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# $^{15}N$ enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon (*Salmo salar*)

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**Abstract**

Nitrogen isotopes were used to study the source of nitrate and its uptake into the food web of a northeastern river in Vermont, USA. In six tributaries of the White River (Vermont) nitrate concentrations were elevated in streams flowing through areas with agricultural land use as compared to streams flowing through pristine forested areas. We observed a strong positive correlation between $\delta^{15}N$ values of stream water nitrate and percent of agricultural land-use within a given catchment. Agricultural sites had relatively high $\delta^{15}N$ values of nitrate (+7.3‰) compared to forested sites (+2.0‰). These relatively high $\delta^{15}N$ values coupled with relatively high concentrations of nitrate in agricultural streams suggest the introduction of $^{15}N$-enriched nitrate draining from agricultural areas. Additionally, elevated $\delta^{15}N$ values of algae, aquatic insects, and Atlantic salmon (*Salmo salar*) in agricultural sites compared to lower $\delta^{15}N$ values of their counterparts in forested sites suggest the uptake of nitrate from agricultural sources by stream organisms. We observe a similar positive correlation between the $\delta^{15}N$ of Atlantic salmon and the percent of agricultural land in a catchment. The nitrogen isotope values of both Atlantic salmon and water nitrate reflect land-use. Thus, nitrogen isotope ratios in salmon, especially when coupled with strontium isotope ratios (which reflect catchment geology), create unique isotopic identities for Atlantic salmon stocking streams and thereby provide a means of determining the natal origin of juvenile salmon. 

*Keywords:* Nitrogen isotopes; Strontium isotopes; Water pollution; Food web; Salmon, Vermont

## 1. Introduction

Nitrogen isotopes can be used to distinguish between natural and anthropogenic sources of nitrate. $\delta^{15}N$ is defined as $\delta^{15}N‰$ (in per mil) = {[($^{15}N/^{14}N$) sample / ($^{15}N/^{14}N$) standard] − 1} × $10^3$, where the standard is atmospheric nitrogen. $\delta^{15}N$ values of nitrate (relative to atmospheric $N_2$) of commercial fertilizer typically range from −2.5 to +2.0‰; organic soil nitrate ranges from −2 to +9‰, and human and animal waste range from +10 to +20‰ (Kreitler and Jones, 1975; Heaton, 1986; Chapelle, 1993; Kendall et al., 1995, 1996). These $\delta^{15}N$ ranges,

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however, are not rigorously defined because isotopic fractionation may occur both in groundwater and in soil (see Macko and Ostrom, 1994 for a review). For example, denitrification in soil and groundwater (Schearer et al., 1974; Bottcher et al., 1990; Aravena et al., 1993); volatile loss of ammonia from manure (Kreitler, 1979); and uptake of nitrate by microbes or algae (Estep and Vigg, 1985) can cause significant isotopic fractionation; which complicates the tracing of nitrate sources with $\delta^{15}N$ values. Despite the complication of isotopic fractionation during the nitrogen cycle, numerous studies have shown that nitrogen isotopes of nitrate in water do, in part, reflect their source (Kohl et al., 1971; Kreitler and Jones, 1975; Gormly and Spaulding, 1979; Kendall et al., 1995). Nitrogen isotopes are now routinely used to monitor pollution of nitrate in groundwaters. A recent study, for example, has shown that $\delta^{15}N$ values of nitrate in groundwater reflects different land-use patterns (Komor and Anderson, 1993).

The purpose of this study was to determine whether nitrogen isotopes vary in a systematic fashion in a watershed that includes both pristine forested and agricultural catchments. Our study focused on the White River (Vermont) and its tributaries because Atlantic salmon (*Salmo salar*) fry are stocked annually in the tributaries of the White River. We were interested in determining whether the nitrogen isotopes of the salmon in these streams reflected the different land-use patterns. Because salmon fry are stocked in tributaries that range from forested to agricultural catchments we suspected that introduction of nitrate from agricultural run-off would influence the nitrogen isotopes of salmon.

Nitrogen isotopes of fish tissues should indirectly reflect the natural variations on nitrogen isotopes of nitrate which is incorporated into the food web. Previous stable isotope research shows that nitrogen has been used effectively to evaluate food web structure, initially in laboratory experiments and later in natural environments. Animals raised on diets with known nitrogen compositions were found to prefer $^{15}N$ over $^{14}N$, producing protein that is enriched in $^{15}N$ relative to the food source (Steele and Daniel, 1978; DeNiro and Epstein, 1981; Minagawa and Wada, 1984). This increased amount of $^{15}N$ results in an approximately 3‰ enrichment in $\delta^{15}N$ values at each trophic level (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). As a result, numerous studies have shown that nitrogen isotopes can be used to trace food web structures in aquatic ecosystems (Estep and Vigg, 1985; Fry, 1988, 1991; Hesslein et al., 1991; Gu et al., 1994; Doucett et al., 1996; Keough et al., 1996). Although these studies have provided a wealth of information on food webs within individual streams and rivers there have been few studies that provide a comparative analysis of freshwater streams in catchments with different land-use patterns. Rounick and Winterbourn (1986) and Rounick et al. (1982) are among the few who have addressed this issue, using stable isotopes to establish changes in stream food webs following forest removal.

In this paper, we expand on these studies and show that nitrogen isotopes of nitrate in stream waters in the White River system vary depending upon land-use patterns. These isotopic variations are reflected in the food web, such that the $\delta^{15}N$ values of Atlantic salmon in agricultural catchments are significantly higher than $\delta^{15}N$ values of salmon in pristine forested catchments. We suggest that the elevated $\delta^{15}N$ of salmon from agricultural catchments reflects the uptake of nitrate at the base of the food chain which is introduced from agricultural sources. Thus, we confirm the importance of nitrogen isotope studies in assessing the uptake of pollution into the food web as previously reported (Rau et al., 1981; Van Dover et al., 1992; Kiriluk et al., 1995). Our results also have important implications with regard to tracking the natal origin of Atlantic salmon. Recently, a study in the White River system in Vermont (Kennedy et al., 1997) has shown that strontium isotopes can be used to distinguish the natal streams of Atlantic salmon. The stream-specific strontium isotope signal in Atlantic salmon bones can potentially identify a returning salmon to a natal stream and thereby assess salmon survival on a per stream basis. However, it has been shown in numerous studies that a multiple isotopic approach (Nelson et al., 1989; Meyer-Rochow et al., 1992; Koch et al., 1995; Chamberlain et al., 1997) provides a more powerful means to discriminate between local populations of animals. Herein we suggest that nitrogen isotopes, like strontium (Kennedy et al., 1997) isotopes can be used to trace natal origin of Atlantic salmon.

## 2. Methods

### 2.1. Study area

Water, plant, and animal samples were collected from second and third order tributaries of the White River and the White River mainstem (a fourth order river), Vermont from August–November, 1995 and September–November, 1996. Fig. 1 shows the location of sampled streams. Streams were chosen on the basis of two criteria: (1) watershed land use and (2) established salmon stocking and censusing locations. Patterns of land use were established using an accuracy-checked GIS land use/land cover data layer (Vermont Center for Geographic Information, 1997) produced from LANDSAT thematic mapper imagery. Maps were made from 1991 to 1993 images produced at a scale of 1:24,000 and a resolution of $25 \times 25$ m cells. Land cover data were collected within a 400 m riparian buffer strip that included all of the watershed area upstream of collection sites within 200 m of either stream bank. Land cover images were classified according to 12 categories, however 8 categories comprised more than 99% of each catchment's areal coverage. Combining the three agricultural categories (row crop, hay farming, and pasture) and the three forested categories (deciduous, coniferous, and mixed forest), accounted for 82–91% of the areal coverage at each site. Two additional categories that were resolved in all watersheds but did not differ significantly between sites were water coverage ($\sim$ 9%) and road coverage ($\sim$ 5%)

The Peavine site is along the mainstem of the White River and adjoins a small dairy farm with open feedlots and manure-fertilized cornfields. Upstream of the Peavine site are additional cornfields and non-sewered residential areas with septic tanks. The First Branch site is a third order tributary flowing through a non-sewered, leach-field residential area with houses located on the lowest river terraces. Agriculture and dairy farms with open feedlot areas are interspersed throughout upstream riparian zones of the First Branch collection site. The Third Branch site is a third order tributary located 0.1 km down-

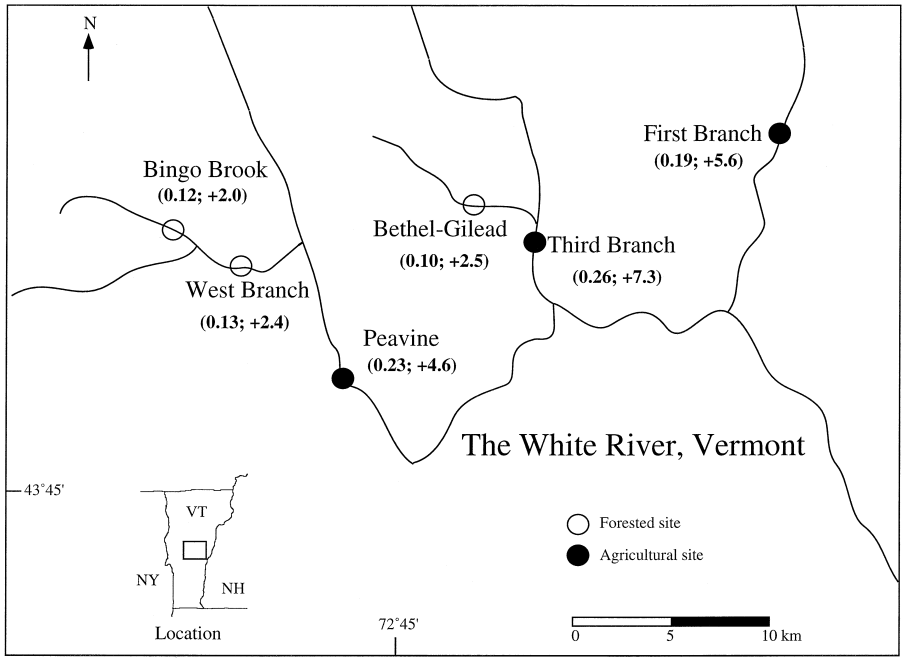


Fig. 1. Map of the study area in Vermont, northeastern United States, showing sampling locations in the White River drainage (Bingo Brook, West Branch, Bethel-Gilead, Peavine, First Branch, and Third Branch). In parentheses are the nitrate concentrations (first number) and $\delta^{15}$N values of nitrate (‰) from Table 2.

stream of an active farm with open feedlots and adjoins corn fields fertilized with manure. The West Branch site is a third order tributary located in a current residential and former cornfield area but is not downstream of present or former farms. Residences along the West Branch are non-sewered, leach-field but located more than 0.2 km away from the stream. The Bingo Brook site is a second order stream located in the Green Mountain National Forest and away from residences. The Bethel-Gilead site is a third order stream located in a forested area with forest interspersed with some non-sewered, leach-field residential and few light agricultural areas upstream.

### 2.2. Collection and preparation of samples

All plant and animal samples were placed on ice in the field and then stored frozen. Samples were thawed in the laboratory and dried in an oven at 65°C. Juvenile Atlantic salmon (*S. salar*) were electroshocked and collected in the field. Fish were weighed wet and measured, a sample of bone-free muscle tissue removed (left filet posterior to the pectoral fin), and dried. We collected four insects present at all sites that included a range of feeding habits: *Perlidae Paragnetina* sp. (predator); *Peltoperlida Tallaperla* sp. (shredder/detritivore); *Pteronarcyidae Pteronarcys* sp. (omnivore); and *Heptageniidae Stenonema* sp. (herbivore/detritivore). Insects were identified, dried, weighed dry and then used whole, or homogenized and used in part. In 1996, only algae identified as *Cladophora* were collected and dried in bulk and then homogenized. In 1995, algae were collected as a filtrate from scrubbed rocks. In both years, decomposing leaf packs were collected from the sides of the streams and from rocks in the stream riffle zones. A representative of 20 leaves were removed from the collection, washed to remove soil and insects, and dried and ground to a fine powder in a Wiley Mill with a #40 sieve.

We collected stream water samples from the center of each stream in a 5 gal LDPE carboy with no headspace. Prior to sample collection the carboy had been rinsed 3 times with deionized water and then rinsed once with stream water. We filtered the sample in our laboratory within 2 h of collection to alleviate biological isotopic fractionation of water nitrate. Water was filtered through several assemblies of 0.45 mm pore diameter Gelman membrane filters in glass funnel supports and 1 l flasks connected to a vacuum pump. Nitrate concentrations of filtered water were determined with both LaChatt and LaMotte colorimeters (American Public Health Association, 1989).

Methods for preparing the nitrate in stream water for isotopic analyses were adapted from Kendall et al. (1996, 1995). To prepare adsorbed nitrate for isotopic analysis, we eluted, neutralized, and freeze-dried the nitrate in the form of $AgNO_3$ as described by Kendall et al. (1996) and Wassenaar (1995). The $AgNO_3$ powder was scraped from the freeze-drying flask and analyzed immediately or stored for later analysis. Throughout processing and storage, $AgNO_3$ was protected from light (to prevent fractionation).

It is important to determine whether the nitrate separation technique caused any isotopic fractionation, especially considering the duration of the separation time (5–10 h) due to low nitrate concentrations. To assess procedural fractionation of nitrate, we started with a pair of crushed dry $KNO_3$ (Baker Analyzed, VWR). One sample was immediately analyzed for nitrogen isotopes. The other sample was mixed with deionized water to produce 10:1 of solution with a nitrate concentration of 0.13 mg/l $NO_3$–N, in the range of our stream water samples. We then processed this standard solution exactly as we did our natural stream waters. We analyzed the resulting $AgNO_3$ (yield = 90%, with some sample visibly adhering on the sides of the flask) and compared this $\delta^{15}N$ value to that of the unprocessed $KNO_3$. The $\delta^{15}N$ values between processed and unprocessed $KNO_3$ differed by $0.23 \pm 0.17$‰ (S.E.).

### 2.3. Isotope analysis

Our sample sizes were 9–10 mg of organic matter, 2–3 mg of animal tissue, or 4–5 mg of $AgNO_3$. For nitrogen isotopic analyses we used the sealed CuO combustion method given in Velinsky et al. (1989). After combustion, nitrogen gas was collected on a 5A 1/16″ molecular sieve trap and analyzed on a Finnigan MAT delta E mass spectrometer in the Dartmouth Laboratory for Isotopic Tracers in the Environment (D-LITE).

Table 1
Replicate $\delta^{15}N$ analyses of individual samples (mean ± 1 S.E.‰) at three sampling sites: Bingo Brook, Bethel-Gilead, and Third Branch

| Material | Bingo Brook | Bethel-Gilead | Third Branch |
|---|---|---|---|
| Water nitrate | +1.83 | | +7.08 |
| | +2.20 | | +7.40 |
| | +1.89 | | +7.33 |
| | +2.13 (+2.01 ± 0.09) | | +7.28 (+7.27 ± 0.07) |
| Detritus | −0.09 | +0.24 | +1.39 |
| | −0.16 | +0.36 | +1.32 |
| | −0.48 | +0.13 | +1.48 |
| | −0.10 (−0.21 ± 0.09) | +0.13 (+0.22 ± 0.05) | +1.14 (+1.33 ± 0.07) |
| *Cladophora* | | +2.63 | +8.65 |
| | | +2.09 | +8.64 |
| | | +2.46 | +8.26 |
| | | +2.14 (+2.33 ± 0.13) | +8.52 (+8.52 ± 0.09) |
| *Pteronarcys* | +1.29 | +1.63 | +10.55 |
| | +0.96 | +1.25 | +9.96 |
| | +1.15 (+1.13 ± 0.10) | +1.68 | +10.24 |
| | | +1.41 (+1.49 ± 0.10) | +10.09 (+10.21 ± 0.13) |
| Salmon 0+ | +5.69 | +8.19 | +8.98 |
| | +5.28 | +7.93 | +8.91 |
| | +6.08 | +8.28 | +9.02 |
| | +5.52 (+5.64 ± 0.17) | +7.81 (+8.05 ± 0.11) | +8.95 (+8.96 ± 0.02) |
| Salmon 1+ | +6.98 | +8.46 | +9.74 |
| | +6.70 | +8.36 | +9.39 |
| | +6.63 | +8.22 | +9.34 |
| | +6.67 (+6.75 ± 0.08) | +8.49 (+8.38 ± 0.06) | +9.34 (+9.45 ± 0.10) |

Replicate analyses were not possible in Bethel-Gilead water nitrate (remaining $AgNO_3$ sample was exposed to light) nor Bingo Brook *Cladophora* (not enough remaining sample).

For each batch of analyses we prepared and analyzed our lab working standard $(NH_4)_2SO_4$ (Baker Analyzed, VWR). The lab standard had a mean $\delta^{15}N$ value ± 1 S.E. of −1.20 ± 0.02‰ for 49 determinations. We also analyzed nitrogen standards with $\delta^{15}N$ values determined by the Carnegie Institution's Geophysical Laboratory (using the same method as the Geophysical Laboratory). We determined a mean value ± 1 S.E. of +7.19 ± 0.06‰ for the Geophysical Laboratory's +7.2‰ $NaNO_3$ over the course of eight analyses. For gelatin with a Geophysical Laboratory value of +7.4‰, we found a mean value ± 1 S.E. of +7.41 ± 0.07‰ for five analyses.

For unknowns, we analyzed replicates of each sample material: water nitrate (after extraction), detritus, algae, insects, and both age groups of fish, at three streams spanning the range of stream types in our study. These results are reported in Table 1. For water nitrate, detritus, *Cladophora* algae, age-class 0+, and age-class 1+ salmon at Bingo Brook, Bethel-Gilead, Third Branch, standard errors ranged

Table 2
Nitrate concentrations and nitrogen isotope ratios for streamwater collected in the fall of 1996 at all sampling sites

| Tributary | Bingo Brook | West Branch | Bethel-Gilead | Peavine site | First Branch | Third Branch |
|---|---|---|---|---|---|---|
| Nitrate concentration (mg/l $NO_3$–N) | 0.12 | 0.13 | 0.10 | 0.23 | 0.19 | 0.26 |
| Nitrate $\delta^{15}N$ (‰) | 2.01 | 2.42 | 2.51 | 4.58 | 5.59 | 7.27 |
| Percent Forested | 85.7 | 80.9 | 87.4 | 75.8 | 67.0 | 68.9 |
| Percent Agricultural | 0.1 | 1.7 | 3.7 | 6.6 | 15.1 | 14.0 |

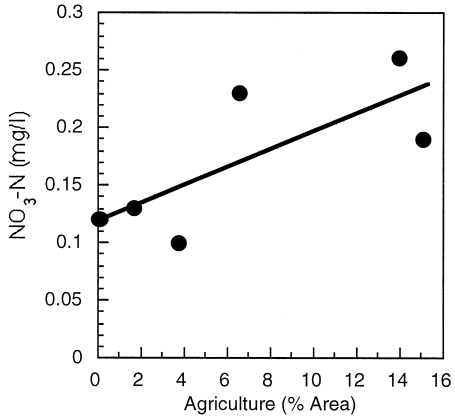


Fig. 2. Nitrate–N concentrations (mg/l) vs. percent agricultural land in catchment.

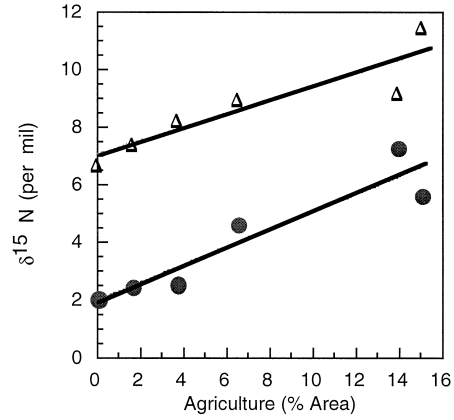


Fig. 3. Average of $\delta^{15}$N values of 0+ and 1+ age class salmon (open triangles), and of nitrate in stream water (solid circles) vs. percent agricultural land in a catchment.

from 0.02 to 0.17‰ for four replicate analyses. To assess the variance of $\delta^{15}$N values within a single individual, we performed replicate analyses of *Pteronarcys* sp. a genus with sufficiently large individuals at these three streams. *Pteronarcys* standard errors were 0.10, 0.10, and 0.13‰ at Bingo Brook, Bethel-Gilead, and Third Branch, respectively.

Strontium isotope analyses of fish backbones and stream water from each site were reported previously

Table 3
Stable nitrogen isotope ratios ($\delta^{15}$N‰) of all samples collected in 1996 with mean values (±1 S.E.) for each type of sample at each site when more than one sample was analyzed

| Material | Bingo Brook | West Branch | Bethel-Gilead | Peavine | First Branch | Third Branch |
|---|---|---|---|---|---|---|
| Water nitrate | +2.01[a] | +2.42 | +2.51 | +4.58 | +5.59 | +7.27[a] |
| Detritus | −0.21[a] | +0.19 | +0.22[a] | +4.70 | +1.91 | +1.33[a] |
| *Cladophora* | +0.89 | +1.04 | +2.33[a] | +3.86 | +3.63 | +8.52[a] |
| *Pteronarcys* | +1.13[a] | +1.98 | +1.49[a] | +4.72 | +6.43 | +10.21[a] |
| | | | | | | +10.72 |
| | | | | | | +10.22 |
| | | | | | | (+10.38 ± 0.17) |
| *Tallaperla* | +1.94 | +2.38 | +2.49 | +6.55 | +5.72 | +6.23 |
| *Stenonema* | +2.56 | +3.07 | +4.62 | +6.43 | +8.39 | +10.00 |
| | +2.43 | | | | | |
| | +2.22 | | | | | |
| | +2.27 | | | | | |
| | (+2.37 ± 0.08) | | | | | |
| *Paragentina* | +5.29 | +5.47 | +7.81 | +7.04 | +10.62 | +12.18 |
| Salmon 0+ | +5.64[a] | +6.34 | +8.05[a] | +8.89 | +11.16 | +8.96[a] |
| | +6.18 | +6.89 | +7.87 | +9.14 | +11.50 | +9.08 |
| | +5.89 | +8.62 | +8.52 | +8.47 | +11.59 | +8.87 |
| | +6.24 | +7.62 | (+8.14 ± 0.19) | +8.84 | (+11.42 ± 0.13) | (+8.97 ± 0.06) |
| | (+5.99 ± 0.14) | (7.37 ± 0.49) | | 8.91 (8.85 ± 0.11) | | |
| Salmon 1+ | +6.75[a] | +9.23 | +8.38[a] | +8.95 | +11.02 | +9.45[a] |
| | +7.97 | +7.21 | +8.20 | +9.52 | +11.87 | +9.56 |
| | +7.91 | +8.27 | +8.40 | (+9.24 ± 0.29) | (+11.44 ± 0.43) | (+9.51 ± 0.06) |
| | (+7.54 ± 0.40) | (+8.23 ± 0.58) | (+8.32 ± 0.06) | | | |

[a]Mean of replicate analyses (see Table 1).

(Kennedy et al., 1997). $\delta^{87}$Sr values are reported relative to a modern $^{87}Sr/^{86}Sr$ seawater value of 0.70918 (Hodell et al., 1989).

## 3. Results

Five results emerge from our analyses. First, streams flowing through agricultural areas have significantly higher nitrate concentrations than those flowing through pristine forested areas. Table 2 shows water nitrate concentrations at each site, reported with a detection limit of 0.01 mg/l $NO_3$–N. Nitrate concentrations are generally low in all streams, ranging from 0.10 to 0.26 mg/l $NO_3$–N. However, we find a weak correlation ($r^2 = 0.57$, $p = 0.08$) between nitrate concentrations in stream water and local land-use patterns (Fig. 2).

Second, $\delta^{15}$N values of nitrate are higher in the agricultural streams than in the pristine streams. The $\delta^{15}$N values of nitrate range from a high of +7.3‰ to a low of +2.0‰ (Table 2). Like nitrate concentrations, $\delta^{15}$N values increase with increasing agricultural use in the surrounding catchment ($r^2 = 0.87$, $p = 0.006$; Fig. 3). In the pristine streams, $\delta^{15}$N values of nitrate are lowest at the national forest site, Bingo Brook, and highest at the sites with intensive agriculture and dairy farming (First Branch and Third Branch).

Third, as expected from other studies (Estep and Vigg, 1985; Fry, 1988, 1991; Hesslein et al., 1991), the $\delta^{15}$N values of algae, insects, and fish within a specific stream increase with increasing trophic level (Table 3). For example in the West Branch, first trophic level detritus and *Cladophora* have $\delta^{15}$N values of +0.2 and +1.0‰, respectively. Second trophic level herbivorous and detritivorous insects *Pteronarcys*, *Tallaperla*, and *Stenonema* have $\delta^{15}$N values of +2.0, +2.4, and +3.1‰, respectively. And, third trophic level predaceous insect *Paragentina* has a $\delta^{15}$N value of +5.5‰. Third trophic level age-class 0+ and 1+ salmon have $\delta^{15}$N

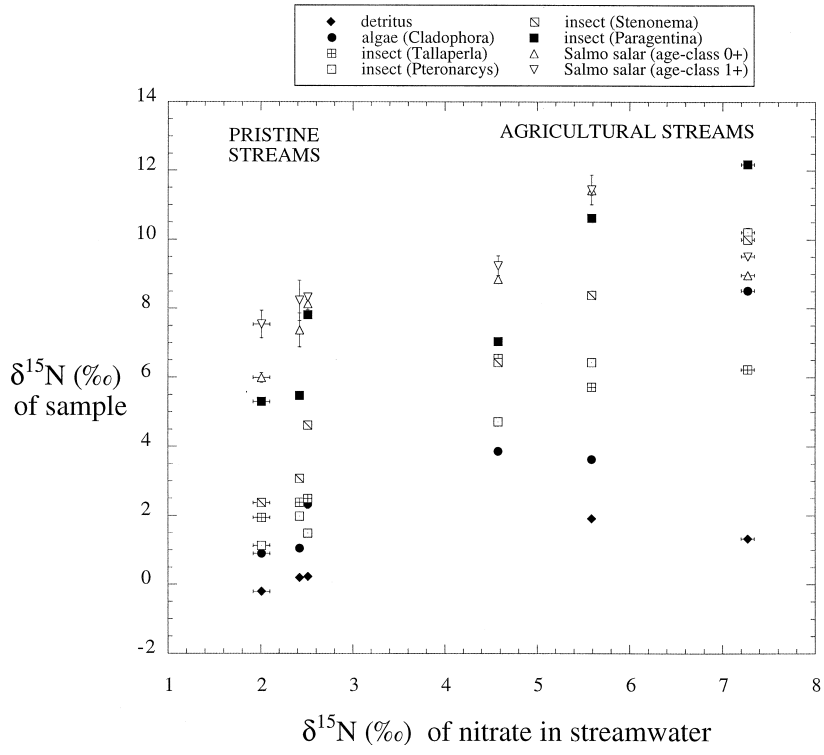


Fig. 4. $\delta^{15}$N of detritus, algae, insects and fish vs. $\delta^{15}$N of nitrate in streamwater for 1996 samples. Values plotted as mean values ± 1 S.E.‰ for replicate analyses or in some cases multiple samples (all *S. salar*, Third Branch *Pteronarcys*, and Bingo Brook *Stenonema*).

Table 4
Comparison of $\delta^{15}N$ values of samples collected in 1995 and 1996

| Material | Bingo brook | | West Branch | | Third Branch | |
|---|---|---|---|---|---|---|
| | 1995 | 1996 | 1995 | 1996 | 1995 | 1996 |
| Detritus | +0.58 | −0.21[a] | +0.39[a] | +0.19 | +2.23 ± 0.77 | +1.33[a] |
| *Cladophora* | +2.49 ± 0.36 | +0.89 | +2.87 ± 0.33 | +1.04 | +7.37 ± 1.49 | +8.52[a] |
| *Pteronarcys* | +1.71 | +1.13[a] | +2.57 | +1.98 | | +10.38 ± 0.17 |
| *Stenonema* | +2.21 | +2.37 ± 0.08 | | +3.07 | +9.13 | +10.00 |
| Salmon 0+ | +6.62 ± 0.18 | +5.99 ± 0.14 | +6.46 ± 0.16 | +7.37 ± 0.49 | | +8.97 ± 0.06 |
| Salmon 1+ | +7.17 | +7.54 ± 0.40 | +7.78 ± 0.15 | +8.23 ± 0.58 | | +9.51 ± 0.06 |

All values represent mean values ± 1 S.E. of multiple samples where possible (see Table 3 for complete 1996 data).
[a]Mean of replicate analyses (see Table 1).

values of +7.4 and +8.3‰, respectively. These values and the corresponding $\delta^{15}N$ values (Table 3 and Fig. 4) at the other sites support the use of nitrogen isotopes to indicate relative trophic level position within each stream food web as shown by Minagawa and Wada (1984). We were unable to determine a detailed food web study of the Atlantic salmon because we did not collect all possible food sources at each of these streams.

Fourth, $\delta^{15}N$ values of both age-class 0+ and age-class 1+ Atlantic salmon have higher $\delta^{15}N$ values in the agricultural streams than in the pristine streams. We found a strong positive correlation ($r^2 = 0.82$, $p = 0.01$; Fig. 3) between percent agricultural area in a catchment and $\delta^{15}N$ values of Atlantic salmon. This correlation suggests that nitrate from agricultural sources is introduced into the food web in the streams draining agricultural areas. Incorporation of nitrate from agricultural sources into the food web is also indicated by the $\delta^{15}N$ values of algae and aquatic insects (Table 3; Fig. 4). Algae, herbivorous, detritivorous, and predacious insects all have significantly higher $\delta^{15}N$ values in the agricultural streams (Third and First Branch) than in the pristine streams (Bingo Brook and West Branch).

Fifth, the $\delta^{15}N$ values of samples collected in two consecutive years is relatively constant. Table 4 compares $\delta^{15}N$ values between 1995 and 1996. Aquatic insects, algae, and detritus, in general, differ by less than 1‰ year to year; and preserve the trend of higher $\delta^{15}N$ values in agricultural streams. The lack of any significant year-to-year difference in $\delta^{15}N$ values is particularly notable considering that collection methods for algae and detritus differed between 1995 and 1996. More importantly, the $\delta^{15}N$ values of salmon is relatively constant year to year. The absolute difference between 1995 and 1996 $\delta^{15}N$ values is 0.6 and 0.9‰ for age-class 0+ salmon and 0.4 and 0.5‰ for age-class 1+ salmon, at Bingo Brook and West Branch, respectively.

## 4. Discussion and conclusions

Our research shows that $\delta^{15}N$ values of water nitrate and food web components vary between tributaries in a single drainage and appear closely linked to local land-use in the catchment. We also observed nitrate concentrations dependent upon land use. However, the isotopic differences between streams have more utility than the nitrate concentration differences: they allow us to assess the ultimate source of nitrate in the streams. In addition, nitrogen isotopes in conjunction with strontium isotopes provide a means for tracing the natal origins of Atlantic salmon.

### *4.1. Source of nitrate*

Nitrate concentrations in filtered water collected from White River tributaries during the fall of 1996 are lower than the median yearly 0.4 mg/l $NO_3$–N water concentration observed at the 383 USGS sampling stations in the United States (USDA Working Group on Water Quality, 1991). Despite these low nitrate concentrations, nitrate in streams draining areas with agricultural activity are higher than nitrate concentrations in streams draining forested areas

(Table 1). The elevated nitrate concentrations at the agricultural sites are a possible indication of introduction of nitrate from fertilizer, or animal and human waste, or introduction of nitrate from the oxidation of soil organic matter (Heaton, 1986). However, it is difficult to identify the source of nitrate in these sites with concentration data alone.

In contrast, nitrogen isotope ratio values can distinguish between natural and anthropogenic nitrate sources. As stated before, $\delta^{15}N$ values of nitrate are generally characteristic of their source but are not rigorously defined because of fractionation effects. However, in general, nitrate from commercial fertilizer ranges from $-2.5$ to $+2.0$‰, organic soil nitrate ranges from $-2.0$ to $+9.0$‰ and human and animal waste nitrate ranges from $+10.0$ to $+20.0$‰ (Kreitler and Jones, 1975; Heaton, 1986; Chapelle, 1993; Kendall et al., 1995, 1996).

Our $\delta^{15}N$ nitrate data suggest that a significant fraction of the nitrate in the streams draining agricultural sites is from anthropogenic sources. For example, in heavily forested relatively pristine areas such as Bingo Brook, West Branch and Bethel-Gilead the $\delta^{15}N$ nitrate values are relatively low ($+1.8$ to $+2.5$‰); and are typical of natural nitrate $\delta^{15}N$ values in streams from other pristine forested areas in Vermont (Kendall et al., 1995). In contrast, the $\delta^{15}N$ values of nitrate from streams in agricultural sites (particularly the Third Branch) are relatively high ($+7.1$‰), which suggests a significant input of nitrate from anthropogenic sources (Komor and Anderson, 1993; Kendall et al., 1995). The increase of $\delta^{15}N$ values of nitrate in waters draining pasture and agricultural lands has been observed elsewhere in Vermont (Kendall et al., 1995). These authors suggest that the increase in $\delta^{15}N$ values of nitrate is the result of contributions of nitrate from animal waste (Kendall et al., 1995).

Because of complications of isotopic fractionation which can occur in both soils and groundwater (see Macko and Ostrom, 1994) we cannot rigorously define the source of nitrate in the agricultural catchments with our isotopic data. It is tempting to suggest that nitrate in the agricultural catchments in the White River drainage is derived, in part, from animal waste because: (1) of the similarity of our $\delta^{15}N$ values of nitrate with the $\delta^{15}N$ values of animal waste (Ritter and Chirnside, 1984; Flipse et al., 1984; Heaton, 1986; Berndt, 1990; Spalding et al., 1993; Komor and Anderson, 1993); and (2) intense dairy farming occurs within these agricultural catchments. However, it has been suggested that the 'typical' $\delta^{15}N$ values of excrement-derived nitrate may be too high. Studies (Aravena et al., 1993; Burg and Heaton, 1997) have shown that $^{15}N$-enrichment is dependent upon loss of volatile ammonia and they suggest that $\delta^{15}N$ of excrement-derived nitrate are at the lowest end of this range from 9‰ (Aravena et al., 1993) to 9.9‰ (Burg and Heaton, 1997). In addition, it has been shown that cultivation of soils in agricultural areas tend to increase $\delta^{15}N$ values of nitrate because of the oxidation of soil organic matter (Heaton, 1986 and references therein). Thus, without further nitrogen isotope studies of agricultural and forested soils in these sites we cannot conclusively determine the source of nitrate in the agricultural catchments.

Nevertheless, our data suggest that nitrate from anthropogenic sources is incorporated into the aquatic food web. As stated earlier, we observe a strong positive correlation between $\delta^{15}N$ values of nitrate and salmon (Fig. 3) and percent agricultural land in the drainage. In addition, we find that the $\delta^{15}N$ values of detritus, algae and aquatic insects are, in general, higher in agricultural catchments (Fig. 4). These correlations and observations suggests that nitrate pollution from agricultural sources is consistently taken up through the food web. Similar observations have been made in marine food webs which show that the $\delta^{15}N$ values of fish (Rau et al., 1981) and benthic fauna (Van Dover et al., 1992) record the uptake of sewage into the food web. As such, our study provides supporting evidence that nitrogen isotope studies provide a useful indicator for the impact of non-point source pollution even when nitrate concentrations are very low.

### 4.2. Natal origin of Atlantic salmon

Previous studies have shown that carbon (Nelson et al., 1989; Meyer-Rochow et al., 1992) and sulfur (Hesslein et al., 1991) isotopes may be useful in determining migrants from nonmigrant fish populations. This work was expanded on by Kennedy et al. (1997) who showed that strontium isotopes can be

used to identify the natal origin of Atlantic salmon. Atlantic salmon in streams of the West and White River drainages in Vermont have distinct $^{87}Sr/^{86}Sr$ isotopic values. The strontium isotopic values of fish were nearly identical to the strontium isotopic composition of stream water values. Each of the streams studied by Kennedy et al. (1997) had distinct strontium isotopic signals which allowed these authors to fingerprint Atlantic salmon. However, the strontium isotopic composition of salmon from other areas could be complicated by mixing of waters from different tributaries and local control of strontium isotopic values by bedrock in each drainage. Unless the strontium isotopic values of waters were measured in all first-, second-, and third-order streams it may be difficult to trace natal origin of salmon using strontium isotopic values alone.

The combination of several isotopes of salmon should allow greater discrimination of local populations. A multiple isotope approach has been used in the study of neotropical migrant birds (Chamberlain et al., 1997), elephants (van der Merwe et al., 1990; Vogel et al., 1990; Koch et al., 1995), and fish (Nelson et al., 1989; Meyer-Rochow et al., 1992; Hesslein et al., 1991). These studies have demonstrated the utility of using a variety of isotopic systems for distinguishing between populations of animals. Like these studies, our study of Atlantic salmon shows more than one isotopic signal provides important and additional information on the natal origin of salmon.

Nitrogen and strontium isotopes are controlled by different processes. The strontium isotopic composition of a stream and its resident fish population is controlled primarily by the type of surficial rocks and bedrock exposed within a drainage basin. Streams draining catchments with metamorphic and granitic rocks typically will have high $^{87}Sr/^{86}Sr$ values, whereas streams draining catchments with carbonate rocks will have lower $^{87}Sr/^{86}Sr$ values. Thus, Atlantic salmon from streams where granites are exposed (Bingo Brook for example) have relatively high $^{87}Sr/^{86}Sr$ values; and salmon from streams where carbonates are a significant rock type (First Branch) will have lower $^{87}Sr/^{86}Sr$ values. Nitrogen isotopes, on the other hand, are controlled by land-use patterns in the catchment. Atlantic salmon from streams within agricultural areas (First Branch) have higher $\delta^{15}N$ values than salmon from forested streams (Bingo Brook).

The relationship between land-use, bedrock and the isotopic composition of fish tissue is illustrated in Fig. 5. Here we plot the strontium vs. nitrogen isotope ratios in age-class 0+ fish collected at stocking sites in the White River (Fig. 5). The strong negative correlation ($r^2 = -0.92$; $p < 0.01$) between the strontium and nitrogen isotopic values of Atlantic salmon (Fig. 5) in the White River system is a direct result of these differing controls of strontium and nitrogen isotopes of streams. Catchments with granitic rocks (high $^{87}Sr/^{86}Sr$ values) have less agriculture (low $^{15}N/^{14}N$ values) because of relatively poor soil development, whereas catchments with carbonate rocks (low $^{87}Sr/^{86}Sr$ values) have higher amounts of agriculture (high $^{15}N/^{14}N$ values) because these catchments have well-developed soils.

These data suggest that nitrogen, like strontium (Koch et al., 1992; Kennedy et al., 1997), carbon (Nelson et al., 1989; Meyer-Rochow et al., 1992) and sulfur (Hesslein et al., 1991) isotopes can be used to track migratory fish populations. We suggest that nitrogen isotopes provide a useful marker for tracing salmon for two reasons. First, nitrogen isotopes provide distinct and additional information about the natal habitat of the fish; and can be used to distinguish between fish originating from streams with different land-use patterns. Although we found little overlap in the Sr isotopic composition of salmon in the streams studied in the White River catchment, in catchments where the bedrock is virtually the same the nitrogen isotopes may be able to distinguish between local populations of fish. Second, the collection of nitrogen isotope data is faster and less expensive than the collection of strontium isotope data. Thus, nitrogen isotope analysis provides a relatively rapid method for determining the numbers of salmon originating from agricultural or forested streams. However, we point out that before nitrogen isotopes can be used to trace natal origin of salmon it is important to establish the structure of the food web and the fractionation of nitrogen during uptake into organisms.

The utility of an isotopic system in tracking animal populations requires knowledge of the turnover times in different tissues and the rate of addition of new tissue in a growing fish. Atlantic salmon back-

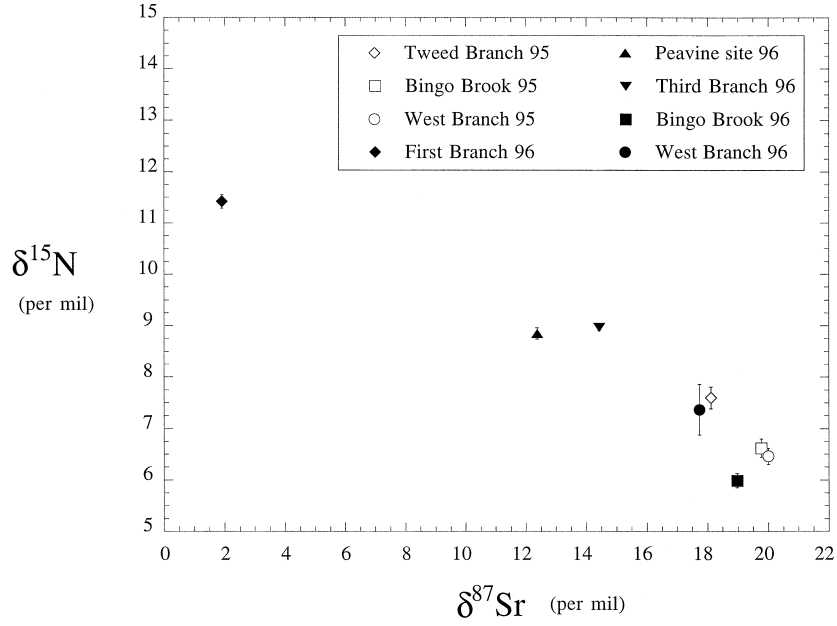


Fig. 5. $\delta^{87}$Sr (‰ vs. modern seawater) of salmon vertebrate vs. $\delta^{15}$N (‰ vs. air) of salmon muscle tissue for 1995 and 1996 samples collected in the White River drainage. N data is from this paper; Sr data is from Kennedy et al. (1997). Values are plotted as mean values $\pm$ 1 S.E.‰.

bones, otoliths, scales, and muscle tissues can vary in their utility for isotopic analysis. It is therefore critical to know the rates of each isotope in each type of material analyzed to derive a natal stream isotopic identity. Bone material retains the strontium isotopic signal, as otolith annuli record the history of the fish's chemical environment (Campana and Nielson, 1985). Because otoliths preserve the chemical signature during growth, it may be possible using strontium isotopes to determine the natal origin of adult salmon long after the fish leave their natal freshwater streams. However, there is relatively little nitrogen in otolith material which eliminates the possibility of using otoliths for a long-term $^{15}N/^{14}N$ signal.

Muscle tissues from Atlantic salmon analyzed in this study show a stream-derived nitrogen signal within three months after stocking (Fig. 6). Hatchery fry were initially enriched in $^{15}N$, reflecting a parentally-derived ocean signal (Bilby et al., 1996). However, within three months after stocking the nitrogen isotope signal reflected that of the natal tributary. A similar rapid rate was observed with strontium isotopes (Kennedy et al., 1997) in the backbones of the same hatchery fry. The rapid incorporation of the stream-derived nitrogen and strontium signals most likely reflects the rapid growth of juvenile salmon after stocking and subsequent generation of new tissue and bone.

It is therefore critical to know how this initial stream-derived signal is retained and what factors control its retention for each isotope utilized in tracing the natal origins of salmon. Recent work by Hesslein et al. (1993) indicates that the C, N, and S stable isotopes in broad whitefish (*Coregonus nasus*) muscle tissue and liver tissue is almost entirely growth rate-dependent rather than metabolically-dependent. They concluded that complete change of the isotope composition of fish tissue would take years in slow-growing adult fish. These results are promising for the long-term retention of nitrogen isotopic signal in salmon, particularly if salmon growth rates are known.

Growth rates of salmon from a similar stream in the Connecticut River watershed (the White River is part of the Connecticut River watershed) show that the rate of length gained per month generally de-

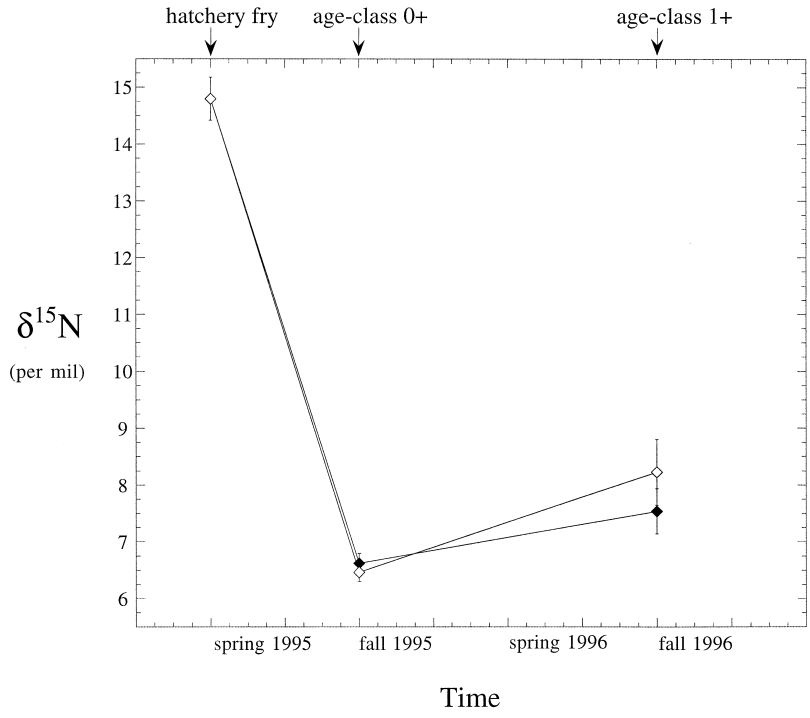


Fig. 6. $\delta^{15}$N in salmon muscle tissue from hatchery fry to age-class 1 + parr in White River tributaries. Filled symbols represent Bingo Brook; open symbols represent West Branch. Values are plotted as mean values ± 1 S.E.‰.

creases after the initial rapid growth rate from hatchery to age-class 0 + (Orciari et al., 1994). Thus, if stable isotopes in fish muscle tissue are controlled by growth (Hesslein et al., 1993), nitrogen isotope values in muscle tissue should be related to the fish's growth rate and rapidly approach the $\delta^{15}$N value of the food source in the stream, as observed in our study (Fig. 6). Retention of the natal $\delta^{15}$N signature after migration is dependent upon the amount of muscle mass accreted from the time the fish is a smolt to the salmon's return to the natal stream to spawn. Therefore, known growth rates before and after salmon migration are critical for constraining the turnover rate of stable isotopes in fish muscle tissue. This information would significantly enhance the utility of stable isotopes as a tool for tracing migratory salmon to their natal streams.

Our study suggests that nitrogen isotopes in Atlantic salmon muscle tissue is potentially useful in tracing natal origins of salmon. As such, nitrogen isotopes of smolt collected downstream of their natal streams can be used to determine the success of different rearing habitats. Although nitrogen isotopes allowed us to discriminate between salmon from agricultural and pristine-forested catchments the nitrogen isotope signal could be complicated by the addition of nitrogen from decaying adult salmon. The addition of nitrogen from adult salmon is not a factor in our study because of the low return rates of Atlantic salmon to the White River. However, in other pristine catchments which have large numbers of spawning salmon the addition of salmon-derived nitrogen has been shown to influence nitrogen isotope values (Bilby et al., 1996).

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