



CrossMark  
click for updates

## Research

**Cite this article:** Low-Décarie E, Fussmann GF, Dumbrell AJ, Bell G. 2016 Communities that thrive in extreme conditions captured from a freshwater lake. *Biol. Lett.* **12**: 20160562.

<http://dx.doi.org/10.1098/rsbl.2016.0562>

Received: 1 July 2016

Accepted: 10 August 2016

### Subject Areas:

ecology, evolution, environmental science

### Keywords:

biogeography, trade-off, limnology, selection, bioreactor, microorganism

### Author for correspondence:

Etienne Low-Décarie

e-mail: [elowde@essex.ac.uk](mailto:elowde@essex.ac.uk)

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.fig-share.c.3457491>.

## Community ecology

# Communities that thrive in extreme conditions captured from a freshwater lake

Etienne Low-Décarie<sup>1</sup>, Gregor F. Fussmann<sup>2</sup>, Alex J. Dumbrell<sup>1</sup> and Graham Bell<sup>2</sup>

<sup>1</sup>School of Biological Sciences, University of Essex, Colchester CO4 3SQ, UK

<sup>2</sup>Department of Biology, McGill University, Montreal, Quebec, Canada H3A 1B1

EL-D, 0000-0002-0413-567X; GB, 0000-0002-7280-6391

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

## 1. Introduction

Microbial life is ubiquitous on the Earth's surface, yet we are only just beginning to understand even basic macroecological patterns for this large portion of the biosphere [1]. An ecosystem would not be expected to contain microorganisms capable of growing in conditions that are far outside the range of those available in that ecosystem. There are nevertheless three processes that might account, individually or in combination, for the occurrence of extremophiles in benign environments: (i) these organisms disperse to the host ecosystem from extreme environments; (ii) the range of conditions available in their host ecosystem is actually larger than the range measured; and (iii) the latent capacity to grow in these extreme conditions does not come at a large cost in terms of growth under average conditions in the host ecosystem.

The expectation that microorganisms capable of growing in extreme conditions will be found in all ecosystems owing to dispersal from extreme environments is based on a classic hypothesis that everything will be found everywhere (the so-called 'Baas Becking hypothesis' [2]), which has recently received support from studies investigating oceanic seed banks [3] and the widespread dispersal of thermophiles across cold seabeds [4]. However, dispersal between extreme environments appears to be very limited, and extremophile communities show clear patterns of geographical endemism [5,6].

Extremophiles may be found in most ecosystems if these contain extreme microhabitats, permanent or ephemeral, that are difficult to detect. For example, soils constitute a highly heterogeneous matrix, in contrast to aquatic ecosystems such as the one investigated in this study, which may explain the large abundance of alkaliphiles [7] and halophiles [8] in neutral and non-saline soils.

Facultative extremophiles may thrive in benign environments if the functional attributes required to grow in extreme conditions come at little or no cost for growth in benign conditions. These functional attributes may be either constitutively expressed or available in the genome and expressed as a

plastic response. Most described extremophiles, however, are obligate forms that cannot grow or grow poorly in non-extreme conditions (e.g. [9,10]).

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

## 2. Material and methods

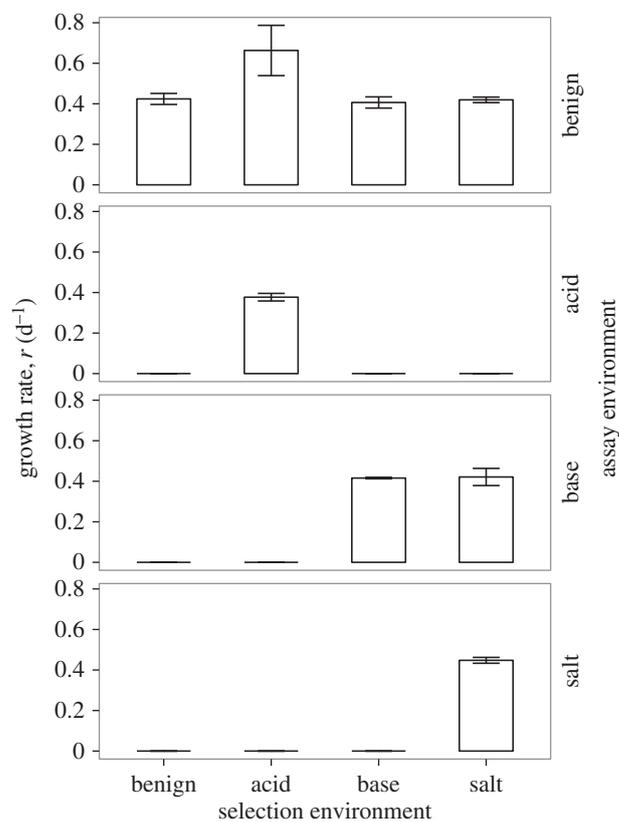
### (a) Enrichment using an amplifying bioreactor

The amplifying bioreactor (ABR; electronic supplementary material, SM1) is a continuous-flow vessel akin to the chemostat [13], except that, in contrast to a chemostat, the ABR receives a constant input of organisms from the environment. An ABR will amplify even extremely rare taxa that can reproduce in the vessel faster than they are washed out. ABRs will potentially amplify any organism present in the source environment based on the rate and duration of operation (electronic supplementary material, SM2).

We set up four ABRs fed from a small mesotrophic lake draining an enclosed watershed of protected old-growth forest (Lake Hertel, 0.34 km<sup>2</sup> surface area, 7 m maximum depth, average chlorophyll-*a* concentration of 2 µg l<sup>-1</sup> [14], average pH is 8.4 ± s.d. 0.5, no detectable salinity 0.00 ppt, conductivity 80.9 ± s.d. 9.1 µS cm<sup>-1</sup> [15]). Our ABRs were set up to enrich for micro-algae through the provision of light (continuous approx. 1000 lx of white light), mineral nutrients (electronic supplementary material, SM3) and air. Micro-algae were targeted because their productivity could sustain a complex community of heterotrophs, and because extremophilic micro-algae have far ranging biotechnological applications [12]. Target treatment conditions (electronic supplementary material, SM1) were control, acidic (addition of HCl to pH 2), basic (addition of NaOH to pH 12) and saline (addition of NaCl 40 ppt). Each 100 l ABR received lake water at an exchange rate of one-third of the volume per day. Temperature in the lake during the experiment was 18.7 ± s.d. 5.1°C and in the amplifying bioreactors 18.9 ± s.d. 4.6°C.

### (b) Isolation of communities

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



**Figure 1.** Growth rate of enriched communities in the reciprocal transplant assay ( $r$  (d<sup>-1</sup>), error bars: 95% CI).

### (c) Reciprocal transplant assay

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

### (d) Community characterization

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

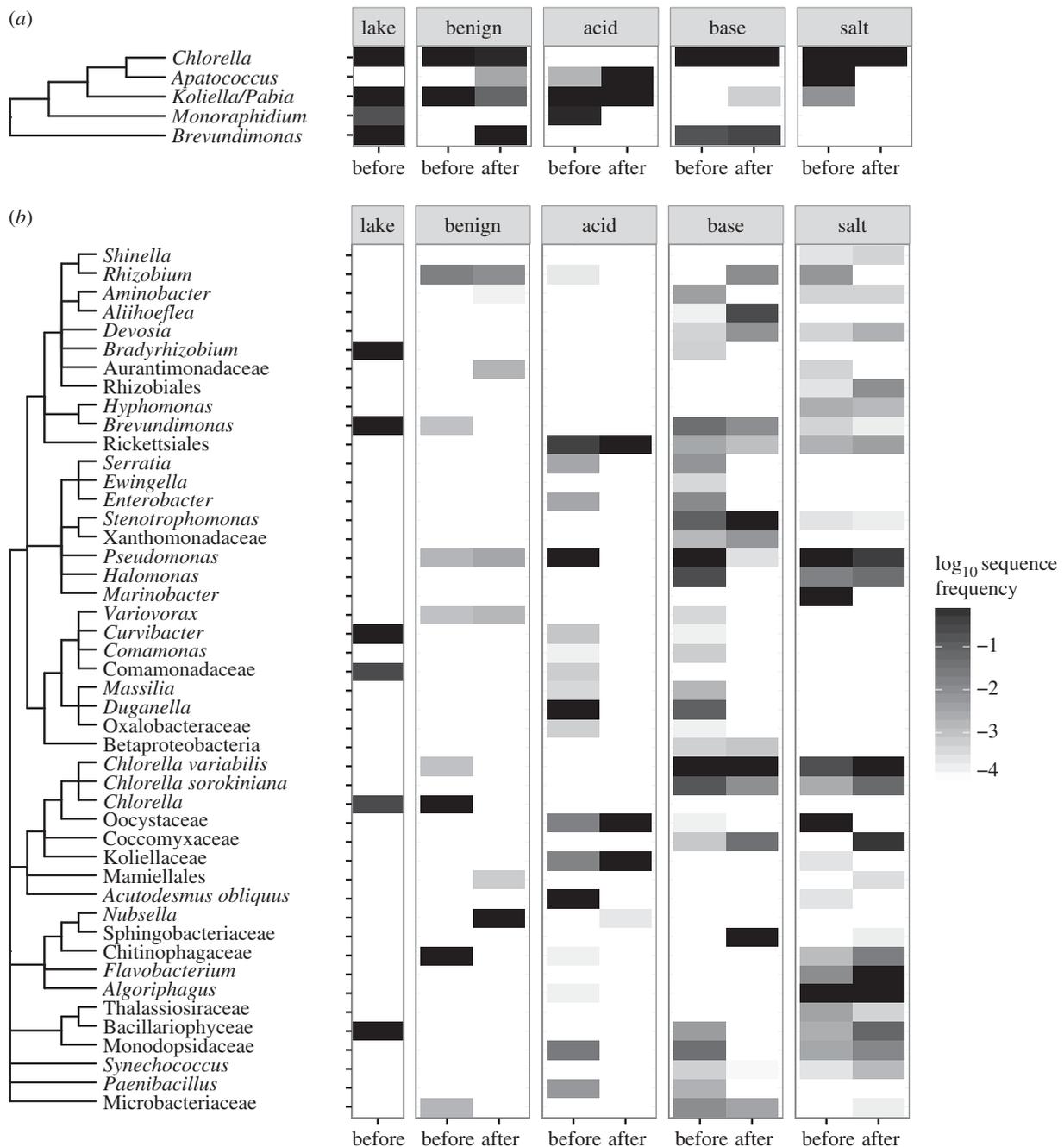
## 3. Results

### (a) Capture

All treatment conditions led to the capture of communities that were highly productive in their selection environment (figure 1 and electronic supplementary material, SM5); average intrinsic growth rate of 0.42 [s.d. 0.03] d<sup>-1</sup> is higher than that of algae being considered for industrial biomass production [17] and is comparable to the dilution rate of the ABRs.

### (b) Reciprocal transplant

With one exception, only communities that were selected for growth in an extreme environment (resident communities) grew in that environment (figure 1, mean difference in growth rate between residents and transplants = 0.49 d<sup>-1</sup>,



**Figure 2.** Taxonomic composition (electronic supplementary material, SM7) using primers (a) 235 and (b) 16S. Tree indicates taxonomic relationship; labels show name of highest identifiable taxonomic level.

Tukey-HSD (honest significant difference)  $p < 0.05$ ). The one exception was that the community selected in the basic environment and the community selected in the saline environment grew equally well in the basic environment (mean growth rate  $0.60$  [s.d.  $0.05$ ]  $\text{d}^{-1}$ , Tukey-HSD  $p = 0.999$ ). All selected communities grew as well in benign control environment as in their environment of selection (mean growth rate in benign environment  $0.71$  [s.d.  $0.22$ ]  $\text{d}^{-1}$ , Tukey-HSD  $p > 0.05$ ) and all communities grew equally well in the benign environment ( $F_{3,8} = 3.568$ ,  $p = 0.125$ ).

### (c) Community characterization

Each extreme environment enriched a different community (figure 2; electronic supplementary material, SM6 and SM7). In total, 61.5% of operational taxonomic units enriched in the extreme environment were not detected in benign culture conditions or in the lake and are thus rare in the lake. In

both saline and basic communities, the dominant autotrophs were chlorophytes of the genus *Chlorella* including *Chlorella variabilis* and *Chlorella sorokiniana*, and both communities also contained Coccomyxaceae. The saline condition also contained a diatom in the family Thalassiosiraceae and the cyanobacterium *Synechococcus*, both known to contain marine species. In the acidic conditions, the dominant autotrophs were chlorophytes from the *Koliella/Pabia* clade, these two genera being closely related phylogenetically [18], the family Oocystaceae and the genus *Apatococcus*. Both *Chlorella* and *Koliella/Pabia* were also enriched by the benign conditions and detected in the lake sample.

## 4. Discussion

The lack of a strong trade-off between growth in the selected extreme conditions and growth in the benign environment

indicates that none of the communities that thrived in extreme conditions was an obligate extremophile (figure 1). This may explain why the functional breadth of biodiversity held in a benign ecosystem includes the capacity to grow in extreme conditions. Although our findings do not preclude the presence of obligate extremophiles in the lake, if present they have a lower fitness in the ABRs than the captured facultative extremophiles.

Some of the taxa captured by the ABRs include species known to have very wide functional breadth or contain extremophile species consistent with their enrichment conditions. However, currently documented functional breadth and plasticity of identified taxa are insufficient to explain growth in the conditions used or to explain the specificity of the capacity to grow in only a single extreme condition (electronic supplementary material, SM7 for discussion of heterotrophs). Strains of *Chlorella* are known to grow across a wide pH range, from pH 3 to pH 10.5 at 25°C [19]. However, the communities assembled at high pH that contained *Chlorella* grew poorly, if at all, in acidic conditions (figures 1 and 2). The salt tolerance of *Chlorella* varies among species and even strains [20]. Some species can grow to between 10 and 50 ppt [21], whereas *C. sorokiniana*, which is found in our saline communities, is inhibited by salt concentrations as low as 11 ppt although it can grow in concentrations as high as 26 ppt [22]. Many of the enriched organisms may depend on ecological interactions for survival, including heterotrophic consumption of algal exudates, and some of the organisms we found, such as the Rickettsiales, may be endosymbionts protected from extreme conditions by living within a host [23].

The treatments used in this experiment are all forms of ionic stress so that the strong trade-offs detected between the

ability to grow in different treatments were not necessarily expected. Though we did not specifically enrich in combinations of stressors that would benefit poly-extremophiles, the existence of strong trade-offs between treatments suggests that poly-extremophiles are outcompeted when a single stressor is applied. The physiochemical boundaries of life may be different when extremes are imposed separately or in combination [24].

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

**Data accessibility.** Raw sequence data have been deposited in the ENA (<http://www.ebi.ac.uk/ena/data/view/PRJEB10729>). Data and analysis scripts are available at dryad.org (<http://dx.doi.org/10.5061/dryad.42r9h>) [25].

**Authors' contributions.** E.L.-D. carried out field/laboratory work and data analysis, led the design of the study and drafted the manuscript; A.J.D. carried out the sequencing analysis; G.B. and G.F.F. contributed to the design of the study. All authors contributed to the editing of the manuscript and gave final approval for publication and all authors agree to be held accountable for the content of the article.

**Competing interests.** We have no competing interests.

**Funding.** McGill University and the Gault Nature Reserve (GNR) funded the hydrology laboratory. This work was supported by the NSERC through grants to G.F.F. and G.B.

**Acknowledgements.** We thank Irene Gregory-Eaves for equipment; staff of the GNR for logistical support; and Paige Homme, Andrea Lofano and Kathy Tallon for laboratory support.

## References

- Martiny JBH *et al.* 2006 Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* **4**, 102–112. (doi:10.1038/nrmicro1341)
- O'Malley MA. 2007 The nineteenth century roots of 'everything is everywhere'. *Nat. Rev. Microbiol.* **5**, 647–651. (doi:10.1038/nrmicro1711)
- Gibbons SM, Caporaso JG, Pirrung M, Field D, Knight R, Gilbert JA. 2013 Evidence for a persistent microbial seed bank throughout the global ocean. *Proc. Natl Acad. Sci. USA* **110**, 4651–4655. (doi:10.1073/pnas.1217767110)
- Hubert C *et al.* 2009 A constant flux of diverse thermophilic bacteria into the cold Arctic seabed. *Science* **325**, 1541–1544. (doi:10.1126/science.1174012)
- Papke RT, Ramsing NB, Bateson MM, Ward DM. 2003 Geographical isolation in hot spring cyanobacteria. *Environ. Microbiol.* **5**, 650–659. (doi:10.1046/j.1462-2920.2003.00460.x)
- Whitaker RJ, Grogan DW, Taylor JW. 2003 Geographic barriers isolate endemic populations of hyperthermophilic archaea. *Science* **301**, 976–978. (doi:10.1126/science.1086909)
- Horikoshi K. 1999 Alkaliphiles: some applications of their products for biotechnology. *Microbiol. Mol. Biol. Rev.* **63**, 735–750. (doi:10.2183/pjab.80.166)
- Usami R, Echigo A, Fukushima T, Mizuki T, Yoshida Y, Kamekura M. 2007 *Alkalibacillus silvisoli* sp. nov., an alkaliphilic moderate halophile isolated from non-saline forest soil in Japan. *Int. J. Syst. Evol. Microbiol.* **57**, 770–774. (doi:10.1099/ijs.0.64713-0)
- Cayol JL, Ollivier B, Patel BK, Prensier G, Guezennec J, Garcia JL. 1994 Isolation and characterization of *Halothermothrix orenii* gen. nov., sp. nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. *Int. J. Syst. Bacteriol.* **44**, 534–540. (doi:10.1099/00207713-45-1-201)
- Khmelenina VN. 1997 Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes. *Curr. Microbiol.* **35**, 257–261. (doi:10.1007/s002849900249)
- Green JL, Bohannan BJM, Whitaker RJ. 2008 Microbial biogeography: from taxonomy to traits. *Science* **320**, 1039–1043. (doi:10.1126/science.1153475)
- Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP. 2015 Extremophilic micro-algae and their potential contribution in biotechnology. *Bioresour. Technol.* **184**, 363–372. (doi:10.1016/j.biortech.2014.11.040)
- Novick A, Szilard L. 1950 Experiments with the chemostat on spontaneous mutations of bacteria. *Proc. Natl Acad. Sci. USA* **36**, 708–719. (doi:10.1073/pnas.36.12.708)
- Rooney N, Kalff J. 2003 Interactions among epilimnetic phosphorus, phytoplankton biomass and bacterioplankton metabolism in lakes of varying submerged macrophyte cover. *Hydrobiologia* **501**, 75–81. (doi:10.1023/A:1026255302443)
- Low-Décarie E, Bell G, Fussmann GF. 2014 CO<sub>2</sub> alters community composition and response to nutrient enrichment of freshwater phytoplankton. *Oecologia* **177**, 875–883. (doi:10.1007/s00442-014-3153-x)
- Low-Décarie E, Kolber M, Homme P, Lofano A, Dumbrell A, Gonzalez A, Bell G. 2015 Community rescue in experimental metacommunities. *Proc. Natl Acad. Sci. USA* **112**, 14 307–14 312. (doi:10.1073/pnas.1513125112)
- Li X, Hu H, Zhang Y. 2011 Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. *Bioresour. Technol.* **102**, 3098–3102. (doi:10.1016/j.biortech.2010.10.055)

18. Lemieux C, Otis C, Turmel M. 2014 Chloroplast phylogenomic analysis resolves deep-level relationships within the green algal class *Trebouxiophyceae*. *BMC Evol. Biol.* **14**, 211. (doi:10.1186/s12862-014-0211-2)
19. Mayo A. 1997 Effects of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water Environ. Res.* **69**, 64–72. (doi:10.2175/106143097X125191)
20. Munns R, Greenway H, Kirst G. 1983 Halotolerant eukaryotes. *Physiol. Plant Ecol. III* **12**, 59–135. (doi:10.1007/978-3-642-68153-0\_4)
21. Meshkini S, Fathi M, Nadiri R. 2013 The effect of extracted salt from Urmia Lake on the growth,  $\beta$ -carotene and chlorophyll *a* content of halophilic alga *Chlorella* sp. *Turkish J. Fish. Aquat. Sci.* **13**, 233–240. (doi:10.4194/1303-2712-v13\_2\_05)
22. Chimiklis PE, Karlander EP. 1973 Light and calcium interactions in *Chlorella* inhibited by sodium chloride. *Plant Physiol.* **51**, 48–56. (doi:10.1104/pp.51.1.48)
23. Baker BJ, Baker BJ, Hugenholtz P, Hugenholtz P, Dawson SC, Dawson SC, Ban JF, Ban JF. 2010 Extremely acidophilic protists from acid mine drainage host. *Appl. Environ. Microbiol.* **69**, 5512–5518. (doi:10.1128/AEM.69.9.5512)
24. Bowers KJ, Mesbah NM, Wiegel J. 2009 Biodiversity of poly-extremophilic *Bacteria*: does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life? *Saline Syst.* **5**, 9. (doi:10.1186/1746-1448-5-9)
25. Low-Decarie E, Fussmann GF, Dumbrell AJ, Bell G. 2016 Data from: Communities that thrive in extreme conditions captured from a freshwater lake. Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.42r9h>.