

The effect of dilution rate and transfer interval on eco-evolutionary dynamics of experimental microbial communities

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Title short: How to design experiments

23 **Abstract**

24 All organisms are susceptible to the environment and changing environmental conditions
25 can infer structural modifications in predator-prey communities. A change in the environment
26 can influence, for example, the mortality rate of both the prey and the predator, or determine
27 how long the interaction between both partners is. This may have a substantial impact on
28 ecological, but also evolutionary dynamics. Experimental studies, in which microbial
29 populations are maintained by a repeated dilution into fresh conditions after a certain period
30 of time, are able to dissipate underlying mechanisms in a controlled way. By design, dilution
31 rate (modifying mortality) and transfer interval (determining the time of interaction) are crucial
32 factors, but they often receive little attention in experimental design. We study data from a
33 live predator-prey (bacteria and ciliates) system used to gain insight into eco-evolutionary
34 principles and apply a mathematical model to predict how various dilution rates and transfer
35 intervals would affect such an experiment. We find the ecological dynamics to be surprisingly
36 robust for both factors. However, the evolutionary rates are expected to be affected. Our
37 work predicts that the evolution of the anti-predator defence in the bacteria, and the
38 evolution of the predation efficiency in the ciliates, both decrease with higher dilution rate,
39 but increase with longer transfer intervals. Our results provide testable hypotheses for future
40 studies of predator-prey systems and we hope this work will help improving our
41 understanding how ecological and evolutionary processes together shape composition of
42 microbial communities.

43

44 Introduction

45 The composition of microbial communities is sensitive to the environment (Alekseeva et al.,
46 2020; Goldford et al., 2018), which changes growth of individual species (Bittleston et al.,
47 2020; de Mazancourt et al., 2008; Scheuerl et al., 2020) and the interaction with other
48 community members (Fiegna et al., 2015b, 2015a; Gibert and Brassil, 2014). Modifications
49 of the environment can affect predator-prey systems (Gilpin, 1972), and a stable predator-
50 prey community may be destabilized due to dwindling densities of a keystone species
51 (Banerjee et al., 2018; Gilljam et al., 2015). For example, a predator may go extinct if the
52 density of the prey becomes too low (Fussmann et al., 2003). Following this, environmental
53 changes can affect community structure and composition and may disrupt vital functions
54 pivotal for ecosystem functioning. Changes of the environment may include the use of
55 antibiotics (Dethlefsen and Relman, 2011), or eutrophication of lake ecosystems (Kearns et
56 al., 2016; Kiersztyn et al., 2019; Kuiper et al., 2015), just as few examples which have been
57 demonstrated to change communities.

58 A common effect of environmental change is the modification of the mortality rate (Abreu et
59 al., 2019) and for how long the community can grow without further disturbance. These two
60 aspects can be easily implemented in laboratory experiments. In fact, a standard method is
61 using microbial communities to study predator-prey dynamics involving periodic transfer to
62 fresh conditions (Hiltunen et al., 2018, 2017; Nair et al., 2019; Scheuerl et al., 2019). In liquid
63 media that contain all nutrients for rapid cell division, microbes can grow extremely quickly,
64 which makes them suitable study organisms for experiments exploring ecological and
65 evolutionary dynamics over several generations (Buckling et al., 2009). This, however,
66 means that populations reach limiting conditions quickly. To keep the growth conditions
67 constant, populations are commonly either maintained in chemostat systems (Fussmann et
68 al., 2003; Scheuerl and Stelzer, 2019; Stelzer, 2009), or a proportion of the population is
69 transferred to fresh conditions regularly (often between 24 hours and 72 hours) (Fiegna et
70 al., 2015b; Good et al., 2017; Hiltunen et al., 2017; Lawrence et al., 2012; Scheuerl et al.,
71 2019; Scheuerl and Stelzer, 2017). Diluting a small part of the populations every few days is
72 a classical approach to keep populations constantly growing and to avoid growth plateaus,
73 e.g. reaching carrying capacity, once nutrient limitation occurs (Bennett et al., 1990). The
74 two key parameters, *dilution rate* and *transfer interval*, are often chosen without further
75 investigation.

76 Theoretically, increasing the dilution rate (e.g. 1% instead of 10%) results in lower initial
77 densities each growth cycle of both partners and prey may grow little constrained by
78 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). Contrary, extending the transfer interval (e.g. every 36 hours instead of every 12 hours), should increase densities so that prey and predator encounter each other more often, which may intensify evolutionary changes in the defence of prey. For predators the converse may be the case. Consider population growth curves of bacteria as prey and ciliates as predators for a single growth period (Fig. 1). Bacteria will start growing exponentially until they reach carrying capacity (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 dilution rate can have major impacts 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 than in the previous round. Thus, the transfer interval determines the ratio between prey and predator for the next growth period, whereas the dilution rate controls the initial densities and how often prey and predator encounter each other. Missing in our knowledge is how modification of both factors, dilution rate 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 dilution rate 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 dilution rate and transfer interval affect predator-prey communities and expand the prior literature by exploring scenarios impractical in experimental studies. Our theoretical findings suggest that both, dilution rate and transfer interval, have effects on the community. First, increasing the dilution rates, we find that coexistence is threatened and evolutionary change is limited, while low dilution rates 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). While the model would allow to simulate a much broader parameters space (e.g. dilution between 0% and 100%), we are missing further information to validate model results. It is worth of noting that the dilution rate 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. Further, 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. Our model represents the growth period of the experiment, which is initiated newly applying a dilution rate by the end of the transfer interval to obtain a semi-continuous system.

Recall that the Lotka-Volterra model is given as

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

$$\frac{dZ(t)}{dt} = b a P(t) Z(t)$$

where P and Z denote the prey and predator populations, r_P is the prey growth rate, K is the carrying capacity, a is the attack rate and b is prey to predator conversion rate.

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

146 $\frac{dP(t)}{dt} = W_p(u, v) P(t)$

147 $\frac{dZ(t)}{dt} = W_z(u, v) Z(t),$

148 where

149 $W_p(u, v) = r_p \left(1 - \frac{P(t)}{K} \right) - a(u, v) Z(t)$

150 and

151 $W_z(u, v) = b(v) a(u, v) P(t)$

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

153

154 The per capita fitness functions are controlled by the trait variables u and v , which
 155 maximize the fitness of the corresponding species. The attack rate and the prey to predator
 156 conversion rate were assumed to be of the form

157 $a(u, v) = a_0 \exp(c_1 v(t)) \exp(-gu(t))$

158 $b(v) = b_0 \exp(-c_2 v(t))$

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

161

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

164

165 $\frac{du(t)}{dt} = G_p \frac{dW_p(u, v, t)}{du} = G_p \left[a_0 g \exp(c_1 v(t)) \exp(-gu(t)) Z(t) \right], u(0) = 0$

166 $\frac{dv(t)}{dt} = G_z \frac{dW_z(u, v, t)}{dv}$

167 $G_z [(c_1 - c_2) b_0 \exp(-c_2 v(t)) a_0 \exp(c_1 v(t)) \exp(-gu(t)) P(t)], v(0) = 0,$

168 where G_P and G_Z are parameters determining the speed of the evolution of the traits. The
 169 evolution of the trait variables then determines the evolution of the attack rate $a(u, v)$ and the
 170 prey to predator conversion rate $b(v)$. In the experimental data studied, the ancestral
 171 individuals in each species did not have any earlier history of occurring together in a
 172 predator-prey interaction. Thus, initial values of the traits u and v are chosen to be equal to
 173 0. Consequently, the initial bacterial and ciliate populations are referred to as “naïve”. Other
 174 parameters are estimated from the experimental data presented elsewhere (Hiltunen et al.,
 175 2018). The model variables are shown in Table 1 and the parameter values with units are
 176 shown in Table 2. For more details about the model please see our previous study (Kaitala
 177 et al., 2020).

178

179 Table 1. Models variables and units

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

180

181

182 Table 2. Model parameter values

r_P	growth rate of the bacterium	3.3/hour
K	carrying capacity of the bacterium	2.58×10^8 Bacterial cells/ml
a_0	attack rate	4.2×10^{-6} ml/Ciliate cells/h
b_0	prey to predator conversion rate	5.75×10^{-4} Ciliate cells/Bacterial cells
g	dimensionless parameter	73347

c_1	dimensionless parameter	0.8568
c_2	dimensionless parameter	0.4745
G_P	dimensionless parameter	0.0017
G_Z	dimensionless parameter	0.0271

183

184 We next study effects on ecological and evolutionary dynamics of modifying the dilution rate
185 or transfer interval while maintaining the original estimated model parameters (Kaitala et al.,
186 2020). The initial condition for the prey is 8.56×10^7 Bacterial cells/ml and for the predator
187 56800 Ciliate cells/ml.

188

189 Results

190 **Model fit and experimental data.** We estimated parameters necessary for our model using
191 data presented in a study exploring ecological and evolutionary dynamics in a live bacteria-
192 ciliate system (Hiltunen et al., 2018). This experiment maintained the organisms using a 1%
193 dilution rate and a transfer interval of 48 hours before starting the next growth cycle for a
194 period of 66 days. The experimental data and our model predictions consistently result in
195 coexisting prey and predator populations under these conditions. Prey densities increase
196 over time because anti-predatory defence evolves and bacteria get less eatable by ciliates
197 (Hiltunen et al., 2018). The predator densities decrease over time as prey becomes better
198 defended against predatory attacks. Coevolution in the predation prevents further decrease
199 in the predator densities (Cairns et al., 2020) and the level of final densities are reached after
200 a few transfers and our model is well equipped to capture these dynamics (Kaitala et al.,
201 2020).

202

203 **Changing dilution rate affects ecological and evolutionary dynamics.** To explore how
204 increased dilution rate affects predator-prey communities we successively modified the
205 dilution rate in our model (Fig. A1) but kept the transfer interval constant at 48 hours.
206 Transferring only 0.3% of the populations (compared to 1% as in the original study), thus
207 increasing the mortality up to 99.7%, results in extinction of the predator. This is the
208 predicted dilution rate where only the prey can survive in this system. A possible explanation
209 is that the dilution rate is so high at this point that there is too little prey available and

predators are unable to catch enough food to grow rapidly enough to compensate mortality. While there is most likely enough prey available (we see 1.5×10^6 bacterial cells per ml) these conditions may simply out-dilute the ciliates and they cannot compensate the loss via growth. Bacteria are dwindling towards extinction at 0.1% dilution rate. Decreasing the dilution rate $>1\%$ has little effect (Fig. 2). When dilution is less severe and the next growth cycle is initiated 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 1% and 2% dilution rates (Fig. A2).

High dilution rates should release prey from predation pressure. This should result in a decrease of anti-predator defence evolution in bacteria, but an increase in predation ability in the ciliates (see Figure 1). Indeed, our results indicate a change in the evolutionary rates. Our model successively predicts that bacteria evolve less anti-predator defence with increasing dilution (Fig. 3a). At high 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 higher change in predator trait v (Fig. 3b and c) and the attack rate a under high dilution rates as we would expect when predators are selected for higher efficiency due to reduced encounter events. The conversion rate b decreases over course of the experiment, but less under lower dilution rates (Fig. 3c). At the extreme high end of dilution rates, when only the bacteria survive, anti-predator defence stops evolving (Fig. A3).

After around 25 transfers, our model predictions are that the prey-predator ratios are the same for all dilution rates (Fig. A2). Before this happens, we see great differences in the bacteria-ciliate ratios with much more bacteria at highest dilution rate. Predators need a prolonged time to catch up and to establish stable populations. The final ratio, however, seems to be robust against different dilution rates.

Changing transfer interval affects ecological and evolutionary dynamics. Because we saw an effect of dilution rate 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 dilution rate 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 and attack rates are expected to intensify under longer antagonistic interaction periods.

Applying different transfer intervals indeed resulted in various ecological dynamics (Fig. 4). In the original case, the populations grow for 48 hours before they are transferred to fresh conditions. The ciliate densities first steadily decrease but levels off towards the end of the experiment at low densities. When the transfer interval is reduced to 36 hours, the ciliates rapidly go extinct. At even lower transfer intervals (24 hours), the bacteria also cannot exist any longer and get out-diluted. Increasing the transfer interval has little effect on the ecological dynamics. The initial dynamics destabilize at longer intervals and show more variation. This may result from a change in the ratio after 48 hours, where predators already start to reduce prey densities significantly. This may further mean, that when the next growth cycle is initiated, proportionally more predators are present. This effect however disappears at later growth cycles.

With increasing interval, we would expect the interaction between prey and predator to intensify, whereas at short intervals any interaction may be weakened because low initial densities and reduced encounter rates. A transfer interval less than 48 hour quickly drives ciliates populations into extinction (Fig. 4) and evolution of anti-predator and attack rates in bacteria and ciliates stop (Fig. 5). Intervals longer than 48 hours result in increasing evolution of trait u in the bacteria (Fig. 5a). Trait v linearly increases, but not more than under 48 hours transfer intervals (Fig. 5b). The attack rate a displays interesting patterns, as it in most cases first decreases slightly, but returns to initial values at later stages (Fig. 5c). Again, conversion rate b linearly decreases but with little differences between transfer intervals (Fig. 5c).

We were also interested how evolutionary dynamics are predicted under very small modifications of transfer intervals. Increasing interval only slightly (only 2-8 hours) has enhanced impact on evolutionary trajectories (Fig. A4). Notably, increasing the interval only initially results in an increase of trait u in the bacteria, while trait v evolves little different compared to the standard transfer interval of 48 hours.

Interaction between mortality and coexistence time. Because we saw both, dilution rate and transfer interval, to affect ecological and evolutionary dynamics individually, we next asked how these two parameters interact. For example, a low dilution rate 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 dilution rate and the transfer interval. Increasing the transfer volume obviously decreases the mortality of both species, thus allowing them to survive better (Fig. 6). Beyond extinction conditions, bacterial and ciliate densities are rather independent from dilution rate and transfer interval. A simultaneous decrease in dilution rate and increase in transfer interval has little overall effect on densities and ecological dynamics are rather robust. Bacterial densities are predicted to be highest at lowest dilution rates and longest transfer intervals (Fig. 6a). Contrary to this, we see highest ciliate densities at long transfer intervals, but at intermediate dilution rates (Fig. 6b).

The evolutionary patterns, however, are predicted to be more depending in the interaction between dilution rate and transfer interval (Fig. 7). Bacterial anti-predator defence traits increase continuously and reach highest levels at maximum simulated coexistence times and lowest dilution rates (Fig. 7a). Please note, the evolutionary change seems to be more pronounced compared to the ecological change in density (Fig. 6a). For the ciliates we observe interesting evolutionary trajectories. While the maximum species densities are predicted for intermediate dilution rates and long transfer intervals (Fig. 6b), the predator trait v initially rapidly increases but suddenly plateaus off (Fig. 7b). Only at long transfer intervals but very high dilution rates there is a change in this trait again. The attack rate a displays a curved mountain ridge pattern with a moving maximum so that the maximum attack rate is observed either under high dilution rate but long transfer intervals, or under low dilutions but shortened transfer intervals (Fig. 7c). The conversion rate b that was estimated for the naïve ciliates seems to be maladaptive and the model predicts constantly that conversion rates reduce independent of dilution rate and transfer interval (Fig. 7d).

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 dilution rate 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, 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 noon 12 pm but the transfer happens any time between 9 am and 3 pm (Fig. A5a)? In this scenario, the ecological dynamics begin to display considerable

variation (Fig. A5b). Particularly the predator densities fluctuate a lot. The evolutionary trajectories seem to be rather robust for this type of variation (Fig. A5c).

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.

Finally, right before transfer, populations may have patchy distribution, which would result in variation of dilution rates when the community is not well mixed. We simulated variable dilution rates by randomizing the transfer volume (Fig. A8a). The result of this is again that ecological dynamics destabilize displaying more variation (Fig. A8b). However, evolutionary dynamics are rather robust (Fig. A8c).

Discussion

The question how communities change in a deteriorating environment is essential to predict future ecosystem functioning and services (Baquero et al., 2008). With progressing global change, acidification and nutrient enrichment of ecosystems, and many other stressful factors, organism's mortalities increase, and interaction networks may be disrupted. We used a mathematical model to simulate ecological and evolutionary dynamics of a life predator-prey system under different mortality rates (dilutions) and interaction times (transfer intervals). 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 are rather robust for changes in dilution rate and transfer interval (Fig. 2, Fig. 4). The densities of bacteria and ciliates only weakly depend on these parameters under regular transfer design. Only when dilution rates become too high or the transfer interval too short, which results in extinction, there are changes in population densities. However, our model suggests that evolutionary dynamics are affected by these two parameters. Decreasing

dilution rates are predicted to increase anti-predator defence evolution in the bacteria and attack efficiency in the ciliates, however in more complex ways for predators (Fig. 3). The transfer interval has also predicted effects, in the sense that longer transfers intensify the evolutionary responses (Fig. 5). With decreasing dilution rates and longer transfer intervals, bacterial defence and ciliate predation both increase which may suggest arms-race dynamics (Brockhurst et al., 2014), as suggested by other studies (Cairns et al., 2020; Kaitala et al., 2020; Klauschies et al., 2016). Those findings are in agreement with other experiments maintaining bacteria and ciliates in high and low density, controlled by nutrient concentration (Friman et al., 2008). An additional advantage of the experimental system we used (Hiltunen et al., 2018) 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 very detailed insight into the starting point how this interaction evolves.

Our model predictions are in line with previous findings suggesting effects of increased mortality rates (high dilution rates) 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 in decreased population sizes of focal bacteria, interacts with predation and results in changed ecological and evolutionary dynamics (Scheuerl et al., 2019). Our finding that evolutionary trajectories are more affected than ecological dynamics is a bit in contrast with other studies, however. Increased dilution rates have been shown to result in the modifications of the compositions in bacterial communities (Abreu et al., 2019), thus more on the ecological side. Maybe it is also worth to mention 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. In this system, increasing or decreasing dilution rates has great impact on the nature of ecological interaction (Fussmann et al., 2000). Changing the dilution rate shifts the rotifer-algal densities between equilibrium and stable limit cycle states. However, this system follows a bit different experimental approach as there is a constant dilution rate because the organisms were grown in chemostat systems. 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 that evolution, and also ecology, depends

on mortality rate and the time species interact with each other, both controlled by dilution rate and transfer interval respectively in laboratory experiments.

The exact mechanisms why ecological dynamics are rather robust cannot be fully explained in our study. It is likely that the bacteria and ciliates reach the environmentally imposed growth maximum quickly enough to result in stable dynamics. The original study used a nutrient limited medium which only supports low overall densities (Hiltunen et al., 2018). Also, it is important to note that this system only reveals end points of each growth cycle, unlike a chemostat system, and within-interval dynamics between growth cycles may be rather different resulting in similar final densities. The exact evolutionary mechanisms also remain open. Whereas evolutionary trajectories look rather clear for the bacteria and are well in line with experimental predictions, the ciliate coevolution is less obvious. Observing comparably little evolutionary change across settings in ciliates may 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 reduced 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.

Our findings suggest that experimental approaches exploring ecological and evolutionary dynamics in microbial communities represent a good way to gain further insight into related questions. However, we call for a careful attention in planning the experimental design. 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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Author contribution: VK and TS designed the study, VK wrote the mathematical model with input from TS and analysed the results, TS wrote the first draft of the manuscript.

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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>).

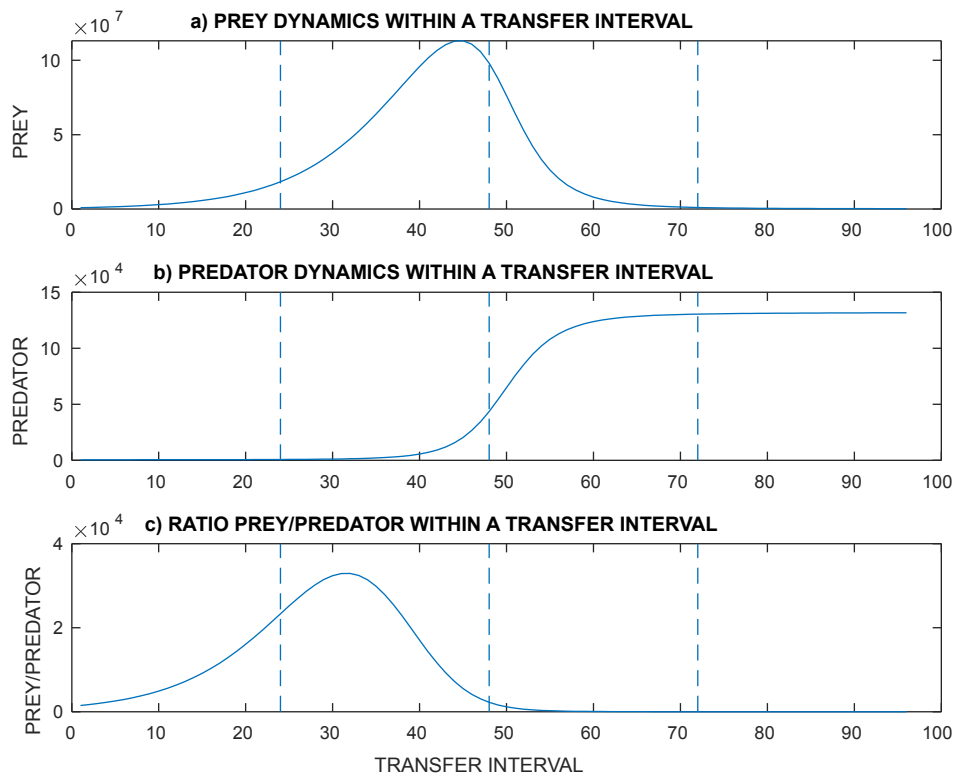
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551 Figure 1



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553 Figure 1. Hypothetical example dynamics of a predator-prey system within a sampling
554 period. a) Prey abundance. b) Predator abundance. c) The ratio of the prey and predator
555 abundances. Three alternative transfer intervals are indicated by vertical lines: 24, 48, and
556 72 hours.

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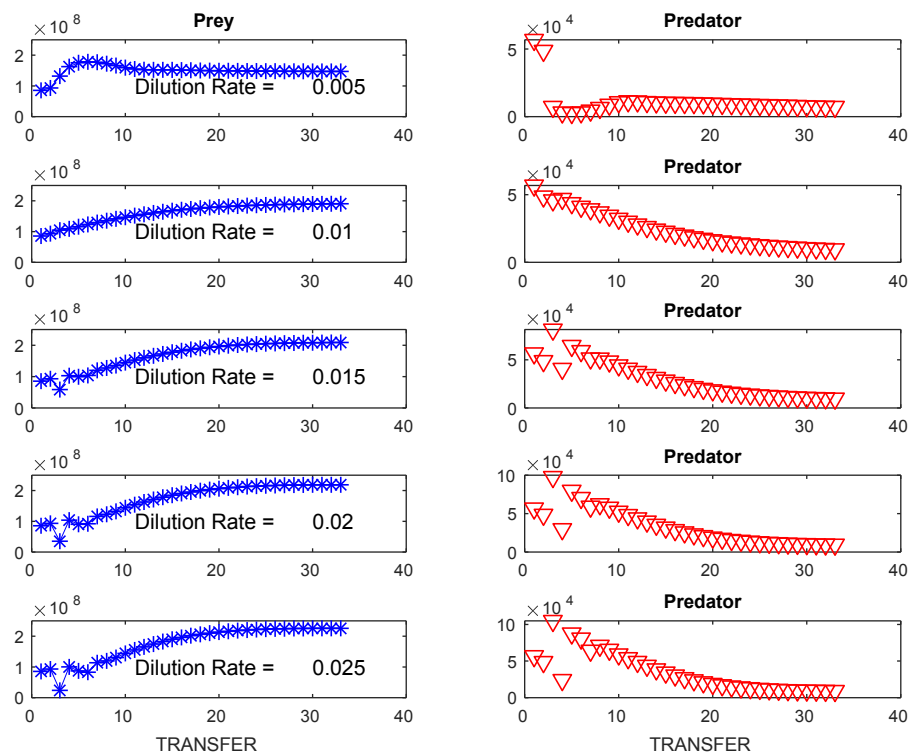
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564 Figure 2



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568 Figure 2. The effect of modifying the dilution rate on population densities controlled by
569 applying different transfer volumes. The transfer interval is kept constant at 48 hours. There
570 are 33 transfers. The mortality rate is ranging between 99.9% and 97.5%. Bacterial
571 population (prey) is denoted by blue stars; the ciliates are represented by red triangles.

Figure 3

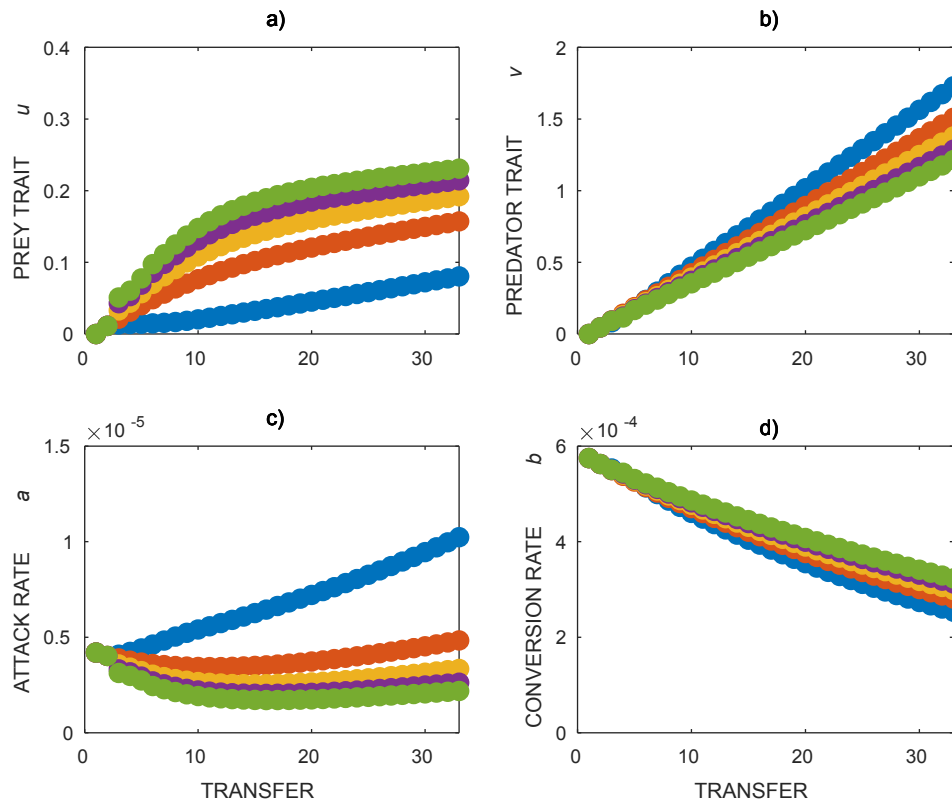


Fig. 3. Evolutionary trajectories for bacteria and ciliates under different dilution rates. a) prey trait u defining the anti-predator defence level, for the ciliates the following parameters are b) predator trait v , c) predator attack rate a and d) predator conversion rate b . Dots in blue, red, yellow, magenta and green denote increasing mortality rates (dilution rate) with 0.5%, 1%, 1.5%, 2% and 2.5%, respectively.

Figure 4

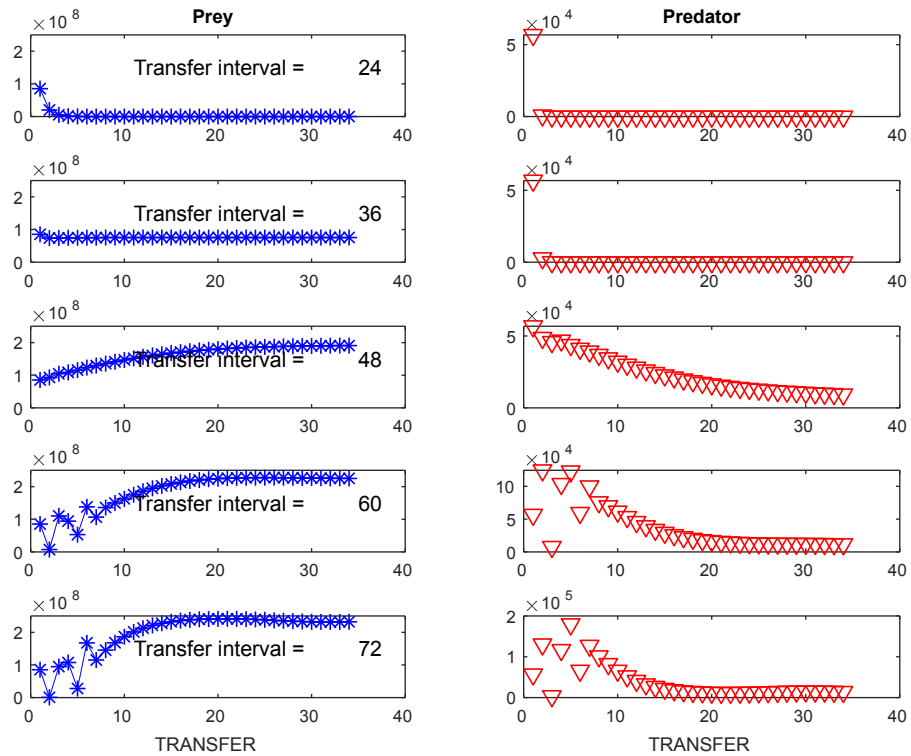


Fig. 4. Effect of transfer interval on predator-prey dynamics. Bacterial densities (blue) and ciliate densities (red) are presented for different transfer intervals ranging from 24-72 hours. The transfer volume was constant with 1% every transfer. There are 33 transfers.

Figure 5

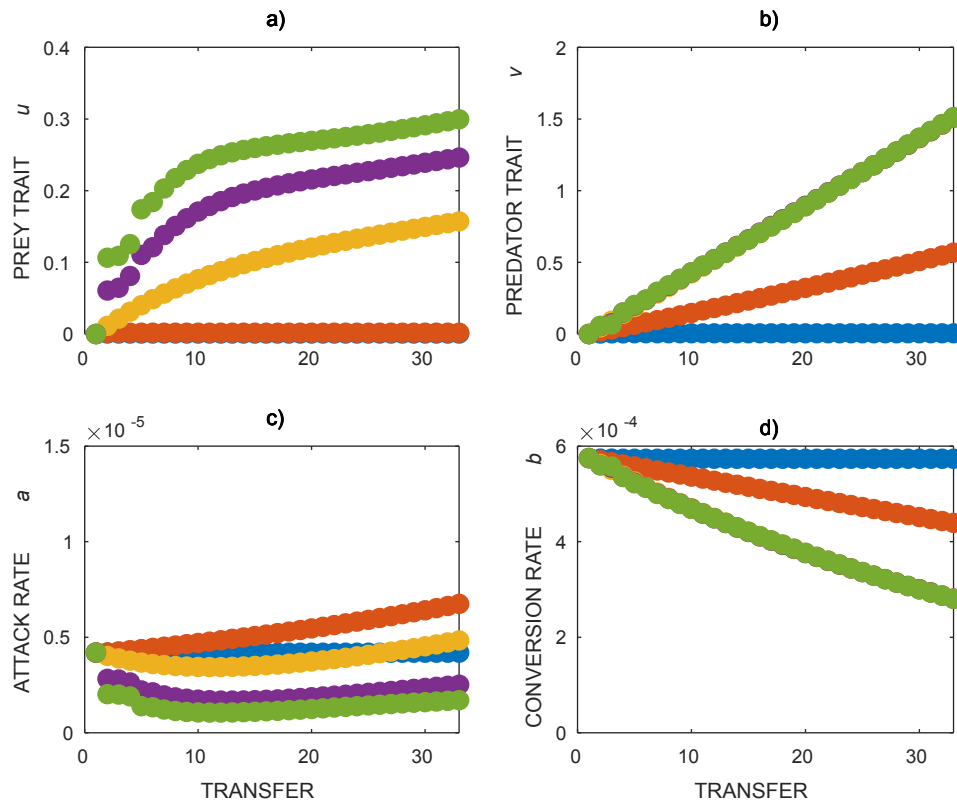


Fig. 5. Evolutionary trajectories for bacteria and ciliates under different transfer intervals. a) prey trait u , b) predator trait v , c) predator attack rate a and d) predator conversion rate b . Dots in blue, red, yellow, magenta and green denote increasing times bacteria and ciliates grow together before next transfer (transfer interval 24h (blue), 36h (red), 48h (yellow), 60h (magenta) and 72h (green) hours). The transfer volume was kept constant at 1%. There were 33 transfers in total.

Figure 6

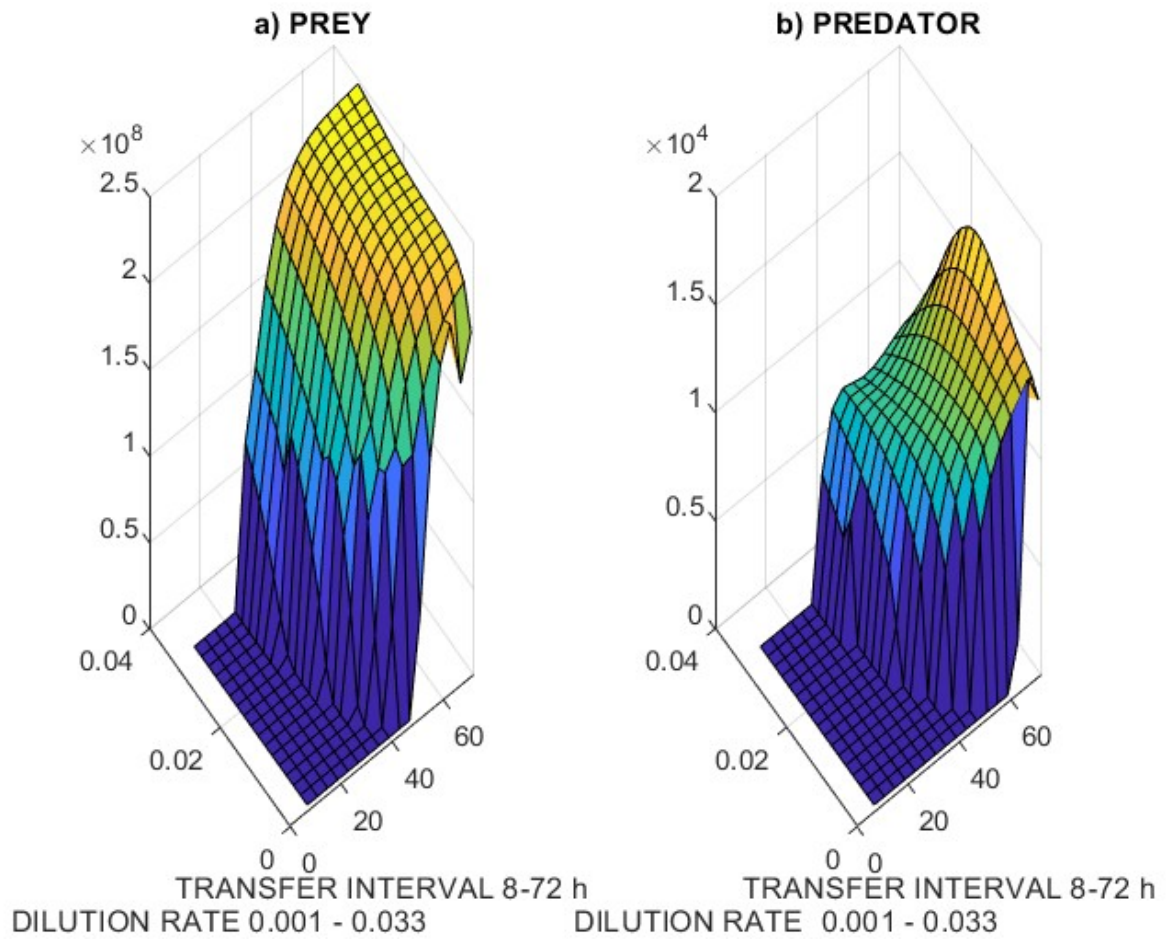
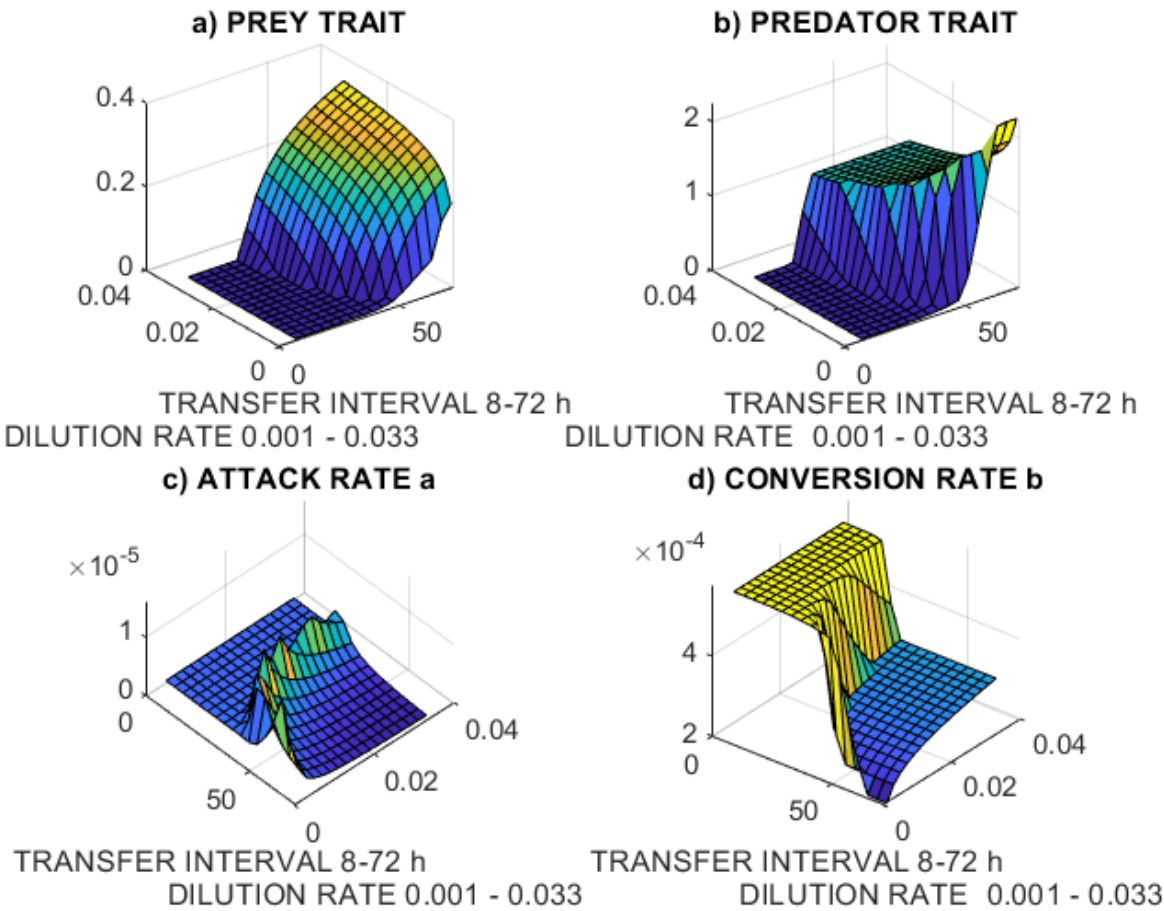


Fig. 6. The combined effect of dilution rate and transfer interval on bacterial and ciliate densities. a) Bacterial densities across various transfer volumes and transfer intervals. b) Corresponding ciliate densities across the different parameters.

Figure 7



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633 Fig. 7. The interaction effect between dilution rate and transfer interval on evolutionary
634 trajectories. Predicted evolutionary trajectories for anti-predator defence trait u of bacteria a),
635 predator traits v b), the attack rate a c) and the conversion rate b d) of the ciliates.

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Appendix

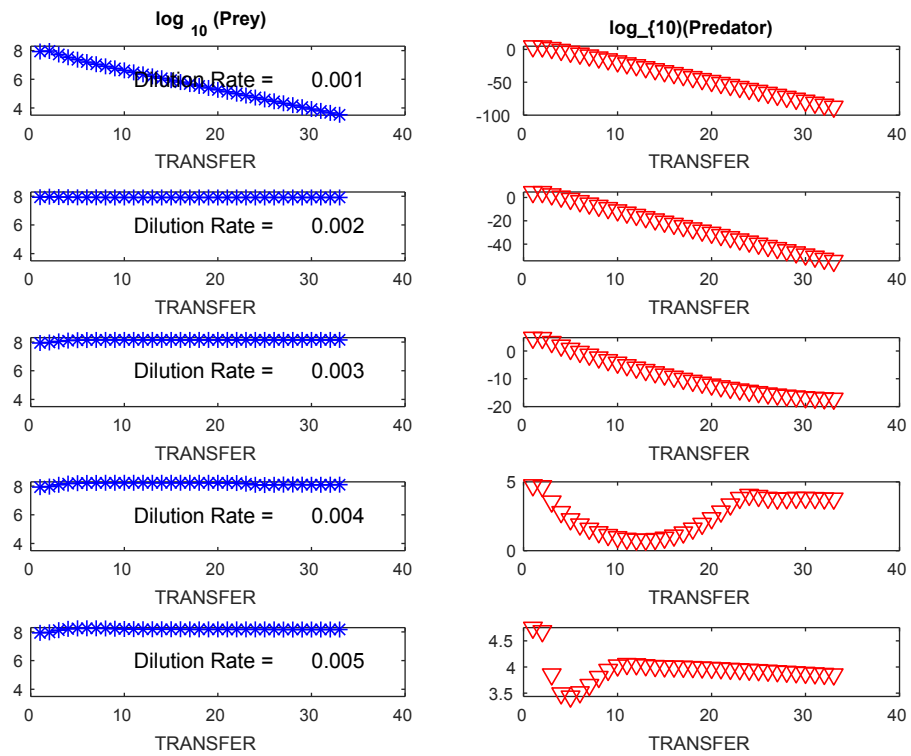
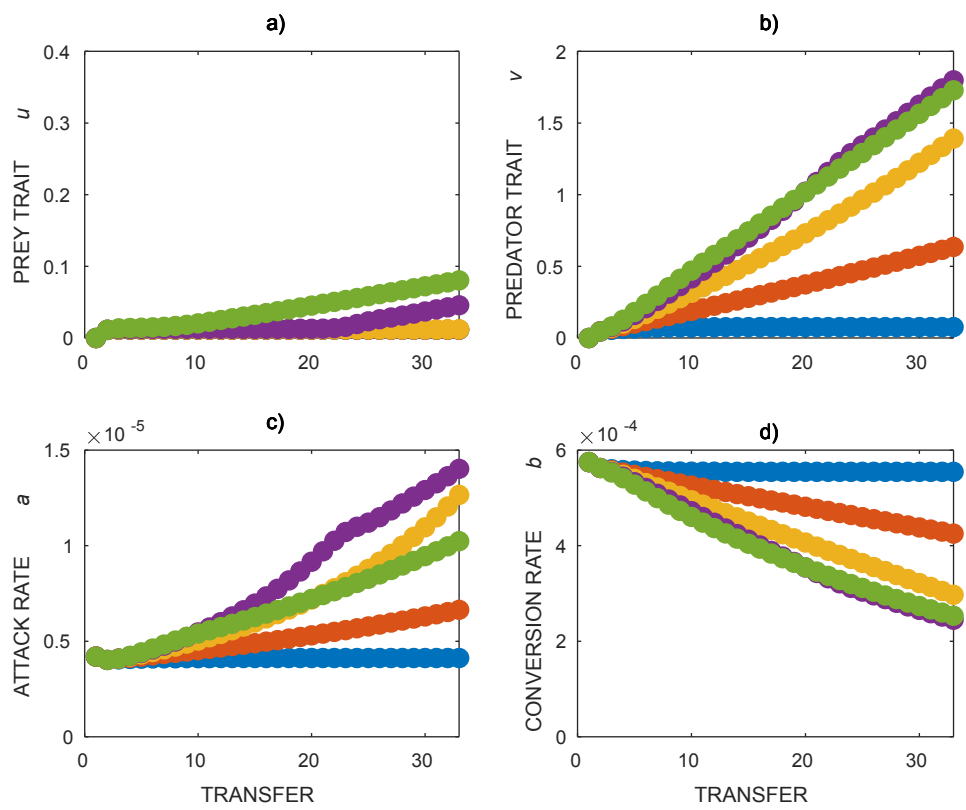


Fig. A1. The effect of extremely high dilution rates at a transfer interval of 48 hour. The experiment is maintained for 33 transfers.

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651 Fig. A2. Evolutionary trajectories under very high dilution rates. a) prey trait u , b) predator trait v , c)
652 predator attack rate a and d) predator conversion rate b . Squares in blue, red, yellow, magenta and
653 green denote increasing dilution rates respectively (dilution rate).

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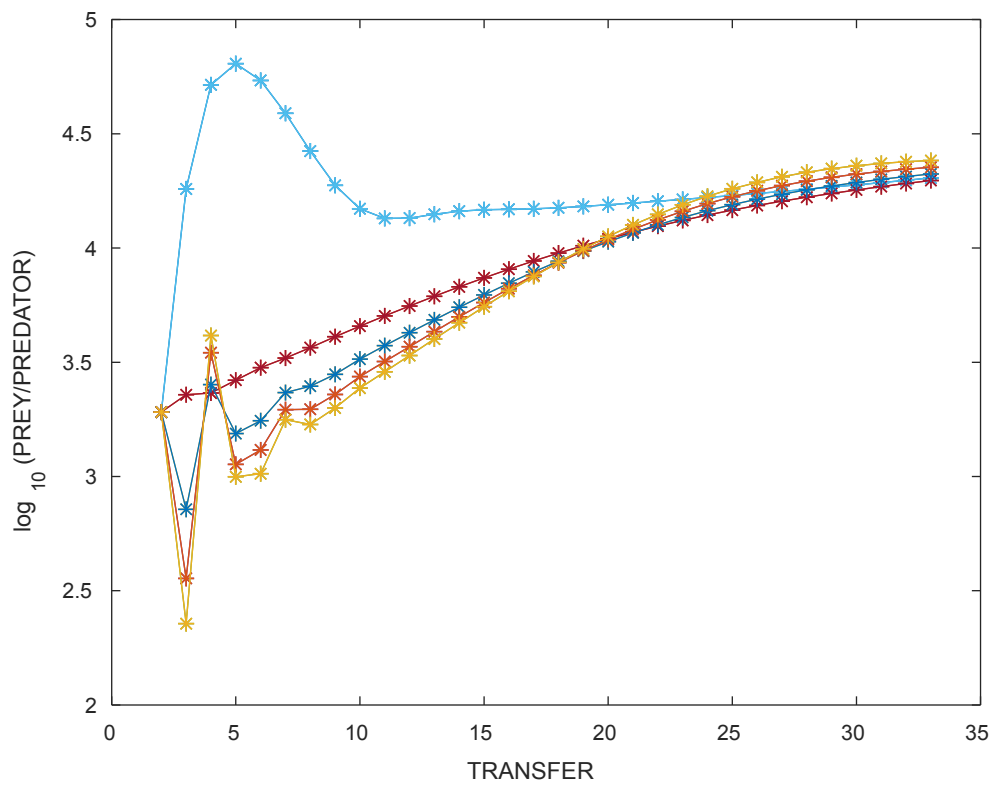
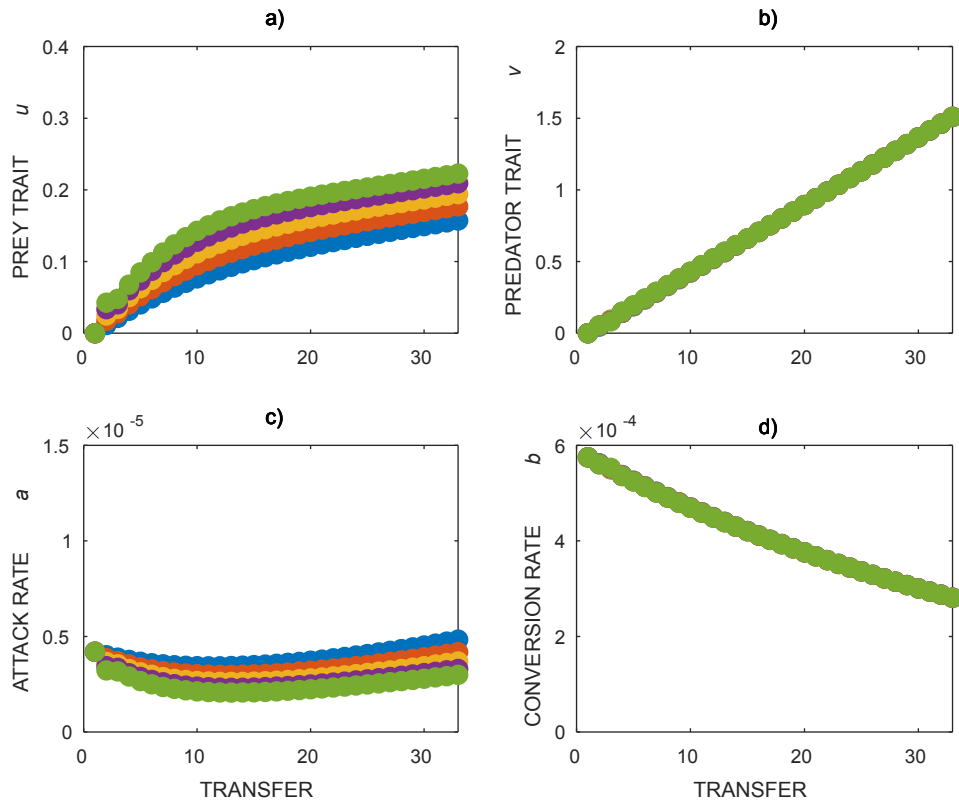


Fig. A3. Level of coexistence between bacteria and ciliates. Blue denotes very high dilution and green the lowest dilution level.



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666 Fig. A4. Evolutionary trajectories for slightly increased transfer intervals. a) prey trait u , b) predator
667 trait v , c) predator attack rate a and d) predator conversion rate b . Dots in blue, red, yellow, magenta
668 and green denote increasing transfer intervals. Here the transfer intervals were increased by 2 hours
669 from 48 to 56 hours.

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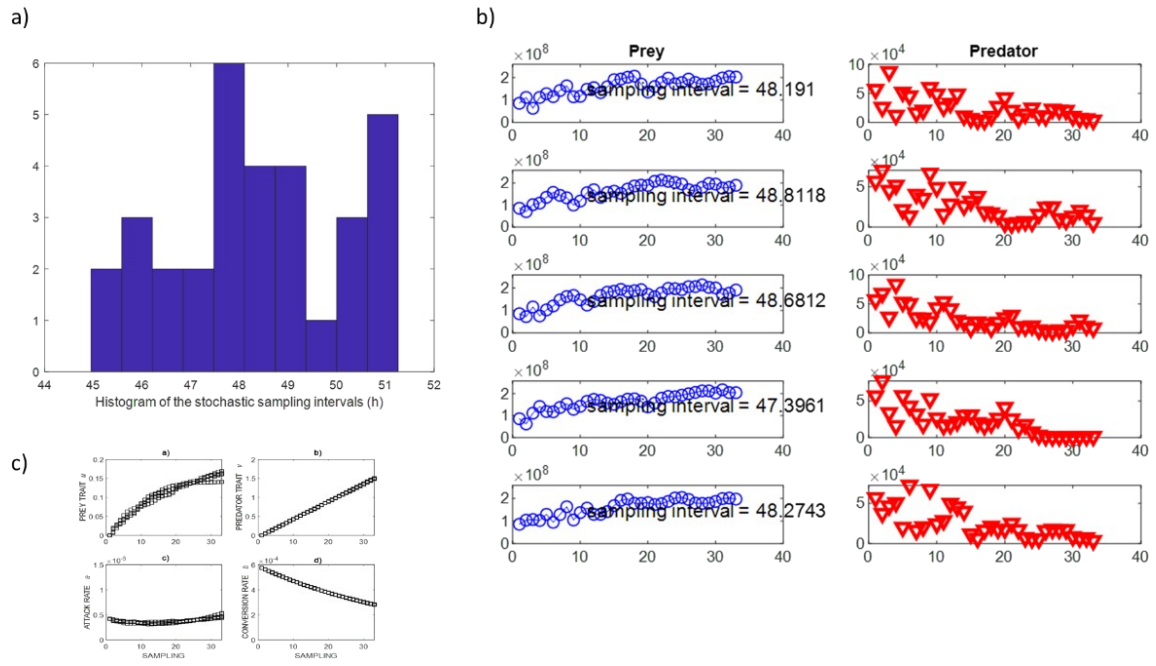
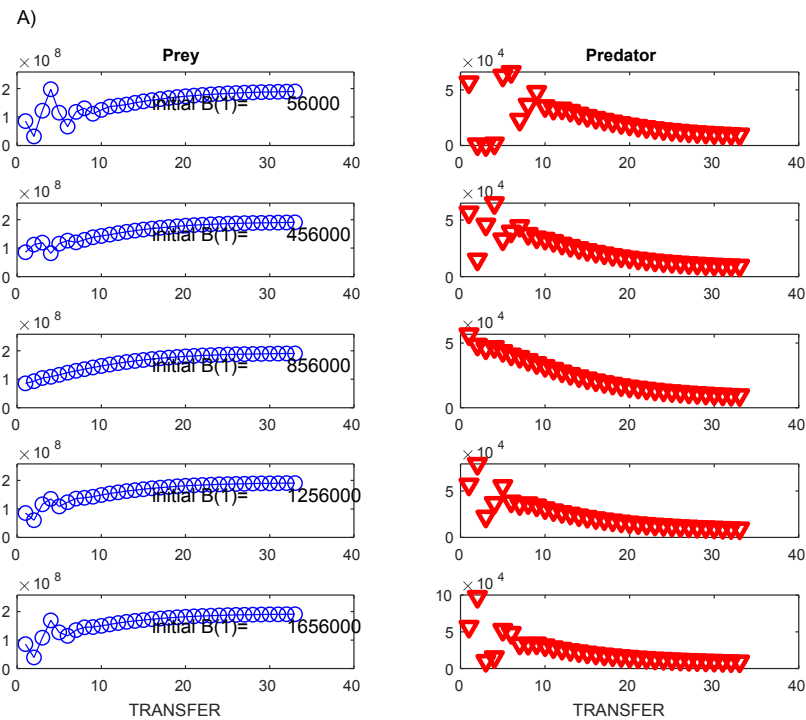
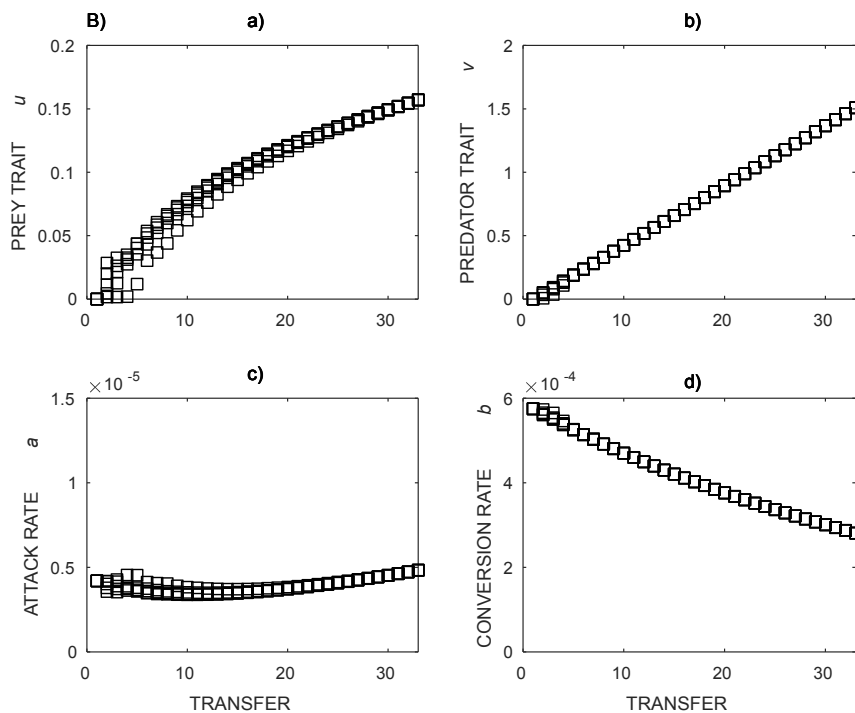


Fig. A5. The effect of variation in transfer intervals. a) Actual transfer happens between 45 and 51 hours and not exactly after 48 hours. b) The ecological dynamics for bacteria (blue) and ciliates (red). c) evolutionary trajectories. Five **replicates** of the experiments with stochastic sampling intervals ($T_2 = 48 + (\text{rand} - 0.5) \times 3$). The expected value is 48 hours. The sampling intervals are independent. The model used a dilution rate of 1%.

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695 Fig. A6. The effect of different initial bacteria concentrations. A) Ecological dynamics. B)
696 Evolutionary dynamics. The model used a transfer interval of 48 hours and a dilution rate of 1%.

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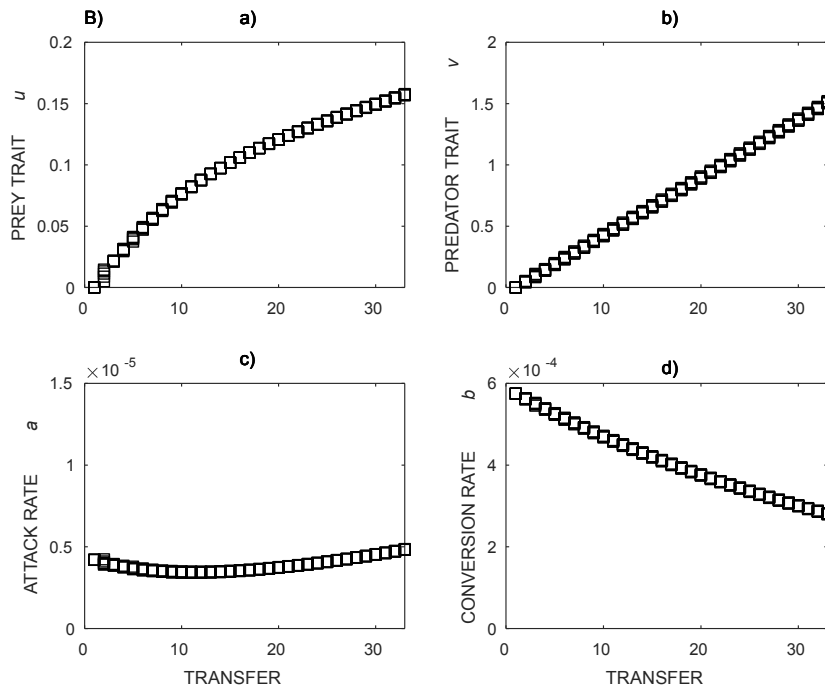
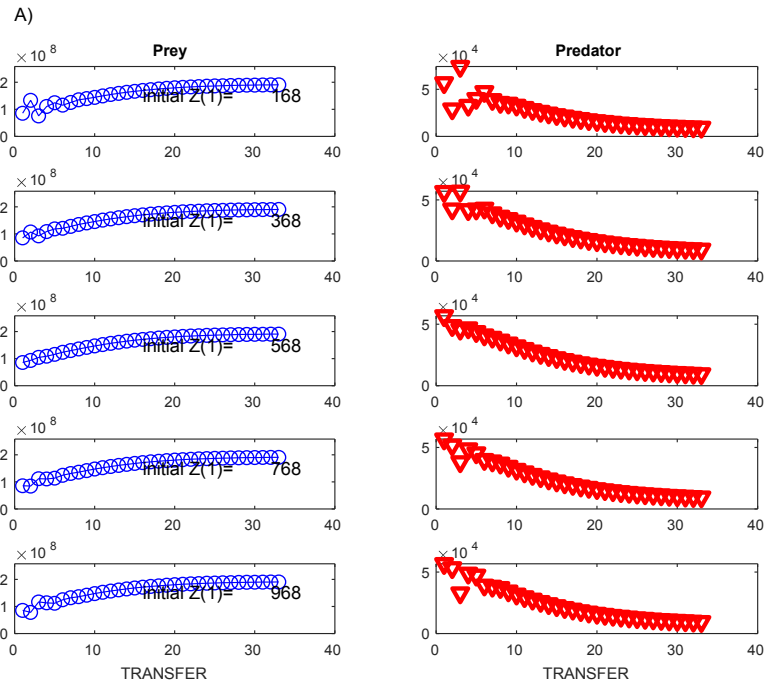


Fig. A7. The effect of different initial ciliate concentrations. A) Ecological dynamics. B) Evolutionary dynamics. The model used a transfer interval of 48 hours and a dilution rate of 1%.

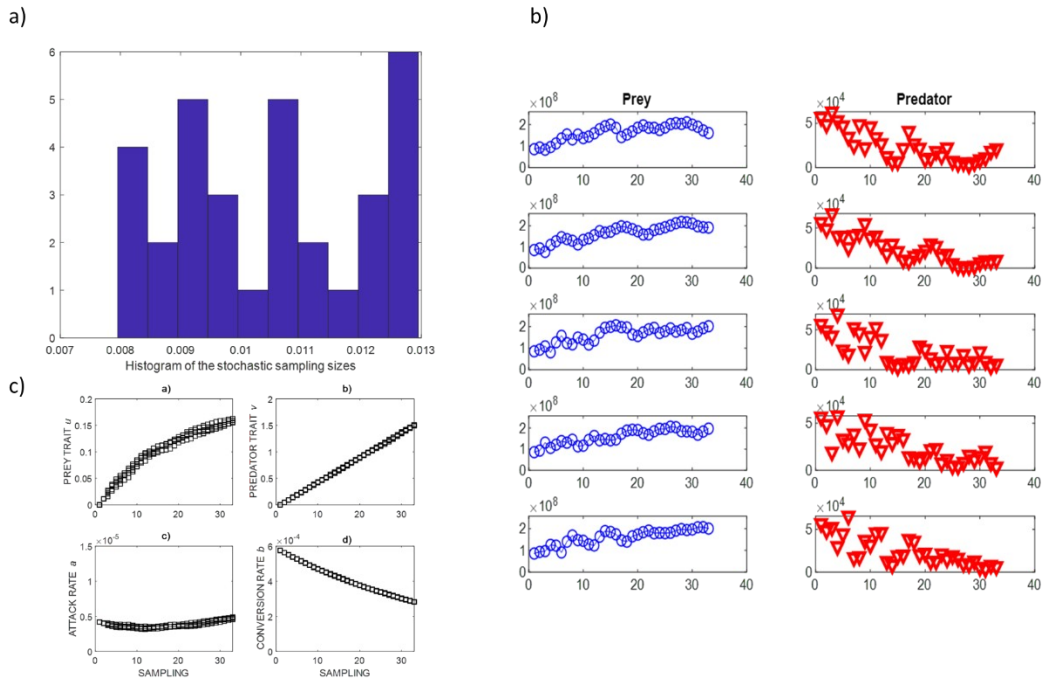


Fig. A8. The effect of different dilution rates implemented by randomized sampling volumes. a) Randomization of dilution rates. b) Bacterial and ciliate densities. c) Evolutionary rates. The model used a transfer interval of 48 hours.