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Establishing an Anthropogenic Nitrogen Baseline Using Native American Shell Middens

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Narragansett Bay, Rhode Island, has been heavily influenced by anthropogenic nutrients for more than 200 years. Recent efforts to improve water quality have cut sewage nitrogen (N) loads to this point source estuary by more than half. Given that the bay has been heavily fertilized for longer than monitoring programs have been in place, we sought additional insight into how N dynamics in the system have historically changed. To do this, we measured the N stable isotope ($\delta^{15}N$) values in clam shells from as early as 3000 BP to the present. Samples from Native American middens were compared with those collected locally from museums, an archeological company, and graduate student thesis projects, during a range of time periods. Overall, $\delta^{15}N$ values in clam shells from Narragansett Bay have increased significantly over time, reflecting known patterns of anthropogenic nutrient enrichment. Pre-colonization midden shell $\delta^{15}N$ values were significantly lower than those post-European contact. While there were no statistical differences among shells dated from the late fifteenth century to 2005, there was a significant difference between 2005 and 2015 shells, which we attribute to the higher $\delta^{15}N$ values in the effluent associated with recent sewage treatment upgrades. In contrast, the $\delta^{15}N$ values of shells from the southern Rhode Island coast remained constant through time; while influenced by human activities, these areas are not directly influenced by point-source sewage discharge. Overall, our results show that this isotope technique for measuring $\delta^{15}N$ values in clam shells provides useful insight into how N dynamics in coastal ecosystems have changed during thousands of years, providing managers vital historical information when setting goals for N reduction.

Keywords: shell, nitrogen, stable isotope, midden, *Mercenaria mercenaria*, *Crassostrea virginica*

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INTRODUCTION

Narragansett Bay (RI, USA) has undergone appreciable change in its physical and biological properties in recent years, some intentional and others indirect. In particular, the bay receives most of its anthropogenic nitrogen (N) from sewage. During the last decade, all of the major sewage treatment plants discharging to the bay and watershed have upgraded to tertiary treatment (Nixon et al., 2008; Krumholz, 2012). These upgrades have reduced sewage N loads to the bay

by more than half, from 16 to 20 mg L^{-1} to less than 8 mg L^{-1} (Krumholz, 2012). The impetus for the upgrades was to improve water quality in the upper portions of the Bay, where bottom water hypoxia is common in summer months (Bergondo et al., 2005; Spaulding and Swanson, 2008; Codiga et al., 2009). The bay also has warmed by ∼1.6°C during the past 50 years, and long term datasets show trends in decreased wind speeds and increased cloudy days (Pilson, 2008; Fulweiler et al., 2015). Others have observed declines in surface water chlorophyll concentrations and there is emerging evidence for decreased benthic-pelagic coupling (Fulweiler and Nixon, 2009). Distinguishing between ecological responses to reduced N conditions and the increasingly apparent effects of climate change, therefore, is difficult and has important implications for future management scenarios.

Ultimately, managers want the best possible water quality in Narragansett Bay, which, ideally is as close to pre-European contact conditions as possible. Giovanni da Verrazzano discovered Narragansett Bay for Europe in 1594 (Wroth, 1970), after which began a period of colonization and extensive land clearing. Nixon (1997) estimated that pre-contact dissolved inorganic nitrogen loads to Narragansett Bay, from the land were on the order of 4.7–23 × 10^6 mol y^{-1}, while loads of dissolved inorganic phosphorous were about 0.3 mol y^{-1}. When considered on a per unit area basis, these estimated N inputs were far lower than those for well-known oligotrophic systems like the Sargasso Sea and North Central Pacific Gyre (Nixon et al., 1996, 2008). With such low pre-contact terrestrial nutrient loads, some 80–90% of the N inputs to the bay may have come from offshore sources (Nixon, 1997). In contrast, Nixon (1997) estimated that, at least in the mid-1990s, the offshore contribution was only 15% of the N load to Narragansett Bay.

Overall, Narragansett Bay has been highly impacted by human activities for more than 200 years (Nixon, 1995). The US Industrial Revolution began in the Narragansett Bay watershed in 1790 and led to rapid development and associated increases in both human populations and manufacturing wastes. There was also massive land-clearing co-occurring and by 1850, 80% of Rhode Island was classified as farmland (Hooker and Compton, 2003). The biggest increase in N inputs to the Bay, however, happened at the end of the nineteenth century, when the city of Providence's sewer system was completed (**Figure 1**; Nixon et al., 2008). Populations served by the sewer systems around Narragansett Bay have been stable since the 1950s, but the methods of wastewater treatment have changed (see Nixon et al., 2008 for a more detailed discussion).

As recent efforts to reduce nutrient loads and improve water quality have been increasingly successful, it seems reasonable to ask what a remediated Narragansett Bay might look like. Unfortunately, we have no direct knowledge of the system prior to large-scale alteration; our water quality and ecological datasets do not extend back that far. Carmichael et al. (2008) and Kovacs et al. (2010) developed methods to measure nitrogen stable isotope ratios ($\delta^{15}N$) recorded in clam and oyster shells during life, where organic N, bound in the shell's matrix in seasonal depositional bands, reflect the bioavailable N at the time of deposition. By looking at the stable isotope ratios of this N, we can understand something about the N sources to the clam or oyster. These methods allow us to measure $\delta^{15}N$ values in shells from a range of time periods, including those from Native American shell middens, to provide data on historical $\delta^{15}N$ values. Such data could provide insight into N dynamics in both modern and pre-European contact environments and establish an anthropogenic baseline from which to compare alterations in $\delta^{15}N$ associated with changes in source N loads (as also demonstrated by Darrow, 2015).

A number of stable isotope studies have been conducted in Narragansett Bay to discern the impact of human sources of N on the ecosystem. Some have focused on the sediment (e.g., Garber, 1982; King et al., 2008), and a core from the Providence River Estuary showed a pronounced 1.7‰ shift after 1950 that was associated with rapid residential development (King et al., 2008). Other studies have focused on how N from sewage treatment plants is taken up and distributed throughout the bay ecosystem (e.g., Chaves, 2004; Oczkowski et al., 2008; DiMilla et al., 2011; Schmidt, 2014; Pruell and Taplin, 2015). Collectively, these studies provide context from which to assess shell $\delta^{15}N$ isotope data. Schmidt (2014), Oczkowski et al. (2008), and Chaves (2004) observed gradients in $\delta^{15}N$ values in particulate material and macroalgae from the urban upper Bay to the more marine dominated lower Bay. Pruell and Taplin (2015) made similar observations in juvenile winter flounder. In contrast, the $\delta^{15}N$ values in hard clams (*Mercenaria mercenaria*) were spatially homogenous, with mean values of 13.2 ± 0.5‰ (n = 485, Oczkowski et al., 2008) in 2006 and 13.7 ± 0.5‰ in 2012 (n = 166, Schmidt, 2014). Because the observed values were characteristic of sewage-enriched systems, Oczkowski et al. (2008) suggested the hard clams might be consuming phytoplankton supported, in part, by sewage effluent. Between 2006 and 2008, the $\delta^{15}N$ values in macroalgae in the Providence River Estuary and upper Narragansett Bay increased by about 1‰, potentially associated with the upgrade from secondary to tertiary treatment. A recent study has demonstrated that, while concentrations of bio-available N in tertiary treated effluent has been greatly reduced, the denitrification process has increased effluent $\delta^{15}N$ values by about 7‰ (Schmidt et al., 2016). Thus, while N inputs have been reduced, the signature of that N has increased substantially.

To better define how N dynamics in the Narragansett Bay system have historically changed, we measured $\delta^{15}N$ values in clam shells from as early as 3000 BP to the present. We hypothesized that the $\delta^{15}N$ values would record significant differences in nitrogen dynamics, both spatially and over time, in shells from Rhode Island waters. We suggest that the pre-European Contact values provide a quantitative metric from which to compare more recent changes in N sources. Also, to calibrate shell data as a proxy for $\delta^{15}N$ values typically measured in tissues, the $\delta^{15}N$ values in clam shells from 2005 and 2015 were compared to values in soft tissues.

MATERIALS AND METHODS

Sample Collection

Hard clam (*M. mercenaria*) and oyster (*Crassostrea virginica*) shells from before 2005 were obtained from museums,

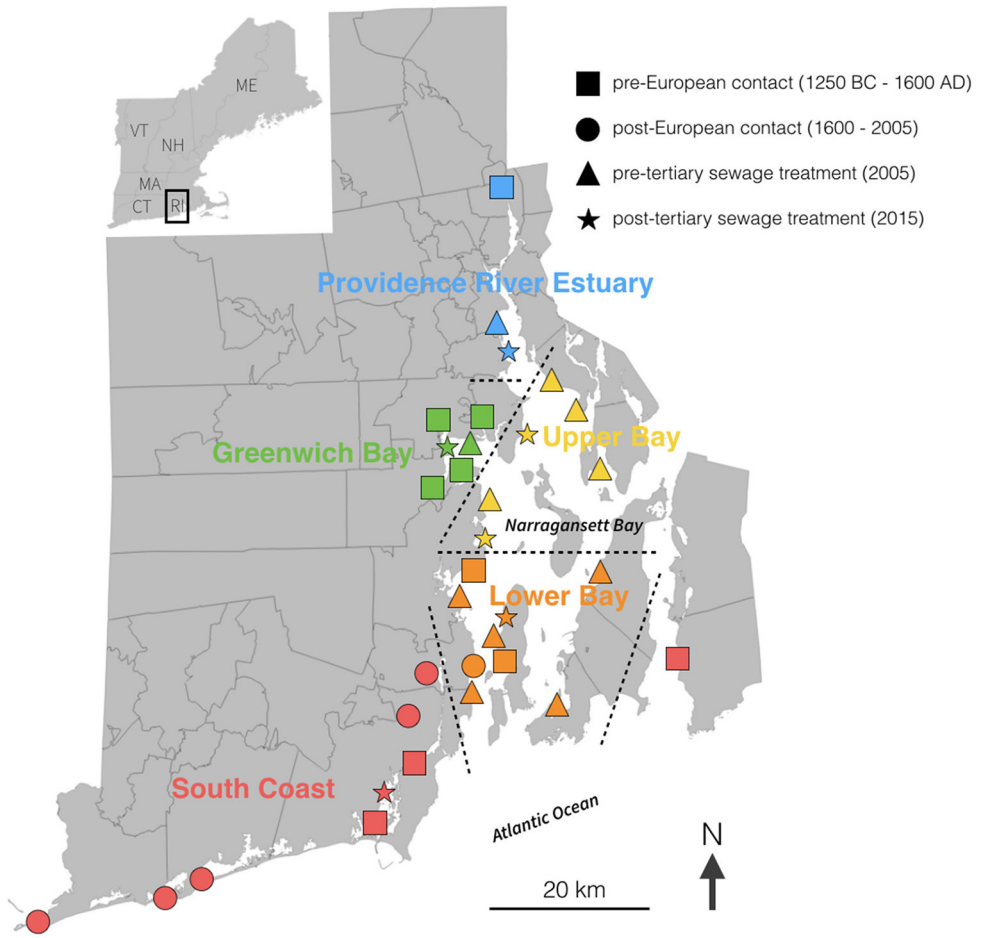

FIGURE 1 | Map of Rhode Island showing sample locations and regional delineations. The shapes of the symbols indicate the time of sampling and colors indicate region. The regional delineations are further illustrated by dashed lines. More detailed information on sample type (clam or oyster) and time period are provided in **Table 1**.

archeological research groups, and colleagues (see **Table 1**), where the next most recent shells were from 1983. All shells were stored dry. While few oyster shells were available, we included them here to provide additional context for the clam shell dataset and to contribute to the research community's understanding of species specific shell stable isotope values. Shells from 2005 were collected in subtidal regions by the Rhode Island Department of Environmental Management (RI DEM) and subsamples were used in a sclerontological project (Henry, 2007). Hard clam samples from 2015 were collected by hand in intertidal regions or obtained from the RI DEM (**Table 1**). Overall, whole shells were similarly sized, with a mean length of 6.2 ± 1.8 cm and width of 4.2 ± 1.3 cm. Tissues (foot) from some of the 2005 Narragansett Bay clams and the 2015 clams were retained for stable isotope analysis (Oczkowski et al., 2008, 2010).

Shell Preparation

Details of shell preparation were described by Kovacs et al. (2010) and Carmichael et al. (2008). Shells were sanded with a Dremel 8220 (Robert Bosch Tool Corporation, Waltham, Massachusetts, USA) until surfaces were smooth. The outer millimeter of shell was removed through this process. The shells were then rinsed with deionized water and dried in a 60°C oven for at least 24 h. Dried shells were ground to a fine powder using a SPEX SamplePrep 8530 ShatterBox (SPEX SamplePrep, Metuchen, New Jersey USA). The powdered shell material was then transferred to acid-washed glass scintillation vials.

It was necessary to remove the carbonate from shell material but leave the N containing organic matter. For shells older than 1950, about 1000 mg of powdered shell was transferred to an acid-washed scintillation vial, but only 500 mg was needed for the more recent shells. Two milliliters of a 1% $PtCl_2$ in 1 N HCl solution was added daily to each vial, until all apparent reaction stopped. About 14 ml of solution was needed for 1000 mg of powdered shell. The vial contents were syringe filtered through a pre-combusted glass-fiber filter. The syringe was then rinsed with 5 ml of deionized water and this water was also pushed through the same filter. The filter was then dried in a 60°C oven for at least 24 h. Dried filters were pelletized in preparation for analysis.

TABLE 1 | Mean $\delta^{15}N$ values of clam and oyster shells, by location.

Location	Time Period[a]	$\delta^{15}N$ (‰)	Std. Dev.	N	Type	Source
PROVIDENCE RIVER ESTUARY						
Covelands	1000–450 BP	10.9		1	Oyster	PAL
	2005	9.8	0.5	6	Clam	RI DEM, (Henry, 2007)
Gaspee point	2015	13.3	1.4	9	Clam	This study
Gaspee point	2015	15.1	0.8	7	Oyster	This study
UPPER BAY						
Exact location unknown	1913	7.4		1	Clam	Roger Williams Museum
Allen's Harbor	2015	12.6	0.1	3	Clam	RI DEM
Rocky point	2015	11.3	0.9	3	Clam	RI DEM
GREENWICH BAY						
Maskerchugg	3000–1600 BP	10.0		1	Clam	Bernstein, 1987
Greenwich Cove	Terminal Archaic (2700 BP)	4.1	3.0	3	Clam	Bernstein, 1987
Greenwich Cove	Early Woodland I (2000 BP)	3.5	1.9	3	Clam	Bernstein, 1987
Greenwich Cove	Early Woodland II (1700 BP)	7.3	0.5	3	Clam	Bernstein, 1987
Greenwich Cove	Middle Woodland (1100 BP)	6.4	1.3	3	Clam	Bernstein, 1987
Greenwich Cove	Late Woodland (1060–850 BP)	3.4	1.6	3	Clam	Bernstein, 1987
Lower south stream	1000–450 BP	5.8		1	Clam	PAL
	1982	11.8	0.6	3	Clam	Bernstein, 1987
	1983	10.8	0.5	3	Clam	Bernstein, 1987
Station 5	2005	11.2	1.9	5	Clam	RI DEM, (Henry, 2007)
Station 6	2005	10.0	1.8	4	Clam	RI DEM, (Henry, 2007)
Greenwich Cove	2015	11.6		1	Clam	This Study
Greenwich Bay	2015	11.9	0.6	6	Clam	This Study
LOWER BAY						
Fort getty	2015	12.6	0.3	6	Clam	This Study
GSO	2005	11.8	1.1	6	Clam	RI DEM, (Henry, 2007)
Hoskins Park, NK	1000–450 BP	8.6	0.5	2	Clam	PAL
Joyner Site, Jamestown	Transitional Archaic or Early Woodland	9.1		1	Clam	PAL
North of Jamestown Bridge	2015	12.7	0.2	6	Clam	This Study
North Kingstown	Late eighteenth century	11.0		1	Oyster	PAL
Saunderstown	1952	12.2		1	Clam	Roger Williams Museum
South Kingstown	Mid- nineteenth Century	9.5		1	Clam	PAL
West Jamestown	2005	11.4	0.4	6	Clam	RI DEM, (Henry, 2007)
Wickford	2005	12.1	0.9	6	Clam	RI DEM, (Henry, 2007)
SOUTH COAST						
Appleby Site, Pt. Judith	Early Woodland ?	9.4	1.9	2	Clam	PAL
Pt. Judith	2015	11.3	1.4	6	Clam	This Study
Quonochontaug	1953	10.6		1	Clam	Roger Williams Museum
Quonochontaug	2015	8.7	0.5	6	Clam	This Study
Quonochontaug	1000–450 BP	8.1		1	Clam	PAL
Quonochontaug	1000–450 BP	7.5		1	Oyster	PAL
Salt Pond Site- 0110	1000–450 BP	8.0		1	Clam	PAL
Salt Pond Site- 0110	Late/Middle Woodland	8.1		1	Clam	PAL
Watch Hill	1953	10.2		1	Clam	Roger Williams Museum
Watch Hill	2015	10.3		1	Clam	This Study
Weekapaug	1953	7.2		1	Clam	Roger Williams Museum
Peckham Farm—Sakonnet	1000–450 BP	11.1		1	Oyster	PAL
Peckham Farm—Sakonnet	1650–1000 BP	10.6		1	Oyster	PAL

The locations are organized by region and the time period sampled indicates either the date of the shell or the year that the shell was collected. Midden shells were from strata dated using ^{14}C and assigned to a period of North American culture (Terminal Archaic, Early Woodland I and II, Middle Woodland, Late Woodland, etc.). We have given the approximate timespan of these periods. The column labeled "Type" distinguishes between clam and oyster shells. The shells come from a range of sources, as noted, where PAL refers to the Public Archaeology Laboratory, Inc. and the RI DEM is the Rhode Island Department of Environmental Management. Archived shells were also used (Bernstein, 1987; Henry, 2007). Tissue samples came from clams collected as part of this study as well as those collected by the DEM for Henry (2007). All archival and collection shell samples were stored dry.

[a] *This refers to either the approximate age of the midden shells, based on ^{14}C dating, or the year of collection.*

Tissue Preparation

The 2015 clam tissue samples were processed in the same manner as described in Oczkowski et al. (2008), where the foot of each clam was removed, rinsed with deionized water, and dried in a 60°C oven for at least 24 h. The intent was to consistently isolate a clearly identifiable portion of muscle tissue and to exclude stomach contents. Dried clam tissue samples were ground to a fine, homogenous powder using a mortar and pestle and stored in acid-washed glass scintillation vials until analysis.

Stable Isotope Analysis

The $\delta^{15}N$ content of the shell and tissue samples was measured using an Isoprime 100 Isotope Ratio Mass Spectrometer interfaced with a Micro Vario Elemental Analyzer (Elementar Americas, Mt. Laurel, New Jersey, USA). The nitrogen isotope composition was expressed as a part per thousand (per mil) deviation ($\delta^{15}N‰$) from air, where $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$ and R is the ratio $^{15}N/^{14}N$ in either the sample or a reference standard (air). Samples were analyzed randomly and laboratory standards were used to check for instrument drift in each run and to correct for instrument offset. Approximately 20% of the shell samples were extracted and analyzed in duplicate, with replicate $\delta^{15}N$ values measured on separate runs. Samples were reproducible to within 0.30‰.

Data Analysis

To look for differences in nitrogen isotope values across regions and at different periods of time (and associated levels of anthropogenic impact), we grouped the $\delta^{15}N$ data for statistical analysis. Data were assigned regional bins of the Providence River Estuary, upper Bay, Greenwich Bay, lower Bay, and South Coast (**Figure 1**). We considered all regional bins except South Coast to be within Narragansett Bay. While receiving some anthropogenic N from non-point sources (Ernst, 1996), nutrient inputs to the South Coast water bodies are lower than the inputs to Narragansett Bay. Thus, the samples collected at these South Coast water bodies were grouped together. Samples were also binned according to time period: (a) pre-European contact from roughly 3000 BP to 350 BP, (b) post-European contact between 1600 (350 BP) and 2005, (c) 2005, and (d) 2015.

We used a linear mixed-effects model (LME) to evaluate the effects of region and time period, and their interaction, on $\delta^{15}N$ values in clam shells. Where there were statistically significant interactions, Tukey *post-hoc* comparisons were used to determine formal relationships. We tested the data for normality using Shapiro–Wilks test and quantile–quantile plots and all data met statistical assumptions (Zuur et al., 2009). We also calculated Pearson's correlation coefficients between clam shell and tissue $\delta^{15}N$ values. Since there were few oyster shell data, we did not formally test these. Statistical analyses were done in R, version 3.1.3. All values are reported as mean $\delta^{15}N$ values.

RESULTS

Clam Shells

The mean $\delta^{15}N$ values measured in clam shells ranged from 3.4 to 13.3‰ across all regions and time periods (**Table 1**). $\delta^{15}N$ varied by region (F-value = 28.23, $p < 0.001$) and time period (F-value = 54.91, $p < 0.001$), as well as their interaction (F-value = 7.27, $p < 0.001$). In general, clam shells collected from all sites in Narragansett Bay in 2015 had higher values than those from earlier time periods, particularly from those dated pre-European contact (**Figure 2**). In contrast to observations from Narragansett Bay, there were no significant differences among $\delta^{15}N$ values in clam shell from different time periods in the Southern Coast region (F-value = 1.129, $p = 0.347$).

The differences across time were consistent in the mainstem of Narragansett Bay (**Figures 3A,B,D**). In Greenwich Bay, where the most pre-European contact samples were available, all clams from time periods since European contact had significantly higher $\delta^{15}N$ values than pre-European contact samples (**Figure 3C**). But, there were no statistically significant differences among the post-European contact categories in Greenwich Bay. Clam shells from 2005 were not different from shells collected between European contact and 2005 in both the overall, whole Bay comparisons, as well as in the regions where samples were available (Greenwich Bay, lower Bay; **Figure 3** and **Table 2**). However, the 2015 clams from both the Providence River Estuary and Lower Narragansett Bay had significantly

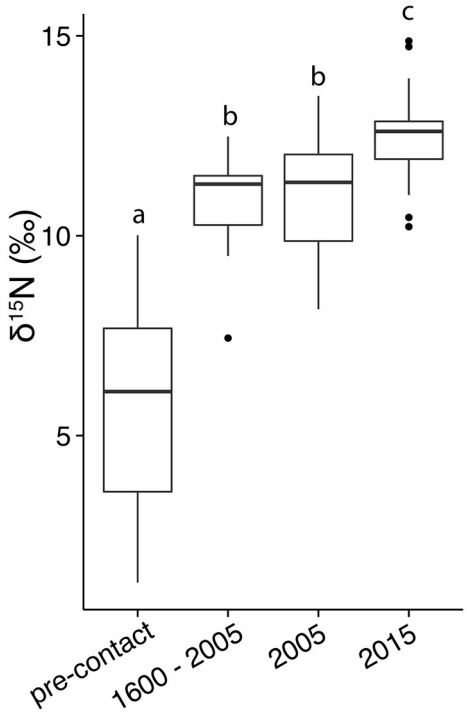

FIGURE 2 | Box and whisker plot of $\delta^{15}N$ values of clam shells from Narragansett Bay (all regions except South Coast) from pre-European contact to present day. Horizontal lines in the middle of the boxes represent the median, the box limits represent the upper and lower quartiles, the vertical lines indicate the maximum and minimum, and the dots lie outside of the 95% confidence interval. Letters indicate homogeneous subgroups using Tukey *post-hoc* comparisons ($p < 0.05$). Sample sizes: pre-contact = 29, 1600–2005 = 13, 2005 = 33, 2015 = 54.

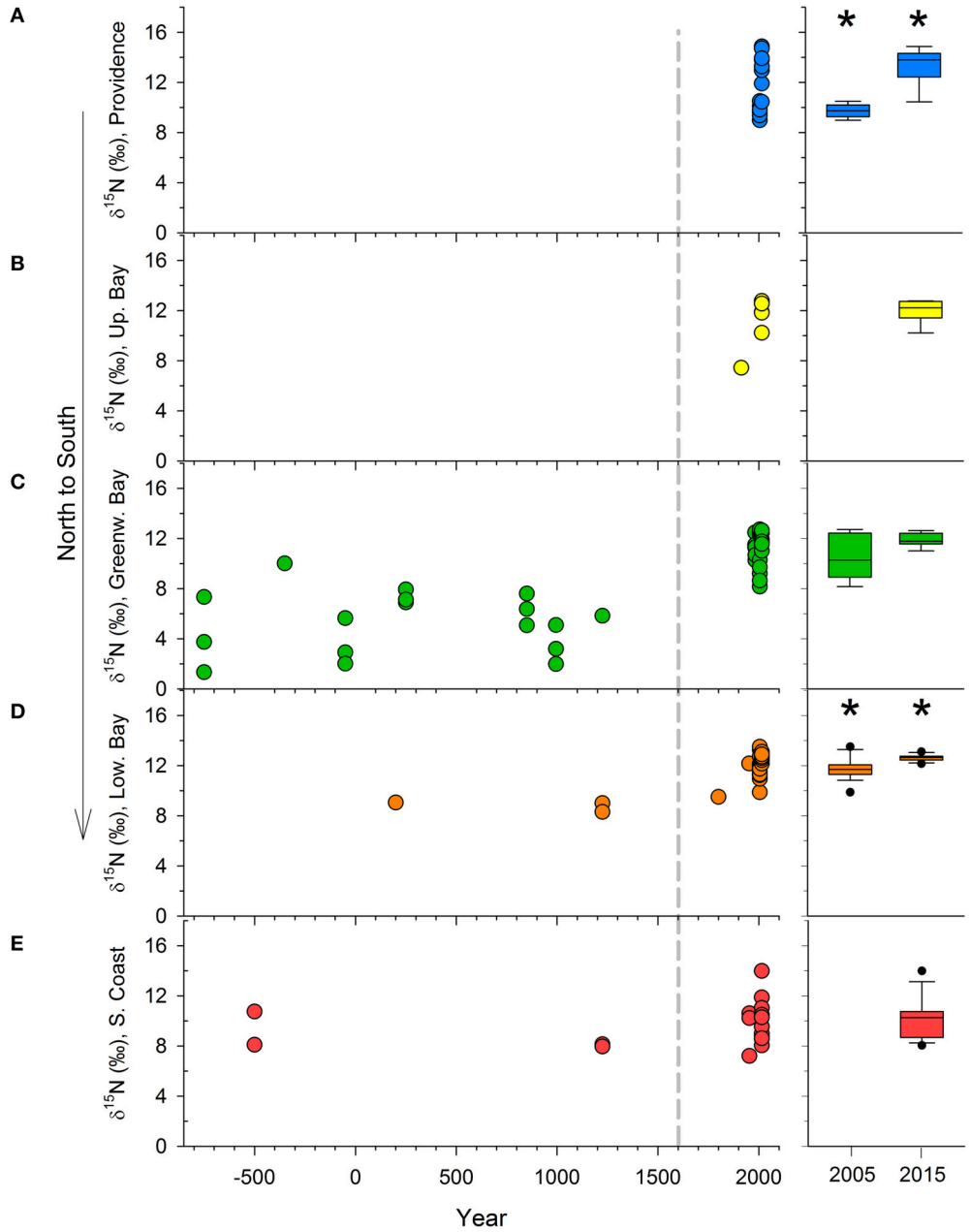

FIGURE 3 | The δ^{15}N values in organic material from clam shells, through time and by region, including the Providence River Estuary (A), upper Bay (B), Greenwich Bay (C), lower Bay (D), and the Southern Rhode Island Coast and Sakonnet (E). The dashed gray line denotes the approximate time of European Contact. For clarity, the 2005 and 2015 data are expanded and given as box and whisker plots in the right column. Asterisks denote significant differences between 2005 and 2015 samples.

higher δ^{15}N values than those from 2005 (**Figures 3A,D**); as with the broader Narragansett Bay dataset.

Regionally, δ^{15}N values in clams from the Providence River Estuary were significantly higher than those from the rest of the Bay and the South Coast regions (**Table 2**, **Figure 3**). Clams from Greenwich Bay and the lower and upper Bay were statistically indistinguishable from one another, but had higher δ^{15}N values than in clam shells from the South Coast (**Table 2**).

Oyster Shells

Oyster shell δ^{15}N values ranged from 7.5 to 15.1‰ among the five midden samples from different places and time periods. The mean δ^{15}N in modern (2015) oyster shells ($n = 7$) was almost 2‰ higher than the δ^{15}N in clam shells ($n = 9$) from the same location (**Table 1**). The two oyster shell samples from middens near the Sakonnet River (on the East side of the bay near the outlet) were ~11‰, similar to midden oyster shells from near the

TABLE 2 | Statistical results from the Tukey *post-hoc* comparisons to determine statistically significant differences among shell $\delta^{15}N$ values over time and across regions, where $p < 0.05$ indicate significance and *t*-values the strength of the significance.

	Providence River Estuary		Upper Bay		Greenwich Bay		Lower Bay	
	t-value	*p*	*t*-value	*p*	*t*-value	*p*	*t*-value	*p*
REGION[a]								
precontact-1600					6.56	< 0.001	3.61	0.005
precontact-2005	−0.79	0.70			6.80	< 0.001	6.68	< 0.001
precontact-2015	2.44	0.06			7.66	< 0.001	8.35	< 0.001
1600–2005					−0.60	0.93	1.96	0.21
1600–2015			4.39	< 0.01	0.58	0.94	3.78	0.002
2005–2015	5.91	< 0.001			1.27	0.59	3.27	0.01
REGIONAL COMPARISONS OF 2015 CLAM SHELLS								
Providence River	–	–	−3.68	0.005	4.10	0.001	3.25	0.02
Upper Bay			–	–	0.18	0.10	−1.04	0.83
Greenwich Bay					–	–	1.30	0.69
Lower Bay							–	–
South Coast	−9.04	< 0.001	3.23	0.015	−3.24	0.02	−5.34	< 0.001

[a]*There were no significant differences among $\delta^{15}N$ values in organic material from clam shells collected outside of Narragansett Bay (e.g., South Coast) in different time categories ($F = 1.13$, $p = 0.35$). Thus, means were not compared.*

Providence River Estuary and North Kingstown (upper Bay). All of these samples were about 3.5‰ enriched compared to shells from middens near Quonochontaug salt pond along the South Coast (**Table 1**).

Shell vs. Tissue

There was a significant correlation between $\delta^{15}N$ values in clam tissue (foot) with organic material in shell ($R = 0.649$, $p < 0.001$; **Figure 4**). Due to sample size, availability of both shell and tissue samples, and distribution at the different sampling locations, these comparisons were only possible for the 2005 and 2015 samples. On average, the difference between tissue and shell ($\delta^{15}N_{tissue-shell}$) was 1.13 ± 1.37‰ for all data, but the mean at each station ranged from −0.8 at Pt. Judith in 2015 to 2.6‰ in the Providence River Estuary in 2005.

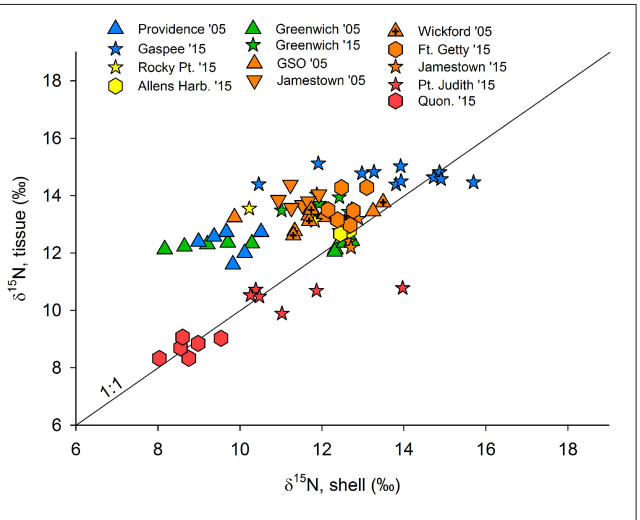

FIGURE 4 | Shell $\delta^{15}N$ values plotted against tissue (clam foot) data, where data are available, namely in 2005 and 2015. Colors indicate sampling regions (shown in **Figure 1**), where blue represents the Providence River estuary, yellow is upper Bay, green is Greenwich Bay, orange is lower Bay, and red indicates the coastal ponds. Quon. is Quonochontaug and a 1:1 line is given.

DISCUSSION

The Anthropogenic Baseline

Our results support the hypothesis that N from clam shells reflects changes in N sources to the bay. The finding that clams from after European contact had higher $\delta^{15}N$ values than those from before contact, for example, is consistent with the ~ five-fold increase in N inputs (predominantly from sewage treatment plants with some degree of advanced treatment) since that time (Nixon, 1997). While the midden $\delta^{15}N$ values were variable, they suggest that baseline, pre-human overprinting of the nitrogen cycle, values were about 6‰. Offshore dissolved inorganic nitrogen concentrations are thought to be <7‰ (Chaves, 2004). In contrast, mean $\delta^{15}N$ values of nitrate (NO_3^-) and ammonium (NH_4^+) were measured as 6.8 ± 0.8‰ and 11.2 ± 0.5‰, respectively, in secondary treatment effluent from the major sewage treatment plants discharging to Narragansett Bay in 2004 and 2005 (DiMilla, 2006). The change in clam shell $\delta^{15}N$ values between 2005 and 2015 reflected the change in $\delta^{15}N$ value of the source N, despite changes in the quantity of N inputs in recent years. When the sewage treatment plants upgraded to tertiary treatment in the late-2000s, the N concentration in effluent decreased by more than half, but the $\delta^{15}N$ values of nitrate in the remaining effluent increased from about 7‰ (2009) to 13.4‰ (2012) (Chaves, 2004; Schmidt et al., 2016). Thus, the source decreased but the $\delta^{15}N$ value of that source increased and was recorded in the shell organic matter of local clams. Although

there are temporal gaps in our dataset and it is likely that the $\delta^{15}N$ values that characterize the anthropogenic N sources to Narragansett Bay were variable through time, our data indicate that those sources have been typically higher than the marine waters that were once the dominant source of N to the bay (Nixon, 1997).

Human sources of N to areas along the Southern Coast are fewer and more diffuse, potentially explaining why clam shell $\delta^{15}N$ values were similar within this region. While there are some water quality concerns in the area, none of the waterbodies receive direct sewage inputs (Bernstein, 1990; Ernst, 1996; Desbonnet et al., 1999). Chlorophyll and nutrient concentrations in Point Judith Pond, one of the Southern Coast ponds, for example, were similar to values in lower portions of Narragansett Bay (Oczkowski et al., 2015), suggesting that the ponds of the Southern Coast were not as enriched by anthropogenic nutrients as the upper portions of Narragansett Bay. As the coastal ponds are within about a 30 mile radius of Lower Narragansett Bay, the chronologically homogenous shell isotope dataset from the South Coast further strengthens the hypothesis that the differences between the South Coast and Narragansett Bay data were due to human-sourced N-loads and not climatological effects, which would be reflected similarly in both regions over this relatively small spatial scale.

The Potential Role of Diagenesis

Diagenesis associated with weathering or breakdown of organic material has also been suggested as a possible source of variation in elemental ratios in ancient shell (Risk et al., 1996; Darrow, 2015). Many stable isotope studies on prehistoric samples, and thus discussions of diagenetic effects, have focused on human and animal remains (e.g., Hare et al., 1991; Larsen et al., 1992; Grupe et al., 2002; Schmidt-Schultz and Schultz, 2004). It is difficult to extrapolate this work to our clam shell data because the organic matter extraction techniques are different and varied. Hence, the effect of diagenesis on the stable isotope values in midden clam shells cannot be known with certainty from the available data. In modeling the impacts on bone, Grupe et al. (2002) found that bacterial degradation could shift the overall $\delta^{15}N$ values higher, on the order of a trophic level. If this were true for clam shells, then ancient midden shells may be even lighter than observed, and the difference between $\delta^{15}N$ values in midden and modern shells could be greater than we reported. Burning alters bone and shell isotope values (DeNiro, 1985; Darrow, 2015), suggesting that the heat associated with cooking also affects $\delta^{15}N$ values (Darrow, 2015). We carefully examined our midden shells for scorch marks and none were present. Other work specific to bivalves, however, suggests organic matter in shell can be well preserved (Risk et al., 1996). Darrow (2015) also found differences in stable isotope ratios between ancient and modern bivalve shell that were distinguishable and dominant due to shifts in N source through time, even when more detailed analyses were conducted to detect the effects of diagenesis. Midden shells analyzed as part of this study were deposited over a period of at least 2000 years, and there were no trends evident (no consistent increase or decrease during that time), suggesting that diagenetic effects were not responsible for the differences observed between midden and more recent shells.

Regional Differences within Narragansett Bay

The 2015 Narragansett Bay shell $\delta^{15}N$ values were consistent with our understanding of anthropogenic nutrient distribution and associated $\delta^{15}N$ values in phytoplankton and macroalgae in the bay (e.g., Krumholz, 2012; Schmidt, 2014), where Providence River clam shells from 2015 had significantly higher $\delta^{15}N$ values than those from the rest of the bay, while the upper and lower Bays were indistinguishable (**Table 1**). Similarly, the 2015 clam tissues had higher $\delta^{15}N$ values in the Providence River Estuary ($\delta^{15}N = 14.7 \pm 0.3‰$, $n = 11$) than the rest of the bay ($\delta^{15}N = 13.2 \pm 0.6‰$, $n = 18$; Oczkowski et al., 2008). We hypothesize that the currently higher $\delta^{15}N$ values in the Providence River Estuary clam shells and tissues reflected the recent tertiary treatment upgrades.

If we assume that anthropogenic N loads did not change substantially during the time period represented by the Greenwich Bay midden samples, particularly compared to more recent fluctuations in N sources, then the observed variability in midden data was unexpected. This variability may reflect inconsistencies in diagenetic processes during the prehistoric period, environmental stress such as periodic hypoxia that affects bivalve condition (Patterson, 2014), localized variability, or large-scale climatic shifts that affected the entire region during the time of deposition. It has been hypothesized that the exploitation of different shellfish species can be linked to paleoecology and, specifically, to cooling water temperatures across the Woodland period (Braun, 1974). It may be possible that the shift in midden $\delta^{15}N$ values over time could be related to some of these shifts. While we cannot elicit a specific mechanism, particularly as there were no comparable midden samples from outside of Greenwich Bay in which to look for similar variability, the effect of climactic conditions on $\delta^{15}N$ values cannot be ruled out. Others have documented shifts in $\delta^{15}N$ values associated with the acidification of sediments, animal tissues, and plants (Bunn et al., 1995; Ryba and Burgess, 2002). But, previous studies on modern clam and oyster shells (Carmichael et al., 2008; Kovacs et al., 2010), indicated the effects of acidification, if any, should be minimal for modern shells when using the method applied for this study. In ancient bivalve shells, acidification was shown to increase variability in stable isotope ratios in some cases, but did not affect mean values (Darrow, 2015), suggesting this may be a factor contributing to variation in our midden shells. For this study, acidification was needed to isolate sufficient organic material for analyses. Because all shells were treated in the same manner, variability due to analytical methods was consistent among shell samples and did not appear to affect broader comparisons among locations or time periods.

The lack of a significant difference in the $\delta^{15}N$ values of the Greenwich Bay post-contact clam shells was not

entirely surprising. The hydrodynamics of Greenwich Bay are quite different from the main bay. Prevailing winds, particularly those that are dominant in the summer, can reduce water exchange between Greenwich Bay and Narragansett Bay (Balt, 2014), contributing to recirculation and, often, poor water quality (Rogers, 2008; Spaulding and Swanson, 2008). Carbon stable isotope data from clam tissues support this observation, where clam tissues reflected a gradient of values in suspended particulate matter from east to west, across Greenwich Bay (**Figure 1**; Oczkowski et al., 2010). The $\delta^{15}N$ values in clam shells from Greenwich Bay may reflect processes like respiration, as well as Providence area municipal sewage δ^{15} N values.

Tissue vs. Shell

To assess the potential for linking our shell-based nitrogen baseline with more recent measurements of isotopes in tissues and primary producers (e.g., food webs), we compared shell and tissue $\delta^{15}N$ values when both were available. While the relationship between clam tissue and shell was statistically significant, it was not highly predictive. The significant, but weak correlation observed between clam tissues and shell appears to be driven by variability in the shell isotope values (**Figure 4**). The average offset of all available tissue-shell pairings was $1.13 \pm 1.37‰$ in the present study, but there were wide variations across stations and between sample years. These ratios are also consistent with ratios observed in a range of bivalve species and tissue types (O'Donnell et al., 2003; Carmichael and Kovacs, 2010). In other studies, however, shell $\delta^{15}N$ has been found to be more highly predictive of tissue $\delta^{15}N$ values (Carmichael et al., 2008; Kovacs et al., 2010), with variation relative to land use among sites greater than variation within sites. These studies, however, sampled bivalves during short periods of time in areas of typically little known change in land use during the period of study. In a system that has recently undergone major shifts in nutrient source abundance and $\delta^{15}N$ signature, the range of $\delta^{15}N$ values in bivalve shell may, at least in part, reflect this recent shift. For example, $\delta^{15}N$ values in individual growth layers of bivalve shell can record wastewater plume entry to an estuary in the form of heavier $\delta^{15}N$ values in subsequent growth layers during the life of a clam (Carmichael unpublished data). Because the turnover time for N in clam tissues is on the order of days to weeks (Carmichael et al., 2008), $\delta^{15}N$ values in soft tissue will consistently reflect N in recently consumed foods. In contrast, N accumulated in shells during the life of the clam is not readily mobilized. Thus, the shell, when analyzed in its entirety, will reflect the mean values of N deposited over the life of the clam. This structural and metabolic difference between shell and soft tissues can result in larger than expected differences in $\delta^{15}N$ values between shell and tissue in systems undergoing change and may explain the within site variation observed among recent shells (2005, 2015) in this study. Comparison of tissue $\delta^{15}N$ values to only the outermost growing region of modern shell could reduce this source of variation. At this point, it is important to acknowledge the ecological uncertainty associated with using clam shell $\delta^{15}N$ values to estimate specific pre-contact food web $\delta^{15}N$ values for this region and dataset.

CONCLUSIONS

The $\delta^{15}N$ values of N deposited in clam and oyster shells, during formation, and collected from historical middens provide useful insight into coastal nutrient dynamics over thousands of years. While such data are not stand-alone metrics of anthropogenic nitrogen inputs, they support evidence and hypotheses posited by others about the long history of N fertilization in Narragansett Bay (e.g., Nixon, 1997; Nixon et al., 2008), where shells harvested before European contact had lower $\delta^{15}N$ values than those harvested more recently. We suggest that the pre-contact shell values represent a baseline from which to compare more recent shell values. Overall, our data support the premise that Narragansett Bay has been heavily and steadily enriched with nutrients for more than a hundred years (Nixon et al., 2008) and recent differences in $\delta^{15}N$ values reflect changes in the source of the fertilization. In Narragansett Bay, where recent upgrades to sewage treatment plants have reduced effluent N concentrations, but increased the $\delta^{15}N$ of the discharge (Schmidt, 2014), 2015 clams had higher $\delta^{15}N$ values than those from 2005 and earlier. This study further illustrates the potential to apply these methods to document anthropogenic N inputs in coastal ecosystems.

AUTHOR CONTRIBUTIONS

AO and AH developed the initial hypothesis, based on the methods developed and contributed by RC. TG, BC, and AO obtained and analyzed samples while AH conducted statistical analyses. AO, RC, and AH contributed to data analysis and all authors contributed to manuscript development.

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