Limnological Analyses of Devils Hole and Point of Rocks Refugium, Ash Meadows Wildlife Refuge, Nye County, Nevada

Kumud Acharya, Ph.D.
Mark Stone, Ph.D.
Candi Schulman

August 2009

Prepared by

Desert Research Institute, Nevada System of Higher Education

Prepared for

United States National Park Service, Death Valley National Park

Great Basin Cooperative Ecosystem Studies Unit
COOPERATIVE AGREEMENT NO.: H8R07060001

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

Table Legends

Figure Legends

Abstract

The Devils Hole pupfish (Cyprinodon diabolis) was one of the first listed species under the Federal Endangered Species Act. The species has been living on the edge of extinction for the past several decades. The Devils Hole pupfish is an important legacy of the Southwest because it is unique to its location. The pupfish have provided clues to the development of the planet over the several million years according to Prof. Emeritus James Deacon (UNLV). Dr. Deacon argues that The Devils Hole pupfish has been an important part of the study into evolutionary biology and has provided valuable insight regarding the pathways followed by early ancestors over millions of years. The fish has ancestral relatives from as far away as the Middle East and Africa. Therefore saving this remarkable creature have vast implications.

The objective of this project was to collect short term (1 year) and targeted data on limnological parameters on the upper shelf of the Devils Hole – where Devils Hole pupfish generally feed and spawn. This data was then compared with previously collected data within the hole and nearby refuge (Point of Rocks). This provides data needed to form a stronger foundation than presently exists regarding Devils Hole and ecosystem pressures on the pupfish. Based on the data, future phases of long term research to perpetuate the existence of the species have been recommended.

1. Introduction

1.1 Purpose of Study

Devils Hole in Ash Meadows National Wildlife Refuge, Nye County, Nevada is a collapsed limestone cavern system which provides an opening to the regions groundwater table. It also serves as the only habitat of the federally endangered Devils Hole pupfish (*Cyprinodon diabolis* Wales) and may be the smallest habitat in the world to support an entire vertebrate population (Deacon et al. 1995). Despite being the head of a vast carbonate aquifer system, a 6.3 m N/S by 3.0 m E/W shelf covered by 0-70 cm of water provides the fishes' entire habitat for foraging, mating, and reproduction (James 1969, Riggs and Deacon 2004). During the late 1960's, the Devils Hole pupfish gained the public's attention when pumping of groundwater from the Ash Meadows regional aquifer by commercial developers began to decrease water levels over the upper shelf (Deacon and Williams 1991). A precipitously declining population size and water level prompted the 1976 United States Supreme Court decision decreeing that a minimal water level was necessary for preservation of the endangered species and commercial activity in the region ended. After the water level was stabilized in the late 1970s, the population appeared to be relatively steady with an average population size of 324 individuals (US Fish and Wildlife Service 2008). Starting in 1997, population surveys at Devils Hole began to record a decline in fish numbers and in April of 2007 the population dipped to the lowest number ever record of 38 individuals (personal communication, NPS).

Since the discovery of this unique desert fish, extensive research has been conducted on its life history (Minckley and Deacon 1973), population dynamics (Deacon and Baugh 1983), and diet (Minckley and Deacon 1975), as well as the water quality, invertebrate and algal communities (Herbst 2003, Shepard 2000), energetics (Wilson and Blinn 2007, Blinn et al. 2000) and habitat structure of Devils Hole (Lyons 2005). The survival of *C. diabolis* is hypothesized to be directly linked to the structure of the upper shelf and the aquatic community it sustains (Riggs and Deacon 2004). Given the cavernous morphology of Devils Hole, the amount of direct sunlight received on the surface waters is limited to 4 hours during the summer (June-August) to none during the winter

(December-January; Wilson and Blinn 2007). Such light limitation severely impacts the amount of photosynthetic organisms in the spring's simple food web. The pupfish have also inured other stresses including high temperatures (on the upper shelf where they feed), fluctuations in dissolved oxygen, and a confined habitat, but the cause of a sudden decline in population size over the past decade has no clear cause.

The purpose of this study was to monitor the abiotic and biotic characteristics of Devils Hole. Basic limnological data collection was performed in monthly intervals from March 2007 to February 2009. Samples were concurrently taken from Point of Rocks Refugium from March 2007 to December 2007, where two Devils Hole pupfish, one male and one female, were inhabitants.

1.2 Site Description

Devils Hole is a collapsed limestone cavern located in Ash Meadows National Wildlife Refuge, Nye County, Nevada, a disjunction of Death Valley National Monument (Minckley and Deacon 1975). Inside the 15 meter deep rock fissure, is the near surface waters of the thermal spring, with estimated dimensions of 22 m by 3.5 m (Anderson and Deacon 2001). At the south end of the spring, a shelf (0-70 cm deep), was created approximately 60,000 years ago after the collapse of the cavern. The shelf extends 3.0 m E/W and 6.3 m N/S (Gustafson and Deacon 1998, Riggs and Deacon 2004) and sustains the entire population of the endemic Devils Hole pupfish (*Cyprinodon diabolis* Wales). Below the upper shelf, lies a slanted lower shelf, with water depths from 5 to 9 m (Gustafson and Deacon 1998). Beyond the northern tip of the lower shelf is a deep cave of an unknown depth. The maximum depth explored via SCUBA has been 133 m (Shepard 2000).

The Point of Rocks Refugium, a part of the Amargosa Pupfish Station, is located at the Ash Meadows Wildlife Refuge, Nye County, Nevada. Created in 1991, the man-made pool is fed by a surrounding spring and was established as a back-up habitat for *C. diabolis*.

From November 2006 to February 2009 monthly grab samples were collected from the upper shelf of Devils Hole. The water samples were analyzed for several limnological parameters including: total nitrogen, total phosphorus, alkalinity, chlorophyll *a*, and organic matter (measured as ash free dry mass). In-situ measurements of pH, temperature, dissolved oxygen, specific conductivity, and turbidity were also taken. Sampling was carried out on: 1-March , 5-April, 11-May, 7-June, 12-July, 18-September, 21-September, 5-October, 31-October, 13-December in 2007; 5-February, 4-April, 6-June, 10-July, 5-October in 2008; and 8-January, 10-February in 2009.

From March 2007 to December 2007 samples were also collected from Point of Rocks Refugium. The water samples were analyzed for the same parameters as the Devils Hole samples; i.e., total nitrogen, total phosphorus, alkalinity, turbidity, chlorophyll *a*, and organic matter. Sampling was carried out on: 18-January, 1-March, 5-April, 11-May, 7-June, 12-July, 15-Septemeber, and 13-December of 2007. Samples were collected with acid-rinsed HDPE bottles, stored on ice and promptly transported to the Eco-Engineering Laboratory at the Desert Research Institute in Las Vegas, Nevada for further analysis.

2. Methods

2.1 Physical Measurements

In-situ physicochemical measurements were taken from a multi-parameter sonde probe (YSI Incorporated) from December 2006 to June 2007 at Devils Hole. Measurements of pH, water temperature, dissolved oxygen (DO), and specific conductivity were taken monthly at 25 cm below the water surface of the upper shelf. Beginning in August 2007, a sonde probe was permanently stationed inside Devils Hole by Death Valley National Park Service personnel. The data obtained and presented here from Death Valley National Park, Resource Management Division are only provisional and are not necessarily calibrated and/or fully corrected; any further use of this data should be made with permission.

Alkalinity (n=1 per site) was determined according to SM-2320. Titrations of sample water were made with hydrogen sulfide (H_2SO_4) to pH 4.3. The volume of acid used was recorded and calculated in relation to calcium carbonate ($CaCO_3$) (APHA 2005).

Turbidity (n=2 per site) was measured on-site using a Hach 2020e Turbidity meter.

Two attempts were made to measure the temperature profile within Devils Hole using continuously recording iButton digital temperature data loggers. The first deployment took place on December 1, 2007 by the Devils Hole Dive team. Due to the complicated nature of deployment and recovery along with corrosion of the instruments, only one iButton (30 m deep) remained functional at the point of recovery on January 18, 2009. A second series of iButtons were deployed on January 18, 2009. However the data from the second deployment was not yet available at the time of this report.

2.2 Chemical Measurements

For the analysis of nitrogen (n=1 per site) sample water was first filtered through a 0.7 μm TCLP glass fiber filters (GF/F). The filtrate was then analyzed for total nitrogen according to the automated SM-4500 F for nitrate (NO_3), nitrite (NO_2), and ammonia (NH_3) with a Lachat QC8000(APHA 1992).

Total phosphorus (TP) (n=3 per site) was analyzed on unfiltered sample water using the colorimetric analysis after persulfate digestion (APHA 2005). Several samples were below the level of detection with this methodology. This data has been excluded from the final results.

Sulfate (SO_4) (n=1 per site) was analyzed on unfiltered sample water according to the ion chromatography method for sulfate (USEPA 1993).

For the analysis of ash free dry mass, sample water was filtered onto pre-weighed ashed 0.7 μm TCLP glass fiber filters (GF/F) and then allowed to dry in a convection oven set at 60°C until a steady dry weight could be obtained (~24 hrs). The filters were then ashed in a furnace set at 500°C for 1 hour. Following ashing, filters were weighed again to obtain the post-ash weight. The method was in accordance with APHA (2005). Due to the low levels of ash free dry mass detected at Devils Hole, analysis ceased after September 2007.

On 1-December 2007 a diving crew surveyed Devils Hole and sampled a vertical gradient starting from the open pool at northern tip of the spring to 120 ft into the cavern. Grab samples were collected at, 0 ft, 30 ft, 75 ft, and 120 ft during the vertical descent. Sample water was tested for a full suite of cations and anions including: nitrite, nitrate, ammonia, silica dioxide (SiO_2), bicarbonate (HCO_3), carbonate (CO_3), chloride (Cl), sulfate, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and total phosphorus, as well as pH and electrical conductivity (EC). On 4-April 2008 a diving crew explored Devils Hole 2, presumably a hydrologically connected cavernous spring, in which water samples were collected from the surface waters and analyzed for the same parameters listed above.

2.3 Biological Measurements

To measure chlorophyll *a* (n=3), approximately 1 liter of sample water was filtered through a 0.7 μm TCLP glass fiber filters (GF/F). The filter was then extracted in 10 mL of 90% acetone and refrigerated for 24 hours. The extract was analyzed using an AquaFlour Handheld Fluorometer (Turner Designs) calibrated for extracted chlorophyll *a* analysis (USEPA 1997).

Composite samples of the phytobenthos and planktonic communities were randomly collected from the upper shelf of Devils Hole on 27-October 2008. The benthic algae samples were sorted to remove any macro-invertebrates resulting for a final volume of 200 mL. A stock solution of 200 mg/L sodium nitrate ($NaNO_3$) was used as a nitrogen source resulting in a nitrogen concentration of 45.16 mg N/L. A stock solution of 1000 mg/L potassium phosphate (KH_2PO_4) was used as a phosphorus source resulting in a phosphorus concentration of 326.32 mg P/L. 10 mL of sorted benthic algae and 140 mL of Devils Hole water (representing the planktonic algae) were added to 150 mL glass flask and amended to 6 different N: P molar ratios: control, 5:1, 10:1, 20:1, 100:1. Phosphorus remained constant at 0.10 mg/L and N varied to reach desired stoichiometric relationship. The experiment was deployed inside an incubator calibrated at 33°C, 4:20 light-dark cycle and placed on an orbital shaker at 150 rpm. The experiment ran for 12 days, after which, the treatments were preserved in 7.5% formaldehyde for identification and enumeration. Algae enumeration was conducted with aid of a Sedgwick rafter cell. The cell chamber was filled with 1 mL of sample and counted under an inverted microscope. Approximately 40 1 mm x 1 mm squares were counted for each repetition for an average of 160 cells per count. Each treatment was counted three times (n=3). Biovolume was estimated (n=3) for the filamentous cyanobacteria, *Oscillatoria*, according to Hillebrand et al. (1999).

During the dive at Devils Hole on 1-December 2007 sediment grab samples were collected from the upper and lower shelves. Upper shelf samples were dried, prepared and coated with carbon (0.025 µm). Lower shelf samples were covered in gold and carbon. Coated samples were analyzed and documented with a scanning electron microscope (SEM).

Data on nitrogen, phosphorus, pH, specific conductivity, dissolved oxygen, turbidity and temperature was intermittently collected from June 1985 to August1999 by the United States Geological Survey and maintained by the National Park Service. For the purpose of this research the previously unreported data was analyzed and is referenced as "historical data" herein.

3. Results and Discussion

3.1 Physical Measurements

(a) Devils Hole

The physicochemical composition of the upper shelf was markedly constant throughout the study period (Table 1). This data is similar to the historical data: temperature 33.1±0.26 °C, specific conductivity 0.682±0.006 µS/cm, dissolved oxygen 2.8±0.10 mg/L, and pH 7.5±0.04 and previously published reports (Wilson and Blinn 2007, Shepard 2000 and references within). Specific conductivity appears to be increasing slightly from the historical records.

Table 1 Mean pH, Temperature, Dissolved Oxygen, and Specific Conductivity from January 2007-February 2009. () indicates standard error

Parameters	January 2007-February 2009
pH	7.37 (0.0009)
Temperature (°C)	33.3 (0.002)
Dissolved Oxygen (mg/L)	2.99 (0.01)
Specific Conductivity (mS/cm)	0.713 (9 E-5)

Dissolved oxygen concentration began to fluctuate diurnally in mid-March when the upper shelf began to receive direct sunlight and ceased in November, when light on the upper shelf become extremely limited. Figure 1 depicts a 48 hour time series from 25-March through 26-March 2008 of dissolved oxygen. Dissolved oxygen ranged from a low of 1.52 mg/L at 12:00 am to a high of 6.0 mg/L at 4:30 pm on March 25. Figure 2 depicts a 48 hour time series during the month of December. As shown, there were no major swings in dissolved oxygen. This rise in dissolved oxygen during the summer months is in response to an increase in photosynthesis during the day and bacterial and plant respiration at night.

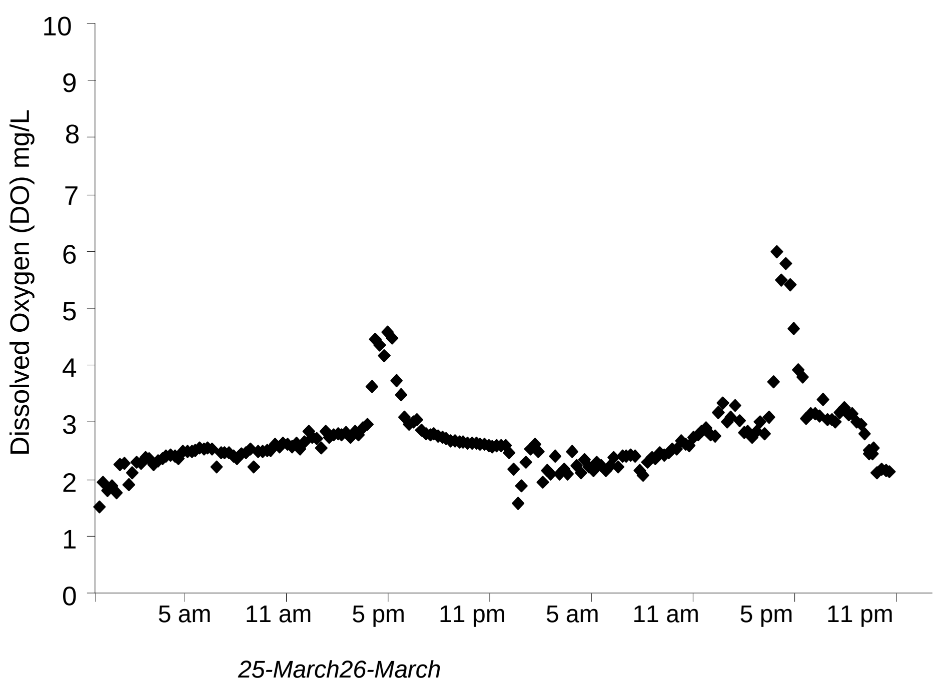


Figure 1 Dissolved Oxygen of Upper Shelf, March, 48 Hour Time Series

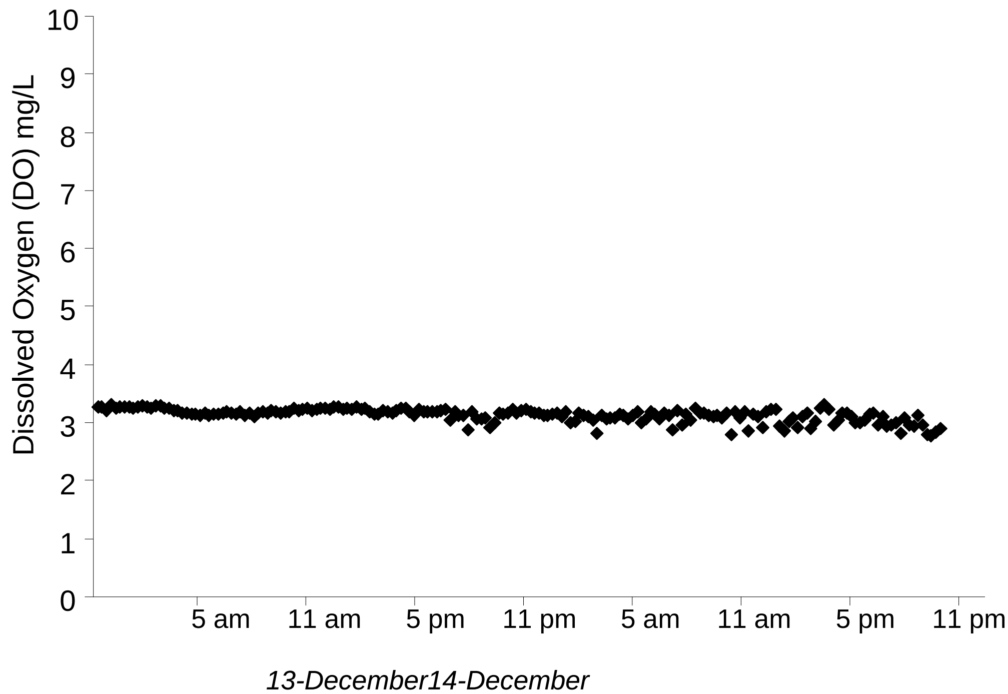


Figure 2 Dissolved Oxygen of Upper Shelf, December, 48 Hour Time Series

Devils Hole is highly buffered spring as the water source comes from the region's deep carbonate aquifer. Alkalinity ranged from 188 to 329 mg $CaCO_3$/L throughout the sampling years with a mean of 260±11 mg $CaCO_3$/L.

Turbidity varied from each sampling date from 0-3 NTU with a mean of 0.4±0.2 NTU.

Continuous temperature data at 30 m depth, as measured with the iButton digital temperature data logger, are included in Figure A-5 which can be found in Appendix A. The observations revealed remarkably stable temperature values ranging from only 34.5 to 34.6 °C over the three month observation period of December 2007 to February 2008.

(b) Point of Rock Refugium

Alkalinity ranged from 125-308 mg $CaCO_3$/L, with a mean of 219±21 mg $CaCO_3$/L.

Turbidity ranged from 0.04-1.75 NTU, with a mean of 0.42±0.2 NTU. The April 2007 measurement recorded the highest level turbidity at 1.75 NTU. Organic carbon (measured as ash free dry mass) is a major constituent of suspended solids, particularly in unpolluted, closed habitats such as Point of Rocks Refugium. Therefore it is reasonable to deduce the spike in turbidity recorded in April 2007 corresponds to the spike in the organic matter during the same month.

3.2 Chemical Measurements

(a) Devils Hole

Throughout the course of the study, total nitrogen concentrations (sum of NO_2, NO_3 and NH_3) were relatively stable with an overall average of 0.165 ±0.007 mg/L (Table B-2). On October 31 of 2007 and February 5 of 2008, total nitrogen concentration peaked significantly above the yearly average to 0.215 mg/L and 0.231 mg/L, respectively. Historically, nitrogen concentration has been significantly higher than present day levels, and has consistently declined from a high of 0.67 mg/L total nitrogen in November 1986 to the current level of 0.17 mg/L measured in February of 2009 (Figure 3). Wilson and Blinn (2007) reported a mean nitrate +ammonia concentration of 0.26 mg/L from 12-October 1999 to 31 July 2001, further demonstrating the gradual decline of nitrogen concentration at Devils Hole as recorded for the past 20 years.

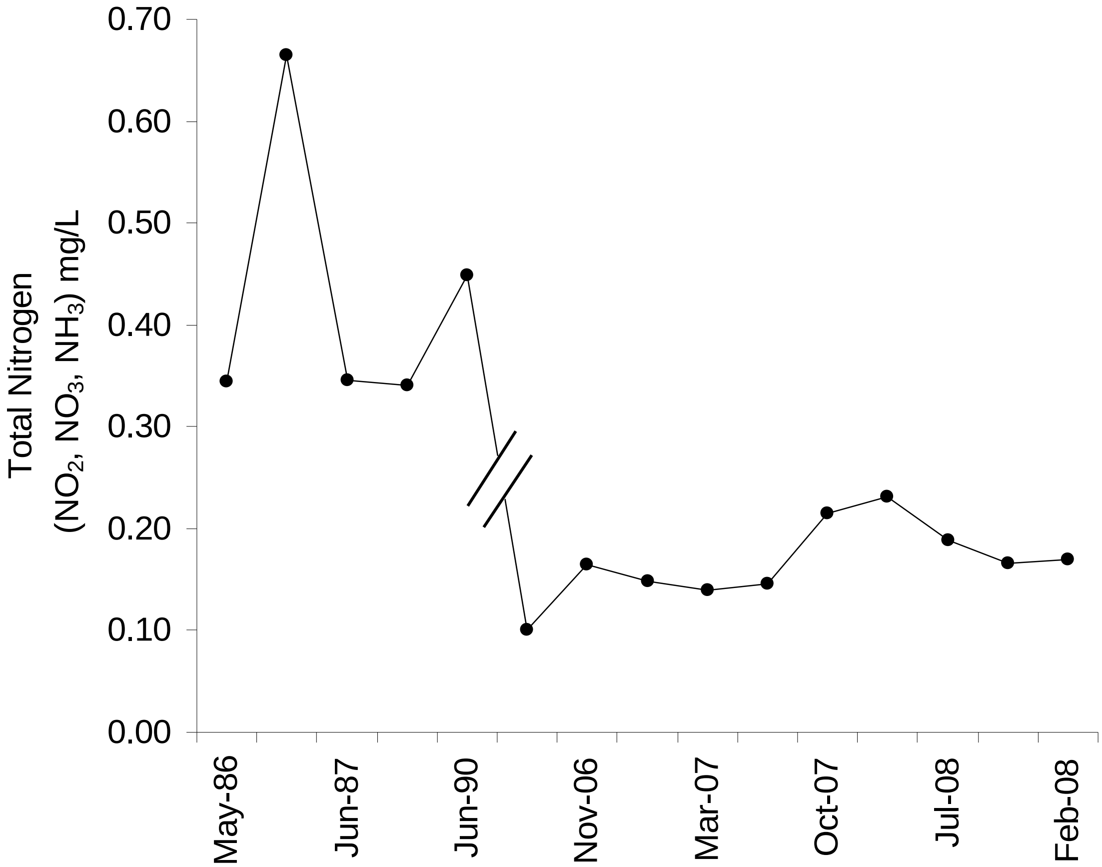


Figure 3 Total Nitrogen: Historical and Recent Data

Total phosphorus concentration at the Devils Hole averaged 7±0.8 μg/L. Overall phosphorus concentrations have remained steady with a historical average of 6±0.001 μg/L (Figure 4).

Sulfate remained stable throughout the study period, with an average of 80±0.7 mg/L.

The ash free dry mass for the shelf water ranged from 0.37-11 mg C/L, with a mean of 1.6±0.5 mg C/L. This amount is significantly larger when compared to the average ash free dry mass documented for oligotophic lakes (0.2 mg/L) (Wetzel 2001).

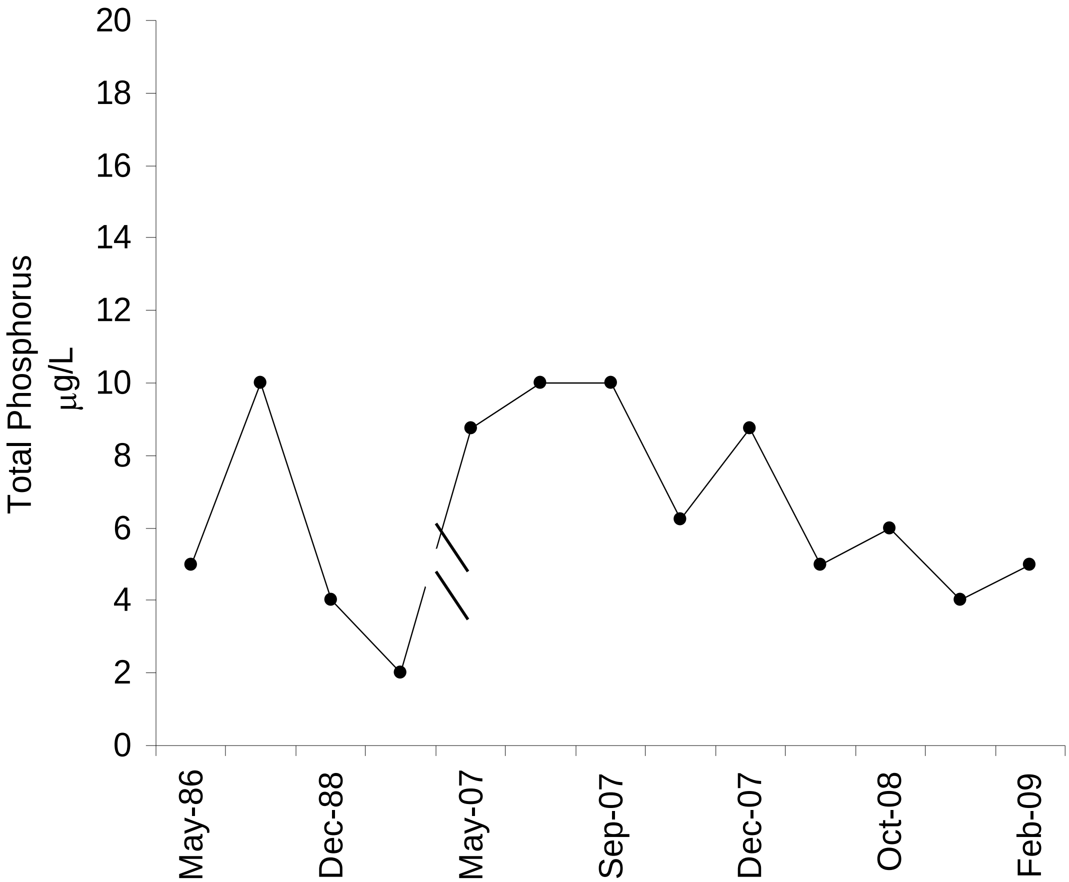


Figure 4 Total Phosphorus: Historical and Recent Data

(b) Devils Hole and Devils Hole Two Diving Events

The physical and chemical measurements taken during the dive at Devils Hole were constant along the vertical gradient. Devils Hole and Devils Hole Two did not differ significantly, except for pH which was slightly higher at Devils Hole Two.

Table 2 Physicochemical data from dive at Devils Hole and Devils Hole Two

(ft)	pH	EC uS/cm	SiO_2 mg/L	HCO_3 mg/L	Cl mg/L	SO_4 mg/L	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	Total P µg/L
0	7.99	670	22.0	309	20.9	80.3	67.9	8.25	49.6	21.4	3
30	8.08	676	21.9	310	20.9	79.6	68.0	8.20	49.5	21.1	2
75	8.08	671	21.9	305	20.9	80.6	67.5	8.12	49.6	21.2	8
120	8.14	668	22.0	307	21.3	80.6	67.8	8.27	50.0	21.3	3
DH Two	8.29	660	23.1	310	22.2	85.6	68.9	8.11	47.9	21.4	5

(c) Point of Rock Refugium

Total nitrogen (sum of NO_2, NO_3 and NH_3) showed a trend of slightly increasing throughout the course of the study. The first measurement taken during the month of March, total nitrogen was measured at 0.087 mg/L. The final measurement taken in December registered at 0.16 mg/L, with a peak seen in October at 0.233 mg/L. Nitrat also followed this trend.

Total phosphorus ranged between 7.5-13.1 µg/L, with a mean of 9.6±1.4.

Sulfate ranged from 78-81 mg/L with a mean of 78±0.8 mg/L.

Ash free dry mass ranged from 0.63-5.9 mg C/L, with a mean of 1.8±0.6 mg C/L. Ash free dry mass at Point of Rocks Refugium peaked at 5.9 mg C/L in April 2007.

3.3 Biological Measurements

(a) Devils Hole

Chlorophyll *a* measurements were taken to determine the primary productivity at Devils Hole. Overall, no clear trend in chlorophyll *a* emerged from our data set (Figure C-1). Chlorophyll *a* ranged from 0.0002-0.674 µg/L with a mean of 0.214±0.06 µg/L. Given the temporal and spatial heterogeneity of the upper shelf, discrete monthly sampling may not be sufficient in capturing the dynamics of primary production; therefore no concise conclusions can be made.

The molar ratio of nitrogen to phosphorus (N: P), the two most limiting macro-nutrients in freshwater ecosystems, has proven to be a key indicator in predicting the phytoplankton biomass, composition and community structure (Kim 2007). Reductions in the N: P ratio commonly selects for new species of phytoplankton, such as cyanobacteria, with competitive advantages in extracting/fixating nutrients from the resource pool (Smith 1983). Phosphorus has generally been considered the most limiting nutrient for algal growth in freshwater lakes (Elser et al. 1990), nevertheless, the trends in the historical and current data analyzed here indicates the nitrogen concentration has declined considerably in the past 20 years, while phosphorus concentration has remained stable. The historical data from Devils Hole reflects N: P molar ratio of 210:1 in 1986 to the lesser, current level of 52:1 (as of February 2009). In order to test the hypothesis that the precipitous decline in nitrogen relative to phosphorus may confer a competitive advantage to one or more resident algal species of the upper shelf, a series of laboratory bioassays was conducted.

Samples were collected in late fall (29-Ocotber 2008), identified and enumerated to obtain the base-line density and composition of the planktonic and benthic algae. Four algal species were found to be dominating the system: *Chroococcus*sp. (class: Cyanophyceae), *Denticula elegans*(class: Bacillariophyceae, dominant diatom at Devils Hole (Shepard et al. 2000), *Monoraphidium*sp.(class: Chlorophyceae), and *Oscillatoria*sp.(class: Cyanophyceae). All of the identifications were consistent with algae previously identified at Devils Hole with the exception of the non-motile, planktonic green alga, *Monoraphidium* (Shepard et al. 2000). *Spirogyra* is known to be a prominent member of the spring and summer flora, but was not captured during our fall bioassay sampling.

For each treatment level, the density of all algae was pooled together to obtain the composite density. Analysis of variance showed there to be a significant variation between the 20:1 and 100:1 treatments ($p=0.001$ and $p=0.01$, respectively). The mean density of the 20:1 treatment (29,625± 3653 cells/mL) was significantly lower than the mean density of the 100:1 treatment (67,336±5470 cells/mL) (Figure5). *D. elegans* differed marginally amongst 20:1 and 100:1 (p=0.07 and 0.06, respectively) with the diatom being 62% more dense in the 100:1 treatment relative to the 20:1 treatment.

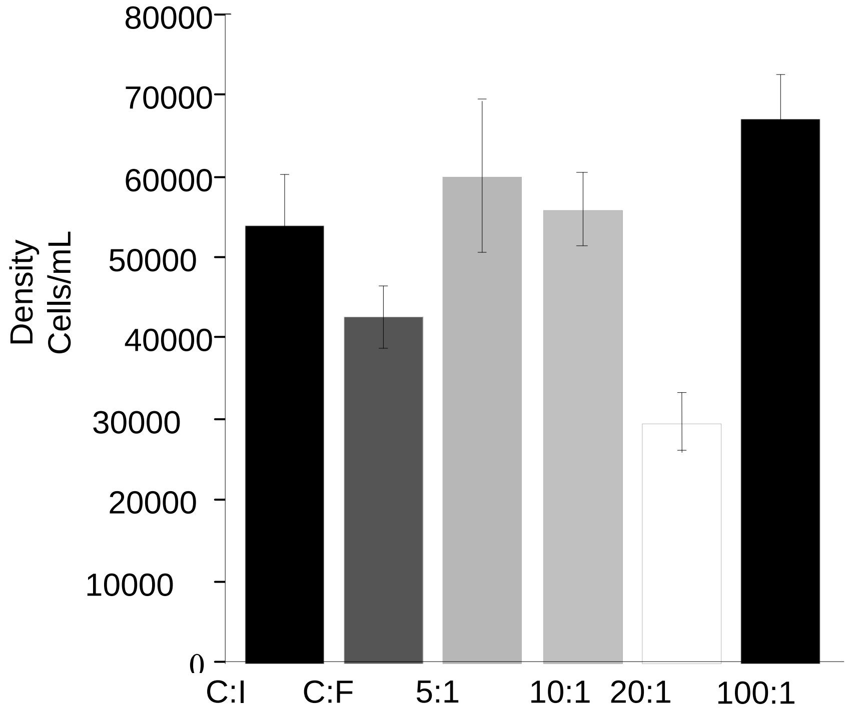


Figure 5 Algae Bioassay Composite Density Results

When different species were compared across treatments the average *Chroococcus* growth, 36620±3787 cell/mL, in the 10:1 treatment was significantly higher than the 20:1 treatment, 21000±3233 cell/mL.

Monoraphidium did not differ amongst treatment levels except for control initial (C:I) (p=0.0002) which had a higher average mean with 386 cells/mL compared to control final (C:F) with 152 cells/mL.

Oscillatoria was significantly different at the 10:1 (p=0.001) and 20:1 (p=0.02) treatment levels with the 10:1 treatment having a higher mean (924 cells/mL) relative to 20:1 with 226 cells/mL.

Biovolume was estimated for *Oscillatoria* only based on observations of differing lengths between treatments not witnessed in the other algal species. Density measurements do not take into account algal biomass and the use of biovolume estimation more accurately accounts for the biomass and overall productivity of a phytoplankton species (Hillebrand 1999). From our biovolume estimates, the only significant treatment level was 10:1 (Figure C-3). This estimates shows 10:1 to have the highest mean biovolume 1.8×10^7 mm^3/L ($p<0.001$). The 10:1 treatment exhibited the lengthiest *Oscilllatoria* filaments as well as the highest density.

The results from our bioassay provide indications to how further reductions in nitrogen at Devils Hole may affect the phytoplankton community composition. Both blue-green species, *Chrococcus* and *Oscillatoria*, thrived better in the 10:1 treatment levels. This supports previous observations in lakes by Tilman et al. (1982) in which blue-green algae that fix nitrogen were more abundant when the N: P ratios are low. The decrease in *Denticula* in the 20:1 treatment relative to the 100:1 treatment indicates that nitrogen limitation may cause a decline in the most abundant diatom at Devils Hole and an important element of both pupfish and riffle beetles, *Stenelmis calida*diets (Shepard 2000).

(b) Sediments of Devils Hole

From the upper shelf several diatoms were positively identified in the sediments: *Denticula elegans*,*Nitzschia amphibian, Achnanthes exigua* var. *heterovalva*,*Nitzschia dissipata*, and *Synedra* sp.

(c) Point of Rocks Refugium

Chlorophyll *a* ranged from 0.008-0.9 µg/L, with an average of 0.3±0.1 µg/L. No clear trend arose from discrete monthly sampling.

4. Conclusions and Recommendations

The physicochemical conditions at Devils Hole have remained markedly constant since data collection began in the 1970s. High temperatures and swings in dissolved oxygen have concurrently placed significant metabolic restraint on the Devils Hole pupfish. Nevertheless, the hearty Cyprinodont has evolved to endure such extreme conditions.

An alarming trend revealed in the study presented here is the precipitous decline in total nitrogen concentrations since the mid-1980s when the United States Geological Survey began measuring nutrient levels at Devils Hole. Total nitrogen went from a high of 0.67 mg/L in November 1986 to the current level of 0.17 mg/L. Although the mixing patterns and hydrologic connectivity of the thermal spring has not been extensively investigated, our cation and anion data at various depths provide anecdotal information on the movement of water within the system. From the top of the pool to 120 feet into the cavern, electrical conductivity, chlorine, and sodium did not vary along the gradient. Additionally, Devils Hole Two had comparable levels of these ions. This indicates that the water within Devils Hole is perhaps well-mixed.

Although many processes control the amount of nitrogen in freshwater systems, several trends have been recorded in relation to nitrogen and desert ecosystems, particularly the American Southwest. Nitrogen deposition from the atmosphere either through dry fallout of particles and gases or wet deposition in the form of precipitation has a significant role in adding nitrogen to terrestrial and aquatic ecosystems (Chadwick et al. 1999). For example in many mountainous regions with oligotrophic lakes precipitation is the major source of nitrogen (Likens 1977). Fenn et al. (2003) have suggested that vast acreages of land in the western US are exposed to low levels of atmospheric nitrogen deposition with interspersed hotspots. They demonstrate that stream water nitrate concentrations are elevated in southern California and in some catchments of the Southwestern Siera Nevada. Wilson and Blinn (2007) further demonstrated the importance of allochothonous inputs (and perhaps nutrients associated with them) and rare rain events on the energy budget of Devils Hole. Beginning in the 1970s a barb wire fence was set in place along the upper perimeter of the cavern opening amidst fear of vandalism. The presence of this structure might constrain the inputs of allochthonous carbon and particulate input (and associated nutrients), slowly changing the energy budget of the hole. Further, the increased occurrence of drought due to global climate change may further restrict allochthonous carbon and particulate inputs.

Alternatively, the extensive carbonate-aquifer system which reaches from Southern Nevada to Eastern California has unknown connectivity (Belcher 2004). Therefore, source water at Devils Hole may be connected to other springs, effectively acting as sinks for nutrients.

As indicated by our bioassay, a reduction in the N: P may have caused valuable algae such as *Denticula* to decline and cyanobacteria species to increase. Cyanobacteria such as *Oscillatoria* are neither significant to the pupfish's diet nor the preferred food choice of food for the riffle beetle. Further declines in N: P ratio on the upper shelf might cause edible foods to diminish, placing further constraints on the pupfish.

It is recommended that the monitoring of the limnological patterns of Devils Hole continue. Detailed analysis of the nutrient profile of the upper shelf both vertically and horizontally with particular attention to the benthos would be beneficial to understand nutrient sources. Further investigation of carbon and particulate inputs as well as diel and seasonal patterns will help in understanding the important of external and internal factor to shelf energetics. Lastly, large scale global changes such as drought and rising CO_2 level should also be looked at to determine how these macro-processes are impacting the local processes of Devils Hole.

Also, to better understand the potential linkages between Devils Hole pupfish population and food quality and quantity, several limnological parameters should be monitored on a regular basis. Some of these include algae and invertebrate community composition and the abundance. Similarly, patterns of primary and secondary production should be investigated, with particular emphasis on the role of nutrient concentrations and stoichiometric ratios.

Wilson and Blinn (2005) showed that the input of allochthonous material is essential to the energetics of the Devils Hole ecosystem and demonstrated a strong positive correlation between total annual precipitation and maximum number of pupfish over a 32-year period, which they concluded was largely from the inputs during rain events. Therefore long term monitoring of allochthonus material is recommended.

5. References

Andersen, M. E. and J. E. Deacon (2001) Population size of Devils Hole pupfish (Cyprinodon diabolis) correlates with water level. Copeia 1:224-228.

APHA (1992) Standard methods for the examination of water and wastewater, 18 ed. American Public Health Association (APHA), Washington, DC.

APHA (2005) Standard methods for the examination of water and wastewater, 21 ed. American Public Health Association (APHA), Washington, DC.

Belcher, W.R., ed., 2004, Death Valley regional ground-water flow system, Nevada and California—Hydrogeologicframework and transient ground-water flow model: U.S. Geological Survey Scientific Investigations Report2004–5205, 408 p.

Blinn, D.W., Wilson, K.P, and D.B. Herbst (2000) Devils Hole energetics/community Relationships: Death Valley National Park, California. Death Valley National Park Annual Report 6 December 2000.

Chadwick, O.A., Derry, L.A., Vitousek, P.M., Hubert, B.J., and L.O. Hedin (1999) Changing sources of nutrients during four million years of ecosystem development. Nature 397**:** 491–497.

Deacon, J.E., and T.M. Baugh (1983) Annual Report on Devils Hole 1983. Report the the National Park Service Death Valley National Monument, pg 18.

Deacon, J.E., and C. Williams (1991) Ash Meadows and the legacy of the Devils Hole pupfish. In Battle against extinction.W.L. Minckley and J.E. Deacon, eds. University of Arizona Press, Tucson pg 69-87.

Deacon, J.E., F.R. Taylor, and J.W. Pedretti (1995) Egg viability and ecology of the Devils Hole pupfish: insights from captive propagation. The Southwestern Naturalist 40(2):216-223.

Elser, J.J., Marzolf E.R., and C.R. Goldman (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences 47: 1468–1477 (1990)

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., Bowman, W. D., Sickman, J. O., Meixner, T., Johnson, D. W. and Neitlich, P. (2003). Ecological effects of nitrogen deposition in the western United States. BioScience 53: 404-420.

Gustafson, E.S., and J.E Deacon (1998) Distribution of larval Devils Hole pupfish, *Cyprinodon diabolis* Wales, in relation to dissolved oxygen concentration in Devils Hole. Final report, Death Valley National Park, 1 April 1998.

Herbst, D.B. (2003) Devils Hole benthic inverterbrate community dynamics: distribution, seasonality and production. Final report to Death Valley National Park. 32 pp

Hillebrand, H, Claus-Dieter, D., Kirschte, D., Pollingher, U. and T. Zohary (1999) Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35:403-424.

James, C.J. (1969) Aspects of the ecology of the Devils Hole pupfish, Cyprinodon diabolis Wales. M.S. thesis, University of Nevada Las Vegas pg 70.

Kim, H., Hwang, S., Shin, J., An, K., and C.G Yoon (2007). Effects of limiting nutrients and N:P ratios on the phytoplankton growth in a shallow hypertrophic reservoir. Hydrobiologia 581:255-267.

Likens, G.E., Bormann, F.H., Pierce, R.S., Eaton, J.S., and N.M Johnson (1977) Biogeochemistry of a forested ecosystem. Springer-Verlag, New York.

Lyons, L.T. (2005) Temporal and spatial variation in larval Devils Hole pupfish (*Cyprinodon diabolis)* abundance and associated microhabitat variable in Devils Hole, Nevada. M.S. Thesis Southern Oregon University.

Minckley, C.O., and J.E. Deacon (1973) Observations on the reproductive cycle of *Cyprinodon diabolis* Copeia 3:610-613.

Minckley, C.O. and J.E Deacon(1975) Food of the Devils Hole pupfish, *Cyprinodon diabolis* (Cyprinodontidae). The Southwestern Naturalist 20(1):105-111.

Riggs, A.C., and J.E. Deacon(2004) Connectivity in desert aquatic ecosystems: the Devils Hole story. Conference Proceedings. Spring-fed Wetlands: Important Scientific and Cultural Resources of the Intermountain Region, 2002. http://www.wetlands.dri.edu

Shepard, W.D., Blinn, D.W., Hoffman, R.J., and P.T. Kantz (2000) Algae of Devils Hole, Nevada, Death Valley National Park. Western North American Naturalist 60(4):410-419.

Smith, V.H. (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221:669-671.

Tilman, D., Kilham, S.S., and P. Kilham (1982) Phytoplankton and community ecology: the role of limiting nutrients. Annual Review of Ecology and Systematics 13:349-372.

USEPA (1993) Determination of sulfate, chloride, bromide, and nitrate by ion chromatography EPA method 300.0 US Environmental Protection Agency Cincinnati, Ohio.

USEPA (1997) In vitro determination of chlorophyll a and pheophytin a in marine and freshwater algae by fluorescence. EPA method 445.0. US Environmental Protection Agency Cincinnati, Ohio.

US Fish and Wildlife Service (2008) The Devils Hole pupfish.
http://www.fws.gov/Nevada/protected_species/fish/species/dhp/dhp.htmL

Wilson, K.P., and D.W. Blinn. (2005). Allochthonous carbon: An important energy source in desert spring ecosystems. Report submitted to Death Valley National Park.

Wilson, K.P, and D.W. Blinn (2007) Food structure, energetics, and importance of allochtonous carbon in a desert cavernous limnocrene, Devils Hole, Nevada. Western North American Naturalist 67(2):185-198.

Wetzel, R.G (2001). Limnology: lakes and river ecosystems. 3rd Ed. Academic Press San Diego, California.

A. Appendix: Physical parameters

Appendix A presents the physical characteristics of the Upper Shelf of Devils Hole and Point of Rocks Refugium.

Figure A-1 Devils Hole: Alkalinity

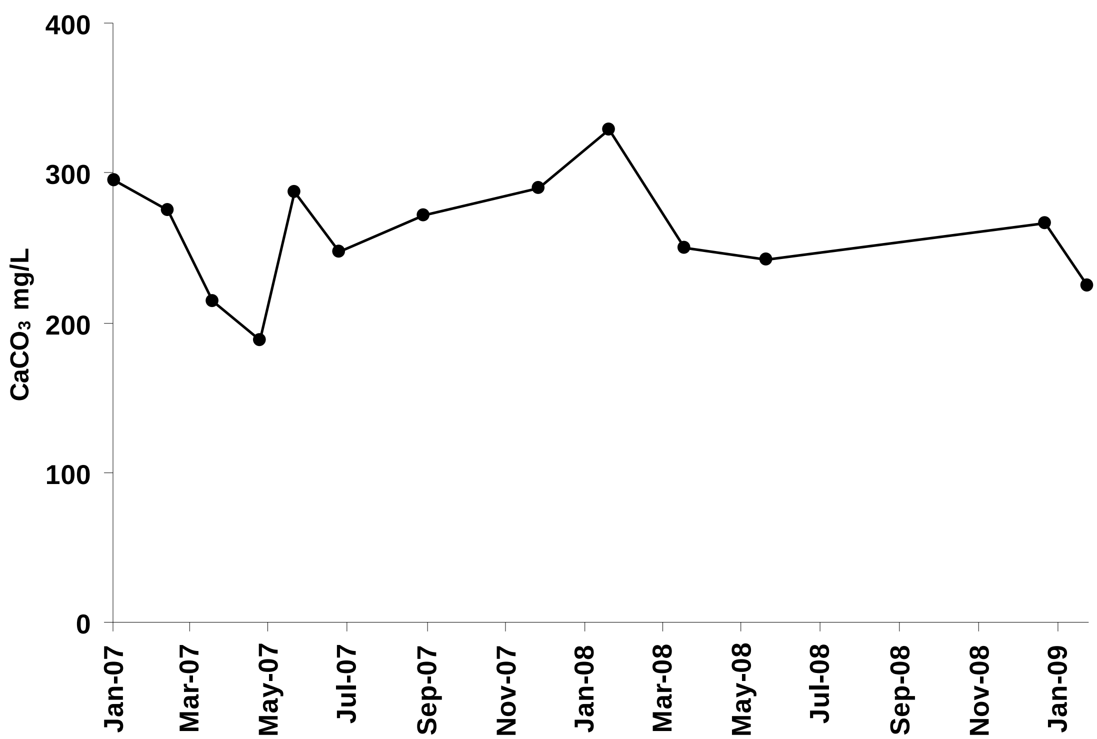


Figure A-2 Devils Hole: Turbidity

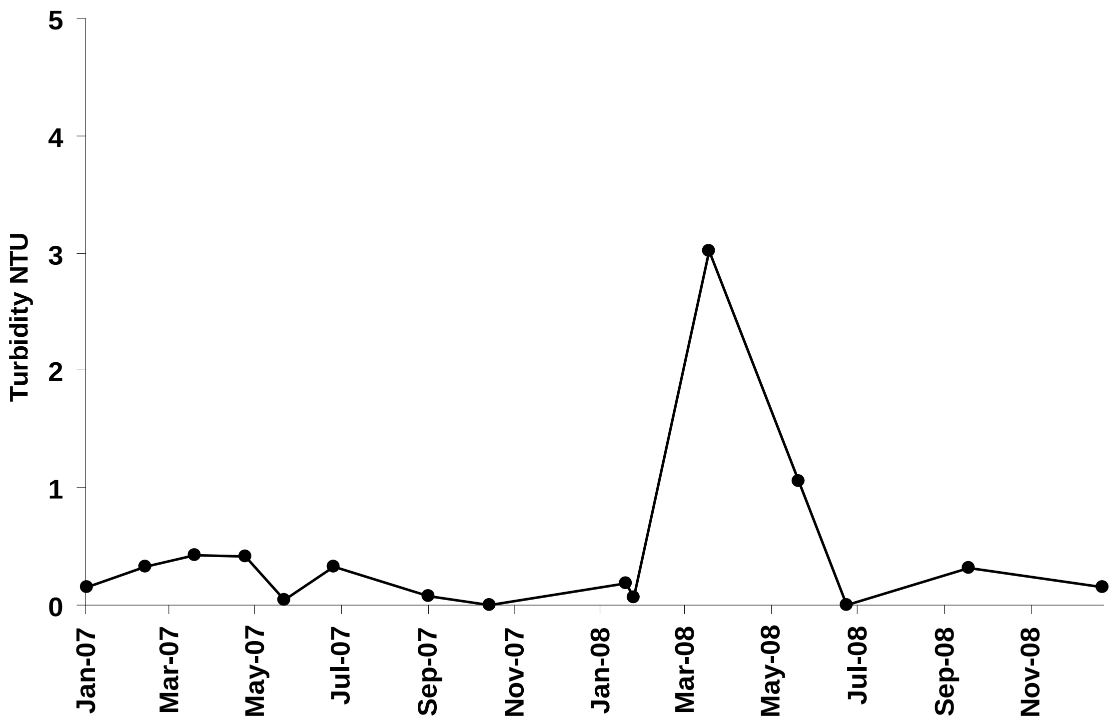


Figure A-3 Point of Rock Refugium: Alkalinity

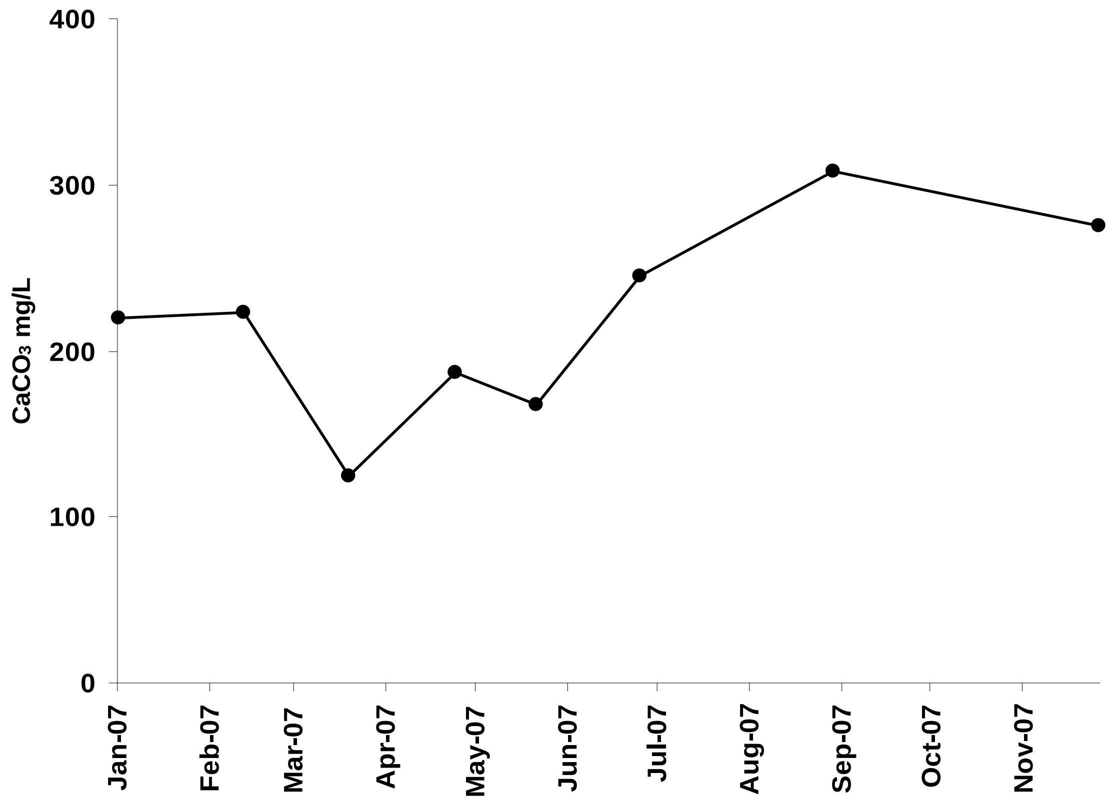


Figure A-4 Point of Rock Refugium: Turbidity

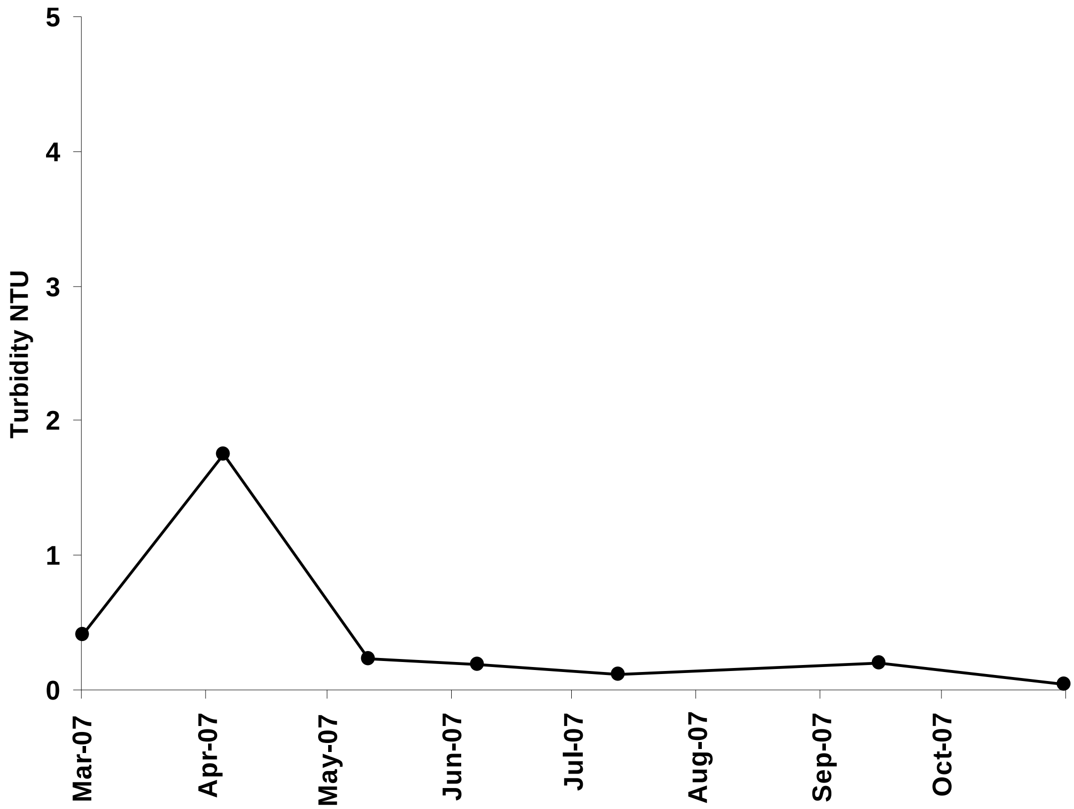
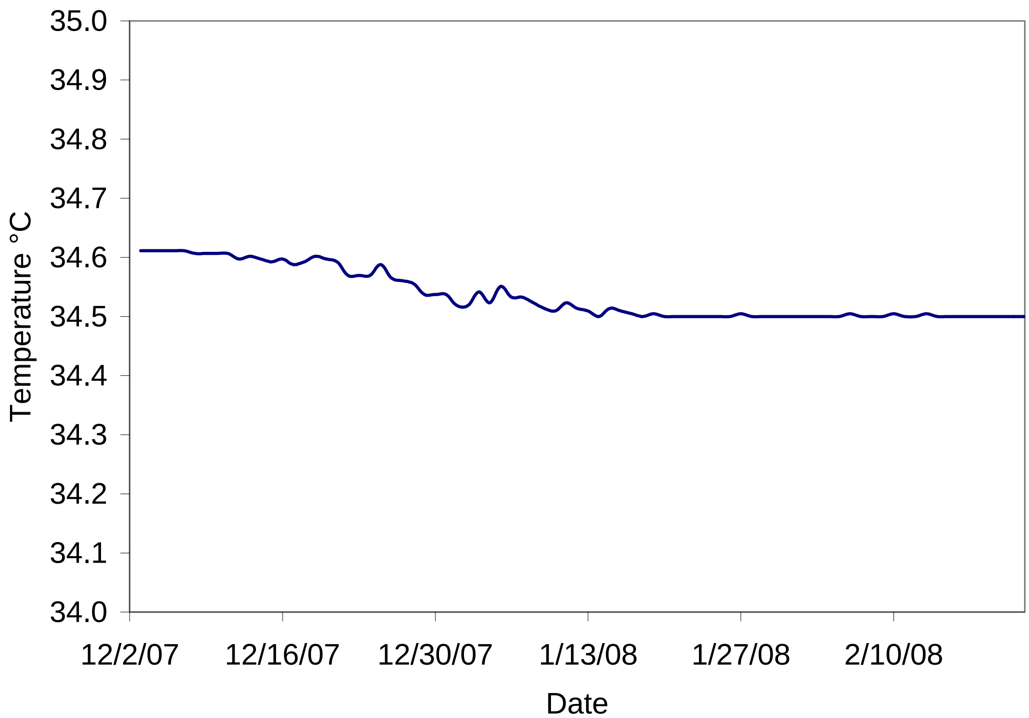


Figure A-5 Devils Hole: Continuous Temperature at 30 m Depth



B. Appendix: Chemical parameters

Appendix B presents the chemical characteristics measured at Devils Hole and Point of Rocks Refugium.

Table B-1 Devils Hole: Nitrogen concentration expressed in mg/L

	NO_2-N	NO_3-N	NH_3-N	Total N
01-Nov-06	0.001	0.154	0.009	0.165
05-Dec-06	0.001	0.143	0.003	0.148
18-Jan-07	0.002	0.151	0.003	0.155
01-Mar-07	0.001	0.135	0.002	0.139
05-Apr-07	0.001	0.142	0.006	0.149
11-May-07	0.001	0.132	0.007	0.140
07-Jun-07	0.001	0.142	0.003	0.146
12-Jul-07	0.001	0.158	0.033	0.192
18-Sep-07	0.001	0.150	0.014	0.166
05-Oct-07	0.001	0.160	0.009	0.171
31-Oct-07	0.001	0.184	0.025	0.215
13-Dec-07	0.003	0.157	0.003	0.161
05-Feb-08	0.001	0.216	0.014	0.231
04-Apr-08	0.001	0.170	0.018	0.190
06-Jun-08	0.001	0.081	0.005	0.087
10-Jul-08	0.001	0.139	0.009	0.188
05-Oct-08	0.002	0.155	0.032	0.166
08-Jan-09	0.001	0.158	0.013	0.167
10-Feb-08	0.001	0.178	n/d	0.169

Table B-2 Point of Rock Refugium: Total nitrogen concentrations expressed in mg/L

	NO_2-N	NO_3-N	NH_3-N	Total N
01-Mar-07	0.001	0.085	0.001	0.087
05-Apr-07	0.001	0.104	0.002	0.107
11-May-07	0.001	0.110	0.008	0.119
07-Jun-07	0.001	0.114	0.005	0.119
12-Jul-07	0.001	0.113	0.016	0.129
18-Sep-07	0.001	0.120	0.013	0.134
31-Oct-07	0.004	0.205	0.024	0.233
13-Dec-07	0.001	0.157	0.003	0.160

Figure B-1 Devils Hole: Nitrite

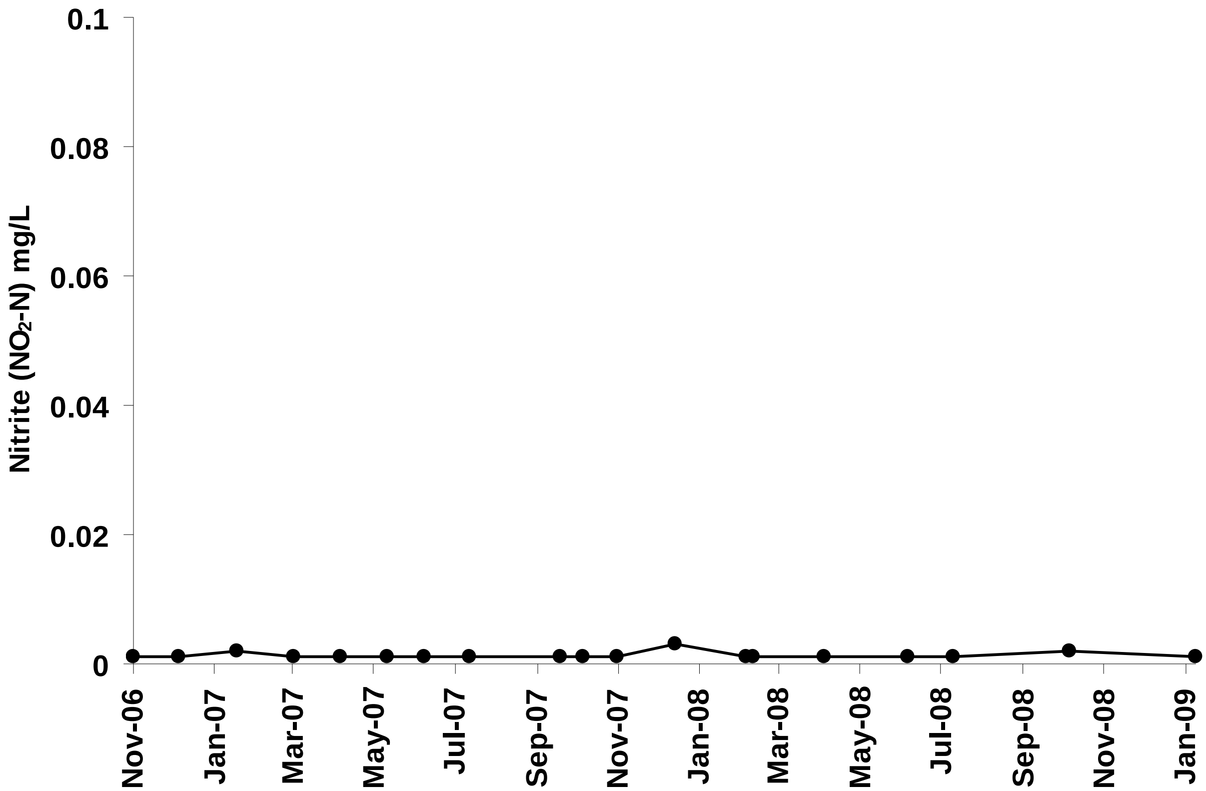


Figure B-2 Devils Hole: Nitrate

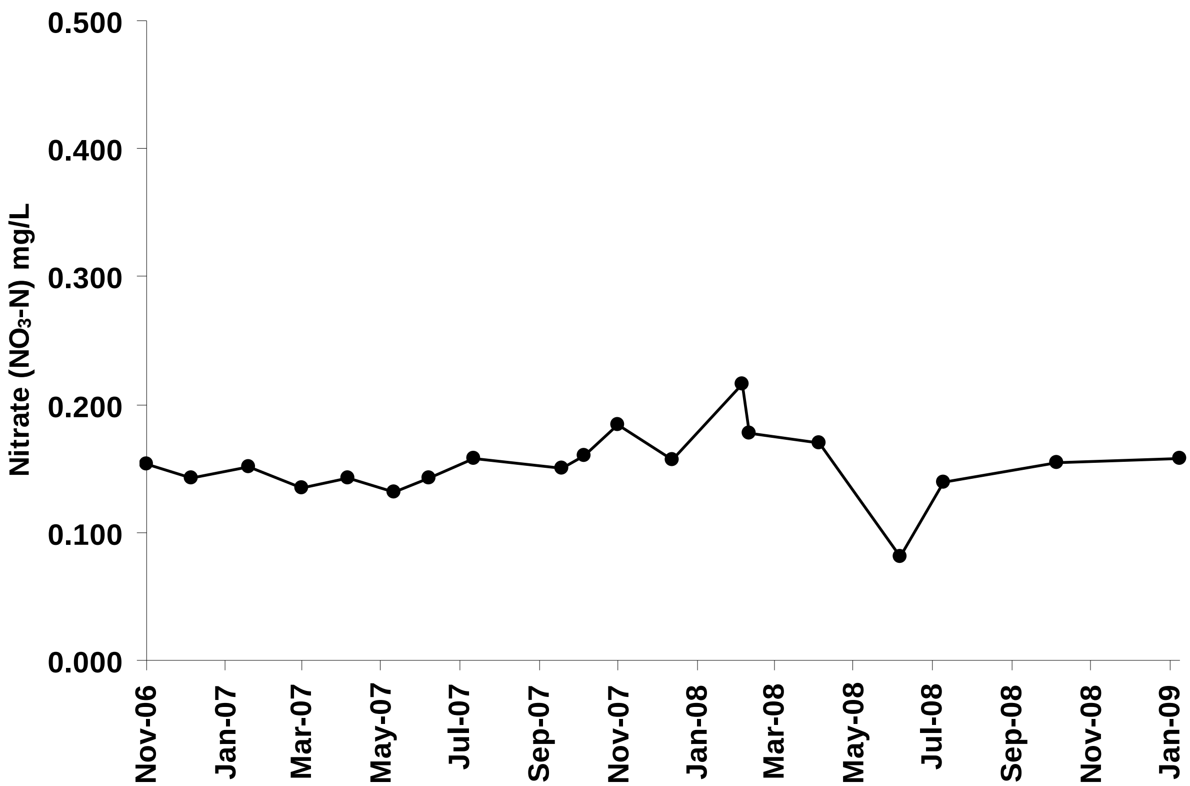


Figure B-3 Devils Hole: Ammonia

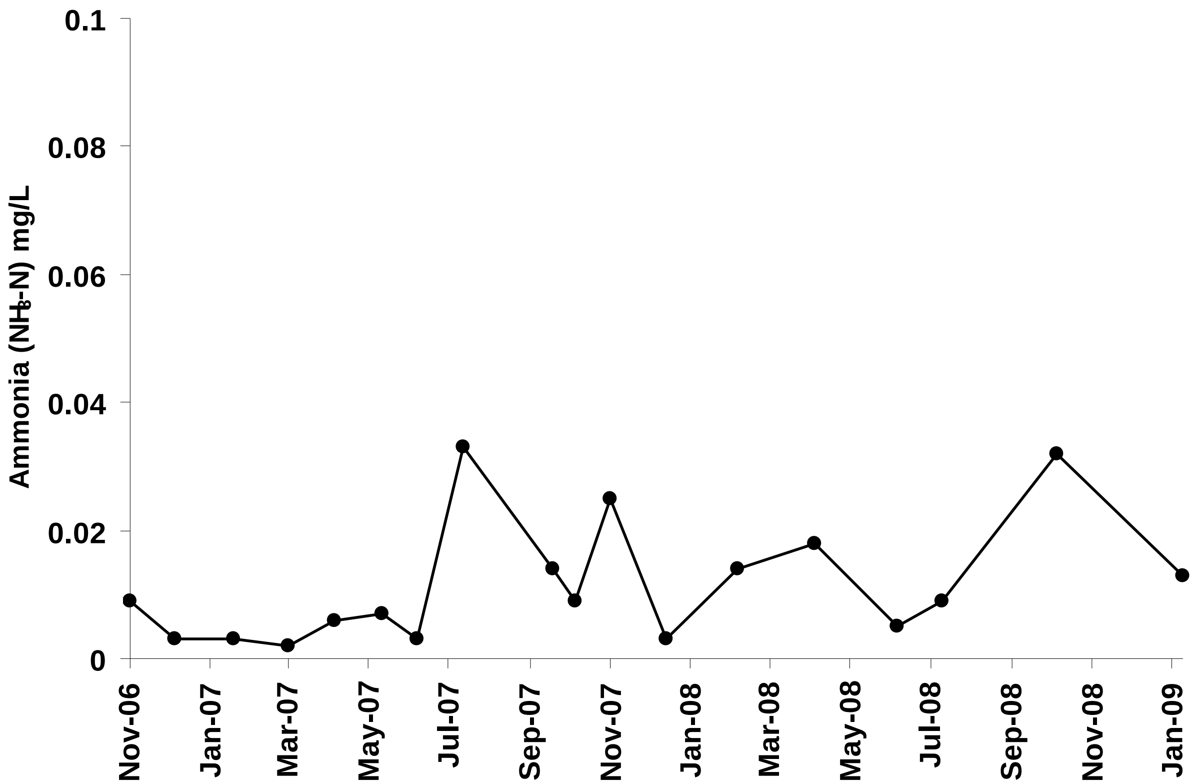


Figure B-4 Devils Hole: Total Nitrogen

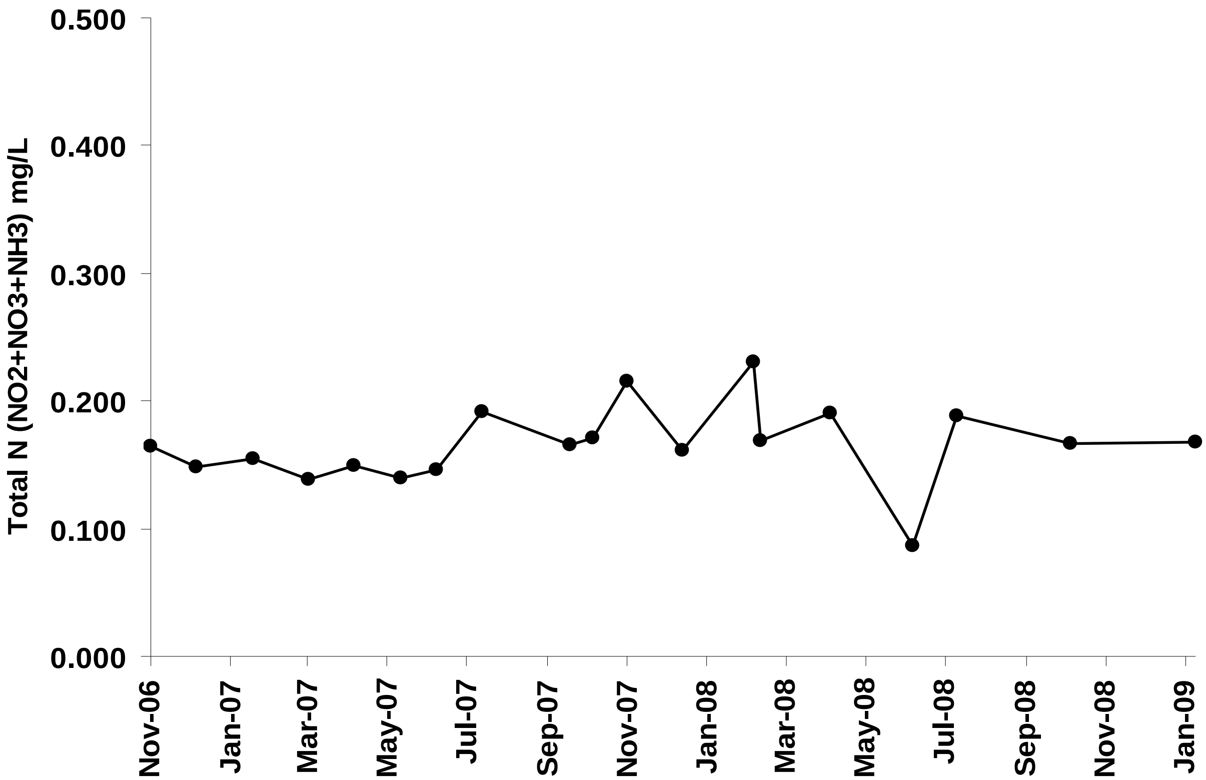


Figure B-5 Devils Hole: Phosphorus

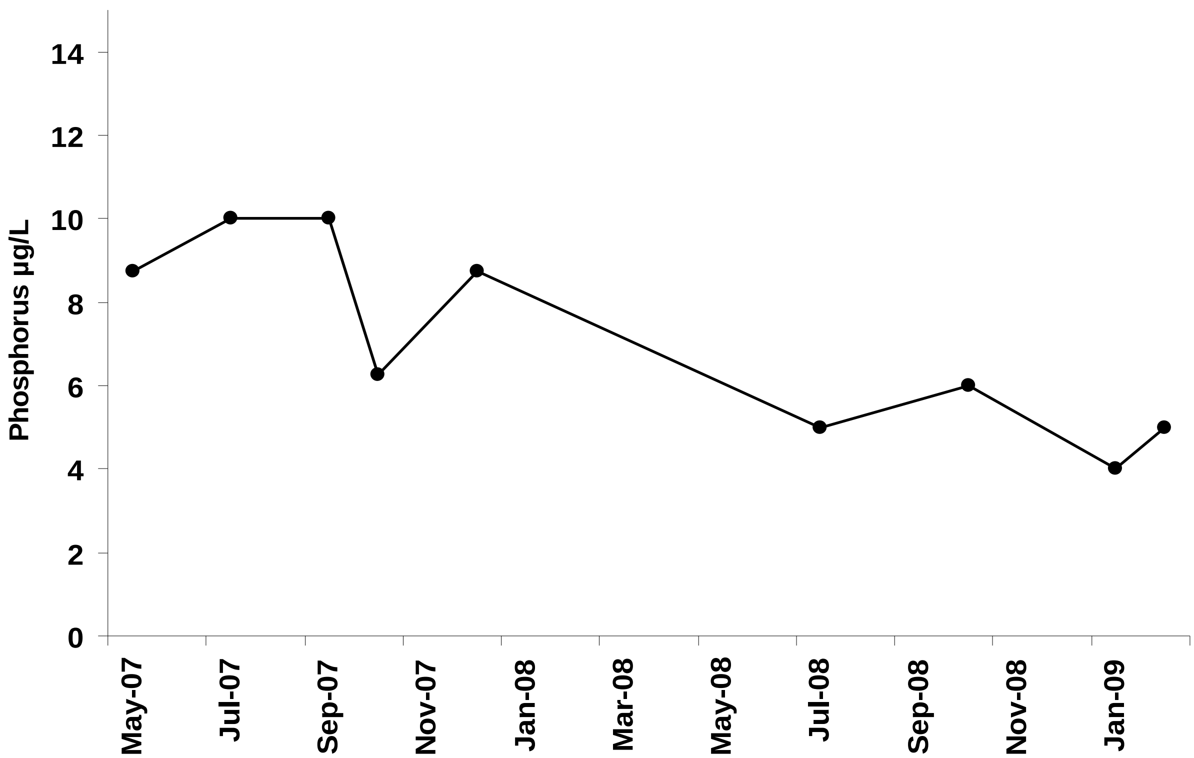


Figure B-6 Devils Hole: Sulfate

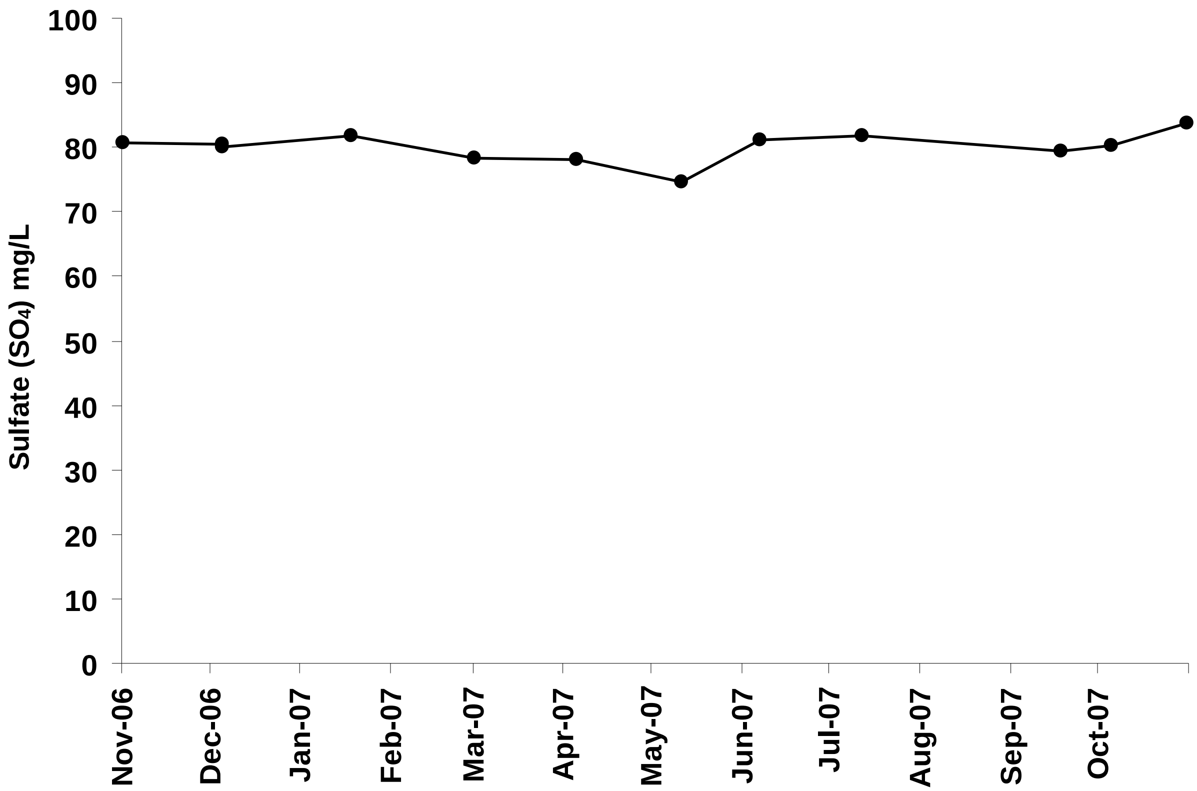


Figure B-7 Devils Hole: Organic Carbon (measured as ash free dry mass)

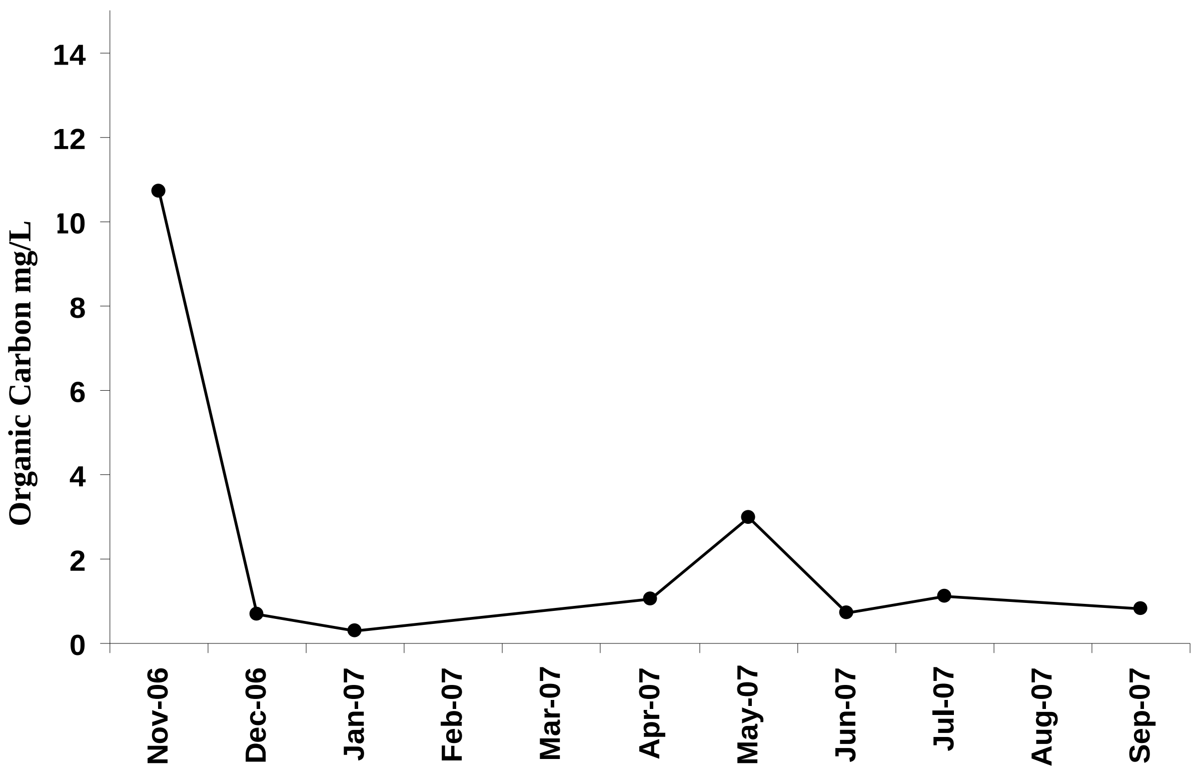


Figure B-8 Point of Rocks Refugium: Nitrite

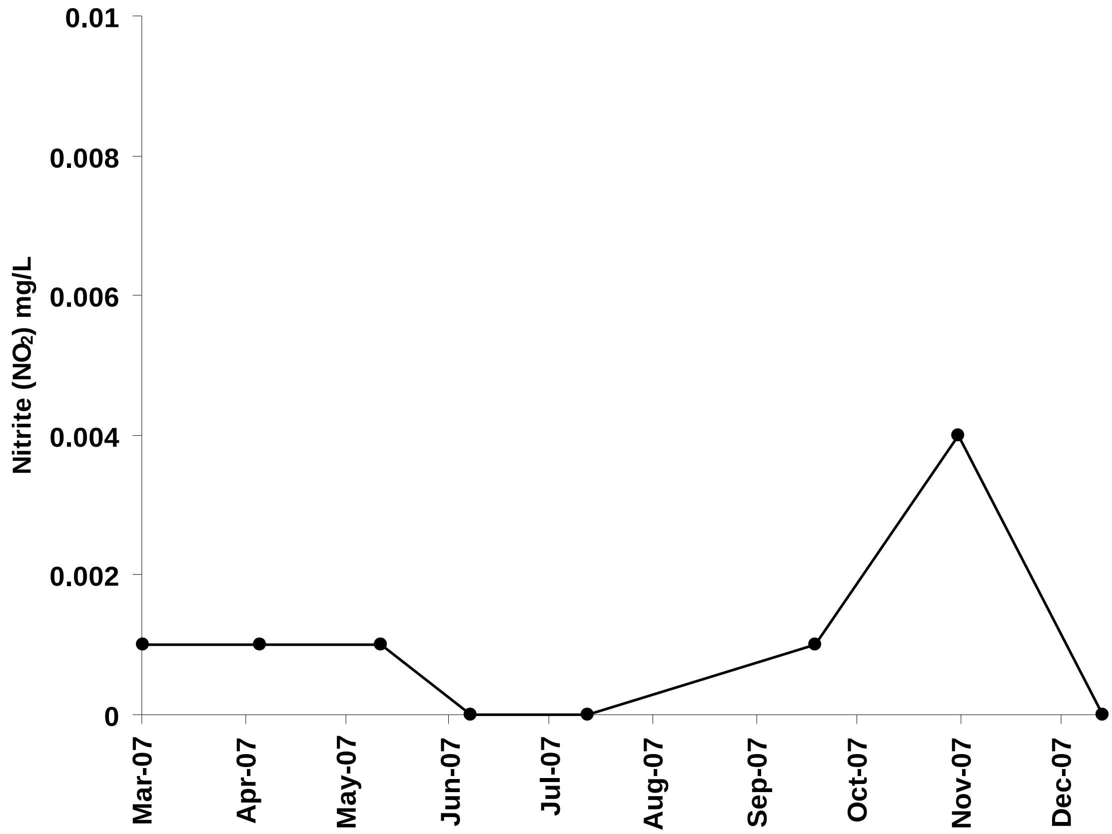


Figure B-9 Point of Rocks Refugium: Nitrate

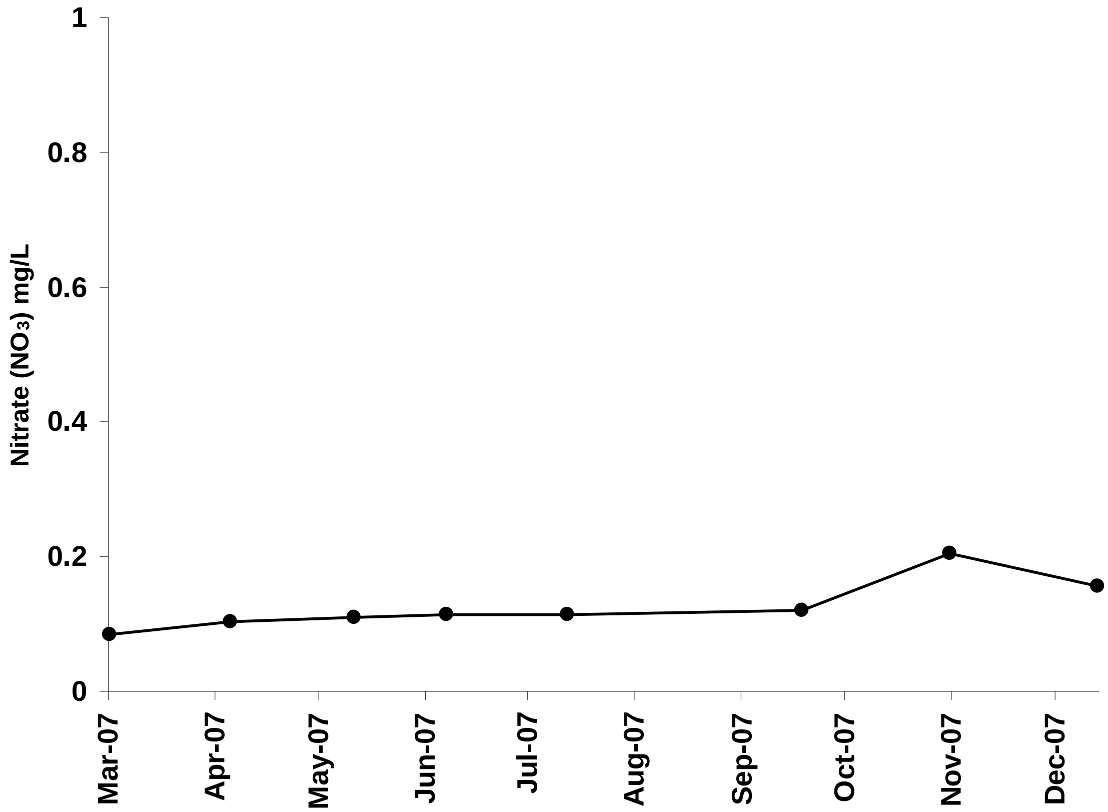


Figure B-10 Point of Rocks Refugium: Ammonia

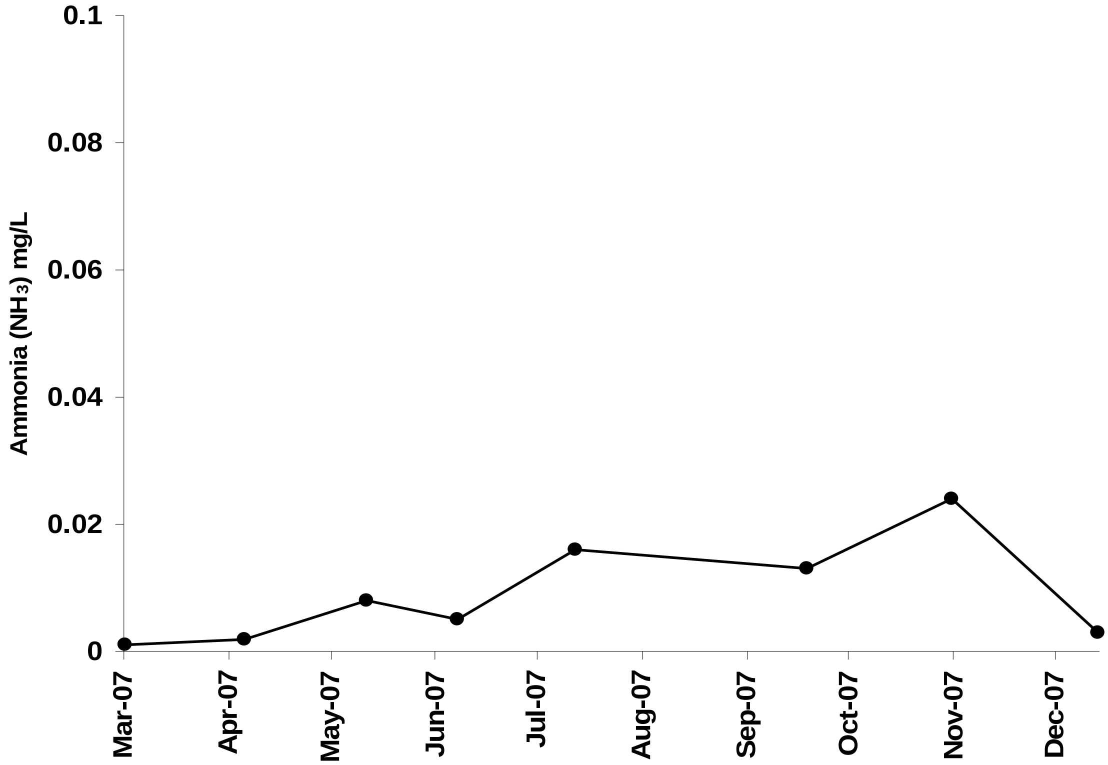


Figure B-11 Point of Rocks Refugium: Total nitroge

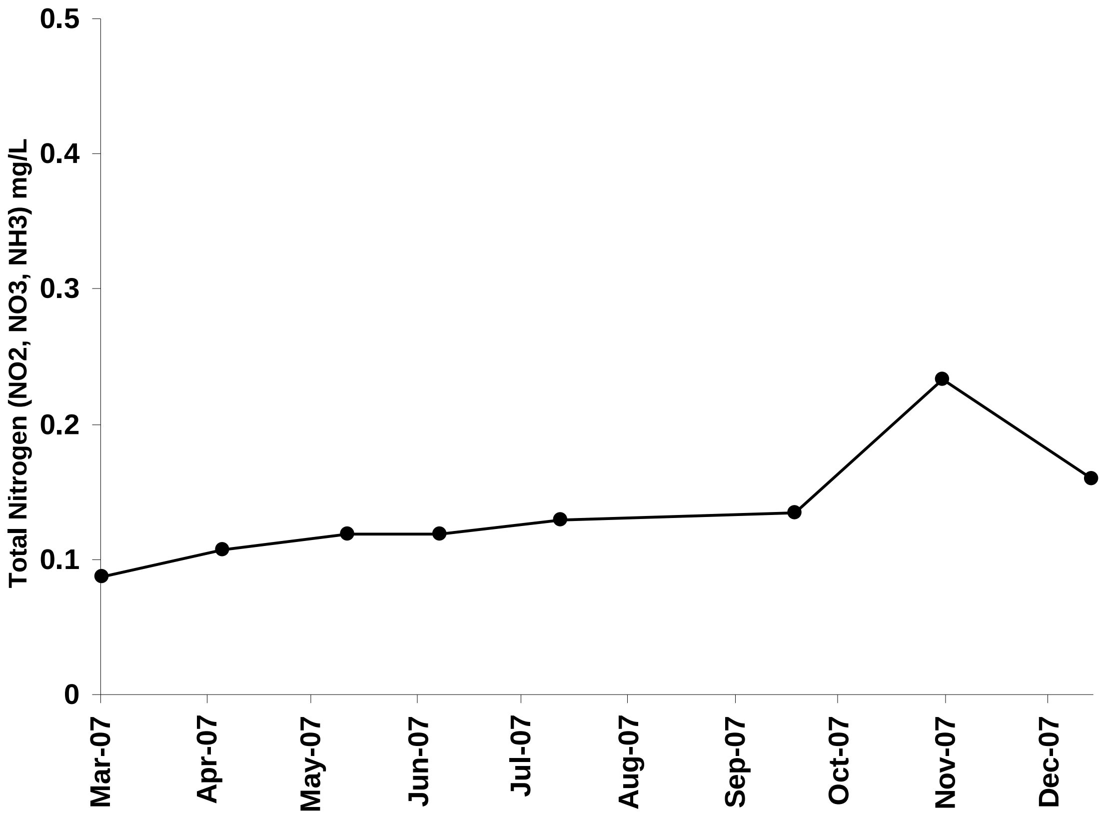


Figure B-12 Point of Rocks Refugium: Total Phosphorus

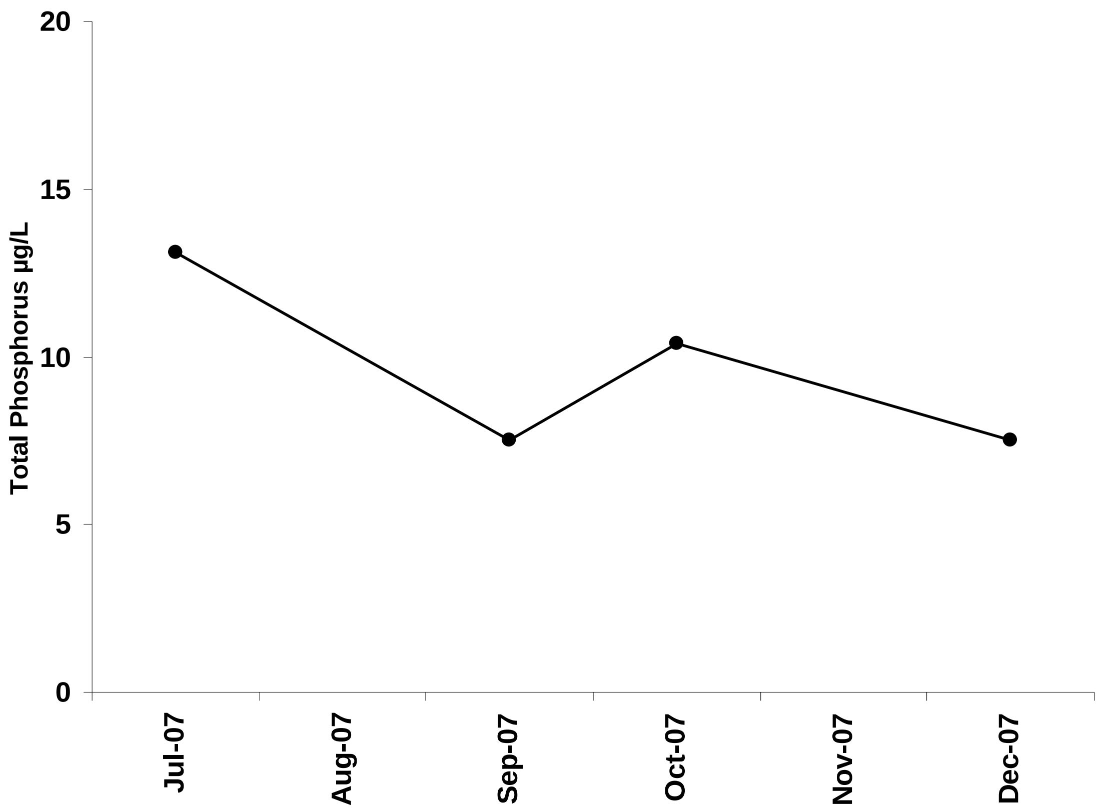


Figure B-13 Point of Rocks Refugium: Sulfate

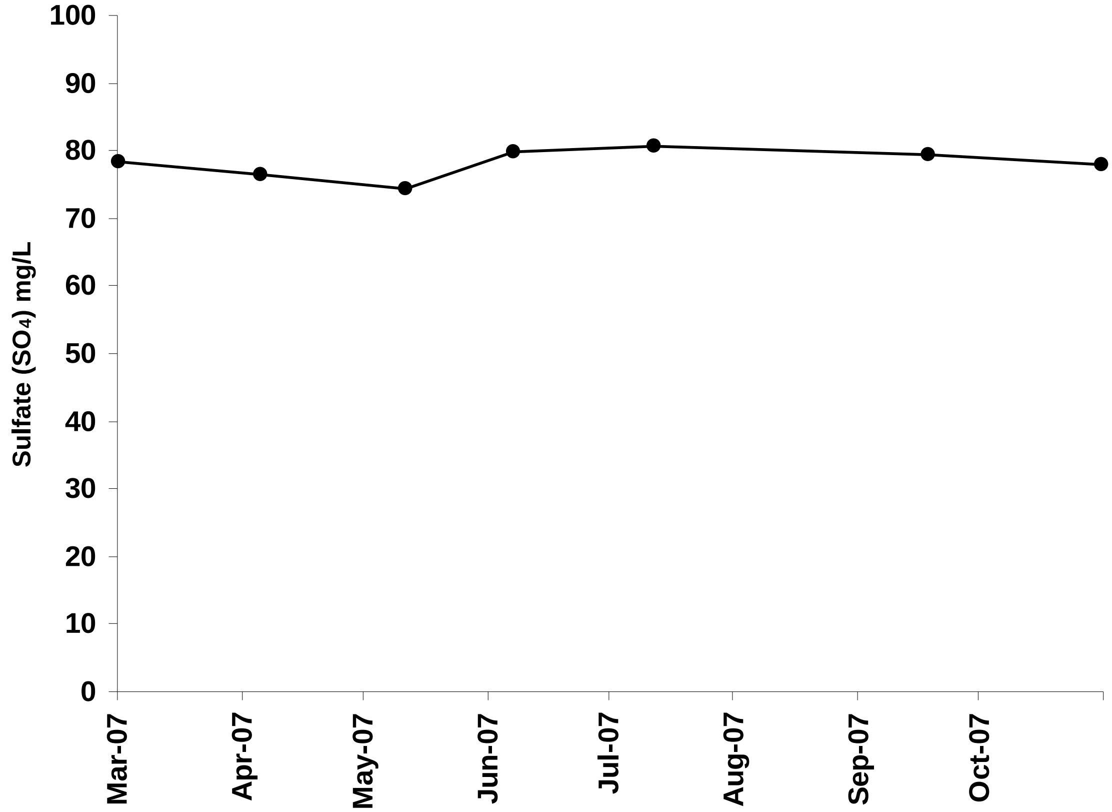
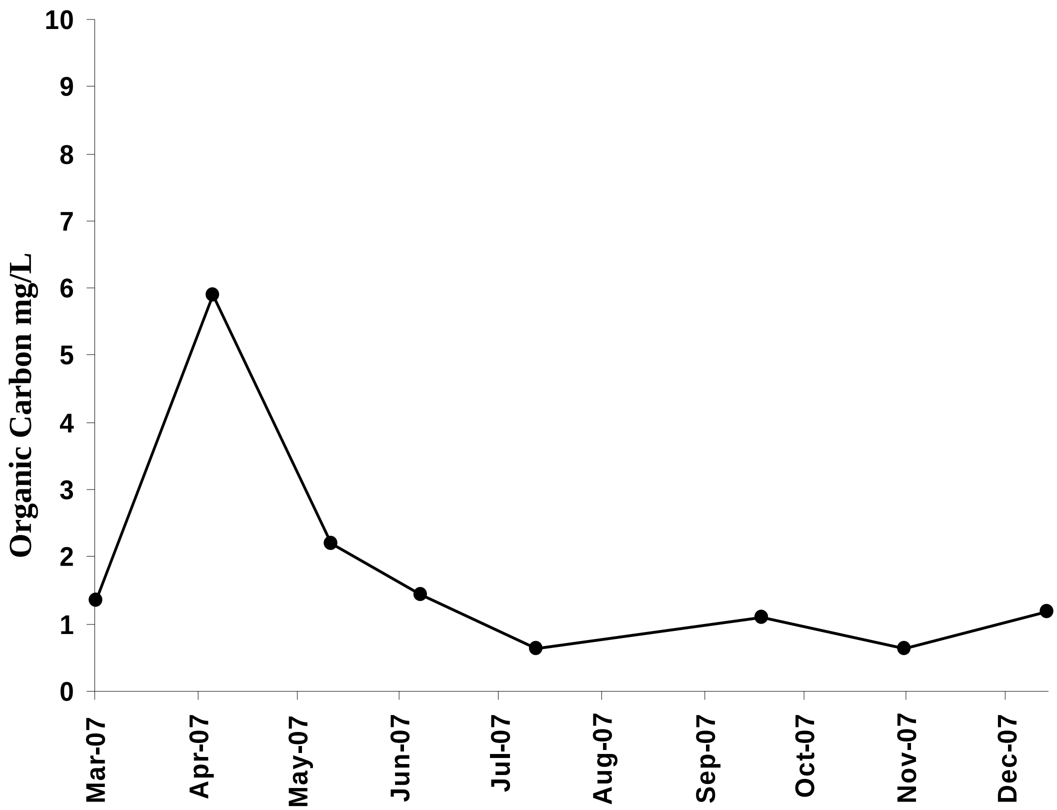


Figure B-14 Point of Rocks Refugium: Organic Carbon (measured as ash free dry mass)



C. Appendix: Biological parameters

Appendix C presents the biological characteristics of the upper shelf at Devils Hole and Point of Rocks Refugium

Figure C-1 Devils Hole: Chlorophyll *a*

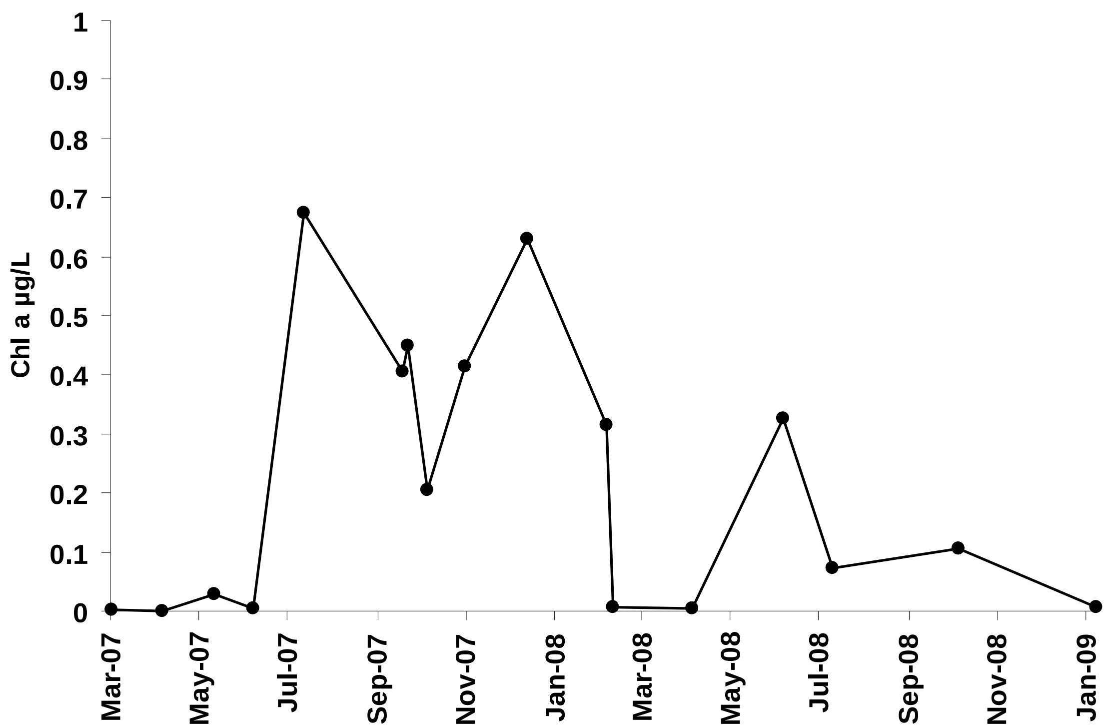


Figure C-2 Devils Hole: Bioassay results, density of Denticula

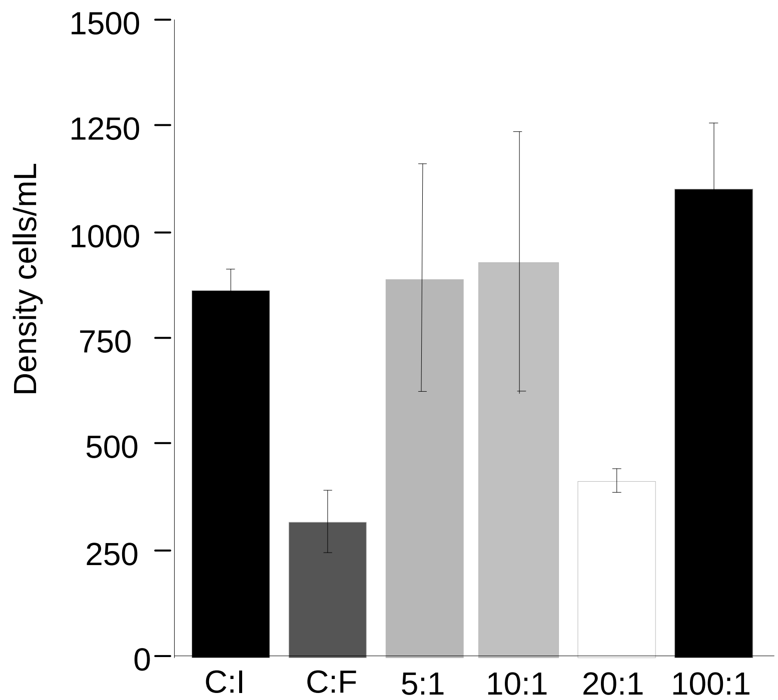


Figure C-3 Devils Hole: Bioassay results, biovolume of Oscillatoria

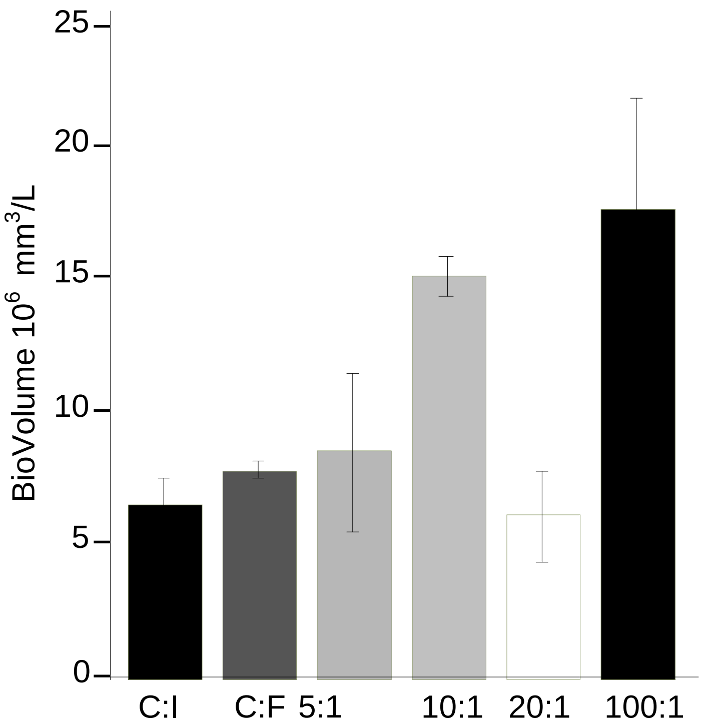
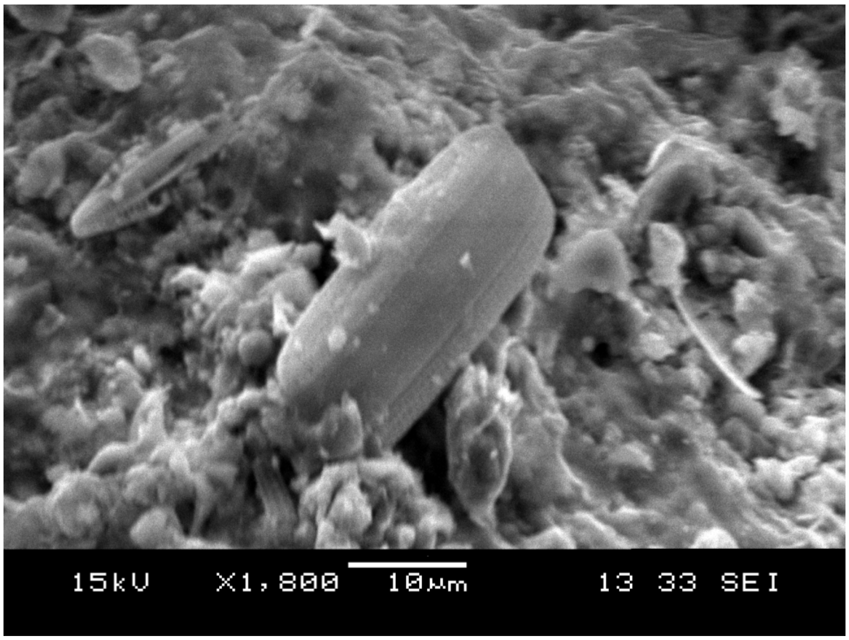
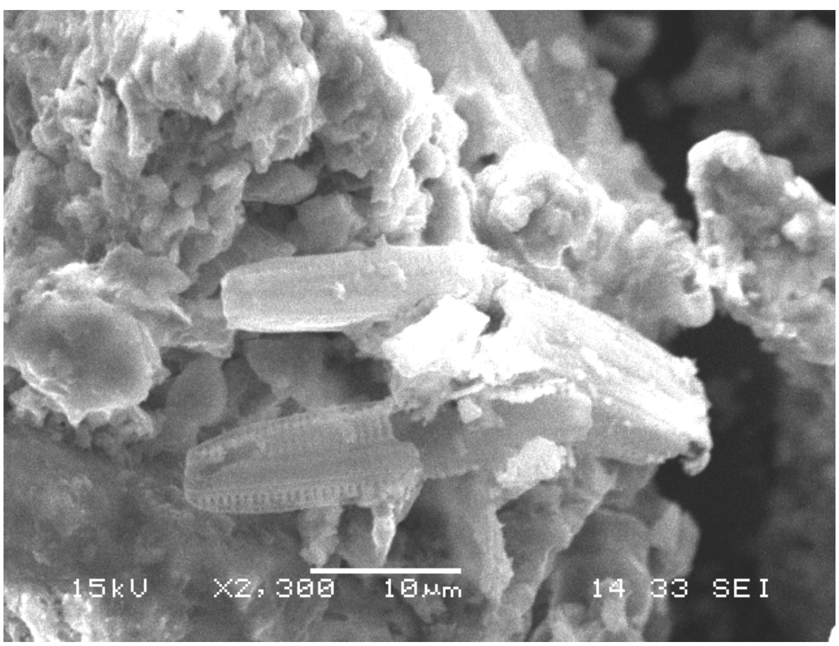


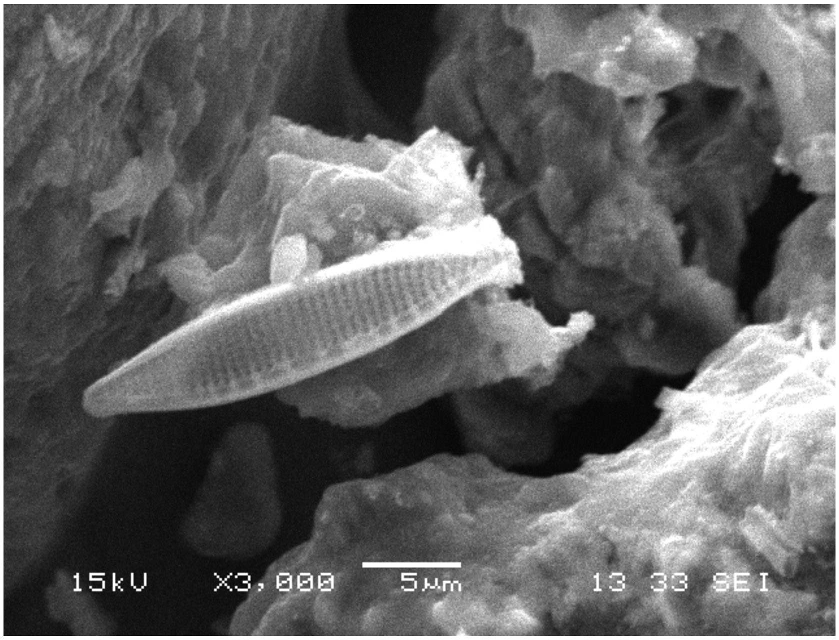
Figure C-4 Devils Hole: Scanning Electron Images

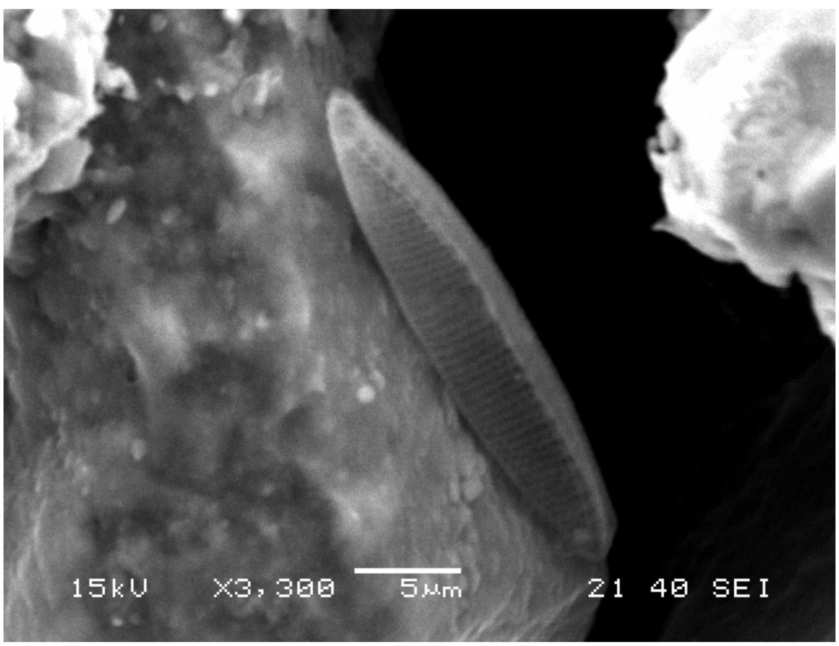
Denticula elegans



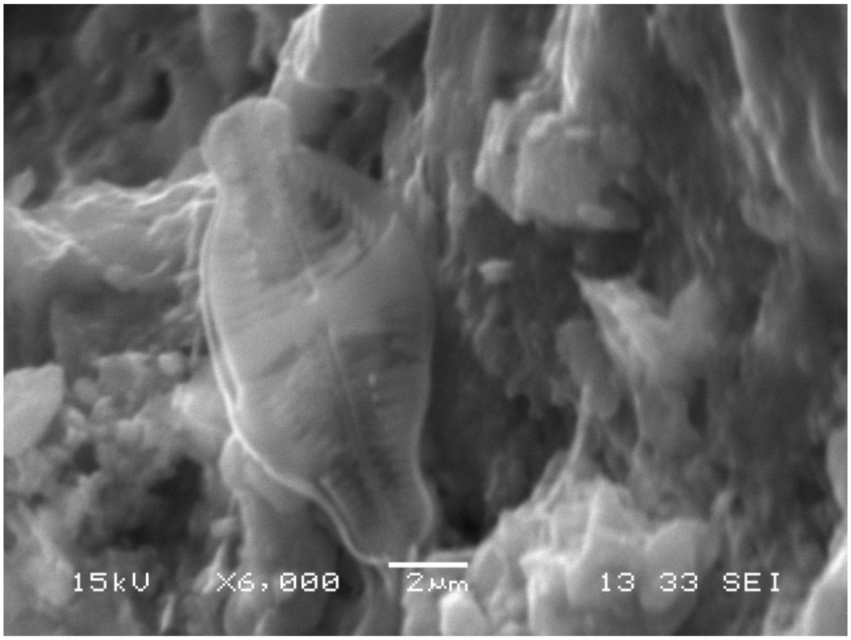


Nitzschia amphibia

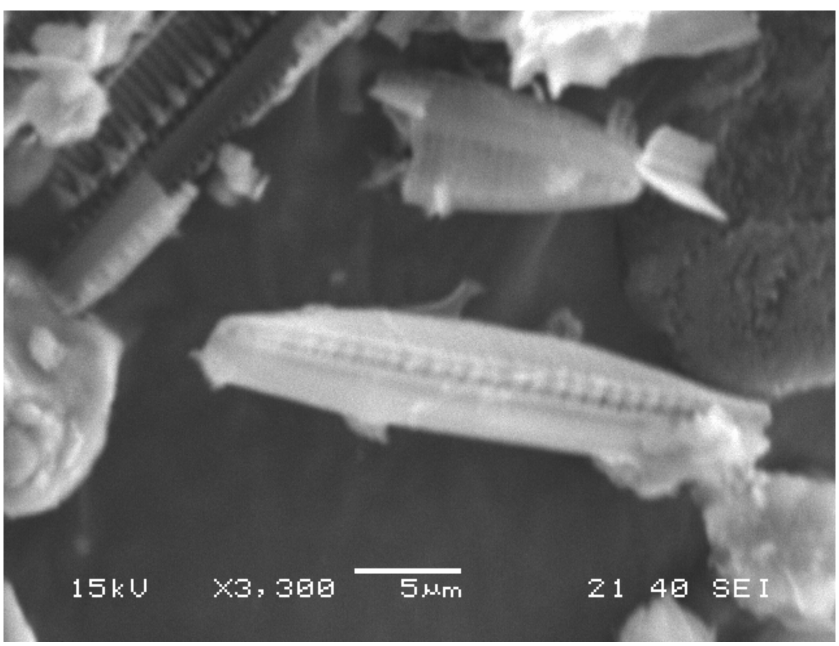




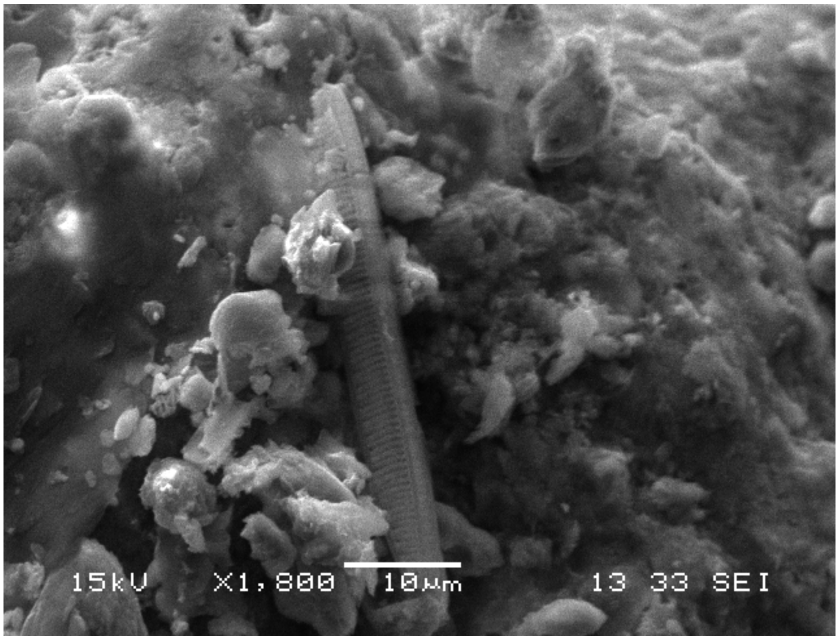
Achnanthes exigua var. heterovalva



Nitzschia dissipata



Synedra sp.



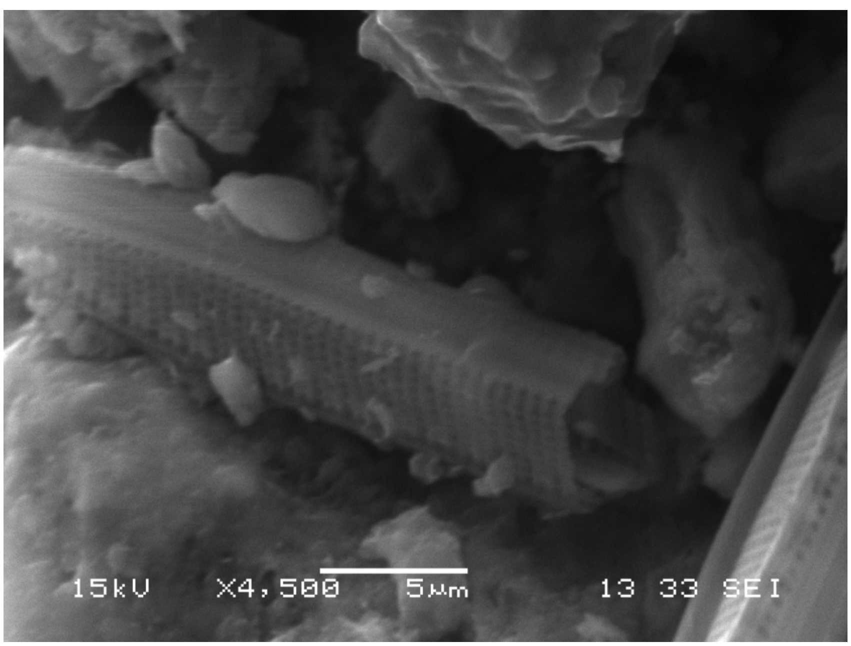


Figure C-5 Point of Rocks Refugium: Chlorophyll *a*