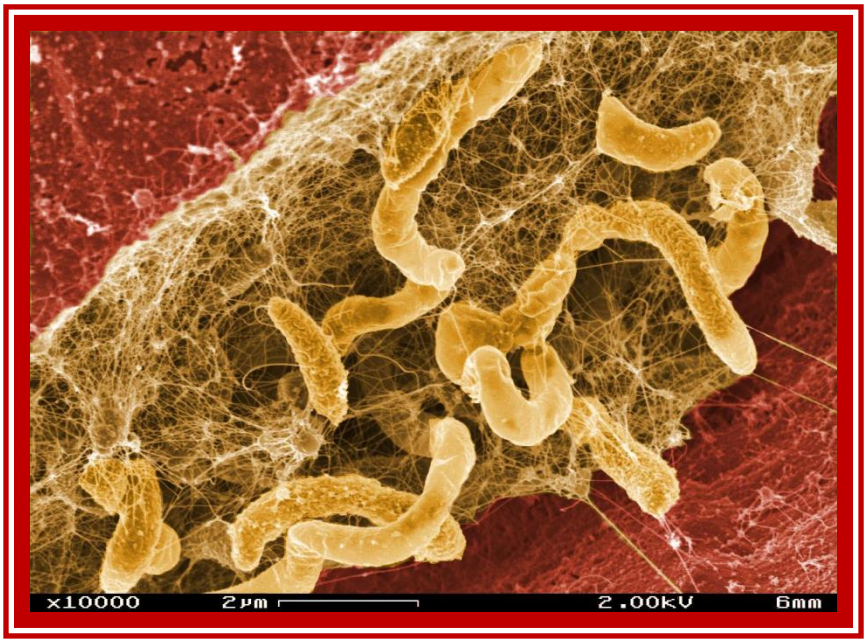
Sulfide Flux as a Function of Temperature in the Severn River



Biofilm of *Desulfovibrio desulfuricans*, anaerobic sulfate-reducing bacteria, cultured on a hematite (Fe_2O_3) surface. Color enhanced scanning electron microscope (SEM) image by Pacific Northwest National Laboratory.

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GEOL394: Geology Senior Thesis

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Honor Pledge:

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1. Abstract

We present a study of sulfide flux from the sediment into the water column at a zone of localized euxinia as a function of temperature in the Severn River. The combination of persistent anoxic conditions and the availability of sulfate generate an environment that allows sulfate reducing bacteria to thrive. The reduction of sulfate to sulfide by sulfate reducers is a metabolic pathway used to obtain energy and secrete the sulfide as waste. In this study, steady state sediment core experiments were conducted at 9 °C and 28 °C to explore changes in sulfide flux between the winter and summer conditions. At steady state the sulfide flux out of the sediment in to the water column is -2.30 ± 0.06 mmol m^{-2} d^{-1} at 28° C in comparison to -0.16 ±0.02 mmol m^{-2} d^{-1} at 9 °C. The results of this study can be used to predict the influence of temperature on the benthic community of the Severn River and similar systems suffering from the effects of eutrophication.

2. Background

2.1. Euxinia

Euxinic conditions are produced by the combination of anoxia, defined as dissolved oxygen levels less than 0.2 mg/l, warm climate and sulfide accumulation via sulfate reduction. Events such as the Cambrian explosion, Late Devonian extinction, and the Late Permian extinction have shaped the history of life on Earth by creating new evolutionary opportunities. The Late Permian extinction has been linked to sulfide buildup in deep oceans and the atmosphere to lethal levels (Kump et al., 2005). Euxinia is not common today because the deep oceans are well oxygenated; consequently sulfate is the second most abundant soluble electron acceptor in seawater, with a concentration of 28 mM (Canfield and Farquhar, 2009). The modern Black Sea is the largest euxinic basin on Earth due to density stratification in the water column. The presence of sulfide in the photic zone of the Black Sea allows for the growth and survival of anoxygenic phototrophs that reduce CO_2 to organic carbon (Repeta et al., 1989). The term euxinia comes from *Euxeinos Pontos*, the Greek name for the Black Sea. Other modern euxinic basins include Switzerland's Lake Cadagno, Norway's Framvaren Fjord, and New York's Fayetteville Green Lake (Meyer and Kump, 2008). Here we examined the effects of localized euxinia termed dead zones in the Severn River.

2.2. Biogeochemical Sulfur Cycle

The biogeochemical sulfur cycle is mainly driven by biotic processes and controlled by redox reactions such as the oxidation of sulfide and the reduction of sulfate (Fig. 1). The processes of the sulfur cycle are preserved in sediments that can be collected and studied. The reduction of sulfate to sulfide is dominant in marine systems with high sulfate content and low dissolved oxygen levels. The sulfide produced can be oxidized back to sulfate if enough oxygen is present in the upper layer of the water column. Sulfide oxidation is carried out by sulfide oxidizers such as *Beggitoa*, a chemolithoautotroph,

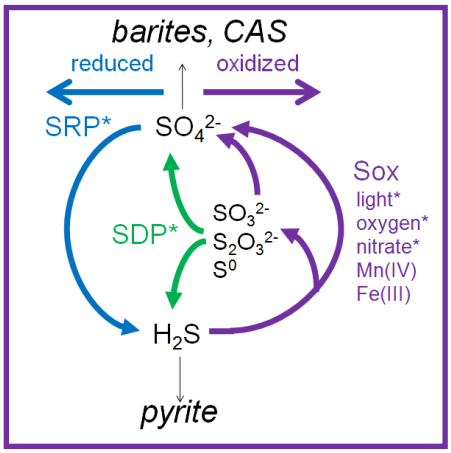

Figure 1: Biogeochemical sulfur cycle. SRP are sulfate reducing prokaryotes.

or anoxygenic phototrophs. Sulfide may be oxidized by both bacterial and abiotic processes utilizing oxygen, Fe(III), Mn(IV), or nitrate (Zopfi et al., 2004 and references therein). Sulfide oxidation in natural aquatic systems can yield intermediates such as elemental sulfur (S^0), thiolsulfate ($S_2O_3^{2}$), and sulfite (SO_3^{2-}) with sulfate (SO_4^{2-}) as the terminal oxidation product (Fig. 1). The products of biological and abiotic oxidation of sulfide are mostly the same. Recently researchers have made advances in understanding the metabolism of sulfur oxidizers and sulfate reducers in various aquatic systems around the world such as Fyetteville Green Lake, Faellestrand and Solar Lake (Zerkle et al., 2010; Canfield, 2001; Habicht et al., 1998). Understanding the biogeochemical sulfur cycle an essential part of exploring ancient and modern marine environments.

2.3. Sulfate Reducers

This study will focus solely on the prokaryotic reduction of sulfate in the biogeochemical sulfur cycle. In the absence of oxygen, sulfate reduction is an important energy source for bacteria (Jørgensen, 1977). Sulfate reducers are delta Proteobacteria that can be found in deep sediments and anoxic waters with abundant carbon content.

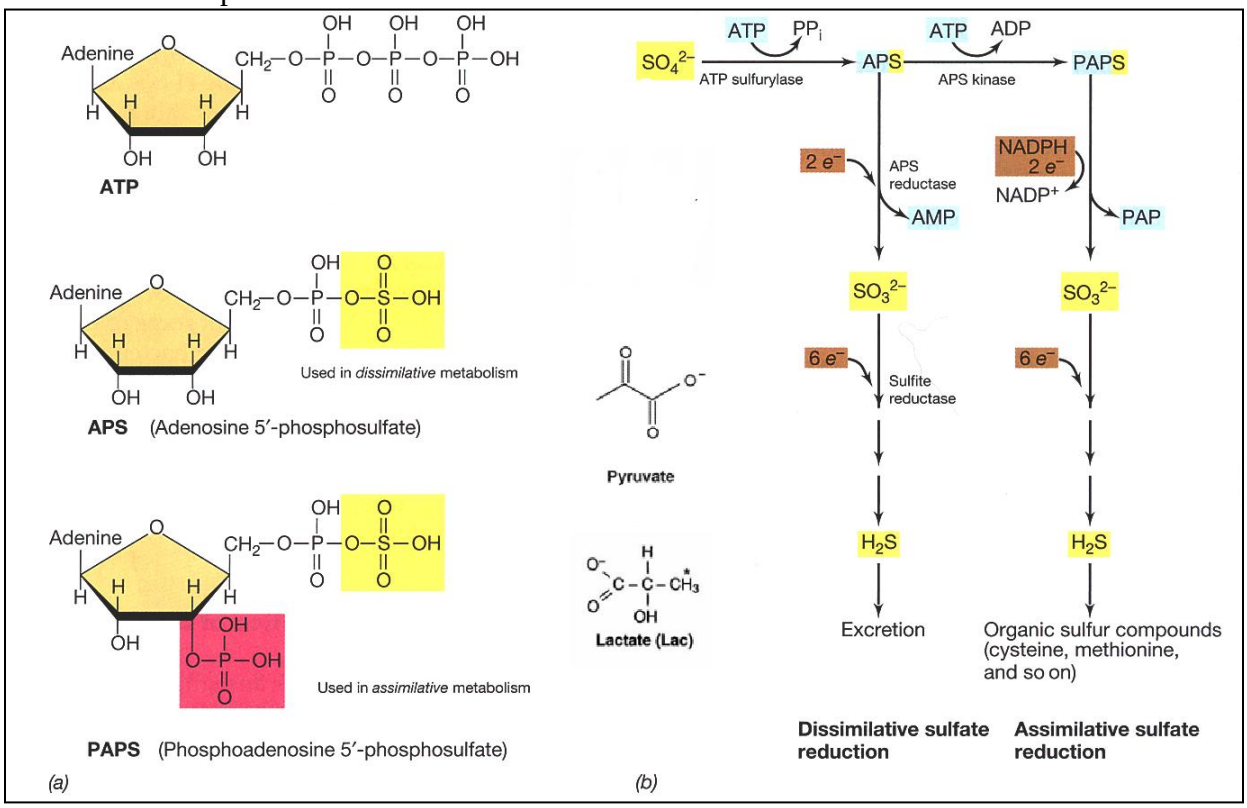

Figure 2: Biogeochemistry of sulfate reduction. Bacteria metabolizing sulfate through dissimilative sulfate reduction in the sediment excrete sulfide that can be quantified. Possible electron donors include pyruvate and lactate shown above (Madigan et al., 2003).

Sulfate reducing bacteria can undergo two different types of sulfate reduction, assimilative and dissimilative. Dissimilative sulfate reduction states that sulfate is reduced to obtain energy and sulfide is excreted as a waste product (Fig. 2). Assimilatory sulfate reduction states that sulfide produced is used to synthesize biological compounds such as cysteine, an amino acid.

Adenosine triphosphate (ATP) is a high energy molecule found in all cell types with various metabolisms whether aerobic or anaerobic. Sulfate reduction occurs in the cytoplasm and the first step is to activate SO_4^{2-} with the removal of two of the three phosphate groups from adenosine triphosphate by enzyme adenosine triphosphate sulfurylase producing adenosine monophosphate (energy releasing reaction-exothermic). The adenosine monophosphate then binds to SO_4^{2-} producing adenosine phosphosulfate allowing for the reaction to proceed as shown in figure 2b. A total of 6 elections are donated from either pyruvate, acetate, or lactate to reduce sulfate to sulfide. Only dissimilative sulfate reduction is relevant for this study because the sulfide excreted can be quantified.

Microbial sulfate reduction produces one mole of hydrogen sulfide for every mole of sulfate ion reduced. In the simplified equation below, CH_2O (organic carbon) can represent either lactate or acetate depending on the sulfate reducing bacteria:

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S. \quad [1]$$

For example, *Desulfobacter* utilizes only acetate molecule as an electron donor and oxidizes it to CO_2. In comparison, *Desulfovibrio* can use lactate, pyruvate, or alcohols as electron donors and produces acetate as an end product (Madigan et al. 2003). Some of the sulfide produced is used to form iron sulfides (FeS and FeS_2) resulting in the black color of the mud (Fig. 8). The remaining sulfide can diffuse from the sediment into the water layer, this flux can be quantified. A field marker of sulfate reduction is the rotten egg smell of hydrogen sulfide, a colorless gas. Humans can detect H_2S in the air at concentrations as low as 0.004 to 0.03 mg/m^{3}depending on their odor threshold (Reiffenstein et al., 1992). Sulfate reducers play an essential role in the continuation of the biogeochemical sulfur cycle.

2.4. Sulfate Reduction in Sediments

The biochemistry of sulfate reduction is well studied but the quantitative importance in natural systems is less understood. A two year study of Limfjorden, a 1500 km^2 river located in northern Denmark, was conducted to understand sulfur cycling in the sediment. Sulfide production was greatest near the surface of the sediment water interface and only 10% of the sulfide formed iron sulfides in the anoxic layer. The remaining sulfide diffuses to a suboxic layer where it oxidized to SO_4^{2-}. In shallow coastal waters about 53% of the organic matter is mineralized in the sediments through sulfate reduction (Jorgensen, 1977).

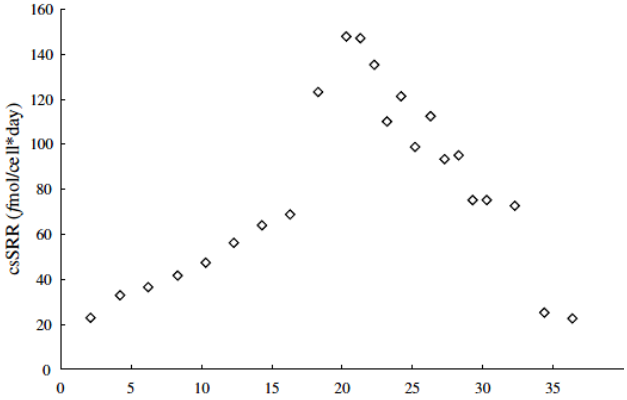

Figure 3: Plot showing the relationship between temperature and cell specific sulfate reduction rate of a pure culture of D. autotrophicum (Johnston et al.2007).

Steady state sulfate reduction rates (SRR) in sediments at Faellestrand lagoon in Denmark were examined by incubating sediment cores in a 25 °C water bath and collecting H_2S as zinc sulfide at varying flow rates. Faellestrand is a shallow oligotrophic lagoon located on the island of Fyn; benthic respiration in the lagoon is controlled by temperature. This study demonstrates that reaching steady state, defined by constant concentration of sulfide in the water collected, is possible in the sediment cores. An interesting observation in this study is that as flow rate varies

from 0.38 to 1.68 ml/hr the sulfate reduction rate was stable at 0.32±5 µmol/cm^3/day suggesting that sulfate reduction is mainly controlled by other factors besides the influx of sulfate (Farquhar et al., 2008). The sulfate reduction rate is principally controlled by temperature, abundance of organic matter, bioturbation, availability of electron donor and acceptors and the SRB metabolizing sulfate (Marvin-Dipasquale and Capone, 1998; Marvin-Dipasquale et al., 2003, and references therein; Canfield and Farquhar, 2009). Pure culture studies demonstrate that each SRB has an optimum temperature at which sulfide productivity is at its maximum (Johnston et al., 2007; Mitchell et al., 2009). As conditions deviate from the optimum temperature the cell specific sulfate reduction rate decreases significantly (Fig. 3). In natural waters, the population of sulfate reducing bacteria will not be pure but instead a mixture different types of bacteria. In this case, the findings from cell specific studies are applicable but the optimum temperature might be a range dependent on the diversity of sulfate reducing bacteria in the system. A long-term study of various sites in Wadden Sea concludes that sulfate reduction rate in sediment is dependent on temperature, oxygen penetration depth, and the availability of organic matter. In figure 4: the dash line represents the relationship between average water surface temperature and month. The highest sulfate reduction rate (SRR) was measured at site D (muddy sediment) due to its lower oxygen penetration depth (OPD) of 0.5 cm below the suface (cmbsf), temperature range of 1 °C to 24 °C and total organic carbon (TOC) range of 1.3 - 3.5 dwt. %. Sites N-I and N-II have similar sediment chemistry but N-II reaches a higher microbial activity because the temperature maximum at N-I is 16 °C while at N-II it is 32 °C. In this natural system optimum productivity is observed during the summer and minimum productivity during the winter (Al Raei et al. 2009) (Fig. 4).

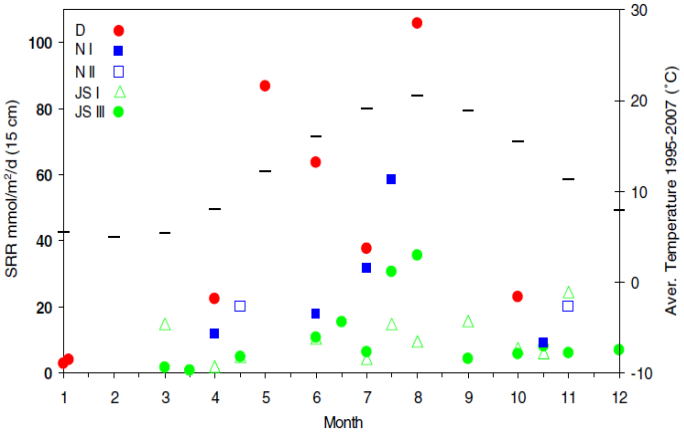

Figure 4: The relationship between temperature and sulfate reduction rates at various sites in Wadden Sea. Each site has different sediment chemistry and microbial activity levels (Al

3. Geological Setting

3.1. Severn River

The Severn River occupies an 81 square mile area that feeds into the western shore of the Chesapeake Bay (Fig. 5). The Severn is bounded by the Magothy and the South River. Since 1997,

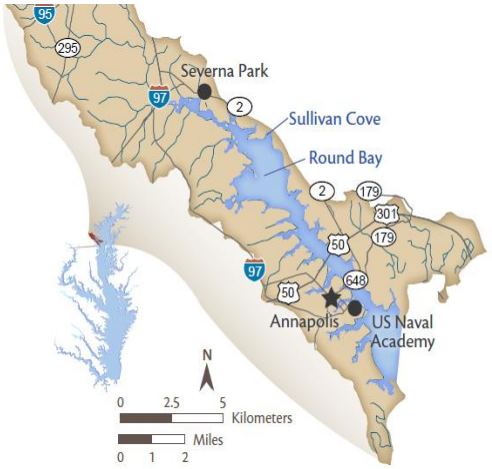

Figure 5: A close up of Severn River from a map of the Chesapeake Bay (lower left).

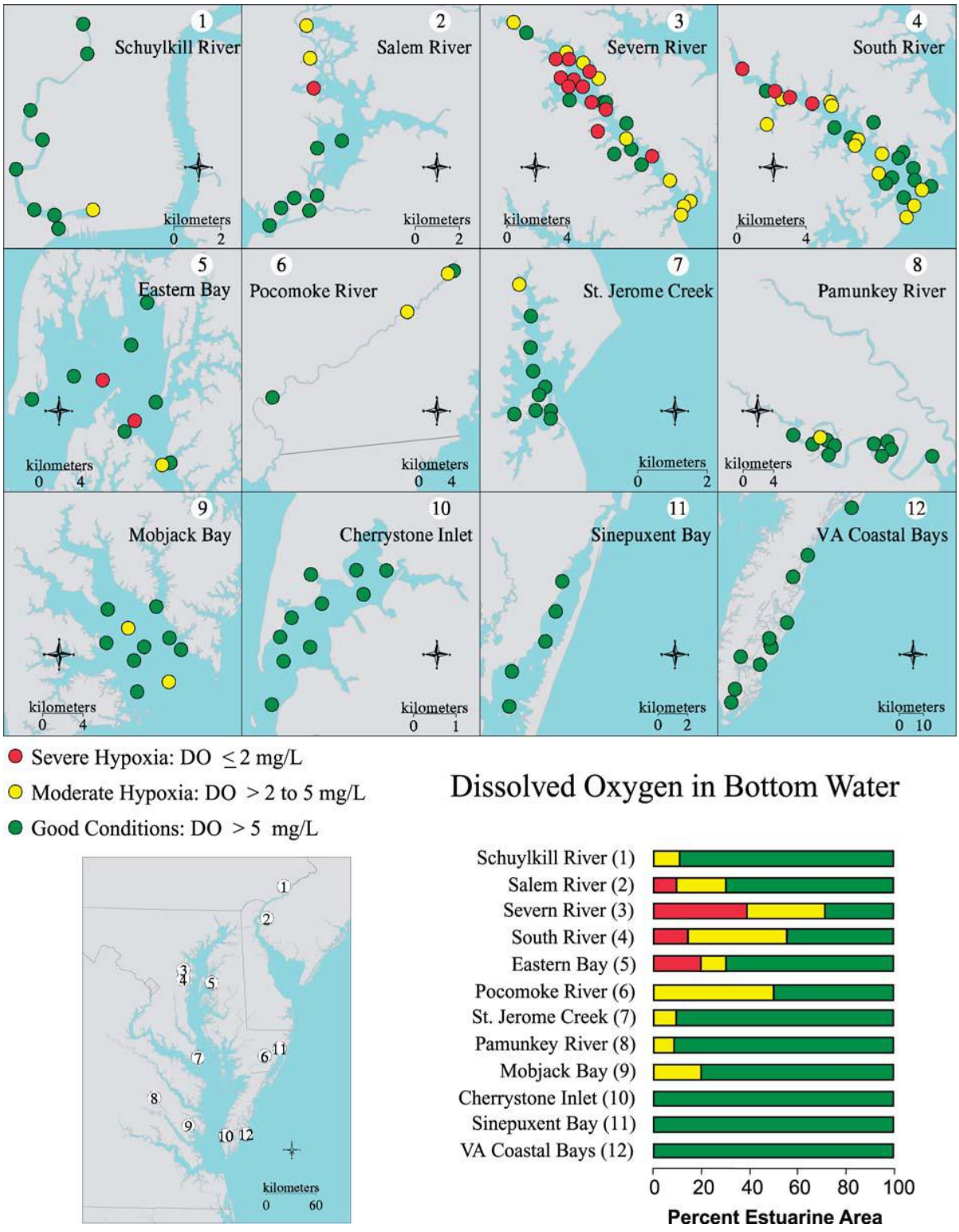

Figure 6: Bottom dissolved oxygen content in rivers along the Chesapeake Bay showing that the Severn and South River are the most oxygen depleted (USEPA, 2002).

eutrophication, defined as an increase in the supply of organic matter to an ecosystem, has been an issue in the Severn. Symptoms of eutrophication include high concentrations of nutrients (nitrogen and phosphorous), low dissolved oxygen levels, and organic rich sediments (USEPA, 2002). Excess nutrient leads to the overgrowth of phytoplankton that sinks to the bottom of the river when they die and are metabolized by oxygen depleting bacteria. This process leads to hypoxia, defined as dissolved oxygen levels ranging from 3.5 to 0.2 mg/l, that over time can worsen to an anoxic state.

A 2002, Environmental Protection Agency (EPA) report states that sediment contamination by pesticides, polycyclic aromatic hydrocarbons, mercury (0.7 ppm), zinc (560 ppm), nickel (82 ppm) and other metals are higher in the Severn than the South and Magothy Rivers. Furthermore, the South and Severn River are some the most organic rich estuaries on the Chesapeake Bay but are also the most depleted in dissolved oxygen in the northwestern shore. The total organic carbon in the sediment of the Severn is approximately 6% (USEPA, 2002). Recent studies by the Severn River Commission suggest that excess nutrients, sewage contamination, and depleted oxygen levels are still issues in the Severn. The Severn Riverkeeper Program was started in 2006 to observe the changes in the Severn to help protect the home of many organisms such as the blue crab and striped bass (Figure 10). The focus on the health of the Severn is a recent topic therefore there have not been any notable publications in journals discussing this matter. The Severn Riverkeeper Program led by Pierre Henkart continues to be the only source yearly reports on the salinity, visibility, oxygen content, and more to keep the community motivated to improve the health of the Severn.

3.2. Round Bay North Station and Asquith Creek

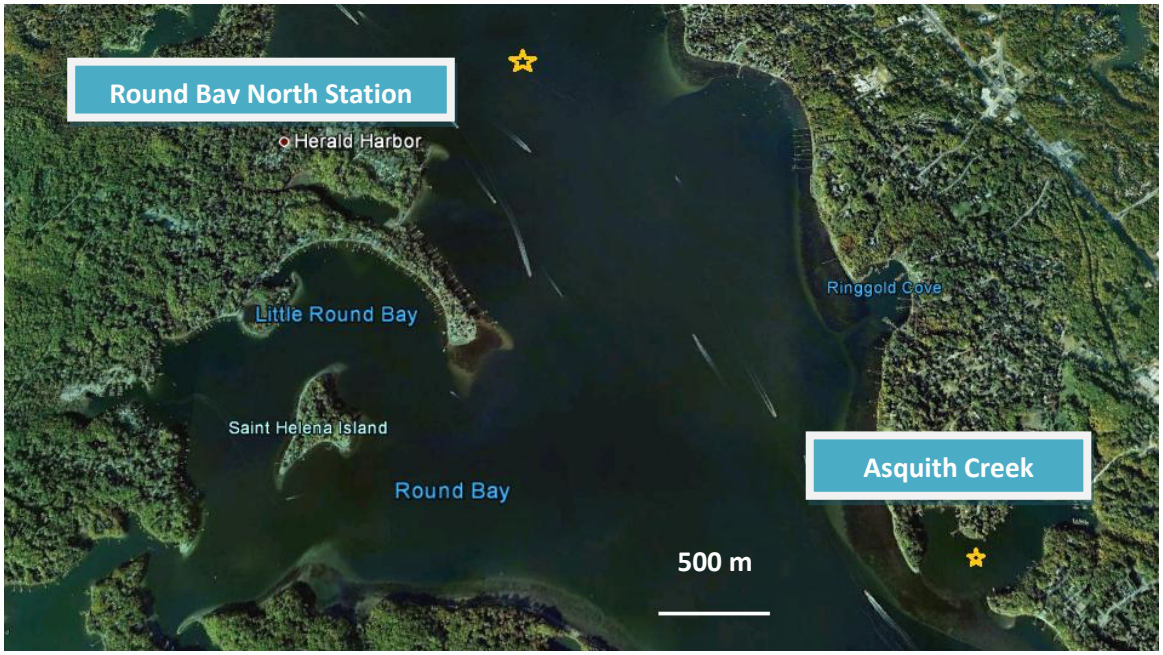

Figure 7: Location of Round Bay North Station (RBN) and Asquith Creek sampling sites in relationship to each other (Google Earth).

All sediment cores for the sulfide flux experiments were sampled from the Round Bay North Station located at the northern center of the Severn River (39°03'23.31" N 76 °33'28.08" W). We chose to sample at Round Bay North (RBN) because the worse effects of eutrophication in the Severn were observed there. RBN is seasonally anoxic during the summer leading to the production of sulfide. The combination of poor levels of bottom dissolved oxygen and sulfide accumulation results in a localized euxinic area in the Severn (Fig. 6). In order for fishes, crabs, soft shell clams, worms, oysters, and other organisms to thrive the dissolved oxygen level should be about 5 mg/l and Round Bay North is below that threshold at lower than 0.2 mg/l. Therefore Round Bay North is unable to support aerobic life in the benthic zone during the summer. As seen in figure 7, Asquith Creek is smaller and more secluded than Round Bay therefore the euxinic cycles are longer than those observed in Round Bay. We sampled sediment cores from Asquith Creek to compare pore water sulfide with Round Bay to understand how the sediment acts as a record of euxinic events in the Severn River.

4. Methods

4.1. Sample Collection

In mid October, the Severn Riverkeeper Program boat was used in the collection of all samples at the Round Bay North station (RBN). On the sampling day the depth of the water column was about 7.2 m, 24.4° C, and dissolved oxygen level was 0.03 mg/l. The water was sampled before the cores to prevent the collection of muddy water because the sediment at RBN is slushy. Water was collected from a depth of 6 m into three 10 L carboys by using a Masterflex Portable Sampler field pump. Next, cylindrical plexiglass sediment cores were collected by using a Wildco Ekman Box Corer attached to 10 m of rope. After anchoring the boat, the box corer was slowly lowered into the water and when the sediment layer was reached two messengers were sent down the rope to close the box corer. The corer was then brought up slowly and placed into a bucket of surface water in the river to prevent the sediment from falling out before being placed in the boat. The Ekman box corer was unclamped and two plexiglass cores were slowly pushed downward not to disturb the layers. A rubber stopper was used to cap the top and bottom of the core before removal from the Ekman corer. Finally both stoppers were carefully exchanged for a 2 inch Qwik Cap for an airtight seal to prevent further oxidation (Fig. 8). These steps were repeated four times in order to collect a total of eight sediment cores. The same method was utilized to collect two sediment cores from Asquith Creek.

Figure 8: Round Bay North Plexiglass Sediment Core.

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4.2. Sediment Core Description

All plexiglass cores had an inner diameter of 2.047 inches and a length of 10 inches. At Round Bay North station the total sediment layer depth ranged from 17.5–18.0 cm in comparison to 21.2–22.1 cm at Asquith Creek. The yellow line in figure 8 separates the top black organic rich layer from the bottom gray organic poor layer in a Round Bay North core. The gray layer occupied 10 cm approximately 56% of the total sediment layer. In the Asquith

Creek cores only about 27% of the sediment layer was gray indicating poor levels of organic content. Loss of ignition test was conducted on all six cores after all sulfide measurements were complete to measure total organic matter and water content.

4.3. Water Sample Preparation

In the lab, a glass gallon jug and diffuser were sterilized by placing them in a Thermo Scientific oven overnight at 175 °C. The water collected from 6 m at Round Bay North was filtered through a fine particle glass fiber filter followed by a 0.20 µm filter into the sterilized gallon jug. The glass filter was used as a pre filter to remove the majority of large particles from the water source. The 0.20 µm filter served as a micro-organism barrier because protozoa size ranges from 2 µm and larger and bacteria size ranges from 0.2 to 10 µm. The filtration method utilized allowed for minimal organic material influence on the experiment. The filtered water was then bubbled with N_2 overnight in an anoxic chamber prior to pumping through sediment cores to ensure anoxic conditions. The sulfate concentration of the water sample was measured by precipitating barium sulfate ($BaSO_4$). A total of 10 ml filtered water sample was pipetted into a 15 ml centrifuge tube and 1 ml of 1 M barium chloride ($BaCl_2$) was added. The mixture was shaken and centrifuged twice at 3500 rpm utilizing an Eppendorf Centrifuge 5810 series. The supernatant was poured out and the remaining content was dried overnight at 55 °C and weighted. The data is shown above in table 1 with the average sulfate concentration of 6.2 ± 0.5 mM which is appropriate because the sulfate concentration in the ocean is 28 mM.

Trial	Dried Weight of $BaSO_4$ (g)	Concentration of Sulfate (mM)
1	0.0132	5.65
2	0.0155	6.64
3	0.0144	6.17
Average Sulfate Concentration = 6.2 ± 0.5 mM		

Table 1: Results for collected Round Bay North station water sulfate concentration.

4.4. 9 °C and 28°C Apparatus

The apparatus used in this study is based on an established laboratory procedure for collecting water column samples as zinc sulfide according to Sorensen and Canfield (2004). Both apparatus were built in an anoxic chamber to prevent the oxidation of the sulfide produced to sulfate. Three cores were used in the 9 °C and 28 °C experiments and were kept in a mostly dark environment to prevent the appearance of anoxygenic phototrophs. In the first experiment three cores were submerged in a 9 °C bath in a styrofoam container with cold water continuously flowing in and out to a temperature controlled water bath. A thermometer was placed into the styrofoam container to ensure that the temperature was always 9±1 °C. A Thermo Scientific FH100 Multichannel Peristaltic Pump was used to transport water containing sulfate from the reservoir into the sediment cores, with a flow rate of 27 ± 1 ml d^{-1}. The water reservoir was kept anoxic by using a diffuser to evenly distribute nitrogen gas throughout the jug. Another tubing transported water from the sediment core into a 50 ml centrifuge tube with 1 ml of 20% zinc acetate to trap H_2S as zinc sulfide (Figure 12). The traps were changed every

two days and the sulfide concentration measured spectrophotometrically according to Cline (1969).

The experimental design for the 28 °C study is identical to the 9 °C study with the exception of the the changes discussed in the subsequent sentences. A Top Fin aquarium water heater was utilized to maintain the temperature of the three sediment cores at the 28 ± 1 °C. All three cores and the water heater were placed in a plastic bucket and covered with foil to contain the heat in the anoxic chamber. Larger diameter tubing was used to transport water from the sediment core into the 50 ml centrifuge tube resulting in the flow rate change from 27 ± 1 ml d^{-1} to 48 ± 1 ml d^{-1}. Due to the limit of 50 ml per centrifuge tube the increase in flow rate meant that sample was collected everyday instead of every two days.

The flow rate was measured by weighing each 50 ml centrifuge tube with 1 ml 20% zinc acetate and then again after water sample was trapped. The difference in weight was calculated and converted from grams to ml using the density of water. The start and end time of the collection of sample was recorded on each centrifuge tube and later converted to hours then day. Finally the calculated ml was divided by the sample collection time to represent the flow rate in ml d^{-1}.

4.5. Cline: Hydrogen Sulfide Concentration

The Cline method is a standard procedure used to measure the concentration of sulfide in water samples. The main components of the Cline reagent are methylene blue, ferric chloride, and concentrated hydrochloric acid. The 3 – 40 μM Cline reagent was mixed and calibrated with a reproducibility error of ±5 % according to Cline (1969). Each sulfide concentration was determined by first diluting the zinc sulfide sample by 10 to 250x depending on predicted sulfide concentration. Then 80 μl Cline/ 1 ml diluted sample or blank were mixed together and placed in the dark for 30 minutes to allow for color stabilization. The spectrophotometer was set to 665 nm because that is the optical absorbance wavelength for methylene blue. Finally the spectrotometer was blanked and each sample absorbance was measured and recorded. Sulfide concentrations were calculated using the following equation where the conversion factor of 27.73 was based on the calibration of the reagent:

$$Sulfide\ Concentration\ (\ ?M) = Dilution \times Conversion\ Factor\ \times Absorbance. \quad [2]$$

4.6. Sediment Pore Water Sulfide Analysis

To understand the difference between euxinia in the open Round Bay North station and the secluded Asquith Creek of the Severn River, pore water sulfide in four sediment cores were analyzed. Depending on the total sediment layer depth several 15 ml centrifuge tubes, spatula, 1cm core sectioning apparatus, and closed sediment core were placed in a glove bag. The glove bag was filled with N_2 and emptied three times to ensure low dissolved oxygen levels. Next the glove bag is closed and filled and sediment material is transferred into a 15 ml centrifuge tube for every 1 cm. After sectioning the core, all 15ml tubes were centrifuged to separate the sediment from the pore water. The pore water was poured into a15 ml centrifuge tube with 1 ml 20% zinc acetate in the glove bag to prevent oxidation. The Cline method was then used to

spectrophotometrically measure the sulfide concentration in 32 Round Bay North and 38 Asquith Creek samples.

4.7. Loss on Ignition Analysis

Loss on ignition is a standard laboratory protocol used to estimate total organic carbon and water content through weight loss measurements after stages of heating. This part of the study was done after all steady state temperature experiments were complete because bulk sediment from the cores was needed. Earlier we discussed the change in color of the sediment in the Severn River from black to grayish brown with increasing depth, so we decided to conduct loss on ignition analyses on the top and bottom sections of the core separately. We measured approximately 30 g of sediment into a foil weigh boat and dried overnight at 80 °C in an oven to remove all water content. The sample was then weighed and ignited in a Thermo Scientific Lindberg Blue M muffle furnace at 550 °C for 4 hours and allowed to cool for 2 hours. At 550 °C all organic matter in the sediment was oxidized to carbon dioxide and ash. The difference in pre/post ignition weight was used to calculate the percent organic matter (Heiri et al., 2001; Santisteban et al., 2004). As shown in fig. 9, the post ignition sediment has a reddish color because all organic carbon has been oxidized and mainly iron oxides remain.

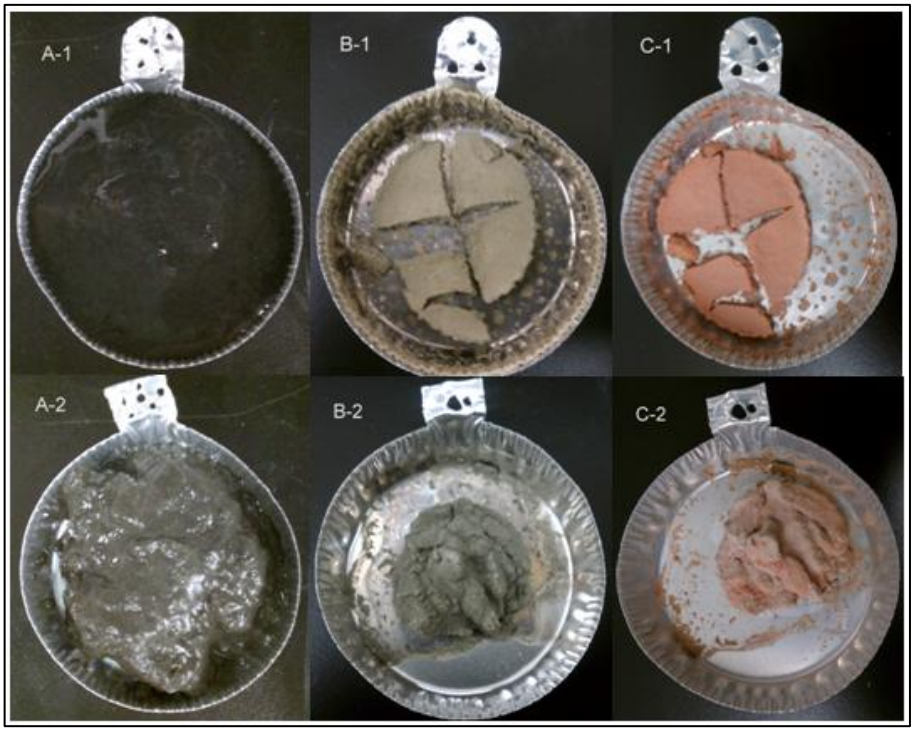

Figure 9: Top row images are from top sediment layer samples and bottom row images are from bottom layer sediment A) wet sample B) sample dried at 80 °C overnight C) sample ignited to 550 °C for 4 hours.

5. Results

The sulfide concentrations and flow rates of each collected sample in the 9 °C and 28 °C experiments are presented in tables 2 and 4. We defined steady state as consistent sulfide concentration measurements. In the cooler temperature cores, steady state was reached within 24 days (labeled with asterisks by the days) with an average flow rate of 27±1 ml d^{-1} and sulfide concentrations ranging from 11.09-14.97 μM. In the warmer temperature cores, steady state was reached in 18 days because the average flow rate was higher than the previous study at 48±1 ml d^{-1} with sulfide concentrations ranging from 98.44-105.37 μM. It is important to note

that cores 1 and 3 had a leak in the 28 °C experiment therefore only data from core 2 was used to calculate sulfide flux. The average 9 °C sulfide flux was -0.16± 0.02 mmol m^{-2} d^{-1} (Table 3) in comparison to -2.30±0.06 mmol m^{-2} d^{-1} in core 2 at 28 °C (Table 5). All sulfide flux calculations produce positive numbers but reported with a negative sign to denote the movement of sulfide from the sediment into the water column.

5.1. Uncertainty and Error

All presented standard deviations are 1 sigma. The uncertainty reported for sulfide concentrations and flow rates are calculated with only steady state data marked with an asterisk. There is a 5% reproducibility error in the spectrophotometer based on 30 measurements during calibration which in turn relates to all sulfide concentrations reported here. The Mettler Toledo AG-204 Digital Balance Scale was used in all measurements of the 50 ml centrifuge tube pre and post sample collection to decrease the error in the flow rate calculation. The AG-204 scale reports weight in grams to the thousandth decimal place.

	9° C Sediment Core Experiment Sulfide Data					
	Core 1		Core 2		Core 3	
Day	Sulfide Concentration (µM)	Flow Rate (ml/day)	Sulfide Concentration (µM)	Flow Rate (ml/day)	Sulfide Concentration (µM)	Flow Rate (ml/day)
3	23.57	27.79	19.41	27.21	26.34	26.97
4	13.87	22.63	19.41	24.87	19.41	22.72
9	6.66	29.59	6.38	26.81	7.49	26.46
11	5.55	27.15	4.44	24.78	3.88	25.50
14	6.93	27.03	5.27	26.58	6.10	27.08
17	8.87	24.67	8.04	27.28	5.82	27.45
18	6.38	27.37	9.43	27.56	5.27	27.32
20	23.02	26.96	5.27	27.27	4.71	27.21
21	22.46	28.49	8.60	26.22	8.04	27.21
24*	14.42	27.72	10.26	24.67	11.09	27.40
28*	13.59	26.52	11.37	27.32	11.65	26.21
34*	14.70	27.03	10.81	25.37	11.92	27.35
39*	14.14	27.58	11.09	27.64	11.65	27.16
42*	14.97	27.53	11.65	27.70	11.09	27.46
Steady State Avg*	14.4±0.5	27.3±0.5	11.0±0.5	26±1	11.5±0.4	27.1±0.5

Table 2: Presented are all sulfide concentrations and flow rate for each core as the 9 °C experiment progresses. Steady state conditions defined as consistent sulfide concentrations are marked by asterisks.

Core #	9° C Sulfide Flux (mmol m^{-2} d^{-1})					Average	Standard Deviation
1	- 0.183	- 0.187	- 0.173	- 0.180	- 0.183	- 0.181	0.005
2	- 0.145	- 0.138	- 0.141	- 0.148	- 0.131	- 0.140	0.007
3	- 0.148	- 0.152	- 0.148	- 0.141	- 0.141	- 0.146	0.005
						- 0.16	0.02

Table 3: Presented are all sulfide fluxes for each day that the core was in steady state and the average for the 9 °C experiment. The negative sign denotes the movement of sulfide from the sediment layer into the water column.

	28° C Sediment Core Experiment Sulfide Data					
	Core 1		Core 2		Core 3	
Day	Sulfide Concentration (µM)	Flow Rate (ml/day)	Sulfide Concentration (µM)	Flow Rate (ml/day)	Sulfide Concentration (µM)	Flow Rate (ml/day)
3	297.54	58.71	191.06	42.09	288.66	48.75
4	51.02	51.59	132.55	48.93	Leak	Leak
5	80.69	44.40	55.46	42.59		
6	3.60	49.90	4.16	48.75		
7	3.33	53.64	6.10	49.82		
10	13.59	64.12	11.92	53.10		
11	16.08	56.10	7.76	48.65		
12	14.42	55.97	19.97	49.51		
13	4.71	47.68	84.30	45.98		
16	3.60	58.41	82.08	46.34		
17	8.87	43.38	112.58	47.11		
18*	0.08	37.29	99.55	46.74		
19*	3.88	46.13	101.32	48.41		
22*	6.10	61.08	103.15	50.41		
23*	1.94	26.96	99.84	47.05		
24*	4.99	56.05	105.37	47.48		
25*	Leak	Leak	98.44	49.54		
26*	Leak	Leak	101.49	47.48		
Steady State Average*			101 ± 2	48 ± 1		

Table 4: Presented are all sulfide concentrations and flow rate for each core as the 28 °C experiment progresses. Steady state conditions are marked by asterisks. The red values in core 1 are due to the tubing turning red because of a leak resulting in the formation of iron oxides. In core 3 there was a leak from day 4 therefore no more measurements were made.

Core #	28° C Sulfide Flux (mmol m^{-2} d^{-1})						Average	Standard Deviation
2	- 2.25	- 2.29	- 2.33	- 2.38	- 2.23	- 2.30	- 2.30	0.06

Table 5: Presented are all sulfide fluxes for each day that the core was in steady state and the average for core 2 in the 28 °C experiment. The negative sign denotes the movement of sulfide from the sediment layer into the water column.

6. Discussion

Moderate levels of organic carbon in the sediments are beneficial for the benthos community of shellfish, worms, and crustaceans. During eutrophication, the nutrient influx is too high and the benthic organisms are deprived of oxygen as the organic matter decomposes, leading to a decrease of the benthic population and restriction of habitable area (Figure 10).

Anoxic conditions allow for the dominance of anaerobic sulfate reducing bacteria in the Severn River that live in the sediment column. Sulfate accumulates in the Atlantic by atmospheric deposition, run off from rivers produced by oxidation sulfide and weathering of sulfur bearing rocks. Some of the sulfur is then transported to the Chesapeake Bay and from there to the Severn River via mixing. The transported sulfate is then metabolized by sulfate reducing bacteria to produce sulfide. The combination of hypoxic conditions and micromolar concentrations of hydrogen sulfide is toxic to benthic organisms reducing their survival time by 30% (Vaquer-Sunyer and Duarte, 2010). Pore water sulfide concentrations in Round Bay range from 0.18-0.69 mM which is enough decrease survival time by at least 50% . The conditions at secluded Asquith Creek are enough to decrease benthic organism survival time by 80% with pore water sulfide concentrations peaking at 4.77 mM (Figure 11, Table 7).

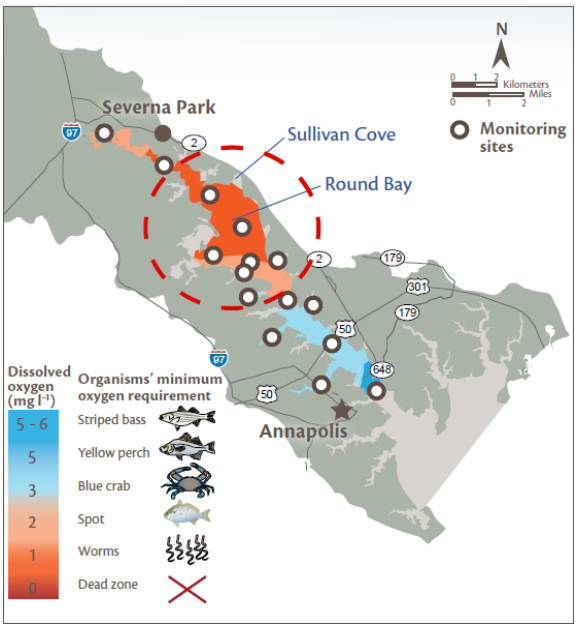

Figure 10: Shows the localized euxinic zones in the Severn and the effects on benthic organisms.

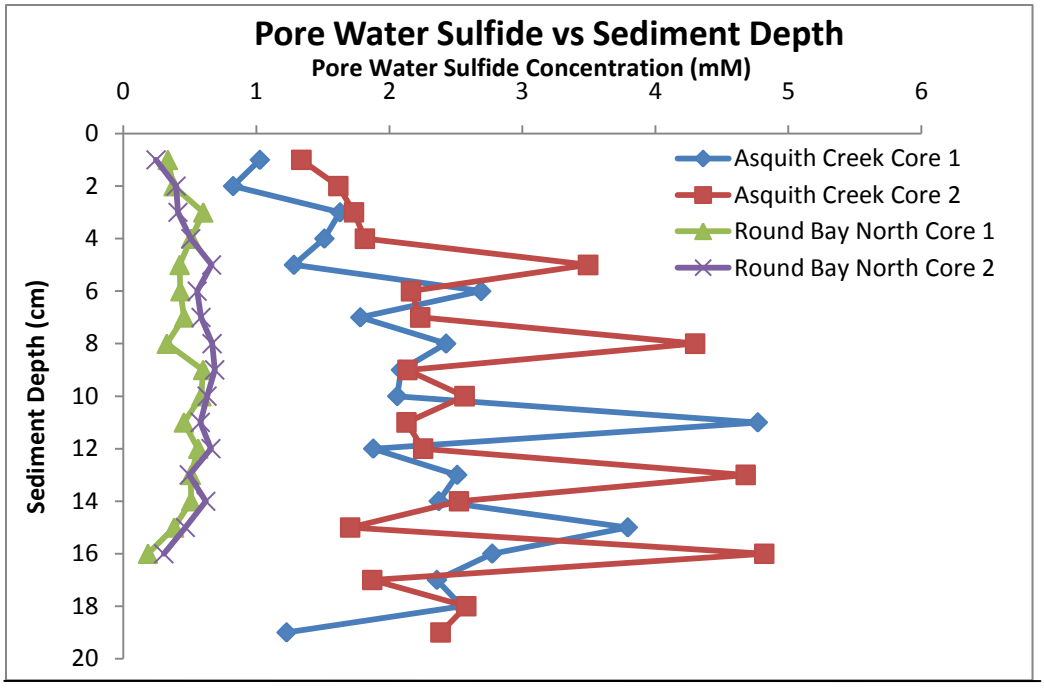

Figure 11: Comparison of sediment pore water sulfide as a function of sediment depth in Round Bay North and Asquith Creek. The sulfide concentrations are higher at Asquith because it is a secluded area allowing for longer euxinic cycles.

Here we present the effect of temperature on sulfide flux at a localized euxinic zone in the Severn River. The data supports the hypothesis that at high temperatures the sulfide flux will increase due to the higher activity levels of the sulfate reducers and low temperatures will result in a decrease of activity and sulfide flux. We tested the hypothesis by incubating sediment cores at 28 °C and 9 °C which are the average summer and winter temperature at the Round Bay North station in the Severn River. As temperature increased sulfide flux increased from -0.16± 0.02 mmol m^{-2} d^{-1} to -2.30±0.06 mmol m^{-2} d^{-1}. The results suggest a direct relationship between sulfide flux and temperature (Table 2, 3, 4, 5). Organic carbon was not a limiting factor in this study because at the end of the experiment the weight percent organic carbon in the bottom sediment layer ranges from 12.9-14.1% and 15.0-17.7% in the top sediment layer (Table 6).

7. Conclusion

In this study we performed temperature controlled experiments, bulk sediment water and organic carbon analysis, and sediment pore water analysis to better understand the living condition of benthic organisms in the Severn River during times of localized euxinia. A direct relationship between sulfide flux and temperature was observed in both the 9 °C and 28 °C sediment core experiments conducted. These results were expected because previous studies have shown a direct relationship between sulfate reduction rates and temperature (Al Raei et al., 2009; Johnston et al., 2007; Mitchell et al., 2009). Results from this study can be applied to other river systems suffering from the effects of eutrophication. A possible solution to the depleted oxygen issue is the removal of algae from the water source decreasing the amount of decomposing organic matter simultaneously allowing for the availability of more oxygen. In the future we plan to conduct further analyses to better understand the effects of eutrophication on river health in the Chesapeake Bay.

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APPENDIX I

Bulk Analysis of Bottom Sediment Layer of Core					
	Wet Weight (g)	Dried at 80° C Weight (g)	Weight % Water Content	Dried at 550° C Weight (g)	Weight % Organic Carbon
9° Core #1	29.8779	4.6614	84.4	4.0045	14.1
9° Core #2	31.3585	6.3232	79.8	5.5301	12.5
9° Core #3	30.3131	5.9294	80.4	5.1664	12.9
28° Core #1	31.7184	5.6748	82.1	4.9130	13.4
28° Core #2	31.8105	5.2317	83.6	4.5300	13.4
28° Core #3	30.2542	5.6239	81.4	4.8618	13.6

Bulk Analysis of Top Sediment Layer of Core					
	Wet Weight (g)	Dried at 80° C Weight (g)	Weight % Water Content	Dried at 550° C Weight (g)	Weight % Organic Carbon
9° Core #1	30.5970	2.6719	91.3	2.2327	16.4
9° Core #2	29.8371	2.9927	90.0	2.5451	15.0
9° Core #3	29.2147	1.9802	93.2	1.6222	18.1
28° Core #1	30.4300	2.4054	92.1	2.0299	15.6
28° Core #2	31.1934	2.2478	92.8	1.8580	17.3
28° Core #3	31.3099	2.3471	92.5	1.9306	17.7

Table 6: Data from loss of ignition analyses. Note that that the weight percent water content is because the sediment is very muddy and not firm. The percent organic carbon values are after the completion of the sulfide flux experiments and the values indicate that organic carbon was not a limiting factor.

Sediment Pore Water Sulfide Concentration (mM)				
Sediment Depth (cm)	**Asquith Creek**		**Round Bay North**	
	Core 1	**Core 2**	**Core 1**	**Core 2**
1	1.03	1.34	0.33	0.25
2	0.82	1.62	0.38	0.40
3	1.63	1.73	0.60	0.41
4	1.51	1.82	0.51	0.51
5	1.28	3.49	0.42	0.66
6	2.69	2.16	0.43	0.55
7	1.78	2.23	0.46	0.58
8	2.43	4.30	0.33	0.67
9	2.09	2.14	0.60	0.69
10	2.06	2.57	0.59	0.63
11	4.77	2.13	0.45	0.58
12	1.88	2.25	0.56	0.66
13	2.51	4.68	0.51	0.49
14	2.37	2.52	0.51	0.62
15	3.79	1.71	0.38	0.47
16	2.77	4.82	0.18	0.30
17	2.36	1.87		
18	2.54	2.58		
19	1.23	2.38		

Table 7: Data on sediment pore water sulfide as a function of sediment depth in Round Bay North and Asquith Creek. The sulfide concentrations are higher at Asquith because it is a secluded area allowing for longer euxinic cycles. Overall Asquith Creek is more dangerous for benthic organisms than Round Bay North.

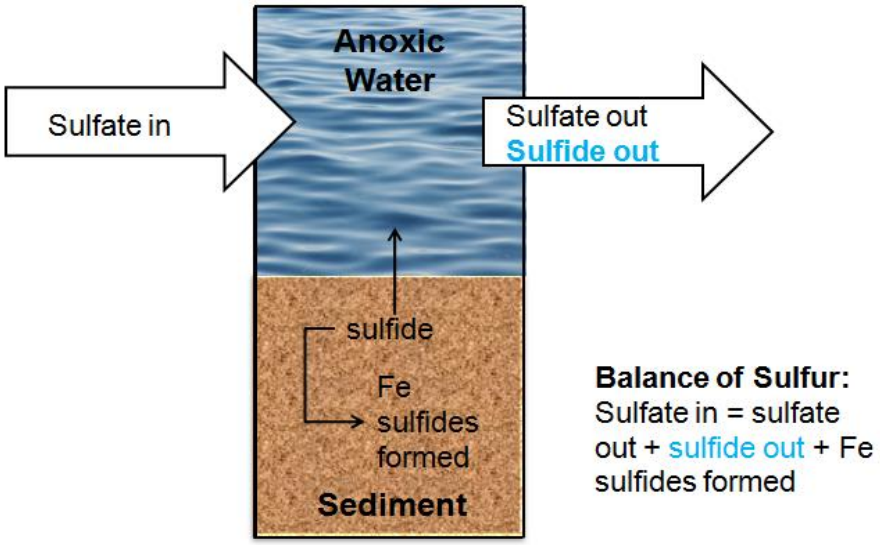

Figure 12: Diagram showing the movement of sulfur in the system. The concentration of sulfate moving into the system was 6.2 ±0.5 mM. In the sediment layer sulfate reducing bacteria reduce sulfate to sulfide. Some of the sulfide is used to form iron sulfides and the remaining sulfide diffuses to the water column and the flux quantifies the amount sulfide out in a given area. For this study we are not concerned about quantifying any other form of sulfur besides sulfide.

APPENDIX II

Example Sulfide Flux Calculations:

$$Flux = \frac{[Sulfide\ measured]\ x\ Flow\ rate\ Measured}{Area\ of\ sediment\ surface}$$

The two variables are concentration of sulfide and flow rate that are based on experimental data. The inner radius of the cylindrical core was .0260 m and that value was used to calculated the area = 3.14 * 0.0260^2 = 0.002122134 m^2

For 9° C:

14.142 µM * 0.001 = 0.0141 mM Sulfide

(0.0141 mM Sulfide * 0.027 L/day)/ 0.002122134 m^2 = 0.180 mmol $m^{-2}d^{-1}$

For 28° C:

105.37 µM * 0.001 = 0.1054 mM Sulfide

(0.1054 mM Sulfide * 0.048 L/day)/ 0.002122134 m^2 = 2.38 mmol $m^{-2}d^{-1}$

Example Sulfate Concentration Calculations:

(Weight of 15mL centrifuge tube and dried $BaSO_4$) – (Weight of empty 15ml centrifuge tube) = Weight of $BaSO_4$

5.5176 g – 5.5032 g = 0.0144 g $BaSO_4$

0.0144 g $BaSO_4$ / 233.43 g/mol BaSO4 = 6.1689E-05 mol $BaSO_4$

6.1689E-05 mol BaSO4/ 0.01 L Round Bay North Water = 0.006169 M SO_4

0.006169 M SO_4 * 1000 mM/ 1 M = 6.18 mM Sulfate