Assessment and Economic Valuation of Nitrogen Mitigation in Texas Coastal Bend Restored Marsh

Final Report

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Table of Contents

EXECUTIVE SUMMARY

The goal of this study is to quantify the nitrogen mitigation ecosystem service provided by natural and restored wetlands in the Texas Coastal Bend. The outcome of this project will help increase awareness of the essential values of wetlands for water quality improvement, as well as justify the restoration and recovery investments throughout the Texas Coastal Bend. Sample collection began in October 2018 and continued through February 2020 from eight sites including three secondary wastewater treatment plants (WWTP), two restored wetlands, and three natural wetlands.

Eutrophication is likely a major contributing factor leading to hypoxia in coastal waters, which has negative ecological and economic consequences. Eutrophication and hypoxia occur frequently in the Gulf of Mexico and its surrounding wetlands and estuaries. Eutrophication is largely caused by excess nutrients, including nitrogen and phosphorous, entering aquatic environments from both point and non-point sources. Secondary wastewater treatment plants are an important source of excess nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) flowing into wetlands and estuaries along the Texas coast. Coastal development connected to rising populations has been causing degradation of natural wetland and has led to an increase in the output of nitrogen-based nutrients into the aquatic environment.

Nitrogen mitigation is an important ecosystem service offered by wetlands, and denitrification is the main pathway for removing excess nitrogen in wetland sediments. In this study, the quantification of potential denitrification in the two restored, and three natural wetlands showed that age is a major factor in regulating denitrification rates. The lowest mean annual denitrification rates were measured in the restored sites, Egery Flats and the Nueces Bay restored marsh (16.81 and 13.45 kg $N{\cdot}ha^{-2}{\cdot}yr^{-1}$, respectively). Natural wetlands sites showed significantly higher denitrification rates of 20.48, 31.33, and 45.73 kg $N{\cdot}ha^{-2}{\cdot}yr^{-1}$ for the Aransas River Estuary, Oso Bay marsh, and the Naval Airbase Bridge, respectively. Seasonality and temperature also influenced denitrification rates in three of the five wetland sites. Measured seasonal denitrification rates were used to quantify the monetary value of nitrogen mitigation in the restored wetland sites. This value was calculated as $\$17,476{\cdot}yr^{-1}$, and $\$3,624{\cdot}yr^{-1}$, for Egery Flats and Nueces Bay restored marsh, respectively. The value of nitrogen mitigation was equivalent to $\$3.85{\cdot}kg\ N^{-1}$ removed.

OUTREACH EFFORTS

This project funded one M.S. student's thesis work and involved two graduate students and 15 total undergraduate students (most of them are female and/or students from underrepresented groups), which participated in several different portions of this project, including field sample collection, data collection using gas chromatography, nutrient analysis, and stable isotope analysis, as well as organizing, analyzing, and interpreting biogeochemical data collected from several locations around the Texas Coastal Bend.

This study has also been incorporated into the graduate course "Coastal and Marine Systems (CMSS 6307)" taught by PI Lin Zhang, where students were able to observe sample collection and sample analysis methods. A list of education and outreach efforts is listed below.

Participating students (Graduate Students):

Lydia Hayes and Charlotte Lee

Participating students (Undergraduate students):

Roslyn Swonke, Jesus Baca, Evelyn Kuhnel, Catherine Shaw, Kaitlin Sams, Sarah RodriguezVazquez, Shahrukh Niazi, Hannah Schulze, Will Mixon, Zoie Bright, Irelyn Lee, Aubrey Dauria, Ames Finley, Austin Fuentes, Alyssa Lucas, April Smith, Jonathon Hoang, Madyson Gilmore, NourEldeen Loubani, Edgardo Jimenez, Daniel Lansidel, Tim Laughbaum, Erik Perez, Gabriela Mondragon, Ryleigh Washerlesky, Morganne Mier, Isabella Moon, Brandon Hodge, Dante Vasquez, Alicia Morales, and Anna-Marie Morse.

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Presentations (public):

Hayes, L. Assessment and valuation of nitrogen mitigation ecosystem services in restored wetlands of the Texas Coastal Bend. Marine Science Graduate Student Organization 8th Annual Research Forum. Texas A&M University-Corpus Christi. Corpus Christi, TX, USA. April 19, 2019.

Hayes, L., Williams, L., and Zhang, L., Assessment and economic valuation of nitrogen mitigation ecosystem services in restored wetland of the Texas coastal bend. Coastal Bend Bays Foundation Coastal Issues Forum. Corpus Christi, TX, USA. October 14, 2019.

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Manuscripts:

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Hayes, L., Sams, K., Shaw, C., Williams, L., Zhang, L. Nitrogen removal and other cycling processes in restored and natural wetlands along the Texas Coastal Bend. (*In Prep*).

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

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There were numerous individuals who helped throughout the duration of this project. This study would not have been possible without their dedication, strong work ethic, and ongoing energy and enthusiasm for their work. We would like to thank the following students for all their hard work with countless field sampling, laboratory sample analysis, data analysis, and interpretation: Jesus Baca, Zoie Bright, Aubrey Dauria, Ames Finley, Austin Fuentes, Madyson Gilmore, Jonathon Hoang, Brandon Hodge, Edgardo Jimenez, Evelyn Kuhnel, Daniel Lansidel, Tim Laughbaum, Charlotte Lee, Irelyn Lee, NourEldeen Loubani, Alyssa Lucas, Morganne Mier, Will Mixon, Gabriela Mondragon, Isabella Moon, Alicia Morales, Anna-Marie Morse, Shahrukh Niazi, Erik Perez, Sarah RodriguezVazquez, Kaitlin Sams, Hannah Schulze, Catherine Shaw, April Smith, Roslyn Swonke, Dante Vasquez, and Ryleigh Washerlesky.

INTRODUCTION

Background Information

Wetlands provide many ecosystem services including water quality improvement, nutrient cycling, sequestration of carbon, fisheries habitat, recreation opportunities and nitrogen mitigation (Gren et al., 1995; Woodward and Wui 2001; Yang et al., 2008; Canfield et al., 2010; Zhang et al., 2016). Ecosystem services are benefits provided by an ecosystem that have a specific purpose with value (Chen et al., 2009; Vymazal 2011). Nitrogen (N) pollution from human activity negatively impacts aquatic environments, making N mitigation, the focus of this study, an essential ecosystem service.

More than 6 million people reside along the Texas coast, according to the 2010 Census. This population is expected to increase by 50 percent by 2050 (Texas Shores, 2013). Development in association with population rise has been causing the degradation of natural wetlands, leading to a loss of ecosystem services. The increase of nitrogen to the environment including agricultural runoff, fossil fuel combustion, and wastewater effluent associated with population rise will take its toll on the economy through the pollution of vital water resources (Shahi et al., 2013).

Wastewater treatment plants (WWTP) without N removal capabilities (secondary treatment) are a substantial source of excess N in the form of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) into important waterways including rivers, streams, and estuaries (Desimone and Howes 1996; Arachana et al., 2016; McLaughlin et al., 2017). This can lead to a multitude of environmental issues including soil acidification, eutrophication, and coastal hypoxia. Hypoxia in coastal zones has increased over the past few decades, specifically along the Texas coast, which is largely related to excess N pollution and a loss of wetlands (Rabalais et al., 2002).

The use of N stable isotope ratios ($\delta^{15}N$) in nitrate, nitrite, and ammonium can help differentiate between N sources and processes in the environment. These sources and processes include synthetic fertilizer, wastewater, soil N, organic matter, atmospheric N, as well as denitrification, nitrification, and ammonification which all have different $\delta^{15}N$ (‰) values (Kendall 1998; Kendal et al., 2007; BryantMason et al., 2013) (Appendix 1). The $\delta^{15}N$ of NO_3^-, NO_2^-, and NH_4^+ can be used to identify specific sources of excess N, as well as point to the major cycling processes in the environment, contributing to our understanding of how N moves through the ecosystem.

Much of the excess nitrogen entering the waterways can be removed in wetlands through microbially-mediated process of denitrification, the reduction of nitrate and nitrite to nitrogen gas (Lindau et al., 2008). Denitrification represents a direct removal of fixed nitrogen from water and sediment, releasing nitrogen gas into the atmosphere, making it an important process for nitrogen mitigation (Groffman 1991; DeLaune et al., 2005; Lindau et al., 2008).

Denitrification has been quantified in wetlands as a nitrogen mitigation service in several studies over the last few decades (Appendix 2). Unfortunately, denitrification in wetlands along the Texas Coastal Bend has been understudied, and even fewer studies have monetized the N mitigation ecosystem service. The quantification of denitrification for several locations in the United States and globally have explored many different types of environments including restored and natural wetlands, as well as marine and freshwater systems (Groffman and Tiedje 1989; Behrendt et al., 1999; Dehnhardt 2002; Richardson et al. 2004; DeLaune et al. 2005; Lindau et al., 2008; Jenkins et al., 2010; Wolf et al., 2011; Theriot et al., 2013; Song et al., 2014; Bruesewitz et al., 2017). These studies have also delved into contributing factors for denitrification including temperature, nutrient availability, carbon availability, water flow rate, soil composition, oxygen content, accumulation of organic matter, and the age of the wetland (Groffman and Tiedje 1989; Behrendt et al., 1999; Dehnhardt 2002; Richardson et al. 2004; Bruesewitz et al., 2017).

Increased episodes of coastal hypoxia can cause the degradation of natural environments, a known cause of these increases is excess nitrogen pollution from agricultural and wastewater runoff. Wetland restoration is a proposed means of improving nitrogen mitigation in areas affected by these hypoxic episodes by reducing the amount of nutrient loading into major waterways by increasing denitrification capacity (Lindau et al., 2008). Restoration projects can include wetland restoration, implementation of created wetlands, river water diversions, and riparian buffer restoration. Most wetland restoration projects cause disturbance to existing sediments which may temporarily reduce denitrifying microbial populations. Studies have shown that created wetlands have less complex soil structure and lower microbial diversity than natural wetlands (Wolf et al., 2011; Song et al., 2014). As time goes on, total organic matter and nitrogen content will accumulate in created wetlands, the sediment will develop a more complex microbial community structure, thus increasing N cycle development. This development may take five to ten years for a created wetland to have similar function to natural wetlands (Wolf et al., 2011). This increased N cycling development will bridge the gap between restored and natural wetlands, yielding restored and created wetlands that are more similar in function to natural wetlands (Richardson et al., 2004; Jenkins et al., 2010; Wolf et al., 2011). Restoration projects can improve nitrate reduction through denitrification and biological assimilation by plants and microorganisms by landscape planning, water depth adjustment, and decreasing velocity to increase contact time between nutrients and sediment allowing more time for denitrification to occur (Mitsch et al., 2005; Bruesewitz et al., 2017).

Studies around the southeast region of the United States and the Gulf of Mexico have provided a monetary value for a variety of ecosystem services (Appendix 3). While denitrification and economic evaluation of different ecosystem services have been done in areas around the Gulf of Mexico as well as globally, a study quantifying and monetizing the N mitigation service for both natural and restored wetlands has not been done for the Texas Coastal Bend. A looming concern with the degradation of ecosystems, is the necessity for making decisions about conservation and restoration projects, as well as cost justification for these types of projects (Chen et al., 2009). Valuing the N mitigation service that is provided by wetland ecosystems can aid in the decision-making process, to inform policymakers and stakeholders of the services and their worth.

Providing an economic evaluation for services that do not have a traditional market value can increase our ability to justify costs for conservation and restoration efforts. Many ecosystem services lack a conventional market, and therefore go undervalued (Salem and Mercer 2012). Many methods exist to determine the monetary value of an ecosystem service, including willingness to pay, contingent valuation, market pricing, and production pricing (Loomis 1992; Breaux et al., 1995; Jenkins et al., 2010; Piehler and Smyth 2011; Pollack et al., 2013; Schmidt et al., 2014).

Commonly used methods for assigning monetary value to the N mitigation ecosystem service are the benefit transfer and replacement cost methods. The benefit transfer method uses an estimate calculated from a previous study and applies it to the current study site. The replacement cost method involves determining the value of the ecosystem service based on the cost to replace the service with a manmade alternative (Salem and Mercer 2012; Pollack et al., 2013). In order to monetize an ecosystem service, the following steps must be completed: 1) identify the ecosystem service, 2) quantify the service, and 3) monetize or assign value to the ecosystem service (Jenkins et al., 2010).

This study assessed N mitigation in restored and natural wetlands of the Texas Coastal Bend under the influence of wastewater discharge and provides an economic valuation of the N mitigation ecosystem service in two restored wetlands and three natural wetlands. This study was completed with the following objectives: 1) identify potential denitrification in wetland sediments, 2) quantify the concentration of nitrogen species and identify nitrogen sources in WWTP effluent and surrounding wetland water columns, 3) quantify denitrification rates for each wetland site and establish seasonality for denitrification, 4) provide a case study to apply economic valuation of N mitigation to two restored and three natural wetlands in the Texas Coastal Bend.

The relevance of this study is tied to many investments that are being made along the Texas Coastal Bend for wetland restoration including the two restored sites incorporated in this project, Egery Flats and Nueces Bay. WWTPs have high manufacturing and energy costs but using healthy wetlands as a system for treating wastewater would reduce those costs, be economically efficient, and decrease environmental pollution (Shahi et al., 2013). Assessment of the N mitigation service offered by wetlands along the Texas coast will improve the understanding of N cycling and N mitigation in restored and natural wetlands in the region, and the monetary valuation of N mitigation services will aid in cost justification for current and future restoration and conservation projects to decision-makers and stakeholders.

Study Areas

Sampling for this project took place at eight sites, three in Corpus Christi, TX, three in Bayside, TX, and two in Portland, TX. Sampling sites included three WWTPs and five wetlands, two restored and three naturals, adjacent to the WWTPs along the Texas Coast (Figure 1). Each of the wetlands are located adjacent to at least one of the following large bodies of water: Oso Bay, Corpus Christi Bay, Nueces Bay, and Copano Bay. All areas were estimated using the polygon feature in Google Earth Pro to outline the perimeter of the sites.

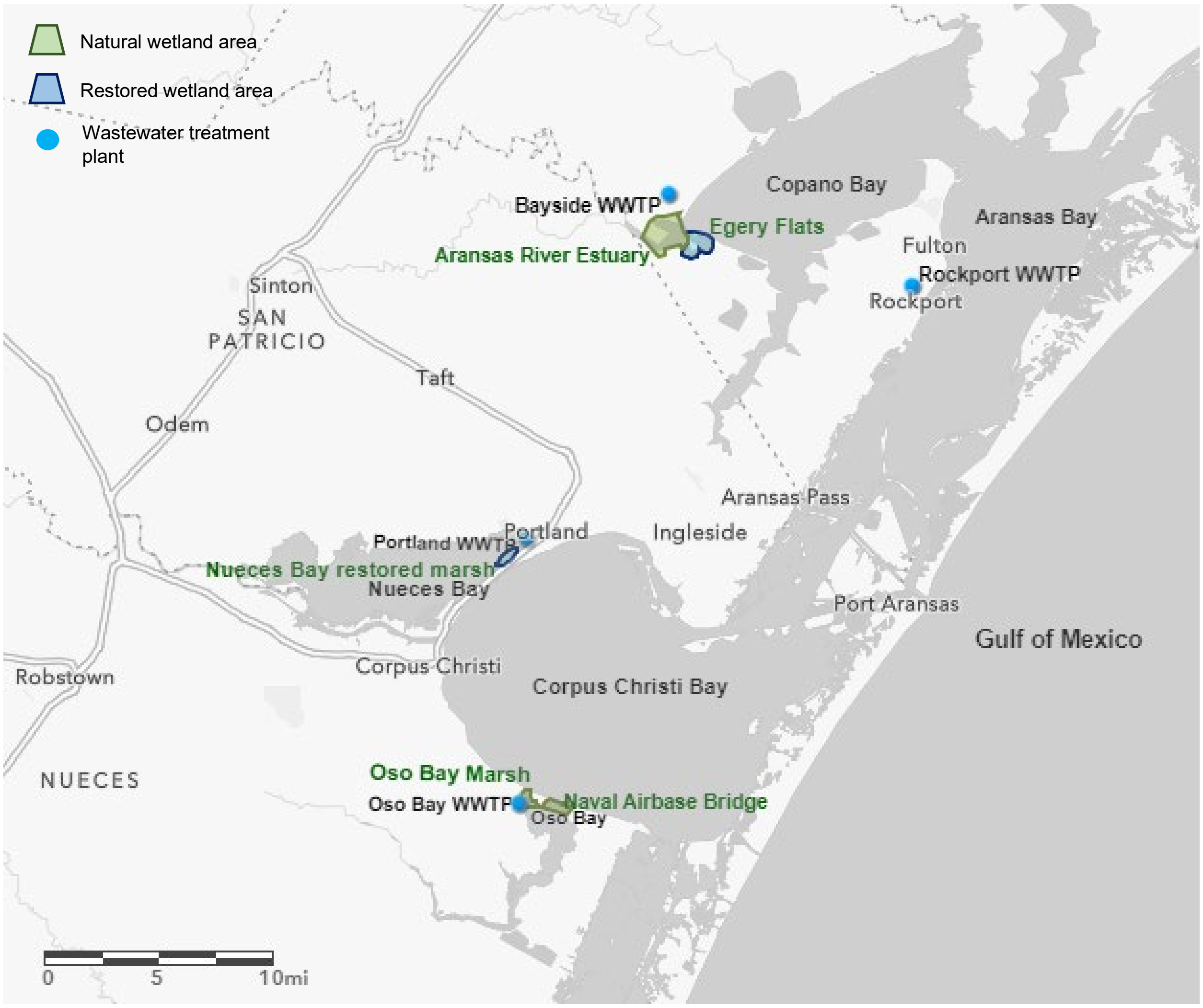


Figure 2. Map of all sampling sites along the Texas Coastal Bend.

Corpus Christi, TX, USA

Corpus Christ, TX, located in Nueces County, is surrounded by agricultural land to the west and northwest, and three large bodies of water: Corpus Christi Bay, Oso Bay, and the Gulf of Mexico. The Oso Bay WWTP sampling site is located in Corpus Christi, TX and the WWTP effluent is discharged into the Oso Bay which connects to the Corpus Christi Bay.

The Oso Bay marsh wetland site is a natural wetland, located in Corpus Christi, TX, which is a 105- ha tidal flat along the northwest shore of Oso Bay influenced by the Oso Bay WWTP effluent. The site is located at the mouth of the stream where the effluent flows into the Oso Bay (Figure 2). The sediment is primarily clay, with a layer of sand less than 1-cm on the surface. The vegetation is primarily *Tamarix ramosissima* (Salt cedar shrubland), *Borrichia frutescens* (sea ox-eye daisy), *Spartina* sp. (cordgrass), and *Prosopis* sp. (mesquite).

Figure 2. Photos of the Oso Bay marsh site.

The Naval Airbase Bridge wetland site is another natural wetland in Corpus Christi, TX. This site is a sub-tidal flat approximately 84-ha located at the connection point of the Oso and Corpus Christi Bays, giving this site influence from two bay systems as well as the Oso Bay WWTP (Figure 3). The sediment is coarse sand and shells. The vegetation in sparse at this site but includes *Borrichia frutescens, Prosopis* sp., *Spartina* sp., and submerged aquatic vegetation including *Cymodocea filiformis, Halophila engelmannii, and Halodule wrightii.*

Figure 3. Photos of the Naval Airbase Bridge site.

Portland, TX, USA

Portland, TX, located within Nueces and San Patricio counties, is surrounded by agricultural land to the north, west, and east, and the Nueces and Corpus Christi Bays to the south. The Portland WWTP is the second WWTP effluent sampled in this project, the outfall flows into the Nueces Bay (Figure 4).

The first restored site is the Nueces Bay restored marsh, which is part of a 70-ha, $5.3 million constructed wetland, which was completed in 2015 by the Coastal Bend Bays and Estuaries Program (CBBEP) (Figure 5). Created sediment mounds at this site were planted with native marsh plants which include *Spartina alterniflora* and *Spartina patens*. Other plants have naturally recruited there, post-construction, including *Spicornia spp.* (Pickleweed), *Batis maritima,* and

Lycium carolinianum (CBBEP, 2014). The sediments are primarily clay with a layer of sand, pebbles, and large broken shells. This site is also adjacent to the Portland WWTP (Figure 6).

Figure 4. Photos of the Portland WWTP effluent outfall from the front (left), and from the top (right).

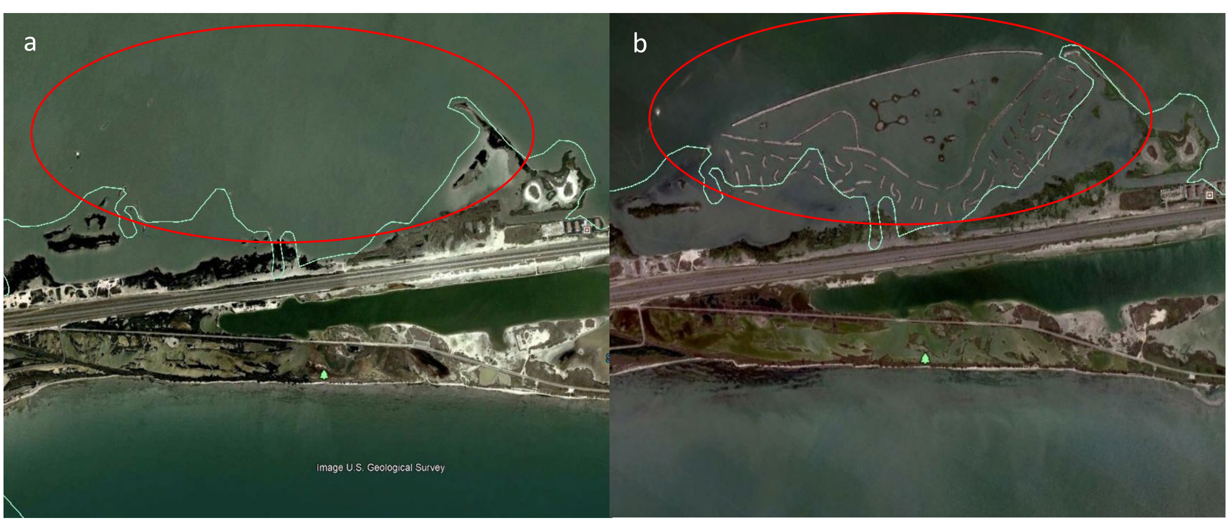


Figure 5. Location of the Nueces Bay restored marsh site. a- Nueces Bay site pre-restoration, 2006, b- Nueces Bay site post-restoration, 2017. Image made using Google Earth.

Figure 6. Photo of the Nueces Bay restored marsh site.

Bayside, TX, USA

The Town of Bayside, TX, located in Refugio County, is surrounded by agricultural land to the north, south, and west, with two bodies of water, the Aransas River, and Copano Bay to the east. The third WWTP sampled in this project is the Bayside WWTP effluent. This is a constructed wetland WWTP with effluent that flows into the Aransas River Estuary, Egery Flats restored marsh, and the out into the Copano Bay (Figure 7). The Bayside WWTP wetland is planted with *Schoenoplectus californicus*, *Typha domingensis, Sagittaria graminea,* and *Pontederia cordata.* The design of this treatment plant allows for the reduction of nitrate through denitrification before the effluent is released into the environment. This process is efficient due to the low flow rate of the Bayside WWTP (O'Malley Engineers 2004).

Figure 7. Photos of the Bayside WWTP effluent outfall.

The final natural wetland included in this project is the Aransas River Estuary, an approximately 630-ha wetland located within Bayside, TX between the mouth of the Aransas River and Copano Bay, and to the west of FM136 (Figure 8). The vegetation at this site includes *Spartina alterniflora, Spartina patens, Borrichia frutescens, Juncus roemerianus, Distichlis spicata,* as well as submerged aquatic vegetation including *Cymodocea filiformis, Halophila engelmannii, and Halodule wrightii.* The sediment at this site is mostly mud, comprised of silt and clay.

Figure 8. Photos of the Aransas River Estuary sampling site.

Another restored wetland is the Egery Flats restored marsh site. Located in Bayside, TX, Egery Flats is a part of a 270-ha $1.5 million marsh reconstruction project that was completed in 2019. This site is located to the east of the Aransas River Estuary and FM136, and near the northwest edge of Copano Bay (Figure 9). The restoration project aims to restore hydrology and reduce salinity in the Egery Flats marsh by replacing previously underperforming culverts with expanded culverts to increase freshwater inflow coming from the Aransas River into the marsh, as well as planting new emergent marsh plants throughout the wetland (Figure 10) (NFWF 2014). The vegetation at this site is very similar to that of the Aransas River Estuary, with much more submerged aquatic vegetation, both the Aransas River Estuary and Egery Flats support emergent marsh, submerged aquatic vegetation, are home to numerous marine life species, waterfowl, and are influenced by the Bayside WWTP. Also like the Aransas River Estuary, the sediment at this site is mostly mud, comprised of silt and clay, with rocks and shells included.

Figure 9. Photos of the Egery Flats restored marsh sampling site.

Figure 10. Images of the culvert restoration project in Egery Flats. **a-** map of Egery Flats showing the two locations of culvert replacement, **b-** photo of 30" pipe culverts, pre-reconstruction in 2018, **c-** photo of 3'x6' box culverts, post-reconstruction, 2019. Map created using Google Earth.

METHODS

Sampling

Each of the eight sites were sampled monthly from October 2018 through February 2020. For all sites environmental parameters including pH, water temperature, salinity, and dissolved oxygen (DO) were measured. A Thermo Orion Star A324 meter was used for pH measurements. Water temperature and salinity were measured using a Thermo Orion model 135A conductivity meter. DO was measured with a Thermo Orion model 835A advanced DO meter. Each instrument was calibrated monthly prior to sampling.

Water samples were collected monthly for nutrient analysis of NO_2^-, NO_3^-, and NH_4^+. Surface water was collected in 10mL centrifuge tubes filter sterilized with 0.22 µm PES syringe filters and collected in triplicate. Water samples for NO_2^-, NO_3^-, and NH_4^+ nitrogen stable isotopes were collected in triplicate quantities of 15mL for each N species. Water was filter sterilized into 15mL centrifuge tubes using 0.22 µm PES syringe filters, where NO_2^-, and NO_3^- were chemically preserved using 6M NaOH, and 2.5 mM sulfamic acid in 25% HCl, respectively (Bourbonnais et al., 2017).

In situ dissolved gas samples were collected using 12mL serum bottles, pre-flushed with N_2 gas and vacuum evacuated. Surface water was collected with a 10mL Hamilton GASTIGHT® syringe and injected into the vacuum evacuated vials, in triplicates for laboratory analysis, using the Gas Chromatograph (GC) headspace equilibrium technique (Hudson 2004; Osburn et al., 2014; Helton et al., 2014; Brazelton et al., 2017).

Sediments were collected from each of the five wetland sites. Sediments were collected manually from the surface sediment layer, up to 10 cm deep, from submerged soil and stored in a

32 oz Mason Jar then sealed. All samples were held on ice for transport back to the laboratory, water and gas sample were then stored at -20°C prior to analysis, sediment samples were stored at 4°C to slow microbial activity prior to analysis (Zhang et al., 2019).

Seasonal Climate Variation

In this study seasons were designated as winter: December, January, February; spring: March, April, May; summer: June, July, August; and fall: September, October, November. To understand climate variation in the study area, data from NOAA National Centers for Environmental Information was collected. The data included daily measurements of total precipitation, and minimum and maximum air temperatures collected from January 2010 through December 2019. The station for the weather data was USW00012926 in Corpus Christi NAS, TX. Statistical analyses including ANOVA and student's t-test were used to determine significant differences between seasons, between the study year and the previous decade, as well as between El Niño, La Niña, and ENSO-neutral years to determine if the measurements in this study are typical, or if the conditions of this year are anomalous.

Nutrient Analysis

For identification and quantification of different N species in water from all sampling locations, nutrient concentrations for NO_3^-, NO_2^-, and NH_4^+ were measured using a SEAL AQ300 Discrete Analyzer. The AQ300 method EPA-148-D Rev 0 was used for NH_4^+ analysis, with a range of 0.21-71 µM. Samples with greater than 71 µM concentration were diluted into the detection range for the method. For this method, 400 µL of water sample reacted with hypochlorite for 40 µL of dichloroisocyanurate. The resulting chloramine reacts with 90 µL of salicylate at alkaline pH in the presence of nitroferricyanide. An indophenol dye, blue-green in color, is formed and measured spectrophotometrically at 660 nm. The concentration is calculated using the measured absorbance unit compared to an eight-point calibration curve (R^2>0.9990).

AQ300 method EPA-115-D Rev A was used to analyze NO_2^- concentrations, this method has a detection range of 0.05 to 107 µM. Samples with concentrations above this range were diluted into the detection range. This method mixes 200 µL of water sample with 200 µL of sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride and 100 µL of a pH buffer solution to form a red-purple dye, measured spectrophotometrically at 520 nm. Concentration is calculated using an eight-point calibration curve (R^2>0.9998).

NO_3^- concentration was measured using the Cadmium reduction method, which is the AQ300 method EPA-126-D Rev. This method yields concentrations of NO_3^- + NO_2^-, previously measured NO_2^- concentrations are subtracted to give final NO_3^- concentrations. This method has a detection range of 0.57-257 µM. The method mixes sample with 290 µL of pH buffer and pulls the 430 µL of sample through a 7-turn copper treated cadmium coil, where NO_3^- is reduced to NO_2^-. The reduced sample reacts with 350 µL of sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride giving the mixture a red-purple color, that is then measured using a spectrophotometer at 520 nm. This test uses an eight-point calibration curve (R^2>0.9990).

Dissolved Gas Concentrations

Gas samples were collected in triplicate monthly from all sites. Vacuum evacuated serum bottles containing dissolved gas samples were injected with helium to fill headspace and set to equilibrate before measurement. Gas concentrations including methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) were determined using a Thermo Scientific Trace 1310 Gas Chromatograph (GC) fitted with a Flame Ionization Detector (FID), Thermal Conductivity

Detector (TCD), and an Electron Capture Detector (ECD). Once balanced with atmospheric pressure, 2 mL of headspace gas from the vial were injected into the GC (Hudson 2004; Brazelton et al., 2017). Dissolved gas concentrations were then calculated using their solubility constants, the analytical temperature and pressure, and calibration curves derived from standard gas mixtures (Hudson 2004; Osburn et al., 2014; Helton et al., 2014; Brazelton et al., 2017). Higher level of N_2O than the ambient concentration may indicate *in situ* denitrification occurring in the surface water.

Stable Isotope Analysis

Nitrogen stable isotopes in NO_3^-, NO_2^-, and NH_4^+ were used to identify nitrogen sources and processes in water samples, which have unique ^{15}N:^{14}N ratios (‰) (Freyer and Republic 1978; Felix et al., 2013). N stable isotopes for NH_4^+ samples were analyzed using an established method (Zhang et al., 2007). Briefly, water samples are treated with sulfamic acid and 10% HCl to remove pre-existing NO_2^-. Once NO_2^- was removed, NH_4^+ was oxidized to NO_2^- using hypobromite. Sodium arsenite was then added to remove any additional hypobromite. Upon completion of this reaction NO_2^- yield was measured on the SEAL AQ300 Discrete Analyzer. The produced NO_2^- was then sent to an external isotope lab where it was further reduced to N_2O and measured $\delta^{15}N$ on a Purge-and-Trap IRMS.

Nitrate samples were reduced to NO_2^- using cadmium and then to N_2O using the azide method following the procedures outlined in McIlvin and Altabet (2005). $\delta^{15}N$ analysis of produced N_2O was conducted in the same external lab. Along with the NO_3^- samples that were reduced, blanks following the same procedure were also analyzed to account for any N in the water used for reagents. All analyses were performed in triplicates. The equation below was used for calculation of $\delta^{15}N$ ratio in the samples:

$$d^{15}N(‰) = \frac{((^{15}N/^{14}N)\ \text{sample}) - ((^{15}N/^{14}N)\ \text{standard})}{((^{15}N/^{14}N)\ \text{standard})}\ X\ 1000$$

Quantification of Nitrogen Mitigation Ecosystem Service Flows

Nitrogen mitigation was quantified through measurement of potential denitrification rates, which was quantified using the acetylene (C_2H_2) blocking method (Figure 11) (Groffman and Tiedje 1989; Groffman et al., 2006). Bulk sediments collected from the upper 10 cm of submerged sediment were well mixed into a slurry, and 70 mL of slurry was funneled into each of three 160 mL serum bottle. The surface of the slurry was covered with 10 mL Milli-Q water. The bottles were sealed with butyl rubber stoppers and flushed with N_2 gas for 10 minutes creating anaerobic conditions. The serum bottles were then incubated overnight at the sampling temperatures to stabilize the water-sediment interface. The bottles were then injected with 20 mL of wastewater, high in NO_3^-, from the adjacent WWTP and then injected with 30 mL of C_2H_2 (Richardson et al., 2004: Schipper et al., 2005). The sediments were then well-mixed and the pressure inside the bottles was balanced with the atmospheric pressure. Headspace was measured on the GC fixed with an ECD using 2 mL injections. The production of N_2O was measured once per hour for six hours. Denitrification rates were calculated by the rate of N_2O accumulation over time (Groffman and Tiedje 1989). Rates were corrected using Henry's Law Constant for N_2O for dissolved gas in the aqueous layer in the serum bottle (Sander 1999; Lindau et al., 2008). The rate of denitrification was then converted to kg $N \cdot ha^{-2} \cdot yr^{-1}$ using the equation below (Rolston 1986; Lindau et al., 2008):

$$Flux = \frac{Headspace\ Volume}{Sediment\ Area} \times \frac{273}{Absolute\ Temperature} \times \frac{\Delta Concentration}{\Delta time}$$

This equation converts denitrification rates into a unit that is suitable to value the nitrogen mitigation ecosystem service.

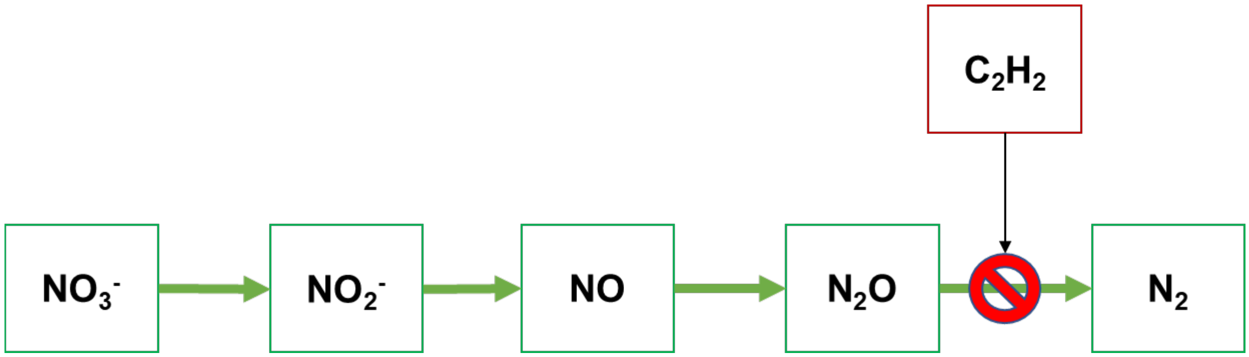


Figure 11. The effects of acetylene gas on the denitrification pathway. Based on Groffman et al., 2006.

Economic Evaluation of the Nitrogen Mitigation Ecosystem Service

This study uses the replacement cost method, where the potential cost equivalent of N removal for two restored and three natural wetlands is quantified by cost estimates for a constructed biological nutrient removal (BNR) addition to a WWTP (Pollack et al., 2013). We estimated the amount of N removed by a constructed BNR addition using the Rockport WWTP located in Rockport, TX, as an example of a manmade nitrogen removal system. The Rockport WWTP was chosen due to its proximity to the study sites. Also, it is a small-scale treatment facility, making it more representative of the needs of cities in this region. All relevant data necessary to apply the replacement cost method was attainable for this WWTP, including volume of water processed by the plant, total N removed from the influent, and capital costs for construction of the BNR system (Pollack et al., EPA ECHO). The engineered system at the Rockport WWTP removes NO_3^- using an anoxic tank with a series of pumps that prevent the waste from settling to the bottom of the tank. Using denitrifying microorganisms under anaerobic conditions, the NO_3^- is reduced to N_2 gas and released to the atmosphere. This WWTP processes 0.97 million-gallons-day (MGD) on average, and removes approximately 62% of total N from influent, a daily average of 21.0 mg $N \cdot L^{-1}$. The capital cost for constructing the BNR system was $1,090,968 US$2012.

The initial step in calculating the N mitigation value was determining the total N removed by the manmade BNR system annually. For the Rockport WWTP this is done with the following equation:

$$Total\ N\ removed = \frac{kg\ N \cdot L^{-1}\ removed}{0.264172\ gal \cdot L^{-1}} \times \frac{gal\ of\ water\ processed}{day} \times \frac{365\ day}{yr}$$

The next step was calculating the amount of nitrogen removed from the wetland per season using measured seasonal denitrification rates. Nitrogen removal per season is calculated using the following equation:

$$kg\ N\ removed \cdot ha^{-2} = \ seasonal\ kg\ N\ removed \cdot ha^{-2} \cdot yr^{-1} \times 0.25 \cdot yr$$

The total sum of N removed seasonally was then multiplied by the total wetland area:

$$annual\ total\ kg\ N\ removed = \ kg\ N\ removed \cdot ha^{-2} \times 0.25 \cdot area\ in\ ha$$

The percent of N removed by the wetland in respect to total N removed by the manmade BNR system was then computed with the following equation:

$$\%\ \ N\ removed = \frac{annual\ total\ N\ removed\ by\ wetland}{annual\ total\ N\ removed\ by\ WWTP} \times 100$$

The processing capacity (PC) for the wetland based on the manmade alternative PC was calculated:

$$Wetland\ PC\ in\ MGD = WWTP\ PC\ in\ MGD \times \%\ N\ removed$$

The Rockport WWTP BNR capital costs were converted from US\$2012 to US\$2020. This is done by inflating the dollar value from October 2012, when the BNR was built, to August 2020 using CPI Inflation Calculator from the U.S. Bureau of Labor statistics, were 1 US\$2012 is equivalent to 1.124 US\$2020:

$$1{,}225{,}859.84\ US\$2020 = \ \$1{,}090{,}968.00\ US\$2012 \times 1.124$$

After adjusting for inflation, the total capital and annual operation and maintenance (O&M) costs were calculated using the capital costs in US$2020, and a highly conservative 2% annual O&M cost estimated by yearly O&M costs at the Rockport WWTP. A 15-yr life span was estimated for the BNR; 15 years is typical for the life span of this type of upgrade (Foley et al., 2007: Pollack et al., 2013):

$$15yr\ unit\ cost = CC\ US\$2020 + (CC\ US\$2020 \times 0.02O\&M \times 15yr)$$

The total unit cost is then divided by the WWTP processing capacity to provide a monetary value in US$2020 per MGD:

$$\$ \cdot MGD^{-1} = \frac{Total\ 15yr\ unit\ cost}{WWTP\ PC\ (MGD)}$$

This value is then used to calculate the value of the equivalent PC for the wetland with the following equation:

$$annualized\ wetland\ unit\ value = \frac{total\ 15\ year\ wetland\ unit\ cost}{15\ years}$$

The annualized unit cost is then multiplied by the 2% annual O&M cost to get the potential annual value for the N mitigation ecosystem service provided by the wetlands with this equation:

$$annualized\ wetland\ unit\ value + (total\ 15yr\ wetland\ unit\ cost \times 0.02)$$
$$= total\ annualized\ wetland\ unit\ value$$

This is the final yearly capital and O&M value of a BNR system to replace the denitrification capabilities of a specific wetland.

Statistical Analysis

All statistical analyses were performed using Microsoft Excel. One- and two-way analysis of variance (ANOVA) was used to compare mean denitrification rates, as well as environmental parameters to determine significance between sampling sites and seasons.

RESULTS

Seasonal Climate Variation

The climate data collected from NOAA Climate Data Online from station USW00012926 in Corpus Christi NAS, TX (https://www.ncdc.noaa.gov/cdo-web/search), showed that for the 10-year period between 2010 to 2019, total precipitation was highest in the fall, significantly higher in the fall compared to both winter and summer (ANOVA, $P>0.05$, Figure 12). Spring precipitation was significantly higher than winter, and higher, though not significantly ($P=0.06$), than summer. Spring and fall were similar in average total precipitation, so were winter and summer. From 2010 to 2019 there was no significant interannual differences in mean total annual precipitation between El Niño, La Niña, and ENSO-neutral years ($P>0.05$). There were also no significant differences in seasonal precipitation patterns between El Niño, La Niña, and ENSO-neutral years (ANOVA, $P>0.05$).

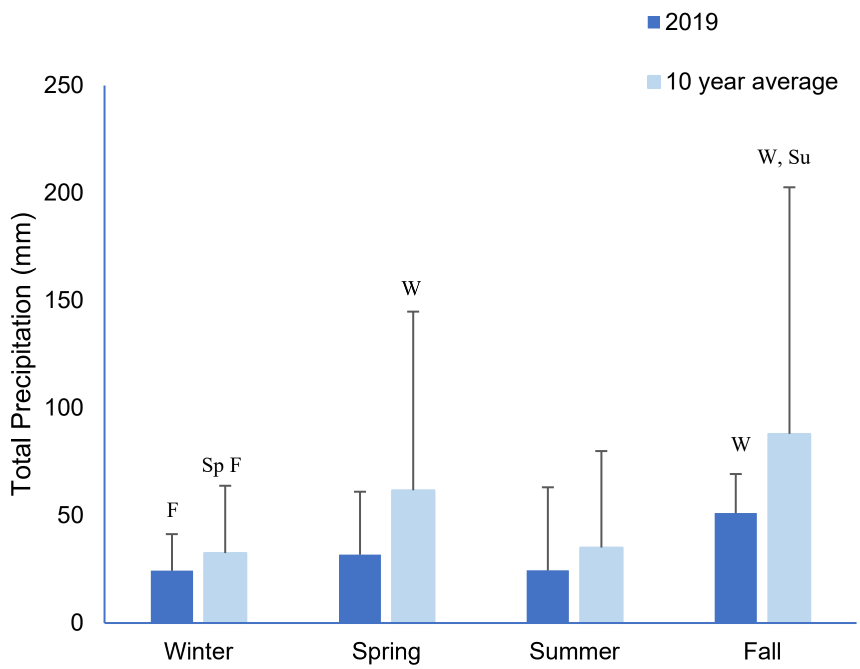


Figure 12. Total precipitation (mm) averaged seasonally for 2019 compared to the 10-year seasonal average from 2010 to 2019. **W-** significant difference from winter, **Sp-** significant difference from spring, **Su-** significant difference from summer, **F-** significant difference from fall.

There were significant differences in seasonal mean maximum air temperatures for all seasons in the 10-yr period. Winter was the coolest, followed by spring, fall, and then summer, respectively (ANOVA, $P<0.05$, Figure 13). There were no significant interannual differences in mean maximum air temperatures between El Niño, La Niña, and ENSO-neutral years ($P>0.05$).

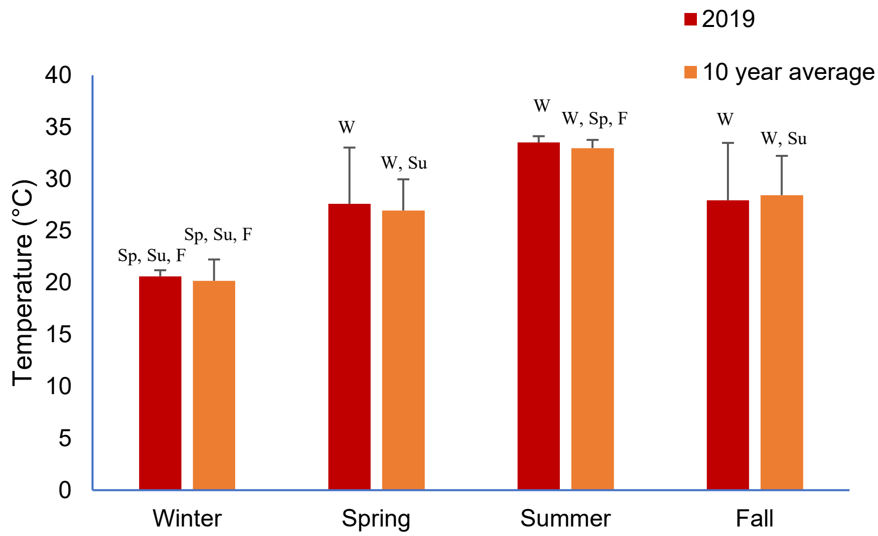


Figure 13. Seasonal mean maximum air temperature in 2019 and averaged for 10-year period from 2010 to 2019. **W-** significant difference from winter, **Sp-** significant difference from spring, **Su-** significant difference from summer, **F-** significant difference from fall.

There were significant seasonal differences seen in mean minimum air temperature between all seasons from 2010 and 2019, except between spring and fall. Winter was the coolest season, and minimum temperature increased with spring, fall, and summer, respectively ($P<0.05$, Figure 14). There were no significant differences in seasonal minimum air temperature patters between El Niño and La Niña years. There were higher minimum air temperatures in the winter during ENSO-neutral years compared to El Niño years, and El Niño summers had warmer minimum air temperatures compared to ENSO-neutral summers ($P<0.05$). Summers in La Niña years had higher minimum air temperatures compared to ENSO-neutral summers. There was no significant difference between La Niña and ENSO-neutral winter, spring, and fall or between El Niño and ENSO-neutral spring and fall.

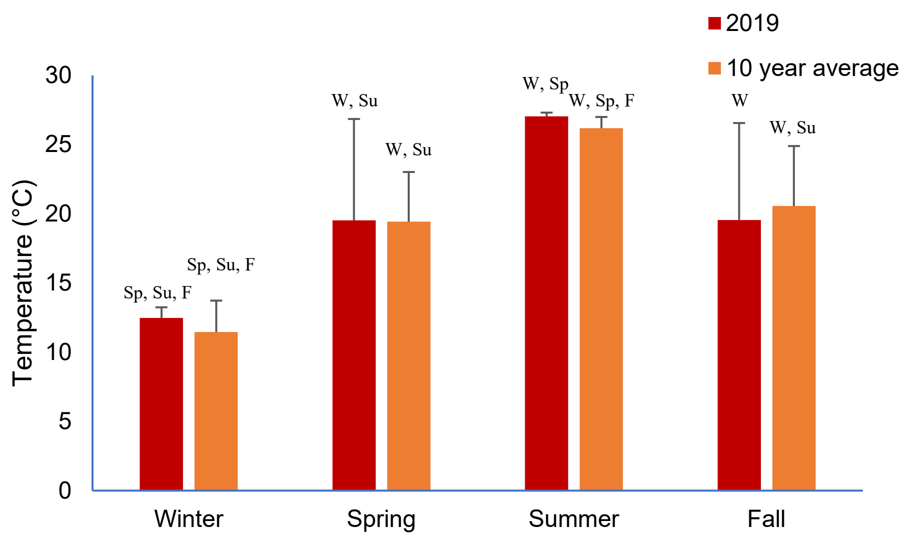


Figure 14. Seasonal mean minimum air temperature in 2019 and averaged for 10-year period from 2010 to 2019. **W-** significant difference from winter, **Sp-** significant difference from spring, **Su-** significant difference from summer, **F-** significant difference from fall.

Environmental Parameters

Environmental conditions varied across all sites (Figure 15). There was no difference between annual mean pH levels between all sampling sites. Mean annual DO concentrations varied between sampling sites, where concentrations were lowest at the Oso Bay WWTP. All wetland sites, as well as Bayside WWTP had significantly higher DO than the Oso Bay and Portland WWTPs (t-test, $P<0.05$). The mean annual temperature was significantly lower at the Bayside WWTP than all other sites. Between other sites there were not significant differences between annual mean water temperatures. Annual mean salinities varied significantly between sites; all wetland sites were significantly higher than all WWTPs. Nueces Bay restored marsh and Naval Airbase Bridge site had significantly higher salinities compared to Oso Bay marsh, Aransas River Estuary, and Egery Flats restored marsh, and all wetlands have higher salinities than the WWTPs, forming a salinity gradient (t-test, $P<0.05$).

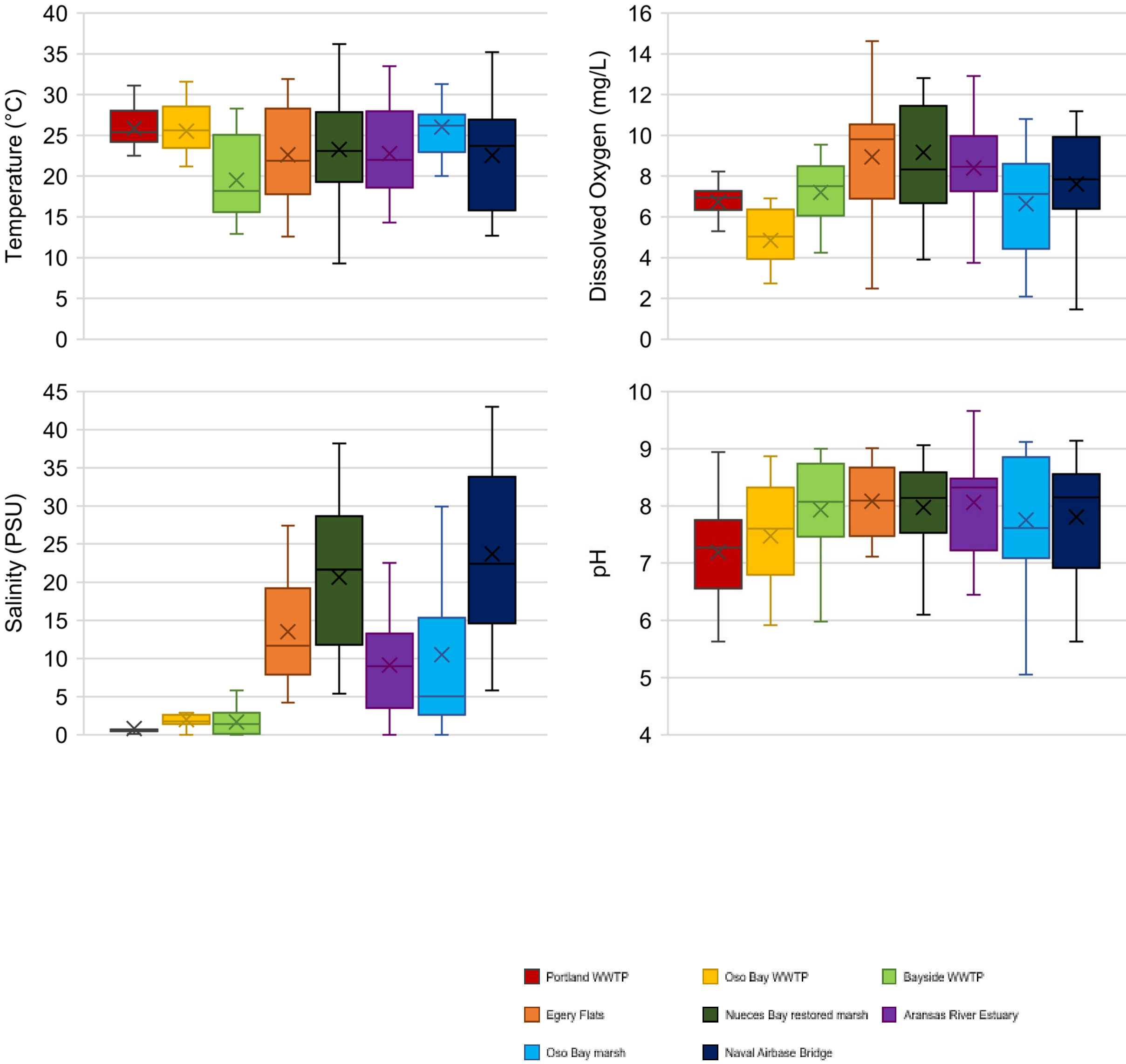


Figure 15. Annual environmental variables at each sampling site including water temperature (upper left), DO concentration (upper right), salinity (lower left), and pH (lower right) for surface water.

Environmental conditions varied by season at some sites (Figure 16). Summer showed highest temperatures, and winter showed the lowest temperatures for all sites. There were no significant differences between fall and spring water temperatures for all sites (t-test, $P>0.05$). DO was significantly lower at the Naval Airbase Bridge site during the summer, and lower in the fall compared to spring or winter. There were no significant differences in DO between seasons in other sites. The highest DO concentrations were found at WWTP sites compared to wetland sites. The Aransas River Estuary was the only site that showed significant seasonal variation with salinity. Fall and winter salinities were significantly lower than spring or summer (ANOVA, t-test, $P<0.05$). There were significant differences in pH levels between seasons at three sites. Bayside WWTP and Egery Flats had higher pH in the winter and spring in comparison to summer and fall, and the Naval Airbase Bridge had lower levels in the fall compared to all other seasons. All sites had low mean annual N_2O and CH_4 concentrations. The mean annual CO_2 concentrations were higher compared to N_2O and CH_4 (Table 1).

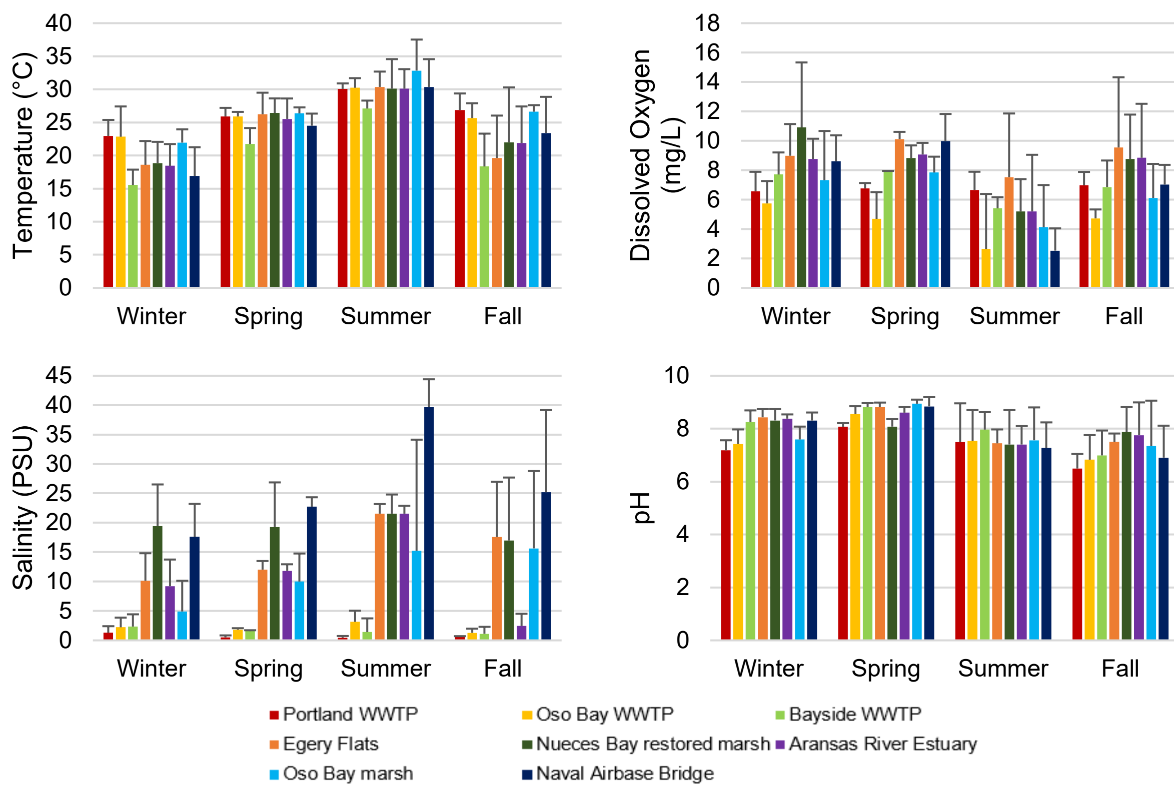


Figure 16. Bar graph depicting seasonal means of environmental variables for each sampling site including water temperature (upper left), dissolved oxygen (upper right), salinity (lower left), and pH (lower right).

Table 1. Annual mean dissolved gas concentrations for nitrous oxide, carbon dioxide, and methane for all sampling sites (mean ± standard deviation).

Site	N_2O (µM)	CO_2 (µM)	CH_4 (µM)
Portland WWTP	1.21 ± 1.49	154.22 ± 119.00	0.24 ± 0.27
Oso Bay WWTP	2.39 ± 1.72	185.65 ± 137.57	0.09 ± 0.07
Bayside WWTP	0.02 ± 0.05	114.42 ± 73.45	2.00 ± 4.00
Egery Flats	0.00 ± 0.01	56.54 ± 67.47	0.14 ± 0.15
Nueces Bay Restored Marsh	0.02 ± 0.07	36.76 ± 49.47	0.10 ± 0.16
Aransas River Estuary	0.01 ± 0.03	49.90 ± 59.02	0.20 ± 0.38
Oso Bay Marsh	0.49 ± 0.78	65.72 ± 74.30	0.13 ± 0.09
Naval Airbase Bridge	0.01 ± 0.01	24.85 ± 17.77	0.06 ± 0.08

Nitrogen Based Nutrients

Nutrient concentrations varied at each site (Table 2, Table 3). Mean annual NH_4^+ concentrations at the Portland WWTP, 418 µM, was significantly higher than all other sites ($P<0.05$, Figure 17). The mean annual NO_3^- concentration at the Portland WWTP, 394 µM, was significantly higher than all other sites ($P<0.05$, Figure 18). Mean annual NO_2^- concentration at the Portland WWTP, 23 µM, was significantly higher than all other sites ($P<0.05$, Figure 19). Mean annual NH_4^+ concentration at the Oso Bay WWTP was significantly higher than all other sites except the Portland WWTP. The annual mean NO_3^- concentration, 161 µM, was higher than all other sites aside from the Portland WWTP ($P>0.05$). The annual mean NO_2^- concentration at the Oso Bay WWTP, 4 µM, showed no significant differences between any of the wetland sites, except for Egery Flats, 0.95 µM. The mean annual NH_4^+ concentration at the Bayside WWTP, 24 µM, was significantly higher than at the Aransas River Estuary, 14 µM, and was significantly lower than the Oso Bay marsh, 24 µM. There were no other differences in mean annual NH_4^+ concentration between Bayside WWTP and the other wetlands sites. The annual mean NO_3^-

concentration, 7.8 μM, was significantly higher than all wetland sites except for the Oso Bay marsh, 124 μM. The annual mean NO_2^- concentration at the Bayside WWTP, 8.6 μM, was significantly higher than all other wetland sites, except for the Oso Bay marsh, 5 μM.

The Oso Bay marsh mean annual NH_4^+ concentration was higher than all other wetland sites. The Oso Bay marsh had significantly higher mean annual NO_3^- concentration compared to all other wetland sites ($P>0.05$). The annual mean NO_2^- concentration at the Oso Bay marsh was significantly higher than all other wetland sites. There were no significant differences in annual mean NO_2^- concentration between any of the other wetlands. The Nueces Bay restored marsh site had higher mean annual NH_4^+ concentration than the Aransas River Estuary. There were no significant differences between the Nueces Bay restored site and Egery Flats or the Naval Airbase Bridge. The mean annual NO_3^- concentration was significantly lower than the concentrations at Oso Bay marsh, and Naval Airbase Bridge, but had no significant difference from Egery Flats and Aransas River Estuary. The mean annual NH_4^+ concentration at the Egery Flats restored marsh, 16 μM, was significantly lower than the Oso Bay marsh, but had no significant differences from any other wetland sites. The annual mean NO_3^- concentration, 1.18 μM, was significantly lower than the Oso Bay marsh as well as the Naval Airbase Bridge.

Nutrient concentrations also varied between seasons for each site. Winter and fall NH_4^+ concentrations at the Portland WWTP were significantly higher than in the spring and summer, spring was significantly higher than the summer NH_4^+ concentration (Figure 20). Winter NO_3^- concentrations, 461 μM, were significantly higher than summer. There were no other significant differences between seasonal NO_3^- concentrations at this site (Figure 22). NO_2^- concentration showed no seasonal variation in the Portland WWTP effluent (Figure 23).

The NH_4^+ concentrations at the Oso Bay WWTP showed significantly higher levels during the winter compared to the spring but showed no other significant seasonal differences. Winter NO_3^- concentrations were significantly higher than spring and summer, but not significantly different from fall. NO_2^- concentrations in the summer were significantly higher in the summer compared to the spring, but there were no other significant seasonal differences in the Oso Bay WWTP effluent.

The NH_4^+ concentrations at the Bayside WWTP were significantly higher in the summer compared to the winter, but there were no other differences between seasons at this site. Fall showed significantly higher NO_3^- concentrations compared to spring, but Bayside WWTP showed no other differences in NO_3^- concentrations across seasons. The NO_2^- concentrations in the fall were significantly higher than in the spring, but there were no other significant differences between seasons at the Bayside WWTP.

At the Egery Flats restored marsh site, NH_4^+ concentrations were significantly higher in the summer compared to spring and fall. There was no significant difference in NH_4^+ concentration between winter and summer. There were no significant seasonal differences in NO_3^- concentrations at the Egery Flats restored marsh site. There were no seasonal variations in NO_2^- concentrations at Egery Flats.

The Nueces Bay restored marsh had highest NH_4^+ concentrations in the summer compared to all seasons. Winter had higher NH_4^+ concentrations than spring, but no significant difference with fall. Fall showed higher concentrations than spring at this site. Winter showed NO_3^- concentrations that were significantly higher than spring and fall. The winter season showed significantly higher NO_2^- concentrations than spring, there were no other significant seasonal differences in NO_2^- concentrations in the Nueces Bay restored marsh surface waters.

At Aransas River Estuary, summer had higher concentration than all other seasons. Winter, and fall both had significantly higher NH_4^+ concentrations compared to spring. There are no significant differences in NO_3^- concentrations across seasons. Fall showed significantly higher NO_2^- concentrations than spring, but the Aransas River Estuary showed no other significant seasonal differences in NO_2^- concentrations.

The Oso Bay marsh showed significantly higher NH_4^+ concentrations in the summer compared to winter and spring, as well as fall, but not significantly. Winter had the lowest NH_4^+ concentration. Winter NO_3^- concentrations were significantly higher than all other seasons. Summer showed significantly higher NO_2^- concentrations than both spring and winter, and fall concentrations were the highest concentrations compared to all seasons.

The NH_4^+ concentrations at Naval Airbase Bridge were highest in the Summer compared to all seasons, and lowest in the spring. There was no significant difference between winter and fall NH_4^+ concentrations. NO_3^- concentrations in the winter were significantly higher than in spring or summer, there were no other significant differences in NO_3^- concentrations between seasons. Summer and fall NO_2^- concentrations were significantly higher than winter and spring but were not significantly different from each other.

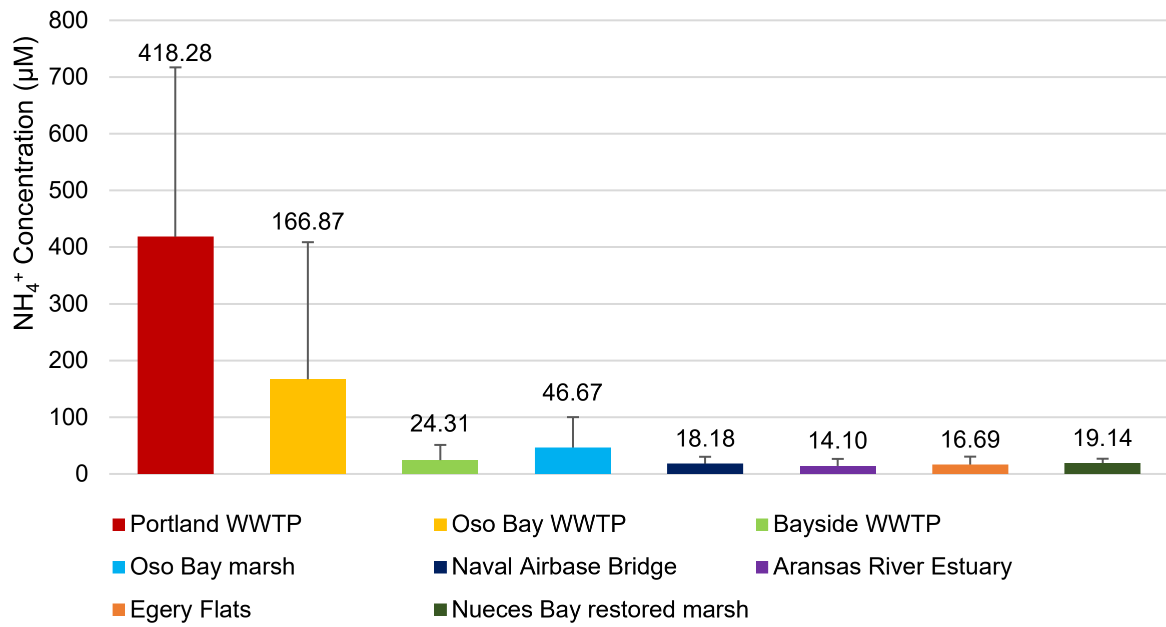


Figure 17. Mean annual ammonium concentrations for all sampling sites.

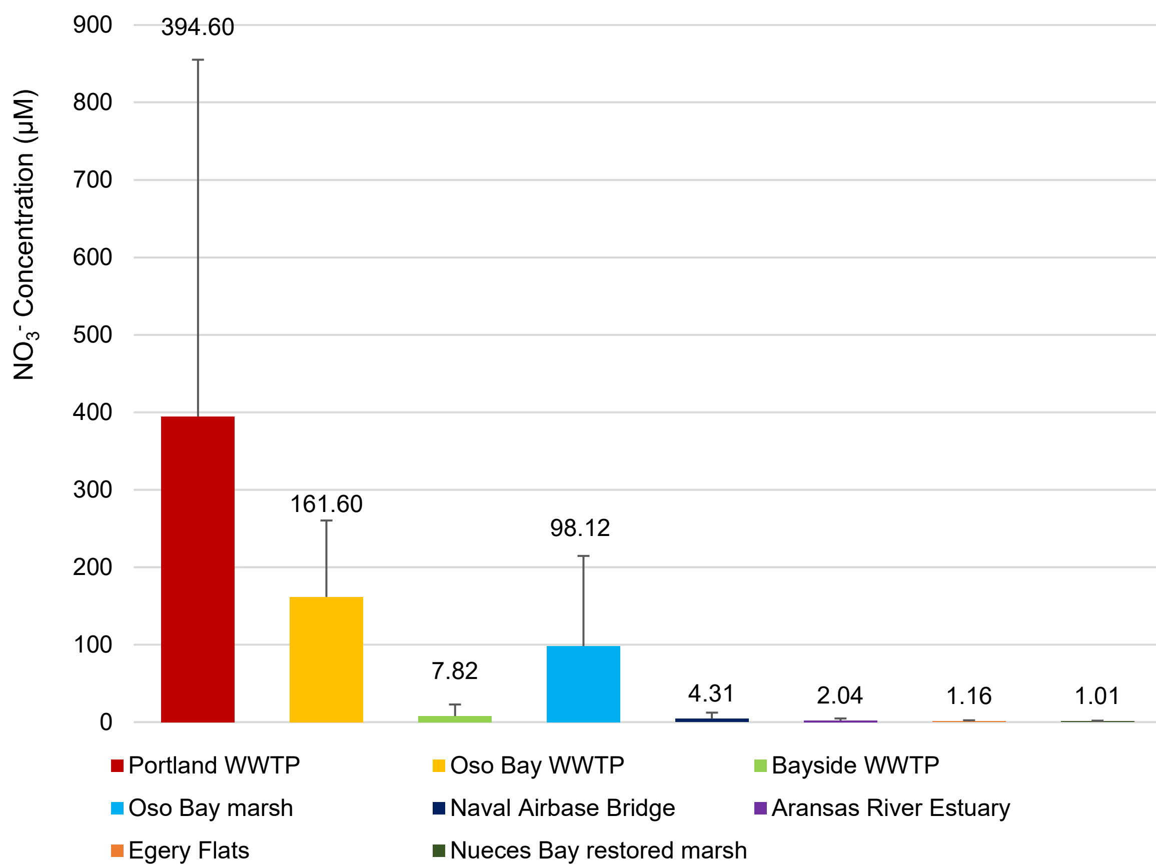


Figure 18. Mean annual nitrate concentrations for all sampling sites.

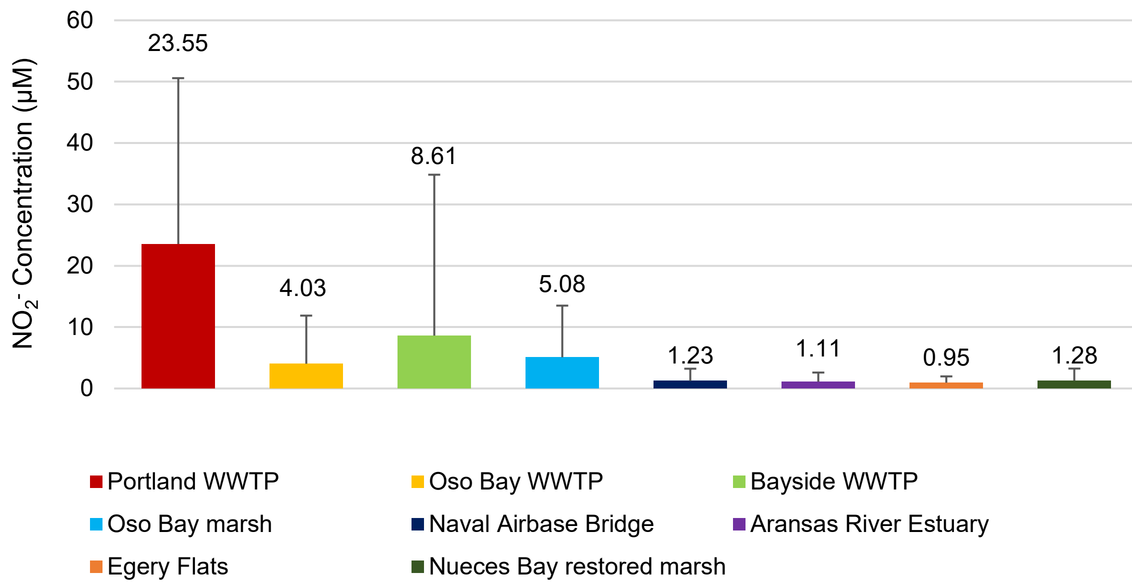
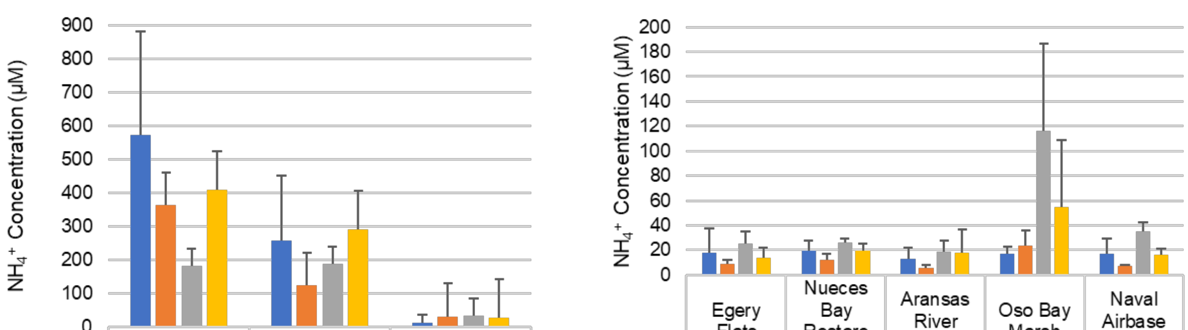


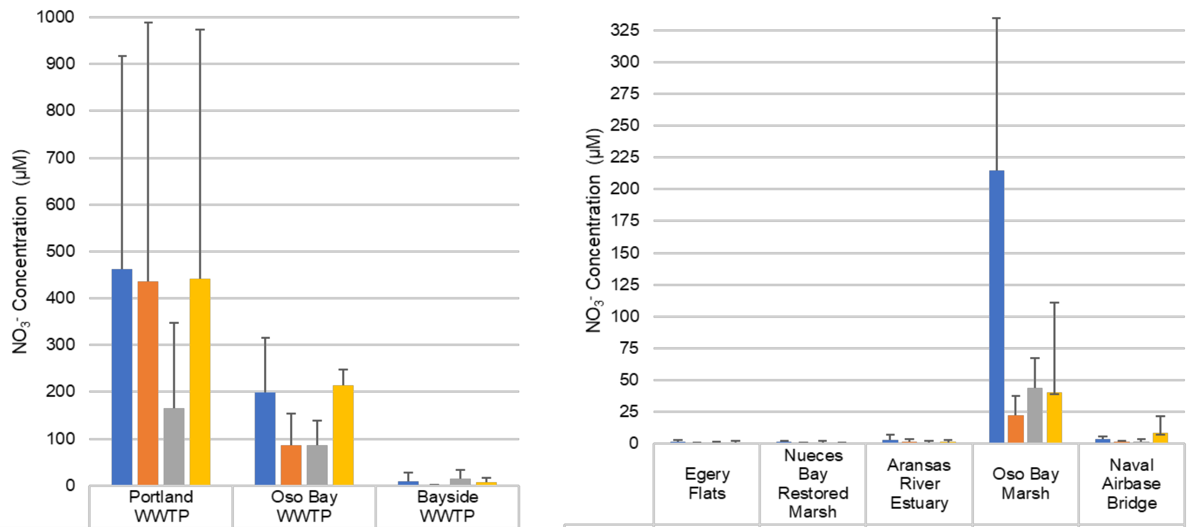
Figure 19. Mean annual nitrite concentrations for all sampling sites.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	572.88	257.66	12.78
Spring	361.97	122.96	31.02
Summer	181.79	187.37	34.54
Fall	409.45	291.80	28.23

	Egery Flats	Nueces Bay Restore d Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	17.82	19.14	12.93	17.00	16.70
Spring	8.57	11.77	5.64	23.95	7.05
Summer	25.29	25.81	18.43	116.32	35.17
Fall	14.13	19.60	18.26	55.00	16.53

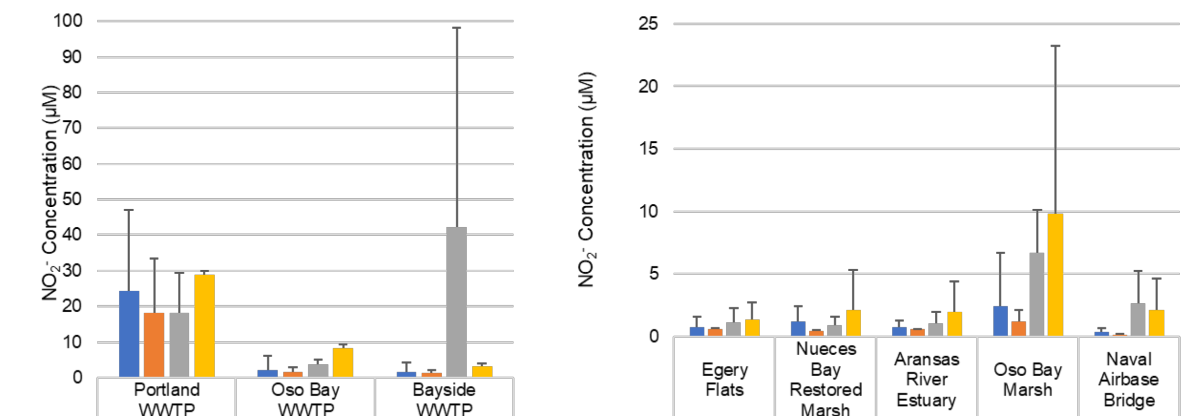
Figure 20. Mean ammonium concentrations by season for all sites, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	461.37	199.50	9.05
Spring	435.68	85.36	0.66
Summer	164.63	85.37	14.49
Fall	442.10	213.88	6.72

	Egery Flats	Nueces Bay Restored Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	1.46	1.41	3.01	215.01	3.71
Spring	0.77	0.44	1.97	22.72	1.60
Summer	1.08	1.23	1.24	43.91	1.55
Fall	1.09	0.65	1.47	40.39	8.28

Figure 21. Mean nitrate concentrations by season for all sites, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	24.45	2.12	1.58
Spring	18.13	1.48	1.26
Summer	18.25	3.85	42.14
Fall	28.97	8.25	3.11

	Egery Flats	Nueces Bay Restored Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	0.75	1.22	0.70	2.39	0.37
Spring	0.60	0.43	0.56	1.21	0.13
Summer	1.11	0.86	1.02	6.71	2.65
Fall	1.32	2.10	1.98	9.84	2.08

Figure 22. Mean nitrite concentrations by season for all sites, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.

Table 2. Average monthly nutrient concentrations for three wastewater treatment plants.

Sampling Site	Sampling Period	NH_4^+ Average (µM)	NH_4^+ Std. Dev.	NO_3^- Average (µM)	NO_3^- Std. Dev.	NO_2^- Average (µM)	NO_2^- Std. Dev.
Portland WWTP	Oct-18	110.38	16.04	1352.79	13.82	6.16	0.29
	Nov-18	330.98	28.21	984.43	64.98	5.60	0.99
	Dec-18	373.31	14.18	748.67	1.64	16.96	0.05
	Jan-19	406.64	18.09	530.93	5.35	20.49	1.99
	Feb-19	468.02	36.10	1201.81	35.85	63.04	8.46
	Mar-19	474.78	0.63	1243.65	29.29	38.14	2.41
	Apr-19	405.29	11.73	170.84	4.36	11.21	2.35
	May-19	257.90	5.64	73.58	4.91	5.03	0.23
	Jun-19	25.25	0.54	3.95	1.20	5.88	1.08
	Jul-19	304.16	3.99	444.34	2.39	31.94	0.35
	Aug-19	204.58	14.42	138.83	2.29	21.48	0.88
	Sep-19	554.12	12.61	17.22	2.73	27.99	1.07
	Oct-19	38.73	1.81	323.49	65.97	124.91	10.06
	Nov-19	937.70	7.53	16.90	1.49	12.16	0.28
	Dec-19	191.57	11.35	745.14	44.97	44.50	2.21
	Jan-20	1006.04	12.95	2.04	0.50	4.30	0.03
	Feb-20	874.87	5.75	0.00	0.00	4.08	0.05
Oso Bay WWTP	Oct-18	29.92	1.32	235.34	37.07	0.46	0.05
	Nov-18	68.31	4.78	216.41	30.49	0.38	0.05
	Dec-18	161.49	8.50	285.34	5.97	0.36	0.03
	Jan-19	84.11	32.65	238.13	1.67	0.45	0.11
	Feb-19	36.54	10.50	106.89	23.13	0.41	0.08
	Mar-19	81.68	8.71	190.70	37.75	3.49	0.66
	Apr-19	211.91	1.37	68.33	8.10	0.59	0.02
	May-19	29.87	4.67	32.15	8.00	0.37	0.03
	Jun-19	65.56	5.08	16.64	0.05	3.12	1.18
	Jul-19	346.66	8.68	135.18	11.21	5.33	0.55
	Aug-19	111.09	5.05	79.39	0.79	3.09	0.32
	Sep-19	1085.07	32.87	192.83	26.37	38.79	3.63
	Oct-19	52.94	7.00	254.77	24.93	2.67	0.29
	Nov-19	333.07	5.76	184.66	15.09	9.12	0.43
	Dec-19	8.96	0.05	107.88	8.51	0.46	0.06
	Jan-20	16.94	2.37	376.15	14.89	0.29	0.01
	Feb-20	334.65	3.65	93.24	1.87	10.73	0.03
Bayside WWTP	Oct-18	107.34	35.38	5.50	1.00	1.95	0.68
	Nov-18	7.04	2.56	23.17	2.04	0.98	0.02
	Dec-18	66.81	3.48	58.62	1.96	9.02	0.20
	Jan-19	9.63	0.18	0.83	0.78	0.86	0.11
	Feb-19	5.93	0.24	4.07	2.00	0.54	0.02
	Mar-19	15.70	1.49	0.73	0.77	0.68	0.12
	Apr-19	23.72	3.78	0.38	0.66	0.82	0.02
	May-19	53.66	5.74	0.86	0.28	2.29	0.12
	Jun-19	35.16	1.42	0.00	0.00	1.76	0.38
	Jul-19	34.23	4.72	3.71	0.17	1.35	0.11
	Aug-19	34.09	4.64	39.77	0.83	109.84	2.78
	Sep-19	13.18	1.17	0.11	0.07	5.84	1.56
	Oct-19	32.03	4.57	2.25	0.21	6.24	0.78
	Nov-19	0.86	0.18	2.17	0.75	0.56	0.12
	Dec-19	4.34	0.35	0.74	0.19	0.50	0.08
	Jan-20	1.90	0.11	4.36	0.49	0.51	0.00
	Feb-20	2.74	0.91	0.53	0.14	0.52	0.01

Table 3. Average monthly nutrient concentrations for five wetland sites.

Sampling Site	Sampling Period	NH_4^+ Average (µM)	NH_4^+ Std. Dev.	NO_3^- Average (µM)	NO_3^- Std. Dev.	NO_2^- Average (µM)	NO_2^- Std. Dev.
Egery Flats restored marsh	Oct-18	13.28	2.04	3.02	0.67	0.65	0.01
	Nov-18	4.24	0.90	0.87	0.16	0.62	0.01
	Dec-18	4.51	0.77	2.36	1.23	2.22	1.08
	Jan-19	3.74	0.48	2.60	0.65	0.62	0.01
	Feb-19	4.46	1.62	4.44	0.54	0.56	0.13
	Mar-19	13.00	2.60	0.89	0.09	0.62	0.02
	Apr-19	7.47	1.56	1.06	0.16	0.62	0.02
	May-19	5.24	0.85	0.35	0.14	0.58	0.03
	Jun-19	11.93	8.73	0.24	0.25	0.38	0.02
	Jul-19	25.28	2.04	2.30	0.14	0.29	0.01
	Aug-19	34.21	3.43	0.70	0.35	2.65	0.32
	Sep-19	26.46	0.98	0.15	0.05	3.63	0.35
	Oct-19	10.31	1.11	0.62	0.18	1.56	1.31
	Nov-19	16.06	0.69	0.80	0.34	0.15	0.01
	Dec-19	59.53	6.35	0.33	0.19	0.28	0.01
	Jan-20	26.19	1.39	0.00	0.00	0.41	0.01
	Feb-20	11.35	0.82	0.00	0.00	0.24	0.01
Nueces Bay restored marsh	Oct-18	16.10	0.84	1.14	1.02	0.42	0.10
	Nov-18	13.44	0.99	0.43	0.16	0.37	0.02
	Dec-18	12.83	1.50	1.81	0.97	0.40	0.02
	Jan-19	12.94	0.46	1.41	0.53	0.45	0.02
	Feb-19	12.82	0.97	1.83	0.36	0.40	0.04
	Mar-19	16.14	2.38	0.91	0.71	0.49	0.13
	Apr-19	13.64	2.92	0.64	0.60	0.43	0.07
	May-19	5.52	1.34	0.00	0.00	0.37	0.01
	Jun-19	23.22	3.40	0.43	0.18	0.43	0.05
	Jul-19	25.47	3.03	2.34	0.04	0.34	0.11
	Aug-19	28.75	1.61	0.93	0.12	1.82	0.23
	Sep-19	29.46	0.26	0.36	0.20	8.30	0.71
	Oct-19	20.86	0.46	0.57	0.05	1.32	0.19
	Nov-19	18.54	2.34	0.74	0.16	0.10	0.02
	Dec-19	13.97	0.97	2.27	0.36	3.37	0.34
	Jan-20	32.35	0.90	1.15	1.94	2.15	0.02
	Feb-20	27.86	1.81	0.00	0.00	0.55	0.01
Aransas River Estuary	Oct-18	1.27	0.71	1.52	0.35	0.81	0.04
	Nov-18	3.19	0.82	3.62	1.45	0.96	0.07
	Dec-18	3.82	0.41	7.00	1.00	1.83	0.01
	Jan-19	9.65	1.04	10.08	0.60	1.38	0.17
	Feb-19	1.67	1.12	2.48	1.00	0.59	0.02
	Mar-19	6.88	1.48	3.73	0.54	0.57	0.03
	Apr-19	7.22	0.31	0.58	0.38	0.55	0.06
	May-19	2.20	0.94	0.88	N/A	0.56	0.04
	Jun-19	6.87	6.53	0.31	0.22	0.41	0.02
	Jul-19	20.94	3.57	2.77	0.23	0.35	0.02
	Aug-19	26.21	0.83	0.65	0.13	2.30	0.28
	Sep-19	26.23	0.51	0.14	0.10	6.54	0.23
	Oct-19	13.66	1.16	1.28	0.32	1.45	0.12
	Nov-19	51.47	7.81	0.78	0.14	0.12	0.01
	Dec-19	16.61	0.20	0.20	0.13	0.27	0.02
	Jan-20	19.28	2.05	0.43	0.12	0.26	0.03
	Feb-20	26.53	3.67	0.00	0.00	0.27	0.01

Oso Bay marsh	Oct-18	1.32	0.22	19.35	4.52	1.21	0.20
	Nov-18	59.31	7.53	194.63	56.46	0.44	0.18
	Dec-18	17.43	4.68	224.00	65.60	0.76	0.36
	Jan-19	16.10	2.47	139.20	8.24	0.35	0.07
	Feb-19	1.36	N/A	66.45	8.52	0.31	0.13
	Mar-19	12.87	8.57	38.97	17.28	2.06	0.76
	Apr-19	34.04	8.64	16.29	6.18	1.49	0.76
	May-19	19.89	4.01	12.91	4.31	0.36	0.08
	Jun-19	24.86	2.62	16.93	1.84	2.24	0.25
	Jul-19	124.09	7.69	34.77	1.48	10.26	0.09
	Aug-19	196.12	4.24	71.04	1.92	6.14	0.95
	Sep-19	147.32	3.87	14.62	2.48	20.78	0.60
	Oct-19	17.77	0.87	13.16	0.89	34.50	3.12
	Nov-19	31.39	2.43	2.53	0.44	0.47	0.04
	Dec-19	24.00	5.08	113.45	17.23	0.56	0.10
	Jan-20	13.86	0.85	242.26	7.14	0.54	0.01
	Feb-20	18.95	0.37	399.05	8.69	11.79	0.22
Naval Airbase Bridge	Oct-18	11.38	0.22	4.27	2.40	0.68	0.35
	Nov-18	10.56	0.71	34.37	3.83	1.99	0.11
	Dec-18	5.41	0.15	3.66	0.20	0.12	0.02
	Jan-19	6.80	0.62	7.05	0.45	0.09	0.02
	Feb-19	6.59	0.89	3.74	0.48	0.15	0.07
	Mar-19	5.83	0.59	2.57	0.56	0.17	0.04
	Apr-19	7.52	0.24	1.14	0.32	0.12	0.03
	May-19	8.18	0.66	1.10	0.55	0.09	0.03
	Jun-19	25.03	1.09	0.06	0.06	0.37	0.01
	Jul-19	36.86	1.21	0.33	0.18	1.52	0.41
	Aug-19	40.24	4.58	4.26	0.82	6.06	0.56
	Sep-19	21.62	1.24	1.17	0.17	6.78	0.43
	Oct-19	15.81	2.46	0.65	0.30	0.83	0.26
	Nov-19	21.28	1.34	0.95	0.15	0.11	0.01
	Dec-19	20.88	0.04	2.22	0.14	0.66	0.02
	Jan-20	35.90	1.28	3.12	2.02	0.53	0.01
	Feb-20	30.64	1.12	2.49	0.10	0.64	0.03

Nitrogen Stable Isotopes

The $\delta^{15}N$- NO_3^- in the Portland WWTP ranged from 1.43 to 10.70‰, and the $\delta^{15}N$- NH_4^+ in the Portland WWTP ranged from -4.70 to 1.30‰ (Figure 23, Table 4, Table 5). The $\delta^{15}N$- NO_2^- in the Portland WWTP effluent ranged from -19.60 to 14.00‰ (Table 6). The Oso Bay WWTP had slightly higher $\delta^{15}N$- NO_3^- values which ranged from 1.90 to 17.84‰, and the $\delta^{15}N$- NH_4^+ values ranged from -8.40 to -0.70‰ of ammonium fertilizers, ammonium volatilization, and soil ammonium. The $\delta^{15}N$- NO_2^- in the Oso Bay WWTP effluent ranged from -7.10 to 2.70‰. The $\delta^{15}N$- NO_3^- in the Bayside WWTP were higher than both Portland and Oso Bay WWTP, ranging from 26.26 to 28.43‰, and the $\delta^{15}N$- NH_4^+ values ranged from 7.40 to 13.00‰, which were significantly higher than both the Portland WWTP and the Oso Bay WWTP values. The $\delta^{15}N$-NO_2^- in the Bayside WWTP effluent ranged from 11.60 to 24.10‰.

Egery Flats had a wide range of $\delta^{15}N$-NO_3^- from -2.60 to 42.69‰. The Nueces Bay restored marsh had very light $\delta^{15}N$-NO_3^- and $\delta^{15}N$-NH_4^+ values, ranging from -21.48 to -17.67‰, and -21.90and -20.20‰, respectively. The $\delta^{15}N$-NO_2^- at the Nueces Bay restored marsh ranged from 6.33 to 6.8‰. The Aransas River Estuary had very light $\delta^{15}N$-NO_3^- values ranging from -9.30 to -2.87‰. The $\delta^{15}N$-NO_2^- at the Aransas River Estuary ranged from -21.8 to -18.6‰. Oso Bay marsh and the Naval Airbase Bridge sites had very similar $\delta^{15}N$-NO_3^- values ranging from -3.00 to 3.30‰, and -3.0 to 1.69‰, respectively. The Oso Bay marsh $\delta^{15}N$-NH_4^+ values range from -7.40 to 6.8‰. The $\delta^{15}N$-NO_2^- at the Oso Bay marsh ranged from -4.00 to -5.60‰. Naval Airbase Bridge $\delta^{15}N$-NH_4^+ ranged from -6.50 to -4.30‰. The $\delta^{15}N$-NO_2^- at the Naval Airbase Bridge ranged from -21.10 to -19.9‰. Due to constraints with the method and interference with dissolved organic nitrogen in the sample the $\delta^{15}N$- NH_4^+ values for Egery Flats and Aransas River Estuary were not able to be reported.

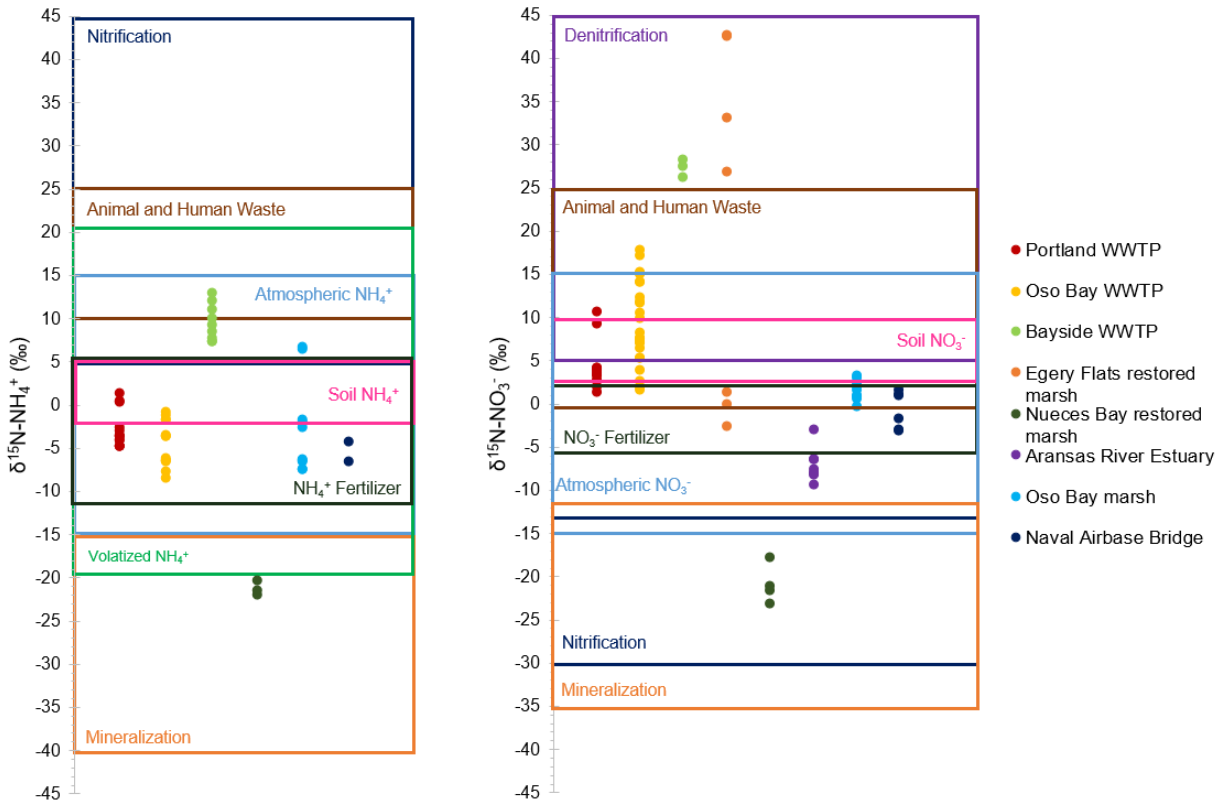


Figure 23. $\delta^{15}N\text{-}NH_4^+$ source plot with ranges reported for various NH_4^+ sources based on Kendall et al., (2007) for all sampling sites (left). $\delta^{15}N\text{-}NO_3^-$ source plot with ranges reported for various NO_3^- sources based on Kendall et al., (2007) for all sampling sites (right). Isotope source and process values adapted from Kendall et al. (2007).

Table 4. Average $\delta^{15}N-NH_4^+$ values for each site by sampling period.

Sampling Site	Sampling Period	NH_4^+ $\delta^{15}N$ Average	Std Dev
Portland WWTP	Oct-18	0.78	0.68
	Feb-19	-3.57	0.14
	May-19	-1.52	2.93
	Jul-19	-4.06	0.04
	Nov-19	-2.80	0.21
	Dec-19	-4.73	0.02
Oso Bay WWTP	Feb-19	-8.06	0.50
	Mar-19	-0.91	0.23
	Apr-19	-1.62	0.01
	Jun-19	-3.54	0.10
	Sep-19	-6.47	0.17
	Feb-20	-6.25	0.31
Bayside WWTP	Oct-18	9.26	0.68
	Dec-18	12.04	0.93
	May-19	7.52	0.23
Nueces Bay restored marsh	Oct-18	-21.18	0.85
Oso Bay marsh	Jun-19	-2.05	0.49
	Aug-19	-6.80	0.55
	Oct-19	-6.86	0.80
	Feb-20	6.62	0.28
Naval Airbase Bridge	Nov-18	-5.40	1.58

Table 5. Average $\delta^{15}N-NO_3^-$ values for each site by sampling period.

Sampling Site	Sampling Period	NO_3^- $\delta^{15}N$ Average	Std Dev
Portland WWTP	Feb-19	3.28	0.44
	May-19	2.02	0.61
	Jul-19	3.50	N/A
	Nov-19	9.96	1.09
	Dec-19	4.08	0.14
Oso Bay WWTP	Oct-18	7.06	0.59
	Dec-18	8.59	1.19
	Feb-19	13.42	1.32
	Mar-19	15.18	0.17
	Apr-19	17.48	0.51
	Jun-19	11.56	0.43
	Sep-19	6.62	1.43
	Oct-19	1.86	0.14
	Feb-20	3.98	1.83
Bayside WWTP	Dec-18	27.41	1.09
Egery Flats restored marsh	Oct-18	42.63	0.08
	Dec-18	-0.40	2.02
	Feb-19	30.06	4.47
Nueces Bay restored marsh	Oct-18	-20.38	3.82
	Mar-19	-21.29	0.27
Aransas River Estuary	Nov-18	-7.76	0.21
	Jan-19	-6.43	2.52
	Mar-19	-7.96	1.44
Oso Bay marsh	Dec-18	2.43	0.54
	Mar-19	0.89	0.18
	Jun-19	1.61	1.79
Naval Airbase Bridge	Nov-18	-2.52	0.74
	Jan-19	1.30	0.36

Table 6. Average $\delta^{15}N$-NO_2^- values for each site by sampling period.

Sampling Site	Sampling Period	NO_2^- $\delta^{15}N$ Average	Std Dev
Portland WWTP	Oct-18	-5.68	4.71
	Feb-19	-12.24	1.11
	May-19	-17.31	3.26
	Jul-19	1.26	0.35
	Nov-19	13.65	0.44
	Dec-19	-1.06	0.06
Oso Bay WWTP	Mar-19	1.75	0.10
	Jun-19	0.36	4.49
	Sep-19	-6.43	0.56
	Feb-20	2.61	0.14
Bayside WWTP	Dec-18	12.42	1.43
	Aug-19	24.00	0.07
Nueces Bay restored marsh	Dec-19	6.58	0.30
Aransas River Estuary	Nov-18	-20.70	0.13
Oso Bay marsh	Mar-19	4.06	0.14
	Jun-19	-3.31	1.04
	Aug-19	-2.71	0.53
	Oct-19	5.44	0.17
	Feb-20	3.12	0.17
Naval Airbase Bridge	Nov-18	-20.67	0.86

Quantification of Nitrogen Mitigation Ecosystem Service Flows

Annual denitrification rates varied significantly between wetland sites ($P<0.05$). The Nueces Bay restored marsh had the lowest annual mean denitrification rate (14.16 ± 15.18 kg $N \cdot ha^{-2} \cdot yr^{-1}$), where the highest rate (44.30 ± 38.26 kg $N \cdot ha^{-2} \cdot yr^{-1}$) was measured at Naval Airbase Bridge (Figure 24). Egery Flats, Nueces Bay restored marsh, and Aransas River Estuary all had significantly lower annual mean denitrification rates compared to Oso Bay marsh and Naval Airbase Bridge ($P<0.05$).

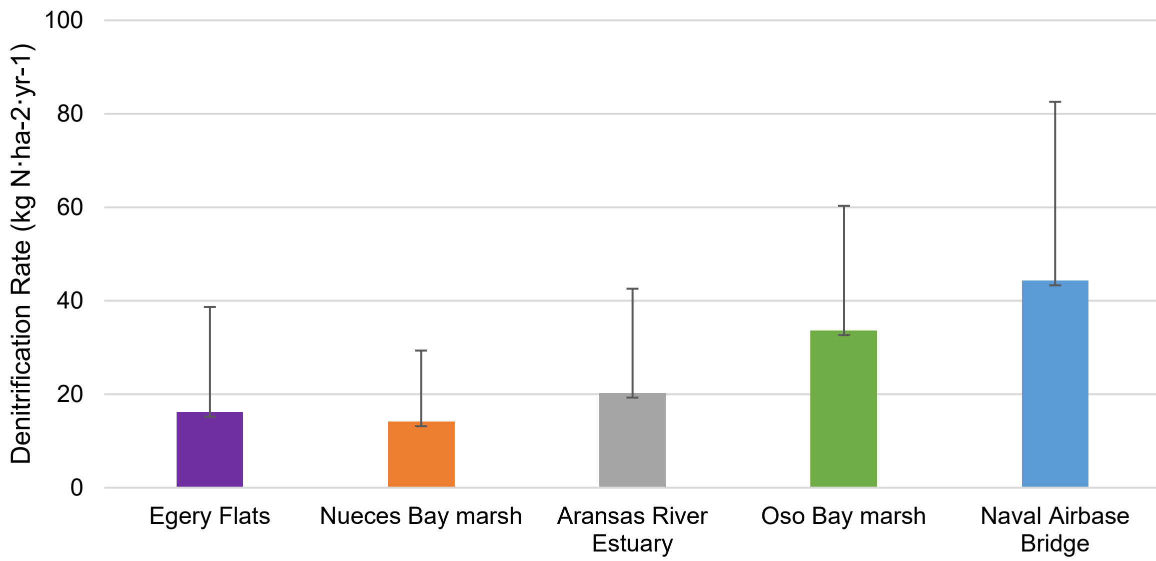


Figure 24. Mean annual denitrification rates for all wetland sites.

Seasonal denitrification rates were calculated for all five wetlands sites (Figure 25). Summer rates were significantly higher than spring and winter but were not significantly different than fall for all sites. There was no significant linear correlation between potential denitrification rates and environmental parameters (pH, water temperature, salinity, and DO) (R^2<0.5).

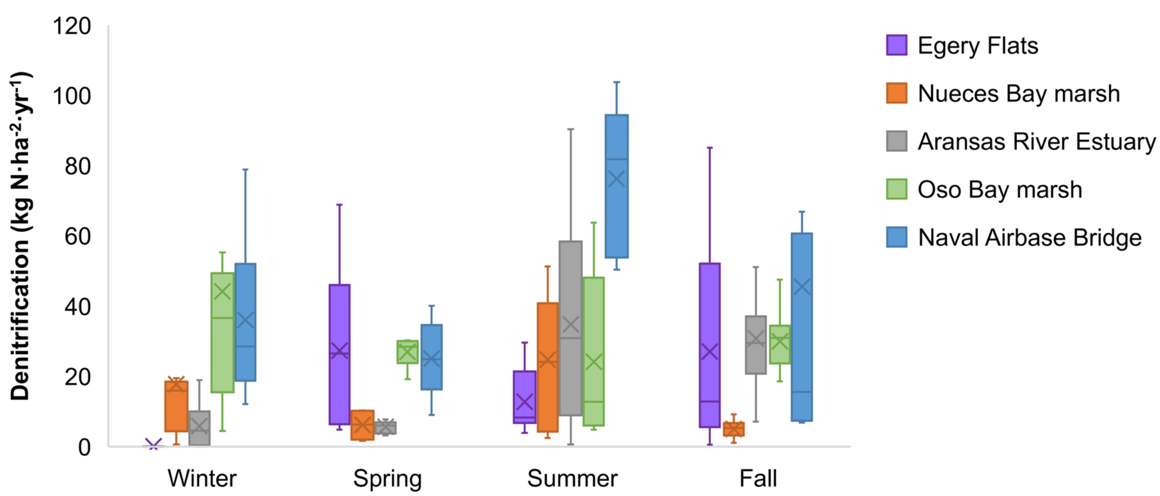


Figure 25. Denitrification rates separated by seasons for all wetland sites.

Economic Evaluation of the Nitrogen Mitigation Ecosystem Service

The replacement cost method was used to value the nitrogen mitigation ecosystem service for the five wetlands using values from the Rockport WWTP (Table 7). The two restored wetlands had the lowest values for nitrogen mitigation. The Egery Flats sediments have the potential to remove an average of 16.81 kg $N \cdot ha^{-2} \cdot yr^{-1}$, over the 270-ha area of the wetland (average 4,539 kg $N \cdot yr^{-1}$). Nitrogen mitigation at Egery Flats has a value of $64.73 per $ha \cdot yr^{-1}$ or $17,476 per year. Over the whole 70-ha area of the Nueces Bay restored marsh can remove an average of 13.45 kg $N \cdot ha^{-2} \cdot yr^{-1}$, or a total of 941 kg $N \cdot yr^{-1}$. The value for the nitrogen mitigation ecosystem service at the Nueces Bay restored marsh was calculated to be $51.78 per $ha \cdot yr^{-1}$ and extrapolated to the whole area is $3,624 annually.

Overall, the natural wetlands had higher values for nitrogen mitigation. Over the 630-ha area, the Aransas River Estuary was calculated to remove an average of 12,903 kg $N \cdot yr^{-1}$. This amount of nitrogen mitigation is valued at $78.85 per $ha \cdot yr^{-1}$ or $49,673 per year. Approximately 3,289 kg $N \cdot yr^{-1}$ is removed by the Oso Bay marsh for the entire 105-ha area, giving it a value of $120.61 per $ha \cdot yr^{-1}$ or $12,664 annually. The last natural wetland site, Naval Airbase Bridge had the highest amount of N removed per hectare, 45.73 kg $N \cdot ha^{-2} \cdot yr^{-1}$, which calculated to be 3,289 kg $N \cdot yr^{-1}$ over the 84-ha area. This was valued to be $176.04 per $ha \cdot yr^{-1}$ or $14,787 annually. Based on this usage of the replacement cost method, the value of the nitrogen mitigation ecosystem service offered by the wetlands is equivalent to $3.85·kg N^{-1} removed.

Table 7. Wetland type (R=restored, N=natural), wetland area, seasonal denitrification rates, and value of nitrogen mitigation for each wetland using the replacement cost method. W=winter, Sp=spring, Su=summer, and F=fall.

Wetland Site	Type	Area (ha)	Seasonal Denitrification rates (kg N·ha^{-2}·yr^{-1})				Total N removed seasonally (kg N·ha^{-2})				Total N removed (kg N·ha^{-2} ·yr^{-1})	N mitigation ecosystem service value	
			W	Sp	Su	F	W	Sp	Su	F		US\$2020·ha^{-2}·yr^{-1}	US\$2020·yr^{-1}
Egery Flats	R	270	0.08	27.36	12.78	27.03	0.02	6.84	3.19	6.76	16.81	$ 64.73	$ 17,476.01
Nueces Bay marsh	R	70	17.80	6.14	24.82	5.04	4.45	1.54	6.20	1.26	13.45	$ 51.78	$ 3,624.27
Aransas River Estuary	N	630	5.83	10.43	34.84	30.82	1.46	2.61	8.71	7.71	20.48	$ 78.85	$ 49,673.11
Oso Bay marsh	N	105	44.18	26.98	24.17	29.99	11.04	6.74	6.04	7.50	31.33	$ 120.61	$ 12,663.84
Naval Airbase Bridge	N	84	36.07	25.10	76.20	45.54	9.02	6.27	19.05	11.39	45.73	$ 176.04	$ 14,787.45

DISCUSSION

Seasonal Climate Variation

The duration of this study (late 2018 to early 2020) occurred during a weak El Niño period, however, after analyzing the 10 years of climate data it was determined that there were no significant differences from La Niña, and ENSO-neutral years. Seasonal trends seen during our study period may be a good representation of trends in other years.

Nitrogen Based Nutrients and $\delta^{15}N$ Stable Isotopes

Nitrogen based nutrients and $\delta^{15}N$ stable isotopes can help illuminate the sources and processes occurring in these systems. The $\delta^{15}N$- NH_4^+ values measured at the Portland WWTP, -4.73 to 0.78 ‰, suggest a mixing of animal and human waste, 10 to 25‰, and the mineralization process of organic N, -40 to -15‰, showing that there is a mixture of signatures between source and process (Kreitler 1975; Kreitler 1979; Heaton 1986; Kendall et al. 2007). Portland WWTP $\delta^{15}N$-NO_3^- values, 2.02 to 9.96‰, suggest that NO_3^- sources are probably human waste, 10 to 20‰, and that nitrification, -38 to -14‰ is also influencing this site (Kendall et al., 2007). The $\delta^{15}N$-NO_2^- increased from -17.31‰ in spring to 1.26‰ in summer which can be an indication of either nitrification of NO_2^- to NO_3^- or denitrification of NO_2^- to N_2 gas since microbes preferentially utilize the N with the lowest $\delta^{15}N$ first. The small increase of $\delta^{15}N$-NO_3^-, 2.02 to 3.5‰, paired with the small decrease in $\delta^{15}N$- NH_4^+, -1.52 to -4.06‰, from spring to summer, respectively, show the possibility that some dissimilatory nitrate reduction to ammonium (DRNA) is occurring, as well as a possibility of denitrification. All three N species concentrations decreased in the summer compared to spring, showing a net removal of N from the system, under the assumption that inflow N concentration does not decrease, which may show more evidence of the occurrence of denitrification.

Oso Bay WWTP $\delta^{15}N$-NO_3^- values, 1.86 to 17.48‰, indicate human waste sources and are slightly higher than the values of the Portland WWTP; this is indicative of water column denitrification. The $\delta^{15}N$-NH_4^+ values at the Oso Bay WWTP ranged from, -8.06 to -0.91‰. This range most likely indicates a mixture of nitrogen cycling processes including organic matter mineralization to NH_4^+, DRNA, and nitrification, as well as influence from human waste. The $\delta^{15}N$-NH_4^+ values are higher than the values of mineralization, -40 to -15‰, and lower than the values of nitrification, 5 to 45‰, and human waste, 10 to 25‰, which shows mixing of these processes and sources (Heaton 1986; Kendall et al., 2007). The more negative values in the $\delta^{15}N$-NH_4^+ shows the possibility of stronger influence from mineralization and DRNA. The increase of NH_4^+ concentration and decrease in $\delta^{15}N$-NH_4^+ during the summer months combined with the decrease of NO_3^- concentration and increase in $\delta^{15}N$-NO_3^- may indicate some DNRA is occurring in the water column (Domangue and Mortazavi 2018).

The Bayside WWTP (a constructed wetland) nutrient concentrations were more similar to the wetland sites with consistently low NH_4^+ and NO_3^- concentrations, which suggests excess nitrogen from influent was efficiently removed as it passed through the created wetland WWTP cells (Coban et al., 2015). The high $\delta^{15}N$-NH_4^+ values, 7.52 to 12.04‰, are indicative of nitrification of NH_4^+ to NO_2^-, and/or human and animal waste. The high $\delta^{15}N$-NO_3^- values, 27.41 ± 1.09‰, show influence from denitrification (Kendall et al., 2007). In the summer there was an increase in concentration for all three nitrogen species. The increase of NO_2^- concentrations were much higher than the increases in the other nitrogen species. Under the assumption that the concentration of nitrogen inflow into the WWTP does not increase, then the concentration mixed with the high $\delta^{15}N$-NO_2^- values, 24‰, may indicate that NH_4^+ is being nitrified fully to NO_3^-, causing the high $\delta^{15}N$-NO_2^- values and then NO_3^- is being denitrified to NO_2^- as an intermediate of the denitrification process.

The wetlands tended to have more mixed sources and processes reflected in the isotope signatures. Egery Flats had higher NH_4^+ concentrations in all seasons compared to both NO_3^- or NO_2^-. The NH_4^+ and NO_2^- concentrations increase in the summer months which may indicate mineralization of organic matter to ammonium, and the nitrification of ammonium to nitrite. Influences from agricultural runoff, human and animal waste, and denitrification were seen in the $\delta^{15}N$-NO_3^- values, -0.40 to 42.63‰ (Heaton 1986; Kendall et al., 2007). This area is surrounded by agricultural land and has inflow from the Bayside WWTP.

The Nueces Bay restored marsh isotope sources were mostly indicative of nitrogen cycling processes, including mineralization of organic matter to ammonium, and nitrification of NO_2^- to NO_3^-. The increase of NH_4^+ in the summer provides further evidence that mineralization of organic matter to ammonium is occurring, the increase in NO_2^- during this season shows than NH_4^+ is also being nitrified to NO_2^- and NO_3^-. This site mostly shows influence of N cycling processes, which indicates that the water from the Portland WWTP effluent is probably diluted before it reaches the site. The low isotopic ratios at this site in $\delta^{15}N\text{-}NH_4^+$, -21.18‰, and $\delta^{15}N\text{-}NO_3^-$, -21.29 to -20.38‰, show mineralization of organic matter to ammonium, and nitrification of NO_2^- to NO_3^-, respectively. The total nitrification of NH_4^+ to NO_3^- would leave high $\delta^{15}N\text{-}NH_4^+$ values. Therefore, since we see negative values, there is a possibility that the nitrifying microbes at this site are dominant in *nor* genes (responsible for NO_2^- to NO_3^- nitrification) rather than *amoA* and *hao* (responsible for NH_4^+ to NO_2^- nitrification), or that *nor* genes are more active, so nitrification of NO_2^- to NO_3^- is more prominent than NH_4^+ to NO_2^- (Cong et al., 2015). The $\delta^{15}N\text{-}NO_3^-$ values do not suggest influence of denitrification.

The Aransas River Estuary $\delta^{15}N\text{-}NO_3^-$ values, -7.96 to -6.43‰, indicate mixing of N sources and processes, including nitrate fertilizers, nitrification processes and atmospheric nitrate (Kendall et al., 2007). The increase in Aransas River Estuary NH_4^+ and NO_2^- concentrations in the summer months may indicate mineralization of organic matter to ammonium and then nitrification to nitrite/nitrate.

The Oso Bay marsh site NO_3^- concentrations were highest in the winter and lower during the other seasons, potentially showing denitrification activity in the warmer temperatures. NO_2^- concentrations increase in the summer and fall which can be indicative of both nitrification and denitrification processes. The increase in $\delta^{15}N\text{-}NO_3^-$ paired with the decrease in $\delta^{15}N\text{-}NO_2^-$ in the summer suggests the stronger possibility of denitrification with the reduction of NO_3^- to NO_2^-, a possibility made stronger by the decrease in $\delta^{15}N\text{-}NH_4^+$ showing that nitrification of NH_4^+ is less likely. The $\delta^{15}N\text{-}NO_3^-$ values, 0.86 to 2.43‰, show a mixture of NO_3^- fertilizers, soil NO_3^-, and human and animal waste, where the $\delta^{15}N\text{-}NH_4^+$ values, -6.86 to -2.05‰, were in line with soil

NH_4^+, NH_4^+ fertilizers, mineralization of organic matter to NH_4^+ and human waste mixing (Kendall et al., 2007). This site is surrounded by agricultural land, a golf course, and a wastewater treatment plant; therefore, this mixing of sources is expected and shows that this site is influenced heavily by anthropogenic N sources. The DO concentrations upstream of the Oso Bay marsh site near the Oso Bay WWTP outfall were lower than many of our other sites, with low averages during the summer of 2.6 $mg \cdot L^{-1}$, and the DO concentrations at the Oso Bay marsh had the lowest average of 4.1 $mg \cdot L^{-1}$ during the summer months. These low DO concentrations are a possible indicator of eutrophication occurring near this site, causing near hypoxic conditions. The Oso Bay marsh site NH_4^+ concentrations increased in the summer months which may indicate mineralization of organic matter, or influence of fertilizers.

The Naval Airbase Bridge had higher NH_4^+ concentrations than either NO_3^- or NO_2^-. The increase of NH_4^+ seen in the summer and fall months are most likely due to either fertilizer influence or mineralization of organic matter by microbes. The NO_3^- concentrations decreasing in the spring and summer months may indicate denitrification occurring in the sediments as a means of NO_3^- reduction. The increase of NO_2^- in the summer and fall months could potentially be due to increased nitrification or denitrification by microbes during the warmer seasons, as NO_2^- is an intermediate oxidation state for both reactions. The $\delta^{15}N$-NO_3^- values, -2.52 to 1.30‰, show NO_3^- fertilizers, human and animal waste, and possible mixing of nitrification and denitrification processes. The $\delta^{15}N$-NH_4^+ values, -5.4‰, suggest a source from NH_4^+ fertilizers, and a possible mixture of nitrification and mineralization of organic matter to ammonium (Heaton 1986; Kendall et al., 2007). This site is influenced by two different large bodies of water, the Oso Bay, and the Corpus Christi Bay, which are both surrounded by agricultural land, this site is also influenced by the Oso Bay WWTP, because the flow of the effluent travels directly around Ward Island and out to the Corpus Christi Bay. This site is heavily influenced by anthropogenic nitrogen sources.

Quantification of Nitrogen Mitigation Ecosystem Service Flows

Hypoxia caused by eutrophication is a worldwide issue that is made worse by excess nitrogen loading into coastal waterways for anthropogenic sources (DeLaune et al., 2005: Rabalais et al., 2014). Restoring and conserving the denitrification ability of natural habitats has been proposed to reduce nutrient loads into different important aquatic habitats (Lindau et al., 2008: Pollack et al., 2013). As shown in this study, wetlands can effectively remove excess nitrogen by means of denitrification. Denitrification is a principal process for direct elimination of excess inorganic nitrogen from a system to the atmosphere (An and Gardner 2002; Koop-Jakobsen and Giblin 2009).

Many studies show that temperature changes strongly influence denitrification, where increased temperature shows and increase in rate of denitrification (Byström et al., 2000; Richardson et al., 2004; Lindau et al., 2008; Vymazal 2011; Song et al., 2014). Our study shows however, that the correlation between water temperature and rate of denitrification were not significant even with visible seasonal trends. This may indicate that there are other factors aside from temperature influencing the rate of potential denitrification in these sites. For example, the diffusion rates of nitrate in different types of sediment soils maybe a limiting factor for denitrifiers to use nitrate as an electron acceptor. Therefore, soil characteristics may be a factor effecting denitrification rates. The soil types vary across the wetlands in this study. The larger pore sizes seen in the sand and shell-based sediment of the Naval Airbase Bridge allows for easier diffusion of NO_3^- deeper into the sediments. This gives microbial communities increased contact with available NO_3^- in the sediments under hypoxic or anoxic conditions, for use as an electron acceptor

(Groffman and Tiedje 1989; DeLaune et al., 2005; Wolf et al., 2011; Theriot et al., 2013). Microbial communities grow more rapidly during warmer summer months. This requires more energy from redox reactions (in other words, more electron acceptors such as nitrate when O_2 is absent, and more electron donors such as organic matter) in a shorter period to maintain growth, denitrifiers with consume NO_3^- quicker during the summer than in other seasons. NO_3^- can become a limiting agent is the concentration is not replenished fast enough due to the diffusion limits in clay/mud sediments. There may be other biogeochemical reactions effecting denitrification rates. Chlorides in high concentration, as well as sulfides can inhibit the reduction of NO_3^- through denitrification (Joye and Hollibaugh 1995; Kendall et al., 2007; Marks et al., 2016).

Wetland age is another major contributing factor to nitrogen mitigation through denitrification. The capacity for denitrification in restored and natural wetlands has been examined in many studies (Jenkins et al., 2010; Wolf et al., 2011; Theriot et al., 2013; Song et al., 2014). A study by Song et al., (2014) showed an increase in NO_3^- removal in created wetlands from 27% to over 50% from the first year after creation of the wetland to year 15. From year 9 to year 15 the rate of nitrogen removal leveled off.

The denitrification rates in the restored wetlands, Egery Flats and Nueces Bay restored marsh, were significantly lower compared to two of the natural wetlands, Oso Bay marsh and Naval Airbase Bridge. These differences suggest a potential age-based trend in denitrification between these sites. The Aransas River Estuary had higher mean denitrification rates than the two restored wetlands, but not significantly, this may be related to the disturbance of sediments with its proximity to the Egery Flats restoration location. It is expected that within the next five to ten years the denitrification rates for the restored wetlands will increase and become more akin to the natural wetlands (Wolf et al., 2011; Song et al., 2014). Diverse microbial communities can be developed with the accumulation of organic matter, therefore, restored wetlands require more time to develop favorable conditions for denitrifiers (Jenkins et al., 2010; Wolf et al., 2011; Mitsch et al., 2012; Song et al., 2014).

Economic Evaluation of the Nitrogen Mitigation Ecosystem Service

Wetlands remove a substantial amount to nitrogen per year through denitrification, which helps to reduce the amount of nitrogen pollution from anthropogenic sources. Egery Flats and Nueces Bay marsh, the two restored wetlands in this study, removed 4,539 and 941 kg $N \cdot yr^{-1}$, respectively. The nitrogen removed by restored wetlands should increase as the wetlands age and increase soil complexity and microbial community structure.

The cost of replacing the nitrogen mitigation ecosystem service provided by the wetlands based on the capital, O&M costs of constructing the engineered BNR system at the Rockport WWTP is equivalent to $\$3.85 \cdot kg\ N^{-1}$. This value is more conservative than those found in other studies. The values for some of these studies are included here adjusted for inflation for ease of comparison, including $\$9.33 \cdot kg\ N^{-1}$ removed in Pollack et al. (2013), $\$31.12 \cdot kg\ N^{-1}$ removed in Jenkins et al. (2010), $\$15.34 \cdot kg\ N^{-1}$ removed seen in Piehler and Smyth (2011), and $\$32.81 \cdot kg\ N^{-1}$ removed in Newell et al. (2005). The method used for the calculation of the dollar value for the nitrogen mitigation ecosystem service can cause variations in the final value. With the benefit transfer method, we applied the values for the nitrogen mitigation ecosystem service found in the other studies listed previously and employed them to our study five wetland sites for the purpose of supplying a value range for the wetlands in this study (Table 8). This benefit transfer shows that the value of nitrogen mitigation can vary greatly depending on the method used, and that the replacement cost method provides a more conservative economic value. Our study focuses on a local example for the economic evaluation of nitrogen mitigation to provide a relevant, yet conservative replacement value for the wetlands of the Texas Coastal Bend.

Table 8. Wetland type, wetland area, total nitrogen removed annually, and value of nitrogen mitigation for each wetland using the benefit transfer method with values from four nitrogen mitigation studies adjusted for inflation.

Wetland Site	Type	Area (ha)	Total N removed annually (kg N^{-1})	N mitigation ecosystem service value: Benefit transfer method (US$2020·$yr^{-1}$)			
				Pollack et al. (2013)	Jenkins et al. (2010)	Piehler and Smyth (2011)	Newell et al. (2005)
Egery Flats	Restored	270	4538.70	$ 42,346.07	$ 141,244.34	$ 69,623.66	$ 148,914.75
Nueces Bay marsh	Restored	70	1176.70	$ 10,978.61	$ 36,618.90	$ 18,050.58	$ 38,607.53
Aransas River Estuary	Natural	630	10590.30	$ 98,807.50	$ 329,570.14	$ 162,455.20	$ 347,467.74
Oso Bay marsh	Natural	105	1765.05	$ 16,467.92	$ 54,928.36	$ 27,075.87	$ 57,911.29
Naval Airbase Bridge	Natural	84	1412.04	$ 13,174.33	$ 43,942.68	$ 21,660.69	$ 46,329.03

Ecosystem services provided by wetlands can have a widespread impact on ecosystem health as well as human health and wellbeing. Nitrogen mitigation provided by wetlands also requires no direct costs for the provision of this service to the community. This service can have many additional benefits to taxpayers in the community. This service may even give respite to taxpayers from additional taxes for the installation of a BNR WWTP system to remove excess nitrogen. Taxpayers can partake in the social benefits provided by the wetlands including recreational activities like fishing, boating, and birdwatching, as well as enjoy the increased wildlife diversity of a healthy ecosystem.

According to the Association of Clean Water Administrators, as of 2012, 55% of states have an active nutrient offset program, while 22% of states are in the process of developing one

(ACWA 2012). This type of program is a common method for controlling point-source nutrient loading to make up for non-point source outputs to limit overall nutrient outputs into aquatic ecosystems. Studies like the one presented here can encourage stakeholder interest in environmental health, leading to formation of markets for ecosystem services, including nutrient trading programs, that can provide opportunities for economic growth.

Conservation and restoration management costs are often a one-time cost that with benefits that will persist long into the future, and in the case of nitrogen mitigation in restored and created wetlands these benefits will increase with time. Engineered alternatives to ecosystem services are important for helping the environment be able to cope with excess number of pollutants entering the environment, but these alternatives require continued O&M costs, and will become more costly over time. They also exclusively offer the service they were made to replace, where the natural ecosystem offers additional services.

The increase of population and development along the Texas Coastal Bend will bring even higher importance to wetland restoration and conservation projects, with the increase in magnitude of nitrogen pollution and land degradation. This type of population change will intensify the importance of studies showing the value of these ecosystems. This study shows the value of nitrogen mitigation in wetlands, as well as emphasizing how restoration projects should be implemented in conjunction with conservation endeavors, since natural wetlands have a higher capacity to removed nitrogen to newly restored wetlands. Restored wetlands take years to acquire comparable ability to remove nitrogen as natural wetlands (Jenkins et al., 2010; Wolf et al., 2011; Mitsch et al., 2012).

The settlement from the 2010 Deep Water Horizon oil spill resulted in the formation of the Gulf Environmental Benefit Fund at the National Fish and Wildlife Foundation (NFWF). In 2014, the CBBEP received $1,587,000 from this fund for the restoration at Egery Flats. The funds were applied in 2018 and 2019 to replace culverts to increase freshwater flow, reducing salinity in the wetlands and plant emergent marsh. This restoration will increase habitability for important fish species and protect endangered waterfowl. The return on investment using the replacement value of nitrogen mitigation alone would take approximately 91 years, or less as the complexity of microbial communities and soil matrix and denitrification increases with the age of the restoration. Using the value for the nitrogen mitigation ecosystem service found in other studies with the benefit transfer method, the time it would take for a full return on investment, without accounting for increase in age of the wetland, would be between 11 and 38 years, using the least and most conservative values, respectively (Table 8) (Newell et al., 2005; Pollack et al., 2013).

The Nueces Bay marsh restoration was much more costly, $5,326,820 was invested for total reconstruction and plantation of the wetland by a multitude of sponsors: Coastal Management Program, U.S. Fish and Wildlife Service, Texas Commission on Environmental Quality, Coastal Impact Assistance Program, Centre for Environmental Research and Policy, Environmental Protection Agency, Coastal Conservation Association, and the Hollomon Price Foundation. This project aimed to increase diversity of fauna and flora, and to restore damaged habitat from dredging and hydrology shifts (Smee 2016). Based on the benefit transfer method using the values in Table 2, the time it would take for a full return on investment, without accounting for the increase in value with age of the wetland, would be between 138 and 486 years. The replacement cost method is a much more conservative way of measuring the value for the nitrogen mitigation ecosystem service at these sites. The low occurrence of denitrification at this site is one reason that the monetary value of nitrogen mitigation based on the replacement cost method at this site is relatively low, $3,624.27 $\cdot yr^{-1}$. The low occurrence of denitrification may also be impacted by the age of the wetland and is likely to increase as the wetlands age and soil structure increases in complexity.

The aim of our study was not to construct a complete cost-benefit analysis of the restored or natural wetlands. Moreover, the aim was to offer an example for the valuation of one single ecosystem service of the many services offered by these habitats and supply evidence of the importance of the conservation and restoration of indispensable ecosystems. Wetlands offer numerous ecosystem services that, if evaluated, would compound the total economic benefit of these ecosystems than considering only a single service. Another ecosystem service that may be evaluated is carbon sequestration. One study revealed that emergent brackish and salt marshes around the Gulf of Mexico can be valued between $1,109-1,309$\cdot ha^{-2} \cdot yr^{-1}$ for the carbon sequestration ecosystem service (Schmidt et al., 2014; Engle 2011). Costanza et al. (2008) presented that storm surge reduction is another valuable ecosystem service offered by wetlands, and, along the Texas Coast, can be valued at $17,353.60$\cdot ha^{-2} \cdot yr^{-1}$ (adjusted to US$2020). Many other ecosystem services can add to the economic value of these ecosystems, including water quality improvements, replenishment of ground water supply, ecotourism, climate regulation, and market price for important commercial fish species. These values aid in cost justification of conservation and restoration of important natural ecosystems, assisting project managers, stakeholders, policy- and decision-makers to reach the best outcome for ecosystem management.

SUMMARY

High nutrient loading leading to eutrophication in aquatic coastal environments is an ongoing global issue, and wetland restoration is a tangible method for removing this excess nutrient loading. Through the study of nutrient concentration and stable isotopes in these systems, we improved better understand nitrogen cycling in these environments.

Using the combination of nitrogen based nutrient concentrations and nitrogen stable isotopes we can interpret the nitrogen sources and processes occurring at each of the study sites. The NO_3^- sources at the Portland WWTP are human waste, and the NH_4^+ values are most similar to human waste with influence of organic matter mineralization. At the Oso Bay WWTP the NO_3^- values also indicate human waste sources, and the NH_4^+ indicate a mixture of organic matter mineralization to ammonium, and nitrification, human waste, and dissimilatory nitrate reduction to ammonium (DNRA). At the Bayside WWTP, the increase in $\delta^{15}N$- NH_4^+ is indicative of nitrification of NH_4^+ to NO_2^-, and/or human and animal waste and the $\delta^{15}N$-NO_3^- values show influence from denitrification. Nitrification is also evident in the $\delta^{15}N$-NO_2^- values at this site, showing that the Bayside WWTP is dominated by human and animal waste sources and multiple nitrogen cycling processes occurring in the wetland cells.

Wetlands also have a variety of mixed sources and processes evident by their combined nitrogen based nutrient concentrations and nitrogen stable isotopes. At Egery Flats we were able to see mineralization of organic matter, nitrification, and denitrification are all occurring within this site. This site is also influenced by agricultural runoff and human/ animal waste. The Nueces Bay restored marsh was mostly influenced by nitrogen cycling processes including mineralization of organic matter, as well as nitrification of NO_2^- to NO_3^-. The Aransas River Estuary showed signatures of nitrate fertilizers, nitrification, and atmospheric nitrate, as well as the mineralization of organic matter to ammonium. At the Oso Bay marsh, the comparison of $\delta^{15}N$-NO_3^-, $\delta^{15}N$-NO_2^- and $\delta^{15}N$-NH_4^+ in the summer shows the occurrence of denitrification. This site also showed a mixture of NH_4^+ and NO_3^- fertilizers, soil NH_4^+ and NO_3^-, mineralization of organic matter to NH_4^+, and human and animal waste. The wide variety of nitrogen sources and processes at this site are most likely due to its proximity to anthropogenic nitrogen sources including agricultural land, a golf course, and a wastewater treatment plant. The Naval Airbase Bridge is influenced by anthropogenic sources including NH_4^+ and NO_3^- fertilizers, human and animal waste, as well as mixture of nitrification, denitrification, and mineralization processes.

Wetlands remove a substantial amount of excess nitrogen from anthropogenic sources. The cost of replacing the nitrogen mitigation ecosystem service provided by the wetlands based on the capital, O&M costs of constructing the engineered BNR system at the Rockport WWTP is equivalent to $\$3.85 \cdot kg\ N^{-1}$. Based on the replacement cost method, the natural wetlands nitrogen removal value on a per area basis was calculated to be 78.85, 120.61, and $176.04 \cdot ha^{-2} \cdot yr^{-1}$ for Aransas River Estuary, Oso Bay marsh, and Naval Airbase Bridge, respectively. The restored wetland nitrogen mitigation values were 64.73, and $51.78 \cdot ha^{-2} \cdot yr^{-1}$, for Egery Flats and Nueces Bay restored marsh, respectively. The monetary value of the nitrogen mitigation ecosystem service in the restored wetlands were low compared to the natural wetlands but are expected to increase with age of the wetland, as microbial communities and soil matrices become more complex.

Studying the removal of nitrogen in restored and natural wetlands can aid in the understanding of how wetland value increases, becoming a valuable tool for ecosystem service management. This study is an example of a multidisciplinary method for assessing ecosystem services to illustrate the importance of nitrogen mitigation in restored and natural wetlands. The results of this study are valuable for cost justification of restoration and conservation projects, and as an instrument for effective ecosystem management.

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APPENDIX 1. Known stable isotope ($\delta^{15}N$) sources and processes.

Source or Process	$\delta^{15}N$-NO_3^- (‰)	$\delta^{15}N$-NH_4^+ (‰)	$\delta^{15}N$-NO_2^- (‰)	Bulk $\delta^{15}N$ (‰)	PON- $\delta^{15}N$ (‰)	Location	Reference
Secondary WWTP Effluent	+5.32	+12.78	-	-	-	Southern California, USA	McLaughlin et al. 2017
Secondary WWTP Effluent	+4.9	-	-	-	-	Ulaanbaatar, Mongolia	Itoh et al. 2011
Animal and Human Waste	+10 to +20	+10 to +25	-	-	-		Kreitler 1975; Kreitler 1979; Heaton 1986; Kendall et al. 2007
River Downstream Secondary WWTP	-	-	-	-	17	Skidmore, Texas	Mooney 2009
Spring Water	-6.7	-	-	-	-	Ulaanbaatar, Mongolia	Itoh et al. 2011
Soil Organic Nitrogen	-2 to +10	-2 to +5	-	+4 to +9	-	Seven Country Average	Heaton 1986; Kendall et al. 2007
Soil Organic Matter	-	-	-	+2 to +7	-	Coastal Sediments	Heaton 1986; Kendall et al. 2007
NH_4^+ Fertilizer	-	-	-	-5 to +5	-	USA, Australia, France, South Africa	Heaton 1986
NO_3^- Fertilizer	-5 to +3	-10 to +5	-	-5 to +7	-	USA, Australia, France, South Africa	Heaton 1986; Kendall et al. 2007
Atmospheric NH_4^+	-	-	-	-15 to 0	-	Jülich, Germany	Freyer 1978; Heaton 1986
Atmospheric NO_3^-	-	-	-	-12 to +2	-	Jülich, Germany	Freyer 1978; Heaton 1986
Wet Deposition	+3.1	-	-	-	-	USA	Hastings et al. 2003; Elliot et al. 2006; Kendall et al. 2007
Nitrification	-38 to -14	+5 to +45	-44.6			Leipzig, Germany	Coban et al. 2015; Kendall et al. 2007
Ammonification	-	-	-	-1 to +1	-		Kendall et al. 2007
Denitrification	+15 to +30	-	-	-	-		Kendall et al. 2007
Nitrate Mineralization	+4 to +9	-	-	-	-		Heaton 1986
Ammonium Mineralization	-	-40 to -15	-	-	-		Kendall et al. 2007
Volatized Ammonia of Urea and Manure	-	-20	-	-	-	Agricultural land	Kendall et al. 2007

Appendix 2. Literature review tables of denitrification studies, including results, methods, and factors affecting denitrification.

Reference	Location	Ecosystem Type	Natural/ Restored/ Constructed	Mean Rates	Converted Units (kg N/ha/yr.)	Factors Affecting Denitrification	Method
Groffman and Tiedje 1989	Michigan USA	Freshwater Wetland	Natural	199 g N/ha/d	72.64	soil texture and drainage	Static Core, acetylene blocking, nitrate amendment
Groffman and Tiedje 1989	Michigan USA	Freshwater Wetland	Natural	1251 g N/ha/d	456.62	soil texture and drainage	Static Core, acetylene blocking, nitrate amendment
Bruesewitz et al 2017	Aransas River, TX, USA	Saltwater Wetland	Natural	3.25 mg N/m2/h	284.70	during drought may decrease anthropogenic N input to coastal systems	sediment slurry, river water as NO3 source, 10uM NO3, MIMS
Bruesewitz et al 2017	Aransas River, TX, USA	Saltwater Wetland	Natural	1.5 mg N/m2/h	131.40	during drought may decrease anthropogenic N input to coastal systems	
DeLaune et al 2005	Louisiana, USA	Freshwater Wetland	Restored	31 mg N/m2/d	113.15	NO_3^- concentration, diffusion rate of NO_3^- to anaerobic soil layer	5 cm soil slurry Acetylene Blockage
Lindau et al 2008	Louisiana, USA	Freshwater Wetland	Restored	386.6 g N/ha/d	141.11	at 22C, temperature dependent	amended 100mg/l NO3, acetylene blockage, sediment slurry
Richardson et al 2004	Wisconsin, USA	Freshwater Wetland	Natural	1.97 ug N/cm2/h	1725.72	temperature dependent, nutrient enrichment, and C availability. High or low flow rates, wetland surface area	slurry, 14mg/L NO3 acetylene blockage
Pollack et al 2013	Mission-Aransas Estuary, TX, USA	Oyster Reef	Natural	502.5 kg N/km2/yr.	5.03		based on a 20% denitrification efficiency from previous laboratory studies
Jenkins et al 2010	MAV, USA	Freshwater Wetland	Restored	28.8 kg N/ha/yr.	28.80	Age	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 3yr old	35 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 4yr old	30 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 7yr old	85 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created-10yr old	60 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Natural	75 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Song et al 2011	Ohio, USA	Freshwater Wetland	Created-15yr old	316 ug N/m2/hr	27.68	Temperature, NO_3^- concentration, vegetation uptake competition	In situ acetylene blocking denitrification in shallow wetlands
Breaux et al 1995	Louisiana, USA	Freshwater Wetland	Natural	14.3 g N/m2/yr.	143.00		Based on 72% denitrification efficiency calculated through biochemical balance analysis
Mitsch et al 2012	Ohio, USA	Freshwater Wetland	Created-15yr old	2.1 g N/m2/yr.	21.00	Age, expected to increase over time with organic matter accumulation	In situ acetylene blocking denitrification
Dehnhardt 2002	Elbe River, Germany	Freshwater Wetland	Natural	200 kg N/ha/yr.	200.00		statistical modeling

Appendix 3. Literature review table of economic evaluation studies for ecosystem services.

Location	Type of Ecosystem	author	Type of study	date	US$ Value	Unit	Converted Value US$ 2019	Converted Unit	Comments
Thibodaux, Louisiana, USA	Freshwater Wetland	Breaux et al., 1995	RCM	1995	$ 64.61	per ha per yr	$ 108.39	per ha per yr	
Sweden	Saltwater Wetland	Byström, O. 2000	RCM	2000	$ 3,913.51	per ha per yr	$ 7,131.28	per ha per yr	used low range converted from 1992 SEK to 1992 US$ from Jan 2, 1992
Elbe River, Germany	Freshwater Wetland	Dehnhardt 2002	RCM	2002	$ 308.45	per ha per yr	$ 438.34	per ha per yr	Converted from 2002€ to 2002 USD from Jan 2, 2002
Stockholm, Sweden	Freshwater Wetland	Gren et al 1995	RCM	1995	$ 1,275.19	per ha per yr	$ 2,139.19	per ha per yr	converted from 1995 ECU to 1995 $US using Hanley and Owen, 2004
Mississippi Alluvial Valley, USA	Freshwater Wetland	Jenkins et al., 2010	Benefit transfer	2010	$ 1,248.04	per ha per yr	$ 1,481.96	per ha per yr	from Ribaudo et al 2005 converted to kg N from lb N, and inflated to $2008
Fiji	Mangroves	Lal, P.N., 1990	RCM	1990	$ 2,125.00	per ha per yr	$ 4,156.63	per ha per yr	
Denver, Colorado, USA	Freshwater Wetland	Loomis et al., 2000	CV	2000	$ 6,180.00	per ha per yr	$ 9,175.16	per ha per yr	Based on if only 26% of households pay for N mitigation
Bogue Sound, North Carolina	Oyster Reef	Piehler and Smyth, 2011	PM	2011	$ 7,330.86	per ha per yr	$ 8,331.99	per ha per yr	
Bogue Sound, North Carolina	Submerged Aquatic Vegetation	Piehler and Smyth, 2011	PM	2011	$ 7,404.94	per ha per yr	$ 8,416.19	per ha per yr	
Bogue Sound, North Carolina	Salt Marsh	Piehler and Smyth, 2011	PM	2011	$ 6,123.46	per ha per yr	$ 6,959.70	per ha per yr	
Bogue Sound, North Carolina	Intertidal Flat	Piehler and Smyth, 2011	PM	2011	$ 3,832.10	per ha per yr	$ 4,355.43	per ha per yr	
Bogue Sound, North Carolina	Subtidal Flat	Piehler and Smyth, 2011	PM	2011	$ 1,022.22	per ha per yr	$ 1,161.82	per ha per yr	
Mission-Aransas Estuary, TX, USA	Oyster Reef	Pollack et al., 2013	RCM	2013	$ 41.29	per ha per yr	$ 45.31	per ha per yr	
Zazari-Cheimaditida, Greece	Freshwater Wetland	Ragkos et al 2006	CV	2006	$ 0.01	per ha per yr	$ 0.01	per ha per yr	converted from 2006€ per person to 2006$/ha/yr from Jan 3, 2006
Mississippi River Delta, USA	Freshwater Wetland	Ribaudo et al., 2005	PM	2005	$ 10.50	per lb N removed	$ 13.75	per lb N removed	
World	Mangroves	Salem and Mercer, 2012	MRA	2012	$ 44.00	per ha per yr	$ 48.99	per ha per yr	
McIntosh County, Georgia, USA	Forested Wetland	Schmidt et al., 2014	BT	2014	$ 1,248.00	per ha per yr	$ 1,347.75	per ha per yr	BT from Jenkins et al 2010, therefore from Ribaudo et al 2005
McIntosh County,	Freshwater Wetland	Schmidt et al., 2014	BT	2014	$ 19.00	per ha per yr	$ 20.52	per ha per yr	BT from Jenkins et al 2010, therefore from Ribaudo et al 2005

Appendix 4. Photos of field sampling.

Photo of students Erik Perez, Tim Laughbaum, and Evelyn Kuhnel sampling in Egery Flats October 2018.

Photo of students, Tim Laughbaum, Ryleigh Washerlesky, and Gabriela Mondragon at the Portland WWTP in January 2019.

Photo of student Catherine Shaw sampling at the Naval Airbase Bridge in May 2019.

Photo of students Erik Perez and Morganne Mier sampling at the Bayside WWTP in September 2019.

Photo of students Morganne Mier and Daniel Lansidel sampling at the Bayside WWTP in November 2019.

Photo of students Shahrukh Niazi, Catherine Shaw, and Jesus Baca collecting samples from the Nueces Bay restored marsh site in December 2019.

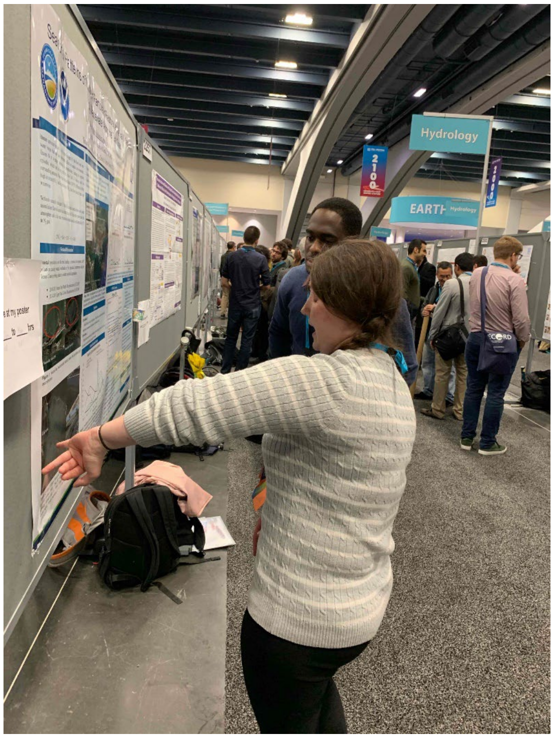
Photo of Catherine Shaw collecting sediment sample from the Oso Bay marsh site in December 2019.

Photo of students Shahrukh Niazi, and Jesus Baca sampling at the Aransas River Estuary in December 2019.

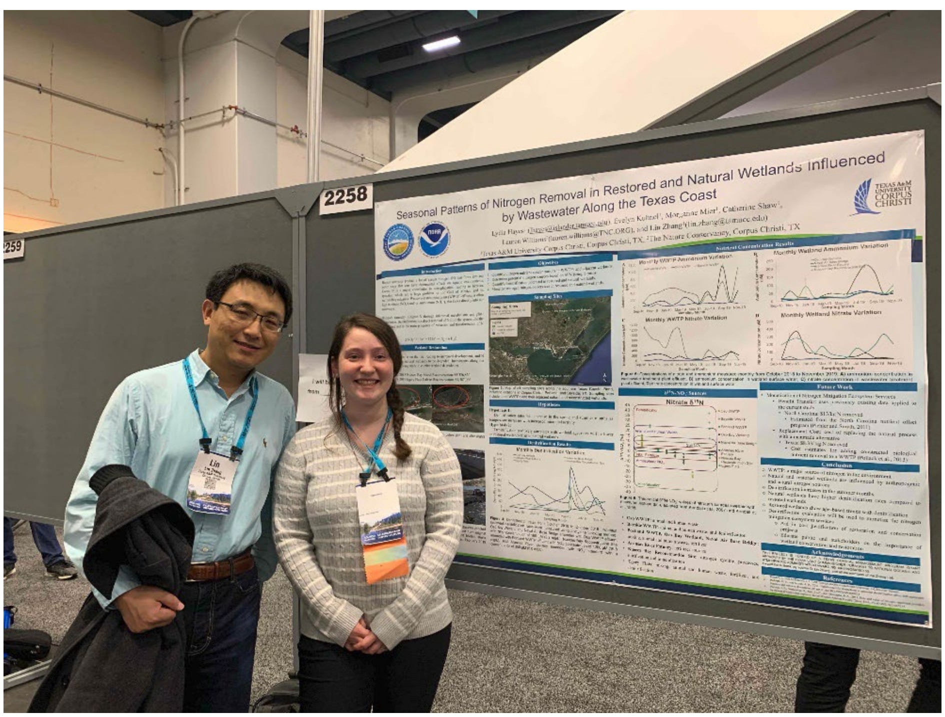
Appendix 5. Photos of outreach events.

Lydia Hayes and Dr. Lin Zhang after presentation at Coastal Bend Bays Foundation Coastal Issues Forum in October 2019.

Lydia Hayes presenting at Coastal Bend Bays Foundation Coastal Issues Forum in October 2019.



Lydia Hayes presenting research at American Geophysical Union annual meeting in December 2019.



Lydia Hayes and Dr. Lin Zhang after presentation at American Geophysical Union annual meeting in December 2019.