The effect of dilution on eco-evolutionary dynamics of experimental microbial communities

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Abstract

Changing environmental conditions can infer structural modifications of predator-prey communities. New conditions often increase mortality which reduces population sizes. Following this, predation pressure may decrease until populations are dense again. Dilution may thus have substantial impact not only on ecological, but also evolutionary dynamics because it amends population densities. Experimental studies, in which microbial populations are maintained by a repeated dilution into fresh conditions after a certain period, are extensively used approaches allowing us to obtain mechanistic insight into fundamental processes. By design, dilution, which depends on transfer volume (modifying mortality) and transfer interval (determining the time of interaction), is an inherent feature of these experiments, but often receives little attention. We further explore previously published data from a live predator-prey (bacteria and ciliates) system which investigated eco-evolutionary principles and apply a mathematical model to predict how various transfer volumes and transfer intervals would affect such an experiment. We not only find the ecological dynamics to be modified by both factors, but also the evolutionary rates to be affected. Our work predicts that the evolution of the anti-predator defence in the bacteria, and the evolution of the predation efficiency in the ciliates, both slow down with lower transfer volume but speed up with longer transfer intervals. Our results provide testable hypotheses for future studies of predator-prey systems, and we hope this work will help improving our understanding how ecological and evolutionary processes together shape composition of microbial communities.

Introduction

The composition of microbial communities is sensitive to the environment (Alekseeva et al., 2020; Goldford et al., 2018; Scheuerl et al., 2020), which changes growth of individual species (Bittleston et al., 2020; de Mazancourt et al., 2008) and the interaction with other community members (Fiegna et al., 2015b, 2015a; Gibert and Brassil, 2014). Modifications of the environment can affect predator-prey systems (Gilpin, 1972), and a stable predator-prey community may be destabilized due to dwindling densities of a keystone species (Banerjee et al., 2018; Gilljam et al., 2015). For example, a predator may go extinct if the density of the prey becomes too low (Fussmann et al., 2003). Following this, environmental changes can affect community structure and composition and may disrupt vital functions pivotal for ecosystem functioning. Changes of the environment may include the use of antibiotics (Dethlefsen and Relman, 2011), or eutrophication of lake ecosystems (Kearns et al., 2016; Kiersztyn et al., 2019; Kuiper et al., 2015), just as few examples which have been demonstrated to change communities.

A common effect of environmental change is the modification of the mortality rate (Abreu et al., 2019) and for how long the community can grow without further disturbance. These two aspects can be easily implemented in laboratory experiments. In fact, a standard method in experimental studies exploring ecological and evolutionary questions is using microbial communities with periodic transfer to fresh conditions (Hiltunen et al., 2018, 2017; Nair et al., 2019; Scheuerl et al., 2019). In such experiments, two or more species are cultivated in batch culture for a certain period of time, after which a subset of the community is transferred to fresh conditions (Barrick and Lenski, 2013). After initiating each growth cycle using serial-dilution, the organisms start growing and deplete the available resources. In predator-prey systems the prey initially growth fast, but at later stages, when the predators are dense enough, the prey population is consumed. Albeit this serial-dilution does rarely reflect conditions found in nature, these approaches allow estimating population densities and traits undergoing evolution, so various hypothesis can be tested to understand principles. In liquid media that contain all nutrients for rapid cell division, microbes can grow extremely quickly, which makes them suitable study organisms for experiments exploring ecological and evolutionary dynamics over several generations (Buckling et al., 2009). This, however, means that populations reach limiting conditions quickly. To keep the growth conditions constant, populations are commonly either maintained in chemostat systems (Fussmann et al., 2003; Scheuerl and Stelzer, 2019; Stelzer, 2009), or a proportion of the population is transferred to fresh conditions regularly (often between 24 hours and 72 hours) (Fiegna et al., 2015b; Good et al., 2017; Hiltunen et al., 2017; Lawrence et al., 2012; Scheuerl et al., 2019; Scheuerl and Stelzer, 2017). Diluting a small part of the populations every few days is a classical approach

to keep populations constantly growing and to avoid growth plateaus, e.g. reaching carrying capacity, once nutrient limitation occurs (Bennett et al., 1990). The two key parameters of dilution, *transfer volume* and *transfer interval*, are often chosen without further investigation. We investigate how dilution, that is transfer volume and transfer interval, affects ecological changes and the speed of grazing resistance/efficiency evolution, by disentangling the two options to realize different dilution terms of a non-chemostat setting. When batch cultures are regularly transferred to fresh conditions, this are fundamentally different conditions compared to a chemostat system, where medium is replenished on a constant rate, which retains populations at the maximum possible density supported by the settings (Barrick and Lenski, 2013). In batch cultures, populations grow rapidly and exploit the resources but then experience fresh conditions after transfer to grow rapidly again.

In a community with predator-prey interaction, theoretically, decreasing the transfer volume to increase dilution (e.g., 1% instead of 10%) results in lower initial densities and prey may initially grow little constrained by predation as predators are rare. Further, prey populations may not be under strong selection to defend because rarely, or only shortly before the next transfer, encounter predators (Friman et al., 2008; Fussmann et al., 2000; Scheuerl and Stelzer, 2019). Contrarily, extending the transfer interval (e.g., every 48 hours instead of every 24 hours), should increase final densities so that prey and predator encounter each other more often, which may intensify evolutionary changes in the defence of prey. Consider growing bacteria as prey and ciliates as predators for a single growth period (Fig. 1). Bacteria will begin growing exponentially until internal density regulation stops this increase. Predation further slows the growth of the prey and may result in a population collapse (Fig. 1a). When bacterial densities are high enough, the ciliates will consume the bacterial cells and will increase in density (Fig. 1b); this way reducing bacterial densities until ciliates can grow no more due to lack of prey. It can be easily seen that the transfer interval and the transfer volume can both have major impact on the next growth period. If the transfer interval is short, only bacterial densities may be high and ciliate densities may still be neglectable. If the transfer interval is long, ciliates may have already consumed most bacteria, and the next growth cycle is initiated at different densities compared to the previous round. Thus, the transfer interval mainly determines the ratio between prey and predator at each transfer for the next growth period (Fig. 1c), whereas transfer volume controls initial conditions for each growth period. Missing in our knowledge is how modification of both factors, transfer volume and transfer interval, together affect ecology and evolution in an experimental predator-prey community. Experimental tests of ecological and evolutionary dynamics in microbial predator-prey systems are extremely laborious and applying more than one transfer volume and transfer

interval is usually not doable. Theoretical modelling offers a convenient approach out of this dilemma.

Here, we explore experimental data of a predator-prey experiment from the literature (see reference (Hiltunen et al., 2018)) and apply mathematical modelling to explore multiple modifications of the original protocols. We use a semi-continuous Lotka-Volterra model (including dilution of populations at regular intervals) and added equations allowing for co-evolutionary change of interaction (Kaitala et al., 2020). Expanding our previous model (Kaitala et al., 2020) we report how transfer volume and transfer interval affect predator-prey communities and expand the prior literature by exploring scenarios impractical in experimental studies. Our theoretical findings suggest that dilution has effects on the community. First, decreasing the transfer volumes, we find that coexistence is threatened, and evolutionary change is limited, while increasing transfer volumes result in more evolution. Second, decreasing transfer interval has similar effects driving populations extinct and decreasing evolutionary rates, while an increase reverses the trend. Our aim was to gain further mechanistic insight into this well-established predator-prey system, thus we focus in our analysis on the similar scenarios as the original study (Hiltunen et al., 2018). In this study, the authors tracked ciliates consuming bacteria, and transferred 1% (transfer volume) of the microorganisms every 48 hours (transfer interval). While the model would allow to simulate a much broader parameters space (e.g., dilution between 0% and 99%), we are missing further information to validate model results. It is worth of noting that the transfer volume or the transfer interval has not been standardized in similar experiments. It is also important to note here that due to the transfer design it is unlikely to see population cycles as any dynamics may be disrupted during transfers. Finally, we can assume that natural mortality rate is rather low because the transfers in the experiments represent a substantial mortality factor for each of the species. We acknowledge that our model simplifies naturally observed dynamics, but we aim for a model easy to understand even by researcher less familiar with mathematical models but conducting related experiments.

Methods

We mathematically modelled the co-evolutionary predator-prey interactions of a published study (Hiltunen et al., 2018) applying an ecological Lotka-Volterra model (Volterra, 1926) modified to explain co-evolution between the prey and predator (Kaitala et al., 2020; Mougi, 2010; Mougi and Iwasa, 2011). In the experimental study 1% of the population was transferred after a 48 hours interval to fresh conditions (Hiltunen et al., 2018). Our model represents the

150 growth period of the experiment, which is initiated newly applying a transfer volume by the end
151 of the transfer interval to obtain a semi-continuous system.

152 We use the following modification of the Lotka-Volterra model

153 $$\frac{dP(t)}{dt} = r_P\left(1 - \frac{P(t)}{K}\right)P(t) - aP(t)Z(t)$$

154 $$\frac{dZ(t)}{dt} = baP(t)Z(t)$$

155 where the linear growth of the prey is replaced by logistic growth and the natural mortality of
156 the predator is omitted, because of the high dilution in the design. *P* and *Z* denote the prey
157 and predator populations, r_P is the prey growth rate, *K* is the carrying capacity, *a* is the attack
158 rate and *b* is prey to predator conversion efficiency.

159 In the co-evolutionary version, the Lotka-Volterra model is revised such that the attack rate *a*
160 and the conversion efficiency *b* are functions of auxiliary trait variables *u* and *v* of the prey
161 and predator, respectively (Kaitala et al., 2020; Mougi, 2010; Mougi and Iwasa, 2011). The
162 trait variables have dynamics of their own, the purpose of which is to maximize the fitness of
163 the corresponding species. Thus, the co-evolutionary model can be presented as follows

164

165 $$\frac{dP(t)}{dt} = W_P(u(t),v(t))P(t),$$

166 $$\frac{dZ(t)}{dt} = W_Z(u(t),v(t))Z(t),$$

167 where

168 $$W_P(u(t),v(t)) = r_P\left(1 - \frac{P(t)}{K}\right) - a(u(t),v(t))Z(t),$$

169 and

170 $$W_Z(u(t),v(t)) = b(v(t))a(u(t),v(t))P(t),$$

171 are the per capita fitness functions of the prey and the predator.

172

173 The per capita fitness functions are controlled by the prey and predator trait variables
174 $u(t)$ and $v(t)$, respectively. The trait dynamics are assumed to be driven by a selection
175 gradient, which ultimately aims to maximize fitness. The attack rate and the prey to predator
176 conversion efficiency were assumed to be of the form

$$a(u(t),v(t)) = a_0 exp(c_1 v(t))\exp(-gu(t)),$$

$$b(v(t)) = b_0 exp(-c_2 v(t)),$$

respectively (Kaitala et al., 2020). Here c_1, c_2 , and g are fixed model parameters estimated from the experimental data (see Kaitala et al., 2020).

The evolutionary dynamics of trait variables *u*(*t*) and *v*(*t*), as defined, e.g., by Abrams et al. (1993) and Mougi (2010), are given as follows

$$\frac{du(t)}{dt} = G_P \frac{dW_P(u(t),v(t))}{du} = G_P[a_0 g exp(c_1 v(t) - gu(t))Z(t)], u(0) = 0,$$

$$\frac{dv(t)}{dt} = G_Z \frac{dW_Z(u(t),v(t))}{dv},$$

$$= G_Z[(c_1 - c_2)b_0 exp(-c_2 v(t))a_0 exp(c_1 v(t) - gu(t))P(t)], v(0) = 0,$$

where G_P and G_Z are parameters determining the speed of the evolution of the traits. The evolution of the trait variables then determines the evolution of the attack rate $a(u,v)$ and the prey to predator conversion efficiency $b(v)$. In the experimental data studied, the ancestral individuals in each species did not have any earlier history of occurring together in a predator-prey interaction. Thus, the initial values of the traits *u*(0) and *v*(0) are chosen to be equal to 0. Consequently, the initial bacterial and ciliate populations are referred to as “naïve”. Other parameters are estimated from the experimental data presented elsewhere (Hiltunen et al., 2018). The model variables are shown in Table 1 and the parameter values with units are shown in Table 2. For more details about the model please see our previous study (Kaitala et al., 2020). The produced evolutionary dynamics are potentially more like evolution from standing genetic variation as traits change continuously. Note also that the bottle-neck effect for small transfer volume cannot be investigated using this model because no discrete units are selected.

Table 1. Model variables and units

P	Bacterial density	Bacterial cells/ml

Z	Ciliate density	Ciliate cells/ml
u	prey trait	dimensionless
v	predator trait	dimensionless

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206 Table 2. Model parameter values

r_P	growth rate of the bacterium	$3.3/h$
K	carrying capacity of the bacterium	2.58×10^{8} Bacterial cells/ml
a_0	Initial value of attack rate	4.2×10^{-6} ml/Ciliate cells/h
b_0	Initial value of prey to predator conversion efficiency	5.75×10^{-4} Ciliate cells/Bacterial cells
g	defence value	7.3347
c_1	offence value	0.8568
c_2	conversion value	0.4745
G_P	speed of prey evolution	0.0017
G_Z	speed of predator evolution	0.0271

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208 We next study effects on ecological and evolutionary dynamics after modifying the transfer
209 volume or transfer interval while maintaining the original estimated model parameters (Kaitala
210 et al., 2020). The initial condition for the prey is 8.56×10^{7} Bacterial cells/ml and for the
211 predator 56800 Ciliate cells/ml. The numerical simulations were performed using ODE solver
212 ode15s in MATLAB R2019a.

213

214 **Model fit and experimental data.** We estimated parameters necessary for our model using
215 data presented in a study exploring ecological and evolutionary dynamics in a live bacteria-

ciliate system (Hiltunen et al., 2018). The experimental data and our model predictions consistently result in coexisting prey and predator populations under these conditions. Prey densities increase over time because anti-predatory defence evolves and bacteria get less eatable by ciliates (Hiltunen et al., 2018). The predator densities decrease over time as prey becomes better defended against predatory attacks. Coevolution in the predation prevents further decrease in the predator densities (Cairns et al., 2020) with the level of final densities reached after a few transfers and our model is well equipped to capture these dynamics (Kaitala et al., 2020).

Results

Changing the transfer volume affects ecological and evolutionary dynamics. To explore how dilution by changed transfer volume affects predator-prey communities, we successively modified the transfer volume in our model (Fig. 2) but kept the transfer interval constant at 48 hours. Transferring only 0.5% of the populations (compared to 1.5%), results in reduced bacterial density (Fig. 2a) and drives the predator very close to extinction (Fig. 2b). Bacterial densities are still observed for a 0.2% transfer volume (Fig. A1). Increasing the transfer volume >1.5% has little effect on densities (Fig. 2). When dilution is less severe and the next growth cycle is started with higher densities, the initial dynamics seem to fluctuate a bit more in the beginning. However, after a few transfers, the fluctuation in predator-prey densities fade away and there is no obvious difference between transfer volumes of 1.5% and 2.5% (Fig. 2).

Low transfer volumes should release prey from predation pressure because the predator density may be too low to initiate selection high enough to have an effect. Indeed, our results indicate a change in the evolutionary rates. Our model successively predicts that bacterial evolution for increased anti-predator defence slows down with increasing dilution (Fig. 2c). At highest dilution, the anti-predator prey trait u only changes moderately, but when dilution is low (high transfer volume) we see a great change in evolution. On the ciliate side, we see a faster change in predator trait v (Fig. 2d) and the attack rate a (Fig. 2e) under low transfer volumes as we would expect when predators are selected for higher attack rate due to reduced encounter events. The conversion efficiency b decreases over course of the experiment, but less under lower transfer volumes (Fig. 2f). At the extreme low end of transfer volumes, when only the bacteria survive, anti-predator defence stops evolving (Fig. A1).

After around 25 transfers, our model predicts that the prey-predator ratios are the same for all transfer volumes (Fig. A2). Before this happens, we see great differences in the bacteria-ciliate ratios with much more bacteria at highest transfer volume. Predators need a

prolonged time to catch up and to establish stable populations. The final ratio, however, seems to be robust against different transfer volumes unless the predator goes extinct.

Changing transfer interval affects ecological and evolutionary dynamics. Because we observed an effect of transfer volume on ecological and evolutionary dynamics in this system, we next addressed the problem whether the transfer interval may have an effect as well. As indicated in Fig. 1, unlike transfer volume which keeps ratios sustained, this should affect the bacterial-ciliate ratio transferred to the next growth cycle. On the ecological side, this means that the transfer interval modifies the initial ratio between bacteria and ciliates for the next growth cycle, which may affect timing when ciliates start to efficiently consume bacteria. On the evolutionary side, anti-predator defence and attack rate are expected to intensify under longer antagonistic interaction periods.

Applying different transfer intervals indeed resulted in various ecological dynamics (Fig. 3). The bacterial and ciliate densities are not strongly affected by the length of the intervals (Fig. 3a,b). For short transfer interval of 24 hours both species become extinct. For the intermediate transfer intervals of 48 hours the bacterial densities steadily increase (Fig. 3a) whereas the ciliate densities first steadily decrease but reach a stable point towards the end of the experiment at low densities (Fig. 3b). When the transfer interval is increased to 72 hours, there will be considerable fluctuations in both species in the beginning after which stable coexistence is reached.

When both species become extinct, no evolution will occur (Figs 3c-e). With increasing transfer intervals, we would expect predation activity to intensify, whereas at shorter intervals predation intensity may be weakened because of low initial densities and reduced encounter rates. A transfer interval less than 48 hour in fact reduces bacterial anti-predation evolution (Fig. 3c), whereas intervals longer than 48 hours result in faster evolution of prey trait *u* in the bacteria (Fig. 3c). Predator trait *v* always increases linearly, for longer transfer intervals (Fig. 3d). The attack rate *a* seems first to decrease slightly, but more under lower dilution (Fig. 3e). Again, conversion efficiency *b* linearly decreases but with no differences between transfer intervals of 48 and 72 hours (Fig. 3f).

We were also interested how evolutionary dynamics are predicted under exceedingly small modifications of transfer intervals. Increasing intervals only slightly (only 2-8 hours) has enhanced impact on evolutionary trajectories (Fig. A3). Notably, increasing the interval only initially results in an increase of prey trait *u* in the bacteria, while for predator trait *v* we see sustained deviations.

Interaction between transfer volume and transfer interval. Because we saw both, transfer volume and transfer interval, to affect ecological and evolutionary dynamics individually, we next asked how these two parameters interact. For example, a low transfer volume and a long transfer interval both result in increased evolutionary rates and we were interested if the effects are additive and evolutionary rates further increase or are dominant and no further change is observed. To explore this question, we simultaneously modified both factors in our model and tracked the dynamics.

Our model predicts an interaction between the transfer volume and the transfer interval. Bacterial densities are predicted to be highest at highest transfer volumes and longest transfer intervals (Fig. 4a). Contrary to this, we see highest ciliate densities at long transfer intervals, but at intermediate transfer volumes (Fig. 4b).

Also, the evolutionary patterns seem to be modified both by transfer volume and transfer interval (Fig. 4 c-f). Bacterial anti-predator defence traits increase continuously and reach highest levels at longest transfer intervals and highest transfer volumes (Fig. 4c). For the ciliates, where the maximum species densities are predicted for intermediate transfer volumes and long transfer intervals (Fig. 4b), the predator trait *v* initially rapidly increases but suddenly plateaus off with a peak at low transfer volumes but long transfer intervals (Fig. 4d). The attack rate *a* displays a curved mountain ridge pattern with a moving maximum so that the maximum attack rate is observed when both, the transfer volume and the transfer interval increase (Fig. 4e). The conversion efficiency *b* is predicted to be stable for a certain transfer volume range, but declines once the transfer interval is too long (Fig. 4f).

Sensitivity analysis and human caused impact. Our modelling approach offers additional insight in how sensitive such a predator-prey experiment is related to protocol changes. In our model, transfer interval and transfer volume are always exact. However, after all, humans are not robots and mistakes can happen. Often there are slight changes in the protocol maybe because of an occupied autoclave that has not finished in time, researcher forget mixing the microcosms, or pipettors work unprecise which remains unnoticed. To explore how a lack in precision affects the dynamics in such a system, we randomized parameters throughout the simulations.

The first parameter we randomized was transfer interval. For various reasons every researcher is aware, the transfer interval may deviate from the experimental protocol. So, what would be the effect if the protocol assumes starting a new growth cycle exactly after 48 hours

with a transfer activity at 12 pm but the transfer happens any time between 9 am and 3 pm (Fig. A4)? In this scenario, the ecological dynamics begin to display considerable variation (Fig. A4a). Particularly the predator densities fluctuate a lot. These dynamics look like predator-prey dynamics however these cycles are not intrinsically induced cycles but induced by the irregular sampling procedure. The evolutionary trajectories seem to be rather robust for this type of variation (Fig. A4b).

Right before transfer, populations may have patchy distribution when the community is not well mixed, which would result in variation of transfer volumes. We simulated variable transfer volumes by randomizing the transfer volume (Fig. A5). The result of this is again that ecological dynamics start fluctuating (Fig. A5a). However, evolutionary dynamics are not affected (Fig. A5b).

Another parameter hard to control when starting the experiment is the effect of initial population densities added to the experiment. Researchers commonly estimate the densities of these microorganisms but, of course, the wanted densities can be added roughly only because of the miniature nature of the study system. To simulate this, we started our model assuming different initial densities for bacteria and ciliates. Differences in initial prey densities have little effect on ecological and evolutionary dynamics (Fig. A6). Increasing or decreasing the bacterial densities to initiate the experiment is predicted to have no impact. Increasing the initial ciliate density also has little ecological and evolutionary effects (Fig. A7). Only the initial predator densities seem to be affected, but after a few growths cycles this initial effect should be lost.

Discussion

Experiments using microorganisms offer great insight into evaluating the underlying mechanisms how evolutionary and ecological forces shape communities (Barraclough, 2015; Barrick and Lenski, 2013). However, the specific protocol used in such experiments is likely to have substantial impact on the interpretation of the findings. We used a mathematical model to simulate ecological and evolutionary dynamics of a life predator-prey system under different transfer volumes and transfer intervals, as this is a common approach in experiments, but the details of the procedures are rarely explored in depth. We feel that our approach making deductions from model predications without further experimental validations turns into a strength as it allows us to explore many core parameters in fine detail.

Our model predicts that ecological dynamics of experimental bacteria-ciliate communities including serial transfers are rather robust for changes in transfer volume and transfer interval (Fig. 2, Fig. 3). The densities of bacteria and ciliates however depend on these parameters under serial transfer design. When transfer volumes become too low or the transfer interval too short, which results in extinction, there are changes in population densities. As could be expected, ciliates become extinct first. A possible explanation is that when the transfer volume is too low, there is not enough prey available and predators are unable to catch enough food to grow rapidly enough to compensate dilution induced mortality. While there is potentially enough prey available (we see 1.5 x 10^6 bacterial cells per ml) these conditions may simply out dilute the ciliates. When there is not enough time to grow, even maximum growth rate may not be high enough to compensate the loss due to dilution. It is further likely that the bacteria and ciliates reach the environmentally imposed growth maximum quickly enough to result in stable densities. Only if dilution results in extinction, this outcome changes, but for most other dilutions biological drivers, e.g., reaching equilibrium, seem to be dominating over experimental procedures.

Our model however suggests that evolutionary dynamics are affected by transfer volume and transfer interval together. Increasing the transfer volume is predicted to accelerate anti-predator defence evolution in the bacteria and attack rate in the ciliates, however in more complex ways for predators (Fig. 2). The transfer interval has also predicted effects, in the sense that longer transfers intensify the evolutionary responses (Fig. 3). With decreasing transfer volumes and longer transfer intervals, bacterial defence and ciliate predation both increase which represents arms-race dynamics (Brockhurst et al., 2014), as suggested by other studies (Cairns et al., 2020; Kaitala et al., 2020; Klauschies et al., 2016). Our model hereby suggests that there is a pronounced change for evolution from low to intermediate transfer volumes, but less obvious change in evolution from intermediate to high transfer volumes. Why the difference between high dilution to medium dilution seems more pronounced than compared from medium to lowest dilution is unfortunately not straight forward to explain. It could reflect that un-protected prey benefits a lot when even small defence trait values evolve, whereas at later stages the effect is not that pronounced anymore, but this is only speculative. A comparison between the transfer volume and the transfer interval suggests that transfer volume may have a little stronger effect on both the ecological and evolutionary dynamics (Figs. 2 and 3). However, the ecological dynamics seem to be more sensitive to changes in the transfer interval than for changes in the transfer volume, especially at the beginning of the experiment. Further, we see more variation in the evolutionary trait changes than changing the transfer interval.

Our findings are in agreement with other experiments maintaining bacteria and ciliates at high and low density, which show how nutrient concentration drives evolution of interactions (Friman et al., 2008). An additional advantage of the experimental system we used is that the ciliates and bacteria have not experienced each other before, a situation commonly referred to as “naïve”. Both partners certainly have a long history of predation but have been maintained in isolation in laboratories for many years and never specifically faced each other. This allows tracking evolutionary changes unbiased to any specific pre-adaptations. So, we can obtain detailed insight into the starting point how this interaction evolves.

When transfer volume and transfer interval are both simultaneously modified, we see highest predator density at long transfer intervals but intermediate transfer volumes. This humped shaped pattern in the predator density is interesting, albeit hard to explain, thus we can only speculate again. It could be that under high transfer volumes anti-predator defence evolution is fastest and thus edible prey may become scarce even when a high bacterial density may be present. This is described by the idea of effective prey biomass, which states that the ratio between edible and inedible prey has effects on population dynamics (van Velzen and Gaedke, 2017, 2018).

Our model predictions are in line with previous findings suggesting effects of increased mortality rates (high transfer volumes) from abiotic change on community structures (Abreu et al., 2019). Increased mortality rates caused by antibiotics affect ecological and evolutionary dynamics in this bacteria-ciliate system (Hiltunen et al., 2018). Similarly, competition, which also weakens under decreased population sizes of bacteria, interacts with predation and results in changed ecological and evolutionary dynamics (Scheuerl et al., 2019). Our finding that evolutionary trajectories are equally affected compared to ecological dynamics is a bit in contrast with other studies, however. Increased transfer volumes have been shown to result in the modifications of the compositions of bacterial communities (Abreu et al., 2019), thus more on the ecological side. It needs to be mentioned here that Abreu et al., (2019) did not explore evolution, thus limited inferences are possible. Our data are also in contrast with a different predator-prey system, namely rotifers grazing on algae, cultivated in chemostats. In this system, increasing or decreasing the dilution has great impact on the nature of ecological interaction (Fussmann et al., 2000). Changing the dilution shifts the rotifer-algal densities between equilibrium and stable limit cycle states. However, this system follows a quite different experimental approach as there is a constant dilution in chemostats. Thus, both protocols, serial batch transfer and chemostats, can hardly be compared. In accordance with our study, the algal population quickly evolves in form of alternating genotype frequencies of contrasting

defence level (Yoshida et al., 2003). Other bacterial studies, inducing high mortality rates at regular intervals, also detect evolutionary changes in interaction (Fiegna et al., 2015a; Lawrence et al., 2012), thus we think our findings represent a general pattern.

Whereas evolutionary trajectories look rather clear for the bacteria and are well in line with experimental predictions, the ciliate coevolution is less obvious (Cairns et al., 2020). Observing comparably little evolutionary change across settings in ciliates may be simply because of slower evolution or depend on the fact that the underlying traits are depending on prey dynamics. This may be reflected by the equal ratios seen under different scenarios (Fig. A2). Perhaps evolutionary forces are similar across settings when ratios between bacteria and ciliates are little changing. From a biological perspective this result makes sense as rate of evolution is expected to decline over time because of imposed costs, which need to be ameliorated before further change can happen.

We however also want to mention again that our approach is limited to specific protocols that are based on experiments using regular dilution of batch cultures. Thus, why helpful to explore principles, comparison to natural dynamics is difficult. We call for a careful attention in planning the experimental design when exploring ecological and evolutionary dynamics in microbial communities. Our modelling study suggests that dilution has effects both on ecological patterns and on evolutionary trajectories. Such experiments will detect ecological and evolutionary dynamics, but the magnitudes may depend on the experimental design. We hope future researchers will take these ideas into account when designing upcoming evolution and ecology experiments.

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Data Availability Statement: This paper is a theoretical work and does not generate any new experimental or field data. We only use parameter values of the model from ref. (Kaitala et al., 2020). All model results can be found within the figures or the Appendix. Original experimental data we used to parametrize our model can be found under (https://ars.els-cdn.com/content/image/1-s2.0-S0022519319304643-mmc1.xlsx).

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596 **Figures**

597 Figure 1

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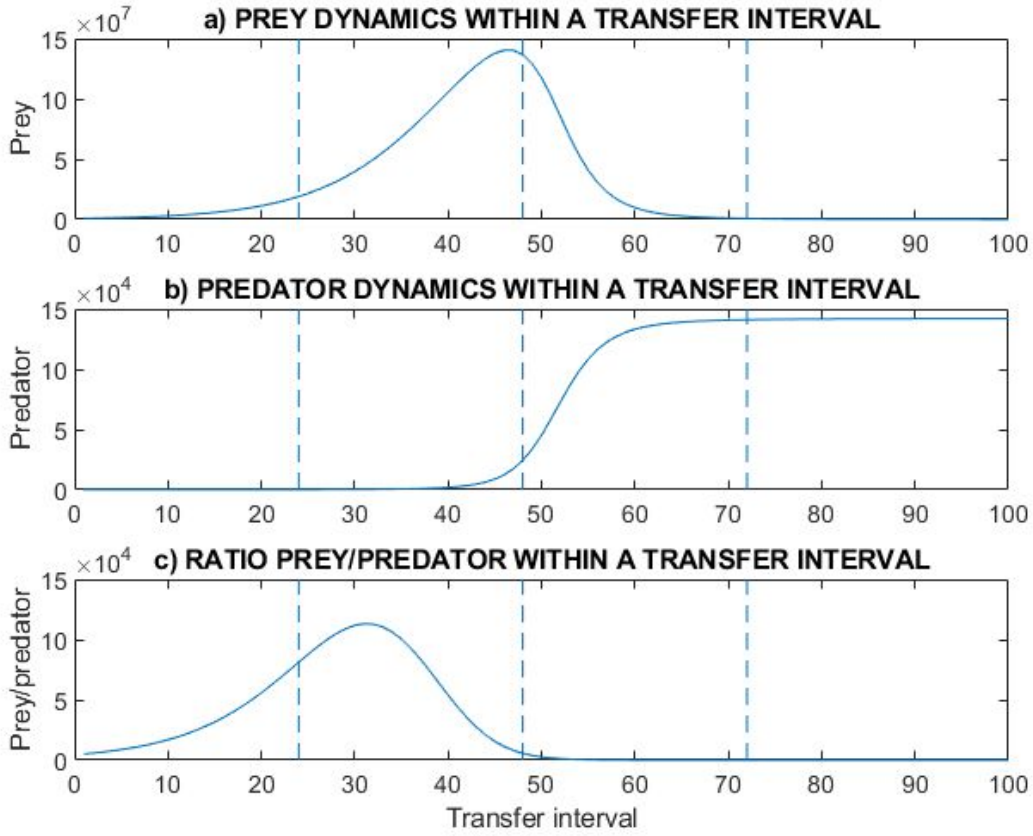

599

600 Fig. 1. Hypothetical example dynamics of a predator-prey system within a transfer interval.
601 The abundances of the prey and the predator may differ massively at the time of a transfer
602 depending on the length of the transfer interval. a) Prey densities; b) Predator densities; and
603 c) The ratio of the prey and predator abundances. Three alternative transfer intervals are
604 indicated by vertical lines: 24, 48, and 72 hours.

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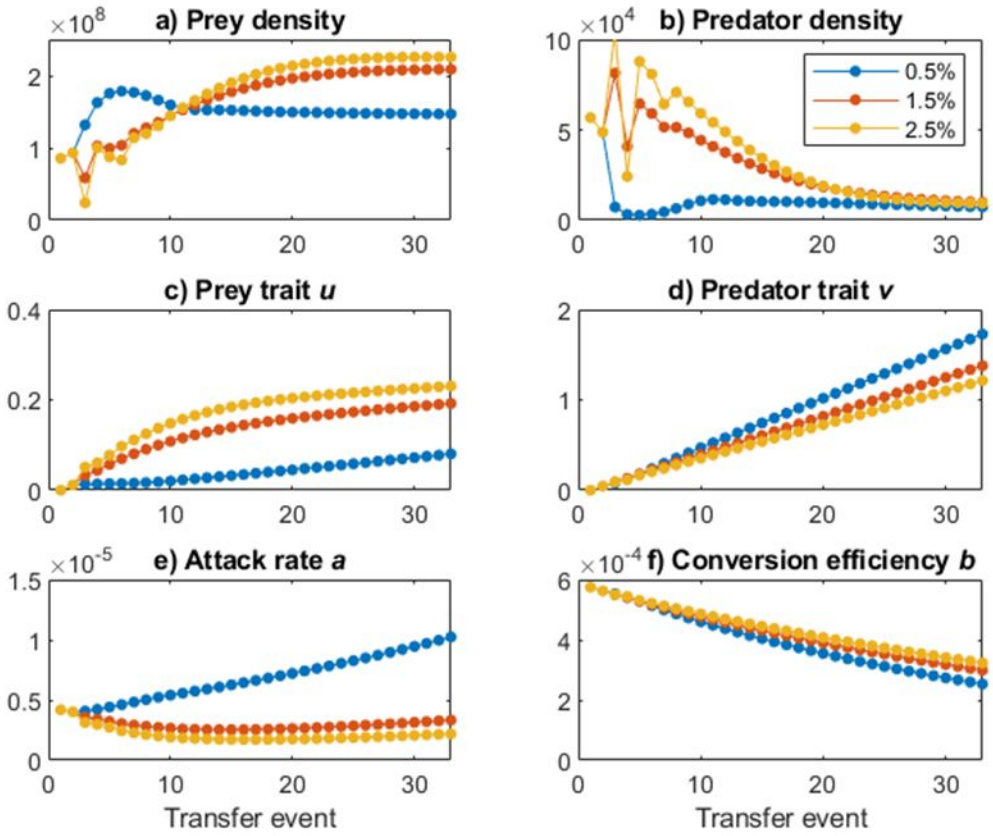
610 Figure 2



611

612 Fig. 2. Effect of transfer volume on predator-prey dynamics. The transfer interval is kept
613 constant at 48 hours. There are 33 transfer events. The transfer volumes are 0.5% (blue), 1.5
614 % (red) and 2.5% (yellow). a) Bacterial population densities (prey); b) the ciliate densities
615 (predator); c) prey trait *u* defining the anti-predator defence level; d) predator trait *v*; e) predator
616 attack rate *a*; and f) predator conversion efficiency *b*.

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624 Figure 3

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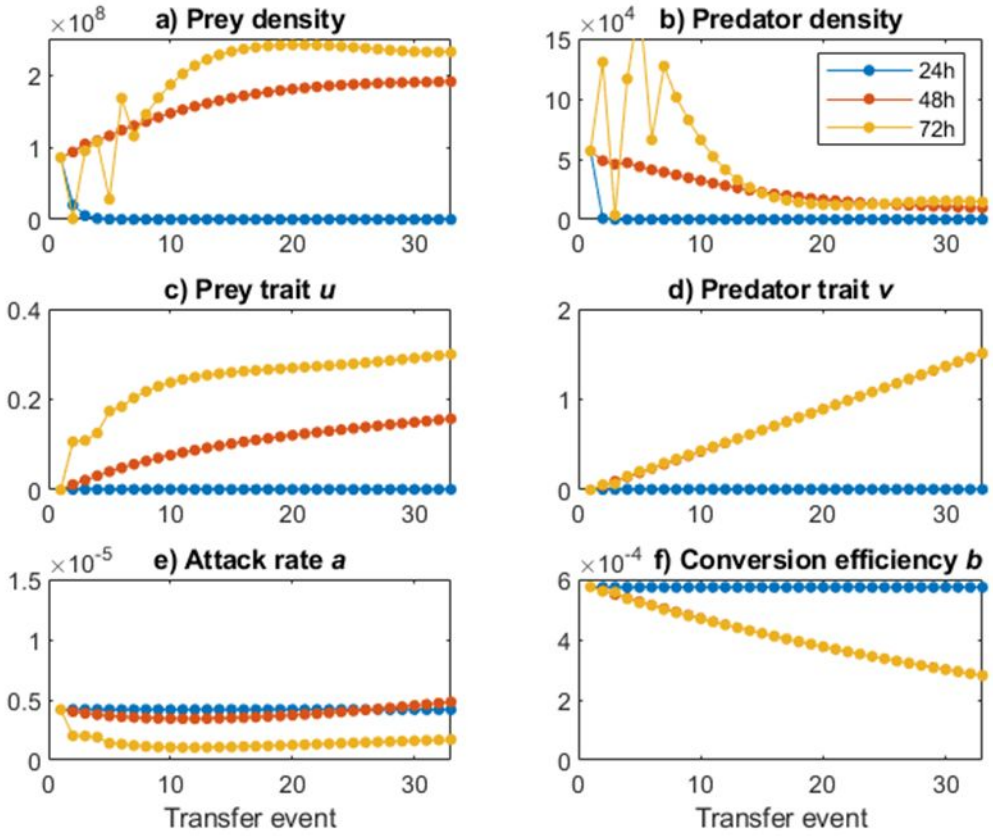

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628 Fig. 3. Effect of transfer interval on predator-prey dynamics. The transfer volume was
629 constant at 1% for all 33 transfers. Transfer intervals are 24 hours (blue), 48 hours (red) and
630 72 hours (yellow). a) Bacterial population (prey) and b) ciliate densities (predator). c) prey
631 trait *u*; d) predator trait *v*; e) predator attack rate *a;* and f) predator conversion efficiency *b*.

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637 Figure 4

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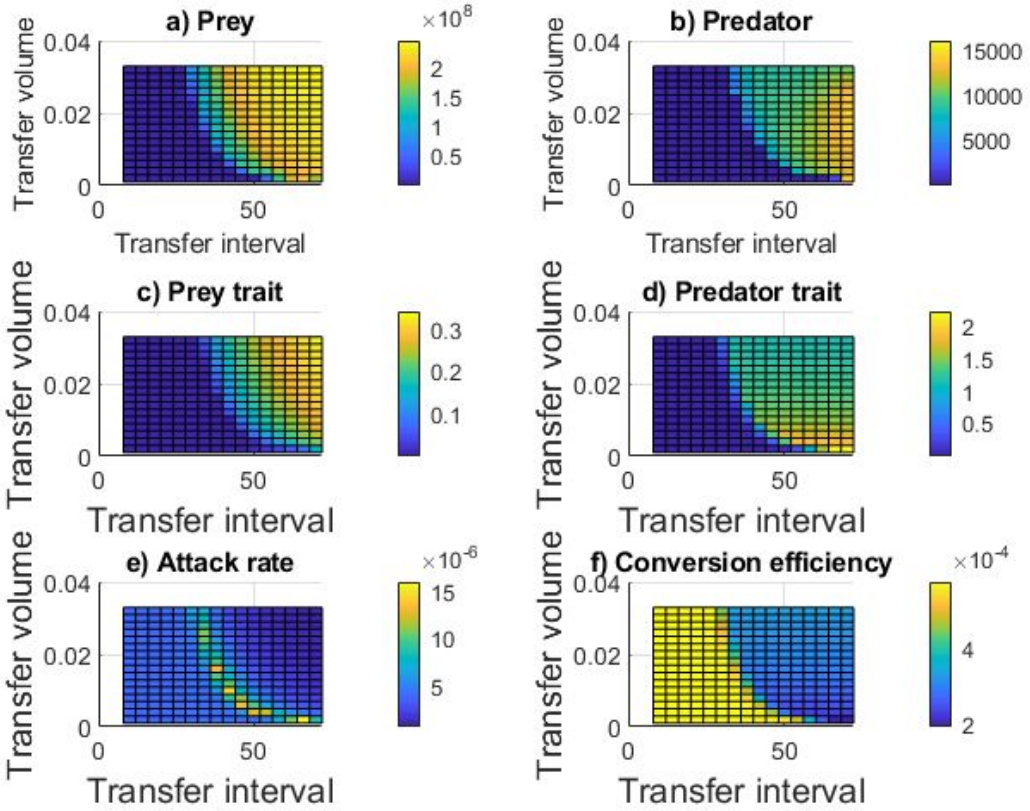

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641 Fig. 4. The combined effect of transfer volume and transfer interval on bacterial and ciliate
642 densities and on evolutionary traits. a) Bacterial densities across various transfer volumes
643 and transfer intervals; b) Ciliate densities; c) anti-predator defence trait *u* of bacteria; d)
644 predator trait *v*; e) attack rate *a*; and f) the conversion efficiency *b* of the ciliates. Vertical
645 bars indicate the value at the end of the experiment (32 transfers).

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649 **Appendix**

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651 Figure A1

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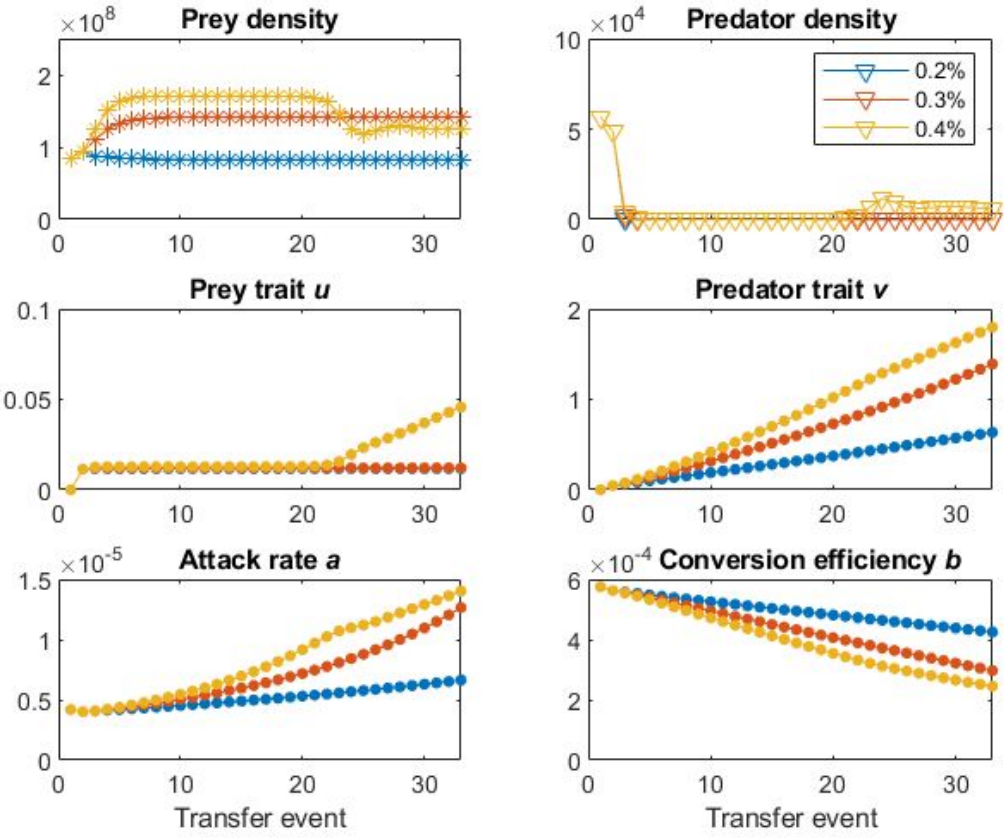

653

654 Fig. A1. The ecological and evolutionary dynamics under low transfer volumes (0.2%, 0.3% and 0.4%).
655 The experiment is maintained for 33 transfers and at a transfer interval is 48 hours. Blue, red and yellow
656 denote increasing transfer volumes (decreasing dilution).

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661 Figure A2

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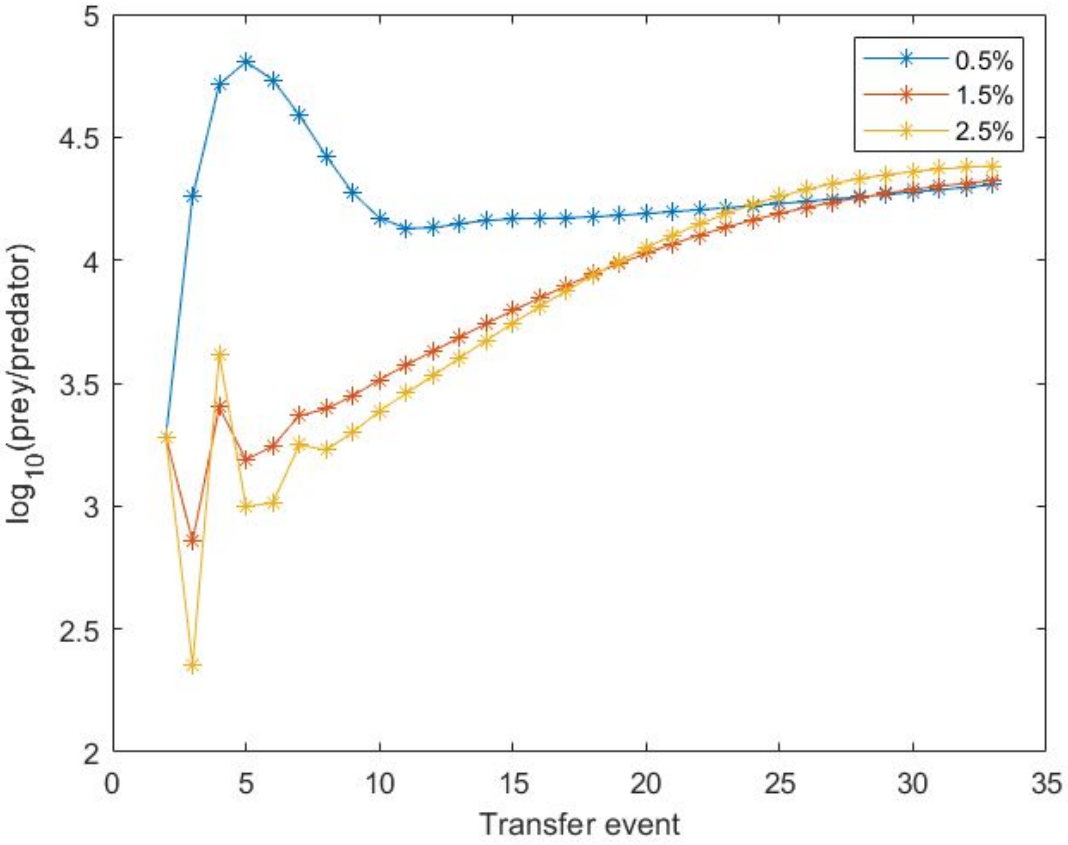

663

664 Fig. A2. Level of coexistence between bacteria and ciliates in the case studied in Fig. 2). The
665 transfer volumes are 0.5% (blue), 1.5 % (red) and 2.5% (yellow). The prey to predator ratio
666 differs notably in the beginning of the experiment. The differences level off with time, after 25
667 transfer events, and with increasing transfer volume.

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673 Figure A3

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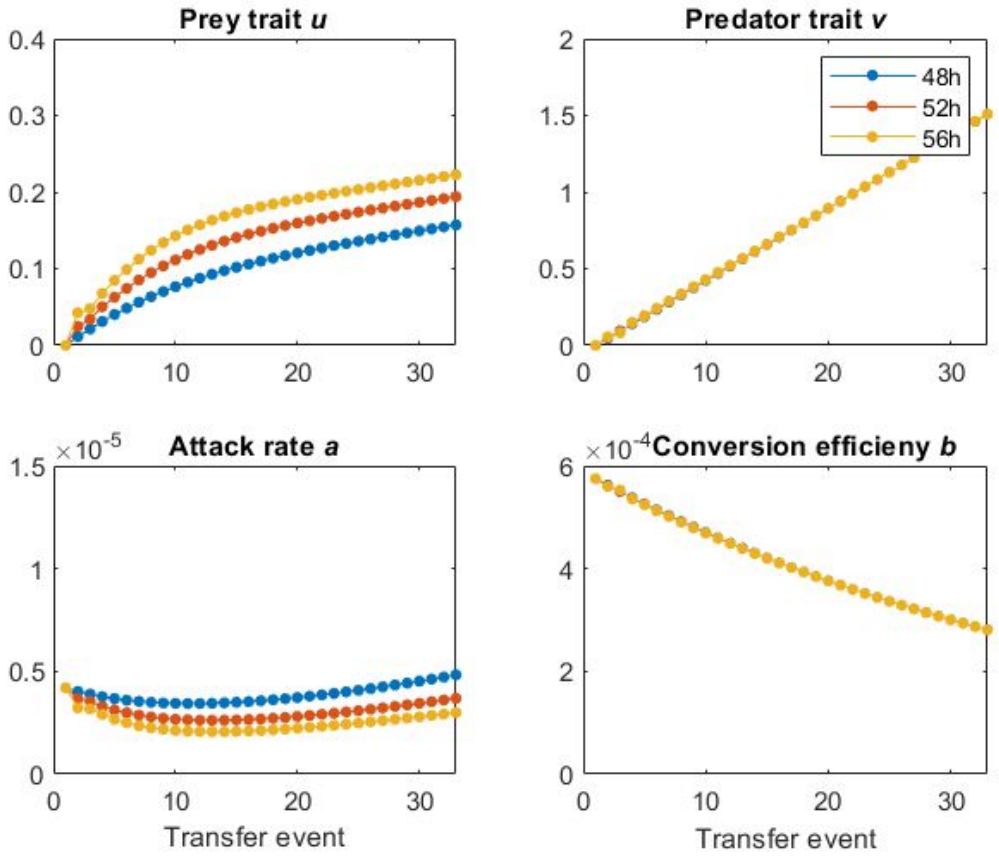

676

677 Fig. A3. Evolutionary trajectories for slightly increased transfer intervals. a) prey trait *u*, b) predator
678 trait *v*, c) predator attack rate *a* and d) predator conversion efficiency *b*. Dots in blue, red and yellow,
679 denote increasing transfer intervals of 48, 52 and 56 hours, respectively.

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688 Figure A4

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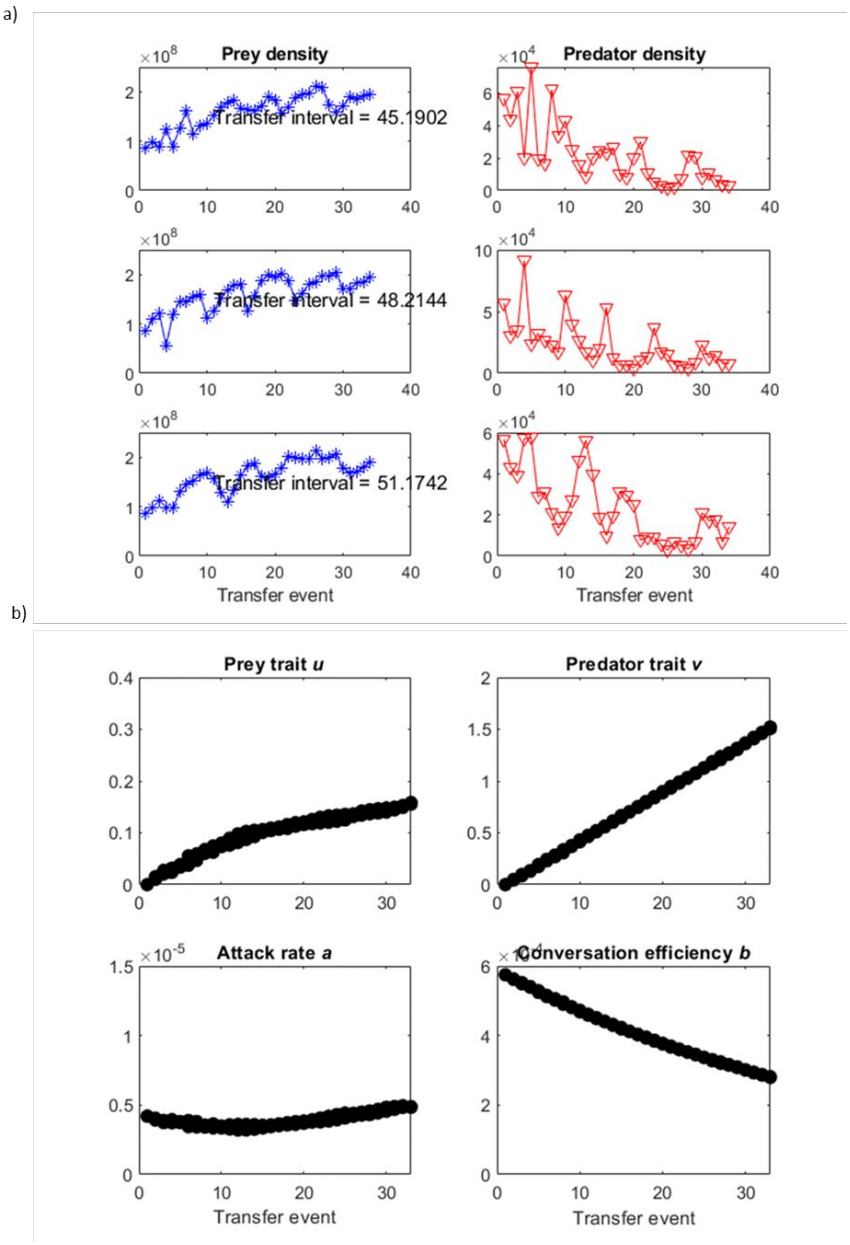

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692 Fig. A4. The effect of variation in the transfer intervals. The stochastic sampling intervals are picked
693 from an even distribution defined as 48h + (rand-0.5)×7h, where rand denotes a random even
694 distribution on [0,1]. The expected value of the transfer interval is 48 hours. The sampling intervals
695 are independent. a) Three **replicates** of the ecological dynamics for bacteria (blue) and ciliates (red)
696 in the experiments with b) Evolutionary trajectories of all three replicates presented in Fig. A4b. The
697 model used a transfer volume of 1%.

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699 Figure A5

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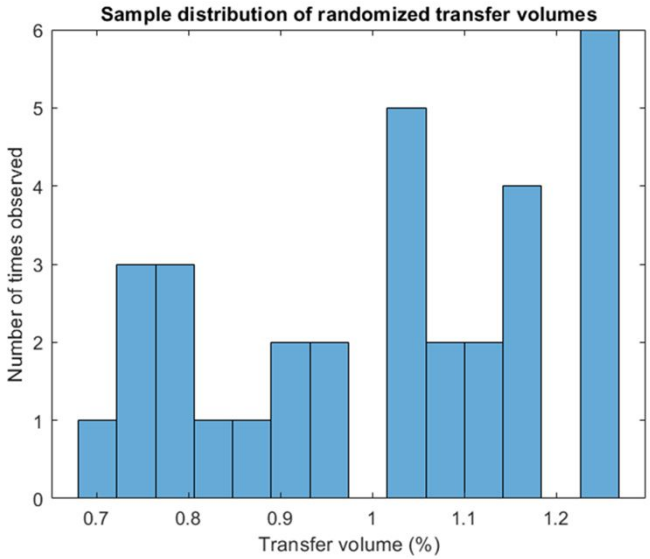
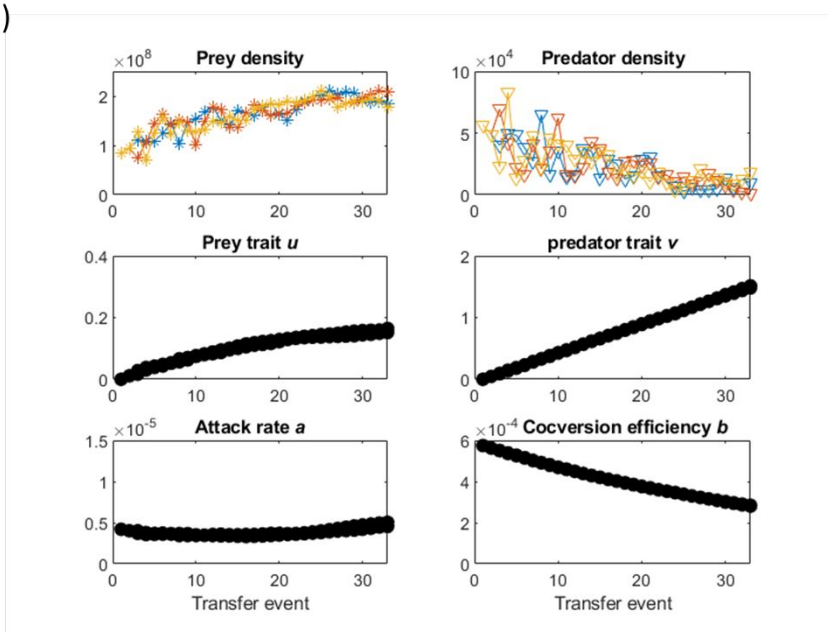
a)



b)



701

702 Fig. A5. The effect of variable transfer volumes. The transfer volume was picked up from an even
703 distribution between 0.7 and 1.3. a) An example of the distribution of the randomized transfer
704 volumes in a single experiment. b) Three examples of the bacterial and ciliate densities in the
705 experiments and corresponding evolutionary dynamics. The evolutionary differences between the
706 three runs were indistinguishable. The model used a transfer interval of 48 hours. Dots in blue, red
707 and yellow, denote increasing bacterial concentrations, respectively.

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Figure A6

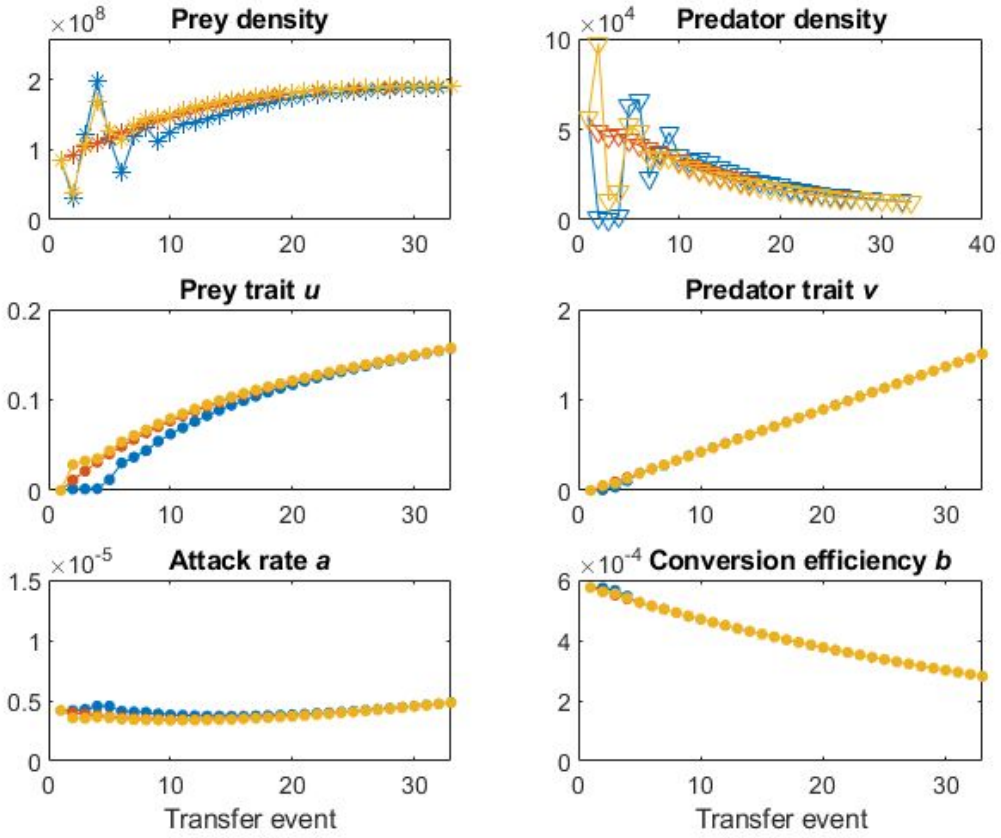

Fig. A6. The impact of different initial bacteria concentrations can be seen in the ecological dynamics of the prey and predator, slightly in the prey evolution, but not in the predator evolution. The model used a transfer interval of 48 hours and a transfer volume of 1%. The initial values of the bacterial populations were 56000, 856000 and 1656000. Dots in blue, red and yellow, denote increasing bacterial concentrations, respectively.

724 Figure A7

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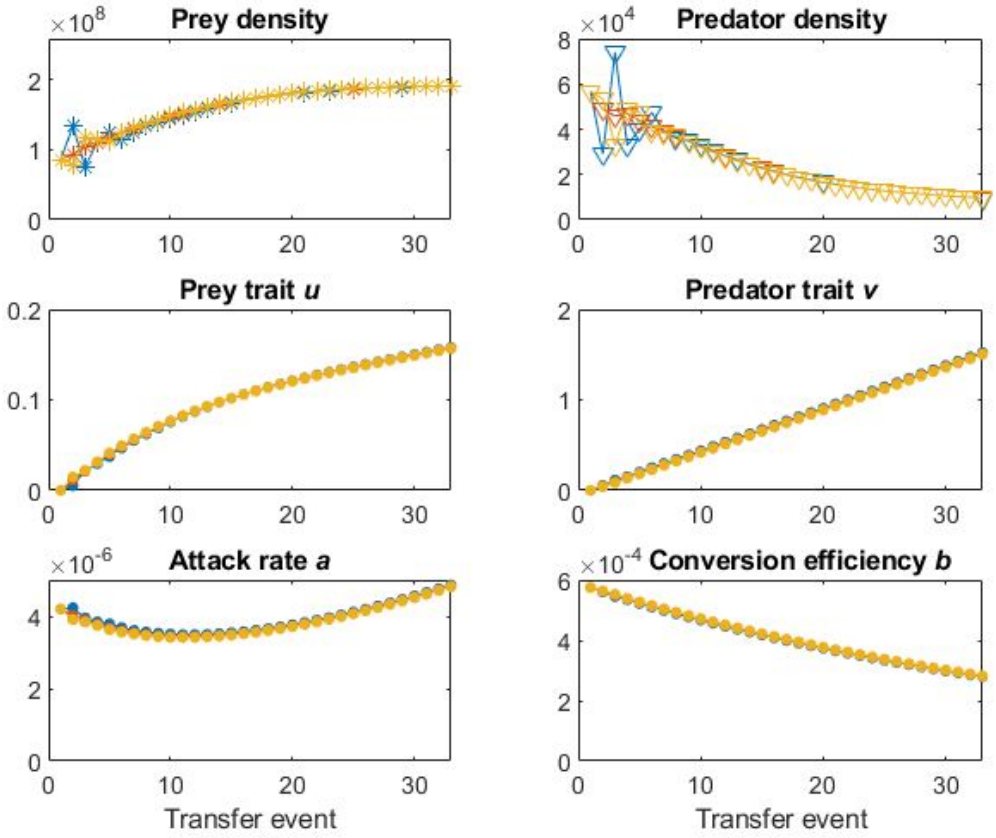

726

727 Fig. A7. The effect of different initial ciliate concentrations. The model used a transfer interval of 48
728 hours and a transfer volume of 1%. The initial values of the ciliate populations were 168, 568 and
729 968. Dots in blue, red and yellow, denote increasing ciliate concentrations, respectively.

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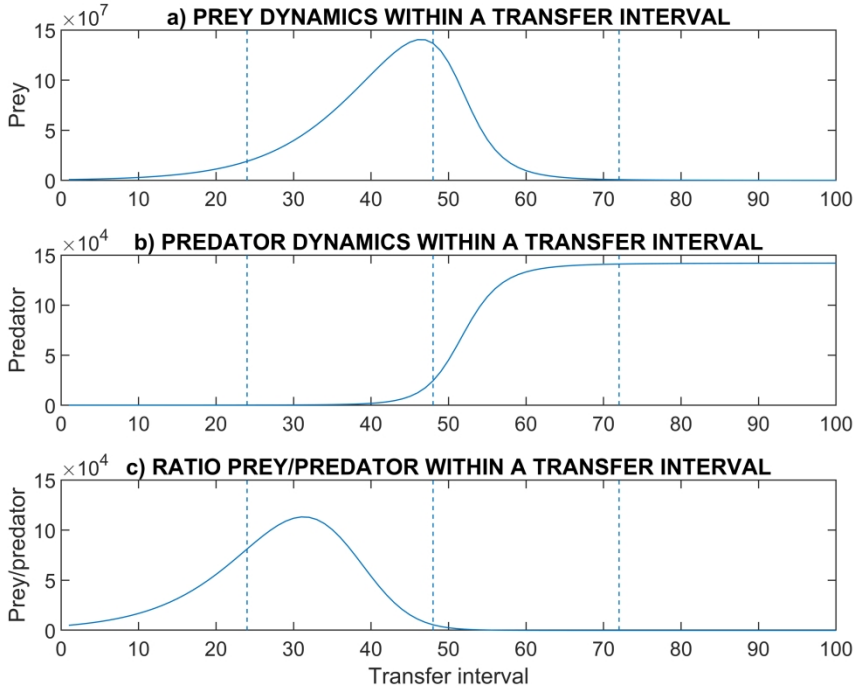

Fig. 1. Hypothetical example dynamics of a predator-prey system within a transfer interval. The abundances of the prey and the predator may differ massively at the time of a transfer depending on the length of the transfer interval. a) Prey densities; b) Predator densities; and c) The ratio of the prey and predator abundances. Three alternative transfer intervals are indicated by vertical lines: 24, 48, and 72 hours.

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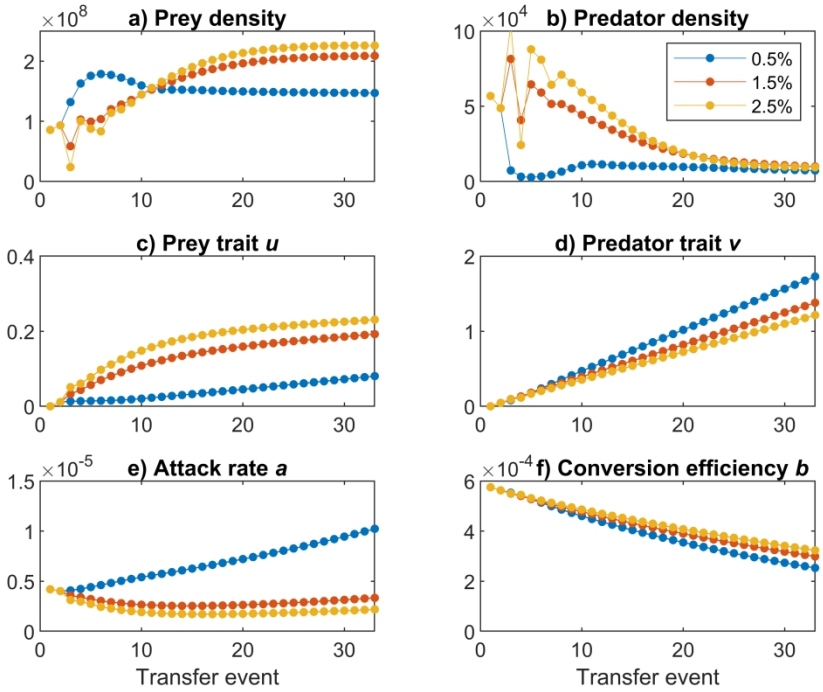

Fig. 2. Effect of transfer volume on predator-prey dynamics. The transfer interval is kept constant at 48 hours. There are 33 transfer events. The transfer volumes are 0.5% (blue), 1.5 % (red) and 2.5% (yellow). a) Bacterial population densities (prey); b) the ciliate densities (predator); c) prey trait u defining the anti-predator defence level; d) predator trait v; e) predator attack rate a; and f) predator conversion efficiency b.

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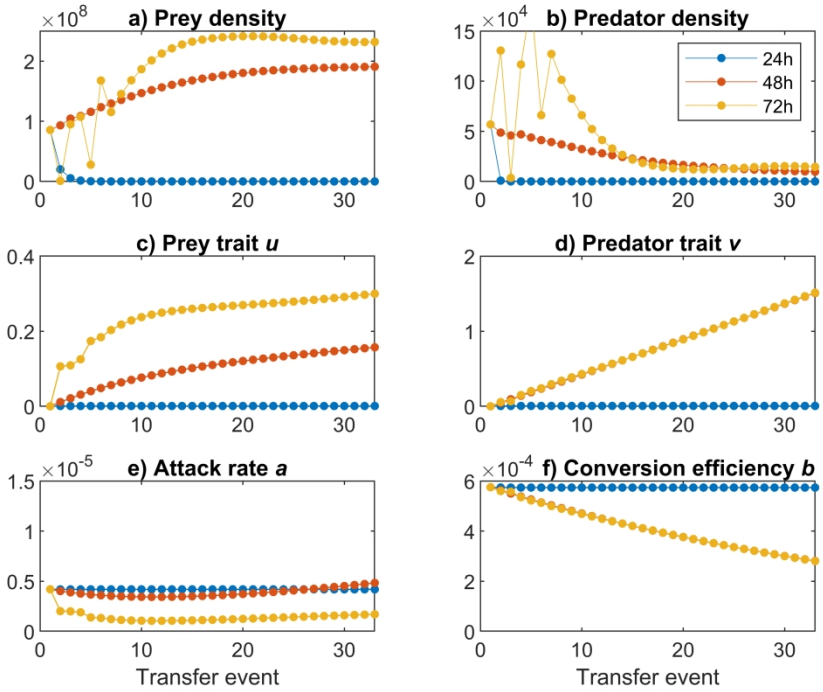

Fig. 3. Effect of transfer interval on predator-prey dynamics. The transfer volume was constant at 1% for all 33 transfers. Transfer intervals are 24 hours (blue), 48 hours (red) and 72 hours (yellow). a) Bacterial population (prey) and b) ciliate densities (predator). c) prey trait u; d) predator trait v; e) predator attack rate a; and f) predator conversion efficiency b.

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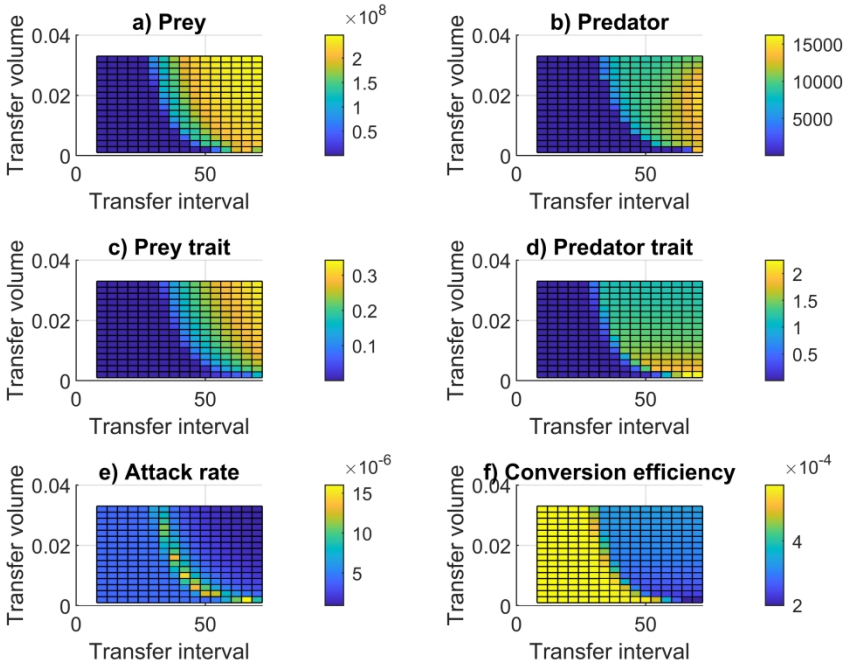

Fig. 4. The combined effect of transfer volume and transfer interval on bacterial and ciliate densities and on evolutionary traits. a) Bacterial densities across various transfer volumes and transfer intervals; b) Ciliate densities; c) anti-predator defence trait u of bacteria; d) predator trait v; e) attack rate a; and f) the conversion efficiency b of the ciliates. Vertical bars indicate the value at the end of the experiment (32 transfers).

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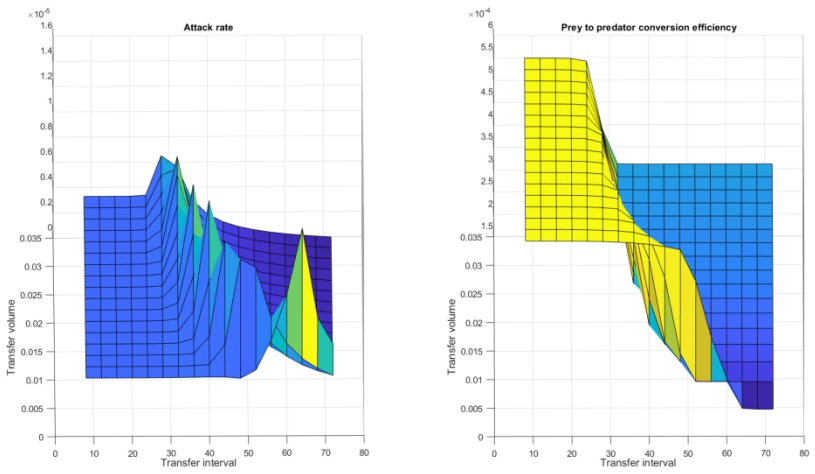

Figure for graphical table of contents

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