June 2008 Final Report

ASSESSMENT OF POTENTIAL TOXICITY AND AQUATIC COMMUNITY IMPACTS ASSOCIATED WITH MEMBRANE AND ION EXCHANGE WATER TREATMENT FACILITY EFFLUENTS IN COASTAL NORTH CAROLINA

U.S. Fish and Wildlife Service
Raleigh, NC

N.C. Division of Water Quality
Raleigh, NC

N.C. Wildlife Resources Commission
Washington, NC

N.C. Division of Marine Fisheries
Columbia, NC

PREFACE

To evaluate the significance of ion imbalance toxicity and the extent of impacts to the aquatic community associated with surface water discharge of concentrate from membrane and ion exchange water treatment processes in coastal North Carolina, the U.S. Fish and Wildlife Service partnered with the North Carolina Division of Water Quality, the North Carolina Division of Marine Fisheries, and the North Carolina Wildlife Resources Commission. This work was coordinated by Sara Ward (Ecologist / Environmental Contaminant Specialist) in the U.S. Fish and Wildlife Service Raleigh Field Office and was funded by the U.S. Fish and Wildlife Service's Environmental Contaminants Program (project number 200440003.1). Toxicity testing and analytical chemistry for the project was performed by EA Engineering, Science, and Technology, Inc. (Sparks, MD) and the North Carolina Division of Water Quality Laboratory Section (Raleigh, NC).

Additional questions, comments, and suggestions related to this final report are encouraged. Inquires can be directed to the U.S. Fish and Wildlife Service at the following address:

Sara Ward
U.S. Fish and Wildlife Service
Raleigh Ecological Services Field Office
P.O. Box 33726
Raleigh, NC 27636-3726

EXECUTIVE SUMMARY

The application of reverse osmosis and ion exchange water treatment of groundwater to meet the growing potable water demand in eastern North Carolina has prompted interest in the potential environmental impacts of these unique water treatment processes. These processes generate a reject water comprised of concentrated salts, metals, and other constituents and major ion toxicity is a important concern in other states where these drinking water production technologies are more prevalent. Effluent and receiving stream samples were collected at three ion exchange and two reverse osmosis membrane water treatment plants (WTP) in North Carolina. Routine water quality characteristics, ion, nutrient and metal concentrations were documented in 15 effluent and 30 receiving stream samples between 2004 and 2005. Facility- and process-specific differences in water quality characteristics of effluents collected from the ion exchange WTPs were apparent. Effluent ion concentrations were typically highest during the sodium regeneration cycle followed by the final rinse and backwash cycles of the ion exchange treatment process. Total residual chlorine concentrations in effluent from one ion exchange facility exceeded the state standard (of 17 μg/L) by 8- to 36-fold. Effluent characteristics differed substantially between the two reverse osmosis WTPs (likely due to source water chemistry); however, effluent samples from each facility were relatively uniform in chemical composition between sampling events. To determine the significance of ion imbalance toxicity associated with process effluents, 15 chronic baseline whole effluent toxicity (WET) tests were conducted using *Ceriodaphnia dubia* (water flea) and *Americamysis bahia* (opossum shrimp) in freshwater and saltwater exposures, respectively. Only one effluent sample was not acutely or chronically toxic to test organisms during the baseline WET tests. The toxicity of ion exchange effluents varied based on the treatment cycle with sodium regeneration samples being most toxic at all three facilities (and rinse and backwash cycles generally associated with lower toxicity). Subsequent toxicity tests using whole effluent and synthetic effluent test mixtures were performed to identify the potential for major ion imbalance as a source of toxicity in the baseline tests. Of the 14 effluent samples where subsequent tests were conducted, results indicated that sublethal toxicity in only one sample appeared to be related to a constituent other than the major ions; the results of testing with another sample were inconclusive due to elevated control mortality. Bioassessment of benthic community structure was also conducted to determine potential aquatic community structure impacts in the vicinity of the WTPs. Quantitative assessment could only be performed in the receiving stream (Filbert Creek) for two ion exchange facilities. Although an ion gradient (potentially related to hypersaline effluent releases or wind/tidal influence) was present in Filbert Creek, increasing taxa richness and decreasing biotic index values were found along this gradient suggesting that ion concentrations present have not uniquely stressed benthic communities. At the remaining three WTP sites, qualitative assessment indicates the presence of limited benthic communities (dominated by species tolerant of low-flow and low-oxygen conditions). Study results provide sufficient evidence that ion concentrations in reverse osmosis and ion exchange water treatment plant effluents are a primary source of the effluent toxicity. Proposed management recommendations focus on more closely matching effluent and receiving stream ion characteristics at existing and proposed WTPs by either diluting effluents prior to discharge (in freshwater environments) and maximizing instream dilution through outfall design and placement.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Tables

Figures

LIST OF ACRONYMS/ABBREVIATIONS

ANOVA	analysis of variance
APHA	American Public Health Association
ATU	Aquatic Toxicology Unit
AWQC	ambient water quality criterion
BI	biotic index
CCC	criterion continuous concentration
ChV	chronic value
EA	EA Engineering, Science, and Technology, Inc.
EPT	Ephemeroptera Plecoptera Trichoptera
EPT S	Ephemeroptera Plecoptera Trichoptera taxa richness
FE	facility effluent
GRI	Gas Research Institute
IC25	25 percent inhibition concentration
LC50	median lethal concentration
LOEC	lowest observed effect concentration
NCDMF	North Carolina Division of Marine Fisheries
NCDWQ	North Carolina Division of Water Quality
NWR	National Wildlife Refuge
NOEC	no observed effect concentration
ppt	parts per thousand
SE	synthetic effluent
TKN	total kjehldahl nitrogen
USEPA	U.S. Environmental Protection Agency
UT	unnamed tributary
WET	whole effluent toxicity
WTP	water treatment plant

Assessment of Potential Toxicity and Aquatic Community Impacts Associated with Membrane and Ion Exchange Water Treatment Facility Effluents in Coastal North Carolina

Introduction

With increasing coastal development in eastern North Carolina, the application of membrane and ion exchange water treatment of groundwater sources to meet the growing potable water demand has prompted regulatory interest in the potential environmental impacts of these unique water treatment processes (NCDEM 1992, NCDENR 2003). To date, about 15 membrane and over 50 ion exchange water treatment plants (WTP) are operational in North Carolina, and state officials anticipate future growth in the number of these plants (particularly reverse osmosis) in the coastal plain given brackish infiltration of groundwater and a scarcity of freshwater sources. Briefly, these technologies remove salts and metals to produce drinking quality water. These processes generate a reject water comprised of concentrated salts, metals, and other constituents which are largely determined by the chemical nature of the raw source water and process additives. Most reject waters are discharged to natural surface waters. As such, toxicity associated with elevated concentrations of major anions (e.g., calcium, potassium, magnesium, and sodium) and or cations (e.g., chloride, sulfate, carbonate, and bicarbonate) associated with these waters has emerged as an important concern in other states where such drinking water production technologies are more prevalent (Mickley and Briceno 2000, Goodfellow et al. 2000, Andrews 2001, Mickley and Briceno 2001).

The potential threats to the aquatic environment of wastewater from WTPs using groundwater sources were the focus of an assessment of selected representative facilities using reverse osmosis membrane and ion exchange technologies in eastern North Carolina (NCDENR 2003). An analysis of discharge monitoring data from five WTPs conducted by North Carolina Division of Water Quality's (NCDWQ) National Pollutant Discharge Elimination System (NPDES) Unit indicated that maximum predicted effluent concentrations exceeded state water quality standards and federal water quality criteria for several parameters (including arsenic, chloride, chromium, copper, fluoride, iron, mercury, nickel, and zinc). Effluent data from the preliminary study also confirm that levels of several pollutants exceed state and federal standards at both membrane (chloride, fluoride, dissolved oxygen, and alkalinity) and ion exchange (chloride, zinc, dissolved oxygen, alkalinity, iron, and total residual chlorine) WTPs (NCDENR 2003). Measured instream concentrations are not reported in discharge monitoring reports for these facilities; however, based on the water dilution capacity, calculated instream waste concentrations ranged from 90 to 100 percent of the receiving stream volume at all three of the membrane facilities examined. The adequacy of this limited dilution capacity to entirely ameliorate concerns over elevated metal and salt concentrations of these effluents is of concern. Results of the initial study indicate that the ionic composition of these wastes substantially differs from that of the receiving stream in many cases; consequently, a multiagency workgroup identified further investigation of ion imbalance toxicity and potential aquatic community impacts as a priority for developing toxicity reduction strategies for these permitted discharges.

In addition to any direct toxicological impacts associated with pollutant levels in excess of state and federal water quality standards, an emerging issue is that the concentration of ions in these wastestreams creates a major ion imbalance that may be physiologically intolerable to aquatic fauna. This may occur when the salinity and / or overall ionic composition of water treatment concentrates substantially differ from that of the ambient water chemistry of the receiving stream (Goodfellow et al. 2000, Mickley and Briceno 2001). Ion imbalance is a condition that adversely effect aquatic organisms through disruption of receiving stream conditions when effluent ion concentrations exceed normal ranges or when the normal ratio of ions is altered by the discharge (SETAC 2004). Ionic imbalance, accordingly, can result in major ion toxicity to aquatic organisms vulnerable to osmotic perturbations when the proportion of specific ions is altered (Ingersoll et al. 1992, Dwyer et al. 1992) or when the high concentration of ions (in combination) exceeds organism tolerance (Goodfellow et al. 2000 and Mount et al. 1997). The latter mechanism of ion imbalance (disproportionate concentration of ions in combination relative to receiving stream conditions) is the focus of the current study. Such toxicity has been widely documented in the state of Florida where failures of whole effluent toxicity (WET) tests at membrane water treatment facilities prompted development of a standardized protocol for determining ion toxicity in WTP concentrates (FDEP 1995). While the composition or ratio of ions potentially contributing to toxicity may be important, the broader management question is whether the combined elevated salt concentrations, or another toxicant, is responsible for toxicity evident in WTP discharges. Accordingly, use of the term ion imbalance throughout this report refers to ion concentrations above the normal range.

The potential for either direct toxicity associated with exceedances of ambient water quality criteria and standards or major ion toxicity associated with ionic imbalance of receiving stream water chemistry is problematic because WTP discharges may prevent free passage of aquatic organisms or impair aquatic communities. Furthermore, hypersaline concentrated effluents from WTPs using ion exchange and reverse osmosis membrane technologies are often associated with elevated temperature and pH conditions and reduced dissolved oxygen concentrations relative to receiving waters. The disparity between effluent characteristics and ambient water quality conditions in receiving streams indicates that the potential for salinity stratification (particularly in low-flow freshwater receiving waters) exists and may subsequently alter existing biological communities. The North Carolina Division of Marine Fisheries (NCDMF) has raised concerns on several occasions (NCDMF 2001a-d) that the salinity of WTP discharges is inconsistent with receiving water ambient conditions and such discharges could considerably alter habitats and adversely affect commercially and recreationally important fishes. Particular concern has been raised regarding the effects of concentrated WTP effluents on spawning and nursery areas. Spawning areas for river herring (alewife and blueback herring) include low-flow freshwater environments where the impacts of concentrated effluents are likely to be most severe due to limited dilution. The NCDWQ Bioassessment Unit also expressed concern that discharge of concentrated WTP reject water and other wastes would cause substantial impacts to the aquatic community structure of freshwater receiving streams. In their assessment of Kendrick Creek (NCDWQ 2000) (a site proposed for discharge of ion exchange process waste), NCDWQ's Bioassessment Unit determined that "the proposed brine discharge from the [facility], while not large (0.015 MGD), is big enough to cause a complete shift in the biological community currently living [in Kendrick Creek] at US 64, including cypress (*Taxodium*) and lily pads (*Nuphar*), as well as the macroinvertebrates."

Based on these concerns, the U.S. Fish and Wildlife Service coordinated an effort to assess potential impacts associated with membrane and ion exchange WTP effluents; the objectives of the effort were to: 1) determine the significance of ion imbalance toxicity associated with process reject water effluents; 2) document water quality conditions both in process effluents and in waters receiving concentrated WTP discharges; and, 3) determine aquatic community structure impacts in the vicinity of existing water treatment facilities with probable ion imbalance toxicity issues.

Methods

Sample Sites

The WTPs included in this assessment were selected based on WET test results and discharge monitoring reports provided by NCDWQ. Facilities discharging to freshwater and estuarine environments with demonstrated toxicity testing failures and/or exceedences of state water quality standards were chosen to determine potential instream impacts. Field reconnaissance of potentially suitable facilities (based on the criteria above) was performed to confirm the location of facility outfalls and the feasibility of instream sampling. Based on field visits, several facilities were eliminated from further consideration (most frequently due to discharge to non-flowing receiving streams, ditches, or swales). The five test facilities selected included the Tyrrell County WTP (Tyrrell Co.), Fairfield WTP (Hyde Co.), Rodanthe/Waves/Salvo WTP (Dare Co.), Freemason WTP (Chowan Co.), and Beaver Hill WTP (Chowan Co.). To assess potential instream impacts under varied receiving stream conditions, facilities discharging to low-flow (Tyrrell Co. and Fairfield WTPs) and moderate flow (Freemason and Beaver Hill WTPs) freshwater streams and estuarine waters (Rodanthe/Waves/Salvo WTP) were targeted. Three of the five facilities are also located within one mile of National Wildlife Refuges (NWRs) in eastern North Carolina; accordingly, refuge management implications of the discharges were also a factor in site selection. Information summarizing facility technologies and receiving stream characteristics is provided in Table 1.

To characterize water quality conditions in effluents and receiving waters, samples were collected directly from each facility (except at Fairfield WTP where samples were obtained from the pipe prior to mixing with the receiving stream) as well as accessible upstream and downstream sites. The Rodanthe/Waves/Salvo WTP outfall is located in Blackmar Gut, a small tidal embayment (originally constructed as an emergency turning basin for ferry operations); in the absence of true upstream and downstream sampling sites, samples were obtained instead from the northernmost and southernmost access points bracketing the discharge point. Figures 1-4 illustrate the sample collection sites for each facility and proximity to NWRs (where applicable).

Table 1. Receiving stream characteristics and treatment technologies for water treatment plants evaluated.

Facility Name	NPDES Permit No.	Permitted Flow (gpd)	Receiving Stream	Receiving Stream Characteristics	Treatment Technology
Tyrrell Co. WTP	NC0087092	53000	UT to Riders Creek	Fresh; low-flow ditch draining to downgradient wetland and creek	ion exchange
Fairfield WTP	NC0068233	100000	UT to Lake Mattamuskeet	Fresh; low-flow canal draining to forested wetland and ultimately reaching Lake Mattamuskeet	reverse osmosis
Rodanthe/Waves/ Salvo WTP	NC0083909	300000	Blackmar Gut	Estuarine; tidal artificially constructed embayment off of Pamlico Sound	reverse osmosis
Freemason WTP	NC0007552	10000	UT to Filbert Creek	Fresh; low-flow stream with drainage area of < 2 mi^2	ion exchange
Beaver Hill WTP	NC0086291	10000	Filbert Creek	Fresh; low-flow stream with drainage area of < 2 mi^2	ion exchange

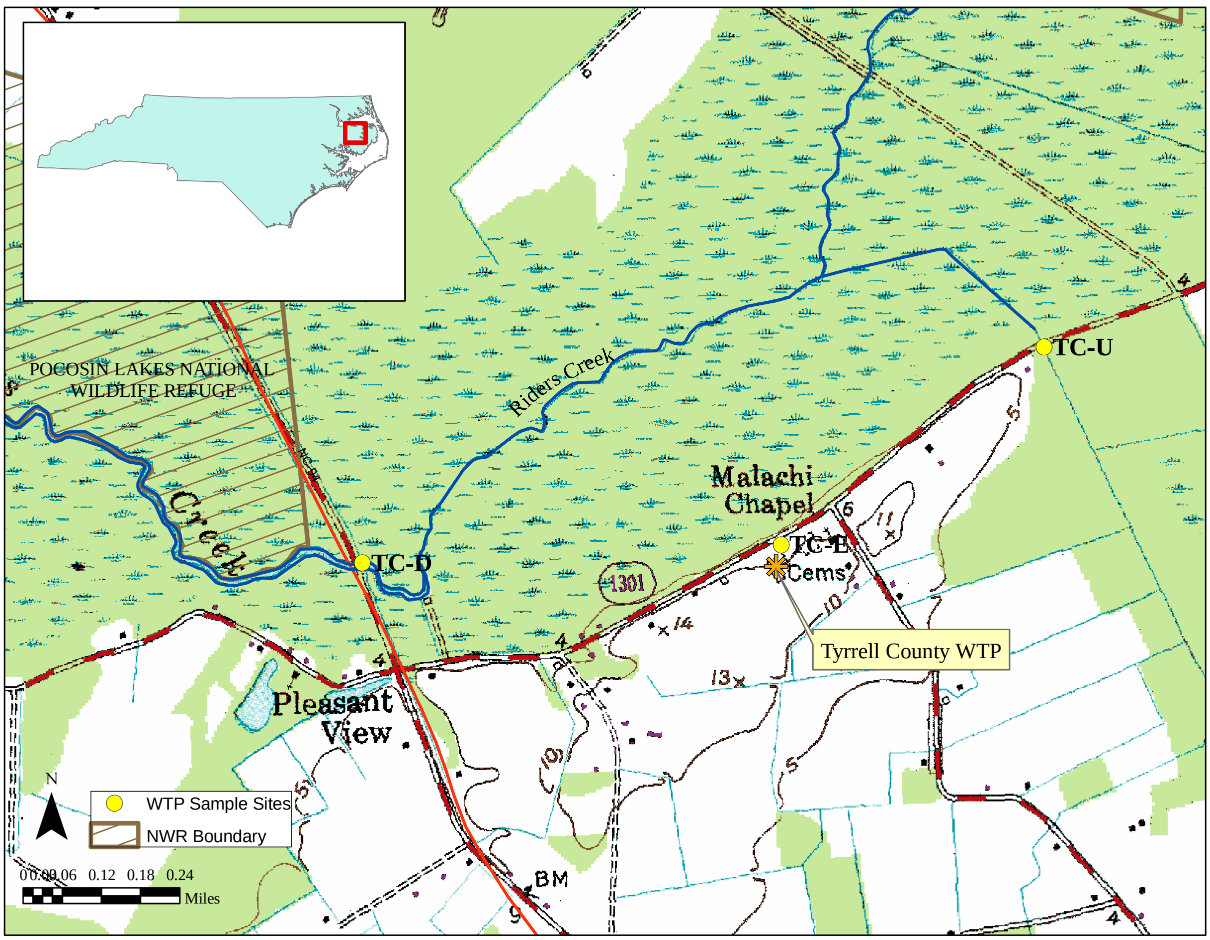

Figure 1. Sample collection sites for the Tyrrell County Water Treatment Plant included upstream (TC-U), effluent (TC-E) in plant, and downstream (TC-D) locations.

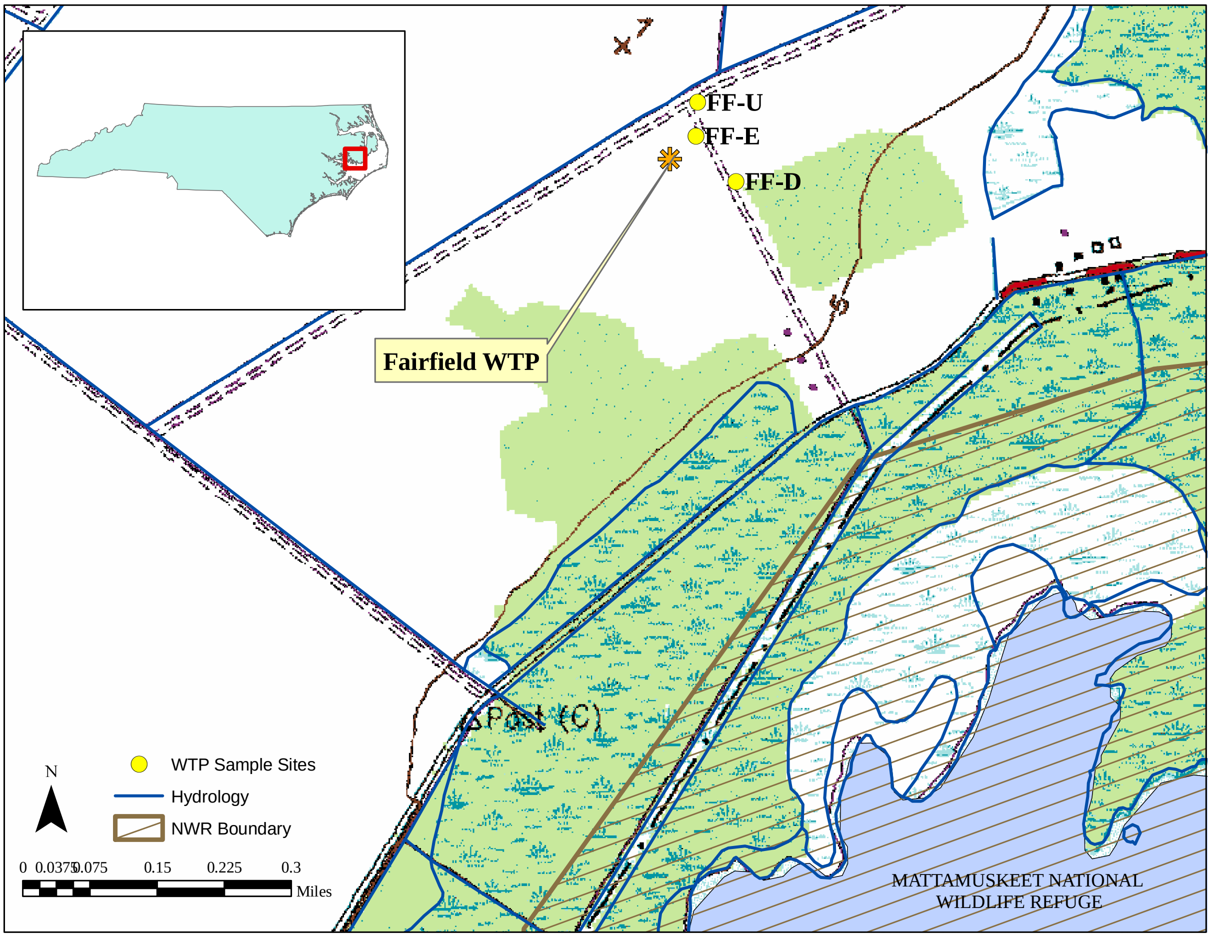

Figure 2. Sample collection sites for the Fairfield Water Treatment Plant included upstream (FF-U), effluent (FF-E) at outfall, and downstream (FF-D) locations.

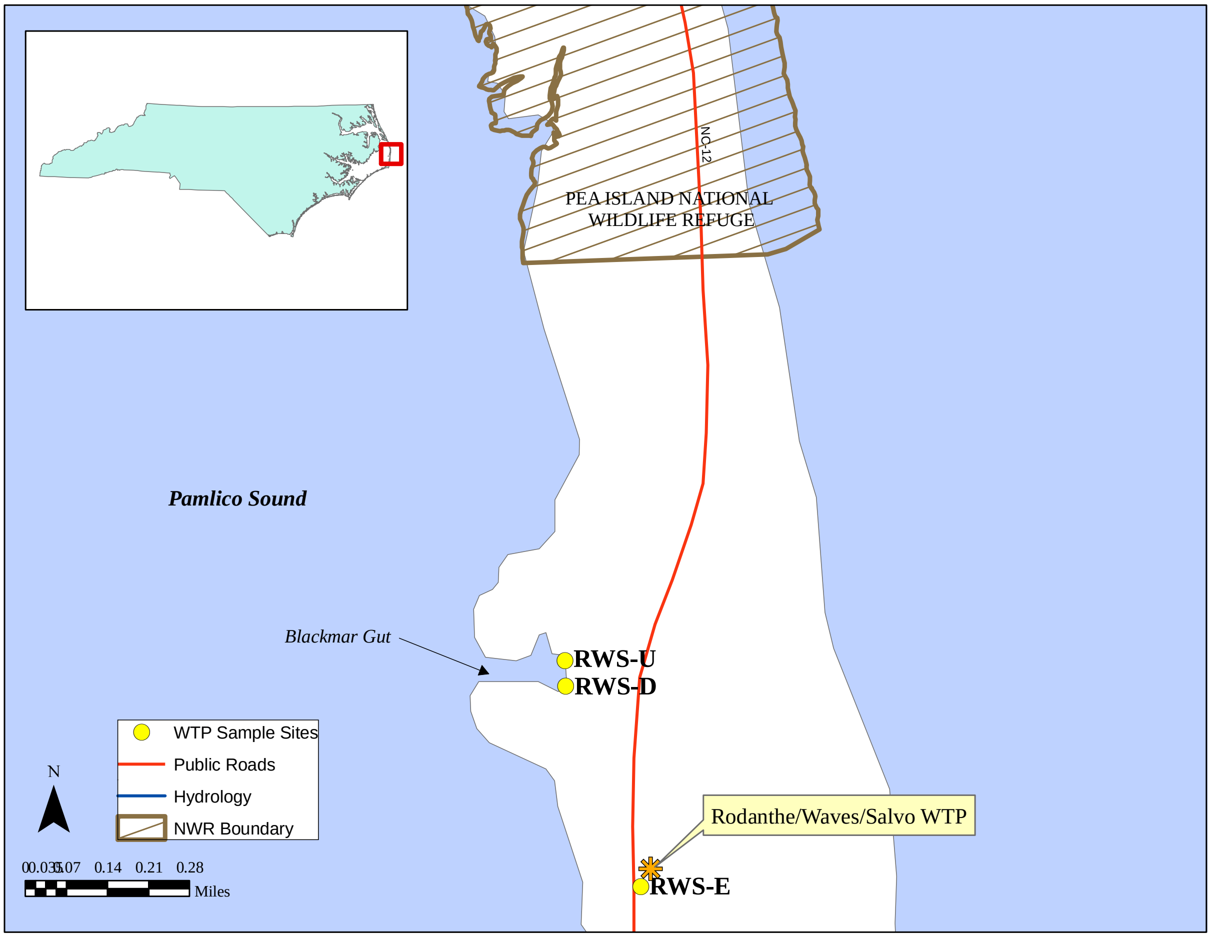

Figure 3. Sample collection sites for the Rodanthe/Waves/Salvo Water Treatment Plant included upstream (RWS-U), effluent (RWS-E) in plant, and downstream (RWS-D) locations.

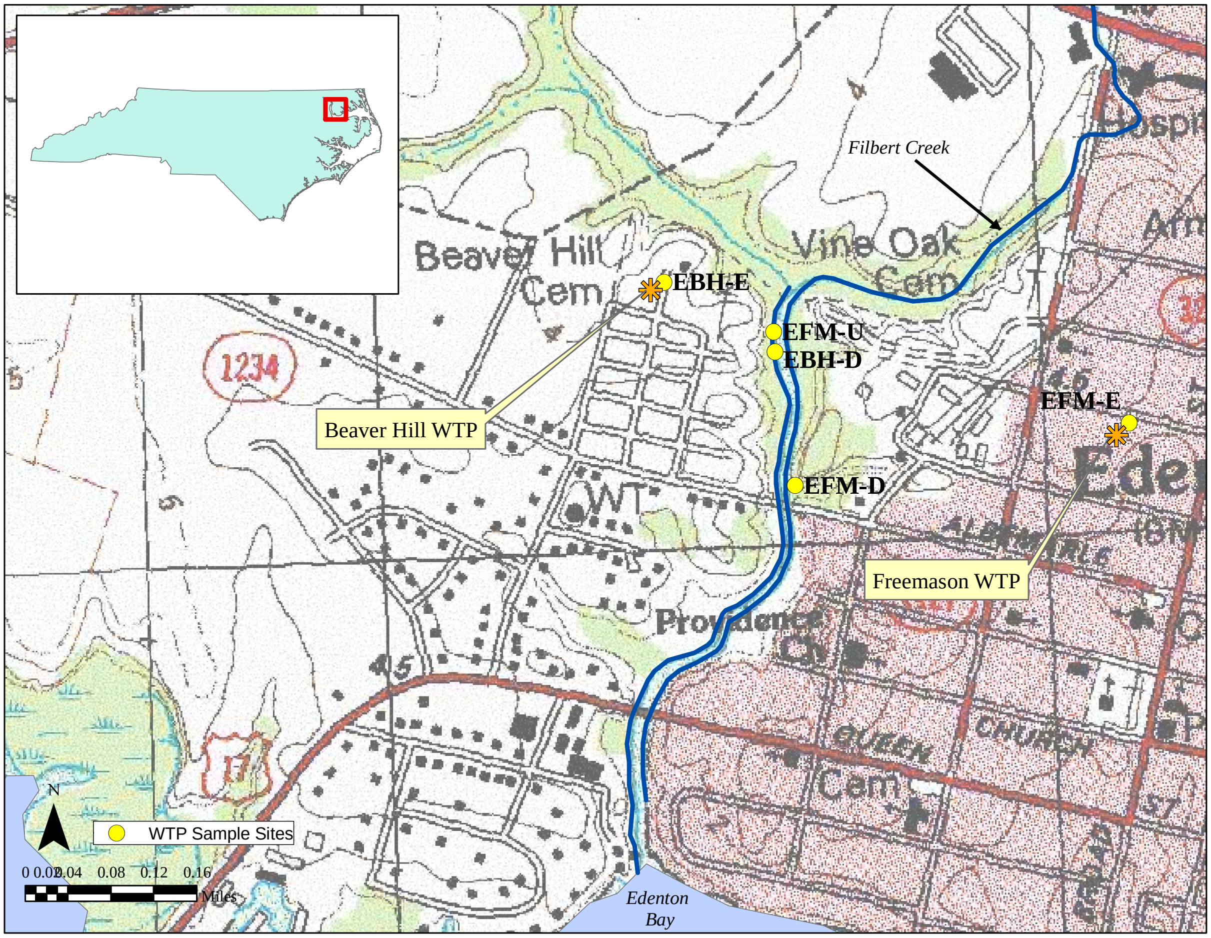

Figure 4. Sample collection sites for Freemason and Beaver Hill Water Treatment Plants included upstream (EFM-U), effluent (EFM-E and EBH-E) in plants, and downstream (EFM-D and EBH-D) locations.

Sample Collection

Samples were collected from each facility on three occasions in order to capture variability in effluent characteristics. Because effluent quality can vary during each phase of the ion exchange water treatment process, sample collection was coordinated with facility operators to assure that the sodium regeneration, iron backwash, and rinse cycles were sampled. Substantial variability in effluent quality resulting from treatment process is not anticipated with reverse osmosis facilities, so no effort was made to target the timing of collection at the two reverse osmosis WTPs.

Water quality characteristics measured included dissolved oxygen, temperature, pH, conductivity, salinity, and total residual chlorine. Dissolved oxygen and temperature were measured using either a YSI Model 51B oxygen meter or a Hydrolab Surveyor 4 with Minisonde 5 multiprobe sensor. Sample pH, conductivity, turbidity and salinity were measured with a Hydrolab Surveyor 4 with Minisonde 5 multiprobe sensor. Total residual chlorine was determined using the HACH DR/2010 spectrophotometer according to American Public Health

Association (APHA) Method 4500-Cl G (N,N-Diethyl-p-Phenylenediamine Colorimetric Method) and HACH Method 10014 (total chlorine, ultra low range for treated wastewater) (APHA et al. 1998). Total residual chlorine was measured immediately after sample collection in the field. Continuous power was provided via a vehicle battery using a cigarette-lighter and power inverter. Blanks and a series of chlorine standards were analyzed in the field for quality assurance / quality control documentation of performance.

Water samples were collected in 500-mL plastic pre-cleaned containers from upstream (U), effluent (E), and downstream (D) sample sites for determination of major ion, metal, and nutrient concentrations. Samples were stored immediately on ice and were maintained at or below 6 ºC; samples were delivered to the NCDWQ's Analytical Chemistry Laboratory in Raleigh, NC for analysis within 24 hours of collection. Protocols for sample collection, preservation, and holding times followed APHA guidance (APHA et al. 1998) and NCDWQ standard operating procedures. Ionic water quality characteristics determined for each sample included chloride, sulfate, carbonate, bicarbonate, potassium, calcium, magnesium, sodium, alkalinity and hardness. Analytical methods for determination of K ,Ca, Mg, Na, Zn, Al, Fe and Mn followed protocols presented in U.S. Environmental Protection Agency's (USEPA) method 200.7 using inductively coupled plasma-atomic emission spectrometry (USEPA 1994). Analytical methods for determination of Cd, Cr, Cu, Ni, Pb, and As followed protocols presented in USEPA method 200.8 using inductively coupled plasma-mass spectrometry (USEPA 1994). Carbonate and bicarbonate concentrations were calculated based on the analysis performed by APHA method 2320 for determination of alkalinity (APHA et al. 1998). Anion concentrations were determined according to USEPA method 300.1 protocols for ion chromatography (USEPA 1997). Analytical methods for determination of nutrient concentrations included USEPA method 350.1 (total ammonia), 351.2 (total kjeldahl nitrogen), 353.2 (nitrate and nitrite), and 365.1 (total phosphorus) (USEPA 1979).

Whole effluent samples were also collected for toxicity testing. Toxicity testing was performed by the NCDWQ's Aquatic Toxicology Unit (ATU, Tyrrell Co. and Fairfield WTPs) and EA Engineering, Science, and Technology, Inc. (EA, Rodanthe/Waves/Salvo, Freemason, and Beaver Hill WTPs); sample collection followed standard operating procedures for the respective laboratories. Effluent samples for toxicity tests performed by NCDWQ were collected in one - gallon polyethylene cubitainers twice (one day prior to test initiation and again on Day 2 of the 7-day tests) for renewal purposes and stored immediately on ice. Grab effluent samples for toxicity testing performed by EA were collected in either one five-gallon or four, one-gallon plastic containers, stored immediately on ice, and shipped via overnight delivery along with chain of custody records to EA's ecotoxicology laboratory in Sparks, MD.

Identification of Ion Imbalance as a Source of Toxicity

A weight-of-evidence approach was used to determine if salinity and / or major ion imbalance contributed to wastewater toxicity. WET tests were conducted by ATU and EA on facility effluents (FE) using 7-day static renewal exposures with *Ceriodaphnia dubia* (water flea) and *Americamysis bahia* (opossum shrimp), organisms for freshwater and estuarine receiving stream environments, respectively, according to USEPA methods (USEPA 2002a-c) and laboratory standard operating protocols (NCDWQ 1998). These test species were selected both because of their use in regulatory toxicity testing requirements for freshwater and saltwater environments and due to their demonstrated sensitivity to ion imbalance (Ingersoll et al. 1992, Dwyer et al. 1992, Mount et al. 1997). Freshwater toxicity test endpoints included survival and reproduction. Survival and growth endpoints were evaluated in estuarine toxicity tests. When whole effluent toxicity was indicated (e.g., No Observed Effect Concentration [NOEC], or the highest toxicant concentration tested for which no statistically significant adverse affect on test organisms relative to control organisms was evident, less than 100%), additional toxicity tests were performed using synthetic effluent (SE) mixtures (developed based on major ion analytical chemistry results). These synthetic or "mock" effluents were used to mimic the ionic composition of FEs for determining whether ion imbalance was a source of wastewater toxicity following previously described methods (Goodfellow et al. 2000, FDEP 1995, McCulloch et al. 1993). Results of toxicity tests using a series of FE and SE mixtures of varied proportions (e.g., 100% FE, 67% FE / 33% SE, 33% FE / 67 % SE, etc.) were compared to results from WET tests conducted to determine if toxicity can be attributed to the presence of major ions or another toxicant present in the wastewater.

Whole Effluent Toxicity Tests

WET tests were conducted by the NCDWQ's ATU and EA on WTP effluents collected prior to discharge to freshwater receiving stream environments using 7-day static renewal exposures with water flea (*Ceriodaphnia dubia*) test organisms following USEPA method 1002.0 (USEPA 2002b, NCDWQ 1998). *C. dubia* used for toxicity tests were obtained from stock cultures maintained at the respective testing facilities. Cultures were maintained at 25±1°C and a 16-hour light, 8-hour dark photoperiod cycle. Test organisms were maintained individually in brood boards and fed algae and a trout chow/yeast/cerophyll suspension daily (USEPA 2002b). Neonates of known age (i.e., less than 24 hours old) were obtained for testing from the individually cultured females in the brood board system. On the day before or the day of the test, neonates were segregated from the parent organisms. All organisms used for testing were taken from the 3rd or later brood, released within one eight-hour period and were taken from broods of eight or more. In EA tests, a moderately hard synthetic fresh water prepared from reagent grade chemicals (US EPA 2002) was used for culturing and testing. Standard soft surface water freshwater culture and dilution water used by the ATU was obtained from Beaver Dam Lake, Falls Lake Reservoir, NC.

Test treatment dilutions for the initial baseline *C. dubia* 7-day chronic reproduction and survival tests were selected based on a standard dilution ratio used by ATU and judged suitable on the basis of initial sample conductivity values. The subsequent baseline *C. dubia* chronic toxicity test treatments for the same facilities on different sample dates were modified slightly and were based on data from the initial tests, as well as sample conductivity measurements. Test treatments were based on a ≤0.5 dilution ratio, using five sample dilutions plus a control. The concentration series for all EA tests consisted of five dilutions (e.g., 6.25, 12.5, 25, 50, and 100 percent effluent plus a control). Test solutions were renewed either twice during the test (ATU, on day 2 and 5) or were renewed daily (EA). All test organisms were fed daily. Vessels were observed daily for the number of surviving organisms, the number of neonates produced, and to monitor the test conditions.

The baseline chronic WET test using a 5/4/04 effluent sample from the Tyrrell Co. WTP resulted in acute toxicity (mortality) to all organisms exposed to effluent treatments in <2.0 hours. As a result, a follow-up *C. dubia* acute 48-hour range-finding toxicity test was performed. All other toxicity tests performed by ATU for this study were chronic 7-day tests. Test organisms for acute tests were <24 hours old, from the third or later brood, and from broods of ≥8 neonates. The acute definitive *C. dubia* test consisted of a control and five treatments, each with four replicates of a minimum of five test organisms. Each test cup received 20.0 mL of test solution. The test was initiated when pooled organisms had been exposed to fresh food (trout chow/yeast/cerophyll suspension) for ≥2 hour, and temperatures were 25.0±1.0°C for all treatments. Mortality was recorded at 24 hours and at test termination (48 hour). *C. dubia* tests were not fed during the 48-hour acute test period.

Mysid (*Americamysis bahia*) test organisms were used in 7-day static renewal toxicity tests performed by EA Laboratory to evaluate effluents collected from the Rodanthe/Waves/Salvo WTP. *A. bahia* were obtained from Aquatic Biosystems (Fort Collins, CO) and were 7-days old at test initiation. Prior to testing, the test organisms were acclimated to 26°C and 20 parts per thousand (ppt) salinity. Cultures were maintained at 25±2°C and a 16 hour light, 8 hour dark photoperiod cycle in static recirculating artificial seawater systems and fed brine shrimp (*Artemia* sp.) nauplii daily (USEPA 2002c). The dilution water used for testing was 20 ppt artificial sea water formulated with Crystal Sea Bioassay Grade sea salts. The test concentration series consisted of 6.25, 12.5, 25, 50, and 100 percent effluent treatments plus a control. Nine-oz. (4-inch diameter) glass culture bowls were used with a final test volume of 150 mL. Tests were conducted using a minimum of eight replicates per concentration, with five organisms per container. The test solutions were renewed daily and were observed for the number of surviving organisms. At test termination, each organism was viewed under a microscope to determine its sex and, in the case of females, the number of individuals with eggs in the oviducts or brood pouch. Growth of the surviving organisms was expressed as average dry weight. Mean dry weights were calculated based on the number of surviving organisms (to evaluate the test acceptability criterion), and based on the original number of exposed organisms (biomass).

Synthetic Effluent Mixture Toxicity Tests

When whole effluent toxicity was indicated in the baseline tests described above, follow-up chronic toxicity tests (following the same protocols) were performed using SE and FE mixtures of varying proportions. SEs were formulated using reagent grade salts based on the concentration of ions measured in the effluent by the NCDWQ. Synthetic formulations were prepared to simulate, as closely as possible, reported ionic composition, conductivity and pH of the FEs. pH was adjusted to ±0.1 SU of the FE pH as needed with dilute HCl or NaOH in synthetic effluents prepared by the ATU. SEs were stirred overnight prior to pH adjustment or use for toxicity tests. Determination of appropriate formulation make-up was generated using an Excel spreadsheet prepared by ATU providing total cation and anion concentrations for specified reagent mixtures. Recipes for SE prepared by EA were based on calculations available in Gas Research Institute (GRI) software (GRI 1999).

In FE/SE mixture toxicity tests performed by the ATU, the FE and SE mixture proportions and the treatment dilution series for each mixture was selected by evaluating baseline toxicity test data. Three sample treatment mixtures plus a SE control were used for these tests. Sample mixtures were intended to bracket the chronic value (ChV) determined in the baseline WET test to provide a range of sub-lethal effects. Portions of FE and SE were then combined such that a minimum of four FE and SE mixtures had ratios ranging from 100% FE / 0% SE to 0% FE / 100% SE were formulated for testing. Test treatments of FE and SE mixtures prepared by EA were diluted with synthetic moderately hard water. ATU diluted test treatments with soft natural surface water (30-50 mg/L hardness as $CaCO_3$). The dilution series for each mixture of FE and SE was selected to bracket the ChV determined in the baseline WET test.

Proportions of FE and SE tested by EA were determined based on the 25 percent inhibition concentration (IC25) values. The IC25 value is the concentration of a toxicant that causes a 25 percent reduction in an endpoint (e.g., biomass, reproduction) in the test population relative to a control population response. Accordingly, lower IC25 values are indicative of a toxicant with greater toxicity because less of the pollutant is required to inhibit a given endpoint. The test concentration series was determined based on IC25 values, the IC25 confidence limits, mean reproduction (or biomass), and percent reduction in mean reproduction (or biomass) in the baseline WET test in order to bracket concentrations associated with chronic toxicity.

Confirmatory baseline toxicity tests were completed concurrent with SE tests when time and staff resources allowed in order to address the time lag (due to the necessary analysis time for ion characterization) that occurred between initial WET tests and subsequent synthetic effluent tests. No confirmatory baseline tests were performed on Tyrrell County and Fairfield WTPs (all sample dates) and Rodanthe/Waves/Salvo WTP (8/24/04 and 9/27/04 effluent samples). The typical time lag between completion of the baseline WET tests and the subsequent tests with FE and SE mixtures was approximately 2 weeks.

Bioassessment of Benthic Macroinvertebrate Community Structure

Freshwater receiving streams were assessed according to NCDWQ Bioassessment Unit standard operation and quality control procedures for benthic macroinvertebrates (NCDENR 2001). The Qual 4 stream method was used to collect three benthic samples from Filbert Creek, the receiving stream for Freemason and Beaver Hill WTP discharges. The Qual 4 sampling method, an abbreviated version of the standard qualitative method, was appropriate because it is typically used for small streams with a drainage area less than 3 square miles. The sample consisted of one riffle kick, one sweep, one leaf pack, and visuals. All samples were sorted in the field per standard operation protocols. The primary output for this sampling method was a taxa list with an indication of relative abundance (Rare, Common, Abundant) for each taxon. For coastal streams with visible flow throughout the year, Ephemeroptera + Plecoptera + Trichoptera (EPT) criteria were used to assign bioclassifications; however, stream conditions prevented rating of the three sites. These facilities were not quantitatively assessed due to the lack of appreciable flow precluding application of bioassessment metrics (total taxa richness, EPT taxa richness, biotic index values, EPT biotic index values and EPT abundance) as planned. Instead, the community compositions at the sites were compared to evaluate potential impacts from the discharges. Field measurements were taken at the time of sampling for temperature, dissolved oxygen, conductivity, and pH using a YSI 85 meter and an Accumet pH meter. Field observations including descriptions of the immediate watershed, substrate, stream width, water characteristics, and benthic community were recorded at the time of collection. Macroinvertebrate sampling at the Rodanthe/Waves/Salvo WTP was conducted in June 2004 according to estuarine sampling protocols (Eaton 2001); however, based on anoxic conditions and a limited benthic community, further enumeration and quantitative analysis of community structure was not performed.

Statistical analyses

The results of the chronic toxicity tests were analyzed using the ToxCalc statistical software package (Version 5.0, Tidepool Scientific Software). Statistical analyses were performed according to US EPA guidance (2002b) on the survival and reproduction (*C. dubia* tests) or survival and biomass (*A. bahia* tests) results to determine if any of the effluent concentrations were significantly ($p \leq 0.05$) different from the control. The short term chronic toxicity test endpoints are expressed as the NOEC, the Lowest Observed Effect Concentration (LOEC, the lowest toxicant concentration tested for which a statistically significant adverse effect on test organisms relative to control organisms was evident), and the ChV (the geometric mean of the LOEC and NOEC). Higher NOEC and LOEC values imply lower toxicity because more of the toxicant is required to result in an adverse effect. The 25 percent inhibition concentration (IC25) values were also calculated. A minimum 20.0% negative effect was also applied to statistical protocols by ATU to define reported endpoints.

Fisher's Exact Test was used to determine statistical significance of the chronic survival data. Analysis of variance (ANOVA) and either Dunnett's Mean Comparison test or Bonferroni's T-test or were used to analyze the data for significance of effects. Depending on the distributional characteristics of the data generated, Steele's Many-One Rank Test or the Wilcoxon Rank Sum Test was used (USEPA 2002b,c). The Shapiro-Wilks test (for datasets with ≤50 datapoints) or the Chi-Square test or Kolmogorov D Test were used to test for normality of the reproduction data. Bartlett's test was used to test for homogeneity of variance of the reproduction data. The IC25s were determined using EPA's ICp program (EA) or ToxCalc (ATU). Chronic lethality (e.g., median lethal concentration [LC50]) was calculated using the following methods (in order of precedence): probit, Spearman-Karber, Trimmed Spearman-Karber, or graphical methods as described by USEPA (2002b,c). The LC50 is the concentration of a toxicant that is lethal to 50 percent of the test organisms relative to the control population; higher LC50 values are associated with lower toxicity because a greater dose of toxicant is required to result in death of test organisms. Depending on the nature of the data, a combination of these statistical methods was used. Specific methods and reporting output for ToxCalc analyses are presented in EA's final reports (Appendix C).

Results and Discussion

Water Quality Conditions in Water Treatment Plant Effluents and Receiving Waters

Tyrrell County WTP

Field-based quality characteristics of effluent and receiving stream samples collected from the Tyrrell County WTP are presented in Table 2. Dissolved oxygen concentrations in Riders Creek were very low (0.40-3.08 mg/L range) during the summer months reflecting the low-flow swamp characteristics of this stream. Conductivity and salinity measurements for effluents collected during the sodium regeneration cycle (5/4/2004) were approximately 400- and 500-fold, respectively, those recorded instream on the same date; however, samples collected from the downstream sampling site did not appear to be influenced by elevated conductivity and salinity in the effluent. Total residual chlorine measured in FEs exceeded the state chlorine standard (of 17 µg/L) by about eight- to 36-fold. During each cycle of the ion exchange process, chlorinated "finish" water is typically used; consequently, chlorine concentrations were elevated during all phases sampled at the Tyrrell County facility. Instream residual chlorine concentrations were typically below the method detection limit (5.7 µg/L). The discharge point for the Tyrrell County WTP is located at the roadside ditch adjacent to the WTP facility. Drainage from the ditch flows diffusely into a forested wetland lining Riders Creek. Although the length of the drainage pathway between the outfall and Riders Creek is indeterminable (due to effluent dissipation in the wetland), approximately 500 meters of wetland separate the outfall ditch and the receiving stream. Limited roadway access, dense wetland vegetation, and the diffuse flow of the effluent through the wetland hampered collection of a sample immediately downstream of the facility. The downstream collection site (TC-D) was located approximately 1200 m below where the wetland adjacent to the facility joins Riders Creek. It is likely that any impacts associated with the hypersaline and highly-chlorinated releases are attenuated by the lengthy overland drainage pathway effluent travels before reaching Riders Creek and downstream environments at TC-D or the Pocosin Lakes NWR boundary (another 150 meters downstream of TC-D). Tree stress and mortality is evident in the wetland that receives wastes from the facility; however, no salt accumulation was present during site reconnaissance and a link between facility releases and vegetation mortality is not certain.

Ionic water quality characteristics reported by the NCDWQ (Table 3) indicate that effluent salts, alkalinity and hardness were consistently elevated relative to receiving stream conditions; however, no discernible downstream impact was evident. Ion effluent concentrations associated with the sodium regeneration cycle of the ion exchange process were typically higher than those measured in the iron backwash and rinse cycles. The federal ambient water quality criterion (AWQC) for alkalinity (criterion continuous concentration [CCC] of 20 mg/L) was exceeded by eight- (sodium regeneration cycle) to 21-fold (iron backwash and rinse cycles) while the chloride CCC (of 230 mg/L) was exceeded by 1.3- (iron backwash and rinse cycles) to 130-fold (sodium regeneration cycle) (USEPA 1986, 1988).

The concentrations of nutrient and elemental contaminants in effluents from the facility and receiving stream waters were also screened against state standards and AWQC (Table 4). The effluent ammonia concentration reported during the sodium regeneration cycle exceeded the USEPA CCC of 5.41 mg/L ammonia nitrogen (adjusted for effluent temperature and pH conditions) by five-fold (USEPA 1999). The effluent arsenic concentration on the same date was nearly three-fold above the state standard for this parameter (of 50 µg/L). State and federal water quality standards were rarely exceeded in effluent samples collected during the iron backwash and rinse cycles of the ion exchange process with the exception of iron (the effluent concentration for this parameter was equivalent to the state action level of 1000 µg/L in a sample collected during the iron backwash cycle). Instream iron concentrations, however, were higher than effluent concentrations reported on all sample collection dates. Although differences in upstream and downstream nutrient and metal concentrations are evident, there does not appear to be a marked influence of the effluent discharge at the downstream sampling site in Riders Creek. Potential impacts of effluent releases to Riders Creek are likely attenuated by vegetative uptake or the ion exchange capacity of surficial sediments along the overland drainage pathway downgradient of the outfall. The Tyrrell County WTP has been discharging to the wetland adjacent to Riders Creek for about 28 years; however, these releases were unpermitted prior to 2002. Therefore, although the discharge is small, the duration of the releases raises concern that the capacity for soil/sediment or vegetation uptake to ameliorate brine effluent impacts may be exhausted over time.

Table 2. Water quality characteristics of Tyrrell County Water Treatment Plant water samples determined at time of collection from upstream (TC-U), effluent (TC-E) in plant, and downstream (TC-D) locations. Effluent samples were obtained during the sodium regeneration cycle (5/4/2004), iron backwash (6/8/2004), and final rinse (7/6/2004) stages of the water treatment process.

Date	Sample ID	Dissolved Oxygen mg/L	Water Temp. °C	pH s.u.	Conductivity mS/cm	Salinity ppt	Cl, total residual µg/L[a]
5/4/2004	TC-U	2.84	17.70	6.94	0.136	0.06	BDL
	TC-E	5.07	16.70	6.84	60.2	39.68	**290**
	TC-D	3.08	17.76	5.88	0.160	0.08	BDL
6/8/2004	TC-U	0.80	24.02	6.66	0.290	0.10	BDL
	TC-E	6.64	17.45	7.99	1.52	0.80	**619**
	TC-D	1.55	26.67	5.60	0.197	0.10	BDL
7/6/2004	TC-U	0.40	27.10	6.69	0.164	0.08	BDL
	TC-E	9.10	17.89	8.27	1.66	0.83	**132**
	TC-D	0.49	27.88	5.16	0.130	0.07	**127***

[a] BDL = below method detection limit of 5.6 µg/L; concentrations exceeding the state standard of 17 µg/L highlighted in bold.

* Highly tannic sample - likely color interference with spectrophotometric method

Table 3. Ionic composition of Tyrrell County Water Treatment Plant water samples collected from upstream (TC-U), effluent (TC-E) in plant, and downstream (TC-D) locations. Effluent samples were obtained during the sodium regeneration cycle (5/4/2004), iron backwash (6/8/2004), and final rinse (7/6/2004) stages of the water treatment process.

Date	Sample ID	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$	Chloride mg/L	Sulfate mg/L	HCO_3^- mg/L	CO_3^{2-} mg/L	K mg/L	Ca mg/L	Mg mg/L	Na mg/L
5/4/2004	TC-U	8	26	48	5 U	8	1 U	8.3	5	3	10
	TC-E	150	25,603	31,000	5 U	150	1 U	1500	3,000	4,400	5,500
	TC-D	5	23	55	5 U	5	1 U	3.3	4	3	53
6/8/2004	TC-U	17	50	52	25	17	1 U	6.1	10	7	24
	TC-E	410	338	300	8	410	1 U	80	48	53	170
	TC-D	4	32	5 U	8	4	1 U	3.8	5	4	21
7/6/2004	TC-U	14	33	24	5 U	14	1 U	4.7	7	4	15
	TC-E	410	32	300	8	40	1 U	32	5	5	620
	TC-D	1 U	20	29	5 U	1U	1 U	2.8	3	3	14

U = the analyte was analyzed for but not detected above the reported practical quantitation limit

Table 4. Nutrient and elemental contaminant concentrations of Tyrrell County Water Treatment Plant water samples collected from upstream (TC-U), effluent (TC-E) in plant, and downstream (TC-D) locations. Effluent samples were obtained during the sodium regeneration cycle (5/4/2004), iron backwash (6/8/2004), and final rinse (7/6/2004) stages of the water treatment process. Results are compared to North Carolina water quality standards (NCWQS) for freshwater; concentrations exceeding state standards are highlighted in bold font.

Date	Sample ID	Total NH_3 as N mg/L	TKN as N mg/L	NO_2 + NO_3 as N mg/L	P: Total as P mg/L	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Al µg/L	Fe µg/L	Mn µg/L	As µg/L
5/4/2004	TC-U	0.47	1.4	0.09	0.75	2.0U	25 U	2.0 U	10 U	10 U	10 U	920	920	34	10 U
	TC-E	27 J3	26 J3	0.65	0.13	10P	25 U	10 P	59	50 P	**100**	50 U	140	1,300	**130**
	TC-D	0.09	1.1	0.66	0.14	2.0U	25 U	2.0 U	10 U	10 U	10 U	520	820	32	10 U
6/8/2004	TC-U	0.33	1.9	0.02 U	0.06	2.0U	25 U	2.0 U	10 U	10 U	27	630	**2,300**	70	NS
	TC-E	0.56	1.3	1.1	0.31	2.0U	25 U	2.7	10 U	10 U	41	50 U	**1,000**	23	NS
	TC-D	0.17	2.1	0.02 U	0.42	2.0U	25 U	2.0 U	10 U	10 U	15	630	**2,400**	56	NS
7/6/2004	TC-U	0.26	1.9	0.02 U	0.37	2.0U	25 U	2.5	10 U	10 U	10 U	650	**2,900**	70	NS
	TC-E	0.17	0.62	1.5	0.33	2.0U	25 U	4.2	10 U	10 U	32	50 U	390	10 U	NS
	TC-D	0.25	2.4	0.02 U	0.13	2.0U	25 U	2.5	10 U	10 U	14	690	**2,600**	33	NS
NCWQS						2.0	50	7 AL	88	25	50 AL		1000 AL		50

NCWQS = North Carolina water quality standard for freshwater
AL = Action Level
U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
J3 = sample matrix interference
P = elevated PQL due to matrix interference and/or sample dilution
NS = not sampled

Fairfield WTP

Water quality characteristics of effluents and receiving waters were determined in the field on three occasions at the Fairfield WTP (Table 5). The facility uses reverse osmosis membrane treatment to eliminate ions and other constituents concentrated in the groundwater. Effluent discharge is not continuous; rather, it occurs only when membrane surfaces require flushing. Effluents from membrane facilities, unlike those from ion exchange plants, are generally expected to have more uniform water quality characteristics dependent on the uniformity of groundwater quality. Effluent samples were collected directly from the pipe discharging to a small unnamed canal that drains eastward towards a forested wetland bordering Lake Mattamuskeet, approximately 550 meters downstream. The channelized flow in the canal is converted to sheet flow upon entering the wetland, and while the exact distance of the drainage pathway in the wetland is not known, there is a minimum separation of about 500 meters between the canal's confluence with the wetland and Lake Mattamuskeet.

An upstream sample could only be retrieved during the first sampling event in May 2004; on subsequent sampling dates, there was no flow in the canal above the discharge point. Instream dissolved oxygen conditions (range of 0.90 to 1.34 mg/L) and the absence of upstream flow during summer months confirm the low-flow nature of the canal system. Total residual chlorine was not detected in the FE during any of the sampling events. Effluent conductivity (range of 2.87 to 3.07 mS/cm) and salinities (range of 1.40 to 1.59 ppt) were substantially elevated relative to measurements taken upstream of the discharge (22- and 23-fold the upstream conductivity and salinity conditions, respectively). Both the conductivity and salinity conditions at a downstream collection site (approximately 100 meters downgradient of the facility outfall) appear to be influenced by the effluent discharge. During reconnaissance of the facility in March 2004, conductivity and salinity measurements were taken along an upstream to downstream gradient to determine the potential influence of the outfall on water quality conditions in the canal. Upstream conductivity (0.120 mS/cm) and salinity (0.1 ppt) were considerably lower than those recorded for effluent (2.70 mS/cm and 1.4 ppt, respectively). Results for both parameters decreased downgradient of the outfall; at 550 meters below the outfall (the point where the canal discharges to the adjacent wetland), surface (0.540 mS/cm and 0.2 ppt) and bottom (1.33 mS/cm and 0.6 ppt) salinity and conductivity readings remained elevated relative to upstream measurements. Although impacts of the discharge are evident in the receiving canal, the overall effect on water quality conditions in the forested wetland and ultimately Lake Mattamuskeet are not known. However, the lengthy overland drainage pathway through the wetland likely limits the potential for surface water quality impacts to Lake Mattamuskeet. Future impacts to downgradient wetlands and Lake Mattamuskeet cannot be ruled out given the potential that the absorptive capacity of the soil, sediment, and vegetation may be exhausted over time.

The ionic characteristics of effluent and receiving water samples collected from the Fairfield WTP are summarized in Table 6. The CCC for alkalinity was exceeded in all samples including a May 2004 sample collected upstream of the discharge point (FF-U); however, effluent alkalinity measurements exceeded the CCC by up to 90-fold and were up to 62-fold those reported at FF-U. The effluent chloride concentration on one occasion was nearly double the CCC of 230 mg/L; chloride screening values were not exceeded in any other samples. Individual ion concentrations were higher in effluent than instream samples during the May collection and downstream ion concentrations were at least an order of magnitude higher than those reported from FF-U on that date. Insufficient surface water flow above the discharge point during June and July sampling events limited interpretation of instream effluent impacts; however, downstream concentrations of several constituents on those dates (e.g., hardness, calcium, potassium and magnesium) were equivalent to or higher than those reported in effluent grab samples.

Nutrient and metal contaminant concentrations in samples collected from Fairfield WTP are reported in Table 7. Ammonia nitrogen concentrations in FEs and downstream samples exceeded calculated ammonia CCC values (ranging from 0.82 to 2.78 mg/L adjusted for sample pH and temperature, Table 5) by up to nine-fold the screening value. On the only collection date that paired upstream and downstream samples were available, the ammonia concentration (5.4 mg/L) downstream was over three-fold higher than at FF-U. Total kjehldahl nitrogen (TKN) and total phosphorus concentrations exhibited similar trends in upstream/downstream samples collected on the same date. The state action level for zinc was exceeded in one effluent sample while iron concentrations in effluent and receiving waters were above the state action level for this parameter in all samples. Concentrations of other pollutants were either not detected or were below screening values.

Table 5. Water quality characteristics of Fairfield Water Treatment Plant water samples determined at time of collection from upstream (FF-U), effluent (FF-E) at outfall, and downstream (FF-D) locations.

Date	Sample ID	Dissolved Oxygen mg/L	Water Temp. ° C	pH s.u.	Conductivity mS/cm	Salinity ppt	Cl, total residual µg/L
5/4/2004	FF-U	0.90	16.85	7.35	0.142	0.07	BDL
	FF-E	3.07	17.93	7.73	3.07	1.59	BDL
	FF-D	1.34	19.24	7.81	1.97	1.00	BDL
6/8/2004	FF-E	2.45	18.50	7.85	2.87	1.40	BDL
	FF-D	1.34	26.41	8.24	2.58	1.30	NS
7/6/2004	FF-E	2.64	18.52	7.96	2.94	1.52	BDL
	FF-D	1.10	25.63	8.24	2.47	1.26	NS

BDL = below method detection limit
NS = not sampled (based on BDL effluent sample)

Table 6. Ionic composition of Fairfield Water Treatment Plant water samples collected from upstream (FF-U), effluent (FF-E) at outfall, and downstream (FF-D) locations.

Date	Sample ID	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$	Chloride mg/L	Sulfate mg/L	HCO_3^- mg/L	CO_3^{2-} mg/L	K mg/L	Ca mg/L	Mg mg/L	Na mg/L
5/4/2004	FF-U	29	62	210	10 U,P	29	1 U	2.5	16	5	10
	FF-E	1,700	1,267	490	5 U	1,700	1 U	74	260	150	310
	FF-D	1,100	782	98	10 U,P	1,100	1 U	50	160	93	200
6/8/2004	FF-E	1,800	585	52	5 U	1,800	1 U	150	20	130	250
	FF-D	1,500	1,060	5 U	5 U	1,500	1 U	160	210	130	260
7/6/2004	FF-E	1,800	1,271	140	5 U	1,800	1 U	140	130	230	480
	FF-D	1,500	1,937	96	5 U	1,500	1 U	140	380	240	450

U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
P = elevated PQL due to matrix interference and/or sample dilution

Rodanthe/Waves/Salvo WTP

The water quality characteristics of effluents and receiving water samples collected at the Rodanthe/Waves/Salvo WTP are presented in Table 8. Effluent dissolved oxygen concentrations were slightly lower than those of the receiving waters during two of the three sampling events. Receiving water salinities (range of 13.5 to 23.6 ppt) confirm the brackish nature of Blackmar Gut and are three- to five-fold higher than effluent salinity concentrations. Effluent conductivity (range of 8.09 to 8.55 mS/cm) was also substantially lower than instream measurements (range of 22.3 to 37.2 mS/cm). Total residual chlorine was not detected in any effluent samples; consequently, chlorine was not sampled in receiving water samples. Ionic water quality conditions were also reported for FEs and receiving water samples (Table 9). Individual ion concentrations in effluent samples were generally much lower than those found in receiving water samples raising concern regarding the potential for ion imbalance toxicity. The effluent alkalinity (range of 2300 to 2400 mg/L as $CaCO_3$) was up to ten-fold higher than instream alkalinity conditions. Although no state or federal alkalinity screening values exist for saltwater environments, these effluent alkalinity conditions exceeded a reported *A. bahia* 48-hour LC50 of 1090 mg/L (Pillard et al. 2000). Nutrient and metal contaminant concentrations for effluent and receiving water samples from the facility are reported in Table 10. The mean estimated effluent TKN concentration (8.8 mg/L) was over five-fold higher than average instream TKN concentration (1.6 mg/L) while the mean effluent phosphorus concentration (0.26 mg/L) was double that of average instream conditions (0.13 mg/L). State saltwater metal standards were infrequently exceeded.

Although a disparity between water quality characteristics of FE and the receiving stream environment exists at the Rodanthe/Waves/Salvo WTP, the degree to which instream environments are affected is not known. Blackmar Gut has previously been dredged to serve as an emergency ferry turning basin and, based on the relatively narrow opening of the embayment, exchange of surface waters in Blackmar Gut with Pamilco Sound waters may be limited.

Regardless, tidal and wind mixing within Blackmar Gut is likely and could promote dissipation of effluent within this confined area. Upstream and downstream samples provide some indication of the mixing potential within Blackmar Gut; given that the system is not a linearly flowing receiving stream, the upstream sample should not be interpreted as a reference site. Because the southernmost boundary of the Pea Island NWR is located approximately one half mile north of Blackmar Gut and the potential for effluent releases in Blackmar Gut to impact waters in Pamilco Sound is limited, no impacts to refuge resources associated with effluent releases from the Rodanthe/Waves/Salvo WTP are anticipated. Likewise, other sensitive habitats in Pamilco Sound are unlikely to be negatively effected by this discharge.

Table 7. Nutrient and elemental contaminant concentrations of Fairfield Water Treatment Plant water samples collected from upstream (FF-U), effluent (FF-E) at outfall, and downstream (FF-D) locations. Results are compared to North Carolina water quality standards (NCWQS) for freshwater; concentrations exceeding state standards are highlighted in bold font.

Date	Sample ID	Total NH_3 as N mg/L	TKN as N mg/L	NO_2 + NO_3 as N mg/L	P: Total as P mg/L	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Al µg/L	Fe µg/L	Mn µg/L	As µg/L
5/4/2004	FF-U	1.6	4.5	0.02 U	0.32	2.0 U	25 U	3.7	10 U	10 U	24	1,300	**1,800**	56	10 U
	FF-E	8.2 J6	10 J6	0.02 U,J6	1.6 J6,J3	2.0 U	25 U	3	10 U	10 U	24	50 U	**3,600**	750	10 U
	FF-D	5.4	8.6	0.02 U	0.92	2.0 U	25 U	2.6	10 U	10 U	11	580	**2,500**	440	10 U
6/8/2004	FF-E	X2	X2	X2	X2	2.0 U	25 U	2.0 U	10 U	10 U	**60**	50 U	**4,300**	530	
	FF-D	X2	X2	X2	X2	2.0 U	25 U	3.3	10 U	10 U	19	61	**2,400**	500	
7/6/2004	FF-E	8.1,J6	10 J6	0.02 U,J6	1.6 J6	2.0 U	25 U	3.5	10 U	10 U	46	50 U	**3,200**	680	
	FF-D	7.4,J6	9.2 J6	0.02 U,J6	1.5 J6	2.0 U	25 U	3.2	10 U	10 U	11	80	**2,700**	460	
NCWQS						2.0	50	7 AL	88	25	50 AL		1000 AL		50

NCWQS = North Carolina water quality standard for freshwater
AL = Action Level
U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
J6 = data may not be accurate; unpreserved or improperly preserved sample
X2 = sampled, but analysis lost or not performed
J3 = sample matrix interference

Table 8. Water quality characteristics of Rodanthe/Waves/Salvo Water Treatment Plant water samples determined at time of collection from upstream (RWS-U), effluent (RWS-E) in plant, and downstream (RWS-D) locations.

Date	Sample ID	Dissolved Oxygen mg/L	Water Temp. °C	pH s.u.	Conductivity mS/cm	Salinity ppt	Cl, total residual µg/L
7/12/2004	RWS-U	3.94	29.15	7.72	30.3	18.8	NS
	RWS-E	4.04	20.72	7.97	8.09	4.46	BDL
	RWS-D	3.97	29.97	7.75	31.3	19.50	NS
8/24/2004	RWS-U	5.65	24.20	7.53	22.3	13.53	NS
	RWS-E	4.30	20.57	7.92	8.55	4.73	BDL
	RWS-D	4.76	24.72	7.44	23.6	14.33	NS
9/27/2004	RWS-U	5.48	23.42	8.09	36.1	22.79	NS
	RWS-E	3.53	20.46	8.34	8.57	4.75	BDL
	RWS-D	5.68	23.54	8.03	37.2	23.63	NS

BDL = below method detection limit
NS = not sampled (based on BDL effluent sample)

Table 9. Ionic composition of Rodanthe/Waves/Salvo Water Treatment Plant water samples collected from upstream (RWS-U), effluent (RWS-E) in plant, and downstream (RWS-D) locations.

Date	Sample ID	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$	Chloride mg/L	Sulfate mg/L	HCO_3^- mg/L	CO_3^{2-} mg/L	K mg/L	Ca mg/L	Mg mg/L	Na mg/L
7/12/2004	RWS-U	280	3,456	15,000	1,400	280	1 U	270	230	700	5,800
	RWS-E	2,300	433	1,800	110	2,300	1 U	140	40	81	2,200
	RWS-D	230	3,579	16,000	1,600	230	1 U	280	230	730	7,100
8/24/2004	RWS-U	310	2,985	12,000	16	310	1 U	260	190	610	4,800
	RWS-E	2,400	466	2000	14 J2	2,400	1 U	130	40	89	2,000
	RWS-D	260	2,903	12,000	10	260	1 U	260	190	590	4,600
9/27/2004	RWS-U	390	4,684	20,000	1,900	390	1 U	420	310	950	7,500
	RWS-E	2,400	442	2,900	5 U	2,400	1 U	140	40	83	1,900
	RWS-D	300	5,352	20,000	2200	300	1 U	450	330	1,100	8,300

U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
J2 = reported value failed to meet the established quality control criteria for either precision or accuracy

Table 10. Nutrient and elemental contaminant concentrations of Rodanthe/Waves/Salvo Water Treatment Plant water samples collected from upstream (RWS-U), effluent (RWS-E) in plant, and downstream (RWS-D) locations. Results are compared to North Carolina water quality standards (NCWQS) for saltwater; concentrations exceeding state standards are highlighted in bold font.

Date	Sample ID	Total NH_3 as N mg/L	TKN as N mg/L	NO_2 + NO_3 as N mg/L	P: Total as P mg/L	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Al µg/L	Fe µg/L	Mn µg/L	As µg/L
7/12/2004	RWS-U	0.50	1.6	0.08	0.14	10 P	25 U	10 P	10 U	10 U	10 U	68	130	42	NS
	RWS-E	8.2 J6	8.4 J6	0.02 U,J6	0.25 J6	2.0 U	25 U	**12**	10 U	10 U	**230**	50 U	66	10 U	NS
	RWS-D	0.25	1.1	0.07	0.09	10 P	25 U	10 P	10 U	10 U	10 U	66	66	37	NS
8/24/2004	RWS-U	0.80	1.9	0.14	0.13	2.0 U	25 U	2.0 U	10 U	10 U	16	100	190	56	NS
	RWS-E	7.6 J6	9.0 J6	0.02 U,J6	0.26 J6	2.0 U	25 U	2.0 U	10 U	10 U	15	50 U	100	10 U	10 U
	RWS-D	0.81	1.9	0.03	0.15	2.0 U	25 U	**4.3**	10 U	10 U	10 U	97	350	57	15
9/27/2004	RWS-U	1.4	1.5	0.03	0.11	2.0 U	25 U	2.0 U	10 U	10 U	18	72	110	33	NS
	RWS-E	7.8 J6	9.0 J6	0.10 J6	0.26 J6	2.0 U	25 U	2.0 U	10 U	10 U	85	50 U	110	10 U	NS
	RWS-D	0.78	1.4	0.10	0.14	2.0 U	25 U	2.0 U	10 U	10 U	42	66	92	36	NS
NCWQS						5.0	20	3 AL	8.3	25	86 AL				50

NCWQS = North Carolina water quality standard for saltwater
AL = Action Level
U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
J6 = data may not be accurate; unpreserved or improperly preserved sample
P = elevated PQL due to matrix interference and/or sample dilution
NS = not sampled

Freemason WTP

Water quality characteristics for effluent and receiving water samples collected from Freemason WTP, an ion exchange facility in Edenton, NC, are summarized in Table 11. Each sampling date targeted a different component of the treatment process in order to determine potential differences in effluent quality during the sodium regeneration (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) cycles. Effluent salinity (10.54 ppt) and conductivity (17.7 mS/cm) during the sodium regeneration cycle were over 65-fold higher than instream conditions for these parameters. Less disparity between effluent and instream salinity and conductivity conditions was evident during the iron backwash and rinse cycles. Total residual chlorine was not detected in FEs; consequently, no receiving stream sampling was conducted. Ionic water quality characteristics (Table 12) of samples collected from the facility indicate that the sodium regeneration cycle effluent is associated with considerably higher ion concentrations than samples collected during the other two cycles. For example, hardness (as $CaCO_3$), chloride, calcium, magnesium, and sodium concentrations measured during the sodium regeneration cycle were all over an order of magnitude higher than those measured in the other two cycles. Despite elevated effluent concentrations of these parameters during the sodium regeneration cycle, impacts were not evident at a downstream sampling site (EFM-D). Although some variability between upstream and downstream sample results was evident on each sampling date, generally ion concentrations at EFM-D did not differ appreciably from upstream conditions. Effluent alkalinity and chloride concentrations exceeded federal CCCs on all sample dates by up to 20- and 25-fold, respectively. Concentrations of nutrients and metals in FEs and receiving stream samples were generally below state and federal standards and substantial differences in concentrations reported for upstream and downstream samples were not evident. Effluent ammonia concentrations never exceeded calculated CCCs for this pollutant. Effluent zinc, iron (during iron backwash cycle only) and manganese concentrations were elevated relative to instream concentrations of these parameters and exceeded state action levels (for zinc and iron).

Table 11. Water quality characteristics of Freemason Water Treatment Plant water samples determined at time of collection from upstream (EFM-U), effluent (EFM-E) in plant, and downstream (EFM-D) locations. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process.

Date	Sample ID	Dissolved Oxygen mg/L	Water Temp. ° C	pH s.u.	Conductivity mS/cm	Salinity ppt	Cl, total residual µg/L
8/24/2005	EFM-U	3.64	26.60	6.21	0.140	0.06	*, NS
	EFM-E	6.27	19.57	5.98	17.7	10.54	*, BDL
	EFM-D	4.82	27.27	6.20	0.270	0.13	*, NS
9/13/2005	EFM-U	2.11	23.83	6.11	0.972	0.50	NS
	EFM-E	7.28	18.79	5.92	1.726	0.92	BDL
	EFM-D	2.73	23.85	6.16	1.740	1.04	NS
11/1/2005	EFM-U	9.02	16.22	6.25	0.994	0.49	NS
	EFM-E	7.86	18.68	5.31	2.87	1.55	BDL
	EFM-D	7.39	16.50	6.06	1.15	0.60	NS

* Not sampled in field; spectrophotometer lamp failure. Grab samples analyzed within 24 hours of collection.
BDL = below method detection limit
NS = not sampled (based on BDL effluent sample)

Table 12. Ionic composition of Freemason Water Treatment Plant water samples collected from upstream (EFM-U), effluent (EFM-E) in plant, and downstream (EFM-D) locations. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process.

Date	Sample ID	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$	Chloride mg/L	Sulfate mg/L	HCO_3^- mg/L	CO_3^{2-} mg/L	K mg/L	Ca mg/L	Mg mg/L	Na mg/L
8/24/2005	EFM-U	29	52	8	13	29	1 U	5.7	15	4	13
	EFM-E	290	4,726	5,800	60	290	1 U	NS	1,200	420	3,000
	EFM-D	36	65	41	15	36	1 U	6.9	18	5	22
9/13/2005	EFM-U	240	186	140	35	240	1 U	14	53	13	130
	EFM-E	370	297	290	60	370	1 U	21	76	26	260
	EFM-D	620	178	260	29	620	1 U	9.0	48	14	140
11/1/2005	EFM-U	160	125	86	30	160	1 U	8.9	35	9	99
	EFM-E	400	282	620	44	400	1 U	20	75	23	510
	EFM-D	95	175	190	34	95	1 U	12	42	17	110

U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
NS = not sampled

Table 13. Nutrient and elemental contaminant concentrations of Freemason Water Treatment Plant water samples collected from upstream (EFM-U), effluent (EFM-E) in plant, and downstream (EFM-D) locations. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process. Results are compared to North Carolina water quality standards (NCWQS) for freshwater; concentrations exceeding state standards are highlighted in bold font.

Date	Sample ID	Total NH_3 as N mg/L	TKN as N mg/L	NO_2 + NO_3 as N mg/L	P: Total as P mg/L	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Al µg/L	Fe µg/L	Mn µg/L	As µg/L
8/24/2005	EFM-U	0.11	0.70	0.17	0.4	2.0 U	25 U	3.6	10 U	10 U	17	890	**1,600**	52	NS
	EFM-E	0.25	0.41	1.6	0.05	2.0 U	25 U	12	10 U	10 U	**65**	420	**1,200**	260	NS
	EFM-D	0.11	0.70	0.25	0.39	2.0 U	25 U	4	10 U	10 U	24	660	**1,000**	83	NS
9/13/2005	EFM-U	0.12	1.1	0.02 U	0.52	2.0 U	25 U	2.1	10 U	10 U	14	410	**1,300**	82	NS
	EFM-E	0.46	1.1	0.20	0.1	2.0 U	25 U	11	10 U	10 U	**60**	170	**6,600**	900	NS
	EFM-D	0.02	0.71	0.02 U	0.19	2.0 U	25 U	2.0 U	10 U	10 U	**51**	160	800	60	NS
11/1/2005	EFM-U	0.20	0.75	0.33	0.24	2.0 U	25 U	2.0 U	10 U	10 U	31	160	**1,600**	93	NS
	EFM-E	0.02 U	0.20 U,J2	0.38	0.04	2.0 U	25 U	5	10 U	10 U	42	69	160	10 U	NS
	EFM-D	0.27	0.96	0.40	0.26	2.0 U	25 U	2.6	10 U	10 U	**50**	210	**1,500**	73	NS
NCWQS						2.0	50	7 AL	88	25	50 AL		1000 AL		50

NCWQS = North Carolina water quality standard for freshwater
AL = Action Level
U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
J2 = reported value failed to meet the established quality control criteria for either precision or accuracy
NS = not sampled

Beaver Hill WTP

Beaver Hill WTP, the second ion exchange facility operated by the Town of Edenton included in the study, was also sampled during each treatment cycle: sodium regeneration (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005). Water quality characteristics of grab effluent and receiving stream samples from the facility are presented in Table 14. Because both the Freemason and Beaver Hill facilities discharge to the same receiving stream (Filbert Creek), one upstream sample (EFM-U) was used as a reference site for both WTPs. The downstream sample site (EBH-D) was located in Filbert Creek approximately 50 feet below the facility outfall. Unlike the other two ion exchange facilities sampled (Freemason and Tyrrell County), the salinity and conductivity of the sodium regeneration cycle effluent sample collected at Beaver Hill WTP was not substantially elevated relative to instream samples. Total residual chlorine was not detected in FEs; therefore, no receiving stream sampling was conducted. Ionic water quality characteristics for Beaver Hill water samples are summarized in Table 15. Although effluent ion concentrations during the sodium regeneration cycle (8/24/2005) were elevated relative to instream concentrations for several parameters (e.g., sodium, bicarbonate, alkalinity and hardness), the ionic disparity between effluent and receiving stream concentrations was not as substantial as was evident at the two other ion exchange facilities. All instream and effluent samples exceeded the chronic federal AWQC for alkalinity (by up to 20-fold in effluent samples and 12-fold in receiving stream samples). Effluent sodium concentrations were at least double that of instream concentrations for samples collected during the iron backwash (9/13/05) and rinse cycles (11/1/2005). However, there was generally no substantial difference in effluent and instream ion concentrations for other parameters and elevated downstream ion concentrations were not evident. Concentrations of nutrient and metal pollutants in Beaver Hill WTP effluents and receiving water samples are shown in Table 16. Effluent nutrient concentrations on all sample dates were similar to those reported for receiving stream samples. Instream and effluent iron concentrations frequently exceeded the state action level, most notably in effluent collected during the iron backwash (55,000 µg/L iron). State action levels were rarely exceeded for copper and zinc. It appears that elevated concentrations of ions and metals in FEs have little impact on instream water quality conditions at the downstream sampling site based on a comparison to baseline conditions at EFM-U.

Table 14. Water quality characteristics of Beaver Hill Water Treatment Plant water samples determined at time of collection from upstream (EFM-U), effluent (EBH-E) in plant, and downstream (EBH-D) locations. One upstream sample (EFM-U) was used for both the Freemason and Beaver Hill WTP facilities due of the close proximity of their outfalls on Filbert Creek. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process.

Date	Sample ID	Dissolved Oxygen mg/L	Water Temp. º C	pH s.u.	Conductivity mS/cm	Salinity ppt	Cl, total residual µg/L
8/24/2005	EFM-U	3.64	26.60	6.21	0.140	0.06	*, NS
	EBH-E	7.12	19.20	5.97	1.16	0.61	*, BDL
	EBH-D	2.36	25.75	6.21	0.150	NS	*, NS
9/13/2005	EFM-U	2.11	23.83	6.11	0.972	0.50	NS
	EBH-E	5.82	19.54	6.03	1.41	0.75	BDL
	EBH-D	2.13	23.59	6.05	1.01	0.53	NS
11/1/2005	EFM-U	9.02	16.22	6.25	0.994	0.49	NS
	EBH-E	8.34	18.25	6.13	1.43	0.76	BDL
	EBH-D	5.31	15.66	6.14	0.941	0.50	NS

* Not sampled in field; spectrophotometer lamp failure. Grab samples analyzed within 24 hours of collection.
BDL = below method detection limit
NS = not sampled (based on BDL effluent sample)

Table 15. Ionic composition of Beaver Hill Water Treatment Plant water samples collected from upstream (EFM-U), effluent (EFM-E) in plant, and downstream (EFM-D) locations. One upstream sample (EFM-U) was used for both the Freemason and Beaver Hill WTP facilities due to the close proximity of their outfalls on Filbert Creek. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process.

Date	Sample ID	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$	Chloride mg/L	Sulfate mg/L	HCO_3^- mg/L	CO_3^{2-} mg/L	K mg/L	Ca mg/L	Mg mg/L	Na mg/L
8/24/2005	EFM-U	29	52	8	13	29	1 U	5.7	15	4	13
	EBH-E	410	100	29	44	410	1 U	21	24	10	270
	EBH-D	30	45	12	17	30	1 U	5.6	13	3	11
9/13/2005	EFM-U	240	186	140	35	240	1 U	14	53	13	130
	EBH-E	90	138	150	41	90	1 U	17	32	14	260
	EBH-D	220	181	140	33	220	1 U	12	51	13	130
11/1/2005	EFM-U	160	125	86	30	160	1 U	8.9	35	9	99
	EBH-E	400	53	170	42	400	1 U	14	12	6	350
	EBH-D	120	139	94	28	120	1 U	8.9	39	10	68

U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)

Table 16. Nutrient and elemental contaminant concentrations of Beaver Hill Water Treatment Plant (WTP) water samples collected from upstream (EFM-U), effluent (EBH-E) in plant, and downstream (EBH-D) locations. One upstream sample (EFM-U) was used for both the Freemason and Beaver Hill WTP facilities because of the close proximity of their outfalls on Filbert Creek. Effluent samples were obtained during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages of the water treatment process. Results are compared to North Carolina water quality standards (NCWQS) for freshwater; concentrations exceeding state standards are highlighted in bold font.

Date	Sample ID	Total NH_3 as N mg/L	TKN as N mg/L	NO_2 + NO_3 as N mg/L	P: Total as P mg/L	Cd µg/L	Cr µg/L	Cu µg/L	Ni µg/L	Pb µg/L	Zn µg/L	Al µg/L	Fe µg/L	Mn µg/L	As µg/L
8/24/2005	EFM-U	0.11	0.70	0.17	0.4	2.0 U	25 U	3.6	10 U	10 U	17	890	**1,600**	52	NS
	EBH-E	0.18	0.41	0.33	0.03	2.0 U	25 U	2.5	10 U	10 U	33	71	470	10 U	NS
	EBH-D	0.09	0.71	0.20	0.44	2.0 U	25 U	3.7	10 U	10 U	26	990	**1,400**	49	NS
9/13/2005	EFM-U	0.12	1.1	0.02 U	0.52	2.0 U	25 U	2.1	10 U	10 U	14	410	**1,300**	82	NS
	EBH-E	0.30	2.0	0.0 2 U	0.61	2.0 U	25 U	**16**	10 U	10 U	34	430	**55,000**	140	NS
	EBH-D	0.10	0.82	0.02 U	0.45	2.0 U	25 U	2.0 U	10 U	10 U	13	220	**1,100**	56	NS
11/1/2005	EFM-U	0.20	0.75	0.33	0.24	2.0 U	25 U	2.0 U	10 U	10 U	31	160	**1,600**	93	NS
	EBH-E	0.06	0.22	0.38	0.21	2.0 U	25 U	3.1	10 U	10 U	**58**	50 U	190	10 U	NS
	EBH-D	0.21	0.66	0.34	0.22	2.0 U	25 U	2.0 U	10 U	10 U	21	180	**1,700**	95	NS
NCWQS						2.0	50	7 AL	88	25	50 AL		1000 AL		50

NCWQS = North Carolina water quality standard for freshwater
AL = Action Level
U = the analyte was analyzed for but not detected above the reported practical quantitation limit (PQL)
NS = not sampled

Identification of Ion Imbalance as a Source of Toxicity

Results of WET tests conducted on WTP effluents from both freshwater and estuarine receiving stream environments with daphnid (*C. dubia*) and mysid (*A. bahia*) organisms using 7-day static renewal exposures are presented in Table 17. Facility-specific discussions of the baseline effluent toxicity test results and FE and SE testing follows.

Tyrrell Co. WTP

A baseline *C. dubia* WET toxicity test on a May 2004 sample collected during the sodium regeneration cycle at the Tyrrell County WTP was acutely toxic to *C. dubia* resulting in 100 percent mortality in all treatments within two hours of test initiation (LC50 < 12.5 percent effluent). A follow up range-finding test conducted using a dilution series ranging from 0.2 to 25% effluent resulted in a *C. dubia* 48-hour LC50 of 7.07 percent effluent. An effluent sample collected during the iron backwash cycle (June 2004) was considerably less toxic based on reported 7-day ChVs of 61.2 percent effluent for survival and reproduction endpoints. Rinse cycle effluent samples (collected in July 2004) were also chronically toxic to *C. dubia*, resulting in 7-day ChVs of 14.1 percent effluent for survival and reproduction endpoints. Based on IC25 values for reproduction, sublethal effluent toxicity varied according to the cycle of treatment with sodium regeneration samples being most toxic followed by rinse cycle and iron backwash cycle samples (IC25s of < 12.5, 15.9, and 52.0 percent effluent, respectively).

The toxicity of baseline tests necessitated follow-up testing using FE and SE mixtures to determine the potential contribution of dissolved ions to sample toxicity using a weight of evidence approach (Table 18). Determination of the appropriate SE formulation make-up was generated using an Excel spreadsheet prepared by ATU providing total cation and anion concentrations for specified reagent mixtures; recipes for SEs used in each test are presented in Appendix A. The IC25 values could not be determined for sodium regeneration cycle effluent samples because of acute toxicity. Conductivity values of whole and synthetic effluents (60.2 and 58.9 mS/cm, respectively), however, suggest elevated salinity as a probable cause of toxicity. The acute NOECs and LOECs were the same for all treatments, and the time to 100 percent mortality in all effluent/synthetic effluent treatments was less than 48 hours. These similarities in acute toxicity test results suggest toxicity due to ions as the causative agent of toxicity (e.g., greater portions of synthetic effluent did not result in a dilution of toxicity as would be expected if a toxicant other than ions was causative).

FE and SE tests conducted using iron backwash effluent samples (collected in June 2004) resulted in IC25 values for reproduction of greater than 70.0 percent sample for all mixtures (Table 18). The IC25 for reproduction in the baseline WET test was 52.0 percent sample; however, a follow up 100% FE/ 0% SE test was not performed for comparison to the mixed effluent treatments. It is possible that the similarity of test results among FE and SE mixtures is indicative of ion toxicity; however, without completion of a confirmatory WET test concurrent with FE and SE mixture testing, interpretation of follow-up test results is less than definitive. For example, the reduced toxicity evident in the FE and SE mixture tests (IC25s greater than 70 percent sample for each treatment) relative to the baseline WET test results (IC25 of 52.0 percent sample) could be indicative of toxicant degradation between the baseline test and initiation of subsequent FE and SE tests; however, it might also suggest that a toxicant other than ions in the original sample was diluted by SE. Synthetic test mixtures were chronically toxic to *C. dubia* in follow-up tests (although less so than in baseline tests), there was no evidence of dilution of a toxicant with increasing portions of synthetic effluent, and ionic water quality characteristics of grab effluent samples for each collection date exceeded levels known to be harmful to aquatic life; therefore, it is reasonable to conclude that ion imbalance toxicity is at least partially responsible for the chronic toxicity initially measured in Tyrrell County WTP effluents.

Table 18 presents chronic toxicity tests using mixtures of rinse cycle effluents (from a July 2004 sample) and synthetic effluents resulted in similar IC25 values among test mixtures (>75, 62.1, and 66.7 percent sample, respectively for 75% FE/25% SE, 50% FE/50% SE, and 25% FE/75% SE mixtures), providing potential evidence of ion toxicity. However, the IC25 for reproduction reported in the baseline WET test (15.9 percent sample, Table 17) was considerably lower than the mixed effluent IC25 values indicating a potential reduction in toxicity of the mixtures relative to the whole effluent. Confirmatory WET testing was not conducted at the time of the follow up effluent/synthetic effluent mixture tests, limiting interpretation of test results. Unacceptable mortality in the dilution water control (60 percent) also confounds test results; consequently, no conclusions regarding the cause of toxicity in the sample can be made.

Fairfield WTP

Baseline WET test results for samples collected between May and July 2004 at the Fairfield WTP are presented in Table 17. Using reproduction as the most sensitive endpoint, NOEC and LOEC values ranged from 12.5 to 20.0 percent effluent and 25.0 to 40.0 percent effluent, respectively. Comparison of IC25s for reproduction indicate similar toxicity among samples from each collection date with the June 2004 sample being slightly more toxic than other samples tested.

An evaluation of the source of toxicity in baseline WET tests was conducted using mixtures of FE and SE (Table 19). Recipes for SE formulations are presented in Appendix A. Proportions of FE and SE in each test mixture were assigned based on ChVs for reproduction reported for the baseline WET tests. Chronic toxicity tests on FE/SE mixtures for samples collected on three separate occasions were all characterized by similar IC25 values among test mixtures on each date (potentially indicating major ion toxicity). However, a reduction in toxicity in the follow up tests was evident relative to baseline WET test results for the same date in all three samples when comparing IC25 values for reproduction (Tables 17 and 19). As noted in the discussion of the Tyrrell County WTP discussion above, the absence of confirmatory WET testing during follow up tests with synthetic effluents makes determination of ion imbalance as a primary contributor to toxicity less definitive. However, due to the chronic toxicity of follow-up tests to *C. dubia* (although less substantial than in baseline tests), lack of evidence of dilution of a toxicant with increasing portions of synthetic effluent, and the ionic water quality characteristics of grab effluent samples for each collection date, it is reasonable to conclude that ion imbalance toxicity is at least partially responsible for the chronic toxicity initially measured in Fairfield WTP effluents.

Table 17. Baseline chronic toxicity test results for *Ceriodaphnia dubia* (Tyrrell County, Fairfield, Freemason, and Beaver Hill WTPs) and *Americamysis bahia* (Rodanthe/Waves/Salvo WTP) in 7-day effluent[a] exposures. Unless otherwise noted, treatment concentrations included 100, 50, 25, 12.5, and 6.25 percent effluent for survival, reproduction, and biomass endpoints.

Facility		7-day NOEC			7-day LOEC			7-day ChV			7-day IC25	
Date	LC50[a]	Survival	Reprod.	Biomass	Survival	Reprod.	Biomass	Survival	Reprod.	Biomass	Reprod.	Biomass
Tyrrell County WTP [b]												
5/4/2004	<12.5	<12.5	<12.5	-----	12.5	12.5	-----	<12.5	<12.5	-----	<12.5	-----
6/8/2004	62.6	50.0	50.0	-----	75.0	75.0	-----	61.2	61.2	-----	52.0	-----
7/6/2004	13.6	10.0	10.0	-----	20.0	20.0	-----	14.1	14.1	-----	15.9	-----
Fairfield WTP [c]												
5/4/2004	39.5	25.0	12.5	-----	50.0	25.0	-----	35.4	17.7	-----	22.7	-----
6/8/2004	30.3	20.0	10.0	-----	40.0	20.0	-----	28.3	14.1	-----	16.6	-----
7/6/2004	55.8	40.0	20.0	-----	80.0	40.0	-----	56.6	28.2[d]	-----	20.9	-----
Rodanthe/Waves/Salvo WTP												
7/12/2004	NR	50.0	-----	6.25	100	-----	12.5	70.7	-----	8.80	-----	21.7
8/24/2004	NR	25.0	-----	25.0	50.0	-----	50.0	35.4	-----	35.4	-----	27.1
9/27/2004	NR	50.0	-----	25.0	100	-----	50.0	70.7	-----	35.4	-----	42.7
Freemason WTP [e]												
8/24/2005	NR	12.5	6.25	-----	25.0	12.5	-----	NR	8.80	-----	9.90	-----
9/13/2005	NR	100	12.5	-----	>100	25.0	-----	NR	17.7	-----	12.5	-----
11/1/2005	NR	100	50.0	-----	>100	100	-----	NR	70.7	-----	66.5	-----
Beaver Hill WTP [e]												
8/24/2005	NR	100	50.0	-----	>100	100	-----	NR	70.7	-----	63.9	-----
9/13/2005	NR	100	100	-----	>100	>100	-----	NR	>100	-----	>100	-----
11/1/2005	NR	100	50.0	-----	>100	100	-----	NR	70.7	-----	84.0	-----

LC50 = median lethal concentration
NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration
NR = not reported

[a] LC50 values expressed as percent effluent. Values reported for Tyrrell Co. and Fairfield WTPs are 7-day LC50s.

[b] Effluent collected during the sodium regeneration cycle (5/4/2004), iron backwash (6/8/2004), and final rinse (7/6/2004) stages. Effluent dilution series: 100, 75, 50, 25, 12.5 % effluent (5/4/04); 75,50,25,12.5,6.25 % effluent (6/8/04); and 80, 60, 40, 20, 10 % effluent (7/6/04)

[c] Effluent dilution series: 100, 75, 50, 25, 12.5 % effluent (5/4/04); and 80, 40, 20, 10, 5 % effluent (6/8/04 and 7/6/04)

[d] Statistically significant ChV calculated at 14.1% effluent, but less than 20.0% effect at specified LOEC (19.6% negative effect at 20.0% treatment). Reporting data adjusted for NCDWQ 20.0% negative effect criteria.

[e] Effluent collected during the sodium regeneration cycle (8/24/2005), iron backwash (9/13/2005), and final rinse (11/1/2005) stages.

Table 18. Toxicity test results for *Ceriodaphnia dubia* using mixtures of facility effluent (FE) collected from the Tyrrell County Water Treatment Plant on three occasions[a] and synthetic effluent (SE). Endpoints included survival and reproduction in 7-day exposures for all tests except those using effluent collected on 5/4/2004 (48-hour survival results presented only). The dilution series for all test mixtures on each sample date is noted and was chosen based on the toxicity of the FE. All results are expressed as percent effluent. The recipe for synthetic effluents was determined based on the ionic composition of whole effluent samples (Appendix A).

Test Mixture	12.5% FE / 87.5% SE		6.25% FE / 93.75% SE		3.125% FE /96.875% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
5/4/2004 (12.5, 6.25, & 3.125% sample and dilution water control)[a]						
NOEC	3.125	-----	3.125	-----	3.125	-----
LOEC	6.25	-----	6.25	-----	6.25	-----
ChV	-----	-----	-----	-----	-----	-----
IC25	-----	-----	-----	-----	-----	-----

Test Mixture	70% FE / 30% SE		55% FE / 45% SE		35% FE / 65% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
6/8/2004 (70, 55, & 35% sample and dilution water control)						
NOEC	70	70	70	70	70	70
LOEC	>70	>70	>70	>70	>70	>70
ChV	>70	>70	>70	>70	>70	>70
IC25	-----	>70	-----	>70	-----	>70

Test Mixture	70% FE / 30% SE		55% FE / 45% SE		35% FE / 65% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
7/6/2004 (75, 50, &25% sample and dilution water control)[b]						
NOEC	75	75	NR	75	NR	75
LOEC	>75	>75	NR	>75	NR	>75
ChV	>75	>75	NR	>75	NR	>75
IC25	-----	>75	-----	62.1	-----	66.7

[a] Baseline toxicity testing on 5/4/2004 effluent sample incidated acute toxicity (mortality) within 2 hours. Subsequent synthetic effluent tests were conducted as a 48-hour acute toxicity test; 48-hour NOEC and LOEC values are reported.

[b] Not reported. Inverse dose-response pattern was evident in 55% FE/45% SE and 35% FE/65% SE text mixtures.

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration

Table 19. Toxicity test results for *Ceriodaphnia dubia* using mixtures of facility effluent (FE) collected from the Fairfield Water Treatment Plant on three occasions and synthetic effluent (SE). Endpoints included survival and reproduction in 7-day exposures for all tests. The dilution series for all test mixtures on each sample date is noted and was chosen based on the toxicity of the FE. All results are expressed as percent effluent. The recipe for synthetic effluents was determined based on the ionic composition of whole effluent samples (see Appendix A).

Test Mixture	35% FE / 65% SE		17.5% FE / 82.5% SE		8.75% FE /91.25% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
5/4/2004 (35, 17.5, & 8.75% sample and dilution water control)						
NOEC	35	35	35	35	35	35
LOEC	>35	>35	>35	>35	>35	>35
ChV	>35	>35	>35	>35	>35	>35
IC25	-----	>35	-----	>35	-----	>35

Test Mixture	30% FE / 70% SE		20% FE / 80% SE		10% FE / 90% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
6/8/2004 (30, 20, & 10% sample and dilution water control)						
NOEC	30	30	30	30	30	30
LOEC	>30	>30	>30	>30	>30	>30
ChV	>30	>30	>30	>30	>30	>30
IC25	-----	>30	-----	>30	-----	>30

Test Mixture	35% FE / 65% SE		25% FE / 75% SE		10% FE / 90% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
7/6/2004 (35, 25, &10% sample and dilution water control)						
NOEC	35	35	35	35	35	35
LOEC	>35	>35	>35	>35	>35	>35
ChV	>35	>35	>35	>35	>35	>35
IC25	-----	>35	-----	>35	-----	31.3

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration

Rodanthe/Waves/Salvo WTP

Results of baseline chronic toxicity tests for effluent samples collected between July and September 2004 at the Rodanthe/Waves/Salvo WTP are summarized in Table 17. Effluent samples collected in July and August 2004 were acutely and chronically toxic while the September effluent sample was only chronically toxic to *Americamysis bahia*. Using mean biomass as the most sensitive test endpoint, the resulting 7-day NOECs and LOECs ranged from 6.25-25% effluent and 12.5-50% effluent, respectively. Seven-day ChVs (8.8 to 35.4% effluent) and IC25s (21.7 to 42.7% effluent) based on biomass indicate that the July 2004 sample was more toxic to test organisms than samples collected in subsequent months.

With chronic toxicity indicated in baseline tests for each sample date, follow up toxicity tests using FE and SE mixtures were conducted to determine the potential for total dissolved solids in the effluent to result in major ion imbalance toxicity; results of these tests are presented in Table 20. The ion composition for SEs used in each test were determined based on FE analytical results using the GRI Marine Salinity Toxicity Relationship (Mount and Gulley 1992); recipes for SE formulations used in each test are presented in Appendix A. Portions of the Rodanthe/Waves/Salvo FE and SE were combined; the FE/SE mixtures were then tested as separate wastewater samples. The concentration series selected for the samples included two test mixture dilutions (determined based on baseline toxicity test results) and a dilution water control.

Toxicity tests on a July effluent sample mixed with SE were chronically toxic to mysid test organisms. The chronic toxicity of the various mixtures did not vastly change despite varying concentrations of FE and SE. Using mean biomass as the most sensitive test endpoint, IC25 values for each test mixture was greater than 30% sample. Recalculating test endpoints based on the IC10 values for FE, 67% FE/33% SE, 33% FE/67% SE and 100% SE were 14.7%, 11.9%, 17.6 % and 26.3 % sample, respectively. This similar response between test mixtures is indicative of toxicity due to dissolved ions as the predominant toxicant in effluent (e.g., if a toxicant other than dissolved ions contributed to baseline whole effluent toxicity, then increasing portions of synthetic effluent would be expected to dilute the toxicant's effect, thus reducing toxicity). Toxicity tests using FE and SE mixtures on an August sample were acutely and chronically toxic to test organisms. The 7-day IC25 values were all >40 percent sample. In the baseline WET test, the IC25 value for whole effluent was 27.1 percent sample. Because the mixtures were less toxic than the initial toxicity test performed on whole effluent, it appears that toxicity was somewhat degraded with storage. Toxicity tests performed with a September 2004 FE sample and SE mixtures were acutely and chronically toxic to mysids and suggesting that total dissolved solids were the principal toxicant. The IC25 value for FE was 42.7 percent sample while IC25 values for 67% FE/33% SE mixture and 33% FE /67% SE mixture were 38.8 and 35.4 percent sample, respectively. The 95 percent confidence limits for each of these IC25 values all overlapped one another, suggesting that the FE/SE mixture test results were not substantially different from each other.

Based on the results of FE/SE toxicity tests coupled with elevated effluent alkalinity (range of 2300 to 2400 mg/L as $CaCO_3$, Table 9) exceeding the reported *A. bahia* 48-hour LC50 value for alkalinity (of 1090 mg/L, Pillard et al. 2000), it appears that the effluent alkalinity is most likely the major contributor to chronic toxicity in all three Rodanthe/Waves/Salvo WTP effluent samples.

Table 20. Toxicity test results for *Amerciamysis bahia* using mixtures of facility effluent (FE) collected from the Rodanthe/Waves/Salvo Water Treatment Plant on three occasions and synthetic effluent (SE). Endpoints included survival and growth. The dilution series for all test mixtures on each sample date is noted and was chosen based on the toxicity of the FE. All results are expressed as percent effluent. The recipe for synthetic effluents was determined based on the ionic composition of whole effluent samples (see Appendix A).

Test Mixture	100% FE / 0% SE		67% FE / 33% SE		33% FE / 67% SE		0% FE / 100% SE	
Endpoint	Survival	Biomass	Survival	Biomass	Survival	Biomass	Survival	Biomass
7/12/2004 (30% & 10% sample and dilution water control)								
NOEC	30	10	30	10	30	30	30	30
LOEC	30	30	30	30	30	30	30	30
ChV	>30	17.3	>30	17.3	>30	>30	>30	>30
IC25	-----	>30	-----	>30	-----	>30	-----	>30
8/24/2004 (40% & 20% sample and dilution water control)								
NOEC	25	25	40	40	40	40	ND	ND
LOEC	50	50	>40	>40	>40	>40	ND	ND
ChV	35.4	35.4	>40	>40	>40	>40	ND	ND
IC25	-----	27.1	-----	>40	-----	>40	-----	ND
9/27/2004 (70% & 35% sample and dilution water control)								
NOEC	50	25	35	<35	<35	<35	ND	ND
LOEC	100	50	70	35	35	35	ND	ND
ChV	70.7	35.4	49.5	<35	<35	<35	ND	ND
IC25	-----	42.7	-----	38.8	-----	35.4	-----	ND

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration
ND = not determined; whole synthetic effluent test was not run

Freemason WTP

Results of the baseline toxicity testing for effluents collected from Freemason WTP between August and November 2005 are presented in Table 17. Effluent collected from Freemason WTP in August (during the sodium regeneration cycle) was acutely and chronically toxic to *C. dubia* while September and November effluent samples were only chronically toxic to test organisms. The conductivity measured in the August sample was sufficient to implicate dissolved ions as a potential toxicant. Using reproduction as the most sensitive endpoint, 7-day NOECs and LOECs ranged from 6.25 to 50 percent sample and 12.5 to 100 percent sample, respectively. ChVs (8.8 to 70.7 percent effluent) and IC25s (9.9 to 66.5 percent effluent) based on reproduction indicate that effluent from the sodium regeneration cycle was more toxic to *C. dubia* followed by the iron backwash and final rinse cycle samples.

Because whole effluent toxicity was indicated for all effluent samples collected from the Freemason WTP, follow up toxicity tests using mixtures of FE and SE were tested to determine the potential for total dissolved solids to be causative; results are presented in Table 21. Toxicity tests on effluent collected during the sodium regeneration cycle were chronically toxic to *C. dubia*. IC25 values for FE, 75% FE/25% SE, 50% FE/50% SE, 25% FE/75% SE, and 100% SE text mixtures were 6.2, 8.9, 8.7, 8.4, and 2.2 percent sample, respectively. The 95 percent confidence limits for these values overlapped for all FE and SE test mixtures. The 100 percent SE was the only sample that was more toxic than the FE/SE mixtures (possibly due to the absence of dissolved organic material, suspended solids and other natural compounds that buffer the toxicity of the ions alone). Despite the differences in toxicity between the SE and the FE/SE mixtures, the similarity of results among the mixtures provides sufficient evidence to implicate ions as the most likely cause of toxicity in this sample of Freemason effluent.

Results of the follow-up toxicity test using effluent collected during the iron backwash (September 2005) cycle indicated that whole effluent was chronically toxic to *C. dubia*; however, based on the IC25 endpoint, mixtures of FE and SE were not toxic (at 25 percent sample). The IC25 of 100% FE in the follow-up test (11.0 percent sample) was similar to the reported IC25 in the baseline WET test (12.5 percent sample, Table 17); however, for all other test mixtures, IC25 values were greater than 25 percent sample suggesting that increasing portions of SE potentially diluted a toxic compound found in the FE. It is possible that the effluent toxicity could be associated with iron (6600 µg/L) which exceeded the state action level standard by 6.6-fold (Table 13); however, the Beaver Hill WTP effluent iron concentration during the iron backwash cycle exceeded the effluent iron concentration during the same cycle of treatment at the Freemason facility by over eight-fold and was not toxic to test organisms in a baseline WET test.

Follow up toxicity tests using final rinse cycle effluent (collected November 2005) and SE mixtures were not chronically toxic to *C. dubia*. IC25 values for all test mixtures (including 100% FE) were greater than 90 percent sample except the 100 % SE mixture (64.7 percent sample) which was similar to the IC25 value reported for the baseline WET test (66.5 percent sample, Table 17). The similarity of IC25 results among the test mixtures as well as the similarity of the 100 % SE and the baseline WET results suggest that total dissolved ions are the most likely cause of toxicity in the November 2005 final rinse cycle effluent sample for the Freemason WTP.

Table 21. 7-day chronic toxicity test results for *Ceriodaphnia dubia* using mixtures of facility effluent (FE) collected from the Freemason Water Treatment Plant on three occasions[a] and synthetic effluent (SE). Endpoints included survival and reproduction. The dilution series for all test mixtures on each sample date is noted and was chosen based on the toxicity of the FE. All results are expressed as percent effluent. The recipe for synthetic effluents was determined based on the ionic composition of whole effluent samples (see Appendix A).

Test Mixture	100% FE / 0% SE		75% FE / 25% SE		50% FE / 50% SE		25% FE / 75% SE		0% FE / 100% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
8/24/2005 (18, 6, & 3% sample and dilution water control)										
NOEC	6	3	18	6	18	6	18	6	18	<3
LOEC	18	6	>18	18	>18	18	>18	18	>18	3
ChV	10.4	4.2	>18	10.4	>18	10.4	>18	10.4	>18	<3
IC25	-----	6.2	-----	8.9	-----	8.7	-----	8.4	-----	2.2

Test Mixture	100% FE / 0% SE		67% FE / 33% SE		33% FE / 67% SE		0% FE / 100% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
9/13/2005 (25, 12.5, & 6.25% sample and dilution water control)								
NOEC	25	6	25	12.5	25	6	25	25
LOEC	>25	12.5	>25	25	>25	12.5	>25	>25
ChV	>25	8.7	>25	17.7	>25	8.7	>25	>25
IC25	-----	11.0	-----	>25	-----	>25	-----	>25
11/1/2005 (90% & 45% sample and dilution water control)								
NOEC	90	90	90	90	90	90	90	45
LOEC	>90	>90	>90	>90	>90	>90	>90	90
ChV	>90	>90	>90	>90	>90	>90	>90	63.6
IC25	-----	>90	-----	>90	-----	>90	-----	64.7

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration

Beaver Hill WTP

Chronic toxicity test results for effluent samples collected from the Beaver Hill WTP between August and November 2005 are presented in Table 17. August and November effluent samples (collected during the sodium regeneration and final rinse cycles, respectively) were chronically toxic to *C. dubia*; a sample collected during the iron backwash cycle in September was not toxic (Table 17). Using reproduction as the most sensitive test endpoint, the 7-day NOECs ranged from 50 to 100 percent effluent; LOEC values were equal to or greater than 100 percent effluent. Seven-day ChVs (70.7 to greater than 100 percent effluent) and IC25 values (63.9 to greater than 100 percent effluent) based on reproduction indicate that the sodium regeneration cycle sample (August) was more toxic to *C. dubia* than effluent collected during the final rinse cycle (November).

Due the to baseline toxicity of effluents collected during the sodium regeneration (August) and final rinse (November) cycles, follow up tests were conducted using mixtures of FE and SE to determine the potential for ion imbalance toxicity (Table 22). The Beaver Hill WTP effluent sample obtained during the iron backwash cycle was not toxic in the baseline WET test, so no further assessment of potential ion toxicity was conducted.

Follow up tests on the sodium regeneration cycle effluent sample and synthetic mixtures were not toxic to *C. dubia* except in the 25% FE/75% SE and the 100% SE mixtures. Results of the chronic toxicity tests performed with FEs and SEs show that the test results were the same among most of the test mixtures. The 25% FE/75% SE mixture had a 7-day IC25 value of 66.1 percent sample, which was similar to the IC25 value measured in the baseline WET test (63.9 percent effluent). The 100% SE was slightly more toxic than the 100% FE (potentially due to the absence of dissolved organic material, suspended solids, etc); however, when the SE was blended with a relatively small portion of FE (25% FE/75% SE), toxicity was almost the same as in the baseline test. The similarity of results among the FE/SE mixtures provides sufficient evidence that ions are the most likely cause of toxicity in this sample of Beaver Hill effluent.

Final rinse cycle effluent (from a November collection) and SE mixtures were not toxic to *C. dubia*, except in the 100 percent SE sample. Test results for FE and SE were the same (IC25s greater than 100 percent sample, Table 22) among most of the test mixtures. The 100 percent SE was the only sample that was more toxic than the FE/SE mixtures. The SE also does not have dissolved organic material, suspended solids and other natural compounds that can buffer the toxicity of the ions alone. When the SE was mixed with portions of FE, toxicity was similar to FE providing evidence that natural compounds in the whole effluent buffer the toxicity of SE. Because FE and SE mixture IC25 values were similar, it is likely that chronic toxicity initially measured in Beaver Hill WTP effluent was related to ion composition.

Table 22. Toxicity test results for *Ceriodaphnia dubia* using mixtures of facility effluent (FE) collected from the Beaver Hill Water Treatment Plant on three occasions and synthetic effluent (SE). Endpoints included survival and reproduction The dilution series for all test mixtures on each sample date is noted and was chosen based on the toxicity of the FE. All results are expressed as percent effluent. The recipe for synthetic effluents was determined based on the ionic composition of whole effluent samples (see Appendix A). A baseline chronic toxicity test on a 9/13/2005 effluent sample was not toxic, so subsequent testing using synthetic effluent mixtures was not performed.

Test Mixture	100% FE / 0% SE		75% FE / 25% SE		50% FE / 50% SE		25% FE / 75% SE		0% FE / 100% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
8/24/2005 (100% & 50% sample and dilution water control)										
NOEC	100	100	100	100	100	100	100	50	100	<50
LOEC	>100	>100	>100	>100	>100	>100	>100	100	>100	50
ChV	>100	>100	>100	>100	>100	>100	>100	70.7	>100	<50
IC25	-----	>100	-----	>100	-----	>100	-----	66.1	-----	26.6

Test Mixture	100% FE / 0% SE		67% FE / 33% SE		33% FE / 67% SE		0% FE / 100% SE	
Endpoint	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.	Survival	Reprod.
11/1/2005 (100% & 50% sample and dilution water control)								
NOEC	100	100	100	50	100	50	100	<50
LOEC	>100	>100	>100	100	>100	100	>100	50
ChV	>100	>100	>100	70.7	>100	70.7	>100	<50
IC25	-----	>100	-----	>100	-----	97.2	-----	63.9

NOEC = no observed effect concentration
LOEC = lowest observed effect concentration
ChV = chronic value
IC25 = 25 percent inhibition concentration

Bioassessment of Benthic Macroinvertebrate Community Structure

Macroinvertebrate sampling was conducted in March 2004 at Tyrrell County and Fairfield WTPs. Both facilities are characterized by freshwater swamp receiving stream environments. Given that collection protocols require positive flow and no appreciable flow at either facility was evident, the swamp stream macroinvertebrate sampling protocol used by the NCDWQ Bioassessment Unit could not be applied. Therefore, metrics typically used to assess aquatic communities in these areas (total taxa richness, EPT taxa richness, biotic index values, EPT biotic index values, and EPT abundance) were not determined. Staff from the Bioassessment Unit conducted a qualitative evaluation of the presence of benthic organisms at the discharge point for the Tyrrell County WTP and indicated that only pollutant tolerant species were found. Macroinvertebrate sampling at the Rodanthe/Waves/Salvo WTP was conducted in June 2004 according to estuarine sampling protocols; however, water quality conditions at depth in Blackmar Gut, the receiving stream for the facility, were typically anoxic and supported a limited benthic community. Enumeration of benthos samples was not performed.

Benthic macroinvertebrate sampling was conducted by NCDWQ Bioassessment Unit staff at sampling sites upstream and downstream of outfalls for Freemason and Beaver Hill WTPs in Filbert Creek in April 2006 (see Appendix D for complete report). Habitat evaluations were conducted at each site using the Biological Assessment Unit's Habitat Assessment Field Data Sheet for Coastal Plain Streams. This assessment assigns a numerical score from 0-100 for the reach of stream sampled, based on channel modification, instream habitat, bottom substrate, pool variety, bank stability and vegetation, light penetration, and width of the riparian zone. Higher scores are indicative of better overall habitat. The diversity of the invertebrate fauna was evaluated using taxa richness counts; the tolerance of the stream community was evaluated using a biotic index (BI). EPT taxa richness (EPT S) criteria have been developed by DWQ to assign water quality ratings (bioclassifications). Higher EPT taxa richness values usually indicate better water quality. Tolerance values for individual species and the final biotic index values have a range of 0-10, with higher numbers indicating more tolerant species or more polluted conditions.

Above Beaver Hill WTP outfall, Filbert Creek was approximately 10 meters wide with a drainage area of 1.17 square miles. Although this stream was located in Edenton, the visible land use for this reach was approximately 80% forest and 20% cleared land (cemetery). The conductivity was 0.418 mS/cm and pH was 6.3. The substrate was predominantly silt (80%) with a small amount of detritus (20%). Very little in-stream habitat was available for macroinvertebrate colonization. Sticks, undercut banks, and root mats were rare and leaf packs, macrophytes, snags, and logs were absent. A large amount of metal scraps and old appliances were present in the stream and along the banks. The site received an overall habitat score of 52. This site received the highest BI score (8.67) of all three samples collected. The taxa richness was the lowest at 37 and the EPT taxa richness was one.

The section of Filbert Creek between the Beaver Hill and Freemason WTP outfalls was approximately 10 meters wide with a drainage area of 1.2 square miles. The visible land use was approximately 80% forest and 20% cleared land (cemetery). The conductivity was 0.860 mS/cm and pH was 6.6. The substrate was predominantly silt (70%) with a small amount of detritus (30%). The site received an overall habitat score of 54. This site had the highest taxa richness (47) of all three sites. Fourteen taxa were from the family Chironomidae with

Cricotopus/Orthocladius sp 41, *Cladopelma*, *Dicrotendipes fumidus*, *Dicrotendipes modestus*, *Parachironomus*, *Procladius*, and *Einfeldia natchitocheae* being the most common midges collected. This sample received a BI of 8.03 and an EPT taxa richness of one.

Below the Freemason WTP outfall, Filbert Creek was approximately 12 meters wide with a drainage area of 1.3 square miles. The visible land use was approximately 50% forest and 50% residential. The conductivity was 1.320 mS/cm and pH was 6.6. The substrate was 50% silt and 50% detritus. The most downstream site (below the Freemason WTP discharge point) received the lowest BI (7.87). Along the right bank, it was narrow with a few breaks present. In-stream habitat was similar to the site located just upstream between the two WTP outfalls. Sticks, undercut banks, and root mats were common; snags and logs were rare; and macrophytes and leaf packs were absent. The site received an overall habitat score of 52. Dragonflies (Odonata) was the most diverse group collected at this site, with a total of 8 taxa. *Enallagma* and *Sympetrum* were the dominant odonate taxa collected. This sample had an EPT taxa richness of one and a total taxa richness of 39. Other dominant taxa included *Caenis*, *Peltodytes*, *Dero*, *Quistadrilus multisetosus*, *Stylaria lacustris*, *Gammarus*, *Sphaerium*, *Amnicola*, *Laevapex*, *Micromenetus*, and *Physella*.

The NCDWQ determined that it is inconclusive whether or not the effluents from Beaver Hill WTP and Freemason WTP have caused negative impacts on the benthic communities of Filbert Creek. The taxa richness increased and the biotic index decreased moving downstream, suggesting improved water quality. However, the communities at all three sites reflected a slow flowing tidal stream with low dissolved oxygen values. Taxa abundant at all sites (*Caenis*, *Enallagma*, *Stylaria lacustris*, *Sphaerium*, and *Physella*) were all very tolerant of low dissolved oxygen conditions. No intolerant taxa were found at any site. Potential additional impact from the WTPs was potentially masked by the natural conditions of the stream or the possible impacts from urban runoff. Conductivity readings recorded during bioassessment sampling indicated that an ion gradient exists and that ion concentrations at the downstream site are three-fold higher than at the upstream site. Because the Chowan River has tidal influences from the Albemarle Sound and can have low salinities at times, this low saline water could be reaching the downstream site. Although the conductivity at the ambient site near Edenton Bay on April 18, 2006 was 0.800 mS/cm, at times these higher conductivities could be a combination of the influence of the discharge and the downstream estuarine waters.

Summary and Management Recommendations

Each of the three ion exchange facilities sampled use a sodium cation exchange (zeolite) resin for divalent cation removal. Water quality characteristics of effluents collected from the three ion exchange water treatment facilities suggest that wastewater from the sodium regeneration treatment cycle is typically the most problematic in terms of elevated ion concentrations and whole effluent toxicity. As the capacity for ion exchange is reached, backwash using finished water (to physically clean the outside of the media) and regeneration with a sodium chloride solution (to restore the contaminant removal capacity of the resin) is necessary. The final rinse cycle follows regeneration and is typically associated with lower levels of salts and other contaminants than at the onset of media regeneration using brine solution. Because the source water requiring treatment differs at each ion exchange facility, it is not surprising that facility- and cycle-specific differences in effluent water quality characteristics were apparent. Of the three facilities evaluated, only the Tyrrell County WTP exhibited routine effluent total residual chlorine exceedances. Based on effluent salinity, conductivity, and hardness characteristics, the ionic strength of facility wastewaters were generally highest at the Tyrrell County facility followed by the Freemason and Beaver Hill WTPs. Highest ion concentrations were typically associated with the sodium regeneration cycle followed by the rinse and backwash cycles. Results of baseline WET testing followed a similar trend where sodium regeneration samples were most toxic at all three facilities (with rinse and backwash cycles generally associated with decreasing toxicity). The baseline toxicity of Tyrrell County WTP effluent was typically more toxic to *C. dubia* than effluents from Freemason and Beaver Hill WTPs.

Two reverse osmosis membrane water treatment facilities were assessed in the study (Fairfield and Rodanthe/Waves/Salvo WTPs). Both use groundwater sources and discharge reject water continuously while source water is being processed. When comparing water quality characteristics, Fairfield effluents were typically associated with substantially lower ion concentrations than at Rodanthe/Waves/Salvo (where the need for source water desalination is likely higher based on its coastal location). There was little variability in water quality characteristics between sampling events at each facility (particularly at the Rodanthe/Waves/Salvo WTP) suggesting wastewater from reverse osmosis facilities may be relatively uniform in chemical composition. Baseline whole effluent toxicity testing using *C. dubia* (Fairfield WTP) and *A. bahia* (Rodanthe/Waves/Salvo WTP) in freshwater and saltwater exposures, respectively, indicate that effluents from both facilities were chronically toxic.

Follow up toxicity tests using whole effluent and synthetic effluent text mixtures were performed to identify ion composition as a source of toxicity in baseline tests. Only one effluent sample (Beaver Hill, September 2007 sample) out of 15 samples was not acutely or chronically toxic to test organisms during the baseline WET tests. Of the 14 effluent samples where subsequent toxicity identification tests were conducted, results indicated that toxicity in only one sample (a September 2005 Freemason WTP effluent sample) appeared to be related to a constituent other than ions. Conclusion regarding the source of toxicity in Tyrrell County and Fairfield WTP samples are not definitive (because confirmatory WET testing was not performed concurrent with synthetic effluent mixture tests); however, due to the similarity of tests results between follow-up test mixtures and the presence of ions in these effluents at levels exceeding those known to be harmful to aquatic life, there is reasonable potential that ion imbalance toxicity is at least partially responsible for sample toxicity. One follow up test (on a July 2004 Tyrrell County WTP effluent sample) proved inconclusive in determining the source of effluent toxicity based on control mortality.

Assessment of biota in receiving streams is important to determine the potential impact of these discharges. Unfortunately, receiving stream conditions precluded a quantitative assessment of the benthic community structure at two of the five facilities (Tyrrell County and Fairfield WTPs). Due to limited evidence of a healthy benthic community at another site (Rodanthe/Waves/Salvo WTP), quantitative assessment of community structure was not performed. Evidence of an ion gradient was present in Filbert Creek, the receiving stream for the remaining two facilities (Freemason and Beaver Hill WTPs), moving from upstream to downstream; however, increasing taxa richness and decreasing biotic index values were found along this gradient suggesting improved water quality conditions. Regardless of whether or not the ion gradient present was related to the presence of two hypersaline discharges on the creek or to wind and/or tidal influence of low salinity water from the Chowan River, it appears that the ion concentrations present have not uniquely stressed benthic communities in this low-flow, low-oxygen stream.

Toxicity testing and analytical results provide sufficient evidence that ion concentrations in reverse osmosis and ion exchange water treatment plant effluents are a primary source of toxicity. The disparity between the water quality of effluents relative to receiving stream characteristics at existing facilities suggests a need to reevaluate discharge options. The following management recommendations are suggested for existing and new/proposed water treatment facilities:

1. Existing facilities routinely fail toxicity testing and exceed state and federal water quality standards for routine monitoring parameters. Field visits to several existing reverse osmosis and ion exchange facilities (beyond those included in this study) also reveal that many WTPs discharge to non-flowing environments with limited to no available dilution capacity. Accordingly, innovative approaches should be considered where the potential for instream impacts exists:
    a. Process changes including dilution of concentrated effluents prior to discharge should be considered. Water sources for effluent dilution may include source water, stored wastewater from process cycles associated with reduced ion loads, or other reclaimed water sources (e.g., other municipal effluents). Use of lined ion equalization basins could be considered for storage of wastewater prior to discharge.
    b. At ion exchange facilities, only non-chlorinated water (e.g., product or finish water prior to disinfectant additions) should be used for pressure filter backwashing.
    c. Existing facilities discharging to non-flowing environments (including ditches, swales, and zero-flow streams or tributaries) should consider piping effluent to receiving streams with suitable dilution (if feasible) or diluting concentrated effluents prior to discharge.
    d. The ion absorption capacity of soil/sediment and vegetation downgradient of facilities discharging to non-flowing environments (e.g., zero-flow waters,

wetlands, swales) should be assessed to determine the potential for habitat impacts (particularly at facilities with a lengthy discharge history).

   e. A review of facility records maintained by the NCDWQ and the NC Public Water Supply Section should be conducted to assure that existing reverse osmosis and ion exchange facilities relying on surface water discharge for wastewater disposal have maintained appropriate environmental permits (e.g., NPDES).
   f. Instream monitoring (upstream and downstream of the facility outfall) should be conducted for conventional water quality parameters (e.g., dissolved oxygen, temperature, pH, salinity, flow, and total dissolved solids) as well as toxicants (e.g., major anions and cations) identified in effluents. Current NPDES guidelines require instream monitoring for dissolved oxygen, salinity, conductivity, temperature, and pH at reverse osmosis facilities only; instream monitoring should be expanded to include both conventional parameters and toxicants at both membrane and ion exchange facilities (at least for a sufficient period to demonstrate that the discharge is not substantially affecting instream conditions).
   g. The results of the present study indicate that ion exchange effluents are not uniform in chemical characteristics. Current NPDES permitting strategies for ion exchange facility monitoring includes collection of composite samples to address fluctuations in effluent quality; however, monitoring targeted to specific process waste flows (e.g., sodium regeneration cycle) is recommended (at least for a demonstration period) to assure that permit requirements are assigned based on reasonable worst case scenarios.
   h. If effluent is directed to a publicly owned wastewater treatment facility, flow equalization will be necessary to prevent bulk loading of hypersaline effluent to the treatment plant. Excessive flows could cause wastewater treatment facility upsets.

2. As recommended in the Water Treatment Plant Workgroup Report (*Assessment and Recommendations for Water Treatment Plant Permitting*, NCDENR 2003), new/proposed facilities should preferentially seek opportunities to discharge to an existing wastewater treatment facility. Where new surface water discharge of wastewater is necessary, new/proposed facilities should be designed and sited such that adequate receiving stream dilution capacity is available and effluent water quality characteristics closely match receiving stream conditions. Specifically:
   a. In freshwater receiving stream environments, dilution of concentrated effluents prior to discharge should be considered.
   b. In estuarine and saltwater receiving stream environments, effluent dilution should be maximized through outfall design (e.g., diffusers) and placement (e.g., target areas where tidal and/or wind mixing promotes effluent dissipation).[1]

[1] In estuarine receiving systems, toxic levels of alkalinity can be reduced through acidification of the effluent followed by a pH neutralization step. This treatment step could eliminate the need for enhanced effluent dilution (when alkalinity is known to drive the effluent toxicity) either through discharge to a wastewater treatment facility or a large body of water.

References

American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. *Standard Methods for the Examination of Water and Wastewater*. Twentieth Ed. American Public Health Association, Washington, DC.

Andrews, L. 2001. Concentrate Disposal. American Membrane Technology Association 2001 Annual Symposium. Camp Dresser & McKee, Inc.

Dwyer, F.J., S.A. Burch, C.G. Ingersoll, and J.B. Hunn. 1992. Toxicity of trace element and salinity mixtures to striped bass (*Morone saxatilis*) and *Daphnia magna*. Environ. Toxicol. Chem. 11(4): 513-520.

Eaton, L. 2001. Development and Validation of Biocriteria Using Benthic Macroinvertebrates for North Carolina Estuarine Waters. Mar. Poll. Bull. 42(1): 23-30.

FDEP. 1995. Protocols for determining major-seawater-ion toxicity in membrane-technology water-treatment concentrate. Florida Department of Environmental Protection, Bureau of Laboratories. Tallahassee, FL.

Gas Research Institute. 1999. Marine Salinity Toxicity Relationship Model. Chicago, Illinois.

Goodfellow, W.L., L.W. Ausley, D.T. Burton, D.L. Denton, P.B. Dorn, D.R. Grothe, M.A. Heber, T.J. Norberg-King, and J.H. Rodgers, Jr. 2000. Major ion toxicity in effluents: a review with permitting recommendations. Environ. Toxicol. Chem. 19(1): 175-82.

Ingersoll, C.G., F.J. Dwyer, S.A. Burch, M.K. Nelson, D.R. Buckler, and J.B. Hunn. 1992. The use of freshwater and saltwater animals to distinguish between the toxic effects of salinity and contaminants in irrigation drain water. Environ. Toxicol. Chem. 11(4): 503-511.

McCulloch, W.L., Goodfellow W.L., and J.A. Black. 1993. Characterization, identification and confirmation of total dissolved solids as effluent toxicants. In Grouch, J.W., Dyer, R.J., Ingersoll, C.G., LaPoint, T.W., eds, *Environmental Toxicology and Risk Assessment*, Vol. 2. STP 1216. American Society for Tesing and Materials, Philadelphia, PA, pp. 213-227.

Mickley, M and J. Briceno. 2000. Environmental concerns: membrane drinking water plants. AWWARF and AWWA. Denver, CO.

Mickley, M and J. Briceno. 2001. Major Ion Toxicity. AWWARF and AWWA. Denver, CO.

Mount D.R., Gulley D.D. 1992. Development of a salinity/toxicity relationship to predict acute toxicity of saline waters to freshwater organisms. Chicago IL, USA: Gas Research Institute (GRI). GRI- 92-0301. 158 p.

Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D., and J.M. Evans. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). Environ. Toxicol. Chem. 16(10): 2009-2019.

NCDEM. 1992. Results of the toxicological and chemical analyses of water treatment facilities. July 27, 1992 memo from Thom Mistele to Steve Tedder through Larry Ausley and Ken Eagleson. North Carolina Division of Environmental Management. Raleigh, NC.

NCDENR. 2003. Assessment and Recommendations for Water Treatment Plant Permitting. Water Treatment Plant Workgroup. January 2003 Draft Report. North Carolina Department of Environment and Natural Resources. Raleigh, NC.

NCDMF. 2001a. Washington County Water Treatment Plant, Draft NPDES Permit #NC0087009. Letter from Sara Winslow, NCDMF, to David Goodrich, NPDES Unit. February 16, 2001.

NCDMF. 2001b. Tyrrell County Reverse Osmosis Treatment Plant, Draft NPDES Permit #NC0086924. Letter from Sara Winslow, NCDMF, to David Goodrich, NPDES Unit. March 1, 2001.

NCDMF. 2001c. Dare County - Stumpy Point Reverse Osmosis Treatment Plant, Draft NPDES Permit. Letter from Sara Winslow, NCDMF, to David Goodrich, NPDES Unit. March 12, 2001.

NCDMF. 2001d. Camden County Reverse Osmosis Treatment Plant, CAMA Permit Application, Pasquotank, NPDES Permit #NC0086681. Letter from Sara Winslow, NCDMF, to David Goodrich, NPDES Unit. July 11, 2001.

NCDWQ. 1998. NC Phase II Chronic Whole Effluent Toxicity Test Procedure, July 1991 (Revised February 1998). NC Division of Water Quality, Water Quality Section. Raleigh, NC.

NCDWQ. 2000. Kendricks Creek, Washington County, Freshwater Survey 10/26/00. Memorandum from Larry Eaton to Jimmy Overton. NCDWQ Bioassessment Unit. October 31, 2000.

NCDENR. 2001. Standard operating procedures for benthic macroinvertebrates, Biological Assessment Unit, April 2001. North Carolina Department of Environment and Natural Resources, Division of Water Quality, Water Quality Section, Environmental Sciences Branch. April 30, 2001. Raleigh, NC.

Pillard, D.A., DuFresene, D.L., Caudle, D.D., Tietge, J.E, and Evans, J.M. Predicting the Toxicity of Major Ions in Seawater to Mysid Shrimp (*Mysidopsis bahia*), Sheepshead minnow (*Cyprinodon variegatus*), and Inland Silverside (*Menidia beryllina*). Environmental Toxicology and Chemistry, Vol. 19, No. 1, pp. 183-191, 2000.

U.S. Environmental Protection Agency. 1979. Method 365.1. Phosphorus by Automated Colorimetry. Methods for the Determination of Inorganic Substances in Environmental Samples (EPA/600/R-93/100).

U.S. Environmental Protection Agency. 1986. Quality Criteria for Water 1986. USEPA, Office of Water. EPA 440/5-86-001, May 1986.

U.S. Environmental Protection Agency. 1988. Ambient Water Quality Criteria for Chloride – 1988. USEPA, Office of Water. EPA 440/5-88-001, February, 1988.

U.S. Environmental Protection Agency. 1994. Method 200.7. Inductively coupled plasma-atomic emission spectrometric method for trace element analysis of water and wastes. Methods for the Determination of Metals in Environmental Samples - Supplement I. EPA 600/R-94-111, May 1994.

U.S. Environmental Protection Agency. 1997. USEPA Method 300.1, Determination of Inorganic Anions in Drinking Water by Ion Chromatography, Revision 1.0. USEPA, EPA-600-R-98-118.

U.S. Environmental Protection Agency. 1999. 1999 Update of Ambient Water Quality Criteria for Ammonia. USEPA, Office of Water. EPA-822-R-99-014, December 1999.

U.S. Environmental Protection Agency. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. 5th ed. USEPA, Office of Water. EPA-821-R-02-012, October 2002.

U.S. Environmental Protection Agency. 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th ed. USEPA, Office of Water. EPA-821-R-02-013, October 2002.

U.S. Environmental Protection Agency. 2002c. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. 3rd ed. USEPA, Office of Water. EPA-821-R-02-014, October 2002.