Division of labor in bacterial populations

Bryan K. Lynn[a], Patrick De Leenheer[*],[1],[b],[c]

[a] Department of Integrative Biology, Oregon State University, United States
[b] Department of Mathematics, Oregon State University, United States
[c] Department of Integrative Biology, Oregon State University, United States

A B S T R A C T

Cooperating behaviors abound across all domains of life, but are vulnerable to invasion by cheaters. An important evolutionary question is to determine mechanisms that stabilize and maintain cooperation levels and prevent population collapse. Policing is one strategy populations may employ to achieve this goal, and it has been observed in many natural populations including microbes. Here we present and analyze a division of labor model to investigate if, when and how policing can be a cooperation-stabilizing mediator. The model represents a chemostat where cooperators produce a public good that benefits all individuals, and where toxin-producers produce a toxin that harms both cooperators and cheaters. We show that in many cases, the mere presence of toxin-producers is not enough to avoid a Tragedy of the Commons in which all individuals go extinct. The main focus of our work is to identify conditions on various model parameters which ensure that a mixed population of cooperators and toxin-producers can stably coexist and can avoid invasion by a cheater population. This happens when all of the following conditions hold: (i) The cost of policing must exceed the cost of cooperation. (ii) There is enough "collateral damage" caused by policing, i.e. the toxicity rate experienced by cooperators is sufficiently high, and (iii) The toxin affects cheaters even more than cooperators, and we provide a precise mathematical condition of how much stronger this effect should be.

1. Introduction

Acts of cooperation are found in a wide variety of species, ranging from bacteria to animals. Many bacteria cooperate by secreting extracellular products, so-called "public goods", such as biosurfactants for swarming [32], extracellular proteases to access food sources [7], and siderophores for the purpose of iron-scavenging [12]. Many higher organisms, including our own, have developed diversely structured societies where individuals take on specific roles to provide goods or services to the benefit of the population.

Despite the ubiquity of cooperation across all domains of life, populations are vulnerable to invasion by non-cooperating cheaters, including in several microbial systems [6,9,26]. Indeed, cheaters that do not invest in cooperation do not incur a fitness cost, and are expected to exhibit a growth advantage compared to cooperators, at least initially. In the long run however, decreased cooperation levels can lead to the collapse of the population, a phenomenon commonly known as the Tragedy of the Commons [4,11,22]. This brings up the important but difficult evolutionary problem to identify mechanisms that maintain cooperative behaviors [10,20].

Various control mechanisms have been proposed that either coerce individuals into cooperating or constrain them from cheating [8,18,21,25]. One such mechanism is policing, which has been found

across biological scales in nature [2] such as in humans, rhesus monkeys, eusocial insects [19,29,30] like ants, bees and wasps (where egg-laying workers are treated aggressively, or have their eggs eaten) and even in symbiotic partnerships like cleaner and cleaning fish, and in nitrogen-fixating rhizobium and plants. Policing strategies have also developed in bacterial populations, as confirmed in experimental work in [14,23,27]. In [27] for example, it is shown that cooperators in *Pseudomonas aeruginosa* can secrete toxins such as cyanide, affecting cheaters but not the toxin-producers because they also activate detoxification genes when producing toxins.

Many, if not most, modern models of cooperation rely on game theoretical concepts such as a Prisoner's Dilemma [13]. The Prisoner's Dilemma is a basic game where two players have a choice between two strategies (cooperate or defect), and a 2 by 2 payoff matrix records the payoffs of all possible pairwise interactions. Now consider a growing bacterial population in a chemostat. Can this growth process really be captured by repeated rounds of individuals playing this game, or even by a multi-player public goods game [1]? Moreover, how are the payoffs defined? And can they be measured? These are essential questions, but very hard -if not impossible- to answer. Unfortunately, they are also rarely addressed in the literature. In contrast, following a mechanistic, first-principles approach which we adopt here, it is very clear how to model this process without having to postulate the existence of an

underlying game. Experiments can and are routinely conducted by microbiologists to accurately measure the population's uptake and growth rates, and the production rates of any public goods. Any biochemical processes taking place inside or outside cells can be measured and modeled in a similar vein. Writing down the mathematical model then boils down to a straightforward exercise in book-keeping.

There are different types of game-theoretical models that have been used in the context of the evolution of cooperation. One class of models is based on the so-called replicator equation [13]. These models take the form of a system of nonlinear ordinary differential equations for the frequencies of players in a population that adopt the pure strategies of the underlying game. One advantage of replicator equations is that they can be analyzed in a mathematically rigorous fashion. The key ingredient for these models is the payoff matrix of the underlying game. Although they are powerful phenomenological tools, they are not built upon mechanistic principles. And as mentioned, defining, let alone measuring the pay-offs is a highly non-trivial task. Another class of models consists of agent-based models, see [15–17] for recent reviews, and [28] for models that incorporate policing. In these types of models, all individuals engaged in the underlying game are tracked over time. Many additional aspects such as the movement of individuals in space, the way in which they interact with each other and with the environment, as well as the impact of public goods are also incorporated in these models. Agent-based models exhibit high degrees of complexity, and this precludes a rigorous mathematical analysis of their behavior. Instead, one has to resort to performing numerical simulations. These can reveal interesting pattern changes in the solutions of the model. However, simulations lack predictive power, and are usually insufficient to explain *why* pattern changes occur. They can also fail to pick up unexpected behavior. This happens when only limited parameter ranges, or a restricted range of initial conditions is explored, which is often inevitable due to the enormous number of parameters and variables in these models.

In contrast, mechanistic models based on first-principles can be analyzed rigorously. The parameter space of these models can be sliced up in regions where distinct patterns are observed. The boundaries between these regions can be determined using powerful tools such as bifurcation theory. Consequently, mechanistic models display very accurate predictive power, and they provide a much clearer path to understanding why behavior changes occur.

Despite recent calls for more mechanistic models based on first principles to try to explain cooperation [3,5,31], little work has been done in this direction. Quoting from the Review of West et al. [31]: *"What we do not need: (i) keep reinventing the wheel with more theoretical models that incorrectly claim to provide a new mechanism for the evolution of cooperation (ii) more convoluted theoretical analyses of games such as the Prisoner's Dilemma,..., which make a large number of extremely specific and often unrealistic assumptions. .... allow the biology to lead the mathematics, rather than contorting real systems into the form of an artificial game. What we need: (i) greater integration between theoretical and empirical work... greater emphasis on the development of models that can be applied to and tested in specific systems. (ii) the possible advantages of less traditional study systems need to be exploited... amazing opportunities offered by bacteria and other microbes have only just been realised, let alone exploited. (iii) greater unification. There is surprisingly little interaction between empirical workers and theoreticians. (iv) emphasize both distinction and interplay between mechanistic and evolutionary approaches ... which are complementary and not competing.... this distinction has led to research on evolutionary questions tending to ignore mechanistic issues."*

This paper is an attempt to contribute to these calls for more mechanistic approaches to the theory of the evolution of cooperation. We propose a conceptual division-of-labor model to investigate if, when, and how policing strategies can stabilize cooperative behavior. The model tracks 3 strains -cooperator, toxin-producer and cheater-, externally supplied growth nutrient, the public good produced by the cooperator that is required for growth, and the toxin that harms cooperators and cheaters. We find that although the mere presence of toxin producers often fails to stabilize cooperation, there are specific circumstances when cooperation is successfully stabilized. We show that when challenged by cheaters, cooperative behavior can indeed be stabilized, provided that the following four conditions hold:

1. Toxin-producers must be present.
2. The cost of toxin production must exceed the cost of public good production. In other words, policing is more expensive than cooperation.
3. The harmful effects of the toxin on the cooperator must be sufficiently high. This is a trade-off to offset that policing is more expensive than cooperation.
4. The effects of the toxin on the cheater must be even higher.

These 4 items will be made precise in terms of inequalities that involve various parameters and functional forms in the model.

Although our model is developed primarily to capture policing in bacterial populations, it provides a framework for modeling the evolution of cooperation via policing strategies in many other populations. In human populations for instance, law enforcement and police agencies specialize to identify and remove criminals, whereas law-abiding citizens contribute to the welfare of the society in various ways. Thus, law-abiding citizens are the cooperators in our model, law enforcement corresponds to the toxin-producers, and criminals represent the cheaters.

2. A division of labor chemostat model

We consider a general chemostat model with positive dilution rate D and positive input nutrient concentration S^0. There are 3 microbial species, the cooperators, toxin producers, and the cheaters whose concentrations are denoted as X_1, X_2 and X_3, respectively. The nutrient concentration in the chemostat has concentration S. The cooperator produces a public good with concentration E, which is required for growth of all 3 species. Public goods in microbial populations are typically enzymes that facilitate nutrient uptake. The toxin producers produce a toxin with concentration T, and the positive toxicity rate constant for cooperators and cheaters is K_1 and K_3, respectively. Toxin producers are resistant to the toxin they produce. Thus, cooperators and toxin producers have specialized tasks, leading to a division-of-labor model below.

Nutrient is consumed by each of the species at per capita rate $F(S, E)/\gamma_i$, for $i = 1, 2, 3$, where γ_i is the yield in the conversion of nutrient into new biomass of species X_i. We assume that $F(S, E)$ is non-negative and twice continuously differentiable for all $S \geq 0$ and $E \geq 0$, and satisfies the following assumptions:

H1: $F(0, E) = F(S, 0) = 0,$
          $F(S, E) > 0$ when $S > 0$ and $E > 0,$
          $\dfrac{\partial F}{\partial S}(S, E) > 0$ and $\dfrac{\partial F}{\partial E}(S, E) > 0$ when $S > 0$ and $E > 0$

These assumptions mean that there is no nutrient uptake when nutrient or public good is missing, that there is nutrient uptake when both are available, and that the uptake rate increases with higher levels of nutrient or public good. Typical examples satisfying **H1** are functions of the form $F(S, E) = F_1(S)F_2(E)$, where $F_1(S)$ is a Michaelis–Menten function (i.e. $mS/(a + S)$ where $m > 0$ and $a > 0$ are parameters) or a linear function (i.e. αS where $\alpha > 0$ is a parameter), and where also $F_2(E)$ is of Michaelis–Menten form, or simply linear.

The theoretically available growth rate for each species is $F(S, E)$, but cooperators and toxin producers divert a fraction q_1 and q_2 (both are numbers in $(0,1)$) to produce the public good E and toxin T, respectively, each with a respective positive conversion efficiency η_E and η_T. The remaining fractions $1 - q_1$ and $1 - q_2$ are allocated to the

growth of cooperators and toxin producers respectively. In contrast, the cheater does not contribute to public good or toxin production and allocates the entirety of the available growth rate $F(S, E)$ to its own growth. Mass-balance for all involved substances is then captured by the following chemostat model:

$$\begin{aligned}
\textbf{nutrient} \quad \dot{S} &= D(S^0 - S) - \left(\frac{X_1}{\gamma_1} + \frac{X_2}{\gamma_2} + \frac{X_3}{\gamma_3} \right) F(S, E) \\
\textbf{public good} \quad \dot{E} &= \eta_E q_1 X_1 F(S, E) - DE \\
\textbf{toxin} \quad \dot{T} &= \eta_T q_2 X_2 F(S, E) - DT \\
\textbf{cooperators} \quad \dot{X_1} &= X_1((1 - q_1)F(S, E) - D - K_1 T) \\
\textbf{toxin producers} \quad \dot{X_2} &= X_2((1 - q_2)F(S, E) - D) \\
\textbf{cheaters} \quad \dot{X_3} &= X_3(F(S, E) - D - K_3 T)
\end{aligned}$$

It is possible to scale out several model parameters. By letting:

$$x_i = X_i/\gamma_i \text{ for } i = 1, 2, 3, \quad s = S, \quad e = E/\eta_E\gamma_1, \quad t = T/\eta_T\gamma_2,$$
$$s^0 = S^0, \quad k_1 = \eta_T\gamma_2 K_1, \quad k_3 = \eta_T\gamma_2 k_3,$$

and setting $f(s, e) = F(s, \eta_E\gamma_1 e)$ (note that $f(s, e)$ also satisfies **H1**), we get the scaled model:

$$\dot{s} = D(s^0 - s) - (x_1 + x_2 + x_3)f(s, e) \tag{1}$$

$$\dot{e} = q_1 x_1 f(s, e) - De \tag{2}$$

$$\dot{t} = q_2 x_2 f(s, e) - Dt \tag{3}$$

$$\dot{x_1} = x_1((1 - q_1)f(s, e) - D - k_1 t) \tag{4}$$

$$\dot{x_2} = x_2((1 - q_2)f(s, e) - D) \tag{5}$$

$$\dot{x_3} = x_3(f(s, e) - D - k_3 t) \tag{6}$$

Our main objective is to understand the behavior of this scaled model, and our main focus lies on identifying conditions which lead to a stable coexistence of cooperators and toxin producers which can resist invasion by mutant cheaters.

We start by showing that this model is well-posed in the following sense:

Lemma 1. *Assume that **H1** holds. All solutions of system (1)–(6) initiated in $\mathbb{R}_+^6$, exist and remain in $\mathbb{R}_+^6$ for all $\tau > 0$, and are bounded. In fact, system (1)–(6) is dissipative.*

Proof. Clearly the non-negative orthant $\mathbb{R}_+^6$ is forward invariant for system (1)–(6). Consider the dynamics of $m := s + e + t + x_1 + x_2 + x_3$. Then

$$\dot{m} = D(s^0 - m) - (k_1 x_1 + k_3 x_3)t \leq D(s^0 - m),$$

and hence

$$\limsup_{\tau \to +\infty} m(\tau) \leq s^0,$$

which implies that system (1)–(6) is dissipative. $\square$

3. Persistence of cooperator-only populations

In this Section we shall establish the fate of the population when no toxin producers (or their toxins), or cheaters are present initially. We will show that a cooperator-only population can persist under reasonable conditions.

To make these assertions more precise, we first note that the set where $x_2 = t = x_3 = 0$ is a forward invariant set for system (1)–(6), motivating an investigation of the restricted system:

$$\dot{s} = D(s^0 - s) - x_1 f(s, e) \tag{7}$$

$$\dot{e} = q_1 x_1 f(s, e) - De \tag{8}$$

$$\dot{x_1} = x_1((1 - q_1)f(s, e) - D) \tag{9}$$

To state our results more succinctly, we define an auxiliary function on the interval $[0, (1 - q_1)s^0]$:

$$h_1(x_1) = f(s^0 - x_1/(1 - q_1), q_1 x_1/(1 - q_1)), \tag{10}$$

and note that $h_1(0) = h_1((1 - q_1)s^0) = 0$, but that $h_1(x_1) > 0$ for all x_1 in $(0, (1 - q_1)s^0)$ when **H1** holds. Furthermore, we introduce the following assumption:

H2: $h_1(x_1)$ is strictly concave, i.e. $h_1''(x_1)$

$$< 0 \text{ for all } x_1 \text{ in } [0, (1 - q_1)s^0]. \tag{11}$$

First, it is easily verified that when $f(s, e) = f_1(s)f_2(e)$, where $f_1(s)$ and $f_2(e)$ are either linear functions, or Monod functions, then **H2** holds because:

$$h_1''(x_1) = \frac{1}{(1 - q_1)^2}(f_1''f_2 - 2q_1 f_1'f_2' + q_1^2 f_1 f_2''),$$

which is negative when f_1 and f_2 are either linear or Monod functions, and more generally when they are both strictly increasing ($f_1' > 0$ and $f_2' > 0$) and concave functions ($f_1'' \leq 0$ and $f_2'' \leq 0$).

Secondly, when **H2** holds, then the equation

$$h_1(x_1) = \frac{D}{1 - q_1},$$

generically either has no, or exactly two solutions x_1^u and x_2^s in $[0, (1 - q_1)s^0]$ with $x_1^u < x_1^s$. The reason for the choice of the superscripts u and s will become clear later, when the stability properties of certain steady states will be investigated. Keeping all model parameters fixed, except for D, no solutions of the equation above exist for all sufficiently large D, and two solutions exist for all sufficiently small D. There is also a non-generic case when there is a unique solution to this equation, but we will never consider this case. This case happens when the maximum of the function $h_1(x_1)$ equals $D/(1 - q_1)$.

We are now ready to show that a cooperator-only population can persist.

Theorem 1. *Assume that **H1** and **H2** hold, and suppose that the equation $h_1(x_1) = D/(1 - q_1)$ has two solutions x_1^u and x_1^s in $(0, (1 - q_1)s^0)$, with $x_1^u < x_1^s$.*

Then system (7)–(9) has exactly 3 steady states: $E_0 = (s^0, 0, 0)$, $E_1^u = (s^0 - x_1^u/(1 - q_1), q_1 x_1^u/(1 - q_1), x_1^u)$ and $E_1^s = (s^0 - x_1^s/(1 - q_1), q_1 x_1^s/(1 - q_1), x_1^s)$.

Every solution of system (7)–(9), converges to one of E_0, E_1^u or E_1^s; E_0 and E_1^s are locally asymptotically stable, and E_1^u is unstable. System (7)–(9) is therefore bi-stable.

Proof. Transforming the state (s, e, x_1) of system (7)–(9) to (m, z_1, x_1), where

$$m = s + e + x_1, \quad \text{and} \quad z_1 = (1 - q_1)e - q_1 x_1,$$

we see that the system is transformed into:

$$\dot{m} = D(s^0 - m) \tag{12}$$

$$\dot{z_1} = -Dz_1 \tag{13}$$

$$\dot{x_1} = x_1((1 - q_1)f(m - (z_1 + x_1)/(1 - q_1), (z_1 + q_1 x_1)/(1 - q_1)) - D), \tag{14}$$

an example of an asymptotically autonomous system [24] because $m(\tau) \to s^0$, and $z_1(\tau) \to 0$ as $\tau \to +\infty$. Recalling the definition of the function $h_1(x_1)$ in (10), we note that the resulting scalar limiting system, obtained by setting $m = s^0$ and $z_1 = 0$ in (14), is given by:

$$\dot{x_1} = x_1((1 - q_1)h_1(x_1) - D), \quad \text{for } 0 \leq x_1 \leq (1 - q_1)s^0. \tag{15}$$

Thus, system (15) has 3 steady states in $[0, (1 - q_1)s^0]$, namely at 0, at x_1^u and at x_2^s. It is easily verified that 0 and x_1^s are asymptotically stable, whereas x_1^u is unstable steady states of system (15), which therefore is an example of a bi-stable system. From the theory of asymptotically

autonomous systems [24], follows that system (12)–(14) also has 3 steady states $(s^0, 0, 0)$, $(s^0, 0, x_1^u)$ and $(s^0, 0, x_1^s)$, of which the former and latter are asymptotically stable, and the middle one is unstable. All solutions of system (12)–(14) converge to one of these steady states.

Consequently, system (7)–(9) has 3 steady states, namely $E_0 = (s^0, 0, 0)$, $E_1^u = (s^0 - x_1^u/(1 - q_1), q_1 x_1^u/(1 - q_1), x_1^u)$ and $E_1^s = (s^0 - x_1^s/(1 - q_1), q_1 x_1^s/(1 - q_1), x_1^s)$; E_0 and E_1^s are asymptotically stable, whereas E_1^u is unstable. Moreover, every solution converges to one of these 3 steady states, and therefore this system is bi-stable. $\square$

4. Tragedy of the Commons

We shall now show that if cheaters are present, but toxin-producing microbes are absent, then the entire population is doomed. This is a manifestation of the famous Tragedy of the Commons (ToC) phenomenon [4,11,22]:

Theorem 2. *Assume that H1 holds. Then every solution of system (1)–(6) with an initial condition such that $x_3(0) > 0$ and $x_2(0) = 0$, converges to the washout steady state $(s^0, 0, 0, 0, 0, 0)$.*

Proof. When $x_2(0) = 0$, then clearly $x_2(\tau) = 0$ for all $\tau \geq 0$, and then $t(\tau) = t(0)e^{-D\tau}$, whence $t(\tau) \to 0$ as $\tau \to +\infty$. Next we explicitly solve the model's differential equations for $x_1(\tau)$ and $x_3(\tau)$:

$$x_1(\tau) = x_1(0)e^{\int_0^\tau (1-q_1)f(s(u),e(u))-D-k_1 t(u)du}$$

$$x_3(\tau) = x_3(0)e^{\int_0^\tau f(s(u),e(u))-D-k_3 t(u)du}$$

We distinguish two possible scenarios, depending on the integrability -or lack thereof- of the function $f(s(u), e(u))$ for u in $(0, +\infty)$.

- Suppose that $\int_0^\infty f(s(u), e(u))du < +\infty$. Then it is immediately clear from the above expressions for $x_1(\tau)$ and $x_3(\tau)$ that $x_1(\tau) \to 0$ and $x_3(\tau) \to 0$ as $\tau \to +\infty$. As all solutions are bounded (by Lemma 1), and exploiting continuity of $f(s, e)$, we obtain from a comparison argument that for any $\epsilon > 0$, $\dot{e}(\tau) \leq \epsilon - De(\tau)$ for all sufficiently large τ. As $\epsilon > 0$ is arbitrary, this implies that $e(\tau) \to 0$ as $\tau \to +\infty$. Finally, a similar comparison argument implies that $s(\tau) \to s^0$ as $\tau \to +\infty$.
- Suppose that $\int_0^\infty f(s(u), e(u))du = +\infty$. As $x_3(0) > 0$, the ratio $x_1(\tau)/x_3(\tau)$ is well-defined for all $\tau > 0$, and

$$\frac{x_1(\tau)}{x_3(\tau)} = \frac{x_1(0)}{x_3(0)}e^{-q_1 \int_0^\tau f(s(u),e(u))du-(k_1-k_3)\int_0^\tau t(u)du}$$

$$= \frac{x_1(0)}{x_3(0)}e^{-(k_1-k_3)(1-e^{-D\tau})t(0)/D}e^{-q_1\int_0^\tau f(s(u),e(u))du} \to 0,$$

as $\tau \to +\infty$.

But as $x_3(\tau)$ remains bounded by Lemma 1, this implies that $x_1(\tau) \to 0$ as $\tau \to +\infty$. Similar comparison arguments as above then show that $e(\tau) \to 0$, and $s(\tau) \to s^0$ as $\tau \to +\infty$. $\square$

Theorem 2 reveals how important toxin producers are: Without them, a ToC cannot be avoided. However, as our next result shows, the mere presence of toxin producers is not sufficient: To avoid a ToC, the toxin must also harm the cheaters at least as much as it harms the cooperators.

Theorem 3. *Assume that H1 holds, and that*

$$k_1 > k_3. \tag{16}$$

Then every solution of system (1)–(6) with an initial condition such that $x_3(0) > 0$, converges to the washout steady state $(s^0, 0, 0, 0, 0, 0)$.

Proof. Again we explicitly solve the model's differential equations for $x_1(\tau)$ and $x_3(\tau)$:

$$x_1(\tau) = x_1(0)e^{\int_0^\tau (1-q_1)f(s(u),e(u))-D-k_1 t(u)du}$$

$$x_3(\tau) = x_3(0)e^{\int_0^\tau f(s(u),e(u))-D-k_3 t(u)du},$$

and distinguish two possible scenarios, depending on the (non-) integrability of the function $f(s(u), e(u))$ for u in $(0, +\infty)$.

- Suppose that $\int_0^\infty f(s(u), e(u))du < +\infty$. Then it is immediately clear that $x_1(\tau) \to 0$ and $x_3(\tau) \to 0$ as $\tau \to +\infty$ from the above expressions for $x_1(\tau)$ and $x_3(\tau)$. From a comparison argument similar to the one used in the proof of Theorem 2 then follows that for any $\epsilon > 0$, $\dot{e}(\tau) \leq \epsilon - De(\tau)$ for all sufficiently large τ. As $\epsilon > 0$ is arbitrary, this implies that $e(\tau) \to 0$ as $\tau \to +\infty$. Finally, similar comparison arguments then imply that $x_2(\tau) \to 0$, $t(\tau) \to 0$, $x_3(\tau) \to 0$ and $s(\tau) \to s^0$ as $\tau \to +\infty$.
- Suppose that $\int_0^\infty f(s(u), e(u))du = +\infty$. As $x_3(0) > 0$, the ratio $x_1(\tau)/x_3(\tau)$ is well-defined for all $\tau > 0$, and since (16) holds, we obtain that:

$$\frac{x_1(\tau)}{x_3(\tau)} = \frac{x_1(0)}{x_3(0)}e^{-q_1\int_0^\tau f(s(u),e(u))du-(k_1-k_3)\int_0^\tau t(u)du} \to 0,$$

as $\tau \to +\infty$.

But as $x_3(\tau)$ remains bounded by Lemma 1, this implies that $x_1(\tau) \to 0$ as $\tau \to +\infty$. Similar comparison arguments as above then show that $e(\tau) \to 0$, $x_2(\tau) \to 0$, $t(\tau) \to 0$, $x_3(\tau) \to 0$ and $s(\tau) \to s^0$ as $\tau \to +\infty$. $\square$

Theorem 3 is not very surprising, because when the toxin affects the cooperators more strongly than the cheaters (i.e. $k_1 > k_3$), then the net per capita growth rate of the cheaters is always higher than that of the cooperators (i.e. $f(s, e) - D - k_3 t > (1 - q_1)f(s, e) - D - k_1 t$, when s and e are positive), which provides cheaters with a net growth advantage. But once cheaters become too abundant, there is no longer a sufficient production of the public good e that is required for growth, and this in turn leads to the demise of the population. Since Theorem 3 clearly indicates that in order to avoid a ToC, the toxin should affect the cheater at least as much as the cooperator, one of the main goals of this paper is to quantify precisely how much more this should be.

5. Persistence of cooperators and toxin producers

In this Section we consider the dynamics of a mixed population that consists of cooperators and toxin producers, but remains unchallenged by cheaters:

$$\dot{s} = D(s^0 - s) - (x_1 + x_2)f(s, e) \tag{17}$$

$$\dot{e} = q_1 x_1 f(s, e) - De \tag{18}$$

$$\dot{t} = q_2 x_2 f(s, e) - Dt \tag{19}$$

$$\dot{x}_1 = x_1((1 - q_1)f(s, e) - D - k_1 t) \tag{20}$$

$$\dot{x}_2 = x_2((1 - q_2)f(s, e) - D) \tag{21}$$

We first show that when the cost of cooperation, as measured by q_1, exceeds the cost of toxin-production, measured by q_2, then this mixed population is doomed:

Theorem 4. *Assume that H1 holds, and that:*

$$q_1 > q_2.$$

Then every solution of system (17)–(21) with an initial condition such that $x_2(0) > 0$, converges to the washout steady state $E_0 = (s^0, 0, 0, 0, 0)$.

Proof. Integrating the x_1 and x_2 equation yields:

$$x_1(\tau) = x_1(0)e^{\int_0^\tau (1-q_1)f(s(u),e(u))-D-k_1 t(u)du}$$

$$x_2(\tau) = x_2(0)e^{\int_0^\tau (1-q_2)f(s(u),e(u))-Ddu}$$

We distinguish two scenarios, depending on the (non-)integrability of the function $f(s(u), e(u))$ for u in $(0, +\infty)$:

- Suppose that $\int_0^\infty f(s(u), e(u))du < +\infty$. Then the above expressions immediately show that $x_1(\tau) \to 0$ and $x_2(\tau) \to 0$ as $\tau \to +\infty$. Three comparison arguments then imply that $e(\tau) \to 0$, $t(\tau) \to 0$ and $s(\tau) \to s^0$ as $\tau \to +\infty$ as well.

- Suppose that $\int_0^\infty f(s(u), e(u))du = +\infty$. Since $x_2(0) > 0$, the following ratio is well-defined:

$$\frac{x_1(\tau)}{x_2(\tau)} = \frac{x_1(0)}{x_2(0)} e^{-(q_1 - q_2)\int_0^\tau f(s(u), e(e))du - k_1 \int_0^\tau t(u)du} \quad \to \quad 0,$$

as $\tau \to +\infty$.

Since $x_2(\tau)$ remains bounded by Lemma 1, there follows that $x_1(\tau) \to 0$ as $t \to +\infty$. Standard comparison arguments then imply that $e(\tau) \to 0$, $x_2(\tau) \to 0$, $t(\tau) \to 0$ and $s(\tau) \to s^0$ as $\tau \to +\infty$.

□

We have just identified a necessary condition for a possible coexistence of cooperators and toxin producers, namely that $q_1 \le q_2$. We shall see that if $q_1 < q_2$ -which means that the cost of toxin production exceeds the cost of cooperation- and if certain additional conditions hold, then a stable coexistence of these 2 species is indeed possible.

We first determine the steady states of system (17)–(21). When **H1** and **H2** hold, and assuming that Eq. (11) has two solutions x_1^u and x_1^s in the interval $[0, (1 - q_1)s^0]$ with $x_1^u < x_1^s$, then by the analysis performed in the previous Section, system (17)–(21) has exactly 3 steady states in the part of the boundary of the system where $x_2 = 0$. By a slight abuse of notation we also denote these respective steady states by $E_0 = (s^0, 0, 0, 0, 0)$, $E_1^u = (s^0 - x_1^u/(1 - q_1), q_1 x_1^u/(1 - q_1), 0, x_1^u, 0)$ and $E_1^s = (s^0 - x_1^s/(1 - q_1), q_1 x_1^s/(1 - q_1), 0, x_1^s, 0)$.

We now turn to the question of the existence of steady states where $x_2 > 0$, i.e. where toxin producers are present. It is easy to see that whenever $x_2 > 0$ at a steady state, then necessarily $x_1 > 0$ as well. Indeed, if $x_2 > 0$ but x_1 were zero, then e would have to be zero, but then the steady state equation corresponding to (21) cannot hold. Thus, we focus on finding steady states where both $x_1 > 0$ and $x_2 > 0$. First, we note that we can transform system (17)–(21) using the transformation (s, e, t, x_1, x_2) to (s, e, z_2, x_1, x_2), where

$$z_2 = (1 - q_2)t - q_2 x_2,$$

into the asymptotically autonomous system:

$$\begin{aligned}
\dot{s} &= D(s^0 - s) - (x_1 + x_2)f(s, e) \\
\dot{e} &= q_1 x_1 f(s, e) - De \\
\dot{z}_2 &= -Dz_2 \\
\dot{x}_1 &= x_1((1 - q_1)f(s, e) - D - k_1(z_2 + q_2 x_2)/(1 - q_2)) \\
\dot{x}_2 &= x_2((1 - q_2)f(s, e) - D)
\end{aligned}$$

Observing that $z_2(\tau) \to 0$ as $\tau \to +\infty$, we can consider the limiting system:

$$\dot{s} = D(s^0 - s) - (x_1 + x_2)f(s, e) \tag{22}$$

$$\dot{e} = q_1 x_1 f(s, e) - De \tag{23}$$

$$\dot{x}_1 = x_1((1 - q_1)f(s, e) - D - k_1 q_2 x_2/(1 - q_2)) \tag{24}$$

$$\dot{x}_2 = x_2((1 - q_2)f(s, e) - D) \tag{25}$$

The steady states of this limiting system for which $x_1 > 0$ and $x_2 > 0$, can be found by finding solutions to the following algebraic equations:

$$\begin{aligned}
f(s, e) &= \frac{D}{1 - q_2} \\
x_2 &= \frac{q_2 - q_1}{q_2} \frac{D}{k_1} \\
e &= \frac{q_1}{1 - q_2} x_1 \\
s &= \left(s^0 - \frac{q_2 - q_1}{q_2(1 - q_2)} \frac{D}{k_1}\right) - \frac{1}{1 - q_2} x_1
\end{aligned}$$

We note that the existence of a solution with $x_1 > 0$ and $x_2 > 0$ requires that:

$$q_2 > q_1, \quad \text{and} \quad c := s^0 - \frac{q_2 - q_1}{q_2(1 - q_2)} \frac{D}{k_1} > 0. \tag{26}$$

The first inequality is not surprising in view of Theorem 4. The second inequality is new, and can be re-written as:

$$k_1 > \frac{q_2 - q_1}{q_2(1 - q_2)} \frac{D}{s^0}, \tag{27}$$

and expresses that the existence of a steady state with $x_1 > 0$ and $x_2 > 0$ requires the toxicity rate k_1 to be sufficiently large.

Assuming that (26) holds, we now introduce a second auxiliary function $h_2(x_1)$, defined on the interval $[0, (1 - q_2)c]$:

$$h_2(x_1) = f(c - x_1/(1 - q_2), q_1 x_1/(1 - q_2)), \tag{28}$$

which is positive in $(0, (1 - q_2)c)$, but zero in the endpoints of this interval. By inserting the last two expressions for e and s of the above steady state equations into the first steady state equation, we see that x_1 at a steady state must satisfy:

$$h_2(x_1) = \frac{D}{1 - q_2}$$

Just like we introduced a concavity assumption for the function $h_1(x_1)$ in **H2**, we now introduce:

H3: $h_2(x_1)$ is strictly concave, i.e. $h_2''(x_1)$

$$< 0 \text{ for all } x_1 \text{ in } [0, (1 - q_2)c]. \tag{29}$$

As explained for the auxiliary function $h_1(x_1)$ in Section 3, **H3** automatically holds when $f(s, e)$ is a product of strictly increasing and concave functions of s and of e, such as linear and/or Monod functions which are commonly used in microbial growth models.

When **H3** holds, the equation $h_2(x_1) = D/(1 - q_2)$ generically either has no, or exactly two solutions $x_{1,2}^s$ and $x_{1,2}^u$ in the interval $[0, (1 - q_2)c]$ with $x_{1,2}^s < x_{1,2}^u$. Once again, the choice of the superscripts s and u will become clear later when the stability of certain steady states is discussed. When there are two solutions to the equation, it follows that the limiting system (22)–(25) has two steady states with $x_1 > 0$ and $x_2 > 0$. Consequently, system (17)–(21) has the following coexistence steady states:

$$E_{1,2}^s = \left(c - \frac{x_{1,2}^s}{1 - q_2}, \frac{q_1 x_{1,2}^s}{1 - q_2}, \frac{(q_2 - q_1)D}{(1 - q_2)k_1}, x_{1,2}^s, \frac{(q_2 - q_1)D}{q_2 k_1}\right) \tag{30}$$

$$E_{1,2}^u = \left(c - \frac{x_{1,2}^u}{1 - q_2}, \frac{q_1 x_{1,2}^u}{1 - q_2}, \frac{(q_2 - q_1)D}{(1 - q_2)k_1}, x_{1,2}^u, \frac{(q_2 - q_1)D}{q_2 k_1}\right) \tag{31}$$

Note in particular that both steady states have the same x_2 and t-values.

Our next result implies that a stable coexistence of cooperators and toxin producers is possible in the absence of cheaters.

Theorem 5. Assume that **H1**, **H2**, (26) and **H3** hold. Suppose that the equation $h_1(x_1) = D/(1 - q_1)$ has two solutions x_1^u and x_1^s in the interval $(0, (1 - q_1)s^0)$, with $x_1^u < x_1^s$. Suppose also that the equation $h_2(x_1) = D/(1 - q_2)$ has two solutions $x_{1,2}^s$ and $x_{1,2}^u$ in the interval $(0, (1 - q_2)c)$, with $x_{1,2}^s < x_{1,2}^u$.

Then system (17)–(21) has exactly 5 steady states: $E_0 = (s^0, 0, 0, 0, 0)$,

$E_1^u = (s^0 - x_1^u/(1 - q_1), q_1 x_1^u/(1 - q_1), 0, x_1^u, 0)$,
$E_1^s = (s^0 - x_1^s/(1 - q_1), q_1 x_1^s/(1 - q_1), 0, x_1^s, 0)$, *and* $E_{1,2}^s$ *and* $E_{1,2}^u$, *defined in* (30) *and* (31).

Moreover, E_0 *and* E_1^s *are locally asymptotically stable, whereas* E_1^u *and* $E_{1,2}^u$ *are unstable.*

If $h_2'(x_{1,2}^s)$ *is sufficiently small, then* $E_{1,2}^s$ *is locally asymptotically stable, and in this case system* (17)–(21) *is tri-stable.*

Proof. Because system (17)–(21) is an asymptotically autonomous system, it suffices to prove that the corresponding steady states of the limiting system (22)–(25) -which by a slight abuse of notation, we shall denote with the same notation- have the same stability properties. Linearizing the vector field of the limiting system yields the Jacobian matrix:

$$
\begin{pmatrix}
-D - (x_1 + x_2)\frac{\partial f}{\partial s} & -(x_1 + x_2)\frac{\partial f}{\partial e} & -f & -f \\
q_1 x_1 \frac{\partial f}{\partial s} & q_1 x_1 \frac{\partial f}{\partial e} - D & q_1 f & 0 \\
(1-q_1)x_1\frac{\partial f}{\partial s} & (1-q_1)x_1\frac{\partial f}{\partial e} & (1-q_1)f - D - \frac{k_1 q_2}{1-q_2}x_2 & -\frac{k_1 q_2}{1-q_2}x_1 \\
(1-q_2)x_2\frac{\partial f}{\partial s} & (1-q_2)x_2\frac{\partial f}{\partial e} & 0 & (1-q_2)f - D
\end{pmatrix}
$$

At E_0, this Jacobian matrix is diagonal with the 4 diagonal entries equal to $-D$. Thus, E_0 is locally asymptotically stable. At E_1^*, where $*$ is either u or s, the Jacobian is

$$
\begin{pmatrix}
-D - x_1^*\frac{\partial f}{\partial s} & -x_1^*\frac{\partial f}{\partial e} & -f & -f \\
q_1 x_1^*\frac{\partial f}{\partial s} & q_1 x_1^*\frac{\partial f}{\partial e} - D & q_1 f & 0 \\
(1-q_1)x_1^*\frac{\partial f}{\partial s} & (1-q_1)x_1^*\frac{\partial f}{\partial e} & 0 & -\frac{k_1 q_2}{1-q_2}x_1^* \\
0 & 0 & 0 & (1-q_2)f - D
\end{pmatrix}
$$

Note that one of the eigenvalues is

$$(1-q_2)f - D = (1-q_2)\left(\frac{D}{1-q_1} - \frac{D}{1-q_2}\right) < 0,$$

because $q_1 < q_2$. This means that both cooperator-only steady states E_1^u and E_1^s are resistant to invasion by toxin producers. The remaining eigenvalues are those of the upper-left 3×3 sub-matrix of the Jacobian. Suppressing a tedious calculation, the characteristic polynomial of this submatrix is given by:

$\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0$, where

$$a_2 = 2D + x_1^*\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right) = 2D - (1-q_1)x_1^* h_1'(x_1^*)$$

$$a_1 = D\left[D + 2x_1^*\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right)\right] = D[D - 2(1-q_1)x_1^* h_1'(x_1^*)]$$

$$a_0 = x_1^* D^2\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right) = -(1-q_1)h_1'(x_1^*)x_1^* D^2$$

and where we have used the fact that

$$h_1'(x_1^*) = -\frac{1}{1-q_1}\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right),$$

which follows when taking the derivative in the definition of $h_1(x_1)$ in (10). Since $x_1^u < x_1^s$ are the two roots of the equation $h_1(x_1) = D/(1 - q_1)$, and since $h_1(x_1)$ is strictly concave by **H2**, there follows that:

$$h_1'(x_1^u) > 0, \quad \text{and} \quad h_1'(x_1^s) < 0.$$

The Routh–Hurwitz test implies that E_1^u is unstable because in this case $a_0 < 0$. For E_1^s, it is clear that $a_2 > 0$ and $a_0 > 0$. Furthermore,

$$
\begin{aligned}
a_1 a_2 & \\
- a_0 &= D[(D - 2(1-q_1)x_1^s h_1'(x_1^s))(2D - (1-q_1)x_1^s h_1'(x_1^s)) \\
&\quad + (1-q_1)h_1'(x_1^s)x_1^s D] \\
&= D[2D^2 - 4D(1-q_1)x_1^s h_1'(x_1^s) + 2(1-q_1)^2(x_1^s)^2(h_1'(x_1^s))^2] \\
&> 0,
\end{aligned}
$$

and the Routh–Hurwitz test implies that E_1^s is asymptotically stable.

We conclude by determining the stability of $E_{1,2}^*$, where $*$ is either u or s. The Jacobian is:

$$
\begin{pmatrix}
-D - (x_{1,2}^* + x_2^*)\frac{\partial f}{\partial s} & -(x_{1,2}^* + x_2^*)\frac{\partial f}{\partial e} & -f & -f \\
q_1 x_{1,2}^* \frac{\partial f}{\partial s} & q_1 x_{1,2}^*\frac{\partial f}{\partial e} - D & q_1 f & 0 \\
(1-q_1)x_{1,2}^*\frac{\partial f}{\partial s} & (1-q_1)x_{1,2}^*\frac{\partial f}{\partial e} & 0 & -\frac{k_1 q_2}{1-q_2}x_{1,2}^* \\
(1-q_2)x_2^*\frac{\partial f}{\partial s} & (1-q_2)x_2^*\frac{\partial f}{\partial e} & 0 & 0
\end{pmatrix},
$$

where $x_2^* = (q_2 - q_1)D/((1-q_2)k_1)$, which is independent of whether $*$ equals u or s, as pointed out earlier. Skipping a very long calculation, the characteristic polynomial of this Jacobian is:

$\lambda^4 + b_3\lambda^3 + b_2\lambda^2 + b_1\lambda + b_0$, where

$$
\begin{aligned}
b_3 &= 2D + x_2^*\frac{\partial f}{\partial s} + x_{1,2}^*\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right) \\
&= 2D + x_2^*\frac{\partial f}{\partial s} - (1-q_2)x_{1,2}^* h_2'(x_{1,2}^*) \\
b_2 &= D\left[D + 2x_2^*\frac{\partial f}{\partial s} + \frac{2 - (q_1 + q_2)}{1-q_2}x_{1,2}^*\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right)\right] \\
&= D\left[D + 2x_2^*\frac{\partial f}{\partial s} - (2 - (q_1 + q_2))x_{1,2}^* h_2'(x_{1,2}^*)\right] \\
b_1 &= D^2\left[x_2^*\frac{\partial f}{\partial s} + x_{1,2}^*\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right)\right] \\
&= D^2\left[x_2^*\frac{\partial f}{\partial s} - (1-q_2)x_{1,2}^* h_2'(x_{1,2}^*)\right] \\
b_0 &= -\frac{q_2}{1-q_2}x_{1,2}^* x_2^* k_1 D^2\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right) \\
&= q_2 x_{1,2}^* x_2^* k_1 D^2 h_2'(x_{1,2}^*)
\end{aligned}
$$

where we have used the fact that

$$h_2'(x_{1,2}^*) = -\frac{1}{1-q_2}\left(\frac{\partial f}{\partial s} - q_1\frac{\partial f}{\partial e}\right),$$

which follows when taking the derivative in the definition of $h_2(x_1)$ in (28). Since $x_{1,2}^s < x_{1,2}^u$ are the two roots of the equation $h_2(x_1) = D/(1 - q_2)$, and since $h_2(x_1)$ is strictly concave by **H3**, there follows that:

$$h_2'(x_{1,2}^s) > 0, \quad \text{and} \quad h_2'(x_{1,2}^u) < 0.$$

The Routh–Hurwitz test implies that $E_{1,2}^u$ is unstable because in this case $b_0 < 0$.

To finish the proof, we shall apply the Routh–Hurwitz test once again and show that if $h_2'(x_{1,2}^s)$ is sufficiently small (which happens when $x_{1,2}^s$ is sufficiently close to the critical point of the function $h_2(x_1)$), then $E_{1,2}^s$ is locally asymptotically stable. First, recall that according to the Routh–Hurwitz test, this steady state is locally asymptotically stable if

$$b_0 > 0, \quad b_3 > 0, \quad b_2 b_3 - b_1 > 0, \quad \text{and} \quad b_1(b_2 b_3 - b_1) - b_0 b_3^2 > 0.$$

When $h_2'(x_{1,2}^s) > 0$, it is clear that $b_0 > 0$. Also, when $h_2'(x_{1,2}^s)$ is positive and sufficiently small, then $b_3 > 0$. This follows from a continuity argument by noticing that if $h_2'(x_{1,2}^s) = 0$, then b_3 is positive. Similar

continuity arguments show that $b_2 b_3 - b_1 > 0$ and $b_1(b_2 b_3 - b_1) - b_0 b_3^2 > 0$ when $h_2'(x_{1,2}^s)$ is sufficiently small. Indeed, if $h_2'(x_{1,2}^s) = 0$, then:

$$
\begin{aligned}
b_2 b_3 - b_1 &= D\left[D + 2x_2^* \frac{\partial f}{\partial s}\right)\right]\left[2D + x_2^* \frac{\partial f}{\partial s}\right] - D^2\left[x_2^* \frac{\partial f}{\partial s}\right] \\
&= 2D\left(D + x_2^* \frac{\partial f}{\partial S}\right)^2 \\
&> 0 \\
b_1(b_2 b_3 - b_1) - b_0 b_3^2 &= D^2\left[x_2^* \frac{\partial f}{\partial s}\right]\left[2D\left(D + x_2^* \frac{\partial f}{\partial S}\right)^2\right] - 0 \\
&> 0
\end{aligned}
$$

$\square$

6. Resisting invasion by cheaters

In Theorem 5 we have identified conditions under which a (locally) stable coexistence of cooperators and toxin producers is possible. This stable coexistence comes in the form of the steady state $E_{1,2}^s$ of system (17)–(21). Here we will show that this steady state is resistant to invasion by cheaters when the rate constant of the toxin acting on the cheater is sufficiently large.

Theorem 6. *Assume that all the assumptions and conditions of Theorem 5 hold, and that*

$$
k_3 \neq \frac{q_2}{q_2 - q_1} k_1. \tag{32}
$$

Then system (1)–(6) has exactly 5 steady states $\mathcal{E}_0 = (E_0, 0)$, $\mathcal{E}_1^u = (E_1^u, 0)$, $\mathcal{E}_1^s = (E_1^s, 0)$, $\mathcal{E}_{1,2}^u = (E_{1,2}^u, 0)$ and $\mathcal{E}_{1,2}^s = (E_{1,2}^s, 0)$. Moreover,

- $\mathcal{E}_0$ *is locally asymptotically stable, but $\mathcal{E}_1^u$, $\mathcal{E}_1^s$ and $\mathcal{E}_{1,2}^u$ are unstable.*
- *If*

$$
k_3 > \frac{q_2}{q_2 - q_1} k_1, \tag{33}
$$

then $\mathcal{E}_{1,2}^s = (E_{1,2}^s, 0)$ is locally asymptotically stable, and system (1)–(6) is bi-stable. If the inequality (33) is reversed, then $\mathcal{E}_{1,2}^s = (E_{1,2}^s, 0)$ is unstable.

Proof. That $\mathcal{E}_0$, $\mathcal{E}_1^u$, $\mathcal{E}_1^s$, $\mathcal{E}_{1,2}^u$ and $\mathcal{E}_{1,2}^s$ are steady states of system (1)–(6) follows from Theorem 5, and the fact that the part of the boundary of the state space where $x_3 = 0$, is an invariant set for the system. Moreover, these 5 steady states are the only steady states in this part of the boundary of the state space. To see that these are the only steady states of the system, it therefore suffices to show that the system cannot have steady states with $x_3 > 0$. By contradiction, suppose there is a steady state with $x_3 > 0$. Then $x_1 > 0$ as well, for if this were not the case, then e would have to be zero, contradicting the steady state equation associated to (6). Thus, if $x_3 > 0$ then $x_1 > 0$ as well. We claim that then $x_2 > 0$ too, for if this were not the case, then t would have to be zero. But then the steady state equations associated to (5) and (6) yield that simultaneously $f(s, e) = D/(1 - q_2)$ and $f(s, e) = D$ for some pair (s, e), which is impossible. Thus, if a steady state with $x_3 > 0$ exists, then $x_1 > 0$ and $x_2 > 0$ as well. But then also $t > 0$. However, the generic condition (32) rules out the existence of such steady states: If such a steady state were to exist, then the steady state equations associated to (4), (5) and (6) imply that $k_3 = q_2 k_1/(q_2 - q_1)$, contradicting (32).

We now investigate the linearization of the system at these 5 steady states. The Jacobian matrix at each of the steady states has the following block-triangular structure:

$$
\begin{pmatrix} J_5 & * \\ 0 & \lambda_6 \end{pmatrix},
$$

where the value of $*$ is irrelevant, where J_5 is a 5×5 matrix, and λ_6 is the real, transversal eigenvalue in the x_3-direction. We now determine the location of the eigenvalues of the Jacobian matrices associated to each of the 5 steady states, from which their stability properties will follow:

1. For $\mathcal{E}_0$, we have that J_5 has 5 real and negative eigenvalues by Theorem 5, and it is easily checked that $\lambda_6 = -D$ is negative. Thus, E_0 is asymptotically stable.
2. For $\mathcal{E}_1^u$, Theorem 5 implies that J_5 has an eigenvalue with positive real part, hence $\mathcal{E}_1^u$ is unstable. Note moreover that here $\lambda_6 = Dq_1/(1 - q_1)$ is positive, implying that this steady state can be invaded by the cheater.
3. For $\mathcal{E}_1^s$, we see that $\lambda_6 = Dq_1/(1 - q_1)$ is positive too. This steady state can be invaded by the cheater, hence it is unstable.
4. For $\mathcal{E}_{1,2}^u$, Theorem 5 implies that J_5 has an eigenvalue with positive real part, and therefore this steady state is unstable.
5. For $\mathcal{E}_{1,2}^s$, it follows from Theorem 5 that all the eigenvalues of J_5 have negative real part. Moreover, the transversal eigenvalue in the x_3-direction equals:

$$
\lambda_6 = \frac{D}{1 - q_2} - D - k_3 \frac{q_2 - q_1}{1 - q_2} \frac{D}{k_1} = \frac{D}{1 - q_2}\left[q_2 - (q_2 - q_1)\frac{k_3}{k_1}\right].
$$

If (33) holds then $\lambda_6 < 0$, and then $\mathcal{E}_{1,2}^s$ is locally asymptotically stable. But if the inequality in (33) is reversed, then $\mathcal{E}_{1,2}^s$ is unstable, and in this case the cheater can successfully invade this steady state.

$\square$

7. Simulations

In this Section we present some numerical results to illustrate the main results obtained earlier.

In all of the following simulations $f(s, e) = ase$ is a linear function, with $a = 1.0$. Additionally, for all simulations we use the parameter values $s^0 = 1.0$, $D = 0.0346$, $k_1 = 0.015$, $q_1 = 0.24$, and $q_2 = 0.25$.

In Fig. 1 we illustrate that a cooperator-only population can persist according to Theorem 1, provided that the system's initial condition is contained in the region of attraction of E_1^s. But notice that washout may also occur, if the initial condition is contained in the region of attraction of E_0.

Fig. 2 illustrates that the Tragedy of the Commons occurs when there are cheaters, but no toxin producers or toxins, as proved in Theorem 2.

In Fig. 3 we show that a stable coexistence of cooperators and toxin producers is possible in the absence of cheaters, as proved in Theorem 5.

Fig. 4 shows two possible outcomes of the full model (1)–(6) when cooperators, toxin producers and cheaters are present, as discussed in Theorem 6. There is resistance to invasion by cheaters when (33) holds, and then the steady state $\mathcal{E}_{1,2}^s$ is locally asymptotically stable. But a Tragedy occurs when the inequality in (33) is reversed.

8. Conclusions

The purpose of this paper was to investigate a division of labor model in a population consisting of cooperators who produce a public good required for growth, and toxin producers who produce a toxin that harms invading cheaters who do not contribute to public good or toxin
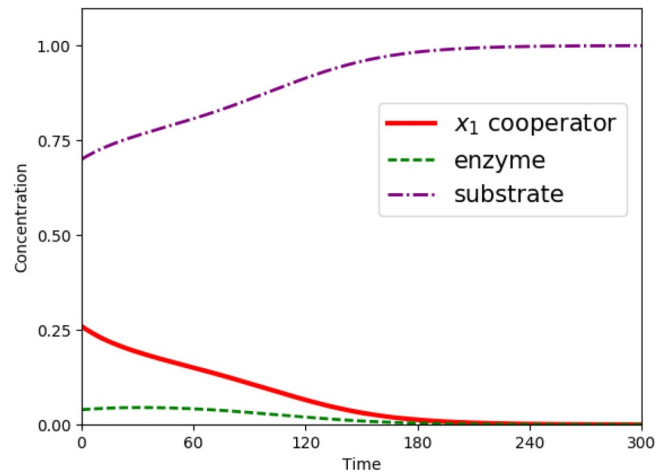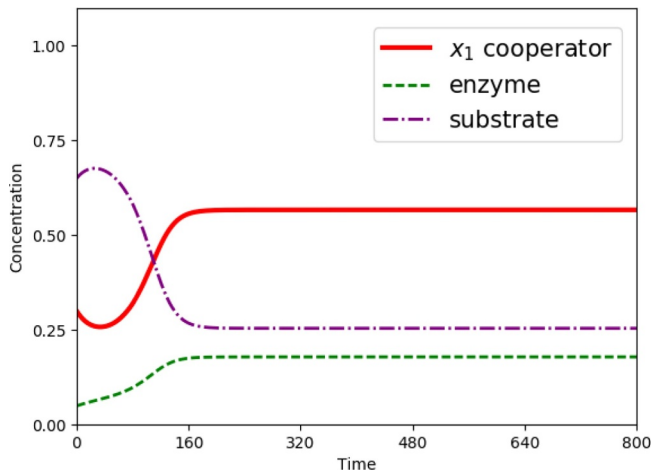
Fig. 1. Time series for system (7)–(9) illustrating the two locally asymptotically stable steady states for that system: The washout steady state E_0 (right panel) and the cooperator persistence steady state E_1^s (left panel). The initial conditions used for the right panel are $s = 0.70$, $e = 0.04$, $x_1 = 0.26$; the initial conditions for the left panel are $s = 0.65$, $e = 0.05$, $x_1 = 0.3$. For both figures $x_2 = t = x_3 = 0$.
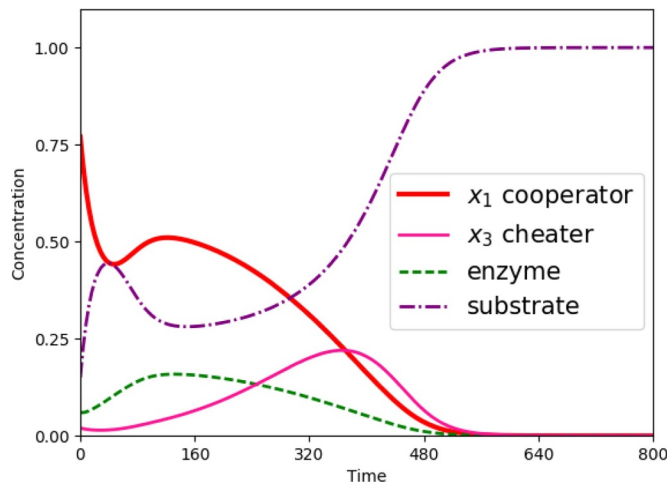

Fig. 2. Time series illustrating the Tragedy that occurs when cheaters are present and toxin-producing microbes are absent. The initial conditions are as follows: $s = 0.15$, $e = 0.06$, $x_1 = 0.77$, $x_3 = 0.02$, and $x_2 = t = 0$.
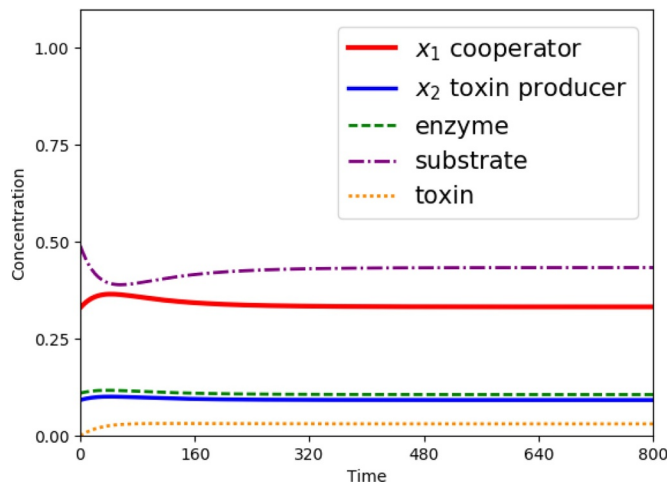

Fig. 3. Time series for the system (22)–(25) illustrating convergence to the locally stable steady state $E_{1,2}^s$. Here the initial conditions are $s = 0.49$, $e = 0.11$, $t = 0$, $x_1 = 0.33$, $x_2 = 0.0923$, $x_3 = 0$.

production. We first established that a cooperator-only population can persist (Theorem 1), but that it is always doomed when it is invaded by mutant cheaters (Theorem 2), a phenomenon known as the Tragedy of the Commons (ToC). Our main goal was therefore to determine if the ToC can be avoided in the presence of toxin-producers. We first showed that the mere presence of toxin producers is not necessarily enough to achieve this. Indeed, when the toxicity rate for cooperators k_1 exceeds the toxicity rate for cheaters k_3, then the entire population will still go extinct, and thus a ToC cannot be avoided (Theorem 3). In the absences of cheaters, a mixture of cooperators and toxin producers will go extinct if the cost of cooperation q_1 exceeds the cost of toxin production q_2 (Theorem 4). But a mixture of cooperators and toxin producers can coexist at a stable steady state in the absence of cheaters (Theorem 5), provided that:

1. The cost of toxin production q_2 exceeds the cost of cooperation q_1, and
2. The toxicity rate for the cooperators k_1 is sufficiently large, made precise in (27).

Theorem 5 was established under additional assumptions **H1, H2** and **H3** imposed on the growth rate function $f(s, e)$, but these are naturally satisfied for commonly used growth rate functions found in the literature. We also had to make the technical assumption that $h_1'(x_{1,2}^s)$ was sufficiently small to prove Theorem 5.

Our final result (Theorem 6) showed that the above mixed stable steady state of cooperators and toxin producers is resistant to invasion by cheaters, provided that the toxicity rate for the cheaters is sufficiently large; more precisely, cheaters cannot invade if

$$k_3 > \frac{q_2}{q_2 - q_1} k_1. \tag{34}$$

We have already mentioned above that to avoid a ToC, the toxicity rate for the cheaters k_3 should exceed the toxicity rate for cooperators k_1. Condition (34) shows exactly how much larger k_3 should be; namely, k_3 should be larger than $q_2/(q_2 - q_1)$ (a number that is strictly larger than 1) times k_1.

Our results contribute support to the idea that policing strategies may have evolved to stabilize and maintain cooperation in populations.

Declaration of Competing Interest

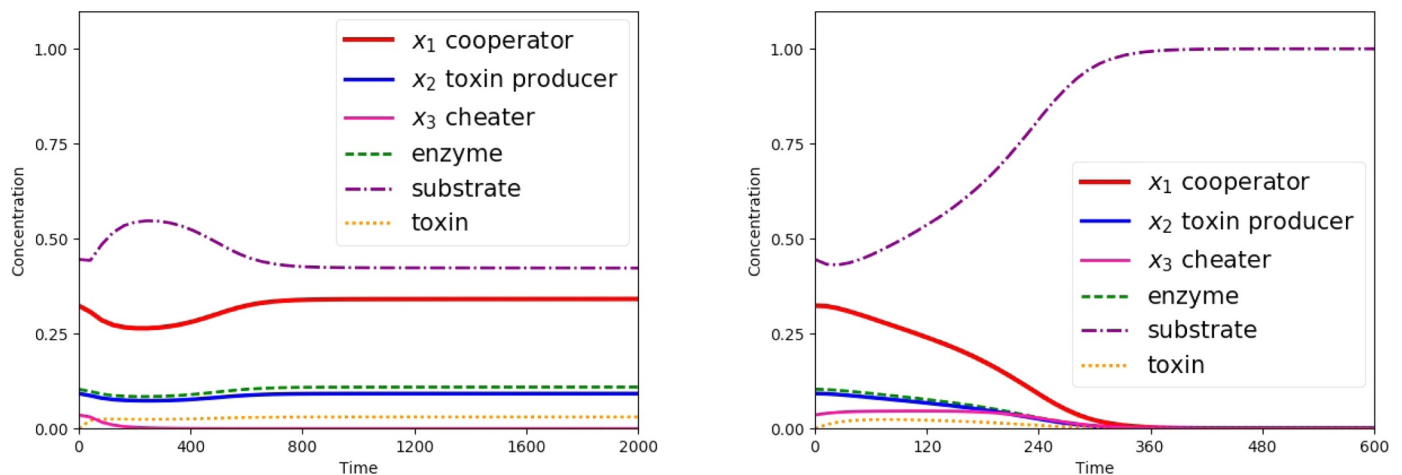Authors declare no conflicts of interest.

Fig. 4. Time series for system (1)–(6) illustrating resistance to the invasion by cheaters (left, $k_3 = 0.99$), or Tragedy (right, $k_3 = 0.30$). The initial conditions for both simulations are $s = 0.4456$, $e = 0.1035$, $t = 0$, $x_1 = 0.3235$, $x_2 = 0.036$, $x_3 = 0.036$.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.mbs.2019.108257

References

[1] M. Archetti, I. Scheuring, Review: Game theory of public goods in one-shot social dilemmas without assortment, J. Theor. Biol. 299 (2012) 9–20.

[2] Cheating (biology), http://www.en.wikipedia.org/wiki/Cheating_(biology).

[3] J.A. Damore, J. Gore, Understanding microbial cooperation, J. Theor. Biol. 299 (2012) 31–41.

[4] P. De Leenheer, H.L. Smith, M. Schuster, Strong cooperation or tragedy of the commons in the chemostat, Mathematical Biosciences and Engineering 16 (2019) 139–149, https://doi.org/10.3934/mbe.2019007.

[5] M. Doebeli, Y. Ispolatov, B. Simon, Towards a mechanistic foundation of evolutionary theory, 2017, ELife 2017;6:e23804.10.7554/eLife.23804.

[6] H.L. Ennis, D.N. Dao, S.U. Pukatzki, R.H. Kessin, Dictyostelium amoebae lacking an f-box protein form spores rather than stalk in chimeras with wild type, Proc. Natl. Acad. Sci. volume 97, (2000), pp. 3292–3297. USA

[7] R.A. Finkelstein, Bacterial extracellular zinc-containing metalloproteases, Microbiol. Rev. 57 (1993) 823–837.

[8] K.R. Foster, K. Parkinson, C.R. Thompson, What can microbial genetics teach sociobiology? Trends Genet. 23 (2007) 74–80.

[9] D. Greig, M. Travisano, The prisoner's dilemma and polymorphism in yeast SUC genes, Proc. Biol. Sci. 271 Suppl. 3 (2004) S25–26.

[10] W.D. Hamilton, The genetical evolution of social behaviour i & 2, J. Theor. Biol. 7 (1964) 1–52.

[11] G.R. Hardin, The tragedy of the commons, Science 162 (3859) (1968) 1243–1248.

[12] F. Harrison, A. Buckling, C.p.o.s.b. Pseudomonas aeruginosa, Front. Biosci. 14 (2009) 4113–4126.

[13] J. Hofbauer, K. Sigmund, Evolutionary games and population dynamics, Cambridge University Press, 1998.

[14] P. Manhes, G.J. Velicer, Experimental evolution of selfish policing in social bacteria, Proc. Natl. Acad. Sci. 108 (2011) 8357–8362.

[15] M. Perc, A. Szolnoki, Coevolutionary games-a mini review, BioSystems 99 (2010) 109–125.

[16] M. Perc, J. Gómez-Gardeñes, A. Szolnoki, L.M. Floría, Y. Moreno, Evolutionary dynamics of group interactions on structured populations: a review, J. R. Soc. Interface 10 (2012) 20120997, https://doi.org/10.1098/rsif.2012.0997.

[17] M. Perc, J.J. Jordan, D.G. Rand, Z. Wang, S. Boccaletti, A. Szolnoki, Statistical physics of human cooperation, Phys. Rep. 687 (2017) 1–51.

[18] F.L. Ratnieks, K.R. Foster, T. Wenseleers, Conflict resolution in insect societies, Annu. Rev. Entomol. 51 (2006) 581–608.

[19] F.L. Ratnieks, T. Wenseleers, Altruism in insect societies and beyond: voluntary or enforced? Trends Ecol. Evol. 23 (2007) 45–52.

[20] J.L. Sachs, U.G. Mueller, T.P. Wilcox, J.J. Bull, The evolution of cooperation, Quart. Rev. Biol. 79 (2004) 135–160.

[21] M. Schuster, D.J. Sexton, S.P. Diggle, E.P. Greenberg, Acyl-homoserine lactone quorum sensing: from evolution to application, Annu. Rev. Microbiol. 67 (2013) 43–63.

[22] M. Schuster, E. Foxall, D. Finch, H. Smith, P. De Leenheer, Tragedy of the commons in the chemostat, PLOS ONE (2017), https://doi.org/10.1371/journal.pone.0186119.

[23] N.E. Smalley, A. Dingding, M.R. Parsek, J.R. Chandler, A.A. Dandekar, Quorum sensing protects Pseudomonas aeruginosa against cheating by other species in a laboratory coculture model, J. Bact. 197 (2015) 3154–3159.

[24] H.L. Smith, P. Waltman, The theory of the chemostat, Cambridge University Press, 1995.

[25] M. Travisano, G.J. Velicer, Strategies of microbial cheater control, Trends Microbiol. 12 (2004) 72–78.

[26] G.J. Velicer, L. Kroos, R.E. Lenski, Developmental cheating in the social bacterium Myxococcus xanthus, Nature 404 (2000) 598–601.

[27] M. Wang, A.L. Schaefer, A.A. Dandekar, E.P. Greenberg, Quorum sensing and policing of Pseudomonas aeruginosa social cheaters, PNAS 112 (2015) 2187–2191.

[28] T. Wechsler, R. Kümmerli, A. Dobay, Understanding policing as a mechanism of cheater control in cooperating bacteria, 2018, BioRxiv. 10.1101/267799.

[29] T. Wenseleers, H. Helantera, A. Hart, F.L.W. Ratnieks, Worker reproduction and policing in insect societies: an ESS analysis, J. Evol. Biol. 17 (2004) 1035–1047.

[30] T. Wenseleers, F.L.W. Ratnieks, Tragedy of the commons in Melipona bees, Proc. R. Soc. Lond. B (Suppl.) 271 (2004) S310–S312.

[31] S.A. West, S. Griffin A., A. Gardner, Evolutionary explanations for cooperation review, Curr. Biol. 17 (2007) R661–R672.

[32] J.B. Xavier, W. Kim, K.R. Foster, A molecular mechanism that stabilizes cooperative secretions in Pseudomonas aeruginosa, Mol. Microbiol. 79 (2011) 166–179.