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PERFORMANCE EVALUATION OF ADVANCED
NITROGEN REMOVAL ONSITE WASTEWATER
TREATMENT SYSTEMS

BY

BRITTANY VICTORIA LANCELLOTTI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

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IN

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MASTER OF SCIENCE THESIS
OF
BRITTANY VICTORIA LANCELLOTTI

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UNIVERSITY OF RHODE ISLAND
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ABSTRACT

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 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. To determine how well these systems reduce influent TN concentration, I monitored the performance of existing advanced N-removal OWTS located within N-sensitive areas of Rhode Island. Additionally, in an effort to provide information that could be used to improve the monitoring of these systems, I assessed the accuracy of rapid tests that can be used to evaluate system performance.

To evaluate the N-removal effectiveness of these systems, I measured a variety of wastewater properties 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 with state regulations ($\text{TN} \leq 19 \text{ mg N/L}$) 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), 11.3 (0.1 - 41.6), respectively.

I 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 CV (56.0%), followed by Advantex (50.4%), and FAST (31.7%). In contrast, median coefficients of variation 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, which prompted examination of the relationship between temperature and TN concentration for each system type. Total N was generally not correlated with temperature: TN concentrations plotted against effluent temperature values resulted in R^2 values of 0.007, 0.001, and 0.04 for Advantex, FAST, and SeptiTech systems, respectively.

Comparison of my findings to results of a similar study in 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, FAST, and SeptiTech systems were lower than ours, with values (mg N/L) of 13.5 for Advantex, 12.7 for FAST, and 20.2 for SeptiTech systems. Similarly, the Cape Cod, MA study data showed that 87% of Advantex, 79% of FAST, and 42% of SeptiTech systems had final effluent TN concentrations less than 19 mg N/L, which are higher percentages than reported in our study.

I identified the combination of wastewater properties that had the strongest correlation with TN to determine the properties that best predicted final effluent TN

concentration. This information can be used to provide ranges of values of wastewater properties that can be expected to result in acceptable TN levels. Final effluent TN concentration was predicted by a different set of variables for each system type: ammonium, nitrate, and alkalinity for Advantex; ammonium, nitrate, average forward flow, and five-day biochemical oxygen demand (BOD₅) for FAST; and, ammonium and effluent temperature for SeptiTech. Service providers were asked to make adjustments to seven underperforming systems to increase N-removal efficiency between December 2015 and March 2016. Total nitrogen was reduced to 19 mg N/L in only two out of seven systems, suggesting final effluent TN concentrations generally did not decrease in response to adjustments. My results suggest that advanced N-removal OWTS can reduce TN to meet regulatory standards, but N-removal effectiveness varies as a function of system type, time, and by individual system. Routine monitoring of advanced N-removal OWTS can enable service providers to proactively manage systems, which may affect their efficiency. However, improvement of performance after adjustment may require repeated visits and long-term monitoring.

In an effort to provide information that could translate into more effective maintenance visits/system adjustments, I evaluated the accuracy of a variety of rapid tests. Rapid tests provide an inexpensive, desirable alternative to standard laboratory analyses for testing advanced onsite wastewater treatment system (OWTS) effluent in the field. Despite their potential utility, their accuracy for analysis of effluent from advanced OWTS has not been assessed. I evaluated the accuracy of an initial suite of rapid tests commonly used to analyze wastewater (test strips for ammonium, pH,

nitrate, and alkalinity; pH pocket meter; titration kit for dissolved oxygen (DO)) using final effluent from 42 study advanced N-removal systems. I compared values obtained using rapid tests to values obtained using standard laboratory methods. Significant differences between field and standard methods were found only for nitrate and pH test strips when the data were analyzed using ANOVA on ranks. However, regression analysis indicated that all test strip-based rapid methods and the DO titration kit produced values that deviated significantly from correspondence with standard 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. 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. My 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.

My findings show that advanced N-removal OWTS in Rhode Island can perform to standard, but their N-removal effectiveness may improve if routine monitoring and effluent TN analysis is required. 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.

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PREFACE

This thesis is presented in manuscript format in accordance with University of Rhode Island Graduate School Guidelines. There are four sections contained within this thesis, an introduction, two chapters, and conclusions. The first chapter is titled “*Evaluation of Advanced N-Removal Onsite Wastewater Treatment System Performance*” and authored by B.V. Lancellotti, G.W. Loomis, K.P. Hoyt, and J.A. Amador, and is in preparation for submission to Science of the Total Environment. The second chapter is titled “*Accuracy of Rapid Tests Used for Analysis of Advanced Onsite Wastewater Treatment System Effluent*” and authored by B.V. Lancellotti, R.J. Bercaw, G.W. Loomis, K.P. Hoyt, E.J. Avizinis, and J.A. Amador, and has been published in Water, Air, and Soil Pollution.

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INTRODUCTION

Nitrogen 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). Residential wastewater, 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 nitrogen (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 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_2O , gases that diffuse to the atmosphere. The conversion of ammonium to nitrogen gas (a biologically inert form of N) helps protect environmental and public health by lowering N loading to ground and surface waters. Incomplete denitrification can produce and release nitrous oxide, a potent greenhouse gas, which 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 applications 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 helps to maintain system mechanical function, but service visits in Rhode Island are not tracked, 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) show that advanced N-removal systems do not always meet standards.

In Manuscript 1, I evaluate the performance of advanced N-removal OWTS; in Manuscript 2, I evaluate the accuracy of rapid tests used to measure wastewater properties from advanced N-removal OWTS. In the first manuscript, I evaluated the performance of these systems by studying 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), between March 2015 and August 2016. I

monitored system performance by measuring a variety of wastewater properties: pH, DO, effluent temperature, TN, NO_3^- , NH_4^+ , pH, alkalinity, five-day biochemical oxygen demand (BOD_5), recirculation ratio, and average forward flow. I examined weekly, monthly, and seasonal variation in effluent TN concentration by system type. I used the performance data to provide best predictors of effluent TN, as well as ranges of values of constituents that corresponded to acceptable N levels.

Using data collected from March to December 2015, I identified the underperforming (median final effluent TN concentrations ≥ 19 mg N/L) systems, notified the O&M service providers responsible for these systems, and encouraged adjustments to be made based on values of wastewater constituents I provided. I 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. This manuscript will be submitted for publication in *Science of the Total Environment*.

In the second manuscript, I identified methods that can be used to monitor advanced N-removal OWTS, evaluating the accuracy of rapid tests used to test effluent from these systems. Rapid tests (test strips and kits) are a desirable alternative to laboratory methods, since they are cost-effective, and provide quick results on-site. If accurate, rapid tests can assist operations and maintenance (O&M) service providers in making adjustments to underperforming systems that may result in more effective N removal. However, despite their potential utility, the accuracy of rapid tests, when used to measure the concentration of constituents in effluent from advanced OWTS, has not been investigated. In an effort to identify reliable rapid tests that could be used to

effectively evaluate system performance on-site, we evaluated the accuracy of rapid tests for NH_4^+ , NO_3^- , pH, and alkalinity, based on their ability to predict standard values. We used the rapid tests in the field, as well as inside 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. The results of this study were published in the journal *Water, Air, and Soil Pollution* (Lancellotti et al., 2016).

**MANUSCRIPT 1: EVALUATION OF ADVANCED N-REMOVAL
ONSITE WASTEWATER TREATMENT SYSTEM PERFORMANCE**

In preparation for submission to Science of the Total Environment

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ABSTRACT

Advanced nitrogen (N) removal onsite wastewater treatment systems (OWTS) are installed in coastal areas throughout the United States to reduce N loading to marine waters. However, once installed, final effluent total nitrogen (TN) concentration from these systems is not always routinely monitored, making it difficult to determine the extent to which they contribute to lowering N loads. To evaluate the N-removal effectiveness of these systems, we monitored the performance of the three most commonly-installed OWTS within the greater Narragansett Bay Watershed, Rhode Island: (i) Orenco Advantex AX20[®] (17 systems), (ii) Bio-Microbics MicroFAST[®] (14 systems), and (iii) SeptiTech D[®] Series (11 systems), between March 2015 and August 2016. The compliance rate ($TN \leq 19 \text{ mg N/L}$) 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), 11.3 (0.1 - 41.6), respectively. Median final effluent TN concentrations for Advantex, FAST, and SeptiTech systems were lowest in fall and winter, and had a coefficient of variation over the course of four weeks of 52.0%, 49.6%, and 62.2%, respectively. Temporal variation in final effluent TN concentration was not driven by temperature: TN concentrations plotted against effluent temperature values resulted in R^2 values of 0.007, 0.001, and 0.04 for Advantex, FAST, and SeptiTech systems, respectively. The median final effluent TN concentration for our study systems (16.7 mg N/L) was greater than Barnstable County, MA systems (13.3 mg N/L), which are monitored quarterly. The wastewater properties that best predicted final effluent TN levels were

NH_4^+ , NO_3^- , alkalinity, average forward flow, biochemical oxygen demand (BOD), and effluent temperature, depending on system type. Service providers made adjustments to seven underperforming systems between December 2015 and March 2016 intended to increase N-removal efficiency, but TN was reduced to 19 mg N/L in only two out seven systems. Our results suggest that advanced N-removal OWTS can reduce TN to meet regulatory standards, and monitoring of advanced N-removal OWTS can enable service providers to proactively manage systems, which may have an effect on their efficiency. However, improvement of performance may require recursive adjustments and long term monitoring.

INTRODUCTION

Residential wastewater (which is transported through ground and surface water) can be a significant source of nitrogen (N) to coastal ecosystems (Valiela et al., 1992). A conventional onsite wastewater treatment system (OWTS; also known as septic system) can contribute about 11 kg of N per year to the ground water (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). 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 discharged to a soil treatment area (STA) in N-sensitive locations.

More than 60 million people in the United States are served by septic systems, which are also commonly used 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 applications 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 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 discharged to the STA. Nitrification occurs inside an oxic tank or chamber where NH_4^+ is oxidized by aerobic, autotrophic bacteria to 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, NO_3^- is reduced to N_2 (nitrogen gas) and N_2O (nitrous oxide), which diffuse to the atmosphere via vents in the system. The conversion of NO_3^- to N_2 (a biologically inert form of N) minimizes N loading to ground water and helps to protect environmental and public health. Incomplete denitrification releases N_2O , a potent greenhouse gas that has received only limited attention in the OWTS industry (Diaz-Valbuena, 2011; Truhlar, 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 do not include measurement of final effluent TN concentration. 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 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 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) show that advanced N-removal OWTS do not always perform to standard.

To determine if these systems are effective at decreasing septic tank effluent 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, DO, effluent temperature) and 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 system 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 treated 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 monitored the performance of all 42 systems between March and August 2016 and compared system performance from the same period in 2015 to March to August 2016 in order 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 denitrification-disabled (i.e. set to minimal recirculation of nitrified effluent to the anoxic tank). The 42 systems are located in the towns of Jamestown, Portsmouth, South Kingstown, North Kingstown, and Charlestown (Figure 1.1). 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 Greater Narragansett Bay watershed. Candidate study systems were identified from those within the Greater Narragansett Bay watershed based on five criteria: (i) year-round occupancy, (ii) system installation between 2006 and 2014, (iii) accessibility of manholes for sampling, (iv) hydraulic flow, and (v) presence of pressurized drainfield pumps. Our 42 study systems were selected based on site visits to visually inspect systems for accessibility, and the homeowners' willingness to participate in the study.

Sample collection. We collected final effluent samples monthly during the first two weeks of each month from March 2015 to June 2016, and in August 2016, from all 42 systems. A subset of nine systems (three of each system type) was sampled for four consecutive weeks every third month to quantify variation on a weekly scale. Final effluent samples were collected in 1-L plastic Nalgene bottles and stored at 4°C

(max. of 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 (referred to as sampling point SP2) (Figure 1.2). Additional field measurements were taken from the systems' anoxic component for pH, DO, and effluent temperature analysis (referred to as sampling point SP1) (Figure 1.2). All samples were transported to the laboratory within 8 h of sampling.

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, dissolved oxygen (DO) and effluent temperature were made in SP1 and SP2 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 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 Instruments, Woonsocket, RI) using a 1 N HCl solution to titrate a 100-mL effluent sample.

Statistical analysis. We used SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA) to complete all statistical analyses. To determine which properties best

predicted effluent TN concentration, we carried out best subsets multiple linear regressions separated by system 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 largest R^2 values and included the fewest independent variables, since monitoring fewer wastewater properties is more cost-effective and less time consuming for service providers. For example, if the addition of an independent variable did not increase the R^2 value appreciably, the subset with fewer variables was chosen. Models that contained independent variables with high variance inflation factors (VIFs) 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.

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 instead.

Daily flow and recirculation ratio calculations. Average daily forward flow and recirculation ratio were calculated as described below. 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 system type. For Advantex systems, we calculated average daily forward flow (F_{ADV} , in liters per day) using the equation:

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

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:

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

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).

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

$$R = V_{recirc} / V_{TOT}$$

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), or 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 (in liters per minute) for Advantex and 91 (in liters per minute) for SeptiTech systems.

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

$$V_{TOTADV} = (C * V_{cycle})$$

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_{TOTST} , in liters per day) using the equation:

$$V_{TOTST} = (t_{pump} * V_{pump})$$

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 drain back after a dose. Recirculation ratio is cannot be determined for FAST systems because nitrified effluent is gravity-fed to the anoxic tank.

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 systems. 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 systems (Figure 1.3). 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) (Figure 1.3), indicating FAST systems performed less consistently. Analysis of TN values by individual system shows the range of values increases with median TN concentration for all technology types (Figure 1.4).

Variation in performance between individual systems could be due to differences in influent (septic tank effluent) TN concentration. The 19 mg N/L standard is based on the expectation that advanced N-removal OWTS reduce influent TN concentration by 50%. Therefore, 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 that receives higher-strength influent will produce effluent with a higher TN concentration than a system that receives lower-strength influent. Since influent TN concentration cannot be measured (due to recirculation that

occurs within the system), perhaps calculating the mass of N produced by each system would provide a more comprehensive picture of system performance.

The high compliance rate and low median TN concentration for SeptiTech systems may be the result of a small sample size ($n=4$), since seven of the 11 SeptiTech systems we sampled were not set to denitrification mode at the onset of the study, prompting us to exclude those systems from this analysis. Nitrified effluent in denitrification-disabled SeptiTech systems recirculates back through the system minimally each day before it is discharged to the STA, which limits N removal. Total N data collected from March to December 2015 show that denitrification-enabled systems removed N more effectively: they had a lower median TN concentration (11.1 mg N/L) (Figure 1.5) 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 (Figure 1.5) and a compliance rate of 14.3%. One denitrification-disabled SeptiTech system was in compliance from March to December 2015, with a median final effluent TN concentration of 16.6 mg N/L, indicating that the system reduced TN to an acceptable concentration with minimal circulation of nitrified effluent back through the anoxic tank. The hydraulic retention time of wastewater inside the anoxic tank may have been sufficient to support denitrification, reducing TN concentration without the need for increased recirculation of nitrified effluent back to the anoxic compartment. The influent TN concentration may also have been low, which would have required less denitrification to lower the final effluent TN concentration to 19 mg N/L or lower.

Variation of effluent TN concentration at different time scales. We sampled a subset of nine systems (three of each technology type) for four consecutive weeks in May, August, and November 2015, and February and May 2016. Effluent TN concentrations varied over the course of four weeks (Figure 1.6): SeptiTech systems had the highest median coefficient of variation (CV) 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 (Figure 1.6). Median coefficients of variation 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 (Figure 1.7). As expected, median CVs for effluent TN concentration were higher at the month scale than the week scale for all technology types (Figure 1.7). Systems are exposed to larger fluctuations in properties that affect N removal (effluent temperature, pH, DO, etc.) throughout the course of a year than over four weeks, which explains higher median CVs at the month scale.

Over the course of four weeks the median CV for effluent temperature was 13.6% and for ambient temperature, 12.9%, indicating these properties are likely not the cause of variation in effluent TN concentrations at the week scale. Water usage throughout the month typically does not change significantly (USGS, 2016), indicating it is probably not responsible for variations in effluent TN concentration. Septic systems have microbial communities that are affected—positively and negatively—by environmental changes (e.g. pH, temperature, osmotic pressure, nutrient

availability) (Handelsman et al., 2007). Variation in effluent TN at the week scale could be explained by a system's low resiliency to these changes, since systems varied in their ability to remove nitrogen at this time scale.

Variation of effluent TN concentration with temperature. We also 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 1.1). 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 is 10 to 20 °C, and 30 to 36 °C for denitrification, although optimal temperature ranges may vary according to the microbial communities present (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 values (Figure 1.8). We also completed linear regressions separated by system type with temperature as the independent variable and final effluent TN concentration as the dependent

variable. Effluent TN concentrations from Advantex and FAST systems were not significantly correlated with effluent temperature, but the opposite was true for SeptiTech systems, which showed a significant positive correlation between effluent TN and temperature, despite having a low R^2 value (0.04) (Table 1.2). 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 likely control nitrification and denitrification rates. Denitrification rates may only be affected by temperature when other properties (i.e. DO, pH, nitrate availability, organic carbon) are within a range that is optimal for denitrification.

Significant predictors of TN

In an effort to provide information that can translate into more 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 system type, since predictors are likely to vary according to system type and treatment process. We used data collected from March 2015 to August 2016 and excluded data from denitrification-disabled SeptiTech systems. We 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 data, 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 Table 1.3-a and Table 1.3-b. 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 1.4). 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 1.4). 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 (to some extent), 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. We also expected the significant inverse relationship between TN and alkalinity observed for FAST systems, since buffering capacity has a significant impact on nitrification and denitrification. The optimal pH range for nitrifying and denitrifying bacteria is 6.5 to 8.0 and 7.0 to 8.5, respectively, although optimal ranges may vary according to the species present (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. A large volume of wastewater entering the system results in high forward flow, as the system needs to discharge all final effluent to the STA. In contrast, a significant positive correlation between TN and effluent temperature 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.

We completed additional best subsets linear regressions separated by system type that included only data from samplings corresponding to TN values of ≤ 19 mg N/L or less in order to determine which wastewater properties best predict acceptable TN concentrations. We also used this dataset to identify ranges of values of wastewater properties for each system type that would result in final effluent TN concentrations of ≤ 19 mg N/L (Figure 1.9). 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 1.5). 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 conversion of NH_4^+ to 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.

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) 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 (513 systems), with 87% of Advantex, 79% of FAST, and 42% of SeptiTech systems in compliance. Similarly, median TN values 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, NH_4^+ , 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 laboratory analysis of effluent TN.

Additionally, systems that do not meet the 19 mg N/L TN standard are revisited until they do. In contrast, RIDEM requires two inspections per year which do not include effluent TN analysis (Rask et al., 2013). 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.

More frequent O&M visits that include analysis of system final effluent allow for 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 tanks 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. Analysis of TN 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 performance evaluation because they remained unutilized (i.e. were not receiving influent) for extended periods of time. In an effort to improve N removal, we provided the O&M service providers responsible for these systems with the median and 25th and 75th percentile values from SP1 and SP2 for pH, DO, and effluent temperature, and from SP2 for alkalinity, ammonium, nitrate, TN, and BOD. Since we were not legally permitted to make adjustments ourselves, service providers were asked to make adjustments they considered appropriate (e.g. pumping of the advanced treatment unit and/or septic tank, recirculation ratio adjustment) to improve N removal in these underperforming systems. 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.

After adjustments, final effluent median TN concentrations decreased in three (one of each system type) of the seven systems. Of these three systems, the median final effluent TN concentration of two systems was reduced below 19 mg N/L (Figure 1.10), and the other system remained out of compliance after adjustments. The TN concentration of the other four systems increased after adjustments (Figure 1.10).

For the majority of systems tested, adjustments did not result in increased N removal. 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. Alternatively, the microbial communities within the underperforming systems may not be able to nitrify and/or denitrify sufficiently because of unfavorable conditions within the system (e.g. dissolved oxygen, pH, alkalinity, supply of C). The concentration of these constituents can limit the activity of nitrifying and denitrifying bacteria, and thus N removal.

To further investigate how adjustments affected underperforming systems, we compared values for each wastewater property before (March to August 2015) and after (March to August 2016) adjustments. The wastewater properties that changed significantly as a result of adjustments varied by individual system (Table 1.6). We couldn't identify a clear trend, since none of the parameters that changed significantly were common among all systems. Although the final effluent TN concentration was reduced to meet the 19 mg N/L standard, we did not detect any significant differences in wastewater properties collected from the SeptiTech system that was adjusted. It is possible that there are other factors associated with reducing TN concentration that we did not measure. Also, although it is assumed that nitrification and denitrification are occurring within these systems, it is possible that other microbial processes are achieving N-removal (i.e. anaerobic ammonium oxidation (ANAMMOX), nitrifier denitrification), in which case, different adjustments may be necessary to increase N-removal.

Denitrification-disabled SeptiTech systems. The seven SeptiTech systems that were not turned on for denitrification mode at the beginning of the project were re-programmed to denitrify in January 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 (Figure 1.11). This is the opposite of what should be observed, given that the denitrification-enabled mode is supposed to result in more effective N removal. Enabling the denitrification mode resulted in the median ratio of ammonium to nitrate concentration in effluent increasing from 0.46 to 1.50. An increased ammonium to nitrate ratio implies ammonium concentrations 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. 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 1.7). As was the case with denitrification-enabled systems, a clear pattern among all denitrification-disabled systems could not be found.

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 system type, time, and by individual system. 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 system type: ammonium, nitrate, and alkalinity for Advantex; ammonium, nitrate, average forward flow, and BOD for FAST; and, ammonium and effluent temperature for SeptiTech. Adjustments made to underperforming systems that were originally set to denitrification-enabled mode resulted in lower final effluent TN concentrations in three out of seven systems, but one of these systems remained out of compliance after adjustments. Paradoxically, final effluent TN concentrations from SeptiTech systems 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 quarterly and recursive site visits, effluent testing for TN, and reporting of results through a county-maintained database. Our results suggest that a single site visit and adjustment to underperforming

systems is not sufficient to improve system performance—additional visits and recurring adjustments may be necessary. In addition, the variability of N removal effectiveness at weekly and monthly scales needs to be taken into account in order to accurately assess system performance. Additional training of 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.

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TABLES

Table 1.1 Median total N concentration in final effluent, and effluent and ambient temperature, separated by season and system type.

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

Table 1.2 Results of linear regression analyses to predict effluent TN concentration based on effluent temperature values. Denitrification-disabled SeptiTech systems were included in this analysis. Significant P values ($\alpha=0.05$) are in bold. N=191, 138, and 123 for Advantex, FAST, and SeptiTech systems, respectively.

System Type	Coefficient	R ²	P-value	
Advantex		0.185	0.007	0.274
FAST		-0.105	0.001	0.709
SeptiTech		0.727	0.040	0.036

Table 1.3-a Summary statistics of wastewater properties sampled from systems between March and December 2015. n= 63-135. Denitrification-disabled SeptiTech systems were excluded from this analysis.

System Type	pH-SP1			pH-SP2			DO-SP1 (mg N/L)			DO-SP2 (mg N/L)			Effluent Temperature- SP1 (°C)		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
Advantex	6.9	5.8	8.4	6.4	2.8	7.2	0.0	0.0	7.0	5.0	0.5	10.5	18.2	5.6	28.4
FAST	7.3	4.5	8.6	7.1	4.0	8.6	5.5	0.0	9.2	2.0	0.0	8.3	18.4	4.8	24.8
SeptiTech	7.2	6.2	8.8	6.8	6.4	7.7	0.0	0.0	3.4	6.1	0.0	8.5	18.3	8.9	25.0

Table 1.3-b Summary statistics of wastewater properties sampled from systems between March and December 2015. n= 63-135. Denitrification-disabled SeptiTech systems were excluded from this analysis.

System Type	Effluent Temperature-SP2 (°C)			Ammonium-SP2 (mg N/L)			Nitrate-SP2 (mg N/L)			Alkalinity-SP2 (mg/L)			Total N-SP2 (mg N/L)			BOD-SP2 (mg/L)		
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.
Advantex	17.8	2.4	28.4	2.5	0.0	49.9	11.3	0.0	29.4	53.5	0.0	24.7	14.9	0.0	61.6	4.0	0.0	10.0
FAST	16.8	3.5	23.6	1.0	0.0	64.7	7.9	0.0	42.2	63.3	0.0	31.3	17.1	0.6	10.4	0.0	0.0	14.2
SeptiTech	18.8	9.1	25.9	3.1	0.1	60.7	10.3	0.0	59.8	59.0	0.0	35.8	11.3	0.1	41.6	0.0	0.0	98.0

Table 1.4 Results of best subsets multiple linear regression analysis to predict effluent total N concentration based on effluent properties using all data collected from March 2015 to August 2016. Denitrification disabled SeptiTech systems were excluded from this analysis.

System type	Property	Coefficient	P	R ²
Advantex	Constant	10.511	0.004	0.442
	Ammonium	0.969	<0.001	
	Nitrate	0.438	0.032	
	Alkalinity	-0.0736	0.017	
FAST	Constant	7.639	<0.001	0.710
	Ammonium	0.519	<0.001	
	Nitrate	0.593	<0.001	
	Avg. Forward Flow	-0.0311	0.003	
	BOD	0.139	<0.001	
SeptiTech	Constant	-78.175	0.028	0.807
	Ammonium	0.548	0.002	
	Effluent	2.189	0.016	
	Temperature			
	Recirculation Ratio	7.674	0.098	

Table 1.5 Results of best subsets multiple linear regression analysis to predict effluent total N concentration based on effluent properties 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.

System type	Property	Coefficient	P	R ²
Advantex	Constant	4.692	0.155	0.083
	Nitrate	0.259	0.057	
	DO – SP1	1.909	0.337	
	DO – SP2	0.595	0.189	
	BOD	0.0573	0.227	
FAST	Constant	4.738	0.067	0.259
	Ammonium	0.224	0.105	
	Nitrate	0.257	0.011	
	DO – SP1	0.589	0.231	
	DO– SP2	-0.384	0.226	
SeptiTech	BOD	-0.0911	0.091	0.335
	Constant	20.546	<0.001	
	Alkalinity	-0.0553	0.006	
	DO	-1.473	0.019	

Table 1.6 Effects of adjustment of underperforming systems on wastewater properties. A t-test ($\alpha= 0.05$) was used to determine statistical significance.

System Type	System	Property	Median		P	t
			Before adjustments	After adjustments		
Advantex	1	Ammonium	43.6	59.8	0.017	-3.3
		Alkalinity	112.0	274.9	0.008	15.0
		Recirc. Ratio	3	4	0.032	11.5
FAST	2	N/A	N/A	N/A	N/A	N/A
	1	pH SP2	4.3	5.7	0.008	-3.5
		pH SP1	4.2	7.0	0.029	-3.0
		Alkalinity	0.4	13.6	0.016	30.0
	2	Nitrate	4.0	13.2	0.002	-4.0
		pH SP2	7.3	7.0	0.016	2.7
		DO SP2	0.6	2.2	0.008	-3.2
		BOD	90.0	31.0	0.006	4.1
	3	Alkalinity	90.4	272.0	0.029	10.0
	4	DO SP1	2.0	5.9	0.028	-3.4
SeptiTech	1	N/A	N/A	N/A	N/A	N/A

*N/A indicates no significant differences were found.

Table 1.7 Wastewater properties that changed significantly ($\alpha = 0.05$) after adjustments to denitrification-disabled SeptiTech systems.

System	Property	Median		P	t
		Before adjustments	After adjustments		
1	TN	28.8	28.7	0.021	-3.7
2	Nitrate	17.4	3.4	0.002	10.2
	pH-SP2	5.9	7.2	0.029	10.0
	Alkalinity	11.3	202.5	0.029	10.0
	BOD	6.0	98.0	0.001	-12.3
3	pH SP2	4.4	7.3	0.002	-5.4
4	N/A	N/A*	N/A	N/A	N/A
5	TN	37.1	72.7	0.013	-3.5
	DO SP2	4.8	6.9	0.007	-6.8
6	Nitrate	30.6	8.1	0.037	2.6
	pH SP2	5.4	6.3	0.032	-2.6
7	TN	34.0	64.0	0.021	-3.7
	pH SP2	6.3	7.1	0.049	-2.5
	Recirc. Ratio	1.0	5.0	0.003	-3.7

*N/A indicates no significant differences were found.

FIGURES

Figure 1.1 Map showing the six towns where study systems were located.

Figure 1.2 Typical layout of Advantex, FAST, and SeptiTech system treatment trains showing sampling locations.

Figure 1.3 Concentration of final effluent total N for all systems within a technology type from March to December 2015. $n = 141, 112,$ and 30 for Advantex, FAST, and SeptiTech systems, respectively. Dashed line represents 19 mg N/L standard. The top bar represents the 90^{th} percentile, followed by the 75^{th} percentile, the median, 25^{th} percentile, and 10^{th} percentile. The dots represent values outside the 10^{th} and 90^{th} percentile.

Figure 1.4 Concentration of final effluent total N for individual Advantex, FAST, and SeptiTech systems between March and December 2015 (n=8-32). Dashed line represents 19 mg N/L standard.

Figure 1.5 Concentration of final effluent total N for denitrification-enabled (n=59) and denitrification-disabled (n=118) SeptiTech systems sampled between March and December 2015. Dashed line represents 19 mg N/L standard.

Figure 1.6 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. Number of systems included in analysis is displayed at the top of each box. All SeptiTech systems in this analysis were denitrification-enabled.

Figure 1.7 Coefficients of variation calculated for monthly data (March 2015 to March 2016) (black bars) compared to median CVs calculated for weekly data (white bars). Data are for a subset of nine systems (3 Advantex, 3 FAST, 3 SeptiTech).

Figure 1.8 Effluent temperature values plotted against effluent TN concentrations for Advantex, FAST, and SeptiTech systems collected from March to December 2015. N=191, 138, and 123 for Advantex, FAST, and SeptiTech systems, respectively. Denitrification-disabled SeptiTech systems were included in this analysis.

Figure 1.9 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, and L/day for average forward flow (FF).

Figure 1.10 Median (n=4-5) final effluent total N concentrations for underperforming denitrification-enabled Advantex, FAST, and SeptiTech systems before adjustments (March 2015 to June 2015; black box) compared to after adjustments (March 2016 to June 2016; white box). The dashed line represents 19 mg N/L standard.

Figure 1.11 Median (n= 4-5) final effluent total N concentrations for denitrification-disabled SeptiTech systems (March 2015 to June 2015; black box) compared to after denitrification mode was enabled (March 2016 to June 2016; white box). The dashed line represents 19 mg N/L standard.

**MANUSCRIPT 2: ACCURACY OF RAPID TESTS USED FOR ANALYSIS
OF ADVANCED ONSITE WASTEWATER TREATMENT SYSTEM
EFFLUENT**

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ABSTRACT

Rapid tests provide an inexpensive, desirable alternative to standard laboratory analyses for testing advanced onsite wastewater treatment system (OWTS) effluent in the field. Despite their potential utility, their accuracy for analysis of effluent from advanced OWTS has not been assessed. We evaluated the accuracy of an initial suite of rapid tests commonly used to analyze wastewater (test strips for ammonium, pH, nitrate and alkalinity; pH pocket meter; titration kit for dissolved oxygen (DO)) by comparing values obtained in the field to values obtained using standard laboratory methods. We tested final effluent from three different advanced nitrogen removal OWTS technologies sampled monthly for seven months at 42 different sites within the greater Narragansett Bay watershed in Rhode Island, USA. Significant differences between values obtained using field and standard methods were found only for nitrate and pH test strips when the data were analyzed using ANOVA on ranks. However, regression analysis indicated that all test strip-based rapid methods and the DO titration kit produced values that deviated significantly from correspondence with standard 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 disappeared for all tests. Evaluation of a suite of alternative rapid tests for ammonium, nitrate, pH and alkalinity indicated that tests kits for NH_4^+ and multi-analysis test strips for pH provide accurate results in the field. Our results indicate that the accuracy of rapid tests needs to be evaluated under field conditions before they are used to assess effluent from advanced N-removing OWTS.

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 NO_3^- -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 NH_3 -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 CaCO_3) 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 O_2 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 $\text{NH}_3/\text{NH}_4^+\text{-N}$ 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 NO_3^- (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 NO_3^- 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 2.1.

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 reanalyzed. For ammonium and nitrate analysis, the

concentration of the method blanks was required to be $\leq 200 \mu\text{g N/L}$. 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.

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 ($\alpha=0.05$) for mean comparisons. A one-way ANOVA on ranks was used to examine whether differences in the accuracy of rapid methods was due to system 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 2.1. Analysis of the data using ANOVA on ranks showed 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 potentially a single test is important.

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 (Figure 2.1 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 R² of 1. All values are represented in mg/L except for pH.). 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 (Figure 2.1 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 R² of 1. All values are represented in mg/L except for pH.), so that a standard value of 120 mg/L was detected by the alkalinity test strip as between 20 and 240 mg/L.

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 (Figure 2.1 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 R² of 1. All values are represented in mg/L except for pH.). Ammonium strips over-predicted at low concentrations and under-predicted at high concentrations. For example, a standard NH₄⁺ value of 300 mg NH₄⁺-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 NO₃⁻-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 NO₃⁻ present. The pH strips detect standard values between 4 and 7 as 4 or 5 (Figure 2.1). 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 2.3 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 ($\alpha=0.05$).). 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 2.3 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 ($\alpha=0.05$).). 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.

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 system 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 2.4). 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 ground water 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.

Differences in accuracy found between locations, month collected, and system 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 2.2), 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.

The accuracy of the initial suite of rapid tests improved under controlled conditions for all the tests evaluated (Table 2.3). Nitrate standard values plotted against rapid test values fit closely to a 1:1 line (Figure 2.2), 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 2.3).

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 2.2), 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 (Figure 2.3). 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 2.5). 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).

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 R^2 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).

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TABLES

Table 2.1 Summary statistics for final effluent properties from different types of advanced nitrogen removal OWTS analyzed using standard methods. Units are mg/L except for pH.

Property	Statistic*	System type		
		AX	FAST	SeptiTech
Alkalinity	Mean	53.0	45.4	57.1
	SD	33.9	36.0	41.1
	Median	48.9	40.8	56.2
	Range	124.7	125.0	123.0
	CV	0.64	0.79	0.72
Ammonium	Mean	8.2	7.9	7.0
	SD	12.8	12.7	8.2
	Median	3.9	1.8	3.5
	Range	49.2	46.8	29.9
	CV	1.56	1.61	1.175
Nitrate	Mean	10.1	11.4	10.9
	SD	5.5	10.1	10.0
	Median	9.2	8.7	8.3
	Range	23.4	41.5	39.6
	CV	0.55	0.89	0.92
Dissolved O ₂	Mean	6.1	2.7	5.8
	SD	2.0	2.4	2.4
	Median	6.2	2.6	6.5
	Range	9.6	7.9	9.5
	CV	0.33	0.88	0.40
pH	Mean	6.4	6.7	6.6
	SD	0.6	0.9	0.8
	Median	6.6	6.9	6.9
	Range	3.3	4.7	4.0
	CV	0.09	0.14	0.12

*SD = standard deviation; CV = coefficient of variation

Table 2.2 Summary statistics for final effluent properties tested using standard methods as well as the initial and alternative suites of rapid tests under field and laboratory conditions. Samples were collected between February and August 2015. Units are in mg/L

Property	Test suite	Method	Test condition	n	Media			
					Mean	n	SD	CV
Alkalinity	Initial	Standard	Laboratory	130	50.6	40.0	39.4	0.78
		Test strip	Field	130	63.6	40.0	70.0	1.10
		Standard	Laboratory	24	50.4	60.0	15.8	0.31
		Test strip	Laboratory	24	55.7	40.0	52.9	0.10
	Alternative	Standard	Laboratory	12	53.3	60.0	9.9	0.18
		Test strip	Laboratory	12	76.7	60.0	51.8	0.67
		Standard	Laboratory	90	72.1	40.0	73.7	1.00
		Test strip	Field	90	67.3	60.0	50.9	0.76
Dissolved	Initial	Standard	Field	284	3.51	3.80	3.1	0.90
O ₂		Titration kit	Field	284	3.8	4.0	2.9	0.75
Ammonium	Initial	Standard	Laboratory	145	17.8	3.0	50.4	2.82
		Test strips	Field	145	13.3	3.0	28.4	2.10
		Standard	Laboratory	24	7.7	2.0	11.6	1.51
		Test strips	Laboratory	24	7.6	4.0	11.1	1.47
	Alternative	Standard	Laboratory	22	8.3	3.0	11.9	1.43
		Test kit	Laboratory	22	8.3	4.0	11.4	1.37
		Standard	Laboratory	170	8.4	2.0	12.0	1.43
		Test kit	Field	170	9.1	2.0	17.6	1.94
Nitrate	Initial	Standard	Laboratory	124	10.2	10.0	11.2	1.10
		Test strip	Field	124	2.8	0.00	5.2	1.83
		Standard	Laboratory	24	11.0	10.0	6.9	0.62
		Test strip	Laboratory	24	14.4	10.0	10.7	0.74
	Alternative	Standard	Laboratory	12	11.7	20.0	10.3	0.88

			Test strip	Laboratory	12	85.0	80.0	29.7	0.35
			Standard	Laboratory	94	12.3	20.0	12.5	1.01
			Test strip	Field	94	31.0	20.0	37.5	1.21
pH	Initial	Standard	Laboratory	39	6.28	7.00	1.02	0.16	
		Test strip	Field	39	4.54	5.00	0.50	0.11	
		Standard	Laboratory	236	6.62	6.80	0.88	0.13	
		Pen	Field	236	6.48	6.70	0.83	0.13	
		Standard	Laboratory	24	6.75	7.00	0.79	0.12	
		Test strip	Laboratory	24	5.29	5.00	0.86	0.16	
	Alternative	Standard	Laboratory	92	6.55	6.50	0.49	0.08	
		Test strip	Field	92	6.90	7.00	0.61	0.09	
		Standard	Laboratory	12	6.96	7.00	0.50	0.70	
		Test strip	Laboratory	12	6.96	7.00	0.69	0.10	

Table 2.3 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 ($\alpha=0.05$).

Property	Test conditions	Regression parameter:		
		Intercept	Slope	R ²
Alkalinity	Field	18.9	0.9	0.43
	Laboratory	19.82	1.64	0.16
Ammonium	Field	4.5	0.5	0.76
	Laboratory	1.23	0.87	0.66
Dissolved O ₂	Field	2.4	0.6	0.46
	Laboratory	--	--	--
Nitrate	Field	1.5	0.1	0.07
	Laboratory	1.16	1.2	0.60
pH – test strips	Field	4.0	0.1	0.02
	Laboratory	0.75	0.67	0.39
pH – pocket meter	Field	0.7	1.0	0.84
	Laboratory	--	--	--

Table 2.4 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	Property:				
		Alkalinit	Dissolved			
		y	Ammonium	O ₂	Nitrate	pH
System	AX	A	A	A	A	A
	FAST	AB	A	B	A	A
	SeptiTech	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	B	A	A	A
	North	A	AB	A	A	A
	Kingstown	A	AB	A	A	A
*NA=Not applicable; no data were collected						

Table 2.5 Parameters of regression lines comparing results of the alternative suite of rapid tests under field conditions (n= 90 – 170) with results of standard analyses. Differences in slope and intercept from hypothesized values (1 for slope, 0 for intercept) were determined using a two-tailed *t*-test. Values in bold are significantly different from ideal values ($\alpha=0.05$).

Property	Method	Regression parameter:		
		Intercept	Slope	R ²
pH	Test strips	0.87	1.17	0.50
Ammonium	Test kit	1.40	0.92	0.39
Nitrate	Test strip	10.61	0.41	0.11
Alkalinity	Test strip	31.58	0.50	0.52

Figure 2.1 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.

Figure 2.2 Standard values plotted against values obtained using the initial suite of rapid tests for alkalinity, pH, 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 except for pH.

Figure 2.3 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.

CONCLUSIONS

My 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 system 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 values, which contradicts the expectation that denitrification rates increase with temperature. My results also show that different variables serve as best predictors of effluent TN concentration, depending on system type: ammonium, nitrate, and alkalinity for Advantex systems; ammonium, nitrate, average forward flow, and BOD for FAST systems; 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. My results also show that, for most underperforming systems, 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 systems remained out of compliance.

I 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. I was able to

identify some 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 improve 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 my 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. My 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.

My 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, it may be that service providers were not adequately trained, and

therefore 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 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.

With regard to rapid tests, my results indicate their accuracy needs to be considered before they are used to evaluate performance of advanced OWTS. My findings show that some rapid tests can be used where lighting conditions are stable in order to increase their ability to predict constituent concentrations without losing the convenience of quick results, whereas others 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 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.

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