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Diatom-inferred records of paleolimnological variability and continental hydrothermal activity in Yellowstone National Park, USA

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DIATOM-INFERRED RECORDS OF PALEOLIMNOLOGICAL VARIABILITY AND CONTINENTAL HYDROTHERMAL ACTIVITY IN YELLOWSTONE NATIONAL PARK, USA

by

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A DISSERTATION

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DIATOM-INFERRED RECORDS OF PALEOLIMNOLOGICAL VARIABILITY AND CONTINENTAL HYDROTHERMAL ACTIVITY IN YELLOWSTONE NATIONAL PARK, USA

Sabrina Renee Brown, Ph.D.

University of Nebraska, 2019

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Fossil diatoms were used to reconstruct paleoclimatic and hydrothermal conditions in Yellowstone National Park. First, an extensive literature review summarizes the current state of knowledge about eukaryotic organisms characteristic of continental hydrothermal environments. Eukaryotes in hydrothermal systems can live at extremes of acidity (pH <4.0) and alkalinity (pH >9.0), and at moderately high temperatures (<62 °C). Silicate and carbonate precipitation in continental hydrothermal environments is mediated by eukaryotic organisms, which are important members of biofilm communities.

A case study of alkaline-chloride sinter deposits in Yellowstone Lake and the Upper Geyser Basin inferred *in-situ* diatom growth rather than post-depositional accumulation of valves settling from the water column. Conical spires from the floor of Yellowstone Lake contained opportunistic fossil diatom species indicative of relatively shallow and alkaline conditions during structure formation. Fossil diatoms of the Upper Geyser Basin were low-nutrient and aerophilic species and in various stages of diagenetic alteration.

An analysis of fossil diatoms showed that assemblages of Yellowstone Lake were sensitive to both direct climate impacts on the lake's physical and chemical structure and indirect impacts on catchment processes. A major shift in species composition occurred at approximately 6475 and 1500 cal years BP. The diatom species composition suggests that the early Holocene winters and springs were cooler than today, with a relatively short interval of spring mixing. In the middle Holocene, the diatoms composition indicates a transition to warmer winters and springs, but colder summer temperatures. Specifically, the diatom assemblage suggests earlier ice-out and delayed stratification that resulted in periods of extended spring mixing. The late-Holocene diatom assemblage is indicative of wetter springs and sustained spring mixing.

The impact of hydrothermal explosion events was assessed on diatom communities using sediment cores from Yellowstone Lake and Cub Creek Pond. The impact of these events was more pronounced in the early Holocene sediment record of the shallower, smaller Cub Creek Pond than in Yellowstone Lake. Overall, diatom assemblages were generally resilient to disturbance via hydrothermal disturbance, with only short-duration changes in diatom assemblage.

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CHAPTER 1

INTRODUCTION

1.1 The Hydrothermal Setting of Yellowstone National Park

Terrestrial hydrothermal systems are dynamic and a critical component of Earth’s thermal budget and geochemical cycles and transport thermal, chemical, and aqueous fluids. Hydrothermal systems, both marine and continental, exert a pronounced impact on the water chemistry in adjoining regions and both store heat and facilitate mineral precipitation (Lowell 1991). Further, hydrothermal vents are centers for diverse faunal communities (Canganella and Wiegel 2011), and the extremophiles commonly encountered associated with hydrothermal systems are models for life processes on early Earth and on extraterrestrial planets. Whereas continental hydrothermal systems account for less than 1% of Earth’s heat flux (Bodvarsson 1982), they hold immense scientific interest for investigating mineral precipitation, geochemical cycles, and exotic ecosystems. Terrestrial hot springs have been utilized for hygienic, medicinal, and spiritual purposes throughout the course of recorded history.

Yellowstone National Park has a long history of research on hydrothermal systems and associated seismic activity, as it is the largest concentration of continental hydrothermal structures in the world and has an extensive history of uplift and subsidence (Hurwitz and Lowenstern 2014). The Yellowstone Plateau Volcanic Field has undergone three major eruptive cycles over the last 2.1 million years, the last of which produced the modern Yellowstone Caldera (Christiansen 2001). The geology of Yellowstone is a mosaic of Precambrian, Paleozoic, and Mesozoic rocks overlain by Tertiary volcanic rocks (rhyolite, andesite, and basalt) and Quaternary glacial deposits (Fritz 1991).

1.2 The Climate Setting of Yellowstone National Park

The eastern Pacific subtropical high-pressure system and monsoonal circulation from the Gulf of Mexico are important climate controls in the northern Rocky Mountains (Brunelle et al. 2005; Whitlock et al. 2012). Two distinct climate regimes occur in modern Yellowstone National Park – the so-called “summer-wet” region, which receives summer precipitation from the Gulf of Mexico, and the “summer-dry” region, which receives the majority of precipitation in winter as influenced by the northeast Pacific subtropical high-pressure system (Whitlock and Bartlein 1993; Curtis and Grimes 2004).

Modern average temperature in Yellowstone ranges between -6.2 °C and 8 °C, with average annual precipitation of 530 mm at the Lake Yellowstone, Wyoming climate station (Figure 1,1; Western Regional Climate Center 2019). In southern Yellowstone the majority of precipitation falls in winter; and snowpack begins to accumulating in October and melts in May (Curtis and Grimes 2004).

1.3 Dissertation Objectives

This dissertation is part of a larger interdisciplinary research project that focuses on evaluating the response of the Yellowstone Lake hydrothermal system to climatic, magmatic, and tectonic processes on time-scales ranging from seconds to thousands of years. The Hydrothermal Dynamics of Yellowstone Lake (HD-YLAKE) project includes a paleoecological component to reconstruct the hydrothermal, limnological, and environmental history of the lake and its catchment during the Holocene. Specific goals of the coring program are twofold: (1) to reconstruct the history of hydrothermal and seismic activity using core lithology, minerology, chemistry, and isotopes, and (2) to

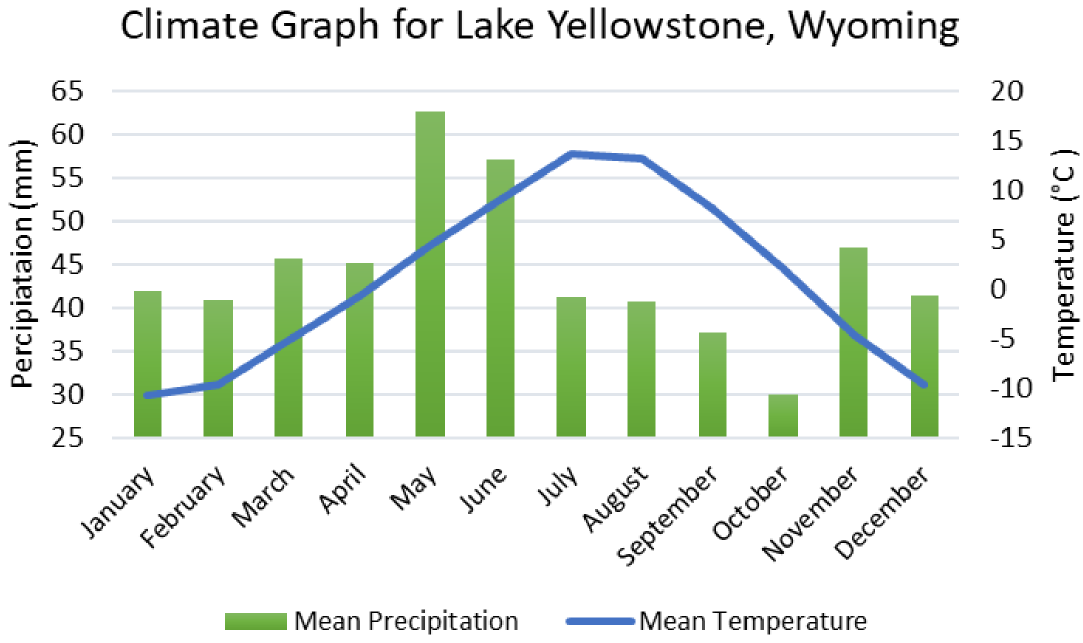

Figure 1.1: Climate graph of average temperature and precipitation for the Lake Yellowstone, Wyoming climate station AD 1981-2010 (data from Western Regional Climate Center 2019).

understand how biogeochemical processes, disturbance regimes, and biotic assemblages of the lake and surrounding landscape are impacted by hydrothermal, climate, and caldera dynamics. The latter involves high-resolution analyses of fossil diatom assemblages, pollen, charcoal, oxygen and silica isotopes, and sediment chemistry from Yellowstone Lake sediments in sites both proximal and distal to modern hydrothermal vents.

This paleoecological research on long time scales is paired with a field program on the dynamics of the modern hydrothermal systems that applies *in-situ* lake floor instruments, including chemical and hydrophone sensors. The aim of the lake floor instrumental network is to measure the system response to geological and environmental instabilities at high-resolution (every 5 seconds) over one year. Specifically, the instrument network includes (1) a dense cluster of instrumentation at the Stevenson Island vent fields, and (2) a lake-wide system of temperature and pressure gauges to monitor hydrostatic pressure. Stochastic models, process models, and systems-level multi-physics and reactive flow models are being developed to understand forcing-response relationships in this hydrothermal system.

This dissertation investigates fossil diatom assemblages of various hydrothermal settings to address the following questions: Which eukaryotic organisms are commonly encountered in continental hydrothermal systems? What do fossil diatom assemblages of siliceous deposits indicate about the hydrothermal environment in Yellowstone Lake and in other hydrothermal systems in the Park? Are the diatom-inferred Holocene climate conditions from Yellowstone Lake sediments consistent with regional records? How did early to middle Holocene hydrothermal explosion events impact fossil diatom communities?

1.4 Preface to the Chapters

Each component of this dissertation investigates an aspect of fossil diatoms in Yellowstone National Park. A brief overview of each chapter in this dissertation, including its relevance to the overall dissertation theme is provided below.

Chapter 2 is an extensive review of extremophilic eukaryotes, including diatoms, encountered in continental hydrothermal systems. It summarizes current knowledge of the eukaryotic microflora characteristic of continental hydrothermal systems and the interaction of these organisms with microbial mats, as well as provides suggestions for the future direction of hydrothermal eukaryote research. I wrote this chapter with guidance from Dr. Sherilyn C. Fritz. This manuscript has been published in *Extremophiles* (Brown, S.R. & S.C. Fritz. 2019. *Extremophiles* **23**(4): 367-376).

Chapter 3 documents the composition of fossil diatoms in siliceous hydrothermal deposits from Upper Geyser Basin and Yellowstone Lake and discusses potential environmental conditions inferred from these diatom assemblages. Because the function of eukaryotic organisms in low-temperature hydrothermal systems is relatively understudied, this chapter provides an important investigation of fossil diatoms within alkaline-chloride environments of the Yellowstone Caldera. I conducted the research presented in this chapter and wrote the initial manuscript draft. Coauthors were involved with initial project conception and provided extensive revisions. This manuscript is accepted in *Diatom Research* (Brown S.R., Fritz S.C., Morgan L.A., Shanks W.C., “Fossilized diatoms of siliceous hydrothermal deposits in Yellowstone National Park, USA”).

Chapter 4 discusses a high-resolution record of diatom-inferred Holocene paleolimnological conditions of Yellowstone Lake. While this chapter focuses on the diatom research that I conducted with guidance from Dr. Sherilyn Fritz, this study is part of a larger collaborative effort within the HD-YLAKE project to understand the Holocene climate and hydrothermal dynamics of Yellowstone Lake. As such, the results presented in this chapter will later be incorporated into a co-authored manuscript detailing a multi-proxy analysis of Yellowstone Lake sediments. This manuscript is currently being written, with myself as lead author.

Chapter 5 discusses the ecological impact of hydrothermal explosion events on diatom assemblages in the Yellowstone Caldera, including deposits from Yellowstone

Lake and Cub Creek Pond. This chapter focuses on inferring post-hydrothermal paleolimnological conditions utilizing fossil diatoms. In the larger HD-YLAKE project, the research presented in this chapter will be integrated with pollen and isotope records to infer landscape and lake dynamic in association with hydrothermal disturbance. The current plan is for me to be a co-author on this manuscript.

Chapter 6 summarizes the important findings from each of the previous chapters and draws overarching conclusions for the dissertation as a whole. This chapter concludes by discussing future research on fossil diatom records of Yellowstone National Park.

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CHAPTER 2

EUKARYOTIC ORGANISMS OF CONTINENTAL HYDROTHERMAL SYSTEMS

Abstract

Continental hydrothermal systems are a dynamic component of global thermal and geochemical cycles, exerting a pronounced impact on water chemistry and heat storage. As such, these environments are commonly classified by temperature, thermal fluid ionic concentration, and pH. Terrestrial hydrothermal systems are a refuge for extremophilic organisms, as extremes in temperature, metal concentration, and pH profoundly impact microorganism assemblage composition. While numerous studies focus on Bacteria and Archaea in these environments, few focus on Eukarya – likely due to lower temperature tolerances and because they are not model organisms for understanding the evolution of early life. However, where present, eukaryotic organisms are significant members of continental hydrothermal microorganism communities. Thus, this manuscript focuses on the eukaryotic occupants of terrestrial hydrothermal systems and provides a review of the current status of research, including microbe-eukaryote interactions and suggestions for future directions.

2.1 Introduction

Both marine and terrestrial hydrothermal systems transport heat and chemical fluids and sequentially induce mineral precipitation via strong chemical and thermal gradients (Lowell 1991). Further, hydrothermal habitats are centers for diverse floral and faunal communities (Cowan et al. 2012), and the organisms commonly associated with geothermal systems provide insights into early Earth and extraterrestrial life processes. While continental hydrothermal systems account for less than 1% of Earth's heat flux (Bodvarsson 1982), they hold immense scientific significance including opportunities for investigating mineral precipitation, geochemical cycles, exotic ecosystems, and advancing biotechnology. Terrestrial hot springs have also been utilized for hygienic, medicinal, and spiritual purposes throughout recorded history (Kearns and Gesler 1998).

This review assesses the spectrum of physical conditions and associated eukaryotic microflora characteristic of continental hydrothermal systems. We discuss in detail (1) the eukaryotic microflora frequently encountered in continental hydrothermal systems, (2) the interaction of microbes and eukaryotes in these environments, and (3) future directions in the field of hydrothermal eukaryote research. Global examples (Figure 1) are presented throughout this review, but Yellowstone National Park is emphasized due to its prominent role in extremophile research.

2.2 Microorganisms in Continental Hydrothermal Systems

Extremophilic organisms (extremophiles) are adapted to either require or tolerate environmental conditions considered hostile in the anthropocentric sense (Horikoshi et al. 2011). Extremophiles (MacElroy 1974) are categorized by optimum growth conditions, including temperature (psychrophiles, thermophiles, and hyperthermophiles), pH (acidophiles and alkaliphiles), pressure (barophiles), and ion strength (halophiles) (Weber et al. 2007; Rampelotto 2013). These classifications are not exclusive; many extremophiles fall into multiple categories. The majority of extremophiles are unicellular

Figure 2.1: Locations of eukaryotic extremophiles found at temperature and/or pH extremes consistent with continental hydrothermal systems reviewed in North America (Stockner 1967; Lynn and Brock 1969; Brock and Boylen 1973; Brock 1978; Tansey and Brock 1978; Redman et al. 1999; Sittenfeld et al. 2002; Bonny and Jones 2003; Sheehan et al. 2003; Sittenfeld et al. 2004; Brown and Wolfe 2006), South America (Van de Vijver and Cocquyt 2009), Africa (Cocquyt 1999; Mpawenayo and Mathooko 2004; Owen et al. 2008), Europe (Owen et al. 2008; Baumgartner et al. 2009; Aguilera et al. 2010; Zirnstein et al. 2012; Aguilera 2013), Asia (Idei and Mayama 2001; Baumgartner et al. 2009; Pan et al. 2010; Yamazaki et al. 2010; Badirzadeh et al. 2011; Kao et al. 2012; Niyyati and Latifi 2017; Pumas et al. 2018), and Australasia (Owen et al. 2008).

(Horikoshi et al. 2011). The phylogenetic origin of extremophilic adaptations is not universal – some lineages appear to be older and others are secondary adaptations by comparable mesophilic taxa (Wiegel and Adams 1998).

Continental hydrothermal systems are refugia for chemophilic and thermophilic microorganisms, and the most common classes of extremophiles in geothermal areas are thermophiles, acidophiles, and alkaliphiles (Canganella and Wiegel 2011). Thermophiles grow at temperatures above 42 °C, and extreme thermophiles grow optimally above 60 °C (Horikoshi and Grant 1998). Acidophiles have an optimal growth range in acidic waters with pH 5.0 or lower; alkaliphilic organisms have an optimum growth rate at pH 9.0 or higher (Kroll 1990) and require high sodium ion concentrations for growth and reproduction (Horikoshi and Grant 1998).

In comparison with marine hydrothermal systems, long-term geochemical variability is reduced in terrestrial hydrothermal environments, enabling the establishment of static, specialized microbial communities (Wilson et al. 2008; Cowan et al. 2012). These communities include a diverse suite of microbes from the three primary phylogenetic domains: Archaea (archaebacteria), Bacteria (eubacteria), and Eucarya (eukaryotes) (Jones et al. 1997; Blank et al. 2002; Brown and Wolfe 2006; Cowan et al. 2012).

The study of microorganisms associated with hydrothermal systems has largely been focused on prokaryotes (Brock 1973; Cowan et al. 2012; Horikoshi 2016), with multiple studies examining extremophile bacteria in hot spring systems (Brock 1978; Stetter 1999; Canganella and Wiegel 2011; Tekere et al. 2015; Horikoshi 2016; Selvarajan et al. 2018), including their relevance to the fields of biotechnology, paleontology, and astrobiology. Although less studied, eukaryotes are encountered actively growing in nearly all types of extreme environments (Horikoshi and Grant 1998) and perform important ecosystem functions, including grazing on acidophilic bacteria and influencing nutrient cycling (Rothschild and Mancinelli 2001; Bottjer 2005; Baker et al. 2009). Although extremophilic eukaryotes are proportionately understudied, recent genomic and metabolic profiling advancements have enabled new research approaches (Weber et al. 2007), increasing the number of studies in recent years.

2.3 Limits for Eukaryotic Life

The abundance and distribution of eukaryotic organisms are mainly determined by temperature-dependent enzyme activity, with the highest recorded temperature for viable eukaryotes 62 °C (Rothschild and Mancinelli 2001). Studies on single-celled protozoa, metazoa, fungi, and algae all indicate that their growth and evolution is hindered at temperatures above 60 °C (Tansey and Brock 1978), in contrast with multicellular plant growth, which is hindered above 50 °C (Horikoshi et al. 2011). The inability of eukaryotes to grow at higher temperatures primarily results from the inability of cells to form functional and thermally stable organelle membranes (Tansey and Brock 1972).

In contrast to temperature, the ability of eukaryotes to thrive at pH, desiccation, and salinity extremes is similar to that of archaea and prokaryotes. Eukaryotic organisms are encountered in both alkaline and acidic environments, but more is known about acidophilic eukaryote diversity, because of their abundance in readily-studied acid mine drainage, geothermal, and acidic lake environments (Hecky and Kilham 1973; Seckbach 2007; Amaral-Zettler 2013). The adaptability of eukaryotes to broad pH ranges appears to be limited and rare (Amaral-Zettler 2013); however, the ability of eukaryotes to adapt to a small, extreme pH range is relatively widespread across clades including *Chlorophyta, Amoebozoa, Alveolata, Rhizaria,* and *Stramenopiles* (Hecky and Kilham

1973; Costas et al. 2007; López-Rodas et al. 2008; Horikoshi et al. 2011; Casamayor et al. 2013).

Eukaryotic photosynthetic microalgae (diatoms, chlorophytes, and rhodophytes) are the dominant organisms in communities at pH values less than 4.0, in which cyanobacteria (prokaryote) cannot survive (Brock 1973). Eukaryotes living in environments with extreme pH have evolved to maintain circumneutral cytoplasm pH through altered internal physiology (Rothschild and Mancinelli 2001). The evolution of extremophilic eukaryotic organisms living in heavy metal-rich, acidic, and hot environments was apparently facilitated through gene transfer of beneficial proteins from bacteria and archaea (Schonknecht et al. 2013).

Extremophile evolution has been documented throughout several eukaryotic clades (Horikoshi et al. 2011). When compared with mesophilic counterparts, genome sequences of extremophilic eukaryotes indicate evolved strategies for survival in extreme environmental conditions (Weber et al. 2007). Additionally, cellular-level metabolic processes differ between extremophilic and mesophilic eukaryotes (Weber et al. 2007). Applications of novel genome sequencing and cellular-level processes, as well as traditional presence-absence studies of extremophiles in terrestrial hydrothermal systems, are discussed below.

2.4 Eukaryote Lineages of Continental Hydrothermal Ecosystems

Multiple eukaryotic lineages (Table 2.1) are known to occur in hydrothermal systems and similarly extreme environments. The acidic pH and temperature extremes of some examples are presented in Figure 2.2. Below we review key studies on the occurrences and tolerance levels of key groups.

2.4.1 Chlorophyta (green algae)

Chlorophyta are a lineage of photosynthetic eukaryotes within the Archeaeplastida supergroup (Simpson et al. 2017) that are characterized by pigments chlorophyll *a* and chlorophyll *b* and have cellulose cell walls (Wehr and Sheath 2003). Several green algal genera (*Zygogonium, Chlamydomonas, Chlorella,* and *Ulothrix*) have been documented in thermal systems. A species of *Zygogonium* forms purple mats in temperatures between 20-31°C and pH of 2.4-3.1 in soils associated with small seeps or springs in Yellowstone National Park (Lynn and Brock 1969). These filamentous *Zygogonium* mats create a damp environment for *Chlamydomonas* to colonize (Tansey and Brock 1978). *Chlorella* and *Ulothrix* have also been documented in eukaryotic mats of thermally active areas of Yellowstone (Brock and Boylen 1973; Brock 1978). In an acidic (pH 1.8) hot (68°C) pool in Lassen Valley National Park, a diverse assemblage in the family Chlamydomonadaceae is established (Brown and Wolfe 2006). In hydrothermal springs in Iceland, Aguilera *et al.* (2010) discovered that phylotypes of *Chlorella* and *Chaetophora* dominated the algae in circumneutral microbial mats. In addition to the acidic environments associated with terrestrial hydrothermal systems, the genera *Zygnemopsis, Chlamydomonas,* and *Chlorella* are also commonly found in acid mine drainage basins (Aguilera 2013).

2.4.2 Rhodophyta (red algae)

Rhodophyta (red algae) are a group of photosynthetic organisms within the Archeaeplastida supergroup of eukaryotes (Simpson et al. 2017) that are red in color due to the presence of phycoerythrin, allophycocyanins, and phycocyanin accessory photosynthetic pigments. The group is also characterized by the absence of flagella and centrioles (Wehr and Sheath 2003). North American freshwater red algal species are overwhelmingly (94%) found in lotic environments (Wehr and Sheath 2003). Cyanidiales, a group of unicellular Rhodophyta that thrive in high-temperature acidic environments, are comprised of three genera- *Galdieria, Cyanidium,* and *Cyanidioschyzon*, and phylogenetic analyses indicate that these genera were ancestrally thermos-acidotolerant (Ciniglia et al. 2004).

Table 2.1 Eukaryotic lineages of extremophile eukaryotes in hydrothermal systems.

Supergroup/Assemblage	Lineage	Group (common name)
Archaeplastida	*Chlorophyta* *Rhodophyta*	green algae red algae
SAR	*Stramenopiles* *Alveolata* *Rhizaria*	diatoms ciliates amoeboflagellates
Discoba	*Euglenozoa*	Euglena
Amorphea	*Amoebozoa*	Amoebae
Obazoa	*Fungi*	fungi and chytrids

Red algae *Galdieria* and *Cyanidium* are documented in hot sulfur springs with an optimum growth temperature of 45 °C and tolerance of up to 57 °C. They are also encountered in highly acidic waters of pH 0.05-4 and sites with elevated heavy metal concentrations, including cadmium, nickel, iron, and arsenic (Seckbach 1994; Ciniglia et al. 2004). The species *Cyanidium caldarium* is present in the hot aquatic systems of Yellowstone National Park (Tansey and Brock 1978). Phylotypes likely belonging to red algae were also extracted from geothermal hot spring biofilms in Seltun, Iceland (Aguilera et al. 2010). *Cyanidioschyzon merolae* was discovered living in a pool with temperature 68 °C and pH 1.8 in Lassen Valley National Park, USA (Brown and Wolfe 2006). The genome sequence for *Cyanidioschyzon merolae* has unique features that include a minimal total number of genes, few introns, and only three ribosomal DNA copies. Thus, this extremophilic red algae has surprisingly simple genomic features (Nozaki et al. 2007).

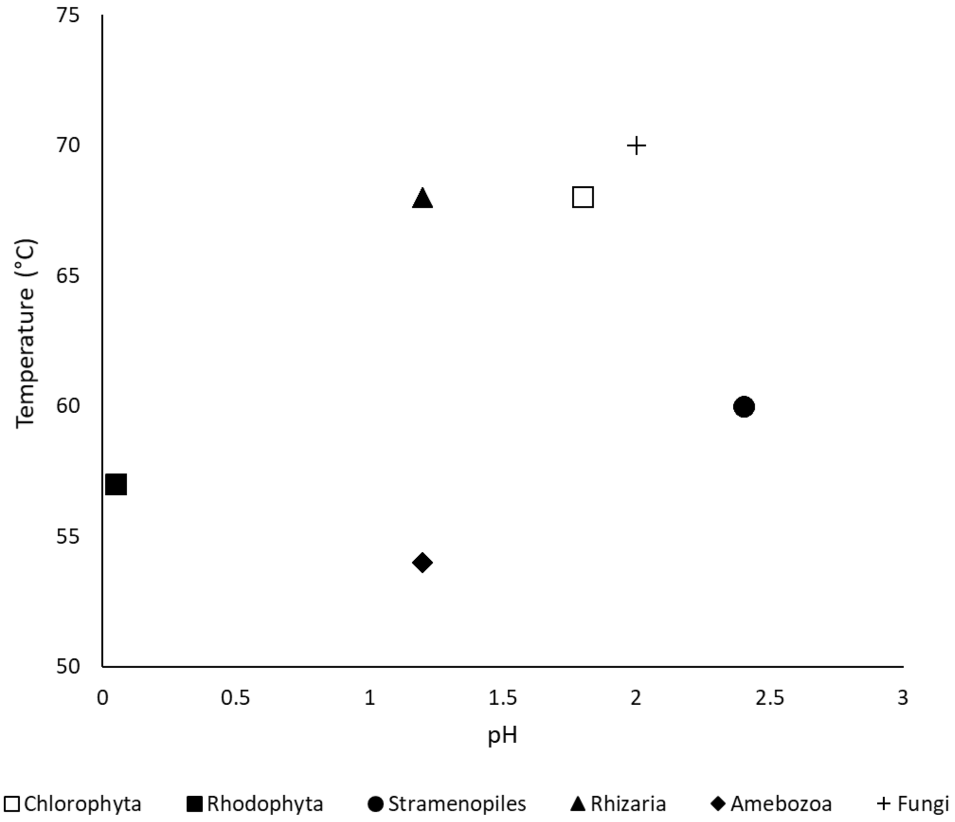

Figure 2.2: Summary of the relative extremes (in temperature and pH) for a number of eukaryote supergroups presented in this review.

2.4.3 Stramenopiles (diatoms)

A lineage of supergroup SAR, stramenopiles are tubulocristate protists that are most often photosynthetic but can be heterotrophic and include unicellular, colonial, and multicellular forms (Simpson et al. 2017). Most stramenopiles have chloroplasts surrounded by four membranes with cholorphyll *a* and *c*, and, usually, they have the accessory pigment fucoxanthin, which imparts a golden-brown color. Bacillariophyta (diatoms) are unicellular stramenopiles that are ubiquitous in both freshwater and marine environments. Most diatom genera have preferences within a small range of pH values, with pH ~3.5 as the threshold below which most cannot survive (Smol et al. 1986). Yet a number of diatom genera have been found in hot springs. For example, Owen *et al.* (2008) observed 251 species preserved with little fragmentation or corrosion from 21 geothermal hot springs in Iceland, New Zealand, and Kenya. Diatoms have also been found in hot springs in Peru, East Africa, and Thailand (Cocquyt 1999; Mpawenayo and Mathooko 2004; Van de Vijver and Cocquyt 2009; Pumas et al. 2018).

One common genus in areas with low pH and high temperatures is *Pinnularia*. An unidentified *Pinnularia* species was recorded in two separate hot springs in New Zealand with extreme temperatures (85°C and 60°C) and low pH values (3.7 and 2.4, respectively), although growth at 85°C is highly unlikely, and its occurrence was attributed to its association with microbial mats that protected the *Pinnularia* from thermal influence (Owen et al. 2008). Species of *Pinnularia* have also been found in the terrestrial hydrothermal systems of Yellowstone and Lassen Volcanic National Parks (Brock 1978; Brown and Wolfe 2006), Río Tinto, Spain (Aguilera 2013), and in strongly acidic waters in Japan (Idei and Mayama 2001). *Pinnularia* is also widespread in acidic waters with pH 3.0 or lower and commonly dominates biofilms in acid mine drainage sites (DeNicola 2000).

Other diatom groups reported in hydrothermal systems include Fragilariaceae in geothermal springs in Iceland (Owen *et al*. 2008), *Navicula* and *Anomoeoneis* in Kenyan springs, and phylotypes of *Bacillaria, Gomphonema,* and *Navicula* in circumneutral microbial mats in Icelandic hydrothermal hot springs (Aguilera et al. 2010). In Jasper National Park (Canada), diatom assemblages within 0.5 m of hot spring vents hosted a diverse (*Cymbella, Mastogloia, Brachysira, Sellaphora, Epithemia* (formerly *Rhopalodia*)*, Nitzschia, Navicula, Pinnularia)* diatom assemblage (Bonny and Jones 2003). *Pinnularia, Achnanthes, Epithemia* (formerly *Rhopalodia*)*, Amphora, Denticula,* and *Navicula* were found alive in hydrothermal streams of Yellowstone National Park and Mount Rainier at approximately 35 °C (Stockner 1967). Dominant genera in northern Thailand hot springs were *Diatomela, Achnanthidium,* and *Anomoeoneis* (Pumas et al. 2018). Four novel species in the genera *Navicula, Ulnaria, Cymbella, and Denticula* were identified from La Caldera, Peru (Van de Vijver and Cocquyt 2009). Dominant genera in Lake Tanganyika hot springs samples were *Caloneis, Diploneis,* and *Sellaphora* (Cocquyt 1999).

2.4.4 Alveolata (ciliates)

Alveolates, included within the eukaryote supergroup SAR, are primarily single-celled organisms (Simpson et al. 2017) characterized by a system of membrane sacs (alveoli). Brown and Wolfe (2006) detected alveolates by conducting molecular surveys on hot spring samples from Lassen Valley National Park. In addition, alveolate lineages have been encountered in low pH and high heavy metal concentration waters of the underground uranium mine in Königstein, Germany (Zirnstein et al. 2012).

2.4.5 Rhizaria (amoeboflagellates)

Cercozoa are heterotrophic protozoa within the Rhizaria lineage of the SAR supergroup and are most abundant in soils, although they are also found in freshwater and marine ecosystems. Cercozoa living in terrestrial hydrothermal environments are found in association with microbial mats – likely preying on the algae and bacteria within the mat matrix (Cowan et al. 2012).

A few Cercozoa have been observed within the terrestrial geothermal system of Lassen Volcanic National Park. These acidophilic protists were sampled from algal mats and biofilms of acidic pools, streams, and mud pots with temperatures up to 68 °C (Brown and Wolfe 2006). A species of amoeboflagellate, *Tetramitus thermacidophilius*, was found living between 28-54 °C and acidities between pH 1.2 and 5 in hot springs of Kamchatka, Russia and Naples, Italy (Baumgartner et al. 2009). Other genera of protozoa, including *Naegleria* and *Achthamoeba*, have been found in association with microbial environments within the active geothermal portions of the Yellowstone region (Sheehan et al. 2003), and phylotypes belonging to small flagellates were extracted from two phototrophic microbial mats in Icelandic hot springs (Aguilera et al. 2010).

2.4.6 Euglenozoa (Euglena)

Euglenozoa, within the assemblage Discoba, are flagellate, unicellular eukaryotes that are commonly free-living (Simpson et al. 2017). Euglenids, one of the two main subgroups of Euglenozoa, especially the genus *Euglena*, are commonly used as environmental indicators in acid mine drainage environments. Additionally, the genus *Euglena* is commonly encountered in acidic geothermal areas. *Euglena* was found through clone sequence analysis in Yellowstone National Park (Sheehan et al. 2003), and the species *Euglena mutabilis* also has been reported from regional studies (Tansey and Brock 1978), as well as from analysis of photosynthetic biofilms in Río Tinto, Spain (Aguilera 2013). Phylotypes of *Euglena* were extracted from microbial mats in Icelandic geothermal hot springs (Aguilera et al. 2010). A non-flagellated photosynthetic *Euglena* strain, closely related to *Euglena mutabilis*, and a novel species, *Euglena pailasensis*, were isolated from an acidic hot mud pool near Rincón de la Vieja volcano, Costa Rica (Sittenfeld et al. 2002; Sittenfeld et al. 2004).

2.4.7 Amebozoa (amoebae)

Amebozoa, within the assemblage Amorphea, are a eukaryote clade consisting of amoeboid protists (Simpson et al. 2017). *Echinamoeba thermarum*, a thermophilic amoeba, was discovered living at temperatures ranging from 50-60°C in the hydrothermal environments of Yellowstone National Park and in Agano Terme, Italy (Baumgartner et al. 2003). In the acidic geothermal pools of Pisciarelli Solfara (Italy) and Kamchatka (Russia), an amoeboflagellate species *Tetramitus thermacidophilus* was encountered in acidic (1.2-5 pH) and warm (28-54 °C) waters (Baumgartner et al. 2009). In addition, a species of *Acanthamoeba* was detected in 15% of water samples collected from thermal springs in southern Taiwan (Kao et al. 2012).

The thermophilic free-living amoebae Vahlkampfiidae live in a variety of hot aquatic environments and are opportunistic amoeba that are pathogenic and can cause disease in human hosts. In Yellowstone National Park, *Vahlkampfia, Acanthamoeba,* and *Naegleria fowleri* were detected in the algal biofilm community (Sheehan et al. 2003). In addition, free-living amoebae *Acanthamoeba* and *Vannella* were isolated from Sarein Hot Springs, Iran (Badirzadeh et al. 2011) and a geothermal spring in Amol City, Iran (Niyyati and Latifi 2017), respectively.

2.4.8 Fungi (fungi and chytrids)

Fungi, within the supergroup Obazoa, are a broad group of eukaryotes encompassing yeasts, molds, and mushrooms (Simpson et al. 2017). Fungi are a very successful phylogenetic lineage and adapt well to extreme environments, with the exception of extreme temperatures (Rampelotto 2013). A variety of fungi have been found thriving in highly acidic acid mine drainage environments (Aguilera 2013); the species *Acontium cylatium,* and *Trichosporon cerebriae,* as well as the genus *Cephalosporium*, are known to grow at pH 0 (Schleper et al. 1995).

In Lassen Valley National Park, two species of fungi were identified in a pool with pH 1.8 and temperature 68 °C (Brown and Wolfe 2006). A new fungal species from the Sainokawara hydrothermal area in Japan, *Teratosphaeria acidoterma*, was found in a wide temperature range (30-70 °C), with an optimal pH range between 2 and 4 (Yamazaki et al. 2010). Additionally, 16 fungal species were found in geothermal soils of Yellowstone National Park, with two species – *Acremonium alabamense* and *Dactylaria constricutum* var. *gallopava* – growing above 50°C (Redman et al. 1999). In the alkaline hydrothermal environments of Tengchong Rehai National Park (China), 102 thermophillic fungal strands have been identified at a temperature range of 47-71 °C (Pan et al. 2010). Specifically, *Thermomyces lanuginosus* and *Scytalidium thermophilum* have been found dominating in extreme temperature conditions (Singh et al. 2003; Cowan et al. 2012).

2.5 Microbial Eukaryote Interactions

Eukaryotic organisms in continental hydrothermal environments appear to be limited to unicellular forms and are typically associated with microbial mats and biofilms in extreme conditions (Cowan et al. 2012; Rampelotto 2013). Thus, within continental hydrothermal systems microbial eukaryote interactions appear extremely beneficial to survival. Microbial mats are an organic matrix in which microorganisms and minerals are imbedded. Eukaryotes, second to *cyanobacteria*, are important in building phototrophic microbial mats – self-sustaining, nearly closed, small-scale ecosystems. Communities in microbial mats are vertically stratified, because microorganisms within the system create physiochemical gradients (Bolhuis et al. 2014).

Microbial mats (Figure 2.3) provide niches for various eukaryotic organisms and, in continental hydrothermal systems, can create refugial microhabitats for organisms that could not survive in the extreme conditions alone (Seilacher 1999). Specifically, adherence to a solid surface allows microorganisms to manage surrounding abiotic factors that present a challenge to survival, including fluid convection. Additionally, proximity and interaction of organisms in microbial mat systems allows higher resource and energy flow than non-mat habitats in the same system. The cyanobacteria in phototrophic microbial mats fix both carbon and nitrogen, which stabilizes the microenvironment for the growth of other organisms (Prieto-Barajas et al. 2017). This stabilization can help buffer the microecosystem against abrupt environmental changes, including high temperatures and elevated heavy metal concentrations.

The precipitation of silicate and carbonate in hydrothermal environments is often mediated by eukaryotes, and precipitation in association with microbial growth has been an important mechanism of carbonate sediment deposition since the Archean (Riding 2000). Eukaryotes that utilize carbonate or silicate in their cell walls effectively precipitate these compounds out of the water column. Additionally, carbonate precipitation is stimulated by various metabolic processes – including photosynthesis, as well as nitrogen and sulfur fixation, which increase alkalinity and, thus, carbonate precipitation (Riding 2000). Microbial mats, including stromatolitic and conical structures (Figure 2.3), composed of amorphous silica are associated with diatom photosynthesis through individually precipitated crystals (Winsborough and Golubic 1987). These structures vary widely in both internal structure and external form – ranging from mats to large conical structures and lacking well-preserved laminations (Cohen 2003) Shape and size is largely controlled by environmental conditions, including light availability and water agitation (Cohen 2003). These microorganism and precipitation associations are more pronounced in hydrothermal systems, where the waters are rich in carbonate or silicate – especially hot, alkaline environments. This is particularly interesting, because carbonate deposits are an important carbon reservoir on Earth, and a substantial portion of insoluble carbon on the surface is formed biogenically (Zhu and Dittrich 2016). Understanding why these processes are prominent in hydrothermal

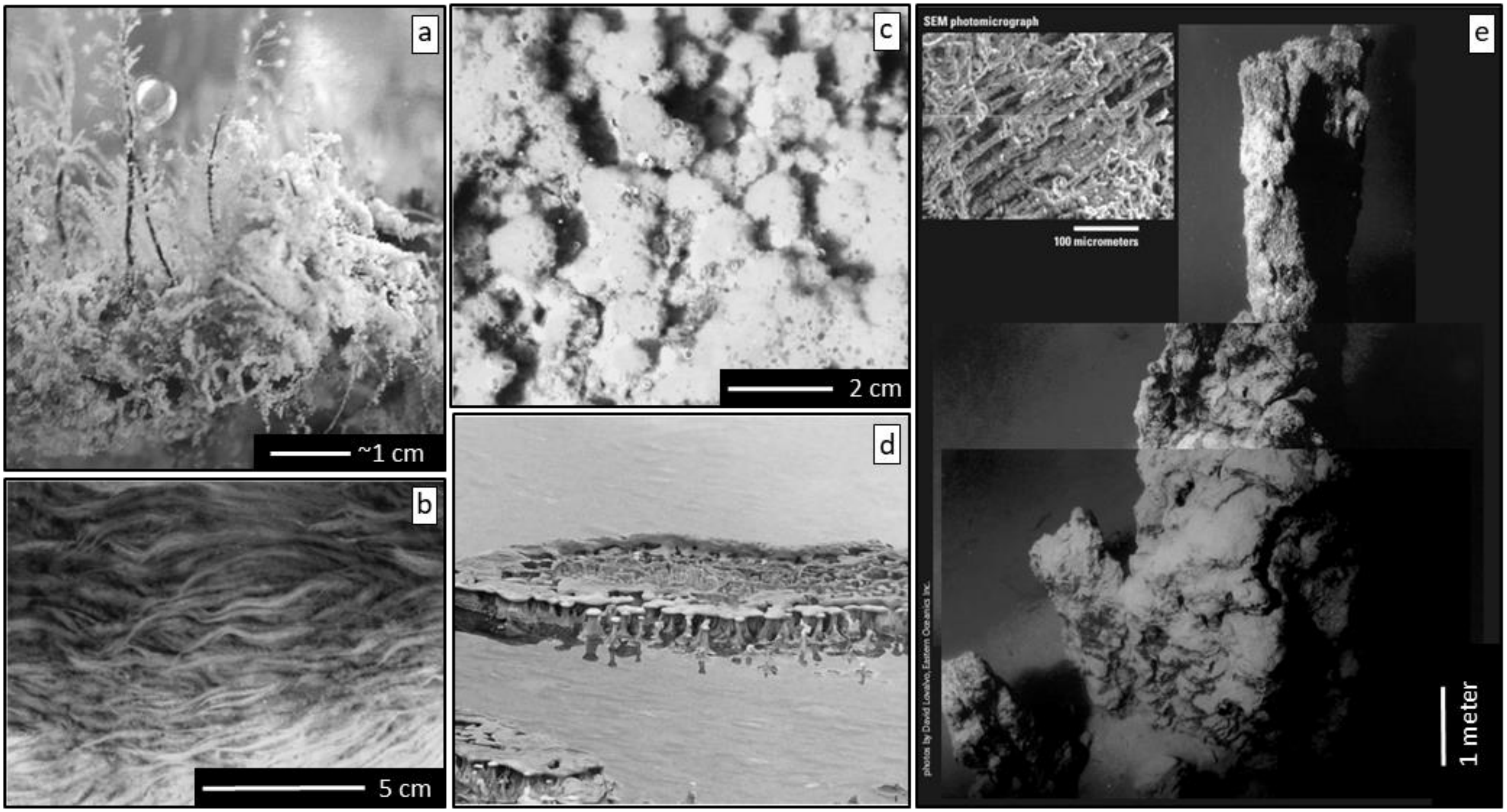

Figure 2.3: Examples of biofilms and microbial mat structures containing eukaryotic organisms. A) Complex mat from a subtidal dome containing green algae and diatoms (used with permission from Riding 2000). B) and C) Microbial biofilms from the acid mine drainage site Rio Tinto, Spain with main components Chorella and fungi (B) and Chlamydomonas and fungi (C), respectively (used with permission from Aguilera et al. 2007). D) Biofilm structures from the Gardener River, Yellowstone National Park (used with permission from Hall-Stoodley et al. 2004). E) Siliceous spire structure on the floor of Yellowstone Lake (from Shanks et al. 2007).

systems and mimicking these natural processes in the laboratory may inspire innovative approaches in biotechnologies and artificial carbon sequestration.

2.6 Future Directions

Although eukaryotic organisms are relatively understudied in hydrothermal systems, the expansion of genetic and genomic tools and their use in phylogenetic analysis has the potential to greatly enhance our understanding of hydrothermal eukaryotic organisms. Applying genomic tools to novel environments will increase scientific knowledge of the diversity and mode of life of extremophilic microbial eukaryotes in hydrothermal systems. For example, a coded 18S ribosomal RNA survey of diatomaceous soil crust in Yellowstone National Park found that community composition dissimilarity between samples was high (Meadow and Zabinski 2012). The use of polymerase chain reaction amplification of mixed population DNA negates the necessity of cultivation for organism identification and therefore may allow the identification of additional eukaryotes capable of living in extreme environments. Molecular approaches still have a fundamental problem – separating genes isolated from organisms actively living within the system of interest from those introduced from nearby, less extreme environments. Currently, novel studies appear limited to genetic studies, because retrieval of identifiable, living microbial specimens is challenging. Additionally, identifying to the highest possible taxonomic level using light and scanning electron microscopy, in conjunction with genetic studies, can supplement our understanding of diversity and life form in hydrothermal environments. Thus, new frontiers in molecular studies should include novel cultivation techniques and microscopy to identify morphologically discrete taxa (Teske 2007).

Many unanswered questions remain regarding how extremophilic eukaryotes work to make their environment more hospitable. While it is clear that microhabitat formation via biofilms and microbial mats are an essential adaptation to living in harsh conditions, the exact mechanisms of the microbial-eukaryote interactions remain unclear. Some recent work has focused on survival strategies of eukaryotes in hydrothermal systems. For example, a thermoacidophilic eukaryotic algae in Yellowstone National Park was discovered biotransforming arsenic (Qin et al. 2009). Cultured samples from hydrothermal vent thermophiles also found biofilms formed with a sulfate-reducing archaeon formed in a variety of laboratory-induced stress conditions, including high metal concentrations, pH, and oxygen exposure (LaPaglia and Hartzell 1997). Additionally, transcriptome analysis provides the potential for understanding which genes are responsible for the functional attributes that enable survival in extreme environments, pinpointing what makes extremophilic eukaryotes vary from their mesophilic counterparts.

Finally, comparisons of eukaryote community composition across continental hydrothermal gradients is also needed. One of the greatest obstacles to large comparative studies of hydrothermal eukaryotic organisms is the limited number of studies, with the majority of studies in Yellowstone National Park, and also the variability in research methods and study design. However, an enhanced understanding of hydrothermal systems may provide clues to the evolution of life on Earth and in extraterrestrial settings. The development of an online database documenting the location of terrestrial hydrothermal environments, including biotic and abiotic characteristics, would be a useful tool for future analyses.

2.7 Summary

Terrestrial hydrothermal systems exert a profound impact on water chemistry and temperature, which in turn determine the growth of microorganisms within these environments. While the field of extremophile research is traditionally focused on Bacteria and Archaea, which can live at higher temperatures, research on eukaryotes has expanded over the last few decades – especially with the advancement of genomic and metabolic profiling methods. Eukaryotic organisms are limited at high temperatures (<62 °C) but can also thrive in highly acidic (pH 0.05-4.0) and alkaline conditions (pH >9.0). Lineages from four eukaryote supergroups (Archeaeplastida, Chromalveolates, Excavata, and Unikonta) are found in terrestrial hydrothermal environments globally. Better understanding of the interaction of eukaryotes with other organisms and with the surrounding environment are vital to their persistence in geothermal conditions. In particular, microbial mats provide microhabitat formation that enables the perseverance of taxa that could not survive otherwise. Additionally, eukaryotes mediate the precipitation of silicate and carbonate in hydrothermal systems, making them important components of major biogeochemical cycles. Thus, eukaryotes provide essential ecosystem functions within the extreme environments created by terrestrial hydrothermal activity.

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CHAPTER 3

FOSSILIZED DIATOMS OF SILICEOUS HYDROTHERMAL DEPOSITS IN YELLOWSTONE NATIONAL PARK, USA

Abstract

The study of eukaryotic extremophiles is relatively novel, and, therefore, documentation of the structure and function of micro-organisms in continental hydrothermal systems globally is limited. In this study, we investigate fossil diatoms in siliceous hydrothermal deposits of the Upper Geyser and Yellowstone Lake hydrothermal basins in Yellowstone National Park and utilize preserved diatom assemblages to infer local environmental conditions. Siliceous sinter from both the Upper Geyser Basin and Yellowstone Lake contain evidence of *in-situ* diatom growth within these environments. At Upper Geyser Basin, the assemblage consisted of saline and high-conductivity species that could grow on moist siliceous sinter and was dominated by *Rhopalodia gibberula*. Diatom valves were found in various preservation states, ranging from nearly pristine to highly diagenetically altered. Diatoms collected from siliceous spires in Yellowstone Lake largely consisted of tychoplanktic and benthic species that were almost certainly growing on the outside of the structure, with an assemblage indicative of relatively shallow alkaline waters. What remains unclear without access to material for high-resolution dating is whether diatoms colonized the spires during hydrothermal activity or after activity ceased. Our results indicate that diatom frustules, to some extent, can survive alteration in low-temperature (<76 °C) hydrothermal environments.

3.1 Introduction

Since Thomas Brock first discovered hyperthermophilic microorganisms in the hot springs of Yellowstone National Park, multiple studies have examined extremophilic bacteria in continental hydrothermal systems (Brock 1978, Stetter 1999, Canganella & Wiegel 2011, Horikoshi 2016), including their relevance to the fields of biotechnology, paleontology, and astrobiology, among others. While it is becoming clear that eukaryotic organisms are important components of the microbiota of hydrothermal communities, documentation of eukaryotic organisms lags behind that of well-studied bacteria and Archea in the Yellowstone region.

Bacillariophyta (diatoms) are eukaryotic unicellular golden-brown algae that have opal-A skeletons and are ubiquitous in both freshwater and marine environments. Diatom growth is inhibited above approximately 70 °C (Beauger et al. 2018, Lai et al. 2019), and these organisms are able to live in both acidic and alkaline environments. Most diatom genera have preferences within a relatively small range of pH values (Hecky & Kilham 1973, Smol et al. 1986). A number of diatom genera have been found in alkaline terrestrial hydrothermal systems of Iceland, New Zealand, Kenya, the United States, and Canada (Stockner 1967, Brock 1978, Gasse 1986, Bonny & Jones 2003, Brown & Wolfe 2006, Owen et al. 2008), as well as in highly acidic waters in Spain (Aguilera 2013) and Japan (Idei & Mayama 2001). Yet, the limited number of studies suggests the potential for more extensive documentation of diatoms in continental hydrothermal systems. Iconic Yellowstone National Park, with its abundant and varied types of continental hydrothermal features, provides an ideal location for expanded documentation of the species inhabiting these unusual systems.

Yellowstone National Park is one of Earth's most geologically dynamic landscapes and has been shaped by forces of late Cenozoic large-scale explosive and effusive volcanism, active tectonism, glaciation, and hydrothermal activity (Morgan et al. 2017 and references within). The interior of Yellowstone National Park is occupied by the 631,000-year-old Yellowstone caldera, an elliptical feature measuring 75 km by 45 km that represents one of the world's largest rhyolitic volcanic structures (Christiansen, 2001). The Yellowstone Plateau is elevated up to 1 km above the northeast front of the Snake River Plain trough and receives abundant annual precipitation due to orographic effects (between 150-180 cm/yr; Pierce et al. 2007). The plateau also experiences high levels of seismicity (averaging 1500-2500 earthquakes/year; Farrell et al. 2014) and has some of the highest heat flow values in the intermountain west (Morgan et al., 1977); all are prerequisites for sustaining hydrothermal systems. In addition, active deformation of the Yellowstone Caldera contributes significantly to the system (Dzurisin et al. 2012).

Yellowstone National Park has a diverse range of hydrothermal features including geysers, hot springs, mud pots, fumaroles, and, in places, travertine terraces. With over 10,000 thermal features, Yellowstone National Park has the largest concentration of continental hydrothermal features on Earth, exceeding all other known hydrothermal features combined (Allen & Day 1935, Fournier 1989, Schreier 1992, Ann Rodman, personal communication, Yellowstone National Park, 2007). The compositions of fluids discharged in continental hydrothermal systems has a profound impact on mineral deposition, hydrothermal alteration, and biotic characteristics of the environment. In Yellowstone National Park, both alkaline-chloride and acid-sulphate geothermal waters are abundant within the Yellowstone caldera and other areas with rhyolite substrate;

location of hydrothermal vents is strongly controlled by the distribution of rhyolite lava flows with low permeability while chemical composition of the springs are, in part, determined by topographic elevation (Fournier 1989, Morgan & Shanks, 2005; Hurwitz & Lowenstern 2014).

Alkaline-chloride waters are saline and characteristic of high-temperature deep geothermal fluids (Fournier 1989, Fournier 2005, Renaut & Jones 2011). Alkaline-chloride hydrothermal fluids are high in dissolved silica and dominated by Na^+ and K^+ cations balanced by Cl^- as the principle anion, and also sometimes contain significant SO_4^{2-} and HCO_3^- (Fournier & Truesdale, 1973). The main dissolved gases are carbon dioxide and hydrogen sulphide (Renaut & Jones 2011). Chloride waters are associated with geysers and hot pools. Because these fluids have high concentrations of dissolved silica, chloride waters are associated with large quantities of sinter precipitation (Guidry & Chafetz 2002). Sinter deposited near hydrothermal pools, flows, and geysers commonly is colonized by diverse and colorful microbial communities. Because silica (opal-A) is abundantly precipitated in these systems, diatoms are commonly found in association with active and inactive sinter deposits (Lynne et al. 2008). As such, the objective of this study is to document the presence, incorporation, and significance of diatom frustules in various siliceous hydrothermal deposits of Yellowstone National Park – specifically from the Upper Geyser Basin and Yellowstone Lake.

3.1.1 Site descriptions

The hydrothermal deposits included in this study are located within the southern portion of the Yellowstone caldera (Figure 3.1). The Upper Geyser Basin is the world's largest concentration of geysers – including iconic Old Faithful and over 150 other geysers concentrated within approximately 2.5 km^2 (Bryan 2018). Hillside Geyser is a thermal feature in the Upper Geyser Basin. The Hillside Geyser rarely erupts, but when it does, it can expel water up to nine meters high for minutes at a time (Bryan 2018). Thus, sinter deposits in the Hillside Group accrue relatively slowly.

Located 35 km East of the Upper Geyser Basin, Yellowstone Lake is the third (after Upper Geyser and Lower Geyser Basins) largest hydrothermal basin in Yellowstone National Park, based on its chloride flux (Shanks et al. 2005). The lake floor has an active hydrothermal system with vents and seeps, which exert an impact on lake water chemistry (Balistrieri et al. 2007). Siliceous conical spire deposits formed as amorphous silica precipitated in the shallow (<25 m water depth) lake bottom associated with now extinct hydrothermal vents (Morgan et al. 2003). These deposits formed hard, porous siliceous chimneys consisting of altered and unaltered sediments. At present, actively forming spires have not been identified in Yellowstone Lake (Shanks et al. 2007). According to stable isotope data collected near vents, sinter and spires form at moderately low temperatures (<76 °C), and diatom frustules are incorporated into the structure along with the amorphous silica matrix (Shanks et al. 2005). In addition to conical spire structures, a number of hydrothermal structures from Yellowstone Lake, including conduits and vents analyzed in Shanks et al. 2007, also were sampled here.

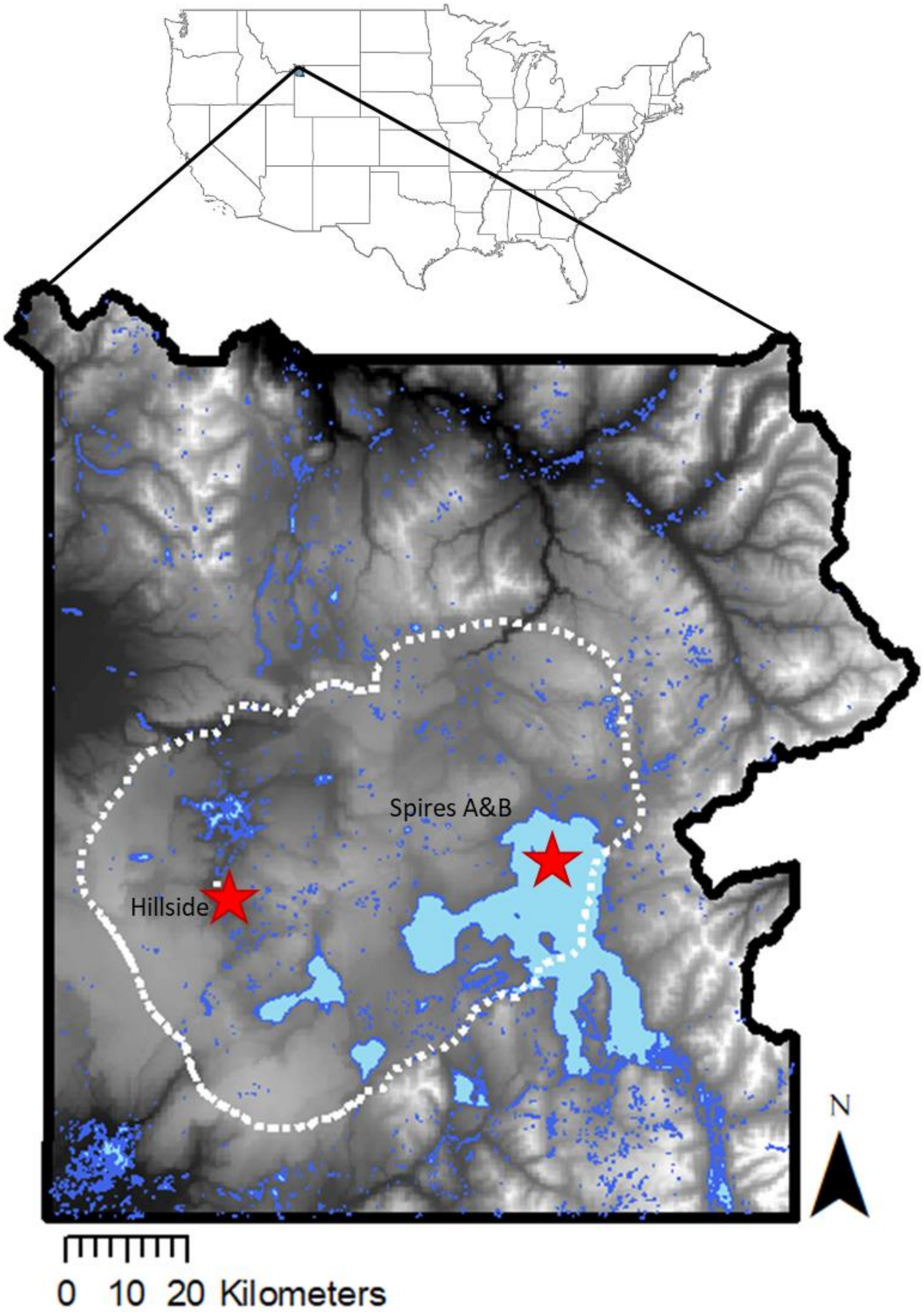


Figure 3.1: Digital Elevation Model map of Yellowstone National Park with the modern caldera boundary indicated by the dashed white line. Approximate sampling locations are indicated by stars.

3.2 Materials and methods

A sinter sample was collected near the top of a cliff from a currently inactive portion of the Hillside Group (Upper Geyser Basin). Two complete siliceous spires (SYL-A and SYL-B) were retrieved from the floor of Yellowstone Lake (depth = 15 m) in 1999 and 2016, respectively, and were sampled at USGS Denver in February 2017. U-series disequilibrium dating on two samples from Spire A yields dates of ~11 ka (Neil Sturchio, written communication, 1998; Morgan et al. 2003). The siliceous spires were sampled at fine resolution (47 total samples) throughout various lithologies for diatom frustule analysis (Figure 3.2). Sediment from core tops collected within the spire field also were sampled to assess modern sediment assemblage. Additionally, four sediment conduits, four vents, and one fissure from Yellowstone Lake were sampled for diatom analysis.

Each individual diatom subsample was added to a vial, weighed to approximately 0.1 gram, and processed with 30% hydrogen peroxide to remove organic matter (Battarbee 2003). A known concentration of polystyrene microspheres was added to each subsample to allow quantitative analyses of diatom concentration. Residual samples were rinsed three times with deionized water to dilute hydrogen peroxide and then mounted to slides with Naphrax. Diatom valves were identified at 1000 X magnification using a Leica DM2500 transmitted light microscope with differential interference contrast (DIC) equipped with a 5-megapixel camera or a Leica DMRX fitted with phase contrast. Diatom species were identified using diverse taxonomic resources relevant to the northern Rocky Mountains (e.g. Bahls 2005, Spaulding and Edlund 2016). Additionally, select diatom samples were analyzed with a Tescan Vega 3 scanning electron microscope

(SEM) operated at 10 kV in high vacuum mode. Presence and absence of diatom valves were assessed for sampled hydrothermal features of Yellowstone Lake (Table 3.1). For slides with diatoms present in abundance (>25 valves), frustules were identified to species and enumerated to a total of 300 diatom valves per slide. A metric of diatom concentration (valves/gram) was calculated using microsphere concentrations, totals, and sample weights. Assemblage counts were converted to percentages and plotted in program C2 (Juggins 2003).

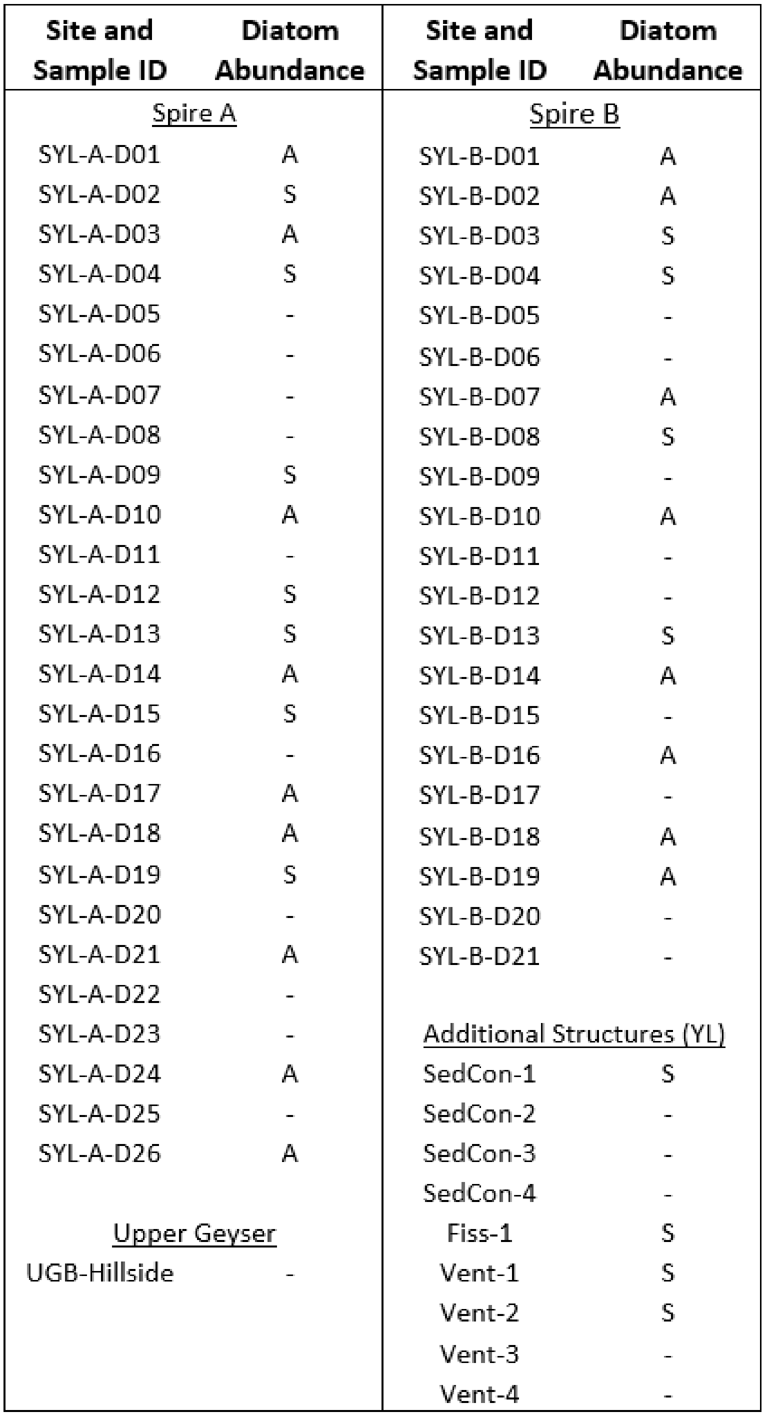
3.3 Results

In total, 57 samples from siliceous hydrothermal deposits were analyzed for diatom presence (Table 3.1). Preserved diatom frustules in traditionally prepared light microscope slides were not present in 25 of these samples. Of the remaining 32, 17 had diatom valves present in abundance (>300 valves/slide), and 15 had sparse (<25 valves/slide) diatom abundance.

3.3.1 Hillside Group

Sinter collected from the Hillside Group contained noticeably abundant fossilized diatom frustules under SEM. Sinter was dominated by *Rhopalodia gibberula* (Ehrenberg) O. Müller and also included valves of *Pinnularia subrostrata* (A. Cleve) Cleve-Euler and *Amphora* Ehrenberg ex Kützing sp. (Figure 3.2). Diatom frustules were found in various stages of diagenetic alteration and incorporation into the surrounding amorphous silica matrix (Figure 3.2). Thus, diatom valves were not visible under traditional light microscope analysis.

Table 3.1: Hydrothermal deposit material analyzed in this study. Diatom abundance in traditionally processed light microscope slides were documented as either abundant (A), sparse (S), or absent (-).



Site and Sample ID	Diatom Abundance	Site and Sample ID	Diatom Abundance
Spire A		Spire B	
SYL-A-D01	A	SYL-B-D01	A
SYL-A-D02	S	SYL-B-D02	A
SYL-A-D03	A	SYL-B-D03	S
SYL-A-D04	S	SYL-B-D04	S
SYL-A-D05	-	SYL-B-D05	-
SYL-A-D06	-	SYL-B-D06	-
SYL-A-D07	-	SYL-B-D07	A
SYL-A-D08	-	SYL-B-D08	S
SYL-A-D09	S	SYL-B-D09	-
SYL-A-D10	A	SYL-B-D10	A
SYL-A-D11	-	SYL-B-D11	-
SYL-A-D12	S	SYL-B-D12	-
SYL-A-D13	S	SYL-B-D13	S
SYL-A-D14	A	SYL-B-D14	A
SYL-A-D15	S	SYL-B-D15	-
SYL-A-D16	-	SYL-B-D16	A
SYL-A-D17	A	SYL-B-D17	-
SYL-A-D18	A	SYL-B-D18	A
SYL-A-D19	S	SYL-B-D19	A
SYL-A-D20	-	SYL-B-D20	-
SYL-A-D21	A	SYL-B-D21	-
SYL-A-D22	-		
SYL-A-D23	-	Additional Structures (YL)	
SYL-A-D24	A	SedCon-1	S
SYL-A-D25	-	SedCon-2	-
SYL-A-D26	A	SedCon-3	-
		SedCon-4	-
Upper Geyser		Fiss-1	S
UGB-Hillside	-	Vent-1	S
		Vent-2	S
		Vent-3	-
		Vent-4	-

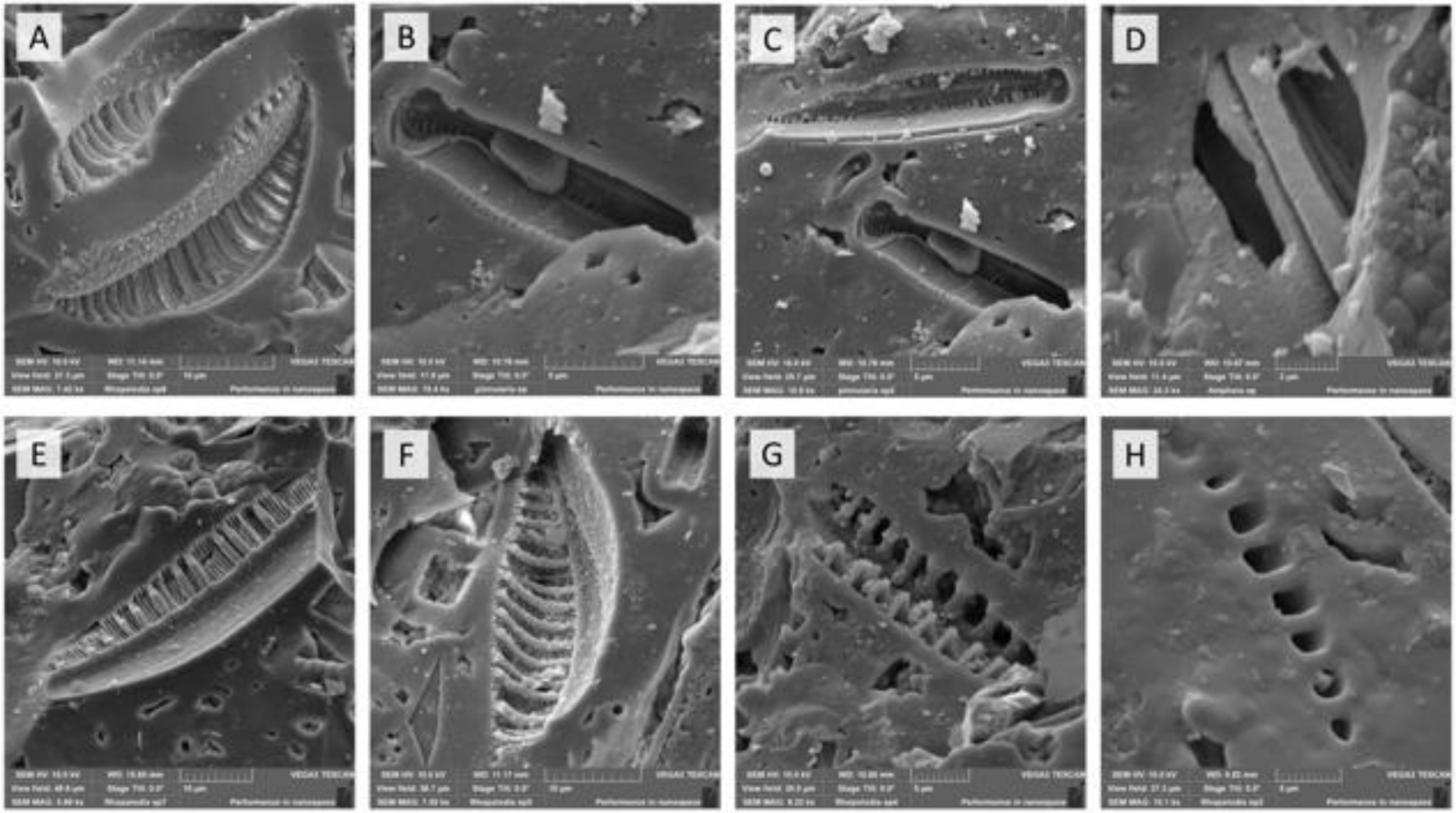


Figure 3.2: SEM photographs of diatoms preserved in the Hillside Group sinter: (A) *Rhopalodia gibberula*, (B, C) *Pinnularia subrostrata*, and (D) *Halamphora* sp.1. Frustules were found in various stages of diagenetic alteration – ranging from high preservation of both fine and coarse features (E), mostly coarse and minimal fine feature preservation (F, G), to minimal preservation of coarse features (H).

3.3.2 Yellowstone Lake hydrothermal structures

Diatoms were concentrated on the outer edge of both siliceous spires retrieved from the floor of Yellowstone Lake (Figure 3.3) and were rare in the interior matrix. Of the 26 samples collected from the once-intact small (~60 cm full length) Spire A, nine contained diatom frustules in abundance, seven sparingly, and ten did not contain diatom frustules (Table 3.1). Of the 21 samples collected from Spire B, abundant diatom valves were present in eight samples, sparingly in four, and absent from nine samples (Table 3.1). Additionally, SEM analysis of samples without diatoms in light microscopy also showed no evidence of diatoms, including no evidence of diagenetic alteration into the surrounding amorphous silica matrix.

The assemblage of Spire A (Figure 3.4) is dominated by *Staurosirella neopinnata* Morales (25-35%), *Amphora micra* Levkov (<50%), and *Amphora inariensis* Krammer (<45%). Fewer numbers of small colonial tychoplanktic *Fragilaria* s.l. species, including *Pseudostaurosira alvareziae* Cejudo-Figueiras, E.A.Morales & Ector (<20%), *Pseudostaurosira brevistriata* (Grunow) D.M. Williams & Round (<5%), and *Pseudostaurosiropsis* E.A. Morales spp. (<5%), benthic *Karayevia suchlandtii* (Hustedt) Buktiyarova (5-15%), and the planktic species *Aulacoseira subarctica* (O. Müller) E.Y.Haworth (<30%), *Stephanodiscus niagarae* Ehrenberg s.l. (<5%), and *Stephanodiscus minutulus* (Kützing) Cleve Möller (<5%) also are present. In total, 13 species had an abundance of >2% for any single Spire A sample.

The assemblage of Spire B (Figure 3.5) is also dominated by tychoplanktic *S. neopinnata* (20-45%) with lesser numbers of *P. alvareziae* (<10%). Planktic species *A. subarctica* (<40%) and *S. minutulus* (<10%) are dominant in two samples (SYL-B D01

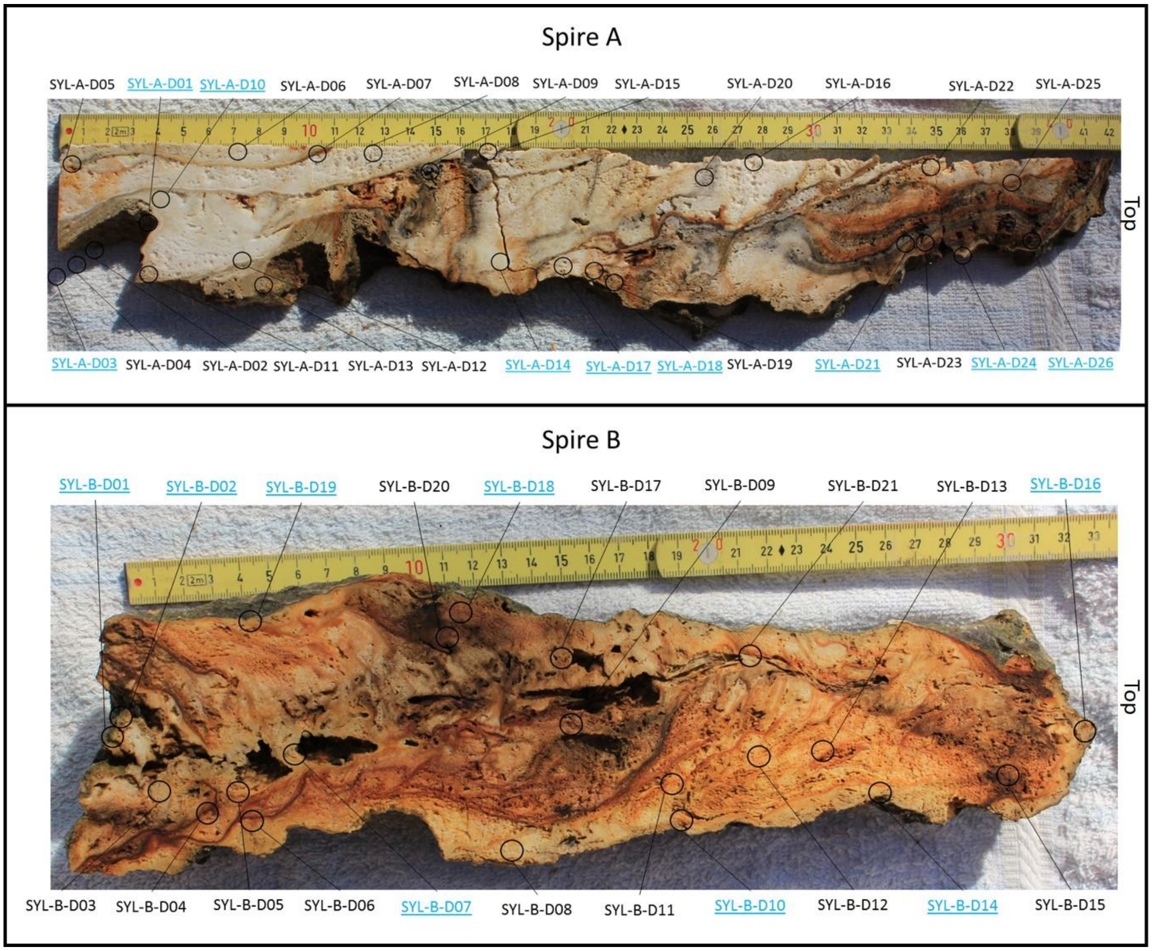


Figure 3.3: Photographs of Spire A (top) and Spire B (bottom) cross-sections with diatom sampling locations circled and labeled with corresponding sample ID. Those samples with abundantly preserved diatom frustules are indicated by blue underlined text. Note that samples SYL-A-D03, SYL-A-D04, and SYL-A-D02 were collected from a block of hydrothermally-altered sediment not pictured in this image (see Morgan et al. 2003).

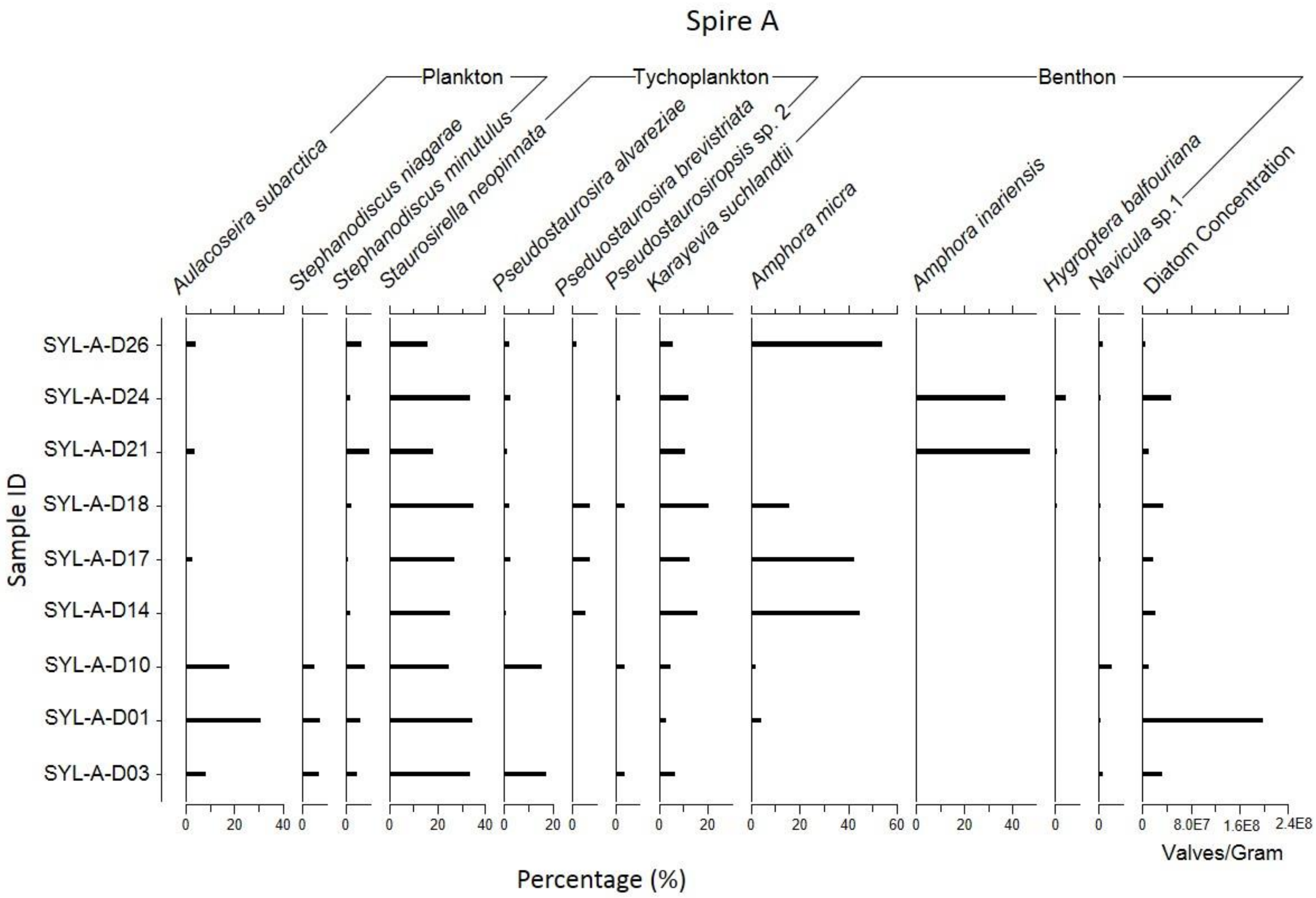


Figure 3.4: Diatom species percentages of abundant (>2%) taxa in Spire A. Samples are organized sequentially with those collected near the base of the spire at the bottom of the diagram

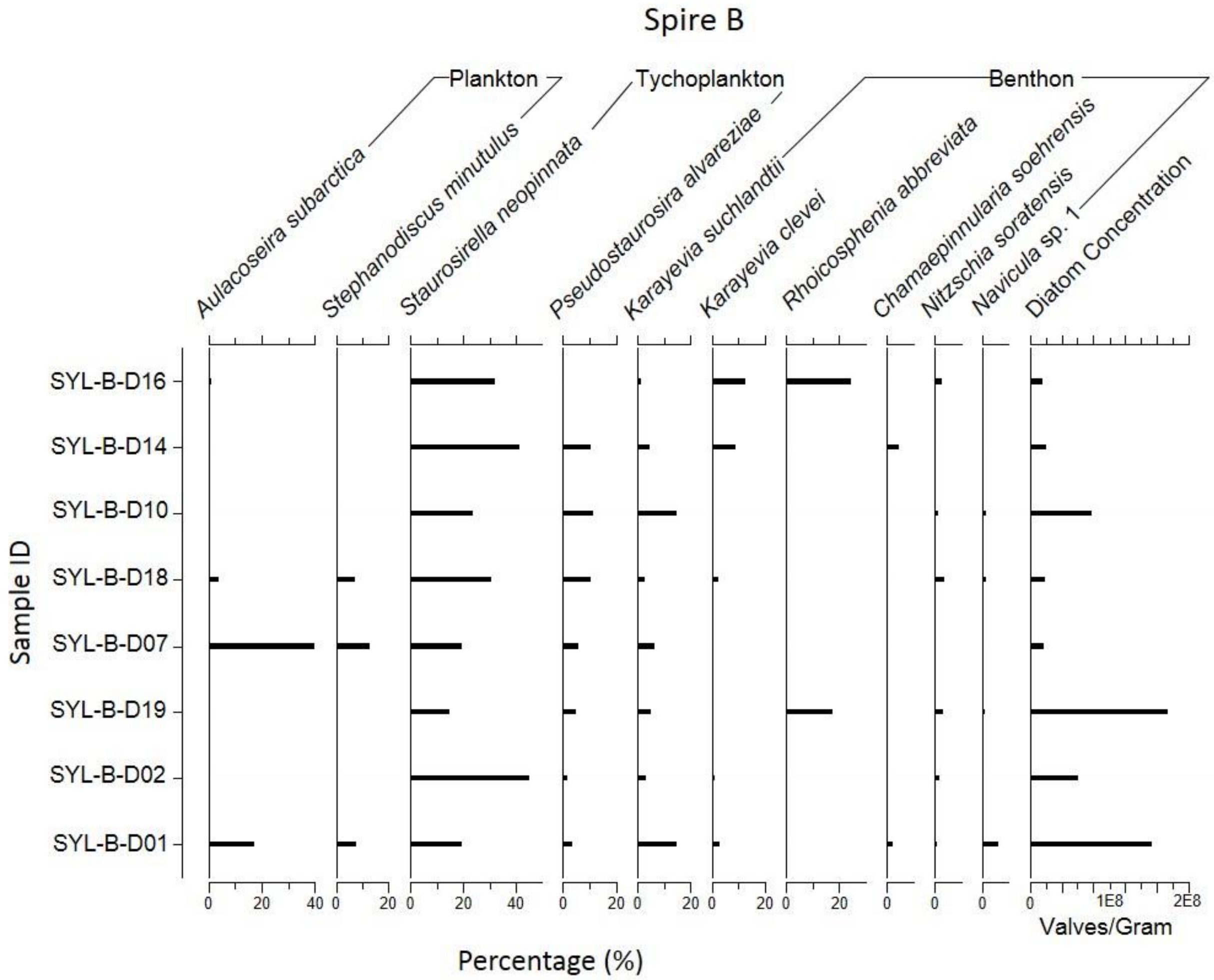


Figure 3.5: Diatom species percentages of abundant (>2%) taxa in Spire B. Samples are organized sequentially with those collected near the base of the spire at the bottom of the diagram

and SYL-B-D07). Benthic species *K. suchlandtii* (<15%), *Rhoicosphenia abbreviata* (C. Agardh) Lange-Bertalot (<20%), and *Karayevia clevei* (Grunow) Bukhtiyarova (<15%) also are present. In Spire B, ten species were found at >2% abundance in any sample. The species composition of spire samples is vastly different from modern sediment collected from the spire field, which is dominated by planktic species *S. minutulus*, *Stephanodiscus yellowstonensis* Theriot & Stoermer s.s., *A. subarctica*, and *Asterionella formosa* Hassall.

Among the other hydrothermal structures sampled from Yellowstone Lake, none had abundantly preserved diatoms. However, two vents, one sediment conduit, and one fissure had sparsely preserved diatom frustules (Table 3.1). The diatom assemblages present in these samples were similar to those of sediment samples elsewhere in Yellowstone Lake – primarily consisting of planktic species *S. minutulus, S. yellowstonensis*, *A. subarctica,* and *A. formosa* (Interlandi et al. 1999). Images of species common in Yellowstone Lake hydrothermal structures, including spires and additional structures, are presented in Figure 3.6.

3.4 Discussion

The fossil diatoms found in association with sinter of Hillside Group in the Upper Geyser Basin are indicative of moist soil or shallow lake environments. Specifically, *Rhopalodia gibberula* has been reported in both benthic periphyton mats and moist soils (Spaulding & Edlund 2016), as well as in low to moderate conductivity environments (Patrick & Reimer 1975). *Rhopalodia gibberula* also has heavily silicified valves (Smol & Stoermer 2010) and, thus, are common in environments where silica is not limiting. Additionally, *R. gibberula* commonly hosts endosybiotic atmospheric nitrogen-fixing cyanobacteria,

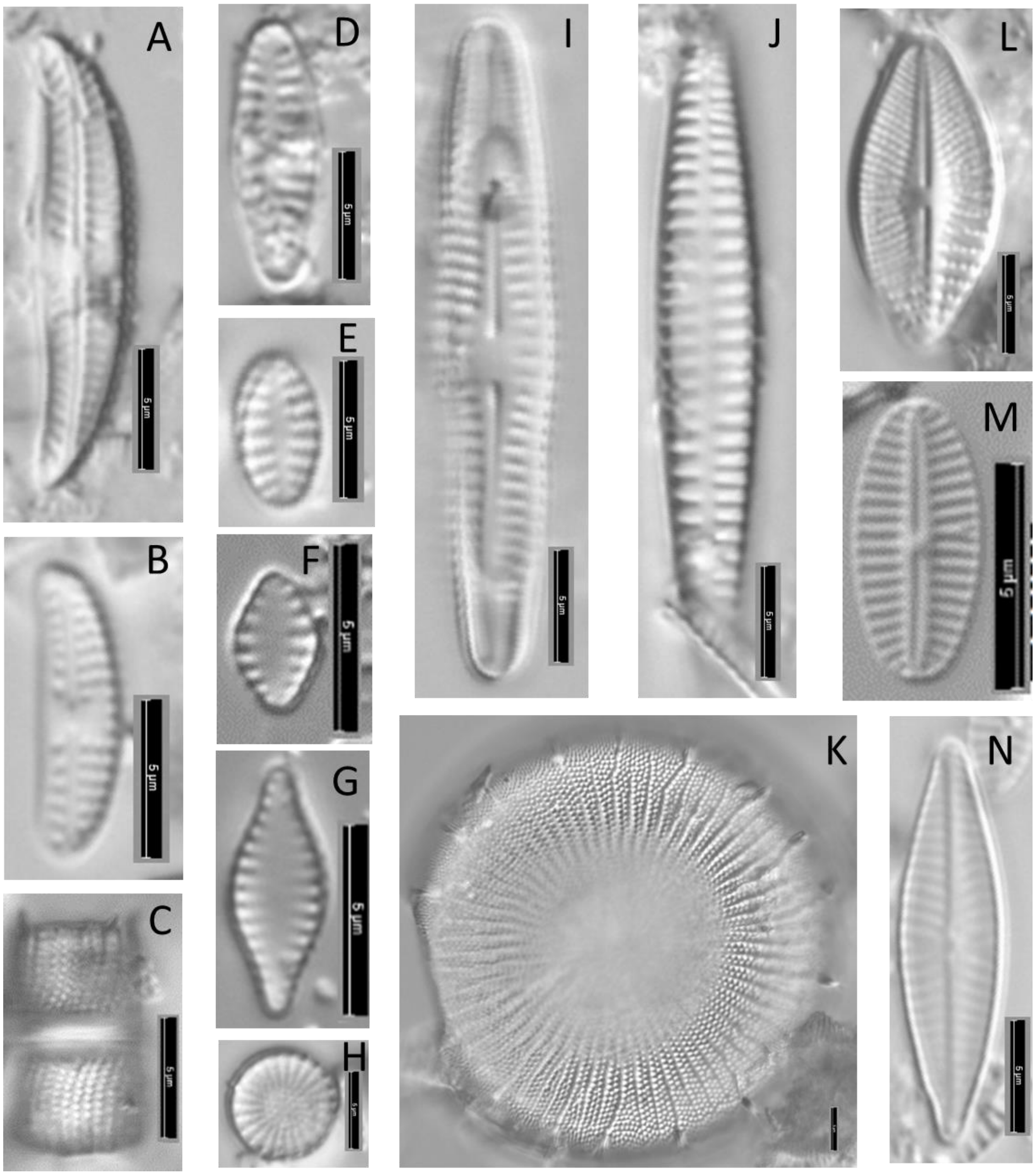


***Figure 3.6**: Light microscope images of common diatoms encountered in Yellowstone Lake hydrothermal structures (length of black bar = 5 µm): (A) Amphora inariensis, (B) Amphora micra, (C) Aulacoseira subarctica, (D) Pseudostaurosira alvareziae, (E) Staurosirella neopinnata, (F) Pseudostaurosira brevistriata,(G) Pseudostaurosiropsis sp. 2, (H) Stephanodiscus minutulus, (I&J) Rhoicosphenia abbreviata, (K) Stephanodiscus yellowstonensis s.s., (L) Karayevia clevei, (M) Karayevia suchlandtii, and (N) Navicula sp. 1.*

enabling growth in low nitrogen environments. *Pinnularia* and *Halamphora* species also are commonly encountered growing in moist soil environments among other environments including fresh and brackish waters (Van Kerckvoorde et al. 2000, Antonelli et al. 2017). Thus, the presence of *Pinnularia* and *Halamphora*, and the overwhelming abundance of *R. gibberula* in the Hillside Group, coupled with its ability to grow in nitrogen-poor, silica-rich environments, and a wide range of salinities, indicate that the preserved diatom assemblage was most likely growing *in-situ* on the moist sinter.

Whereas Shanks et al. (2005, 2007) hypothesized that diatoms entered Yellowstone Lake siliceous spire structures through overhead water column fallout, assessment of diatom assemblage habitat preferences indicate *in-situ* diatom growth. Specifically, *S. neopinnata*, *P. alvareziae*, and *Pseudostaurosiropsis* spp. are species within the small colonial *Fragilaria* s.l. complex that are commonly found living attached to substrates in alpine lake systems (Saros et al. 2003, Spaulding 2016). The presence of *S. neopinnata* likely indicates persistence of generally shallow-water conditions or high light penetration to the site; *S. neopinnata* and *P. alvareziae* prefer alkaline pH (Bradbury et al. 2004). Further, the benthic assemblage contains several benthic species that are tolerant of alkaline or saline conditions. For example, the small *Amphora* that dominate the Spire A assemblage, and *R. abbreviata,* present in Spire B, are commonly encountered in moderately saline waters <32 ppt (Herbst & Blinn 1998, Wehr & Sheath 2003). *Karayevia* is euryhaline, growing equally well in a range of salinities (Yamamoto et al. 2017). As such, the tychoplanktic and benthic diatom assemblages of Yellowstone Lake siliceous spires indicate growth in a relatively shallow (20-25 m), alkaline environment during the early Holocene.

Spire diatom species assemblages differed greatly from modern sediments collected within the spire field and from shallow sediments collected from a paleoshoreline dated at the ~9.3 ka (see Chapter 5). The diatom assemblage of nearby sediment is overwhelmingly dominated (>95%) by planktic species (*S. minutulus, S. yellowstonensis* s.s., *A. subarctica*, and *A. formosa*), which have been characteristic of the Yellowstone Lake diatom flora from ~10 ka to the present (Kilham et al. 1996, Theriot et al. 1997, 2006). Thus, the predominantly tychoplanktic and benthic forms preserved in Yellowstone Lake siliceous spires indicate diatoms likely grew directly on the structures, rather than settling out of the water column or becoming entrained from nearby sediment.

A few exceptions include three samples (SYL-A-D03, SYL-A-D01, and SYL-A-D10) from Spire A that correspond to a block of hydrothermally altered sediment attached to the base of the structure (Figure 3.3). This sediment appears to have been altered at approximately the same time as spire formation, as a transitional form of the *S. niagarae/yellowstonensis* complex is present. The species *S. niagarae* was present in Yellowstone Lake in the late-Pleistocene but evolved into *S. yellowstonensis* between 13.7 and 10.0 ka (Theriot et al. 2006). The transitional form of *S. niagarae* s.l., which has one or more regions of three spineless costae, was present in the lake between ~11.7 and 11.2 ka (Theriot et al. 2006).

Samples in Spire B with higher planktic abundance (SYL-B-D01 and SYB-B-D07) do not correspond directly with a block of hydrothermally altered sediment. However, these samples are located in the open central conduit of the spire. It is probable that existing sediment was leached or that diatoms from overlying water were incorporated during initial vent and spire growth, entering the central cavity (Shanks et al. 2005, 2007). This also could be an artifact of post-hydrothermal activity sediment contamination. Spire B was collected from the lake floor, not intact and lying on its side. Thus, it is possible that sediment accrued in this conduit after the spire fell. The diatom assemblages of other Yellowstone Lake hydrothermal structures, including vents and conduits, indicate that fossil frustules can survive various forms of low-temperature alteration of *in-situ* sediments. However, the absence of abundantly preserved diatoms in these other structures is an indication that hydrothermal alteration modifies the original sediment – either including diatoms into the amorphous silica beyond visual recognition or by dissolving the majority of diatom valves, leaving behind only heavily silicified individuals.

Clustering of diatoms on spire edges may offer additional insight into the structure growth process. Whereas it is clear that diatoms were growing attached to the spires – either during the mature venting stage (24-76° C) or after hydrothermal activity ceased – what remains uncertain is the exact timing of growth in the absence of high-resolution dating. However, because the diatoms were found preserved up to ~2 cm into the amorphous silica matrix and not simply on the outer crust, it is likely that growth occurred before the spires ceased all hydrothermal activity. Additionally, because the spires are inactive today with no modern analogs, it is unclear whether the structures accrued incrementally during periods of higher hydrothermal activity, as evidenced by apparent growth fronts (Figure 3.3) or accrued quickly in more or less a single event. As such, the absence of diatom frustules in central samples with the exception of the open conduits of Spire B – both in light microscope and SEM analyses – suggests two alternative interpretations, assuming diatoms were able to grow proximal to near-boiling vent fluids: (1) The spires grew, more or less, in a single period of hydrothermal activity without long periods of stasis that allowed diatom colonization, or (2) there were hiatuses in growth that allowed for diatom community establishment, but these diatoms, if incorporated into the structure with additional growth, were hydrothermally altered beyond recognition.

3.5 Conclusions

Fossil diatom frustules were found in association with several alkaline-chloride hydrothermal deposits in Yellowstone National Park, specifically from the Upper Geyser and Yellowstone Lake Basins. Diatoms preserved in Hillside Group sinter were aerophilic, low-nitrogen diatoms in various stages of diagenetic alteration, which suggests growth directly upon moist substrate. Diatom frustules in Yellowstone Lake structures were likely growing on the outside of the structures before cessation of hydrothermal activity in the spire field. Diatom assemblages of these structures are indicative of a shallow, alkaline, and high conductivity microhabitats. Hydrothermal alteration of sediment and sinter in Yellowstone hydrothermal structures indicates that fossil diatoms are able to withstand some low-temperature alteration. Additionally, the absence of diatoms in traditional light microscope analyses and their presence in sinter material analyzed under scanning electron microscope serves as a call for innovative sampling and analysis methods for diatoms in continental hydrothermal regions. Additionally, this research suggests the utility of fossil diatoms to infer general environmental conditions during past periods of hydrothermal activity in inactive and long return-interval geyser deposits.

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CHAPTER 4

HIGH-RESOLUTION RECORD OF HOLOCENE PALEOLIMNOLOGICAL CONDITIONS IN YELLOWSTONE LAKE, WYOMING

Abstract

Yellowstone Lake is a major hydrothermal basin in Yellowstone National Park, where limnologic processes are influenced by both regional climate and hydrothermal activity. Here I utilize fossil diatoms to infer early- to late-Holocene (9876 calendar years BP to -37 cal years BP) ecosystem dynamics and climate conditions from Yellowstone Lake sediments. The abundant fossil diatoms in the fossil assemblage include *Stephanodiscus minutulus*, *Aulacoseira subarctica*, *Asterionella formosa*, *Stephanodiscus yellowstonensis*, and *Fragilaria* (sensu lato). Constrained cluster analysis (CONISS) defines two diatom assemblage shifts at ca. 6475 and 1500 cal years BP. At the beginning of the record (9876-6475 cal years BP), the assemblage is dominated by *S. minutulus,* a species that requires high concentrations of phosphorus and blooms just after ice off. The overall species composition is consistent with a period when spring turnover was relatively short due to warmer summers and possibly also cooler winters. In the middle section of the record (6475-1500 cal years BP), the diatom assemblage includes the increased abundance of *A. subarctica* and *A. formosa* – species that bloom later in the spring or in early summer during periods of extended isothermal mixing. This suggests either a transition to earlier ice-out and/or cooler summers. Around 1500 cal years BP, the diatoms show an increase in *A. formosa*, which responds to increased nitrogen concentrations, consistent with a transition to wetter winters and springs with an associated increase in nitrogen (N) inputs from runoff. Thus, the diatom assemblage of

Yellowstone Lake is largely controlled by the direct climate impacts of temperature and precipitation on stratification dynamics and nutrient inputs.

4.1 Introduction

Regional climate in the northern Rocky Mountains is affected by solar radiation and its direct impacts on temperature and atmospheric circulation, including the influence of the eastern Pacific subtropical high-pressure system and monsoonal circulation from the Gulf of Mexico (Brunelle et al. 2005, Whitlock et al. 2012). Yellowstone National Park has traditionally been classified into two climate regimes: summer-dry in southern Yellowstone and summer-wet in northern Yellowstone. Summer-wet regimes receive substantive precipitation from spring and summer monsoonal circulation, whereas summer-dry regimes receive the majority of precipitation as winter snowfall under the associated influence of the northeast Pacific subtropical high-pressure system on the jet stream (Whitlock 1993). The boundary between these two climate regimes is controlled by topography and, therefore, was originally suggested to have remained constant throughout the Holocene (Whitlock and Bartlein 1993). More recent multi-proxy analyses of sediment records in the Greater Yellowstone Ecosystem indicate that although coherent regional patterns are evident in Holocene paleoclimate data, that the summer-wet/summer-dry hypothesis is likely overly simplified (Whitlock et al. 2012).

Previous fossil diatom research in the Greater Yellowstone Ecosystem also demonstrated that lake response to paleoclimatic conditions is spatially variable. For example, a comparison of three lakes in the Greater Yellowstone Ecosystem found synchronous diatom assemblage changes in the early Holocene, indicating that large-scale changes in climate largely controlled diatom responses (Lu et al. 2017). However, species composition varied among lakes, indicating the importance of localized factors, including water chemistry and basin size (Lu et al. 2017). Similarly, a comparison of four lakes in western Montana, including Crevice Lake in Yellowstone, found synchronous changes in the late-Holocene diatom stratigraphy, yet the nature of the changes in diatom species composition indicated that two of the lakes were more sensitive to seasonal precipitation changes and two were more sensitive to changes in energy balance (Bracht-Flyr and Fritz 2012). Thus, previous research in Yellowstone National Park indicates that while diatom communities have the ability to record large-scale Holocene climate change, lakes with different basin morphometries and water chemistries are sensitive to different regional climate factors. As such, further developing a network of paleoclimate reconstructions from lakes of different sizes and locations is imperative to refining scientific understanding of past climate variability in the Greater Yellowstone Ecosystem.

A hierarchy of climate influences – regional to local – that vary with time affect fossil diatom stratigraphy on decadal to millenial scales (Theriot et al. 2006, Smol and Stoermer 2010, Fritz and Anderson 2013). Specifically, climate directly impacts lake thermal and hydrologic budgets, which in turn impact ice cover, mixing, and stratification timing, all of which are important in deteriming diatom assembalge dynamics in Yellowstone Lake (Wolin and Stone 2010, Saros et al. 2012, Fritz and Anderson 2013). Additionally, climate forcings also control catchment processes, which indirectly impact diatom assemblages. Specifically, climate influences soil development, hydrology, and catchment vegetation, in turn controling both water chemistry and nutrient availibility, important drivers of diatom assemblage composition (Harris et al. 2006, Fritz and

Anderson 2013). Thus, fossil diatom assemblages can be used to infer past direct and indirect climatic conditions.

The objective of this research is to utilize fossil diatoms to infer early- to late-Holocene (9876 to $^{-}$37 cal years BP) ecosystem dynamics of Yellowstone Lake. Thus, this research investigates the direct and indirect Holocene climate forcings on Yellowstone Lake via analysis of its fossil diatom record.

4.1.1 Site Description

Yellowstone Lake (Figure 4.1; 44°30'N, 110°20'; 2,350 m elevation) is the largest alpine lake in North America, with an area of 344 square kilometers and a maximum depth of 107 meters (Interlandi et al. 2003). In most years, it stratifies in both winter and summer with periods of mixing in spring and autumn (dimictic). The lake has low to moderate nutrient concentrations (Table 4.1) and consists of four primary basins: two formed as part of a volcanic caldera (West Thumb and the Central Basin), and two formed through glacial processes (South Arm and Southeast Arm).

Table 4.1: Limnologic characteristics of Yellowstone Lake (modified from Theriot et al. 1997).

pH	**Secchi Depth (m)**	**Total PO$_4$ (mg/L)**	**Kjeldahl-N (mg/L)**	**Conductivity (uS/cm)**	**Total Alkalinity (ueq/L)**
7.4	8.1	0.2	0.2	86	64

Modern climate for the study area is Continental Subarctic, with 530 mm of average annual precipitation and an average annual temperature range between -6.2 and 8 °C (Western Regional Climate Center 2019). Modern vegetation in the Yellowstone Lake catchment is a mosaic of lodgepole pine (*Pinus contorta*) and mixed conifer (*Abies lasiocarpa*, *Picea engelmanii*, and *Pinus albicaulis*) forest. Forest type is largely related to soils of the area, with lodgepole pine dominating on rhyolitic soils and mixed conifer forests dominating in andesitic and basaltic soils (Whitlock 1993).

4.2 Methods

An 11.99-m sediment core (HD-YLAKE-2C) was retrieved with a Kullenberg corer (Kelts et al. 1986) from 61 m water depth in the northern portion of the Yellowstone Lake Central Basin in September 2016 (Figure 4.1). Cores were shipped to the LacCore facility at the University of Minnesota – Twin Cities for initial core description, sampling, and archiving. Initial core description included core splitting, high-resolution photography, and collection of a suite of elemental data with a Geoteck Multi-Sensor Core Logger (MSCL). Sediments were laminated throughout, and a thin (~0.5 cm) lens of Mazama Ash was visible at 9.4 m in the core.

Ten macrofossil and pollen samples were collected from discrete locations throughout the core for radiocarbon dating, and radiocarbon measurements were made using Accelerator Mass Spectrometry at Woods Hole Oceanographic Institution (NOSAMS), calibrated to IntCal13 (Reimer and Bayliss 2004). An age model was developed using the statistical package Bacon in R (Blaauw and Christen 2011), which uses Bayesian methods to reconstruct accumulation histories using prior assumptions about accumulation rate and its variability through time.

Subsamples (~0.5 cm^3) for diatom analysis were collected every 4 cm throughout the length of the core, for a total of 289 samples. A portion of each individual diatom subsample was added to a vial, weighed to approximately 0.1 gram, and processed with 30% hydrogen peroxide to remove organic matter (Battarbee 2003). Residual samples were rinsed thrice with deionized water to dilute hydrogen peroxide. A known concentration of polystyrene microspheres was added to each subsample to allow quantitative analyses of diatom concentration. After processing, each subsample was mounted to a slide with Naphrax. Diatom valves were identified at 1000 X magnification using a Leica DM2500 transmitted light microscope with differential interference contrast (DIC) equipped with a 5-megapixel camera or a Leica DMRX fitted with phase contrast. At least 300 diatom valves were identified to species and enumerated per slide.

Assemblage counts were converted to percentages and plotted in the program C2 (Juggins 2003). A metric of diatom concentration (valves/gram) was calculated using microsphere concentrations, diatom valve totals, and sample weights. Constrained cluster analysis (CONISS) was conducted on species assemblage percentage data using R package Rioja (Juggins 2017). A broken stick model, which compares cluster results against a randomly-generated curve representing background noise, was used to determine the appropriate number of zonations. A ratio of plankton:tychplankton:benthon percentages and diatom concentration (valves/gram) were calculated. A transient simulation climate model of Holocene seasonal temperature and precipitation anomalies for the Greater Yellowstone Ecosystem (Hostetler, unpublished data) was smoothed with a 200-year moving average window in the statistical program PAST (Hammer et al. 2001).

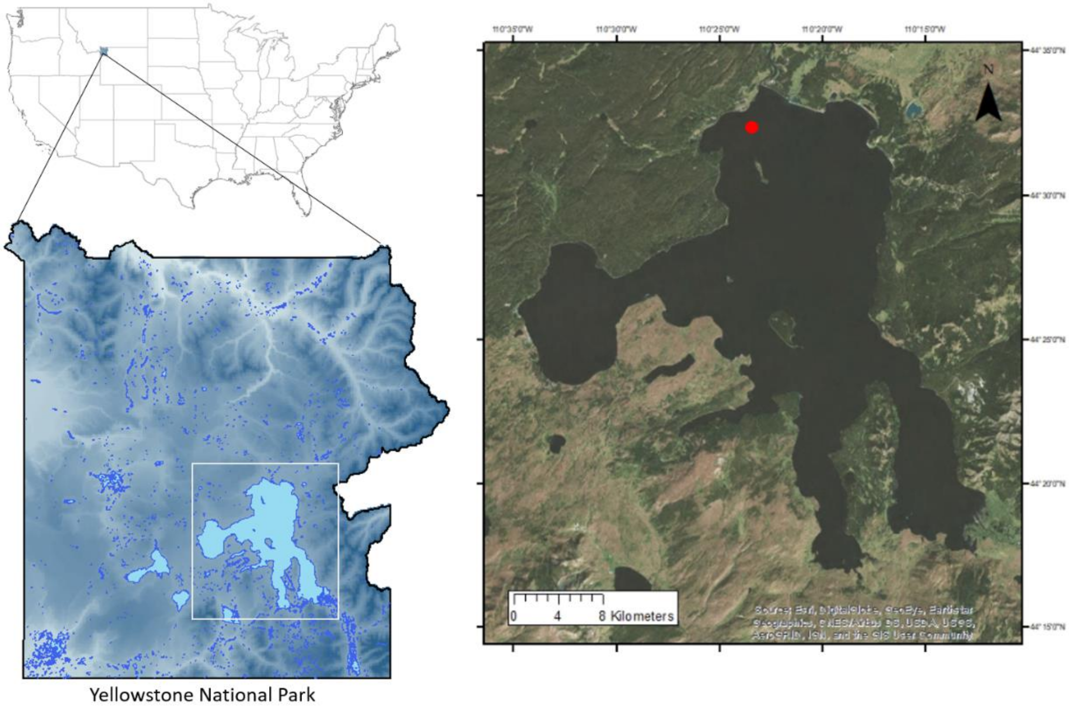

Figure 4.1: Digital Elevation Model inset of Yellowstone National Park (left) within the contiguous United States, with the location of Yellowstone Lake (white box). Satellite image (right) of the study site with coring location indicated by the red circle.

4.3 Results

Calibrated radiocarbon ages were systematically older for pollen extracts than for macrofossil samples, likely as a result of Yellowstone hydrothermal system carbon cycling dynamics (Schiller et al., manuscript in preparation). Thus, a subset of calibrated macrofossil ages and the Mazama Ash (Table 4.2) (Zdanowicz et al. 1999) were used to create the age-depth model. The age-depth model generates a minimum age (-37 cal years BP) for the surface, which is consistent with the absence of a large-magnitude charcoal peak from the 1988 Yellowstone fires and with the observed over-penetration of the Kullenberg corer. The calculated maximum age (9876 cal years BP) for the core base also is consistent with the regional Yellowstone National Park pollen stratigraphy

(Whitlock 1993). Additionally, this model generates an average sedimentation rate of 0.1 cm/year, which is similar to those previously recorded for Yellowstone Lake (Tiller 1995, Johnson et al. 2003, Morgan et al. 2007).

Table 4.2: Section ID and depth of samples used in creating an age-depth model, with corresponding composite depth, lab accession number, material type, ^{14}C age and error, and median probability calibrated age.

Section ID	Section depth (cm)	Composite depth (cm)	^{14}C Lab #	Type	^{14}C age (years BP)	^{14}C error	Calibrated age (cal years BP)
YLAKE-YL16-2C-1K-3-W	132	328	OS-135957	Plant/Wood	2590	20	2743
YLAKE-YL16-2C-1K-4-W	71.5	402	OS-135958	Plant/Wood	3150	25	3378
YLAKE-YL16-2C-1K-6-W	3.5	624		Plant/Wood	4510	20	5156
YLAKE-YL16-2C-1K-8-W	85	940	-	tephra	-	-	7627

The Yellowstone Lake fossil diatom assemblage (>5% abundance) is comprised of four planktic species (*Stephanodiscus minutulus*, *Aulacoseira subarctica*, *Asterionella formosa*, and *Stephanodiscus yellowstonensis*) and one tychoplanktic species complex (*Fragilaria* (sensu lato)) (Figure 4.3). CONISS and associated broken stick model (Figure 4.4) results were used to group the percent abundance data into three major zones (Figure 4.5): – Zone 1 (9876-6475 cal years BP), Zone 2 (6475-1500 cal years BP), and Zone 3 (1500-$^{-}$37 cal years BP) – with visually-determined subzones (Figure 4.4). Seasonal (Figure 4.4) and annual (Figure 4.6) temperature and precipitation anomalies are presented stratigraphically.

4.3.1 Zone 1 (9876-6475 cal years BP)

Zone 1 is dominated by *S. minutulus* (>50%), sometimes in excess of 90%, with peaks in the relative abundance of *A. subarctica* (<35%) and *A. formosa* (<40%), often in

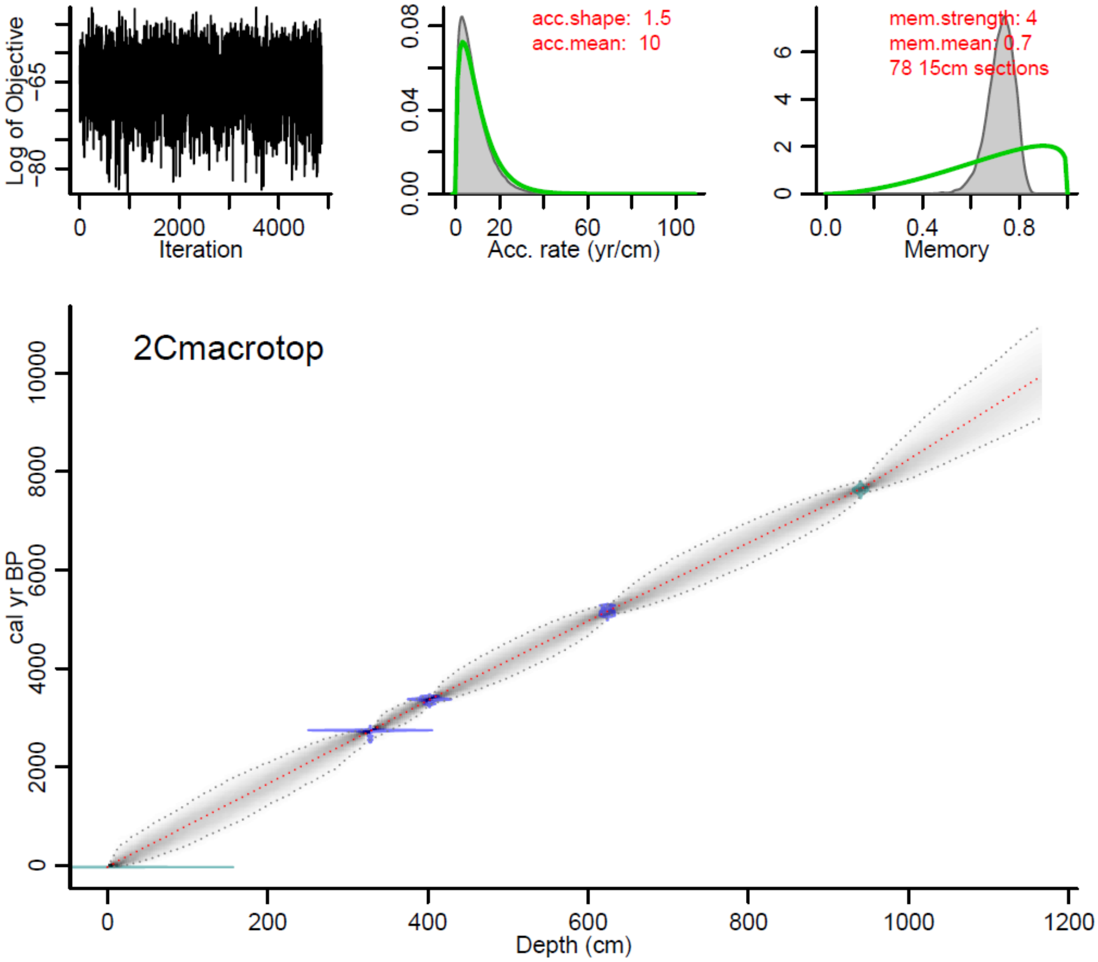

Figure 4.2: Bayesian age-depth model for Yellowstone Lake sediment core HD-YLAKE-2C. Blue diamonds and associated error bars represent dated material input into the model. The grayscale cloud, with boundaries outlined in dotted lines by the 95% confidence interval, represents age model probability with darker shades indicating higher probability. The red dotted line designates the weighted mean age-depth model. Inset figures (left to right) show iteration history and the prior (green curve) and posterior (grey curve) densities for the mean accumulation rate and memory.

tandem, with corresponding decreases in *S. minutulus*. This zone routinely has high percentages of plankton and very low relative abundances of (<5%) tychoplankton and benthon. *S. yellowstonenesis* and *S. oregonicus* are at their lowest abundance (<2%) in Zone 1. Within Zone 1 are several intervals with distinctive increases in *A. subarctica, A. formosa*, and/or *S. yellowstonensis* relative to *S. minutulus*: ~8900, 8375-8150, 8050-7925, 7675-7525, and 6700 cal yr BP. Zone 1 is further subdivided into two subzones – 1A (9876-8950 cal years BP), 1B (8950-6475 cal years BP). Determination of sub-zones within Zone 1 was based upon slightly higher percentages of *S. yellowstonensis* and *A. formosa.* Small *Fragilaria* (sensu lato) are less than 5% of the assemblage in subzone 1A.

During the latter portion of the early Holocene (after 9500 cal years BP), reconstructed winter and spring temperature anomalies (Figure 4.6) for the Greater Yellowstone Ecosystem fall below the pre-Industrial mean, whereas summer anomalies fall above the pre-Industrial mean. Precipitation anomalies decreased in the early Holocene (10000-9000 cal years BP) and then increased, with sustained increased precipitation 8500-7500 cal years BP. Spring precipitation anomaly values are around the pre-Industrial mean.

4.3.2 Zone 2 (6475-1500 cal years BP)

Zone 2 is also dominated by *S. minutulus* (30-90%), but with greater variability in percentages than Zone 1, primarily driven by changes in the relative abundance of *A. subarctica* (5-50%) and *A. formosa* (5-30%). In addition, the relative abundances of *A. subarctica*, as well as *S. yellowstonensis* (<15%), *S. oregonicus* (<15%), and small

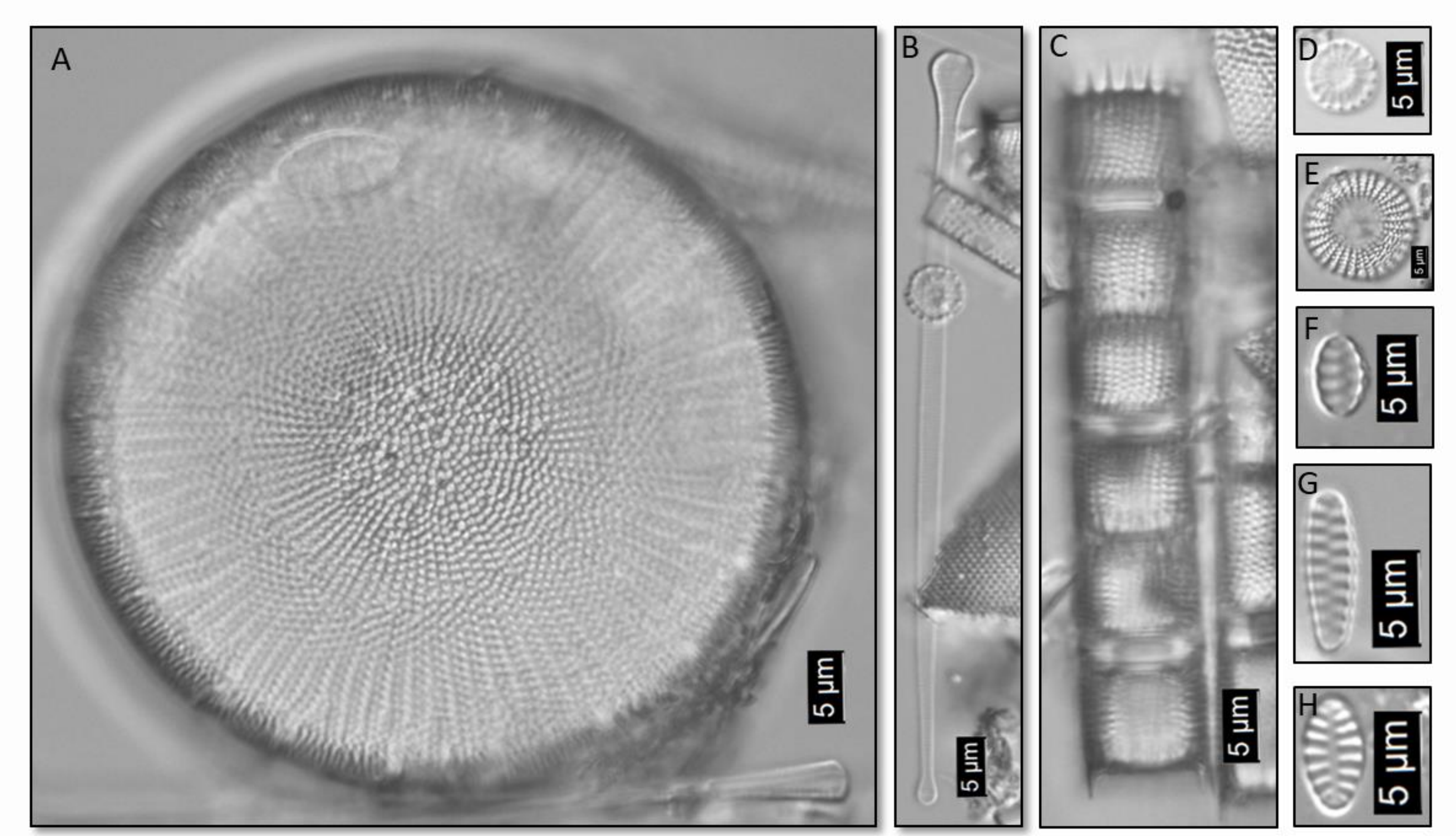

Figure 4.3: Light microscope images of abundant (>5%) diatom taxa encountered in Yellowstone Lake sediments (black bar = 5 µm): (A) *Stephanodiscus yellowstonensis*, (B) *Asterionella formosa*, (C) *Aulacoseira subarctica*, (D) *Stephanodiscus minutulus*, (E) *Stephanodiscus oregonicus*, (F-H) small colonial *Fragilariaceae*.

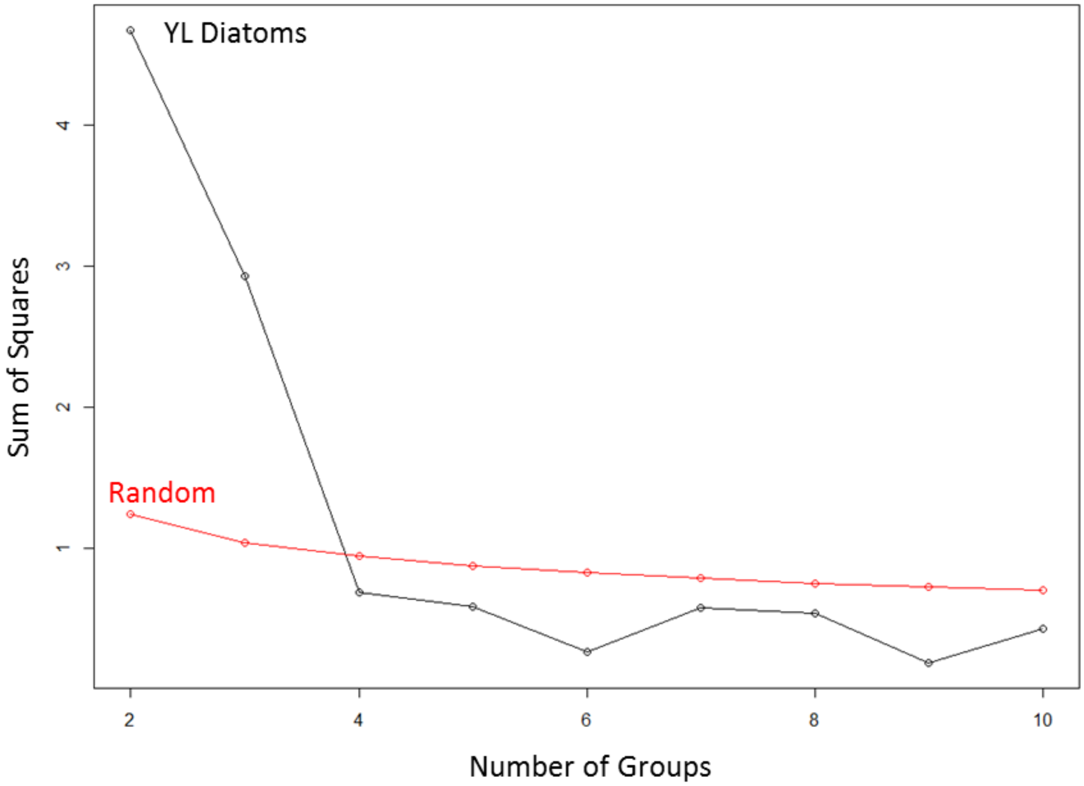

Figure 4.4: Broken stick model derived from CONISS (Juggins 2017) of Yellowstone Lake fossil diatom species percentages. The black line represents the diatom assemblage data, while the black line represents a randomly-generated dataset.

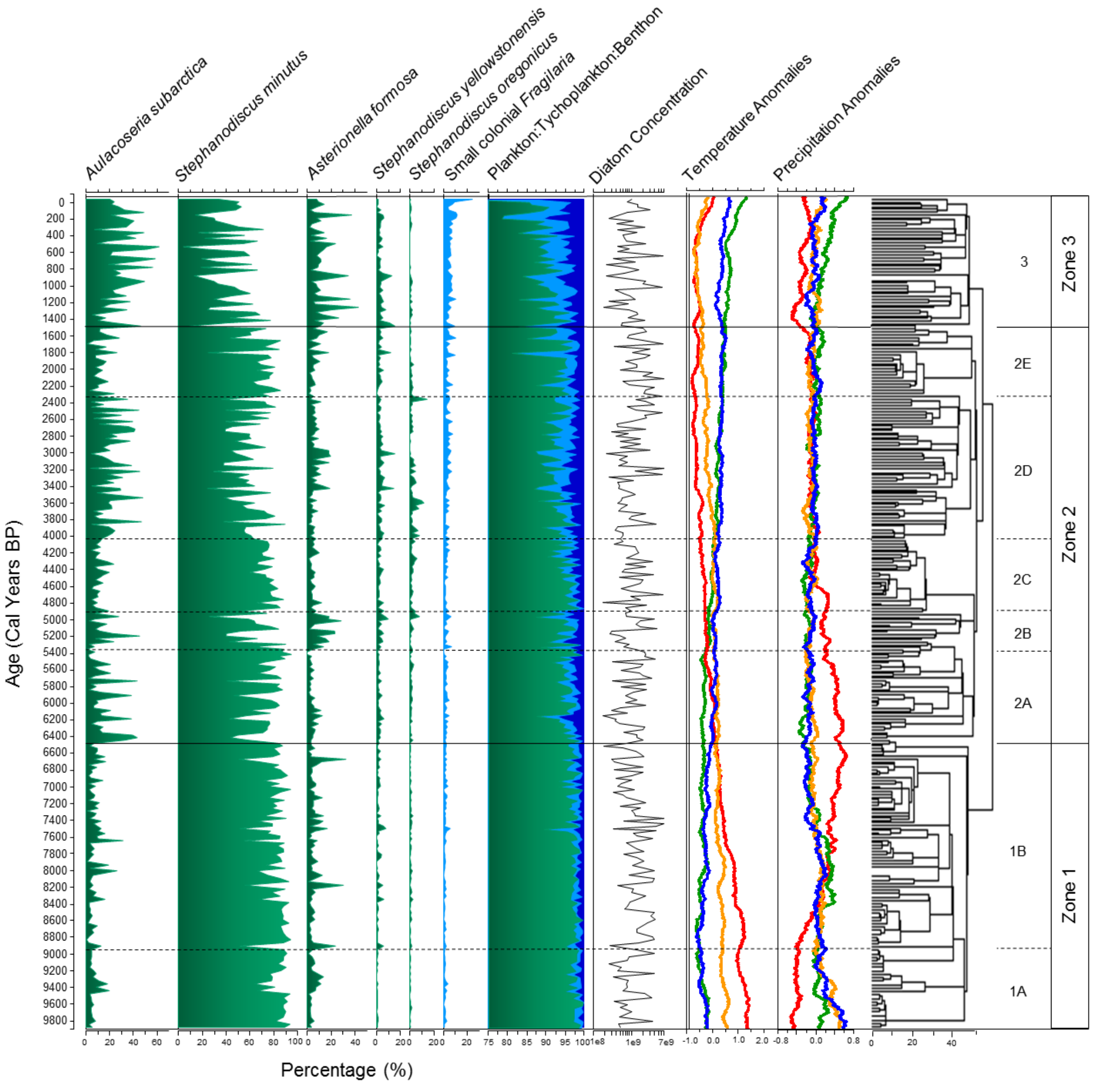

Figure 4.5: Stratigraphic plot by age of abundant (>5%) diatom species, plankton:tychoplankton:benthon ratio, log-transformed diatom concentration (valves/gram), and 200-year smoothed GENMOM temperature and precipitation anomaly seasonal models with constrained cluster analysis (CONISS) tree and corresponding zones. The GENMOM model curves are colored blue for winter (December, January, February), green for spring (March, April, May), red for summer (June, July, August), and orange for autumn (September, October, November).

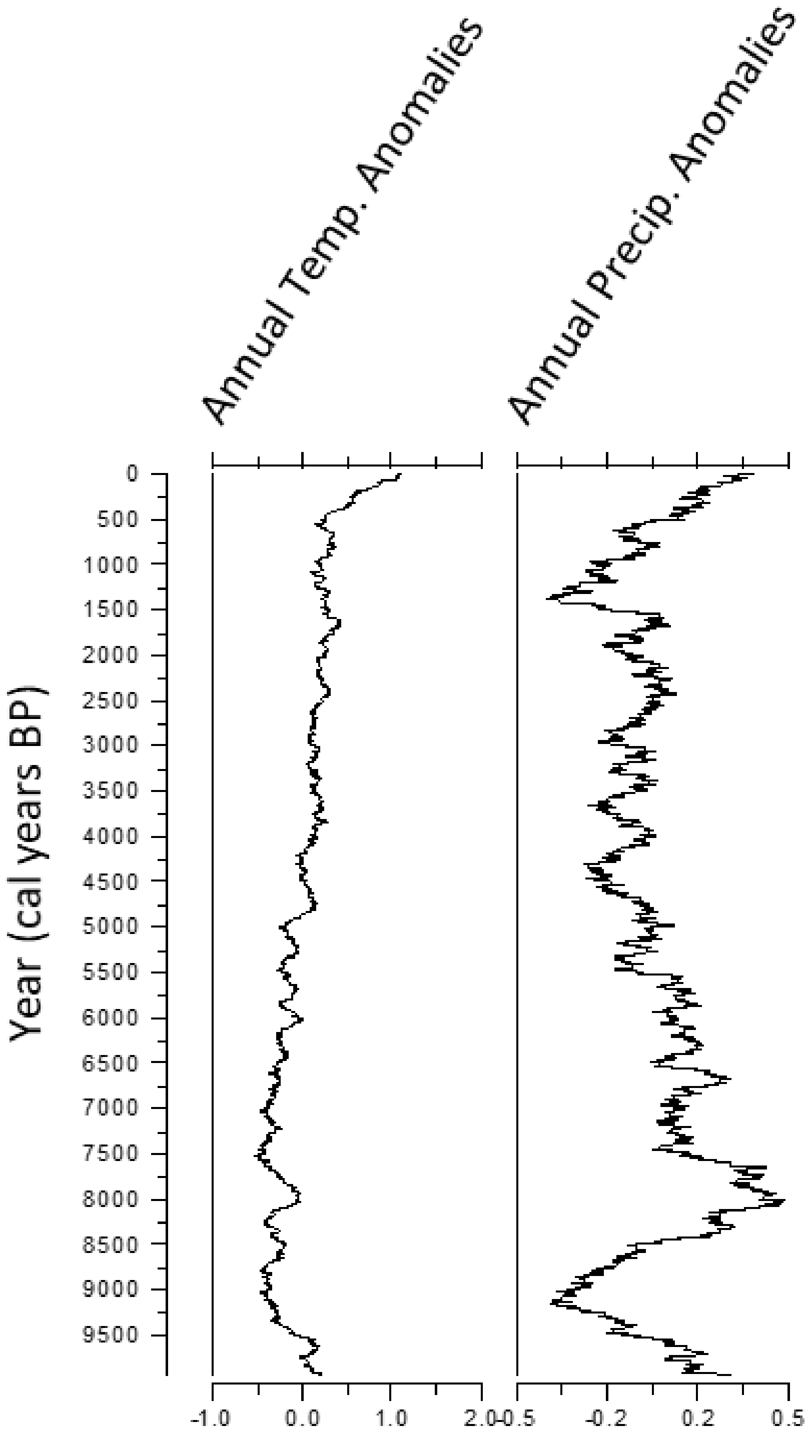

Figure 4.6: GENMOM reconstructed annual Holocene temperature and precipitation anomalies relative to a pre-Industrial base period.

Fragilaria (sensu lato) (<10%) are generally higher than Zone 1. Although plankton still dominate the assemblage (80-95%), tychoplankton and benthon percentages (<7.5%) begin to increase. Zone 2 is subdivided into five subzones based on shifts in the relative abundance of the three most abundance species (*S. minutulus, A. subarctica, A. formosa*) – 2A (6475-5350 cal years BP), 2B (5350-4900 cal years BP), 2C (4900-4050 cal years BP), 2D (4050-2325 cal years BP), and 2E (2325-1500 cal years BP). Throughout this zone, summer temperature anomalies decrease steadily, and winter temperature anomalies increase by approximately by ~1.0. Reconstructed precipitation anomalies remain moderate, fluctuating between -0.2 and 0.2 in Zone 2.

4.3.3 Zone 3 (1500-$^{-}$67 cal years BP)

Zone 3 continues to be co-dominated by *A. subarctica* (5-60%), *S. minutulus* (5-80%), and *A. formosa* (2-40%) and also has some *S. yellowstonensis* (<20%), low *S. oregonicus* (<2%), and higher small colonial *Fragilaria* (5-25%) than previous zones. It is distinctive in consistently higher relative abundances of *A. subarctica* and A. formosa and a higher proportion of tychoplankton and benthon. This zone is not divided into any additional subzones. The amount of modeled warming that occurs from 500 cal years BP to present (~1.0 change in anomaly value) is twice the amount that occurs between 7500 and 500 cal years BP. Winter and spring temperature anomalies are higher than the pre-Industrial mean; autumn and winter are below or near the pre-Industrial base value.

4.4 Discussion

Dominance of the diatom assemblage by planktic species and one tychoplanktic species complex indicates that Yellowstone Lake has been a deep system throughout the record, with limited changes in lake depth affecting diatom assemblage composition. Overall, analysis of fossil diatoms during the Holocene indicates that the system has been relatively stable throughout this period. However, small shifts in diatom assemblage relative abundances provide insight into paleoclimatic changes in Yellowstone National Park over the last ~10000 years as suggested by the varying ecological preferences of the dominant taxa, including nutrients, climate seasonality, and depth within the water column of the most abundant species (*A. subarctica, S. minutulus, A. formosa*¸ and *S. yellowstonensis*) (Table 4.2). Modern ecological research in Yellowstone Lake found that population maxima of two species rarely occurred in the same space and time, indicating that the diatom community structure is differentiated (Interlandi et al. 1999). In general, the diatom stratigraphy features trade-offs between *S. minutulus* and *A. subarctica*.

Stephanodiscus minutulus is a high-phosphorus specialist, with low nitrogen and silica requirements (Lynn et al. 2000, Interlandi et al. 2003). Thus, *S. minutulus* is commonly an opportunistic species that responds quickly to phosphorus enrichment. In Yellowstone Lake *S. minutulus* blooms earliest in spring during isothermal mixing (Interlandi et al. 1999, Theriot et al. 2006).

Aulacoseira subarctica blooms in spring after *S. minutulus* and before the onset of stratification and can persist at depth (>20 m) into early summer in Yellowstone Lake (Interlandi et al. 1999). As a heavily silicified species, it also requires high silica concentrations (Kilham et al. 1996). Larger population of *A. subarctica* are indicative of cool, early summers that extend the duration of isothermal mixing and allow the population to grow and persist into summer.

Asterionella formosa blooms in spring at approximately the same time as *A. subarctica*, but slightly deeper in the water column. It requires high nitrogen and moderate to high silica relative to phosphorus (Kilham et al. 1996, Michel et al. 2006, Berthon et al. 2014). Populations of *A. formosa* decrease at the onset of stratification. *A. formosa* is an opportunistic species that is one of the first to respond to nitrogen enrichment (Mcknight et al. 1990, Wolfe et al. 2001, Reavie et al. 2002, Saros et al. 2005, Michel et al. 2006). Thus, factors that increase N and/or Si availability during spring months, including increased N in runoff, would be expected to favor increased *A. formosa*.

Stephanodiscus yellowstonenesis, endemic to Yellowstone Lake, is a characteristic summer species with the ability to grow at depth and in low light conditions (Kilham et al. 1996, Theriot et al. 2006). In historical records, *S. yellowstonenesis* was most abundant during intervals of drought.

4.4.1 Zone 1 (9876-6475 cal years BP)

Zone 1 is overwhelmingly dominated by *S. minutulus*. Portions of the record in Zone 1 are punctuated by synchronous or individual increases in *A. subarctica* and *A. formosa*, and the arrival of *S. yellowstonensis* at significant (>5%) abundances. Thus, increases in the relative abundance of *S. yellowstonensis* suggest periods with better-developed summer stratification. The dominance of *S. minutulus* during the early portion of the record (9876-6475 cal years BP) suggests generally low nitrogen and silica concentrations and high phosphorus availability. Intervals with higher *A. formosa* and *A. subarctica* abundances suggest higher silica availability and likely indicate extended water column instability as a result of extended spring mixing. The virtual absence of *S. yellowstonensis* in the basal part of this interval, from 9476-9225 cal years BP (Zone 1A) is indicative of short summers with minimal stratification, whereas the increased abundance of *S. yellowstonensis* beginning approximately 8950 cal years BP (Zone 1B) is indicative of longer intervals of summer stratification continuing throughout the remainder of the Holocene.

Table 4.3: Ecological preferences of abundant Yellowstone Lake diatom species (Kilham et al. 1996, Interlandi et al. 1999).

Species	***S. minutulus***	***A. subarctica***	***A. formosa***	***S. yellowstonensis***
Nitrogen	Low	Low	High	Low
Silica	Low	High	High	Moderate/High
Phosphorous	High	High	Low	Low
Seasonality	Spring	Spring to Early Summer	Spring	Summer
Depth	More abundant at depth (>15 m)	More abundant in surface during spring (<15 m), at depth (>15 m) in summer	Abundant at 0-10 m and 35-45 m	More abundant at depth
Ecological Notes	Opportunistic	Can maintain high population in summer hypolimnion	Opportunistic - responds rapidly to nutrient enrichment	Most abundant during historical droughts

4.4.2 Zone 2 (6475-1500 cal years BP)

A marked increase in *A. subarctica* differentiates Zone 2 after ~6475 cal years BP. Because *A. subarctica* requires higher Si:P than *S. minutulus*, and its blooms extend from the onset of early spring ice-off into early summer, the increase of this species suggest longer periods of isothermal mixing than in the previous zone – which would allow the development of both *S. minutulus* and *A. subarctica* populations before the onset of stratification. This could occur in years of early ice out or alternatively in years where early summer is cool and stratification is delayed. In either case, the shifts between various subzones in the relative abundance of *A. subarctica* versus *S. minutulus* suggest shifts in the duration of isothermal mixing. As discussed above, intervals with increased *A. formosa* may result from higher N input from increased runoff in spring. *S. oregonicus* is most abundant in Zone 2, generally with an inverse relationship to *S. yellowstonenesis*. Unfortunately little is known about the ecological differences of these two taxa.

The synchronous occurrence of increased *A. subartica*, *A. formosa,* and *S. yellowstonensis* is rare, but subzone 2B (5350-4900 cal years BP) is characterized by a sustained, synchronous elevation of these three planktic species, which suggests warm wet winters resulting in earlier ice-off and increased nutrient influx into Yellowstone Lake, coupled with some stratification and thus the increased abundance of *S. yellowstonensis*.

4.4.3 Zone 3 (1500-$^{-}$67 cal years BP)

In Zone 3 (1500-$^{-}$37 cal years BP), a shift occurs from *S. minutulus* dominance to a co-dominance of *A. subarctica* and *S. minutulus* and higher overall *A. formosa* abundance. Increasing trends of *A. subarctica* and *A. formosa* are indicative of overall extended spring turnover, allowing the establishment of large early- and late-spring blooms. Additionally, higher abundance of *A. formosa* is indicative of higher nitrogen availability in the catchment (Wolfe et al. 2001). In this setting, elevated nitrogen inputs are likely the result of wetter winters or springs that increase N loading to the lake (Kilham et al. 1996).

4.4.4 Synthesis

Dominance of *S. minutulus* throughout the entire record, particularly in the early Holocene, is indicative of high phosphorus availability. Phosphorus is abundant in the Yellowstone Lake basin due to andesitic rock weathering within the catchment (Morzel et al. 2017, Theriot et al. 1997). Higher abundance of *S. minutulus* is most likely related to water column stability, including the duration of spring turnover and the resulting internal phosphorus regeneration from the hypolimnion during mixing, rather than direct input from the catchment (Bracht et al. 2008, Theriot et al 1997, Interlandi et al 1999).

A. subarctica can persist into summer during cool years when stratification is delayed. Yellowstone Lake's deep chlorophyll maximum and the general low light requirements of this species allow significant populations to photosynthesize during mixing and also at the base of the thermocline during stratification. Thus, it is particularly challenging to delineate the environmental conditions or seasonality (spring versus summer) controlling the species abundance in the fossil diatom assemblage of Yellowstone Lake.

Overall, the Yellowstone Lake diatom assemblage is controlled by climatological changes – temperature and precipitation – throughout the Holocene (Figure 4.7). Specifically, the trade-off between *A. subarctica* and *S. minutulus* populations appears to be largely controlled by temperature. Higher populations of *S. minutulus* are favored by cool springs with phosphorus regeneration from the hypolimnion during mixing, but warmer summers, because a shortened mixing period created by the co-occurrence of later ice-off and stronger stratification truncated the duration of isothermal mixing before large populations of later spring species *A. subarctica* and *A. formosa* could become established.

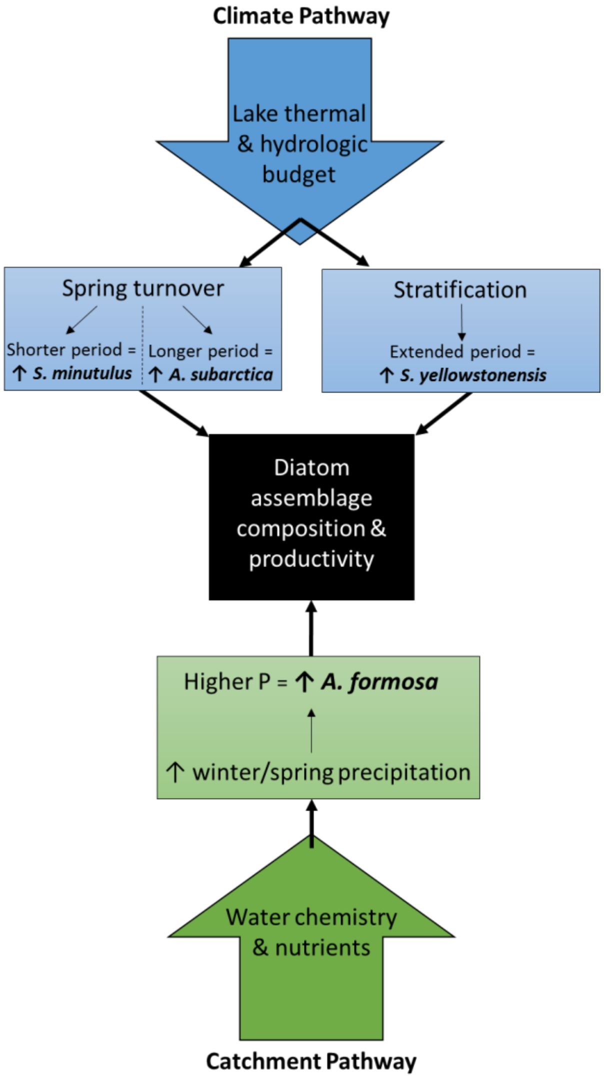

Figure 4.7: Conceptual diagram of direct (climate pathway) and indirect (catchment pathway) climate impacts on diatom assemblage composition and productivity including diatom-inferred Yellowstone Lake conditions (modified from Fritz & Anderson 2013).

Because *A. subarctica* blooms later in spring, it benefits from earlier ice-off and, hence, an extended spring mixing period. Alternatively, below-average summer temperatures in the late Holocene may have also favored *A. subarctica* by either delaying the onset of stratification or weakening stratification. Surprisingly, the highest abundances of the summer species *S. yellowstonenesis* also appear to occur when summers were getting progressively colder. Thus, warmer winters and springs with cooler summers effectively extended spring turnover and resulted in larger populations of *A. subarctica,* which blooms later in spring. In Yellowstone Lake, delayed or weaker stratification favored species with higher silica requirements.

The population of *A. formosa* in Yellowstone Lake appears to be controlled by winter and/or spring precipitation, with higher abundances generally occurring with more positive precipitation anomaly values and in general tandem with *A. subarctica* increases. Because *A. formosa* is a high-nitrogen specialist, increases in its population are likely related to an influx of runoff from the surrounding landscape during spring turnover.

Relatively warm summer temperatures are reconstructed from other early Holocene paleolimnological records of the Yellowstone region. Warm, dry summers were inferred during the early Holocene (~11000 cal years BP) from three lake records (Blacktail Pond, Cub Creek Pond, and Hendrick Pond) in the Yellowstone region (Lu et al. 2017), and the lakes underwent a synchronous shift approximately 8800-8700 cal years BP, attributed to increased convective moisture (Lu et al. 2017). However, steadily drier conditions after 8500 cal years BP were inferred from the $\delta^{18}O$ record of Crevice Lake in northern Yellowstone National Park (Whitlock et al. 2012). This transition period in the early Holocene is consistent with the interpretation of increasing winter/spring precipitation and a slight increase in the duration of the spring mixing period (~8950 cal years BP) in the Yellowstone Lake record inferred from increases in *A. formosa* throughout Zone 1B (8950-6475 cal years BP).

In the Greater Yellowstone Ecosystem, decreasing summer insolation and increasing winter and spring insolation are characteristic of middle Holocene climate controls (Whitlock and Brunelle 2006). Drier than present early-Holocene conditions are inferred at Crevice Lake, Slough Creek Lake, Cygnet Lake, and Blacktail Pond (Whitlock 1993, Whitlock et al. 2012). Dry conditions also are inferred from the Foy Lake fossil diatom record 7000-3500 cal years BP (Stone and Fritz 2006). This period in the Yellowstone Lake record is marked by increasing *A. subarctica* and an oscillatory relationship between *S. yellowstonenesis* and *S. oregonicus*, particularly from approximately 5,400-2,400 years BP. The Yellowstone Lake fossil diatom assemblage records the transition into cooler summers and warmer winters and springs. Particularly, Zone 2D (4050-2325 cal years BP) is a period of longer spring mixing. Thus, while precipitation changes were driving the diatom assemblages of lakes in the northern Greater Yellowstone Ecosystem, the diatom assemblage of Yellowstone Lake in southern Yellowstone National Park appears more sensitive to temperature changes in the middle Holocene.

Late-Holocene changes constructed from diatom assemblages of four lakes in Western Montana (Foy Lake, Crevice Lake, Morrison Lake, and Reservoir Lake) showed distinct synchronous changes 2200-250 cal years BP (Bracht-Flyr and Fritz 2012) indicative of large-scale climatic forcing. Crevice Lake and Morrison Lake were more sensitive to changes in spring mixing duration, whereas Foy Lake and Reservoir Lake recorded lake-level fluctuations. The Reservoir Lake record had a sudden increase in *A. formosa* beginning 1500-1200 cal years BP (Bracht-Flyr and Fritz 2012), as does Yellowstone Lake. This shift to *A. formosa* co-dominance in both lakes is inferred as a sustained increase in winter/spring precipitation and resultant runoff during the late Holocene (Bracht-Flyr and Fritz 2012).

4.5 Summary

The fossil diatom assemblage records Holocene paleolimnologic changes in Yellowstone Lake. Early-Holocene (9876-6475 cal years BP) conditions had generally high phosphorous availability with generally shorter periods of spring mixing. The mid- to early late-Holocene (6475-1500 cal years BP) was a transition period with increasing winter and spring temperatures, as well as decreasing summer temperatures, which affected the relative abundance of species within the diatom assemblage. The late-Holocene (1500 cal years BP-$^{-}$67 cal years BP) was wetter than the mid-Holocene, resulting in increased nitrogen input. The diatom assemblages of Yellowstone National Park are largely controlled by changes in the duration of the ice-free period and onset of summer stratification, indicating the record is most sensitive to direct climate changes in lake seasonality and energy balance. Yet, fluctuations in *A. formosa* appear to be linked to precipitation dynamics and, thus, indirect climate impacts on the lake via fluctuations in regional hydrologic budget.

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CHAPTER 5

THE ECOLOGICAL IMPACT OF HYDROTHERMAL EXPLOSION EVENTS ON DIATOM COMMUNITIES IN THE YELLOWSTONE CALDERA

Abstract

Hydrothermal explosions have perturbed the Yellowstone hydrothermal landscape since the Late Pleistocene. Macroscopic evidence of past hydrothermal explosion events is preserved in a number of lakes in Yellowstone National Park as deposits of breccia formed during hydrothermal explosion events. This study explores the impact of localized hydrothermal explosions on fossil diatom assemblages of Yellowstone Lake and Cub Creek Pond. Pre- and post-eruption assemblages were assessed for a total of five early to middle Holocene hydrothermal explosion events. The stratigraphic records indicate that fossil diatom assemblages were resilient to hydrothermal disturbance and the associated changes in limnological conditions. However, localized hydrothermal explosion events do appear to impact environmental conditions severely enough to be recorded in time-averaged fossil diatom assemblages in shallow systems. Diatom assemblages of Cub Creek Pond – a small, shallow basin – recorded a sustained shift in diatom assemblage composition, whereas any observed changes in diatom assemblages of the large, deep Yellowstone Lake record did not persist. These diatom assemblage shifts were largely restricted to biodiversity within species complexes, indicating resilience via functional compensation.

5.1 Introduction

Terrestrial hydrothermal systems provide critical inputs to Earth's biogeochemical cycles and thermal budget, as well as insights regarding how life functions in harsh environments (Lowell 1991). Large craters (>400 meters in diameter), one type of hydrothermal landscape feature, are present in numerous locations within Yellowstone National Park (Muffler et al. 1971), which has the largest concentration of terrestrial hydrothermal features on Earth (Hurwitz & Lowenstern 2014). These craters were formed by hydrothermal explosions – a catastrophic response to forcing where fluids trapped in near-surface rocks flash to steam and perturb confining rock. During hydrothermal explosion events, water and steam are expelled along with solid rock, which is potentially deposited and preserved *in situ* as breccia. Numerous large hydrothermal explosion craters have formed since the Late Pleistocene (~14,000 years BP) (Morgan et al. 2009), primarily within the Yellowstone Caldera.

A number of outcrops in the vicinity of Yellowstone Lake include lacustrine units that contain layers of breccia indicative of past hydrothermal explosions. These include the Mary Bay (13.0 Ka), Indian Pond (2.9 Ka), and Turbid Lake (9.4 Ka) events (Morgan et al. 2009 and references therein). Additionally, several large explosion craters have been mapped on the floor of Yellowstone Lake. Smaller craters exist within the basins created by large explosions, indicating subsequent, less-intense events that occurred after the initial explosions. Whereas subaerial craters are rimed with breccia deposits, sub-lacustrine craters lack apparent rims – suggesting that ejecta from underwater explosion events was widely dispersed into lake water, with the potential to cause considerable water column disturbance (Muffler et al. 1971, Hamilton 1987, Morgan et al. 2009).

Disturbances that disrupt ecosystem balance can have significant impacts on ecological function and community composition (White & Jentsch 2001). The objective of this study is to assess the effect of localized hydrothermal explosions on diatom ecological dynamics in Yellowstone Lake and Cub Creek Pond in Yellowstone National Park. Specifically, this research evaluates the resilience of diatom assemblages to high-magnitude disturbances.

5.1.1 Study Area

Three sampling locations – modern Yellowstone Lake, a paleoshoreline outcrop of Yellowstone Lake, and Cub Creek Pond – with preserved hydrothermal explosion breccia were selected for analysis (Figure 5.1). The Yellowstone Lake outcrop (44.55466 N, -110.32510 W) is located on the northern shore of the lake, with a total height of 56 m above the modern shore, and is directly west of Mary Bay. The outcrop includes alternating strata of clay and sand, with three matrix-supported explosion breccia deposits with angular to sub-angular clasts.

Yellowstone Lake (44.50782 N, -110.32685 W, 2356 m) is a large high-altitude lake, with a surface area of 344 square kilometers (Morgan et al. 2003) and a maximum depth of 107 meters (Tiller 1995). Yellowstone Lake is dimictic and oligotrophic (Table 5.1, Theriot et al. 1997). Yellowstone Lake inflow is dominated by the Yellowstone River, which flows into the southern portion of the Southeast Basin, although other small rivers and streams also contribute water and sediment. The Yellowstone River is the only outlet, located in the northern Central Basin of Yellowstone Lake.

During the Last Glacial Maximum (~21 ka), the Yellowstone region was covered by a large independent ice field, with ice reaching a height of 920 meters above base level (Pierce 1979). Glacial retreat began at 14,490 +/- 350 cal years BP and created the first direct ancestor of modern Yellowstone Lake (Pierce et al. 2003). Ancestral Yellowstone Lake was significantly larger than the modern basin, and numerous paleo shoreline terraces exist, documenting post-glacial lake decline. Cub Creek Pond (44.58917 N, -110.45861 W, 2051 m) is a small (<5 ha) shallow lake basin with an extensive sedge fen surrounding the margin and is located ~4 km directly east of Yellowstone Lake.

Table 5.1: Limnologic characteristics of Yellowstone Lake (Theriot *et al* 1997) including P (total PO_4) and N (Kjeldahl-N) and Cub Creek Pond (Lu et al. 2017) including P (TP) and N (TN).

	Depth (m)	**P (mg/L)**	**N (mg/L)**	**DOC (mg/L)**
Yellowstone Lake	107	0.2	0.2	4.7
Cub Creek Pond	4	0.126	1.553	14.5

Modern vegetation in the Yellowstone Lake region consists of a mosaic of lodgepole pine (*Pinus contorta*) and mixed conifer (*Abies lasiocarpa, Picea engelmanii*, and *Pinus albicaulis*) forest. Soils of the area are mainly andesitic and rhyolitic. Modern climate for the study area is Continental Subarctic, with 390 mm of average annual precipitation and an average annual temperature range between -2.4 and 11.5 °C (National Park Service 2019).

5.2 Methods

A sedimentary outcrop described previously by Morgan (2009) was measured and described in July 2016 using standard methods. A total of 26 high-resolution (continuous 1-cm) subsamples were collected with a brass cuvette from the 31-cm section of diatomaceous lagoonal sediments that occur immediately before and after the Turbid Lake (9.3 ka) explosion breccia. The Turbid Lake breccia was too silicified to sample.

An 11.5-m long sediment core (HD-YLAKE5A) was collected from Elliot’s Crater (44.50782 N, -110.32685 W, 2365 m) in 102 m of water, near the center of hydrothermal activity in the northern basin of Yellowstone Lake, using a Kullenberg corer in September 2016. A 4.31-m long sediment core was collected from the fen on the southern side of Cub Creek Pond in August 2017 with a Livingstone corer. For Yellowstone Lake and Cub Creek Pond, high-resolution samples (every ½ cm continuously) were collected before and after suspected hydrothermal explosion deposits to enable sufficient stratigraphic time (~100 years) to assess the pre-explosion “normal” conditions, immediate post-explosion response, and any longer term community impacts.

A portion of each individual diatom subsample was added to a vial, weighed to approximately 0.1 gram, and processed with 30% hydrogen peroxide to remove organic matter (Battarbee 2003). Residual samples were rinsed thrice with deionized water to dilute hydrogen peroxide. A known concentration of polystyrene microspheres was added to each subsample to allow quantitative analyses of diatom concentration. After processing, each subsample was mounted to a slide with Naphrax for archival purposes. Diatom valves were identified at 1000 X magnification using a Leica DM2500 transmitted light microscope with differential interference contrast (DIC) equipped with a

5 Megapixel camera or a Leica DMRX fitted with phase contrast. At least 300 diatom valves were identified to species and enumerated per slide. In addition to counts of species relative abundance, the ratio of initial to linking valves was calculated after assessing the morphology of 300 A*ulacoseira subarctica* valves per sample. The ratio of diatom frustules to chrysophyte cysts also was enumerated for each slide.

Diatom assemblage counts were converted to percentages and plotted in program C2 (Juggins 2003). A metric to infer relative productivity (valves/gram) was calculated using microsphere concentrations, diatom valve totals, and sample weights. Constrained cluster analysis (CONISS) was conducted on species assemblage percentage data using the R package Rioja (Juggins 2017). Program Past3 (Hammer et al. 2001) was used to calculate the Shannon Diversity Index.

5.3 Results

In total, pre- and post-hydrothermal explosion event paleolimnological conditions were assessed for five Holocene deposits (Figure 5.2). These deposits are a series of upward-grading beds, some with matrix-supported angular clasts. One hydrothermal explosion deposit (9965 ^{14}C yr BP) was found in the Cub Creek Pond core. One hydrothermal explosion deposit was apparent in the Yellowstone Lake paleoshoreline outcrop (Turbid Lake, 9.3 ka). Three hydrothermal explosion deposits, two pre- plus one post-Mazama Ash (7627 cal yr BP, Zdanowicz et al. 1999) event were apparent in the Yellowstone Lake core lithology.

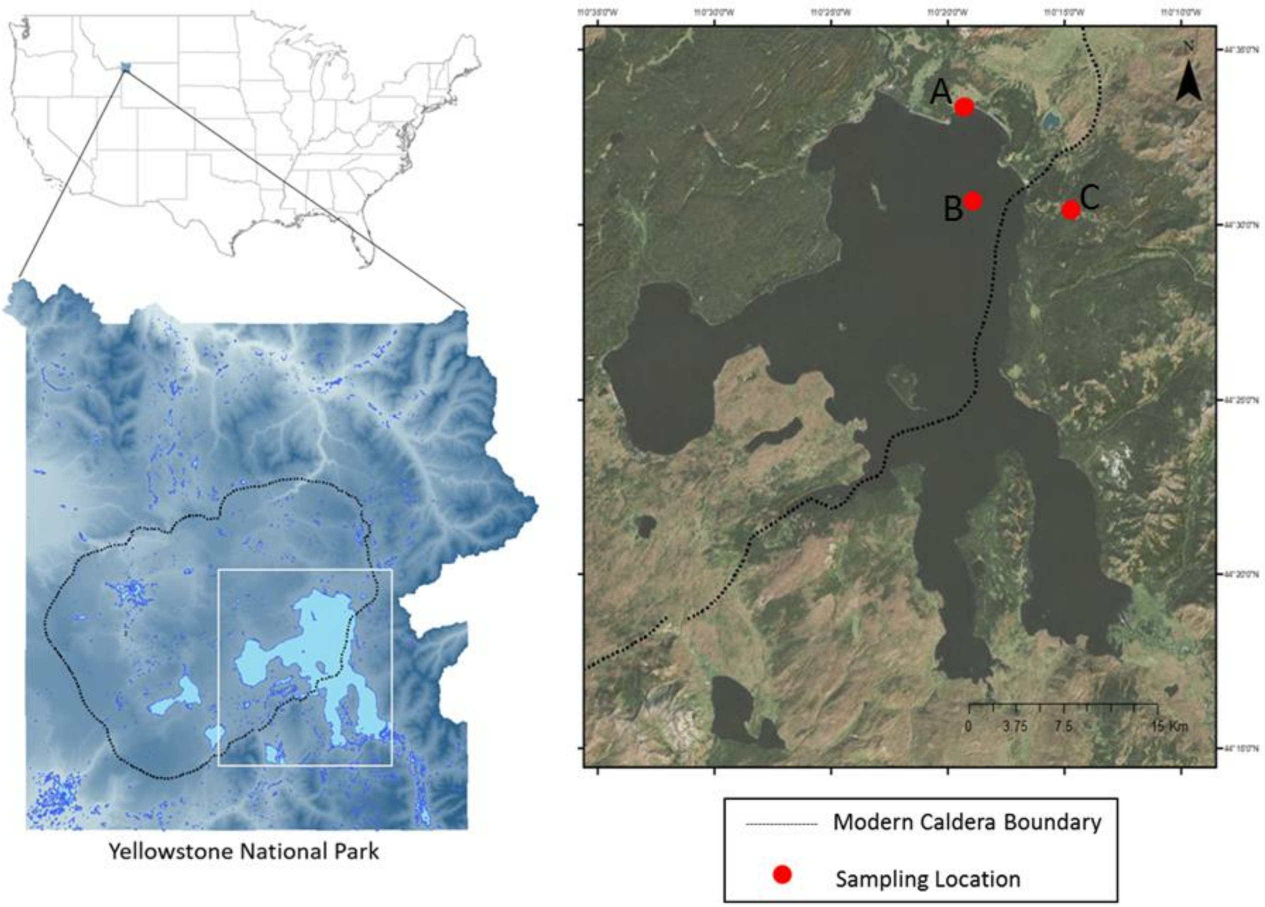

Figure 5.1: Digital Elevation Model inset of Yellowstone National Park (left). Satellite image of the study site with red dots indicating sampling locations (A) Yellowstone Lake paleoshoreline outcrop, (B) Yellowstone Lake sediment, and (C) Cub Creek Pond (right).

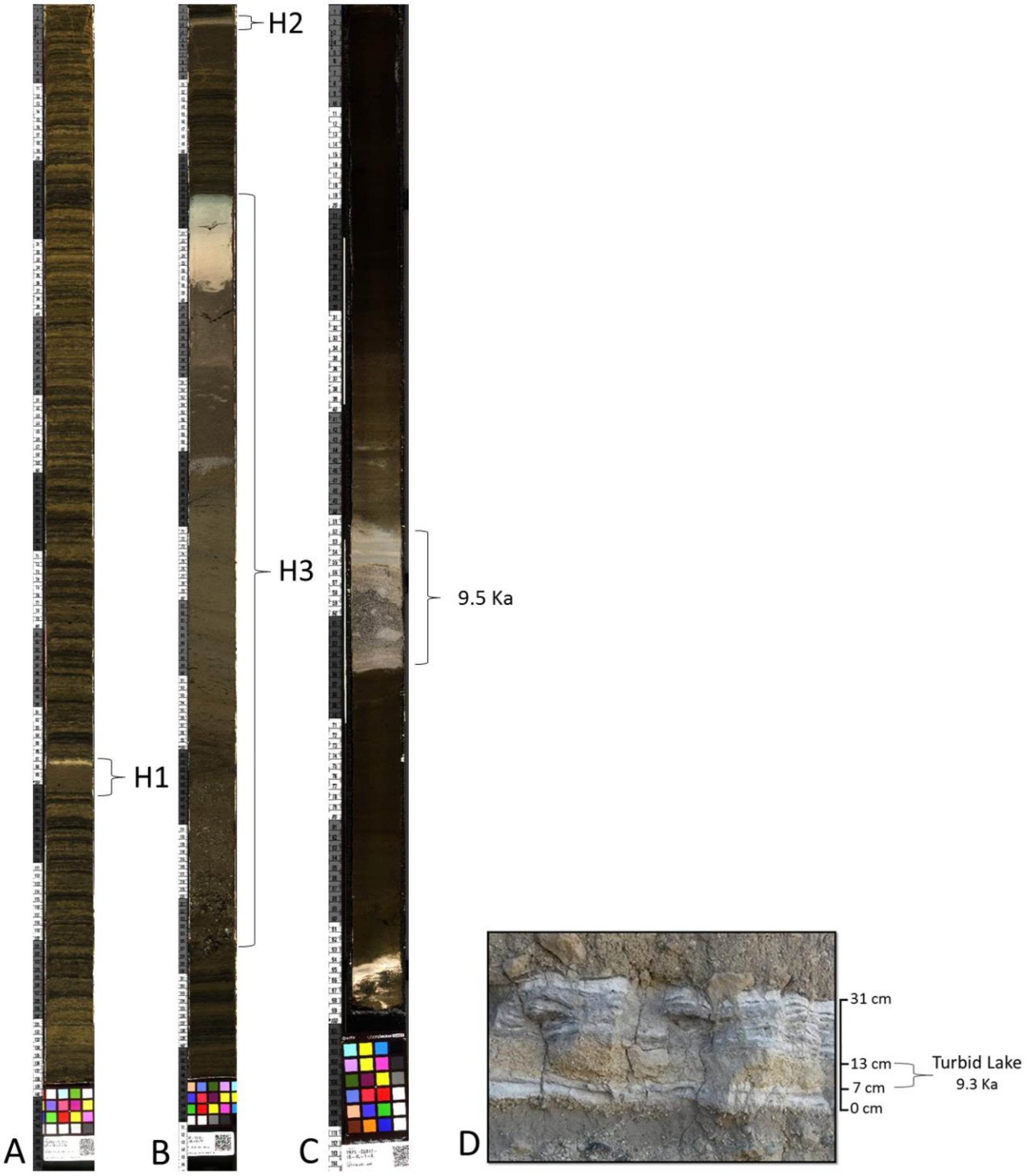

Figure 5.2: Photographic images depicting the lithology surrounding hydrothermal events in (A,B) Yellowstone Lake sediment cores, (C) Cub Creek Pond sediment core, and (D) Yellowstone Lake paleoshoreline outcrop.

5.3.1 Cub Creek Pond

The hydrothermal explosion deposit recorded in the Cub Creek Pond sediment lithology dates to 9532 cal yr BP (median probability, 9881-9250 cal yr BP (95% probability)). Abundant (>5%) diatoms in the assemblage of Cub Creek Pond (Figure 5.3) include five tychoplanktic (*Pseudostaurosiropsis pseudoconstruens, Pseudostaurosiropsis* sp. 1, *Pseudostaurosiropsis* sp. 2, *Stauroforma exiguiformis*, *Staurosirella neopinnata*, and *Staurosirella* sp. 2) taxa of the small colonial *Fragilaria* (sensu lato) complex. The pre-explosion breccia deposits are dominated by *S. neopinnata* (30-85%) and *Pseudostaurosiropsis* sp. 2 (<40%), with lower abundances of *Pseudostaurosiropsis* sp. 1 (15%) and *P. pseudoconstruens* (<20%). Shannon diversity index values vary widely, and diatom concentrations (valves/gram) increase steadily throughout the pre-explosion assemblage. The post-explosion assemblage is co-dominated by *Pseudostaurosiropsis* sp. 2 (40-70%), *S. neopinnata* (<30%), and *Staurosirella* sp. 2 (10-40%). Diatom concentration is low in the sample immediately above the explosion breccia and subsequently increases to the highest values of the record at 47.5 cm, followed by a decrease to moderate levels. The Shannon diversity values decrease in the sample directly above the explosion breccia, and they remain at moderate values with reduced variability compared with pre-explosion conditions.

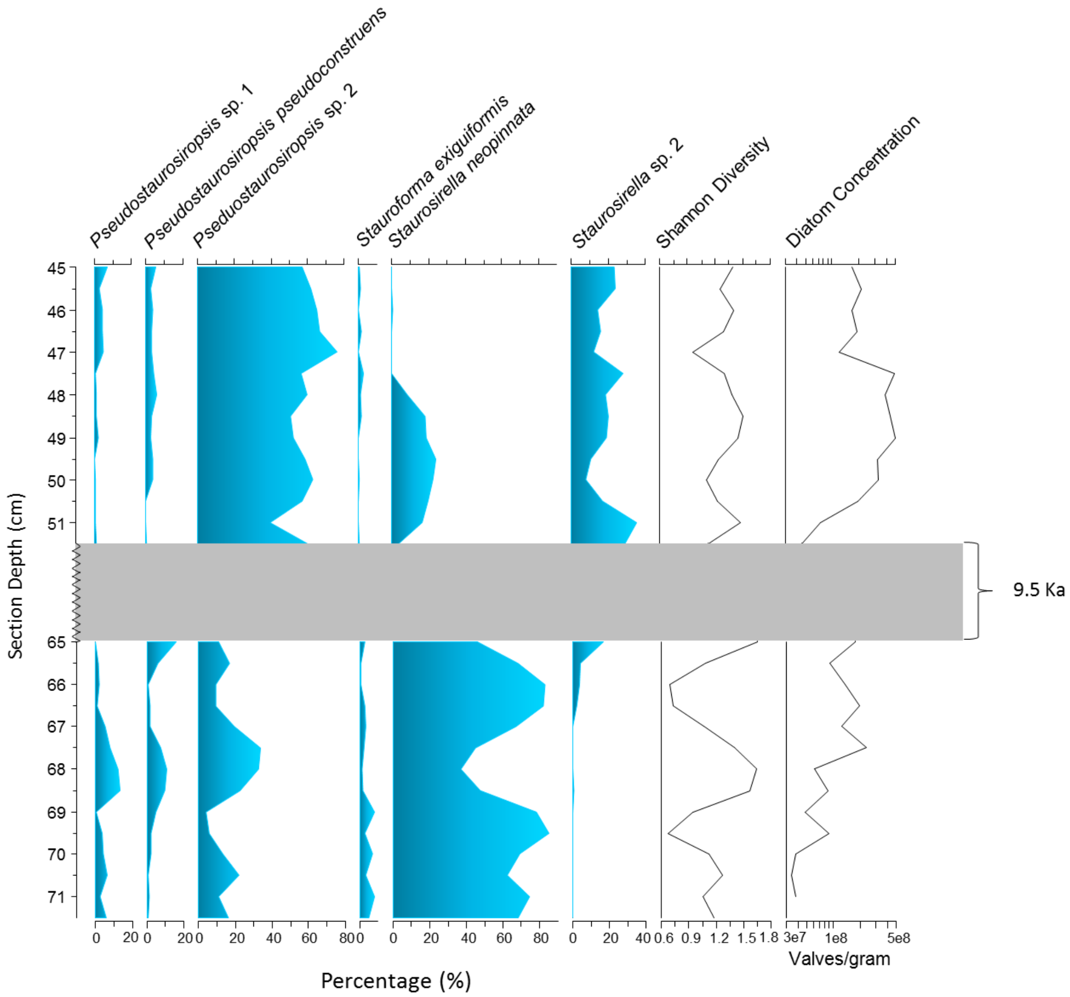

Figure 5.3: Stratigraphic plot of abundant (>5%) diatom species, Shannon diversity index, and diatom concentrations for Cub Creek Pond. Gray bar indicates the explosion deposit, which does not contain diatoms.

5.3.2 Yellowstone Paleoshoreline Outcrop

The hydrothermal explosion breccia deposited in the Yellowstone Lake paleoshoreline outcrop is dated to the early Holocene event that created the Turbid Lake Basin (9.3 Ka, Morgan et al. 2009). Abundant diatoms (>5%) in the Yellowstone Lake paleoshoreline outcrop (Figure 5.4) include one planktic (*Aulacoseira subarctica*) and seven benthic (*Stauroforma exiguiformis*, *Pinnularia microstauron, Pinnularia viridiformis, Pinnularia sudetica, Nitzschia liebethruthii* v. *siamensis*, *Luticula yellowstonensis*, and *Gomphonema parvulius*) species. The pre-explosion (1.0-6.5 cm) assemblage is co-dominated by *A. subarctica* (20-40%) and *S. exiguiformis* (15-30%), with lesser relative abundances of *P. viridiformis* and *G. parvulius*. The diatom valve to chrysophyte cyst ratio is low (<2.3) at the beginning of the record and reaches moderate levels (~2) in the sample directly below the explosion breccia. The diatom concentration is low at the bottom of the record (1.0 cm) and in the sample directly below the explosion deposit (6.5 cm). The ratio of initial/linking *A. subarctica* valves is low throughout the pre-explosion zone.

The post-explosion (12.5-31.0 cm) assemblage is co-dominated by *A. subarctica* (20-40%), *S. exiguiformis* (1-30%), and *P. viridiformis* (1-20%) directly above the explosion breccia (12.5-16.0 cm). *P. microstauron* (1-20%) and *P. sudetica* (0-20%) increase in abundance later in the post-explosion zone (16.0-31.0 cm), along with *L. yellowstonensis* (<10%) and a short interval with *N. liebethruthii* v. *siamensis* (20.0-22.0 cm). Directly above the explosion breccia, diatom concentration is low (12.5-14.5 cm), then steadily increases throughout the post-explosion zone. Diatom valve to chrysophyte cyst ratio is moderate (~2) in the sample immediately above the explosion breccia (12.5

cm) and then increases slightly (~3) in the post-explosion zone. In the sample directly above the explosion breccia, initial/linking valves attain the second-highest value in the record, but subsequently decline until much later in the record (29 cm). Shannon diversity values increase slightly in the post-hydrothermal explosion diatom assemblage in comparison with pre-explosion sediment.

5.3.3 Yellowstone Lake Sediment Cores

The three hydrothermal explosion breccia deposits in Yellowstone Lake core lithology have not been dated. However, the presence of the well-dated Mazama Ash deposit (Zdanowicz et al. 1999) provides some age constraint – with two hydrothermal explosion deposits occurring below (H3 and H2) and one (H1) above the ash. The abundant (>5%) diatom taxa of the Yellowstone Lake sediment cores (Figures 5.5, 5.6) include five planktic (*Aulacoseira subarctica, Stephanodiscus yellowstonensis, Stephanodiscus minutulus, Stephanodiscus oregonicus*, and *Asterionella formosa*) and one tychoplanktic species complex (*Fragilaria* (sensu lato)).

In the sample immediately preceding the H3 deposit (Figure 5.5, 163 cm), *A. subarctica* is locally absent, with >90% *S. minutulus* and a low relative abundance *A. formosa.* Diatom concentrations are high, with a peak at 166 cm core depth. In the sample immediately post-H3 breccia (44.5 cm), *S. minutulus* is low, *A. subarctica* increases slightly, and *A. formosa* is increased in comparison with the pre-explosion (163 cm) assemblage. However, this pre- to post- shift in diatom abundance is not outside the natural variability of the system. Diatom concentrations decrease and Shannon diversity increases in the sample directly post-H3.

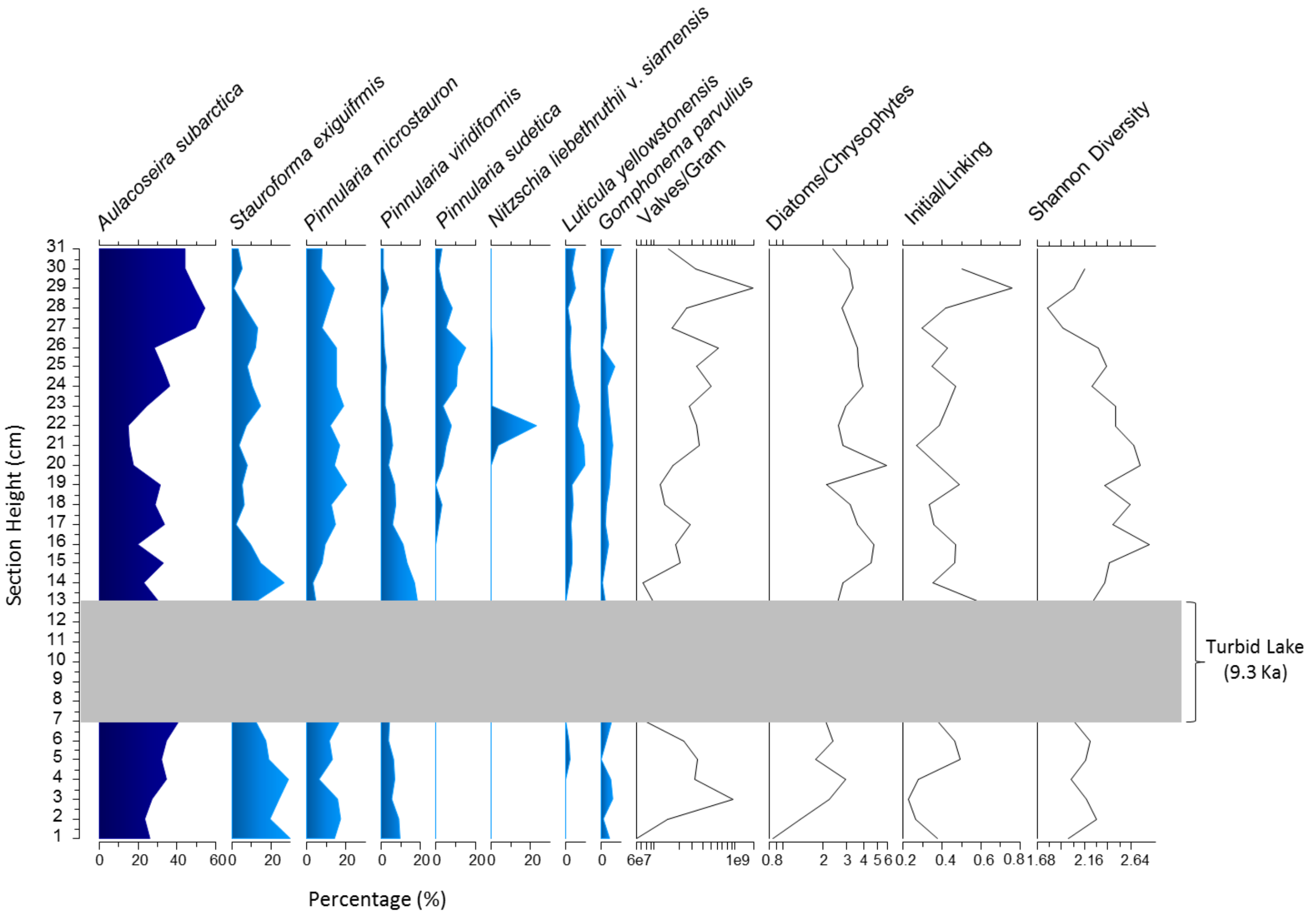

Figure 5.4: Stratigraphic plot of abundant (>5%) diatom species, diatom concentrations, diatoms/chrysophytes, initial/linking valves of *A. subarctica*, and Shannon diversity index values for the Yellowstone Lake paleoshoreline outcrop samples. Gray bar indicates the explosion deposit, which does not contain diatoms.

In the sample immediately preceding the H3 deposit (Figure 5.5, 163 cm), *A. subarctica* is locally absent, with >90% *S. minutulus* and a low relative abundance *A. formosa*. Diatom concentrations are high, with a peak at 166 cm core depth. In the sample immediately post-H3 breccia (44.5 cm), *S. minutulus* is low, *A. subarctica* increases slightly, and *A. formosa* is increased in comparison with the pre-explosion (163 cm) assemblage. However, this pre- to post- shift in diatom abundance is not outside the natural variability of the system. Diatom concentrations decrease and Shannon diversity increases in the sample directly post-H3.

In the sample immediately below the H2 breccia (29 cm), *S. minulutus* relative abundance is high (~90%). Shannon diversity is relatively low, and the diatom concentration is moderate. Immediately above the H2 breccia (21 cm), *S. minutulus* is slightly lower in abundance, with *A. subarctica* and *Fragilaria* (sensu lato) increased relative to pre-explosion conditions. Shannon diversity increases and diatom concentration decreases in comparison to pre-H2 breccia assemblage. However, this pre- to post- shift in diatom abundance is not outside the natural system variability.

Diatom assemblage composition pre-H1 explosion (Figure 5.6, 100.5 cm) consists of relatively increased *S. minutulus*, with relatively low abundances of *A. subarctica* and *Fragilaria* (sensu lato). Shannon diversity is moderate, and diatom concentration is moderate. Immediately post-H1 (97 cm), *S. minutulus* decreases, and *A. subarctica*, as well as *Fragilaria* (sensu lato), increase relative to the pre-explosion assemblage

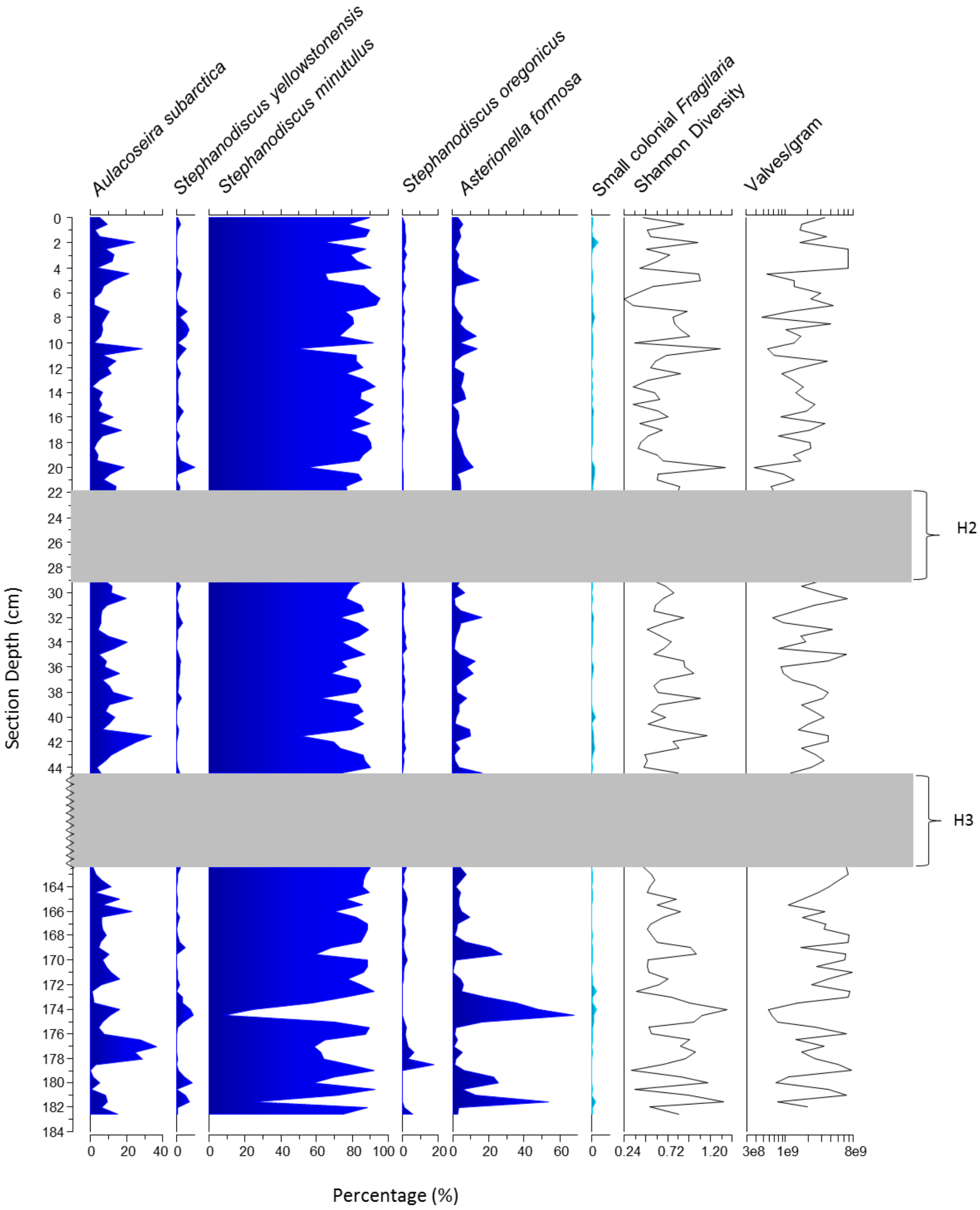

Figure 5.5: Stratigraphic plot of abundant (>5%) diatom species, Shannon diversity index, and valves/gram values surrounding H3 and H2 breccia deposits in Yellowstone Lake sediment core samples. Gray bar indicates the explosion deposits, which do not contain diatoms.

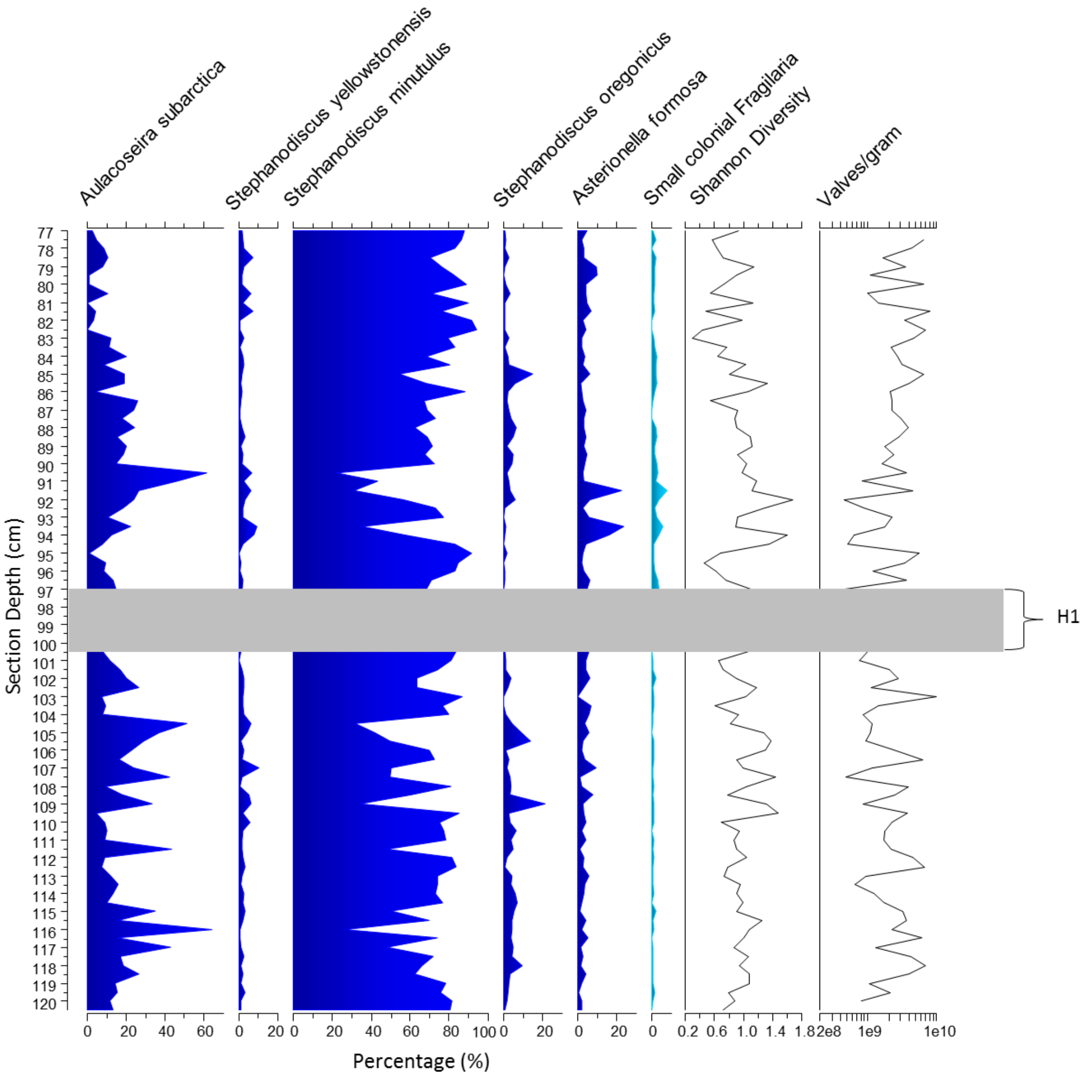

Figure 5.6: Stratigraphic plot of abundant (>5%) diatom species, Shannon diversity index, and valves/gram values surrounding the H1 breccia deposit in Yellowstone Lake sediment core. Gray bar indicates the explosion deposit, which does not contain diatoms.

percentages. Additionally, post-explosion Shannon diversity minimally increases and valves/gram decreases. Again, this pre- to post- shift in diatom abundance is not outside the natural variability of this system.

5.4 Discussion

Fossil diatoms preserved in Cub Creek Pond sediments are indicative of shallow, low-nutrient conditions. The diatom assemblage of Cub Creek pond shows a marked change after the hydrothermal explosion deposit (9.5 Ka). Specifically, *P. pseudoconstruens*, *Pseudostaurosiropsis* sp. 2, and *S. neopinnata* show substantive changes in relative abundance (>40%) before and after the hydrothermal explosion deposit. Whereas *S. neopinnata* dominates the pre-explosion assemblage, it is completely absent from the sample directly above the hydrothermal explosion deposit (47.5-45 cm). The relative abundance of *S. neopinnata* does recover to somewhat higher values, but not to pre-explosion levels. In contrast, *Pseudostaurosiropsis* sp. 2 increases from <35% below to 40-75% above the hydrothermal explosion deposit. Additionally, *Pseudostaurosiropsis* sp. 1, *P. pseudoconstruens,* and *S. exiguiformis* are locally absent in post- hydrothermal deposit diatom assemblages (51.5-50.5 cm).

These changes in fossil diatom assemblages indicate a shift in paleoenvironmental conditions in association with the hydrothermal explosion event. As such, the shift in dominance from *S. neopinnata* to *Pseudostaurosiropsis* sp. 2 indicates a sustained change in environmental conditions to favor *Pseudostaurosiropsis*. Decreased diatom concentrations in post-hydrothermal event sediments indicate an extended impact on diatom abundance in the sediments. Small colonial *Fragilaria* (sensu lato) species are low-nutrient, opportunistic, and disturbance-tolerant (Saros et al. 2003). However, whereas diatom ecologists have documented the environmental preferences of the species complex, the challenging taxonomy of this group has created obstacles for inferring the ecological conditions that favor one species over another (Westover et al. 2006). Thus, we can only speculate on the nature of environmental change. One potential implication of a hydrothermal explosion could be that the graded breccia deposit creates a functional difference in the substrate texture or stability that favors some species of small *Fragilaria* (sensu lato) over others.

The fossil diatom assemblage of Yellowstone Lake changes minimally in association with the hydrothermal explosion deposits. The benthon-dominated assemblage of the Yellowstone Lake outcrop indicates relatively shallow conditions in this portion of the northern Central Basin during the early Holocene. In contrast, the dominance of plankton in the Yellowstone Lake sediment core indicates deep conditions in the Central Basin during the early to middle Holocene period when the eruptions occurred.

The most drastic change in diatom assemblage surrounding the Turbid Lake (9.3 Ka) breccia in the Yellowstone Lake outcrop is a decrease in *P. microstauron* and an increase in *P. viridiformis* across the breccia threshold. Increased abundance of *P. viridiformis* is relatively extended (13-17 cm), before percentages return to pre-explosion values. *P. viridiformis* is larger and more heavily silicified than *P. microstauron* (Bahls et al. 2018), indicating an influx of silica into the system in association with the hydrothermal explosion. The shift to higher post-explosion values of the diatoms:chrysophyte ratio may be indicative of higher nutrient availability as higher diatom abundance relative to chrysophytes is typical in lakes of higher trophic status (Smol 1985). The peak in initial/linking *A. subarctica* valves in the sample immediately post-explosion deposit designates an increase in auxospores and, thus, sexual reproduction perhaps indicating higher nutrient availability or elevated environmental stress.

In Yellowstone Lake sediment cores, the fossil diatom assemblages record minimal changes in association with hydrothermal explosion events. Decreases in diatom concentrations (valves/gram) occur with all three hydrothermal explosion deposits in the Yellowstone Lake sediment cores. Yellowstone Lake core species assemblage shifts are short-lived, only occurring in the sample immediately above the explosion event and are not outside of the normal-condition fluctuations documented in non-disturbed periods of the record.

Holistically, diatoms appear resilient to hydrothermal explosion disturbance, particularly in time-averaged sediment records. However, changes in the fossil diatom record indicate that hydrothermal explosions were able to alter paleolimnological conditions more effectively in shallow conditions in contrast to deeper systems and had a larger impact on smaller basins. Thus, hydrothermal explosion disturbance impacted small, shallow Cub Creek Pond diatom assemblage more severely than that of the larger Yellowstone Lake. Specifically, a long-lasting assemblage shift occurred in Cub Creek Pond, while assemblage changes in Yellowstone Lake were relatively short-lived. Variations in diatom concentration (valves/gram) were also more pronounced in Cub Creek Pond than Yellowstone Lake. Changes in diatom concentration are impacted by diatom productivity, sedimentation rate, and valve preservation. In this instance, diatom concentration is most likely a measure of changes in water clarity or substrate disturbance. Shallow-water diatom flora were impacted more than deep-water assemblages in Yellowstone Lake. As such, decreased diatom productivity is likely related to decreased water clarity. Additionally, inferred increased sexual reproduction and decreased diversity in Yellowstone Lake and Cub Creek Pond fossil diatom assemblages indicate harsh limnologic conditions immediately following hydrothermal explosion events, although these are not sustained.

Biodiversity is an important ecosystem component that affects a system's resilience to disturbance (Carpenter & Cottingham 1997). Specifically, diversity at the species level can result in functional compensation (Frost et al. 1995), where the availability of a high species pool can facilitate resistance to disturbance. As such, the post-disturbance shift in the dominant taxa of a small system (Cub Creek Pond) from one small colonial *Fragilaria* (sensu lato) to another is a compensatory shift in species that may stabilize ecosystem process rates (Carpenter & Cottingham 1997). In the larger lake system, there was a short-lived response to disturbance in the diatom assemblage in the shallow water samples, but not in deeper samples. Substrate disturbance in the area could be a contributing factor explaining how planktic species were unaffected, but the benthic species assemblage changed. Additionally, the entire Cub Creek Pond basin was impacted by the explosion deposit, whereas only northern Yellowstone Lake shallow environments were impacted. As such, functional compensation appears to be key factor to the resilience of shallow-water species, while planktic species are resilient to local disturbance via input from the surrounding species pool.

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CHAPTER 6

SUMMARY

6.1 Important Findings

Investigation of fossil diatom assemblages from various hydrothermal settings yielded a number of important findings and conclusions; an overview of these are presented below:

Extensive literature review in Chapter 2 suggests that eukaryotic extremophile research in continental hydrothermal systems – particularly in the fields of genomic and metabolic profiling – has drastically increased in recent decades. While eukaryotic growth is hindered at high temperatures (>62 °C), they can grow in low-temperature continental hydrothermal systems globally, even at extreme acidity (pH <4.0) and alkalinity (pH >9.0). Further, eukaryotes are important organisms in microbial mat communities, and also mediate silicate and carbonate precipitation in continental hydrothermal activity.

Sinter from Yellowstone National Park alkaline-chloride systems contained evidence of *in-situ* diatom growth, as described in Chapter 3. The fossil diatoms of Upper Geyser Basin, found in various stages of diagenetic alteration, were aerophilic and indicative of low nutrient availability. Fossil diatoms of Yellowstone Lake structures suggest alkaline, relatively shallow conditions. The presence of diatoms in these materials indicates that silica frustules can maintain an identifiable shape under some low-temperature hydrothermal alteration.

Chapter 4 reconstructs Holocene climate from Yellowstone Lake sediment. Diatom assemblages of Yellowstone Lake were sensitive to changes in the duration of the ice-free period and onset of summer stratification. This indicates that the record is mainly recording Holocene temperature changes and their influence on lake energy balance and the seasonality of water-column mixing and stratification. This record details how a large lake responded to climatic change in the spatially variable climate regimes of the Greater Yellowstone Ecosystem.

Chapter 5 investigates how early to middle Holocene hydrothermal explosion events affected paleolimnological conditions. The impact of early Holocene explosions is more pronounced – due to the relative instability of lake and coupled landscape dynamics in the post-glacial environment or as a function of lake morphology. Preference for R-selected species were apparent in post-hydrothermal explosion periods. However, diatom assemblages were generally resilient to hydrothermal explosion disturbance.

6.2 Ongoing Research

High-resolution, multi-proxy studies intrinsically require abundant data collection and analysis. As such, despite the extensive data presented within this dissertation, this research in Yellowstone National Park is still ongoing. Future research is largely related to data integration and interpretation of multi-proxy records.

Whereas the results documenting fossil diatom presence and incorporation into sinter deposits of the Yellowstone hydrothermal system presented in Chapter 3 were compelling enough to merit publication of a diatom-focused manuscript, additional samples of Yellowstone Lake spires were collected for chemistry and isotope analysis. As such, the continuation of this research focuses on integrating fossil diatom results with chemistry, silica isotopes, and a more detailed lithology to gain additional insights into spire formation including the environmental conditions in which the structures formed.

Similarly, while Chapter 4 focuses on diatom-inferred Holocene paleoclimate conditions from Yellowstone Lake, this research is only a contribution to a collaboration on the sediment core that includes fossil pollen, charcoal, oxygen and silica isotopes, elemental chemistry, and detailed lithologic analyses. The hierarchy of climate forcing mechanisms and related variable sensitivity of lake records, as well as the spatial heterogeneity of Holocene climatic change, necessitates the development of a network of lake sites in the Greater Yellowstone Ecosystem. As such, the HD-YLAKE paleolimnology research group is currently working on synthesizing these data to provide a high-resolution paleoclimate reconstruction of the largest alpine lake in North America.

In addition to reconstructing Holocene climate from Yellowstone Lake sediment cores, a main objective of the HD-YLAKE project is to assess the impact of hydrothermal explosion events on landscape and lake processes. As such, the fossil diatom data presented in Chapter 5 are preliminary. Additional diatom analysis from Goose Lake in the Lower Geyser Basin will be conducted to expand the range of systems beyond the Lake Geyser Basin. Statistical analyses and synthesis of fossil diatom, fossil pollen, and silica isotopes for Yellowstone Lake, Cub Creek Pond, and Goose Lake will be the final result of this project.