Predation strategies of the bacterium *Bdellovibrio bacteriovorus* result in bottlenecks, overexploitation, minimal and optimal prey sizes

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Running title: Predation strategies of *Bdellovibrio*

Abstract

With increasing antimicrobial resistance, alternatives for treating infections or removing resistant bacteria are urgently needed, such as the bacterial predator *Bdellovibrio bacteriovorus* or bacteriophage. Therefore, we need to better understand microbial predator-prey dynamics. We developed mass-action mathematical models of predation for chemostats, which capture the low substrate concentration and slow growth typical for intended application areas of the predators such as wastewater treatment, aquaculture or the gut. Our model predicted a minimal prey size required for predator survival, explaining why *Bdellovibrio* is much smaller than its prey. A too good predator (attack rate too high, mortality too low) overexploited its prey leading to extinction (tragedy of the commons). Surprisingly, a predator taking longer to produce more offspring outcompeted a predator producing fewer offspring more rapidly (rate versus yield trade-off). Predation was only efficient in a narrow region around optimal parameters. Moreover, extreme oscillations under a wide range of conditions led to severe bottlenecks. A bacteriophage outcompeted *Bdellovibrio* due to its higher burst size and faster life cycle. Together, this suggests that *Bdellovibrio* would struggle to survive on a single prey, explaining why it is a generalist predator and suggesting it is better suited to environments with multiple prey than phage.

Highlights

- Model prediction that *Bdellovibrio* has to be much smaller than its prey to survive is consistent with empirical knowledge
- Co-existence of two predators is possible based on a prey affinity prey attack rate trade-off
- A predator that is too good (too high attack rates, too low mortality) overexploits its prey and becomes extinct (tragedy of the commons)
- Therefore, *Bdellovibrio* has an optimal attack rate constant, optimal mortality and optimal prey size
- *Bdellovibrio* preying on a single prey is prone to extinction because of very narrow parameter ranges where predation is effective and a tendency to extreme oscillations leading to bottlenecks
- A bacteriophage has several advantages over *Bdellovibrio* when preying on a single, non-resistant prey and outcompetes *Bdellovibrio*
- For the above reasons, the model predicts that preying on a single prey risks extinction of *Bdellovibrio*, explaining why *Bdellovibrio* is a generalist predator

Introduction

Predator-prey relationships are some of the oldest and most important interactions in nature and occur at every level from the smallest virus infecting a bacterium to lions attacking wildebeest. Predators are often keystone species in natural ecosystems (Paine 1969). Investigations into predator-prey dynamics in natural settings are, however, complicated by an array of confounding factors (Krebs et al. 2001). Microbes by contrast make attractive models for studying predator-prey interactions in a controlled environment, with millions of individuals in a drop of liquid that can go through many generations in a day. A completely separate but increasingly important reason for the interest in microbial predators is the antimicrobial resistance crisis, with resistance to even last resort antibiotics such as colistin rising (Zhi et al. 2016). There is an urgent need for 'living antibiotics' as alternatives to antibiotics, such as phage therapy and bacterial predators.

The best studied bacterial predator is *Bdellovibrio bacteriovorus*. It is a Gram-negative bacterium that predates a wide range of other Gram-negative bacteria (Stolp & Starr 1963), regardless of their antimicrobial resistance or pathogenicity. *Bdellovibrio* alternates between two phases in its lifecycle. In the free-living attack phase, it does not grow but hunts prey. It swims faster than most bacteria, at speeds of up to 160 µm s^{-1} or ~100 body lengths s^{-1} (Lambert et al. 2006). This requires a high metabolic rate, resulting in a loss of viability within 10 hours if prey is not encountered (Hespell et al. 1974). Once a cell is encountered, its suitability is investigated for several minutes (Hobley et al. 2006). It suitable, it enters the prey's periplasm by creating and squeezing through a pore in the outer membrane and peptidoglycan layer, shedding its flagellum in the process (Starr & Baigent 1966). Once inside the periplasm, *Bdellovibrio* kills the prey and lets the cytoplasmic nutrients leak into the periplasm (Romo et al. 1992). It also alters the prey's peptidoglycan, causing the prey cell to round up into a "bdelloplast". In this bdelloplast phase, *Bdellovibrio* uses these nutrients to grow into a long filament rather than dividing (Lambert et al. 2006). When the nutrients have been used up, this filament septates into as many new cells as resources allow for, typically between 3 and 6 new predators per *E. coli* cell (Seidler & Starr 1969). If it were using normal binary fission, it could only produce 2^n offspring, potentially forsaking prey resources if they would only suffice for say 5 rather than 8 offspring. The new *Bdellovibrio* grow flagella and lyse the remains of the prey cell, allowing them to burst out in search of fresh prey.

Mathematical models explain how predator-prey interactions can generate stable oscillations (Berryman 1992). Most models of microbial predator prey interactions focussed on protist predation of bacteria or bacteriophage infection. Only a few models considered predatory bacteria (see Table S1 for an overview of models). Varon & Zeigler (1978) fitted a Lotka-Volterra model to their experimental results, which led them to propose that a minimal prey density is necessary for the survival of the predator. Crowley's (1980) model tracked substrate levels and used Monod kinetics for prey growth, as well as included a delay

between prey attack and the production of new predators to reflect the ~3 h long bdelloplast phase. The dynamics were destabilised by higher nutrient concentrations, especially at low dilution rates, leading to extreme oscillations. Wilkinson (2001) developed two *Bdellovibrio* models, one based on a Holling type II functional response, but without specifically modelling the bdelloplast stage. The other model did include a combined predator-prey complex, but used a Holling type I functional response (Wilkinson 2001). Both models again showed a destabilising effect of nutrient enrichment, albeit somewhat ameliorated by the presence of a decoy species. Similarly, Hobley et al. (2006), Baker et al. (2017) and Said et al. (2019) included a predator-prey complex and a Holling type I functional response. Two further models have been developed by Hol et al. (2016) and Dattner et al (2017), both including spatial structure. These previous models focussed on the effects of dilution rate and substrate inflow on the dynamics of the chemostat systems. However, the effects of prey and predator characteristics such as prey size, attack kinetics and predation efficiency have not been studied.

In this study, we developed a family of models based on ingredients from previous models and examined their behaviour, identifying a unique combination of ingredients that generates realistic outcomes from realistic model assumptions. This model predicts that *Bdellovibrio* has to be much smaller than its prey for survival, which is in agreement with empirical evidence. We also found that high values for the attack rate constant and prey size and low values for predator mortality, which might be thought of as advantageous for the predator, are in fact detrimental for predator survival. Instead, these parameters have optimal values that maximize predator density. These optima occurred at the tipping point into oscillations and were often very narrow relative to the natural variation of these parameters. Moreover, we found that the system was prone to extreme oscillations in bacterial densities, leading to bottlenecks that would result in stochastic extinction. Additionally, *Bdellovibrio* would easily be outcompeted by a bacteriophage since the phage has several key advantages and only one minor disadvantage. Together, these three key predictions (narrow optima, population bottlenecks and phage superiority) suggest that *Bdellovibrio* would struggle to survive on a single prey species in a natural environment full of phages and where conditions are unlikely to be optimal and if so, not for long. Our findings are consistent with the fact that *Bdellovibrio* is a generalist predator so we posit that our model explains why *Bdellovibrio* is a generalist predator and why it has to be as small as it is.

Model development

We developed models to investigate the effects of a consumer, the predatory bacterium *Bdellovibrio bacteriovorus* or a bacteriophage, on bacterial populations under continuous culture conditions in a chemostat. The chemostat captures the low substrate concentrations and slow growth rates typical of the natural environment (Jannasch 1969). Also, the prey on its own will reach a steady state population density in the chemostat, which facilitates the investigation of oscillations caused by predation. Oscillations could not be observed in a batch culture model. We developed a family of models to investigate the effect of changes in the structure of the model on the predator prey dynamics. This structural sensitivity analysis (Hellweger et al. 2016) identified Model 6 as the most realistic in terms of assumptions and predictions (see SI text, Table S2 and Fig. S4), so we explored Model 6 further and describe it in Figure 1 and list its parameters and their values in Table 1. Model 6 included a single abiotic resource (substrate S), single prey species (N) and a single obligate predator (P). The prey species grew by consuming the substrate according to Monod kinetics. The predator had a Holling type II functional response (predation rate proportional to predator density but saturating at high predator density), bdelloplast (for *Bdellovibrio*) or infected cell (for bacteriophage) stage and mortality. The bdelloplast (B) is a distinct stage in the *Bdellovibrio* life cycle that usually lasts for 2 to 4 hours. There are distinct parallels between this bdelloplast stage and a bacteriophage infected cell. The prey cell does not grow and replicate when infected by a bacteriophage or consumed by *Bdellovibrio*. The predator or phage does not prey on or infect further prey. We modelled the bdelloplast or infected cell stage as a separate entity to account for the delay in producing offspring and the combining of the consumer and its prey. For simplicity, these entities will be referred to as a bdelloplast from now on, but the principles apply equally to an infected cell.

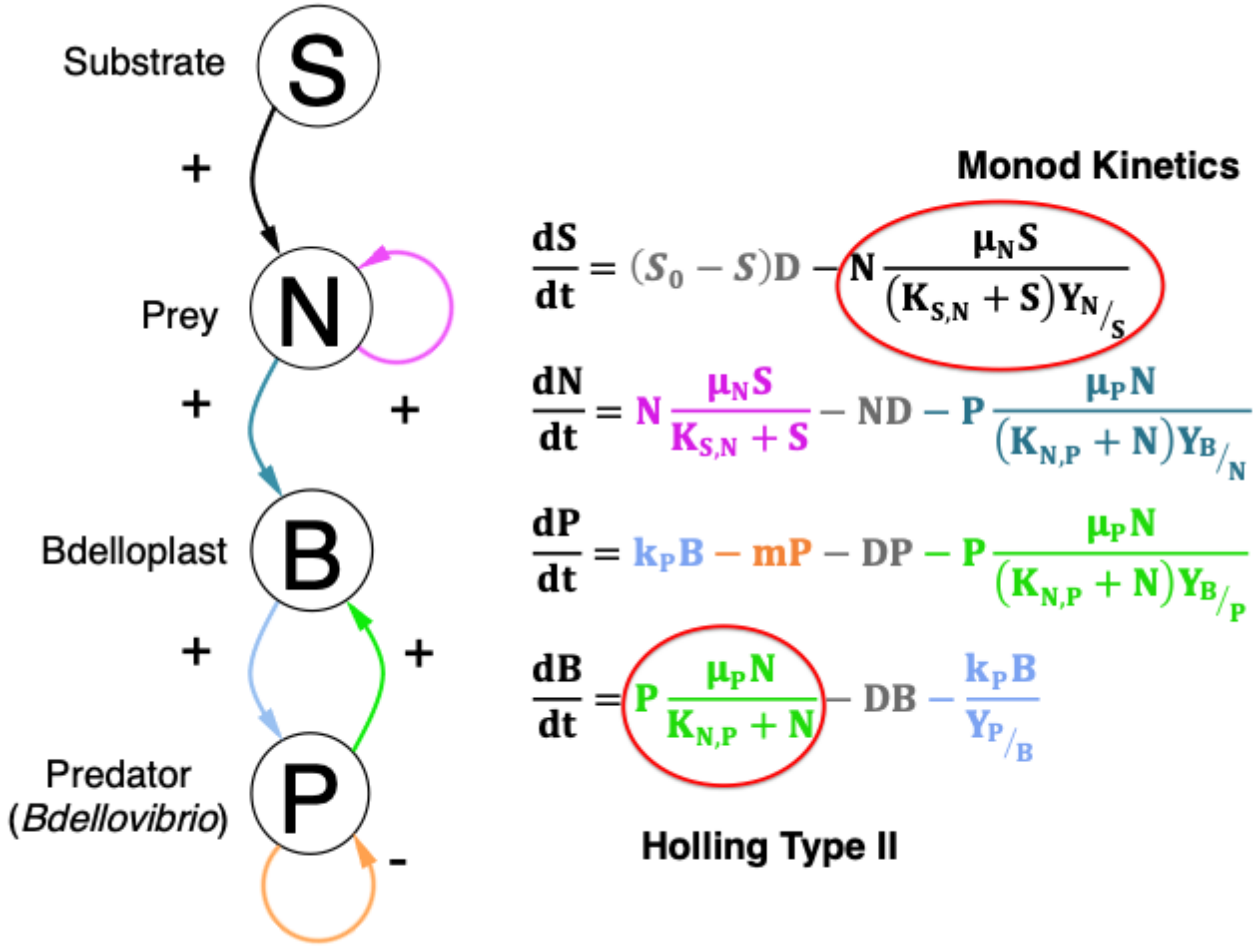

Figure 1 – Principal model used to track predator and prey densities under chemostat conditions (Model 6 in Table S2). Substrate is consumed by prey to fuel growth. Prey and predators combine to form a bdelloplast. The bdelloplast matures to give new predators. Predators have mortality. This is the model used unless stated otherwise.

Table 1 – Baseline parameters for the principal model and range over which global sensitivity analysis was performed (Model 6). All yields are expressed in the form $\boldsymbol{Y_{A/B}}$, which is the yield of consumer A per resource B consumed. The affinity of consumer B for resource A is expressed as $\boldsymbol{K_{A,B}}$.

Parameter	Units	Value	Minimum	Maximum	Source
Inflow substrate concentration (S_0)	g l^{-1}	5 x 10^{-3}	5 x 10^{-3}	2.5	Assumed to be ~2 × $K_{S,N}$
Prey maximum specific growth rate (μ_N)	h^{-1}	1.23	¾ ln(2)	3 ln(2)	(Kreft et al. 1998)
Predator attack rate constant (μ_P)	g bdelloplast g $predator^{-1}$ h^{-1}	0.38	0.3	6	(Hobley et al. 2018)
Predator affinity for prey ($K_{N,P}$)	g prey l^{-1}	8.6 x 10^{-4}	1.5 x 10^{-6}	6.5 x 10^{-6}	(Hobley et al. 2018)
Yield of bdelloplasts from predators ($Y_{B/P}$)	g bdelloplast g $predator^{-1}$	8	5	20	Relative sizes of *E. coli* and *Bdellovibrio* cells (Stolp & Starr 1963; Kubitschek & Friske 1986).
Bdelloplast maturation rate (k_P)	g predator g $bdelloplast^{-1}$ h^{-1}	0.208	0.075	0.3	(Seidler & Starr 1969)
Predator mortality rate (m)	h^{-1}	0.06	0.03	0.09	(Hespell et al. 1974)
Dilution rate (D)	h^{-1}	Always 0.05			
Prey affinity for substrate ($K_{S,N}$)	g l^{-1}	Always 2.34 x 10^{-3}			(Kreft et al. 1998)
Yield of prey from substrate ($Y_{N/S}$)	g dry mass prey g $substrate^{-1}$	Always 0.4444			(Kreft et al. 1998)
Yield of bdelloplasts from prey ($Y_{B/N}$)	g $bdelloplast^{-1}$ g $prey^{-1}$	Always $\frac{Y_{B/P}}{Y_{B/P}-1}$			Relative sizes of *E. coli* and *Bdellovibrio* cells (Stolp & Starr 1963; Kubitschek & Friske 1986).
Yield of predators from bdelloplasts ($Y_{P/B}$)	g predator g $bdelloplast^{-1}$	Always 0.625			Relative sizes of *E. coli* and *Bdellovibrio* cells and burst size (Stolp & Starr 1963; Kubitschek & Friske 1986; Seidler & Starr 1969).

Results

Model implementation and validation shows the system to be brittle

Before fully exploring model behaviour we ensured that the numerical results were reliable using a set of test simulations covering the dynamical regimes (Figure 2). Firstly, we tested all MatLab Ordinary Differential Equation (ODE) solvers and found that the Runge-Kutta ode45 solver was the only one that could handle the extreme oscillations in the linearly unstable region of parameter space correctly (Fig. S1). It was also necessary to set both absolute and relative tolerances of this solver to the unusually low value of 1×10^{-9} and to constrain variables to be non-negative to get accurate results (Fig. S2). Secondly, we used the same test set to compare simulations using biomass-based units with those using particle-based units that we needed to introduce to obtain numerically stable simulations of bacteriophage predation. The results were in agreement (SI text and Figure S3). These difficulties of obtaining correct numerical results highlight that the system tends to undergo extremely rapid changes followed by periods of stasis and is very sensitive to initial conditions and parameter settings.

Structural sensitivity analysis identifies most appropriate model

To gain a better understanding of the impact of various modelling choices, we compared a number of ordinary differential equation (ODE) and delay differential equation (DDE) models (Table S2, Fig. S4). We first ran the simulation without predators until the prey had reached a steady state (same for all models). (Model 1) Addition of a predator without a bdelloplast stage (or other form of delay between prey killing and predator birth) gave rise to stable but extreme oscillations. (Model 2) Incorporating an explicit delay of 4 hours, approximately doubled the oscillatory period from ~150 hours to ~300 hours. (Model 3) Adding mortality reduced the oscillatory period to approximately 100 hours. (Models 4-6) Addition of an explicit bdelloplast stage stabilised the system, resulting in a stable steady state of co-existing predator and prey, regardless of the predator's Holling type functional response. (Model 4 vs. 6) With a Holling type I predator functional response (Model 4), the final prey density was more than 3 times lower, and the predator density higher, than with the saturating type II response (Model 6). (Model 5) Constant input of prey to the system, this is akin to growing the prey on its own in one chemostat, which feeds into a second chemostat with prey and predator. This gave a very similar response to the single chemostat with constant input of substrate. Model 6 was chosen as the most biologically appropriate model for all further work, for several reasons. Firstly, the time taken for a *Bdellovibrio* from attaching to entering prey is in the region of ten minutes (Hespell 1976). This would be the minimum time for a successful attack in a prey-saturated environment. By contrast, the time required for a *Bdellovibrio* to consume the contents of a prey cell, convert these into new *Bdellovibrio* predators, septate and finally lyse the prey cell to release new predators is in the region of four hours (Seidler & Starr 1969). As the prey is killed very shortly after penetration (Rittenberg & Shilo 1970), there is a significant delay between prey killing and the birth of new predators that is best modelled by treating the bdelloplast stage as a separate entity. Secondly,

Bdellovibrio is known to have a high endogenous respiration rate and a correspondingly low life span in the absence of suitable prey (Hespell et al. 1974). Hence, it is appropriate to include predator mortality in the model. Thirdly, the saturating Holling type II functional response has been shown to result from a 'handling time' of the predator (Jeschke et al. 2002), i.e., the attachment and penetration time of *Bdellovibrio*. Moreover, in other work we found that a Holling type II functional response fitted much better to experimental data from batch cultures than a Holling type I response (Hobley et al. 2019).

Dynamic regimes from steady states to extreme oscillations

Model 6 has 12 parameters and six possible dynamic regimes (Fig. 2A). In order to gain a better understanding of the factors determining which regimes were observed, we swept through a range of inflow substrate concentrations (S_0) and dilution rates and evaluated the steady state and its stability analytically (see model analysis in SI). As expected, at the highest dilution rates and lowest S_0, all biological species washed out. Reducing the dilution rate or increasing S_0 enabled survival of first the prey and then the predator. Further increases in S_0 destabilised the system, resulting first in damped and then sustained oscillations, before finally reaching a linearly unstable state. We ran simulations in each of the regimes and found that they agreed with the outcomes predicted from the model analysis (Fig. 2B-G), apart from giving sustained, extreme oscillations where the analytical results predicted a linearly unstable state (Fig. 2D).

Dimensional analysis was then performed to deduce which combinations of parameters determine the qualitative behaviour of the system, yielding 7 independent parameter combinations. Essentially, rates were relative to the dilution rate, inflow substrate concentration relative to the prey's substrate affinity and predator affinity replaced by its ratio to prey affinity (for details see SI text). The next 7 sections show the effect of these 7 dimensionless parameter combinations that determine qualitative behaviour.

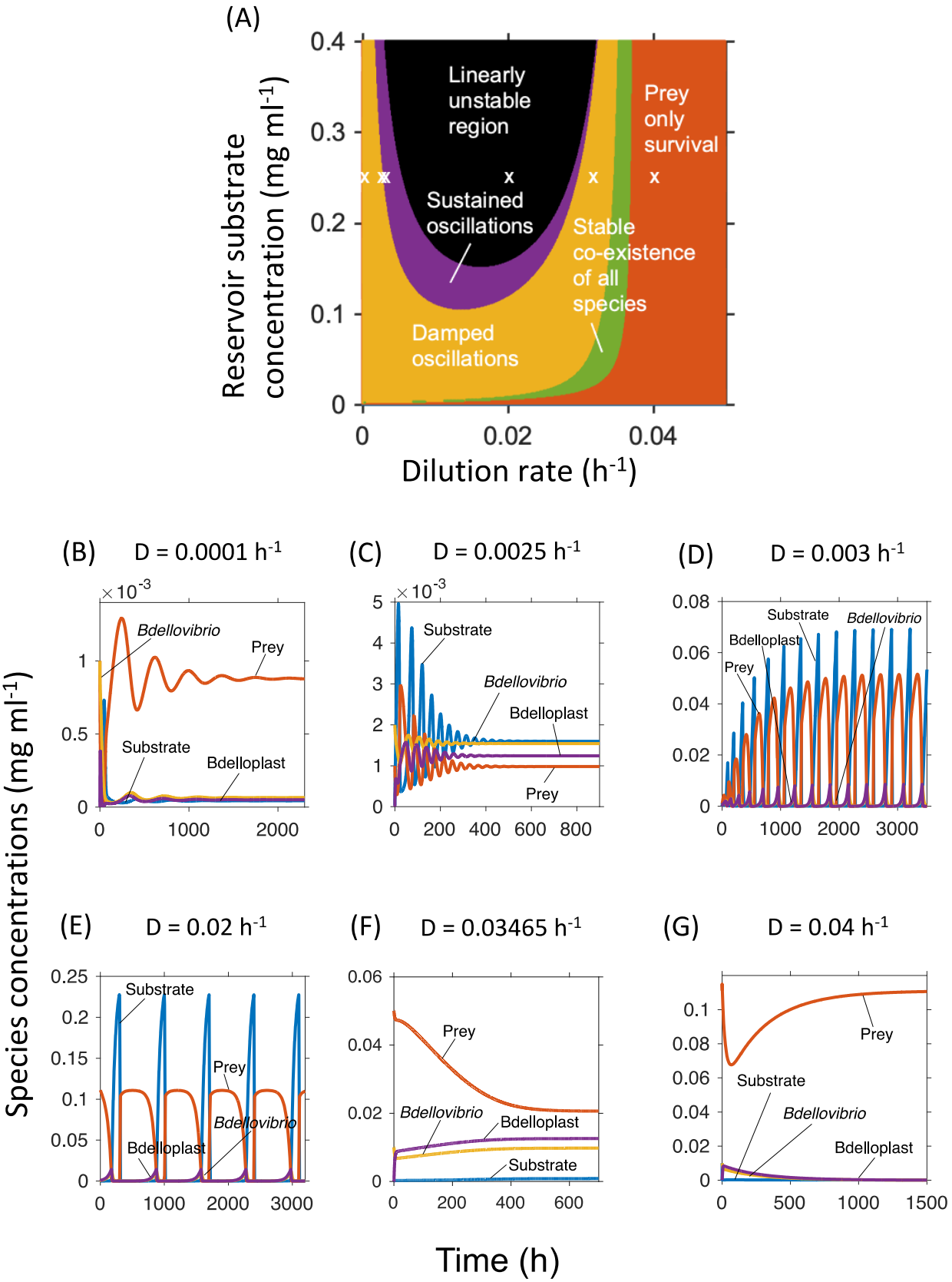

Figure 2 – Dynamic regimes of Model 6. (A) Analytically calculated regimes for varying S_0 and dilution rate. (B-G) Simulations at S_0 = 0.25 mg ml^{-1} and increasing dilution rates D, indicated by white crosses in (A). (B) Damped oscillations that ended in steady state co-existence. (C) Damped oscillations of much shorter period than (B). (D) Amplifying oscillations that ended in sustained, extreme oscillations. (E) Predicted linearly unstable region gave sustained, extreme oscillations. (F) Stable co-existence of predator and prey. (G) Predator extinction.

Bdellovibrio has a minimal and optimal attack rate constant

To our knowledge, Varon & Zeigler (1978) is the only study of predation kinetics of a relative of *Bdellovibrio*, the marine strain BM4, and *Photobacterium leiognathi* as prey. We used this study to calculate our default values for the attack kinetics. Since different *Bdellovibrio* And Like Organisms and prey combinations may have different kinetics, we investigated the effect of varying the attack rate constant. The attack rate constant in the Holling type II functional response corresponds to the catalytic rate constant of a Michaelis-Menten type saturating enzyme reaction. The ratio of attack rate constant (μ_P) and half-saturation constant ($K_{N,P}$) gives the initial slope of the Holling type II function, which determines the predation rate at low prey densities. We found that the highest attack rate constant was not best for the predator. Instead, a lower attack rate constant was optimal for the abundance of the predator and conversely for the prey (Fig. 3). The position and width of this optimum varied with dilution rate (Fig. 3C, D), and was just above a minimal attack rate constant that was necessary for the predator to survive. Increasing levels of S_0 caused the peak to narrow towards the minimal attack rate constant, which hardly changed (Fig. 3A-C), thus decreasing the range of μ_P in which the predator could achieve near maximal density. Below the optimal μ_P, there was a sharp drop to predator extinction. The optimal μ_P was also the rate at which the system underwent a Hopf bifurcation (Hopf 1948) from a stable steady state of co-existence into an oscillatory regime (Fig. 3E-G).

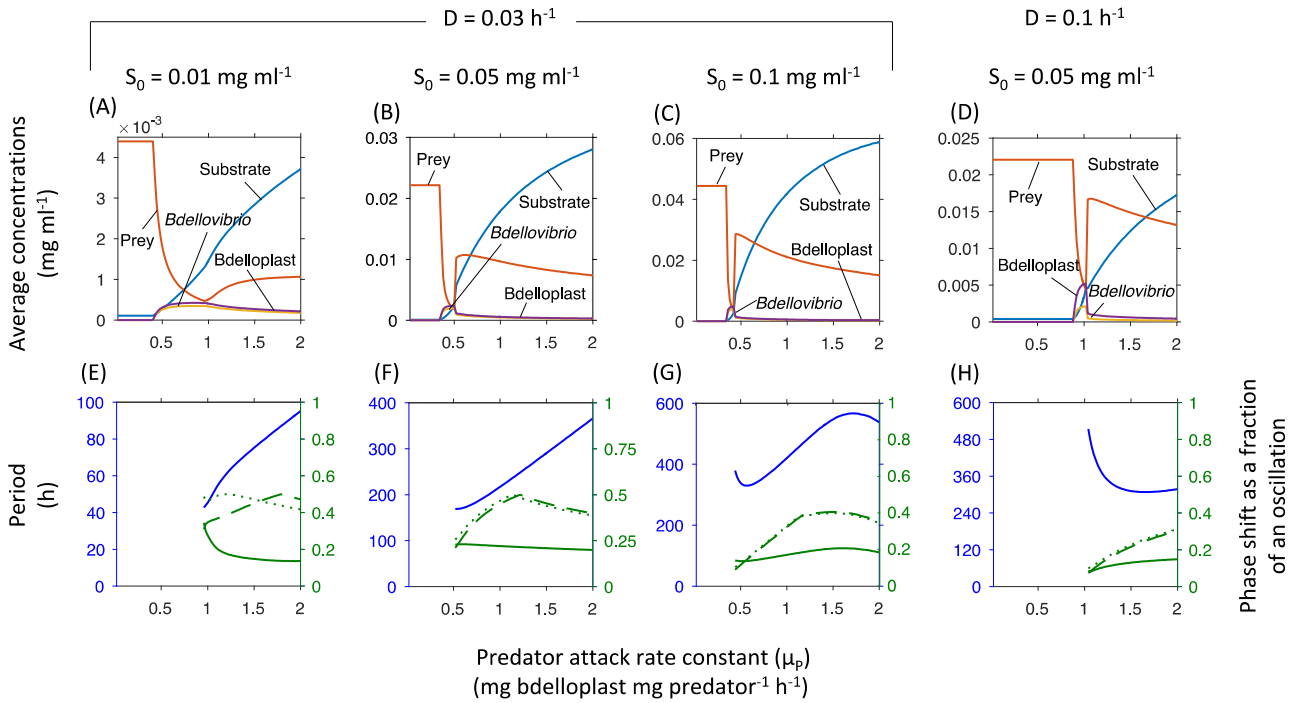

Figure 3 – Minimal and optimal attack rate constant (μ_P). The average population densities and substrate concentrations, oscillatory periods and phase shifts strongly depend on the attack rate constant, shown at increasing inflow substrate concentrations (S_0) and two dilution rates (cf. phase diagram in Fig. 2A). Top row shows concentrations at steady state or averaged over one oscillatory cycle. Bottom row shows the oscillatory period (blue, left axis) and phase shifts (green, right axis) from substrate peak to peak of prey (solid line), free *Bdellovibrio* (dashed line) or bdelloplast (dotted line). Note that oscillations start above the optimal attack rate constant. In order to obtain accurate simulation results at all parameter values, the absolute tolerance of the ode45 solver had to be reduced from 1 x 10^{-9} to 1 x 10^{-12}.

Higher prey growth rate does not benefit prey

Our default prey maximum specific growth rate is based on *E. coli* growing on glucose as its sole carbon and energy source. Since *Bdellovibrio* can prey on a wide range of Gram-negative bacteria with a wide range of maximum specific growth rates, we investigated the effects of changing the prey's maximal specific growth rate parameter (μ_N). At low inflow substrate concentration (S_0), populations were co-existing in a stable steady state and prey growth rate had no effect on prey and predator density (Fig. 4B, cf. phase diagram in Fig. 2A). At high S_0, populations were co-existing in sustained oscillations and increasing prey growth rate led to decreasing prey abundance and then a sharp drop in prey abundance at the bifurcation point where the system became stable (Fig. 4A,C). Overall, higher prey growth rate did not benefit the prey.

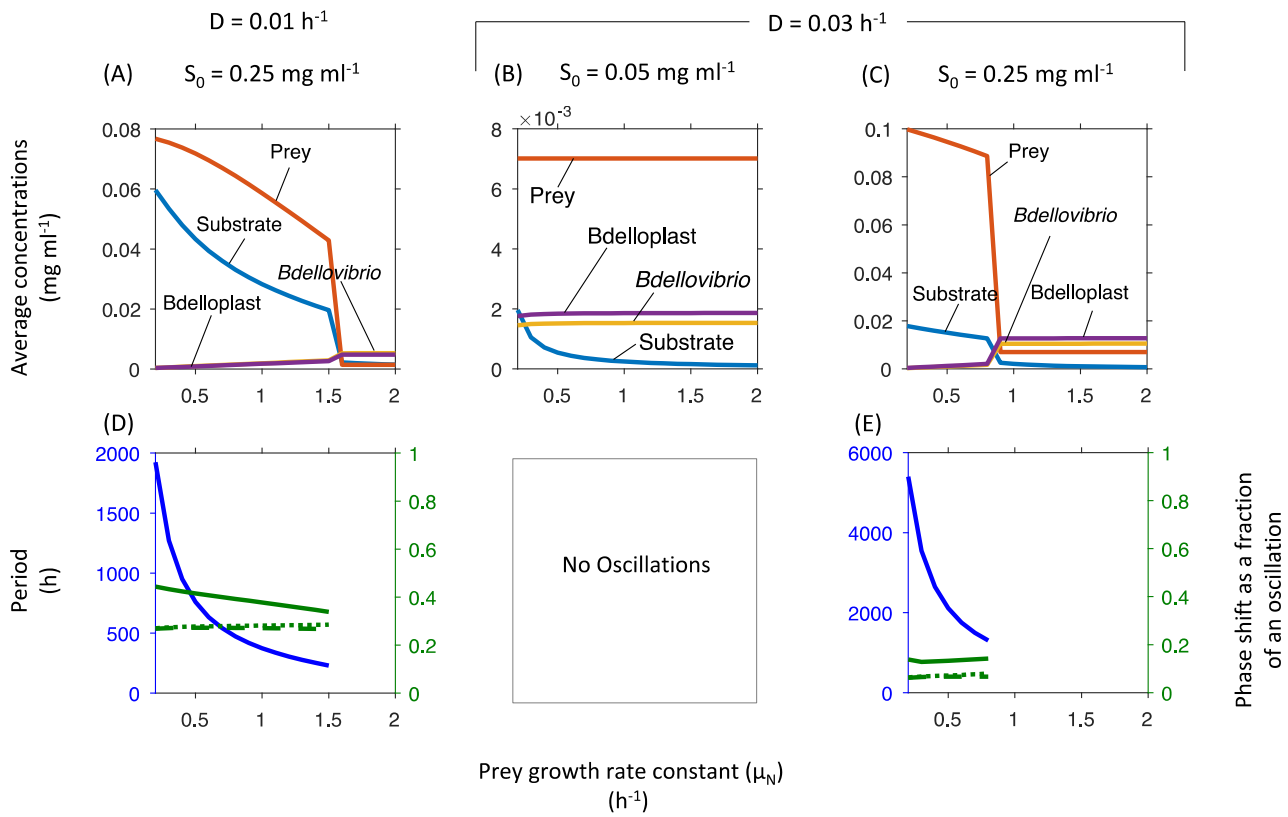

Figure 4 – Increasing the maximal specific growth rate of the prey (μ_N) leads to a sharp drop of prey density and stabilizes the system at high inflow substrate concentrations (S_0). The system was more stable at low S_0 (cf. phase diagram in Fig. 2A). Top row shows concentrations at steady state or averaged over one oscillatory cycle. Bottom row shows the oscillatory period (blue, left axis) and phase shifts (green, right axis) from substrate peak to peak of prey (solid line), free *Bdellovibrio* (dashed line) or bdelloplast (dotted line). Note that oscillations occur at the higher inflow substrate concentration and below a critical μ_N. Prey density is much higher in the oscillatory regimes where it decreases with increasing prey growth rates (prey density will of course be 0 if μ_N is zero so there is an optimal prey growth rate).

Predators benefit from mortality

Experimental observations of *Bdellovibrio* show that its mortality is much higher than the usually negligible intrinsic mortality of bacteria. Therefore, we included predator death in our model and swept mortality rates from zero to 0.2 h^{-1} – substantially more than the 0.06 h^{-1} reported for *Bdellovibrio* (Hespell et al. 1974). Surprisingly, predator death was beneficial for the predator with an optimal death rate just above a critical mortality where oscillations were replaced with stable co-existence (Fig. S5). Further increases in mortality caused predator extinction. Increasing inflow substrate concentration (S_0) and dilution rate (D) narrowed the predator peak (Fig. S5).

There is an optimal maturation rate for bdelloplasts

The bdelloplast stage, where the predator grows inside the prey periplasm with a certain specific growth rate (maturation rate) until prey resources are exhausted and offspring is produced, takes ~3 hours for *E. coli*. We found as with most other parameters that there was a minimal maturation rate required for predator survival and an optimal maturation rate (Fig. S6). This optimal rate was just below a critical rate above which populations oscillated at higher inflow substrate concentration.

Highest affinity of the predator for its prey is not always optimal

The dimensional analysis identified that the system behaviour depends on the affinity of the predator for its prey relative to the prey affinity for its substrate (see SI text). Here, we swept through a range of predator affinities. At low inflow substrate concentrations (S_0), populations did not cycle and the highest affinity of the predator for its prey (lowest $K_{N,P}$) was optimal (Fig. S7A). At high S_0, in contrast, too high prey affinities (too low $K_{N,P}$) resulted in oscillations with reduced average predator levels (Fig. S7B-E). Lowering the affinity (increasing $K_{N,P}$) resulted in a bifurcation to a stable co-existence that benefited the predator at the expense of the prey. The optimal affinity for the predator was just above this critical $K_{N,P}$ (see SI text for more details).

Increasing prey productivity benefits predator until an optimum is reached

Substrate inflow determines prey productivity. Increasing the substrate concentration in the inflow from 0 at the outset benefits the predator much more than the prey, which remains at very low levels, until a predator abundance maximum is reached. Further increasing inflow concentrations now benefits the prey more than the predator. For more details on this and bifurcations see the SI text.

Bdelloplast burst size

The last of the 7 dimensionless parameter combinations is the burst size, which increases with prey size. It is not surprising that there was a minimal burst size for predator survival but it was not expected that this minimal burst size was higher than the experimentally determined burst size for *E. coli* of 3.5 at higher dilution rates or inflow substrate concentrations (Fig. S9). We also found an optimal burst size, after which the predator abundance declined, i.e., the largest prey was not optimal. The lower the dilution rate, the lower the minimal and optimal burst size and the broader the optimum such that the optimum was in the range corresponding to typical prey sizes (Fig. S9). Increasing the burst size above the optimal value resulted in a bifurcation to extreme oscillations, corresponding to a sharp rise in prey and drop in predator average densities, but only at low inflow substrate concentration. At higher S_0, the optimum was very narrow, above values corresponding to typical prey and oscillations did not occur (Fig. S9).

Bdellovibrio versus bacteriophages

We asked whether bacteriophages outcompeted *Bdellovibrio* on single prey populations and if so, under which conditions and why. The bacteriophage was parameterised based on the well-studied T4 phage infecting *E. coli*. The bacteriophage caused oscillations and outcompeted *Bdellovibrio* (Fig. 5). Why did the phage win? There are three processes where the two predators differ. Firstly, the attack kinetics, where the phage had a higher attack rate constant (μ_P), but a lower affinity for prey (higher $K_{N,P}$). Secondly, the kinetics of prey consumption, where the phage had a higher burst size ($Y_{P/B}$) and a faster maturation rate (k_P). Thirdly, the phage did not have mortality like *Bdellovibrio*. When two species compete in a chemostat, any single advantage (no matter how small) between two otherwise identical competitors will result in the extinction of the disadvantaged competitor. To find out which advantage(s) allowed the phage to overcome the disadvantage of a lower affinity for prey, we ran competitions where the phage kept the prey affinity disadvantage and had one or more of the advantages. We found that increased burst size ($Y_{P/B}$) alone was sufficient to allow the phage to outcompete the *Bdellovibrio* (Fig. S10D). A combination of increased attack rate constant (μ_P) and reduced mortality was also sufficient (Fig. S10A,B,E). Increased maturation rate (k_P) was insufficient even in the presence of either an increased attack rate constant (μ_P) or reduced mortality (Fig. S10C,F,G).

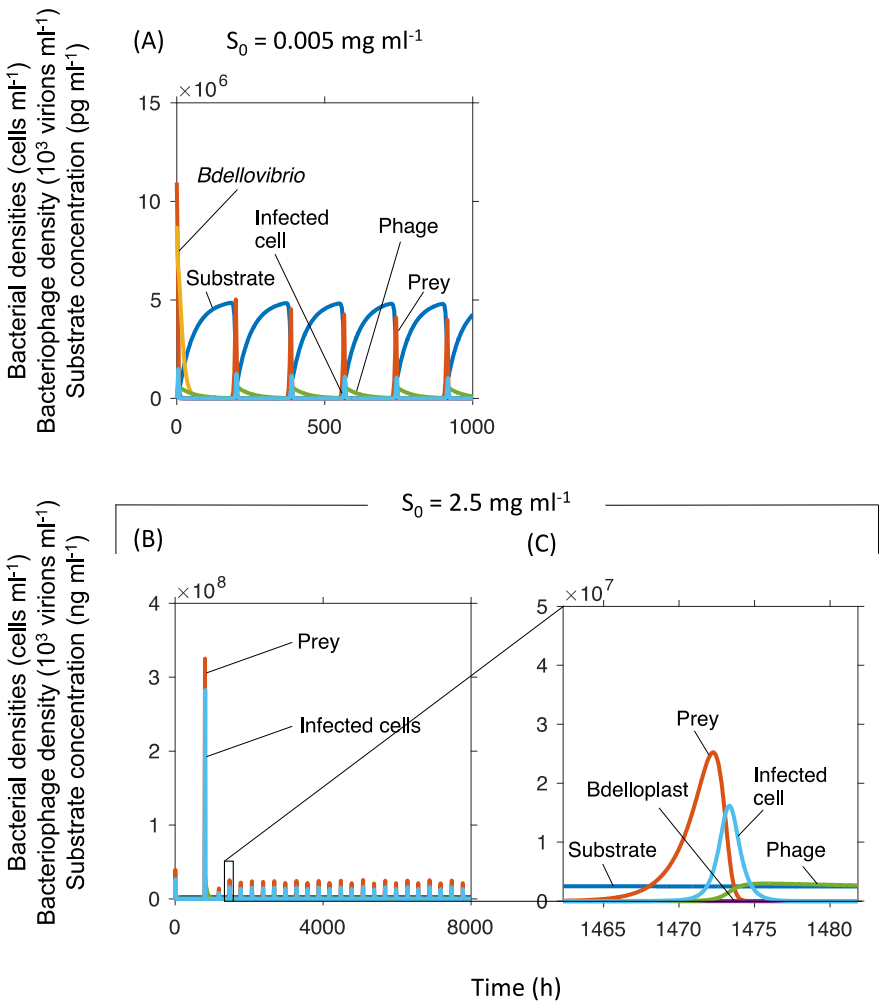

Figure 5 – T4 phage caused oscillations and outcompeted *Bdellovibrio* at two very different inflow substrate concentrations. Dilution rate was 0.02 h^{-1}. Note that units had to be changed to particle densities rather than the biomass densities used in the other sections. Panel C shows a zoomed in version of a peak in panel B, the substrate concentration appears to be constant on the scale needed to show the other variables.

Trade-off between high rate versus high yield for predators

Since it takes time to convert prey resources into predator biomass, more complete exploitation of prey resources (higher yield or burst size) should come at the cost of a longer maturation time (lower maturation rate) before offspring will emerge. We competed two predators with different strategies according to this rate yield trade-off. The fast but wasteful type of predator was capable of converting bdelloplasts into offspring at a higher rate (k_P one third higher), but had a lower burst size ($Y_{P/B}$ halved) to reflect a reduced yield. The high yield strategy outcompeted the high rate strategy (Fig. 6). Note that the phage had both higher burst size and faster maturation rate, considering this, it is not surprising that the phage won.

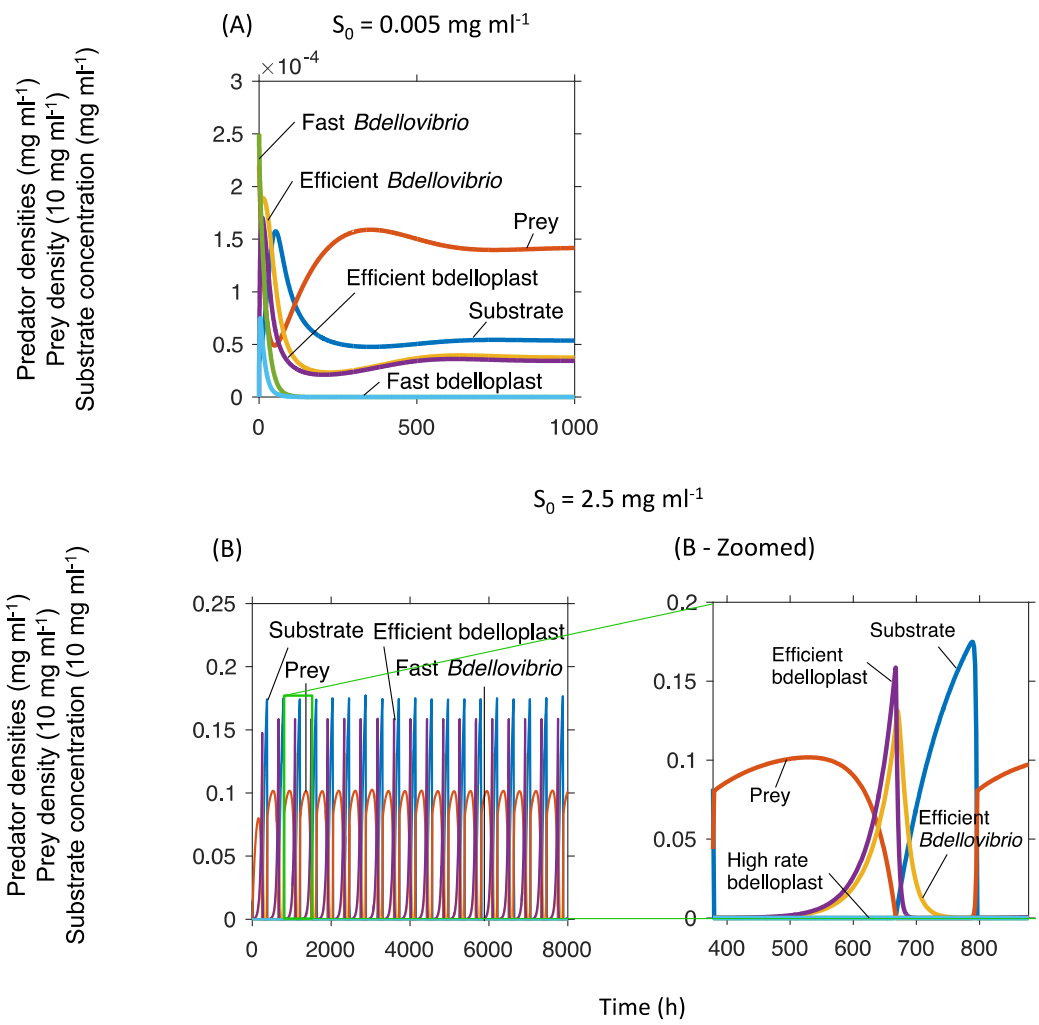

Figure 6 – A slow but efficient predator (high $Y_{P/B}$), outcompetes a fast but wasteful predator (high k_P) at different inflow substrate concentrations (S_0). Dilution rate = 0.02 h^{-1}.

Co-existence of two predators on one prey

We assumed that a trade-off between attack rate constant and affinity for prey exists because time has to be invested for finding prey as well as for binding and examining potential prey. We found that this trade-off would allow coexistence of a 'fast' predator with a higher attack rate constant and a 'high affinity' predator with lower $K_{N,P}$ on a single prey (Fig. S11).

Prey cell size

Bacterial cells have a large range of sizes (Schulz & Jørgensen 2001). Clearly there is a minimum size for a prey cell that *Bdellovibrio* as a periplasmic predator could successfully enter and consume. *Bdellovibrio* also needs to produce more than one offspring per prey cell. There may be an upper size limit as well. Cells of *B. bacteriovorus* are around seven times smaller than *E. coli* cells; this small size might enable *Bdellovibrio* to prey on a wider range of cell sizes. While consuming a larger prey cell will produce more offspring, it will take longer and larger prey cells will mean fewer prey are available if prey growth is limited

by the substrate entering the system as in our chemostat case and most environments. It is not obvious whether the higher number of offspring per prey would offset the disadvantages of longer maturation times and fewer cells to hunt. We found that there was both a minimum prey to predator size ratio required for predator persistence and an optimal value for maximal predator biomass (Fig. 7). Fat prey caused the system to display extreme oscillations. The optimal prey size was just below the size causing these oscillations. Increases in S_0 narrowed the optimum towards the minimal prey size (Fig. 7A-C). Increases in dilution rate also narrowed the peak, but increased the optimal prey size (Fig. 7C,D).

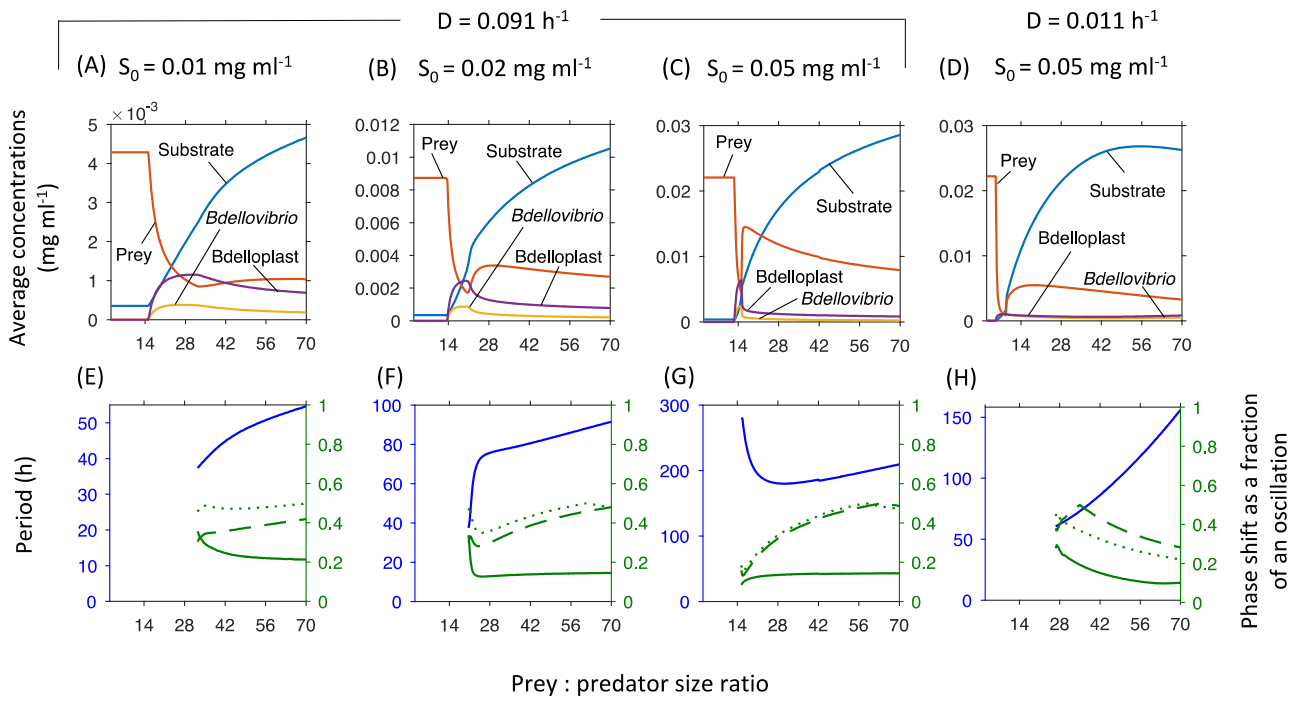

Figure 7 – Minimal and optimal prey size, relative to predator size. The average predator density showed a broad optimum at low inflow substrate concentrations (S_0) that became narrower at higher S_0 and dilution rate. Too large prey caused oscillations. Top row shows concentrations at steady state or averaged over one oscillatory cycle. Bottom row shows the oscillatory period (blue, left axis) and phase shifts (green, right axis) from substrate peak to peak of prey (solid line), free *Bdellovibrio* (dashed line) or bdelloplast (dotted line).

Bottlenecks, permanence and paradox of enrichment

When sweeping parameters, it became clear that the system oscillated with long periods of hundreds of hours and extreme amplitudes for many of the conditions tested, with extremely low minimum values (< 1 x 10^{-30} mg dry mass ml^{-1}). Such bottlenecks would result in the extinction of the predator (or both predator and prey) in any natural system prone to extrinsic fluctuations and governed by stochastic processes. We therefore examined how prey size (previous section) in combination with dilution rate and inflow substrate concentration (cf. Fig. 2A) would affect robust predator persistence (union of regions of stable co-existence and damped oscillations). Robust persistence is known as permanence and is the case when population

densities do not become arbitrarily close to zero or infinity, i.e., when the boundaries of $\mathbb{R}_+^n$ are repellers (Jansen & Sigmund 1998). The prey size range allowing permanence narrowed with increasing inflow substrate concentration (Fig. S12). This effect of increased productivity (higher influx of resource for prey) destabilising the system is known as paradox of enrichment (Rosenzweig 1971) and is typical for predator prey systems. Prey also had to be unrealistically large to enable permanence at higher dilution rates and lower productivity.

Tragedy of the commons

Similarly to increasing productivity, the prey size range enabling permanence shrank with increasing attack rate constant of the predator (Fig. S13). This is an example of the tragedy of the commons, where overexploitation of a shared resource known as the commons is to the detriment of the users of the resource, such as overfishing (Hardin 1968). Here, a too effective predator that consumed its prey faster than it could regrow led to extinction of the predator. Increases in the inflow substrate concentration (S_0) narrowed the prey size range for permanence further (Fig. S13A-C), whilst increases in dilution rate expanded the range (Fig. S13A,D-E).

Global parameter sensitivity analysis

When parameterising our model, we based our values on literature data from the best studied predator strain (*B. bacteriovorus* strain HD100) predating the best studied prey (*E. coli* MG1655) growing on glucose as the sole carbon and energy source (Table 1). To understand how much the system behaviour would change if the parameters were different due to changes in substrate, prey or predator species, we conducted a global sensitivity analysis for each of the parameters identified from the dimensional analysis (SI text and Figs. S14, S15). Each parameter was changed by 1% for 10,000 settings of the other parameters over a range given in Table 1. Substrate concentration was only sensitive to the inflow substrate concentration (proportional response, Figs. S14A, S15A). Prey density was not sensitive to substrate concentration and prey growth kinetics (Monod parameters) but sensitive to predator parameters, particularly attack rate constant and biomass burst size where the response was more than proportional (Figs. S14B, S15B). Predator densities were also particularly sensitive to the attack rate constant but also to the maximal specific prey growth rate and less so to the yield of bdelloplast per predator, $Y_{B/P}$ (Figs. S14C, S15C). Predator and bdelloplast densities responded similarly to parameter changes apart from two parameters governing the conversion between bdelloplast and predator (bdelloplast maturation rate k_P and yield of bdelloplast per predator, $Y_{B/P}$, Figs. S14C,D, S15C,D).

Discussion

Our results show that *Bdellovibrio* consuming a single prey species can only survive permanently within a very narrow range of conditions. We therefore characterise this predator prey system as 'brittle'. For example, over a wide range of conditions, the system is prone to extreme oscillations with periods of over a hundred hours and bacterial densities dropping below 0.1 pg ml^{-1}. In a deterministic mathematical model, species densities can eventually recover from even the smallest positive number but in a biological system, there has to be at least a single cell left (~1 pg). More importantly, when a system contains just a few cells over a long time, stochastic fluctuations will almost inevitably result in the loss of those few cells, leading to local extinction. For most system parameters, there was a narrow range of values that allowed the predator to reproduce fast enough to avoid being washed out without triggering these oscillations, and the optimal value to maximise predator numbers occurred just above or below the threshold triggering these oscillations. Increasing nutrient concentrations narrowed this survival range. Previous models of microbial predator prey interactions in chemostats, including those with *Bdellovibrio* as the predator, also showed a tendency to extreme oscillations, especially for higher nutrient concentrations (Crowley et al. 1980; Nisbet et al. 1983; Wilkinson 2001). Only the group of Varon studied this in chemostats experimentally with a *Bdellovibrio* like predator and *Photobacterium leiognathi* as prey. They did observe oscillations, albeit less extreme ones than those seen in our model (Varon 1979; Varon et al. 1984).

Given the brittle behaviour predicted by the model it is surprising that *Bdellovibrio* is ubiquitous in non-marine and *Bdellovibrio* like organisms are ubiquitous in marine environments (Shilo & Bruff 1965; Williams 1987; Williams, Schoeffield, et al. 1995; Shemesh & Jurkevitch 2004). This suggests that there must be other forces at work stabilising population numbers. One possibility is that *Bdellovibrio* is a hotspot organism targeting habitats of high prey density, or structured environments containing areas of high prey density such as biofilms, and that these hotspots are the sources of *Bdellovibrio* in other habitats. Biofilms represent a lump of prey for *Bdellovibrio*. It possesses the lytic enzymes needed to chew the extracellular matrix that holds cells within the biofilm and can likely derive valuable nutrients from this (Im et al. 2018). *Bdellovibrio* can predate the metabolically inactive cells found deeper within biofilms and its gliding motility allows it to move within the biofilm (Lambert et al. 2011). *Bdellovibrio* is also highly motile and contains chemotaxis genes that might enable it to locate these prey rich areas (Hobley et al. 2012). Indeed, there is ample evidence that *Bdellovibrio* is a hotspot organism. Higher numbers are found in sewage than in rivers (Dias & Bhat 1965; Seliavko & Lambina 1985), and downstream sewage treatment plants than upstream (Staples & Fry 1973) or in sewage treated soils (Klein & Casida

1967). The prey rich rhizosphere sustains higher predator numbers than the bulk soil (Jurkevitch et al. 2000). Eutrophic lakes support higher numbers than oligotrophic lakes (Chauhan et al. 2009). *Bdellovibrio* like organisms are more abundant in marine sediments than the water column and much higher in oyster shell biofilms (Williams, Kelley, et al. 1995). Bdellovibrio are likewise more common in trickling filter biofilms than in their inflow (Fry & Staples 1976).

However, the models predict a more narrow range of conditions for survival at the higher nutrient concentrations expected in hotspots. Therefore, it is unlikely that these hotspots would sustain *Bdellovibrio* if it were preying only on a single prey species. Moreover, our model also suggests that bacteriophage would outcompete *Bdellovibrio* under all conditions tested. This is because phage, which are specialist predators, have several big advantages. Indeed, bacteriophage are far more numerous in nature than *Bdellovibrio* (Kutter & Sulakvelidze 2004; Stolp 1973). This is despite the fact that phage have several disadvantages that we did not consider. First, half of the sequenced bacterial genomes contain a CRISPR-CAS system providing adaptive immunity against phage (Koonin & Wolf 2015); restriction enzymes cutting up phage DNA are also common (Bayliss et al. 2006). Second, bacteria can rapidly evolve resistance to phages (Luria & Delbrück 1943), in contrast to *Bdellovibrio*, although phenotypic plasticity of prey can afford temporary protection against *Bdellovibrio* (Shemesh & Jurkevitch 2004). Third, phage require a metabolically active host cell to replicate whereas *Bdellovibrio* can consume dormant prey (Stolp & Starr 1963). Fourth, phage do not benefit from chemotaxis as *Bdellovibrio* likely does. Nevertheless, phage outnumber *Bdellovibrio*, suggesting that these four factors are not that important. Overall, our results suggest that *Bdellovibrio* must prey on several prey species to survive. Indeed, all known *Bdellovibrio* and like organisms have a wide prey range (Stolp & Starr 1963; Jurkevitch et al. 2000; Dashiff et al. 2011; Feng et al. 2016).

One might expect that prey cells have to be large enough to give rise to two predator offspring. Surprisingly, our model predicts that *Bdellovibrio* needs prey that is at least 7 times larger than itself. Indeed, this is about the difference in size between *Bdellovibrio* and *E. coli* (Cover et al. 1984) and other typical prey are of similar size. However, the predicted optimal prey size is considerably larger. Maybe *Bdellovibrio* cannot be much smaller than it already is.

Our model also predicts that a predator that kills its prey too efficiently (has a too high attack rate, or too little mortality) will drive its prey to extinction to become extinct itself, resulting in a tragedy of the commons (Hardin 1968). Indeed, *Bdellovibrio* has a much higher mortality rate than other bacteria. While this may make over-exploitation of prey less likely, the high mortality is likely caused by its high energy expenditure when swimming fast and not feeding at the same time, also its small size prevents storage of large energy reserves (Hespell et al. 1974).

In conclusion, our model results suggest that *Bdellovibrio* and like organisms are unlikely to survive in most natural environments if they were preying only on single prey species. They would also be outcompeted by phage. In line with empirical evidence, *Bdellovibrio* ought to be a generalist predator and would only thrive in prey density hotspots – which it should be able to find by chemotaxis. For application as a living antibiotic to reduce the abundance of pathogens or antimicrobial resistant bacteria in aquaculture or plant and animal agriculture, *Bdellovibrio* would be expected to be more effective where multiple prey species are available, not only the target species.

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