Microbial community dynamics in polar hypersaline springs: viral ecology and sulfur cycling

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Abstract

As a field, astrobiology spans a range of disciplines, intertwining the tools and foundation that each can bring to a common table of inquiry: Where did we come from? Are we alone? Grand in aspiration, the experimental work behind these questions is of an exacting nature less prone to superlatives, but essential to constraining the likelihood of life's origin, evolution and detectability on scales spanning galactic to the molecules on which life depends. The work described in this thesis focuses on an *extreme environment* and the microbial ecology therein. Microbes that inhabit such environments are of fundamental interest to astrobiologists, as they open windows to observe deviations on biological processes such as might be found under conditions disparate from what we have traditionally thought of as hospitable conditions for life. These conditions are informative to considering life on other planetary bodies, or on early Earth, when the chemistry of the oceans and atmosphere were substantially different than they are today. The environments on which this work focuses are two polar hypersaline springs in the Canadian High Arctic, considered extreme by their low temperature, high salinity and low oxygen. Within those springs, this work focuses on two processes: 1) microbial mortality at the hands of tiny and ubiquitous predators: viruses; and 2) biosignatures of microbial metabolism that can endure in the rock record: isotopes of sulfur.

The abundance of viruses from Arctic hypersaline spring water and sediment environments was investigated and from those findings the first estimates of contact rates between viruses and bacteria in sediments were made. The extremely oligotrophic nature of these springs maintains some of the lowest virus-bacteria ratios and contact rates observed in natural environments to date, indicating that viruses do not play a major lytic role in these springs and that alternative viral replication strategies maintain the free virion population. Comparison of these results to reports from marine sediments identifies a viral biogeographic divide in deep-sea sediments that separates shallower bacterial communities controlled by lytic viruses from deeper ones that are not. Paper 1 is titled *Low viral predation pressure in cold hypersaline springs of Axel Heiberg Island*.

Complementary viral dynamics experiments were performed in cold, oligotropic sediments of polar hypersaline spring sediments in the Canadian High Arctic. This work specifically addresses 1) the trophic hypothesis of viral influence on microbial growth:

that there is a direct relationship between trophic conditions and the predatory influence exerted by viruses on their microbial hosts, and 2) the relationship between oligotrophy and lysogenic replication: that in oligotrophic environments, viruses capable of this replication strategy are more common. By nature of their low temperature, high salinity and low carbon availability, the springs investigated in this study represent an experimentally accessible, low energy environmental outlier in which to test these hypotheses. These sediments maintain extremely low rates of microbial growth and viral production and a substantial fraction of viruses are extremely resistant to decay. These viruses do contribute to microbial mortality, but are not the primary cause of such. A substantial fraction of microbes in these sediments appear to be lysogens, harboring inducible provirus, yet temperate viruses do not seem to account for a large fraction of the in situ population of virions. These findings support the trophic hypothesis, but offer limited support for an increasing frequency of lysogens in oligotrophic environments. These findings extend the range of geochemical conditions under which viral dynamics have been explored and suggest that viruses are able to maintain a role in microbial mortality even in extremely low biomass environments, potentially by means of resisting decay (providing a longer period for them to contact a host). Paper 2 is titled *Sluggish viral dynamics in Arctic hypersaline spring sediments*.

Finally the preserved signals of biological isotopic fractionation of sulfur in one of the two springs was described, as were the accompanying sulfate reducing microorganisms that inhabit its cold, oligotropic sediments. This work specifically addresses the influence that environmental chemistry and genomic diversity have on the fractionation of sulfur isotopes across a small spatial scale with variations in redox, pH, temperature, dissolved oxygen and dissolved sulfide, as well as microbial taxonomic composition and diversity in the final gene in the sulfate reduction pathway. These sediments show little variation in sulfur fractionation, despite differences in several of the characters known to contribute to the extent of fractionation by cultured sulfate reducing isolates. To address the discrepancy between measured and modeled cell-specific sulfate reduction rates (csSRR) and isotopic fractionation; environmental data from the spring was incorporated into a previously reported thermodynamic-based model. The model indicated the most likely cause of deviation from predicted fractionation values was a

greater than measured csSRR, suggesting heterogeneous activity among that group. Employing the same model to address the unexpected lack of correlation between *dsrB* alpha diversity metrics and relatively constant sulfur isotope fractionation between stations, kinetic parameters associated with the *apr* gene in the sulfate reduction pathway were found to have greater influence on expressed fractionation factor than the *dsr* gene. Paper 3 is titled *Diversity-signal disconnect between dsrB sequencing and sulfur isotope fractionation signals.*

Résumé

L’astrobiologie est un domaine qui rassemble plusieurs disciplines, entrelaçant les outils et la base que chacune apportent à une table commune d’enquête: D’où venons-nous? Sommes-nous seuls? Le travail expérimental derrière ces questions d’envergure est très exigeant et, bien qu’il appelle moins aux superlatifs, il est essentiel pour contraindre la probabilité de l’origine de la vie, son évolution et son dépistage à des dimensions allant des galaxies aux molécules dont la vie elle-même dépend. Le travail décris dans cette thèse se concentre sur un environnement extrême et l’écologie microbienne qui existe dans celui-ci. Les microbes qui habitent des tels environnements sont d’un intérêt fondamental aux astrobiologistes car ils ouvrent des fenêtres nous permettant d’observer les différences entre dans les processus biologiques que l'on peut trouver dans des conditions diverses de celles auxquelles on réfère traditionnellement comme étant propices à la vie. Ces conditions extrêmes nous informent sur la possibilité de la vie sur d’autre corps planétaire, ou sur la terre primitive, a un moment ou la chimie des océans et l'atmosphère était sensiblement différente de ce qu'elles sont aujourd'hui. Ce travail se concentre sur deux sources d’eau hypersaline dans l'Extrême-Arctique, considéré comme extrême d*û* à leur température basse, une salinité très élevé, et une faible teneur d’oxygène. Au sein de ces sources d’eau ce travail se concentre sur deux processus : 1) la mortalité microbienne sous l’influence de prédateurs minuscules et omniprésents : les virus ; et 2) les biosignatures de métabolisme microbien qui peuvent survivre à long terme dans la roche : les isotopes du soufre.

L'abondance de virus qui proviennent de sources d'eau hypersaline et de leurs sédiments a été étudiée et de ces constatations les premières estimations des taux de contact entre les virus et les bactéries dans les sédiments ont été faites. La nature extrêmement oligotrophe de ces sources contribue à maintenir un des plus bas ratios virus-bactéries et taux de contact observés dans les milieux naturels à ce jour, ce qui indique que les virus ne jouent pas un rôle majeur d’infection lytique dans ces sources et que des stratégies alternatives de réplication virale doive maintenir la population de virion libre. En comparant ces résultats et ceux des sédiments marins, on peut identifier une division virale biogéographique dans les sédiments d'eau profonde qui sépare les communautés bactériennes d’eau peu profondes, contrôlées par des virus lytiques, des

communautés d'eau plus profondes, qui ne le sont pas. Le premier article est intitulé *Faible prédation virale dans les sources d'eau froide et hypersaline de l'île Axel Heiberg*.

Des expériences virales complémentaires ont été conduites dans les sédiments froids et oligotrophiques des sources hypersalines polaires de l'arctique Canadien. Le travail addresse spécifiquement 1) l'hypothèse trophique de l'influence virale sur la croissance microbienne: il y a une relation directe entre les relations trophiques et l'influence de prédation excercée par les virus sur les hôtes. 2) La relation entre oligotrophie et réplication lysogénique: dans des milieu oligotrophiques, les virus qui sont capable de cette forme de replication sont plus fréquents. Les conditions environnmentales froides, à haute salinité, oligotrophiques des sources étudiés représentent un milieu expérimentale accessible et plutôt rare dans lequel cette hypothèse a pu être testée. Ces sediments maintiennent des taux de croissances microbiens et viraux extrêmement bas et une proportion importante des virus sont résistants à la décomposition. Ces virus contribuent à la mortalité microbienne mais n'en sont pas la cause primaire. Une fraction importante des microbes dans ces sédiments semblent êtres lysogènes, contenant des provirus inducibles. Par contre, les virus tempérés ne semblent pas d'une grande importance dans la population de virions. Ces résultats semblent supporter l'hypothèse trophique mais offre aussi un support pour un fraction augmentante de lysogènes en environnement oligotrophiques. Ces observations étendent les conditions géochimiques sous lesquelles les dynamiques virales ont été explores et suggère que les virus sont capables de maintenir un role dans la mortalité microbienne, même en environnements avec une très basse biomasse et ceci, potentiellement par la résistance à la decomposition (augmentant la période possible pour ceux-ci d'arriver en contact avec un hôte). Le deuxième article est intitulé *Sluggish viral dynamics in Arctic hypersaline spring sediments*.

Finalement, les signatures isotopiques biologiques dans un des deux sources a été étudiée en conjonction avec la composition de la communauté de micorganismes sulfato-réducteurs qui habitent les sédiments froids et oligotrophiques. Le travail, décrit l'influence que la géochimie et la diversité génomique peut avoir sur le fractionnement isotopique du souffre dans un échelle spatiale petite avec variations de rédox, de pH, température, oxygène, matière organique, sulfate, le sulfure ainsi que la composition

taxonomique microbienne et la diversité nucléotidique du système génétique responsable de la sulfato-réduction. Ces sédiements suggèrent peut de variations en fractionnement du soufre malgré que la variation mesurée de plusieurs des caractères génétiques qui affectent le fractionnement isotopique en cultures pures. Pour résoudre une différence entre les taux de sulfato-réduction mesurées et ceux modélisées, les paramètres environnementaux des sources ont été incorporées dans un modèl thermodynamique. Le modèl suggère que la cause la cause la plus probable de la déviation des valeurs isotopiques est que le taux de sulfato-réduction par cellule, est sous estimé. Ces conclusions suggèrent que le taux de sulfato-réduction est variable parmis les microorganismes identifiés. En employant le même modèl pour addresser la différence entre la diversité du gène *dsrB* et le fractionnement isotopique constant, les paramètres cinétiques associés au gène *apr*, critique pour la sulfato-réduction et responsable pour une plus grande variation du fractionnement isotopique que le gène *dsr*. Le troisième article est intitulé: *Diversity-signal disconnect between dsrB sequencing and sulfur isotope fractionation signals.*

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Preface & contributions of authors

The core of this thesis consists of three papers with multiple authors as contributors. Each manuscript provides original contributions to knowledge.

Paper 1 is titled *Low viral predation pressure in cold hypersaline springs of Axel Heiberg Island* and is authored by Jesse Colangelo-Lillis, Boswell Wing and Lyle Whyte. It was published in the journal *Environmental Microbiology Reports*. Colangelo-Lillis and Whyte designed the study. Experiments for this paper consisted of fieldwork (measurements and sample collection), epifluorescent and transmission electron microscopy; these were conducted by Colangelo-Lillis. Developing models of contact rates was by Colangelo-Lillis and Wing and the results were interpreted and the manuscript written by Colangelo-Lillis, Wing, and Whyte. Original contributions of this work include 1) the identification and enumeration of viruses from the highest latitude non-marine environment yet studied, 2) development of the first model of contact rates between microbes and viruses in sediments, and 3) providing evidence that contact rates between viruses and microbes in the global ocean's deep sediments my be too low for viral predation to have a significant impact on microbial mortality there.

Paper 2 is titled *Sluggish viral dynamics in Arctic hypersaline spring sediments* and is authored by Jesse Colangelo-Lillis, Boswell Wing, Isabelle Raymond-Bouchard and Lyle Whyte. It is submitted for publication to the journal *Frontiers in Microbiology*. Colangelo-Lillis and Whyte designed the study. Field experiments for this paper consisted of environmental measurements, sample collection and in situ incubation experiments; these were conducted by Colangelo-Lillis and Raymond-Bouchard. Wet lab and microscopy and data analyses and were by Colangelo-Lillis. The results were interpreted and the manuscript written by Colangelo-Lillis, Wing, and Whyte. Original contributions of this work include 1) making the first measurements of in situ microbial growth and viral dynamics from an extremely low biomass environment, 2) demonstrating that despite low numbers of viruses and microbes, that viruses do contribute to microbial mortality and are able to compensate for low contact rates with attenuated rates of decay of viral particles, and 3) demonstrating that a substantial portion of the microbes in these spring sediments harbor prophage within their genomes, lending support to the hypothesis that cold and hypersaline environments promote lysogenic viral

replication.

Paper 3 is titled *Diversity-signal disconnect between dsrB sequencing and sulfur isotope fractionation signals* and is authored by Jesse Colangelo-Lillis, Claus Pelikan, Ianina Altshuler, Alexander Loy, Lyle Whyte and Boswell Wing. It is to be submitted for publication to the journal *International Society for Microbial Ecology Journal*. The study was designed by Colangelo-Lillis and Wing. Field experiments for this paper consisted of environmental measurements, sample collection and in situ incubation experiments; Colangelo-Lillis and Altshuler conducted these. Wet lab, molecular biology and isotopic analyses were by Colangelo-Lillis and Pelikan. Bioinformatics work was by Pelikan. The results were interpreted and the manuscript written by Colangelo-Lillis, Pelikan, Whyte, Loy and Wing. Original contributions of this work include 1) performing the first simultaneous characterization of microbial diversity, activity and sulfur isotope fractionation signal from an environmental system, 2) identifying a disconnect between the molecular diversity of the *dsrB* gene, typically used for assigning phylogeny to sulfate reducers in the environment, and the net fractionation signals associated with those communities, and 3) identifying the probable role of heterogeneous activity by members of the sulfate reducing community, which likely influences the difference between predicted and observed fractionation effects.

Jesse Colangelo-Lillis is responsible for the full content of this thesis.

Introduction

Astrobiology

Typically fields of scientific inquiry trend towards specialization. Each experiment provides new directions and new, finer resolution questions lead to finer resolution experiments. Occasionally, one, or more often several, findings in one of these specialization spirals requires the discipline raise its head to seek the counsel of another. Such was the case for the field of molecular biology several decades ago, when microbiology, genetics, physical and organic chemistry combined facets of their disciplines to elucidate the structure, function and regulation of the nucleic acids that dictate life's structure and function (Chen *et al.* 2014). So again is the case with astrobiology in the past 20 years (Brazelton and Sullivan 2009). A number of findings, in different fields, provoking the emergence of this discipline include an expansion in our understanding of the metabolic versatility of microbial life (microbiology; Wrighton *et al.*, 2012), an abundance of extrasolar planets (astronomy; Howard 2013), the detection of indications of liquid water on numerous bodies within our solar system (planetary science; Russell *et al.*, 2014), and the [controversial] report of signs of microbial life in fragments of a meteor of Martian origin recovered on earth (geology; McKay *et al.*, 1996). In light of a heightened appreciation for the diversity of life, its potential habitats and our ability to detect signatures of that life in the rock record, coalesced a field of study: astrobiology. Formally that field seeks to address the origin, evolution, distribution and future of life in our universe (Des Marais *et al.* 2008). The work contained in this thesis addresses a number of themes in astrobiology: extreme environments, evolution, and detecting and interpreting signs of life.

Extreme Environments

Extreme environments are defined by their deviation in some physical or chemical property from those in which eukaryotic life thrives. Examples of these parameters include temperature, pressure, oxygen availability, radiation, acidity, water availability, salinity, or presence of any of a number of substances that are toxic to many (primarily eukaryotic) forms of life (e.g. arsenic). Extreme environments are of interest to

astrobiologists and microbiologists in that they offer contemporary, natural environments in which to study the diversity of (primarily microbial) life and the adaptations that facilitate its existence in conditions inhospitable to multicellular life (Des Marais *et al.* 2008). Paleo-reconstructions of the conditions of the early Earth's atmosphere and ocean allude to a very different planet than the one we now inhabit (Lyons, Reinhard and Planavsky 2014). Life persisted through these changes and the manner in which it did can inform what record (e.g. biosignatures) that life may have left behind. Further, the most proximate bodies in our solar system are unlike Earth, but not all are (definitively) beyond the realm of the conditions that are permissible for *some* forms of life. As such, studying the microbes that live in cold, dark, anoxic, saline environments may inform what signatures of that life we might search for on Jupiter's moon Europa, or studying those that live below surface in aquifers or caves may inform the character of life or microbial fossils we might find on Mars. Further, extreme environments may require unique metabolisms for energy acquisition, and these metabolisms may lend themselves to the production of biosignatures that could be detected beyond our solar system (e.g. oxygen or methane).

Hypersaline springs of Axel Heiberg Island

This work focuses on hypersaline springs in the Canadian High Arctic. These springs are extreme environments in their deviations in temperature (i.e. they are cold), salinity (i.e. they are hypersaline), and oxygen availability (i.e. they are near anoxic at their outlets). Both spring systems: Gypsum Hill and Lost Hammer, are located on Axel Heiberg Island of the High Arctic, flow through deep permafrost (~400 m), and are among the only known cold springs in thick permafrost on Earth (Andersen 2002). Because of their location in a polar desert environment and presumed discharge from a substantial subterranean reservoir, and because of their salinity and freezing point depression, these springs are considered amongst the best Earth analog environments for potential ground water systems and aquatic habitats on Mars (Pollard *et al.* 2009). The perennial Gypsum Hill (GH) spring system consists of approximately forty springs and seeps located at 79°24'30"N, 90°43'05"W, discharging into Expedition River ~7 km upstream from the head of Expedition Fjord (Pollard *et al.* 2009). The work reported on

in this thesis was conducted on the spring with the largest outlet of the GH spring system: GH4. Lost Hammer spring, located in a valley south of Strand Fjord (79°07'N, 90°21'W) flows from a single outlet and forms a distinct 2 m high halite tufa. Both springs are perennial, LH spring outlet water is colder (~−5°C) than GH4 (~6°C), and more saline (24% compared to 8%)(Perreault *et al.* 2007; Lay *et al.* 2012). At their outlets, the water of both springs is at near-neutral pH, reduced, nearly anoxic, and enriched in sulfate. Mineralogy of the spring sediments are similar and include abundant gypsum, halite, elemental sulfur, quartz, calcite, and plagioclase/clays (Battler, Osinski and Banerjee 2013).

Three microbiological studies have been undertaken at GH4. The first two performed clone library and isolation characterization on GH4 outlet sediments and found representatives from the families Proteobacteria, Firmicutes, and Bacteroidetes, and that the dominant proportion of sequences belonged to one species, *Thiomicrospira psychrophila* (Perreault *et al.* 2007). Based on taxonomy, sulfur oxidizing and sulfate reducing metabolisms were inferred to be active. Isolates included members of phyla Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Halothiobacillus and Thiomicrospira; common isolates were bacteria of the genera *Gillisia*, *Psychrobacter*, *Marinobacter*, and *Sporosarcina* (Perreault *et al.* 2008). The third study focused on a microbial streamer (anchored bacterial filaments) community found in the spring channel water (Niederberger *et al.* 2009a). Microscopy revealed the filament material to be composed of extracellular polymeric substance (EPS), bacterial cells and gypsum crystallites. Using clone libraries and fluorescent in situ hybridization technique, the bacteria were identified to consist entirely of *Thiomicrospira*. That these microbial filaments can persist under sub-zero temperatures via chemolithotrophy and in the absence of light is of astrobiological interest, particularly for its relevance to subsurface aqueous environments hypothesized to exist on Mars. Notably these streamer communities have received further examination for the development of techniques and tools for extraterrestrial life detection (Rogers *et al.* 2010).

Four microbiological studies have been undertaken at Lost Hammer spring. The first of these studies focused on the spring outlet sediments, reported low biomass, and using clone libraries established low microbial 16S rRNA gene diversity, identified

Loktanella, *Gillisia*, *Halomonas* and *Marinobacter spp*. and cultured *Marinobacter* and *Halomonas spp.* at −5°C (Niederberger *et al.* 2010). Lay et al. (2012) reported microbial diversity and activity from the channel sediments of the LH spring, and found 100X higher biomass compared to outlet, greater microbial diversity, and phylotypes containing members capable of methanogenesis, methanotrophy, sulfur oxidation and sulfate reduction. Psychrotolerant and halotolerant *Marinobacter*, *Planococcus*, and *Nesterenkonia spp*. were isolated from these sediments and were capable of growth at −5°C. The first meta-omics study of this system was reported in 2013 (Lay *et al.* 2013) in a metagenome and pyrosequencing of 16S rRNA cDNA from LH outlet sediments. Cyanobacteria, Bacteroidetes, and Proteobacteria represented the dominant phyla most similar to the classified sequences origins. cDNA indicated activity predominantly by ammonia oxidizing Archaea, denitrifying *Pseudomonas spp*., sulfate reducing *Desulfobulbus spp.*, and sulfur oxidizing *Thermoprotei*. A final study on succession (Lamarche-Gagnon *et al.* 2015) found microbial communities in LH outlet sediment to remain relatively constant between late winter and summer and that hydrogenotrophy and short chain alkane degradation were likely metabolic processes. In tandem this work from both springs demonstrated the presence and potential activity of a wide array astrobiologically interesting metabolisms under extreme conditions of low temperature, high salinity and low oxygen, extending the range of conditions under which a number of these metabolisms were known to be active.

Building off a foundational knowledge of the chemistry and diversity of these extreme environment Arctic springs, this work aimed to focus on ecological processes. Among the most fundamental of these processes are 1) What influence do predators have on microbial populations?, and 2) What are the signatures of a microbial population's activity in its environment? The work described in this thesis focuses on the influence of viruses on the microbial populations that inhabit these springs, and also on one signature of the sulfur metabolisms that play a large role in the microbial ecology of these springs: sulfur isotopes.

Viruses of microbes

Viruses are as ubiquitous as life. In every place life of any sort has been found, when examined, viruses of that life have been found in association. To the best approximation of virologists, viruses infect every living thing on our planet (Forterre 2010) and may be an inevitable consequence of cellular life (Iranzo *et al.* 2016). However their diversity and mechanisms of infection and replication are poorly understood for reasons that are both methodological and an outcome of sheer numbers. Because viruses are thought to have narrow host range (a decades old dogma being brought back into question), and because the majority of organisms that have been studied appear to have multiple viruses, viral diversity is expected to be much greater than the diversity of living things (Sime-Ngando 2014). Speaking to that diversity, viruses outnumber microbes (the most abundant living things on our planet) by an estimated order of magnitude, putting their numbers in the global ocean on the order of 10^{31} (Suttle 2005). Based on a smaller set of studies, viral abundances in ocean sediments are expected to equal the abundance of microbes there, and viruses in terrestrial environments (the least well studied environment) are expect to exceed microbes by one to two orders of magnitude (Danovaro *et al.* 2008a). Complementing these numbers, based on the novel sequences identified in contemporary viral metagenomes, compared to the database of all genomic sequences, viral diversity is currently estimated to be on the order of 3.9 million protein cluster types (Cesar Ignacio-Espinoza, Solonenko and Sullivan 2013). As a result of this abundance, this diversity, and the difficulty of studying viruses in culture- which requires a host isolate (99% of Earth's microbes are currently resistant to cultivation)- the viral infective and replicative mechanisms that are known are expected to represent a small fraction of those in existence (Brum and Sullivan 2015). However, even the small number that are well studied are fantastical in their range of molecular versatility (Rohwer *et al.* 2014). The simplicity of these 'biological entities'—nucleic acids wrapped in protein—coupled with this extensive diversity of form and function make viruses an appealing entity for astrobiologically informative study.

As a density dependent predator, through predation viruses maintain microbial biodiversity (Weinbauer and Rassoulzadegan 2004). This role has been described as 'kill the winner' (Thingstad 2000) though it harks back to ecological processes (antagonistic

coexistence) described for animals several decades prior. As implied by their abundances, viruses are extremely effective predators, in the global ocean turning over 20-40% of microbial biomass every day (Suttle 2007). There are biogeochemical implications of this massive transformation of particulate C, N and P in the biologically rich surface ocean to dissolved pools of those elements which serve as nutrients for growth, as dissolved nutrients resist export to the deep ocean (Fuhrman 1999). Free from the molecular burdens of independent activity and reproduction (and the maintenance of genes for such), viruses play another role, as a vector of gene exchange. As a mechanism of overcoming microbial defenses to infection, some viruses employ low fidelity polymerases or forego the molecular proofreading and editing machinery of cellular life (Steinhauer, Domingo and Holland 1992). As a result they are much more likely to introduce diversity through replication error, and even to incorporate fragments of host nucleic acids into viable virion particles (Roux *et al.* 2015b). The extent to which genes carried by viruses are able to express novel proteins within their hosts is poorly constrained, but in instances in which such translation has been studied, the impacts on the host's ecology can be dramatic (Li *et al.* 2003; Sullivan *et al.* 2006).

Microbes and viruses are often described as engaged in an arms race: microbes trying to avoid viral predation, viruses dependent on cellular machinery for their own replication and each trying to outmaneuver the other. In no environment have microbes been found to evade these ubiquitous predators. However, given that individual viral particles have a finite time to contact and infect an appropriate host before decaying to an non-infective state, there may be biomass or microbial activity thresholds below which viruses cannot replicate. Under such conditions, their role in maintaining biodiversity would be undermined. It is this hypothesis that is addressed in the low biomass hypersaline springs of the High Arctic. This hypothesis is specifically investigated by 1) developing a model of contact rates between viruses and microbes in sediments, taking into account a number of physical parameters [Paper 1], and 2) making in situ measurements of rates of microbial growth and viral decay, allowing calculations of the impact that viral predation has on microbial mortality [Paper 2].

Isotopic biosignatures and sulfate reducing bacteria

Both the origin and early evolutionary history of life on Earth are poorly constrained, as is the corresponding record of early Earth's environmental conditions. The reasons for this are tied to the fact that clues to this information persists only in the geologic record, and rocks older than 2.5 billion years are quite rare. Of those ancient rocks, only a small subset are likely to yield evidence of life, and even then, the fidelity with which they preserve information as to the time of their deposition (and biological or geological processes) is related to the extent of their alteration by metamorphism or deformation. Indicators of life include microfossils, biological organic molecules and stable isotopes. Many microfossils offer contentious evidence, as life on early Earth was exclusively microbial, of simple shape and lacking organelles. The combination of small size and unremarkably morphology makes distinguishing true fossils from abiogenic structures of similar size and form extremely difficult (Brasier *et al.* 2002). The skeletons of molecular biomarkers- recognizable derivatives of biological molecules (mostly lipids)- can serve as long-lived diagnostic indicators of taxonomic activity, in the case where the parent molecule is specific to some group of organisms (Brocks 1999). These molecules often persist only in extremely low abundance, are subject to degradation and are difficult to assess for syngenicity. Stable isotopes of a number of the elements essential for life (i.e. C, H, N, O, S) have proven to be the most robust and continuous record of environment and biology. Each of these elements occurs naturally in multiple isotopes. Biological processes metabolize lighter isotopes at a faster rate, resulting in metabolic products isotopically depleted in heavier isotopes compared to the substrates metabolized. Despite their relatively continuous record, the accurate and predictive interpretation of isotopic signatures requires mechanistic understanding of the multiple processes influencing the extent of fractionation. Microbial dissimilatory reduction of sulfate plays the largest role in the fractionation of sulfur isotopes.

Microbial dissimilatory sulfate reduction is a geologically ancient, anaerobic, energy-yielding metabolic process in which sulfate reducing microorganisms (SRM) reduce sulfate to hydrogen sulfide while oxidizing organic molecules. Sulfate reduction is a dominant pathway for organic degradation in marine sediments (D'Hondt, Rutherford and Spivack 2002) as well as in some terrestrial subsurface settings where sulfur-bearing

minerals dominate over iron(III)-bearing minerals. Diversity of SRM extends across the domains Archaea and Bacteria (Rabus, Hansen and Widdel 2006). Described SRM can be divided into seven phylogenetic lineages: five Bacterial and two Archaeal. Most SRM fall into 23 Bacterial genera, within the Gram-negative Deltaproteobacteria and the Gram-positive Clostridia. They are extremely versatile with respect to the electron donors and electron acceptors used for growth. SRM inhabit in a broad range of environmental conditions and are ubiquitous in anoxic habitats: they have been identified and cultured from both marine and freshwater sediments and from deep subsurface environments, including oil wells and hydrothermal vents. They additionally persist in environments with extremes of pH, temperature and salt concentrations. Microbial sulfate reduction has a substantial influence on global biogeochemical cycles and is responsible for the respiration of up to 30-50% of organic matter in marine sediments (Bowles *et al.* 2014). During the reduction of sulfate to sulfide, lighter isotopes of sulfur are reduced at a faster rate than heavier isotopes, resulting in the metabolic waste being sulfide isotopically enriched in the light isotopes of sulfur relative to the source sulfate (Harrison and Thode 1958). This sulfide is preserved in sediments as metal sulfides and can persist as evidence of metabolism on the scale of billions of years (Shen and Buick 2004). Given their contemporary role in carbon and sulfur cycling, their antiquity on Earth, and the sulfur isotope signature that results from their metabolism, the specific role that the environment plays on that fractionation has been of interest to paleo-geologists and biologists for decades (Berner and Canfield 1989).

Recent work has taken a reductionist approach to investigating mechanisms of microbial sulfate reduction, focusing on culturing isolates in the laboratory and experimentally challenging those cultures with variations in temperature, pH, electron donor and acceptor types and concentrations. Occasionally several isolates will be subjected to the same challenges in tandem (e.g. Bradley *et al.* 2016) to give an idea of the variation between species (or domains, e.g. Detmers *et al.* 2001) but to date no study of fractionation of multiple sulfur isotopes has been conducted on environmental communities –in situ or in enrichment culture- where the sum of the diversity of species effects result in a net observed fractionation signal. In contrast to the paleo-geologists and climatologists whose focus on interpreting S isotope signatures is a reconstruction of the

conditions of the ocean and atmosphere of the early Earth, astrobiologists and evolutionary biologists are as interested in extracting as much biological information as possible from the isotope record. Increasing resolving power of mass spectrometry, and the recent development of rare isotopes of S as a diagnostic tool of oxidative processes (Farquhar, Bao and Thiemens 2000; Johnston *et al.* 2005), made apparent that biologically relevant information in the S isotope record remains to be explored. The objective of the final paper of this thesis is to simultaneously assess the influences of taxonomy, gene diversity and environment on isotopic biosignatures in an extreme environment.

Paper 1

Low viral predation pressure in cold hypersaline Arctic sediments and limits on lytic replication

Low viral predation pressure in cold hypersaline Arctic sediments and limits on lytic replication

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Abstract

Viruses are ubiquitous drivers of microbial ecology and evolution and contribute to biogeochemical cycling. Attention to these attributes has been more substantial for marine viruses than viruses of other environments. Microscopy-based investigation of the viral communities from two cold, hypersaline Arctic springs was undertaken to explore the effects of these conditions on microbe-viral ecology. Sediments and water samples were collected along transects from each spring, from anoxic spring outlets through oxygenated downstream channels. Viral abundance, virus-microbe ratios, and modeled virus-microbe contact rates were lower than comparable aqueous and sedimentary environments and most similar to deep subsurface sediments. No individual cell from either spring was visibly infected. Viruses in these springs appear to play a smaller role in controlling microbial populations through lytic activity than in marine water column or surface sedimentary environments. Relief from viral predation indicates the microbial communities are primarily controlled by nutrient limitation. The similarity of these springs to deep subsurface sediments suggests a biogeographic divide in viral replication strategy in marine sediments.

Introduction

Viruses are ubiquitous with microbial life and act as a control on microbial growth and community diversity (Suttle 2005). They harbor vast, largely uncharacterized, genetic diversity (Rosario and Breitbart 2011), and play a role in the transmission and expression of foreign genes to and within their hosts (Lindell *et al.* 2007). These conclusions derive from work conducted in marine environments: open ocean, coastal, and benthic waters and sediments (e.g. Evans and Brussaard, 2012; Parsons et al., 2012; Danovaro et al., 2008; Engelhardt et al., 2014). Whether viruses of non-marine environments serve the same roles as marine viruses in controlling microbial growth, diversity, and carbon supply are only more recently being addressed. Fewer studies have explored viral diversity and interactions in other aqueous (e.g. Hewson et al., 2001; Winget et al., 2011), or terrestrial environments (e.g. Williamson et al., 2007), and fewer still in environments categorized as extreme (e.g. Rice et al., 2001; Fancello et al., 2012). As a result, large tracts of the virosphere await exploration. While not absent (e.g. Maranger et al., 1994; Wells and Deming, 2006a, 2006c, 2006d; Collins and Deming, 2011), Arctic environments in particular are underrepresented in environmental virology literature. Given the strong control of salinity and temperature on viral distribution and replication strategy, Arctic environments should provide new insight into the distribution of viruses and impacts on their microbial hosts.

The influence of salinity has been extensively characterized in solar salterns, where small and stable mesocosms can be compared to their close neighbors over salinities ranging from seawater (35‰) to saturation (~350‰; Oren, 2009). Viral abundances increase along salinity gradients, with substantial increases at salinities exceeding 240‰ (Guixa-Boixareu *et al.* 1996; Bettarel *et al.* 2011). In addition, lytic replication becomes less prevalent compared to non-lethal replication strategies (Guixa-Boixareu *et al.* 1996; Oren, Bratbak and Heldal 1997; Bettarel *et al.* 2011; Boujelben *et al.* 2012). Hypersaline environments contain the highest concentrations of virions reported for natural environments (10^{10} ml^{-1}; Boujelben et al., 2012). Maintenance of such a large viral population is puzzling in light of the diminished role played by lytic replication, and may be due to increased virion stability (Bettarel *et al.* 2011). Alternatively, enhanced viral production may keep virion levels high, through large burst

sizes or chronic infections wherein virions are produced without destroying their hosts (Porter, Russ and Dyall-Smith 2007; Bettarel *et al.* 2011). Larger scale ecological factors could also be important, as high concentrations of salt preclude the presence of microbial predators (e.g. protists), leaving viruses to fill that niche (Santos *et al.* 2012). Enhanced viral abundances in briny environments often result in virus-microbe ratios (VMR) between 40 and 100 (Santos *et al.* 2012), significantly greater than those typically found in marine environments (3 to 10; Wommack and Colwell, 2000).

Similar to high salinity, cold temperatures (≤ 4°C; Deming, 2010) increase virion persistence (Wells and Deming 2006d), favor temperate replication strategies (Angly *et al.* 2006) and inhibit protist grazing (Steward, Smith and Azam 1996; Wells and Deming 2006b). High concentrations of virions have been measured (up to 10^7 ml^{-1}; Madan et al., 2005) and extrapolated (up to 10^9 ml^{-1}; Collins and Deming, 2011) from low-temperature environments including sea ice, permafrost and cryopegs, perennially cold lakes, polar seawater and marine sediments (Tables 2 and 3). When these environments are icy, high virion concentrations can result from physical occlusion, as solutes (including microbes) are progressively concentrated as they are rejected by newly forming ice (Deming, 2010). The influence of cold on lytic replication dynamics is less clear. Long latent periods, low virion burst sizes, and slow production rates appear to be common traits at cold temperatures (Olsen and Metcalf 1968; Whitman and Marshall 1971; Sillankorva *et al.* 2004). Alternatively, some phages appear to be cold-active, with production of the uniquely well-characterized *Pseudomonas* phage 27 five orders of magnitude greater at cold temperatures (between −5 and 4°C) compared to 10°C (Delisle and Levin 1972) and both burst size and production rate of cold-active phage 9A greater at −1°C than 8°C (Wells and Deming 2006a).

Strong controls exerted by salinity and temperature influence the role of viruses in controlling microbial growth, diversity, carbon turnover (through lysis) and evolution (through transduction, phage conversion and selection for defense mechanisms). In order to examine the combined impact of salinity and temperature, we undertook an investigation of viruses inhabiting two cold, perennial, hypersaline springs of Axel Heiberg Island (80°N): Gypsum Hill 4 (GH4; Pollard et al., 1999) and Lost Hammer (LH; Niederberger et al., 2010). No viruses have yet been reported from these springs, or

from any other non-marine Arctic environment at this latitude. Both GH4 and LH outlets are annually consistent in their geochemical and geophysical character (Table 1). As environmental stability confers stable metabolic potential and consistent microbial ecology (Angly *et al.* 2009), the microbial communities found in these springs are model systems for isolating environmental controls on viral-microbe dynamics. They also facilitate biogeographic comparison with Antarctic systems that share their cold, hypersaline nature (e.g. Vestford Hills and Taylor Valley lakes; Kepner et al., 1998; Laybourn-Parry et al., 2001). The abundance of virions in each spring system were characterized along transects extending away from their outlets. These results were interpreted in the context of novel contact rate models that considered spatial distributions of viruses and microbes in the water and sediments of each spring. These results were used to address the constancy of, and controls on, viral abundance and dynamics in cold, hypersaline aqueous and sedimentary environments.

Experimental Procedures

Site description

All samples were collected on Axel Heiberg Island (Fig. 1). The geology of this site has been thoroughly described (Pollard *et al.* 2009). The source of these springs' waters is uncertain; ground-penetrating radar indicates the waters ascend through 400 m of Carboniferous anhydrite evaporite permafrost (Jackson and Harrison 2006). The water's major ions derive from dissolution of minerals as the water travels through subterranean salt diapirs (Andersen 2002). Further details on the physical and chemical and character of each study site can be found in Table 1.

Spring transects and sample collection

Sampling of spring water and shallow sediments (uppermost 3 cm) from both springs (Fig. 2) was performed at the center of the spring outlet, at the edge of the outlet pool, and at five to ten meter intervals downstream for a total of nine sampling sites from GH4 and five from LH. Spring water was collected into sterile 15 ml tubes from 1 cm above the sediment surface. Sediments were collected using 10-ml cut-off syringes (forming sediment corers) and transferred to sterile 15 ml tubes. All samples were fixed to final concentration 1.5% with 0.02 μm-filtered aldehydes. Samples were collected in July 2013, stored at −10°C on site, and transported at that temperature to McGill University for analyses.

Microbe and virus extraction and enumeration

Sediment samples were disrupted by sonication (Heat Systems Ultrasonics W185D, 100 W) in 10 mM sodium pyrophosphate (Danovaro and Middelboe, 2010). Samples were centrifuged (1,000 x g, 2 min, 4°C) and aliquots of supernatant were filtered in the same manner as spring water samples. Sediment extracts and spring water samples were filtered through 0.02 μm-pore size filters, stained with SYBR Green I, and enumerated at 1000X magnification (Nikon Eclipse 80i, NIS-Elements BR imaging software v3.2). Between 200 and 500 cells and viruses were counted from 960 to 2400 10 μm^2 fields (Suttle and Fuhrman 2010; Fig. 3).

Decay of fixed virus-like particles

To evaluate the potential loss of chemically-fixed spring and sediment VLP in cold storage, VLP enumeration from four representative samples (spring water and sediments from the outlet and one channel station of GH4) were repeatedly conducted over a period of 62 days, starting on the day of collection (day 0, 2, 4, 7, 11, 24, 31, 45, 62). VLP counts were fit to a model of VLP decay that had three free parameters: an exponential VLP decay rate expressed as a half life (days), a constant production rate (% day^{-1}) between the start of the experiment and the first time point, and a steady state fraction of recalcitrant VLP (%) that did not decay over the timeframe of the experiment. An initial increase in VLP concentrations required the short-lived constant VLP production rate to be included in the model.

Frequency of visibly infected cells

Transmission electron microscopy (TEM) was used to evaluate frequency of visibly infected cells. Cells were interrogated from eight representative samples: sediments and spring water from the outlet and one channel station for each spring: 200 cells were examined per sample. Samples were centrifuged (90,000 x g, 90 min) onto 200 mesh, formvar coated, copper TEM grids, visualized with a FEI Tecnai 12 instrument, and photographed using an AMT XR80C CCD camera system at the McGill Facility for Electron Microscope Research.

Virus-microbe contact rates

Spring water virus-microbe contact rates (J_{VM}, contacts cm^{-3} s^{-1}) were modeled using the equation of Murray and Jackson (1992), where $J_{VM} = 2\pi \cdot d_M \cdot D_V \cdot V_W \cdot M_W$. d_M is the spherical diameter of the average microbe (cm), D_V is viral diffusivity (cm^2 s^{-1}), and V_W and M_W are in situ concentrations of viruses and microbes (cm^{-3}). Viral diffusivity (D_V) = $k \cdot T / 3\pi \cdot \mu \cdot d_V$, where k is Boltzmann's constant, T is temperature (K), μ is viscosity (g cm^{-1} s^{-1}), and d_v is the diameter of the average virus. Viscosity (μ_{SW}) of spring water was calculated using the most appropriate algorithm for the temperature and salinity of the samples (Phillips *et al.* 1981). Microbe-specific contact rates (J_M) for spring water were calculated as $J_M = J_{VM} / M \cdot$ 86,400 s d^{-1} to yield daily

average virus contacts per microbe. Values employed for each variable are given in Table 2.

Sediment pore water contact rates were modeled considering four possible spatial distributions of viruses and microbes. Each scenario assumes dynamic equilibrium where viruses are replaced at the same rate as they are removed by either sediment or microbe contact. In the first scenario, both viruses and microbes are homogeneously distributed in the pore water (*Homogenous Distribution*, all scenarios illustrated in Fig. 4), with no influence of the sediment grains on either population. This scenario is modeled with the same equation as spring water (above), requiring only a conversion of viruses and microbes cm^{-3} of sediment (as measured for enumeration) to viruses and microbes ml^{-1} of sediment pore water by calculating porosity of sediments from sediment wet mass, dry mass and density. In the second scenario, both viruses and microbes are homogeneously distributed in the pore water and viruses are removed following contact with sediment surfaces (*Homogenous Distribution+Virus Sediment Adsorption*) by subtracting the virus-sediment contact rate (J_{VS}) from J_{VM}. J_{VS} is determined by replacing d_M and M_W in the equation of Murray and Jackson (1992) with sediment diameter d_s and concentration S. In the third scenario, viruses are homogeneously distributed in pore waters but microbes are permanently adsorbed to sediment particles (*Microbes Sediment-Adsorbed*). Here, multiplying J_{VS} by a relative sediment surface area covered by microbes yields J_{VM}. In the fourth scenario, both microbes and viruses are adsorbed to the sediment surface (*Biofilm*), and viruses diffuse within a biofilm coating each sediment particle. This scenario requires conversion of viral and microbial concentrations to concentration per sediment biofilm volume. Biofilm thickness was modeled at four times the diameter of a microbe. New viscosity (μ) values were employed from estimates for low and high viscosity exopolysaccharides (Calvo et al., 1998). All sediment grains were modeled as perfect spheres, with an assumed mode of packing consistent with calculated porosity (Cooke and Rowe 1999).

Results

Chemical and physical parameters of the springs

Physical and chemical measurements from Gypsum Hill (GH4) and Lost Hammer (LH) springs were consistent with previous measurements (Table 1). The springs differed in that Lost Hammer outlet water emerged at subzero temperature, had a greater flow rate compared to GH4, three times the salinity, less negative oxidation-reduction potential (ORP), and a lower concentration of aqueous sulfide. The waters from both springs increased in temperature and oxygen concentration with distance from outlet. GH4 outlet sediment pore water chemistry is expected to be equivalent to outlet spring water measurements made at the depth of the outlet pool, as the sediments are roiling. GH4 channel sediments had higher pH, comparable ORP, decreased alkalinity, and decreased sulfide compared to the spring outlet.

Abundance of microbes and virus-like particles

Our methods did not distinguish between members of the domains Bacteria, Archaea, and small single-celled Eukaryota. Members of each domain have been reported in both springs (Perreault *et al.* 2007, 2008; Niederberger *et al.* 2010; Lay *et al.* 2012, 2013) and are herein collectively referred to as microbes. Similarly, we follow the convention of Schoenfeld *et al.* (2008) and report virus-microbe ratios (VMR), rather than the more commonly reported virus-bacteria or virus-prokaryote ratios.

Virus-like particles (VLP) and microbes were present in all samples. In both springs, VLP and microbes were more abundant in the sediments than in the spring waters (Fig. 5). VLP and microbial abundances correlated positively in all transects, albeit more strongly in GH4 sediments (r=0.97) and spring water (r=0.82) and than in LH sediments (r=0.77) or spring water (r=0.16).

VLP concentrations from GH4 sediments ranged from 4 to $97 \cdot 10^6$ ml^{-1}, increasing by over an order of magnitude with distance from the spring outlet (Fig. 5), and then decreasing under the influence of Expedition River (>≈20 m). Sediment microbial abundances followed a similar pattern, leading to virus-microbe ratios (VMR) that were consistently less than 1 (0.2 to 0.9). VLP concentrations from GH4 spring water

ranged from 4 to 250 · 10^4 ml^{-1} and were lowest at the spring outlet, roughly constant within the channel, and highest when influenced by Expedition River. VMRs were greater than 1 (less than 10), with no trend with distance from the spring outlet.

LH sediment VLP and microbial concentrations were lower than those in GH4 by ≈10X, increasing from 2 to 11 · 10^6 cm^{-3} along the sampling transect (Fig. 5). VMR in LH sediments ranged from 0.9 to 5.3. Spring water VLP concentrations at LH ranged from 5 to 53 · 10^4 ml^{-1}, between 10 and 50% of GH4 channel water concentrations. LH spring water VLP abundance did not increase with distance from the outlet. VMR in LH spring water ranged from 0.6 to 6.6.

Frequency of visibly infected cells

No visibly infected cells (VIC) were observed from any water or sediment sample from either spring. In verification of our methods, visibly infected *E.coli* were detected in *E.coli*-T4 positive controls. While assessing sediments for the presence of VICs, numerous cells exuding or embedded within exopolysaccharides were encountered (Fig. 6). This character was considered in evaluating our virus-microbe contact rate models.

Decay of fixed virus-like particles

Significant viral decay has been reported for pelagic viruses following storage in aldehydes (reaching 35% in 4 hours; Wen et al., 2004). VLP concentrations from the GH4 spring outlet and channel decreased with storage time, though at a greater rate and to greater extent in spring water compared to sediments (Fig. 7). Substantial fractions of sediment VLP appear recalcitrant to decay. Storage decay rates measured from GH4 were used to correct for storage time for both springs.

Discussion

In this study, we sought to identify what influence the combination of low temperature, high salinity, anoxia and low organic carbon would have on the viral communities of Gypsum Hill (GH4) and Lost Hammer (LH) springs.

Oligotrophic springs limit microbial growth and viral abundance

The most striking characters of the virus and microbial communities of GH4 and LH springs are their low population sizes. Compared to other cold and/or hypersaline environments (Table 3), the paucity of microbes in AHI spring waters ($<2.5 \cdot 10^5$ ml^{-1}) is approached by the lower ranges found in brines of Arctic sea ice and matched only by the lakes of Vestford Hills, Antarctica (Fig. 8). The virus concentrations are lower than any comparable aqueous environment. Sediment microbial and virus concentrations are comparable to marine sediments at tens to hundreds of meters depth (Fig. 9). Lower concentrations are found only in oligotrophic sediments of the South Pacific Gyre (Engelhardt *et al.* 2014). While the majority of reports of viruses from hypersaline environments have found concentrations in excess of marine environments (Tables 3 and 4), these Arctic hypersaline springs do not conform to this trend. The low virus concentrations may be due to limited microbial growth.

Environmental parameters control microbial growth. The abundance of microbes within both springs' sediments increases substantially (>20X) with distance from spring outlet, concomitantly with factors associated with increasing rates of community population growth (oxygen, temperature, nutrient inputs from surface runoff; Fig. 5). In both springs, aqueous microbial concentrations are consistently one to two orders of magnitude lower than sedimentary ones (Fig. 5): the spring water populations most likely reflect an efflux from the sediments. Generally microbial abundances increase with distance from outlet, but this trend is not consistent between each sampling station (Fig. 1). Deviations are attributed to pockets of organic matter delivered to the sediment from allochotonous sources, or permafrost carbon mobilized by the transient flow paths of the channels. Microbial abundance in sediment is dependent upon the amount of available organic matter (Danovaro and Serresi 2000), which is known to be low in GH and LH springs (e.g. GH dissolved organic carbon undetectable, Andersen, 2004; LH sediment

total organic carbon 0.45%, Niederberger et al., 2010). While surface aqueous environments are typically populated by photosynthetic organisms, a lack of photosynthetically derived organic carbon in other hypersaline systems has been proposed as an explanation for low biomass (Elevi Bardavid, Khristo and Oren 2008). Chlorophyll has not been detected at GH4 (Andersen 2004; Perreault *et al.* 2007), and the greater salinity and colder temperature of LH may similarly inhibit photosynthesis. As organic carbon is more consistently associated with low biomass than either hypersalinity or low temperature, it is likely to be the limiting factor on microbial growth in GH4 and LH springs, which in turn is the ultimate cause of the low viral abundances documented here.

Low virus-microbe ratios result from low rates of viral production

High virus concentrations and virus-microbe ratios (VMR) have been suggested to indicate a substantial role of viruses in controlling microbial mortality, especially in eutrophic environments (Wommack and Colwell, 2000), where viral-induced mortality of marine microbes is estimated at 50% of total mortality (Suttle, 2005). VMRs in both AHI springs are consistently below ten and at times below one (Fig. 5). Ten is the most often cited VMR for marine systems, though VMRs are known to vary considerably (Fig. 8 and 9; Wommack and Colwell, 2000). In solar salterns and other hypersaline systems of comparable salinity to AHI springs, the VMR is typically much greater (oftentimes >100). Although exceptions to this pattern exist – in hypersaline Lake Retba VMR is 2 (Sime-Ngando *et al.* 2011) – other characteristics of the AHI springs must counteract the impact of salinity on VMR. For example, the microbial concentration in AHI spring sediments might be below the minimum threshold required for viral infection (Wiggins and Alexander 1985), though our documented persistence of a viral population suggests otherwise. Low VMRs have been correlated with low rates of microbial metabolism (as a microbe must be metabolically active in order to replicate a viral genome) nearly exclusively in sedimentary environments (Fig. 9; Danovaro et al., 2002). In support of this control, in-situ metabolic activity is low for GH spring outlet water heterotrophs (leucine uptake: 0.3 pmol l^{-1} h^{-1}) and chemoautotrophs (dark CO_2 uptake: 0.1 nmol C l^{-1} hr^{-1}; Perreault et al., 2008).

Microbial growth rates are predicted to be very low in oligotrophic sub-seafloor sediments, with population turnover on the scale of 10s to 1000s of years (Hoehler and Jørgensen 2013). Although viral numbers are low, VMRs from these sediments have been reported as consistently greater than one and often greater than ten (Engelhardt *et al.* 2014), exceeding the results reported here. Given the increasing VMR in progressively more oligotrophic layers, the measurements in sub-seafloor sediments may indicate increasing viral persistence or a strong role in controlling microbial mortality in the marine deep biosphere (Engelhardt *et al.* 2014). The increased stability of virions in cold and hypersaline environments (Wells 2008; Bettarel, Desnues and Rochelle-Newall 2010) suggests that viral persistence in oligotrophic GH4 and LH sediments should be even greater than in oligotrophic seafloor sediments. The low VMRs in AHI springs, then, are most likely sustained by diminished rates of viral production. In tandem, low biomass and low rates of metabolism in the AHI springs limit the production of viruses, resulting in both the low VMR and low concentration of free virions. Support for this dynamic could be provided by experimentally monitoring rates of production and decay in situ.

Low virus-microbe contact rates restrict lytic control

A first-order control on the spread of infections is the rate of contact between viruses and their hosts. Aqueous contact rates have been reported from sea water (4 to 20 contacts microbe^{-1} d^{-1}) and sea ice brines (30 to 1000; Collins and Deming, 2011), eutrophic lakes (50 to 250; Weinbauer and Höfle, 1998; Fischer and Velimirov, 2002), and hypersaline Mono Lake (100 to 1000; Brum et al., 2005). Contact rates from both GH4 and LH springs were calculated with the same equations as these studies, and are one and two orders of magnitude less frequent (Fig. 10), suggesting lower rates of infection, predation, and carbon turnover compared to other aqueous environments.

Virus-microbe contact rates have yet to be estimated in sediments. Sediments oftentimes contain higher abundances of both microbes and viruses than the overlying water. Contact rates have thus been predicted to be greater, with limited consideration of factors that might impede interaction in the sediment matrix. Using equations modified for modeling aqueous rates (Murray and Jackson 1992), we estimated contact rates in

GH4 and LH sediments employing several spatial arrangements of microbes and viruses. Each contact rate model showed trends similar to microbial and viral concentrations from each spring, illustrating the first-order control of population sizes on contact rates (Fig. 10). However the spatial distribution of microbial and viral populations can have a substantial impact, with contact rates spanning four orders of magnitude for a given sampling site. Physical occlusion can enhance contact rates, as seen in sea ice brines (Wells and Deming 2006c; Collins and Deming 2011), and in models with microbes and viruses distributed homogeneously in pore waters, or located within low viscosity biofilms (Fig. 4 and 10). Among the models, the highest microbe-specific contact rates (J_M) in sediments (≈500 contacts microbe^{-1} d^{-1}, GH4, 20 m from outlet) were found when viruses and microbes were constrained within a low viscosity biofilm. The lowest contact rates (≈0.001 contacts microbe^{-1} d^{-1}, LH outlet) were found when microbes were sediment-adsorbed and viruses were homogeneously distributed in the pore water.

Experimental work in a variety of environments has described microbial and viral adsorption to sediments (Bitton 1975; Suttle and Chen 1992; Bettarel, Bouvier and Bouvy 2009). TEM imaging of LH and GH4 sediments frequently revealed microbe-associated exopolysaccharides (Fig. 6). Viruses are known to move through biofilms largely composed of such exopolysaccharides (Sutherland *et al.* 2004). A virion from GH4 outlet sediment might only encounter a microbe once every day (virus-specific contact rate (J_V) = J_{VM} / V · 86,400 s d^{-1}) in a thin, high-viscosity biofilm. The likelihood of contacting a host microbe, successful adsorption, and infection is likely to be one to two orders of magnitude less frequent. Even with attenuated rates of decay (Bettarel, Desnues and Rochelle-Newall 2010; Bettarel *et al.* 2011), in the absence of large burst sizes and short latent periods (Wells 2008), calculated contact rates appear to preclude maintenance of the pool of virions encountered by predominantly lytic replication. Contacts between virions and sediments are likely to result in absorption (Gerba 1984). However, in vitro experiments demonstrated that only a small fraction (≈5%) of contacts between a virion and its host result in adsorption (Son *et al.* 2014). Our model assumes 100% adsorption. Further, all environments contain multiple viral types and non-host microbes. If one in ten virus-microbe contacts are between a virus and a suitable host, in tandem with sediment heterogeneity and 5% host-specific adsorption (and assuming

adsorption to host is equivalent to infection), infection rates drop to <0.5% of the calculated contact rates. These factors all suggest that the modeled contact rates presented are upper limits on viral infectivity. Even the rates experienced in spring sites with the greatest contact rates are still orders of magnitude lower than those encountered in the open ocean, where lysis accounts for 50% of microbial mortality (Suttle 2007).

Limits to lysis and biogeography of non-lethal replication strategies

We observed no visibly infected cells (VIC) from over 1600 cells from eight outlet and channel stations. In contrast, 0.7 to 16% of marine microbes have been estimated to contain mature phage (Suttle and Chen 1992). Our findings are similar to those of Bettarel et al. (2011) who also reported no VIC from salterns at salinities 190 to 360‰ and the highest frequency of lysogens (63 and 30%) at similar salinity ranges. Similar to GH4 and LH, reports from lake sediments (including hypersaline Lake Retba and anoxic freshwater Lake Pavin) found an absence, or very low abundance of VIC (Bettarel *et al.* 2006; Borrel *et al.* 2012) indicating that viruses of hypersaline sediments may not regulate the size of their associated microbial communities (Guixa-Boixareu *et al.* 1996; Pedrós-Alió *et al.* 2000).

Viral presence in the absence of lytic activity suggests non-lethal replication. Alternative replication strategies include lysogeny, wherein the viral genome is temporarily housed within the genome of its host, and chronic infection, wherein virions are persistently produced and released without host mortality. Several other studies have found the incidence of lysogeny to increase in oligotrophic, hypersaline, low microbial density and/or slow growing environments (Jiang and Paul 1994; Jiang 1998; Weinbauer, Brettar and Höfle 2003; Lisle and Priscu 2004; Laybourn-Parry, Marshall and Madan 2007; Bettarel *et al.* 2011). In these environments, natural selection may favor viral replication strategies that exert minimal selective pressure on fragile hosts: either replicating non-destructively or only when a host is likely to be destroyed by changes in its environment (Porter, Russ and Dyall-Smith 2007).

In order to explore the biogeographic application of these hypotheses, we applied the *Biofilm* contact rate model to virus and microbe sediment concentrations reported for a set of sediments diverse in their geography, depth, sedimentation rate, and the nutrient

content of the overlying water (Engelhardt *et al.* 2014). In doing so, similar sediment size, porosity, microbe and virus size, temperature of 4°C and salinity of 35‰ were employed (Table 2). Contact rates from sub-seafloor sediments from the Bering Sea (8 to 300 m) and the continental margin of Peru (1 to 500 m) were most similar to GH4 and LH outlet sediments while rates in the South Pacific Gyre sediments were significantly lower, and those in tidal flats were significantly higher (Fig. 11). The similarly low contact rates in deep-sea sediments and AHI spring sediments supports the interpretation that lytic replication is unlikely to account for the free virion pool in the deep subsurface. As lytic viruses appear to be responsible for enhancing organic carbon turnover in shallow marine sediments (Engelhardt *et al.* 2014), the contact rate estimates made here point toward a fundamental divide in sub-seafloor viral biogeography. As this lytic/non-lytic divide is ultimately controlled by oligotrophy, the AHI spring sediments may facilitate experimental investigations of virus-microbe interactions comparable to those occurring in deeper, less accessible marine sediments.

Conclusion

The springs of Axel Heiberg Island provide unique opportunity for the study of viral ecology in cold, hypersaline environments. This work demonstrated low concentrations of viruses and microbes in two springs and their sediments, suggesting low microbial growth and viral production, extended persistence of virions in the environment, and low lytic control on these microbial populations compared to other aqueous and surface sediment environments. Contact rate models suggest that viruses and microbes encounter one another much less frequently than in comparably cold or salty aqueous or surface sedimentary environments, and that contact rates are similar to those in deep subsurface sediments. Viral decay must be attenuated to maintain a standing low concentration pool of free virions. A lytic strategy is less likely to sustain the virion population than are host-preserving replication strategies such as chronic infection or lysogeny.

The sediments of these springs are more amenable to experimentation than deep subsurface marine sediments. Cold, saline, anoxic and oligotrophic sediments are infrequently found close to the surface; sampling subsurface marine sediments is expensive and logistically challenging, in situ experimentation complicated, and annually revisiting those sites unprecedented. The hypersaline springs of AHI lend themselves to experimental exploration and may serve as geochemical and microbiological analogs for less accessible systems (e.g. deep subsurface sediments, marine anoxic basins) or systems associated with astrobiological targets (e.g. transient hypersaline brines on Mars). Future work will quantitatively explore the dynamics of virus-microbe interactions and support or refute hypotheses presented in this paper regarding viral control of microbial populations and the frequency of non-lytic replication strategies.

Acknowledgments

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Figure 1. Map of Axel Heiberg Island showing locations of Gypsum Hill and Lost Hammer springs.

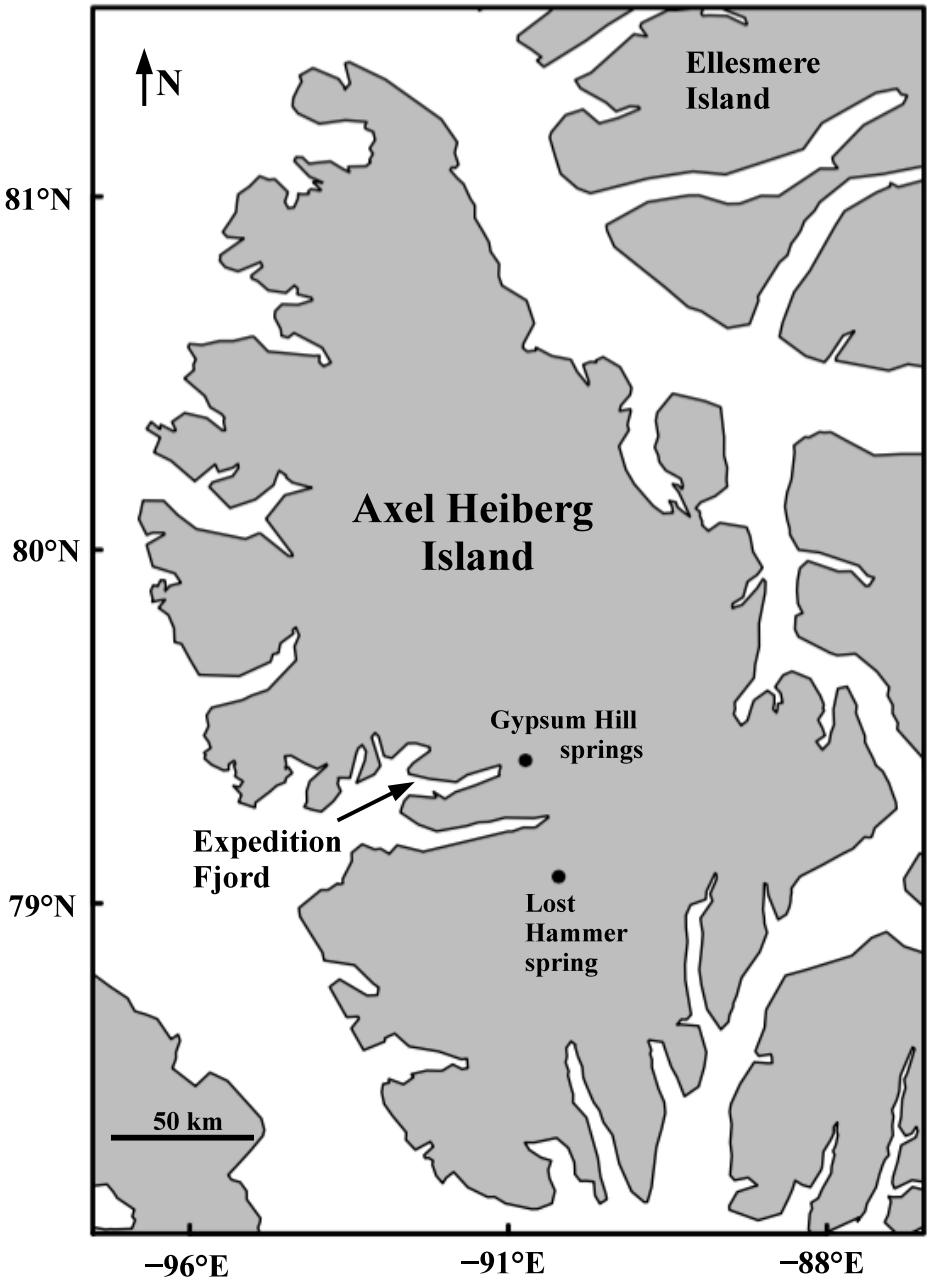
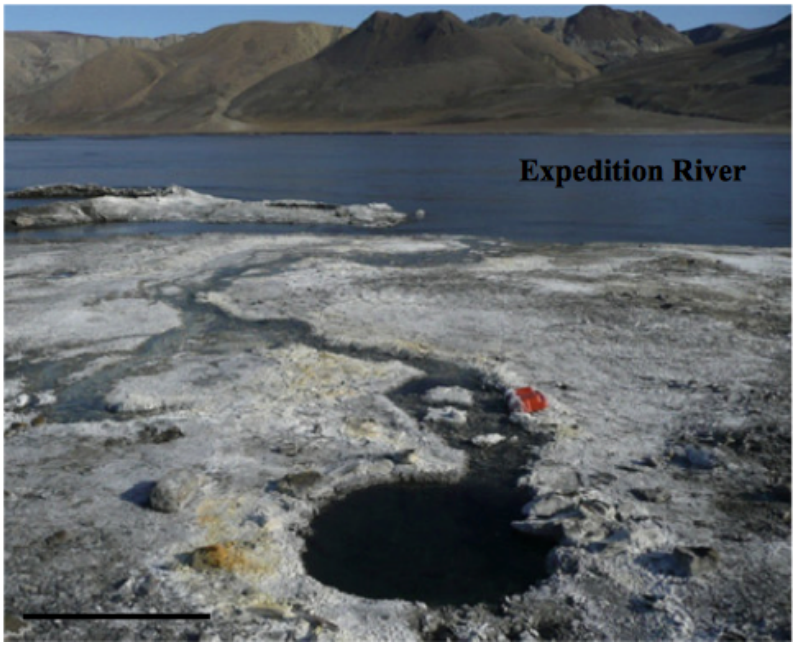


Figure 2. Gypsum Hill and Lost Hammer spring sampling site photographs. Scale bars are 2 m. Spring water and sediment samples were collected in July 2013 from outlet pools, spring channel initiation point, and at progressive 5-10 m intervals.

Gypsum Hill



Lost Hammer

Figure 3. Epifluorescent microscopy of SYBR Green I-stained microbes and virus-like particles (VLP) from Gypsum Hill spring water (a) and sediments (b). Bacteria indicated with blue arrows, VLP with red arrows.

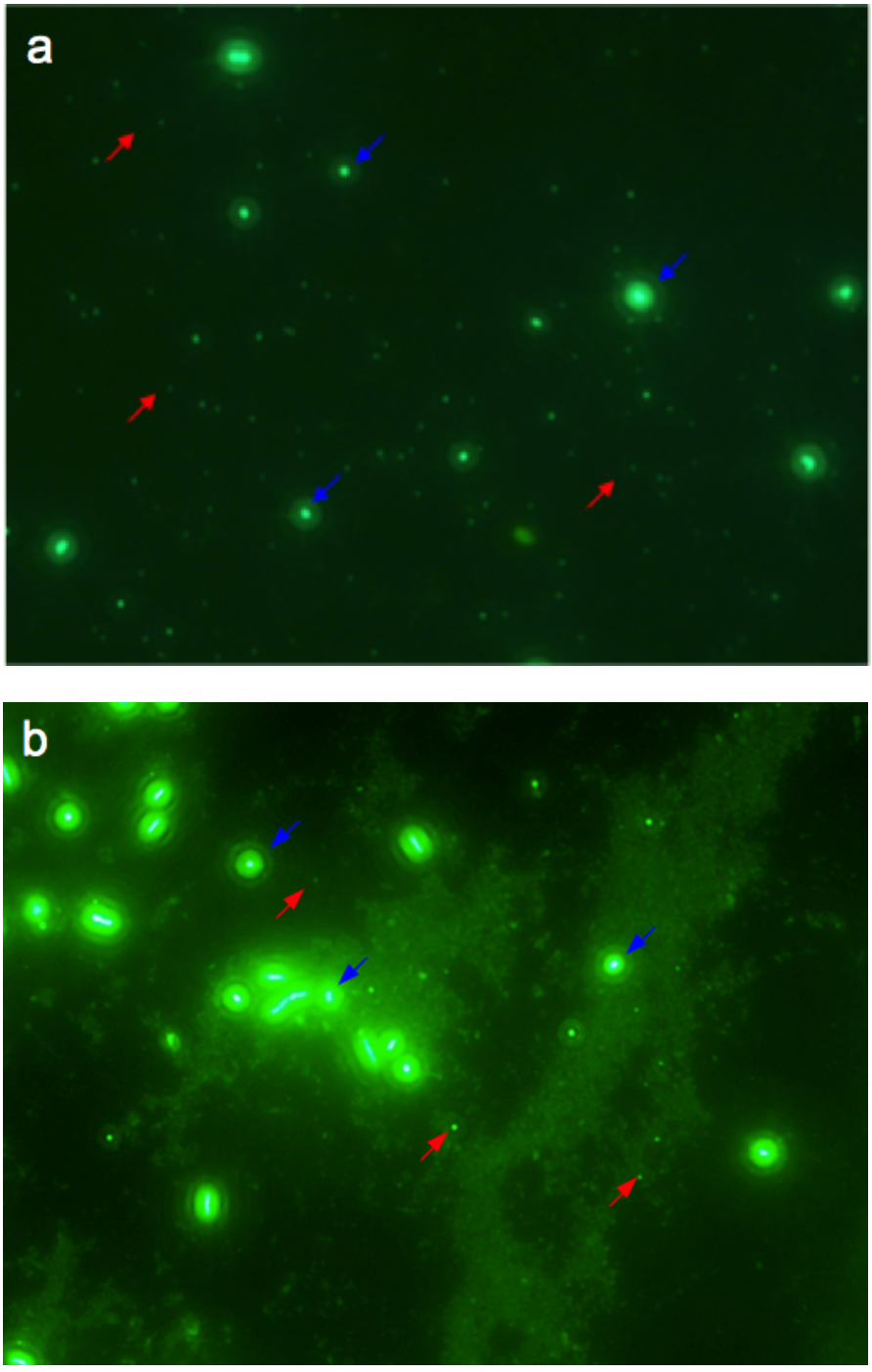


Figure 4. Sediment spatial distribution scenarios for contact rate calculations.

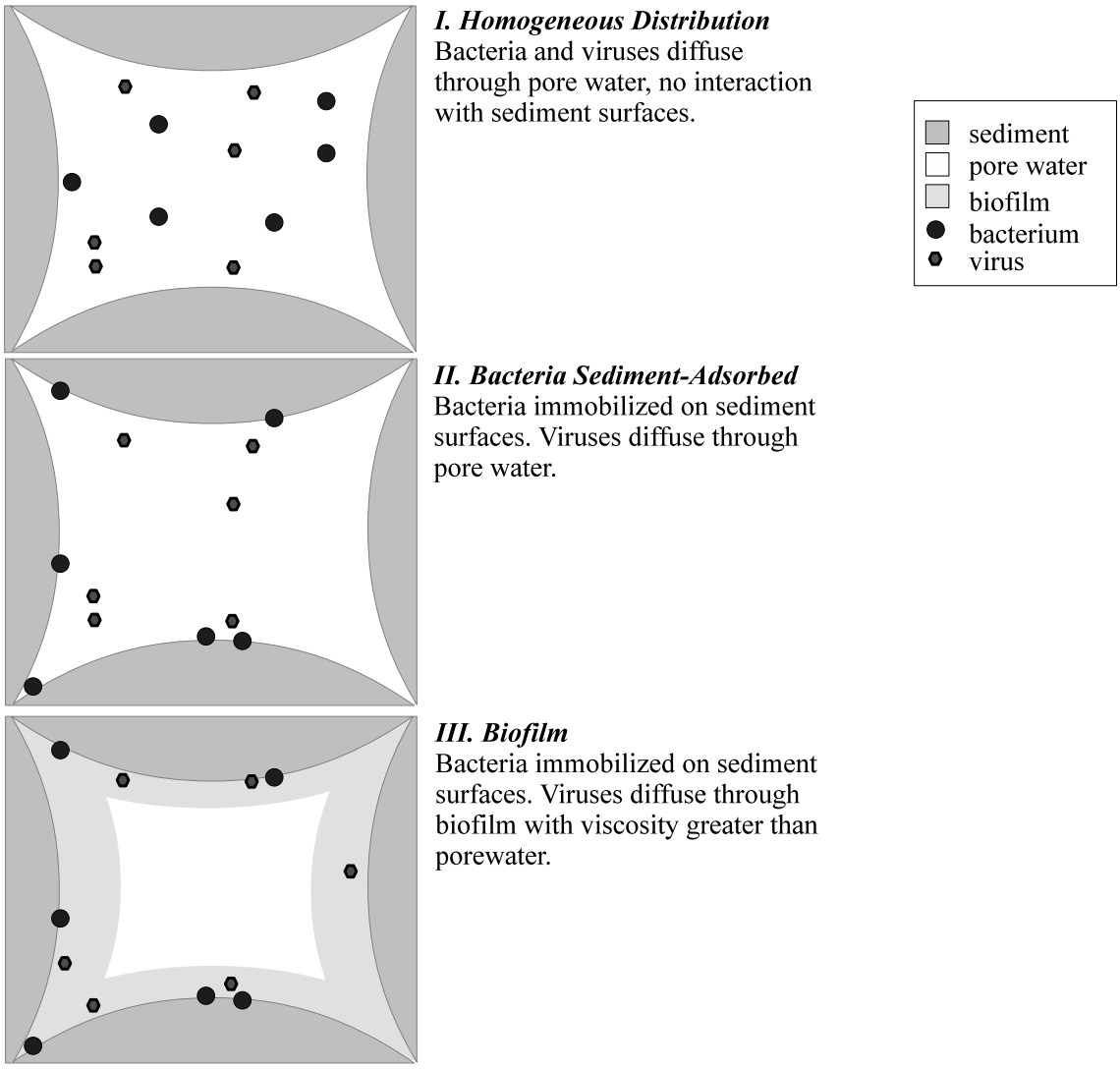


Figure 5. Gypsum Hill and Lost Hammer Springs' water and sediment microbial and virus-like particle (VLP) abundances and virus-microbe ratios (VMR). Circles represent virus-like particles, squares represent microbes and triangles represent VMRs. Shaded shapes are sediment values, open shapes are spring water values. Horizontal lines are at VMR=1 (solid), 0.5, and 2 (dashed). Gypsum Hill sampling sites influenced by Expedition River indicated by grey background shading; sampling sites within Expedition River indicated by a dot within larger symbols.

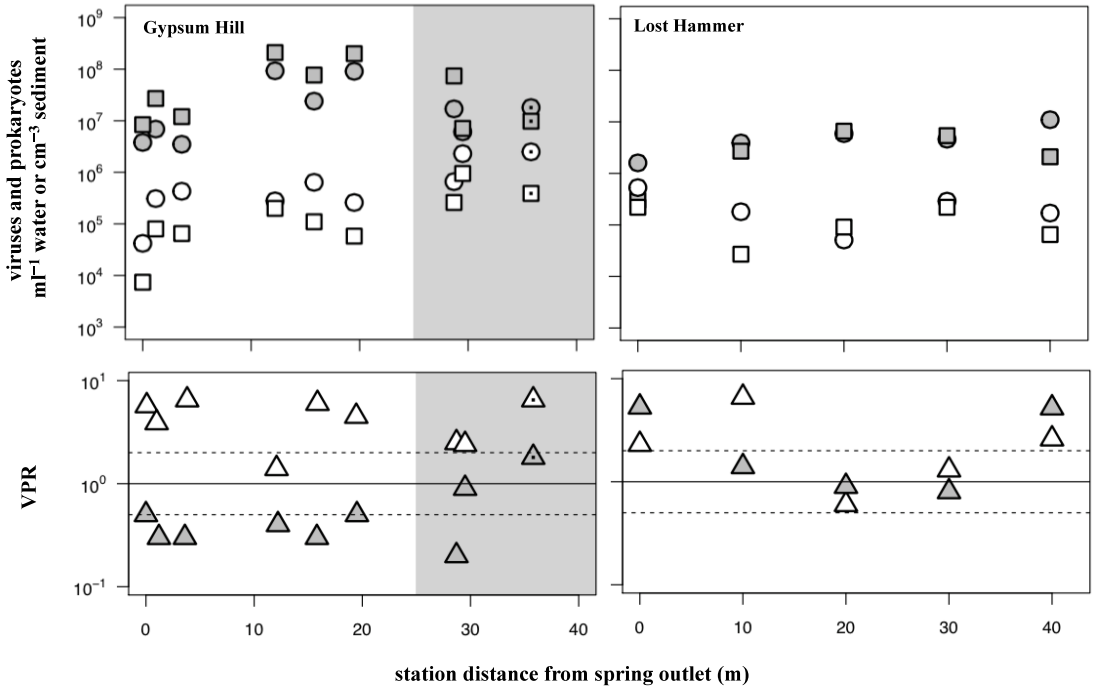


Figure 6. Microbes and exopolysaccharides from Gypsum Hill Spring. Scale bars 500 nm.

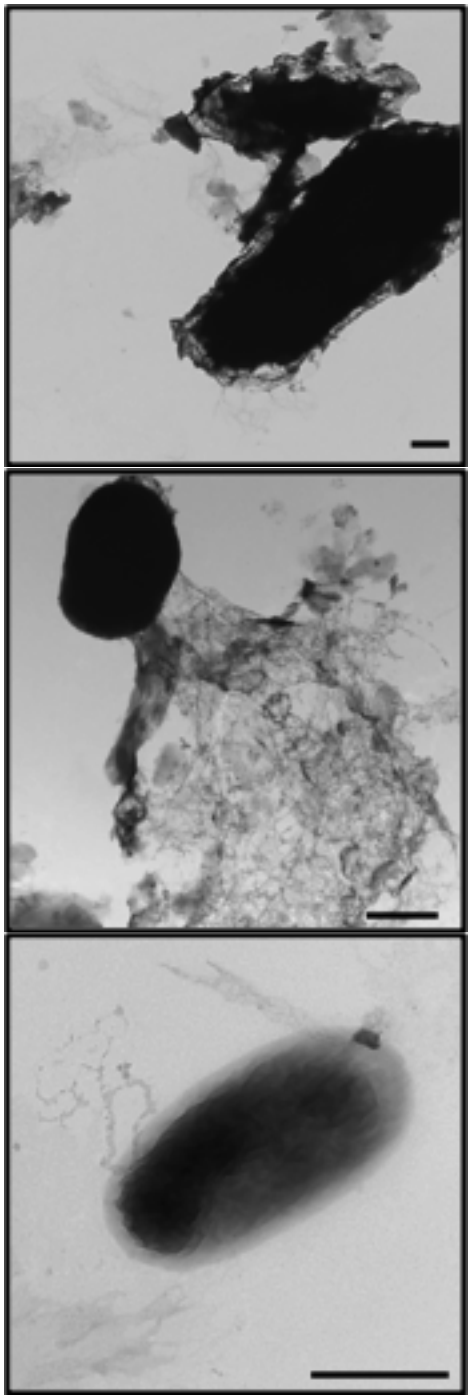


Figure 7. Decay of Gypsum Hill Spring formaldehyde-fixed virus-like particles at −10°C.

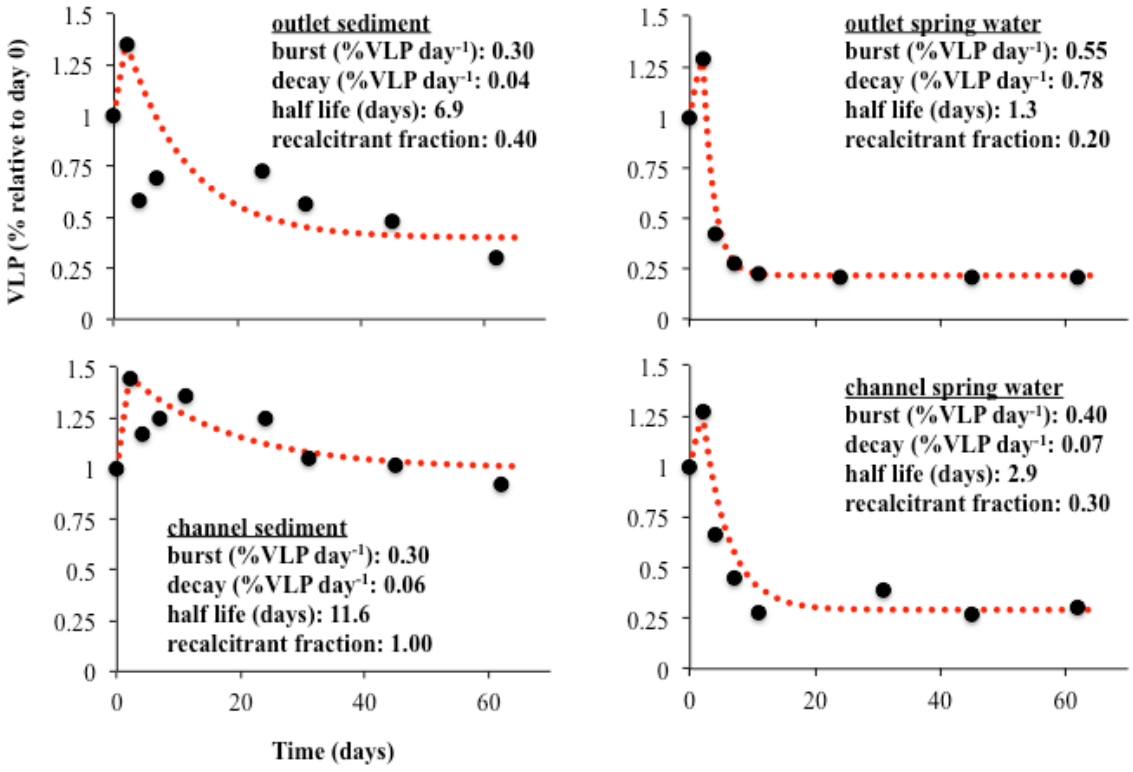


Figure 8. Comparative viral and microbial abundances and VMRs in AHI springs' water and comparable aqueous environments.

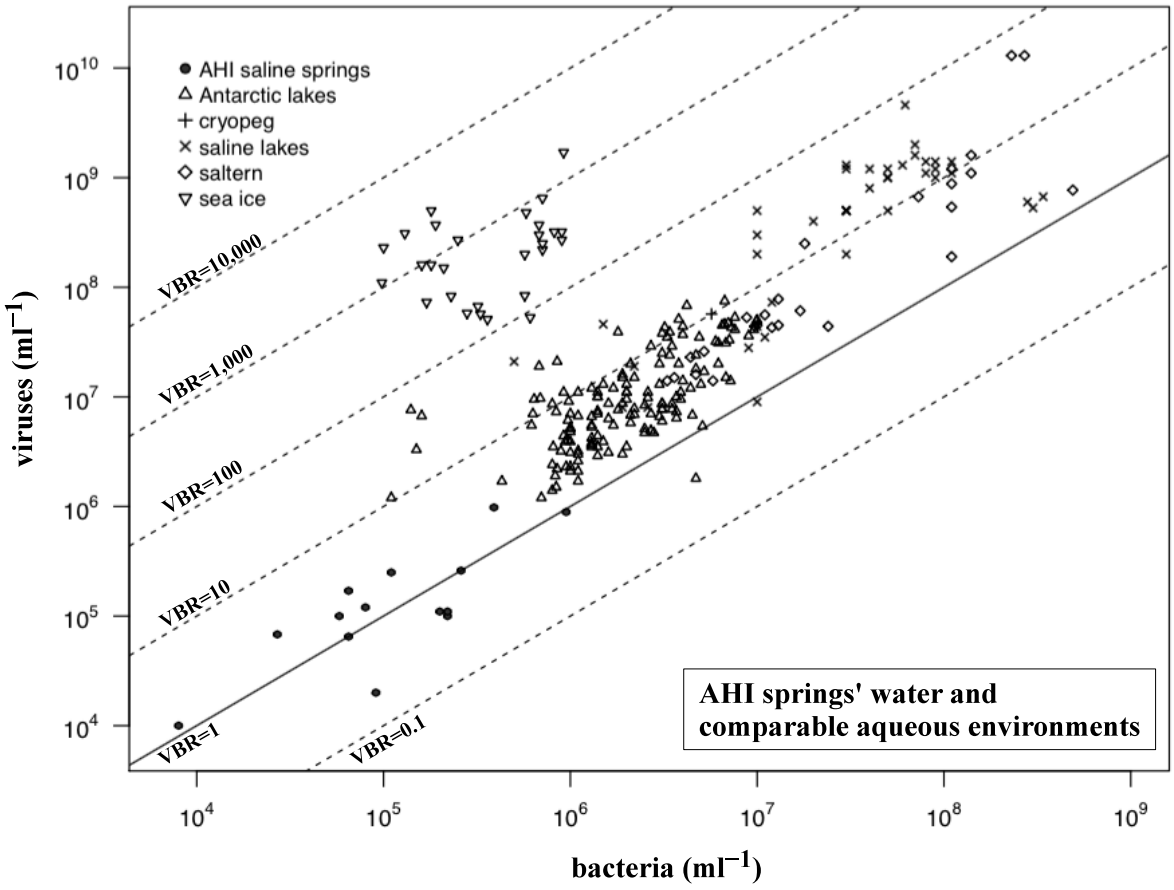


Figure 9. Comparative viral and microbial abundances and VMRs in AHI springs' sediments and comparable sedimentary environments.

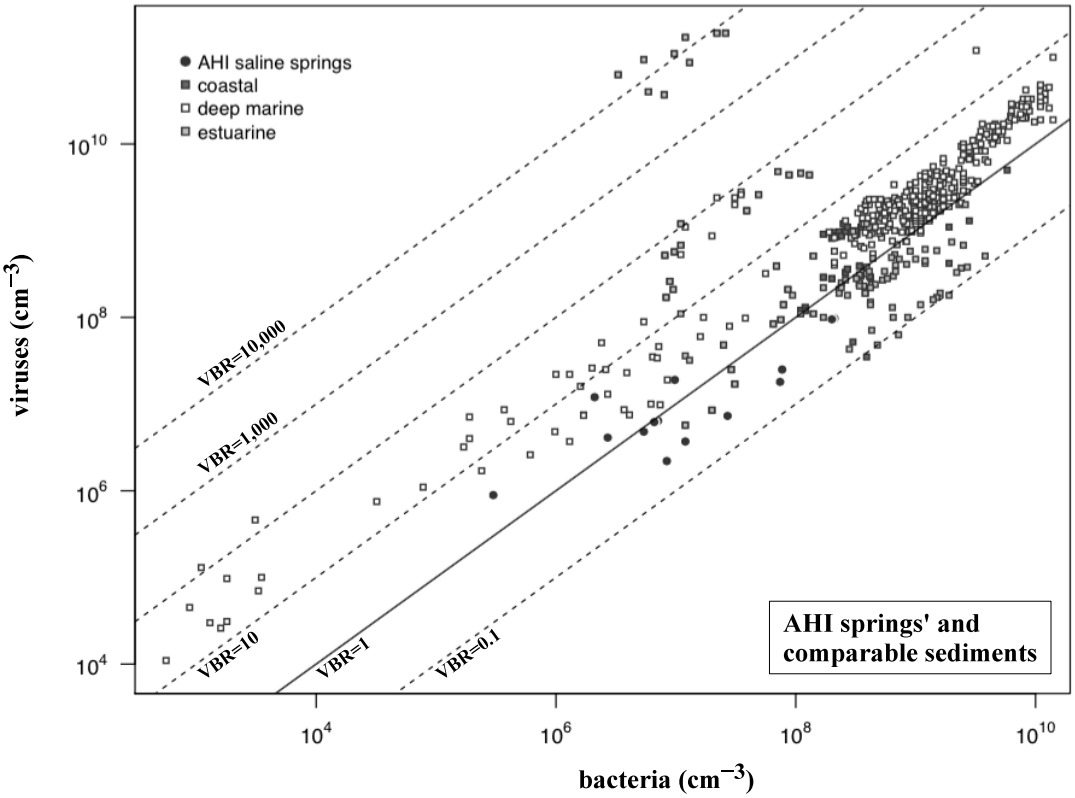


Figure 10. Modeled microbe-specific viral contact rates (J_M) in Gypsum Hill and Lost Hammer Springs' water and sediments. Gypsum Hill sampling sites influenced by Expedition River indicated by grey background shading; sampling sites within Expedition River indicated by a dot within larger symbols.

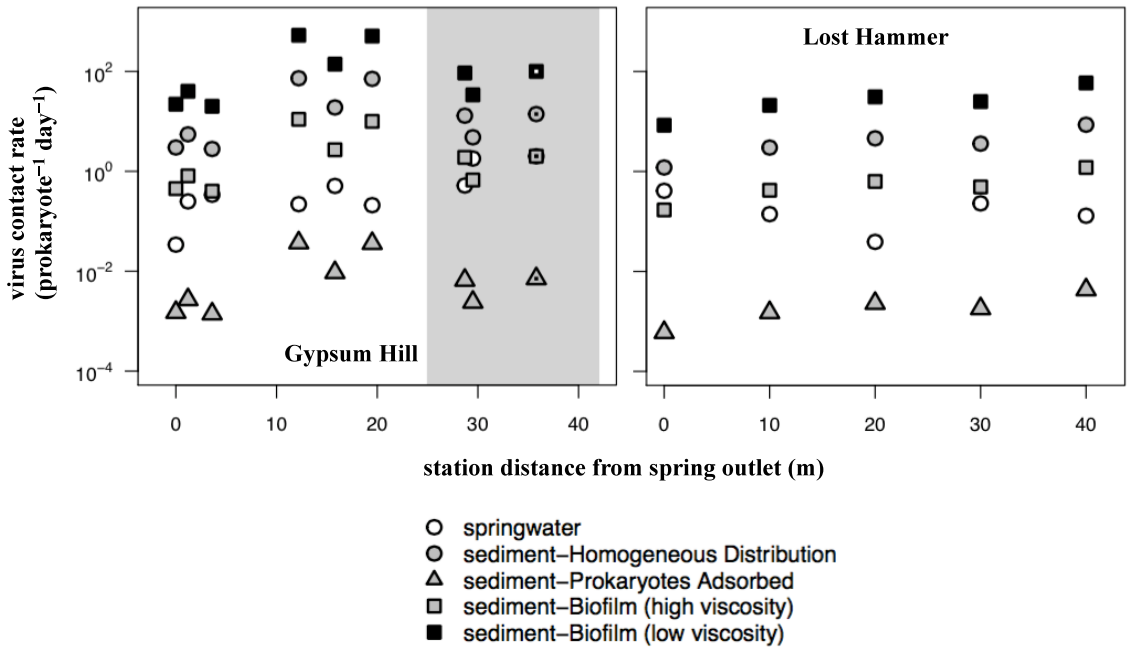


Figure 11. Microbe-specific contact rates modeled from diverse marine sediments ($J_{M\_\text{marine-sed}}$) compared to AHI springs' sediments ($J_{M\_\text{GH-outlet}}$ and $J_{M\_\text{LH-outlet}}$). The left y-axis represents rates from marine sediments compared to GH outlet sediments (rates are equal at y=1, solid horizontal line). Contact rates compared to LH outlet sediments are shown on the right y-axis (horizontal dashed line represents contact rates equal to LH outlet sediments). Depth of sediments indicated by shading of points. Points for each site column are jittered for clarity.

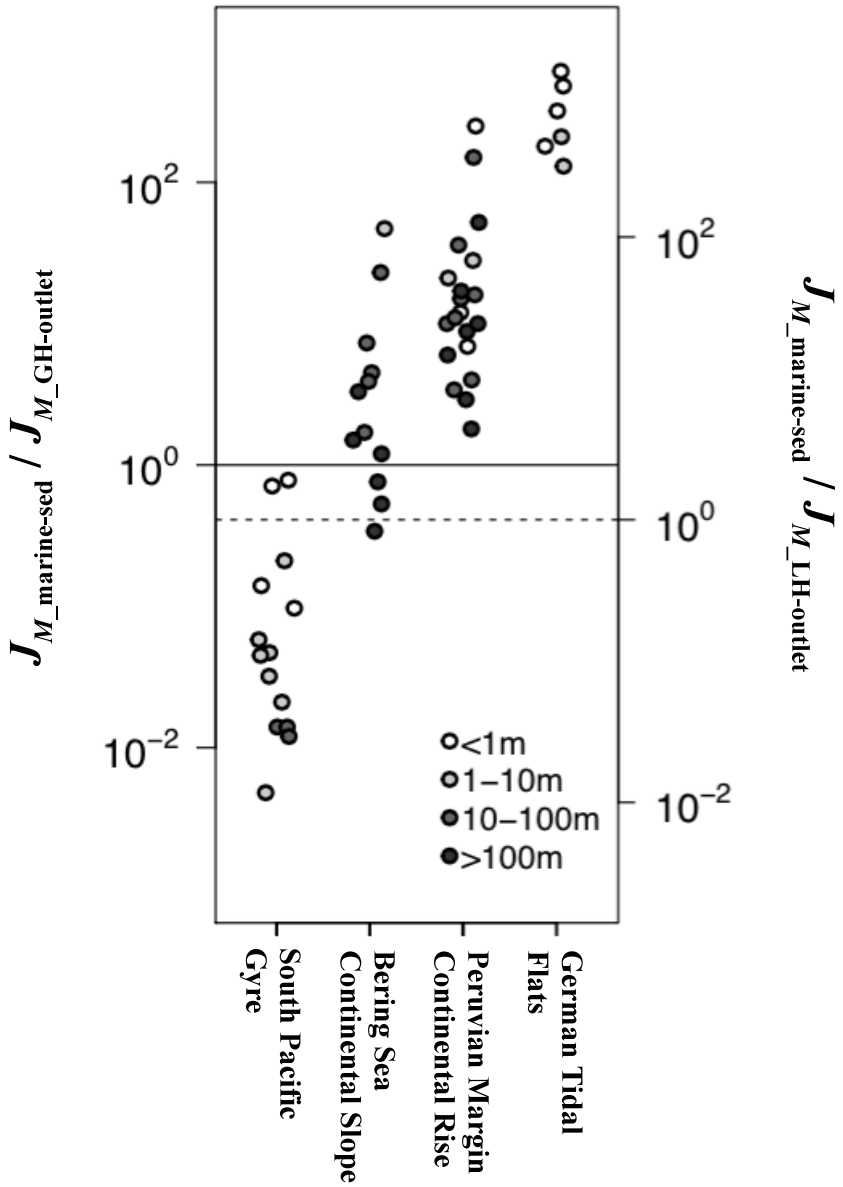


Table 1. Axel Heiberg springs: water and sediment chemistry measurements.

Measurement	Gypsum Hill			Lost Hammer			
	outlet	channel		outlet		channel	
	water	water	sediment	water	sediment	water	sediment
Temperature (ºC)	4.0, 6.6 [1,2,7]	5, 12 [2,7]	6, 12 [2,7]	−5.9, −4.7 [4,7]	NM	−18.0, 9.2 [6]	−18.0, 9.2 [6]
Salinity (%)	8, 9 [1,3,7]	8, 9 [1,5,7]	7.2, 13.3 [7]	22, 26 [4]	NM	25 [6]	NM
Flow rate (L s^{-1})	1.0 [1,2,3,7]	1.0 [1,5,7]	1.0 [8]	4 [5]	NM	4 [5]	NM
pH	6.9, 7.4 [2,5]	7.5, 8.9 [2]	7.7, 8.0 [2,7]	6.0, 7.4 [4]	NM	6.5, 7.2 [6]	NM
Oxidation-Reduction Potential (mV)	−325, −291 [3,7]	NM	−331, −237 [7]	−187, −154 [4,6]	NM	−29, 125 [6]	NM
Alkalinity (ppm)	62, 63 2 [2,7]	46, 62 [2]	15, 30 [7]		NM	NM	NM
dissolved Oxygen (ppm)	0.05, 0.20 [5,7]	1.0 3 [3,7]	5.85 [7]	0.0, 1.0 [4,6]	NM	>1 [6]	>1 [6]
Anions							
Chloride (mM)	1100, 1200 [1,2,3]	1200 [1,3]	NM	3900 [4,6]	1100 [4]	NM	NM
Sulfate (mM)	32, 38 [2,5,7]	32, 38 [5,7]	NM	54 [5]	1040 [4]	NM	NM
Sulfide (mM)	0.40, 3.1 [2,5,7]	0.01, 0.10 [7]	0.31, 0.47 [7]	0.0, 1.6 [4]	NM	0.0, 0.6 [6]	NM
Cations							
Calcium (mM)	48, 52 [1,2,3]	48 [1,3]	NM	42 [4,6]	1470 [4]	NM	NM
Sodium (mM)	1200, 1900 [1,2,3]	1900 [1,3]	NM	2900 [4,6]	2390 [4]	NM	NM
Primary mineralogy	gypsum, halite, elemental sulfur, detrital quartz, calcite, detrital plagioclase [2,5]			halite, thenardite, gypsum, mirabilite, detrital quartz, plagioclase, clays [5]			

NM Not Measured

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[7] This study

Table 2. Equations, variables and example values used to model virus-microbe contact rates in spring water and sediments.

Equation	Definition	Scenario	Units
$J_M = J_{VM} / M \cdot 86{,}400$	microbe specific contact rate	All	microbe^{-1} day^{-1}
$D_V = k\,T / 3\,\pi\,\mu\,d_V$	viral diffusivity equation	All (μ varies)	cm^2 s^{-1}
$J_{VM} = 2\pi \cdot d_M \cdot D_V \cdot V_W \cdot M_W$	virus-microbe contact rate	Spring Water	cm^{-3} s^{-1}
$J_{VM} = 2\pi \cdot d_M \cdot D_V \cdot V_{PW} \cdot M_{PW}$	virus-microbe contact rate	Sediments: *Homogeneous Distribution*	cm^{-3} s^{-1}
$J_{VM} = J_{VS} \cdot SA_M / SA_S$	virus-microbe contact rate	Sediments: *Microbes Adsorbed*	cm^{-3} s^{-1}
$J_{VM} = 2\pi \cdot d_M \cdot D_V \cdot V_B \cdot M_B$	virus-microbe contact rate	Sediments: *Biofilm*	cm^{-3} s^{-1}
$J_{VS} = 2\pi \cdot d_S \cdot D_V \cdot V_{PW} \cdot S$	virus-sediment contact rate	Sediments: *Homogeneous Distribution+ Sediment Adsorption; Bacteria Adsorbed*	cm^{-3} s^{-1}
$\mu = \mu_W [\,1 + 0.0816\,m - 0.0122\,m^2 + 0.000128\,m^3 + 0.000629\,T\,(1 - e^{-0.7\,m})]$	brine viscosity equation	Spring Water, Sediments: *Homogeneous Distribution (+Sediment Absorption), Microbes Adsorbed*	g cm^{-1} s^{-1}

Variable	Definition	Units	Example value: GH4 outlet
d_B	diameter of average microbe	cm	5.0 x 10^{-5}
d_V	diameter of average virus	cm	6.0 x 10^{-6}
d_S	diameter of average sediment particle	cm	0.1
V_B	virus concentration (biofilm)	cm^{-3}	5.2 x 10^{9}
V_W	virus concentration (spring water)	cm^{-3}	4.2 x 10^{4}
V_{PW}	virus concentration (pore water)	cm^{-3}	1.6 x 10^{7}
M_B	microbial concentration (biofilm)	cm^{-3}	2.0 x 10^{10}
M_W	microbial concentration (spring water)	cm^{-3}	7.4 x 10^{3}
M_{PW}	microbial concentration (pore water)	cm^{-3}	6.1 x 10^{7}
k	Boltzmann constant	g cm^2 K^{-1} s^{-2}	1.4 x 10^{-16}
m	solute concentration	mol	3.2
S	sediment particle concentration	cm^{-3}	1.2 x 10^{2}
SA_M	microbe surface area	cm^2 cm^{-3}	0.27
SA_S	sediment surface area	cm^2 cm^{-3}	15
T	temperature	K	2.8 x 10^{2}
μ_W	viscosity of freshwater at the corresponding temperature	g cm^{-1} s^{-1}	1.8 x 10^{-2}

Table 3. Viral and microbial abundances and virus microbe ratios (VMR) from cold and hypersaline aqueous environments. Where ranges of values were reported, minimum and maximum values are listed, separated by a comma. Values for sea ice brines are calculated based on bulk measurements of salinity, microbial and VLP counts and partitioning coefficients for salt.

Environment	Location	Coordinates (N, E)	Water depth (m)	Temperature (°C)	Salinity (ppt)	Microbes (x 10^6 ml^{-1})	Viruses (x 10^7 ml^{-1})	VMR	Reference
Salterns	La Trinitat salterns, Tarragona, Spain	40.58, 0.68	0-0.5	NR	37	10	4.5	4.5	Guixa-Boixareu et al. 1996
			0-0.5	"	150	15	5.0	5.0	"
			0-0.5	"	250	70	65	9.3	"
			0-0.5	"	370	140	180	12.9	"
	Sfax solar salterns, Tunisia	34.65, 10.70	0-0.2	21, 24	138, 147	110	19, 54	1.7, 4.8	Boujelben et al. 2012
			0-0.2	23, 27	185, 190	110, 490	80, 120	1.6, 11	"
			0-0.2	27, 34	340, 360	230, 270	1300	49, 54	"
	Kaolack salterns, Senegal	14.10, −16.00	0.5	29	140, 240	10, 20	3.0, 8.0	1.5, 8.0	Bettarel et al. 2011
Saline lakes	Dead Sea, Israel	31.50, 35.50	0-20	22, 33	340	0.5, 11.6	0.9, 7.3	0.9, 42	Oren et al. 1997
	Lake Retba, Senegal	14.80, −17.20	0.5	27	290, 360	280, 350	55, 68	1.8, 2.1	Betteral et al. 2011
	Great Salt Lake, USA	41.17, −112.58	0-0.3	NR	240, 300	62	460	74	Baxter et al. 2011
	Mono Lake, USA	38.02, −119.00	2-35	3, 20	70, 85	10, 120	14, 190	5.8, 47	Brum et al. 2005
Antarctic lakes	Crooked Lake, Vestford Hills	−68.62, 78.37	0-6	NR	0	0.16	0.14	8.4	Laybourn-Parry et al. 2001
	Highway Lake, Vestford Hills	−68.23, 78.47	0-6	NR	4	0.18	0.83	50	"
	Pendent Lake, Vestford Hills	−68.48, 78.25	0-6	NR	15	0.70	1.9	28	"
	Ace Lake, Vestford Hills	−68.47, 78.18	0-6	NR	18	0.19	0.72	40	"
	Williams Lake, Vestford Hills	−68.48, 78.17	0-6	NR	56	4.1	3.7	9.2	"
	Bonney Lake, Taylor Valley	−77.00, 162.87	5-37	−2, 7	0, 150	NR	0.1, 1.1	1.5, 5.5	Kepner et al. 1998
	Fryxell Lake, Taylor Valley	−77.62, 163.18	4-31	0, 5	0, 1	0.2, 8	0.12, 9	1.9, 121	"
	Hoare Lake, Taylor Valley	−77.63, 162.87	5-30	0, 2	0, 1	0.1, 1	0.9, 5.1	1.7, 141	"
Sea Ice	Arctic Sea Ice Amundsen Gulf	71.08, −123.44	<0.10 m thick	−7.3, −4.7	83, 116	0.32, 0.60	5.5, 7.1	90, 230	Collins and Deming. 2011
		70.13, −125.08	0.30-0.4 m thick	−11.3, −2.2	41, 153	0.16, 0.80	5.9, 68	150, 2800	"
		71.45, −125.96	0.6-0.8 m thick	−11.3, −2.4	46, 153	0.096, 0.92	11, 170	310, 2400	"
Cryopeg	Barrow Permafrost Tunnel Alaska, USA	71.29, −156.72	7 mbs	−6	115	5.7	5.7	10	Colangelo-Lillis, Eicken, Carpenter & Deming *unpublished*

VMR virus-microbe ratio; *mbs* meters below surface
" indicates value equivalent to that listed immediately above

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Table 4. Viral and microbial abundances and virus-microbe ratios (VMR) from subaqueous sedimentary environments. Where ranges of values were reported, minimum and maximum values are listed, separated by a comma.

Environment	Location	Coordinates (N, E)	Depth (mbss)	Temperature (°C)	Salinity (ppt)	Microbes (x 10^6 cm^{-3})*	Viruses (x 10^7 cm^{-3})*	VMR	Reference
Marine sediments	Bering Sea Continental Slope	59.05, −179.20	8-300	NR	NR	0.03, 17	0.8, 10	1.3, 11	Engelhardt et al. 2014
	South Pacific Gyre	−27.90, −123.15	0-20	NR	NR	0.0005, 0.01	0.0004, 0.17	7, 200	"
	Peru margin	−9.00, −79.97	1-500	NR	NR	0.2, 140	0.4, 60	2, 20	"
	Challenger Mound, Ireland	51.38, −11.72	4-96	11, 14	NR	0.98, 38	0.49, 10	2.4, 5.6	Middelboe et al. 2011
	Atlantic Ocean	various**	0.01	NR	NR	260, 3330	83, 490	0.9, 5.2	Danovaro et al. 2008
	Mediterranean	"	"	NR	NR	190, 2400	40, 330	0.9, 5.1	"
	Black Sea	"	"	NR	NR	1700, 3800	260, 660	1.4, 2.2	"
	South Pacific	"	"	NR	NR	210, 1500	43, 480	1.7, 5.6	"
Coastal sediments	Øresund,Denmark	50.97, 12.68	0.01-0.10	6, 13	30, 33	110, 390	11, 120	0.8, 5.4	Siem-Jørgensen et al. 2008
	Saanich Inlet, BC	48.59, −123.50	0-118	NR	NR	310, 15000	100, 12000	1.3, 38	Bird et al. 2001
	Adriatic sea	43.63, 13.50	0.01	15	NR	950	21	0.2	Mei et al. 2004
	Thermaikos Gulf	38.97, 22.72	0.01	11	NR	940	56	0.6	"
	Manfredonia Gulf	41.65, 16.26	10-100	21, 24	NR	440, 530	50, 68	1.5, 3.3	"
Tidal flats & estuaries	Janssand, Germany	53.74, 7.70	0-3	NR	NR	40, 170	27, 140	8, 10	Engelhardt et al. 2014
	Queensland, Australia	−27.42, 153.83	0-0.02	NR	0, 36	3.3, 140	0.57, 19000	0.4, 19000	Hewson et al. 2001
		−26.25, 153.00	0-0.02	NR	0, 36	8.1, 11	17, 120	21, 109	"
		59.93, 12.58	0-0.16	15	5	9.4, 380	4.3, 74	0.9, 19	Middelboe et al. 2003

VMR virus-bacteria ratio; *mbss* meters below sediment surface.
" indicates a value equivalent to that listed immediately above.
*concentrations reported as g dry $sediment^{-1}$ were converted cm^{-3} assuming porosity of 50 % and sediment density 3.0 g cm^{-3}
** numerous samples collected within +/- 2 degrees N and E

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Connection 1

The first paper included in this thesis described the first examination of the abundance of microbes and viruses in two hypersaline springs of the Canadian High Arctic. Despite measuring very low concentrations of microbes in both the spring water and sediments of each springs' outlet, viruses were also identified from these—and all downstream—stations. This finding does not support one of the central hypotheses of this thesis, that there may be environments on Earth with sufficiently low biomass to preclude successful viral replication, resulting in their absence. These environments may still exist, but this study serves as a lower limit of microbial abundance at which viruses *are* still capable of persistence. Beyond persistence, whether viruses play a significant role in microbial mortality and the maintenance of microbial diversity was another, complementary hypothesis of this thesis. As a first order approach to this question, we developed the first model of contact rates between viruses and microbes in cold, hypersaline sediments. Using variations on this model, very low contact rates were calculated between viruses and microbes in each spring's outlet—on the order of contact rates modeled for abundances of microbes and viruses enumerated from sediments of the South Pacific Gyre (amongst the ocean's most oligotrophic water and sediments). Utilizing measurements of viral decay rates from other aqueous environments, the study concluded that viruses were unlikely to play a substantial role in microbial mortality, as contacting a suitable host before decaying was unlikely to be the fate of the majority of individual virions.

The logical extension of this first paper was to make the necessary measurements to assess the impact of viruses on microbial mortality in these spring sediments. These measurements include microbial growth rate, viral production rate and viral burst size. Such measurements have never been made from the combined conditions of cold, oligotrophic, hypersaline and anoxic, as encountered in these springs' sediments. These parameters in turn can be used to directly calculate the fraction of microbial mortality for which viruses are responsible. It is these measurements and calculations that make up the second paper of this thesis.

Paper 2

Sluggish viral dynamics in Arctic hypersaline spring sediments

Sluggish viral dynamics in Arctic hypersaline spring sediments

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Abstract

Viruses are a primary influence on microbial mortality and carbon cycling in the global ocean. The impacts of viruses on their microbial hosts and elemental cycling in low energy environments are less well explored. To investigate viral community dynamics in low-energy environments, we conducted a set of in situ time series incubations in the outlet and channel sediments of two cold, hypersaline springs of the Canadian High Arctic. We found microbial and viral populations in dynamic equilibrium, indicating approximately equal birth and death rates for each population. In situ rates of microbial growth were low (0.5 to 50 x 10^3 cells cm^{-3} h^{-1}) as were rates of viral decay (0.09 to 170 x 10^4 virions cm^{-3} h^{-1}). A large fraction of the springs' viral communities (49 to 100%) were refractory to decay over the timescales of our experiments. Microcosms amended with lactate or acetate exhibited increased microbial growth rates (up to three-fold) indicating organic carbon as one limiting resource for the microbial communities in these environments. A substantial fraction (15 to 71%) of the microbial population contained inducible proviruses that were released in multiple pulses over eight days following chemical induction. Our findings indicate that viruses in low energy systems maintain low rates of production and activity, have a small but notable impact on microbial mortality (8 to 29% attenuation of growth) and that successful viral replication in low energy environments may primarily proceed by non-lethal strategies. In cold, low energy marine systems of similar character (e.g. deep subsurface sediments), viruses may be a relatively minor driver of community mortality and diversity compared to less energy-limited environments such as the marine water column or surface sediments.

Introduction

Sunlight and photosynthesis are the drivers for surface life and environments sufficiently connected to utilize macromolecules synthesized in the photic zone. In environments decoupled from either sunlight or photosynthesis, the source of electron potential can limit microbial growth. Examples of these environments include aquifers, subsurface lakes, glacial beds, permafrost environments, marine environments below the photic zone, and any surface environment whose chemistry inhibits the growth of photosynthetic life (e.g. Don Juan pond; Siegel *et al.*, 1979): collectively, these environments can be thought of as 'low energy' habitats (Hoehler and Jørgensen 2013). Microbes in these environments must capitalize on energetic resources other than organic carbon and their investigation provides an opportunity to explore the metabolic diversity and microbial ecology of habitats that make up a substantial fraction of the contemporary biosphere (e.g. the deep subsurface; Fry et al., 2008). Additionally, such environments may serve as analogs for early Earth microbial habitats (Canfield, Rosing and Bjerrum 2006), prior to the advent of photosynthesis, or as models to facilitate understanding of potential metabolic redox couplings (i.e. chemoautotrophic metabolisms) that might allow life to persist on other planets and moons in and beyond our solar system (Russell *et al.* 2014).

Viral dynamics, including rates of production and decay, combined with rates of microbial growth, inform a variety of processes in microbial ecology. Since the recognition of vast number of viruses in the ocean, research has focused on the impact of these entities on their microbial hosts (Bergh *et al.* 1989; Fuhrman 1999). Initial dynamics research correlated TEM enumeration of virus-like particles (VLP) with changes in microbial abundances across space and time, demonstrating environmental viral activity and primarily infection of microbes (Bratbak *et al.* 1990, 1996; Hennes and Simon 1995). Recognition of the extent of microbial infection and lysis as a control on microbial abundance, maintaining populations far below resource-controlled levels was a milestone in microbial oceanography (Lenski 1988; Proctor and Fuhrman 1992). Following characterization of community-scale rates of viral lysis, came an appreciation of the role of viruses in both maintaining microbial diversity and in shunting organic carbon from a particulate to a dissolved state, thereby maintaining that carbon in oceanic

surface waters (Proctor and Fuhrman 1990; Waterbury and Valois 1993; Hennes, Suttle and Chan 1995; Middelboe, Jørgensen and Kroer 1996; Gobler *et al.* 1997). Investigations exploring whether high viral abundances might be maintained by resistance to decay found that viruses in sea water are quick to decay, implying that their production is similarly rapid in the ocean and that interactions between microbes and viruses are dynamic on short time scales (Suttle and Chen 1992; Wilhelm *et al.* 1998).

As an alternative to lytic replication, a significant fraction of viruses are capable of lysogenic replication, wherein the viral genome is integrated into that of its host, replicating along with host until a time (indicated by intracellular signals) that the virus genome excises and reverts to lysis (Burnett 1934). The frequency of lysogens (microbes with at least one viral genome integrated into their own) in the aqueous marine environment has been found to vary widely between environments and seasons (Paul 2008). Significantly, despite a high frequency of lysogens in the environment, natural induction of lysogens is rare, and the vast majority (97% or more) of viruses observed in sea water are the result of lytic infections (Wilcox and Fuhrman 1994). Provirus (a viral genome integrated into its host's) genes are known to occasionally be expressed, altering the phenotype of their microbial host in a process termed lysogenic conversion (Barksdale and Arden 1974). Genomics research indicates viruses are responsible for a substantial amount of genetic exchange in marine microbial communities (McDaniel *et al.* 2010). In tandem, viruses simultaneously significantly impact both community scale processes (mortality and diversity) as well as genetic composition and gene flow within the microbial community (Rohwer and Thurber 2009).

A longstanding hypothesis proposes that viral dynamics are controlled by trophic state, as assessed by the rate of primary productivity and the abundances of biologically essential nutrients (Weinbauer, Fuks and Peduzzi 1993). The rationale behind this hypothesis is that eutrophic environments support higher concentrations of microbes (and consequently viruses) than oligotrophic systems, and that viruses will have greater impact at higher concentrations. This hypothesis has been addressed across trophic gradients in aqueous environments (Corinaldesi *et al.* 2003; Bongiorni *et al.* 2005; Cheng *et al.* 2010). Investigating habitats that extend the range of these parameters may expand our

understanding of the variability in tempo and mode of viral genetic and predatory influence on microbial communities.

Small-scale environments with gradients in physical, chemical, and biological parameters are attractive systems in which to investigate factors that influence viral dynamics. In order to constrain the relative impact of physical and chemical variability on viral and microbial dynamics in polar aqueous environments, we studied two perennial cold hypersaline springs on Axel Heiberg Island in the Canadian High Arctic. These springs have a combination of physical and chemical variability between their near-anoxic, cold outlets and progressively warmer and more oxygenated channels and serve as a natural extension of the conditions in which the trophic control hypothesis has been addressed. Further, these systems are characterized by low viral abundance, virus to microbe ratios, and virus-microbe contact rates, suggesting that viruses in these springs play less of a role in controlling microbial populations through lytic activity than in the marine environment, but a comparable role to that in deep subsurface sediments (Colangelo-Lillis, Wing and Whyte 2016). Here we evaluate this suggestion with in situ time series experiments to monitor microbial growth, viral production and provirus induction from lysogens.

Methods

Site description

All samples were collected from spring sediments in the proximity of the McGill Arctic Research Station on Axel Heiberg Island (Pollard et al., 2009). Prior to surface exposure, spring water ascends through > 400 m of Carboniferous anhydrite evaporites and permafrost (Jackson and Harrison 2006), thereby setting the Na-, Ca-, Cl-, SO_4-rich geochemistry of the springs. We studied two springs (Gypsum Hill and Lost Hammer) that have exhibited stable geochemistry over the last 10 years (Table 1).

Gypsum Hill springs emerge from the southern base of Gypsum Hill via ≈40 outlets that flow over ≈30 m of permafrost, in shallow, dispersive channels terminating into Expedition River (Fig. 1). The outlet of the largest spring (GH4) discharges at a rate of ≈1 L s^{-1}, forming a 1 m deep, 2 m wide, circular pool with roiling sediments as a result of water and gas discharge. Outlet water is perennially constant at ≈4-7°C, pH ≈7, 80‰ salinity and is microoxic (1.3 μM dissolved oxygen) and reducing (Eh −285 mV). Spring water exiting the outlet pool is quickly oxygenated as it flows over the permafrost. These channels contain mixed sediments (fine to coarse sand and pebbles) that are variably coated with a veneer of travertine precipitate (Omelon, Pollard and Andersen 2006). Major spring water ions include chloride, sodium, sulfate, and calcium (Pollard *et al.* 1999). Microbial and molecular characterization has identified Bacterial and Archaeal genes associated with aerobic and anaerobic heterotrophic and autotrophic metabolisms, including sulfur- and sulfate- reducing bacteria, methanogens, sulfur oxidizing bacteria and methanotrophs (Perreault *et al.* 2007, 2008; Niederberger *et al.* 2009b).

Lost Hammer spring (LH) emerges from a single outlet and has formed a 3 m high halite tufa (Fig. 1). In summer months, water flows through the base of this structure; in winter months the hydrohalite structure freezes and outlet water overflows the tufa lip. Outlet water is perennially constant at ≈−5°C, pH ≈6.5, 240‰ salinity, and is microoxic (0.6 μM dissolved oxygen) and reducing (Eh −187 mV). The abundant ions of LH are the same as GH4, in similar ratios but greater concentrations (Niederberger *et al.* 2010). Microbial and molecular characterization from both the spring outlet and channel indicate a low diversity community and the presence of anaerobic methanotrophs,

methanogens, sulfur oxidizing bacteria and ammonia oxidizers (Niederberger *et al.* 2010; Lay *et al.* 2012, 2013; Lamarche-Gagnon *et al.* 2015). The biomass of both springs are very low, with the channel sediments of each inhabited by an order of magnitude more microbes than the outlet of the same springs (Colangelo-Lillis, Wing and Whyte 2016).

Sediment measurements

Pore water measurements included temperature and concentrations of dissolved oxygen, nitrate, nitrite, ammonia, sulfide, sulfate, and dissolved and solid phase organic carbon. Dissolved oxygen was measured at 3 cm below sediment surface using a Picollo2 fiber-optic oxygen meter (Pyroscience, Aachen, Germany). Pore water was collected into 15 mL syringes through Rhizon (Rhizosphere, Wageningen, NL) tubing inserted 3 cm into the sediment, and measured for aqueous chemistry immediately upon recovery using CHEMetrics kits (Midland, Virginia; K6923, K7003, K1503, K9523, K9203) and a portable spectrometer (V-2000, CHEMetrics). Sediment porosity was calculated from sediment density and mass reduction following drying at 100°C for 24 h. Total organic carbon and total nitrogen content was measured on an Elemental Analyzer (NC 2500, CE Instruments, Wigan, UK), following acid removal of inorganic carbonates as carbon dioxide at the GEOTOP stable isotope laboratory at UQAM (Montreal, QC). Dissolved organic carbon was measured on a Total Organic Carbon Analyzer (TOC-V CHS; Shimadzu, Tokyo) at the GEOTOP Environmental Organic Geochemistry laboratory at Concordia University (Montreal, QC).

Microcosms

Microcosms for all dynamics experiments were assembled on site in July 2015. Microcosm experiments were conducted in 6 mL serum vials. All vials were filled with surface (0-5 cm depth) sediment (80% volume) and spring water (20% volume), and immediately capped with butyl rubber stoppers underwater. Microcosm experiments were established at four sampling stations (Fig. 1). Incubations were performed in situ in sediments of GH4 in both the spring outlet and channel (5 m downstream of the spring outlet) sediments. LH microcosms were taken from the spring site and incubated in a permafrost freezer at the McGill Arctic Research Station at −3±1°C. Following

incubation, microbial processes were terminated by the addition of formaldehyde (final concentration 2%) using sterile syringes. Formaldehyde and all other treatment reagents were 0.02 μm-filtered prior to use. Fixed samples were maintained at 4°C through transport and storage until enumeration.

Microbial growth

Microbial growth and viral production microcosms employed non-native nucleotide 5-ethynyl-2'-deoxyuridine (EdU; Invitrogen A10044). This synthetic nucleotide was incorporated into newly replicated nucleic acids, which could later be identified using click chemistry between the nucleotide and a fluorescent azide (Alexafluor488-azide, Invitrogen A10266; Ferullo *et al.*, 2009; Qu *et al.*, 2011; Wang *et al.*, 2013; Cieślar-Pobuda and Los, 2013). At each spring site, 60 μL of 5 mM EdU (final concentration 50 μM) was injected into nine 6 mL microcosms using sterile syringes. Two additional vials served as no treatment controls to normalize for any effects of EdU addition or bottling effects. EdU-treated vials were formaldehyde fixed at time points: 0, 1, 2, 4, 8, 12, 24, 48, and 96 hours. Untreated vials were fixed at time points 0 and 96 hours. At each spring site, eight additional 6 mL microcosms were assembled and EdU treated as above. Four of these vials were amended with lactate and another four with acetate (60 μL of 1 M carbon source, final concentration 10 mM). Per spring site, EdU+/−carbon treated vials were formaldehyde fixed at time points: 0, 8, 24 and 96 hours. Lactate was chosen because it is can be utilized by many sulfate-reducers, known to be present and active in these sediments (Perreault *et al.* 2007; Lay *et al.* 2013). Acetate was chosen as it is among the most simple and ubiquitously utilized carbon sources, and has been employed in culturing isolates from LH (Niederberger *et al.* 2010).

Microbial growth rates (μ) were calculated both from linear best fits to plots of microbial abundances with time ($\mu_{\Delta cells}$, cells cm^{-3} h^{-1}), and separately from linear best fits to plots of percentages of new cells having incorporated EdU ($\mu_{\Delta\%EdU\text{-}cells}$, % new cells cm^{-3} h^{-1}). EdU incorporation is less than 100% efficient; its efficiency was calculated as the ratio of the growth rate determined from changes in % cells with incorporated EdU to the growth rate determined from changes in cell numbers. This efficiency factor (calculated for carbon amended microcosms) facilitated conversion from

% cells with incorporated EdU to in situ growth rate in non-carbon amended microcosms, where the growth and death rates were equal and no changes in absolute cell numbers could be observed. Microbial turnover rates were estimated by dividing μ by microbial abundance. Theoretically EdU incorporation should be detectable in viruses generated from microbes that had incorporated the nucleotide; however, high background fluorescence resulting from click-chemistry reactions on Anodisc 0.02 μm filters, made assessment of viral production rates infeasible using this method.

Viral decay

Endogenous viral production was terminated by addition of the bacteriostatic KCN, and decay was measured as the disappearance over time of directly countable viral particles (Heldal and Bratbak 1991). At each spring site, 60 μL of 0.2 M KCN pH 7.0 (final concentration 2 mM) was injected into ten 6 mL microcosms using sterile syringes; two additional vials per site served as no treatment controls to normalize for any immediate effects of KCN addition or bottling effects. KCN-treated microcosms were formaldehyde fixed at time points: 0, 1, 2, 4, 8, 12, 24, 48, 96 and 144 hours. Untreated vials were fixed at time points 0 and 96 hours. Decay rates were calculated from linear best fits to a plot of viral abundance with time.

Provirus induction

Provirus induction microcosms employed DNA-damaging agent mytomycin C to induce provirus excision from their host genomes, replication and lysis (Ackermann and DuBow 1987). At each spring site, 0.1 mL of 60 μg mL^{-1} (final concentration 1 μg mL^{-1}) was injected into six 6 mL microcosms using sterile syringes; two additional vials served as no treatment controls to normalize for any immediate effects of mytomycin C addition or bottling effects. Mytomycin C-treated microcosms were formaldehyde fixed at time points: 0, 8, 24, 48, and 96 and 144 hours. Untreated vials were fixed at time points 0 and 96 hours. The fraction of lysogens was calculated as the reduction in microbial abundance associated with the induction divided by the starting population abundance. Temperate (capable of lysogenic replication) viral burst sizes were determined from the ratio of increase in VLP abundance (VLP cm^{-3}) to the decrease in microbial abundance

(cells cm^{-3}) over the apparent duration of the induction event. When possible, the decay rate of the induced viral community was calculated as above.

Sediment extraction

Prior to filtration, 3.0 cm^3 aliquots of sediments were washed 2X with 1X PBS to remove excess salt, then disrupted in extraction buffer (0.1% sodium pyrophosphate, 0.5% Tween20, in 1X PBS) first by agitation on a shaker (Scientific Instruments Vortex Genie 2, setting 7, 20 min) and then by sonication (Heat Systems Ultrasonics W185D, 100 W). This extracted sample was underlain with 2 mL 50% Histodenz (Sigma D2158; 1.3 g mL^{-1}) and centrifuged at 500 g for 15 minutes at 4°C. Supernatant was retained for filtration and enumeration of microbes and viruses, and extracted sediments were dried overnight at 60°C and weighed to determine sediment density and porosity. All chemicals were 0.02 μm-filtered prior to use.

Microbial and viral abundance

Enumeration followed Suttle and Fuhrman (2010). Sediment extracts were filtered through 0.02 μm-pore size Anodisc filters, stained with 40X SYBR Green I, and enumerated at 1000X magnification using a Nikon Eclipse 80i microscope and NIS-Elements BR imaging software (v3.2). Per sample, between 200 and 500 microbes and virus-like particles (VLP) were counted from 1140 10-μm^2 fields. Visibly dividing cells were also enumerated. Viral counts were back-corrected to in situ concentrations using the storage decay relationships described in Colangelo-Lillis *et al.* (2016). Methods employed here did not distinguish between Bacteria, Archaea, and small single-celled Eukaryota. Members of each domain have been reported in both springs (Perreault *et al.* 2007, 2008; Niederberger *et al.* 2010; Lay *et al.* 2012, 2013) and are herein collectively referred to as microbes.

Click-chemistry fluorescent tagging of EdU

Microbes and viruses from samples incubated with EdU were prepared as described above for viral and microbial abundances. Parallel samples were prepared on 0.2 μm-pore size, black nucleopore filters for determining percentage of cells with

incorporated EdU. Samples prepared on nucelopore filters retained only microbes only, and not VLPs. These samples were more thoroughly washed of extraction supernatant before filtration, and all washes, EdU-labeling and counter stain were performed in microcentrifuge tubes. Fixed microbes were pelleted from sediment extractions by centrifugation at 5000 x g at 4°C. The supernatant was decanted and the cells were resuspended in 1.5 mL 1X PBS and transferred to microcentrifuge tubes. After pelleting, the supernatant was again removed and the pellet was resuspended gently in 100 µL Triton X-100 (0.5% in PBS) and incubated at room temperature for 20 min. Following incubation, cells were centrifuged and washed once in 1.5 mL PBS. The cell pellet was resuspended in 250 µL PBS to which 250 µL 2X fluorophore-azide reaction mixture was added. Microbes were incubated at room temperature (protected from light) for 20 min and then collected by centrifugation. The pellet was washed with 1.5 mL PBS, resuspended in 250 µL PBS to which 250 µL Hoechst 33342 (4 µg mL^{-1}) was added. Microbes were incubated again at room temperature (protected from light) for 15 min and then collected by centrifugation. The pellet was washed with 1.5 mL PBS and finally resuspended in 1.5 mL PBS and filtered on to a 0.2 µm pore size nucleopore filter for enumeration. Similar methods were employed on 0.02 µm pore size anodiscs to attempt a determination of viruses with incorporated Ed. However, background fluorescence prevented meaningful enumeration.

Results

Sediment characteristics

Measurements of sediment physical and chemical characters are consistent with those made from the same springs in previous years (Tables 1 and 2): pH was circumneutral (6.8 to 7.2); temperature was −3.6 to 7.6 °C; dissolved oxygen was 0 to 110 μM; and ORP was −287 to −120 mV. These parameters were greater in channel sediments compared to outlet sediments. Solid phase organic carbon (0.17 to 0.50%) and nitrogen (0.02 to 0.04%) and dissolved organic carbon (233 to 283 μM) were similar in both outlet and channel sediments.

In situ microbial and viral abundances

Treated and untreated samples fixed at time 0 and 96 h for each experiment (i.e. growth, decay, and induction) yielded consistent abundances of both microbes (8.0±0.7 x 10^6, 7.7±0.3 x 10^7 cells cm^{-3} for Gypsum Hill outlet and channel and 3.4±0.8 x 10^5, and 3.0±0.8 x 10^6 cells cm^{-3} for Lost Hammer outlet and channel) and viruses (8.6±1.6 x 10^6, 8.2±1.2 x 10^7, 1.5±0.3 x 10^6, and 4.0±0.8 x 10^6 VLP cm^{-3}; Fig. 2 and Table 3). The standard deviations of all values within each set (e.g. microbes or viruses per spring station) were smaller than the standard deviation between the microscope field of view counts used to determine the abundance of any individual sample (<25% of mean). The percentage of visibly dividing cells (VDC) was between 0 and 5% for all samples (2.2±1.3, 1.1±0.4% for Gypsum Hill outlet and channel and 1.4±0.4, and 1.0±0.3% for Lost Hammer outlet and channel). Untreated samples, chemically-fixed for enumeration at both time 0 and at 96 hours for each experiment, within each spring station, indicate that no discernible 'bottle effects' (wherein the in situ conditions and processes are influenced by the artificial replication of their environment; Pernthaler and Amann, 2005), resulting from either establishing the microcosms, or from adding experimental treatment chemicals. Thus no corrections for these influences were made to counts for any of the growth, decay or provirus induction experiments (Fig. 2).

Microbial growth

We used C-amended microcosms to calculate the efficiency of EdU incorporation into new microbes and the intrinsic growth rates at each station. The efficiency of EdU incorporation into new cells in acetate-amended microcosms was 2 to 19% and 6 to 27% in lactate-amended microcosms (Table 4). Intrinsic microbial growth ranged from 5.4 x 10^2 to 4.3 x 10^5 microbes cm^{-3} h^{-1} (Fig. 3 and Table 3). In each spring growth rates were higher in the channel sediments compared to the outlet, and growth rates in GH4 were greater than those in LH. Microbial growth rates in microcosms amended with organic carbon were greater than the intrinsic growth rates and varied from 5.6 x 10^2 to 1.1 x 10^6 microbes cm^{-3} h^{-1} (Fig. 3 and Table 4). In GH4 sediments acetate increased growth rates more so than lactate (by an average factor of 2.5). In LH outlet sediments lactate increased growth rate more than acetate (factor of 2.7), and in LH channel sediments carbon addition apparently exerted a small inhibitory affect on growth (3 to 20%; Table 4).

Viral decay

Viral decay rates varied from 9.1 x 10^2 to 1.7 x 10^6 viruses cm^{-3} h^{-1} (Fig. 4 and Table 3). In GH4 outlet, GH4 channel and LH channel, decay was initially relatively rapid, then quickly subsided, leaving a refractory viral pool that decayed at a slower or unobservable rate (Fig. 4 and Table 3). The refractory viral population made up 49 to 58% of the initial populations of these spring stations. The entire viral population of LH outlet exhibited a decay rate comparable or lower than the refractory populations of other stations. Given evidence that the viral and microbial communities were in dynamic equilibrium, initial decay rates were taken as equivalent to in situ viral production rates, and following numerous reports (Heldal and Bratbak 1991; Guixa-Boixereu *et al.* 1999; Fischer and Velimirov 2002), the viral production rates that balanced the initial (labile) viral pool decay rate were employed in calculations of viral-induced microbial mortality.

Provirus induction and fraction of lysogens

In each induction time series, microbial abundance decreased and viral abundance increased following exposure to mytomycin C. The apparent time to respond to the

inducing agent and duration of induction varied between experimental stations (Fig. 5); these induction periods (24 to 144 h) were used to assess the fraction of lysogens in each station. Lysogens made up 15 to 71% of the microbial populations, with the largest proportion in the coldest and highest salinity spring station (LH outlet; Table 3). Lysis of induced lysogens may have stimulated microbial growth (see Fig. 5, channel station time series), and our time series did not capture the full extent of the induction event in all spring stations (Fig. 5, outlet station time series). As a result, estimates of the fraction of microbes that are lysogens should be considered minimum values. Temperate virus burst sizes were similar between stations within each spring: 22 to 21 viruses $cell^{-1}$ in GH4 and 9 to 12 viruses $cell^{-1}$ in LH. The microbial response to induction was sufficiently rapid and uniform in channel sediments to allow for estimates of decay rates of induced temperate phage. Compared to in situ populations, induced temperate phages decayed more slowly in GH4 channel sediments (20% in situ rate), and more quickly in LH channel sediments (200% in situ rate; Table 5).

Discussion

This work describes viral dynamics experiments performed in cold, oligotropic sediments of polar hypersaline spring sediments in the Canadian High Arctic in order to address two questions: (1) is there is a direct relationship between trophic conditions and the predatory influence exerted by viruses on their microbial hosts? and (2) are viruses capable of lysogenic replication more common in oligotrophic environments? These questions form the basis for the trophic hypothesis of viral influence on microbial growth (Corinaldesi *et al.* 2003) and have implications for microbial ecology and evolution. By nature of their low temperature, high salinity and low carbon availability, the springs investigated in this study represent an experimentally accessible, low energy environmental outlier in which to test the hypotheses above.

Microbial and viral populations are in dynamic equilibrium

Constant in situ abundances of microbes and viruses and dividing cells are consistent with microbial and viral abundances measured from the same spring sediments in summer of 2013 (Colangelo-Lillis, Wing and Whyte 2016). These measurements suggest that microbial and viral populations in these sediments are each in dynamic equilibrium, both throughout our sampling period (July 11-18, 2015) and potentially across annual seasons (July; 2013 and 2015). Further, this dynamic equilibrium indicates that endogenous rates of viral production and decay are balanced. The abundances of viruses measured ($\approx 10^6$ to 10^8 viruses cm^{-3}; Fig. 2) were approximately 100-fold lower than those generally found in marine surface sediments (i.e. 10^8 to 10^{10} viruses cm^{-3}; Danovaro et al., 2008a), indicating different controlling influences on the communities in the surface sediments of these polar springs.

Slow microbial growth is limited by organic carbon

In situ microbial growth varied from 0.05 to 43.0 x 10^4 microbes cm^{-3} h^{-1} (Table 3). Growth in GH4 channel sediments was one order of magnitude greater than in its outlet, and two orders of magnitude greater in LH channel sediments compared to its outlet. Notably, in each spring a greater fraction of cells were found to be dividing from

outlet stations compared to channel stations (Fig. 2), indicating longer durations of the visible phase of cell division in the colder, less oxygenated outlet sediments. These differences in abundances and duration of cell division may be related to the availability of organic carbon or nitrogen as these nutrients varied ≈two-fold between sites within a given spring (Table 2) and as carbon amended experiments resulted in increased growth (Fig. 3). These differences are unlikely to be related to viral predation pressures, which are greater in channels compared to outlets, or to protist predations, as protists were not observed in any sediment samples. Nutrient limited trophic conditions influence viral production by changing the size, metabolism, and growth rate of host cells (Hewson *et al.* 2001; Corinaldesi *et al.* 2003), primarily by decreasing the overall energy available to support the microbial community. Microbial production in marine surface sediments leads to between 2 and 130 ng C cm^{-3} h^{-1} (converted from reported g^{-1} values, 50% porosity and sediment density 1.8 g cm^{-3}; Danovaro et al., 2008b) from sediments spanning a wide range of water depths and trophic conditions. Using a representative cell radius of 0.25 μm (based on TEM images of cells from these sediments; Colangelo-Lillis et al., 2016b) and a cellular carbon content of 310 fg C μm^{-3} (Danovaro *et al.* 2008a), production rates from GH4 and LH sediments fell between 1 and 872 pg C cm^{-3} h^{-1}, underlying the limited energy availability in these sediments relative to the marine surface environment.

Low rates of viral production and decay indicate sluggish viral dynamics

In even the most prolific of spring station sediments (GH4 channel, 1.7 x 10^6 cm^{-3} h^{-1}), viral production was lower than that seen in marine surface sediments (viral production from lowest 5% of dataset was 9.8 ± 3.9 x 10^6 cm^{-3} h^{-1}; Danovaro et al., 2008b) or deep sea sediments (5.7 ± 1.4 x 10^7 cm^{-3} h^{-1}; Corinaldesi et al., 2007). These low viral production rates of GH4 and LH are consistent with the observed low microbial growth rates. In contrast to both shallow and deep-sea sediment viruses, described as highly dynamic and an active component of sediment ecosystems, with production rates ranging from 0.1 to 5 x 10^8 cm^{-3} h^{-1} (Hewson and Fuhrman 2003; Mei and Danovaro 2004; Danovaro *et al.* 2008b), the viruses of these cold hypersaline spring sediment environments appear to be active, but at a greatly reduced rate.

In each spring the viral decay profile was interpreted to represent two viral communities: the first appeared prone to decay (labile) and the second refractory (Fig. 4). A similar trend of rapid viral decay, followed by much more gradual or negligible decay has been reported from both water column (Heldal and Bratbak 1991; Guixa-Boixereu *et al.* 1999; Fischer and Velimirov 2002) and sediments (Fischer *et al.* 2004). In each spring, a substantial proportion of viruses (49 to 100%) fell into the refractory category (Table 3). The viral community from the LH outlet station was subject to a single decay rate, but given that rate was lower than the refractory viral decay rates from other spring stations, the entire viral population was considered refractory. Measurements of viral decay in sea water have led to estimates of viral turnover times ranging between one hour and a few days (Suttle and Chen 1992; Fuhrman and Noble 1995; Noble and Fuhrman 1997). These decay rates were correlated with the biological richness of the water, with the most rapid decay occurring in coastal waters and the slowest decay in oligotrophic offshore waters (Fuhrman 1999). Investigations of viral decay in sediments have reported both rapid and very slow rates, leading authors to alternatively propose that virus preservation in sediments is either very poor (Mei and Danovaro 2004) or very good (Middelboe, Glud and Filippini 2011; Dell'Anno, Corinaldesi and Danovaro 2015). Viruses from GH4 and LH spring sediments appear to be in the former category, with turnover rates of the labile pool on the order of tens of hours, and weeks to months for the refractory pool. The mechanism of this decay-resistance is unknown but could be related to the high concentration of salt, low concentrations of extracellular proteolytic enzymes, clay-adsorption and stabilization of viral particles, or structural modifications to viral protein coats specifically adapted to persist in environments with low host abundance and encounter rates.

Viruses are not the dominant cause of microbial mortality

In order to evaluate viral induced microbial mortality, a measure of the viral burst size of a lysing cell is required. This value is frequently determined by direct assessment of visibly infected cells, but no such cells have been detected from these sediments (Colangelo-Lillis, Wing and Whyte 2016). Here, burst size was inferred from provirus induction experiments, by dividing the number of temperate virions released by the

concomitant decreases in microbial abundances (Figure 5 and Table 3; Weinbauer and Suttle, 1999). These burst sizes are expected minimums; increases in burst size would force estimates of viral induced microbial mortality downwards. These estimates of burst sizes are comparable to those found in other cold, hypersaline and/or oligotrophic environments (e.g. 15 to 28, Weinbauer and Suttle, 1999; 6 to 324, median = 16, Wells, 2008; 6 to 35, mean = 22, Guixa-Boixareu et al., 1996), but much lower than virus burst sizes reported from eutrophic environments (e.g. 33 to 64, Weinbauer and Suttle, 1999). Increasing latent periods and decreasing burst sizes (even the apparent cessation of infection) are associated with suboptimal nutrient concentrations (Proctor, Okubo and Fuhrman 1993; Moebus 1996). Low nutrient concentration also correlates with small cell size, another factor observed to decrease burst size, and observed in GH4 and LH sediments (Choi *et al.* 2010).

Virus-induced microbial mortality (M_v, expressed as percent) was calculated from viral production (P_v, virions cm^{-3} hr^{-1}), burst size (B, virions $cell^{-1}$) and microbial growth rate (μ, cells cm^{-3} hr^{-1}), as $M_v = P_v \cdot B^{-1} \cdot \mu^{-1} \cdot 100$. M_v ranged from 8 to 29%, with greater values in LH compared to GH4 (Table 3). With notable exceptions (Hewson and Fuhrman 2003; Mei and Danovaro 2004) these values are lower than are typically reported from marine sediments. The highest M_vs (up to 100%) were found in coastal sediments off Chile (Middelboe and Glud 2006) and in coastal Adriatic sediments (57%; Mei and Danovaro, 2004). Danovaro et al.'s (2008b) assessment of globally distributed marine surface sediments estimated 80% M_v. Similarly, studies of M_v in deep-sea sediments (Middelboe *et al.* 2006; Corinaldesi *et al.* 2010) reported that viruses play a crucial role in the mortality of deep benthic microbes, and are responsible for up to 60% of microbial mortality. Prior to this work, we had hypothesized that low biomass, slow microbial growth, and infrequent viral-microbe contact rates would result in limited viral-induced microbial mortality, possibly alluding to a trophic lower limit to viral activity. However in this system, viruses may have adapted to their hosts' relatively slow growth rates and low densities by adopting both physical characters that resist decay, prolonging their opportunity to contact a suitable host, and by reducing their burst size, necessitating fewer of their host's energetic resources in order to propagate. Similarly to cold-active phage 9A, there may also be selection for broad host range under the low contact

frequency conditions in these sediments (Wells and Deming 2006a; Colangelo-Lillis and Deming 2013). Viruses are thus unlikely to be the dominant cause of microbial mortality in these cold hypersaline oligotrophic sediments but do appear to be active and may yet have a significant impact on carbon and nutrient flow in these sediments.

Dissolved organic carbon and the viral shunt

Viral infection has the potential to stimulate microbial growth by increasing nutrient availability through cell lysis and the liberation of soluble cytoplasmic components. This viral shunt has important ecological and biogeochemical consequences in the surface ocean (Suttle 2007), marine surface sediments (Danovaro *et al.* 2008b), and potentially subsurface sediments (Engelhardt *et al.* 2014). Employing a microbial N/C- and P/C-ratio of 0.26 and 0.04 (Weinbauer and Höfle 1998b), lysis of cells in GH4 and LH sediments released organic carbon at rates between 0.18 and 140 x 10^{-2} ng C cm^{-3} h^{-1}; organic nitrogen at rates between 0.48 to 370 x 10^{-3} ng N cm^{-3} h^{-1}; and phosphorous at rates between 0.74 to 560 x 10^{-4} ng P cm^{-3} h^{-1} (Fischer et al., 2006). These rates are one to three orders of magnitude lower than the lowest rates reported for surface (1 cm below sea floor) sediments (Danovaro *et al.* 2008b), and thus are not expected to stimulate microbial growth to a degree comparable to those sediments. Extending these findings, the sediments investigated here more closely resemble the bulk volume of deep subsurface marine sediments globally in organic carbon content, biomass, temperature, and oxygenation than do many of the other sediments yet investigated for viral dynamics, and the dynamic values here may serve as proxies for similar cold and/or hypersaline environments where these measurements have not been made (Colangelo-Lillis *et al.* 2016).

Large fraction of lysogens from coldest, most hypersaline sediments

Lysogenic replication is more frequent in conditions unfavorable to microbial growth, while lytic replication dominates in highly productive environments (Wilcox and Fuhrman 1994; Weinbauer and Suttle 1996; Tapper and Hicks 1998; Paul 2008). Concordantly, Danovaro (2008a) proposed that lysogeny might be less important than the lytic cycle in surface sediments, as those sediments generally provide abundant resources for the growth of heterotrophic microbes. However, the environmental frequency of

lysogens varies widely (3 to 52%; Cochran and Paul, 1998; Cochran et al., 1998; Paul, 2008; Weinbauer and Suttle, 1996; Wilcox and Fuhrman, 1994; Wommack and Colwell, 2000), and recently the stringency of this trophic condition-replication strategy relationship has been called into question (Knowles *et al.* 2016). In surface sediments, low frequencies of lysogens have been reported, but those frequencies increase (up to five-fold) as nutrients decrease with depth (Mei and Danovaro 2004). A hypothesis of this investigation was that lysogenic replication would be favored as a replication strategy in cold hypersaline oligotrophic sediments with low microbial growth, possibly to a greater extent than yet reported, reflecting the extremely low biomass and viral-host encounter rates (Colangelo-Lillis, Wing and Whyte 2016). Inducible lysogens represented substantial fractions of the microbial community of each spring station investigated here (15 to 71%; Table 3); these notably exceed those found in other sedimentary environments (Glud and Middelboe 2004; Danovaro *et al.* 2008b). The greatest fraction of lysogens was found in the outlet sediments of LH, the spring station characterized by the lowest microbial and viral abundances, lowest microbial growth and viral production rates, and relatively low concentrations of total and organic carbon. This may have implications for microbial evolution in these sediments as proviruses enhance host fitness (Edlin, Lin and Kudrna 1975), and hyperhalophilic viruses display a greater rate of gene transfer and recombination (Roux *et al.* 2015a). Compared to the decay rates of the in situ viral communities, induced temperate viruses decayed six-fold more slowly in GH4 channel and three-fold more quickly in LH channel. The discrepancies between these rates of decay suggest that the in situ labile viral populations are not the result of lysis by the substantial population of inducible temperate viruses.

Conclusions

This work was undertaken to identify the role of viruses in a sedimentary environment at the boundaries of physical and geochemical parameters yet examined, and to test the hypothesis that a combination of cold and subzero temperatures, low nutrient, low biomass and high salinity would inhibit viral replication by lysis and promote replication by lysogenic replication. Our findings indicate that these low energy sediments maintain extremely low rates of microbial growth and viral production and that a substantial fraction of viruses are extremely resistant to decay. These viruses do contribute to microbial mortality, but are not the primary cause of such, leaving the questions of mechanism of control on growth and ultimate cause of mortality open. The relatively low rates of viral-induced mortality released amounts of dissolved nutrients into the sediments insufficient to stimulate microbial growth. A substantial fraction of microbes in these sediments appear to be lysogens, harboring inducible provirus yet based on their distinct decay rates these temperate viruses do not seem to make up a substantial fraction of the in situ population of virions. The similarity in physical character, aqueous chemistry and abundances of microbes and viruses between these cold, oligotrophic, hypersaline sediments and deep subsurface marine sediments indicate that viruses may have a substantially tempered role in influencing microbial ecology in the deep subsurface sediments of the global ocean, and that these interactions are operating on substantially greater time scales.

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Table 1. Historical physical and spring and pore water chemistry measurements from Gypsum Hill and Lost Hammer springs. For each parameter, a comma separates minimum and maximum values. *Where no season is indicated, report covers multiple seasons- see reference for further details. bdl- Below detection limits. Single quotation ' indicates value equivalent to cell immediately above.

Spring	Station	Study 1st Author. Year	year.season*	substrate	temperature (°C)	salinity (‰)	pH	ORP (mV)	dissolved oxygen (µM)	organic carbon (%)	dissolved organic carbon (mM)	sulfate (mM)	sulfide (µM)	nitrate (µM)	nitrite (µM)	ammonia (µM)	CO_2 (µM)
Gypsum Hill	**Outlet**	Pollard. 1999	1993-1998	water	6.0	138	8.4	na	na	na	na	39, 41	0.8	na	na	na	na
'	'	Anderson. 2004	nr, < 2004	water	6.6	100	7.7	−97.1	na	na	bdl	39	na	bdl	bdl	na	na
'	'	Perreault. 2007	2004/5.summer	water	6.9	75	7.4	−287	6.3	na	na	na	na	810	na	na	na
'	'	Colangelo-Lillis. 2016	2013.summer	water	4.2	82	7.4	−318	2.5	na	na	36	0.8	na	na	na	na
'	'	'	'	sediment	4.2	82	7	−287	na	na	na	na	na	na	na	na	na
'	'	this report	2015. summer	water	7.0	na	6.5	na	2.8, 6.6	na	0.2, 0.8	33	151	15	0.14	3.3	329
'	'	'	'	sediment	7.0	na	na	na	0, 1.3	0.15, 0.18	na	41	53	na	0.29	10.6	na
'	**Channel**	Perreault. 2007	2004/5.summer	sediment	na	na	na	na	na	0.37	na	20	na	na	na	na	na
'	'	Niederberger. 2009	2007.spring	water	5.0, 6.9	na	na	na	31	na	na	na	625	na	na	na	na
'	'	Colangelo-Lillis. 2016	2013.summer	water	na	na	na	na	na	na	na	na	na	na	na	na	na
'	'	'	'	sediment	7.8	na	na	na	na	na	na	na	na	na	na	na	na
'	'	this report	2015. summer	water	7.5	na	6.0, 6.5	na	31, 228	na	0.3, 0.5	21	299	na	2.46	3	< 227
'	'	'	'	sediment	7.5, 8.7	na	7.7	−330	0, 109	0.42, 0.45	na	39	179	26	0.43	0.3	343
Lost Hammer	**Outlet**	Niederberger. 2010	2005-2008	water	−5.9, −4.7	220, 260	6.0, 7.4	−187, 154	3.1, 31	na	na	54	0, 1560	na	na	na	na
'	'	'	'	sediment	na	na	na	na	na	0.45	na	1000	na	bdl	bdl	na	na
'	'	Lay. 2012	2008-2010	water	na	na	na	na	na	na	na	na	na	0, 2.6	0, 2.4	504	na
'	'	'	'	sediment	na	na	na	na	na	na	na	na	na	0, 58	0, 52	187	na
'	'	Lamarche-Gagnon. 2015	2011-2012	water	−7.7, −3.1	220, 260	6.0, 6.8	−224, −165	1.3, 35	na	na	na	0.5, 18	na	na	na	na
'	'	Colangelo-Lillis. 2016	2013.summer	water	−4.8	225	6.8	−181	0.6	na	na	na	na	na	na	na	na
'	'	this report	2015. summer	water	−4.8	na	6.1	−391	5.0	na	na	na	na	na	na	na	852
'	'	'	'	sediment	na	na	na	na	na	0.33	na	na	na	na	na	na	na
'	**Channel**	Lay. 2012	2008-2010	water	−18, −9.2	220, 260	6.5, 7.3	na	> 31	na	na	na	0, 625	0, 3.42	0, 3.42	46, 482	na
'	'	'	'	sediment	−18, 0	na	na	−30, 126	na	0.77, 0.93	na	na	na	0, 2.42	0, 2.17	56, 65	na
'	'	this report	2015. summer	water	−1.1	na	na	na	313	na	na	46, 156	4, 12	3	0.14, 0.32	300	1988
'	'	'	'	sediment	0.3, 1.6	na	na	na	35, 58	0.46, 0.54	na	75	19	na	na	na	na

Table 2. Spring chemistry.

Spring	Station	Distance from outlet (m)	Temp. (°C)	pH	ORP (mV)	Density (g cm^{-3})	Porosity (%)	Dissovled Oxygen (µM)	Total Carbon (wt %)	Total organic Carbon (wt %)	Dissolved organic Carbon (µM)	Total Nitrogen (wt %)	Sulfate (mM)	Sulfide (µM)	Ammonium (mM)	Nitrate (µM)	Nitrite (µM)
GH4	Outlet	0	4.2	7.0	−287	3.02	48	0 - 1.3	0.74	0.17	233	0.02	41	53	10.6	15	0.29
GH4	Channel	5	7.6	7.2	−330	3.51	54	38 - 110	0.72	0.44	283	0.04	39	179	0.3	26	0.43
LH	Outlet	0	−3.6	6.8	−181	2.67	42	0 - 0.63	0.29	0.33	na	0.02	75	75	0.2	*	*
LH	Channel	5	−0.6	7.0	−120	2.82	49	34 - 69	0.76	0.50	na	0.03	75	<19	0.06	*	*

*sum of nitrate and nitrite values reported in Lay et al. 2012 as 58 mM for LH outlet sediments and 1.8 - 2.4 mM for LH channel sediments.

Table 3. Viral dynamics measurements. VDC- visibly dividing cells; VMR- virus:microbe ratio.

Spring	Station	Microbes (cm^{-3})	VDC (%)	Viruses (cm^{-3})	VMR	Microbial growth (cm^{-3} h^{-1})	Microbial turnover (h)	Labile viral population (%)	Labile viral decay (cm^{-3} h^{-1})	Labile viral turnover (h)	Refractory viral population (%)	Refractory viral decay (cm^{-3} h^{-1})	Refractory viral turnover (h)	Burst size	Microbial mortality (%)	Inducible microbes (%)
GH	Outlet	8.0×10^{6}	2.2	8.6×10^{6}	1.1	5.0×10^{4}	160	47	8.3×10^{4}	49	53	3.4×10^{3}	1335	21	8	15
GH	Channel	7.7×10^{7}	1.1	8.2×10^{7}	1.1	4.3×10^{5}	180	51	1.7×10^{6}	24	49	8.3×10^{4}	483	22	18	34
LH	Outlet	3.4×10^{5}	1.4	1.5×10^{6}	4.4	5.4×10^{2}	630	0	na	na	100	9.1×10^{2}	1648	9	19	71
LH	Channel	3.0×10^{6}	1.0	4.0×10^{6}	1.3	2.1×10^{4}	140	42	7.4×10^{4}	23	58	0	∞	12	29	42

Table 4. Growth rate calculations.

Spring	Station	Growth evaluated by EdU-Alexafluor (# new cells cm^{-3} h^{-1})			Growth evaluated by total cell counts (# new cells cm^{-3} h^{-1})			Corrected growth evaluated by total cell counts* (# new cells cm^{-3} h^{-1})			EdU incorporation efficiency (%)		In situ growth (# new cells cm^{-3} h^{-1})	C-appended growth rate factor	
		EdU	EdU+L	EdU+A	EdU	EdU+L	EdU+A	EdU	EdU+L	EdU+A	EdU+L	EdU+A		L	A
GH4	Outlet	4190	8320	7140	22900	74900	150000	0	52000	127000	6.3	17.8	5.04E+04	1.04	2.53
GH4	Channel	34900	71300	59600	293000	731000	1400000	0	438000	1100000	6.1	18.5	4.30E+05	1.02	2.57
LH	Outlet	56	112	96	117	1600	676	0	1480	559	13.3	5.8	5.36E+02	2.77	1.04
LH	Channel	900	633	1020	−4136	12700	16300	0	16800	20500	26.5	2.0	2.10E+04	0.80	0.97

* growth values in microcosms unamended with carbon and in dynamic equilibrium were corrected to 0. This correction is based on the presumption that EdU can serve as a nutrient (personal correspondence with Invitrogen technical representative)

Table 5. Provirus induction calculations.

Spring	Station	Induction period (h)	Microbial reduction (cells cm^{-3})	Microbial reduction (%)	Induced viral production (VLP cm^{-3})	Burst size (VLP $cell^{-1}$)	Induced temperate viral decay rate (VLP cm^{-3} h^{-1})	Endogenous viral decay rate (VLP cm^{-3} h^{-1})	Induced/ Endogenous decay ratio
GH	Outlet	24	1.2E+06	15	2.7E+07	22.1	na	8.3E+04	na
GH	Channel	24	2.6E+07	34	5.6E+08	21.6	2.9E+05	1.7E+06	0.17
LH	Outlet	144	2.4E+05	71	2.2E+06	9.1	na	na	na
LH	Channel	48	1.3E+06	42	1.2E+07	9.1	1.4E+05	7.4E+04	1.95

Figure. 1. Sampling sites. Hypersaline arctic springs Gypsum Hill 4 (GH4) and Lost Hammer (LH) investigated for viral dynamics. Outlet (O) and channel (C) experimental stations are indicated for each spring. Top down photos of each outlet are inset.

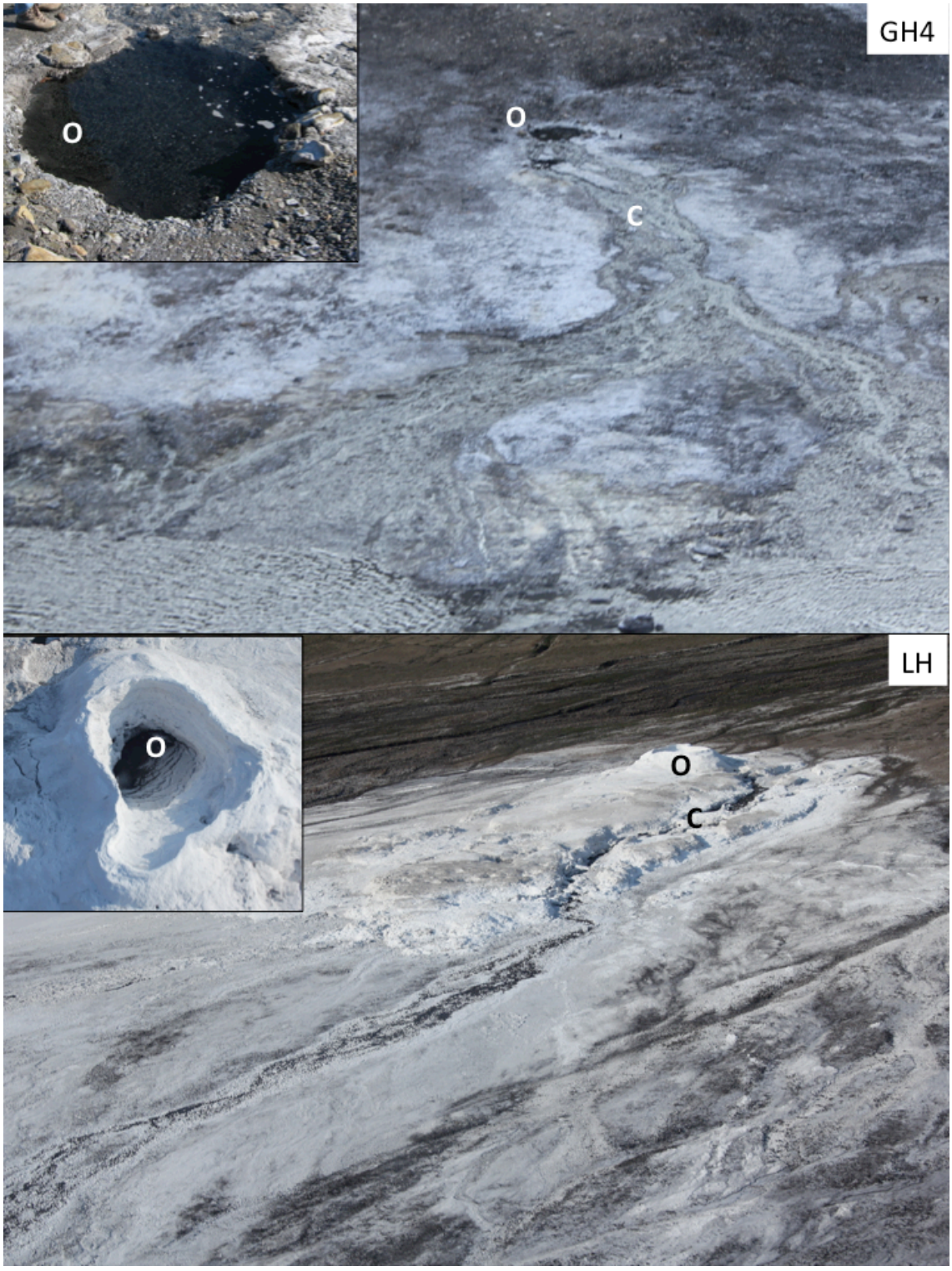


Figure. 2. In situ microbial and VLP abundances collated from growth, decay and induction experiments. Measured values from no-treatment controls (time-0 and -96 h) and time-0 h experimental treatment microcosms are shown. Microbial and VLP abundances are indicated by circles and triangles, respectively; the percentage of visibly diving cells is indicated by squares (right hand y axis values). Open shapes indicate no treatment, shaded shapes indicate treatment (KCN for decay experiments, mytomycin C for induction experiments, EdU±lactate or acetate for growth experiments). Experimental treatment and incubation times (0 or 96 h) are specified by text in line with column of measured values. Time 0 h for GH4 incubations corresponds to 1200 July 15, 2015; time 0 h for LH incubations corresponds to 1200 July 11, 2015. Note logarithmic left hand y-axis.

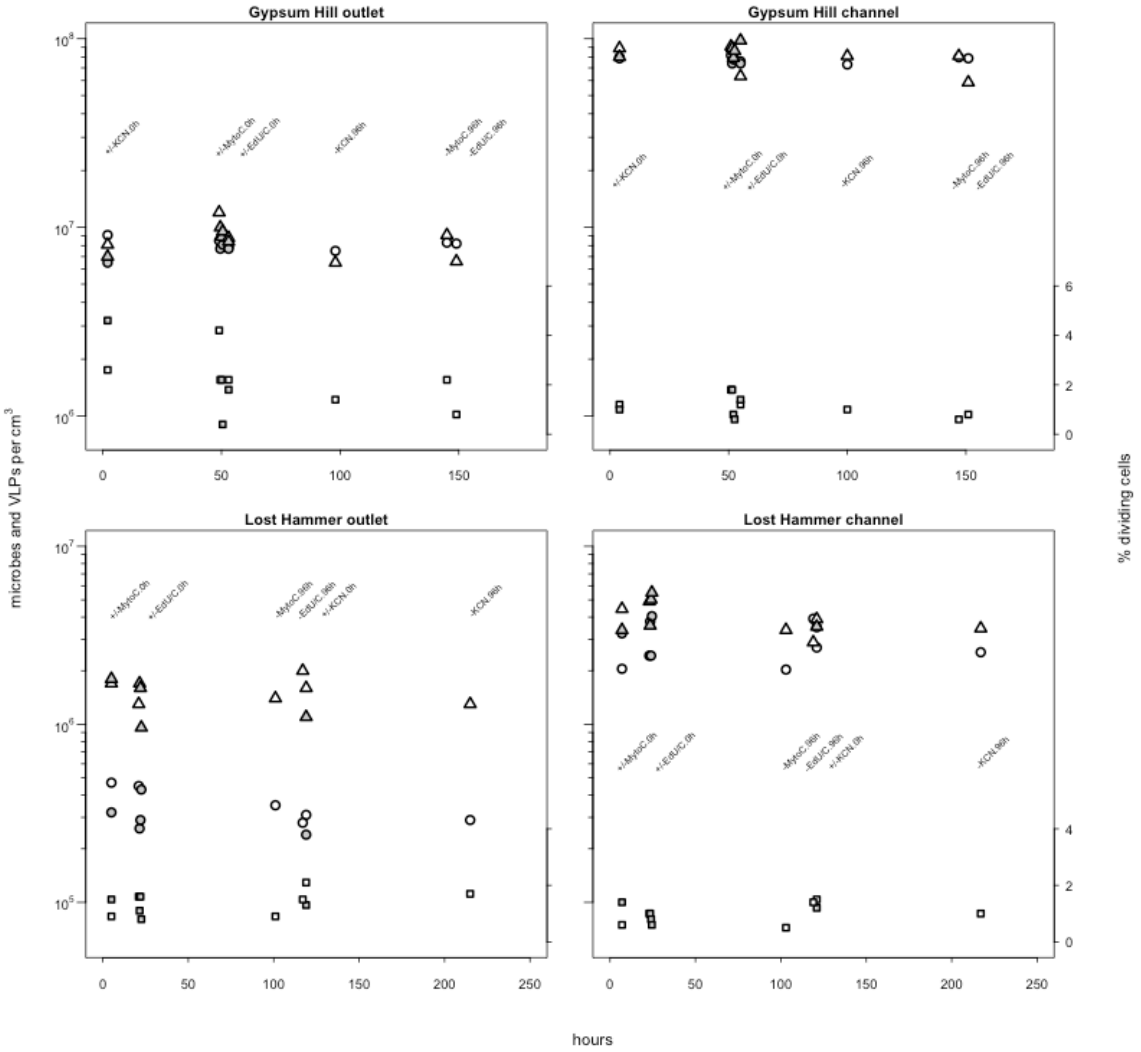


Figure. 3. Microbial growth in unamended and carbon amended microcosms. Circles indicate total microbial abundances, the percentages of microbes with incorporated EdU are indicated by squares (right hand y axis values). Unshaded shapes indicate EdU treated samples; shaded shapes indicate samples additionally treated with lactate (grey) or acetate (black). Best-fit line equations and associated R^2 values for each treatment time series are indicated (dashed: EdU, grey: EdU+lactate, black: EdU+acetate). Note linear left hand y-axis.

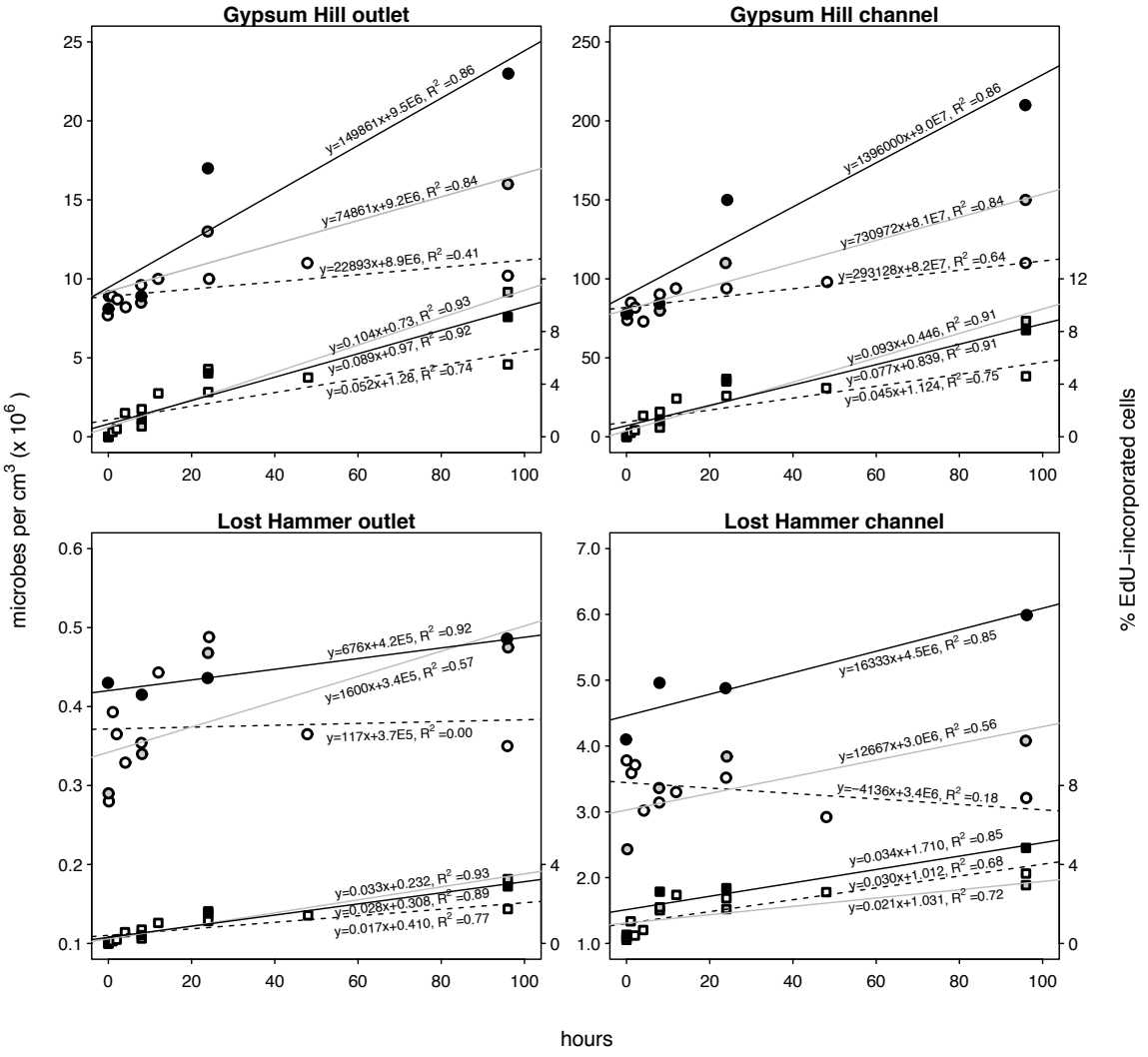


Figure. 4. Viral decay microcosms. Circles indicate microbial abundances, VLPs are indicated by triangles. Unshaded shapes indicate untreated control samples; shaded shapes indicate samples treated with KCN. Grey and black shading are only for clarity in distinguishing VLP from microbial abundances. Solid best-fit lines indicate labile viral decay rates; dashed lines indicate refractory viral decay rates (best fit line equations and associated R^2 values indicated). For each station the intersection of the best-fit lines indicates the abundance of viruses refractory to decay. Note linear left hand y-axis.

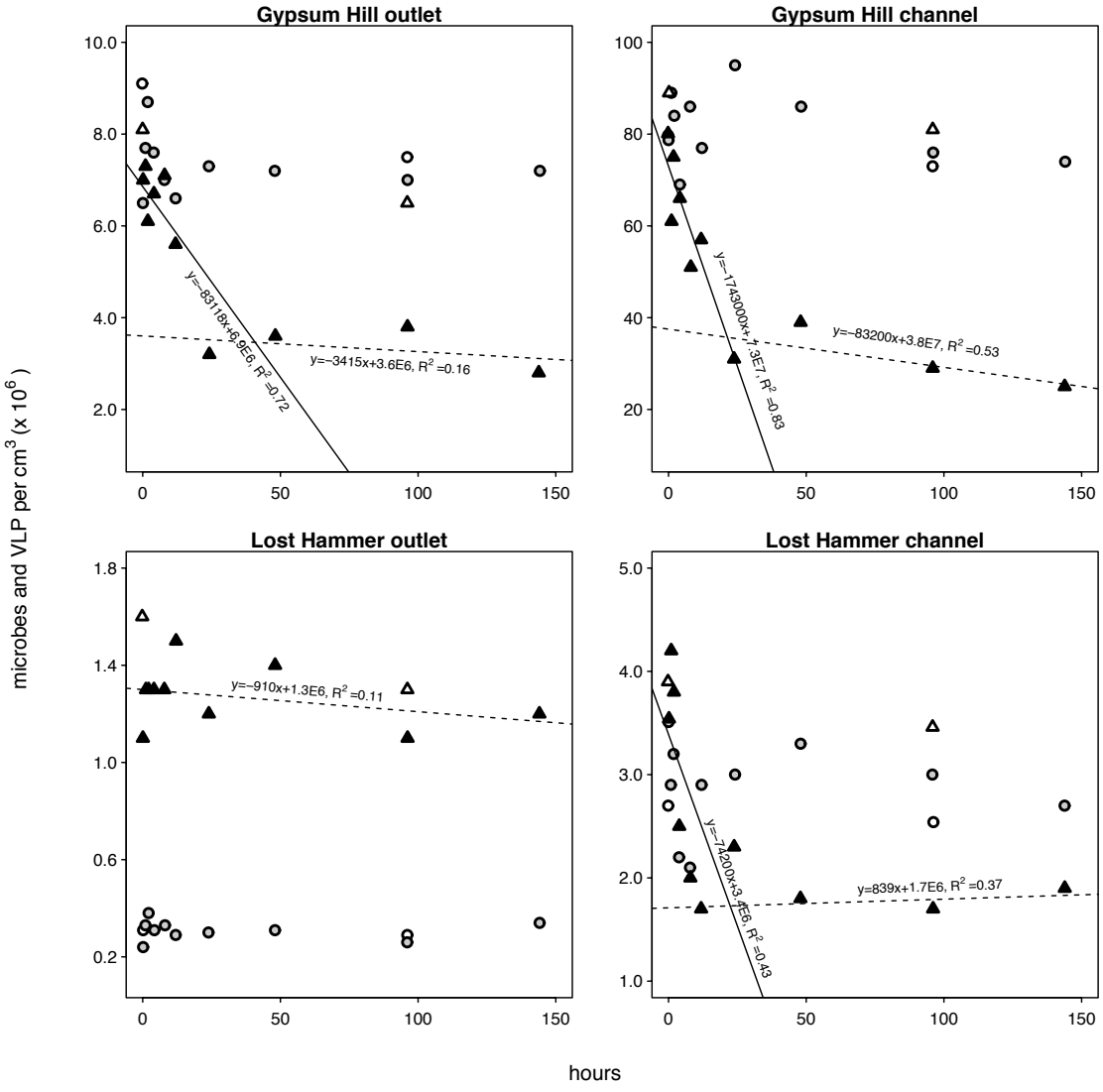
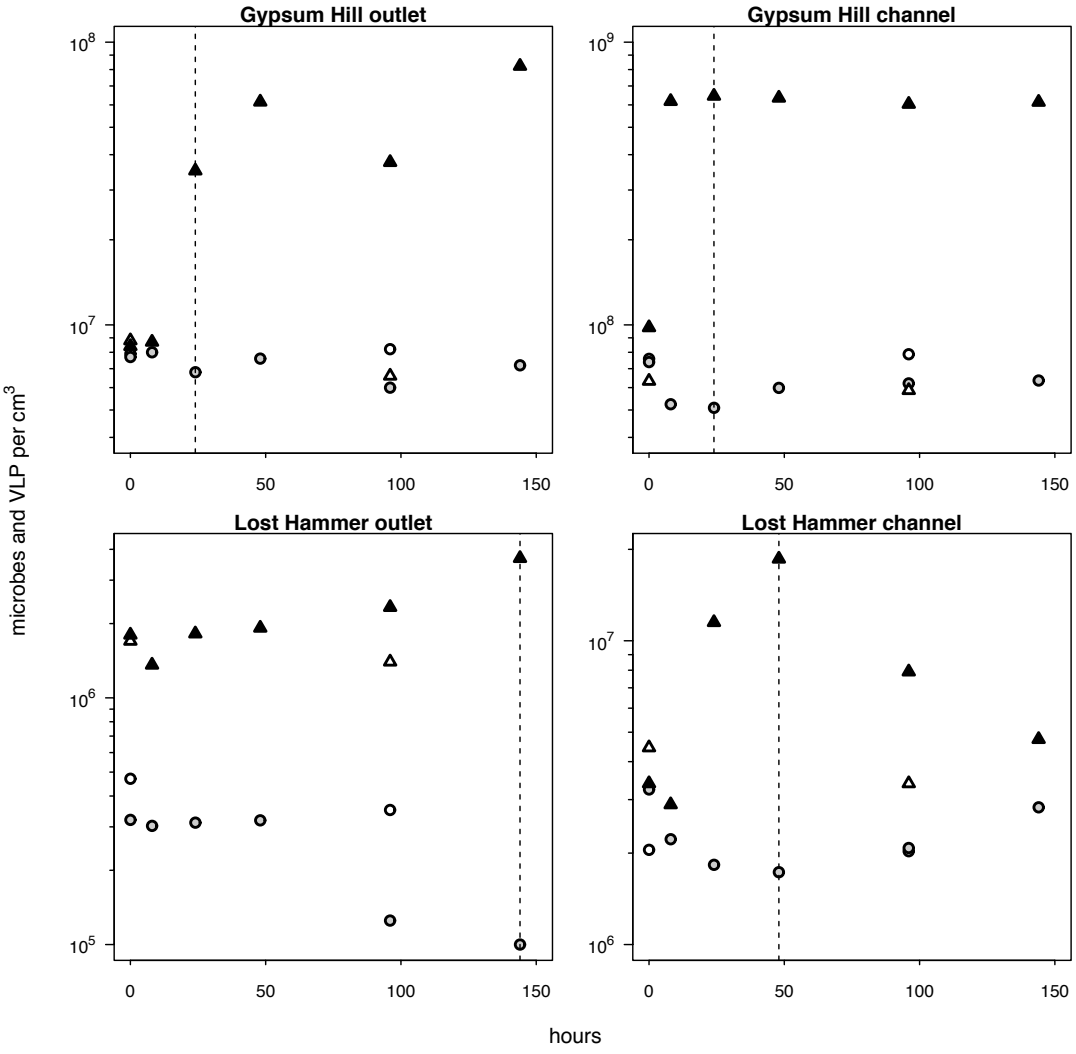


Figure. 5. Provirus induction microcosms. Shapes and shading as in Fig. 4; shaded shapes indicate values from mytomycin C treated samples. For each station, the duration of the induction period considered in calculations of percentage of lysogens and lysogen burst size is indicated by a vertical dashed line. Note logarithmic left hand y-axis.



Connection 2

The second paper included in this thesis described the first experimental measurements of viral dynamics and calculated the impact of viral predation on microbial growth in the energy limited environments found in the sediments of two polar hypersaline springs. Despite calculating very low contact rates between viruses and microbes in these springs' sediments, viruses were found to contribute to microbial mortality, though to less than 50% of that mortality. Experiments to assess the rate of viral decay in these sediments suggest that viruses are able to compensate for the relatively long period to contact a suitable host by adaptations that retard their susceptibility to decay. Additionally a high incidence of lysogens- microbes harboring prophage genomes- was detected. In similar fashion to the first paper in this thesis, these findings support a role of viruses in microbial ecological processes (including both mortality and maintaining diversity, as well as potentially genetic exchange) even under these extreme environment conditions that limit biomass and microbial growth.

At this point, the focus of this thesis shifts from a focus on one element of astrobiologically relevant ecological factors (microbial growth and viral predation) to another: microbial metabolism and biosignatures of in situ activity. Biosignatures of extreme environments are of particular interest to astrobiology, as extreme environments on Earth best approximate contemporary conditions of many of the astrobiologically relevant targets in our solar system. Considering the dominance of sulfur metabolisms previously identified in these springs, and the longevity and fidelity of isotopic biomarkers, our focus shifts to the genetic diversity of sulfur metabolizing microbes, their capacity for sulfate respiration in situ and the fractionation of sulfur isotopes that results from their metabolism, and that serves as a biomarker preserved in sediments. In recent years substantial effort has been made to ascertain the specific effects of environmental parameters on cultured sulfate reducing isolates in vitro. The work in this third paper is the first to approach the interpretation of sulfur isotope signatures preserved in the rock record using both modern genomic tools and a natural system including a community of organisms (rather than an isolate) metabolizing sulfur at the very low rates encountered in nature.

Paper 3

Diversity-signal disconnect between *dsrB* sequencing and sulfur isotope fractionation signals

Diversity-signal disconnect between *dsrB* sequencing and sulfur isotope fractionation signals

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Abstract

Sulfur metabolizing microbes fractionate sulfur isotopes. Metabolic byproducts are sequestered in sediments as metal sulfides and preserved isotopic signals inform atmospheric and oceanic chemistry over >3 billion years. The extent of fractionation is dictated by genetic, phenotypic and environmental variance, the later being disproportionately studied, as biological characteristics of extinct microbial populations leave no fossil record. To explore the relationship between environmental and genetic variance and isotopic fractionation in situ, we assessed environmental chemistry, sulfate reduction rates, diversity of sulfur metabolizing organisms (16S rRNA gene) and the dissimilatory sulfite reductase gene, and net fractionation of sulfur isotopes, along a hypersaline Arctic spring. Sediments were sulfate-rich (39 to 75 mM), and sulfide- (< 20 to 120 μM) and organic carbon- (0.2 to 0.5 wt %) poor. In situ sulfate reduction rates were very low (< 2 x 10^{-9} moles cm^{-3} day^{-1}); minimum cell-specific sulfate reduction rates were < 0.3 x 10^{-15} moles $cell^{-1}$ day^{-1}. Neither 16S rRNA nor *dsrB* gene diversity correlated with net isotope fractionation ($\varepsilon^{34}_{sulfate-sulfide}$; range −38 to −45‰). $\varepsilon^{34}S$ values predicted by a mechanistic model describing fractionation by microbial sulfate reduction could be matched by invoking 1-2% of the microbial community as engaged in sulfate respiration, indicating heterotrophic activity within the populations capable of this metabolism. Using the same model we found that enzymatic kinetic diversity of apr was more likely to correlate with sulfur fractionation than dsrB. This study was the first to simultaneously interrogate fractionation of multiple sulfur isotopes and microbial composition over an environmental gradient. This work suggests future work investigating microbial diversity-fractionation relationships focus on the *apr* gene rather than the *dsrB* gene, and given the difficulty in assessing within-functional-taxa activity, focus on sulfate reduction enzyme proteomics or transcriptomics rather than measurements of sulfate reduction rate and extrapolation to cell specific rates.

Introduction

Microbial dissimilatory sulfate reduction is a geologically ancient, anaerobic, energy-yielding metabolic process in which sulfate reducing microorganisms (SRM) reduce sulfate to hydrogen sulfide while oxidizing organic carbon or hydrogen. Diversity of SRM extends across the domains Archaea and Bacteria with the majority of species in 23 genera, primarily in classes Deltaproteobacteria and Clostridia (Rabus, Hansen and Widdel 2006). They are extremely versatile with respect to the electron donors and electron acceptors used for growth. SRM inhabit a broad range of environmental conditions and are ubiquitous in anoxic habitats. They occur in environments with extremely low or high pH, temperature and salt concentrations. Microbial sulfate reduction is responsible for the respiration of up to 50% of organic matter in marine sediments (Bowles *et al.* 2014), thus playing a key role the carbon and sulfur cycles. Their range, antiquity, metabolic versatility and influence on geochemical cycling underpin an interest in the role of SRM in fractionating isotopes of sulfur (Canfield 2001a).

During the reduction of sulfate to sulfide, lighter isotopes of sulfur are reduced at a faster rate than heavier isotopes, resulting in sulfide isotopically enriched in the light isotopes of sulfur relative to the source sulfate (Harrison and Thode 1958). A fraction of this sulfide is preserved in sediments as metal sulfides and can persist as evidence of metabolism on the scale of billions of years (Shen and Buick 2004). The extent of this fractionation of isotopes is influenced by genetic, phenotypic and environmental variance (Wing and Halevy 2014). Batch culture experiments with sulfate reducing isolates and natural populations have yielded kinetic isotopic enrichment ($\varepsilon^{34}S$) values ranging from −2‰ to −46‰ (Kaplan and Rittenberg 1964; Kemp and Thode 1968; Chambers *et al.* 1975; Habicht and Canfield 1997, 2001; Bolliger *et al.* 2001; Brüchert, Knoblauch and Jorgensen 2001; Canfield 2001b; Detmers *et al.* 2001; Habicht *et al.* 2005). Variation in these observed $\varepsilon^{34}S$ values has been attributed to sulfate concentration, electron donor type and concentration, cell specific sulfate reduction rate (csSRR), temperature, and species-specific isotope enrichment effects (Kemp and Thode 1968; Chambers *et al.* 1975; Brüchert, Knoblauch and Jorgensen 2001; Detmers *et al.* 2001; Kleikemper *et al.* 2004; Habicht *et al.* 2005; Canfield, Olesen and Cox 2006; Hoek *et al.* 2006). However

both due to methodological constraints, and to facilitate reasonable experimental duration, laboratory culture csSRRs range from to 0.1 to 18 fmol $cell^{-1}$ h^{-1}, notably greater than rates expected in many contemporary (where only bulk sulfate reduction rates are commonly measured) or ancient sediments (Hoehler and Jørgensen 2013; Leavitt *et al.* 2013). In addition to the parameters influencing fractionation by SRM isolates, in natural communities a complementary metabolism, microbial sulfur disproportionation (MSD), can generate an isotopically depleted source of sulfate which can then again be reduced by SRM, resulting in even greater depletion of ^{34}S in sulfide waste product and increased apparent net fractionation. Biological sulfur oxidation does not cause significant fractionation (Habicht, Canfield and Rethmeier 1998).

Tracking biological fractionations of sulfur isotopes, through enrichment of ^{32}S in biogenic sulfides, with respect to the source sulfate, provides a metric of biogeochemical activity and the role of microbial metabolisms in the cycling of sulfur, both in modern environments and in ancient environments. This metric extends back as far as the rock record, and suggests that SRM emerged more than 3.4 billion years ago (Canfield 1998; Shen and Buick 2004). Because the cycling of sulfur is involved in atmospheric oxygen regulation, sulfur isotopes have also been used to decipher the history of atmospheric oxygen and the oxidation state of Earth's surface environments (Canfield and Teske 1996). Prior to the past two decades this work focused on fractionation of the dominant isotopes ^{34}S and ^{32}S. For example, this signature has been used to indicate a low concentration of sulfate in the Archaean ocean (Habicht 2002), progressive oxygenation of the atmosphere and ocean (Canfield and Teske 1996; Canfield, Habicht and Thamdrup 2000), and influence of bioturbating organisms at Precambrian/Cambrian boundary (Canfield and Farquhar 2009).

Following improvements in analytical methods, the rare isotope ^{33}S and ^{36}S have provided additional information to the interpretation of preserved signals (Farquhar, Bao and Thiemens 2000; Farquhar *et al.* 2003; Ono *et al.* 2006). Microbial sulfate reduction, sulfide oxidation, and sulfur disproportionation all produce characteristic relationships between ^{33}S–^{32}S and ^{34}S–^{32}S ratios of their respective products and reactants (Johnston *et al.* 2005; Farquhar, Johnston and Wing 2007; Zerkle *et al.* 2009). As a result, multiple S isotopes can be used to differentiate among a variety of biogeochemical S pathways.

These relationships have been used to demonstrate a substantial contribution of MSD to global sulfur cycle 1.3 billion years ago (Wu *et al.* 2010). Combined with reaction–diffusion modeling these signals can also provide quantitative constraints on the relative influence of reduction and reoxidation in geologically contemporary environments (Zerkle *et al.* 2010; Pellerin *et al.* 2015).

The goal of this study was to apply a foundation of S isotope fractionation measurements associated with culturing to an environmental system, which can be more finely characterized through genomic technology than was possible at the time much of the work cited above was conducted. While improvements in application of mass spectrometry facilitated greater understanding of sulfur disproportionation, we aim to probe the multiple sulfur isotope signal further, employing improvements in sequencing technology to address the question: What is the role of a microbial *community*, in a *natural setting*, on the observed fractionation of sulfur isotopes? Specifically are changes in community diversity correlated with changes in net fractionation? To this end, we collected geochemical data from the sediments of a polar hypersaline spring, along a transect extending from the spring's outlet to 20 m downstream. This spring was chosen to explore this relationship for two reasons: 1) its high concentration of sulfate allows us to ignore Rayleigh fractionation effects (i.e. source sulfate has of constant $\delta^{34}S$ value); 2) previous reports of microbial taxonomy indicated low diversity (Perreault *et al.* 2008), simplifying observing changes in community structure. From the same sediments we measured in situ rates of sulfate reduction and analyzed multiple isotopes of sulfur and genomic diversity of sulfate reducing microbes. A standard approach for determining microbial diversity and phylogenetic dissimilarities between communities employs the 16S rRNA gene, though other reports have proposed this gene is less useful to infer functional relationships among closely related taxa (Lan and Reeves, 2000; Rocap et al., 2003). We focus on a highly conserved gene, ubiquitous in SRM: dissimilatory sulfite reductase (*dsrB*), which catalyzes the reduction of sulfite to sulfide (Wagner et al., 1998; De Wit and Bouvier, 2006). In addition to its utility in phylogeny, amplification and annotation of this gene from environmental communities has been optimized (Pelikan *et al.* 2016). Finally, *dsrB* has been proposed to act as a isotopic bottleneck in sulfate reduction, suggesting changes in this gene might disproportionately influence

fractionation (Harrison and Thode 1958; Brunner and Bernasconi 2005). Our findings were analyzed in the context of empirical data and a previously described mechanistic model of microbial sulfur fractionation.

Methods

Site description

Samples were collected from a spring in the proximity of the McGill Arctic Research Station on Axel Heiberg Island, Nunavut. The geologic context of this site has been thoroughly described (Pollard *et al.* 2009). Gypsum Hill springs emerge from the southern base of Gypsum Hill (GH) via tens of outlets that flow across ≈30 m of permafrost, in shallow (2 to 5 cm water depth), dispersive channels into Expedition River (Fig. 1). The source of the springs' water is uncertain, however prior to surface exposure, the water ascends through > 400 m of Carboniferous anhydrite evaporite diapir permafrost (Jackson and Harrison 2006). The water's major ions derive from dissolution of minerals as the water travels through a subterranean salt diapir (Andersen 2002). The geochemistry of the spring from which sediment samples were collected for this work has been measured over more than a decade and is relatively constant. The outlet of the largest spring, and the one investigated for this report (GH4), discharges at a rate of ≈1 L s^{-1}, forming a 1 m deep, 2 m wide, circular pool with roiling sediments as a result of water and gas discharge. Emerging spring water is perennially constant at 3-6°C, pH 7.1, 80‰ salinity, and is anoxic and reducing (Eh −285 mV). Spring water exiting the outlet pool is progressively oxygenated as it flows in channels over the permafrost. These channels contain mixed sediments (fine to coarse sand and pebbles) that are variably coated with a veneer of travertine precipitate (Omelon, Pollard and Andersen 2006). Major spring water ions include chloride, sodium, sulfate, and calcium (Perreault *et al.* 2007). Biological culturing and molecular characterization has identified Bacterial and Archaeal genes associated with aerobic and anaerobic heterotrophic and autotrophic metabolisms, including sulfur and sulfate reducing bacteria, methanogens, sulfur oxidizing bacteria and methanotrophs (Perreault *et al.* 2007, 2008; Niederberger *et al.* 2009b). Notably, GH4 intermittently hosts a sulfur oxidizing streamer community, primarily composed of *Thiomicrospira* (Niederberger *et al.* 2009a). Samples and measurements for this study were taken from the spring outlet (0 m) and from channel stations at 1, 4, 8, 12, and 18 m downstream (Channel stations 1-5).

Sediment and pore water measurements

Pore water measurements relevant to sulfur metabolism were taken at 3 cm sediment depth from each sampling station and included temperature, pH, oxidation-reduction potential (ORP), and concentration of dissolved oxygen and sulfide. Concentrations of nitrate, nitrite, ammonia, thiosulfate, ferric iron, phosphate, and total carbon and nitrogen were assessed from the outlet and a single channel station. pH and ORP were measured with a YSI 556 Multi Probe System (YSI Incorporated, Yellow Springs, OH, USA). Temperature was measured with a handheld thermometer. Dissolved oxygen was measured at 3 cm below sediment surface using a Picollo2 fiber-optic oxygen meter (Pyroscience, Aachen, Germany). Sediment pore water was collected into 15 mL syringes through microfiltration (0.12-0.18 μm) membrane tubing (Rhizosphere, Wageningen, NL) inserted 5 cm into the sediment, and assessed for dissolved ions immediately and without exposure to atmosphere upon recovery using CHEMetrics (Midland, Virginia) kits (K6923, K7003, K1503, K9523, K9203, K9705, K6210, K8513) and portable spectrometer (V-2000). Pore water samples for dissolved organic carbon measurement were filtered through GF/F filters, acidified with 12N HCl and transported in pre-combusted glass vials. Sediments for solid phase carbon and nitrogen were collected with a sterile spatula into 7 ml PE tubes. Following acid conversion of inorganic carbonates to carbon dioxide, total organic carbon and nitrogen were measured on an Elemental Analyzer (NC 2500, CE Instruments, Wigan, UK) at the GEOTOP stable isotope laboratory at UQAM (Montreal, QC). Dissolved organic carbon was measured on a Total Organic Carbon Analyzer (TOC-V CHS; Shimadzu, Tokyo) at the GEOTOP Environmental Organic Geochemistry laboratory at Concordia (Montreal, QC). Sediment porosity was calculated from sediment density and mass reduction following drying at 100°C for 24 h.

Sediment collection for sulfur isotopes and DNA

Sediments were collected for a variety of analyses, in the same fashion as those collected for total carbon and nitrogen. Sediments for analysis of sulfur isotopes were treated with 20% wt/vol acidified Zinc Acetate and stored and transported frozen. Spring water samples were also collected and similarly treated, and additional water samples

were treated with $BaCl_2$ for precipitation of aqueous sulfate. Sediments for DNA extraction were treated with RNALifeguard (MoBio Laboratories, Inc., Carlsbad, CA) and stored and transported at −5°C.

In situ sulfate reduction rate

Dissimilatory sulfate reduction rates were determined using radiotracer $^{35}SO_4$, following Røy et al. (2014). From GH4 outlet and channel stations six 5-cm replicate sediment cores were taken using cut-tip 30 ml PE syringes, the barrel of which was manually pressed into the sediments while the plunger was held in constant position at the water-sediment interface. Immediately following collection, each core was capped with a butyl stopper, wrapped in electrical tape, and 120 µl of $^{35}SO_4^{2-}$ (1480 kBq, in carrier solution of similar NaCl and SO_4^{2-} concentrations to the pore water) was injected along the central vertical axis of each core. All cores were incubated in situ (syringe cores were returned to the holes from which they were derived). Expecting lower SRR than are typically found in coastal sediments (20 nm SO_4^{2-} cm^{-3} d^{-1}, when $[SO_4^{2-}]$ is 20 µmol cm^{-3}), higher amounts of tracer and longer incubations were employed than those typical for coastal surface sediments (Røy *et al.* 2014). Microbial reduction was terminated at time points 0, 24 and 72 hours from each station by expelling the sediment core material into 35 mL 20% wt/wt Zinc Acetate (ZnAce), precipitating $H_2{}^{35}S$ as $Zn^{35}S$. Samples were stored and transported frozen. Subsamples of the ZnAce from fixed samples were taken to determine ^{35}S in sulfate. Reduced sulfur and unreacted sulfate were separated by hot acidic (12N HCl) distillation in the presence of 1M Cr^{2+} ($CrCl_3(OH_2)_6$). Radioactive sulfide was captured into ZnAce, the volume was reduced by centrifugation and 5 mL of ZnAce and the precipitated ZnS was added to a 20 mL scintillation vial with 15 mL of scintillation cocktail (Ecoscint XR, National Diagnostics). Radioactivity of all samples was quantified on a multipurpose liquid scintillation counter (LS6500; Beckman Coulter); the fraction (F) of sulfate reduced was determined by the ratio of measured total activity to measured activity of total reducible inorganic sulfur (TRIS), as $F = A_{TRIS} / A_{SO42-} + A_{TRIS}$ and sulfate reduction rate (SRR) was calculates as $SRR = F \cdot [SO_4^{2-}] \cdot 1.06 \cdot \phi / t$, where $[SO_4^{2-}]$ is the sulfate concentration in the pore water, ϕ is porosity and

t is incubation time. The factor 1.06 is an estimate isotope fractionation between ^{32}S and ^{35}S during bacterial sulfate reduction (Røy *et al.* 2014).

Sulfur isotope composition of spring water and sediments

Mineralized forms of various oxidation states of sulfur were serially extracted for isotopic analysis. Sediments were first extracted (overnight shaker, 200 rpm) with Milli-Q water, centrifuged and decanted. Soluble sulfate was precipitated from the supernatant with $BaCl_2$. Sulfate was reduced to sulfide by reacting 10 mg of $BaSO_4$ with 15 mL of Thode solution (32:15:53 HI:H_3PO_2:HCl) at 100°C under a stream of pure N_2 for 90 min (Thode, Monster and Dunford 1961). N_2 carried generated H_2S through a zinc acetate solution to quantitatively precipitate H_2S as ZnS. Five drops of silver nitrate (0.1 N) were added to the zinc acetate trap to convert ZnS to Ag_2S. This reaction was carried out overnight in the dark. Ag_2S was separated from the solution by filtration on a 0.2 μm nitrocellulose filter, rinsed with 2 mL of ammonium hydroxide and three times with 15 mL Milli-Q water, scraped from the filter, and dried for 24 h at 50°C. The same distillation apparatus and conversion to Ag_2S was employed in each serial extraction of a given sample. Water-extracted sediments were then extracted (overnight shaker, 200 rpm) with 100% MeOH to extract elemental sulfur (S^0). MeOH was decanted and evaporated over 12 hours. Residual S^0 was converted to H_2S by hot HCl (12N) distillation in the presence of 1M Cr^{2+}. MeOH-extracted sediments were washed 2x in Milli-Q water. Acid volatile sulfides were extracted with hot HCl and precipitated as above. Following collection of trapped ZnS from the acid distillation, and without removal from the distillation apparatus, sediments were extracted for chromium reducible sulfides as above. Both decanted supernatant from this acidic chromium extraction, as well as residual (Milli-Q washed) sediments were separately extracted with Thode solution in the same manner as the soluble sulfate $BaSO_4$ precipitated above. The Ag_2S from all samples was reacted in the presence of excess fluorine gas for 12 h in a Ni reaction vessel heated to 250°C. The SF_6 generated by the reaction was first purified by removing non-condensable by-products of the reaction by cryo-separation at −120°C. A second purification was carried out by passing the SF_6 through a GC column (~2 m Haysep Q and ~2 m Molsieve 5A) with ultrapure He as the carrier gas at a rate of 20 mL

min^{-1}. The SF_6 peak was isolated from residual contaminants and the carrier gas by trapping the SF_6 on a cold finger at −192°C as the carrier gas was pumped out. The isotopic composition of the purified SF_6 was then determined on a ThermoElectron MAT 253 dual inlet isotope ratio mass spectrometer in the Stable Isotope Laboratory of the Earth and Planetary Sciences Department at McGill University.

***Isotope* notation**

Isotopic compositions are reported using the delta notation $\delta^{3i}S = ({}^{3i}R_{sample}/{}^{3i}R_{V\text{-}CDT} - 1) \cdot 1000$; where ${}^{3i}R = {}^{3i}S/{}^{32}S$, *i* is 3 or 4 and V-CDT refers to the Vienna-Canon Diablo Troilite international reference scale. On the V-CDT scale, the $\delta^{34}S$ value of the Ag_2S reference material, IAEA-S-1, is defined as −0.3‰ (Ding *et al.* 2001). The uncertainty on the measured $\delta^{34}S$ values is less than ±0.2‰. The capital delta notation is used to report deviations among the fractionation relationships of ${}^{33}S–{}^{32}S$ and ${}^{34}S–{}^{32}S$ ratios: $\Delta^{33}S = \delta^{33}S - 1000 \cdot ((1 + \delta^{34}S/1000)^{0.515} - 1)$ (Hulston and Thode 1965; Farquhar, Bao and Thiemens 2000). We take the $\Delta^{33}S$ value of IAEA-S-1 to be 0.094‰ V-CDT. The uncertainty on the measured $\Delta^{33}S$ values is less than ±0.01‰. Fractionation factors (${}^{3i}\alpha$) between reactant (r) and product (p) are given by ${}^{3i}\alpha = {}^{3i}R_p/{}^{3i}R_r$; and ${}^{3i}R = 1 + \delta^{3i}S/1000$. These are expressed as isotopic enrichment factors in ${}^{3i}\varepsilon$ notation, where ${}^{3i}\varepsilon$ (‰) = $({}^{3i}\alpha - 1) \cdot 1000$. The fractionation factors for the heavy isotopologues are related by: ${}^{33}\lambda = \ln {}^{33}\alpha / \ln {}^{34}\alpha$; where ${}^{33}\alpha$ is the fractionation factor for ${}^{33}S–{}^{32}S$ ratios and ${}^{34}\alpha$ is the fractionation factor for ${}^{34}S–{}^{32}S$ ratios.

DNA extraction, amplification and sequencing

DNA was extracted from 0.5 g sediments from each sampling station in Tris-HCl, EDTA, sodium phosphate, NaCl and cetyltrimethylammonium bromide on a shaker at 37°C for 30 min. These slurries were incubated with SDS at 65°C for 60 min and mixed by inversion. Cells were dislodged by bead beating, slurries were centrifuged (6,000 x g, 10 min, 25°C) and supernatant was decanted. This solution was mixed with equal volume PCI, mixed by inversion and centrifuged (16000 x g, 10 min, 25°C). DNA was precipitated from the aqueous phase with isopropanol overnight at 4°C. DNA was pelleted (16,000 x g, 30 min, 4°C), washed with 70% ethanol, pelleted again, air dried

and resuspended in 50 µL of 10 mM Tries buffer (pH 8.0). DNA was initially amplified using target-head for 16S rRNA genes and *dsrB* genes and then barcoded with unique barcode-head primers for unambiguous identification of samples according to a recently established workflow (Herbold *et al.* 2015). 16S rRNA genes were first amplified in triplicates (12.5 µL) with the modified primer pair H_ArBa519F/H_ArBa785R. The PCR mix contained 1x DreamTaq buffer (Thermo Scientific), 0.2 mM dNTP mix (Thermo Scientific), 1 U DreamTaq (Thermo Scientific), 0.2 mg ml^{-1} bovine serum albumin (Thermo Scientific) and 2 µM of each forward and reverse primer. The first PCR was performed with initial denaturation at 95°C for 3 min; 30 cycles of 30 s denaturation at 95°C, 30 s annealing at 50°C and elongation for 1 min at 72°C; and final elongation at 72°C for 10 min. *dsrB* genes were first amplified in triplicates (12.5µL) as previously described (Pelikan *et al.* 2016). However, thermal cycling was not performed in 'touch down' mode, but with annealing for 30 cylces at 50°C. Barcoding was performed with the barcode-head primers described in (Pelikan *et al.* 2016). The barcoding PCR mix (50 µL) contained for the amplification of both genes 1x DreamTaq buffer (Thermo Scientific), 0.2 mM dNTP mix (Thermo Scientific), 0.4 U DreamTaq (Thermo Scientific) and 2 µM of the barcode-head primer. Thermal cycling was performed with initial denaturation at 95°C for 3 min; 30 cycles of 30 s denaturation at 95°C, 16 s annealing at 50°C and elongation for 30s at 72°C; and final elongation at 72°C for 2 min. Negative controls of the whole barcoding procedure were performed with ddH_2O and amplicons were included in all further processing steps. Amplicons were purified with AMPure XP purification kit (Beckman Coulter Genomics) and quantified with Quant-iT™ PicoGreen® dsDNA Assay (invitrogen). The libraries were then pooled and sequenced on a MiSeq system (Illulmina) as previously described (Herbold *et al.* 2015). Sequenced datasets will be available in the NCBI Sequence Read Archive following publication.

Bioinformatics analyses

16S rRNA and *dsrB* raw sequencing reads were demultiplexed and quality filtered as previously described in Herbold *et al.*, 2015 and Pelikan *et al.*, 2016, respectively. OTU clustering of 16S rRNA amplicons was performed in USEARCH (Edgar 2013) in a two step process with initial pre-clustering (-cluster_otus) at 99 % sequence identity for

optimal chimera detection followed by OTU clustering at 97 %. These OTUs were classified using the Ribosomal Database Project naïve Bayesian classifier (Wang *et al.* 2007). OTU clustering of *dsrB* amplicons and taxonomic classification of OTUs was performed as previously described (Pelikan *et al.* 2016). OTU tables and classification tables were imported into the R software environment (R core team, 2014) and analyzed there using native functions and the sequence data processing software package phyloseq (McMurdie and Holmes 2013). First both datasets were filtered for samples with more than 100 reads. Then relative abundances were calculated and OTUs with the highest relative abundance in the negative controls were removed from the datasets. Additionally, samples with a Bray-Curtis distance of less than 0.8 to the negative control were removed from the datasets. Only samples present in 16S rRNA and *dsrB* dataset were kept for further analyses. The "clean" OTU tables were then rarefied at the smallest library size.

Results

Sediment and pore water chemistry

Physical and chemical measurements are listed in Table 1. Moving downstream from the anoxic outlet sediments, sediments were progressively more oxygenated, warmer, higher pH, and had less negative redox potential and lower concentration of dissolved sulfide.

Sulfur isotopic composition of water and sediments

The majority of sulfur from the sediments of GH4 was chromium-reducible sulfur (CRS; Fig. 2). Sulfur isotope values ($\delta^{33}S$ and $\delta^{34}S$) derived from spring water, mineral gypsum from Gypsum Hills, and sediments collected from GH4 are listed in Table 3. Soluble sulfate $\delta^{34}S$ values were 17.6‰ for GH4 spring water, 18.6‰ for gypsum, and ranged between 16.7 and 20.2‰ in GH4 sediment pore water. Elemental sulfur (S^0) is insoluble and was not recovered from spring water. S^0 $\delta^{34}S$ values ranged between −29.0 and −25.4‰ in GH4 sediments. Acid volatile sulfides (AVS) were in insufficient concentration (< 40 µg S g wet sediment^{-1}) to recover from several sediment samples (Fig. 2); $\delta^{34}S_{AVS}$ values were −29.9‰ for GH4 spring water, and ranged between −26.2 and −24.3‰ in GH4 sediments. CRS $\delta^{34}S$ values were between −27.9 and −22.30‰ in GH4 sediments. No increasing $\delta^{34}S_{sulfate}$ trend with distance from outlet was observed suggesting that reduction of spring water sulfate was inconsequential to its isotopic signature.

In situ rates of sulfate reduction

In situ sulfate reduction rates were measured from the outlet and a single channel sampling station 8 m downstream (Channel-3). Replicate one-day incubation periods yielded sulfate reduction rates (SRR) of 0.05 and 0.08 x 10^{-9} moles cm^{-3} d^{-1} from the outlet station and 0.24 and 0.27 x 10^{-9} moles cm^{-3} d^{-1} from the channel station. Replicate three-day incubations yielded SRRs of 0.12 and 0.21 x 10^{-9} moles cm^{-3} d^{-1} from the outlet station and 1.32 and 1.91 x 10^{-9} moles cm^{-3} d^{-1} from the channel station. SRRs

from replicate outlet and channel control incubations, which were fixed immediately following addition of 35S tracer, were $\leq 0.03 \times 10^{-9}$ moles cm^{-3} d^{-1}.

Relative abundance and diversity of 16S and dsrB genes

A total of 357 494 high quality 16S rRNA sequences were obtained, with an average of 44 687 sequences per sample replicate. Overall analysis of operational taxonomic units (OTUs) at the 97% similarity level showed that across all samples the sediments were moderately diverse (Chao1 139-279, Shannon 2.07-3.53; Table 2) and dominated by the classes Gammaproteobacteria (18-66% of sequences), Deltaproteobacteria (4-14%) and Clostridia (2-26%; Fig. 3A). Within the known sulfate-reducing taxa Deltaproteobacteria, the majority of sequences belonged to families Desulfuromonadaceae (23-62% of Deltaproteobacteria sequences), Desulfobulbaceae (19-42%) and Desulfobacteraceae (3-44%; Fig. 3B). Remaining Deltaproteobacteria families each made up less than 2% of the class. Desulfuromonadaceae exhibited a marked increase in abundance with distance from outlet, while Desulfobacteraceae exhibited a marked decrease along the same gradient.

A total of 140 441 high quality *dsr*B sequences were obtained, with an average of 17 555 sequences per sample. Overall analysis of OTUs at the 97% similarity level showed lower diversity compared to 16S OTUs (Chao1 14-41, Shannon 0.99-2.34; Table 2) and that sediments were dominated by Desulfobacteraceae (6-84%) and Desulfobulbaceae (9-94%; Fig. 3C). The relative abundance of these *dsr*B sequence types showed notable trends in the spring sediments, Desulfobacteraceae dominating the outlet and most upstream channel sediments (77-84%) and Desulfobulbaceae dominating downstream channel sediments (87-94%). Remaining sequences were most closely related to families that composed less than 2% of all *dsr*B sequences average across all stations. Sequences related to the Desulfobacca acetoxidans lineage were the only other *dsr*B sequences to exceed to 5% composition of any individual station (GH outlet; 8.7%). *dsrB* OTU distributions were more even for the outlet station compared to all channel stations (Fig. 3D and Table 2).

Discussion

The purpose of this study was to describe the sulfate reducing microbial community and the geochemical parameters expected to influence that community, along an Arctic spring transect exhibiting gradients in a number of its characters. We analyzed the isotopic signature of sulfur fractionation along the same transect seeking evidence of a relationship between microbial diversity and the magnitude of that signal.

Fractionation signal consistent with sulfate reduction

The fate of sulfide produced by dissimilatory reduction in the sediments of GH4 has not been evaluated. In order to constrain the fractionation associated with reduction, we take as possible fates: 1) remaining soluble in sediment pore water, subject to export, 2) reaction with metals and precipitation as metal sulfides, 3) biological or abiotic oxidation (Fig. 4). The flux (φ) to each pool is unknown, and so we plot the outlier values, assuming φ to each pool is 1 (totality), using the $\delta^{34}S$ values separately for each recovered pool of AVS, CRS and S^0 per sampling station (Fig. 5). By considering each case of φ=1, the fractionation to that pool from sulfate is equal to the fractionation between sulfate and the sulfide produced by microbial sulfate reduction. In cases where each of these pools was recoverable, this generates a triangle of boundary fraction values. In the case of sediments from each of the sampling stations of GH4, these values are consistent with those measured in cultures of microbes solely reducing sulfate (i.e. not disproportionating sulfur; Fig. 5).). These values however fall just outside the $^{33}\lambda$ range in which disproportionation can be empirically evaluated to have played a role (Pellerin *et al.* 2015).

Neither 16S nor dsrB diversity metrics correlated with environmental parameters or observed net fractionation

As a first step in evaluating the role of a community of sulfate reducers on the net fractionation of sulfur isotopes in the environment, we queried a number of measured environmental factors for correlation by linear regression with both the Chao1 and

Shannon metrics of diversity for both the 16S and *dsrB* gene (Fig. 6). Each of these factors has been demonstrated to play a role in shaping microbial communities in other environments; e.g. temperature: (Redmond and Valentine 2012), pH: (Liu *et al.* 2015), oxygen: (Meyerhof *et al.* 2016), sulfide: (Skirnisdottir *et al.* 2000). Each relationship between an environmental parameter and diversity metric produced an inverse correlation (save one: [sulfide]-Shannon). Correlations were greatest between oxygen and 16S Chao 1 diversity (R^2=0.86, $p<0.05$) and pH and 16S Shannon diversity (R^2=0.88, $p<0.05$); all other correlation R^2 values were less than 0.70 and p values were greater than 0.08. This suggests taxonomic species numbers may be most influenced by oxygen, and evenness by pH; while *dsrB* gene diversity did not correlate with any of the environmental parameters investigated. A driving hypothesis of this work was that changes in sulfate reducing community diversity (e.g. Chao1) and evenness (e.g. Shannon index) would have observable influence on net fractionation of sulfur isotopes. This hypothesis is based on the known diversity of isotope phenotypes (e.g. Detmers *et al.* 2001). These authors examined the relationship between fractionation and taxonomic phylogeny; while they found an array of isolate specific isotope phenotypes, they could not correlate them with 16S phylogeny. Assuming that in any environment the most abundant SRM taxa will also exhibit the greatest rate of respiration (and impart the smallest fractionation), increasing either diversity or evenness should increase net fractionation. No correlation between any diversity metric and net fractionation was observed (Fig. 7).

GH4 sulfur fractionation values were lower than predicted

The inverse relationship between cell specific sulfate reduction and observed fractionation of sulfur isotopes by SRM has been among the most enduring of mechanistic explanations for variation in fractionation signals (Harrison and Thode 1958; Kaplan and Rittenberg 1964; Chambers *et al.* 1975; Habicht *et al.* 2005; Hoek *et al.* 2006; Johnston, Farquhar and Canfield 2007; Sim, Bosak and Shuhei 2011; Sim, Ono and Bosak 2012; Leavitt *et al.* 2013). It has been proposed that environmental factors influence fractionation only to the extent that they influence csSRR. Notably other studies have not found the inverse relationship to be ubiquitous (Detmers *et al.* 2001; Davidson *et al.* 2009). Empirical data underlying this relationship, as well as csSRR and

sulfur fractionation data from GH4 sediments are plotted in Figure 8. Minimum csSRR for GH4 are plotted here by dividing measured [bulk] in situ sulfate reduction rate by the total number of cells in the same sample volume. Actual csSRR should be higher, as only a fraction of the microbial community is capable of sulfate reduction, and are simultaneously active. To constrain the fraction of cells falling into this category (i.e. SRM+active), we employed a mechanistic model of sulfate fractionation by SRM that relies on measured environmental parameters, equilibrium and kinetic fractionation factors, and the thermodynamics that determine the reversibility of each step of the reduction pathway (Wing and Halevy 2014). Each of these parameters has been empirically evaluated for at least one- and often several- SRM. Using this model and the environmental parameters measured from two GH4 sediment stations (Outlet and Channel-3); we find that csSRR predicted to generate the fractionations ($^{34}\varepsilon$) measured from GH4 to be 46X and 112X greater than measured. This is consistent with the finding that only 3.5% and 8.7% of the microbes from each station are Deltaproteobacteria- a first order proxy for the abundance of SRM, and validated by the taxonomic assignment of sequenced *dsrB* genes entirely to Deltaproteobacteria families. However even by assigning the bulk sulfate reduction to these putative fractions of SRM from each population, measured csSRR would still be 1.6X and 9.7X lower than expected to generate the observed signal. The simplest explanation to resolve this discrepancy is that only 62% and 10% of the SRM from Outlet and Channel-3 sediments, respectively, are active in situ. Heterogeneous activity within isolate cultures is well documented (Avery 2006). Though a distribution of activity within functional groups is expected in situ, the methods to evaluate this activity on such a scale are only starting to be developed (Hatzenpichler *et al.* 2014). However as is evident by the discrepancy between GH4 measured values and those predicted by the leading mechanistic model for sulfur isotope fractionation by SRM, this influence of heterogeneous activity on apparent csSRR has substantial implications for developing predictive models. To better address the relationship between csSRR and fractionation, a more accurate and standardized method of assessing the fraction of active cells in isolate culture and environmental populations is necessary.

Enzymatic kinetic explanations for model-data discrepancy, and the utility of dsrB

In addition to the community activity that underlies csSRR, microbial physiological parameters influencing fractionation we also unmeasured. As an additional test of our assertion that heterogeneous activity was responsible for the differences between measured and modeled fractionation values, we manipulated the enzymatic kinetic parameters V_{max} (the enzyme's maximum rate) and K_m (the enzyme's half saturation constant) associated with each enzyme, its substrates and products, in the sulfate reduction pathway (Fig. 8). These manipulations treat the microbial community diversity of each enzyme type as a single enzyme, but give an idea of the deviation for each parameter from those utilized in the model (based on empirical values) that would be required to yield the fractionations values measured in GH4. Of 19 parameters, 16 required a 1000X or greater manipulation from the empirical values employed in the model in order to generate the measured fractionation values. Notably all three parameters requiring a smaller degree of manipulation (10-100X) we associated with the apr enzyme, which is responsible for the reduction of activated sulfate to sulfite. Two conclusions can be drawn from this exercise. First given the known diversity of activity within cultured isolates, and the expectation of similar and greater diversity of activity within a functional class of enzyme, it is more likely that heterogeneous activity is responsible for the difference between measured and modeled fractionations than enzymatic parameters at three-four orders of magnitude the measured rates. Second, variations in parameters associated with the dsr enzyme complex are more resilient to influencing net fractionation through the sulfate reduction pathway than are comparable variations associated with the apr enzyme. Drawing a parallel between sequence diversity and enzymatic kinetic diversity, we expect that only very large changes in kinetic diversity of dsr would impart observable changes in fractionation. Thus, observed diversity in *dsrB* may be neutrally selected and another iteration of testing the sulfate reduction pathway diversity-fractionation hypothesis might employ *apr* rather than *dsrB*.

Conclusion

The findings of this study did not support our hypothesis that increased diversity, either in Deltaproteobacterial 16S, or sulfate reduction pathway gene *dsrB* would correlate with greater measured fractionation. This work does allude to the importance of ascertaining an accurate measurement of csSRR, and that a transcriptomics or proteomics approach might be more suitable methods to evaluate this activity from environmental samples. Further, employing a recently developed mechanistic model of sulfur fractionation during sulfate reduction, we find that *apr* is a more suitable gene to examine than *dsrB* in looking for genomic-fractionation correlation. Future work might take advantage of the many environmental studies that have already characterized metagenomic or proteomic diversity and couple those datasets to newly generated multiple sulfur isotope datasets from the same environments.

Acknowledgements

This research was supported by the Canadian Astrobiology Training Program (NSERC CREATE *371308-09*; BAW, LGW) through a PhD fellowship to JCL, the Polar and Continental Shelf Program (PCSP; LGW) through logistical support in the field, and NSERC Discovery grants to BAW (RGPIN-2014-06626) and LGW (RGPNS 305490-2012). We acknowledge advising by Hans Røy regarding ^{35}S radiotracer experimental set up and sulfate extraction.

Table 1. Gypsum Hill Spring chemistry measurements.

sampling station	distance from outlet (m)	temperature (°C)	pH	redox. (mV)	dissolved oxygen (µM)	sulfide (µM)
Outlet	0	7.1	7.63	-287.0	0.07	127.8
Channel 1	1.0	7.4	7.88	na	0.00	35
Channel 2	4.0	8.2	8.70	na	0.30	na
Channel 3	8.0	8.5	8.74	na	0.06	*bdl*
Channel 4	12.0	9.6	8.65	-230.0	0.24	58.7
Channel 5	18.0	8.7	9.16	na	0.20	*bdl*

na data not available.
bdl below detection limit.
For Chemetrics sulfide kit K9523, detection limits are 0.00 to 6.00 ppm (0.00 to 187.5 µM).

Table 2. Sediment 16S and *dsrB* diversity metrics. Fractionation ($\varepsilon^{34}_{sulfate-sulfide}$) values listed for reference.

Sampling station	Gene	Homologous coverage	Observed OTU	Chao1	Shannon diversity	Library size	$\varepsilon^{34}_{sulfate-sulfide}$
Outlet	16S	0.99	178	198	3.48	11378	-44.33
Channel 1	16S	0.98	225	275	3.51	10331	-38.10
Channel 3	16S	0.98	183	231	2.17	24679	-44.36
Channel 4	16S	0.99	119	139	2.73	3373	-40.04
Channel 5	16S	0.99	146	183	2.28	136363	-45.44
Outlet	*dsrB*	0.99	26	28	2.34	11378	-44.33
Channel 1	*dsrB*	0.98	32	38	1.31	10331	-38.10
Channel 3	*dsrB*	0.98	28	34	1.32	24679	-44.36
Channel 4	*dsrB*	0.99	13	14	0.99	3373	-40.04
Channel 5	*dsrB*	0.99	26	30	1.28	136363	-45.44

Table 3. Sulfur isotope values ($\delta^{33}S$ and $\delta^{34}S$) derived from spring water, mineral gypsum from Gypsum Hills, and sediments collected from GH4.

spring	station	sample	extraction fraction	extraction reagent	$\delta^{33}S$	$\delta^{34}S$	$\Delta^{33}S$
GH	na	gypsum	$BaSO_4$.rep1	THODE	9.57	18.58	0.04
GH	na	gypsum	$BaSO_4$.rep2	THODE	9.99	19.44	0.03
GH	Outlet	spr-water	water (ZnAce precip)	AVS	-15.38	-29.91	0.13
GH	Outlet	spr-water	water (CdAce precip)	AVS	-14.96	-29.37	0.27
GH	Outlet	spr-water	water (BaCl precip)	THODE	9.06	17.61	0.03
GH	Outlet	sed	SO_4-sol (aq.)	THODE	9.70	18.86	0.03
GH	Outlet	sed	S^0 (precip)	CRS	-13.27	-25.82	0.11
GH	Outlet	sed	dry sed	AVS	-12.46	-24.26	0.11
GH	Outlet	sed	dry sed (AVS extracted)	CRS	-13.48	-26.28	0.14
GH	Outlet	sed	CRS-sn	THODE	na	na	na
GH	Outlet	sed	SO_4-insol (solid)	THODE	na	na	na
GH	Channel1	sed	SO_4-sol (aq.)	THODE	8.60	16.74	0.01
GH	Channel1	sed	S^0 (precip)	CRS	-13.92	-27.10	0.13
GH	Channel1	sed	dry sed	AVS	na	na	na
GH	Channel1	sed	dry sed (AVS extracted)	CRS	-11.31	-22.03	0.10
GH	Channel1	sed	CRS-sn	THODE	na	na	na
GH	Channel1	sed	SO_4-insol (solid)	THODE	na	na	na
GH	Channel2	sed	SO_4-sol (aq.)	THODE	8.59	16.70	0.02
GH	Channel2	sed	S^0 (precip)	CRS	-14.66	-28.56	0.15
GH	Channel2	sed	dry sed	AVS	na	na	na
GH	Channel2	sed	dry sed (AVS extracted)	CRS	-12.41	-24.15	0.10
GH	Channel2	sed	CRS-sn	THODE	na	na	na
GH	Channel2	sed	SO_4-insol (solid)	THODE	na	na	na
GH	Channel3	sed	SO_4-sol (aq.)	THODE	8.86	17.22	0.03
GH	Channel3	sed	S^0 (precip)	CRS	-13.09	-25.45	0.10
GH	Channel3	sed	dry sed	AVS	na	na	na
GH	Channel3	sed	dry sed (AVS extracted)	CRS	-14.31	-27.85	0.13
GH	Channel3	sed	CRS-sn	THODE	na	na	na
GH	Channel3	sed	SO_4-insol (solid)	THODE	na	na	na
GH	Channel4	sed	SO_4-sol (aq.)	THODE	9.12	17.75	0.02
GH	Channel4	sed	S^0 (precip)	CRS	-13.57	-26.47	0.15
GH	Channel4	sed	dry sed	AVS	na	na	na
GH	Channel4	sed	dry sed (AVS extracted)	CRS	-11.83	-23.02	0.09
GH	Channel4	sed	CRS-sn	THODE	9.83	19.13	0.02
GH	Channel4	sed	SO_4-insol (solid)	THODE	na	na	na
GH	Channel5	sed	SO_4-sol (aq.)	THODE	10.34	20.16	0.01
GH	Channel5	sed	S^0 (precip)	CRS	-14.90	-28.97	0.13
GH	Channel5	sed	dry sed	AVS	-13.46	-26.23	0.13
GH	Channel5	sed	dry sed (AVS extracted)	CRS	-13.47	-26.24	0.13
GH	Channel5	sed	CRS-sn	THODE	7.74	15.03	0.03
GH	Channel5	sed	SO_4-insol (solid)	THODE	-14.60	-28.47	0.16

Figure 1. Gypsum Hill Spring sampling stations. Sediment collection stations indicated by asterisks (white: outlet; yellow: channel). Outlet pool is ~2 m in diameter. Distance from outlet pool to spring terminus (Expedition River) is ~ 30 m.

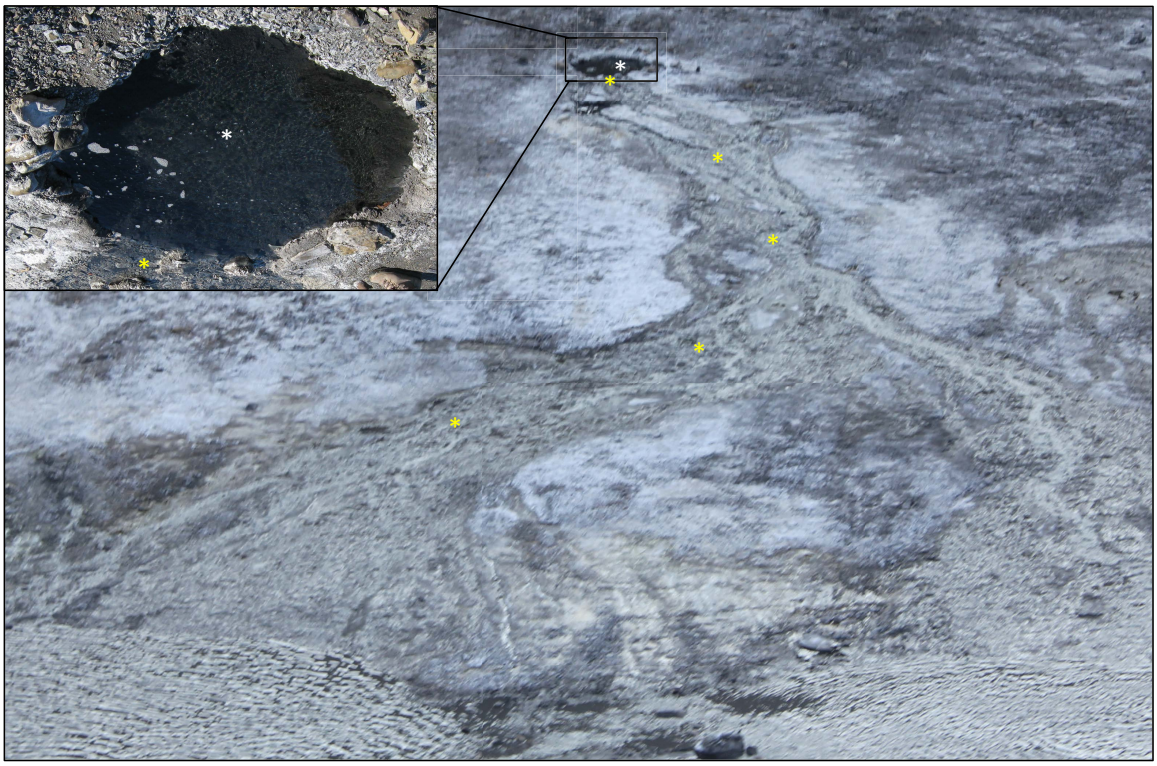


Figure 2. Mineralized sulfur fractions from Gypsum Hill sediments. Y-axis extraction fractions include Milli-Q water extraction of aqueous sulfates (SO4-sol), MeOH extraction of elemental sulfur (S0), hydrochloric acid extraction of acid volatile sulfides (AVS), acidic chromium extraction of chromium reducible sulfur (CRS), Thode solution extraction of CRS-soluble sulfur (CRS-sn), and Thode solution extraction of CRS-insoluble sulfates (SO4-insol).

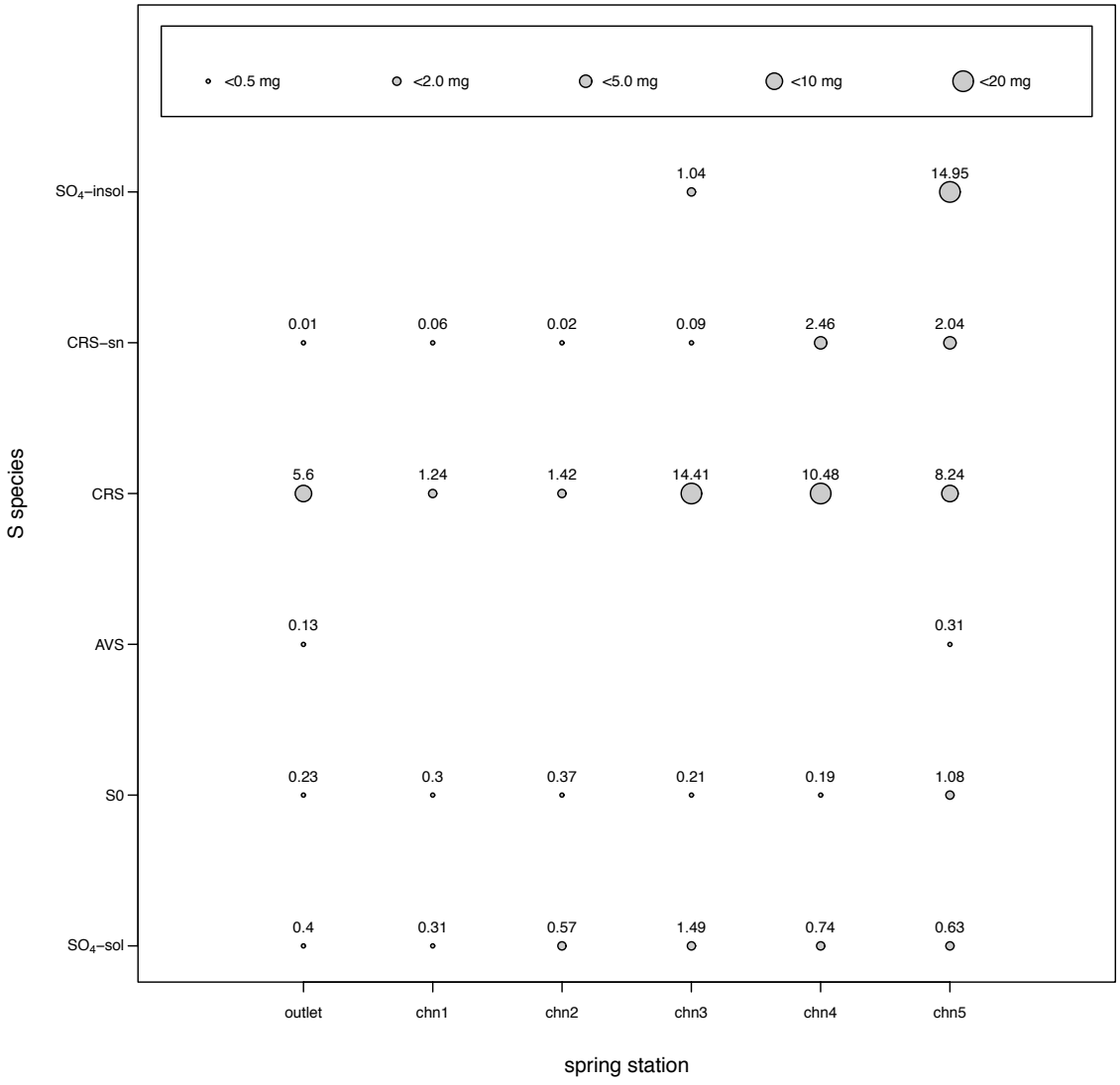
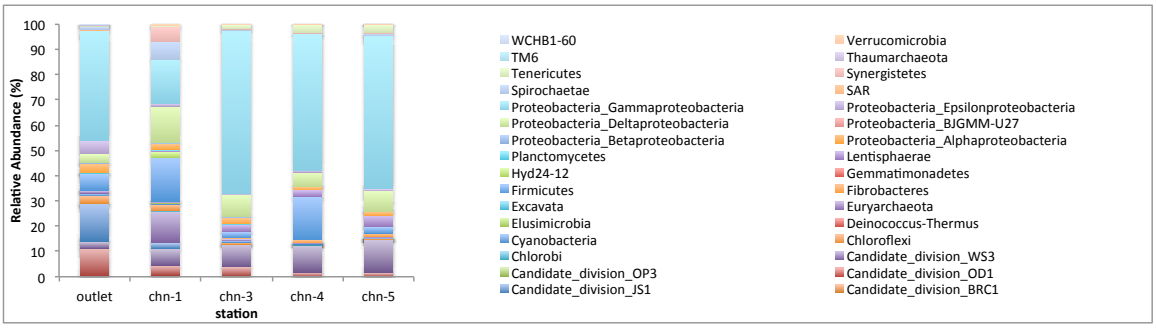
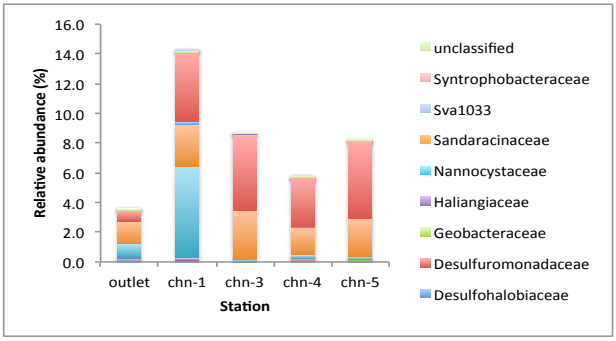


Figure 3. 16S and *dsrB* taxonomy and relative abundance. A) 16S Phyla. B) 16S Deltaproteobacteria Families. C) *dsrB* Families. D) *dsrB* OTU (taxonomy not assigned, plot only demonstrates relative OTU-level diversity distributions).

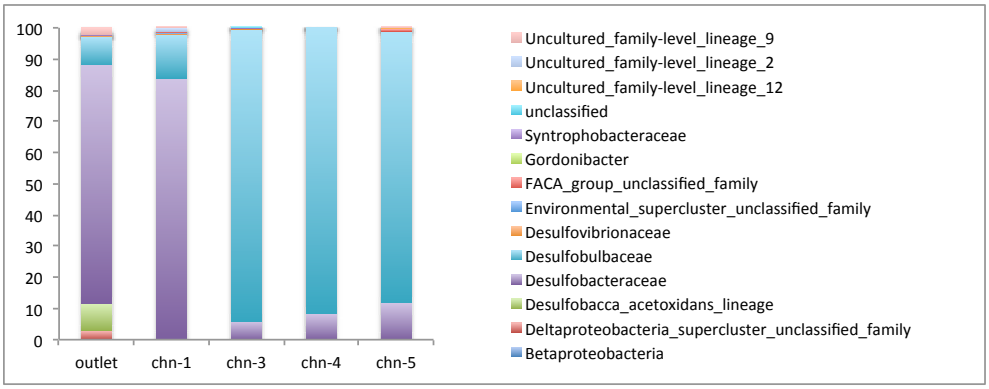
A



B



C



D

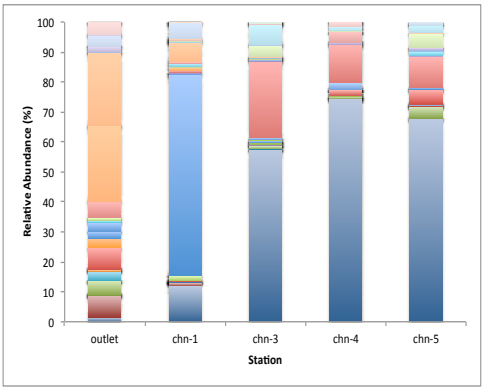
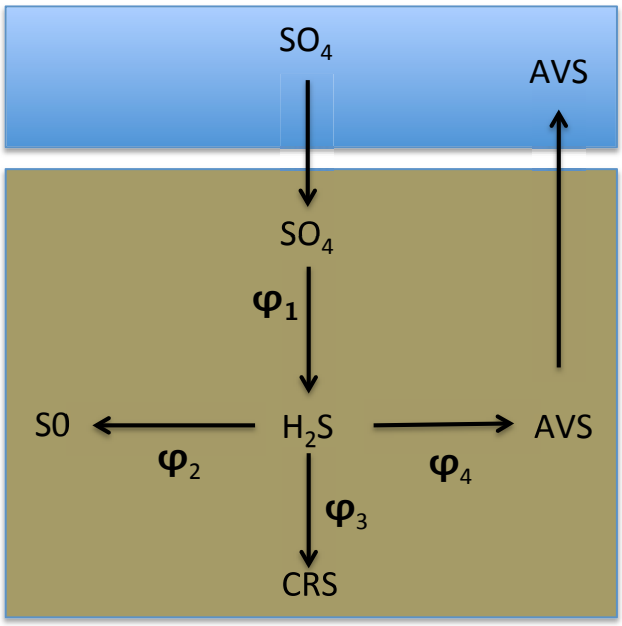


Figure 4. Possible fates of sulfide waste product following microbial sulfate reduction. Blue box represents spring water; brown box represents sediments.



$\varphi_1 = \varphi_{SO4-H2S}$

$\varphi_2 = \varphi_{H2S-S0}$

$\varphi_3 = \varphi_{H2S-CRS}$

$\varphi_4 = \varphi_{H2S-AVS}$

Figure 5. Microbial sulfate reduction boundary fractionations. Triangle apices represent $^{33}\lambda$, $^{34}\varepsilon$ fractionation values if flux of AVS to product x is complete (x is elemental sulfur, chromium reducible sulfur, or hydrogen sulfide). Background points are assembled from literature of cultured sulfate reducers and all values are $^{34}\varepsilon_{sulfate\text{-}sulfide}$.

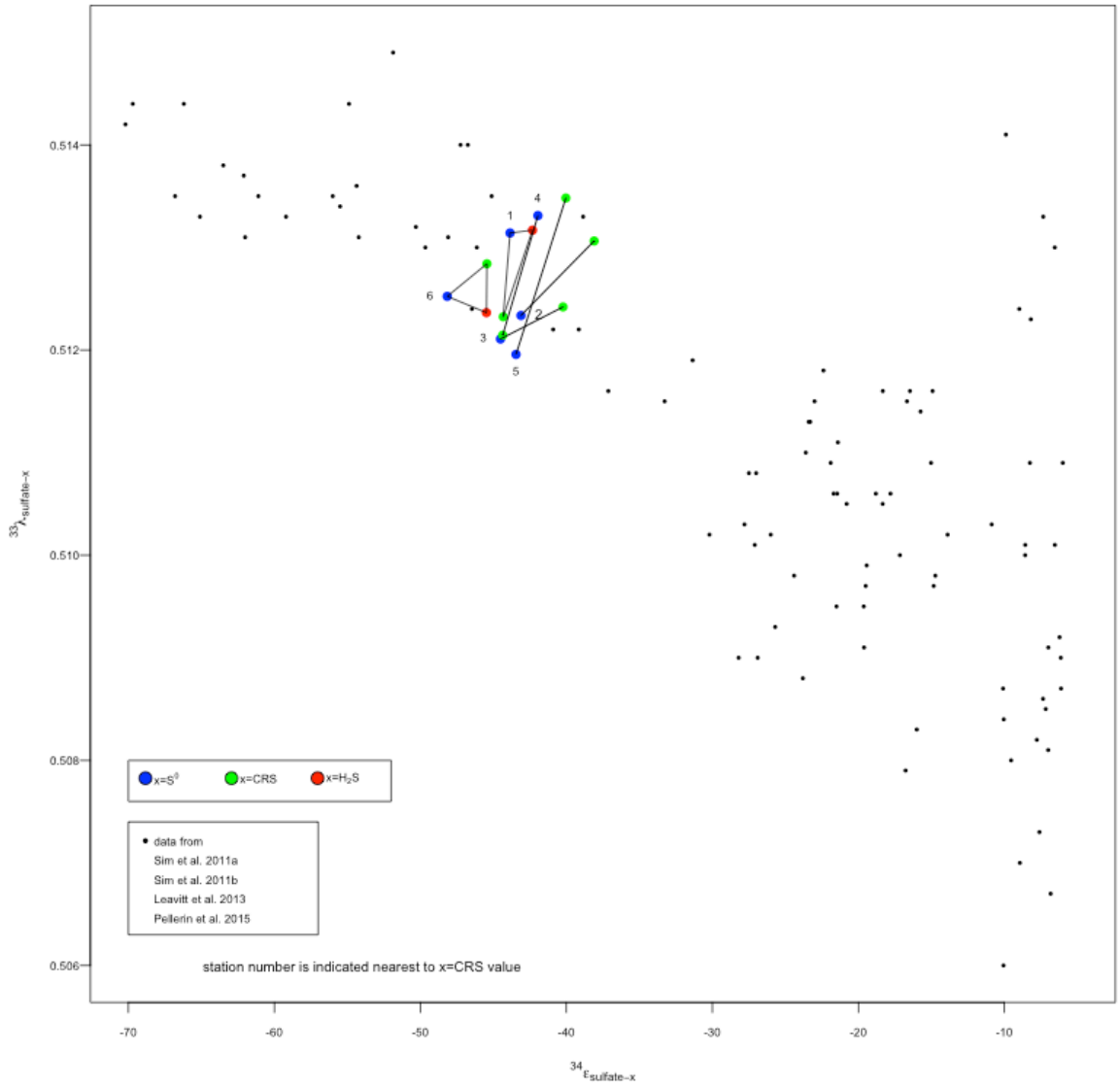


Figure 6. Environmental-diversity correlations. Chao1 (top row) and Shannon (bottom row) diversity metrics are plotted against environmental variables pH (column 1), temperature (column 2), dissolved oxygen (column 3) and dissolved sulfide (column 4). 16S Chao1 diversity (grey triangles) values correspond to primary (left-hand) y-axis of top row, *dsrB* Chao1 diversity (black circles) values correspond to secondary (right-hand) y-axis of top row. 16S and *dsrB* Shannon diversity values are plotted on the same scale.

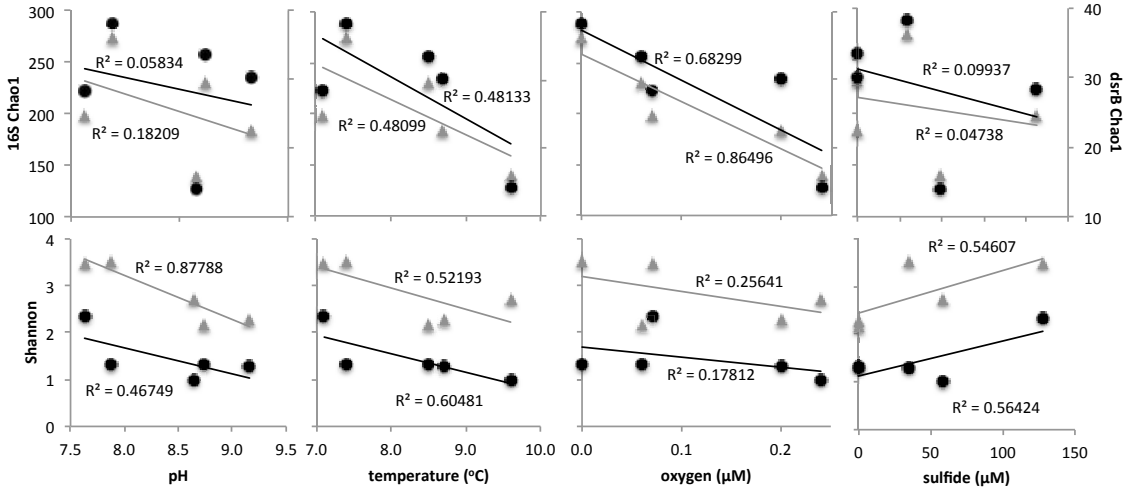


Figure 7. Diversity-fractionation correlations. Chao1 (left column) and Shannon (right column) diversity metrics are plotted against net fractionation from each GH4 sediment sampling station. 16S diversity represented with grey triangles, *dsrB* diversity represented with black circles.

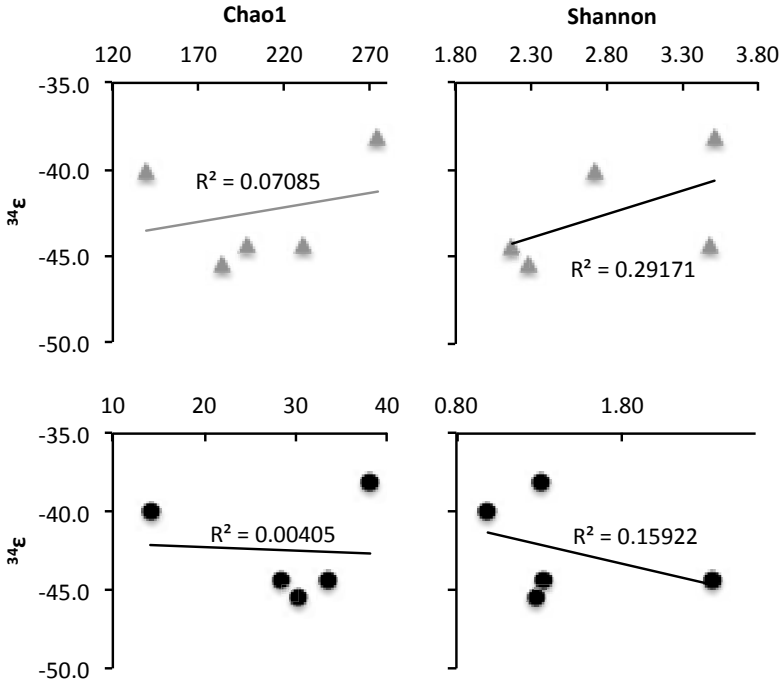


Figure 8. Sulfate fractionation ($^{34}\varepsilon_{MSR}$) associated with variable reduction rate. csSRR for GH sediments are calculated from total cell counts. Note logarithmic x-axis.

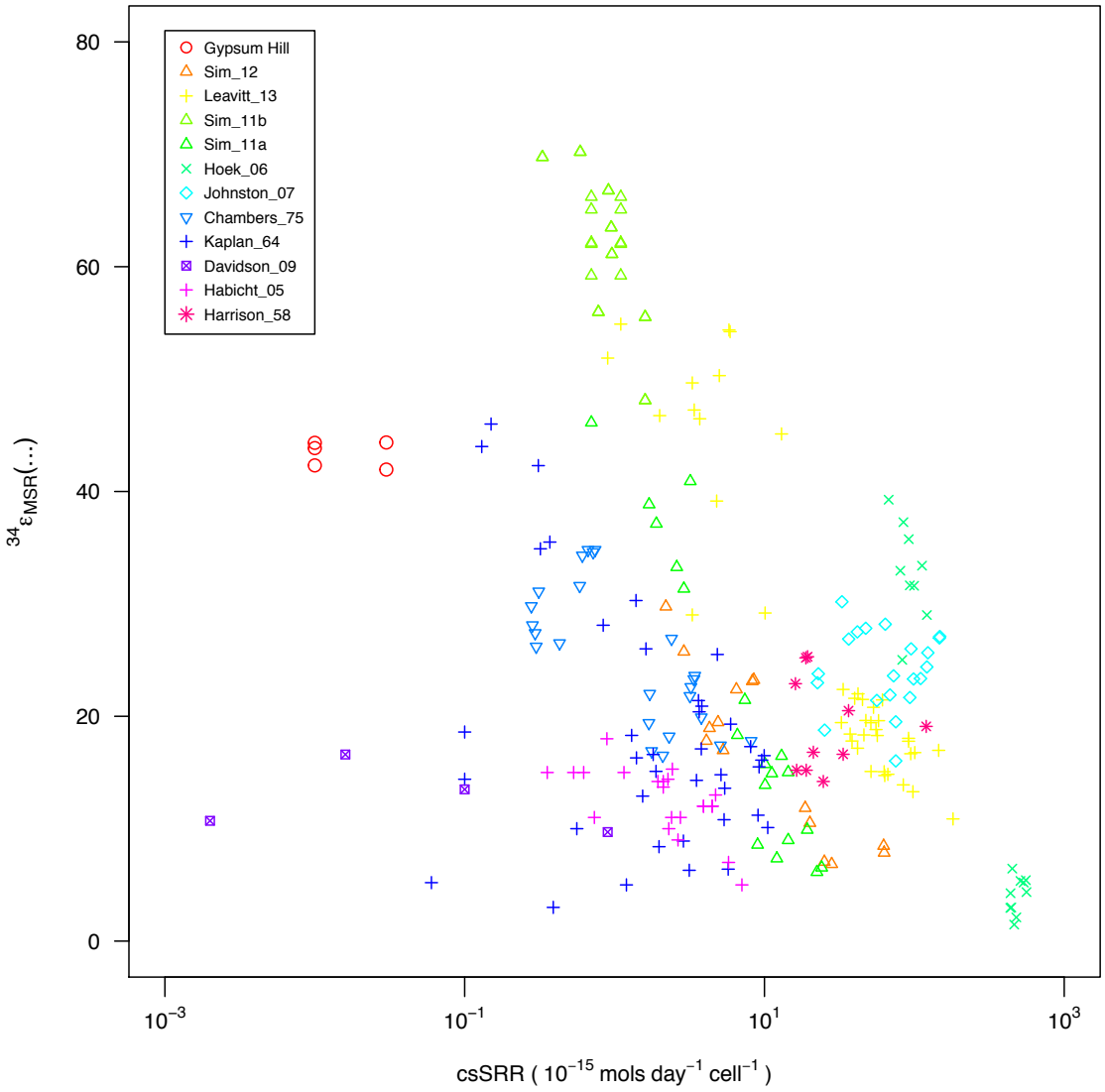
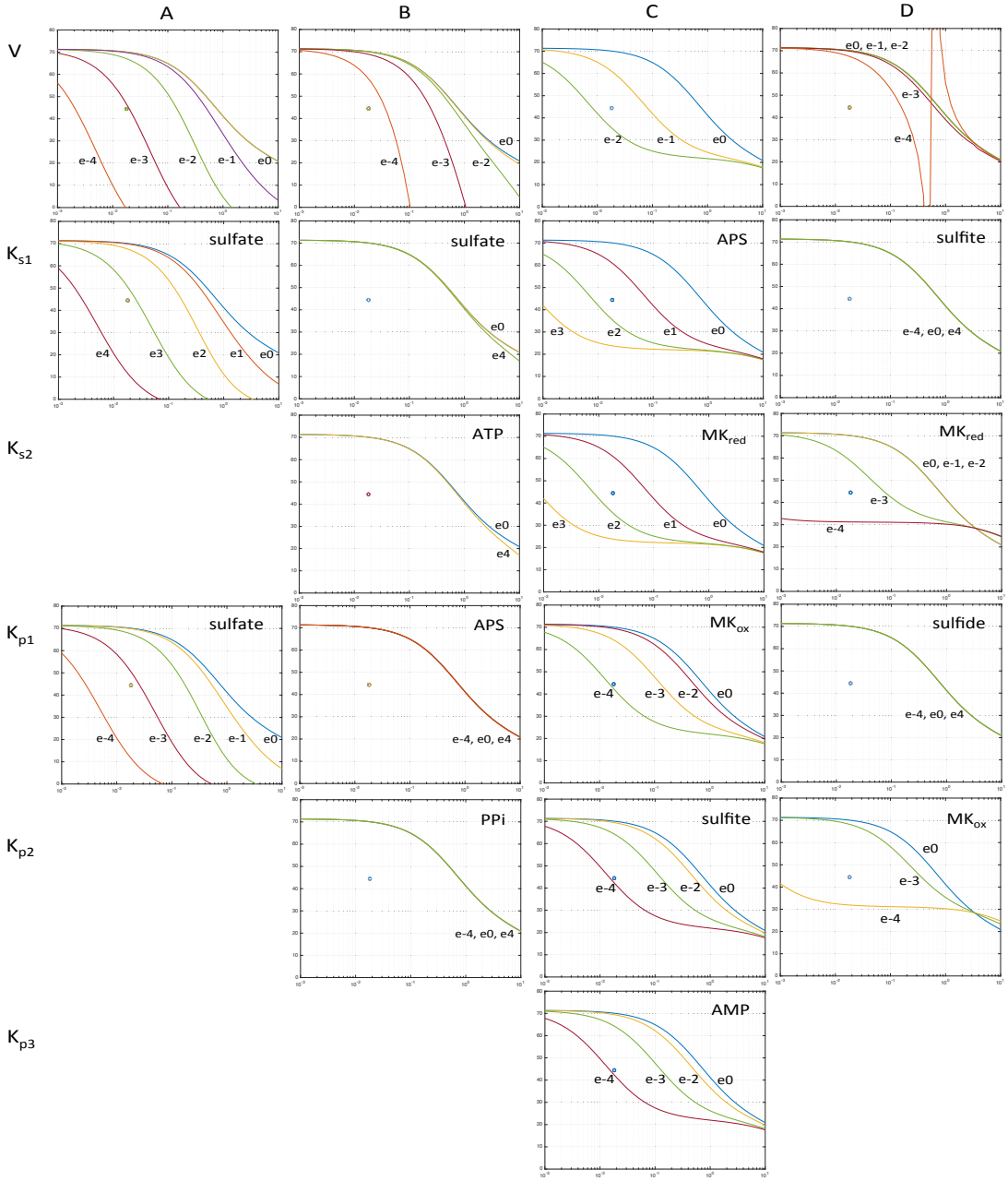


Figure 9. Influence of changing sulfate respiration pathway enzymatic kinetic parameters (V- max rate ; K- half saturation constant) on modeled csSRR-fractionation ($^{34}\varepsilon_{sulfate\text{-}sulfide}$) relationship. Values measured from GH indicated by circle. E-value labels indicate change in relationship by changing parameter value by the indicated order of magnitude. Uppercase column letters designate steps in the sulfate reduction pathway (A- sulfate uptake, B- sulfate activation, C-sulfate reduction, D-sulfite reduction), lowercase subscripts indicate substrate or product; these are given in each plot.



Conclusion

This thesis set out to address aspects of microbial ecology in an extreme environment that are of interest within the field of astrobiology. Specifically the focus was on the role of viral predation in maintaining microbial community diversity, and the influence of that same community diversity on shaping sulfur isotope biosignatures that serve as a record of microbial activity. Identifying the mechanisms influencing microbial diversity, and the preserved signatures of that diversity inform the most basic questions in astrobiology: *How did we (i.e. life on our planet) get to where we are*?, and *How might the biological factors that determined how we arrived at this place differ for life on early Earth or other planets*? Cold, salty and low oxygen environments are particularly interesting environments to explore given the frequency of these conditions in our solar system. In Paper 1 initial inventories of the microbial and viral communities of two hypersaline springs in the Canadian Arctic were made. Finding very low abundances, the first model of contact rates between viruses and microbes in sediments was constructed, and utilized to demonstrate the contact rates in the outlet of each spring to be comparable to the extremely oligotrophic marine sediments of the South Pacific Gyre. Considering these low frequency contacts, it is unlikely that viruses are able to regulate the growth of the most dominant members of the microbial community, as is found in marine surface waters. Ergo, the diversity of very low microbial biomass environments may not be subject to regulation by viral predation. In Paper 2, a complete set of microbial dynamics experiments was performed to quantitatively address the influence of viral predation on microbial mortality. Contrary to expectation shaped by the contact-rate model described in Paper 1, viruses did play a substantial role in microbial mortality, but all elements of community dynamics (microbial growth, viral production, and viral decay) were attenuated. Further, a substantial fraction of microbes were found to be lysogens- suggesting some selection for this replication strategy in these low energy environments. Paper 3 shifted from examining a driver of microbial diversity, to examining the mechanisms behind a biosignature of microbial activity, and whether that biosignature might serve to inform the biological diversity of past communities. This work did not support the hypothesis that community diversity was reflected in the multiple sulfur

isotope fractionation signal. It did indicate that accurate assessment of in situ csSRR is essential to matching model predictions to community fractionation, and that *apr* may be a more informative gene to examine with respect to sulfur fractionation than either 16S or *dsrB*. The findings of each of these papers suggest directions forward.

Future work

Viral ecology

Among the more striking findings of the viral-microbial dynamics work described in this thesis is that the causes of mortality for the majority of microbes in the sediments of both Gypsum Hill and Lost Hammer hypersaline springs, are unknown (Paper 2). In the environments characterized for such, microbial mortality is attributed nearly entirely to protist and viral predation (Azam *et al.* 1983; Fuhrman and Noble 1995). Microbial mortality attributed to viral predation in the sediments investigated here ranged from 8 to 29%. The number of protists encountered while making counts of viruses and microbes from these sediments was essentially 0 (Paper 1). Understanding the mechanisms of microbial mortality inform biogeochemical cycling, the food web, and the factors on which natural selection acts and from which evolution of microbial communities derives. Other mechanisms of mortality include resource limitation (though cultured strains thought to be representative of the pelagic environment are often oligocarbophilic and can survive extended periods of starvation; Finkel 2006; Hoehler and Jørgensen 2013), destruction by physical processes (e.g. radiation or osmotic stress), or programmed cell death (Engelberg-Kulka *et al.* 2006). Finding any of these factors to be a primary cause of community-wide microbial mortality in a natural environment would be novel and inform a broader appreciation of the role of biotic and abiotic processes on mortality between habitats.

The ability for viruses to impact microbial mortality in the low biomass sediments of both Gypsum Hill and Lost Hammer springs (Paper 2), despite low rates of contact between the two (Paper 1), is contingent on decay rates orders of magnitude lower than those encountered in the surface ocean. The mechanism of attenuated decay is virtually

unexplored. Particularly in light of a substantial reservoir of VLP apparently resistant to decay over very large time scales (Paper 2) these mechanisms are interesting not only as a question of biophysics and protein stability, but also raise the question as to what extent viruses may preserve a record of microbial activity and infection in the sedimentary record. Logical work to follow includes characterization of the bonding environment in the tertiary structure of capsid coat proteins from viruses isolated from these spring environments. Similarly to ascertaining the features on which natural selection acts with respect to microbial mortality in extreme environments, a systematic evaluation of protein coat stability may inform the features on which natural selection of viral types acts across biomes. Complementing such work, extended in situ decay experiments would shed light on the 'true' decay rates associated with the pool described as recalcitrant in Paper 2 (49-100% of the in situ VLP population). Such experiments could also serve as a sorting mechanism to address the hypothesis that the viral particles most resistant to decay had the most stable protein structure. Finally, first steps towards addressing the ability for preserved virions to serve as a proxy for microbial activity could be taken by characterizing sediment depth profiles. From each depth, profiles of microbial and viral abundances, microbial activity, and genomic characterization of microbial taxa and viral types could inform whether certain viral types are more resistant to decay, or whether the types identified are associated with the presence of their putative hosts. Findings of low microbial biomass, low microbial activity, high virus-bacteria ratios, and viral types unrelated to co-localized microbial taxa would support the hypothesis that some viral types can resist decay for geologically relevant periods, and serve as indicators of prior microbial activity.

Due to the difficulty in attributing specific outcomes to specific causal agents in environmental settings, much of what is inferred to take place in microbial ecology is based on genomic and peptide sequencing and the potential activities and interactions of the genes, RNA and proteins identified. Viral ecology research has followed the same trend, perhaps even more so than microbial ecology due to the difficulty of establishing ecologically relevant virus-host culturable isolate systems. One result of such efforts was the initial identification of photosynthesis genes in viruses of cyanobacteria (Lindell *et al.* 2004), and subsequent confirmation that the genes were expressed in host following

infection (Lindell *et al.* 2005) and capable of increasing the number of viral progeny at time of lysis (i.e. fitness; Hellweger 2009). This body of work stimulated a more extensive search for viral auxiliary metabolic genes, carried by viruses, expressed in host and capable of increasing host or viral fitness (Thompson *et al.* 2011). Continuation of this work in extreme environments such as the hypersaline springs of Axel Heiberg Island will facilitate a greater understanding of these genetic exchanges. Environmental virology is undergoing a revolution in interest, primarily as a result of accessible sequencing technology and methods developed specifically for the isolation and bioinformatic characterization of viruses. While viruses have classically been thought of primary as a pathogenic agent, or predator of microbes, their role in shaping diversity though gene exchange and expression is giving cause for reconsideration of the advantages their presence can bring to a microbial community (Rohwer and Thurber 2009). Extending the reach of such studies to extreme environments will inform the breadth of such interactions and their relationship to diverse environmental factors.

Sulfur isotope biosignatures

The sulfur isotopes preserved in the rock record are an information rich signal. However incomplete understanding of the mechanisms and diversity of fractionation result in inconclusive interpretation of that signal. Much of the mechanistic understanding of the process of fractionation resulting from sulfate reducing microbial metabolism is derived from experimental work with isolate cultures. In the natural environment, the majority of sulfate reducers are expected to persist under conditions substantially different from the exponential growth conditions typically employed in culture. As such, application of these experimental results is of limited utility in interpreting the geological isotope record. Complementary work on additional isolates, enrichment cultures and in situ incubations could fill in some of the gaps regarding species specific effects on fractionation as well as employing representative microbial communities and environmental conditions. Such work can only complement intensively focused culture work, as the environmental and biological parameters of environmental samples can never be known or controlled to the same extent.

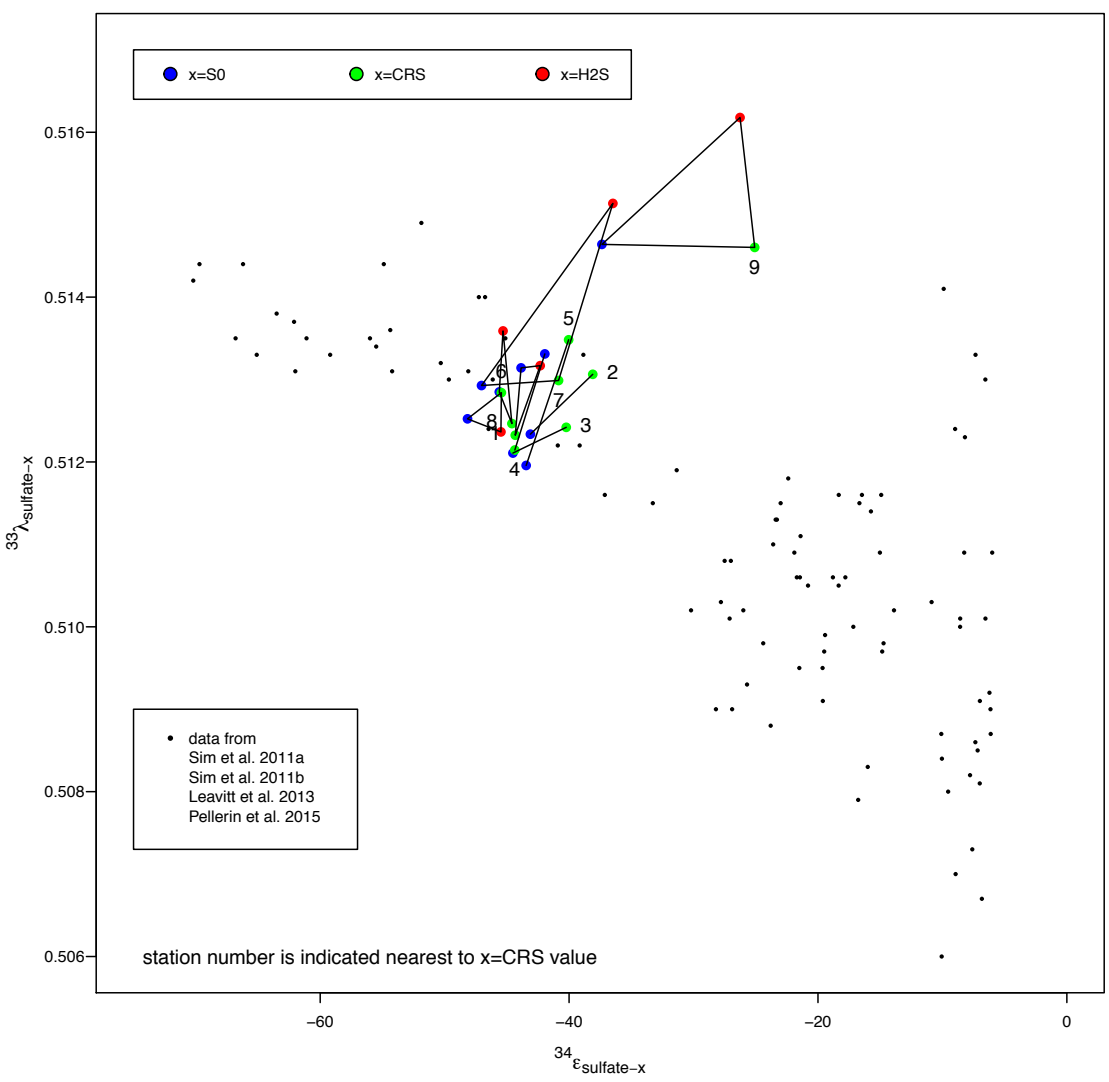
The diversity of sulfate reducing bacteria in the environment has been described for several systems (Rabus, Hansen and Widdel 2006), including the springs in this study (Paper 3). Based on *dsrB* diversity, there are at least tens of SRM types present in each of the sediments investigated in this thesis. Detmers et al. (2001) provided a first inventory of the diversity of species-specific effects on isotope fraction, by measuring fractionation by 32 different SRM under defined conditions. This work was performed before the utilization of the rare isotopes of sulfur, and before the widespread use of metagenomics. Mechanistic experiments in the past decade have focused on tightly controlled conditions and employed one of four or five model sulfate reducing isolates in culture. With the availability of methods and instruments to measure the relative abundances of rare isotopes, and parallel sequencing of the sulfate reduction pathway genes from each organism, it is time to revisit the basic question of isotope phenotype diversity within the sulfate reducing taxa. Making these measurements (under standard conditions including csSRR) will lay a much broader foundation for the interpretation of environmental signals, both by further informing the mechanistic model we rely on in this work (Wing and Halevy 2014), and by potentially providing diagnostic $^{33}\lambda$ and $^{36}\lambda$ values that could assist in identifying the major contributors of contemporary systems to in situ fractionation and the signal preserved.

Complementing the work proposed above, and addressing a concern outlined in Paper 3, would be a reassessment of the most accurate and relevant manner in which to evaluate csSRR. Current methods are based on culture density, or in the work described here, environmental microbial density adjusted based on sequence-justified expectation of functional taxa. These methods do not take into consideration heterogeneous activity on the part of a community of clonal or disparate cells, but the ramifications of only a subset of the expected community actively respiring are significant in the interpretation of the measured net fractionation, especially at the low bulk activities encountered in natural environments (Sim, Bosak and Shuhei 2011). The cost of high throughput sequencing continues to decrease, and the accessibility of sequencing facilities continues to increase. Complementary measurements of quantitative proteomics and transcriptomics associated with the enzymes and gene expression of the sulfate reduction pathway may provide an alternative, and more quantitative metric of the reduction taking

place in vitro and in situ, and facilitate more constrained interpretation of the sulfur isotope biosignature.

Returning to the system at hand, one of the foremost revelations of the utilization of the rare ^{33}S isotope, was that it does not fractionate as predicted with respect to ^{34}S fractionation and the mass difference between these isotopes. These findings eventually were utilized to distinguish between signals produced by sulfate reduction and signals that were produced by both sulfate reduction and sulfur disproportionation in tandem (Farquhar *et al.* 2003). To date, no study of multiple sulfur isotopes and in situ microbial composition has explored the relationship between presence, abundance and activity of sulfur disproportionating microbes and the S isotope signal in the same environment. Additional multiple sulfur isotope measurements made on Gypsum Hill Spring sediments in the course of the work described in this thesis indicated that sulfur disproportionation was in fact contributing to the sulfur isotope signal downstream of the stations discussed in Paper 3 (Fig. 1). Genomic characterization the sulfate reducing community of those stations has not been undertaken, nor has a targeted interrogation for versions of the same enzymes utilized in the sulfate reduction pathway, but known to be active in sulfur oxidation- a biological precursor to disproportionation (Loy *et al.* 2009). This system is an obvious target for exploring the stringency of the association between the two, with ramifications for making the deduction that an absence of the isotopic disproportionation signal is equivalent to the absence of the microbes capable of that metabolism, or indicating a lower limit of in situ activity for members of that functional group to leave an isotopic imprint in the sedimentary record.

Fig 1. Microbial sulfate reduction boundary fractionations from station included in Paper 3 *and three downstream stations* (station labeled 7-9). Triangle apices represent $^{33}\lambda$, $^{34}\varepsilon$ fractionation values if flux of AVS to product x is complete (x is elemental sulfur (S0), chromium reducible sulfur (CRS) or hydrogen sulfide (H2S)). Background points are assembled from literature of cultured sulfate reducers and all values are $^{34}\varepsilon_{\text{sulfate-sulfide}}$.



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