 δ^{15}N (‰)	2012 δ^{15}N (‰)
Bucklin & Field's Point			*WWTFs (all)*	
Winter	9.0	15.2	9.0	15.2
Spring	7.4	11.2	7.5	11.2
Summer	2.9	13.8	4.6	11.9
Fall	10.8	15.6	9.2	14.7
St. Dev.	*3.4*	*2.1*	*2.1*	*2.0*
Rivers				
Winter	5.9	13.0		
Spring	7.9	12.9		
Summer	10.6	13.4		
Fall	10.8	12.7		
St. Dev.	*2.3*	*0.3*		
Combined			*Combined (all)*	
Winter	6.3	13.4	6.3	13.4
Spring	7.9	12.7	7.9	12.7
Summer	8.4	13.3	8.3	13.5
Fall	10.5	13.1	10.6	13.3
St. Dev.	*1.8*	*0.3*	*1.8*	*0.4*

Legend:
- Wastewater Treatment Facilities
- Rivers

Axis labels:
- x-axis: $\delta^{15}N\text{-}NO_3^-$
- y-axis: $\delta^{18}O\text{-}NO_3^-$

Implementation of 3° treatment