THE ROLE OF MACROALGAL SPECIES AS BIOINDICATORS OF WATER QUALITY IN BERMUDIAN KARSTIC CAVE POOLS

A Thesis

by

BRIDGET MARIE MALONEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Wildlife and Fisheries Sciences

THE ROLE OF MACROALGAL SPECIES AS BIOINDICATORS OF WATER QUALITY IN BERMUDIAN KARSTIC CAVE POOLS

A Thesis

by

BRIDGET MARIE MALONEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Thomas M. Iliffe
Committee Members,	Andrew Klein
	Antonietta Quigg
Head of Department,	Thomas E. Lacher, Jr.

December 2008

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

The Role of Macroalgal Species as Bioindicators of Water Quality in Bermudian Karstic Cave Pools. (December 2008)

Bridget Marie Maloney, B.S., The University of Arizona

Chair of Advisory Committee: Dr. Thomas Iliffe

Bermuda has one of the highest concentrations of cave systems of any country in the world, but as the resident human population and tourism expand, this unique habitat is becoming increasingly threatened by development and water pollution. A water quality assessment was performed in six of Bermuda's anchialine cave pools during summer 2007. Vertical water profiles were collected at each site to determine temperature, salinity, dissolved oxygen, and pH levels throughout the water column. Each cave pool had its own unique hydrological patterns. Additionally, water samples were collected at the surface and at the maximum depth to determine the concentration of nitrate, nitrite, ammonium, phosphate, and urea in the water. Further samples were collected to establish the fecal bacteria content in the surface waters. High nitrate levels were measured in two of the cave pools, while unsafe bacterial levels were measured in three pools.

In situ nutrient addition experiments were used to examine effects of nutrient enrichment on algae naturally occurring in the six cave pool. Initial assessments of the pools determined distribution of foliose species. Six algal species (*Ulva* sp., *Caulerpa mexicana*, *C. racemosa*, *C. sertularioides*, *Halymenia floresii*, and *Cryptonemia* sp.) inhabited multiple sites, but not all species occurred at all sites. Changes in the biomass of individual plants were recorded for 6 to 12 days in response to N (nitrate), P (phosphate), and NP additions. The effect of nutrient addition on increase in biomass was significant over a 6 day period for *Ulva* sp. and *H. floresii*. Intermediate to no growth was seen in *C. mexicana* and *Cryptonemia* sp. *C. mexicana* exhibited both increases and in biomass depending on the site. *Cryptonemia* sp. had low but positive growth. A significant decrease in biomass occurred with *C. racemosa* and *C. sertularioides*. Primary productivity was measured using the traditional light dark bottle method. Differences in net productivity and respiration may explain why some plants responded positively and other negatively to nutrient additions. *Ulva* sp. and *H. floresii* showed potential as bioindicators for groundwater quality.

DEDICATION

This thesis is dedicated to my husband, Brendan.

ACKNOWLEDGEMENTS

Many people have assisted me as I worked through the academic process to complete my thesis research project. First, I would like to express my gratitude for the professors at Texas A&M University. Thank you, Dr. Iliffe, for introducing me to the unique world of both terrestrial and submarine caves in Bermuda. I very much enjoyed the months I was able to spend on this beautiful archipelago learning about the natural environment and the local culture. Dr. Quigg, I appreciate your guidance and support as I explored the different realms of Phycology. I would also like to extend my thanks to Dr Klein for your assistance as I wrote and edited this thesis.

My research would not have been possible without the extensive help and support extended to me by the wonderful people of Bermuda. Successful data collection would not have been possible without the generous help of Dr. Anne Glasspool from the Bermuda Biodiversity Project (BBP). Thank you for volunteering many hours of your time to provide logistical support. Without your assistance, my field work would not have run as smoothly as it did. I would also like to extend my appreciation to Dr. Thad Murdoch and Mandy Shailer from the BBP for tracking down equipment and information that was critical for my project. Jack Ward from the Department of Conservation Services and Lisa Greene from the Bermuda, Aquarium, and Zoo (BAMZ) provided additional support whenever it was required. Data acquisition from the cave pools would not have been possible without the extensive time and patience provided by Gil Nolan and Leon Kemp. Thank you for hauling dive equipment to more remote sites without expecting anything in return. I would also like to express my gratitude to Elizabeth Foster and Kimberly Janusaitis, the undergraduate field assistants from Texas A&M University at Galveston. Without your help day in and out during the beautiful but hot days of summer I would not have been able to collect the volume of data that was acquired.

Funding for this thesis research project came from many sources. All on site expenses during the summer of 2007 was provided through an internship granted by

BAMZ and the Bermuda Zoological Society. Without this funding, the longer term experiments would not have been possible. I was able to purchase field supplies and airfare with the generous support provided by the Erma Lee and Luke Mooney travel grant and the Galveston Graduate Student Association (GGSA). Funding support for my field assistant was graciously provided by the Research Advisory Council (RAC) committee from Texas A&M University at Galveston.

I would also like to express my immense gratitude to my encouraging network of friends and family. Amy Garrett, thank you for taking time out of your very hectic life to act as my nonacademic editor. Your invaluable insights showed me the significance of my project. Mom and Dad, I would like thank you for always believing in me. Your never-ending patience and guidance motivated me each and every day. My importance in this world was evident in your interest in my career path even though it was very different from your own. Finally, thanks to the love of my life, Brendan. I will be forever grateful for your ability to keep me grounded as I worked my way through the process of designing and executing my research project. Your understanding and support enabled me to complete this portion of my academic career with my sanity intact. I am never a disappointment in your eyes yet you inspire me to do the best I can each day. Thank you for the laughter and preventing me from ever being too serious.

TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGEMENTS vi

TABLE OF CONTENTS vii

LIST OF FIGURES x

LIST OF TABLES xiii

CHAPTER

I GENERAL INTRODUCTION 1

Introduction to Bermuda 1
Bermuda Geology 2
Bermuda Hydrology 6
Bermuda Caves 10
Seaweeds of Bermuda 12
Effect of Human Activities on Bermudian Caves and Seaweeds .. 14
Purpose of Research 17
Study Sites 18
Objectives 26
Hypotheses 27

II CAVE POOL WATER CHARACTERISTICS AND QUALITY 28

Introduction 28
    Groundwater Pollution in Bermuda 28
    Historical Groundwater Nutrient and Bacterial Levels 30
    Nitrogen Cycle 31
    Phosphorus Cycle 33
    Use of Fecal Bacteria as Indicators of Groundwater Pollution . 34
    Purpose 36
Materials and Methods 36
    Environmental Water Parameter Measurements 36

CHAPTER Page

Nutrient and Bacterial Sampling 37
Statistical Analyses 38
Results 38
Vertical Profiles of Environmental Water Parameters 38
Nutrient Analysis 56
Bacterial Analysis 59
PCA Analysis 59
Discussion 61

III ALGAL GROWTH AND NUTRIENT EXPERIMENTS 66

Introduction 66
Macroalgae as Bioindicators 66
Purpose 67
Materials and Methods 68
Nutrient Enrichment Experiments 68
Primary Productivity Experiments 69
Statistical Analyses 70
Results 72
Nutrient Enrichment 72
Primary Productivity 77
Discussion 80

IV FINAL SUMMARY AND CONCLUSIONS 84

Vertical Profiling of Environmental Water Parameters 84
Nutrient and Bacterial Analysis 85
Nutrient Enrichment Experiments 86
Primary Productivity Experiments 87
Overall Conclusion 88
Recommendations for Future Research 89

REFERENCES 91

APPENDIX 99

VITA 116

LIST OF FIGURES

FIGURE Page

1.1 Map of Bermuda 2

1.2 Geologic Map of Bermuda 6

1.3 Speleothems Found in Sibley’s Cave 11

1.4 Wilkinson Quarry in Bermuda 15

1.5 Oil Barrels Dumped in Bitumen’s Cave, Bermuda 16

1.6 Trash Dumped in Sear’s Cave, Bermuda 17

1.7 Location of Cave Pool Study Sites in Bermuda 19

1.8 Photograph of Calabash 21

1.9 Photograph of Harbour Pool 22

1.10 Photograph of Deep Blue 23

1.11 Photograph of Grenadier 24

1.12 Photograph of Cliff Pool 25

1.13 Photograph of Emerald 26

2.1 Nitrogen Cycle 32

2.2 Phosphorus Cycle 33

2.3 Temperature versus Depth at Calabash 39

2.4 Salinity versus Depth at Calabash 40

2.5 Dissolved Oxygen versus Depth at Calabash 40

2.6 pH versus Depth at Calabash 41

FIGURE Page

2.7 Temperature versus Depth at Harbour Pool 42

2.8 Salinity versus Depth at Harbour Pool 43

2.9 Dissolved Oxygen versus Depth at Harbour Pool 43

2.10 pH versus Depth at Harbour Pool 44

2.11 Temperature versus Depth at Deep Blue 45

2.12 Salinity versus Depth at Deep Blue 46

2.13 Dissolved Oxygen versus Depth at Deep Blue 46

2.14 pH versus Depth at Deep Blue 47

2.15 Temperature versus Depth at Grenadier 48

2.16 Salinity versus Depth at Grenadier 49

2.17 Dissolved Oxygen versus Depth at Grenadier 49

2.18 pH versus Depth at Grenadier 50

2.19 Temperature versus Depth at Cliff Pool 51

2.20 Salinity versus Depth at Cliff Pool 52

2.21 Dissolved Oxygen versus Depth at Cliff Pool 52

2.22 pH versus Depth at Cliff Pool 53

2.23 Temperature versus Depth at Emerald 54

2.24 Salinity versus Depth at Emerald 55

2.25 Dissolved Oxygen versus Depth at Emerald 55

2.26 pH versus Depth at Emerald 56

FIGURE Page

2.27 Principle Component Analysis of Water Parameters at the Six Cave Pools 60

3.1 Growth of Algal Species from Day 0 to Day 6 with Addition of No Nutrients (C), Nitrogen (N), Phosphorus (P), and Nitrogen and Phosphorus (NP) across Six Sites 73

3.2 Canonical Correspondence Analysis (CANOCO 4.5 CCA) for Nutrient Enrichment Experiments 75

3.3 Post-hoc Plot of Relationships due to Supplemental (Alternative) Explanatory Variables (Background Environmental Water Parameters and Cave Sites) 76

3.4 Net Photosynthetic Rate, Gross Photosynthetic Rate, and Dark Respiration Rate for the Six Species of Algae Used for Nutrient Addition Experiments 78

3.5 Net Photosynthetic Rate, Gross Photosynthetic Rate, and Dark Respiration Rate for *Ulva* sp. and *C. mexicana* Using Different Combinations of Source Ambient Water and *in situ* Location 79

LIST OF TABLES

TABLE Page

1.1 Characterization of six study sites.. 20

2.1 Mean concentrations (uM) nutrients from analyzed water column data.... 57

2.2 One-way ANOVA results for nutrient data.. 58

2.3 Concentration (CFU/100 ml) of the bacteria, *Enterococcus*, in surface waters 59

3.1 Presence and abundance of algal species utilized for nutrient addition and productivity experiments at each cave pool site.. 68

3.2 Variance decomposition for changes in algal biomass that were correlated with nutrient treatments, background water chemistry, and algal species among sites. 77

CHAPTER I

GENERAL INTRODUCTION

Introduction to Bermuda

Situated at 32°20'N latitude and 64°45'W longitude in the northwestern Atlantic Ocean, Bermuda is an archipelago composed of 138 small islands that occupy only 53.3 km^2 (Fig. 1.1). It lies approximately 960 km (600 miles) southeast of Cape Hatteras, North Carolina and about 1280 km (800 miles) south of Nova Scotia, Canada. This series of small islands is one of the most densely populated countries on earth (Census Office 2005). In 1950, only 37,000 people were permanent residents, but that number grew to 65,773 by 2006 (CIA 2006). Additionally, hundreds of thousands of foreign tourists visit Bermuda each year. This rapid growth in human presence has led to increased development of and encroachment on the natural environment.

Although the islands of Bermuda lie closest to the shores of the United States, this country is the oldest remaining British overseas territory. Much of the control of Bermuda remains with the United Kingdom. The executive authority of Bermuda is held by the Queen of England who appoints and gives her power to the Governor of Bermuda. The United Kingdom retains control over Bermuda’s defense and foreign affairs and must approve any changes to the Constitution of Bermuda. The Bermudians are given power to self-govern, making all of their own laws. The head of the government in Bermuda, the Premier, is elected by the eligible voting populace in Bermuda. The legislative branch of government consists of a bicamel parliament. The members of the upper house are appointed by the governor, while the members of the lower house are elected by the voting populace of Bermuda.

This thesis follows the style of *Phycologia*.

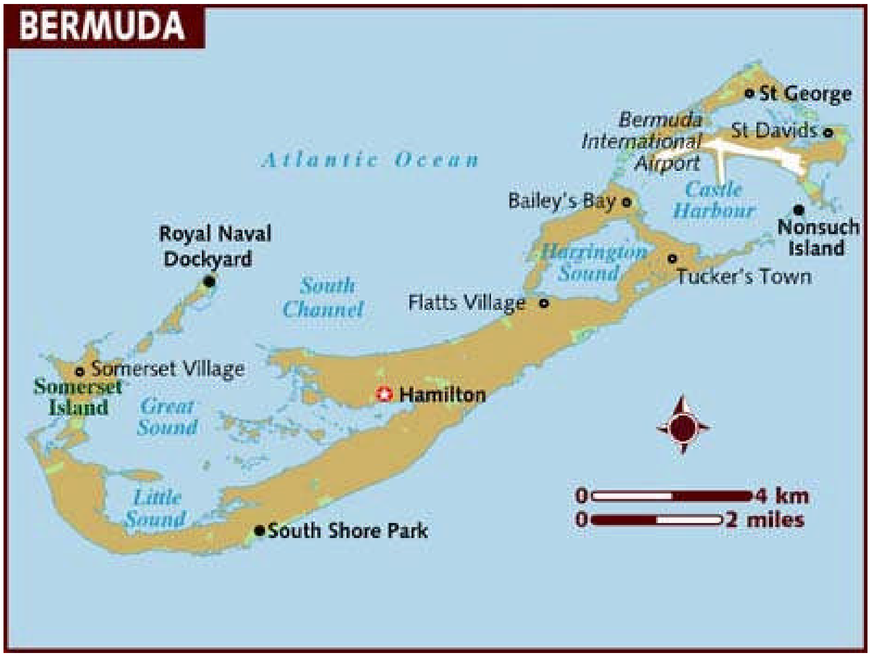

Fig. 1.1. Map of Bermuda (http://static.lonelyplanet.com/worldguide/maps/wg-bermuda-608-400x300.gif)

In recent decades the driving force behind the Bermudian economy has shifted from tourism to international business. Beginning in the 1950s and continuing until the 1980s, tourism dominated the economy. In 1978 tourism contributed two fifths of the gross domestic product (GDP) and was the largest source of employment (Murphy & Gomez 1981). This market reached its maximum influence in the early 1980s. With the introduction of the internet in the early 1990s, Bermuda became more connected with the rest of the world. Today, international business is the largest industry driving the Bermudian economy, followed by tourism. Together, these industries have secured this small country with the highest per capita GDP in the world.

Bermuda Geology

The archipelago of Bermuda is located on a seamount that resides on the North Atlantic Plate. The oceanic crust at the base of the rise is estimated to have formed 123-124 mya (Vogt & Jung 2007) at the Mid-Atlantic Ridge. Swelling off the plains of oceanic crust is a 1500 km long, 500-1000 km wide bulge called the Bermuda Rise. Extending towards the ocean's surface from this rise are four northeast to southwest trending volcanic peaks, including the emergent Bermuda Pedestal and the submerged Challenger, Plantagenet, and Bowditch Seamounts. The islands of Bermuda are located along the southeast margin of the largest and most northeasterly peak, the Bermuda Pedestal (Vogt & Jung 2007).

Various theories proposed as to the geophysical origins of the Bermuda Rise and its four volcanic peaks are still being debated among scientists. Through much of the 20th century, it was believed that the foundation for Bermuda originated during a large, underwater volcanic eruption along the Mid –Atlantic Ridge approximately 110 mya (Reynolds & Aumento 1974). This first explosion established a raised platform. Over time, the exposed portion of the platform began to erode as the tectonic plate moved westward. Since this interpretation is not well supported by drill cores and by theories arising after 1974 concerning shallow water (<100 m) volcanic eruptions, it is not considered a likely geological event (Vogt 1979).

During the middle Eocene (49-41.3 mya), the pre-existing oceanic crust began lifting to form the Bermuda Rise (Jaroslow & Tucholke 1994). Most of this uplift occurred by the early Oligocene (33.7-28.5 mya), before the major volcanic peaks were created on top of the rise (Jaroslow & Tucholke 1994). It is widely accepted that approximately 33 mya, extensive volcanic activity occurred over the Bermuda Rise creating four peaks (Vogt & Jung 2007). Why this activity occurred is still being widely discussed by geologists. An older theory proposed by Morgan and Crough (1979) hypothesizes that the volcanism occurred when the Rise passed over a hot spot or plume in the Earth's crust (40-45 mya), while a newer theory presented by Vogt and Jung (2007) proposes that the volcanic activity occurred due to a worldwide reorganization of the planet's tectonic plates. This reorganization may have occurred due to the closing of the Tethys Ocean when Arabia collided with Eurasia during the Cenozoic Era (35-40 mya) (Rona & Richardson 1978).

A limestone cap, mainly of Quaternary age (1.8 mya-present), formed on top of the volcanic platform. This calcium carbonate top was created from calcareous algae and corals when the volcanic peaks were submerged at shallow depths. The thin cap extends 15-100 m (49-328 ft) from its volcanic base (Vogt & Jung 2007). During the Pleistocene (1.8 mya to 10,000 ya), periods of continental glaciation and interglacial led to changes in sea-level, resulting in deposition of a series of stratigraphic layers predominantly composed of carbonate eolianites, with a few marine deposits, calcarenite paleosols, and terra-rossa paleosols (Vacher & Hearty 1989). The carbonate eolianites and marine limestones would have formed during periods of higher sea levels when the volcanic peak was submerged, while the paleosols formed during times of extensive glaciation and low sea-levels (Vacher *et al.* 1995). Cycles of cementation, erosion, and re-cementation occurred due the fluctuations in sea-level. Six to nine interglacial stands are represented in the rock layers that vary in their structure and time of formation (Hearty *et al.* 2004).

Due to the processes that created the limestone cap, the terrain of Bermuda is considered karstic (Bretz 1960). Karst is a landform where solution rather that erosion is the principle geomorphic weathering process. Land with this type of topographic feature typically has drainage through subterranean passages. The surface karst in Bermuda is irregularly pitted with complex dissolution channels that extend through the first few meters of rock. This zone is then partially to completely covered by a thin layer of soil and weathered material (Mylroie *et al.* 1995)

The most widely accepted theory of cave formation in Bermuda states that cave formation occurred during periods of glaciation when sea levels were lower (Palmer *et al.* 1977, Mylorie *et al.* 1995). During these periods, the emergent portion of the platform and fresh groundwater lens was much more extensive than that of present day Bermuda. The limestone platform is composed of calcium carbonate (from the skeletons of marine plants and animals) sand grains that have been compacted and cemented together. Rain water soaks through soils rich with CO_2 to form carbonic acid in the groundwater. The acidic nature of this groundwater acts as a solvent to dissolve the calcite in the limestone as it soaked through the vadose zone into the phreatic zone. The movement of the acidic water resulted in lateral growth of phreatic voids. Over time, the dissolution process enlarged the crevices to form conduits (caves), caverns, and underground lakes (Palmer *et al.* 1977). Further drops in sea level placed these solutionally formed voids above the water table where substantial collapse of roof rock gave rise to large break down chambers and fissure entrances characteristics of Bermuda caves. Once post glacial sea levels rose, the air spaces filled with sea water that were in turn overlain by less dense fresh groundwater and cave formation ceased.

The age and degree of lithification of Bermuda's major limestone layers affects the level of karstification and cave development (Vacher *et al.* 1995). Cave development was mainly confined to the geological layer known as the Walsingham formation (Fig. 1.2). This limestone is the oldest and hardest found in Bermuda and thus the most porous and weathered (Vacher 1978). This rock is characterized by larger crevices, caverns, and caves that were formed from dissolution activity within the voids of the vadose zone. The rock surrounding these channels is tightly cemented together so that water flow largely occurs within the channels (Morris *et al.* 1977). Postdating the Walsingham are the Town Hill, Belmont, Rocky Bay and Southampton formations, listed in order of decreasing age (Vacher *et al.* 1995). Interspersed between and in the middle of the various formations are terra-rossa paleosols. These soils were deposited on top of the rock formations during periods of low sea level and separate the carbonate deposits (Mylroie & Carew 1995). The Walsingham formation is predominantly found in the interior of Bermuda near inshore bodies of water and forms a core surrounded by the younger formations (Vacher *et al.* 1995). The largest outcropping of the Walsingham formation occurs along the northeastern portion of the islands between Castle Harbour and Harrington Sound. More isolated caves occur around the islands in areas of Walsingham outcropping or younger formations.

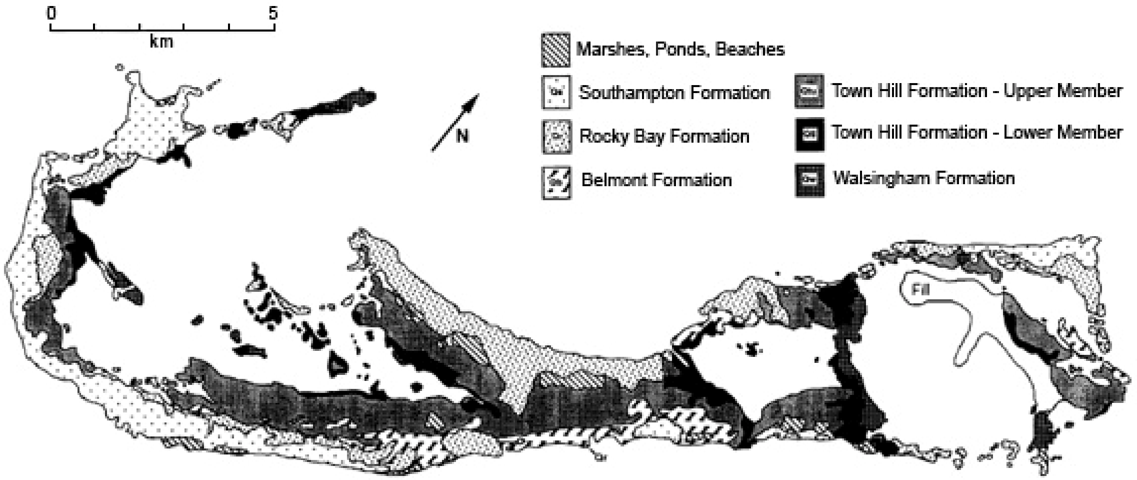

Fig. 1.2. Geologic Map of Bermuda (Vacher *et al.* 1995)

Besides caves, depositional closed depressions are characteristics land formations typically found on karstic, carbonate islands. The depressions are located between elevated areas on the outside edge of the island. Their expression is a result of subterranean drainage by karst processes. Bermuda's annual precipitation exceeds its evapotranspiration providing the islands with a positive water budget. During repeated periods of sea level change, rain water collected within the depressions basins and percolated down through the soils into the groundwater and outwards towards the ocean. Through this process the acidic freshwater expanded and deepened the depressions by dissolution (Mylroie & Carew 1995). Thus the topographic features of Bermuda continually evolved over time.

Bermuda Hydrology

The islands of Bermuda, seemingly isolated in the Atlantic Ocean, are dominated by the oceanic waters that surround them. The extensive coastline created by the islands encloses a number of saline, inshore water bodies, but the islands lack surface rivers and streams (Mylroie & Carew 1995). Except for the inland pools and ponds, the water associated with the interior of Bermuda is predominantly groundwater.

The groundwater is composed of layers of fresh, brackish and fully marine waters. The distribution of the groundwater layers is dictated by the limestone layers that contain it. The porosity and permeability of the surrounding limestone formation determine the nature of the groundwater layers present and the extent of mixing that occurs between the different layers. The size of the pore spaces between individual grains that make up the limestone vary between formations. In the youngest limestone layers, the water is not able to move freely between pore spaces since this rock does not contain cracks, crevices, or channels, and thus is confined within the interstitial space between the grains. It is in these areas of limited water movement that the five fresh water lenses of the islands occur.

The accumulation of freshwater within these lenses mainly occurs from rain water which percolates down from the land surface and is termed Ghyben-Herzberg lens (Todd 1959). In such lenses, fresh groundwater floats on the denser saline groundwater. The transition zone between fresh and saline water varies in depth throughout the islands. This zone occurs when the two layers physically mix. The mixing of the layers and thickness of the transition zone is dictated by the permeability of the surrounding limestone layers and tidal mixing (Vacher 1978). Due to the variation in permeability across the island of Bermuda, much of the inland groundwater is brackish to fully marine. In the highly cavernous Walsingham Formation, the rock is too permeable and thus no freshwater occurs (Vacher *et al.* 1995). This area, with its abundant subterranean channels and caves, has the greatest amount of mixing and exchange between the groundwater and the ocean.

The cave waters of Bermuda are characterized as an anchialine habitat. Holthuis (1973) originally defined the anchialine habitat as “pools with no surface connection with the sea, containing salt or brackish water, which fluctuates with the tides.” Later this definition was altered by Stock *et al.* (1986) to habitats that consist of “bodies of haline waters, usually with restricted exposure to open air, always with more or less extensive subterranean connections to the sea, and showing noticeable marine as well as terrestrial influences.” This second definition expanded the use of the term anchialine to include those caves, crevices, and entirely submerged passages that were excluded by the narrow perception of the term pool in the original definition.

The larger pools (>0.5 ha) in Bermuda have received the greatest amount of attention in the literature. Thomas *et al.* (1991) documented the physical characteristics of Bermuda's larger ponds (Mangrove Lake, Trotts Pond, Evans Pond, Lovers Lake, and Walsingham Pond) across all seasons from 1980-1989. They found that each pond had different salinity and temperature patterns that were heavily influenced by the different ocean connections and somewhat influenced by the season. In some of the ponds there was a vertical stratification of salinity and temperature. The ponds with deeper, subterranean connections tended to have the most stratified waters. In these waters, surface salinities were brackish (14-36.5 ppt) while underlying waters were fully marine (34.5-36.5 ppt). Typically, there was a marked halocline (0.5-1 m) between the layers due to lack of mixing. In ponds without stratification salinities were fairly constant throughout depth and averaged 29.7-34.1 ppt, depending on the amount of freshwater input from rain. While surface salinities were highly variable between the ponds, measurements below 2 m never fell below 35 ppt.

Bermuda's anchialine pools and caves do not typically contain freshwater. The pools have different salinity patterns that are heavily influenced by the different ocean connections and somewhat influenced by the season. The pools with deeper, subterranean connections tend to have the most stratified waters (Thomas *et al.* 1991). These waters have a thin (0.5-2 m) brackish surface layer (0-25 ppt salinity) that overlies a bottom layer of nearly to fully marine waters (25-35 ppt salinity) (Iliffe 2000). The most inland, isolated pools and caves normally have the lowest surface salinities. This is typically a result of increased freshwater input due to precipitation and decreased tidal influence due to the distance from the sea. The boundary layer between the brackish layer and the fully marine water is termed a halocline. The high degree of stratification and distinct boundary between water masses is usually a result of the lack of mixing between layers in the absence of wind and waves. In pools and caves without stratification, salinities are fairly constant throughout depth and tend to be fully marine

(34.5-36.5 ppt) throughout the water column (0-25 m) (Thomas *et al*. 1991, Iliffe 2000). At some locations, a seasonal appearance and subsequent disappearance of the stratified layers does occur (Iliffe 2000).

The temperature in the pools and caves show a similar pattern to that of salinity. Surface waters tend show the greatest variations. Depending on the position of cave in relation cave entrances, surface waters can range from 16 to 28ºC throughout the year (Iliffe 2003). In the pools, temperatures tend to show seasonal fluctuations. The lowest temperatures occur during the winter months of January and February, while the highest temperatures occur during July and August (Thomas *et al.* 1991). In summer months, pools with stratified layer have surface waters that exceed bottom temperature by >5ºC (Thomas *et al.* 1991). In winter months, the surface layers can be cooler. When this occurs the temperature tends to increase sharply at the halocline and then more gradually increase and stabilize with depth, within the fully marine waters (19-20ºC) (Iliffe 2003). It is believed that this gradual increase in temperature may be a result of the seamount's documented geothermal gradient, which warms waters at depth (Iliffe 2000).

The tidal cycle seen in Bermuda water's is semidiurnal – that is there are two fairly equal high and two fairly equal low tides each day. The caves of Bermuda typically have a reduced amplitude (33-87% reduction) and delayed tidal effect compared to the open ocean (Iliffe 2003). Pools and caves that are on or near the coast usually display the largest fluctuations in tidal volume, have the shortest tidal delay, and most similarly resemble the patterns of the coast. Pools and caves further inland have a smaller tidal range and a longer delay of up to 171 minutes (Iliffe 2000). Currents within the pools and caves are directly influenced by the tidal water movements and determine the amount of mixing that occurring within the subterranean water masses. Pools located farther inland that have reduced tidal amplitude and long time delays indicate little connection with the sea. These areas tend to have the least amount of mixing and thus the longest residence time of the water masses (Iliffe 2000). These areas also tend to have crystal clear water as very little particulate matter is suspended in the water column.

The dissolved oxygen (DO) levels in caves are typically particularly low. Most portions of the caves and many pools are not exposed to any sunlight. In these areas, there is a lack of photosynthetic oxygen production due to the absence phytoplankton or algae. In these areas, the highest concentration of DO generally occurs at the surface (90-100% saturation) due to gas exchange with atmospheric oxygen. Levels then fall off sharply at the halocline and remain at consistently low levels (56 to 78% saturation) throughout depth (>2 m) (Iliffe 2003). The lowest value recorded in a non polluted Bermudian cave system was 55% of saturation (3.75 mg O_2/l) (Iliffe *et al.* 1984). In cave pools that are partially to fully illuminated by sunlight during portions of the day, algae and phytoplankton are present. Due to the heavy vegetation, the daily values are typically >50% saturation throughout the year and in some locations routinely exceed 100% (Thomas *et al.* 1991). During a 10 year study through the 1980s, only once, at Evans Pond, did DO levels fall to 2% saturation during August, but anoxic levels were never recorded (Thomas *et al.* 1991). Due to the presence of photosynthesis these pools have higher than normal DO levels during the day when photosynthesis is occurring, but the levels decrease as the distance from the light zone increases (Iliffe 2003).

Bermuda Caves

As long as people have been visiting Bermuda either to explore or colonize, there has been a fascination with the mystery of the caves found on the island. The first written reference to a Bermudian cave occurred in 1664 by Captain John Smith who described the caves as “varye strange, darke, and cumbersome” (Smith 1964). Since the 15th century, people have continued to observe and utilize the caves. These actions have been in some cases destructive, while in others have come to appreciate the historical, aesthetic, and biological value of this habitat.

Due to the karstic landscape of Bermuda, the islands have one of the highest concentrations of cave systems in the world (Iliffe 1979, Thomas 2004). While some cave systems are completely dry and filled with air, others begin at the surface and then submerge beneath the surface of water, and still others exist only underwater. Bermuda cave systems include inland entrances, interior cave pools, underwater passages, and tidal spring outlets to the ocean.

Bermuda's caves are highly decorated by delicate speleothems, solutionally formed mineral deposits. These naturally created rock formations slowly developed over thousands of years and are exclusively formed in the moist, humid environment of a dry cave. Among the most well known speleothems are the stalactites and stalagmites. Stalactites are icicle-like deposits that extend down from the ceilings. They form by precipitation of calcium carbonate from super-saturated drip waters. Stalagmites are the counterpart to stalagtites, but rise from the cave floor. They form from the drips of water falling from the stalactites. Columns may form when a stalactite and stalagmite meet (Fig 1.3). Many other speleothems occur in Bermuda such as draperies, flowstone, cave pearls, and helectites.

Fig. 1.3. Speleothems Found in Sibley's Cave (Photo courtesy of Tamara Thomsen 2008)

Entrepreneurs quickly realized that the beauty and mystery of the caves with their accompanying speleothems could turn a profit. Since the 19th century, a number of

Bermudian caves, including Devil's Hole (opened 1843 and still used as a natural fish pool), Leamington (now closed and on private property), Blue Grotto (once a site for dolphin shows), Island (at the Grotto Bay Hotel and once disco club), Cathedral (also at Grotto Bay), Crystal (a major tourist attraction), Fantasy (formerly known as Wonderland and also currently open to tourists), Tucker's Island (destroyed when the US Naval Annex was built at west end island), and Admiral's (behind Swizzle Inn, once part of Grotto Bay caves; now closed) Caves have been developed into commercial caves one time or another.

These ecosystems are in rich there biological and ecological value, containing many rare species of plants and animals. There are currently 75 stygobitic invertebrates which are living within the confines of Bermudian caves (Iliffe 2003). Stygobites are aquatic organisms that show some sort of specialization to the underground environment (Iliffe & Bishop 2007). 80% of identified cave species are endemic to Bermuda (Iliffe *et al.* 1984). Most of these invertebrates are crustaceans, but mites, ciliates, gastropod mollusks, and segmented worms have also been discovered. Most of these species occur in limited numbers and have a very limited known geographic distribution, often limited to a single cave (Iliffe 2003). Although these endemic anchialine species are found exclusively on the secluded islands of Bermuda, they show close taxonomic affinities to species inhabiting caves across the western and eastern Atlantic as well as the Pacific and even deep sea (Iliffe 2000, 2003).

Seaweeds of Bermuda

The many algal species present around and in the Bermudian cave pools include all three major macroalgal phyla: Chlorophyta, Rhodophyta, and Phaeophyta. The species diversity varies greatly from cave pool to cave pool due mostly to differences in illumination (Schwede, unpublished notes). The first specific references to cave and marine flora on Bermuda appears in Collins and Hervey's The Algae of Bermuda in 1917. Howe contributed a section on algae to Britton's Flora of Bermuda (1918).

In 1949, Taylor first visited Bermuda with his student Bernatowicz. Their records and observations dating from 1949 were included in Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas (Taylor 1960). Taylor and Bernatowicz (1969) further created an annotated list of the distribution of Bermuda's macroalgae. Frederick (1963) wrote a dissertation on the deep water algae of the Argus and Challenger Banks of Bermuda. These works produced the framework detailing the macroalgae present on and around Bermuda. In 1983 and 1985, Searles and Schneider collected marine algae from 12-50 m water depths around the Bermuda islands (Searles & Schneider 1987). They subsequently published a number of articles from 1987-2003. In 2003, Schneider published an annotated checklist and bibliography of the macroalgae of Bermuda. During these years, they reported 30 species new to Bermuda from both offshore and inland habitats. While many of these species are found elsewhere in the Atlantic, others such as *Antithamnionella bermudica* and *Polysiphonia plectocarpa* represent newly described endemics to Bermuda (Searles & Schneider 1987, Schneider 2000, Schneider 2003). Several field guides were published in the 1990's to aid in identification of common species. In 1994, Cavaliere published a field guide for species found in inshore and shallow waters around Bermuda (Cavaliere 1994), while Sterrer and Cavaliere (1998) published a manual on Bermuda's seashore plants and seaweeds.

Between 1979 and 1985, Schwede, a medical physician whose hobby was algal taxonomy and ecology, visited Bermuda on numerous occasions making detailed observations and measurements in several of Bermuda's karstic cave pools, and thoroughly mapped the algal distribution in 18 of the pools. Within each pool, he recorded abiotic conditions such as the direction of sunlight exposure, percentage of pool area that received direct sunlight, salinity, and tidal fluctuations. He also made abundance estimates and noted the reproductive status of the algal populations, preserving representative species from different cave pools in an extensive herbarium and slide collection. This work has been preserved and is currently being reviewed for publication. In, addition, the work has been documented on the website: http://www.tamug.edu/cavebiology/Bermudaflora/index.html.

Effect of Human Activities on Bermudian Caves and Seaweeds

The karst topography of Bermuda provides a direct link between the human activities on the surface and the groundwater and cave systems below. A number of environmental threats have been identified as caves have disappeared or become heavily polluted due to the encroachment of man. Major threats to Bermudan caves include construction projects, limestone quarrying, water pollution (particularly cesspits and liquid waste injection boreholes) and dumping of trash and other wastes into sinkholes (Iliffe 1981, Glasspool 2007).

Ongoing land development pressures from new construction and quarrying pose the biggest threat, both geologically and hydrologically to cave habitat. Many new homes, golf courses, and hotels continue to be constructed in areas prone to cave development. Numerous caves lack natural entrances and hence remained undiscovered beneath the earth's surface. Most of newly discovered caves in Bermuda occur when developers break through a cave passage during construction activities.

Two quarries, the privately owned Wilkinson Quarry (Fig. 1.4) and the government run Public Works Department Quarry, operate in the Walsingham rock formation. This hard, tightly cemented limestone can only be quarried by blasting and is crushed to produce aggregate for cement and asphalt used during construction (Department of Planning 1998). During the course of quarrying operations, a number of caves have been completely destroyed including Government Quarry Cave, one of the deepest but still unexplored in Bermuda (Iliffe *et al.* 1984, Iliffe & Bishop 2007). The caves are destroyed either directly by the blasting efforts or when the open chambers of the caves are intentionally filled in before harvesting the top layer of rock.

Fig.1. 4. Wilkinson Quarry in Bermuda

Another major threat to the cave environment is pollution of its water. Household sewage is disposed of in individual, unlined cesspits. Effluent from these pits seeps through the limestone and into crevices and channels within the cave systems. Larger developments such as hotels dispose of their sewage and wastewater through ‘deep injection’ boreholes. These disposal holes are only required to extend 30 m below the surface at depths in the limestone where caves have formed (Glasspool 2007). The seepage of these systems allows sewage, detergents, pharmaceuticals, and other contaminants to enter the cave systems through underground passages and fissures (Iliffe & Bishop 2007).

Fig. 1.5. Oil Barrels Dumped in Bitumen’s Cave, Bermuda (Photo courtesy of Tamara Thomsen 2008)

Dumping and vandalism are other major destructive behaviors that threaten the caves of Bermuda. Bassett’s Cave is situated on the former US Naval Annex. Large amounts of waste fuel-oil and raw sewage were dumped into a lake in this cave (Iliffe 1979). Hundreds of rusting oil drums and piles of glass bottles and other trash were dumped in Bitumen’s Cave (Fig. 1.5) located on the grounds of the Tucker’s Point Resort. Today, this cave is gated to prevent any further destruction or trespassing, but no attempts have been made to clean up the oil seeping out of the rusting oil drums. Considerable dumping has also occurred at Sear’s Cave where a rare fern grows. Stoves, refrigerators, beer cans, glass bottles, shoes, and other litter cover the lower slope of this sink (Fig. 1.6). This cave has been purchased by the Audubon Society and has been completely enclosed by a wire fence to preserve the rare fern, but again the trash dumped in past years remains. The use of caves as a public dumping site is common in the non-commercial caves of Bermuda.

Fig.1. 6. Trash Dumped in Sear's Cave, Bermuda (Photo courtesy of Tamara Thomsen 2008)

The Development and Planning Act of 1974 was the first piece of Bermudian legislation to mention protection of caves. It categorized caves as a portion of land that possessed natural features of environmental value that and as such designated caves as protected areas. Today, Bermuda caves are primarily protected under the Planning Act of 1999 and the Bermuda 1992 Plan. Additionally, 23 cave species have been submitted for protection under the Protected Species Act 2003, which will necessitate recovery plans to be developed and implemented within one year for all accepted species.

Purpose of Research

The destruction or pollution of the cave environment has a negative impact on the algae utilizing the cave pool waters. Cave pools with lower water quality due to higher nutrient loading are expected to exhibit different algal distributions and show higher primary productivity and growth characteristics than cave pools with high water quality and less or no nutrient loading relative to background levels. Different algal species utilize nutrients in a variety of ways. Some species use available nutrients immediately, while others store nutrients. Species of both microalgae and macroalgae have the ability to rapidly uptake nutrients and may exhibit prolific growth. This expansive growth, coupled with excessive nutrients increase the turbidity in the water column and decreases the quality and clarity of the water. By determining if there is a correlation between water quality and the presence and distribution of certain algal species in the cave pools, the environmental health of various inland cave pools and the groundwater can be monitored. Cave pools that have extensive growth of opportunistic, fast growing algal species may indicate the water quality in that pool is poor and nutrient loaded. Cave pools with widespread growth of slower growing species may be a sign of higher water quality and no nutrient loading.

Study Sites

Anchialine pools or ponds are land-locked, saline bodies of water with permanent connections to the ocean (Por 1985). A large number of anchialine pools occur in Bermuda. The pools vary greatly in size from just a few square meters up to 12 ha. The connections to the ocean within each pool range from small fissures to small caves, which occur solely at the surface or through submerged passages (Thomas *et al.* 1991). Due to their connection with the sea, the pools are affected to some extent by the tides. The range and time delay are determined by their distance from the ocean and the nature of the subterranean connections (Thomas *et al.* 1991). There is also a great amount of variation in the amount of bottom sediments within the pools. With higher organic input tend to have thicker sediment layers.

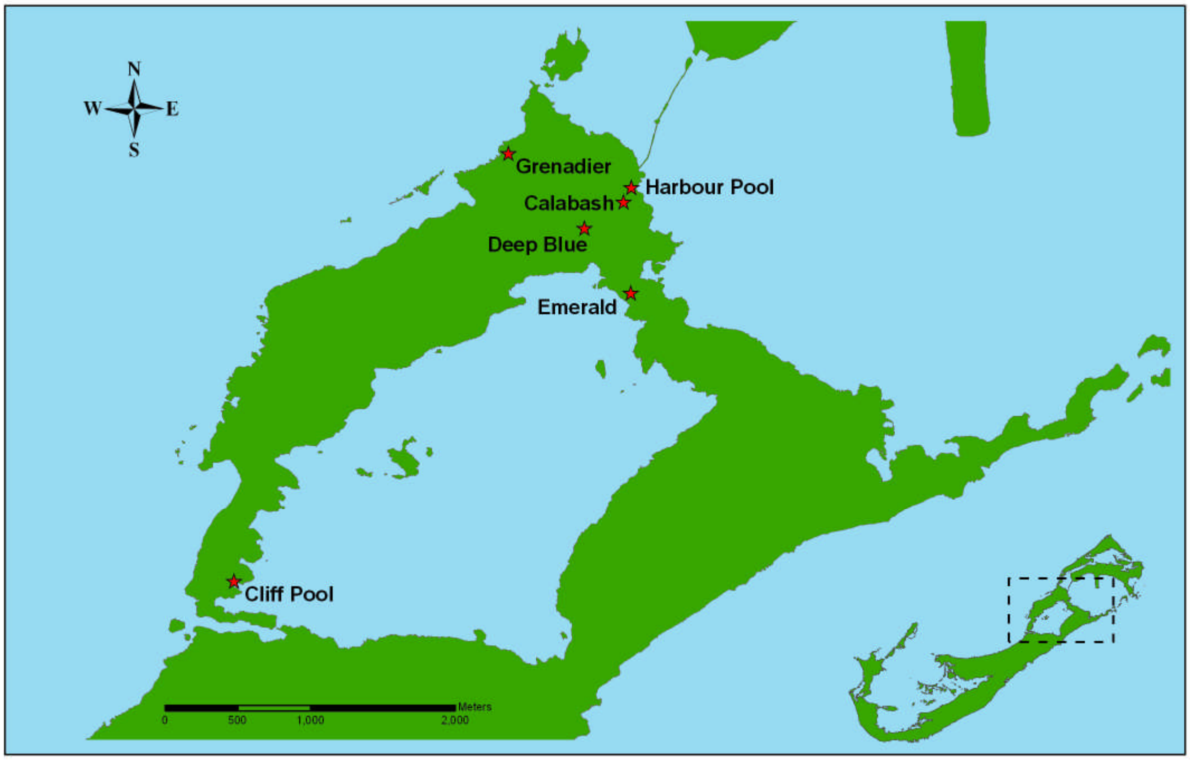

Fig.1. 7. Location of Cave Pool Study Sites in Bermuda

Six cave pools were selected as sites for this study (Table 1.1). All of the pools were located around Castle Harbour and Harrington Sound at sites indicated on Fig. 1.7. Each pool has subterranean connections to the open ocean and is thus tidally influenced. Harbour Pool (HP), Calabash (CB), and Deep Blue (DB) are located within the boundaries of the Walsingham Trust Nature Reserve. This reserve remains privately owned but opened to the public for recreation activities. Although the Bermuda Government Department of Conservation Services is carrying out a reforestation program in this area to eliminate introduced vegetation and restore the native forest, human activities there are not strictly monitored. The remaining three pools, Grenadier (G), Cliff Pool (CP), and Emerald (E), are located on private land in residential areas.

Table 1.1. Characterization of six study sites

Site (Abbr.)	Location	Urbanized vs Reserve[1]	Turbid Surface Layer	Deep vs Shallow[2]	Artificial Clearing Around Site	Artificial Side or Stairway
Calabash (CB)	32º20.51.15” N 64º42’35.07” W	Reserve	No	Shallow	Yes	Yes
Harbour Pool (HP)	32º20’52.25” N 64º42’33.05” W	Reserve	No	Shallow	Yes	Yes
Deep Blue (DB)	32º20’54.10” N 64º42’41.24” W	Reserve	No	Deep	Yes	Yes
Grenadier (G)	32º21’09.41” N 64º43’08.92” W	Urbanized	Yes	Shallow	Yes	Yes
Cliff Pool (CP)	32º19’33.29” N 64º44’22.50” W	Urbanized	Yes	Deep	Yes	No
Emerald (E)	32º20’36.06” N 64º42’35.41” W	Urbanized	No	Deep	Yes	No

[1] Urbanized cave pools were those located close to roadways near developed buildings. Reserve cave pools were located within an area of undeveloped land set aside for recreational purposes by private foundations.

[2] Shallow cave pools are characterized as being <3 m in depth. Deep cave pools are characterized as being >3 m in depth.

Calabash is a small (2.7 m by 5.8 m) oval-shaped pool shallow pool located off a main walking trail in the reserve. Most of the pool is only about 0.5 m deep, although it does deepen to 1.3 m on one side. A cliff face shades the north edge and vegetation shades the northeastern side of the pool (Fig. 1.8). A manmade wall surrounds the southern and western side of the pool that borders a large clearing. The bottom of the pool is composed of small rocks that are covered with various species of green, brown, and red algae. Occasion shrimp are the only fauna commonly found in the open portion of the pool.

Fig.1. 8. Photograph of Calabash (Photo courtesy of Tamara Thomsen 2008)

Harbour Pool is a shallow (maximum depth 1.2 m), circular (13.4 m by 14.6) pool located near Calabash on the southeastern side of the clearing. It is shaded by vegetation on all but the southern side, with the greatest overhang occurring on the north and eastern sides (Fig. 1.9). Species of green, brown, and red algae cover rocks along the outer edges, while the center is composed of sand and devoid of any flora. The greatest diversity of fauna occurs in this pool as it has a large connection to Castle Harbour. Sessile organisms such a few giant anemone and two colonies of brain coral are found along the eastern and northern edges, whereas juvenile damsel fish, parrot fish, small sea slugs, and large sea cucumbers are found occasionally throughout the pool. Often during the summer, children can be seen using this pool as a swimming hole.

Fig.1. 9. Photograph of Harbour Pool (Photo courtesy of Tamara Thomsen 2008)

Deep Blue is located in a valley with an overhanging cliff wall on the western side and a steep hill covered with vegetation on the eastern side (Fig. 1.10). It is composed of a large main pool (25.6 m by 7 m) separated from two smaller pools by rock wall collapse and extensive leaf litter. It is the deepest (8.9 m) of the cave pools sites within the reserve and has an extensive cavern zone that extends westward from the pool. The collapsed rocks within the pool are covered in green and red algae as well as anemones. Due to its remoteness from the ocean, a few open water fish species are rarely seen.

Fig. 1.10. Photograph of Deep Blue (Photo courtesy of Tamara Thomsen 2008)

Grenadier is a shallow (3.1 m deep), circular pool (18.9 m by 17.8 m) located in a densely populated area off of the major road on the North Shore of the island. It is situated in the side yard of a Bermudian residence. Landscaping occurs directly around all sides of this pool. Thick vegetation over hangs a portion of Grenadier on the southwestern and western edges, while the eastern side is highly exposed as it is surrounded by a manicured lawn and cement walkway (Fig. 1.11). The surface water is not as clear as the other pools, having a brownish cast, and is often covered with leaves and branches that have blown into the pool. Green algae and anemones are attached to the rocks in the pool with white, segmented worms living in the bare, sediment patches between them. A red algal mat covers the algae throughout the pool.

Fig. 1.11. Photograph of Grenadier (Photo courtesy of Tamara Thomsen 2008)

Cliff Pool is also located in a side yard of a Bermudian residence in a densely populated area around Harrington Sound. It is an oval shaped pool (19.8 m by 10 m) that narrows at the southwestern and northeastern points of the pool (Fig. 1.12). It is one of the deeper pools (7.9 m) and has an extensive cavern zone like Deep Blue that extends westward from the open pool. Cliff Pool is shaded by an overhanging cliff wall on its northwestern side and by vegetative growth on its southern and northern corners. A cleared, landscaped area occurs on the southeastern side of the pool. During the winter months the waters of Cliff Pool are crystal clear, but during the summer, the surface waters become more turbid and have a greenish brown color. The collapsed rocks within the pool are covered in green and red algae. Thousands of small, black snails are present across the eastern side of the pool with the algae. Larger fish, such as snapper and jack, are often seen swimming throughout the pool.

Fig. 1.12. Photograph of Cliff Pool (Photo courtesy of Tamara Thomsen 2008)

Emerald Sink is a large collapse sinkhole (9.0 m deep) located directly off a main road and surrounded by residences and open land that may be developed in the future (Fig 1.13). It is composed of one large pool and four smaller pools. At its longest point, the roughly oval main pool is 15.7 m long and 6.7 m wide. A cliff face begins at the western edge and extends up and over the pool. The land to the east of the pool rises up a steep hill that was cleared of vegetation between May and June 2007. Due to these two features, Emerald receives the least direct sunlight of any of the cave pools. Green and red algae cover the rocks on the western side of the pool, whereas the eastern side of the pool is mostly sandy sediment that lacks any algal growth. Large lobsters are the only fauna consistently found in this pool under rock ledges.

Fig.1. 13. Photograph of Emerald (Photo courtesy of Tamara Thomsen 2008)

Objectives

The overall objective of this study was to determine if the different algal species present in Bermudian inland cave pools could be used as bioindicators for water quality in the increasingly urbanized and developing environment. There are a number of more specific project objectives that needed to be satisfied in order to address the overarching objective. The findings helped determine how variations in water quality and cave morphology effect algal location, diversity, primary productivity, and growth rates. Statistical comparisons were used to determine if the algal species found in each cave pool could serve as bioindicators for the health of the Bermudian karst waters.

A detailed list of sub-objectives follows:

(i) Record cave pools locations according to their GPS coordinates, and determine their position relative to adjacent inshore water bodies.

(ii) Make an initial assessment and survey of individual cave pools. Determine the relative presence and abundance of opportunistic algal species that have been previously used to study nutrient effects in marine systems.

(iii) Photo document each cave pool to record zonation patterns, locations, and relative abundances of species present as well as document visual anthropogenic impacts at the surface.

(iv) Document environmental water parameters across six of the Bermudan cave pools.

(v) Determine the background nutrient content and fecal bacteria content at each cave pool to determine initial levels and amount of nutrient loading naturally present within each system.

(vi) Conduct *in situ* bottled nutrient addition experiments at each site on opportunistic, fast growing algal species.

(vii) Measure gross and net photosynthetic as well as respiration rates for all algal species used in the bottled nutrient addition experiment. Use these measurements to determine the natural growth rates of the studied algal species within bottles.

Hypotheses

The data collected were used to evaluate the following two null hypotheses:

H_0: Each cave pool has similar environmental water parameters and does not show any significant difference in background nutrient or fecal bacteria levels. (Examined in Chapter II)

H_0: There is no significant difference in biomass accumulation between algal specimens living in nutrient enriched water compared to those in waters without enrichment. (Examined in Chapter III)

CHAPTER II

CAVE POOL WATER CHARACTERISTICS AND QUALITY

Introduction

Groundwater Pollution in Bermuda

Due to its complex connections, the karst topography of Bermuda tends to make the groundwater (fresh, brackish, and saline) more susceptible to pollutants. As previously discussed in chapter I, Bermuda is composed of different rock formations that vary in their age, porosity, and permeability. These characteristics determine the nature of the groundwater layers present. They also influence the quantity and type of pollutants that can enter the underlying groundwaters. In the youngest limestone layers, the rock does not contain cracks, crevices, or channels and thus acts as a filter, removing contaminants and absorbing non-soluble contaminants from percolating water (Rowe 2005). In the older limestone layers, conduits are surrounded by tightly cemented rock that channel pollutants rapidly downward into the phreatic zone (Sterrer & Barnes 1982, Thomson & Foster 1986).

The greatest risks for groundwater quality include sewage disposal, oil discharge, and contamination from agricultural practices (Thomson & Foster 1986). The primary means for domestic waste water disposal in Bermuda is through cesspits (Sterrer & Barnes 1982, Rowe 2005). Sewage and gray water from showers, sinks, dishwashers, and washing machines drain through pipes into unlined subsurface containments which drain by percolation through the base and walls (Thomson & Foster 1986). These cesspits are specifically designed for maximum seepage into the ground (Simmons *et al.* 1985). Organic nitrogen in sewage is mineralized to highly soluble ammonia with nitrite. The dissolved oxygen in the unsaturated zone is used by microorganisms to oxidize the ammonium and nitrite in cesspit percolate to nitrate, which then makes it into the groundwater (Simmons *et al.* 1985, Thomson & Foster 1986).

A significant portion of the fresh groundwater comes from waste waters. In the Central Lens, it has been estimated that the contribution of recharge from cesspits ranges from 15 to 27% (Simmons et al. 1985, Rowe 2005). The average human contribution to wastewater is about 5 kg N/capita annually. If 90-95% of the nitrogen from human excreta in an area with 15 persons/ha (medium housing density) were to enter the groundwater, concentrations should average 30-40 mg N/l (Thomson & Foster 1986). As of 2000, the average population density in Bermuda was 12.60 persons/ha, but this figure includes all land in Bermuda, even areas restricted from the public (Census Office 2005). High nitrate levels in the groundwater from cesspits have been continually documented since the 1980s and have been associated with densely populated areas (Thomson & Foster 1986, Rowe 2005). In some areas, the seepage of these systems allow not only sewage, but also detergents, pharmaceuticals, and other contaminants to enter the cave systems through underground passages and fissures and transport them for considerable distances with little or no filtration (Simmons *et al*. 1985, Simmons and Lyons 1994).

Inorganic and organic fertilizers are applied to the soil to promote growth in plants growing in gardens, lawns, agricultural fields, and golf courses. They introduce both nitrates and phosphates into the natural environment. In Bermuda, significant nutrient leeching has been detected in areas of cultivated and cropped land. Concentrations up to 15 mg N/l have been recorded in areas of intense cultivation in Paget Limestone (Thomson & Foster 1986). In recent studies, herbicides and pesticides, often associated with cropped areas and golf course, have not been detected in freshwater samples collected within the Paget Formation (Rowe 2005). Saline groundwaters in close proximity to golf courses and crop lands have not been extensive studied. Due to this lack of information, the effects of fertilizer application on nutrient concentrations in the older rock formations cannot be determined.

Another source of groundwater pollution is oil spillage. A fuel oil pipeline traverses the Central Lens. It is frequently monitored and has not yet posed a problem in the surrounding waters (Thomson & Foster 1986). Most of the potential risk stems from oil disposal by garages, car owners, and private businesses. Currently, only sporadic localized groundwater contamination has occurred (Thomson & Foster 1986). A few caves have been heavily impacted due to dumping or oil drums, but with proper disposal this threat to groundwater can be minimized.

Bermuda Historical Groundwater Nutrient and Bacterial Levels

Levels of bacteria and nutrients have been well documented by the Bermuda government in the fresh groundwater lenses since 1980 (Rowe 2005). Having a low permeability, the youngest limestone formations accumulate rain water as it percolates down form the land's surface. In the 1980s the Health Department set the limit of nitrates in the fresh groundwater lenses, measured as nitrogen, at 10 mg/l. Historically, in areas to the northeast of Hamilton, the capital city, nitrogen levels average 25-35 mg/l in the groundwater (Thomson & Foster 1986). During 2005, the average nitrate level from a survey of 25 freshwater wells was 11.4 mg/l, with 2 wells exceeding 20 mg/l (Rowe 2005). Bacterial levels were also elevated at points within the freshwater lenses. In the 2005 study, *Escherichia coli* exceeded the EPA limit fro drinking water (126 CFU/100 ml in freshwater) in 6 out of 25 sites (Rowe 2005). Previous studies found that levels were predominantly lower, with only minor contamination (10-200 CFU/100 ml) in areas composed of the younger Paget formation, while very contaminated levels (>500 CFU/100 ml) were concentrated where the unsaturated zone consisted of the slightly older, Belmont Formation (Thomson & Foster 1986). These levels make the raw water unsuitable for drinking and thus must be disinfected before use (Rowe 2005). Ammonia, nitrite, and phosphate levels are fairly low through the freshwater lenses. Typical concentrations of phosphate range from <1 to 4 µM and tend to increase with depth (Simmons & Lyons 1994). Ammonia and nitrite levels tend to be lower as they are often oxidized to nitrate before entering the groundwater supply.

The saline groundwaters found within Bermudian caves show similar characteristics to those of the fresh groundwater. The cave water contains high levels of nitrate (5-600 mg/l) but low ammonia, nitrite, and phosphate (Iliffe *et al.* 1984). The nitrate concentrations in these waters decrease with increasing salinity in a linear manner, although vary extensively in different locations (Iliffe 2000). Ammonia (<1 µM) and phosphate (<1 to 4 µM) levels are typically low. In areas with higher levels of organic decomposition by microorganisms, elevated maximum readings do occur (ammonia up to 56.5 µM and phosphate up to 8.68 µM), indicating these ions are released during the oxidation of the organic matter (Iliffe *et al.*1984). Nitrite levels (0-0.23 mg/l) are negligible if present at all in the cave waters across the island (Gibbons 2003). In a study of 20 caves, the fecal bacteria, *Enterococcus*, was only detected in surface waters of 8 caves (10-74 CFU/100 ml) (Gibbons 2003). These levels do not exceed EPA's one-time reading scale of 104 CFU/100ml (in marine waters) for beach waters (U.S. EPA 2003).

Nitrogen Cycle

Nitrogen is a key element that exists in nature as a cycle that moves back and forth between the physical environment and organisms within the many ecosystems on earth (Fig. 2.1). The largest concentration of nitrogen occurs in the atmosphere as a dissolved gas (N_2), composing 78% of air. It can be found in the bodies of organisms and as dissolved organic matter (DOM). Nitrogen is critical component of amino acids, proteins, chlorophyll, and nucleic acids. Nitrogen also commonly exists as the dissolved inorganic ions nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+).

In the ocean, dissolved nitrogen gas accounts for 48% of the dissolved gas in the water. Most of the organisms that utilize nitrogen cannot use the free, gaseous form of nitrogen, but instead must fix it into useable chemical forms with either oxygen or hydrogen. In nature, bacteria and cyanobacteria bind nitrogen with hydrogen to produce ammonia (NH_3). Nitrogen can also be fixed into useable compounds by anthropogenic processes. The industrial process of Haber-Bosch, binds nitrogen gas with hydrogen gas to create ammonia to be used in fertilizers and explosives. The combustion of fossil fuels by automobiles and thermal power plants converts nitrogen to nitrogen oxides (NO_x). Sewage facilities such as septic tanks, holding tanks, and cesspits release large amounts of nitrogen from human waste. These useable forms of nitrogen typically enter aquatic systems through rivers, terrestrial runoff, and groundwater (Morris *et al.* 1977, Capone & Bautista 1985, Vacher *et al.* 1990).

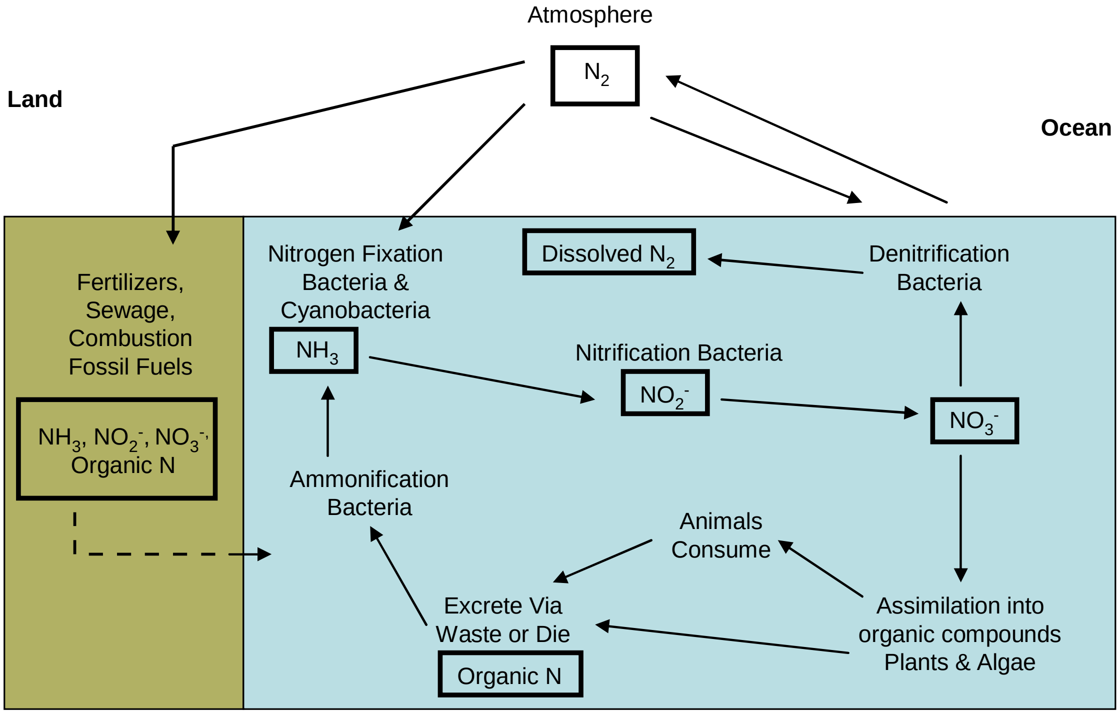

Fig. 2.1. Nitrogen Cycle

Marine plants assimilate nitrogen primarily as nitrate, but also as nitrite and ammonium as well as organic nitrogen forms such as urea (Garrison 2002). Through this process, the plants absorb the nitrate, reducing the ion to its nitrite and ammonium forms, to be incorporated into amino acids, nucleic acids, and chlorophyll. Heterotrophic organisms can then consume the plants and utilize the nitrogen within them. The nitrogen is subsequently recycled back to the environment by either the death of the plant or animal or by the excreted matter of the animals as organic nitrogen such as urea ($(NH_2)_2CO$). Bacteria can convert the organic nitrogen back into a reduced form of nitrogen, ammonia, through a process called ammonification or mineralization. In aerobic conditions, bacteria use these reduced forms of nitrogen as an energy source. During this process of nitrification, the nitrogen is oxidized back into nitrate, via nitrite, and can again be utilized by plants. The nitrogen cycle is completed by denitrification under anaerobic conditions. During this process, bacteria use nitrate as a terminal electron acceptor to breakdown organic matter in respiration. The nitrate is converted to nitrite and then into nitrogen gas, which is lost back into the water and atmosphere. Additional nitrogen becomes lost in the aquatic environments during the burial of nitrogen-containing organisms and debris in sediments.

Phosphorus Cycle

Phosphorus is another key element that exists in nature with a cycle that moves back and forth between the physical environment and organisms within the many ecosystems on earth (Fig. 2.2). Unlike nitrogen, it is not a major atmospheric component as it typically exists as a solid on earth. The major reservoir on earth is found in phosphate rock. Additional anthropogenic sources of phosphorus come from fertilizers and sewage (Lapointe *et al.* 1990). Phosphorus is a major component of nucleic acids, ATP, and phosolipids of cell membranes. Calcium phosphate is used the formation of bones, teeth, and some shells. Phosphorus is utilized by organism in the ionic forms of orthophosphate (PO_4^{-3}) and HPO_4^{-2}.

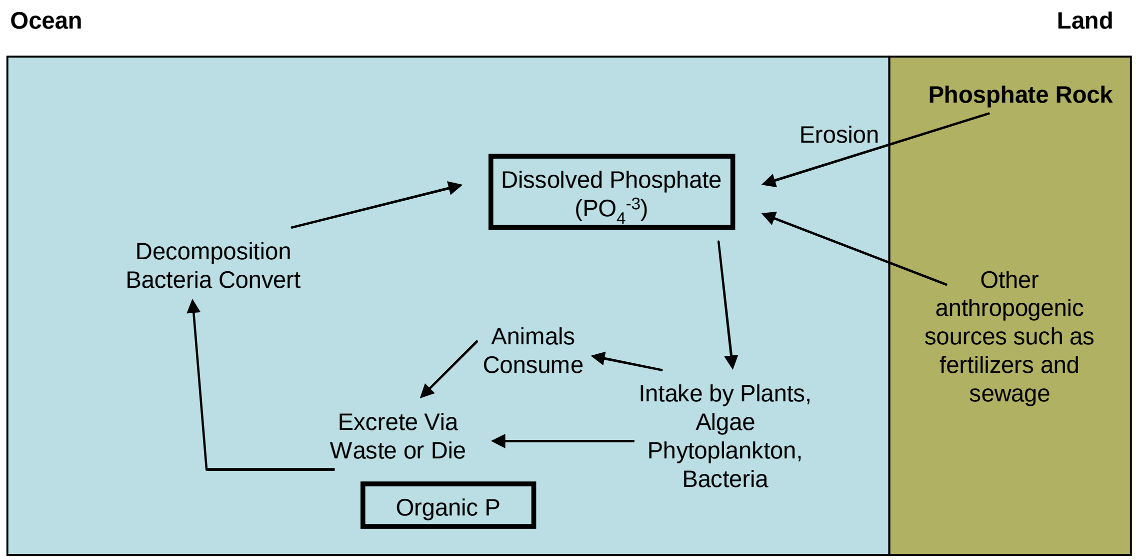

Fig. 2.2. Phosphorus Cycle

Phosphorus exists in a relatively simple cycle. It becomes available from minerals through the process of erosion and enters aquatic systems from rivers, terrestrial runoff, precipitation, and groundwater. In nature, phosphorus is normally present as a phosphate ion; the most abundant being orthophosphate (PO_4^{-3}). Bacteria, phytoplankton, and marine plants use the phosphates present in the water and sediments and convert it into organic forms. Heterotrophic organisms can then consume the plants and utilize the phosphorus within them. The phosphorus is subsequently recycled back to the environment by either the death of the plant or animal or by the excreted matter of the animals. At the pH of seawater, most of this phosphorus is quickly converted to phosphate, while the remainder ends up in the sediments.

Dissolved phosphate has a natural affinity to calcium carbonate. In aquatic systems such as those found around Bermuda where limestone is present, phosphate is readily absorbed by the rock through the formation of calcium carbonate-phosphate minerals (Kitano *et al.* 1978, Freeman & Rowell 1981). As the limestone dissolves, phosphate is re-released into the marine environment. It is then returned to the phosphorus cycle where bacteria and autotrophic organisms can utilize it.

The Use of Fecal Bacteria as Indicators of Groundwater Pollution

There are many different pathogens composed of bacteria, viruses, protozoans, fungi, and parasites found in sewage. These pathogens are normally associated with gastrointestinal disorders and hepatitis that occur upon ingestion. Other problems including ear or skin infections and respiratory diseases arise just from contact or inhalation of contaminated waters. Due to the difficulties of testing for all the potential pathogens, a small subset of fecal bacteria have been selected in many water quality monitoring programs as indicators of sewage pollution in fresh, brackish, and marine waters.

The most commonly tested fecal bacteria are total coliforms, fecal coliforms, *E. coli*, and fecal streptococci, and *Enterococcus* species (U.S. EPA 1986). Total coliform and fecal coliform counts are composed of bacteria that have similar characteristics and are typically found in feces. However, genera within these groups are not necessarily fecal in origin. In recent years, the U.S. EPA and other agencies have begun recommending the sole use of *E. coli* and *Enterococcus* as indicators of fecal contamination in water supplies. These two indicators are primarily found in the digestive tract of warm-blooded animals. *Enterococcus* is a subgroup within the fecal streptococcus group. Species within this genus are resistant to harsh environmental factors and can survive in saline waters. Due to this characteristic, the U.S. EPA recommends *Enterococcus* as the sole, reliable indicator of detecting fecal contamination in saltwater and using both *E.coli* and *Enterococcus* as indictors in freshwater (U.S. EPA 2003). A study on the survival, transport, and dissemination for *E. coli* compared to *Enterococcus* in a karstic system similarly concluded that *Enterococcus* is a better indicator of sewage because it had better resilience in water and soil conditions (Personné *et al.* 1998).

The EPA has published a set of standards for tolerable levels of *Enterococcus* in marine waters. Measured counts should not exceed a geometric mean of 35 CFU/ 100 ml, calculated from 5 or more samples evenly spaced over a 30 day period. If only a single sample is collected, levels should not exceed 104 CFU/100 ml for a designated beach area, 158 CFU/100 ml for moderate full body contact, 276 CFU/100 ml for lightly used full body contact, and 501 CFU/100 ml for infrequently used full body contact (U.S. EPA 1986).

Fecal groundwater pollution risk in Bermuda is highly dependent on the thickness of the unsaturated zone and the degree of cementation, fissuring, and karstification of the limestone around the cesspits. The youngest limestone found in the Paget Formation is effective at eliminating a large portion of the fecal bacteria, but the older formations are unable to filter the bacteria due to their increased fissure development and lower matrix porosity, making groundwater in these areas much more susceptible to fecal pollution (Thomson & Foster 1986).

Purpose

The objectives of this chapter were to document the environmental water parameters and determine the nutrient and bacterial content in each of the cave pools. The collected data was used to analyze the following null hypothesis: each cave pool had similar environmental water quality parameters and did not show any significant difference in background nutrient or bacterial levels. By testing this hypothesis, the uniqueness of each cave pool was evaluated. The results were used to determine if the cave pools can be grouped based on the measurements. A number of parameters were documented through out the water column including salinity, temperature, dissolved oxygen (DO), and pH. Background nutrient content and fecal bacteria were also measured at each site. Light is a variable that may be an important factor in distinguishing the pools but did not contribute to the overall results in this thesis. Due to this the light data is included in the appendix.

Materials and Methods

Environmental Water Parameter Measurements

A multi-parameter water quality sonde (YSI 600 XLM) was used to collect data on environmental water parameters in each cave pool. It was deployed by SCUBA divers during the initial assessment of each pool. The sonde measured depth, temperature, pH, salinity, and DO in each cave. Each probe was calibrated according to YSI standards before each deployment with the program Eco-Watch. Prior to each run, the sonde was held steady just below the surface of the pool, in undisturbed water for 30 sec. The diver then held the sonde in front of their body, angling the sensors so they extended as far as possible from the body. It order to obtain accurate readings, the sonde must penetrate through water that has been undisturbed by the diver. The descent occurred at a steady, slow rate with the sonde measuring surrounding parameters continuously ever 4 sec. All data was stored in the sonde’s internal memory pack. Upon completion of the dive, data was downloaded into an Excel spreadsheet and each parameter graphed with respect to depth.

Nitrogen to phosphate (N:P) ratios were calculated to determine if cave pools were either nitrogen or phosphate limited. Limitation in this sense was first defined by the Redfield ratio (Redfield at al. 1963). Redfield's ratio was based on extensive analysis of marine phytoplankton. A N:P ratio of <16 indicates N limitation while ratios of N:P > 16 indicate P limitation. A number of studies have been conducted since that time and have determined that macroalgae deviate from this ratio. For macroalgae, a modified N:P ratio range of 35-50:1 rather than 16:1 is used to distinguish between N and P limitation (Atkinson & Smith 1983, Duarte 1992). An acceptable range is used within this study to acknowledge the variation in the literature. The two papers reviewed a large sample group (92 and 46 species respectively) from multiple phyla and locations to determine this range.

Nutrient and Bacterial Sampling

Two duplicate water samples were collected at depths of 0, 3, and 6 m or the maximum depth for nutrient analysis. Samples were collected in acid-washed falcon tubes (50 ml) and transported from each site in a dark cooler on ice. They were stored frozen until they could be shipped and analyzed by personnel of the Geochemical and Environmental Research Group (GERG) at Texas A&M University. Each sample was tested for levels of phosphate ($HPO_4^=$), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_4^+), and urea.

Additional water samples were collected for bacterial analysis. Samples were collected from surface waters in sterilized, polyethylene containers (250 ml). The bottles were stored in a dark cooler and transported directly to the Central Government Laboratory in Bermuda. This facility is operated by the Department of Health at the King Edward VII Memorial Hospital. The samples were analyzed within 6 hrs of collection for *Enterococcus* using the membrane filtration method (U.S. EPA 1985). Replicates and subsurface samples were not collected due to the restrictions of the local laboratory. Due to these constraints, the Enterococcus samples were compared to the EPA's recommended, less conservative one-time reading scale. The EPA one reading exposure limit for moderate full body contact is 158 CFU/100 ml (U.S. EPA 2003).

Statistical Analyses

To determine if differences in mean background nutrient concentrations occurred between the three nature reserve and three urbanized cave pool sites, a one-way analysis of variance (ANOVA) was run using SPSS Version 13.0 (SPSS Inc., Chicago, IL). Data was logarithmically transformed when necessary to conform to the assumption of ANOVA. The concentration of each nutrient ($HPO_4^{=}$, NO_2^{-}, NO_3^{-}, NH_4^{+}, and urea) was separately compared between the two groups, reserve (Calabash, Harbour Pool, Deep Blue) and urbanized (Grenadier, Cliff Pool, Emerald). Separate ANOVAs were run for surface and bottom water samples between the reserve and urbanized pools. Results from only the first six days of the nutrient addition were included in the statistical analysis. The ANOVAs run for this analysis were not extremely power due to the low replication. To be more statistically robust, additional water samples should have been collected (that is, at least 3-5 at each depth within each cave pool). This could not be done due to the remoteness of the sampling sites and due to sampling expense.

Principal Components Analysis (PCA), a multivariate method of indirect ordination, was run on background environmental data across the six cave pool site using CANOCO Version 4.5 (ter Braak and Smilauer 2002). This nonparametric, linear transformation was used to ascertain which main environmental parameters differentiated the cave pool sites. The parameters included in this analysis were surface and bottom nutrient levels, surface bacterial levels, temperature, salinity, dissolved oxygen, and maximum depth. Vectors in the PCA diagrams point toward increasing values for an environmental variable. Location of a site relative to vector arrows show which water quality variables are positively or negatively associated with each cave pool site. Longer arrows indicate an increase in variation within the variable.

Results

Vertical Profiles of Environmental Water Parameters

Environmental water parameter profiles were taken for all six cave pools that were studied, including the three reserve site (Calabash, Harbour Pool, and Deep Blue) and the three urbanized sites (Grenadier, Cliff Pool, and Emerald). The vertical profiles presented below show how temperature, salinity, dissolved oxygen, and pH behaved throughout the different depths of the pool during a single dive in June or July 2007. Calabash is a shallow pool located within the boundaries of the Walsingham Trust Nature Reserve. Most of the pool is approximately 0.5 m deep, but deepens to 1.3 m on one side against a cliff wall where it connects to a submerged passage. The vertical profiles for temperature, salinity, dissolved oxygen, and pH were taken through the shallower to the deeper section in June 2007 and presented in the following figures 2.3-2.6.

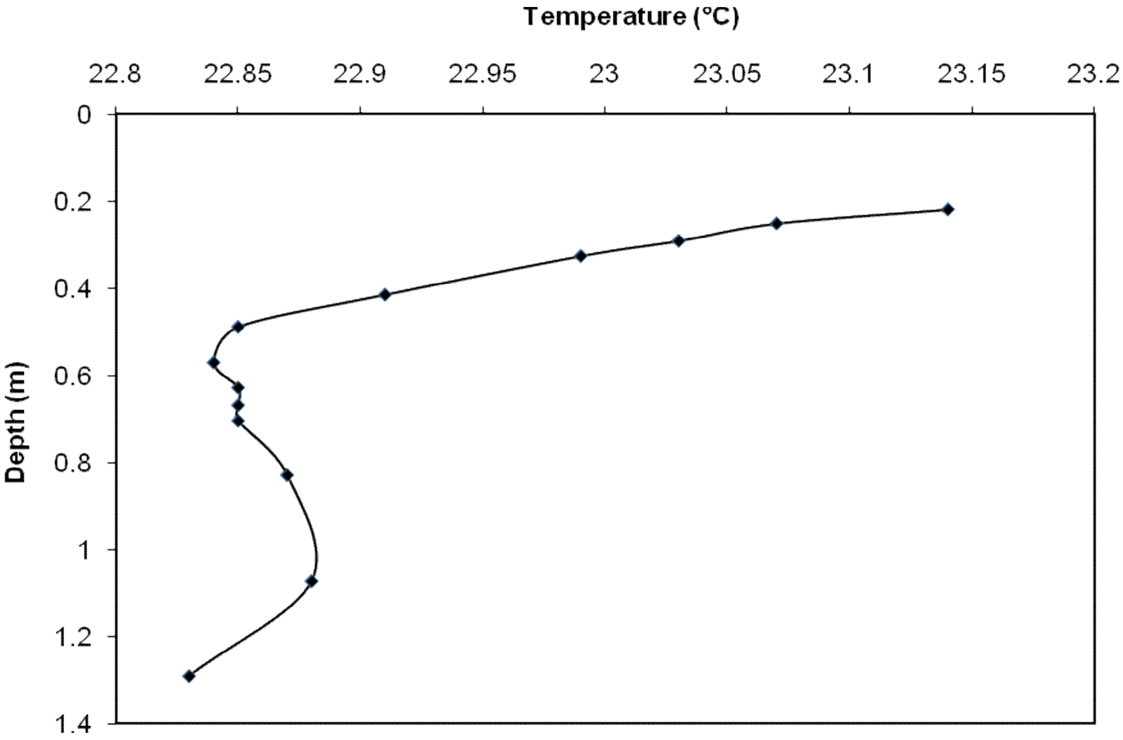

Fig 2.3. Temperature versus Depth at Calabash

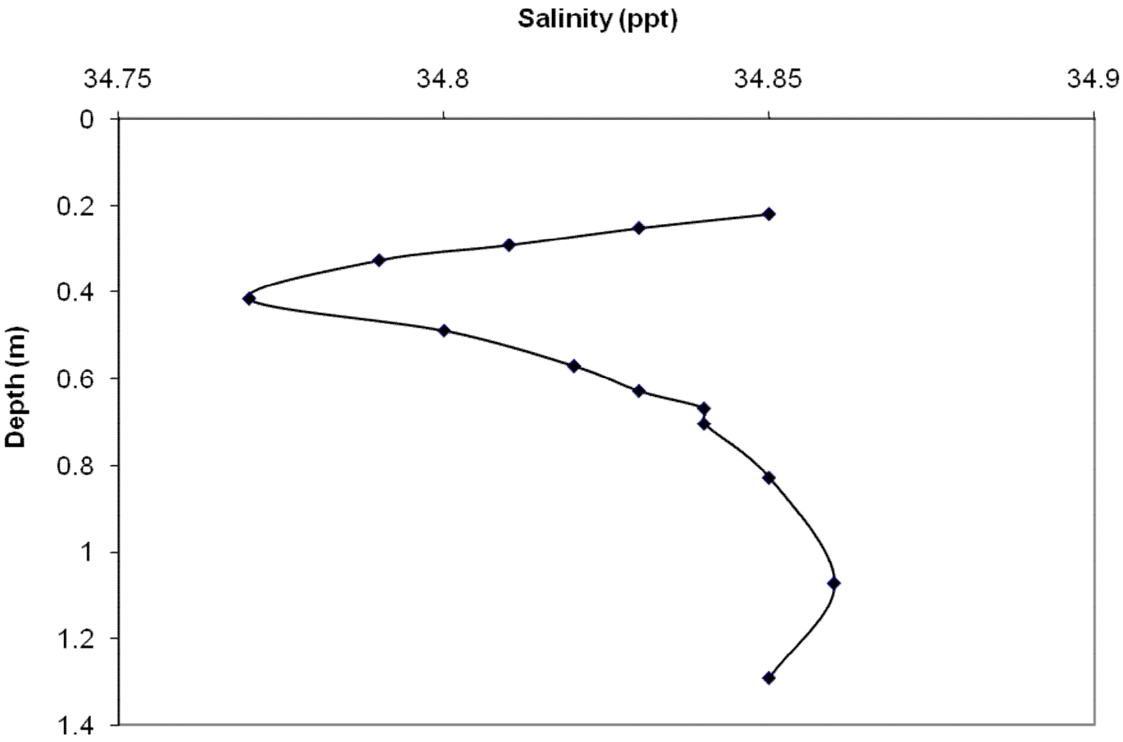

Fig 2.4. Salinity versus Depth at Calabash

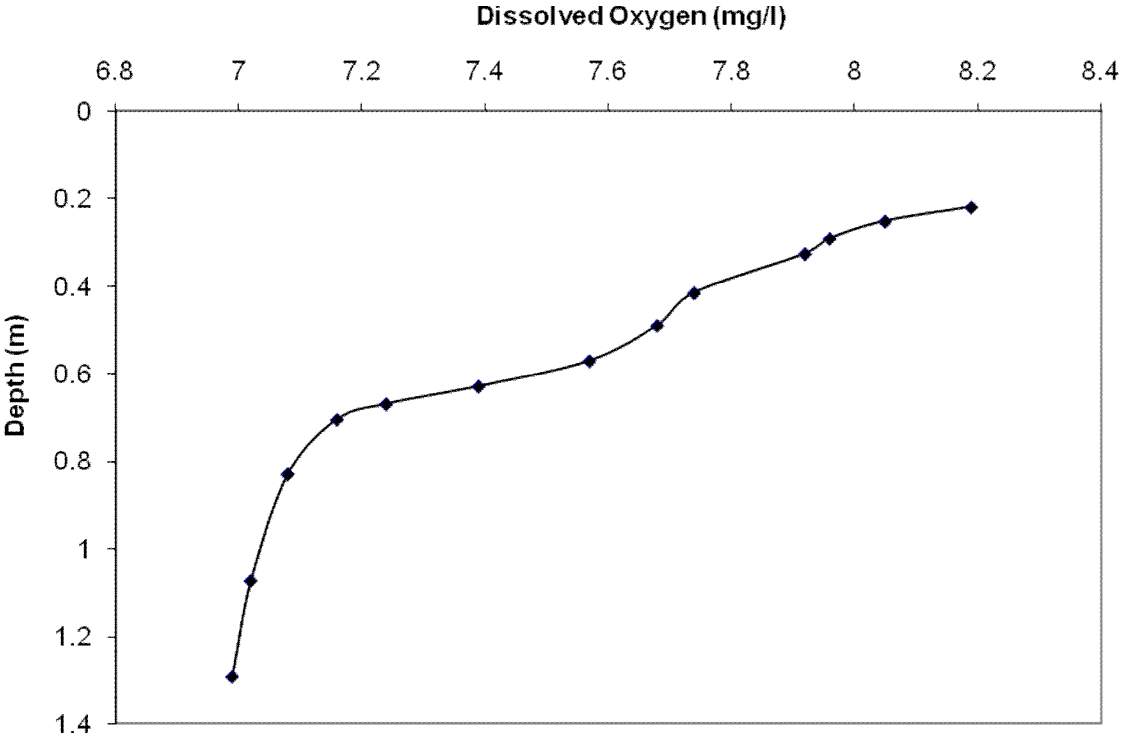

Fig 2.5. Dissolved Oxygen versus Depth at Calabash

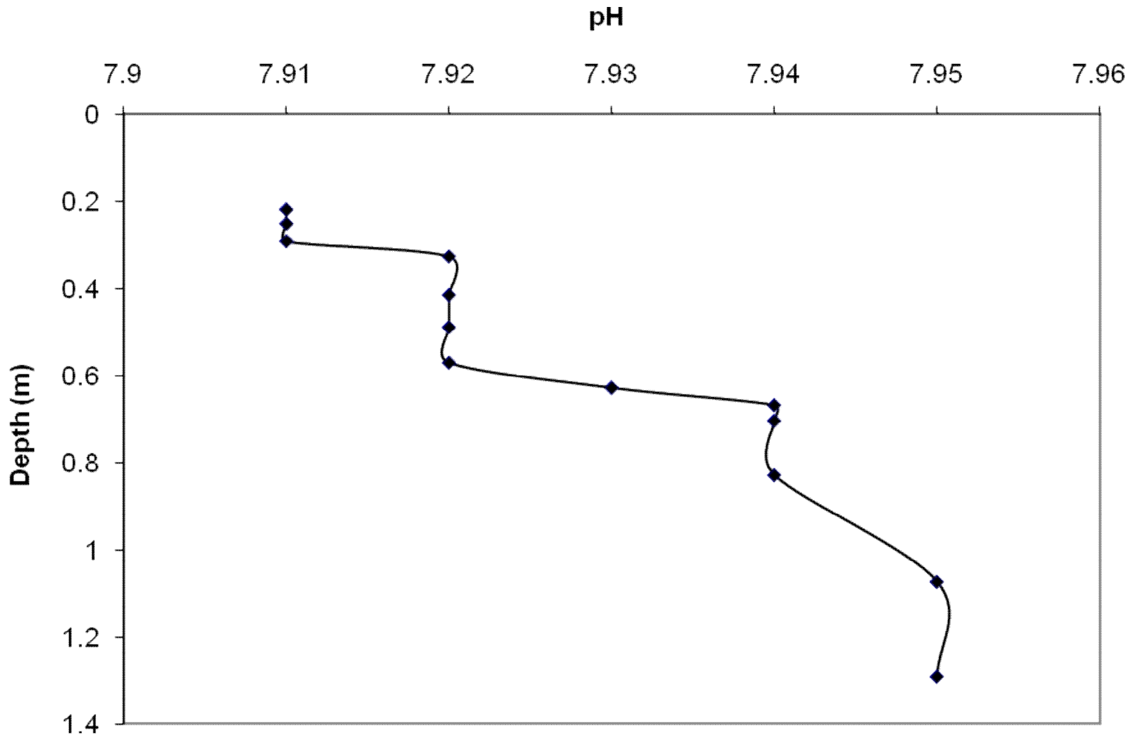

Fig 2.6. pH versus Depth at Calabash

The profiles all exhibit certain trends as they move from the surface to deeper water. The surface temperature was 23.14°C, but conditions cooled slightly with depth (22.83 °C). There was a very slight increase in the declining temperature at 0.5 m, before continuing to decrease. The salinity was fully marine (34.85 ppt) with only a slight dip (<0.1 ppt) at 0.4 m before increasing back to surface concentrations. The dissolved oxygen showed a similar pattern as that seen with temperature. Levels were highest at the surface (8.19 mg/l) and lowest at 1.3 m (6.99 mg/l). The pH ranged from 7.91-7.95 but the behavior could not be further interpreted due to the +/- 0.2 accuracy of the pH sensor. The measured changes were very small in this pool due to the shallowness of the pool.

Harbour Pool is located just to the east of Calabash within the reserve and is slightly deeper throughout most of the pool. It has a short, subterranean connect to the more open waters of Castle Harbour. The vertical profiles, taken in June 2007 for the four water parameters, are presented in figures 2.7-2.10.

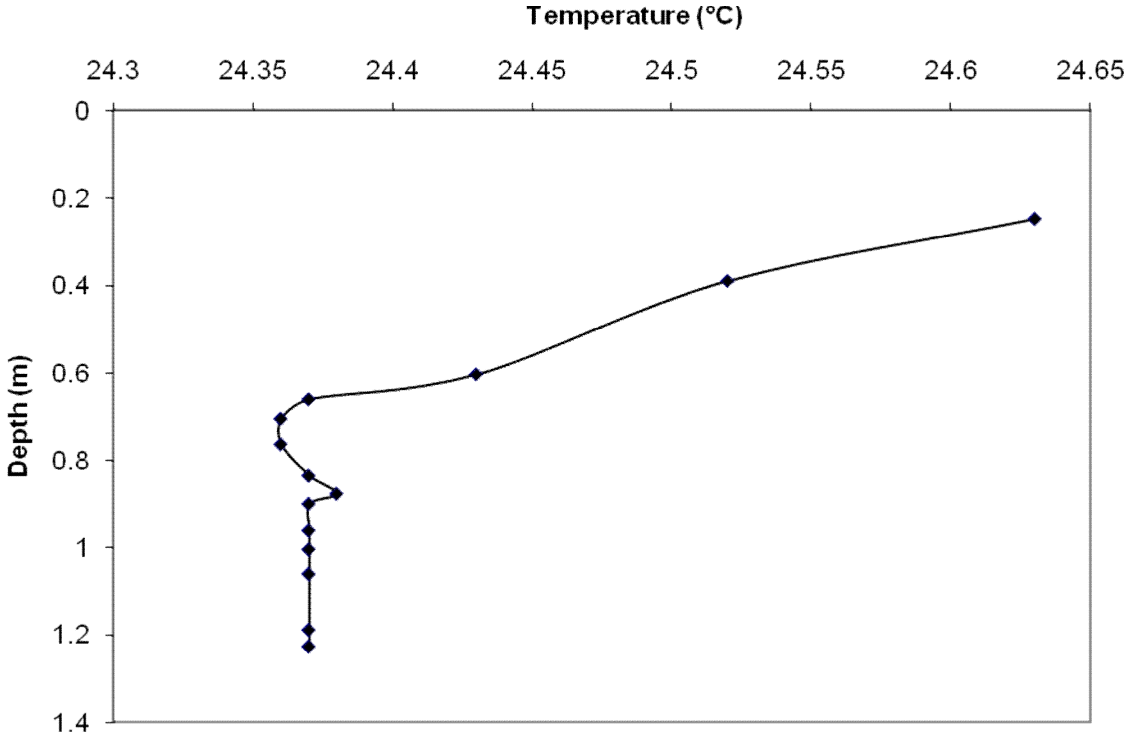

Fig 2.7. Temperature versus Depth at Harbour Pool

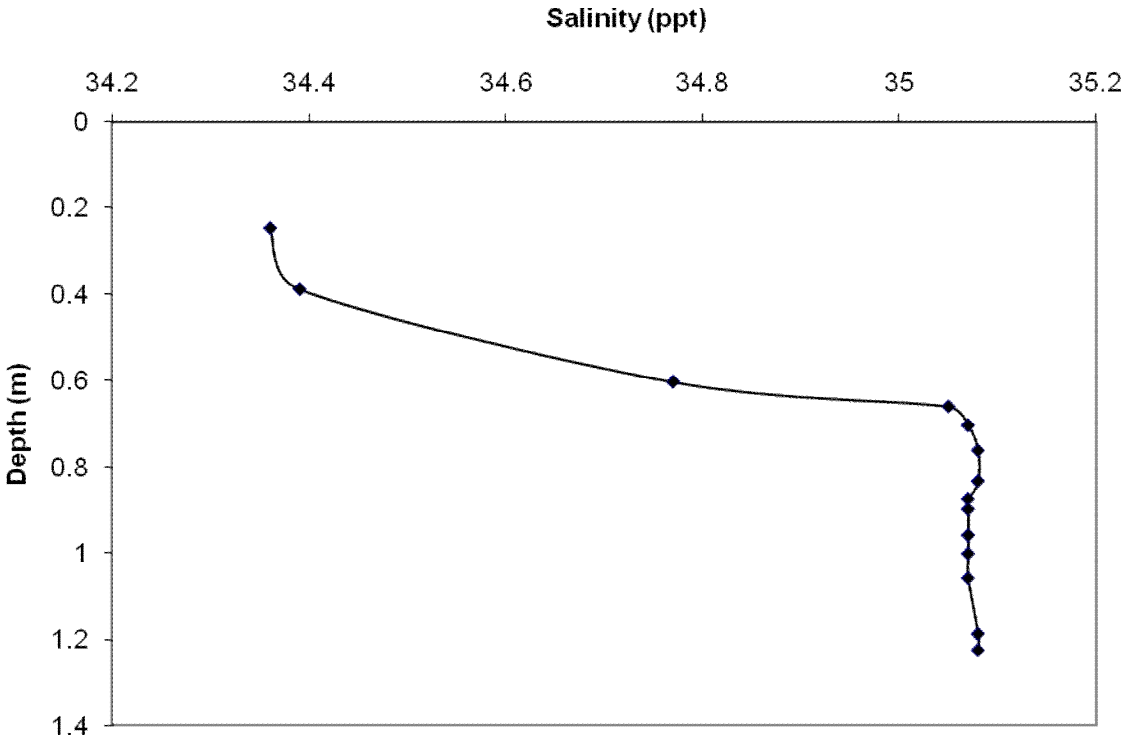

Fig 2.8. Salinity versus Depth at Harbour Pool

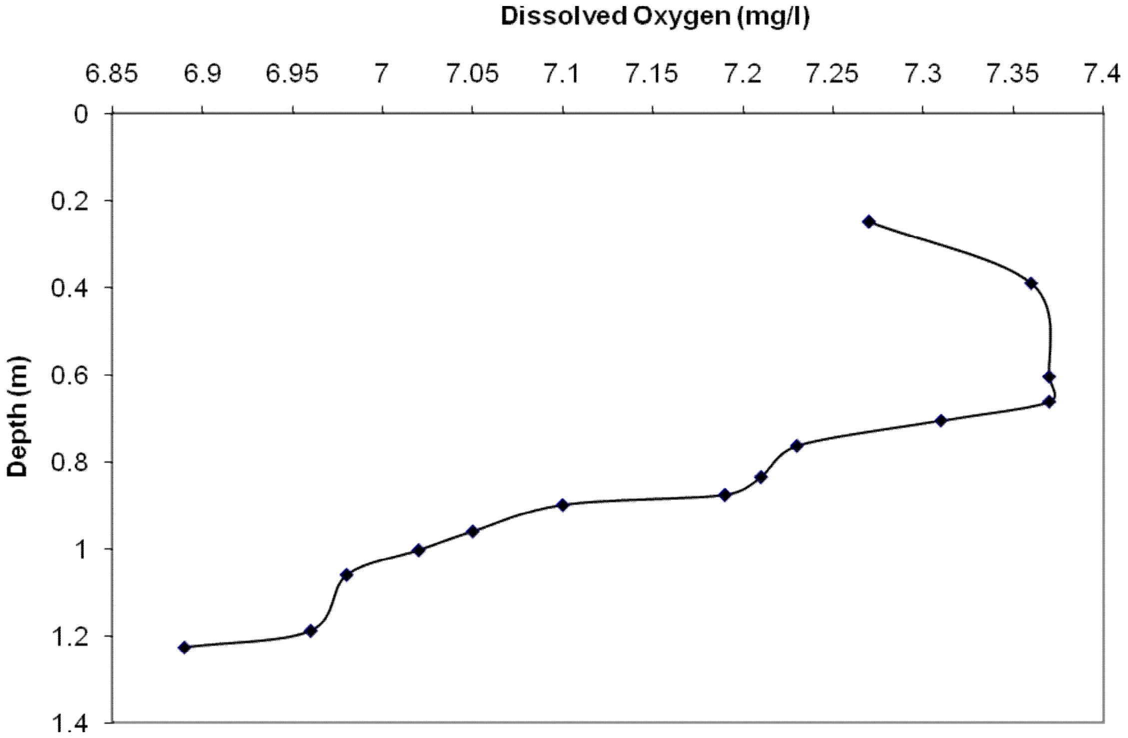

Fig 2.9. Dissolved Oxygen versus Depth at Harbour Pool

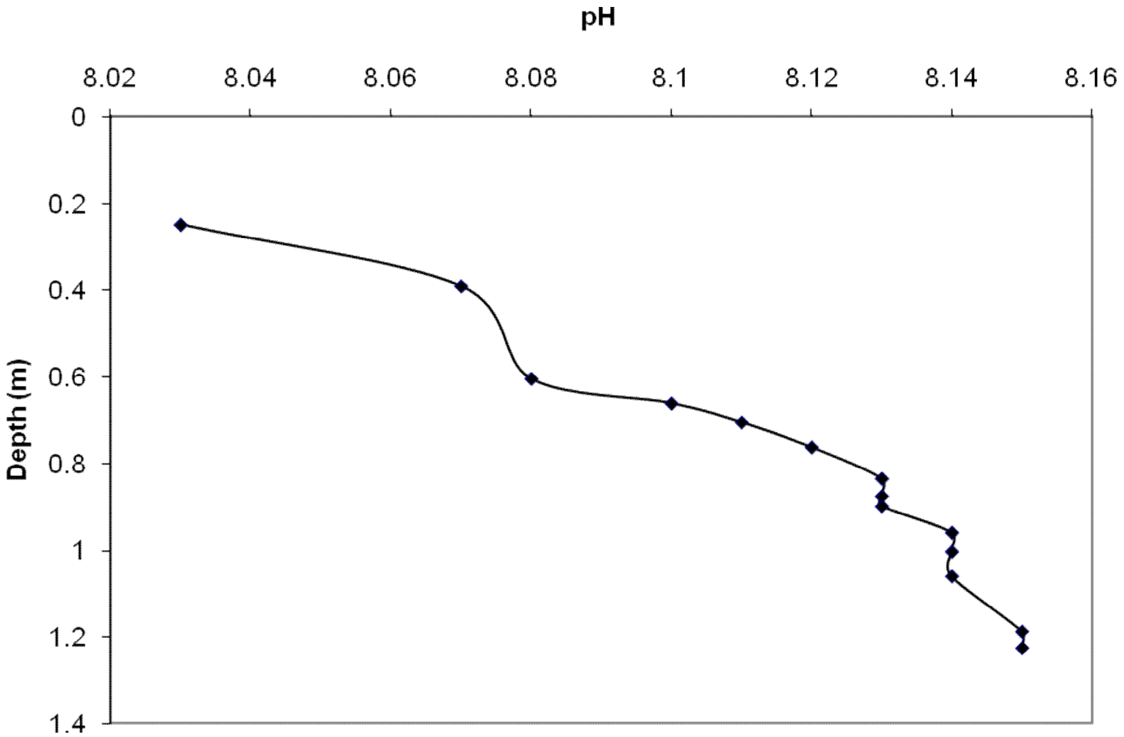

Fig 2.10. pH versus Depth at Harbour Pool

The patterns shown in these graphs are similar to those seen in Calabash. The temperature was highest at the surface (24.63°C) and quickly dropped by 0.3°C at the maximum depth of 1.2 m. As with Calabash, the waters within this pool were fully marine. The salinity profile had an opposite trend, with the lowest salinity level at the surface (34.36 ppt) that stabilized at 35.08 ppt at depth. The dissolved oxygen increased for the first 0.6 m to 7.37 mg/l, before decreasing to 6.89 mg/l at maximum depth. The pH increased from 8.03 at the surface to 8.15 at 1.2 m. As with Calabash, this range was within the error of the instrument and could not be interpreted strongly.

Deep Blue, also in the Walsingham reserve, has one large and two smaller surface pools that connect under water into a large cavern that extends to depths of 16.5 m. The cavern is connected by submerged passages to at least six other pools in the

Walsingham area. Profiles were measured in the main pool to the entrance of the cavern at 8.9 m in July 2007 and are presented in figures 2.11-2.14.

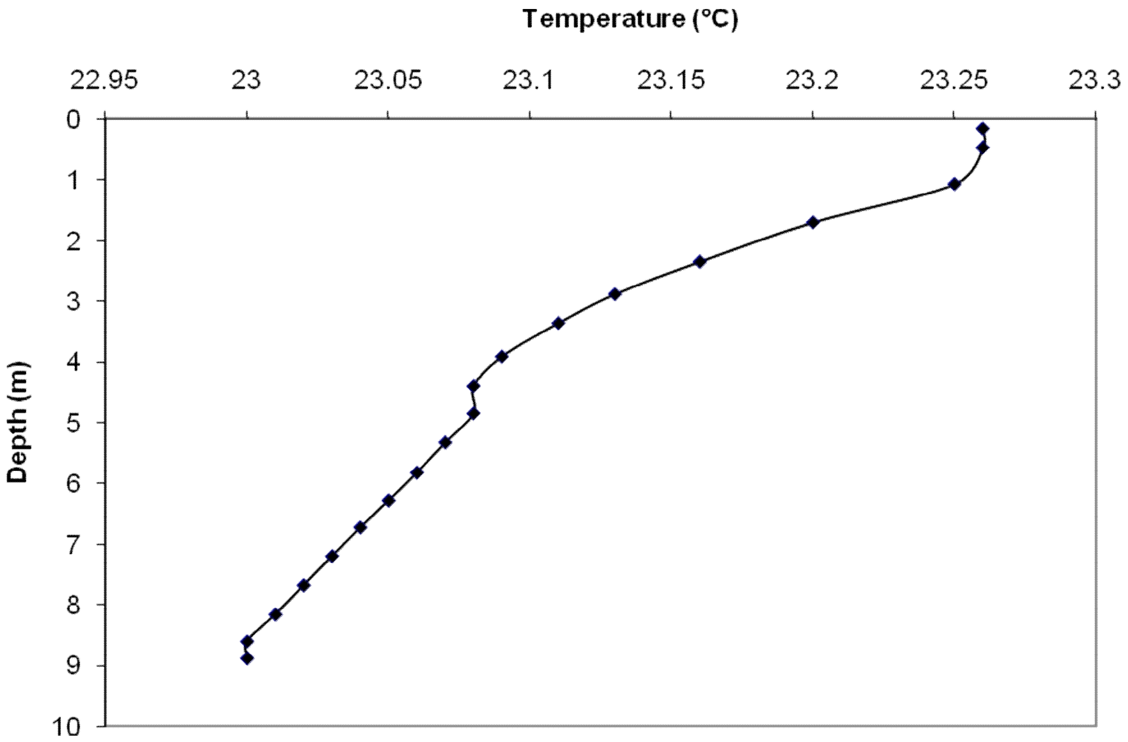

Fig 2.11. Temperature versus Depth at Deep Blue

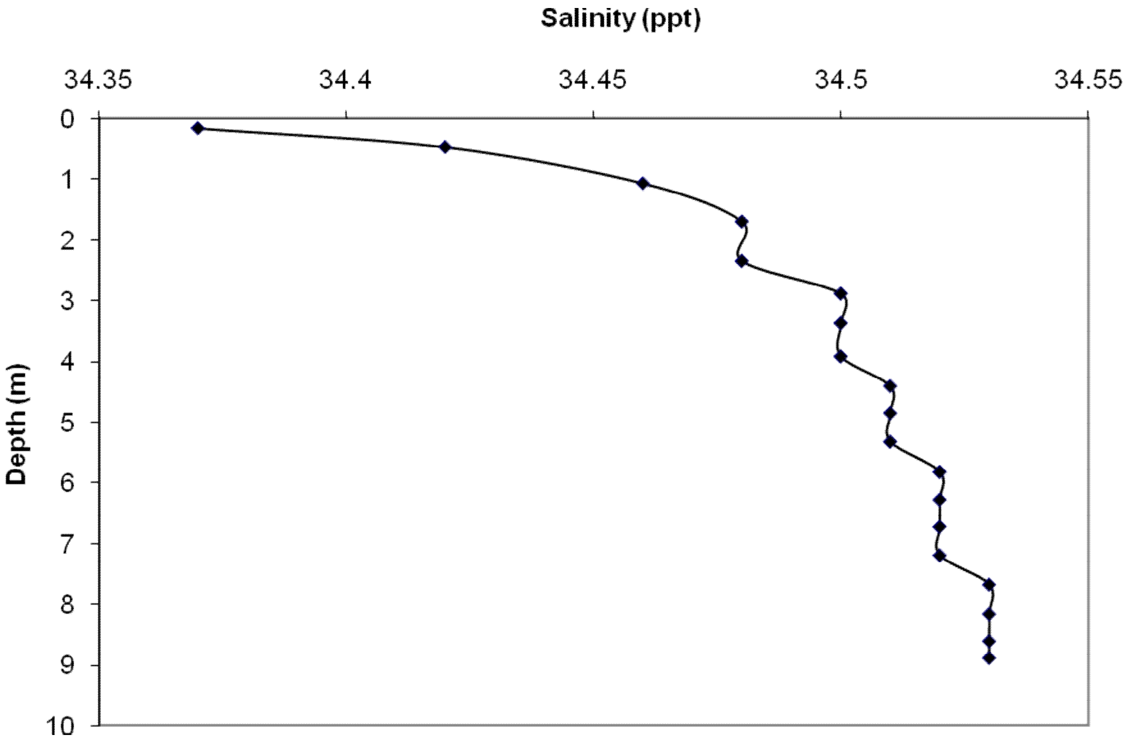

Fig 2.12. Salinity versus Depth at Deep Blue

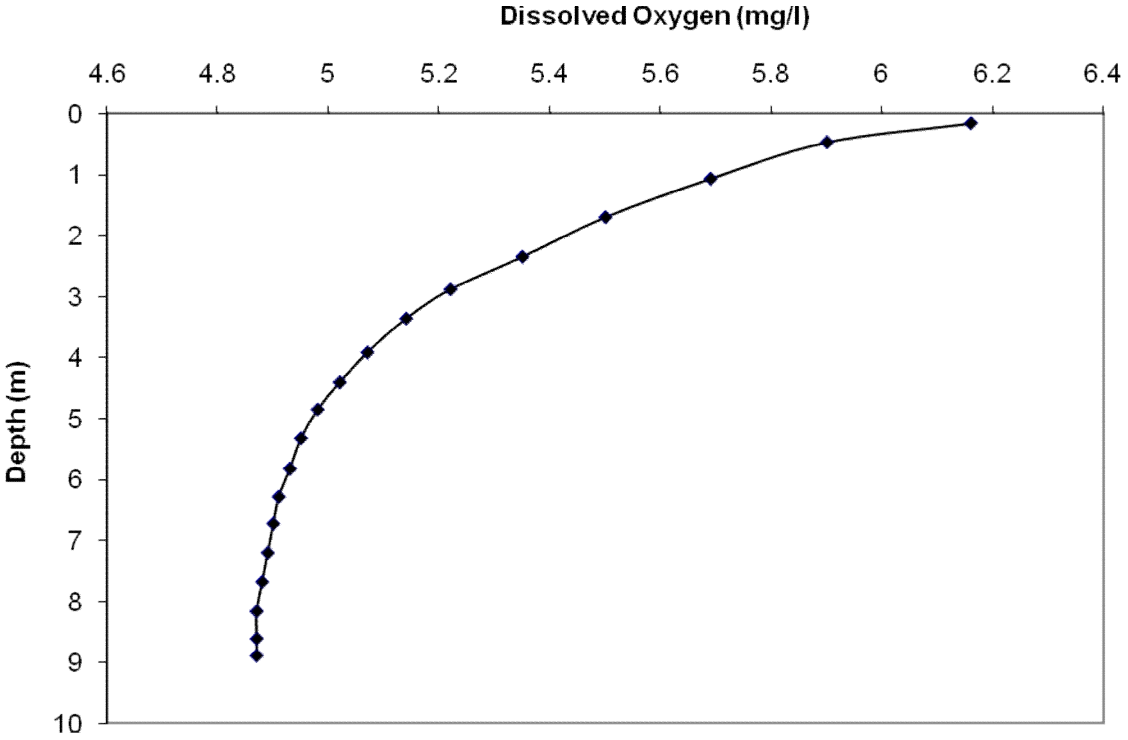

Fig 2.13. Dissolved Oxygen versus Depth at Deep Blue

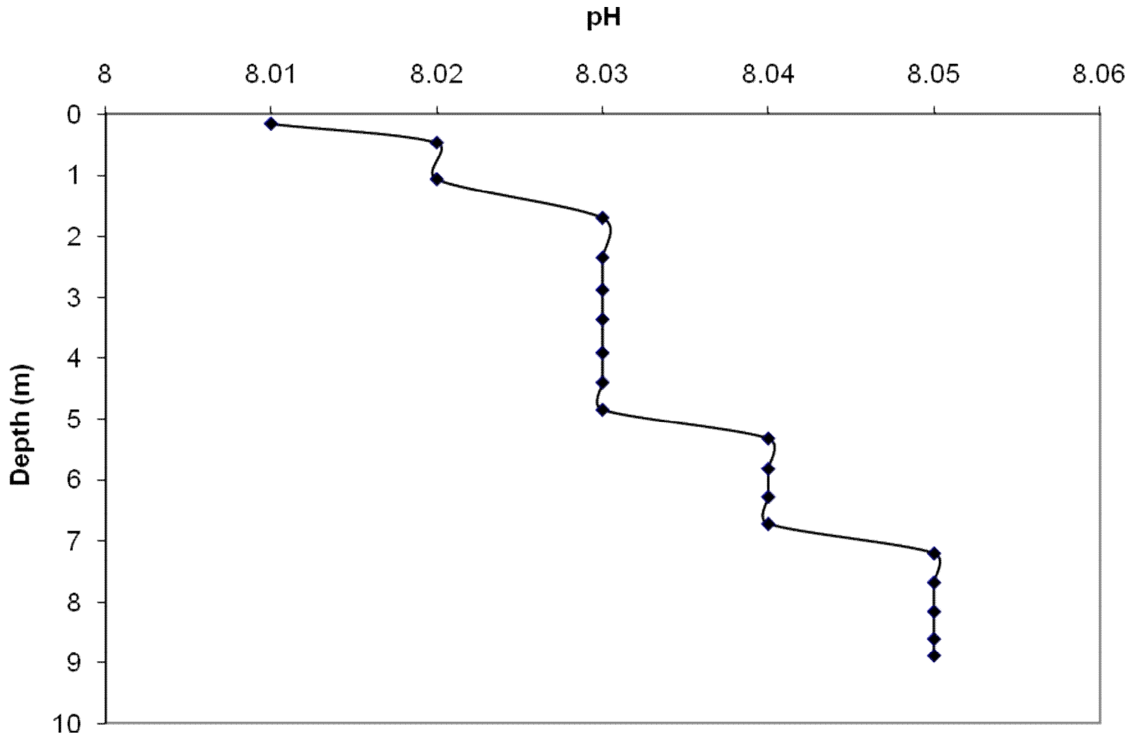

Fig 2.14.. pH versus Depth at Deep Blue

The hydrological trends displayed within the above profiles, show that the patterns seen within the shallower pools of Calabash and Harbour Pool are similar to those in the deeper pool of Deep Blue. The temperature gradually declined from 23.26°C at the surface to 23.0°C at 8.9 m. As with the other two pools, the waters within Deep Blue were fully marine and just slightly increased from the surface (34.37 ppt) to deep (34.53 ppt) waters. The dissolved oxygen levels were started out lower in the surface water of this deeper pool than the shallower pools and behaved similar to the temperature. Levels started out at 6.2 mg/l at 0.1 m and decreased to 4.8 mg/l at 8.9 m. The lower surface levels are perhaps due to the more reduced tidal influence and thus less turbulent water exchange as seen in the pools located closer to the coast. The pH ranged from 8.01 to 8.05. This 0.04 difference can not be interpreted strongly due to the +/- 0.2 accuracy of the pH sensor.

Grenadier is located within the backyard of a privately owned residence on the North Shore of the island. On one side of the pool, an underwater ledge extends 0.5-1.5 m into the pool at a depth of ~1 m. Past the ledge, the pool deepens to 3.1 m. A submerged cavern run opposite the ledge and extends to 7.6 m. Passages too small for divers to enter connect off the cavern and show evidence of tidal exchange as indicated by sponge growth. The vertical profiles were taken through the main section of the pool in June 2007 and are presented in figures 2.15-2.18.

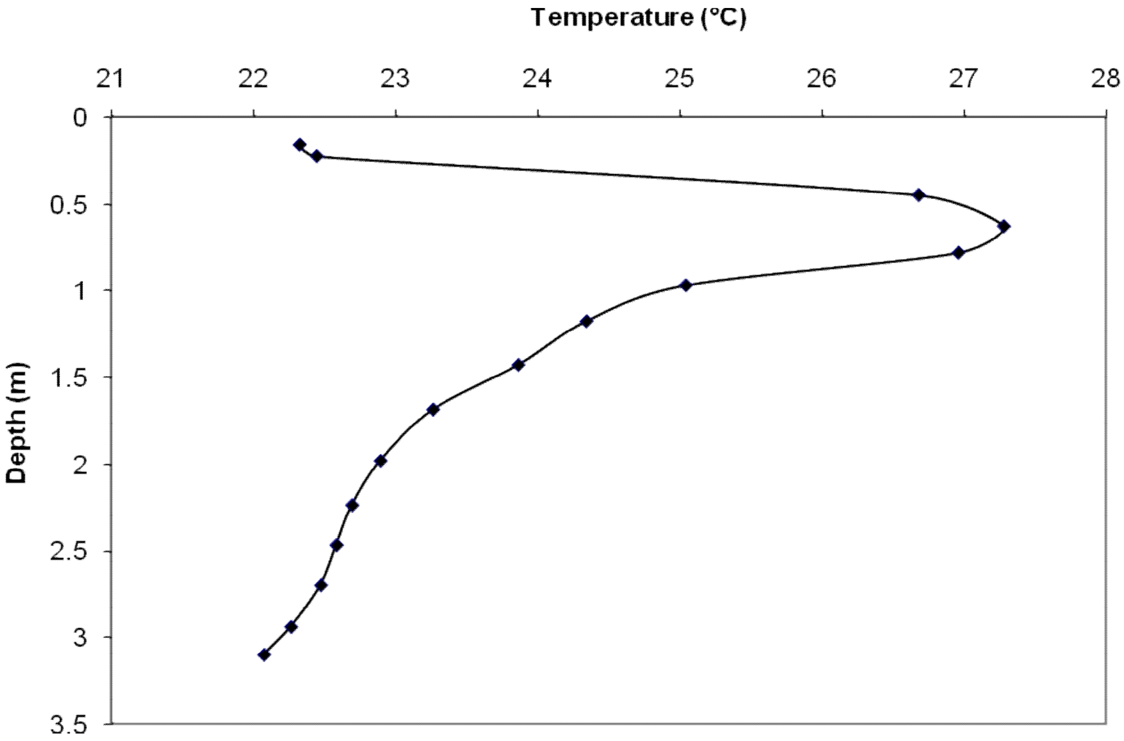

Fig 2.15. Temperature versus Depth at Grenadier

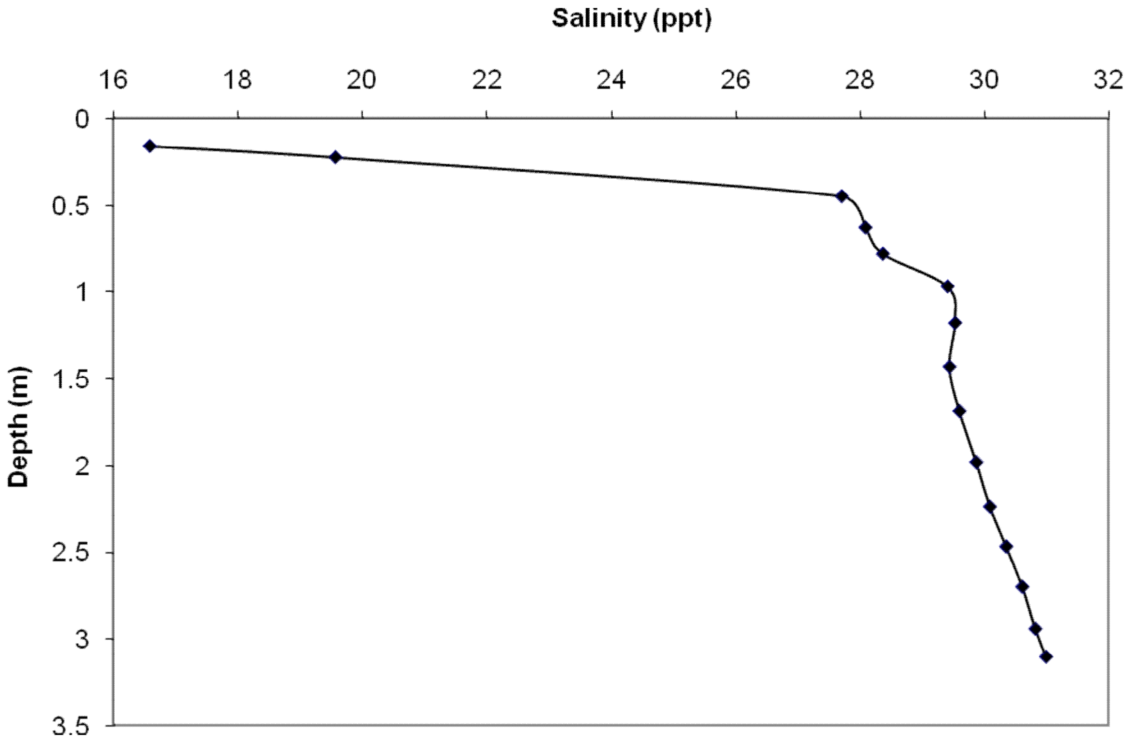

Fig 2.16. Salinity versus Depth at Grenadier

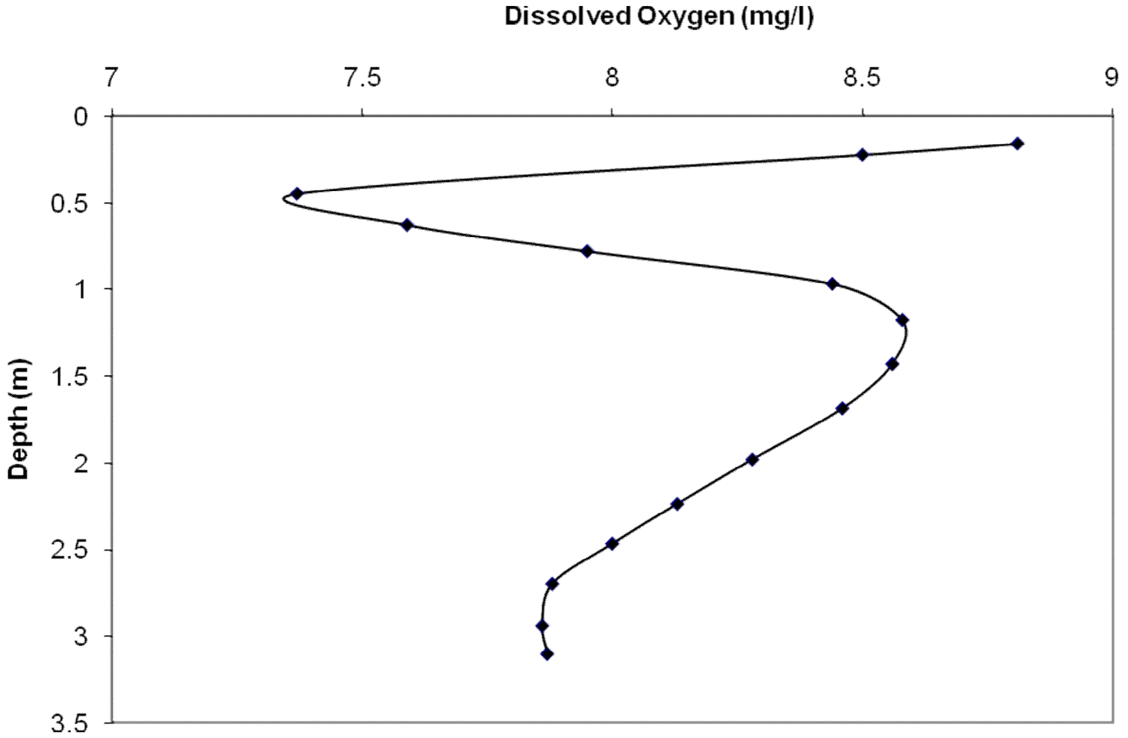

Fig 2.17. Dissolved Oxygen versus Depth at Grenadier

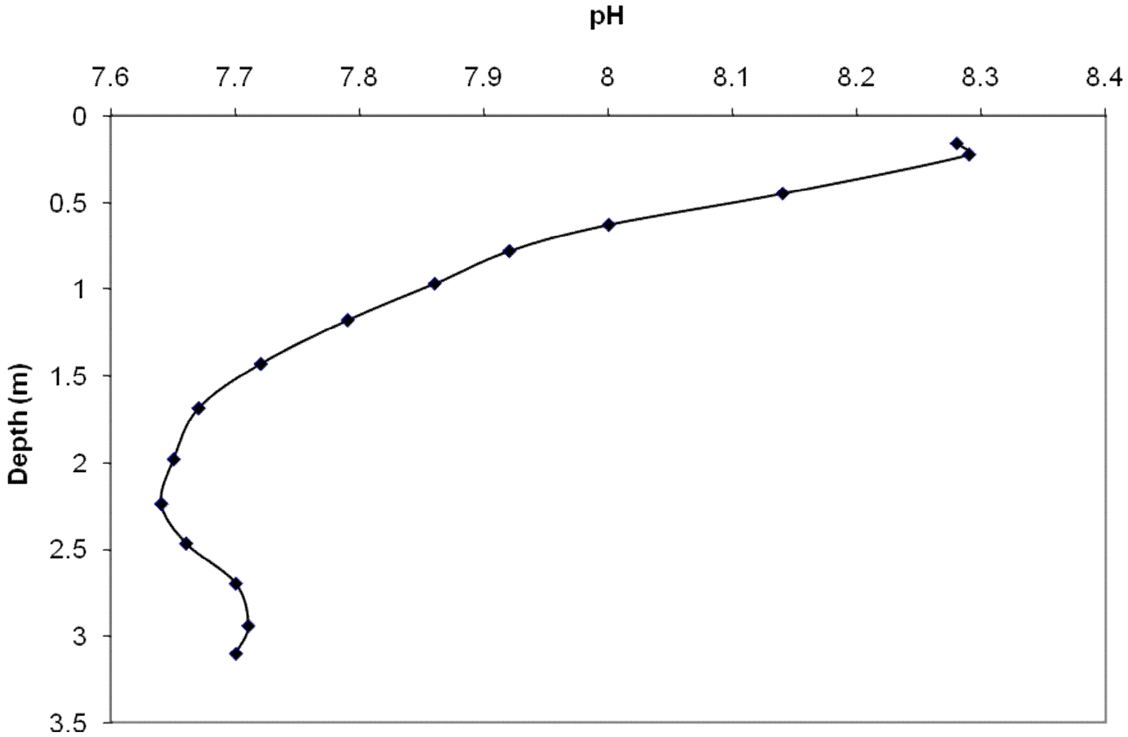

Fig 2.18. pH versus Depth at Grenadier

These profiles depict different trends in the water parameters than those previously illustrated. Unlike the other pools, temperature quickly increased from 22.32°C at the surface to 27.28°C at 0.5 m. Once past this point, it gradually declined back to surface levels (22.07°C). A brackish surface layer extended from 0-0.5 m. Salinity levels within this layer quickly increased from 16.59 ppt to a noticeable halocline at 0.5 m (27.7 ppt). Below the halocline, salinity levels gradually rose to 30.98 ppt. Dissolved oxygen had a markedly different pattern than previous pools and was affected by the two water layers. A measurement of 8.81 mg/l was recorded at the surface. It then sharply decreased to 7.37 mg/l at 0.5 m before spiking back to 8.58 mg/l at 1.2 m, and then declining for a second time to 7.87 mg/l at 3.1 m. The pH decreased from 8.29 at the surface to 7.7 at maximum depth.

Cliff Pool is located in the sideyard of a private residence, just inland of the inshore water body of Harrington Sound. Its configuration is similar to Deep Blue. A steep cliff face makes up the northwest side of the pool. Under this cliff, the pool descends into a large cavern at a depth of 18 m. Large underground passages connect to the Green Bay cave system which has a direct opening to Harrington Sound. The environmental water parameters profiles shown in figures 2.19-2.22 were taken from the main pool to the steep drop off into the cavern area in June 2007.

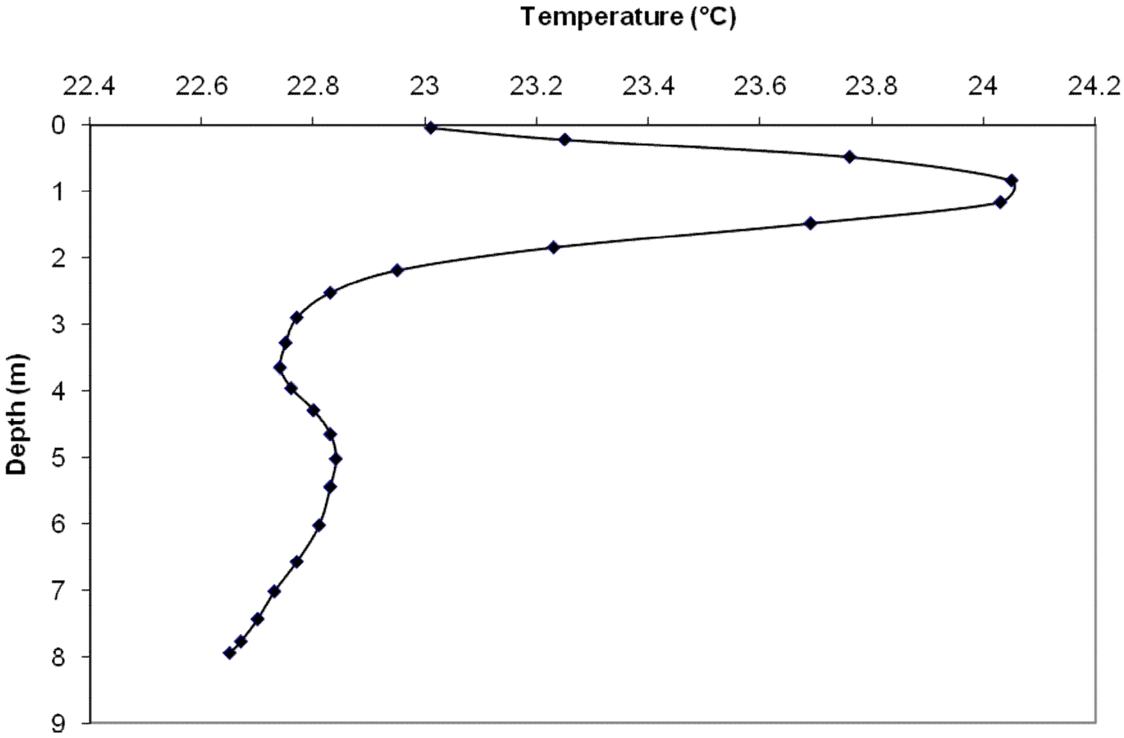

Fig 2.19. Temperature versus Depth at Cliff Pool

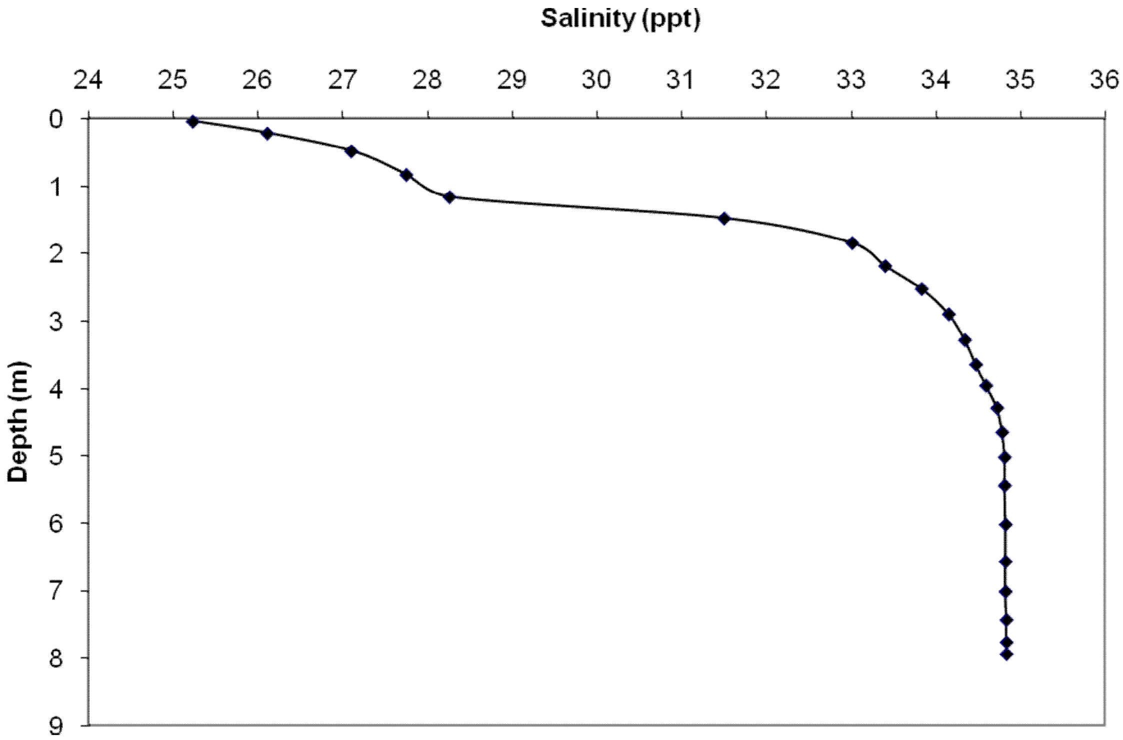

Fig 2.20. Salinity versus Depth at Cliff Pool

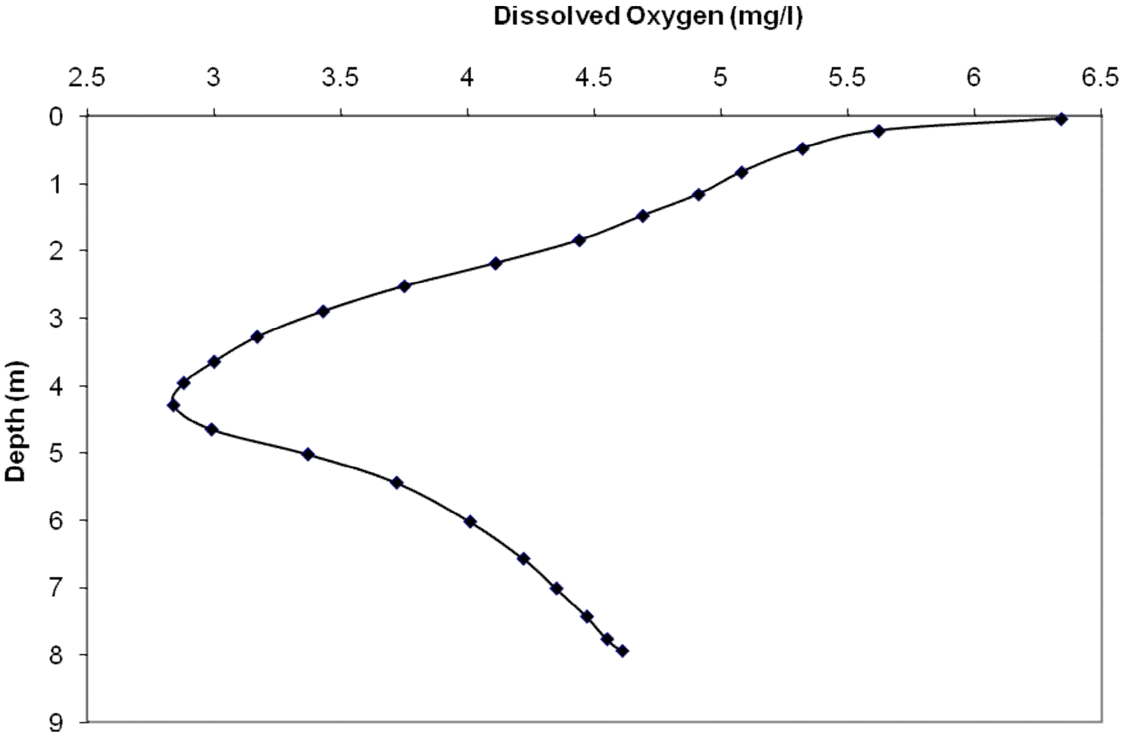

Fig 2.21. Dissolved Oxygen versus Depth at Cliff Pool

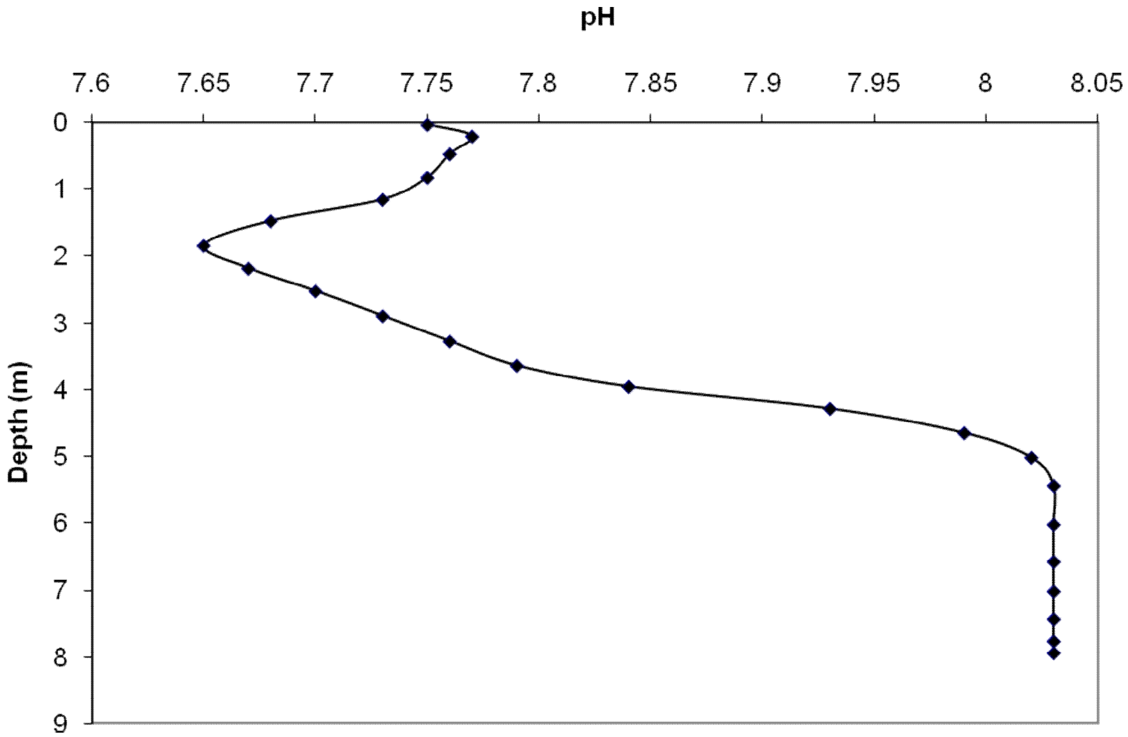

Fig 2.22. pH versus Depth at Cliff Pool

Although the pool had a cavern and connecting subterranean passages similar to Deep Blue, the profiles had many different qualities to them. As with Grenadier, the temperature increased from 23°C to 24°C in the first meter. Below 1m, the temperature quickly decreased to 22.7°C by 3 m, before stabilizing at 22.6°C at 7.9 m. Similarly, there was a brackish layer (0-1.0 m) of water overlying fully marine. The surface water had a higher salinity than Grenadier, ranging from 25-28 ppt within the first meter. At the halocline (1.1-1.4 m), salinity jumped sharply to 33 ppt, which slightly increased further to 34.7 ppt at 7.9 m. The dissolved oxygen profile was different than all the other above pools. At the surface, it measured 6.34 mg/l, then decrease to 2.84 mg/l at 4.3 m, before beginning to increase. The dissolved oxygen continued to climb with depth to 4.61 mg/l at 7.9 m. This differing pattern of increased dissolved oxygen in deeper waters could have been a result of stronger tidal influence between the cave and open water of

Harrington Sound bringing in oxygen into the system compared to the other pools. The pH moved in the opposite direction as temperature in the surface and deeper waters. It started off at 7.74 at the surface, rapidly decreased to 7.65 at 2 m, then increased to 8.03 at 5 m and remained steady to 7.9 m.

Emerald is located on a privately owned portion of land in close proximity to other homesteads and a major road, Harrington Sound Road. Currently, there is not a house built directly next to the site but construction of one have been submitted to the Planning Department. There are a series of five pools located in a horseshoe shape under a cliff face. The pools slope back under the cliff to submerged passages at depths of 19.8 m. Several of the pools have been found to connect by diving, but the system as a whole remains relatively unexplored. The profiles displayed in figures 2.23- 2.26 were taken in the largest, most western pool to the edge of the cliff face as a depth of 9.4 m in July 2007.

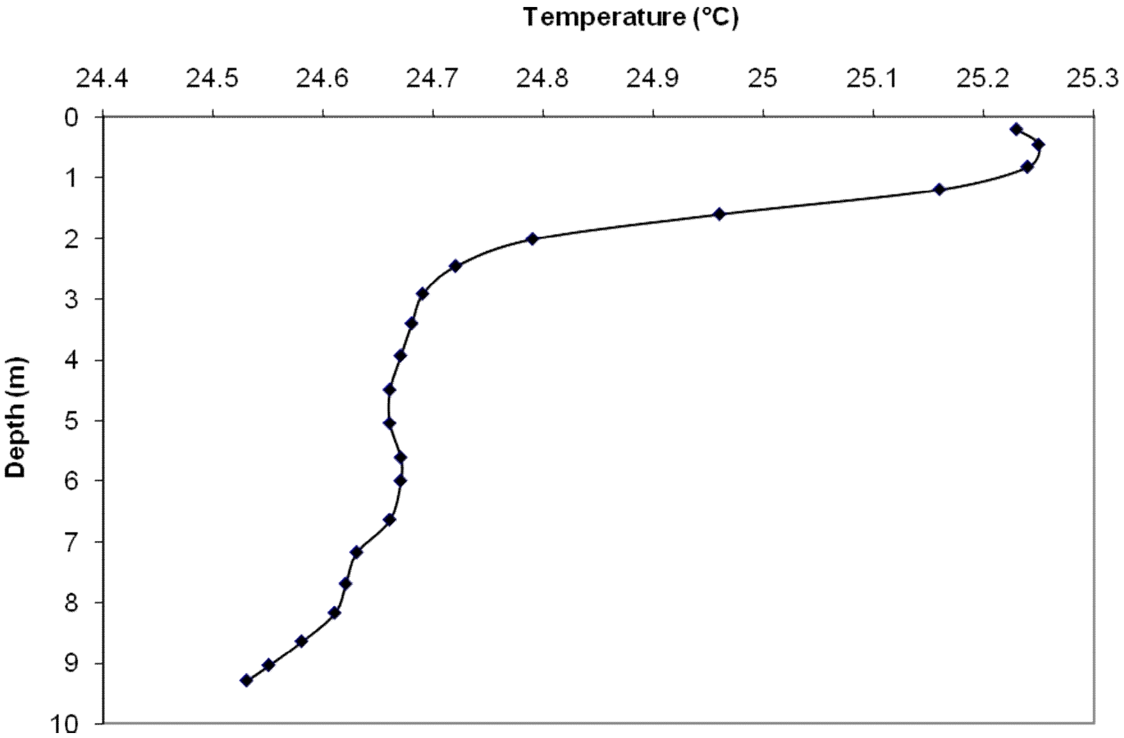

Fig 2.23. Temperature versus Depth at Emerald

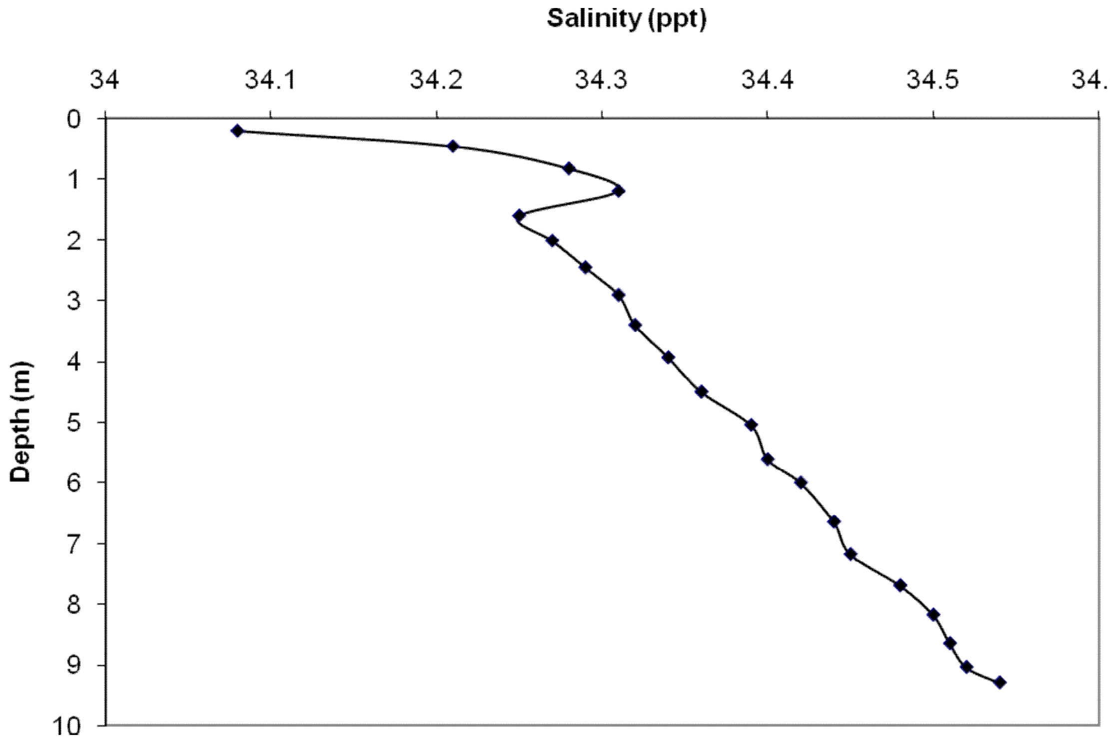

Fig 2.24. Salinity versus Depth at Emerald

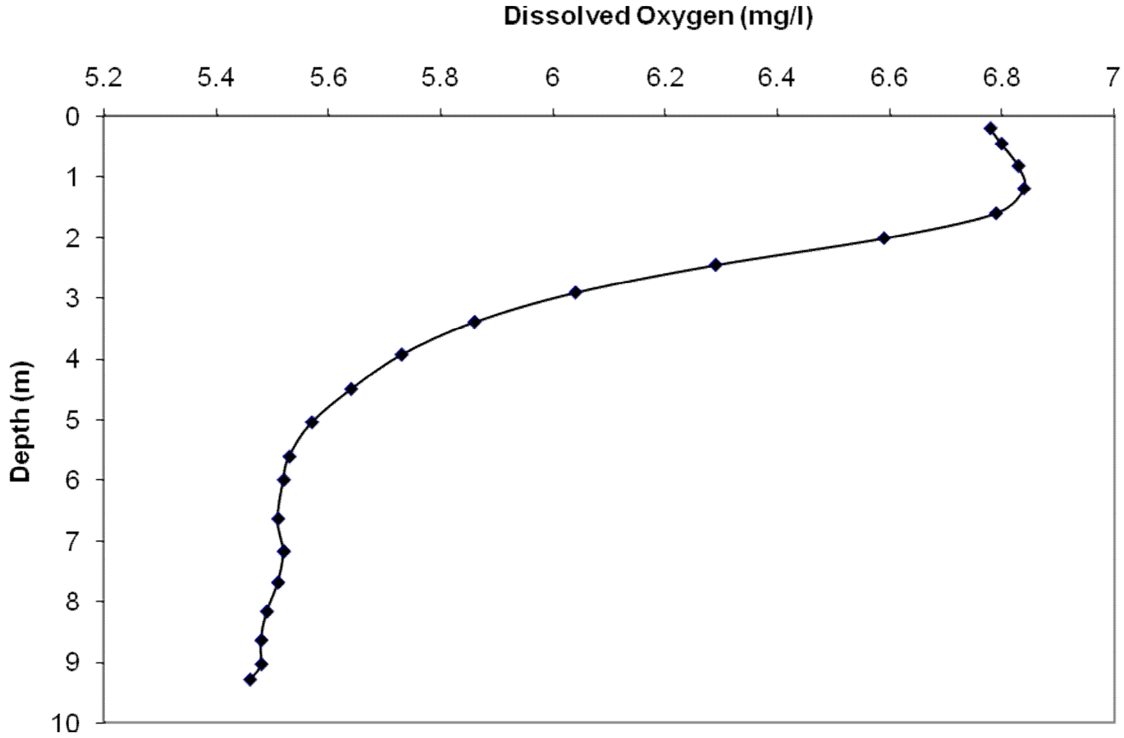

Fig 2.25. Dissolved Oxygen versus Depth at Emerald

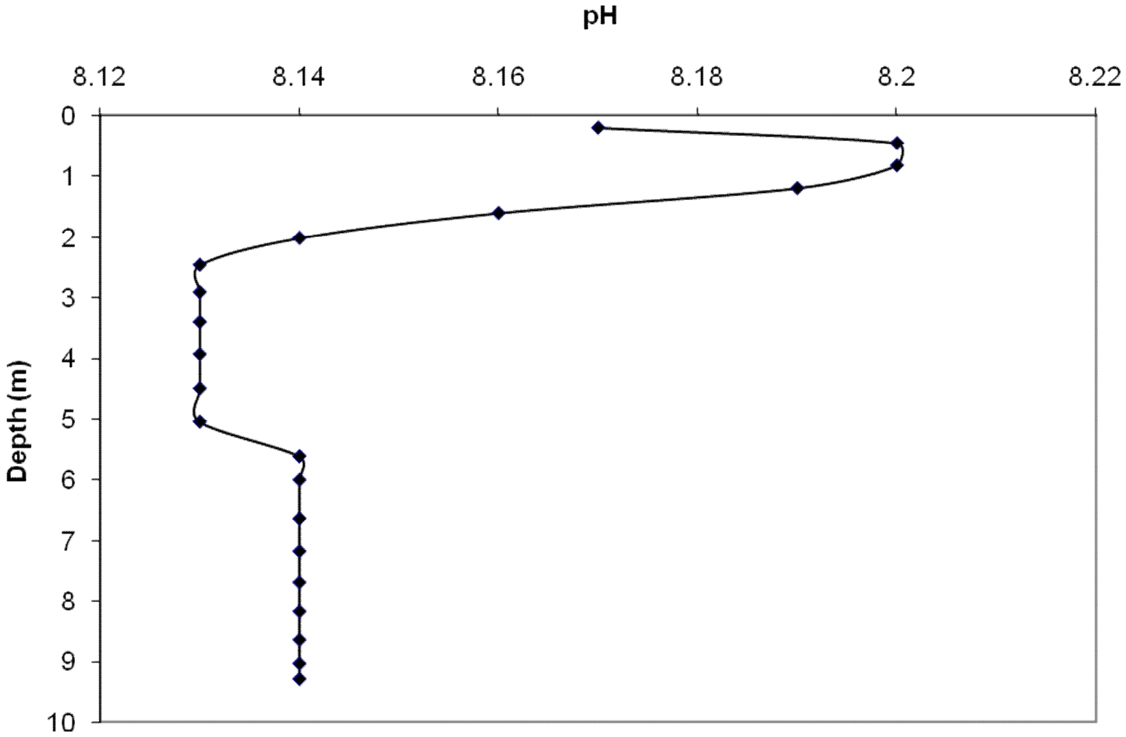

Fig 2.26.. pH versus Depth at Emerald

Temperature in Emerald decreased from 25.28°C at the surface to 24.52°C at 9.4 m. In contrast, salinity increased from 34.94 ppt at 0 m to 34.31 at 1.2 m before making a slight decrease to 34.25 at 1.6 m and then gradually increasing to 34.54 ppt at 9.4 m. Dissolved oxygen behaved similar to temperature, decreasing rapidly in the first 5 m (6.77 to 5.57 mg/l) before roughly stabilizing for the final 4.4 m. As with some of the previous sites, pH ranged from 8.13 to 8.2 which is within the error of measurement for the probe and so conclusive patterns could not be determined.

Nutrient Analysis

In order to detect levels of nutrient enrichment in the cave pools, samples were analyzed from surface and bottom waters. The following table includes the averaged concentrations of nutrients and bacteria measured in each pool: nitrate, nitrite, ammonium, urea, phosphate, and *Enterococcus* (Table 2.1). Surface samples were collected from 5 cm (2 in) below the surface from the center of the pool. Samples were also taken at the maximum depth of each pool 15 cm (6 in) from the bottom.

Of the six caves, Cliff Pool and Grenadier had the highest levels of nitrogen and phosphate. Cliff Pool had higher levels of nitrate (137 uM), nitrite (3.3 uM), ammonium (2.2 uM), and phosphate (1.3 uM) in surface waters than any other site. Grenadier had higher levels of nitrate (31.6 uM) and phosphate (0.79 uM) in surface waters relative to Calabash, Harbour Pool, Deep Blue, and Emerald but lower levels than Cliff Pool. Grenadier had higher levels of nitrate (81.2 uM), nitrite (1.1 uM), ammonium (1.3 uM), and phosphate (0.82 uM) in its deepest waters than all other sites.

Table 2.1. Mean concentrations (uM) nutrients from analyzed water column data. Samples were collected in June and July 2007 from the six experimental sites.

Site	Depth (m)	Nitrate (NO_3^-) (uM)	Nitrite (NO_2^-) (uM)	Ammonium (NH_4^+) (uM)	Urea ($(NH_2)_2CO$) (uM)	Phosphate ($HPO_4^=$) (uM)	N:P Ratio[1]
CB							
	0	4.1 (± 0.30)	0.20 (± 0.06)	0.82 (± 0.21)	1.6 (± 0.47)	0.31 (± 0.04)	14:1
	1.3	4.4 (± 0.13)	0.18 (± 0.01)	0.98 (± 0.21)	1.0 (± 0.01)	0.43 (± 0.02)	11:1
HP							
	0	1.3 (± 0.36)	0.41 (± 0.24)	4.0 (± 2.84)	2.7 (± 1.79)	0.33 (± 0.07)	5:1
	1.2	0.57 (± 0.02)	0.15 (± 0.02)	0.61 (± 0.19)	0.94 (± 0.03)	0.23 (± 0.01)	3:1
G							
	0	31.6 (± 0.30)	0.98 (± 0.02)	0.48 (± 0.04)	1.4 (± 0.02)	0.79 (± 0.01)	41:1
	3.1	81.2 (± 0.48)	1.1 (± 0.02)	1.3 (± 0.11)	0.95 (± 0.06)	0.82 (± 0.01)	100:1
CP							
	0	137 (± 1.57)	3.3 (± 0.07)	2.2 (± 0.05)	1.3 (± 0.02)	1.3 (± 0.04)	108:1
	7.9	8.4 (± 0.26)	0.19 (± 0.02)	1.1 (± 0.17)	1.2 (± 0.01)	0.44 (± 0.05)	20:1
DB							
	0	7.1 (± 0)	0.09 (± 0.01)	0.79 (± 0.61)	1.0 (± 0.05)	0.37 (± 0.01)	19:1
	8.9	7.6 (± 0.09)	0.09 (± 0)	0.77 (± 0.34)	0.91 (± 0.08)	0.34 (± 0)	23:1
E							
	0	2.3 (± 0.14)	0.18 (± 0)	0.54 (± 0.22)	0.91 (± 0)	0.25 (± 0.01)	10:1
	9.0	4.3 (± 0.08)	0.20 (0.02)	0.69 (± 0.18)	0.95 (± 0.02)	0.28 (± 0.01)	16:1

[1] Nitrogen in N:P ratios calculated at Nitrate + Nitrite.

One-way ANOVAs were used to determine which nutrients showed statistically significant variation between the reserve and urbanized sites (Table 2.2). A significant level of p = 0.05 was set. From surface water samples, only nitrate (p=0.042), nitrite (p=0.042), and phosphate (p=0.046) were found to differ statistically between the two groups of sites. From samples collected at the maximum depth of each pool, none of nutrient concentrations were found to be statistically significant at the 0.05 level, although the levels of significance for nitrates (p=0.055) and nitrite (p=0.078) were less than the 0.10 level (Table 2.2). The maximum depth at each of the pools was different, making the comparison at this variable depth difficult. Based on this analysis, Grenadier and Cliff Pool, the sites most closely correlated with urbanized development, show the highest level of nutrient enrichments. In contrast, Emerald had levels similar to the unpolluted sites of Harbour Pool and Calabash, which were located in the reserves. Construction has not occurred directly around this cave pool nor has there been heavy traffic around this site. Deep Blue’s nutrient levels fall between these two groups. Some nutrients such as nitrite were comparable to Emerald, Harbour Pool, and Calabash, while nitrate levels were similar to Grenadier and Cliff Pool.

Table 2.2. One-way ANOVA results for nutrient data. Analysis compared nutrient levels between the three reserve sites and the three urbanized sites.

	Surface		Maximum Depth	
	F Value	P-Value	F Value	P-Value
Nitrate	5.436	0.042	4.727	0.055
Nitrite	5.404	0.042	3.869	0.078
Ammonia	0.318	0.585	1.502	0.248
Urea	0.723	0.415	1.523	0.245
Phosphate	5.190	0.046	2.760	0.125

Nitrogen to phosphate (N:P) ratios were calculated to determine if cave pools were either nitrogen or phosphate limited (Table 2.1). Nitrogen refers to the sum of nitrate and nitrite for this analysis. Cliff Pool showed severe phosphate limitation in surface waters (108:1), while G had phosphate limitation in waters at the maximum depth (100:1). Nitrogen was extremely limited in the surface (5:1) and at the maximum depth (3:1) of HP. Less extreme nitrogen limitation was measured in the surface water of CB (14:1), DB (19:1), and E (10:1) and the maximum depth waters of CB (11:1) and E (16:1).

Bacterial Analysis

The bacterial samples were only collected for surface waters during June and July 2007 (Table 2.3). The EPA one reading exposure limit for moderate full body contact is 158 CFU/100 ml (U.S. EPA 2003). People occasionally swim or dive in the Bermuda cave pools. This type of exposure most accurately reflects the typical use of these pools. *Entercococcus* levels exceeded the EPA standard at the two urbanized sites, Grenadier (>=500 CFU/100 ml) and Cliff Pool (218 CFU/100 ml), and were just below the standard at the reserve site, Deep Blue (152 CFU/100 ml). Based on these findings, half of the cave pools studied show high fecal bacteria levels that are not safe for recreational use by swimmers and divers.

Table 2.3. Concentration (CFU/100 ml) of the bacteria, *Enterococcus*, in surface waters. Samples were collected in June and July 2007 from the six cave pool sites.

Site	*Enterococcus* (CFU/100 ml)
CB	7
HP	1
G	>=500
CP	218
DB	152
E	6

PCA Analysis

Relationships among sites based on salinity, temperature, surface bacterial levels and nutrient levels are plotted for Principle Component Axes 1 and 2 (Fig. 2.27). Vectors point toward increasing values for an environmental variable. Location of a site relative to vector arrows showed which water quality variables were positively or negatively associated with each cave pool site. Longer arrows indicated an increase in variation within the variable. Cave pools Emerald, Calabash, and Harbour Pool all cluster close together in the same quadrant implying they have similar relationships to salinity, temperature, and nutrients. Deep Blue, Cliff Pool, Grenadier all fell in different quadrants, illustrating their unique correlations with the water quality variables. Interestingly, all the cave pools located in the Walsingham Trust Nature Reserve did not cluster together, nor did those caves perceived to be possibly polluted. This diagram demonstrates the diverse aquatic environments that exist between cave pools.

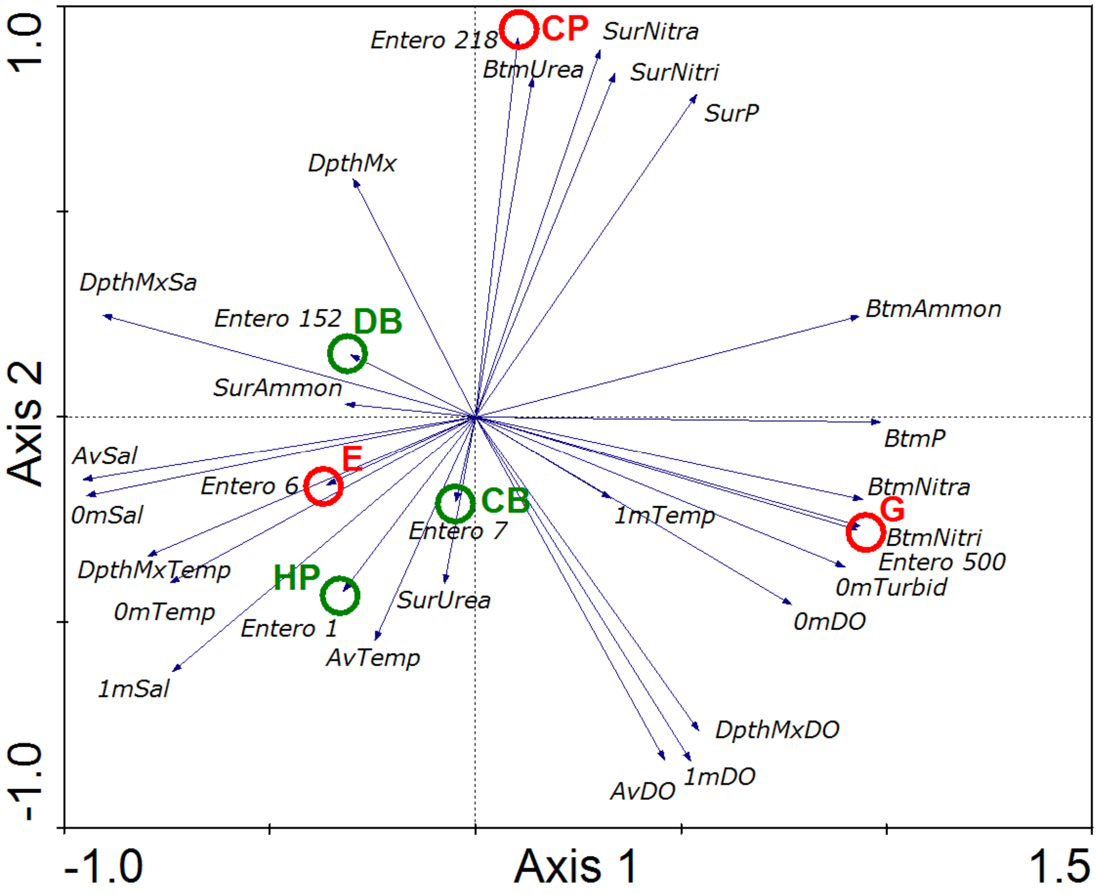

Fig 2.27. Principle Component Analysis of Water Parameters at the Six Cave Pool. The output shows which environmental and background nutrient levels are positively and negatively correlated with one another. Sites plotted post-hoc as supplemental variables to visualize these relationships among sites. Axis 1 explains 40.7% of the variation at each site, while Axis 2 explains 27.1%. Nature reserve sites are displayed in green. Urbanized sites are shown in red.

Discussion

The karst topography of Bermuda provides a direct link between the human activities on the surface and underlying groundwater and cave systems. Seepage from cesspits, boreholes, and unlined land fills allow sewage, detergents, pharmaceuticals, and other contaminants to enter the cave systems through underground passages and fissures and transport them for considerable distances with little or no filtration (Simmons *et al*. 1985; Simmons and Lyons 1994, Glasspool 2007). The pools located further inland have reduced tidal amplitude (33-87% reduction) and long time delays (up to 171 minutes), which indicates there is restricted connection with the sea (Iliffe 2000, Iliffe 2003). These areas tend to have the least amount of mixing and thus the longest residence time of the water masses (Iliffe 2000). Thus, groundwaters high in nutrient content that seep through the soil and limestone could provide a significant cumulative nutrient source in the confined pools.

Based on the nutrient analysis in this study, all the cave pool nutrient levels exceeded the values typically measured in Bermuda's inshore waters (Bondungen *et al.* 1982, Jickells & Knap 1984), but fell within the ranges of the fresh and saline groundwater (Iliffe *et al.* 1984, Simmons & Lyons 1994). The inorganic nitrogen within the cave pools was dominated by nitrate. In comparison, concentrations of ammonium, nitrite, and urea were much lower than that of nitrate due to the conversion of these nitrogen compounds during the process of nitrification. High levels of nitrates have continually been documented in the fresh and saline groundwaters of Bermuda (Iliffe *et al.* 1984, Thomsen & Foster 1986, Rowe 2005). Cesspits are the primary source of this organic nitrogen and form when organic waste breaks down in the pits (Simmons & Lyons 1994, Rowe 2005). With Bermuda's ever increasing population, construction of new homes continues to increase the number of cesspits. As elevated levels of nitrates are associated with cesspits, nutrient concentrations in groundwater will follow population trends and densities (Rowe 2005). Although this study did not directly link the high levels of nitrates in the urbanized site, Grenadier and Cliff Pool, to nearby cesspits, it appears they are the primary point of contamination as other natural sources of nitrogen could not produce these concentrations. From this study it can by hypothesized that groundwater seepage and nutrient contamination of cave systems will continue to rise as additional homes are built. Currently, nitrate levels in Emerald are similar to those found within the reserve pools. Once a residence is built on this property, nitrate levels can be compared to this baseline to help evaluate and further support this hypothesis.

Capone and Bautisa (1985) found that groundwaters adjacent to areas undergoing rapid population growth and urbanization are often enriched with nutrients that through seepage can enrich nearby coastal ecosystems. In Bermuda, it was found that nutrients slowing seeping into Harrington Sound (an inshore body of water in Bermuda that has little flushing and residence times estimated at 130 days) from enriched groundwaters caused a massive bloom of *Cladophora prolifera* (Lapointe & O’Connell 1989). Marine plants and animals in Bermuda have evolved under relatively poor nutrient conditions and may be particularly vulnerable to changes. Large inputs of nutrients into Bermuda’s inshore and near shore waters could upset the balance of the ecosystems (Sterrer & Barnes 1982, Simmons & Lyons 1994). Areas where dilution is slow and residence times are long may be more heavily impacted.

Nitrogen added to the marine environment is a primary cause of eutrophication (Lapointe *et al.*1990). This excess nitrogen contributes to changes in marine communities. Phytoplankton and opportunistic algae have the ability to utilize excess nutrient more rapidly other marine autotrophs. The blooms cased by these phytoplankton and algae can create large volumes of biomass that block out light for photosynthetic organisms occurring below the bloom. Flora which favor nitrogen rich waters displace seagrasses, corals, and other, less opportunistic algae (Lapointe & O’Connell 1989, Valiela *et al.* 1997, McGlathery 2001, Diaz *et al.* 2002, Armitage *et al.* 2005). When plants die, bacteria use oxygen to decompose the dead matter. If the rate of oxidation exceeds the amount of oxygen present, the environment can quickly become depleted of oxygen. The submerged caves are particularly susceptible due to the limited amount of dissolved oxygen present (Iliffe & Bishop 2007). Lack of oxygen, which may generate toxic levels of hydrogen sulfide, can kill fish, cave stygobites, and other marine organisms.

Concentrations of fecal bacteria can be used to establish that sewage is present in the aquatic environment. *Enterococcus*, a bacteria that has evolved to deal with the higher salinities of sea water, can be used to identify waste from warm blooded mammals. In Bermuda, there are no native wild mammals so these bacteria must be originating from anthropogenic sources. Groundwater that has been contaminated with sewage contains potentially infectious human pathogens. Although *Enterococcus* itself is not an infectious agent, it is strongly correlated with gastrointestinal illness, skin rashness, ear infections, and respiratory problems in people that come in contact with contaminated waters. In Bermuda, cesspits located in older limestone layers with greater fissure development, do not filter out the fecal contaminants as they were designed to do (Thomson & Foster 1986). All of the cave pools in this study were located within the oldest limestone, the Walsingham Formation. The one-time samples collected during this study already exceed the U.S. EPA standards of 158 CFU/100 ml in Grenadier and Cliff Pool. These results support the hypothesis that contamination from sewage is entering the cave systems. The primary source is believed to be coming from cesspit seepage as other mammalian sources are not present on Bermuda. In addition, levels were very close to the one-time EPA standard at the reserve pool, Deep Blue. With additional samples, levels would probably exceed the geometric mean standard of 35 CFU/100 ml. This result suggests that pools not directly located in urbanized areas can still be threatened by sewage contamination and nutrient enrichment due to lateral transport of sub-surface waters in subterranean fissures and passages.

As with the nutrient analysis, the vertical profiling of different environmental water parameters in each cave pools showed that while pools may have some similar individual characteristics, they each have their own individual trends. Water temperature did not show much variation among pools, but salinity showed two distinct patterns. In four of the pools, salinities were fully marine and fairly homogenous throughout the water column, but the other two pools had a distinct brackish layer on top of the marine waters. These two patterns are common in the anchialine caves of Bermuda; highly stratified waters in these pools result from the absence of wind and wave induced mixing (Iliffe *et al.* 2000). Similar variations between sites were observed in a study of Bermuda's ponds; researchers found that each pond had different salinity and temperature patterns that were heavily influenced by the different ocean connections. The also found that they were both somewhat influenced by the season (Thomas *et al.* 1991). The fairly uniform temperatures observed in this study could have resulted from measurements only being made during the summer months. The study of the ponds recorded the lowest temperatures during the winter months of January and February, while the highest temperature occurred during July and August (Thomas *et al.* 1991). Dissolved oxygen showed different trends at various sites. At some locations, it declined with depth, while others had spikes in the middle of the water column, and still another had increased levels at deeper depths. In the cave systems of Bermuda, Iliffe et al. (2000) found dissolved oxygen was typically greatest in the surface waters, dropped off sharply at the halocline (if one is present) and remained fairly constant through depth. In contrast, Gibbons (2003) found that the dissolved oxygen in different cave systems had highly variable patterns as was found in this study. It is believed that the amount of water exchange with the inshore bodies have a strong influence on the type of pattern observed. The pH showed to be just as variable, with levels increasing with depth at some sites, but decreasing at others. This variability is consistent with that seen throughout the cave systems of Bermuda (Iliffe *et al.* 2000). The profiles presented in this study present new data that help show the characteristic water chemistry and hydrology present within the entrance cave pools.

It should be noted that the karstic environment that compose the cave pools creates a complex network of minute cracks and fissures as well as larger conduits. The location of cave pools in close proximity to highly urbanized areas can only partially act as an indicator of water pollution. Each pool had varying, but mostly unknown connections to other water bodies. Some had extensive, large submerged passages to other pools, while others had short connections to the open ocean or very small cracks and fissures with unknown connections. Cliff Pool and Grenadier have higher levels of nitrate, nitrite, ammonium, and phosphate as was initially predicted based on their proximity to urbanized development, but Emerald, although located directly off a major road in Bermuda in close proximity to many private homes, had very low nutrient levels similar to those found at Calabash and Harbour Pool located in the nature reserves. Neither construction nor heavy traffic has occurred directly around Emerald. Deep Blue's nutrient levels fall between the two groups with nitrate levels similar to Grenadier and Cliff Pool. Hence, sites that are labeled as "pristine" or "polluted" on first examination due to location and visible disturbances might actually have very different characteristics on closer inspection and water quality analysis.

CHAPTER III

ALGAL GROWTH AND NUTRIENT EXPERIMENTS

Introduction

Macroalgae as Bioindicators

Macroalgae are autotrophic, photosynthesizing organisms at the base of the trophic food web. As such, they rely directly on sunlight and nutrients in their environment for survival, growth, and reproduction. Algae were first studied for their value as bioindicators in the mid 19th century by Ferdinand Cohn (Dokulil 2003). Cohn was one of the first botanists to study the life histories of certain genera of algae as well as growth and contents of algal cells in relation to water analysis. Since that time, algal species from around the world and from a variety of habitats have been tested and used as indicators of waste water, trace metal and other forms of pollution (Dokulil 2003, Quigg *et al.* 2008a,b).

There are a number of advantages for using macroalgae over other organisms in the aquatic environment as bioindicators. Macroalgae are sessile making them easy to collect and observe. They can be used to characterize a specific location over time. In near shore waters, relative abundances of species can be quantified using aerial photography and/or ground surveys. Due to their prolonged presence at a particular site, they can reflect environmental conditions over extended periods (Dokulil 2003). They are very sensitive to pollution and nutrient enrichment. In eutrophic waters, they show rapid and quantifiable morphological changes (Karez *et al.* 2004). With these advantages, studies have been used to determine if the presence of certain algal species provide information about the water quality parameters in which they live. Macroalgae have been used to study nutrient effects on growth, productivity, and morphology. Studies on macroalgae have utilized opportunistic, fast growing, foliose species with large surface to area ratios that show rapid changes to nutrient input (Rosenberg 1984, Fong *et al.* 1998, Diaz *et al.* 2002). *Ulva*, *Enteromorpha*, *Cladophora*, *Gracilaria*, *Codium*, *Fucus*, *Ascophyllum*, *Laminaria*, and *Macrocystis* have all shown promise as indicators of water quality in areas of nutrient enrichment (Costanzo *et al.* 2000, Dokulil 2003, Karez *et al.* 2004, Lapointe *et al.* 2005).

Various techniques and assays have been developed over the past few decades to measure quantifiable changes in macroalgae in response to nutrient levels. Each aquatic environment has different ecosystem dynamics. Due to this diversity, specialized procedures have been established for use in specific environments. Assays that measure growth and productivity relative to water column nutrients have proven useful in enclosed settings such as lakes and ponds (Fong *et al.* 1998). However, these same assays have produced inconclusive results when used in systems with pulsed, mixed waters such as estuaries and coastal ecosystems (Fong *et al.* 1998, Fong *et al.* 2001, Lapointe *et al.* 2005). In the latter systems, concentrations of nutrients in the tissues have been useful for detecting nutrient enrichment. *In situ* experiments have utilized both local, naturally occurring species as well as cultured specimens, while laboratory conducted experiments make use of only cultured species. Each experimental type has its own advantages and disadvantages, but combining multiple bioindicators increases the power of an individual assay (Cohen & Fong 2006).

Purpose

The objectives of this chapter were to determine the effects of nutrient enrichment on naturally occurring algal species growing in Bermuda's cave entrance pools and determine if they could act as bioindicators of cave pool health. Based on the environmental conditions, it was hypothesized that all treatments with phosphorus enrichment would stimulate the positive growth of macroalgae given phosphorus limitation of Bermuda waters (Simmons *et al.* 1985, Lapointe & O'Connell 1989). It was also hypothesized that the most opportunistic, green algae would be the best indicator of cave pool enrichment based on previous surveys and enrichment studies conducted in marine waters (Díaz *et al.* 2002, Cohen & Fong 2006). This type of nutrient enrichment study had not been done previously in Bermuda cave pools.

Materials and Methods

Nutrient Enrichment Experiments

Nutrient addition experiments were conducted on macroalgae at the six cave pools to test for effects of nutrient enrichment. An initial survey of each pool was conducted to determine the presence and abundance of opportunistic, foliose species. Species occurring at multiple sites were selected preferentially, but not all species were found at all sites (Table 3.1). Specimens from each site were collected from a single depth and only intact plants were used. Plants were cleaned with filtered (0.45 µm), ambient water collected from the same depth as the plants to remove any resident fauna, epiphytes, and sediment. Initial wet weights (± 0.01 g) were determined after blotting plants for five minutes with paper towels.

Table 3.1. Presence and abundance of algal species utilized for nutrient addition and productivity experiments at each cave pool site. Species were selected after completion of initial surveys conducted in June 2007. The following scale was used to rank species abundance at each site: (0) absent, (1) rare, (2) few, (3) many, and (4) abundant. Species that exhibited abundances rated as a 3 or 4 were plentiful enough to conduct experiments.

Site	*Caulerpa mexicana*	*Caulerpa racemosa*	*Caulerpa sertularioides*	*Ulva* sp.	*Halymenia floresii*	*Cryptonemia* sp.
CB	2	4	1	3	1	0
HP	1	4	2	3	1	0
G	0	0	4	0	0	0
CP	4	0	4	0	0	1
DB	4	3[1]	0	2	1	3
E	0	0	0	0	3	1

[1] Most individuals were >20 g. This size was too large to use in experiments due to the constraints of bottle size.

Seven individual plants of each species were placed in acid-washed, 1.89 L polyethylene terephthalate (PETE) bottles filled with filtered cave pool water. Each bottle was randomly assigned to one of four nutrient treatments: either addition of nitrate alone (30 µM), phosphate alone (2 µM), nitrate (30 µM) plus phosphate (2 µM), or no nutrient addition (control). All the experimental bottles were placed in an open mesh bag. Each bag was secured at the collection depth. The total wet weight of plants ranged from 1.4 to 10.1 g in the bottles at the start of the experiments. The change in biomass of individual plants was monitored and recorded as a wet weight. Each site was revisited over the course of six to twelve days. During each visit (at 2 day intervals), plants were blotted dry and weighed. Freshly collected cave water supplemented with respective nutrient was replaced in the bottles. A growth curve was plotted through each measurement period. When growth reached a plateau or the individual plant died, the experiment was terminated. At the conclusion of the experiment, a final wet weight was recorded.

Primary Productivity Experiments

Primary productivity was measured using the traditional light dark bottle method to determine gross and net photosynthesis and respiration (Strickland and Parsons 1972). This method was performed on the same species and site combinations selected for use in the nutrient addition experiments. Freshly collected macroalgal specimens were harvested immediately before the start of each experiment. All individuals from a single species were collected from a uniform depth, removed from any attached strata, and cleaned and weighed as described above. Only fully intact plant specimens were placed in individually numbered, duplicate, 0.74 L high density polyethelene (HDPE) bottles filled with filtered ambient water. One control bottle and two light bottles were placed in a mesh bag and anchored at the collection depth. Duplicate dark bottles were placed in doubled, black bags within a separate mesh sack, and secured within 1 m of the light bottles. The experiments began at 10:00 a.m. (-05:00 GMT). Initial DO was measured in the control bottle, a light bottle, and a dark bottle in mg/L with a calibrated YSI dissolved oxygen meter. The wet weight was recorded before being placed in each container. Each bottle was capped immediately. After 3-4 hours (1:00-2:00 p.m.), the DO was measured in all bottles and the plants reweighed. The DO meter had an upper limit reading of 16 mg/L and dictated the duration of the experiments.

An additional primary productivity experiment was run to determine the effects of site-specific light levels and background water-column nutrients on rates of photosynthesis and respiration. Two species of green algae and two sites were selected –

one species from site A and one from site B. Three combinations of water and light source were then established. A complete set of bottles were set up for each of the following combinations: (1) site A water located at site A or site B water located at site B, (2) site A water located at site B, and (3) site B water located at site A.

Gross and net productivities and the respiration rates for the plants were calculated using the following equations:

Gross Productivity = [Light Bottle (mL O_2/L) – Dark Bottle (mL O_2/L) / time in hours]
Net Productivity = [Light Bottle (mL O_2/L) – Initial Bottle (mL O_2/L) / time in hours]
Respiration Rate = [Initial Bottle (mL O_2/L) – Dark Bottle (mL O_2/L) / time in hours]

Once rates were calculated, the wet weights of the respective treatments were divided by the last number so that the findings could be expressed per wet weight.

Statistical Analyses

Variance partitioning was used according to Borcard *et al.* (1992) to quantify the variation among our response (dependent) variables (i.e., change in biomass of macroalgae on days 2, 4, and 6) that was related to each group of our explanatory variables (i.e., species, site, background environmental variables, and nutrient addition). Due to colinearity among some of explanatory variables, some of the variation observed in the biomass data could be simultaneously explained by multiple variables. Variance partitioning is similar to partial regression, in that by running multiple ordinations of our algal biomass data, with each run constrained by different groups of explanatory variables, the variance among the species biomass data could be quantified for each group. We calculated the unique variation explained solely by (a) the group of background environmental parameters, (b) macroalgal species identity, and (c) nutrient addition treatment, as well as the variation in algal biomass that could be equally explained (shared) among them.

The total percentage of explained variation in algal biomass among the samples was computed by running a Canonical Correspondence Analysis (CCA), a multivariate method of direct ordination (ter Braak 1986). This technique combines the use of weighted averages with multiple regression to determine a linear combination of explanatory values (the canonical axes). We used data matrices for algal biomass and explanatory variables in order to calculate the percentage of explained (i.e., the canonical eigenvalues were divided by the total variance, calculated as inertia of the biomass). The residual variation is unexplained. The variation uniquely explained by each group of explanatory was determined by running partial CCAs. In separate partial CCAs, each group of target explanatory variables was the only constraining matrix, while the other explanatory variables were included as covariables, thus removing the variation due to those other variables plus any variation that they shared with the target variables. F-tests of the canonical axes obtained by each partial CCA determined if the unique variance explained by the target group was significant ($P<0.05$). This method enabled the non-mutually exclusive explanatory relationships (i.e., those that overlap in nature and in out experimental treatments) to be separated into their individual components and quantified (Økland & Eilertsen 1994).

To visualize this shared variation in the algal biomass data that also was related to the alternative background nutrient parameters among our sites, but such that it did not directly influence the analysis, a CCA was run with the sites and background nutrient parameters as supplemental variables, whose post-hoc correlations with scores for biomass were determined by the constrained ordination. These were plotted separately along with biomass scores, for the same canonical axes constrained by the target explanatory variables. The background nutrient concentrations were $\log(x + 1)$ transformed to normalize their distribution. Monte Carlo simulations were run and F-tests of significance ($P<0.05$) values were used to test for variation in biomass that was due to algal species identity and nutrient treatment for the first canonical (major explanatory) axis, as well as for all the canonical axes combined.

Results

Nutrient Enrichment

The growth of the individual algal species with nutrient addition(s) across the six different cave pool sites is presented in Figure 3.1. Beginning biomasses (1-10 g) varied due to species morphology and natural species abundance at each site. To facilitate comparison, each individual's growth was normalized to one based on the initial wet weight. Standard deviation error bars are included to illustrate the variation in replicate response by individuals to treatments. Individual species experiments were run from six to twelve days. After six days, the different species either showed a plateau in positive growth or showed a rapid decline (negative growth). Therefore only days 0-6 are included in the graphs and statistical analysis.

Ulva sp. exhibited the largest positive increase in biomass over a six day period in the two locations it was tested. At Calabash similar patterns of biomass accumulation (defined as % increase) occurred with all three nutrient treatments (N 28.6%, P 18.5%, and NP 20.9%); the water column N:P ratio showed nitrogen limitation at this site. (Refer to Table 2.1 presented in Chapter II for a complete summary of the N:P ratios at each site.) At Harbour Pool, a larger response was shown by *Ulva* sp. with N addition (20.0%) than with P (10.8%) or NP (5.4%) addition. This parallels the low nitrogen levels compared to phosphorus levels in the water column of this pool. One of the foliose, red algal species, *H. floresii*, showed less substantial positive growth with all nutrient treatment levels demonstrating that the waters if Emerald are colimiting. This decrease in response by the red algae is consistent with the lower productivity rates of red algae when compared with green species.

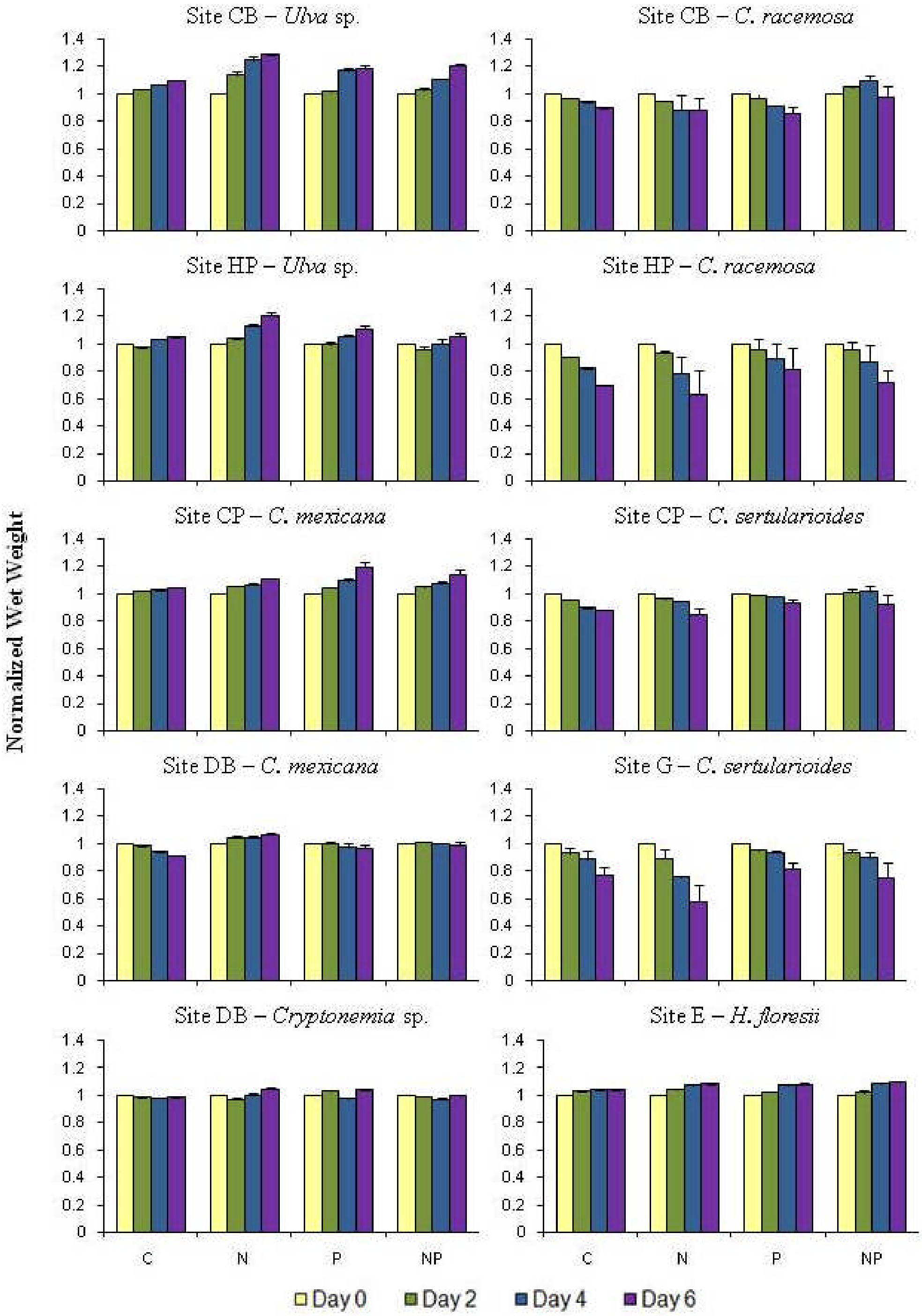

Fig. 3.1. Growth of Algal Species from Day 0 to Day 6 with Addition of No Nutrients (C), Nitrogen (N), Phosphorus (P), and Nitrogen and Phosphorus (NP) across Six Sites. The initial wet weight of each individual within a species ranged from 1-10 g, therefore the wet weights were normalized to their initial weight at day 0. Errors bars are standard deviations. In those cases where the error bars are not visible, standard deviations were less than 0.02.

For *C. mexicana* and *Cryptonemia* sp., little to no growth occurred with nutrient treatments. *C. mexicana* had a positive growth response to nutrient addition only at Cliff Pool, and most strongly to P addition (27.0%). Collection of this species occurred within the first 2 m and was correlated with the severe phosphate limitation in the surface waters. At Deep Blue, the both species of algae showed little to no response; *C. mexicana* had maximum growth of 8% with N addition and *Cryptonemia* sp. had maximum growth of 5.4% with N addition. The lack of response corresponded to the more preferable N:P ratio of the waters. *C. racemosa* and *C. sertularioides* did not respond well to he bottled nutrient experiments and exhibited a decline in biomass at all sites. Both species were similar in appearance between day 0 and 2, but quickly deteriorated by day 4.

Results for the CCA analysis supported the observed daily growth patterns (described above) of the algae. Canonical axis 1 (P=0.002) explained 51.5% of the variation, while canonical axis 2 explained an additional 1.6% of the variation. The difference in the biomass on days 2, 4, and 6 from day 0 appeared to be explained by nutrients and algal species (Fig 3.2). The grouping of the nutrient centroids close to the origin of axes 1 and 2 indicates a weak effect of the individual treatments on growth. At each site, the algae responded differently to each nutrient treatment. This outcome is supported by the highly variable N:P ratios measured at each site.

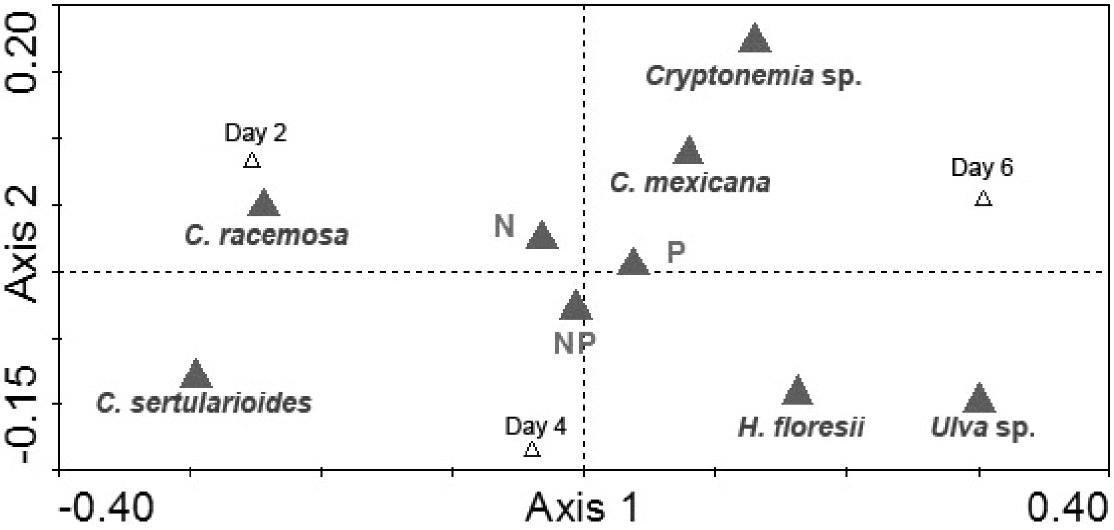

Fig. 3.2. Canonical Correspondence Analysis (CANOCO 4.5 CCA) for Nutrient Enrichment Experiments. Analysis was used determine which algal species and nutrient treatments were correlated with algal biomass on days 2, 4, and 6. Centroids closest to the origin indicate a weak association with the canonical axes. Axis 1 explains 51.5% of the variation at each site, while Axis 2 explains 1.6%. First canonical axis is significant (F-ratio = 67.93, P-value = 0.002).

Ulva sp. and *H. floresii* are most closely associated with days 4 and 6 (Fig 3.2). The significant relationship between these species with biomass matched their higher positive growth as the experiments progressed. In addition, the growth of *Ulva* sp. and *H. floresii* were related to the background water variables of ammonia, urea, and salinity (indicated by the environmental vectors in Fig. 3.3) Distribution of *Ulva* sp. and *H. floresii* along these parameter indicated that the higher biomass response in days 4 and 6 may not be solely attributed to species-specific responses. *C. mexicana* and *Cryptonemia* sp. were most correlated with days 2 and 6, but were not statistically significant. Although, these two algal species had their highest cumulative growth on day 6, they had a very small response. Due to this their relationships cannot be interpreted as strongly as *Ulva* sp. and *H. floresii*. *C. racemosa* and *C. sertularioides* had a significant correlation with biomass levels on day 2. Both species are positioned on the negative side of canonical axis one in the CCA analysis (Fig. 3.2, left side); this location is opposite of days 4 and 6, illustrating their decline in biomass as the experiments advanced.

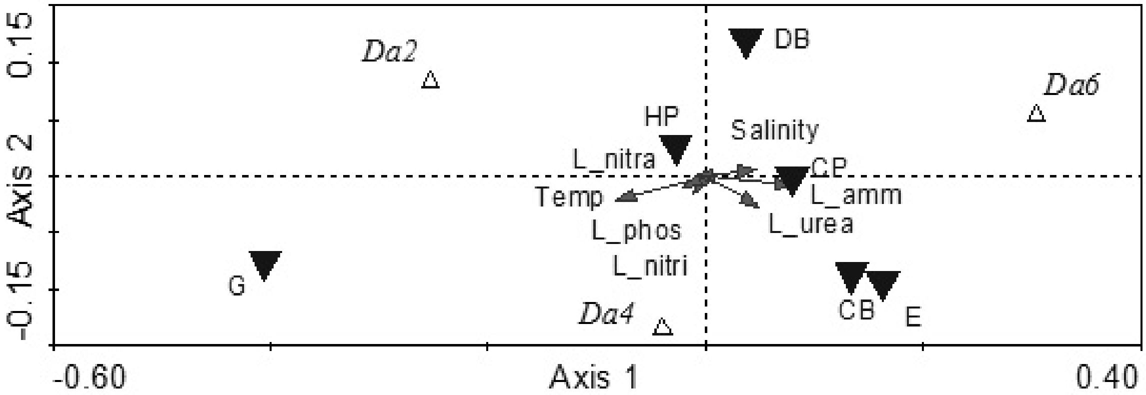

Fig. 3.3. Post-hoc Plot of Relationships due to Supplemental (Alternative) Explanatory Variables (Background Environmental Water Parameters and Cave Sites). Plotted on the same graph as presented in Fig. 3.2 for algal biomass on days 2, 4, and 6. The arrows indicate the direction of increasing value for the explanatory variables. Longer vectors signify a greater range of variation in the observed values for the variable. More acute angles between vectors and axes indicate a stronger correlation.

A statistical analysis of the variation in plant biomass response was to the three sets of explanatory variables (background environmental variables, algal species, and nutrient treatment) is summarized in Table 3.2. In the partial CCAs, our explanatory variables combined to explain 60% of the total amount of variation present within the biomass measurements. The remaining 40% of variation was unexplained and could be attributed to the many background variables not measured in the *in situ* experiment design. Of the explained variation, 44% was uniquely attributed among the three groups. The background environmental water variables, based on 15 parameters, uniquely explained 27% of the variation in the measurements and were significant at the level P=0.002. The algal species identity were significant at the level P=0.008 and uniquely explained 13% of the variation. The remainder of the unique variation, 4%, was explained by the nutrient treatments, but was not significant (P=0.320). These results were consistent with the results observed in the daily growth patterns of the algae (Fig. 3.1). The strongest (first) canonical axis (P = 0.002), explained 51.5% of the variation, whereas canonical axis 2 explained an additional 1.6% of the variation. The difference from day 0 for biomass on days 2, 4, and 6 was related most strongly to ambient nutrients and algal species (Fig. 3.2). As discussed above, the weak effect of the nutrient treatment is illustrated in the grouping of the centroids close to the origin of axes 1 and 2.

Table 3.2. Variance decomposition for changes in algal biomass that were correlated with nutrient treatments, background water chemistry, and algal species among sites.

	Eigeen Value (λ)	Percent of Variation Explained (%)	P-Value
Total Variation	0.005	100	-
Total Explained (Unique + Shared) Variation	0.003	60	0.001
Unique to Background Variables	0.001	27	0.002
Unique to Species of Algae	0.0007	13	0.008
Unique to Nutrient Treatment	0.0002	4	0.320
Residual Unexplained Variation	0.002	40	-

Primary Productivity

Patterns of the net and gross photosynthetic and respiration rates (Figs 3.4 and 3.5) were consistent with results of the nutrient enrichment experiment. Higher net photosynthetic rates typically were associated with species that also achieved the largest positive response to nutrient enrichment, while highest respiration rates corresponded to the species that actually lost biomass during the nutrient addition experiments. *Ulva* had the highest net photosynthetic rate (2.2 ml O_2 L^{-1} hr^{-1}) at Harbour Pool, as compared to Calabash (0.54 ml O_2 L^{-1} hr^{-1}). At Harbour Pool, the net photosynthetic rate for *C. mexicana* (0.70-0.89 ml O_2 L^{-1} hr^{-1}) was less than for *Ulva* but similar between the two cave pools. The additional productivity experiments with *Ulva* and *C. mexicana*, conducted in different natural light regimes and ambient nutrient levels (Fig. 3.5), showed similar rates for both species regardless of experimental conditions of cave and water source.

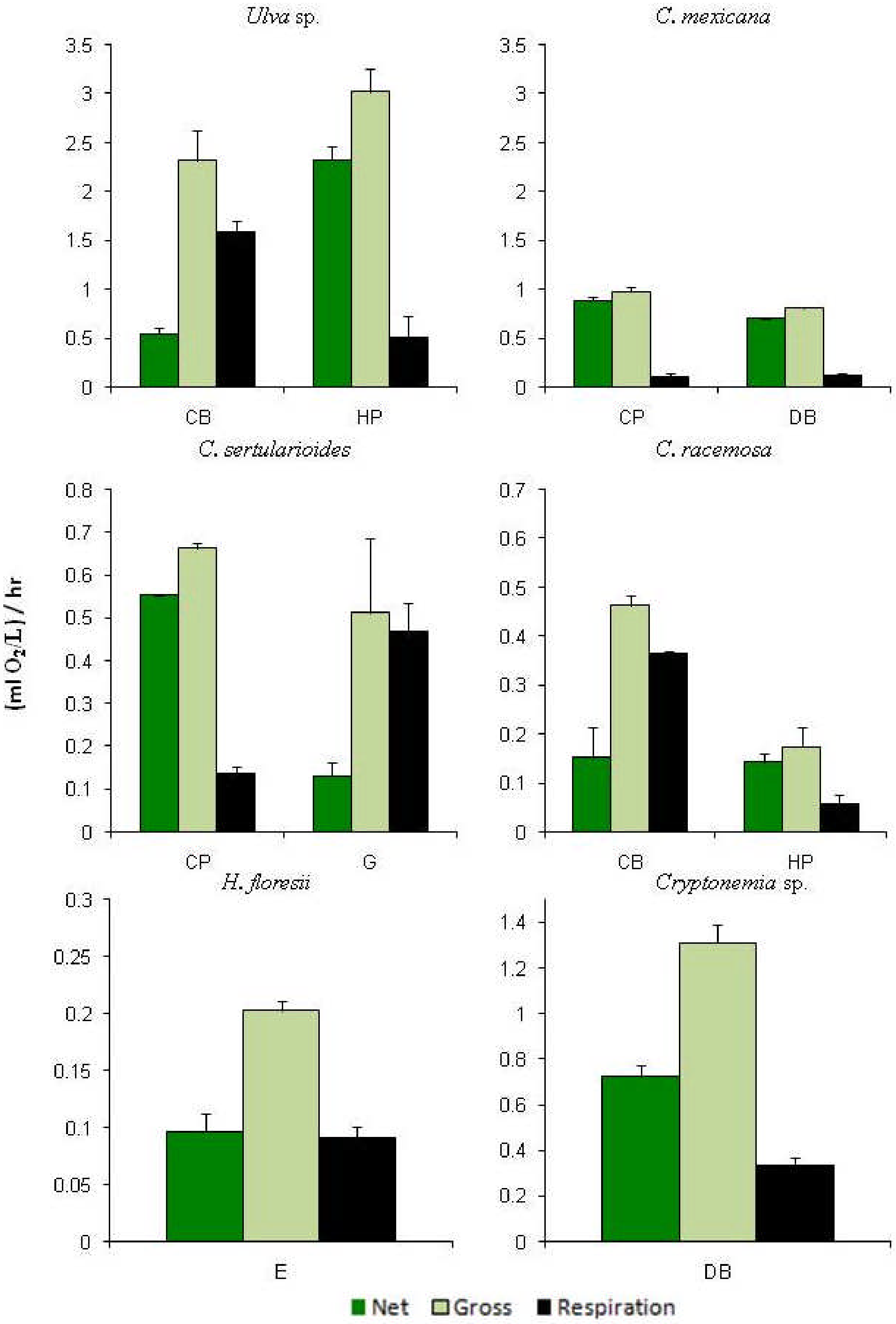

Fig. 3.4. Net Photosynthetic Rate (dark green bars), Gross Photosynthetic Rate (light green bars), and Dark Respiration Rate (black bars) for the Six Species of Algae Used for the Nutrient Addition Experiments. Initial and final dissolved oxygen concentrations were measured over a 3-4 hour period. To account for the slight variation in individual algae size, the individual rates were normalized by dividing the ml of O_2 per liter by the initial wet weight. Standard deviations are indicated by error bars. In those cases where the error bars are not visible, standard deviations were less than the following values: 0.02 for *Ulva* sp. and *C.mexicana* and 0.009 for *C. sertularioides* and C. *racemosa*.

Respiration rates were 35-250% of the net photosynthetic rates with the other four species studied. *C. sertularioides* (0.13-0.55 ml O_2 L^{-1} hr^{-1}) and *C. racemosa* (0.14-0.15 ml O_2 L^{-1} hr^{-1}) exhibited notably lower net photosynthetic rates as compared to the two other green algal species. *C. sertularioides* and *C. racemosa* exhibited very high respiration rates relative to the net photosynthetic rate (by more than 200% at Grenadier and Calabash) suggesting that the algae were severely stressed under these experimental conditions. The exception to this pattern was observed for *C. sertularioides* at Cliff Pool, which did not behave as expected, but rather it gained biomass with NP addition.

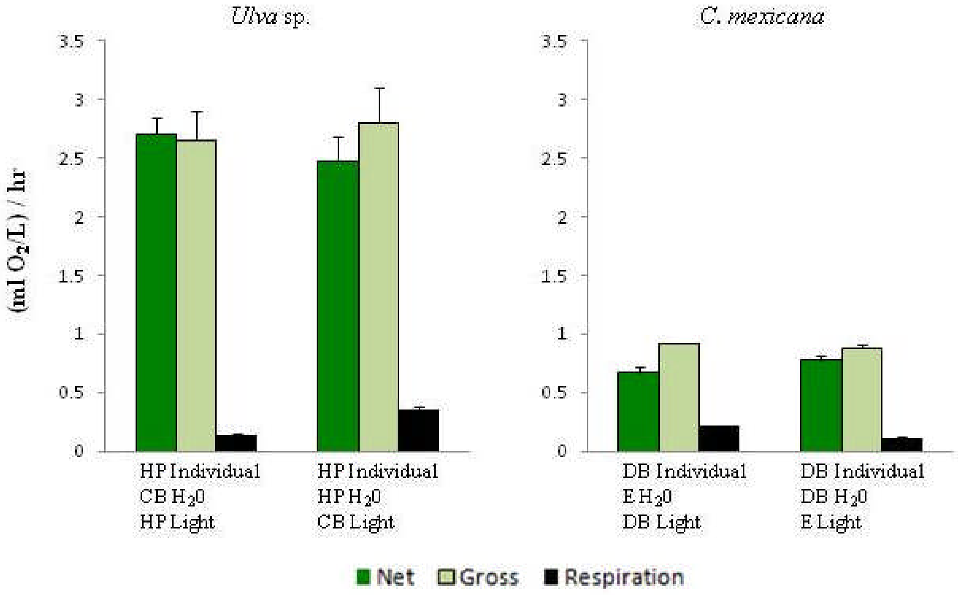

Fig. 3.5. Net Photosynthetic Rate (dark green bars), Gross Photosynthetic Rate (light green bars), and Dark Respiration Rate (black bars) for *Ulva* sp. and *C. mexicana* Using Different Combinations of Source Ambient Water and *in situ* Location. Initial and final dissolved oxygen concentrations were measured over a 3-4 hour period. To account for the slight variation in individual algae size, the individual rates were normalized by dividing the ml of O_2 per liter by the initial wet weight. Standard deviations, indicated by error bars, are included to illustrate the variation in replicate response to light of dark bottle treatments. In those cases where the error bars are not visible, standard deviations were less than 0.02.

Discussion

The results presented in this study support the hypothesis that opportunistic, foliose species of macroalgae can be used as indicators of nutrient enrichment due to their ability to quickly absorb nutrients for growth in marine environments (Duarte 1995, Valiela *et al.* 1997, Fong *et al.* 1998, Diaz *et al.* 2002). In these Bermudian cave pools, *Ulva* sp. and *H. floresii* could potentially act as bioindicators of water quality. While both species showed significant increases in biomass with nutrient addition, *Ulva* sp. had the most substantial growth due to its higher net photosynthetic rate. This genus has routinely been characterized in the literature as an opportunistic species with a high photosynthetic rate that proliferates in nutrient enriched waters (Waite & Mitchell 1972, Morand & Briand 1996, Viaroli *et al.*1996). Although, *C. mexicana* and *Cryptonemia* sp. did not produce significant responses to nutrient enrichment, they did accrue biomass and should be further studied. The results of this study show that even though red algae typically have a lower net photosynthetic rate compared to green algae, both phyla have species of algae that show a significant growth response to nutrient enrichment. Individual species within a phylum utilize nutrients differently based on their specific morphology and physiology; only species that exhibit a measurable response with nutrient enrichment can be utilized as bioindicators. This conclusion is supported by previous studies that have found when looking for macroalgal species that can act as bioindicators, organisms from all the different phyla of algae need to be considered (Morad & Briand 1996).

The measured growth responses to different nutrient treatments among algal species did not follow the hypothesized response under an assumption that waters in Bermuda are P-limited. Algal species in N, P, and N plus P nutrient treatments increased in growth at different sites. Nutrient water analysis showed that the waters within the cave pools had highly variable N:P ratios throughout the water column. For macroalgae, a N:P ratio range of 35-50:1 is optimal for growth (Atkinson & Smith 1983, Duarte 1992). The ratios within the cave pools deviated from this range so that some sites were N-limitation while other sites showed P-limitation. In a laboratory experiment, uptake of nutrients by a red alga were proportional to the surrounding water column concentrations, demonstrating that different N:P will elicit different responses in growth (Pedersen & Borum 1997). This illustrates the importance of evaluating each cave pool individually for nutrient availability. Sites whose waters are N-limited will have the most growth with N addition, whereas sites with P-limited waters will grow best with P addition.

Multiple abiotic and experimental factors could have led to the responses of *C. mexicana* and *Cryptonemia* sp. and the deaths of *C. racemosa* and *C. sertularioides*. The limitation of light can prevent primary producers from responding to nutrient enrichment. Algal species that are light-limited can increase their internal nutrient reserves, which could impact the results from a nutrient assay (Lapointe & Duke 1984, Duke *et al.* 1986). Cliff Pool and Grenadier, having surface brackish layers, were generally more turbid than the other cave pools. A red algal mat also covered a large portion of the *C. sertularioides* growing in Grenadier. Although, algal densities were high at these sites, the stress of the bottle environment coupled with a lower light intensity could have inhibited the growth of the algae.

Lack of a growth response to addition of N or P in known nutrient-limited waters at some of the cave pool sites suggested that another nutrient may have been influencing algal growth. Iron availability can limit algal growth, especially in carbonate sediments (Duarte *et al.* 1995). Levels of iron were not measured at any of the cave pool sites, but previous studies conducted in the inshore waters of Bermuda did not find levels of iron that would appear to limit the growth of algal (Morris *et al.* 1977).

Measuring a change in biomass and N:P ratios may have not been the most appropriate way to measure a response to nutrient enrichment and indicate nutrient supply to the macroalgae in the cave pools. Predictions of nutrient limitation based solely on N:P ratios may not apply uniformly to all primary producers within a community (Ferdie & Fourqurean 2004). Various species of algae can store excess nutrients in different areas; they can then utilize these nutrients to sustain positive growth despite low water column nutrient levels. Several studies have found that internal tissue nutrient content can be an indicatation of nutrient availability (Björnsäter & Wheeler 1990, Fong *et al.* 1998, 2001). Thus, in future investigations, the measurements performed in this study could be used in conjunction with tissue concentrations to determine nutrient effects on macroalgae.

The absence of a nutrient effect in some of the species could have been due to the ambient nutrients already present in the water of the cave pool. The concentrations of N and P addition used in this study may not have been sufficient enough to elicit a response by species over background levels in certain cave pools. This may explain why *C. mexicana* achieved a larger increase in biomass at CP but showed little response at DB. This finding is supported in other studies on macroalgae that have shown group-specific responses with significant variation between regions and sites (Armitage *et al.* 2005). Macroalgae are adapted to local habitats and thus have different thresholds for growth and nutrient storage (Dalsgaard & Krause-Jensen 2006).

A few additional effects due to experimental design should be noted as they may have influenced the data collected. The placement of the macroalgae in individual bottles created a microcosm environment for the algae that had slightly different characteristics that the actual environment in the pools. The capped bottles prevented continual gas exchange with surrounding waters. The algae would have utilized the fixed amount of dissolved oxygen within the bottles for photosynthesis. To help off set this effect, the bottles were uncapped every other day and new water was added as needed, but as the oxygen was not measured throughout the experiment, lower levels could have affected the growth rates of the species. The closed bottles would have also altered the turbulence of the water. Within the bottles the water was stagnant while anchored in the cave pools. This lack of movement could have altered the rate of nutrient uptake and also led to a decrease in dissolved oxygen levels within the bottle. The bottles also prevented grazers (such as snails and fish) from consuming the algae. Due to this the growth rates measured in this study may not translate to actual growth with nutrient enrichment of the pools. The bottles also had a fixed volume, 1.89 L in the nutrient enrichment experiments and 0.74 L in the productivity experiments. It has been found that bottle volume directly affects photosynthetic rates; larger volumes may be preferable to reduce the potential impact (Littler 1979). Multiple volume container sizes were not used in these experiments so their potential impact was not measured. Larger thallus weights can also produce lower photosynthetic levels and thus reduce growth (Littler 1979); to try to offset this effect initial wet weights were limited to 10 g. Productivity experiments were run with algae limited to 5 g and algae limited to 10 g; results were similar between the two size classes so the maximum limit of 10g was mostly likely sufficient for the size of the bottles used for these experiments. One additional effect was the overheating of the light dark bottles at Calabash. Due to its shallower depth, the bottles were very close to the surface compared to the other five cave pools. During the productivity experiments, the water temperature spiked 5°C within the containers, inducing a stress response. This resulted in the notably higher respiration rates of both *Ulva* sp. and *C. racemosa* at Calabash compared to Harbour Pool.

CHAPTER IV

FINAL SUMMARY AND CONCLUSIONS

The purpose of this study was to examine the response of marine plants in Bermudian cave pools in order evaluate the following hypotheses: 1) each pool has similar environmental water parameters and does not show any significant difference in background nutrient or fecal bacteria levels (Chapter II) and 2) biomass accumulation between algal specimens living in nutrient enriched water compared to those in waters without enrichment show no significant difference (Chapter III). Different instrumentation and experimental procedures were utilized to collect the data necessary to test these hypotheses.

Vertical Profiling of Environmental Water Parameters

Vertical profiles of temperature, salinity, dissolved oxygen, and pH were measured at all six of the cave pools (Chapter II). While the pools may have some similar individual characteristics, they each had their own individual trends (Fig. 2.27). Water temperature did not show much variation among pools (Fig. 2.3, 2.7, 2.11, 2.15, 2.19, and 2.23), because the measurements only being made during the summer months. In contrast, salinity showed two distinct patterns; waters were either fully marine (Fig. 2.4, 2.8, 2.12, and 2.24) or had a distinct brackish layer overlying the marine layer (Fig. 2.16 and 2.20). The variation that was seen in temperature seems to be linked the two different salinity profiles. In fully marine water, the temperatures were warmest at the surface and gradually cooled with depth, whereas in pools with the brackish layer, surface water was cooler down to the halocline where it sharply increased before decreasing with depth similar to the fully marine pools. The slightly cooler surface water was most likely due to the trapped rainwater in the less saline layer floating on top of the fully marine layer. Dissolved oxygen also showed different trends at various sites. At some locations, it declined with depth (Fig. 2.5, 2.13 and 2.25), while others had spikes in the middle of the water column (Fig. 2.9 and 2.17), and still another had increased levels at deeper depths (Fig. 2.21). These variations at different depths with salinity and dissolved oxygen could be influenced by their relative distance from the shoreline, the absence of wind and wave-induced mixing, and the amount of tidal mixing with ocean waters, which is heavily influenced by the different ocean connections at each site.

Nutrient and Bacterial Analysis

Water samples were collected at the surface and the maximum depth in each of the cave pools. They were analyzed for levels of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+), urea, and phosphate ($HPO_4^=$) (Chapter II). Statistical analysis showed that nutrient concentrations varied between the reserve and urbanized pools; nitrate, nitrite, and phosphate varied significantly among the surface waters, while none of the waters at maximum depth were significant at the 0.05 level (Table 2.2). More detailed comparison between the sites could not be done due to the low replication (i.e., only two samples per site per water level), but some observations could be made (Table 2.1). Inorganic nitrogen was dominated by nitrate. Elevated levels (>30 µM) were detected in the surface waters of Cliff Pool and throughout the water column at Grenadier. High levels of nitrates have continually been documented in the fresh and saline groundwaters of Bermuda (Iliffe *et al.* 1984, Thomsen & Foster 1986, Rowe 2005).

Although the experimental design used in this project did not determine the source of the nitrate in these two urbanized caves, it is believed that nearby cesspits were major contributing factors. In studies of the fresh groundwater, elevated levels of nitrates have been associated with cesspits, as nutrient concentrations in groundwater have followed population trends and densities (Rowe 2005). Low phosphate values (<2 µM) were detected at al of the sites. Phosphate concentrations in this study ranged from 0.25 to 1.3 µM, and were similar levels in the fresh groundwater (0.1-4 µM) and near shore waters (<2 µM) (Simmons & Lyons 1994, Morris *et al.* 1977). The low phosphate concentrations in Bermudian waters are attributed to phosphate uptake by the limestone forming calcium carbonate-phosphate minerals (Kitano *et al.* 1978, Freeman & Rowell 1981).

Fecal bacteria are present in the surface waters of Bermuda cave pools (Chapter II). The U.S. EPA’s recommendation for a one-time sample for moderate body contact is

158 CFU/100 ml. This level of contact is consistent with the swimming and diving activities that occur within the cave pool waters. While *Enterococcus* was detected at all six sites, high levels (>150 CFU/100 ml) were only detected at Deep Blue, Cliff Pool, and Grenadier (Table 2.3). The latter two sites had readings considerably higher than that recommended by the EPA. Although the levels at Deep Blue did not exceed the standard for moderate use, the level does exceed the recommendation for beach waters of 104 CFU/100 ml (U.S. EPA 1986). These high levels are indicative of sewage contamination from anthropogenic sources as no other significant mammalian source of effluent is present in Bermuda. Cliff Pool and Grenadier are located in highly urbanized areas, and although a definitive point source cannot be proven with this study, the likely source of contamination is cesspit seepage. Deep Blue, on the other hand, is located in a nature reserve suggesting that pools not directly located in urbanized areas can still be threatened by sewage contamination. Previous studies have found that the older limestone layers are unable to filter fecal bacteria due to lateral transport of sub-surface waters through subterranean fissures and passages (Thomson & Foster 1986). The one-time samples analyzed in this study, while only providing a snapshot, provide further support to this past study.

Nutrient Enrichment Experiments

Nutrient addition experiments were conducted on naturally occurring macroalgae in the pools to test for effects of nutrient enrichment (Chapter III). Six species of algae (*Ulva* sp, *C. racemosa*, *C. mexicana*, *C. sertularioides*, *H. floresii*, and *Cryptonemia* sp.) were studied at one of more sites (Table 3.1). Individuals within a species were assigned to one of four nutrient treatments: N addition, P addition, N and P addition, or no nutrient treatment. Among sites, the algae responded differently to the nutrient treatments due to the highly variable N:P ratios (calculated from the background water column nutrient levels in Table 2.1). At sites that were nitrogen limited, algae typically responded most positively to N addition, whereas at sites that showed phosphorus limitation in the water column, algae usually responded most positively to P addition (Fig 3.1).

The effects of three sets of explanatory variables (background environmental variables, algal species, and nutrient treatment) on the biomass response were examined with variance partitioning. Both the background nutrients and the algal species explained a significant amount of the variation in biomass measured (Table 3.2). This suggests nutrient enrichment can be detected through changes in growth of certain algal species. This corresponds with other studies that have found macroalgae can indicate nutrient enrichment in various aquatic systems (Duarte 1995, Valiela *et al.* 1997, Fong *et al.* 1998, Diaz *et al.* 2002).

In the Bermudian cave pools, higher positive growth was measured in *Ulva* sp. and *H. floresii*. With CCA analysis, a significant relationship was found between these two species and growth response (Fig 3.2). An additional post-hoc plot of the analysis also indicated their growth was related to the background water levels of ammonia, urea, and salinity (Fig. 3.3). This analysis coincides with the variance partitioning and indicates that the higher biomass response may not be solely attributed to species-specific effects. For *C. mexicana* and *Cryptonemia* sp., little to no growth occurred with nutrient treatments and did not show a significant relationship with biomass in the CCA analysis. Thus their relationships could not be interpreted strongly. *C. racemosa* and *C. sertularioides* did not respond well to the bottled nutrient experiments and exhibited a decline in biomass at all sites. Due to these results, it can be concluded that *Ulva* sp. and *H. floresii* have the potential to act as bioindicators for water quality in the cave pools. Further studies should be conducted with Since *C. mexicana* and *Cryptonemia* sp. did have a small biomass response, they should be further studied to determine their potential as bioindicators.

Primary Productivity Experiments

Primary productivity was measured for each of the six macroalgal species studied (Chapter III). The traditional light dark method was used to determine gross and net photosynthesis and respiration (Fig. 3.4). Patterns of these different rates were consistent with the findings of the nutrient enrichment experiments. *Ulva* sp. had the highest net photosynthetic rate regardless of ambient water or light conditions at different cave sites

(Fig 3.5). *C. mexicana* had a higher net photosynthetic rate compared to the other species, although had very little growth at Deep Blue. This could have been a result of the less extreme nutrient limitation in background levels at this site. This is consistent with previous studies have found that uptake of nutrients were proportional to water column nutrients (Pederson & Borum 1997).

The net photosynthetic rates of both red algal species and those of *C. sertularioides* and *C. racemosa* were notably lower net photosynthetic rates in comparison of the two other green algal species. For *C. sertularioides* and *C. racemosa*, respiration rates exceeded net photosynthetic rate by more than 200% at Grenadier and Calabash. This result suggested that the algae were under severe stress under the experimental conditions and could have lead to the decline in growth of these two species in the nutrient enrichment experiments.

Overall Conclusion

The cave systems in Bermuda are a unique aquatic habitat and are under increased threat from rising urbanization and tourism (Iliffe 1981, Glasspool 2007). Presently, this island group is one of the most densely populated countries on Earth (Census Office 2005). The islands, however, do not have sufficient legislation, management practices, and enforcement to deal with the pollution input so that caves are receiving an undocumented increase in nutrient and sediment loading (Glasspool 2007). Major threats to Bermudan caves include construction projects, limestone quarrying, water pollution (particularly cesspits and liquid waste injection boreholes) and dumping of trash and other wastes into sinkholes, but revenue from both tourism and quarrying take priority over conservation (Glasspool 2007).

The karstic terrain of Bermuda creates a complex network of minute cracks and fissures as well as larger cave conduits. Therefore, it is very difficult to determine sources of nutrient and bacterial contamination in the subterranean ecosystems. The location of cave pools in close proximity to highly urbanized areas can only partially act as an indicator of water pollution. This study collected data on water quality in cave pools to provide a baseline of conditions currently present in the pools. By filling in gaps of available data, researchers and the Bermudian government can more accurately asses and predict human impacts to the pools and cave system. This study also attempted to provide naturally occurring macroalgae species that can indicate decline in cave pool water quality due to nutrient enrichment. From the results, both *Ulva* sp. and *H. floresii* can be used by local scientists to continually monitor the entrances to many of the cave systems and provides a solid foundation for future research of finding bioindicators local to Bermuda.

Recommendations for Future Research

A number of questions were raised when conducting these experiments and analyzing the data. Further studies are recommended in order to better understand the questions. Temporal studies are needed to quantify the variation in light incidence, water chemistry, nutrient levels, and fecal bacterial levels in Bermudian cave pools to detect seasonal and annual patterns. Studies in the Florida Keys have found that groundwater and surface nutrient concentrations vary throughout the year due to different water flow regimes during the wet and dry seasons (Lapointe *et al.* 1990). Due to its location in the higher latitudes, Bermuda has more of a semi-tropical climate that does not have a rainy season, but some months are much wetter than others. These periods of higher rainfall could impact nutrient concentrations in the cave pools. Current data on caves is very limited and inadequate; there is not a clear baseline for which the government can develop action plans (Glasspool 2007).

This study has provided new data only on summer trends that occur between June and July. During these periods, nitrate and *Enterococcus* levels are high, but an annual standard level in the pools cannot be determined. Vertical profiles and nutrient analysis should be conducted monthly over at least a four to five year period. This prolonged duration would not only give a complete annual profile, but should also illustrate different trends in rainy and dry years. Additionally, more detailed spatial studies across the surface and depth of each cave pool should be conducted instead of just at a single point. It has not been determined if the contamination is from a single point source or multiple sources; certain areas of the pool could have more concentrated levels if they are nearest to a single point source. These types of studies will help locate any nutrient pockets existing within the pools. Each pool should be divided up into a grid. Water samples should be collected across the grid at 0.5 m increments from the surface waters to maximum depth and analyzed for nutrient and bacterial content. This would provide a more complete picture of the levels within the entire pool. Once these supplementary studies have been conducted, the knowledge base will be expanded so that adequate resource management and developmental plans can be further developed.

Additional work is needed to develop biological indicators of nutrient enrichment in Bermudian cave pools. At the present time, it has only been determined that certain species of macroalgae (*Ulva* sp. and *H. floresii*) show a quick response in growth after nutrient addition. *In situ* experiments cannot control for the wide range of environmental factors; the complex response by macroalgae to these factors may lead to a weak response to nutrient enrichment (Armitage *et al.* 2005). To avoid the complexity of the natural environment, laboratory experiments need to be conducted to investigate the effect of various physical and chemical conditions on the algae’s growth and tissue nutrient content. The conditions that should be studied include variations in light intensity, temperature, and nutrient loads. This would help determine the unique effects individual cave pools are imposing on the macroalgae. Additional temporal studies needed to be conducted to look at natural changes in community composition and species abundance. The strength of growth responses to nutrients may vary and be more pronounced during periods of the year when conditions are most favorable (Karez *et al.* 2004). A long term study would be able to determine species-specific seasonal fluctuations (Fong *et al.* 1998). Once these additional studies have been conducted, a uniform set of techniques may be developed to use the naturally occurring algae as an indicator of nutrient enrichment.

REFERENCES

Armitage A.R., Frankovich T.A., Heck Jr. K.L. & Fourqurean J.W. 2005. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. *Estuaries* 28: 422-434.

Atkinson M.J. & Smith S.V. 1983. C:N:P ratios of benthic marine plants. *Limnology and Oceanography* 28: 568-574.

Bertz J.H. 1960. Bermuda – A partially drowned, late mature, Pleistocene Karst. *GSA Bulletin* 71: 1729-1754.

Björnsäter B.R. & Wheeler P.A. 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenetrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). *Journal of Phycology* 26: 603-611.

Bodungen B.V., Jickells T.D., Smith S.R., Ward J.A. & Hillier G.B. 1982. *The Bermuda marine environment: the final report of the Bermuda inshore waters investigations 1975-1980.* Bermuda Biological Station for Research, Inc. Special Publication No. 18, St. George, Bermuda, 123 pp.

Bordcard D., Legendre P. & Drapeau P. 1992. Partialling out the spatial component of ecological variation. *Ecology* 73: 1054-1055.

Capone D.G. & Bautista M.F. 1985. A groundwater source of nitrate in nearshore marine sediments. *Nature* 313: 214-216.

Census Office. 2005. *Report on the 2000 census of population and housing.* Census Office, Hamilton, Bermuda. 296 pp.

Cohen R.A. & Fong P. 2006. Using opportunistic green macroalgae as indicators of nitrogen supply and sources to estuaries. *Ecological Applications* 16: 1405-1420.

Costanzo S.D., O’Donohue M.J.M & Dennison W.C. 2000. *Gracilaria edulis* (Rhodophyta) as a biological indicator of pulsed nutrients in oligotrophic waters. *Journal of Phycology* 36: 680-685.

Dalsgaard T. & Krause-Jensen D. 2006. Monitoring nutrient release from fish farms with macroalgal and phytoplankton bioassays. *Aquaculture* 256: 302-310.

Department of Planning. 1988. *The supply of building materials in Bermuda.* Discussion Paper Number 1, Department of Planning, Bermuda.

Diaz P., Lopez Gapp J.J. & Piriz M.L. 2002. Symptoms of eutrophication in intertidal macroalgal assemblages of Nuevo Gulf (Patagonia, Argentina). *Botanica Marina* 25: 267-273.

Dokulil M.T. 2003. Algae as ecological bio-indicators. In: *Bioindicators and biomonitors: principles, concepts, and applications* (Ed. by B.A. Markert, A.M. Breure & H.G. Zechmeister), pp. 285-328. Elsevier, New York.

Duarte C.M. 1992. Nutrient concentration of aquatic plants: patterns across species. *Limnology and Oceanography* 37: 882-889.

Duarte C.M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41: 87-112.

Duke C.S., LaPointe B.E. & Ramus J. 1986. Effects of light on growth, RuBPCase activity and chemical composition of *Ulva* species (Chlorophyta). *Journal of Phycology* 22: 362-370.

Ferdie M. & Fourqurean J.W. 2004. Responses of seagrass communities to fertilization along a gradient of relative availability of nitrogen and phosphorus in a carbonate environment. *Limnology and Oceanography* 49: 2082-2094.

Fong P., Boyer K.E. & Zedler J.B. 1998. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of opportunistic alga, *Enteromorpha intestinalis* (L. Link). *Journal of Experimental Marine Biology and Ecology* 231: 63-70.

Fong P.F., Kamer K., Boyer K.E. & Boyle K.A. 2001. Nutrient content of macroalgae with differing morphologies may indicate sources of nutrients for tropical marine systems. *Marine Ecology Progress Series* 220: 137-152.

Freeman J.S. & Rowell D.L. 1981. The absorption and precipitation of the phosphate onto calcite. *Journal of Soil Sciences* 32: 75-84.

Garrison T. 2002. *Oceanography: an invitation to marine science.* Thomas Learning, Inc, Stamford, CT. 554 pp.

Gibbons D.A. 2003. *An environmental assessment of Bermuda's caves*. M.S. Thesis, Texas A&M University, College Station, TX. 150 pp.

Glasspool A.F. 2007. *Proceedings of a workshop to develop a strategic action plan of research or Bermuda's caves.* BBP Special Publication 2007-001. Bermuda Biodiversity Project, Flatts, Bermuda. 47 pp.

Hearty P.J., Olson S.L., Kaufman D.S., Edwards R.L. & Cheng H. 2004. Stratigraphy and geochronology of pitfall accumulations in caves and fissures, Bermuda. *Quaternary Science Reviews* 23: 1151-1171.

Iliffe T.M. 1979. Bermuda's caves: A nonrenewable resource. *Environmental Conservation* 6: 181-186.

Iliffe T. M. 1981. Caves. In: *Bermuda's delicate balance: people and the environment* (Ed. by S.J. Hayward, V.H. Gomez & W. Sterrer), pp. 59-76. Bermuda Biological Station for Research, Inc. Special Publication 20. Bermuda National Trust, Hamilton, Bermuda.

Iliffe T.M. 2000. Anchialine cave ecology. In: *Ecosystems of the world* (Ed. by H. Wilkens, D.C. Culver, & W.F. Humphreys), pp. 59-76. Elsevier, New York.

Iliffe T.M. 2003. Submarine caves and cave biology of Bermuda. *NSS News* 61: 217-224.

Iliffe T.M. & Bishop R.E. 2007. Adaptations to life in marine caves. In: *Encyclopedia of life support systems (EOLSS)* (Ed. P. Safran), pp. 1-25. UNESCO, Eolss Publishers, Oxford, UK.

Iliffe T.M., Jickells T.D. & Brewer M.S. 1984. Organic pollution of an island marine cave from Bermuda. *Marine Environmental Research* 12:173-189.

Jaroslow G.E. & Tucholke B.E. 1994. Mesozoic-Cenozoic sedimentation in the Kane Fracture Zone, western North Atlantic, and uplift history of the Bermuda Rise. *Geological Society of America Bulletin* 106: 319-337.

Jickells T.D. & Knap A.H. 1984. The distribution and geochemistry of some trace metals in the Bermuda coastal environment. *Estuarine, Coastal and Shelf Science* 18: 245-262.

Jin G., Englande A.J., Bradford H. & Jeng H. 2004. Comparison of *E. coli*, *Enterococci*, and fecal coliform as indicators for brackish water quality. *Water Environment Research* 76: 245-255.

Karez R., Engelbert S., Kraufvelin P., Pedersen M.F. & Sommer U. 2004. Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. *Aquatic Botany* 78: 103-117.

Kitano Y., Okumura M. & Idogaki M. 1978. Uptake of phosphate ions by calcium carbonate. *Geochemical Journal* 12: 29-37.

Lapointe B.E., Barile P.J., Littler M.M., Littler D.S., Bedford B.J. & Gasque C. 2005. Macroalgal blooms on southeast Florida coral reefs I. Nutrient stoichiometry of the invasive green alga *Codium isthmacladum* in the wider Caribbean indicates nutrient enrichment. *Harmful Algae* 4: 1092-1105.

Lapointe B.E. & Duke C.S. 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* in relation to light intensity and nitrogen availability. *Journal of Phycology* 20: 488-495.

Lapointe B.E. & O'Connell J. 1989. Nutrient-enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: eutrophication of a confined, phosphorus-limited marine ecosystem. *Estuarine, Coastal, and Shelf Science* 28: 347-360.

Lapointe, B.E., O'Connell J.D. & Garrett G.S. 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. *Biochemistry* 10: 289-307.

Littler M.M. 1979. The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae. *Aquatic Botany* 7: 21-34.

McGlathery K.J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* 37: 453-456.

Morand P. & Briand X. 1996. Excessive growth of macroalgae: a symptom of environmental disturbance. *Botanica Marina* 39: 491-516.

Morgan W.J. & Crough S.T. 1979. Bermuda hotspot and the Cape Fear Arch. *Eos Trans America Geophysical Union* 60: 392-393.

Morris B., Barnes J., Brown F. & Markham J. 1977. *The Bermuda marine environment: a report of the Bermuda inshore waters investigations 1976-1977.* Bermuda Biological Station for Research, Inc. Special Publication No. 15, St. George, Bermuda, pp.120.

Mylroie J.E. & Carew J.L. 1995. Karst development on carbonate islands. In: *Unconformities in carbonate strata* (Ed. by D.A. Budd, A.H. Saller, & P.A. Harris), pp. 55-76. American Association of Petroleum Geologists, Tulsa, OK.

Mylroie J.E., Carew J.L. & Vacher H.L. 1995. Karst development in the Bahamas and Bermuda. In: *Terresstrial and shallow marein geology of the Bahamas and Bermuda* (Ed. by H.A. Curran & B. White), pp. 251-267. Geological Society of America Special Paper 300, Boulder, CO.

Økland R.H. & Eilertsen O. 1994. Canonical correspondence analysis with variation partitioning: some comments and an application. *Journal of Vegetation Science* 5: 117-126.

Palmer A.N., Palmer M.V. & Queen J.M. 1977. Geology and the origin of the caves in Bermuda. In: *Proceedings of the 7th International Speleological Congress* (Ed. by T. D. Ford), pp. 336-338. British Cave Research Association, Sheffield, England.

Pederson M.F. & Borum J. 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series* 161: 155-163.

Personné J.C., Poty F., Vaute L. & Drogue C. 1998. Survival, transport and dissemination of *Escherichia coli* and enterococci in a fissured environment, study of a flood in a karstic aquifer. *Journal of Applied Microbiology* 84: 431-438.

Por F.D. 1985. Anchialine pools – comparative hydrobiology. In: *Hypersaline ecosystems* (Ed. by G.M. Friedman & W.E. Krumbein), pp. 136-144. Ecological Studies 53, Springer-Verlag, New York.

Quigg A. 2008. Ecological stoichiometry: trace elements. In: *Encyclopedia of ecology* 1st version (Ed. by S.E. Jorgensen & B.D. Fath), pp. 3564-73. Elsevier, Oxford.

Quigg A., Kevekordes K. & Phillips J.A. 2008. Ecophysiological status of 10 species of macroalgae and seagrasses examined in Moreton Bay (Queensland, Australia). *Memoirs of the Queensland Museum* (In press).

Redfield A.C., Ketchum B.H., & Richards F.A. 1963.The influence of organisms on the composition of sea-water. In: *The sea* V. 2 (Ed. by M.N. Hill), pp. 26-77. John Wiley & Sons, New York.

Reynolds P.R. & Aumento F.A. 1974. Deep drill 1972: potassium-argon dating of the Bermuda drill core. *Canadian Journal of Earth Sciences* 11: 1269-1273.

Rona P.R. & Richardson E.S. 1978. Early Cenozoic global plate reorganization. *Earth and Planetary Science Letters* 40: 1-11.

Rosenberg G. & Ramus J. 1984. Uptake of inorganic nitrogen and seaweed surface area:volume ratios. *Aquatic Botany* 19: 65-72.

Rowe M. 2005. *Ground water quality survey: Bermuda's fresh water lenses.* Department of Environmental Protection, Hamilton, Bermuda, pp. 15.

Simmons J.A.K., Jickells T.D., Knap A.H. & Lyons W.B. 1985. Nutrient concentrations in groundwaters from Bermuda: anthropogenic effects. In: *Planetary ecology* (Ed. by D.E. Caldwell, J.A. Brierly, & C.L. Brierly), pp.383-398. Van Nostrand Reinhold Company, Inc., New York.

Simmons J.A.K. & Lyons W.B. 1994. The ground water flux of nitrogen and phosphorus to Bermuda's coastal waters. *Water Resource Bulletin* 30: 983-991.

Smith J. 1624. (reprinted 1876). In: *Memorials of the Bermudas or Somer Isles 1515-1685, I* (By J.H. Lefroy), pp. 327-333. The Bermuda Library, Bermuda.

Sterrer W. & Barnes J.A. 1981. Sewage. In: *Bermuda's delicate balance: people and the environment* (Ed. by S.J. Hayward, V.H. Gomez & W. Sterrer), pp. 266-276. Bermuda Biological Station for Research, Inc. Special Publication 20. Bermuda National Trust, Hamilton, Bermuda.

Stock J.H., Iliffe T.M., & Williams D. 1986. The concept "anchialine" reconsidered. *Stygologia* 2: 90-92.

Strickland J.D.H. & Parsons T.R. 1972. *A practical handbook of seawater analysis*. Fisheries Research Board of Canada, Ottawa. 310 pp.

ter Braak C.J.F. 1986. Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67: 1167-1179.

ter Braak C.J.F. & Smilauer P. 1998. *CANOCO reference manual and user's guide to Canoco for Windows: Software for Canonical Community Ordination* (version 4). Microcomputer Power, Ithaca, NY.

ter Braak C.J.F. & Smilauer P. 2002. *CANONO reference manual and CanoDraw for Window's user's guide: software for Canonical Communitiy Ordination* (version 4.5). Microcomputer Power, Ithaca, NY.

Thimijan R.W. & Heins R.D. 1983. Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *HortScience* 18: 818-822.

Thomas L.H., Eakins K.E. & Logan A. 1991. Physical characteristics of the anchialine ponds of Bermuda. *Bulletin of Marine Science* 48: 125-136.

Thomas L.H., Logan A., Eakins K.E. & Mathers S.M. 1992. Biotic characteristics of the anchialine ponds of Bermuda. *Bulletin of Marine Science* 50: 133-157.

Thomas M.L. 2004. *The natural history of Bermuda.* Bermuda Zoological Society, Brimstone Media Ltd., Bermuda. 255pp.

Thomson J.A.M. & Foster S.S.D. 1986. Effect of urbanization on groundwater of limestone islands: an analysis of the Bermuda case. *Journal of the Institution of Water Engineers and Scientists* 40: 527-540.

Thomsen, T. 2008. Personal communication. Atlas Archaeology Lab, Madison, WI.

Todd D.K. 1959. *Ground water hydrology*. Wiley, New York. 336 pp.

U.S. Environmental Protection Agency (EPA) 1985. Test methods for *Escherichia coli* and *enterococci* in water by the membrane filter procedure (Method #1101.1), EPA 600/4-85-076. EPA Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency (EPA) 1986. Bacteriological ambient water quality criteria for marine and fresh recreational waters, EPA-440/5-84-002. EPA Office of Research and Development, Cincinnati, OH.

U.S. Environmental Protection Agency (EPA). 2003. Bacterial water quality standards for recreational waters (freshwater and marine waters) - status report, EPA-823-R-03-008. EPA, Washington, DC.

Vacher H.L. 1978. Hydrogeology of Bermuda – significance of an across-the-island variation in permeability. *Journal of Hydrology* 39: 207-226.

Vacher H.L., Bengtsson T.O. & C.N. Plummer. 1990. Hydrology of meteoric diagenesis: residence time of meteoric groundwater in island fresh lenses with application to aragonite-calcite stabilization in Bermuda. *GSA Bulletin* 102: 223-232.

Vacher H.L., Heary P.J. & Rowe M.P. 1995. Stratigraphy of Bermuda: nomenclature, concepts, and status of multiple systems of classification. In: *Terrestrial and Shallow Marine Geology of the Bahamas and Bermuda Geological Society of America Special Paper 30* (Curran, H.A. & White, B.), pp. 271-294. Geological Society of America, Boulder, CO.

Vacher H.L. & Rowe M.P. 1997. Geology and hydrogeology of Bermuda. In: *Geology and hydrogeology of carbonate islands. Developments in sedimentology, 54* (Ed by H.L. Vacher & T.M. Quinn) pp. 36-90. Elsevier, Amsterdam.

Valiela I., McClelland J., Hauxwell J., Behr P.J., Heresh D. & Foreman K. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105-1118.

Viaroli P., Naldi, M. Bondavalli, C. & Bencivelli S. 1996. Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass denstities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia* 329: 93-103.

Vogt P.R. & Jung W.Y. 2007. Origin of the Bermuda volcanoes and the Bermuda Rise: history, observations, models, and puzzles. In: *Plates, Plumes, and Planetary Process Geological Society of America Special Paper 430* (Ed by G.R. Foulger & D.M. Jurdy), pp.1-47. The Geological Society of America, Boulder, CO.

Waite T. & Mitchell R. 1972. The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. *Botanica Marina* 15: 151-156.

APPENDIX

LIGHT DATA

Light Meter Set Up

Incident light was measured at each study site using submersible data loggers (HOBO, Onset Computer Corp., Pocasset, MA). Data was collected continuously throughout a single cloudless day over 2 second intervals from 9:00 a.m. to 7:00 p.m. (-05:00 GMT). Five stations were established around each pool to determine incidence at the surface and at various depths throughout the volume of the cave pool. At each station, a data logger was secured to the bottom portion of an oval float on the surface. Nylon line was then run from the float to a 2.7 kg (6 lb) tubular weight placed on the bottom of the pool. A second data logger was affixed on the line 15-45 cm (6-18 in) above the weight to ensure the light sensor was not shaded by surrounding foliage. Both data loggers were positioned horizontally in the water column at a 90 degree angle to the float and line.

Results

Light levels were measured in each of the cave pools during June 2007. Summary values (minimum, maximum, and average) are presented in Table A.1. Light levels were highly variable at each station due to differences in shading both above and within the pools. Cliff faces and vegetation over hung portions of the pools preventing direct light from reaching the meters during all or part of the day. Within the pools, large boulders and rock ledges shaded some of the meters. The set up of the meters to free-floating bouys also affected the readings. Wind and water movement turned the surface floats so that instead of being directed at the light source, the meters were sometimes partially shaded by the floats. Comparisons across the surface stations within a cave pool and between cave pools can be made, but just general observations can be made about the light meters in deeper water due to their highly variable depths within and between cave pools.

Table A.1. Incident light levels across the surface and depths at five selected points within each of the six cave pools (Data collected in June 2007).

Site	Station	Depth (m)	Minimum Value (lux)	Maximum Value (lux)	Average Value (lux)
CB					
	1	0	3.8	404.3	63.2.
		0.86	1.1	178.4	28.7
	2	0	2.2	570.8	79.7
		0.66	1.5	214.0	31.3
	3	0	3.3	451.9	81.0
		0.30	0.3	35.7	9.0
	4	0	7.5	856.2	199.6
		0.33	4.3	499.4	151.9
	5	0	5.4	475.7	91.1
		0.30	3.2	142.7	56.5
HP					
	1	0	11.9	1712.4	309.5
		0.61	2.8	475.7	125.6
	2	0	17.8	1284.3	358.0 [1]
		1.13	1.2	523.2	127.7
	3	0	11.9	856.2	247.8
		1.24	1.6	309.2	71.6
	4	0	7.2	1617.2	315.8
		0.64	4.9	547.0	109.2
	5	0	26.8	1379.4	395.0
		1.08	4.5	428.1	142.5
DP					
	1	0	1.0	713.5	8.3
		0.69	0	4.9	0.8
	2	0	0.4	1331.9	108.9
		2.81 [2]	na	na	na
	3	0 [2]	na	na	na
		2.79	0.1	14.1	1.9
	4	0	0.5	1189.2	72.9
		2.74 [2]	na	na	na
	5	0	3.6	1427.0	93.5
		0.48	1.2	249.7	9.4

Table A.1. Continued.

Site	Station	Depth (m)	Minimum Value (lux)	Maximum Value (lux)	Average Value
G					
	1 [3]	0	1.0	1712.4	380.8
		1.17	0.3	92.2	15.7
	2 [3]	0	1.5	1094.0	172.6
		0.58	1.3	404.3	76.8
	3	0	3.4	1902.6	384.3
		2.47	0.5	124.9	40.7
	4	0	7.9	1522.1	465.6
		2.30	0.9	130.8	58.9
	5	0	2.7	1807.5	535.4
		2.68	1.2	142.7	53.9
CP					
	1	0	2.8	998.9	34.5
		2.77	0.1	18.6	1.0
	2	0	0.7	761.1	29.2
		2.76	0	28.2	3.8
	3	0	2	1046.5	144.5
		2.91	0.5	130.8	33.5
	4	0	4.5	1284.3	176.6
		0.69	3.6	689.7	105.6
	5	0	5.3	1807.5	579.9
		0.72	2.8	665.9	144.3
E					
	1	0	0.7	107	11.6
		2.82	0.2	62.4	1.6
	2	0	0.3	34.2	4.4
		2.51 [2]	na	na	na
	3	0	0.5	38.6	10.0
		2.59	0	3.8	1.0
	4	0	2.7	249.7	25.7
		0.97	0.4	142.7	7.6
	5	0	0.1	10.2	1.9
		0.61	0.1	10.2	1.9

[1] Meter was no longer attached to float at 90° angle at the 7 pm.
[2] Meters malfunctioned and did not record accurate data.
[3] Meters were covered by floating leaf litter at 7 pm.

Calabash has a cliff face that shades the north edge and vegetation that shades the northeastern side of the pool. The locations of the five stations are presented below in Fig. A.1. Readings were consistently high between the hours of 9:00 am and 4:00 pm (Fig. A.2). After this time, the sunlight was too low on the horizon to reach the pool through surrounding forest cover. Station 4, located in the southern edge of the pool received the highest incidence of light with an average value of 199.6 lux at the surface and 151.9 lux at 0.33 m.

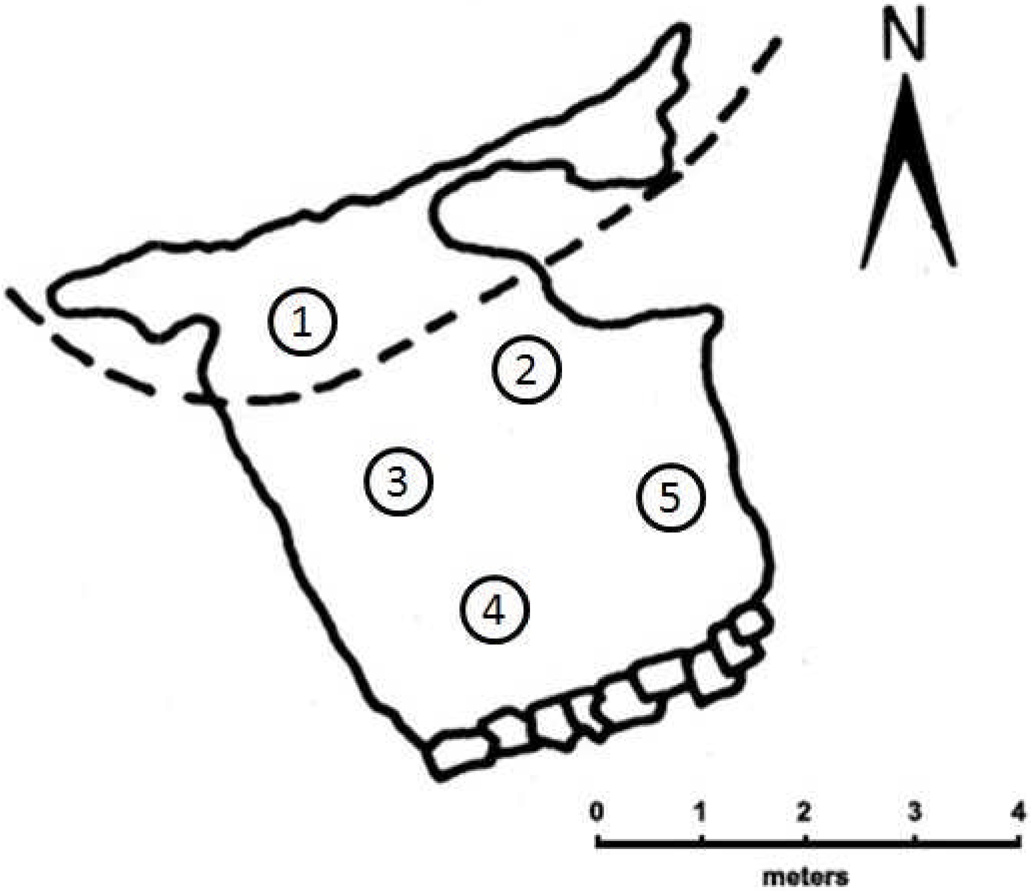

Fig.A.1. Plan view of Calabash (Schwede unpublished collection). Locations of light meter stations are marked.

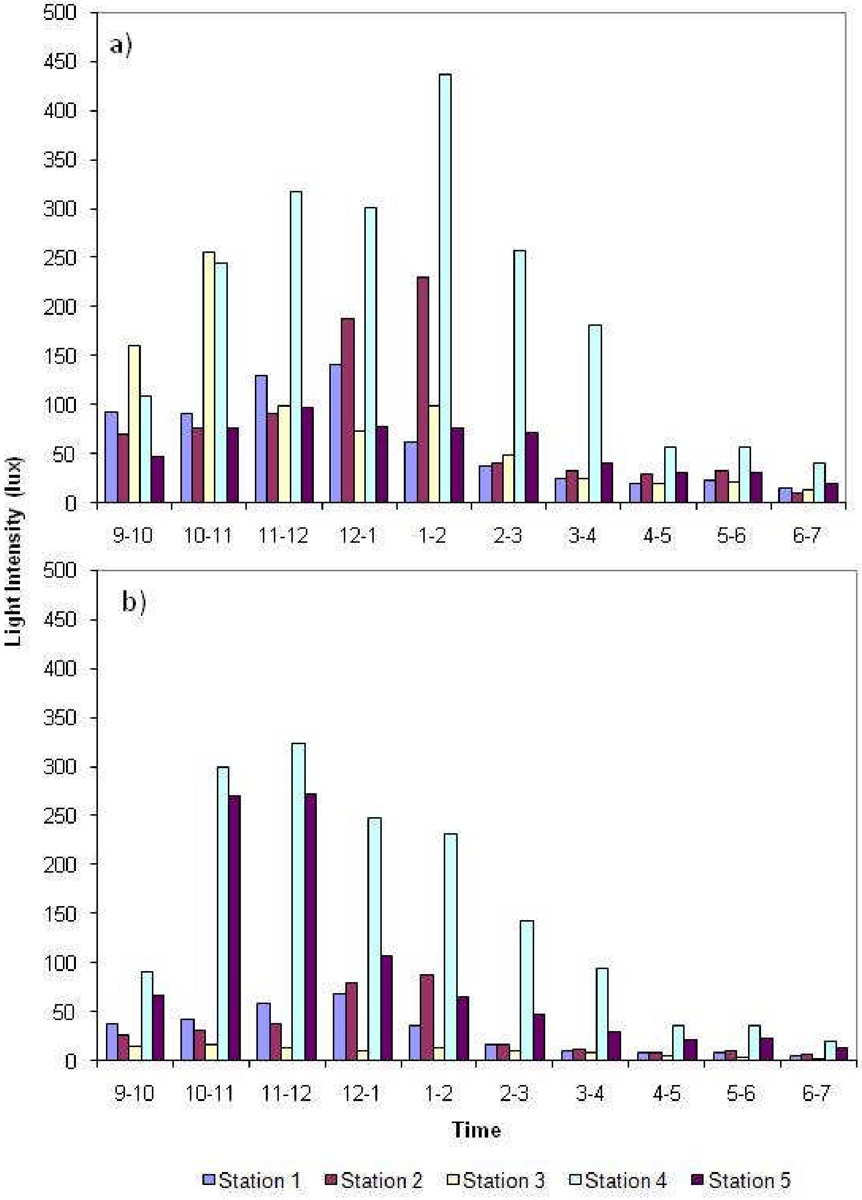

Fig. A.2. Average hourly light at five stations in waters of Calabash a) surface meters b) depth meters (ranged from 0.30-0.86 m).

Harbour Pool is shaded by vegetation on all but the southern side, with the greatest overhang occurring on the north and east sides. The locations of the five stations are presented below in Fig. A.3. Harbour Pool consistently had high readings between 9:00 am and 6:00 pm in the center of the pool (Fig. A.4). Station 5 was center of the pool on the south side where no shading occurred. It had a maximal reading of 1379.4 lux and an average reading of 395.0 lux at the surface and 428.9 lux maximum,142.5 lux average at 1.08 m. In areas of vegetative over hang such as Station 3, surface measurements dropped to an average 247.8 lux at the surface and 71.6 lux at 1.24 m.

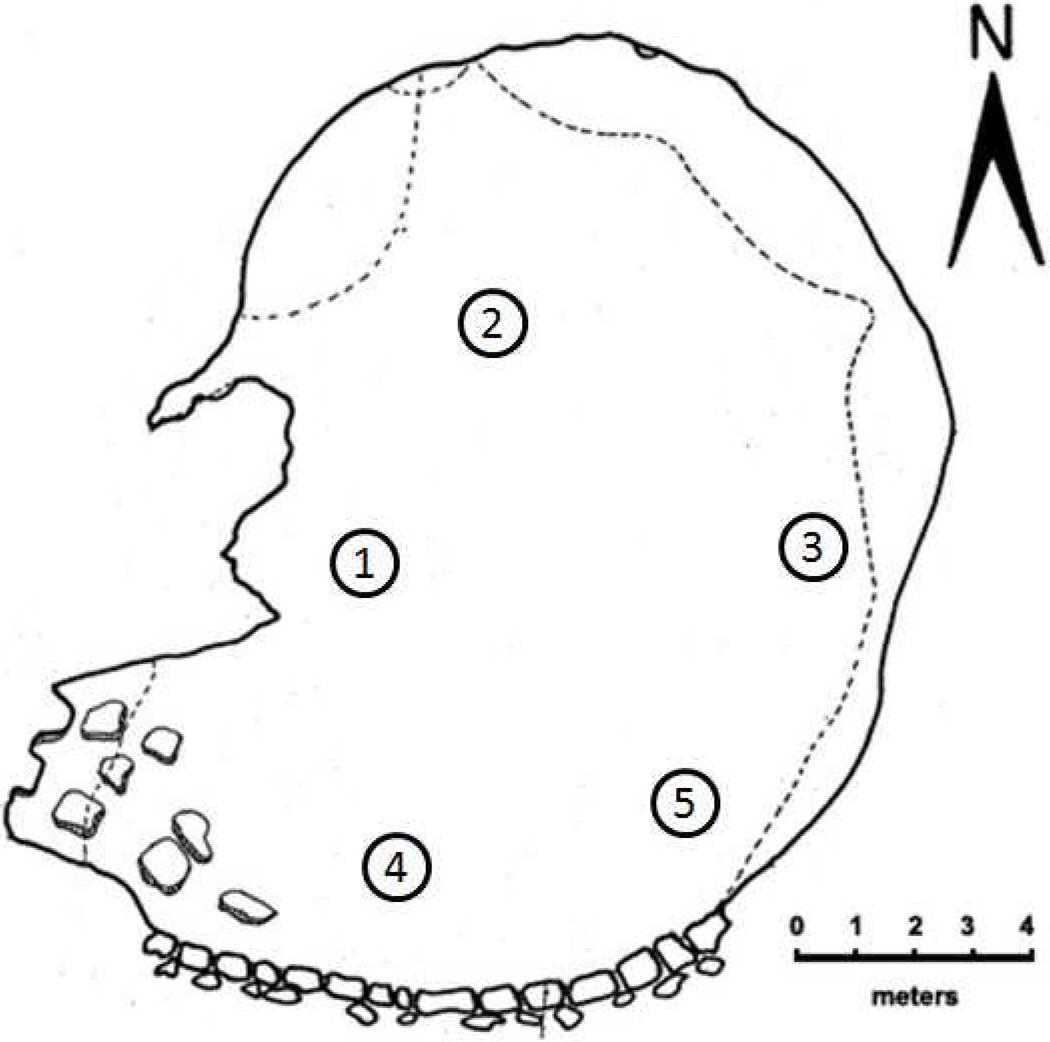

Fig.A.3. Plan view of Harbour Pool (Schwede unpublished collection). Locations of light meter stations are marked.

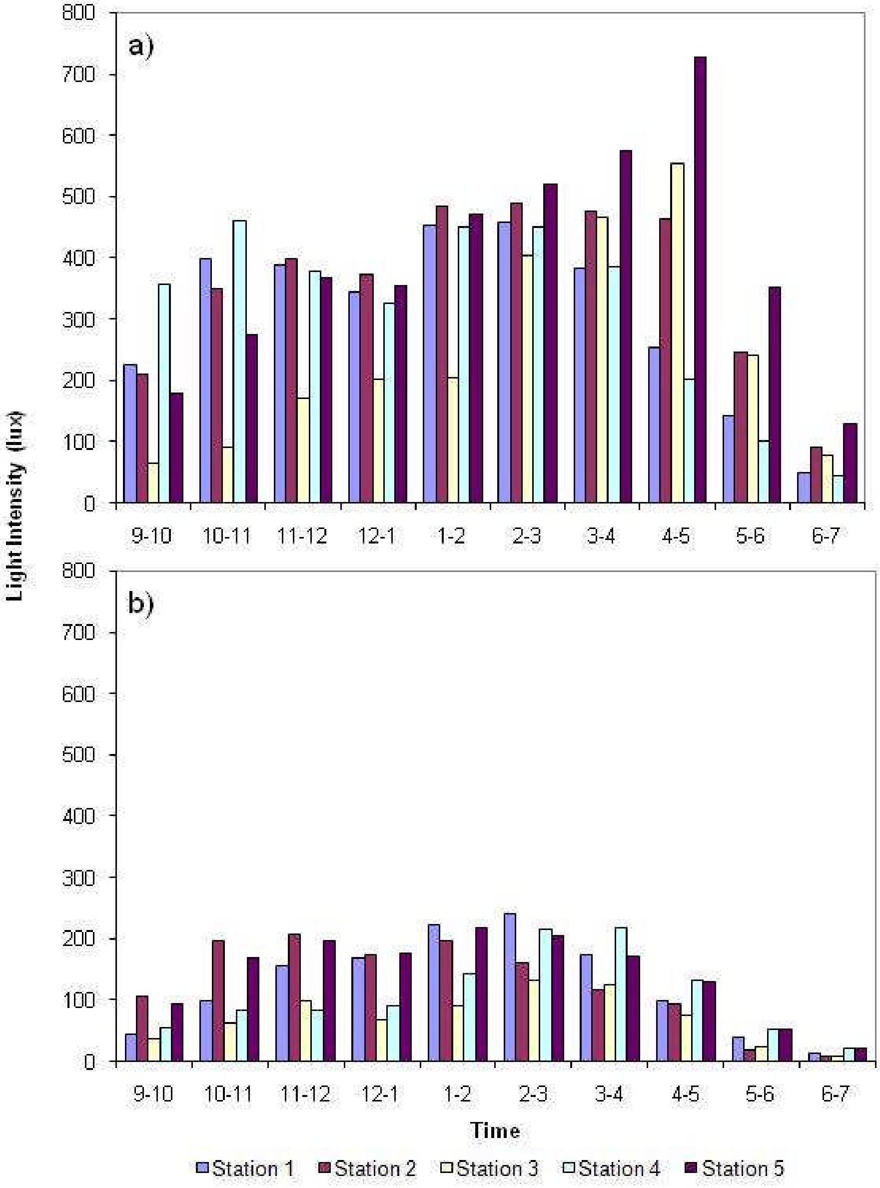

Fig. A.4. Average hourly light at five stations in waters of Harbour Pool a) surface meters b) depth meters (ranged from 0.61-1.24 m).

Deep Blue is located in a karstic depression with an overhanging cliff wall on the western side and a steep hill covered with vegetation on the eastern side. The locations of the five stations are presented below in Fig. A.5. Due to these obstructions, direct light only reached meters located on the eastern side of the pool between 10:00 am and 1:00 pm (Fig. A.6). Stations 2-5 were located through the larger, main pool, while station 1 was located in a smaller pool on the southern side than is shaded during the whole day. Station 5 was located on the most exposed, eastern edge and during the hours of direct sunlight received readings up to 1331.9 lux that averaged 108.9 lux over the entire ten hour period.

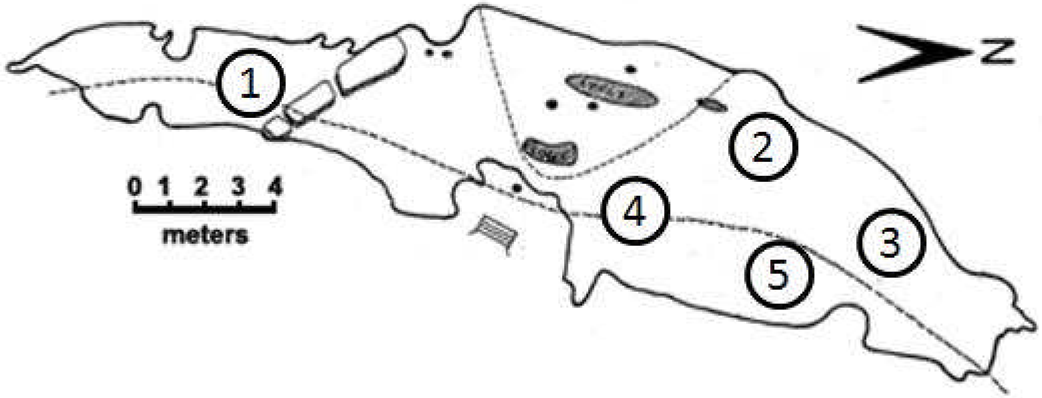

Fig.A.5. Plan view of Deep Blue (Schwede unpublished collection). Locations of light meter stations are marked.

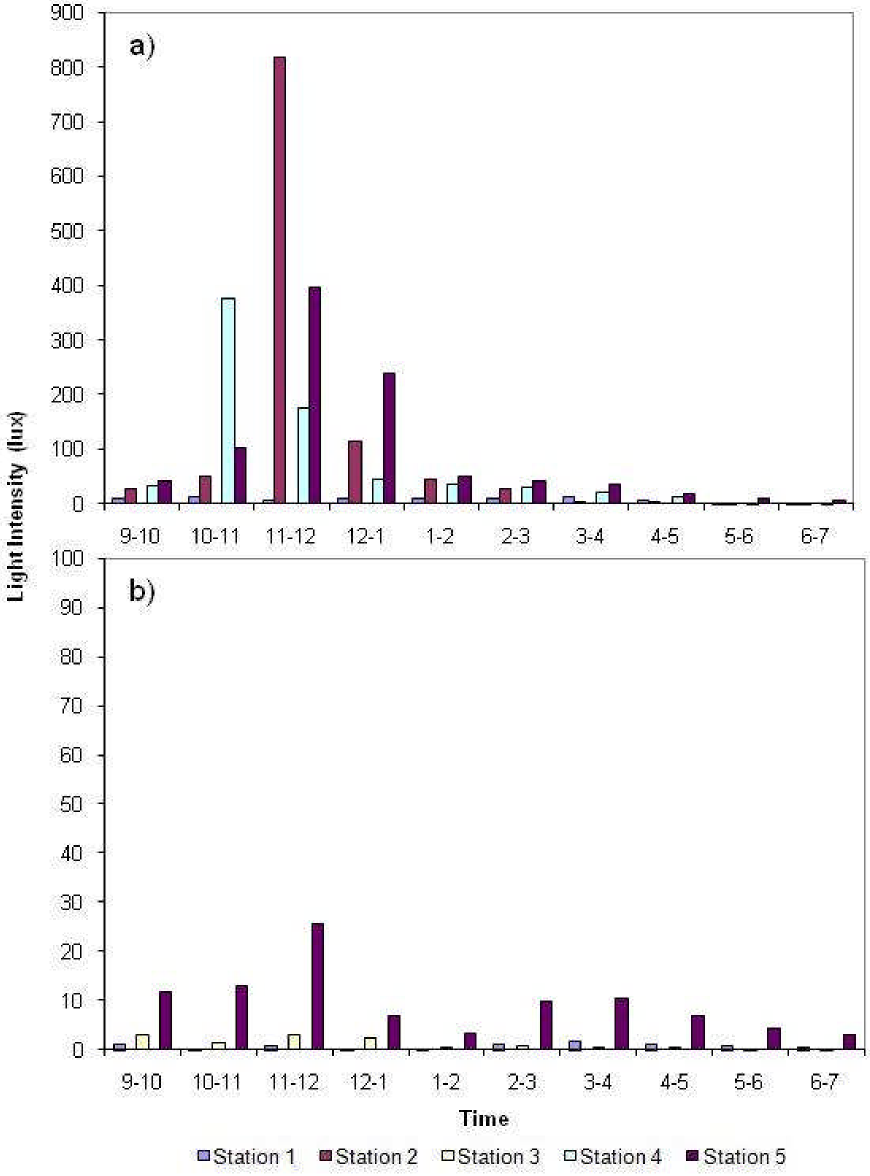

Fig. A.6. Average hourly light at five stations in waters of Deep Blue a) surface meters b) depth meters (ranged from 0.48-2.81 m).

Thick vegetation over hangs a portion of Grenadier on the southwestern and western edges, while the eastern side is highly exposed as it is surrounded by a manicured lawn and cement walkway. The locations of the five stations are presented below in Fig. A.7. The day the light meters were placed in this pool, it was mostly sunny throughout the day before thick clouds covered the skies at 5:00 pm. This resulted in the high light levels measured consistently from 9:00am to 5:00 pm to quickly decline (Fig. A.8). Maximum readings, occurring at mid-day, ranged from 1522.1-1902.6 lux at the surface and 92.2-142.7 lux from 1.17-2.68 m. Station 5 was the most exposed to direct sunlight throughout the day and had an average incidence level of 535.4 lux at the surface and 53.9 at 2.68 m; Station 2 was received the most shading, averaging 172.6 lux at 0 m. Floating leaf litter collected around the float at Stations 1 and 2 as the day progressed and may have led to a lower average.

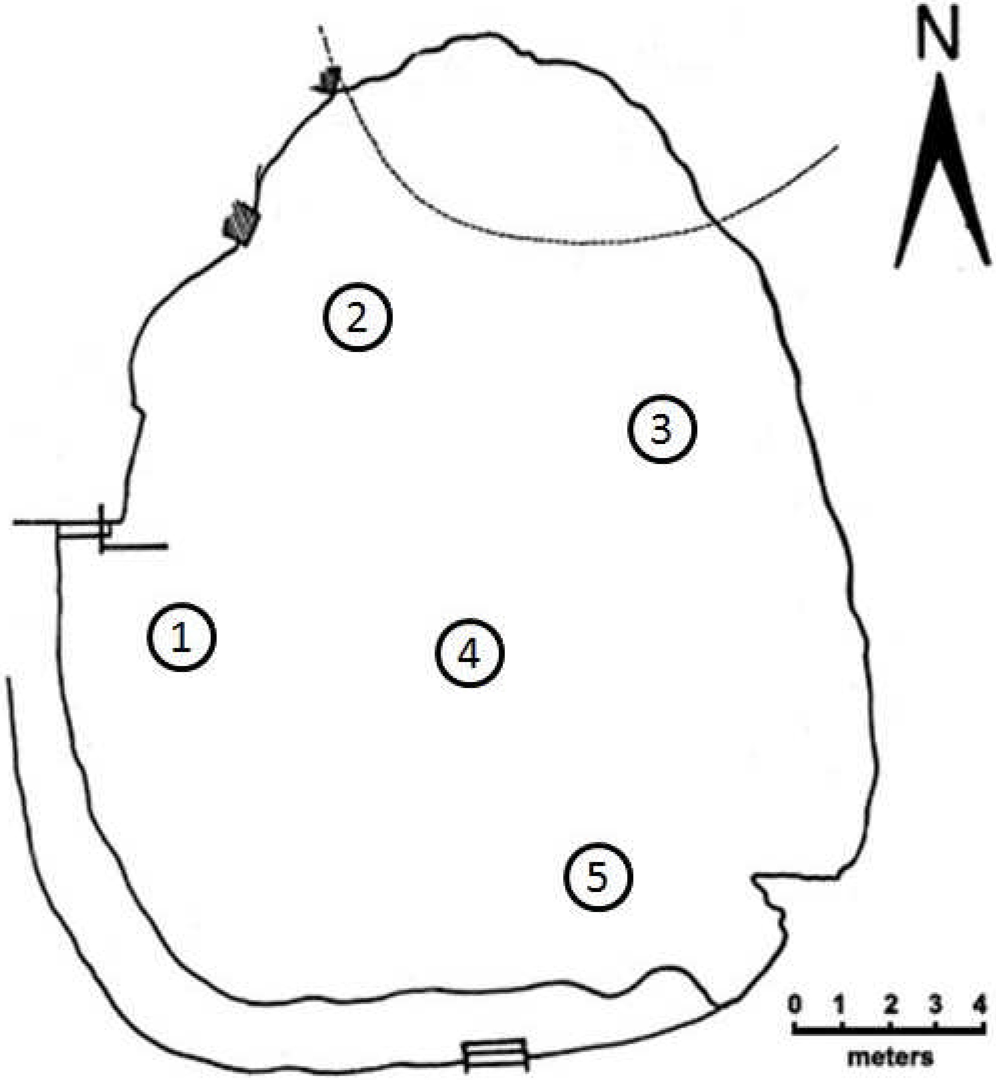

Fig.A.7. Plan view of Grenadier (Schwede unpublished collection). Locations of light meter stations are marked.

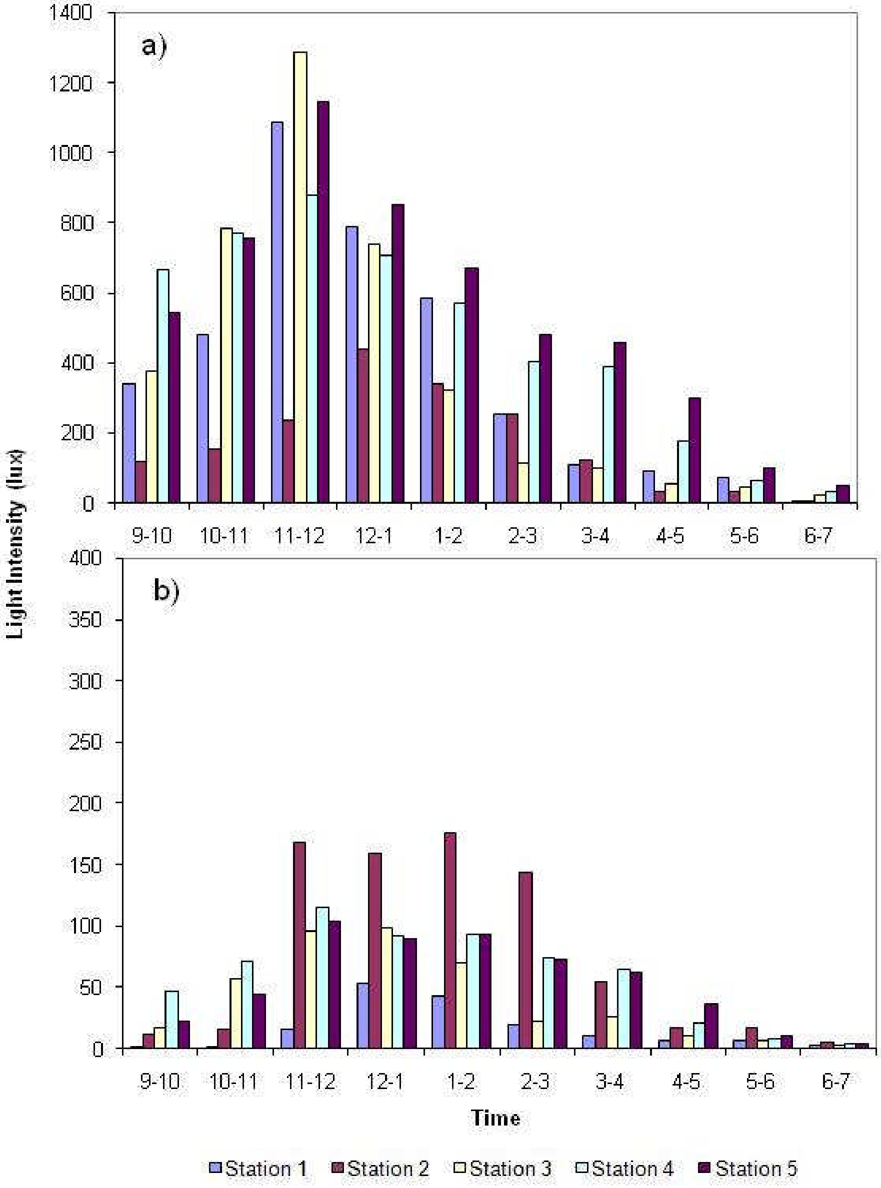

Fig. A.8. Average hourly light at five stations in waters of Grenadier a) surface meters b) depth meters (ranged from 0.58-2.68 m).

Cliff Pool's northwestern side is composed by an overhanging cliff face that shaded part of the pool. There is also vegetative growth that overhangs the southern and northern corners of the pool. The locations of the five stations are presented below in Fig. A.9. As with many of the other more open pools, constant high light levels were measured between the hours of 10:00 am and 4:00 pm (Fig A.10). Stations 3-5 received the most direct light during the day with maximum incidence readings ranging from 1046.5-1284.3 lux at 0 m and averaged 144.5, 579.9, and 176.5 lux respectively. At a depth of 2.91 m, Station 3 averaged 33.5 lux. Station 5, in shallower water, averaged 144.3 lux in 0.72 m of water. Much lower values were measured at Stations 1 and 2 due to the combined effect of the overhanging cliff and vegetation.

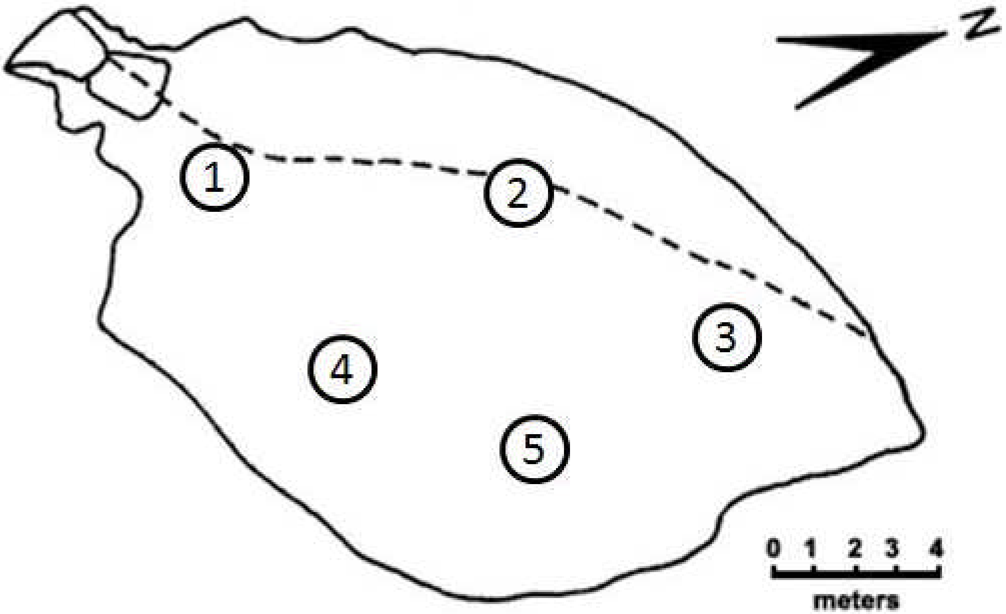

Fig.A.9. Plan view of Cliff Pool (Schwede unpublished collection). Locations of light meter stations are marked.

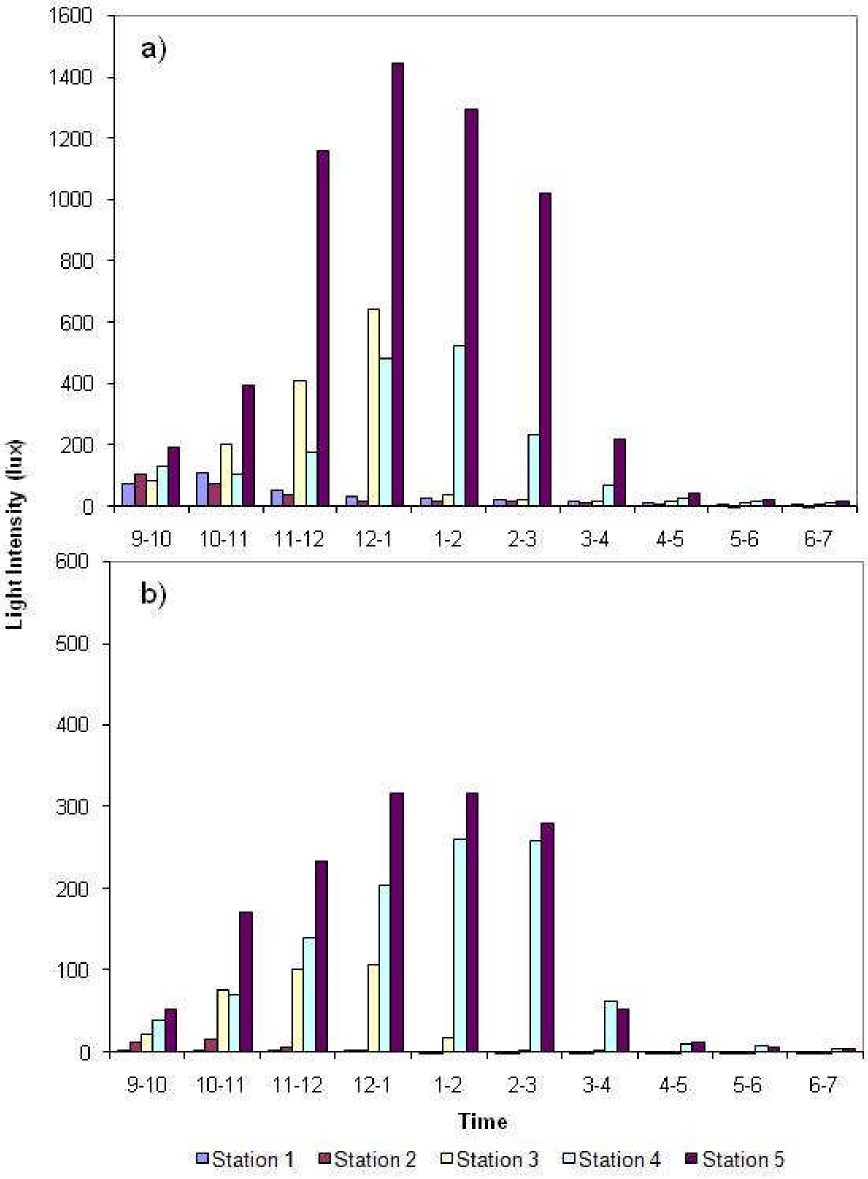

Fig. A.10. Average hourly light at five stations in waters of Cliff Pool a) surface meters b) depth meters (ranged from 0.69-2.91 m).

Emerald receives the least direct sunlight of any of the cave pools sites. All five stations averaged less than 26 lux over the course of the day. A cliff face begins at the western edge and extends up and over the pool. The locations of the five stations are presented below in Fig. A.11. The land to the east of the pool rises up a steep hill that was cleared of vegetation between May and June 2007. Due to the narrow, eastern opening of the pool, it only received direct light over a small portion of the pool from 9:00-10:00 am (Fig. A.12). The highest reading of 249.7 lux was recorded at the surface for Station 4.

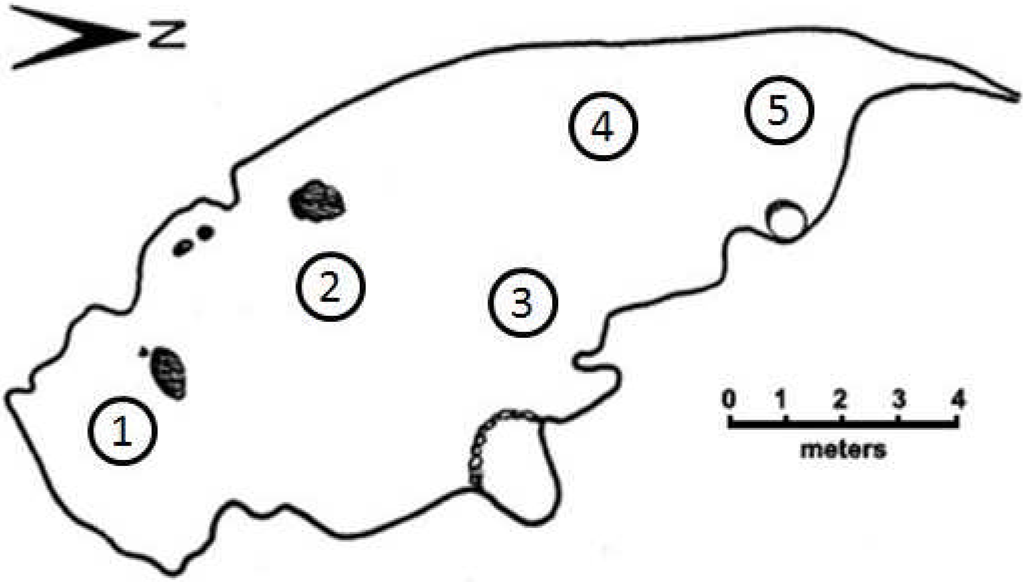

Fig.A.11. Plan view of Emerald (Schwede unpublished collection). Locations of light meter stations are marked.

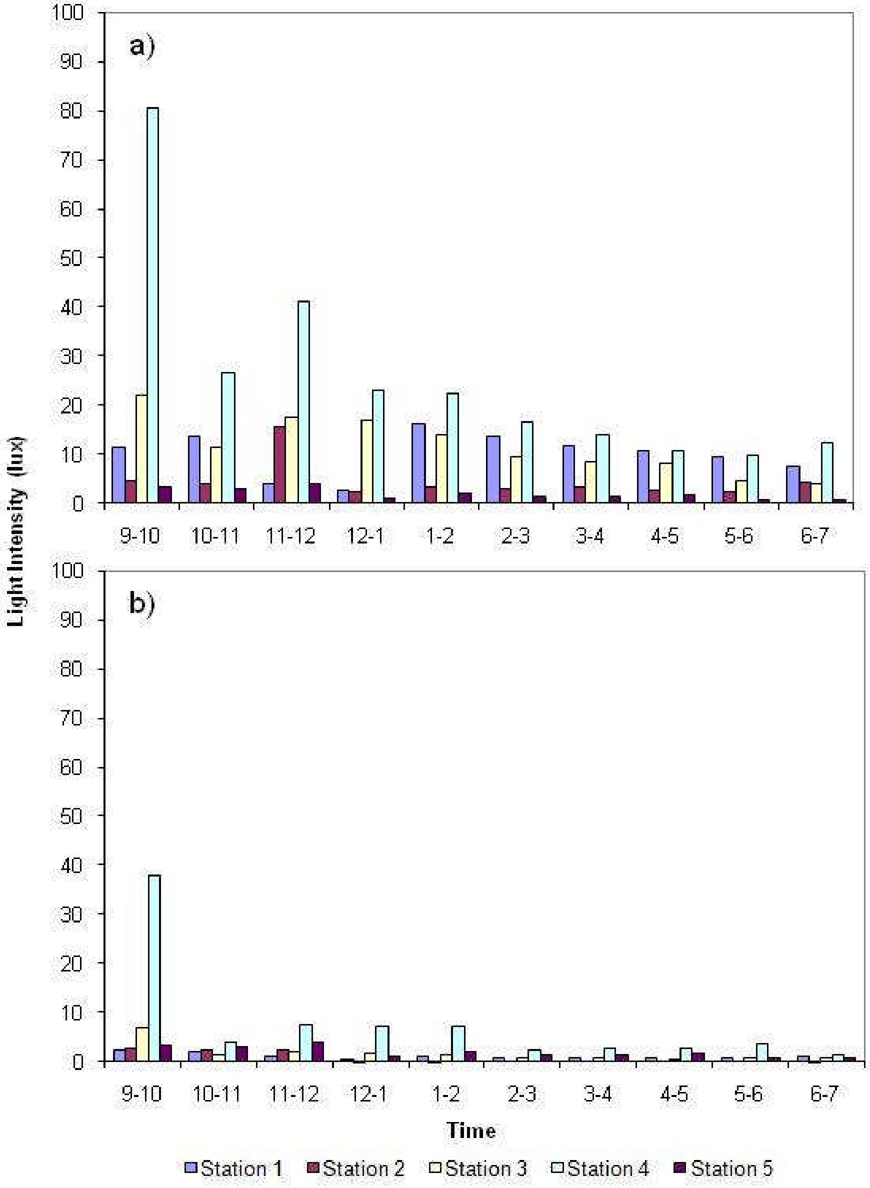

Fig. A.12. Average hourly light at five stations in waters of Emerald a) surface meters b) depth meters (ranged from 0.61-2.82 m).

The affect of light on flora of the pools cannot be determined using this data. The light stations were not linked to any algal distributions or environmental water parameters in the cave pools. This data is included in the appendix for comparison to future studies.

VITA

Bridget Marie Maloney
Texas A&M University at Galveston
5001 Avenue U
Galveston, TX 77551
Phone: 409-740-4875
Email: maloneyb@tamug.edu

Education

- B.S., General Biology; University of Arizona, Tucson, AZ 2005
- M.S., Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 2008

Research Experience

- Field Research Assistant: survey of Murex's aggregations, snail measurements, habitat collections, and juvenile preservation. Puerto Peñasco, Sonora, Mexico. 2003-2004
- Laboratory Research Assistant: microscopic division of marine species, species preservation, and comparison statistical analysis techniques. Tucson, AZ. 2003-2005
- Biodiversity Internship: Cave exploration and comprehensive study. Bermuda Aquarium, Musuem and Zoo. Flatts, Bermuda. 2007
- Research Assistant: Yucatan cave exploration, water quality monitoring, and cave species collection, sorting, and preservation. Quintana Roo, Mexico. 2008

Academic Professional Employment

- Teaching Assistant: Introduction to Scientific Diving, Spring 2007 & Spring 2008
- Research Assistant: Marine Biology Department, Fall 2007
- Field Guild: Galveston Graduate Student Association, 2007-2008
- Field Guide: Sea Camp, 2007-2008
- Teaching Assistant: Methods in Scientific Diving, Summer 2008
- Teaching Assistant: Natural History of Vertebrates, Fall 2008
- General Academics Lecturer: Scuba I & Health & Fitness, Fall 2008

Awards/Honors

- Graduate Merit Fellowship to Texas A&M University, 2005-2007
- Scuba Made Easy Scholarship, 2006
- McCord Scholarship, 2006
- Bermuda Zoological Society Research Grant, 2007
- Erma Lee & Luke Mooney Travel Grant, 2007
- Texas A&M University Academic Excellence Award, 2008