1-1-2014

Karst Estuaries: A newly described ecosystem governed by aquifer hydrology

Damian M. Menning
University of South Florida, dmenning@usf.edu

Scholar Commons Citation
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Karst Estuaries: A newly described ecosystem governed by aquifer hydrology

by

Damian M. Menning

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Cell Biology, Microbiology, and Molecular Biology
College of Arts and Sciences
University of South Florida

Major Professor: James R. Garey, Ph.D.
Kathleen M. Scott, Ph.D.
Jonathan G. Wynn, Ph.D.
James T. Riordan, Ph.D.

Date of Approval:
August 21, 2014

Keywords: anchialine, aquifer, microbial, hydrology, geochemistry

DEDICATION

I dedicate this work to my wife and my parents without whose love, support, and encouragement this would not have been possible.

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ABSTRACT

The overall goal of this dissertation is to define the hydrological, geochemical, and biological characteristics of a Karst Estuary. These types of estuaries represent a unique ecosystem created by freshwater inputs from direct flow through karst conduits and/or diffuse flow through a karst matrix. In order to determine the characteristics of a Karst Estuary we monitored short-term tidal fluctuations, long-term rainfall patterns, aquifer levels, spring discharge, multiple geochemical parameters, microbial communities in the water column and sediment, and macrofaunal communities in the sediment along a transect from a submarine spring through the Gulf of Mexico. Four sites were selected along a spring/marine transect and one nearby freshwater spring was used as a reference site. Datasondes were deployed in the nearshore brackish submarine spring to measure discharge volume, tidal fluctuations, and physical water parameters for two years. Water column and sediment samples were collected quarterly from both springs and the surrounding surface sites over the same time period. An isotopic/trace element mass balance method was used to determine the hydrogeological conditions of the spring discharge with three possible sources: 1) freshwater from the upper portion of the Upper Floridan aquifer, 2) freshwater from the lower portion of the Upper Floridan aquifer, and 3) saltwater from the Gulf of Mexico. Archaea, Bacteria, and microbial eukaryote communities were analyzed using molecular techniques, and macrofauna communities were determined using light microscopy. Correlation analyses were conducted to compare all studied biological communities to the hydrological and geochemical data in order to determine the influence of aquifer discharge. Within the water column of the submarine spring conduit, there were no significant differences of the sampled parameters over short sampling distances (<400 m) and periods (<1 hr). Spring discharge was found to be negatively correlated with tidal level and directly correlated with aquifer level. The brackish nature of the spring discharge is primarily due to simple mixing between the Gulf of Mexico saltwater and freshwater from the lower portion of the Upper Floridan aquifer originating from the mixing zone beneath the estuary. The composition of the spring discharge varied seasonally, showing increased marine influence at the beginning of the wet season. Tropical Storm Debby, June 2012, resulted in measurable freshwater inputs to spring discharge from the upper portion of the Upper Floridan aquifer. The number of spring reversals (salt water intrusion events) increased as the dry season progressed, stopped reversing immediately after Tropical Storm Debby, and then gradually increased into the next dry season. Statistically significant geochemical differences were found along the spring/marine transect on each collection date and seasonally at the individual sites. The major finding was that the primary driver of change in all of the studied biological communities of this Karst Estuary is the volume of aquifer discharge and the gradients formed by aquifer discharge and not the geochemical fluctuations within the system. Events that result in shifting the mixing zone inland have dramatic impacts on the biological communities of these environments. Karst Estuaries are a newly discovered type of ecosystem that are different from surface estuaries in that they are formed by aquifer discharge which is more stable in terms of geochemistry than water discharged to the sea via surface rivers.

CHAPTER ONE: INTRODUCTION

Submarine groundwater discharge (SGD) through subterranean estuaries has been recognized as a major source of nutrients to coastal ecosystems (Burnett et al., 2006; Moore, 2010). This influx of nutrients may have dramatic impacts on the ecology of the surrounding estuaries (Johannes, 1980; Kotwicki et al., 2014) and can be seen by the geochemical gradients they form (Burnett et al., 2003; Kim et al., 2005). Increased aquifer usage and/or increased sea levels may result in increased salt water intrusion resulting in the permanent inland shift of the mixing zone underneath estuaries turning them from estuarine to salt marsh habitats and having dramatic effects on the biological communities of the area which currently serve as nurseries for numerous invertebrate and fish species (Beck et al., 2001). The primary goal of this study is to define the hydrological, geochemical, and biological characteristics of a Karst Estuary.

Estuaries are semi-enclosed bodies of water which have a free connection to the open sea and within which sea water is measurably diluted with freshwater derived from land drainage (Pritchard, 1967). This definition generally applies to freshwater inputs from surface rivers and streams which may be secondary to subterranean drainage in karst regions. Moore (1999) defined the subterranean estuary as a coastal aquifer where terrestrial groundwater measurably dilutes seawater that has invaded the aquifer through a free underground connection to the sea. In karst regions, these subterranean estuaries are the primary source of freshwater and nutrient inputs to the sea. I refine these definitions as they apply to karst regions and define a Karst

Estuary as a semi-enclosed body of surface water, which has a free connection to the open sea and within which seawater is measurably diluted by freshwater from direct flow through karst conduits and/or diffuse flow through a karst matrix.

The study of SGD has primarily focused on diffuse flow over large regions (Moore, 1996; Santos et al., 2008) but less research has been conducted on point-source discharges such as conduit flow through submarine springs (Swarzenski et al., 2001; Peterson et al., 2009). In Florida there are over 700 documented springs (http://www.dep.state.fl.us/springs/), most of which are inland, but there are also numerous undocumented springs along the Florida coast (Fig. 1-1). Some of these springs discharge aquifer water directly to the Gulf of Mexico forming Karst Estuaries and act similarly to surface rivers bringing freshwater and nutrients to the estuary (Harrington et al., 2010).

Submarine springs may also be potential points for salt water intrusion to the aquifer that could be harmful to the freshwater sources used by many coastal communities (Fleury et al., 2007; Vera et al., 2012). Terrestrial coastal springs typically discharge freshwater but are also tidally influenced because of underlying marine water. Nearshore and offshore springs may discharge fresh or brackish water depending on inland hydrological conditions (Michael et al., 2005). Changes in long term weather patterns and human water use can have deleterious impacts on coastal springs (Wetland-Solutions-Inc., 2007; Quinlan et al., 2008), and thus these springs can act as sentinels of hydrological change in coastal regions. For example, an offshore submarine spring known as Jewfish Sink (Garman & Garey, 2005), approximately one km offshore of West Central Florida, ceased flow completely in 1962 following a prolonged drought and increased aquifer use by a nearby citrus industry.

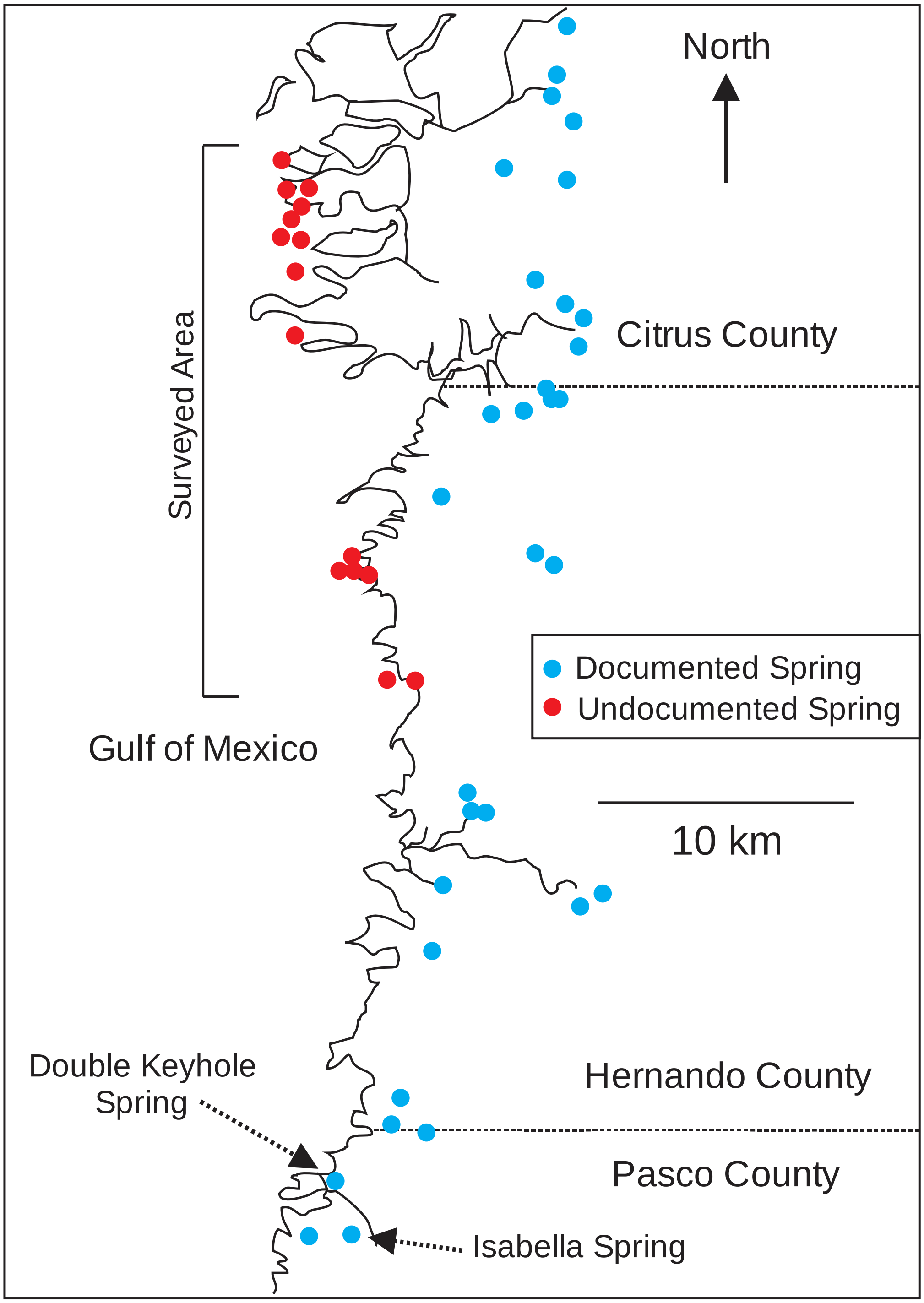


Figure 1-1. Location of documented and undocumented springs along the west central gulf coast of Florida. The blue circles are springs documented by the Florida Department of Environmental Protection (http://www.floridasprings.org/). The red circles are coastal submarine springs not documented by the Florida DEP but identified by a local dive team (Brett Hempill, personal communication). Only the top half of the map has been surveyed to date.

Storm events play an important role in determining the hydrological conditions of coastal springs (Lerner et al., 1990). A few studies have been conducted on the influences that tidal fluctuations and rainfall patterns have on submarine spring discharge but more are needed (Ozyurt, 2008; Valle-Levinson et al., 2011; Exposito-Diaz et al., 2013). In karst regions, rainfall events are the primary source of direct aquifer recharge (Lerner et al., 1990). These events result in raising the water table and subsequently increasing aquifer discharge through springs (reviewed in de Vries and Simmers, 2002). Heavy rainfall due to storm events can create a surge of freshwater through the system that may contribute as much as 25 % of the total spring discharge (Lakey & Krothe, 1996). This surge plays an important role in not only aquifer recharge but in defining the geochemical gradients formed by aquifer discharge through coastal springs.

River-fed estuaries are well known for the many ecosystem services they provide (Luisetti et al., 2014). They are areas of high primary productivity (MacIntyre et al., 1996; Underwood & Kromkamp, 1999), they serve as nursery habitats for organisms ranging from microbes to vertebrates (Beck et al., 2001), they protect coastal communities from storms events (Turner et al., 2007), and they provide important commercial and economic benefits (Lenanton & Potter, 1987). In karst regions, estuaries can also indicate the health of the aquifer, are indicators of sea level change and aquifer overuse, and can be points of saltwater intrusion to an aquifer. Unlike river-fed estuaries, little is known about the characteristics of Karst Estuaries or how they function.

Nutrient fluctuations in river-fed estuaries are well known and have been shown to impact biological communities (for reviews see Vitousek et al., 1997; Carpenter et al., 1998; and Kemp et al., 2005). For example, in a study of phytoplankton in Chesapeake Bay, Fisher el al. (1992)

found that during periods of high runoff phytoplankton biomass was limited by the amount of phosphorus in the system but during periods of low runoff phytoplankton biomass was limited by the amount of nitrogen in the system. In a more recent study of dinoflagellates in Kochi estuary, Kerala, Kumar at al. (2014) found that changing salinity and nitrogen values due to rainfall may be responsible for the observed succession of species resulting in a change of the phytoplankton community structure. Less well studied are the impacts of submarine springs on biological communities. Water from spring discharge is more geochemically stabile in terms of nutrient concentrations (Knight et al., 2008) and less prone to the fluctuations observed in surface rivers and streams (Tsiaras et al., 2014). In a study comparing a near-shore sink to an active submarine spring, Garman et al. (2011) found decreased benthic richness and diversity in the vicinity of the spring compared to the sink most likely due to increased spring discharge during the rainy season and not nutrient fluctuations.

Ecosystems comprising Karst Estuaries could be controlled by inland hydrological conditions and sea level. Events that shift the balance between these two forces may have a dramatic impact on the biological communities of these environments. The overall goal of this dissertation is to define the hydrological, geochemical, and biological characteristics of a Karst Estuary. In order to do this I 1) describe the influence of tidal fluctuations on spring discharge, 2) demonstrate the impact rainfall patterns have on aquifer level and spring discharge, 3) define the hydrogeological conditions of the nearshore brackish submarine spring discharge, 4) show the geochemical gradients formed by spring discharge within the surrounding Karst Estuary, 5) estimate biological richness and relative abundance of microbial communities using length heterogeneity PCR and macrofaunal communities by morphological measurements, 6) estimate relative change of biological abundance of microbial communities using quantitative PCR, 7) evaluate community structure using multidimensional scaling plots, and 8) correlate changes of the biological communities to multiple geochemical and hydrological parameters.

Site Description

Approximately one km south of the town of Aripeka on the west coast of central Florida is Double Keyhole Spring, a nearshore brackish submarine spring that discharges directly into the Gulf of Mexico forming a surface estuary (Figs. 1-1 & 1-2). I initially expected that the brackish discharge was the result of two conduits (one with inland fresh groundwater, and one with offshore saltwater) that merged at some point below Double Keyhole Spring. Five sites were monitored in the area, Double Keyhole Spring, Double Keyhole Pond directly outside of Double Keyhole Spring, an ‘estuary’ site 100 m west of Double Keyhole Spring, a ‘marine’ site two km west-southwest of Double Keyhole Spring in the Gulf of Mexico, and an inland freshwater spring, Isabella Spring, one km south-southeast of Double Keyhole Spring (Fig. 1-2). These sites were chosen to investigate geochemical gradients between freshwater and marine sources associated with Double Keyhole Spring in order to determine the amount of mixing between the Upper Floridan aquifer and the Gulf of Mexico.

Geological and Hydrological Background

The Floridan aquifer system encompasses approximately 160,000 km^2 and underlies the southern parts of Alabama, Georgia, and South Carolina, and all of Florida (Miller, 1986). The system is split into two parts based on the permeability of the rock (Upper and Lower Floridan aquifer) which are separated by a Middle Confining Unit. The Upper Floridan aquifer is composed of Tertiary karstic rocks of which the thickest and most productive are the Eocene Avon Park Formation and Ocala Limestone (Miller, 1986). Near the town of Aripeka, Florida the Upper Floridan aquifer is unconfined at the surface (Sprinkle, 1989) allowing for rapid recharge

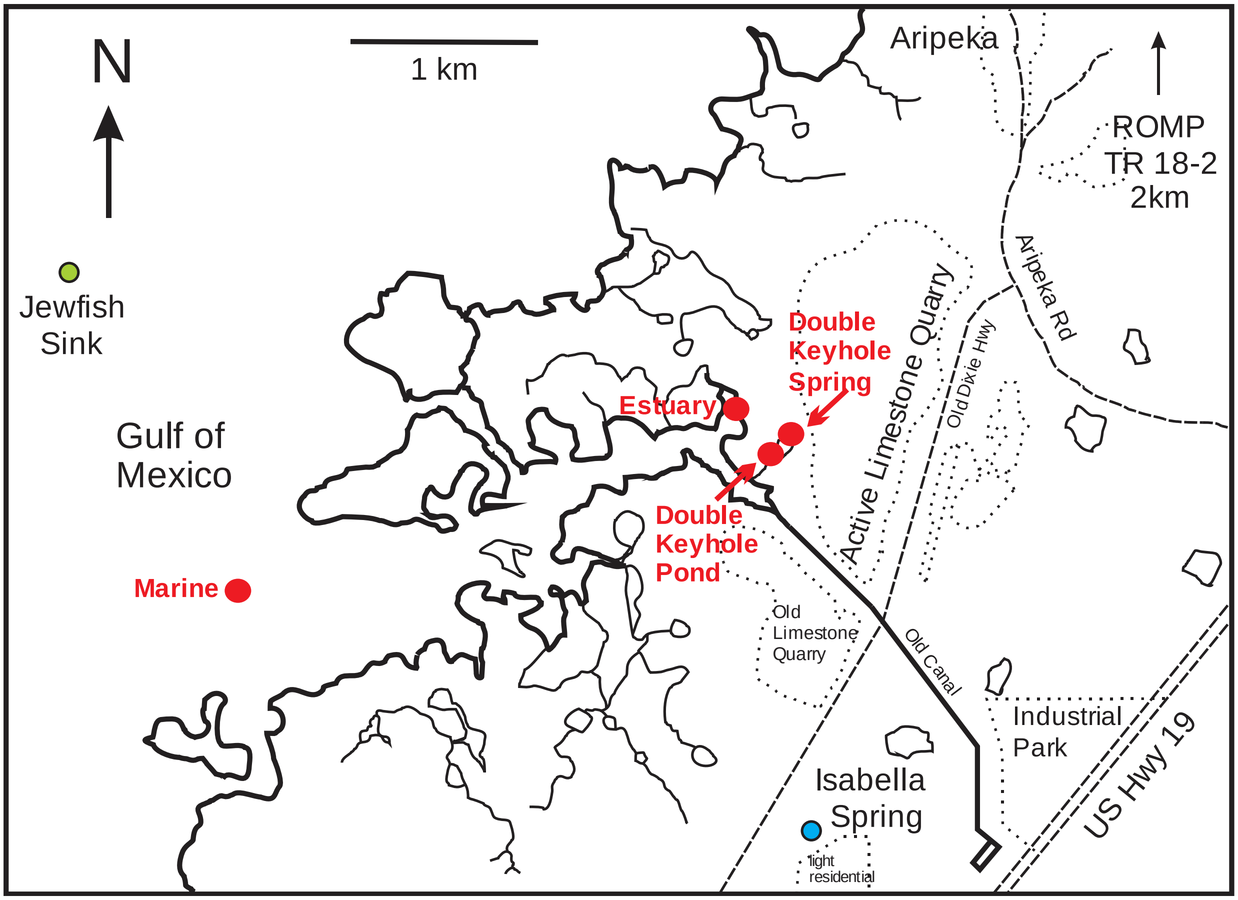


Figure 1-2. Site map showing the studies sites in west central Florida. ROMP TR 18-2 is approximately two km north of the town of Aripeka.

and increased discharge soon after rainfall events. In this area the Upper Floridan aquifer consists of undifferentiated sands near the surface, limestone from one – 151 m, followed by dolostone and limestone layers from 151 – 248 m (Fig. 1-3). The lower portion below 151 m is interspersed with multiple layers of clay (Decker, 1983).

The hydrogeological framework of the area can be divided into three sections, the upper portion of the Upper Floridan aquifer consisting of low salinity, low sulfate water, the lower portion of the Upper Floridan aquifer consisting of low salinity, high sulfate water, and the Gulf of Mexico consisting of high salinity, high sulfate water. Each section has distinct geochemical signatures distinguished by strontium concentrations and $\delta^{18}O$ values that can be used to determine the extent of mixing found in Double Keyhole Spring discharge. Strontium concentration and $\delta^{18}O$ values were used as they are well known conservative tracers that are able to differentiate different sources of water (Epstein & Mayeda, 1953; Craig & Gordon, 1965; Peterman et al., 1970; Veizer et al., 1999; Bigg & Rohling, 2000). The $\delta^{18}O$ values reported are a measure of the ratio of two oxygen isotopes, ^{18}O:^{16}O. This ratio changes over time due to mechanisms such as biologically mediated isotopic fractionation (Kendall & McDonnell, 1999) and evaporation (Dansgaard, 1964). The $\delta^{18}O$ values and strontium concentrations present during the formation of the Floridan aquifer system due to $CaCO_3$ deposition are reflected in the water discharged from the aquifer due to the dissolution of the karst. This results in a chemical signature found in the water that can be used to differentiate the different levels of the Floridan aquifer system from each other and from the Gulf of Mexico.

Pump tests of wells in the area around Aripeka indicate that the karstic rocks from the surface to 157 m are filled with low salinity, low sulfate freshwater. A transition zone between freshwater and saltwater was determined to be between 157 – 166 m below which the water increases in salinity and sulfate concentration. High sulfate concentrations (3,155 mg/L) were found at a depth of 280 m near the highest concentration of gypsum in the lower portion of the Upper Floridan aquifer (Decker, 1983). Further inland from this site, Sacks et al. (1995) found low salinity, high sulfate waters at the bottom of the Upper Floridan aquifer near the Middle Confining Unit.

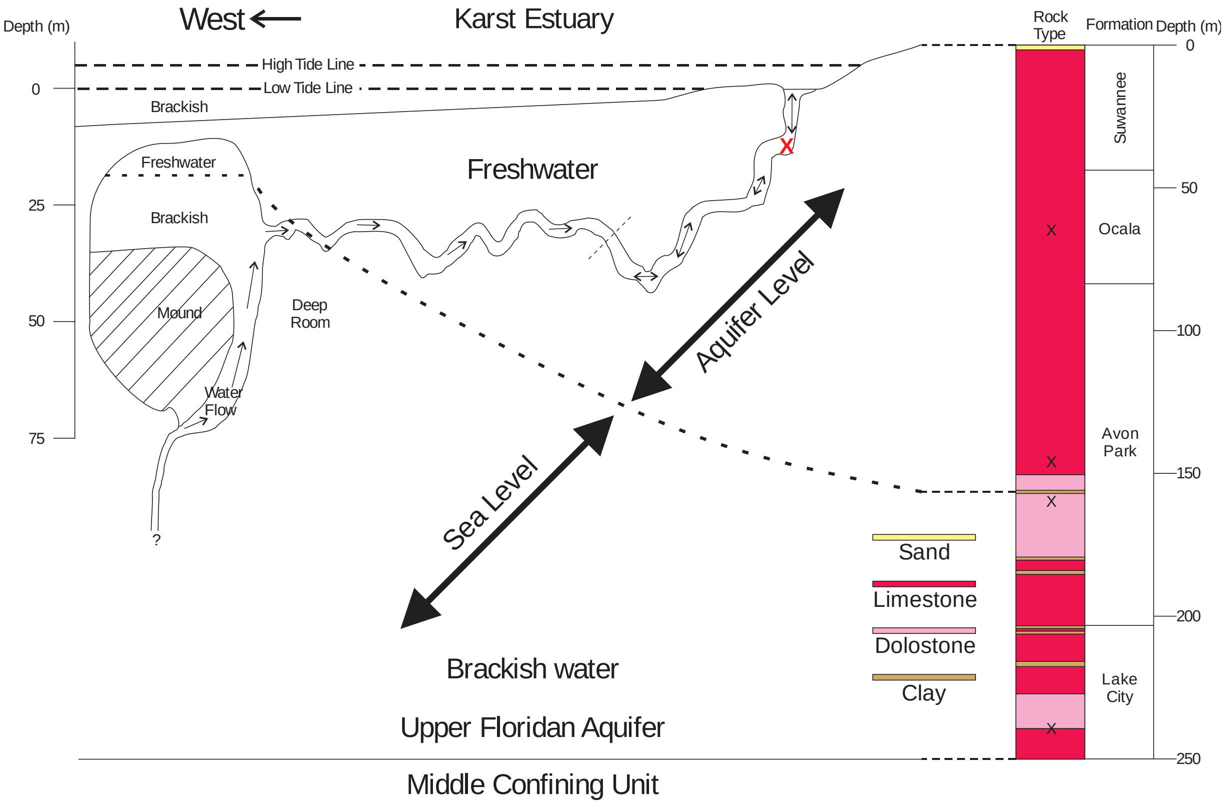


Figure 1-3. Double Keyhole Spring profile and the geology of the nearby area. On the left side is the cross section of Double Keyhole Spring showing the direction of water flow. The red X near the entrance indicates the datasonde deployment location. The dashed line across the conduit path estimates the location of the deepest penetration of surface water during reversals noted during the study period. The right side of the figure shows the geology of the nearby area with data from Decker (1983). Black X's indicate the approximate depth of SWFWMD hydraulic head gauges. The dashed line across the middle of the figure indicates the edge of the mixing zone between the Gulf of Mexico and the Upper Floridan aquifer.

CHAPTER TWO: HYDROLOGY AND GEOCHEMISTRY

Materials and Methods

Sample Collection

Monitoring of the study sites was conducted from September 2011 through September 2013 to examine spring discharge patterns and to establish correlations to local rainfall and aquifer hydrology. Datasondes were deployed nearly continuously within Double Keyhole Spring to measure water velocity and direction using an Argonaut-Acoustic Doppler Velocimeter (ADV) (SonTek, USA) as well as water temperature, dissolved oxygen (DO), pH, and salinity using a HACH Hydrolab DS3 or DS5X multi-parameter datasonde (HACH, USA). All datasondes were serviced annually and calibrated prior to each deployment. Datasondes deployed in the spring conduit were set to record measurements every 30 minutes. Spring discharge volumes were calculated by multiplying the area of the conduit at the datasonde deployment location (6.12 m) by the water velocity determined by the ADV. Five 500 mL replicate water samples were collected from the springs and surface sites quarterly for geochemical, stable isotopic, and trace element analyses. Spring samples were collected by scientific divers using closed-circuit rebreathers under the auspices of the University of South Florida (USF) scientific diving program. Within the spring conduit, previously sterilized 500 mL bottles filled with autoclaved deionized water were purged three times with helium to prevent the introduction of surface water and air into the spring samples. All samples were kept on ice for transport to the lab. Concurrent with all surface water sample collections, a HACH Hydrolab DS3 or DS5X multi-parameter datasonde was used to measure the same parameters as those in spring conduit. Strontium concentrations (12.2 ppb) and $\delta^{18}O$ values (-2.08‰) for the lower portion of the Upper Floridan aquifer were used from Sacks et al. (1995). Aquifer hydraulic head from a nearby well, ROMP TR 18-2, and total daily rainfall data from the Engle Park, Shady Hills, and Summer Tree sites was retrieved from the Southwest Florida Water Management District (SWFWMD) Water Management Information System (WMIS) website (http://www18.swfwmd.state.fl.us/ResData/Search/ExtDefault.aspx).

Water Sample Analysis

All water samples were filtered through GTTP 04700 0.2 µm membrane filters (Millipore, USA). Samples were divided into 50 mL tubes for trace element analysis, 10 mL bottles for stable isotope analysis, and the remainder was transferred to a 500 mL bottle for major ion analysis. Trace element concentrations were determined using an ELAN DRC II ICP Mass Spectrometer (PerkinElmer SCIEX, USA) following the procedures described by Eggins et al. (1997). Standard curves were created using serial dilutions of known concentrations of trace elements obtained from High-Purity Standards (USA) and Fisher-Scientific (USA). NIST 1640a and CRM-SW (High-Purity Standards, USA) were used to verify the efficiency of the measurements and to account for drift resulting from measuring multiple samples consecutively. The average standard deviation for trace element concentration was less than 0.15% of the values based on replicate measurements of internal standards in each run. Oxygen isotopic composition was determined by the USF Stable Isotope Laboratory using a Finnigan Delta V 3 keV Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA) and a Gasbench II (Thermo Fisher Scientific, USA) preparation device using the equilibration method. The ratio of ^{18}O:^{16}O in the water samples was compared to the Vienna Standard Mean Ocean water standard ratio of ^{18}O:^{16}O and is reported as $\delta^{18}O$ values where $\delta^{18}O = (((^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{standard}) - 1) * 1000$

‰. The average standard deviation of the δ^{18}O values was less than 0.25% of the values based on replicate measurements of internal standards in each run. An isotopic/trace element mass balance analysis of the trace element strontium and δ^{18}O values was conducted to determine the hydrogeological composition of Double Keyhole Spring discharge and the surrounding estuarine waters assuming three end-members following the methods described by Lazareva and Pichler (2011). Strontium concentration and δ^{18}O values were used in conjunction as they are well known conservative tracers (Epstein & Mayeda, 1953; Craig & Gordon, 1965; Peterman et al., 1970; Veizer et al., 1999; Bigg & Rohling, 2000). The three end-members used in the mixing calculations consisted of Gulf of Mexico water represented by samples collected from the marine site (Mar), the upper portion of the Upper Floridan aquifer represented by samples collected from Isabella Spring (Isa), and the lower portion of the Upper Floridan aquifer (LFA) represented from samples collected by Sacks et al. (1995). The following equation was used to determine the individual contribution from each source at each site where x represents the individual sites along the transect (Double Keyhole Spring, Double Keyhole Pond, and estuary): $m_x = m_{Mar} + m_{Isa} + m_{LFA} = 1$.

Water samples were analyzed for ammonia, nitrate, phosphate, sulfate, and total hardness using a HACH DR/2400 spectrophotometer (HACH, USA). The average standard deviations for geochemical parameters based on replicate samples were 0.01 mg/L, 0.1 mg/L, 0.08 mg/L, 68 mg/L, and 4.4 µg/L, respectively. Total alkalinity was measured using an Orion Total Alkalinity kit (Thermo Fisher Scientific, USA) and pH measured using a Jenco 6250 pH meter (Jenco, USA). The average standard deviation for total alkalinity based on replicate samples was 4.1 mg/L. Field measurements of DO, pH, water temperature, and salinity were conducted using a HACH Hydrolab DS3 or DS5X multi-parameter datasonde (HACH, USA). The average standard deviations for DO (mg/L), pH (units), water temperature (°C), and salinity (psu) based on replicate samples were all 0.01. All sampled geochemical parameters were plotted using Microsoft Excel.

Statistical Analyses

Statistical analyses were performed using IBM SPSS 22.0 (IBM, USA). One-Way ANOVAs were conducted on all geochemical data to determine statistical significance ($p < 0.05$) between sample sites collected on the same date and between sample dates at the same site. Partial correlation analyses were conducted to determine statistically significant correlations between the studied parameters controlling for study site or collection date (Bai et al., 2010).

Results

Double Keyhole Spring Profile

The Double Keyhole Spring profile (Fig. 1-3) shows that the depth of the conduit generally stays near 33 m until the ‘Deep Room’ at the end of the diver accessible conduit. The linear distance from the spring entrance to the ‘Deep Room’ is approximately 150 m although divers must travel approximately 400 m due to the meandering of the conduit. During a 50 min dive, datasonde measurements of temperature, salinity, pH, and DO taken every five seconds along the conduit path from the beginning of the spring to the ‘Deep Room’ show no significant difference in any of the variables collected (Fig. 2-1).

Double Keyhole Spring ‘Deep Room’ Profile

The ‘Deep Room’ in Double Keyhole Spring extends from 10m below the surface of the estuary to a depth of 76 m (Fig. 1-3) and is only accessible by divers through the main conduit at 27 m depth. The water within the ‘Deep Room’ is well mixed from the conduit entrance up to a halo/chemo/thermocline at an approximate depth of 19 m where DO and pH increase and temperature and salinity decrease (Fig. 2-2). Datasonde measurements of temperature, salinity, pH, and DO of the brackish water discharging from the ‘Deep Room’ show no significant difference in any of the variables collected until reaching the halo/chemo/thermocline (Fig. 2-2).

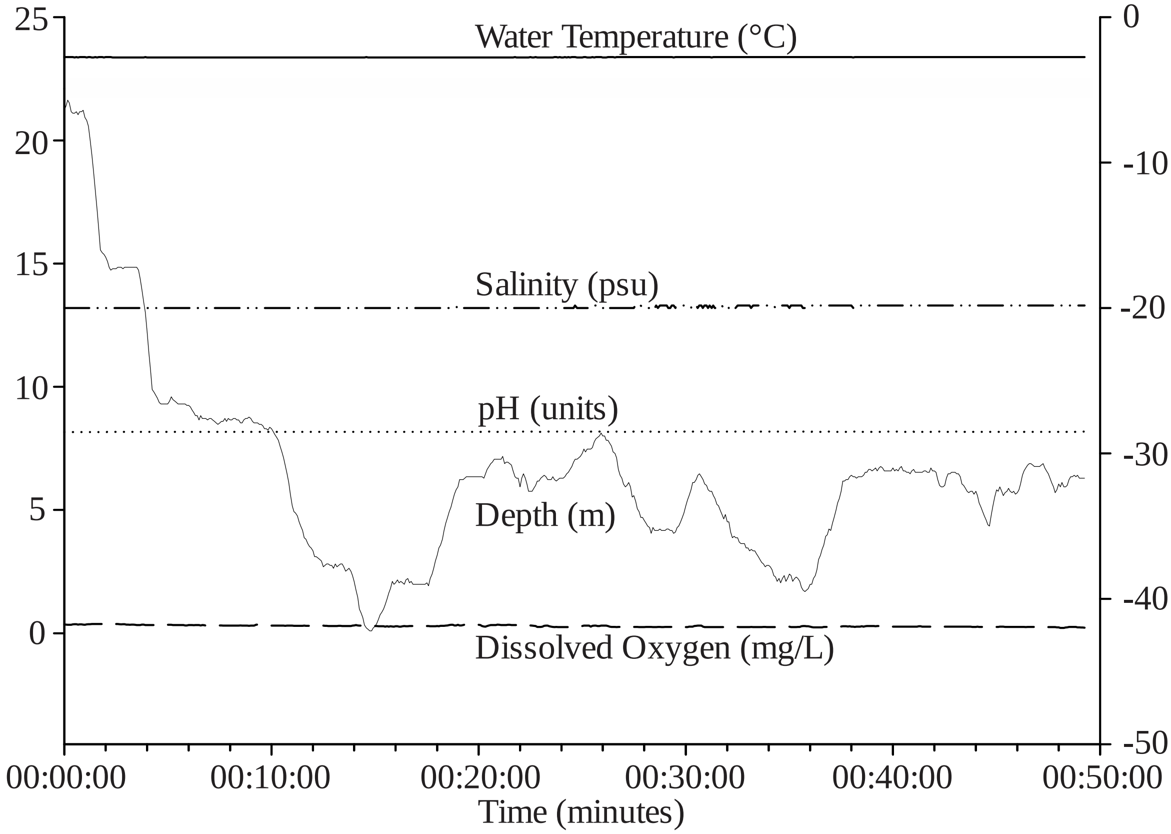


Figure 2-1. Datasonde profile along the Double Keyhole Spring conduit path from the entrance of the spring through the ‘Deep Room’ over the course of a 50 minute dive.

Rainfall Patterns

Rainfall data was retrieved from three SWFWMD data collections sites (Engle Park, Shady Hills, and Summer Tree, site IDs 20546, 20552, and 20461 respectively) located within a two km radius of Double Keyhole Spring. The average daily rainfall between these three sites ranged from nil – 20 cm (Fig. 2-3). Rainfall in Florida generally follows a wet/dry season pattern with the wet season coinciding with hurricane season from June through November, and the data follows this pattern. Maximum average daily rainfall (20 cm) was observed during Tropical Storm Debby in June 2012.

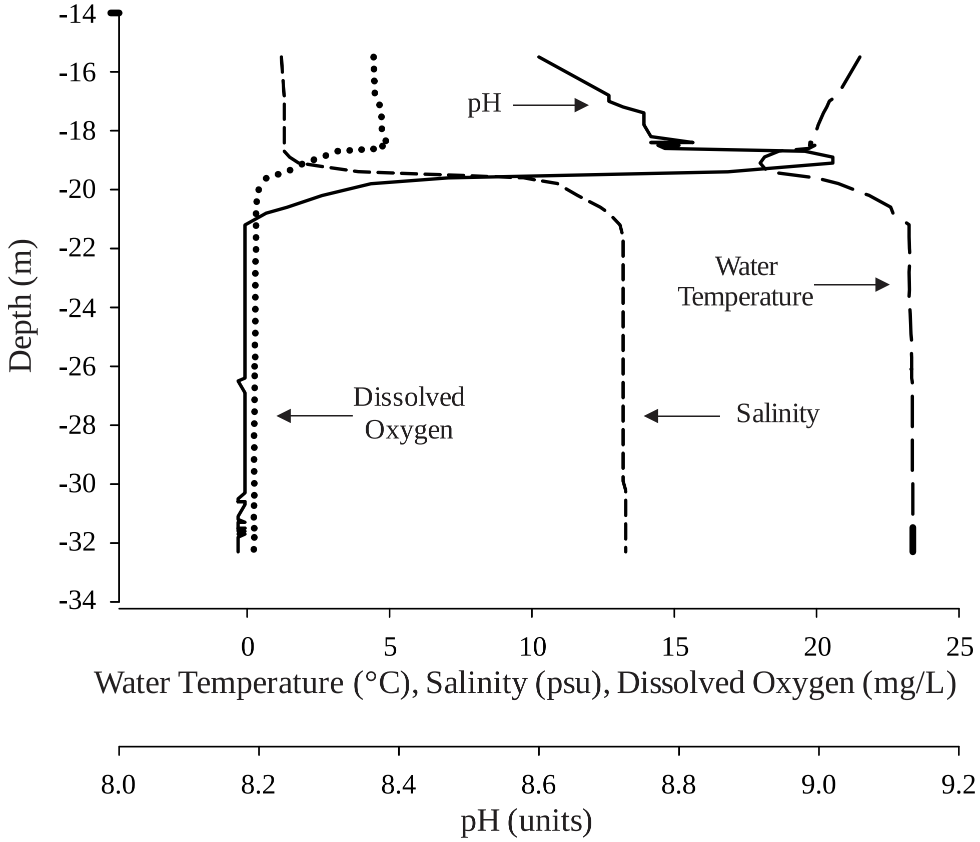


Figure 2-2. Datasonde profile of the ‘Deep Room’ in Double Keyhole Spring.

Tidal Fluctuations and Flow Reversals

Short-term monitoring of Double Keyhole Spring indicates that spring discharge is inversely related to tidal level (Fig. 2-4). The data shows regularly timed salinity increases that correspond to increased aquifer discharge at low tide. It also shows irregularly timed salinity increases that correspond to spring reversals at some high tides. Spring reversals are accompanied by increases in DO and salinity. Reversals of aquifer discharge (saltwater intrusion events) can be seen on Figure 2-4 as all points that fall below zero L/s Estimated Aquifer Discharge. The frequency of reversals increased throughout the dry season with a maximum occurring just before Tropical Storm Debby (Table 2-1). Immediately after Tropical Storm Debby, spring reversals stopped but gradually increased through the dry season until the next rainy season in May 2013. Datasondes

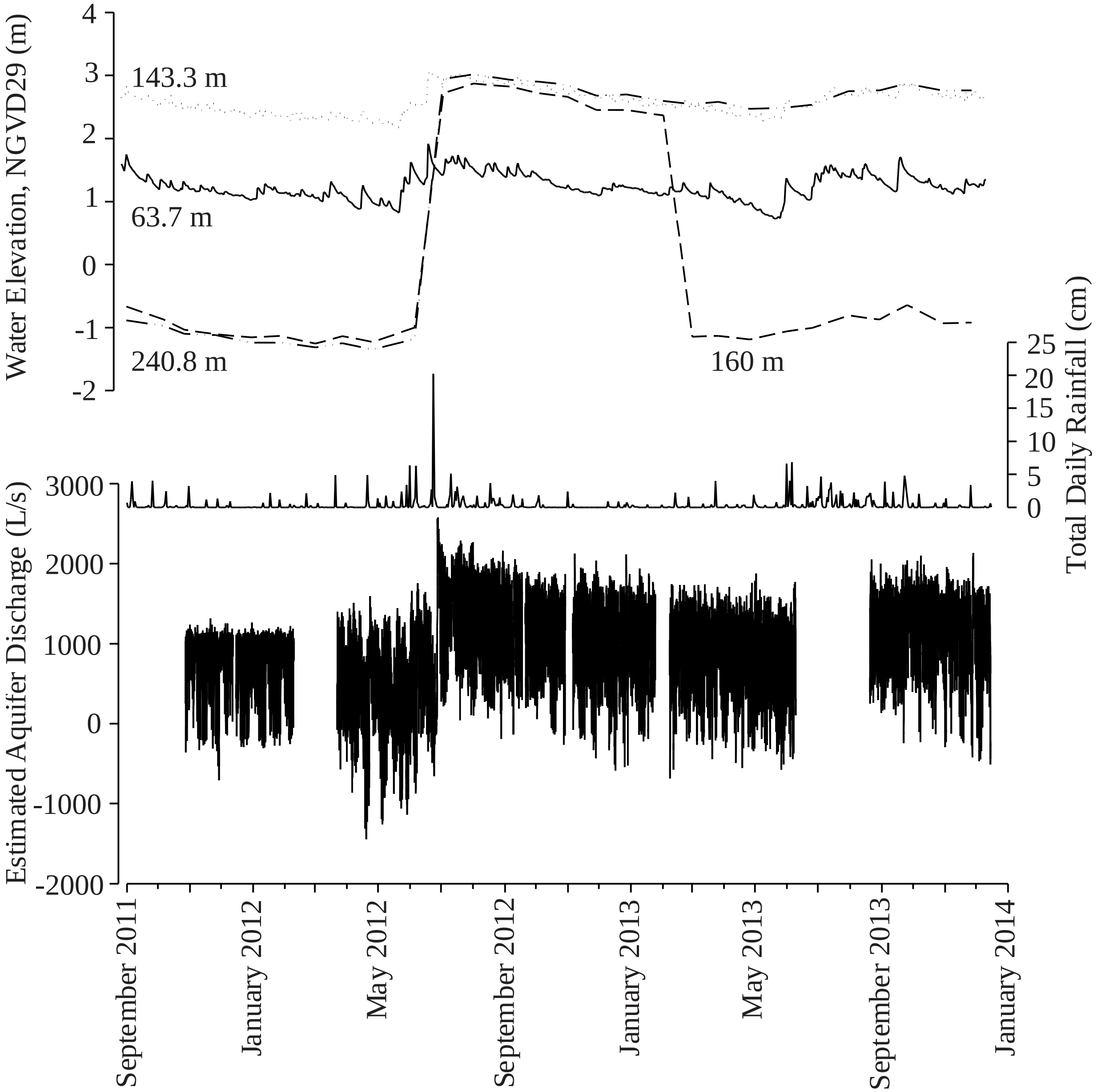


Figure 2-3. Hydraulic head data from four depths within ROMP TR 18-2 in the Upper Floridan aquifer, total daily rainfall around the study sites, and estimated aquifer discharge from fall 2011 through winter 2013.

were deployed from February 12th through March 16th 2013 at different depths (16 m and 40 m) in Double Keyhole Spring. During that time there were a total of 39 high tides that included 10 reversal events of which seven reached a depth of 16 m (24 m from the entrance) and four reached a depth of 40 m (110 m from the entrance).

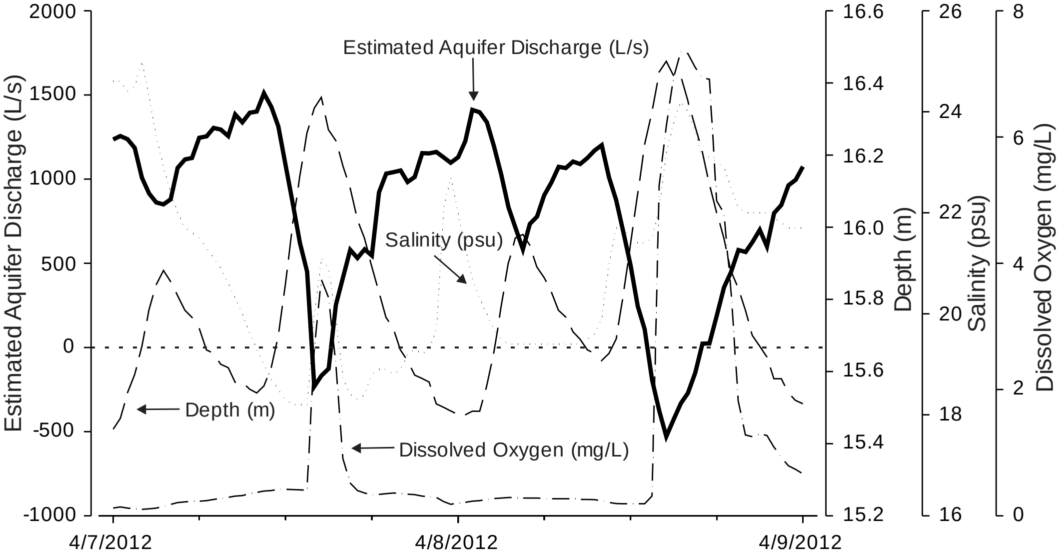


Figure 2-4. Estimated aquifer discharge, tidal height (as datasonde depth at deployment location in meters), salinity, and dissolved oxygen concentration over a two day period in April 2012.

Table 2-1. Discharge data of Double Keyhole Spring from fall 2011 through winter 2013.

Sample dates	Average discharge (L/s)	Maximum discharge (L/s)	Minimum discharge (L/s)	Discharge vs. Tide r^2	Total data points	Total data points reversing	Percent reversing
10/27/2011 3:00:00 PM - 12/12/2011 4:00:00 PM	765.56	1313.04	-708.78	0.6279	2211	114	5.16
12/15/2011 4:00:00 PM - 2/9/2012 2:00:00 PM	754.81	1260.54	-307.13	0.7758	2685	162	6.03
3/22/2012 1:00:00 PM - 5/3/2012 11:00:00 AM	552.64	1591.19	-1449.11	0.81	2013	294	14.61
5/3/2012 12:30:00 PM - 6/25/2012 12:30:00 AM	505.99	1753.87	-1261.05	0.7036	2521	465	18.45
6/27/2012 2:00:00 PM - 7/19/2012 2:00:00 PM	1548.65	2576.68	40.26	No data	1057	0	0.00
7/19/2012 2:00:00 PM - 9/17/2012 2:30:00 PM	1279.47	2288.32	-190.25	0.6515	2882	10	0.35
9/20/2012 4:30:00 PM - 10/29/2012 1:00:00 PM	1082.07	1894.91	-264.73	0.7571	1866	16	0.86
11/5/2012 4:00:00 PM - 1/24/2013 12:30:00 PM	1028.90	2127.03	-588.26	0.7544	3834	92	2.40
2/7/2013 1:30:00 PM - 4/25/2013 11:30:00 AM	896.88	1742.41	-689.19	0.739	3693	107	2.90
4/25/2013 1:30:00 PM - 5/9/2013 11:30:00 AM	786.36	1874.65	-347.77	0.8306	669	32	4.78
5/9/2013 2:00:00 PM - 6/9/2013 4:30:00 PM	775.57	1766.78	-575.07	0.815	1494	91	6.09
8/20/2013 12:30:00 PM - 12/15/2013 2:30:00 AM	1154.17	2130.85	-510.74	0.7189	5597	91	1.63

Aquifer Hydraulic Head Measurements

Aquifer hydraulic head data relative to mean sea level (National Geodetic Vertical Datum of 1929) were retrieved from the SWFWMD WMIS website (Fig. 2-3). Four depths (63.7 m, 143 m, 160 m, and 240 m) within the same well (ROMP TR 18-2) located approximately two km northeast of Double Keyhole Spring in the Upper Floridan aquifer (Fig. 1-2) were used to compare long-term hydraulic head changes to rainfall patterns and aquifer geochemistry. The hydraulic head at 143 m was higher (2.17 - 3.08 m) than any of the other three locations monitored, 63.7 m (0.73 - 1.92 m), 160 m (-1.27 - 2.88 m), and 240 m (-1.37 - 3.02 m). Tropical Storm Debby in June 2012 resulted in a noticeable increase at all locations but the greatest changes were seen at 160 m and 240 m, +3.34 m and +4.15 m respectively. Approximately nine months after Tropical Storm Debby hydraulic head at 160 m showed a 3.53 m decrease.

Aquifer Discharge

Monitoring of Double Keyhole Spring discharge over a two year period shows that spring discharge ranges from approximately -1449 L/s to 2576 L/s (Table 2-1). Average daily discharge and maximum daily discharge both correlated to all four aquifer levels but not to total rainfall the week prior to or total rainfall between sampling dates (Table 2-2). The lowest discharge rate observed (-1449 L/s) was in April 2012 during the dry season. The highest discharge rate observed (2576 L/s) was immediately following Tropical Storm Debby in June 2012. Approximately nine hours after Tropical Storm Debby aquifer discharge from Double Keyhole Spring increased from an average of 506 L/s to 1549 L/s (Table 2-1). Correlations of aquifer discharge to tidal height are shown in Table 2-1. The highest correlations were seen during the dry seasons (r^2 = 0.81-0.83) while the lowest were seen in the wet seasons (r^2 = 0.63-0.65).

Table 2-2. Correlations of rainfall, Double Keyhole Spring discharge, and aquifer hydraulic head.

		Total rainfall the week prior to sampling	Total rainfall between sample collections	Average Daily Discharge	Maximum Daily Discharge	Aquifer water elevation guage @ 63.7m	Aquifer water elevation guage @ 143m	Aquifer water elevation guage @ 160m
Total rainfall between sample	Correlation Significance (2-tailed) df	.643 .000 47						
Average Daily Discharge	Correlation Significance (2-tailed) df							
Maximum Daily Discharge	Correlation Significance (2-tailed) df			.721 .000 47				
Aquifer water elevation guage @ 63.7m	Correlation Significance (2-tailed) df	.543 .000 47	.815 .000 47	.388 .006 47	.573 .000 47			
Aquifer water elevation guage @ 143m	Correlation Significance (2-tailed) df		.592 .000 47	.615 .000 47	.764 .000 47	.888 .000 47		
Aquifer water elevation guage @ 160m	Correlation Significance (2-tailed) df			.565 .000 47	.868 .000 47	.499 .000 47	.804 .000 47	
Aquifer water elevation guage @ 240m	Correlation Significance (2-tailed) df			.694 .000 47	.799 .000 47	.635 .000 47	.871 .000 47	.870 .000 47

Strontium Concentration and $\delta^{18}O$ Values

Strontium concentration and $\delta^{18}O$ values are shown in Table 2-3 and plotted versus salinity in Figure 2-5. Strontium concentrations ranged from a high of 8916 ppb at the Double Keyhole Spring site in summer 2013 to a low of 3008 ppb at the estuary site in late-summer 2012. Samples taken from Isabella Spring are representative of the upper portion of the Upper Floridan aquifer and ranged from 262 - 866 ppb. The $\delta^{18}O$ values ranged from a low of -1.5 ‰ at Double Keyhole Spring in fall 2012 to a high of 2.4 ‰ at the marine site in summer 2013. Samples taken from Isabella Spring are representative of the upper portion of the Upper Floridan aquifer and range from -2.8 to - 3.2 ‰ which is indicative of more evaporatively ^{18}O-enriched water from surficial aquifers.

Table 2-3. Strontium concentrations and $\delta^{18}O$ values at all sites from fall 2011 through fall 2013.

Strontium (ppb)	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine - Average	2107	3883	4234	4270	3642	5442	4974	4370	7434	5074
Marine - Std Dev	300	446	231	372	514	697	536	234	483	217
Estuary - Average	3655	5632	4418	4549	3008	4230	4151	4834	4771	4229
Estuary - Std Dev	229	37	614	446	309	159	126	268	319	177
Double Keyhole Pond - Average	4603	4775	5481	5109	3906	3755	4051	4211	5146	4577
Double Keyhole Pond - Std Dev	269	639	816	67	357	197	439	76	490	34
Double Keyhole Spring - Average	3794	4392	5130	8916	3062	3603	3534	4287	5470	4852
Double Keyhole Spring - Std Dev	441	223	235	1337	346	388	514	430	394	492
Isabella - Average	425	262	350	867	496	499	369	480	519	494
Isabella - Std Dev	30	25	22	101	16	29	18	19	0	4
$\delta^{18}O$ (‰)	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine - Average	0.8	0.0	1.3	2.4	-0.3	1.0	0.6	0.8	1.1	0.9
Marine - Std Dev	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Estuary - Average	-0.8	-0.4	0.1	0.8	-0.9	-0.3	-0.9	-0.7	-0.1	-0.3
Estuary - Std Dev	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Double Keyhole Pond - Average	-1.0	-0.8	-0.2	-0.1	-1.0	-0.8	-0.9	-0.8	-0.6	-0.9
Double Keyhole Pond - Std Dev	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1
Double Keyhole Spring - Average	-1.0	-0.6	-0.6	0.3	-1.4	-1.5	-1.0	-1.0	-0.6	-1.0
Double Keyhole Spring - Std Dev	0.3	0.1	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.1
Isabella - Average	-2.9	-2.9	-2.9	-2.8	-3.0	-2.9	-3.2	-3.0	-3.1	-3.0
Isabella - Std Dev	0.3	0.3	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.1

Water Quality Along the Estuarine Transect

Monitored water quality parameters are shown in Figures 2-6 & 2-7. Water temperature, salinity, pH, and DO showed statistically significant differences between all sites and dates. Water temperature remained close to 24 °C in Double Keyhole Spring throughout the sampling period. Seasonal variations were observed at the marine site where water temperatures fluctuated from 17 °C during the fall and winter to 28 °C during the summer. Salinity values ranged from a low of 13.1 psu at the Double Keyhole Pond site in late-summer 2013 to a high of 23.9 psu during summer 2013 at the marine site. There was a general linear increase of pH from Double Keyhole Spring through the marine site. The pH ranged from a low of 6.2 at Double Keyhole Spring in winter 2011 to a high of 8.03 in fall 2013 at the marine site. Dissolved oxygen concentrations displayed a general increasing linear trend from nil - 2.62 mg/L at Double Keyhole Spring to nil - 2.62 mg/L at the marine site. Total hardness concentrations ranged from 5.8 µg/L in late-summer 2012 at Double Keyhole Spring to 31.4 µg/L in late-summer 2012 at the estuary site. Alkalinity ranged from 28 mg/L in spring 2012 at Double Keyhole Spring to 136 mg/L in winter 2011 at the estuary site. Ammonia concentrations ranged from 0.002 mg/L during summer 2012 at the marine site to 0.056 mg/L during fall 2011 at Double Keyhole Spring. Nitrate concentrations ranged from zero mg/L at the estuary and Double Keyhole Pond sites (late-summer and summer 2012 respectively) to one mg/L in fall 2012 at the marine site. Sulfate concentrations ranged from 550 mg/L in summer 2013 at Double Keyhole Spring to 1350 mg/L in summer 2012 at the marine site. Phosphate concentrations ranged from 0.01 mg/L at the marine and estuary sites (fall 2013 and winter 2011 respectively) to 1.26 mg/L in summer 2012 at the estuary site. Correlations of rainfall, aquifer discharge, and aquifer hydraulic head to the studied geochemical parameters at all sites along the spring/marine transect are shown in Tables 2-4 to 2-7. Ammonia concentration was the only measured variable that did not show significant correlation to the hydrologic parameters monitored during the study period. There were generally more significant and higher correlations of the monitored geochemical parameters to rainfall, aquifer discharge, and aquifer hydraulic head at sites closer to Double Keyhole Spring than the marine site.

Discussion

Double Keyhole Spring Profile

Datasonde results from Double Keyhole Spring suggest that once water enters the conduit system from the ‘Deep Room’ it has already been well mixed and remains constant over distances of 400 m or less and in time frames of less than one hour (Fig. 2-1). In anchialine and submarine caves in Bermuda, Van Hengstum and Scott (2011) found differences of salinity, organic matter, $\delta^{13}C$ values, and C:N ratios near cave entrances but once inside those parameters remained relatively constant throughout the conduit. Liu et al. (2004) found similar results in a karst conduit system in China where prior to major rainfall events there were no short-term

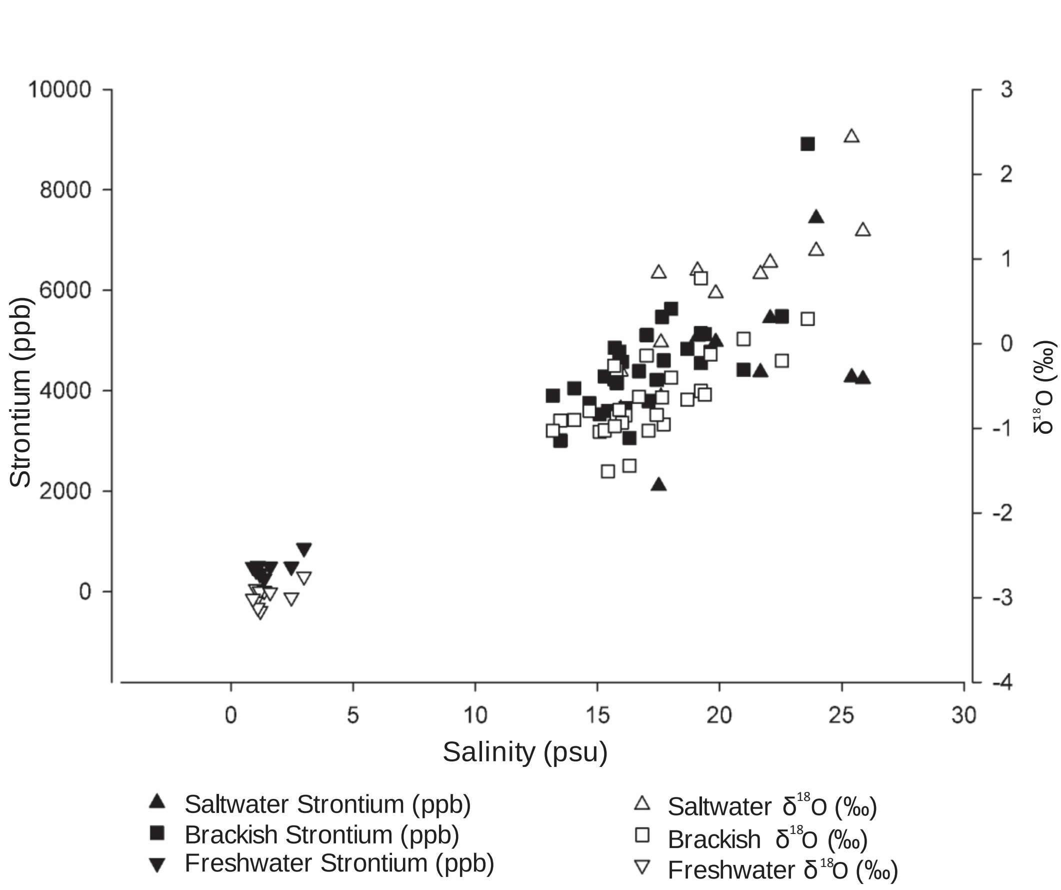
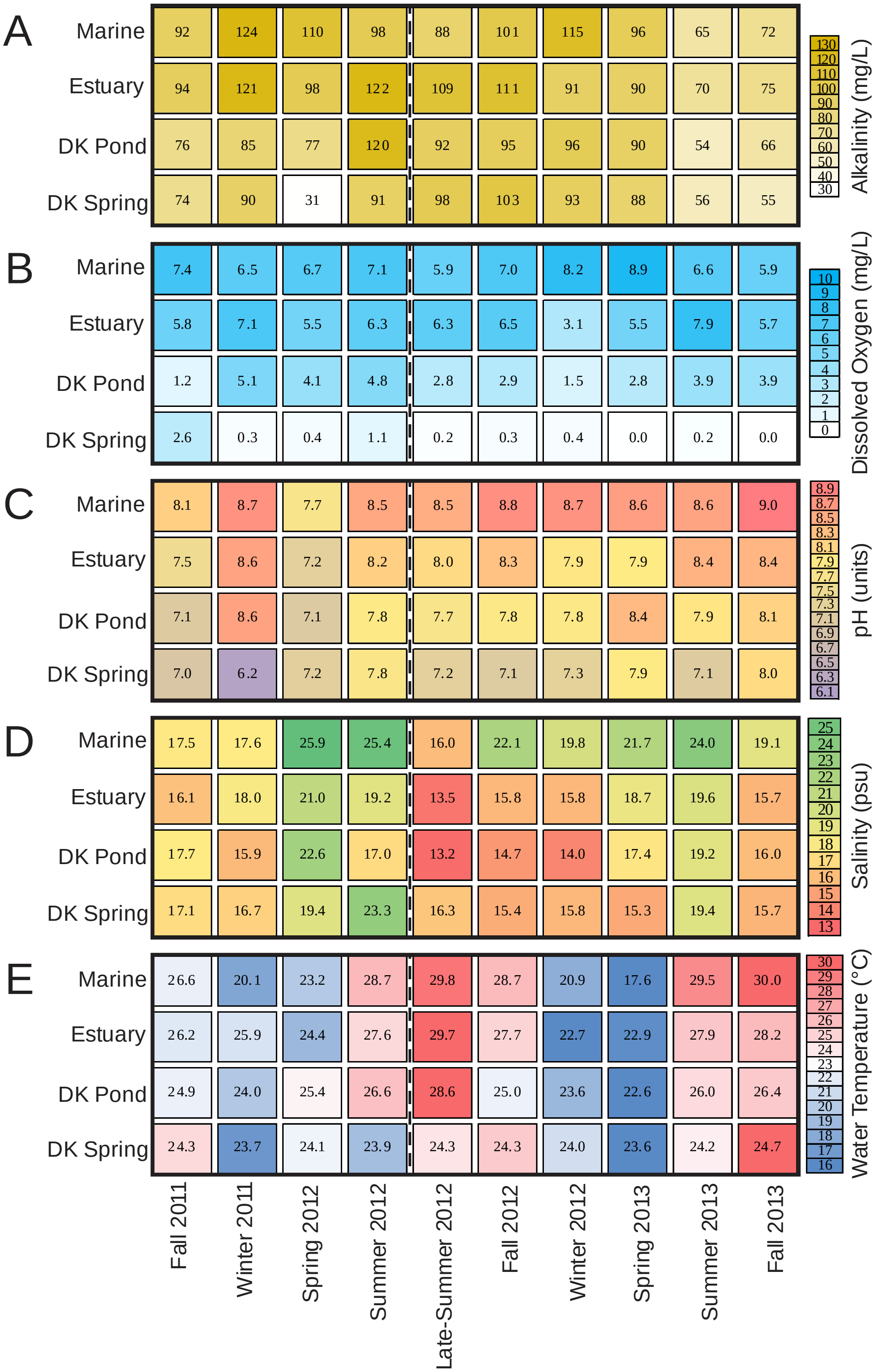


Figure 2-5. Strontium concentration and $\delta^{18}O$ values plotted versus salinity. Saltwater samples were collected from the marine site in the Gulf of Mexico. Brackish samples are from the estuary, Double Keyhole Pond, and Double Keyhole Spring sites. Freshwater samples are from Isabella Spring.

changes observed in water temperature or pH of spring discharge. The data suggests that there are no other water sources entering the conduit path between the surface and 'Deep Room' or if so, their impacts on the geochemistry of the spring discharge are negligible over short time scales.

Double Keyhole 'Deep Room' Profile

The depth profile of the 'Deep Room' in Double Keyhole Spring indicates that the water is well mixed from the conduit entrance up to the halo/chemo/thermocline at the top of the room (Fig. 2-2). I suspect that the level of the halo/chemo/thermocline in the 'Deep Room' of Double



A	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	92	124	110	98	88	101	115	96	65	72
Estuary	94	121	98	122	109	111	91	90	70	75
DK Pond	76	85	77	120	92	95	96	90	54	66
DK Spring	74	90	31	91	98	103	93	88	56	55

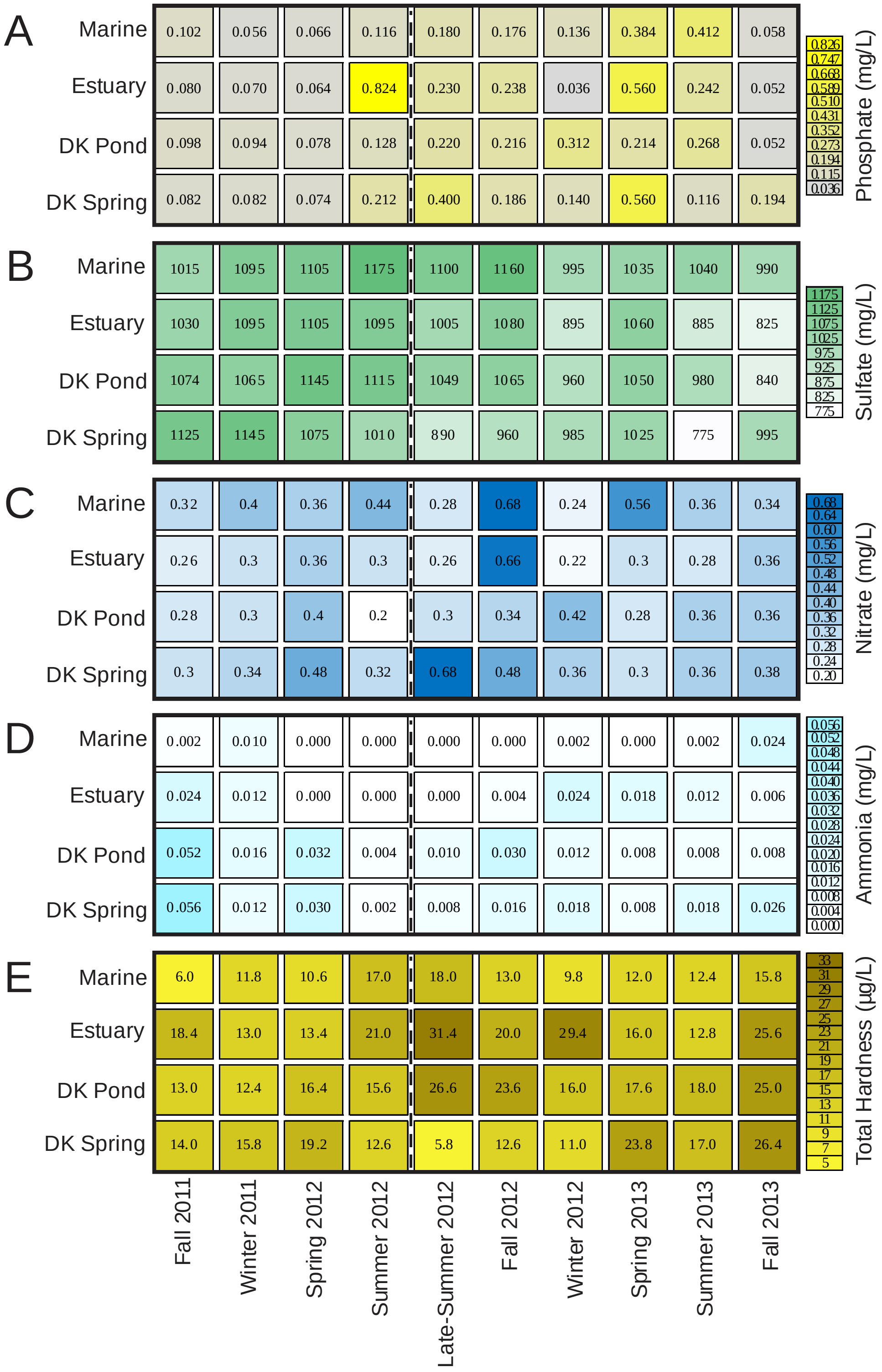
B	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	7.4	6.5	6.7	7.1	5.9	7.0	8.2	8.9	6.6	5.9
Estuary	5.8	7.1	5.5	6.3	6.3	6.5	3.1	5.5	7.9	5.7
DK Pond	1.2	5.1	4.1	4.8	2.8	2.9	1.5	2.8	3.9	3.9
DK Spring	2.6	0.3	0.4	1.1	0.2	0.3	0.4	0.0	0.2	0.0

C	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	8.1	8.7	7.7	8.5	8.5	8.8	8.7	8.6	8.6	9.0
Estuary	7.5	8.6	7.2	8.2	8.0	8.3	7.9	7.9	8.4	8.4
DK Pond	7.1	8.6	7.1	7.8	7.7	7.8	7.8	8.4	7.9	8.1
DK Spring	7.0	6.2	7.2	7.8	7.2	7.1	7.3	7.9	7.1	8.0

D	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	17.5	17.6	25.9	25.4	16.0	22.1	19.8	21.7	24.0	19.1
Estuary	16.1	18.0	21.0	19.2	13.5	15.8	15.8	18.7	19.6	15.7
DK Pond	17.7	15.9	22.6	17.0	13.2	14.7	14.0	17.4	19.2	16.0
DK Spring	17.1	16.7	19.4	23.3	16.3	15.4	15.8	15.3	19.4	15.7

E	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	26.6	20.1	23.2	28.7	29.8	28.7	20.9	17.6	29.5	30.0
Estuary	26.2	25.9	24.4	27.6	29.7	27.7	22.7	22.9	27.9	28.2
DK Pond	24.9	24.0	25.4	26.6	28.6	25.0	23.6	22.6	26.0	26.4
DK Spring	24.3	23.7	24.1	23.9	24.3	24.3	24.0	23.6	24.2	24.7

Figure 2-6. Plots of alkalinity, dissolved oxygen, pH, salinity, and water temperature at all sites from fall 2011 through fall 2013. The dashed line indicates the occurrence of Tropical Storm Debby.



A	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.102	0.056	0.066	0.116	0.180	0.176	0.136	0.384	0.412	0.058
Estuary	0.080	0.070	0.064	0.824	0.230	0.238	0.036	0.560	0.242	0.052
DK Pond	0.098	0.094	0.078	0.128	0.220	0.216	0.312	0.214	0.268	0.052
DK Spring	0.082	0.082	0.074	0.212	0.400	0.186	0.140	0.560	0.116	0.194

B	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	1015	1095	1105	1175	1100	1160	995	1035	1040	990
Estuary	1030	1095	1105	1095	1005	1080	895	1060	885	825
DK Pond	1074	1065	1145	1115	1049	1065	960	1050	980	840
DK Spring	1125	1145	1075	1010	890	960	985	1025	775	995

C	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.32	0.4	0.36	0.44	0.28	0.68	0.24	0.56	0.36	0.34
Estuary	0.26	0.3	0.36	0.3	0.26	0.66	0.22	0.3	0.28	0.36
DK Pond	0.28	0.3	0.4	0.2	0.3	0.34	0.42	0.28	0.36	0.36
DK Spring	0.3	0.34	0.48	0.32	0.68	0.48	0.36	0.3	0.36	0.38

D	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.002	0.010	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.024
Estuary	0.024	0.012	0.000	0.000	0.000	0.004	0.024	0.018	0.012	0.006
DK Pond	0.052	0.016	0.032	0.004	0.010	0.030	0.012	0.008	0.008	0.008
DK Spring	0.056	0.012	0.030	0.002	0.008	0.016	0.018	0.008	0.018	0.026

E	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	6.0	11.8	10.6	17.0	18.0	13.0	9.8	12.0	12.4	15.8
Estuary	18.4	13.0	13.4	21.0	31.4	20.0	29.4	16.0	12.8	25.6
DK Pond	13.0	12.4	16.4	15.6	26.6	23.6	16.0	17.6	18.0	25.0
DK Spring	14.0	15.8	19.2	12.6	5.8	12.6	11.0	23.8	17.0	26.4

Figure 2-7. Plots of phosphate, sulfate, nitrate, ammonia, and total hardness at all sites from fall 2011 through fall 2013. The dashed line indicates the occurrence of Tropical Storm Debby.

Table 2-4. Correlations of rainfall, Double Keyhole Spring discharge, and aquifer hydraulic head to studied geochemical parameters at the marine site.

Marine		Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Total rainfall the week prior to sampling	Correlation	.510				-.497					
	Significance (2-tailed)	.000				.000					
	df	47				47					
Total rainfall between sample collections	Correlation	.736		-.527	-.544	-.748					
	Significance (2-tailed)	.000		.000	.000	.000					
	df	47		47	47	47					
Average Daily Discharge	Correlation			-.538	-.494						
	Significance (2-tailed)			.000	.000						
	df			47	47						
Maximum Daily Discharge	Correlation	.456									
	Significance (2-tailed)	.001									
	df	47									
Aquifer water elevation guage @ 63.7m	Correlation	.583		-.591		-.504					
	Significance (2-tailed)	.000		.000		.000					
	df	47		47		47					
Aquifer water elevation guage @ 143m	Correlation	.443		-.693							
	Significance (2-tailed)	.001		.000							
	df	47		47							
Aquifer water elevation guage @ 160m	Correlation			-.396							
	Significance (2-tailed)			.005							
	df			47							
Aquifer water elevation guage @ 240m	Correlation			-.623							
	Significance (2-tailed)			.000							
	df			47							

Table 2-5. Correlations of rainfall, Double Keyhole Spring discharge, and aquifer hydraulic head to studied geochemical parameters at the estuary site.

Estuary		Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Total rainfall the week prior to sampling	Correlation	.444					.337			.474	
	Significance (2-tailed)	.001					.018			.001	
	df	47					47			47	
Total rainfall between sample	Correlation	.730		-.567			.338				-.395
	Significance (2-tailed)	.000		.000			.017				.005
	df	47		47			47				47
Average Daily Discharge	Correlation	.411		-.562			.352			-.345	
	Significance (2-tailed)	.003		.000			.013			.015	
	df	47		47			47			47	
Maximum Daily Discharge	Correlation	.497		-.623		.386	.517				
	Significance (2-tailed)	.000		.000		.006	.000				
	df	47		47		47	47				
Aquifer water elevation guage @ 63.7m	Correlation	.560		-.815			.533				
	Significance (2-tailed)	.000		.000			.000				
	df	47		47			47				
Aquifer water elevation guage @ 143m	Correlation	.459		-.951			.625				
	Significance (2-tailed)	.001		.000			.000				
	df	47		47			47				
Aquifer water elevation guage @ 160m	Correlation			-.749	-.320	.373	.599				
	Significance (2-tailed)			.000	.025	.008	.000				
	df			47	47	47	47				
Aquifer water elevation guage @ 240m	Correlation			-.801			.461				
	Significance (2-tailed)			.000			.001				
	df			47			47				

Table 2-6. Correlations of rainfall, Double Keyhole Spring discharge, and aquifer hydraulic head to studied geochemical parameters at the Double Keyhole Pond site.

Double Keyhole Pond		Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Total rainfall the week prior to sampling	Correlation	.498				.333		-.375			
	Significance (2-tailed)	.000				.019		.008			
	df	47				47		47			
Total rainfall between sample	Correlation	.675			-.431						-.355
	Significance (2-tailed)	.000			.002						.012
	df	47			47						47
Average Daily Discharge	Correlation	.499		-.369			.481				
	Significance (2-tailed)	.000		.009			.000				
	df	47		47			47				
Maximum Daily Discharge	Correlation	.571		-.572	-.420		.479			.430	
	Significance (2-tailed)	.000		.000	.003		.000			.002	
	df	47		47	47		47			47	
Aquifer water elevation guage @ 63.7m	Correlation	.495		-.579	-.405		.402				
	Significance (2-tailed)	.000		.000	.004		.004				
	df	47		47	47		47				
Aquifer water elevation guage @ 143m	Correlation	.407		-.754	-.535		.464				
	Significance (2-tailed)	.004		.000	.000		.001				
	df	47		47	47		47				
Aquifer water elevation guage @ 160m	Correlation			-.737	-.533	.376	.367			.492	
	Significance (2-tailed)			.000	.000	.008	.010			.000	
	df			47	47	47	47			47	
Aquifer water elevation guage @ 240m	Correlation			-.699	-.332		.366			.465	
	Significance (2-tailed)			.000	.020		.010			.001	
	df			47	47		47			47	

Table 2-7. Correlations of rainfall, Double Keyhole Spring discharge, and aquifer hydraulic head to studied geochemical parameters at the Double Keyhole Spring site.

Double Keyhole Spring		Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Total rainfall the week prior to sampling	Correlation			.777							-.536
	Significance (2-tailed)			.000							.000
	df			47							47
Total rainfall between sample collections	Correlation	.377			.515						
	Significance (2-tailed)	.008			.000						
	df	47			47						
Average Daily Discharge	Correlation	.455	-.531	-.690	-.402		-.339	.448			
	Significance (2-tailed)	.001	.000	.000	.004		.017	.001			
	df	47	47	47	47		47	47			
Maximum Daily Discharge	Correlation	.595	-.461	-.698			-.416	.439			
	Significance (2-tailed)	.000	.001	.000			.003	.002			
	df	47	47	47			47	47			
Aquifer water elevation guage @ 63.7m	Correlation	.675		-.370				.453			
	Significance (2-tailed)	.000		.009				.001			
	df	47		47				47			
Aquifer water elevation guage @ 143m	Correlation	.600		-.614		.447	-.465	.420			
	Significance (2-tailed)	.000		.000		.001	.001	.003			
	df	47		47		47	47	47			
Aquifer water elevation guage @ 160m	Correlation	.538		-.433		.604	-.654	.407			
	Significance (2-tailed)	.000		.002		.000	.000	.004			
	df	47		47		47	47	47			
Aquifer water elevation guage @ 240m	Correlation	.491		-.677		.598	-.530	.382			
	Significance (2-tailed)	.000		.000		.000	.000	.007			
	df	47		47		47	47	47			

Keyhole Spring varies daily with tidal fluctuations and seasonally with rainfall patterns and aquifer levels although long term monitoring has not yet been conducted. In a nearby off- shore sink Garman et al. (2011) found seasonal variations of temperature, salinity, pH, and DO with depth indicating the upward and downward movement of the halo/chemocline over time which may also be occurring in the ‘Deep Room’ of Double Keyhole Spring. The mixing diagram showing the ratio of salinity to $\delta^{18}O$ values versus salinity (Fig. 2-8) suggests that the water found at the top of the ‘Deep Room’ underneath the estuary inside Double Keyhole Spring ($\delta^{18}O$ = 1.2‰) has a composition more similar to evaporatively ^{18}O-enriched water from surficial aquifers and surface water ($\delta^{18}O$ = -1.04‰ to 2.93‰ (Sacks et al., 1998)) than to Isabella Spring ($\delta^{18}O$ = -2.8 to -3.2‰). This suggests that the freshwater at the top of the ‘Deep Room’ in Double Keyhole Spring is terrestrial runoff that followed the topologic gradient through the karst, which is distinct from the water from Isabella Spring that has made its way through the Upper Floridan aquifer system. This is further supported by the increase of pH shown in Figure 2-2. Kempe (1990) found that in anaerobic carbonate basins, sulfate reduction in the presence of organic matter results in the production of bicarbonate (HCO_3^-) which in turn increases the alkalinity and pH of the surrounding water at the chemocline.

Tidal Fluctuations

Monitoring of Double Keyhole Spring discharge shows a direct correlation of tidal height to spring discharge where spring discharge increases at low tide (Fig. 2-4, Table 2-1). These correlations were generally higher during the dry season than the wet season. The lower correlations observed during the wet season are most likely due to the added variable of rainfall which recharges the aquifer and results in increased aquifer discharge. Koizumi (1993) also found tidal influences on groundwater discharge from an artesian well in Japan where discharge increased at low tide. Numerous papers modeling SGD also support this observation (Ataie-Ashtiani et al., 1999; Li et al., 1999; Robinson et al., 2007; Abarca et al., 2013).

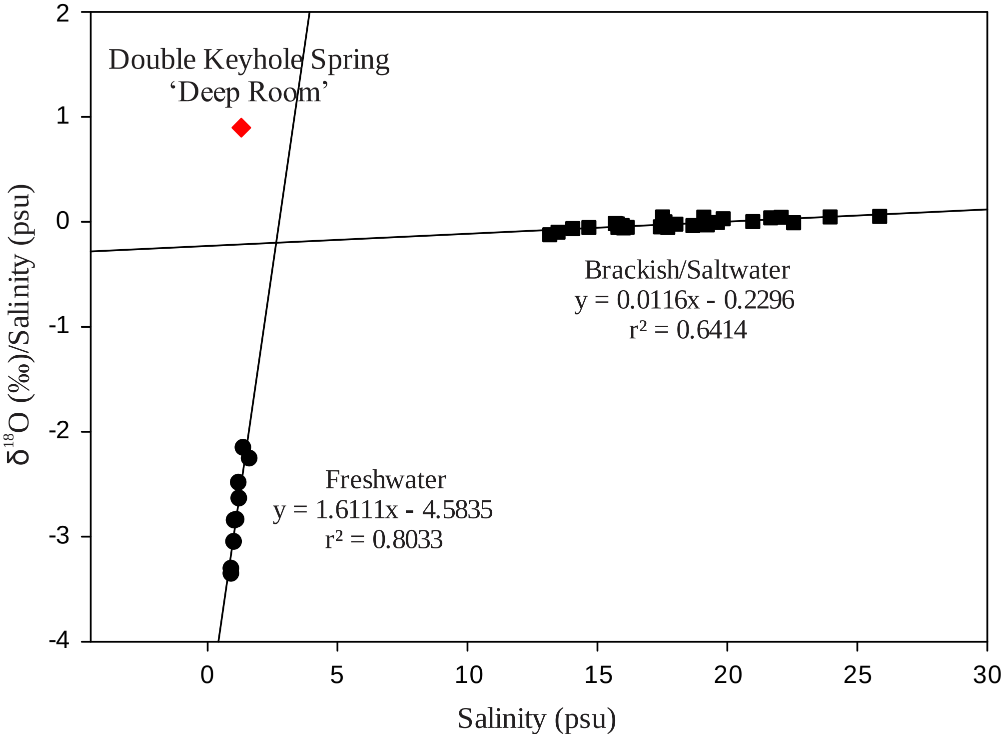


Figure 2-8. Mixing diagram showing the ratio of δ^{18}O values to salinity versus salinity from water samples. Freshwater samples were collected from Isabella Spring. Saltwater samples include all samples collected from the Double Keyhole Spring, Double Keyhole Pond, estuary, and marine sites. The red diamond indicates the sample collected from the top of the 'Deep Room' in Double Keyhole Spring. Freshwater lakes in central Florida have salinity concentrations near zero and δ^{18}O values ranging from -1.04‰ to 2.93‰ (Sacks et al., 1998).

The observed salinity increase during periods of both low tide and increased spring discharge may be due to reduction of overhead tidal pressure allowing more input from the mixing zone and less input from the freshwater portion of the 'Deep Room' in Double Keyhole Spring or a venturi-type effect where increased aquifer discharge draws higher salinity water from the surface estuary down through the karst into the spring conduit (Maramathas et al., 2006) (Fig. 2-4). Maramathas et al. (2006) assume that two conduits (one containing fresh groundwater and one containing sea water) mix at a single point inland of the spring entrance resulting in brackish spring discharge. I originally assumed that there were two conduits (one with inland fresh groundwater, and one with offshore saltwater) that merged at some point below Double Keyhole Spring forming the brackish spring discharge. If this were the case, I would have expected a large decrease in salinity following Tropical Storm Debby which was not observed. Another explanation is that the inland movement of the mixing zone (Michael et al., 2005) that occurs during high tide forces more saline mixing zone water into Double Keyhole Spring (Fig. 1-3). These salinity increases were not due to saltwater intrusion events as this situation is indicated by increased DO concentrations and negative discharge volumes. The DO concentrations and discharge volume data demonstrate that estuarine water brought in during reversal events is washed out within the same approximate amount of time in which the reversal occurred.

Rainfall Patterns

Analysis of rainfall patterns shows the connectivity of Double Keyhole Spring to the Upper Floridan aquifer (Fig. 2-3, Table 2-2). Hydraulic head data from ROMP TR 18-2 at a depth of 63.7 m shows significant correlations ($r^2 = 0.543$) to total rainfall the week prior to sampling. Hydraulic head data at both 63.7 m and 143 m show significant correlations ($r^2 = 0.815$ and 0.592, respectively) to the total amount of rainfall between sample collections. These data suggest a rapid recharge of the upper portion of the Upper Floridan aquifer as was seen in June 2012 when Tropical Storm Debby produced over 20 cm of rainfall in the area surrounding the study sites. After Tropical Storm Debby, aquifer discharge from Double Keyhole Spring and the hydraulic head at all four monitored well depths within ROMP TR 18-2 increased. Studies by Lakey and Krothe (1996), Doctor et al. (2006), and Herman et al. (2009) all show similar responses of storm events to spring discharge although the lag times vary depending on the amount of rainfall and geological setting. In a study of the Santa Fe river basin in north-central

Florida where the geology is very similar to the area around Double Keyhole Spring, Ritorto et al. (2009) found that diffuse recharge following storm events results in increased river and conduit discharge. They also found that in between storm events, river discharge generally decreased with decreasing hydraulic heads in wells nearby. Although the study only focused on conduit discharge, the same patterns can be seen between the Santa Fe river basin and Double Keyhole Spring in that discharge amounts increased following storm events and generally decreased with decreasing hydraulic heads in nearby monitoring wells (Fig. 2-3) that accompany drought or overuse of the aquifer.

Aquifer Hydraulic Head Measurements

There were no significant correlations between hydraulic head in the lower portions of the Upper Floridan aquifer to rainfall indicating a slower recharge of this part of the Floridan aquifer. This is most likely due to the differences in porosity and permeability between the limestone and dolostone layers as well as the multiple layers of clay that may act as semi-confining units (Table 2-2). Ehrenberg et al. (2006) showed that buried dolostone tends to have higher porosity than buried limestone in the same area and that the buried limestone may act as a barrier to water flow due to chemical compaction and cementation. The slower recharge may also be due to multiple layers of clay in the lower portion of the Upper Floridan aquifer that creates semi-confining units. A review by Back (1986) describes how semi-confining units can determine the rates of infiltration and discharge in aquifers. The hydraulic head at 160 m shows a sudden decrease in May 2013 but no concurrent change at 240 m (Fig. 2-3). There are several possible explanations for the observed decline in hydraulic head at 160 m but not at 240 m. The most likely explanation, given the geology of the aquifer layers (Fig. 1-3), is that the multiple layers of clay between 160 m and 240 m are acting as a semi-confining unit preventing the rapid discharge of water from 240 m. Other possible explanations for the rapid discharge seen at a depth of 160 m but not at 240 m include: the permeability of the rocks around 160 m may be greater than 240 m; there may be a break in the single layer of clay above 160 m; there could be a connection to a conduit at 160 m; or there could be increased pumping of freshwater from the same depth as 160 m but at more inland locations. All locations appear to increase with wet season rainfall and decrease during the dry season. Only the hydraulic head at 63.7 m had a significant correlation ($r^2 = 0.543$) to total rainfall the week prior to sample collection but the hydraulic head at both 63.7 m and 143 m had significant correlations to the total rainfall between sample collection dates ($r^2 = 0.815$ and 0.592, respectively) (Table 2-2).

Aquifer Discharge

The mass balance analysis indicates that the brackish water found in Double Keyhole Spring originates from the mixing zone created by saltwater from the Gulf of Mexico and high sulfate freshwater from the lower portion of the Upper Floridan aquifer (Fig. 2-9). It also indicates seasonal variations in the amount of mixing between the Gulf of Mexico, the lower portion of the Upper Floridan aquifer, and the upper portion of the Upper Floridan aquifer. Two sample sets collected after Tropical Storm Debby in late-summer and fall 2012 contained all three end members while the others contained only water from the Gulf of Mexico and the lower portion of the Upper Floridan aquifer. The marine portion of the brackish water found in Double Keyhole Spring ranged from a low of 22% during fall 2011 to a high of 63% during summer 2013. Generally there was more of a marine influence following rainfall events. This suggests that during the wet season, increased hydraulic head of the aquifer is increasing the hydraulic head of the mixing zone, pushing it offshore and resulting in increased water from the mixing zone to discharge through Double Keyhole Spring (Fig. 1-3) thereby protecting inland freshwater

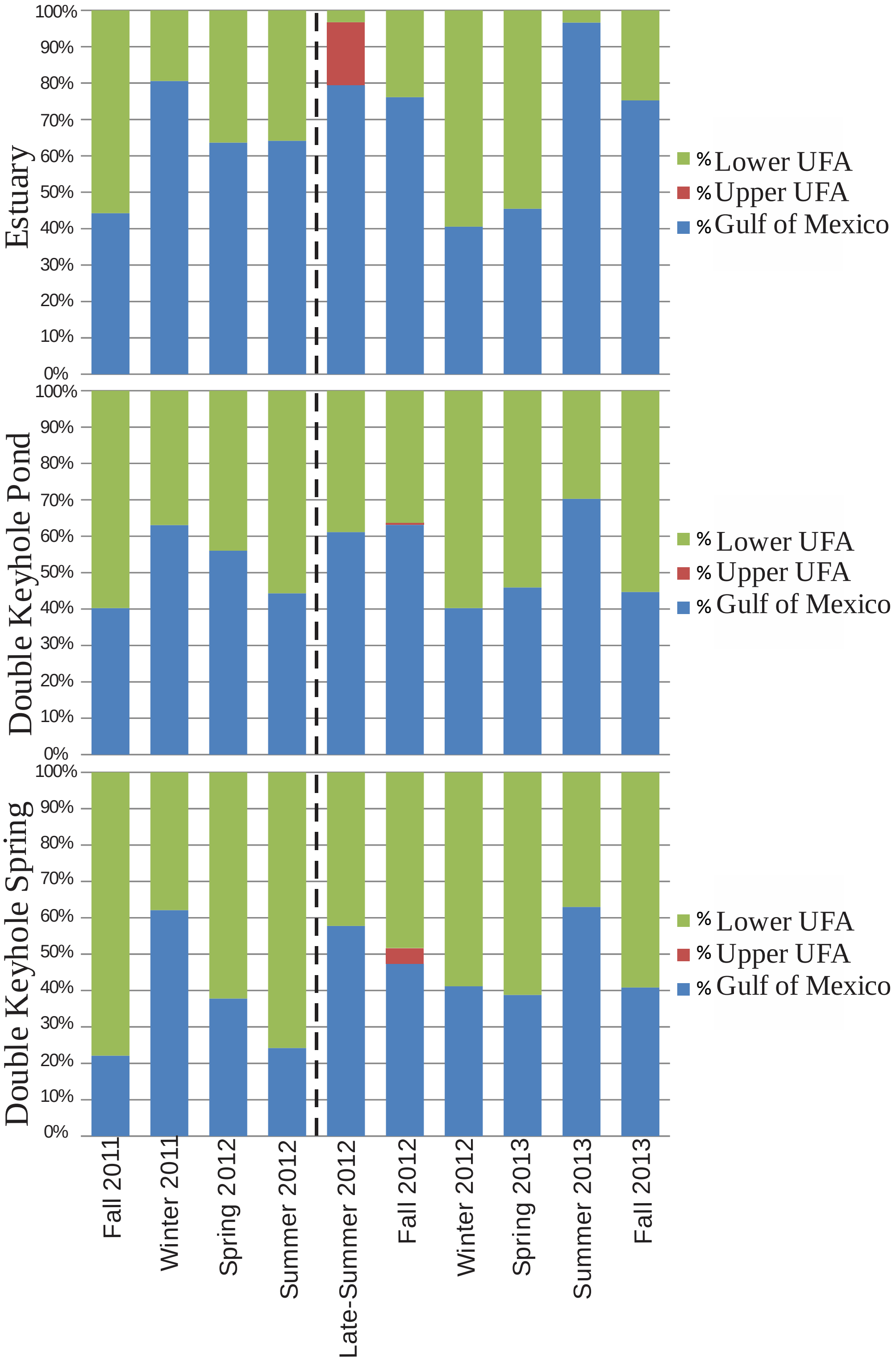


Figure 2-9. Hydrogeological conditions of the Double Keyhole Spring, Double Keyhole Pond, and the estuary sites showing the proportions of Upper Floridan and Gulf of Mexico water from fall 2011 through fall 2013. The dashed line indicates the occurrence of Tropical Storm Debby.

resources. A study by Michael et al. (2005) of Waquoit Bay, Massachusetts, also found seasonal movement of the mixing zone that is dependent on the inland hydrologic cycle. They noted that during the dry season there is an inland shift of the mixing zone as the water table falls, and during the wet season, when it reverses, moving off-shore as the water table rises. Seasonal shifts such as this can also be seen at all of the sites where the proportion of marine water changes with rainfall and aquifer hydraulic head.

The effects of Tropical Storm Debby were apparent during the late-summer and fall 2012 collections. In late-summer 2012, approximately 17% of the estuarine water was from the upper portion of the Upper Floridan aquifer. This was the only time during the two year study when water from the upper portion of the Upper Floridan aquifer was detected in the estuary. This indicates that during times of heavy rainfall, freshwater bypasses the Double Keyhole Spring conduit and discharges directly into the estuary by diffuse flow through the karst or through small conduits. In fall 2012 there was a measureable percentage of water (4%) from the upper portion of the Upper Floridan aquifer found within Double Keyhole Spring discharge and a smaller percentage (0.5%) found within Double Keyhole Pond water (Fig. 2-9) that occured 3-4 months after Tropical Storm Debby. This was the only time that water from the upper portion of the Upper Floridan aquifer was detected in Double Keyhole Spring discharge indicating the minor influence this portion of the aquifer has on the composition of the spring discharge. Although this suggests that it takes three to four months for rainwater from tropical storms to pass through the aquifer and discharge from the spring, time series analysis of total daily rainfall to average daily discharge and maximum daily discharge found the most significant correlations ($r^2 = 0.208$ and 0.197 respectively) at six weeks. This suggests that is takes six weeks for rainfall to pass through the aquifer and push water already in the mixing zone through the conduit that feeds Double Keyhole Spring. The smaller percentage of upper Floridan aquifer water found at the Double Keyhole Pond site compared to the Double Keyhole Spring site suggests that the discharge from the spring is quickly diluted as it moves into the estuary.

Table 2-8: Correlations of total daily rainfall, average daily discharge, and maximum daily discharge at Double Keyhole Spring with a six week delay.

Control Variables		Aveage Daily Discharge	Maximum Daily Discharge
Total Daily Rainfall	Correlation	.208	.197
	Significance (2-tailed)	.000	.000
	df	629	629

Water Quality Along the Estuarine Transect

Geochemical profiles along the transect from Double Keyhole Spring through the marine site in the Gulf of Mexico show the influences of aquifer fed spring discharge on geochemical gradients in the surrounding estuary (Figs. 2-6 & 2-7). In an examination of submarine groundwater discharge near the island of Hawaii, Grossman et al. (2010) found that SGD extended between 100 m and 1,000 m offshore showing the range of impact SGD may have on the surrounding area. I found the effects of Double Keyhole Spring discharge two km from the spring at the marine site.

Water temperature appears to be the most seasonally influenced factor along the transect (Fig. 2-6E, Tables 2-4 to 2-7). In the summer, the cool discharge water warms up to Gulf temperatures by the time it reaches the estuary site while in the winter the discharge water cools to Gulf temperature well past the estuary site. Water temperature at the surface of the pond, estuary and marine sites correlated significantly ($r^2 = 0.444$ to 0.510) to total rainfall in the week prior to the sample but water temperature within the spring did not correlate significantly. Even higher correlations were found when comparing water temperatures to total rainfall over the three months prior to sampling (r^2 = 0.675 to 0.736) and these correlations were also significant to water temperature within the spring (r^2 = 0.377). These correlations support the hypothesis that there is a three to four month lag between a major rainfall event and discharge of that water from the spring, despite the fact that increased discharge from the spring occurs in less than a day. Therefore the immediate increase in discharge after a major rainfall event is due to existing water being pushed out by increased inland hydraulic head but it takes months for the actual rainwater to be discharged from the spring.

Dissolved oxygen, pH, and salinity also show seasonal variations (Fig. 2-6B-D). During the dry season, DO and pH gradients extend further out from Double Keyhole Spring toward the marine site than during the wet season. Dissolved oxygen concentration only correlated to hydraulic head at the estuary and Double Keyhole Pond sites suggesting the presence of additional SGD from diffuse flow or small conduits in these areas that bypass the Double Keyhole Spring conduit (Tables 2-4 to 2-7). Salinity had the highest correlation to aquifer level at all sites except for within Double Keyhole Spring which had the highest correlation (r^2 = 0.777) to rainfall the week prior to sampling. This suggests the importance of aquifer discharge in creating the salinity gradient found along the estuarine transect.

The effects of Tropical Storm Debby can be seen in the changes of geochemistry at the estuary and Double Keyhole Pond sites. Some parameters showed extensive differences both before and after Tropical Storm Debby (Fig. 2-6). Prior to Tropical Storm Debby, there were more changes of some geochemical parameters over time and space close to Double Keyhole Spring than there were at the Pond, estuary, and marine sites. After Tropical Storm Debby, there appeared to be more differences between geochemical parameters at the sites farther away from Double Keyhole Spring. This was most apparent with phosphate, sulfate, and nitrate (Fig. 2-7A -

C) where there were fewer significant differences prior to Debby and more significant differences after Tropical Storm Debby. Other parameters (DO, pH, salinity, and water temperature) showed fewer differences before and after Tropical Storm Debby, but the patterns of distribution changed dramatically most likely due do increase aquifer discharge (Fig. 2-6B - E).

At the estuary and Double Keyhole Pond sites total hardness and ammonia concentrations increased while salinity decreased immediately after Tropical Storm Debby (Figs. 2-6D & 2-7DE). This is most likely due to increased submarine groundwater discharge diffusely flowing through the karst or small conduits that bypass the Double Keyhole Spring conduit and discharge directly into the area surrounding the Double Keyhole Pond and estuary sites.

CHAPTER THREE: MICROBIAL AND MACROFAUNAL COMMUNITIES

Materials and Methods

Sample Collection

Water column, sediment, and macrofauna samples were collected quarterly from September 2011 through September 2013 along the transect from Double Keyhole Spring through the marine site. Water column samples consisted of five replicates of 500 ml collected from mid-water column. Sediment samples for microbial community analysis consisted of five replicates of one ml by volume of surface sediment (approximately one g wet weight). Five sediment cores (3.8 cm internal diameter) were collected randomly within a one m^2 area to a maximum depth of 30 cm for macrofauna community analysis. All spring samples were collected by scientific divers using closed-circuit rebreathers under the auspices of the University of South Florida Scientific Diving Program. Water column samples within the spring were purged three times with helium prior to sample collection. All samples were kept on ice during transport to the lab for processing. No sediment or macrofauna samples were collected from Double Keyhole Spring in late-summer 2012 due to logistical constraints.

Sample Preparation

All sediment and water column samples were filtered through 47 mm diameter 0.2 µm pore size filters (Millipore, USA). The filters were stored at -20 °C until processing. Microorganisms were extracted from the filters using a sterile DNA-free spatula in 1.5 ml of pH 7 phosphate buffered saline, followed by 30 seconds of vortexing in a 15 ml conical tube. The filters were washed two times in this manner. DNA extractions were performed using an Ultraclean Fecal

DNA Kit (MoBIO, USA) as described by Menning et al. (2014a). The concentrations of purified environmental DNA were determined using a Thermo Scientific Nanodrop 2000 Spectrophotometer (Fisher Scientific, USA).

Quantitative Polymerase Chain Reaction

Quantitative PCR was conducted separately on all environmental DNA samples to estimate the total abundance of Archaea, Bacteria, and microbial eukaryotes in the water column and sediment using SYBR Premix Ex Taq II (Takara, USA) and a Realplex^2 Mastercycler (Eppendorf, USA). The universal primers used for each domain are listed in Table 3-1. The PCR conditions have been described previously in Menning et al. (2014a). Five replicate samples were run for each primer set. A positive control (DNA from a pure culture, see Table 3-1) and a negative control (no DNA) were run to verify PCR efficiency. Log-linear standard curves were made using 1:10 serial dilutions of DNA of control organisms from full concentration to 10^{-5} dilution (Table 3-1). Estimated abundance provided in this study was determined by comparing the cycle threshold (Ct) values generated from the samples to a standard curve created at the same time from a pure culture of a known organism with a known DNA concentration and extrapolating the estimated abundance from the standard curve.

Table 3-1. List of primers used for LH-PCR and q-PCR with associated data.

Primer	Sequence (5'-3')	Variable region covered	Annealing Temperature (°C)	Positive Control	Source
Archaea					
A1098F	CNGGCAACGAGCGMGACCC	7-8	50 °C	*Sulfolobus solfataricus*	Reysenbach and Pace, 1995
UA1406R	ACGGGCGGTGWGTRCAA				Baker et al., 2003
Bacteria					
27F	AGAGTTTGATCCTGGCTCAG	1-2	50 °C	*Escherichia coli*	Lane, 1991
355R	GCTGCCTCCCGTAGGAGT				Giovannoni, 1991
Eukaryote					
1961F	TGGTGCATGGCCGTTCTTAG	5-6	55 °C	*Caenorhabditis elegans*	Modified from Sogin and Gunderson, 1987
2532R	CGGTGTGTACAAAGGGCAGGG				Modified from Sogin and Gunderson, 1987

I tested multiple organisms with each primer set to validate the use of q-PCR as a proxy for determining changes in the size of environmental microbial communities (Ollivier et al., 2014)

(Table 3-2). *Halobacterium salinarum* (ATCC 700922D-5), *Archaeoglobus fulgidus* (ATCC 49558D-5), and *Sulfolobus solfataricus* (ATCC 35092D-5) were used for Archaea; *Escherichia coli* K-12 (ATCC 700926), *Staphylococcus epidermidis* (ATCC 14990p), and *Bacillus subtilis* (ATCC 6051p) were used for Bacteria; and *Caenorhabditis elegans* N-2, *Penicillium notatum* (ATCC 85w4702), and *Saccharomycyes cerevisiae* (ATCC 85w5000) were used for microbial eukaryotes. DNA from each organism was serially diluted from full concentration to 10^{-5} and log-linear standards curves were made. A Ct value was determined for 10 ng of DNA from each standard curve. The average of the Ct values for the three species each of Archaea, Bacteria and eukaryotes was then used to determine a mean and standard deviation (Table 3-3). To test for differences of primer affinity and potential bias due to mixed environmental samples I combined equal portions of DNA from each pure archaeal and bacterial species in three molar ratios of archaeal: bacterial DNA; 1:1, 1:10 and 10:1 (Table 3-4). Each of the mixed samples was subjected to q-PCR measurements using archaeal primers in one experiment and bacterial primers in another experiment.

Table 3-2. List of organisms used for q-PCR validation experiment, standard curves produced for each organism and associated r^2 values.

Domain	Organism	Standard curve	r^2
Archaea	*Halobacterium salinarum*	$Y = -1.496 \ln(x) + 16.549$	0.9973
	Archaeoglobus fulgidus	$Y = -1.592 \ln(x) + 18.015$	0.9901
	Sulfolobus solfataricus	$Y = -1.516 \ln(x) + 17.504$	0.9988
Bacteria	*Escherichia coli*	$Y = -1.595 \ln(x) + 12.495$	0.9993
	Staphylococcus epidermidis	$Y = -1.723 \ln(x) + 12.693$	0.9992
	Bacillus subtilis	$Y = -1.614 \ln(x) + 12.591$	0.9904
Eukaryota	*Caenorhabditis elegans*	$Y = -1.677 \ln(x) + 13.433$	0.9988
	Penicillium notatum	$Y = -1.442 \ln(x) + 13.450$	0.9915
	Saccharomycyes cerevisiae	$Y = -1.508 \ln(x) + 10.159$	0.9979

Length Heterogeneity Polymerase Chain Reaction

Length heterogeneity PCR was conducted on all sediment and water column DNA samples to estimate the species richness and relative species abundance of Archaea, Bacteria, and

Table 3-3. Estimated amount of DNA determined from the standard curves when using 10ng of DNA.

Archaea	Estimated DNA
Halobacterium salinarum	6.19
Archaeoglobus fulgidus	13.92
Sulfolobus solfataricus	11.34
DNA Average	10.48
DNA Standard Deviation	3.94
Bacteria	Estimated DNA
Escherichia coli	10.09
Staphylococcus epidermidis	9.54
Bacillus subtilis	10.42
DNA Average	10.02
DNA Standard Deviation	0.45
Eukaryota	Estimated DNA
Caenorhabditis elegans	15.94
Penicillium notatum	25.16
Saccharomycyes cerevisiae	2.46
DNA Average	14.52
DNA Standard Deviation	11.41

Table 3-4. Prokaryotic DNA concentrations when using three different mass ratios.

	Mix 1	Mix 2	Mix 3
	2ng Archaea + 2ng Bacteria	2ng Archaea + 0.2ng Bacteria	0.2ng Archaea + 2ng Bacteria
Average Archaeal DNA (ng/µL)	10.7860	9.9841	2.0669
Archaeal Standard Deviation	0.8095	0.6927	0.2036
Average Bacterial DNA (ng/µL)	0.8089	0.0788	0.7702
Bacterial Standard Deviation	0.1090	0.0122	0.0796
Expected ratio	1:1	10:1	1:10
Actual ratio	13:1	127:1	3:1

microbial eukaryotes in the water column and sediment using an ABI 3130 four-capillary Genetic Analyzer (Applied Biosystems, USA). Ten ng of DNA was used in each PCR reaction. The universal primers used were selected to amplify two variable regions of the target 16S or 18S rRNA genes (Table 3-1). Each forward primer contained a 56/FAM fluorescent tag for fragment detection. A positive control (DNA from a pure culture, see Table 3-1) and negative control (no DNA) were run to verify PCR efficiency. Length heterogeneity PCR conditions have been previously described in Menning et al. (2014a). Electropherograms were analyzed using Gene Mapper v4.0 (Applied Biosystems, USA). The expected amplicons contain two conserved flanking regions and two internal variable regions. Peaks from fragments of a size representing only the conserved flanking regions or less (250 bp for Archaea, 300 bp for Bacteria and microbial eukaryotes) were omitted from further analysis (Suzuki et al., 1998). Species richness was determined by calculating the total number of peaks from each electropherogram. Relative species abundance was determined by dividing the area of each individual peak by the total area of all peaks in the sample.

Macrofauna

Sediment samples used for macrofauna collection were washed through a 500 µm sieve using pre-filtered water from the sample collection sites. Samples were placed in 10 % formaldehyde Rose Bengal solution for three days after which the samples were washed and transferred to a 70 % ethyl alcohol Rose Bengal solution. Sorting and identification of macrofaunal organisms to the lowest practical taxonomic unit was conducted using light microscopy.

Statistical Analyses

Partial correlation analyses were conducted using IBM SPSS 22.0 (IBM, USA) to determine statistically significant correlations ($0 < 0.025$) between the biological communities and the hydrological and geochemical parameters, described by Menning et al. (2014b), controlling for study site or collection date (Bai et al., 2010). Relative species abundance and estimated total abundance of each dataset were combined to calculate estimated absolute abundance of Archaea, Bacteria, and microbial eukaryotes (Yarwood et al., 2010; Menning et al., 2014a). The estimated absolute abundance of the archaeal, bacterial, microbial eukaryotes, and macrofauna data were combined and analyzed by non-parametric multivariate analysis of multidimensional scaling

(MDS) based on Bray-Curtis similarity using Primer v6 statistical software (Primer-E Ltd, UK) (Clarke & Ainsworth, 1993). The biological portion of the MDS dataset was square root transformed and correlated to the log transformed geochemical and hydrological data (Clarke, 1993).

Results

Testing q-PCR Methods Using Known DNA

The r^2 values for all standard curves made from DNA of a single species was greater than 0.98 in all cases (Table 3-2). For Archaea, with a theoretical DNA concentration of 10 ng/µL, the mean calculated DNA concentration from the three species was 10.48 ± 3.94 ng/µL. For Bacteria the mean calculated DNA concentration was 10.02 ± 0.45 ng/µL and for eukaryotes was 14.52 ± 11.41 ng/µL (Table 3-3).When an equimolar mixture of DNA from three archaeal species was mixed in varying proportions (1:1, 10:1, 1:10) with an equimolar mixture of DNA from three bacterial species, the archaeal DNA concentration was approximately ten times higher than bacterial DNA concentration (Table 3-4).

Estimated Abundance

The q-PCR data of environmental DNA samples shows that the highest concentrations of extractable bacterial and microbial eukaryote DNA in the water column were at the Double Keyhole Pond site in summer 2012 (10.06 ng/L and 1.92 ng/L respectively) while archaeal DNA concentrations were highest (110.58 ng/L) at the estuary site in fall 2012 (Fig. 3-1). DNA concentrations were highest in the sediment at the Double Keyhole Pond site (48777 ng/L, 6187 ng/L, 2036 ng/L for Archaea, Bacteria and microbial eukaryotes) in fall 2013, fall 2011, and summer 2013 respectively. Macrofauna were most abundant (487 organisms/L) at the estuary site in winter 2012 (Fig. 3-2).

Species Richness

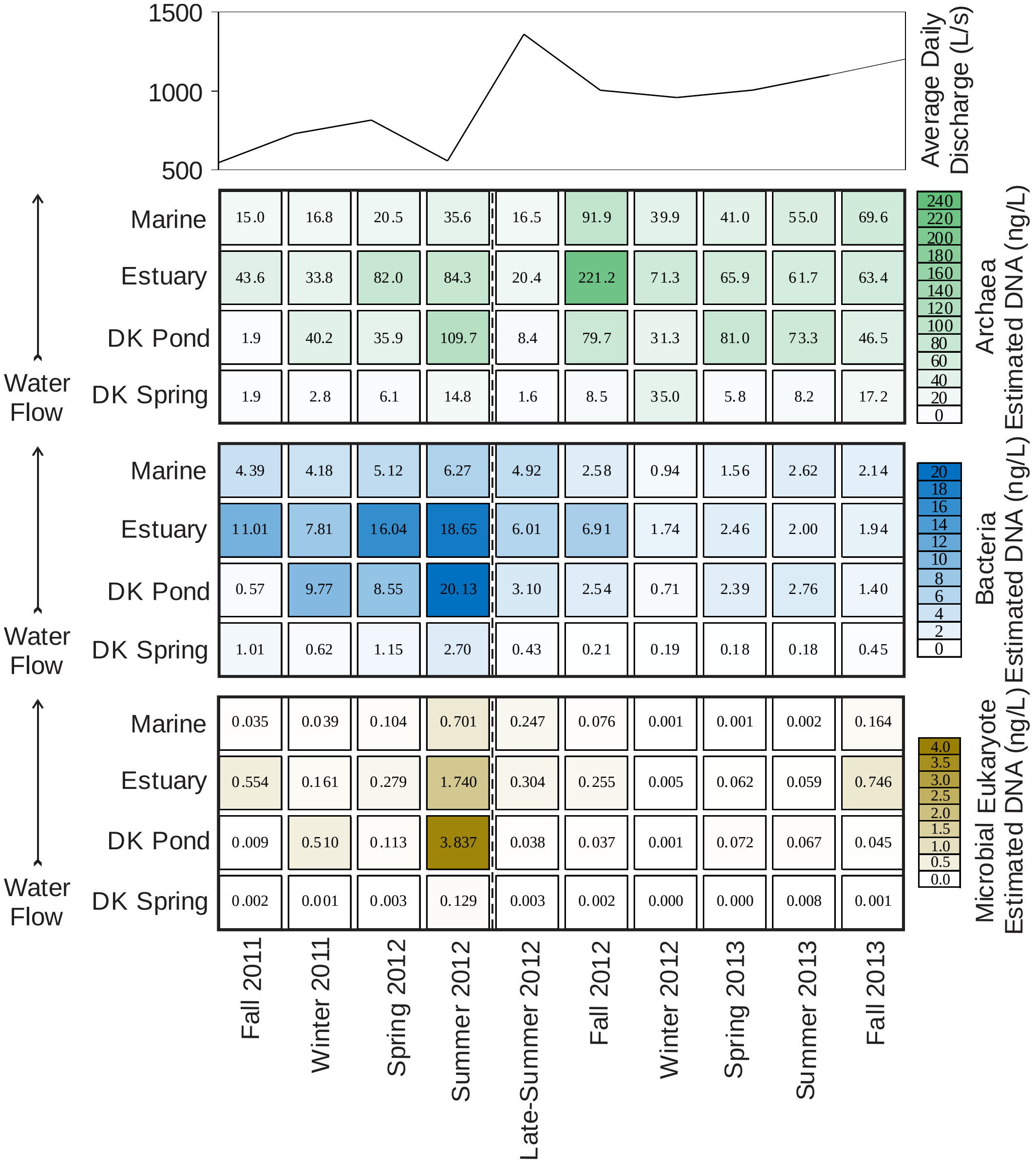
Species richness data indicate that average archaeal and bacterial richness in the water column (21.2 and 31.2 peaks respectively) were highest in late-summer 2012 at the Double Keyhole Spring and Pond sites respectively while average microbial eukaryote richness was highest in summer 2012 at the estuary site (35.8 peaks) (Fig. 3-3). In the sediment, average archaeal and bacterial richness (27.6 and 37 peaks respectively) were highest in spring 2013 at the Double Keyhole Pond and Spring sites respectively (Fig. 3-4). Average microbial eukaryote diversity was highest in winter 2012 at Double Keyhole Pond (42.4 peaks) and macrofaunal diversity was highest (12.2 peaks) in summer 2013 at the estuary site.

Macrofauna

A total of 62 different macrofaunal organisms were found along the transect from Double Keyhole Spring through the marine site. The most abundant organisms identified over the course of the collection period were an amphipod *Americorophium ellisi,* a bivalve *Parastarte triquetra,* an annelid from the family Turbificidae and an isopod *Xenanthura brevitelson* (Table 3-5). All of the macrofauna found within Double Keyhole Spring were also found at one or more of the other sites and were less numerous inside the spring with the exception of a polychaete *Stenoninereis martini*.

Multi-Dimensional Scaling Plots

Multi-Dimensional Scaling Plots of estimated absolute species abundance for the combined communities are shown in Figures 3-5 – 3-8. Examination of communities along the transect during the same collection period indicate that the Double Keyhole Spring samples are generally distinct from the other three sites but during certain times of the year overlap with the other sites along the transect (Figs. 3-5 & 3-6). Generally, the samples fall into two categories, samples

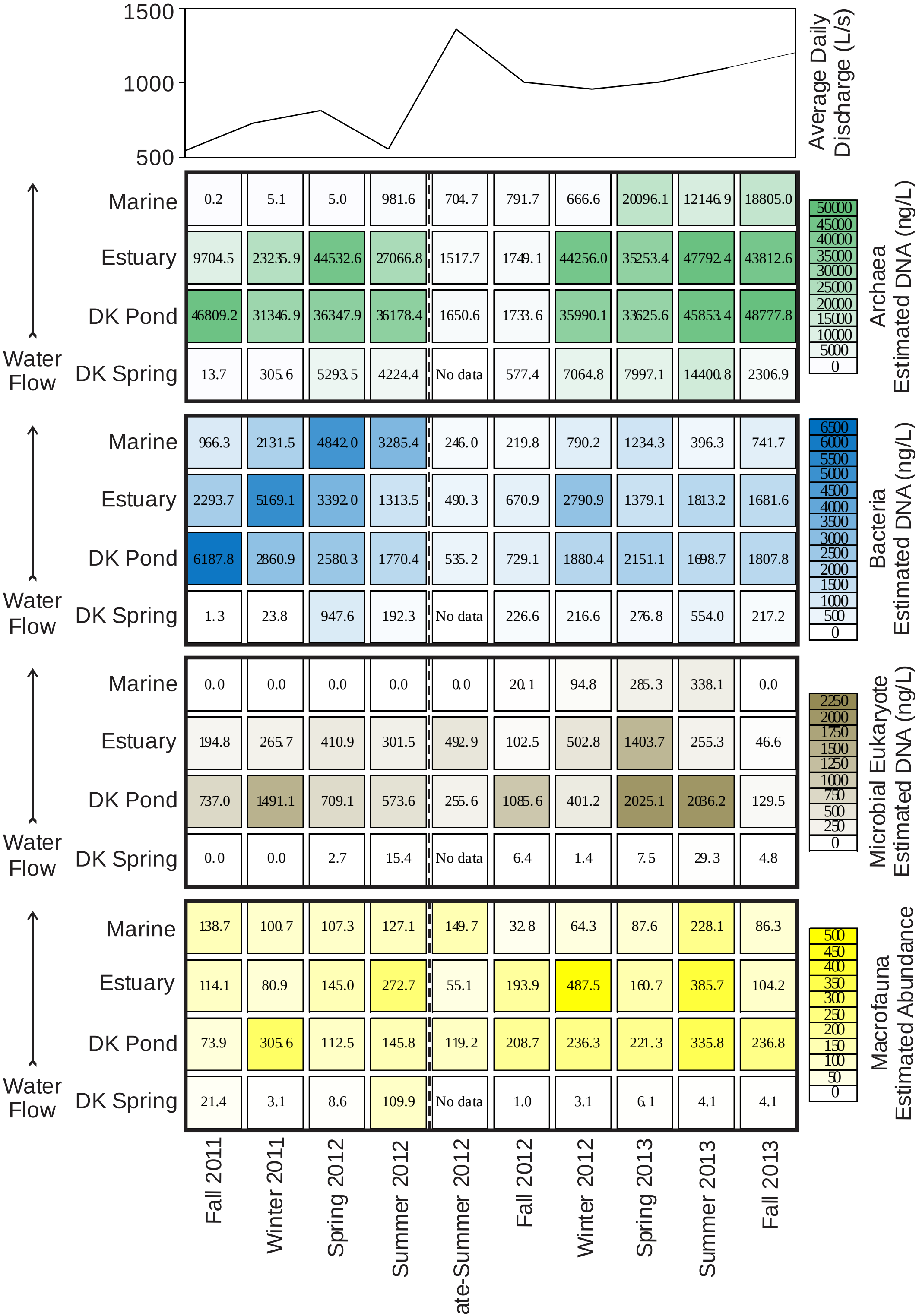


	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	15.0	16.8	20.5	35.6	16.5	91.9	39.9	41.0	55.0	69.6
Estuary	43.6	33.8	82.0	84.3	20.4	221.2	71.3	65.9	61.7	63.4
DK Pond	1.9	40.2	35.9	109.7	8.4	79.7	31.3	81.0	73.3	46.5
DK Spring	1.9	2.8	6.1	14.8	1.6	8.5	35.0	5.8	8.2	17.2

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	4.39	4.18	5.12	6.27	4.92	2.58	0.94	1.56	2.62	2.14
Estuary	11.01	7.81	16.04	18.65	6.01	6.91	1.74	2.46	2.00	1.94
DK Pond	0.57	9.77	8.55	20.13	3.10	2.54	0.71	2.39	2.76	1.40
DK Spring	1.01	0.62	1.15	2.70	0.43	0.21	0.19	0.18	0.18	0.45

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.035	0.039	0.104	0.701	0.247	0.076	0.001	0.001	0.002	0.164
Estuary	0.554	0.161	0.279	1.740	0.304	0.255	0.005	0.062	0.059	0.746
DK Pond	0.009	0.510	0.113	3.837	0.038	0.037	0.001	0.072	0.067	0.045
DK Spring	0.002	0.001	0.003	0.129	0.003	0.002	0.000	0.000	0.008	0.001

Figure 3-1. Estimated abundance of Archaea, Bacteria, and microbial eukaryotes in the water column as determined by q-PCR. The dashed line indicates the occurrence of Tropical Storm Debby. The top pane shows average daily discharge from Double Keyhole Spring on the sample collection date. Arrows on the far left indicate the general direction of water flow.



Archaea Estimated DNA (ng/L)

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.2	5.1	5.0	981.6	704.7	791.7	666.6	20096.1	12146.9	18805.0
Estuary	9704.5	23235.9	44532.6	27066.8	1517.7	1749.1	44256.0	35253.4	47792.4	43812.6
DK Pond	46809.2	31346.9	36347.9	36178.4	1650.6	1733.6	35990.1	33625.6	45853.4	48777.8
DK Spring	13.7	305.6	5293.5	4224.4	No data	577.4	7064.8	7997.1	14400.8	2306.9

Bacteria Estimated DNA (ng/L)

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	966.3	2131.5	4842.0	3285.4	246.0	219.8	790.2	1234.3	396.3	741.7
Estuary	2293.7	5169.1	3392.0	1313.5	490.3	670.9	2790.9	1379.1	1813.2	1681.6
DK Pond	6187.8	2860.9	2580.3	1770.4	535.2	729.1	1880.4	2151.1	1698.7	1807.8
DK Spring	1.3	23.8	947.6	192.3	No data	226.6	216.6	276.8	554.0	217.2

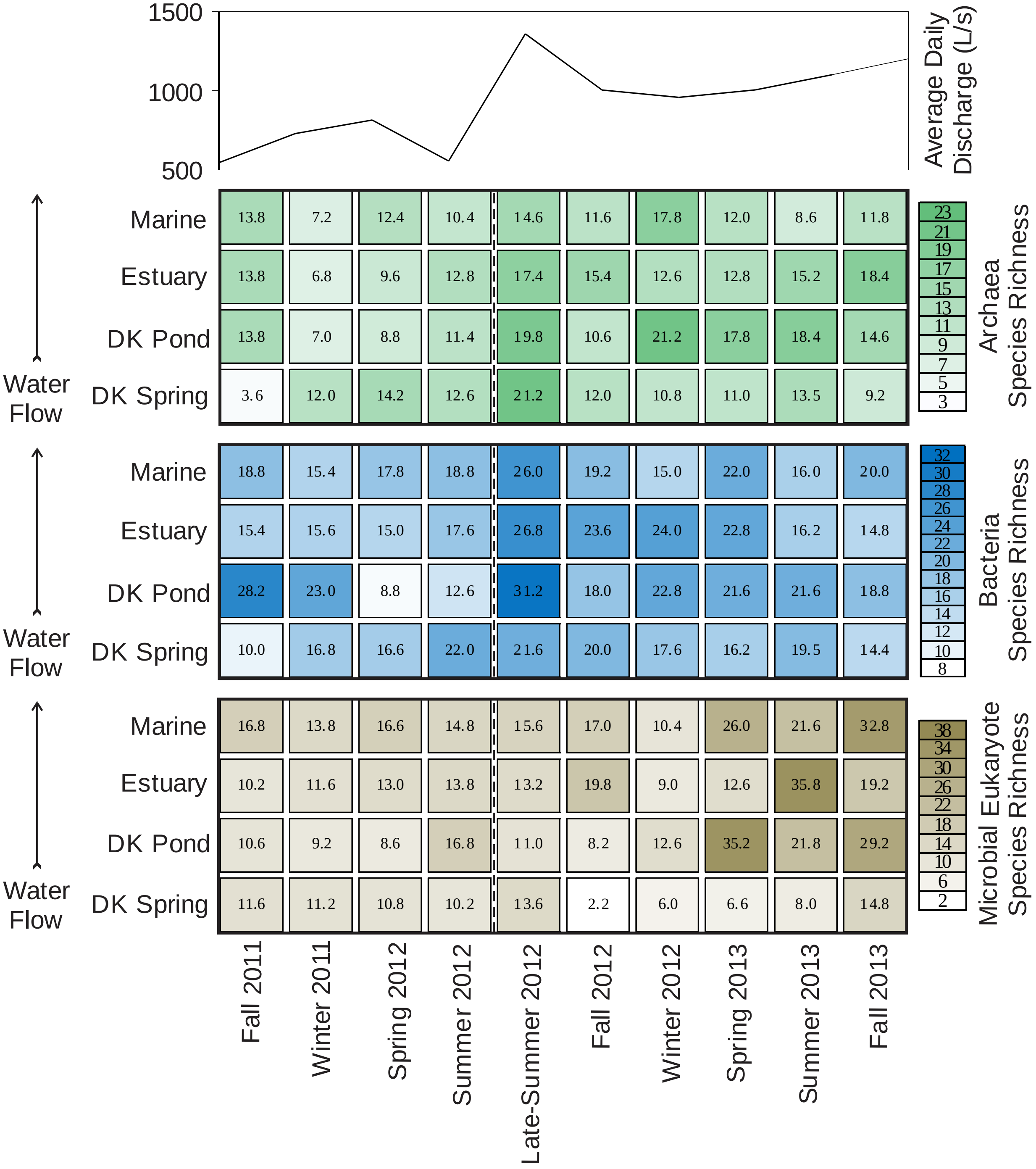
Microbial Eukaryote Estimated DNA (ng/L)

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	0.0	0.0	0.0	0.0	0.0	20.1	94.8	285.3	338.1	0.0
Estuary	194.8	265.7	410.9	301.5	492.9	102.5	502.8	1403.7	255.3	46.6
DK Pond	737.0	1491.1	709.1	573.6	255.6	1085.6	401.2	2025.1	2036.2	129.5
DK Spring	0.0	0.0	2.7	15.4	No data	6.4	1.4	7.5	29.3	4.8

Macrofauna Estimated Abundance

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	138.7	100.7	107.3	127.1	149.7	32.8	64.3	87.6	228.1	86.3
Estuary	114.1	80.9	145.0	272.7	55.1	193.9	487.5	160.7	385.7	104.2
DK Pond	73.9	305.6	112.5	145.8	119.2	208.7	236.3	221.3	335.8	236.8
DK Spring	21.4	3.1	8.6	109.9	No data	1.0	3.1	6.1	4.1	4.1

Figure 3-2. Estimated abundance of Archaea, Bacteria, microbial eukaryotes, and macrofauna in the sediment as determined by q-PCR and light microscopy. The dashed line indicates the occurrence of Tropical Storm Debby. The top pane shows average daily discharge from Double Keyhole Spring on the sample collection date. Arrows on the far left indicate the general direction of water flow.

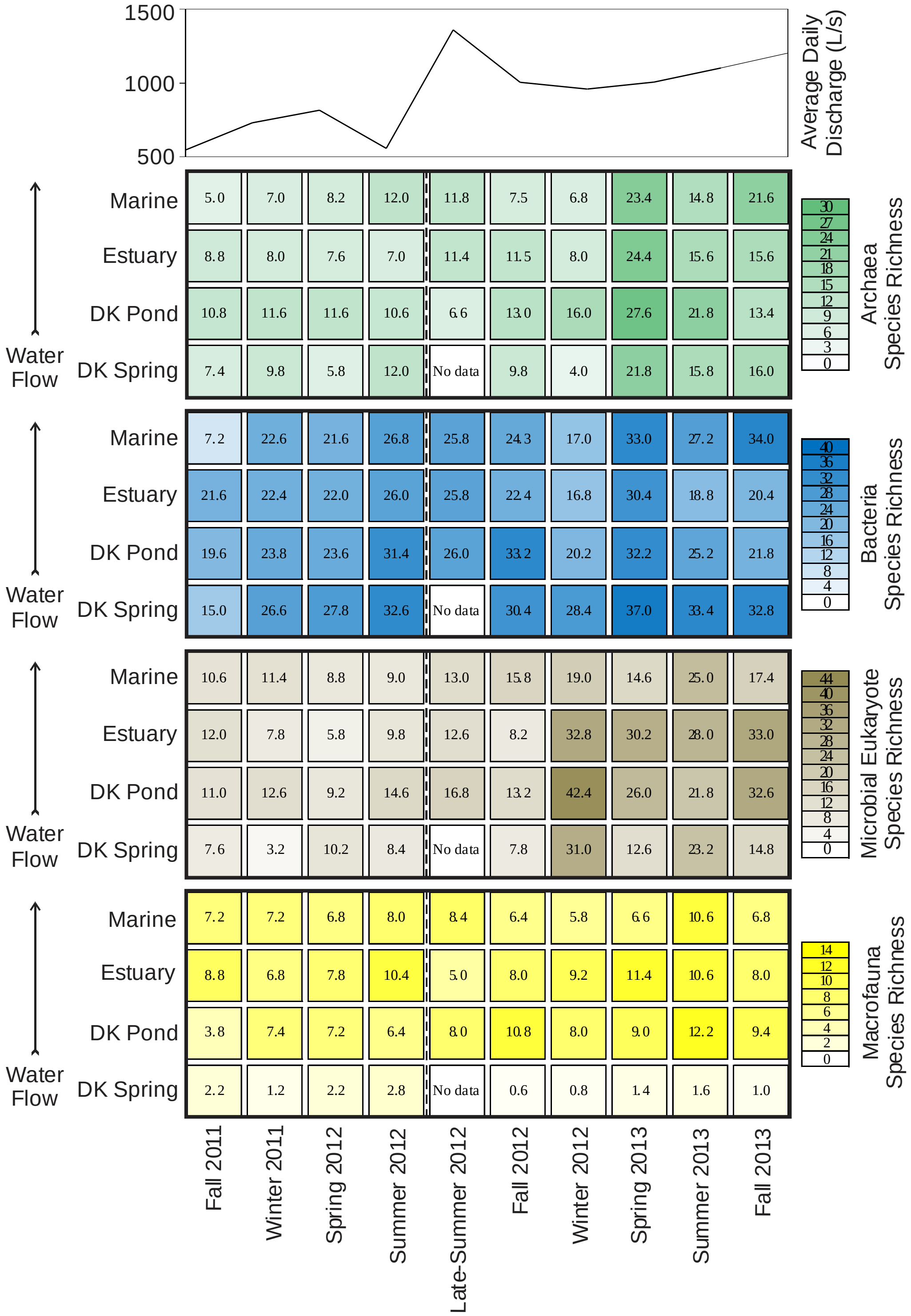


	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	13.8	7.2	12.4	10.4	14.6	11.6	17.8	12.0	8.6	11.8
Estuary	13.8	6.8	9.6	12.8	17.4	15.4	12.6	12.8	15.2	18.4
DK Pond	13.8	7.0	8.8	11.4	19.8	10.6	21.2	17.8	18.4	14.6
DK Spring	3.6	12.0	14.2	12.6	21.2	12.0	10.8	11.0	13.5	9.2

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	18.8	15.4	17.8	18.8	26.0	19.2	15.0	22.0	16.0	20.0
Estuary	15.4	15.6	15.0	17.6	26.8	23.6	24.0	22.8	16.2	14.8
DK Pond	28.2	23.0	8.8	12.6	31.2	18.0	22.8	21.6	21.6	18.8
DK Spring	10.0	16.8	16.6	22.0	21.6	20.0	17.6	16.2	19.5	14.4

	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	16.8	13.8	16.6	14.8	15.6	17.0	10.4	26.0	21.6	32.8
Estuary	10.2	11.6	13.0	13.8	13.2	19.8	9.0	12.6	35.8	19.2
DK Pond	10.6	9.2	8.6	16.8	11.0	8.2	12.6	35.2	21.8	29.2
DK Spring	11.6	11.2	10.8	10.2	13.6	2.2	6.0	6.6	8.0	14.8

Figure 3-3. Species richness of Archaea, Bacteria, and microbial eukaryotes in the water column as determined by LH-PCR. The dashed line indicates occurrence time of Tropical Storm Debby. The top pane shows average daily discharge from Double Keyhole Spring on the sample collection date. Arrows on the far left indicate the general direction of water flow.



Archaea Species Richness	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	5.0	7.0	8.2	12.0	11.8	7.5	6.8	23.4	14.8	21.6
Estuary	8.8	8.0	7.6	7.0	11.4	11.5	8.0	24.4	15.6	15.6
DK Pond	10.8	11.6	11.6	10.6	6.6	13.0	16.0	27.6	21.8	13.4
DK Spring	7.4	9.8	5.8	12.0	No data	9.8	4.0	21.8	15.8	16.0

Bacteria Species Richness	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	7.2	22.6	21.6	26.8	25.8	24.3	17.0	33.0	27.2	34.0
Estuary	21.6	22.4	22.0	26.0	25.8	22.4	16.8	30.4	18.8	20.4
DK Pond	19.6	23.8	23.6	31.4	26.0	33.2	20.2	32.2	25.2	21.8
DK Spring	15.0	26.6	27.8	32.6	No data	30.4	28.4	37.0	33.4	32.8

Microbial Eukaryote Species Richness	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	10.6	11.4	8.8	9.0	13.0	15.8	19.0	14.6	25.0	17.4
Estuary	12.0	7.8	5.8	9.8	12.6	8.2	32.8	30.2	28.0	33.0
DK Pond	11.0	12.6	9.2	14.6	16.8	13.2	42.4	26.0	21.8	32.6
DK Spring	7.6	3.2	10.2	8.4	No data	7.8	31.0	12.6	23.2	14.8

Macrofauna Species Richness	Fall 2011	Winter 2011	Spring 2012	Summer 2012	Late-Summer 2012	Fall 2012	Winter 2012	Spring 2013	Summer 2013	Fall 2013
Marine	7.2	7.2	6.8	8.0	8.4	6.4	5.8	6.6	10.6	6.8
Estuary	8.8	6.8	7.8	10.4	5.0	8.0	9.2	11.4	10.6	8.0
DK Pond	3.8	7.4	7.2	6.4	8.0	10.8	8.0	9.0	12.2	9.4
DK Spring	2.2	1.2	2.2	2.8	No data	0.6	0.8	1.4	1.6	1.0

Figure 3-4. Species richness of Archaea, Bacteria, microbial eukaryotes, and macrofauna in the sediment as determined by LH-PCR and light microscopy. The dashed line indicates the occurrence of Tropical Storm Debby. The top pane shows average daily discharge from Double Keyhole Spring on the sample collection date. Arrows on the far left indicate the general direction of water flow.

Table 3-5. List of macrofauna identified to the lowest practical taxonomic unit in the sediment from Double Keyhole Spring through the marine site. Numbers indicate the total number of organisms found from September 2011 through September 2013.

Phylum	Class	Order	Family	Genus	Species	Marine	Estuary	DK Pond	DK Spring	Scale
Annelida	Clitellata	Haplotaxida	Tubificidae			1058	1425	871	130	4000
Arthropoda	Malacostraca	Amphipoda	Aoridae	*Grandidierella*	*bonnieroides*	647	749	226	114	3500
Annelida	Polychaeta		Paraonidae	*Aricidea*	*taylori*	437	136	117	7	3000
Annelida	Polychaeta	Phyllodocida	Nereididae	*Stenoninereis*	*martini*	300	168	85	464	2500
Annelida	Polychaeta		Capitellidae	*Capitella*		212	298	465	33	2000
Annelida	Polychaeta		Paraonidae	*Paraonis*	*fulgens*	146	27	11	7	1500
Cnidaria	Hydrozoa					53	20	5	13	1000
Arthropoda	Maxillopoda	Copepoda				37	100	62	3	500
Nemertea						34	51	7	10	0
Annelida	Polychaeta		Capitellidae	*Mediomastus*	*ambiseta*	20	349	189	2	
Arthropoda	Malacostraca	Isopoda	Idoteidae	*Edotia*	*triloba*	20	5	35	23	
Nematoda						344	178	83	0	
Arthropoda	Malacostraca	Tanaidacea				273	588	108	0	
Annelida	Polychaeta	Sabellida	Sabellidae	*Fabricinuda*	*trilobata*	260	21	3	0	
Arthropoda	Ostracoda					227	106	3	0	
Annelida	Polychaeta	Phyllodocida	Nereididae			178	189	393	0	
Mollusca	Bivalvia	Veneroida	Tellinidae	*Macoma*	*constricta*	113	148	6	0	
Arthropoda	Malacostraca	Isopoda	Hyssuridae	*Xenanthura*	*brevitelson*	105	700	1036	0	
Annelida	Polychaeta		Paraonidae	*Aricidea*	*philbinae*	92	4	4	0	
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Americorophium*	*ellisi*	78	2969	3647	0	
Mollusca	Bivalvia	Veneroida	Veneridae	*Parastarte*	*triquetra*	62	501	1528	0	
Annelida	Polychaeta		Orbiniidae	*Leitoscoloplos*		59	302	532	0	
Arthropoda	Malacostraca	Cumacea	Leuconidae	*Leucon*	*americanus*	54	26	31	0	
Mollusca	Gastropoda	Neogastropoda	Marginellidae	*Prunum*	*apicinum*	53	49	141	0	
Annelida	Polychaeta		Maldanidae			39	165	54	0	
Sipuncula	Sipunculidea	Golfingiida	Phascolionidae	*Phascolion*		34	42	12	0	
Annelida	Polychaeta	Eunicida	Onuphidae	*Kinbergonuphis*	*simoni*	33	95	21	0	
Mollusca	Gastropoda	Cephalaspidea	Bulloidea	*Bullidae*	*bulla*	29	65	49	0	
Annelida	Polychaeta		Capitellidae	*Heteromastus*	*filiformis*	10	40	26	0	
Mollusca	Gastropoda	Neogastropoda	Marginellidae	*Granulina*	*ovuliformis*	8	22	6	0	
Mollusca	Gastropoda	Neogastropoda	Marginellidae	*Hyalina*	*pallida*	7	17	28	0	
Annelida	Polychaeta	Spionida	Magelonidae	*Magelona*	*pettiboneae*	21	5	0	0	
Arthropoda	Malacostraca	Mysidacea				19	27	0	0	
Annelida	Polychaeta	Terebellida	Cirratulidae	*Monticellina*		16	353	0	0	
Arthropoda	Malacostraca	Cumacea	Nannastacidae	*Almyracuma*	*bacescui*	11	7	0	0	
Mollusca	Gastropoda	Neogastropoda	Nassariidae	*Nassarius*	*vibex*	4	7	0	0	
Mollusca	Gastropoda	Pulmonata	Ellobiidae	*Melampus*	*bullaoides*	15	20	0	2	
Echinodermata	Ophiuroidea					50	0	17	0	
Annelida	Polychaeta	Phyllodocida	Goniadidae	*Glycinde*	*solitaria*	17	0	14	0	
Annelida	Clitellata	Haplotaxida	Tubificidae	*Tubificoides*		10	0	3	0	
Mollusca	Gastropoda	Pulmonata	Ellobiidae	*Melampus*	*bidentatus*	9	0	21	0	
Arthropoda	Malacostraca	Amphipoda	Oedicerotidae	*Hartmanodes*	*nyei*	103	0	0	0	
Arthropoda	Malacostraca	Cumacea	Diastylidae	*Oxyurostylis*		92	0	0	0	
Annelida	Polychaeta	Sabellida	Sabellidae	*Augeneriella*		71	0	0	0	
Annelida	Polychaeta	Sabellida	Serpulidae	*Spirorbides*		45	0	0	0	
Arthropoda	Malacostraca	Amphipoda	Caprellidae	*Deutella*	*incerta*	42	0	0	0	
Arthropoda	Malacostraca	Isopoda	Idoteidae	*Erichsonella*	*attenuata*	14	0	0	0	
Annelida	Polychaeta	Sabellida	Sabellidae	*Chone*		13	0	0	0	
Arthropoda	Malacostraca	Amphipoda	Corophiidae			12	0	0	0	
Mollusca	Gastropoda	Pulmonata	Ellobiidae	*Ellobium*	*dominicense*	8	0	0	0	
Mollusca	Bivalvia	Veneroida	Semelidae	*Semele*	*proficua*	6	0	0	0	
Mollusca	Gastropoda	Neogastropoda	Columbellidae	*paravanachis*	*obesa*	5	0	0	0	
Mollusca	Gastropoda	Pyramidelloidea	Pyramidellidae	*Turbonilla*	*vinidaria*	5	0	0	0	
Mollusca	Gastropoda	Neogastropoda	Buccinidae	*Gemophos*	*tinctus*	0	13	0	0	
Mollusca	Bivalvia	Veneroida	Veneridae	*Anomalocardia*	*cuneimeris*	0	3	0	0	
Mollusca	Gastropoda	Cephalaspidea	Cylichnidae	*Acteocina*	*canaliculata*	0	6	6	0	
Mollusca	Gastropoda	Caenogastropoda	Janthinidae	*Recluzia*	*rollandiana*	0	4	22	0	
Mollusca	Gastropoda	Littorinimorpha	Littorinidae	*Littoraria*	*angulifera*	0	0	53	0	
Mollusca	Gastropoda	Caenogastropoda	Litiopidae	*Alaba*	*incerta*	0	0	45	0	
Mollusca	Gastropoda	Littorinimorpha	Rissoinidae	*Rissoina*	*angleli*	0	0	6	0	
Mollusca	Gastropoda	Neogastropoda	Olividae	*Oliva*	*fulgerator*	0	0	5	0	
Mollusca	Gastropoda	Neogastropoda	Muricidae	*Calotrophan*	*ostrearum*	0	0	3	0	

collected prior to and samples collected after Tropical Storm Debby, which occurred shortly after the summer 2012 collection (Figs. 3-7 & 3-8).

Statistical Analyses

Partial correlation analyses of biological abundance and richness data to hydrological and geochemical data described in Chapter Two are shown in Tables 3-6 – 3-11. Analyses indicate that across sites during the same collection period, water temperature, pH, salinity, and dissolved

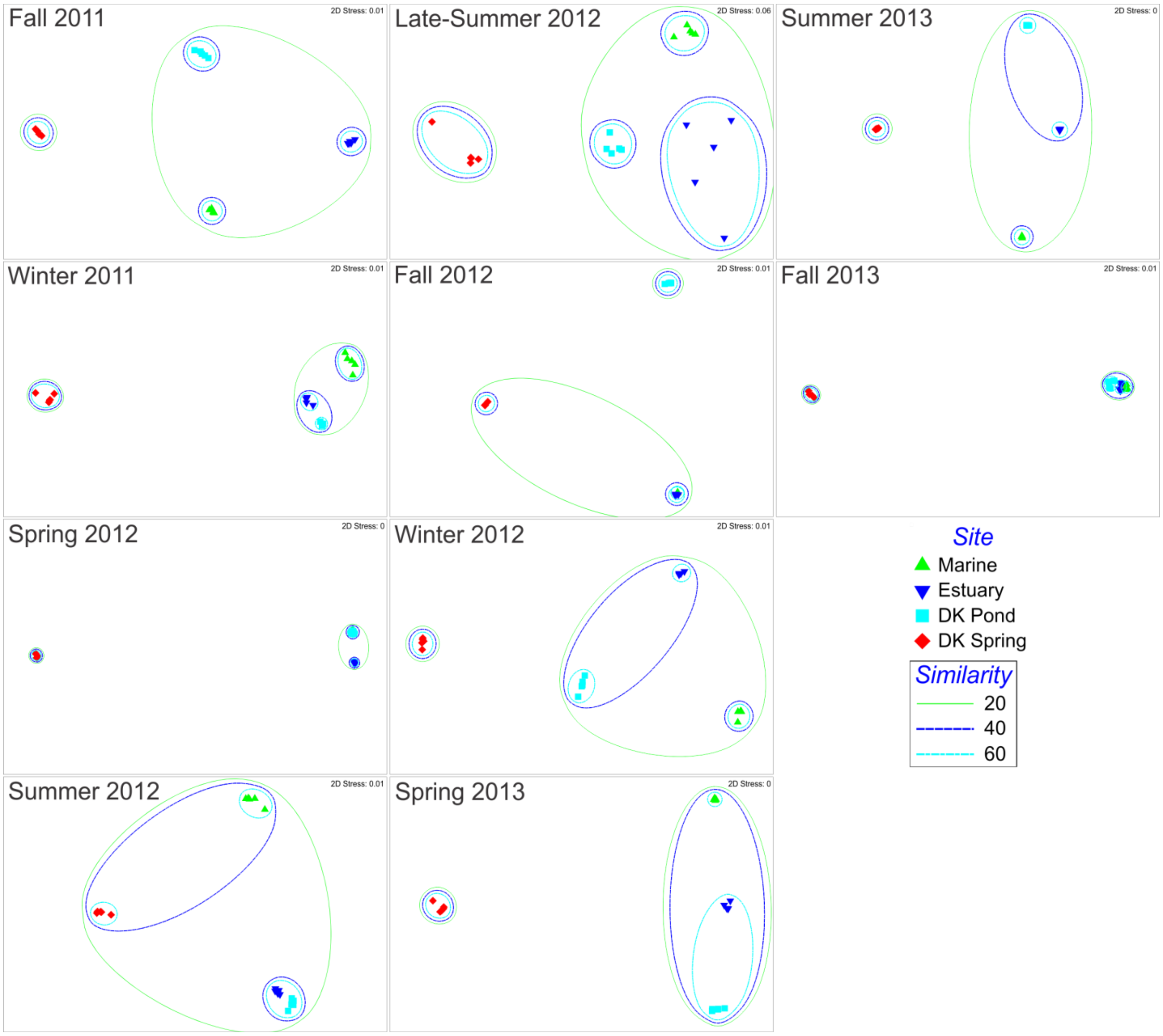


Figure 3-5. Multi-Dimensional Scaling plots of the combined archaeal, bacterial, and microbial eukaryote communities in the water column on each collection date at each site. Each of the five replicate samples from each site are indicated by different colored shapes. Colored circles around the samples represent the percentage of similarity between samples.

oxygen have the highest number of statistically significant correlations (Tables 3-6, 3-7, 3-9 & 3-10). The same analyses of biological data at each site over time indicate that aquifer level had the most numerous significant correlations followed by aquifer discharge and rainfall (Tables 3-8 & 3-11). Other geochemical data collected indicate sporadic correlations and but no distinct patterns. Correlations of community structure (MDS analyses) to geochemical and hydrological

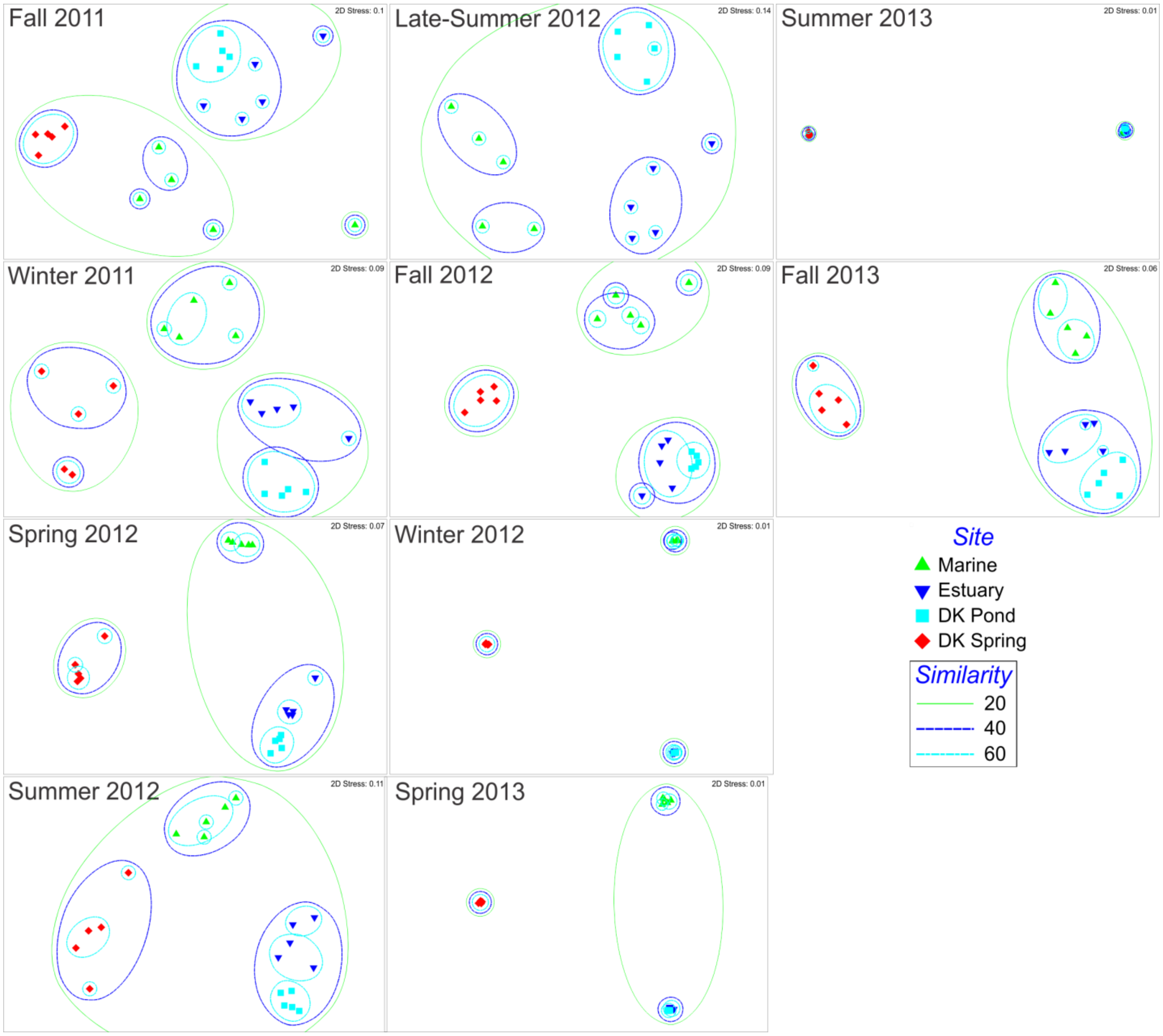


Figure 3-6. Multi-Dimensional Scaling plots of the combined archaeal, bacterial, microbial eukaryote, and macrofaunal communities in the sediment on each collection date at each site. Each of the five replicate samples from each site are indicated by different colored shapes. Colored circles around the samples represent the percentage of similarity between samples.

data are shown in Tables 3-12 – 3-15. Only correlations with r^2 values greater than 0.20 are shown in the tables.

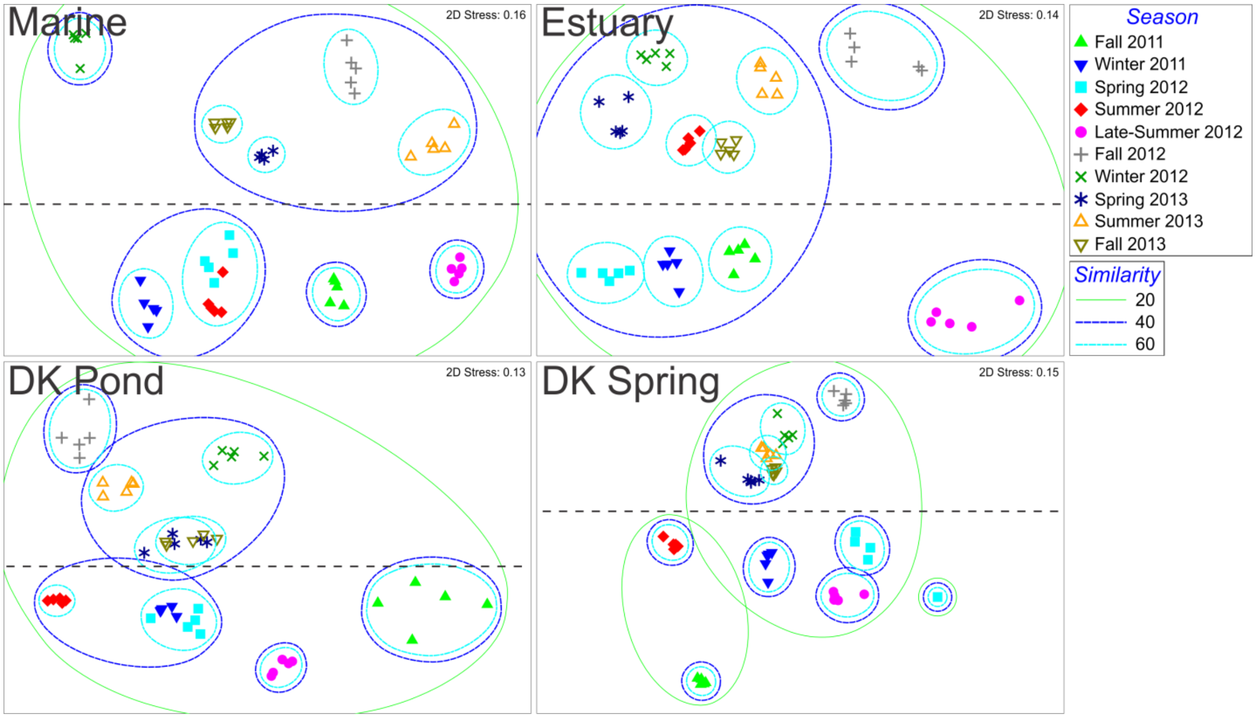


Figure 3-7. Multi-Dimensional Scaling plots of the combined archaeal, bacterial, and microbial eukaryote communities in the water column at each site from Fall 2011 through Fall 2013. Each of the five replicate samples from each site are indicated by different colored shapes. Colored circles around the samples represent the percentage of similarity between samples. The dashed lined separates samples before and after Tropical Storm Debby with samples collected after Tropical Storm Debby on top.

Discussion

The purpose of this study is to explore the interactions between the biotic and abiotic factors within Karst Estuaries. Results from this study indicate that there are complex interrelationships among hydrological, geochemical, and biological components of this Karst Estuary. Previous work by Menning et al. (2014b) has shown that the volume of aquifer discharge directly correlates to rainfall and aquifer levels. They also showed that the water temperature, pH, dissolved oxygen, salinity, and alkalinity gradients along the transect from Double Keyhole Spring through the marine site are directly related to the volume of aquifer discharge. Results

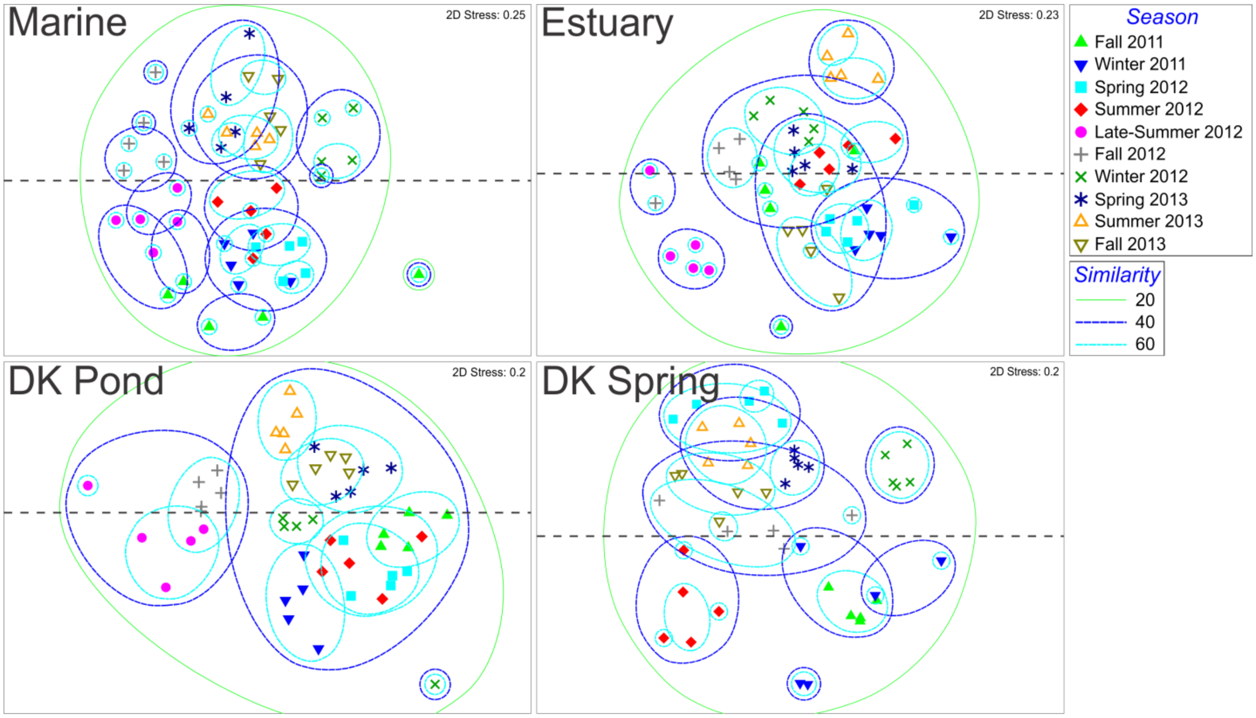


Figure 3-8. Multi-Dimensional Scaling plots of the combined archaeal, bacterial, microbial eukaryote, and macrofaunal communities in the sediment at each site from Fall 2011 through Fall 2013. Each of the five replicate samples from each site are indicated by different colored shapes. Colored circles around the samples represent the percentage of similarity between samples. The dashed lined separates samples before and after Tropical Storm Debby with samples collected after Tropical Storm Debby generally on top.

from this study show that the primary driver of biological change in this Karst Estuary is the volume of aquifer discharge and the geochemical gradients formed by aquifer discharge.

In June 2012, Tropical Storm Debby produced over 20 cm of rain in the area surrounding the study sites. This caused a three meter increase in the aquifer water level and resulted in an almost three-fold increase in aquifer discharge from Double Keyhole Spring (Menning et al., 2014b). All of the data in this study suggests two distinct patterns based on aquifer discharge conditions; 1) increased abundance and decreased species richness during the low aquifer discharge conditions observed prior to Tropical Storm Debby, and 2) decreased abundance and increased species richness during the high aquifer discharge conditions observed after Tropical Storm Debby. No clear seasonal cycles were noted because Tropical Storm Debby occurred during the

Table 3-6. Partial correlation analyses of estimated microbial abundance in the water column to hydrological and geochemical data described in Chapter Two controlling for sample collection date. All values shown are statistically significant (p<0.025).

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	.971	---	-.968	---	.832	.806	---	-.791	-.589	---
Winter 2011	.628	.949	---	.940	---	---	---	---	---	---
Spring 2012	.602	-.856	-.749	.617	.692	---	---	---	---	---
Summer 2012	.967	-.981	-.974	.980	.883	---	-.533	---	---	---
Late-Summer 2012	---	---	-.553	.810	.718	.635	---	---	---	---
Fall 2012	---	---	-.888	.980	.637	---	---	---	---	---
Winter 2012	.758	-.823	-.618	-.813	-.544	.724	---	---	---	---
Spring 2013	.514	---	---	.732	---	---	---	---	---	---
Summer 2013	---	.785	-.636	.537	---	---	---	---	---	---
Fall 2013	.598	-.653	-.548	.659	---	---	---	-.528	---	---

Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	.883	---	-.881	---	.780	.693	---	-.727	-.559	---
Winter 2011	.586	.954	---	.922	---	---	---	---	---	---
Spring 2012	.687	-.900	-.665	.699	.749	---	---	---	---	---
Summer 2012	.924	-.979	-.984	.980	.920	---	-.517	---	---	---
Late-Summer 2012	---	---	-.546	.639	---	.601	---	---	---	---
Fall 2012	---	---	-.897	.977	.587	---	---	---	---	---
Winter 2012	.754	-.799	-.622	-.805	-.589	.614	---	---	---	---
Spring 2013	.608	---	---	.598	---	---	---	---	---	---
Summer 2013	---	.545	---	---	---	---	---	---	---	---
Fall 2013	---	-.534	---	.540	---	---	---	---	---	---

Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	.945	-.514	-.940	---	.810	.811	---	-.756	-.578	---
Winter 2011	---	.966	-.711	.787	-.622	---	---	---	---	---
Spring 2012	---	-.731	-.652	.523	.566	---	---	---	---	---
Summer 2012	.945	-.895	-.870	.890	.730	---	-.531	---	---	---
Late-Summer 2012	---	-.516	---	.582	.726	---	---	---	---	---
Fall 2012	.567	---	-.749	.942	.755	---	---	---	---	---
Winter 2012	---	-.628	---	-.553	-.522	---	---	---	---	---
Spring 2013	.700	---	---	.596	---	---	---	---	---	---
Summer 2013	.526	.910	-.847	.787	---	---	---	---	---	---
Fall 2013	---	-.584	-.808	---	---	---	---	---	---	-.515

middle of the study period, although the biological data near the end of the study (Fall 2013) appeared to be similar to the data collected at the beginning of the study (Fall 2011) (Figs. 3-2 – 3-4).

Testing q-PCR Methods Using Known DNA

The use of q-PCR to measure changes in abundance using DNA from a single organism for the standard curve (see Methods) was validated by the experiment using DNA from pure cultures of different archaeal, bacterial, and microbial eukaryote species separately for the standard curve. The results show that using DNA from different species used to calculate abundance of rDNA by q-PCR introduces some variance, but the variance was small for the prokaryotes (Table 3-3). I found that when DNA from three different eukaryotes were used for standard curves the variance was unacceptably large, suggesting that comparisons should only be made among studies that use DNA from the same eukaryote species for the standard curve (Table 3-3). The large variance measured with eukaryotes species could be due to the large range of 18S rRNA gene copy number between different eukaryotic species (Koid et al., 2012).

I also wanted to know if I could compare the abundance of archaeal species to bacterial species. According to the q-PCR results, there appeared to be far more archaeal DNA than bacterial DNA in the samples. The experiments using different equimass mixtures of archaeal and bacterial DNA in q-PCR experiments (Table 3-4) suggests that the archaeal rDNA primers are approximately ten times more efficient at binding to archaeal DNA than the bacterial primers bind to bacterial DNA which may be due to the degenerate bases incorporated into the archaeal primers. Therefore, the DNA concentrations I report are estimates of community size and are used to reflect general increases/decreases in population size and should not be considered absolute values.

Estimated Abundance

The estimated species abundance data indicates differences of microbial abundance between all sites but was generally highest at the Double Keyhole Pond and estuary sites (Figs. 3-2 & 3-3). Aquifer discharge had significant negative correlations (r^2 = -0.321 to -0.776) to the

Table 3-7. Partial correlation analyses of estimated microbial and macrofaunal abundance in the sediment to hydrological and geochemical data described in Chapter Two controlling for sample collection date. All values shown are statistically significant ($p<0.025$).

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.802	---	-.949	---	---	---	---	---	---
Winter 2011	.685	.868	---	.915	---	---	---	---	---	---
Spring 2012	---	---	-.684	---	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	.666	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	.696	---	---	---	.600	---	---	---	---	---
Winter 2012	.744	---	-.773	-.707	---	.614	---	---	---	---
Spring 2013	---	---	---	-.642	---	---	---	---	---	---
Summer 2013	---	---	---	---	---	---	---	---	---	---
Fall 2013	-.583	---	---	---	---	---	---	---	---	---

Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.830	---	-.829	---	---	---	---	---	---
Winter 2011	.561	---	---	---	---	---	---	---	---	---
Spring 2012	---	---	---	---	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	---	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	---	---	---	---	---	---	---	---	---	---
Winter 2012	.870	-.618	-.821	-.872	-.654	.671	---	---	---	-.617
Spring 2013	---	---	---	---	---	---	---	---	---	---
Summer 2013	---	---	---	---	---	---	---	---	---	---
Fall 2013	-.574	---	---	---	---	---	---	---	---	---

Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.735	---	-.850	---	---	---	---	---	---
Winter 2011	---	.667	-.546	.513	---	---	---	---	---	---
Spring 2012	---	---	---	---	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	.611	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	---	---	---	.584	---	---	---	---	---	---
Winter 2012	---	---	-.787	-.788	-.541	.554	---	---	---	---
Spring 2013	.518	-.731	-.643	-.543	---	---	---	---	---	---
Summer 2013	.902	---	-.668	.752	.749	---	---	---	---	---
Fall 2013	---	---	-.605	---	---	---	---	---	---	---

Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	---	---	---	---	---	---	---	---	---
Winter 2011	---	.843	-.791	.593	-.718	---	---	---	---	---
Spring 2012	---	---	---	---	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	.617	---
Late-Summer 2012	-.636	.636	.636	-.636	---	---	---	---	---	---
Fall 2012	---	.719	-.739	.539	---	.597	---	---	---	---
Winter 2012	.832	-.711	-.743	-.856	-.665	.755	---	---	---	---
Spring 2013	---	---	---	.635	---	---	---	---	---	---
Summer 2013	---	.621	-.579	.539	---	---	---	---	---	---
Fall 2013	.815	-.576	---	.665	---	---	---	---	---	-.523

Table 3-8. Partial correlation analyses of estimated microbial and macrofaunal abundance in the water column and sediment to hydrological and geochemical data described in Chapter Two controlling for collection site. All values shown are statistically significant (p<0.025).

Water Column																		
Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	---	.382	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	.352	---	.657	---	---	.370	---	---	---	---	---	---	.339	---
DK Pond	---	---	---	.469	.482	---	---	---	---	.416	---	-.410	-.567	---	-.350	-.481	---	-.441
DK Spring	---	.396	.444	---	---	---	---	---	---	---	---	---	-.586	-.525	-.340	-.347	---	-.492
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	.559	---	.320	-.510	-.370	.496	---	---	---	---	.508	---	---	---	---	---	---	---
Estuary	---	---	.478	---	---	---	---	---	.499	---	.403	---	-.358	---	---	-.374	---	-.557
DK Pond	---	---	---	.356	.506	---	---	-.464	---	---	.389	---	-.403	---	-.398	-.536	-.356	-.630
DK Spring	---	.606	.780	---	---	---	---	---	---	---	.392	---	-.757	-.728	---	-.457	-.346	-.593
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	.448	---	---	---	---	.444	---	---	---	.339	.727	---	---	---	---	---	---	---
Estuary	.375	---	---	---	---	---	---	---	.503	---	.809	---	-.420	---	---	---	---	-.456
DK Pond	---	---	---	---	.609	---	---	-.387	---	---	.647	---	-.515	---	---	---	---	-.504
DK Spring	---	.523	.814	---	---	---	---	-.347	---	---	.437	---	-.776	-.718	-.321	-.416	---	-.482
Sediment																		
Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	-.625	.628	---	---	---	---	---	---	---	---	---	-.353	-.533	-.511	-.713	-.714	-.728
Estuary	-.338	---	.634	---	---	---	---	---	---	---	---	---	-.501	-.667	-.618	-.733	-.652	-.774
DK Pond	-.491	---	---	---	---	-.388	---	---	---	-.422	---	---	---	-.324	---	---	---	---
DK Spring	-.518	.454	---	---	---	---	---	---	.473	---	---	---	-.453	-.521	---	-.382	-.535	-.379
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	.332	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	.381	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DK Pond	---	---	---	-.327	-.473	---	---	.322	---	-.450	---	.345	---	-.321	---	---	---	---
DK Spring	---	.362	.651	---	---	---	---	---	---	---	---	-.370	-.634	-.594	---	-.503	-.349	-.641
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	-.347	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DK Pond	---	.475	---	---	---	---	---	---	---	-.393	---	---	---	---	---	---	---	---
DK Spring	---	---	.345	---	---	---	---	---	---	---	.415	---	---	---	---	---	---	---
Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63m	Aquifer Level @ 140m	Aquifer Level @ 160m	Aquifer Level @ 250m
Marine	---	---	---	---	-.470	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	-.331	---	---	---	---	---
DK Pond	---	.515	---	---	---	---	---	---	---	---	-.348	---	---	---	-.416	---	---	---
DK Spring	---	.412	.518	---	---	---	---	---	.346	---	.369	---	-.604	-.543	---	---	---	-.345

Table 3-9. Partial correlation analyses of microbial species richness in the water column to hydrological and geochemical data described in Chapter Two controlling for sample collection date. All values shown are statistically significant (p<0.025).

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.881	---	-.824	---	---	---	---	---	---
Winter 2011	-.570	-.949	---	-.910	---	---	---	---	---	---
Spring 2012	-.904	.877	---	-.906	-.839	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	.651	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	.783	---	---	.821	.835	---	---	---	---	---
Winter 2012	---	.634	---	---	---	---	---	---	.803	---
Spring 2013	---	---	---	.934	---	---	---	---	---	---
Summer 2013	---	.946	-.852	.776	---	---	---	---	---	---
Fall 2013	.695	-.959	-.952	.915	.731	---	---	-.528	---	-.773
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	-.586	-.640	.599	-.993	-.642	---	---	.641	---	---
Winter 2011	---	.874	-.892	.574	-.834	---	---	---	---	---
Spring 2012	-.931	.751	---	-.926	-.847	---	---	---	---	---
Summer 2012	-.976	.906	.875	-.899	-.715	---	.543	---	---	---
Late-Summer 2012	.878	.824	-.828	---	---	.638	---	---	-.735	.581
Fall 2012	.803	---	---	.789	.828	---	---	---	---	---
Winter 2012	.985	-.650	-.946	-.978	-.652	.742	---	---	---	---
Spring 2013	---	---	---	---	---	---	---	---	---	---
Summer 2013	---	---	---	---	-.666	---	---	---	---	---
Fall 2013	---	---	.601	---	---	---	---	---	---	---
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	.706	---	---	---	-.628	---	---	---	---
Winter 2011	---	-.574	---	-.520	---	---	---	---	---	---
Spring 2012	-.734	.626	---	-.731	-.648	---	---	---	---	---
Summer 2012	.764	-.676	-.642	.668	.535	---	---	---	---	---
Late-Summer 2012	-.703	---	.685	---	---	-.518	---	---	.666	---
Fall 2012	---	---	-.769	.919	.709	---	---	---	---	---
Winter 2012	---	---	-.577	---	---	---	---	---	---	---
Spring 2013	---	.782	---	.932	---	---	---	---	-.537	---
Summer 2013	.831	.861	-.922	.927	---	---	---	---	---	---
Fall 2013	---	---	---	---	---	---	---	---	---	---

abundance of all microbial communities within the water column and sediment at the study sites (Table 3-8). This is evident in the reduction of prokaryote abundance within the sediment immediately following Tropical Storm Debby in late-summer and fall 2012 (Fig. 3-3).

Examination of individual peaks during the periods of increased abundance in the water column, summer and fall 2012, indicate that the majority of the increases observed were due to relatively few species (Fig. 3-9). In summer 2012, before Tropical Storm Debby, when aquifer

Table 3-10. Partial correlation analyses of microbial and macrofaunal species richness in the sediment to hydrological and geochemical data described in Chapter Two controlling for sample collection date. All values shown are statistically significant (p<0.025).

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.729	---	-.548	---	---	---	---	---	---
Winter 2011	---	.698	-.670	---	-.563	---	---	---	---	---
Spring 2012	---	-.675	-.873	---	---	---	---	---	---	---
Summer 2012	.606	-.681	-.695	.684	.658	---	---	---	---	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	---	---	-.620	.638	---	---	---	---	---	---
Winter 2012	.585	---	-.760	---	---	---	---	---	.635	---
Spring 2013	.571	-.763	-.690	-.534	---	---	---	---	---	---
Summer 2013	.900	.647	-.793	.846	.653	---	---	---	---	---
Fall 2013	-.685	.812	.730	-.803	-.650	---	---	.512	---	.745

Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	-.661	---	---	---	.577	---	---	---	---
Winter 2011	---	---	---	---	---	---	---	---	---	---
Spring 2012	.904	---	---	.899	-.833	---	---	---	---	---
Summer 2012	.879	-.932	-.938	.934	.761	---	-.630	---	---	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	---	.616	-.733	.609	---	---	---	---	---	---
Winter 2012	-.867	---	.891	.828	---	-.570	---	---	---	---
Spring 2013	---	---	---	---	---	---	---	---	---	---
Summer 2013	---	.822	-.650	.538	---	---	---	---	---	---
Fall 2013	---	---	---	---	---	---	---	---	---	---

Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	---	---	---	---	---	---	---	---	---
Winter 2011	---	.675	-.749	---	-.690	---	---	---	---	---
Spring 2012	---	---	---	---	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	.669	---
Late-Summer 2012	---	---	---	---	---	---	---	---	---	---
Fall 2012	---	---	---	---	---	---	---	---	---	---
Winter 2102	.708	---	-.776	-.651	---	---	---	---	---	---
Spring 2013	---	---	---	-.525	---	---	---	---	---	---
Summer 2013	---	---	---	---	---	---	---	---	---	---
Fall 2013	---	---	---	---	---	---	---	---	---	---

Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	---	---	---	---	---	---	---	---	---	---
Winter 2011	---	.636	---	.552	---	---	---	---	---	---
Spring 2012	.535	-.524	---	.537	---	---	---	---	---	---
Summer 2012	---	---	---	---	---	---	---	---	---	---
Late-Summer 2012	-.692	.692	.692	-.692	-.734	---	---	---	-.690	.701
Fall 2012	-.594	.883	-.674	---	---	.711	---	---	---	---
Winter 2012	.750	---	-.770	-.717	-.552	---	---	---	---	-.553
Spring 2013	.760	---	-.596	---	---	---	-.596	---	---	---
Summer 2013	---	.752	-.593	---	---	---	---	---	---	.524
Fall 2013	.787	-.674	---	.730	---	---	---	---	---	-.583

Table 3-11. Partial correlation analyses of microbial and macrofaunal species richness in the water column and sediment to hydrological and geochemical data described in Chapter Two controlling for collection site. All values shown are statistically significant ($p<0.025$).

Water Column

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	.341	---	---	-.327	---	---	---	---	---	---	.384	.394	.516	.592	.489
Estuary	.593	---	-.577	---	---	.410	---	---	---	---	.497	.745	.326	.503	.815	.690	.363	.414
DK Pond	---	---	-.339	---	---	---	---	---	.502	---	---	---	---	.414	.449	.447	.422	.515
DK Spring	---	---	---	-.693	---	-.418	.509	-.416	---	-.398	.529	---	.431	.338	---	---	.379	---

Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	-.337	---	---	---	---	---	---	---	.387	.479	.593	.537	.661	.562	---	.469
Estuary	---	---	-.560	---	.477	.477	---	---	---	---	---	---	.503	.773	.400	.620	.826	.816
DK Pond	---	---	-.601	---	---	---	---	---	.326	---	---	.496	.473	.371	.618	.649	.392	.677
DK Spring	---	---	.441	-.494	.391	-.535	---	-.549	---	-.440	.545	---	---	---	---	---	.387	---

Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	-.347	---	---	.375	---	---	---	.375	---	-.424	---	---	-.544	---
Estuary	.480	---	.342	.769	---	-.457	---	---	---	---	---	---	---	---	---	---	---	---
DK Pond	---	---	---	.724	---	---	---	---	-.363	---	.382	---	-.391	-.604	---	-.384	-.689	-.430
DK Spring	---	---	---	---	-.383	---	---	---	---	---	---	.565	---	---	---	---	---	---

Sediment

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	-.374	---	---	---	---	---	---	---	.459	---	---	---	---	---	---	---	-.326	---
Estuary	---	---	.487	.356	---	-.533	---	---	.386	.377	---	---	---	---	---	-.358	-.441	---
DK Pond	-.703	.432	---	.482	---	-.426	---	---	---	---	---	---	-.584	-.601	---	-.351	-.413	---
DK Spring	-.584	.385	---	---	-.355	.620	---	---	.372	---	---	---	-.469	-.564	---	-.401	-.786	-.463

Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	.395	---	---	---	.431	---	-.406	---	---	---	---	---	---
Estuary	---	---	---	.443	.518	---	.383	---	.647	.650	---	---	---	---	---	---	---	---
DK Pond	---	---	.383	.635	---	---	---	---	---	.450	.168	---	---	---	---	-.393	-.447	-.381
DK Spring	-.633	---	---	---	---	---	---	---	---	---	---	---	---	-.389	-.491	-.467	---	-.369

Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	---	---	---	---	-.407	---	---	---	-.464	---	---	-.450	---
Estuary	-.459	---	---	-.647	---	---	-.366	---	---	-.402	---	---	---	---	---	---	---	---
DK Pond	---	---	---	-.440	---	---	---	---	.401	---	---	---	---	---	---	---	.346	---
DK Spring	.426	---	---	---	---	-.466	---	---	---	---	---	---	.348	.405	---	---	.515	.367

Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	-.369	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	.384	---	---	---	---	---	---	---	---	---	-.524	-.348	---	-.361	-.320	---
DK Pond	---	---	---	---	---	---	---	---	---	---	-.396	---	---	---	---	---	---	---
DK Spring	---	.355	.442	---	---	---	---	---	---	---	---	---	-.413	-.388	---	---	-.345	-.383

Table 3-12. Correlations of microbial community structure in the water column (MDS analyses) across all sites on the same collection date to geochemical and hydrological data described in Chapter Two. Only correlations with r^2 values greater than 0.20 are shown and all are statistically significant ($p<0.01$).

	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Total Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	0.834	0.455	0.469	0.447	0.652	---	---	---	---	0.526
Winter 2011	0.414	0.964	0.308	0.820	0.302	---	---	---	---	---
Spring 2012	---	---	0.598	0.815	0.805	0.271	0.396	---	---	---
Summer 2012	0.627	0.474	0.867	0.565	0.670	---	---	---	---	---
Late-Summer 2012	0.891	0.880	0.428	0.857	0.203	0.485	0.359	---	0.570	0.532
Fall 2012	0.424	0.641	0.327	0.693	0.367	0.210	0.207	---	---	---
Winter 2012	0.666	0.738	0.406	0.974	0.208	---	---	---	---	---
Spring 2013	0.663	0.601	0.731	0.864	---	---	---	---	---	---
Summer 2013	0.661	0.709	0.582	0.603	0.278	---	---	---	0.226	---
Fall 2013	0.953	0.619	0.323	0.953	0.714	---	---	---	---	---

Table 3-13. Correlations of microbial and macrofaunal community structure in the sediment (MDS analyses) across all sites on the same collection date to geochemical and hydrological data described in Chapter Two. Only correlations with r^2 values greater than 0.20 are shown and all are statistically significant ($p<0.01$).

	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Total Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)
Fall 2011	0.464	0.388	0.219	0.205	0.280	---	---	---	---	0.268
Winter 2011	0.497	0.540	0.422	0.703	0.251	0.248	0.247	---	0.330	---
Spring 2012	0.436	0.593	0.506	0.805	0.232	0.205	0.251	---	0.522	---
Summer 2012	0.714	0.482	0.712	0.663	0.641	---	---	---	---	0.228
Late-Summer 2012	0.665	0.725	0.725	0.508	0.274	---	---	---	---	0.212
Fall 2012	0.365	0.618	0.306	0.688	0.200	0.380	---	---	---	0.339
Winter 2012	0.433	0.568	0.376	0.844	---	0.218	---	---	---	---
Spring 2013	0.503	0.377	0.589	0.737	---	---	---	---	---	---
Summer 2013	0.687	0.865	0.299	0.844	---	---	---	---	0.355	0.297
Fall 2013	0.771	0.442	0.272	0.902	0.617	---	---	---	---	0.362

level and discharge were at seasonal lows, bacterial and microbial eukaryote abundance increased at the Double Keyhole Pond site. Of the 48 bacterial fragments detected in the water column in summer 2012 by LH-PCR, only two fragments (311 and 317 bps), were responsible for over half of the community. During that same period there were 57 microbial eukaryote fragments detected in the water column. Of those microbial eukaryote fragments, only two (370 and 372 bps) were responsible for over 80 % of the community. In fall 2012, when there was an increase in archaeal populations in the estuary, 39 peaks were detected but only three (286, 299, and 327 bps) were responsible for over 75 % of the community. Partial correlation analyses of

Table 3-14. Correlations of microbial community structure in the water column (MDS analyses) across all dates at the same collection site to geochemical and hydrological data described in Chapter Two. Only correlations with r^2 values greater than 0.20 are shown and all are statistically significant ($p<0.01$).

	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	0.266	---	0.205	---	---	---	---	---	---	0.212	0.356	0.451	0.370	0.422	0.422	0.432
Estuary	0.436	0.230	0.444	---	---	---	---	---	---	---	0.244	0.273	0.300	0.548	0.531	0.566	0.551	0.438
DK Pond	---	0.275	---	0.495	---	---	---	---	---	---	0.274	0.269	0.517	0.484	0.539	0.345	0.387	0.368
DK Spring	---	---	0.331	---	---	---	---	---	---	---	---	---	0.646	0.569	0.317	0.330	0.343	0.467

Table 3-15. Correlations of microbial and macrofaunal community structure in the sediment (MDS analyses) across all dates at the same collection site to geochemical and hydrological data described in Chapter Two. Only correlations with r^2 values greater than 0.20 are shown and all are statistically significant ($p<0.01$).

	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	0.234	---	---	---	0.208	---	---	---	---	---	---	0.345	0.439	0.311	0.252	0.334	0.368
Estuary	---	---	0.380	---	0.275	---	---	---	---	---	---	---	0.288	0.360	0.373	0.432	0.372	0.295
DK Pond	0.284	0.258	0.355	---	---	---	---	---	---	---	---	---	0.401	0.518	0.396	0.454	0.452	0.438
DK Spring	---	0.214	0.377	---	---	---	---	---	---	---	---	0.212	0.440	0.417	0.288	0.302	0.245	0.371

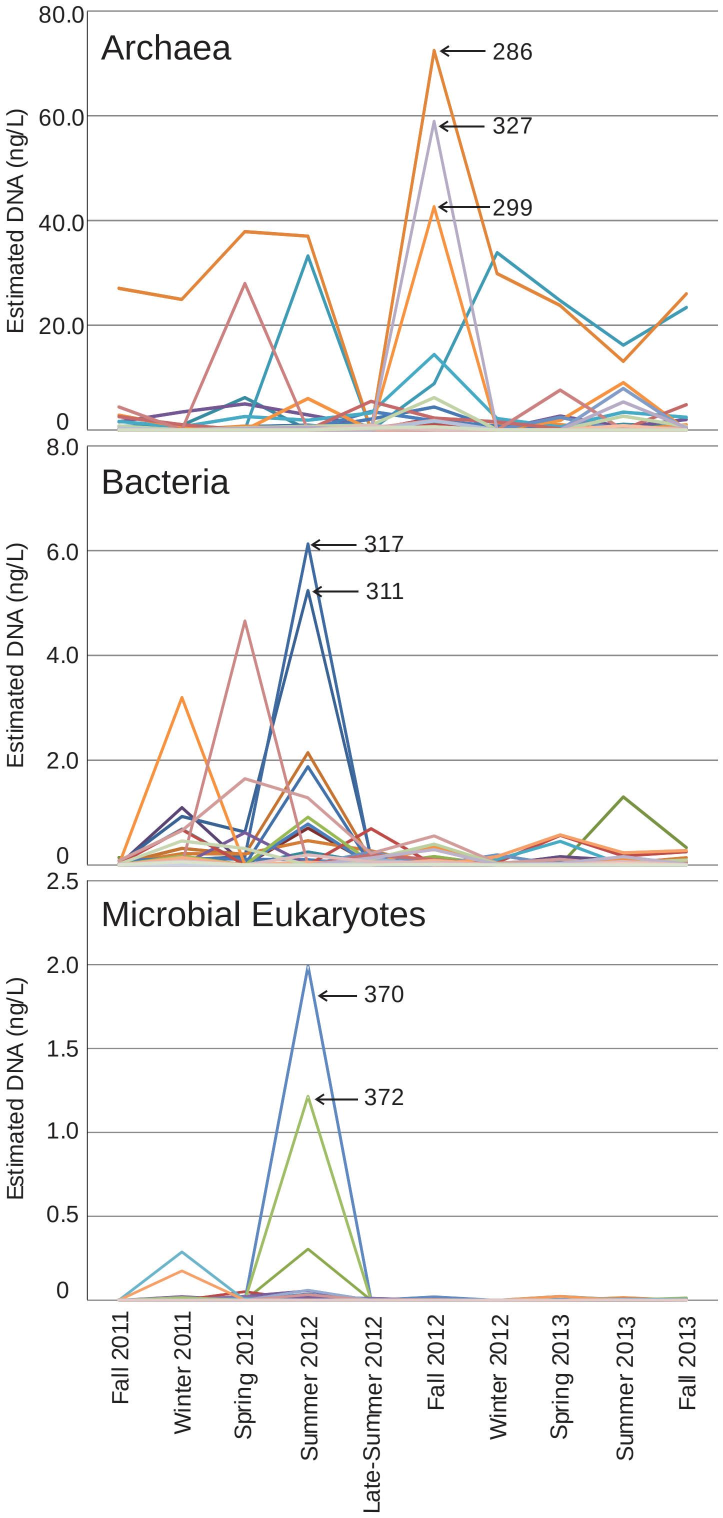


Figure 3-9. Estimated DNA of individual peaks during the periods of increased abundance in the water column. Archaeal peaks are from the estuary site during the fall 2012 collection. Bacterial and microbial eukaryote peaks are from the Double Keyhole Pond site during the summer 2012 collection.

these peaks to hydrological and geochemical data indicate strong negative correlations of the bacterial and microbial eukaryote populations to average daily discharge (r^2 = -0.506 to -0.517) and strong positive correlations to alkalinity (r^2 = 0.477 to 0.614) and rainfall the week prior to sample collection (r^2 = 0.619 to 0.692) (Table 3-16). There were multiple factors that impacted the archaeal community in the estuary, but the strongest correlation to all fragments was to nitrate concentrations (r^2 = 0.631 to 0.688). In a study of the Parker River estuary of Massachusetts, Crump et al. (2004) noted seasonal increases in bacterial communities that correlated to longer residence times of the water within the estuary. I find similar results at the study sites where microbial abundance in the water column increased when aquifer discharge was at seasonal low volumes. This suggests that the increased aquifer discharge reduces the amount of microbes throughout the system and prevents the build-up of microbes as was seen before Tropical Storm Debby. The peak of archaeal abundance in the water column in fall 2012 after Tropical Storm Debby indicates that while increased aquifer discharge reduced the abundance of bacterial and microbial eukaryotes it increased the archaeal abundance possibly due to changes of nitrate concentration (Table 3-16). I expect that when drought conditions return or if there is an increase in aquifer use, microbial abundance in the estuary will again increase due to the increased residence time of the water in the estuary.

Species Richness

Aquifer discharge also has a major influence on species richness within the system. Generally, species richness increased during periods of high aquifer discharge in both the water column and sediment with the exception of macrofauna in the sediment at the estuary site (Figs. 3-4 & 3-5). The loss of macrofaunal species richness at the estuary site following tropical Storm

Table 3-16. Partial correlation analyses of DNA from individual microbial peaks in the water column (Fig. 3-9) to hydrological and geochemical data described in Chapter Two controlling for sample collection date. Only hydrological and geochemical data with statistically significant correlations are shown. All values shown are statistically significant ($p<0.025$).

	Alkalinity (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Archaea 286	---	0.633	---	---	---	-0.386	-0.375	---	---	---	---
Archaea 299	0.357	0.631	---	---	---	---	---	0.370	---	0.413	0.345
Archaea 327	0.369	0.688	---	---	-0.321	---	---	0.406	0.351	0.488	0.419
Bacteria 311	0.609	---	-0.393	---	0.619	---	-0.506	---	-0.371	---	-0.549
Bacteria 317	0.614	-0.358	-0.334	---	0.679	---	-0.506	---	---	---	-0.469
Eukaryota 370	0.481	---	---	0.700	0.680	---	-0.512	---	---	---	-0.464
Eukatyota 372	0.477	---	---	0.702	0.692	---	-0.517	---	---	---	-0.474

Debby highlights the impacts of submarine groundwater discharge in the area. Menning et al. (2014b) showed that following Tropical Storm Debby there was increased submarine groundwater discharge in the estuary that bypassed Double Keyhole Spring. This water was hypothesized to be the result of a freshwater wedge following the topological gradient through the karst discharging in the estuary. This event coincides with a transient loss of macrofaunal diversity in the estuary suggesting that increased submarine groundwater discharge and subsequently lower salinity have negative impacts on macrofaunal species richness.

Macrofauna

Macrofaunal species richness was greatest at the marine site (53 species) compared to the other sites (41, 42, and 12 species, at the estuary, Double Keyhole Pond and Spring sites respectively) (Table 3-5) and species abundance was greatest at the estuary and Double Keyhole Pond sites (9998 and 9979 total organisms respectively). A notable event occurred at the estuary site following Tropical Storm Debby when macrofaunal abundance and richness decreased from an average of 272 organisms per sample representing 22 species prior to TS Debby to an average of 55 organisms per sample representing 16 species after TS Debby (Fig. 3-4). This reduction in abundance (217 organisms) and richness (six species total, 13 species were lost and seven species were gained) coincided with increased aquifer discharge from Double Keyhole Spring and through the karst matrix that also resulted in decreased salinity at the site (Figs. 2-6D and 2-9). Approximately 77% of the abundance lost (167 organisms) was from 15 species that either do not tolerate physical perturbations or do not tolerate very low salinity water (Lloyd, 1964; Bloom et al., 1972; Santos & Simon, 1974; Swennen et al., 1982; Hsieh & Simon, 1991; Mikkelsen et al., 1995; Sarda et al., 1995; Mannino & Montagna, 1997; Gamenick et al., 1998; Brewster-Wingard & Ishman, 1999; Castanedo et al., 2012). In general, the rapid decrease of salinity after

Tropical Storm Debby could not be tolerated by the stenohaline members of the macrofaunal community and other species could not tolerate the increased rate of discharge of the spring.

Species richness and abundance was lowest within Double Keyhole Spring with the exception of one polychaete, *Stenoninereis martini*. This is a nereid polychaete known to live in a variety of habitats throughout the Gulf of Mexico (Williams et al., 1976). Originally, *S. martini* was thought to only inhabit brackish sinkholes as it was first found in tidally influenced ponds or sinkholes in the West Indies and Sarasota County, Florida. It was later found in Tampa Bay, Florida and Cedar Bayou, Texas, with more variable environments than sinkholes. The data suggests that the environment within Double Keyhole Spring is more favorable for *S. martini* than the surrounding estuary. Although not formally studied, there is a large population of juvenile game fish present within the estuary. At low tide they congregated at high density in the pond near the mouth of the spring, appearing to take refuge from shallower water and in winter, from the much colder water in the main part of the estuary.

Multi-Dimensional Scaling Plots

Examination of the MDS plots of the communities at all four sites at each collection time (Figs. 3-6 & 3-7) show spatial variations between sites with the Double Keyhole Spring samples generally grouped away of the other sites. The exception to this pattern can be seen in both the water column and sediment communities in summer 2012 when all four sites showed a 20% similarity. This event coincides with the lowest aquifer discharge noted during the two year study. The correlations of abundance, species richness, and community structure along the transect to the corresponding geochemical data (Tables 3-6, 3-7, 3-9 & 3-10) consistently show the greatest number of significant correlations are to the geochemical gradients formed by aquifer discharge (water temperature, pH, salinity, dissolved oxygen, and alkalinity). This indicates that the primary driver of change between sites in this Karst Estuary are the geochemical gradients formed by aquifer discharge.

The MDS plots of the communities at each site over the two-year study also indicate variations within the communities are determined by aquifer discharge (Figs. 3-8 & 3-9). The communities within this Karst Estuary generally divide into two groups; 1) low aquifer discharge, as seen prior to Tropical Storm Debby, and 2) high aquifer discharge, as seen after Tropical Storm Debby. There was one exception found in the estuarine community when the low aquifer discharge summer 2012 samples grouped with the high aquifer discharge communities observed after Tropical Storm Debby. The sediment communities showed less distinct groupings than the water column communities. In a study of the Chesapeake Bay, USA, Malone et al. (1988) found seasonal variations of phytoplankton biomass that were associated with variations in freshwater flow and fluctuations in nitrate concentrations. Although the results do not show seasonal variations over time it does show variations based on aquifer discharge which varies seasonally with local rainfall. The correlations of abundance, species richness, and community structure at each site over time to hydrological and geochemical data (Tables 3-8 & 3-11) consistently show the greatest number of significant correlations to aquifer level, aquifer discharge, and rainfall which are all interrelated. This suggests that the primary driver of change at each site over time is the volume of aquifer discharge. Both the sediment and water column microbial communities appear to be robust and responsive to environmental changes within the system suggesting that they are likely active in nutrient cycling.

The effects of aquifer discharge on community interactions indicate a clear connection between aquifer discharge and community structure. The MDS plots of community structure (Figs. 3-5 and 3-6) indicate the greatest amount of similarity between all sites occurred during the period of lowest aquifer discharge noted in the collection prior to Tropical Storm Debby (Summer 2012). The remainder of the MDS plots also indicate high similarity between the communities only if the Double Keyhole Spring site is excluded. These similarities occur during times of both low aquifer discharge and high aquifer discharge. I suspect that during periods of low aquifer discharge the communities are dominated by organisms from the marine environment due to the general flow of water towards Double Keyhole Spring and during times of high aquifer discharge the communities are dominated by organisms from the Double Keyhole Pond environment due to the general flow of water towards the Gulf of Mexico.

To determine if the observed correlations were due the disturbance caused by Tropical Storm Debby the biological data was correlated to the geochemical parameters in this study without the two samples immediately following Tropical Storm Debby (Fall and Winter 2012) (Tables 3-17 and 3-18). This data indicates minor differences from the previous analyses but the general pattern of highest and most numerous correlations continued to be due to aquifer discharge. This indicates that while Tropical Storm Debby had a major influence on the communities in the karst estuary surrounding Double Keyhole Spring the storm itself was not the primary driver of the observed change throughout the system over time.

Table 3-17. Partial correlation analyses of estimated microbial and macrofaunal abundance in the water column and sediment to hydrological and geochemical data described in Chapter Two controlling for collection site without Fall and Winter 2012 collections. All values shown are statistically significant (p<0.025).

Water Column DNA

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	0.667	---	---	-0.545	---	0.414	---	---	---	---	0.561	0.582	---	---	0.364	---	---	-0.408
Estuary	---	-0.483	0.427	---	---	---	---	---	0.360	---	---	---	---	0.411	---	---	---	-0.428
DK Pond	---	---	---	0.531	0.579	---	---	-0.520	---	0.438	0.376	---	-0.617	---	---	-0.596	-0.379	-0.591
DK Spring	---	0.445	0.425	---	---	---	---	---	---	---	---	---	-0.490	-0.442	---	---	---	-0.401
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	0.591	---	0.608	-0.465	---	0.541	---	---	---	0.364	0.469	---	-0.371	---	---	-0.442	-0.535	-0.835
Estuary	---	---	0.519	---	---	---	---	---	0.514	---	0.478	---	-0.467	---	---	-0.442	---	-0.796
DK Pond	0.442	---	---	---	0.599	---	---	-0.559	---	---	0.409	---	-0.532	---	---	-0.613	---	-0.802
DK Spring	---	0.601	0.762	---	---	---	---	---	---	---	0.419	---	-0.824	-0.802	---	-0.448	---	-0.678
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	0.470	---	0.476	---	---	0.484	---	---	---	0.408	0.740	---	-0.730	---	---	---	---	-0.679
Estuary	0.550	---	---	---	0.390	---	---	---	0.508	---	0.858	0.394	-0.713	---	---	---	---	-0.611
DK Pond	0.452	---	---	---	0.693	---	---	-0.470	---	---	0.691	---	-0.808	---	---	---	---	-0.612
DK Spring	---	0.518	0.820	---	---	---	---	-0.417	---	---	0.475	---	-0.906	-0.877	---	-0.522	---	-0.617

Sediment DNA

Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	-.650	.671	---	---	---	---	---	---	---	---	---	---	---	---	-.571	-.538	-.590
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	---	---	-.373	-.380	---	-.434
DK Pond	-.415	---	-.564	-.489	---	---	---	---	.372	-.416	---	---	---	---	---	.676	.668	.857
DK Spring	-.364	.434	---	---	---	---	---	---	.533	---	---	---	---	---	---	---	---	---
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	.431	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DK Pond	---	---	---	-.463	-.429	---	---	.427	---	-.441	---	.523	---	---	.648	.805	---	.744
DK Spring	---	---	.630	---	---	---	.438	---	---	---	---	-.401	-.611	-.586	---	-.497	---	-.684
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DK Pond	---	.471	-.425	---	---	---	---	---	---	-.381	---	---	---	---	---	---	---	---
DK Spring	---	---	---	---	---	---	---	---	---	---	.402	---	---	---	---	---	---	---
Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	-.460	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	---	.658	---	---	.480	---
DK Pond	---	.541	---	---	---	---	---	---	---	---	---	---	---	---	-.363	---	---	---
DK Spring	---	.400	.511	---	---	---	---	---	---	---	---	---	-.608	-.571	---	---	---	-.364

Table 3-18. Partial correlation analyses of microbial and macrofaunal species richness in the water column and sediment to hydrological and geochemical data described in Chapter Two controlling for collection site without Fall and Winter 2012 collections. All values shown are statistically significant (p<0.025).

Water Column Peaks											---							
Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	0.530	---	---	---	---	---	---	---	---	---	0.489	---	0.571	0.768	0.564
Estuary	0.496	---	-0.423	---	-0.445	---	---	---	---	---	0.701	0.868	-0.374	---	0.868	0.637	---	---
DK Pond	-0.406	---	---	---	---	---	---	---	0.621	---	---	---	---	0.604	0.385	0.523	0.660	0.754
DK Spring	---	---	0.506	-0.759	---	---	---	-0.436	---	---	0.585	-0.709	---	---	-0.657	-0.868	---	-0.548
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	---	---	0.371	---	---	---	0.459	---	---	-0.564	0.408	---	-0.514	---
Estuary	-0.813	---	---	-0.657	---	---	---	0.368	---	---	---	-0.593	---	0.404	---	---	0.640	0.578
DK Pond	-0.429	---	-0.537	---	---	---	---	---	---	-0.382	---	---	---	---	0.509	0.653	---	0.774
DK Spring	---	---	0.743	-0.421	---	-0.381	---	-0.553	---	---	0.653	-0.525	-0.441	-0.529	-0.764	-0.824	---	-0.539
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	-0.389	---	---	---	---	---	---	0.490	---	-0.736	---	---	-0.653	---
Estuary	0.613	---	0.455	0.783	---	-0.508	---	---	---	---	---	---	---	---	---	---	-0.468	---
DK Pond	-0.252	---	---	0.734	---	---	-0.390	---	---	---	---	---	---	-0.628	---	---	-0.549	---
DK Spring	---	---	---	---	-0.376	---	---	---	---	0.405	---	0.542	---	---	---	---	-0.359	-0.369
Sediment Peaks	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Archaea	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	-.449	---	---	.370	---	---	.478	---	.471	---	---	---	---	-.639	---	---	-.598	---
Estuary	---	---	.557	.394	---	-.580	---	---	.395	.430	---	---	---	---	---	-.435	-.578	---
DK Pond	-.656	.486	-.501	.443	---	---	---	---	---	---	---	---	---	---	---	---	---	.523
DK Spring	-.495	---	---	---	---	.593	---	---	.395	---	---	---	-.374	-.460	---	---	-.792	---
Bacteria	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	-.440	---	.452	---	.404	.375	.464	---	---	.442	---	-.621	---	---	-.599	-.749	-.386	-.490
Estuary	---	---	.585	.421	.401	---	---	---	.763	.603	.371	---	-.530	---	---	-.536	-.642	-.490
DK Pond	---	---	.443	.669	---	---	---	---	---	.461	---	---	---	-.385	---	-.594	-.729	-.618
DK Spring	-.670	---	---	-.454	.374	---	---	-.452	---	---	---	---	---	---	-.436	---	---	---
Eukaryota	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	-.445	---	---	---	---	---	---	---	---	---	---	-.408	---	---	---	---
Estuary	-.382	---	-.676	-.721	---	.443	---	---	---	-.419	---	---	---	---	.402	.657	.685	.562
DK Pond	---	---	-.407	-.534	---	---	---	---	.515	---	---	---	---	.667	---	---	.851	.600
DK Spring	.502	---	---	---	---	-.472	---	---	---	---	---	---	.388	.501	---	---	.736	.502
Macrofauna	Water Temperature (°C)	pH (units)	Salinity (psu)	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (µg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphate (mg/L)	Sulfate (mg/L)	Rainfall Week Prior (cm)	Total Rainfall Between collections (cm)	Average Daily Discharge (L/s)	Maximum Daily Discharge (L/s)	Aquifer Level @ 63 m	Aquifer Level @ 146 m	Aquifer Level @ 160 m	Aquifer Level @ 240 m
Marine	---	---	---	---	-.362	---	---	---	---	---	---	---	---	---	---	---	---	---
Estuary	---	---	---	---	---	---	---	---	---	---	---	---	-.362	---	---	---	---	---
DK Pond	---	---	---	---	---	---	---	---	---	---	-.368	---	---	---	-.446	-.438	---	---
DK Spring	---	---	.396	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

CHAPTER FOUR: CONCLUSIONS

The major findings from this dissertation are that: 1) the primary driver of the geochemical gradients in this Karst Estuary appears to be the rate at which water discharges from the spring, 2) the primary drivers of biological change within this Karst Estuary is the volume of aquifer discharge and the geochemical gradients formed by aquifer discharge, 3) estimated abundance varies inversely and species richness varies directly to aquifer discharge, and 4) community structure varied over the two year study with no observable seasonal trends but a clear delineation was observed between low and high aquifer discharge patterns noted before and after Tropical Storm Debby.

Double Keyhole Spring is located in a transition zone between an inland nearshore freshwater spring (Isabella Spring) and an offshore inactive spring (Jewfish Sink) (Fig. 1-2). The spring discharges brackish water directly into the Gulf of Mexico forming a Karst Estuary which represents a previously undescribed type of ecosystem that links the nearshore Gulf of Mexico to the Floridan aquifer. This type of estuary differs from surface estuaries in that the hydrological factors that create Karst Estuaries are driven by the fluctuating hydrology of the aquifer and not surface rivers and streams which drive surface estuaries. The original assumption was that there were two conduits (one with inland fresh groundwater, and one with offshore saltwater) that merged at some point below Double Keyhole Spring forming the brackish nature of Double Keyhole Spring discharge. Instead, I discovered that the brackish water discharging from Double Keyhole Spring originates from the mixing zone of the Upper Floridan aquifer and Gulf of Mexico below the estuary (Fig. 2-9). The increased inland aquifer head resulting from periods of heavy rainfall push downward on the mixing zone, so that more mixing zone water enters the deep conduit that feeds Double Keyhole Spring. It seems that the storm recharged the aquifer which caused a sustained increase in aquifer discharge resulting in long-term changes in the estuary communities after Tropical Storm Debby. The impacts of tidal fluctuations, rainfall, and aquifer level on submarine spring discharge can be used as a baseline for the impacts of future development in the area as well as in other Karst Estuaries around the world.

The datasonde data from Double Keyhole Spring suggests unique hydrological patterns within the system (Figs. 2-1 & 2-2). Over short time frames along the conduit path there are no statistically significant differences of collected parameters. The ‘Deep Room’ showed no geochemical differences until the halocline at a depth of 19 m. At the top of the ‘Deep Room’ is a layer of freshwater that is isotopically distinct from the freshwater found at Isabella Spring and is more similar to surface water than aquifer water suggesting that a shallow freshwater lens extends offshore under the estuary.

The composition of the Double Keyhole Spring water is most often the result of simple mixing between water from the Gulf of Mexico and the lower portions of the Upper Floridan aquifer (Fig. 2-9). The amount of each portion (Floridan Aquifer vs. Gulf of Mexico) varies seasonally and is determined by inland hydrological conditions. During the dry season, when aquifer levels fall, the mixing zone moves inland resulting in increased amounts of the salt water component from the Gulf of Mexico. I expected that storm events, such as Tropical Storm Debby, would result in a higher volume of less brackish water being discharged, but instead I measured a higher volume of brackish water of nearly the same salinity as prior to the storm. I hypothesize that the increased hydraulic head inland caused by Tropical Storm Debby pushed brackish water already in the mixing zone out through the spring within a day or two. It appears that a limited amount of freshwater was discharged into the estuary several months after Tropical Storm Debby.

Aquifer discharge through Double Keyhole Spring varies daily with tidal fluctuations and seasonally with local rainfall and aquifer levels (Figs. 2-3 & 2-4). The frequency of reversals increases as the inland water table decreases. Reversals were observed to a depth of 40 m approximately 110 m within the Double Keyhole Spring conduit from the entrance (Fig. 1-3). Future sea level rise and/or increased aquifer use may increase this distance with the potential to reach the freshwater portion of the Floridan aquifer. Should this situation arise in conjunction with a surface water contamination event such as an oil spill, the conduit would provide a direct path for contamination to enter the Upper Floridan aquifer.

Correlations of hydraulic head from the Floridan aquifer and Double Keyhole Spring discharge to the geochemical parameters in this study (Tables 2-4 to 2-7) indicate that the primary driver of the geochemical gradients seen between Double Keyhole Spring and the surrounding Karst Estuary appears to be the rate at which water discharges from the spring. These differences are due to volume of Double Keyhole Spring discharge and were observed at the marine site almost two km away. With the exceptions of ammonia and alkalinity, there were more differences between the geochemical parameters along the transect from Double Keyhole Spring through the marine site after Tropical Storm Debby than before Tropical Storm Debby with more differences at the sites farther away from the spring.

The geochemical variations observed, indicate that during periods of heavy rainfall, such as Tropical Storm Debby, diffuse SGD from the upper freshwater portion of the Upper Floridan aquifer bypass the Double Keyhole Spring conduit and discharge directly into the surrounding estuary (Figs. 2-6D & 2-7E). This water was characterized by higher total hardness and lower salinity concentrations at the estuary and Double Keyhole Pond sites than either the marine or Double Keyhole Spring sites. During the dry seasons when aquifer discharge was at a minimum, the observed gradients were less partitioned, indicating more water input from the Gulf of Mexico. This was evidenced by the lack of statistically significant differences in the geochemical parameters along the transect prior to Tropical Storm Debby (Fig. 2-7).

The correlation analyses of microbial and macrofaunal communities in the water column and sediment to hydrological and geochemical parameters collected (Tables 3-6 to 3-16) show that although there are some geochemical parameters that impact microbial abundance at various sites and times, the primary factors influencing microbial and macrofaunal communities in this Karst Estuary are the amount of aquifer discharge and geochemical gradients formed by that discharge (water temperature, pH, salinity, dissolved oxygen, and alkalinity). A major implication of this conclusion is that reduced aquifer discharge during the dry season may be detrimental to the ecology of the estuary in that the majority of increased abundance of the bacterial and microbial eukaryote communities was due to two or three species. Generally, algal blooms are the result of a dramatic increase of only a few species (for a review see Hallegraeff, 1993). The species found in this study may be a contributing factor to the development of harmful algal blooms (Anderson et al., 2002) in Karst Estuaries in Florida and around the world.

This study suggests that this Karst Estuary ecosystem was relatively stable over the two year study period. There were only a few changes of some geochemical parameters (nitrate, ammonia, phosphate, and sulfate) (Tables 3-6 to 3-16) during the study period and subsequently few significant correlations of these parameters to the studied biological communities. However, this stability could be greatly impacted by minor changes to the geochemical concentrations due to aquifer overuse and/or contamination (Sophocleous, 2005), coastal pollution (Kennish, 2002), sea level rise (Gornitz, 1991), and/or development in the area (Mallin et al., 2000).

In its current state the estuary surrounding Double Keyhole Spring is considered pristine according to the conditions set by the Florida Department of State Rule Chapter 62 - 302 (http://www.dep.state.fl.us/legal/Rules/shared/62-302/62-302.pdf). These types of spring/estuary systems appear to be common along the Florida Gulf Coast (Fig. 1-1). However, if drought, increased aquifer usage, and/or increased sea levels cause the mixing zone underneath Double Keyhole Spring to permanently move inland it could result in turning the area around Double Keyhole Spring from a Karst Estuary to a salt marsh having dramatic effects on the biological communities of the area which currently serves as a nursery for a number of invertebrate and game fish species. A model of this Karst Estuary depicting the general geochemical and hydrological patterns, and biological interactions between the different sites is shown in Figure 4-1.

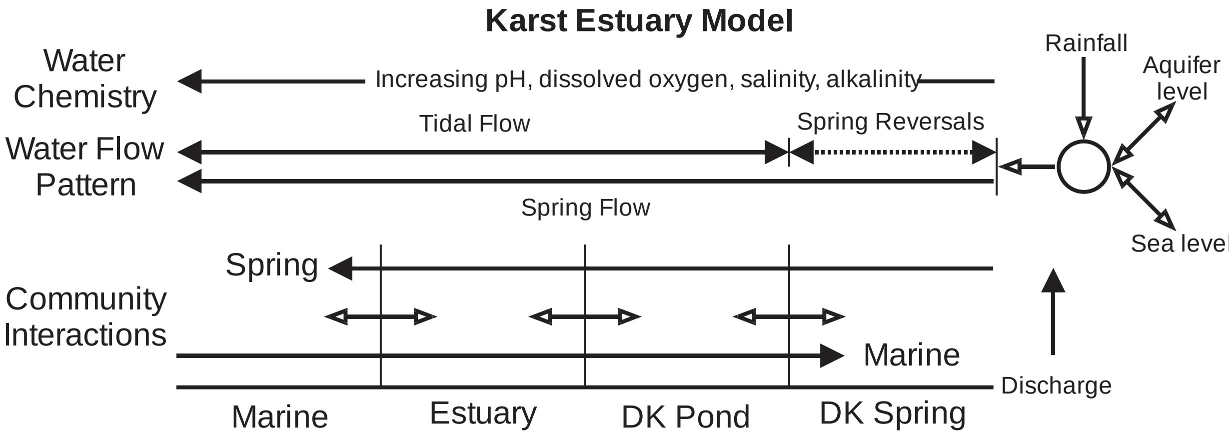


Figure 4-1. Karst Estuary model representing the general geochemical and hydrological patterns, and biological interactions between the study sites. Solid lines in the Community Interactions portion represent the extent of community similarity between sites during low aquifer discharge conditions. Dashed lines in the Community Interactions portion represent the extent of community similarity between sites during high aquifer discharge conditions.

Globally, karst covers approximately 20% of the land surface with between 20-25% of the world’s population dependent on water found within karst aquifers (Ford & Williams, 2007). In coastal regions, much of this water discharges directly to the sea through submarine springs (see Fleury et al. (2007) for a review). My study shows that submarine spring discharge forms Karst Estuaries in Florida and likely in coastal karst regions worldwide (Menning et al., 2014b). Karst Estuaries represent unique ecosystems that are controlled by inland hydrological conditions and sea level. While river-fed estuaries are well known and well documented for the many ecosystem services they provide, submarine spring fed Karst Estuaries also provide such services. In particular, the Karst Estuary of Double Keyhole Spring carries out nutrient dispersal, nutrient cycling, primary production, as well as habitat for juvenile game fish and is a sentinel of saltwater intrusion to the aquifer and sea level change.

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