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# Interaction of temperature, salinity and extracellular polymeric substances controls trace element incorporation into tufa calcite.

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## Abstract

The influence of extracellular polymeric substances on carbonate mineral growth in natural settings remains one of the most poorly understood contributors to the growth of non-marine carbonate sediments. The influences of these materials are complicated by their association with living cells creating local microenvironments via metabolism and enzyme production, and by our uncertainty about the extracellular polymeric substances materials themselves. Different mixtures of extracellular polymeric substance molecules may behave in different ways, and differences in the local physical environment may alter how the mixtures influence mineral formation, and even result in different patterns of polymerisation. Here, the influence of extracellular polymeric substances on calcite precipitation rate and  $Mg/Ca_{\text{calcite}}$  in the absence of cells is investigated using

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extracts of extracellular polymeric substances from temperate fluvial tufa biofilm. The influence is complex, with the concentration of extracellular polymeric substances in solution altering deposition rate and trace element incorporation. Moreover, the results show interaction of EPS presence/ absence and both temperature and salinity. However, despite extracting extracellular polymeric substances from the same parent sample, a uniform influence was not found in these experiments, implying that the mixture is sufficiently variable within a sample for microenvironments within the biofilm to either promote or inhibit mineralisation. As sedimentologists, we can no longer take the view that extracellular polymeric substances is a bystander material, or that it has a single set of coherent and predictable or intuitive influences. Rather, the emphasis must be on investigating the specific mixtures present in nature, and their complex and dynamic interaction with both mineral surfaces and hydrochemical conditions.

# 1 Introduction

2  
3 The growth of calcite crystals can be manipulated by the addition of a range of organic additives  
4 (Meldrum and Cölfen, 2008), permitting specific growth forms to be created (Meldrum and  
5 Ludwigs, 2007), and a range of materials to be incorporated into the lattice, including fully-  
6 tuneable fluorescence (Green et al., 2016). This concept has been successful in explaining  
7 biomineralisation and skeletal growth, to the extent that it has been proposed that hydrogel  
8 scaffolds could be used to grow artificial bone grafts in humans (Kurian et al., 2019). The  
9 structure and trace element composition of carbonate skeletons (Allemand et al., 2004) and tests  
10 (De Nooijer et al., 2014) depends on similar organic-calcite interaction. It has long been  
11 established that similar processes occurred in freshwater limestones (tufa) (Braissant et al., 2003).  
12 Increasingly, it is recognised that organic-calcite interactions are the primary control determining  
13 the trigger for precipitation of tufa (Pedley et al., 2009), its structure (Mercedes-Martin et al., 2015)  
14 and trace element incorporation (Saunders et al., 2014). As tufa precipitates are generally  
15 considered bio-influenced rather than biomediated (Dupraz and Visscher, 2005), there is less  
16 control on the sites of mineral nucleation in tufa than in comparable sites in coral skeleton growth.  
17 Consequently, the chemistry of fluids at these sites is far more able to vary, and little is known  
18 about how local conditions will alter the properties of the mineral formed. This paper takes the  
19 first steps towards establishing the joint control of organic influence and local environment, by  
20 investigating the combined impact of naturally occurring organic additives, salinity and  
21 temperature on calcite precipitation in the absence of metabolic processes.

## 22 1.1 Extracellular Polymeric Substances and their role in tufa formation.

23 The precipitation of calcite in karstic streams generally occurs in the presence of microbial  
24 biofilms. It is well accepted that microorganisms have some influence on calcite precipitation  
25 through varying mechanisms such as metabolic processes (Merz-Preiss and Riding, 1999, Bissett  
26 et al., 2008, Shiraishi et al., 2010) and acting as nucleation sites to enhance precipitation rates  
27 (Bosak and Newman, 2003). Nucleation happens on the surface of cells (Brasier et al., 2018), but  
28 also on hydrogels of extracellular polymeric substances (EPS). These materials generally account  
29 for between 50 and 90% of the total organic matter of a biofilm (Wingender et al., 1999), so an  
30 understanding of its role is a crucial step in the quest to discover the full role of biofilms in calcite  
31 precipitation. It has been reported that the chelation of  $\text{Ca}^{2+}$  ions to EPS is initially an inhibiting

32 factor in carbonate mineral precipitation in seawater (Kawaguchi and Decho, 2002), however *in*  
33 *vitro* experiments have shown this may not be the case in a freshwater setting (Rogerson *et al.*,  
34 2008 and Pedley *et al.*, 2009) and the presence of anionic functional groups on EPS molecules  
35 may help initiate calcite precipitation through the unidentate bonding of  $\text{Ca}^{2+}$  to the anionic  
36 groups (Decho, 2010). Furthermore, the binding of  $\text{Ca}^{2+}$  to EPS molecules can reduce the  
37 activation energy barrier which usually limits spontaneous precipitation (Dittrich and Sibling,  
38 2010), indicating that the presence of EPS may result in greater precipitation rates. The ability of  
39 isolated EPS molecules to precipitate calcite in the absence of the microbial processes of the  
40 associated organisms has been observed experimentally (Kawaguchi and Decho, 2002, Ercole *et*  
41 *al.*, 2007, Tourney and Ngwenya, 2009, Dittrich and Sibling, 2010). These processes likely operate  
42 via electrochemical influences operating between dissolved or hydrated EPS molecules and ions  
43 within ambient water, and consequently are sensitive to the ionic strength of the solution  
44 (Rogerson *et al.*, 2014).

## 45 **1.2 Trace element incorporation into tufa carbonate**

46 Incorporation of trace elements into carbonates frequently provide critically important information  
47 about past environments, from ocean surface conditions in foraminiferal tests (Allen *et al.*, 2016)  
48 and coral skeletons (Fowell *et al.*, 2016) to recharge processes overlying speleothems (Rossi and  
49 Lozano, 2016). Similar ratios of trace elements to calcium have been used in tufa geochemistry  
50 studies over the last 20 years (Ihlenfeld *et al.*, 2003, Dabkowski *et al.*, 2019, Wróblewski *et al.*,  
51 2017, Sironić *et al.*, 2017, Garnett *et al.*, 2004), but use of these proxies is complicated by the  
52 organic-calcite interactions described above (Saunders *et al.*, 2014). Clearly, full development of  
53 these proxies will require effective biomineralisation models comparable to those that have been  
54 found to be necessary in foraminifera (Evans *et al.*, 2018) and corals (Marchitto *et al.*, 2018).  
55 Understanding how the organic components in the tufa system cause trace element incorporation  
56 to deviate from the inorganic thermodynamic equilibrium will be a key step towards this. As there  
57 is a continuum of depositional contexts from strictly freshwater “tufa” to geothermal “travertine”  
58 and even marine biofilm deposition, this investigation centres on the roles played in controlling  
59 carbonate Mg/Ca from temperature, concentration of EPS and salinity.

60

## 61 **2 Methods**

62



63 Tufa biofilm was collected from artificial substrates put into the River Lathkill (Derbyshire, UK grid  
64 reference SK 225 645; Figure 1) for >2 years. These substrates had accumulated substantial carbonate  
65 growth associated with a diverse diatom-cyanobacterial biofilm, similar to that used in previous  
66 experiments (Pedley et al., 2009, Rogerson et al., 2008). From this material, an aliquot of  $10 \pm 0.2$  g  
67 was removed for extraction of organic components following the heat extraction approach of  
68 Barranguet et al, (2004). To this, 24 mL of ultrapure water (18 M $\Omega$ ) was added in a 50 mL conical  
69 flask. The mixture was shaken vigorously to ensure mixing and then placed in a preheated oven at  
70 130 °C for 2 hours (Barranguet et al., 2004). The solution was gently shaken every 20 minutes whilst  
71 in the oven. After heating, the solution was allowed to cool before being centrifuged at 3,300 rpm for  
72 15 minutes. The supernatant was filtered through 11  $\mu$ m filter papers and then through 0.2  $\mu$ m filter  
73 papers into sterilised sterilin tubes to ensure the EPS solution was free from cells. This yielded an EPS  
74 solution with a dissolved organic component of  $0.925 \pm 0.025$  mg L<sup>-1</sup>.

75  
76 Experimental treatments were created by adding volumes of EPS solution (or no solution) to 25 mL of  
77 natural-analog water supersaturated with respect to calcium carbonate. Experiments investigating the  
78 influence of temperature were conducted in clear conical flasks mounted on a Stuart SF1 flask shaker  
79 (Bibby Scientific Limited, Staffordshire, UK) which was set to 100 oscillations per minute, with  
80 temperature controlled via a water bath using the approach of Saunders et al. (2014). Otherwise,  
81 experiments were conducted in clear plastic Petri dishes to reduce sample volumes. In both cases, each  
82 contained two frosted glass microscope slides. The flasks, dishes and slides were sterilised with pure  
83 ethanol. Where the ionic strength of the solution was manipulated, this was done by adding heat  
84 sterilised NaCl<sub>(s)</sub> directly to the mixed solution. Lids were placed on each dish, which were sealed by  
85 applying a thin coating of clear silicone gel to ensure no infection occurred during the experiment. The  
86 dishes were then covered to exclude light and left under controlled conditions for 27 days within an  
87 air-conditioned laboratory at 16°C. The standard deviation of temperature variability through all  
88 experiments was <0.5 °C.

89  
90 At the end of the experiment, the lids were carefully removed and a sample of the dish solution was  
91 taken from each dish and immediately acidified by dilution with 5 % ultrapure HNO<sub>3</sub> for  
92 determination of Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations by inductively coupled plasma-atomic emission  
93 spectroscopy (ICP – OES). The slides were gently rinsed with ultra high quality water and returned to  
94 new Petri dishes and placed in an oven at 60 °C to dry. The washing of the slides was done to ensure  
95 no further precipitation could take place during the drying process. Ultrapure 10 % HNO<sub>3</sub> was

96 gravimetrically added to each dried dish to dissolve the precipitates on the slides. Each dish was left  
97 for two hours and shaken gently every 20 minutes to aid dissolution.

98

99 Experimental water was collected from a spring sourced by a Cretaceous chalk aquifer at Welton Beck,  
100 East Yorkshire (UK grid reference SE 965 275). It was analysed for its  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations  
101 by ICP – OES. Acetates of calcium ( $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ ) and magnesium ( $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2$ ) (Alfa Aesar,  
102 Massachusetts, USA) were added to the spring water to bring the concentrations of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  to  
103 a constant  $51 \pm 1.8$  and  $1544 \pm 19.8$   $\text{mg L}^{-1}$  respectively, giving a Mg/Ca molar ratio of  $0.054 \pm 0.002$ .  
104 Using a natural water provides a more suitable matrix in which to perform these experiments than a  
105 solution based on UltraPure water, which would lack the range of minor ions present in nature. In  
106 addition, using a stock water already rich in Ca and Mg permits us to achieve a constant concentration  
107 and ratio of these metals, while adding a minimum concentration of counter-ions. The saturation state  
108 of the experimental solution was determined using the aqueous geochemical modelling software  
109 PHREEQC. The saturation index for the experimental solution was 1.64 at 16 °C. In the case of flask  
110 experiments, 50 mL solution was used and in the case of petri dishes 25 mL solution was used.

111

112 The analyses were carried out on a Perkin Elmer Optima 5300DV ICP – OES instrument at the  
113 University of Hull. The selection of the analytical lines used in the results was based on the Perkin  
114 Elmer recommendations for the Optima 5300 DV spectrometer, 393.366 nm for calcium and 280.271  
115 nm for magnesium. Calibration standards were prepared using 1,000 ppm standard stock solutions  
116 (99.9% pure or greater, PrimAg, Xtra, Romil, Cambridge) of calcium and magnesium. Mixed  
117 standards of calcium and magnesium were prepared through dilution with 2% ultrapure  $\text{HNO}_3$  to give  
118 calibration standards of 1, 2, 3, 4 and 5 ppm for calcium and 0.1, 0.2, 0.3, 0.4, and 0.5 ppm for  
119 magnesium. Samples for analysis were diluted with 5 % ultrapure  $\text{HNO}_3$  to bring the expected  
120 concentrations to within or very near the linear calibration of the standards.

121

## 122 **3 Results**

123

### 124 **3.1 Influence of the presence of EPS**

125 For this experiment, petri dishes were used to house the solution. Four replicates were run for each  
126 treatment, and for a control with no EPS addition. Figure 2A shows the influence of EPS on the  
127 Mg/Ca of calcite, and Figure 2B shows the precipitation rate, calculated from the loss of calcium from  
128 the parent solution. It is likely a small mass of calcium and magnesium are chelated to EPS during

129 these experiments in addition to that deposited as calcite, but masses released during slide washing  
130 were minor compared to mineral accumulation. These data reveal a simultaneous reduction in calcite  
131 precipitation and rise in the Mg/Ca of the mineral precipitated. Both trends appear to be quasi-linear  
132 across the range investigated in these experiments. Figure 2C shows the interaction between the  
133 controls, emphasising that addition of EPS tends to result in slower precipitation, and higher Mg/Ca in  
134 the precipitate.

135

### 136 **3.2 Influence of Salinity**

137 For this experiment, petri dishes were used to house the solution. Two replicates were run for each  
138 treatment for control (no EPS) and treatment (3 mL EPS solution, forming a  $0.05 \text{ mg L}^{-1}$   
139 concentration of EPS) respectively. A concentration of  $0.05 \text{ mg L}^{-1}$  EPS was selected for this  
140 experiment, as this placed it in the middle of the range used for the previous experiment, helping  
141 integration of results. Figure 3A shows the Mg/Ca of control and treatment experiments respectively,  
142 and Figure 3B shows the precipitation rate for the two experiments. No systematic change in Mg/Ca  
143 and only a slight reduction in precipitation rate is found in the absence of EPS, but both change  
144 significantly with rising salinity with EPS present. Precipitation was also higher in most experiments  
145 where EPS was present, with the gain over control reducing as salinity increased. Figure 3C shows the  
146 increasing Mg/Ca with falling precipitation for EPS-treated solutions, and the overall control from  
147 salinity. With EPS present, Mg/Ca rises as precipitation rate falls in a similar manner to that seen in  
148 the concentration experiment (Figure 2C), meaning that high salinity tends to lead to raised Mg/Ca.

### 149 **3.3 Influence of temperature.**

150 For this experiment, flasks were used to house the solution, so that a controlled temperature different  
151 to the ambient laboratory ( $16 \text{ }^\circ\text{C}$ ) could be maintained. Both control (no EPS) and treatments (3 mL  
152 EPS solution, forming a  $0.05 \text{ mg L}^{-1}$  concentration of EPS which lies at the middle of the range of  
153 conditions used for the variable EPS concentration experiment) were run, with two replicates at  $12.1 \pm$   
154  $0.5$ ,  $14.3 \pm 0.2$ ,  $16.3 \pm 0.2$ ,  $18.3 \pm 0.3$  and  $20.5 \pm 0.5 \text{ }^\circ\text{C}$ . Figure 4A shows Mg/Ca for both control and  
155 EPS treated solutions, and Figure 4B shows precipitation rate for EPS treated samples. From 12 to 18  
156  $^\circ\text{C}$ , precipitation rate falls and Mg/Ca rises with temperature, and there is little difference between  
157 control and EPS treated flasks. However, at  $20 \text{ }^\circ\text{C}$ , there is a dramatic change, with an order of  
158 magnitude increase in precipitation rate and a matching decrease in Mg/Ca. Figure 4C shows the  
159 relationship between precipitation rate and Mg/Ca for the EPS-treated samples, showing the same  
160 tendency to a non-linear inverse relationship. The fit shown in Figure 4C has the form  $\text{Mg/Ca} =$

161 0.00069 x precipitation rate<sup>-0.804</sup>, which is very comparable to the value previously published for living  
162 biofilms in similar experiments (Saunders et al., 2014).

163

## 164 **4 Discussion**

165

### 166 **4.1 Control of calcite precipitation rate by EPS**

167 The results shown in Figures 2 and 3 provide further evidence that the presence of dissolved organic  
168 matter at sites of precipitation will influence carbonate precipitation kinetics, even in the absence of  
169 metabolism from living cells. This has been established both for laboratory systems where  
170 manufactured molecules are provided to the precipitation solution (Meldrum and Cölfen, 2008) and  
171 for natural extracts of organic matter from biofilms (Kawaguchi and Decho, 2002, Dittrich and Sibling,  
172 2010). However, the presented data is ambiguous whether the presence of EPS will enhance or impede  
173 precipitation, with different extracts from the original biofilm sometimes raising precipitation rate  
174 (Figure 3B) and sometimes reducing it (Figure 2B). It is worth considering that the EPS concentration  
175 used in the zero ppm salinity experiment (Figure 3B) is in the middle of the range used in the EPS  
176 concentration experiment (Figure 2B). All of these extracts were from a single parent sample from the  
177 River Lathkill, and extracted in the same way. Clearly, the parent material is heterogeneous, and the  
178 specific characteristics of the EPS extracted have widely different consequences for carbonate  
179 precipitation.

180

181 These EPS mixtures are challenging to characterise. They comprise a mixture of polar and non-polar  
182 organic components, each assembled from a relatively limited range of carbon chain and functional  
183 group components (Dittrich and Sibling, 2010). However, the way in which the molecule is assembled  
184 can alter its behaviour considerably, even to the level of the chirality (i.e. symmetry) of the molecule  
185 potentially changing its behaviour. They may also change their behaviour depending on the state of the  
186 solution (Decho et al., 2005), making this an especially difficult problem to solve. The most likely  
187 active agents in the organic-carbonate system are polar, as these components are most able to bind  
188 either onto dissolved ions or onto the growing surface of the crystal (Meldrum and Cölfen, 2008). The  
189 functional groups providing polar characteristics to these molecules are generally polysaccharides  
190 such as amino or fatty acids and glycoconjugates with reactive functional groups including carboxylic,  
191 phosphoric, sulphhydryl, amin-phenolic and hydroxyl bonds (Dittrich and Sibling, 2010). Indeed, the  
192 balance of amino and hydroxyl groups in otherwise similar EPS has been shown to strongly influence

193 precipitation rate in a manner analogous to the findings reported here (Li et al., 2017, Shiraishi et al.,  
194 2017).

195

196 The different extractions made differed in composition, revealing that the precipitation environment  
197 within the biofilm itself is highly heterogeneous. Assuming that the likely cause of differences in these  
198 experiments arise from change in the functional group ratio (cf. Li et al, 2017), it is possible to  
199 conclude that the influence of the organic component of biofilms varies on a millimetre scale and is  
200 independent of influences arising from metabolism. Research needs to move on from the established  
201 perspective of sedimentologists to consider “how EPS influences precipitation”, or to explicitly link  
202 precipitation processes to organism metabolisms. A dedicated survey of the composition, variance and  
203 influence of the mixtures of EPS arising in nature are urgently needed.

204

205 The changing influence of EPS as the salinity (Figure 3) and temperature (Figure 4) of the solution is  
206 altered demonstrates that there is important interaction between the organic and inorganic components  
207 of the solution. The abrupt and spectacular change in the system behaviour at the highest temperature  
208 investigated (20°C, Figure 4) may reflect a protein thermally denaturing, and a consequent change in  
209 the behaviour of one or more components of the EPS present in this experiment. It is interesting that  
210 the EPS extracted in this experiment has “cooked” at a temperature low enough to be within the  
211 envelope of natural environmental variance. This limit on the stability of the EPS components may  
212 reflect the natural variability of water temperature in their habitat, as 20°C represents the most extreme  
213 warm days recorded in monitoring efforts of adjacent catchments in Derbyshire UK (Dove and  
214 Manifold) (Toone et al., 2011). However, monitoring reveals that water average temperature in  
215 English rivers is rising by *ca* 0.03 °C yr<sup>-1</sup> (Orr et al., 2015). It is thus possible that riverine tufa  
216 formation may be vulnerable to contemporary and future climate change, and further investigation of  
217 EPS composition will also be needed to elucidate this possibility. Regardless, temperatures >20 °C  
218 will certainly be routinely achieved on hot days in shallow / still pools and river margins, so transient  
219 changes in EPS-calcite interaction may be a generic feature of tufa systems which has yet to be  
220 investigated.

221

#### 222 **4.2 Influence of isolated EPS on Mg/Ca**

223

224 The results presented here show that, generally, the Mg/Ca of calcite precipitated is enhanced in the  
225 presence of EPS (Figures 2A and 3A). This is consistent with previous experiments, which have

226 demonstrated that polar EPS molecules bind metal ions, and that ions with low charge density are  
227 selectively bound (Rogerson et al., 2008). The consequence of this binding can be that calcite  
228 precipitation is favoured due to immobilisation of calcium ions (Zippel and Neu, 2011) or impeded by  
229 reduction of calcium concentration in solution (Decho et al., 2005). Here, the emphasis is on the latter  
230 (Figures 2A and 3A) and the rise in  $Mg/Ca_{(calcite)}$  can be considered a direct consequence of the rise in  
231  $Mg/Ca_{(solution)}$  caused by calcium chelation by EPS locally at sites of precipitation. The observation that  
232  $Mg/Ca$  increases with falling precipitation rate gives further support that this is the primary control, as  
233 generally incorporation of the less abundant ion would increase with precipitation rate if this were a  
234 kinetic fractionation.

235

236 Combining the results of the isolated EPS experiments presented here with previously published  
237 experiments with living biofilms (Saunders et al., 2014) show similar behaviour, although with some  
238 scatter arising from the different conditions of the experiment (Figure 5). The link between low  
239 precipitation rate (i.e. high influence of EPS-bound calcium) and high  $Mg/Ca$  is persistent between  
240 these experiments, demonstrating that this is a metabolism-independent process comparable to what is  
241 found in metazoans (Pérez-Huerta et al., 2008).

242

## 243 **5 Conclusions**

244

245 Extracellular Polymeric Substances regulate the rate of precipitation and trace element incorporation  
246 into tufa calcite, even in the absence of living cells. The control on trace element incorporation into  
247 tufa calcite, including  $Mg/Ca$ , largely arises from organic-metal interaction before precipitation,  
248 overcoming inorganic thermodynamic and metabolic controls. However, the impact will be highly  
249 context dependent. Where there is a net production of EPS molecules, it is likely that precipitation will  
250 have increased  $Mg/Ca$  as calcium is bound to the organic components and becomes unavailable for  
251 precipitation. Where there is a net consumption of EPS, the release of bound calcium is likely to cause  
252 decreased  $Mg/Ca$ .

253

254 However, the specific impact depends on the composition of the EPS at the site of mineral formation.  
255 This composition is variable at millimetre scale, to the extent that different microsites within a biofilm  
256 may exhibit opposite tendencies. Clearly, the assumptions that 1) EPS is essentially a “bystander” to  
257 processes which are either inorganic or metabolically forced and 2) that the impact of EPS can be  
258 established as a single, homogenous feature of tufa sedimentology is false. Process-based

259 understanding of these systems will require detailed and wide-ranging assessment of the organic  
260 geochemistry of the materials which make up this complex material. Moreover, the observed  
261 interaction with environmental conditions (e.g. salinity) which may be non-linear (e.g. high  
262 temperature “cooking” EPS molecules) indicate that even where the local recipe for EPS is known, its  
263 sedimentological impact may still vary between and within sites, and at different times at the same site.  
264  
265 We need more knowledge of the polysaccharides present at sites of tufa carbonate formation, their  
266 functional groups and the stability of those functional groups to environmental variance. The  
267 possibility that where a biofilm material is taken beyond its normal envelope of conditions (e.g. “high”  
268 temperature, in this case  $\geq 20$  °C) it may substantially change its influence on calcite formation implies  
269 that tufa formation may be significantly altered by contemporary climate change. This adds urgency to  
270 the need to close the gap in our knowledge addressed by this study.

## 272 **6 Data Availability and Acknowledgments**

273  
274 The data that support the findings of this study are available from the corresponding author upon  
275 reasonable request.

276  
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### Captions

Figure 1. Location of biofilm sampling site. Numbers in inset indicate UK National Grid reference within the SK block.

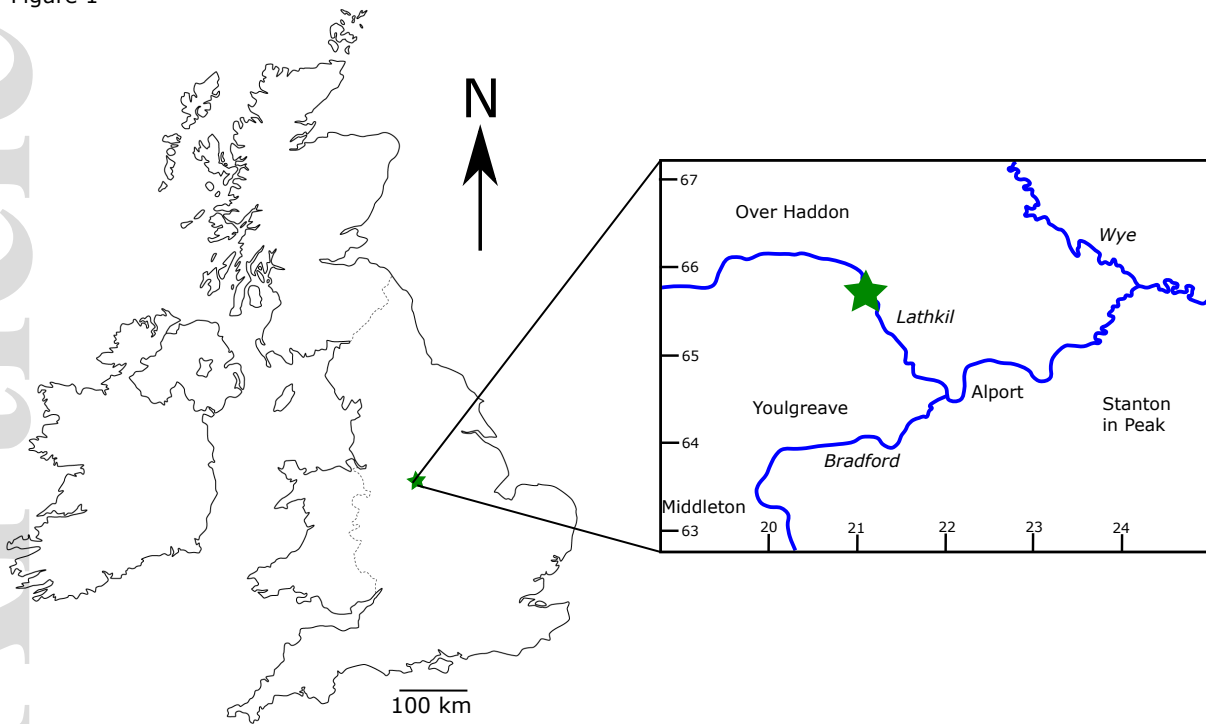
Figure 2. A) relationship of Mg/Ca and addition of EPS. Error bars are measurement uncertainty. B) Relationship of precipitation rate and addition of EPS. C) Interaction of EPS addition, precipitation rate and Mg/Ca. Error bars are measurement uncertainty.

Figure 3. A) relationship of Mg/Ca and salinity. Filled circles are in the presence of EPS, and open circles are in its absence. Error bars are measurement uncertainty. B) Relationship of precipitation rate and salinity. Filled circles are in the presence of EPS, and open circles are in its absence. C) Interaction of salinity, precipitation rate and Mg/Ca in the presence (circles) and absence (diamonds) of EPS. Error bars are measurement uncertainty.

Figure 4. A) relationship of Mg/Ca and temperature. Filled circles are in the presence of EPS, and open circles are in its absence. Error bars are measurement uncertainty. B) relationship of precipitation rate and temperature. C) interaction of salinity, precipitation rate and Mg/Ca in the presence of EPS. Error bars are measurement uncertainty. Line shows fit with regression  $0.00069*x^{-0.804}$

Figure 5. Precipitation rate against Mg/Ca for isolated EPS and experiments with living biofilm. Note logarithmic axes.

Figure 1



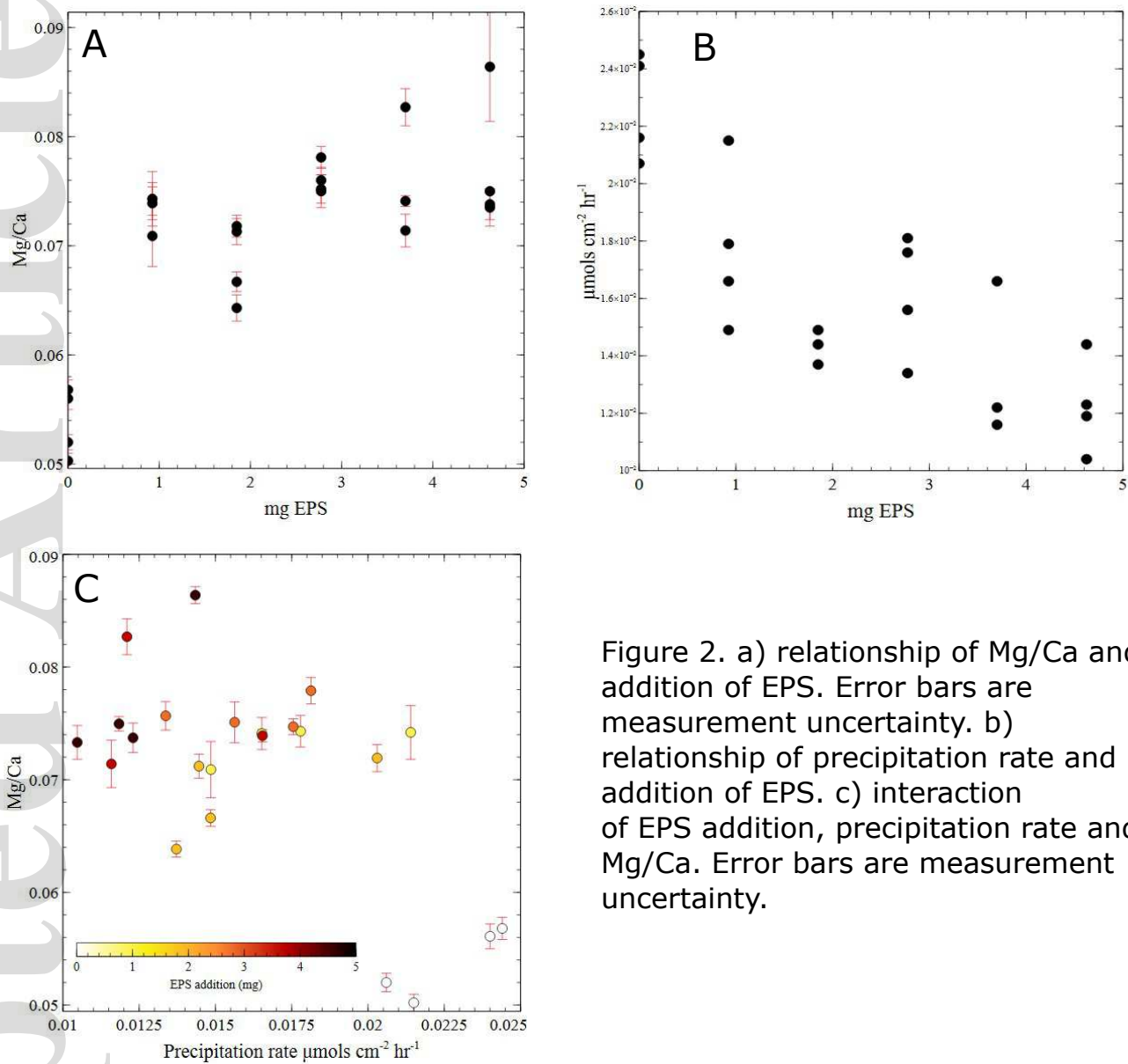


Figure 2. a) relationship of Mg/Ca and addition of EPS. Error bars are measurement uncertainty. b) relationship of precipitation rate and addition of EPS. c) interaction of EPS addition, precipitation rate and Mg/Ca. Error bars are measurement uncertainty.

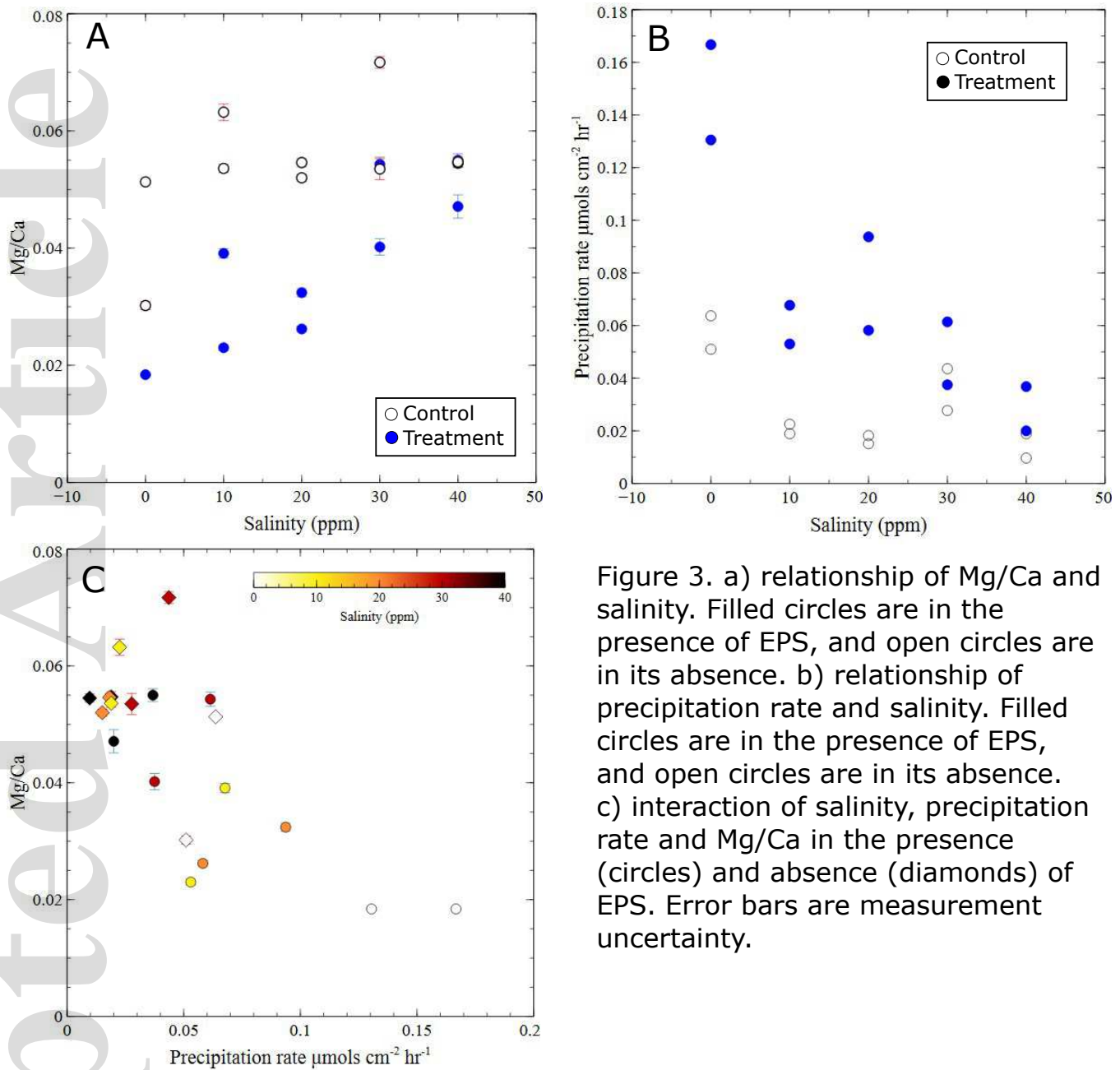


Figure 3. a) relationship of Mg/Ca and salinity. Filled circles are in the presence of EPS, and open circles are in its absence. b) relationship of precipitation rate and salinity. Filled circles are in the presence of EPS, and open circles are in its absence. c) interaction of salinity, precipitation rate and Mg/Ca in the presence (circles) and absence (diamonds) of EPS. Error bars are measurement uncertainty.

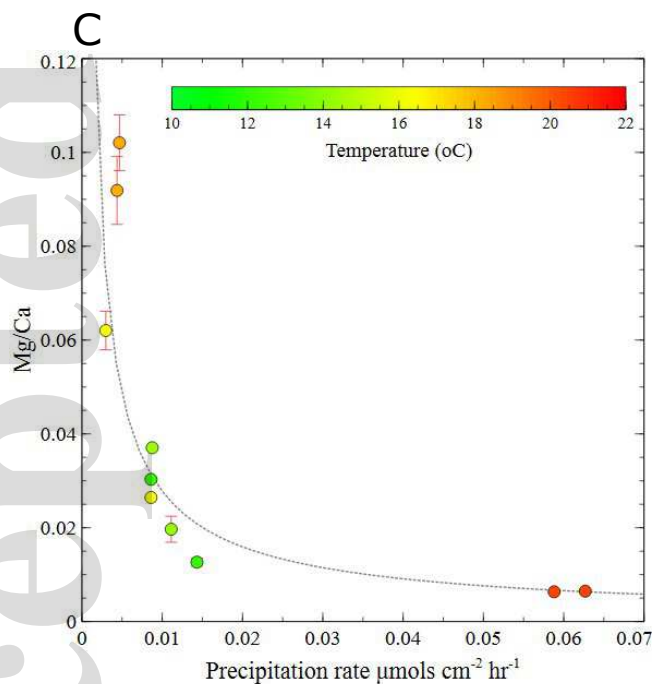
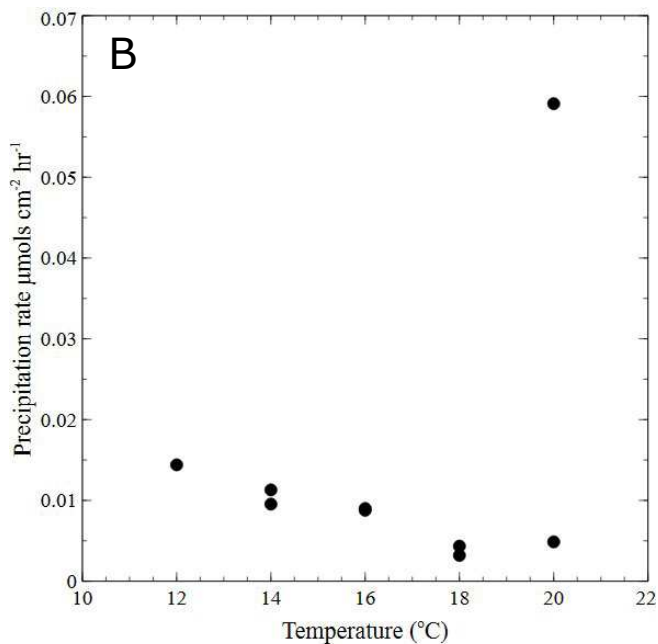
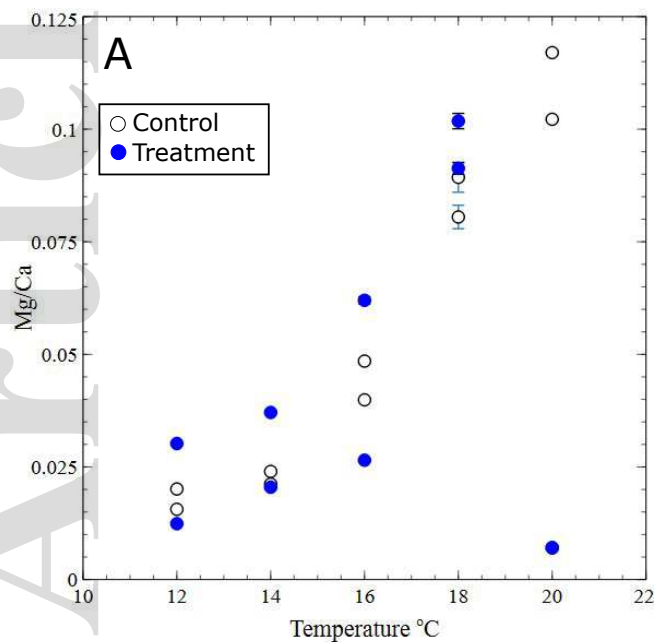


Figure 4. a) relationship of Mg/Ca and temperature. Filled circles are in the presence of EPS, and open circles are in its absence. Error bars are measurement uncertainty. b) relationship of precipitation rate and temperature. c) interaction of salinity, precipitation rate and Mg/Ca in the presence of EPS. Error bars are measurement uncertainty. Line shows fit regression  $0.00069 * \text{precip. rate}^{-0.804}$

