1 **The effect of desiccation on the emission of volatile bromocarbons from two common temperate**
2 **macroalgae**
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12 **Abstract**
13 Exposure of intertidal macroalgae during low tide has been linked to the emission of a variety
14 atmospherically-important trace gases into the coastal atmosphere. In recent years, several studies
15 have investigated the role of inorganic iodine and organoiodides as antioxidants and their emission
16 during exposure to combat oxidative stress, yet the role of organic bromine species during desiccation
17 is less well understood. In this study the emission of dibromomethane (CH_2Br_2) and bromoform
18 ($CHBr_3$) during exposure and desiccation of two common temperate macroalgae, *Fucus vesiculosus*
19 and *Ulva intestinalis*, is reported. Determination of the impact exposure may have on algal
20 physiological processes is difficult as intertidal species are adapted to desiccation and may undergo
21 varying degrees of desiccation before their physiology is affected. For this reason we include
22 comparisons between photosynthetic capacity (F_v/F_m) and halocarbon emissions during a desiccation
23 time series. In addition, the role of rewetting with freshwater to simulate exposure to rain was also
24 investigated. Our results show that an immediate flux of bromocarbons occurs upon exposure,
25 followed by a decline in bromocarbon emissions. We suggest that this immediate bromocarbon pulse
26 may be linked to volatilisation or emissions of existing bromocarbon stores from the algal surface
27 rather than the production of bromocarbons as an antioxidant response.
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38 **1. Introduction**
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40 Seaweeds in intertidal habitats exhibit zonation patterns influenced by multiple abiotic and biotic
41 factors. This includes the ability to tolerate desiccation during tidal emersion, which tends to
42 determine the upper shore limit of a species. Tidal variations in exposure are natural and to survive in
43 the intertidal region sessile organisms, including seaweeds, have evolved mechanisms to withstand the
44 rapid fluctuations in temperature, light, salinity and nutrient availability that occur in the intertidal
45 region. Studies have shown that seaweeds grow faster when continually submerged compared to those
46 that are exposed during the daily tidal cycle (Williams & Dethier, 2005) strongly suggesting that
47 emersion causes a metabolic cost to the algae. A common physiological response to stress is an
48 increase in reactive oxygen species, ROS, and if these are produced at a rate faster than the alga can
49 quench them this can lead to oxidative stress (Lesser, 2006). Variation in environmental conditions
50 during exposure may also combine to enhance the impact on the algae. For example, a reduction in
51 photosynthesis due to inorganic carbon limitation and damage to photosystem II (PSII) reduces the
52 energy available to regenerate antioxidants (Burritt et al., 2002), thereby hindering the response to
53 oxidative stress and reducing the ability to cope with prolonged desiccation.
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55 Desiccation may form part of post-harvest processing for both wild and farmed seaweed species as in
56 some cases biomass is left to dehydrate before further processing. As this process often occurs in the
57 open potential emissions of volatile halocarbons are likely to have atmospheric impacts. The impact of
58 emissions during this dehydration process is an important consideration when estimating emission
59 budgets from seaweed aquaculture (Leedham et al., 2013).
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75 Recent studies provide evidence that a balanced stratospheric inorganic bromine (Br_y) budget requires
76 a contribution to stratospheric Br_y from short-lived bromocarbons of mainly biogenic origin, such as
77 dibromomethane (CH_2Br_2) and bromoform ($CHBr_3$). This suggests that biogenic bromine compounds
78 may impact on tropospheric and stratospheric ozone chemistry (Montzka et al., 2010). As algae
79 accumulate halides from seawater and emit a range of organic halogenated species they are important
80 sources of CH_2Br_2 and $CHBr_3$ (e.g. Carpenter & Liss, 2000; Gschwend et al., 1984; Küpper et al.,
81 2013; Leedham et al., 2013). It is believed that organic and inorganic halides, in their role as
82 antioxidants, may play a role in mitigating ROS damage (Collén et al., 1994) and therefore the
83 macroalgal adaptation to tidal exposure. In several incubation experiments, production of
84 polyhalogenated compounds was enhanced in the light compared to the dark – evidence that
85 halocarbon emissions could be linked to ROS production during photosynthesis (Collén et al., 1994;
86 Keng et al., 2013; Klick, 1993; Nightingale et al., 1995; Pedersén et al., 1996). Other studies report
87 increased bromocarbon production with the addition of H_2O_2 (Collén et al. 1994; Küpper et al. 2013)
88 or decreased production with the addition of photosynthesis inhibitors (Goodwin et al., 1997). Methyl
89 halides, which do not scavenge H_2O_2, were not affected by light in the Collén et al. (1994) study.
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91 It has been shown that variations in atmospheric abundances of polyhalomethane concentrations
92 (including bromocarbons) over seaweed beds correspond to tidal cycles, together with bursts of
93 iodine-containing particles at low tide (Carpenter et al., 1999; Jones et al., 2009; Mäkelä et al., 2002;
94 Nightingale et al., 1995). This was linked to increased halocarbon production due to oxidative stress
95 and an increased sea-air flux due to increased seawater concentrations as the water level decreased
96 (McFiggans et al., 2004). Much of the recent work in this field has focused on emissions of molecular
97 iodine, I_2, (e.g. Cainey et al., 2007; McFiggans et al., 2004; Palmer et al., 2005) and, until recently, the
98 role of brominated compounds in the antioxidant/stress response to desiccation remained poorly
99 understood. A recent study by Küpper et al. (2013) found that *Laminaria digitata* sequesters bromine
100 from seawater (mostly as bromide) but its accumulation is far less pronounced than for iodine. It is a
101 less suitable as an antioxidant and there was no detectable bromide flux under oxidative stress. This is
102 perhaps expected: although *Laminaria* spp. do release volatile bromocarbons (Carpenter & Liss, 2000)
103 they are known to be stronger emitters of iodinated compounds and to use iodide as an inorganic
104 antioxidant (Küpper et al., 2008). However, the recent Küpper et al. (2013) study highlighted the
105 complexity of the role bromine and bromocarbons may play in macroalgae and that this role is not yet
106 fully understood, in particular from species that may release larger quantities of brominated
107 compounds. A better understanding of these processes is important for accurate quantification of
108 coastal emission budgets, especially in intertidal regions where algae are exposed for several hours
109 each day and, moreover, in the case of seaweed harvesting. The latter is particularly pertinent given
110 global interest in seaweed farming as a source of chemical products and feedstocks, biofuels, food and
111 for carbon sequestration (Schlarb-Ridley & Parker, 2013).

112 Here we report the results of a suite of laboratory experiments aimed at improving our understanding
113 of CH_2Br_2 and $CHBr_3$ emissions during seaweed exposure and desiccation. The difference in
114 emissions between two common temperate seaweed species, *Fucus vesiculosus* and *Ulva intestinalis*,
115 was investigated, as was the effect of rewetting seaweeds with freshwater to mimic exposure to
116 rainwater. This study also provides the first time series of photosynthetic capacity alongside
117 halocarbon emissions during desiccation with the aim of increasing our understanding of the links
118 between photosynthetic stress and bromocarbon emissions.
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120 **2. Methodology**
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122 **2.1 Sample collection**
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124 *F. vesiculosus* (whole, individual specimens, 5 – 13 g dry weight) and *U. intestinalis* (groups of fronds,
125 3 – 6 g dry weight) were collected at low tide from the intertidal region of West Runton beach,
126 Norfolk, UK (52° 56' N 1° 15' E). These species were selected for their contrasting morphologies: *F.*
127 *vesiculosus* is a perennial species with a differentiated frond including tough, leathery blades and *U.*
128 *intestinalis* is an annual with thin, tubular fronds only a couple of cells thick. As the ability to cope
129 with desiccation is a strong determinant in zonal positioning and the extent of an individual species'
130 range within the tidal region (Lobban et al., 1985), samples were collected from the same 2 m strip of
131 the shore on each visit. For both species, care was taken to select intact specimens with a healthy
132 appearance free from visible wounding (e.g. grazing damage) or epiphytes. Samples were returned to
133 the laboratory, rinsed gently in artificial seawater (Seachem Marine Salt™ at a salinity of 32-34) and
134 placed in a 35 l tank of aerated artificial seawater within 2 hours. The tank was housed in a constant
135 temperature room held at 13 °C (±0.5 °C) with a light level of ~180 μmol photons m^{-1} s^{-1} and a 14:10
136 hour light:dark cycle. All samples were left to acclimatise to these conditions for 24-48 hours before
137 the first experiment began. Samples were used within one week of collection. Biological replicates
138 were always collected on the same date. Before experiments, samples were removed from the tank
139 with a small volume of seawater and placed in the laboratory until the seawater temperature stabilised
140 to laboratory temperatures. In all experiments samples were weighed at the start and end of the
141 experiment to determine 'wet weight' (after careful blotting to remove excess water). Dry weights
142 were also measured after drying samples for three days in a 60 °C oven followed by one day in a
143 desiccator.
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149 **2.2 Desiccation apparatus**

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151 Halocarbon emission experiments were conducted using an in-house built system shown in Fig. 1. The
152 air flow (commercial cylinder scrubbed using a hydrocarbon trap) to each flask was controlled
153 individually via a series of Luer taps and flow control valves (C and F in Fig. 1). These allowed for
154 two flow rates to be established before the experiment began and then selected via a switch of a valve
155 during the experiment. Two flow rates were used to provide a balance between the higher flow rates
156 needed to desiccate the sample (250 ml min^{-1}, referred to henceforth as 'desiccating flow') and flow
157 rates suitable for sorbent tube sampling (70 ml min^{-1}, referred to henceforth as 'sampling flow').

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159 At the start of each experiment the air supply was used to flush air from the system for at least 10
160 minutes. Flow to each flask was checked every 10 minutes during the first hour of the experiment and
161 at least every 30 minutes to 1 hour thereafter using an electronic flow meter. The 70 ml min^{-1} flow rate
162 and 700 ml total sample volume were within the quoted ‘safe sampling volumes’ and recommended
163 flow rate ranges given by Markes International (2012; 2008) for their sorbent sampling tubes. A
164 thermometer attached to the frame provided daily temperature readings and during the ~5 month
165 spread of experiments temperature varied between 19 – 22 °C. One flask was always used as a control.
166 As tube sorption efficiency may be affected by air moisture levels (Markes International, 2012) a
167 small volume of artificial seawater was added to the control flask and this flask was observed to still
168 contain signs of moisture at the end of each experiment.

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170 **2.3 Sorbent tube sampling and analysis**

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172 To quantify halocarbon emissions at high resolution, thermal desorption tubes (Markes, UK) were
173 used. These contained three sorbents (in order of increasing sorbent strength): Tenax TA, Carbograph
174 1TD and Carboxen 1000. As previous work (Hughes et al. 2012; 2009) established that trapping
175 efficiency was improved with the use of cold tubes, tubes were stored at -18 °C before use and were
176 wrapped in reusable frozen gel packs (as used for sports injuries) to keep them cold for the duration of
177 sampling. The temperature within the gel packs was usually 0-2 °C with an occasional maximum of
178 5 °C. To prevent post-sampling migration of bromocarbons between sorbents the tubes were returned
179 to the freezer until analysis. The majority of samples were analysed within 7 days of collection and all
180 were analysed within 2 weeks. Sample stability of up to 16 months was reported by Hughes et al.
181 (2009).

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186 Air samples were preconcentrated using an automated Markes ULTRA^{TM} multi-tube autosampler and
187 UNITY^{TM} thermal desorption/sample preconcentration system following standard Markes protocols.
188 Briefly, the ULTRA^{TM} desorbed analytes from each tube at 300 °C for 5 minutes and transferred them
189 in a flow of high purity helium along a short, insulated transfer line to the UNITY^{TM} where they were
190 concentrated on a trap (commercially packed by Markes with glass wool, Tenax TA, Carbograph 1TD
191 and Carboxen 1000) held at -10 °C. The cold trap was then heated to 300 °C for 15 minutes to desorb
192 the analytes into a flow of helium and transfer them along a 200 °C heated transfer line to an Agilent
193 6890 gas chromatograph (GC) fitted with a 60 m DB-VRX capillary column (J&W Ltd.; 0.32 mm
194 diameter, film thickness 1.8 µm). A 5973 Agilent mass spectrometer (MS) in electron impact single ion
195 mode provided quantification, and identification of each halocarbon was via retention time
196 comparison with a known standard using at least two known mass fragments. ULTRA^{TM} systems can
197 hold up to 100 tubes, but as the tubes are held at ambient temperature we loaded a maximum of 10
198 tubes at any one time to minimise losses or migration of halocarbons within the tubes. Each batch of
199 10 tubes included one blank, 2-3 standards and 6-7 samples. Blank tubes were used to verify the
200 effectiveness of tube cleaning and storage and to monitor changes in background bromocarbon levels
201 in the system over time. No significant contamination was observed in blank tubes during this study.
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203 Sample concentrations were calculated relative to a UEA calibration flask comprised of background
204 air calibrated to the NOAA scale. A discussion of UEA-NOAA intercomparisons can be seen in
205 Leedham-Elvidge et al. (2014). Due to the high range of bromocarbon concentrations observed during
206 our experiments we also performed tests to ensure the detector was linear in excess of the range of
207 detector responses we observed. To do this we purged aqueous standards of concentrations between 0-
208 950 pmol L^{-1} onto tubes (see Hughes et al., 2009), a range that gave a detector response that exceeded
209 the maximum response we saw in our experiments, and observed a linear detector response (R^2=0.98,
210 p=<0.01). Sorbent tubes charged with labelled surrogate analytes, deuterated methyl iodide (CD_3I) and
211 ^{13}C-labelled dibromoethane ($^{13}C_2H_4Br_2$), purged from aqueous samples (Leedham et al., 2013), were
212 also used to monitor and account for any drift in system sensitivity. The standards were trapped onto
213 chilled sorbent tubes exactly as for sample collection to provide a calibration of the entire analytical
214 system and standards were run roughly every three tubes for a point concentration calibration. The
215 overall error on the calculated concentrations was 6.66% for CH_2Br_2 and 7.43% for $CHBr_3$.
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223 **2.4 Experiments**

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225 Table 1 provides details of individual experiments conducted as part of this work. Codes given in the
226 first column will be used throughout the text for brevity. Experiments included:
227 **1. Mass loss during desiccation.** Seaweed samples were placed in the desiccation system (Fig. 1) for
228 varying periods of time. Specimens were removed at regular intervals to be weighed. For this reason
229 halocarbon emissions were not measured concurrently. Mass loss experiments were designed to
230 replicate the pattern of desiccating and sampling flow rates of both short (FS/US) and long (FL/UL)
231 halocarbon experiments with a sampling rate of ~3 samples an hour up for the first 3 hours of the
232 experiment and ~1 sample an hour for the remainder.
233 **2. Photosynthesis experiments**. A Walz PHYTO-PAM (Pulse-Amplitude-Modulation fluorometer)
234 with an Emitter-Detector-Fiberoptics Unit (EDF) attachment (commonly used for periphyton/
235 microphytobenthos measurements) was used to provide a measure of how stress affected PSII by
236 comparing the dark-adapted fluorescence state with a light saturated state (achieved by application of a
237 saturating light pulse to the dark-adapted sample so that its reaction centres close). The resulting value,
238 the maximum potential quantum efficiency (F_v/F_m), is lower in stressed samples where more reaction
239 centres are already shut prior to light saturation and so there is less difference between the two states.
240 Due to the need to dark adapt samples before fluorescence measurements were taken it was
241 impractical to desiccate samples within the incubation chambers. Instead, samples were dried under
242 the same light and temperature conditions but in shallow glass petri dishes (coated in black tape to
243 block light from the sides) on a lab bench. A household fan was used to provide movement of air to aid
244 desiccation and the temperature remained within the range of laboratory temperatures given above.
245 The first F_v/F_m measurement for each sample was made when the specimen was submerged in a small
246 volume of water from the seaweed storage tank. This water was then removed and the alga weighed.
247 Periods of desiccation were interspersed with periods of 15 minute dark adaptation followed
248 immediately by F_v/F_m measurements. During UP2, light and temperature fluctuations in the lab were
249 recorded (78 to 110 μmol photons m^{-2} s^{-1} and 22.5-23.5 °C respectively. Samples were weighed
250 periodically to provide an indication of mass loss.
251 **3. Halocarbon emission during desiccation.** Seaweed samples were placed in the desiccation system
252 (Fig. 1) for varying periods of time. In UR1 and UR2 *U. intestinalis* specimens were rewetted with
253 ~50 ml of deionised water after a period of desiccation varying from ~3-8 hours.

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260 **3. Results**
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262 **3.1 Mass/water loss during desiccation**
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264 Results of the mass loss experiments are depicted in Fig. 2. We assume mass loss is equivalent to
265 water loss (as in Bravo-Linares et al., 2010) and take changes in mass as a measure of the rate of
266 desiccation. Two mass loss experiments (FM1 and FM2, n=5) were performed on *F. vesiculosus* and
267 one on *U. intestinalis* (n=3). Of the 5 *F. vesiculosus* replicates 3 showed a relatively constant loss of
268 water, with rates of 0.22 (SD=0.06), 0.18 (SD=0.03) and 0.18 (SD=0.06) % min^{-1} respectively.
269 Corresponding linear fits (Fig. 2) for these 3 replicates had R^2 values of 0.995, 0.994 and 0.981
270 respectively. The final two replicates did not show a linear decay, the loss rate slowed during the latter
271 half of the experiment. This change in loss rate occurred around the time that the experimental
272 procedure switched from ~ 3 samples an hour to ~ 1 sample an hour (see Section 2.4 – this was to
273 replicate ‘short’ (e.g. FS1) and ‘long’ (e.g. FL1) halocarbon emission experiments). As the change
274 occurred around the time of a switch in experimental procedure we did not fit a decay curve to these
275 replicates and have added a marker to Fig. 2 to show where the experimental change occurred. *U.*
276 *intestinalis* lost water faster and to a larger extent than *F. vesiculosus*: both the total percentage water
277 loss and the percentage loss rate per hour were greater for *U. intestinalis* than *F. vesiculosus*. At the
278 end of the experiment *U. intestinalis* weighed between 31-68 % more than its dry mass compared to
279 66-82% for *F. vesiculosus*. As a consequence, water loss in *U. intestinalis* slowed toward the end of the
280 experiment as the amount of water available within the algae decreases substantially and the rate of
281 water loss was not as linear as for *F. vesiculosus* (exponential fits have been applied to the *U.*
282 *intestinalis* mass loss plots in Fig. 2).
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284 **3.2 F_v/F_m changes during desiccation**
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286 Changes in F_v/F_m during desiccation of both species can be seen in Fig. 3. As F_v/F_m experiments were
287 conducted under different conditions to mass loss and halocarbon experiments (Section 2.4) sample
288 mass was also measured at several time points during the F_v/F_m experiments and F_v/F_m changes
289 relative to mass (water) loss are also shown in Fig. 3. Initial measurements of all replicates in our
290 experiments, made when the specimens were submerged in a small volume of seawater, were ~0.7. As
291 environmental factors and history can play a role in determining F_v/F_m (Walz, 1998) care must be
292 taken when comparing F_v/F_m results between studies. Our starting F_v/F_m values, nevertheless, compare
293 well with previous studies which report F_v/F_m values for healthy *F. vesiculosus* and *U. intestinalis*
294 samples of ~0.7-0.8 (Lewis et al., 2001; Magnusson, 1997; Pearson et al., 2000).
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297 In FP1 and UP2 F_v/F_m remained stable for some time before beginning to decline in hours 3-5 (Fig. 3a
298 and c). In UP1 (Fig. 3b) F_v/F_m began to decrease earlier but still remained fairly constant within the
299 first hour of the experiment. The difference between UP1 and UP2 may be attributable to different
300 light levels on the sampling day, inherent biological variability or different environmental histories of
301 the samples collected. Previous studies investigating oxidative bursts show the initial burst to be the
302 largest (Küpper et al., 2001), suggesting that samples with different stress histories may respond
303 differently to laboratory stresses. Mass loss measured during the F_v/F_m experiments showed substantial
304 water loss within this first hour, supporting the results from the FM and UM mass loss experiments.
305 These results show that, despite significant water losses within the first hour of desiccation (19-25%
306 for FP1 and 15-31% for UP1, mass of UP2 was not re-measured until 2 hours into the experiment),
307 photosynthetic capacity was unaffected. As shown by the F_v/F_m vs mass loss plots (Fig. 3) mass losses
308 of around 40-60% were observed before F_v/F_m values in FP1 and UP2 began to decline. This could
309 indicate that a certain threshold, with respect to water loss or physiological stress, must be reached
310 before F_v/F_m is affected. However, we did not see this response during UP2 where the F_v/F_m values
311 declined steadily throughout the experiment. Due to this variation, and as we did not measure
312 specimen mass at every F_v/F_m measurement point, further research into threshold stress levels during
313 the desiccation of algae are warranted. The delay in the decline in the F_v/F_m response suggests that the
314 initial burst in halocarbon emissions (Section 3.3) are not related to the photosynthetic health of the
315 plants, this will be discussed further in Section 4. The overall pattern of decrease observed during
316 these experiments fits with previous studies that report decreasing photosynthesis during desiccation
317 (e.g. Peña et al., 1999).
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319 **3.3 Bromocarbon emissions during desiccation**
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321 The bromocarbon concentrations observed in the desiccation flasks during the FS, FL, US and UL
322 experiments, and production rates calculated from these concentrations, are shown in Figs. 4 (FS and
323 FL) and 5 (US and UL). Refer to Table 1 for descriptions of individual experiments and replicates.
324 Experiments are displayed relative to total flow volume (not time) to standardise between experiments
325 with different sampling procedures and therefore exposures to different volumes of air. All specimens
326 demonstrated bromocarbon emissions whilst control flasks maintained low concentrations, 0-2 ppt for
327 CH_2Br_2 and 0-3.7 ppt for $CHBr_3$, for the duration of the incubations. Variation in bromocarbon
328 production rate varied considerably between some algal specimens, even those collected from the
329 same location at the same time. For example the maximum production rate (rates were calculated for
330 each sampling period) observed for replicate FL1a was around three times higher than that seen in
331 replicate FL1b (~0.01 pmol g DW^{-1} min^{-1} compared to ~0.03 pmol g DW^{-1} min^{-1}, Fig. 4). Variation
332 between individual algal specimens is not unexpected as it has been reported in previous desiccation
333 studies (Ball et al., 2010) and was discussed in detail in Leedham et al. (2013).

334 Five experiments (FS1 to 3 and US1 and 2) started with the algal specimen in the incubation flask still
335 submerged in seawater so that the immediate effect of exposure could be observed. In FS1 to 3 (Fig. 4)
336 concentrations increased after exposure and began to plateau or decrease within the 2-3 hour duration
337 of these experiments. The extremely low concentrations when the algae were submerged are likely due
338 to the fact that a constant flow of gas passed through the flask headspace and the flux of bromocarbons
339 from the seawater to the air was not sufficient to cause an increase in bromocarbons during the
340 residence time of the air. Upon exposure, bromocarbons on or close to the surface of the algae could
341 flux directly to the headspace, leading to an increase in observed concentrations as well as the
342 immediate peak seen in experiments where samples were exposed from the start of the experiment
343 (FL1 and FL2, Fig. 4). In FL1 and FL2 bromocarbon concentrations began to decline after the first
344 sample (within the first hour). In FL2 a second peak was observed after about 4 hours of desiccation,
345 although only in one sample. In both FL experiments the majority of samples concentrations had
346 declined to, or were close to, control levels within 5 hours of exposure (Fig. 4). Short (FS1 to FS3) and
347 long (FL1 and FL2) experiments differed in that FL1 and FL2 exposed the algal specimens to longer
348 periods of high 'desiccating' flow rates as samples were taken once per hour (Table 1). This difference
349 may play a role in the different bromocarbon responses seen between FS and FL experiments. No
350 differences in the range of concentrations were observed between FS and FL experiments (or US and
351 UL experiments). For example, the range of observed CH_2Br_2 concentrations was around 0-100 ppt in
352 all *F. vesiculosus* experiments (FS1 to FS3 and FL1 and FL2, Fig. 4). US1 and US2 (Fig. 5) showed
353 similar patterns to *F. vesiculosus* experiments, with a peak in bromocarbon emissions within the first
354 couple of hours. UL1 (Fig. 5) showed sustained halocarbon concentrations that were not seen in the
355 FL experiments with concentrations of both CH_2Br_2 and $CHBr_3$ remaining similar to starting
356 concentrations up to 5-7 hours after the experiment began. U. intestinalis produced more
357 bromocarbons that *F. vesiculosus* with production rates of up to 0.3 pmol g DW^{-1} min^{-1} for CH_2Br_2 and
358 up to 3 pmol g DW^{-1} min^{-1} for $CHBr_3$. In comparison, F. vesiculosus production rates reached
359 maximum values of 0.2 pmol g DW^{-1} min^{-1} and 0.9 pmol g DW^{-1} min^{-1} for CH_2Br_2 and $CHBr_3$
360 respectively.
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371 All experiments showed similarities between CH_2Br_2 and $CHBr_3$ emission patterns. This is not
372 unexpected given that many field and laboratory experiments have demonstrated highly correlated
373 concentrations of CH2Br2 and CHBr3 in seaweed emissions or air influenced by said emissions (e.g.
374 Carpenter & Liss, 2000; Leedham et al., 2013). This strong correlation is considered to be due to these
375 bromocarbons sharing the same production mechanism (Manley, 2002). The emission ratio,
376 CH2Br2:CHBr3 calculated from a linear regression plot (not shown) was calculated for each
377 experiment with n = >5 data points (all except FS1). A strong correlation (R^2=0.57-0.95) between
378 these two gases was seen for all experiments except FS2. For these experiments (FS3, FL1 and Fl2)
379 the CH_2Br_2:$CHBr_3$ ratio fell between 0.12-0.40. A range that falls within the existing range of values
380 for this ratio, the lower value being similar to a ratio of 0.14 observed by Carpenter & Liss (2000) in
381 coastal air samples and the upper value being similar to a ratio of 0.46 observed in measurements of
382 seawater samples from laboratory cultures by Leedham et al. (2013).
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384 Our results show that, for many of the replicates, the majority of the halocarbon 'response' (increasing
385 concentrations or peaks in bromocarbon emissions) began immediately or within the first hour of
386 exposure. This does not correlate with the F_v/F_m response, outlined in the previous section and will be
387 discussed further in Section 4.
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408 **3.4 Rewetting experiments**

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410 To investigate the impact of freshwater (e.g. rainfall) rewetting on bromocarbon emissions two
411 experiments (UR1-2) were conducted on *U. intestinalis*. The addition of water will impede the
412 halocarbon flux as halocarbons partition first to the aqueous phase and then flux to the headspace. We
413 attempted to minimise this factor by adding enough water to fully rewet each specimen without
414 creating a large depth of water above the algae. The results for CH_2Br_2 and $CHBr_3$ can be seen in Fig.
415 6. The length of desiccation prior to rewetting varied from 3.5 hours for UR1 and ~8 hours for UR2,
416 and this possibly contributed to the differences in the magnitude of halocarbon response observed
417 upon rewetting. In UR2 a slight increase in both CH_2Br_2 and $CHBr_3$ was observed, however, this is of
418 no greater magnitude than other fluctuations observed during the desiccation process (Fig. 5). UR1,
419 however, demonstrated a larger increase in both CH_2Br_2 and $CHBr_3$ emissions after freshwater
420 rewetting. The increase in emissions was observed over several samples and rose to over half the
421 maximum emission near the start of the experiment, showing a response above the variation seen in
422 the previous *U. intestinalis* experiments (US1 and 2, UL1, Fig. 5). At the end of the UR1 sampling
423 period concentrations of CH_2Br_2 and $CHBr_3$ in the flasks appeared to still be increasing. However, due
424 to the natural variability in algal emissions, as demonstrated in Figs. 4 and 5, further experiments are
425 needed to determine a conclusive response to freshwater rewetting. Emission patterns pre-rewetting in
426 UR1 and UR2 (Fig. 6) act as replicates for UL1 (Fig. 5) as they were conducted in the same manner.
427 They also show emissions taking longer to decrease than seen in the *F. vesiculosus* experiments.

428

429 **4. Discussion**

430

431 Previously published bromocarbon desiccation studies focused on natural halocarbon production
432 mechanisms in coastal waters and did not concentrate on the timescale of emissions. Two studies
433 (Bravo-Linares et al., 2010; Nightingale et al., 1995) desiccated algae for several hours but
434 measurements were made to monitor the resubmergence of seaweeds after exposure and not during
435 exposure itself. A pulse of halocarbon emissions into seawater upon reimmersion was reported by
436 Nightingale et al., whereas Bravo-Linares et al. reported a general increase in iodinated compounds
437 but a general decrease in brominated compounds compared to samples that had not undergone
438 desiccation. As physiological stress or damage can be caused by reimmersion as well as exposure
439 (because rehydration alters the cell membrane leading to a flux of ROS upon reimmersion (Collén &
440 Davison, 1999)), this response could be linked to stresses associated with reimmersion as well as
441 desiccation.

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445 Both *F. vesiculosus* and *U. intestinalis* showed relatively linear patterns of water loss, as seen in
446 previous studies (Bravo-Linares et al., 2010; Ji & Tanaka, 2002). *U. intestinalis* dried faster, losing
447 ~50% of its water after 4 hours compared to ~35% for *F. vesiculosus,* in line with Lüning (1990) who
448 reported 20-30% water loss for fucoid species after 4 hours. This may be due to its thinner thallus form
449 allowing for faster water loss. In contrast to our results, Bravo-Linares et al. (2010) found that *U.*
450 *intestinalis* was better than *F. vesiculosus* at retaining water due to its structure, trapping water
451 between its fronds to prevent it drying out. In our study, *U. intestinalis* was spread out to form a thin
452 mat, potentially negating the benefits conveyed by the multiple fronds trapping water. The *U.*
453 *intestinalis* mat could increase the surface area of the alga exposed to desiccation (Davison & Pearson,
454 1996), making the *U. intestinalis* in our study more like flatter *Ulva* species, such as *U. lactuca* and *U.*
455 *pertusa,* which had higher rates of water loss in previous studies due to their larger surface area
456 (Bravo-Linares et al., 2010; Ji & Tanaka, 2002). This result demonstrates that differences between in
457 situ and laboratory conditions may affect experimental outcomes. It also provides a potentially
458 interesting example of how artificial desiccation, for example during drying of harvested algae to
459 create a market product, may vary from natural tidal desiccation. During commercial drying processes
460 the algae are often spread into thin mats to increase the speed of drying and ensure drying occurs
461 before onset of decay. This will increase the rate at which the algal biomass dries and so increase the
462 rate of exposure to stress.
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482 Our results are novel in combining F_v/F_m (photosynthetic capacity) time series measurements with
483 halocarbon emissions. Overall patterns in F_v/F_m were similar between *F. vesiculosus* (FP1) and *U.*
484 *intestinalis* (UP1 and UP2). During the first hour F_v/F_m values for all FP and the majority of UP1 and
485 UP2 replicates showed F_v/F_m values that remained relatively constant or even increased slightly. This
486 slight increase has been reported by others (Kumar et al., 2011; Peña et al., 1999), and has been
487 attributed to a greater demand for energy by desiccation tolerance mechanisms or an increased
488 availability of CO_2 as diffusion into the cell is enhanced. Decreases in F_v/F_m began after an hour for
489 some replicates (e.g. in UP1). In other replicates large decreases were not noted until several hours
490 into the experiment. One prior study reported F_v/F_m measurements during trace gas emission
491 desiccation experiments, although their study focused on emissions of iodinated compounds from *L.*
492 *digitata* (Nitschke et al., 2011). They measured at two time points only (t=0 and 180 minutes). A
493 significant decrease was seen in this time, although the magnitude of the decrease was small, 3%,
494 compared to the decline we observed after further desiccation, supporting the idea that larger decreases
495 in F_v/F_m do not begin until several hours of desiccating conditions. A decrease in F_v/F_m occurs as
496 inorganic carbon becomes limited, oxidative damage affects the photosynthetic apparatus and electron
497 flows between photosystem 1 (PSI) and PSII are interrupted (Kumar et al., 2011; Sampath-Wiley et
498 al., 2008). Although *U. intestinalis* dried quicker than *F. vesiculosus,* decreases in F_v/F_m varied
499 between experiments; decreasing quicker for *U. intestinalis* compared to *F. vesiculosus* in UP1 but not
500 in UP2.
501
502 In many of the experiments the bromocarbon response to desiccation was a short-lived pulse of
503 emissions that occurred within the first few hours of exposure and was not linked to declines in F_v/F_m.
504 These results may suggests that existing bromocarbon stores are released during exposure rather than
505 being produced as a direct response to oxidative stress. This supports recent findings by Küpper et al.
506 (2013) who found iodide, not bromide, to be the major antioxidant in *L. digitata*. The prolonged
507 emissions from *U. intestinalis* may be due to the fact it is known to produce higher quantities of
508 CH_2Br_2 and $CHBr_3$ compared to *F. vesiculosus* and so might have greater bromocarbon reserves to
509 release (Carpenter & Liss, 2000). As *U. intestinalis* has a faster rate of water loss during desiccation,
510 volatilisation of halocarbons from the algal surface or surface water layer are likely to be greater. Also,
511 as *U. intestinalis* may be subjected to a higher level of oxidative stress (due to the faster rate of water
512 loss) and if bromocarbons do contribute to the antioxidant response their emissions are likely to be
513 greater and more prolonged from *U. intestinalis* compared to *F. vesiculosus*.
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519 Other possible causes for the decrease in bromocarbon emissions after the initial pulse, mainly halide
520 or carbon/energy limitation, are unlikely to play a major role on the timescale of our experiments. As
521 seaweeds concentrate halides from seawater in high concentrations (Saenko et al., 1978) halide
522 limitation probably does not drive the observed decrease. A previous study on the rhodophyte
523 *Stictosiphonia arbuscula* reported a decreasing ability to regenerate antioxidants (specifically the
524 ascorbate-glutathione antioxidant response) when desiccation persisted for 12 hours or more due to
525 nutrient limitation (Burritt et al., 2002), a longer timescale than used in our experiments.
526
527 Rewetting in freshwater causes an extra osmotic stress to the cells (Lobban et al., 1985) and the results
528 of the rewetting experiment UR1 suggest that bromocarbon emissions increase upon freshwater
529 rewetting. It should be noted that *U. intestinalis* is a salinity-tolerant species found in a wide range of
530 salinities in the natural environment (Edwards et al., 1988). Therefore the response of *U. intestinalis* to
531 freshwater rewetting may not represent that of all species.
532
533 **5. Conclusions**
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535 The emission of two important biogenic bromocarbons, CH_2Br_2 and $CHBr_3$, was observed during the
536 desiccation of two common temperate macroalgae species, *F. vesiculosus* and *U. intestinalis*. A rapid
537 pulse in bromocarbon emissions was seen within 10 minutes of exposure and, in most cases, either
538 peaked or came to a plateau within 1-3 hours or decreased immediately. In contrast, decreases in F_v/F_m
539 only began 2.5 hours into the desiccation period, and mass loss was steady throughout the
540 experiments. From these results, we attribute the immediate pulse in bromocarbons to an emission or
541 volatilisation of existing halocarbon stores from on or near the surface of the alga upon exposure. The
542 rapid decrease in emissions suggests that bromocarbons may not be actively produced as a response to
543 oxidative stress, in the same manner as iodocarbons and I_2, supporting previous studies (Küpper et al.,
544 2013; Küpper et al., 2008). The prolonged emissions of bromocarbons from *U. intestinalis* over 6-8
545 hours could be due to a greater production of bromocarbons compared to *F. vesiculosus* (Carpenter &
546 Liss, 2000) creating higher concentrations of bromocarbons at/near surface to be volatilised/emitted
547 during desiccation.
548
549 Preliminary experiments investigating the impact of freshwater rewetting on bromocarbon emissions
550 suggest that rewetting may lead to an increased emission of bromocarbons dependent on the length of
551 exposure time prior to rewetting. However, due to the high variability in natural emissions further
552 experiments in this area are required.
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Previously, we (Leedham et al., 2013) estimated annual emissions from tropical macroalgae aquaculture without taking into account emissions post-harvesting (mainly emissions from open-air drying). The results from this study suggest post-harvest desiccation may not greatly increase bromocarbon emission budgets from aquaculture. However, as our experiments ran for a maximum of 8 hours, compared to several days of desiccation in an aquaculture environment, further investigation into the effects of long-term exposure is warranted.

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753 **Table 1. A summary of the desiccation experiments conducted as part of this study.**

Code[a]	Description	Experiment date[b]	# Replicates	# control samples	Specimen mass / g[c]	Description
			Mass loss during desiccation experiments			
FM1	*F. vesiculosus* mass loss	21st Mar	3	n/a	6.0, 10.7 & 9.7	As for halocarbon production experiments but specimens weighed 3 times an hour.
FM2		23rd Mar	2	n/a	9.7 & 12.1	
UM	*U. intestinalis* mass loss	22nd Mar	3	n/a	4.3, 4.8, 3.8	
			F_v/F_m experiments			
FP	*F. vesiculosus* photosynthetic performance during desiccation	19th Jun	3	n/a	13.8, 10.5 & 12.1	Specimens dried on laboratory bench in petri dishes. F_v/F_m samples taken twice an hour.
UP1	*U. intestinalis* photosynthetic performance during desiccation	19th Jun	3	n/a	4.6, 2.4 & 3.0	
UP2		21st Jun	3	n/a	3.0, 3.6 & 3.2	
			Halocarbon production			
FS1	*F. vesiculosus* short desiccation	31st Jan	1	1	7.9	Samples taken every 10 minutes for 2 hours; submerged for first 3 samples.
FS2		3rd Feb	1	1	13.0	Samples taken every 10 minutes for 3 hours; submerged for first 2 samples.
FS3		14th Feb	1	1	6.6	As FS2.
FL1	*F. vesiculosus* long desiccation	12th Mar	2	1	5.2 & 7.9	Samples taken every 20 minutes for 8 hours.
FL2		20th Mar	2	1	6.6 & 7.2	Samples taken every 20 minutes for 5 hours
US1	*U. intestinalis* short desiccation	23rd Feb	1	1	6.0	Samples taken every 10 minutes for 2 hours; submerged for first 2 samples.
US2		2nd Mar	1	1	4.0	Samples taken every 10 minutes for 3 hours; submerged for first 2 samples.
UL1	*U. intestinalis* long desiccation	14th Mar	2	1	3.2 & 3.0	Samples taken every 20 minutes for 8 hours.
UR1	*U. intestinalis* desiccation followed by rewetting in freshwater	12th Apr	2	1	4.4, 4.4	Samples taken hourly for 3.5 hours > specimens rewetted > samples taken every 10 minutes for 2.5 hours.
UR2		26th Apr	2	1	3.4, 5.0	Samples taken every 20 minutes for 8 hours >specimens rewetted > samples taken every 10 minutes for 2 hours.

754 [a]Code describes experiment as follows: first letter = species (F = F. vesiculosus, U = U. intestinalis), second letter = experiment type (S = short (3 hours or less) desiccation, L = long

755 desiccation, R = rewetting, M = mass loss and P = F_v/F_m experiment), number = individual experiments conducted at different times. Lower-case letters used in the main body of the
756 text (e.g. FL1a, FL1b) refer to replicates within these individual experiments. [b] All experiments conducted in 2012. All samples used within 1 week of collection. [c] Mass is fresh
757 weight at start of experiment.

FIGURES

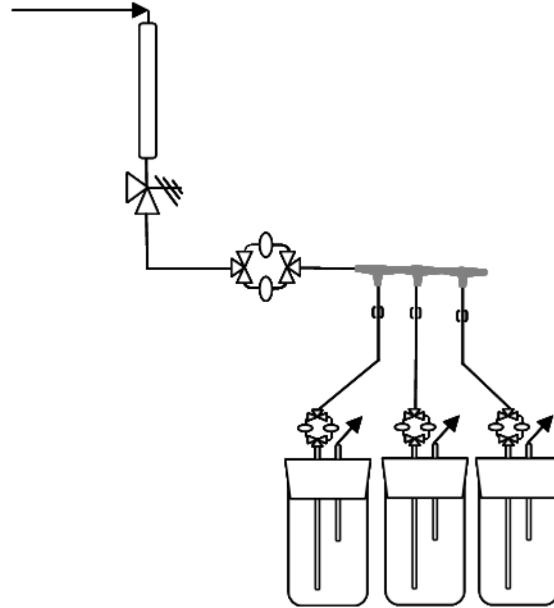


Figure 1. Desiccation system, comprising: A – hydrocarbon trap. B – pressure release valve. C – flow control system; system comprises two three-way valves and two needle valves allowing either a high or low flow to be selected. D – Luer taps to turn flow on/off to individual flasks. E – mini hose clamp to control flow through Tygon® tubing to each flask. F – (smaller) flow control system 2, see C. G – rubber bung and glass inlet and outlet tubes (arranged at different heights to ensure air circulation through the whole flask). H – 1 l wide-necked glass Duran® incubation vessel (seaweed sample placed on bottom of flask). I – ¼ inch Ultra-torr fitting outflow, sorbent tubes or flowmeter connected here.

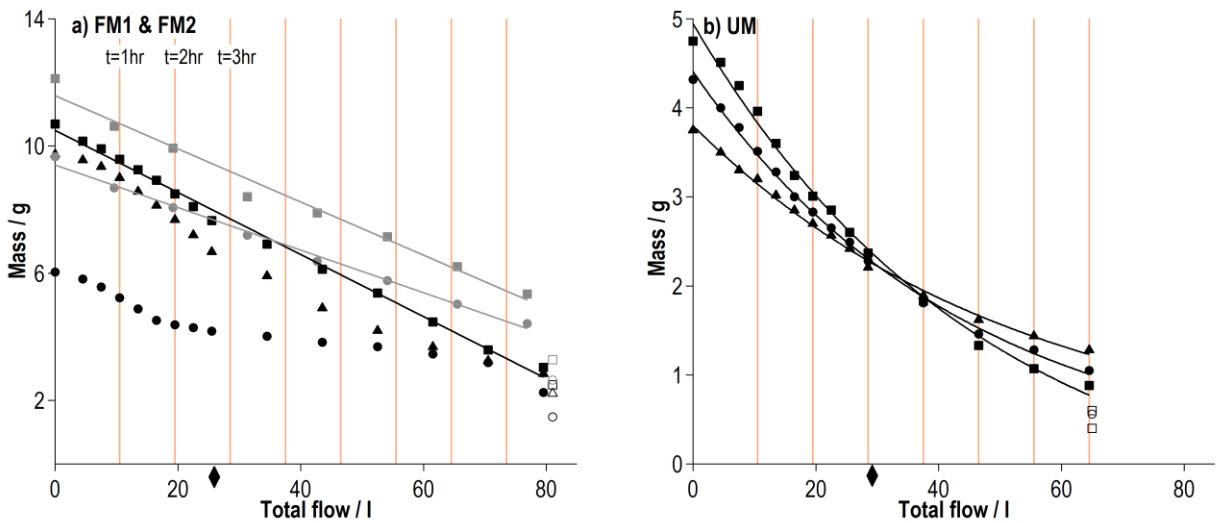
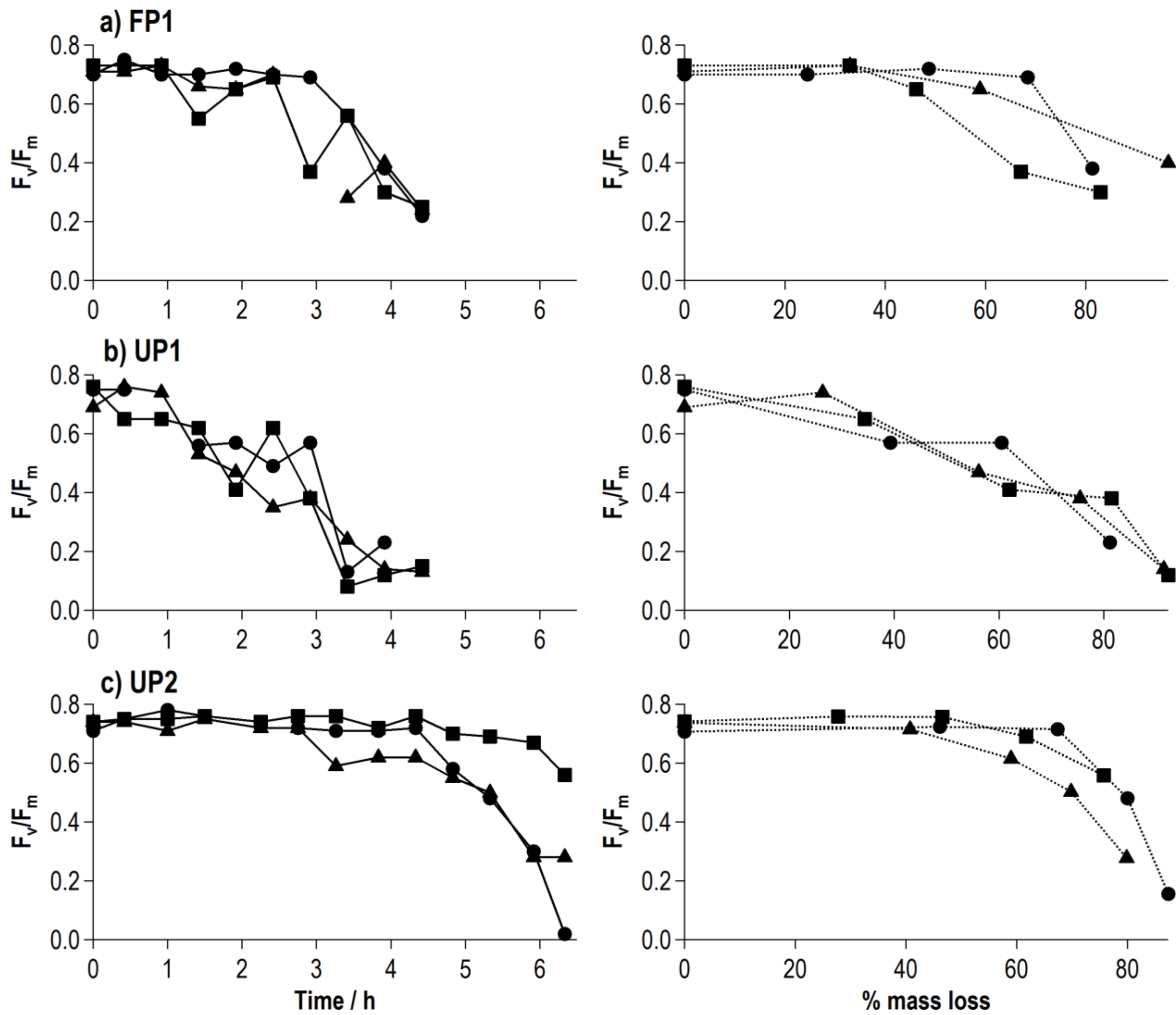


Figure 2. Mass (water) loss during desiccation of a) *F. vesiculosus* (FM1 in black and FM2 in grey) and b) *U. intestinalis* (UM). For details of replicates see Table 1, individual replicates are denoted by different marker shapes. Open shapes show dry mass of each sample (see Section 2.1). Vertical lines = 1 hour intervals from start (0 hours) of desiccation. ◆ = time experimental procedure switched from sampling 3 times an hour to sampling once per hour (see Section 2.4). Patterns in mass loss are discussed further in Section 3.1; loss in *U.*

774 *intestinalis* (b) has been represented with an exponential decay curve. Loss in *F. vesiculosus* was more
775 varied, however some replicates showed a linear loss of mass (see Section 3.1 for more details).



776

777 Figure 3. F_v/F_m changes relative to time (left column) and % mass loss (right column) during desiccation of
778 (a) *F. vesiculosus* and (b, c) *U. intestinalis*. Sample mass was not measured at every time point, see Section
779 3.2 for further details. Symbols represent the three biological replicates used in each experiment, see Table 1
780 for further details.

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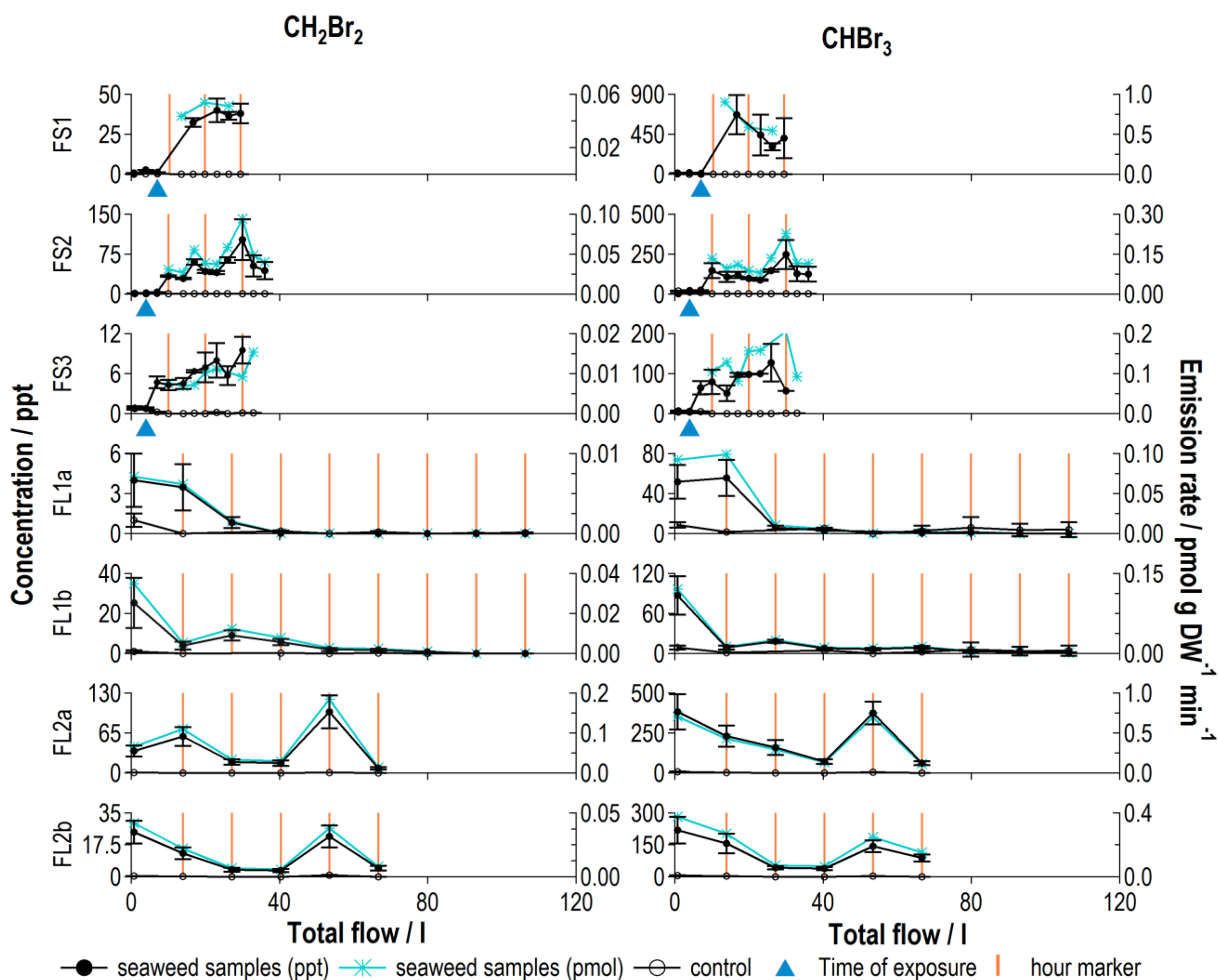

783 Figure 4. Bromocarbon concentrations (ppt, left axis) and production rates (pmol g DW^{-1} min^{-1}, right axis)
784 observed in flasks during desiccation of *F. vesiculosus*. Changes are shown relative to total flow (l) for easier
785 comparison between experiments that used different flow regimes (Section 2.2/Table 1). Experiments
786 conducted at different times are denoted by individual numbers (e.g. FS1, FS2). Within these experiments
787 replicates in different flasks (Fig. 1) are denoted by individual letters (FL1a, FL1b), see Table 1 for further
788 details. Error bars are taken from the daily variations in calibration standards. Vertical lines = 1 hour intervals
789 from start (0 hours) of desiccation. For experiments that began with submerged seaweed samples the
790 exposure point is marked with a triangle.

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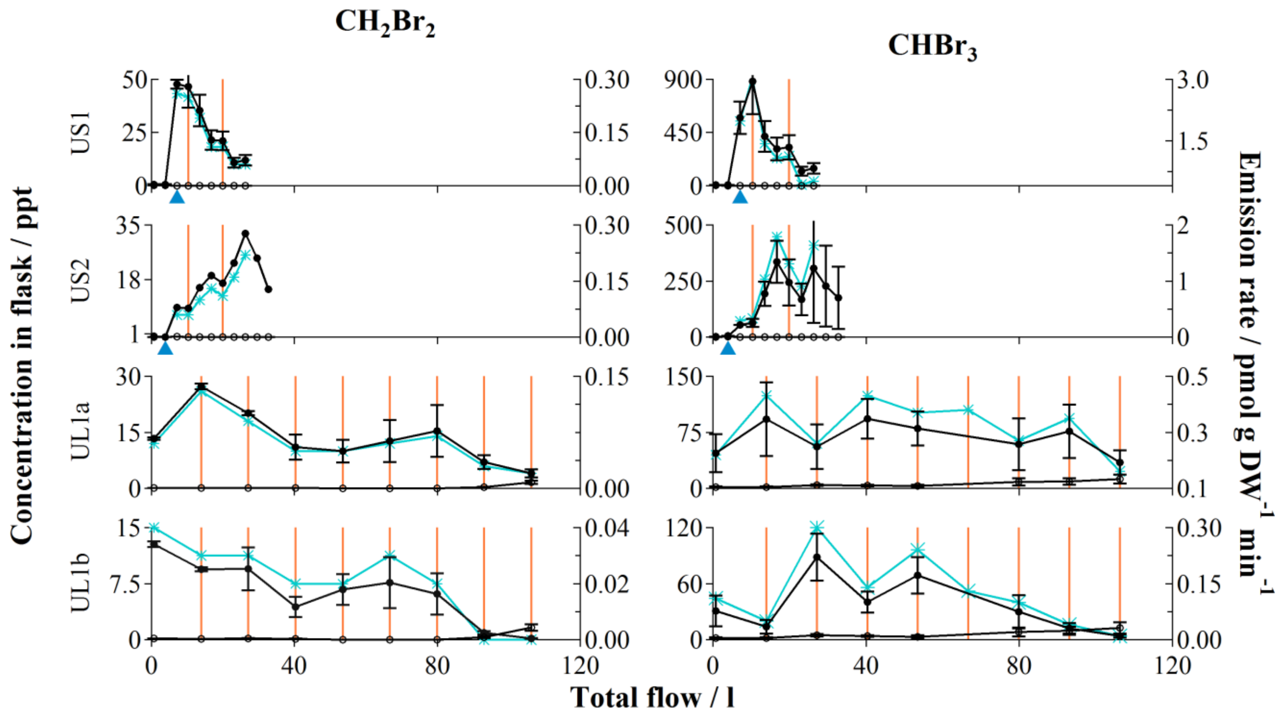


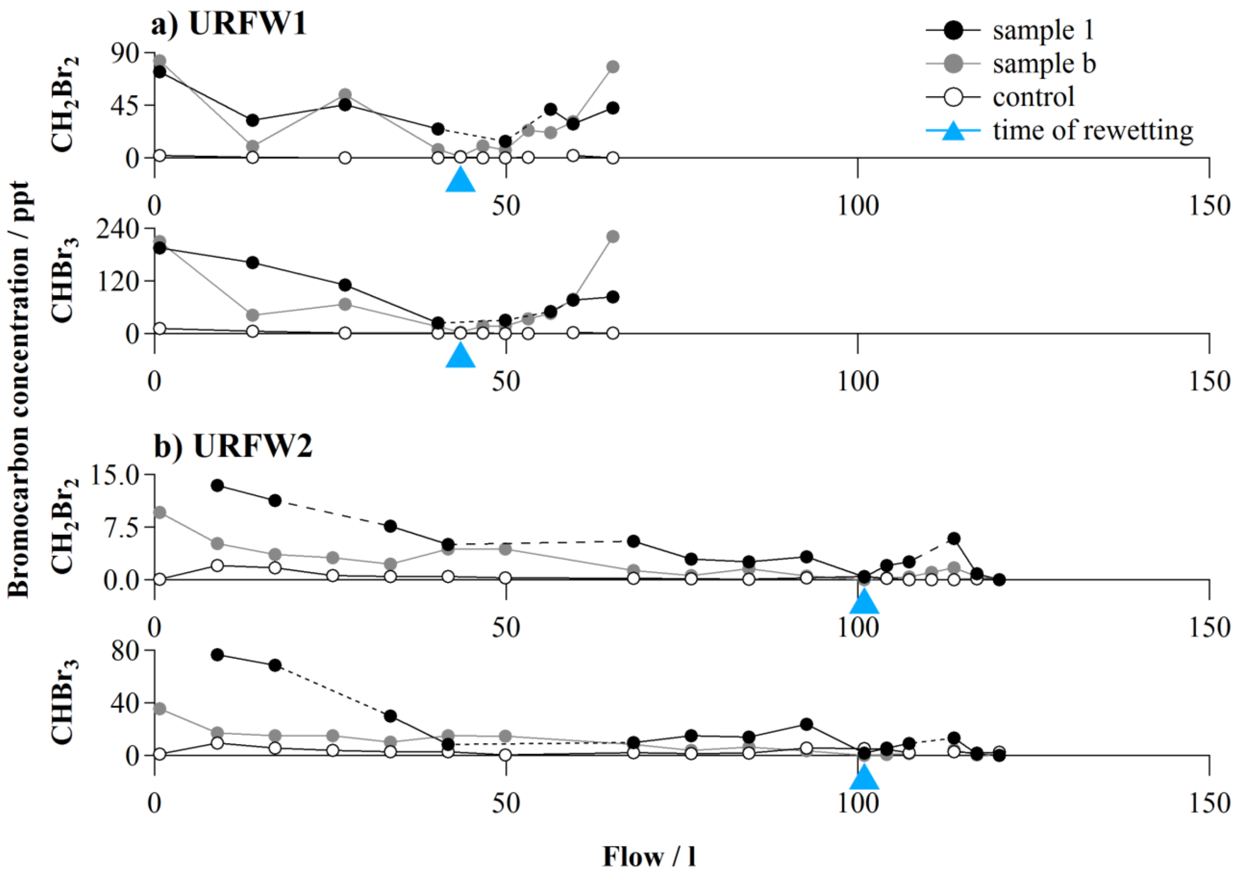
794

795 Figure 5. Bromocarbon concentrations (ppt, left axis) and production rates (pmol g DW^{-1} min^{-1}, right axis)

796 observed in flasks during desiccation of *U. intestinalis* (US and UL experiments). All other details are as for

797 Fig. 4.

798



799

800 Figure 6. Desiccation of *U. intestinalis* followed by rewetting (URFW experiments). Changes are shown

801 relative to total flow (l). Experiments conducted at different times are denoted by individual numbers (e.g.
802 US1, US2). Within these experiments replicates in different flasks (Fig. 1) are denoted by individual letters
803 (UL1a, UL1b), see Table 1 for further details. Error bars are taken from the daily variations in calibration
804 standards.