

Predation in the Presence of Decoys: An Inhibitory Factor on Pathogen Control by Bacteriophages or Bdellovibrios in Dense and Diverse Ecosystems

Running title:

Predation in the Presence of Decoys

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Abstract

Several attempts have been made at the removal of specific pathogens from the intestinal microflora using either bacteriophages or "predatory" bacteria such as *Bdellovibrio* spp. To date these attempts have had mixed success. A mechanism explaining these findings based on competitive hindrance by non-prey, or decoy species is put forward. It is shown that this hindrance tends to damp out predator-prey oscillations, and therefore reduces the probability of prey extinction. Possible experiments to verify this theory are discussed. The decoy effect may play a role in any system with high densities of bacteria or other particulate matter, such as activated sludge or biofilms.

List of symbols

Species

X_0	concentration of limiting substrate for X_1
X_1	prey species concentration
X_2	non-prey, or decoy species concentration
Y	total predator species concentration
Y_{free}	free predator species concentration
$[X_1Y], [X_2Y]$	concentrations of complexes of predator bound to X_1 and X_2

Parameters

A_{col}	collisional cross-section
D	dilution rate
d_y	starvation rate, or maintenance energy term for species Y
K_1	Michaelis-Menten constant for growth of X_1 on X_0
K_X	Michaelis-Menten constant for growth of Y on X_1 in the absence of X_2
K_X^*	Michaelis-Menten constant for growth of Y on X_1 in the presence of X_2
K_{inh}	inhibition constant due to decoy effect
k_1, k_2	dissociation rate constants of complexes $[X_1Y]$ and $[X_2Y]$
μ_1	maximum specific growth rate of X_1 on X_0
μ_y	maximum specific growth rate of Y on X_1
r	collision rate constant
S	input concentration of X_0
V_1	maximum specific uptake rate of X_0 by X_1
V_y	maximum specific uptake rate of X_1 by Y
v_{pred}	mean swimming velocity of Y
y_x	net yield of Y biomass per unit of X_1

Introduction

The members of the genus *Bdellovibrio* are obligately predatorial bacteria, which prey on other gram-negative bacteria. Though they are less selective than bacteriophages in their prey selection, each strain preying on a range of gram-negative species (Drutz, 1976; Myamoto and Kuroda, 1975; Westergaard and Kramer, 1977), they are by no means generalists. Bdellovibrios are small, aerobic, highly motile curved rods. The free swimming "attack phase" of these bacteria can reach speeds of some 100 $\mu\text{m/s}$, or seventy body lengths per second. They appear to shoot randomly through the medium, and collide with prey species by chance, rather than using chemotaxis (Straley and Conti, 1977). After colliding with their gram-negative prey, they penetrate the outer membrane and develop in the periplasmic space. The prey cell becomes a rounded "bdelloplast", and finally lyses after division of the bdellovibrios. This lifestyle bears some similarities to that of bacteriophages, which find their host by diffusion, inject their genetic material, proliferate within the cell and emerge after cell lysis. Bdellovibrios occur in a wide range of habitats, ranging from low density, aquatic, ecosystems (Richardson, 1990; Schoeffield and Williams, 1990; Rice *et al.*, 1998), to high density ecosystems such as rhizosphere soil communities (Elsherif and Grossmann, 1996) and the gut flora of some vertebrates (Imbragimov, 1980). There are indications that they may associate with surface biofilms in aquatic environments (Kelley *et al.*, 1997). Some sources state that a prey density of at least $10^4/\text{ml}$ is required to support a bdellovibrio population (Rice *et al.*, 1998). However, bdellovibrios may survive starvation periods in stable bdelloplasts (Sanchez-Amat and Torrella, 1990).

In line with other forms of biological pest control, bdellovibrios (Jackson and Whiting, 1992; Fratamico and Whiting, 1995) and other predatory bacteria (Casida and Lukezic, 1992) have been proposed as agents for biological control of pathogenic or food spoilage bacteria. However, the few attempts to control the numbers of gram-negative pathogens using *Bdellovibrio* spp. in the intestinal microflora have met with no or limited success to date (Imbragimov, 1980; Westergaard and Kramer, 1977). On the face of it this may seem curious: introducing a predator into an ecosystem in which there is plentiful prey, it fails to

colonize. One simple explanation could lie in the aerobic nature of these predatory bacteria (Westergaard and Kramer, 1977), though survival and growth under anaerobic and microaerobic conditions respectively have been reported (Schoeffield, *et al.* 1996). However, obligate aerobes such as *Pseudomonas* spp. can grow in the intestine, and isolation of *Bdellovibrio bacteriovorus* from the intestines and faeces of mammals has been reported (Imbragimov, 1980). In a similar vein, attempts at using lytic phages to eliminate pathogens have met with varying degrees of success, ranging from a total lack of prophylactic effect (Sarkar *et al.*, 1996) to a good measure of protective, and even curative effect during acute diarrhoea (Smith and Huggins, 1983).

In this article, a mechanism is proposed which may explain some of these observations. The theory is based on interactions between the predator and non-prey species, which coexist with the prey in the ecosystem. Such interactions have been found by Drutz (1976), who observed that *B. bacteriovorus* strains could not predate on *Neisseria gonorrhoeae*, but that occasional, transient attachment was observed. Furthermore, though the violent collisions seen between predator and prey were not observed between predator and non-prey bacteria, the predators were observed moving around the non-prey cells as if attempting to find a binding site for some time, before "losing interest" in the non-prey cell. The observations of Drutz were made in population densities of 10^7 - 10^9 per ml. It is shown that at higher densities, such as found in the gut microflora, this behaviour can have profound consequences on the population dynamics of the predator-prey system.

Of course, non-specialist, protozoan predators thrive in the rumen of livestock (Newbold *et al.*, 1996). Any theory attempting to explain the failure of treatment by specialist predators must also account for the fact that these generalists survive, or even thrive in similar conditions.

Though the gut microflora is highly complex both in its composition and in the fluid dynamics of the system, a chemostat model formalism is used in this paper throughout, for two reasons. Firstly, chemostat models have been used extensively as *in vitro* models of the gut ecosystem. Though multicompartiment and intermittent flow systems perform better than single compartment, constant flow systems, the latter category is capable of reproducing a

number of important properties of especially the luminal flora of the large intestine (Nisbet *et al.* 1993, Nuotio and Mead, 1993, Marteau *et al.* 1997). Since this is the compartment with the highest densities, this is the most important compartment to model well in view of the current model. A similar situation is seen in the very few computer simulation models of the gut ecosystem, where chemostat-like models (Freter *et al.* 1983, Coleman *et al.* 1996) are used in all cases but the MIMICS simulator developed in our department (Wilkinson, 1997), which uses a reaction/diffusion system with bulk motion included.

The second, more important reason for using the chemostat formalism is that analytical results can be obtained easily, and stability analysis is straightforward. The partial differential equations involved in the reaction/diffusion/bulk-motion system used in the MIMICS simulator should give a more accurate simulation in the gut microflora case, but analytical solutions cannot be obtained.

The Decoy Mechanism

Predation in the presence of decoys

In the following discussion we will use the double-Monod model (Canale, 1969) with and without extensions for maintenance energy costs (Nisbet *et al.*, 1983) to study the effect of decoys on the dynamics of predator prey interactions. Though the dynamic energy budget model (Kooi and Kooijman, 1994a, 1994b) models certain features of microbial predator prey system more accurately, the models of uptake of prey by predators (by Michaelis-Menten terms) are identical for both models. As will be shown, the effect of decoys lies in altering the Michaelis-Menten constant of this uptake process, so the impact of this effect should be similar in both models. Besides, the model of Kooi and Kooijman (1994a), like that of Jost *et al.* (1973), is designed to take the size distribution of the predator into account. They pertain to predators larger than their prey, which divide into smaller (juvenile) cells after reaching a certain size. Bdellovibrios and bacteriophages are smaller than their hosts, and divide inside the host, emerging as full-grown predators. Therefore, the population size structure is not expected to be important. In the case of bdellovibrios, maintenance energy must be taken into

account, but not in the case of bacteriophages. Therefore, I use the simpler double-Monod model, with extensions with maintenance energy as in (Nisbet *et al.*, 1983). The model of Marchand and Gabignon (1981) for the *B. bacteriovorus* - *Escherichia coli* system differs from the models discussed above, in that they model infected and non-infected prey cells explicitly. This will also be done in the discussion of the decoy effect. However, Marchand and Gabignon do not introduce a third species into the interaction.

Thus, the growth of microbial predators Y on prey species X_1 is modelled as:

$$\frac{dY}{dt} = \frac{\mu_y X_1}{K_X + X_1} Y - (D + d_y) Y \quad (1)$$

in which μ_y is the maximum specific growth rate, K_X the saturation constant, D is the dilution rate of the chemostat, and d_y is the starvation rate, or maintenance energy term (Nisbet *et al.*, 1983). The differential equation for species X_1 is

$$\frac{dX_1}{dt} = \frac{\mu_1 X_0}{K_1 + X_0} X_1 - \frac{V_y X_1}{K_X + X_1} Y - DX_1 \quad (2)$$

In which X_0 is the concentration of the limiting substrate, μ_1 is the maximum specific growth rate, K_1 is the saturation constant, and V_y is the maximum specific uptake rate of prey by predator. The differential equation for the limiting substrate becomes

$$\frac{dX_0}{dt} = D(S - X_0) - V_1 \frac{X_0}{K_1 + X_0} X_1 \quad (3)$$

in which S is the input substrate concentration, and V_1 is the maximum specific uptake rate of X_0 by X_1 .

We will now take the case of a three species ecosystem: one prey species X_1 , one non-prey species X_2 , and a predator Y . Species X_2 is not modelled explicitly using differential equations of its own, since we assume it uses a different limiting substrate than species X_1 .

This is done to study the effect of the simple presence of a "decoy" species independently of any other competition effect. Though the word "decoy" might be thought of as implying attraction of the predator or parasite species, this is not intended. The presence of the decoy species simply leads to collisions with the predator, which is assumed to move through the medium randomly.

Disregarding starvation, the predator can be in three states: free, bound to X_1 , and bound to X_2 . These complexes are denoted as $[X_1Y]$ and $[X_2Y]$. Assume the rate of collisions is r per unit of prey or non-prey species per unit of predator. Since chemotaxis towards prey has not been observed in *Bdellovibrio* spp. (Straley and Conti, 1977) and bacteriophages are not motile, this rate is assumed to be identical for prey and non-prey species. Furthermore, the prey/predator complex dissociates at a rate of k_1 , and non-prey/predator complex dissociates at a rate of k_2 . However, only the dissociation of the first complex yields new predators, with a yield of $y_x + 1$. Since one phage or predator is lost in the forming of this complex, the net yield per unit of prey biomass is y_x .

The central assumption of this theory is that after colliding with a non-prey cell, a predator will briefly attach, before detaching, as observed in *N. gonorrhoeae* - *B. bacteriovorus* systems (Drutz, 1976). The rate constant k_2 will therefore be finite. We also assume that all reaction rates are an order of magnitude higher than the dilution rate of the entire system, for the sake of quasi-steady state analysis. Since the dilution rate of the large intestine is in the order of once per day, this assumption should hold reasonably well there. This leads to the following set of differential equations:

$$\frac{dY_{free}}{dt} = (y_x + 1)k_1[X_1Y] + k_2[X_2Y] - r(X_1 + X_2)Y_{free} \quad (4a)$$

$$\frac{d[X_1Y]}{dt} = -k_1[X_1Y] + rX_1Y_{free} \quad (4b)$$

$$\frac{d[X_2Y]}{dt} = -k_2[X_2Y] + rX_2Y_{free}. \quad (4c)$$

The term At steady state we have

$$[X_1Y] = \frac{r}{k_1} X_1 Y_{free} \quad \text{and} \quad [X_2Y] = \frac{r}{k_2} X_2 Y_{free}.$$

Remembering that the total predator content Y is

$$Y = [X_1Y] + [X_2Y] + Y_{free} = \left(1 + \frac{r}{k_1} X_1 + \frac{r}{k_2} X_2 \right) Y_{free}$$

and summing the equations (4a,b,c) we find a growth rate of:

$$\frac{dY}{dt} = \frac{y_x k_1 X_1 Y}{k_1/r + X_1 + k_1 X_2/k_2} = \frac{\mu_y X_1 Y}{K_X + X_1 + K_{inh} X_2}, \quad (5)$$

in which we recognize the standard form of competitive inhibition. Therefore, in an extremely densely populated *and* diverse ecosystem, such as the intestinal microflora, specialist predators would be in a serious disadvantage compared to generalists.

The reasoning (and differential equations) used above can be used without adaptation for lytic bacteriophages.

The consequences for the ecosystem

In the following discussion we use the differential equations given by equations (1) through (3). The decoy species is not considered to be a competitor of the prey, so its actions can be absorbed into the saturation constant

$$K_X^* = K_X + K_{inh} X_2. \quad (6)$$

This set of differential equations was studied in detail by Canale (1969). Four different "modes" of operation can be identified (Kooi and Kooijman 1994a): (0) total washout of both species; (I) stable prey population with washout of predator; (II) stable coexistence of predator and prey, and (III) unstable coexistence (limit cycle behaviour). If both species coexist, the (stable or unstable) focus is given by:

$$X_0 = \frac{1}{2} \left(S - K_1 - \frac{V_1 K_X^*}{\mu_y - D - d_y} \pm \sqrt{\left(S - K_1 - \frac{V_1 K_X^*}{\mu_y - D - d_y} \right)^2 + 4K_1 S} \right) \quad (7a)$$

$$X_1 = \frac{(D + d_y) K_X^*}{\mu_y - D - d_y} \quad (7b)$$

$$Y = \frac{D}{D + d_y} \frac{\mu_y}{V_y} \left(\frac{\mu_1}{V_1} (S - X_0) - X_1 \right) \quad (7c)$$

Thus it can be seen that the equilibrium concentration of prey is directly proportional to saturation constant K_X^* , and should therefore vary linearly with the concentration of decoys (of course, provided that the solution of Y is positive). The boundaries between modes 0 and I and between I and II as a function of the chemostat's control parameters (dilution rate D and input concentration of the limiting substrate S) can be obtained analytically. The first boundary is given by

$$S = \frac{DK_1}{\mu_1 - D}. \quad (8)$$

The boundary between (I) and (II) is

$$S = \frac{DK_1}{\mu_1 - D} + \frac{V_1 K_X^*}{\mu_1} \frac{D + d_y}{\mu_y - D - d_y} \quad (9)$$

As can be seen, increasing K_X^* means that, for a given D , the predator can only be present in the ecosystem *at all* at a higher input substrate concentration than in the absence of decoys. Figure 1 shows the zone boundaries as a function of the control parameters, using the same values for the metabolic parameters of predator and prey as were used by Nisbet *et al.* (1983) and Kooi and Kooijman (1994a) (i.e., $K_1 = 8 \text{ mg/l}$, $K_X = 9 \text{ mg/l}$, $\mu_1 = 0.5 \text{ h}^{-1}$, $\mu_y = 0.2 \text{ h}^{-1}$, $V_1 = 1.25 \text{ h}^{-1}$, and $V_y = 0.3333 \text{ h}^{-1}$). The zone boundary between zones II and III was obtained by local stability analysis of the steady-state solution. Figures 1(a) and 1(b)

show the results for the double Monod model without metabolic costs, whereas 1(c) and 1(d) show the results for $d_y = 0.01 \text{ h}^{-1}$. Figures 1(b) and 1(d) show the stabilizing effect of decoys, with $K_X^* = 2K_X$.

Figure 2 shows the transient dynamical behaviour of these systems when a small number of predators is introduced in a system in which the prey is present at its equilibrium value *in the absence of predators*, with $D = 0.1 \text{ h}^{-1}$ and $S = 200 \text{ mg/l}$. The same parameters were used as in Figure 1. The damping effect of decoys on the predator-prey oscillations is evident. In the absence of decoys, the prey is almost driven to extinction. In practice, total extinction may occur. By contrast, if $K_X^* = 2K_X$, the prey is only reduced by a factor of 3.6 or 0.56 $^{10}\log$ steps, so prey extinction does not occur.

Estimating the magnitude of the effect.

One shortcoming of the theory presented here so far is that no estimate of the magnitude of the inhibitory effect can be given *a priori*. In the case of actively swimming predators such as *Bdellovibrios*, a lower limit can be given. Assume that collisions with decoys are completely non-elastic, and that the decoys are far more massive than the predator. Furthermore, assume that the predator immediately starts a tumble (without any time needed for signal transduction). A typical tumble lasts in the order of 0.1s (Singleton, 1995), yielding a value of 10 s^{-1} for dissociation constant k_2 . Furthermore, we can estimate the collision rate constant r as

$$r = A_{col}v_{pred}$$

in which v_{pred} is the mean swimming velocity of the predator, and A_{col} is the collisional cross-section. Setting A_{col} to $1 \mu\text{m}^2$ and v_{pred} to $100 \mu\text{m/s}$, we find $r = 100 \mu\text{m}^3/\text{s}$, or $1 \times 10^{-10} \text{ cm}^3/\text{s}$. Writing the effective Michaelis-Menten constant as

$$K_X^* = k_1(1/r + [X_2])/k_2 \quad (10)$$

we find that at a decoy concentration of $10^{11}/\text{cm}^3$, K_X^* has doubled compared to the case without decoys. Since numbers of bacteria in the large intestine can be in the order of $3 \cdot 10^{11}/\text{cm}^3$, the effect of decoys may be appreciable. Remember that this is a lower limit of the magnitude of the effect. Drutz (1976) noted that bdellovibrios swim around *N. gonorrhoeae* cells as if trying to find a suitable point of entry, and occasionally transient attachment was observed. Unfortunately for our purposes, he did not measure the time bdellovibrios took to "investigate" the non-prey cells, but we may assume it would be in the order of 1 second at least for it to be described as Drutz does. This means that the effect can be ten times larger than the above estimate, and doubling of K_X^* should take place at a concentration of $10^{10}/\text{cm}^3$, and the K_X^* in the large intestine could be 30 times greater than that measured in the absence of decoys. Using an intermediate value of $K_X^* = 10K_X$, and the same parameters as in figure 2, the prey (pathogen) would only be reduced by 1.1%.

For bacteriophages the situation is different. In this case the collision rate is limited by diffusion, rather than active propulsion. It will most certainly be an order of magnitude lower than in the case of bdellovibrio. Estimating the dissociation constant k_2 is also problematic. However, if we assume the collisions are inelastic, and some non-specific binding takes place, it might become sufficiently large to have an effect similar to the case of bdellovibrios. I am not aware of any experiments which provide direct evidence of transient binding of phages to non-prey bacteria, but it is unlikely that no such binding occurs.

Discussion

A proposal for testing the hypothesis

A continuous flow co-culture of *E. coli* serving as prey, *Bdellovibrio bacteriovorus* as predator, and some aerotolerant *Lactobacillus* sp. as decoy could be an ideal model system to measure the proposed decoy effect. Lactobacilli are gram-positive, and therefore immune to attack from *B. bacteriovorus*. Besides, *E. coli* and the lactobacilli can be grown without direct competition if glucose and lactose are used as limiting substrates for the two species respectively. Alternatively, heat killed bacteria of a suitable gram-positive species could be

added at various concentration to the inflowing medium. Given the observations of Drutz (1976), *N. gonorrhoeae* would seem an ideal candidate. The steady state prey population should vary linearly with the decoy population according to (7b).

Conclusions

The theory presented in this paper may go some way to explain certain failures of pathogen elimination by specialist predators, and possibly bacteriophages in the intestine. The theory does allow colonization of the intestinal flora, and some reduction of numbers of prey should occur in such cases. More importantly from a therapeutic point of view, the ecosystem becomes more stable by the presence of decoys, so that the oscillatory behaviour needed to drive the prey to (near) extinction is reduced. Only when the prey (or host) is abundant compared to other species can the predators such as *B. bacteriovorus* or lytic phages reach the same levels at steady state as in the absence of decoys. The protective and curative effect of phage treatment obtained by Smith and Huggins (1983), can be explained in this way, since their experimental animals were infected between 6 and 16 hr after birth, by which time no complex intestinal microflora is expected to be present yet, and pathogens as *E. coli* may rapidly dominate (Schaedler, 1972). Besides, during acute bacterial diarrhoea, large numbers of pathogens may be present, and dilution of the ecosystem occurs (Miskovitz and Rochwarger, 1993).

A large number of alternative mechanisms for the failure of pathogen control by bdellovibrios or phages can be put forward (Westergaard and Kramer, 1977). They include the stomach acidity, anaerobic nature of the intestine, the action of enzymes, and adhesion of immunoglobulins, especially IgA. All these factors, and possibly many more may indeed play a role in limiting the success of biological pathogen control in the gut. In the case of *Bdellovibrio* spp. immunoglobulins in particular may cause problem, since these species incorporate complete cell wall structures and membrane proteins fragments of the prey in their own outer envelope (Diedrich, 1988, Stein *et al.* 1992). If the host has mounted an immune response to the pathogen (prey) it is likely to affect the predator as well in such cases. It is harder to see why stomach acidity should have an effect, since Westergaard and

Kramer (1977) successfully administered bdellovibrios orally. Also, the anaerobic environment of the gut should not affect bacteriophages.

The effect described here is the opposite of the "alternative prey" effect observed by Mallory *et al.* (1983). This effect occurs when the predator is not too specific. Mallory *et al.* studied elimination of salmonellas by predators in sewage. They found that addition of an alternative, harmless prey species to the ecosystem caused increased predation of the salmonellas. This is explained by assuming that the alternative prey increased the mean number of more-or-less generalist predators. Quite contrary to introduction of decoys, addition of alternative prey should also lead to an increased likelihood of Lotka-Volterra oscillations, as is often observed in more hypertrophic systems (DeAngelis, 1992). However, adding generalist predators to the intestinal microflora for therapeutic reasons may have an effect similar to broad spectrum antibiotics, and cause overgrowth by resistant pathogens.

The situation may be different for non-obligate predators, similar to *Pseudomonas* strain 679-2 (Casida, 1992). If suitable, non-obligate, specialist predators could be supported in sufficiently large numbers by nutrients in the intestine, they may be able to reduce the numbers of bacteria of certain species in the intestine effectively.

In the previous discussion, the decoy effect is attributed only to other bacteria. However, other particulate matter such as fibre could also act as decoy. Furthermore, this effect should not be limited to the gut microbial ecosystem. The decoy effect may play a role in any system with high densities of bacteria, such as activated sludge or biofilms, or indeed to other predator-prey systems altogether. In any system where a predator may be hindered in any way by the presence of another, inedible species, the decoy effect will come into effect.

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Figures

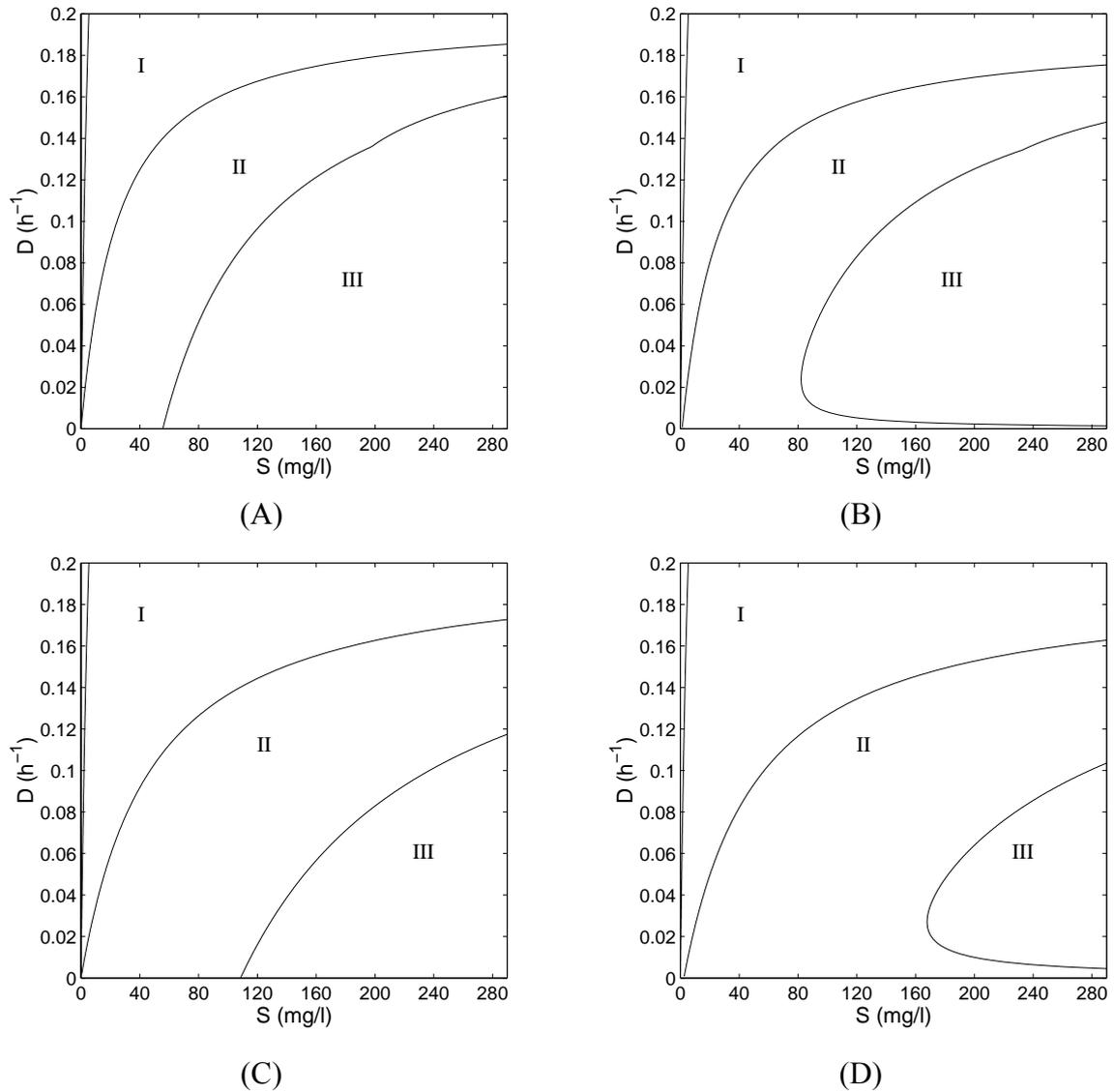


Figure 1. Operating diagrams for chemostats containing microbial predator-prey systems using the double Monod model with different numbers of decoys: (A): double Monod model without maintenance energy, using $K_X = K_X^* = 9$; (B) same as (A) but with $K_X^* = 18$; (C) and (D): same as (A) and (B), but with maintenance energy term $d_y = 0.01$. In zone I, only prey survive, in zone II both predator and prey coexist in a stable equilibrium, and in zone III the system becomes unstable. Increasing K_X^* reduces zone III and increases zone I.

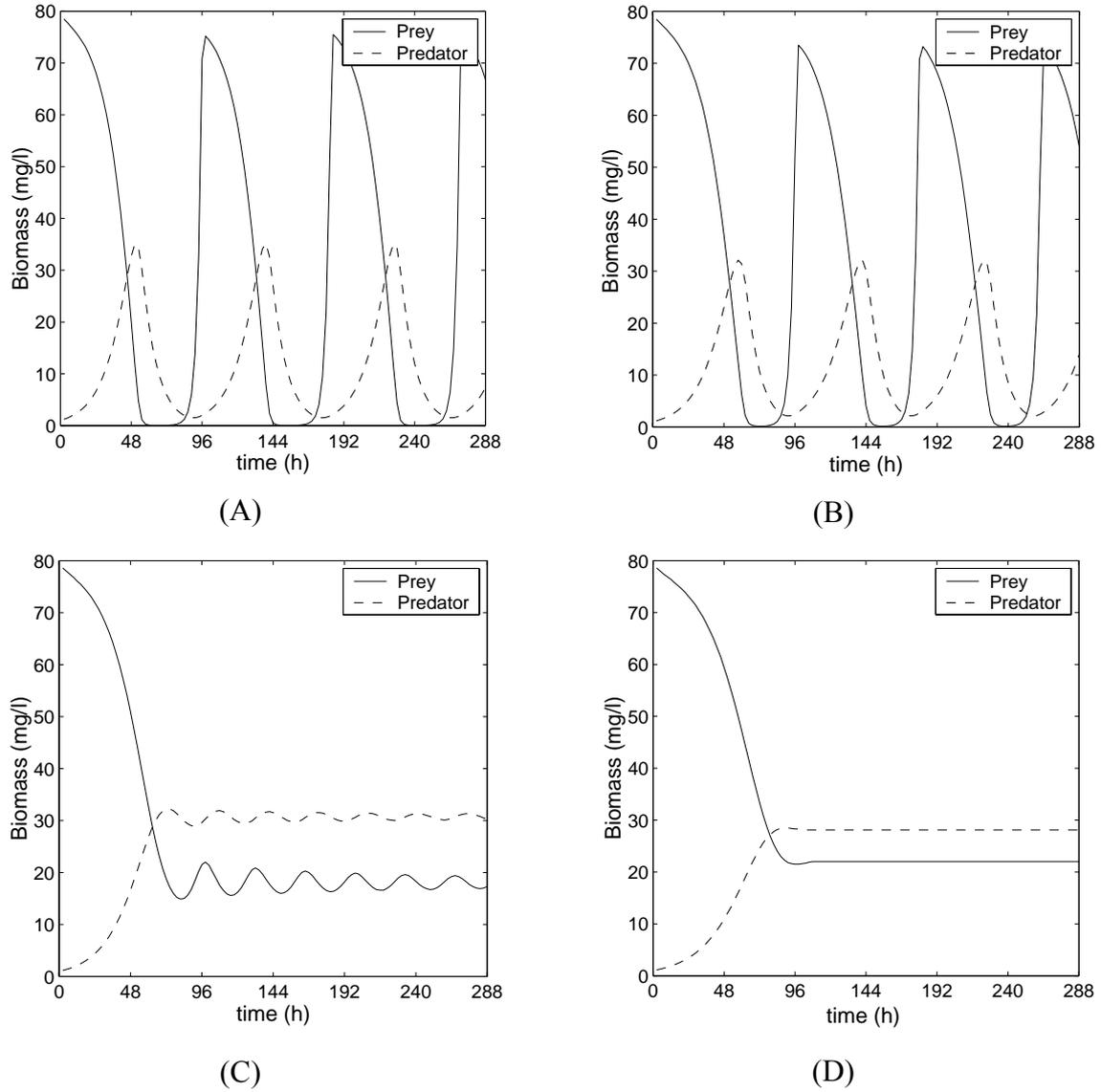


Figure 2. Transient behaviour of chemostats containing microbial predator-prey systems using the double Monod model with different numbers of decoys: (A): double Monod model without maintenance energy, using $K_X = K_X^* = 9$; (B) same as (A) but with $K_X^* = 18$; (C) and (D): same as (A) and (B), but with maintenance energy term $d_y = 0.01$. In the absence of decoys, the prey is driven to near extinction, whereas the presence of decoys leads to a reduction of only a factor of 3.6 (or 0.56 on a $^{10}\log$ scale).