ELEVATED δ^{15}N LINKED TO SEPTIC SYSTEM USE

COMMUNICATION: ELEVATED δ^{15}N IN STREAM BIOTA IN AREAS WITH SEPTIC TANK SYSTEMS IN AN URBAN WATERSHED, CHESTER COUNTY, PA.

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Abstract

Anthropogenic inputs of nitrogen from human sewage are a central concern in urban watershed management. However, identifying the locations of these inputs, whether from improperly functioning or ill-maintained septic tanks or from leaking sewer lines, is difficult. We used nitrogen stable isotope analysis of aquatic food webs as indicators of sewage-derived nitrogen in Valley Creek watershed. Stable nitrogen isotope analysis revealed elevated $\delta^{15}N$ in all trophic levels at stations located downstream of the divide between sewered and non-sewered (septic tanks) neighborhoods in the watershed. Stations not located in the septic system area or on the other branch of the creek did not show this elevated level of $\delta^{15}N$. Allochthonus inputs, that do not derive nitrogen from aquatic sources, such as detrital leaf material, showed no difference in $\delta^{15}N$ between Valley Creek and Little Valley Creek. Particular fish species that are found throughout both branches, such as blacknose dace and creek chub, had as much as a 10 ‰ enrichment in $\delta^{15}N$ in septic versus sewered areas. These results indicate that stable isotope analysis can be a useful tool for determining the location of inputs of human sewage and may have broad applications in watershed planning and management, especially in urban systems.

Key words: anthropogenic nitrogen sources, food webs, Pennsylvania, septic systems, stable isotope analysis, streams, urban watershed, watershed planning

Introduction

Stable isotope analysis has been used increasingly in recent years to quantify food webs (Peterson and Fry 1987, Wada et al. 1993, Lajtha and Michener 1994, O'Reilly et al. 2002) by examining the fractionation of various elements as they cycle through the biosphere. Because elements such as nitrogen and carbon fractionate in predictable ways in aquatic organisms, it is possible to construct food webs based on these data. The ratio of ^{15}N to ^{14}N is used to determine trophic position. Consumers become enriched in δ^{15}N relative to their food by 3-4 ‰, with a mean enrichment value of 3.4 ‰. This predictable isotope partitioning has been well documented in the literature (Peterson and Fry 1987, Wada et al. 1993, Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). As a result of this stepwise trophic level enrichment, nitrogen stable isotopes have become a valuable tool in food web analysis.

Comparisons using large primary consumers, which have relatively stable tissue isotope signatures because of their slower nitrogen turnover, show that δ^{15}N increases markedly with human population density (Cabana and Rasmussen 1996). This shift likely reflects the higher δ^{15}N in human sewage. There has been some debate about the mechanism behind this connection between human activity and enriched δ^{15}N. Cabana and Rasmussen (1996) reported that the empirically derived δ^{15}N value of primary consumers from aquatic systems that are dominated by anthropogenic nitrogen input was 11.0 ‰ while for pristine systems this value averaged 3.3 ‰. In one case, organisms downstream of a sewage treatment plant had lower δ^{15}N (DeBruyn and Rasmussen 2002), but this was likely due to the microbial activity during the treatment process.

In contrast, Wainright et al. (1996) documented an elevated δ^{15}N in juvenile striped bass below sewage effluent outfalls, which were not indicative of an increase in food chain length or varying trophic position of the species. They suggested the cause of this enrichment may have been sewage-derived nitrogen. Cabana and Rasmussen (1996) suggested that δ^{15}N signatures at the base of the food chain can be a useful tool in the assessment of anthropogenic nutrient inputs. Lajtha and Michener (1994) confirmed the probability of contamination from sewage being the cause of high $\delta^{15}N$ in groundwater and sediments. It follows that the enriched $\delta^{15}N$ signal will be seen in aquatic food webs where there is a sewage contamination in the groundwater, surface water and sediments. As such, nitrogen stable isotope analysis can be a useful tool for identifying anthropogenic sources of $\delta^{15}N$ as an indicator of sewage inputs into an aquatic system. However, little is known about the suitability of this application, nor the temporal or spatial scales at which stable isotope analysis of higher consumers might detect sewage inputs.

Valley Creek watershed in Chester County, PA is a 64 km^2 urbanized watershed in the suburbs of Philadelphia. The creek is composed of two main branches Valley Creek and Little Valley Creek, which converge about 5 km from the mouth of the stream before emptying into the Schuylkill River. Valley Creek watershed is unusual in that the amount of urbanization decreases downstream, with the last 3 km of stream being relatively undisturbed in Valley Forge National Historic Park. Land use in Valley Creek watershed is 41% residential by area and approximately 25% of these residential parcels are using private septic tank systems and not public sewer lines. A large majority of the septic tank use is along the Valley Creek branch (Figure 1).

Methods

Carbon and nitrogen stable isotope analysis was performed to quantify food webs at fifteen stations in the Valley Creek watershed. Samples were collected seasonally and frozen until analysis. Details of sample locations and sample collection methods can be found in Steffy (2003). Samples were dried in an oven at 65°- 70°C for 24-48 hours depending on type and size of sample. Dried samples were powdered using a mortar and pestle and a Polytron homogenizer (Brinkman PT 10/35) and stored in glass vials in a dessiccator. Powdered samples were loaded into 4 x 6 mm or 5 x 8 mm tin capsules (Elemental MicroAnalysis) and placed in 96-well plates. Replicate samples were analyzed for every fifth sample.

Stable isotopes were analyzed on a Europa Scientific continuous-flow, elemental analysis system. The system consisted of an ANCA-GSL elemental analyzer which combusted the samples at 1000°C, and separated the combustion gases chromatographically before sending them sequentially to the 20-20 stable isotope ratio mass spectrometer. Instrument precision was 0.3 ‰ for $\delta^{15}N$ isotope values (S. Wainright, pers. comm.).

Sewer maps were obtained from the two major townships within the watershed. Areas that currently use septic tanks were selected on a land parcels GIS layer using ArcView 8.1 so that the locations of septic systems could be easily mapped in relation to the sampling station locations (Figure 1).

Background levels of $\delta^{15}N$ were calculated for individual sample types by finding the mean value of each sample located in sewered areas in Little Valley Creek and Valley Creek. Enrichment values were defined as the difference between the mean $\delta^{15}N$ value for samples from septic areas and the determined background value. One-way analysis of variance (ANOVA) was used to test for significant variation in $\delta^{15}N$ from septic and sewered regions. The indicated mean and standard deviation values (Table 1) are based on the mean of the site-specific mean values. These stations were sub-categorized into one of four classifications (septic, downstream (ds) of septic, sewered Valley Creek (VC) and sewered Little Valley Creek (LVC)) and the means of each classification were compared using Student's t-test.

Results

Stable isotope analysis revealed enrichment above background values in $\delta^{15}N$ in all trophic levels in the Valley Creek watershed when sampling sites were located within the 25% of the watershed that uses septic tank systems for human sewage removal (Table 1). Primary producers sampled included macroalgae and periphyton. $\delta^{15}N$ values for macroalgae varied significantly ($p< 0.0001$) among septic and sewered stations and explained 61% of the variance. Additionally, mean $\delta^{15}N$ for macroalgae in septic sites significantly differed from those samples taken from downstream septic, sewered VC and sewered LVC sites. Macroalgae had an elevated (above background) mean value of 4.3 ‰ with a maximum $\delta^{15}N$ of 17.9 ‰. Periphyton also showed a significant variability ($p<0.0001$) between septic and sewered regions of the watershed, which explained 35% of the variation in $\delta^{15}N$. Like macroalgae, the mean values from the septic system areas were significantly different in $\delta^{15}N$ from the other three categories. Periphyton had an elevated mean value of 2.9 ‰ and a maximum value of 16.9 ‰. Allocthonous autotrophic stream inputs such as leaves that presumably do not derive their nitrogen from aquatic sources showed only a 0.86 ‰ higher mean $\delta^{15}N$ in septic areas and this was due to a very high value (9.73 ‰) at station #9. There was no significant difference in the variability ($p=0.31$) and Student's t-test revealed no significant difference in the means from any of the four regions for leaves.

Macroinvertebrates and small fish showed the highest enrichment above background values of any trophic level. Crayfish had a mean enrichment value of 4.8‰ with a maximum of 17.4 ‰, while all other macroinvertebrates showed 3.6 ‰ enrichment above background in septic areas with a maximum value of 17.7 ‰. Crayfish ($p<0.0001$) and other macroinvertebrates ($p<0.0001$) both varied significantly in $\delta^{15}N$ with type of sewage disposal accounting for 67% and 44% of the variation respectively. Small omnivorous fish such as the blacknose dace (*Rhinichthys atratulus*) and creek chub (*Semotilus atramaculatus*) make up a large percentage of the fish in the Valley Creek system. Creek chub $\delta^{15}N$ showed a significant variation ($p<0.0001$) between septic system and sewered areas, which explained 78% of the variability (Figure 2).

Creek chub were chosen to represent the general pattern seen throughout the food web (Figure 2) mainly because there were very few other potential species in this low diversity urban stream. Very few snails and no unionid mussels, which would perhaps be better indicators, were present in the stream system. Also, creek chub was the only species found widely throughout the watershed. Creek chub had an average enrichment of 5.1 ‰ and a maximum value of 20.9 ‰ while blacknose dace showed a 4.2 ‰ higher mean $\delta^{15}N$ in septic areas with a maximum value of 20.7‰. Blacknose dace also had a significant ($p<0.0001$) difference in $\delta^{15}N$ stations located in sewered or septic system areas, accounting for 77% of the variability, indicating that the $\delta^{15}N$ enrichment effect was not confined to creek chub.

Larger fish in the Valley Creek system, such as white sucker, *Catostomus commersoni*, and brown trout, *Salmo trutta*, had similar mean enrichment in $\delta^{15}N$ at 2.9 ‰ and 3.2 ‰ respectively and maximum values of 17.1 ‰ and 16.1 ‰. Both species also showed significant ($p<0.0001$) differences in $\delta^{15}N$ values between the sewered and septic system locations, which also explained 77% and 80% of the total variability.

Each consumer trophic level differed from the autotrophs in that the means for the septic system areas and the downstream septic stations were significantly different from the sewered areas in both the Valley Creek and Little Valley Creek branch. All maximum values were samples collected at station #9, which is the first station downstream of the septic/sewered divide except for brown trout which had a maximum $\delta^{15}N$ value at the station #4.

Discussion

In all observed food webs in Valley Creek, $\delta^{15}N$ increased with trophic level as expected (Table 1). However, in septic regions the $\delta^{15}N$ values at the base of the food web were much higher than in sewered areas. At the upstream septic stations (#8 and #9) the macroalgae, periphyton and aquatic macrophytes had a $\delta^{15}N$ signature that was higher than the primary and secondary consumers (data not shown). This indicates some degree of temporal variation in nitrogen supply, since autotrophs have a much higher turnover rate for nitrogen and can more rapidly respond to changes in supply. We recognize the importance of time averaging in using stable isotopes to quantify food webs (O'Reilly et al. 2002). However, that kind of detailed temporal data was not included in this more general application summary.

Station #9 appeared to have the greatest input of anthropogenic ^{15}N and perhaps at this location there were not only septic leakages into the creek but over land flow as well that could explain the higher $\delta^{15}N$ in the leaves of the trees bordering the stream at that station. The enrichment in $\delta^{15}N$ revealed by stable isotope analyses in septic system areas within the Valley Creek watershed does not signify that the fish are occupying a higher trophic level in these regions. By looking at the entire food web at each station it is obvious that where there are septic systems being used, the whole food web at these locations is shifted upwards in $\delta^{15}N$. No new species were added or deleted and no other physical differences were observed between these stations.

The very high $\delta^{15}N$ signal at the station #9 decreased downstream but some enrichment was still evident in samples taken at the mouth of the watershed (10 km away). This downstream decrease is not a clear dilution because two other stations (# 8 and # 4) are also located in septic areas (Figure 2). It is difficult to separate what might be coming from upstream and what is coming in from the septic systems surrounding these other stations. Land parcels in the entire catchment of the other branch of the stream, Little Valley Creek, are connected to public sewers and elevation in $\delta^{15}N$ is not evident at any trophic level. Furthermore, the upstream stations on the Valley Creek branch, in sewered areas did not show any $\delta^{15}N$ enrichment. It should be noted that the most upstream station in Valley Creek (#13) is located in an area of septic use; however this station was dry for a majority of this study so no samples were collected. Also of interest, station 14 (Figure 2) shows a slight enrichment in $\delta^{15}N$ but it is not in a septic system area. This station is located in the middle of an old landfill and is partially fed by a tributary receiving discharge from a local, sewage treatment plant.

The two branches of Valley Creek watershed are generally similar except for the high amount of septic tank usage in the Valley Creek catchment. Land use and geology are nearly identical for both branches. Many of the land parcels in these areas are large lot residential plots interspersed with some commercial properties. Background $\delta^{15}N$ values for primary producers were slightly higher than what has been reported for pristine streams. This is likely a function of widespread urbanization throughout the watershed. Cabana and Rasmussen (1996) also found elevated $\delta^{15}N$ at the base of food webs with anthropogenic inputs. The link between elevated $\delta^{15}N$ in the food web and areas of the Valley Creek watershed that use septic tank systems is quite clear. Stable isotope analysis has proved to be a valuable tool in locating and evaluating potential sources of sewage contamination into a stream system.

Implications

This research provides evidence of the applicability of stable isotope analysis as an indicator of anthropogenically based inputs of excess N in aquatic systems. There was a marked difference in the $\delta^{15}N$ at all trophic levels in food webs in Valley Creek watershed between areas that used septic tank systems and those areas that were connected to public sewers. Stream sites located in areas of septic systems had higher $\delta^{15}N$ values in each trophic level by as much as 10 ‰. We conclude that improperly functioning septic systems in these regions are contributing large amounts of N carrying the ^{15}N signature of human sewage. This type of stable isotope analysis can be a very useful tool in studies of the effects of urbanization on watersheds. The implications of this research for watershed managers and urban planners are great, pointing to the importance of making informed decisions about sewage disposal and the upkeep of private septic tank systems. Numerous watershed groups, both large and small, indicate on their websites that the prevention of public health problems by maintaining septic systems is a major goal of their management plans. However, it is difficult to achieve this goal without an efficient and cost-effective way to locate the potential source of contamination. This study offers a viable option for addressing this problem as well as providing scientists with a valuable tool for quantifying the impact of human activity on N cycling and food webs in urban ecosystems.

Acknowledgements

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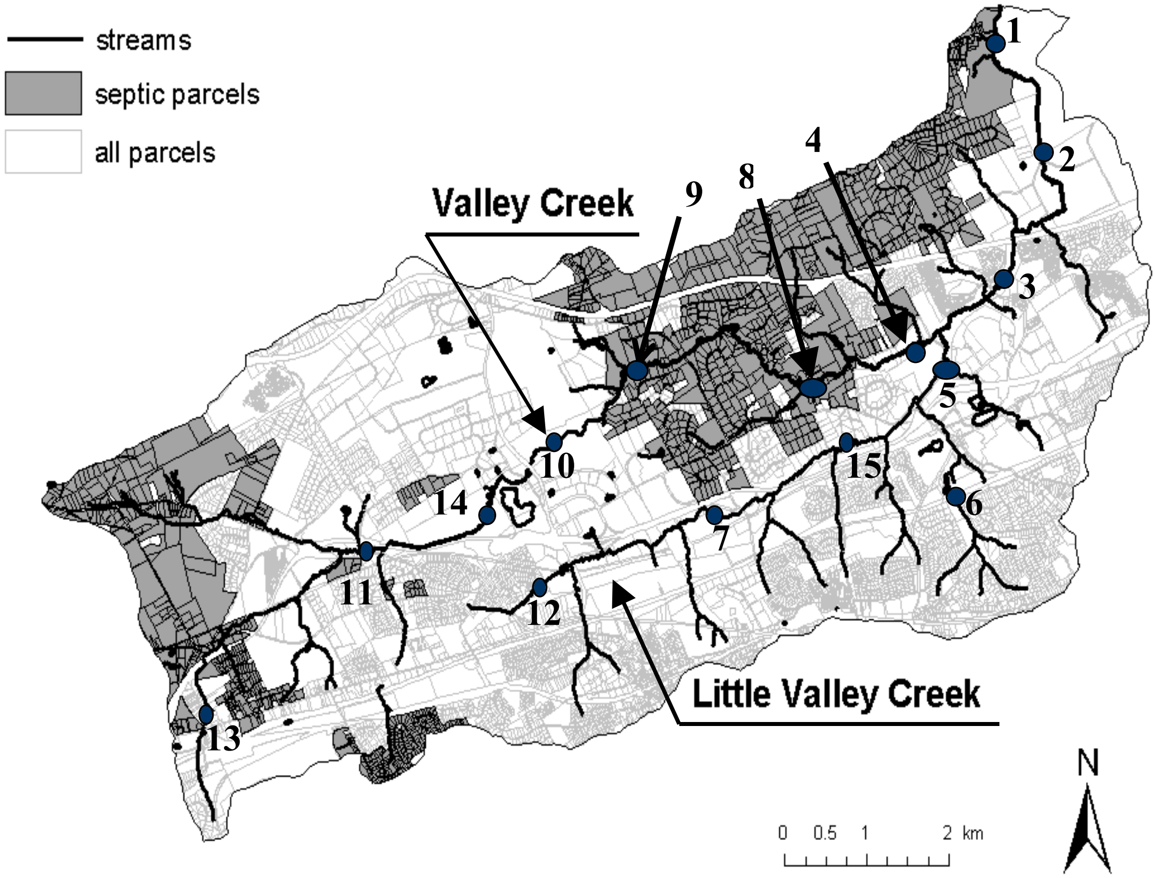
Table 1. Mean δ ^{15}N values, [± one standard deviation shown] for stations located in septic (including downstream stations; stations 9, 8, 4, 3, 2, and 1) and sewered (stations 11, 10, 12, 7, 15, 6 and 5) areas of Valley Creek Watershed, Chester County, PA.

Sample	**SEPTIC**	**SEWERED**	
	δ ^{15}N (‰)	**δ ^{15}N (‰)**	**δ ^{15}N enrichment**
Leaves	4.5 ± 1.7 (n=8)	3.7 ± 1.1 (n=13)	0.8
Periphyton	9.6 ± 4.1 (n=31)	6.7 ± 1.5 (n=33)	2.9
Macroalgae	12.3 ± 5.2 (n=21)	6.7 ± 0.9 (n=21)	4.3
Macroinvertebrates	10.5 ± 3.5 (n=43)	7.1 ± 0.8 (n=62)	3.6
Crayfish Cambaridae	12.7 ± 2.7 (n=23)	7.9 ± 0.9 (n=42)	4.8
Blacknose dace *Rhinichthys atratulus*	15.3 ± 3.0 (n=30)	11.1 ± 0.5 (n=32)	4.2
Creek chub *Semotilus atramaculatus*	16.3 ± 3.1 (n=13)	11.2 ± 1.1 (n=31)	5.1
White sucker *Catostomus commersoni*	14.2 ± 2.1 (n=25)	11.3 ± 0.9 (n=17)	2.9
Brown trout *Salmo trutta*	14.3 ± 1.6 (n=18)	11.1 ± 1.2 (n=22)	3.2

Figure legends

Figure 1: Map of Valley Creek watershed indicating areas of septic system use. Numbers designate the fifteen sampling locations.

Figure 2: Elevated $\delta^{15}N$ signal in creek chub (*Semotilus atramaculatus*) in Valley Creek watershed. Stations are arranged from upstream to downstream in the Little Valley Creek (LVC) branch and the Valley Creek (VC) branch. The two branches join just upstream of station 3.


streams
septic parcels
all parcels
Valley Creek
Little Valley Creek
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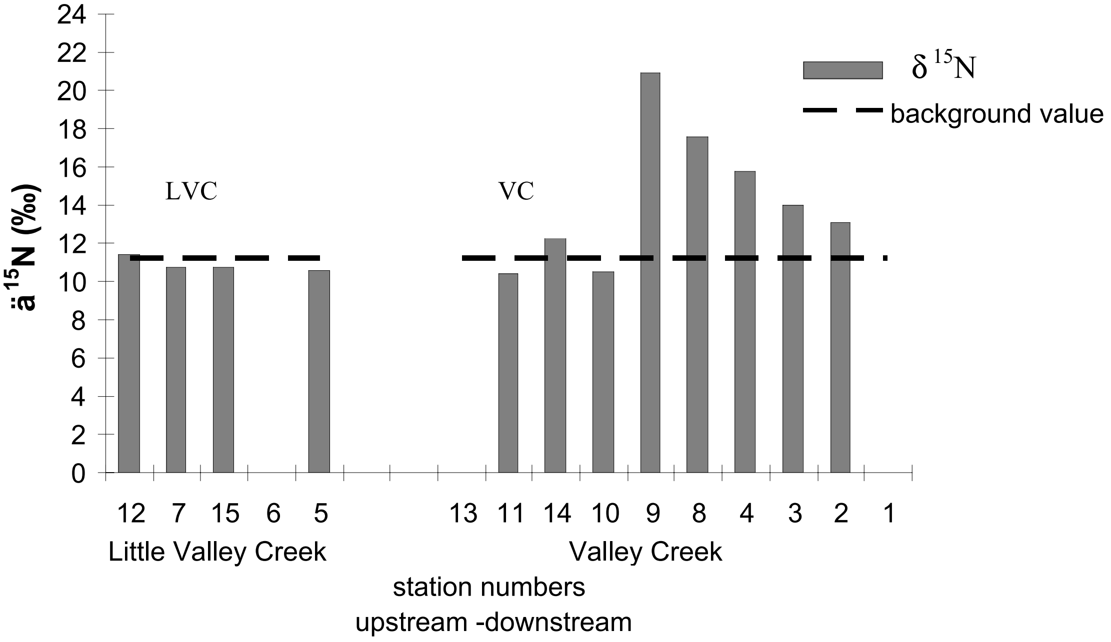
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Little Valley Creek
Valley Creek
station numbers
upstream -downstream