Microbial grazers can control chytridiomycosis caused by aquatic zoosporic fungi

Hazel N. Farthing[2, *] Jiamei Jiang[1, *], Alexandra J. Henwood[2], Andy Fenton[2], Trent Garner[3], David R. Daversa[2,3] Matthew C. Fisher[4], David J. S. Montagnes[2]

[1] Shanghai Universities Key Laboratory of Marine Animal Taxonomy and Evolution, Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306 China

[2] Institute of Integrative Biology, BioSciences Building, University of Liverpool, Liverpool L69 7ZB UK

[3]Institute of Zoology, Zoological Society of London, London, England, UK

[4]MRC Centre for Global Infectious Disease Analysis, Imperial College London

[*] These authors contributed equally to this work.

Corresponding author: David Montagnes, dmontag@liv.ac.uk

Abstract

Metazoan disease control may require an understanding and inclusion of microbial ecology. We evaluated the ability of micrograzers (primarily protozoa) to control chytridiomycosis, a disease caused by the chytrid *Batrachochytrium dendrobatidis*, a devastating panzootic pathogen of amphibians. Although micrograzers consumed zoospores (~3 µm), the dispersal stage of chytrids, not all species grew monoxenically on zoospores; but the ubiquitous ciliate *Tetrahymena pyrifomis*, exhibited a growth rate of 1.7 d^{-1} and approached its maximum rate of growth. A functional response (ingestion vs. prey abundance) of *T. pyrifomis* measured on spore-surrogates (microspheres) revealed maximum ingestion (I_{max}) of 1.63 x 10^3 spores d^{-1}, with a half saturation constant (k) of 5.75 x 10^3 spores ml^{-1}. We then developed and assessed a population model that incorporated chytrid-host and micrograzer dynamics over 100 days. Simulations using *T. pyrifomis* data and realistic parameters obtained from the literature, suggested that micrograzers can control *B. dendrobatidis* and prevent chytridiomycosis (defined as 10^4 sporangia per host). However, inferior micrograzers (0.7 x I_{max} and 1.5 x k) were unable to prevent chytridiomycosis, although they did reduce pathogen abundance to negligible levels. These findings strongly argue that micrograzers should be considered when evaluating disease dynamics for *B. dendrobatidis* and other zoosporic fungi.

Key words: *Batrachochytrium dendrobatidis*, ciliates, disease, fungi, microbial loop, protozoa, *Tetrahymena*

Introduction

Although the traditional microbial food web (i.e. prokaryotes and protists, *sensu* Azam et al. [1] is well-established as a driver of aquatic productivity [2], micro-fungi are only now being appreciated as integral aquatic microbes. A dominant group of micro-fungi, the chytrids, are parasites of phytoplankton, zooplankton, and vertebrates [3]. Zoospores, the chytrid dispersal stage, are also nutritious and of an appropriate size for protozoan grazers [2, 3]. Hence, grazers within the microbial food web have the potential to reduce the likelihood or severity of, or even prevent, disease outbreaks caused by these pathogens, through top-down control [3-5]. Here, we examine these processes by assessing the potential for microbial grazers to control the chytrid *Batrachochytrium dendrobatidis*, a panzootic pathogen of amphibians that is argued to have caused the greatest loss of biodiversity attributed to any disease [6].

Batrachochytrium dendrobatidis (henceforth, *Bd*) infects amphibian hosts through the dispersal of microscopic (3-5 µm), motile zoospores (Fig. 1). This spore-pool is instrumental in driving infection dynamics, as host costs of infection accrue in a dose-dependent manner [7]. As the disease, chytridiomycosis, is dependent on zoospore dose, the size of the zoospore pool determines the longer-term consequences for host population survival or extinction [8]. It follows that processes that reduce the pool of zoospores will potentially reduce the probability and intensity of disease outbreaks. Consumption of spores by naturally occurring grazers has been suggested to result in significant losses. Experiments show that some grazers may reduce the likelihood of *Bd* infections, and field data indicate a negative relationship between grazer abundance and both prevalence of infection and host mortality from disease [9-12].

Most work to date on the consumption of *Bd* spores has focused on large zooplankton, especially cladocerans [10, 13-15]. However, experiments on cladocerans have used

unrealistically high grazer abundances (>10-100 times higher than natural levels), and at natural levels large zooplankton seem to have little impact on *Bd* infections [16]. Micrograzers, in contrast, are highly abundant in shallow waters and are often found near the bottom, where infected hosts tend to occur. Furthermore, micrograzers have generation times on the order of hours; by reproducing asexually when nutritious spores are abundant, these populations could increase several fold, consuming spores as they are released from the host. We, therefore, argue that main consumers of spores will be the eukaryotic microbes (i.e. protozoa). This is supported by Schmeller et al. [12] who showed the ciliate *Paramecium* can significantly reduce the number of hosts infected by 65% when it is introduced at naturally occurring abundances.

We also suggest that the main impact of grazers on *Bd* spore loads will be in the water directly surrounding the host, where spores will be most abundant. Spore densities in the water column are low, ranging from ~0.5 to 500 L^{-1} [17, 18]. In contrast, spores originating from hosts, at up to 250 spores min^{-1} [19], disperse only ~1 cm [20] and survive only ~1-3 days [9]. This limited movement and survival of rapidly produced spores suggests we should expect dense aggregations in localized regions. Hence, recognising the likelihood of these local abundances and the well-established density-dependent feeding and growth responses of micrograzers [2], we therefore assess the impact of micrograzers on *Bd* dynamics around the host. We achieve this by: investigating which grazers will grow on a diet of *Bd* spores; measuring ingestion and growth rates of a species that thrives on *Bd*; and developing and employing a model that couples the *Bd* life cycle with grazer-control on spores. In doing so, we provide substantive evidence that micrograzers may control *Bd* and that this component of the microbial food web should be included in any further predictions of disease dynamics.

Materials and methods

Culture maintenance

Batrachochytrium dendrobatidis (*Bd*) cultures (strain #262 IA 9'13, Imperial Collage London) were maintained at 18 °C (at which all experiments were conducted) on H-broth medium [21] and were regularly transferred (every ~5 d) to maintain exponential growth. Bacterial growth was prevented by the addition of antibiotics (Ampicillin at 100 µg ml^{-1}; Kanamycin at 50 µg ml^{-1}; Chloramphenicol at 34 µg ml^{-1}). Grazers were obtained from Sciento (Manchester, UK): the ciliates *Blepharisma* sp., *Oxytrichia* sp., *Paramecium aurelia*, *Paramecium caudatum*, *Tetrahymena pyriformis*, and *Urocentrum turbo* and the rotifers *Brachionus calcyciflorous* and *Philodina* sp. *Tetrahymena pyriformis* was maintained for extended periods, axenically on H-broth. All other species were maintained prior to experiments on a mixed assemblage of bacteria in medium provided by Sciento.

Assessing growth of grazer species on Bd

Prior to introducing grazers to *Bd*, the fungus was isolated from its growth medium, to prevent the medium being a source of nutrients for the grazers. To do so, a *Bd* suspension (in exponential phase) was centrifuged (50 ml tubes, 5000 rpm, 6 min), the supernatant removed, and the pellet resuspended in autoclaved Volvic® mineral water to a concentration > 1.50 x 10^5 ml^{-1} (determined microscopically). Bacterial growth was prevented with antibiotics, as above.

To assess growth rate, we followed our previous methods [22]. Individual grazers were passed five times through autoclaved Volvic® water, to remove bacteria, and then a defined number (5 to 8) were added to a 10 ml well containing the *Bd* suspension. Parallel treatments containing only sterile Volvic® water were used to assess mortality rate in the absence of prey. All treatments were replicated (n = 3). To assess growth rate (r, d^{-1}), after two or three days (depending on the species), the number of gazers in each well was determined,

microscopically. Then, new *Bd* suspensions were prepared (as above), and grazers were transferred to these, i.e. maintaining *Bd* abundance. If grazer numbers decreased (i.e. net death occurred) over the incubation, then all individuals were transferred, but if numbers increased (i.e. net growth occurred) then the initial number was transferred. This procedure was continued for 14 days or until all grazers had died. Cultures were routinely checked to ensure there was no bacterial contamination.

When numbers increased between transfers, growth rate (r, d^{-1}) was determined over each incubation period, as $r = ln(N_t/N_0)/t$, where N_t and N_0 are grazer abundance on the initial and final day, and t is the incubation period (i.e. 2 or 3 days); to determine growth rate across all transfers (up to 14 d), the average of these was obtained. When grazer numbers decreased between transfers, mortality rates ($-r$, d^{-1}) were determined as slope of *ln* numbers over the entire incubation period. To assess if growth (or death) rate differed between treatments (i.e. with or without *Bd*) a two tailed t-test two was conducted ($\alpha = 0.05$).

Tetrahymena pyriformis *ingestion rate*

As *T. pyriformis* grew rapidly on *Bd* spores (see Results), grazing experiments focused on this species. Prior to experiments, *T. pyriformis* was acclimated with *Bd* spores for >10 h. To do so, the ciliates were first removed from H-broth by centrifugation (50 ml tubes, 8000 rpm, 8 min) and then resuspended in 10 ml of autoclaved Volvic® water. To obtain only *Bd* spores, a centrifuged *Bd* culture (as above) was filtered through a 5 µm Nitex® mesh. Spores were then added to the water containing ciliates, to a total volume of 20 ml, with antibiotics (as above). Spore concentration was initially ~10^6 ml^{-1}. After 10 h, few spores were present, and the ciliates were active; i.e. in a similar condition to that when they were first introduced to the spores.

To determine ingestion rate on spore-sized particles, 3 µm fluorescent polymer microspheres (henceforth beads, Fluoro-Max™, Thermo Fisher Scientific, USA,) acted as a

surrogate for *Bd* spores which are 3 - 5 µm [23]. Bead concentrations were prepared in autoclaved Volvic® water and vortexed prior to use, ensuring mono-dispersion. An aliquot (0.5 ml) of the ciliate culture (> 30 grazers) was added to 1 ml of Volvic® water with beads, at various concentrations (with more measurements at low abundances [24], see Results), and incubated for 5 or 10 minutes, depending on the bead concentration (preliminary experiments deemed these to be appropriate incubation periods). Incubations were terminated by fixing cells with ethanol (final concertation 70%). Via fluorescent microscopy the average number of beads ingested per ciliate (>30 cells) was used to determine ingestion rate (I, prey d^{-1}) at each prey concentration.

The functional response, the relationship between ingestion rate and spore abundance (Z ml^{-1}), was determined by fitting a Type II functional response to the data: $I = I_{max}*Z/(k + Z)$, where I_{max} (Z min^{-1}) is the maximum ingestion rate, and k is the half saturation constant (Z ml^{-1}). The response was fit using the Marquardt-Levenberg algorithm (SigmaPlot, Systat, Germany); this algorithm is appropriate for describing such biological data sets [25].

Modelling grazer impacts on Bd populations

To assess the extent to which grazing pressure might impact *Bd* infection dynamics we applied the following model, based on a reduced version of the *Bd*-load model [8] and Rosenzweig-MacArthur predator-prey model [26]. Data for *T. pyriformis* were used to represent grazers as this ciliate grows on *Bd* spores (see Results). Following logic outlined in the Introduction, the model describes the infection load on a single host and only considers a volume of 10 ml around that host, where spores and grazers reside. It assumes that a sporangia load of 10^4 per host results in host mortality [8]. It does not include reduction of spore numbers by emigration as spores are unlikely to move much further before dying, and we assume grazers remain near their food source. The model is described by the following equations,

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

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

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

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

Then, Eq. 2 and Eq. 3 were coupled to include spore loss by grazers (G), where loss by grazing (I) is dictated by the functional response (see *Tetrahymena pyriformis* ingestion, above); e is the abundance-based conversion efficiency, determined assuming a biomass-based estimate of e is ~0.1 [27] and biovolumes of *Bd* spores and *T. pyriformis*; and d is the grazer per capita death rate. We assume here that *Bd* spores are not the only food source for the grazers, and so incorporate a minimum grazer abundance (Kl) that implicitly assumes the grazer population us maintained by the presence of other potential food sources; hence we model potential increases in grazer abundance over and above this minimum, dependent on consumption of *Bd* spores. Estimates of d and Kl are based on our personal observations (Table 1).

All model runs (100 d) were initiated with 10 sporangia host^{-1} (1 sporangium ml^{-1}), 100 spores ml^{-1}, and 1 grazer ml^{-1} (again assumed to be the minimal population size, maintained by other resources in the environment). For *Bd*, we applied parameter values that were within the range explored by Briggs et al. [8] (Table 1). We first performed simulations to assess the ability of *T. pyriformis* to control *Bd*. Then, through exploration of grazer parameter space, we assessed the extent to which grazers that are inferior to *T. pyriformis* could control *Bd*. Inferior grazers had reduced maximum ingestion rate (up to 0.5 × I_{max} of *T.*

pyriformis) and increased half saturation constant (up to 2 × *k* of *T. pyriformis*); see Fig. 4 for an indication of the range of these responses. To indicate the impact of grazers on *Bd*, we reported on the maximum abundance (over the 100 days) and the abundances at 50 and 100 days of *S*, *Z*, and *G*.

Results

Assessing growth of grazer species on Bd

The growth rate of all grazers was negative when exposed to water alone, wheras when fed *Bd* they exhibited four distinct responses (Fig. 2): 1) for the ciliate *Stentor coeruleus* death that was significantly (and substantially) higher than death rate in water alone; 2) for the ciliates *Urocentrum turbo, Blepharisma* sp., and *Oxytrichia* sp. and the rotifer *Philodina* sp. there was no significant difference between death rate with or without *Bd*; 3) for the ciliates *Paramecium aurelia, P. caudatum* (Fig. 3) and the rotifer *Brachionus calcyciflorous* (data not shown) growth rate was initially positive when *Bd* was present followed by a negative growth rate (death) as time progressed (on average over the incubation *P. aurelia* exhibited negative growth while *P. caudatum* exhibited zero growth, Fig. 3); and 4) for the ciliate *Tetrahymena pyriformis* there was sustained positive growth rate (Fig. 2), which was significantly higher than the negative growth rate on water alone; this growth rate of ~1.7 ± 0.23 (SE) d^{-1} was equivalent to that when the ciliate was grown axenically on H-broth and near its maximum rate under any conditions (data not shown).

Tetrahymena pyriformis ***bead ingestion experiments***

Ingestion rate followed typical Type II functional response (Fig. 4, adjusted $R^2 = 0.82$), with $I_{max} = 1.63 \times 10^3 \pm 98$ (SE) prey d^{-1} and $k = 5.75 \times 10^3 \pm 1.38 \times 10^3$ (SE) prey ml^{-1}.

Modelling grazer impacts on **Bd** ***populations***

Disease control by micrograzers

Control of *Bd* occurred when the grazer (*T. pyriformis*) was included in the model (Fig. 5): in the absence of the grazer, sporangia per host reached lethal levels (>10^4 host^{-1} [8]) by ~55 days (Fig. 5a), but when grazers were included their population rose from 1 to ~35 ml^{-1}, with the result that sporangia were limited to a maximum of 3 x 10^3 per host (i.e. the host would survive), and *Bd* was virtually eradicated by 100 days (Fig. 5b).

We then assessed the ability of grazers that were inferior to *T. pyriformis* to control *Bd*, through exploration of grazer parameter space: i.e. up to twice the half saturation (k) and half the maximum ingestion rate (I_{max}) of *T. pyriformis* (Fig. 4). Fig. 5c illustrates population dynamics when the most inferior grazer was included (highest half-saturation constant and lowest maximum ingestion rate): the general pattern remained similar to that when *T. pyriformis* parameters were applied, with the grazers controlling *Bd* over 100 d, but the abundances zoospores, sporangia, and grazers were more than 10 times greater (leading to host death at ~55 days), and abundances peaked ~20 days later. We then illustrate the pattern of the temporal dynamics across a wider range of parameter space by reporting the maximum abundance and the abundances at 50 and 100 days of spores, sporangia, and grazers. Across all parameters explored, the grazer population provided top-down control of *Bd*, as over the entire range, *Bd* was virtually eradicated by 100 days (Fig. 6 c, f). However, the quantitative levels and rates of control varied considerably with grazer efficiency: with reduced I_{max} and increased k, spores and sporangia reached higher maximum abundances (Fig. 6a,d) and persisted longer (Fig. 6b,e), indicating a decrease in the control of *Bd*. In particular, grazers with < 0.7 I_{max} (~10^3 prey d^{-1}) and >1.5 x k (~9 x 10^4 ml^{-1}) were not capable of preventing sporangia per host exceeding lethal levels (yellow-to-red region on Fig. 6 d, representing ≥10^4 sporangia per host). Decreased I_{max} and increased k also led to increases in grazer abundance (Fig. g-i), in response to the increased spore levels available under these grazing regimes.

Discussion

Control of a wide range of diseases caused by zoosporic fungi may be achieved through consumption on aquatic, motile spores by grazers [3-5]. Here, we show that micrograzers will play a pivotal role in controlling the devastating amphibian disease chytridiomycosis, which is caused by the micro-fungus *Batrachochytrium dendrobatidis* (*Bd*). We do so by first indicating that some protozoa can grow on *Bd* spores, and that the ciliate *Tetrahymena pyriformis* grows at near maximal rates on spores alone. We then provide the first assessment of a functional response of *T. pyriformis* feeding on spore-sized prey. Finally, using our data and literature estimates, we develop and employ a novel dynamic model that couples the *Bd* life cycle with grazer-control on spores. This synthesis provides strong arguments that micrograzers are capable at supressing *Bd* loads, and so any further assessment of *Bd* dynamics should consider their importance in the top-down control of disease risk.

Micrograzer growth on Bd

Our initial observations suggested that all the micrograzers we examined could ingest *Bd*, but they exhibited a range of growth-responses. For one ciliate, *S. coeruleus*, *Bd* appeared to be toxic, while other species seemed to obtain no nutritional benefit (Fig. 2). However, several species benefited from ingesting *Bd*. Both *Paramecium aurelia* and *P. caudatum* exhibited initial growth, although it was not sustained (Fig. 3), suggesting that while *Bd* may be nutritionally valuable, it lacks essential nutrients for these ciliates. In contrast, *Tetrahymena pyriformis* sustained positive growth, indicating that *Bd* can provide a complete diet for some species. These observations are supported by previous work on ciliates: *T. pyriformis* and a closely related species, *Colpidium striatum*, also grow on yeast (*Saccharomyces*), while *P. aurelia*, and *P. caudatum* cannot, again possibly due to a lack of nutrients such as essential fatty acids and B-vitamins [28, 29]. Critically, these data suggest

that not all micrograzers would be equally proficient at controlling *Bd* through an increase in population numbers.

However, with additional prey sources to sustain the consumers, there may be selective feeding on *Bd*. For instance, *T. pyriformis* differentiates between prey, leading to a more efficient assimilation of prey biomass and a greater cell yield of ciliates [30]. Likely, in the mesocosm experiments conducted by Schmeller et al. [12], where *Paramecium* controlled *Bd*, this ciliate's diet was supplemented by naturally occurring bacteria. In fact, in our initial growth-experiments (unpublished), where antibiotics were not included, bacteria grew, and both *Paramecium* species consumed spores and maintained extended positive growth. Our analysis here focused on *Bd* as the sole food source, but given the recognition that *Bd* may be controlled by micrograzers (see below), we suggest that further experiments should examine mixed, natural diets to assess the extent to which a wider range of micrograzers might be sustained at densities that impact dynamics of *Bd* infections.

Tetrahymena grazing on Bd

Globally, in shallow waters where *Bd* occurs, *Tetrahymena* is common, where it consumes bacteria and other microbes [31, 32]. *Tetrahymena* is also associated with amphibians where it may be an opportunistic ectoparasite [33, 34], but possibly also a consumer of *Bd* spores as they emerge from sporangia. Considering its habitat and ability to rapidly reproduce on *Bd* alone (see above), we focused on *T. pyriformis'* ingestion of *Bd* spores. Initially, attempts were made to stain *Bd* spores with calcofluor-white [12], but this was not successful; calcofluor stains chitin [35], and although *Bd* sporangia have a chitin wall, spores do not [23]. We then explored vital stains (e.g. cell tracker green), but again we were not successful. Consequently, ingestion estimates relied on the uptake of fluorescent beads as surrogates for *Bd*, which may underestimate rates (e.g. [36]). We, therefore, see our predictions as conservative. From our data, a clear Type II functional response was obtained

for *T. pyriformis* (Fig. 4), providing parameters for modelling *Bd*-grazer dynamics. To our knowledge, this is the first such response for any *Tetrahymena* species: the estimates of I_{max} and k are within the range of those obtained for other ciliates, although the k-values are on the lower end of the spectrum [29, 37, 38]; our modelling, therefore, includes grazers that are inferior to *Tetrahymena*.

Modelling grazer impacts on* Bd *populations

Empirical evidence suggests that *Paramecium* can reduce *Bd* infections, through examining end point estimates of host infection [12]. Here we explore the temporal dynamics of such control and the potential for grazers to prevent host death. Our analysis is reductionist and hence illustrative, but it reveals that by applying plausible parameters for both the parasite and grazer, chytridiomycosis may be prevented and *Bd* virtually eradicated, or at least reduced to negligible levels (Fig. 6). Admittedly, we indicate that grazers that are inferior to *T. pyriformis* are less likely to prevent host death, yet they still, ultimately, reduce *Bd* populations to negligible numbers, potentially preventing further disease outbreaks (Fig, 6). These model predictions strongly argue for the continued exploration of micrograzers in *Bd* research, specifically, and in the control of a range of diseases that spread through zoospores or other similarly sized dispersal stages [3-5].

To date, models of *Bd*-dynamics [8, 9, 39, 40] have not included estimates of spore loss by micrograzers. As indicated above, the modelling provided here is instructive, but it could benefit from elaboration. Given the ubiquity of protozoa in natural waters [2], and the clear indication of their potential impact (Fig. 6, [3-5]), we suggest there is now a need for better parameterization of micrograzer-*Bd* responses. For instance, *Tetrahymena* exhibits chemosensory behaviour [41]; the extent to which ciliates are attracted to amphibian hosts and *Bd* requires evaluation. Likewise, the role of *Bd* as a supplement rather than a sole dietary component deserves attention. Finally, the Rosenzweig-MacArthur predator-prey

model, which we used, has limitations, and other structures such as the Independent Response model [42] that relies, independently, on growth and ingestion responses provides better predictions [43]. To this end, we suggest that both functional (ingestion) and numerical (growth) responses to *Bd*-concentrations are established for a range of micrograzers.

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

Both *Tetrahymena* and *Paramecium* are common species in shallow waters where *Bd* occurs [31, 32, 44]. These ciliates survive on a diet of bacteria and slightly larger prey, and as we have shown here they will consume and grow on *Bd* zoospores for limited to extended times. Other, but not all, protozoa will also, undoubtedly consume and grow on *Bd* spores. We, therefore, suggest that the role of these grazers is considered when evaluating *Bd*-dynamics and the dynamics of other zoosporotic diseases. For instance, chytridiomycosis is more prevalent at higher altitudes [45]. High altitude waters will often be both cooler and oligotrophic. While temperature has been shown to determine infection burdens [46], there will likely be an interaction with the trophic status of the water. If in oligotrophic waters bacteria are reduced below levels sufficient to support ciliates ($<10^6$ ml^{-1}), our analysis suggests that *Bd* may thrive, resulting in chytridiomycosis. Consequently, assessing the abundance of micrograzers in waters where chytridiomycosis occurs or is predicted seems warranted.

We end with some speculations on the potential for biomanipulation. Traditional approaches for managing wildlife diseases have proven ineffectual or impractical for *Bd*. Treating amphibians with probiotic bacteria directly has generally failed as a conservation strategy (but see [45]), and, while theoretically plausible, culling hosts to below the critical threshold for disease transmission contravenes conservation objectives [47-49]. This means that alternative mitigation and management strategies must be perused. To date, the only successful effort towards in situ *Bd*-mitigation relied on dosing animals with antifungal

chemicals alongside applying chemical disinfectants directly to the environment to reduce spore survival [47]. Fungicides and chemical disinfection, however, have shortcomings, not least of which are ethical issues associated with indiscriminate toxic effects. As both *Tetrahymena* and *Paramecium* are ubiquitous in aquatic environments and are simple and inexpensive to grow in large quantities [32, 44], they may be tractable target species for biomanipulation. We, therefore, suggest that by augmenting natural densities of these species, through addition or supplementary feeding, it may be possible to reduce zoospore densities for *Bd in situ.* Clearly though, further evaluation of the role of microgazers is required before we can definitively understand their likely impact in natural conditions, and advocate the implementation of such approaches.

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Competing Interests

The authors declare no competing financial interests.

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Figure Legends

Fig. 1 *Bd* infectious life cycle including the potential grazing pressure by grazers. Zoospores (*Z*) move using a flagellum, and on contact infect the amphibian host. Within the host epidermal cells, a spore then forms a sporangium (*S*) that releases further spores through asexual reproduction (*n*), after which the sporangium dies (σ). Released spores may die naturally (μ) or be ingested (*I*) by grazers (*G*).

Fig. 2 Average growth rates (r d-1) for grazers fed *Bd* (black) or no prey (grey); error bars are one standard error, and * indicates where significant ($\alpha = 0.05$) differences occurred between fed and unfed treatments. Note that for *P. caudatum* growth rate was zero when fed *Bd*.

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

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

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

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

Table 1 Parameters used to assess *Bd* dynamics (Eq. 1-3). *Bd* parameter estimate are from Briggs et al. [8] who present a wide range of possible values; we have chosen one set of these that provide an illustration of Bd-grazer dynamics. Grazer, data are from our functional response (Fig. 4). Conversion efficiency (*e*) was estimated as described in the text. The minimum number of grazers (*Kl*) and grazer death 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	Grazer death rate	0.01		t^{-1}
Kl	Minimum number of grazers	1		ml^{-1}

Fig. 1

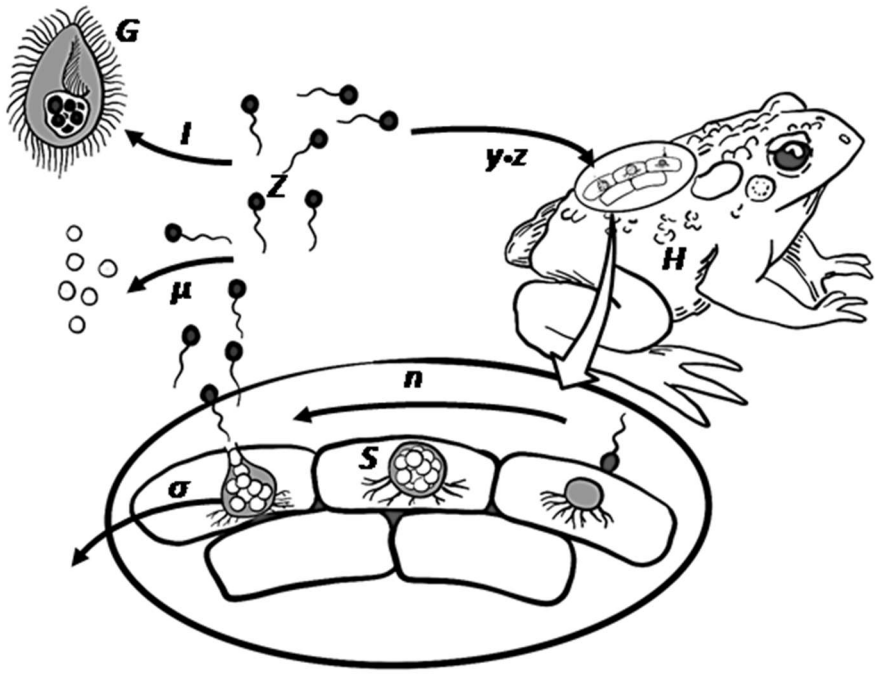


Fig. 2

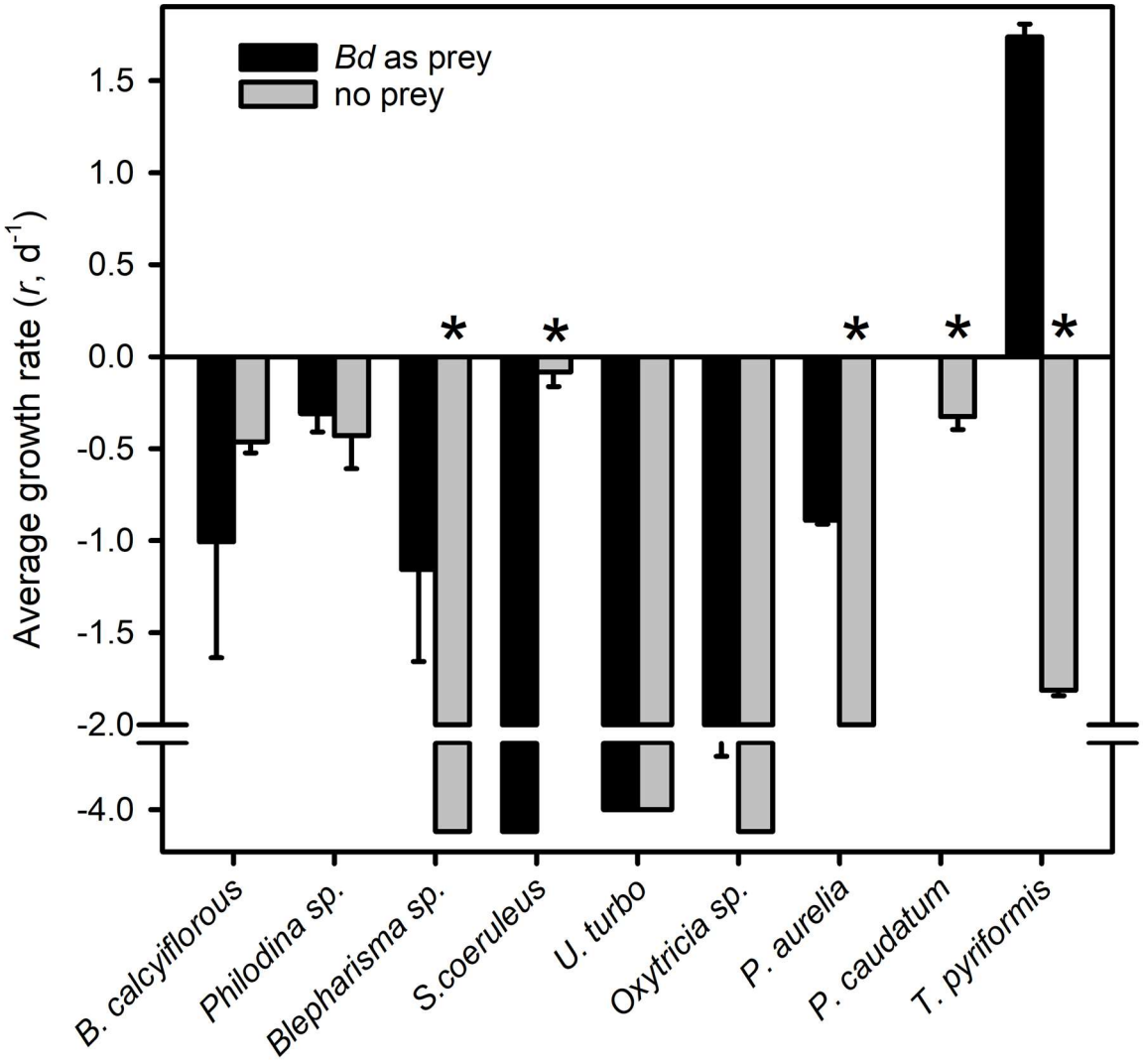

Bd as prey
no prey
Average growth rate (r, d-1)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-4.0
*
*
*
*
*
B. calcyiflorous
Philodina sp.
Blepharisma sp.
S.coeruleus
U. turbo
Oxytricia sp.
P. aurelia
P. caudatum
T. pyriformis

Fig. 3

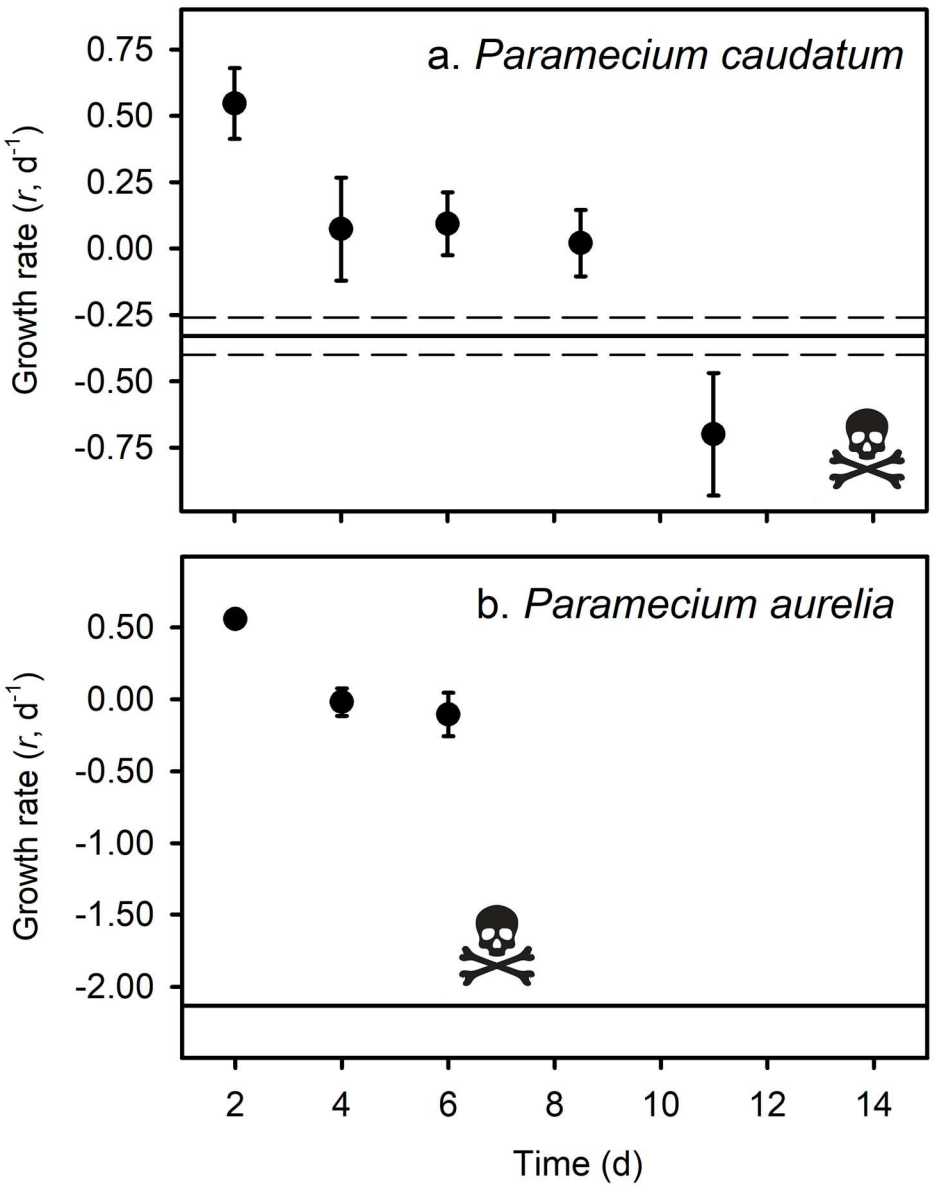

a. Paramecium caudatum
b. Paramecium aurelia
Growth rate (r, d-1)
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.50
-2.00
2
4
6
8
10
12
14
Time (d)

Fig. 4

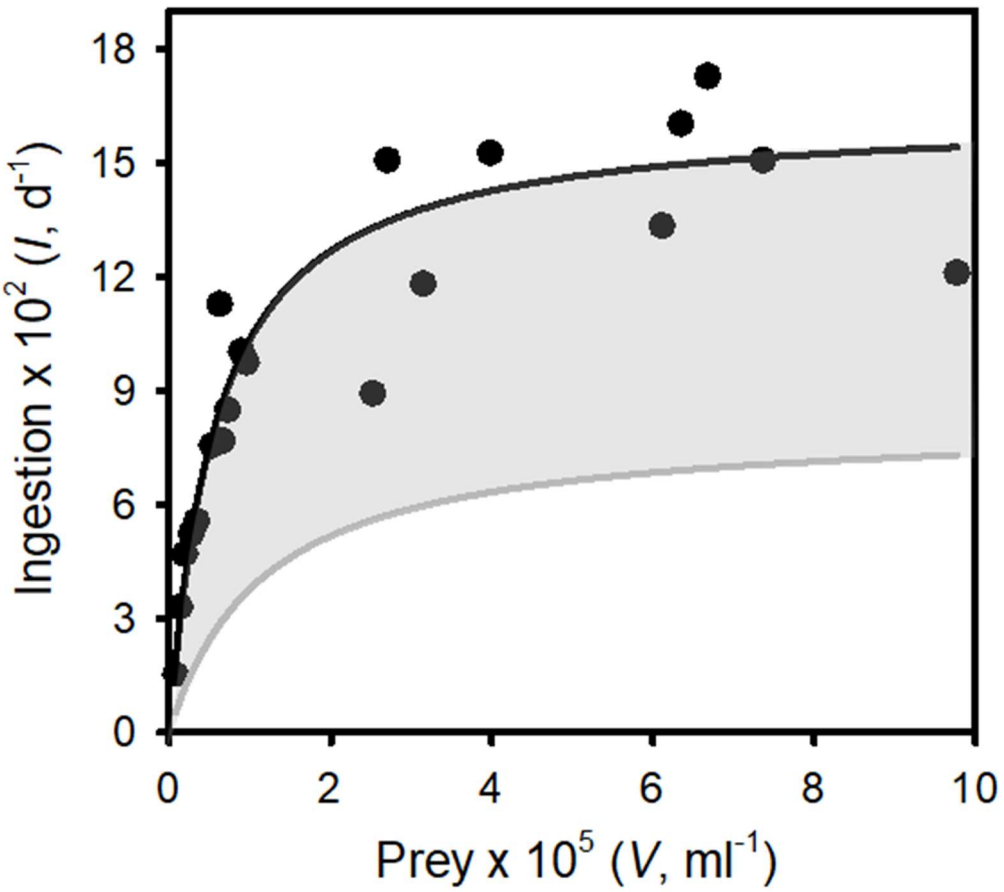


Fig. 5

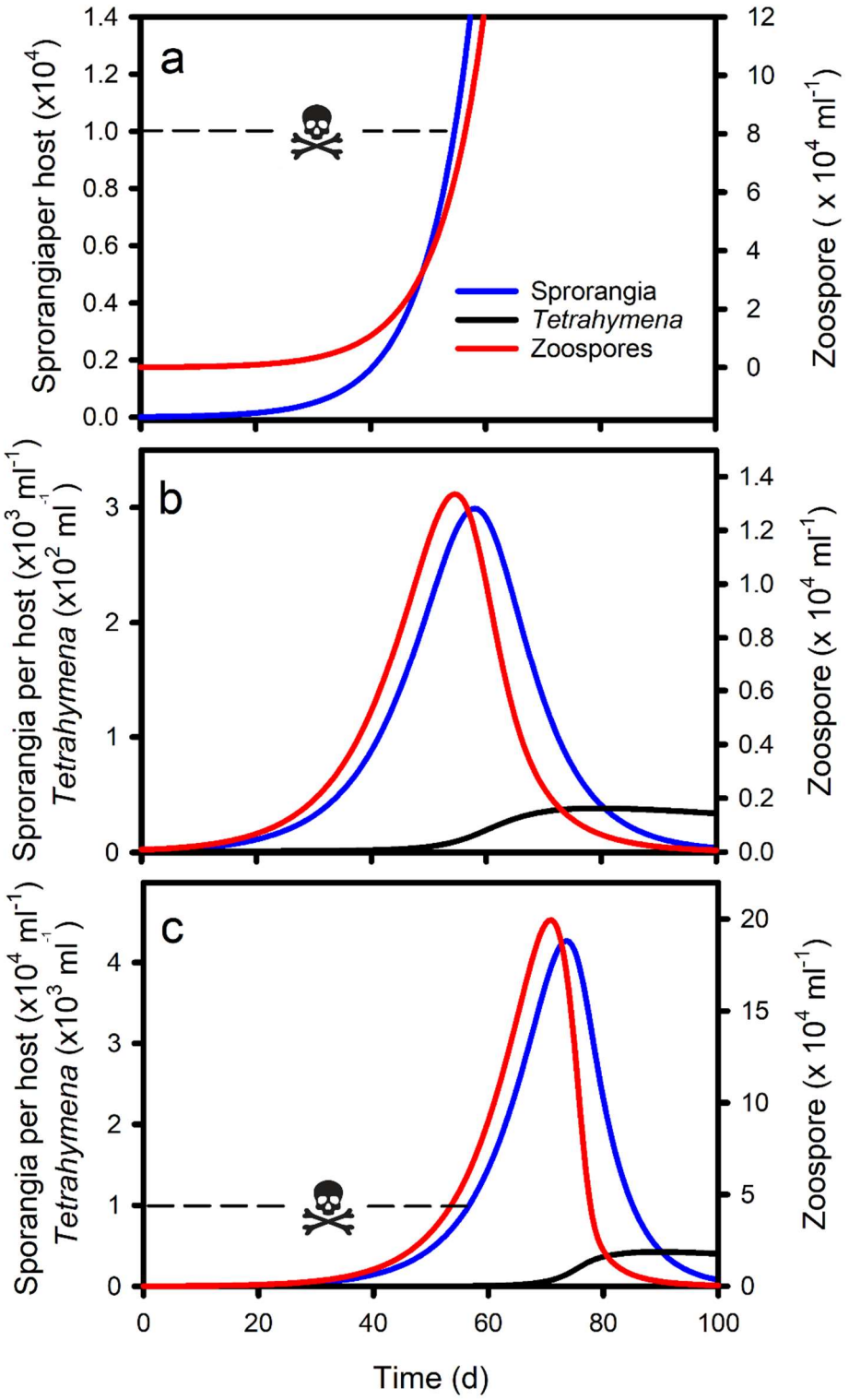
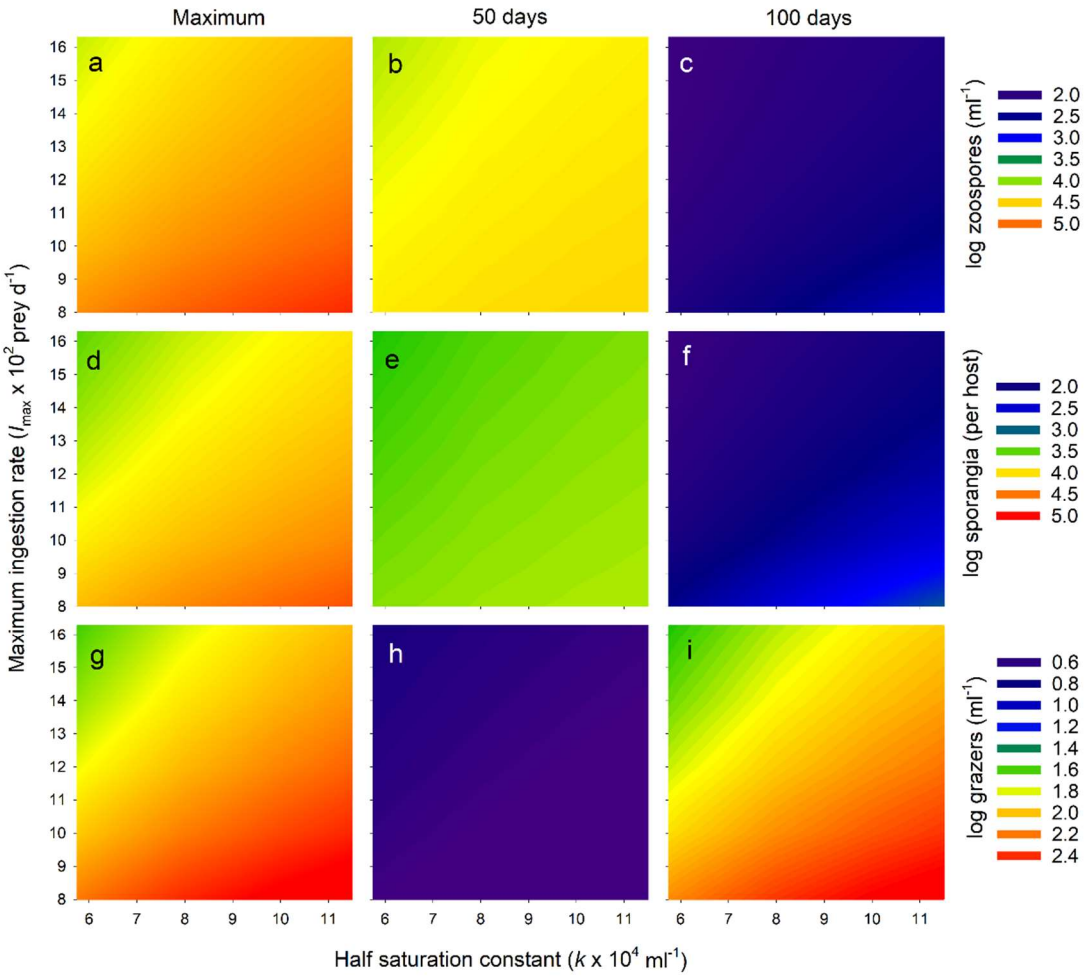

a
b
c
Sprorangiaper host (x10^4)
Zoospore (x 10^4 ml^-1)
Sprorangia
Tetrahymena
Zoospores
Sprorangia per host (x10^3 ml^-1)
Tetrahymena (x10^2 ml^-1)
Zoospore (x 10^4 ml^-1)
Sporangia per host (x10^4 ml^-1)
Tetrahymena (x10^3 ml^-1)
Zoospore (x 10^4 ml^-1)
Time (d)

Fig. 6


Maximum
50 days
100 days
a
b
c
d
e
f
g
h
i
Maximum ingestion rate (I_{max} x 10^2 prey d^{-1})
Half saturation constant (k x 10^4 ml^{-1})
log zoospores (ml^{-1})
2.0
2.5
3.0
3.5
4.0
4.5
5.0
log sporangia (per host)
2.0
2.5
3.0
3.5
4.0
4.5
5.0
log grazers (ml^{-1})
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4