Christian-Albrechts-Universität zu Kiel

Masterarbeit

Eco-evolutionary effects on infectious disease dynamics in metacommunities

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“Eco-evolutionary effects on infectious disease dynamics in metacommunities.”

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The Community dynamics group, for all their helping hands, open ears and intellectual input in the progress of my master thesis.

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And Kathrin, who will ever have my gratitude and love.

Table of Contents

Zusammenfassung

Ansteckende Krankheiten sind allgegenwärtig und im Forschungsbereich der Epidemiologie sind die Entstehung, die Häufigkeit, die Verbreitung, das Fortbestehen und die mögliche Kontrolle von Krankheiten von besonderem Interesse. Forschung im Bereich der experimentellen Evolution kann bedeutend sein, um einen tieferen Einblick in diese Themen zu erhalten und um infektiöse Krankheiten und ihre Dynamiken besser zu verstehen. Hierfür haben wir experimentell die öko-evolutionären Effekte auf infektiöse Krankheitsdynamiken in einem koevolvierenden Wirts-Virus-System, bestehend aus der asexuellen, einzelligen Grünalge *Chlorella variabilis* und ihrem wirtsspezifischem Virus, den Chlorovirus Pbcv-1, untersucht. Wir haben ein neues System mit zwei verbundenen Kulturflaschen (Patches) etabliert, um herauszufinden, ob und wie sich ökologische und evolutionäre Dynamiken in einem räumlich strukturierten System beeinflussen. Nachdem die Algenpopulation mit dem Virus infiziert wurde, sank diese schnell ab, wohingegen die Virenpopulation stark anstieg. Aufgrund der fehlenden Anwesenheit von Wirten sank die Virenpopulation über die Zeit hin ab, und die Algenpopulation erholte sich daraufhin nach der Infektion langsam wieder (25,87 ± 2,99 Tage). Dieser Beobachtung folgte ein erneuter Abfall der Algenpopulationsdichte verbunden mit einem wiederholten Anstieg der Virenpopulation. Unter der Verwendung von Time-shift-Experimenten haben wir überprüft, ob und wann Resistenz von Alge gegenüber dem Virus evolviert ist, oder umgekehrt, ob und wann der Virus koevolviert ist. Die Time-shift-Experimente zeigten, dass eine rasche Evolution von Resistenz der Alge gegenüber dem Virus innerhalb von circa vier Tagen nach Infektion stattgefunden hat. Als wichtigstes Ergebnis unserer Studie lässt sich festhalten, dass die räumliche Struktur einen großen Einfluss auf die öko-evolutionären Effekte und somit auch auf die infektiösen Krankheitsdynamiken in natürlichen Populationen hat. In diesem Zusammenhang kann räumliche Heterogenität oder Patchiness, wie sie in der Natur üblich ist, einen großen Einfluss auf die infektiösen Krankheitsdynamiken haben.

Abstract

Infectious diseases are omnipresent and in the research field of epidemiology the emergence, incidence, distribution, persistence and possible control of diseases are of special interest. Research in experimental evolution can be crucial to get further insights in these subjects and to better understand infectious diseases and its dynamics. We experimentally studied the eco-evolutionary effects on infectious disease dynamics in a coevolving host-virus system consisting of the asexual reproducing, unicellular green algae *Chlorella variabilis* and its hostspecific dsDNA Virus, the Chlorovirus Pbcv-1. We established a novel system of two connected batch cultures (patches) to ascertain whether and how ecological and evolutionary dynamics might interfere in a spatial structured system. After infection of the algae population, the population density decreases rapidly, whereas the virus population density increased. Due to lack of hosts the virus populations decreased over time and the algae populations recovered slowly after some time of infection (25.87 ± 2.99 days), followed by a repeated decrease of algae population and an increase of virus population. Using time-shift experiments, we tested whether and when resistance of algae to virus evolved, or vice versa whether and when the virus counter adapted to the host. The time-shift experiments showed a rapid evolution of resistance of algae populations within approximately four days after infection with virus. Most importantly, our study revealed that spatial structure has a profound impact on the eco-evolutionary effects and therefore on the infectious disease dynamics in natural populations. In this context spatial heterogeneity or patchiness, which is common in nature, can have a major influence on the infectious disease dynamics.

1. Introduction

In marine ecosystems 10^{23} viral infections are occurring every second (Suttle 2007). These infections cause diseases in a wide range of organisms and have a high potential of mortality (Suttle 2005, 2007). This makes marine viruses to one of the strongest forces dominating the global ecosystem (Suttle 2007; Grimsley et al. 2012). Marine viruses are often suggested as being responsible for termination of algal blooms and thus playing a key role in shaping algal biodiversity (Bratbak et al. 1993; Fuhrman 1999; Tarutani et al. 2000; Brussaard 2004, Brussaard et al. 2005; Sandaa 2008).

Previous studies revealed that there is a positive correlation between abundance of marine viruses and temperature (reviewed in Danovaro et al. 2011). A higher abundance of marine viruses can harbor a higher risk of extinction for the marine phytoplankton. As marine phytoalgae are responsible for 50 % of the NPP (Field et al. 1998) this would have enormous consequences for the whole ecosystem. As global climate warming has now become unambiguous and is still ongoing, it is especially important to understand the infectious disease dynamics between marine viruses and its hosts and how they get affected.

In principle, the dynamics of infectious diseases depend on several factors, such as encounter rate of host and pathogen, coevolution, temporal dynamics and spatial dynamics. A positive correlation is typically found between disease prevalence and host density, which can be related to the encounter rate of host and pathogen (Anderson & May 1982, 1992; Hanski 1999). A low host population density can result in a higher extinction rate or rather extinction risk of the pathogen, as compared to a host population with high density (reviewed in Grenfell & Harwood 1997; Hanski 1999). With a higher encounter rate, increased host resistance and pathogen infectivity can evolve more rapidly (Flor 1971; Thompson & Burdon 1992). Therefore ongoing evolution, which can be density dependent and tightly linked with ecology, is an important factor that needs to be taken into account. Additionally, temporal dynamics, such as fluctuating population sizes, can affect infectious disease dynamics. Furthermore, spatial dynamics can be crucial for infectious disease dynamics. Increased connectivity between host populations (metapopulations) can result in increased pathogen resistance and decreased pathogen colonization success, as a consequence of higher gene flow among the host populations (Gandon & Michalakis 2002; Carlsson-Granér & Thrall 2002; Jousimo et al. 2014). Alternatively, isolated host populations with reduced gene flow can have increased susceptibility to pathogen infection (Granér & Thrall 2002; Jousimo et al.

2014). Overall, pathogen success or host survival and resulting infectious disease dynamics depend on ecological factors, e.g. population densities and fluctuations, as well as evolutionary factors, e.g. coevolution and gene flow. Consequently, spatiotemporal dynamics as well as eco-evolutionary dynamics can have a great impact on infectious disease dynamics.

It has been shown recently, that eco-evolutionary dynamics are also occurring in a coevolving host-virus system (Frickel et al. 2016). Generally, coevolutionary dynamics can be described as spatial processes not only depending on the traits of the interacting species but also on the environment in which those interactions take place (Thompson 1999; Forde et al. 2004; Sieber et al. 2014). Frickel et al. (2016) studied the eco-evolutionary dynamics in a chemostat, representing an enclosed environment, which is not common in nature. Therefore the impact of spatial structure on eco-evolutionary dynamics and thus on infectious disease dynamics is missing in our knowledge so far. Consequently, in order to enhance the understanding of the combination of temporal dynamics with spatial dynamics as well as the interplay of both, which affect infectious disease dynamics, we need to come up with a spatial structured (patched) system.

For the study we used the coevolving host virus system of the asexual reproducing, unicellular green algae *Chlorella variabilis* and its host specific dsDNA Virus, the Chlorovirus Pbcv-1. We established a novel system of two connected batch cultures (patches) to see if and how ecological and evolutionary dynamics might interfere in a patched system. For the comparison of different spatial and temporal effects we used three different treatments. The patches were inoculated with different combinations of *Chlorella variabilis* and Pbcv-1 of either algae + virus in both patches, or algae + virus in only one patch, or algae + virus in one patch and only algae in the other patch. With this approach we tested whether there are differences in the infectious disease dynamics due to different compositions of the communities. We examined the interaction between ecology and evolution on spatial and temporal scales. Ecological dynamics were followed by population densities of host and virus. Evolutionary insights were gathered by performing time-shift experiments to assess whether and when host evolved resistance, and whether and when the virus evolved counter adaptations in return (Frickel et al. 2016).

We expect that different eco-evolutionary dynamics occur because of different compositions of the communities.

2. Materials & Methods

2.1 Study system

Chlorella variabilis (NC64A)

Chlorella variabilis is a unicellular, photosynthetic microalga (Trebouxiophyceae) with a size of 2-12 µm (Shihira & Krauss 1965; Friedl 1995). It reproduces asexually, has a short generation time and is nonmotile (Van Etten et al. 1991). It is a facultative intracellular photobiont of the ciliate *Paramecium bursaria* and a model system for studying virus-algal interactions (Blanc et al. 2010).

Paramecium bursaria chlorella virus (Pbcv-1)

The large plaque forming dsDNA Pbcv-1 has a genome size of ca. 300 kbp and is nonmotile (Van Etten et al. 1982; Skrdla et al. 1984). After host specific attachment the algal cell wall gets digested and the virion DNA is injected before a lytic infection cycle starts (Meints et al. 1984; Grimsley et al. 2012).

2.2 Experimental design

Experiments were performed in a connected batch culture system (Fig. 1) with bold's basal medium (BBM; Bischoff & Bold 1963). Because both virus and algae are nonmotile, previous pilot experiments were executed to find the optimal length between the two patches and at which length the two patches can be treated as independent of each other (Fig. 36 Appendix). Two Corning batch culture flasks (125 ml) were then connected with an 8 cm silicon tube (ID = 3.175 mm).

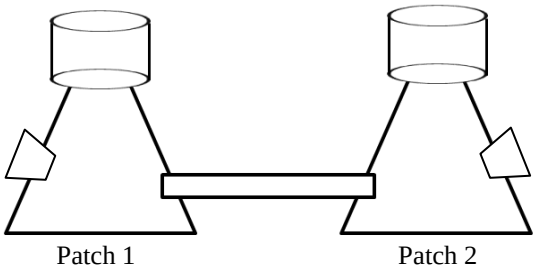

Figure 1 Schema of the experimental setup of the patched population dynamics experiment. Both Corning batch culture flasks (125 ml) are connected with an 8 cm silicon tube (ID = 3.175 mm). A silicon plug was attached to each patch for daily sampling.

One isolated algal clone of *Chlorella variabilis* was used to inoculate the batch cultures in order to minimize the initial genetic variability. To inoculate each batch culture separately a

clamp was attached at the silicon tube. For the experiment five different treatments with different combinations of algae and virus populations were used (Tab. 1).

Table 1 Treatments used for the experiment. For different combinations of the communities the patches were inoculated with different combinations of *Chlorella variabilis* and Pbcv-1 of either algae + virus in both patches, or algae + virus in only one patch, or algae + virus in one patch and only algae in the other patch. The empty batch (dashed line) was filled with BBM.

Treatment	**Patch 1**	**Patch 2**
A + V – 0	Algae + Virus	----------
A + V – A	Algae + Virus	Algae
A + V – A + V	Algae + Virus	Algae + Virus
A – A (control 1)	Algae	Algae
A – 0 (control 2)	Algae	----------

The batch cultures were started with an algae population of 2×10^5 algae cells / ml. At day 4 three out of five treatments were inoculated with 100 µl of purified and concentrated virus (9.68×10^7 virus particles / ml). Hence the starting concentration of the virus population after inoculation was 7.74×10^5 virus particles / ml. The clamps were detached one hour after inoculation. Each treatment was replicated three times. The different treatments and replicates were allocated randomly in order to exclude variation due to surrounding conditions e.g. light intensity, temperature gradient.

Before sampling, clamps were attached in the middle of the silicon tubes to avoid interaction between the patches related to sampling procedure. After shaking the set-up, samples were taken with a syringe through a silicon plug. Population densities were followed daily by counting virus (Brussaard 2004) with FACS (BD Biosciences, FACSCalibur HTS, San Jose, California) and algae (2.5 % Lugol preserved) with FlowCam (Fluid Imaging Technologies, FlowCam VS Series, Yarmouth, Maine, USA). Samples of virus and algae populations were stored twice per week by plating algae on BBM agar plates and storing virus at 4 °C (Van Etten et al. 1983) after filtering (0.45 µm cellulose syringe filter). The daily removed total sample volume of 12.5 ml (10 %) was directly replaced with fresh BBM. After sampling, the different treatments and replicates were arranged randomly again to increase independence of locality. The experiment was performed for 37 days, at 21°C and at continuous light.

2.3 Time-shift experiments

To examine the evolution of resistance and infectivity of algae and virus a time-shift experiment (Gaba & Ebert 2009; Frickel et al. 2016) was executed. For the time-shift experiment five time-points (grey vertical lines: Fig. 2 A, 3 A, 4 A, 6, 9, 12, 16, 19, 22) per batch culture were selected. These selected time-points were supposed to represent different stages of the algae populations: pre-infection, post-infection, minimum, increasing and second maximum. In some cases there were no algae colonies growing on BBM-plates of Patch 2 at the desired time-points of Patch 1 and were therefore missing for further analysis (indicated by missing grey vertical lines at these time-points: Fig. 2 B, 3 B, 4 B).

Instead of single clones, the entire population of each time-point was isolated from the agar plates and re-grown in batch cultures separately. To compare this method with the one used by Frickel et al. (2016), 10 clones of one batch culture from the same time-points as in the entire population assay were isolated and re-grown in batch cultures separately. Each host population was separately exposed to each virus population from relative past, present and relative future time-points from which the host population was isolated. This was only done within the same batch culture. An exception of this is the treatment where no virus could be detected in Patch 2 (Algae + Virus – 0). In this case the combinations where done with the viruses of Patch 1. Furthermore, algae populations of the latest time-point of the control treatments were tested against the virus used for inoculation of the other treatments to verify that resistance is not occurring spontaneously without selection force.

The fitness of algae population was measured by optical density (OD) at a wavelength of 680 nm (Tecan, Infinite M200PRO, Männedorf, Switzerland). Each algae population was diluted to the same starting OD of 0.045 and the virus populations were diluted to a resulting multiplicity of infection (MOI) of 0.01 particles / algal cell based on the dilution curve (Algal cells / OD, Fig. 37 Appendix).

(1) $$MOI = \frac{Virus\ particles}{Algal\ cells}$$

The algae-virus and algae without virus (control) combinations with four technical replicates each were incubated in 96-well-plates for 72 hours at continuous light and 21 °C. For each combination growth rates per day were calculated based on ODs measured at 0 hours and after 72 hours. This was done using the formula:

$$(2) \qquad Growth\ rate = \frac{Ln(N_t\ algae\ cells) - Ln(N_{t-1}\ algae\ cells)}{3}$$

To assess whether an algal population was resistant or susceptible to a particular virus population, we compared the mean growth rate per day plus 2 standard deviations of the four technical replicates to the mean growth rate per day minus 2 standard deviations of the control (growth without virus) and also vice versa (Frickel et al. 2016). An overlap of the means of the algae with virus and algae without virus assays ± 2 standard deviations would mean that the algae population is not affected by the virus indicating resistance. Accordingly a non-overlap of the growth rates can be interpreted as algae population susceptible to the virus because the algae population cannot grow as good as without virus.

2.4 Data analysis

All data analyses were performed in Rstudio 0.99.491 (Rstudio 2015) and R 3.1.3 (RCoreTeam 2015) using the packages astsa (Stoffer 2014), reshape2 (Wickham 2014) and ggplot2 (Wickham & Winston 2015). For the algae population densities the software of FlowCam (Visualspreadsheet 4.0.27) was used for reprocessing the data by checking the captured pictures of the particles. Pictures which could not be classified as alga cell or colony were excluded.

2.4.1 Differences within treatment

Smoothing of algae and virus population densities was used at the beginning to minimize short term fluctuations (function: smooth.spline, smoothing parameter = 0.3). The daily growth rate of algae population was calculated using the following formula:

$$(3) \qquad Growth\ rate = \frac{Ln(N_t\ algae\ cells) - Ln(N_{t-1}\ algae\ cells)}{1}$$

Additionally, the MOI, as a proxy for force of infection, was calculated (see formula above (1)). Within each treatment the algae population densities, virus population densities and MOI were compared between the patches using one-way ANOVA and Tukey post hoc test. Furthermore, the lag of algae and virus populations between the two patches was determined per replicate by cross correlation. The lag of algae and virus populations between the patches was compared using one-way ANOVA.

2.4.2 Differences between patches and treatments

Differences in algae population densities, virus population densities, growth rates and MOI were compared between the treatments using two-way ANOVA and Tukey post hoc test. Within each patch the maximum algal growth rate per day, the maximum population density of algae and virus and the maximum MOI was determined. For each patch the time of decrease of the algae population, as an indicator for infection by virus, was detected by the first value of negative growth rate per day greater than 0.5. The time-point of evolutionary rescue, as the time how long it takes until the algae population recovers the first time after infection, was also ascertained. It was calculated by the difference of the time-point of the second maximum and the time-point of decrease. The values of each factor were compared by using two-way ANOVA and Tukey post hoc test.

3. Results

3.1 Population dynamics

One distinct pattern could be observed in all the treatments where virus was added. After infection of the algae population, the population density decreased rapidly, whereas the virus population density increased. Nevertheless, the virus populations decreased over time and the algae populations recovered slowly after some time of infection (25.87 ± 2.99 days) followed by a repeated decrease of algae population and an increase of virus population (Fig. 2 A, 3 A, 4 A, 6 A, 9 A, 12 A, 16, 19, 22). In the treatment *Algae + Virus – 0* no viruses were found in patch 2, whereas the algae population increased over time (Fig. 2 B, 3 B, 4 B). In the treatment *Algae + Virus – Algae* infections of algae populations in patch 2 occurred over all replicates within 4.33 ± 3.3 days, following the same pattern than the patches inoculated with virus (Fig. 6 B, 7, 8, 9 B, 10, 11, 12 B, 13, 14). There was no difference in the population dynamics between the two patches of treatment *Algae + Virus – Algae + Virus*, as there was no lag in population densities between them (Fig. 17, 18, 20, 21, 23, 24). The control batch cultures with only algae showed stable densities after initial growth (Fig. 26, 27).

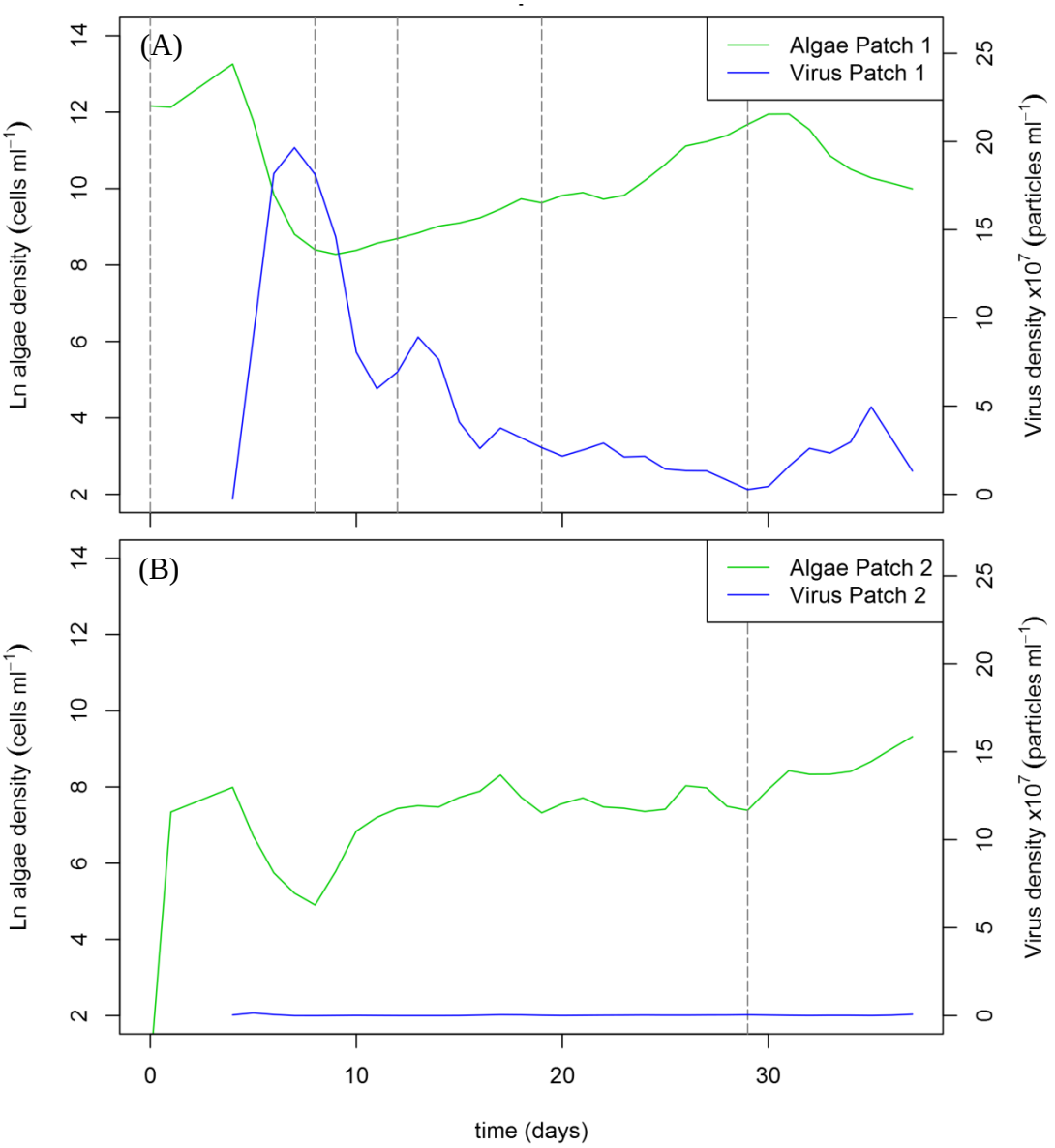

Figure 2 Population dynamics of *Algae + Virus – 0* replicate 1 of patch 1 (A) and patch 2 (B). Green line: algal densities (natural logarithm); blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Missing grey lines in B indicate that no colonies were grown on the agar-BBM plates at earlier time-points. Because no virus in patch 2 were present, algae-virus combinations were done with the virus of patch 1 during the time-shift experiment.

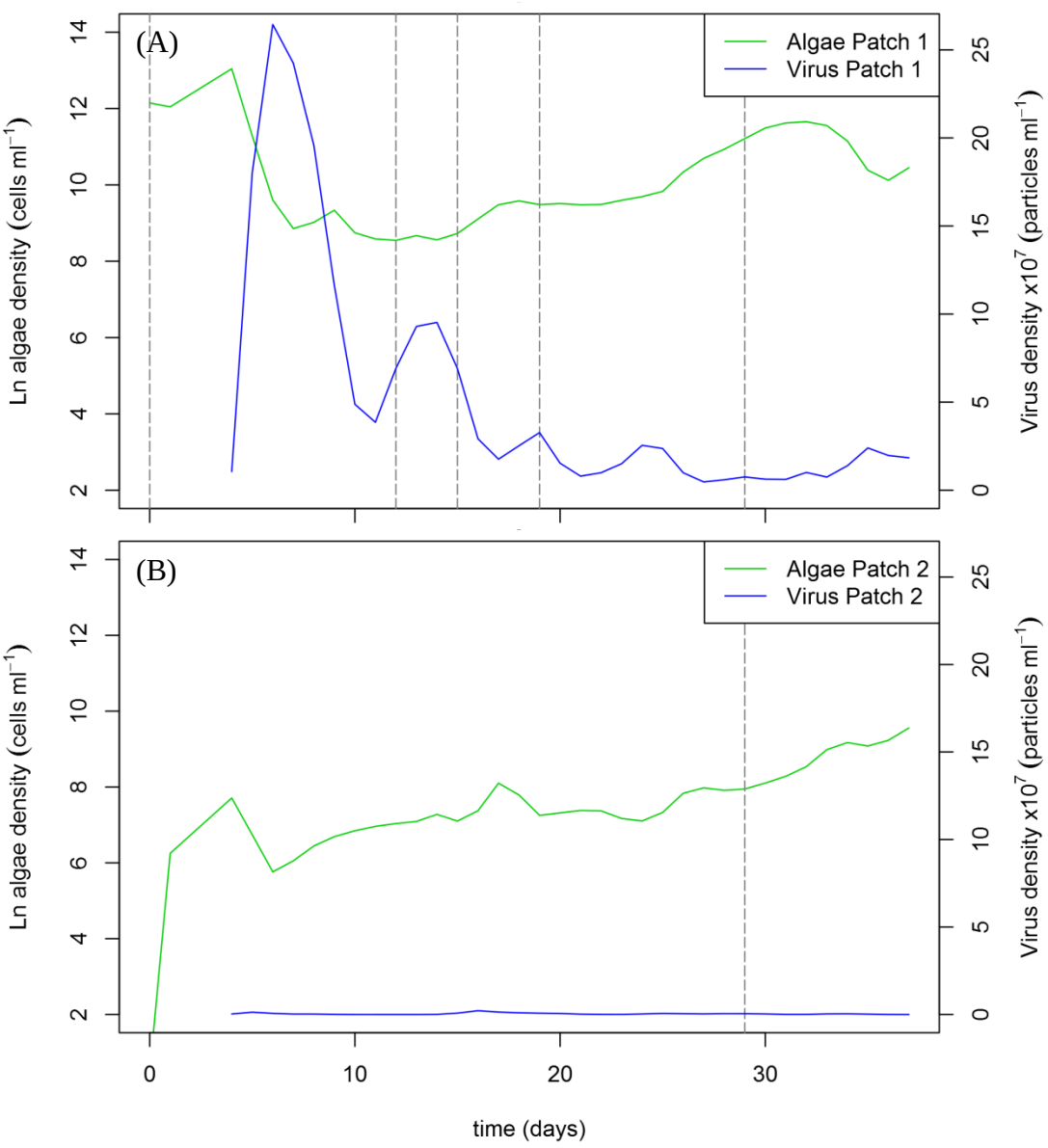

Figure 3 Population dynamics of *Algae + Virus – 0* replicate 2 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Missing grey lines in B indicate that no colonies were grown on the agar-BBM plates at earlier time-points. Because no virus in patch 2 were present, algae-virus combinations were done with the virus of patch 1 during the time-shift experiment.

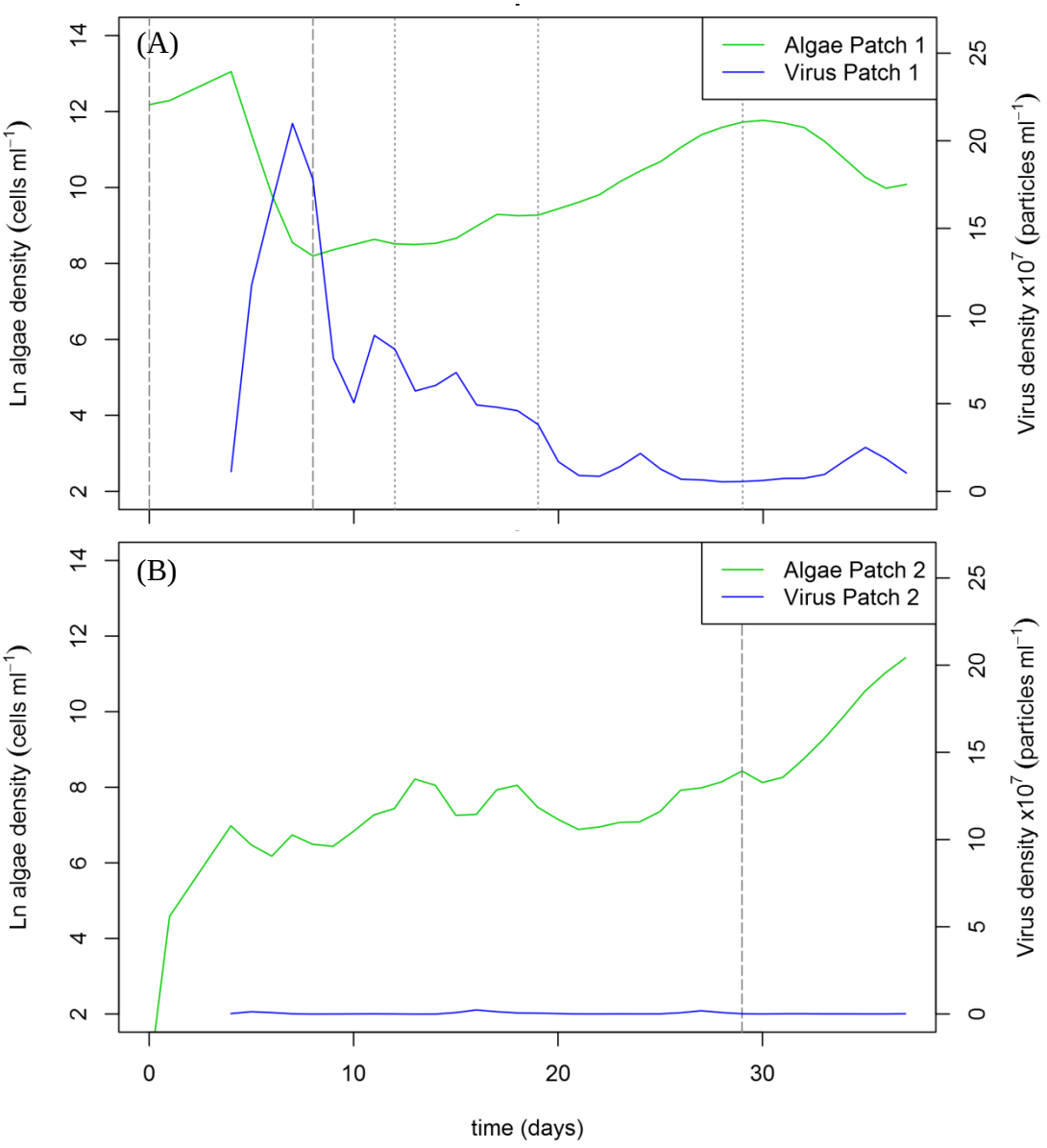

Figure 4 Population dynamics of *Algae + Virus – 0* replicate 3 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Missing grey lines in B indicate that no colonies were grown on the agar-BBM plates at earlier time-points. Because no virus in patch 2 were present, algae-virus combinations were done with the virus of patch 1 during the time-shift experiment.

There was neither a correlation between the algae density of patch 1 and patch 2 (Fig. 5 A, ANOVA: $F_{1,106} = 0.02$, p = 0.88, adj. R^2 = -0.01), nor a correlation between the virus density of patch 1 and patch 2 (Fig. 5 B, ANOVA: $F_{1,100} = 0.03$, p = 0.86, adj. R^2 = -0.01), nor a correlation between the MOI of patch 1 and patch 2 (Fig. 5 C, ANOVA: $F_{1,100} = 0.07$, p = 0.79, adj. R^2 = -0.01). The interaction between the algae and virus populations in patch 1 represents a typical cycle of consumer-resource dynamics (Fig. 5 D).

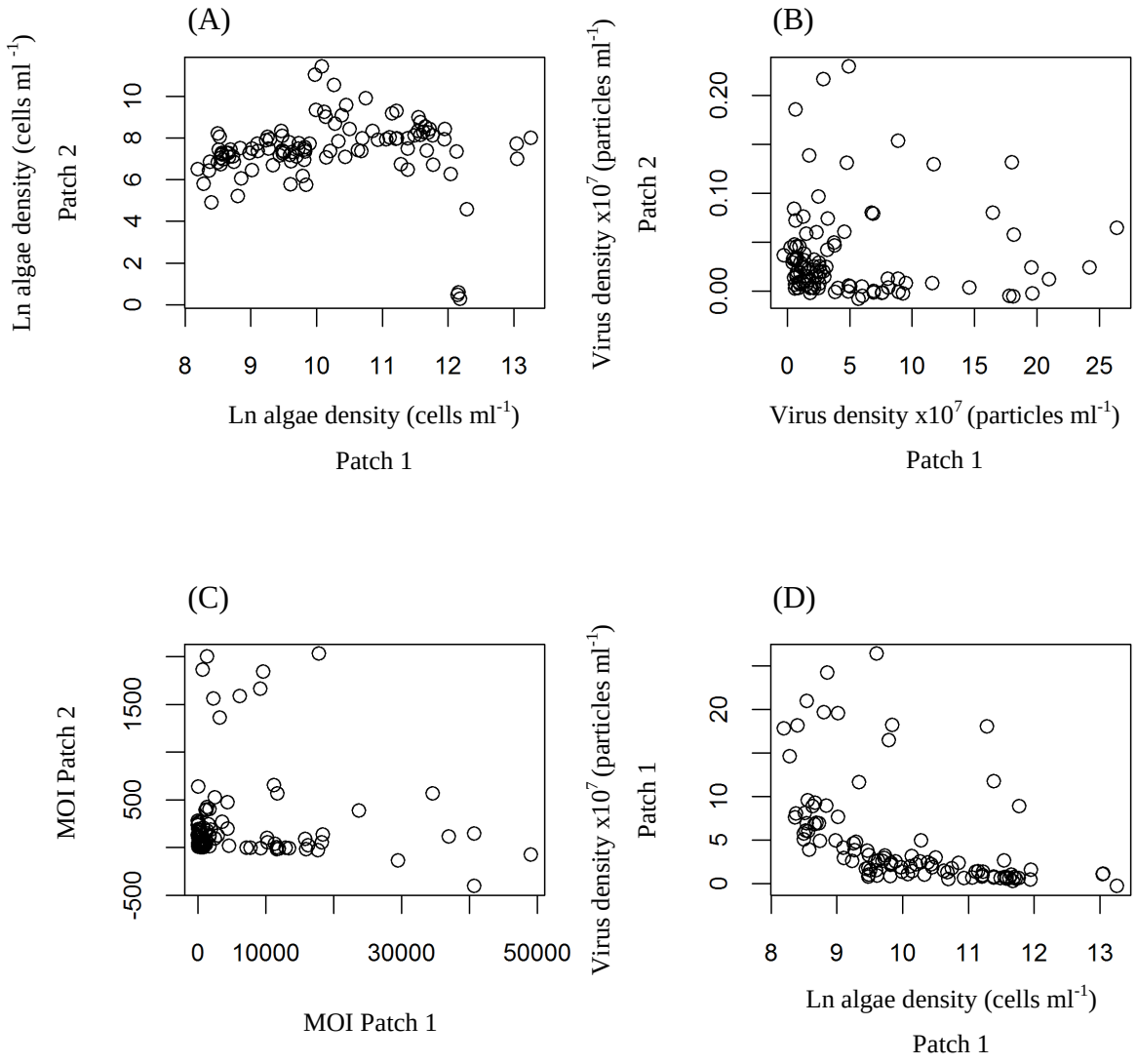

Figure 5 Correlations of patch 1 against patch 2 of algae density (A), virus density (B) and MOI (C) for treatment *Algae + Virus – 0*. Additionally, a correlation for the virus density against the algae density in patch 1 is shown (D). Algae density patch 1 x patch 2 (ANOVA: $F_{1,106} = 0.02$, p = 0.88, adj. R^2 = -0.01), virus density patch 1 x patch 2 (ANOVA: $F_{1,100} = 0.03$, p = 0.86, adj. R^2 = -0.01), MOI patch 1 x patch 2 (ANOVA: $F_{1,100} = 0.07$, p = 0.79, adj. R^2 = -0.01), algae patch 1 x virus patch 1 followed typical consumer-resource cycle.

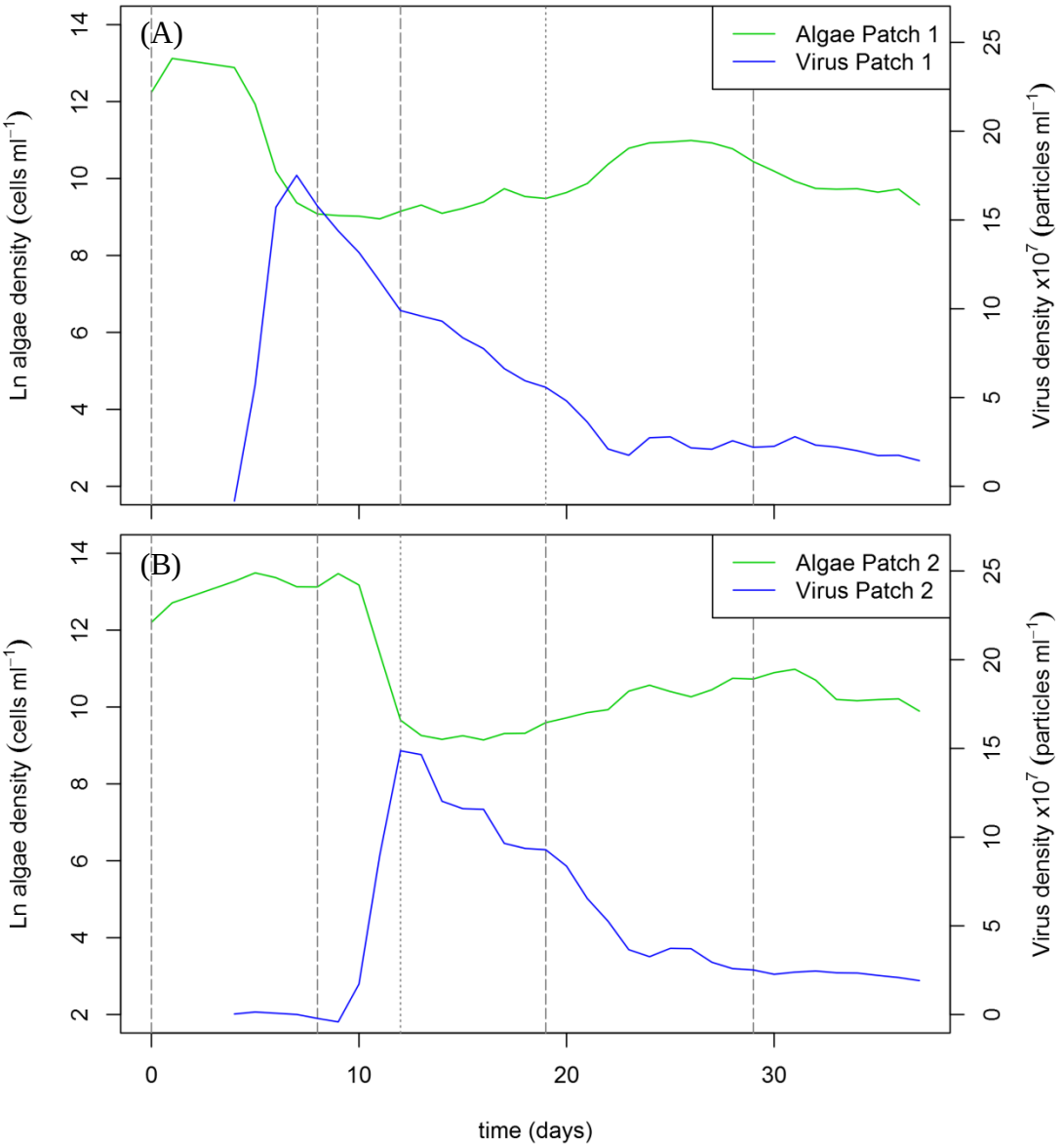

Figure 6 Population dynamics of *Algae + Virus – Algae* replicate 1 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Grey dotted lines indicate missing algae populations for time-shift experiments due to technical errors.

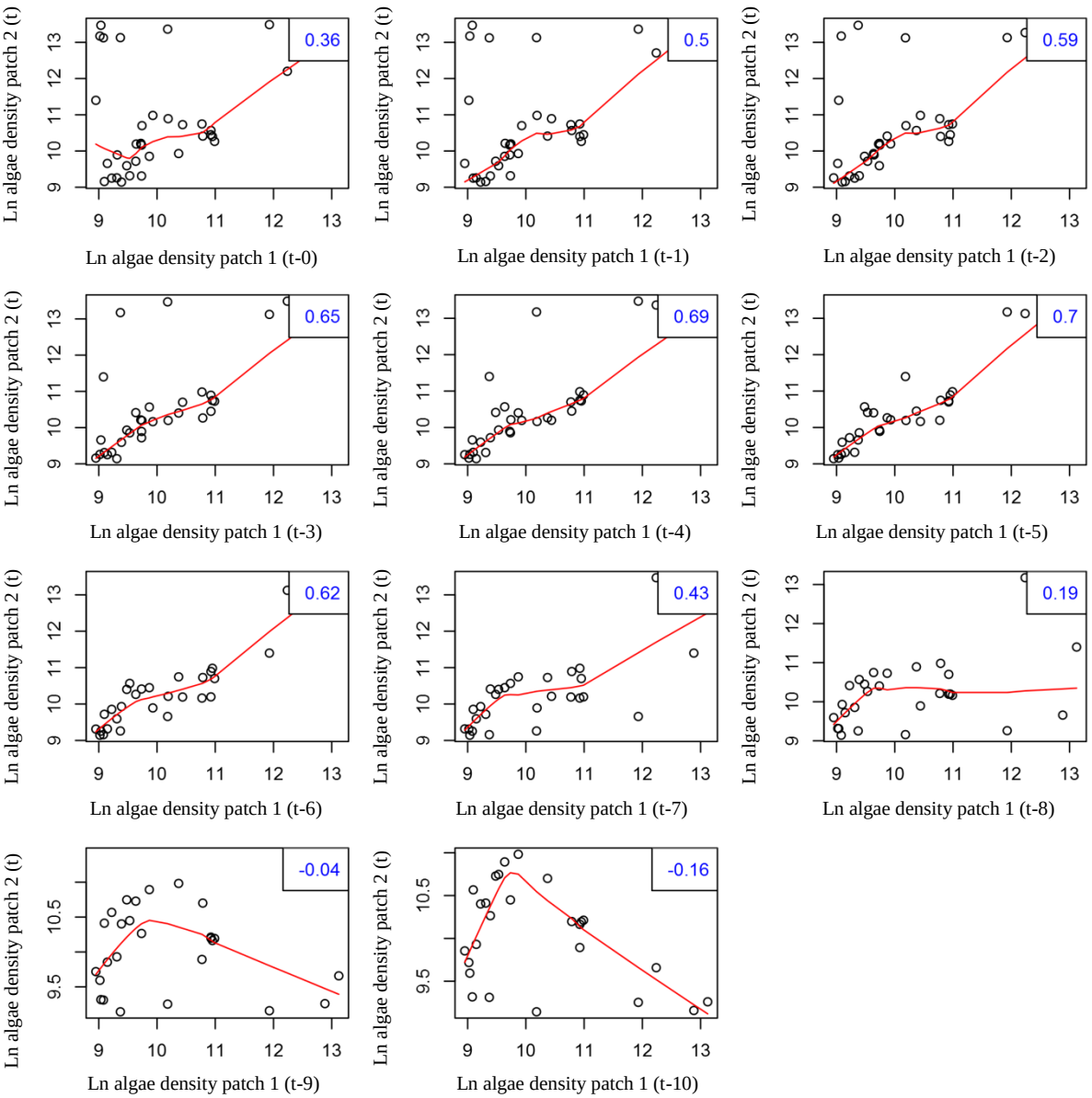

Figure 7 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags (days) for treatment *Algae + Virus – Algae* replicate 1. In each plot algae density (natural logarithm) patch 2 is on the vertical and a past lag of algae density (natural logarithm) patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

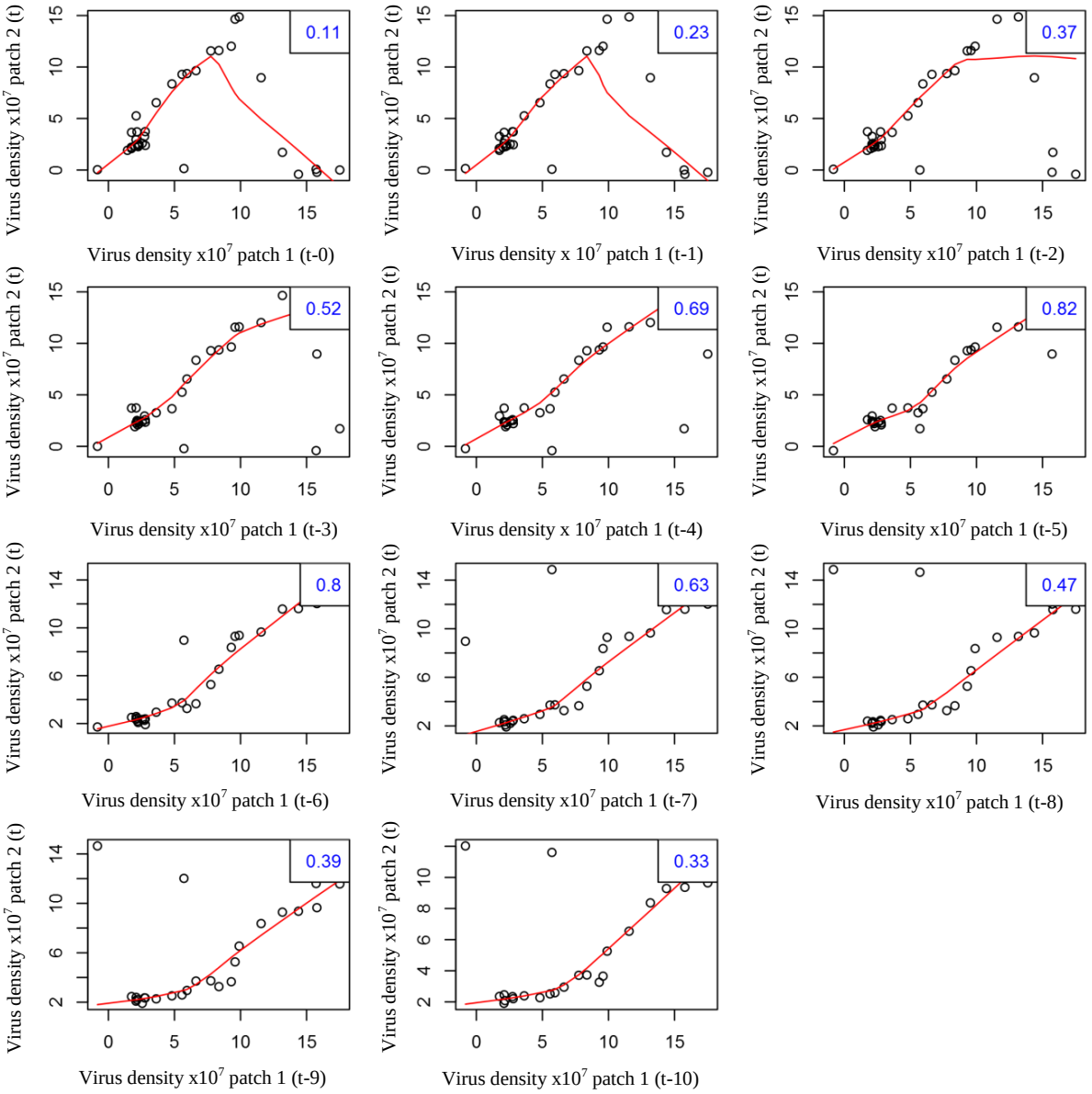

Figure 8 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags (days) for treatment *Algae + Virus – Algae* replicate 1. In each plot virus density patch 2 is on the vertical and a past lag of virus density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

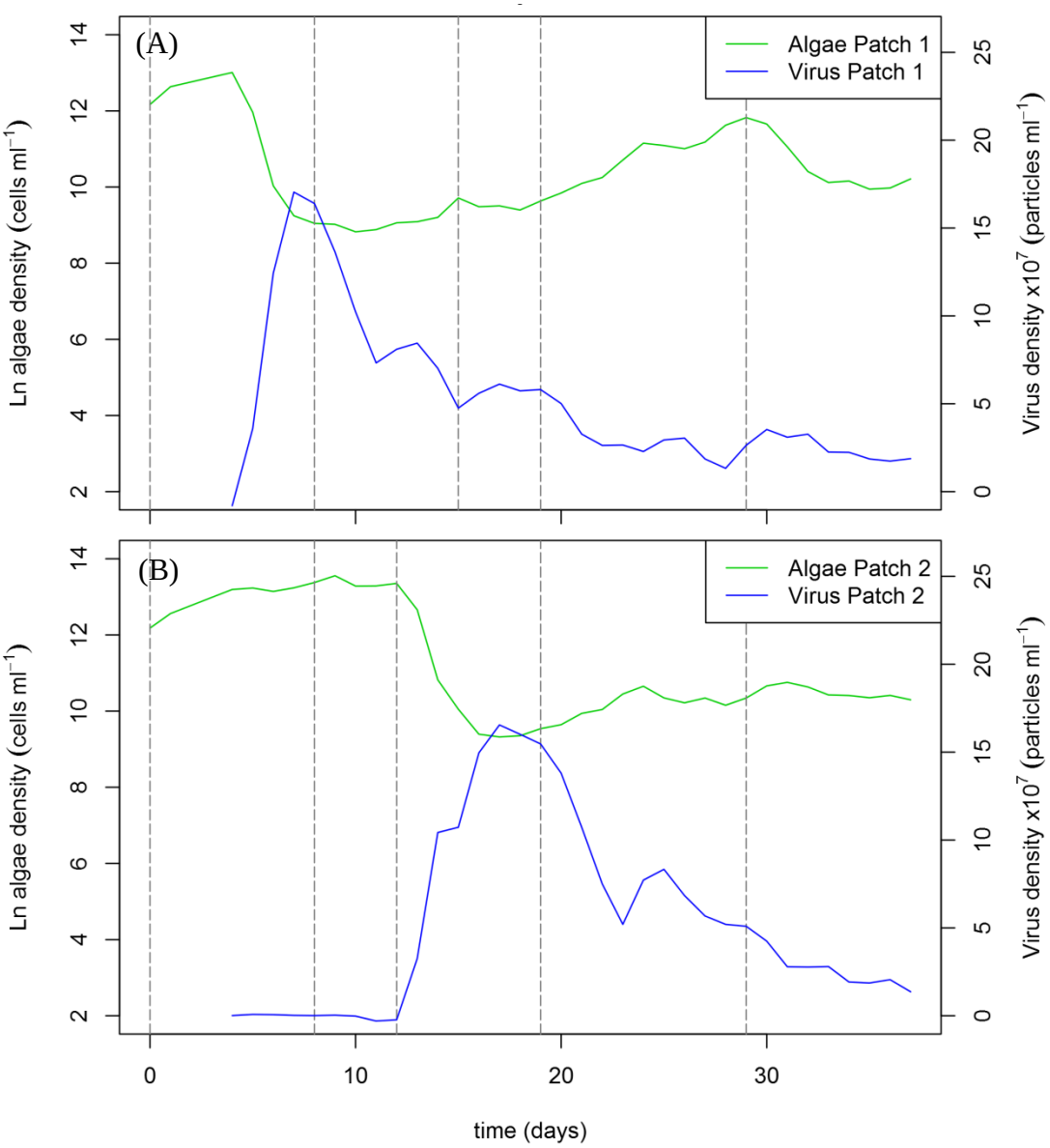

Figure 9 Population dynamics of *Algae + Virus – Algae* replicate 2 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments.

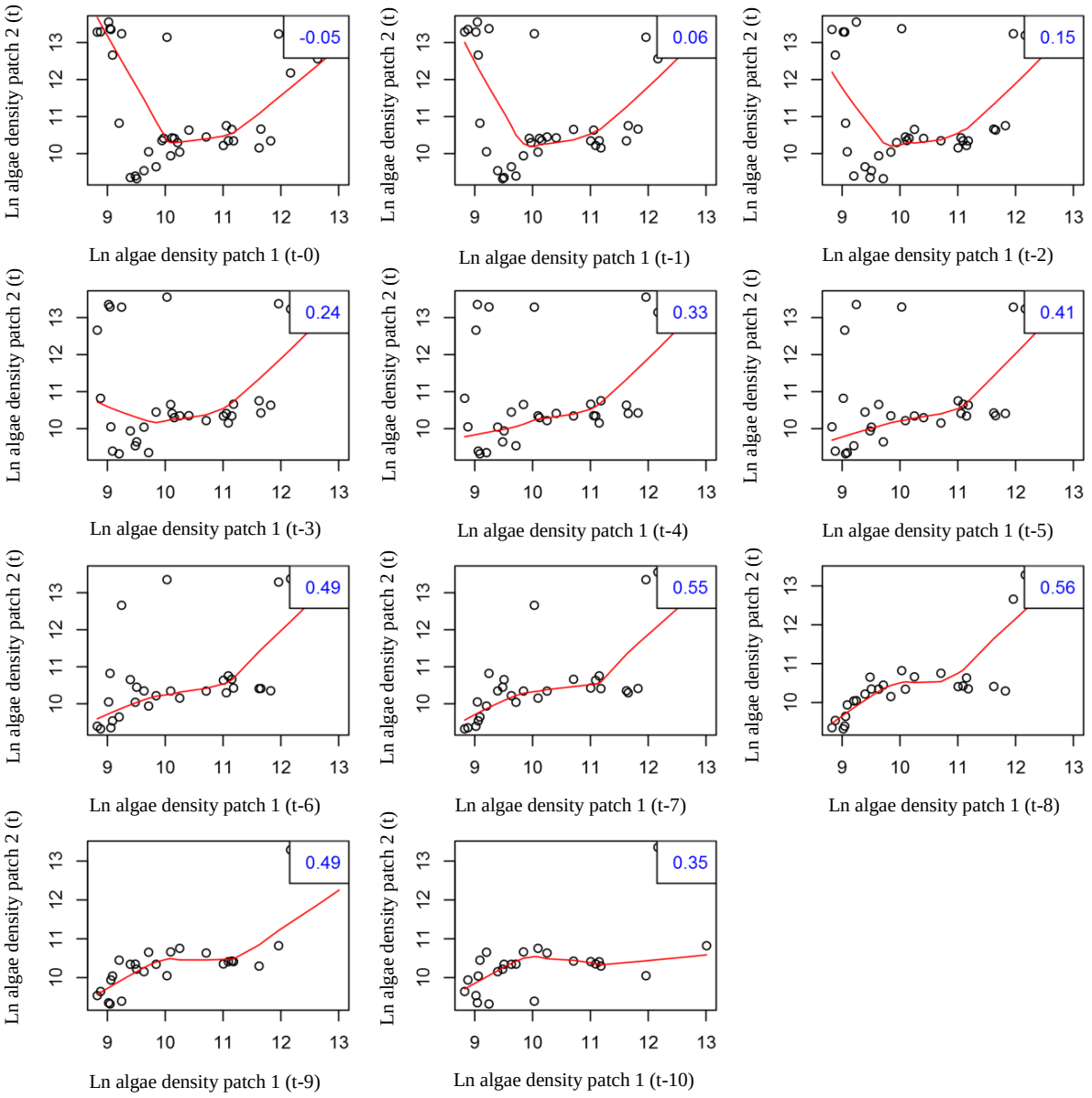

Figure 10 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae* replicate 2. In each plot algae density patch 2 is on the vertical and a past lag of algae density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

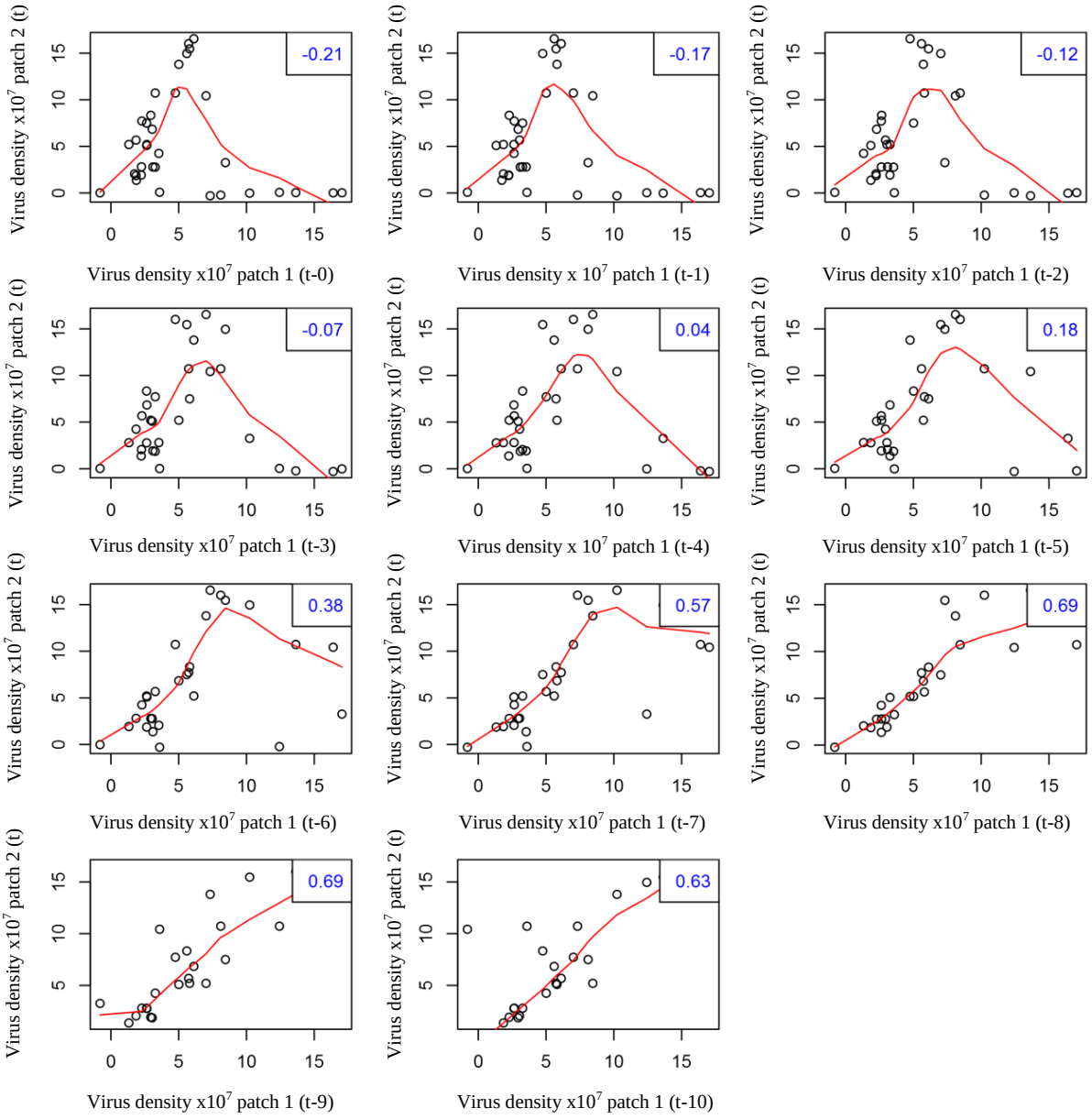

Figure 11 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae* replicate 2. In each plot virus density patch 2 is on the vertical and a past lag of virus density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

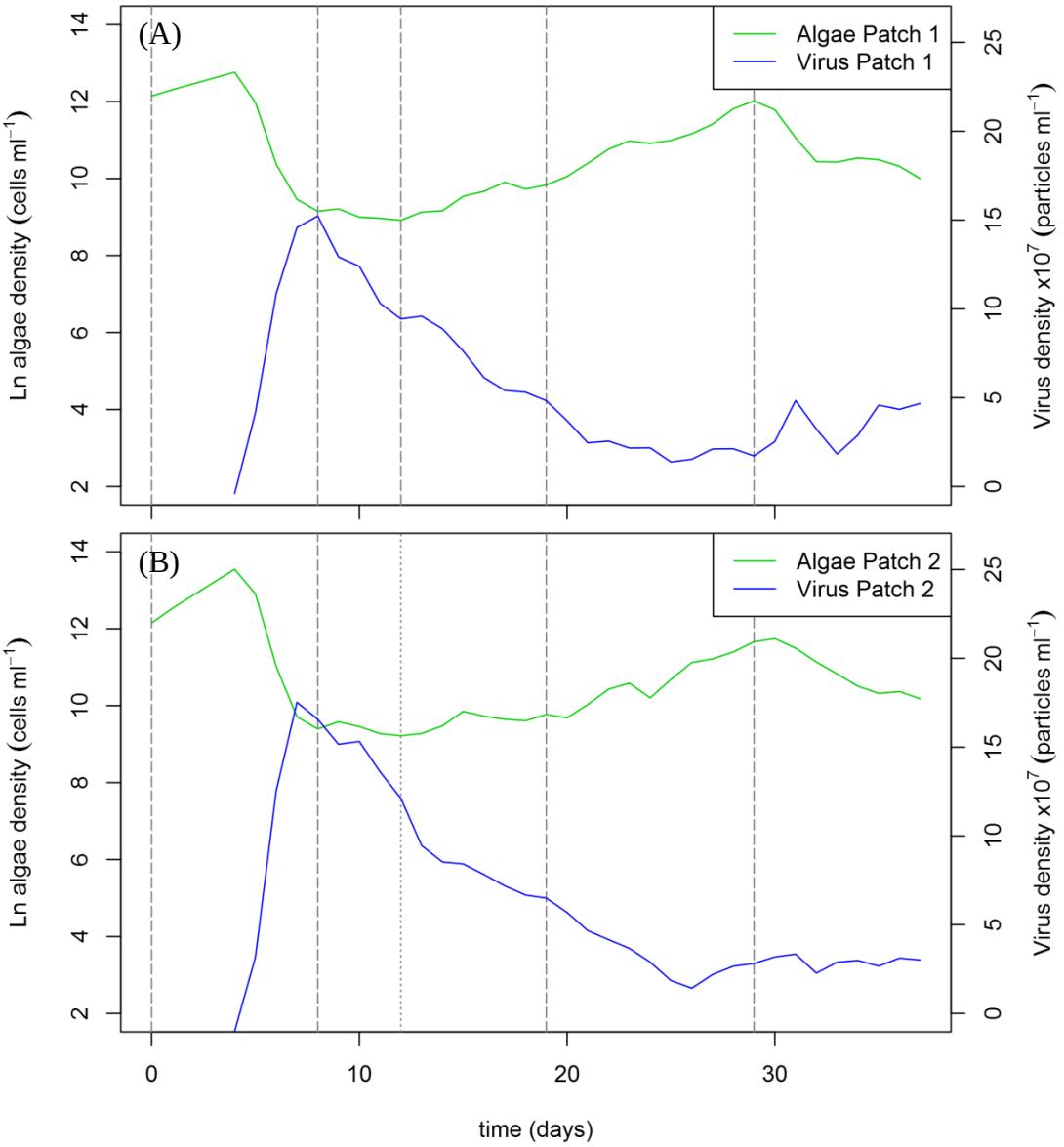

Figure 12 Population dynamics of *Algae + Virus – Algae* replicate 3 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Grey dotted lines indicate missing algae populations for time-shift experiments due to technical errors.

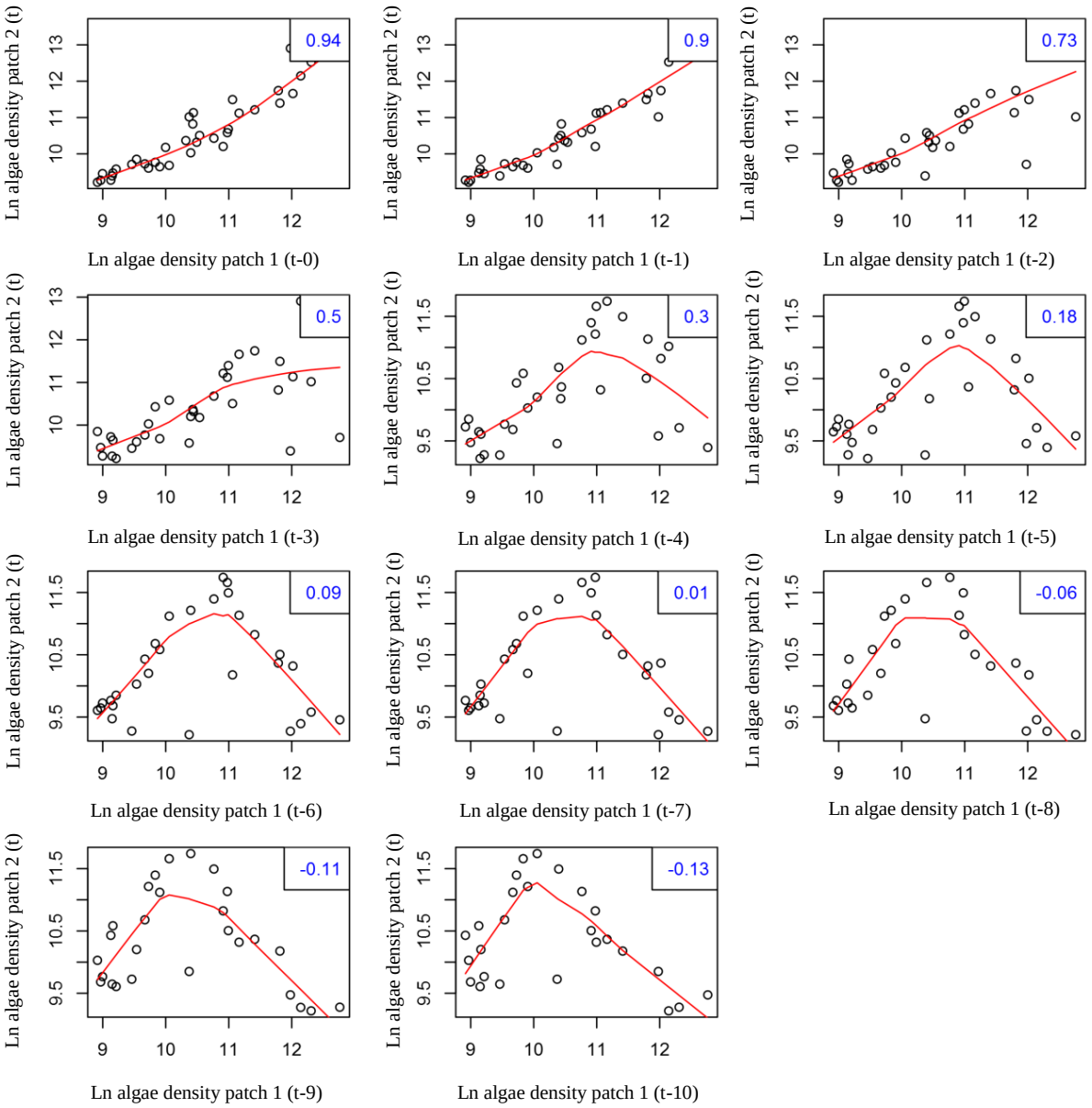

Figure 13 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae* replicate 3. In each plot algae density patch 2 is on the vertical and a past lag of algae density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

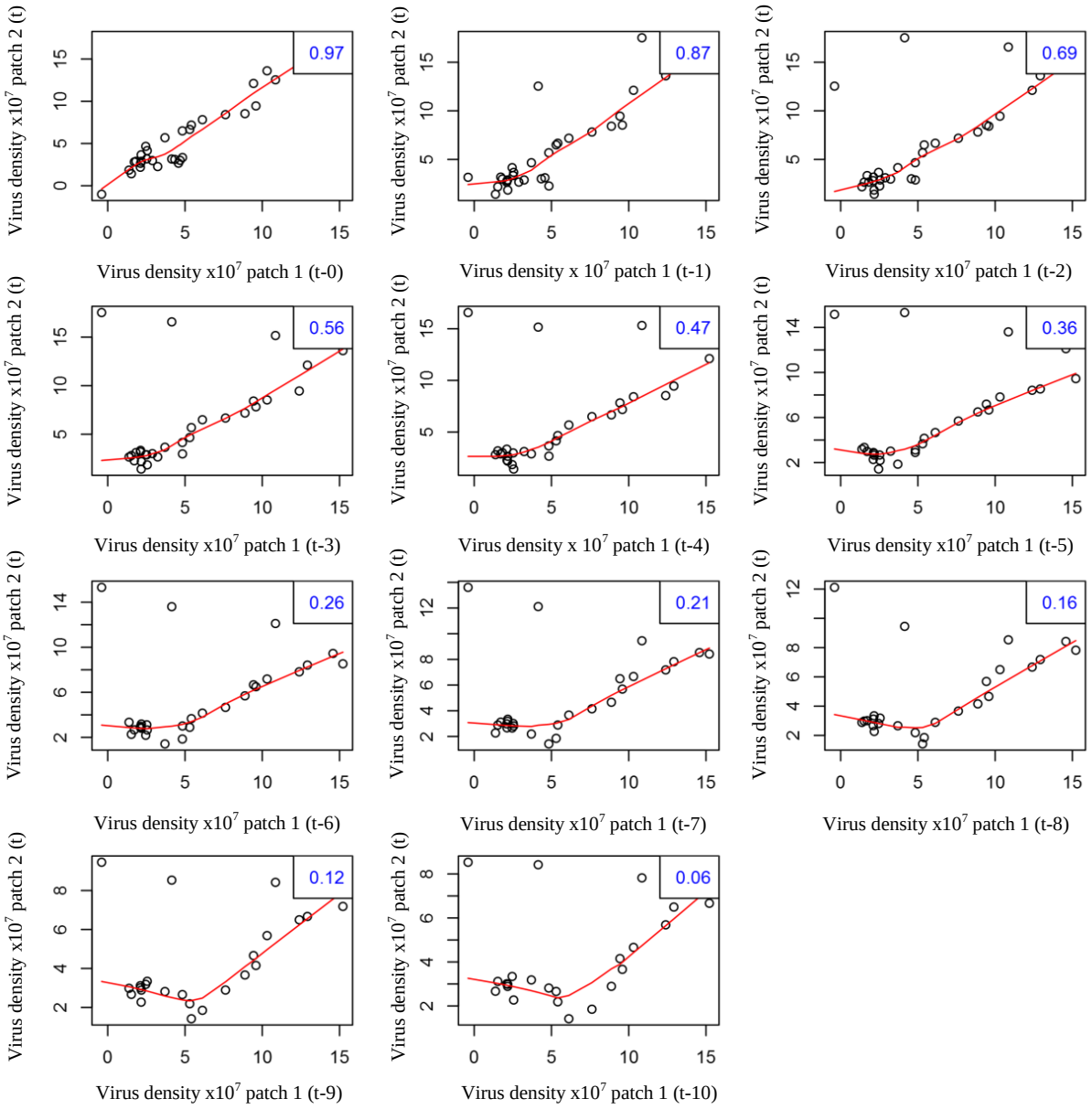

Figure 14 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae* replicate 3. In each plot virus density patch 2 is on the vertical and a past lag of virus density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

There were several positive correlations between patch 1 and patch 2 shown in algae density (Fig. 15 A, ANOVA: $F_{1,106} = 14.07$, $p < 0.001$, adj. $R^2 = 0.11$), virus density (Fig. 15 B, ANOVA: $F_{1,100} = 6.97$, $p < 0.001$, adj. $R^2 = 0.056$) as well as in MOI (Fig. 15 C, ANOVA: $F_{1,100} = 12.48$, $p < 0.001$, adj. $R^2 = 0.1$). The interaction between the algae and virus populations in patch 1 are showing once again a typical cycle of consumer-resource dynamics (Fig. 15 D).

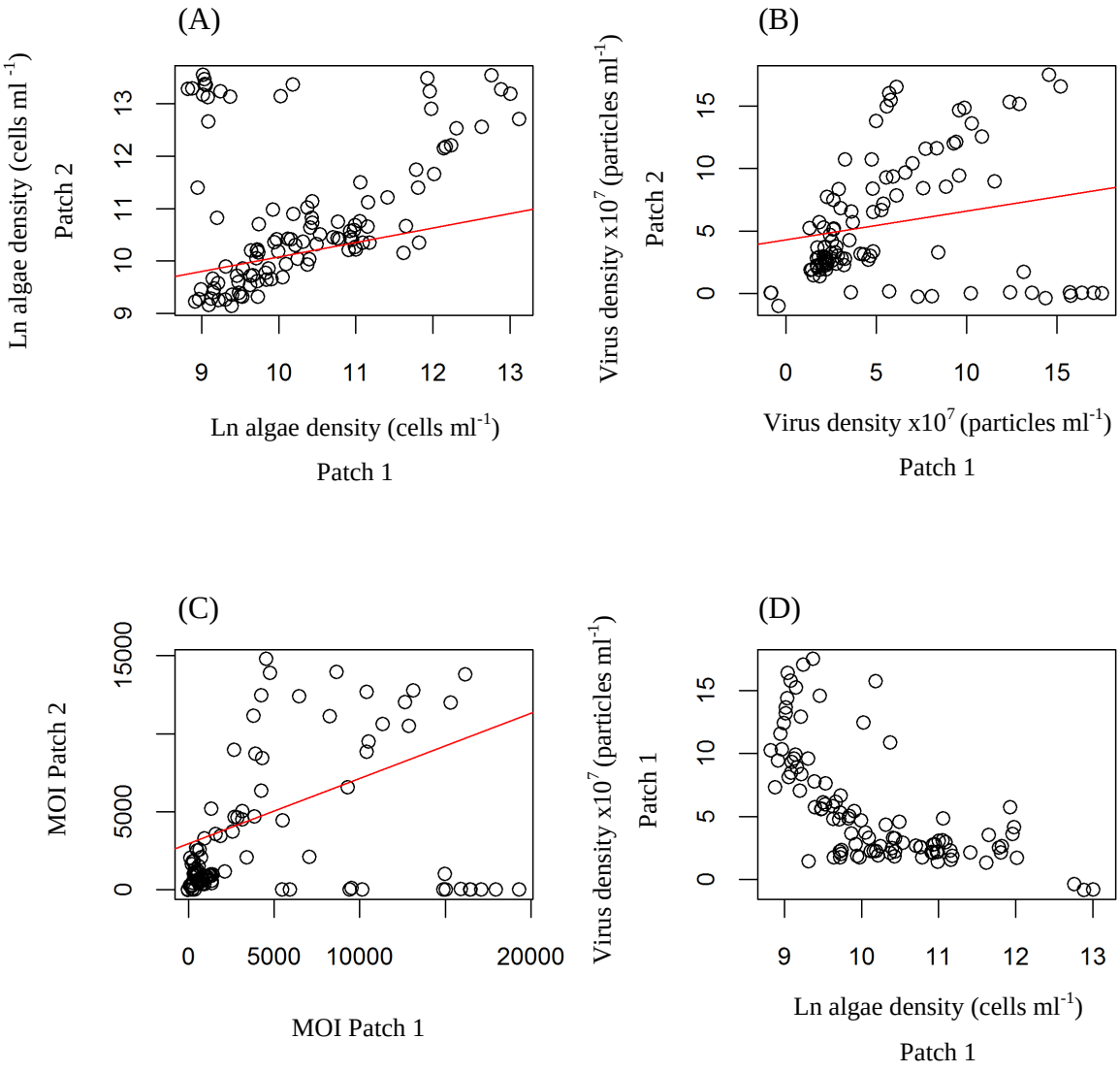

Figure 15 Correlations of patch 1 against patch 2 of algae density (A), virus density (B) and MOI (C) for treatment *Algae + Virus – Algae*. Additionally, a correlation for the virus density against the algae density in patch 1 is shown (D). The red line indicates a significant correlation. Algae density patch 1 x patch 2 (ANOVA: $F_{1,106} = 14.07$, $p < 0.001$, adj. $R^2 = 0.11$), virus density patch 1 x patch 2 (ANOVA: $F_{1,100} = 6.97$, $p < 0.001$, adj. $R^2 = 0.056$), MOI patch 1 x patch 2 (ANOVA: $F_{1,100} = 12.48$, $p < 0.001$, adj. $R^2 = 0.1$), algae patch 1 x virus patch 1 followed typical consumer-resource cycle.

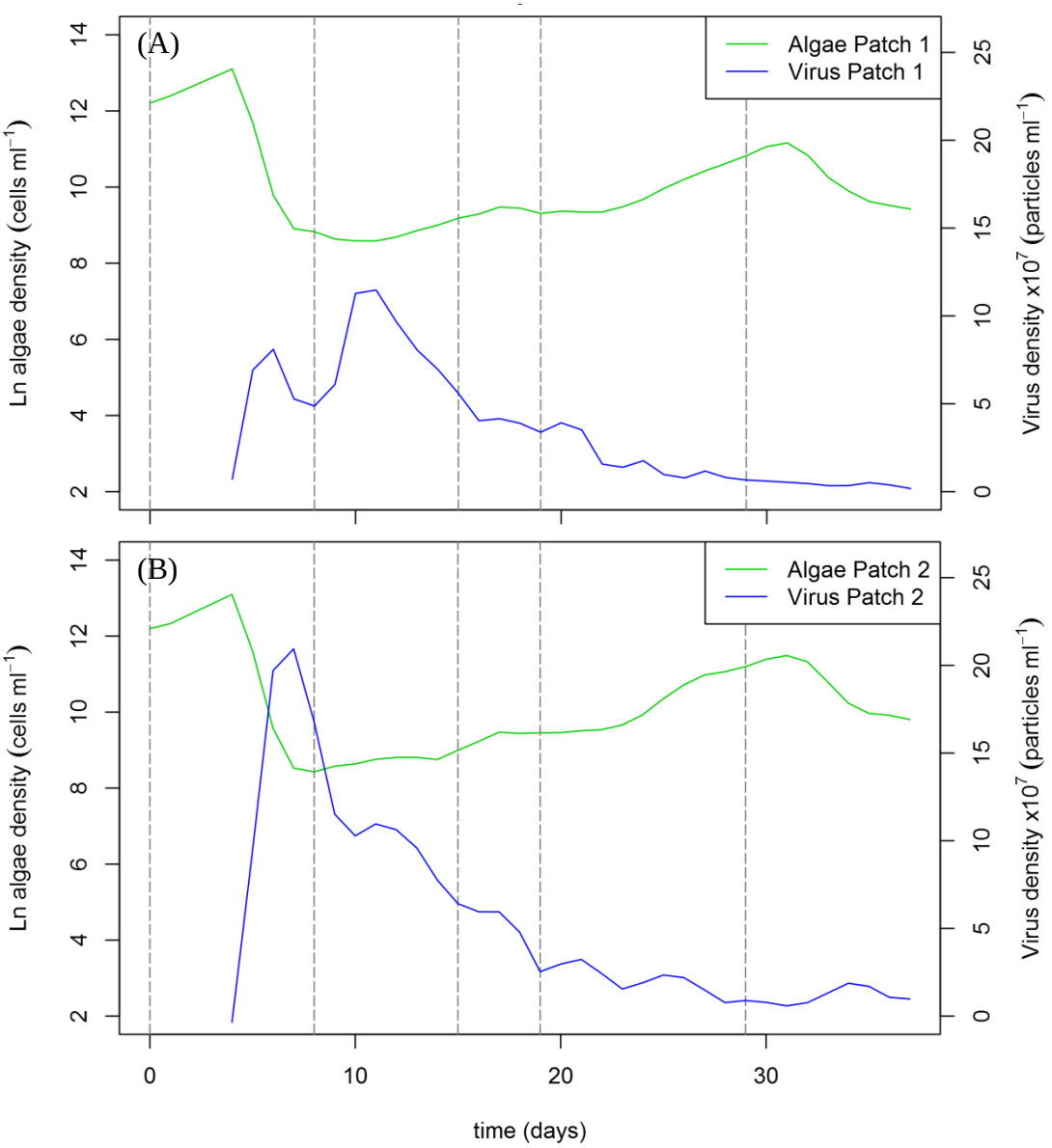

Figure 16 Population dynamics of *Algae + Virus – Algae + Virus* replicate 1 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments.

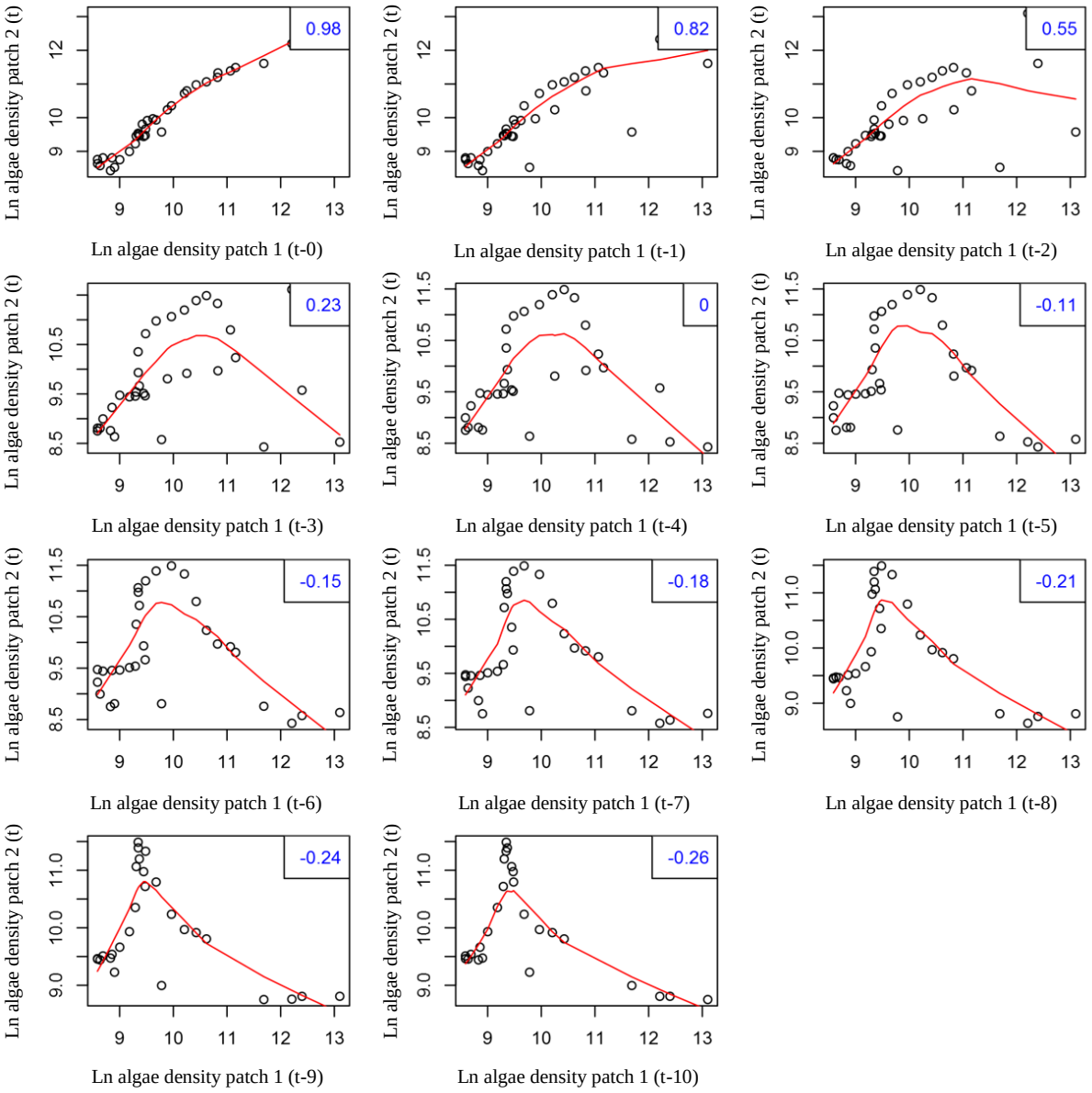

Figure 17 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 1. In each plot, algae density patch 2 is on the vertical and a past lag of algae density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

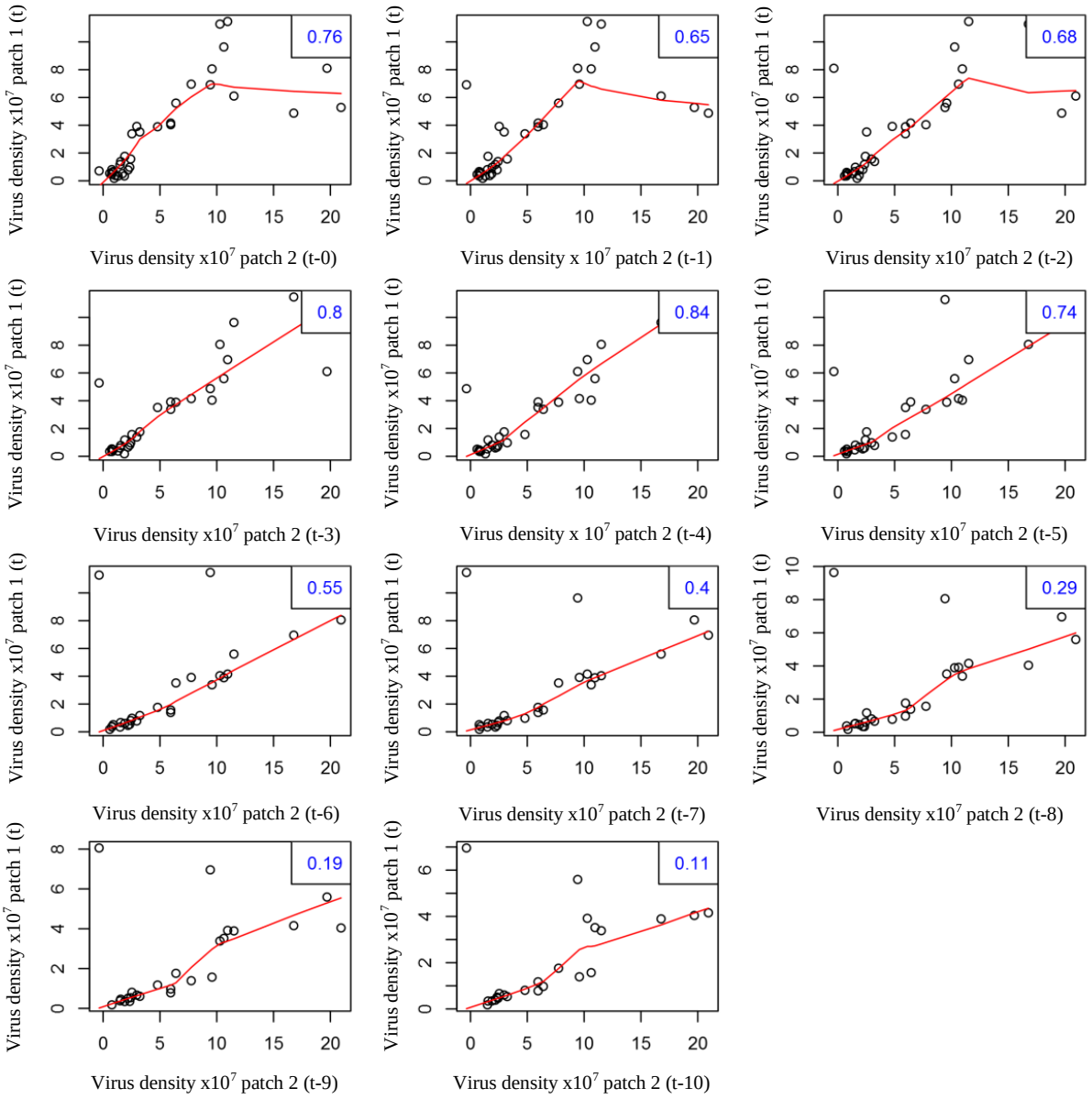

Figure 18 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 1. In each plot, virus density patch 1 is on the vertical and a past lag of virus density patch 2 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

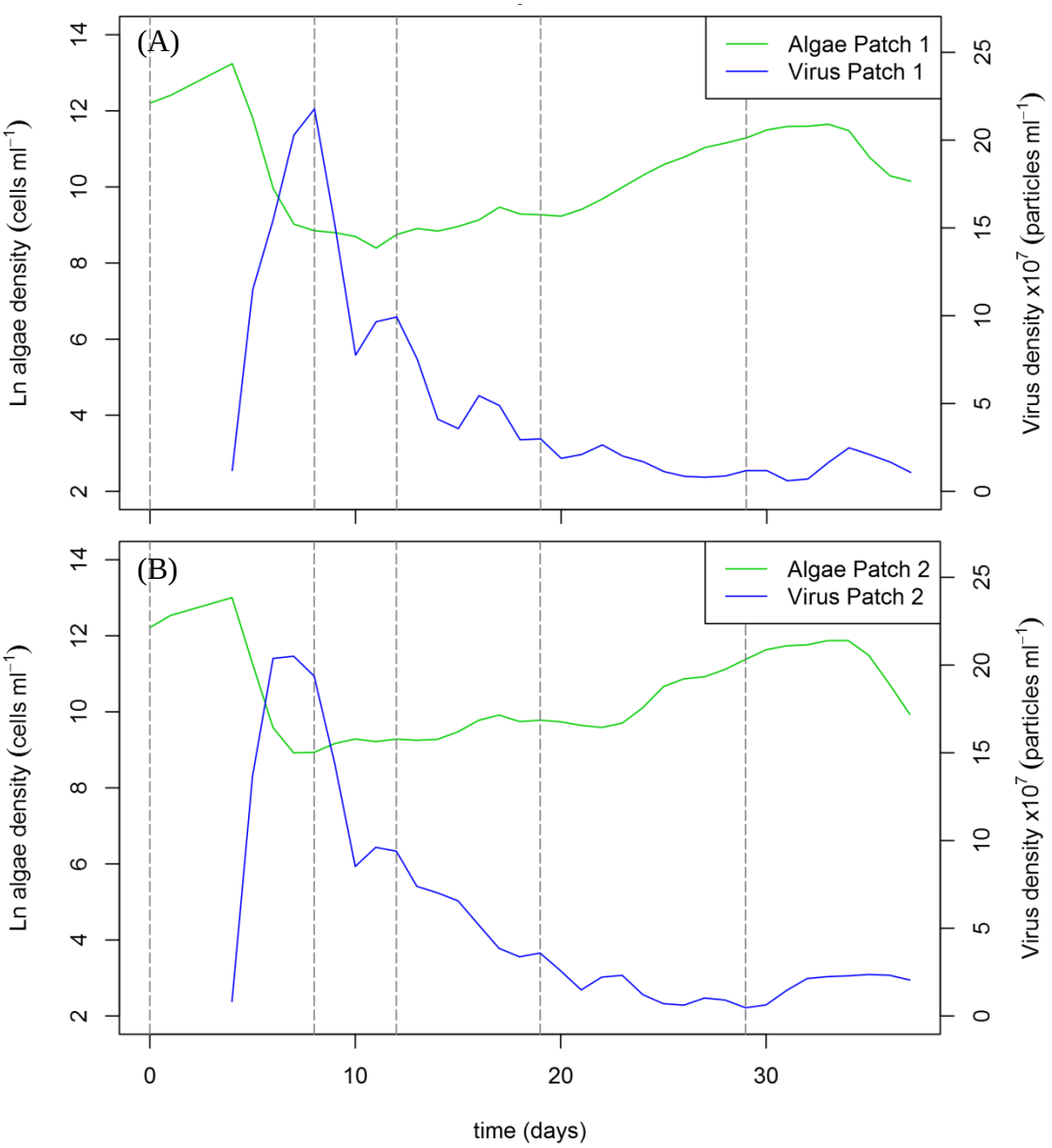

Figure 19 Population dynamics of *Algae + Virus – Algae + Virus* replicate 2 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments.

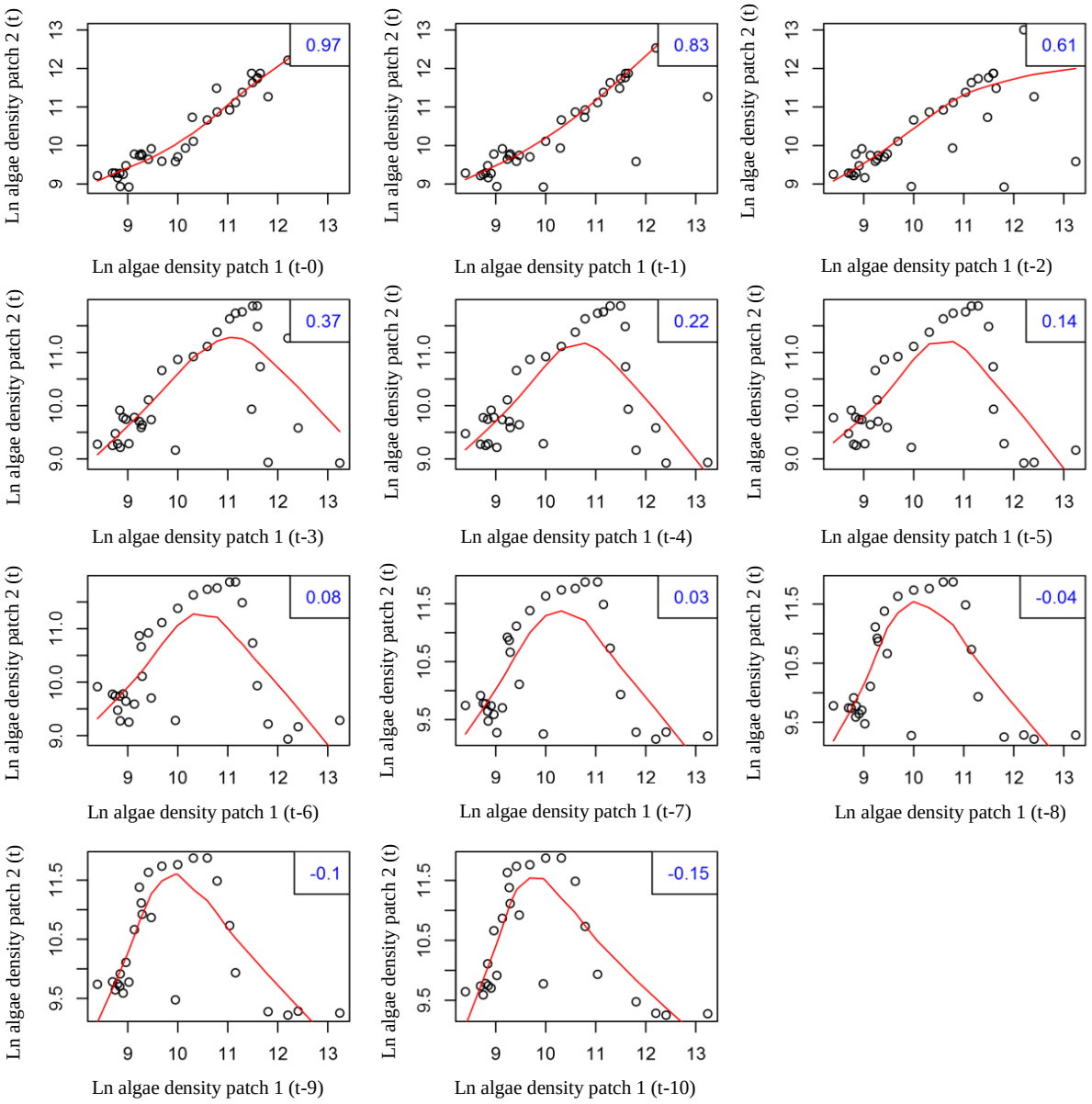

Figure 20 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 2. In each plot, algae density patch 2 is on the vertical and a past lag of algae density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

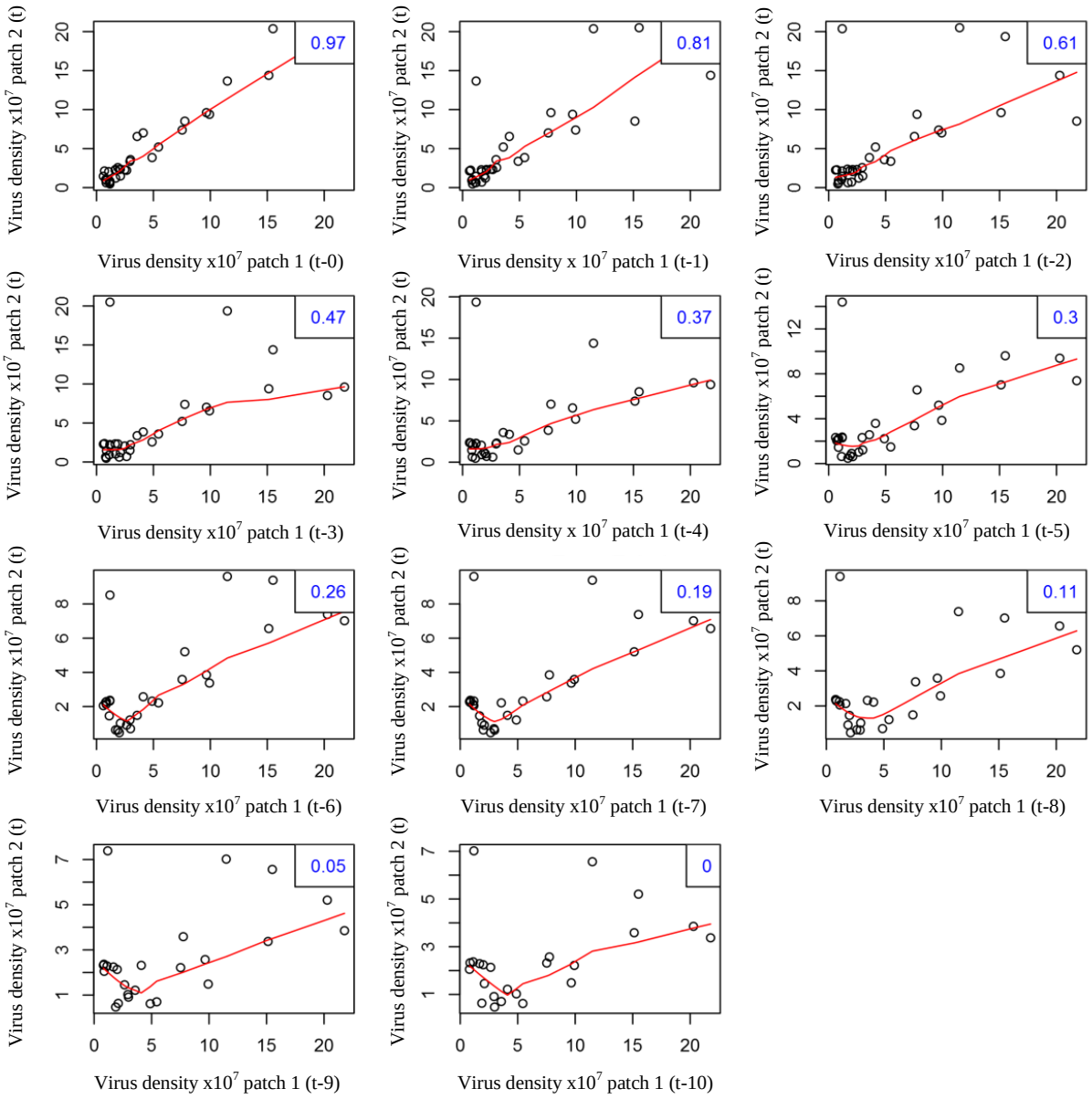

Figure 21 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 2. In each plot, virus density patch 2 is on the vertical and a past lag of virus density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

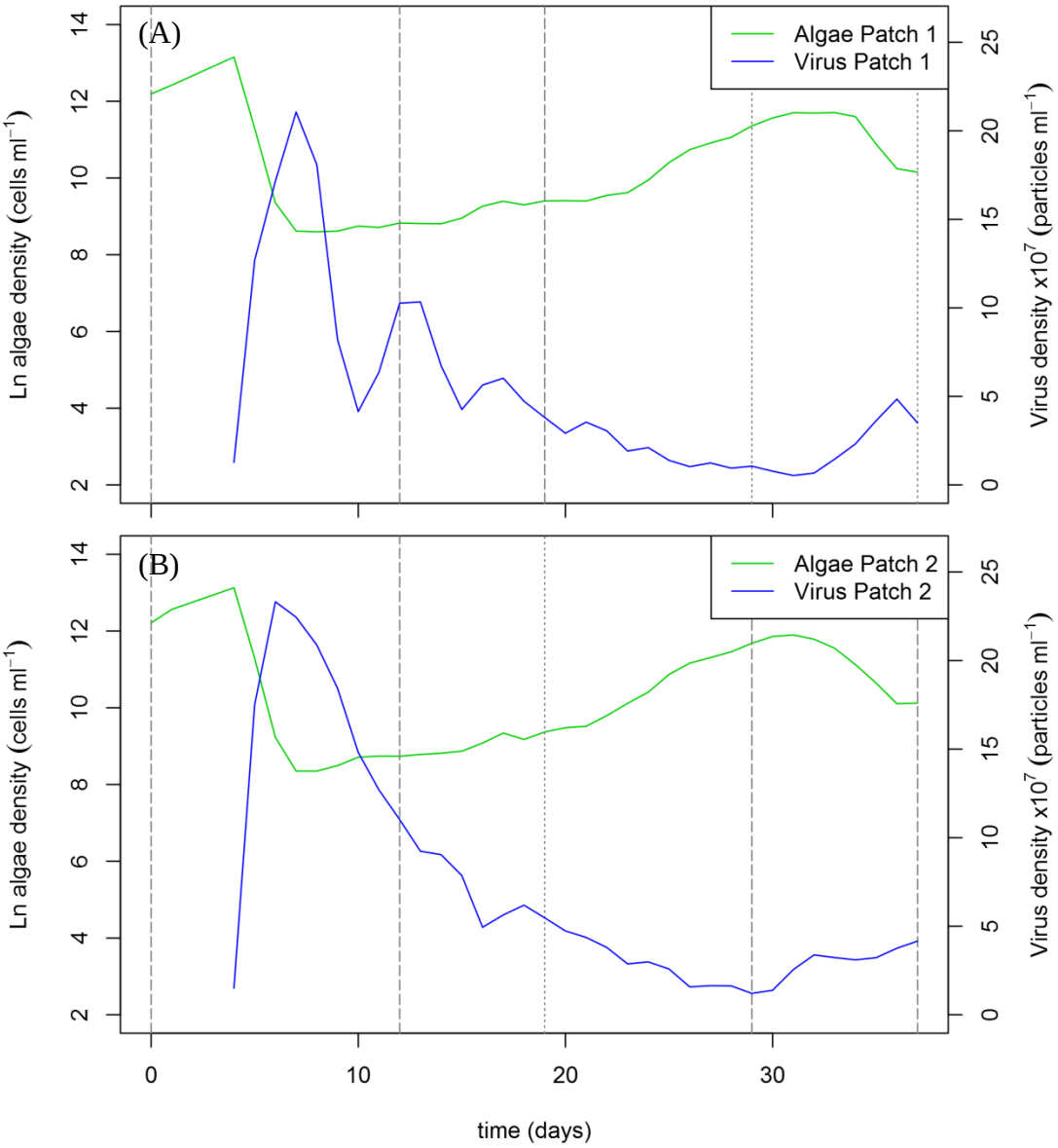

Figure 22 Population dynamics of *Algae + Virus – Algae + Virus* replicate 3 of patch 1 (A) and patch 2 (B). Green line: algal densities; blue line: virus densities. Grey dashed lines indicate days of time-shift experiments. Grey dotted lines indicate missing algae populations for time-shift experiments due to technical errors.

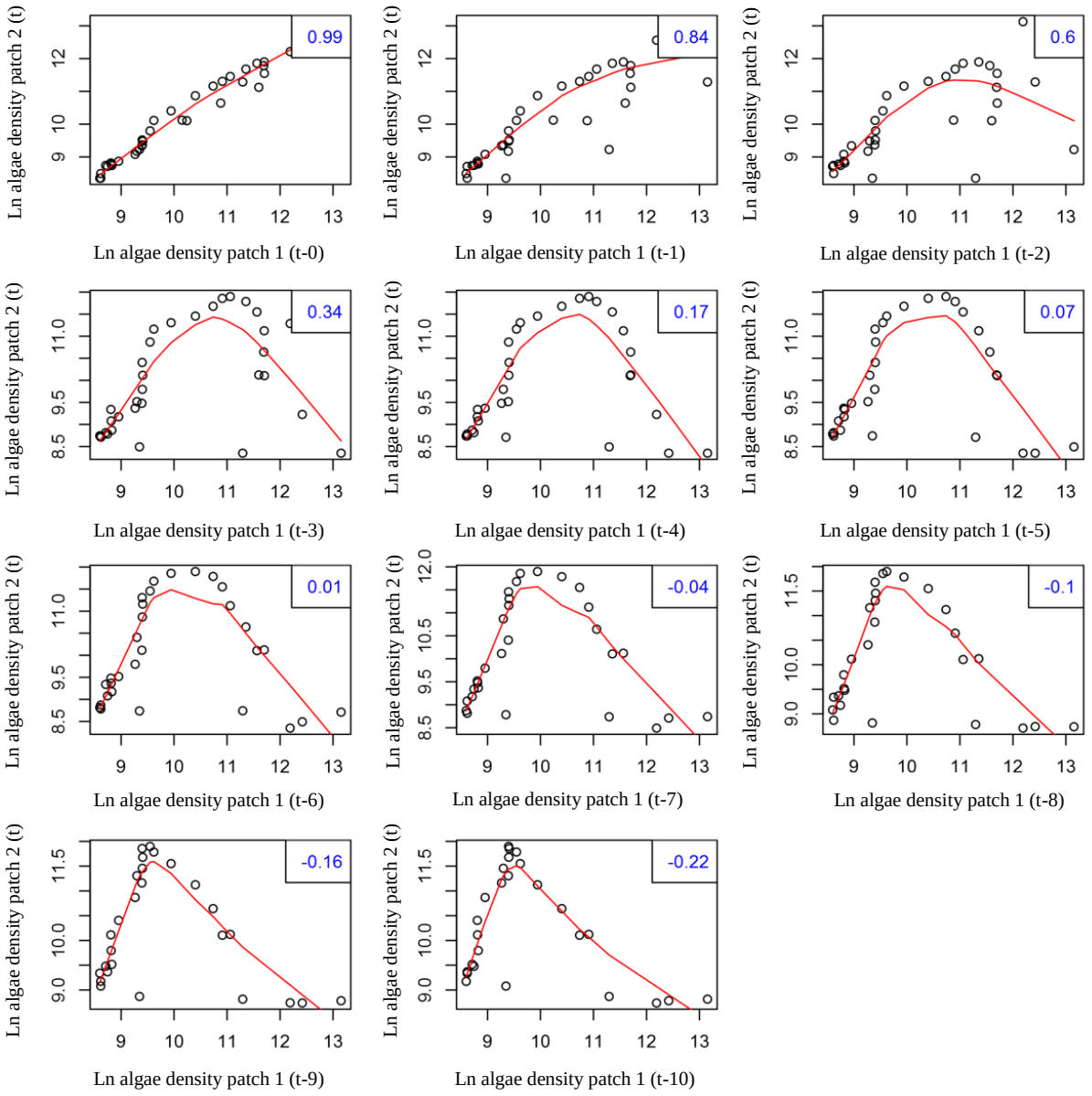

Figure 23 Correlations of algae population densities of patch 1 and algae population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 3. In each plot, algae density patch 2 is on the vertical and a past lag of algae density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

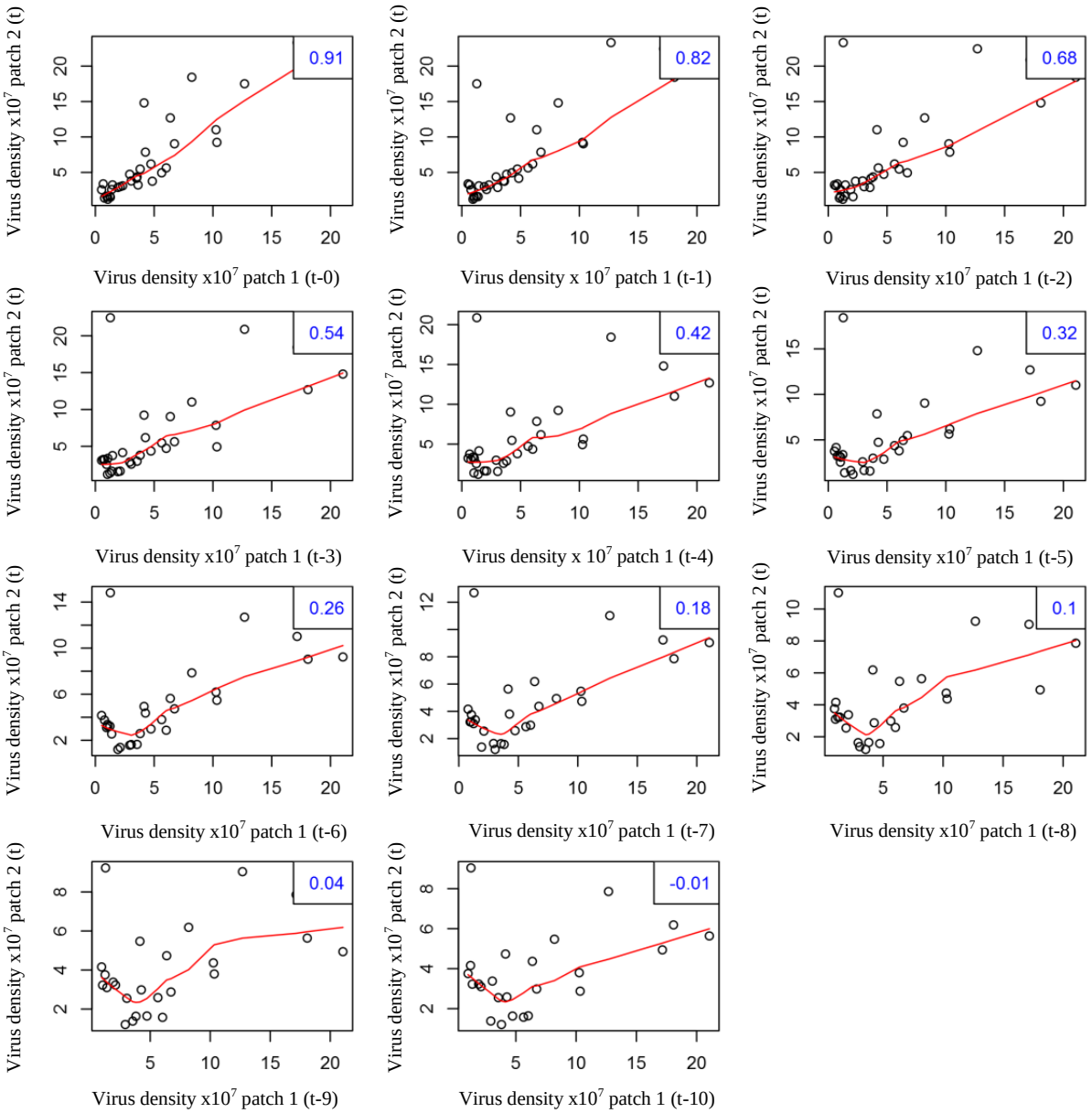

Figure 24 Correlations of virus population densities of patch 1 and virus population densities of patch 2 with different time lags for treatment *Algae + Virus – Algae + Virus* replicate 3. In each plot, virus density patch 2 is on the vertical and a past lag of virus density patch 1 is on the horizontal. Correlation lines (red) and values (blue) are given on each plot.

There was a positive correlation between patch 1 and patch 2 regarding the algae density (Fig. 25 A, ANOVA: $F_{1,106} = 1945$, $p < 0.001$, adj. $R^2 = 0.95$), the virus density (Fig. 25 B, ANOVA: $F_{1,100} = 349.3$, $p < 0.001$, adj. $R^2 = 0.78$) and also the MOI (Fig. 25 C, ANOVA: $F_{1,100} = 208.2$, $p < 0.001$, adj. $R^2 = 0.67$). The interaction between the algae and virus populations in patch 1 can be described again as a typical cycle of consumer-resource dynamics (Fig. 25 D).

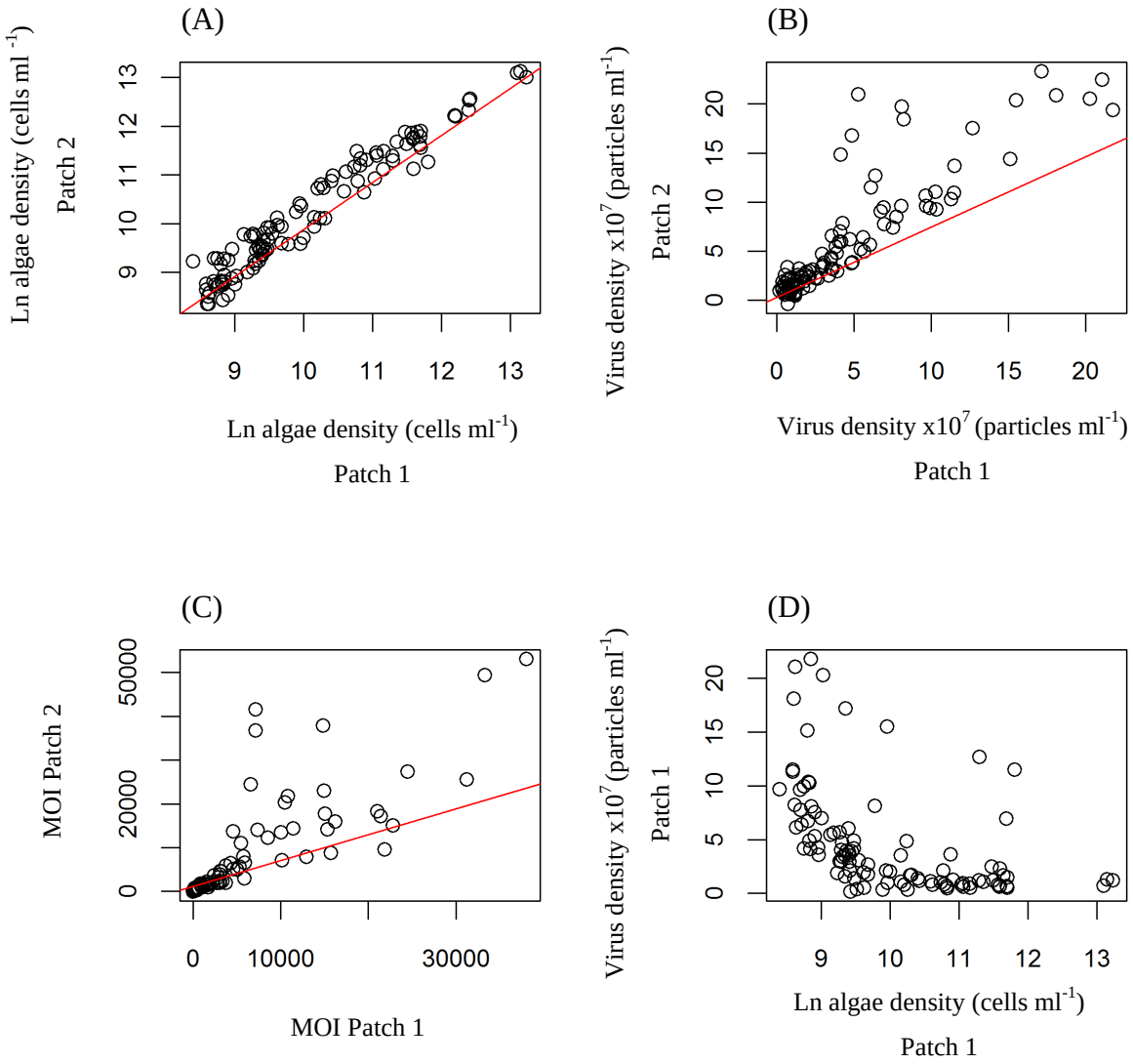

Figure 25 Correlations of patch 1 against patch 2 of algae density (A), virus density (B) and MOI (C) for treatment *Algae + Virus – Algae + Virus*. Additionally, a correlation for the virus density against the algae density in patch 1 is shown (D). The red line indicates a significant correlation. Algae density patch 1 x patch 2 (ANOVA: $F_{1,106} = 1945$, $p < 0.001$, adj. $R^2 = 0.95$), virus density patch 1 x patch 2 (ANOVA: $F_{1,100} = 349.3$, $p < 0.001$, adj. $R^2 = 0.78$), MOI patch 1 x patch 2 (ANOVA: $F_{1,100} = 208.2$, $p < 0.001$, adj. $R^2 = 0.67$), algae patch 1 x virus patch 1 followed typical consumer-resource cycle.

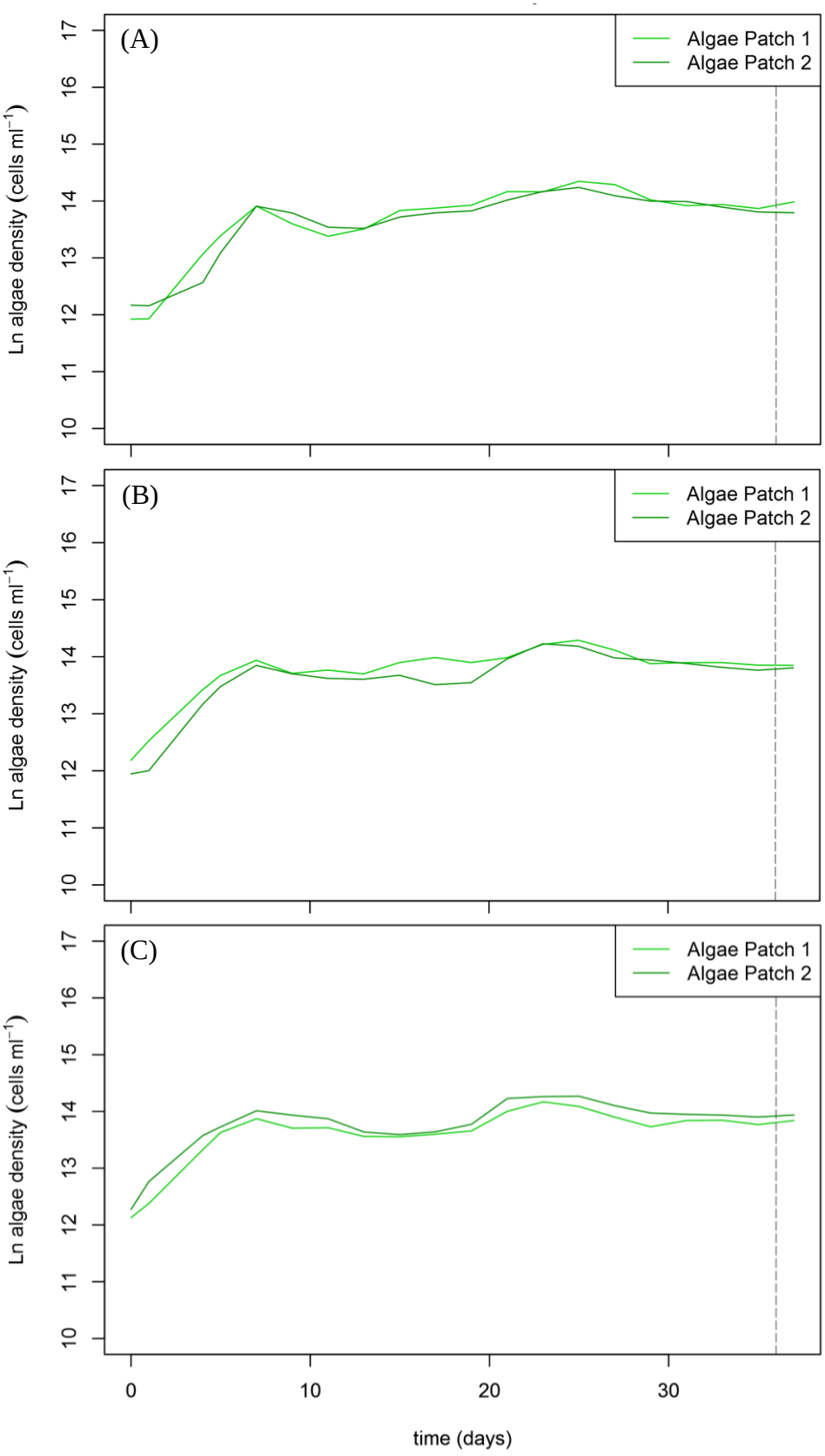

Figure 26 Population dynamics of the control 1 *Algae – Algae* replicate 1-3 (A, B, C). Lightgreen line: algal densities in patch 1; darkgreen line: algal densities in patch 2. Grey dashed lines indicate days of time-shift experiments. Latest time-point was tested against virus used for inoculation (day 4) of the other treatments.

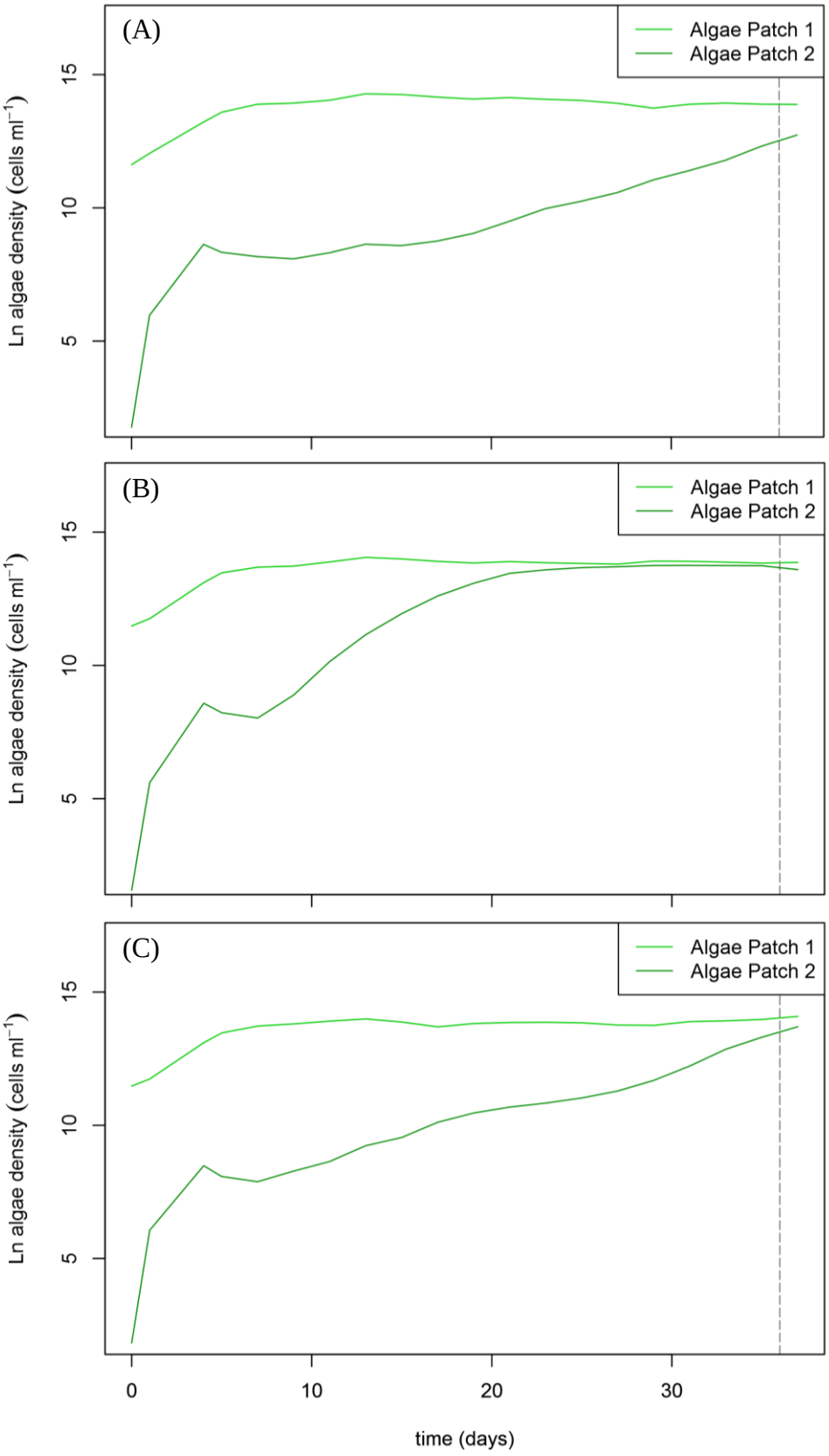

Figure 27 Population dynamics of the control 2 *Algae – 0* replicate 1-3 (A, B, C). Lightgreen line: algal densities in patch 1; darkgreen line: algal densities in patch 2. Grey dashed lines indicate days of time-shift experiments. Latest time-point was tested against virus used for inoculation (day 4) of the other treatments.

3.2 Differences between patches and treatments

The population dynamics over all time-points were compared concerning different response variables between the two patches as well as between the treatments taking patch 1 and patch 2 together, using two-way ANOVA followed by Tukey post hoc test. Based on the Tukey contrasts, it was also possible to observe a potential interaction between patch and treatment.

The algae population density was significantly higher in patch 1 than patch 2 (ANOVA: $F_{1,642} = 45.36$, $p < 0.001$, Fig. 28 A). In addition, the algae population density was considerably lower in treatment A + V – 0 compared to treatment A + V – A and A + V – A + V (ANOVA: $F_{2,642} = 111.27$, $p < 0.001$, Tukey post hoc test: A + V – 0 and A + V – A + V : $p < 0.001$, A + V – 0 and A + V – A : $p < 0.001$). Besides that, it was significantly higher in the treatment A + V – A than in A + V – A + V (Tukey post hoc test: $p = 0.004$). We further observed a significant interaction between treatment and patch, with a lower algae population density in patch 2 of treatment A + V – 0 compared to all the other patches of each treatment (ANOVA: $F_{2,642} = 100.86$, $p < 0.001$, Tukey post hoc test results shown in Tab. 2). Patch 2 of treatment A + V – A showed a significantly higher algae population density compared to all the other patches of each treatment.

Table 2 Results of the Tukey post hoc test for the interaction effects of treatment (both patches together) and patch on algae population density comparing all time-points (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – A patch 1 x A + V – A patch 2	0.03
A + V – A patch 1 x A + V – 0 patch 2	< 0.001
A + V – A patch 2 x A + V – 0 patch 1	0.002
A + V – A patch 2 x A + V – 0 patch 2	< 0.001
A + V – A patch 2 x A + V – A + V patch 1	< 0.001
A + V – A patch 2 x A + V – A + V patch 2	0.008
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – A + V patch 1 x A + V – 0 patch 2	< 0.001
A + V – A + V patch 2 x A + V – 0 patch 2	< 0.001

Also the virus population density was significantly higher in patch 1 compared to patch 2 (ANOVA: $F_{1,606} = 9.325$, $p = 0.002$, Fig. 28 B). In fact, there was a lower virus population density in treatment A + V – 0 compared to treatment A + V – A and A + V – A + V (ANOVA: $F_{2,606} = 25.4$, $p < 0.001$, Tukey post hoc test: A + V – 0 and A + V – A + V : $p < 0.001$, A + V – 0 and A + V – A : $p < 0.001$). Furthermore, a combined patch and treatment effect, with a lower virus population density in patch 2 of treatment A + V – 0 compared to all the other patches of each treatment was present (ANOVA: $F_{2,606} = 23.59$, $p < 0.001$, Tukey post hoc test results shown in Tab. 3). We could not observe a difference in the virus population density between the treatments A + V – A and A + V – A + V (Tukey post hoc test: $p = 0.85$).

Table 3 Results of the Tukey post hoc test for the interaction effects of treatment and patch on virus population density comparing all time-points (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	< 0.001
A + V – 0 patch 2 x A + V – A patch 2	< 0.001
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

Interestingly, daily algal population growth rate was significantly higher in patch 2 than in patch 1 (ANOVA: $F_{1,624} = 5.24$, $p = 0.022$, Fig. 28 C). The experiment showed, that the growth rate was indeed significantly higher in treatment A + V – 0 in contrast to treatment A + V – A and A + V – A + V (ANOVA: $F_{2,624} = 5.38$, $p = 0.005$, Tukey post hoc test: A + V – 0 and A + V – A + V : $p = 0.01$, A + V – 0 and A + V – A : $p = 0.01$). Patch 2 of treatment A + V – 0 showed an overall higher growth rate in compared to all the other patches of each treatment (ANOVA: $F_{2,624} = 4.82$, $p = 0.008$, Tukey post hoc test results shown in Tab. 4). However, no difference was observable in the growth rate between the treatments A + V – A and A + V – A + V (Tukey post hoc test: $p = 1.0$).

Table 4 Results of the Tukey post hoc test for the interaction effects of treatment and patch on calculated algae growth rate per day comparing all time-points (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	0.001
A + V – 0 patch 2 x A + V – A patch 2	0.002
A + V – 0 patch 2 x A + V – 0 patch 1	0.002
A + V – 0 patch 2 x A + V – A + V patch 1	0.001
A + V – 0 patch 2 x A + V – A + V patch 2	0.001

In general, MOI, as a proxy for force of infection, was significantly higher in patch 1 than in patch 2 (ANOVA: $F_{1,606} = 8.6$, $p = 0.003$, Fig. 28 D). The MOI was significantly higher between the treatments A + V – A + V contrasted with treatment A + V – 0 and A + V – A (ANOVA: $F_{2,606} = 7.95$, $p < 0.001$, Tukey post hoc test: A + V – A + V and A + V – 0 : $p < 0.001$, A + V – A + V and A + V – A : $p = 0.01$). Moreover, or study revealed a significant interaction between treatment and patch with a lower MOI in patch 2 of treatment A + V – 0 compared to all the other patches of each treatment (ANOVA: $F_{2,606} = 12.72$, $p < 0.001$, Tukey post hoc test results shown in Tab. 5). Additionally, patch 2 of treatment A + V – A showed a significantly lower MOI than in patch 2 of treatment A + V –A + V (Tukey post hoc test: $p = 0.01$).

Table 5 Results of the Tukey post hoc test for the interaction effects of treatment and patch on MOI comparing all time-points. MOI, as a proxy for force of infection, was calculated as virus particles per algae cells (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	0.001
A + V – 0 patch 2 x A + V – A patch 2	0.046
A + V – A + V patch 2 x A + V – A patch 2	0.012
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

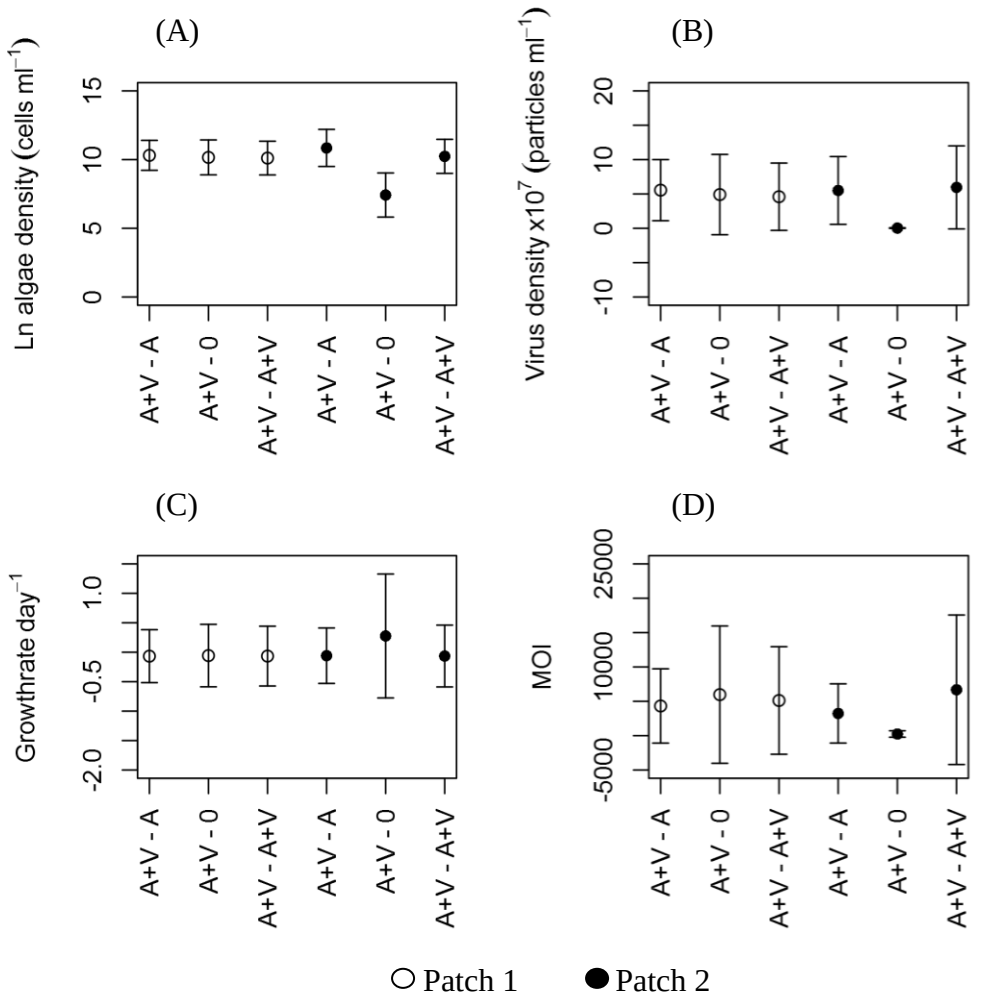

Figure 28 Means (±SD) of algae density (A), virus density (B), algal growth rate per day (C) and MOI (D) per patch and treatment. The means (±SD) were calculated as the mean values of the three replicates of the treatments per patch.

Overall, the maximum algae density was significantly lower in patch 2 than in patch 1 (ANOVA: $F_{1,12} = 13.94$, $p = 0.003$, Fig. 29 A). In particular, it was considerably lower in treatment A + V – 0 compared to treatment A + V – A and A + V – A + V, but not different between the treatments A + V – A and A + V – A + V (ANOVA: $F_{2,12} = 21.51$, $p < 0.001$, Tukey post hoc test: A + V – 0 and A + V – A + V : $p < 0.001$; A + V – 0 and A + V – A : $p < 0.001$; A + V – A and A + V – A + V: $p = 0.89$). A significant interaction between treatment and patch could be detected, shown in a lower maximum algae density in patch 2 of treatment A + V – 0 compared to all the other patches of each treatment (ANOVA: $F_{2,12} = 23.59$, $p < 0.001$, Tukey post hoc test results shown in Tab. 6).

Table 6 Results of the Tukey post hoc test for the interaction effects of treatment and patch on overall maximum algae density (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	< 0.001
A + V – 0 patch 2 x A + V – A patch 2	< 0.001
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

Besides that, maximum virus density was significantly lower in patch 2 than in patch 1 (ANOVA: $F_{1,12} = 20.91$, $p < 0.001$, Fig. 29 B). Moreover, maximum virus density was vastly lower in treatment A + V – 0 compared to treatment A + V – A and A + V – A + V, even though it was not different between the treatments A + V – A and A + V – A + V (ANOVA: $F_{2,12} = 12.98$, $p < 0.001$, Tukey post hoc test: A + V – 0 and A + V – A + V : $p < 0.001$, A + V – 0 and A + V – A : $p = 0.03$, A + V – A and A + V – A + V: $p = 0.15$). Furthermore, there was a significant combined effect of treatment and patch, with a lower maximum virus density in patch 2 of treatment A + V – 0 compared to all the other patches of each treatment (ANOVA: $F_{2,12} = 33.47$, $p < 0.001$, Tukey post hoc test results shown in Tab. 7).

Table 7 Results of the Tukey post hoc test for the interaction effects of treatment and patch on overall maximum virus density (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	< 0.001
A + V – 0 patch 2 x A + V – A patch 2	< 0.001
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

Maximum algae growth rate per day was significantly higher in patch 2 than in patch 1 (ANOVA: $F_{1,12}$ = 13.71, p = 0.003, Fig. 29 C). Interestingly, maximum growth rate, comparing at treatment level, was higher in treatment A + V – 0 than in treatment A + V – A and as well as in A + V – A + V but not different between the treatments A + V – A and A + V – A + V (ANOVA: $F_{2,12}$ = 10.33, p = 0.002, Tukey post hoc test: A + V – 0 and A + V – A + V : p = 0.003, A + V – 0 and A + V – A : p = 0.01, A + V – A and A + V – A + V: p = 0.74). Overall, Patch 2 of treatment A + V – 0 showed the greatest maximum growth rate compared to all the other patches of each treatment, whereas there was no difference among those patches (ANOVA: $F_{2,12}$ = 7.53, p = 0.008, Tukey post hoc test results shown in Tab. 8).

Table 8 Results of the Tukey post hoc test for the interaction effects of treatment and patch on overall maximum algae growth rate (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	0.003
A + V – 0 patch 2 x A + V – A patch 2	0.002
A + V – 0 patch 2 x A + V – 0 patch 1	0.003
A + V – 0 patch 2 x A + V – A + V patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

In general, maximum MOI was significantly higher in patch 1 than in patch 2 (ANOVA: $F_{1,12}$ = 11.03, p = 0.006, Fig. 29 D). Furthermore, maximum MOI was significantly lower in treatment A + V – 0 and A + V – A compared to treatment A + V – A + V, though not different between the treatments A + V – 0 and A + V – A (ANOVA: $F_{2,12}$ = 12.31, p = 0.001, Tukey post hoc test: A + V – 0 and A + V – A + V : p = 0.01 , A + V – A + V and A + V – A : p = 0.001, A + V – 0 and A + V – A: p = 0.36). We also observed a significant interaction between treatment and patch (ANOVA: $F_{2,12}$ = 20.41, p < 0.001), with a lower maximum MOI in patch 1 and 2 of treatment A + V – A compared to patch 1 of treatment A + V – 0 and to patch 2 of treatment A + V – A + V. Additionally, the maximum MOI in patch 2 of treatment A + V – 0 was lower than in patch 1 and also comparatively lower than in both patches of treatment A + V – A + V (Tukey post hoc test results shown in Tab. 9).

Table 9 Results of the Tukey post hoc test for the interaction effects of treatment and patch on overall maximum MOI (Fig. 2, 3, 4, 6, 7, 8, 12, 13, 14).

Groups compared	**p adj.**
A + V – 0 patch 2 x A + V – A patch 1	0.013
A + V – A + V patch 2 x A + V – A patch 1	0.017
A + V – 0 patch 1 x A + V – A patch 2	0.005
A + V – A + V patch 2 x A + V – A patch 2	0.006
A + V – 0 patch 2 x A + V – 0 patch 1	< 0.001
A + V – 0 patch 2 x A + V – A + V patch 1	0.003
A + V – 0 patch 2 x A + V – A + V patch 2	< 0.001

The algae population in patch 1 decreased significantly faster than in patch (ANOVA: $F_{1,10}$ = 5.654, p = 0.04, Fig. 29 E). However, there was no difference in the time of decrease of algae population between the treatments A + V – A and A + V – A + V taking both patches together (Tukey post hoc test: p = 0.13). Although, the time of evolutionary rescue was not different between the patches (ANOVA: $F_{1,10}$ = 1.76, p = 0.21, Fig. 29 F), the algae population of treatment A + V – A was recovering faster after the infection than in treatment A + V – A + V (ANOVA: $F_{2,10}$ = 9.55, p = 0.005, Tukey post hoc test: p = 0.004).

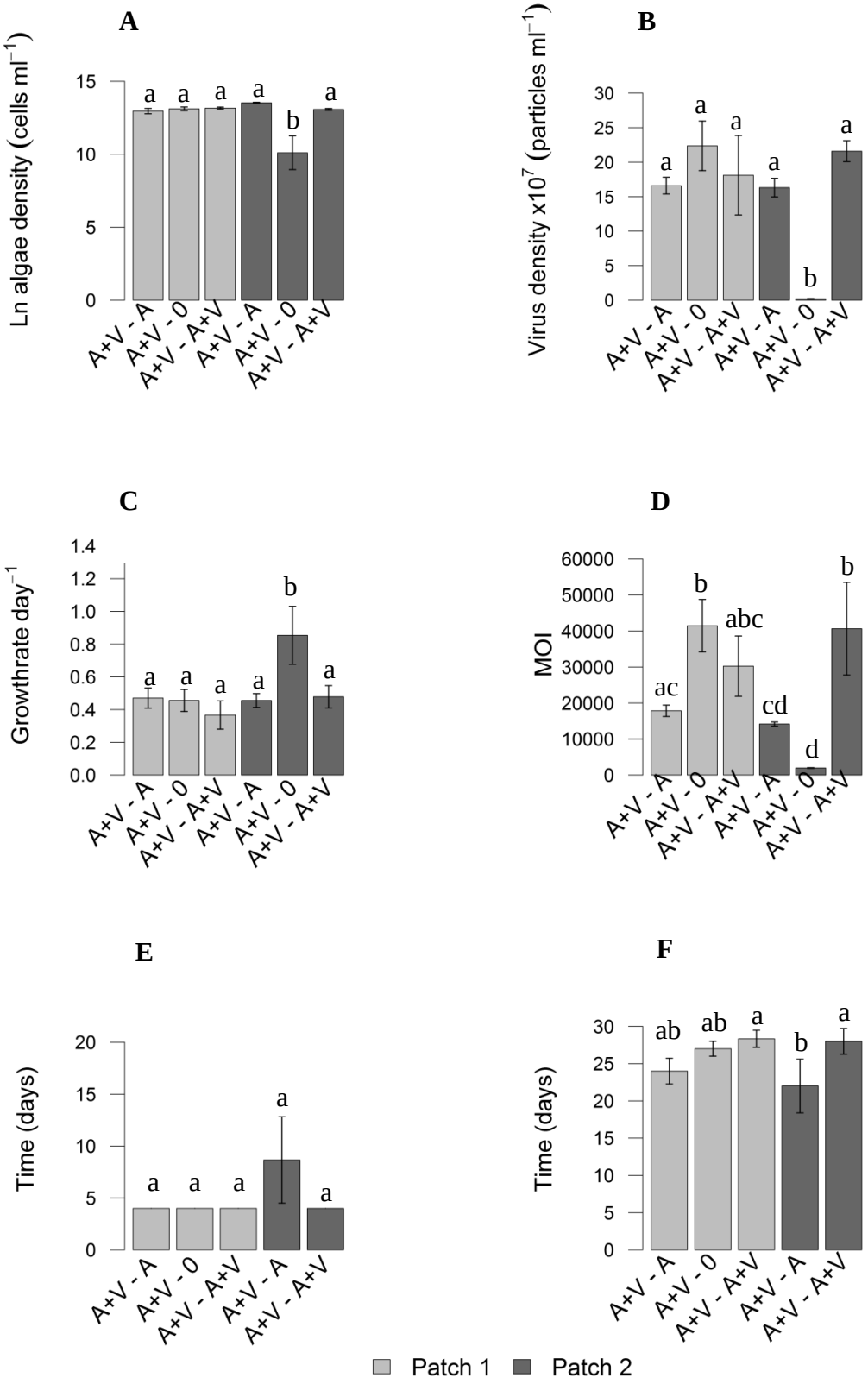

Figure 29 Means (+SD) of maximum algae density (A), maximum virus density (B), maximum algal growth rate per day (C), maximum MOI (D). For the time of decrease of algae population (E) and the time of evolutionary rescue (F) patch 2 of A + V – 0 was excluded, because no virus was present. The means (+SD) were calculated as the mean values of the three replicates of the treatments per patch.

We calculated the mean lag (between patch 1 and patch 2) of algae and the mean lag of virus per treatment (A + V – A and A + V – A + V) resulted from the significant lagplot time-points. There was no difference neither in the lag of algae populations (ANOVA: $F_{1,4} = 3.449$, $p = 0.137$, Fig. 30 A) nor in the lag of virus populations (ANOVA: $F_{1,4} = 3.449$, $p = 0.137$, Fig. 30 B) between the two treatments.

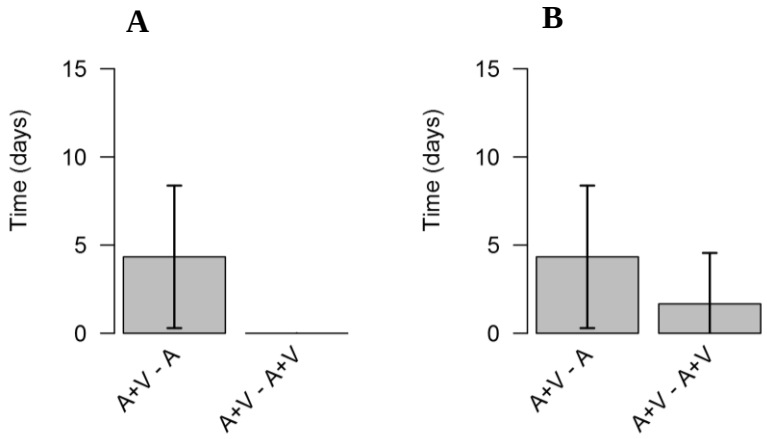

Figure 30 Mean time of lag algae (A) and mean time of lag virus (B) of the treatments *Algae + Virus – Algae* and *Algae + Virus – Algae + Virus* (+SD).

3.3 Time-shift experiment

Using time-shift experiments, we wanted to test whether and when resistance of algae to virus evolved, or vice versa whether and when the virus counter adapted to the host. The time-shift experiments revealed a resistance within approximately four days after infection. Additionally, the single clone-testing results were not different to the results of the whole population infection assays, following the same pattern (Fig. 34), although statistical tests were not performed. In particular, ancestor population clones got infected by the viruses from all time-points, whereas the algae population clones of later time-points were resistant to viruses from all time-points. However, some of the replicates of the control populations (day 37) showed resistance to the virus used for inoculation of the other treatments (Fig. 35). Furthermore, there were some inconsistencies which are further discussed in detail.

In patch 1 of treatment A + V – 0 the algae populations of day 0 were not resistant to any viruses of any time-point (Fig. 31). After day 8 (replicate 1) and day 12 (replicate 2) the algae populations were resistant to viruses from all time-points. The same holds for the algae population of day 8, replicate 3. In patch 2 the algae populations of day 29 showed resistance to all viruses of patch 1 independently from time-point.

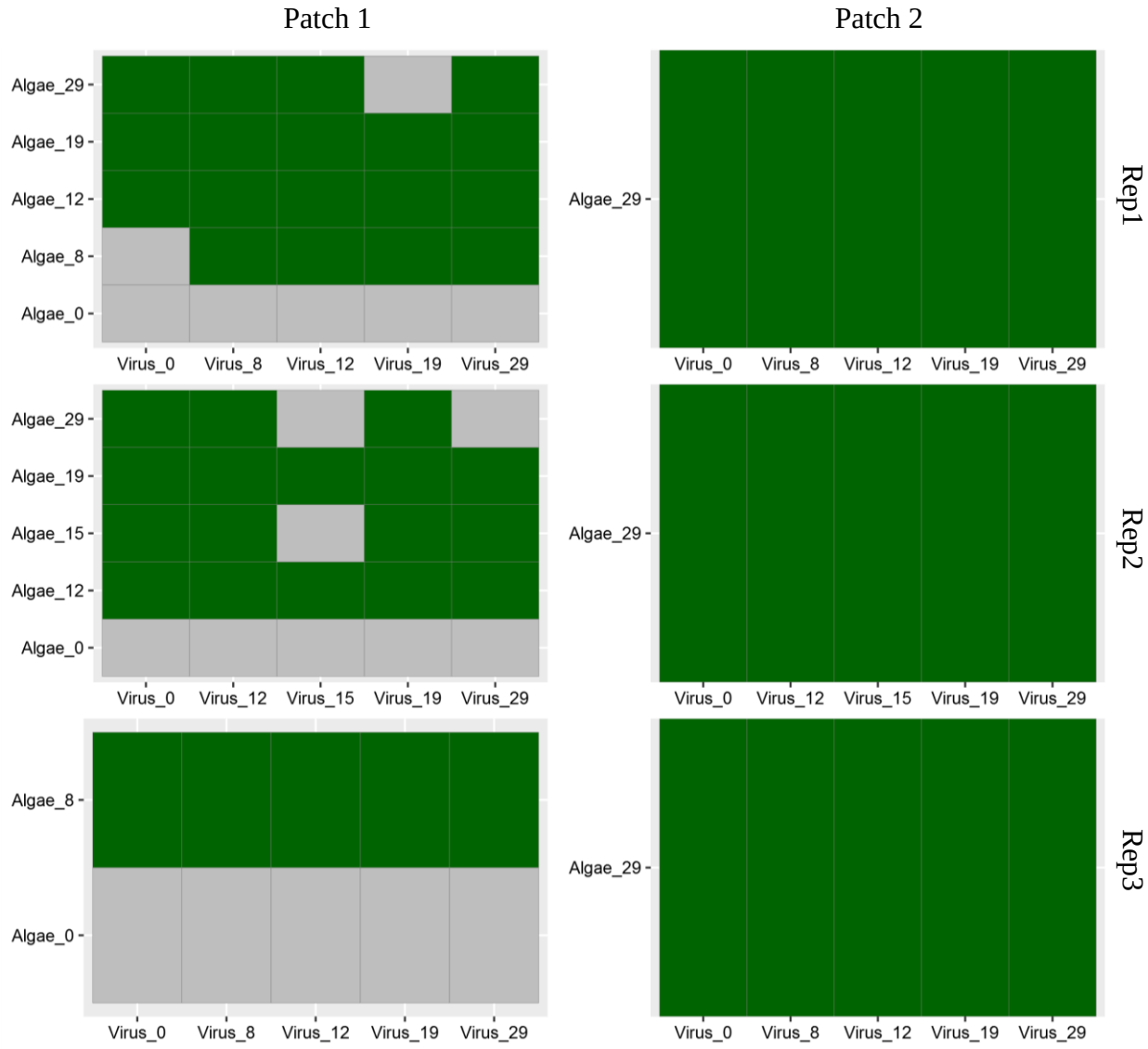

Figure 31 Infection matrix time-shift experiment for treatment *Algae + Virus – 0* (replicate 1-3). Resistance (green) and susceptibility (grey) of algae populations to virus populations over all time-points (days) per patch.

In both patches of treatment A + V – A the algae populations of day 0 were not resistant to any virus from any time-point (Fig. 32). After day 8 all of the algae populations in patch 1 showed resistance to virus of all time-points. In contrast the algae populations of day 8 in patch 2 (replicate 1, 2) were susceptible to all viruses of all time-points. Here, resistance to viruses from all time-points could be observed at day 19 (replicate 1) and day 15 (replicate 2). In patch 2 replicate 3 all of the algae populations showed resistance after day 8.

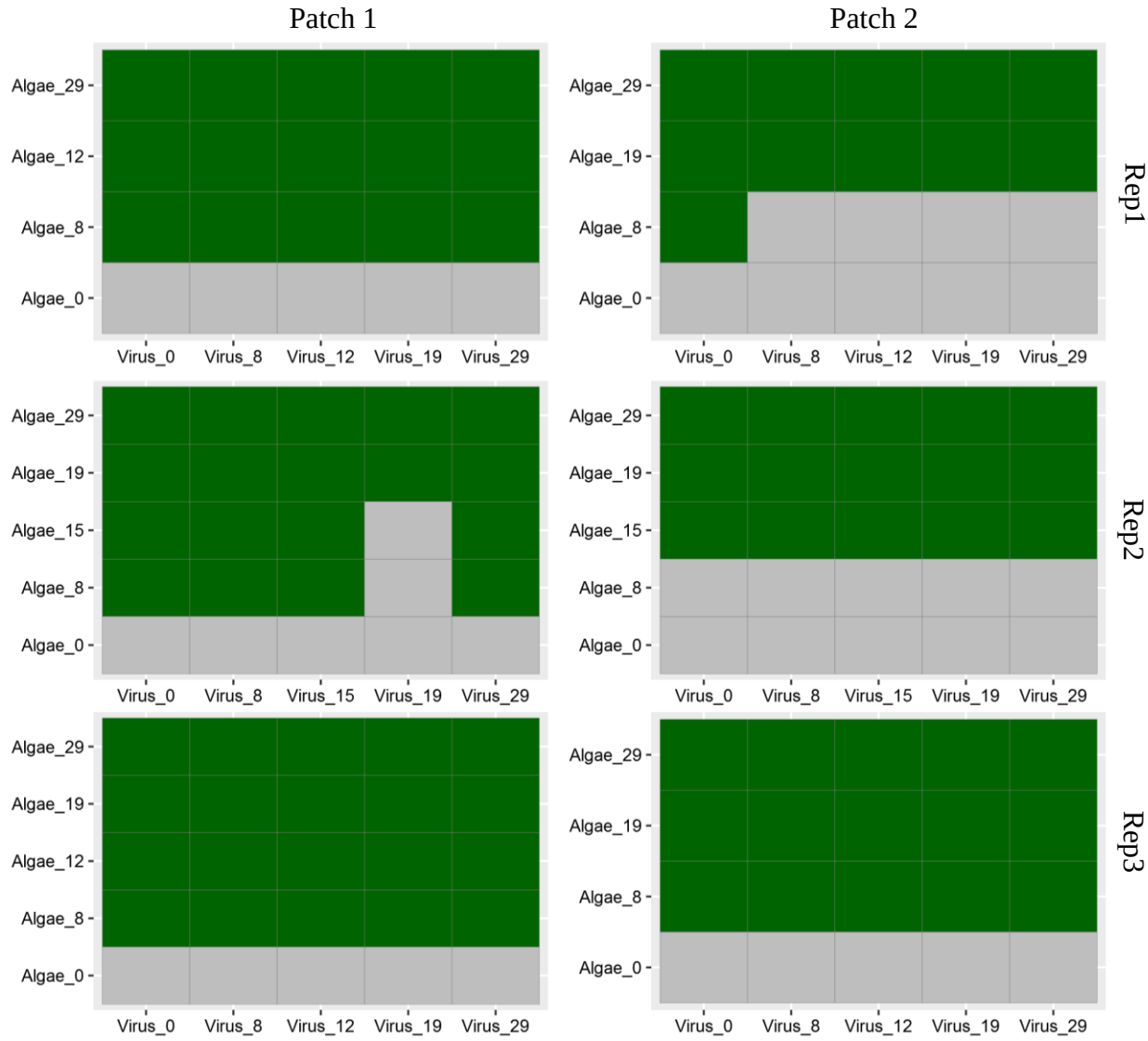

Figure 32 Infection matrix time-shift experiment for treatment *Algae + Virus – Algae* (replicate 1-3). Resistance (green) and susceptibility (grey) of algae populations to virus populations over all time-points per patch.

In both patches of treatment A + V – A + V the algae populations of day 0 were not resistant to any viruses of any time-point (Fig. 33). After day 8 (replicate 1, 2) and day 12 (replicate 3) all of the algae populations in patch 1 and 2 showed resistance to viruses of all time-points.

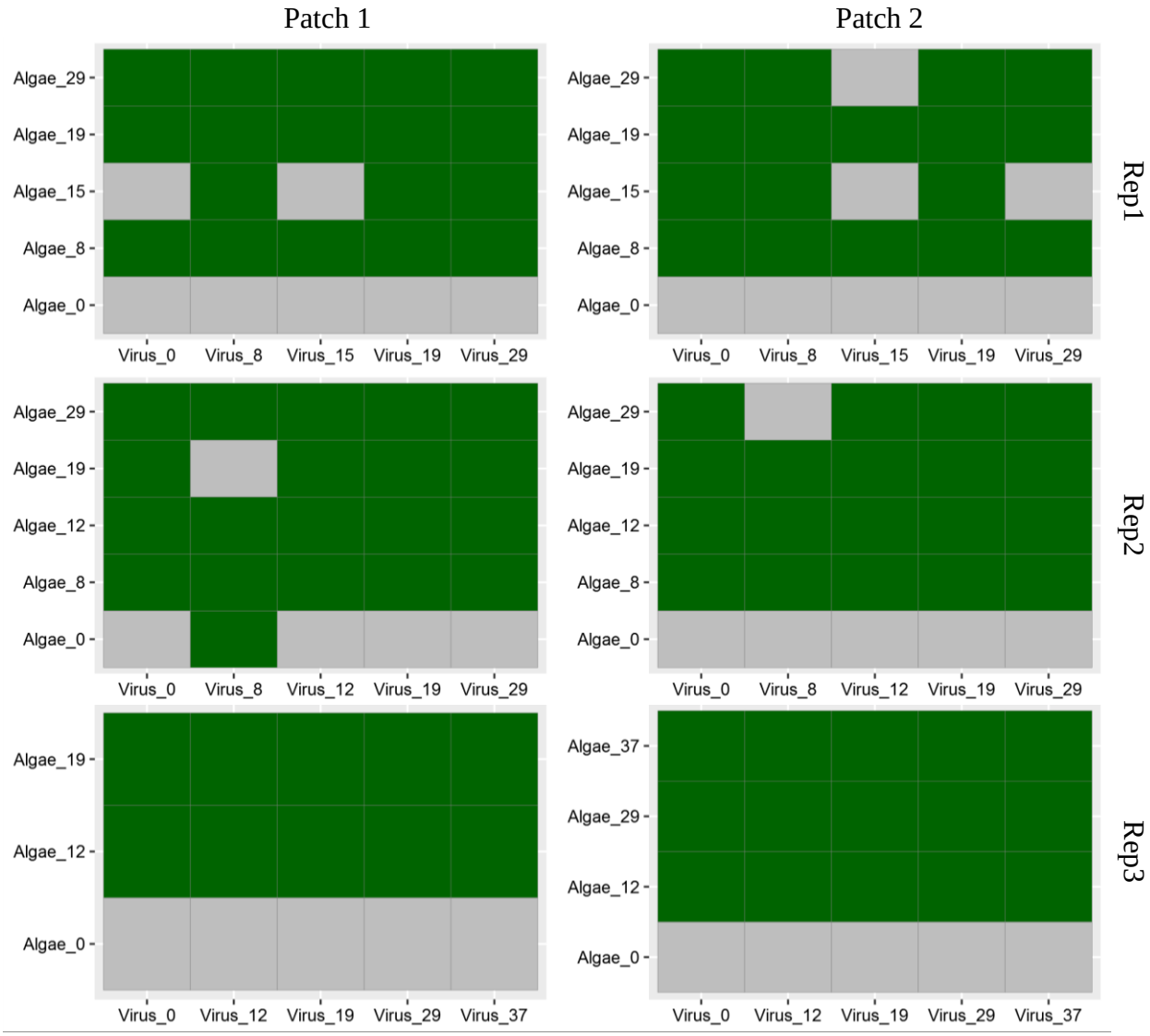

Figure 33 Infection matrix time-shift experiment for treatment *Algae + Virus – Algae + Virus* (replicate 1-3). Resistance (green) and susceptibility (grey) of algae populations to virus populations over all time-points per patch.

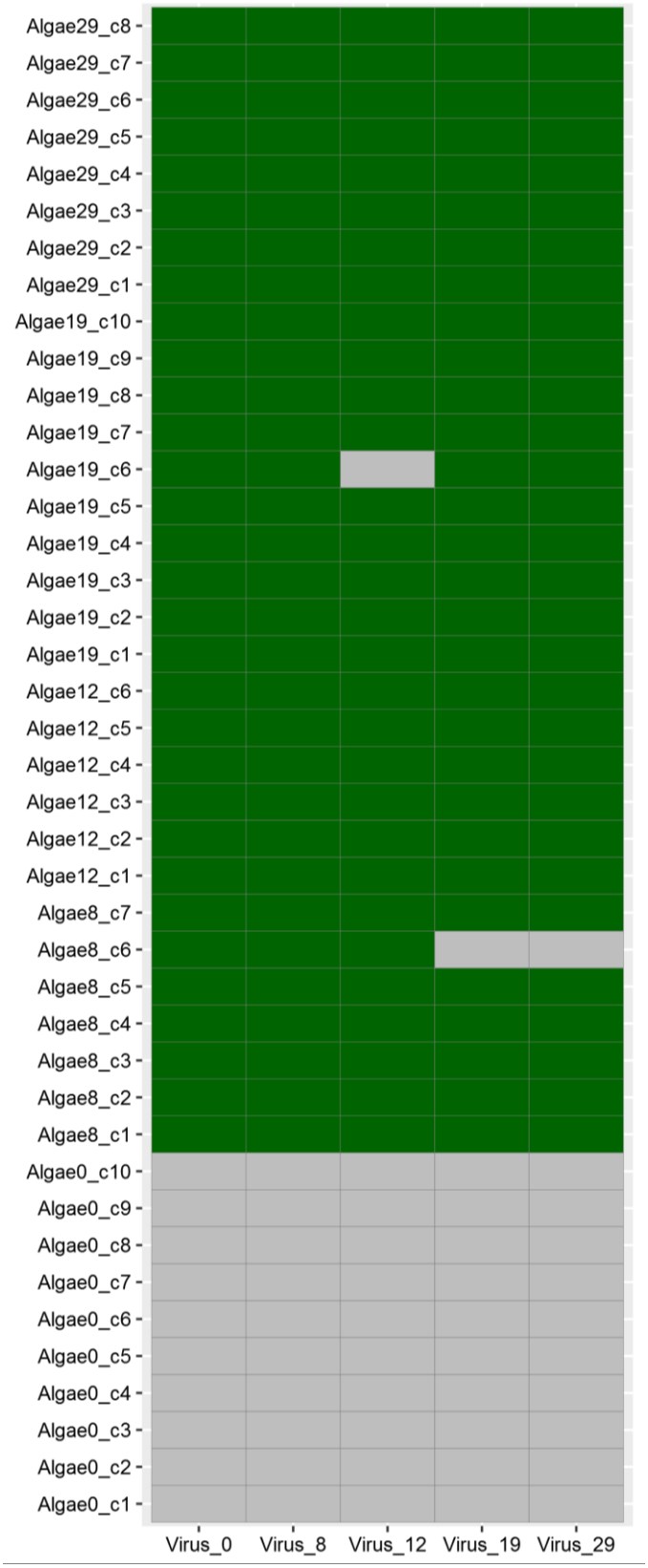

Figure 34 Infection matrix time-shift experiment. Resistance (green) and susceptibility (grey) of algal host clones of treatment *Algae + Virus – Algae* (patch 1 replicate 1) to virus populations over all time-points per patch.

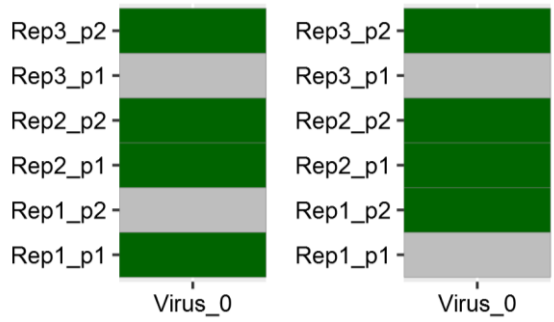

Figure 35 Infection matrix time-shift experiment for control 1 (Algae-Algae) left, and control 2 (Algae – 0) right. Resistance (green) and susceptibility (grey) of the latest time-point of algae population to ancestor virus population.

4. Discussion

We experimentally studied the eco-evolutionary effects on infectious disease dynamics in a coevolving host-virus system. Overall, we confirmed in our experiment that spatial structure and different community compositions alter the eco-evolutionary dynamics and thus lead to different infectious disease dynamics. The most important factors, which affected the infectious disease dynamics, were population densities and fluctuations (ecological) as well as coevolution and gene flow (evolutionary).

4.1 Population dynmics

Host and virus populations showed fluctuating population densities, which were different among the community compositions. The algae populations that were inoculated with virus decreased rapidly, whereas the virus population density increased. Nevertheless, the virus population decreased over time and the algae populations recovered slowly after some time of infection. In treatment A + V – 0 no viruses were found in patch 2, whereas the algae population density increased over time. It could be suggested that the virus population density was low, due to lack of susceptible hosts and that resistant algal clones were able to colonize the patch (Fig. 2 B, 3 B, 4 B). This corresponds to Anderson & May (1982), who stated that the persistence of virus population is host density dependent. In treatment A + V – A cross infections of alga populations occurred over all replicates, following the same pattern as in the patches inoculated with virus. Although algae populations of patch 2 were cross infected later, unless it was not significantly later due to large variation between replicates (Fig. 29,

30), they recovered strikingly faster than the patches of treatment A + V – A + V. It could be suggested that an overall higher virus density at the beginning leads to a delay in algae population recovery (Fig. 6, 9, 12, 16, 19, 22, 29). In addition, there was no difference in population dynamics between the two patches in treatment A + V – A + V. Generally, we could observe low virus population densities when the algae population density was low. This pattern could be recognized in all patches inoculated with virus, as virus population decreased rapidly during low algae population density (Fig. 6, 9, 12, 16, 19, 22). This could be related to the declining encounter rate between host cells and virus particles, which is fundamental for the virus persistence (Anderson & May 1982).

The observed differences in population dynamics between the patches can enable gene flow among patches. In addition, differences in algae population recovery after infection with virus indicate ongoing evolutionary forces (evolutionary rescue). Our time-shift experiment revealed that differences in population dynamics among the treatments resulted in different strengths in response of resistance of hosts to virus.

4.2 Coevolutionary dynamics

In our time-shift experiment the algae populations evolved resistant to all viruses populations tested within approximately four days after infection. This can be attributed to arms race dynamics, as a short term response, because of high selection pressure of virus on hosts. However, we cannot conclude general resistance in this case, as the virus might evolve further if the experiment had been run for longer (Frickel et al. 2016). The algae population at day 29 of treatment A + V – 0 patch 2 showed resistance to all virus time-points tested. This suggests that colonization of an evolved resistant algal clone from the other patch occurred, because no virus, which could have forced evolution of resistance, was present in patch 2. Unfortunately we cannot determine for sure when this resistant clone arrived in the other patch because of missing earlier time-points. Nevertheless, in consideration that the algae population of day 8 in patch 1 evolved resistant to all tested virus time-points, it could be assumed that the early colonizing algal cells in patch 2 were resistant. Interestingly, we could observe a shift in evolved resistance between the patches in treatment A + V – A. This could be attributed to a later cross infection. The observed faster algae population recovery in patch 2 of treatment A + V – A was not detectable due to the sampled time-point intervals of algae population. Moreover, no differences in evolution of resistance were observed between the patches in the treatment where both patches with algae populations were inoculated with

virus (A + V – A + V). Because of missing earlier time-points, i.e. before day 8 for the other treatments, we could make no statements whether evolution of resistance occurred faster or slower. Looking at the observed later algae population recovery suggests that evolution is happening more slowly in this treatment. In addition, it could be suggested that we cannot detect the differences in time of arising of resistance to virus among the treatments because we were using whole algae population. By testing single clones we may have been able to see differences in the ratio of resistant and susceptible algal clones and thus strength of evolution among the treatments. This may have made it possible to verify the observed differences in population dynamics among the treatments. For instance, we would expect a higher ratio (resistant algal clones / susceptible algal clones) in treatment A + V – A at day 8 than in the treatment A + V – A + V due to faster algae population recovery in treatment A + V – A than in treatment A + V – A + V.

Contrary to the results shown by Frickel et al. (2016), some replicates of the control populations were resistant to ancestral virus, contradicting evolution only occurring from algae virus interactions and therefore due to selection pressure. Furthermore, some inconsistencies were observed within the time-shift experiments. This might be the case because the method used for determining resistance (overlap / non-overlap of means ± 2*SD) was perhaps not suitable. For future consideration, conducting time-shift experiments at population level an adjustment of the threshold value for example comparing the means ± 1.5*SD is more appropriate. It is also possible that these inconsistencies are due to the usage of whole algae population instead of single algal clones. One algal genotype might be favored in one environment but not in the other. Furthermore, one resistant algal clone within a susceptible algae population could be outcompeted, whilst growing the algae population culture because of an existing trade-off between growth rate and resistance (Lenski 1988; Weinbauer 2004; Frickel et al. 2016). In this context, using a whole population method was not a problem in our case, as the results between those two methods were not different (Fig. 34). This might be the case because only ARD were present during the duration of our experiment and therefore trade-offs were playing a minor role (Frickel et al. 2016)

Our results indicate that evolutionary dynamics were present and also different among spatiotemporal scales as indicated by the algae populations which got infected later and exhibited a shift in the time-point of resistance (Fig. 32). Overall, our study shows that there is a tight link between ecology and evolution. Our study provides direct evidence of spatial

heterogeneity having a direct effect on the ecological dynamics as well as on the evolutionary dynamics and that the ongoing feedback loops of both of them are affecting infectious disease dynamics (Post & Palkovacs 2009).

Indeed, we observed in our study rapid genetic change that occurred in some algal clones, which could be for example lead to phenotypical changes in cell wall structure. These algal clones were then favored in the presence of viruses and enabled algal population recovery (Fig. 2 A, 3 A, 4 A, 6, 9, 12, 16, 19, 22). As these changes in cell wall structure are possibly costly to maintain, indicated by a trade-off between growth rate and resistance, susceptible algal cells are favored in the absence of the pathogen, resulting in a dynamic equilibrium between hosts and viruses in the environment (Lenski 1988; Weinbauer 2004; Thomas et al. 2012; Frickel et al. 2016).

A critical issue in our study is how the algae populations, which were not inoculated with virus, got infected by virus particles, or how the algal cells moved to the other patch as both are immobile. We suggest that this may have occurred as a result of diffusion. In this case we would have expected that the virus particles, which are smaller, are diffusing faster than algal cells following basic physical laws (Jost 1960). However, we did not observe virus colonization in the patch, which was not inoculated with algae, possibly due to low density of susceptible hosts (Anderson & May 1982). Furthermore, the sampling procedure, consisting of attachment of the clamp in the middle of the connecting tube and shaking before sampling, might be one possible explanation of how either virus particles or algal cells were moving to the other patch. A possible solution for future experiments might be to attach two clamps at both ends of the connecting tube.

5. Conclusion

Our study revealed that spatial structure has a profound impact on the eco-evolutionary effects and on infectious disease dynamics. In this context spatial heterogeneity or patchiness, which is common in nature, can have a major influence on the infectious disease dynamics. Previous studies reported that viruses play a major role in termination of algal blooms and thus in shaping algal biodiversity (Bratbak et al. 1993; Fuhrman 1999; Tarutani et al. 2000; Brussaard 2004, Brussaard et al. 2005; Sandaa 2008). Indeed, our experiment showed that rapid evolution of resistance lead to algae population recovery and minors the effect of virus on algal mortality. In accordance with the results of our experiment, other studies report that algal 'escape' strategies are related with a narrow host specifity associated with a high host diversity resulting in a large dilution of susceptible hosts, which reduces their accessibility by virus (Suttle & Chan 1994; Suttle 2005).

For future experiments it would be interesting to repeat the experiment with more replicates and for a longer period. Furthermore, more time-points are useful to inspect the ongoing evolution, which affects infectious disease dynamics. For instance, earlier time-points of algae populations after infection with virus can give us a closer insight when resistance firstly arises. Besides that, observation of later time-points of algae populations can help us to assess whether and when a switch between arms race dynamics and fluctuating selection dynamics is appearing. Once again we would also be able to compare evolution at clone level and population level to infer if dynamics get masked at population level, e.g. arms race dynamics, trade-off driven dynamics and fluctuating selection dynamics.

Furthermore, a higher number of connected patches would be a possible approach to follow infectious disease dynamics, as highly connected patches are expected to result in higher resistance due to higher gene flow among these patches (Gandon & Michalakis 2002; Granér & Thrall 2002; Jousimo et al. 2014). In addition, the success of a virus can also depend on vectors, which could, with their existence and movement direction, greatly influence infectious disease dynamics (Johnson et al. 2015). Some interesting vectors could be for example the rotifer *Brachionus calicyflorus* or the ciliate *Paramecium bursaria*. The special feature about *Paramecium bursaria* is that it lives in facultative symbiosis with *Chlorella variabilis* and represents a possible refuge against viruses (Lenski 1988; Fujishima 2009), whereas *Brachionus calyciflorus* depicts a predator (Fussmann et al. 2007). Both species

might greatly, albeit differently, influence the eco-evolutionary effects on the infectious disease dynamics in a coevolving host-virus system.

In conclusion, future work needs to be done to get further insights on infectious disease dynamics. Nevertheless, using this novel experimental set-up we are able to entangle ecological and evolutionary dynamics and how they affect infectious disease dynamics. In addition, future experiments in the field of experimental evolution can be crucial for epidemiology research to better understand ubiquitous infectious disease dynamics in general, e.g. in plants or humans.

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7. Appendix

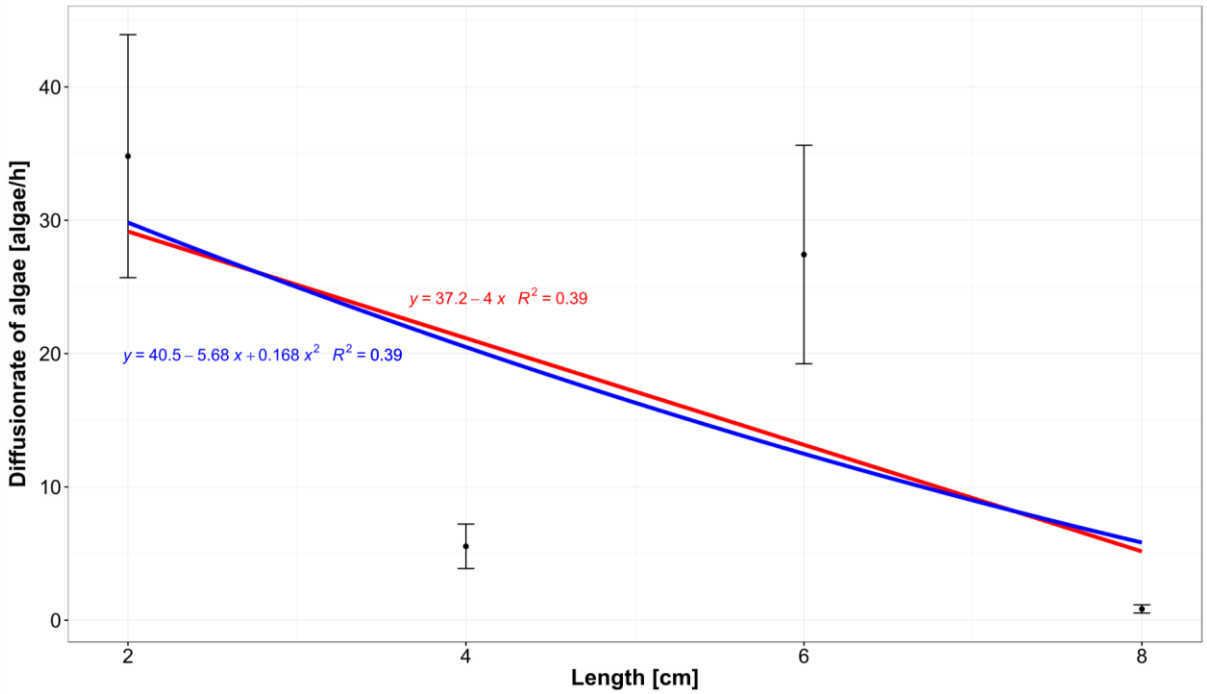

Figure 36 Diffusionrate of algae [algal cells / h] depending on tube-length [cm]. Means (+SD) result of three times replication of the pilot experiment. Red line: linear regression line, blue line: exponential regression line. At a tube-length of 8 cm the two patches can be treated as independent of each other because the diffusionrate of algal cells / h is very low.

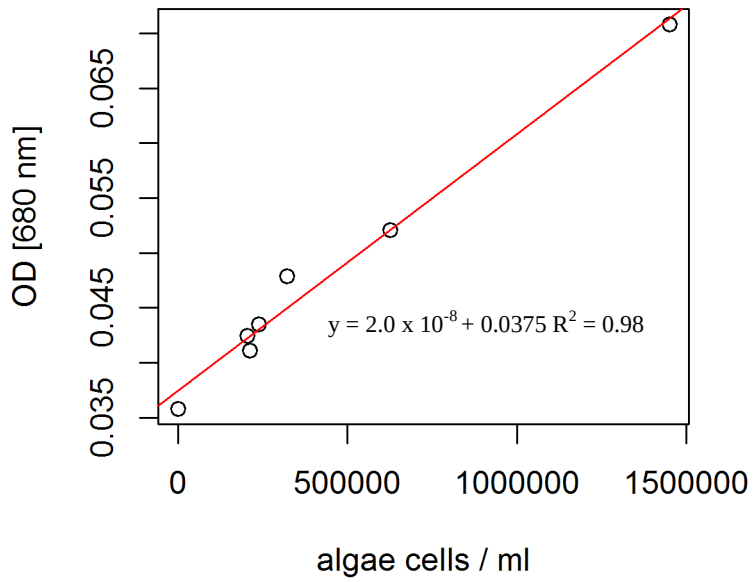

Figure 37 Dilutioncurve of algae cells / ml against the optical density (OD) at a wavelength of 680 nm. The concentration of *C.variabilis* cells was counted with FlowCam. With the formula and a given OD we are able to calculate the density of *C.variabilis* cells / ml.

Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Die eingereichte schriftliche Fassung der Arbeit entspricht der auf dem elektronischen Speichermedium.

Weiterhin versichere ich, dass diese Arbeit noch nicht als Abschlussarbeit an anderer Stelle vorgelegen hat.

Ort, Datum, Unterschrift