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High Salinity Stabilizes Bacterial Community Composition and Activity Through Time

Tylan Wayne Magnusson
Brigham Young University - Provo

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Tylan Wayne Magnusson

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Alan R. Harker, Chair
Donald P. Breakwell
Zachary T. Aanderud

Department of Microbiology and Molecular Biology

Brigham Young University

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ABSTRACT

High Salinity Stabilizes Bacterial Community Composition and Activity Through Time

Tylan Wayne Magnusson
Department of Microbiology and Molecular Biology, BYU
Master of Science

Dormancy is a plausible strategy for bacteria to overcome the effects of temporal fluctuations in resources or stresses and await more "optimal" conditions to resume metabolic activity and growth. Seasonal changes in environmental conditions force microbes to adjust their metabolic activity accordingly, and community composition drastically shifts. In extreme environments, however, the overriding effects of a constant stress may constrain the need or benefit of bacteria entering dormancy. In hypersaline lakes, high metabolic activity is required to maintain adaptations that permit survival. Sampling from six lakes on a salinity gradient (0.05% – 30.3%), we measured seasonal fluctuations in bacterial dormancy patterns in summer, fall, winter, and spring of 2013-14. Dormancy was calculated based on ratios of OTU recovery between 16S rRNA-based communities (only the active bacteria) and 16S rRNA gene-based communities (all bacteria present in the community) from lake water. Dormancy was linked to lake chemistry shifts through time. We found that salinity was strongly related to relative bacterial dormancy. There was a negative linear relationship ($R^2 = .89$ $P < 0.01$) between total dormancy and salinity. Total phosphorus ($R^2 = .63$, $P < .001$) and relative community contribution by rare taxa ($R^2 = .89$, $P < .001$) were also important in structuring dormancy. Our findings suggest that temporal nutrient flux is highly influential on bacterial community composition and activity, but that the presence of an extreme variable decreases change in both through time.

Key words: salinity, dormancy, Great Salt Lake, bacteria

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1. BACKGROUND

Ecological studies are based on the observed association of, and changes in, relationships between organisms with external influences (Kingsland 2004). Alexander Humboldt first used "biogeography" in 1807 when he characterized the types of plants that could grow at certain latitudinal zones and elevations (Jackson 2009). He hypothesized that all natural phenomena could be explained by thorough and consistent observation. Scientists quickly recognized that his ideas could be integrated into their own fields of study. For example, Charles Darwin's analyses of the geographical distribution of wildlife led to the theory of natural selection (Darwin 1859); Daniel Drake observed the geographic distribution of diseases and their insect vectors (Drake 1832); Ellen Swallow Richard's early 20th century studies linked the advent of industrialization and its pollutants to environmental and human health (Richards 1910). These large-scale ecological studies led to astounding paradigm shifts across many fields of science, and the methods by which their findings were made paved the way for modern studies in microbial ecology.

Microorganisms are ubiquitous on Earth, having been found over 3.6km beneath the Earth's surface (Borgonie 2011), throughout soils (Torsvik 1996), in the atmosphere (Jaenicke 2007), and at all depths of the ocean (Kirchman 2008). Early studies in microbial ecology were limited to isolated cultures from the environment, but even today, nearly half of known phyla have no successfully cultivated representatives (Yarza 2014, Rappé 2003). Only with the development and application of next-generation sequencing technologies has the breadth, in both form and function, of the microbial world begun to unfold (Mardis 2008). Deep sequencing techniques have yielded phylogenetic constructs that have revolutionized understanding of microbial evolution and adaptation (Brockhurst 2010), enabled the use of a wider array of

genetic markers for ecological studies (Ekblom 2011), and illuminated the unculturable microbial world (Schloss 2005).

2. INTRODUCTION

2.1 Extreme Environments and their Microbiota

Most of life on Earth is best adapted to moderate conditions (0.9-3% salinity, 7-42°C, pH 5-8.4, ~1 atm) (Canganella 2011), but abundant life is also found among hypersaline lakes, dry hot and cold deserts, at the bottom of the Marianas Trench, and in acidic mine drainage sites (Tazi 2014, Friedmann 1980, Kato 1998, Baker 2003). These extreme environments present conditions hostile to humans and the majority of life on Earth, but the extremophilic bacteria found therein have evolved to tolerate or even thrive (Canganella 2011). Such environments contain relatively simplified microbial communities that have evolved specific life history strategies to survive in their environment (Fiser 2012, Oren 2002). Thermophiles, for example, have amino acid substitutions in many of their most important proteins that decrease their flexibility and increase their resilience (Aguilar 1997). Psychrophiles, in contrast, have experienced mutations that increase protein flexibility to maintain stable active sites in very cold environments (Lonhienne 2000). In the hot deserts, *Deinococcus radiodurans* has evolved sets of polymerases capable of reassembling the entire genome after being fragmented by years of UV radiation and cellular dessication, and reviving when water becomes available again (Zahradka 2006). In order to combat the osmotic pressure of high-saline environments, halophile genomes may encode multiple Na^+/H^+ antiporters (Mesbah 2009), Na^+ gradient-powered ATPases (Mesbah 2011), cytoplasmic accumulation of K+, or even the synthesis of organic osmolytes to control osmotic pressure (Ciulla 1997).

Hypersaline lakes offer an especially convenient environment in which extremophiles may be studied because many of them share similar geological histories with freshwater analogs that experience the same temporal shifts in environmental characteristics. Hypersaline lakes

share a common core microbiome, filled with halophilic and halo-tolerant bacteria and archaea (Horikoshi 1998). Although other resources (such nitrogen and phosphorous) tend to fluctuate widely, salinity is constantly high throughout the year, overriding the effects of other resources as environmental drivers on bacterial community composition (Cytryn 2000, Verschuren 2004). Changes in the community composition of hypersaline lakes have been found to correlate with salinity alone, and no other abiotic factors, further streamlining methods used to analyze them (Logares 2013, Jiang 2007). The microbiota of hypersaline lakes have been found to be a reservoir of various metabolic genes, encoding enzymes capable of degrading a wide range of substrates, such as starch, glycogen, pectin, cellulose, chitin, and various hydrocarbons due to the limited input of carbon sources (Ollivier 1994). Actinomycetes, an abundant and highly active member of the hypersaline microbiota, found in soils, are the source of dozens of important antibiotics (e.g. streptomycin, gentamicin, vancomycin, erythromycin, tetracycline), pyrostatins, sporolides, tetrodotoxin, and many other chemicals of medical interest (Jose 2014). An examination of the response in cellular activity to environmental fluctuations is the first step towards understanding temporal interactions between microbes and their environment.

2.2 Bacterial Dormancy

Often when an environment experiences fluctuations of environmental characteristics or limiting factors, microbes must enter into a state of reduced metabolic activity in order to survive until conditions become favorable once again (Jones 2010). Under this dormant state, microbes measurably decrease rRNA production, a primary component of ribosomes; rRNA production is proportional to total RNA production and serves as an indicator of metabolic activity (Segev 2013). Microbes that are capable of entering and exiting dormancy under fluctuating conditions

have a competitive advantage over bacteria using fast growth strategies because their long-term survival is independent of static drivers. They are also more capable of coexisting with multiple, competing taxa, due to their ability to switch on and off from the active state (Stolpovsky 2011). Dormancy also contributes to the diversity and long-term stability of the system as a whole because dormant bacteria act as seeds in the ecosystem that can endure periods of adversity and later revive (Lennon 2011). Once they resuscitate, these microbes often become extremely active and may become dominant in the ecosystem. Dormant members of the rare biosphere, for example, who are considered extremely low abundance organisms have been found to become metabolically active following environmental triggers that lead to massive oceanic blooms (Alonso-Saez 2014), dramatic shifts in carbon use and production (Wang 2015), and even the activation of pathogenic disease in otherwise benign communities (Su 2013, Carvalhais 2014).

Bacteria may employ a number of different methods to enter into a dormant state, such as morphological changes through sporulation, implantation of the nucleoid into a host cell, or through persistence in which endonucleases cleave mRNAs to inhibit protein synthesis (Dworkin 2010). While in this dormant state, bacteria are able to evade nutrient deficiencies, harsh and growth-limiting conditions, and even antibiotics. Upon detection of necessary nutrients and peptides released by other growing organisms, bacteria emerge from dormancy and continue regular growth patterns (Dworkin 2010). In a prior study, it was found that hypersaline lakes experience significantly less bacterial dormancy than do freshwater lakes, and that dormancy in hypersaline lakes correlated with degree of salinity ($P < .001$); there, the extent to which fluctuating environmental drivers control the entrance and exit of bacteria into dormancy was poorly understood (Vert 2013)

2.3 Experimental Approach

In this study, bacterial dormancy was evaluated and linked to environmental conditions in 6 lakes in Utah on a continuum of salinity (0.52 - 303.22 PSU). DNA and RNA were extracted from the aquatic bacteria at 4 equally spaced time points throughout the year. The 16s rRNA genes (referred to as rDNA) were amplified; 16s rRNA transcripts were reverse transcribed and then amplified, and both were sequenced on the 454 pyrosequencing platform. Sequences were analyzed in Mothur, and dormancy for each OTU was determined. Total dormancy for a given lake was then calculated by summing relative abundances of all dormant OTUs. Dormancy was then associated with multiple environmental characteristics using linear regression and model selection.

It was hypothesized that bacteria in hypersaline lake communities exhibit lower levels of dormancy than bacterial communities in freshwater lakes; that bacteria in hypersaline lake communities undergo little change in composition, despite fluctuations in lake characteristics throughout the year; that both bacterial dormancy and community composition in freshwater lakes will widely fluctuate in response to temporal lake characteristics. This study serves to test whether salinity remains the sole driver of bacterial dormancy in hypersaline lakes over time. In addition to salinity, we measured other environmental characteristics known to structure microbial communities, including pH (Lauber 2009), temperature (Nadarajah 2007), dissolved oxygen (Zheng 2014), total nitrogen and phosphorus (Li 2015), and dissolved organic carbon (Johnson 2012).

3. MATERIALS AND METHODS

3.1 Lakes, salinity gradient, and water chemistry

Our study was conducted across a salinity gradient consisting of six lakes measured over four seasons (fall: 1-2 November 2013, winter: 30-31 January 2014, spring: 2-3 May 2014, and summer: 1-2 August 2014) along the Wasatch Front, UT, USA. Three of six lakes were considered hypersaline, exceeding 3.5% salinity at least once in the year (Grant 1998). Specifically, they averaged 30.3%, 19.4%, and 2.5% salinity for the North Great Salt Lake (GSL), South GSL, and Farmington Bay, respectively. The other three lakes (i.e., Willard Bay, Utah Lake, and Deer Creek Reservoir) were considered freshwater possessing average salinity concentrations from .05% to .16% (see Supplemental Table 1 for general characteristics and locations of lakes). All lakes possessed similar average depths and geological origins, having formed from the remains of ancient pluvial Lake Bonneville (Eardley 1973; i.e., North GSL, South GSL, and Utah Lake) or result from built infrastructure and snow-derived inputs from montane systems (i.e., Deer Creek Reservoir, Willard Bay, and Farmington Bay).

Water was collected for chemistry analysis 1.0 m below the lake surface approximately 200 m from the shoreline in the limnetic zone. We measured pH, temperature (ºC), salinity (PSU), dissolved oxygen (%, mg/L) with a YSI EXO1 Water Quality Sonde (YSI, Yellow Springs, OH, USA). Total dissolved N (mg/L) and total dissolved P (mg/L) was measured with a potassium persulfate digestion (Valderrama 1981) followed by cadmium reduction for measurement of NO_3^- and NO_2, and an ascorbic acid molybdenum reaction for soluble reactive phosphorus (Franson 1998). We filtered a subset of the sample and, following acidification to a pH of approximately 2 with HCl, measured dissolved organic C with a Shimadzu TOC-L analyzer via catalytic oxidation combustion at 720°C (Shimadzu Corp., Kyoto, Japan). We

examined differences in chemistry among the lakes and variation through time using boxplots to measure means and 95% confidence intervals in R (R Development Core Team 2015).

3.*2 Active and total bacterial communities*

Inferences were made about the temporal dynamics of dormancy in lake bacterial communities using relativized rRNA:rRNA gene ratios of all recovered OTUs. We assumed that OTUs identified from RNA transcripts where there was at least 1 copy of the transcript for every 2 copies of the gene were metabolically active, while the bacteria recovered from 16S rDNA genes reflected the total assemblage of bacteria (Hugoni 2013; Campbell 2013). As a result, we refer to communities based on the 16S rRNA gene as "rDNA" and 16S rRNA transcripts as "rRNA." We isolated bacteria from 1 L of water onto 142 mm 0.2 micron Supor® PES membrane disc filters (Pall Life Sciences, Port Washington, New York, USA) using a pressure filtration system (Advantec MFS Inc., Tokyo, Japan). Water samples for community analyses were collected concurrently with those for chemistry analyses; samples were flash-frozen in liquid nitrogen and stored at -80°C in the laboratory. To prevent cross-contamination of samples, the filtration system was cleaned and rinsed with 10% bleach solution, water, and 70% ethanol between each collection.

DNA and RNA were extracted from the filter discs using the PowerSoil DNA Isolation Kit and the RNA PowerSoil Total RNA Isolation Kit (MoBio Corporation, Carlsbad, CA, USA). A cDNA library was made of each RNA template with SuperScript III, One-step RT-PCR System with Platinum Taq (Invitrogen Corporation, Carlsbad, California, USA). The V4 regions of 16s rDNA and rRNA were amplified using the bacterial specific primer set 515F and 806R

with unique 12-nt Golay barcodes. All barcode sequences and full primer constructs are summarized in Table 2.

For the target amplification of rDNA and rRNA reverse transcripts, the following thermal cycle conditions were used: an initial denaturation step at 94ºC for 3 minutes followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 57.5˚C for 30 seconds, and a 45 second extension at 72ºC for 90 seconds. The amplified DNA and cDNA samples were purified using an Agencourt AMPure XP PCR Purification (Beckman Coulter Inc., Brea, California, USA) and quantified using a Quant-iT^{TM} PicoGreen dsDNA Kit (Invitrogen Corporation, Carlsbad, California, USA). Samples were adjusted to approximately equimolar concentrations, and sequenced at the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/) in a 454 Life Sciences Genome Sequence FLX instrument (Roche, Branford, Connecticut, USA). Sequences were cleaned, sorted, and analyzed using Mothur open-source software (http://www.mothur.org/wiki/Main_Page). Only sequences that were >300bp in length were included in the analysis in order to eliminate partial alignments that prevent proper OTU identification. All sequences were trimmed and sorted by barcode into their corresponding samples. They were then aligned to the SILVA database (http://www.arb-silva.de), matching identified OTUs with classified taxa. A 97% similarity cutoff was used to identify OTUs.

3.3 Estimates of Bacterial Dormancy

To estimate dormancy for individual taxa, the following equation was used:

$$\text{Dormancy} = 1 - \frac{\text{relative recovery of rRNA}}{\text{relative recovery of rDNA}}$$

We then estimated total dormancy in each lake across the four seasons by summing the relative recovery of rDNA-based OTUs that were considered dormant. To investigate changes of individual taxa, we grouped OTUs by phyla and related dormancy and relative abundance to

salinity and phosphorus through time. We produced a coordinate plane and assigned each dependent variable a different measure. Increasing relative abundance was demonstrated by proportionately larger points, while increasing dormancy was shown on a color gradient. We then generated heatmaps in R using bacterial families that represented at least 1% of the rDNA- or rRNA-based community of at least one sample. Intensity indices were based on the relative combined proportion of all OTUs for a given family.

3.4 Correlations with Bacterial Dormancy

The influence of temporal fluctuations of lake characteristics on bacterial dormancy was then evaluated using linear mixed-effects modeling, and model selection using AIC as a selection criteria. All environmental characteristics were tested for normality in R using the Shapiro-Wilks normality test and evaluated with QQ plots (Winther 2015, Shapiro 1965). Salinity was the only variable represented by a non-normal distribution; all analyses used a $\log_{10}$ transformation of salinity. Models were chosen for their known biological relevance and tested for collinearity. Those containing multicollinear variables (salinity and total nitrogen) were eliminated. Each regression of dormancy and lake characteristic was evaluated for linearity and significance. Generalized linear models were used due to the non-normal distribution of salinity values.

We used linear regression to examine whether rare or abundant OTUs primarily contributed to total dormancy. OTUs were considered rare if they represented less than 0.1% of the bacterial community, and abundant if they represented >1% of the community. Relative abundance of all rare and abundant OTUs were summed to give a value for each sample. The relationships between total dormancy and the proportion of the community considered rare, abundant, rare and dormant, and abundant and dormant were all examined for significance.

3.5 16s rDNA and rRNA bacterial phyla and communities

To evaluate similarities in bacterial community structure among the lakes and changes they underwent through time, a principle coordinate analysis (PCoA) of rDNA and rRNA communities from all samples was used to generate a relativized abundance-based distance matrix. The PCoA used Bray-Curtis similarity coefficient for the ordination of treatments. To reveal relationships between community structure and environmental chemistry variables through time, permutational multivariate analysis of variance (PERMANOVA) and redundancy analysis (RDA) were used via the *Adonis* function of the Vegan package in R (R Development Core Team 2008).

4. RESULTS

4.1 Lake chemistry

The lakes had a range of salinities, and also constituted a robust variety of nutrient concentrations (Figure 1). Mean salinity ranged from 0.05% (0.52 PSU) to 30.3% (303.22 PSU), mean P from 0.01 mg/L to 0.87 mg/L, mean N from 0.51 mg/L to 6.81 mg/L, mean DO from 50.53% to 154.98%, mean DOC from 1.83 μM/L to 55.93 μM/L, mean pH from 7.67 to 9.37. There was no difference in average temperature between lakes ($P = .98$). Average P, N, and DOC scaled with average salinity. pH demonstrated the opposite trend, and dissolved oxygen (DO) generally increased with salinity but was lowest when salinity was at its highest (in the North GSL). These values generally varied little throughout the year, but Farmington Bay experienced large temporal oscillations in each of the lake characteristics examined.

4.2 Bacterial Dormancy

Low salinity and low phosphorus availability were strongly associated with an increase in bacterial dormancy. Mixed-effects models associating dormancy with environmental parameters suggested that salinity alone ($AIC_c = -50.958$, $\Delta AIC_c = 0$, $w_i = 1.000$) and total phosphorus in addition to salinity ($AIC_c = -50.780$, $\Delta AIC_c = 0.178$, $w_i = 0.915$) structured bacterial dormancy. No other lake characteristics were found to be influential. Models with their associated AIC values are summarized in Table 1.

From the sequence analysis, 16,780 OTUs were identified and sorted by phylum and family. Neither rare (<0.1% recovery; $R^2 = .15$, $F = 3.75$, $P = .07$, $df = 1$) nor abundant (>1% recovery; $R^2 = .17$, $F = 4.34$, $P = .06$, $df = 1$) OTUs significantly contributed to total dormancy. However, generalized linear models demonstrated a strong linear relationship between dormancy

and relative abundance of rare OTUs (R^2 = .74, F = 54, P < .001, *df* = 1), dormancy and salinity (R^2 = .89, F = 175.3, P < .001, *df* = 1), and dormancy and total phosphorus (R^2 = .63, F = 37.07, P < .001, *df* = 1) (Figure 2). As salinity and total phosphorus decreased, dormancy increased. Where relative abundance of rare OTUs was high, dormancy was also high. Regression of the three most prominent drivers of dormancy were combined to show relationships between dormancy and total relative abundance of all rare taxa, salinity, and total phosphorus.

4.3 Dormancy Dynamics of Dominant Phyla

Some phyla were prone to enter and exit dormancy frequently throughout the year (i.e. Actinobacteria, Bacteroidetes), while others were consistently active (i.e. Betaproteobacteria, Cyanobacteria, Gammaproteobacteria) or dormant (i.e. Verrucomicrobia) (Figure 3). Actinobacteria were ubiquitous and abundant throughout all lakes (7.9%-37.8% average relative rDNA recovery), and temporal dynamics of relative abundance and dormancy were clearly correlated to seasonal changes. Where salinity and phosphorus were low throughout the year, Actinobacteria remained dormant. However, under highly saline conditions, members of this phylum were perpetually active. Bacteroidetes populations (9.2%-48.2% average relative rDNA recovery) were also highly responsive to seasonal changes, being especially active at high salinity and phosphorus in the summer, but entering into dormancy in the winter. Between these periods of elevated activity and dormancy, activity was moderate, but relative abundance was very high (80.4%, 57.1% of rDNA recovered from the North Great Salt Lake in spring, fall).

The relative abundance of cyanobacterial populations (0.2%-16.9% average relative rDNA recovery) varied widely throughout the year, contributing up to 14.5% of rDNA recovered in the summer and down to 0.4% of recovery in the winter at the same site (Willard Bay).

Cyanobacterial dormancy remained low throughout the year at all sites (average 3.7% recovery of dormant communities across all sites). Betaproteobacteria (0.9%-20.5% average relative rDNA recovery) were prominent contributors to the active community of freshwater lakes. They appeared to thrive in low nutrient (TP, TN, DOC) conditions, being relatively few but highly active members of the community. For example, in the Utah Lake winter extraction, they contributed to 24% of the total community, but 86.1% of the active community. Decreasing relative amounts of Betaproteobacterial rDNA coincided with increases in both activity and relative abundance of Cyanoabacteria and Verrucomicrobia. Gammaproteobacteria (0.7%-41.1% average relative rDNA recovery) were most abundant at the highly saline and moderate phosphorus conditions of the South Great Salt Lake. Dormancy was low throughout the year and consistent seasonal trends were absent.

Verrucomicrobia (0.6%-19.8% average relative rDNA recovery) were consistently highly abundant but dormant (0.01%-1.2% average relative rRNA recovery) throughout most of the year under low salt/phosphorus conditions. In the spring, however, activity increased significantly in all lakes. In the spring extraction of Deer Creek Reservoir, for example, Verrucomicrobia contributed to 8.3% of the total community, and 18.1% of the active community.

4.4 Dormancy Dynamics of Dominant Families

Several families were closely tied to high salinity while others were only found in the most freshwater lakes (Figure 4). Families within phylum Betaproteobacteria were found to be ubiquitous, highly active members of the freshwater community. In Utah Lake, for example, 23.0%, 7.3%, 53.3%, and 23.2%, (in summer, fall, winter and spring, respectively) of total RNA

came from Betaproteobacterial genus Rhodoferax, while genera Hydrogenophaga, Polynucleobacter and various Oxalobacteraceae were also prominent. Similar trends were found in Deer Creek Reservoir and Willard Bay, as well as Farmington Bay in the winter extraction when salinity was more similar to the freshwater lakes than the hypersaline lakes (5.3 PSU).

In Farmington Bay, Bacteroidetes families Saprospiraceae and Chitinophagaceae, as well as Actinobacteria families Actinomycetales and Nitriliruptorales were highly represented in the spring and summer extractions. Data from February showed community structure more similar to the freshwater lakes, while November gave rise to Bacteroidetes family Flavobacteriaceae and Planctomycetes genus *Rhodopirellula*.

Bacterial community composition of the South Great Salt Lake was much less fluid than the more freshwater lakes previously discussed. At all extraction times, members of Gammaproteobacteria genera Halomonas, Marinobacter and Thiomicrospira were highly prevalent, as were Actinobacteria genus Micrococcineae and Bacteroidetes genus *Gracilimonas*. These Gammaproteobacteria were especially dominant during the spring and summer, while fall and winter saw relative increases in all present Actinobacterial families.

The North Great Salt Lake likewise experienced little change in community composition throughout the year, being continuously dominated by the Bacteroidetes genus *Salinibacter* and a large number of unclassified bacteria (16.0%-27.8% rDNA recovery). Additionally Actinobacteria families Frankineae, Solirubrobacteriaceae, Pseudonocardineae, and Micromonosporineae represented omnipresent and highly active community members throughout the year. Bacteroidetes family Chitinophagaceae rose to prominence in the summer and fall sampling periods, while Deltaproteobacterial genus *Desulfobacter* became highly active, but relatively inactive in winter.

4.5 Environmental Influence on Community Composition

The analysis of similarities using the Bray-Curtis distance matrix of rDNA-based communities revealed a large set of variables structuring community composition. Salinity ($F(1,23) = 6.12$, $P < .001$), dissolved oxygen ($F(1,23) = 3.25$, $P < .001$), and total nitrogen ($F(1,23) = 2.82$, $P < .005$) were most significant. Where salinity was > 35 PSU, relative recoveries of Verrucomicrobia and Betaproteobacteria were low or nonexistent. Bacteroidetes appeared to be highly affected by dissolved oxygen, thriving (31.8-46.0% relative recovery) in low-oxygen conditions (<4mg/L) and blooming (>78.9% relative recovery) in hypoxic conditions in the North GSL (<2mg/L). As salinity remained high throughout all seasons in the South and North arms of the Great Salt Lake, community composition varied little, and appeared to be tied to changes in dissolved oxygen concentrations. In the more freshwater lakes, however, seasonal oscillations of all tested lake characteristics influenced the relative recovery of different bacterial taxa.

Furthermore the rRNA-based communities of bacteria in Deer Creek Reservoir, Utah Lake, and Willard Bay were more similar to one another than to their own rDNA-based counterparts throughout the year (Figure 5). In the three most saline lakes, rRNA-based communities were structured more similarly to their rDNA-based counterparts and experienced far less dramatic changes in community structure during seasonal shifts (Figure 4, 5).

5. DISCUSSION

5.1 Community Composition and Dormancy

Although extreme environments are considered to be arduous places for life to survive, this study demonstrates that they may also provide for decreased temporal changes in microbial community composition and decreased dormancy. We found that high salinity was associated with lower total bacterial dormancy and reduced magnitude of influence that seasonal changes in lake chemistry had on bacterial dormancy. Bacteria living in freshwater environments that experience seasonal oscillations of nutrients and oxygen must carry a versatile genome in order to adapt to circumstance, or lie dormant during times of duress (Comte 2011, Jones 2010). It follows, then, that natural selection favors those that are able to recognize changing variables and regulate cellular activity accordingly. Furthermore, such a dynamic system favors large diversity of bacteria with an array of "optima", leading to a bacterial community in a state of constant change as it responds to the environment (Dykhuizen 1990). As hypothesized, in hypersaline lakes, although the same variables also experienced temporal oscillations, high salinity was far more influential in shaping community structure and dormancy than any other variable or set of variables. Additionally, total phosphorus highly influenced bacterial dormancy, especially where salinity was not characteristically high.

Community structure varied greatly throughout the year in the more freshwater systems, as taxa responded to different variables. For example, Betaproteobacteria were dominant of the active community throughout the year in all of the freshwater lakes. Verrucomicrobia were consistently dormant, except for two instances of emergence in the May samplings of Deer Creek Reservoir and Utah Lake. These two samples represented the lowest values of dissolved oxygen, total nitrogen, and total phosphorus, suggesting verrucomicrobial seed banks may revive in times

of metabolic stress, either because they must increase in activity to survive or because the limiting conditions give them a competitive advantage over other taxa. Due to their low activity, but immense presence in these samples, Verrucomicrobia may have an under-recognized dominance in freshwater bacterial communities, similar to what has recently been discovered in soils (Bergmann 2011). Actinobacteria were also a consistent and predominant member of the dormant freshwater community, especially members of the suborders Micrococcineae and Frankineae, bacteria known for versatile genomes and environmental persistence in a wide range of soils and waters (Hamada 2010, Chaudhary 2013).

In the hypersaline environments, where most taxa that were present were also active, Bacteroidetes classes Sphingobacteria and Flavobacteria were consistently dominant, regardless of season. The nearest relative (~93% sequence similarity) of the most predominant Sphingobacter OTU was *Salinibacter ruber*, a red-pigmented, extreme halophile found in saltern crystallizer ponds (Antón 2002). These bacteria produce pigments that protect the cell from UV radiation and K^+ importers, which may partially contribute to their need for constant activity. Another predominant Sphingobacter OTU in the hypersaline lakes was from the family Chitinophagaceae. There was a four-fold increase in the relative community contribution of this family from May to August. These taxa likely represent the primary degraders of the immense brine shrimp and brine fly population living in these hypersaline lakes. As the shrimp and flies die off at the end of the summer, their chitinous exoskeletons may be hydrolyzed into chitin oligosaccharides, which then provide an abundant nitrogen and carbon source for microbes (Del Rio 2010). This constant influx of degradable biomatter may help to explain the high levels of nitrogen, phosphorus, and dissolved organic carbon found in the more hypersaline lakes.

5.2 Contributions of Common and Rare Taxa to Total Dormancy

All of the lakes examined in this study maintained some degree of core microbiome throughout the year. Although proportions of represented taxa fluctuated, the actual OTUs being represented changed little in any of the lakes. It could follow, then, that fluctuations in dormancy could be best attributed to a limited number of highly-represented OTUs. However, there was no relationship found between total dormancy and dormancy of rare taxa ($P = .53$), nor between total dormancy and dormancy of common taxa ($P = .88$). There was, however, a very strong relationship between total dormancy and the percent of the community representing rare taxa. This suggests that neither rare nor abundant taxa use dormancy as a life-history strategy more than the other. Rather, total dormancy and the abundance of rare and diverse bacteria are connected. Freshwater lakes provide a neutral environment capable of hosting an immense range of bacteria (Newton 2011). Hypersaline lakes, like all extreme environments, tend to select for organisms carrying specific adaptations (Oren 2002). Although all of the tested lakes receive input from similar sources (i.e., snow melt, farm runoff, treated industrial water), freshwater environments are more permissive to incoming microorganisms. With the increase in diversity comes an inevitable increase in competition among taxa for resources. It follows, then, that profuse interspecific competition may promote the use of dormancy as substantially as, or perhaps more substantially than, environmental stressors.

5.3 Environmental Structuring of Bacterial Communities

As the seasons change, all of the environmental characteristics included in this study were expected to fluctuate, and bacterial communities restructure accordingly. We found that high salinity reduces the magnitude of influence that other variables have on community

structure. Communities based almost entirely on a single, constant variable are inherently more stable for the microbes that tolerate or thrive in such conditions. By living in an environment that fails to support the proliferation of introduced taxa, existing community members are free from many of the microbe-microbe competitive interactions faced by bacteria living in freshwater. Thus, the use of dormancy as a tool for overcoming stresses decreases and bacterial communities remain stable in both community composition and activity.

We found that the most highly active taxa were often found in low relative abundance, suggesting that rRNA production is highest when bacteria are attempting to recover dominance or actively increase in abundance, relative to other taxa. As most large populations of specific taxa, particularly in freshwater lakes, were often found to be relatively dormant, decreases in activity may be associated with quorum sensing, as has been found in other environments (Maddula 2006, Zhang 2014, Leung 2012). Like other bacteria in which dormancy has been studied, i.e. human pathogens, dormancy of lake bacteria may be primarily driven by microbe-microbe interactions, which are simply limited by the extreme environmental parameters of hypersaline lakes. Acting as a robust filter for microbes with niche-specific adaptations, these select taxa with an extensive history of coexistence do not require dormancy to bet-hedge fluctuating conditions.

TABLES AND FIGURES

Table 1. Salinity and total phosphorus influenced seasonal fluctuations in the relative recovery of dormant bacteria based on forward-selection mixed effects models using Akaike's information criterion (AIC). The best-fitted model was determined by the highest Akaike weight (w_i) with AIC (log-likelihood term) and ΔAIC_c (difference from lowest AIC, penalized by the number of parameters) also shown. Dormancy in lakes was estimated from rRNA: rDNA ratios of the recovery of OTUs in active (rRNA) and total (rDNA) 16S community libraries, and calculated as the percent relative recovery of dormant OTUs occurring in each lake. Models including both salinity and total nitrogen were excluded from the final set due to collinearity.

ID	*Parameters*	*Log-likelihood*	*ΔAIC_c*	*Akaike weight*
1	Dissolved Organic Carbon (DOC)	-31.186	20.343	0.000
2	Dissolved Oxygen (DO)	1.115	52.644	0.000
3	pH	-4.604	46.925	0.000
4	Salinity	-51.529	0.000	1.000
5	Temperature	0.889	52.418	0.000
6	Total Nitrogen (TN)	-41.002	10.527	0.005
7	Total Phosphorus (TP)	-22.583	28.946	0.000
8	DOC + TP	-35.771	16.387	0.000
9	Salinity + TP	-51.980	0.178	0.915
10	Season + Salinity	-46.941	5.217	0.074
11	Temperature + DO + pH	-9.516	43.547	0.000
12	Temperature + DOC + pH + Salinity	-45.741	8.550	0.014

Figure 1.

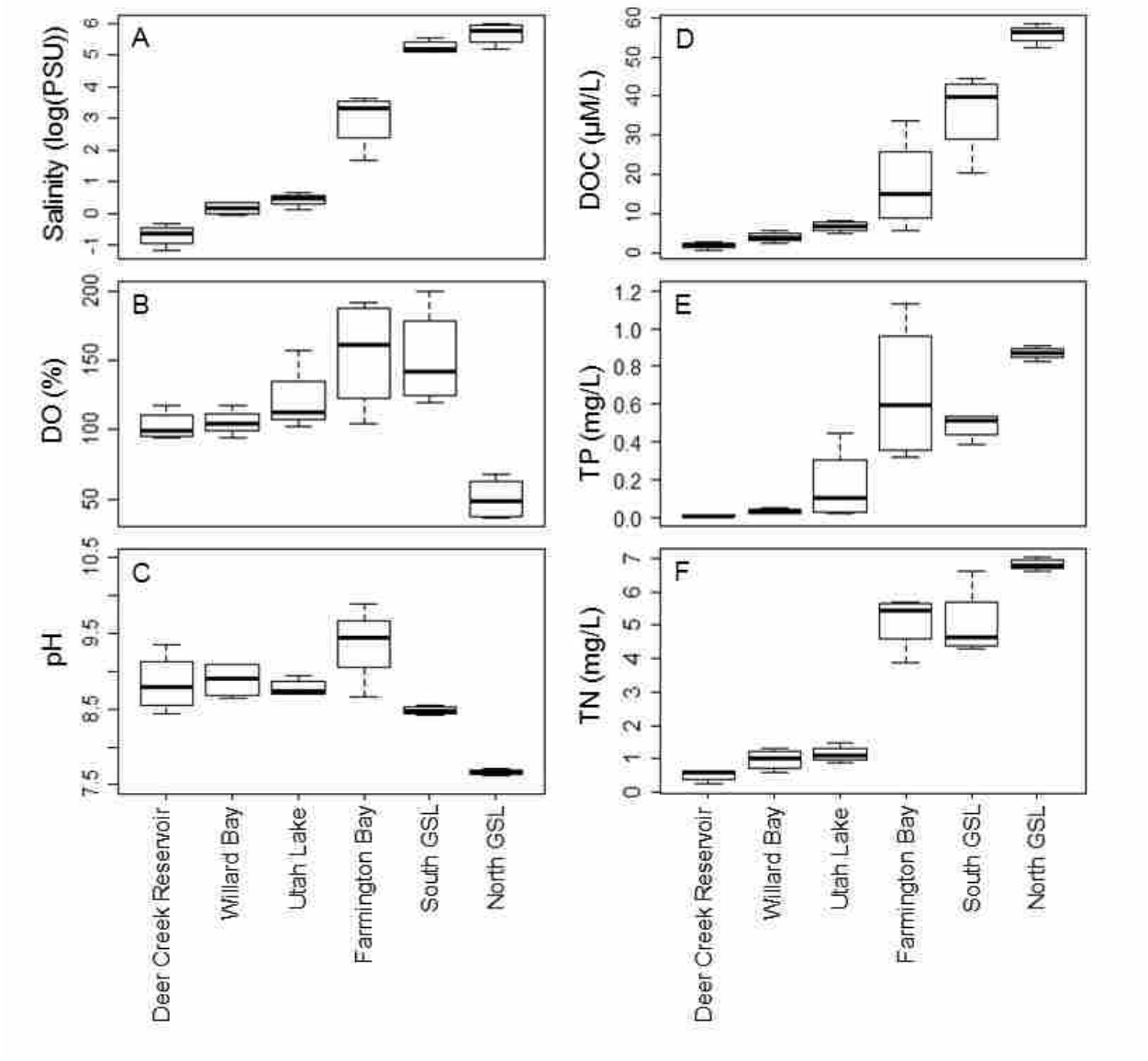


Figure 1. Box-and-whisker diagrams of lake chemistry across a salinity gradient consisting of six lakes, Utah, USA. Data in boxes are the mean (n = 4) and minimum and maximum values, with attending whiskers representing 95% confidence intervals, from four seasonal measurements.

Figure 2.

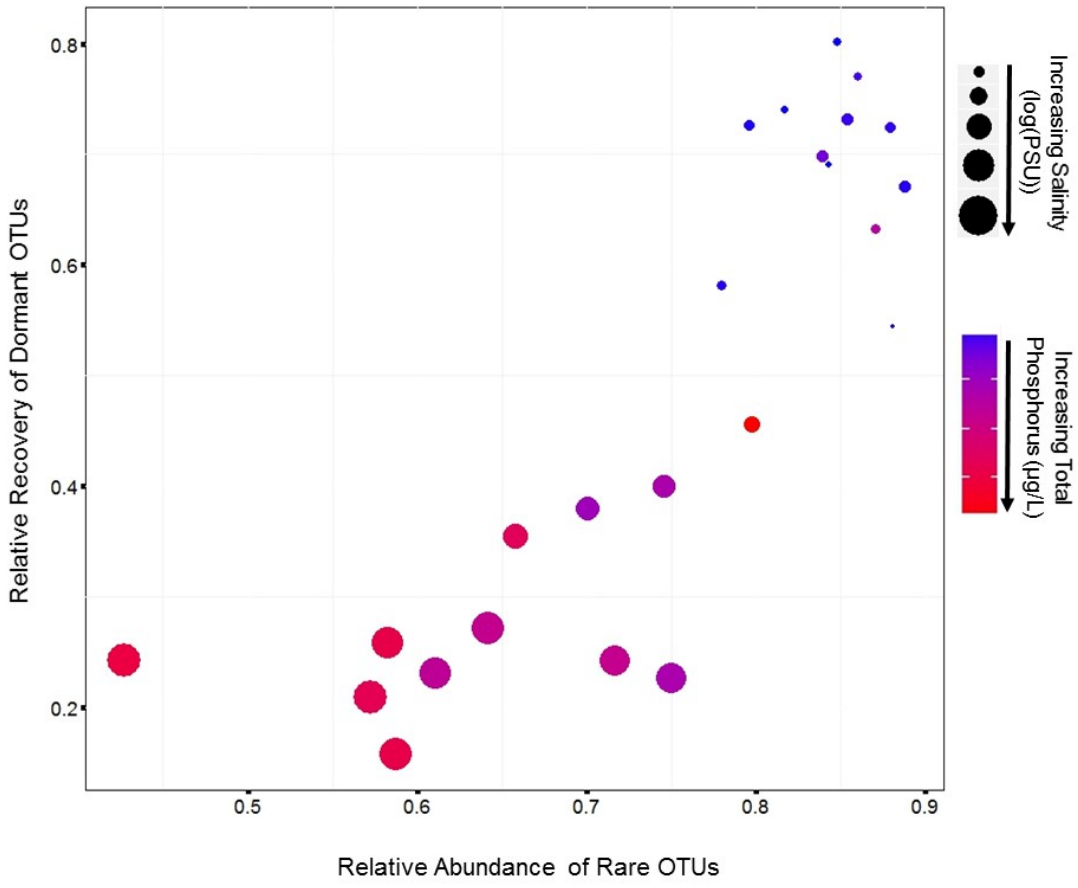


Figure 2. Dormancy increased as rare OTUs comprised more of the community, especially as salinity and total P declined over a year. The size of circles represents salinity (log (PSU)), while the color gradient represents TP availability (mg/L). Dormancy was estimated from 16S rRNA:rDNA ratios of total relative recovery within community libraries (97% similarity cut-off). OTUs were considered rare if they represented <0.1% in a given community.

Figure 3.

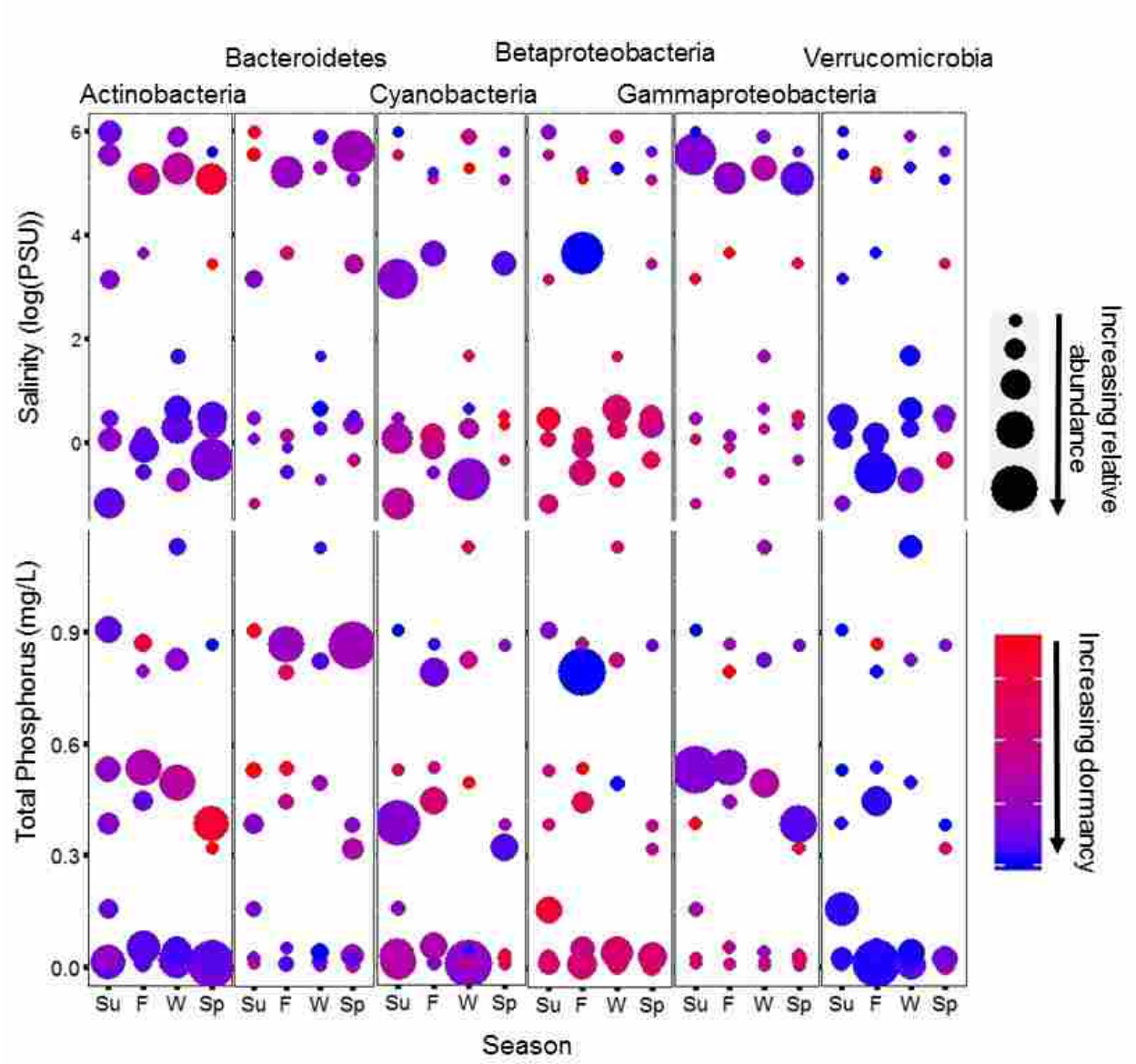
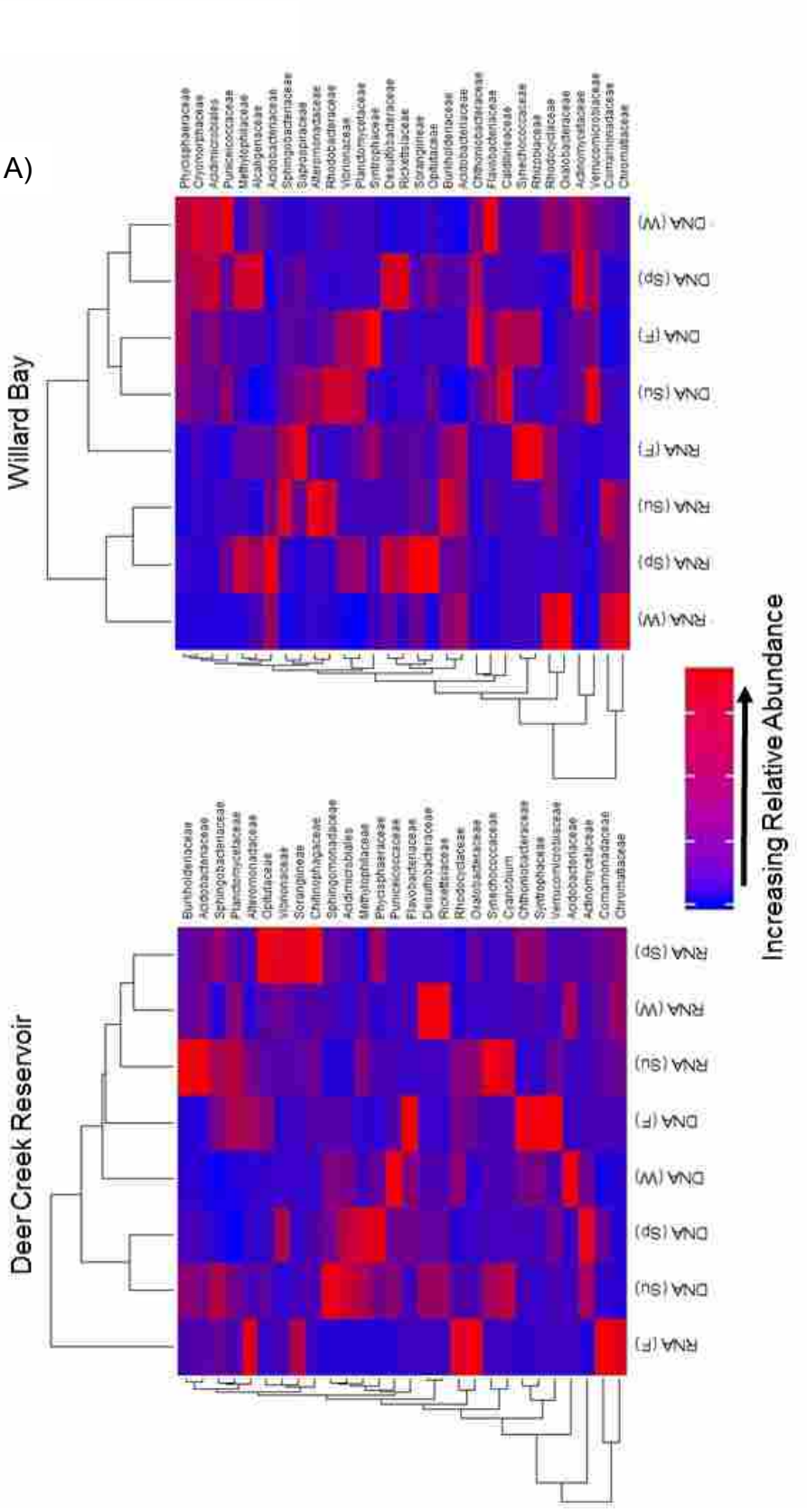
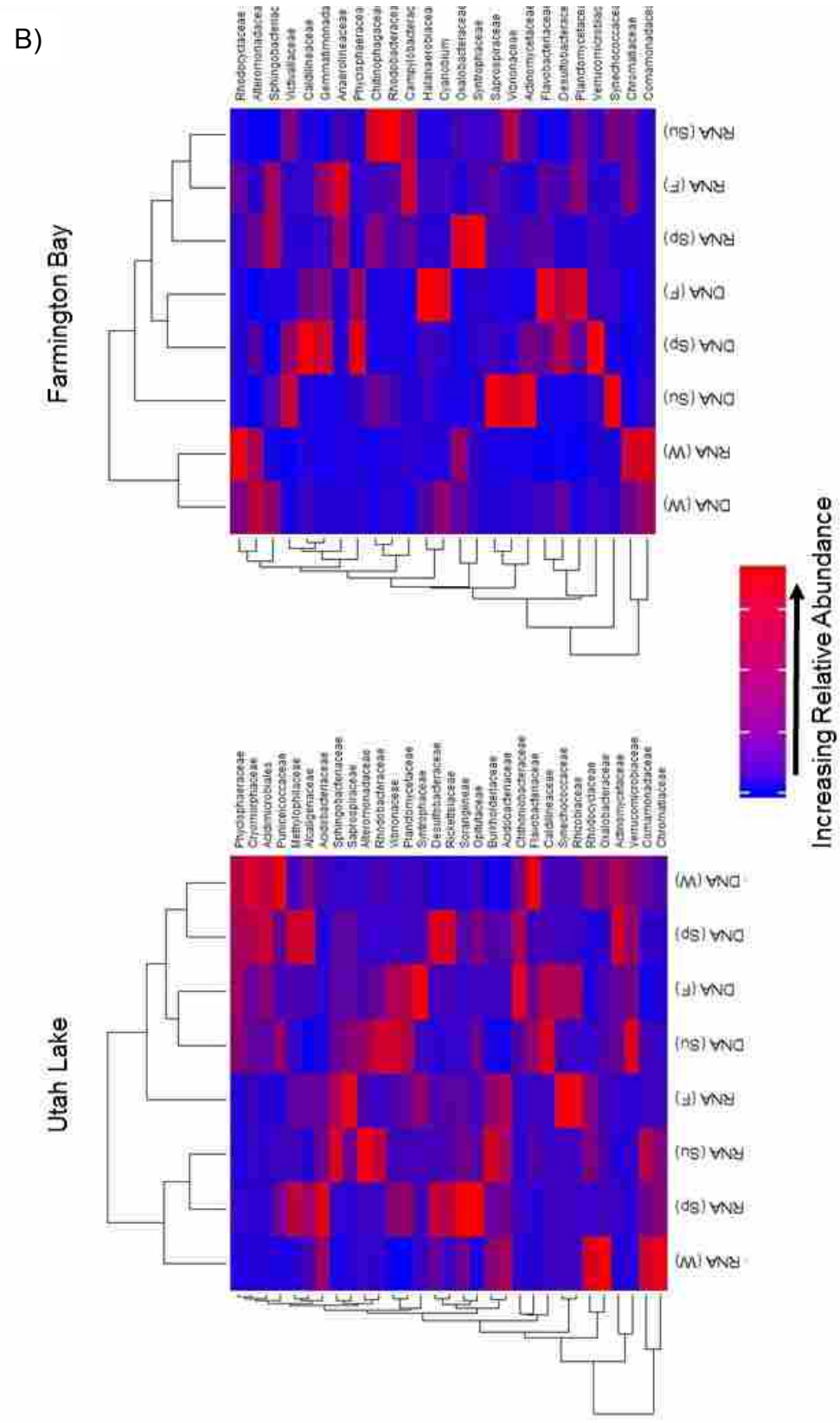


Figure 3. Seasonal dormancy patterns of the six most dominant phyla and Proteobacteria subclasses (>3% mean rDNA relative recovery across all lakes) highlighting interactions with salinity (log (PSU)) and TP (mg/L). While some taxa were consistently active (Betaproteobacteria) or dormant (Verrucomicrobia), the dormancy of Actinobacteria, Cyanobacteria, and Gammaproteobacteria fluctuated in response to seasonal changes of salinity and phosphorus. The four seasons included Summer (Su), Fall (F), Winter (W), Spring (Sp).

Figure 4.


A)
Willard Bay
Deer Creek Reservoir
Increasing Relative Abundance
DNA (W)
DNA (Sp)
DNA (F)
DNA (Su)
RNA (F)
RNA (Su)
RNA (Sp)
RNA (W)


B)
Farmington Bay
Utah Lake
Increasing Relative Abundance
DNA (W)
RNA (W)
DNA (Su)
DNA (Sp)
DNA (F)
RNA (Su)
RNA (Sp)
RNA (F)

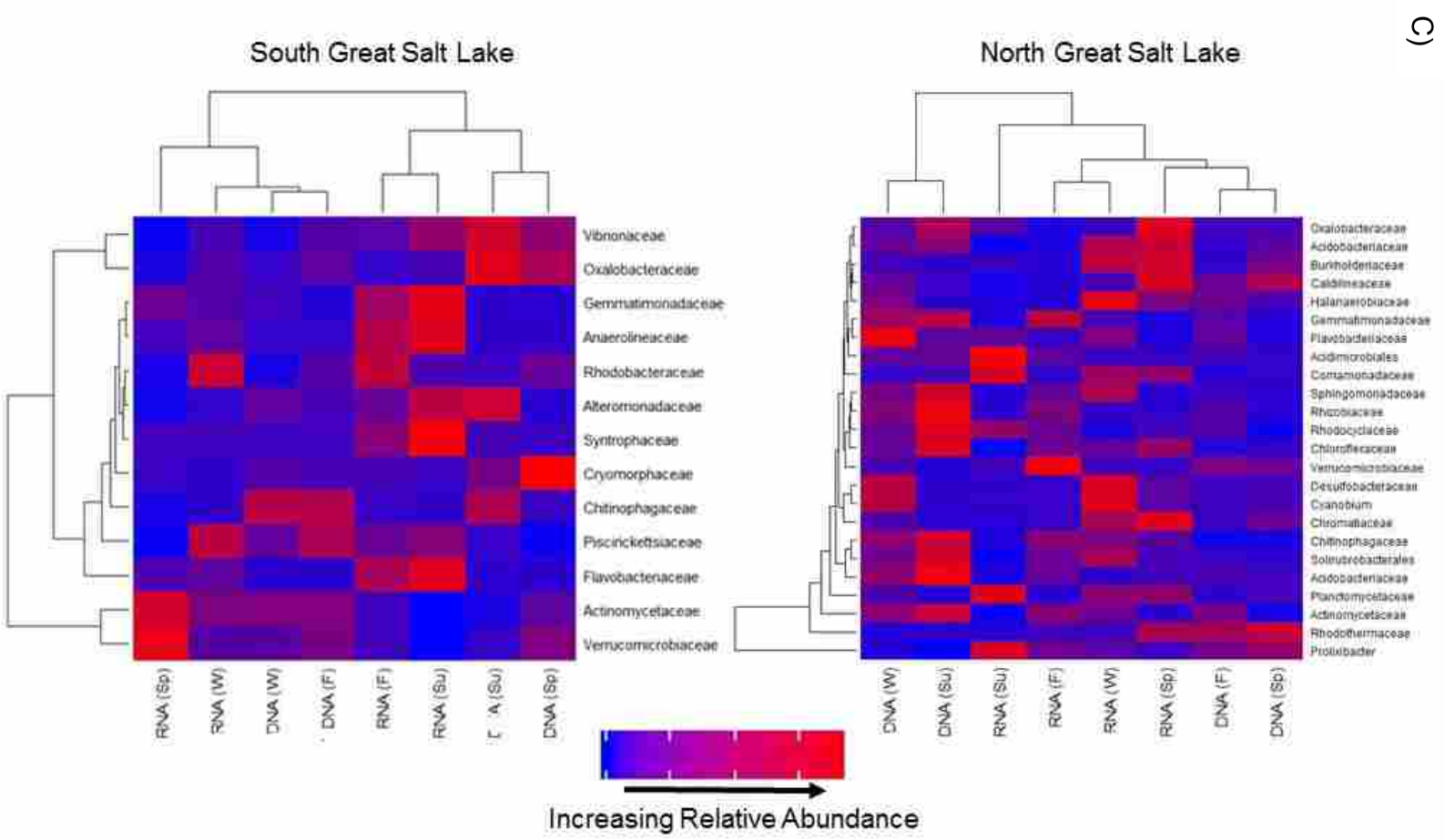


Figure 4. Heatmaps showing the distribution of 45 different families that contributed ≥1% to the total recovery of the 16S rRNA gene in each lake across the salinity gradient. Dendrograms are hierarchal clusterings by season and nucleic acid type (bottom) and bacterial families (right). A) Deer Creek Reservoir and Willard Bay; B) Utah Lake and Farmington Bay; C) South Great Salt Lake and North Great Salt Lake.

Figure 5.

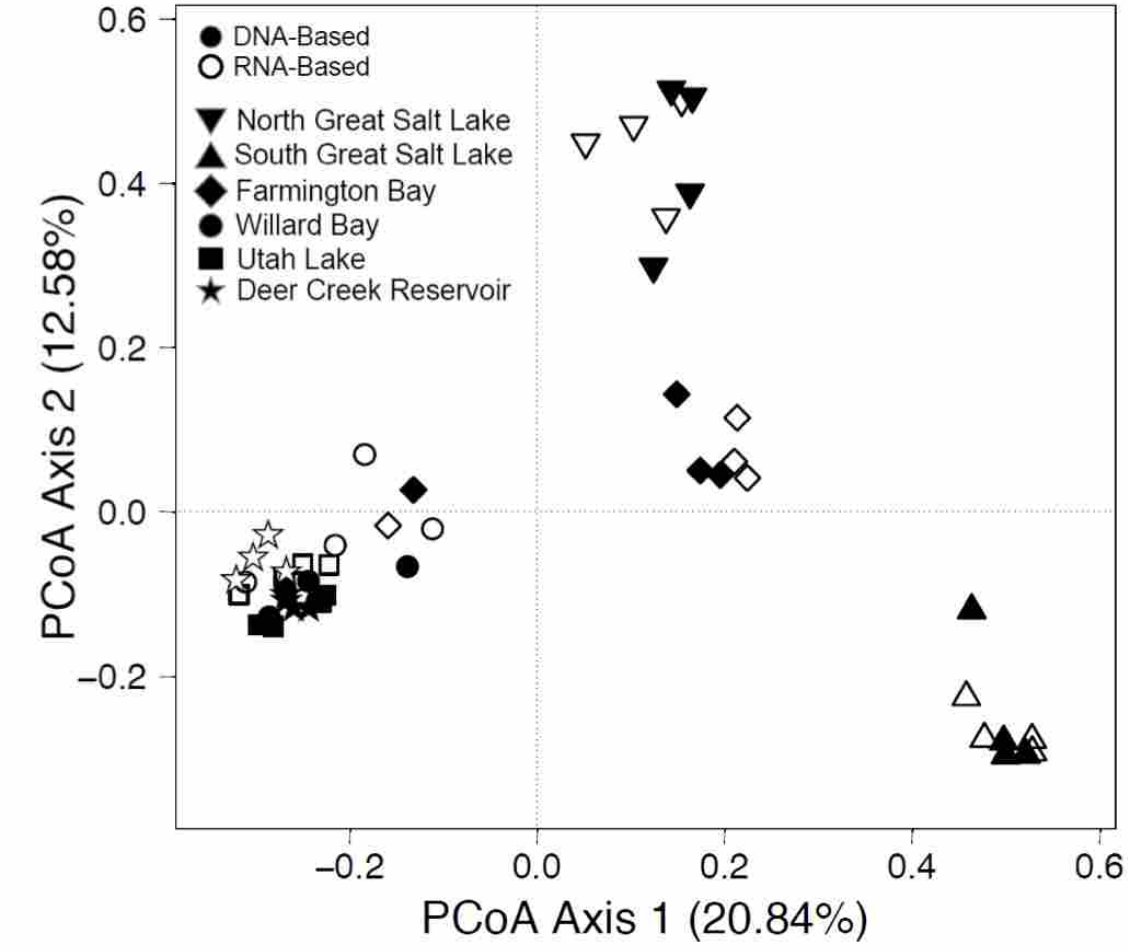
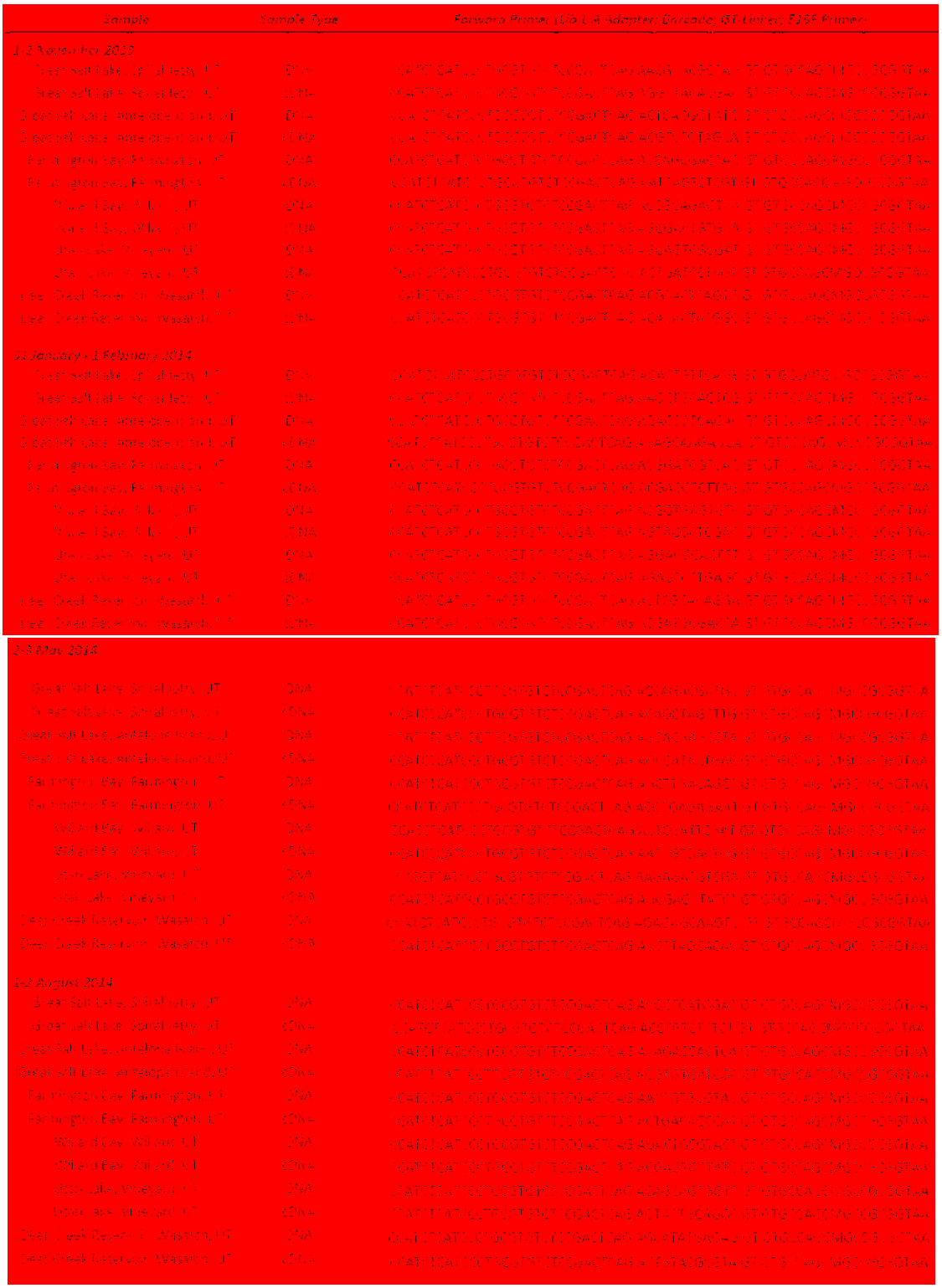


Figure 5. Bacterial communities differed by lake, season, and multiple lake characteristics. The multivariate ordination was generated using principle coordinate analysis (PCoA) on a sample × OTU matrix of active (rRNA) and total (rDNA) community libraries (97% similarity cut-off).

Supplementary Table 1. Locations and elevations of lakes

Sample	*Elevation (m)*	*Location*
Great Salt Lake, Spiral Jetty, UT	1285	N 41°25'54.43" W 112°40'0.05"
Great Salt Lake, Antelope Island, UT	1280	N 41° 3'5.98" W 112°15'27.00"
Farmington Bay, Farmington, UT	1300	N 41° 3'40.35" W 112°14'2.71"
Willard Bay, Willard, UT	1300	N 41°23'43.72" W 112° 3'17.59"
Utah Lake, Vineyard, UT	1368	N 40° 8'48.09" W 111°48'13.63"
Deer Creek Reservoir, Wasatchy, UT	1651	N 40°25'34.53" W 111°29'34.22"

Supplementary Table 2. Primer, adapter, and barcode sequences and constructs



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