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The Biogeochemistry of Submerged Coastal Karst Features in West Central Florida

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The biogeochemistry of submerged coastal karst features in West Central Florida

by

Keith Michael Garman

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Cell Biology, Microbiology and Molecular Biology
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DEDICATION

This dissertation is dedicated to my wife, Sherry. Without her tireless support and participation in hundreds of exploration and research dives, this work would not have been possible. I have been very fortunate to have her in my life and to share so many adventures with her. Many of these adventures were part of obtaining the data on which this paper was based.

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ABSTRACT

West Central Florida is a complex karst environment with numerous sinkholes, springs, and submerged cave systems. Many of these karst features are anchialine, located within the subterranean estuary where freshwater and saltwater mix. Water quality data and/or fauna data were obtained from twenty-one karst features and their associated cave systems. The anchialine karst environment of the study area has a wide range of habitats with measured salinities ranging from freshwater at <0.2 ppt to sulfidic, hypersaline water at 38.5 ppt and measured pH readings ranging from 6.39 in water impacted by sulfur oxidizing bacteria to 10.3 in an isolated room of a cave. Stygobitic crustaceans were identified in conduits extending beneath the Gulf of Mexico supporting the hypotheses that freshwater crustaceans could survive higher sea levels in freshwater conduits beneath saltwater. The fauna associated with the anchialine cave systems included Sabellidae and Polychaeta worms, hydroids, cnidarians and hydrobiid snails. Jewfish Sink, like other anaerobic marine basins that were submarine springs, has four zones: oxic zone, transition zone, upper anoxic zone and anoxic bottom water. The upper zones have seasonal water quality variations from winter cooling and sinking of surface water and changes in the microbial communities. Activity of sulfate reducing bacteria is carbon limited in the anoxic zones, where sulfate reduction is the major metabolic

process, and primary production is phosphate limited in the oxic zones. Organic input from the Gulf of Mexico drives the bacterial anaerobic ecosystem, resulting in a “sulfide pump”, in which sulfide percolates upward removing oxygen from the overlying sediment.

PREFACE

This paper discusses the biogeochemistry of submerged coastal karst features in West Central Florida. These karst features include cave systems, anchialine cave systems, and anoxic marine basins. Each of these features has a unique set of environmental conditions. Anchialine cave systems are characterized by the presence of saltwater or brackish water, a halocline that forms between waters of different salinities, and subterranean connections to a saltwater body (Iliffe 1992). Such mixing zones between salt water and freshwater in cave systems and other groundwater circulation systems have been referred to as the subterranean estuary by Moore (1999). Similar features are common to karst and non-karst terranes in many areas of the world.

Along Florida's Gulf of Mexico coastline, some cave systems discharge water as submarine springs. Over the last 50 years, many submarine springs have ceased flowing, creating anoxic marine basins at the former spring locations. In this paper, the diversity of flora and fauna in an anoxic marine basin, which was previously a submarine spring, is examined using molecular techniques. The composition of the shallow marine benthic macrofauna communities in the Gulf of Mexico at an anoxic marine basin are also compared to the community at an active submarine spring to evaluate environmental impacts from the cessation spring flow.

Cave Systems

Cave systems are generally considered aphotic, oligotrophic ecosystems that are isolated from interaction with the atmosphere and surface ecosystems (Parzefall 1992; Pohlman et al. 1997). Despite these conditions, cave systems support complex microbial ecosystems (Caumartin 1963). The development of microbial ecosystems within cave systems is influenced by the relatively stable environment including constant temperature (Caumartin 1963; Pohlman et al. 1997). Beyond the entrance where surface conditions can influence cave conditions, cave systems show minimal daily, seasonal, and annual changes compared to surface conditions at the same location (Parzefall 1992).

Some cave ecosystems are influenced by chemosynthesis or chemolithoautotrophic activity (Sarbu and Popa 1992; Sarbu et al. 1994a; Pohlman et al. 1997). The evidence for chemosynthesis in cave systems includes differences in $\delta^{13}\text{C}$ signatures between surface samples and cave samples, the isolation of sulfur oxidizing bacteria from cave systems, the presence of RuBisCO activity in cave systems, and carbon fixation in the cave systems (Sarbu and Popa 1992; Sarbu et al. 1994a; Vlasceanu et al. 1997; Mattison et al. 1998). Pohlman (1997) suggested that nitrification was the major source of chemosynthesis based on the presence of a nitrate maximum and oxygen minimum at the pycnocline in profiles of anchialine cave water in Yucatan, Mexico. In Florida, groundwater in the caves of many spring systems has elevated nitrate levels, which is believed to be the result of anthropogenic activities in the recharge area, and not the result of in situ nitrification (Jones and Upchurch 1993).

The contribution of sulfur-oxidizing bacteria to a cave ecosystem has been documented in Movile Cave in the karst terrane of Southern Dobrogea, Romania, where

several sulfide-oxidizing chemolithoautotrophs were isolated from microbial mats in the cave system. The mats support a rich and diverse invertebrate community that includes an abundance of predators (Sarbu et al. 1994a; Sarbu et al. 1994b; Sarbu et al. 1996). Sulfur oxidizing bacteria are also known to be present in submerged cave systems in Florida (Brigmon et al. 1994).

Anchialine Cave Systems

Anchialine cave systems are typically characterized by marine and freshwater ecosystem influences. Anchialine cave systems formed in limestone are found along the Gulf of Mexico coast of Florida, Bermuda, Cuba, the islands of the West Indies including the Bahamas, the Yucatan Peninsula, and Central America including Belize (Ilfie 1992). The marine waters of anchialine cave systems often contain relict fauna that were once widespread and are now cave-limited and endemic to specific cave system(s) (Ilfie et al. 1984). Anchialine cave systems share the same stable, aphotic, oligotrophic, conditions that are characteristic of other submerged cave systems. Anchialine cave systems are also becoming recognized as habitats in which primary production by chemolithoautotrophs can be a significant energy source for heterotrophic members of the ecosystem (Pohlman et al. 1997; Mattison et al. 1998).

Anoxic Marine Basins

Sulfur Cycling in Anoxic Marine Basins

Marine basins that have minimal exchange with oxygenated waters become anaerobic from the decay organic material. Once the oxygen has been depleted, anaerobic sulfate-reducing bacteria become the primary oxidizers of organic carbon using sulfate as an electron acceptor. These bacteria produce hydrogen sulfide, alkalinity in the form of

bicarbonate, ammonia, hydroxide, and reactive phosphorous as significant byproducts (Kempe and Degens 1985; Kempe and Kazmierczak 1994).

The sulfide produced by sulfate reduction readily reacts with reduced metals to form insoluble metal sulfide compounds such as the iron-sulfide pyrite. When sulfide reacts with metals, escapes the water column, or is converted to elemental sulfur by anoxic photoautotrophic sulfide bacteria, the water column remains basic favoring the precipitation of calcium carbonate (Castanier et al. 1999). The sulfide can also support communities of chemolithoautotrophic sulfur-oxidizing bacteria at the chemocline where oxygen is present and in zones where nitrate is present (Moon et al. 2010). The end product of the bacterial mediated sulfide oxidation is often sulfuric acid, which reduces the pH creating conditions in which calcium carbonate precipitation does not occur (Castanier et al. 1999). By these processes, the composition of microbial communities and the reactions of sulfide in anoxic marine basins are indicated by pH and by the presence or absence of calcium carbonate precipitation.

In anoxic marine basins, sulfide is produced by the oxidation of organic carbon by sulfur reducing bacteria using sulfate as an electron acceptor. The sulfide fluxes are directly related to organic carbon input, which is typically from particulate organic matter (POM) contributed from phytoplankton blooms in marine systems (Monteiro and Roychoudhury 2005). In some basins, the sulfide fluxes are seasonal, as summer eutrophication leads to increases in POM and the resulting anoxic, sulfidic conditions in bottom waters. The anoxic, sulfidic bottom waters in turn have an adverse affect on benthic communities (Diaz and Rosenberg 1995; Gray et al. 2002; Wu 2002; Karim et al. 2002).

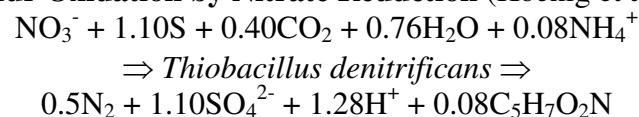
In the Black Sea, variations in the depth of the anoxic zone are related to the flux of sinking POM into the anoxic zone and the corresponding upward flux of sulfide from the anoxic zone (Konovalov et al. 2001; Glazer et al. 2006). Off the west coast of South Africa at times of increased POM fluxes to the anoxic zone, corresponding increases in sulfide production have resulted in oxygen depletion in shallow coastal waters causing mortality events for lobsters, fish, and mussels (Chapman and Shannon 1985; Cockcroft et al. 2000). In Ise Bay and Osaka Bay, Japan, similar anoxic bottom water conditions from increased summer POM fluxes and the resulting sulfide production have reduced the number of benthic macrofauna species (Yamochi and Oda 2002; Ganmanee et al. 2004).

The anoxic marine basins in West Central Florida included in this study are sinkholes with relatively small openings compared to their depths and bottom diameters. As a result, when there are increased organic carbon inputs to the basins, there is potential for the flux of sulfide upward from the anoxic zone through the limestone to overlying oxic zones. This sulfide flux from the anoxic zone to the overlying oxic zone is referred to as the sulfide pump.

Nitrogen Cycling in Anoxic Marine Basins

Below the chemocline, where oxygen is not available, a community of facultative and obligate anaerobic sulfur-oxidizing bacteria such as *Thiobacillus denitrificans* that use nitrate as an electron receptor may be present. This nitrate reaction creates sulfuric acid from the oxidation of sulfide and nitrogen gas from the reduction of nitrate (see Equation P-1).

Equation P-1: Sulfur Oxidation by Nitrate Reduction (Koenig et al. 2005)



Sulfur-oxidizing bacteria that actively use nitrate as an electron acceptor are not found in abundance at the chemocline because the sulfide oxidation reaction that uses oxygen as an electron acceptor generates more energy than the corresponding reaction that uses nitrate. Therefore, bacteria using nitrate to reduce sulfide are found in abundance only below the chemocline where oxygen is absent (Focht and Verstraete 1977; Thauer et al. 1977).

Although nitrate is typically present in minor concentrations in seawater, it can be produced in larger amounts within anaerobic marine basins. In these basins, the source of nitrate is assumed to be nitrification of ammonium ions released from the oxidation of organic material below the chemocline by sulfate reduction. Nitrification is an aerobic process predominately performed by chemolithoautotrophic nitrifying bacteria (reviewed in Atlas and Bartha 1998). Therefore, in anoxic marine basins, nitrate is produced at or above the chemocline where oxygen is present. In the presence of oxygen, ammonium and nitrite are used as electron sources, ultimately fueling the reduction bicarbonate to organic carbon. Nitrate is typically at its maximum concentration at or above the chemocline where it is produced (Oguz et al. 2000). As nitrate diffuses downward away from the chemocline, it becomes available to the nitrate-reducing, sulfur-oxidizing bacteria community.

Examples of Anoxic Marine Basins

Chemical gradients and the cycling of elements between oxic surface water and anoxic bottom water in stratified, anoxic marine basins have been studied in the Black Sea, crater lakes, Bahamian blue holes, and deeper cenotes in the Yucatan State of Mexico.

Black Sea

The Black Sea is the largest anoxic basin in the world with a total sulfide pool of about 4.6×10^3 Tg. This sulfide is oxidized most actively at the chemocline (Neretin et al. 2001) where chemolithoautotrophic sulfur-oxidizing bacteria have been identified (Jannasch et al. 1991). A nitrate maximum has also been reported at the chemocline (Jorgensen et al. 1991). Oguz et al. (2000) linked anaerobic sulfide oxidation to nitrate consumption below the chemocline.

Crater Lakes

Satonda Crater Lake in Indonesia evolved from a freshwater lake to the present day slightly alkaline, anaerobic, marine basin with a chemocline at 23 m. Today, the main difference between seawater and Satonda Crater Lake is increased alkalinity, depletion of alkaline earth ions versus seawater, and decreasing pH with depth. Data from the lake also show a nitrate peak in the oxic zone above the chemocline (Kempe and Kazmierczak 1993).

At Kauhako Crater Lake on the Kalaupapa Peninsula in Molokai, Hawaii, Donachie et al. (1999) reported a distinct chemocline at 4.5 m with a nitrate peak at the chemocline and significantly increased phosphorous and ammonia concentrations below the chemocline.

Exploration dives in Kauhako Crater Lake revealed a dark gray biofilm that forms fingers about 20 cm long coating the walls of the crater in the anoxic zone below the chemocline (Figure P-1). This biofilm is visually similar to biofilms on the walls of anoxic zones in West Central Florida karst features including anoxic marine basins. Between the biofilm and the volcanic rock in which the crater lake formed, there is a

coating of calcium carbonate (Figure P-2). Calcium carbonate is a common deposit in anoxic marine basins as sulfate reduction leads to increased alkalinity and pH creating favorable conditions for calcium carbonate precipitation (Castanier et al. 1999).

Blue Holes and Cenotés

Bottrell et al. (1991) studied Bahamian blue holes in which an oxic freshwater lens overlies anoxic saline water. They documented bacterially mediated sulfate reduction, which produces sulfide in the anoxic saline zone. They also identified bacterially mediated sulfide oxidation at the chemocline, which reduces pH and may enhance dissolution of the carbonate rock. Similar processes were also documented in cenotés of the Yucatan Peninsula (Stoessell et al. 1993; Schmitter-Soto et al. 2002).



Figure P-1: Example of biofilm on walls of Kauhako Crater Lake below the chemocline. Biofilm fingers are about 20 cm long. Photos were taken at depths of 60 to 90 m.

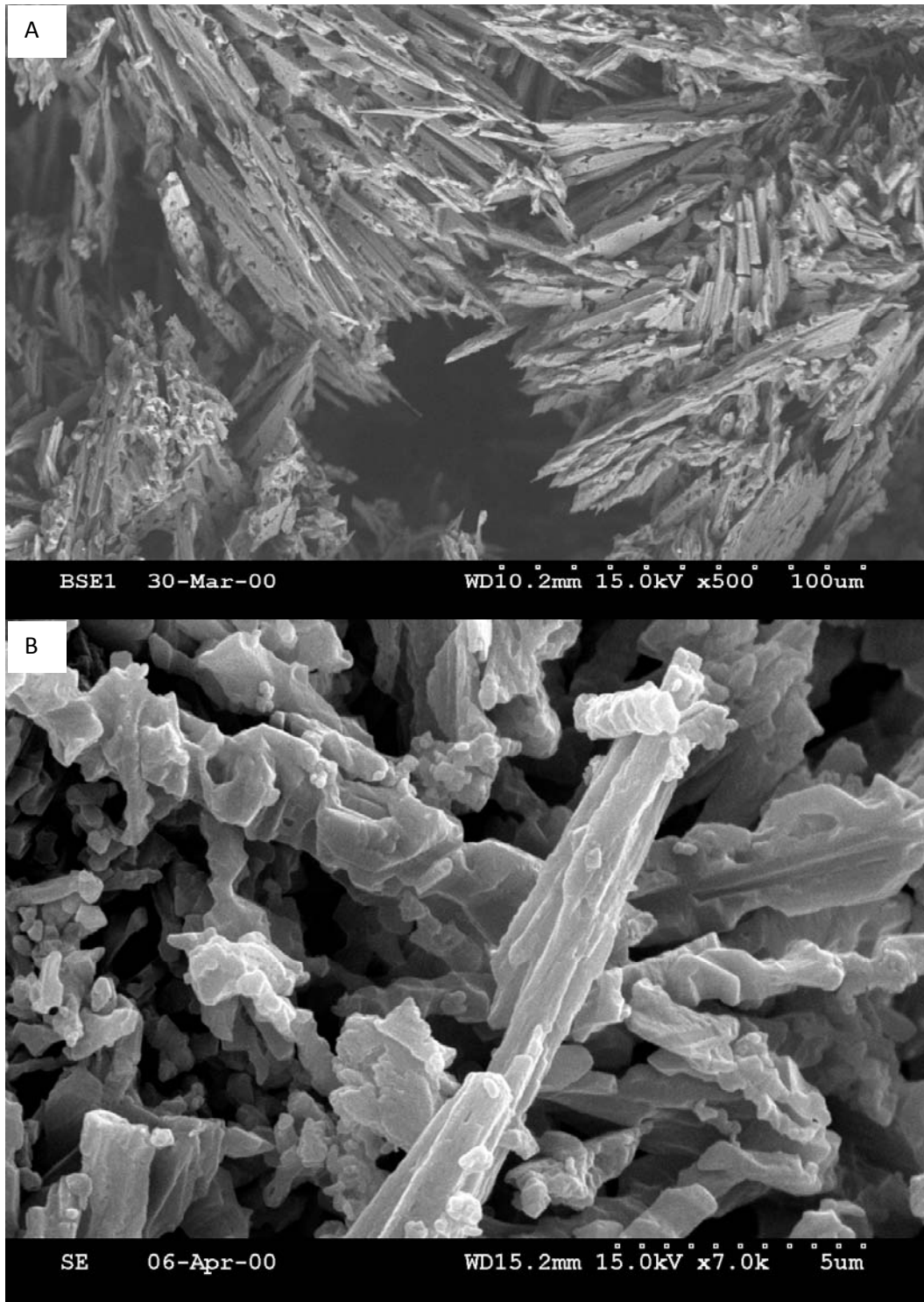


Figure P-2: SEM photographs of calcium carbonate precipitate deposited on walls of Kauhako Crater Lake between the biofilm and volcanic rock wall at depths of 40 m (A) and 24 m (B).

Benthic Macrofauna in the Gulf of Mexico

Engle and Summers (2000) examined benthic macroinvertebrates from 870 estuarine sites along the Gulf of Mexico and western Atlantic coasts of the United States to evaluate fauna provinces. The West Central Florida study area of this paper was included in the Florida West Coast province. The top ten species contributing to the dissimilarity between the Florida West Coast benthic macroinvertebrate province and the Florida Bay province to the south are shown in Table P-1.

Table P-1. Benthic Macroinvertebrate Species Contributing to Dissimilarity between Florida West Coast Province and the Province to the South.

- Polychaeta
 - *Aricidea taylori* (Pettibone 1965)
 - *Cirrophorus lyra* (Southern 1914)
 - *Exogone lourei* (Berkeley & Berkeley 1938)
 - *Fabricinuda trilobata* (Fitzhugh 1990)
 - *Lumbrineris (Scoletoma) verrilli* (Perkins 1979)
 - *Syllis (Typosyllis) lutea* (Harmann-Schroder 1960)
- Gastropoda
 - *Caecum pulchellum* (Stimpson 1851)
- Amphipoda
 - *Ampelisca abdita* (Mills 1964),
 - *Ampelisca holmesi* (Pearse 1908)
 - *Cerapus benthophilus* (Thomas & Heard 1979)

From Engle and Summers (2000).

The top ten species contributing to the dissimilarity between the Florida West Coast benthic macroinvertebrate province and the Louisianian province to the west are shown in Table P-2.

Table P-2. Benthic Macroinvertebrate Species Contributing to Dissimilarity between Florida West Coast Province and the Province to the West.

- Polychaeta
 - *F. trilobata*
 - Bivalvia
 - *Mulinia lateralis* (Say 1822)
 - *Nucula crenulata* (Adams 1856)
 - Gastropoda
 - *C. pulchellum*
 - *Caecum johnsoni* (Winkley 1908)
 - *Texadina sphinctostoma* (Abbott & Ladd 1951)
 - Amphipoda
 - *A. abdata*
 - *A. holmesi*
 - *C. benthophilus*
 - Arthropoda
 - *Balanus improvisus* (Darwin, 1854)
- From Engle and Summers (2000).

In a literature review of benthic macrofauna assemblages that included the Gulf of Mexico, Brooks et al. (2006) identified polychaetes, specifically *Prionospio cristata*, *Nephtys incisa*, *N. picta*, and *Spiophanes bombyx*, as the principal taxa common to all assemblages. A study of estuaries in the northern Gulf of Mexico by Rakocinski et al. (1997) determined that the influence of natural gradients in depth, salinity and sediment composition made evaluating the impacts of sediment pollution difficult to identify in benthic macrofauna communities. During storm events in the Gulf of Mexico, scouring of the sediment and salinity changes have been identified as the primary factors contributing to changes in diversity and abundance of benthic macrofauna (Malin et al. 1999; Engle et al. 2009). Seasonal hypoxic zones were also found to decrease benthic macrofauna abundance, diversity, richness, and evenness (Rabalais et al. 2001; Montagna and Ritter 2006).

Current Study

This dissertation is organized in three independent chapters. The first chapter presents a broad survey of numerous coastal springs and sinks located mostly in west central Florida. The second chapter focuses on the geochemistry of Jewfish Sink, an anoxic marine basin that was once an active, freshwater submarine spring located in the Gulf of Mexico. The third chapter is a detailed study of the biogeochemistry and biodiversity within Jewfish Sink and compares the biodiversity in the ecosystem around Jewfish Sink to that of Crystal Spring, an active freshwater submarine spring located to the south of Jewfish Sink.

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CHAPTER ONE: FAUNA AND WATER QUALITY OF HABITATS IN THE SUBMERGED KARST FEATURES OF WEST CENTRAL FLORIDA

Abstract

West Central Florida is a complex karst hydrogeologic environment with numerous sinkholes, springs, and submerged cave systems. Water quality and/or cave fauna data were obtained from twenty-one karst features in West Central Florida. Much of the data were from submerged cave systems beyond the light zone in areas of limited previous studies. Away from the coast, these karst features are filled with freshwater and are part of the Floridan Aquifer. Along the coast, many of these karst features are anchialine within the zone in which freshwater and saltwater mix. The saltwater mixing with the Floridan Aquifer water includes saltwater intrusion from the Gulf of Mexico and geothermal circulation.

The known range and locations of the stygobitic fauna in the Gulf Coastal Lowlands assemblage of the Ocala fauna group was expanded and a new species of stygobitic crayfish from Pinellas may identify a new assemblage. Stygobitic crustaceans were also identified in brackish water environments including submerged cave conduits beneath the Gulf of Mexico. In anchialine cave conduits, the fauna included Sabellidae and Polychaeta worms, hydroids, cnidarians and hydrobiid snails. The anchialine cave systems contain variable salinity niches from near fresh brackish water to saltwater with salinities greater than those in the Gulf of Mexico. The diverse environmental niches

provide opportunities for isolation and the development of new species, as indicated by the identification of a new species of Sabellidae.

The biogeochemistry of anoxic zones are strongly influenced by the respiration of organic matter by sulfur reducing bacteria and the subsequent oxidation of sulfide by sulfur oxidizing bacteria, which produces acidic conditions. Biofilms and microbial consortia are abundant in many of the cave systems, especially in anoxic zones.

Introduction

West Central Florida is a complex karst hydrogeologic environment with numerous sinkholes, springs, and submerged cave systems. Many of these karst features are anchialine, located within the subterranean estuary where freshwater and saltwater mix. Other features are located in the productive zone of the Floridan Aquifer, the primary source of potable water in the region. The hydrology of these karst systems is poorly understood and new karst features are found in the region every year (Florea 2006). Wilson (1994) estimates that there are at least 21 km of hydraulically active underwater cave passages per 2.6 km² of land surface area based on ground penetrating radar studies and the examination of cavities encountered in boreholes (Stewart 1966; Lichtler et al. 1968). Few water quality or biological surveys have been performed beneath the surface waters of these features. This report presents a survey of selected sinkholes, springs, and submerged cave systems in West Central Florida for which water quality and biological data were obtained from beneath the surface pool. These data provide a general background of the nature and complexity of these karst systems, which contain environmental niches of varying salinity, pH, and oxygen availability, and of the flora and fauna that inhabit them.

General Hydrogeology of West Central Florida

West Central Florida is a karst terrane in which groundwater movements in the carbonate rock are controlled by secondary porosity features such as caves. The groundwater system in West Central Florida consists of two major aquifers typically separated by a confining layer of Miocene clays. The surficial aquifer consists of unconsolidated sediments that lie above Miocene and older limestones, whereas the Floridan Aquifer consists of limestones of Miocene age and older. The clays in the confining layer are from clastic deposits carried to Florida by rivers originating in the Appalachian Mountains during the Miocene Epoch (Heath and Smith 1954; Wetterhall 1964; Rosenau et al. 1977).

The main source of freshwater in West Central Florida is the Floridan Aquifer. The top of the Floridan Aquifer is defined by confining clay units of the Hawthorn Group of Miocene age (Hickey 1982; Ryder 1985). This confined aquifer consists, in descending order, of the Tampa Member (Limestone) of the Arcadia Formation of Miocene age, the Suwannee Limestone of Oligocene age, and the Ocala Group and Avon Park Formation of Eocene age. The base of the aquifer is defined as the first occurrence of vertically persistent evaporites at a depth of about 183 m National Geodetic Vertical Datum (NGVD). These marine carbonate deposits comprise one of the most productive potable aquifer systems in the world (Stringfield 1936; Parker et al. 1955; Swarzenski et al. 2001). The Suwannee Limestone is the source of most of the springs and potable water used in West Central Florida (Wetterhall 1964; Yobbi 1992). The Floridan Aquifer contains groundwater under sufficient pressure to flow out at the land surface and creates

springs where there are discontinuities in the confining beds (Rosenau et al. 1977). The overlying confining unit is absent in many areas along the coast (Yobbi 1992).

Recharge to the Floridan Aquifer occurs inland by infiltration through surficial deposits where confining units are thin or absent, and through sinkholes and karst windows that breach the confining units. Recharge to the Upper Floridan Aquifer has been estimated at 25 to 76 cm year⁻¹ (Yobbi 1989). A surficial aquifer occurs above the Floridan Aquifer (Fretwell 1983). The undifferentiated sands and clays that compose this aquifer are highly variable in composition laterally and vertically. As a result, the sands forming the permeable portion of this aquifer are discontinuous and clayey, making its extent limited (Fretwell 1983; Knochenmus and Thompson 1991; Yobbi 1992). Rivers generally are absent in the study area except where major springs discharge and where areas of impermeable strata overlie the Floridan Aquifer (Beck et al. 1985; Yobbi 1992; Wilson 1996).

The near offshore area of the Gulf of Mexico in the study area is a shallow (less than 6m average depth) karst platform characterized by limestone outcrops, sinkholes, anchialine cave systems, and a few submarine springs (Yobbi 1992). During a period of lower sea level 10 to 12 thousand years ago, the west Florida coastline was located about 160 km west of the present shore. At that time, karst features formed on the extended land surface (Beck 1986). Some karst features may have formed as submarine features from groundwater circulation and the mixing of fresh and saline waters within the continental margin (Land et al. 1995). The number of karst features observed today decreases with increasing distance from shore (Brooks 1973).

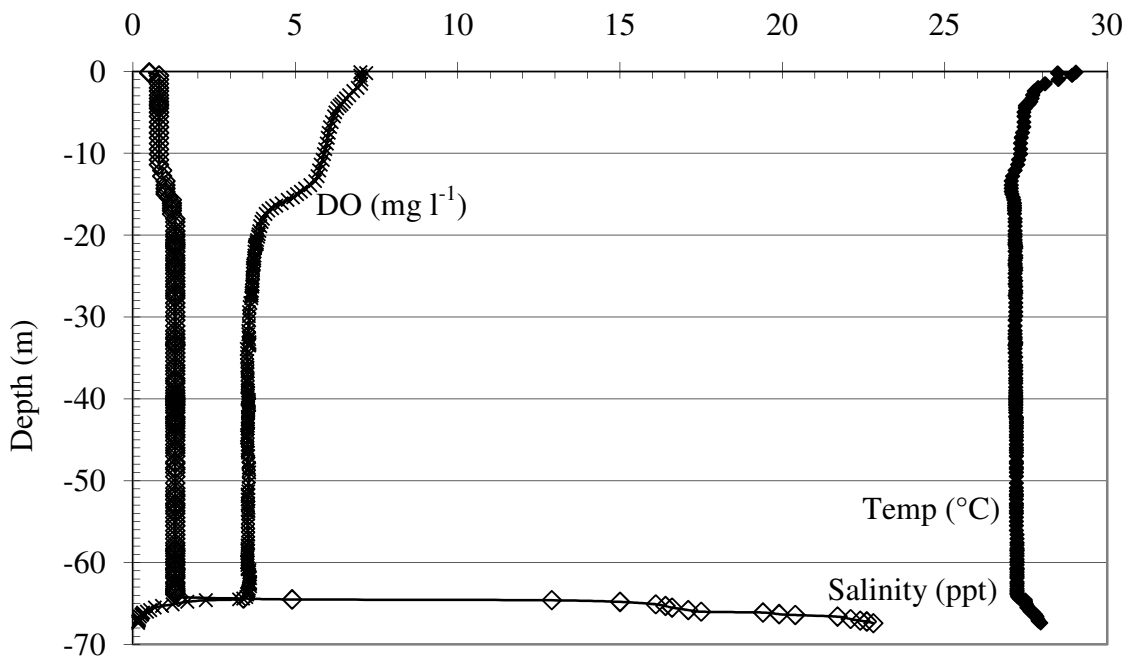
Submarine Groundwater Discharge

Along the Gulf of Mexico coast of West Central Florida, the confining units over the Floridan Aquifer thin making the Aquifer unconfined. Like other unconfined coastal aquifers, the Floridan Aquifer in West Central Florida is density stratified with a freshwater lens floating on saline water with a chemocline in between where the two waters mix. The conventional model of groundwater circulation at the coast involves the entrainment of saltwater in the base of the westward flowing freshwater lens with compensatory inland flow of saline water at depth (Henry 1964). This conventional type of flow model assumes a homogeneous aquifer, but karst aquifers like the Floridan are highly heterogeneous with porosity features spanning five orders of magnitude from intergranular spaces to large caves (Quinlan et al. 1996; Worthington et al. 2000; Beddows et al. 2007). The presence of large caves creates turbulent flow conditions in which density dependent flow structures similar to those observed in open channel flow are created and the thickness of the freshwater lens is reduced near the coast compared to predictions based on conventional models (Wilson 1989).

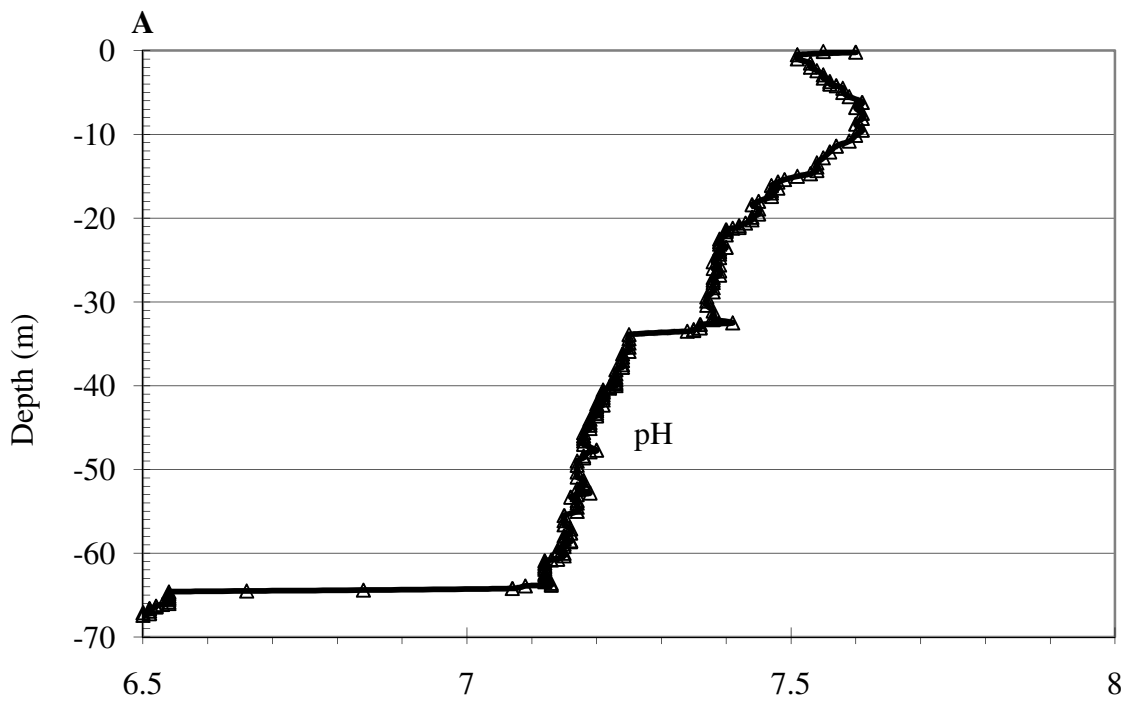
Because of the influence of turbulent conduit flow, groundwater movement in coastal Florida aquifers can be rapid, exceeding laminar flow rates in areas of primary porosity by several orders of magnitude. Studies in the Florida Keys have shown rapid movement of tracers from on-shore sewage systems and injection wells to offshore sampling stations with some rates exceeding 100 m hr^{-1} in a karst limestone, demonstrating that secondary porosity features can dramatically affect the transport of nutrients and contaminants in the subterranean estuary (Paul et al 1995; 1997; 2000).

In some Florida springs and submerged anchialine cave systems, there is evidence of geothermal circulation of heated, anoxic, mineralized water. At Warm Mineral Springs in Sarasota County, Florida, geothermally heated water reaches the surface with very little mixing (Kohout et al. 1977; Rupert 1994). The main source vent in the spring basin at a depth of 63 m discharges anoxic, sulfidic water with an average temperature of 30 °C, sulfate concentrations of 1,600 to 1,700 mg l⁻¹, and chloride concentrations of 9,200 to 9,600 mg l⁻¹ (Rosenau et al. 1977; Rupert 1994). This water is hypothesized to be seawater that seeps beneath the Floridan Plateau where it is heated at depths of about 1 km before migrating upward through the aquifer (Kohout et al. 1977). The Floridan Plateau is a predominately carbonate platform that includes peninsular Florida and the surrounding continental shelf, which extends to a depth of 91 m NGVD about 160 to 240 km from Florida's west coast (Beck et al. 1985).

The Yucatan Peninsula, where it meets the Caribbean Sea, is a karst environment similar to the West Central Florida coast at the Gulf of Mexico. Studies of the Yucatan Peninsula show the presence of a density stratified aquifer with cooler freshwater overlying warmer saline water (Beddows et al. 2007). These density-stratified aquifer profiles are also found in the Floridan Aquifer in West Central Florida. In areas of conduit flow in the Yucatan, turbulent mixing between fresh and saline waters occurs because of differential movement within 4 km of the coast. Farther inland the mixing zone lies below the floor of cave conduits accessible to divers (Beddows et al. 2007). At one unusually deep interior cenoté (a karst window over 150 m deep), Sabak-Ha in Sacalum in the Yucatan, the chemocline between fresh and saline water has been observed at a depth of 64 m, where a cave system has also formed (Figure 1-1).



A



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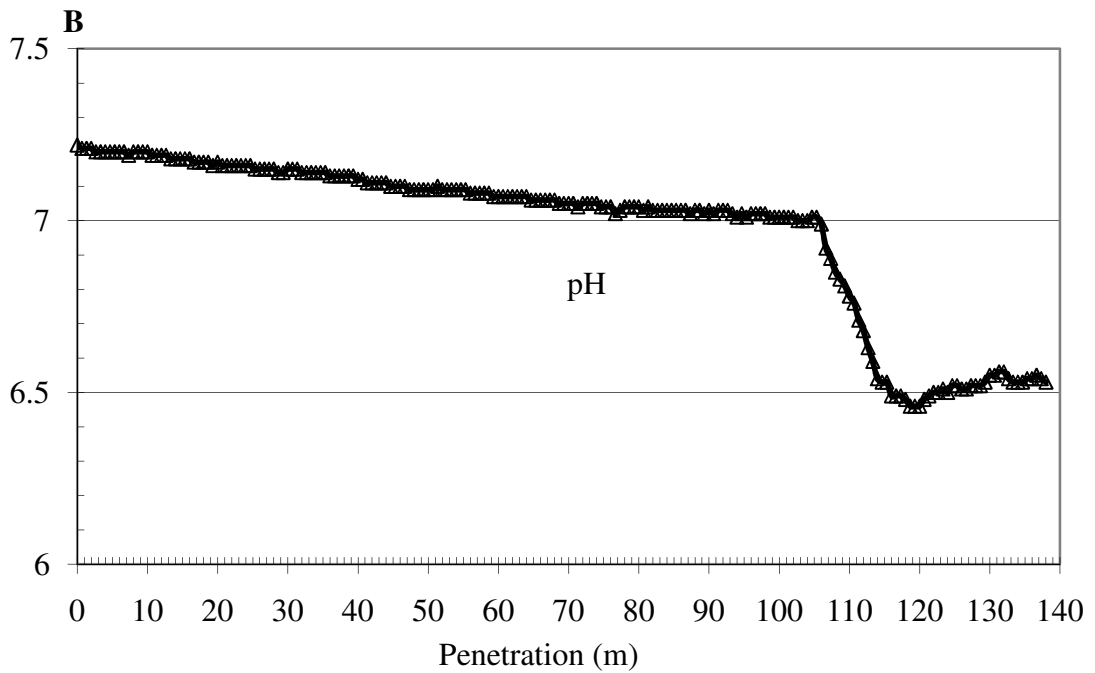
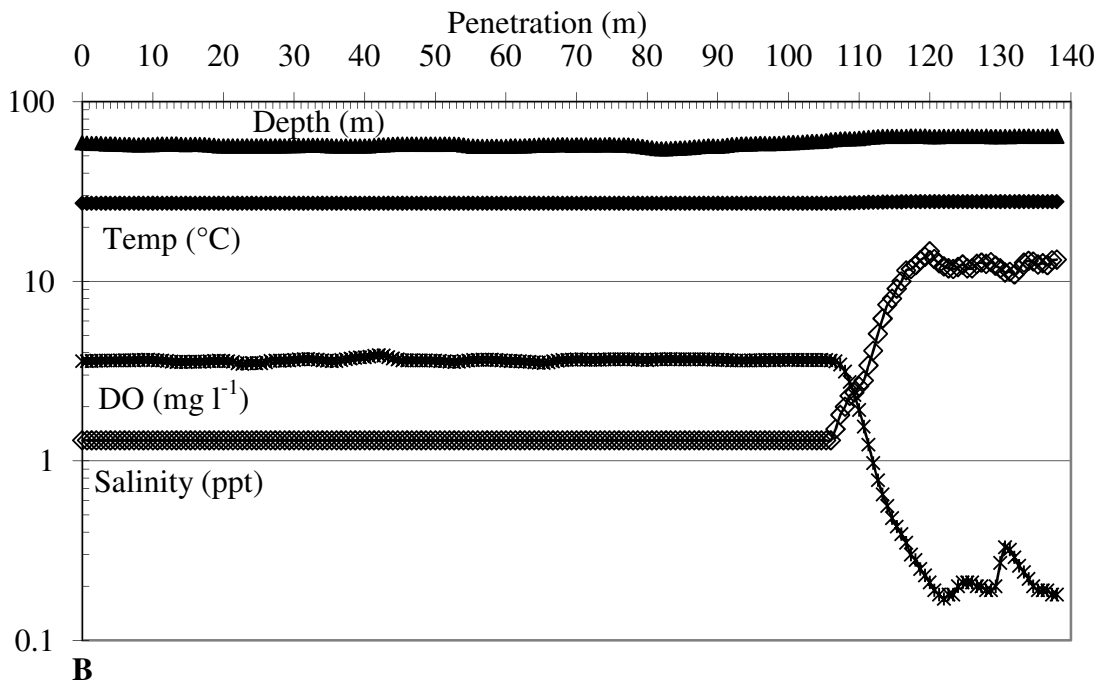
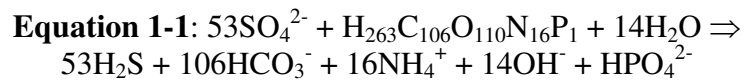


Figure 1-1: Cenoté Sabak-Ha Water Quality Profiles March 18, 2003.

A) Open water profile in sinkhole. B) Cave system profile. Penetration refers to the distance traveled by a diver in the cave system from the cave entrance, which is the distance from a possible ascent to the surface.

Anoxic Marine Basins

Marine basins with minimal flow and minimal exchange with oxygenated waters become anaerobic as the decay of the organic material by aerobic respiration quickly uses the available oxygen. Once the oxygen has been depleted, anaerobic sulfate-reducing bacteria begin metabolizing organic debris by anaerobic respiration. These bacteria use sulfate that is abundant in seawater as an electron acceptor and produce hydrogen sulfide, alkalinity in the form of bicarbonate, ammonia, hydroxide, and reactive phosphorous as significant byproducts (Kempe and Degens 1985; Kempe and Kazmierczak 1994) Equation 1-1 shows the oxidation of a Redfield ratio organic compound by sulfate reduction.



By these reactions, anoxic marine basins are characterized by a prominent sulfur cycle. Today, most of the sinkholes and karst windows located offshore of West Central Florida in the Gulf of Mexico are anoxic marine basins. Increased water demands by development from 1965 to 1990 have seen groundwater withdrawals in one three-county area of West Central Florida increase from 227 to 852 million liters per day from 1965 to 1990 (Marella 1995). As a result, near shore springs have become anoxic marine basins (Garman and Garey 2005).

The sulfide produced by sulfate reduction readily reacts with reduced metals to form insoluble metal sulfide compounds such as the iron-sulfide mineral pyrite. The sulfide can also support communities of chemolithotrophic sulfur-oxidizing bacteria at the chemocline where oxygen is present. The end product of the bacterial mediated

sulfide oxidation is sulfuric acid, which reduces the pH creating acidic conditions (Castanier et al. 1999).

Bottrell et al. (1991) studied Bahamian blue holes in which an oxic freshwater lens overlies anoxic saline water. They documented bacterially mediated sulfate reduction that produces sulfide in the anoxic saline zone. They also identified bacterially mediated sulfide oxidation at the chemocline that reduces pH and may enhance dissolution of the carbonate rock. Similar processes were also documented in cenotés of the Yucatan peninsula (Stoessell et al. 1993; Schmitter-Soto et al. 2002).

Water Quality of Florida Springs

Historically, the water quality of springs and sinks in West Central Florida has been characterized by the sampling of water at the spring discharge or at the surface of the sink (Ferguson et al 1947; Wetterhall 1965; Rosenau et al 1977). Information on saltwater intrusion into the aquifer has been evaluated using data collected from wells (Heath and Smith 1954; Wetterhall 1964). Little water quality information has been available from the cave systems that feed the springs and connect karst features. DeWitt (2003) obtained water quality data from below the surface in a study of submarine springs and sinks in West Central Florida. These data indicated that the submarine karst features without active freshwater flow were anoxic at the bottom.

Cave Fauna

The variety of macrofauna and diverse salinity niches in coastal anchialine cave systems may provide opportunity and isolation for marine macrofauna to evolve into new species (Iliffe 1986; Iliffe 1992; Coineau and Boutin 1992). A survey of cenotés in the Yucatan, Mexico, performed by Schmitter-Soto et al (2002) included water quality and

macrofaunal diversity data. This survey concentrated on the open water area of the cenotés and identified endemic crustaceans and fish.

The distribution of stygobitic (cave-adapted) crayfishes in Florida was discussed by Franz and Lee (1982). Most of these crayfishes are endemic to a single geologic formation and, in some cases, a single cave system. A more comprehensive survey of cave-adapted macrofaunal diversity that included underwater cave systems in Florida was performed by Franz et al. (1994). Their survey of 267 terrestrial and submerged caves in South Georgia and Florida identified troglobitic and stygobitic faunas that included 27 invertebrates and one vertebrate. (Troglobitic fauna are cave adapted terrestrial fauna and stygobitic fauna are cave-adapted aquatic fauna.) Their survey included eleven submerged caves in West Central Florida: six in Hernando County; four in Pasco County; and one in Pinellas County. In Hernando County, Eagle's Nest Sink cave system fauna included: the stygobitic crayfish *Procambarus leitheuseri* and *Troglocambarus maclanei*; and the stygobitic amphipods *Crangonyx grandimanus* and *Crangonyx hobbsi*. In Pasco County, Arch Sink cave system fauna included *P. leitheuseri* and Nexxus Sink cave system fauna included the *P. leitheuseri*, *C. grandimanus*, and *C. hobbsi*. Nexxus Sink is part of the Beacon Woods cave system that includes Round Sink. In Pinellas County, a stygobitic crayfish was observed from Knight's Sink; but no voucher was collected.

In the survey performed by Franz et al. (1994), the obligate cave fauna of the South Georgia and Florida region are divided into six cave fauna groups: Ecofina Creek; Apalachicola; Woodville; Ocala; St. Johns River; and Miami, each characterized by precinctive taxa. The Ocala group is the largest with twelve taxa and covers the largest geographic area. West Central Florida is included in the Ocala fauna group in the Gulf

Coastal Lowlands assemblage and includes: the crayfish *P. leitheuseri*, and *T. maclanei*; and the amphipods *C. grandimanus* and *C. hobbsi*. The crayfish *P. leitheuseri* is the unique species that identifies this assemblage.

Because cave systems are oligotrophic, some researchers have hypothesized that cave-adapted faunas may concentrate near openings, which are a source of allochthonous organic carbon. A study by Hale and Streever (1994) found no significant difference in the abundance of stygobitic crayfish near cave openings compared to the abundance away from cave openings in submerged cave systems in Florida.

Current Survey

For the survey of West Central Florida karst features, water quality and/or cave fauna data were obtained from twenty-one karst features. These features included:

- Three freshwater sinkholes and karst windows
 - Eagle's Nest Sink
 - Ward's Sink
 - Arch Sink
- Four transition sinkholes and karst windows
 - Jewel Sink
 - Wayne's World Sink
 - Palm Sink
 - Round Sink
- Eight coastal springs
 - Wall Spring
 - Salt Spring
 - Gar Spring
 - Cauldron Spring
 - Spring 822-241A
 - Hudson Spring
 - Sulphur Spring
 - Double Keyhole Spring
- Two submarine springs
 - Crystal Beach Spring
 - Indian Island Spring

- Four anoxic marine basins
 - Jewfish Sink
 - Horseshoe Crab Sink
 - Cedar Island Spring East
 - Cedar Island Spring West

The locations of these features are shown on Figure 1-2.

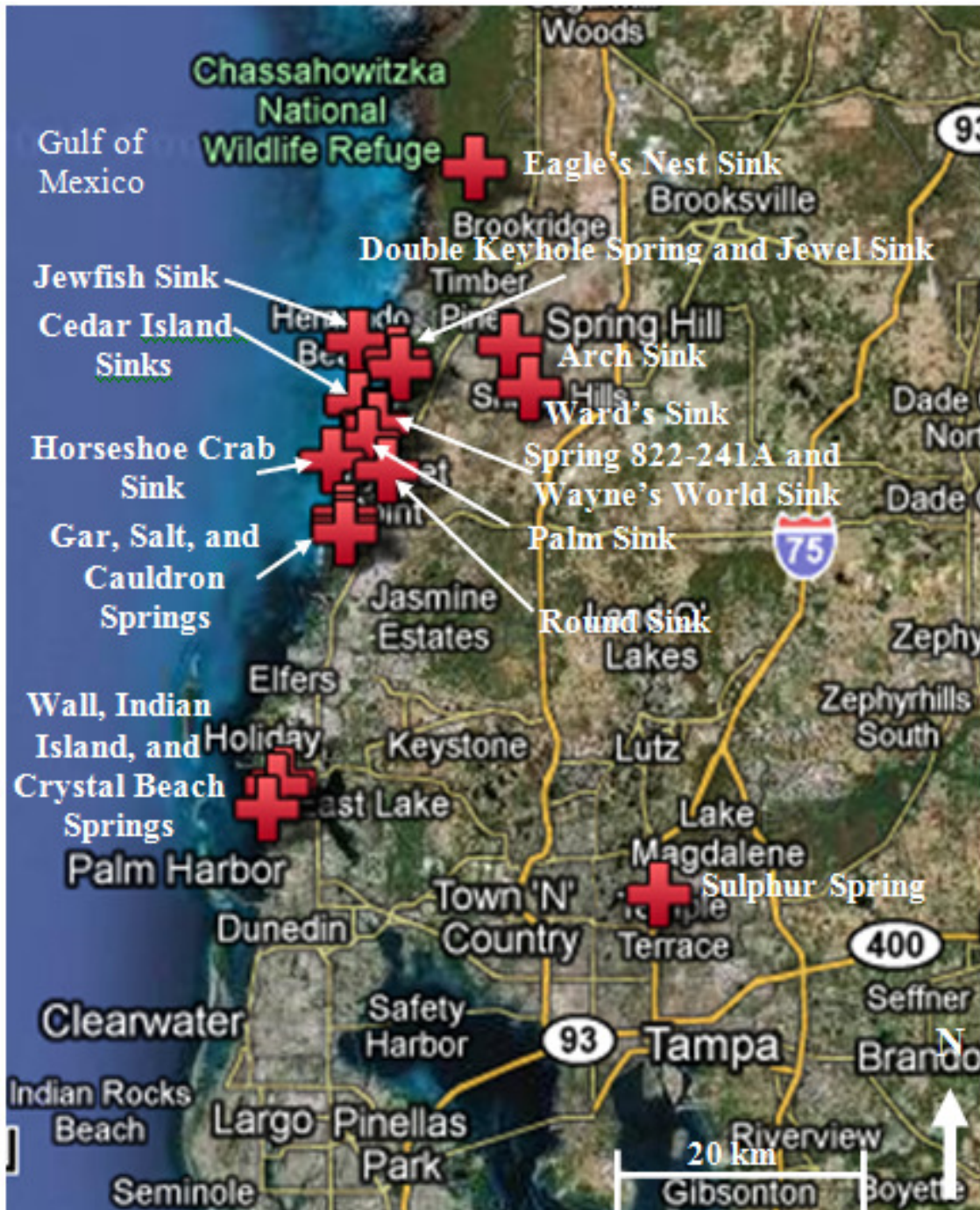


Figure 1-2: West Central Florida Karst Features.

Materials and Methods

Certified divers affiliated with the National Association for Cave Diving, the National Speleological Society Cave Diving Section, the American Association of Underwater Scientists, and the Academic Diving Program at the University of South Florida performed the research dives for this study.

Water Quality Measurements

A Datasonde (Hydrolab-Hach Company, Loveland, Colorado) was used to record temperature, pH, specific conductivity, salinity, dissolved oxygen, and depth readings during dives. The instrument was calibrated prior to each dive using standard pH solutions of 7.00 and 10.00 standard units, a specific conductivity standard of 41.62 milliSiemens per centimeter, and the water saturated air method at known temperature and atmospheric pressure for the dissolved oxygen calibration. The Datasonde was serviced and calibrated by the manufacturer on a regular basis. The diver carrying the Datasonde descended first with the Datasonde carried below the diver to prevent disturbance of the water column and interference from exhaust gases.

Water samples for on-site analyses were collected by divers using 60-milliliter (ml), capped syringes, using the method described by Brigmon et al. (1994). Water samples were analyzed for sulfide, alkalinity, ammonia, and nitrate by colorimetric methods using kits supplied by CHEMetrics (CHEMetrics, Inc. Calverton, Virginia).

Water samples for laboratory analyses were collected in containers supplied by the laboratory performing the analyses or constructed of material recommended by the laboratory performing the analyses. Containers were filled with water from the surface pool at the start of the dive. At the sampling location, the containers was purged with

analytical grade helium (99.999% pure) and then filled. Purging and filling of the containers was performed with the container held in front of and below the diver to prevent contamination by the diver's exhaust gases.

Seepage Rates and Velocity

For measuring vertical hydraulic gradient in the Dragon's Lair of Crystal Beach Spring cave system, a piezometer was constructed from two sections of 107 cm long, 1.27 cm diameter solid PVC pipe and a single section of 10 cm long, 1.27 cm diameter, slotted, PVC pipe. The piezometer sections and a submersible manometer were attached to a diver propulsion vehicle (DPV). Once at the Dragon's Lair, the piezometer sections were removed from the DPV and connected using slip couplings. The base of the slotted section was covered with an end cap. The assembled piezometer was pushed into the sediment by hand so that the top of the slotted section was approximately 214 cm below the sediment surface. The submersible manometer was then attached to the top of the piezometer. The manometer was held vertical by attaching a lead weight to the bottom and a float ball to the top. The water level difference between the two sides of the manometer was then recorded (Lee and Cherry 1978).

The velocity of the freshwater flow through the Dragon's Lair was estimated using a ruler and cotton fibers. An area of the Dragon's Lair closely resembling the typical cross section, 2 meters by 3 meters, was selected. A research diver then positioned herself against the ceiling by inflating her buoyancy compensator. Then, while holding a ruler below with her arm fully extended, pieces of neutrally buoyant cotton fiber were released upstream of the ruler. The time it took the fibers to travel the length of the ruler

was measured by videotaping the experiment. The digital video tape was played following the dive with the time codes shown.

Particulate Analyses

In the Dragon's Lair of the Crystal Beach Spring cave system, water samples were collected with 60 ml syringes for particulate analyses. Glass slides were also placed in the water column for a period of six months to evaluate biofilm formation (Allen et al. 1998). Water samples and glass slides with potential biological specimens were fixed with 5% formaldehyde. Geologic samples were filtered onto a 0.1 or 0.2 μm polycarbonate filter and bacterial samples were filtered on a 0.02 μm glass fiber filter. Filtered bacterial samples and slides were fixed in 1% osmium tetroxide in 0.1 M Sorenson's buffer solution, rinsed in deionized water, and dehydrated in an ethanol series. Following dehydration, the filters and slides were covered with hexamethyldisiazane for two hours and dried in a fume hood (Dykstra 1992; Privett 2000).

Filters and glass slides were examined in environmental mode of a scanning electron microscope (Hitachi S-3500N, Schaumburg, IL). Elemental analysis was performed using an energy dispersive X-ray analysis system with a light element prism detector (Princeton Gamma Technology, Rocky Hill, NJ). Dried filter samples were also sputter coated with gold-palladium alloy for microphotography.

Cave Flora and Fauna Collection and Identification

Crustaceans were collected from cave systems by divers using small hand-held nets. The specimens were preserved in isopropanol. Hydrobiid snails were collected by setting cotton mop heads in the cave system and leaving them for a period of 1 to 4

weeks. The mop heads were collected by being placed in plastic bags. Following the dive, snails were rinsed from the mop head over a sieve and sent for identification in water from the cave system. Other benthic fauna were collected by hand in glass containers containing cave water and shipped in chilled containers for identification.

Identification of Bacteria Types

Samples of biofilm from selected cave systems were inserted into biological activity reaction test (BART™) reactors (LaMotte Company, Chestertown, Maryland) for identification of bacteria types present in the biofilm. These reactors have nutrient and oxygen gradients. Byproducts of bacterial metabolism create color changes in the reactor, which indicate the type of bacteria present. Samples from the water column at the chemocline at selected sites were placed in sodium thiosulfate media containing a colored pH indicator. A drop in pH of the media was an indicator of the presence of sulfur oxidizing bacteria (Stubner et al. 1998).

Bacteria Counts

Samples of from the water column in the Dragon's Lair of the Crystal Beach Spring cave system were used for direct counts of the number of bacteria by the SYBR Gold (Invitrogen, Carlsbad, California) stain method. SYBR Gold stock was diluted working solution with 2X SYBR Gold using 0.02 μm filtered TE buffer (10 mM Tris-Cl; 1 mM EDTA, pH 7.4-7.6). A 0.02 μm pore-size Anodisc filter was placed over a pre-wetted 0.8-2.0 μm Millipore filter and 1-2 ml of sample was vacuum filtered. 300 μl of 2X SYBR Gold solution was added to the filter and covered with aluminum foil to avoid light for 15 min. Vacuum was applied to remove the stain and the filter was mounted on a drop of mounting solution with mounting solution between the cover slip and the slide.

Immersion oil was added to the top of the cover slip and the slides were examined under blue-green light excitation (Noble and Fuhrman 1998; Chen et al. 2001).

Results and Discussion

Freshwater Sinkholes and Karst Windows

Eagle's Nest Sink (aka Lost Sink)

Description

Eagle's Nest Sink is located at 28°33'17"N and 82°36'33"W in Hernando County north of Weeki Wachee Spring; this sinkhole is a karst window to a large cave system. The sinkhole and surrounding land is part of an undeveloped wildlife management area. The surface pool for the sinkhole has a diameter of about 60 to 90 m depending on water levels from recent rain events. At a depth of about 10 m, three shafts with diameters of 3 m or less in the bottom of the surface pool lead to a large room with a diameter of about 60 m. Upstream and downstream cave passages continue from this room at an average depth of 75 m (see Figure 1-3). The sinkhole and cavern are formed in the Suwannee Limestone and the cave system is formed primarily in the Ocala Group limestones (Yon and Hendry 1972; Campbell and Scott 1993).

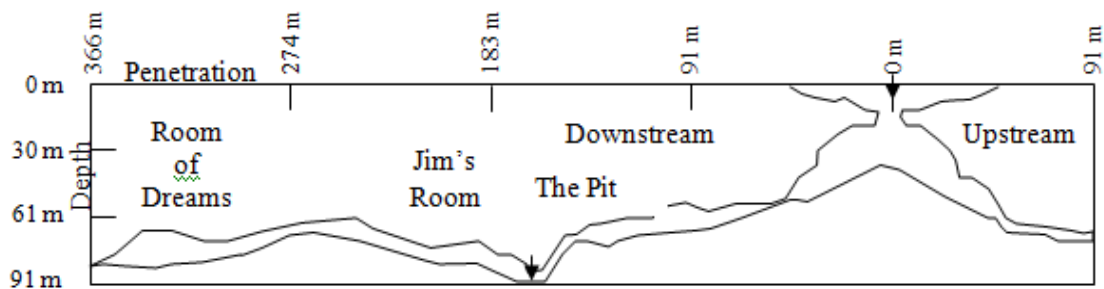


Figure 1-3: Sketch of Eagle's Nest Sink Cave System.

Arrows denote the start and stop of the water quality profile collected on November 11, 2003. Sketch is based on map by National Speleological Society Cave Diving Section.

Water Quality

Water quality data for Eagle's Nest Sink are summarized in Table 1-1. See Figure 1-4 for details.

TABLE 1-1: Eagle's Nest Sink Water Quality Summary November 11, 2003

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Surface Pool (5 m)	23.1	7.35	0.2	2.91
Downstream Cave Tunnel (90 m)	23.2	7.37	0.2	2.29

The water quality data from Eagle's Nest Sink are representative of typical Floridan Aquifer water quality in an area that has not experienced degradation of groundwater quality from development or saltwater intrusion. The water column was well-mixed, as values of the water quality parameters showed little variability with depth.

Flora and Fauna

Reported stygobitic fauna included: *P. leitheuseri*, *T. maclanei*, *C. grandimanus*, and *C. hobbsi* (Franz et al. 1994). No new fauna were identified during the dives performed as part of this study.

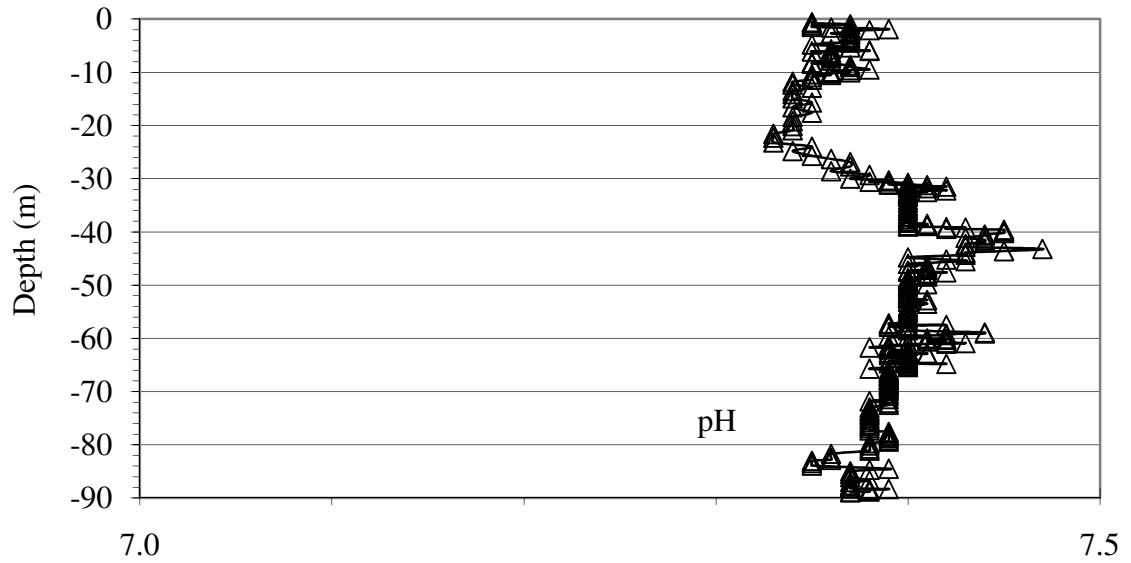
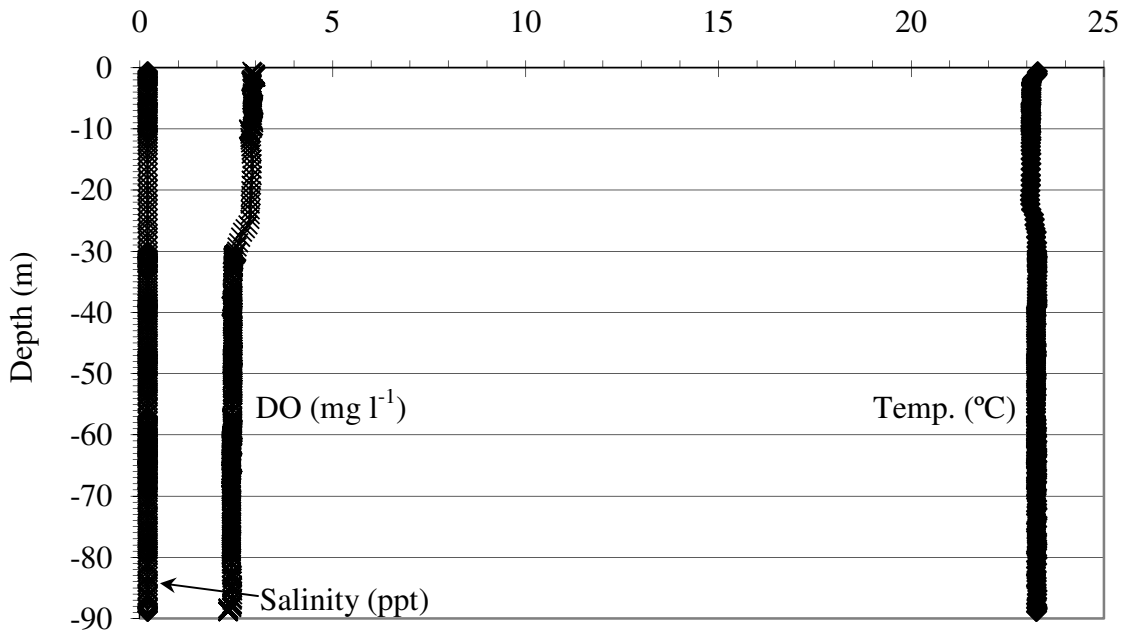


Figure 1-4: Eagle's Nest Sink Water Quality Data, November 11, 2003

Ward's Sink

Description

Ward's Sink is located at 28°23'39"N and 82°33'45"W in Pasco County. The sinkhole and surrounding land is on private property that is part of the lightly developed, rural residential area of Shady Hills. This sinkhole has a surface pool with a diameter of about 20 m. The silt mound begins at a depth of about 38m and a maximum depth of about 62 m. The diameter of the sink at the bottom is about 69 m. There are no cave systems penetrable by divers in the sinkhole. Ward's Sink occurs in the Suwannee Limestone with Ocala Group limestone at the bottom few meters of the sinkhole (Wetterhall, 1965; Yon and Hendry 1972; Arthur 1993).

Water Quality

Water quality data for Ward's Sink are summarized in Table 1-2. See Figure 1-5 for details.

TABLE 1-2: Ward's Sink Water Quality Summary February 15, 2004

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
5 m	22.9	7.61	0.2	6.77
59 m	22.0	7.63	0.2	3.98

The water quality data from Ward's Sink are representative of typical Floridan Aquifer water quality in an area that has not experienced degradation of groundwater quality from development or saltwater intrusion. The water column was well-mixed, as values of the water quality parameters showed little variability with depth except for dissolved oxygen concentrations. Dissolved oxygen levels were higher than typical for Floridan Aquifer water because of the use of the sinkhole for extensive recreational scuba

diving at the time the water quality profile was collected. Dissolved oxygen concentrations increase from exposure to divers' exhaust gases.

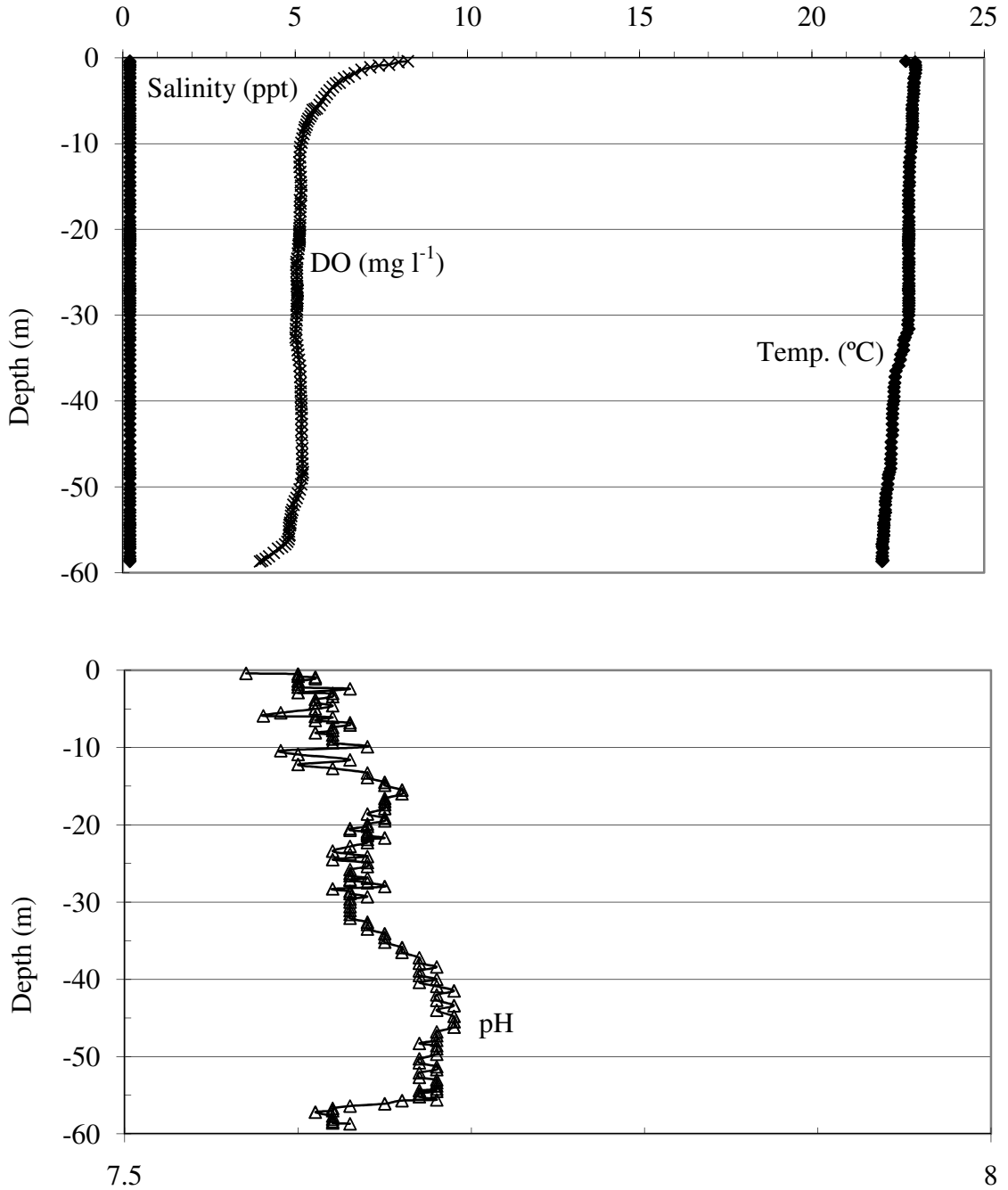


Figure 1-5: Ward's Sink Water Quality Data February 15, 2004

Flora and Fauna

Stygobitic amphipods have been observed near the bottom of the sinkhole in crevices. Amphipod vouchers were collected on May 31, 1998, and have been tentatively identified as genus *Crangonyx*. Ward's Sink represents a new location for stygobitic fauna from the Gulf Coastal Lowlands assemblage of the Ocala fauna group.

Arch Sink

Description

Located at 28°25'32"N and 82°34'42"W in Pasco County. The sinkhole and surrounding land is on private property that is part of the lightly developed, rural residential area of Shady Hills. This sinkhole has an oval-shaped surface pool with axes of about 12 m and 6 m. Beneath the surface pool at a depth of about 13 m, cave passages extend in three directions (see Figure 1-6). The maximum horizontal distance of a single cave passages is 75 m and the maximum depth of the cave is 59 m. A dome-shaped room above the passage to the deepest part of the cave system has a diameter of about 25 m. Arch Sink occurs in the Suwannee Limestone (Wetterhall 1965; Yon and Hendry 1972; Arthur 1993).

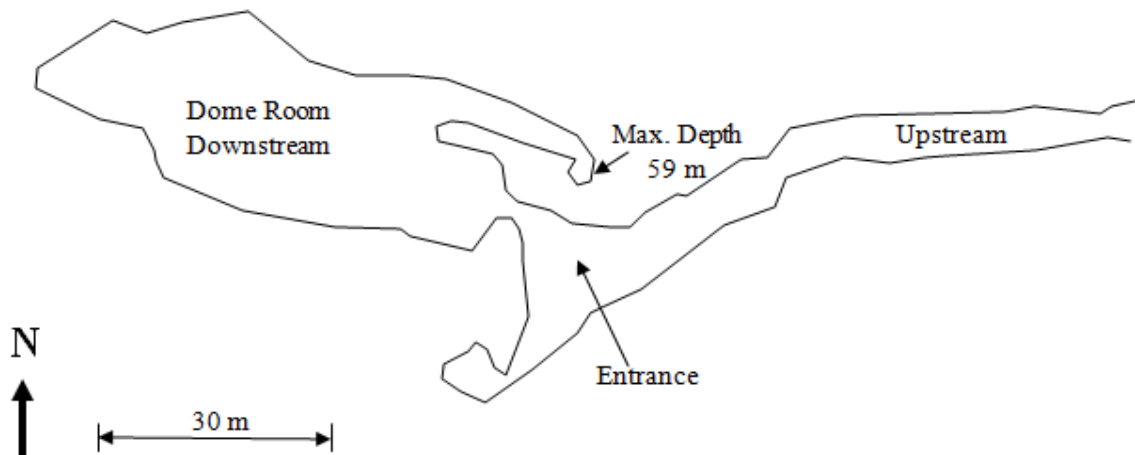


Figure 1-6: Sketch of Arch Sink Cave System. Sketch is based on map by Mike Emanuel.

Water Quality

Water quality data for Arch Sink are summarized in Table 1-3.

TABLE 1-3: Arch Sink Water Quality Summary May 1998

Location (depth)	Temperature (°C)	Salinity (ppt)
Surface Pool 5 m	23.5	0.2

The water quality data from Arch Sink are representative of typical Floridan Aquifer water quality in an area that has not experienced degradation of groundwater quality from development or saltwater intrusion.

Flora and Fauna

The stygobitic crayfish *P. leitheuseri* was previously identified in the Arch Sink cave system (Franz et al. 1994). Stygobitic amphipods were observed in this cave system during this study. Amphipod vouchers were collected in May 1998 and have been tentatively identified as genus *Crangonyx* (Figure 1-7). Amphipods from this genus are part of the Gulf Coastal Lowlands assemblage of stygobitic fauna for which *P. leitheuseri* is the identifying species.



Figure 1-7: Amphipod *Crangonyx* sp. from Arch Sink cave system Dome Room. Actual body size is 2 cm.

Jewel Sink

Description

Jewel Sink is located at 28°24'29"N, 82°40'19"W in the former Sunwest Mine, which was used as a limestone quarry, in Hudson, Pasco County, Florida. In one location, the mining operations penetrated the top of a large cavern. The submerged cavern extends to a depth of 37 m. A cave system extends from the bottom of the cavern to large rooms and depths over 90 m. The 6 m deep mined basin in which the sink is located is full of brackish water and is separated from Fillman Bayou on the Gulf of Mexico by embankments composed of mine tailings. The sink and upper passages of the cave system have formed in the Suwannee Limestone (Yon and Hendry 1972; Arthur 1993).

Water Quality

Water quality data for Jewel Sink are summarized in Table 1-4 See Figure 1-8 for details.

TABLE 1-4: Jewel Sink Water Quality Summary January 25, 2004

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Surface (2.3 m)	16.1	7.67	4.4	9.36
Sink (20 m)	14.8	7.75	4.5	8.99
Start Cave System (37 m)	19.2	6.49	7.3	2.30

Jewel Sink contains brackish groundwater that has been influenced by saltwater intrusion from the Gulf of Mexico. The low temperatures and high dissolved oxygen concentrations in the sink are from the sinking of chilled winter surface water. The drop in pH to acidic conditions at the start of the cave may be an indication of sulfide oxidation by sulfur oxidizing bacteria.

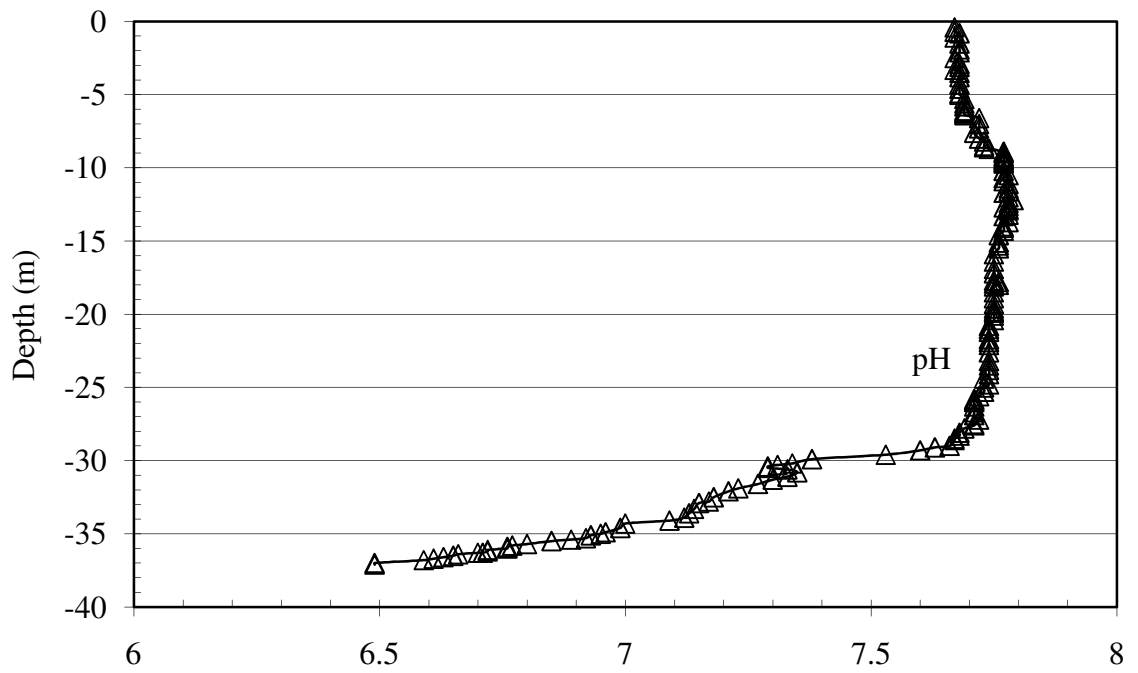
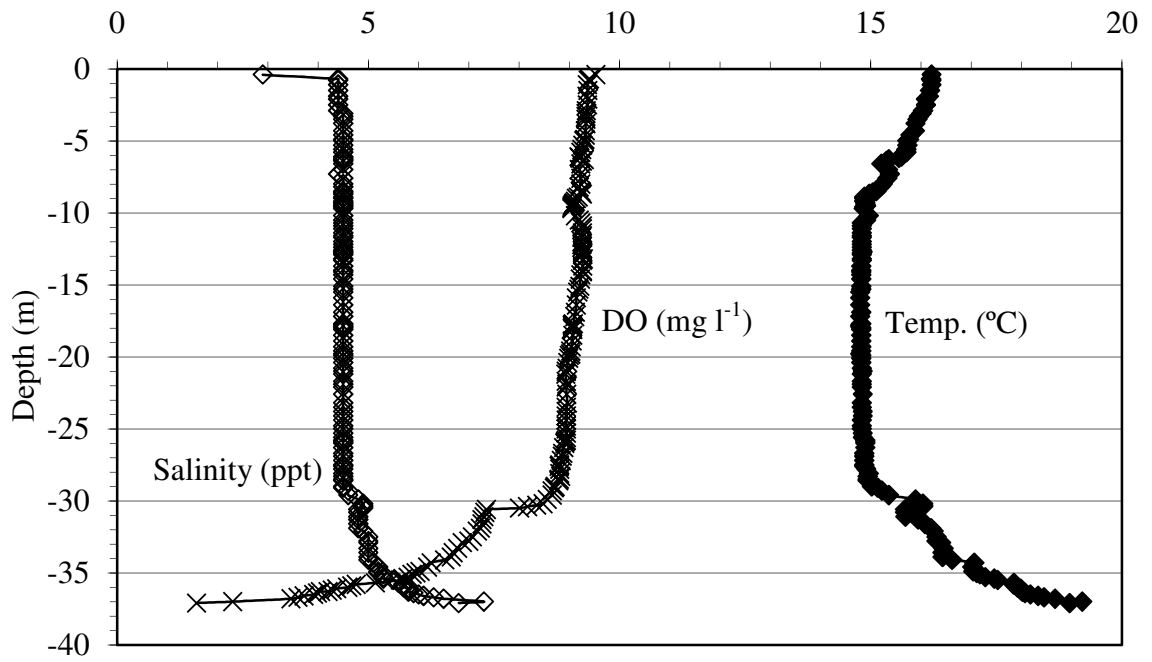


Figure 1-8: Jewel Sink Water Quality Data January 25, 2004

Flora and Fauna

Poor visibility during dives performed as part of this study prevented the identification of fauna in the cave system.

Coastal Springs

Wall Spring (aka Health Spring)

Description

Wall Spring is located on the edge of Boggy Bayou in Pinellas County Florida at 28°06'22"N and 82°46'21"W. The spring is located in a county park and the land surrounding the park is a heavily developed, residential area of Pinellas County. Diving in the spring was performed by written permission and signed agreement between the research divers and Pinellas County Parks Department.

The spring discharges to a manmade 10 m diameter pool before discharging to Boggy Bayou on the eastern edge of the Gulf of Mexico (Wetterhall 1965; Rosenau et al. 1977). At high tide, the spring flow usually reverses and brackish to saline water flows into the spring. Siphoning events are typically short at the end of the wet season in the Fall and lengthy at the end of the dry season in the Spring. The cave system has formed in the Tampa Member (Limestone) of the Arcadia Formation of the Hawthorn Group (Heath and Smith 1954; Wetterhall 1965; Arthur and Campbell 1993).

The spring vent is less than 0.5 m in diameter at a depth of 9 m. The cave system is developed along a bedding plane at an average depth of 9 m. The length of explored cave is about 55 m. The first 36 m of the cave system is a series of small rooms about 2 m wide and 1 to 1.5 m tall. The rooms are separated by restrictions that are generally 1.25 m wide and 0.6m tall (Figure 1-9). At a penetration of 36 m into the cave system, the main

spring flow splits with a significant amount of the flow (approximately 25%) coming from a side room off the main tunnel. This area of the cave system is called the Junction Room. The floor of the cave system is covered by silica sand and shell from the entrance to the Junction Room.



Figure 1-9: Diver in entrance restriction at Wall Spring cave system with Datasonde.

Beyond the Junction Room, the cave continues an additional 19 m in a narrow tunnel that has an average height of 0.6 m without taller rooms. In this area, the floor of the cave system is covered by clayey sediment instead of sand. Water issues from several small vents in this area of the cave, but there are no additional passages large enough for a diver to explore.

Water Quality

Historical water quality data include May 3, 1946, data from the spring pool with a pH of 7.9 and salinity of 0.3 ppt. On December 12, 1972, the salinity was 1.5 ppt.

Discharge was estimated at 168 l s^{-1} on December 12, 1972 (Rosenau et al. 1977). During the current study, the spring pool at Wall Spring was sampled on a monthly basis by Pinellas County because of its proximity to the County's spray effluent discharge field. Typical nitrate values in the spring pool were 2 to 10 mg l^{-1} (personal communication, Dave Slonena, Pinellas County Utilities Department).

The typical water quality of the cave system during this study is characterized by data collected on January 2, 1999, and summarized in Table 1-5.

TABLE 1-5: Wall Spring Water Quality Summary January 1999

Location	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
Spring Pool	24.0	7.09	2.80	6.17
Junction Room	24.0	7.03	2.48	0.00
Side Room	24.0	6.98	2.43	0.00

The data in Table 1-5 show that the spring water in the cave system is hypoxic to anoxic and that the spring discharge is influenced by saltwater intrusion, as the discharge is brackish. In June 2000, the Pinellas County Parks Department requested additional water quality data from the spring to evaluate whether relatively fresh brackish water from the aquifer was still flowing from the spring after several months of severe drought. The dive was performed near low tide to maximize the possibility of finding freshwater in the cave system. There was no flow from the spring pool. From the entrance to the Junction Room the cave system contained saltwater with salinity values less than half of typical coastal Gulf of Mexico values of 25 to 30 ppt. Beyond the Junction Room, the upper part of the cave system contained brackish water and the lower part contained saltwater. No dissolved oxygen was detected in the cave system. The water quality data from this dive are summarized in Table 1-6.

TABLE 1-6: Wall Spring Salinity Summary

Location	Salinity (ppt)	
	January 02, 1999	June 11, 2000
Junction Room - Upper	2.48	3.3
Lower	2.48	11.1
Side Room	2.43	13.4

In spite of the severe drought conditions, brackish water of similar salinity to the water found in the cave system during studies performed in 1999 was found in the upper part of the cave passages from the Junction Room to the back of the cave indicating flow from the aquifer was present during the drought.

Flora and Fauna

Live hydrobiid snails were found within the cave system. These were identified as *Littridinops palustris* (Thompson 1968). Shells from another hydrobiid snail were found within the cave system and were identified as *L. monroensis* (Frauenfeld 1863) (personal communication, Dr. Fred Thompson, Florida Museum of Natural History).

Stygobitic amphipods tentatively identified as genus *Crangonyx* were also observed in the cave system (personal communication, Richard Franz, Florida Museum of Natural History). The ceiling and walls of the cave system have a thin coating (1 to 3 mm) of a rust-colored biofilm. Mike Emanuel explored the cave system in the early 1990s and reported the observation of stygobitic crayfish (personal communication, Mike Emanuel). No crayfish were observed during studies performed in 1998 through 2000.

Apparently in the past, conditions within the Wall Spring cave system were sufficient to support crayfish, a relatively large scavenger/predator. Conditions in the cave system have changed and it is no longer a hospitable environment for crayfish. The most likely reason that crayfish are no longer present is the lack of dissolved oxygen in

the cave system. This condition is probably a result of the introduction to the groundwater system of compounds that increase the biochemical oxygen demand (BOD). Groundwater in West Central Florida cave systems typically contains 2 to 3 mg l⁻¹ dissolved oxygen as in the Eagle's Nest Sink cave system. It only takes a small amount of organic material to make a cave system anoxic. Once the system becomes anoxic, reduced compounds are available to support the growth and metabolism of microbial biofilms, creating more biomass, and increasing the BOD of the water in the cave system. The amphipods are more tolerant of hypoxic conditions and brief periods of anoxia compared to crayfish (Gannon et al. 1999; Bishop et al. 2004); and may be able to survive on the dissolved oxygen injected into the cave system during siphoning events when siphoning occurs at regular intervals.

In June 2000, the cave system contained algae washed in by inflow of saltwater from the adjacent bayou at high tide. The ceiling and walls of the cave system had a thick coating (1 to 2 cm) of rust-colored biofilm (Figure 1-10). No amphipods were observed.



Figure 1-10: Biofilm coating the walls of Wall Spring cave system from June 2000 when biofilm was 1 to 2 cm thick. Left to right field of view is approximately 1 m.

Salt Spring

Description

Salt Spring is located at 28°17'29"N, 82°43'09"W in the tidal marsh of the Gulf of Mexico about 2.5 km north of the town of Port Richey. The spring is located in a state park and the land surrounding the park is a heavily developed, commercial and residential area of Pasco County along the U.S. Hwy 19 corridor. Diving in the spring was performed by permission from Salt Spring State Park.

The spring discharges to a spring run formed in limestone. In 1960, the spring siphoned at high tide, as was observed during this study (Wetterhall 1964). The spring vent for Salt Spring has a diameter of about 1 m and the spring run discharges to the Gulf of Mexico.

The spring vent leads to a small cave system with passages 3 m or less than diameter. At a depth of 40 m, the passages open to a large room over 30 m diameter, which in turn connects to a second large room through a 5 m opening at a depth of 56 m. The explored depth of the cave system is 100 m (see Figure 1-11). The upper passages of the cave system and upper large room are developed in the Suwannee Limestone and the lower large room is developed in the Ocala Group limestones (Wetterhall 1964; Yon and Hendry 1972; Arthur 1993). The cave system is a spongework pattern, as it is a series of large interconnected rooms.

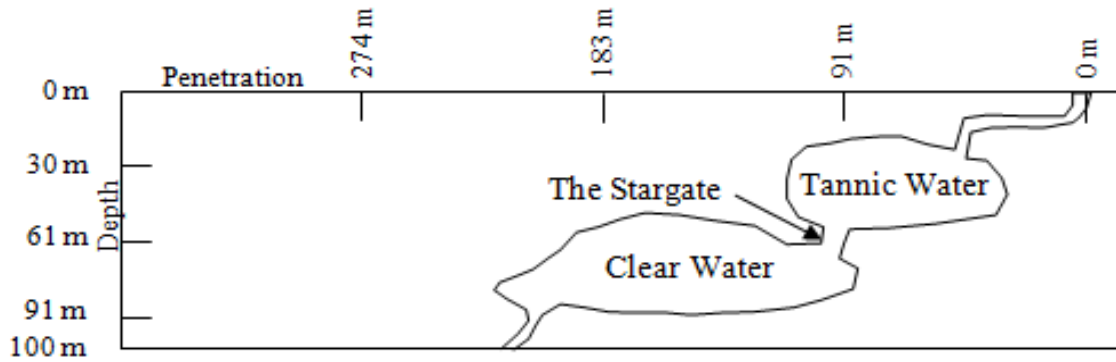


Figure 1-11: Sketch of Salt Spring Cave System.

Water Quality

Water with a salinity of 14.1 ppt and a pH of 7.1 was discharged from the spring in 1960, 14.1 ppt and 7.6 in 1964, and 18.2 ppt in 1972. When these measurements were made, the flow from the spring vent was estimated to be 283 l s^{-1} (Rosenau et al. 1977). The discharge from the spring vent during this study was tannic saltwater at 20 ppt with a temperature of $24.6 \text{ }^\circ\text{C}$ and pH of 6.76. As shown in Table 1-7, the salinity of the discharge from the spring vent has been increasing over time, reflecting increased saltwater intrusion into the aquifer.

TABLE 1-7: Salt Spring Salinity Summary

Location	Salinity (ppt)			
	1960	1964	1972	2003
Spring Vent	14.1	14.1	18.2	19.9
Below Chemocline	-	-	-	38.6

The water discharging from the spring vent has less than 1 mg l^{-1} of dissolved oxygen. In the cave system beyond the first few meters, the water is anoxic. The chemocline occurs in the opening between the two large rooms at a depth of 56.2 m. There is a pH minimum and a particulate “cloud” at the chemocline (Figure 1-12). The bottom water below the chemocline is hypersaline with a maximum salinity of 38.6 ppt

and a maximum temperature of 24.7 °C. The water quality data collected from Salt Spring are summarized in Table 1-8. See Figure 1-13 for details.

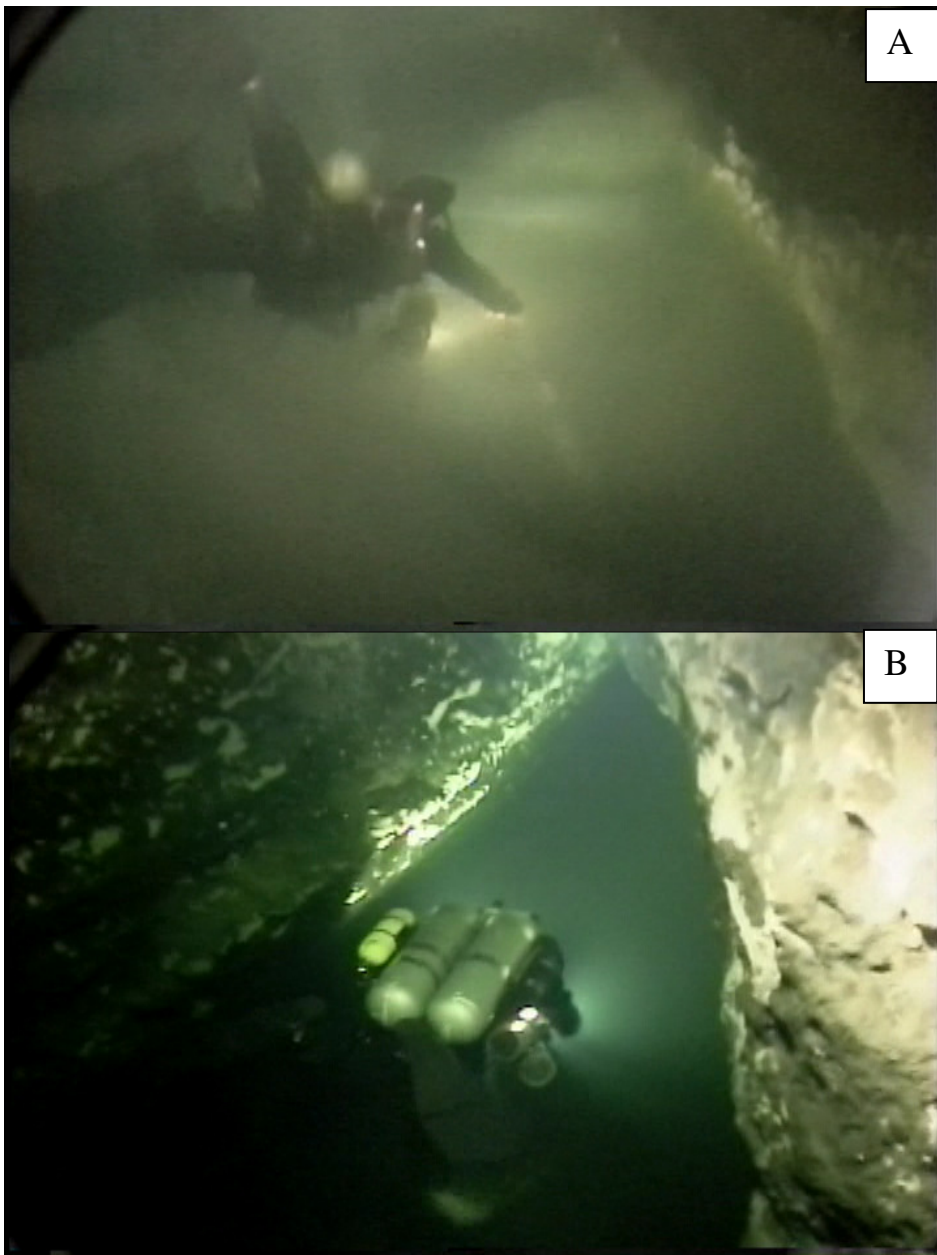


Figure 1-12: Particulate cloud at the chemocline in Salt Spring cave system. Diver is descending through particulate cloud in Stargate restriction (A). Same camera position shows diver just below particulate cloud (B).

TABLE 1-8: Salt Spring Water Quality Summary
Datasonde Data - December 29, 2001

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Spring Vent (0.7 m)	23.3	7.03	19.4	1.00
Tannic Cave Passages (24 m)	23.5	7.02	19.4	0.37
Tannic Upper Room (46 m)	23.3	7.04	19.4	0.45
Clear Lower Room (61 m)	24.7	7.01	33.6	0.07
Clear Lower Room (87 m)	24.6	7.06	33.9	0.04

Datasonde Data - February 16, 2002

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Spring Vent (0.7 m)	23.4	6.99	20.0	1.01
Tannic Cave Passages (24 m)	24.1	6.93	20.4	0.02
Tannic Upper Room (46 m)	24.1	6.95	20.4	0.02
Clear Lower Room (61 m)	24.7	6.95	35.4	0.01
Clear Lower Room (87 m)	24.6	7.00	35.7	0.01

Datasonde Data - October 18, 2003

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Spring Vent (0.7 m)	24.8	6.76	19.9	0.69
Tannic Cave Passages (24 m)	24.6	6.69	20.1	0.00
Tannic Upper Room (46 m)	24.6	6.69	20.2	0.00
Clear Lower Room (61 m)	24.8	6.72	38.2	0.00
Clear Lower Room (87 m)	24.7	6.80	38.6	0.00

On-Site Analyses – October 18, 2003

Location (depth)	H₂S (mg l ⁻¹)	Alkalinity (mg l ⁻¹)	NH₃ (mg l ⁻¹)	NO₃⁻ (mg l ⁻¹)	PO₄³⁻ (mg l ⁻¹)	Fe (mg l ⁻¹)
0.7 m	0.0	130	0.2	0.0	0.20	0.20
24 m	0.0	100	1.0	0.0	0.15	0.15
46 m	0.0	150	0.4	0.0	0.30	0.25
61 m	0.0	115	0.1	0.0	0.20	0.40
87 m	0.0	125	0.0	0.0	0.15	0.40

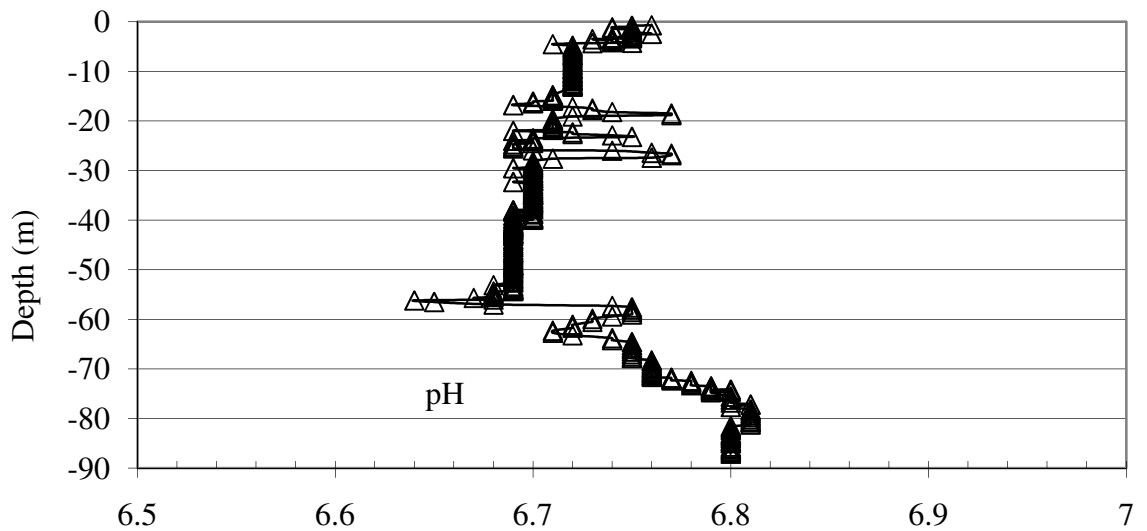
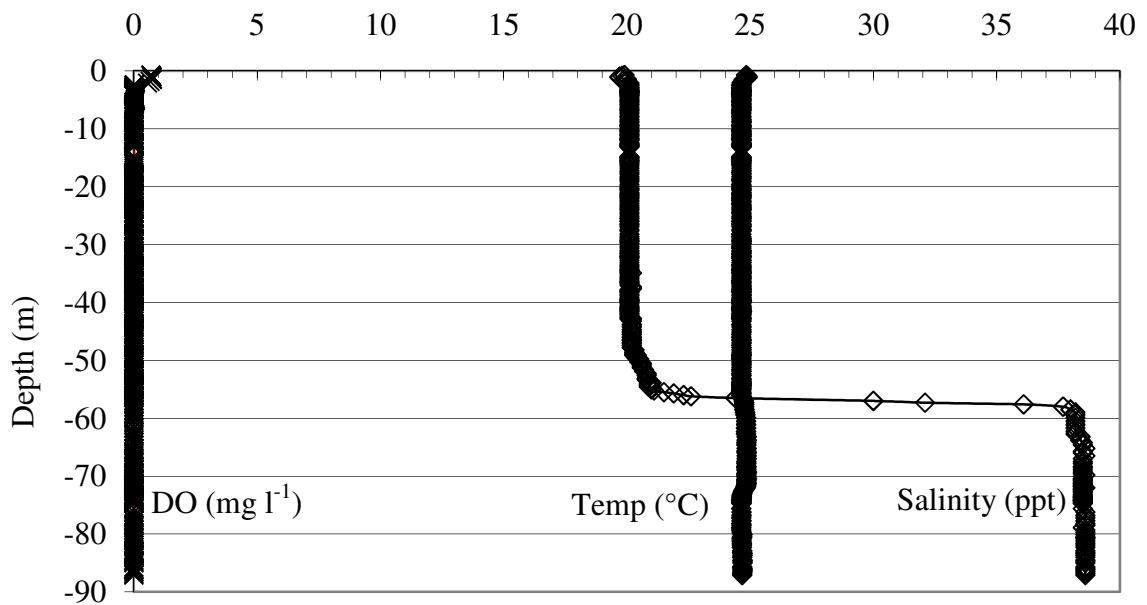


Figure 1-13: Salt Spring Water Quality Profile October 18, 2003

The temperature of the water in the Salt Spring cave system is slightly higher than expected for Floridan Aquifer water as the freshwater sinkholes and cave systems in West Central Florida typically have water temperatures of 22.0 to 24.0 °C. The shallower tannic water in the cave system shows some seasonal temperature variability with cooler temperatures in the winter. With temperatures greater than 24.6 °C and hypersaline

conditions with salinities higher than normal sea water from the Gulf of Mexico in the bottom water, there may be some influence from geothermal circulation of deep saline water. This hypothesis is supported by the clarity of the bottom water with over 60 m of visibility compared to the water in the shallower part of the cave, which is tannic and has a visibility of less than 3 m. It is likely that the salt spring cave system contains a shallow tannic water component that reflects the influence of saltwater intrusion into the unconfined coastal Floridan Aquifer and a deeper hypersaline component that reflects the influence of geothermal circulation.

The pH profile for Salt Spring shows neutral to slightly acidic conditions that vary from 6.64 to 7.06 with a pH minimum at the chemocline density interface between the tannic water and hypersaline bottom water. Often in anoxic marine basins, the pH is acidic from activity of chemolithotrophic sulfur oxidizing bacteria that convert sulfide, released from the degradation of organic matter by sulfur reducing bacteria, to sulfate (Castanier et al. 1999; Garman and Garey 2005). This process may not be as significant in Salt Spring as it is in other anoxic marine basins because sulfide was below detection limits in water samples from the cave system and traces of oxygen were recorded in the bottom water from two of the three Datasonde dives. Geothermal circulation may be bringing the slightly acidic, hypersaline water into the cave system without processes in the cave system creating the acidic conditions in the bottom water.

Alkalinity, ammonia and phosphate concentrations were highest in the upper large room with tannic water. This room has extensive microbial biofilm growth on the walls and ceiling (Figure 1-14). The higher concentrations of these parameters in this area suggest that the microbial growth is accompanied by the release of products from the

oxidation of organic material by sulfate reduction. The sulfide released by this process may be used rapidly by sulfur oxidizing bacteria creating the pH minimum. The lower alkalinity, ammonia, and phosphate concentrations in the hypersaline bottom water indicate that the oxidation of organic matter is not as prominent in the bottom water as in the overlying tannic water.



Figure 1-14: Biofilm growth on the walls of the Salt Spring cave system upper large room with tannic water. Biofilm fingers are 20 to 30 cm long.

Flora and Fauna

The walls and ceiling of the tannic water cave passages and upper room are coated by a biofilm. The biofilm is thick and forms fingers about 20 to 30 cm long above the chemocline. Below the chemocline in the hypersaline clear water, the biofilm is a spotty, thin coating on the walls and ceiling of the cave system less than 1 cm thick and is generally absent below 75 m (Figure 1-15). At the entrance to the cave system, a white filamentous bacteria has been observed (Figure 1-16). White filamentous bacteria are often sulfur oxidizing bacteria; however, no samples from Salt Spring have been analyzed for the presence of sulfur oxidizing bacteria.



Figure 1-15: Deeper room of Salt Spring cave system showing diver in front of wall without biofilm coating.



Figure 1-16: White filamentous bacteria on rocks at entrance to Salt Spring cave system.

Gar Spring

Description

Gar Spring is located on the edge of the same saltwater marsh that contains Salt Spring in Pasco County Florida at 28°17'56"N and 82°43'4"W. The spring is located in a state park and the land surrounding the park is a heavily developed, commercial and residential area of Pasco County along the U.S. Hwy 19 corridor. Diving in the spring was performed by permission from Salt Spring State Park.

The spring discharges to a 5 m diameter pool before discharging to a spring run that discharges to Double Hammock Creek and the Gulf of Mexico. The spring run and pool contain a school of alligator gar, giving the spring its name. No record of this spring was found in published literature. The spring was identified by Park Rangers working at Salt Springs State Park after the property was purchased by the State of Florida.

The spring vent is a vertical shaft about 1m in diameter that extends to a depth of 7.6 m before the shaft is blocked by large tree branches. The spring discharges from the Suwannee Limestone (Wetterhall 1964; Yon and Hendry 1972).

Water Quality

The discharge from the spring is clear, brackish water. The spring flow was estimated to be 150 l s^{-1} and was not observed to reverse at high tide. The typical water quality of the spring during this study is summarized in Table 1-9.

TABLE 1-9: Gar Spring Water Quality Summary December 2001

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
Spring Pool (0.7 m)	23.8	7.09	6.0	0.71
Cave Shaft (7.6 m)	23.8	7.10	6.0	0.13

Gar Spring provides insight into the complexities of the coastal Floridan Aquifer. Although it is about 800 m north of and in the same marsh as Salt Spring, Gar Spring discharges clear, brackish water with a temperature 1 °C lower and a salinity almost 13 ppt lower compared to the discharge from Salt Spring. Like Eagle’s Nest Sink and Ward’s Sink, the water in Gar Spring is slightly basic compared to the acidic water in Salt Spring. While the water quality of Salt Spring is likely influenced by shallow tannic saltwater from the coastal marsh and deep geothermal circulation, Gar Spring appears to be Floridan Aquifer freshwater with some subterranean mixing of Gulf of Mexico saltwater from saltwater intrusion into the aquifer.

Flora and Fauna

The cave system was not accessible preventing the identification of fauna in the cave system during dives performed for this study.

Cauldron Spring

Description

Cauldron Spring is located on the edge of the same saltwater marsh that contains Salt Spring about 300 m south of Salt Spring in Pasco County Florida at 28°17'20"N and 82°43'09"W. The spring is located in a state park and the land surrounding the park is a heavily developed, commercial and residential area of Pasco County along the U.S. Hwy 19 corridor. Diving in the spring was performed by permission from Salt Spring State Park.

The spring discharges to a small spring run that discharges to Salt Springs Run and the Gulf of Mexico. The spring discharge is tannic. No record of this spring was found in published literature.

The spring vent is an opening about 3 m in diameter in the creek bank at the edge of a dirt road. The cave system is a 3 to 5 m diameter tunnel trending north-northwest in the general direction of Salt Spring. The cave system has been explored to a penetration of about 100 m and a maximum depth of about 15 m. The spring discharges from the Suwannee Limestone (Wetterhall 1964; Yon and Hendry 1972).

Water Quality

The discharge from the spring is tannic saltwater. The spring discharge was estimated to be 150 l s^{-1} and was not observed to reverse at high tide. The water quality of the spring cave system was measured in February 1996 at 14.4 °C and 18.8 ppt.

Cauldron Spring discharges tannic water of similar salinity as Salt Spring. The cave system feeding the spring trends toward Salt Spring and it is likely that Cauldron Spring shares a common source of shallow tannic saltwater from the coastal marsh with

Salt Spring. The low temperature of the discharge from Cauldron Spring indicates that the recharge source is likely nearby in the salt marsh as the water did not have time to equilibrate to groundwater temperature before discharging from the spring.

Flora and Fauna

The tannic and low visibility conditions of the spring cave system prevented the identification of fauna in the cave system during dives performed for this study.

Spring #822-241A

Description

This spring is located in a tannic saltwater canal in Hudson Florida about 160 m west of US Highway 19 (28°21'19"N and 82°41'57"W). The land surrounding the spring is a heavily developed, commercial and residential area of Pasco County along the U.S. Highway 19 corridor. The spring has a distinct boil even at high tide. The discharge of this Spring has been estimated at 850 l s^{-1} at low tide (Wetterhall 1965). There is no access to the cave system feeding the spring through the rubble at the spring vent; however, exploration from a nearby sinkhole (Wayne's World Sink, aka School Sink, 28°22'07"N and 82°41'40"W) identified a cave network to which the spring is connected. See Figure 1-17 for Wayne's World Sink cave system map. The land on which Wayne's World Sink is located is currently owned by the National Speleological Society Cave Diving Section. The cave system at Wayne's World Sink has been connected to the Beacon Woods Cave System that includes Round Sink (28°20'02"N, 82°40'50"W) and the Bear Creek submergence in Bear Sink, creating one of the largest cave systems in Florida (personal communication, Alex Warren). See Figure 1-18 for Beacon Woods cave system map. This cave system has been connected to Horseshoe Crab Sink (aka

Spring 820-243A or Heart Spring) by a dye trace study originating in Round Sink in 1961 (Wetterhall 1965).

Round Sink is a karst window in the Beacon Woods cave system up-gradient of the Wayne's World Sink cave system. The sink has a diameter of about 30 m with nearly vertical walls that extend to a depth of about 20 m. At 20 m, there is a steeply sloping passage that meets the cave system. The total depth at the sink is 45 m.

Palm Sink (aka Hazel Sink) is a sinkhole in the vicinity of the Wayne's World Sink cave system (see Figure 1-18). At the present time, there are no cave passages leading from the sink. The sink has a diameter of about 76 m with nearly vertical walls that extend to a depth of about 42 m.

The Wayne's World Sink and Beacon Woods cave systems have formed primarily in the Suwannee Limestone, which is at or near land surface in the vicinity of the Sink (Yon and Hendry 1972; Arthur 1993). The water in cave systems is brackish and tannic with saltwater intruding into the system through a deeper saltwater tunnel. The sink and cave system are tidally influenced with a direct connection to the Gulf, as the high and low tides in the Sink are of approximately the same magnitude and at approximately the same time as corresponding tides in the Gulf. Water flow in the cave system reverses with changing tides.

The typical cave passage is approximately 27 to 34 m deep with thick reddish brown silt on the floor. Visibility is usually 1 to 2 m because of tannic conditions. The Deep Salt Tunnel contains water with higher salinities that is not tannic. On occasion during periods of very low rainfall, visibility may reach 6 to 9 m. At depths shallower than 27 m, the system is characterized as a spongework cave with porous walls that look

like Swiss cheese and wider passages. Below 27 m, the limestone becomes dense and the system is an anastomatic cave, consisting of arrays of curvilinear tubes that commonly intersect forming closed loops.

The cave systems have a complex hydrology. The Main Street Tunnel has considerably less silt than other brackish water tunnels in the cave. Currents in the passage are a strong siphon toward the Gulf at low tide and a strong spring inland at high tide. Swimming toward the entrance to the Main Street Tunnel, the direction of flow can abruptly change from spring to siphon. This may be a groundwater divide created at low tide with some water flowing toward the Gulf of Mexico through the Main Street Tunnel and some water flowing toward Spring #822-241A.

The G Tunnel is one of numerous side tunnels off the Main Street Tunnel. The G Tunnel current flows toward the Main Street Tunnel and the water is less tannic than the rest of the system. The G Tunnel travels beneath a section of canal northeast of Wayne's World Sink that is usually clear and is fed by several small spring vents.

The Deep Salt Tunnel occurs where the cave system drops below a depth of 40 m and the salinity of the water increases abruptly at a chemocline. Beyond the Deep Salt Tunnel, the cave system returns to tannic brackish water at an average depth of 34 m. This area known as the Beyond is near an office building on U.S. Highway 19. Residents in the neighborhood stated that a sinkhole opened in the vicinity of the office building and was filled in by the County. When the sink was filled, flow at Hudson Spring at the head of Hudson Creek (28°21'53"N, 82°42'10"W) decreased dramatically. It is possible that the Beyond is pirating flow that once discharged to Hudson Spring. The entrance to Hudson Spring has been intentionally filled with boulders and debris (Wetterhall 1965);

and is not passable by divers. Exploration of the spring entrance reached a maximum penetration of 50 m and a maximum depth of 10 m. Wetterhall reported Hudson Spring had continual discharge but likely reversed at the higher high tides associated with full and new moons in 1965. Similar flow at Hudson Spring was observed during this study.



Figure 1-17: Map of the Wayne's World Sink cave system showing the major tunnels. Cave survey data compiled by Alex Warren of the Southeast Exploration Team. Used by permission.

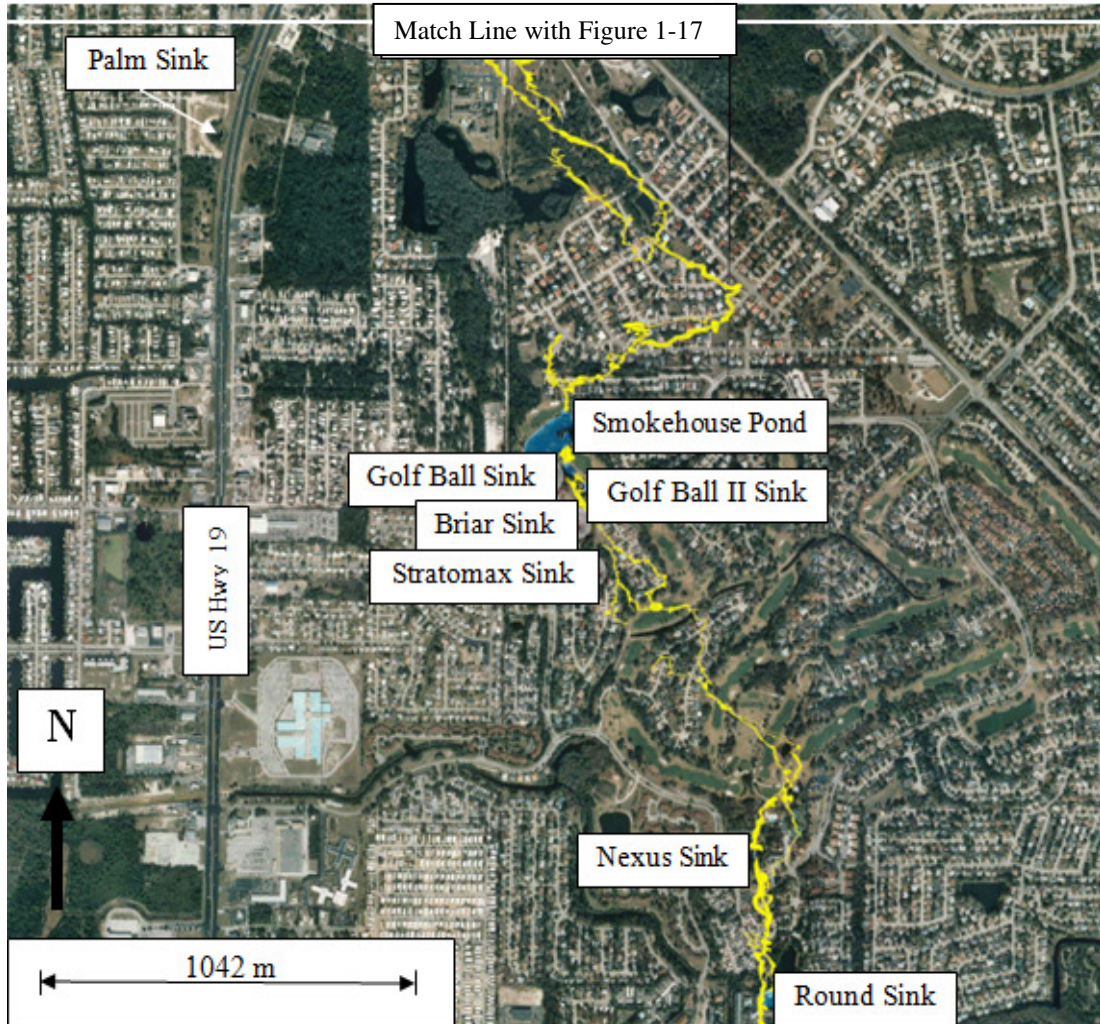


Figure 1-18: Map of the Beacon Woods cave system showing the major tunnels. This cave system connects to the Wayne’s World Sink cave system at the north end of the map. Cave survey data compiled by Alex Warren of the Southeast Exploration Team. Used by permission.

Water Quality

In January 1997, water quality readings were collected from the Wayne’s World Sink cave system and are summarized in Table 1-10. Salinity was the only parameter that changed significantly in the cave system passages. The lowest salinity value was 4.1 ppt from the surface of the Sink. The shallow part of the cave system (depth less than 27 m) had salinity readings ranging from 9 to 16 ppt. Salinity readings between 16 and 19 ppt were recorded in the deeper parts (33.5 to 34.5 m deep) of Gary’s tunnel, which leads to

the Main Street tunnel. Salinity readings up to 25.9 ppt were recorded in the tannic water tunnels. Salinities were greater than 17 ppt in areas of Main Street where Sabellidae worms were observed. The highest salinity readings in the tannic water tunnels were 25.8 ppt near a penetration of 610 m in the Main Street Tunnel where cnidarians were observed.

The other parameters measured showed very little change in the cave system. Typically the pH in the system was between 7.2 and 7.6 and the temperature 23.5 to 24 °C. Dissolved oxygen concentrations were typically 0.5 to 1 mg l⁻¹ in the cave system. The Deep (43 to 46 m deep) Salt Tunnel separates two tannic water tunnels. The Deep Salt Tunnel has salinity up to 31 ppt and a variable temperature from 23 °C in the winter to 26 °C in the summer. The Deep Salt Tunnel water is always warmer than the surrounding tannic water tunnels.

TABLE 1-10: Wayne's World Sink Cave System Water Quality Data January 1997

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Sink (5 m)	21.2	7.45	4.1	2.19
Cave Passage near sink (24 m)	23.5	7.47	12.0	1.52
Gary's Tunnel (32 m)	23.8	7.48	25.9	0.97
Main St. at G Tunnel	23.6	7.30	16.2	0.76
G Tunnel (28 m)	23.6	7.59	8.2	0.99
Main St. at Sabellidae (27 m)	23.5	7.33	17.4	0.61
Main St. at cnidarians (33 m)	23.7	7.28	25.8	0.53
Start Deep Salt Tunnel (31 m)	23.5	7.52	12.0	0.76
Deep Salt Tunnel (44 m)	23.9	7.48	31.0	0.65
End Deep Salt Tunnel (40 m)	23.8	7.51	22.5	0.48
Beyond Tunnel (35 m)	23.6	7.59	10.0	0.66

In Round Sink there is a chemocline a few meters above the floor in the cave system. The water quality data for Round Sink are summarized in Table 1-11. See Figure 1-19 for details.

TABLE 1-11: Round Sink Water Quality Summary March 3, 2002

Location	Temperature (°C)	pH	Salinity (ppt)	DO (mg/L)
Above Chemocline (4 m)	22.8	7.17	0.9	0.73
Below Chemocline (45 m)	22.0	6.95	4.0	0.00

In Palm Sink, there is also a chemocline a few meters above the bottom of the sink. Water quality data for Palm Sink are summarized in Table 1-12. See Figure 1-20 for details.

TABLE 1-12: Palm Sink Water Quality Summary January 11, 2003

Location	Temperature (°C)	pH	Salinity (ppt)	DO (mg/L)
Above Chemocline (5m)	15.8	7.37	0.3	3.83
pH Minimum (34m)	21.4	6.23	32.6	0.09
Bottom Water (42m)	23.5	6.47	36.5	0.00

The Wayne's World Sink and Beacon Woods cave systems contain tannic brackish groundwater. The tannic water is derived from the submergence of Bear Creek and the water from the hardwood wetlands in which the sinks connected to the cave system are located. Saltwater intrusion from the Gulf of Mexico is observed throughout the cave systems and the sinkholes in the area with water equal to Gulf of Mexico salinity in the Deep Salt Tunnel of the Wayne's World cave system. At Round Sink, the farthest point inland from which data were obtained, the influence of saltwater intrusion is less and freshwater overlies brackish water below the chemocline. Both Round and Palm Sinks show that saltwater intrusion leads to the degradation of organic matter entering the sinkhole by sulfate reduction and the corresponding oxidation of the sulfide by sulfur oxidizing bacteria, creating acidic pH conditions at depth.

The bottom water of Palm Sink is hypersaline at 36.5 ppt but the temperature is normal for the aquifer at 23.5 °C. It is possible that there is some geothermal circulation

influencing the water quality of the bottom water and bringing hypersaline water into the sink. The current isolation of the sink from cave passages may account for the cool water compared to other karst features in the region by the sinking of cold winter surface water.

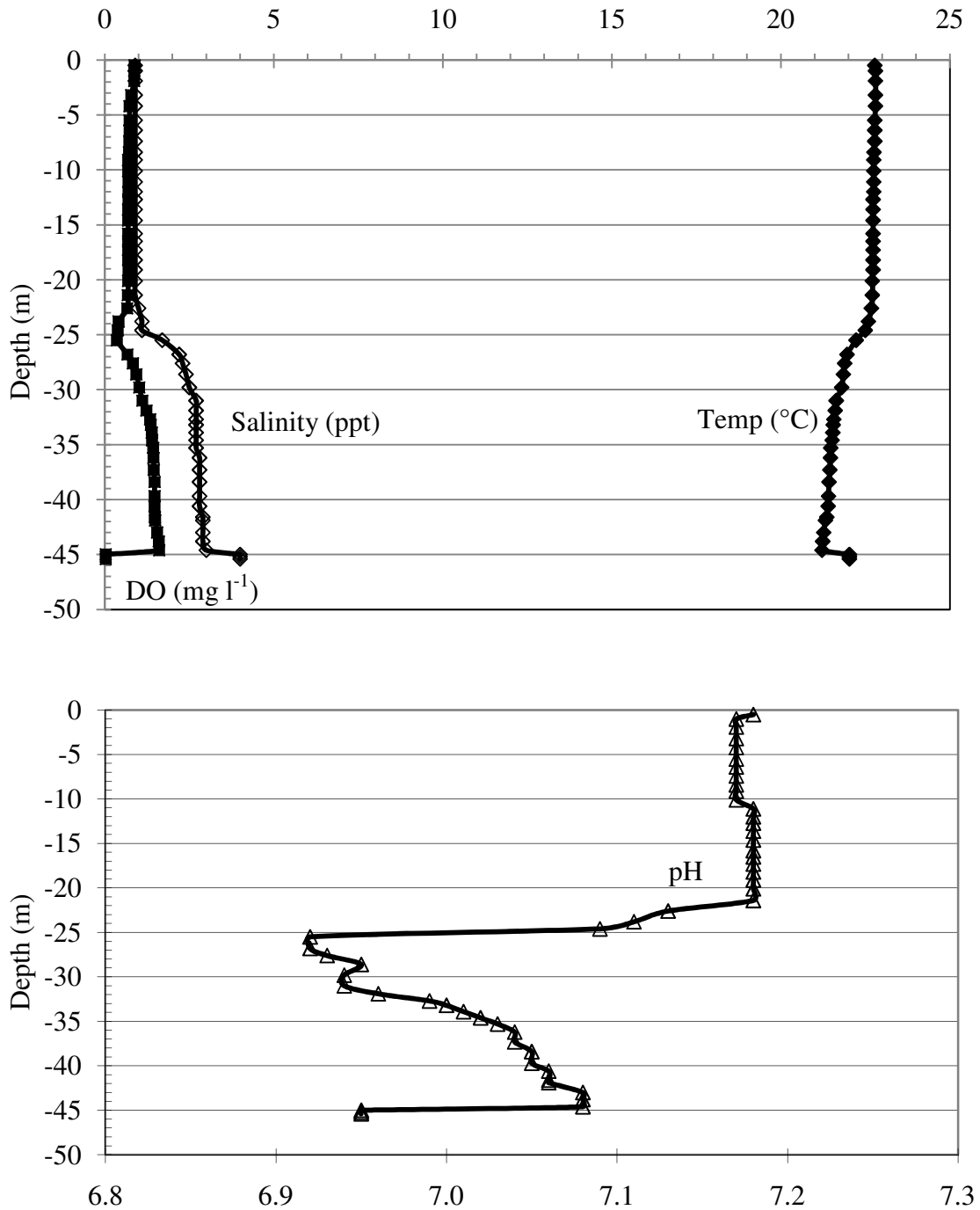


Figure 1-19: Round Sink Water Quality Profile March 3, 2002

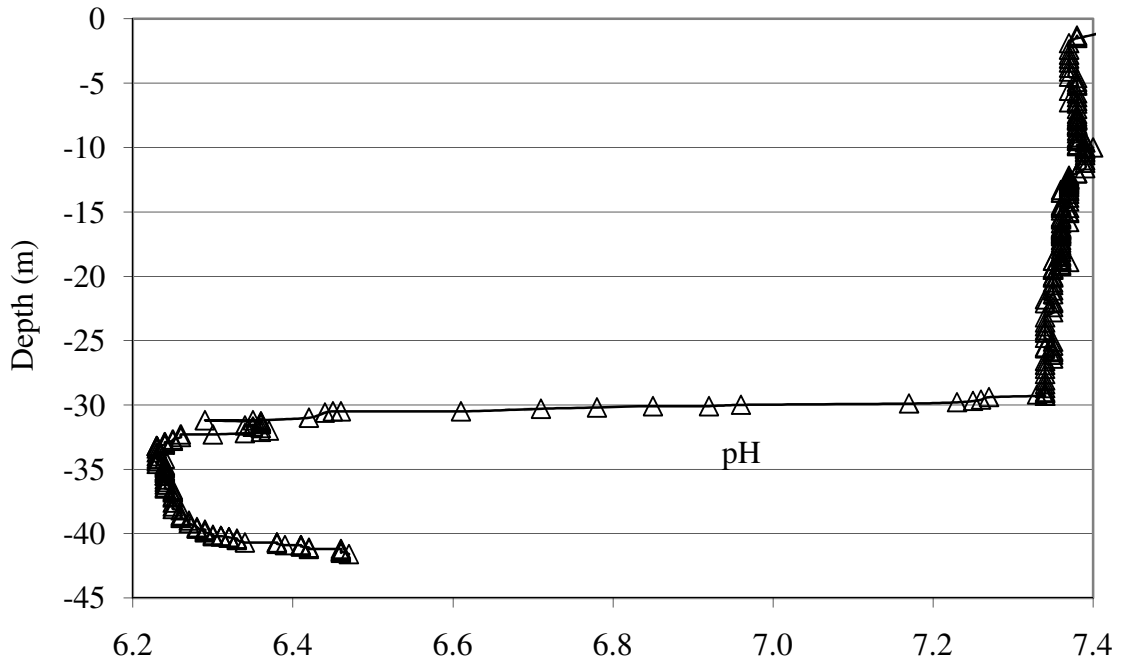
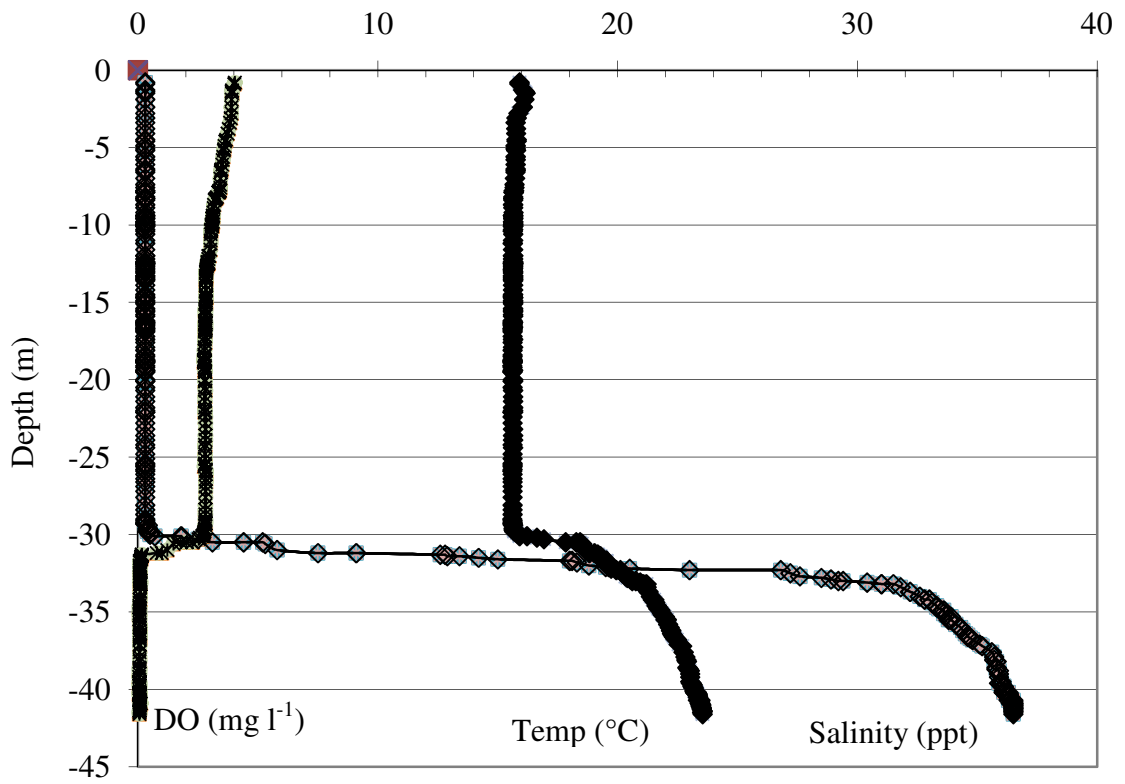


Figure 1-20: Palm Sink Water Quality Data January 11, 2003

Flora and Fauna

Previous studies had identified three stygobitic crustaceans from the Beacon Woods cave system in the vicinity of Round Sink: the crayfish *P. leitheuseri* and the amphipods *C. grandimanus* and *C. hobbsi* (Franz et al. 1994). During this study, crayfish of genus *Troglocambarus* were observed, but no vouchers were collected.

Four stygobitic crustaceans have been found in the tannic, brackish to saline waters of the Wayne's World Sink cave system. The crustaceans are found in tunnels with salinities ranging from 1 to 20 ppt, pH 7.3 to 7.6 standard units, and dissolved oxygen 0.4 to 1.5 mg l⁻¹. During periods of normal rainfall, these tunnels with stygobites had salinities of 1 to 5 ppt. The stygobites include: a crayfish, *P. leitheuseri*; an isopod, *Caecidotea* sp.; and two amphipods, *C. hobbsi* and *C. grandimanus* (personal communication, Richard Franz, Florida Museum of Natural History). Adult isopods have been observed carrying up to forty offspring.

Hydrobiid snails collected from the cave system in the same areas in which stygobitic crustaceans were found were identified as *Littoridinops monroensis* (Frauenfeld). These vouchers are likely the first for this species from a cave system (personal communication, Fred Thompson, Florida Museum of Natural History).

Near the explored limits of the Main Street Tunnel, small (1 cm tall) Sabellidae worms were observed at depths greater than 30 m in areas with salinities that range from 15 to 25 ppt. Sediment samples were collected in this area and several specimens were identified including: Sabellidae, subfamily Fabriciinae, *Fabriciola* sp.; Nereididae; Polychaeta, Ampharetidae, *Hobsonia florida* (Bansae 1979); Polychaeta, Ampharetidae, *Paramphicteis* sp. (Caullery 1944); and Polychaeta, Nerillidae, *Nerilla digitata* (Wieser

1957 in Kristeuer 1969). The *Fabriciola* sp. specimens were the first recorded from Florida for this genus (personal communication, Tom Perkins, Florida Marine Research Institute). The *Fabriciola* sp. were further identified as a new species and the first record of the subfamily from a subterranean habitat (personal communication, Kirk Fitzhugh, Los Angeles County Museum of Natural History).

Sediment samples from areas with the worms also contained specimens of hydroids and clams. The hydroid is *Cordylophora caspia*. This species typically inhabits water with salinity up to 7 ppt; but has been maintained in laboratories at 30 ppt. This voucher may be the first from a natural environment with such high salinities (personal communication, Dale Calder, Royal Ontario Museum). The clams were identified as *Mytilopsis leacophaea* (personal communication, Fred Thompson, Florida Museum of Natural History).

American eels (*Anguilla rostrata*), which breed in saltwater, have been observed in the Beacon Wood's cave system near Round Sink. Blue crabs (*Callinectes sapidus*) and striped mullet (*Mugil cephalus*) have been observed in Wayne's World Sink. These observations indicate the Beacon Woods and Wayne's World cave systems are still connected to the Gulf of Mexico by passages large enough for the eels and fish to navigate.

Orange bacterial "jellyballs" are common throughout the system. They are most often observed near the halocline as they are denser than brackish tannic water and lighter than saltwater. At the entrance to the Deep Salt Tunnel just below the average halocline depth of 30.5 m, the rocks in the cave are coated with a layer of orange bacteria, which may be coming loose in currents to form the jellyballs. Gray bacterial mats are found in

Palm sink below the chemocline (Figure 1-21). A gray biofilm that forms small fingers about 5 cm long is present on the ceiling at the entrance to Hudson Spring in the small volume of cave accessible to divers.

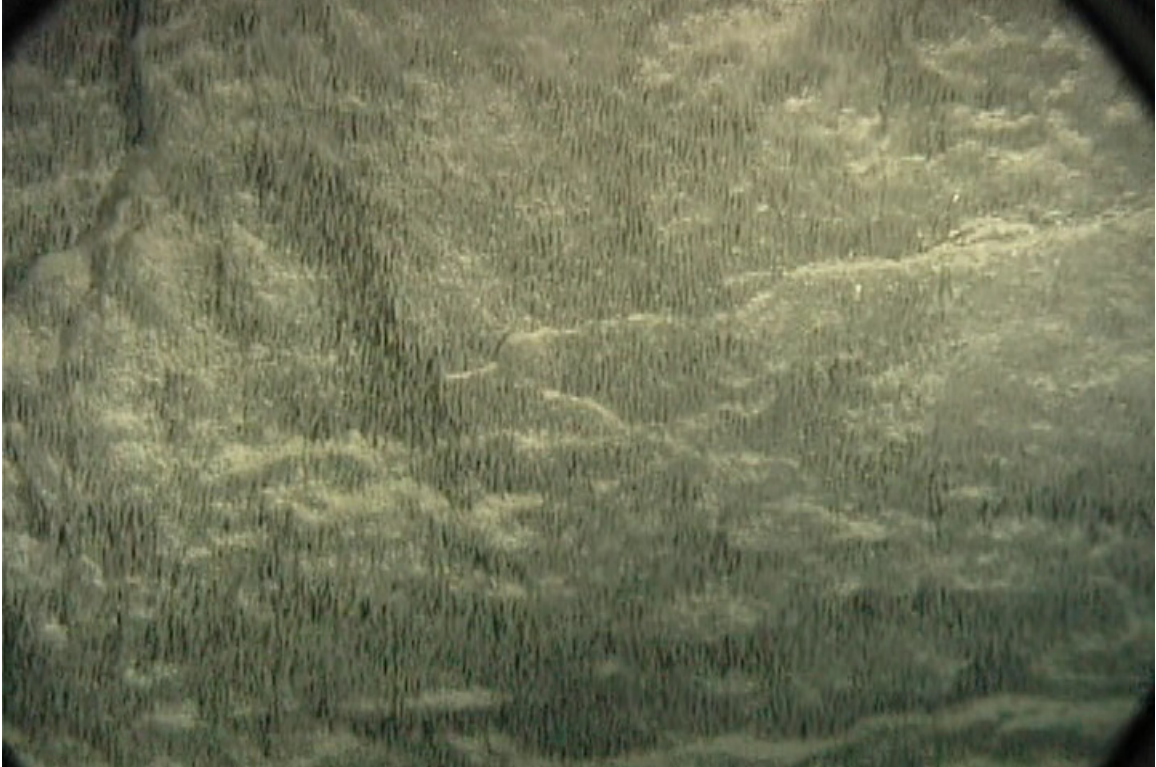


Figure 1-21: Biofilm on the walls of Palm Sink below the chemocline. The biofilm fingers are 5 to 10 cm long and the left to right field of view is about 3 m.

Double Keyhole Spring

Description

Double Keyhole Spring is located at 28°24'55"N, 82°40'31"W at the edge eastern edge of Fillman Bayou near the town of Aripeka. The spring is located in a rural area of Pasco County between a limestone mine and the Gulf of Mexico. The main spring vent is connected to two additional vents by a tunnel at an average depth of 3 m. Below the tunnel connecting the vents, the spring is connected to an extensive cave system with passages that extend to depths over 95 m. The upper passages of the cave system have formed in the Suwannee Limestone (Yon and Hendry 1972; Arthur 1993). The spring

generally has continuous discharge but reverses at the higher high tides associated with full and new moons during the dry season.

Water Quality

The discharge from the spring is tannic, saline water. The typical water quality of the spring is summarized in Table 1-13. See Figure 1-22 for details.

TABLE 1-13: Double Keyhole Spring Water Quality Summary June 2004

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
Spring Vent (2.4 m)	24.3	7.52	17.5	0.59
Cave System (20 m)	23.8	7.55	18.1	0.11
Cave System (40 m)	23.8	7.66	18.2	0.12

The Double Keyhole Spring cave system contains tannic saline groundwater. The tannic water is derived from the coastal wetlands and the salinity of the water reflects the influence of saltwater intrusion from the Gulf of Mexico. The constant temperature with depth is an indication that the water in the cave system has equilibrated with normal groundwater temperatures for the area and is not influenced by rapid influx of Gulf of Mexico saltwater.

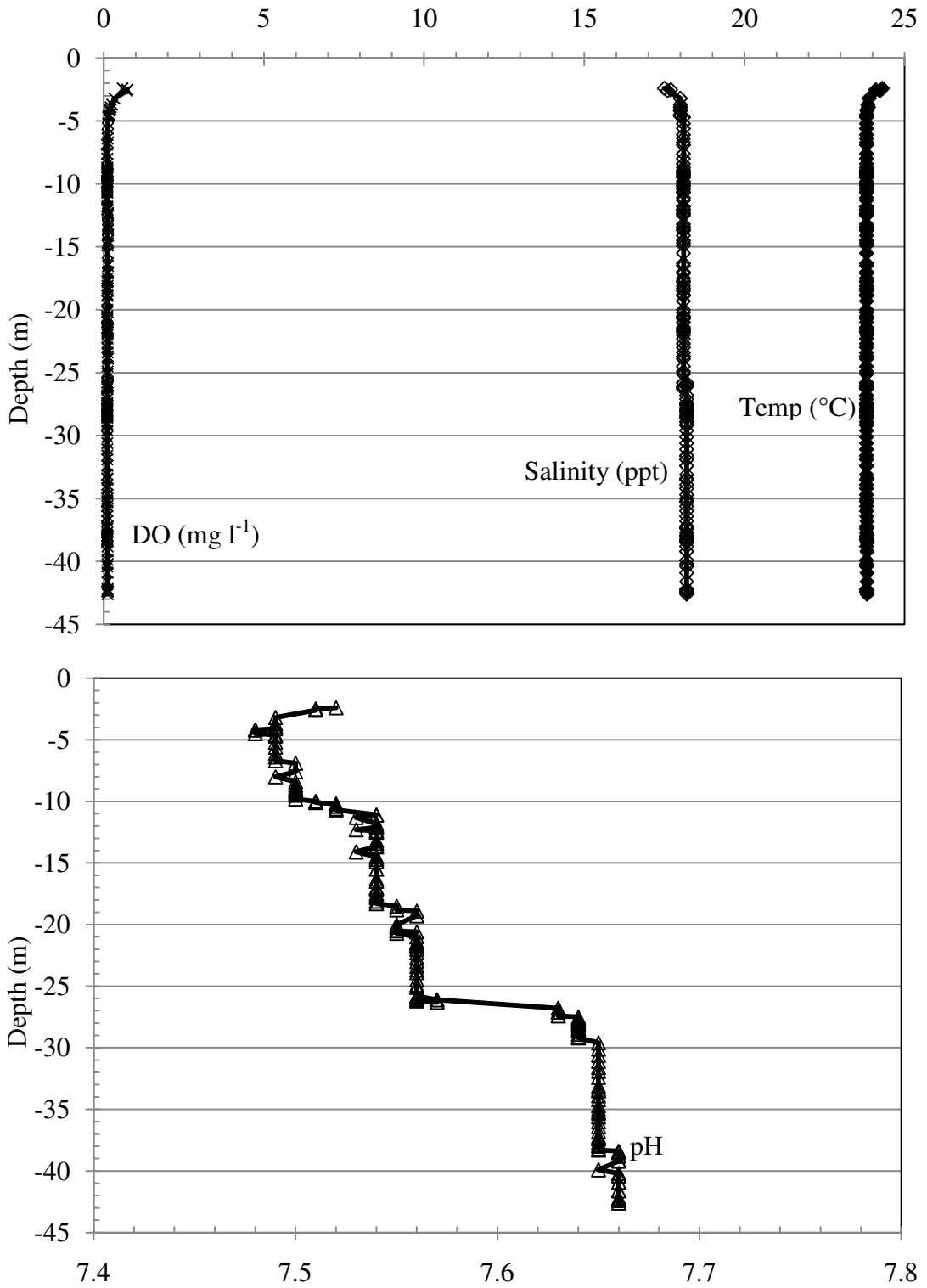


Figure 1-22: Double Keyhole Spring Water Quality Data June 5, 2004

Flora and Fauna

Hydroids and cnidarians have been observed in the cave passages. No vouchers have been identified to date.

Sulphur Spring

Description

Located in Tampa, Florida, at 28°01'15"N and 82°27'05"W, the spring was historically used as a public swimming pool and a manmade pool with a diameter of 15 m was constructed around the spring in the early 1900s. The spring is located in a City of Tampa park and the land surrounding the park is a heavily developed, urban commercial and residential area of Tampa. Diving in the spring was performed by written permission and signed agreement between the research divers and City of Tampa Parks Department.

The pool surrounding the spring was closed for swimming in June 1986 because of bacterial contamination that exceeded Class III recreational water standards. The bacterial contamination of the spring discharge has been attributed to storm-water runoff (Environmental Engineering Consultants, Inc. 1990). Dye trace studies have shown that Sulphur Spring is hydraulically connected to several sinkholes that receive runoff from the streets of Tampa, including: Curiosity Sink, Blue Sinks, Poinsettia Sink, Orchid Sink, and Alaska Sink (aka Tenth Street Sink). Travel times between the sinks and Sulphur Spring were calculated at 55 to 164 m hr⁻¹ (City of Tampa 1958; Stewart and Mills 1984; Burwell 1987; and Environmental Engineering Consultants, Inc. 1990). Burwell (1987) reported that the City of Tampa used clay fill during the construction of a sanitary sewer lift station and the construction had isolated Curiosity Sink from Sulphur Spring so that there was no longer a hydraulic connection.

Currently, the City of Tampa uses the Spring as an emergency water source when the water level in the Hillsborough River is too low to meet demand. Three sinkholes in Tampa that receive storm water runoff from City streets and residences have been hydraulically connected to Sulphur Spring by dye trace studies: Alaska Sink; Orchid Sink; and Poinsetta Sink (Environmental Engineering Consultants, Inc. 1990). The spring emanates from a small vent about 2 m wide by 0.5 m tall at a depth of 7.5 m.

The cave system is developed in the Tampa Member (Limestone) of the Arcadia Formation (Parker 1975). Green clays associated with the confining clay units of the Hawthorn Group are visible in the ceiling of the cave system at joints in the limestone.

Because the cave system is located beneath a developed area of Tampa, Florida, detailed survey information was obtained (Figure 1-23). The 1,029 m of survey data show that the cave system generally trends north. At the main split in the cave passage in the Terminal Room 853 m from the entrance, the Orchid Tunnel continues north while the Alaska tunnel trends southeast.

At a penetration of about 84 m from the cave system entrance, a small side tunnel, the Weak Spring Tunnel, begins. This short and narrow passage includes the Black Room, a room with a chemocline and black deposits. The black deposits have been identified as palygorskite by X-ray diffraction. Beyond the Black Room above the chemocline, the tunnel ends in the Crystal Room, a room with elevated pH and active low magnesium calcite precipitation as identified by X-ray diffraction (personal communication, Chris Elmore, University of South Carolina) (Figure 1-24).

The cave conduit (passage) heights and widths were measured at regular intervals throughout the cave system using a 100 m fiberglass tape. The conduit height to width

ratio was calculated from these data. More than 25% of the surveyed passages in the Sulphur Spring cave system have a height to width ratio greater than 1 compared to less than 10% for phreatic caves in Florida. In fact, the median height to width ratio of the Sulphur Spring cave system is 0.64 compared to 0.33 for phreatic caves in Florida (Wilson 1994). The unusually high height to width ratio of the Sulphur Spring cave system may be related to the upwelling of the sulfidic, saline water into the cave system.



Figure 1-23: Map of Sulphur Spring cave system showing the major tunnels.

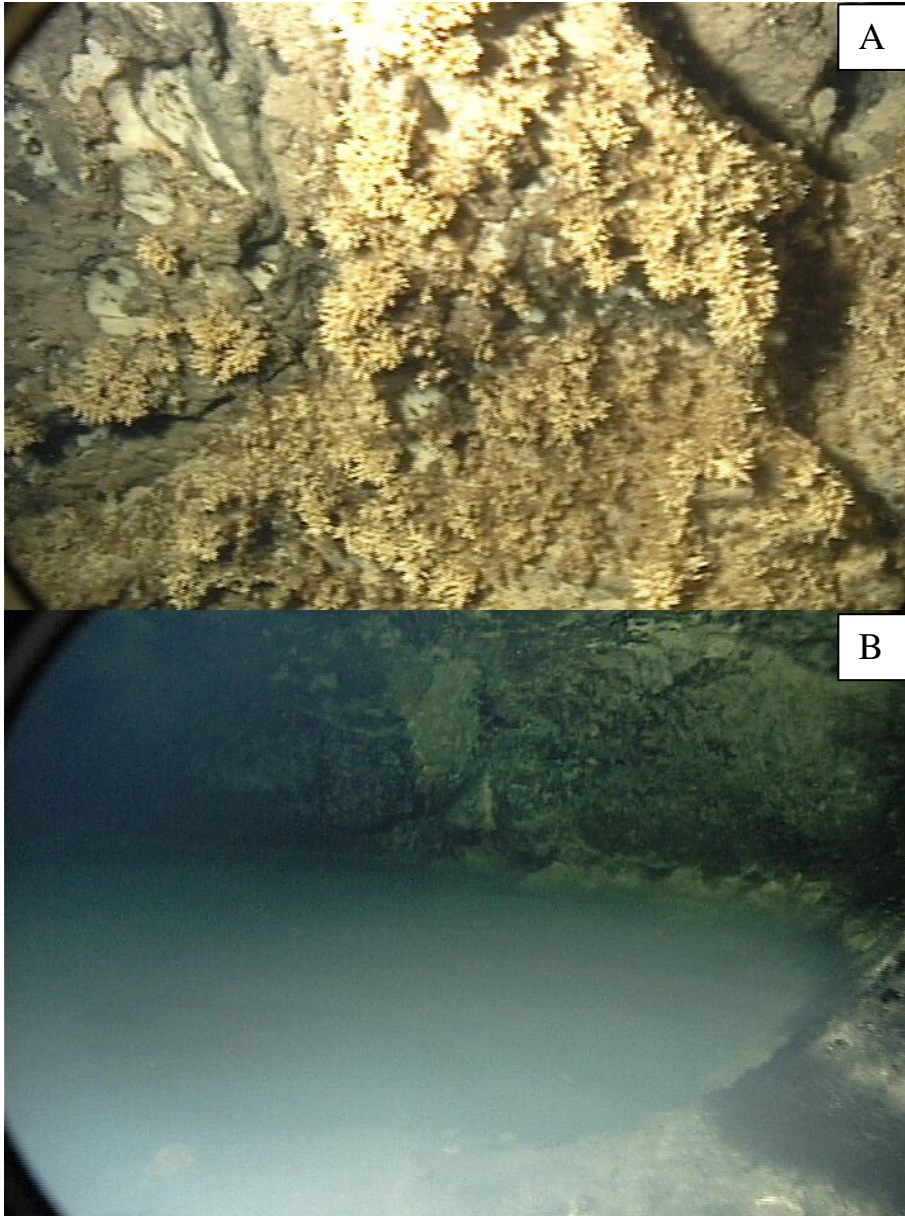


Figure 1-24: Rooms in the Weak Spring Tunnel of Sulphur Spring. Crystal Room has low magnesium calcite crystals on the walls (A). Black Room has particulates in the water at the chemocline and black deposits on wall (B). Field of view is 1 m in (A) and 3 m in (B).

Water Quality

When the Spring discharge pool is pumped for the City water supply, the hydraulic head in the pool is drawn down and the chloride concentration of the Spring water increases. As a result, water can only be pumped from the spring intermittently to prevent excessive chloride levels in the City's drinking water. Average spring discharge over a 15 year period was 1246 l s^{-1} (Rosenau et al. 1977).

Because the spring is used as a public water supply, the City of Tampa tests the water in the spring pool on a monthly basis. The monthly sampling program shows that BOD values in the spring pool are typically less than 1 mg l^{-1} . A water sample collected from the main cave tunnel down gradient of the intersection of the Orchid and Alaska tunnels on November 5, 1997, showed a BOD value of 31.2 mg l^{-1} , indicating that any dissolved oxygen in the cave system would be quickly consumed.

A summary of water quality data collected from the Sulphur Spring cave system is shown in Table 1-14.

TABLE 1-14: Sulphur Spring Cave System Water Quality Summary 1998 to 2001

Parameter	Main Tunnel	Orchid Tunnel	Alaska Tunnel
Temperature (°C)	24.3 to 25.2	24.3 to 25.2	24.3 to 25.3
pH	6.73 to 7.12	6.73 to 7.25	6.62 to 6.97
Salinity (ppt)	1.1 to 2.2	0.9 to 1.4	1.8 to 4.4
DO (mg l^{-1})	0.00 to 0.08	0.00 to 0.08	0.00

From the Spring discharge pool to the Terminal Room, the water in the cave system is well mixed. The only significant change occurs where the Main Tunnel splits into the Orchid Tunnel and the Alaska Tunnel at the Terminal Room. The Alaska Tunnel generally has higher temperature, lower pH, and higher salinity than the Orchid Tunnel. There are discreet saltwater vents scattered throughout the cave system and these vents

are more common in the Alaska Tunnel than other areas of the cave system. The water quality values, especially dissolved oxygen, vary some depending upon the influx of rainwater. The cave system is generally anoxic; but trace dissolved oxygen concentrations were observed within 24 to 48 hours following larger rain events during this study.

Water quality data show that the flows from the Orchid and Alaska Tunnels completely mix within about 60 m of the junction because of the turbulent flow in the cave system. Based on salinity and conservative mixing of the two flows, about 70 to 80% of the flow is from the Orchid Tunnel and 20 to 30% is from the Alaska Tunnel. Following rain events, about 10% of the flow in the cave system appears to be storm water runoff based on the presence of dissolved oxygen at concentrations of 0.6 to 0.8 mg l⁻¹ following heavy rains.

The pH values in the cave system are typically slightly basic when the head in the spring pool is high. When the head in the spring pool is drawn down by pumping for the City water supply, flow from saltwater vents increases and the pH becomes slightly acidic.

One larger saltwater vent (0.2 m diameter) was identified in the Alaska Tunnel approximately 920 m from the entrance. The vent did not flow continuously and when saltwater was observed in the vent there was no measurable flow. The saltwater accumulated in the vent on the floor and was slowly mixed into the flow of the tunnel by the passing turbulent flow. The water quality from the vent was measured on October 14, 2001, and January 6, 2002. The water quality data are summarized in Table 1-15.

TABLE 1-15: Sulphur Spring Cave System Vent Water Quality Summary

Parameter	Vent
Temperature (°C)	25.7 to 26.0
pH	6.39 to 6.47
Salinity (ppt)	14.0 to 17.7
Dissolved oxygen (mg l ⁻¹)	0.00

The saltwater vents discharge acidic saltwater that is significantly warmer than the water in the cave system. It is likely that the vents derive water from deep geothermal circulation.

At the termination of the Weak Spring Tunnel in the Crystal Room, alkaline water with pH of 10.2 to 10.3 is present. This highly alkaline value was confirmed by using two different Datasondes. The very low pCO₂ of 6.7 ppmv is a result of the high pH compared to pCO₂ of 5000 ppmv outside the room in slightly acidic conditions. In this room, low magnesium calcite is being deposited as the high pH has caused the water to become oversaturated with respect to calcite. Ion analyses indicate that the Crystal Room water is depleted in Mg and HCO₃ and contains excess Ca compared to the water below a chemocline in the Black Room outside and downstream from the Crystal Room (personal communication, Stephan Kempe, University of Darmstadt). The water quality readings from this side tunnel are summarized in Table 1-16.

TABLE 1-16: Sulphur Spring Side Tunnels Water Quality Summary

In-Situ Measured Parameter	Weak Spring Tunnel	Crystal Room	Black Room
Temperature (°C)	26.6	25.0	25.5
pH	6.93	10.30	6.81
Salinity (ppt)	3.1	0.7	7.0
DO (mg l ⁻¹)	0.00 to 0.06	0.00	0.00
Laboratory Measured Parameter (mg l⁻¹)		Crystal Room	Black Room
Sodium		491.25	1922.80
Potassium		17.01	60.09
Calcium		116.32	331.40
Magnesium		16.64	160.15
Iron		0.00	0.00
Manganese		0.00	0.00
Strontium		1.11	3.77
Chloride		708.96	2920.05
Fluoride		0.00	0.00
Bicarbonate		31.12	158.64
Bromide		1.47	17.22
Sulfate		149.94	581.28
Nitrate		0.00	0.00
Nitrite		0.00	0.00
Phosphate		0.00	0.00

Flora and Fauna

The walls of the cave system are lined with a dark biofilm about 5 cm thick.

Testing of the biofilm using BART™ reactors showed the presence of sulfur reducing and iron-related bacteria. Iron-related bacteria include: heterotrophic bacteria that create oxygen for use in the oxidation of organic matter by the reduction of ferric oxide to ferrous hydroxide; and chemolithotrophic bacteria that derive energy from the oxidation of ferrous iron (Sawyer and McCarty 1967; Snoeyink and Jenkins 1980).

Evaluation of the biofilm from Sulphur Spring with scanning electron microscopy revealed the presence of freshwater diatoms, bacilli, filaments, and unusual phosphorus- and copper-rich bacteria (Nankivell and Andre 1999). Some of the diatoms likely originated in one of the upstream sinkholes and were flushed into the cave system

where they were entrained within the biofilm. Other diatoms may be heterotrophic and living in the biofilm. Around saltwater vents, the dark biofilm is absent and a white bacterial mat forms (Figure 1-25). The white bacterial mat likely contains sulfur oxidizing bacteria as samples caused a drop in pH when added to a thiosulfate media.



Figure 1-25: Saltwater vent is surrounded by a white bacterial mat. Dark biofilm covering the walls of most of the cave system is visible around the vent. Vent opening is 10 cm across.

Stygobitic crustaceans have been observed only in one small section of the cave system, the Weak Spring Tunnel near the entrance. This side tunnel typically has measureable dissolved oxygen concentrations up to 0.60 mg l^{-1} . Amphipods were observed in this tunnel and vouchers were collected on June 10, 1998. The amphipods have been tentatively identified as genus *Crangonyx* (personal communication, Richard Franz, Florida Museum of Natural History). Stygobitic isopods were also observed in this tunnel but no vouchers have been collected.

Submarine Springs

Crystal Beach Spring

Description

Crystal Beach Spring is located at 28°05'00"N, 82°47'07"W in the intracoastal waterway of the Gulf of Mexico between the community of Crystal Beach and Honeymoon Island. The land in the vicinity of the spring is a heavily developed, residential area of Pinellas County. The Gulf of Mexico near Crystal Beach Spring ranges from 0.6 to 2 m deep and supports a shallow marine ecosystem. Crystal Beach Spring begins as a 0.5 m opening at a depth of 6 m in a cone shaped basin with a diameter of about 15 m in the floor of the Intracoastal Waterway. The spring opening is connected to cave passages that extend to the east beneath dry land and have a mapped length of over 2 km. See Figure 1-26 for a map of the cave system. The cave system has formed in the Tampa Member (Limestone) of the Arcadia Formation of the Hawthorn Group (Heath and Smith 1954; Wetterhall 1965; Arthur and Campbell 1993).

The main tunnel is 3 m tall and 3 m wide at an average depth of 15 m for the first 381 m of penetration. At a penetration of 381 m, the cave opens into a large room, the R and B Room, which is 18 m tall and 34 m in diameter. Near the floor of the R and B Room at a depth of 29 m, there is a halocline and a 1 m layer of water that has salinity two times that of the main flow (5.8 ppt compared to 2.7 ppt). On the floor of the R and B Room below a depth of 30 m, there are several saltwater vents.

Beyond the R and B Room, the cave passage continues at an average depth of 26 m through three smaller chamber rooms to a bedding plane restriction at a penetration of 503 m and a depth of 25 m. At 549 m penetration, the restriction opens to a 4 m tall

tunnel at an average depth of 35 m. Observations made during siphoning events indicate that 579 m penetration is the farthest point normally reached by Gulf saltwater siphoning into the cave system during high tides at the end of the dry season.

At a penetration of 671 m, the main tunnel splits. The right tunnel (as seen swimming into the system) continues at an average depth of 35 m. The right tunnel is a series of rooms separated by bedding planes and includes some large rooms similar to the R and B Room. This tunnel has been explored to a penetration of 945 m and continues beyond that point.

The left tunnel drops down to an average depth of 39 m. At this depth, there is a chemocline separating cooler freshwater from warmer saltwater. This area of the cave is known as the Dragon's Lair. The cooler freshwater on top is flowing toward the cave entrance and the warmer saltwater below is stagnant.

At a penetration of approximately 823 m the left tunnel goes up a chimney to a depth of approximately 18 m. A large room, the Bacteria Room is encountered at a penetration of 975 m. In the Bacteria Room the ceiling is at a depth of 12 m and the only lead out of the room is on the floor at a depth of 34 m. The Bacteria Room gets its name from the fact that many of the recesses in the walls and ceiling are filled with orange bacterial mats. The lead from the Bacteria Room has been explored to a penetration of 1128 m and continues beyond that point.

The general trends of the cave system are to the east and east-northeast. The cave is below dry land at a penetration of approximately 427 m.

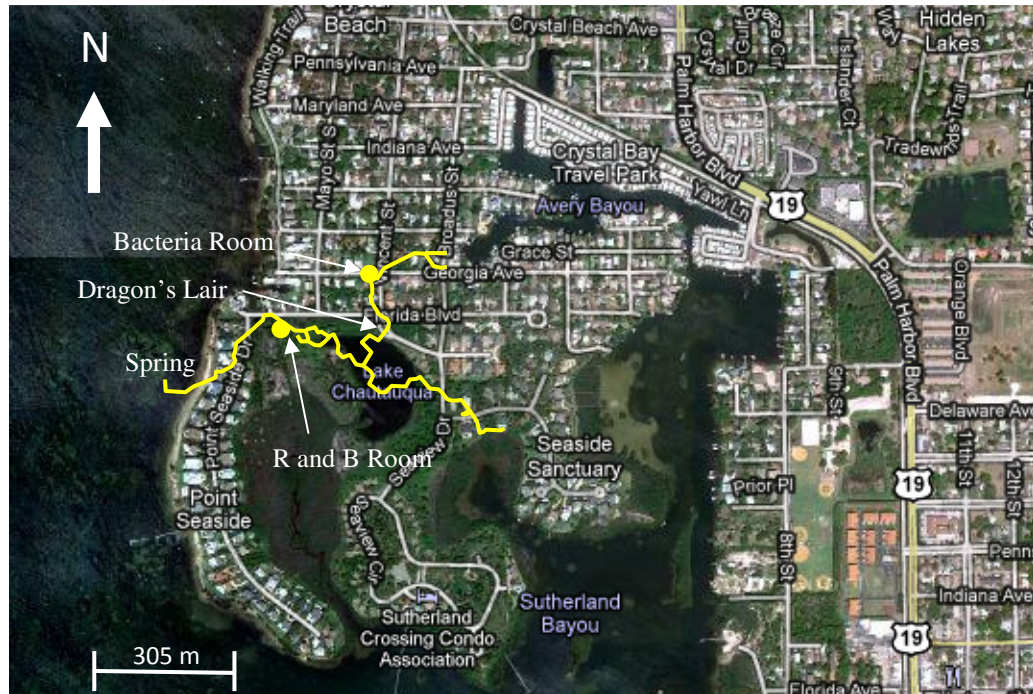


Figure 1-26: Map of Crystal Beach Spring cave system showing the major tunnels.

Water Quality

At peak discharge at the end of the wet season (typically September and October), the spring siphons only during the highest high tides that occur during the full and new moon; and in years of above normal rainfall it may not siphon at all. Discharge from the spring during the wet season in July 2002 showed brackish water discharge with a steady salinity reading of 2.9 ppt with minor siphoning only at the highest high tides associated with the full and new moon phases. Low tide flow rates were measured at 164 to 235 l s⁻¹ (DeWitt 2003). In contrast, at low discharge at the end of the dry season (typically April and May), the spring generally experiences significant siphoning events at least once daily at the higher high tide. Discharge from the spring during the dry season was measured at 4 ppt from the main vent during low tide in December 2001 in the middle of the dry season (DeWitt 2003).

On February 15, 1997, water quality data were collected from the cave system during a 36 hour period in which siphoning events would not affect the readings. In the cave system near the entrance, the temperature was 24.5 °C, pH was 7.16, salinity was 3.3 ppt, and DO was 1.36 mg l⁻¹ compared to readings in the Gulf near the boil of 21.0 °C, 8.09, 26.9 ppt, and 7.45 mg l⁻¹, respectively. Salinity in the cave system dropped from 3.2 ppt to 2.9 ppt across the R and B Room. This is probably because of the presence of saltwater vents in the floor of the room. The freshest water in the system with salinity of 1.6 ppt was in the Dragon's Lair above the chemocline. As the fresher water flows over the interface with the saltwater, turbulent flow and friction causes some mixing of the saltwater into the main flow of the cave system.

On February 22, 1997, a siphoning event occurred prior to the dive. Near the entrance to the cave system water quality readings were 23.6 °C, pH of 7.75, 13.3 ppt, and 5.05 mg l⁻¹, which demonstrates the effects of the siphoning saltwater by elevated pH, salinity, and DO readings and a decreased temperature reading compared to the February 15 data. The effects of the saltwater intrusion on temperature, pH, salinity, and DO were evident until a penetration of approximately 579 m into the cave system. In the Dragon's Lair, the Datasonde readings and laboratory data showing cooler fresher water flowing over warmer more saline water are summarized in Table 1-17. See Figure 1-27 for details.

TABLE 1-17: Dragon's Lair Water Quality Summary

Parameter	Above Chemocline	Below Chemocline
Temperature (°C)	24.3	24.9
pH	7.19	6.61
Salinity (ppt)	2.29	33.3
DO (mg l ⁻¹)	1.23	0.00
Ammonia as N (mg l ⁻¹)	0.047	0.112
Nitrate as N (mg l ⁻¹)	2.691	2.391
Nitrite as N (mg l ⁻¹)	0.0	0.0
Total Organic Carbon (mg l ⁻¹)	1.64	1.82
Orthophosphate (mg l ⁻¹)	0.066	0.069
Total Phosphate (mg l ⁻¹)	0.077	0.092
Iron (mg l ⁻¹)	0.22	0.57
Calcium (mg l ⁻¹)	65.1	546
Magnesium (mg l ⁻¹)	70.3	1470
Alkalinity (mg l ⁻¹)	200	300
Sulfate (mg l ⁻¹)	4.2	5100

The fresher water flow toward the spring in the Dragon's Lair is characteristic of Floridan Aquifer water in a coastal area except for the elevated nitrate values. The elevated temperature and salinity and acidic pH of the saltwater in the Dragon's Lair are an indication of deep geothermal circulation (Kohout et al. 1977). The elevated nitrate concentrations are an indication of anthropogenic contamination of the shallow fresh to brackish water aquifer and the deeper saline aquifer in which there is a component of geothermal circulation. The spring recharge likely includes water impacted by the Pinellas County spray effluent discharge field. The dissolved calcium values are lower than the dissolved magnesium values, as expected with active calcium carbonate precipitation at the chemocline.

Dissolved iron is present at relatively low concentrations less than 1 mg l⁻¹ above and below the chemocline; but it is very important in biological and chemical processes in the Dragon's Lair. Pyrite framboids have been observed in sediment samples from the Dragon's Lair indicating that iron is reacting with sulfide in an abiotic precipitation

process. Iron is also involved in the biologic reactions with iron-related bacteria in the biofilm below the chemocline and with the formation of phreatite in the brackish water above the chemocline.

A submersible manometer was used to measure the vertical hydraulic gradient of the saltwater in the Dragon’s Lair. The vertical hydraulic gradient was positive, indicating upward head, each time a measurement was made. There also appears to be some correlation between tidal height and the vertical hydraulic gradient as the vertical hydraulic gradient increased with decreasing tidal height (Table 1-18).

TABLE 1-18: Dragon’s Lair Vertical Hydraulic Gradient Measurements

Date	Δh (cm)	$\Delta h/L$	Tidal Height (m)
March 27, 1999	1.9	0.0089	0.39
March 28, 1999	1.3	0.0061	0.43
April 24, 1999	6.0	0.0280	0.30

The water quality data show that the reduced saltwater seeping into the Dragon’s Lair is distinct from the Gulf of Mexico water at the spring entrance (Table 1-19). These data indicate that the Dragon’s Lair saltwater is not simply from saltwater intrusion but may have a significant geothermal circulation component.

TABLE 1-19: Comparison between Gulf of Mexico Water and Dragon’s Lair Saltwater March 28, 1999

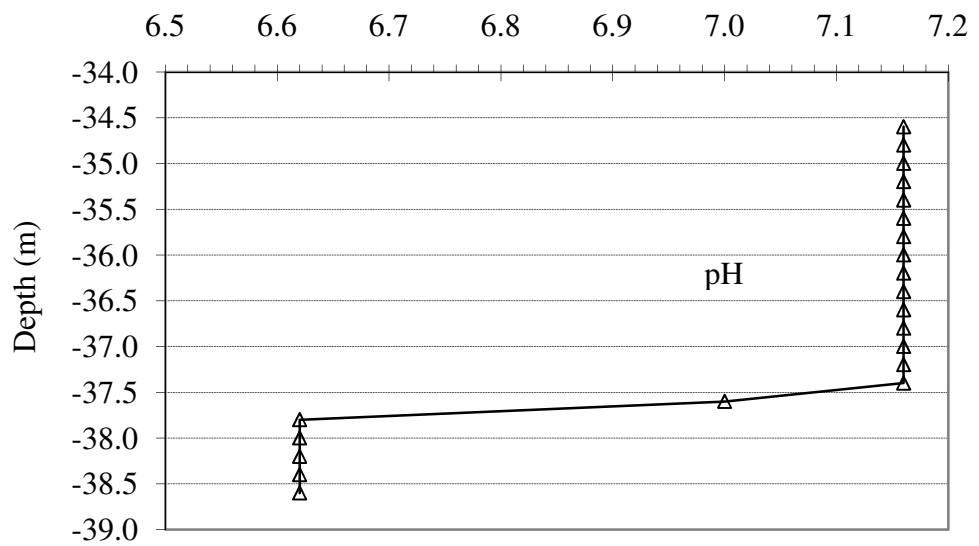
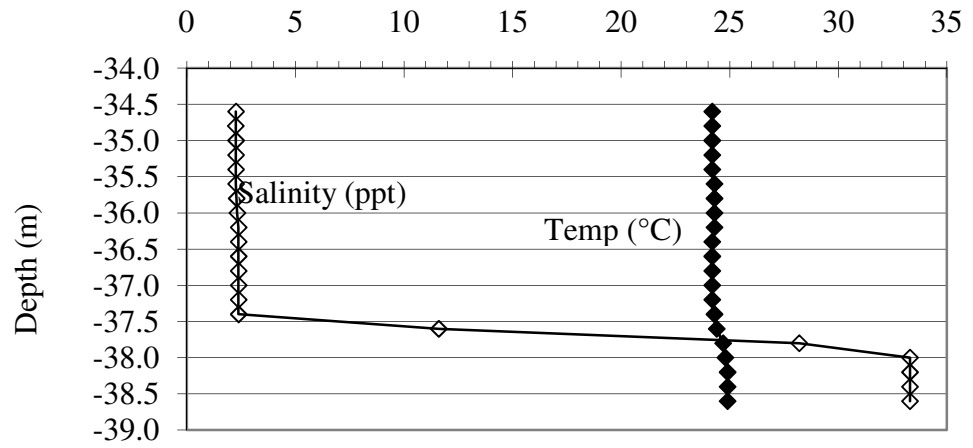
Location	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
Gulf Water	19.4	8.14	33.7	8.24
Dragon’s Lair	24.8	6.60	34.4	0.00

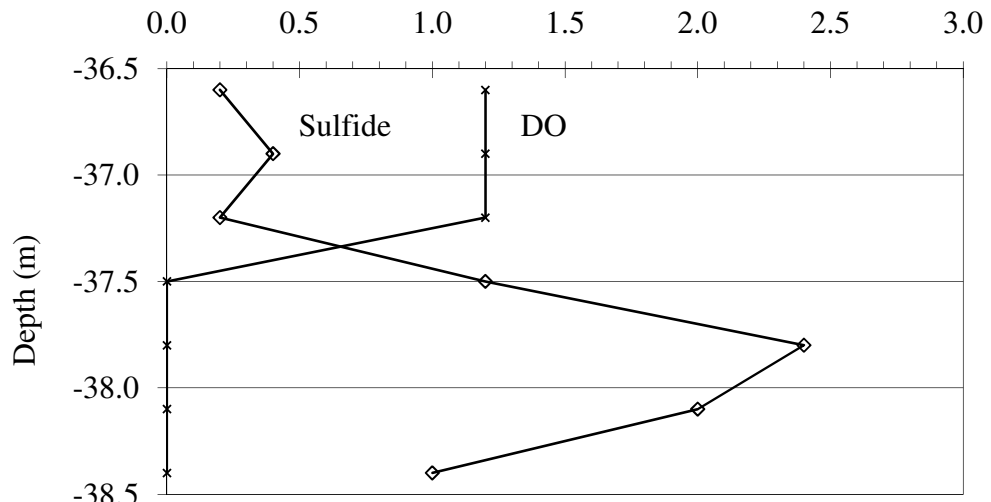
The spring flow in the cave system is influenced by the saltwater in the Dragon’s Lair. The turbulent flow in the cave system creates friction at the chemocline and some of the saltwater is mixed into the spring flow as shown in Table 1-20.

TABLE 1-20: Changes in Water Quality across Dragon's Lair

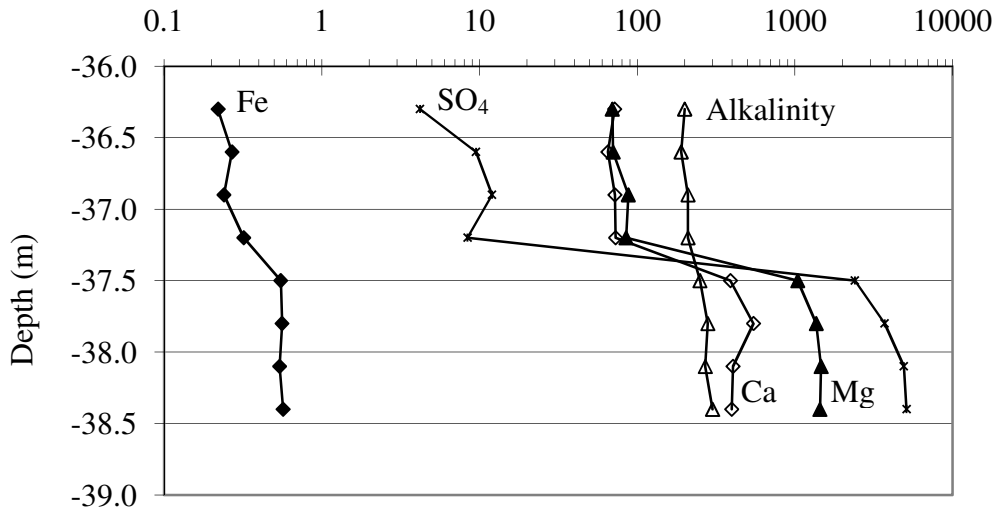
Location	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
Upstream	24.2	7.21	1.7	0.80
Downstream	24.3	7.01	3.5	0.27
Dragon's Lair saltwater	24.8	6.60	34.4	0.00

Flow measurements in the Dragon's Lair were measured at 0.034 m s^{-1} . This value is reasonable when compared to data from karst conduits, as Wilson (1994) reports that water flowing through phreatic cave systems in karst environments has a median velocity of 0.022 m s^{-1} . The total freshwater flow across the Dragon's Lair is calculated to be $0.20 \text{ m}^3 \text{ s}^{-1}$ or 200 l s^{-1} by multiplying the velocity by the average cross sectional area of 2 m tall by 3 m wide. Using a conservative mixing model and a change in total dissolved solids content of the flow across the Dragon's Lair from 2,250 to 4,000 mg l^{-1} , the contribution of the upwelling saltwater with a total dissolved solids content of 33,300 mg l^{-1} to the flow in the cave system is 10.5 l s^{-1} or $0.0105 \text{ m}^3 \text{ s}^{-1}$. Given a floor area of approximately 120 m long by 3 m wide, the specific upwelling saltwater discharge is estimated to be: $q_s = Q_s/A = 2.92 \times 10^{-5} \text{ m s}^{-1}$.





Dissolved oxygen and sulfide concentrations mg l⁻¹.



Calcium, iron, magnesium, alkalinity, and sulfate concentrations mg l⁻¹.

Figure 1-27: Water quality profiles from the Dragon's Lair in Crystal Beach Spring cave system.

Flora and Fauna

Five stygobitic crustaceans have been identified in the cave system: the crayfish *Procambarus* sp., which is found throughout the fresher water in the cave system and *Troglocambarus* sp., which is found in the Dragon's Lair area of the cave system. The *Procambarus* sp. crayfish may be a new species as it does not have the dark pigment eyespots of *P. leitheuseri*, commonly found in West Central Florida cave systems (Figure 1-28). Vouchers of an isopod (*Caecidotea* sp.) and two amphipods (*C. hobbsi* and *C. grandimanus*) have also been collected. These were the first stygobitic crustacean vouchers identified from Pinellas County (personal communication, Richard Franz, Florida Museum of Natural History).

The presence of stygobitic crustaceans in the Crystal Beach Spring cave system, where the low salinity conduits extend beneath the saline waters of the Intracoastal Waterway of the Gulf of Mexico, is significant because it has been previously hypothesized that freshwater crayfish and other crustaceans could survive higher sea levels during interglacial periods of the Pleistocene in freshwater conduits beneath the Gulf of Mexico (personal communication, Richard Franz, Florida Museum of Natural History).

A hydroid, *Garveia franciscana*, is found at a penetration of 30 to 580 m in the cave system. This hydroid is considered an estuarine species normally found in salinities of 0.5 to 15 ppt but briefly tolerating salinities of over 30 ppt (personal communication, Dale Calder, Royal Ontario Museum). Normally hydroids feed on zooplankton indicating that the hydroids in this cave system probably depend upon the periodic siphoning of saltwater for a food supply. The hydroid colonies become dormant during the wet season

when freshwater flow is high enough to prevent the siphoning of saltwater into the system. Some of the largest hydroid colonies are found near saltwater vents on the floor of the R and B Room. Entoprocts have been identified with hydroid colonies found near saltwater vents on the floor of the R and B Room.

Two species of clams, identified as *Mytilopsis leucophaeata* (Conrad 1831) and *Brachydontes exustus* (Linnaeus 1758) are found in the cave system. *M. leucophaeata* are found at a penetration of 60 to 625 m. *B. exustus* is found near the floor of the R and B Room where the water generally has a salinity of 10 ppt, which is higher than the average salinity of the main line part of the cave at 3 to 5 ppt. Hydrobiid snails have also been found in the cave system at penetrations of 60 to 625 m. The snails were identified as *Littoridinops palustris* (Thompson 1968) (personal communication, Fred Thompson, Florida Museum of Natural History).

Sediment samples were sieved to check for the presence of worms. Specimens of *Stenoninereis martini* (Wesenburg-Lund 1958) were identified in the sediment (personal communication, Tom Perkins, Florida Marine Research Institute).

Significant microbial communities exist within the Crystal Beach Spring cave system. Where a halocline forms near the floor of the R and B Room, orange bacterial “jellyballs” collect and stygobitic crayfish congregate around the “jellyballs.” Samples of the “jellyballs” were found to contain fungi and filamentous iron bacteria (personal communication, Robin Brigmon, Westinghouse Savannah River Company Environmental Sciences Section).

Orange bacterial biofilms coat the walls of the cave below the chemocline in the Dragon’s Lair and are also found in isolated pockets within rooms (e.g. Orange Room

and Bacteria Room) in the cave system (Figure 1-29). Samples of the biofilm were analyzed in BART™ reactors and found to include sulfur reducing and iron-related bacteria. Diatoms and diatom tests were observed by scanning electron microscopy in biofilm samples from the Orange Room.

At the chemocline in the Dragon's Lair, there is a white "cloud" of mineral and bacterial particles in the water column (Figure 1-30). Water samples from the "cloud" tested positive for sulfur oxidizing bacteria as the samples caused a drop in pH when added to a thiosulfate media. Dinoflagellates have been observed in water samples from the "cloud" observed under light microscopy. Calcium carbonate particles have been identified by electron microscopy of filtered water samples collected from the "cloud." See Figure 1-31 for scanning electron microscope photographs of calcium carbonate crystals precipitated in the Dragon's Lair.

Scanning electron microscope examination of glass slides placed in the Dragon's Lair showed distinctly different bacterial populations above and below the chemocline. Slides placed above the chemocline and "cloud" were primarily coated by filamentous bacteria with some spherical bacteria. Slides placed above the chemocline in the "cloud" were primarily coated by rod-shaped bacteria. These rod-shaped bacteria are presumed to be sulfur oxidizing bacteria because of their similarity to bacteria grown in and filtered from thiosulfate media. Slides placed below the chemocline were primarily coated by spherical bacteria and filamentous bacteria were absent. See Figure 1-32 for scanning electron microscope photographs of the slides.

Throughout the cave system except in saltwater zones, scattered phreatite formations are found on the walls of the cave system. Phreatite is an iron oxide based

mineral that precipitates underwater and may be deposited by iron-oxidizing bacteria. The pure iron oxide end member is goethite, which is reddish brown. Common accessory minerals include manganese dioxide, sulfates, and alumina (Wilson 1992; Martin 1990). Manganese dioxide is black. The dark brown to black phreatite deposits in the Crystal Beach Spring cave system likely contain manganese dioxide.

Examination of phreatite samples from the upper brackish water zone of the Dragon’s Lair show a granular structure (Figure 1-31). The granular structure suggests possible microbial mediated precipitation, as the mineral appears to be a conglomerate of particles rather than crystals that have grown together in an abiotic precipitation process. Filaments that are possible fungal hyphae are visible in some of the samples.

Total cell counts from the water column in the Dragon’s Lair were generally higher from water samples collected at the chemocline and within 60 cm of the chemocline compared to the remainder of the water column as summarized in Table 1-21. See Figure 1-33 for details.

Table 1-21: Total Cell Counts for Bacteria and Viruses

Location	Bacteria	Viruses
Fresher Water above Chemocline	0.008 to 0.755 x 10 ⁶	0.006 to 0.205 x 10 ⁶
“Cloud” at Chemocline	0.792 to 2.98 x 10 ⁶	0.108 to 1.62 x 10 ⁶
Saltwater below Chemocline	0.045 to 5.13 x 10 ⁶	0.051 to 2.37 x 10 ⁶

The mussels in the cave system are not found beyond the Dragon’s Lair. The farthest point at which mussels have been observed is a penetration of 625 m before the Dragon’s Lair starts. No mussels have been observed beyond the split in the main line in the right tunnel either, possibly indicating that the mussels depend on nutrients from the “cloud” for survival.



Figure 1-28: Stygobitic crayfish of genus *Procambarus* from West Central Florida. A) *Procambarus leitheuseri* from Wayne's World Sink cave system. This species has distinct eyespots. Photo is actual size. B) *Procambarus* sp. from Crystal Beach Spring cave system. This species has no eyespots. Photo is 1.5 times actual size.



Figure 1-29: Biofilm below the chemocline in the Dragon's Lair. The biofilm fingers are about 10 cm long and the field of view is about 1 m.

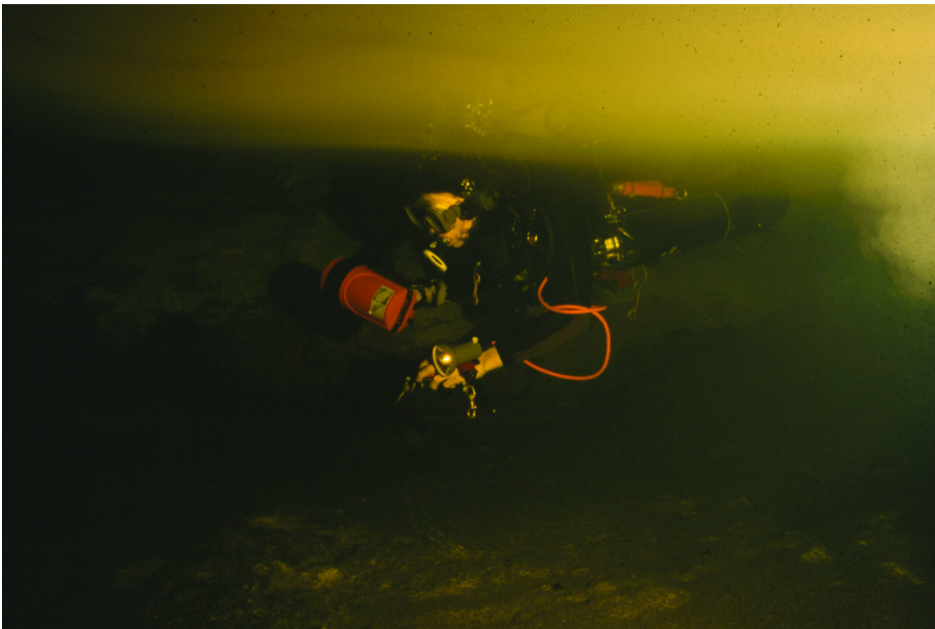


Figure 1-30: Diver below the chemocline in the Dragon's Lair. The particulates in the water column are obscuring the view of the diver's tanks.

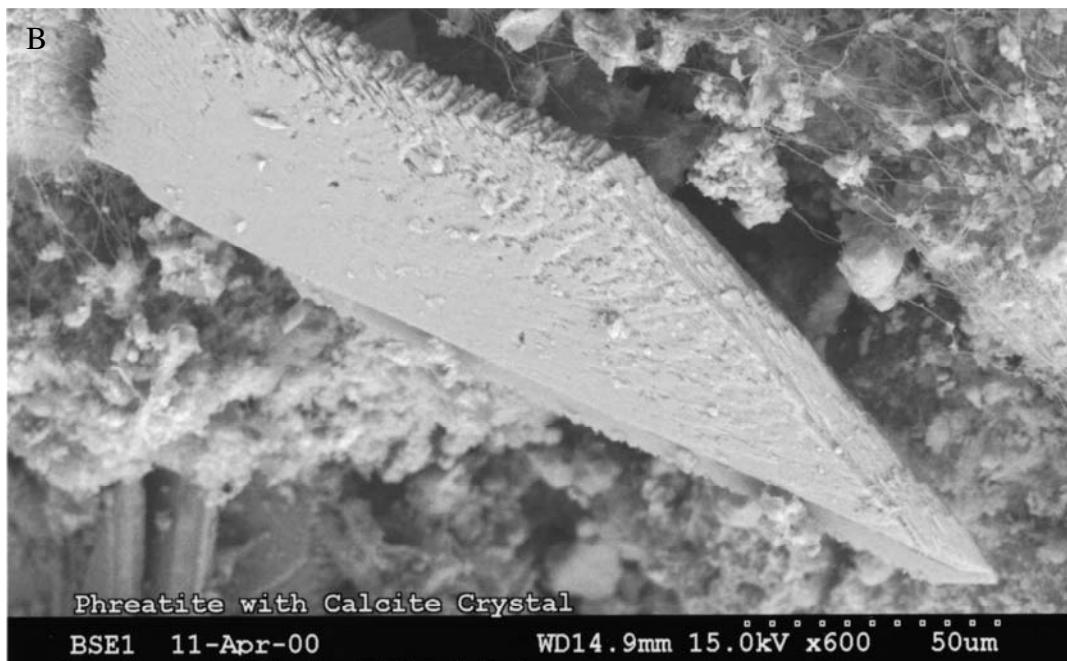
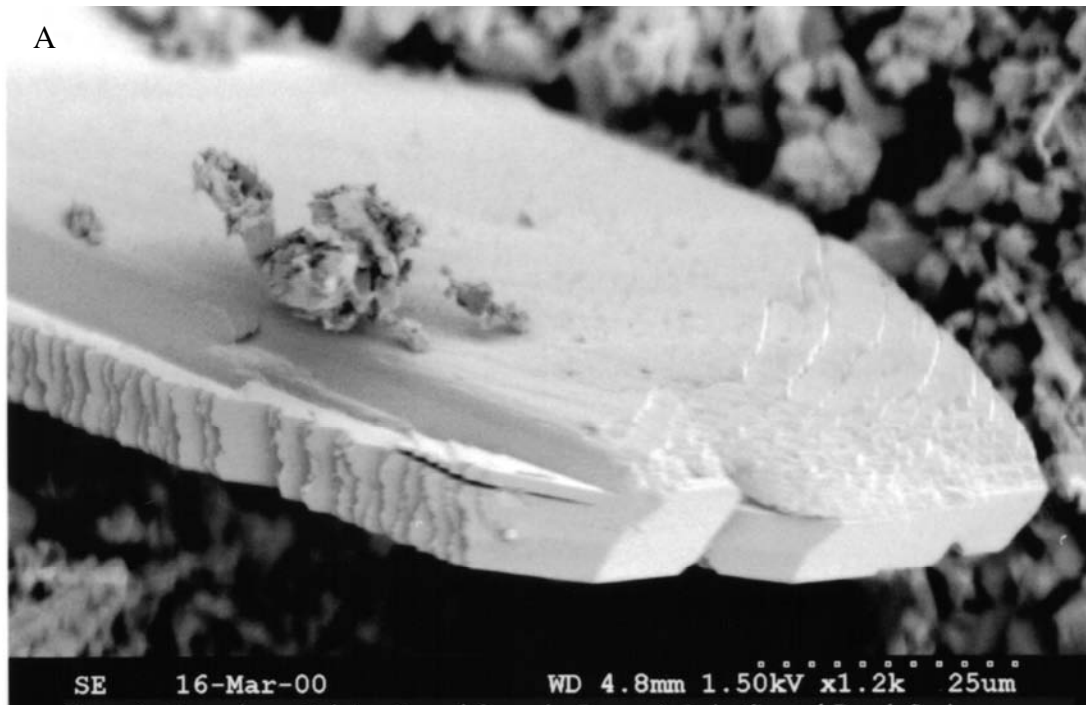
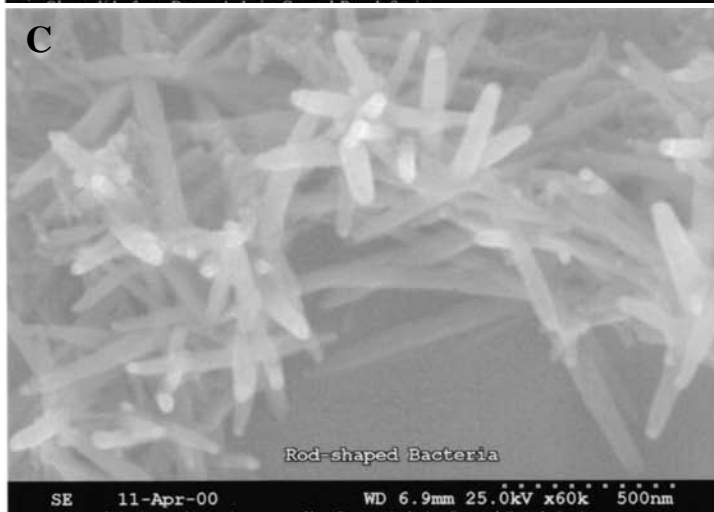
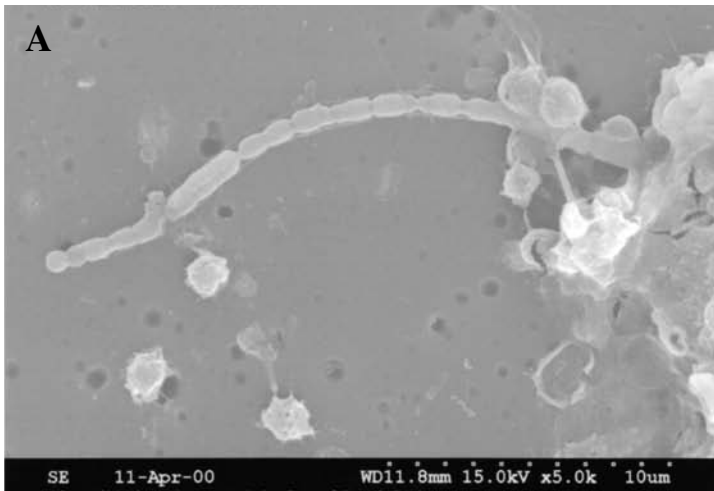


Figure 1-31: Scanning electron microscope photographs of calcium carbonate crystals and phreatite from the Dragon's Lair, Crystal Beach Spring cave system. A) Calcium carbonate crystal deposited on phreatite formation. In foreground, phreatite granules are on top of the calcium carbonate crystal. B) Calcium carbonate crystal on phreatite formation. Filaments of possible fungi hyphae are visible.



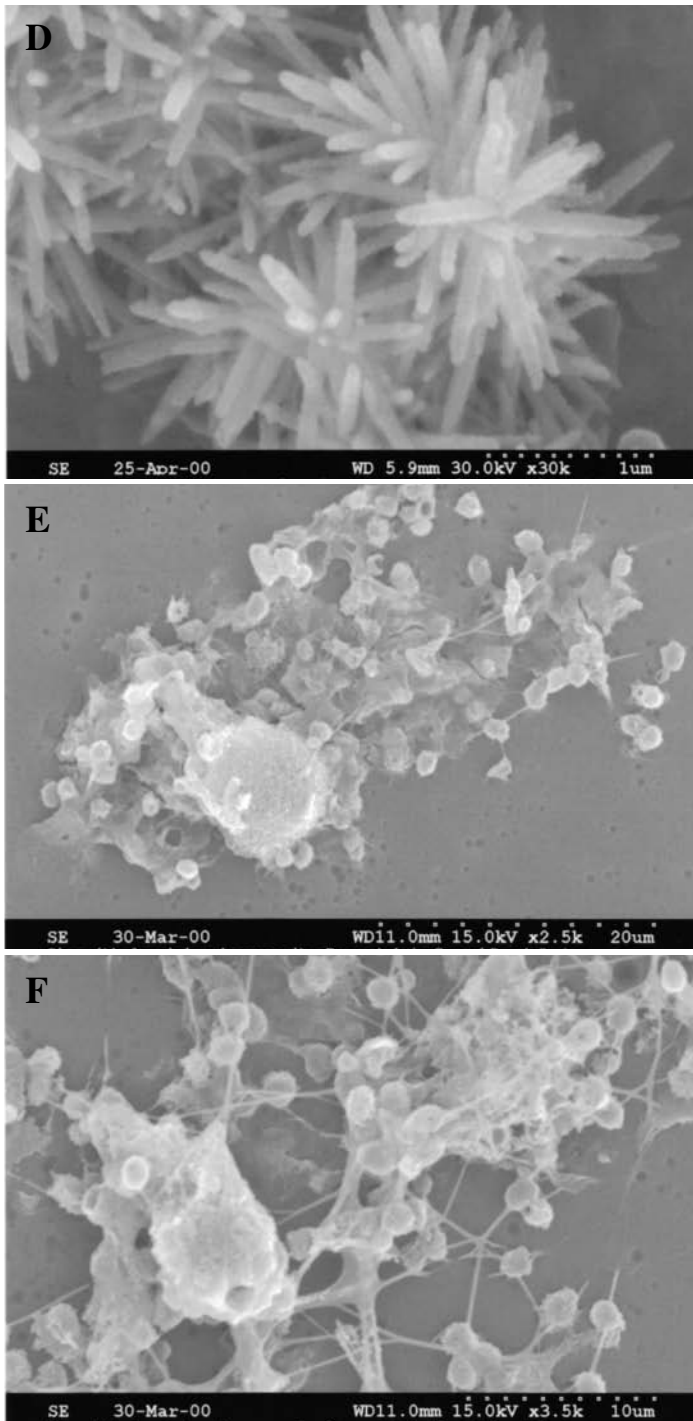


Figure 1-32: Scanning electron microscope photos of bacteria collected from the Dragon's Lair tunnel of the Crystal Beach Spring cave system. A and B) Samples from glass slides placed above chemocline and "cloud" showing filamentous and spherical bacteria. C) Sample from glass slide placed above chemocline in "cloud" showing presumed sulfur oxidizing bacteria rods. D) Sample from filtered thiosulfate media seeded with water from the "cloud." E and F) Samples from glass slides placed below chemocline showing spherical bacteria and no filamentous bacteria.

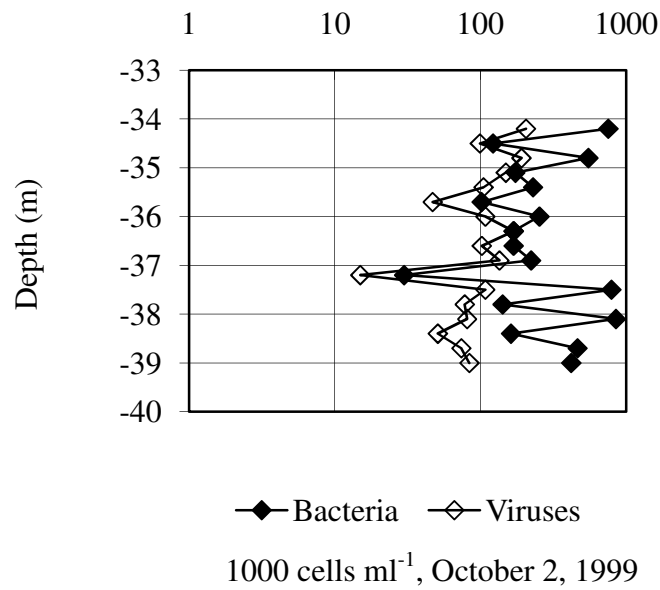
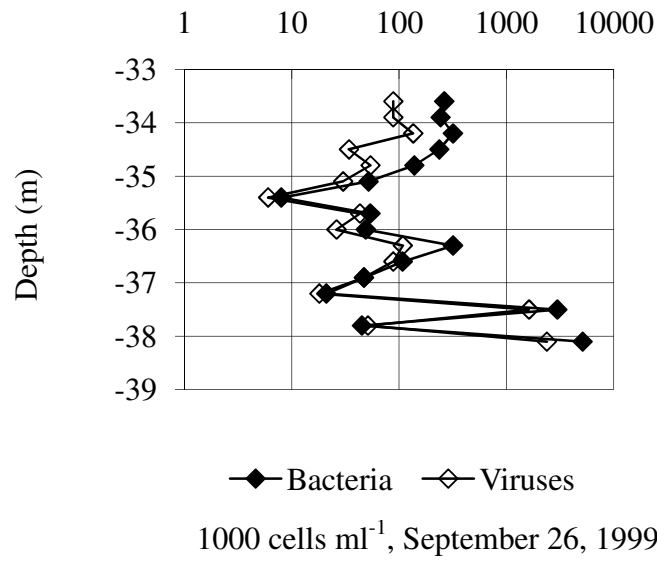


Figure 1-33: Direct cell counts from Dragon's Lair tunnel of Crystal Beach Spring cave system. Sampling stations were spaced 30 cm apart. Chemocline is at -37.5 m.

Indian Island Spring

Description

Indian Island Spring is located at 28°06'06"N, 82°46'38"W in the intracoastal waterway of the Gulf of Mexico between Indian Island and the community of Palm Harbor, Florida. The spring is located between Crystal Beach Spring and Wall Spring. The land in the vicinity of the spring is a heavily developed, residential area of Pinellas County. The Gulf of Mexico near Indian Island Spring ranges from 0.6 to 2 m deep and supports a shallow marine ecosystem. Indian Island Spring begins as a 0.25 m diameter opening in bedrock at a depth of 3 m. The spring opening narrows quickly and there is no cave system accessible to divers beyond the spring vent. The spring discharges from the Tampa Member (Limestone) of the Arcadia Formation of the Hawthorn Group (Heath and Smith 1954; Wetterhall 1965; Arthur and Campbell 1993).

Water Quality

At peak discharge at the end of the wet season (typically September and October), the spring siphons only during the highest high tides that occur during the full and new moon; and in years of above normal rainfall it may not siphon at all. Discharge from the spring during the dry season in March 1998 showed clear, brackish water discharge with a steady salinity reading of 6 ppt and a temperature of 23.3 °C.

Flora and Fauna

The cave system was not accessible preventing the identification of fauna in the cave system during dives performed for this study.

Anoxic Marine Basins

Jewfish Sink

Description

Jewfish Sink has been previously described as a spring (Wetterhall 1965). The basin is located 28°25'43"N, 82°42'30"W in the sandy shallow waters of the Gulf of Mexico about 1 m deep and about 3 km south of the town of Aripeka. In December 1960, discharge from the spring was measured at 14.4 °C and 8 ppt (Wetterhall 1965; Rosenau et al. 1977). The spring probably ceased flowing during the drought of 1961-1962 (Waller 1985). Jewfish Sink begins as round opening with a diameter of about 6 m in the limestone at a depth of 2 m. The sink has a diameter of about 76 m at the maximum depth of 63 m where the rock walls meet the silt mound. The sink is developed in the Suwannee Limestone (Yon and Hendry 1972; Arthur 1993).

Water Quality

Water quality data were obtained from the sink using a Datasonde on 21 dives from 2001 through 2004 (Garman and Garey 2005). The data show dissolved oxygen saturated surface water with salinity of 15 to 27 ppt with a chemocline near 10 m in the summer and down to 30 m in the winter when cooler surface water sinks. Below the chemocline, the sink contains anoxic, sulfidic bottom water. The water quality data are summarized in Table 1-22. See Figure 1-34 for details.

**TABLE 1-22: Jewfish Sink Water Quality Summary
October 25, 2003**

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
2 m	23.9	8.79	19.0	7.55
5 m	23.9	8.76	18.9	7.38
10 m	23.2	8.10	27.4	2.55
20 m	17.9	8.64	29.8	0.19
30 m	16.6	8.76	33.3	0.05
40 m	16.5	8.41	33.4	0.03
50 m	16.6	8.11	33.5	0.00
60 m	17.1	7.80	34.7	0.00

December 26, 2003

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
2 m	14.2	7.90	23.8	8.56
5 m	14.2	7.89	23.8	8.44
10 m	13.3	7.78	27.3	7.30
20 m	13.9	7.71	28.4	5.53
30 m	16.9	7.07	32.7	0.61
40 m	16.6	6.67	33.0	0.28
50 m	16.6	6.57	33.0	0.19
60 m	17.1	6.38	34.1	0.15

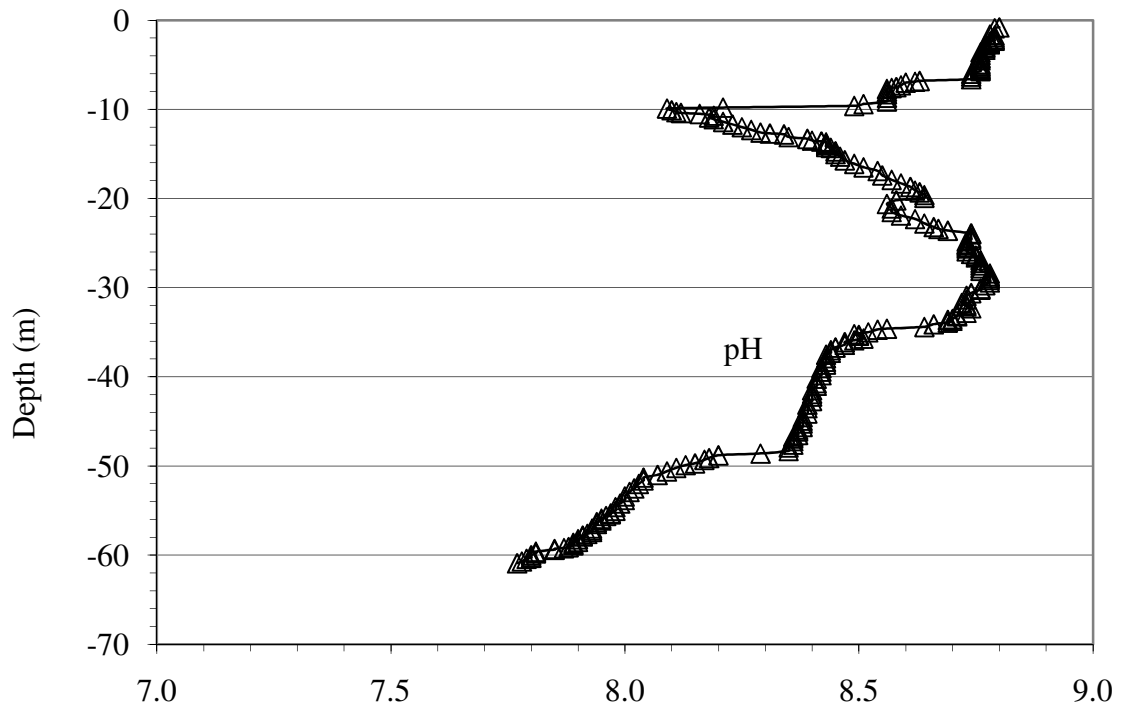
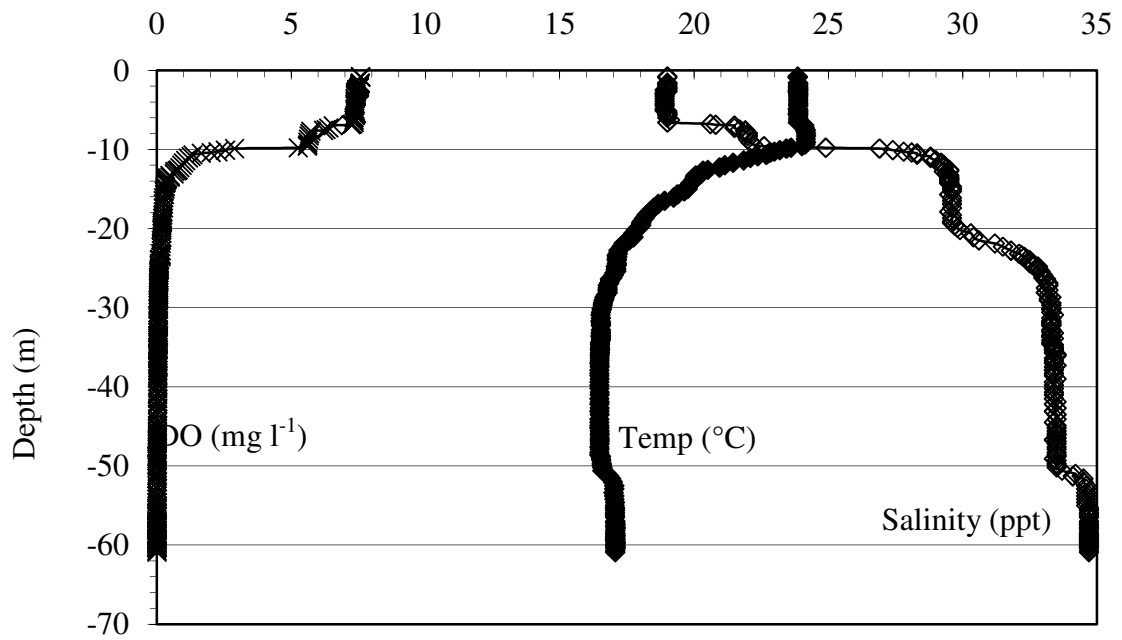
The bottom water in Jewfish Sink typically has a sulfide concentration high enough to cause tearing of the eyes of most divers. The bottom water has temperatures and salinities similar to those at the continental shelf at depths of 100 to 200 m (Garman and Garey 2005). The temperatures in the upper 30 m of the water column show the influence of seasonal heating and cooling while the bottom water temperature is very stable. Salinity values in the upper 10 m also show variability with lower values at the end of the summer rainy season in October compared to dry season values in December.

The acidic pH profile in December is considered a typical winter profile. The low pH values are likely from sinking oxygenated surface water creating a habitat that promotes growth of chemolithotrophic sulfur oxidizing bacteria over a greater depth range in the water column. The chemolithotrophic sulfur oxidizing bacteria convert

sulfide to sulfate decreasing the pH. In contrast, the October profile is considered a typical summer profile in which oxygen is limited to the top 10 m of the water column and there is a distinct chemocline that occurs within a narrow depth range. These conditions limit the depth range of the habitat for chemolithotrophic bacteria resulting in an increase in pH with depth from the accumulation of the byproducts of sulfate reduction (Garman and Garey 2005; Castenier et al. 1999).

Flora and Fauna

The oxic zone in the top 10 m of the water column contains flora and fauna typical of a shallow Gulf of Mexico rock ledge community. A biofilm coats the walls of the sink in the anoxic zones below a depth of about 23 m. The biofilm is thick and forms fingers about 20 cm long.



October 25, 2003.

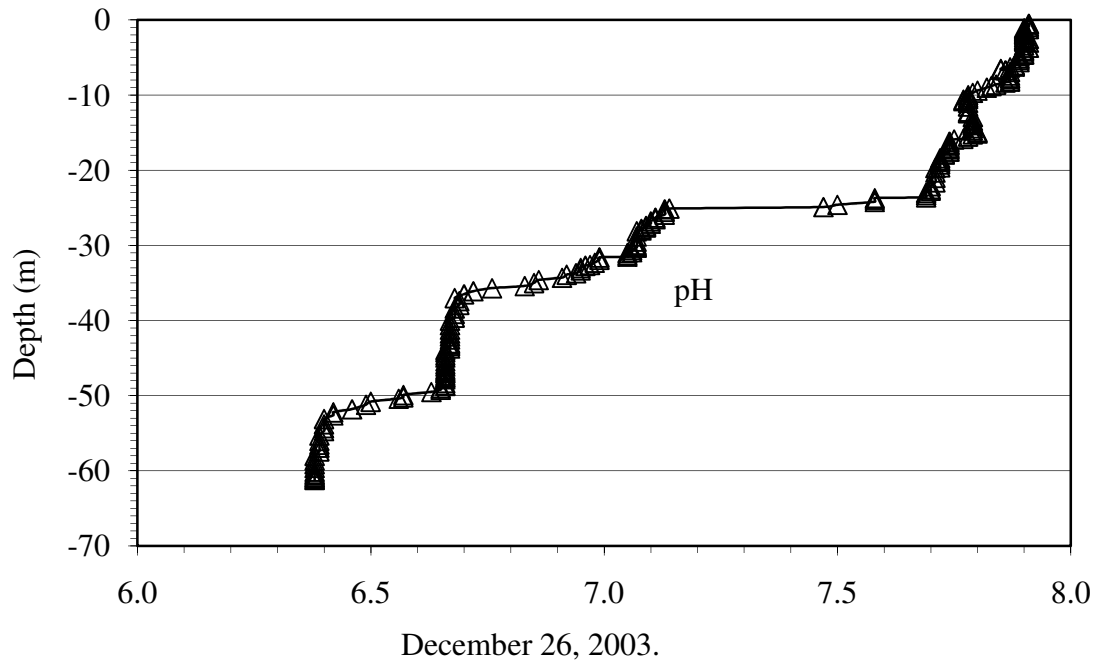
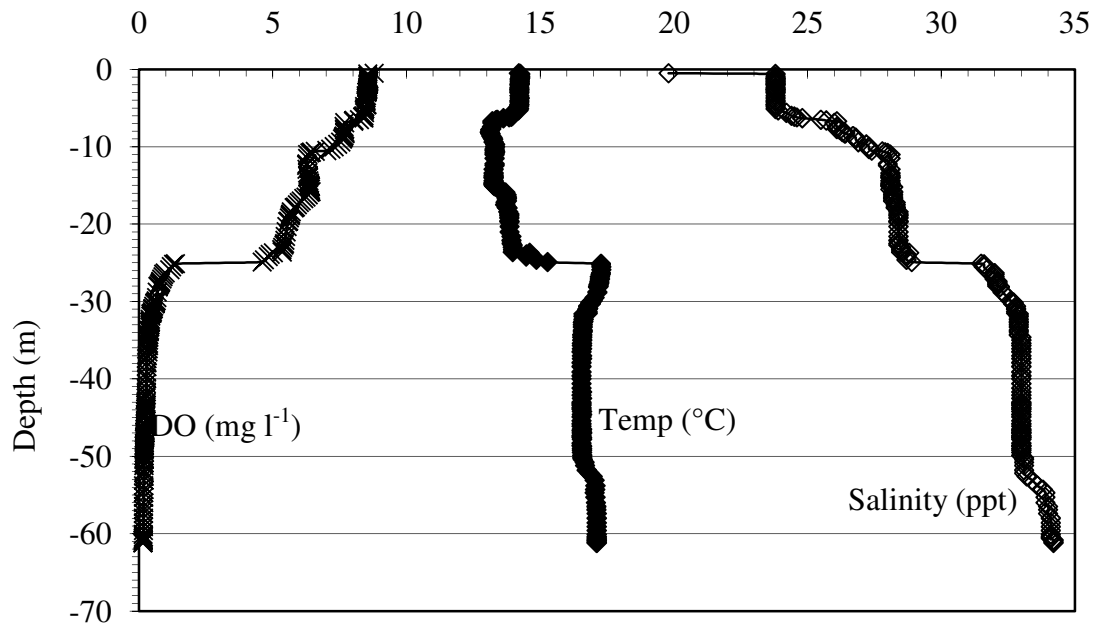


Figure 1-34: Jewfish Sink Water Quality Profiles

Horseshoe Crab Sink

Description

Horseshoe Crab Sink has been previously described as a spring (820-243A, Wetterhall 1965). The basin is located 28°20'25"N, 82°43'45"W in the sandy shallow waters of the Gulf of Mexico about 1 m deep and about 4.2 km southwest of the town of Hudson. In December 1960, discharge from the spring was measured at 15 °C and 15 ppt (Wetterhall 1965; Rosenau et al. 1977). A dye study performed in June 1961 demonstrated a connection between Round Sink and Horseshoe Crab Sink when it was still an active spring. Round Sink is about 5 km from Horseshoe Crab Sink and the straight line travel time was about 152 m day⁻¹ (Wetterhall 1965). The spring probably ceased flowing during the drought of 1961-1962 (Waller 1985). Horseshoe Crab Sink begins as an oval opening with major and minor axes of about 7 m by 4 m in the limestone at a depth of 2 m (DeWitt 2003). The sink basin extends to a depth of 80 m before ending at a silt mound. The sink is developed in the Suwannee Limestone (Yon and Hendry 1972; Arthur 1993).

Water Quality

Water quality data from the sink in October and December 2003 showed dissolved oxygen saturated surface water with salinity of 21.1 to 24.0 ppt with a sharp chemocline between 9 and 13 m and anoxic, sulfidic bottom water. The water quality data are summarized in Table 1-23. See Figure 1-35 for details.

**TABLE 1-23: Horseshoe Crab Sink Water Quality Summary
October 5, 2003**

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
1.9 m	26.0	8.36	21.1	7.18
9.2 m	25.7	8.38	21.7	7.00
12.0 m	24.3	7.05	32.0	0.64
16.6 m (pH minimum)	24.2	7.00	33.5	0.18
62.2 m	23.4	7.14	38.2	0.01
80.1 m	23.8	7.03	38.5	0.00

December 30, 2003

Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l⁻¹)
1.9 m	17.3	8.01	24.0	7.82
12.4 m	16.8	7.32	26.4	5.84
12.6 m	16.9	7.26	26.5	0.16
18.4 m (pH minimum)	22.6	6.40	32.2	0.16
62.2 m	23.4	6.51	37.4	0.07
74.8 m	23.8	6.54	37.7	0.05

The bottom water in Horseshoe Crab Sink typically has a sulfide concentration high enough to cause tearing of the eyes of most divers. The bottom water is hypersaline with salinity greater than normal Gulf of Mexico saltwater possibly indicating the influence of hydrothermal circulation even though temperature readings were within the normal range for aquifer water in the area. The temperatures in the upper 20 m of the water column show the influence of seasonal heating and cooling while the bottom water temperature is very stable. Salinity values in the upper 20 m also show variability with lower values at the end of the summer rainy season in October compared to dry season values in December.

The drop in pH in December compared to October is likely from increased sulfide production from the input of organic matter into the sinkhole as bottom feeding horseshoe crabs and sting rays fall into the sink in large numbers in early spring and late fall (Garman and Garey 2005). The increased sulfide production leads to increased

activity by sulfur oxidizing bacteria, which generate sulfuric acid and decrease pH, near the chemocline.

Flora and Fauna

Below the oxic zone, a biofilm coats the walls and ceiling of the cave system. The biofilm is thick and forms fingers about 20 cm long.

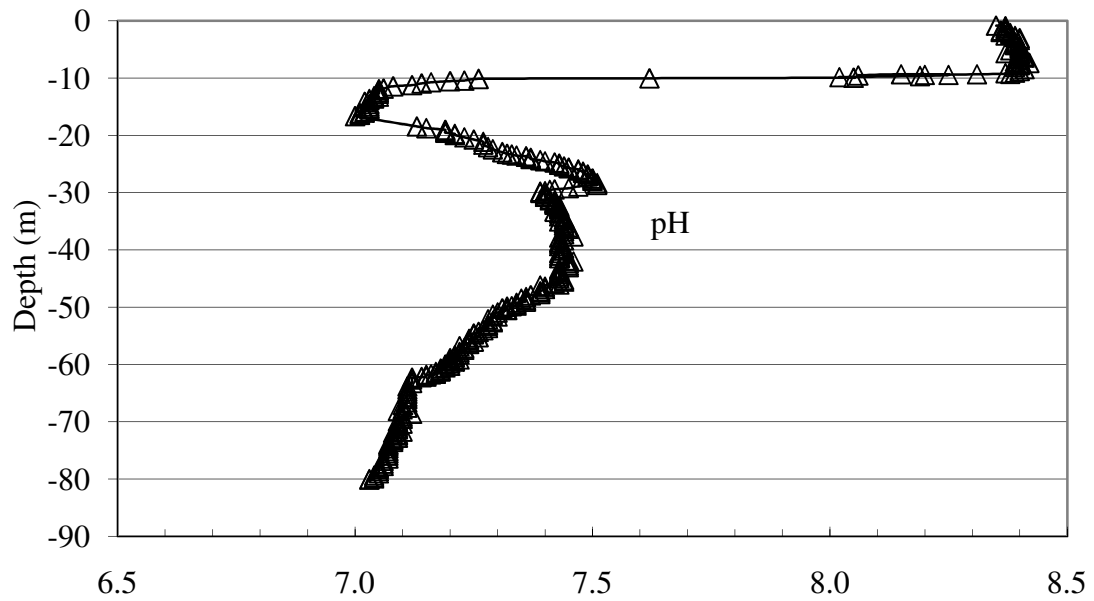
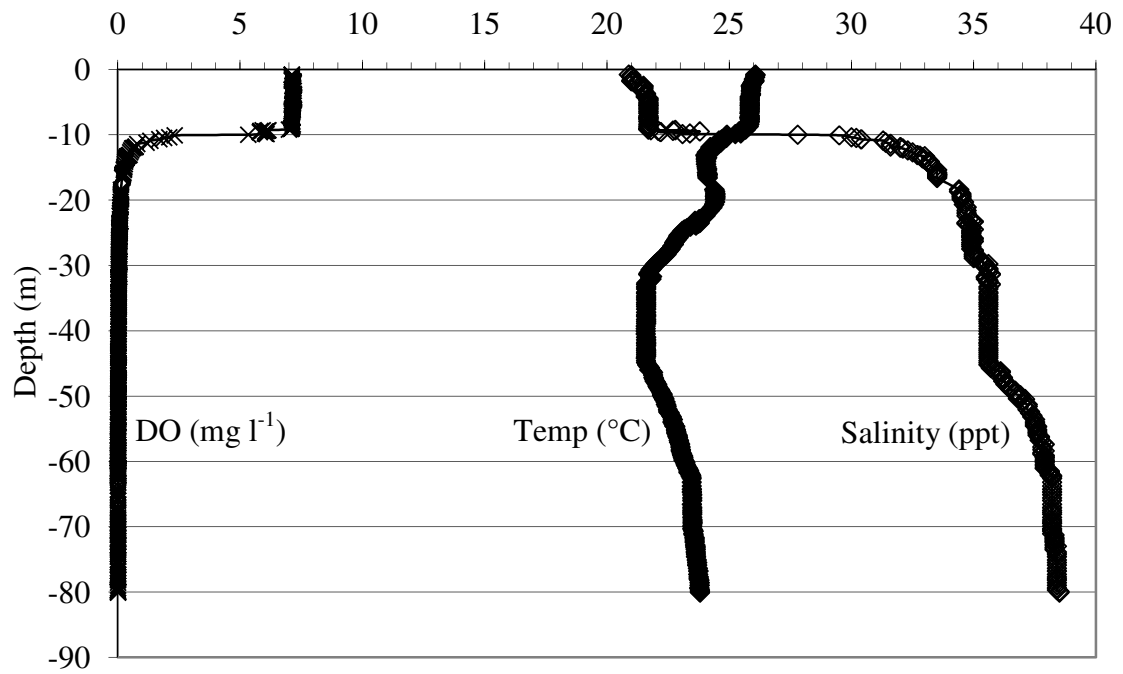


Figure 1-35: Horseshoe Crab Sink Water Quality Profile October 5, 2003

Cedar Island Sinks

Description

Cedar Island Sinks have been previously described as springs. The sinks are located 28°22'52"N, 82°42'26"W in the sandy shallow waters of the Gulf of Mexico about 0.7 m deep and about 1.6 km north of the town of Hudson. In December 1960, discharge from the spring was not measurable; but some flow was indicated in both sinks by a bottom water temperature of 26.2 °C compared to surface water temperatures of 16 to 17 °C. Surface water salinities were 12 ppt at the east spring and 15 ppt at the west spring (Wetterhall 1965). The springs probably ceased flowing during the drought of 1961-1962 (Waller 1985). Cedar Island Sink east begins as an oval opening with major and minor axes of about 8 m by 4.5 m in the limestone and Cedar Island Sink west begins as an oval opening with major and minor axes of about 8 m by 2 m in the limestone. The two sinks are about 13 m apart (Wetterhall 1965). The basins extend to depths of 15 m. The sinks are developed in the Suwannee Limestone (Yon and Hendry 1972; Arthur 1993).

Water Quality

Water quality data from the sinks are summarized in Table 1-24. See Figures 1-36 and 1-37 for details.

TABLE 1-24: Cedar Island Sinks Water Quality Summary May 29, 2004

East Sink				
Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
1.0 m	27.9	8.30	19.6	4.06
5.0 m	19.4	7.78	19.2	1.88
10.0 m	15.6	6.91	21.6	0.00
15.0 m	17.1	6.82	25.6	0.00
West Sink				
Location (depth)	Temperature (°C)	pH	Salinity (ppt)	DO (mg l ⁻¹)
1.0 m	28.0	8.31	19.4	4.05
5.0 m	19.7	7.73	19.4	0.58
10.0 m	15.6	6.95	21.7	0.00
15.0 m	17.1	6.84	25.6	0.00

The water quality data show bottom water in the sinks is likely derived from the sinking of overlying Gulf of Mexico water in the winter. The bottom water is anoxic and sulfidic. The oxidation of sulfide by sulfur oxidizing bacteria has likely created the acidic pH conditions in the sinks.

Flora and Fauna

Below the oxic zone, a biofilm coats the walls of the sinkholes.

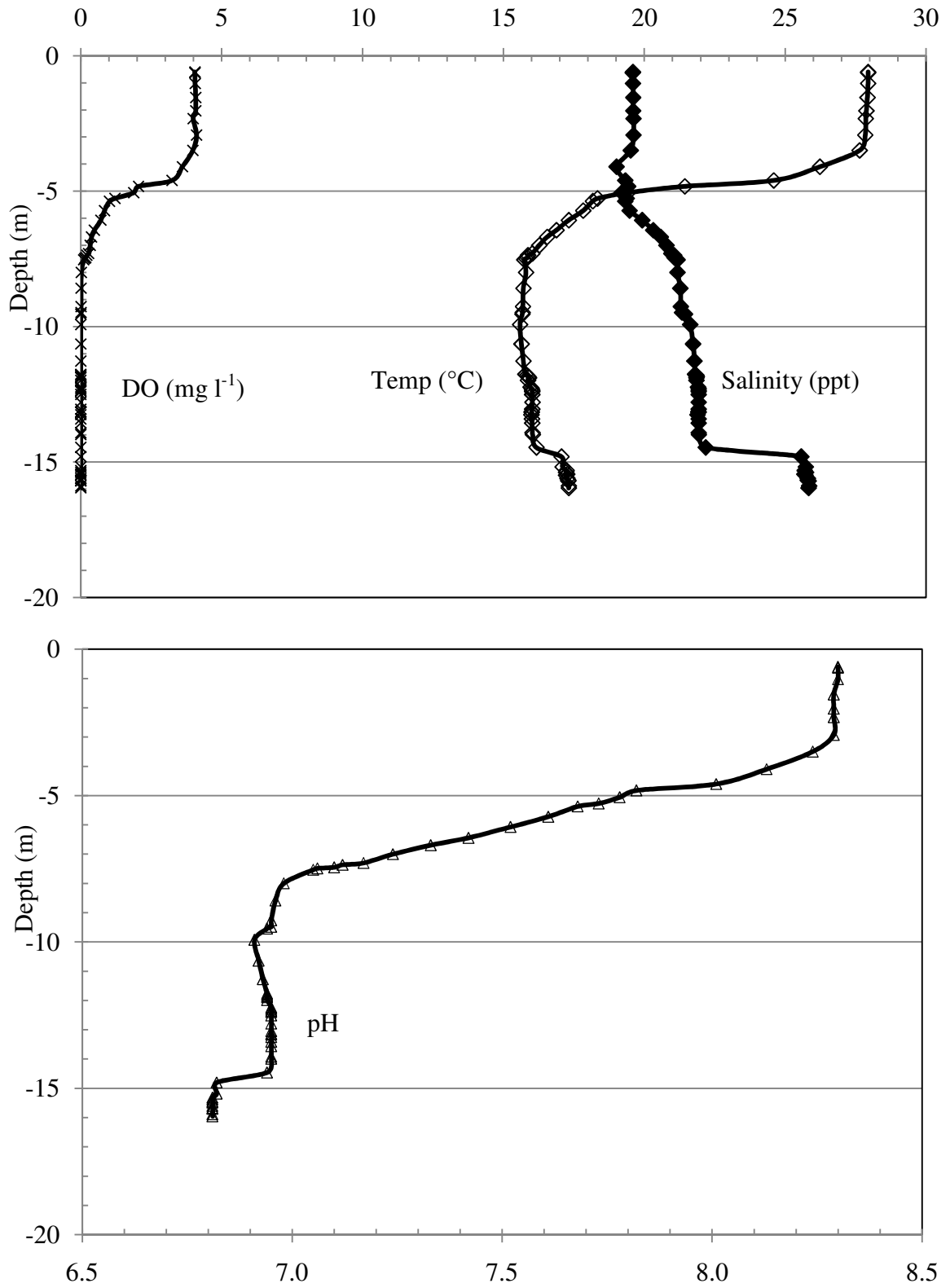


Figure 1-36: Cedar Island Sink East Water Quality Profile May 29, 2004

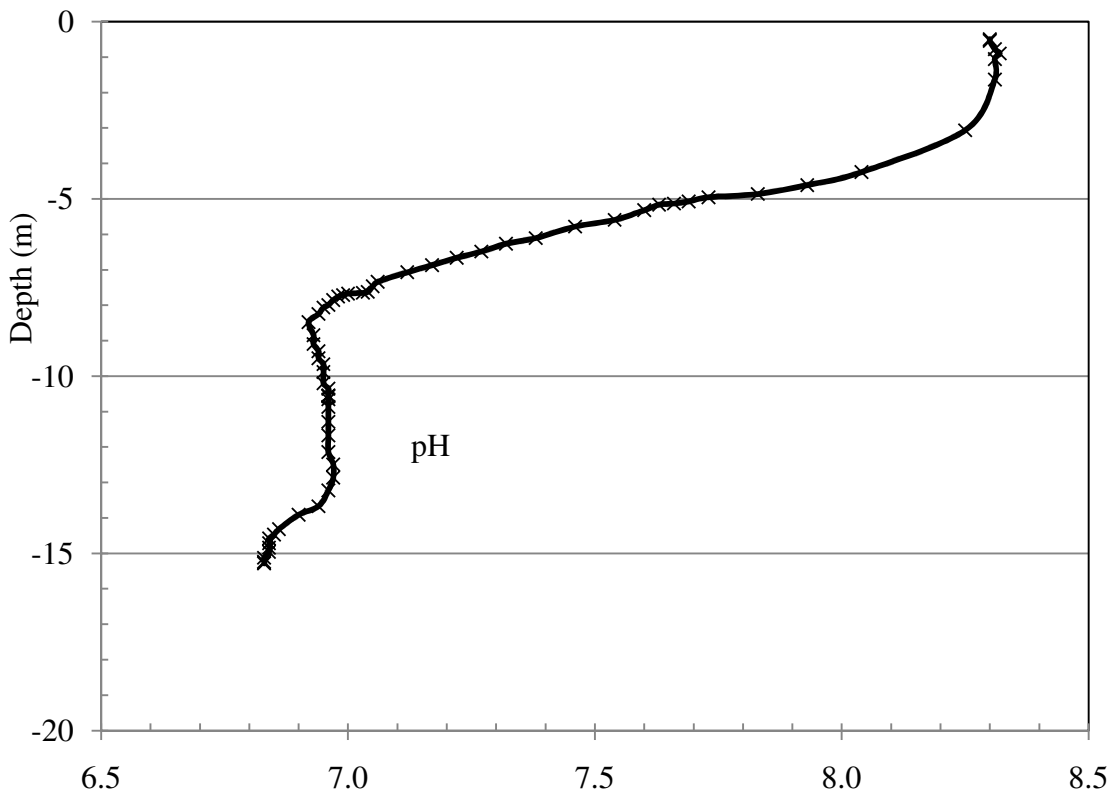
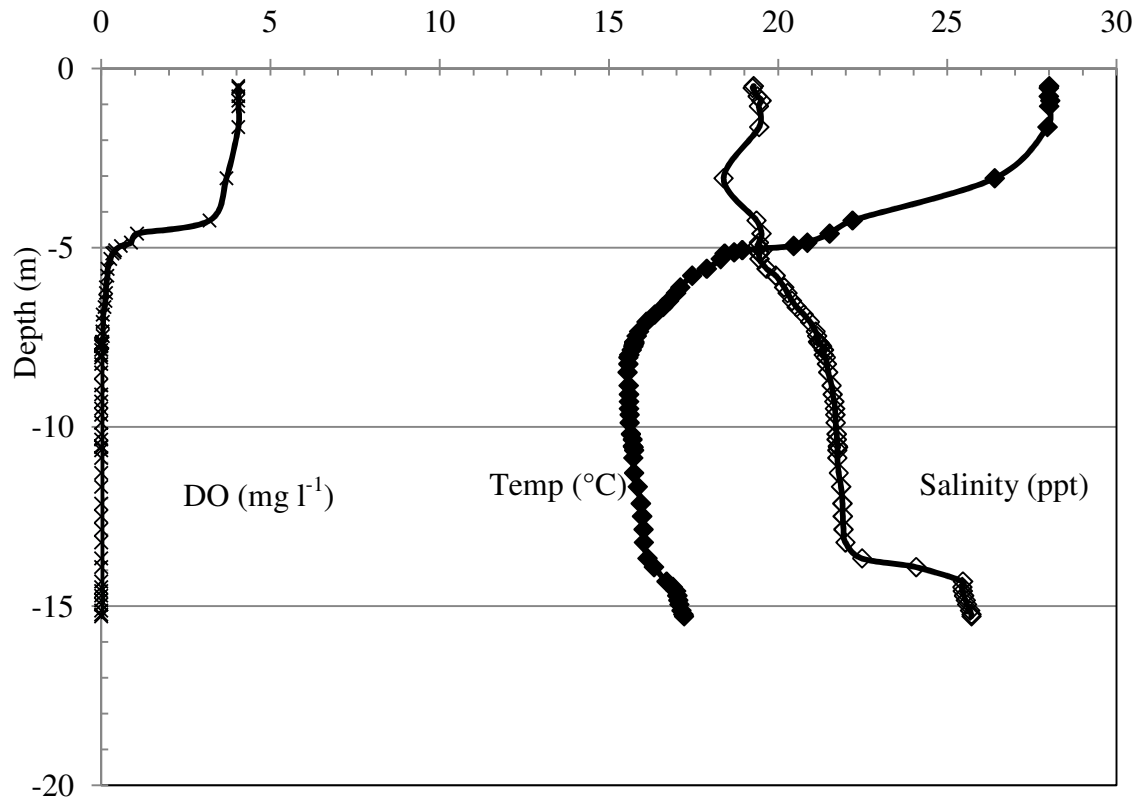


Figure 1-37: Cedar Island Sink West Water Quality Profile May 29, 2004

Summary

The data collected during this study have expanded the known range and locations of the stygobitic fauna in the Gulf Coastal Lowlands assemblage of the Ocala fauna group. The possible new species of crayfish in the Crystal Beach Spring cave system in Pinellas County immediately south of Pasco County may indicate that Pasco County is the southern extent of the Gulf Coastal Lowlands assemblage (Figure 1-38). The crayfish *P. leitheuseri* is the unique species that identifies this assemblage and its absence in Crystal Beach Spring cave system indicates Pinellas County may have its own assemblage of the Ocala fauna group. The stygobitic amphipod and isopod observed in Sulphur Spring have wide ranges and, without a crayfish associated with them, the stygobitic fauna assemblage associated with Sulphur Spring is not defined.

The submerged karst environment of West Central Florida is very complex with coastal mixing of saltwater intruding from the Gulf of Mexico with Floridan Aquifer fresh water combined with potential geothermal circulation of saltwater (Kohout et al. 1977). This complexity is demonstrated by spring discharge and near surface salinity values (see Figure 1-39) and bottom water salinities and temperatures (see Figures 1-40 and 1-41, respectively).

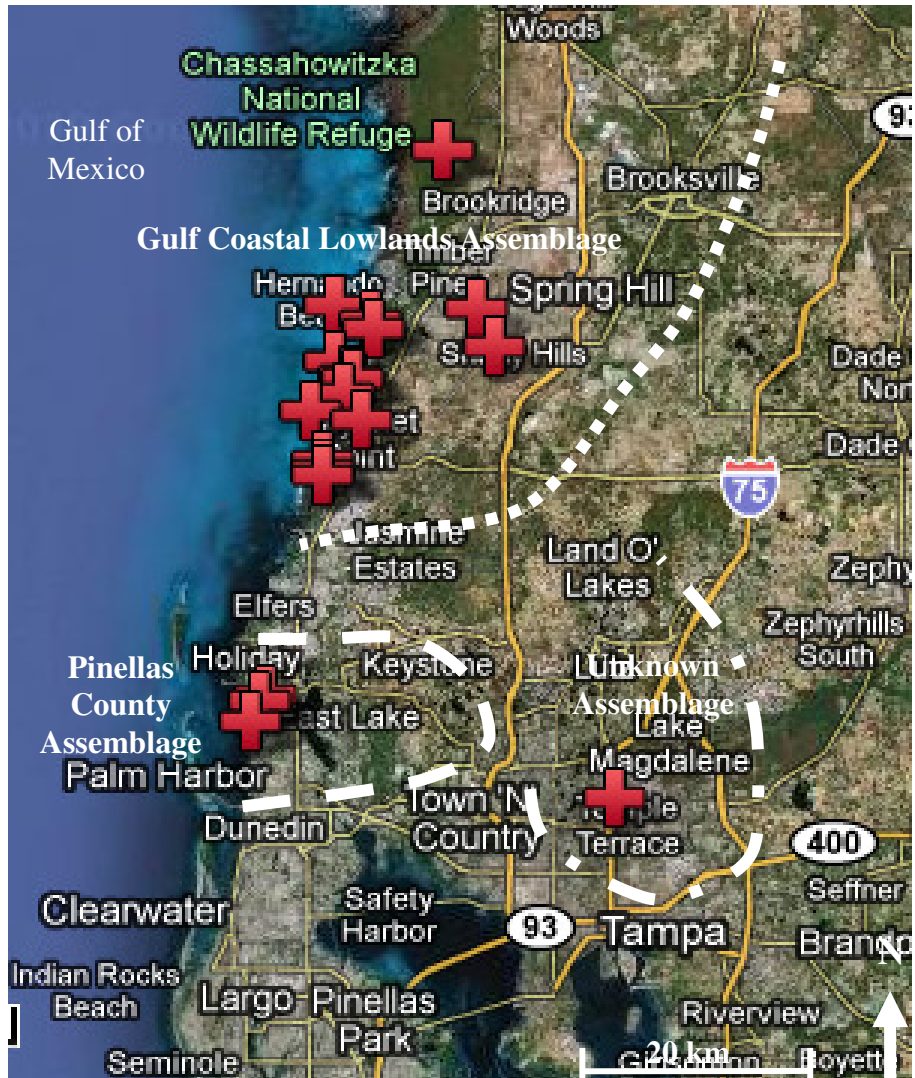


Figure 1-38: West Central Florida Stygobitic Fauna Assemblages. The assemblage within the Ocala fauna group is defined by the crayfish. The Gulf Coastal Lowlands assemblage is defined by *P. leitheuseri*. The crayfish from Crystal Beach Spring cave system in Pinellas County is a new species and *P. leitheuseri* is absent making a new Pinellas County assemblage likely. The amphipod and isopod genera from Sulphur Spring are widespread throughout the Ocala fauna group assemblages. Without a defining stygobitic crustacean, the assemblage in the Sulphur Spring cave system is not defined.



Figure 1-39: Salinity of Spring Discharge and Aquifer Water at 10 m Depth. Values are ppt. Fresh Floridan Aquifer water (<1 ppt) is present at Eagle's Nest Sink, Arch Sink and Ward's Sink. The near surface waters in Palm Sink and Round Sink are also fresh. Sulphur Spring in Tampa and Wall, Indian Island and Crystal Beach Springs in Pinellas County are brackish (1 to 10 ppt). The coastal area of Pasco County is a complex mixture of brackish and saline (>10 ppt) water with shallow Gulf of Mexico water at the anoxic marine basins typically at 19 to 27 ppt compared to coastal spring discharges of 19 to 20 ppt at Salt and Cauldron Springs and 6 ppt at Gar Spring.

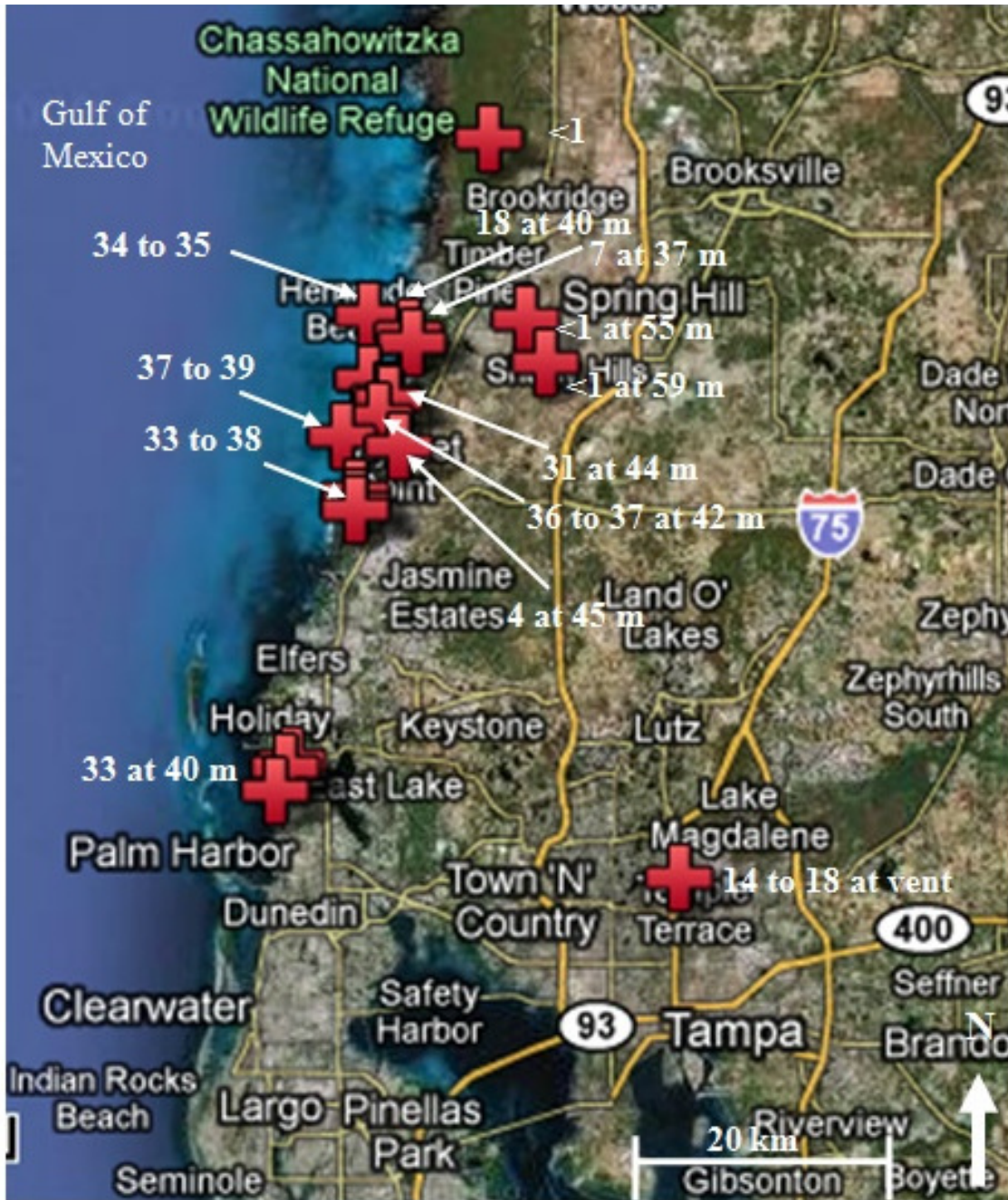


Figure 1-40: Salinity of Bottom Water. Measurement is in ppt at 61 m unless otherwise noted. Fresh Floridan Aquifer water (<1 ppt) is present at Eagle's Nest Sink, Arch Sink and Ward's Sink. The bottom waters in Palm Sink, Salt Spring, Horseshoe Crab Sink, and Crystal Beach Spring are hypersaline compared to shallow Gulf of Mexico water and indicate possible influence by geothermal circulation. Jewfish Sink also contains hypesaline bottom water but the temperatures are much colder than water influenced by geothermal circulation (see Figure 1-41). High salinity values from discrete vents in Sulphur Spring cave system also indicate potential geothermal circulation. Round Sink is inland of the other coastal karst features in Pasco County and has brackish bottom water.

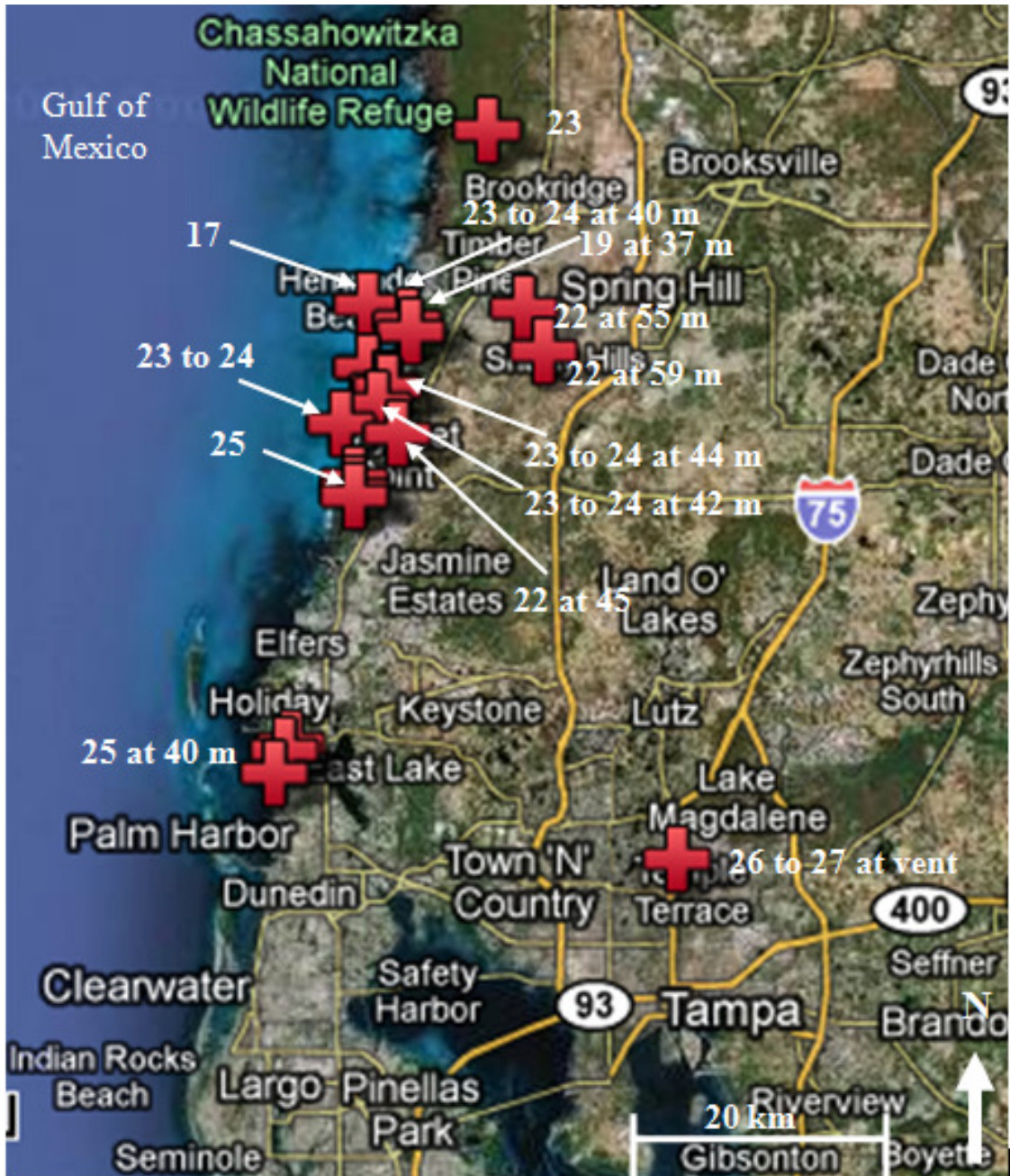


Figure 1-41: Temperature of Bottom Water. Measurement is in °C at 61 m unless otherwise noted. Normal Floridan Aquifer temperatures for the area (22 to 24 °C) are present in all but five of the karst features. The bottom waters of the Crystal Beach Spring cave system and Horseshoe Crab Sink, and the water from the Sulphur Spring cave system vents is significantly warmer than typical Floridan Aquifer groundwater and is likely influenced by geothermal circulation. The bottom waters in Jewfish and Jewel Sinks are much cooler than typical Floridan Aquifer water. The Jewfish Sink bottom water may be influenced by circulation of Gulf of Mexico shelf water through conduits. Jewel Sink may simply be an isolated sink that traps sinking winter surface water.

Conclusions

The type of fauna associated with cave systems in the area have been expanded with the identification of Sabellidae and Polychaeta worms, hydroids, cnidarians and hydrobiid snails in the coastal cave systems. Areas of saltwater intrusion such as found in the Wayne's World Sink cave system contain diverse environments with variable salinity niches from near fresh brackish water to saltwater with salinities equal to those in the Gulf of Mexico. The diverse environmental niches may provide opportunities for isolation and the evolution of new species, as indicated by the new species of Sabellidae identified in the Wayne's World Sink cave system. More detailed macrofauna sampling of coastal cave systems and the varying environments in the systems will likely greatly expand the known diversity of the fauna in these complex environments.

Evidence of geothermal circulation was found in the Salt Spring cave system, the Sulphur Spring cave system, the Crystal Beach Spring cave system, Horseshoe Crab Sink, and Palm Sink. The upwelling of warm, hypersaline, sulfidic water with reduced pH can increase the rate of cave formation by increasing the rate of limestone dissolution. As freshwater use increases, adverse impacts to the Floridan Aquifer will come from increased upwelling of geothermally circulated seawater as well as saltwater intrusion from the Gulf of Mexico.

Geothermal circulation of reduced groundwater and the production of sulfide in anoxic marine basins create conditions in which the sulfur cycle is prominent and the subsequent oxidation of sulfide by sulfur oxidizing bacteria creates acidic conditions accelerating the rate of cave formation. This karst environment is rapidly changing with the overuse of groundwater resources in the area and the potential for rising sea levels

with climate change. The impact of changes to this environment cannot be understood until the hydrology and biology is better known.

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CHAPTER TWO: THE TRANSITION OF A FRESHWATER KARST AQUIFER TO AN ANOXIC MARINE SYSTEM

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Abstract

Jewfish Sink is located in the shallow seagrass flats of the Gulf of Mexico in west central Florida. Jewfish Sink was a submarine spring until the drought of 1961-62 when it ceased flowing. Today, the sink is an anaerobic marine basin and provides the opportunity to study the implications of saltwater intrusion in coastal karst areas. The biogeochemistry of Jewfish sink was studied from summer 2001 through spring 2004. A distinct feature of the sink is the uniform cold temperature (16-17 °C) of the deeper anoxic water that does not match ground water found near shore or onshore (22-24 °C). There are four zones within the sink: oxic zone, transition zone, upper anoxic zone and anoxic bottom water. The anoxic bottom water does not mix with water from above but may be linked to deep Gulf shelf water through ancient aquifer conduits. The other three zones vary seasonally in oxygen, salinity and temperature because of limited mixing in the winter due to cooling and sinking of surface water. The walls of the anoxic zones have characteristic microbial mats that are found in other sulfidic karst features in the area. Bacterial activity appears to be carbon limited in the anoxic zones where sulfate reduction appears to be the major metabolic process. The reduction of sulfate to sulfide appears to be driven by irregular influxes of organic matter including macroalgae,

horseshoe crabs and stingrays that become entrapped within the sink. In contrast, bacterial activity in the oxic zones appears to be phosphate limited.

Although the system is partially isolated from the overlying marine ecosystem, organic input from above drives the bacterial anaerobic ecosystem, resulting in a “sulfide pump”. In this model, sulfide percolates up through the karst and removes oxygen from the overlying sediment, which has likely caused changes in the shallow benthic ecosystem. Jewfish Sink appears to be part of an extensive anoxic subterranean estuary that extends under parts of at least three coastal counties in Florida and can serve as a model for the effects of rising sea levels or aquifer mining.

Introduction

Jewfish Sink is located in the shallow seagrass flats of the Gulf of Mexico approximately 1 kilometer offshore and 3 km south of Aripeka, Pasco County, Florida (Figure 2-1). This sink is one of numerous karst windows that are characteristic of the coastal area of west-central Florida. There are at least fifty other karst features in the region (Wetterhall, 1965; Garman, unpublished data), seven of which were utilized in this study (Figure. 2-1).

The Gulf of Mexico near Jewfish Sink ranges from 0.6 to 2 meters (m) deep and supports a shallow marine ecosystem. The sink begins as a 6-m diameter rim of limestone on the floor of the Gulf. The silt mound at the bottom of the sink begins at a depth of 40 m and slopes to a maximum depth of about 64 m. The diameter of the sink increases to about 76 m at the bottom (Figure 2-2).

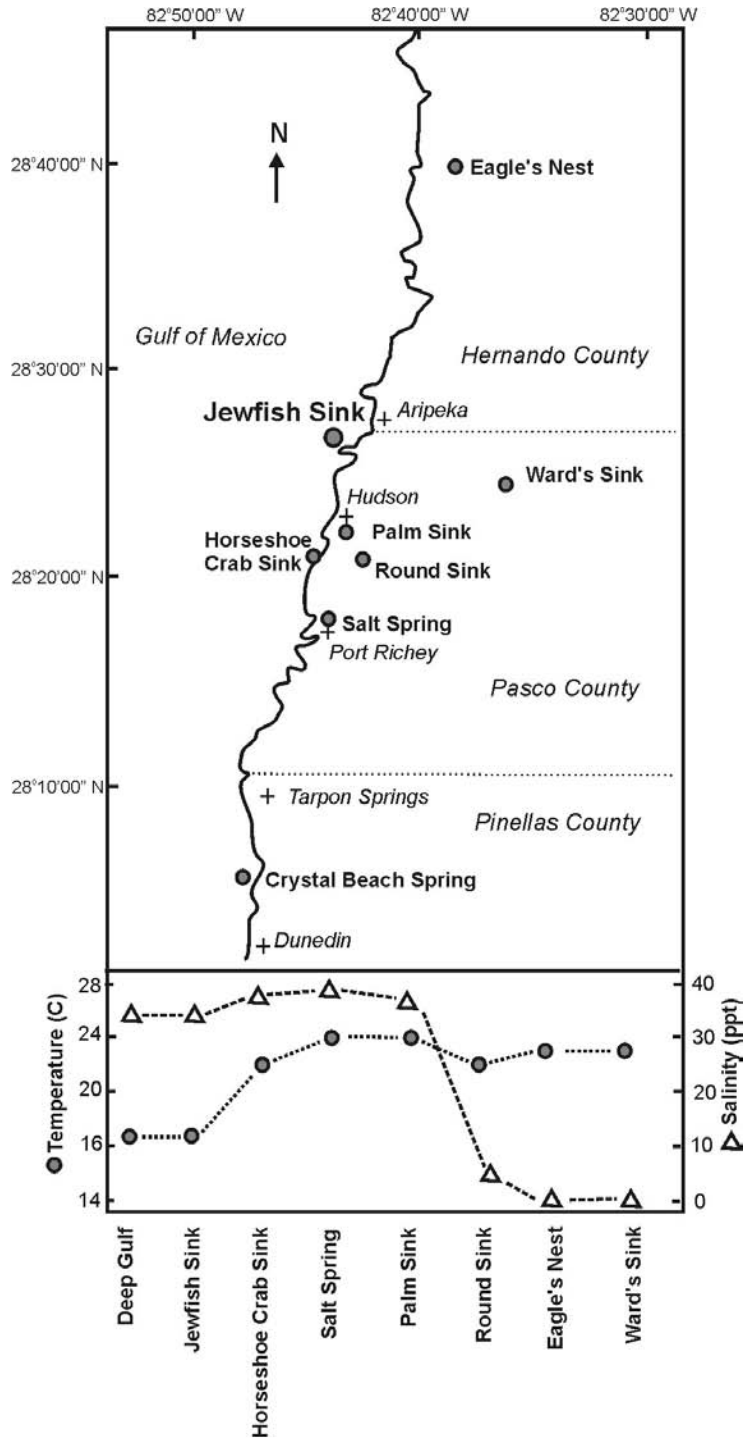


Figure 2-1: Map of study area with bottom water temperature and salinity. A) Map showing locations of sinks and springs utilized for this study in UTM 17 North coordinate system (meters). B) The relationship between temperature and salinity of bottom water in sinks and springs. The data are shown in order from farthest offshore to farthest inland. The temperature of the bottom water in Jewfish Sink matches the temperature of deep water in the Gulf of Mexico (16-17°C), while all other sources are near ground water temperature for this region of Florida (22-24°C).

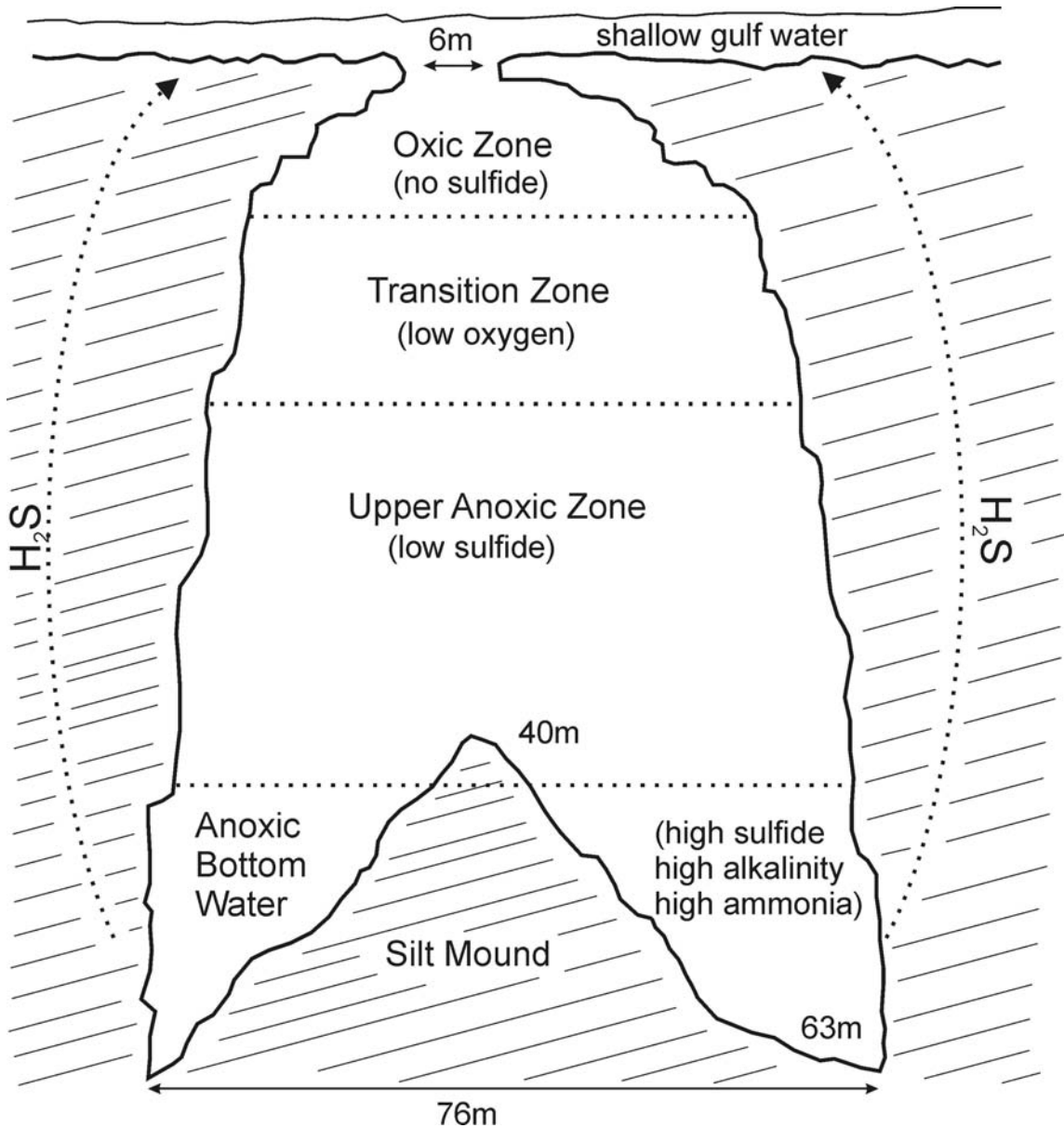


Figure 2-2: Schematic of Jewfish Sink. Jewfish sink is located in a shallow coastal region of the Gulf of Mexico. The opening to the sink is 6 m wide and approximately 2 m below the surface of the Gulf. An oxidic zone extends to approximately 10 m and a transition zone in which dissolved oxygen concentrations decrease to near zero or zero extends to 13 m in the summer and as deep as 31 m in the winter. An upper anoxic zone contains low levels of sulfide and an anaerobic bacterial mat is present on the walls. The anoxic bottom water is defined by relatively constant temperature of 16-17 °C and salinity of 33-35 ppt and by elevated concentrations of ammonia, alkalinity and sulfide. Sulfide percolates up from the sink and through the porous limestone to the surface sediment where it removes oxygen from the sediment. This “sulfide pump” results from overuse of the aquifer and may be responsible for changes in the benthic ecology of the region.

General Geology of Jewfish Sink

The Eocene to Miocene marine carbonate deposits that form the Floridan aquifer comprise one of the most productive potable aquifer systems in the world. Relatively impermeable Miocene and younger sediments overlie and confine the aquifer in many parts of the state (Stringfield 1936; Parker et al. 1955; Swarzenski et al. 2001). This confined Floridan aquifer contains groundwater under sufficient pressure to flow out at the land surface, creating springs where there are discontinuities in the confining beds (Rosenau et al. 1977).

Near the town of Aripeka at the Hernando-Pasco County border, the Oligocene Suwannee Limestone is found near the ground surface and outcrops in some locations. The Suwannee Limestone is thickest near the coast at the Hernando-Pasco County border where a thickness of 71 m has been measured from well WPs26S-18E-28db (Yon and Hendry, 1972). Jewfish Sink appears to be developed entirely within the Suwannee Limestone (Wetterhall 1965).

Jewfish Sink

Jewfish Sink was described as a spring with a distinct boil at low tide and a surface slick at high tide. A surface water salinity measurement from the spring on December 5, 1960, was 8 parts per thousand (ppt) (Wetterhall 1965). A severe drought occurred 1961-62 during which runoff was 21% of normal (Waller 1985). Jewfish Spring ceased flowing during this drought (Wetterhall 1965; Rosenau et al. 1977). During the same time period in which Jewfish Spring ceased flowing freshwater, potentiometric maps of the Floridan aquifer for West Central Florida showed significant permanent decreases in groundwater levels that have been attributed to increasing demand for water

largely from the citrus and phosphate industries (Parker 1975). Groundwater withdrawals in the three-county area surrounding Jewfish Sink have increased from 227 to 852 million liters per day from 1965 to 1990 (Marella 1995). With the cessation of freshwater flow, the spring became a stagnant basin that turned anaerobic and sulfidic due to sulfate reduction. Today, the sink receives oxygenated water only in the winter when cold surface water sinks into the basin.

Jewfish Sink is unique in that it offers the opportunity to examine the fate of a submarine freshwater spring, which has ceased flowing due to the mining of groundwater. Although there are a number of other marine influenced karst features in the region, there is little or no documentation in the literature that describes their biogeochemistry in detail. The objective of this study is to examine what happens to freshwater springs and the aquifer conduits leading to them when they cease flowing and are inundated by seawater. This not only has implications related to saltwater intrusion in coastal areas from over-pumping of groundwater but also potential effects of sea level rise. To accomplish this we observed fluctuations in chemical constituents over time at Jewfish Sink and selected nearby sinks.

Materials and Methods

Divers affiliated with the academic diving program at the University of South Florida performed the research dives performed as part of this study. Divers measured water quality parameters using a Datasonde (Hydrolab-Hach Company Brand, Loveland, Colorado) and collected samples.

Water Quality Measurements

The Datasonde was used to record temperature, pH, specific conductivity, salinity, dissolved oxygen, and depth readings during the dive. The instrument was calibrated prior to each dive using standard pH solutions of 7.00 and 10.00 standard units, a specific conductivity standard of 41.62 milliSiemens per centimeter (mS cm⁻¹), and the water saturated air method at known temperature and atmospheric pressure for the dissolved oxygen calibration. The Datasonde was serviced and calibrated by the manufacturer on a regular basis. The diver carrying the Datasonde descended first with the Datasonde carried below the diver to prevent disturbance of the water column and interference from exhaust gases.

Water samples were collected by divers using 60-milliliter (ml), capped syringes, using the method described by Brigmon et al. (1994). The water samples were collected from the surface to a depth of 30 m at 6 m intervals and a sample of the anoxic bottom water was collected at a depth of 61 m. Water samples were analyzed for sulfide, alkalinity, and ammonia by colorimetric methods using kits supplied by CHEMetrics (CHEMetrics, Inc. Calverton, Virginia). Some samples were analyzed at a commercial laboratory using standard EPA methods 325.2 for chloride, 350.3 for ammonia, 353.2 for nitrate/nitrite, 365.2 for ortho-phosphate, 375.4 for sulfate, 9060 for dissolved organic carbon and 6010 for calcium and magnesium (Nelson 2003).

Pore water sulfide within the sediment overlying the area around Jewfish sink was measured in two ways. The openings of plastic beakers (100 ml) were covered with 50 micrometer (μm) plankton netting using a rubber band. The beakers were filled with seawater and placed within the sediment on their sides and left in place. After a week, the

beakers were located and water samples withdrawn while the beakers were still in place using a 50 ml syringe and a long needle that was inserted through the plankton netting. Sulfide from the pore water was analyzed as described above. In addition, sealed seepage meters constructed from 55-gallon drums (Lee 1977) were placed over the sediment. Water was sampled a week later and sulfide content measured.

Particulate Analysis

Divers using 4 L containers collected samples of the particulate cloud. The containers were submerged filled with distilled water. At the depth where the particulate cloud was observed to be the densest, the containers were purged with inert gas and filled with water. The samples were filtered onto a 0.2 μm filter and dried under a vacuum. Pieces of the filter (5 mm square) were examined in environmental mode of a scanning electron microscope (Hitachi S-3500N, Schaumburg, IL). Elemental analysis was performed using an energy dispersive X-ray analysis system with a light element prism detector (Princeton Gamma Technology, Rocky Hill, NJ). Dried filter samples were also sputter coated with gold for microphotography.

Results

Observations

Jewfish Sink is a rocky oasis in a shallow grass flat. Typically, schools of baitfish surround the sinkhole and the shallow rocks are inhabited by a school of snapper and an occasional grouper. Within the rocks, there are stone crabs and octopods. Encrusting sponges and tunicates cover the rocks. Commonly, the animals are found to a depth of about 5 m, the extent of the surface oxic zone; but the encrusting sponges may be found to a depth of about 13 m.

Within the anoxic zones, a microbial mat covers the walls of the sinkhole (Figure 2-3). The mat grows in finger-like projections that hang down from the wall. In the summer of 2001, the microbial mat began at a depth of about 13 m. Over the winter of 2001/2002, the microbial mat died back. By spring 2002, the microbial mat began at a depth of about 23 m. Over the remainder of the study, the microbial mat thinned and thickened but was not observed shallower than 23 m. Similar microbial mats have been observed in the anoxic zones of other karst features in the area, including Horseshoe Crab Sink, Palm Sink and Salt Spring (see Figure 2-1).

The water column in the sinkhole is also characterized by the presence of “clouds” of white particulate matter (Figure 2-4). In fall 2001, particulate matter was condensed in one prominent layer about 2 m thick that ranged in depth between 27 and 43 m. By winter 2001/2002, particulate matter was dispersed throughout the water column from a depth of about 15 m to about 50 m. In spring 2003, particulate matter was present between 15 and 50 m but was condensed into a series of four layers about 2 m thick. In spring 2003, particulate matter had dispersed again. It was present between 9 m and 50 m but was thickest between 9 m and 12 m. similar patterns of particulate distribution were present through summer 2003. During the fall of 2003, particulate matter was condensed into two layers at depths of 9 and 28 m. A third layer of particulate matter was observed at a depth of 45 m in the winter of 2003/2004 and two layers at 9 and 28 m were again present in the spring of 2004.

Electron microscopy revealed two kinds of particles in the cloud (Figure 2-5). The first type of particles was 10 to 20 μm in length and flat in appearance. They were composed primarily of aluminum and silicon and are most likely to be particles of

aluminum silicate clay minerals (Figure 2-5 a, b). The second type was 2 to 5 μm long and appeared to be an aggregate of organic and inorganic particles rich in iron, sulfur and sea salts (Figure 2-5 c, d).

Two major sources of organic carbon input into the bottom water were observed during the study. The first was bottom feeding horseshoe crabs and stingrays that fall into the sink and are unable to escape. These animals succumb to the sulfide in the bottom water and die, providing a source of reduced organic carbon for anaerobic respiration. The largest flux of these animals into the sinkhole was observed in early spring 2002 and another large flux was observed in late fall 2002.

The second major source of organic carbon was macro-algae washed into the sinkhole by storms and currents. On June 27, 2003, the silt mound was clean. Two weeks later, a layer of macro-algae about 0.5 m thick covered the silt mound and the following week the macro-algae were completely degraded.

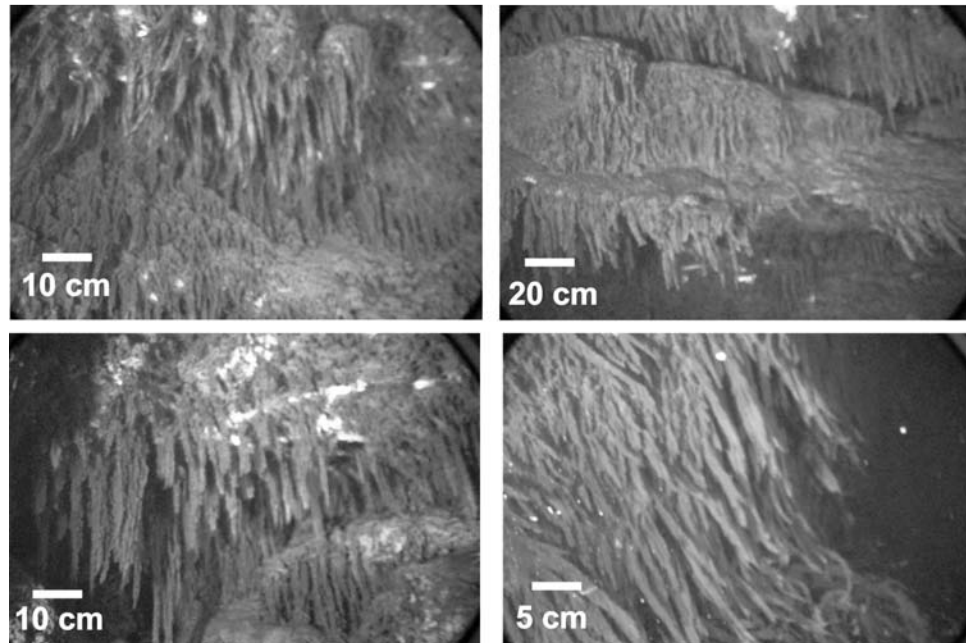


Figure 2-3: Bacterial Mat. Several views of the thick bacterial mat found on the wall of Jewfish sink in the anoxic zone. Note the crevices that suggest the sink is connected to small horizontal conduits that were once part of the aquifer.

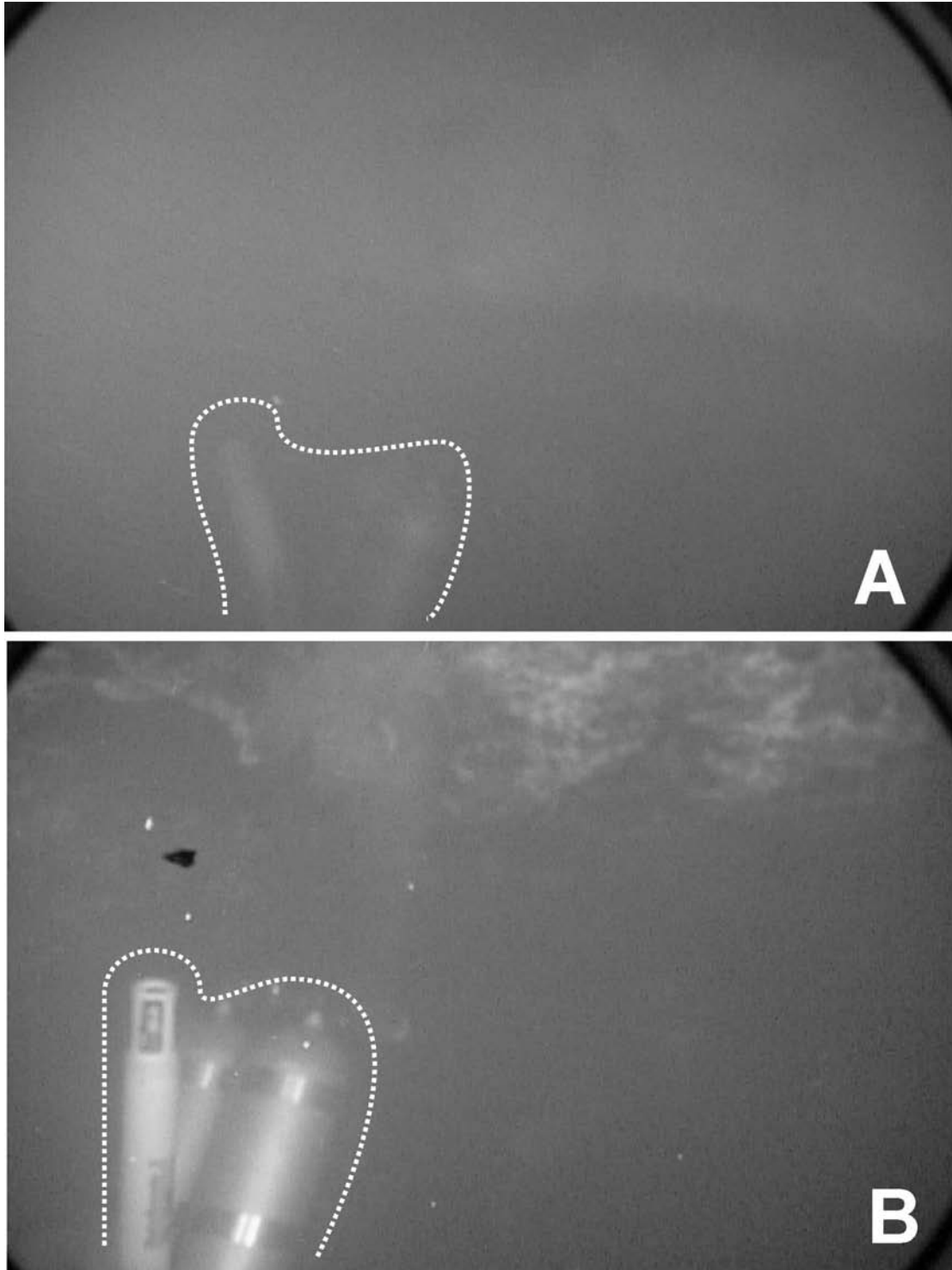


Figure 2-4: Particulate Cloud. Two photographs of a diver taken within the particulate cloud (A) and below the cloud (B). Note that the scuba tanks and Datasonde outlined with the dotted line are difficult to make out in panel A but can be seen clearly in panel B. The rock face in the upper portion of panel B cannot be seen in panel A. The photographs were made from two video frames where the subject and the photographer were 2 m deeper in panel B than panel A.

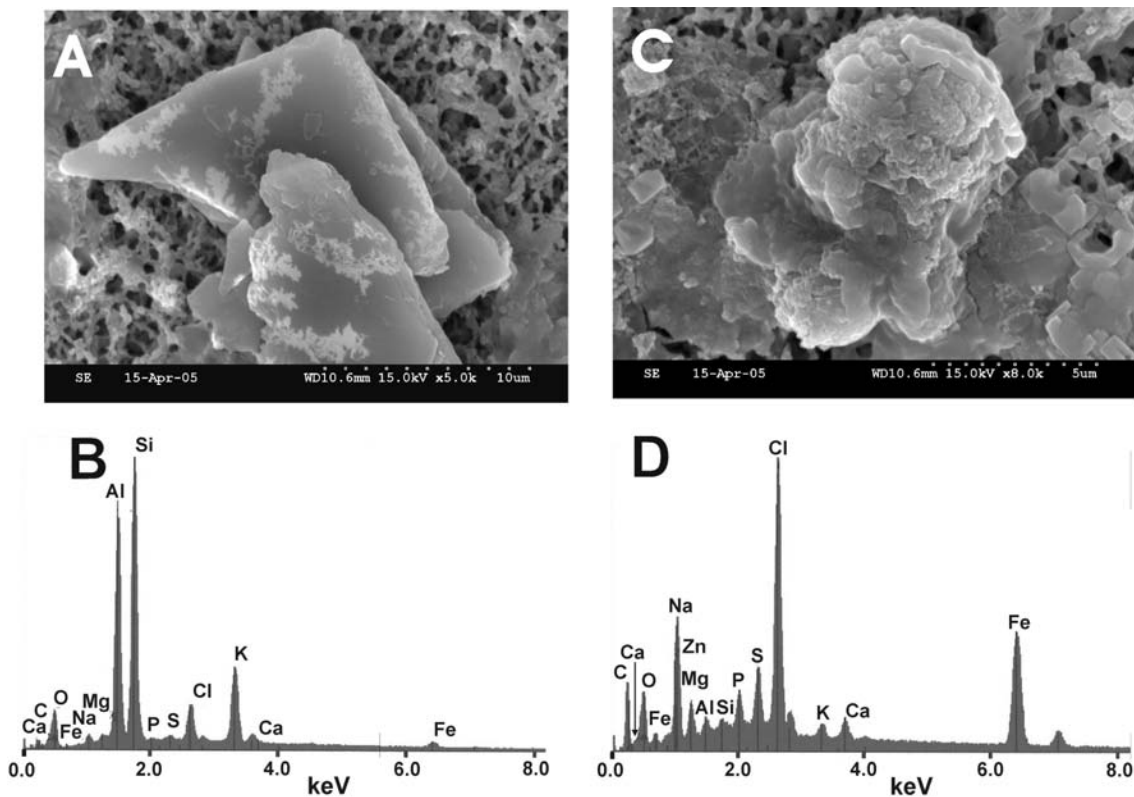


Figure 2-5: Scanning Electron Micrograph of Cloud Particulates. Panel A: Scanning electron micrograph of flat particles 10 to 20 μm in size from the particulate cloud. The light dendritic pattern is from salts that crystallized when the sample was dried. Panel B: X-ray analysis of a flat particle similar to the one shown in Panel A. The elemental analysis indicates that the flat particles are probably aluminum silicate clay minerals. Panel C: Scanning electron micrograph of aggregate particles 2 to 5 μm in size from the particulate cloud. Panel D: X-ray analysis of an aggregate particle similar to the one shown in Panel A. The elemental analysis indicates that the aggregate particles are a mixture of organic and inorganic particles rich in iron, sulfur and salts. No pyrite crystals were observed indicating that the iron and sulfur are probably not bound together in iron sulfide minerals.

Water Quality Data

Datasonde Profiles

Between summer 2001 and summer 2004, water quality data were obtained using the Datasonde from twenty-one dives in Jewfish Sink. Representative profiles of temperature, salinity, dissolved oxygen, and pH versus depth are shown in Figure 2-6. The profiles show a surface oxic zone only a few meters thick. The oxic zone overlies a transition zone where dissolved oxygen drops abruptly to zero or near zero. Temperature and salinity also change abruptly in the transition zone. Time series of pH, temperature, salinity and dissolved oxygen are shown in Figure 2-7.

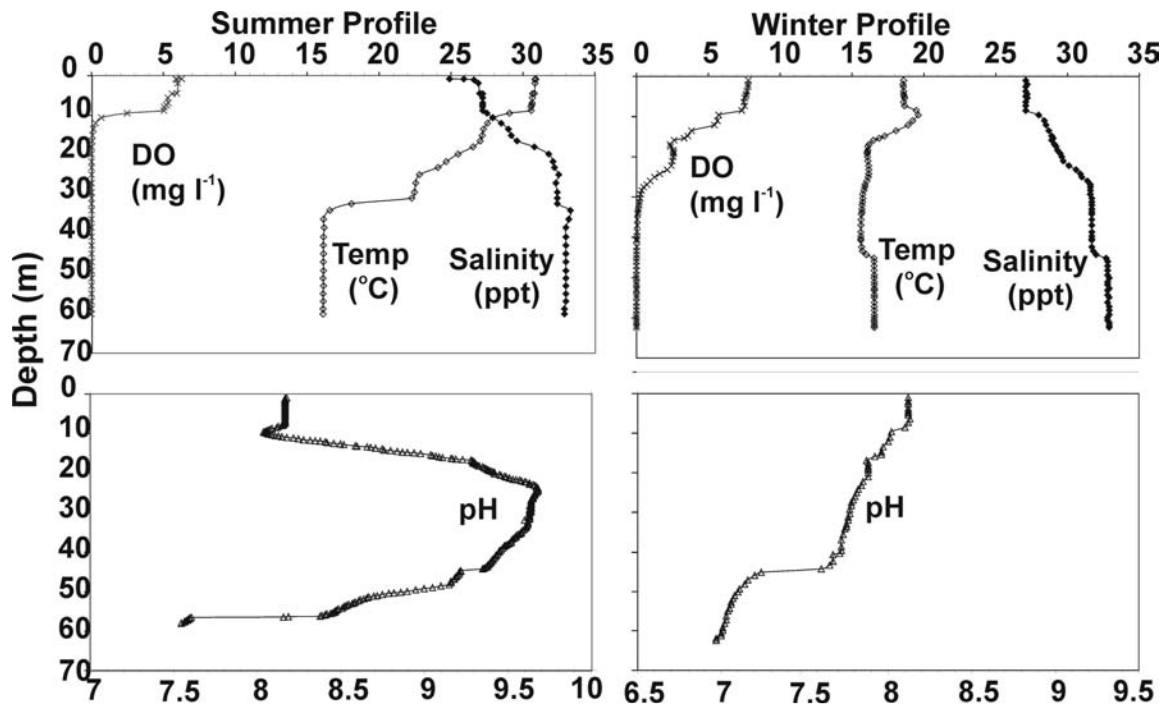


Figure 2-6: Datasonde data of a typical summer profile (left) show a pH minimum in the transition zone and a pH maximum in the upper anoxic zone. Summer temperature is lowest in the bottom water. The corresponding data for the winter (right) show a deeper transition zone from sinking surface water. Winter temperature is lower in the upper anoxic zone than in the bottom water. Salinity is highest in the bottom water year round. The extreme increase of pH seen in the upper anoxic zone in the summer profile is absent in the winter profile. These two patterns (summer and winter) were observed each year of the study.

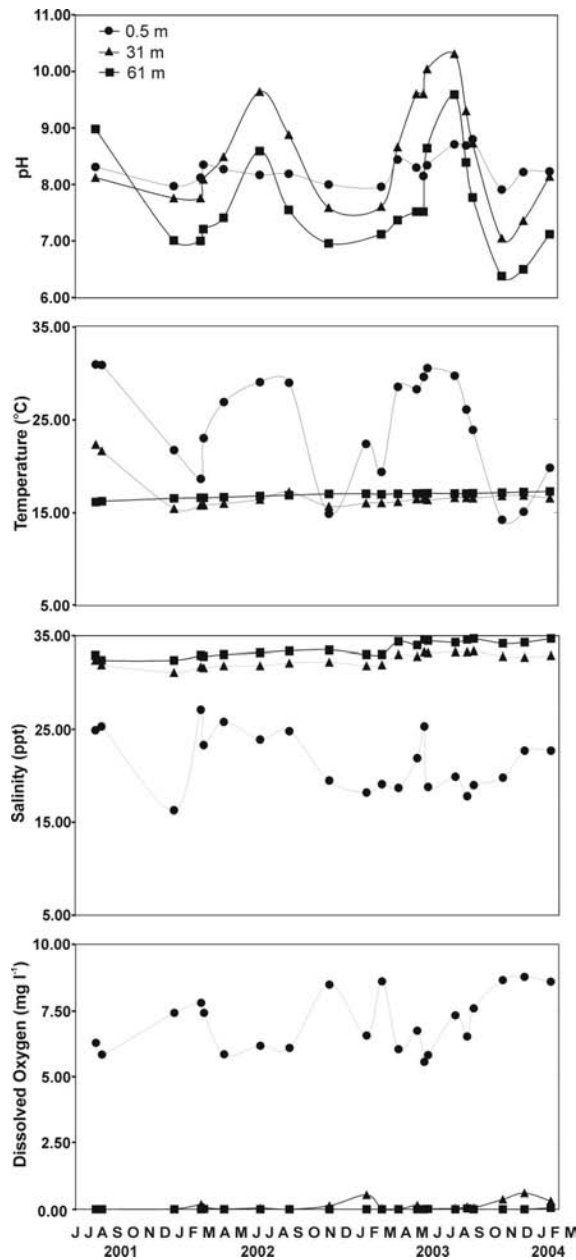


Figure 2-7: Water Quality Data vs Time. Panel A: pH at various depths is plotted against time. There is a periodic maximum pH every summer and minimum pH every winter at depth that probably relates to seasonal changes in microbial communities. The surface water varies the least. Panel B: Temperature at various depths plotted against time. Note that the bottom water (61 m) temperature does not change seasonally while the shallower depths change profoundly between summer and winter. Panel C: Salinity at various depths plotted against time. The deeper water (31 m and 61 m) changes very little while the surface water (0.5 m) changes seasonally. Panel D: Dissolved oxygen at various depths plotted against time. Note that the sinking oxygenated surface water reaches 31 m each winter. No oxygen was detected in the bottom water (61 m).

Chemical Constituents

Figure 2-8 shows the results of laboratory chemical analysis of water samples at various depths from Jewfish sink from February 2004. Similar results were obtained throughout the study using field test kits. The laboratory analytical data show four general trends of concentration of chemical constituents: dissolved oxygen and dissolved organic carbon concentrations decreased with depth and were below detection limits in the bottom water; chloride, sulfate, and alkalinity concentrations increased with depth and were detectable throughout the water column; ortho-phosphate, ammonia, and sulfide concentrations increased with depth but were not detectable above the anoxic zone; and the nitrate/nitrite concentration showed a peak at the transition zone.

Sulfide measurements in the bottom water were as high as 75 mg l^{-1} . Preliminary sulfide measurements of surface sediment pore waters and seepage meter samples around the sinkhole are in the same range as those measured in the water column below the transition zone of the sink.

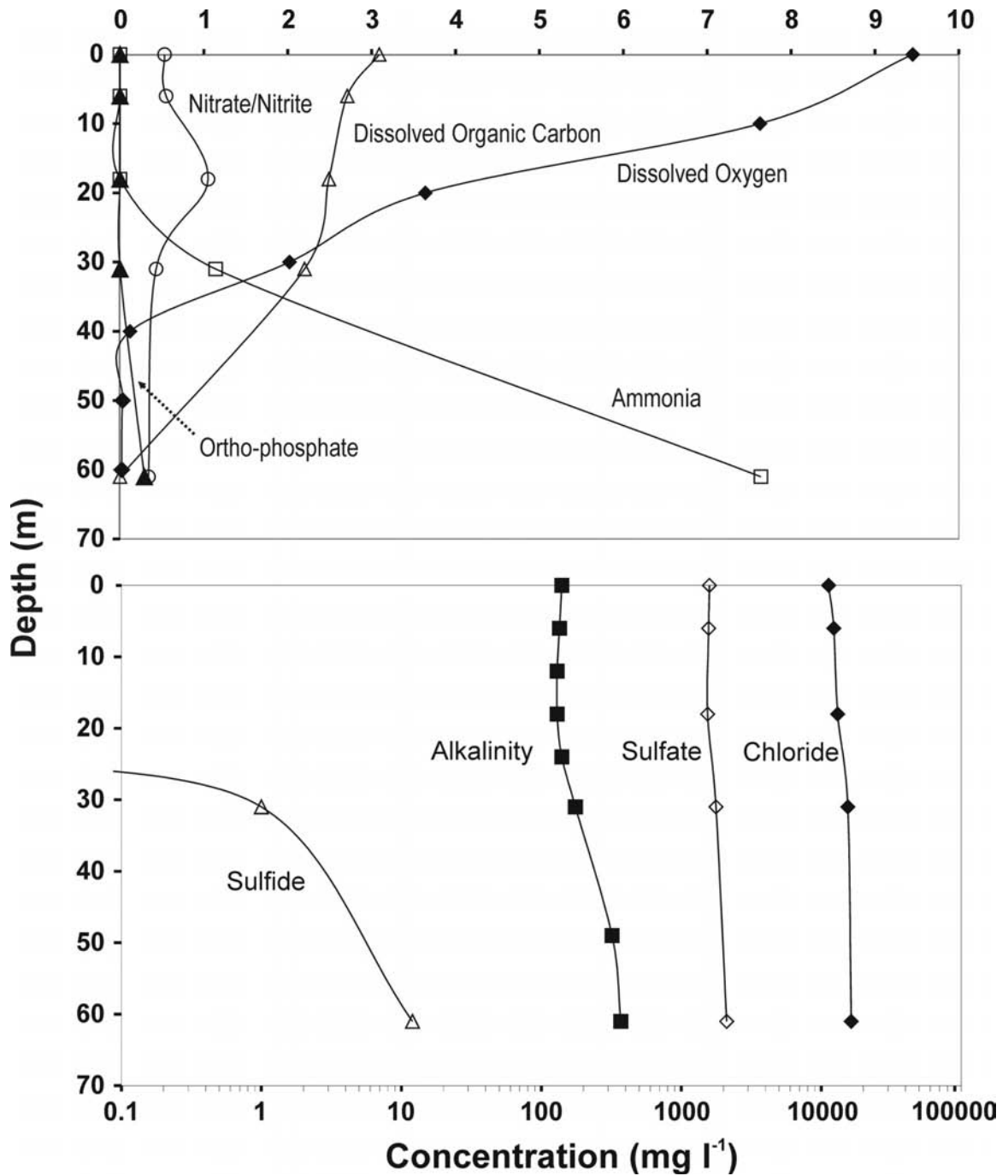


Figure 2-8: Chemical constituents of the sink water at various depths during the winter of 2003/2004). Panel A shows nutrients. The oxic and transition zones appear to be phosphate limited and the anoxic zones appear to be organic carbon limited. Panel B shows other major components, which increase in concentration with depth.

Bottom Water

The temperature, salinity, ammonia, alkalinity and sulfide data combined show a distinct bottom water different from the overlying anoxic zone and surface seawater.

Bottom water is defined by relatively constant temperature of 16-17 °C and salinity of 33-35 ppt and by elevated concentrations of ammonia, alkalinity and sulfide.

Discussion

Freshwater Discharge

No evidence of freshwater flow into Jewfish sink was found. The lowest surface salinities measured were 16 ppt, typical for shallow coastal regions of the Gulf of Mexico in the winter. This is significantly greater than the 8 ppt measured by Wetterhall in 1960 when the spring was still flowing.

Bottom Water

The bottom water in Jewfish Sink is a mystery. The measured temperature of 16-17°C and the high salinity of 33-35 ppt appear to be distinct from the surrounding shallow Gulf water and nearby saline ground water. Salinity of Gulf water is lower (16-27 ppt at the surface) and the temperature of the ground water (23-24°C) is higher. Sinking winter surface water reaches 31 m, the location of the upper anoxic zone during most of the year, as shown by small dissolved oxygen peaks and temperatures below 16°C in late winter (Figure 2-7). However, the salinity and high concentrations of sulfate reduction products make the density of the bottom water too high for sinking winter surface water to mix with it.

One possible hypothesis to explain the temperature and salinity of the bottom water is that the bottom water is derived from shelf water (Figure 2-9). This shelf water could be drawn in through ancient aquifer conduits by current induced pressure gradients (Burnett et al. 2003) and the cyclic flow of seawater caused by salt dispersion (Kohout 1966). This assumes that the aquifer reached the current continental shelf, which would have been the shoreline when sea levels were lower. Gulf shelf water between 100 and 200 m has similar temperature and salinity values to the bottom water of Jewfish sink (16-17°C and 33-35 ppt, Herring et al. unpublished data).

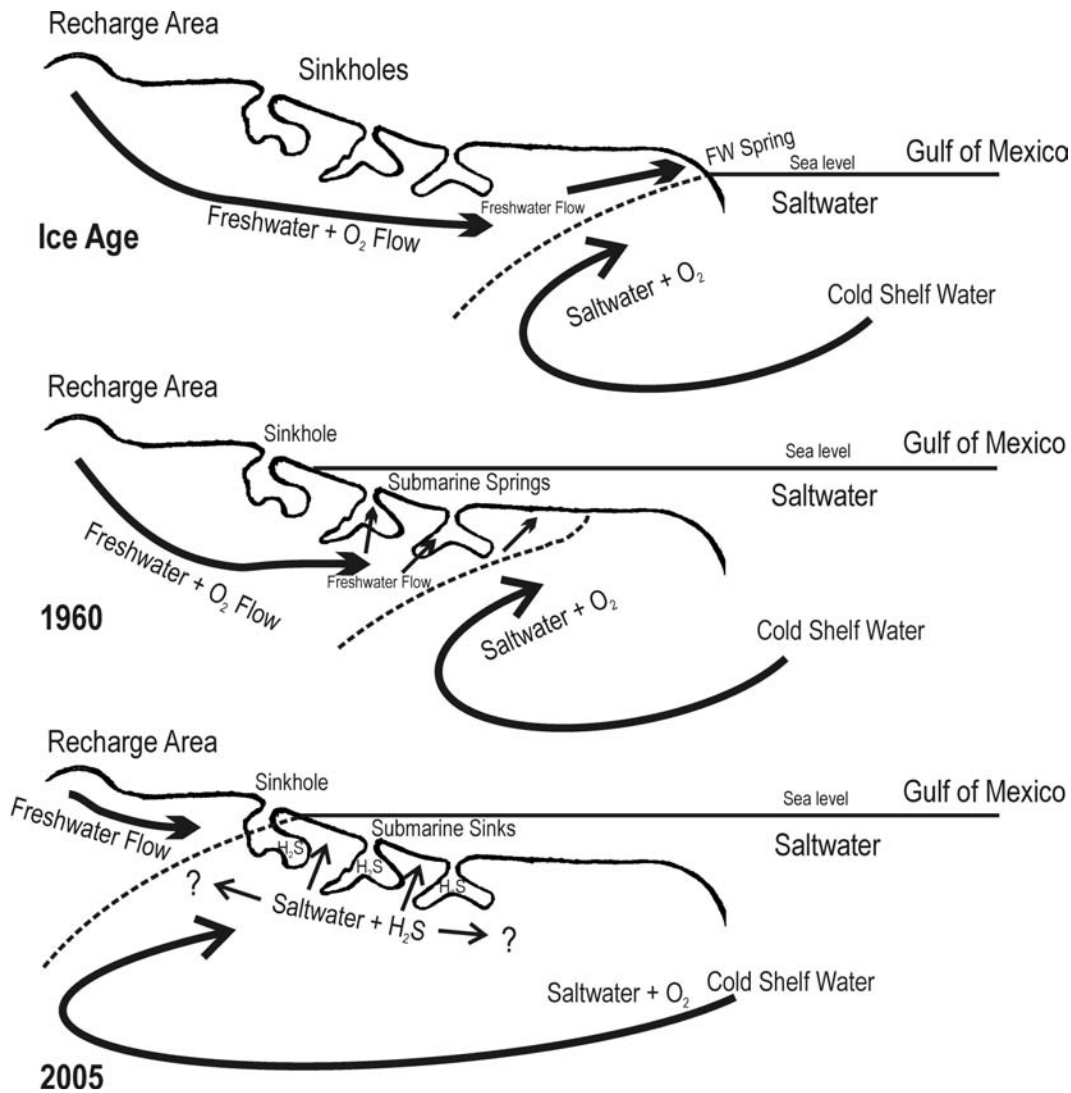


Figure 2- 9: The Sulfide Pump. Panel A: At lower sea levels, the freshwater aquifer and its conduits probably extended to the shelf. Panel B: Prior to aquifer overuse, the freshwater aquifer extended under the shallow water of the Gulf of Mexico. Conduits interconnect submarine springs like Jewfish spring to the aquifer and inland freshwater sinkholes. The flow of freshwater through the conduits kept them clear of seawater preventing the biological production of sulfide, which requires sulfate from seawater. At that time, Jewfish Sink was an active submarine freshwater spring and the sediment above the aquifer was normoxic and supported a normal benthic faunal community. Panel C: As the aquifer was overused, freshwater ceased to flow through the aquifer conduits below the Gulf of Mexico. The freshwater spring in Jewfish Sink ceased flowing. The conduits and karst features filled with seawater and bacterial action produced sulfide. Sulfide now percolates through the sediment, scrubbing oxygen from the sediments, which can no longer support the former benthic faunal community. The karst conduits may extend to the shelf, which would have been shoreline during times of low sea level. Flow of shelf water through conduits may explain the anomalously cold and hypersaline bottom water in Jewfish Sink.

Biogeochemistry

Today, without freshwater flow, the former spring is an anaerobic marine basin in which sulfide is generated in the bottom water by anaerobic metabolism through sulfate reduction. The analytical data show the bottom water is hypersaline as evidenced by the increasing salinity, chloride, and sulfate values with depth. The analytical data also show that products of sulfate reduction, ortho-phosphate, ammonia, alkalinity, and sulfide, impact the bottom water as the concentrations of these compounds are much higher in the bottom water compared to the upper water column. The bottom water is a distinct fluid that may affect the water quality of the coastal aquifer by more than simply raising chloride concentrations (Burt 1993, Burnett et al. 2003).

The nitrate/nitrite profile with a peak concentration at the transition zone is evidence of the chemolithotrophic nitrification of ammonia. The ammonia is created by the oxidation of organic material by sulfate reduction in the bottom water. The oxygen required for the oxidation of ammonia to nitrate is available within the transition zone. Therefore, a nitrate peak is expected at the transition zone when there is nitrification (reviewed in Atlas and Bartha 1998; Oguz et al. 2000).

The pH profiles from Jewfish Sink show distinct seasonal variability. In the summer, there is typically a pH minimum within the transition zone and a pH maximum in the upper anoxic zone. In contrast, the winter profiles show a trend of decreasing pH with depth (Fig. 2.6). We hypothesize that seasonal variability of pH in the transition and upper anoxic zones is related to the winter sinking of oxygenated surface water. The sinking surface water carries oxygen to greater depths creating a broader depth range with habitat suitable for chemolithotrophic sulfur-oxidizing bacteria that use oxygen as

an electron acceptor and convert sulfide to sulfate. This reaction causes a drop in pH, creating the summer pH minimum in the transition zone. As the chemolithotrophic sulfur oxidizing bacteria follow the sinking oxygenated surface water in the winter, they reduce the pH over a broad depth range. In the summer, with oxygen limited to the near surface waters, the byproducts of sulfate reduction buildup in the anoxic zones leading to pH increases (Castanier et al. 1999).

The observation of the rapid decay of macro algae in the bottom water and the chemical data provide evidence (see Figure 2-8) that sulfate reduction in the bottom water is limited by the availability of reduced organic carbon (Capone and Kiene 1988). The nutrients nitrate and ortho-phosphate are both available in the bottom water but dissolved organic carbon was below laboratory detection limits. In contrast, primary production in the surface oxic zone and transition zone appear to be phosphate limited as both nitrate and dissolved organic carbon are available but ortho-phosphate is below laboratory detection limits.

The white particulate clouds in the water column are composed of clay minerals and aggregates of organic and inorganic matter (Figure 2-5). The clay mineral component likely washed into the sink from the surface water. The aggregate particles were rich in iron and sulfur but crystals of pyrite (iron sulfide) were not observed. This suggests that the concentration of iron and sulfur in the aggregate particles results from bacterial activity within the sink. It appears that these particles concentrate at different density interfaces within the sink. For example in Figs. 2.6 and 2.7, there are complex changes in salinity and temperature, causing density changes that are related to depth and vary seasonally.

Effects of the Sulfide Pump

The production of sulfide in the anoxic zone and bottom water of the sinkhole indicates the presence of sulfur reducing bacteria that use sulfate as an electron acceptor in the anaerobic metabolism of organic carbon. The production of sulfide is the major factor influencing the ecosystem within the sinkhole and may affect the shallow marine community in the surrounding area.

In normal sandy shallow marine systems, an oxic zone extends several cm or more below the sediment/water interface due to the diffusion of oxygen from the water column. The sediments overlying Jewfish sink and the surrounding area have a thinner oxic zone that extends only a few mm below the sediment/water interface. This is based on observations that the sediment appears black and sulfidic below the first two mm and on the detection of sulfide in shallow sediment pore water. This is evidence of the sulfide pump in which sulfide generated in the bottom water of Jewfish Sink seeps upward through the overlying rock and sediments, removing the oxygen that diffuses downward from the overlying water column (Figures 2-2 and 2-9).

Summary and Conclusions

The conduits in the karst underlying the coastal regions of the Gulf of Mexico form a system of subterranean estuaries (Moore 1999). These estuaries can bypass the Ghyben-Herzberg relationship for the fresh groundwater and saline groundwater boundary in karst terrane, where conduit flow produces submarine freshwater springs (Bonacci and RojeBonnacci 1997). Historically, the subterranean estuaries were flushed by the flow of fresh water from the aquifer so that the conditions within the submarine conduits resembled those within the aquifer, which contains oxygen but very little

organic matter. The main result of saltwater intrusion into an aquifer was thought to be increasing salt content, simply the replacement of freshwater with saltwater. Our investigation of Jewfish Sink suggests that another result is the biogenic production of sulfide, ammonia and excess alkalinity, which could have a major effect on the aquifer community and the overlying benthic marine community (Fig. 2.9).

With overuse of the aquifer, the oligotrophic freshwater retreats and is replaced by seawater rich in dissolved and particulate organic matter. The seawater becomes anaerobic due to bacterial action, leading to a bacterially driven anoxic ecosystem. Jewfish Sink appears to be one window into this anoxic ecosystem. Although the system is partially isolated from the overlying marine ecosystem, organic input from above drives the anaerobic bacterial ecosystem, producing sulfide. The sulfide percolates up through the karst and scrubs the oxygen from the overlying sediment, which could cause changes in the benthic ecosystem above.

This anoxic coastal system appears to be extensive and is driven by the input of organic matter through many coastal karst windows combined with reduced freshwater flow in the coastal conduits. Jewfish Sink is only one example of these karst windows. At least three other nearby marine-influenced karst features (see Fig. 2.1: Horseshoe Crab Sink, Palm Sink, Salt Spring) have remarkably similar biogeochemistry, suggesting that this system extends under parts of at least three coastal counties in Florida. Crystal Beach Spring is an active submarine freshwater spring that may represent the southern boundary of the anoxic subterranean estuary, and the anoxic subterranean estuary may extend north at least to the Weeki Wachee river/spring basin (Eagle's Nest in Figure 2-1).

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CHAPTER THREE: BIODIVERSITY AND THE TRANSITION OF AN ANCHIALINE SYSTEM TO A MARINE SYSTEM

This chapter is currently in review at the journal *Hydrobiologia* under the same title:
Garman, K. M., H. Rubelmann, D. J. Karlen, T. Wu and J. R. Garey.

Abstract

Jewfish Sink is a former anchialine karst feature located in the shallow grass flats within the Gulf of Mexico off the coast of West Central Florida. Freshwater flowed from the feature until 1962, coinciding with drought and aquifer overuse. It is now an anoxic marine basin with no freshwater influence. One purpose of this study was to examine the effect of the change in freshwater flow on the surrounding marine ecosystem by comparing the macrofauna around Jewfish Sink to that of Crystal Beach Spring, a nearby karst feature that is still an active submarine spring. The other purpose was to characterize the prokaryote and eukaryote biodiversity within Jewfish Sink and correlate its biodiversity to its geochemistry. Transects were established adjacent to each site. We found significantly higher species richness, abundance, and diversity of benthic fauna at the Jewfish Sink site compared to the Crystal Beach Spring Site. Cluster analysis revealed three main groups when the sampling sites from both transects were compared: one group composed of two replicates from the Jewfish Sink 1km site, one replicate from the Jewfish Sink 5 m site and the replicates from the Crystal Beach Spring 1 km site; one group of Jewfish Sink sites; and one group of Crystal Beach Spring sites. This study suggests that active submarine groundwater discharge is a significant factor reducing the richness and diversity of the benthic community structure in the near shore, shallow

marine environment. We also surveyed the biodiversity within Jewfish sink in terms of Bacteria, Archaea and Eukaryota using a combination of 16S and 18S ribosomal RNA analysis from environmental samples collected inside Jewfish Sink. Sequence analysis of 836 18S clones revealed that fungi and dinoflagellate sequences appeared to dominate shallow oxic and deeper anoxic zones. Analysis of 16S rRNA sequences from microbial mats in the anoxic zone revealed a broad diversity of bacteria (320 clones) and archaea (412 clones), many of which had been previously identified in anoxic environmental samples and likely involved with sulfur, nitrogen and methane metabolism. The transition of Jewfish Sink from a freshwater spring environment to an anoxic basin appears to have increased the macrofaunal biodiversity of the surrounding ecosystem, and by providing a unique anoxic environment, has allowed a unique ecosystem containing sulfide tolerant eukaryotes, bacteria and archaea to thrive.

Introduction

Jewfish Sink and Crystal Beach Spring are located in the shallow seagrass flats of the Gulf of Mexico off the coast of West Central Florida. Average depth at both sites is 0.6 to 2 m. Jewfish Sink is located at 28°25'43"N, 82°42'30"W about 1km offshore from rural Pasco County, Florida, near the town of Aripeka. Crystal Beach Spring is located at 28°05'00"N, 82°47'07"W in the intracoastal waterway of the Gulf of Mexico about 200 m offshore from the residential neighborhood of Crystal Beach in highly developed Pinellas County.

Jewfish Sink

Jewfish Sink was a submarine spring that represented a unique anchialine environment with flow at all tidal stages until the drought of 1961-62 when the spring ceased flowing (Wetterhall 1965; Rosenau et al. 1977; Waller 1985). Today, the former spring is an anoxic marine basin with sulfidic bottom water. The basin has a maximum depth of about 64 m and a diameter of about 76 m at the bottom compared to a diameter of about 6 m on the floor of the Gulf of Mexico (Garman and Garey 2005). There are no visible conduits passable by a diver leading from the sinkhole to the aquifer today, so either the spring had numerous smaller conduits or the main conduits have filled in with sediment since flow ceased.

The Jewfish Sink water column has four zones: the shallow oxic zone; the transition zone; an upper anoxic zone; and relatively stable anoxic bottom water. The oxic zone is influenced by sulfide seepage from the anoxic zones and contains a variety of fish and macroinvertebrates while the deeper anoxic zones are sulfide rich and contain no living fish or macroinvertebrates. One of the more interesting findings from previous studies is a dramatic increase in the pH of the upper anoxic zone during the summer months that are likely of biological origin. Analyses of particulate clouds collected during the winter from the water column in the transition and upper anoxic zones indicated the presence of two types of particles: aluminum silicate clay minerals; and aggregates of organic and inorganic particles rich in iron, sulfur and sea salts (Garman and Garey 2005).

Crystal Beach Spring

Crystal Beach Spring is an active freshwater water spring with salinity values as low as 1.6 ppt in the cave passages unaffected by siphoning of Gulf water and the influx of saline groundwater. The spring opening is connected to anchialine cave passages that extend to the east beneath dry land and have a mapped length of over 2 km. Crystal Beach Spring begins as a 0.5 m opening at a depth of 6 m in a cone shaped basin with a diameter of about 15 m in the floor of the Intracoastal Waterway. Cave passages are developed along bedding planes and are primarily located at depths between 15 and 42 m. Where the cave passages drop to a depth of 39 m there is a stable pycnocline in the water column with warmer, anoxic, saline groundwater (24.86 °C, salinity 33.3 ppt) below the freshwater spring flow (24.26 °C, salinity 2.29 ppt) (Garman et al. 1999; Garman 2000).

Peak discharge occurs at the end of the wet season (typically September and October). At this time of the year, the spring siphons only during the highest high tides that occur during the full and new moon, if at all. Low discharge occurs at the end of the dry season (typically April and May). At this time of year, the spring generally experiences significant siphoning events at least once daily at the higher high tide (DeWitt 2003) when the net flow out of the spring reverses and seawater flows into the spring.

Five stygobitic crustaceans have been identified in the Crystal Beach Spring cave system: two crayfish, two amphipods, and one isopod. These stygobites represent the first cave-adapted animals found in a freshwater cave system flowing beneath a body of salt water in Florida (Garman 1997).

Background

Jewfish Sink is located offshore from rural Pasco County without large point sources of environmental pollutants. In contrast, Pinellas County operates a spray irrigation and sludge disposal facility for sewage about 5 km north-northeast of Crystal Beach Spring. This facility has treated about 11,355,000 cubic meters of water per year since 1976. The disposal operations have created potentiometric highs in both the surficial and Floridan aquifers at the disposal facility and elevated nitrate concentrations that exceed 10 mg/l have been detected in wells to the west of the facility (Trommer 1992). The geologic investigation for the Trommer study showed a high degree of secondary porosity development in the Floridan aquifer allowing rapid movement of effluent impacted groundwater to coastal discharge points. Studies in the Florida Keys where there is also a high degree of secondary porosity measured groundwater velocities exceeding 100m/hr to discharge points offshore (Paul et al. 1995; 1997; 2000).

Benthic macrofauna are useful indicators of environmental stress. Previous studies have focused on changes in benthic abundance and diversity due to natural or anthropogenic disturbance within a localized area (Zajac and Whitlatch 2003), or along an environmental or pollution gradient (Weston 1990; Simboursa et al. 1995; Rakocinski et al. 1997; Rosenberg et al. 2001; Je et al. 2003; Stark et al. 2005; Karlen et al. 2010). The primary goal of this macrofaunal study was to look at the effect of the cessation of freshwater flow from a submarine spring and the formation of a sulfidic, anaerobic marine basin on the benthic macrofauna community structure.

This study examines the benthic macrofauna community structure at an anaerobic marine basin that was formerly a submarine freshwater spring compared to the benthic

macrofauna community structure at an active submarine freshwater spring. The shallow marine environment around Jewfish Sink is subject to potential impacts from sulfide production in the bottom water of the sinkhole that creates a sulfide pump by diffusion of sulfide upward into the overlying sediments (Garman and Garey 2005). In contrast, the shallow marine environment around Crystal Beach Spring is subject to potential impacts from the freshwater discharge from the spring and submarine groundwater discharge in the vicinity of the spring. We also measured some physical parameters including temperature, seepage and particulate composition to provide a more complete evaluation of the biogeochemistry of the sites.

Another goal of this project was to survey the prokaryotic and eukaryotic biodiversity within Jewfish Sink to test the hypothesis that the geochemical properties of the sink water are related to biological activity. The organisms within the sediments in the oxic and transition zones of the sinkhole, the water column at the chemocline, and bacterial mats in the anoxic zones are quite diverse and generally microscopic. Therefore we used a molecular profiling method (Wu et al. 2009) to characterize the prokaryotic and eukaryotic microfauna using 16S and 18S rRNA gene sequences from DNA extracted directly from environmental samples.

Materials and Methods

Certified divers affiliated with the National Association for Cave Diving, the National Speleological Society Cave Diving Section, and the Academic Diving Program at the University of South Florida performed the research dives for this study.

Physical characteristics

HOBO recording thermometers (Onset Computer Corporation 470 MacArthur Blvd., Bourne, Massachusetts) were installed in Jewfish Sink at ten fixed depths (3, 6, 9, 12, 18, 24, 31, 37, 46, and 61 meters) to record the water temperature every 4 hours from October 11, 2003 to July 26, 2004. HOBO thermometers were encased in watertight 5-cm diameter plastic spheres weighted with lead to have slight negative buoyancy in saltwater. Each sphere was labeled with the desired installation depth. A nylon line was attached to a rock outcrop at a depth of 3.1 m where the first thermometer was installed. The thermometers were attached to the nylon line at each monitoring depth and the nylon line was attached to rock outcrops of opportunity so that the depth was fixed.

Divers using 4 L containers collected samples of the particulate cloud. The containers were submerged filled with distilled water. At the depth where the particulate cloud was observed to be the densest, the containers were purged with inert gas and filled with water. The samples were filtered onto a 0.2 μm filter and dried under a vacuum. Pieces of the filter (5 mm square) were examined in environmental mode of a scanning electron microscope (Hitachi S-3500N, Schaumburg, IL). Elemental analysis was performed using an energy dispersive X-ray analysis system with a light element prism detector (Princeton Gamma Technology, Rocky Hill, NJ). Dried filter samples were also sputter coated with gold for microphotography.

Seepage rates were measured at Jewfish Sink and Crystal Beach Spring in June 2006 using the method described by Lee (1977) and sampling procedures discussed in similar studies (Simmons and Netherton 1987; Shaw and Prepas 1990). The top third of a 55-gallon drum was buried with the edge 5 to 10 cm deep into the sediments in May

2005. At Jewfish Sink and Crystal Beach Spring, the seepage meters were installed 5 m and 15 m east (shoreward) of the rim of the sinkhole. Seepage rates were computed from the volume of water in the collection bag, the surface area of sediment enclosed by the meter, and the time that the collection bag was deployed. Local tide charts for the sampling period were obtained.

Macrofauna Sample Collection and Processing

At each location on August 8, 2004, benthic macrofauna samples were collected at four sites: 5 m, 50 m, 100 m, and 1 km from the edge of each basin. At Jewfish Sink, the 5, 50, and 100 m samples were collected along a transect running from the eastern edge of the sink eastward toward shore. The 1 km sample was collected to the west of the sink basin. At Crystal Beach Spring, the 5, 50, and 100 m samples were collected along a transect running from the eastern edge of the spring basin eastward toward shore. The 1 km sample was collected to the north of the spring basin. At each site, a 1 m² grid was placed next to the transect line. Three core samples (diameter = 7.62 cm; area = 45.6 cm²) were collected from the grid at random positions. The core samples were sieved through a 500 µm mesh sieve and the retained animals were fixed in 10% formalin with Rose Bengal stain for a minimum of 72 hours then transferred into 70% methanol for preservation. Organisms were sorted from the sediment under a dissecting microscope, identified to the lowest practical taxonomic level and enumerated.

Macrofauna Data Analysis

All data was initially entered into a Microsoft Excel spreadsheet. Statistical analysis was conducted using SigmaStat ver. 3.5 (Systat Software, Richmond CA). Multivariate statistics, SIMPER analysis, and basic community indices were calculated

using PRIMER ver 6 (Plymouth Marine Laboratory, U.K.). Abundance and richness between transects were analyzed using a Mann-Whitney rank sum test. Abundance among sites along transects was analyzed using a Kruskal-Wallis one-way analysis of variance on ranks. Shannon-Weiner diversity index and evenness between transects were analyzed using a t-test. Shannon-Weiner diversity indices, evenness and richness among sites along transects were analyzed using a one-way analysis of variance with a Holm-Sidak pairwise multiple comparison procedure. Cluster analyses were performed using the Bray-Curtis similarity index that was calculated using a square root transform of the abundance data with PRIMER ver 6. Multi-dimensional scaling (MDS) using the Bray-Curtis similarity index was also used to show the community structure between transects and among sites along each transect.

Molecular Analyses

Samples were collected from the bacterial mats in the anoxic zones for analysis for bacterial and archaeal 16S rRNA and eukaryotic 18SrRNA, and sediment samples were collected from rock ledges within the sinkhole in the oxic zone for eukaryotic 18SrRNA.

Samples from the bacterial mat and water column were collected on March 30, 2002, by divers using sterile 60 mL capped syringes held below the diver during descent into the sinkhole to avoid contamination by exhaust gases, as described by Brigmon et al. (1994). The samples were stored at -20°C. Sediment samples were collected by divers on July 22, 2002, and stored at -20°C. Prior to DNA extraction, the water samples were vacuum filtered onto a 0.2 µm filter and the sediment samples were passed through a 500 µm sieve onto a 50 µm sieve.

DNA was extracted (Hempstead et al. 1990) by adding 5 mL extraction buffer (1M Tris-HCl, pH 8.0, 100mM EDTA, 3.5% SDS) to each sample and rotating for 10 to 20 min. A phenol extraction and ethanol precipitation was performed. The DNA pellet was dried and re-suspended in 500 μ L water.

The samples for eukaryotic 18S rRNA gene analysis were PCR amplified using conserved primers 18S4 (5' CCGGAATTCAAGCTTGCTTGTCTCAAAGATTAAGCC 3') and 18S5 (5' CCGGAATTCAAGCTTACCATACTCCCCCGGAACC 3') representing approximately 800 base pairs of the 5 prime portion of the gene (Mackey et al., 1996). The samples for prokaryotic 16S rRNA gene analysis were PCR amplified using conserved primers 8F (5' AGAGTTTGATCCTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3') (Baker et al., 2003, Reysenbach et al. 1994). The samples for archaeal 16S rRNA gene analysis were PCR amplified using conserved primers Arch21F (5' TTCCGCTTGATCCYGCCGGA 3') and Arch958R (5' YCCGGCGTTGAMTCCAATT 3') (Baker et al., 2003). For PCR amplification the following components were used: 39.5 μ L nanopure water; 5 μ L 10X reaction buffer (200mM Tris-HCl pH 8.75, 100 mM KCl, 1mg/mL BSA); 1 μ L dXTP mixture with 12.5 mM of each nucleotide; 0.5 μ L (2.5 units) Taq polymerase; 0.5 μ L of 5 μ M of each primer; 2 μ L 50 mM MgCl₂; and 1 μ L 1:20 dilution of environmental DNA. PCR amplification was carried out using a 95°C 2-min initial denaturing followed by 35 cycles: 95°C 15 sec, 55°C 1 min. The samples were then heated to 72°C for 2 min and stored at 4°C. Libraries of PCR amplified 18S rRNA gene fragments from each site were cloned using a TOPO TA Cloning Kit (Invitrogen Inc., Carlsbad, California). Individual clones were sequenced with a Beckman CEQ 8000 instrument (Beckman Co., Palo Alto,

California). For sequencing, archaeal primer Arch21F, prokaryotic primer 27F (5' AGAGTTTGATCCTGGCTCAG 3') (DeLong 1992), and eukaryotic primer 18S4 were used.

Raw sequences were processed with custom software developed in our lab that finds a highly conserved region of the sequence as an anchor point and then trims upstream and downstream from that point. We optimized the trim points so that only the most accurate part of the sequences were used, and employed a filter that removed poor quality sequences. This produced highly accurate sequences by reducing the length of the read to 500 bp and discarding poor sequences (Wu et al. 2009).

The filtered sequences were analyzed using the program Sequencher (Gene Codes, Ann Arbor, MI) to group sequences into operational taxonomic units (OTUs) with 97% identity for prokaryotes (Schloss and Handelsman 2005) and 99% identity for eukaryotes (Wu et al. 2009). A representative sequence from each OTU was used as a query in a Genbank search and the nearest identified sequence match used to provisional identify each OTU. The sorting of prokaryote OTUs into groups with similar metabolic function was carried out by reviewing the literature for each provisionally identified prokaryote. Rarefaction curves and estimates of Shannon Diversity were calculated using EcoSim Software (Gotelli 2009).

Results

Physical Characteristics

Temperature data was recorded every 4 hours in from Jewfish Sink from October 11, 2003 to July 26, 2004. The data show that the temperature in the upper 9 m varies in close relationship to the surface air temperature with a delay of about 10 days at 6 and 9

m compared to 3 m. The coldest temperatures in the upper 9 m were 10.6 °C on December 12, 2003, and 10.2 °C on January 1, 2004, at 3 m; 10.2 °C on December 21, 2003, and on January 12, 2004, at 6 m; and 10.9 °C on December 22, 2003, at 9m. The sinking of denser, colder water was observed in the data from the recording thermometers at depths of 12, 18, and 24m. At a depth of 12m, the temperature dropped 3.8 °C in 8 hours on December 3, 2003, and another 3 °C in 20 hours on December 6 and 7, 2003. At a depth of 18m, the temperature dropped 4.2 °C in 8 hours on December 7 and 8, 2003. At a depth of 24m, the temperature dropped 3.4 °C over 16 days from December 28, 2003, to January 13, 2004. The sinking surface water was not dense enough to influence temperatures at depths of 31m and deeper. At these deeper depths, the temperature was generally constant with a slight increase over the monitoring period. Time series from the HOBO recording thermometers are shown in Figure 3-1.

Previous analyses of the particulate cloud in Jewfish Sink were from samples collected in late winter 2004-2005 from the upper anoxic zone. Additional samples were collected during the summer of 2005 to determine whether the composition of the particulate cloud changes in the summer when the pH of the water in the upper anoxic zone undergoes a dramatic increase. Electron microscopy revealed two kinds of particles in the cloud from the summer samples: iron sulfide minerals including pyrite framboids and a mineral composed of calcium, carbon, and oxygen identified as calcium carbonate (Figure 3-2). The summer composition of the particulate clouds is different from the particles of aluminum silicate clay minerals and aggregates of organic and inorganic particles observed in the winter (Garman and Garey, 2005).

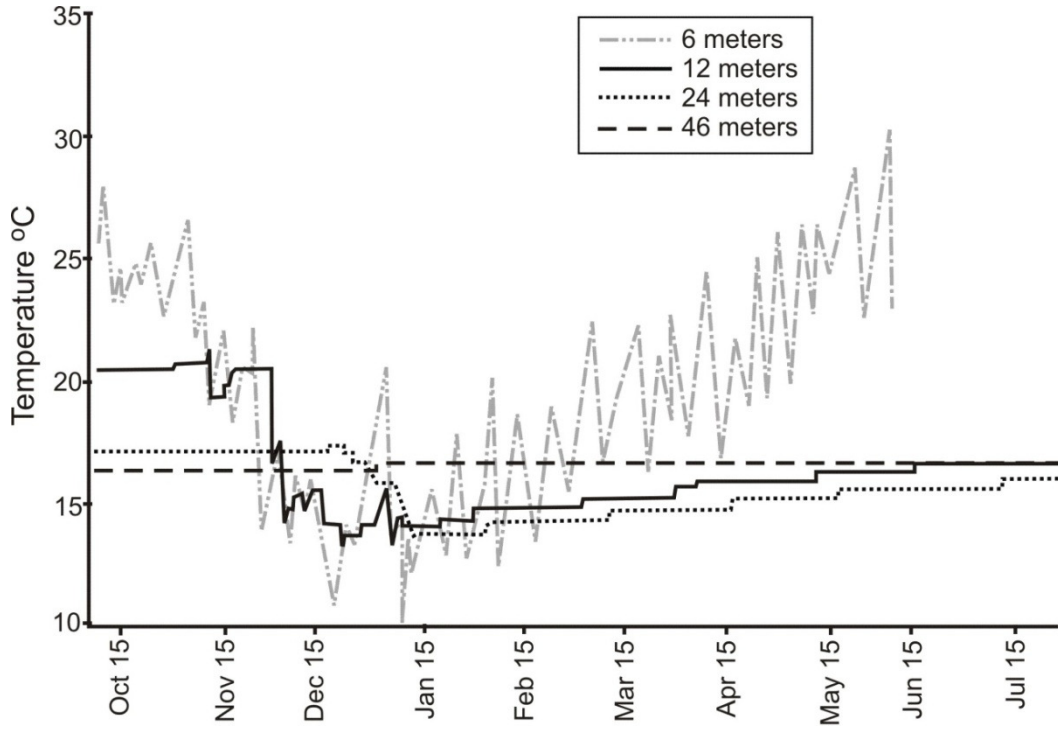


Figure 3-1: Temperature profiles of Jewfish Sink at different depths measured every four hours from October through July 2004. Measurements were taken at 3, 6, 9, 12, 18, 24, 37, 46 and 61m but only selected depths are shown for clarity.

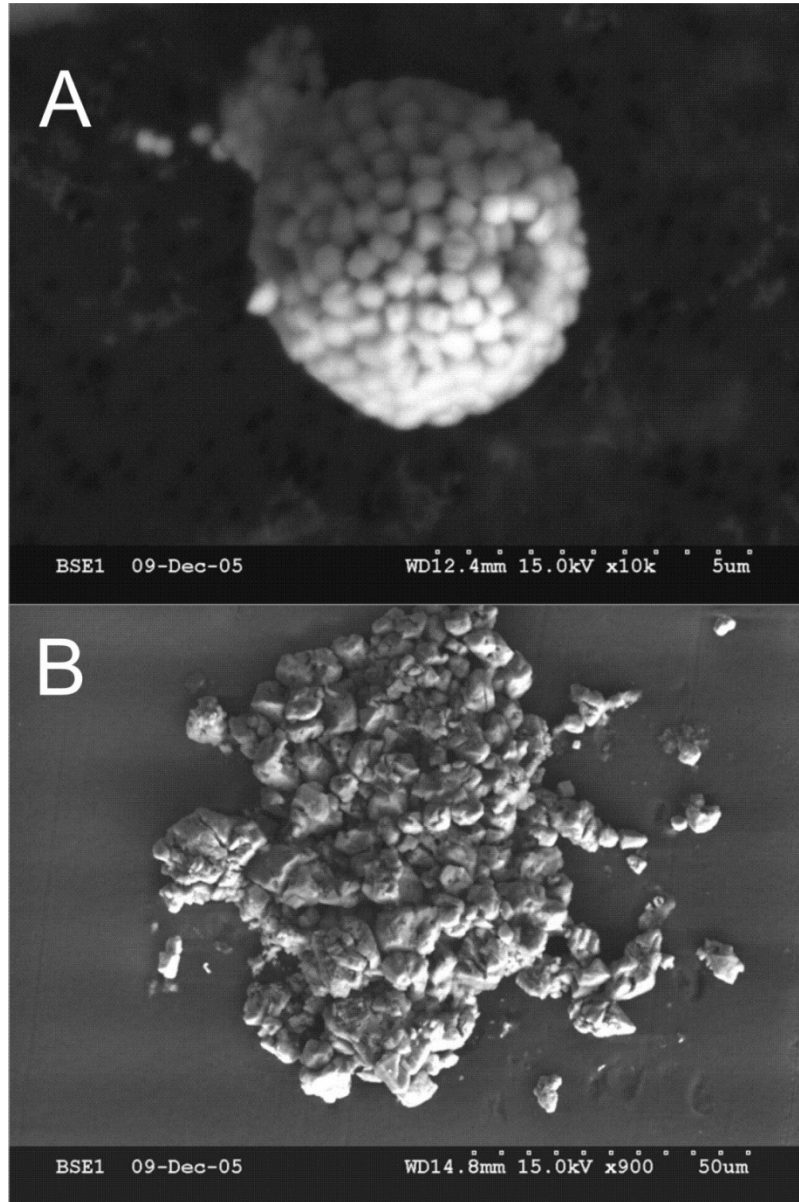


Figure 3-2: Two major types of particles found in Jewfish Sink Summer water: A. Pyrite framboid particle. B. Calcium Carbonate particles.

The surface water salinity at Jewfish Sink was 22 to 22.5 ppt compared to 28 to 30 ppt at Crystal Beach Spring. The samples collected from the Jewfish Sink area were greater than 50% coarse sand (>500 μm). At Crystal Beach Spring, the 5 m sample next to the spring basin was washed by the spring discharge and contained greater than 50% coarse sand. The low flow in the intracoastal waterway behind the barrier island allows the accumulation of finer sediments away from the spring basin and the other sampling locations contained less than 10% coarse sand.

Two seepage readings were taken at Jewfish Sink. The first was over a period of 1500 min from a falling tide just below high tide through two high tides ending at a medium tide before a low tide. The second was over a period of 1215 minutes beginning at a medium tide before a low tide through two low tides and ending just before high tide. Seepage rates were between 0.008 and 0.020 l/min- m^2 at locations 5 m and 15 m east (shoreward) of the rim of the sinkhole, respectively. Surface salinity at Jewfish Sink was 22 to 22.5 ppt; the same as the salinity of the seepage.

A seepage reading was taken at Crystal Beach Spring beginning at low tide through one low tide and ending on a rising tide near high tide. At Crystal Beach Spring, which is closer to shore and still actively discharging low salinity water, seepage rates were 0.08 l/min- m^2 at locations 5 m and 15 m east (shoreward) of the rim of the sinkhole. Surface salinity at Crystal Beach Spring was 28 ppt at low tide and 30 ppt at high tide compared to the seepage salinity of 27 to 28.5 ppt and spring salinity of 6 ppt.

Macrofauna in the vicinity of Jewfish Sink and Crystal Beach Spring

A total of 833 individual specimens were identified from the two transects with 664 individuals from the Jewfish Sink transect and 169 individuals from the Crystal Beach transect.

The macrofauna abundance and richness from each sample are shown in Figure 3-3. Both the number of taxa and overall abundance were greater at Jewfish Sink compared to Crystal Beach Spring ($p < 0.001$). The 1 km site at Crystal Beach Spring included more taxa than the samples collected closer to Crystal Beach Spring. Species richness was significantly higher at the Jewfish Sink transect compared to the Crystal Beach Spring transect ($p = 0.003$). There were no statistically significant differences in species richness when sites within a transect were analyzed, but there were some statistical differences in richness for two sites at Jewfish Sink (50m and 100m) compared to three sites at Crystal Beach Spring (5m, 50m, and 100m).

The Shannon-Weiner diversity index (H' ; \log_e) was higher at the Jewfish Sink transect compared to the Crystal Beach Spring transect ($p = 0.014$). There were no significant differences among individual sites within either transect (Figure 3-4a). The evenness was significantly higher at the Crystal Beach Spring transect compared to the Jewfish Sink transect ($p = 0.006$) (Figure 3-4b).

The cluster analysis of abundance data showed four groups at 30% Bray-Curtis similarity (Figure 3-5). The Crystal Beach Spring 1 km samples and one of the Crystal Beach Spring 5 m replicates grouped with two replicates from the Jewfish Sink 1 km site and one replicate from the Jewfish Sink 5 m site (Group A). The remaining Jewfish Sink samples form Group B and the remaining Crystal Beach Spring samples excluding one

replicate from the 100 m site form Group D. Replicate 2 from the Crystal Beach 5 m site was in its own group (Group C) but closest to Group D. The same groupings were observed in an MDS plot (not shown).

SIMPER analysis (Table 3-1) of the cluster groups showed that the Group A sites had an average similarity of 36%, with Tubificidae and *Tectidrilus squalidus* accounting for 68% of the similarity. Group B sites had an average similarity of 36%, with Tubificidae and *Aricidea philbinae* accounting for 50% of the similarity. Group D sites had an average similarity of 22%, with *Cirrophorus* sp. accounting for 66% of the similarity.

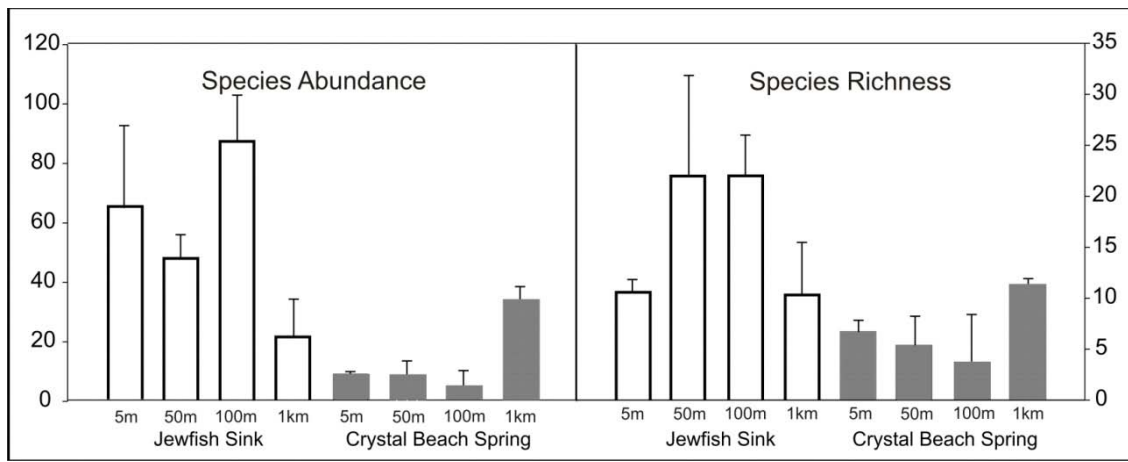


Figure 3-3: Species Abundance and Species Richness of macrofauna from transects at Jewfish Sink (white bars) and Crystal Beach Spring (gray bars). Distance along each transect from the entrance are shown.

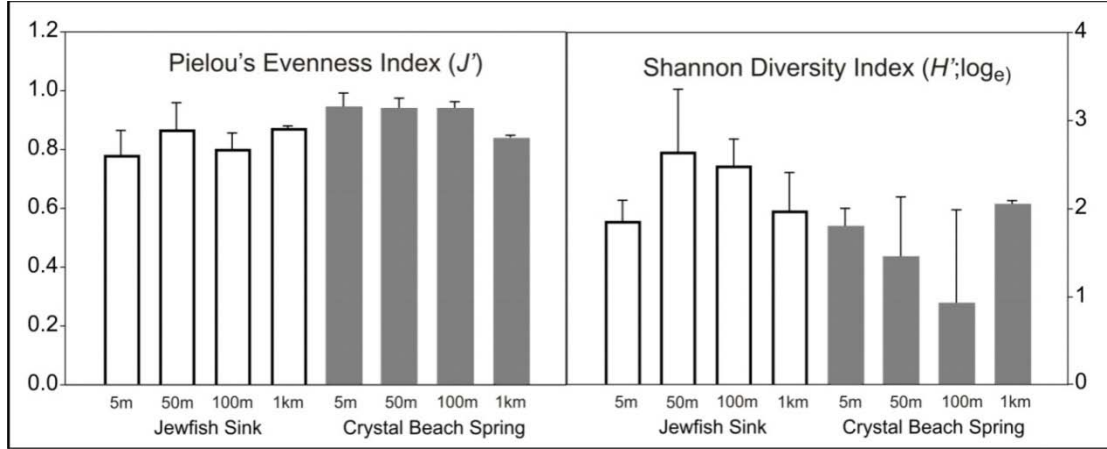


Figure 3-4: Evenness and Shannon Diversity of macrofauna from transects at Jewfish Sink (white bars) and Crystal Beach Spring (gray bars). Distance along each transect from the entrance are shown.

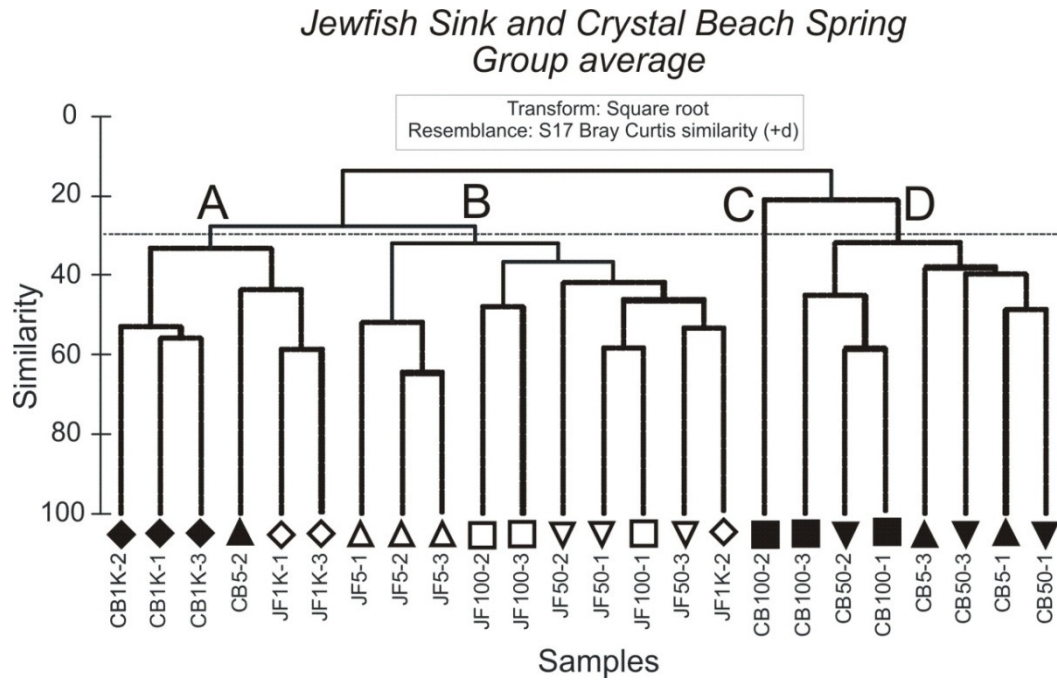


Figure 3-5: Cluster analysis of similarity among macrofauna samples along the transect from Jewfish sink (JF, open symbols) and Crystal Beach Spring (CB, filled symbols). Distances (5=5m, 50=50m, 100=100m, 1K=1000m) and the replicate number (1, 2 or 3) are shown underneath each symbol. The letters A, B, C and D represent the major groups discussed in the text. The dotted horizontal line indicates 30% similarity.

Table 3-1. Simper Analysis		
Group A: Average Similarity: 35.91		
Macrofauna Identification	Contribution %	Cumulative %
Tubificidae	40.85	40.85
<i>Tectidrilus squalidus</i>	27.58	68.42
<i>Prionospio heterobranchia</i>	10.86	79.28
Mysidacea	6.51	85.79
<i>Cirrophorus</i> sp.	5.06	90.86
Group B: Average Similarity: 36.20		
Macrofauna Identification	Contribution %	Cumulative %
Tubificidae	26.74	26.74
<i>Aricidea philbinae</i>	22.97	49.71
<i>Tectidrilus squalidus</i>	8.60	58.30
<i>Kinbergonuphis</i> sp.	6.15	64.45
<i>Chone americana</i>	5.86	70.31
Group C: Average Similarity: 21.57		
Macrofauna Identification	Contribution %	Cumulative %
<i>Cirrophorus</i> sp.	66.22	66.22
Nemertea	15.96	82.18
<i>Ampelisca</i> sp. A	4.69	86.87
<i>Magelona pettiboneae</i>	3.93	90.80

Molecular Biodiversity

The 413 archaeal sequences clustered into 205 OTUs based on 97% similarity and the results summarized in Table 3-2. Shannon Diversity of archaeal sequences were lower (4.64) than bacterial sequences (4.81). Rarefaction curves suggest that sampling of both archaeal and bacterial sequences was incomplete (Figure 3-6). The closest sequences in Genbank for each OTU varied widely but included environmental sequences from hypersaline waters, microbial mats, anoxic sediments, methane cold seeps and hydrothermal sediments. Archaeal sequences represented a variety of groups that included anaerobes, ammonia oxidizers, methanogens and sulfur reducers (Table 3-3).

Table 3-2: Archaeal Sequences found at Jewfish Sink by Phylum and Genus.

Phyla	Abundance	Richness	Match Percentage
	# Species	# OTUs	
Crenarchaeota	276	139	78-89
Euryarchaeota	135	64	80-97
Korarchaeota	1	1	77
Genera	# Species	# OTUs	Match Percentage
<i>Staphylothermus</i>	218	92	81-88
<i>Methanobrevibacter</i>	62	26	81-87
<i>Nitrosocaldus</i>	31	24	80-85
<i>Methanobacterium</i>	26	4	82-83
<i>Aciduliprofundum</i>	24	13	81-85
<i>Thermofermentum</i>	15	15	81-85
<i>Methanothermobacter</i>	13	12	80-83
<i>Thermofilum</i>	6	6	82-85
<i>Methanlobus</i>	5	4	95-97
<i>Caldisphaera</i>	2	2	78-83
<i>Fervidococcus</i>	2	2	85-87
<i>Aeropyrum</i>	1	1	89
<i>Desulfurococcus</i>	1	1	86
<i>Geoglobus</i>	1	1	82
<i>Korarchaeum</i>	1	1	77
<i>Methanococcoides</i>	1	1	85
<i>Methanoplanus</i>	1	1	92
<i>Methanopyrus</i>	1	1	89
<i>Methanosarcina</i>	1	1	97

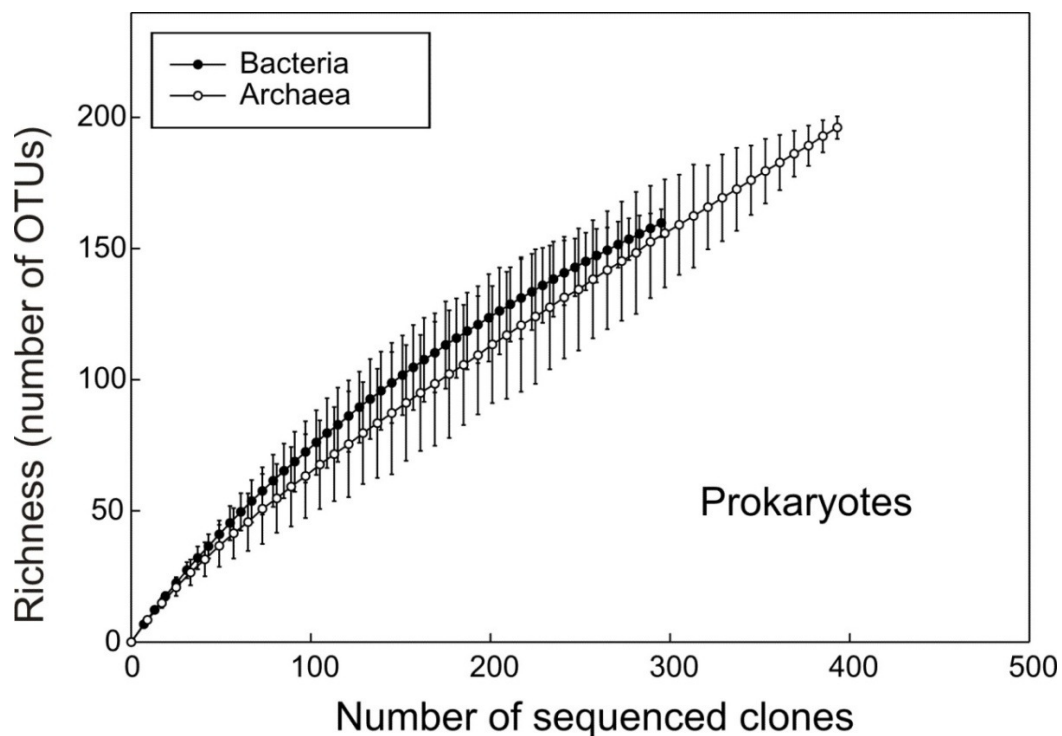


Figure 3-6: Rarefaction analyses of prokaryote 16S rRNA richness based on the number of OTUs. Mat samples from the vertical wall of Jewfish Sink were collected from the anaerobic zone. Error bars represent variance.

Table 3-3: Potential Functional Properties of Prokaryotes at Jewfish Sink.

The percentages add up to more than 100% because some prokaryotes fit into multiple categories.

	<u>Archaea</u>		Bacteria		<i>Total Percentage</i>
	<i>Sequences</i>	<i>Percentage</i>	<i>Sequences</i>	<i>Percentage</i>	
Anaerobes	341	82.77	183	57.19	71.58
Aerobes	32	7.77	110	36.25	19.40
<u>Methanogens</u>	110	26.70	0	0.00	15.03
Obligate Methane Oxidizers	0	0.00	14	4.38	1.91
Sulfur Reducers	247	59.95	116	36.25	49.59
Sulfur Oxidizers	0	0.00	70	21.88	9.56
Unknown	39	9.47	27	6.56	9.02
Nitrogen Reducers	26	6.31	3	0.93	3.96
Nitrogen Oxidizers	93	22.57	37	11.56	17.76
Iron Reducers	1	0.24	7	2.19	1.09

The 320 bacterial sequences clustered into 164 OTUs based on 97% similarity. Results are summarized in Table 3-4. The closest sequences in Genbank for each OTU included environmental sequences from hypersaline microbial mats and waters, deep sea microbial mats, anoxic sediments, methane cold seep sediments, salt marshes, landfill leachate, hydrothermal sediments, seagrass sediments, and whale fall ecosystems. Bacterial sequences represented groups that included anaerobes, methane oxidizers, ammonia oxidizers, sulfur reducers and oxidizers.

Table 3-4: Bacterial Sequences found at Jewfish Sink by Phylum and Genus.

	Abundance	Richness	
Phyla	# Species	# OTUs	Match Percentage
Proteobacteria	179	164	80-99
Firmicutes	58	34	78-99
Bacteroidetes	14	14	82-98
Actinobacteria	13	9	78-85
Spirochaetes	21	9	84-100
Chloroflexi	12	7	82-100
Verrucomicrobia	9	6	79-84
Planctomycetes	7	5	83-88
Nitrospirae	2	2	80-81
Aquificae	1	1	80
Cyanobacteria	1	1	88
Cyanophora	2	1	83
Synergistetes	1	1	84
Genera	# Species	# OTUs	Match Percentage
<i>Arcobacter</i>	40	10	85-93
<i>Clostridium</i>	28	14	87-99
<i>Desulfobacterium</i>	26	11	82-91
<i>Spirochaeta</i>	19	8	84-100
<i>Dehalococcoides</i>	15	7	84-100
<i>Desulfosarcina</i>	12	4	91-94
<i>Pseudomonas</i>	8	1	98
<i>Thiomicrospira</i>	8	5	91-97
<i>Verrucomicrobia</i>	7	5	79-84
<i>Unkown</i>	6	5	
<i>Catabacter</i>	5	4	89-93
<i>Cytophaga</i>	5	3	88
<i>Desulfomonile</i>	5	2	83-89
<i>Desulfovibrio</i>	5	5	83-88
<i>Methylobacter</i>	5	2	93-99
<i>Pelobacter</i>	5	2	85-91
<i>Planctomycetales</i>	5	3	88
<i>Sulfurimonas</i>	5	2	80-91
<i>Sulfurovum</i>	5	1	93
<i>Desulfatibacillum</i>	4	2	83-89
<i>Syntrophomonas</i>	4	1	81

The 836 eukaryotic sequences clustered into 149 distinct (OTUs) based on 99% similarity and the results are summarized in Table 3-5. Shannon Diversity of eukaryotic sequences within the anoxic mat is higher (3.42) than in the oxic sediment (2.53) while rarefaction curves suggested that sampling was incomplete (Figure 3-7). These sequences represent six eukaryotic supergroups (based on Baldauf, 2003): Alveolates, Amoebozoa, Cercozoa, Heterokonts, Opisthokonts, and Plantae. The two most prominent groups are Alveolates (39% of OTUs and 34% of sequences) and Opisthokonts (34% of OTUs and 49% of sequences). The Opisthokont sequences were dominated by fungi (64% of OTUs and 93% of sequences) and metazoans comprised 18% of the OTUs and 3% of the sequences. The Alveolate sequences consisted of: Apicomplexa, Ciliophora, Cryptosporidium and Dinophyceae. The Heterokont sequences included diatoms and three other groups of algae: Bolidophyceae, Raphidophyceae, and Thraustochytriidae. The Plantae included Chlorophyta and Streptophyta. The Cercozoa included Ascetosporea, Cercomonadida, Cryomonad, Ebridea, and Thaumatomonadida.

Super Group Summary	Abundance			Richness			
	# Sequences			# OTUs			
	Total	Mat	Sediment	Total	Mat	Sediment	Both
Opisthokonts	409	102	307	50	17	36	3
Alveolates	272	199	73	58	37	28	9
Heterokonts	65	32	33	21	6	15	0
Cercozoa	54	43	11	10	5	6	1
Plantae	35	18	17	8	4	6	1
Amoebozoa	1	0	1	1	0	1	0
Alveolates Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Dinophyceae	144	94	50	30	16	19	5
Ciliophora	61	61	0	12	12	0	0
Apicomplexa	44	33	11	10	4	8	2
Cryptosporidium	8	8	0	3	3	0	0
Unknown	15	3	12	3	2	1	1
Heterokonts Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Diatoms	26	2	24	13	2	11	0
Algae, Bolidophyceae	19	18	1	2	1	1	0
Algae, Thraustochytridae	13	11	2	3	2	1	0
Algae, Raphidophyceae	6	0	6	2	0	2	0
Unknown	1	1	0	1	1	0	0
Opisthokonts Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Fungi, Sordariomycetes, saprobe degrades algal polysaccharides	297	31	266	13	2	13	2
Fungi, Tremellomycetes, deep-sea methane cold seep sediment	40	29	11	7	3	5	1
Fungi, Saccharomycetes, fermentation	18	18	0	3	3	0	0
Fungi, Ustilaginomycetes, plant parasite	12	12	0	1	1	0	0
Fungi, Dothideomycetes, plant pathogen or saprobe	9	0	9	3	0	3	0
Protist, Ichthyosporean	9	6	3	5	3	2	0
Metazoa, Mollusk	5	0	5	1	0	1	0
Fungi, Mucoromycotina, oxygen depleted marine	4	4	0	3	3	0	0
Fungi, Eurotiomycetes	2	2	0	2	2	0	0
Metazoa, Platyhelminthes marine	2	0	2	2	0	2	0
Metazoa, Annelid-2	2	0	2	2	0	2	0
Protist, Apusomonadidae, marine flagellate	2	0	2	2	0	2	0
Metazoa, Gnathostomulid	2	0	2	1	0	1	0
Glomeromycetes	1	0	1	1	0	1	0
Metazoa, Annelid-1, Capitella capitata	1	0	1	1	0	1	0
Metazoa, Copepod	1	0	1	1	0	1	0
Metazoa, Nemertea	1	0	1	1	0	1	0
Protist, Eccrinales	1	0	1	1	0	1	0
Cercozoa Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Ebriidae	35	35	5	1	1	0	0
Thaumatomastigidae	7	0	7	2	0	2	0
Cryothecomonas	6	5	1	2	2	1	1
Cercomonadidae	4	3	1	3	2	1	0
Paradinium	2	0	2	2	0	2	0
Plantae Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Pycnococcaceae	15	3	12	4	1	4	1
Pyramimonadales	12	0	0	2	2	0	0
Chlorellaceae	4	0	4	1	0	1	0
Alliaceae	3	3	0	1	1	0	0
Polypodiaceae	1	0	1	1	0	1	0
Amoebozoa Detail	Total	Mat	Sediment	Total	Mat	Sediment	Both
Hartmannellidae	1	0	1	1	0	1	0

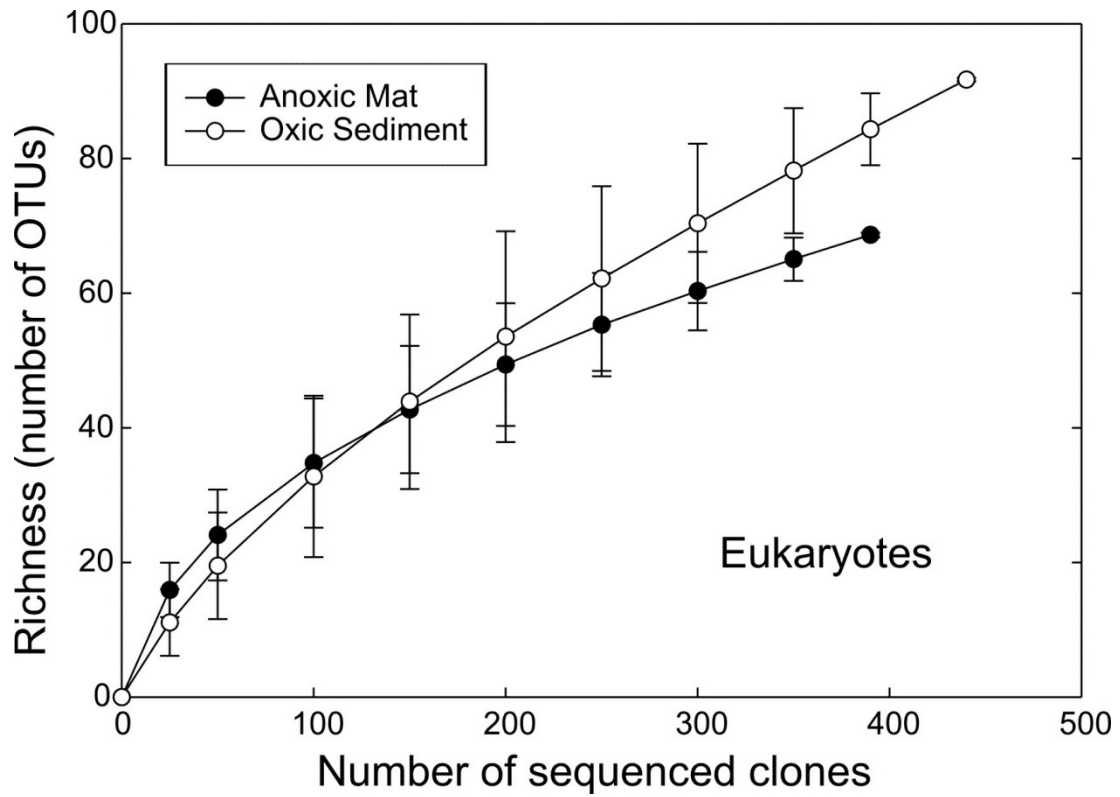


Figure 3-7: Rarefaction analyses of eukaryote 18S rRNA richness based on the number of OTUs. Mat samples from the vertical wall of Jewfish Sink were collected deep within the anaerobic zone. Sediment samples were collected from horizontal ledges in the shallow oxic zone. Error bars represent variance.

Discussion

Macrofauna in the vicinity of Jewfish Sink and Crystal Beach Spring

The original hypothesis based on previous work at Jewfish Sink (Garman and Garey 2005) was that the benthic macrofauna community at the Jewfish Sink transects would be influenced by the effects of the sulfide pump creating a community with lower abundance and diversity compared to Crystal Beach Spring, an active freshwater submarine spring.

At Crystal Beach Spring, the sites near the basin had relatively few taxa and low overall abundance. This condition is likely a result of freshwater influx through the spring conduit and vent, which drives seepage at rates that are an order of magnitude greater at Crystal Beach Spring compared to Jewfish Sink. Studies by Johannes (1980), D'Elia et al. (1981), and Simmons (1992) indicate that groundwater seepage into shallow marine environments has higher levels of ions including nitrate than surface water discharges. This increase in ion concentration is thought to have a significant impact on benthic communities. The benthic macrofauna data from Jewfish Sink and Crystal Beach Spring support this hypothesis, as there is less diversity and abundance of taxa at Crystal Beach Spring where freshwater flow still discharges offshore and drives high seepage rates in the near shore marine sediments. At Jewfish Sink where freshwater flow has ceased and seepage rates are an order of magnitude lower, there was higher species abundance and diversity. It should also be noted that Pinellas County in the vicinity of Crystal Beach Spring is much more highly developed than the rural, sparsely populated area near Jewfish Sink.

Physical Characteristics of Jewfish Sink

There was no evidence of groundwater seepage into Jewfish sink from the HOBO temperature data. This would have appeared as transient temperature spikes of 24 to 25 °C and were not found at 9 m or deeper (Figure 3-1) confirming that Jewfish sink is not an active freshwater spring. The sinking of cold surface water did not influence the bottom water of the sinkhole during the study period. The salinity and high concentrations of other solutes make the bottom water too dense for sinking winter surface water to mix with it.

Biodiversity and related processes within Jewfish Sink

The seasonal changes in the composition of the particulate clouds in the water column appear to be related to the composition of the microbial communities. In the winter, the sinking of oxygenated surface water combined with the limited sunlight penetrating into the sinkhole opening from the low angle of the winter sun would allow sulfur-oxidizing bacteria in the upper anoxic zone to out-compete the anaerobic sulfide phototrophic bacteria. Sulfur oxidizers comprise 22% of the bacteria present in Jewfish sink winter water (Table 3-3) with *Thiomicrospira* and *Sulfurimonas* representing the most common genera (Table 3-4). The chemoautotrophic bacteria use sulfide and convert it to sulfate, thereby reducing pH, as observed in the winter water column profile. The reduced pH favors bicarbonate and dissolved carbon dioxide rather than carbonate, and calcium carbonate precipitation does not occur (Castanier et al. 1999).

In the summer, when the bottom water pH is at a maximum and calcium carbonate is one of the principal components of the particulate clouds, the light from the overhead sun penetrates deeper into the sinkhole through the narrow opening. At this

time of the year, anoxygenic phototrophic bacteria can be expected to out-compete chemoautotrophic bacteria for sulfide. The anoxygenic phototrophic bacteria use sulfide and convert it to elemental sulfur, removing sulfide without creating sulfate resulting in a pH increase. Although we examined water collected in late winter, we found at least one example of anoxygenic phototrophic bacteria (*Thi alkalivibrio*) in low abundance as would be expected in the winter. Similarly, because the sink is predominantly anoxic during the summer, prokaryotes using dissimilatory sulfate reduction to produce hydrogen sulfide are expected to increase, resulting in a higher pH. In our winter sample, we found numerous prokaryotes capable of dissimilatory sulfate reduction (e.g. *Desulfovibrio*, *Desulfococcus*, *Desulfatibacillum*, *Moorella*), some at high abundance. An increase in pH favors the presence of carbonate anion in solution instead of bicarbonate anion or dissolved carbon dioxide. The presence of both carbonate anion and calcium results in the passive precipitation of calcium carbonate (Castanier et al., 1999). It has been demonstrated that the cell wall of some prokaryotes plays a role in carbonate precipitation. The increased reactivity of cations adsorbed to the cell wall promotes nucleation of carbonate minerals such as calcium carbonate (Merz-Preiß 2000). Cave divers in Florida often encounter particulate clouds associated with a strong sulfide odor, particularly at the freshwater/saltwater interface of anchialine environments as well as in anoxic marine sinks. This provides anecdotal evidence that the link between calcium carbonate precipitation and bacterial mediated sulfide production is widespread in marine-influenced anoxic environments.

In addition to the bacteria that contribute to calcium carbonate precipitation and sulfur reduction, 15% of archaeal sequences were potentially methanogenic (Table 3-3).

Methanogenesis leads to an increase in alkalinity as well as a depletion of carbon dioxide. Subsequently, an increase in calcium carbonate precipitation is observed (Castanier 1999). The known syntrophy of certain methanogens with sulfur reducing bacteria suggests that the sulfide peaks corresponding to an increase in sulfur reduction may also correlate to an increase in those methanogens. Indeed, both *Methanosarcinales* and *Desulfosarcina*, two genera known to cluster in tight association in this type of syntrophy (Konhauser 2007), were found in the samples collected. Lastly, the production of carbonate and bicarbonate ions as a result of nitrogen cycling favors calcium carbonate precipitation. Ammonification, anoxic dissimilatory nitrate reduction and urea degradation not only produce the carbonate ions, but also lead to an increase in pH which induces the precipitation (Castanier et al. 1999). Roughly 22% of the prokaryotic sequences found represent taxa potentially involved in the cycling of nitrogen (Table 3-3).

The presence of iron sulfide minerals in the particulate cloud in the summer indicates that some sulfide is reacting with iron in an inorganic reaction. This reaction is mediated indirectly by both the high sulfide concentration caused by microbial sulfur reduction and the iron (II) that results from microbial iron reduction (Konhauser 2007). Sequences similar to known iron reducing prokaryotes were represented by approximately 2% of the bacterial sequences and 0.24% of the archaeal sequences found in Jewfish Sink. Bacteria within the water column may serve as a surface for the precipitation of iron sulfide, the precursor to the framboidal pyrite (Schoonen 2004). The sulfide concentration in the bottom water showed peaks in late winter (March) and late summer (August/September). The sulfide peaks occurred at times of large inputs of

organic carbon into the sinkhole: the influx of bottom feeding horseshoe crabs and stingrays in late winter and the influx of macroalgae from storm surges during the summer. The spikes in sulfide production are believed to correspond to the development of condensed particulate clouds that were periodically observed in the sinkhole. The particulates created in conjunction with the sulfide spikes are biomineral aggregates in the winter and calcium carbonate and pyrite in the summer. It appears that seasonal biogeochemical cycles in the sinkhole cause large variations in pH and the onset and cessation of calcium carbonate precipitation.

The largest prokaryotic contributors found within the sink in our late winter samples were dominated by sulfur metabolizers. Members of the genera *Arcobacter*, *Clostridium*, *Desulfobacterium*, *Staphylothermus* (Tables 3-2, 3-4) are associated with either sulfur reduction or sulfur oxidation. Both *Arcobacter* and *Clostridium*, the two dominant bacterial taxa found (21% of bacterial sequences), are genera known to contain human pathogens. Among the Archaea, *Methanobrevibacter* and *Nitrosocaldus*, both ammonia oxidizers, were also abundant. While the percentage of nitrogen oxidizers exceeds that of nitrogen reducers, the anoxic environment is primarily composed of anaerobic organisms using terminal electron acceptors other than oxygen, with sulfur reducers comprising half of all prokaryotes sequenced (Table 3-3). The sink, never becoming completely oxic or anoxic, sustains a functionally diverse consortium of prokaryotes; the successions of which manifest the geochemical cycles observed.

Bacterial Shannon Diversity was slightly higher (4.81) than Archaea (4.64). Rarefaction curves (Fig. 3-6) demonstrate that sampling was incomplete in that the number of OTUs continues to increase with the addition of new sequences. Rarefaction

curves using Shannon Diversity (not shown) did not increase much with the addition of new sequences, suggesting that most of the new sequences being added were rare. This is consistent in that most of the bacterial and archaeal OTUs (with 97% match criteria) were represented only one time in our data.

The eukaryotes within Jewfish Sink were diverse, representing 6 super groups, ophisthokonts, avleolates, heterokonts, plants, and one amoebozoan (Table 3-5). The 836 sequences clustered into 164 OTUs, most of which were provisionally identified using GenBank Blast searches. In some cases, the sequence identity to known GenBank entries was low, so the provisional identification was sometimes imprecise as seen in the Plantae detail in Table 3-5 where a fern (Polypodiaceae) and flowering plant (Alliaceae) sequence were “identified.” However, provisional identifications did provide an overview of the eukaryotic community in Jewfish Sink.

The eukaryote sequences originated from two locations. There were 394 sequences from microbial mat collected from the vertical wall of the sink deep in the anoxic zone (see Fig. 2-3 in Chapter Two), and 442 sequences from sediment on horizontal ledges in the shallow, aerobic throat of the sinkhole. No metazoan sequences were found in the anoxic zone, and only 14 of 442 sequences from the aerobic sediment (3%) represented metazoans. Table 3-5 indicates that 9 provisionally identified eukaryote groups were found only in the microbial mat sample, 17 were found only in the aerobic sediment samples, while 8 groups were found in both environments. The dominant eukaryote group in terms of sequence number was fungi that composed 65% of the sediment sequences. Although fungi were also the most common type of sequences in the microbial mat, they represented only 24% of those sequences. Dinoflagellates were

equally predominant in the microbial mat (24%), followed by ciliophorans (16%). An analysis of the Shannon Diversity revealed that the diversity of eukaryotic sequences in the anaerobic microbial mats (3.42) was higher than in the aerobic sediment (2.53), most likely due to the predominance of a few fungi OTUs in the aerobic sediment. Rarefaction analysis revealed that although Shannon Diversity of eukaryotes was higher in the microbial mat sample, more OTUs were present in the oxic sediment than in the anoxic mat. The rarefaction analysis also suggested that sampling was far from complete because the number of OTUs continued to increase with increasing numbers of sequences (Figure 3-7). As with the prokaryote sequences, Shannon diversity became relatively stable with increasing number of sequences (not shown).

Examination of fresh samples of microbial mat under a microscope revealed numerous dinoflagellates and ciliates that appeared to be grazing on the mat material. Several provisionally identified taxa including some alveolates and cercozoans in Table 3-5 have been reported to feed on diatoms and dinoflagellates (Massana and Pedros 1994; Schnepf and Kühn 2000; Taylor 1987), so our analysis suggests that Jewfish Sink provides a thriving and diverse environment for anaerobic eukaryotes.

Conclusions

Coastal anchialine ecosystems are highly sensitive to long term ground water fluctuations that arise from anthropogenic overuse of aquifers or from changing sea levels. Jewfish sink represents a former anchialine system that has transitioned to an anoxic sulfur-cycling marine basin. Although the biota within Jewfish sink has likely changed considerably with the transition, the overall influence of the sink's geochemistry on the surrounding benthic marine ecosystem is significantly lower than the influence of

Crystal Beach Spring, an anchialine spring that discharges water that is low in salinity and high in nutrients.

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