

Hypersaline lakes harbor more active bacterial communities

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ABSTRACT

18 Extremophiles employ a diverse array of resistance strategies to thrive under harsh
environmental conditions but maintaining these adaptations comes at an energetic cost. If
20 energy reserves to drop too low, extremophiles may enter a dormant state of reduced
metabolic activity to survive. Dormancy is frequently offered as a plausible explanation
22 for the persistence of bacteria under suboptimal environmental conditions with the
prevalence of this mechanism only expected to rise as stressful conditions intensify. We
24 estimated dormancy in ten hypersaline and freshwater lakes across the Western United
States. To our surprise, we found that extreme environmental conditions did not induce
26 higher levels of bacterial dormancy. Based on our approach using rRNA:rDNA gene
ratios to estimate activity, halophilic and halotolerant bacteria were classified as inactive
28 at a similar percentage as freshwater bacteria, and the proportion of the community
exhibiting dormancy was considerably lower (16%) in hypersaline than freshwater lakes
30 across a range of cutoffs estimating activity. Of the multiple chemical characteristics we
evaluated, salinity and, to a lesser extent, total phosphorus concentrations influenced
32 activity. But instead of dormancy being more common as stressful conditions intensified,
the percentage of the community residing in an inactive state decreased with increasing
34 salinity in freshwater and hypersaline lakes, suggesting that salinity acts as a strong
environmental filter selecting for bacteria that persist and thrive under saltier conditions.
36 Within the compositionally distinct and less diverse hypersaline communities, abundant
taxa were disproportionately active and localized in families Microbacteriaceae
38 (Actinobacteria), Nitrospiraceae (Actinobacteria), and Rhodobacteraceae
(Alphaproteobacteria). Our results demonstrate that extreme environments may not

40 necessarily be stressful or suboptimal for highly adapted extremophiles causing them to
need dormancy less often to survive.

42

INTRODUCTION

44 Bacteria in extreme environments survive and often thrive in environmental conditions
that are outside the range experienced by the majority of life (Wardle et al., 2004).

46 Extremotolerant and extremophilic bacteria, which are found in virtually all harsh
environments, have motivated a wide range of research including the metabolic functions
48 that have contributed to the evolution of Earth's biosphere (Javaux 2006; Pikuta et al.,
2007); novel enzymes for biotechnological applications in chemical, food,
50 pharmaceutical industries (van den Burg 2003; Ferrer et al., 2007); and astrobiological
clues for discovering life elsewhere in the universe (Rothschild and Mancinelli, 2001). In
52 addition, extremophiles provide insight into the physiological adaptations and functional
traits that affect microbial performance along environmental gradients (Feller and
54 Gerday, 1997; Nealson and Conrad, 1999; Pakchung et al., 2006). For example,
extremotolerant and extremophilic bacteria have evolved a diverse array of resistance
56 mechanisms, such as the upregulation of organic osmolytes to deal with hypersalinity
(Detkova and Boltyanskaya, 2007), heat-shock proteins to combat high temperatures
58 (Solow and Somkuti, 2000; Pakchung et al., 2006), and antifreezes to survive in subzero
conditions (D'Amico et al., 2006; Struvay and Feller, 2012). However, all of these
60 adaptations come at an energetic cost, and if environmental conditions cause energy
reserves to drop too low, extremophiles may need to rely on other strategies to ensure
62 survival.

One mechanism, dormancy, is frequently offered as a plausible explanation for the
64 persistence of bacterial populations under suboptimal or harsh conditions (Stevenson
1978; Nicholson et al., 2000; Dworkin and Shah, 2010; Lennon and Jones, 2011). As a

66 bet-hedging strategy, dormancy builds “seed banks” or reservoirs of inactive individuals
that may resuscitated in the future under a different set of conditions (Lennon and Jones,
68 2011). This mechanism not only protects taxa from extinction (Kalisz and McPeck, 1992;
Honnay et al., 2008), alters species interactions (Chesson and Warner, 1981), and
70 influences ecosystem processes (Aanderud et al., 2015), but is prolific, with >90% of
biomass and >50% of all bacterial taxa residing in a state of inactivity at any time
72 (Alvarez et al., 1998; Lennon and Jones, 2011; Wang et al., 2014). However, the
empirical evidence for this mechanism is lacking under the harshest and most adverse
74 conditions—extreme environments. In general, the prevalence of dormancy is expected to
rise as stressful conditions intensify (Lennon and Jones, 2011). Therefore, bacteria in
76 environments at the margins of life should be overly dormant. Even though
extremophiles may not just *tolerate* their extreme condition but actually *require* it for
78 optimal growth and metabolism (Madigan and Marrs, 1997; Harrison et al., 2013), the
activity of extremophiles and extremotolerant bacteria is sensitive to abiotic factors with
80 many of these taxa only become metabolically active when a specific set of
environmental conditions are met (Pikuta et al., 2007; Zeldovich et al., 2007; Canganella
82 and Wiegel, 2011). Thus, if conditions stray outside these boundaries, extremophiles may
employ dormancy to survive.

84 Hypersaline lakes and their more benign analogs, freshwater lakes, not only offer an
ideal setting to identify the extent extremophiles employ dormancy but also the abiotic
86 cues structuring bacterial activity. In extreme environments, the primary adverse
conditions defining the environment (e.g, salinity, acidity, temperature) vary both
88 seasonal or episodically in intensity (Ferris et al., 2003; Detkova and Boltyanskaya, 2007;

Yucel et al., 2013). There is evidence that halophilic organisms are capable of using
90 dormancy as a way of contending with hypersalinity and the osmotic stress that it
induces. For example, an experimental reduction of hypersaline conditions in lagoon
92 water allowed previously undetected protozoa species to emerge from seed banks
(Esteban and Finlay, 2003). Also, as ancient hypersaline lakes disappear, haloarchaea
94 may survive rising hypersalinity levels in subterranean salt-remains in a dormant state
lasting thousands of years (Grant et al., 1998). Further, with the high energetic costs of
96 maintaining resistance strategies to combat osmotic stress (Oren 1999), halophiles and
halotolerant bacteria may be poorly suited to weather seasonal or episodic changes in
98 other abiotic conditions, thus increasing the need for dormancy. In freshwater lakes, the
activity of bacteria may track nutrient cues. Dormancy in freshwater systems is common
100 with >50% of freshwater biomass and >40% of bacterial taxa potentially residing in a
state of reduced metabolic activity (Lennon and Jones, 2011). P availability, in particular,
102 influences both bacterial activity (Schindler, 1978; Cole et al., 1993; Jones et al., 1998)
and dormancy (Jones and Lennon, 2010). Therefore, under periods of nutrient limitation
104 bacteria may decrease their metabolic activity to avoid competition, starvation, and
potentially death (Jones and Lennon, 2010).

106 In this study, we tested whether dormancy was more prevalent in extreme hypersaline
than freshwater environments and identified the differences in lake chemistry that
108 influenced activity. We estimated the dormancy of individual taxa from the recovery of
16S rRNA transcripts of metabolically active bacteria and 16S rRNA genes of all
110 potentially active bacteria (Jones and Lennon, 2010) in five freshwater and five
hypersaline lakes across the Western United States. We employed two different

112 measurements of dormancy: the percentage of bacterial taxa exhibiting dormancy in each
lake and the total relative recovery represented by these dormant taxa within the
114 community. We related our dormancy metrics to a suite of chemical characteristics
including dissolved O₂, pH, salinity, total nitrogen (TN), and total phosphorus (TP), and
116 temperature.

118 MATERIALS AND METHODS

Lakes and water chemistry

120 We sampled water from five hypersaline and five freshwater lakes located in seven states
(i.e., AZ, CA, CO, ID, OR, UT, WA) across the Western United States in the early
122 summer (17 May - 23 June 2012). We selected hypersaline lakes based on salinity (\geq
3.0%) and freshwater lakes that were comparable to at least one of the hypersaline lakes
124 in terms of mean depth. The hypersaline lakes included: Great Salt Lake, North Arm
(UT); Great Salt Lake, South Arm (UT); Salton Sea (CA); Abert Lake (OR); Mono Lake
126 (CA); and the freshwater lakes included: Mormon Lake (ID); Riffe Lake (WA); Arivaca
Lake (AZ); Lily Lake (CO); and Silverwood Lake (CA). Supplemental Table 1 provides
128 additional information on the elevation, surface area, mean depth, and location of the
lakes. Water samples were removed 1.0 m below the lake surface approximately 200 m
130 from the shoreline. We measured electrical conductivity, dissolved oxygen (O₂), and
temperature *in situ* with an OAKTON EcoTestr EC Low Meter (Oakton Instruments Inc.,
132 Vernon Hills, Illinois, USA) and YSI EcoSense DO 200 meter (YSI Inc., Yellow Springs,
OH, USA). All other data were collected on lake water samples after being transported on
134 ice back to the laboratory. We measured salinity with a conductivity bridge (Beckman,

Brea, CA, USA) and pH with a Thermo Orion Model 410 pH meter (Thermo Scientific,
136 Beverly, MA, USA). Total nitrogen (TN) was measured by oxidation and subsequent
chemiluminescence using a Shimadzu TOC-V equipped with a TNM-1 unit (Shimadzu,
138 Kyoto, Japan). We measured total phosphorus (TP) by persulfate oxidation of organic
phosphorus to phosphate followed by colorimetric analysis (Koroleff 1983). We tested
140 for differences between hypersaline and freshwater lake chemistry using multiple t-tests
and a Benjamini-Hochberg correction to control for the false discovery rate associated
142 with multiple comparisons (Benjamini and Hochberg, 1995). Last, in the field, we
collected bacterial biomass for molecular analyses from 2.0 L of water on 142 mm 0.2
144 μm filters (Supor[®] PES membrane, Pall Life Sciences, Port Washington, New York,
USA) using a pressure filtration system (Advantec MFS Inc., Tokyo, Japan). Filters were
146 immediately flash frozen with liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$.

148 **rDNA and rRNA bacterial communities**

We characterized lake bacterial communities using RNA- and DNA-based approaches to
150 make inferences about the activity of bacterial taxa. Because ribosomal RNA has a
relatively short half-life and is required for protein synthesis (Flardh et al., 1992;
152 Bernstein et al., 2002; Steglich et al., 2010), we assumed that bacteria identified from
RNA transcripts were metabolically active, while the bacteria recovered from 16S rDNA
154 genes reflect the taxa with varying levels of activity, including organisms that are slow
growing and/or dormant (Hugoni et al., 2013, Campbell and Kirchman, 2013). For the
156 remainder of the paper, we refer to communities based on the 16S rRNA gene as “rDNA”
and 16S rRNA transcripts as “rRNA.” Nucleic acids were extracted from filters using a

158 PowerSoil DNA Isolation Kit and a RNA PowerSoil Total RNA Isolation Kit (MoBio
Corporation, Carlsbad, CA, USA). We reverse transcribed RNA transcripts to cDNA
160 using a SuperScript III, one-step RT-PCR kit (Invitrogen Corporation, Carlsbad, CA,
USA). We PCR amplified the V3-V4 region of the 16S rRNA gene and cDNA using
162 bacterial specific primer set 515F and 806R with unique 12-nt error correcting Golay
barcodes (Aanderud and Lennon, 2011). The thermal cycle conditions were: an initial
164 denaturation step at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 45
sec, annealing at 50 °C for 30 sec, and an extension at 72 °C for 90 sec. After purifying
166 (Agencourt AMPure XP PCR Purification Beckman Coulter Inc., Brea, CA, USA) and
pooling PCR amplicons at approximately equimolar concentrations, samples were
168 sequenced at the Brigham Young University DNA Sequencing Center
(<http://dnac.byu.edu/>) using a 454 Life Sciences genome sequence FLX (Roche,
170 Branford, CT, USA). We analyzed all sequences using mothur (v. 1.29.2), an open-
source, expandable software pipeline for microbial community analysis (Schloss et al.,
172 2009). After removing barcodes and primers, we screened sequences to remove short
reads, chimeras, and non-bacterial sequences. First, we eliminated sequences < 250 bp in
174 length and sequences with homopolymers longer than 8 bp. Second, we removed
chimeras using UCHIME (Edgar et al., 2011) and eliminated chloroplast, mitochondria,
176 archaeal, and eukaryotic 16S rRNA gene sequences based on reference sequences from
the Ribosomal Database Project (Cole et al., 2009). We then aligned sequences against
178 the SILVA database (Pruesse et al., 2007) with the SEED aligner, created operational
taxonomic units (OTUs) based on uncorrected pairwise distances at 97% sequence
180 similarity, and determined the phylogenetic identity of OTUs with the SILVA database.

To characterize variability in bacterial community composition among lakes, first, we
182 used Principal Coordinates Analysis (PCoA) and permutational multivariate analyses of
variance (PERMANOVA, Anderson 2001). The PCoA was based on a Bray-Curtis
184 distance matrix using the 'vegan' package in R (R Development Core Team 2013). While
the PCoA aided in the visualization of communities, we tested for the main effects and
186 interactions between lake type (hypersaline versus freshwater) and nucleotide type
(rDNA and rRNA) with PERMANOVA using the *adonis* function also in the 'vegan'
188 package of R. Second, we quantified the alpha diversity of communities as the inverse
Simpson index (Haegeman et al., 2013) after rarefaction by a common sequence number
190 (5,846) to remove any bias due to differences in sequencing depth among samples
(Nipperess and Matsen 2013). We examined differences in alpha diversity between lake
192 (hypersaline vs. freshwater) and nucleotide (rDNA vs. rRNA) type using two-way
ANOVA with a Tukey's HSD test. Third, we calculated the relative recovery of eleven
194 phyla and four subclasses in rDNA communities to identify differences in the distribution
of major taxonomical groups (recovery $\geq 1.0\%$) between hypersaline and freshwater
196 lakes. Taxonomic trends were shown with a heat map with hierarchal clustering using the
heatmap function in the 'gplot' package in R (Oksanen et al., 2013). Last, to evaluate if
198 hypersaline and freshwater environments supported similar numbers of bacteria, we
estimated abundance as the number of 16S rRNA gene copies in lakes using quantitative
200 PCR and the bacterial specific primer set 515F and 806R (Aanderud and Lennon, 2011).
We tested for differences between hypersaline and freshwater lakes using a t-test.

202

Bacterial dormancy estimates

204 We used rRNA: rDNA ratios as a proxy to estimate if a given taxa was dormant or active
(Franklin et al., 2013, Jones and Lennon, 2010). Specifically, in each lake, we estimated
206 the dormancy of individual OTUs as $1 - (\text{rRNA recovery} / \text{rDNA recovery})$. From each
of the resulting values, we classified OTUs as either dormant or active based on a cutoff.
208 The classification of dormant versus active OTUs is sensitive to the specific cutoff
selected (Franklin et al., 2013). Therefore, we estimated dormancy across a range of
210 cutoffs from 0.1 - 0.9. From each of these cutoffs, we estimated bacterial dormancy as the
percentage of dormant OTUs occurring in each lake and as the total relative recovery
212 represented by these dormant taxa within the community. Specifically, the percentage of
dormant OTUs was calculated as the number of dormant OTUs divided by the total
214 OTUs present in a given lake $\times 100$, while the relative recovery of dormant OTUs was
calculated as the sum of all dormant OTUs in each lake. To determine if dormancy was
216 more prevalent in hypersaline environments, we used an indicator variable in multiple
regression where lake type (hypersaline vs. freshwater) was treated as a categorical
218 predictor variable. Differences in the slopes or intercepts between lake type suggest that
hypersalinity differentially affected dormancy responses across the cutoffs (Lennon and
220 Pfaff, 2005).

222 **Environmental drivers of bacterial dormancy**

We identified the lake chemical characteristics that influenced bacterial dormancy in
224 hypersaline and freshwater lakes using multiple regression with lake as a categorical
predictor variable (Neter et al., 1996, Lennon and Pfaff, 2005; Lennon et al., 2013). We
226 tested whether or not a variable (i.e., dissolved oxygen, pH, salinity, TN and TP) related

to the percentage of dormant bacteria occurring in the five hypersaline and five
228 freshwater lakes and the recovery of dormant taxa using forward selection procedure and
Akaike's information criterion (AIC; Akaike 1998). For these analyses, we used the
230 median cutoff value to classify OTUs as either dormant (≥ 0.5) or active (< 0.5). At this
cutoff ratio, the total recovery of an OTU (active and inactive cells) was at least double
232 the recovery of RNA transcripts being produced. Therefore, we assumed that in dormant
OTUs no more than half of the bacteria were metabolically active and producing RNA
234 transcripts. In indicator multiple regression, lake chemistry variables were treated as
continuous predictor variable and lake type (hypersaline vs. freshwater) was treated as a
236 categorical predictor variable. Differences in the slopes or intercepts between lake type
suggest that hypersalinity differentially affected dormancy responses to the chemistry
238 variables. The chemical characteristics were checked for collinearity using the *vif*
function in the 'car' package in R.

240

Rare and abundance bacteria and dormancy

242 We classified dormant and active OTUs into abundance categories to gain insight into the
contribution of rare and abundant taxa to bacterial dormancy. In our study, rare OTUs
244 were defined as OTUs with a relative recovery $\leq 0.1\%$ and all other OTUs were
considered abundant with a relative recovery $> 0.1\%$ in rDNA communities. Justification
246 for this is based on rank abundance curves of bacterial communities from sequencing
efforts. In these curves, the bacterial recovery of 0.1% often represents a visible
248 demarcation between the few abundant OTUs with relatively high recoveries and the
thousands of rare OTUs with relatively low recoveries (Pedros-Alio 2012). Similar to

250 indicator variables multiple regression analyses, OTUs were classified as either dormant
(≥ 0.5) or active (< 0.5). We tested for the effects of lake type (hypersaline versus
252 freshwater) and activity (dormant versus active) on the percentage and recovery of rare
and abundant OTUs in communities using two-way ANOVA and Tukey's HSD tests.
254 Further, to evaluate whether dormancy was restricted to specific OTUs, we estimated the
number and recovery of dormant rare and abundant OTUs in forty-five bacterial families.
256 Similar to dormancy in lakes, we estimated dormancy in families as the percentage of
dormant OTUs occurring in a given family in each lake and summed the relative recovery
258 of dormant OTUs for these taxonomical groups. Differences in dormancy among
taxonomical groups and lakes were shown in heat maps with hierarchal clustering using
260 the *heatmap* function in the 'gplot' package in R (Oksanen et al., 2013).

262 RESULTS

Water chemistry

264 Salinity clearly distinguished the extreme conditions in hypersaline lakes from the more
benign environmental conditions in freshwater lakes, but other chemical variables also
266 differed between lake types (**Table 1**). On average, salinity was twenty-four-times higher
in hypersaline than in freshwater lakes. In addition, electrical conductivity was nineteen-
268 times higher in hypersaline lakes, and pH was 8.7 ± 0.47 in hypersaline and 7.0 ± 0.18 in
freshwater lakes (mean \pm SEM). Conversely, O₂ levels were 23% lower in hypersaline
270 than freshwater lakes. Based on concentrations of TN and TP, the trophic status of
freshwater and hypersaline lakes varied widely from oligotrophic to hypereutrophic,

272 resulting in no differences in total resources between lake types (Vollenweider and
Kerekes, 1980; Bachmann et al., 2013).

274

Bacterial communities in hypersaline and freshwater lakes

276 Hypersaline environments had strong effects on the composition of active and total
bacterial communities. This inference was based on the recovery of 570,013 quality
278 sequences and 4,212 unique OTUs with samples possessing an average sequencing
coverage of $97\% \pm 0.01$. The PCoA results distinctly separated hypersaline from
280 freshwater bacterial communities in ordination space along PCoA axis 1, which
explained 32.4% of the variation (**Figure 1**). Hypersaline communities were further
282 separated along PCoA axis 2, which explained 18% of the variation. The PERMANOVA
results supported the ordination demonstrating a compositional difference between
284 hypersaline and freshwater communities (PERMANOVA, lake type, $F = 5.33$, $P = 0.005$,
 $df = 1$), and also revealed a significant difference between active and total bacterial
286 communities (PERMANOVA, nucleotide type, $F = 1.9$, $P = 0.03$, $df = 1$).

Despite having similar bacterial densities as freshwater communities, hypersaline
288 communities were less diverse and compositionally similar. Specifically, bacterial
diversity was 58% lower in hypersaline than freshwater rDNA communities (two-way
290 ANOVA, lake \times nucleotide type, $F = 15.1$, $P = 0.001$, $df = 1$, **Supplemental Figure 1**).
The distribution of six phyla and three Proteobacteria subclasses distinguished
292 hypersaline from freshwater communities; while rDNA and rRNA communities closely
grouped together only within hypersaline lake (**Figure 2**). The recovery of
294 Alphaproteobacteria and Cyanobacteria was at least 2.5- and 1.7-times higher in

hypersaline than freshwater rDNA and rRNA communities, respectively. Alternatively,
296 the recovery of Bacteroidetes was 7.1-times lower in hypersaline rDNA and rRNA
communities. Based on qPCR of rDNA, hypersaline ($5.8 \times 10^6 \pm 4.41 \times 10^6$ copies 16S
298 rDNA L⁻¹ water) and freshwater lakes ($1.1 \times 10^7 \pm 1.00 \times 10^7$ copies 16S rDNA L⁻¹
water) bacterial densities were comparable (t-test, $t = 0.24$, $P = 0.64$, $df = 1$).

300

Bacterial dormancy estimates in lakes

302 Dormant bacteria were detected in hypersaline and freshwater lakes and dormancy was
more prevalent in freshwater than extreme hypersaline environments. Based on indicator
304 linear regression results describing the relative recovery of dormant OTUs ($R^2 = 0.82$,
 $F_{86,8} = 133$, $P < 0.001$, **Figure 3**), the effect of lake type on dormant OTUs was reflected
306 in a difference between the y-intercepts in the equations for each lake type (equation 1
and 2; $P < 0.001$) where dormancy was 16% lower (percent decrease based on y-
308 intercepts) in hypersaline than freshwater lakes across a wide range of cutoffs.

310 Freshwater: recovery of dormant OTUs (%) = $80.4 - 59.2$ (cutoff) (1)

Hypersaline: recovery of dormant OTUs (%) = $67.6 - 62.4$ (cutoff) (2)

312

In general, the recovery of bacteria exhibiting dormancy decreased linearly as the
314 cutoff estimating dormancy increased, and there were no interactions between the slopes
and intercepts, suggesting that the dormancy conclusions were robust across the entire
316 range of cutoffs. Alternatively, the effect of lake type on the number of dormant OTUs
was similar leading to the overall model:

318

$$\% \text{ Dormant OTUs} = 74.0 - 60.8 (\text{cutoff}) \quad (3)$$

320

($R^2 = 0.68$, $F_{86,8} = 186$, $P < 0.001$, **Supplemental Figure 2**). As the cutoff increased or
322 became more stringent, the number of OTUs exhibiting dormancy decreased with values
ranging from $59.3 \% \pm 6.92$ to $39.6\% \pm 1.22$.

324

Environmental drivers of bacterial dormancy

326 Salinity influenced dormancy in both hypersaline and freshwater lakes. The multiple
regression model that best predicted the relative recovery of dormant OTUs differed by
328 lake type for salinity (equation 4 and 5; $P < 0.05$) but also included TP to a lesser extent
($P < 0.09$, $R^2 = 0.96$, $F_{8,1} = 50.0$, $P < 0.001$; **Figure 4**). Of all possible models, this one
330 generated the lowest AIC score (54) with a ΔAIC of 4.4 units, and resulted in the
following equations:

332

$$\text{Hypersaline: recovery of dormant OTUs (\%)} = 57.6 - 9.10 (\text{salinity \%}) + 0.04 (\text{TP}) \quad (4)$$

$$\text{Freshwater: recovery of dormant OTUs (\%)} = 33.0 - 0.58 (\text{salinity \%}) + 0.04 (\text{TP}) \quad (5)$$

336 Dormancy decreased with increasing salinity across both lake types, but the effect of
salinity on dormancy was more pronounced in freshwater systems where the slope
338 describing salinity's impact on dormancy was 16-times higher in freshwater than
hypersaline lakes, substantially contributing to a 43% decline in dormancy (percent
340 decrease based on y-intercepts, $P < 0.001$) in freshwater to hypersaline lakes. We found

no significant model describing the impact of environmental drivers on the number of
342 dormant OTUs.

344 **Abundance and dormancy**

A greater percentage of abundant bacteria were classified as active rather than dormant in
346 hypersaline lakes. Specifically, the recovery of abundant OTUs ($> 0.1\%$ relative
recovery) comprised the majority of rDNA in extreme communities ($95\% \pm 1.1$) with
348 2.4-times more of this recovery being dormant than active (two-way ANOVA, lake \times
active versus dormant, $F = 78.1$, $P < 0.0001$, $df = 1$, **Figure 5**). Further, the recovery of
350 abundant and active taxa was 61% higher in extreme than freshwater communities. The
relative recovery of rare active and dormant OTUs were similar across lakes (two-way
352 ANOVA lake \times active versus dormant, $F = 0.32$, $P = 0.58$, $df = 1$), with values ranging
from $6.9\% \pm 2.5 - 1.7\% \pm 0.40$.

354 Between hypersaline and freshwater lakes, there were robust taxonomical differences
in abundant active and dormant taxa. Differences in abundant and active bacteria between
356 lake types were localized in five families within two phyla (i.e., Actinobacteria and
Proteobacteria), which accounted for upwards of 46% of the recovery in any lake (**Figure**
358 **6**). For example, the percentage of abundant and active OTUs in the Microbacteriaceae
(Actinobacteria, hypersaline = $23\% \pm 7.5$, freshwater = $0.86\% \pm 0.84$), Nitrospiraceae
360 (Actinobacteria, hypersaline = $13\% \pm 7.6$, freshwater = 0%), and Rhodobacteraceae
(Alphaproteobacteria, hypersaline = $2.0\% \pm 0.68$, freshwater = $0.49\% \pm 0.28$) were at
362 least 26-times higher in hypersaline than freshwater lakes, while Burkholderiaceae and
Comamonadaceae (Betaproteobacteria) were absent in hypersaline lakes but accounted

364 for $4.6\% \pm 2.1$ and $12\% \pm 3.5$ of the community in freshwater environments,
respectively. Alternatively, taxonomical patterns among abundant and dormant taxa were
366 apparent in freshwater systems where the recovery of families: Verrucomicrobiaceae
(Verrucomicrobia, hypersaline = $0.20\% \pm 0.11$, freshwater = $13\% \pm 6.0$),
368 Flavobacteriaceae (Bacteriodetes, hypersaline = $0.41\% \pm 0.33$, freshwater = $6.1\% \pm 4.8$),
and an unclassified Frankineae family (Actinobacteria, hypersaline = $0.50\% \pm 0.40$,
370 freshwater = $8.5\% \pm 3.9$) were at least 14-times higher in freshwater than hypersaline
lakes.

372

DISCUSSION

374 Bacterial dormancy is often assumed to be a survival mechanism allowing taxa to
contend with harsher environmental conditions (Stevenson 1978; Nicholson et al., 2000;
376 Dworkin and Shah, 2010; Lennon and Jones, 2011). However, in this study, we found
bacterial dormancy to be less common in extreme hypersaline habitats than seemingly
378 more benign freshwater habitats. To our surprise, bacteria in hypersaline lakes were
classified as dormant at a similar percentage as freshwater bacteria, and the proportion of
380 the community exhibiting dormancy was 16% lower in hypersaline than freshwater lakes
across a range of cutoffs describing activity. In both lakes, activity was influenced by
382 salinity and to a lesser extent P. Instead of dormancy being more common as stressful
conditions intensified, the percentage of the community residing in an inactive state
384 decreased with increasing salinity. Taken together, dormancy might not be an
advantageous mechanism to weather extreme conditions if bacteria are adapted to harsh
386 conditions that are consistently stressful.

388 Hypersaline bacterial communities

Hypersaline bacterial communities supported relatively low levels of bacterial diversity
390 consisting of taxa from one dominant phylum and two Proteobacteria subclasses.
Hypersaline environments are generated as waters containing high concentrations of salts
392 flow into an endorheic lake and are concentrated as evaporation outputs exceed
precipitation inputs (Ollivier et al., 1994; Boutaiba et al., 2011). Across these lakes, as
394 with all extreme environments from acid seeps and deep-sea thermal vents to glacial ice
and acid mine drainage, adverse abiotic conditions select for bacterial communities
396 composed of extremotolerant bacteria and extremophiles (Baker and Banfield, 2003;
Miroshnichenko and Bonch-Osmolovskaya, 2006; Seufferheld et al., 2008; Anesio and
398 Laybourn-Parry, 2012). In hypersaline lakes, the primary adverse condition, hypersalinity
and the osmotic stress that it induces, selects a subset of halophilic taxa from regional
400 species pool (Wu et al., 2006; Wang et al., 2011; Logares et al., 2013; Tazi et al., 2014).
We found evidence supporting this as hypersaline conditions selected for unique
402 assemblages of bacteria that were compositionally distinct from freshwater communities.
Our lakes contained a range of salinity from 3.0 – 15%, and, thus communities contained
404 both “salt loving,” halophilic and halotolerant bacteria, which can exist in water up to
15% salinity (Pikuta et al., 2007). Specialized halophiles contributed to hypersaline
406 communities that were 50% less diverse than their more benign analog with
Cyanobacteria, Alphaproteobacteria, and Gammaproteobacteria contributing upwards of
408 70% of the sequences. All three of these taxonomical groups are consistently dominant in

other saline environments as well (Tourova et al., 2007; Jiang et al., 2010; Lefort and
410 Gasol, 2013).

412 **Hypersaline lakes contain more active microbes**

The prevalence of dormancy did not rise as stressful conditions intensified as expected.
414 Based on our approach using rRNA: rDNA ratios to classify the activity of bacterial taxa,
we found that the proportion of the community exhibiting dormancy was 16% lower in
416 hypersaline than freshwater lakes across a range of cutoffs, and species were classified as
dormant at a similar percentage in hypersaline as freshwater lakes. Harsher or more
418 extreme saline conditions did not select for higher levels of dormancy. The reverse was
actually true. As extreme environments became more hypersaline, a greater proportion of
420 the community was active. An explanation for this result may stem from halophiles being
highly adapted to hypersalinity for optimal metabolism and growth (Madigan and Mairs,
422 1997; Harrison et al., 2013). Extreme environments may not necessarily be stressful or
suboptimal for highly adapted extremophiles causing them to need dormancy less often to
424 survive. For example, hypersaline environments generally select for a wide range of
metabolic diversity, such as oxygenic and anoxygenic phototrophs, obligate and
426 facultative aerobic heterotrophs, fermenters, denitrifiers, sulfate reducers, and
methanogens (Ollivier et al., 1994; Burke and Knott 1997; Ciulla et al., 1997). However,
428 as salinity continues to rise, metabolic diversity dramatically decreases (Oren 2002;
Pikuta et al., 2007). Thus, as hypersalinity intensifies, the resulting bacterial communities
430 may become more specialized, perform fewer functions but remain predominately active.

432 Salinity and P drive bacterial dormancy

Surprisingly, saltier conditions in both freshwater and hypersaline lakes increased
434 activity. A steep rise in salinity spanning less than a single percent (0.01 – 0.87%) across
freshwater lakes corresponded to a 17% decrease in the recovery of dormant taxa
436 exhibiting dormancy. Additionally, a five-fold increase in salinity (3.0 – 15%) among
hypersaline lakes corresponded to a 30% decrease in the recovery of dormant taxa. Thus,
438 salinity seemed to act as a strong environmental filter selecting for not only active
extremophiles but also active freshwater bacteria able to contend with and thrive under
440 saltier conditions. The immense effects of salinity on activity may be best explained or
mirrored by the importance of salt concentrations to community composition. For
442 example, across multiple biomes and in freshwater lakes, microbial community structure
and diversity are primarily structured by salinity rather than temperature, pH, or other
444 physical and chemical factors (Lozupone and Knight, 2007). As for hypersaline lakes,
salinity levels exert immense selective pressure on bacterial species (Canganella and
446 Wiegel, 2011). To remain active under saltier conditions, we expect most bacteria to cope
with increasing or toxic levels of Na^+ and potential desiccation stress by using “salting
448 out” osmoregulation resistance strategies (Oren 2002; Oren 2008; Canganella and
Wiegel, 2011). This coping mechanism requires energy to actively export Na^+ ions and
450 synthesize and/or accumulate organic compatible solutes such as polyols, sugars, amino
acids and amines (Detkova and Bolyanskaya, 2007; Canganella and Wiegel, 2011). In
452 light of these costs, it may be more beneficial/efficient for salt tolerant or salt loving taxa
to just remain active and cope with osmotic stresses instead of entering a state of
454 dormancy.

P availability appeared to regulate bacterial activity even in extreme environments.

456 Regardless of lake type, we found a trend ($P < 0.09$ marginally significant) where higher
TP concentrations related to a lower percentage of the community exhibiting dormancy.
458 Our results support previous evidence that low concentrations of TP influence dormancy
in freshwater systems (Jones and Lennon, 2010). In more benign systems, like freshwater
460 lakes, selection pressures may drive bacteria to develop different patterns of resource use
or functional traits allowing them to occupy different niches. Under low resource
462 availability, dormancy confers a competitive advantage to bacteria as they avoid
starvation and escape death. As nutrient levels decline, bacteria enter dormancy by
464 forming cysts or endospores (Sussman and Douthit, 1973; Segev et al., 2012), creating
persister cells (Rotem et al., 2010; Maisonneuve et al., 2011), or, simply suspending
466 normal metabolic activity (Lennon et al., 2011). Although the role competition is
controversial in extreme environments and the impact of resources availability on activity
468 is remains unclear, there is a tendency for competition to decline as conditions become
more stressful (Fiser et al., 2012) due to extreme conditions limiting the number of niches
470 for bacteria to occupy (Oren 2002). In addition to stresses, we propose that competition
for essential resources in extreme environments exerts some pressure on competition and,
472 ultimately, activity.

474 **Abundant taxa are disproportionately active**

Communities are unevenly distributed with a few dominant species being numerically
476 abundant and contributing overwhelmingly to the overall community, while rare taxa are
thousands in number and contribute little in terms of abundance (Pedros-Alio 2012).

478 However, overlaid on top of this seemingly universal shape to bacterial rank abundance
curves lies the uncertainty of activity (Shade et al., 2014; Aanderud et al., 2015). We
480 found the same ubiquitous rank abundance curve in both hypersaline and freshwater
lakes, but hypersaline abundant taxa were disproportionately active. These dominant and
482 active taxa were localized in families adapted to a range of salinities. For example,
Microbacteriaceae are predominantly aerobic, planktonic, and halotolerant bacteria with
484 the potential to persist and thrive at multiple salinity levels (Han et al., 2003; Jiang et al.,
2010; Jang et al., 2013), and Rhodobacteraceae of the Alphaproteobacteria are
486 halotolerant, moderately thermophilic chemoorganotrophs and photoheterotrophs
common in water, biofilms, and microbial mats that withstand fluctuations in salinity
488 (Denner et al., 2006; Farias et al., 2013; Lindermann et al., 2013). Abundant and active
Nitriliruptoraceae are haloalkaliphic bacteria that require high salt levels to decompose
490 organic C containing nitrile groups (Sorokin et al., 2009). The activity of abundant
freshwater taxa may relate to differences in C source availability. For example, the
492 Comamonadaceae, which were abundant and active in freshwater lakes, are associated
with the decomposition of cyanobacteria biomass, particularly *Microcystis* species, and
494 wastewater streams (Li et al., 2012; Krustok et al., 2015). Alternatively,
Verrucomicrobiaceae and Flavobacteriaceae, which were abundant but dormant in
496 freshwater lakes, are associated with high levels of laminarin and xylan from algal
sources. Collectively, Verrucomicrobiaceae and Flavobacteriaceae produce six endo-
498 acting polysaccharide hydrolases facilitating the decomposition of polysaccharides and
cell wall constituents (Cardman et al., 2014). Unfortunately, we did not measure C

500 sources in our water samples. Our results suggest that dormancy is unnecessary for taxa
to achieve dominance in extreme conditions.

502

Reasonable and robust dormancy estimates

504 Our estimates of dormancy represent an approximation of bacterial activity with the
cutoffs separating active from dormant taxa conserved over a wide range of values. We
506 classified taxa as either active or dormant bacteria based on rRNA: rDNA ratios using the
recovery of individual taxa in active (rRNA) and total (rDNA) bacterial communities.
508 Inferring activity based on rRNA is a commonly applied approach to characterize
growing or active bacteria (Campbell and Kirchman, 2013; Hugoni et al., 2013);
510 however, rRNA alone may not be a reliable indicator of the metabolic state of a
bacterium (Blazewicz et al., 2013). In the case of dormancy, inactive bacteria may
512 contain measureable amounts of rRNA, and in specific cases, specialized cells (e.g.,
akinetes of some Cyanobacteria), may contain more rRNA in an inactive than active state
514 (Sukenic et al., 2012). By estimating bacterial dormancy for each taxa independently
using rRNA: rDNA ratios, we compensated for potential taxonomic discrepancies
516 associated with rRNA and activity (Blazewicz et al., 2013). Our results are based on a
rRNA: rDNA ratio cutoff of ≥ 0.5 . But even if the cutoff was more strict, where the total
518 recovery of an OTU (active and inactive cells) was at least 10-times the recovery of RNA
transcripts being produced (cutoff = 0.9), our major findings were the same. We do
520 concede that our approach does not perfectly discriminate between dormant and
extremely slow-growing bacteria populations (Jones and Lennon, 2010). But slow-
522 growers and dormant individuals may respond, grow, and resuscitate similarly following

changes in environmental cues (Kjelleberg et al., 1987; Choi et al., 1999). Thus, we feel
524 our estimate is an appropriate metric quantifying the baseline effects of dominant
ecosystem characteristics on activity.

526

CONCLUSION

528 Halophilic and halotolerant bacteria may employ dormancy to facilitate their long-term
persistence and maintain bacterial diversity in extreme environments, but a lower
530 proportion of extreme communities utilize dormancy. The overarching adverse condition,
hypersalinity, not only structured the less diverse and distinct bacterial communities, but
532 also activity levels. However, instead of dormancy being more common as extreme
conditions intensified, the percentage of the community residing in an inactive state
534 decreased with increasing hypersalinity. Environmentally adapted halophiles seem to be
able to capitalize on their “extreme,” yet highly selective set of conditions, allowing them
536 to thrive and employ dormancy less often to survive.

538 AUTHOR CONTRIBUTIONS

ZTA, JCV, DPB, and ARH designed the study. ZTA, JCV, and TWM conducted the
540 experiments. ZTA, JCV, JTL, TWM, DPB, and ARH analyzed and interpreted the data,
ZTA, JCV, JTL, TWM, DPB, and ARH helped write and review the manuscript. ZTA
542 agrees to be accountable for all aspects of the work in ensuring that questions related to
the accuracy or integrity of any part of the work are appropriately investigated and
544 resolved.

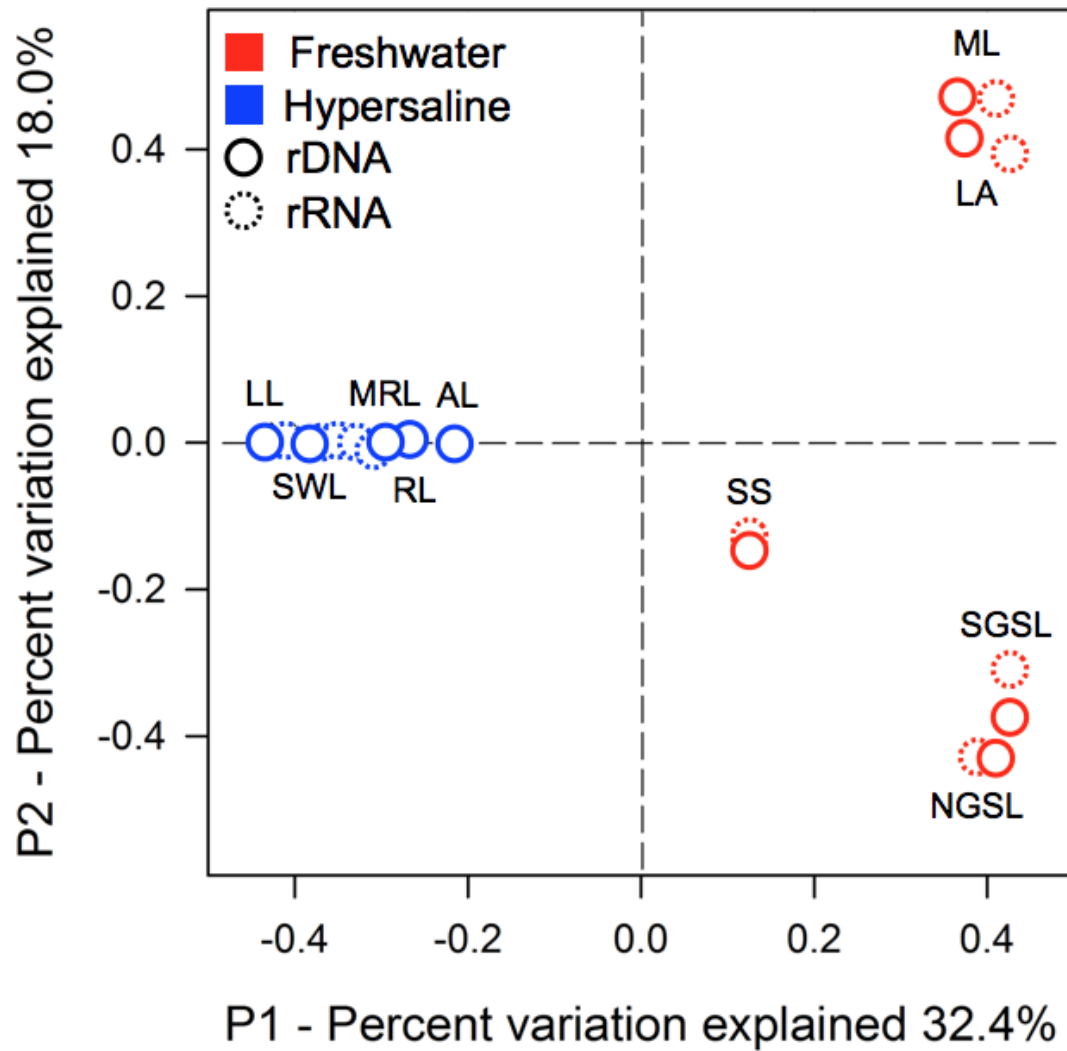
546 **ACKNOWLEDGMENTS**

We would like to thank Dr. Bonnie Baxter for her assistance in registering our Great Salt

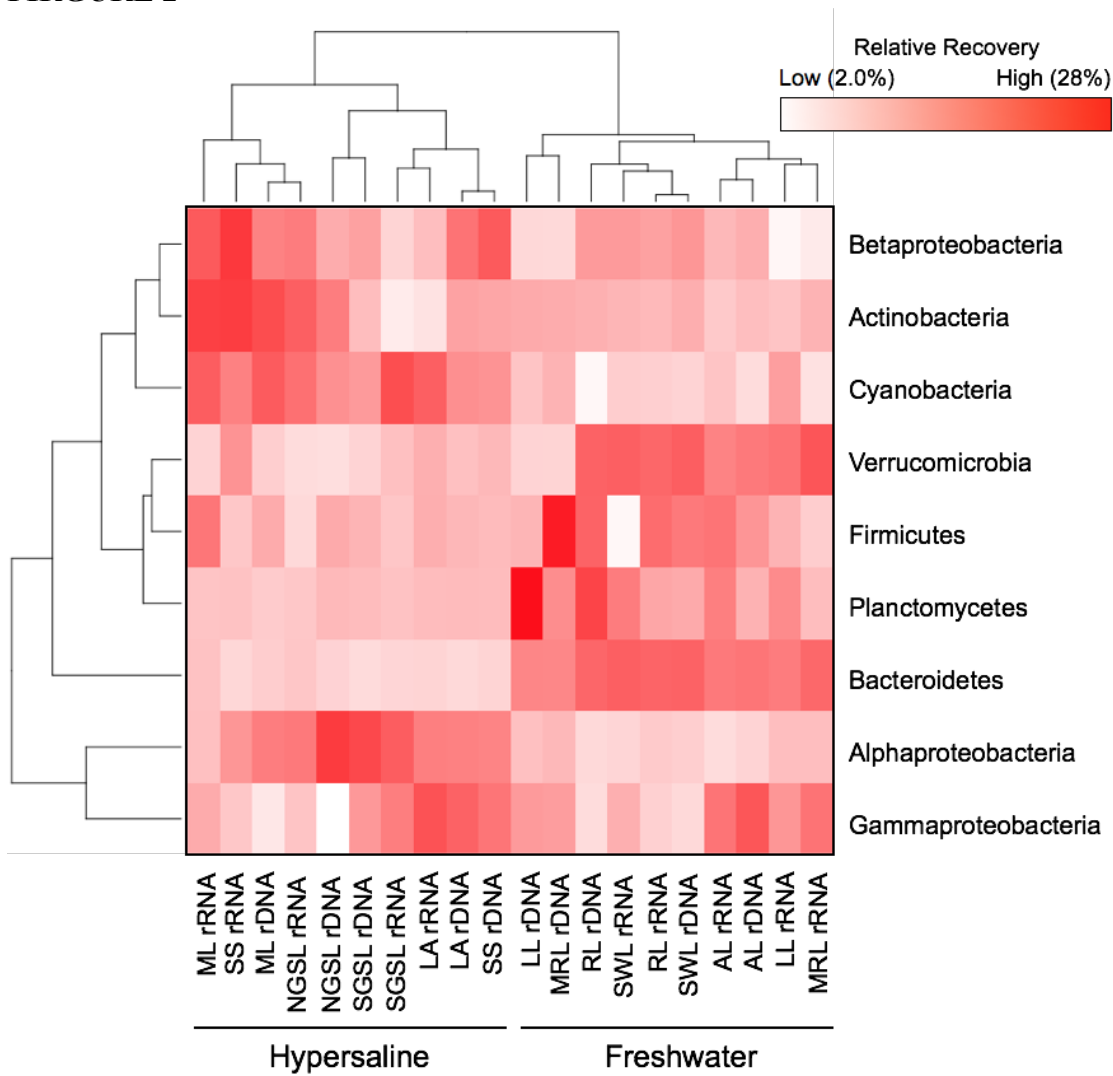
548 Lake samples with the Great Salt Lake Institute.

550 **CONFLICT OF INTEREST:** The authors declare no conflict of interest.

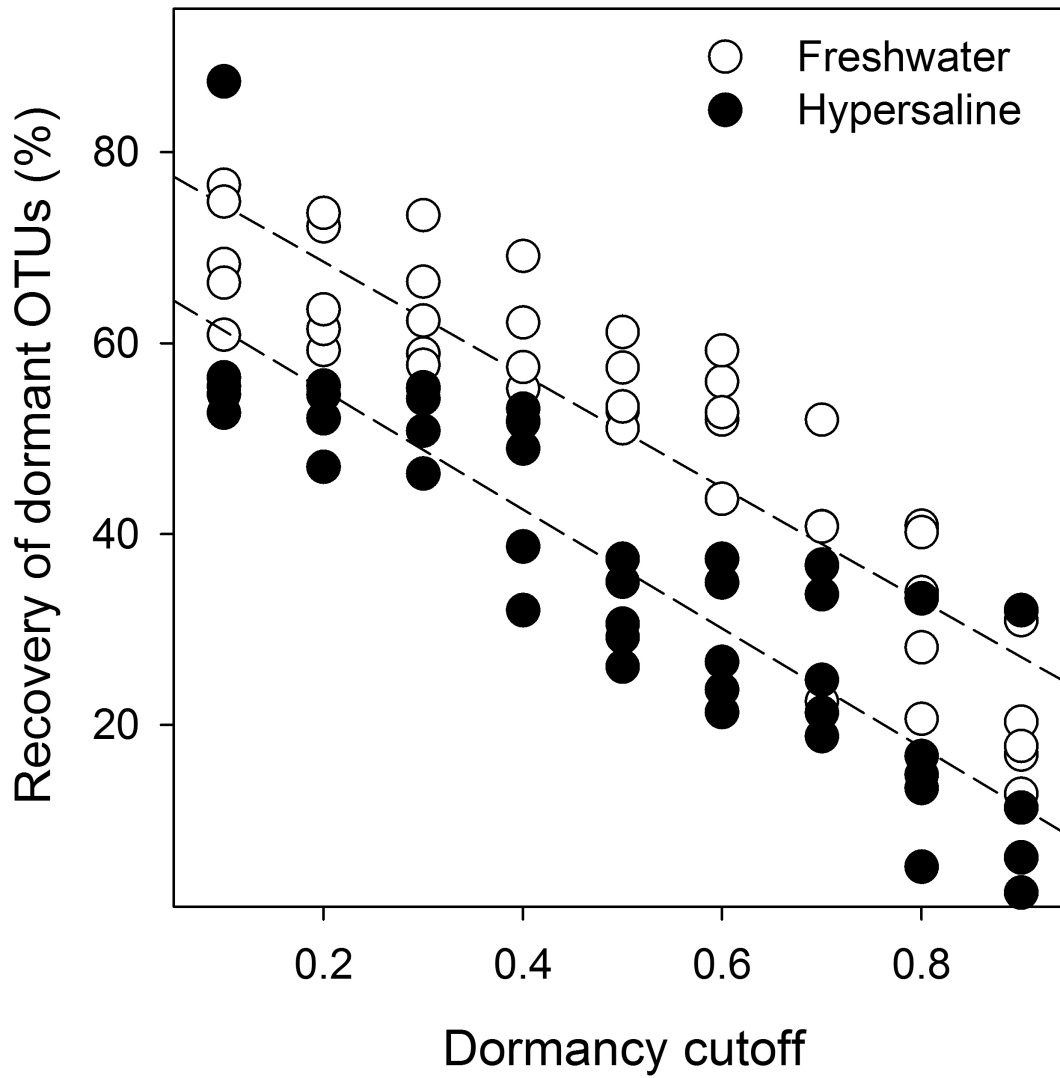
552 FIGURE 1



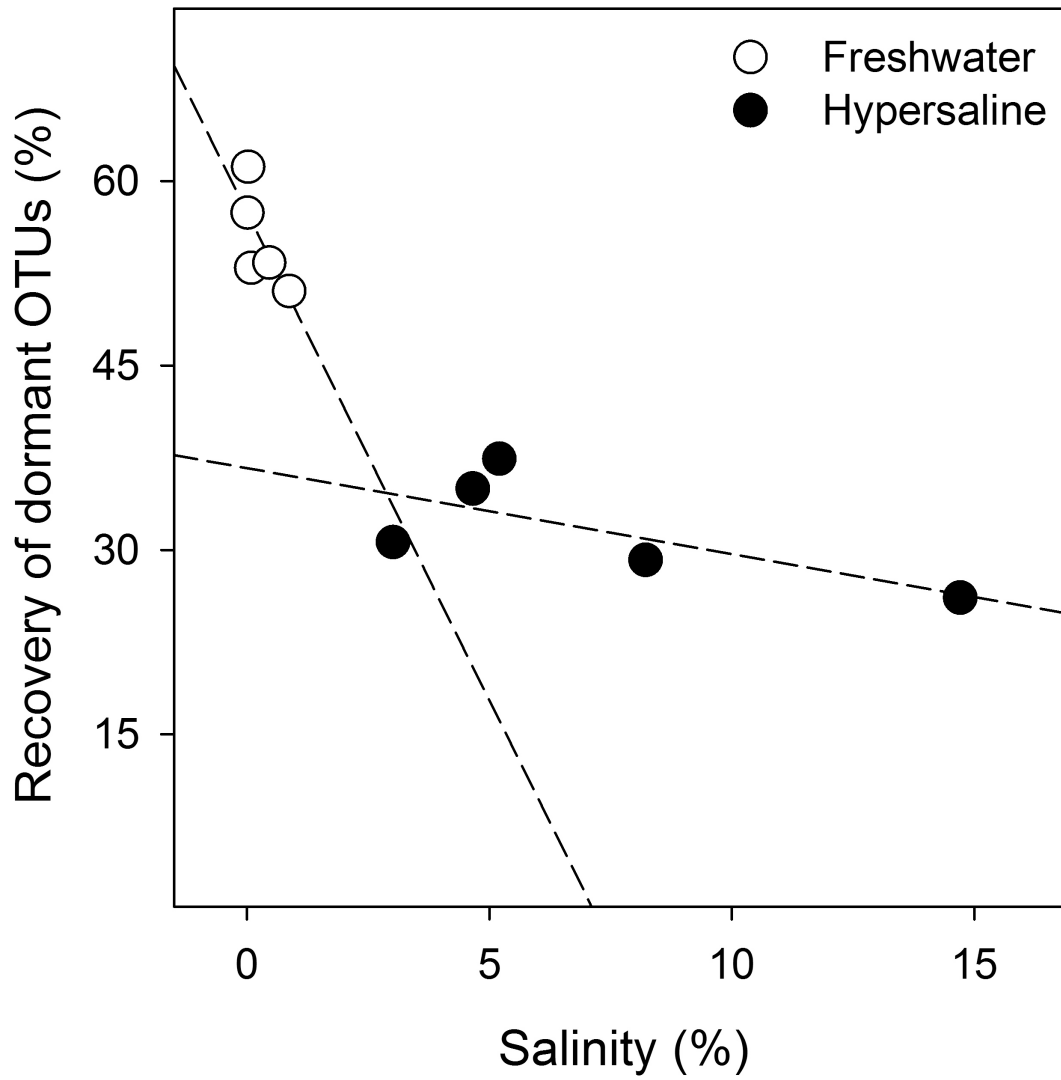
554

556 **FIGURE 2**

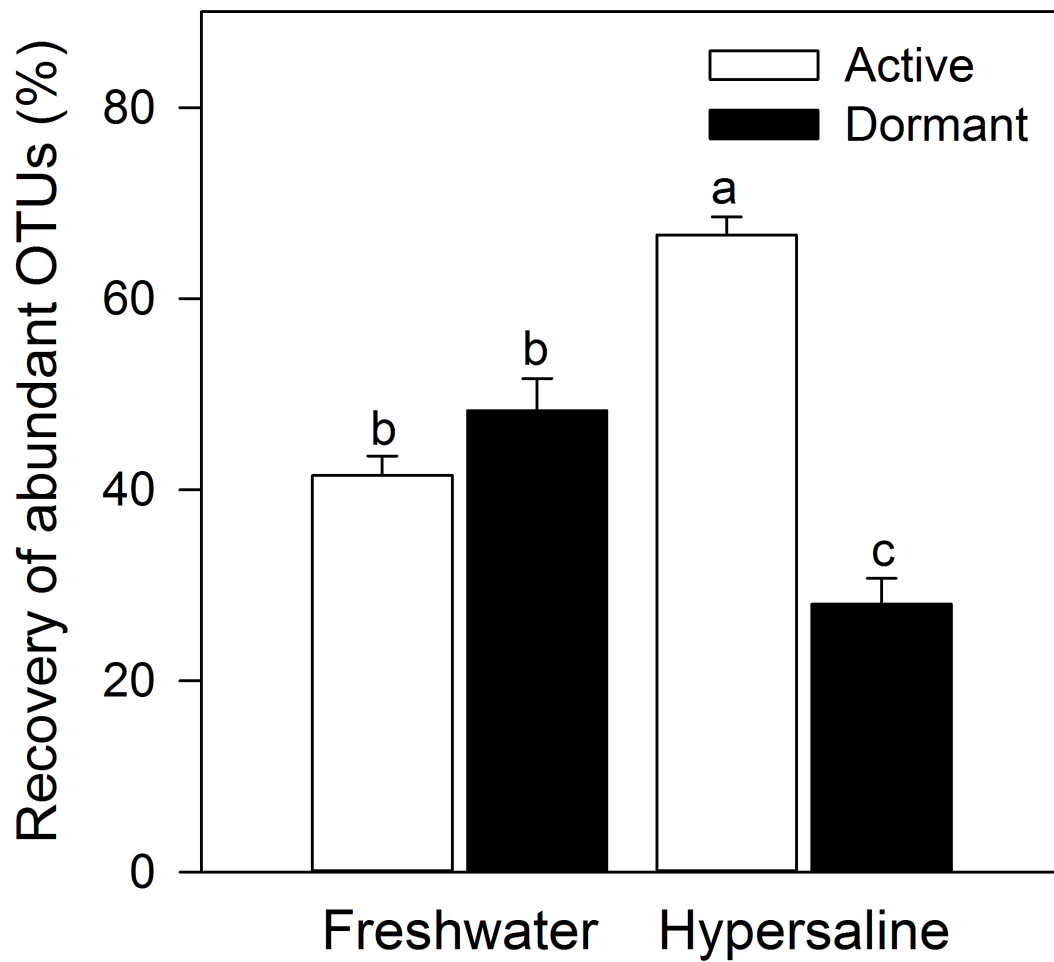
558 FIGURE 3

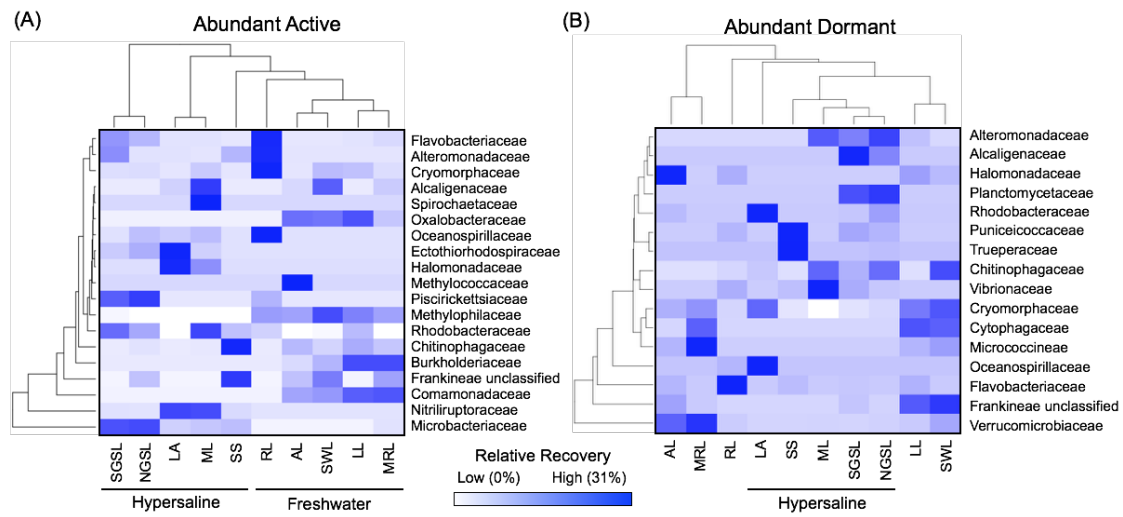


560 FIGURE 4



562 FIGURE 5



564 **FIGURE 6**

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842

844 **TABLE 1 | Chemistry in freshwater and hypersaline lakes.** Data are mean \pm SEM (n
 = 5) between freshwater and hypersaline lakes with significant differences based on t-
 846 tests and a Benjamini-Hochberg adjustment for multiple comparisons ($P < 0.05$), which
 resulted in no false discoveries among significant variables.

	Freshwater	Hypersaline	<i>P</i> value
Dissolved O ₂ ($\mu\text{mol L}^{-1}$)	233 \pm 15.0	174 \pm 9.01	0.01
Electrical conductivity (dS m ⁻¹)	4.5 \pm 2.5	85 \pm 19	0.01
pH	7.0 \pm 0.18	8.7 \pm 0.47	0.02
Salinity (%)	0.29 \pm 0.17	7.2 \pm 2.1	0.03
Temperature ($^{\circ}\text{C}$)	18.0 \pm 1.40	20.5 \pm 2.12	0.36
Total N ($\mu\text{mol L}^{-1}$)	30.6 \pm 8.99	125 \pm 57.9	0.18
Total P ($\mu\text{mol L}^{-1}$)	6.57 \pm 5.40	70.4 \pm 33.3	0.14

848

FIGURE CAPTIONS

850 **FIGURE 1 | Extreme hypersaline lakes influenced the composition of active and**
total bacterial communities. The multivariate ordination was generated using principle
852 coordinate analysis (PCoA) on a sample \times OTU matrix of rDNA and rRNA (indicated by
dashed lines) community libraries (97% similarity cutoff). Lake abbreviations are as
854 follows: hypersaline lakes—Great Salt Lake, North Arm (NGSL); Great Salt Lake, South
Arm (SGSL); Salton Sea (SS); Abert Lake (LA); Mono Lake (ML); and freshwater
856 lakes—Mormon Lake (MRL); Riffe Lake (RL); Arivaca Lake (AL); Lily Lake (LL); and
Silverwood Lake (SWL).

858

FIGURE 2 | Heat map showing the distribution of six phyla and three
860 **Proteobacteria subclasses that contributed $\geq 1\%$ of the relative recovery to rDNA**
and rRNA lake communities. Values are based on means ($n = 5$) with hierarchal
862 clustering of ecosystem (bottom) and phylum (left).

864 **FIGURE 3 | Bacterial dormancy decreased linearly as the cutoffs estimating**
dormancy increased or became more stringent and was more prevalent in
866 **freshwater lakes.** Indicator linear regression analysis ($R^2 = 0.82$, $F_{86,8} = 133$, $P < 0.001$,
 $n = 10$) was based on the relative recovery of dormant OTUs across a range of cutoffs
868 (0.1 – 0.9) calculated as $1 - (\text{rRNA recovery} / \text{rDNA recovery})$ for each OTU from
rDNA and rRNA community libraries. Dormancy was 16% lower in hypersaline than
870 freshwater lakes measured as the percent decrease between the significantly different y-
intercepts ($P < 0.001$) from the equations for each lake.

872

FIGURE 4 | Bacterial dormancy decreases as lake salinity increases. The indicator regression analysis ($R^2 = 0.96$, $F_{8,1} = 50.0$, $P < 0.001$, $n = 10$) was based on the relative recovery of dormant OTUs at the cutoff of 0.5 from the equation $1 - (\text{rRNA recovery} / \text{rDNA recovery})$. Dormancy was calculated for each OTU from rDNA and rRNA community libraries.

878

FIGURE 5 | Abundant bacteria were more likely to be dormant than active in hypersaline lakes. OTUs with a relative recovery $\leq 0.1\%$ were considered rare, while OTUs with a relative recovery > 0.1 were considered abundant based on rDNA community libraries (97% similarity cutoff). Values are means \pm SEM ($n = 5$) with different letters indicating significant differences ($P < 0.05$) based on a two-way ANOVA and a Tukey's HSD test.

FIGURE 6 | Heat map showing the distribution of abundant active (A) and dormant (B) lake taxa in 16-19 bacterial families. Values are based on means ($n = 5$) with hierarchical clustering of lakes (bottom) and families (left) that contributed $\geq 1\%$ of the relative recovery to any rDNA lake community.

890