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1 # Microbial grazers may aid in controlling infections caused by aquatic
2 # zoosporic fungi

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22 **Abstract**

23 Free-living eukaryotic microbes may reduce animal diseases. We evaluated the dynamics by
24 which micrograzers (primarily protozoa) apply top-down control on the chytrid
25 *Batrachochytrium dendrobatidis* (*Bd*) a devastating, panzootic pathogen of amphibians.
26 Although micrograzers consumed zoospores (~3 µm), the dispersal stage of chytrids, not all
27 species grew monoxenically on zoospores. However, the ubiquitous ciliate *Tetrahymena*
28 *pyriformis*, which likely co-occurs with *Bd*, grew at near its maximum rate ($r = 1.7$ d^{-1}). A
29 functional response (ingestion vs. prey abundance) for *T. pyriformis*, measured using spore-
30 surrogates (microspheres) revealed maximum ingestion (I_{max}) of 1.63×10^3 zoospores d^{-1},
31 with a half saturation constant (k) of 5.75×10^3 zoospores ml^{-1}. Using these growth and
32 grazing data we developed and assessed a population model that incorporated chytrid-host
33 and micrograzer dynamics. Simulations using our data and realistic parameters obtained
34 from the literature suggested that micrograzers could control *Bd* and potentially prevent
35 chytridiomycosis (defined as 10^4 sporangia $host^{-1}$). However, simulated inferior micrograzers
36 ($0.7 \times I_{max}$ and $1.5 \times k$) did not prevent chytridiomycosis, although they ultimately reduced
37 pathogen abundance to below levels resulting in disease. These findings indicate how
38 micrograzer responses can be applied when modelling disease dynamics for *Bd* and other
39 zoosporic fungi.

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41 **Introduction**

42 Although the traditional microbial food web (i.e. prokaryotes and protists, *sensu* Azam et
43 al. [1] is well-established as a driver of aquatic productivity [2], micro-fungi are only now
44 being appreciated as integral aquatic microbes. A dominant group of micro-fungi, the
45 chytrids, are parasites of phytoplankton, zooplankton, and vertebrates [3], and zoospores, the
46 chytrid dispersal stage, are argued to be nutritious and of an appropriate size for protozoan
47 grazers [2, 3]. Hence, through top-down control micrograzers within the microbial food web
48 have the potential to reduce the likelihood or severity of, or even prevent, disease outbreaks
49 caused by these pathogens [3-5]. Here, by developing and parameterizing a population
50 model we explore the dynamics by which microbial grazers may control the chytrid
51 *Batrachochytrium dendrobatidis*, a panzootic pathogen of amphibians that is argued to have
52 caused the greatest loss of biodiversity attributed to any disease [6].

53 *Batrachochytrium dendrobatidis* (henceforth, *Bd*) infects amphibian hosts through the
54 dispersal of motile, 3-5 µm zoospores (Fig. 1). The environmental pool of zoospores is
55 instrumental in driving infection dynamics, as these can accrue in a dose-dependent manner
56 [7], with for some hosts the size of the zoospore pool influencing long-term consequences for
57 population survival or extinction [8]. It follows that processes that reduce the zoospore-pool
58 should reduce the probability and intensity of disease outbreaks. Consumption of zoospores
59 by naturally occurring micrograzers has been suggested to result in losses sufficient to reduce
60 infections. Experiments show that some micrograzers may reduce the likelihood of *Bd*
61 infections, and field data indicate a negative relationship between potential-grazer abundance
62 and both the prevalence of infection and host mortality from disease [9-12]. There is now a
63 need to build on these observations and investigate in more depth the dynamics by which
64 consumers may impact on *Bd*.

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65 Most work to date on the consumption of *Bd* zoospores has focused on large
66 zooplankton, especially cladocerans [10, 13-15]. However, experiments on cladocerans have
67 used unrealistically high micrograzer abundances (>10-100 times higher than natural levels),
68 and at natural levels large zooplankton seem to have little impact on *Bd* infections [16].
69 Micrograzers, in contrast, are abundant in shallow waters and are often near the bottom of
70 ponds where infected hosts (e.g. benthic, grazing tadpoles) spend time resting and grazing on
71 the substrate [17-19]. Furthermore, as many protozoa have generation times on the order of
72 hours, by reproducing asexually when zoospores are abundant, micrograzer populations may
73 increase several fold, consuming zoospores as they are released from the host. We, therefore,
74 argue that protozoa will be more important than cladocerans in reducing the abundance of
75 chytrid zoospores. This is supported by Schmeller et al. [12] who, using mesocosms, showed
76 the ciliate *Paramecium* can significantly reduce the number of hosts infected with *Bd* by up
77 to 65% when it is introduced at naturally occurring abundances.

78 We also suggest that the main impact of micrograzers on *Bd* spore-load will be in the
79 water directly surrounding the host, where zoospores will be most abundant. Field studies
80 suggest that in water bodies where *Bd* occurs, zoospore densities in the water column are low,
81 ranging from ~0.5 to 500 L^{-1} [20, 21]. In contrast, zoospores are shed from hosts at up to 250
82 zoospores min^{-1} [22], surviving only ~1-3 days [9]. Additionally, zoospores mostly disperse
83 on the order of 1 cm [23], demonstrating chemotaxis towards keratinised skin over this
84 distance [24, 25]. Although these laboratory-based rates will be dependent on environmental
85 factors such as temperature [9], they suggest that the limited movement and survival of the
86 rapidly produced zoospores will lead to dense aggregations in localized regions around the
87 host. Recognising the likelihood of these local abundances and the well-established density-
88 dependent feeding and growth responses of micrograzers [2], in this study we focused
89 attention on the impact of micrograzers on *Bd* dynamics around the host. We achieved this

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90 by: first, investigating a range of potential micrograzers, determining which survived on a
91 diet of only *Bd* zoospores, and concentrating on those that grew; second, measuring ingestion
92 and growth rates of a common species that thrives on *Bd*; and third, using these data,
93 developing and exploring a model that couples the *Bd* life-cycle with micrograzer-control on
94 zoospores. In doing so, we indicate the dynamics by which micrograzers may reduce *Bd*
95 populations – potentially preventing disease – and provide a mechanism by which chytrid-
96 diseases can be incorporated into microbial food web models.
97

98 **Materials and methods**

99 ***Culture maintenance***

100 *Batrachochytrium dendrobatidis* (*Bd*) cultures (strain #262 IA 9'13, Imperial Collage
101 London) were maintained at 18 °C (at which all experiments were conducted) on H-broth
102 medium (500 mL: 5 g Tryptone and 16 g glucose) and were regularly transferred (every ~5 d)
103 to maintain exponential growth. Bacterial growth was prevented by the addition of
104 antibiotics (Ampicillin at 100 μg ml^{-1}; Kanamycin at 50 μg ml^{-1}; Chloramphenicol at 34 μg
105 ml^{-1}). Micrograzers were obtained from Sciento (Manchester, UK): the ciliates *Blepharisma*
106 sp., *Oxytrichia* sp., *Paramecium aurelia*, *Paramecium caudatum*, *Stentor coeruleus,*
107 *Tetrahymena pyriformis*, and *Urocentrum turbo* and the rotifers *Brachionus calcyciflorous*
108 and *Philodina* sp. *Tetrahymena pyriformis* was maintained axenically for extended periods
109 on H-broth. All other species were maintained prior to experiments on a natural assemblage
110 of bacteria in Chalkley's medium enriched with cereal grains, as provided by Sciento [26].

111 ***Assessing growth of micrograzer species on* Bd *zoospores***

112 We tested the hypothesis that *Bd* zoospores were of nutritional benefit to the
113 micrograzer. To do so, we compared growth with zoospores to when no food was available.

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114 We also compared growth on zoospores to maximal rate of growth of the micrograzers,
115 obtained from literature estimates (Fig 2).

116 Prior to introducing micrograzers, *Bd* was isolated from its growth medium to ensure that
117 the medium was not a source of nutrients for the micrograzers. To do so, a *Bd* suspension (in
118 exponential phase) was centrifuged (50 ml tubes, 5000 rpm, 6 min), the supernatant removed,
119 and the pellet resuspended in autoclaved Volvic® mineral water to a concentration > 1.50 x
120 10^5 ml^{-1} (determined microscopically). Bacterial growth was prevented with antibiotics, as
121 above.

122 To assess growth rate, we followed our previous methods [27]. Micrograzers (9 species)
123 were passed 5 times through autoclaved Volvic® water to remove bacteria. Then 5 to 8
124 individuals, dependent on micrograzer size, were added to a 10 ml well containing the *Bd*
125 suspension. Parallel treatments containing only sterile Volvic® water were used to assess
126 mortality rate in the absence of prey. All treatments (i.e. species incubations with or without
127 *Bd*) were replicated in triplicate (i.e. three 10 ml wells). To assess growth rate (r, d^{-1}), the
128 number of gazers in each well was determined microscopically after 2 or 3 days (depending
129 on the observed change in abundance). Then, new *Bd* suspensions were prepared (as above),
130 and micrograzers were transferred to these, maintaining *Bd* abundance. If micrograzer
131 numbers decreased (net death occurred) over the incubation, then all individuals were
132 transferred, but if numbers increased (net growth occurred) then the initial number was
133 transferred. This procedure was continued for 14 days or until all micrograzers had died.
134 Cultures were routinely checked to ensure there was no bacterial contamination.

135 When numbers increased between transfers, growth rate (r, d^{-1}) was determined over
136 each incubation period, as $r = ln(N_t/N_0)/t$, where N_0 and N_t are the micrograzer abundance on
137 the initial and final day respectively, and t is the incubation period (2 or 3 days); to determine
138 growth rate across all transfers (up to 14 d), the average of these was obtained. When

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139 micrograzer numbers decreased between transfers, mortality rates ($-r$, d^{-1}) were determined as
140 slope of *ln* numbers over the entire incubation period. To assess if growth (or death) rate
141 differed between treatments (i.e. with or without *Bd*) a two tailed t-test was conducted (α =
142 0.05).

143 ***Quantifying the functional response of* Tetrahymena *grazing on* Bd**

144 Our study focused on *Tetrahymena pyriformis* as: 1) it grew rapidly on *Bd* zoospores (see
145 Results) and therefore clearly consumed and assimilated zoospores; 2) it is a model organism
146 for which there are substantial data (see Discussion), and 3) it is common, globally, in
147 habitats where *Bd* may occur (see Discussion). Prior to determining ingestion rate, *T.*
148 *pyriformis* was acclimated with zoospores for >10 h. To do so, the ciliates were first
149 removed from H-broth by centrifugation (50 ml tubes, 8000 rpm, 8 min) and then
150 resuspended in 10 ml of autoclaved Volvic® water. To obtain only zoospores, a centrifuged
151 *Bd* culture (as above) was filtered through a 5 µm Nitex® mesh. Zoospores were then added
152 to the water containing ciliates, to a total volume of 20 ml (resulting in ~10^6 zoospores ml^{-1}),
153 with antibiotics (as above). This acclimation had no negative effects on the ciliates: after 10
154 h, zoospore abundance had substantially decreased and ciliate abundance increased
155 (indicating the ciliates were feeding and growing), the ciliates behaved similarly to when
156 grown on H-broth (i.e. similar swimming pattern), and their cell size and shape were similar
157 to when grown on H-broth.

158 To determine ingestion rate on spore-sized particles, 3 µm fluorescent polymer
159 microspheres (henceforth beads, Fluoro-Max™, Thermo Fisher Scientific, USA,) acted as a
160 surrogate for *Bd* zoospores which are 3 - 5 µm [28]. Bead concentrations, ~8 x 10^3 ml^{-1} to
161 10^6 ml^{-1} (see Results), were prepared in autoclaved Volvic® water and vortexed prior to use,
162 ensuring mono-dispersion. An aliquot (0.5 ml) of the acclimated ciliate culture (> 30
163 micrograzers) was added to 1 ml of Volvic® water with beads, at various concentrations (with

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164 more measurements at low abundances [29], see Results), and incubated for 5 or 10 min,
165 depending on the bead concentration (preliminary experiments deemed these to be
166 appropriate incubation periods). Incubations were terminated by fixing cells with ethanol
167 (final concertation 70%). The average number of beads ingested per ciliate (>30 cells) was
168 determined via fluorescent microscopy and was subsequently used to determine ingestion rate
169 (I, prey d^{-1}) at each prey concentration.

170 The relationship between ingestion rate and zoospore abundance (Z ml^{-1}), was
171 determined by fitting a Type II functional response to the data: $I = I_{max}*Z/(k + Z)$, where I_{max}
172 (Z min^{-1}) is the maximum ingestion rate and k is the half saturation constant (Z ml^{-1}). The
173 response was fit using the Marquardt-Levenberg algorithm (SigmaPlot, Systat, Germany);
174 this algorithm is appropriate for describing such biological data sets [30].

175 ***Modelling micrograzer impacts on* Bd *populations***

176 To assess the dynamics by which grazing pressure impacts on *Bd* infection populations
177 we developed and applied the following model that couples a reduced version of the *Bd*-load
178 model [8] with the Rosenzweig-MacArthur predator-prey model [31]. Data for *T. pyriformis*
179 were used to represent micrograzers (see the Discussion for a justification to focus on this
180 species). Following logic outlined in the Introduction, the model describes the infection load
181 on a single host and, as a proxy for the waters surrounding the host, only considers a volume
182 of 10 ml around that host, where zoospores and micrograzers reside. As a metric to predict
183 chytridiomycosis, it assumes that a sporangia load of 10^4 per host results in host mortality [8],
184 with the recognition that this will vary between hosts and *Bd* strains [32, 33]. It does not
185 include reduction of spore numbers by emigration as zoospores are unlikely to disperse far
186 before dying [23], and we assume through chemokinesis, micrograzers remain near their food
187 source[34-36]. The model is described by the following equations,
188

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189 $$\frac{dS}{dt} = yvZ - \sigma S \quad (1)$$

190 $$\frac{dZ}{dt} = \eta S - yZ - \mu Z - \frac{I_{max}Z}{k+Z}G \quad (2)$$

191 $$\frac{dG}{dt} = e\frac{I_{max}Z}{k+Z}G - dG(1 - \frac{b}{G}) \quad (3)$$

192 where for Eq. 1 and Eq. 2, *S* is the number of sporangia ml^{-1} (note for per host measurements
193 this value is multiplied by 10); *Z* is the zoospore abundance (ml^{-1}); *y* is the per capita spore-
194 host encounter rate; *v* is the fractional likelihood of spore infection per encounter; *σ* is the per
195 capita sporangia mortality rate; *η* is the per sporangia spore-release rate; and *μ* is the per
196 capita spore mortality rate (see Table 1).

197 Then, Eq. 2 and Eq. 3 were coupled to include spore loss by micrograzers (*G*), where
198 grazing (*I*) is dictated by the functional response (see *Tetrahymena pyriformis* ingestion,
199 above); *e* is the abundance-based conversion efficiency, determined assuming a biomass-
200 based estimate of *e* is ~0.1 [37] and biovolumes of *Bd* zoospores and *T. pyriformis*; and *d* is
201 the micrograzer per capita death rate. We assume here that *Bd* zoospores are not the only
202 potential food source for the micrograzers, and so incorporate a minimum micrograzer
203 abundance (*b*) that implicitly assumes that in the absence of zoospores the micrograzer
204 population is maintained by the presence of other potential food sources; hence we model
205 potential increases in micrograzer abundance over and above this minimum, dependent on
206 consumption of *Bd* zoospores. Estimates of *d* and *b* are based on our unpublished
207 observations. Table 1 summarises the above parameters and the estimates used.

208 All model runs (100 d) were initiated with 10 sporangia $host^{-1}$ (1 sporangium ml^{-1}), 100
209 zoospores ml^{-1}, and 1 micrograzer ml^{-1} (again assumed to be the minimal population size,
210 maintained by other resources in the environment). For *Bd*, we applied parameter values that
211 were within the range explored by Briggs *et al.* [8] (Table 1).

212 We first performed simulations to assess the ability of *T. pyriformis* to control *Bd*. Then,
213 we assessed the extent to which micrograzers that are inferior to *T. pyriformis* could control

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214 *Bd*, through exploration of micrograzer parameter space. Inferior micrograzers had reduced
215 maximum ingestion rate (up to 0.5 × I_{max} of *T. pyriformis*) and increased half saturation
216 constant (up to 2 × *k* of *T. pyriformis*); see Fig. 4 for an indication of the range of these
217 responses. To quantify the impact of micrograzers on *Bd*, we examined the maximum
218 abundance (over the 100 days) and the abundances at 50 and 100 days of *S*, *Z*, and *G*.
219

220 **Results**

221 ***Assessing growth of micrograzer species on*** **Bd**

222 All of the micrograzers can be maintained in laboratory cultures, with maximum growth
223 rates ranging from ~0.4 to 2 d^{-1} (Fig 2), and all died when maintained on water alone,
224 indicating their relative mortality rates when starved (Fig. 2). When fed *Bd*, micrograzers
225 exhibited four distinct responses (Fig. 2): 1) for the ciliate *Stentor coeruleus* the death rate
226 was significantly (and substantially) higher than in water alone; 2) for the ciliates
227 *Urocentrum turbo, Blepharisma* sp., and *Oxytrichia* sp. and the rotifer *Philodina* sp. there
228 was no significant difference between death rate with or without *Bd*; 3) for the rotifer
229 *Brachionus calcyciflorous* growth rate initially increased (i.e. after 48 h) followed by death,
230 and for the ciliates *Paramecium aurelia* and *P. caudatum* (Fig. 3) the growth rate was
231 initially positive when *Bd* was present followed by a negative growth rate as time progressed
232 – on average over the incubation *P. aurelia* exhibited negative growth while *P. caudatum*
233 exhibited zero growth (Fig. 3); and 4) for the ciliate *Tetrahymena pyriformis* there was a
234 sustained positive growth rate (Fig. 2), which was significantly higher than the negative
235 growth rate on water alone; this growth rate of ~1.7 ± 0.23 (SE) d^{-1} was equal to that when
236 the ciliate was grown axenically on H-broth (unpublished data) and near its maximum rate
237 under any conditions.

238 ***Quantifying the functional response of*** **Tetrahymena** ***grazing on*** **Bd**

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239 As *T. pyriformis* grew on zoospores alone it was clear that this ciliate ingested *Bd*
240 zoospores. Zoospores were also observed in *T. pyriformis,* in the food vacuoles of the ciliate,
241 under 40 x magnification. Ingestion rate followed a typical Type II (rectangular hyperbolic)
242 functional response (Fig. 4, adjusted $R^2 = 0.82$), with $I_{max} = 1.63 \times 10^3 \pm 98$ (SE) prey d^{-1} and
243 $k = 5.75 \times 10^3 \pm 1.38 \times 10^3$ (SE) prey ml^{-1}.

244 ***Modelling micrograzer impacts on* Bd *populations***

245 Control of *Bd* occurred when parameters for the micrograzer (*T. pyriformis*) were
246 included in the model (Fig. 5). In the absence of the micrograzer, sporangia per host reached
247 lethal levels (>10^4 $host^{-1}$ [8]) by ~55 days (Fig. 5a). However, when micrograzers were
248 included their population rose from 1 to ~35 ml^{-1}, with the result that sporangia were limited
249 to a maximum of 3×10^3 per host (i.e. based on the assumption that 10^4 sporangia is a lethal
250 limit, the host would survive), and *Bd* was virtually eradicated by 100 days (Fig. 5b).
251 We then assessed the ability of micrograzers that were inferior to *T. pyriformis* to control
252 *Bd*, through exploration of micrograzer parameter space: i.e. up to twice the half saturation
253 (k) and half the maximum ingestion rate (I_{max}) of *T. pyriformis* (Fig. 4). Fig. 5c illustrates
254 population dynamics when the most inferior micrograzer was included (highest half-
255 saturation constant and lowest maximum ingestion rate): the general pattern remained similar
256 to that when *T. pyriformis* parameters were applied, with the micrograzers controlling *Bd*
257 over 100 d, but the abundance of zoospores, sporangia, and micrograzers were more than 10
258 times greater than the simulation including *T. pyriformis*, leading to predicted host death at
259 ~55 days and a peak in abundance at ~70 days.
260 We then examined the pattern of the temporal dynamics across a wider range of
261 parameter space (representing a range of predators-types) by reporting the maximum
262 abundance and the abundances at 50 and 100 days of zoospores, sporangia, and micrograzers.
263 Across all parameters explored, the micrograzer population provided top-down control of *Bd*,

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264 as over the entire range *Bd* was virtually eradicated by 100 days (Fig. 6 c, f). However, the
265 quantitative levels and rates of control varied considerably with micrograzer efficiency: with
266 reduced I_{max} and increased k, zoospores and sporangia reached higher maximum abundances
267 (Fig. 6a, d) and persisted longer (Fig. 6b,e), indicating a decrease in the control of *Bd*. In
268 particular, micrograzers with < 0.7 I_{max} (~10^3 prey d^{-1}) and >1.5 x k (~9 x 10^4 ml^{-1}) were not
269 capable of preventing sporangia per host exceeding lethal levels of 10^4 per host (the yellow-
270 to-red region on Fig. 6 d). Decreased I_{max} and increased k also led to increases in micrograzer
271 abundance (Fig 6. g-i), in response to the increased spore levels available under these grazing
272 regimes.
273

274 **Discussion**

275 Control of a wide range of diseases caused by zoosporic fungi may be achieved through
276 consumption on aquatic, motile zoospores by micrograzers [3-5]. Here, we explore the
277 dynamics by which micrograzers may play a pivotal role in controlling the devastating
278 amphibian disease chytridiomycosis, caused by the micro-fungus *Batrachochytrium*
279 *dendrobatidis* (*Bd*). Previously, it has been shown that several micrograzers may consume
280 *Bd* zoospores [12]. We expand on this by first indicating that some protozoa can also grow
281 on *Bd* zoospores for short periods, and that the ubiquitous ciliate *Tetrahymena pyriformis*
282 grows at near maximal rates on zoospores alone for extended periods. Recognising that
283 micrograzers will grow on *Bd*, provides essential information for modelling population
284 dynamics. We then provide an assessment of the functional response of *T. pyriformis* feeding
285 on spore-sized prey, also required for establishing a population model. Finally, using our
286 data and literature estimates, we develop and employ a novel model that couples the *Bd* life
287 cycle with micrograzer-control on zoospores. This synthesis reveals the dynamics by which

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288 micrograzers may supress *Bd* loads and argues that for theoretical predictions of *Bd*-host
289 interactions it will be useful to consider embedding these into the larger microbial food web.

290 ***Micrograzer growth on* Bd**

291 Previous work has suggested that a large range of micrograzers will consume *Bd*
292 zoospores and may reduce *Bd* viability[12]. We have extended this observation by assessing
293 if *Bd* provides nutritional benefits, that support micrograzer growth. All of the micrograzers
294 we examined could ingest *Bd*, but they exhibited a range of growth-responses. For one
295 ciliate, *S. coeruleus*, *Bd* appeared to be toxic (possibly explaining previous reports that *S.*
296 *coeruleus* does not reduce *Bd* viability;[12]), while other species seemed to obtain no
297 nutritional benefit (Fig. 2). However, several species benefited from ingesting *Bd*. Both
298 *Paramecium aurelia* and *P. caudatum* initially grew, although this was not sustained (Fig. 3),
299 suggesting that while *Bd* is of some value, it may lack essential nutrients for these ciliates. In
300 contrast, *Tetrahymena pyriformis* sustained positive growth, indicating that *Bd* can provide a
301 complete diet for certain species. These observations are supported by previous work on
302 ciliates: *T. pyriformis* and a closely related species, *Colpidium striatum*, also grow on yeast
303 (*Saccharomyces*), while *P. aurelia*, and *P. caudatum* cannot, again possibly due to a lack of
304 nutrients such as essential fatty acids and B-vitamins [38, 39].

305 Our analysis, therefore, suggest that not all micrograzers would be capable of or equally
306 proficient at controlling *Bd*. However, with additional prey sources to sustain the consumers,
307 there may be selective feeding on *Bd*. For instance, *T. pyriformis* differentiates between prey,
308 leading to a more efficient assimilation of prey biomass and a greater cell yield of ciliates
309 [40]. Likely, in the mesocosm experiments conducted by Schmeller et al. [12], where
310 *Paramecium* controlled *Bd*, this ciliate's diet was supplemented by naturally occurring
311 bacteria. In fact, in our initial growth-experiments, where antibiotics were not included,
312 bacteria grew, and *Paramecium caudatum* consumed zoospores in addition to bacteria and

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313 maintained extended positive growth (Supplement 1). Our analysis here has focused on *Bd*
314 as the sole food source and indicates that micrograzer dynamics (growth and ingestion) lead
315 to control of *Bd* populations. These findings argue that *Bd*-host dynamics should now be
316 examined in a wider food-web context, with mixed an assemblage of microgazers sustained
317 by *Bd* and a wider range of natural food sources.

318 ***Tetrahymena grazing on* Bd**

319 Globally, *Tetrahymena* is common in shallow waters, living near sediments, where it
320 consumes bacteria and other microbes [41, 42]. These are the same habitats that *Bd* occupies.
321 *Tetrahymena* is also associated with amphibians where it may be an opportunistic
322 ectoparasite [43, 44], but possibly also a consumer of *Bd* zoospores as they emerge from
323 sporangia. Considering its habitat and ability to rapidly reproduce on *Bd* alone, we focused
324 on *T. pyriformis'* ingestion of *Bd* zoospores. In contradiction to Schmeller et al., attempts to
325 stain *Bd* zoospores with calcofluor-white [12] were not successful; calcofluor stains chitin
326 [45], and although *Bd* sporangia have a chitin wall, zoospores do not [28]. Therefore, this
327 staining method seems inappropriate for *Bd* zoospores. We then explored vital stains (e.g.
328 cell tracker green), but again we were not successful. Consequently, ingestion estimates
329 relied on the uptake of fluorescent beads as surrogates for *Bd*, which may underestimate rates
330 (e.g. [46]). We, therefore, see our predictions as conservative. From our data, a clear Type II
331 functional response was obtained for *T. pyriformis* (Fig. 4), providing essential parameters for
332 modelling *Bd*-micrograzer dynamics (see Methods). To our knowledge, this is the first time a
333 functional response on *Bd* sized particles has been obtained for any *Tetrahymena* species: the
334 estimates of I_{max} and k are within the range of those obtained for other ciliates, although the k-
335 values are on the lower end of the spectrum [39, 47, 48]; our modelling, therefore, includes
336 micrograzers that are inferior to *Tetrahymena*.

337 ***Modelling micrograzer impacts on* Bd *populations***

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Disease control by micrograzers

338 Empirical evidence suggests that *Paramecium* can reduce *Bd* infections, through
339 examining end point estimates of host infection [12]. Here we explore the temporal
340 dynamics of such control and the potential for micrograzers to prevent host death. Our
341 analysis is reductionist and hence more qualitative than quantitative in its predictions.
342 However, it clearly reveals that by applying plausible parameters for both the parasite and
343 micrograzer, in a local environment, chytridiomycosis may at times be prevented and *Bd*
344 virtually eradicated, or at least reduced to negligible levels (Fig. 6). Critically, it suggests the
345 time scales over which such dynamics may occur. Admittedly, we indicate that micrograzers
346 that are inferior to *T. pyriformis* are less likely to prevent host death, yet they still, ultimately,
347 reduce *Bd* populations to negligible numbers, potentially preventing further disease outbreaks
348 (Fig, 6). Our model, therefore, provides a mechanism to evaluate *Bd*-micrograzer dynamics,
349 and its predictions strongly argue for the continued exploration of micrograzers in *Bd*
350 research, specifically, and in the control of a range of diseases that spread through zoospores
351 or other similarly sized dispersal stages [3-5].
352 To date, models of *Bd*-dynamics [8, 9, 49, 50] have not included estimates of spore loss
353 by micrograzers. As indicated above, the modelling provided here is instructive and could
354 benefit from elaboration. Given the ubiquity of protozoa in natural waters [2], and the clear
355 indication of their potential impact (Fig. 6, [3-5]), we suggest there is now a need for better
356 parameterization of micrograzer-*Bd* responses. We suggest three main directions. First,
357 micrograzers, and specifically *Tetrahymena*, exhibit chemosensory behaviour [35]; the extent
358 to which protozoa are attracted to amphibian hosts and *Bd* requires evaluation. Second, as
359 indicated above, the role of *Bd* as a supplement rather than a sole dietary component deserves
360 attention. Finally, the Rosenzweig-MacArthur predator-prey model, which we used, has
361 limitations. Model structures such as the independent response model [51] that rely,
362 independently, on growth and ingestion responses provide better predictions [52]. To this

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363 end, we suggest that both functional (ingestion) and numerical (growth) responses associated
364 with *Bd* abundance are established for a range of micrograzers.

365 ***Future directions for microbial ecology and* Bd**

366 Both *Tetrahymena* and *Paramecium* are common species in shallow waters [41, 42, 53],
367 that, as we have shown, are capable of growth on *Bd* zoospores for limited to extended times.
368 Undoubtedly, other protozoa will also consume and grow on *Bd* zoospores. We, therefore,
369 suggest that the role of micrograzers is considered when evaluating *Bd*-dynamics and the
370 dynamics of other zoosporic diseases. Contributions of micrograzers to disease dynamics are
371 also likely to have a highly site-specific component, due to their dependence on
372 environmental factors [12]. For instance, chytridiomycosis is more prevalent at higher
373 altitudes [34], which will often be both cooler and oligotrophic. While temperature may, in
374 part, determine infection burdens [54], there will likely be an interaction with the trophic
375 status of the water. If in oligotrophic waters bacteria are reduced below levels sufficient to
376 support ciliates ($<10^6$ ml^{-1}), top-down control may be absent, and our analysis suggests that
377 *Bd* may thrive, resulting in chytridiomycosis. Consequently, assessing the abundance of
378 micrograzers in waters where chytridiomycosis occurs or is predicted seems warranted.

379 We end with some speculations on the potential for biomanipulation using micrograzers,
380 as this has been considered by others [12]. Traditional approaches for managing wildlife
381 diseases have proven ineffectual or impractical for *Bd*. Treating amphibians with probiotic
382 bacteria directly has generally been unsuccessful as a conservation strategy (but see [34]),
383 and, while theoretically plausible, culling hosts to below the critical threshold for disease
384 transmission contravenes conservation objectives [55-57]. This means that alternative
385 mitigation and management strategies must be perused. To date, the only successful effort
386 towards in situ *Bd*-mitigation relied on dosing animals with antifungal chemicals alongside
387 applying chemical disinfectants directly to the environment to reduce spore survival [55].

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388 Fungicides and chemical disinfection, however, have shortcomings, not least of which are
389 ethical issues associated with indiscriminate toxic effects. As both *Tetrahymena* and
390 *Paramecium* are already common if not ubiquitous in aquatic environments and are simple
391 and inexpensive to grow in large quantities [42, 53], they may be tractable target species for
392 biomanipulation. We, therefore, support previous suggestions Schmeller et al. [12] that by
393 augmenting natural densities of these species, through addition or supplementary feeding, it
394 may be possible to reduce zoospore densities for *Bd* in situ. However, an introduction or
395 population increase of any species could have detrimental, ecosystem-changing effects that
396 require in depth evaluation before application [58]. Further evaluation of the role of
397 microgazers is, therefore, required before we can understand their likely impact in natural
398 conditions, and advocate the implementation of such approaches.
399

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408 **Conflict of Interest**

409 The authors declare that the research was conducted in the absence of any commercial or
410 financial relationships that could be construed as a potential conflict of interest.

411

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568 **Figure Legends**

569 **Fig. 1** *Bd* infectious life cycle including the potential grazing pressure by micrograzers.
570 Zoospores (*Z*) move using a flagellum, and on contact infect the amphibian host. Within the
571 host epidermal cells, a spore then forms a sporangium (*S*) that releases further zoospores

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572 through asexual reproduction (n), after which the sporangium dies (σ). Released zoospores
573 may die naturally (μ) or be ingested (I) by micrograzers (G).

574 **Fig. 2** Average growth rates (r, d^{-1}) for micrograzers fed *Bd* (black) or no prey (grey); error
575 bars are one standard error, and * indicates where significant ($\alpha = 0.05$) differences occurred
576 between fed and unfed treatments. Note that for *P. caudatum* growth rate was zero when fed
577 *Bd*. The horizontal lines connected by a vertical line represent the range of predicted
578 maximum growth rate (i.e. food saturated on suitable prey) at 18 °C, for each species. Data
579 were obtained from a various sources at a range of temperatures [59-66] and were converted
580 to rates at 18 °C by two methods, either assuming a Q_{10} of 2 or that growth rate varies
581 linearly with temperature at a rate of 0.07 r (d^{-1}) °C^{-1} [67].

582 **Fig. 3** Average growth rates (r d^{-1}) of three replicates of *Paramecium caudatum* (a) and
583 *Paramecium aurelia* (b) in the *Bd* treatment, with standard error bars. The skull and
584 crossbones indicate the time point where all individuals had died. The solid black line
585 represents the average death rate of the micrograzers when no prey were present, and the
586 dotted black line indicates the standard error of the control groups.

587 **Fig. 4** The functional response**:** ingestion rates of *Tetrahymena pyriformis* on surrogate
588 zoospores (prey) vs prey concentration. Points are ingestion rates at defined prey
589 abundances. The solid line represents the best fit of a Type II functional response to the data
590 (see Results for the parameter estimates). The grey region represents the range of functional
591 responses used to assess the ability of "inferior micrograzers" to control *Bd* (i.e. reduced
592 maximum ingestion rate and increased half saturation constant; see Methods, Modelling
593 micrograzer impacts on *Bd* populations).

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594 **Fig. 5** Simulations of micrograzer (*T. pyriformis*) control of *Bd*, based on Eq. 1-3 and
595 parameters presented in Table 1. a. *Bd* (zoospore and sporangia) dynamics in the absence of
596 micrograzers, indicating that by ~55 days sporangia per host approach lethal limits (skull and
597 crossbones, 10^4 sporangia per host Briggs et al. [8]). b. *Bd* and micrograzer dynamics,
598 indicating control of zoospores and sporangia, maintaining sporangia numbers below the
599 lethal limit. c. *Bd* and micrograzer dynamics based on an inferior micrograzer to *T.*
600 *pyriformis* (0.5 x I_{max}; 2 x k presented in Table 1), indicating the micrograzers inability to
601 prevent host death at ~55 days (skull and crossbones) but its ability to ultimately reduce *Bd*
602 levels by 100 d.

603 **Fig. 6** Exploration of *Bd*-micrograzer dynamics (Eq. 1-3), through varying two key
604 micrograzer parameters: the half saturation constant (k) and the maximum ingestion rate
605 (I_{max}); see Methods for details. To characterise dynamics, we provide the log numbers of
606 zoospores, sporangia, and micrograzers. For each, we present the maximum number reached
607 over the 100 days, the number at 50 days, and the number at 100 days.

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610 **Table 1** Parameters used to assess *Bd* dynamics (Eq. 1-3). *Bd* parameter estimates are from
611 Briggs et al. [8] who present a wide range of possible values; we have chosen one set of
612 these that provide an illustration of Bd-micrograzer dynamics. Micrograzer data are
613 from our functional response (Fig. 4). Conversion efficiency (e) was estimated as
614 described in the text. The minimum number of micrograzers (b) and micrograzer death
615 rate (d) were derived from personal observations.

Symbol	**Parameter**	**Estimate**	**Range explored by Briggs et al. [7]**	**Dimension**
y	Rate of zoospores encounter with hosts	(0.05)	Large range of values	d^{-1}
v	Fraction of successful *Bd* spore infections	0.1	0-1	dimensionless
η	Production rate of zoospores from sporangium	17.5	--	d^{-1}
σ	Sporangia loss rate	0.2	0.1-0.3	d^{-1}
μ	Spore death rate	0.1 (1)	0.02-1	t^{-1}
E	Conversion efficiency	5×10^{-4}		dimensionless
I_{max}	Maximum ingestion rate	1630		$S\ d^{-1}$
K	Half saturation constant	5.75×10^{4}		$S\ ml^{-1}$
d	Micrograzer death rate	0.01		t^{-1}
b	Minimum number of micrograzers	1		ml^{-1}

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620 Fig. 1

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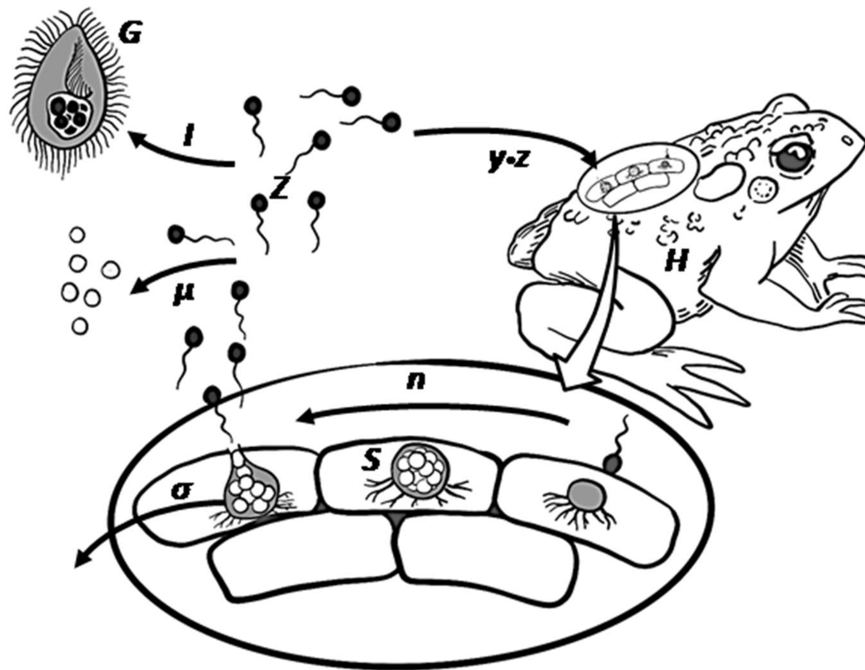

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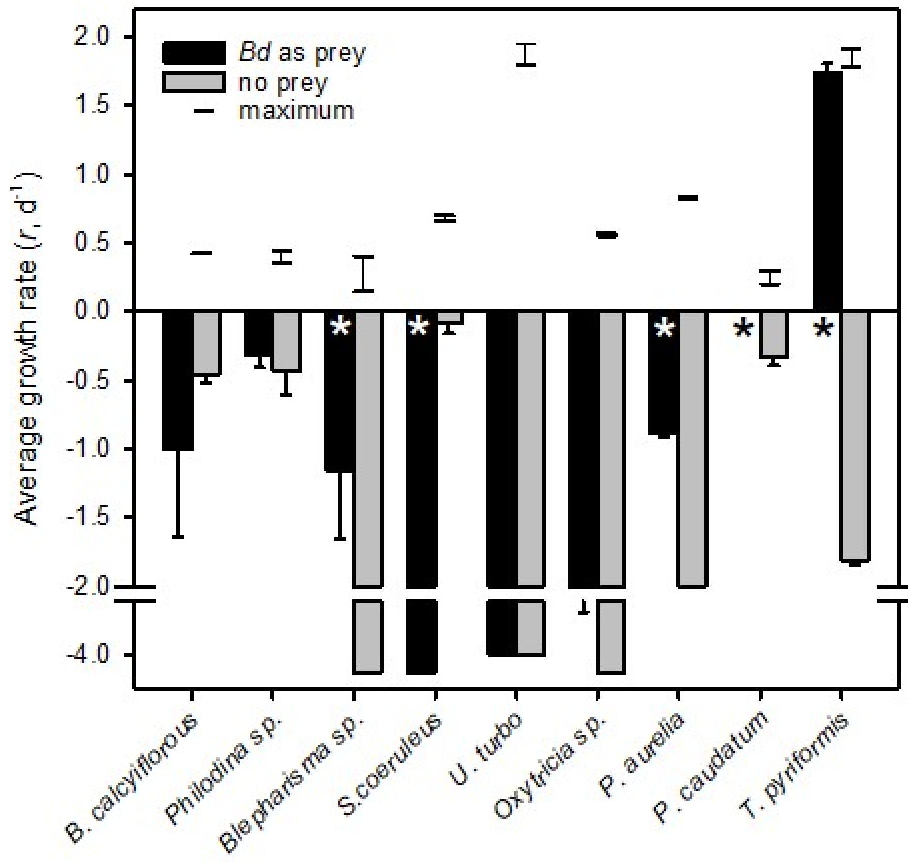
623 Fig. 2



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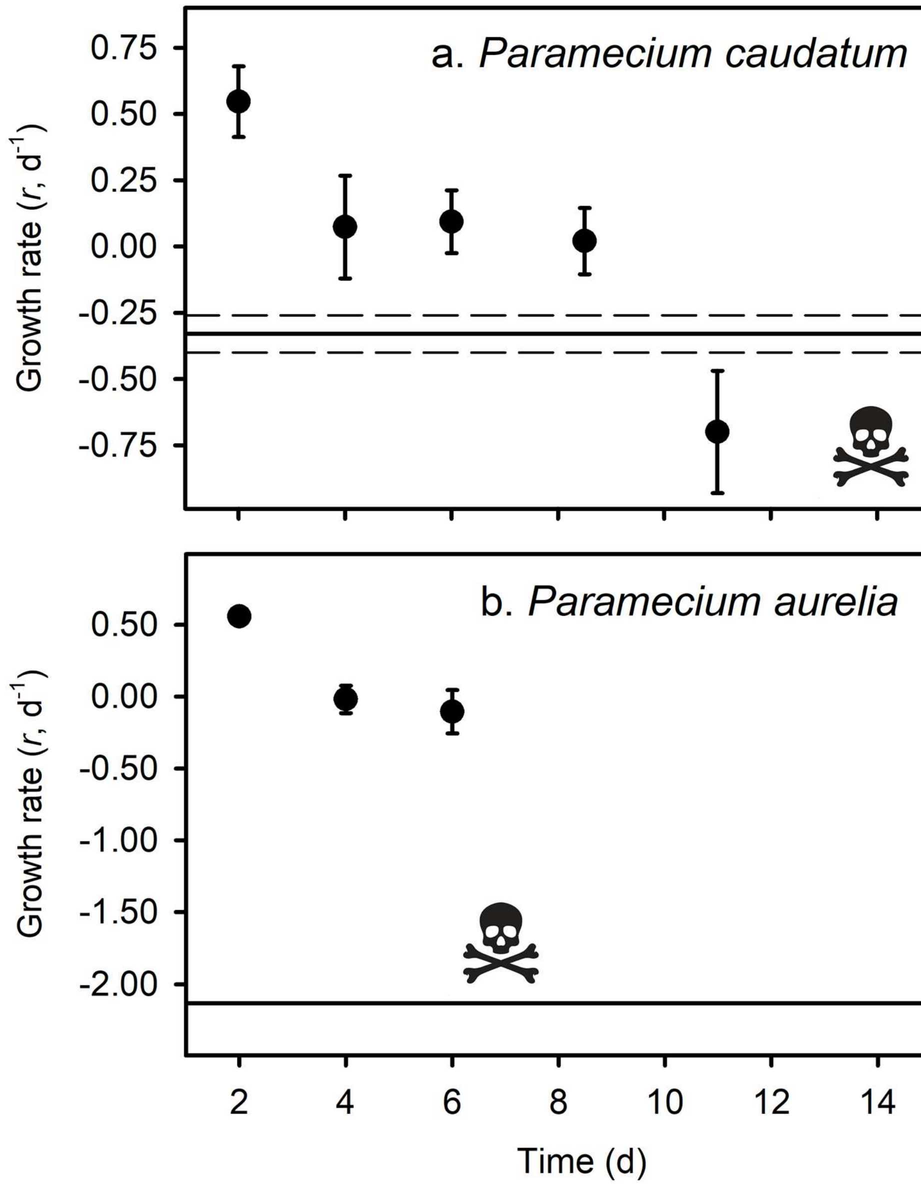
626 Fig. 3



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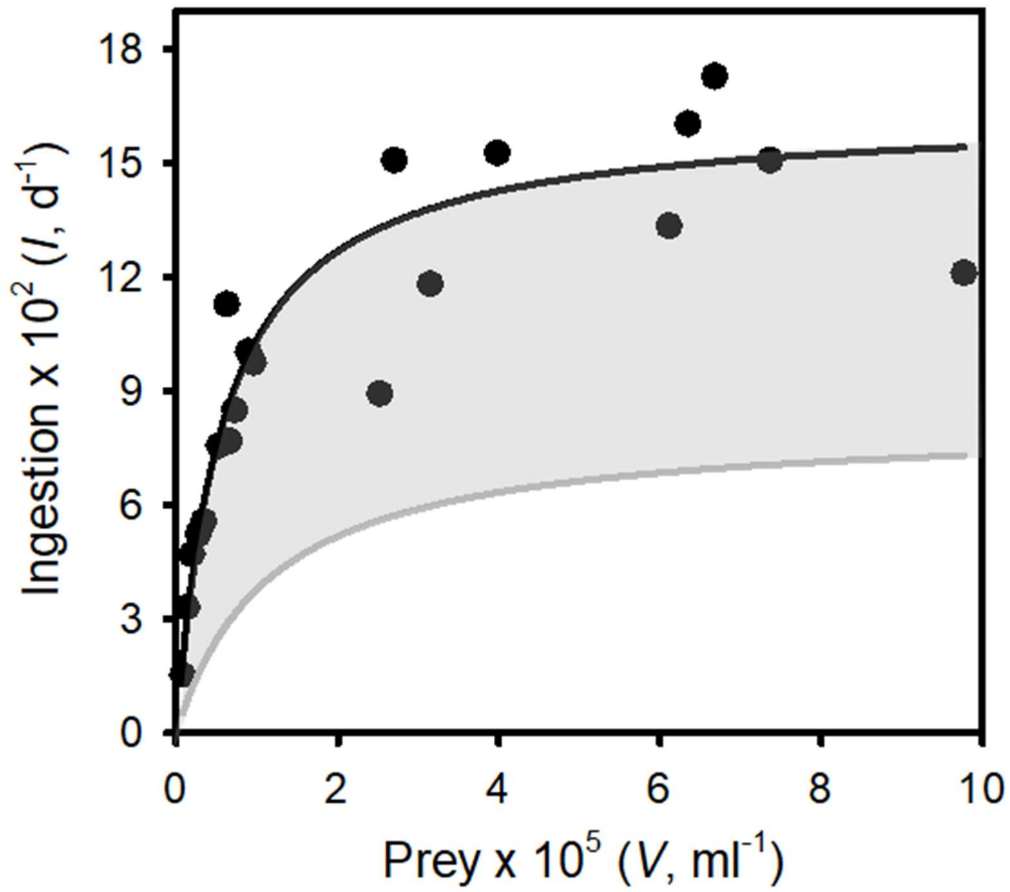
627 Fig. 4



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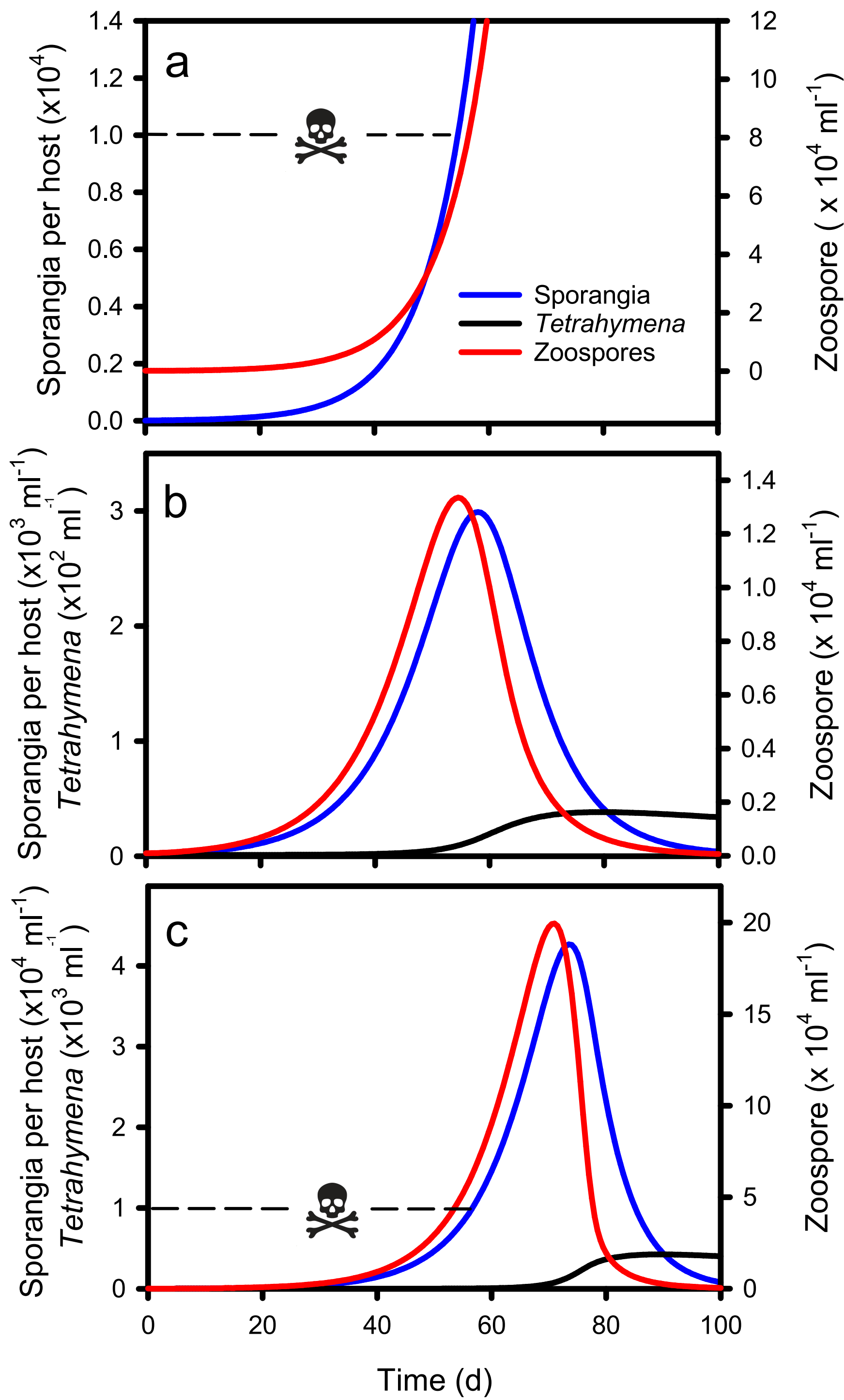
628 Fig. 5



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630 Fig. 6

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