Reducing Nitrogen Inputs to Narragansett Bay: Optimizing the Performance of Existing Onsite Wastewater Treatment Technologies

Final Report to the Narragansett Bay Estuary Program and the New England Interstate Water Pollution Control Commission
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Executive Summary

Wastewater from onsite wastewater treatment systems (OWTS; also known as septic systems) can be a significant source of nitrogen (N) to coastal ecosystems. Because N limits primary production in coastal ecosystems, excessive inputs can cause eutrophication, which results in the loss of ecosystem services. To reduce N loading to marine waters, advanced N-removal OWTS – which rely on the microbial processes of nitrification and denitrification for N removal – are installed in N-sensitive areas. However, once installed, final effluent total nitrogen (TN) concentration from these systems is not always monitored, making it difficult to determine the extent to which they contribute to lowering N loads.

Our study addressed four aspects of advanced N-removal OWTS within the Greater Narragansett Bay watershed in Rhode Island: (1) determine the performance of existing advanced N-removal OWTS and the impact of adjusting non-compliant systems on performance, (2) measure the relationship between system performance and the concentration of genes for nitrification and denitrification, (3) assess the accuracy of rapid tests that can be used to evaluate system performance, and (4) conduct outreach activities and developed outreach education materials for OWTS professionals, regulators, decision-makers, and scientists focused on monitoring and evaluating the performance of advanced N-removal OWTS.

Performance of existing systems and impact of adjustments. We measured TN and other properties in final effluent from three of the most commonly-installed advanced N-removal OWTS within Rhode Island’s Greater Narragansett Bay Watershed: (i) Orenco Advantex AX20® (17 systems), (ii) Bio-Microbics MicroFAST® (14 systems), and (iii) SeptiTech D® Series (11 systems). Sampling was carried out monthly between March 2015 and August 2016. The compliance rate of our study systems with the Rhode Island Department of Environmental Management standard of TN ≤ 19 mg N/L was 70.6%, 64.3%, and 75.0% for Advantex, FAST, and SeptiTech systems, respectively. The median (range) final effluent TN concentration (mg N/L) for Advantex, FAST, and SeptiTech systems was 14.9 (0.6 - 61.6), 17.1 (0.6 - 104.9), and 11.3 (0.1 - 41.6), respectively.

We investigated changes in effluent TN concentration at different time scales to determine how consistently the systems performed. Over the course of five, four-week sampling periods, SeptiTech systems had the highest median coefficients of variation (CV; 56.0%), followed by Advantex (50.4%), and FAST (31.7%). In contrast, median CV calculated at the month scale followed the order: 62.8% (Advantex), 59.0% (SeptiTech), and 56.6% (FAST). Median final effluent TN concentrations for Advantex, FAST, and SeptiTech systems were lowest in fall and winter; however, TN was not significantly correlated with temperature.

Comparison of our values to values reported for Barnstable County, MA – where systems are monitored quarterly and sampling and reporting of effluent TN is required – showed that the median final effluent TN concentration for Advantex and FAST systems were lower than in our study, with values (mg N/L) of 13.5 for Advantex, 12.7 for FAST, and 20.2 for SeptiTech systems. Furthermore, compliance with the 19 mg TN/L standard in Barnstable County, MA
systems was generally higher than in Rhode Island, with 87% of Advantex, 79% of FAST, and 42% of SeptiTech systems meeting or exceeding the standard.

Multiple regression analysis showed that final effluent TN concentration was predicted by a different set of variables for each technology: ammonium, nitrate, and alkalinity for Advantex; ammonium, nitrate, average forward flow, and five-day biochemical oxygen demand (BOD$_5$) for FAST; and, ammonium and effluent temperature for SeptiTech.

Service providers were asked between December 2015 and March 2016 to make adjustments to seven underperforming systems to increase N-removal. Total N was reduced to 19 mg N/L in two out of seven systems, suggesting that recursive adjustments may be needed to increase the number of systems in compliance. Our results show that advanced N-removal OWTS can reduce TN to meet regulatory standards, but N-removal effectiveness varies as a function of technology, time, and by individual system. Monitoring and recursive adjustment of advanced N-removal OWTS can allow service providers to proactively manage systems to optimize system performance and minimize N inputs to the Greater Narragansett Bay watershed.

**Concentration of nitrification and denitrification genes.** Advanced N-removal OWTS rely on sequential microbial processes – nitrification and denitrification – to remove N from wastewater. The size of the microbial populations that carry out these processes, their presence in different parts of the treatment train, and their relationship to TN concentration in final effluent and other wastewater properties may help explain differences in performance. We used quantitative polymerase chain reaction (qPCR) to determine the concentration of genes coding for ammonia monooxygenase (*amoA*), which carries out the first step in the oxidation of NH$_4^+$ to NO$_2^-$ during nitrification and nitrous oxide reductase (*nosZ*), which reduces N$_2$O to N$_2$ during denitrification. We measured gene concentrations in the anoxic and oxic compartments of 38 systems representing three advanced N-removal OWTS technologies (Orenco Advantex AX-20, Bio-Microbics MicroFAST, SeptiTech D Series) within the Greater Narragansett Bay watershed (Rhode Island, USA).

The concentration of *nosZ* genes, a measure of the presence of denitrifying bacteria, was not significantly different among technology types, but was higher in the anoxic compartment for all technologies. *amoA* gene concentration, a measure of the presence of nitrifying bacteria, was significantly different among technology types, following the order: AX-20 = SeptiTech > FAST and was higher in the oxic compartment for all technologies. The concentration of nucleic acids – a proxy for the size of the microbial community – was not significantly different among technologies, but was higher in the anoxic compartments than in the oxic zones of each technology. The number of copies of *amoA*/ng nucleic acid was significantly different among technology types, following the order: FAST > AX-20 = SeptiTech, and was higher in the oxic compartment for AX-20 and SeptiTech systems, but not in FAST systems. In contrast, there were no significant differences among system types or between compartments in the number of copies of *nosZ*/ng nucleic acid. The oxic compartment of systems with final effluent TN ≤ 19 mg/L (the state treatment standard) had a significantly higher concentration of *amoA* genes (AX-20) or *nosZ* genes (FAST) than systems with TN > 19 mg N/L.
Our results show that the concentration of two key genes – amoA and nosZ – involved in N removal in advanced OWTS differ as a function of technology type and location within the treatment train (oxic vs. anoxic compartments). Assessment of the size and structure of the microbial communities responsible for N-removal, the factors that drive differences in these communities, and their relationship to system performance will help develop management strategies that enhance N-removal and reduce variability among systems, ultimately resulting in greater compliance with treatment standards.

**Accuracy of rapid tests to evaluate system performance.** Although a variety of quick and inexpensive field tests are used by operation and maintenance (O&M) service providers to determine system treatment performance, the accuracy of these tests has not been evaluated relative to standard laboratory analyses. In an effort to provide information that could translate into more effective O&M visits and proactive system adjustments, we evaluated the accuracy of a variety of rapid tests that can be used for field evaluation of system performance. We evaluated the accuracy of an initial suite of rapid tests commonly used to analyze wastewater using final effluent from our study’s 42 N-removal systems by comparing to values obtained using standard laboratory methods. Regression analysis indicated that all test strip-based rapid methods and the DO titration kit produced values that deviated significantly from correspondence with standard laboratory analyses. When effluent samples were analyzed in the laboratory (to minimize sources of variability) using the same rapid tests, significant differences between rapid tests and standard analysis were not found, indicating that field conditions affected the accuracy of rapid tests.

Subsequent evaluation of a suite of alternative rapid tests for ammonium, nitrate, pH, and alkalinity showed that test kits for ammonium and multi-analysis test strips for pH produced accurate results in the field. Our results show that rapid tests may be used for field analysis of effluent, but their accuracy in the field needs to be considered before they are used to provide data to evaluate the function and treatment performance of advanced N-removal OWTS. Accurate rapid tests are available and can be used to quickly and cost-effectively evaluate advanced N-removal OWTS performance, which may result in more effective monitoring, and in turn, increase N-removal efficiency.

**Outreach.** We disseminated the results of our study to technical and general audiences at the local and national levels via two peer-reviewed papers, twelve webinars, talks and poster presentations, and inclusion of study results in two training courses for OWTS professionals. We developed outreach education materials targeting OWTS professionals, including a document summarizing the performance of advanced N-removal OWTS, and a PowerPoint presentation for educating policy makers, regulators and practitioners on the current performance, monitoring, and approaches to improve performance, to be used in NEOWTP classes. We also sent a letter to homeowners that participated in the study informing them of the performance of advanced N-removal systems.

The results of our study show that advanced N-removal OWTS within the Greater Narragansett Bay watershed are capable of lowering effluent TN to meet regulatory standards. Although the level of performance in Rhode Island systems is lower than in nearby Barnstable County, MA, monitoring and recursive and iterative adjustments are likely to increase the fraction of systems that perform to standards. We found that different wastewater properties
predict TN for different technology types, and this information should be considered in evaluating and adjusting systems to improve performance. Differences in performance may be associated with differences in the concentration of genes for ammonia oxidation and nitrous oxide reduction among individual systems and technologies, and between treatment train compartments we observed. We also identified a series of rapid tests to evaluate performance in the field, which should help O&M personnel to improve performance with minimal time and financial investments. This information has been synthesized into outreach materials being used to educate wastewater professionals, policy-makers, regulators and the general public about improving the capacity of advanced N-removal OWTS to lower N inputs from wastewater to the Greater Narragansett Bay watershed.
Acknowledgements

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Disclaimer

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Project Background and Overview

Nitrogen (N) limits primary production in poorly flushed coastal ecosystems, and excessive N inputs to marine environments can lead to eutrophication, which results in the loss of various ecosystem services (Bergondo et al., 2005). Final effluent from OWTS, which is transported through ground or surface water, has been identified as the third largest contributor to groundwater pollution in the United States (USEPA, 2002), and can be a significant source of N to coastal ecosystems. More than 60 million people in the U.S. are served by onsite wastewater treatment systems (OWTS) (USEPA, 2002), and a conventional, single-family OWTS can contribute about 11 kg per year to the ground water (MDE, 2011). This has prompted the installation of advanced N-removal OWTS in N-sensitive locations that are designed to reduce 50-75% of N inputs before final effluent is discharged to the soil treatment area (STA) (Oakley et al., 2010).

Advanced N-removal OWTS reduce N inputs by coupling microbial nitrification and denitrification to convert NH$_4^+$ to N$_2$ and N$_2$O, gases that diffuse to the atmosphere. The conversion of ammonium to N$_2$ (a biologically inert form of N) helps protect environmental and public health by lowering N loading to ground and surface waters. Incomplete nitrification and denitrification produce N$_2$O, a potent greenhouse gas, an issue that has received limited attention in the OWTS industry (Diaz-Valbuena et al., 2011; Truhlar et al., 2016).

About 51% of New England households and 30% of Rhode Island households are served by OWTS (USEPA, 2002). Approximately 30% of all OWTS permit applications annually submitted to the State of Rhode Island Department of Environmental Management (RIDEM) are for advanced N-removal technologies (RIDEM, 2014). Rhode Island’s Narragansett Bay has repeatedly experienced the negative effects of N overloading (Bergondo et al., 2005). To reduce N loads to this area, the State of Rhode Island requires the use of advanced N-removal OWTS within N-sensitive areas of the Greater Narragansett Bay Watershed, and limits the final effluent total nitrogen (TN) concentration to 19 mg N/L (RIDEM, 2009).

Servicing of advanced N-removal OWTS by operations and maintenance (O&M) service providers is required by RIDEM and helps to maintain system mechanical function. However, service visits in Rhode Island are not tracked by RIDEM, typically occur less frequently than the required twice per year, and do not include effluent testing. The lack of data on final effluent N concentrations, on O&M visit tracking and verification by state regulators, and of performance optimization based on measurable parameters, may result in underperforming systems. This, combined with the possibility of improper system installation, leads to uncertainty surrounding the extent to which these systems reduce N loading to watersheds. Data collected from advanced N-removal OWTS in Massachusetts (BCDHE, 2012), where systems are monitored quarterly and sampling and reporting of effluent TN is required, show that advanced N-removal systems do not always meet standards.

We conducted a study to assess the performance of advanced N-removal OWTS within the Greater Narragansett Bay watershed and their response to adjustments, identify the factors associated with compliance of TN levels in final effluent, and identify field tests to assess system performance accurately. We also carried out a variety of outreach activities and developed
outreach education materials to disseminate the results of our study to technical and general audiences. Our study involved three of the most commonly-installed advanced N-removal OWTS within the Greater Narragansett Bay watershed in RI: (i) Orenco Advantex AX20® (17 systems), (ii) Bio-Microbics MicroFAST® (14 systems), and (iii) SeptiTech D® Series (11 systems).

In Chapter 1, we report on the performance of advanced N-removal OWTS between March 2015 and August 2016. We monitored system performance by measuring a variety of wastewater properties: pH, dissolved oxygen (DO), effluent temperature, TN, NO₃⁻, NH₄⁺, pH, alkalinity, five-day biochemical oxygen demand (BOD₅), recirculation ratio, and average forward flow. We examined weekly, monthly, and seasonal variation in effluent TN concentration by technology. We used the performance data to identify the best predictors of effluent TN, as well as ranges of constituent values that corresponded to acceptable N levels. We identified underperforming (TN ≥19 mg N/L) systems, notified the responsible O&M service providers, and asked them to make adjustments based on values of wastewater properties we provided. We subsequently monitored the performance of all 42 systems between March and August 2016 and compared it to performance data from March to August 2015 to assess changes in N removal efficiency in response to adjustments.

In Chapter 2, we report on the concentration of advanced N-removal OWTS: nosZ, which codes for the enzyme that reduces N₂O to N₂ in denitrifying microorganisms, and amoA, which codes for the enzyme that oxidizes NH₄⁺ to NO₂⁻ in ammonia-oxidizing bacteria. We compared the concentration of these genes in oxic and anoxic compartments of the treatment train within and among technologies, and assessed the relationship between gene abundance and effluent TN.

In Chapter 3, we report on methods that can be used to monitor advanced N-removal OWTS, evaluating the accuracy of rapid tests used to test effluent from these systems. If accurate, rapid tests can assist operation and maintenance (O&M) service providers in making adjustments to underperforming systems, which may result in more effective N removal. We evaluated the accuracy of rapid tests for NH₄⁺, NO₃⁻, pH, dissolved oxygen (DO) and alkalinity, based on their ability to predict values obtained using standard methods. We tested rapid tests in the field and the laboratory to determine the effects of environmental conditions on accuracy. We also evaluated the accuracy of an alternative suite of rapid tests to provide additional accurate test methods.

In Chapter 4, we list the outreach activities we carried out to educate OWTS professionals, policy makers, and scientists of project results. We also describe the outreach education materials developed to inform OWTS practitioners and the general public of our findings.

References


RIDEM. Rhode Island Department of Environmental Management. (2014). Innovative and alternative nitrogen removal system use in Rhode Island. Personal communication with Brian Moore, P.E., Chief of Office of Water Protection, Rhode Island Dept. of Environmental Management, Providence, RI.


Chapter 1: Performance Evaluation of Advanced Nitrogen-Removal Onsite Wastewater Treatment System Performance

Introduction

Residential wastewater can be a significant source of nitrogen (N) to coastal ecosystems (Valiela et al., 1992), and has been identified as the third largest contributor to groundwater pollution in the United States (USEPA, 2002). A conventional, single-family onsite wastewater treatment system (OWTS; also known as septic system—a gravity-flow septic tank and soil treatment area (STA; also referred to as drainfield or leachfield) can contribute about 11 kg of N per year to the groundwater (MDE, 2011). Nitrogen limits primary production in coastal ecosystems, and excessive N inputs to marine environments can lead to eutrophication, which results in the loss of various ecosystem services (Carpenter et al., 1998). Wastewater from OWTS can also contaminate drinking and recreational water with bacterial and viral pathogens, and nitrate, posing public health risks (USEPA, 2002). This has prompted the development and installation of advanced N-removal OWTS, which provide additional removal of N from septic tank effluent before it is dispersed to a soil treatment area in N-sensitive locations.

More than 60 million people in the United States are served by OWTS, which are also common in rural areas of Canada, Australia, and Europe (Vedachalam, 2015). In Rhode Island, 30% of households are served by OWTS (USEPA, 2002), and approximately 30% of all OWTS permit applications submitted yearly to the State of Rhode Island Department of Environmental Management (RIDEM) are for advanced N-removal technologies (RIDEM, 2014). Rhode Island’s Narragansett Bay has repeatedly experienced the negative effects of N pollution, including hypoxia and anoxia, alterations to food web dynamics, loss of biodiversity and habitat, and increased frequency of algal blooms (Bergondo et al., 2005). To reduce N loads to this area, the State of Rhode Island requires the use of advanced OWTS within N-sensitive areas of the Greater Narragansett Bay watershed, and limits the final effluent total nitrogen (TN) concentration to ≤ 19 mg N/L (RIDEM, 2009).

Advanced N-removal OWTS can eliminate 50-75% of N inputs by promoting conditions for nitrification and denitrification (Oakley et al., 2010) before effluent is dispersed to the STA. Nitrification occurs inside an oxic tank or chamber where $\text{NH}_4^+$ is oxidized by aerobic, autotrophic bacteria to $\text{NO}_3^-$. Following the nitrification step, wastewater is diverted to an anoxic zone in the treatment train where denitrification is carried out by heterotrophic bacteria. Here, $\text{NO}_3^-$ is reduced to $\text{N}_2$ (nitrogen gas) and $\text{N}_2\text{O}$ (nitrous oxide), which diffuse to the atmosphere via vents in the system. The conversion of $\text{NO}_3^-$ to $\text{N}_2$ (a biologically inert form of N) minimizes N loading to groundwater and helps to protect environmental and public health. Incomplete denitrification releases $\text{N}_2\text{O}$, a potent greenhouse gas that has received only limited attention in the OWTS industry (Diaz-Valbuena et al., 2011; Truhlar et al., 2016).

Servicing of advanced N-removal OWTS by operation and maintenance (O&M) service providers helps to maintain system mechanical function, but monitoring for N-removal performance is typically not done in Rhode Island. Although O&M visits are required twice per year for residential systems (RIDEM, 2016), they typically occur less frequently, may not include a determination of forward flow and recirculation ratio and, more importantly, do not
include measurement of final effluent TN concentration. The lack of data on final effluent N concentration, on O&M visit tracking and verification by state regulators, and of performance optimization based on measurable parameters, may result in systems that exceed the 19 mg N/L standard. This, combined with the possibility of improper system installation, may translate into N inputs from OWTS to the Greater Narragansett Bay watershed in Rhode Island that are higher than those based on original assessments of system performance made by RIDEM. Performance data collected from advanced N-removal OWTS installed in Massachusetts (BCDHE, 2012) where systems are monitored quarterly and sampling and reporting of effluent TN is required, show that advanced N-removal OWTS do not always perform to standard.

To determine if these systems are effective at decreasing influent TN concentrations, we monitored a subset of existing advanced N-removal OWTS located within the Greater Narragansett Bay watershed. We measured wastewater properties in the field (pH, dissolved O\(_2\) (DO), effluent temperature) and in the laboratory (TN, NO\(_3^-\), NH\(_4^+\), pH, alkalinity, five-day biochemical oxygen demand (BOD\(_5\))) (Lancellotti et al., 2016). We assessed system compliance with the TN standard, and investigated weekly, monthly, and seasonal variation in concentration of TN by technology type. We also compared final effluent TN data from systems that were set to denitrify to systems that were not set to this mode. We used best subsets multiple linear regression to identify the wastewater properties (NO\(_3^-\), NH\(_4^+\), alkalinity, BOD\(_5\), and DO, effluent temperature, recirculation ratio, and average forward flow) that best predict final effluent TN concentration. We used data collected from March to December 2015 to identify the systems that were operating outside Rhode Island’s compliance standard (median TN concentration ≤ 19 mg N/L) and worked with O&M service providers to adjust underperforming systems from January 2016 to March 2016 to increase N removal. We then compared the performance of all 42 systems between March and August 2015 to the same period in 2016 to assess changes in N removal efficiency in response to system adjustments.

**Methods**

**Study systems.** We studied the three most commonly-installed advanced N-removal OWTS within the Greater Narragansett Bay watershed in Rhode Island: (i) Orenco Advantex AX20° (17 systems), (ii) Bio-Microbics MicroFAST° (14 systems), and (iii) SeptiTech D° Series (11 systems). Of the 11 SeptiTech systems, seven were initially not required to denitrify (i.e. systems programmed to denitrification-disabled mode, set to minimal recirculation of nitrified effluent to the anoxic tank). These seven Septitech systems were latter converted to denitrification mode during the adjustment period of the project. The 42 systems are located in the towns of Jamestown, Portsmouth, South Kingstown, North Kingstown, and Charlestown (Fig. 1). Our study systems were chosen in collaboration with RIDEM, from 5,125 systems that existed statewide in 2014. Geographic information system (GIS) maps were generated to show system locations by sub-watersheds and determine which systems were in locations representative of the watershed. Candidate study systems were identified based on five criteria: (i) year-round occupancy, (ii) system installation between 2006 and 2014, (iii) accessibility of manholes for sampling, (iv) suitable hydraulic flow for the system design (≤ 435 L/day per bedroom), and (v) presence of pressurized drainfield pumps. The final 42 study systems were selected based on site visits to inspect systems for accessibility, and the homeowners’ willingness to participate in the study.
Sample collection. We collected final effluent samples monthly from all 42 systems during the first two weeks of each month from March 2015 to June 2016, and in August 2016. A subset of nine systems (three of each technology) was sampled for four consecutive weeks every third month to quantify variation on a weekly scale. The Advantex, FAST, and SeptiTech technologies were sampled at the recirculating splitter valve assembly, drain field pump basin, and discharge pump basin within the processor tank, respectively (referred to as sampling point SP2) (Fig. 2). Additional field measurements were taken from the systems’ anoxic component for pH, DO, and effluent temperature analysis (referred to as sampling point SP1; Fig. 2). Final effluent samples were collected in 1-L plastic Nalgene bottles and stored at 4°C and transported to the laboratory within 8 h of collection.

Field measurements and standard laboratory methods. Field and laboratory analyses, as well as quality control criteria for laboratory data are described in detail in Lancellotti et al. (2016). Field measurements of pH, DO and effluent temperature were made in SP1 and SP2 locations using a Hanna Instruments HI9828 Multiparameter Meter (Woonsocket, RI). The concentration of ammonium (Doane and Horwath, 2003) and nitrate (Weatherburn, 1967) were measured colorimetrically using a Bio Tek Powerwave 340 microplate reader (Winooski, VT). Total nitrogen concentration was determined using the persulfate oxidation method (APHA, 1998), and nitrate in the digestate was measured colorimetrically (Weatherburn, 1967) using the Bio Tek microplate reader. Five-day biochemical oxygen demand (BODs) was measured using an OxiTop BOD measurement system (WTW, Weilheim, Germany). pH was determined in the laboratory using a Denver Instruments (Bohemia, NY) Ultrabasic10 meter equipped with a pH/ATC electrode. Alkalinity was measured with an Automatic Titration System (Hanna

Figure 1. Map of Rhode Island showing the towns where study systems were located (yellow stars).
Instruments, Woonsocket, RI) using a 1 N HCl solution to titrate a 100-mL effluent sample.

To determine which properties best predicted effluent TN concentration, we carried out best subsets multiple linear regressions (SigmaPlot 11.0, Systat, San Jose, CA) separated by technology type (Advantex, FAST, and SeptiTech) with TN as the dependent variable, and NO$_3^-$, NH$_4^+$, alkalinity, and BOD$_5$ collected from SP2; pH, DO and effluent temperature collected from SP1 and SP2; and average forward flow and recirculation ratio as independent variables. We chose models that provided the highest R$^2$ values and included the fewest independent variables, since monitoring fewer wastewater properties is more

**Figure 2.** Schematic diagram of Advantex, FAST, and SeptiTech technology treatment trains showing sampling locations. P = pump; SP1=anoxic compartment; SP2=oxic compartment and final effluent.

**Statistical analysis.** To determine which properties best predicted effluent TN concentration, we carried out best subsets multiple linear regressions (SigmaPlot 11.0, Systat, San Jose, CA) separated by technology type (Advantex, FAST, and SeptiTech) with TN as the dependent variable, and NO$_3^-$, NH$_4^+$, alkalinity, and BOD$_5$ collected from SP2; pH, DO and effluent temperature collected from SP1 and SP2; and average forward flow and recirculation ratio as independent variables. We chose models that provided the highest R$^2$ values and included the fewest independent variables, since monitoring fewer wastewater properties is more
cost-effective and less time consuming for system service providers. For example, if the addition of an independent variable did not increase the $R^2$ value more than 10%, the subset with fewer variables was chosen. Models that contained independent variables with high variance inflation factors (VIFs) (i.e. variables that were highly correlated with each other) were not considered, since the addition of these variables results in a less robust model. Independent variables with a P value < 0.05 were considered significant and therefore the best predictors of effluent TN. Linear regression analysis was used to investigate the relationship between effluent temperature and TN concentration for each technology type. Technology types with a P value < 0.05 (i.e. the slope of the regression line was statistically significantly different from zero) were considered to have a statistically significant linear relationship between effluent temperature and TN. Student’s $t$-test was used to compare values of wastewater properties before and after adjustments ($\alpha= 0.05$). If the data failed the normality test ($\alpha= 0.05$), a Mann-Whitney rank sum test was used.

**Daily flow and recirculation ratio calculations.** Average daily forward flow and recirculation ratio were calculated as described in the Supplementary Information section.

**Results and Discussion**

**Total nitrogen**

**Evaluation of system performance prior to adjustments.** The percentage of systems in compliance with the 19 mg N/L TN standard from March to December 2015 (prior to system adjustments) was 70.6% for Advantex, 64.3% for FAST, and 75.0% for SeptiTech. The median TN concentration (mg N/L) of final effluent over this sampling period was 14.9 for Advantex, 17.1 for FAST, and 11.3 for SeptiTech (Fig. 3). The range of TN values (mg N/L) from all FAST systems (0.6 to 104.9) was much larger than Advantex (0 to 61.6) and SeptiTech (0.1 to 41.6) (Fig. 3), indicating FAST system performance was less consistent. Analysis of TN values by individual systems shows the range of values increases with median TN concentration for all technology types (Fig. 4).

Influent TN concentration cannot accurately be determined for these advanced N-removal OWTS due to recirculation of wastewater that occurs within the treatment train; therefore, we cannot calculate the percentage of N removed by these technologies. Because of this, variation in performance among individual systems could be due to differences in influent (raw sewage) TN concentration. Differences in influent TN concentration between households could explain differences in compliance with the TN standard between individual systems. For example, if 50% reduction of influent TN concentration is assumed, a system receiving higher-strength influent will produce effluent with a higher TN concentration than a system receiving lower-strength influent.
SeptiTech systems had a high compliance rate and low median TN concentration, despite a small sample size (n=4). Seven of the 11 SeptiTech systems we sampled were not set to denitrification mode at the onset of the study, prompting us to evaluate systems set to these different modes separately (Fig. 5). Nitrified effluent in denitrification-disabled SeptiTech systems recirculates back through the system minimally each day before it is dispersed to the STA, which limits N removal. Total N data collected from March to December 2015 show that denitrification-enabled systems had a lower median TN concentration (11.1 mg N/L) (Fig. 5) and a higher compliance rate (50.0%) than denitrification disabled systems, which had a median final effluent TN concentration of 33.8 mg N/L and a compliance rate of 14.3%.
Variation of effluent TN concentration at different time scales. We sampled a subset of nine systems (three of each technology) for four consecutive weeks in May, August, and November 2015, and February and May 2016. SeptiTech systems had the highest median coefficient of variation (CV) for effluent TN over the course of four-week time periods (56.0%), followed by Advantex (50.4%), and FAST (31.7%), indicating that FAST systems perform most consistently at the week scale, and SeptiTech systems reduce effluent TN less consistently at this time scale (Fig. 6).

Median CVs calculated at the month scale using TN data collected from March 2015 to March 2016 followed a different trend, with values of 62.8%, 59.0%, and 56.6% for Advantex, SeptiTech, and FAST, respectively (Fig. 7). As expected, median CVs for effluent TN concentration were higher at the month scale than the week scale for all technology types. Systems are exposed to larger fluctuations in properties that may affect N removal (effluent temperature, pH, DO, inputs of toxic substances, etc.) throughout the course of a year than over four weeks, which explains higher median CVs at the month scale.

Variation of effluent TN concentration with temperature. We examined how median effluent TN concentrations changed at the seasonal scale: January-March (winter), April-June (spring), July-September (summer), and October-December (fall). We expected a negative relationship between effluent temperature and TN concentration, since nitrification and denitrification rates increase with temperature (Seitzinger, 1998; Shammas, 1986). Each system type was affected differently by season: median effluent TN concentrations for Advantex (10.1 mg N/L) and SeptiTech systems (9.1 mg N/L) were lowest in fall, when median sample and ambient temperatures were low (Table 1).
Figure 6. Final effluent total N concentration from a subset of nine systems (3 Advantex, 3 FAST, 3 SeptiTech) sampled quarterly for four consecutive weeks from May 2015 to May 2016. All SeptiTech systems in this analysis were denitrification-enabled. Number of systems included in analysis is displayed at the top of each box.

Figure 7. Median coefficient of variation calculated for monthly data (March 2015 to March 2016) (black bars) compared to median coefficients of variations calculated for weekly data (white bars). Data are for a subset of nine systems (3 Advantex, 3 FAST, 3 SeptiTech).
### Table 1. Seasonal and technology type differences in median total N concentration in final effluent, and effluent and ambient temperatures.

<table>
<thead>
<tr>
<th>System type</th>
<th>Seasona</th>
<th>n</th>
<th>Total N concn. (mg N/L)</th>
<th>Effluent sample temperature (°C)</th>
<th>Ambient temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantex</td>
<td>Winter</td>
<td>24</td>
<td>18.6</td>
<td>7.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>66</td>
<td>15.5</td>
<td>16.2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>64</td>
<td>15.7</td>
<td>23.2</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>63</td>
<td>10.1</td>
<td>18.8</td>
<td>17</td>
</tr>
<tr>
<td>FAST</td>
<td>Winter</td>
<td>21</td>
<td>12.1</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>54</td>
<td>20.8</td>
<td>14.9</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>52</td>
<td>14.4</td>
<td>21.7</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>50</td>
<td>12.7</td>
<td>17.1</td>
<td>17</td>
</tr>
<tr>
<td>SeptiTech</td>
<td>Winter</td>
<td>10</td>
<td>12.7</td>
<td>9.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>19</td>
<td>21.2</td>
<td>16.4</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>24</td>
<td>26.5</td>
<td>24.6</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>19</td>
<td>9.1</td>
<td>20.3</td>
<td>17</td>
</tr>
</tbody>
</table>

a January-March (winter), April-June (spring), July-September (summer), and October-December (fall).

In contrast, the median final effluent TN concentration for FAST systems was lowest in winter (12.1 mg N/L) when median sample and ambient temperatures were lowest. Low median final effluent TN concentrations during the colder seasons contradict the expectation that denitrification rates increase with temperature (Carrera et al., 2004), and data showing that the optimal temperature for nitrification ranges from 10 to 20 °C, and from 30 to 36 °C for denitrification, depending on microbial community composition (Balmelle et al., 1992; Ford et al., 1980).

We investigated the relationship between TN and temperature further by plotting effluent TN concentrations against effluent temperature between March and December 2015 separated by technology (Fig. 8). Effluent TN concentrations from Advantex and FAST systems were not significantly correlated with effluent temperature ($R^2 = 0.007$ and 0.001 for Advantex and FAST, respectively). In contrast, SeptiTech systems showed a significant positive correlation between effluent TN and temperature ($R^2 = 0.039$). The lack of significant correlation between temperature and TN for most systems likely explains why we do not see clear temporal trends in the TN data, and suggests that other factors may control N removal. Denitrification may only be affected by temperature when other properties (i.e. DO, pH, nitrate availability, organic carbon) are within optimal ranges.

### Significant predictors of TN

In an effort to provide information that can translate into more proactive and effective O&M site visits that may increase N-removal efficiency, we identified the wastewater properties that best predicted effluent TN concentration by carrying out best subsets linear regressions. The regressions were performed separately, by technology type, since predictors are likely to vary by technology and treatment process. We used data collected from March 2015 to August 2016 (excluding SeptiTech systems in denitrification-disabled mode) and included values of wastewater properties that corresponded to TN concentrations ≥19 mg N/L to identify the best
predictors for all systems, regardless of performance.

The independent variables examined included average forward flow and recirculation ratio, values collected from SP2 for ammonium, alkalinity, nitrate, BOD (based on laboratory analysis), and values from SP1 and SP2 for effluent temperature, DO, and pH (based on field analysis). Median values and ranges of values of these properties are shown in Supplementary Table 1. Because the FAST technology does not allow for adjustment of recirculation ratio, this variable was not included.

For Advantex systems, TN had a significant positive correlation with ammonium and nitrate, and a significant negative correlation with alkalinity (Table 2). For FAST systems, TN had a significant positive correlation with ammonium, nitrate, and BOD, and a negative correlation with average forward flow. For SeptiTech systems, TN was significantly positively correlated with ammonium and effluent temperature (Table 2). The independent variables accounted for 44.2, 71.0, and 80.7% of the variation in effluent TN concentrations for Advantex, FAST, and SeptiTech systems, respectively.

We expected the significant positive correlation between ammonium, nitrate, BOD and TN observed in Advantex, FAST, and SeptiTech systems, since TN is comprised of inorganic species (NH$_4^+$ and NO$_3^-$), as well as organic N (measured as BOD$_5$); therefore, TN must increase if its constituents (NH$_4^+$, NO$_3^-$, and organic N) increase. The inverse relationship between TN and alkalinity observed for Advantex systems suggests that buffering capacity has a significant impact on nitrification and/or denitrification for this technology. The optimal pH range for nitrifying and denitrifying bacteria is 6.5 to 8.0 and 7.0 to 8.5, respectively (Haandel and Lubbe, 2007). If alkalinity decreases, pH is more likely to drop below this optimal range, and nitrifying and denitrifying bacteria will not reduce nitrogen optimally (Painter and Loveless, 1983). A negative correlation between TN and average forward flow observed for FAST systems is expected, because an increased amount of wastewater entering and circulating within the system can dilute the final effluent TN concentration. Because it has a gravity flow treatment train and no surge flow storage capacity, a large volume of wastewater entering the FAST system results in a corresponding volume of final effluent forward flow being displaced from the FAST tank to the STA. In contrast, a significant positive correlation between TN and effluent temperature.

Figure 8. Effluent temperature values plotted against effluent TN concentrations for Advantex (n=191), FAST (n=138), and SeptiTech systems (n=123). Denitrification-disabled SeptiTech systems were included in this analysis. The equation of the regression line, $R^2$, and P values are displayed.
observed for SeptiTech contradicts expectations, since denitrification rates typically increase with temperature. It is likely that the independent variables that were not included in the best subsets regression models, like pH and recirculation ratio, did not have a linear relationship with TN, and therefore did not contribute to increasing the $R^2$ value. Variations in the configuration of advanced treatment trains between technology types may explain why significant predictors were different for each technology.

We completed additional best subsets linear regressions separated by technology that included only data from sampling events corresponding to TN values of $\leq$ 19 mg N/L in order to determine which wastewater properties best predict acceptable TN concentrations. These models accounted for less variation in TN than when all data were included: 8.3%, 25.9%, and 33.5% for Advantex, FAST, and SeptiTech, respectively (Table 3).

Table 3. Results of best subsets multiple linear regression analysis to predict effluent total N concentration based on effluent parameters using only data from sampling points with TN values of 19 mg N/L or less. Denitrification-disabled SeptiTech systems were excluded from this analysis. Properties with significant P-values ($\alpha = 0.05$) are bolded.

<table>
<thead>
<tr>
<th>System type</th>
<th>Property</th>
<th>Coefficient $^a$</th>
<th>P</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantex</td>
<td>Nitrate</td>
<td>0.259</td>
<td>0.057</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>DO – SP1</td>
<td>1.909</td>
<td>0.337</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DO – SP2</td>
<td>0.595</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOD</td>
<td>0.0573</td>
<td>0.227</td>
<td></td>
</tr>
<tr>
<td>FAST</td>
<td>Ammonium</td>
<td>0.224</td>
<td>0.105</td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td>Nitrate</td>
<td>0.257</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DO – SP1</td>
<td>0.589</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DO – SP2</td>
<td>-0.384</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BOD</td>
<td>-0.0911</td>
<td>0.091</td>
<td></td>
</tr>
<tr>
<td>SeptiTech</td>
<td>Alkalinity</td>
<td>-0.0553</td>
<td>0.006</td>
<td>0.335</td>
</tr>
<tr>
<td></td>
<td>DO</td>
<td>-1.473</td>
<td>0.019</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values for constant of regression equation for Advantex, FAST, and SeptiTech systems = 10.511, 7.639, and 78.175, respectively.
For Advantex, none of the independent variables were significantly correlated with TN. For FAST, TN had a significant positive correlation with nitrate, and for SeptiTech, TN had a significant negative correlation with alkalinity and DO. A significant negative correlation with DO observed for SeptiTech systems is expected, since a decrease in DO would limit nitrification, which is required for ultimate conversion of $\text{NH}_4^+$ to $\text{N}_2$. It is possible that, for Advantex systems, the independent variables do not have a linear relationship with TN, which could explain why the regression model did not identify any independent variables as significant.

We also used this dataset to identify ranges of values of wastewater properties for each technology type that would result in final effluent TN concentrations of $\leq 19$ mg N/L (Fig. 9). Operation and maintenance service providers can use this information to evaluate system performance on-site. For some wastewater properties, such as nitrate (mg N/L), a large range of values (0.6-29.4, 0.2-37.3, and 0.4-36.1 for Advantex, FAST, and SeptiTech systems, respectively) is associated with an acceptable TN concentration. In contrast, for other properties, like pH from sampled from SP1, a smaller range of values (6.1-9.6, 5.1-8.2, 6.4-8.2) for Advantex, FAST, and SeptiTech systems, respectively) is associated with an acceptable TN concentration. This could be because systems are more sensitive to changes in pH. Although pH sampled from SP1 or SP2 are not significant predictors for any system type when only data points for TN $\leq 19$ mg N/L are included (Table 3), it is known that nitrifying and denitrifying bacteria are sensitive to changes in pH.

**Comparisons to other studies**

We compared final effluent TN concentrations and compliance rates from March to December 2015 (before adjustments) to those published by the Barnstable County Department of Health and Environment (BCDHE) for 513 systems in Cape Cod, MA (BCDHE, 2012), where a 19 mg N/L final effluent TN standard is enforced. SeptiTech systems that were not turned on for denitrification mode were included in this analysis, since they were also included in the Cape Cod data. Compliance rates (median effluent TN concentration $\leq 19$ mg N/L) were higher in Cape Cod, with 87% of Advantex, 79% of FAST, and 42% of SeptiTech systems in compliance. Similarly, median TN concentration for the systems in the Cape Cod study were lower than ours, with values (mg N/L) of 13.5 for Advantex, 12.7 for FAST, and 20.2 for SeptiTech systems. Differences in system performance between the Cape Cod data and ours are likely due to differing regulatory requirements in the two states. Advanced N-removal OWTS must be serviced frequently and proactively in order for N removal to be optimized (Bounds et al., 2004). Proper maintenance of these systems includes an assessment of recirculation ratios, as well as monitoring of wastewater properties (e.g. pH, $\text{NH}_4^+$, $\text{NO}_3^-$, DO), which can be performed on-site. The Massachusetts Department of Environmental Protection (MADEP) requires quarterly inspections of advanced N-removal OWTS which include analysis of effluent TN and other wastewater properties. Additionally, systems that do not meet the 19 mg N/L TN standard continue to be visited and adjusted until they do (Rask et al., 2013). In contrast, RIDEM requires two inspections per year which do not include effluent TN analysis (RIDEM, 2009). The MADEP also requires O&M service providers to enter their findings into an online database-
accessible to all service providers and regulators that tracks system performance and maintenance scheduling.

**Figure 9.** Values (n = 8-32) of wastewater properties associated with total N concentrations ≤ 19 mg N/L. Data are for samples collected from March 2015 to August 2016. Units for ammonium and nitrate are mg N/L; mg/L for alkalinity, DO and BOD; °C for temperature; L/day for average forward flow (FF).

More frequent O&M visits that include analysis of system final effluent allow service providers to make adjustments necessary to facilitate effective system performance. Analysis of wastewater properties likely translates into more proactive system maintenance because service providers can learn more about how the system is performing internally, rather than rely solely
on visual observations to make assessments. For example, if during a site visit a service provider encounters a system with a high effluent TN concentration, the system’s recirculation ratio could be adjusted accordingly during the next visit (using the control panel), since altering the residence time of wastewater in the oxic and anoxic zones can increase N removal. Additionally, recursive site visitation ensures that underperforming systems are adjusted to meet the TN standard. Analysis of effluent TN combined with more frequent O&M site visits and recursive site visits to underperforming systems required by the MADEP likely lead to improved N removal, resulting in better performance of Advantex, FAST, and SeptiTech systems compared to those in our study.

**Evaluation of system performance after adjustments**

*Denitrification-enabled systems.* We determined the response of underperforming systems to adjustments made by O&M service providers. Data collected between March and December 2015 from all denitrification-enabled systems indicated nine systems (2 Advantex, 6 FAST, 1 SeptiTech) had median final effluent TN concentrations greater than 19 mg N/L. Two of the FAST systems were excluded from this evaluation because they were not receiving influent for extended periods of time.

We provided the O&M service providers responsible for these seven remaining systems with approximate values (we were not legally permitted to provide exact values) from SP1 and SP2 for pH, DO, and effluent temperature, and from SP2 for alkalinity, ammonium, nitrate, TN, and BOD₅. Since we were not legally permitted to make adjustments ourselves, we asked service providers to make the adjustments to their client’s systems that they considered necessary (e.g. pumping of the advanced treatment unit and/or septic tank, recirculation ratio adjustment, dose adjustment, cleaning media, venting, fixing recirculation troughs, etc.) to improve N removal. Adjustments were made between December 2015 and February 2016. To evaluate the effectiveness of adjustments, we compared median final effluent TN concentrations between March-August 2015 to the same period in 2016.

Final effluent median TN concentrations decreased in three (one of each technology type) of the seven systems after adjustments (Fig. 10). Of these three systems, the median final effluent TN concentration of two systems was reduced below 19 mg N/L, whereas the third system remained out of compliance. In contrast, the TN concentration of the other four systems increased after adjustments were made by the service providers (Fig. 10).

Adjustments did not result in a consistent increase in N removal for underperforming systems. This could be because the O&M service providers were unable to correctly assess why the systems were failing to achieve compliance and determine which adjustments needed to be made. Some physical conditions of system components (e.g. tank settling, condition of trough in aeration insert, and liquid level within the FAST technology) are not easily corrected and would require more involved repair work. In the case of the FAST technology, this may have prevented service providers from taking corrective actions to improve treatment performance. In addition, the microbial communities within the underperforming systems may not be able to nitrify and/or denitrify at a sufficiently high rate because of unfavorable conditions (e.g. DO, pH, alkalinity, supply of C), which can limit the activity of nitrifying and denitrifying bacteria, and thus N removal. It is also possible that inputs of N are higher in those systems that did not respond to adjustments, making it more difficult to achieve compliance.
We also compared values for each wastewater property before (March to August 2015) and after (March to August 2016) adjustments were made. There was no clear response pattern, since the properties that changed significantly after adjustments differed by system (Table 4) and none of the parameters that changed significantly were common to all seven systems. Surprisingly, although the final effluent TN concentration was reduced to meet the 19 mg N/L standard in the SeptiTech system, we did not detect any significant differences in any of the wastewater properties.

Table 4. Effects of adjustment of underperforming systems on wastewater properties. A t-test ($\alpha = 0.05$) was used to determine statistical significance. All units are in mg/L, except for pH and recirculation ratio.

<table>
<thead>
<tr>
<th>System type</th>
<th>System</th>
<th>Property</th>
<th>Before adjustment</th>
<th>After adjustment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantex</td>
<td>1</td>
<td>Ammonium</td>
<td>43.6</td>
<td>59.8</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkalinity</td>
<td>112.0</td>
<td>274.9</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Recirculation ratio</td>
<td>3</td>
<td>4</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>N/A a</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>FAST</td>
<td>1</td>
<td>pH SP2</td>
<td>4.3</td>
<td>5.7</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH SP1</td>
<td>4.2</td>
<td>7.0</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alkalinity</td>
<td>0.4</td>
<td>13.6</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Nitrate</td>
<td>4.0</td>
<td>13.2</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH SP2</td>
<td>7.3</td>
<td>7.0</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DO SP2</td>
<td>0.6</td>
<td>2.2</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BOD5</td>
<td>90.0</td>
<td>31.0</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Alkalinity</td>
<td>90.4</td>
<td>272.0</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>DO SP1</td>
<td>2.0</td>
<td>5.9</td>
<td>0.028</td>
</tr>
<tr>
<td>SeptiTech</td>
<td>1</td>
<td>N/A a</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

a None of the independent variables were significantly correlated with TN.
The absence of a common response to adjustments within a technology type, or of any response for the system that reached compliance, is puzzling. Although we measured wastewater properties widely considered to affect treatment (e.g. temperature, DO, pH, alkalinity, BOD$_5$), it is possible that there are other properties associated with TN removal that we did not measure. Furthermore, it is generally assumed that heterotrophic denitrification is responsible for N removal in these systems. However, other microbial processes, such as autotrophic nitrification, nitrifier denitrification and anaerobic ammonium oxidation (anammox), may either contribute significantly to, or be solely responsible for, N removal. There are no peer-reviewed studies on the mechanisms by which N is removed in the type of advanced N-removal technologies tested in our study. If mechanisms other than heterotrophic denitrification are important, different adjustments may be necessary to increase N-removal.

**Denitrification-disabled SeptiTech systems.** The seven SeptiTech systems that were not initially turned on for denitrification mode at the beginning of the project were programmed to denitrify in February 2016. After this adjustment, the median final effluent TN concentration (mg N/L) of these seven systems increased from 29.4 to 35.3, and the compliance rate for this group of systems (14.3%) did not change (Fig. 11). This is the opposite of what we expected to observe, given that the denitrification-enabled mode is supposed to result in more effective N removal. Enabling the denitrification mode resulted in an increase in the median ratio of ammonium to nitrate concentration in effluent from 0.46 to 1.50, suggesting the ammonium concentration in system final effluent increased relative to nitrate concentrations after systems were enabled for denitrification. This is the opposite of what we expected, since denitrification mode should have increased rates of ammonium oxidation as well as nitrate reduction, resulting in lower ammonium levels. Without sufficient NO$_3^-$, denitrification cannot proceed, resulting in high final effluent TN concentrations largely due to the presence ammonium that did not get oxidized to nitrate in the nitrification step. Switching the system to denitrification mode may have increased recirculation rates beyond optimal values, limiting the retention time of wastewater in the oxic treatment train, thus limiting nitrification.

We determined which wastewater properties changed significantly due to adjustments to denitrification-disabled systems and found that they varied by individual system (Table 5). As was the case with denitrification-enabled systems, a clear pattern among all denitrification-disabled systems could not be found. The reasons for the absence of a common response may be the same for denitrification-enabled and–disabled systems.

**Conclusions**

Our results show that, although they are capable of producing effluent that complies with the 19 mg TN/L standard, the performance of advanced N-removal OWTS within Rhode Island’s Greater Narragansett Bay watershed varies as a function of technology type, time, and by individual system within a technology type. SeptiTech systems had the lowest median final effluent TN concentration (mg N/L) (11.3) and the highest percentage of systems in compliance with the 19 mg N/L standard (75%), followed by Advantex (14.9; 70.6%) and FAST (17.1; 64.3%). Median final effluent TN concentrations were lowest in fall and winter when effluent and ambient temperatures were lowest; however, linear regression analysis indicates that, for Advantex and FAST systems, effluent TN concentration is not significantly correlated with
temperature. Final effluent TN concentration was predicted by a different set of variables for each technology type: ammonium, nitrate, and alkalinity for Advantex; ammonium, nitrate, average forward flow, and BOD for FAST; and, ammonium and effluent temperature for SeptiTech.

Table 5. Wastewater properties that changed significantly (α=0.05) after adjustments to denitrification-disabled SeptiTech systems. All units are in mg/L, except for pH and recirculation ratio.

<table>
<thead>
<tr>
<th>System</th>
<th>Property</th>
<th>Before adjustment</th>
<th>After adjustment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total N</td>
<td>28.8</td>
<td>28.7</td>
<td>0.021</td>
</tr>
<tr>
<td>2</td>
<td>Nitrate</td>
<td>17.4</td>
<td>3.4</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>pH-SP2</td>
<td>5.9</td>
<td>7.2</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Alkalinity</td>
<td>11.3</td>
<td>202.5</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>BOD₂</td>
<td>6.0</td>
<td>98.0</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>pH-SP2</td>
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<td>7.3</td>
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</tr>
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<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
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<td>72.7</td>
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<td>6.3</td>
<td>0.032</td>
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<tr>
<td></td>
<td>pH-SP2</td>
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<td>7.1</td>
<td>0.049</td>
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<td>Recirculation ratio</td>
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<td>0.003</td>
</tr>
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</table>

a None of the independent variables were significantly correlated with TN.

Figure 11. Final effluent total N concentrations (n=4-5) for denitrification-disabled (March to June 2015; black box) and denitrification-enabled SeptiTech systems (March to June 2016; white box). The dashed line represents 19 mg N/L standard.

Adjustments made to seven underperforming systems that were originally set to denitrification-enabled mode resulted in lower final effluent TN concentrations in three systems, although one of these remained out of compliance after adjustments. Paradoxically, final effluent
TN concentrations from SeptiTech systems that were initially denitrification-disabled increased after they were turned on for denitrification.

Advanced N-removal systems in Barnstable County, MA (BCDHE, 2012) perform better than those in our study, likely the result of required quarterly and recursive site visits, effluent testing for TN, and reporting of results through a county-maintained electronic database. Our results indicate that a single site visit and adjustment to underperforming systems results in limited improvement in system performance. Additional visits and recurring adjustments may be necessary to achieve widespread improvement in performance, and these should take into consideration the variability of N removal effectiveness at weekly and monthly scales. Additional training of O&M service providers in adjusting systems to improve N-removal efficiency may also lead to more effective management, improved system performance, and lower N inputs to the Greater Narragansett Bay watershed.

References


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Technologieshttp://www.dem.ri.gov/programs/benviron/water/permits/isds/pdfs/ialist.pdf


Supplementary Information

Forward Flow
Average daily forward flow is the average volume of wastewater generated by a dwelling in a day that is delivered to the STA. We calculated average daily forward flow differently depending on technology type. For Advantex systems, we calculated average daily forward flow ($F_{ADV}$, in liters per day) using the equation:

$$F_{ADV} = \frac{(C*V_{cycle})}{30}$$

where $C$ is the number of times wastewater was discharged to the STA over the course of 30 days (discharge cycles are tallied by the system as 30-day totals), $V_{cycle}$ is the volume of water discharged per cycle (in liters), and 30 is the number of days over which each cycle is tallied.

For FAST and SeptiTech systems, average daily forward flow ($F_{FAST, ST}$, in liters per day) was calculated using the equation:

$$F_{FAST, ST} = \frac{(t_{pump}*V_{pump})}{t_{samp}}$$

where $t_{pump}$ is run time of the discharge pump (in minutes), $V_{pump}$ is the volume of water pumped to the STA per unit time (in liters per minute), and $t_{samp}$ is the time between sampling dates (in days).

**Recirculation Ratio**

We calculated recirculation ratio for SeptiTech and Advantex systems ($R$) using the equation:

$$R = \frac{V_{recirc}}{V_{TOT}}$$

where $V_{recirc}$ is the volume of wastewater returned to the processing tank (in liters) and $V_{TOT}$ is the total forward flow volume (in liters), i.e. the volume of water pumped to the STA between sampling dates.

$V_{recirc}$ was calculated using the equation:

$$V_{recirc} = t_{recirc}*P_{flow}$$

where $t_{recirc}$ is the time the recirculation pump was on and $P_{flow}$ is the pump delivery rate (established by the manufacturer), which was 121 (liters per minute) for Advantex and 91 (liters per minute) for SeptiTech systems.

For Advantex systems, we calculated total forward flow ($V_{TOT}$, in liters per day) using the equation:

$$V_{TOT ADV} = (C*V_{cycle})$$

where $C$ is the number of times wastewater was discharged to the STA over the course of 30 days, $V_{cycle}$ is the volume of water discharged per cycle (in liters).

For SeptiTech systems we calculated total forward flow ($V_{TOT ST}$, in liters per day) using the equation:

$$V_{TOT ST} = (t_{pump}*V_{pump})$$

where $t_{pump}$ is run time of the discharge pump (in minutes), and $V_{pump}$ is the volume of water pumped to the STA per unit time (in liters per minute).

All calculations accounted for pipe fill up and drain back for STA discharge pumps after a dose. Recirculation ratio cannot be determined for FAST systems because nitrified effluent is gravity-fed from the oxic zone in the aeration insert to the anoxic zone of the tank.
**Supplementary Table 1.** Median (minimum–maximum) values of wastewater properties for systems sampled between March and December 2015 (n= 63-135). Denitrification-disabled SeptiTech systems were excluded from this analysis.

<table>
<thead>
<tr>
<th>System type</th>
<th>pH–SP1</th>
<th>pH–SP2</th>
<th>DO–SP1</th>
<th>DO–SP2</th>
<th>Temp.–SP1</th>
<th>Temp.–SP2</th>
<th>NH₃–SP2</th>
<th>NO₃–SP2</th>
<th>Alkalinity–SP2</th>
<th>Total N–SP2</th>
<th>BOD₅–SP2</th>
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</thead>
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<tr>
<td>Advantex</td>
<td>6.9</td>
<td>6.4</td>
<td>0.0</td>
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<td>18.2</td>
<td>17.8</td>
<td>2.5</td>
<td>11.3</td>
<td>54</td>
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<td>4</td>
</tr>
<tr>
<td></td>
<td>(5.8–8.4)</td>
<td>(2.8–7.2)</td>
<td>(0.0–7.0)</td>
<td>(0.5–10.5)</td>
<td>(5.6–28.4)</td>
<td>(2.4–28.4)</td>
<td>(0.0–49.9)</td>
<td>(0.0–29.4)</td>
<td>(0.0–247)</td>
<td>(0.0–61.6)</td>
<td>(0–100)</td>
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<td>FAST</td>
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<td>7.1</td>
<td>5.5</td>
<td>2.0</td>
<td>18.4</td>
<td>16.8</td>
<td>1.0</td>
<td>7.9</td>
<td>63</td>
<td>17.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(4.5–8.6)</td>
<td>(4.0–8.6)</td>
<td>(0.0–9.2)</td>
<td>(0.0–8.3)</td>
<td>(4.8–24.8)</td>
<td>(3.5–23.6)</td>
<td>(0.0–64.7)</td>
<td>(0.0–42.2)</td>
<td>(0–314)</td>
<td>(0.6–104.9)</td>
<td>(0–142)</td>
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<td>SeptiTech</td>
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<td>0.0</td>
<td>6.1</td>
<td>18.3</td>
<td>18.8</td>
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<td>10.3</td>
<td>59</td>
<td>11.3</td>
<td>0</td>
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<tr>
<td></td>
<td>(6.2–8.8)</td>
<td>(6.4–7.7)</td>
<td>(0.0–3.4)</td>
<td>(0.0–8.5)</td>
<td>(8.9–25.0)</td>
<td>(9.1–25.9)</td>
<td>(0.1–60.7)</td>
<td>(0.0–59.8)</td>
<td>(0–359)</td>
<td>(0–41.6)</td>
<td>(0–98)</td>
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Chapter 2: Concentration of Genes Coding for Nitrous Oxide Reductase (nosZ) and Ammonia Monoxygenase (amoA) in Advanced N-Removal Onsite Wastewater Treatment Systems

Introduction

Advanced N-removal onsite wastewater treatment systems (OWTS) are used to lower inputs of N where ground and surface water resources are sensitive to excess N levels in the northeastern U.S. (Oakley et al., 2010). Analysis of the effectiveness of a range of advanced N-removal systems has shown that, although they can lower final N concentration to meet standards, the compliance rate can vary considerably, ranging from 42 to 87% (Lancellotti et al., 2017; BCDHE, 2012, and vary among technology types (Lancellotti et al., 2017; BCDHE, 2012). Although recursive adjustment of underperforming systems has been shown to improve performance in Barnstable County, MA, compliance rates remain below 100% (BCDHE, 2012).

Autotrophic bacterial nitrification and heterotrophic bacterial denitrification are assumed to be responsible for N removal in advanced OWTS (Oakley et al., 2010). Nitrifying bacteria oxidize ammonium in septic tank effluent to nitrate, and reduction of nitrate to nitrogen gases (N₂O and N₂) by heterotrophic denitrifiers results in removal of N. These processes are separated in space, with a treatment train that includes an aerated compartment or zone to promote nitrification, and an anoxic compartment or zone to promote heterotrophic denitrification. Differences in treatment performance among technology types and among the same technology type may be linked to differences in microbial communities and how they respond to wastewater properties and environmental factors.

Despite the widespread use of N-removal OWTS to lower N inputs to sensitive ecosystems for nearly three decades, there is no information on the identity or diversity of the microorganisms involved, the size of their populations, or their activity. This is in contrast with municipal wastewater treatment plants (MWTP), where information on the size, structure, and function of relevant microbial communities is used to understand and improve plant function (e.g. Shu et al., 2015; Wang et al., 2014; Lu et al., 2014; Kim et al., 2013; Jaranowska et al., 2013) and lower greenhouse gas emissions (Song et al., 2014; Yan et al., 2016).

We used real-time quantitative PCR (qPCR) to determine the concentration of genes coding for nitrous oxide reductase (nosZ) from denitrifying bacteria and ammonia monooxygenase (amoA) from ammonia-oxidizing bacteria (AOB). nosZ genes are present in most denitrifying bacteria, which are generally heterotrophic, facultative anaerobes. The capacity to denitrify is widespread among taxa (Zumft, 1997). A high concentration of nosZ genes within a treatment component indicate that a large number of heterotrophic denitrifying bacteria are present to reduce nitrate to N₂O and N₂. amoA genes are found in aerobic, autotrophic AOB which oxidize NH₄ to NO₂. Because AOB are physiologically constrained by the availability of O₂ (Geets et al., 2006), we would expect to see a higher concentration of amoA genes in components where oxic conditions prevail. Our study was conducted using the three most frequently installed advanced N-removal OWTS (Orenco AdvantexAX-20, SeptiTech D Series and Bio-Microbics MicroFAST) systems within the Greater Narragansett Bay watershed in Rhode Island, USA. Measurements were made on samples from the oxic and anoxic
compartments of 38 systems in August 2016. In an effort to better understand the microbial processes responsible for N-removal in these advanced systems, we examined differences in the concentration of these genes in oxic and anoxic compartments as a function of technology type.

**Methods**

We used three of the most commonly installed advanced OWTS in Rhode Island, USA for this study: Orenco Advantex AX-20 (recirculating textile media filter), BioMicrobics MicroFAST (aerobic treatment unit), and SeptiTech D Series (recirculating trickling filter) (Fig. 1). Each system included an anoxic component for denitrification (referred to as SP1) and an aerated component for nitrification (referred to as SP2). The sample from SP2 represented final effluent dispersed to the soil treatment area. These systems were part of a larger study evaluating the capacity of advanced N-removal OWTS within the Greater Narragansett Bay watershed (Rhode Island, USA) to remove N from domestic wastewater (Lancellotti et al., 2017).

**Sample collection.** We collected samples from SP1 and SP2 in August 2016 from all 38 systems. Samples were collected in sterilized, 1-L plastic Nalgene bottles and stored at 4°C (max. 8 h). The Advantex, FAST, and SeptiTech technologies were sampled at the recirculating splitter valve assembly, drain field pump basin, and discharge pump basin within the processor, respectively (Fig. 1). We measured TN, ammonium, nitrate, pH, DO, effluent temperature, BOD5 and alkalinity. Median, maximum and minimum values of wastewater properties for samples from the oxic and anoxic compartments of these systems can be found in Table 1.

**DNA extraction.** Approximately 100 mL of sample was vacuum filtered onto sterile 0.22-μm-pore size nitrocellulose membrane filters (Millipore Corporation, Darmstadt, Germany). Non-sterile filters were used for 30 of 86 samples. Blanks were analyzed to confirm the lack of contamination. The filter was used for genomic DNA extraction using a PowerWater DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA). The concentration quality of all extracted DNA was determined with a NanoDrop 8000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and stored at -20°C until qPCR analysis.

**Quantitative PCR (qPCR).** The concentration of ammonia monooxygenase genes (amoA) and nitrous oxide reductase genes (nosZ) was quantified using real-time polymerase chain reaction (qPCR) with the primer sets in Table 2. Individual standard curves were prepared for each gene from a sample that presented a single clear band of the correct size after PCR amplification, and was purified with a QIAGen PCR Purification Kit (Qiagen, Gemantown, MD). The concentration (ng/μL) of the purified products to be used as standards was determined using an Invitrogen Qubit 2.0 (Thermo Fisher Scientific, Waltham, MA) and converted to number of copies/μL using their molecular weight and Avogadro’s number. Ten-fold serial dilutions of the purified product were prepared from 10^7 to 10^1 copies/μL.
Figure 1. Schematic diagram of Advantex, FAST, and SeptiTech technology treatment trains showing sampling locations. P = pump; SP1=anoxic compartment; SP2=oxic compartment and final effluent.
Table 1. Properties of wastewater samples from anoxic (SP1) and oxic (SP2) compartments in AX-20 (n=15), FAST (n=12) and SeptiTech (n=11) systems. Systems were sampled in August 2016. Units are mg/L except for temperature (°C) and pH.

<table>
<thead>
<tr>
<th>Property</th>
<th>Compartment</th>
<th>Parameter</th>
<th>AX-20</th>
<th>FAST</th>
<th>SeptiTech</th>
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<tr>
<td>Total N</td>
<td>SP1</td>
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<td>19</td>
<td>21</td>
<td>29</td>
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<tr>
<td></td>
<td>SP1</td>
<td>Max - Min</td>
<td>4 - 44</td>
<td>5 - 44</td>
<td>5 - 75</td>
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<td></td>
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<td>20</td>
<td>12</td>
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<tr>
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<tr>
<td></td>
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<td>Max - Min</td>
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<td>1 - 7</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
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<td>11</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
<td>Max - Min</td>
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<td>1 - 22</td>
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<tr>
<td>Ammonium</td>
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<td>13</td>
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<td></td>
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<td>1 - 58</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
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<td>0 - 55</td>
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</tr>
<tr>
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<td>0</td>
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<tr>
<td></td>
<td>SP1</td>
<td>Max - Min</td>
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<td>4 - 8</td>
<td>0 - 0</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
<td>Median</td>
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<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
<td>Max - Min</td>
<td>0 - 7</td>
<td>0 - 6</td>
<td>3 - 7</td>
</tr>
<tr>
<td>pH</td>
<td>SP1</td>
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<td></td>
<td>SP1</td>
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<td>6.8 - 7.9</td>
<td>6.7 - 7.7</td>
</tr>
<tr>
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</tr>
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<td>6.7 - 7.9</td>
<td>3.4 - 7.7</td>
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<td>SP1</td>
<td>Max - Min</td>
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<td>22.2 - 25.0</td>
<td>22.7 - 27.2</td>
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<td>25.5</td>
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<td>19.0 - 25.0</td>
<td>24.8 - 28.0</td>
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<td>Median</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>SP1</td>
<td>Max - Min</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
<td>Median</td>
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<td>0</td>
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<tr>
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<td>SP2</td>
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<td>0 - 12</td>
<td>0 - 4</td>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>SP1</td>
<td>Max - Min</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>SP2</td>
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<td>SP2</td>
<td>Max - Min</td>
<td>3 - 274</td>
<td>17 - 249</td>
<td>24 - 176</td>
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</table>

*Not determined.
We performed real-time PCR quantification using a Lightcycler 480 (Roche Diagnostics, Indianapolis, IN) with SYBR Green I Master (Roche Diagnostics, Indianapolis, IN). All standards and samples were analyzed in triplicate, and at least one triplicate negative control containing no template DNA was analyzed in each qPCR run to detect contamination. For both genes a total reaction volume of 20 μL was used and contained 5 μL DNA template, 0.5 μL of each primer, 10 μL of the SYBR master mix, and 4 μL of water. The thermocycler settings for nosZ were as follows: 94°C for 10 min, 40 cycles at 94°C for 10 sec, 61°C for 15 sec, and 72°C for 20 sec. The thermocycler settings for amoA were as follows: 94°C for 10 min, 45 cycles at 94°C for 10 sec, 54°C for 10 sec, and 72°C for 14 sec. For both genes, a melt curve analysis was performed to confirm the amplification of a single product. In addition, the qPCR product for one of each triplicate was examined on 1% (w/v) ethidium bromide-stained agarose gels. Amplification efficiencies for both genes ranged from 78 to 100%.

**Wastewater properties.** Field measurements of pH, DO and effluent temperature were made using a Hanna Instruments HI9828 Multiparameter Meter (Woonsocket, RI) (Lancellotti et al., 2017). The concentration of TN, ammonium, nitrate, alkalinity and BOD$_5$ were measured as described in Lancellotti et al. (2017).

**Statistical analyses.** We used a two-way ANOVA on log-transformed data to determine statistical differences in the concentration of genes, gene ratios, concentration of nucleic acids, and number of copies of genes per mass of nucleic acids among technology types and treatment train compartments. Gene concentrations below the detection limit of 10 copies/µL were assigned a value of zero. Where data sets contained values of zero, we added “1” to all values before log transformation. Mean separation was accomplished using the Student-Newman-Keuls method for pairwise multiple comparisons. Differences between technologies with final effluent TN values in regulatory compliance (TN ≤19 mg/L) and technologies with TN concentration >19 mg/L were determined using a Rank Sum Test. Analyses were performed using SigmaPlot Version 12.5 (Systat Software, San Jose, CA). Statistical significance was assessed at p<0.05 for all tests.

**Results and Discussion**

**Concentration of nosZ and amoA genes.** nosZ genes were present above the detection limit (10 copies/µL) in samples from the anoxic (SP1) and oxic (SP2) compartments from all 38
systems (Fig. 2). The median concentration of \textit{nosZ} varied by an order of magnitude, from 3,056 copies/µL for SP1 to 306 copies/µL for SP2, both in FAST systems. There were no significant differences in \textit{nosZ} concentration among technology types, but levels of \textit{nosZ} were significantly higher in SP1 than SP2 for all technology types. There was no significant interaction between technology and compartment.

**Figure 2.** Concentration of \textit{nosZ} and \textit{amoA} genes and \textit{nosZ/amoA} ratio in water samples from the anoxic (SP1) and oxic (SP2) treatment train compartments of AX-20 (n=15), FAST (n=12), and SeptiTech (n=11) advanced N-removal onsite wastewater treatment systems. Samples were taken in August, 2016 from systems within the Greater Narragansett Bay watershed in Rhode Island, USA. Treatments and compartments with the same letter were not significantly different.

The median specific abundance of \textit{nosZ} – the fraction of the microbial community accounted for by denitrifiers – varied by more than an order of magnitude, from 16.5 copies/ng nucleic acid in the oxic compartment of FAST systems to 227 copies/ng nucleic acid in the oxic compartment of AX-20 systems (Fig. 3). There were no significant differences in specific abundance of \textit{nosZ} among technology types or between compartments within technology type.

\textit{amoA} genes were present at detectable levels (>10 copies/µL) in the SP1 compartment in
24 of 38 systems, and in SP2 in 30 of 38 systems. The median concentration of amoA genes ranged from 0 copies/µL in SP2 to 263 in SP2 copies/µL, both in SeptiTech systems (Fig. 2). The concentration of amoA was significantly lower in AX-20 and SeptiTech than FAST systems, and the level of amoA was higher in SP2 than in SP1 for all technology types. There was no significant interaction between compartment and technology type.

The median specific abundance of amoA ranged from 0 copies/ng nucleic acid in SP1 to 30.9 copies/ng nucleic acid in SP2, both in SeptiTech systems (Fig. 3). Specific abundance of amoA genes was significantly higher in AX-20 and SeptiTech than in FAST systems, and was significantly higher in SP2 than in SP1 in AX-20 and SeptiTech technologies.

**Figure 3.** Specific abundance of nosZ and amoA genes in water samples from the anoxic (SP1) and oxic (SP2) treatment train compartments of AX-20 (n=15), FAST (n=12), and SeptiTech (n=11) advanced N-removal onsite wastewater treatment systems. Samples were taken in August, 2016 from systems within the Greater Narragansett Bay watershed in Rhode Island, USA. Treatments and compartments with the same letter were not significantly different.

The median ratio of copies of amoA to nosZ ranged from 0 in SP1 to 0.31 in SP2, both in SeptiTech systems. There were no significant differences in amoA:nosZ among technology types or between compartments within a particular technology type.

Our results show that nosZ genes were ubiquitous, present in both the oxic and anoxic compartments of all the systems we tested. This is not particularly surprising, given that denitrifying bacteria are generally facultative anaerobes, and the capacity to denitrify is widespread among taxa (Zumft, 1997). The concentration of nosZ genes in advanced N-removal
OWTS (Fig. 2) was within the range of values reported for MWTP with biological N removal (Geets et al., 2007; Wang et al., 2014). The median concentration of amoA genes was generally lower than that of nosZ genes (Fig. 2), but was in the range of values reported for MWTP with biological N removal (Awolusi et al., 2015; Geets et al., 2007). Although amoA genes were present in at least one system of each technology type, and in oxic and anoxic compartments, they were detected in fewer systems than nosZ genes: the concentration of amoA genes was below the detection limit of 10 copies/µL in 36% of SP1 samples and 21% of SP2 samples.

As aerobes, autotrophic AOB are physiologically constrained by the concentration of O₂ (Geets et al., 2006), so that the absence of amoA genes in anoxic wastewater is not surprising, since DO was absent from water in SP1 in AX-20 and SeptiTech systems (Table 1). However, the absence of amoA in the oxic compartment of some systems is unexpected, given that DO levels were above the requirements for ammonia oxidation (Geets et al., 2006) and ammonium was present, albeit over a range of concentrations (Table 1).

The absence of amoA genes in some systems is troubling from a practical standpoint, since advanced N-removal systems rely on sequential nitrification and denitrification for N removal. It is possible that ammonia oxidation is active despite our inability to detect amoA genes. Ammonia oxidation could, for example, take place within the anoxic compartment, with AOB at the air-water interface oxidizing ammonia, albeit at a slower rate because of limited O₂ diffusion. Our samples were taken from ~50 cm below the water surface, and was not designed to capture spatial variations in gene concentration. We observed only one instance of amoA genes being absent from both the oxic and anoxic compartments – in a single AX-20 system. Although the final effluent TN for that system was 26.8 mg/L, the concentration of ammonium was lower, and the concentration of nitrate higher in the oxic than the anoxic compartment, indicating that ammonia oxidation was taking place, possibly at the air-water interface in the anoxic compartment. Even if AOB were present in low numbers (estimated at <10,000 cells/mL, assuming one copy of the amoA gene per cell), they may have maintained a rate of ammonia oxidation sufficient to support N removal if they had a high specific activity. Method limitations may also have affected detection of the amoA gene. For example, DNA from less prevalent taxa may be amplified less efficiently, or the primers used may be less likely to bind to certain taxa, since primer sequences generally target the most common taxa. Both cases would result in underestimation of the size of the AOB community.

There were significant differences in the concentration of amoA genes, but not nosZ genes, among technology types, and between compartments within a technology for amoA and nosZ (Fig. 2). Levels of amoA were similar in AX-20 and SeptiTech systems, both of which were significantly lower than FAST systems. As stated previously, the ubiquity of nosZ among various taxa, and the physiological versatility of denitrifiers, likely accounts for the absence of differences among technology types. Differences in amoA levels among technology types may reflect differences in technology configuration (Fig. 1): both AX-20 and SeptiTech are media filters that have discrete oxic and anoxic compartments separated in space, whereas FAST systems rely on an oxic-anoxic gradient within a zone that promotes nitrification and denitrification in different locations, which may provide AOB with better growing conditions.
Differences in levels of amoA and nosZ genes between compartments in all technology types were likely driven by differences in levels of dissolved O₂, which exert a substantial selective pressure, particularly on AOB (Geets et al., 2006). AOB accounted for a different fraction of the microbial community in different technology types and compartments, as indicated by differences in the specific abundance of amoA genes (Fig. 3). These differences are likely driven by the concentration of O₂ and NH₄, and by system configuration, as indicated previously. In contrast, the fraction of the microbial community made up of denitrifiers was relatively constant, as indicated by the absence of differences in specific abundance of the nosZ gene among technology types or between compartments, likely resulting from the ubiquitous distribution of the nosZ gene among bacteria in different taxa, and their physiological versatility.

The absence of differences in the ratio of amoA:nosZ among technology types or compartments suggests that, although the magnitude of the concentration of these genes differs significantly, the factors controlling the relative numbers of these organisms were similar among technology types and compartments.

**Differences between compliant and non-compliant systems.** We examined whether there were differences in concentration of nucleic acids, amoA or nosZ, amoA:nosZ, or the specific abundance of amoA or nosZ from either compartment between systems that had final effluent TN values in compliance with state regulations (≤ 19 mg/L) (RIDEM, 2009) and those that were non-compliant. Comparisons were made only for AX-20 and FAST systems, which had 4 out of 15 and 6 of 12 non-compliant systems, respectively, whereas only 1 out of 10 SeptiTech systems was out of compliance, precluding statistical comparisons. For AX-20 systems, the concentration of amoA in SP2 was significantly higher in compliant systems, whereas for FAST systems, the concentration of nosZ in SP2 was significantly higher in compliant systems. No other variables were significantly different among compliant and non-compliant systems.

Although compliant AX-20 systems had a higher concentration of amoA in SP2 than non-compliant systems, and FAST systems had higher levels of nosZ in SP2 than non-compliant systems, we did not observe significant correlations between effluent TN concentration and levels of amoA or nosZ genes in either case (data not shown). The relationship between the concentration of nosZ and amoA genes and effluent TN may be more complex than can be revealed by our data, since the presence of a gene is not synonymous with its expression or the associated enzymatic activity (Kim et al., 2013).

**Nucleic acid concentration.** The median concentration of extracted nucleic acids (double and/or single-stranded) – a proxy for the size of the microbial community – varied by an order of magnitude among systems, from 6.2 ng/µL in the oxic compartment of AX-20 systems to 42.7 ng/µL in the anoxic compartment of SeptiTech systems (Fig. 4). There were no significant differences in the concentration of nucleic acids among technology types, but the concentration was significantly higher in the anoxic than the oxic compartment for all technology types.

The absence of differences in the size of the microbial community, reflected in the concentration of nucleic acids, among technology types, suggests that the factors controlling the size of the microbial community (e.g. nutrients, substrate availability, temperature) were similar among systems. This is not unexpected, since all the systems in our study received sewage from
households – which tend to have wastewater with similar properties – and were found within a relatively small geographic area. Higher concentration of nucleic acids in anoxic than oxic compartments, observed in all technology types, is likely driven by a higher concentration of carbon substrates in the anoxic compartment, which receives septic tank effluent with a high BODs. Organic C is consumed by denitrifiers and other microorganisms in the anoxic compartment, which lowers the amount of C that is transferred to the oxic compartment (Table 1) that may be available for growth of heterotrophic microorganisms.

![Figure 4](image-url)  

**Figure 4.** Concentration of nucleic acids in water samples from the anoxic (SP1) and oxic (SP2) treatment train compartments of AX-20 (n=15), FAST (n=12), and SeptiTech (n=11) advanced N-removal onsite wastewater treatment systems. Samples were taken in August, 2016 from systems within the Greater Narragansett Bay watershed in Rhode Island, USA. Treatments and compartments with the same letter were not significantly different.

**Conclusions**

We provide the first report on the concentration of the genes for ammonia oxidation – amoA – and nitrous oxide reduction – nosZ – in advanced N-removal OWTS. Our results show that the concentration of both genes is in the range reported for MWTP with biological N removal. Furthermore, the levels of these genes differ as a function of technology type and/or compartment within the treatment train, depending on the gene. In addition, amoA genes were below detection levels for a number of systems, whereas nosZ genes were detected in all 38 systems tested. Finally, compliant and non-compliant systems differed in the concentration of amoA or nosZ genes.

Our results point to the need for further examination of the functioning of these systems at the molecular level. For example, improved understanding of the level of expression of these genes – using qPCR targeting mRNA instead of genomic DNA – rather than just their concentration, may lead to improved understanding of the relationship between system properties and N removal. Similarly, knowledge of the identity of the microorganisms involved can help us identify optimal conditions to promote the growth of species that support system function. Finally, we need to explore the possibility that processes other than autotrophic nitrification and heterotrophic denitrification may contribute significantly to N removal in these systems.
References


Chapter 3: Accuracy of Rapid Tests Used for Analysis of Advanced Onsite Wastewater Treatment System Effluent

Introduction

Water quality data from advanced onsite wastewater treatment systems (OWTS) can be used to evaluate their function and treatment performance. These data can be obtained in situ, quickly and inexpensively, using rapid tests. This can be especially advantageous when monitoring advanced OWTS because it allows for quick assessment of system treatment efficiency (Bounds et al., 2004; SeptiTech, 2009), enabling real-time system adjustment without having to wait for the results of laboratory analyses. For example, DO concentrations serve as an indicator of conditions favorable for nitrification and denitrification in advanced N removal systems (Heger, 2015). Rapid measurement of DO in the field allows for operational adjustments to be made promptly, improving the nitrogen removal efficiency of the system.

Rapid tests are commercially available for a number of water quality parameters, including DO, alkalinity, nitrate, ammonium, and pH using test strips, test kits and portable instruments. Although most rapid tests are not sold explicitly for field use or testing of wastewater, their ease of use, low cost, and availability makes them desirable for this purpose.

Test strips for chemical constituents are equipped with a chemically-sensitive pad that is submerged into a water sample for a specific time; the pad changes color according to the concentration present, which is interpreted by comparing the test pad to a color chart (Isbell et al., 2006). The values are discontinuous, representing ranges of values. Field test kits for DO employ a modified Winkler titration method, with the volume of titrant required equal to the concentration of dissolved oxygen in the sample (USEPA, 2007). This produces a series of discontinuous values limited by the ability to measure the volume of titrant added. Colorimetric test kits for nutrients involve mixing of reactants with the water sample, waiting for a specific amount of time, and comparing the color of the reaction mixture to a color chart. As with test strips, the resulting values are discontinuous. In contrast, the values from portable instruments, such as a pocket pH meter, are continuous and are not subjective, because they do not involve interpretation by the user.

Despite the advantages of rapid tests, their accuracy when used to measure the concentration of constituents in effluent from advanced OWTS has not been investigated. Published reports on their accuracy when used with freshwater samples are mixed. For example, Isbell et al. (2006) concluded that nitrate test strips, when used to test freshwater samples, are an acceptable field method compared to standard laboratory methods, although they provide more accurate results at higher nitrate concentrations. In contrast, Murphy et al. (2014) reported that nitrate test strips are inaccurate compared to standard analysis values when used to test freshwater samples, and concluded that they are not a reliable method. A number of factors may contribute to inaccurate values, particularly for those that rely on color changes. For example, the absence of masking agents to reduce interferences by other constituents can result in distorted colors (Sweazy, 2009). Insufficient buffering capacity, resulting in pH values that are suboptimal for a reaction (Isbell et al., 2006; Russell, 1944) can result in poor color development.
Field conditions present a number of challenges to the use of rapid tests for monitoring the functioning of advanced OWTS, including temporal and spatial variations in temperature, humidity and light. Many rapid methods require visual comparison to provided color standards, which is affected by the quality and intensity of sunlight, which differs over the course of a day and over the seasons, and with weather conditions (Taylor and Kerr, 1941). Furthermore, color standards are limited to a few possible values, with colors that may be difficult to differentiate because they are close on the visible light spectrum, differences in the quality of ambient light, and variations in an individual’s ability to perceive color differences (Post et al., 1993; Fellers et al., 2015). Other sources of error include interference from compounds present in effluent, as well as inadvertent contamination of reagents, reaction vessels and measuring devices, which is more likely to be experienced under field conditions.

The lack of information surrounding the performance of rapid tests – and their potential utility to monitor treated wastewater – prompted us to assess their accuracy. We used treated wastewater effluent samples collected monthly from 42 different advanced nitrogen removal OWTS within the Greater Narragansett Bay watershed in southern Rhode Island, USA. We analyzed the samples in the field using an initial suite of rapid tests that included test strips for alkalinity, ammonium, and nitrate, a pocket meter for pH, and a DO titration test kit. We analyzed the same samples for the same analytes using standard (laboratory) methods. We carried out a variety of statistical analyses on values obtained using rapid tests and standard methods in order to assess the accuracy of the rapid tests based on their ability to predict actual constituent levels determined using standard methods. Poor agreement between field and standard values for most tests in the initial suite prompted us to evaluate the accuracy of alternative rapid tests.

Methods

Study Systems

We sampled effluent from the three most commonly installed advanced nitrogen removal OWTS within the greater Narragansett Bay watershed in Rhode Island, USA (RIDEM, 2013): (i) Orenco Advantex AX20® (17 systems), (ii) Bio-Microbics MicroFAST® (14 systems), and (iii) SeptiTech D® Series (11 systems). These 42 systems are located in the towns of Jamestown, Portsmouth, South Kingstown, North Kingstown, and Charlestown.

Sample Collection

Effluent samples were collected once a month during the first two weeks of February, March, April, May, June, July and August 2015 from 42 different advanced nitrogen removal OWTS. The Advantex, FAST, and SeptiTech technologies were sampled at the recirculating splitter valve assembly, drainfield pump basin, and discharge pump basin within the processor, respectively. To avoid contamination, samples were collected using a deep sampler or in a separate clean, new, disposable plastic cup for shallow sampling locations. Samples were collected in site-dedicated 1-L plastic Nalgene bottles and stored in the dark at 4°C. Samples for alkalinity analysis were stored in 250-mL plastic Nalgene bottles that were overfilled with sample to minimize exchange of CO₂ with the atmosphere. All samples were brought back to the
laboratory within 8 h of sampling and promptly analyzed for pH on the same day of collection. A portion of the sample was passed through a 0.45-μm-pore filter and frozen prior to NH₄⁺ and NO₃⁻ analysis using standard methods.

Analyses

**Initial Suite of Rapid Tests.** Evaluation of the accuracy of the initial suite of rapid tests was carried out using effluent samples from February, March, April and May 2015. The manufacturer-provided instructions were followed for all rapid tests. All analyses were performed on unfiltered samples. Nitrate was analyzed using Insta-Test nitrate strips (Lamotte, Chestertown, MD), which measure NO₃⁻-N over a range of 0 to 50 mg/L and gradations of 0, 5, 10, 25, and 50 mg/L. Ammonia was measured using Insta-Test ammonia strips (Lamotte), which have a detection range for NH₃-N of 0 to 6.0 mg/L and gradations of 0, 0.5, 1.0, 3.0, and 6.0 mg/L. If necessary, samples were diluted with deionized distilled water to ensure that values were within the detection range. Total alkalinity (as CaCO₃) was measured using Aquacheck total alkalinity test strips (Hach, Loveland, CO), with a detection range of 0 to 240 mg/L, measured in increments of 40 mg/L. pH was measured using pH indicator strips (Fisherbrand, Pittsburgh, PA) which measure pH from 0 to 14 in increments of 1 pH unit. We also determined pH using an EcoTestr pH 2 pocket meter (Oakton, Vernon Hills, IL) that measures pH from 0 to 14.0 with a resolution of 0.1 pH unit. Dissolved oxygen (DO) was measured exclusively in the field using a titration test kit (rapid test) and a handheld meter (considered the standard method for the purposes of our study, described below) to minimize exchange of O₂ with the atmosphere. We used a model OX-2P Dissolved Oxygen Test Kit (Hach), which measures DO over a range of 0.1 to 4 and 1 to 20 mg/L. The test has a resolution of 1 mg/L in the 1-20 mg/L range, and of 0.1 mg/L in the 0.1-4 mg/L range.

Test strips were dipped directly into the plastic cup, with a single test strip used per test site. For analysis of DO by titration, an aliquot of the sample was taken from the plastic cup and placed into a glass titration vial that was thoroughly rinsed with deionized, distilled water between samples. For analysis of pH using the pocket meter, the meter was calibrated with pH 4, pH 7, and pH 10 buffer before each sampling event, and the probe was rinsed with deionized, distilled water in between samples. To avoid individual differences in interpretation, the same person performed a rapid test during a sampling event. For all rapid tests, one set of duplicate analyses was carried out for every 10 analyses. For test strips, field replicates and duplicate were required to be in the same category as the initial sample (e.g. no difference between the two results); for the pocket pH meter duplicates were required to be within 1.0 standard unit.

**Alternative Suite of Rapid Tests.** We also evaluated a suite of alternative rapid tests for ammonium, pH, nitrate, and alkalinity using effluent samples from May, June, July and August 2015, with analyses carried out on unfiltered samples. A test kit (Aquarium Pharmaceuticals, Chalfont, PA) was used to measure NH₃/NH₄⁺-N from 0 to 8 mg/L, with gradations of 0, 0.25, 0.5, 1, 2, 4, and 8 mg/L. A “5-in-1” test strip (Aquarium Pharmaceuticals) was used to measure pH (range of 6 to 9, in increments of 0.5 pH units), as well as NO₃⁻ (range of 0 to 200 mg/L, with gradations of 0, 20, 40, 80, 160, and 200 mg/L), and carbonate alkalinity (range of 0 to 240 mg/L, with gradations of 40, 80, 120, 180 and 240 mg/L). Nitrate was also measured using a test kit (Aquarium Pharmaceuticals) that detects NO₃⁻ from 0 to 160 mg/L, with gradations of 0, 5,
10, 20, 40, 80, and 160 mg/L. Nitrate values obtained using the Aquarium Pharmaceuticals test strips and test kit were divided by 4.43 in order to be expressed as NO$_3$-N.

Analyses using test strips were performed as described above. For analyses using test kits, an aliquot of effluent was placed into a reaction vessel that had been thoroughly rinsed with deionized, distilled water between samples.

**Standard Laboratory Methods.** Analysis for pH and alkalinity was carried out on unfiltered samples, whereas samples analyzed for NH$_4^+$ and NO$_3^-$ were passed through a 0.45-μm-pore-size membrane filter before analysis. Filtered and unfiltered samples were stored frozen before analysis. pH was determined using a Denver Instruments (Bohemia, NY) Ultrabasic10 meter equipped with a pH/ATC electrode. Alkalinity was measured with an Automatic Titration System (Hanna Instruments, Woonsocket, RI) using a 1 N HCl solution to titrate a 100-mL effluent sample. Dissolved oxygen measurements were made in the field with a model HI9828 Multiparameter Meter (Hanna) fitted with a polarographic electrode that measures DO from 0 to 50.00 mg/L. The concentration of ammonium (Doane and Horwath, 2003) and nitrate (Weatherburn, 1967) were measured colorimetrically using a Bio Tek Powerwave 340 microplate reader (Winooski, VT). The values obtained by standard methods were within the range of expected values (NSF International/US EPA, 2003; Ohio Environmental Protection Agency, 2010) and are listed by technology in Table 1.

Data collected from laboratory analyses were required to meet performance criteria to ensure accuracy and precision, and eliminate bias, and data that did not meet these criteria were eliminated and the samples were reanalyzed. For ammonium and nitrate analysis, the concentration of the method blanks was required to be ≤200 μg N/L. Sample analyses were performed in triplicate, and the coefficient of variation among the triplicates could not vary more than 20%. The calibration curve was required to have an $R^2$ of at least 0.99, and the concentration of standards used to create the calibration curve could not deviate more than 20% from the true value. An externally purchased laboratory control standard was also analyzed, and its concentration could not deviate more than 20% from the true value. A matrix spike was analyzed, which was required to have a recovery of 80-100%. The electrodes for pH and alkalinity analysis were calibrated before each use, and the electrode efficiency was required to be at least 95%. Laboratory control standards (4.0, 7.0, and 10.0) were required to be within 0.2 standard units of the true value. The handheld meter used to measure DO was calibrated using a saturation standard before each sampling event. The DO meter did not accept the calibration if the standard concentration deviated more than 20% from the true value.

**Statistical Analyses**

Prior to performing statistical analyses we binned values obtained using standard methods into intervals reflecting the possible values obtained using rapid methods. This eliminates error from differences in resolution between the two methods. For data obtained using Hach alkalinity strips, the results were binned to include values between the gradations (0 to 240 mg/L in increments of 40 mg/L), since the directions instruct the user to interpolate.
### Table 1. Summary statistics for final effluent properties from different types of advanced nitrogen removal technologies analyzed using standard methods. Units are mg/L except for pH.

<table>
<thead>
<tr>
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<th>SeptiTech</th>
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*SD = standard deviation; CV = coefficient of variation

Analysis of variance (ANOVA) and regression analysis were used to determine differences between values produced by rapid and standard methods, and to evaluate the accuracy of rapid methods. We used a one-way ANOVA on ranks – which makes no assumptions about normality of data – to determine whether there were statistical differences between values obtained using rapid and standard methods. We used Tukey’s test (α=0.05) for mean comparisons. A one-way ANOVA on ranks was used to examine whether differences in the accuracy of rapid methods was due to technology type, location, and/or sampling month.
Comparing measures of central tendency may yield results obscured by the variability of parameter levels themselves, unrelated to experimental error or differences between methods, as well as differences in accuracy as a function of constituent concentration. To address this, we carried out a linear regression of the data, with the values obtained using standard methods as the independent variable and values from rapid methods as the dependent variable. We compared the resulting line to the line representing perfect correspondence between field and standard methods, with a slope of 1, an intercept of 0, and an $R^2$ value of 1. This method is more robust to high variation in data and yields a more appropriate representation of accuracy. Statistically significant deviation of regression parameters from ideal values was assessed using a two-tailed $t$-test ($\alpha=0.05$).

**Results and Discussion**

**Comparison of Initial Suite of Rapid Tests and Standard Methods**

_Evaluation of accuracy using ANOVA._ Values of alkalinity, DO, ammonium, nitrate and pH determined using the initial suite of rapid tests in the field and values obtained using standard methods are shown in Table 2. Analysis of the data using ANOVA on ranks showed

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<th>Property</th>
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statistically significant differences in the median values using rapid tests and standard methods for the nitrate and pH strips, but not for ammonium, alkalinity, DO, or the pH pocket meter (data not shown). However, the absence of significant differences using ANOVA does not assure the accuracy of these rapid tests, because measures of central tendency are affected by high variability of observed values unrelated to inaccuracy between methods, or interaction between standard factor level and accuracy. Furthermore, rapid tests are often performed once for a particular sample. A measure of central tendency chooses a single representative point for all the data, whereas a regression test gives a better assessment of all data points, making it a better choice for statistical assessment when the accuracy of a single test can make a difference.

**Evaluation of accuracy using linear regression.** In order to assess accuracy in a more comprehensive manner, we compared the regression parameters of rapid and standard methods (intercept, slope and R\(^2\) values) with ideal values of 0, 1 and 1, respectively. This test is more robust to variability of data and interaction factors than ANOVA.

When standard values were plotted against rapid test values, the resulting regression lines showed a clear deviation from a 1:1 line, except for the pH pocket meter (Fig. 1). This indicates poor correlation between standard and field values, which translates into inaccuracy of rapid tests used in the field. For example, when values obtained using alkalinity test strips were plotted against standard values, the resulting regression line appeared to compare well with the 1:1 line, but the values obtained using strips were very variable compared to standard values. The strips over-predicted values within the 0 to 160 mg/L range (Fig. 1), so that a standard value of 120 mg/L was detected by the alkalinity test strip as between 20 and 240 mg/L.

**Figure 1.** Standard values plotted against values obtained using the initial suite of rapid tests for alkalinity, pH test strips, pH pocket meter, dissolved oxygen, ammonium-N, and nitrate-N tested under field conditions (n = 39 – 284). The dashed line represents complete correspondence between values obtained using rapid tests and standard methods, with an intercept of 0, a slope of 1, and an R\(^2\) of 1. All values are represented in mg/L except for pH.
Dissolved oxygen values obtained by titration were highly variable throughout the entire range of values tested, although the regression line matched well with the 1:1 line over the 4 to 6 mg/L range (Fig. 1). Ammonium strips over-predicted at low concentrations and under-predicted at high concentrations. For example, a standard NH$_4^+$ value of 300 mg NH$_4^+$-N/L was detected by the strips as half that. Nitrate strips under-predicted values at high concentrations, with a standard value of 50 mg NO$_3^-$-N/L detected as between 0 and 25 mg N/L by the test strips. They appeared to be more accurate in the 5-10 mg N/L range, but detected between 0 and 10 mg N/L when there was no NO$_3^-$ present. The pH strips detect standard values between 4 and 7 as 4 or 5 (Fig. 1). In contrast, the values produced by the pocket pH meter corresponded closely to the 1:1 line, indicating that it accurately represents effluent pH.

The results of statistical analysis of our data underscore the lack of accuracy of most rapid tests used in the field (Table 3). The fraction of total variation explained by a proportional relationship between field and standard values, as indicated by R$^2$ values, ranged from 0.02 for pH strips to 0.84 for the pH pocket meter. The slope of the regression line for all rapid tests – with the exception of the pH pocket meter and alkalinity strips – deviated significantly from correspondence with standard values (Table 3). The intercept of the regression line for all rapid tests – except for the pH pocket meter – deviated significantly from 0, indicating that mean differences between field and standard values were greater than 0 for all parameters after accounting for the relationship between standard and rapid test values.

Table 3. Parameters of regression lines comparing results of the initial suite of rapid tests under field (n = 120 – 284) and laboratory (n = 24) conditions with results of standard analyses. Differences in slope and intercept from ideal values (1 for slope, 0 for intercept) were determined using a two-tailed t-test. Values in bold are significantly different from ideal values (α=0.05).

<table>
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<tr>
<th>Property</th>
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<th>Intercept</th>
<th>Slope</th>
<th>R$^2$</th>
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<td>0.66</td>
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<tr>
<td>Dissolved O$_2$</td>
<td>Field</td>
<td>2.4</td>
<td>0.6</td>
<td>0.46</td>
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<td>--</td>
<td>--</td>
</tr>
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<tr>
<td></td>
<td>Laboratory</td>
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</table>

Identification of factors that affect accuracy. We analyzed the data to identify factors that may influence the accuracy of rapid tests. The difference between field and standard values was determined for all observations – as a measure of accuracy – and sorted by technology type, sampling month, and the town where the system was located, allowing us to determine whether
any of these factors could account for differences in accuracy (Table 4). There was a significant
difference in the accuracy of alkalinity measurements using rapid tests between Advantex and
SeptiTech systems. For DO, the accuracy of rapid tests was significantly lower for FAST
systems than for Advantex and SeptiTech. Accuracy of ammonium rapid tests differed
significantly between April and May, and for pH between February and March. The accuracy of
ammonium rapid tests also differed significantly between Portsmouth and South Kingstown.
Differences in the treatment processes within a technology and in water use patterns within a
home may result in final effluent properties that produce variations in the accuracy of rapid tests.
Similarly, variations in physicochemical properties of potable water to the home (individual
drinking water wells vs municipal water supplies) may be responsible for differences in accuracy
as a function of location.

To determine if some wastewater properties affect the accuracy of rapid tests on other
properties (for instance, if a high pH correlated with lower accuracy for a nitrate test), we
performed multiple regressions analysis using values obtained using standard methods as
predictors for the absolute value of differences between standard values and rapid test values – a
measure of accuracy. Values of pH obtained using test strips were less accurate at lower
ammonium levels, and the inaccuracy of ammonium, pH, and nitrate rapid testing was positively
correlated with standard values (data not shown). We did not observe other significant
correlations. The differences in accuracy at extreme values are further indication that regression
testing is more effective than ANOVA in this case, as ANOVA testing would not account for a
linear relationship between field and standard values that differs in proportionality from what is
expected.

| Table 4. Results of ANOVA to determine effects of system type, sampling month, and sampling location on the accuracy of rapid tests. Properties with the same letter within the same level of a factor were not statistically different. |
|---|---|---|---|---|---|
| Factor | Level | Alkalinity | Ammonium | Dissolved O₂ | Nitrate | pH |
| Technology | FAST | AX | A | A | A | A |
| SeptiTech | AB | B | A | A | A | A |
| Month | February | A | AB | A | A | A |
| March | A | AB | A | A | B |
| April | A | A | A | A | NA* |
| May | A | B | A | A | NA* |
| Location | Jamestown | A | AB | A | A | A |
| Portsmouth | A | A | A | A | A |
| Charlestown | A | AB | A | A | A |
| South | A | B | A | A | A |
| Kingstown | A | AB | A | A | A |
| North | South Kingstown | A | AB | A | A | A |

*NA=Not applicable; no data were collected
Differences in accuracy found between locations, month collected, and technology type can obscure statistical results; however, the high variability of rapid test values also makes detection of statistical differences between standard and rapid methods more difficult. The average coefficient of variation (CV) for rapid tests in the field was 0.95, with values as high as 2.14 for ammonium (Table 2), indicating that the standard deviation was almost as high or higher than the mean of datasets. This level of variability makes statistical differences difficult to detect. For example, based on the standard deviation of the difference between standard and rapid test values to estimate the population standard deviation, an approximate sample size of 206 is needed to detect an ammonium difference of 4 mg/L, and a sample size of approximately 131 for an alkalinity difference of 10 mg/L.

**Evaluation of Initial Suite of Rapid Tests Under Controlled Conditions**

The low accuracy of most rapid tests under field conditions led us to examine their performance under controlled conditions. To exclude the influence of field conditions on the accuracy of tests, we carried out the same rapid tests procedures for nitrate, ammonium, pH and alkalinity in a controlled laboratory setting under consistent fluorescent lighting, and stable temperature and moisture conditions.

![Graphs showing the accuracy of rapid tests](image)

**Figure 2.** Standard values plotted against values obtained using the initial suite of rapid tests for alkalinity, pH test strips, ammonium-N, and nitrate-N conducted under controlled laboratory conditions (n = 24). The dashed line represents complete correspondence between values obtained using rapid tests and standard methods, with an intercept of 0, a slope of 1, and an $R^2$ of 1. All values are represented in mg/L.

The accuracy of the initial suite of rapid tests improved under controlled conditions for all the tests evaluated (Table 3). Nitrate standard values plotted against rapid test values fit closely to a 1:1 line (Fig. 2), and the strips detected nitrate at low (0 to 10 mg/L) concentrations more accurately. Data from pH strips were more variable than for nitrate, and produced variable results for standard values of 7. Ammonium strips were least accurate at high concentrations, as
was the case in field conditions. Alkalinity strips produced more accurate results at low (0-40 mg/L) concentrations under controlled conditions than in the field. None of the regression lines deviated significantly from a 1:1 line; nevertheless, $R^2$ values were generally low, ranging from 0.16 for alkalinity to 0.66 for ammonium (Table 3).

Excluding the variability imparted by field conditions (e.g. differences in moisture, temperature and lighting) improved the accuracy of rapid tests, likely for various reasons. For example, the color on the test pad of test strips is difficult to pinpoint when compared to the provided chart, which subjectively influences the results, rendering them inaccurate. Variations in the intensity and quality of sunlight in the field can exacerbate this issue, which is controlled for in the laboratory by consistent, artificial lighting. Additionally, exposure to moisture, heat, or cold conditions in situ can interfere with color development in test strips, causing anomalous color changes to occur. Under these conditions the chemical reaction on the test pad may produce colors with significantly less pigmentation than those on the color chart, making it more difficult to differentiate between color blocks (Sweazy, 2009). The effects of variations in moisture and temperature are eliminated in the laboratory, where test strips are stored and used at a constant temperature (19 – 21°C) and a constant, low relative humidity (~60%).

The presence of particles in field-tested samples may also have affected the ability to distinguish colors, therefore affecting the accuracy of the results. This could be addressed by filtering samples in the field, but this may introduce another, more significant source of error from contaminated filters as a result of handling. Filtering samples in the field would also add to the costs of testing and require longer site visits. Thus, although filtration in the field may, in theory, improve accuracy, it may not be practical and may in practice reduce accuracy.

The absence of statistical differences from an ideal regression line could be partly due to using a smaller sample size for the evaluation of accuracy in the laboratory. Although the average CV for rapid tests conducted in the laboratory (0.85) is lower than the average CV for the same tests used in the field (1.32) (Table 2), the smaller sample size makes determination of statistical differences more difficult. This could explain, in part, the lack of statistical differences observed for rapid tests conducted under controlled laboratory conditions when these tests were determined to be inaccurate in the field. The smaller sample size results in wider confidence intervals, and thus a lower likelihood of identifying statistical differences, if they exist.

**Evaluation of Alternative Suite of Rapid Tests Under Field Conditions**

In view of the poor accuracy of most of the rapid tests in the initial suite under field conditions, we examined an alternative suite of rapid tests to try to identify other accurate methods. We evaluated a “5-in-1” test strip that tested for alkalinity, pH and nitrate, and a test kit for ammonium. The alternative rapid tests are manufactured by a different company than the initial suite, and the test kit employs a different method than the test strips.

The ammonium test kit produced variable results for standard values of 20 and 40 mg N/L, with a standard value of 20 mg N/L detected as between 2 to 120 mg N/L using the test kit (Fig. 3). The nitrate test strips underestimated nitrate concentrations in the range of 0 to 40 mg N/L; for example, a true value of 40 mg N/L was detected as 0 mg N/L using the test strips. The
pH strips also produced variable results for a standard value of 7. The “5-in-1” test strips for alkalinity detect up to 120 mg/L when there was no alkalinity present in the sample. Rapid tests for pH and ammonium of the alternative suite yielded regressions with a slope and intercept that did not differ significantly from a 1:1 line under field conditions, but the opposite was true for nitrate and alkalinity tests (Table 5). Values of $R^2$ ranged from 0.11 for nitrate to 0.52 for alkalinity. Although the alkalinity test strips yielded the highest $R^2$ value, the intercept and slope differ significantly from ideal values, indicating that the $R^2$ value alone is not a good measure of accuracy. As was the case for the initial suite of rapid tests, evaluation of the alternative suite of rapid tests under controlled conditions resulted in improved accuracy relative to results obtained under field conditions (data not shown).

**Figure 3.** Standard values plotted against values obtained using the alternative suite of rapid tests for alkalinity, pH, ammonium-N, and nitrate-N under field conditions ($n = 90 – 170$). The dashed line represents complete correspondence between values obtained using rapid tests and standard methods, with an intercept of 0, a slope of 1, and an $R^2$ of 1. All values are represented in mg/L except for pH.

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Conclusions

All the rapid tests in the initial suite evaluated in the field – other than the pH pocket meter – showed a considerable lack of accuracy, evidenced by poor correspondence with standard values, low R² values, and statistical differences from an ideal (1:1) relationship of slope and intercept. In addition, some tests in the alternative suite of rapid tests evaluated in the field also yielded inaccurate results, as evidenced by poor correspondence with standard values, even in the absence of statistically significant deviation from an ideal relationship. Our results show that sampling date, system technology type and location, as well as the concentration of other wastewater constituents, can influence the accuracy of rapid tests. In addition, comparison of results obtained under field and laboratory conditions indicated that the controlled conditions of the laboratory resulted in accurate values, suggesting that field conditions can be a considerable source of error in the measurement of effluent properties. These factors need to be considered when evaluating the suitability of rapid tests for effluent analysis. Our results point to the need to evaluate the accuracy of rapid tests under field conditions before they are adopted for monitoring purposes. Nevertheless, our data suggest that the pH pocket meter, “5-in-1” test strips for pH, and the ammonium test kit may be suitable for testing of septic system final effluent in the field.

The accuracy of rapid tests involving color changes could be improved significantly by performing the tests in a more controlled environment, such as an indoor work space where light, temperature and humidity are more consistent. Test strip readers, smartphone applications that detect color changes, and pocket colorimeters may be used to further minimize the subjectivity of rapid tests involving colorimetric determinations. Although these approaches add to the cost of analysis, they should increase the accuracy of rapid tests by removing the need for interpretation of color changes (Webb, 2008; Cooper, 2012).

References


Ohio Environmental Protection Agency. (2010). Summary Report of Sample Results Obtained under the Household Sewage Treatment Systems General National Pollutant Discharge Elimination System Permit Program (Revised). Available at: https://www.odh.ohio.gov/~media/ODH/ASSETS/Files/eh/STS/NPDES-2010-SampleRept.pdf


Chapter 4: Outreach Activities and Materials

The outreach component of our project consisted of three elements: (i) general outreach, (ii) practitioner PowerPoint presentation, and (iii) results summary for project participating homeowners.

General Outreach

During the project lifespan we published articles in peer-reviewed journals and conducted outreach efforts to raise awareness of our findings with the onsite wastewater industry. Outreach activities occurred in the form of webinars, and presentations to Rhode Island Department of Environmental Management (RIDEM) staff and to practitioners and scientists at several professional conferences. These were as follows:

Peer-reviewed articles and conference proceedings:


Webinars:

*Evaluation of Advanced Nitrogen Removal Onsite Wastewater Treatment Systems Within the Greater Narragansett Bay Watershed* - webinar presented by Brittany Lancellotti as part of the New England Interstate Water Pollution Control Commission Research Webinar Series (June 2016).

Presentations to Rhode Island Department of Environmental Management:

*Evaluation of Nitrogen Removal by Advanced Onsite Wastewater Treatment Systems Within the Greater Narragansett Bay Watershed* - invited talk presented by Brittany Lancellotti to the Office of Water Resources, Rhode Island Dept. of Environmental Management, Providence, RI (February 2016).

Presentations and posters at professional conferences:

*Accuracy of Rapid Field Testing of Advanced Onsite Wastewater Treatment System Effluent* - poster presented by Brittany Lancellotti at the Annual Meeting of the Soil Science Society of America, Phoenix, AZ (November 2016)
Evaluation of Advanced Nitrogen Removal OWTS Within the Greater Narragansett Bay Watershed - poster presented by Brittany Lancellotti at the Annual Meeting of the Soil Science Society of America, Phoenix, AZ (November 2016)

Optimizing Nitrogen Removal in Advanced Onsite Wastewater Treatment Systems within the Greater Narragansett Bay Watershed - thesis defense talk presented by Brittany Lancellotti, University of Rhode Island, Kingston, RI (October 2016)

Optimizing Nitrogen Removal in Advanced OWTS - invited talk presented by Brittany Lancellotti, Massachusetts Health Officers Association Annual Conference, Cape Cod, MA (October 2016)

Optimizing Nitrogen Removal in Advanced Onsite Wastewater Treatment Systems within the Greater Narragansett Bay Watershed - invited talk presented by Brittany Lancellotti at the 54th Annual Yankee Conference on Environmental Health, Mystic, CT (September 2016)

Evaluation of Advanced Nitrogen Removal OWTS Within the Greater Narragansett Bay Watershed - poster presented by Jose Amador at the EPA Southeast New England Program Forum, Rhode Island Audubon Society, Bristol, RI (September 2016)

Evaluation of Advanced Nitrogen Removal OWTS Within the Greater Narragansett Bay Watershed - talk presented by Brittany Lancellotti at the 5th Northeast Onsite Wastewater Treatment Short Course and Equipment Exhibition, Taunton, MA (April 2016)

Accuracy of Rapid Field Testing of Advanced Onsite Wastewater Treatment System Effluent - talk presented by Jose Amador at the 5th Northeast Onsite Wastewater Treatment Short Course and Equipment Exhibition, Taunton, MA (April 2016)

Evaluation of Nitrogen Removal by Advanced Onsite Wastewater Treatment Systems Within the Greater Narragansett Bay Watershed - talk presented by Brittany Lancellotti, Dept. of Natural Resources Science Graduate Seminar, University of Rhode Island, Kingston, RI (March 2016)

Evaluation of Nitrogen Removal by Advanced Onsite Wastewater Treatment Systems Within the Greater Narragansett Bay Watershed - talk presented by Brittany Lancellotti at the 2015 NOWRA Onsite Wastewater Mega Conference, Virginia Beach, VA (November 2015)

Accuracy of Field Testing for Advanced Onsite Wastewater Treatment System Effluent - poster presented by Brittany Lancellotti and Robert Bercaw at the 2015 New England Graduate Student Water Symposium, Univ. of Massachusetts, Amherst, MA (September 2015)
We developed a general outreach document entitled “Advanced N-removal OWTS in Rhode Island: How well are they performing, and how can we optimize their N-removal efficiency?” (Appendix A). The document was designed to raise awareness of the topic among wastewater practitioners and decision makers and convey the main findings and implications of the project in a comprehensive way. It consists of five sections: (i) purpose of the project, (ii) methods, (iii) major findings, (iv) recommendations, and (v) current projects. We used a combination of text, tables, figures, and graphics to concisely summarize each section. As many New England Onsite Wastewater Training Program (NEOWTP) class attendees deal with advanced nitrogen removal technologies in New England, the document will be distributed to all class participants as a handout (the NEOWTP typically reaches between 500 – 700 practitioners annually). Those interested in more details will be directed to peer-reviewed articles on the URI – Laboratory of Soil Ecology and Microbiology (LSEM) and NEOWTP websites and to specific training classes that will highlight the findings (INSP 200 and OWT 160 – see next section).

We also integrated a key project field observation that relates to technology installation practices into existing NEOWTP classes – OWT 105 (Innovative and Alternative Technologies) and OWT 185 (Designing Nitrogen Removal Technologies). During our field sampling visits we observed that poor compaction at the time of installation have resulted in ground settling around system components – specifically outlet pipe settling (BioMicrobics FAST technology), final effluent pressurized discharge pipe breakage (SeptiTech technology), and textile pod settling (Orenco AX-20 technology) on several of the systems. These issues may influence treatment performance and, in the case of broken discharge pipes with the SeptiTech technology, may cause effluent to come to the ground surface, posing a public health risk.

We will integrate the results of the qPCR research into an existing NEOWTP class - OWT 170 Wastewater Microbiology. This will include a brief overview of the relationship of genes to microbial activities and how the concentration of genes is measured using qPCR, as well as a description of qPCR results and their meaning in the context of the mechanisms by which advanced N-removal OWTS work.

**Practitioner Power Point**

We designed a PowerPoint presentation to educate policy and decision-makers, practitioners, and O&M service providers about the main findings of our project, the importance of monitoring advanced OWTS to improve performance, and methods to increase the effectiveness of routine site visits (Appendix B). The presentation will be incorporated into two NEOWTP classes: INSP 200 (Innovative and Alternative Systems Service Provider Program) course, and OWT 160 (Nitrogen in the Environment and OWTS), and will be presented by a member of the project team. The INSP 200 course is designed to teach O&M service providers how to inspect and maintain commonly-installed I/A technologies used in New England. The OWT 160 class focuses on the impact of nitrogen on receiving waters and public health, nitrogen transformations in OWTS, state-approved nitrogen removal technologies, state and local regulatory requirements, and expected treatment performance from these technologies.

The practitioner presentation focuses on four topics: (i) performance of advanced nitrogen removal OWTS, (ii) evaluation of rapid field tests, (iii) identifying predictors of total
nitrogen, and (iv) recommendations. Service providers will gain an understanding of how reducing N inputs to the STA protects groundwater and coastal ecosystems, why O&M is key to achieving better N-removal performance, how to effectively conduct in-field rapid tests, and how to optimize N removal in these technologies.

The practitioner Power Point presentation shows TN data separated by technology type, and then by individual system, to emphasize that the median effluent TN concentration of all three technologies was below the standard. We highlight that there are differences in the consistency of performance between technology types, and therefore there is room for improvement. To introduce the idea that the analysis of effluent may be necessary to improve performance, we show that proactively managed (sampled and monitored) Barnstable County, MA systems perform better than Rhode Island systems, which are not sampled for performance. To help educate service providers on methods to measure wastewater properties on-site, we provide the brand names and typical prices of accurate rapid tests, as well where they can be purchased. We include a step-by-step tutorial to highlight how quickly, easily, and correctly the rapid tests can be performed.

Working with RIDEM and local communities, it is our objective to make this particular training mandatory for all O&M service providers working in Rhode Island (see the Influencing Policy section for more details). This effort may also be effective in other states in the region, should the interest exist.

**Project Summary for Participating Homeowners**

A summary document describing the project findings was sent to homeowners participating in the project (Appendix C). Although participating homeowners cannot see how their individual system performed, they can see how their type of technology performed as an aggregate, as well as the performance of the other two technologies studied. In addition, we included performance information from a similar study in Barnstable County, MA. This homeowner outreach document will also be posted to LSEM and NEOWTP websites for other homeowners to access.

**Influencing Policy**

Although not a deliverable product of the project, we expect that our findings will influence regulatory policy at the State level. In recent discussions, the RIDEM OWTS section staff expressed interest in requiring O&M service providers to take training classes to learn how to effectively perform in-field rapid tests. RIDEM has also expressed an interest in requiring biannual in-field monitoring using rapid tests to help optimize nitrogen removal technology treatment. We are also discussing with decision-makers to require that rapid field test results be reported to local wastewater management specialist so these values can become part of the system record. One town wastewater management authority has expressed an interested in moving forward with this to better manage N-removal technologies and more accurately assess nitrogen loading to their coastal ponds.
Study Conclusions and Recommendations

Our results show that the performance of advanced N-removal OWTS installed within the Greater Narragansett Bay watershed in Rhode Island varies as a function of technology type and time. SeptiTech systems had the lowest median final effluent TN concentration (mg N/L) (11.3) and the highest percentage of systems in compliance with the 19 mg N/L standard (75%), followed by Advantex (14.9; 70.6%), and FAST (17.1; 64.3%). Regression analysis revealed that effluent TN concentrations from Advantex, FAST, and SeptiTech systems did not have a significant negative correlation with effluent temperature, which contradicts the expectation that denitrification rates increase with temperature. Our results also show that different variables serve as best predictors of effluent TN concentration, depending on technology type: ammonium, nitrate, and alkalinity for Advantex systems; ammonium, nitrate, average forward flow, and BOD for FAST systems; and, ammonium and effluent temperature for SeptiTech systems. Median effluent TN concentrations of Rhode Island systems were higher than systems in Barnstable County, MA, where systems are inspected quarterly and inspections include analysis and reporting of effluent TN concentration. Our results also show that, for most underperforming systems, service provider-performed adjustments did not result in lower median effluent TN concentrations: of a total of 14 systems, median effluent TN concentrations increased after adjustments in 11 systems and decreased in three systems, but one of these three systems still remained out of compliance.

We found that most rapid tests, when used in the field to measure effluent from advanced OWTS, were inaccurate. When the same rapid tests were used inside the laboratory, where lighting conditions are stable, accuracy improved. We were able to identify some alternative rapid tests that were accurate when used in the field: a test kit for ammonium, which is a significant predictor of effluent TN concentration, and a multi-analysis strip for pH.

Monitoring may affect the performance of advanced N-removal OWTS. Proper maintenance of these systems includes an assessment of recirculation ratios, as well as monitoring of wastewater properties, which are incorporated into quarterly site visits in Barnstable County, MA, where the same systems perform better (BCDHE, 2012). Analysis of effluent TN concentration during site visits gives the service provider a better understanding of how the system is performing, as well as how to adjust the system to optimize N-removal. Requiring these data to be reported to an easily accessible database ensures that effluent is analyzed for TN, and allows for data from different towns to be compiled and examined. More frequent site visits that include effluent TN analysis provide a more accurate picture of system performance, given that our results also show that performance varies at weekly and monthly scales. Towns in Rhode Island with advanced N-removal OWTS may consider requiring quarterly site visits that include analysis and reporting of effluent TN. Our results show that Rhode Island systems are capable of lowering effluent N to acceptable levels (≤ 19 mg N/L) – implementing stricter regulations may improve N removal in underperforming systems and optimize N-removal in compliant systems, thus lowering the N load to Narragansett Bay.

Our findings also showed that system adjustments did not result in increased N removal. In addition to the possibility that the effects of adjustments may take longer to be apparent, and therefore, an iterative adjustment monitoring approach is needed (following that in Barnstable
County, MA), it may be that service providers were not adequately trained, and consequently were unable to make effective adjustments. Since analysis and reporting of effluent TN, and quarterly site visits are not required in Rhode Island, service providers may likely have little experience optimizing these systems for N removal. Towns that wish to implement stricter monitoring requirements should consider requiring training in identifying underperforming systems and making effective adjustments to improve performance. Additional, longer-term monitoring of a larger number of underperforming systems and their response to adjustments may be helpful to better understand how systems respond to adjustments, provide information to service providers and regulators on what works and does not work, and on the time it takes to see improvements.

Examination of the concentration of genes for ammonia oxidation – *amoA* – and nitrous oxide reduction – *nosZ* – showed that it differed as a function of technology type and/or location within the treatment train (oxic vs anoxic component). Furthermore, our results suggest that the concentration of these genes may affect compliance with regulatory standards, depending on the technology. To the best of our knowledge, ours is the first study quantifying genes involved in nitrification and denitrification in advanced N-removal systems. Our data indicate that, although the mechanisms for N removal – sequential nitrification and denitrification – may be similar for all technologies at the system level, there are likely differences at the level of microbial communities that carry out these processes. Current understanding of the microbial communities that carry out N removal is based entirely on circumstantial evidence and speculation as to the processes and microorganisms involved. This has left us with a limited set of parameters, such as recirculation ratio, to adjust in order to optimize system performance. A better understanding of these microbial communities and their requirements – for electron donors and acceptors and physicochemical conditions – will help us identify the mechanisms behind variation among systems of a particular type, and among technology types, which may not be captured in routine analysis of wastewater properties. Molecular analyses of microbial communities has become increasingly accessible in terms of necessary technical expertise and cost, and has resulted in an improved understanding and efficiency of treatment processes in wastewater treatment plants. This approach should benefit advanced N-removal OWTS in a similar manner.

With regard to rapid tests, our results indicate their accuracy needs to be considered before they are used to evaluate performance of advanced OWTS. Our findings show that some rapid tests can be used where lighting, temperature, and moisture conditions are stable in order to increase their ability to predict constituent concentrations without losing the convenience of quick results, whereas other rapid tests provide accurate results in the field. These results will be useful to O&M service providers and OWTS regulators, as it can translate into more proactive and, therefore, effective monitoring. Service providers can use accurate rapid tests to measure wastewater properties of advanced OWTS effluent which are associated with N-removal effectiveness. Requiring analysis of wastewater properties during site visits does not have to increase supply or labor costs significantly, since accurate rapid tests are inexpensive and provide results quickly in the field.

Installation practices influence system function, operation and maintenance, and treatment performance. We saw evidence of improper soil compaction at the time of installation that resulted in ground settling around several systems’ components. This settling caused FAST
tank outlet invert elevations to rise in five of the project systems, which raised liquid level in the units to where the recirculation troughs in the FAST inserts where partially (3 systems) to fully (2 systems) submerged. In addition, we observed little to no recirculation from troughs in two other FAST systems, possibly due to damaged or deflected troughs.

Ground settling, due to improper soil compaction at installation, was also a problem with SeptiTech systems. The junction of the PVC coupler cast into the SeptiTech processor tank wall and final effluent transport pipe (to the pressurized drainfield) is subject to breakage if the ground settles. This was evident in two SeptiTech systems and resulted in final effluent surfacing at the ground level.

Advantex textile filter pods can also settle if they are installed on ground that is not sufficiently compacted. This may result in AX-20 pods being sufficiently out-of-level to cause some degree of ponding in the pod base, which may influence treatment performance. We noticed differential settling of Advantex pods in two systems.

When notified, the SeptiTech service providers repaired the broken discharge pipes promptly. The outlet issues on the FAST systems and the out-of-level Advantex pods would require more attention and be more costly to repair, and no corrective action was taken during the project.

The use of proper compaction techniques at the time of installation would minimize the risk of setting of the outlet in FAST systems and pipe breakage in SeptiTech systems. The use of flexible PVC pipe and/or the use a rubber outlet boot at the tank and drainfield transport pipe junction would further reduce the risk of pipe breakage in the SeptiTech technology. Careful attention to installation is needed to assure that these issues do not become obstacles to achieving nitrogen removal goals.
Advanced N-removal OWTS in Rhode Island: How well are they performing, and how can we optimize their N-removal efficiency?

**Appendix A: General Outreach Document**

### Why are advanced N-removal OWTS required in some areas of Rhode Island?

Once dispersed to the soil treatment area (STA), final effluent from advanced N-removal OWTS can enter our coastal ecosystems through groundwater. Final effluent contains forms of nitrogen (N) called ammonium and nitrate that are harmful to coastal ecosystems. Advanced N-removal OWTS are designed to reduce the concentration of these forms of N to protect our coastal waters.

### Concerns with Advanced N-Removal OWTS in RI

- **Effluent N concentrations no longer monitored after installation.**
- **Instillation and maintenance may affect system performance.**
- **Uncertainty surrounding performance.**
- **N inputs to coastal systems could be higher than expected.**

Although final effluent total nitrogen (TN) concentrations are limited to ≤ 19 mg N/L in RI, effluent N is not monitored from these systems. This makes it difficult to assess how effectively they are reducing influent N concentration and contributing to lowering N inputs to coastal ecosystems.

### What did we do?

- **17 Systems**
- **14 Systems**
- **11 Systems**

We evaluated the performance of existing advanced N-removal OWTS in 5 towns within the Narragansett Bay and coastal ponds watersheds in RI.

We collected wastewater samples from the three most commonly-installed advanced N-removal OWTS in Rhode Island. We sampled 42 systems each month from March 2015 to August 2016.

We also assessed the accuracy of rapid tests that can be used to evaluate OWTS performance. We statistically compared values from rapid tests to values from standard laboratory methods. We analyzed for pH, DO, nitrate, and ammonium.

### Distribution of Effluent TN Concentrations

- **Some systems produced effluent with high TN concentration.**
- **Median final effluent TN concentrations were <10 mg N/L for all three technology types, but wide ranges of effluent TN suggest inconsistent treatment performance.**
- **Our results show that systems are capable of producing effluent with low effluent TN concentration.**

### Comparison of Rhode Island to Barnstable County Study

- Median effluent TN concentrations for all technology types were higher in Rhode Island than Barnstable County, MA, where systems are monitored quarterly, effluent is analyzed for wastewater properties, and results reported to county.

---

65
Performance Comparison Among Technology Types

| Technology Type | Percentage of Systems in Compliance
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Narraganset Bay Systems</td>
<td>65% (11 out of 17 systems)</td>
</tr>
<tr>
<td>Barnstable County Systems</td>
<td>87% (23 out of 25 systems)</td>
</tr>
<tr>
<td>Advantex AX-20</td>
<td>64% (8 out of 14 systems)</td>
</tr>
<tr>
<td>MicroFAST</td>
<td>79% (349 out of 442 systems)</td>
</tr>
<tr>
<td>SeptiTech D</td>
<td>50% (2 out of 4 systems)</td>
</tr>
<tr>
<td></td>
<td>42% (19 out of 44 systems)</td>
</tr>
</tbody>
</table>

*In compliance - median effluent TN concentration ≤ 10 mg N/L.

Performance varied by technology type and could be improved. Advantex systems had the highest compliance rate, followed by FAST, and SeptiTech.

Best Predictors of Effluent TN

- Ammonium
- Nitrate
- Alkalinity
- BOD
- Avg Forward Flow

These wastewater properties best predict effluent TN concentration. Values of these wastewater properties can provide clues as to how well a system is reducing influent N.

Accuracy of Rapid Tests

- Most rapid tests were not accurate when used in the field.
- Don’t rely on rapid tests for critical decisions - go with actual certified laboratory results.
- Accuracy increased when rapid tests were used indoors, where lighting conditions are stable.
- Accurate rapid tests are available for two significant predictors of TN (ammonium and nitrate).

What do we recommend?

Advanced N-removal OWTS Performance

- Monitoring of advanced N-removal OWTS may affect their performance.
- We recommend effluent testing for wastewater properties and reporting this data to state and local decision makers.
- Knowing system performance and reporting data will enable more proactive maintenance and management.

Rapid Tests

- Our results indicate variations in sunlight can complicate visual comparisons to color charts when using test strips and kits outdoors.
- To increase their accuracy, we recommend using rapid tests inside, where lighting conditions are stable.
What do we recommend? (cont.)

Accurate Rapid Tests

- Nitrate test strip
- Ammonium test kit
- Ammonium & Nitrate photometer
- pH pocket meter

- Accurate rapid tests are available for nitrate, ammonium, and pH.
- The test strips (Aquarium Pharmaceuticals) and pH pocket meter (Oakton) can be purchased online on Amazon.com.
- The Hanna photometer can be purchased online at HannaInst.com.

What's new in the Laboratory of Soil Ecology and Microbiology?

**Greenhouse Gas Fluxes**

- The microbial processes that remove N in advanced N-removal CWTS can produce greenhouse gases (GHG).
- Since GHG contribute to global warming, we measured GHG fluxes from advanced N-removal CWTS.

**OWTS & Sea Level Rise**

- We are using ground penetrating radar (GPR) to predict how sea level rise will affect the distance between the STA and the groundwater table of systems installed within close proximity to the coast.
- We’ll also investigate the effectiveness of perennial plants to absorb nutrients from wastewater leached by OWTS, which could potentially make up for a loss of separation distance due to sea level rise.

**Microbial Community Assessment**

- We are quantifying nitrification and denitrification genes in wastewater from advanced N-removal CWTS.
- This will help us manage the microbial communities that carry out N-removal.
- We can also investigate the relationship between GHG fluxes and the abundance of N-removal genes.

**Charlestown, RI & Barnstable County, MA**

- Our laboratory is working with the town of Charlestown, RI to help them evaluate and optimize treatment performance of their advanced N-removal systems.
- We are working with Barnstable County, MA to evaluate the performance of a non-proprietary passive N-removal system.
Appendix B: Practitioner Presentation

Optimizing Nitrogen Removal in Advanced OWTS Within the Greater Narragansett Bay Watershed

Objectives
1. Monitor performance of advanced N removal OWTS in the Narragansett Bay Watershed
2. Evaluate rapid field tests
3. Identify predictors of effluent total nitrogen
4. Recommendations

Nitrogen Loading and Coastal Ecosystems: Rhode Island

Addressing Eutrophication
Advanced Nitrogen Removal OWTS
19 mg/L Total Nitrogen final effluent concentration standard

Advanced N Removal OWTS
Advanced OWTS in Rhode Island: Considerations

Site Locations: Greater Narragansett Bay Watershed

Technology and Sampling Design

Objective 1:
Monitor performance of Advanced N removal OWTS in the Narragansett Bay Watershed

Our Approach:

Evaluation of N Removal in Advanced OWTS

- Final effluent total nitrogen concentrations
- Collected from March 2015 to August 2016
- Standard laboratory values reported

<table>
<thead>
<tr>
<th>17 Systems</th>
<th>14 Systems</th>
<th>4 Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orenco Advantex AX20®</td>
<td>BioMicrobics FAST®</td>
<td>SeptiTech D®</td>
</tr>
</tbody>
</table>
Visualizing the Data

Distribution of TN Concentrations: March 2015 - August 2016


55% (11 of 17) of systems meet the 15 mg N/L TN standard


64% (9 of 14) of systems meet the 19 mg N/L TN standard


50% (2 of 4) of systems meet the 19 mg N/L TN standard

Scaling Compliance Rates, State of RI
Based on Applications as of January 2016

3,740 Total Applications 35% Out of Compliance (1,309)
231 Total Applications 53% Out of Compliance (115)
720 Total Applications 36% Out of Compliance (255)
System Adjustments

1. Identified systems with median TN values > 19 mg/L (March-December 2019)
2. Notified service providers & gave them performance data
3. Service providers made system adjustments
4. Compared median TN values from March-August 2019 to same time period in 2018

Advantex Pod settling concern
- Improper compaction of area beneath Advantex pod can result in ground settling after construction.
- This may lead to textile pod becoming out of level.
- This may result in pooling/ponding in base of pod.
- May influence treatment within the system.

System Adjustments: FAST

System Adjustments: SeptiTech

FAST System Performance
The trough located within a FAST insert is a key component for nitrogen reduction
- Damaged, submerged or bent troughs may limit recirculation of nitrified effluent.
- Settling of the FAST unit outlet pipe causes the tank outlet invert elevation to rise.
- This results in a liquid level that submerges the trough.
- This will likely hamper trough function, and treatment performance.
- Corrective action is not a simple process.

System Adjustments: Advantex

Neither system was in compliance after adjustments by service provider.

System Adjustments: FAST

System adjustments only decreased TN in one FAST system.

Out of the 4 systems originally in denitrification mode, one was out of compliance, but went into compliance after adjustments.
SeptiTech Systems: Switched to Denitrification Mode

- Switching SeptiTech systems to denitrification mode decreased TN in only one of the systems.

SeptiTech System faulty controller

- Systems switched to denitrification mode
- No response detected during monitoring
- Service provider detected PLC software issue
- PLCs on all systems were then reprogrammed

This shows the importance of monitoring!

SeptiTech System outlet concern

- The final effluent pressurized discharge line exits the concrete tank wall, though a PVC coupler cast into the tank wall.
- A PVC pipe (transport line to pressure drainfield) is glued to the exterior of this coupler.
- If area is not compacted well at installation, ground settles, and this junction is subject to breakage.
- A broken pipe would result in effluent surfacing at ground level.
- This would pose a public health risk.

Median TN Concentration: Narragansett Bay vs. Barnstable County Systems

- Comparison of TN concentrations between Narragansett Bay and Barnstable County systems.

Percentage of Systems in Compliance: Narragansett Bay vs. Cape Cod

- Percentage of systems in compliance across different systems and locations.

Differences in Regulations

Rhode Island vs. Barnstable County, Massachusetts

- Rhode Island:
  - 2 maintenance visits required per year
  - Focus on mechanical function
  - Do not include effluent sampling
  - 19 mg N/L TN standard

- Barnstable County, Massachusetts:
  - 4 maintenance visits required per year
  - Effluent sampling required
  - 19 mg N/L TN standard
  - Tracks effluent constituent levels
  - Alerts when effluent does not meet standards
  - Triggers corrective action
Findings: Objective 1

OWTS performance in Narragansett Bay Watershed

- All systems are capable of meeting compliance
- We need to observe, adjust, and verify
- If we do not observe, we cannot verify
- Regulations affect effluent TN
- Proactive management reduces nitrogen
- Monitoring helps identify system malfunctions
- Installation affects system function and treatment performance

Objective 2:

Assess accuracy of rapid tests for Advanced OWTS effluent analysis

Evaluation of Rapid Field Tests

Methodology: Operational Parameters

- Field Analysis
- Lab Analysis
- DO
- NML
- Total Nitrogen
- Ammonia N
- Nitrite N
- Nitrate N
- pH
- Water Temperature
- Reoxygenation Ratio
- Avg. Daily Forward Flow

Rapid Tests Initially Evaluated

- Used by O&M service providers to evaluate system performance
- Provide quick results on-site
- Do they provide accurate results?
How is Accuracy Defined

**Slope** is rise over run, i.e., "steepness."

**R** describes data variance. How close do the data fit the slope? i.e., how well does the line represent the actual data?

- Slope = 0.97
- R² = 0.9

This is still a very strong relationship with only slight variation and would indicate a very close relationship between Lab and Rapid tests.

How is Accuracy Defined (Cont.)

**Slope** = 0.6
**R²** = 0.3

This is not a very strong relationship with high variation. Using this rapid test could cause you to over or under estimate data points.

Is our Data Accurate?

**Slope** = 0.5
**R²** = 0.8

The agreement between Rapid and Lab tests is not strong, but the variation from the relationship is low. This means that Rapid test readings for Ammonium were underestimated, but consistently underestimated.

Results: Rapid Field Test Methods (done outdoors)

Rapid Field Test Methods (done outdoors)
Rapid Field Test Methods:
Done in the Laboratory

| Key | 5 | 0 |

Field Conditions and Error
Varying light conditions in the field complicate visual comparisons to color charts.

Alternative Rapid Field Test Methods
(performed outside)

Accurately measuring dissolved oxygen (DO)

Findings: Objective 2
“Accurately measuring predictors of TN”

- Accuracy of rapid tests improves indoors
- Test strips do not require calibration and are low cost, but are less accurate
- pH pen and photometer must be calibrated, are more expensive, but are more accurate
Objective 3
Determine Predictors of TN

Why is a predictor of TN important?

- All forms of N combined = TN. Excess nitrogen is dangerous to the environment and human health
  
  HOWEVER...
  
  - Measuring TN cannot be done in the field. TN is measured using a lab test involving dangerous reagents and an expensive instrument
  
  BECAUSE OF THIS...
  
  - We need good predictors of TN that can be measured easily by practitioners

Which Parameters Best Predict Effluent Total Nitrogen Concentration?

- How well do these parameters correlate with TN?
- Ammonium
- Nitrate
- Alkalinity
- pH
- DO
- Sample Temperature
- Average Forward Flow
- Recirculation Ratio
- BOD
- Total Nitrogen

Which parameters can serve as indicators for effluent TN?

How to Predict TN

<table>
<thead>
<tr>
<th>Predictors of TN/Technology Type</th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Alkalinity</th>
<th>DO</th>
<th>Average Forward Flow</th>
<th>Effluent Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denis Alkaline RPE™</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>RoboMatic RFS™</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aquafloic CF™</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive correlation
Negative correlation
No correlation

Predicting TN using our data

Findings: Objecting 3
“How to predict TN”

- Ammonium is significantly positively correlated with TN in all technologies
- When Ammonium increases, so does TN
- We have multiple rapid tests (in field and in lab) that can accurately measure Ammonium
- Nitrate, Alkalinity, BOD, Effluent temperature, and Average Forward Flow predict TN in some technologies
**Recommendations**

- Reduce variability
- Provide consistency
- Improve accuracy
- Assure confidence in data
- Optimize treatment

**Recommended tests**

- Accurate inside only
  - Nitrate
  - Ammonium

- Accurate in field and inside
  - pH
  - Alkalinity
  - Ammonium
  - Nitrate

**Performing test strip analysis**

Alkalinity, Nitrate, pH, Ammonia

Always perform inside to prevent inaccurate readings

**EcoTest pH 2 Pocket Meter**

- Step 1: Set cu to high (12 pH), neutral (7 pH), and low (6 pH)
- Step 2: Turn on pen, immerse sensor and stir probe to 7 pH
- Step 3: Rinse probe
- Step 4: Repeat Step 2 for low pH and standard

Can be performed in field or in lab

**EcoTest pH 2 Pocket Meter (Cont.)**

- Step 5: Rinse probe
- Step 6: Repeat Step 2 for low pH and standard
- Step 7: Rinse probe
- Step 8: Dip sensor in sample, stir for 30 sec

Can be performed in field or in lab
Aquarium Pharmaceuticals Ammonium Test Kit

Step 1: Fill clean test tube with $5.0$ mL of sample (to the line on the tube). Can be performed in field or in lab

Step 2: Add $8$ drops of Ammonia Test Solution Bottle #1.

Step 3: Add $8$ drops of Ammonia Test Solution Bottle #2.

Can be performed in field or in lab

Aquarium Pharmaceuticals Ammonium Test Kit (Cont.)

Step 4: Cap tube and shake vigorously for $5$ seconds.

Can be performed in field or in lab

Step 5: Wait $5$ minutes.

Step 6: Compare sample to chart and record value.

Hanna Photometer - Ammonium

Step 1: Select Ammonia HR method from the "Method Selection" menu.

Can be performed in field or in lab

Step 2: Measure $1$ mL of sample.

Can be performed in field or in lab

Step 3: Carefully transfer sample to a clean reagent vial.

Can be performed in field or in lab

Hanna Photometer - Ammonium (Cont.)

Step 4: Cap vial and invert to mix.

Can be performed in field or in lab

Step 5: Wipe outside of vial before placing it in the instrument.

Can be performed in field or in lab

Step 6: Push the vial completely into the holder and press "Zero". This zeros the instrument.

Hanna Photometer - Ammonium (Cont.)

Step 7: Remove vial and add $4$ drops of HI 7376A-9 Nessler Reagent.

Can be performed in field or in lab

Step 8: Replace cap and invert sample several times to mix.

Can be performed in field or in lab

Step 9: Return vial to the holder and push it completely down. Wait for $3$ min. and $30$ sec. and press "Read". Record value.

Can be performed in field or in lab

Hanna Photometer - Nitrate

Step 1: Select nitrate method from the "Method Selection" menu.

Can be performed in field or in lab

Step 2: Measure $1$ mL of sample.

Can be performed in field or in lab

Step 3: Carefully transfer sample to a clean reagent vial.

Can be performed in field or in lab
Hanna Photometer - Nitrate (Cont.)

Step 4: Cap tube and invert 10 times.
Step 5: Wipe outside of vial before placing in the instrument.
Step 6: Push the vial completely into the holder and press “ Zero.” This zeros the instrument.

Can be performed in field or in lab

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Hanna Photometer - Nitrate (Cont.)

Step 7: Carefully open one packet of nit/93706-0 Nitrate Reagent.
Step 8: Add reagent to sample vial.
Step 9: Invert vial 10 times.
Step 10: Return vial to the holder and push it completely down. Wait for 5 min. and press “Read.” Record value.

Can be performed in field or in lab

---

Recommended Ammonium and Nitrate Tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rapid Test</th>
<th>Where to purchase</th>
<th>Apparatus Cost</th>
<th>Where to use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>Test kit</td>
<td>Hanna Instruments</td>
<td>$49.00 (40 tests)</td>
<td>Field or lab</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Test kit</td>
<td>Hanna Instruments</td>
<td>$49.00 (40 tests)</td>
<td>Field or lab</td>
</tr>
</tbody>
</table>

Recommended pH and Alkalinity Tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rapid Test</th>
<th>Where to purchase</th>
<th>Apparatus Cost</th>
<th>Where to use</th>
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<td>pH</td>
<td>Test kit</td>
<td>Hanna Instruments</td>
<td>$49.00 (40 tests)</td>
<td>Field or lab</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>Test kit</td>
<td>Hanna Instruments</td>
<td>$49.00 (40 tests)</td>
<td>Field or lab</td>
</tr>
</tbody>
</table>

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In conclusion...

- 40% of all the systems monitored did not comply with State standard.
- Could translate to nearly 1700 systems statewide.
- Differences in regulatory requirements for monitoring influences management and may affect performance.

- Nitrate, ammonium, DO, alkalinity, etc.
- Implications, and exfiltration of flow are critical to understanding.

---

Where are we now?

- Greenhouse gas fluxes (N₂O, CH₄)
- Quantifying microbes using qPCR targeting nitrification and denitrification genes
- Continued monitoring
We are hopeful!

- Monitored Massachusetts systems are performing better, but with the same level of management, RI systems can perform to standard.
- One town in RI is considering requiring effluent sampling during O&M visits.
- Accurate rapid field tests are available.

Special thanks to...

The Amador Lab:
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- Gina Collette
- Jennifer Cooper
- Ashely Wagoner

THE UNIVERSITY OF RHODE ISLAND
National Institute of Food and Agriculture

NEIWPCC
New England States Water Pollution Control Federation
Appendix C: Homeowner Letter

Advanced N-removal OWTS in Rhode Island: How well are they performing, and how can we optimize their N-removal efficiency?

Why are advanced N-removal OWTS required in some areas?

- Wastewater from conventional onsite wastewater treatment systems (OWTS) adds harmful nitrogen (N) to our groundwater and surface waters, contaminating wells and causing excess algal growth in coastal waters
- The RIDEM requires that advanced N-removal OWTS be used to protect these waters
- Once installed, these systems are not tested for N removal performance
- Nitrogen inputs from these systems could be higher than expected

What did our study do to address the performance question?

We evaluated the performance of existing advanced N-removal OWTS in 5 towns within the Narragansett Bay and coastal ponds watersheds.

We collected wastewater samples from the three most commonly-installed advanced N-removal OWTS in Rhode Island. We sampled 42 systems each month from March 2015 to August 2016.

What did we find?

Some systems produced effluent with high total nitrogen (TN) concentration.

The median (bars within the boxes) final effluent TN concentrations were <19 mg N/L (same as parts per million – ppm) for all three technology types, but wide ranges of effluent TN suggest inconsistent treatment performance.

Our results show that systems are capable of producing effluent with low effluent TN concentration.
We compared our findings to a similar study in Barnstable County, MA

<table>
<thead>
<tr>
<th>Technology Type</th>
<th>Percentage of Systems in Compliance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Narragansett Bay Systems</td>
</tr>
<tr>
<td>Advantex AX-20</td>
<td>65% (11 out of 17 systems)</td>
</tr>
<tr>
<td>MicroFAST</td>
<td>64% (9 out of 14 systems)</td>
</tr>
<tr>
<td>SeptiTech D</td>
<td>50% (2 out of 4 systems)</td>
</tr>
</tbody>
</table>

*In compliance = median effluent TN concentration ≤ 19 mg N/L (the RI and MA standard)

- Performance varied by technology type in both states.
- Advantex systems had the highest compliance rate, followed by FAST, and SeptiTech.
- More systems were in compliance in Barnstable, where systems are monitored quarterly, effluent is analyzed for wastewater properties, results are reported to county, and system adjustments are made.
- Median effluent TN concentrations for all technologies were higher in Rhode Island than Barnstable County, MA.
- Suggests that sampling, testing, and proactive management leads to improved performance of systems.

What does this all mean?

- Continued use of advanced N-removal OWTS in Rhode Island is important.
- These systems are removing nitrogen, but they can do better.
- Treatment performance can be improved with testing and proactive management.
- Knowing system performance will enable your service provider to adjust your system.
- We have new low cost techniques to help service providers test system performance.
- We are working with the state and towns to improve system performance.
- This will improve treatment, and help protect drinking water and surface water quality.

What do we recommend?

- Keep your contract with your system service provider current – maintenance is important.
- Effluent testing of all advanced N-removal OWTS.
- Reporting and tracking this data in existing town septic systems data bases.
- Proactive management of all systems.