Interactive effects of warming and species loss on model Antarctic microbial food webs

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Running title *Warming and species loss effects on food webs*

Summary

1. Predicting the effects of environmental warming and species loss on ecosystems are two significant challenges currently facing ecologists. However little is known of the interactive effects of these two factors. We hence tested whether or not warming and species loss interact to influence productivity and dissolved nitrogen concentrations in model Antarctic microbial food webs. Food webs, consisting of a uniform bacterial community and mixtures of six, four, two and zero bacterivorous flagellate species, drawn randomly from a pool of six flagellate species isolated from an Antarctic freshwater lake, were grown in soil extract suspension medium held in microcosms for 252 h. Half of the microcosms were kept at 4 ºC and half were warmed to 8 ºC over the first 36 h and then held at this temperature.

2. After 252 h there were significant interactive effects of flagellate species loss and warming on the abundance of bacterial prey and the concentration of ammonium in the medium: bacterial abundances were reduced by 75% and NH_4-N concentrations were doubled in mixtures inoculated with six and four flagellate species, compared with those inoculated with two species, but only in warmed microcosms. This difference in response was apparently largely owing to the absence of *Bodo saltans* and *Spumella putida*, species with high grazing activities and growth rates, from most replicates of the warmed two species mixtures.

3. Evidence for an apparent complementarity effect was also found, with *B. saltans* and *Spongomonas uvella* growing more rapidly at 4 ºC in mixtures of six species than in those of four species.

4. Data from a separate experiment, in which the flagellate species were grown in single-species culture under food-saturated conditions, confirmed that the logarithmic growth rates of *B. saltans* and *S. putida* were the highest of each of the six species at both 4 ºC and 8 ºC.

5. We broadly conclude from our data that random species loss from food webs or communities is likely to alter their responses to environmental change, largely owing to interspecific differences in responses to change.

Key-words: ammonium, bacterivorous flagellates, complementarity, productivity, sampling effect

Introduction

Ecosystems collectively influence the biogeochemical processes that regulate the Earth System (Loreau *et al.* 2001). The effects of the current rapid loss of biodiversity from the Earth's ecosystems on their functioning are hence a major focus for ecological research (Tilman 1999; Loreau, Naeem & Inchausti 2002). Typically, studies have examined the influence of reduced biodiversity on synthetically-constructed communities of plants (e.g. Naeem *et al*. 1996; Tilman, Wedin & Knops 1996; Hector *et al.* 1999) or, less commonly, of protists (e.g. Petchey *et al.* 1999) or metazoans (e.g. Mikola & Setälä 1998). These studies have often found that 'ecosystem functions', typically measures of productivity and nutrient turnover, are diminished by lowering the number of species in experimentally-assembled communities (e.g. Tilman *et al.* 1996; van der Heijden *et al*. 1998; Hector *et al.* 1999). Unpredictable or null responses to diminished biodiversity have, however, also been recorded (Mikola & Setälä 1998; Laakso & Setälä 1999).

In addition to the rapid loss of biodiversity from ecosystems, environmental change, in the form of warming, increased atmospheric CO_2 or nitrogen deposition, has the potential to alter radically the performance of ecosystems. However, the interactive effects of biodiversity loss and environmental change on ecosystems are, at present, poorly understood (Loreau *et al.* 2001). It is reasonable to assume that the loss of species from habitats subjected to environmental change will affect ecosystem performance, because there are likely to be differences between species in the way in which they respond to abiotic factors such as warming or CO_2. This has recently been demonstrated in synthetically-constructed plant communities (Reich *et al.* 2001). Similarly, microcosm studies demonstrate that the biomass accumulation of consumers is higher in species-rich assemblages of microbes exposed to warming than in more depauperate ones (Petchey *et al.* 1999). However, other than the study by Petchey *et al.* (1999), we are unaware of any studies in the literature showing interactions between species loss and environmental change in microbial food webs.

Here we report a laboratory study that aimed to determine the interactive effects of rapid warming and loss of flagellate species diversity on productivity and nitrogen concentrations in model Antarctic microbial food webs. Antarctic terrestrial and freshwater ecosystems are ideal habitats on which to make this type of study: they are typically dominated by organisms with small body sizes, and generally exhibit low levels of both diversity and trophic complexity (Smith 1996), making them tractable systems to study in the laboratory. Their inherently low species richness also allows a significant proportion of the species present in the field to be incorporated into laboratory experiments, allowing more accurate predictions about the consequences of change in the natural environment than the study of ecosystems with higher species richness. We tested for the effects of warming on increasingly depauperate mixtures of bacterivorous flagellate species, with the aim of determining how food webs that have undergone the loss of consumer species might respond to warming. We hypothesized that changes to the productivities of specific flagellate species as a consequence of warming would alter the abundance of bacteria in food webs. We also predicted that the resulting changes in flagellate grazing would modify the concentrations of dissolved inorganic nitrogen present in the growth medium.

Material and Methods

ISOLATION OF MICROBES

The microbes used in this study were isolated from Sombre Lake on Signy Island in the Maritime Antarctic (60º 42' S, 45º 38' W), a freshwater lake with a maximum depth of 11.2 m and an area of 2.7×10^4 m^2 (Butler 1999a). The lake is ice-free for approximately three months each year, and has mean water column temperatures of 1.5 - 2 °C during the winter and *c*. 4 °C during the summer months (Butler 1999a). Cultures of the bacterivorous flagellates *Bodo saliens* (Larsen and Patterson), *B. saltans* (Ehrenberg), *Goniomonas truncata* (Stein), *Rhynchomonas nasuta* (Klebs), *Spongomonas uvella* (Stein) and *Spumella putida* (Cienkowski) were isolated and purified to single species either by serial dilution or micropipetting. These are typical representatives of the protozooplankton of other freshwater lakes in the Maritime Antarctic (Butler 1999b; Butler, Edworthy & Ellis-Evans 2000). The flagellates were cultured in tissue culture flasks, vented with 0.2 μm air filters and containing sterile soil extract suspension medium (SESM; 5 ml extract from autoclaved Signy Island soil, 10 mg KNO_3, 1 mg K_2HPO_4 and 1 mg $MgSO_3.7H_2O$ L^{-1}). Bacteria from the lake were co-isolated into SESM. Cultures were transferred to the UK, kept at 4 ºC and subcultured every 28 d into fresh sterile SESM.

EXPERIMENT 1: FOOD WEB RESPONSES TO WARMING

Inoculates of each flagellate species were pre-grown at 4 °C for 10 d in 25 ml SESM and then for a further 10 d in 300 ml of SESM. A flagellate-free inoculum of all bacterial strains growing in the initial isolates was obtained by passing 150 ml of each flagellate culture twice through sterile 1.2 μm cellulose nitrate filters (Whatman Int. Ltd., Maidstone, UK) under low (< 12 cm Mg) vacuum pressure, checking for flagellate contamination, and combining the filtrates. It was necessary to use a combined bacterial inoculum in order to ensure that the initial bacterial community was identical between treatments. The bacterial inoculum was distributed evenly under a sterile hood amongst 32

106 microcosms, consisting of 1 L capacity sterile glass jars vented with 0.2 μm air filters (Pall Gelman,
107 Ann Arbor, MI, USA), each containing filtered (Whatman GF/C) sterile SESM (600 ml). This gave
108 an initial bacterial density of *c*. 0.4×10^6 cells ml^{-1}. A uniform community biovolume (2.1×10^3
109 μm^3 ml^{-1}) of flagellates was then inoculated into 24 of the microcosms, with eight microcosms each
110 receiving a mixture of six, four or two flagellate species. Six, four and two species mixtures hence
111 received respectively 0.350, 0.525 and 1.050×10^3 μm^3 ml^{-1} of each individual flagellate species.
112 Eight microcosms did not receive flagellates. Flagellates for inoculation into the four and two
113 species mixtures were selected randomly from the six species pool. This took into account the view
114 of Huston (1997) that increasingly depauperate subsets of high diversity treatments should not be
115 identical. The biovolume of each flagellate species to be inoculated into the microcosms was
116 calculated from the product of the abundances of cells in the inoculates and the mean volume of live
117 cells of each species. Cell abundances were measured several hours before inoculation under
118 epifluorescence at 1000 × magnification after fixation (1.5% glutaraldehyde solution), staining with
119 the fluorochrome DAPI (4', 6-diamidino-2-phenylindole; 10 μg ml^{-1}; Sigma-Aldrich, Gillingham,
120 UK) and filtration onto 0.2 μm nitrocellulose membranes (Millipore, Bedford, MA, USA). The
121 mean (n = 20) volumes of individual live cells were estimated using standard geometric formulae.
122 Those of *B. saliens*, *R. nasuta*, *S. putida, B. saltans*, *G. truncata* and *S. uvella* were estimated to be
123 17, 18, 22, 27, 58 and 124 μm^3, respectively.
124 The 32 microcosms were placed into a purpose-built system consisting of two sets of four
125 open-topped coolant baths, each with internal dimensions of 500 × 135 × 95 mm (l × w × d), with
126 each set attached by PVC braided tubing to one of two 400 W thermocirculators (RC 400G, Grant
127 Instruments Ltd., Shepreth, Cambridge, UK) fitted with eight bit set point programmers. The
128 microcosm bases were submerged to a depth of 85 mm in an ethanediol solution (7.5%) in the
129 coolant baths. Four each of the six, four, two and zero flagellate species microcosms were held at 4
130 ºC for the duration of the experiment, and the remainder were held at 4 ºC for 1 h to equilibrate,

131 before warming to 8 ºC over the following 36 h (at 0.11 ºC h^{-1}), at which temperature they were
132 held for the remainder of the experiment. Sampling took place at 0, 36, 108, 180 and 252 h after the
133 1 h equilibration period. At each sampling, SESM (40 ml) was removed from each microcosm under
134 a sterile hood, 25 ml was fixed with 1.5% glutaraldehyde and 15 ml was frozen for further analyses.
135 Fresh SESM (40 ml) was added to each microcosm after sampling. At the end of the experiment, the
136 remaining SESM was also filtered (Whatman GF/C) and analysed for concentrations of NH_4-N and
137 NO_3-N, using colorimetry (indophenol blue reaction, modular Skalar, Breda, Netherlands) and ion
138 chromatography (DX100, Dionex Corp., Sunnyvale, CA, USA), respectively. Fixed samples were
139 stained with DAPI solution, as above, and the abundances of each flagellate species and of total
140 bacteria were measured under epifluorescence at 1000 × magnification. There were sufficient
141 morphological differences between flagellate species to distinguish each species accurately. Total
142 flagellate volumes were then estimated as above. At 252 h, 1 ml of SESM from each microcosm was
143 inoculated into multiwell tissue culture plates containing sterile SESM. After 10 d the plates were
144 checked at 1000 × magnification for the presence of each flagellate species. All species that were
145 inoculated were found to be present and no flagellates were observed in the zero species treatment.
146
147 EXPERIMENT 2: SINGLE SPECIES' RESPONSES TO WARMING
148 The responses of the six flagellate species to warming were examined in single species culture
149 under food-saturated conditions. Each species was pre-grown at 4 ºC, first for 10 d in 25 ml of SESM
150 and then for a further 10 d in 300 ml of SESM. Subsequently, each species was inoculated into six
151 microcosms containing 200 ml of SESM at an initial biovolume of 2.1×10^3 μm^3 ml^{-1}. Three of the
152 microcosms inoculated with each species were placed into one set of coolant baths in the
153 experimental system described above, and the remainder were placed in the other set of baths. All
154 microcosms were equilibrated for 1 h at 4 ºC. Over the following 36 h, the temperature of the
155 coolant circulating in one set of baths was raised to 8 ºC. That of the coolant in the other set

156 remained at 4 ºC. At 24, 72, 120 and 192 h after the medium had reached its target temperature,
157 cells were sampled, fixed and counted as described above to estimate abundances. In order to avoid
158 food limitation of flagellate population growth, mixed bacteria isolated from the flagellate cultures
159 were added to culture flasks when bacterial abundances, checked regularly with a haemocytometer,
160 fell below *c.* 10^8 cells ml^{-1}. Data were used to calculate logarithmic growth rates (μ, cells d^{-1}), using
161 the following formula, applied to cell abundances between each of the four samplings:
162
163 $\mu = (\ln N_2 - \ln N_1) / \Delta t$
164
165 , where N_2 and N_1 were cell densities at different sampling times and Δt was time (d) between
166 samplings.
167

168 **Results**

169 There was a significant main effect of time on flagellate community biovolume over the course of
170 experiment 1 (Table 1): the volume of flagellate communities increased between 36 and 252 h (Fig.
171 1). Main effects of species loss and warming were also recorded on this variate over the course of
172 the experiment (Table 1): species loss reduced flagellate biovolume accumulation while warming
173 increased it (Fig. 1). At the end of experiment 1, there were significant main effects of species loss
174 and warming on flagellate community biovolume (Table 1): total flagellate biovolume in warmed
175 mixtures at 252 h was higher than that in control mixtures, and was lower in mixtures inoculated
176 with fewer species (Fig. 1).

177 *Bodo saltans* and *Spumella putida*, and to a lesser extent *Spongomonas uvella*, contributed
178 substantially to flagellate community biovolume in warmed microcosms in experiment 1 (Fig. 2).
179 The population biovolumes of the former two flagellates were increased by warming in six species
180 mixtures. In control and warmed mixtures of six species, the respective mean biovolumes (10^6 ×
181 μm^3 ml^{-1}) ± S.E.M. of *B. saltans* were 0.18 ± 0.02 and 0.52 ± 0.10 (one-way ANOVA: $F_{1,6} = 10.3$,
182 $P<0.05$) and those of *S. putida* were 0.11 ± 0.04 and 2.19 ± 0.43 ($F_{1,6} = 22.3$, $P<0.01$). The
183 population biovolume of one other species, *Rhynchomonas nasuta*, was also increased by warming:
184 the mean biovolumes (10^6 × μm^3 ml^{-1}) ± S.E.M. of this species were 0.01 ± 0.01 and 0.11 ± 0.01 in
185 control and warmed six species mixtures, respectively ($F_{1,6} = 113.8$, $P<0.001$). That of *S. uvella*
186 remained unaffected by warming ($F_{1,6} = 2.78$, $P>0.1$). Analyses of logarithmic growth rates under
187 food-saturated conditions in experiment 2 in part corroborated the data from the food web
188 experiment: *B. saltans* and *S. putida* exhibited the highest logarithmic growth rates of the six
189 flagellate species at both 4 ºC and 8 ºC (Table 2). However, at both temperatures the growth rates of
190 *R. nasuta* were the lowest of all the species used in the experiment and those of *S. uvella* were
191 intermediate (Table 2).

A comparison of the growth rates of individual flagellates in mixtures of different numbers of species in experiment 1 indicated that two species grew less rapidly when fewer flagellate species were present. There was only a limited number of species for which we could test for such effects, owing to the random species loss design employed in the experiment. However, at 4 ºC, the logarithmic growth rates of *B. saltans* and *S. uvella* were lower in more depauperate mixtures. In four and six species mixtures, the mean logarithmic growth rates ± S.E.M. of *B. saltans* were 0.90 ± 0.02 and 1.03 ± 0.03 (one-way ANOVA: $F_{1,5} = 7.80$, $P<0.05$) and those of *S. uvella* were 1.10 ± 0.03 and 1.26 ± 0.02 ($F_{1,5} = 8.21$, $P<0.05$), respectively.

There were significant interactive effects of species loss and warming on bacterial abundance (Table 1). Between 36 and 252 h and at 252 h, mixtures that had been inoculated with six and four species of flagellates contained fewer bacteria than those inoculated with two and zero species, but only when microcosms had been warmed (Fig. 3). The interaction term was significant at 252 h when data for zero species mixtures were eliminated from analyses (one-way ANOVA: $F_{2,15} = 5.2$, $P = 0.017$): there were 75% fewer bacterial cells in mixtures inoculated with six and four species of flagellates, compared with those inoculated with two species, but only when mixtures had been warmed (Fig. 3). A main effect of time was also recorded on bacterial abundance over the course of experiment 1 (Table 1), owing to bacterial growth between 36 and 252 h (Fig. 3). At 252 h, there were significant main effects of warming and species loss on bacterial abundance (Table 1): numbers of bacteria were lower in warmed microcosms and were generally higher in mixtures inoculated with fewer species (Fig. 3). Warming increased bacterial abundance in mixtures not inoculated with flagellates at 252 h (control = 17.7×10^6 cells ml^{-1}; warmed = 22.4×10^6 cells ml^{-1}, one-way ANOVA: $F_{1,6} = 7.14$, $P<0.05$).

Nitrate concentrations in SESM were unaffected by species loss and warming at 252 h in experiment 1 (data not shown). However, there was a highly significant interactive effect of species loss and warming on ammonium concentrations (two-way ANOVA: $F_{3,24} = 44.48$, $P<0.001$):

concentrations of the ion were more than halved in SESM that had been inoculated with two or zero flagellate species, compared with SESM inoculated with four or six species, but only when the medium had been warmed (Fig. 4). There were also highly significant main effects of species loss and warming on ammonium concentrations in SESM at the final sampling (two-way ANOVA: $F_{3,24}$ = 45.22 and $F_{1,24}$ = 186.78, respectively, both P<0.001): species loss reduced NH_4-N concentrations but warming increased concentrations of the ion (Fig. 4).

Discussion

Our study recorded significant interactive effects of warming and flagellate species loss on bacterial abundances and ammonium concentrations in model Antarctic microbial food webs. These interactions indicated that the loss of consumer species from the food webs altered their responses to the warming treatment. Our data hence broadly suggest that the response to environmental change of food webs or communities that have undergone the loss of species may be different to that of food webs or communities with a full complement of species. A similar conclusion was reached by Reich *et al.* (2001), who showed that plant biomass accumulation in response to elevated CO_2 or nitrogen addition was greater in species-rich than in species-poor assemblages. In a remarkably similar response to that recorded in our study, this accumulation of plant biomass was owing to the enhanced growth of four out of 16 plant species in response to the CO_2 or nitrogen treatment (Reich *et al.* 2001). Similarly, reducing the number of species present in synthetically-constructed microbial food webs altered the response of bacterivore biomass to a long-term warming treatment, with increased biomass of consumers in warmed species-rich food webs, but unpredictable responses to warming at low diversity (Petchey *et al.* 1999).

Two mechanisms apparently accounted for the effects observed in our study: these were the sampling (or selection probability) effect (Huston 1997) and a niche (or complementarity) effect (Loreau *et al.* 2001). The sampling effect, which diminishes the productivity of depauperate synthetically-constructed communities because they have a reduced probability of containing a productive species, appears to have had a strong influence of the outcome of the experiment. The interactive effects of warming and species loss on bacterial abundance and ammonium concentration were apparently largely owing to the absence of two species that exhibited rapid growth rates (*Bodo saltans* and *Spumella putida*) from replicates of the warmed two species mixtures. *B. saltans* and *S. putida* respectively were present in three and four replicates of the warmed four species mixtures, but were present in only two and one of the warmed two species

mixtures (Fig. 2). The absence of these species from most replicates of the latter mixtures apparently had subsequent effects on bacterial abundance, which remained higher in warmed two, compared with four and six, species mixtures, most probably because of the absence of grazing pressure exerted by the two flagellate species. Furthermore, ammonium concentration in SESM was lower in warmed species-poor mixtures, apparently because of diminished excretion of the ion by the flagellates into the growth medium (cf. Güde 1985). The influence of warming on the metabolism of two consumer species thus appears to have had wide effects on food web functioning, as predicted by metabolic theory (Brown *et al*. 2004). Although it has been widely reported in the literature that the productivity of depauperate mixtures of species is diminished (e.g. Naeem *et al*. 1995; Tilman *et al*. 1996; van der Heijden *et al*. 1998), apparently largely because of the sampling effect (Wardle 1999), our data indicate that the sampling effect can also have a significant influence on the outcome of experiments by interacting with an applied treatment, in this case warming.

There has been considerable debate in the literature about whether the sampling effect is relevant in the natural environment or not. The argument against the effect occurring in nature is that species are rarely lost randomly from ecosystems because extinction occurs in a defined order as a consequence of, for example, species' differences in body size, trophic position or sensitivity to stress (McKinney 1997; Solan *et al*. 2004). However, while Wardle (1999) and Leps *et al.* (2001) argue that the sampling effect is purely an experimental artefact, other workers cite scenarios in which species may be lost randomly from ecosystems. These authors point out that real and experimental random species loss patterns may be similar, particularly in fragmented or isolated habitats subjected to extreme abiotic conditions (Loreau *et al.* 2001). Such scenarios may occur in the Antarctic natural environment: protistan communities on this isolated continent inhabit an extreme abiotic environment and are subjected to significant seasonal and intraseasonal variability (Heywood 1968; Butler 1999a, b; Butler *et al.* 2000). Thus, although we do not know at present

whether the flagellate species used in our study might become extinct in a defined order, limiting our ability to generalise the results to the natural environment, it is possible that random temporal species loss, including the loss of rapidly-growing taxa, occurs in the extreme environment inhabited by these species.

Some ecologists argue that the loss of microbial species cannot occur from natural habitats because of massive local species pools and unlimited dispersal of microbial inocula across geographical boundaries (Finlay, Maberly & Cooper 1997; Finlay 2002). Recent studies in Antarctica have, however, challenged this view. For example, Boenigk *et al*. (2006) found that isolates of *Spumella* from Antarctic habitats, including Signy Island lakes, exhibited lower tolerance of warming (to > 30 ºC) than isolates from four other continents, suggesting geographical isolation and possible endemism within the morphospecies. Furthermore, in a study of eukaryotic microbial diversity in Antarctic soils, Lawley *et al*. (2004) found little overlap between the diversity of small subunit *r*DNA clone libraries between six locations across a 3,350 km transect from Signy Island to the La Gorce Mountains (86º 30' S, 147º 00' W). Both of these studies suggest that the dispersal of microbes within, and to, geographically-isolated Antarctic ecosystems may not readily occur, corroborating the view that endemism may be present in isolated microbial populations inhabiting extreme environments (Papke & Ward 2004). Microbes lost from such environments may hence not be immediately replaced.

In addition to the sampling effect, a further, and more subtle, main effect of species loss on community biovolume in our study was caused by the slower growth rates of two species, *B. saltans* and *Spongomonas uvella,* in more depauperate mixtures of flagellates. At present the mechanism responsible for this effect remains unclear. However, we hypothesize that by reducing the number of consumer species, we would have decreased trophic diversity by reducing the number of feeding modes and degrees of surface association of the flagellate community. Its grazing efficiency on a diverse bacterial prey community would hence have been diminished, and

299 mechanisms used by bacteria to avoid grazing, such as changes in morphology, would have become
300 more efficient at decreased consumer diversity (Pernthaler *et al.* 1996; Simek *et al.* 1999). In a
301 bottom-up controlled situation, this could have removed parts of the bacterial community from the
302 growth-limiting food resource pool available to bacterivores and hence diminished the growth of
303 flagellate species in the more depauperate mixtures. Niche differentiation, or complementarity
304 (Loreau *et al.* 2001), may therefore have played a role in determining the grazing impact of the
305 flagellate species on their resources.

306 The present study used one fifth of the 30 heterotrophic flagellate species known to occur in
307 the plankton of Sombre Lake (Butler 1999a; T. Garstecki, unpubl. data), and thus allowed more
308 accurate predictions about the effects of species loss and environmental change on this habitat than
309 the study of more diverse ecosystems from lower latitudes. Bacterial abundance, an important factor
310 limiting the productivity of heterotrophic flagellates in Sombre Lake, was similar in SESM in the
311 current study (0.4 - 22 × 10^6 cells ml^{-1}) to that recorded in the lake (2 – 16 × 10^6 cells ml^{-1}; Butler
312 1999a). However we are cautious about extrapolating from our data. The microcosms used in the
313 present study were a closed batch system and, because of this, our experiment was conducted over a
314 short time scale. The experimental communities hence did not reach equilibrium, limiting the
315 conclusions that could be drawn from the data. We also applied a rapid change in temperature, to
316 several degrees above that recorded in the lake, to the microbial food webs, which, although
317 frequently occurring at soil surfaces in Antarctic habitats (Smith 1996), is not experienced by
318 microbes inhabiting Antarctic lakes. A more realistic treatment would therefore be to grow
319 flagellates under food-saturated conditions and to raise temperature at a slower rate, enabling
320 experiments over longer time scales (*c.f.* Petchey *et al.* 1999). This, together with experiments
321 examining the influence of elevated UV-B radiation on food webs at different levels of species
322 richness, will be foci for further studies. However, it is clear from the present study that reducing
323 the number of consumer species in model microbial food webs alters their responses to rapid

environmental warming, and that interspecific differences in responses to warming, as well as complementarity, are likely to explain most of the observed effects.

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443 **Figure legends**

444 **Fig. 1.** Log_{10} flagellate community biovolume (BV) in control and warmed mixtures of two, four
445 and six flagellate species over the course of experiment 1. Values are means of four replicates. Error
446 bars have been omitted for clarity.

447

448 **Fig. 2.** Biovolumes of flagellate species in control (top row) and warmed (bottom row) mixtures of
449 two (left column), four (middle column) and six (right column) species at 252 h in experiment 1.
450 Note that the bars for each species in the Figure are stacked in the same order as in the key and that
451 *y*-axes are identically scaled.

452

453 **Fig. 3.** Bacterial abundance in control and warmed mixtures of zero, two, four and six flagellate
454 species over the course of experiment 1. Values are means of four replicates. Bars are LSD.

455

456 **Fig. 4.** Ammonium concentrations in SESM at 252 h in control and warmed mixtures of zero, two,
457 four and six flagellate species in experiment 1. Values are means of four replicates + S.E.M.

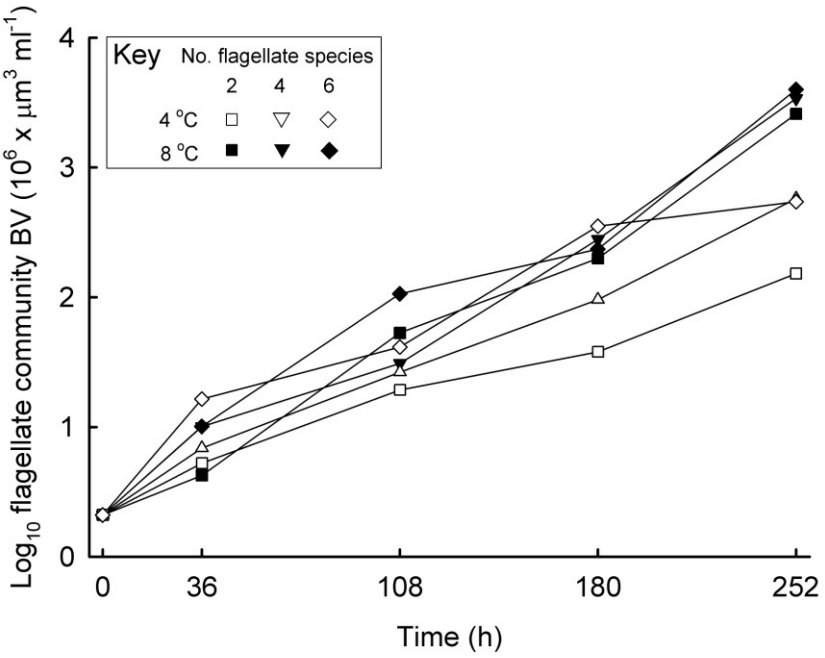
458

Table 1. Main and interactive effects of flagellate species loss and warming, and effects of time, on flagellate community biovolume and bacterial abundance between 36 and 252 h and at 252 h in experiment 1. Repeated measures ANOVA was used to analyse data between 36 and 252 h. All data were $\log_{10}$ transformed prior to analysis. Values for zero species mixtures were eliminated from analyses on flagellate community biovolume.

	Flagellate community biovolume			Bacterial abundance		
	d.f.	*F* ratio	*P* value	*d.f.*	*F* ratio	*P* value
36-252 h						
Time	3,45	229.12	<0.001	3,63	606.04	<0.001
Species loss	2,15	11.98	<0.001	3,21	0.63	0.604
Warming	1,15	23.15	<0.001	1,21	0.50	0.488
Species loss × warming	2,15	0.76	0.483	3,21	7.40	0.002
252 h						
Species loss	2,15	6.30	0.010	3,21	10.39	<0.001
Warming	1,15	54.78	<0.001	1,21	52.03	<0.001
Species loss × warming	2,15	0.40	0.676	3,21	13.20	<0.001

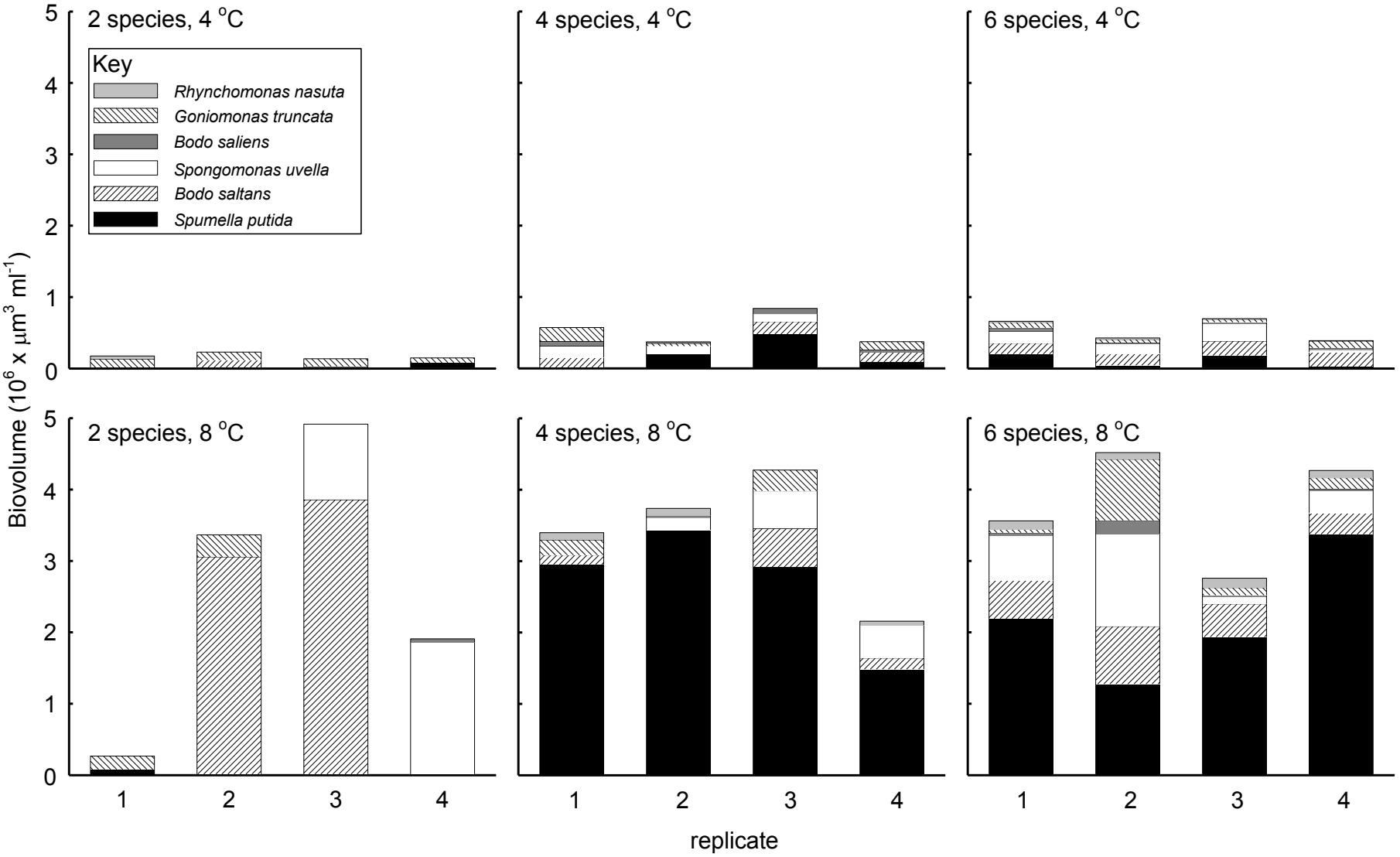
Table 2. Logarithmic growth rates (μ, cells d^{-1}) at 4 ºC and 8 ºC of flagellate species in experiment 2. Data are means of three replicates ± S.E.M. and are the maximum growth rates recorded between each of the four samplings.

Species	Temperature (º C)	
	4	8
Bodo saliens	0.696 ± 0.026	1.053 ± 0.104
Bodo saltans	0.943 ± 0.075	1.267 ± 0.086
Goniomonas truncata	0.656 ± 0.032	0.813 ± 0.056
Rhynchomonas nasuta	0.237 ± 0.033	0.357 ± 0.027
Spongomonas uvella	0.667 ± 0.165	0.860 ± 0.145
Spumella putida	0.873 ± 0.084	1.313 ± 0.050


Key
No. flagellate species
2
4
6
4 °C
8 °C
Log10 flagellate community BV (10^6 x µm^3 ml^-1)
0
1
2
3
4
0
36
108
180
252
Time (h)

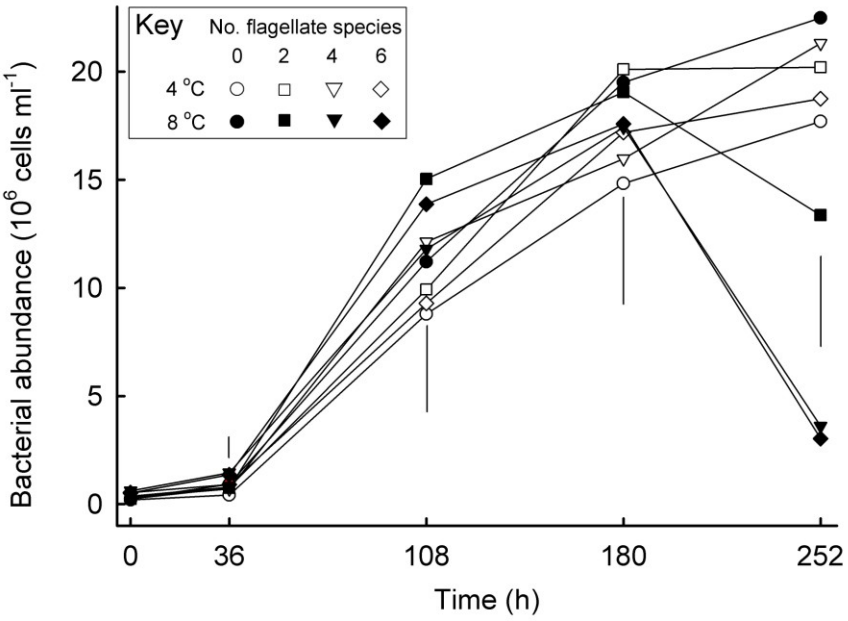
471

472 Fig. 1


2 species, 4 °C
4 species, 4 °C
6 species, 4 °C
2 species, 8 °C
4 species, 8 °C
6 species, 8 °C
Key
Rhynchomonas nasuta
Goniomonas truncata
Bodo saliens
Spongomonas uvella
Bodo saltans
Spumella putida
Biovolume (10^6 x µm^3 ml^-1)
0
1
2
3
4
5
1
2
3
4
replicate

473

474 Fig. 2



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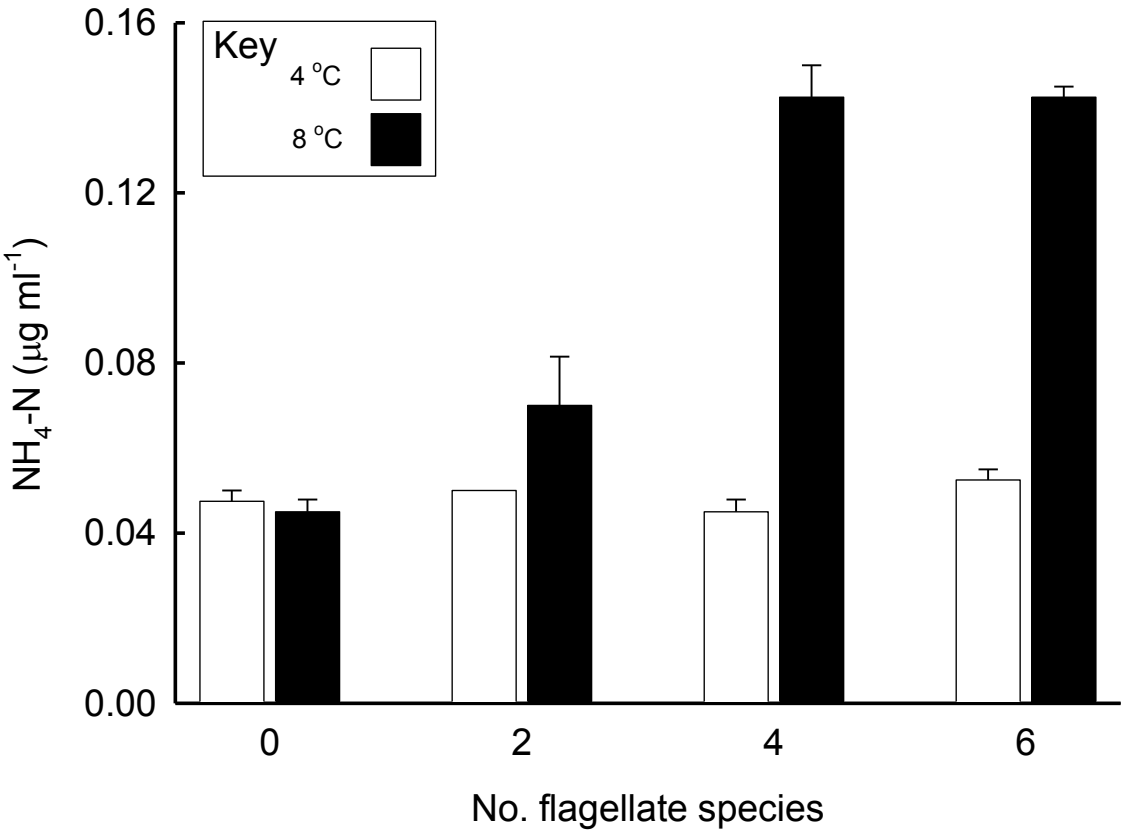
477 Fig. 3


Key
4 °C
8 °C
0.16
0.12
0.08
0.04
0.00
NH4-N (µg ml-1)
0
2
4
6
No. flagellate species

478

479 Fig. 4