

1 **Communities that thrive in extreme conditions captured from a freshwater lake**

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12

13 **Abstract**

14 **Organisms that can grow in extreme conditions would be expected to be confined to**  
15 **extreme environments. However, we were able to capture highly productive**  
16 **communities of algae and bacteria, capable of growing in acidic (pH 2), basic (pH**  
17 **12) and saline (40 ppt) conditions, from an ordinary freshwater lake. Microbial**  
18 **communities may thus include taxa that are highly productive in conditions that are**  
19 **far outside the range of conditions experienced in their host ecosystem. The**  
20 **organisms we captured were not obligate extremophiles, but were capable of**  
21 **growing in both extreme and benign conditions. The ability to grow in extreme**  
22 **conditions may thus be a common functional attribute in microbial communities.**

23

24

25 **Introduction**

26 Microbial life is ubiquitous on the Earth's surface, yet we are only just beginning to  
27 understand even basic macroecological patterns for this large portion of the biosphere [1].

28 An ecosystem would not be expected to contain microorganisms capable of growing in  
29 conditions that are far outside the range of those available in that ecosystem. There are  
30 nevertheless three processes that might account, individually or in combination, for the  
31 occurrence of extremophiles in benign environments: **1)** these organisms disperse to the  
32 host ecosystem from extreme environments; **2)** the range of conditions available in their  
33 host ecosystem is actually larger than the range measured; **3)** the latent capacity to grow  
34 in these extreme conditions does not come at a large cost in terms of growth under  
35 average conditions in the host ecosystem.

36

37 The expectation that microorganisms capable of growing in extreme conditions will be  
38 found in all ecosystems due to dispersal from extreme environments is based on a classic  
39 hypothesis that everything will be found everywhere (the so-called "*Baas Becking*  
40 *hypothesis*" [2]), which has recently received support from studies investigating oceanic  
41 seed banks [3] and the widespread dispersal of thermophiles across cold seabeds [4].

42 However, dispersal between extreme environments appears to be very limited, and  
43 extremophile communities show clear patterns of geographic endemism [5,6].

44

45 Extremophiles may be found in most ecosystems if these contain extreme microhabitats,  
46 permanent or ephemeral, that are difficult to detect. For example, soils constitute a highly  
47 heterogeneous matrix, in contrast to aquatic ecosystems such as the one investigated in

48 this study, which may explain the large abundance of alkaliphiles [7] and halophiles [8]  
49 in neutral and non-saline soils.

50

51 Facultative extremophiles may thrive in benign environments if the functional attributes  
52 required to grow in extreme conditions come at little or no cost for growth in benign  
53 conditions. These functional attributes may be either constitutively expressed or  
54 available in the genome and expressed as a plastic response. Most described  
55 extremophiles, however, are obligate forms that cannot grow or grow poorly in non-  
56 extreme conditions (examples [9,10]).

57

58 Finding extremophiles and identifying the mechanism for their presence in benign  
59 environments provides insight into our nascent understanding of microbial functional  
60 biogeography [11] and, as extremophiles are used in a number of industrial processes  
61 [12], may highlight the potential of benign environments for bio-prospecting. If the only  
62 extremophiles present are obligate extremophiles because of a trade-off between growth  
63 in extreme and benign environments, their presence would be due to dispersal or local  
64 heterogeneity and they would be expected to be very rare. This potential rarity requires an  
65 innovative approach for their enrichment. To find out whether an ordinary freshwater  
66 lake contained extremophiles, we used a novel method to enrich organisms in acidic,  
67 basic and saline conditions from this system. To measure the cost of the ability of  
68 enriched communities to grow in extreme conditions, we measured the ability of these  
69 organisms to grow in both extreme and benign conditions.

70

71 **Methods**

72

73 Enrichment using an amplifying bioreactor

74 The amplifying bioreactor (ABR, Supplemental Material [SM] 1) is a continuous-flow  
75 vessel akin to the chemostat [13], except that, in contrast to a chemostat, the ABR  
76 receives a constant input of organisms from the environment. An ABR will amplify even  
77 extremely rare taxa that can reproduce in the vessel faster than they are washed out.  
78 ABRs will potentially amplify any organism present in the source environment based on  
79 its rate and duration of operation (SM2).

80

81 We set up 4 ABRs fed from a small mesotrophic lake draining an enclosed watershed of  
82 protected old-growth forest (Lake Hertel, 0.34 km<sup>2</sup> surface area; 7 m maximum depth,  
83 average chlorophyll-a concentration of 2 µg/L [14], average pH is 8.4 ± SD 0.5, no  
84 detectable salinity 0.00 ppt, conductivity 80.9 ± SD 9.1 µS/cm [15]). Our ABRs were  
85 setup to enrich for micro-algae through the provision of light (continuous ~1000 Lux of  
86 white light), mineral nutrients [\(SM 3\)](#) and air. Micro-algae were targeted because their  
87 productivity could sustain a complex community of heterotrophs, and because  
88 extremophilic micro-algae have far ranging biotechnological applications [12]. Target  
89 treatment conditions (SM1) were control, acidic (addition of HCl to pH 2), basic  
90 (addition of NaOH to pH 12) and saline (addition of NaCl 40 ppt). Each 100 L ABR  
91 received lake water at an exchange rate of 1/3 of the volume per day. Temperature in the  
92 lake during the experiment was 18.7 ±SD 5.1 °C and in the amplifying bioreactors 18.9  
93 ±SD 4.6 °C.

94

95 Isolation of communities

96 After 10 weeks, 50 mL samples were taken from the ABRs. A sample for benign  
97 conditions was taken directly from the lake water inlet. These sampled communities were  
98 maintained in batch cultures using aseptic techniques, shaken at 350 rpm under 1000 Lux  
99 of continuous light at 20°C, by transferring 0.5 mL of inoculum into 50 mL of BBM  
100 medium adjusted to the target treatment conditions every ten days. To ensure that the  
101 organisms identified were growing in the treatment conditions rather than being  
102 continuously brought in from the environment, community sequencing was performed  
103 immediately following transfers to flasks (“before”) and repeated after 1.5 years of  
104 culture in the lab (“after”) with the reciprocal transplants.

105

106 Reciprocal transplant assay

107 Flasks of each treatment condition were inoculated with each of the enriched  
108 communities. Cultures were transferred once into replicate flasks in all treatment  
109 conditions before measurements started. Optical density at 660 nm was recorded daily.  
110 An exponential model was fitted to the first 8 days of optical density measurements for  
111 the calculation of growth rate (optical density was not linked to abundance after 8 days).

112

113 Community characterisation

114 Community composition was established by amplicon sequencing using primers for 16S,  
115 18S and 23S regions on 454 GS FLX Titanium platform (as per [16] and presented in  
116 SM4).

117

118 Raw sequence data has been deposited in the ENA

119 (<http://www.ebi.ac.uk/ena/data/view/PRJEB10729>). Data and analysis scripts are

120 available at dryad.org (10.5061/dryad.42r9h [17]).

121

## 122 **Results**

### 123 Capture

124 All treatment conditions led to the capture of communities that were highly productive in

125 their selection environment (Fig. 1 and SM5); average intrinsic growth rates of 0.42 [SD

126 0.03] day<sup>-1</sup> is higher than that of algae being considered for industrial biomass production

127 [18] and is comparable to the dilution rate of the ABRs.

128

### 129 Reciprocal transplant

130 With one exception, only communities that were selected for growth in an extreme

131 environment (resident communities) grew in that environment (Fig. 1, mean difference in

132 growth rate between residents and transplants = 0.49 day<sup>-1</sup>, Tukey-HSD- P<0.05). The

133 one exception was that the community selected in the basic environment and the

134 community selected in the saline environment grew equally well in the basic environment

135 (mean growth rate 0.60 [SD 0.05] day<sup>-1</sup>, Tukey-HSD- P= 0.999). All selected

136 communities grew as well in benign control environment as in their environment of

137 selection (mean growth rate in benign environment 0.71 [SD 0.22] day<sup>-1</sup>, Tukey-HSD-

138 P>0.05) and all communities grew equally well in the benign environment (F<sub>3,8</sub>=3.568,

139 P=0.125).

140

141 Community characterisation

142 Each extreme environment enriched a different community (Fig 2, SM7). 61.5 % of  
143 OTUs enriched in the extreme environment were not detected in benign culture  
144 conditions or in the lake and are thus rare in the lake. In both saline and basic  
145 communities, the dominant autotrophs were chlorophytes of the genus *Chlorella*  
146 including *Chlorella variabilis* and *Chlorella sorokiniana*, and also contained  
147 Coccomyxaceae. The saline condition also contained a diatom in the family  
148 Thalassiosiraceae and the cyanobacterium *Synechococcus*, both known to contain marine  
149 species. In the acidic conditions, the dominant autotrophs were chlorophytes from the  
150 *Koliella/Pabia* clade, these two genera being closely related phylogenetically [19], the  
151 family Oocystaceae and the genus *Apatococcus*. Both *Chlorella* and *Koliella/Pabia* were  
152 also enriched by the benign conditions and detected in the lake sample.

153

154 **Discussion**

155 The lack of a strong trade-off between growth in the selected extreme conditions and  
156 growth in the benign environment indicates that none of the communities that thrived in  
157 extreme conditions were obligate extremophiles (Fig. 1). This may explain why the  
158 functional breadth of biodiversity held in a benign ecosystem includes the capacity to  
159 grow in extreme conditions. Although our findings do not preclude the presence of  
160 obligate-extremophiles in the lake, if present they have a lower fitness in the ABRs than  
161 the captured facultative extremophiles.

162



163 Some of the taxa captured by the ABRs include species known to have very wide  
164 functional breadth or contain extremophile species consistent with their enrichment  
165 conditions. However, currently documented functional breadth and plasticity of identified  
166 taxa are insufficient to explain growth in the conditions used or to explain the specificity  
167 of the capacity to grow in only a single extreme condition (SM7 for discussion of  
168 heterotrophs). Strains of *Chlorella* are known to grow across a wide pH range, from pH 3  
169 to pH 10.5 at 25°C [20]. However, the communities assembled at high pH that contained  
170 *Chlorella* grew poorly, if at all, in acidic conditions (Fig. 1 and 2). The salt tolerance of  
171 *Chlorella* varies among species and even strains [21]. Some species can grow between 10  
172 to 50 ppt [22], whereas *Chlorella sorokiniana*, which is found in our saline communities,  
173 is inhibited by salt concentrations as low as 11 ppt although it can grow in concentrations  
174 as high as 26 ppt [23]. Many of the enriched organisms may depend on ecological  
175 interactions for survival, including heterotrophic consumption of algal exudates, and  
176 some of the organisms we found, such as the Rickettsiales, may be endosymbionts  
177 protected from extreme conditions by living within a host [24].  
178

179 The treatments used in this experiment are all forms of ionic stress so that the strong  
180 trade-offs detected between the ability to grow in different treatments were not  
181 necessarily expected. Though we did not specifically enrich in combinations of stressors  
182 that would benefit poly-extremophiles, the existence of a strong trade-offs between  
183 treatments suggests that poly-extremophiles are outcompeted when a single stressor is  
184 applied. The physiochemical boundaries of life may be different when extremes are  
185 imposed separately or in combination [25].

186

187 The ability of ABRs to sort extremely large and diverse communities efficiently suggests  
188 that the systematic deployment of ABRs would allow us to probe the functional breadth  
189 of biodiversity held in a range of ecosystems and to describe the biogeography of  
190 extremophiles [11]. Finding organisms that can thrive in extreme conditions in an  
191 ordinary lake suggests that organisms of biotechnological importance may even be found  
192 in a backyard pond.

193

194

195 **Authors' contributions**

196 ELD carried out field/lab work, data analysis, led the design of the study and drafted the  
197 manuscript; AJD carried out the sequencing analysis; GB and GFF contributed to the  
198 design of the study. All authors contributed to the editing of the manuscript and gave  
199 final approval for publication and all authors agree to be held accountable for the content  
200 of the article.

201 **Competing interests**

202 We have no competing interests.

203 **Ethical statement**

204 None of the activities described in this article required ethical approval.

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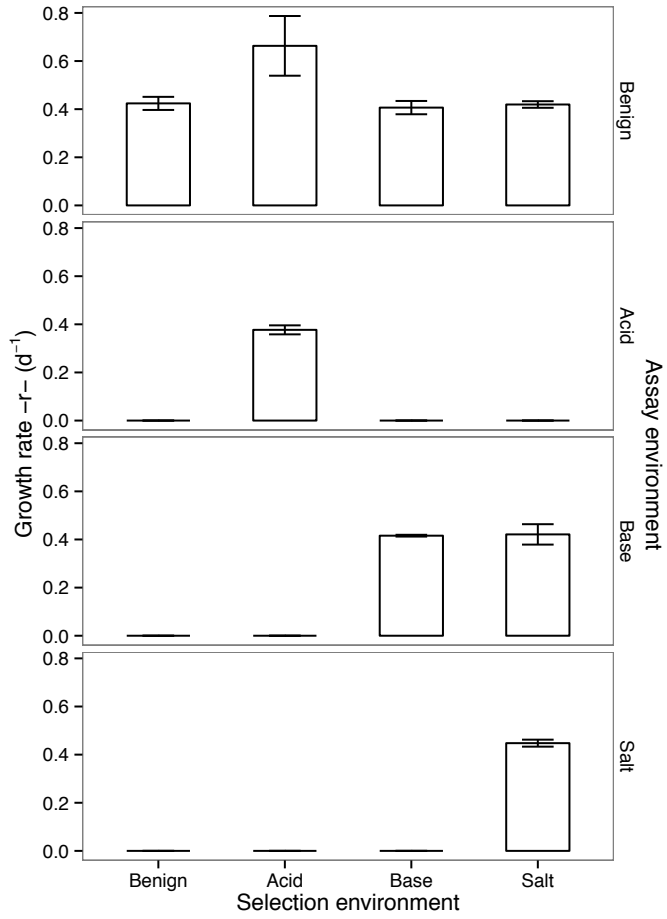
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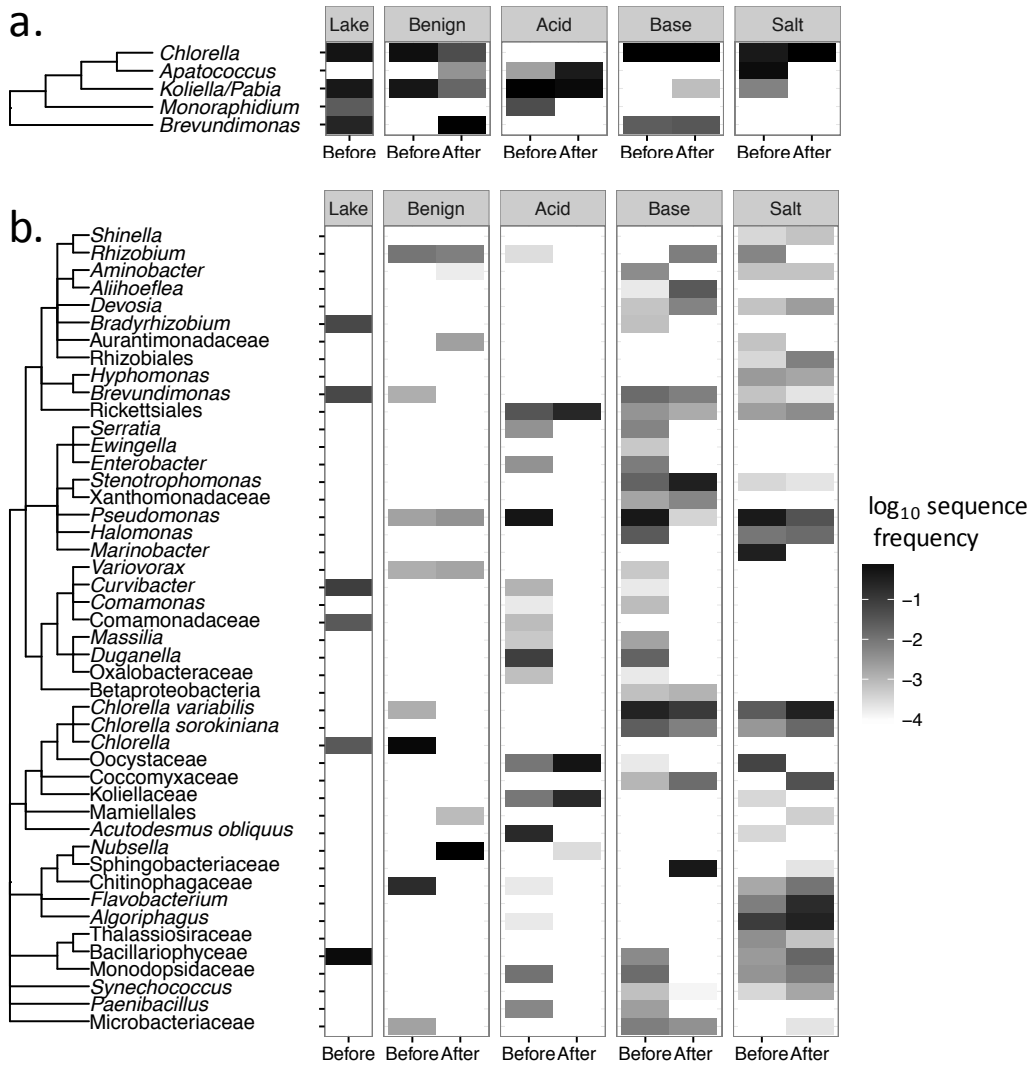
215

216 **Figure 1:** Growth rate of enriched communities in the reciprocal transplant assay ( $-r-$

217  $\text{day}^{-1}$ , error bars: 95 % confidence interval).

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221 Figure 2: Taxonomic composition (SM7) using primers a. 23S and b. 16S. Tree indicates

222 taxonomic relationship; labels are name of highest identifiable taxonomic level.

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224

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