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How the distribution of anthropogenic nitrogen has changed in Narragansett Bay (RI, USA) following major reductions in nutrient loads

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Abstract

Over the past decade, nitrogen (N) loads to Narragansett Bay have decreased by more than 50%. These reductions were, in large part, the direct result of multiple wastewater treatment facility upgrades to tertiary treatment, a process which employs N removal. Here we document ecosystem response to the N reductions and assess how the distribution of sewage N in Narragansett Bay has changed from before, during, and shortly after the upgrades. While others have observed clear responses when data were considered annually, our seasonal and regional comparisons of pre- and post-tertiary treatment dissolved inorganic nitrogen (DIN) concentrations and Secchi depth data, from bay-wide surveys conducted periodically from the early 1970s through 2016, resulted in only a few subtle differences. Thus we sought to use stable isotope data to assess how sewage N is incorporated into the ecology of the Bay and how its distribution may have changed after the upgrades. The nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotope measurements of particulate matter served as a proxy for phytoplankton, while macroalgae served as short-term integrators of water column bio-available N, and hard clams (*Mercentaria mercenaria*) as integrators of water column production. In contrast to other estuarine stable isotope studies that have observed an increased influence of isotopically lower marine N when sewage N is reduced, the opposite has occurred in Narragansett Bay. The tertiary treatment upgrades have increased the effluent $\delta^{15}\text{N}$ values by at least 2‰. The plants and animals throughout Narragansett Bay have similarly increased by 1–2‰, on average. In contrast, the $\delta^{13}\text{C}$ values measured in particulate matter and hard clams have declined by about the same amount. The $\delta^{15}\text{N}$ results indicated that, even after the N-reductions, sewage N still plays an important role in supporting primary and secondary

production throughout the Bay. However, the $\delta^{13}\text{C}$ suggest that overall net production in Narragansett Bay has decreased. In the five years after the major wastewater treatment facilities came on-line for nutrient removal, oligotrophication has begun but sewage remains the dominant source of N to Narragansett Bay.

Keywords

stable isotope; $\delta^{15}\text{N}$; eutrophication; sewage treatment plant; wastewater treatment facility; tertiary treatment; ecosystem response; oligotrophication

Introduction

In what has been labelled as a “Grand Ecological Experiment” (Nixon et al. 2008) for the improvement of water quality, anthropogenic nitrogen loadings have been drastically reduced into Narragansett Bay (Rhode Island, USA) over the past decade. Starting in 2006, all major wastewater treatment facilities (WWTFs) began removing nitrogen (N) from effluent by upgrading from secondary to tertiary treatment (NBEP 2017). The initial target of 50% N reduction was met in 2012, and between 2013 and 2015 additional facilities have upgraded for a total reduction of 62% in sewage N loads from rivers and a 47% reduction in direct WWTF discharges into the Bay since 2000. A recent, post-upgrade budget calculated the total N load from rivers and sewage to this N-limited system at $3,570 \times 10^3 \text{ kg year}^{-1}$ (NBEP 2017), down from an estimated $8,131 \times 10^3 \text{ kg year}^{-1}$ for 1982 to 1983 (Nixon et al. 2008). Total phosphorous (P) loads have similarly declined from $1,053 \times 10^3 \text{ kg year}^{-1}$ in 1982–1983 to $332 \times 10^3 \text{ kg year}^{-1}$ in 2013–2015 (NBEP 2017). Recent work by Oviatt et al. (2017) has documented reductions in mean annual nutrient concentrations along the length of Narragansett Bay as well as decreases in the duration of hypoxia and in system apparent production. Narragansett Bay has a long history of human influence (Nixon et al. 2008) and the recent large-scale reduction in N loading provides a unique opportunity to continue to document recovery of the system.

Narragansett Bay (Figure 1) is a fairly straightforward research estuary. The Bay has a narrow salinity gradient. From Conimicut Point at the top of the Upper Bay to its mouth, it ranges from about 29 to 32 and is generally considered to be partially stratified to well-mixed (Kremer and Nixon 1978; Spaulding and Swanson 2008). The relatively small freshwater inputs primarily enter at the head of the Bay via the Providence River Estuary. Sewage remains the largest single source of nutrients to Narragansett Bay, with 12 WWTFs discharging directly to the Bay and 25 others that discharge to tributaries (NBEP 2017). The bulk of the sewage enters the Providence-Seekonk River Estuary and Upper Bay because the northern portion of the Bay watershed is more urban. Between 2000 and 2010, the sewage N loads that discharged directly to the Providence River Estuary and its tributaries were about 60–70% of the total sewage loads to the Bay (NBEP 2017). Most geochemical and ecological parameters that we measure reflect a gradient from the urban head of the Bay to the mouth. This includes chlorophyll and nutrient concentrations (e.g. Oviatt et al. 2002), metals (in sediment, Wells et al. 2000), benthic community structure (Perez et al. 1999) and a suite of emerging contaminants (Cantwell et al. 2017).

There have been measurements of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) made periodically in Narragansett Bay from the early 1980s (e.g. Garber 1982; Gearing et al. 1991) to the present in both coastal salt marshes (e.g., McKinney et al. 2001; Pruett et al. 2006) and open estuarine waters (Chaves 2004; Oczkowski et al. 2008; Schmidt 2014). Measurements of $\delta^{15}\text{N}$ often reflect anthropogenic inputs, where higher values are indicative of human or animal sources and lower values are associated with lower impact (e.g., Fry 2006). Because of this, the highest $\delta^{15}\text{N}$ values in Narragansett Bay would be expected in the Providence River—the area of the Bay most closely associated with direct and indirect sewage input (Figure 1). However, Chaves (2004) reported higher values in the Upper Bay, measured in particulate matter (PM), than in either the Providence River Estuary or Lower Bay. The isotopically lighter values measured in the Providence River Estuary could have been reflecting preferential uptake of the lighter ^{14}N over ^{15}N in this nutrient rich environment. Lower $\delta^{15}\text{N}$ values in the Providence River Estuary, relative to Upper Narragansett Bay, were also measured in PM, macroalgae, fish, and shellfish prior to the WWTF upgrades (e.g., Oczkowski et al. 2008; Pruett and Taplin 2015; Pruett et al. 2017). Chaves (2004) also suggested that the lower $\delta^{15}\text{N}$ values measured at the mouth of Narragansett Bay were reflecting shelf N influences in this N-limited environment. In contrast to the observed trends in PM- $\delta^{15}\text{N}$, Oczkowski et al. (2008) did not observe a gradient in the mainstem of Narragansett Bay in either $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values measured in the tissues of hard clams (*Mercenaria mercenaria*), which are located in the sediment and filtering food as it passes. They did, however, find a clear gradient in macroalgae where $\delta^{15}\text{N}$ values decreased down the Bay. It was hypothesized that the clams in Narragansett Bay were largely consuming sewage-supported phytoplankton formed in the Upper Bay. The macroalgae integrated $\delta^{15}\text{N}$ values associated with the in-situ bio-available dissolved N, which reflected a mixing gradient from isotopically high ($\delta^{15}\text{N}$) sewage-enriched Upper Bay waters to isotopically lower shelf water nitrogen. Pruett and Taplin (2015) also observed patterns similar to those seen in the macroalgae in young-of-year winter flounder (*Pseudopleuronectes americanus*) collected from nearshore locations.

Stable isotopes are tools that, when considered in conjunction with other water quality and ecological data, allow one to track how the anthropogenic N is distributed through an ecosystem and to quantify responses to major perturbations. There are only a few studies that have used a stable isotope approach to assess the ecological response to nutrient reduction. In a Baltic Sea embayment, a major WWTF was upgraded to tertiary treatment by 1998 (Savage and Elmgren 2004). The $\delta^{15}\text{N}$ in the macroalga *Fucus vesiculosus* declined with distance from the sewage outfall, and there was a substantial decrease in $\delta^{15}\text{N}$ values throughout the embayment after a tertiary treatment upgrade reduced N loads by about 85% (Savage and Elmgren 2004). The post-reduction $\delta^{15}\text{N}$ values were roughly 4‰ lower than earlier measurements, reflecting proportionally more of a marine influence. Similar observations occurred in Moreton Bay, Australia, after a 50% reduction in N loads, for the red macroalga *Catenella nipa* (Costanzo et al. 2005) and in the Moreton Bay estuarine tributaries as measured in filamentous algae and shore crabs (Pitt et al. 2009). While only a few studies have been completed, they provide insight into what might be expected in Narragansett Bay in response to its N load reductions.

Given the foundation of stable isotope studies in this region and our ongoing research, we used these tools to understand how the Bay responded to the nutrient reductions in sewage effluent in the first five years after the improvements. Prior to the upgrades, secondary treated effluent had $\delta^{15}\text{N}$ values of about $6.1 \pm 0.8\text{‰}$ and $11.2 \pm 0.5\text{‰}$ in nitrate and ammonium, respectively (DiMilla 2011). While the dissolved inorganic N (DIN) in the secondary effluent shifted back and forth from being ammonia dominated to nitrate dominated, the DIN in the tertiary effluent is predominantly nitrate with an average value of 13.4‰ (Schmidt et al. 2016). While the N load from the WWTFs decreased by more than half, the $\delta^{15}\text{N}$ of the DIN load increased. Recent work has shown that $\delta^{15}\text{N}$ values in juvenile flounder in Upper Narragansett Bay increased significantly between 2002–2004 and 2012–2014 (Pruell et al. 2017).

We used stable isotopes of N and C to identify how the distribution of sewage N has changed in Narragansett Bay since the WWTF upgrades. In addition to nutrient concentrations and measures of Secchi depth, we focused on water column PM, which is a mix of phytoplankton and other water column particulates, and macroalgae, which integrates water column nutrients over longer time periods. We also used filter feeding hard clams as a sentinel of the food web by measuring $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in their tissues. We hypothesized that the $\delta^{15}\text{N}$ values of primary and secondary producers in the Upper Bay would increase, to reflect the shift in source $\delta^{15}\text{N}$ and that the Middle and Lower Bay $\delta^{15}\text{N}$ values in the plant and animal tissues would decrease to reflect offshore N contributions. We also expected to see declines in $\delta^{13}\text{C}$ values in particulate matter and hard clams in those samples collected after the N reductions as they would reflect decreases in net production associated with lower N inputs (e.g., Oczkowski et al. 2010; Oczkowski et al. 2014). Our results were inconsistent with our hypothesis that the $\delta^{15}\text{N}$ in the estuary would look more marine following the sewage N reductions and support the observation that sewage is still the dominant N source to the Bay's food web (NBEP 2017). But, the $\delta^{13}\text{C}$ data from the clams and particulate matter support observations of an overall decrease in production (e.g., Oviatt et al. 2017). Given how different Narragansett Bay looks from other coastal ecosystems that have experienced similar nutrient reductions, we provide important context for how ecosystem responses to large-scale source reductions should be considered.

Methods

Water clarity measurements

We compiled Secchi depth measurements (in m), as a proxy for water clarity, from a number of different sources (Table 1). All of the monthly values from 1997–1998 were converted from light attenuation coefficient data (K_d , m^{-1}) to estimates of Secchi depth, where Secchi depth was approximated as $1.7 \div K_d$ (Poole and Atkins 1929; Oviatt et al. 2002). About 10% of values from the Narragansett Bay Commission (NBC) dataset for the Providence River Estuary were estimated from K_d (NBEP 2017). As part of the Narragansett Bay Fixed Site Monitoring Network (NBFSMN), the Graduate School of Oceanography (GSO) at the University of Rhode Island also recorded K_d values from 9 stations in our sampling area from 2006–2015, which were converted to measurements of water column clarity (from m^{-1}

to m). Given that we were focused on the impacts during the growing season, we excluded winter Secchi data from our analyses.

Particulate matter & nutrients

Recent samples for particulate matter and nutrients were collected as part of the Bay Ecosystem Time Series (BETS) efforts led by the US EPA's Atlantic Ecology Division. Additional nitrate plus nitrite ($\text{NO}_3^- + \text{NO}_2^-$) and ammonium (NH_4^+) concentration data, collectively known as dissolved inorganic N or DIN, were available from several additional sources, where Narragansett Bay was sampled over a range of locations and at different frequencies (see Table 1). Samples were collected approximately monthly, although extensive ice cover prevented sampling in some winter months, at eight stations (Tables 1, 2) from January 17, 2014 to December 7, 2016. Using a 5L Niskin bottle, the surface, middle and bottom of the water column were sampled. Water was decanted into acid-washed 1L bottles, which were then stored in the dark, on ice, until they were transported back to the laboratory. There, two replicates of approximately 400ml of sample water were filtered through pre-combusted glass fiber filters. The exact volume of water filtered was noted as it varied with water particulate content.

Some of the filtrate was decanted into acid-stripped scintillation vials which were then frozen until samples could be analyzed for inorganic nutrient concentrations. We used an Astoria-Pacific Astoria 2 continuous-flow analyzer (Astoria-Pacific, Clackamas, Oregon, USA) to measure nitrate plus nitrite ($\text{NO}_3^- + \text{NO}_2^-$) and ammonium (NH_4^+) concentrations. The $\text{NO}_3^- + \text{NO}_2^-$ was measured via cadmium reduction (U.S. EPA method 353.2) and the NH_4^+ concentrations were measured using EPA method 350.1. Samples were calibrated against a five-point standard curve, check standards were run every 15 samples and Milli-Q blanks were run every 10 samples.

To determine stable isotope values of the particulate matter, filters were dried in a 60° C oven for at least 24 hours. Once dry, they were pelletized and C and N isotope compositions were determined using a Elementar Vario Micro elemental analyzer connected to a continuous flow Isoprime 100 isotope ratio mass spectrometer (Elementar Americas, Mt. Laurel, NJ). Replicate analyses of isotopic standard reference materials USGS 40 ($\delta^{13}\text{C} = -26.39\text{‰}$; $\delta^{15}\text{N} = -4.52\text{‰}$) and USGS 41 ($\delta^{13}\text{C} = 37.63\text{‰}$; $\delta^{15}\text{N} = 47.57\text{‰}$) were used to normalize isotopic values of working standards (blue mussel homogenate) to the air ($\delta^{15}\text{N}$) and Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) scales (Paul et al. 2007). Isotope values are expressed in δ notation following the formula $\delta X (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ isotopic ratio, respectively. Working standards were analyzed after every 24 samples to monitor instrument performance and check data normalization. The precision of the laboratory standards was better than $\pm 0.3\text{‰}$ for C and N.

Particulate $\delta^{15}\text{N}$ values given in Chaves (2004) and DiMilla (2006) were measured via the US EPA Atlantic Ecology Division's Carlo-Erba NA 1500 Series II elemental analyzer interfaced with a Micromass Optima mass spectrometer. Further, while particulate samples were also collected by Schmidt (2014), the sampling methods and analyses were different

enough that direct comparisons to the other data were not appropriate, thus they were not included in these analyses.

Macroalgae

Samples were collected at low tide from rocky outcrops and the bases of lighthouses positioned mid-channel throughout Narragansett Bay. The goal was to collect samples from below the water line and away from the coast, and any associated seeps, so that the $\delta^{15}\text{N}$ values of the macroalgae would be representative of the water column $\delta^{15}\text{N}$. Collected samples were stored in plastic bags, in the dark and on ice, until they were processed in the lab. Samples were separated by genus and, where possible, species, rinsed with deionized water, and all visible epiphytes were removed. In some cases, when we were unsure of the genus or species, subsamples were frozen and later identified by macroalgal experts (Carol Thornber or Lindsay Green, Personal Communication). Clean samples were dried in a 60° C oven for at least 24 hours and then ground to a fine powder using a mortar and pestle. Ground samples were stored in acid-washed scintillation vials until analysis on the Carlo-Erba NA 1500 Series II elemental analyzer interfaced with a Micromass Optima mass spectrometer, as described for particulate matter.

Analytical methods were also the same across all sampling periods, except the 2015 and 2016 samples were analyzed on an Isoprime 100 Isotope Ratio Mass Spectrometer interfaced with a Micro Vario Elemental Analyzer. In some cases, macroalgal samples were frozen until they could be processed. Freezing macroalgae does not have an impact on $\delta^{15}\text{N}$ values (Oczkowski et al. 2015).

Hard clams

Hard clams (*Mercenaria mercenaria*), known locally as quahogs, were first collected subtidally in 2005 and 2006 from throughout Narragansett Bay. Resultant data were presented in Oczkowski et al. (2008) and DiMilla (2006). Briefly, the foot muscle was removed from individual clams, rinsed with deionized water, and dried in a 60° C oven. Dried tissues were ground to a fine powder using a mortar and pestle and stored in acid-washed scintillation vials until analysis. These methods were repeated again by Schmidt (2014) and in 2015 and 2016, as part of this study (Table 1). Note that the hard clams collected in 2015 were collected manually from shallow, subtidal near-shore waters. In contrast, the other clams were collected via dredge by the Rhode Island Department of Environmental Management and, in a few cases in 2005, by scuba divers (DiMilla 2006; Oczkowski et al. 2008; Schmidt 2014).

Hard clam samples collected prior to 2015 were analyzed on the Carlo-Erba NA 1500 Series II elemental analyzer interfaced with a Micromass Optima mass spectrometer and the more recent samples were measured on the Isoprime 100 Isotope Ratio Mass Spectrometer interfaced with a Micro Vario Elemental Analyzer. Analytical techniques were the same as those described in the macroalgae methods section.

Statistics

To explore the impact of the WWTF upgrades on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ spatially, seasonally, and over time, we used a series of linear regression models; with the exception of the comparison of our particulate matter data to those of Chaves (2004). In this latter case, an analysis of variance (ANOVA) was used because we did not include a continuous variable for latitude in our analysis. The sewage plant upgrade effect was examined in the models by categorizing sampling events as pre-, post-, and during upgrade, where the pre- category included all data from before 2012, before most of the WWTFs upgraded to tertiary treatment, during included data from the transitional period of 2012–2013, and post- included any data from 2014 to the present. When supported by the model fit and data, a three-level ordinal variable was used for the upgrade variable; otherwise, two indicator (binary) variables were used, with pre-upgrade used as a reference category. When sampling occurred throughout the year, seasonality was included in the models using multiple indicator variables. Because the head and mouth of the Bay are oriented north to south, spatial effects were first evaluated by including the latitude of the sampling location in the models. This allowed spatial effects to be quantified as a function of distance. For the Secchi and DIN evaluations, we did not assume a linear effect with latitude. Instead, locations were categorized as Providence (PVD), Upper, Middle, and Lower Bay and fit as separate indicator variables within the model. Additional explanatory variables specific to a given sample type, such as the macroalgae color and water column position of particulate matter samples were included as indicator variables in the associated models. Interactions among explanatory variables were included in all models; when interactions were statistically significant, pairwise comparisons were made between combinations of the two interacting variables using the Bonferroni correction. Data were evaluated for compliance with the normal distribution assumption using the Shapiro-Wilk statistical test and graphical evaluations such as histograms and quantile-quantile plots. Based on these results, the assumption of a normal distribution was determined to be valid for stable isotope results for each of the measured matrices. For Secchi and DIN, the assumption of a normal distribution was not met; however, the assumption of a lognormal distribution was, and therefore these results were natural log-transformed prior to performing the analyses for these parameters. Median and mean Secchi and DIN values of untransformed data are presented for descriptive purposes (Table 3). All analyses were performed at the 95% confidence level, and all analyses were performed using SAS Version 9.2 software.

Results

Water clarity

We compared Secchi data from before 2012 to data after 2014. The WWTF upgrades were ongoing over the course of a few years, but two of the largest dischargers upgraded in 2012 and 2013, making 2013 the first full year of achievement (NBEP 2017). Given the skewedness of the dataset, we looked for statistical differences only in the LN-transformed data. Based on a three-factor ANOVA model with upgrade status (pre vs. post), region, and season (spring, summer, fall), all effects were significant ($p < 0.001$), but differences in the LN-transformed means were small (~ 0.2), especially given the standard deviations (~ 0.4). In the Lower Bay, Secchi depth was significantly more shallow (lower values indicating a less

clear water column) than it was before the upgrades (Table 3) and Secchi depth was also significantly shallower after the upgrades in both the spring and summer.

Nutrients

We used the same statistical ANOVA model to evaluate the nutrient data that was used to analyze the Secchi dataset. All effects, including all two-factor interactions, of LN-transformed data were significant ($p < 0.001$, based on the model term F-tests) in surface water DIN concentrations. Only in the Providence River Estuary was there a significant decline in DIN concentrations after the upgrades (Table 3). In Mid Narragansett Bay, DIN concentrations were significantly higher after 2014. These differences may have been biased by very different numbers of samples, where $n=390$ in the Mid Bay prior to and $n=169$ after upgrades, which could be influenced by inter-annual variability. Only in spring were post-upgrade DIN concentrations throughout the Bay significantly higher than those measured before 2012. In the fall, post-upgrade DIN concentrations were lower when compared to before the nutrient reductions took place.

Particulate matter

The $\delta^{15}\text{N}$ values of the PM were homogenous throughout the water column, with surface, middle, and bottom samples similar for most of the sampling periods (e.g., Figure 2). Our statistical multiple linear regression model found an interaction ($p = 0.042$ for the model interaction term significance test) between latitude and depth, where the change in $\delta^{15}\text{N}$ was greater in the bottom waters, with increasing latitude. Estimated differences among depths were less than 0.3‰. While values throughout the water column generally ranged from 5 to 15‰, there were some notable outliers. Samples collected on 1 May 15 had elevated $\delta^{15}\text{N}$ values at the Lower Bay stations 5, 7, and 8, where the highest values were measured in the bottom waters. Also, on 28 June 16, there was one very high bottom water $\delta^{15}\text{N}$ value observed at station 8. We suggest that this last exceptionally high value may have reflected some sort of contamination, but have included it in our results (although not in our statistical analyses).

There were significant seasonal differences, where $\delta^{15}\text{N}$ values were greater in the summer and fall, compared to winter, based on the above model's season indicator variables' significance tests (Figure 3). To explore the magnitude of these differences, we used the results of our regression analysis to estimate mean $\delta^{15}\text{N}$ above winter values for each season (including spring) and for each region of the Bay (Providence River Estuary, Upper Bay, Mid Bay, and Lower Bay; Figure 3). The magnitude of difference between spring and winter and between summer and winter decreased down the Bay, while the difference between fall and winter showed the opposite trend. To explore whether this down bay increase in fall $\delta^{15}\text{N}$ values might be attributable to production or recycled particulate N, we performed the same statistical analyses with the $\delta^{13}\text{C}$ data and observed trends that were consistent with those seen for $\delta^{15}\text{N}$ (Figure 3).

When comparing the PM data collected in 2000–2002 to those collected in 2014–2016, our statistical model identified a number of interactions, namely between the two time periods and season and between latitude and season. Positive seasonal differences between the

2014–2016 dataset and the 2000–2002 dataset were clear, both in space and time (Figure 4). In general, the most pronounced differences were observed in the fall. These differences were on the order of 2.2‰. Summer and spring differences ranged from about 1.8 to 2.0‰, with a subtle gradient from upper to lower Bay. Differences between datasets were < 0.3‰ in the winter.

High resolution (approximately weekly) PM data collected from 2000 to 2002 (Chaves 2004) at a station in the lower west passage of Narragansett Bay were compared to data collected between 2014 and 2016. When a separate statistical two-factor ANOVA model was fit using data from this location, a significant time period/season interaction was observed ($p=0.0382$ for the model interaction term F-test). Bonferroni pairwise comparisons of the period-specific seasonal difference indicated that there were no significant seasonal differences in the 2000–2002 dataset, but that fall $\delta^{15}\text{N}$ values were greater than spring and winter in the more recent dataset. When comparing the two datasets on a seasonal basis, there were significant increases in $\delta^{15}\text{N}$ values in the spring, summer, and fall, but no significant differences in the winter. Overall, while there were gaps in the datasets, they do illustrate the differences between $\delta^{15}\text{N}$ values measured in particulate matter from two time-series collected fifteen years apart and before and after major perturbations to the nutrient regime of Narragansett Bay (Figure 5).

Macroalgae

The $\delta^{15}\text{N}$ values ranged from about 6 to 16‰ over the length of Narragansett Bay (Figure 6). Because we used attached macroalgae as a passive sampler and integrator of bioavailable water column N, we grouped the macroalgae, which were identified to the genus or species level, by color: brown (Phaeophyta), green (Chlorophyta), red (Rhodophyta). The brown algae consisted primarily of fucoids (e.g., *Fucus spiralis*, *Fucus vesiculosus*), the green primarily of ulvoids, and the red primarily of *Grateloupia turuturu* and *Polysiphonia* sp. The green macroalgae were the most readily available throughout the Bay and Providence River Estuary, although all three groups were present at all sampling locations in the Bay proper. When considering the overall means of all macroalgae collected in the Bay as a whole, there were clear increases in $\delta^{15}\text{N}$ values among the three time periods (Figures 6 and 7).

As macroalgae were collected in the late summer and early fall, no seasonal comparisons could be made. However, based on a multiple linear regression model including latitude and separate indicator variables for color and time period, there were significant differences among colors (red, green, brown), time periods (2005–2007, 2012, 2015–2016), and with latitude ($p<0.001$ for all comparisons based on the regression parameter estimate significance tests). Overall, the $\delta^{15}\text{N}$ of the macroalgae decreased significantly with latitude, with the statistical model estimating a decrease of 0.7‰ per 0.1° latitude, or an overall 1.4‰ decrease down the Bay. Values for $\delta^{15}\text{N}$ increased over time. Between consecutive time periods, there was an average increase of 1.4‰ for brown macroalgae, 0.6‰ for green macroalgae, and 0.8‰ for red macroalgae from pre-2012 to 2012–2013 and 2012–2013 to post-2013. This translates to overall increases of 2.8‰, 1.2‰, and 1.5‰ for brown, green, and red macroalgae over the decade between the first and last sampling periods (Figure 7).

Hard clams

Tissue $\delta^{15}\text{N}$ values ranged from approximately 11 to 16‰, where the lowest measured values were from clams collected in the Providence River Estuary in 2005–2006. Conversely, the highest measured values were also from clams collected in the Providence River Estuary in 2015–2016. Throughout the rest of the Bay, differences among regional means were subtle, with mean values ranging from 12.9‰ (Upper Bay, pre-2012) to 13.9‰ (Upper Bay, post-2013) (Figure 8). While the periods during and after the upgrades were not significantly different, both were different from pre-upgrade (pre-2012). The magnitudes of these differences varied significantly depending on location in the Bay, where the differences were the most pronounced in the Providence River Estuary and Upper Narragansett Bay (Figure 9). Differences between time periods were negligible in the Lower Bay. In contrast to the increased $\delta^{15}\text{N}$ values over time observed throughout the rest of our datasets, the statistical model estimated a slight decrease (0.3‰) in $\delta^{15}\text{N}$ between the earliest (pre-2012) and most recent (post-2013) clam datasets in the Lower Bay (Figure 9).

Carbon stable isotope data were available for some of the 2005–2006 clams as well as for the 2015–2016 clams. Because $\delta^{13}\text{C}$ data were only available for two of the time periods, and due to the spatial patterns observed for $\delta^{15}\text{N}$, separate two-sample t-test comparisons of $\delta^{13}\text{C}$ between the two periods were run for each subarea. There were clear differences between periods for each region, where the more recent data had significantly lower $\delta^{13}\text{C}$ values than the 2005–2006 clams ($p < 0.0001$ for each region) (Figure 8). Overall, clams from 2015–2016 were at least 1‰ lower, on average, than they were in 2005–2006 where mean differences between the two time periods ranged from 1.2‰ in the Upper Bay to 2.2‰ in the Providence River Estuary.

Discussion

Narragansett Bay has been highly impacted by human activities for more than 200 years, with the bulk of the nutrient loads entering at the head of the Bay from sewage (Nixon 2008). Given the timespan of fertilization, the recent WWTF N reductions have been akin to turning off the tap, reducing loads to the lowest ever measured in the system. However, in the first four years following the reductions, the impact of the WWTF reductions on nutrient concentrations in the Bay has been complex. Oviatt et al. (2017) observed pronounced declines in annual concentrations of nitrogen, particularly in the Providence River Estuary and our seasonal analyses found significant, albeit small, declines in surface water DIN concentrations in the Providence River Estuary, coincident with the upgrades. But these water column reductions did not persist throughout the Bay proper in our dataset. In one region mean concentrations significantly increased. Overall, median DIN concentrations were very low south of the Providence River Estuary ($< 5 \mu\text{M}$), both before and after upgrades (Table 3). Nutrient concentrations in Narragansett Bay have always been low during the growing season, even when the Bay was heavily fertilized by secondary treated waste (Oviatt 2008; Krumholz 2012; Schmidt 2014). While the water column DIN concentrations in our analyses did not clearly reflect the source reductions, probably because they have always been very low during the growing season, this does not mean that the upgrades are not impacting the overall net production of this N-limited ecosystem.

Comparisons of Secchi depth measurements from before and after the upgrades also showed small differences, again with counter-intuitive results, where in some locations and in some seasons Secchi depth was slightly shallower after the upgrades. While these differences were statistically significant, we suggest that they may not be ecologically significant, especially given the regional-scale of the analyses, the heterogeneity of sampling frequencies and sites among the different data sources, as well as imprecise conversions of some of the data from light attenuation coefficients to Secchi depths. However, the results are consistent with the more detailed analyses presented in NBEP (2017). That report documents a steady improvement in water column clarity in the Lower Bay from 1972 to 1997, coincident with the decline in chlorophyll concentrations (e.g., Nixon et al. 2009). However, no improvement was seen between 2004 and 2014 (the time spanning the N reductions). In the northern part of the Bay there was a strong inter-annual variability in water clarity, but with no trends. In the summer months, variability in water column clarity was attributed to rainfall, where wet summers were associated with lower water clarity (NBEP 2017). Climate and other variables may mask the influence of water quality improvements associated with sewage upgrades and as responses are ongoing, clear discernment of ecosystem response to the nutrient reductions will likely occur over a longer timescale than we report here (e.g., Boesch 2002; Greening and Janicki 2006).

Narragansett Bay is N-limited, particularly during the growing season (e.g., Oviatt et al. 1995; Kremer and Nixon 1978; Pilson 1985a) and despite eutrophication in the Upper Bay and Providence River Estuary, secondary production is often food limited in the late summer and fall (e.g., Durbin and Durbin 1981; Grassle and Grassle 1984; Rudnick et al. 1985; Campbell 1993). Water column DIN concentrations are very low during this time, frequently <2 μM , throughout the Bay. If bio-available N is quickly taken up by primary producers, then measurements of remaining water column N concentrations would not be the best indicator of anthropogenic N distribution in Narragansett Bay, particularly during the growing season. Further, while measurements of water clarity are useful for monitoring Bay changes, particulate matter can be stirred up during storms, temporarily reducing water column visibility. Also, plankton (and particulate matter) at a station are not necessarily formed at that location, as they drift with the tides. While the residence time of water in Narragansett Bay is on the order of a month (Pilson 1985b), near-surface residual currents are ~ 5 cm s^{-1} (Kincaid et al. 2008). We calculate transport times on the order of seven days from Upper to Lower Bay (distance ~ 30 km). Thus, it is possible, and even probable, that water clarity measurements at a Mid or Lower Bay station are impacted by phytoplankton formed in the Upper Bay, supported by sewage N (e.g., Melrose et al. In Review). Changes in water clarity and production, or lack thereof, may also be influenced by longer term incremental drivers associated with changes in climate. Surface water chlorophyll concentrations in the Bay have been declining for more than 50 years (Borkman and Smayda 2009; Nixon et al. 2009; Borkman and Smayda 2016; NBEP 2017). The decreases have been attributed to increased water column stratification, and subsequently reduced benthic-pelagic coupling due to warmer waters, decreased winds, and warmer, cloudier winters (Pilson 1991; Oviatt 2004; Nixon et al. 2009; Fulweiler et al. 2015). While direct measurements of nutrient concentrations and water clarity can indicate the net impact of these factors, stable isotopes can tease out changing N sources from more long-term stressors associated with

climate change. The $\delta^{15}\text{N}$ values have changed as the source has changed (Schmidt et al. 2016) while any changes in $\delta^{13}\text{C}$ could reflect changes in net production (Oczkowski et al. 2014; Oczkowski et al. 2018).

We propose that the stable isotope data provide the necessary insight to assess changes as bioavailable N is quickly incorporated into primary and secondary production. Spatial trends in $\delta^{15}\text{N}$ values in juvenile winter flounder (*Pseudopleuronectes americanus*) are consistent with our hypothesis that biota in the Providence River Estuary would have elevated $\delta^{15}\text{N}$ values after the treatment plant upgrades (Pruell et al. 2017). Higher $\delta^{15}\text{N}$ values in the Providence River Estuary where DIN concentrations were also high ($>10\ \mu\text{M}$) were likely due to both the elevated $\delta^{15}\text{N}$ values of the source (sewage) N and the decrease in the abundance of bioavailable N. In the latter case, less N available would reduce the ability of the primary producers to preferentially select the metabolically more favorable ^{14}N over ^{15}N (e.g., York et al. 2007; Oczkowski et al. 2008; Thornber et al. 2008; Pruell et al. 2017). We also observed elevated macroalgal $\delta^{15}\text{N}$ values in the Providence River Estuary (e.g., Figure 6).

In fact, macroalgal $\delta^{15}\text{N}$ values increased throughout the Bay across the three different sampling periods (2005–2007, 2012, 2015–2016). The macroalgae, which were fixed to rocky outcrops, integrated *in situ* water column bio-available N; they were autochthonous samplers of water column DIN- $\delta^{15}\text{N}$. The clear down-bay gradients in macroalgae $\delta^{15}\text{N}$ values were consistent over our sampling period, even though the overall values increased over time (Figure 6). While there were differences among color, they all reflected a mixing gradient of high sewage $\delta^{15}\text{N}$ values at the head of the Bay to lower $\delta^{15}\text{N}$ values at the mouth. Most of the green macroalgae (*Ulva* sp.) were annuals and integrated $\delta^{15}\text{N}$ values in water column DIN over a period of days. Some red species, as well as all the brown species sampled, were perennial, with tissue turnover times and N uptake rates that were slower than they were for *Ulva* sp. (Pedersen and Borum 1996; Thornber et al. 2008; Raimonet et al. 2013), perhaps explaining differences among divisions. While the magnitude of change in $\delta^{15}\text{N}$ values over time differed among the divisions, they presented a consistent picture of N-dynamics in Narragansett Bay, with $\delta^{15}\text{N}$ values indicative of tertiary treated sewage. A 2013–2015 nutrient budget for Narragansett Bay calculated that WWTFs are still the dominant source of N to the Bay, accounting for 55% of the total N loading (NBEP 2017).

The particulate matter (PM) dataset further supports these observations. Our statistical model looked at differences among seasons and regions of the Bay (Providence River Estuary, Upper Bay, Mid Bay, Lower Bay) and indicated that there were some clear differences in the spring, summer, and fall PM $\delta^{15}\text{N}$ values among the different regions, across the whole dataset. Winter PM $\delta^{15}\text{N}$ values didn't differ spatially, presumably because low temperatures and shorter day lengths reduced both primary production and WWTF N-removal. The statistical model estimated differences between $\delta^{15}\text{N}$ values in the spring and winter, summer and winter, and fall and winter. There were significant down-bay gradients in the spring and summer data, where the difference between spring and winter ranged from 0.9‰ in the Providence River Estuary to 0.2‰ in the Lower Bay and the difference between summer and winter ranged from 2.2‰ to 0.7‰ (Figure 3). In contrast to these expected down-bay gradients, differences between fall and winter displayed the opposite trend, with

the Providence River Estuary being essentially 0‰ and increasing to 1.5‰ in the Lower Bay. To better understand why the fall data trends were so different, we performed the same analyses using available $\delta^{13}\text{C}$ measurements as they can reflect net ecosystem production, where higher values are associated with high levels of production (e.g., Oczkowski et al. 2014; Oczkowski et al. 2018). The $\delta^{13}\text{C}$ data exhibited the same spatial trends as the $\delta^{15}\text{N}$. We speculate that the fall gradient towards higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the Lower Bay may reflect resuspension of recently deposited organic matter associated with rougher fall weather which would likely show a down-bay gradient like the down-bay gradient in vertical mixing (e.g., Spaulding and Swanson 2008). Or they could be reflecting a seasonal shift as tertiary treatment supported Upper Bay plankton were transported down the Bay during the same season that N removal rates decreased in the WWTFs. The N-removal in the facilities is temperature dependent, with lower rates associated with colder weather (NBEP 2017). The spring $\delta^{15}\text{N}$ values were closest to the winter values. In contrast, the summer values, for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, were much higher than winter and occurred when Narragansett Bay was food limited and DIN concentrations were near zero (Oviatt 2008; Schmidt 2014, Figure 4). Overall, $\delta^{15}\text{N}$ values have increased more than 1.5‰ throughout the Bay during the spring, summer, and fall between 2000–2002 and 2014–2016 (Figures 4, 5).

Based on their study of clam and macroalgae samples collected in 2006, Oczkowski et al. (2008) suggested that sewage-supported plankton, grown in the Upper Bay and transported down-bay, were a significant food source for hard clams (*Mercenaria mercenaria*) throughout Narragansett Bay. The clam $\delta^{15}\text{N}$ values were homogenous throughout the Bay and the authors estimated that as much as half of the N in the clam tissues could be of sewage origin. The clams collected as part of this study, as well as those collected in 2012, support this hypothesis. Clams, like the macroalgae, showed substantial increases in $\delta^{15}\text{N}$ values in the Providence River Estuary. The statistical model-estimated mean values for this system were 12.7‰, 13.8‰, and 14.2‰, for the periods 2005, 2012, and 2015–2016, respectively (Figure 8). Differences among sampling periods, with higher values associated with more recent years, narrowed down the Bay (from Upper Bay to Mid Bay to Lower Bay). The Lower Bay means ranged from 13.2‰ in 2015–2016 to 13.6‰ in 2005–2006. The slight decrease in Lower Bay $\delta^{15}\text{N}$ values could be reflecting increased contributions of shelf N. If this is the case then differences could be more pronounced over time (Figures 8, 9).

While the $\delta^{15}\text{N}$ values in clams, as well as in particulate matter and macroalgae, suggest that sewage is still an important source of N to Narragansett Bay, an observation supported by current nutrient budgets (NBEP 2017), the $\delta^{13}\text{C}$ values in the clams indicate that the net production in the Bay has declined. There were substantial decreases in $\delta^{13}\text{C}$ values between 2005–2006 and 2015–2016 (Figure 8), on the order of 1–2‰, on average. Others have demonstrated that $\delta^{13}\text{C}$ values can reflect net ecosystem production, with higher $\delta^{13}\text{C}$ values indicating more productive systems and vice versa (e.g., Oczkowski et al. 2010; Oczkowski et al. 2018). As the decreases in $\delta^{13}\text{C}$ values were also consistent with longer-term observations of declining chlorophyll values in the Bay (Nixon et al. 2009; Fulweiler and Heiss 2014), we can't specifically identify the WWTF upgrades as the only driver of the changes in $\delta^{13}\text{C}$ values.

A synthesis of ecological responses

While sewage N loads have been reduced by more than half, the predominant source of N to Narragansett Bay is still from WWTFs (NBEP 2017). Our analyses found that, when considered seasonally and regionally, water column DIN concentrations have decreased and water clarity appears to have (slightly) improved in the Providence River Estuary. The stable isotope results illustrate how sewage N still plays an important role in supporting the plants and animals in the ecosystem. While we had expected that the 2015–2016 samples collected in the Lower Bay to more closely reflect marine N sources, the only evidence of this was in the hard clam data where the decrease in $\delta^{15}\text{N}$ values between the earliest and most recent data was $\sim 0.3\text{‰}$. Except for the Lower Bay clams, the $\delta^{15}\text{N}$ values throughout Narragansett Bay have increased over time. This is particularly pronounced in the Providence River Estuary, where increases were typically $>1\text{‰}$. Of course, the upgrades were recent and it is too soon to fully assess the legacy effects of more than a century of heavy nutrient fertilization on the ecosystem. However, dramatic declines occurred in nutrients, turbidity, and chlorophyll concentrations in the year after sewage diversion in Kaneohe Bay, Hawaii (Smith et al. 1981), although the response of Tampa Bay to nutrient reductions occurred over a longer time-period (years to decades) and response was also influenced by weather conditions (Greening and Janicki 2006). A mesocosm study by Oviatt et al. (1984), concluded that water column recovery rates in the “grossly polluted” Providence River Estuary could occur on a short timescale (< 1 year). Mesocosms designed to replicate Mid-Narragansett Bay conditions were outfitted with intact, structurally undisturbed sediments from the Providence River Estuary, a Mid Bay location, and offshore. After five months, the behavior of the Providence River treatments, including seasonal dynamics of plankton and benthic fauna, were more similar to the other treatments than they were to the *in situ* Providence River Estuary conditions (Oviatt et al. 1984). A partially to well-mixed water column and fairly short residence times surely contribute to more efficient recoveries.

Recycling of N, and any associated isotope transformations are impossible to tease out of our bulk analyses, but it is unlikely that benthic N cycling is driving the observed isotope changes. Surface water chlorophyll concentrations and the amount of freshly deposited organic matter are the best predictors of sediment N-cycling in Narragansett Bay (Fulweiler and Heiss 2014). Chlorophyll concentrations have been declining over the past 50 years, indicating long term declines in plankton production (Fulweiler and Heiss 2014) and further declines in nutrient loads associated with the WWTF upgrades also appear to be decreasing Bay-wide production (e.g., Oviatt et al. 2017), observations supported by our $\delta^{13}\text{C}$ dataset. Declines in chlorophyll and production are frequently observed after major nutrient reductions (Boesch 2002), but the challenge in this system is separating responses from long-term trends. There are no apparent long-term temporal trends in N_2 fluxes or rates of sediment oxygen demand (the latter is a good indicator of organic matter loading) at two stations in Narragansett Bay (Fulweiler et al. 2016; Fulweiler, Personal Communication). The work of Oviatt et al. (1984) suggests that legacy nutrient regeneration is unlikely to be the primary drivers of the observed stable isotope trends while the observations of Fulweiler and Heiss (2014) indicate that rates of N-cycling have not increased. Lower N loads could lead to more tightly recycled bio-available N and associated elevated $\delta^{15}\text{N}$ values (e.g., Brandes and Devol 1997; Granger et al. 2004; Granger et al. 2010). But, if this were the

primary driver, we would expect to see down-bay gradients in $\delta^{15}\text{N}$ values lessen or even reverse as N was continuously recycled as it moves through the Bay.

The results presented here are like the few other studies that have used stable isotopes to document estuarine ecological response to WWTF upgrades to tertiary treatment (and N-removal) in the sense that responses were swift (within years) and distinct (e.g., Smith et al. 1981). But, this study also differs from those studies. For example, in Moreton Bay, researchers observed that $\delta^{15}\text{N}$ values decreased after the WWTF upgrades, reflecting more marine sources (Costanzo et al. 2005; Pitt et al. 2009). Savage and Elmgren (2004) made similar observations in the Himmerfjärden Bay of the Baltic Sea. In both examples, declines in $\delta^{15}\text{N}$ values were substantial, about 4–6‰. In contrast, we saw $\delta^{15}\text{N}$ values increase throughout the Bay by 1‰ or more. While sewage N-loads to Narragansett Bay have decreased considerably, they remain the largest source of N to the estuary. This, in conjunction with the documented increases in sewage effluent $\delta^{15}\text{N}$ values, support our suggestion that Narragansett Bay is still heavily influenced by sewage N loads (Figure 10, NBEP 2017). Our study supports the hypothesis put forth in Oczkowski et al. (2008) that sewage supported production in the Upper Bay is an important food source to the mid and lower portions of the Bay.

The reduction in N loads was a relatively discrete (few year) event that co-occurred during other long-term changes in the ecology and phenology of Narragansett Bay. As Duarte et al. (2009) cautioned, the results of this study should be considered in the context of shifting baselines and concurrent changes in other stressors. There is substantial documentation of increased variability in seasonal plankton dynamics, including shifts in the timing of some annual phytoplankton blooms, from the late-winter to the summer, as well as declines in the magnitude of blooms since the 1960s and particularly after the nutrient reductions (Nixon et al. 2009; Oviatt et al. 2017). Zooplankton are better positioned to graze down these warmer weather phytoplankton blooms, which decreases the amount of organic matter reaching the benthos (Oviatt et al. 2002). Others have documented declines in benthic nutrient fluxes and speculate on the potentially important role of N-fixation in the future, particularly in the lower parts of the Bay (see Nixon et al. 2009). While N-fixation could supply the Narragansett Bay water column with a significant amount of ‘new’ N, which would have $\delta^{15}\text{N}$ values close to 0‰, we do not see evidence of this in the stable isotope data, but recognize that the system is ever-changing and the response to the reductions is probably ongoing. Our current assessment could serve as a recovery baseline from which to observe N-dynamics as ecosystem oligotrophication continues. While the $\delta^{15}\text{N}$ data do not reflect the lower values associated with marine contributions or N-fixation, the $\delta^{13}\text{C}$ data, particularly for the clams, are consistent with the observations of declining productivity (e.g., Li and Smayda 2001; Nixon et al. 2009; Fulweiler and Heiss 2014; Oviatt et al. 2017).

In the five years after the major WWTFs came on-line for nutrient removal, and just over a decade since the process began, net production may have decreased, and the process of oligotrophication begun. But, current nutrient budgets show that sewage remains the dominant source of N to Narragansett Bay (NBEP 2017) and our stable isotope data demonstrate that it still plays an important role in supporting primary and secondary ecosystem production.

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The late coastal ecologist Scott Nixon was fond of saying that the wise studied lakes, the less-wise studied rivers, where water flowed in one direction, and only the most foolish studied estuaries where water moved in all directions, changing over the course of a day. This has never been truer when trying to assess the impacts of oligotrophication. We would like to thank the Rhode Island Department of Environmental Management for providing hard clams to us for more than a decade. We would also like to thank Mike Charpentier for creating Figure 1, Steve Granger for assistance in the field during those first collections in the mid-2000s, and to Scott Nixon and Candace Oviatt for all of the valuable data that they have collected, and information and wisdom that they have provided, over the years. Thanks to Lindsey Fields for measuring DIN concentrations and Rebecca Robinson for support to C. Schmidt. We are grateful to Carol Thornber and Lindsay Green of the University of Rhode Island for their help with identifying macroalgae. This manuscript has been improved by input from Roxanne Johnson, Rose Martin, and Brenda Rashleigh. This is ORD tracking number ORD-023673. The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency, NEIWPCC, nor of the McLaughlin Research Corporation. Any mention of trade names, products, or services does not imply an endorsement by the U.S. Government or the U.S. Environmental Protection Agency. The EPA does not endorse any commercial products, services, or enterprises.

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Figure 1. Map showing the Providence River Estuary and the Upper, Mid, and Lower regions of Narragansett Bay. Markers on the map depict the volume of discharge (in millions of gallons per day or MGD) from wastewater treatment facilities (WWTF).

Figure 2.

The $\delta^{15}\text{N}$ values of particulate matter (PM) from January 2014 through November 2016 are given for eight stations in Narragansett Bay. PM was sampled from water collected at the surface, middle, and bottom of the water column at each location. Station locations are given in the inset map.

Figure 3. Seasonal differences among PM $\delta^{15}\text{N}$ data (A) and $\delta^{13}\text{C}$ values (B) are presented as the statistical model estimated mean difference between spring, summer, or fall and winter values. Data were divided into regions of the Bay where PVD is the Providence River Estuary and Upper, Mid, and Lower refer to the regions of Narragansett Bay (see Figure 1). WWTFs are not practicing tertiary treatment in the winter months, when temperatures are cold.

Figure 4.

The statistical model estimated mean differences between PM $\delta^{15}\text{N}$ values measured in 2014–2016 and in 2000–2002 (collected by Chaves 2004) broken out by season and region of Narragansett Bay.

Figure 5.

Individual $\delta^{15}\text{N}$ PM values for samples collected by Chaves (2004), just off the GSO dock, identified in Figure 1, and as part of this study at Station 5, identified in Figure 2. Closed circles represent data collected as part of this study (2014–2016) while open circles represent samples collected by Chaves (2004; 2000–2002).

Figure 6.

Macroalgae $\delta^{15}\text{N}$ values from samples attached to rocky outcrops, organized by latitude. The higher latitude samples were collected from the head of the Bay and lower latitude samples are from the mouth. Samples were organized by color where brown macroalgae are shown in the top panel, green in the middle, and red in the bottom panel. Samples were collected over three different sampling periods: before (2006–2008), during (2012), and after (2015–2016) the WWTF upgrades. Details on data sources are given in Table 1.

Figure 7. Statistical model based estimates of mean estimated macroalgal $\delta^{15}\text{N}$ values in tissues, by color, over time.

Figure 8.

Hard clam (*Mercenaria mercenaria*) $\delta^{15}\text{N}$ values, with distance down Narragansett Bay expressed as latitude. Individual clam measurements are given as circles and horizontal bars represent statistical model-estimated mean $\delta^{15}\text{N}$ values for each region of the Bay. PVD is the Providence River Estuary and Upper, Mid, and Lower refer to the regions of the Bay as defined in Figure 1.

Figure 9.

Model estimated mean differences between periods for hard clam (*Mercenaria mercenaria*) $\delta^{15}\text{N}$ values where the pre-upgrade period (Period 1) is represented by the 2005 clams, during the upgrades by the 2012 (Period 2), and post-upgrades by the clams collected in 2015–2016 (Period 3). Differences are given by region of the Bay where PVD is the Providence River Estuary and Upper, Lower, and Mid represent regions of the Bay, from north to south.

Figure 10.

Conceptual model of $\delta^{15}\text{N}$ dynamics based on the results of this study. The light gray line represents the $\delta^{15}\text{N}$ values in plants and animals in Narragansett Bay before the sewage upgrades. The black line represents the situation with tertiary treatment. The dashed line denotes the mouth of the Providence River Estuary (to the left) and downstream Narragansett Bay proper (to the right). The arrows were designed to represent the primary N loads. Their size is roughly proportional to the size of the annual loads (gray arrow = pre-upgrade sewage N loads, black arrow = post-upgrade, white arrow = shelf contributions). The points of the arrows represent the approximate $\delta^{15}\text{N}$ values of the sources.

Table 1.

The timespans in which data were collected are given for each parameter, as well as the source of each dataset.

Timespan	Frequency	No. Stations	Water Column Position	Source
<i>Secchi</i>				
Apr 1997 – Apr 1998	Twice monthly	15 ^a		Oviatt et al. 2002
Jul 2007 – Nov 2015	Variable, but roughly weekly	10 ^b		NBEP 2017
Aug 2008 – Aug 2009	Variable, but roughly twice monthly from June to October	9 ^c		GSO/NBFSMN
Jul 2013 – Feb 2016				
Jan 2014 – Dec 2016	Monthly	8		This study
<i>Dissolved Inorganic Nitrogen</i>				
Jul 1972 – Aug 1973	Twice monthly	13	Surface & bottom	Kremer & Nixon 1978
Sep 1979 – Sep 1980	Twice monthly	6	Surface	Oviatt & Pastore 1980
Jun 2000 – Dec 2002	Variable ^a	Variable ^d	Surface	Chaves 2004
Nov 2011 – Nov 2012	Monthly	9	Surface & bottom	Schmidt 2014
Mar 2006 – Dec 2012	Monthly & twice monthly in Jun, Jul, Aug, Sep	12	Surface	Krumholz 2012
Jan 2013 – Jun 2016				Oviatt, pers. comm.
Jan 2014 – Dec 2016	Monthly	8	Surface, middle, and bottom	This study
<i>Particulate Matter</i>				
Jun 2000 – Sep 2001	Variable ^a	Variable ^d	Surface	Chaves 2004
Aug 2004 – Nov 2005	Five sampling events	Ranged from 1–10	Surface	DiMilla 2006
Jan 2014 – Dec 2016	Monthly	8	Surface, mid, and bottom	This study
<i>Macroalgae</i> ^e				
Sep 2006		16		Oczkowski et al. 2008
8/1/2007, 8/1/2008		24		This study
Jul 2012		23		Schmidt 2014
Sep 2015		21		This study
Aug 2016		12		This study
<i>Hard Clams</i> ^e				
Jun – Sep 2005		10		Oczkowski et al. 2008, DiMilla 2006
Aug – Oct 2012		15		Schmidt 2014
Jun 2015 – Dec 2016		10		This study

^aSixteen stations were sampled. We included 15 in our analysis as we excluded a Greenwich Bay station.

^bAll stations are in the Providence River, with the exception of one station that was immediately to the south.

^cOmitting two Greenwich Bay stations and one station in Mt. Hope Bay. One station, included in the analysis, had only three data points.

^dSampling frequencies across stations ranged from single events at some stations to four times per week at select stations.

^eDiscrete sampling events.

Table 2.

Station locations for water samples collected from the top, middle, and bottom of the water column at each of eight locations as part of the ongoing Bay Ecosystem Time Series (BETS) research effort. Resultant water clarity and dissolved inorganic nitrogen concentration data, as well as particulate stable isotope data, from these stations, are presented in this manuscript.

Station No.	Latitude	Longitude	Spatial Category
1	41.76109	-71.3783	Providence River Estuary
2	41.69886	-71.3263	Upper Bay
3	41.65884	-71.38687	Upper Bay
4	41.59823	-71.38174	Mid Bay
5	41.49254	-71.41158	Lower Bay
6	41.64164	-71.31014	Upper Bay
7	41.56658	-71.31102	Mid Bay
8	41.49481	-71.35384	Lower Bay

Table 3.

Results of a comparison between Secchi depth (m) and DIN concentrations (μM) from before the upgrades (“pre,” before 2012) and after (“post,” 2014 and after). The number of samples are given under the “n” column. We present the median, means, and standard deviations (SD) of the raw (untransformed) data as well as the means and SD of the LN-transformed means. When differences in the LN-transformed means were significantly different, they were noted under the column “Signif.” Comparisons are presented for both region (Lower Bay, Mid Bay, Upper Bay, Providence River Estuary) (Fig. 1) as well as by season, where spring is April through June, summer is July through September, and Fall is October through December. Winter data were excluded from the Bonferroni correction pairwise comparisons.

Period	Region	Secchi				DIN							
		Raw values		LN-transformed		Raw values		LN-transformed ¹					
		n	Median	Mean	SD	Signif.	n	Median	Mean	SD	Signif.		
Region													
Pre	Lower	88	4.15	4.40	1.49	1.43	0.30	507	1.08	4.94	9.89	0.22	1.76
Post		69	3.50	3.60	1.15	1.23	0.32	pre>post	195	1.87	2.90	2.81	0.59
Pre	Mid	138	2.99	3.24	0.79	1.15	0.23	390	0.80	5.09	8.84	0.14	1.93
Post		102	2.89	3.12	1.42	1.05	0.42	169	2.44	3.63	3.76	0.67	1.23
Pre	Upper	72	2.56	2.77	0.81	0.98	0.29	22	4.00	9.35	11.34	0.66	2.53
Post		53	2.60	2.92	1.44	0.96	0.49	22	4.27	5.76	4.23	1.52	0.69
Pre	PVD	474	2.02	2.06	0.83	0.64	0.42	232	17.11	20.03	17.75	2.14	1.73
Post		500	1.77	1.96	0.86	0.58	0.44	101	8.36	10.25	9.15	1.54	1.63
Season													
Pre	Spring	287	2.60	2.74	1.27	0.90	0.48	385	0.53	5.82	12.53	-0.21	2.02
Post		214	1.88	2.35	1.19	0.74	0.46	pre>post	196	1.72	3.50	5.16	0.61
Pre	Summer	288	2.11	2.20	0.98	0.69	0.47	522	1.12	4.91	9.56	0.27	1.71
Post		307	1.67	1.82	0.92	0.50	0.44	pre>post	172	1.84	3.10	3.76	0.41
Pre	Autumn	197	2.77	2.99	1.26	1.02	0.39	244	13.78	18.59	15.04	2.53	1.01
Post		203	3.00	3.15	1.12	1.08	0.38	119	7.69	9.42	6.90	1.92	0.93

¹ Surface water concentrations only. Values in μM .