Accelerating returns from public goods favor quorum sensing in bacteria

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Abstract

Many bacteria excrete compounds, which may act in their environment as public goods. It has been observed that such excreted compounds often are under quorum sensing regulation, yet its is not well understood exactly when and why a bacterial population may gain from having a specific public good under quorum sensing regulation. Here, using mathematical modeling, we have determined how the functional forms of the benefit and cost curves can influence the need (or lack of same) for quorum sensing regulation. We found that the shape of cost and benefit functions crucially determines the optimal way to regulate production of a public good. In particular, we found that if the public good provides accelerating returns then the optimal strategy is to ramp up production sharply at a precise population size.

We further compared the shape of the analytically determined optimal production curves, to production curves which can be obtained by regulating production using a quorum sensing signal feeding back on its own production. We determined the quorum sensing parameters that maximize fitness in different scenarios, which revealed that the optimal parameters are very different for public goods with accelerating and decelerating returns respectively. Most importantly we found that public goods which provides accelerating returns calls for stronger positive signal feedback.

1 Introduction

Bacterial cells live complex lives, constantly adjusting to the fluctuating presence or absence of nutrients, toxins, competitors and other environmental factors. They do this by regulating the production of different molecules that can perform the functions required to give them a fitness advantage in the current environment. Some of these molecules, like membrane bound nutrient receptors, are "private goods" which provide a benefit only to the individual cell that produced them. Others are molecules that are secreted by the cells and perform their function outside. Once secreted these molecules can diffuse away and potentially benefit other cells making them "public goods". Microbes which produce extracellular molecules that can be thought of as public goods are ubiquitous. Examples of such products are: extracellular enzymes [21], exopolysaccharides [28, 30], surfactants [10, 32], antibiotics [1, 13, 16, 17], virulence factors [23, 11, 33], siderophores [19, 8, 12]. In some of these cases, the good produced is public "by necessity", constrained by the chemistry of the required function. In other cases, it may be public "by choice", i.e. it is essential to the overall benefit that there are other cells around that can use the public good and themselves produce it too. In the latter case it may make sense to call the act of production and excretion cooperation. Although such cooperation in bacteria has received a lot of attention, the exact way benefit depends on the extracellular concentration of public good (hereafter referred to as the benefit function of the public good) has in general not been studied in great detail experimentally. Results from a recent theoretical paper [3] however suggest that the shape of the benefit function will influence whether a population may benefit from having a certain public good under quorum sensing[1] (QS) regulation. Bacterial public goods

[1] Quorum sensing (QS) is a bacterial behavior ubiquitously present in the microbial world and most bacteria possess at least one quorum-sensing system, [15, 7, 25]. This term covers all types of behavior where bacteria produce, excrete, and subsequently respond to diffusable signal molecules. Typically, the signal molecules are small and relatively cheap to produce; often an acyl homoserine lactone [7].

are in fact often under quorum sensing regulation, in for example *Pseudomonas aeruginosa* the most represented functional class in the list of quorum sensing regulated gene products are excreted compounds [27]. It is often assumed that public goods provide more benefit at higher population densities (e.g. [5, 29]) and this has also recently been shown to be true experimentally for a specific public good produced by *Pseudomonas aeruginosa* [4]. There is however stil a lack of general analytical arguments concerning when and why a bacterial population may benefit from having a public good under QS regulation. Here we use simple mathematical models, to determine how the functional form of the benefit function of a public good affects the optimal way to regulate the production of the public good. Specifically, we have determined how the optimal public good production rate depends on the population size, for public goods with either accelerating or diminishing returns. Further we have determined how well a QS system can generate production curves which mimic the analytically determined optimal curves, and we discuss whether there might be evolutionary tradeoffs associated with "choosing" the right QS parameters for regulating certain public goods.

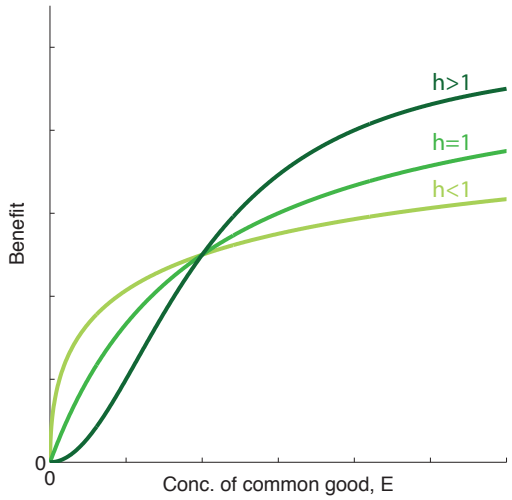

Figure 1: **Concave/convex sigmoidal benefit function:** 'Benefit' could for example be the increase in growth rate due to the presence of the public good (cost of production is not considered here). For example the benefit function could be a sigmoidal function: $benefit = b\frac{(E/K_E)^h}{1+(E/K_E)^h}$, plotted here for three different values of the exponent $h = [1/2, 1, 2]$. The parameter h can be tweaked so that benefit initially decelerates ($h \leq 1$) or accelerates ($h > 1$) with increasing concentration of public good.

2 Models and Results

Optimal production of public goods in a well mixed population

We consider a well mixed[2] population of isogenic cells that produce a public good, E. Let Δg denote the change in growth rate of such a population due to the act of producing the public good. Δg, which could be negative or positive, can be decomposed into the benefits accrued from having the public good present and the costs of producing it. We make the following assumptions about the costs and benefits:

1. The cost is an increasing function of the rate of production of the public good.

[2]Using a well-mixed system is a simplification that allows us to treat each cell as identical to all others and to ignore spatial variations in the public good concentration.

2. The cost is zero when no public good is being produced.

3. The benefit is an increasing function of the concentration of the public good.

4. The benefit does not increase indefinitely as the concentration of the public good increases; it saturates at some finite value.

5. The benefit is zero when there is no public good present.

For now, we will assume that the cost is a linear function [3] of the rate of production, σ_E, of the public good, and the benefit a sigmoidal function of the concentration, E, of public good:

$$\begin{aligned} \Delta g &= benefit - cost \\ &= b\frac{(E/K_E)^h}{1+(E/K_E)^h} - c\sigma_E \end{aligned} \quad (1)$$

We will focus (for now) in particular on the shape of the first term of eq. (1), i.e. the benefit function of the public good, (see fig. 1). When $h \leq 1$, the benefit function is always concave, i.e. its slope is maximum at $E = 0$ and steadily decreases as E increases. In other words, increasing public goods results in decelerating returns. In contrast, when $h > 1$, increasing public goods initially results in accelerating returns; i.e. the benefit function is initially convex. In figure 2 we propose a concrete example of a class of public goods that can have differently shaped benefit functions depending on the precise molecular mechanism by which the public good works. Exoenzymes that work by degrading proteins in the environment into smaller metabolizable pieces can work in two different ways: Exoproteases break the polymer peptide bonds starting from the end of the polymer and endoproteases target specific peptide bonds effectively breaking the polymers at random sites causing the benefit function to be either convex or concave (providing accelerating or decelerating returns, respectively) (see supplementary for the full length derivation which lead to this conclusion).

We now wish to determine how the convexity/concavity of the benefit function influences the optimal production strategy for a public good when

[3] More on general nonlinear cost and benefit functions in the discussion.

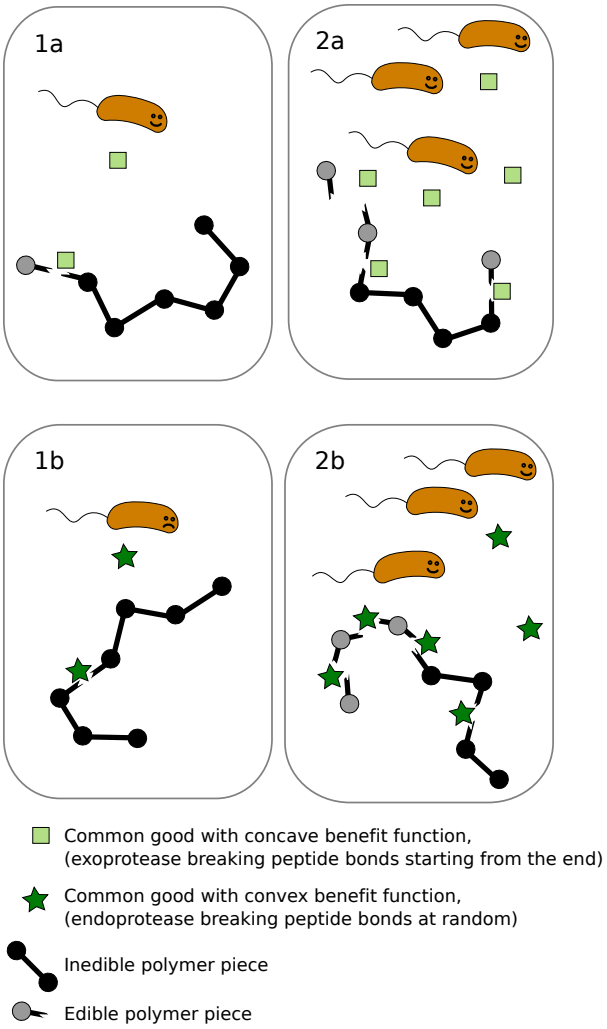

Figure 2: **Example of public goods with differently shaped benefit functions:** Proteases which works extracellularly by degrading polymers that are otherwise too large to be transported over the cell membrane can either break the peptide bonds of the polymer starting from the end of the polymer (exoprotease) or target a range of specific types of peptide bond within the chain, effectively breaking bonds at random (endoprotease). In this scenario benefit is proportional to the probability of yielding "edible" pieces of polymer. **1a and 2a:** Benefit increases approximately linearly with exoprotease concentration resulting in a concave benefit function. **1b and 2b:** The probability of breaking the polymer at a site producing an piece small enough to transport over the cells membrane is low when enzyme concentration is low, but accelerates as endoprotease concentration increases, resulting in a convex benefit function. (See supplementary materials for full derivation).

the cost function is linear. The rate of change of the concentration of a public good E in a wellmixed system with N cells wil be given by:

$$\frac{dE}{dt} = N\sigma_E - E\gamma_E, \tag{2}$$

where σ_E is the public good production rate per cell and γ_E is the degradation rate of the public good. For the sake of simplicity we will for now make the assumption that the timescales of public good production and degradation are much faster than the timescale for growth of cells. This allows us to assume that the public good concentration is always at steady state value E^* for a given population size:

$$E^* = N\frac{\sigma_E}{\gamma_E}, \tag{3}$$

and thus we can express E^* as a function of σ_E When this assumption holds we can, by replacing E in eq. 1 with E^* from eq. 3 write the effect of the public good on the growth rate in terms of population size N and production rate per cell σ_E only:

$$\Delta g = b\frac{(N\sigma_E/K_E\gamma_E)^h}{1+(N\sigma_E/K_E\gamma_E)^h} - c\sigma_E. \tag{4}$$

In this situation, it is evident that cells which produce public good at a rate, σ_E^{opt} that maximizes eq. (4), above, for each value of N, will grow fastest.

In fig. 3 we have plotted this optimal rate $\sigma_E^{opt}(N)$ as a function of N. We see that the function $\sigma_E^{opt}(N)$ has very different properties when the benefit function is always concave ($h < 1$), compared to when it is initially convex ($h > 1$). When $h < 1$, $\sigma_E^{opt}(N)$ smoothly rises from zero at $N = 0$ to a maximum and then smoothly back to zero as $N \to \infty$. In contrast, when $h > 1$, i.e. with accelerating returns, $\sigma_E^{opt}(N) = 0$ for N smaller than a critical value N_{crit}, after which it jumps discontinuously to a non-zero value. After the jump the production rate may rise further to a maximum before decreasing to zero, or may simply decrease to zero, as $N \to \infty$, depending on the values of other parameters such as h, b, c. The $h = 1$ case is a marginal one. The production rate is zero for $N < N_{crit}$ and then rises, but not discontinuously; only the slope is discontinuous at $N = N_{crit}$.

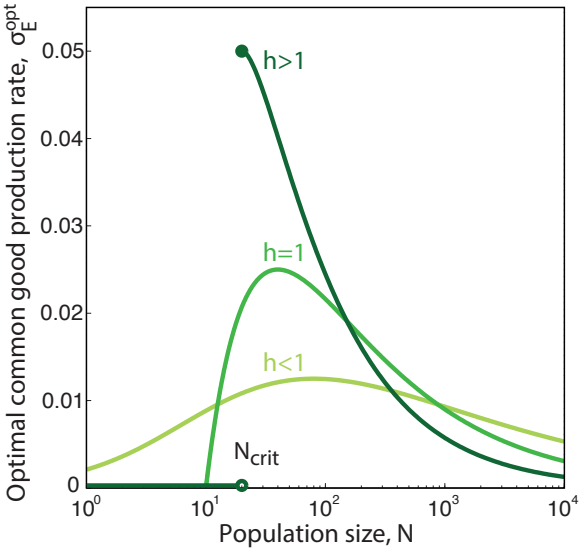

Figure 3: **Public goods with accelerating benefits should not be produced below a critical population size:** Optimal production rate, σ_E^{opt} of public good as a function of population size, N for three different values of the public good benefit function hill-factor, $h = [0.5, 1.0, 2.0]$. The value, $\sigma_E^{opt}(N')$, corresponds to the σ_E which maximizes Δg for N'. In the marginal case $h = 1$ the optimal production function can be put in closed form: $\sigma_{E,h=1}^{opt}(N) = \sqrt{\frac{bK_E\gamma_E}{cN}} - \frac{K_E\gamma_E}{N}$. The critical population size above which public good production should commence, is for $h = 1$: $N_{crit} = \frac{c}{b}\gamma_E K_E$, see supplement for derivation. In this figure σ_E is given in units of γ_E, whic was set to one. $b/c = 1/10$ and $K_E = 1$).

The take home message is that when the public goods provide accelerating returns the optimal behavior is to turn on production to a finite non-zero value only at a critical population size. In contrast, for decelerating returns even a single cell may benefits from producing the public good.[4]

2.1 Regulating public goods using a quorum sensing system

In the previous paragraph we saw that the convexity/concavity of the public good benefit function is important for determining the shape of the optimal public good production curve, i.e. the way the optimal production rate depends on population size. Although the curves are very different it is clear that for both $h \leq 1$ and $h > 1$) the optimal production rate varies with the population size, (see fig. 3) and that in all cases it thus looks like cells would benefit from having production linked to a mechanism which senses the cell density; a property which QS systems posses. We now wish to determine just how closely a QS regulation mechanism realistically could come to generating production curves matching the optimal curves in fig. 3.

2.1.1 A simple well mixed model of QS regulated public good production

We formulate a simple ODE model of a well mixed population producing signal and public good with a positive feedback of signal on its own production:

$$\frac{dS}{dt} = N\left(1+\sigma_S^{max}\frac{(S/K_S)^\alpha}{1+(S/K_S)^\alpha}\right) - S \quad (5)$$

$$\frac{dE}{dt} = N\sigma_E^{max}\frac{(S/K_S)^\alpha}{1+(S/K_S)^\alpha} - \gamma_E E \quad (6)$$

Here S is the concentration of quorum sensing signal molecule[5], E, the concentration of public good and N is the number of cells. The equations are non-dimensionalized by measuring all rates in units of the basal rate of signal production (i.e. the rate of signal production when signal concentration is very low) and by measuring time in units of the mean lifetime of a signal molecule. The rate of signal production when the population is fully induced is $\sigma_S^{max}+1$ and[6] σ_E^{max} is the maximum rate of public good production per cell. The mean lifetime of a public good molecule is $1/\gamma_E$ and K_S is the signal concentration where both signal production and public good production is at half of the maximum rate. The exponent α captures the strength of the positive feedback of the signal on its own production; for example this could be the cooperativity of the DNA binding by the transcription factor which activates production of signal synthase. In this model signal receptor molecules are not modeled explicitly for the sake of simplicity. The parameter, α, does thus strictly not need to be interpreted just as the cooperativity of the transcription factor DNA binding but could also be influenced by the effect of signal positively feeding back on production of receptor molecules, (as discussed in e.g. [9, 22]). Assuming that both signal and public good have the same half-saturation constant K_S and feedback exponent α is probably a crude assumption which we will nonetheless make for the sake of simplicity. Once again we make the assumption that production and decay of quorum sensing molecules and public good molecules happen at a much faster timescale than growth of cells. This means that we can assume that for each population size N signal concentration, S, will reach steady state, S^*. In fig. 4 the steady state concentration of QS signal S^* is plotted as function of the population size N for different values of the feedback exponent α. We see that when α goes above a certain critical value α_C (see supplement for an analytical expression for α_C) the system will have a bistable region with two stable fix points, S^*_{low} and

[4] See supplementary materials for a further discussion on 1) the case of a spatially structured system, 2) situations where the assumption that timescales of production and decay of public good are much faster than the time scale of growth and 3) situations where cost does not increase linearly with production rate, but have an initial convex or concave shape.

[5] Actually the feedback acts not on the signal production but on the production of the signal synthase, an enzyme which facilitates the production of signal intracellualarly, but since concentration of signal synthase and signal has been found to be approx. proportional in eg. the Las system of *Pseudomonas aeruginosa* [6], we can model signal and signal synthase by one symbol S.

[6] Note that because all rates are given in units of the basal signal production rate ($\sigma_S^{basal} \equiv 1$), σ_S^{max} is in fact the ratio between the basal rate and the maximum rate.

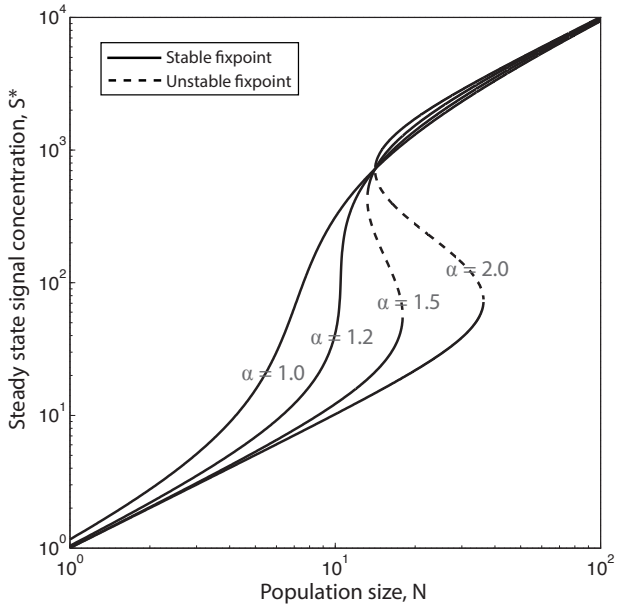

Figure 4: **Increasing the QS signal feedback exponent α causes hysteric response to population changes:** Steady state QS signal concentration as function of the population size N, plotted for four different values of the QS feedback exponent $\alpha = [1.0, 1.2, 1.5, 2.0]$. When $\alpha > \alpha_c = \frac{2+\sigma_s^{max}+2\sqrt{1+\sigma_s^{max}}}{\sigma_s^{max}} \approx 1.2210$ the system will be bistable for a certain range of N's. ($\sigma_S^{max} = 100$, concentration of S is here given in units of $\sigma_S^{basal}/\gamma_S$. σ_S^{basal} is the basal production rate and γ_S is the degradation rate of the of public good.)

S^*_{high} (and one unstable, $S^*_{unstable}$ in between). (Such bistability has indeed been observed in e.g. Vibrio fischeri [31]). The public good production rate per cell, σ_E, for a given population size N will be given by the first right hand side term in eq. 6 and thus depend on the steady state concentration of signal, $S^*(N)$:

$$\sigma_E(N) \equiv \sigma_E^{max} \frac{(S^*/K_S)^\alpha}{1+(S^*/K_S)^\alpha}. \tag{7}$$

When the system is bistable this means that there will be two different production curves; one which involves the lower stable fix point (S^*_{low}) that the system will follow when going from low to high numbers and another curve which involves the the higher stable fix point (S^*_{high}) which the system will follow when going from high numbers to low. Fig. 5-A, -B and -C (upper panels) show examples of such QS production curves (with and without bistability) plotted together with the matching optimal curves from fig. 3 for comparison.

2.2 Quantifying the fitness of a public good regulation strategy

Different choices of QS parameters α, K_S and σ_E^{max} result in different public good production curves. These curves $\sigma_E(N)$, may resemble the optimal curves from fig. 3 more or less. In order to determine which QS parameters optimize the regulation of a public good with a specific benefit function, we need a way to assess the fitness of a population of cells which utilize a specific production curve. Equation 4 expresses how Δg depend on the production rate, σ_E, so for a given QS regulated production curve $\sigma_E(N)$, eq. 4 gives a corresponding function $\Delta g(N,h) \leq \Delta g^{opt}(N,h)$ which shows how growth is affected by public good production as population size, N, varies. In the lower panels of fig. 5-A, -B and -C, the corresponding $\Delta g(N,h)$ curve of the production curve $\sigma_E(N)$ from the upper panel is plotted.
The fitness/performance of a given production curve, $\sigma_E(N)$, can be quantified by the following expression:

$$w \equiv \frac{\int_0^{N_{max}} \Delta g(N,h) dN}{\int_0^{N_{max}} \Delta g^{opt}(N,h) dN}. \tag{8}$$

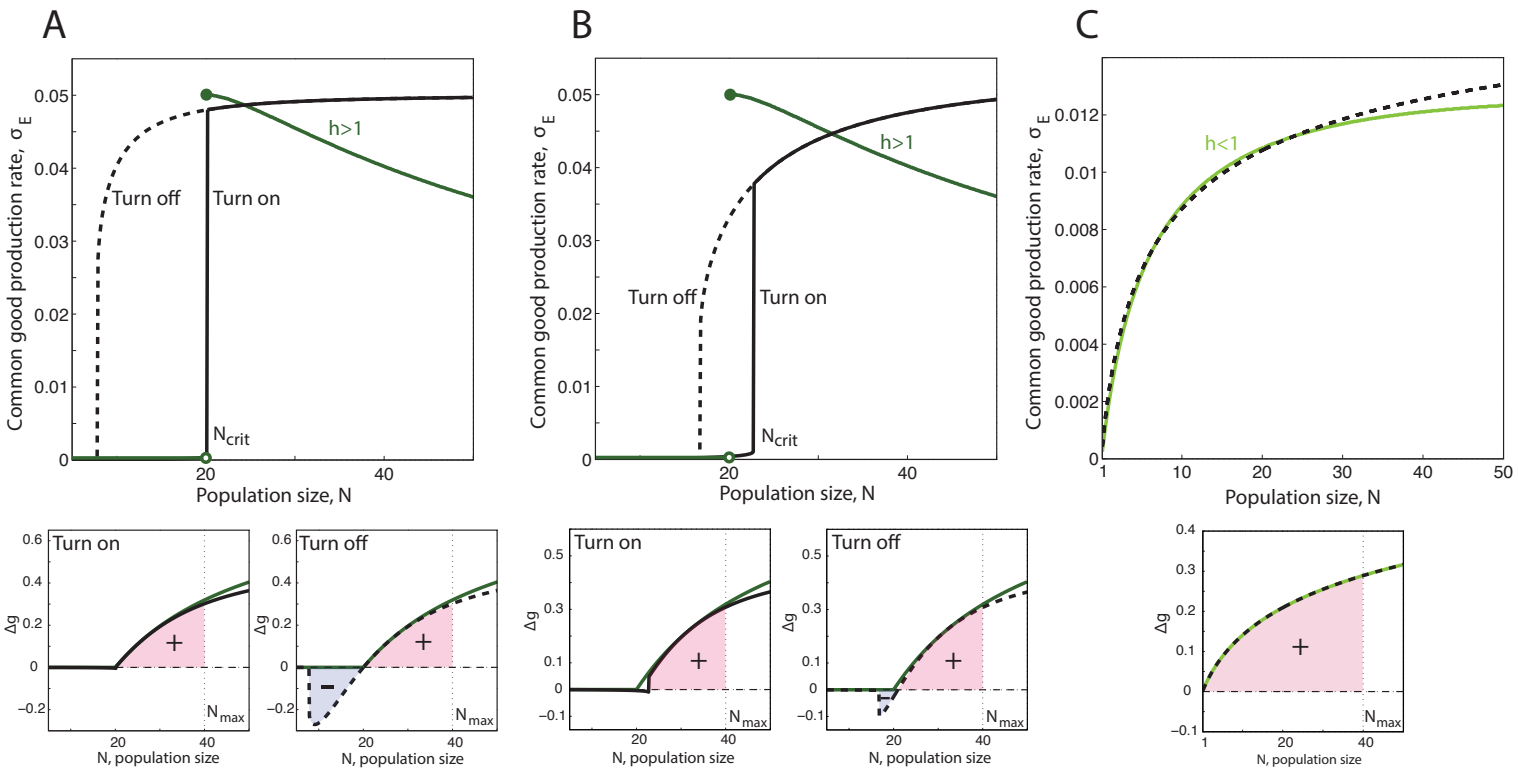

Figure 5: **Quorum sensing parameters optimized for different ecological scenarios and different types of public goods: Upper panels:** Green line shows optimal production rate, $\sigma_E^{opt}(N,h)$, as a function of population size N. Black full line and dashed line show the production rate as a function of population size, N, when production is regulated by **A**: a QS system with the parameters optimized for a public good with a convex benefit function in turn on situations only ($\alpha = 2$, $K_S = 400$, $\sigma_E^{max} = 0.055$), **B**: a system with QS parameters for a public good with a convex benefit function maximizing fitness in both turn on and turn off scenarios, ($\alpha = 1.5$, $K_S = 913$, and $\sigma_E^{max} = 0.05364$), and **C**: a QS system with parameters that maximize fitness for a public good with a concave benefit function, ($\alpha = 0.5$, WRONG NUMBERS CORRECT!!$K_S = 913$, and $\sigma_E^{max} = 0.05364$) **Lower panels:** Green lines show Δg^{opt}, the increase in growth rate achieved, as a function of N when producing public good using the optimal production curve σ_E^{opt}. Full black line shows Δg as a function of N for the QS regulated production curve marked 'Turn on' in the plot above. Dashed black line shows Δg as a function of N for the QS regulated production curve marked 'Turn off' in the plot above. The colored area underneath the Δg curves in the range $N = [0, N_{max}]$ is proportional to the fitness, w (defined in eq. 8 and 9).

This fitness measure quantifies how growth is affected by public good production over a range of different population sizes (the range set by the limits[7] of the integral, $[0, N_{max}]$). Fitness, w, is normalized with respect to the performance of a population which follows the optimal production strategy. This means that fitness will be one ($w = 1$) if the QS production curve perfectly mimics the optimal curve $\sigma_E^{opt}(N, h)$ and fitness will be zero ($w = 0$) for a population which does not produce public good at all. (Negative fitness corresponds to having a lower fitness than a non-producer).

2.2.1 'Turn on' and 'turn off' of public good production in the wild

It has been observed in several experimental studies (e.g. [23]) that, very often non-producing cheater mutants will arise over time in populations which excretes a public good. Invasion of a faster growing cheater mutant could fragment the producer population and thus lead to an effective dilution of the wild type population. It seems that in this type of situation, having a mechanism which would down-regulate production of public good in response to the decreasing cell density would be crucial for the wild type cells' ability to avoid population collapse (this scenario is also discussed in [14]). It thus seems there could exists ecological settings where both 'turn on' and 'turn off' scenarios are important for the fitness of the population.

Very little is known about actual ecological situations in the wild where bacteria use quorum sensing to regulate gene expression. In most of the quorum sensing literature the emphasis has been on the process of turning *on* QS regulated genes when population size increases, not *off* when it decreases. This bias might stem from the fact that 'turn off' scenarios are probably not of great importance in the organism where QS was first discovered: *Vibrio fischeri* a bacterium which lives in symbiosis with the bobtailed squid, an ecological setting where the bacteria period-

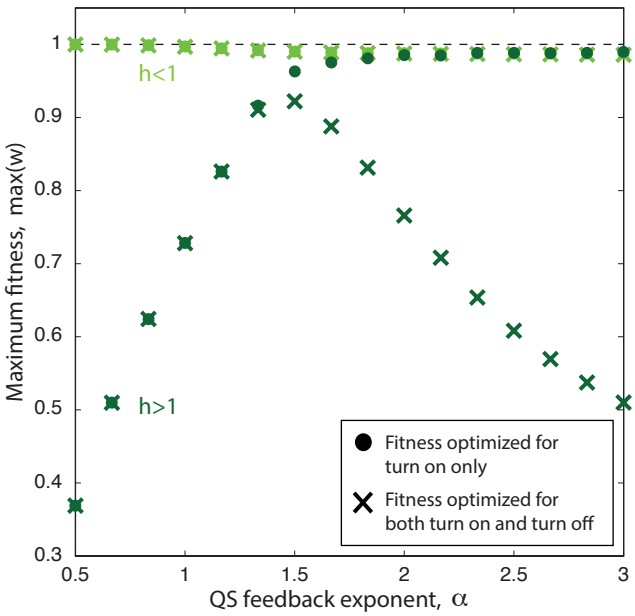

Figure 6: **Fitness as a function of the QS feedback exponent, α:** Each point in fig. 6 corresponds to the height of the tallest peak in a 2D fitness landscape with σ_E^{max} and K_S on the xy-axes for the given value of α. Light green is $h = 0.5$ and dark green is $h = 2.0$. Fitness w is defined in eq. 8 and 9. A fitness of 1 corresponds to the fitness of a population following the optimal curves in fig. 3 perfectly and a fitness of 0 corresponds to the fitness of a non producer. For values of α where the system is bistable (here when $\alpha > \alpha_c \approx 1.2210$) fitness can be calculated either such that only the turn on curve is considered (marked by full circles) or such that both the turn on curve and the turn off curve is considered (marked by crosses). Parameters where $\sigma_S^{max} = 100$, $b/c = 1/10$ and $K_E = 1$.

[7] For this study we are mostly interested in the behavior of the system in the vicinity of the critical turn on point, N_{crit} and not so much in the behavior in the limit of high N, so we set the integral limit N_{max} to a value just above N_{crit}.

ically goes through stages of population increase and decrease. The bacteria slowly grow to high density inside the light organ of the squid, reach the point where they turn on light production at nightfall only to be quickly diluted and turn off light production when the squid spurts out the majority of the bacteria by morning. In this situation, the dilution of signal and decrease in the population density happen so fast when the squid vents its light organ, there is probably no need to have an accurate mechanism for down regulating light production as the population density decreases, since very little time is spent at intermediate densities.

2.2.2 Quantifying fitness in both "turn on" and "turn off" scenarios

As we saw earlier, there exist parameters for which the QS signaling system is bistable and thus where a set of QS parameters, $(\alpha, K_S, \sigma_E^{max})$, correspond to not one production curve but to two: one 'turn on' curve which the population will follow when going from low to high numbers and one 'turn off' curve which it will follow when going from high to low numbers. For a bacteria population that encounters both situations where they need to regulate public good expression when the population is increasing and when it is decreasing and which has such a bistable set of parameters, we thus need to assess the performance in both situations and include both in the fitness measure. One way of doing this is simply by using a weighted average of the fitness, w, of the two individual production curves:

$$w \equiv x_{on} w_{on} + x_{off} w_{off} \tag{9}$$

(where the weights add up to one $(x_{on} + x_{off} = 1)$). Depending on how often a population encounters 'turn on' and 'turn off' situations and on the relative importance of these situations the weight would be distributed differently. Since we do not have any information about what exactly these weights are for any actual ecological setting we will here, for the sake of argument, just compare the two extreme cases of $(x_{on} = 1,\ x_{off} = 0)$ where strictly 'turn on' scenarios are important for fitness (possibly an ecological setting like the case of *Vibrio fischeri* living in the bobtailed squid where population dilution happens very fast) and the case where 'turn on' scenarios and 'turn off' scenarios are equally important for fitness $(x_{on} = 0.5,\ x_{off} = 0.5)$, (possibly an ecological setting where the population relatively often faces the risk of slow dilution due to the appearance of a cheater mutant).

2.2.3 Fitness as a function of the QS feedback exponent, α

For a public good with a certain benefit function there will be a specific set of QS parameters, (α, K_S, σ_E^{max}), which maximizes the fitness, w. This is, roughly speaking, the set of parameters that within the range $[0, N_{max}]$ manage to best mimic the optimal production curve (e.g. fig. 5A), or in the case where there is bistability and both 'turn on' and 'turn off' curve are considered to be equally important, it is the set of parameters which allows both curves to best mimic the optimal curve, (e.g. fig. 5B) at the same time.

Often a single QS system will regulate the expression of many different excreted products which can be very diverse in nature and potentially have differently shaped benefit functions. Since the parameter K_S quantifies the binding strength and σ_E^{max} the overall promoter strength of the promoter regulating a specific gene product, it seems likely that these two parameters in most cases can be fine tuned by evolution to suit the regulation needs of individual QS products. The parameter α, on the other hand, characterizes the way the QS signal molecule feeds back on its own production and will thus in most cases be a parameter which has to be the same for all the QS regulated products unless there is more than one QS system in the cell[8]. In fig. 6 the maximum fitness obtainable with any choice of the parameters σ_E^{max} and K_S is plotted as a function of the signal feedback exponent α, (see supplementary materials for example plots of the σ_E^{max}, K_S fitness landscapes, for different values of α and h). Interestingly, we see in fig. 6

[8] Is is however possible to have different promoter sequences for signal synthase expression and public good production. For example LasR can bind either cooperatively or noncooperatively depending on the promoter sequence, [26]

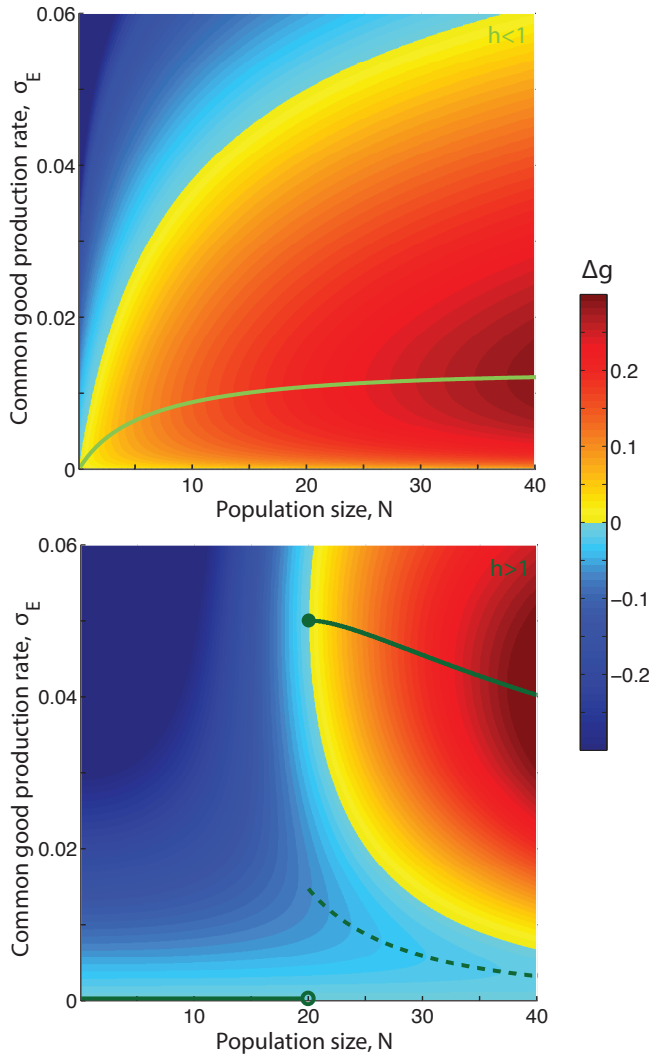

Figure 7: **The impact on growth of public good production:** Red/blue colors show Δg as a function of the population size N and the public good production rate σ_E. **Upper panel:** The benefit function of the public good is concave ($h = 1/2$). The light green line marks the optimal public good production curve (same as plotted in fig. 3). Note that there are many ways to plot production curves in this Δg landscape without ever having $\Delta g < 0$. **Lower panel:** The benefit function of the public good is convex ($h = 2$). The dark green full line marks the optimal public good production curve (same as plotted in fig. 3). The dark green dashed line marks the values of the public good production rate, σ_E^{min}, which locally minimizes Δg for each N. Note that it is virtually impossible to plot a continuous production curve without having parts of it going through regions where $\Delta g < 0$.

that the feedback exponent α which optimizes fitness for a population regulating a convex good ($h > 1$) and a concave good ($h < 1$) are very different. For $h = 1/2$ the optimal[9] α value is around $\alpha \approx 0.5$ both when fitness is optimized for 'turn on' only and when it is optimized for both 'turn on' and 'turn off' scenarios. For $h = 2$ on the other hand, when fitness is optimized for 'turn on' only, fitness increases monotonically with increasing α, slowly approaching the maximal value of one, (note however that beyond $\alpha = 2$ there is virtually no extra advantage to be had from increasing α further). When $h = 2$ and fitness is optimized for both 'turn on' and 'turn off' the optimal feedback exponent is at an intermediate value $\alpha \approx 1.5$. The reason for this is that at low values of α the QS production curves do not ramp up production fast enough while at higher values of α, the system becomes highly bistable and 'turn on' and 'turn off' curves thus very different which means they cannot both resemble the optimal curve, (more on this in the discussion).

2.2.4 Regulating expression of both convex and concave public goods with one QS system

In fig. 6 it is clear that the α-values which optimize fitness are very different for public goods with convex and concave benefit functions. It is thus interesting to think about what a bacterial population which needs to regulate several different types of public good with the same QS system would do. Note in fig. 6 that while the fitness peak is very broad when $h < 1$, it is quite narrow when $h > 1$, signifying that the evolutionary pressure which will drive the system towards the optimal α value will be far stronger in the case of $h > 1$ than $h < 1$.

In fig. 7 we have plotted Δg as a function of the population size, N and public good production rate, σ_E. It is clear that for $h < 1$ and $h > 1$ the topology of this Δg- 'landscape' differs dramatically. It is this difference which explains the broad and narrow fitness peaks for $h < 1$ and $h > 1$ respectively. Recall that different sets of QS parameters, (α, K_S

[9] In fig. 6 the x-axis only goes down to 0.5 but we did check fitness at lower values of α.

and σ_E^{max}), correspond to different production curves: When we look at figure 7 it becomes apparent that for $h < 1$ there are many ways to draw suboptimal production curves through the Δg 'landscape' without encountering regions where $\Delta g < 0$, i.e. many choices of QS parameters which would give a positive fitness. For $h > 1$ on the other hand we see that only curves which keep production very low for low N and then ramp up production very rapidly later can avoid going through major regions where $\Delta g < 0$. This means that for $h > 1$, the range of QS parameters α, K_S and σ_E^{max} that result in positive fitness is relatively narrow.

3 Discussion

3.0.5 Results can be generalized to all cases of nonlinear cost and benefit function pairs

For the sake of simplicity we have throughout this paper considered the specific case of a linear cost function and a sigmoidal benefit function. There is however obviously no reason to believe that actual public goods will have costs and benefits which fit these arbitrarily chosen functions exactly. Fortunately it turns out that more general cases of nonlinear cost and benefit function pairs can be mapped onto the results presented earlier. When cost is linear, we saw that convex ($h > 1$) and concave ($h < 1$) sigmoidal benefit functions fall into two major categories: Either they have continuous or discontinuous optimal production curves. It turns out that general pairs of (monotonically increasing) cost and benefit functions all fall into either of these two classes. We will refer to common goods with cost and benefit functions that cause them to have continuous optimal production curves as belonging to class 1 and common goods with cost and benefit functions which cause them to have discontinuous optimal production curves as belonging to class 2. Note that class 1 thus resembles the case of linear cost and sigmoidal benefit function with $h < 1$ while class 2 resembles the case of a linear cost and a sigmoidal benefit curve with $h > 1$. (In figure 2 in the supplementary materials we show examples of pairs of nonlinear cost and benefit functions belonging to these two classes).

If the benefit function $b(\sigma_E)$ and the cost function $c(\sigma_E)$ for a certain common good are known it is possible to determine whether it belong to class 1 or class 2. If production pays of even at single cell level it means that the optimal production curve will be continuos, (class 1). The requirement for this to happen is simply that:

$$\left.\frac{dc}{d\sigma_E}\right|_{\sigma_E=0} \leq \left.\frac{db}{d\sigma_E}\right|_{\sigma_E=0,N=1} \tag{10}$$

The requirement for production only to pay off after a critical population size N_c has been reached is that:

$$\left.\frac{dc}{d\sigma_E}\right|_{\sigma_E=0} > \left.\frac{db}{d\sigma_E}\right|_{\sigma_E=0,N=1} \tag{11}$$

but this criteria is not necessarily enough to produce a discontinuous optimal production curve (a class 2 case). Functions which satisfy eq. 11 can have a optimal production function which rises continuously from zero at population size $N_c > 1$, (behaving like the limiting case where $h = 1$ with linear cost and sigmoidal benefit function). The additional requirement which ensures a discontinuous optimal production curve is that the second derivative of the benefit and cost functions have a relative shift in convexity at a non zero value of $\sigma_E^* > 0$, such that:

$$\left.\frac{d^2c}{d\sigma_E^2}\right|_{\sigma_E<\sigma_{*E}} < \left.\frac{d^2b}{d\sigma_E^2}\right|_{\sigma_E<\sigma_{*E}} \tag{12}$$

and

$$\left.\frac{d^2c}{d\sigma_E^2}\right|_{\sigma_E>\sigma_{*E}} > \left.\frac{d^2b}{d\sigma_E^2}\right|_{\sigma_E>\sigma_{*E}} \tag{13}$$

Note that this can happen even if both functions are strictly concave, (see eg. figure 2 lower panel in the supplementary materials).

Finally it must be emphasized that as we expect the shape and magnitude of the cost and benefit functions to depend sensitively on specific growth conditions, we thus predict that the optimal production curves, and particularly the critical population size

where production need to be ramped up, to vary in different conditions too. This prediction fits well with the observation by Duan et al. [6], that Las and Rhl expression profiles can vary greatly for different growth conditions. Such environmentally sensitive responce could for example be achieaved by making QS parameters like K_S (binding strength of signal-receptor complex to promoters) and σ_E^{max}, (common good promoter strength) dependent on enviromental cues such as nutrient availability and stresses.

3.0.6 Class 2 public goods require density dependent regulation

Overall we can draw the conclusion that class 2 public goods (which resembles the case of a convex benefit curve and liniar cost) require density dependent regulation more so than class 1 public goods, based on the properties of the optimal production curves. To reiterate, these properties are for a class 2 good:

1. Production below a certain critical population size N_{crit}, will give a negative impact on fitness (this was also suggested in a study [3] by Cornforth et al.)
2. The optimal production curve has a discontinuous jump from low to high production rate at N_{crit}.
3. Even when $N > N_{crit}$ production at a rate lower than the optimal one can cause a negative impact on fitness. (see fig. 7).

The optimal production curve of a class 1 good on the other hand have quite different properties:

1. Public good production can yield a fitness advantage even at the single cell level.
2. It is possible to impact fitness negatively by producing a class 1 good, but only by producing at too high a rate compared to the optimum, never when producing at a rate which is less than the optimum.
3. A group can indeed benefit from letting production of a class 1 good depend of population size but deviating from the optimal curve does not necessarily come at a great fitness cost.

Because of the very different properties of the optimal production curves for class 2 and class 2 goods respectively the QS parameters which would be needed to regulate them differ too. In the case of a class 2 good, fitness can be negatively impacted if the QS feedback exponent is not appropriate. Conversely for a class 1 good fitness is approximately constant for a broad range of QS feedback exponents and one could thus argue that constant constitutive expression would be a better choice for regulation of a class 1 good than a costly elaborate QS regulation mechanism.
Interestingly in Schuster et al. [26] they find that LasR can bind either cooperatively or non-cooperatively depending on the promoter sequence. So although the overall signal feedback is characterized by a single exponent α_S, the individual public goods, E_i, can have different responses to the signal-receptor complex characterized by different exponents α_{E_i} [26]. The reason for these differences could perhaps be that the various excreted products have different cost/benefit functions and thus different optimal production curves and resultantly different regulation needs.

3.0.7 A class 2 good calls for QS regulation with strong positive feedback - a novel explanation for the existence of QS signal feedback

Quorum sensing is a mechanism usually assumed to give individual cells information about the density of the population. It is thus paradoxical that one feature found in many quorum sensing systems, the positive signal feedback, actually makes a system less accurate for sensing population size changes. Roughly speaking a strong positive signal feedback makes a QS system more appropriate for answering the binary question "are we many or few?" than for providing information about the precise population size over a broad range of densities. The reason usually given for why QS signals often feed back positively on their own production, is that this feedback ensures a synchronized response across a population.

Our analysis inspires another explanation for the existence of positive QS signal feedback, (which does however not exclude the existing one). Recall that for

a class 2 public good the optimal production strategy calls for a sudden discontinuous jump in production rate at a critical population size N_{crit}. After this critical population size has been reached a substantial growth increase can be gained by producing public good at a specific optimal rate, but there will exist a production rate lower than the optimal one which will result in a growth rate decrease (see fig. 7, for $N > N_{crit}$ there exists a local fitness minima below the optimal rate $\sigma_E^{min} < \sigma_E^{opt}$). When ramping up production from zero to the optimal rate, the population thus necessarily has to pass through a local fitness minimum. A reason for having a strong positive signal feedback could thus be to ensure a sharper turn on of the public good production in order to minimize the time spent at the production rates where fitness of the population is impacted negatively.

3.0.8 Optimizing QS parameters for a class 2 common good both in 'turn on' and 'turn off' scenarios - A trade off situation?

When only 'turn on' scenarios are important for fitness it is just the production curve that the population follows when going from low to high numbers, which needs to mimic the optimal curve, and bistability thus does not matter. If however both 'turn on' and 'turn off' scenarios are important for fitness, this changes. Now both the production curve which the population follows going from low to high numbers *and* the curve it follows from high to low numbers needs to mimic the optimal one simultaneously. For higher values of the feedback exponent this becomes problematic since the two curves will differ more and more due to the increasing width of the bistable range. This suggests that a bacterium which needs to accurately regulate the production of a class 2 good both in situations where the population increases and decreases, will face an evolutionary trade off between '*presicion*' and '*sharpness*' of the production curves. Too high a feedback exponent will mean *imprecise* turn on/off points for either (or both curves) due to the bistability and too low a feedback will mean that the turn on/turn off of the public good does not happen *sharply* enough. We thus predict that a bacteria living in such an ecological settings will have a feedback exponent bound at intermediate values - relatively close to the point where the system starts to display bistability.

3.0.9 Ideas for experiments

With few exceptions [24, 14]) There is a general tendency in the QS literature to focus only on the 'turn on' of QS genes and only few studies have looked thoroughly at the effect of positive signal feedback (in fact it is disregarded in some theoretical studies, [20, 18, 2]. So far no one to our knowledge has attempted to assess the fitness cost of bistability when both 'turn on' and 'turn off' scenarios are important. Our analysis suggest thats it could be interesting to explore experimental setups which examine how QS regulated genes are turned off in response to a decrease in population density, and setups which probe the effect of signal feedback on bistability.

Measuring the shape of public good cost and benefit functions The benefit function of a public good (e.g. elastase produced by *P. aeruginosa*) could be quantified by measuring the growth rates of signal-blind cheats (e.g. the lasRrhlR mutant) in a chemostat, as a function of the concentration of externally added public good (e.g. LasB). The cost function could be quantified by measuring the growth rate of an inducible constitutive producer mutant at different expression rates. It would be interesting to automate these types of measurements so that they could be done for a wide range of different molecules thought to be public goods, from different bacterial species. The shape/convexity of the measured benefit and cost functions could then be compared with already known information about whether the molecules are QS regulated or not, to determine whether class 2 public goods are overrepresented among QS regulated compounds.

Manipulating the shape of the benefit function experimentally The way the specific public good LasB of *P. aeruginosa* works might provide a way to manipulate the convexity of its benefit function. When provided solely with a diet of casein polymers,

P. aeruginosa growth depends on the production of LasB (and similar proteases) that degrade the casein polymers into smaller importable units, which can be transported through the cell membrane and metabolized. In the supplementary materials we show that the benefit function becomes increasingly convex if the maximum length of the polymers in the environment is increased. This suggests that one way of experimentally tweaking the benefit function would be to pre-digest casein polymers to varying degrees before providing them to *P. aeruginosa*. Media with undigested casein should result in a more convex benefit function than media with pre-digested casein. Growth of constitutive producer mutants could then be compared with growth of wild-type *P. aeruginosa* in these media to test our results regarding the importance of the convexity of the benefit function for QS regulation.

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