Using Mussel Isotope Ratios to Assess Anthropogenic Nitrogen Inputs to Freshwater Ecosystems

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USING MUSSEL ISOTOPE RATIOS TO ASSESS ANTHROPOGENIC NITROGEN INPUTS TO FRESHWATER ECOSYSTEMS

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Abstract. Stable nitrogen isotope ratios (δ^{15}N) of freshwater mussels from a series of lakes and ponds were related to watershed land use characteristics to assess their utility in determining the source of nitrogen inputs to inland water bodies. Nitrogen isotope ratios measured in freshwater mussels from 19 lakes and ponds in Rhode Island, U.S.A., ranged from 4.9–12.6‰ and were found to significantly correlate with the fraction of residential development in 100 and 200 m buffer zones around the ponds. Mussel δ^{15}N values in 12 of the 19 ponds also showed significant correlation with average dissolved nitrate concentrations, which ranged from 23–327 μg L^{-1}. These observations, in light of previous studies which link elevated δ^{15}N values of nitrogen derived from septic wastewater with those seen in biota, suggest that mussel isotope ratios may reflect nitrogen source in freshwater ecosystems. We followed an iterative approach using multiple regression analysis to assess the relationship between mussel δ^{15}N and the land use categories fraction residential development, fraction feedlot agriculture, fraction row-crop agriculture, and fraction natural vegetation in 100 and 200 m buffer zones and pond watersheds. From this we developed a simple regression model to predict mussel δ^{15}N from the fraction of residential development in the 200 m buffer zone around the pond. Subsequent testing with data from 16 additional sites in the same ecoregion led us to refine the model by incorporating the fraction of natural vegetation. The overall average absolute difference between measured and predicted δ^{15}N values using the two-parameter model was 1.6‰. Potential sources of error in the model include differences in the scale and categorization of land-use data used to generate and test the model, differences in physical characteristics, such as retention time and range of residential development, and exclusion of sources of enriched nitrogen such as runoff from feedlot operations or increased nitrogen loading from inefficient or failed septic systems.

Keywords: eutrophication, isotopes, nitrogen, nitrogen source, unionid mussels

1. Introduction

Despite progress made in the past several decades towards limiting nutrient inputs, human-induced eutrophication in lakes, ponds, and reservoirs continues to be of concern. Inland water bodies are particularly vulnerable to the effects of over-enrichment because of potentially long residence times of nutrients in these systems. Although many successes in reversing the effects of nutrient enrichment were realized by regulating phosphorous inputs to inland water bodies, investigations into the relationship between nutrient loading and productivity suggest the need to consider nitrogen when assessing eutrophication in natural systems (Walker, 1984; Reckhow, 1988; Smith, 1990). Even though evidence suggests that primary production is phosphorous limited in a majority of freshwater systems, there are increasing numbers of examples of nitrogen limitation in lakes, ponds and reservoirs (Morris and Lewis, 1988; Reckhow, 1988; Downing and McCauley, 1992; Schelske, 1994). Furthermore, trends towards increasing nitrogen limitation with eutrophication have been reported (Forsberg, 1977; Downing and McCauley, 1992; Jaervinen and Salonen, 1998).

Assessing the impact of nitrogen enrichment in inland waters is made difficult by the greater complexity of the biogeochemical cycle of nitrogen relative to that of phosphorous (Schindler, 1985), for example, nitrogen in lakes and ponds can be lost to the atmosphere via microbial denitrification, but can also accumulate as a result of biological nitrogen fixation (Schindler, 1977; Howarth *et al.*, 1988; Seitzinger, 1988). Still relatively little is known about the natural regulation of these processes and their effect on nitrogen dynamics, however, it is clear that human inputs, from both point-source discharges and diffuse or non-point sources are increasing nitrogen concentrations in inland waters (Howarth *et al.*, 1996). This increase, coupled with the potential of nitrogen enrichment to exacerbate impairment of inland water bodies, has led to continuing efforts to assess the extent and source of nitrogen inputs (USEPA, 1992, 1994).

Stable isotope analysis provides a potential tool to identify the source of nitrogen inputs to natural waters. Several studies have demonstrated that the stable isotopic ratio of nitrogen (expressed as $\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1] \times 1000‰$ relative to the standard of N_2 in atmospheric air) from anthropogenic sources is reflected in the biota of ecosystems (McClelland *et al.*, 1997; McClelland and Valiela, 1998). When dissolved nitrogen is processed and incorporated into the biomass of primary producers, the $\delta^{15}N$ of the source nitrogen is reflected in that of the organism. Because the nitrogen isotope ratio increases in a fairly predictable fashion as it passes through the food chain, anthropogenic nitrogen inputs can influence the isotopic composition of organisms in an ecosystem (DeNiro and Epstein, 1981; Minigawa and Wada, 1984; Fry, 1988). Dissolved nitrogen in a freshwater system may be enriched or depleted in ^{15}N by dilution with nitrogen from anthropogenic sources as a result of physical processes or biological transformations which, through mass discrimination, result in greater relative losses of one of the isotopes. For example, dissolved nitrogen derived from human septic wastewater is relatively enriched in ^{15}N when it reaches takes and ponds ($\delta^{15}N$ values in the range of 10 to 20‰), owing both to the high trophic position of humans and to fractionation resulting from greater proportionate loss of ^{14}N during ammonification and volatilization of nitrogen waste products. Organisms which incorporate this enriched nitrogen will reflect elevated tissue isotope ratios (Kreitler, 1979; Gormley and Spalding, 1979; Kreitler and Browning, 1983; Aravena *et al.*, 1993). Conversely, nitrogen derived from synthetic fertilizer which, as a result of manufacturing processes, is relatively depleted in ^{15}N ($\delta^{15}N$ values in the range of

–3 to 3‰), would be reflected in lower δ^{15}N values in organisms in an ecosystem (Freyer and Aly, 1974). Linking anthropogenic sources in watersheds to nitrogen in lakes and ponds could provide important information for managing and regulating nutrient enrichment.

Determining a nitrogen isotope ratio which accurately reflects that of biota in a particular system is an important component in the use of δ^{15}N values to trace nitrogen from source to ecosystem. The best reflection of ecosystem δ^{15}N may be the isotope ratio of biotic components at or near the base of the food chain, referred to as base level δ^{15}N. One approach to determining base level δ^{15}N is to measure primary producer δ^{15}N; however, this measurement is complicated by significant temporal and spatial variability. For example, differences in nitrogen fractionation associated with seasonal changes in the dominant species of primary producers in freshwater and marine systems can result in δ^{15}N values that vary up to 6–10‰ during the course of a year (Yoshioka and Wada, 1994; Cabana and Rasmussen, 1996; Cifuentes *et al*., 1996; Fourqurean *et al*., 1997). In addition, significant spatial variability may result from habitat differences within a system (Vander Zanden and Rasmussen, 1999). Difficulties may also arise when trying to identify nitrogen source from the isotope ratio of higher-level consumers, because food chains vary in length and complexity (Kling and Fry, 1992; Cabana and Rasmussen, 1994; Vander Zanden and Rasmussen, 1999).

Several studies have suggested the use of filter feeding bivalves to assign a base level δ^{15}N to an ecosystem (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999). These organisms occupy a fixed trophic position near the base of the food chain, are consistently found in the littoral zone, and have been shown to reflect base level δ^{15}N in smaller freshwater systems such as shallow lakes and ponds (McKinney *et al*., 1999). Mussels in marine systems integrate the δ^{15}N of primary producers over time, incorporating seasonal fluctuations in an average δ^{15}N value (McKinney *et al*., 2001). Freshwater mussels should also provide an integrated, base level δ^{15}N of an ecosystem, and this isotope ratio could potentially be used to provide information about the source of nitrogen to the system.

Certain watershed land-use practices have been shown to result in the discharge of nutrients to receiving water bodies. For example, residential land use will result in the movement of septic wastewater nitrogen in groundwater which in turn feeds lakes and ponds, and agricultural land will lead to surface runoff of nitrogen from excess fertilizer application (Valiela *et al*., 1992; Jordan *et al*., 1997). In order to more effectively manage nutrient enrichment in freshwater systems, models are needed which link watershed land use to water quality (Summer *et al*., 1990; Carpenter *et al*., 1998; Smith, 1998). Ecosystem base level δ^{15}N as reflected in the isotope ratio of freshwater mussels could help to provide this link and in doing so provide information about the source of nitrogen to inland waters which would be of value to managers and regulators in developing water management policies.

In this study we investigate the use of unionid mussel δ^{15}N values as indicators of the source of nitrogen input to ponds and small lakes. Mussels were initially

TABLE I

Locations and physical characteristics of 19 study lakes/ponds in Rhode Island used to develop the predictive model

Lake/Pond	Pond No.[a]	Lat. (°N)	Long. (°W)	Water area (km^2)	Max. depth[b] (m)	Avg. depth[b] (m)	Watershed area (km^2)
Boon Lake	12	41°35′	71°41′	0.18	6.8	3.7	6.67
Browning Mill Pond	2	41°34′	71°41′	0.18	1.4	1.2	12.9
Eisenhower Lake	1	41°37′	71°43′	0.22	2.0	–	10.0
Gorton Pond	15	41°42′	71°27′	0.25	13.7	5.0	3.57
Hundred Acre Pond	8	41°30′	71°33′	0.34	10.9	5.5	24.2
J.L. Curran Reservoir	6	41°45′	71°33′	0.38	6.0	3.0	2.21
Larkin Pond	10	41°28′	71°33′	0.10	10.3	4.2	0.46
Locustville Pond	13	41°31′	71°43′	0.33	4.0	2.4	28.7
Mashpaug Pond	11	41°48′	71°26′	0.31	5.2	2.1	4.98
Mishnock Lake	17	41°39′	71°36′	0.19	5.4	2.4	1.08
Oak Swamp Reservoir	14	41°50′	71°33′	0.44	3.0	1.5	2.40
Quidnick Reservoir	7	41°41′	71°41′	0.70	10.8	3.6	6.51
Tiogue Lake	18	41°41′	71°33′	0.92	3.3	1.8	7.49
Tucker Pond	4	41°25′	71°33′	0.38	9.8	3.4	2.93
Upper Pawtuxet	9	41°43′	71°32′	0.18	–	–	258
Warwick Pond	19	41°44′	71°25′	0.34	7.9	4.4	4.23
Worden Pond	5	41°26′	71°35′	4.26	2.1	1.2	66.8
Wyoming Pond	16	41°31′	71°42′	0.14	3.6	–	130
Yawgoo Pond	3	41°31′	71°34′	0.58	10.0	4.0	3.65

[a] Pond number refers to Figure 1.

[b] Data from Guthrie and Stolgitis (1977) and Herron and Green (1996).

collected from 19 small lakes and ponds in the Northeast Coastal Zone ecoregion located along a gradient of human activity ranging from highly developed urban to sparsely developed rural. We examined various correlations between mussel δ^{15}N values and land use patterns within buffer zones of varying size, and also within the entire lake or pond watershed. These data were used to develop a regression model to predict mussel isotope ratio based on watershed land use characteristics. We then tested the model with mussel δ^{15}N values and land use data from a set of 16 lakes and ponds located within the same ecoregion, compared differences between the predicted and measured δ^{15}N values, and refined the model by adding variables identified by the test data to improve model performance.

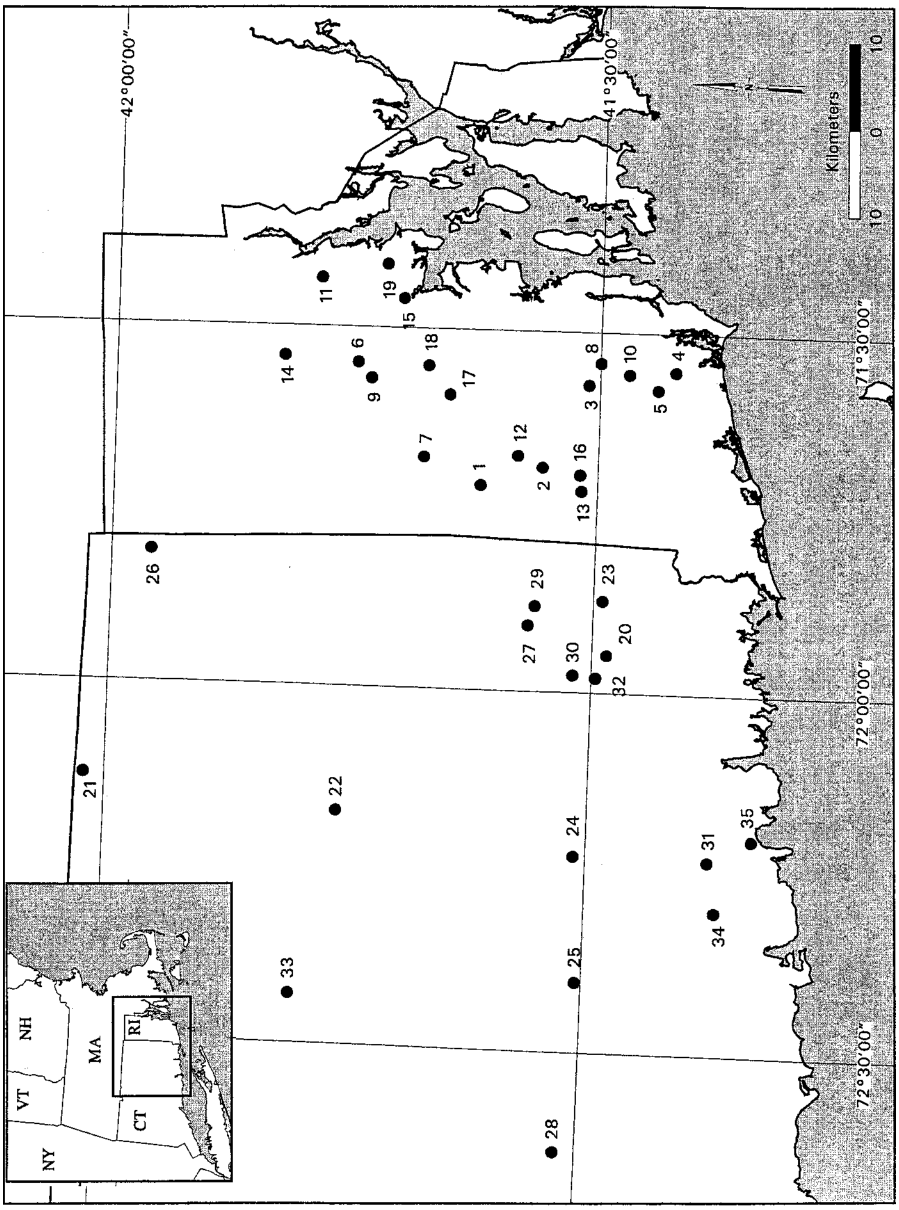

Figure 1. Study sites, in the Northeastern Coastal Zone ecoregion, consisting of Rhode Island and Connecticut lakes and ponds. Numbers refer to ponds listed in Tables I and II.

2. Methods

2.1. Study sites

A model to predict mussel $\delta^{15}N$ from land use characteristics was developed using data from nineteen shallow lakes and ponds located within a 80 km radius in central Rhode Island, U.S.A. (Figure 1). These ponds are all located within the Northeastern Coastal Zone ecoregion, which is characterized by irregular plains with low to high hills, Appalachian oak forests, inceptisol soils, and predominantly urban and woodland/forest land use (Omernik, 1987). Pond water area ranged from 0.10 to 4.26 km^2 with an average value of 0.55 km^2, maximum water depth ranged from 1.4 to 13.7 m^2 with an average value of 6.5 m^2, and average water depth ranged from 1.2 to 5.5 m^2 (Table I). The watershed area of the ponds ranged from 0.46 to 258 km^2 with an average of 30.4 km^2. The ponds are influenced by human activities to varying degrees as measured by fraction residential development and fraction agricultural land use in the pond watersheds. According to 1990 GIS data and information obtained from town public works departments, none of the residences in the 100 or 200 m buffer zones around the ponds were serviced by public sewers (i.e., all residences had individual septic systems). However, several of the ponds in the urban centers (Mashpaug Pond, Warwick Pond, Gorton Pond) showed sewer coverage in the pond watersheds.

In order to test the model, we collected mussels from 16 ponds in Connecticut, U.S.A., which are also located within the Northeastern Coastal Zone ecoregion, and at roughly the same range of latitude as the Rhode Island ponds. The Connecticut ponds exhibited a similar range of depths and areas as the Rhode Island ponds (Table II). Pond accessibility and mussel availability limited the pool of possible sample locations and resulted in sites with lower overall average extent of residential development (4% versus 20% for the RI ponds) and a more narrow range of residential land use (0–19% versus 3–58% for the RI ponds).

2.2. Sample collection and analysis

Unionidae mussels (*Elliptio* spp.) were collected in mid to late summer 1998 and 1999 by hand or by hand rake, generally in 1–2 m of water. Between 3 and 6 individual mussels were collected from each site and the mussel tissue $\delta^{15}N$ values were averaged. In some cases we collected samples at multiple locations within a site, and in these cases a site average $\delta^{15}N$ is reported. The mussels were transported to the laboratory on ice, depurated for 24 hr, and frozen pending analysis. Samples were prepared for isotope analysis by removing a sub-sample of the foot tissue which was dried at 75 °C and then ground and homogenized using a mortar and pestle. The nitrogen isotopic composition was determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) employing a Carlo-Erba NA 1500 Series II Elemental Analyzer interfaced to a Micromass Optima Mass Spectrometer. The nitrogen isotope ratio of the tissue is expressed as a part per thousand

TABLE II

Locations and physical characteristics of 16 study lakes/ponds in Connecticut used to test the predictive model

Lake/Pond	Pond No.[a]	Lat. (°N)	Long. (°W)	Water area (km^2)	Max. depth[b] (m)	Avg. depth[b] (m)	Watershed area (km^2)
Amos Lake	30	41°31′	71°59′	0.46	14.6	5.8	6.00
Avery Pond	32	41°30′	71°59′	0.18	–	–	2.04
Crystal Lake	28	41°31′	72°38′	0.13	7.3	2.4	0.71
Dodge Pond	35	41°19′	72°12′	0.12	14.6	3.1	1.49
Gardner Lake	24	41°31′	72°14′	2.14	13.1	4.2	14.1
Glasgo Pond	29	41°34′	71°53′	0.33	7.6	3.1	93.9
Lake of Isles	20	41°29′	71°57′	0.37	3.0	1.9	1.74
Bolton Lake	33	41°48′	72°26′	0.71	7.9	3.0	9.64
Mansfield Hollow Lake	22	41°46′	72°11′	1.77	6.9	4.9	417
Mashapaug Pond	21	42°01′	72°08′	1.21	13.1	2.8	10.1
Moodus Reservoir	25	41°30′	72°24′	1.81	3.1	1.5	34.0
Pachaug Pond	27	41°34′	71°54′	3.39	5.5	1.9	130
Patagansett Lake	31	41°22′	72°14′	0.51	10.4	3.8	9.98
Quaddick Reservoir	26	41°58′	71°49′	1.35	7.6	2.0	35.9
Rogers Lake	34	41°22′	72°18′	1.12	20.1	6.1	19.5
Wyassup Lake	23	41°29′	71°52′	0.40	8.5	2.7	2.20

[a] Pond number refers to Figures 1 and 4.
[b] Data from Hanten *et al*. (1997).

(‰) difference from the composition of a recognized reference material, which by convention is N_2 in air (Mariotti, 1983). We used a DORM-1 powdered dogfish reference material (National Research Council, Institute for Environmental Chemistry, Ottawa, Canada) as a working standard. Our measured value for this standard is 11.2±0.1‰, after correcting our values to the standard reference material IAEA-NI (Ammonium Sulfate, INIST # 8547, National Institute of Standards and Technology, Gaithersburg, MD). All samples were analyzed in duplicate with a typical difference of about 0.1‰. Sample material analyzed periodically over a several month period exhibited a precision of ±0.30‰ calculated as a single standard deviation of all replicate values.

2.3. Dissolved Inorganic Nitrate Concentrations

Correlations between mussel isotope ratios and dissolved inorganic nitrate [NO_3^-] concentrations were made using data from 12 of the 19 Rhode Island ponds generated from citizen monitoring data provided by the University of Rhode Island

Watershed Watch Program (Herron and Green, 1996). Sites were sampled in May, June and July of each year. Results of sampling from 1993 to 1998 were combined into a single average value for each pond; a value of one-half the detection limit was used for concentrations that were reported as being below the detection limit.

2.4. MUSSEL LAND USE CORRELATIONS

The Geographic Information System (GIS) data were processed with the Environmental Systems Research Institute (ESRI) ARC/INFO software package. Rhode Island land use and land cover data was obtained from the Rhode Island Geographic Information System (RIGIS) database. This data was developed from 1988 aerial photography (1:24 000 scale) which was then updated using 1992–1995 ortho-photography. The data was classified by RIGIS using a modified USGS system (modified Anderson level 3), which resulted in 38 land use and land cover categories classified with one half acre (21 780 square feet) minimum polygon resolution (Anderson, 1976). We delineated pond watersheds using data from 15 min (1:24 000 scale) United States Geological Survey (USGS) topographic maps.

The RIGIS land use and land cover data were aggregated for use in mussel δ^{15}N land use correlations. For the Rhode Island ponds, the categories High Density Residential, Medium High Density Residential, Medium Density Residential, Medium Low Density Residential, and Low Density Residential were combined as residential development (RES); the categories Pasture and Confined Feeding Operations were combined as feedlot agriculture (LVSTK), the category Cropland was considered row-crop agriculture (CROP), and the categories Deciduous Forest, Evergreen Forest, Mixed Deciduous Forest, Mixed Evergreen Forest, Brushland, and Wetland were combined as natural vegetation (VEG). The GIS data were used to generate the fraction of each category within each buffer zone or watershed.

A series of regressions between land use characteristics and mussel δ^{15}N values in the Rhode Island lakes and ponds were performed to determine which land use type would best correlate with mussel nitrogen isotope ratio. We first looked at single regressions of fraction residential, fraction feedlot agriculture, fraction row-crop agriculture, and fraction natural vegetation versus mussel δ^{15}N for the 100 m buffer, 200 m buffer, and the entire watershed to determine whether there were any significant correlations. We then examined multiple regressions of the fractions of each of the four land use categories in the 100 m buffer, 200 m buffer, and watershed versus mussel δ^{15}N (i.e., land use types grouped by size of buffer zone). By comparing the r^2 values of these three regressions we were able to determine which multiple regression explained most of the variance in the data. Based on the results of both the single and multiple variable regressions, we then proceeded to eliminate variables that did not significantly improve the correlation to arrive at the best-fit empirical model relating mussel isotope ratio to land use characteristics for this data set.

2.5. Testing the predictive model

The model was tested with mussel isotope and land use data from 16 Connecticut, U.S.A., lakes and ponds located within the same ecoregion as the Rhode Island sites. The water depths, areas, and watershed areas of these ponds were in the same range as those of the Rhode Island ponds (Table II). Pond water area ranged from 0.12 to 3.39 km^2 with an average value of 1.00 km^2, maximum water depth ranged from 3.0 to 20.1 m^2 with an average value of 9.6 m^2, and average water depth ranged from 1.5 to 6.1 m^2. The watershed area of the ponds ranged from 0.71 to 417 km^2 with an average of 49.3 km^2.

The land use and land cover data layer for Connecticut was obtained from the University of Connecticut's Map and Geographic Information Center (MAGIC) database. This data was developed using LANDSAT Thematic Mapper Satellite Imagery information obtained in 1987, 1988 and 1990. The data contained 23 categories of land use and land cover which were classified with a one hectare (107 600 square feet) minimum polygon resolution. Although this land use and land cover data was on a more coarse scale and used a different classification method than the Rhode Island data, it was the best available GIS data for this region. The Connecticut land use and land cover data was also aggregated to help reduce the inherent differences between the two GIS data sets. For this study, the categories Residential/Commercial High Density and Residential Medium Density were combined as residential development (RES), the category Grass/Hay/Pasture was labeled feedlot agriculture (LVSTK); the categories Soil/Corn and Grass/Corn were combined as row-crop agriculture (CROP); and the categories Forest Deciduous, Forest Evergreen, Wetland Forested and Wetland Non-Forested were combined as natural vegetation (VEG). The GIS data was then used to generate the fraction of each category within each buffer zone or watershed. A plot of the predicted versus measured $\delta^{15}N$ values was generated, and comparisons were made to a line of slope = 1 which is representative of the model.

3. Results

3.1. Dissolved inorganic nitrate concentrations

Average concentrations of dissolved inorganic nitrate for 12 of the 19 Rhode Islands ponds over the period from 1993 to 1998 ranged from 23–327 $\mu g\ L^{-1}$ (Table III), with concentrations of individual samples ranging from below detection (50 or 40 $\mu g\ L^{-1}$ depending on the sampling year) to 880 $\mu g\ L^{-1}$ at Tiogue Lake in May of 1997 (data not shown, Herron and Green, 1996). Nitrate concentrations in the ponds showed a significant positive correlation with the fraction of residential development in a 200 m buffer zone around the pond ($r^2 = 0.50$, $p = 0.01$, Figure 2a), and also with mussel nitrogen isotope ratio ($r^2 = 0.70$, $p = 0.001$, Figure 2b).

TABLE III

Mussel nitrogen isotope ratios and dissolved inorganic nitrate concentrations, where available, for the 35 lakes and ponds

	Pond No.[a]	$\delta^{15}N$ (‰)	$[NO_3^-]$ ($\mu g\ L^{-1}$)[b]
RI Lakes and Ponds			
Boon Lake	12	8.1	100
Browning Mill Pond	2	6.2	–
Eisenhower Lake	1	6.1	–
Gorton Pond	15	11.9	265
Hundred Acre Pond	8	8.8	179
J.L. Curran Reservoir	6	8.3	–
Larkin Pond	10	8.5	–
Locustville Pond	13	8.7	41
Mashpaug Pond	11	8.8	–
Mishnock Lake	17	9.1	286
Oak Swamp Reservoir	14	7.8	–
Quidnick Reservoir	7	4.9	41
Tiogue Lake	18	11.9	327
Tucker Pond	4	5.3	23
Upper Pawtuxet	9	7.5	–
Warwick Pond	19	12.6	306
Worden Pond	5	7.8	79
Wyoming Pond	16	9.6	71
Yawgoo Pond	3	6.1	23
CT Lakes and Ponds			
Amos Lake	30	10.1	–
Avery Pond	32	8.9	–
Crystal Lake	28	7.5	–
Dodge Pond	35	10.1	–
Gardner Lake	24	9.3	–
Glasgo Pond	29	11.3	–
Lake of Isles	20	3.8	–
Bolton Lake	33	7.6	–
Mansfield Hollow Lake	22	8.1	–
Mashapaug Pond	21	4.1	–
Moodus Reservoir	25	7.0	–
Pachaug Pond	27	10.2	–
Patagansett Lake	31	7.6	–
Quaddick Reservoir	26	6.2	–
Rogers Lake	34	5.9	–
Wyassup Lake	23	5.5	–

[a] Pond numbers refer to Figure 1.

[a] Nitrate concentrations from Herron and Green (1996).

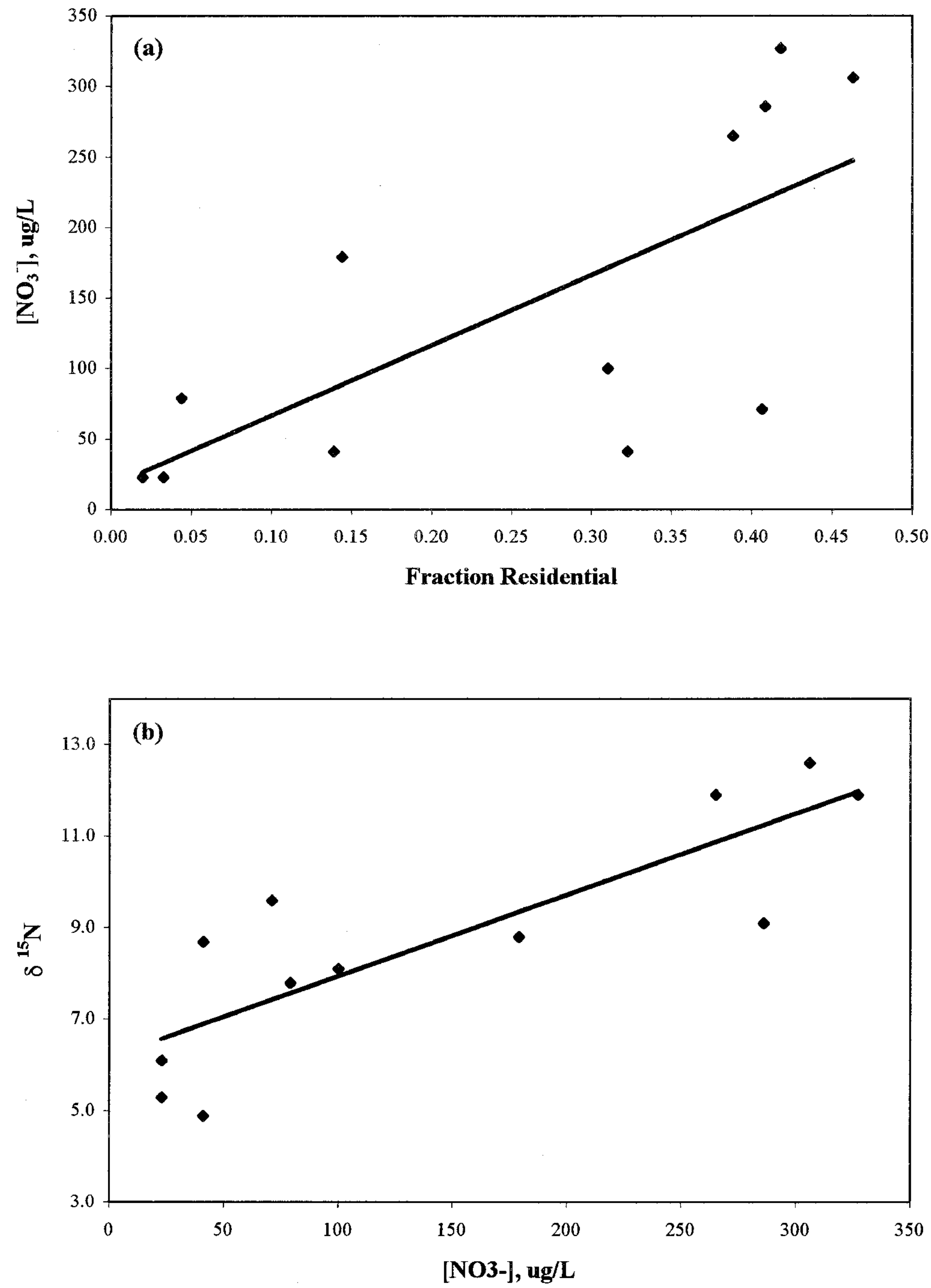

Figure 2. (a) Concentration of dissolved nitrate versus fraction residential development in a 200 m buffer zone, and (b) mussel δ^{15}N versus concentration of dissolved nitrate for 12 of the 19 Rhode Island ponds.

TABLE IV

Fraction of residential development, agricultural land use, and natural vegetation (combined forest and wetlands) in the 100 m buffer zone, 200 m buffer zone, and watershed for the Rhode Island lakes and ponds

Lake/Pond[a]	Frac. RES			Frac. LVSTK			Frac. CROP			Frac. VEG		
	100 m	200 m	Watershed	100 m	200 m	Watershed	100 m	200 m	Watershed	100 m	200 m	Watershed
Boon Lake	0.64	0.39	0.14	0.00	0.00	0.00	0.00	0.00	0.02	0.25	0.55	0.71
Browning Mill Pond	0.00	0.01	0.06	0.00	0.00	0.00	0.10	0.08	0.01	0.79	0.83	0.87
Eisenhower Lake	0.00	0.00	0.05	0.00	0.00	0.00	0.11	0.11	0.02	0.87	0.87	0.87
Gorton Pond	0.55	0.53	0.44	0.00	0.00	0.00	0.00	0.02	0.00	0.21	0.19	0.25
Hundred Acre Pond	0.32	0.20	0.13	0.00	0.00	0.00	0.01	0.10	0.16	0.66	0.69	0.61
J.L. Curran Reservoir	0.06	0.11	0.06	0.05	0.01	0.01	0.30	0.30	0.43	0.64	0.60	0.38
Larkin Pond	0.21	0.22	0.09	0.00	0.00	0.00	0.00	0.04	0.00	0.30	0.40	0.41
Locustville Pond	0.44	0.42	0.07	0.00	0.00	0.00	0.08	0.12	0.02	0.41	0.37	0.81
Mashpaug Pond	0.26	0.32	0.48	0.00	0.00	0.00	0.05	0.05	0.00	0.11	0.06	0.01
Mishnock Lake	0.60	0.54	0.44	0.00	0.00	0.00	0.05	0.04	0.00	0.35	0.35	0.29
Oak Swamp Reservoir	0.51	0.52	0.45	0.00	0.00	0.00	0.12	0.07	0.01	o 40	0.43	0.46
Quidnick Reservoir	0.26	0.23	0.15	0.00	0.00	0.00	0.04	0.06	0.01	0.66	0.69	0.78
Tiogue Lake	0.73	0.68	0.30	0.00	0.00	0.00	0.02	0.01	0.00	0.21	0.23	0.26
Tucker Pond	0.08	0.05	0.03	0.00	0.00	0.00	0.07	0.12	0.09	0.77	0.73	0.78
Upper Pawtuxet	0.19	0.20	0.10	0.02	0.01	0.00	0.05	0.07	0.02	0.73	0.64	0.76
Warwick Pond	0.82	0.70	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.29	0.32
Worden Pond	0.14	0.13	0.12	0.00	0.00	0.00	0.00	0.02	0.11	0.69	0.77	0.65
Wyoming Pond	0.46	0.50	0.05	0.00	0.00	0.00	0.05	0.12	0.02	0.24	0.26	0.86
Yawgoo Pond	0.06	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	1.02	1.00	0.91

[a] Land use fractions are based on the total area of buffer or watershed minus that of the pond or lake itself. The fraction pond or lake area ranged from 0.20–0.63 for the 100 m buffer, 0.17–0.48 for the 200 m buffer, and 0.01–0.21 for the watershed.

TABLE Va

Statistics from the regressions of the fractions of each of the land use types with mussel δ^{15}N values within the different buffer zones for the Rhode Island ponds

Buffer zone	RES[a]		LVSTK		CROP		VEG	
	r	*p*	r	*p*	r	*p*	r	*p*
100 m	0.78	7.6×10^{-5}	0.04	0.88	–0.24	0.31	–0.76	0.0002
200 m	0.83	7.6×10^{-5}	0.18	0.45	–0.14	0.58	–0.77	0.0001
Watershed	0.63	0.003	0.16	0.52	–0.09	0.72	–0.67	0.0020

[a] RES = fraction residential land use, CROP = fraction agricultural row-crop land, LVST = fraction agricultural feedlot land, VEG = fraction natural vegetation.

TABLE Vb

Statistics from the stepwise multiple regressions of the fractions of the four land use types with mussel. δ^{15}N values within each of the buffer zones for the Rhode Island ponds

Land use	200 m buffer			
	r	r^2	Overall *p*	Single variable p[b]
RES +	0.87	0.75	0.0004	0.02
LVSTK +				0.40
CROP +				0.44
VEG				0.23
RES +	0.84	0.71	0.0002	0.04
LVSTK +				0.78
VEG				0.26
RES +	0.84	0.71	5.5×10^{-5}	0.02
VEG				0.26
RES	0.84	0.71	1.4×10^{-5}	–

[b] *p* value for the conditional test of individual variables on top of all other variables.

3.2. Mussel land use correlations

Land use practices in the three zones (i.e., 100 m buffer, 200 m buffer, and watershed) varied widely among the lakes and ponds (Table IV). The fraction of residential land use ranged from 0 to 0.82, and decreased when moving from 100 m buffer to watershed for all but three of the ponds with an average decrease of 44.3%. The fraction of natural vegetation increased from the 100 m buffer to the watershed in a majority of the ponds (14 of 19) with an average increase of 8.3% across all ponds.

Mussel nitrogen isotope values ranged from 4.9 to 12.6‰ (Table III). The highest three isotope values were found at sites in heavily populated urban areas (Warwick Pond, Gorton Pond, and Tiogue Lake) and the three lowest values were measured at sparsely populated sites in heavily wooded areas (Quidnick Reservoir, Tucker Pond, and Eisenhower Lake). Regressions of the fraction of residential land use with mussel δ^{15}N values showed significant positive correlations ($p < 0.05$) for each of the zones around the Rhode Island lakes and ponds (Table Va). Significant negative correlations were also found for regressions of fraction of natural vegetation versus mussel δ^{15}N values in each of the zones. Regressions of the fraction of both feedlot and row-crop agriculture with mussel δ^{15}N showed no significant correlations.

To develop the predictive model, multiple regressions of the fraction residential, feedlot and row-crop agriculture, and natural vegetation with mussel δ^{15}N were performed for each of the three zones around the ponds. Although all of the regressions were significant at $p < 0.05$, the regression for the 200 m buffer zone showed the highest r^2 value, and was therefore selected for further investigation. In this regression, the fraction of row-crop agriculture showed the highest single component p value, and was therefore eliminated and the regression re-run (Table Vb). Examination of the single component p values of the resulting correlation indicated that the addition of the fraction of feedlot agriculture did not significantly improve the regression ($p > 0.05$), and therefore this component was eliminated. At this point, the regression was re-run, and the result indicated that fraction natural vegetation did not significantly improve the regression. We eliminated this fraction, and the resulting correlation of fraction residential land use with mussel δ^{15}N ($r^2 = 0.71$, $p < 0.0001$) was used as the predictive model:

$$\delta^{15}N_{pred} = 7.8 \times (RES) + 5.9‰ .$$

3.3. Testing the Predictive Model

Fraction land use categories showed predominantly natural vegetation in all of the zones around the Connecticut ponds used to test the model, with an average value of 0.69 across all ponds (Table VI). Fraction residential development ranged from 0 to 0.50, and decreased when increasing the buffer size from 100 m to the watershed for all but three of the ponds (Lake of Isles, Mashapaug Pond, and Quaddick Reservoir) with an average decrease of 65.9%. Feedlot and row-crop agricultural land was less than 10% of each zone for all of the sites except Amos Lake and Avery Pond. The fraction of natural vegetation increased with increasing buffer size for all of the ponds except Lake of Isles, with an average increase of 25.9%.

The fraction of residential development in the 200 m buffer was used in the model to generate predicted δ^{15}N values, which were then compared to the

TABLE VI

Fraction of residential development, agricultural land use, and natural vegetation (combined forest and wetlands) in the 100 m buffer zone, 200 m buffer zone, and watershed for Connecticut lakes and ponds

Lake/Pond[a]	Frac. RES			Frac. LVSTK			Frac. CROP			Frac. VEG		
	100 m	200 m	Watershed	100 m	200 m	Watershed	100 m	200 m	Watershed	100 m	200 m	Watershed
Amos Lake	0.15	0.12	0.07	0.08	0.14	0.12	0.00	0.00	0.01	0.62	0.59	0.66
Avery Pond	0.13	0.13	0.06	0.00	0.00	0.08	0.09	0.11	0.01	0.45	0 47	0.68
Crystal Lake	0.14	0.09	0.08	0.03	0.08	0.06	0.00	0.01	0.00	0.58	0.62	0.68
Dodge Pond	0.50	0.51	0.19	0.03	0.01	0.01	0.00	0.00	0.00	0.34	0.28	0.72
Gardner Lake	0.03	0.02	0.02	0.00	0.01	0.06	0.00	0.00	0.01	0.70	0.74	0.78
Glasgo Pond	0.05	0.09	0.01	0.02	0.08	0.02	0.00	0.00	0.00	0.44	0.56	0.88
Lake of Isles	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03[a]	1.02[a]	1.01[a]
Bolton Lake	0.17	0.16	0.04	0.03	0.05	0.04	0.00	0.00	0.00	0.48	0.56	0.74
Mansfield Hollow Lake	0.02	0.02	0.01	0.01	0.01	0.04	0.00	0.00	0.00	0.61	0.65	0.84
Mashapaug Pond	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.81	0.88
Moodus Reservoir	0.04	0.04	0.01	0.04	0.05	0.04	0.03	0.03	0.00	0.68	0.73	0.80
Pachaug Pond	0.07	0.06	0.01	0.03	0.05	0.03	0.00	0.01	0.01	0.64	0.70	0.85
Patagansett Lake	0.15	0.12	0.03	0.08	0.06	0.01	0.00	0.00	0.00	0.63	0.65	0.80
Quaddick Reservoir	0.04	0.04	0.05	0.01	0.01	0.02	0.00	0.00	0.00	0.54	0.64	0.77
Rogers Lake	0.23	0.18	0.02	0.01	0.01	0.01	0.00	0.00	0.00	0.45	0.58	0.91
Wyassup Lake	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.86	0.93

[a] Land use fractions are based on the total area of buffer or watershed minus that of the pond or lake itself. The fraction pond or lake area ranged from 0.20–0.63 for the 100 m buffer, 0.17–0.48 for the 200 m buffer, and 0.01–0.21 for the watershed.

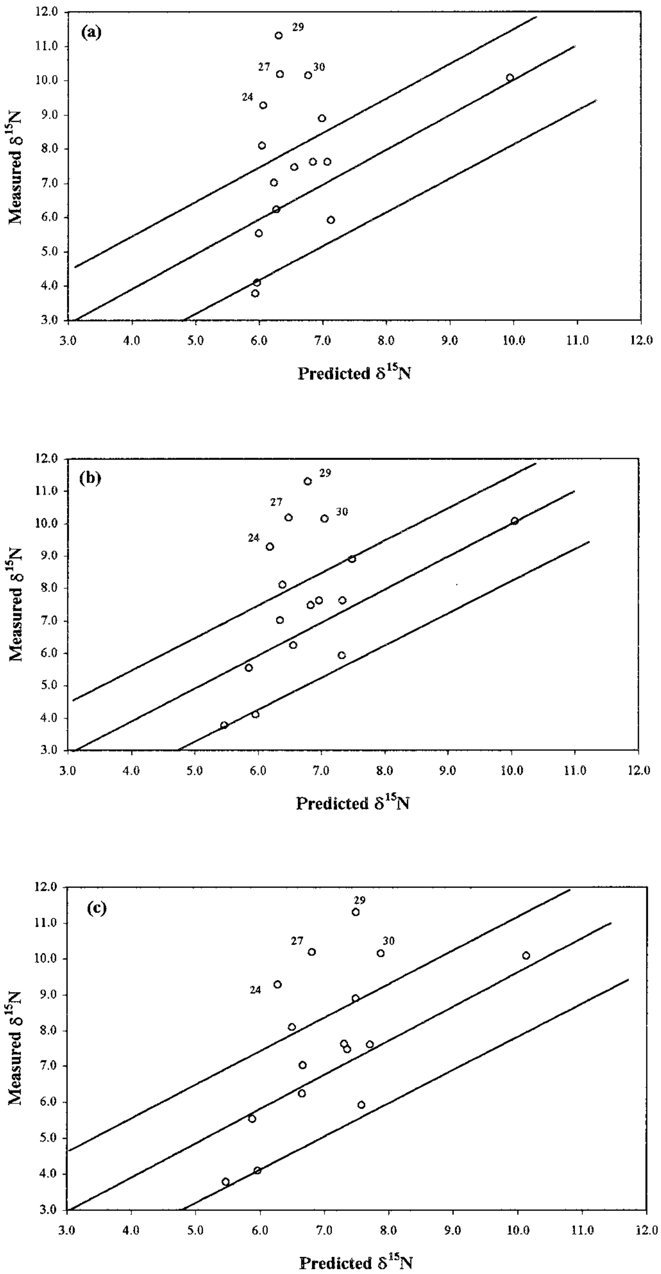

Figure 3. Measured versus predicted mussel δ^{15}N for the 16 Connecticut ponds used to test the model. a) Model based on the fraction of residential development (δ^{15}N = 7.80 × (frac res) + 5.94‰), b) model based on fraction residential development and fraction natural vegetation (δ^{15}N = 7.80 × (frac res) – 2.29 × (frac veg) + 7.80‰), c) model based on fraction residential development plus fraction of livestock agriculture and fraction natural vegetation (δ^{15}N = 7.80 × (frac res + lvstk agr) – 2.29 × (frac veg) + 7.80‰). Pond numbers: 24 = Gardner Lake, 27 = Pauchaug Pond, 29 = Glasgo Pond, 30 = Amos Lake.

measured δ^{15}N values from mussels collected in the Connecticut ponds (Figure 3). All differences reported between measured and predicted values are absolute, and reported in permil (‰). For comparative purposes, a line of slope equal to one, which represents the model, and lines representing one average difference above and below the model predicted values were drawn on the plots. The average difference between predicted and measured values for the one-parameter model was 1.8‰. Six of the sixteen ponds fall above the upper average difference line, and one pond falls below the lower average difference line for this model (Figure 3a). Because of the significant negative correlation between the fraction of natural vegetation and mussel δ^{15}N in the original data, we added this variable and assessed a two-parameter model in an attempt to improve model performance:

$$\delta^{15}N_{pred} = 5.6 \times (RES) - 2.3 \times (VEG) + 7.8‰ .$$

The average difference between predicted and measured values for this model was 1.6‰ and five of the sixteen ponds fell above the upper average difference line (Figure 3b).

4. Discussion

4.1. Dissolved Inorganic Nitrate Concentrations

The significant correlation of the dissolved nitrate concentrations with both the fraction of residential development in the pond 200 m buffer and with the mussel δ^{15}N values suggests that nitrogen derived from anthropogenic sources is influencing the mussel isotope ratios. However, the nitrate data used in this correlation was the average of three measurements per year for five years. Nitrate concentrations varied considerably with sample time, with highest values typically seen in the spring. Using data from a single sampling date averaged over five years or from a single year did not result in significant correlations. Mussel isotope values only correlated with the long term average concentrations of nitrate which provides evidence that freshwater mussels may integrate the δ^{15}N of primary producers in a manner similar to that of marine mussels in estuarine systems (Fry, 1999; McKinney *et al.*, 2001).

4.2. Mussel Land Use Correlations

Studies have shown that nitrogen derived from residential sources has a distinct range of isotope ratio values. Nitrogen derived from septic wastewater and altered in isotopic composition by volatilization and bacterial transformations that occur during transport via groundwater and surface waters has δ^{15}N values in the range of 10 to 20‰ (Kreitler and Browning, 1983; Gormley and Spalding, 1979; Aravena *et al.*, 1993; Macko and Ostrum, 1994). Based on studies linking nitrogen in primary producers to land-derived sources, nitrogen derived from wastewater which is processed and incorporated into biota in an ecosystem would result in a more positive isotope ratio in the biota (McClelland *et al*., 1997; McClelland and Valiela, 1998; Aguilar *et al*., 1999). We report mussel δ^{15}N values that range from 4.9 to 12.6‰ and increase with the fraction of residential development within the pond buffer zones. Our results are similar to those reported in mussels in Canadian lakes by Cabana and Rasmussen (1996), who found that mussel δ^{15}N ranged from 1.2‰ in pristine areas to 9.0‰ in areas of high population density. These findings confirm the influence of septic wastewater nitrogen to aquatic systems, and agree with recent studies that report a similar increase in filter feeder δ^{15}N with increasing influx of wastewater-derived nitrogen (Yelenik *et al*., 1996; McClelland *et al*., 1997; Fry, 1999; McKinney *et al*., 2001). Correlations with land use practices in the present study suggest that the nitrogen isotope value in lake and pond mussels is influenced by nitrogen derived from anthropogenic activities within the pond watershed.

Significant correlations of mussel δ^{15}N with watershed land use characteristics in this study may be evident in part because of the ability of mussels, as filter feeding primary consumers with relatively long tissue turnover rates, to assimilate and integrate the δ^{15}N of primary producers (McMahon, 1991). Seasonal and species related fluctuations in primary producer δ^{15}N values have been reported to range as high as 10‰ which would potentially obscure any apparent trends in our study (Cabana and Rasmussen, 1996). Laboratory experiments have shown that the marine mussel *Geukensia demissa* reflects the δ^{15}N of its food source slowly, taking up to 1 yr to come to equilibrium with source nitrogen isotope ratio (McKinney *et al*., 2001). These mussels will therefore incorporate fluctuations in primary producer δ^{15}N into an integrated, average δ^{15}N of their food source. Because of their similar physiology and feeding habits, the freshwater mussels in this study should also reflect an average, integrated near-base level δ^{15}N of the pond ecosystem, and thus allow trends in nitrogen isotope values with changing land use characteristics in the pond watersheds to be recognized.

Mussel nitrogen isotope ratios also showed a significant negative correlation with the fraction of natural vegetation in the buffer zones around the pond and in the pond watersheds (Table Va). Natural vegetation in watersheds, in the form of riparian forests, freshwater swamps, and tidal marshes, has been shown to mitigate the transport of nitrogen to receiving aquatic ecosystems by acting as a nutrient sink (Correll and Weller, 1989; Brinson *et al*., 1984; Jansson *et al*., 1994; Vought *et al*., 1994). For example, a riparian deciduous forest was shown to remove up to 85% of the nitrate in both overland flows and shallow groundwater drainage flowing from cropland to hydrologically-linked ecosystems (Correll *et al*., 1992). Our results suggest that natural vegetation in the pond buffer zones and watersheds may be having a similar effect, preventing nitrogen derived from predominately wastewater sources from reaching the ponds and allowing other sources, such as atmospheric deposition, to have a greater influence on the base level isotope ratio seen in our mussel δ^{15}N values. However, we were concerned that in the urban northeast, with its lack of large-scale agriculture, the fraction natural vegetation may essentially equal the difference of the fraction residential from one (i.e., VEG = 1 – RES). We tested for this confounding effect and found that the fraction natural vegetation is significantly different from the difference of the fraction residential in the ponds from one (ANOVA, $p = 0.04$). These results led us to conclude that natural vegetation in the 200 m buffer zones may be influencing nitrogen reaching the ponds.

The land use in the buffer zones and watersheds of the Rhode Islands ponds used to develop the predictive model showed patterns characteristic of the Northeastern Coastal Zone ecoregion (Table IV). Lakes and ponds located within the urban centers (e.g. Warwick Pond, Gorton Pond, Tiogue Lake, Oak Swamp Reservoir, Mashpaug Pond) generally had a higher fraction of residential land use and little agricultural land within each of the buffer zones and watersheds. In general the fraction of residential land use decreased with increasing buffer size from the 100 m buffer zone to the watershed, which demonstrates the tendency of residential development to favor shoreline areas. Several of the ponds and lakes located in rural, forested areas (Boon Lake, Mishnock Lake, Wyoming Pond) had substantial residential development around the pond shoreline, and showed the highest decrease in fraction residential when moving from the 100 m buffer to the watershed. Most of the sites showed a concurrent increase in the fraction of natural vegetation as the buffer zone size increased.

Regressions between mussel δ^{15}N and land use used to develop the predictive model indicated that the fraction of residential development and fraction of natural vegetation explained most of the variance in the data for all zone sizes (Table Vb). A comparison of r^2 values showed that the 200 m buffer zone gave the best fit relationship between mussel δ^{15}N and land use, and this zone was selected for further refinement. This finding is consistent with the hypothesis that the influence of nitrogen is proportionally larger from septic systems located near receiving water bodies (Valiela *et al.*, 1992). Anthropogenic nitrogen transported through groundwater is subject to transformation and attenuation; the transport time required for nitrogen of distant origin may result in less of that nitrogen reaching the receiving water body. Based on data from studies in an estuarine system in which nitrogen is primarily transported via groundwater, Valiela *et al.* (1997) suggest that septic systems within 200 m of the shore are likely to make significantly greater contributions to nitrogen loading.

After eliminating the fractions of feedlot and row-crop agriculture from the 200 m regression, analysis of single component p values indicated that the fraction of natural vegetation did not significantly improve the relationship between mussel δ^{15}N and land use, and this variable was dropped from the regression to arrive at a predictive model based on fraction residential development.

4.3. Testing the predictive model

The model shows a relatively poor fit between predicted and measured mussel δ^{15}N values, with those from six of the ponds above the average difference between measured and predicted values. Of these, four of the ponds (the four highest outlier ponds), Glasgo, Amos, Pauchaug and Gardner show greater than 3‰ difference between measured and predicted (average value 3.9‰). Based on the significant negative correlation of fraction natural vegetation with mussel δ^{15}N and evidence that natural lands can influence the delivery of nitrogen to receiving water bodies, we refined the model by adding the fraction of natural vegetation to the regression. This improved the model performance somewhat, and decreased the average difference of the four highest outlier ponds to an average of 3.0‰ (Figure 3b). The differences between measured and predicted mussel δ^{15}N values may result from i) errors inherent in the model itself or in the applicability of the model, including differences in the scale and categorization of available land use data, ii) other anthropogenic inputs or sources of nitrogen, not accounted for by GIS land use data, and iii) differences in physical characteristics of the lakes and ponds, that may influence the ecosystem base level isotope ratio as represented by mussel δ^{15}N.

The selection of lakes and ponds used to test the model was limited by accessibility and the availability of mussels. Freshwater mussel populations can be influenced by natural factors including the availability of fish stocks as reproductive hosts and the presence of suitable habitat (Lee and DeAngelis, 1997; Sietman *et al.*, 1998). As a result, the test ponds exhibit a somewhat more narrow and lower range of residential land use than the Rhode Island ponds use to develop the model. This difference in the range of general land use between data sets may also be a source of model variability. Other potential sources of error are differences in data sources, classification systems, and scale in the GIS data used to describe land use around the Connecticut and Rhode Island ponds. Generally, the RI data were developed at a larger scale using more recent information. Errors resulting from these factors may be minimized by restricting the model to narrower ranges of parameters such as land use, or by including more ponds in the model.

Inherent variability in the model may arise from its being developed using data from ponds which exhibit a range of water surface areas and depths, or water retention time. These factors may influence how nitrogen is processed, which may result in differences in the measured mussel isotope values used to develop the model. In addition, pond watershed areas may influence the amount and source of nitrogen to the ponds, and differences in the flow path of surface or groundwater through areas of natural vegetation in the pond buffer may result in variability between predicted measured mussel δ^{15}N values. However, in this study the ranges of water surface areas and depths. water retention times and watershed area for the test ponds was similar to those of the ponds used to develop the model. Normalizing mussel δ^{15}N to each of these parameters did not improve the fit between predicted and measured isotope ratios.

Other inputs, sources, or land use characteristics that are not included in the model may influence the degree to which nitrogen impacts the lake or pond. Examples include nitrogen from livestock and related farm operations (i.e., feedlot operations) that may be have elevated δ^{15}N values, inputs from recent residential development not reflected in GIS data, and increased inputs from failed or inefficient septic systems. Of these sources, nitrogen from livestock can be related to the fraction of feedlot agriculture determined from GIS data. Field surveys conducted as part of this study revealed that several of the test ponds, including Amos Pond, had feedlot operations in close proximity to the ponds. During transport via groundwater to the receiving water body, livestock nitrogen may be presumed to be subject to the same transformations and processes that affect nitrogen from human septic wastewater (Wada and Hattori, 1991; Valiela *et al.*, 1997), and hence may have elevated δ^{15}N values. Therefore nitrogen derived from agricultural land which primarily consists of livestock operations may actually cause an increase in base level δ^{15}N of an ecosystem, as opposed to nitrogen from agricultural land which employs synthetic fertilizers which would tend to decrease the ecosystem base level isotope ratio (Freyer and Aly, 1974). To account for this input, we added the fraction of feedlot agriculture to the fraction residential development and recalculated predicted isotope ratios using the two-parameter model, resulting in a slight decrease in the overall average difference between measure and predicted (from 1.6 to 1.4‰), and a decrease in difference of the four highest outlier ponds to an average of 2.6‰ (Figure 3c).

Other sources of nitrogen input to lakes and ponds include that from recent residential development not represented in the available GIS data. We saw examples of this in Pauchaug Pond, which has extensive new development, with many new houses built in the last 5–10 yr concentrated along its shoreline, Amos and Avery ponds and Gardner Lake. This development would not be included in the 1987–1990 GIS data from which the predicted values are derived, which may result in erroneously low predicted values.

Failing septic systems may also increase the amount of septic nitrogen input from residential development. For example, the development that surrounds Glasgo Pond is comprised of mostly older houses (majority of the structures built before 1960), with some dating back to the early 20th or late 19th century. It has been demonstrated that some percentage of septic systems do fail outright or lose efficiency over time, often in as little as 12 yr (Cotteral and Norris, 1969). Older developments such as those surrounding Glasgo Pond may have septic systems that, through loss of efficiency or failure, may release more nitrogen that will ultimately be transported to and impact the pond.

Using the proposed model to assess an ecosystem base level isotope ratio as reflected in mussel isotope values can result in information about the source of nitrogen to inland water bodies which can be used in conjunction with GIS land use data. Mussel δ^{15}N values may be useful in providing insight into anthropogenic nitrogen inputs that may not be immediately apparent such as those that result from nitrogen from livestock and related farm operations, recent residential development, and increased inputs from failed or inefficient septic systems. This information could potentially be of use to managers and regulators when assessing the impact of human activities in pond watersheds. However, further refinement of the model is needed and may require inclusion of more extensive data from aquatic systems exhibiting a broader range of physical and land use characteristics. In addition, relatively little is known about rates of denitrification and nitrogen fixation and their effect on ecosystem nitrogen isotope values, and these processes may have to be accounted for and incorporated into subsequent models for broader applicability. The model will also need to be tested with data from sites located in different ecoregions to discern differences related to soil type, atmospheric nitrogen input, and type of natural vegetation.

5. Conclusions

Nitrogen isotope ratio values of mussels from a set of Rhode Island ponds located within the Northeastern Coastal Zone ecoregion correlated significantly with the average nitrate concentrations in the ponds. This suggests that the mussels may be acting as long term integrators of the isotope ratios of primary producers, and were therefore reflecting an average base level δ^{15}N for the ponds. Significant correlations of mussel δ^{15}N and fraction of residential land use in 100 and 200 m buffer zones around the ponds, as well as in the pond watersheds, suggest that nitrogen from septic wastewater is impacting the biota in the pond. The mussel isotope values also show a significant negative correlation with the fraction of natural vegetation in the 200 m buffer zone, which indicates that forested lands and wetlands in close proximity to the ponds may be acting as nitrogen sinks. Multiple regressions of fraction residential development, fraction agricultural land, and fraction natural vegetation with mussel δ^{15}N in the Rhode Island ponds showed that residential development in the 200 m buffer zone explained most of the variance in the data, and this regression equation was used to develop a model to predict mussel δ^{15}N from residential land use data.

Data from a separate set of lakes and ponds within the same ecoregion showed that the model based solely on the fraction of residential development underestimated the mussel δ^{15}N in several of the test sites. Including the fraction of natural vegetation and feedlot agriculture improved model performance, and resulted in the best fit between predicted and measured isotope values for these test data.

Possible sources of error in the model include differences in the range of residential land use between the sites used to develop the model and the test sites, and differences in the scale and categorization of land use data used to generate the predicted values. Physical characteristics of the lakes and ponds and chemical or biological processing of nitrogen (e.g., rates of denitrification and nitrogen fixation) may also influence the measured mussel δ^{15}N values.

However, other inputs, those that the proposed model would ultimately help to elucidate, may in this test case actually be adding to differences we observed between predicted and measured values. These include nitrogen from recent development not included in available land use data, and from sources such as run-off from livestock operations and failed septic systems. By pointing out differences between available land use data and measured isotope ratios, the model may give insight into problems resulting from anthropogenic stress within a specific ecosystem, and could be of value to managers and regulators in developing general policies or strategies regarding monitoring and assessment of eutrophication in lakes and ponds.

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