universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

VIRAL ABUNDANCE, LYTIC LIFE CYCLES AND LYSOGENY IN A RIVERINE ENVIRONMENT (DANUBE, AUSTRIA)

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag. rer. nat.)

Verfasserin:	Jana Tvarogová
Studienrichtung /Studienzweig (lt. Studienblatt):	Biologie / Ökologie
Betreuer:	Ao. Univ. Prof. Dr. Peter Peduzzi

Wien, im Juli 2010

Danksagung

Im Rahmen des Projektes im Donau Nationalpark wurde es mir möglich gemacht an dieser experimentellen Studie teilzunehmen.

Ich möchte mich bei all jenen bedanken, die mich bei der Realisierung meiner Diplomarbeit unterstützt haben.

Besonders bedanke ich mich bei meinem Betreuer **Prof. Dr. Peter Peduzzi**, der es mir ermöglicht hat, an diesem interessanten Thema zu arbeiten und der mir während der gesamten Arbeit alle nötigen Mittel zu Verfügung gestellt hat.

Weiteres möchte ich mich für die Unterstützung bei **Martin Agis** von der Universität de Montpellier II, **Tim Ulrich** von der Abteilung Ökogenetik, bei **Christian Winter** von der Abteilung Meeresbiologie, **Kilian Stöcker** und **Christian Baranyi** von der Abteilung Mikrobielle Ökologie, **Wolfgang Postl** und **Anne-Mette Hanak** von der Abteilung Molekulare Systembiologie, sowie bei der Abteilung Limnologie, und speziell bei **Birgit Luef**, **Andrea Funk, Anna Sieczko**, **Maria Maschek,** sowie bei **Birgit Görnet** bedanken. Sie haben mich sowohl im praktischen als auch im theoretischen Teil meiner Diplomarbeit unterstützt und mir viele meiner Fragen beantworten können. Ich durfte die Daten Ihrer chemischen Analysen weiter verwenden.

Außerdem möchte ich Herrn **Hubert Kraill** danken, der mir Chemikalien zur Verfügung gestellt hat.

Ein Dankeschön geht an meine Freunde von meiner Arbeitsstätte Tiergarten Schönbrunn und aus meinem Freundeskreis, die mich unterstützt haben. Besonders bedanke ich mich bei Jaja, Mirka, Lenka, Verena und Marlen, die mir auch in schlechten Zeiten geholfen haben.

Weiter, möchte ich mich bei meiner Familie bedanken, die mich während der gesamten Arbeit finanziell unterstützt hat und mir somit ermöglichte an diesem spannenden Thema zu arbeiten.

Content:

Zusammenfassung

In dem 9.300 ha großen Donau Nationalpark liegt das Aussystem Lobau, das im Zeitabschnitt von Juni bis Oktober 2009 untersucht wurde.

In meiner Diplomarbeit wurden die hydrologischen und chemischen Parameter eines Fluss-Ausystem mit über das Programm VIPCAL kalkulierten Viren-Parametern verglichen (Luef et al. 2009).

Während der Studie kam es im Juni zu Hochwasser, wobei eine intensive Probennahme erfolgte. Leider war es nicht möglich während dieser Zeit die Virenparameter zu errechnen.

Veränderungen in einem Fluss-Aussystem sind unter anderem abhängig von Veränderungen innerhalb des Systems, als auch von externen Parametern. Um dieses dynamische Fließgewässer-Ökosystem zu verstehen, wurden während dieser Studie einige Wasserkörper des Fluss-Aussystems im Donau Nationalpark (Stationen TLM1, 2007 und Hauptkanal der Donau) sowie ein süd-östlich gelegener Wasserkörper in Regelsbrunn analysiert.

Da die Wechselwirkungen in den mikrobiellen Lebensgemeinschaften dieses Gewässers noch nicht ausreichend bekannt sind, sollte damit neue Information erlangt werden. Die vier oben angeführten Stationen wurden näher auf frei lebende Viren untersucht, neben den Bakterien die zahlreichsten biologischen Einheiten in Gewässern.

Hinsichtlich der Abhängigkeit der Mikroorganismen von Parametern dieses Systems inkludierte man in dieser Studie Wetterveränderungen, Unterschiede in der Zusammensetzung von organischem, anorganischem Material sowie die Wechselwirkungen der Bakterien und Viren in und mit ihrer Umwelt.

Da möglicherweise hydrologische und chemische Parameter wie Temperatur, pH, Sauerstoff, Chlorophyll *a*, bakterielle Sekundärproduktion (BSP), partikulär anorganisches Material (PIM), partikulär organisches Material (POM) und total suspendiertes Material (TSS) sowie Lichtintensität und Wasserstand der Donau die bakterielle und virale Abundanz und Diversität beeinflussen, wurden diese mit dem Vorkommen der Viren mittels Regressions- und Korrelationsanalysen (SPSS 11.0) verglichen und auf ihre Gemeinsamkeiten untersucht. Vermutet wurde hohe Diversität und hohe Anzahl an Viren bei hohem Anteil an organischem Material, hoher Temperatur und niedrigem Wasserspiegel. Um

maximale und minimale Abundanz und Diversität der frei lebenden DNA-Viren festzustellen, wurden folgende Methoden verwendet: Filtration, Epifluoreszenzmikroskopie (Nikon E 800), SYBR-Färbemethode, Viren-Reduktionsansatz (VRA) und molekular biologische Methoden. Die Veränderungen in der viralen Diversität über die Zeitspanne von 5 Monaten konnten nicht festgestellt werden, da die Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) Technik noch nicht ausreichend etabliert werden konnte.

Um die Veränderungen der bakteriellen und viralen Abundanzen zu unterschiedlichen Zeiten zu prüfen, wurde Oberflächenwasser durch 3 µm-Filter und auf 0.02 µm-Filter abgefiltert, mit SYBR Gold gefärbt (Weinbauer et al. 2002) und im Epifluoreszenzmikroskop bei 1250-facher Vergrößerung gezählt. Basierend auf älteren Studien des Ausystems im Donau Nationalpark konnte gezeigt werden, dass die bakterielle > 3 µm Fraktion mit dem organischen Material positiv korreliert. Es konnte nachgewiesen werden, dass der virale Parameter bakterielle Mortalität pro Tag mit der Temperatur negativ korrelieren. Weiteres konnte ein positiver Zusammenhang zwischen POM und TSS mit dem bakteriellen Verlust pro Tag festgestellt werden.

Abstract

The Danube National Park downstream of Vienna, with a of 9.300 ha river-floodplain system, was the location for this study in the period from June to October 2009. In my study, hydrological and chemical parameters of the water bodies were compared with virus parameters and values using the online tool program VIPCAL (Luef et al. 2009).

The sampled areas were frequently investigated during the flood event in June. In the various locations, the observed changes were linked to changes inside and outside of the aquatic systems.

To cover both isolated and dynamically connected water bodies, these four stations of the Danube National Park and its back waters were selected: stations TLM1 and 2007 in the floodplain system and the stations side arm Regelsbrunn and Main Channel of the River Danube. At these stations viruses (bacteriophages), the most abundant biological agents of the aquatic environments, which infect the communities of the microbial food loop, were investigated.

This work attempted to learn more about the influence of the river-floodplain system, the changes of weather, as well as differences of the concentrations of the organic matter on virus-related parameters. In order to determine biotic and abiotic parameters of each water body the samples were prepared by performing several filtration steps (GF/C, GF/F Whatman filter; 3 µm pore-size GSWP Millipore filter and 0.22 µm pore-size WP Millipore filter), centrifugation (0.22 µm viva spin tubes), and viva flow ultrafiltration cartridges (30.000 DA). To quantify the viral and bacterial abundance the following methods were also used: SYBR Gold DNA staining method (Weinbauer et al. 2002), epifluorescence microscopy (Nikon E 800) at 1250 x magnification for counting.

In our results, large differences in bacterial and viral abundance of the free-living fraction were detected at each sampled station. We also observed significant differences in the bacterial and viral abundances associated with particles and organic matter. Only the attached bacterial abundance increased with the increase of the organic matter. The bacterial abundance did not evidence any relation with bacterial secondary production (BSP).

On the other hand, no characteristic associations of attached viruses with organic matter and BSP were detected. We also noted large differences in the lytical and lysogenic life style of viruses. To show which of the most common viral life cycles is more important, the percentage of infected bacterial cells was calculated through the online tool program VIPCAL with highest value of 73% at station TLM1 (Luef et al. 2009).

The abiotic parameters temperature, oxygen concentration, particulate inorganic matter (PIM) and the biotic parameters chlorophyll *a* concentration, bacterial secondary production (BSP), particulate organic matter (POM) and total suspended solids (TSS) were compared with the viral parameters viral lytical production, lysogenically infected cells, lytically infected cells, lysis rate of bacteria and bacterial loss/mortality per day using analyses of regression and correlation via the program SPPS 11.0.

Overall, in our study, the bacterial mortality per day increased with the increase of POM and TSS as well as with the decrease of temperature.

It would require more data to underline our hypotheses about the importance of viruses in riverine systems.

Introduction

River-floodplain systems and their geomorphology play a significant role influencing biogeochemical cycles, transport of elements as well as the hydrological cycle. The surrounding regions of a river system, its active side arms and also its dead arms have effects on thermal heterogeneity, biodiversity, regulation, fluctuation and natural transport of sediments and also of organic matter, related to the river's hydrological connectivity (Push et al. 1998). A complex temporal heterogeneity of a river system depends also on the presence of floodplain systems and its surrounding habitats. Changes in a river-floodplain system, also in transported elements from the catchment, are important parameters involved in the river's environment and development.

River-floodplain systems are still less studied regarding their biodiversity, richness, changes in sediment load, growth of terrestrial zones as well as changes in trophic level of the water columns. Alterations in the trophic status of a floodplain system as well as changes in precipitation and transported nutrients, surface and groundwater flow and increase/decrease of biota leads to changes in both terrestrial and aquatic zones of a river floodplain ecosystem (Tockner et al. 1997).

In order, to close gaps in our knowledge of river systems and its temporal changes, we selected for this study the Danube National Park involving freshwater water bodies of the 2.300 ha large floodplain Lobau. The Danube National Park is located near the capital city of Austria, Vienna, and is a freshwater system with different turbidity, different steepness and fluctuation in discharge. In the 18th century the first regulation scheme of the River Danube for protection of the city of Vienna against flooding was developed and is modernized till today. Since 1996, the river floodplain system southeast of Vienna was selected as a National Park and plays today a significant role in European environments. The River Danube is the second largest river in Europe with a total length of 2850 km and originates from two streams Brigach and Breg in the Black Forest (Bavaria, Germany, Hohensinner et al. 2005, Jungwirth et al. 2002, Tockner et al. 1998, 2001 and Ward et al. 2002).

In the urban part and downstream of Vienna a floodplain landscape Lobau within the Danube National Park consists of a mosaic of dynamic areas with hot

spots in biodiversity, with some temporal heterogeneity, which is also controlling ecological processes (de Groot et al. 2002).

Anthropogenic changes in water quality as well as changes in water level enhanced eutrophication, and led to negative diversification of the floodplain's biota (Tockner et al. 1998). The importance in the migration of biota, exchange and transport of organic matter as well as the degree of hydrological connectivity underlines why a floodplain ecosystem should be analysed to know more about its ecological settings. Floodplains also influence the riverine landscape together with the associated aquatic communities (Tockner et al. 1998).

We aimed to analyse one of the most important, still less known element of aquatic ecosystems, the viruses. Another important component are bacteria, Kirchmann et al. (1982) describes prokaryotes as a key factor in trophic dynamics of an aquatic ecosystem. Further, bacteria are seen as important consumers of dissolved organic matter in the water column (Pusch et al. 1998). Produced dissolved organic matter is metabolised by bacteria, which themselves are consumed by nanoplankton, zooplankton and so on. This process, called microbial loop, is explained e.g. by Wilhelm et al. (1999).

In another studies of the floodplain system of the Danube National Park the importance of prokaryotes in ecological processes was documented (Besemer et al. 2004, 2005).

For getting information on the richness of the virus community in the floodplain system Lobau one group of viruses was analysed, the free-living DNA-viruses.

Based on former studies it was shown by pulsed field gel electrophoresis (PFGE) that viruses are highly variable in their diversity, and change in their richness in periodical intervals (Agis et al. in prep.).

Viral diversity and abundance are not only high in the water column but, also in sediments as well as in soils. They are 5 to 25 times more abundant than bacteria. In order to know more about viral diversity we used Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR), one of the newest molecular biological techniques. Unfortunately, we could not establish the changes in the viral diversity over a period of 5 months, because the RAPD-PCR technique did not work as expected. Further, the viral

abundance was analysed with the SYBR Gold DNA staining method and enumerated with epifluorescence microscopy (Luef et al. 2007, 2009, Suttle et al. 2007 and Winget et al. 2008)

The most common viral life cycles are the lysogenic and lytic one. We still don't know which of them is more important. Lytic viruses infect host cells and lyse them immediately. Temperate viruses integrate their nucleic acids in the host genome. During stress conditions lysogenic viruses lyse their hosts and release themselves into the environment. In a biologically dynamic system, as the floodplain section Lobau, lysogenic and lytic viruses are fluctuating in their abundance and diversity in periodical interval. The fluctuation of their abundance was monitored with the Virus Reduction Approach (VRA) technique, which is used for identification of the respective life style in aquatic ecosystems. To calculate lytic viral production and lysogenic cells based on this approach, we used the online tool program VIPCAL (Luef et al. 2009). From our investigation we found that viruses play an important role in this aquatic system (Luef et al. 2009 and Weinbauer et al. 2002).

Methods

Ecological methods

Monitoring (location and sampling)

For the experimental part of the diploma thesis, four stations of the River Danube and its back waters of the Danube National Park were investigated for abiotic and biotic parameters with emphasis on the microbial communities over the period from June to October 2009 (Tab.1).

An isolated water body (TLM1) with less macrophytes was selected in the floodplain system of the Danube National Park. The second station (REG), located in Regelsbrunn, is a dynamically connected side arm of the River Danube. The Main Channel of the River Danube with usually high transport of particulate matter was also investigated. Finally, the station 2007 with low transport of water and sediment was selected.

Due to a flood event in June, the four stations were more frequently sampled and more closely investigated regarding the hydrology and chemistry. The monitoring data of the stations, relevant for this study, are shown in Tab.1.

Furthermore, the bacterial activity at the locations was measured in a concomitant study by Sieczko (unpublished data).

Processing of viral concentrates (filtration methods)

The surface water of each of the four locations was filtered using several filtration steps (Fig.2).

First, up to 1000 mL of the surface water were used for the experimental part. 10 mL of the surface water were fixed immediately with formaldehyde (2% final concentration) and cooled for 20 minutes to fix most of the cells. 800 µL of the formaldehyde fixed surface water were filled up to 1 mL with 0.02 µm-dionised water. Therefore, 1 mL sample was prepared as described in the Virus Reduction Approach (VRA) experiments part (p.12).

Furthermore, the remaining 990 mL were filtered using a peristaltic pump at 2 bar pressure through 3 µm pore-size filters (GSWP Millipore). 1 mL of the 3 µm filtrate was prepared in the same way as described in the Virus Reduction Approach (VRA) experimental part (p.12, Weinbauer et al. 2002).

The last filtration using 0.22 µm pore-size membrane filters (Millipore) should remove the remaining particulate matter

Virus Reduction Approach (VRA) experiments

The viruses in aquatic environments propagate typically via the lysogenic or lytic life cycle. Surface water was handled applying the Virus Reduction Approach (VRA) experiments as reported by Weinbauer et al. (2002) to investigate which of the viral life cycles, the lysogenic or lytic one, is more important in a riverine system such as the Danube River and its back waters.

200 mL of the 3 µm filtrate of each of the stations were centrifuged at 3000 g for 3 minutes to produce 2 mL of a bacterial concentrate. Furthermore, 800 mL of the 0.22 µm filtrate were ultrafiltered using a 30.000 Da cartridge at 2.5 bar pressure. With this, we were able to produce a virus-free size fraction and a virus concentrate. The virus concentrate was stored at -20°C for later analyses of changes in viral diversity over time. 49 mL of the virus-free size fraction were amended with 1 mL of the bacterial concentrate and with the antibiotic Mitomycin-C (5 µg/mL solution). Concurrently, we established a series of tests of untreated samples which contained 49 mL of the virus-free water and 1 mL of the bacterial concentrate alone. Each treatment was done in duplicates.

The treated and untreated samples were incubated in darkness for 24 hours at temperatures as measured in the aquatic environment. The antibiotic Mitomycin-C should stress the bacterial cells. This induces the lytic cycle in lysogenic cells. In the experiment, the abundance of viruses should increase during the time course of the experiment, thus representing an estimate of lysogeny.

To investigate the viral abundance we took 1 mL of the treated and untreated sample at the beginning of the experiment (time zero). Every 6 hour, after the beginning of the experiment till 24 hours, we sampled 1 mL of the treated and untreated samples (time 1, 2, 3, 4).

Afterwards, the treated and untreated samples were fixed with a drop/mL formaldehyde, stained for 20 minutes in a SYBR Gold solution (1:3000), filtered through a 0.02 µm pore-size AnoDisc filter (Whatman, a 0.45 µm pore-size Nitrocellulose filter (Millipore) used as a supporting filter) at 200 mbar.

Afterwards, the filters were air-dried. The dry filters received a drop of CITIFLUOR on finally covered slides. For later enumeration of the viruses and bacteria using epifluorescence microscopy (Nikon E 800) at 1250-fold magnification the slides were stored at -20°C.

Furthermore, the viral parameters, such as lytical viral production, the frequency of lytically infected and lysogenic cells as well as the bacterial lysis rate and mortality per day were calculated using the online tool program VIPCAL as reported by Luef et al. (2009). To calculate the viral parameters, we assumed a burst size of 50 and used the BSP rates measured by Sieczko (Tab.1, 2, 3, 4, 5, 6, Fig. 5, unpublished data).

Bacterial and viral abundance in the original sample

Surface water was filtered, fixed with formaldehyde and stained with a DNA SYBR Gold solution as described in the Virus Reduction Approach (VRA) above (Noble and Fuhrman, 1998). One difference should be noted: 800 µL of the formaldehyde fixed surface water were filled up to 1 mL with 0.02 µm- filtered (Whatman) dionised water to dilute the bacteria and viruses in the surface water. Afterwards, the enumeration of bacterial and viral abundances was easier; they did not overlap each other.

Parameters taken during monitoring

During monitoring abiotic and biotic parameters were measured and analysed, such as – temperature, pH, oxygen concentration, dissolved organic carbon (DOC), bacterial secondary production (BSP), chlorophyll *a* concentration, particulate organic matter (POM), particulate inorganic matter (PIM), total suspended solids (TSS), water level of the River Danube, particulate primary production and light intensity (Sieczko, pers.comm.).

Molecular methods

The changes of the viral diversity were investigated using a virus concentrate (p.12) prepared by ultracentrifugation (300.000 g for 90 minutes, rpm = 90.000, Beckman). 50 µL of PCR-water were added to dissolve the pellet of viruses.

We used the Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) method for a fingerprint of the free-living DNA-viruses community (Winget et al. 2008).

Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR)

The RAPD-PCR (Winget & Wommack 2008) method was used for the determination of changes of viral diversity over a time period. The ultracentrifuged virus concentrate was not extracted for the DNA, but added directly in 1:1, 1:10, 1:100 and 1:1000 dilution steps to the PCR mixture as discussed by Helton et al. (2009).

The primer used for the PCR reaction was a decamer primer OPA-9 with the sequence 5`-GGG TAA CGC C-3´ (synthetised by Invitrogen).

Each of 10/15/30 µL of the PCR mixture contained 1/5/10x PCR buffer (Fermentas), 0.16/0.4/0.8 mM of each deoxynucleotide triphosphate (Fermentas), 0.2/0.4 µM of decamer primer (Invitrogen), different concentrations of $MgCl_2$, 2.5 U of Taq polymerase (Fermentas), 0.5 µL up to 2 µL of virus concentrate and was filled with DEPC-water (Fermentas) up to 10, 15 or 30 µL of final volume.

The samples were amplified according to the protocol by Winget (2008) with an initial denaturation step at 94°C for 10 minutes, followed by 30 cycles of denaturing at 94°C for 30 seconds, annealing at 35°C for 3 minutes, extension at 72°C for 1 minute and a final extension step at 72°C for 10 minutes.

Gel Electrophoresis

Five grams of Biozym Sieve 3:1 Agarose (Biozym Scientific) were added to 100 mL of 1x TBE buffer (TRIS, Boric acid, EDTA, pH = 8.0) and filled with dionised water up to 500 mL. After boiling up of the 1.5 % agarose by mixing with a magnetic stirrer the agarose gel was prepared.

PCR products were submitted to electrophoresis in 1.5 % agarose gel at different volts for up to 10 hours. PCR products were stained for 20 min with an Ethidum Bromide solution (20 µL Ethidium Bromide were added to 200 mL dionised water) and visualized using a UV-transilluminator.

The PCR products of each location should be submitted to electrophoresis in the agarose gel and compared to detect differences or similarities of the viral diversity over a time period of 5 months (Tab.1). Unfortunately, the RAPD-PCR did not work during the experimental part as expected.

Statistical analyses

The bacterial and viral abundances from the VRA experiments were compared and calculated using the program VIPCAL (Luef et al. 2009). The observed viral parameters were correlated with the abiotic and biotic parameters using SigmaPlot 11.0 and SPSS 12.0 used for correlation and regression analyses.

Results

Characterisation of abiotic parameters

Characterisation of temperature

The abiotic parameter temperature ranged from 16.3°C to 20.2°C at the station TLM1 (Tab.2). The maximum temperature was measured on 18 August at station 2007 with 24.4°C, the lowest value of 10.5°C on 13 October (Tab.4). In Main Channel the temperature varied from 12.3°C to 19.6°C (Tab.5). Temperature in the dynamically connected site Regelsbrunn varied in a range from 10.1°C to 22.4°C (Tab.6). Overall, in each sampled station the maximum values were measured on 18 August, the lowest on 13 October except at TLM1 (Tab.2).

Characterisation of oxygen

The maximum oxygen concentration of 15.2 mg/L was detected on 18 August in Regelsbrunn (Tab.6) and the lowest with 1.19 mg/L on 13 October at station TLM1 (Tab.2). At station 2007 the values fluctuated from 4.7 mg/L on 3 July to 9.9 mg/L on 13 October (Tab.4). Main Channel noted values between 8.7 mg/L and 11.7 mg/L (Tab.5).

Characterisation of particulate inorganic matter (PIM)

The highest value of particulate inorganic matter (PIM) was measured with 212.4 mg/L in Main Channel on 26 June 2009 and the lowest of 2.45 mg/L on 13 October (Tab.5), in comparison to more than 708 times lower value of PIM assessed at station 2007 (0.3 mg/L, Tab.4), whereas the highest values at station 2007 and TLM1 were similar, 19.44 mg/L and 22.85 mg/L, respectively (Tab.2).

Characterisation of biotic parameters

For the experiments the surface water of the four selected stations of the River Danube and the back waters of the Danube National Park (Fig.1) was taken in the interval from June to October 2009 (Tab.1). After a flood event in June the

four stations were frequently sampled and analysed (Tab.1) to determine changes in hydrology, chemistry and the dynamics of the microbial communities.

The free-living bacterial abundance varied between 0.2 and 10.8 $x10^6$ cells/mL. The maximum values were detected on 15 September in TLM1 (Fig.4a) and the lowest on 15 September in Regelsbrunn (Fig.29a). At the station 2007 (Fig.13a) the range of the free-living bacterial abundance was noted from 2.6 $x10^6$ cells/mL to 6.1 $x10^6$ cells/mL. In Main Channel values were determined from 1.5 $x10^6$ cells/mL to 3 $x10^6$ cells/mL (Fig.21a).

The maximum value of particle-associated bacteria was 6.6 $x10^6$ cells/mL on 13 October at the station TLM1 (Fig.4a) and the lowest of 1 $x10^5$ cells/mL on 18 August in Regelsbrunn (Fig.29a). At station 2007, the particle-associated bacteria were from 1.4 $x10^6$ cells/mL to 5.7 $x10^6$ cells/mL (Fig.13a). In Main Channel the bacterial numbers varied from 4 $x10^5$ cells/mL to 2 $x10^6$ cells/mL (Fig.21a).

Furthermore, the free-living viral abundances filtered samples ranged from 0.5 to 51.8 $x10^6$ viruses/mL with a maximum in TLM1 on 13 October (Fig.4b) and lowest on 18 August in Regelsbrunn (Fig.29b).

The viruses of the unfiltered water fluctuated in their abundance from 16.5 $x10^6$ viruses/mL to 36 $x10^6$ viruses/mL at station 2007 (Fig.13b). In Main Channel of the River Danube the values varied from 5 $x10^6$ viruses/mL to 18 $x10^7$ viruses/mL.

The maximum abundance of particle-associated viruses of 3.1 $x10^7$ viruses/mL was observed at the same station as the highest value of free-living viruses at TLM1 (13 October, Fig.4b). The lowest numbers of 4 $x10^5$ viruses/mL were counted in Regelsbrunn (Fig.29b). At station 2007 we detected values of 9.8 $x10^6$ viruses/mL to 2.8 $x10^7$ viruses/mL (Fig. 13b). In Main Channel of the River Danube the viral abundance was from 2.4 $x10^6$ viruses/mL to 8.9 $x10^6$ viruses/mL (Fig.21b).

The parameters bacterial secondary production (BSP), Chlorophyll *a* concentration, particulate inorganic matter (PIM) together with particulate organic matter (POM), total suspended solids (TSS) and dissolved organic matter (DOC) revealed variability at each sampled station.

The lowest value of the bacterial secondary production was noted at station 2007 with 0.29 µg C/L/h on 26 June (Tab.4, maximum production of 1.502 µg C/L/h on 3 July) in comparison to more than 6 times higher activity assessed at the station Regelsbrunn (1.87 µg C/L/h, Tab.6). The BSP at Main Channel of the River Danube was 3.4 higher (0.99 µg C/L/h, Tab.5) than BSP noted at station 2007 with 0.291 µg C/L/h (Tab.4). The highest value was detected at TLM1 with 2.45 µg C/L/h (Tab.2).

The concentrations of chlorophyll *a* showed large differences between the stations with the highest value of 29.18 µg/L on 16 June 2009 in Regelsbrunn (Tab.6) and minimum of 1.35 µg/L on 3 July 2009 at station TLM1 (Tab2).

TLM1 noted a maximum in chlorophyll *a* concentration of 12.73 µg/L on 30 July (Tab.2). At station 2007 the concentration ranged from 3.32 µg/L to 23.68 µg/L (Tab.4). In Main Channel values from 2.49 µg/L to 9.97 µg/L were observed (Tab.5).

Overall, the particulate organic matter (POM, Tab. 2, 4, 6) noted large difference in its concentration on each sampled station. The highest concentration of POM of 98.44 mg/L was measured in Regelsbrunn on 13 October (lowest of 2.75 mg/L on 21 July, Tab.6). At the station Regelsbrunn the concentration was 17.6 times higher on 13 October than the noted lowest POM concentration detected on 16 June with 5.59 mg/L (Tab.6). In Main Channel the concentrations of POM ranged from 2.12 mg/L to 16.65 mg/L (Tab.5). At TLM1 POM concentration varied from 1.45 mg/L to 15.93 mg/L (Tab.2).

Comparison of attached to the free-living abundance of planktonic microorganisms

The attached bacterial abundance to the total number of bacteria varied from 13.4 (Fig.29a) to 89.9% (Fig.21a). The maximum percentage of the free-living bacteria of 89.9% was noted in Regelsbrunn, where bacterial abundance was 4 times higher than counted in Main Channel (Fig. 21b). The lowest measured percentage of 13.4 was determined in Main Channel of the River Danube.

The viral abundance on particles compared to the free-living viruses showed a range of 13.4 (Fig.13b) to 99.7% (Fig.21b). The maximum percentage of 99.7

was observed in TLM1, whereas in Regelsbrunn the second highest percentage of 97.4, with more than 3 times higher ratio of attached bacteria to the total number of bacteria, was detected.

The percentage of viruses on particles compared to the free-living viral abundance was higher than the attached bacterial abundance to the total number of bacteria in each sampled station.

Nevertheless, low total numbers of bacterial and viral abundances were detected at high percentage of attached bacteria and viruses enumerated in Regelsbrunn (Fig.29a, b). TLM1 with the maximum value of free-living bacteria and viruses exhibited more than 3 times higher averages of bacterial and viral abundance on particles than the station with the lowest detected number of bacteria and viruses associated on particulate matter.

VBR

The Virus to Bacterium Ratio (VBR) of unfiltered sampled surface water was higher than VBR on particles. At station 2007 the maximum VBR was more than 200 times (13 October, Fig.13d) lower than the minimum VBR at TLM1 (15 September, Fig.4d). Quite low VBR was measured in Regelsbrunn with 0.9 (18 August, Fig.29) and the highest value of 6.7 on 16 June. The VBR in Main Channel varied from 3.4 to 6.9. The maximum VBR was detected with 11.6 at station 2007 and minimum of 0.1 at TLM1.

On particles, the lowest VBR of 2.4 was measured in Main Channel and the maximum of 9.6 at station 2007. At TLM1 the virus to bacterium ratio was noted from 3.7 to 7.9, whereas the VBR in Regelsbrunn varied from 2.6 to 8.7.

In general, large differences between the virus concentrations in each station were detected on every sampling date (Tab.1). The maximum VBR of 11.6 at station 2007 was measured at lowest temperature (10.5°C), but at highest oxygen concentration of 9.9 mg/L. At TLM1, the lowest value of VBR with 0.06 was noted at the lowest temperature of 16.3°C, highest BSP of 2.45 µg C/L/h and highest PIM concentration of 22.85 mg/L (Tab.4).

Experiments with planktonic microorganisms – Virus Reduction Approach (VRA)

To investigate the frequency of lysogenic or lytically infected cells and the lytical viral production of this fresh water system we applied the VRA experiment. The treated and untreated samples were analysed for changes in their bacterial and viral abundances. In the with Mitomycin-C treated samples, the bacterial cells should be lysed due to prophage induction. Mitomycin-C is an antibiotic which stressed the cells. The lytical viral production should increase if the bacterial cells were infected by viruses.

At the Main Channel station, the largest recover-efficiency of bacteria in the experimental setup, sometimes even a concentration at the beginning of the experiment was noted in June 2009 with a percentage of 452.6 compared to more than 2 times lower values assessed at the station TLM1 (213.3%). The recover-efficiency of bacteria in Regelsbrunn was lower (287.8%) than in Main Channel. At station 2007 we determined the lowest bacterial recover-efficiency with 35.3%. The lowest recover-efficiency of all experiments was noted with 2.03 on 18 August at TLM1.

The viral parameters lytical viral production, lysogenic cells, lytically infected cells, lysis rate of bacteria and bacterial loss/mortality per day were calculated using the online tool program VIPCAL (Luef et al. 2009, Tab.3, Fig.5).

In Regelsbrunn, the lytical viral production varied from 0.2 to 5.9 $x10^2$ viruses/mL/h (Fig.30a). The values of the other sampled stations revealed ranges of 0.2 and 0.01 $x10^6$ viruses/mL/h in TLM1 (Fig.6a), 0.03 and 1.1 $x10^3$ viruses/mL/h at station 2007 (Fig.14a). In Main Channel of the River Danube the range of lytical viral production reached 0.018 to 5.1 $x10^2$ viruses/mL/h (Fig.22a).

The calculated lysis rate of bacteria was noted with the maximum of 9 $x10^5$ cells/mL/h in TLM1 (Fig.6d) and the minimum of 0.4 $x10^5$ cells/mL/h in Regelsbrunn (Fig.30d). The values of lysis rate of bacteria varied from 7.3 $x10^{-3}$ to 15 cells/mL/h at Main Channel (Fig.22d). The value of 0.2 $x10^2$ cells/mL/h at station 2007 (Fig.14c) was in comparison more than 2 times lower than at station TLM1 station (Fig.6d).

The minimum and maximum percentage of lysogenic cells was measured with 0.02 to 99% in Regelsbrunn (Fig.30b). Second highest value was noted with 73% in TLM1 in July, lowest percentage of 0.6 in June (Fig.6c), whereas in Main Channel the values varied from 0.2 to 38% (Fig.22b). At station 2007 we detected, at the coldest sampling date on 13 October, 12% of the lysogenic cells and the lowest of 0.15% on 18 August at the hottest sampling date with highest water temperature (Tab.4, Fig.14b).

The range from 0.01% to 44% of the lytically infected cells was measured with maximum values at TLM1 (Fig.6c) and minimum in Main Channel (Fig.22c). At station 2007 a value of 0.02 and 1% was noted (Fig.14b), whereas the percentage of lytically infected cells in Regelsbrunn (Fig.30b) was more than 11 times higher than in Main Channel (1%, Fig.22c).

The loss of bacteria per day ranged from 0.01% in Regelsbrunn (Fig.30e) to 44.1% in Main Channel (Fig.22e). At the station 2007, the percentage of bacterial loss per day varied from 0.02 to 1.3 (Fig.14e). TLM1 exhibited 6.6 times higher maximum value than at station 2007 with 8.8% of bacterial loss per day. The lowest value of 0.6% at 2007 was in comparison 60 times lower than in Main Channel (Fig. 14e, Fig.22e).

Comparing different parameters from all experiments

At the station TLM1 the lytical viral production of 0.2 $x10^6$ viruses/mL/h was measured on 18 August at the maximum in temperature (20.2°C, Tab.2, Fig.7a). Overall, no connection was revealed between the oxygen concentrations (Fig.8a), BSP (Fig.9a), chlorophyll *a* concentrations (Fig.10a), PIM (Fig.11a), POM (Fig.12a) and the water level of the River Danube (Tab.2,4,5,6) with the lytical viral production.

The maximum value of lysis rate of bacteria with 0.039 $x10^6$ cells/mL/h was determined at highest BSP with 2.45 µg C/L/h (Fig.9d) and at a maximum concentration of PIM of 22.85 mg/L (Fig.11d). The percentage of the lytically infected cells and of the lysogenic cells showed no correlations with the abiotic parameters temperature (Fig.7b,c), oxygen concentration (Fig.8b,c) and the biotic parameters BSP (Fig. 9b,c) and chlorophyll *a* concentration (Fig.10b,c).

The higest percentage of bacterial loss per day was noted at a maximum value of BSP (Fig.9e) and PIM (Fig.11e).

At station 2007, the lowest values of the viral parameters lytical viral production (1.1×10^{-3} viruses/mL/h, Fig.15a), the lytically infected cells (0.02%, Fig.15c) and the bacterial loss per day (0.02%, Fig.15e) were calculated at lowest temperature of 10.5°C on 13 October. At the maximum oxygen concentration of 9.9 mg/L and of POM with 6.07mg/L the lowest values of the lytical viral productions (Fig.16a), of the lytically infected cells (Fig.16c) and of the bacterial loss per day (Fig.16e) were detected.

In Main Channel, no interdependencies between all measured abiotic and biotic parameters with calculated viral parameters were observed.

At the station Regelsbrunn, the maximum bacterial loss per day was found with 44.1% per day (Fig.31e) at the lowest value of temperature of 10.1°C. The maximum concentration of POM with 98.44 mg/L (Tab.6) was noted at the highest value of bacterial loss per day with 44.1% (Fig.36e) and of the bacterial lysis rate of 4.5×10^3 cells/mL/h (Fig.36d).

To investigate the interdependence of the production of lytical and lysogenic viruses with the hydrological data (Tab. 2, 4, 5, 6), the calculated viral parameters were correlated with the abiotic and biotic parameters.

Positive correlations were detected with bacterial loss per day ($p < 0.05$) and the parameters temperature ($p < 0.049$, Fig.42e), particulate organic matter ($p < 0.001$, Fig.47e) and total suspendid solids ($p < 0.001$, Fig.48e).

To assess the microbial metabolic activity, the hydrolytic enzymes glucosidase and aminopeptidase were fluorometrically measured and calculated as disscused in the report of Sieczko (pers.comm.). The enzyme alpha-glucosidase was in positive correlation with the frequency of lytically infected cells ($p < 0.02$, Fig.49c) and with the bacterial lysis rate ($p < 0.001$, Fig.49 d). We could also prove positve relations between the activity of beta- glucosidase and the lytically infected cells ($p < 0.018$, Fig.50c) and the lysis rate of bacteria ($p < 0.003$, Fig.50d).

The parameters water level of the River Danube (Fig.41a,b,d), temperature (Fig.42a,b,d), oxygen concentration (Fig.43a,b,d), chlorophyll *a* concentration

(Fig.45a,b,d), PIM (Fig.46a,b,d), POM (Fig.47a,b, d), TSS (Fig.48.a,b,d), alpha-glucosidase activity (Fig.49a,b,d) and beta-glucosidase activity (Fig.50a,b,d) were negatively correlated to the lytical viral productions, lysogenic cells and lysis rate of bacteria. The correlation analyses revealed an increase of the bacterial secondary production (BSP) together with the increase of the bacterial lysis rate ($p < 0.012$, Fig.44d).

Viral shunting in aquatic environments

The viral shunting is the transfer of lysis products of the cells into the pool of dissolved organic carbon (DOC) and particulate organic carbon (POC). Furthermore, viruses play an important role in the bacterial mortality and the release of carbon.

The efficiency of viral shunting on the basis of the report from Motegi et al. 2009 was assesed with:

Shunting efficiency (v) = VS/ (BP+VS)

VS = (Lytical viral production [$x10^6$ viruses/L/d]/ Burst size) x Release of dissolved organic carbon [µg C/L/d])

BP = Bacterial secondary production [µg C/L/d]

Overall, a maximum value of 4.1 $x10^{-4}$% was determined at Regelsbrunn (Fig.40d) and the minimum of 8.8 $x10^{-7}$% in TLM1 (Fig.40a). The efficiency of viral shunting in Main Channel was more than 700 x lower as e.g. observed at the station Regelsbrunn (Fig.40c). At station 2007, the values ranged from 7.3 $x10^{-6}$ % to 1.4 $x10^{-5}$ % (Fig.40b).

Viral diversity

In our study we could not detect changes in viral diversity over the period of 5 months. Unfortunately, the Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique did not work as expected. One of the reasons might have been a too low volume of processed water. For the production of virus free-size water as well as of the virus concetrate, we used 1000 mL of the surface water. On the other hand, on some sampling dates we could detect low total number of viral as well as of bacterial abundances in each of the locations. The second problem was the high appearance of particulate

matter on which viruses were attachted. Therefore, viruses were lost during the first filtration steps. We produced a virus concetrate with a low total number of viral abundance and with a high fraction of humic substances. In further studies, we should produce a virus concetrate from more than 1000 mL water as well as we need to treat the humic substances.

Discussion

Characterisation of abiotic and biotic parameters under varying hydrology

Our study was conducted in the interval from June to October 2009, when changes in the hydrology and chemistry as well as the microbial parameters were observed.

The hydrological dynamics of fresh waters together with the general biodiversity give the opportunity to establish dynamic microbial communities, primary and secondary producer as well as influence the quality and the quantity of particulate matter (Tockner et al. 1998).

Surface waters typically display large differences in abiotic and biotic parameters over time. In freshwater ecosystems dissolved organic matter (DOM) constitutes a major fraction of the organic matter pool. It is the main source which supports growth and respiration of bacteria. Since the DOM pool is comprised of different compounds, it varies in its bioavailability, thus the differences in DOM composition can alter the activity of microbial communities (Schuiwang et al. 2007). A factor which decides about the amount of DOM released by phytoplankton is light intensity, which can enhance the above process (Findlay et al. 2004). The highest light intensity was noted at the Main Channel station with 1389.1 µmol/m^2/s (the lowest value of 1.7 µmol/m^2/s in station TLM1), which could also cause elevated release of DOM (Urabe et al. 2002 and Sieczko, pers.comm.).

In our investigation high chlorophyll *a* concentrations were observed on some monitoring dates (Tab.1). We investigated the chlorophyll *a* concentrations over a short time interval, whereas the study of Schiemer et al (2006) monitored a longer period, where concentration of chlorophyll *a* was lower when the system was completely disconnected in comparison to connected locations. Therefore, we could not define perticular connected or disconnected locations, but we detected during the flood event the lowest chlorophyll *a* concentrations in each of the stations in June and July. Aspetsberger et al. (2002) determined occasionally high chlorophyll *a* concentrations in the same floodplain area. The same tendency was noted for the particulate organic matter (POM) with high

values reported by Hein et al. (2004). In our results, we observed the highest value with 98.44 mg/L dry mass in Regelsbrunn (Tab.6).

Based on the survey reported by Aspetsberger et al. (2002), the BSP (bacterial secondary production) showed linear increase with water age, which couldn´t be found during our study. In our results, we detected dissimilarities in the rates of BSP whith the highest value of 2.45 µg C/L/h at the location Regelsbrunn which was more than 5 times higher than the lowest measured BSP value at station 2007.

A parameter influencing the bacterial activity (reflected as bacterial production and enzymatic activity) is DOC (dissolved organic carbon) concentration. Biddandah et at. (1994) evidenced that with the increase of DOC the bacterial production increases. Furthermore, these changes depend also on other factors such as temperature and nutrients (Findlay and Sinsabaugh et al. 1998). Nervertheless, in our study, the highest concentration of DOC (38.09 mg C/L) was observed in station TLM1, where the lowest BSP rate of 0.474 µg C/L/h was detected.

Comparison of free-living and attached microbial abundance

As discussed by Kirchman et al. (1982), about 10% of the bacterial population may be particle-associated. Furthermore, based on the survey conducted in the Danube by Luef et al. (2007), the attached bacterial abundance reached 30.3% in the location Regelsbrunn and 41.6% in the station Main Channel. On average, it has been found that the proportion of particle-associated bacteria, in comparison to the free-living bacteria, was 30%. The ratio of attached viruses to the total number of viral abundance showed a percentage between 0.4 to 35% (Luef et al. 2007).

However, in our study, the ratio of particle-associated bacteria to the overall bacterial abundance was on average 15% in station TLM1 and 17% in station 2007. In Main Channel, we could observe a ratio of 15% of attached bacteria. Furthermore, in Regelsbrunn an average of 19% was noted. Overall, considerable differences between the two size fractions were established on each station.

From fresh water studies, focusing on particles, organic matter seems to be important for bacterial abundance. Typically, the > 3µm size fraction was more active than the free-living size fraction as discussed by Kirchman et al. (1982) and Peduzzi & Schiemer et al. (2008). Cammen et al. (1982) reported that the attached bacteria play an important role for particulate matter. In our study, however, the bacterial abundance associated with particles showed large differences in each of the locations with the maximum of 89.9% (Fig.21a).

In Regelsbrunn the > 3µm bacterial size fraction increased directly with the concentration of the particulate organic matter. In the stations 2007 and TLM1, there were no relations between the bacteria associated on particles and the concentration of particulate matter. Our findings underline inconsistent differences between attached bacterial abundance at each station. Moreover, the bacteria attached on particles did not evidence any relations to the BSP increase.

At the investigated locations, the free viral abundance of the surface water was 0.1 (Fig.4a, b) to 11.6 times the bacterial population (Fig.13a, b). Our data indicated maximum value of 99.7% (station TLM1), 94.4% (station 2007), 69.8% (Main Channel) and 97.4% (Regelsbrunn) of viruses associated with particles (original data are shown in Tab.1). In all water bodies, the virus- attached fraction reached more than 60% of the overall abundance.

Furthermore, the particulate matter concentration was positively related with the viruses which colonized 16% (TLM1), 16% (2007), 12% (Main Channel) and 17% (Regelsbrunn) of the particulate matter.

We could detect a maximum value of viruses attached to particles of 30.9 $x10^6$ viruses/mL in station TLM1 (Fig.4b) and the lowest of 4 $x10^5$ viruses/mL in Regelsbrunn (Fig.29b). As discussed by Wilhelm et al. (1999), in coastal marine environments the viruses reached abundances of 10^{10} per liter and in other marine habitats 10^7 to 10^{11} per liter on particles. In our study the viral abundance varied from 10^9 to 10^{11} per liter on particles, which was similar to the results from this report.

Also, we tried to detect the total number of viruses in the free-living fraction. Therefore, we removed the > 3µm size-fraction by filtration. There were dissimilarities of the free-living viral fractions at each location with 0.5 $x10^6$

viruses/mL in Regelsbrunn, 17.9 x10^6 viruses/mL in Main Channel, 51.8 x10^6 viruses/mL in station TLM1 and 36 x10^6 viruses/mL in the location 2007.

VBR

The Virus to Bacterium Ratio (VBR) is documenting the general predominance of the viral over the bacterial abundance (Danovaro and Serresi et al. 2000). Danovaro et al. 2008 reported that viruses influence the microbial dynamic also in aquatic sediments. In marine (Bergh et al. 1989), coastal (Suttle et al. 1990) and fresh waters (Klut, Stockner et al. 1990) there were high pelagic viral abundances observed.

In fresh water the VBR ranged from 0.7 to 1.2 as discussed by Lemke et al. (1997). In our study the VBRs were higher than reported by Lemke (original data of our study shown in Fig.4c,d, 13c,d, 21c,d, 29c,d). Typical values in freshwater of around 2.0 are reported in Peduzzi and Luef (2009).

Mean abundance of the viruses associated with particles increased together with the bacterial attached fraction. The predominance of viruses over bacteria on particles ranged from 0.4 (Fig.29c,d) to 27.5 (Fig.13c,d) in comparison to the lower VBRs of 0.01 and 1.2 reported by Taylor (2003).

According to our values, we could not establish any relations between the chlorophyll *a* concentration and VBRs. Furthermore, we could not detect any correlations between the variability of the viral and bacterial abundances of either the attached or the free-living fraction. In the literature, a negative relation of the chlorophyll *a* concentration with viruses was found (Luef et al. 2007). In lake snow, the VBRs ranged from 0.3 to 8.5 as reported by Simon (2002), which was lower than detected in our study.

Virus Reduction Approach (VRA) experiments

Viruses, infecting all components of the microbial food web, play an important role also in running waters (Peduzzi and Luef et al. 2009). To investigate which of the most common viral life cycles, the lytic or lysogenic one, is more important, we compared the viral abundances of the surface waters of several locations of the River Danube and its back waters, as well as the viral parameters lytical viral production, lytically infected cells, lysogenic cells,

bacterial loss per day and lysis rate of bacteria. The viral parameters were calculated using the online tool program VIPCAL based on the VRA as reported by Luef et al. (2009). Lytic viral production is the difference between viral abundance in the stationary phase of the control incubations and viral abundance at the start of the experiment. Lysogenic viral production is the difference between viral abundance in the Mitomycin-C treated and viral abundance in the control incubations (Weinbauer et al. 2002).

We compared the viral abundances of the with and without Mitomycin-C treated samples to investigate which of the viral life cycles is more important in the investigated riverine waters. Our results were also compared with references of viral abundances from marine samples. There are no results from freshwater environments so far. As reported by e.g. Ripp and Miller et al. (1997), there is a third common viral life cycle, the pseudolysogeny, which is a phenomen of viruses between lysogenic or lytic production. It's possible that there are such viruses in our samples, therefore we should take into acount this third possibility, the pseudolysogeny as biasing our results.

In our study, large differences in the lytical viral production were determined as well as in the frequency of lysogenic and lytically infected cells over time in each of the stations. Overall, in all water bodies the lytical viral production (Fig.6a, 14a, 22a, 30a) exhibited major changes among the stations on each sampling date (Tab.1).

In station Regelsbrunn, we could detect a maximum of lysogenic cells of 99% and a minimum of total bacterial abundance with 2 $x10^5$ cells/mL of surface water. In station TLM1, we observed 73% of lysogenic cells which was 2 times higher than in Main Channel. In each of the locations, there were found dissimilarities in the lysogenic cells over time, maybe because of the weather changes as well as of the flood event in June 2009.

It was hypothesized that the lysogenic viral production is an important survival mechanism of viruses in low host cell populations, and there are typically up to 40% of lysogenic bacteria in marine oligotophic environments (Williamson and Paul et al. 2004). There are no results about the lysogenic viral production in an aquatic environment as the River Danube and its back waters. Maybe, over a

longer period, we would observe a consistent percentage of lysogenic infected cells in each of the locations, but more analyses are required.

However, in another study, from marine environments, often 90% of bacteriophages are reported as temperate viruses (Freifelder, 1987). They may contribute to nutrient cycling and may act as factors for genetic exchange and horizontal gene transfer (Chiura et al. 1997). We found maximum and minimum of viral lytical production in stations TLM1 and Regelsbrunn. In stations 2007 and Main Channel, we illustrated a low percentage of lytically (0.3%, 3.5%) and lysogenic (5.4%, 12.8%) infected cells.

Furhter, we found a ratio between PIM and POM of 2:1 in station 2007 and 10:1 in Main Channel. Therefore, most of the viruses could be bound to the particulate matter. However, there are other major abiotic and biotic parameters which could control the viral production.

Moreover, the viruses may impact the nutrient cycle in aquatic systems. Carbon is an important element reflecting the energy flux of organisms. The organic carbon is separated into dissolved organic carbon (DOC) and particulate organic carbon (POC). Much of the DOC is recycled in the microbial loop (Falkowski al. 1992), and the lysis rate of bacteria is directly responsible for some of the DOC (Fuhrman et al. 1992, Wilhelm et al. 1998). In our study, however, the highest value of the release of DOC due to viruses was 7.96 pg C/mL/h in station TLM1, where we observed the highest rate of BSP with 2.45 µg C/L/h and the maximum of bacterial abundance of the free-living fraction with 10.8 $x10^6$ cells/mL.

To establish any relations between viruses and the aquatic environment, we compared the viral parameters of the VRA experiments with the following abiotic and biotic parameters: temperature, oxygen concentration, and chlorophyll *a* concentration, BSP, PIM, POM and TSS.

We could detect negative correlations between the viral parameters bacterial lysis rate, lytically infected and lysogenic cells with oxygen concentration, chlorophyll *a* concentration, and PIM. In our study, the oxygen concentration increased with lower percentage of lysogenic and lytically infected cells as well as with low lysis rates of bacteria ($p < 0.911$, Fig.45b, $p < 0.146$, Fig.45c, $p < 0.251$, Fig.45d). On the other hand, the viral production decreased with the

increase of chlorophyll *a* concentration and PIM ($p < 0.359$, Fig.45a, $p < 0.521$, Fig.46a).

Moreover, we could not observe any correlation between the water level of the River Danube and the viral parameters (Fig.41a,b,c,d,e). The reason for such results could be the flood event, which is responsible for extreme changes in the water level of the River Danube and its back waters.

Also positive correlations have been determined between alpha- and beta-glucosidase activity data from Sieczko (unpublished) and the frequency of lytically infected cells ($p < 0.002$, Fig.49c, $p < 0.018$, Fig.50c) as well as the bacterial lysis rate ($p < 0.001$, Fig. 49d, $p < 0.003$, Fig.50d). The reason for such results is still unclear. Up to now, we are sure that the activity of enzymes for beta-linked substrate usually exceeded the activity of alpha-glucosidase as also reported by Bhaskar et al. (2008). Elevated enzymatic activity may be triggered by the increased availability of lysis products.

Moreover, in our investigation, the viral parameters lytically infected cells and bacterial loss per day decreased together with increase of temperature ($p < 0.651$, Fig.42c, $p < 0.049$, Fig.42e). In sediments, as reported by Baker et al. (2004), 18% of bacterial mortality/loss due to viruses was detected. In our results, we established an increase of bacterial loss per day together with POM and TSS ($p < 0.001$, Fig.47e, $p < 0.001$, Fig.48e).

On the basis of the report from Noble et al. (2000), there is a contribution of viruses to bacterial mortality that enhances the bacterial production and production of the organic matter.

Viral shunting in aquatic environments

As discussed by Suttle et al. (2005), viruses of marine environments are important catalists for the biogeochemical cycles, and responsible for shunting off a part of the flux from the pool of the dissolved organic carbon (DOC) to the pool of the particulate organic carbon (POC). Moreover, the efficiency of the transfer of DOC decreases in higher trophic levels, and more carbon should be respired in the surface water.

Overall, the viral shunting is reported as an important phenomenon contributing to the carbon flux to the atmosphere. Danovaro (2008) reported that the viral

shunting is a significant source of organic detritus in the deep-sea ecosystem, and responsible for the release of 0.37 to 0.63 gt of carbon per year from the sea.

We investigated the possibility of the influence of viruses on the microbial loop in a riverine system and detected many dissimilarities in the viral shunting efficiency at each of the stations with a maximum of 4.1 $x10^{-4}$% in Regelsbrunn (Fig.40d) and the minimum of 8.8 $x10^{-7}$% in station TLM1 (Fig.40a). There are no such previous results for inland water environments. Potentially, the DOC is consumed by bacteria, some of them lysed by viruses . (Wilhelm et al. 1999). Therefore, one part of the DOC is shunted off by viruses and lost for other consumers of the microbial food web. However, more and detailed studies are necessary for a better understanding of such riverine systems.

Viral diversity

In our study, we could not detect changes in viral diversity over the period of 5 months, the Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique did not work as expected. We will have to improve this technique to study the changes in viral communities. We still don`t know the exact reasons for the malfunction of the RAPD-PCR for later analyses of a freshwater environment such as the River Danube and its back waters.

References

1. **Aspetsberger, F., Huber, F., Kargl, S., Scharinger, B, Peduzzi, P., and Hein, T.** (2002) Particulate organic matter dynamics in a river floodplain system: impact of hydrological connectivity. *Hydrobiology* 156: 23-42

2. **Baker, W., P., and Lef, G., L.** (2004) Seasonal patterns of a abundance of viruses and bacteria in a Northeast Ohio (USA) stream. *Archiv für Hydrobiologie* 161: 225-233

3. **Bergh, Ø., and Børsheim, Y., K.** (1989) High abundance of viruses found in aquatic environments. *Nature* 340: 467-468

4. **Besemer, K., Moeseneder, M., Arrieta, M. J., Herndl, J. G., and Peduzzi, P.** (2005) Complexity of Bacterial Communities in a River-Floodplain System (Danube, Austria). *Applied and Environmental Microbiology* 71: 609-620

5. **Besemer, K., and Peduzzi, P.** (2004) The importance of microorganisms and particles in the Danube River floodplains: an experimental Approach. *Diplom thesis*

6. **Biddandah, B, Opsahl, S., and Benner, R.** (1994) Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf. *Limnology and Oceanography* 39: 1259-1275

7. **Cammen, L., M., and Walker, J., A.** (1982) Distribution and activity of attached and free-living suspended bacteria in the Bay of Fundy. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 2655-1663

8. **Chiura, X., H.** (1997) Generalized gene transfer by virus-like particles from marine bacteria. *Aquatic Microbial Ecology* 13: 75-83

9. **Danovaro, R., Dell`Anno, A., Corinaldesi, C., Magagnini, M, Noble, R., Tamburini, C., and Weinbauer, M.** (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature* 454:
1084-1089

10. **Danovaro, R., and Serresi, M.** (2000) Viral Density and Virus-to-Bacterium Ratio in Deep-Sea Sediments of the Eastern Mediterranean. *Applied amd Environmental Microbiology* 66: 1857-1861

11. **de Groot, S. R., Wilson, A. M., and Bourmans, M. J. R.** (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecological Economics* 41: 393-408

12. **Falkowski, G., P, and Woodhead, D., A.** (1992) Primary production and biogeochemical cycles in the sea. *Environmental Science Research* 43

13. **Findlay, E., G., S., and Sinsabaugh, L., R.** (2004) Aquatic ecosystems: interactivity of dissolved organic matter. *Journal of the North American Benthological Society* 23: 662-663

14. **Findlay, E., G., S., Sinsabaugh, L., R., Fischer, D., T., and Franchini, P.** (1998) Soruces of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* 1: 227-239

15. **Freifelder, D.** (1987) Molecular biology. *Jones and Bartlett, Inc., Boston*

16. **Fuhrman, A., J.** (1992) Bacterioplankton roles in cycling of organic matter: The microbial food web. In: Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York: 361-383

17. **Hein, T., Baranayi, C., Reckendorfer, W., and Schiemer, F.** (2004) The impact of surface water exchange on the nutrient and particle dynamics in side-arms along the River Danube, Austria. *Science of the Total Environment* 328: 207-218

18. **Helton, R. R., and Wommack, K. E.** (2009) Seasonal Dynamics and Metagenomic Characterization of Estuarine Viriobenthos Assemblages by Randomly Amplified Polymorphic DNA PCR. *Applied and Environmental Microbiology* 75: 2259-2265

19. **Hohensinner, S., Haidvogl, G., Jungwirth, M., Muhar, S., Preis, S., and Schmutz., S.** (2005) Historical analysis of habitat turnover and age distributions as a reference for restoration of Austrian Danue floodplains. *River Basin Managment III*: 489-502

20. **Jungwirth, M., Muhar, S., and Schmutz, S.** (2002) Re-establishing and assesing ecological integrity in riverine landscapes. *Freshwater Biology* 47: 867-887

21. **Kirchmann, D., and Mitchell, R.** (1982) Contribution of Particle-Bound Bacteria to Total Microheterotrophic Activity in Five Ponds and two Marches. *Applied and Environmental Microbiology* 43: 200-209

22. **Klut, E., M., and Stockner, G., J.** (1990) Virus-like particles in an ultra-oligotrophic lake on Vancouver Island. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 7225-730

23. **Lemke, M., Wickstrom, C., and Leff, L.** (1997) Preliminary study on the distribution of viruses and bacteria in lotic environments. *Archiv für Hydrobiologie* 141: 67-74

24. **Luef, B., Aspentberger, F. Hein, T., Huber, F., and Peduzzi, P.** (2007) Impact of hydrology on free-living and particle-associated microorganisms in a river floodplain system (Danube, Austria). *Freshwater Biology* 52: 1043-1057

25. **Luef, B., Luef, F., and Peduzzi, P.** (2009) Online program 'vipcal' for calculating lytic viral production and lysogenic cells based on a viral reduction approach. *Environmental Microbiology* 1: 78-85

26. **Luef, B., Neu, R.T., and Peduzzi, P.** (2009) Imaging and quantifying virus fluorescence signals on aquatic aggregates: a new method and its implication for aquatic microbial ecology. *Microbial Ecolology* 68: 372-380

27. **Motegi, C., Nagata, T., Miki, T., Weinbauer, G. M., Legendre, L., and Rassoulzadegan, F.** (2009) Viral control of bacterial growth efficiency in marine pelagic environments. *Limnology and Oceanography* 54: 1901-1910

28. **Noble, T. R., and Fuhrman, A. J.** (1998) Use of SYBR Green I for rapid epifluorescence counts if marine viruses and bacteria. *Aquatic Micrbial Ecology* 14: 113-118

29. **Noble, T. R., and Fuhrman, A. J.** (2000) Rapid Virus Production and Removal as Measured with Fluorescently Labled Viruses as Tracers. *Applied and Environmental Microbiology* 66: 3790-3797

30. **Peduzzi, P., and Luef, B. (**2009) Viruses. *Gene E. Likens* 3: 279-294

31. **Peduzzi, P., and Schiemer, F.** (2008) Bacterial dynamics, nutrients and organic matter in the water column of tropical freshwater reservoirs of Sri

Lanka. In Schiemer, F., Simon, D., Amarasinghe, U., and Moreau, J. (eds): Aquatic ecosystem and development: Comparative Asian perspectives; Backhuys Publishers, Leiden, Margraf Publishers, Weikersheim; 135-152

32. **Push, M.** (1998) The role of micro-organisms in the ecological connectivity of running waters. *Freshwater Ecology* 40: 453-495

33. **Ripp, S., and Miller, V., R.** (1997) The role of pseudolysogeny in bacteriophage-host interactions in anatural freshwater environment. *Microbiology* 143: 2065-2070

34. **Schiemer, F. Hein, T., and Peduzzi, P.** (2006) Hydrological control of system characteristics of floodplain lakes. *Ecohydrology* 6: 1-4, 7-18

35. **Schuiwang, D., Bianchi, S. T, and Sampere, S. P.** (2007) Temporal variability in the composition and abundance of terrestrially-derived dissolved organic matter in the lower Mississippi and Pearl Rivers. *Marine Chemistry* 103: 172-184

36. **Simon, M., Grossart, H.,-P., Schweitzer, B., and Ploug, H.** (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquatic Microbial Ecology* 28: 175-211

37. **Suttle, C., A.** (2007) Marine viruses – major players in the global ecosystem. *Nature* 5: 801-812

38. **Suttle, C., A.** (2005) Viruses in the sea. *Nature* 437: 356-361

39. **Suttle, C., A., Chan, M., A., and Cottrell, T., M.** (1990) Infection of phytoplankton by viruses and reduction of primary productivity. *Nature* 347: 467-469

40. **Tockner, K., Pennetzdorfer, D., Reiner, N., Schiemer, F., and Ward, V. J.** (2001) Hyrdological connectivity, and the exchange of organic matter and nutrients in a dynamic-river-floodplain system (Danube, Austria). *Freshwater Biology* 41: 521-535

41. **Tockner, K., and Schiemer, F.** (1997) Ecological aspects of the restoration strategy for a river-floodplain system in the Danube River in Austria. *Global Ecology and Biogeography Letters* 6: 321-329

42. **Tockner, K., Schiemer, F., and Ward, V. J.** (1998) Conservation by restoration: the management concept for a river-floodplain system on the DanubeRiver in Austria. *Aquatic Conservation: Marine and Freshwater Ecosystems* 8: 71-86

43. **Urabe, J., Kyle, M., Makino, W., Yoshida, T., Andersen, T., and Elser, J., J.** (2002) Reduces light increases herbivore production due to stochiometric effects of light/nutrient balance. *Ecological Society of America* 83: 619-627

44. **Ward, V., J., Malard, F., and Tockner, K.** (2002) Landscape ecology: a framework for integrating pattern and process in river corridors. *Lanscape Ecology* 17: 35-45

45. **Weinbauer, G. M., Winter, C., and Höfle, G. M**. (2002) Reconsidering transmission electron microscopy based estimates of viral infection of bacterioplankton using conversion factors derived from natural communities. *Aquatic Microbial Ecology* 27: 103-110

46. **Wilhelm, W. S., and Suttle, A. C**. (1999) Viruses and Nutrient Cycles in the Sea: Viruses play critical roles in the structure and function of aquatic food webs. *BioScience* 49: 781-78

47. **Wilhelm, W., S., Weinbauer, G., M., Suttle, A., C., and Jeffrey, H., W.** (1998) The role of sunlight in the removal and repair of viruses in the sea. *American Society of Limnology and Oceanography* 43: 586-592

48. **Williamson, J., S., and Paul, H., J.** (2004) Nutrient stimulation of lytic phage production in bacterial populations of the Gulf of Mexico. Aquatic Microbial Ecology 36: 9-17

49. **Winget, M. D., and Wommack, K. E.** (2008) Randomly Amplified Polymorphic DNA PCR as a Tool for Assesment of Marine Viral Richness. *Applied and Environmental Microbiology* 74: 2612-2618

Figures and Tables

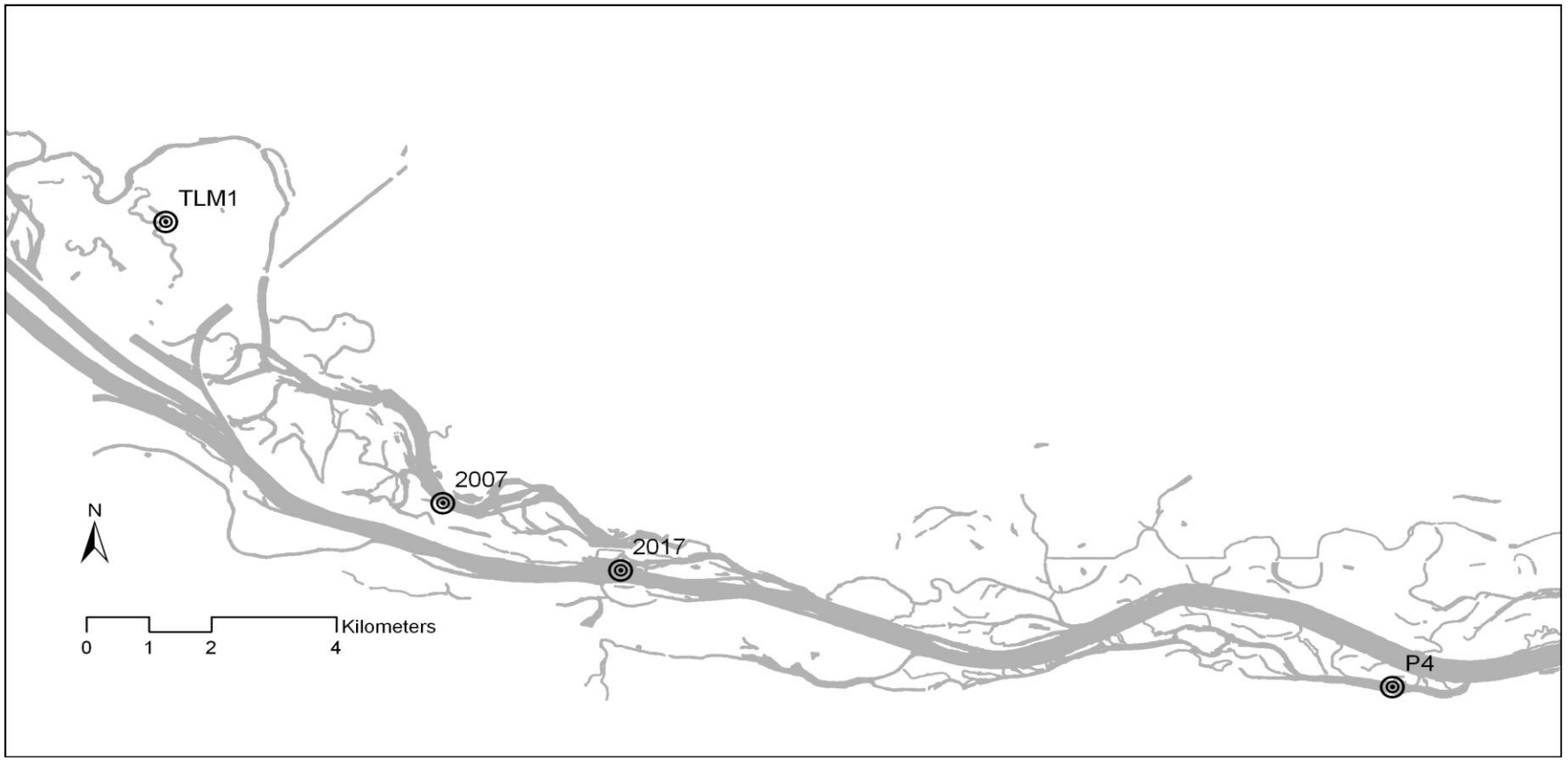


Figure 1. The four stations located in back waters of the National Park of the River Danube; the station 2007 has a low transport of water and sediment, TLM1 is a water body without macrophytes and the dynamic water body Main Channel of the River Danube (2017). Outside this area is the station Regelsbrunn, a dynamically connected side arm (P4).

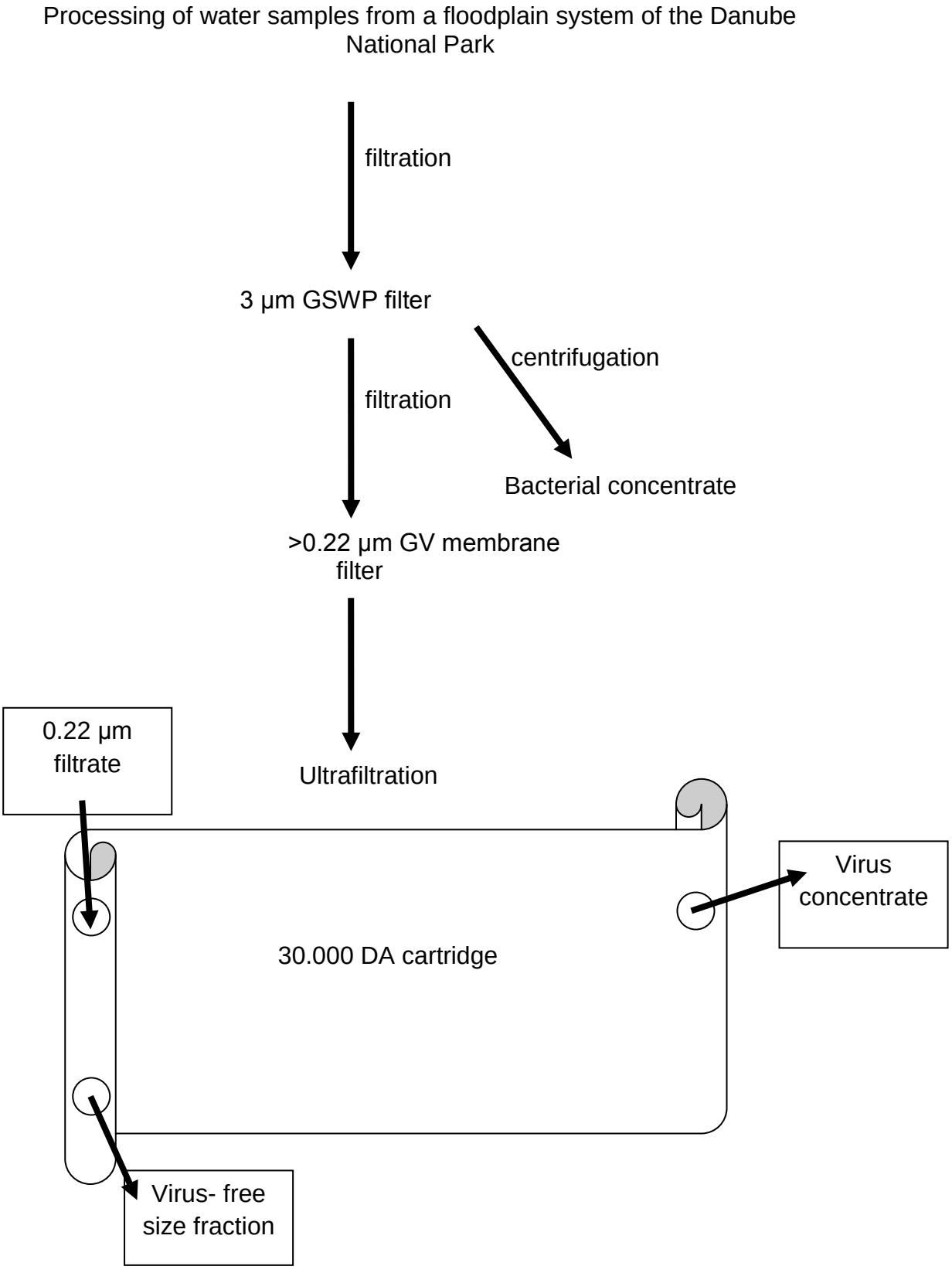


Figure 2. Filtration steps for Virus Reduction Approach (VRA) experiments

Days of sampling			
d1	16062009	d6	21072009
d2	26062009	d7	30072009
d3	30062009	d8	18082009
d4	03072009	d9	15092009
d5	09072009	d10	13102009

Table 1. Sampling dates of each station in the interval from June to October 2009. In June a flood event led to frequent sampling of the fresh waters in the Danube National Park and its back waters. The days of the frequent monitoring were days of sampling of the surface water over a long period of some years. During the experimental part of my diploma thesis the surface water was sampled during the frequent monitoring over a short period of 5 months. (d= days; the yellow marked dates = dates of frequently sampling; the underlined dates = dates of monitoring).

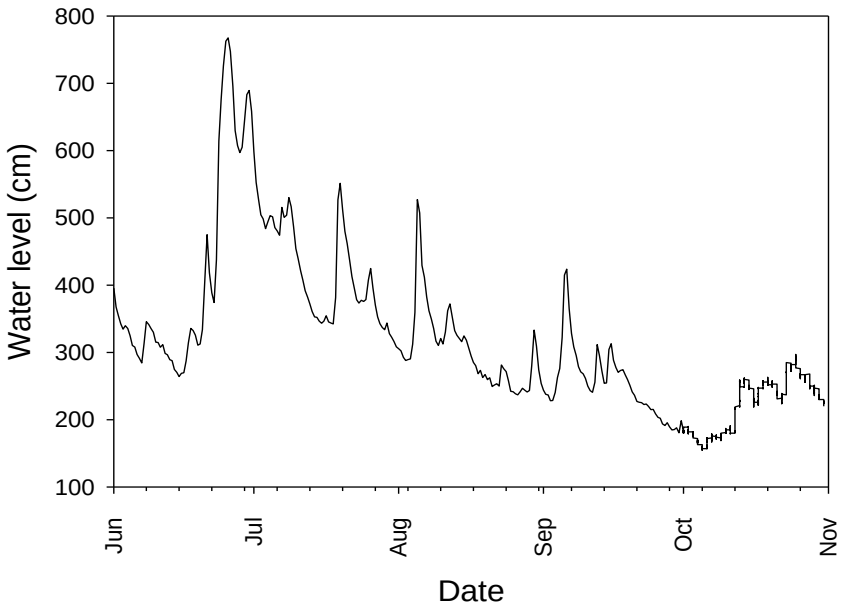


Figure 3. Water level of the River Danube in the interval from June to October 2009; orignal data from via Donau.

station TLM1/Lobau											
days	**T [C°]**	**pH**	**O_2 [mg/L]**	**DOC [mg C/L]**	**BSP [µg C/L/h]**	**Chl *a* [µg/L]**	**PIM [mg/L]**	**POM [mg/L]**	**TSS [mg/L]**	**AGA [nm/h]**	**BGA [nm/h]**
16062009	16.7	7.75	3.0	22.87	0.771	3.26	4.48	5.53	10.01	50.20	51.02
26062009	16.6	7.8	4.5	21.22	0.680	1.60	2.33	2.12	4.45	32.20	39.53
30062009	19.1	8.01	5.0	22.93	0.558	3.19	0.71	1.76	2.47	40.15	47.57
03072009	19.4	7.96	2.9	28.64	0.531	1.35	0.71	1.45	2.16	27.28	29.03
09072009	17.7	8.04	4.0	30.42	0.761	4.35	4.26	2.70	6.96	26.83	35.16
21072009	18.1	8.10	3.2	29.88	0.376	7.95	0.69	3.6	4.29	16.55	21.62
30072009	18.8	8.08	3.08	32.03	0.772	12.73	0.98	4.04	5.02	11.49	20.04
18082009	20.2	8.0	2.12	30.48	1.013	7.86	17.12	8.57	25.69	30.12	36.46
15092009	16.3	7.8	4.0	27.81	2.446	5.24	22.85	9.37	32.22	86.54	143.13
13102009	9.6	7.80	1.19	38.09	0.474	5.86	11.57	15.93	27.50	28.14	149.69

Table 2. Measured parameters of the station TLM1/Lobau; T = Temperature; DOC = Dissolved organic matter; pH; O_2 = Oxygen concentration; BSP = Bacterial secondary production; Chl *a* = Chlorophyll *a* concentration; PIM = Particulate inorganic matter; POM = Particulate organic matter, TSS = Total suspendid solids, AGA = Alpha-glucosidase activity and BGA = Beta-glucosidase activity

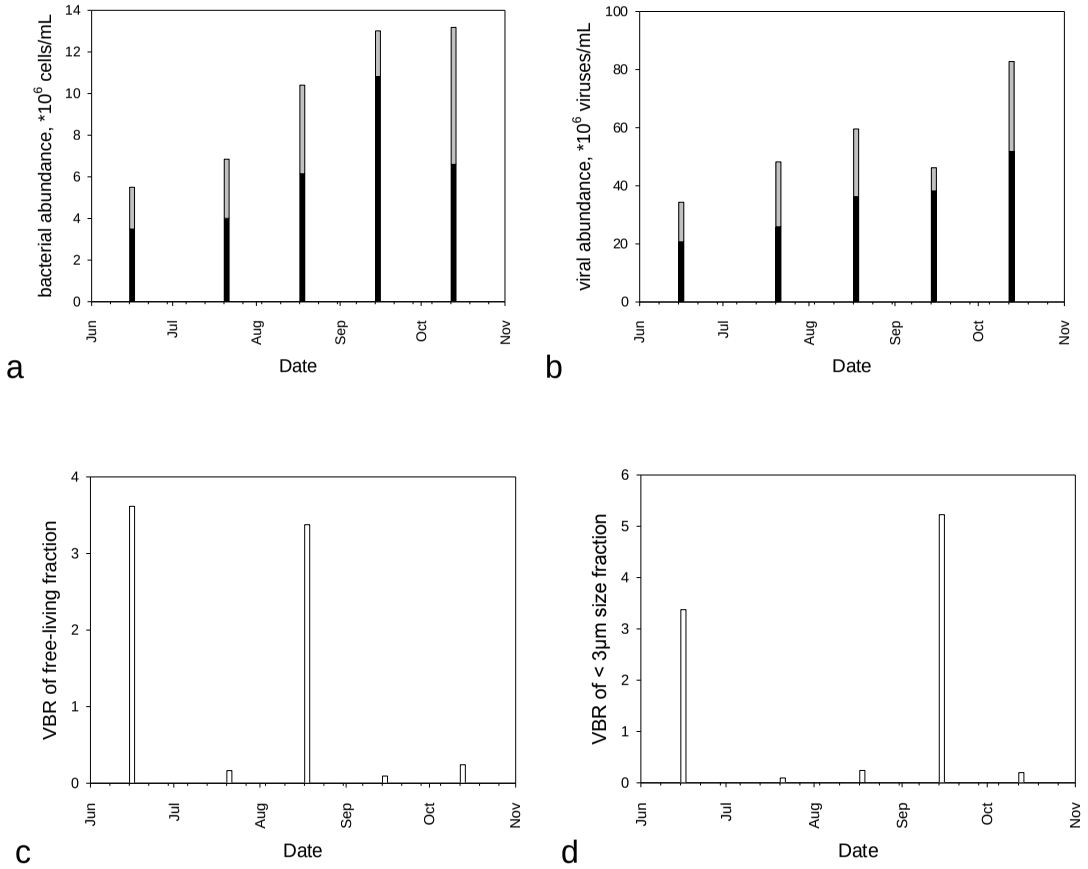


Figure 4. Free-living and attached bacterial (a) and viral abundance (b), Virus to Bacterium Ratio (VBR) of free-living (c) and attached fraction (d) of the station TLM1/Lobau in the interval from June to October 2009 (grey bar = free-living bacterial and viral abundance; black bar = attached bacterial and viral abundance; white bar = VBR of free-living and > 3µm size fraction; sampling dates are shown in Tab.1).

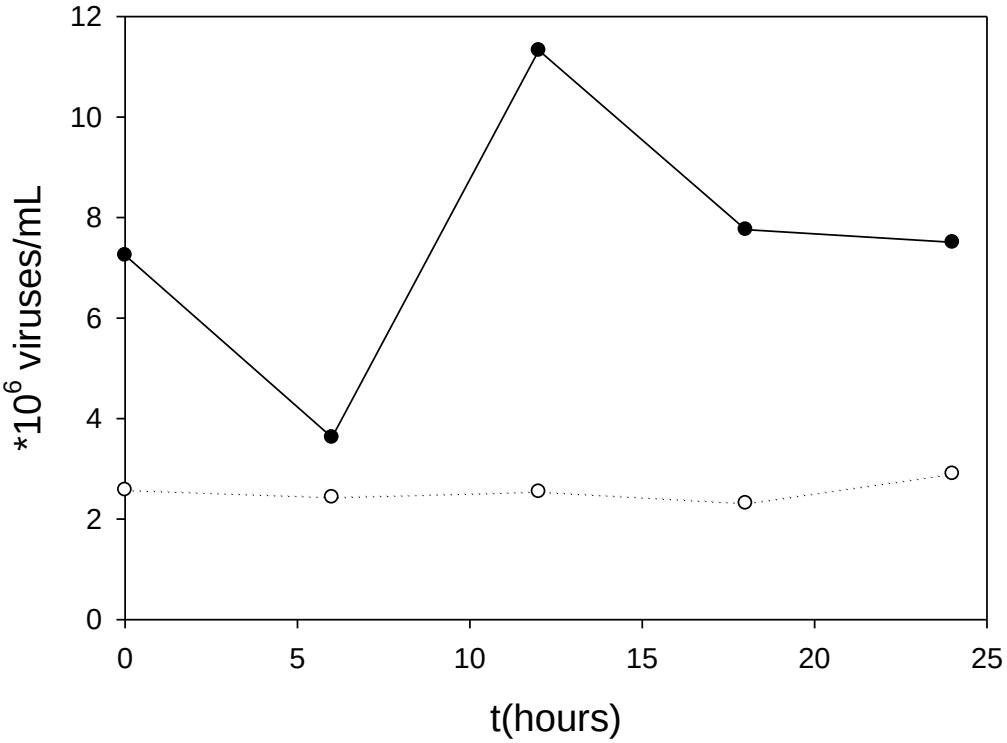


Figure 5. Lysogeny and lytic viral production from Virus Reduction Approach (VRA) of the station TLM1/Lobau in June 2009; black dots = Mitomyicin-C treated sample, open dots = untreated sample

VIPCAL: Viral Production Calculator	
lytic viral production (*10^6 viruses/mL/h)	**0.0577**
% of lytically infected cells	**0.3236**
lysis rate of bacteria (*10^6 cells/mL/h)	**0.0009**
% of bacterial loss per day	**0.6471**
% of lysogenic cells	**3.5497**
viral turnover times (h^{-1})	**0.0023**
DOC release (g C mL/h)	**1.8831** *10^{-11}
DON release (g N/mL/h)	**3.7661** *10^{-12}

Table 3. Calculated parameters from the online tool program VIPCAL

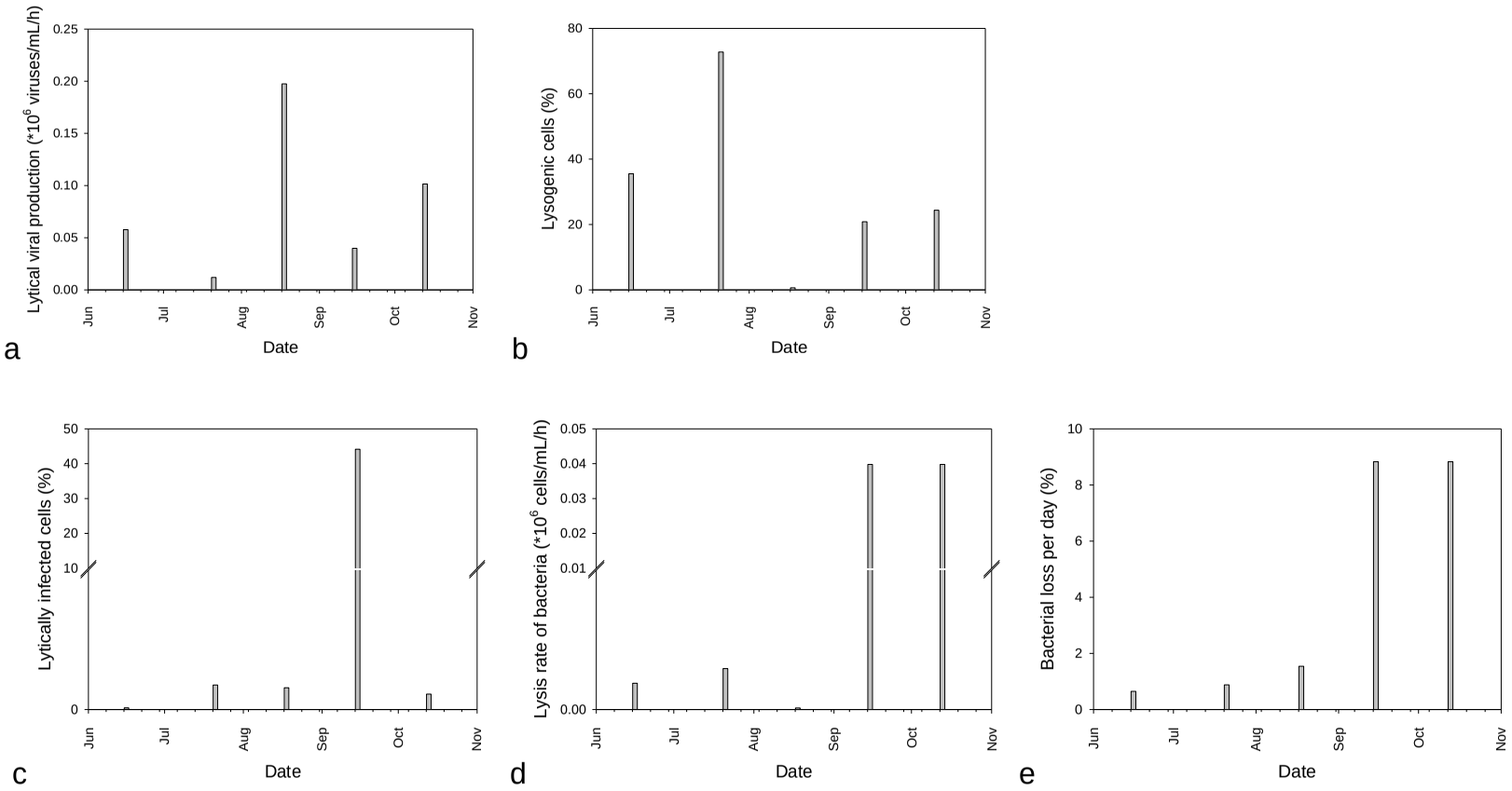


Figure 6. Lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments at the station TLM1/Lobau in the interval from June to October 2009 (grey bar = viral parameters from VRA; sampling dates are shown in Tab.1).

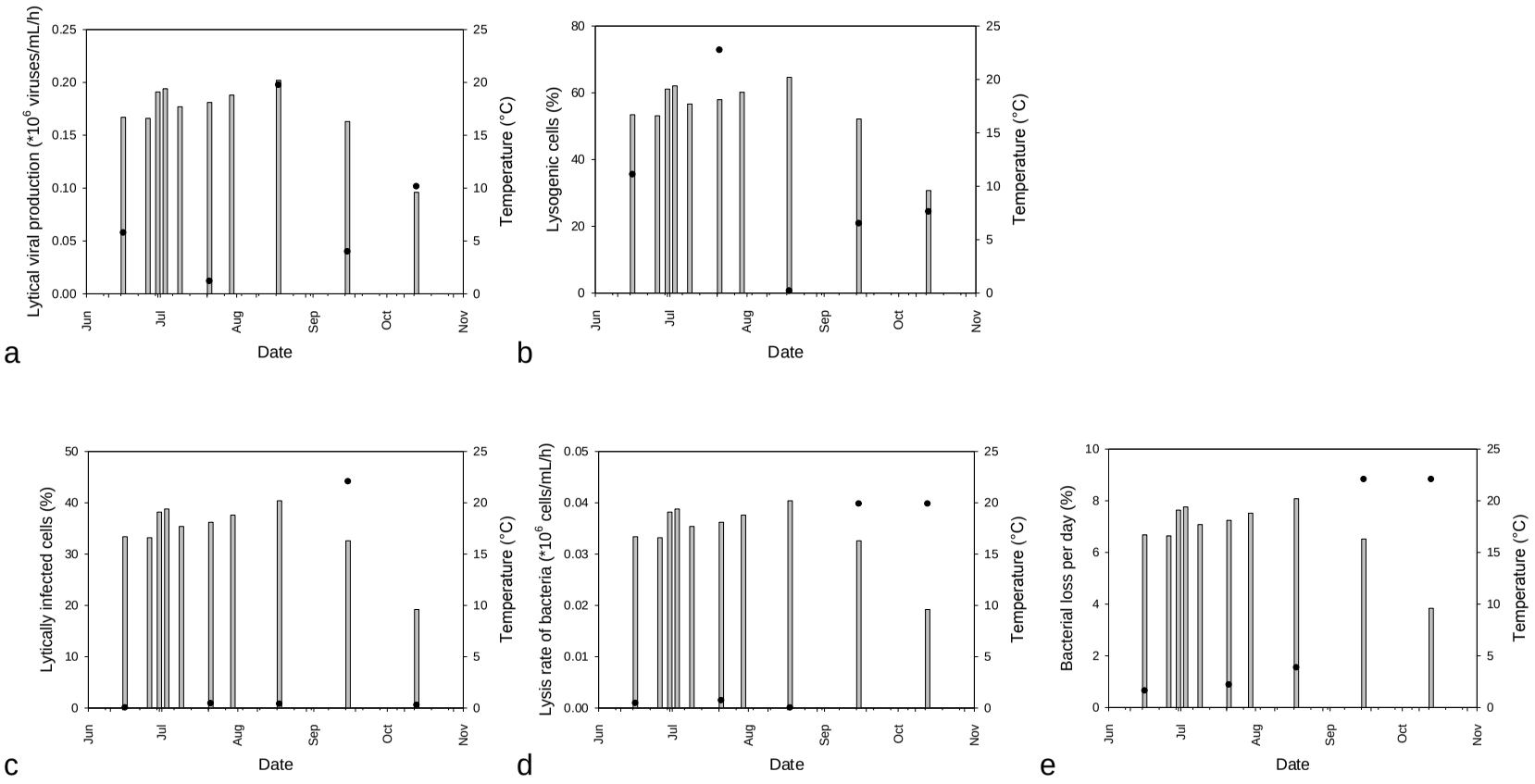


Figure 7. The abiotic parameter temperature is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

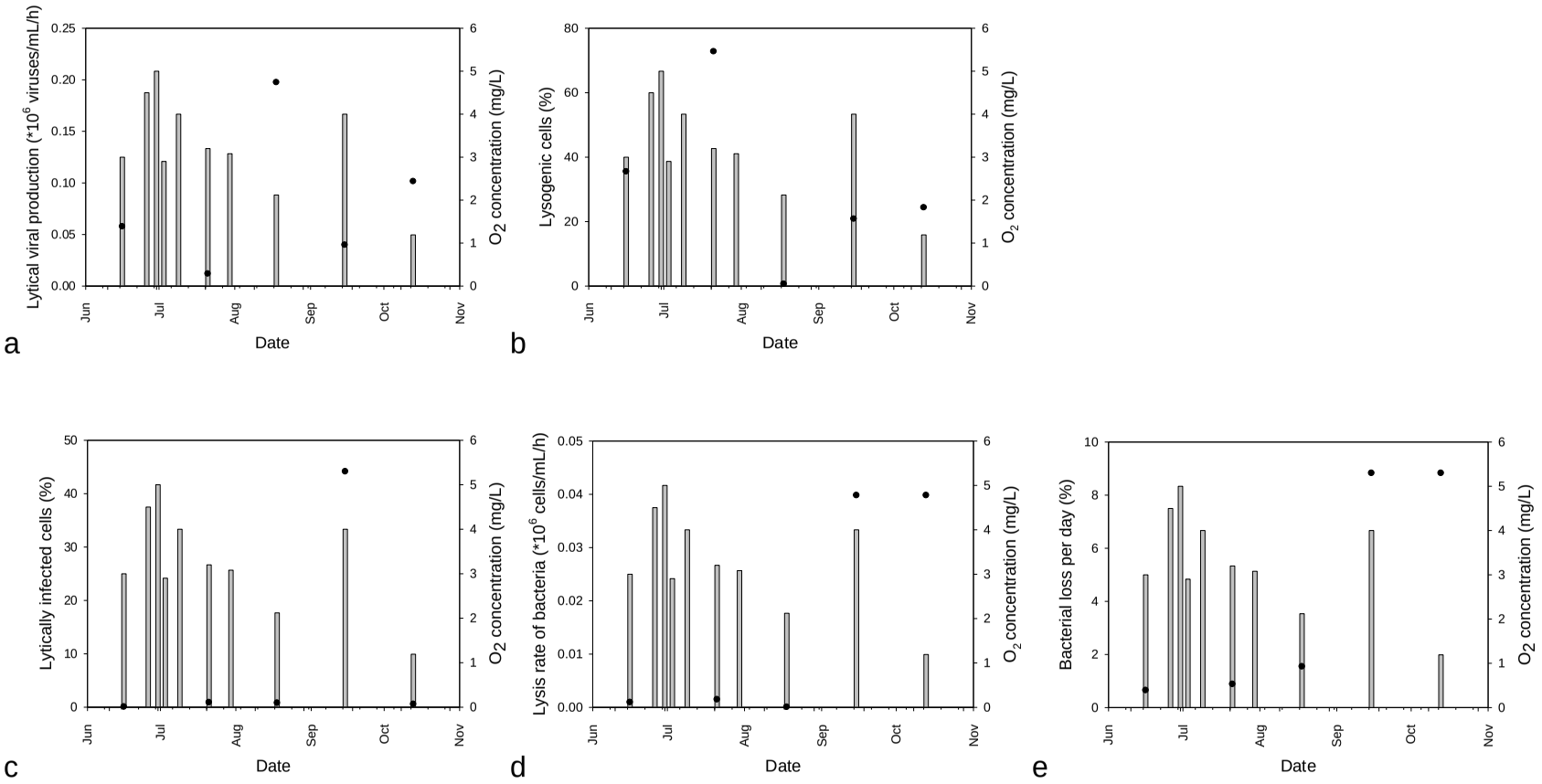


Figure 8. The abiotic parameter oxygen is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

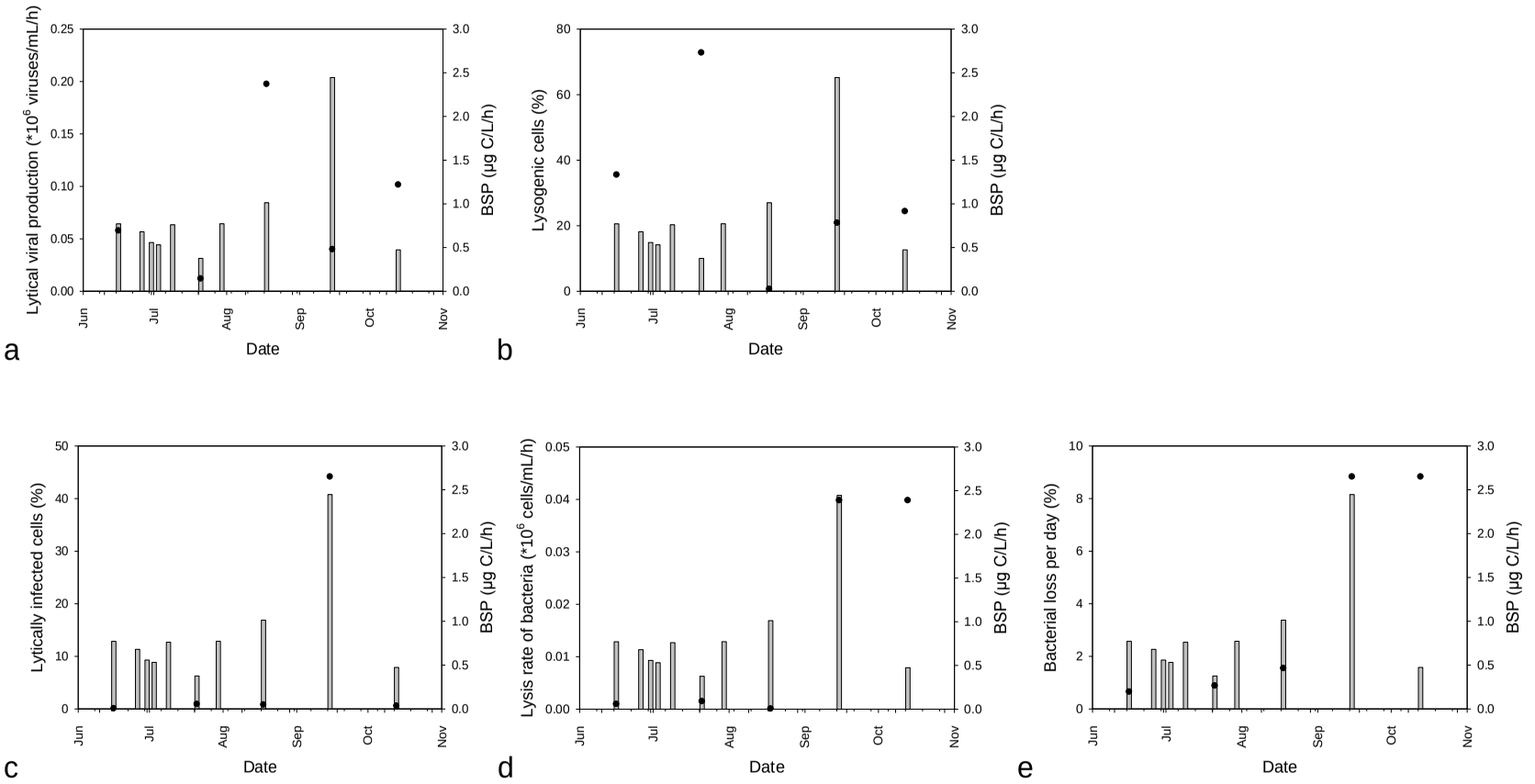


Figure 9. The biotic parameter bacterial secondary production (BSP) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

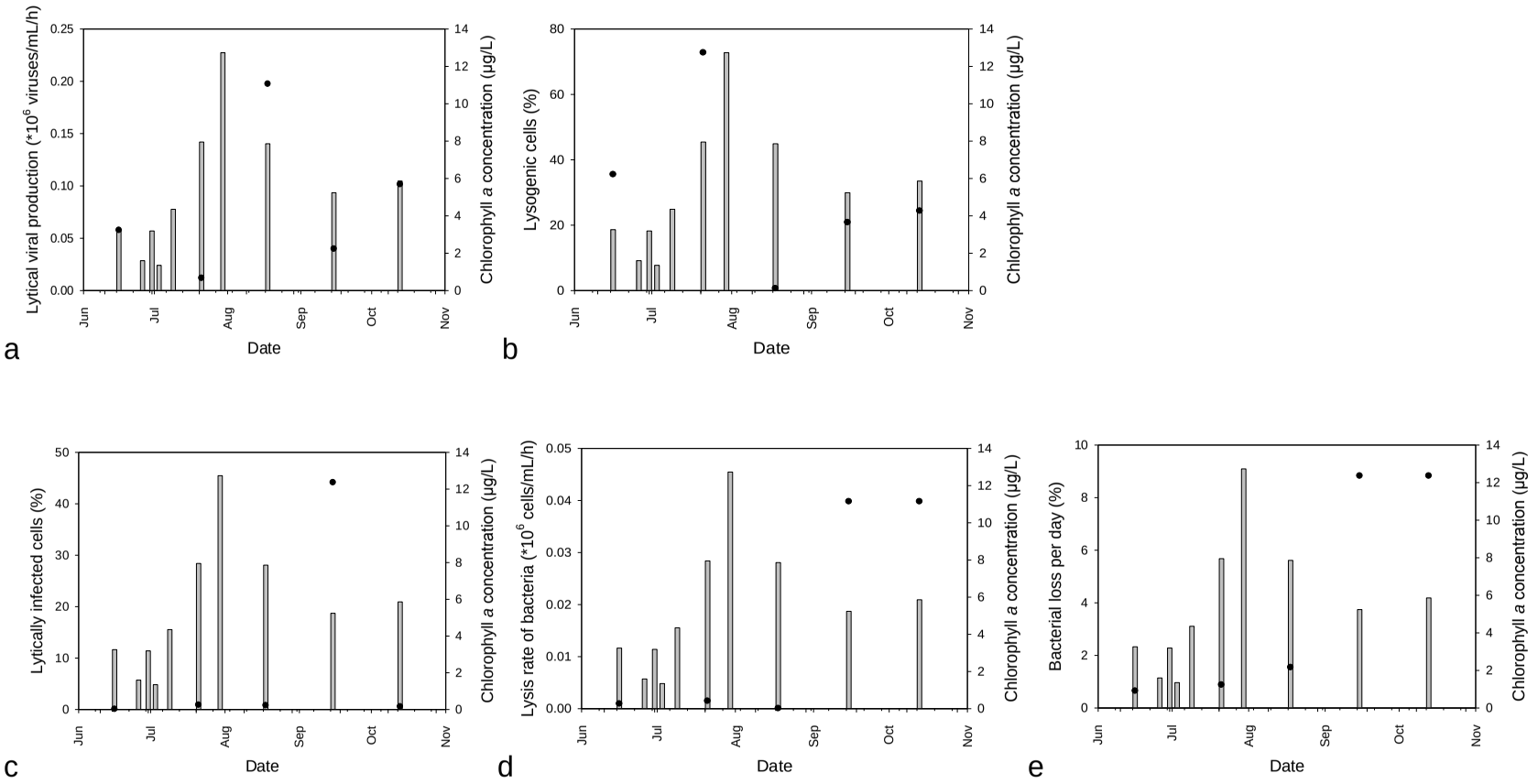


Figure 10. The biotic parameter chlorophyll *a* concentration is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

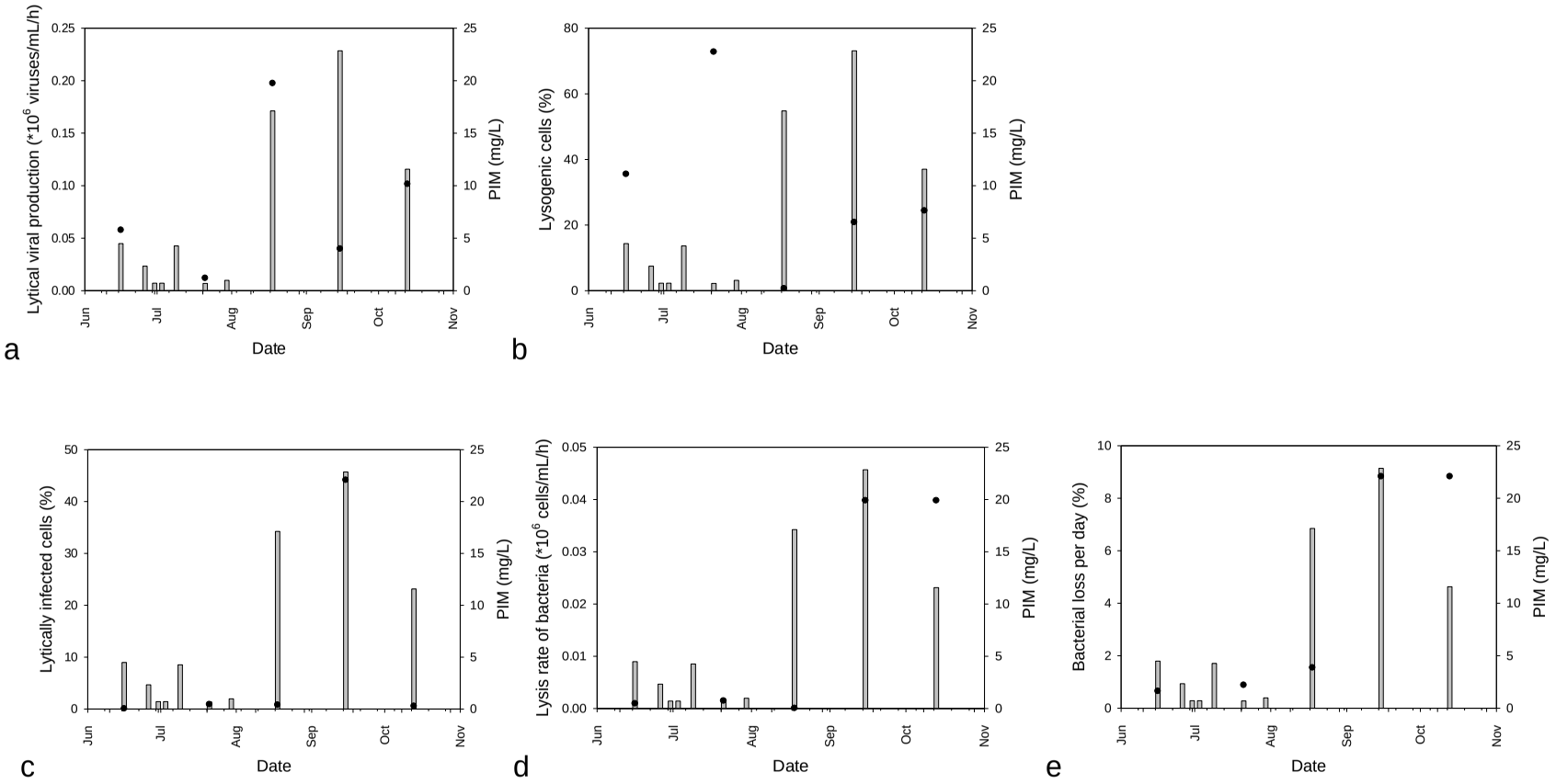


Figure 11. The abiotic parameter particulate inorganic matter (PIM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

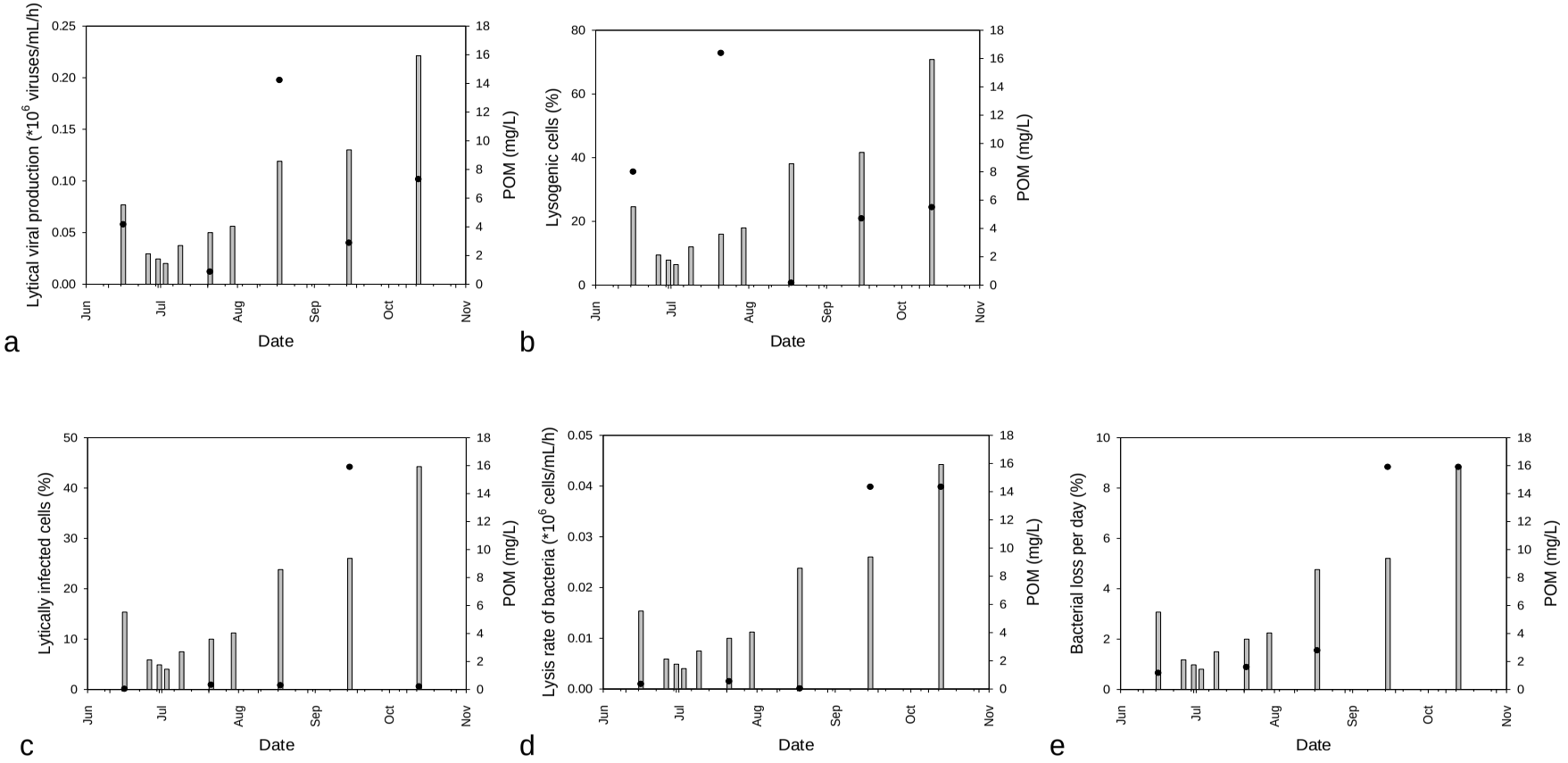


Figure 12. The biotic parameter particulate organic matter (POM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station TLM1/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

station 2007/Lobau											
days	**T [C°]**	**pH**	**O_2 [mg/L]**	**DOC [mg C/L]**	**BSP [µg C/L/h]**	**Chl *a* [µg/L]**	**PIM [mg/L]**	**POM [mg/L]**	**TSS [mg/L]**	**AGA [nm/h]**	**BGA [nm/h]**
16062009	20.3	8.06	8.1	1.77	0.987	5.4	2.21	1.26	3.47	25.43	25.43
26062009	16.9	7.86	8.8	2.62	0.291	3.32	19.44	2.26	21.70	9.39	13.43
30062009	18.1	7.85	6.7	3.08	1.395	23.68	8.19	3.09	11.28	8.14	20.46
03072009	21.4	7.6	4.7	4.37	1.502	8.63	0	0.12	0.12	39.91	49.58
09072009	19.9	7.79	6.5	3.25	0.975	3.53	0.3	1.26	1.56	7.18	9.63
21072009	20.6	8.07	8.8	2.33	1.143	10.75	0.75	1.99	2.74	16.09	20.16
30072009	22.9	8.12	8.4	2.33	1.432	6.32	2.14	2.43	4.57	12.19	15.17
18082009	24.4	7.8	8.1	2.24	1.095	12.52	1.66	3.03	4.69	14.81	20.49
15092009	19.0	8.02	8.4	2.06	1.413	15.14	1.86	2.7	4.56	9.86	14.82
13102009	10.5	8.11	9.9	1.98	0.661	15.84	3.6	6.07	9.67	5.20	8.33

Table 4. Measured parameters of the station 2007/Lobau; T = Temperature; DOC = Dissolved organic matter; pH; O_2 = Oxygen concentration; BSP = Bacterial secondary production; Chl *a* = Chlorophyll *a* concentration; PIM = Particulate inorganic matter; POM = Particulate organic matter, TSS = Total suspendid solids, AGA = Alpha-glucosidase activity and BGA = Beta-glucosidase activity

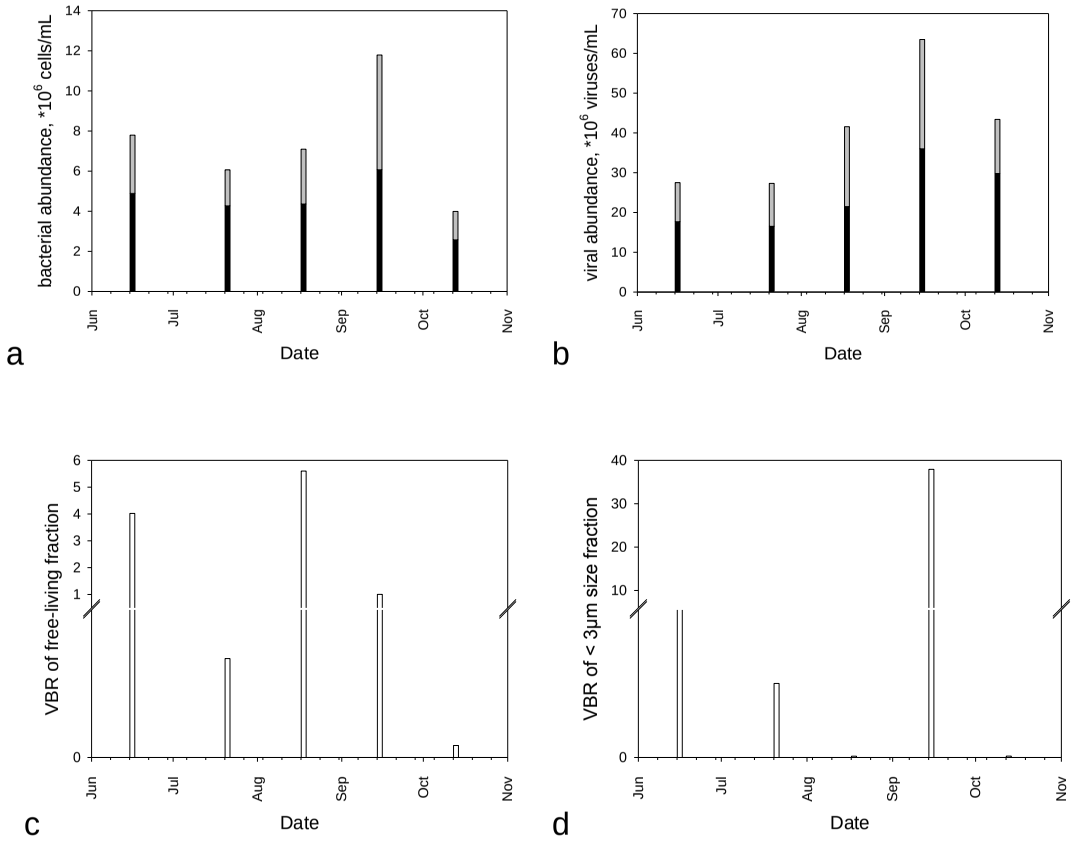


Figure 13. Free-living and attached bacterial (a) and viral abundance (b), Virus to Bacterium Ratio (VBR) of free-living (c) and attached fraction (d) of the station 2007/Lobau in the interval from June to October 2009 (grey bar = free-living bacterial and viral abundance; black bar = attached bacterial and viral abundance; white bar = VBR of free-living and > 3µm size fraction; sampling dates are shown in Tab.1).

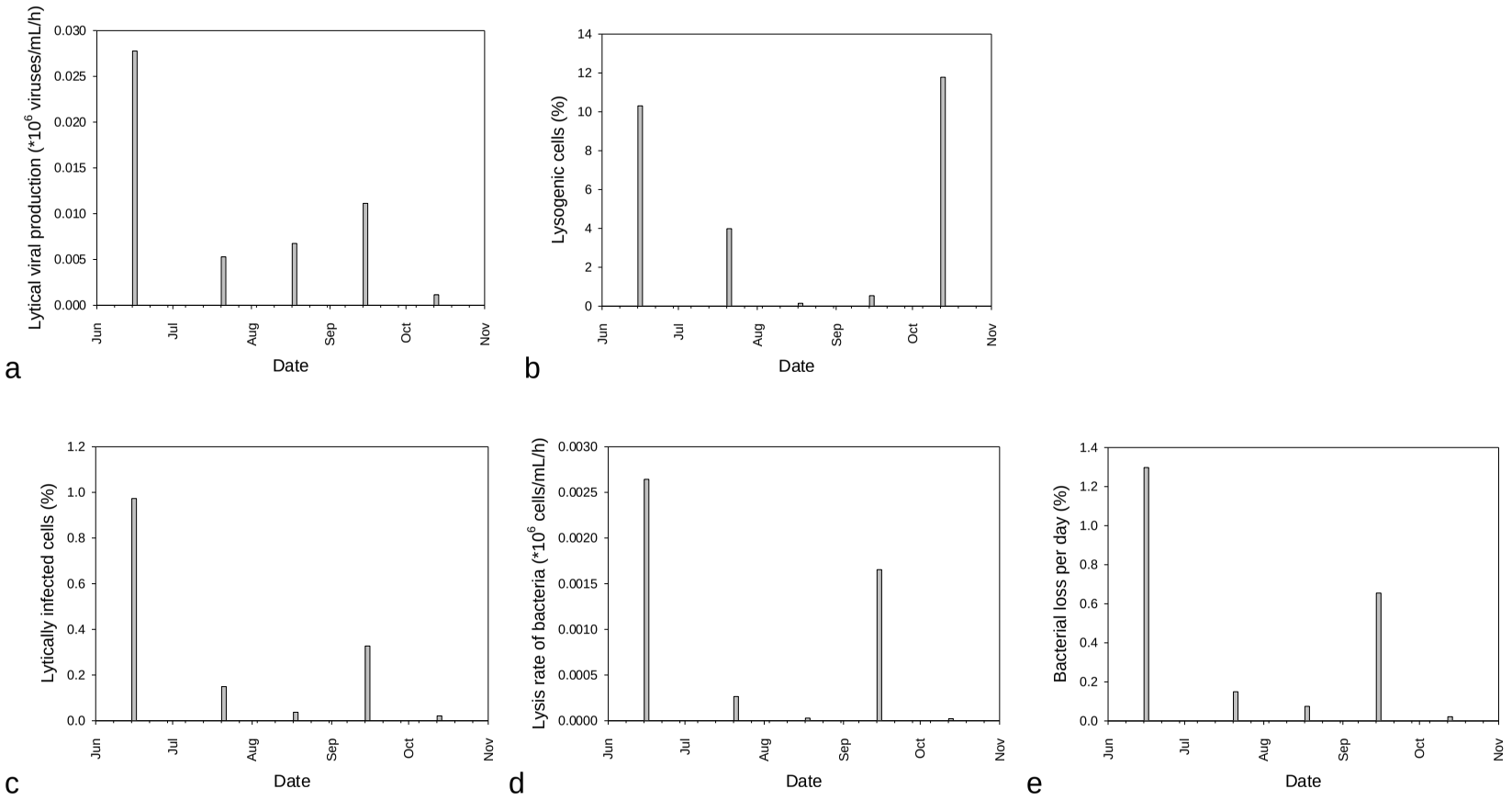


Figure 14. Lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments at the station 2007/Lobau in the interval from June to October 2009 (grey bar = viral parameters from VRA; sampling dates are shown in Tab.1).

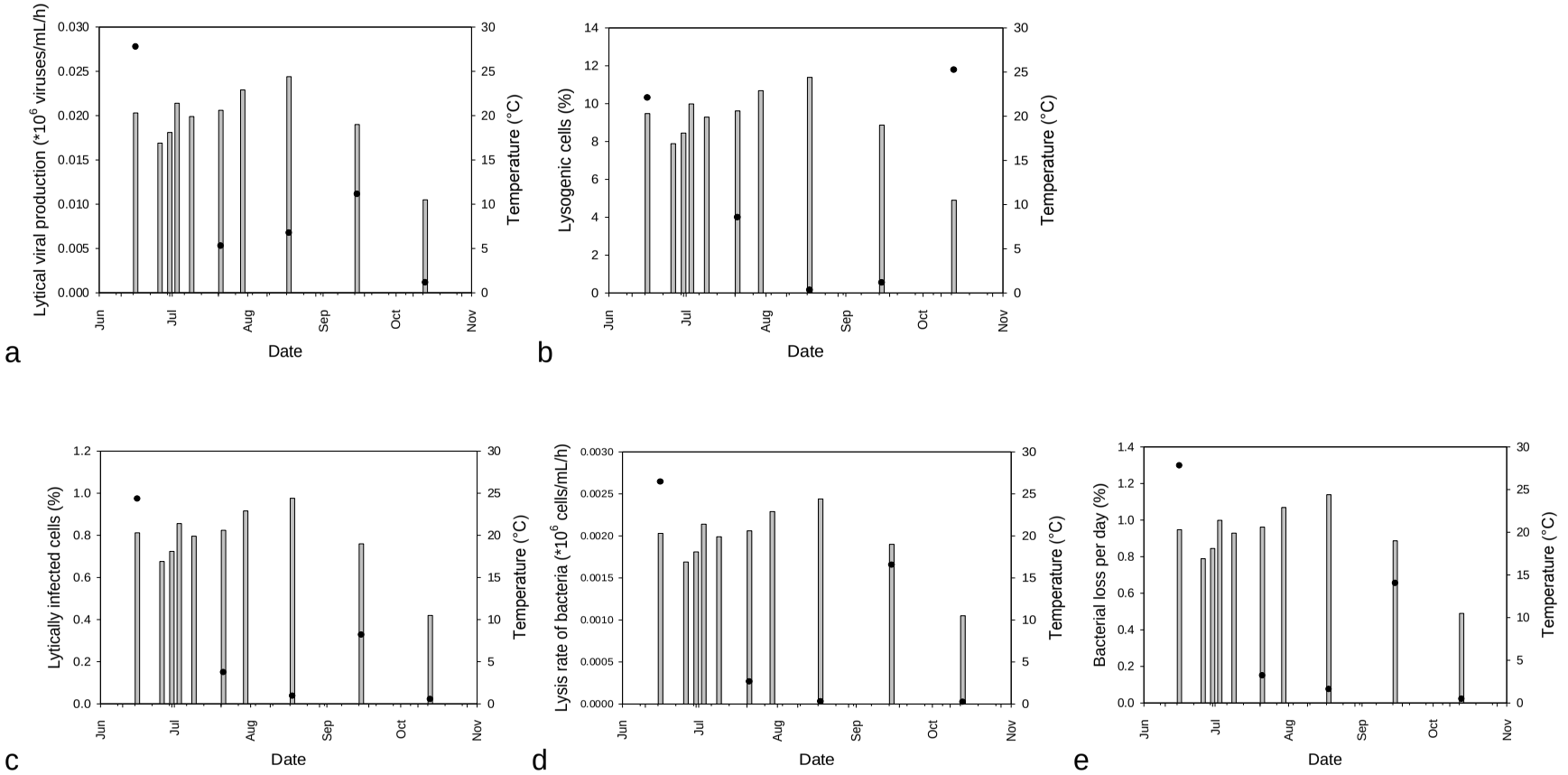


Figure 15. The abiotic parameter temperature is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

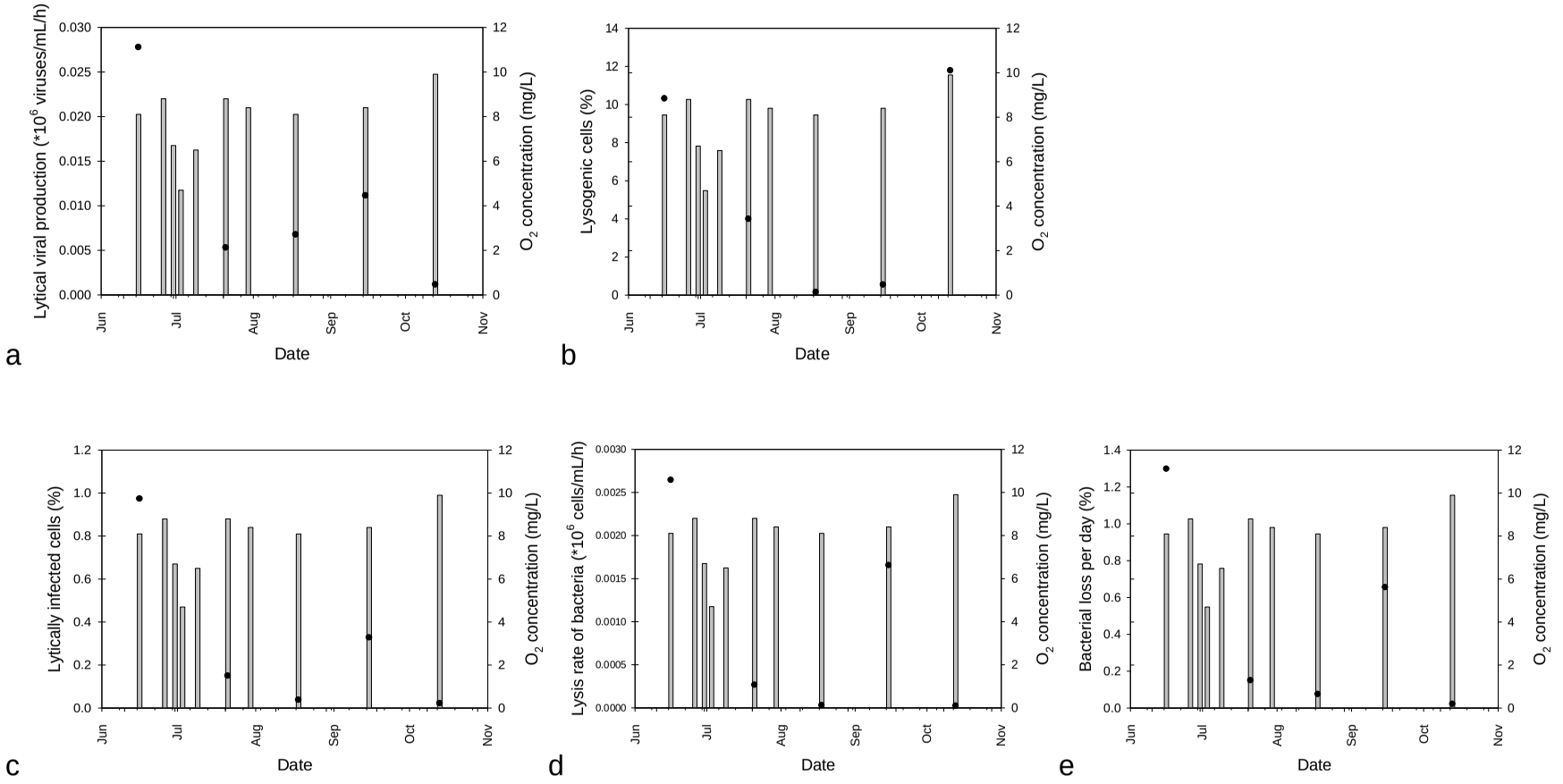


Figure 16. The abiotic parameter oxygen is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

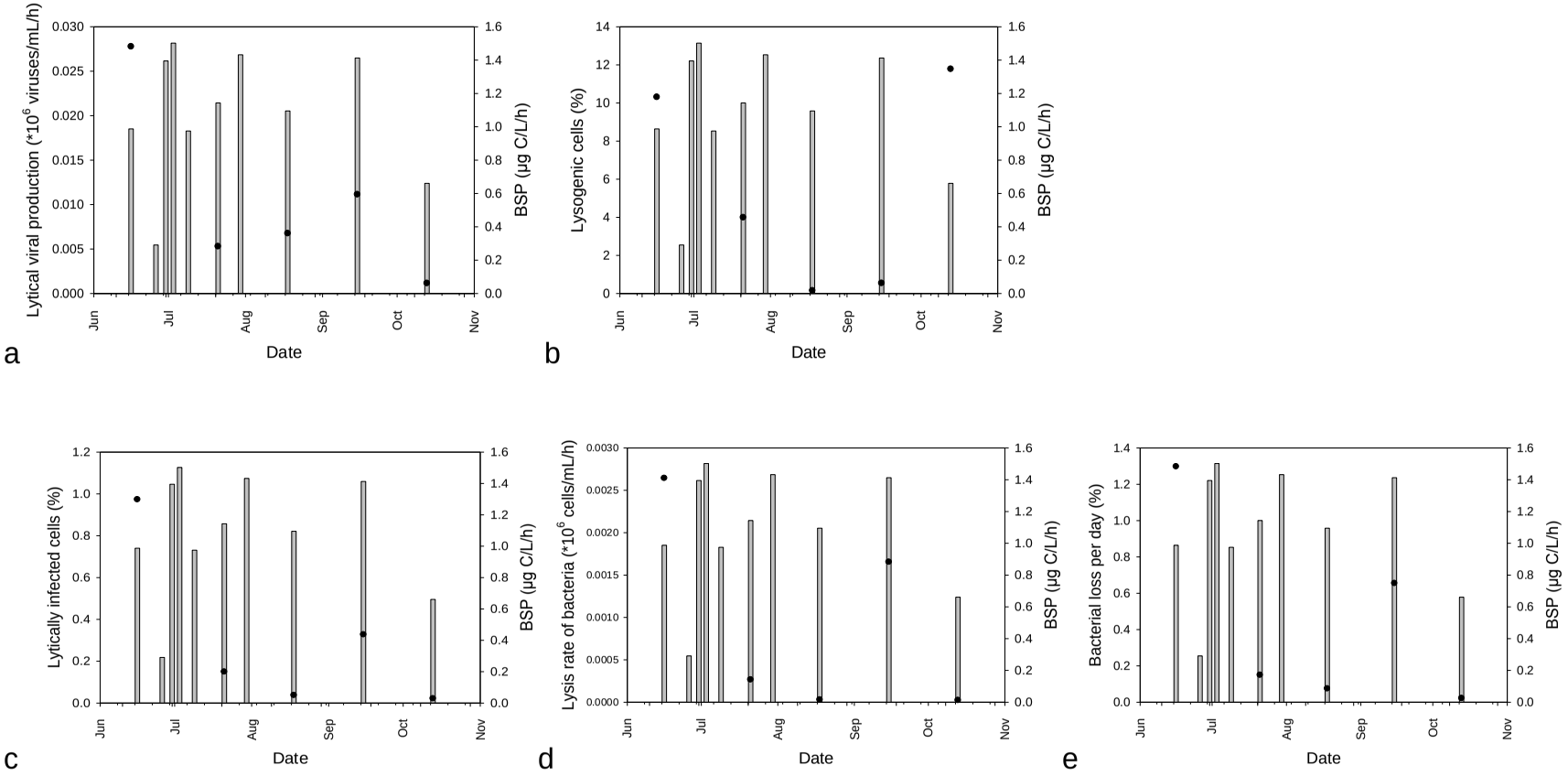


Figure 17. The biotic parameter bacterial secondary production (BSP) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

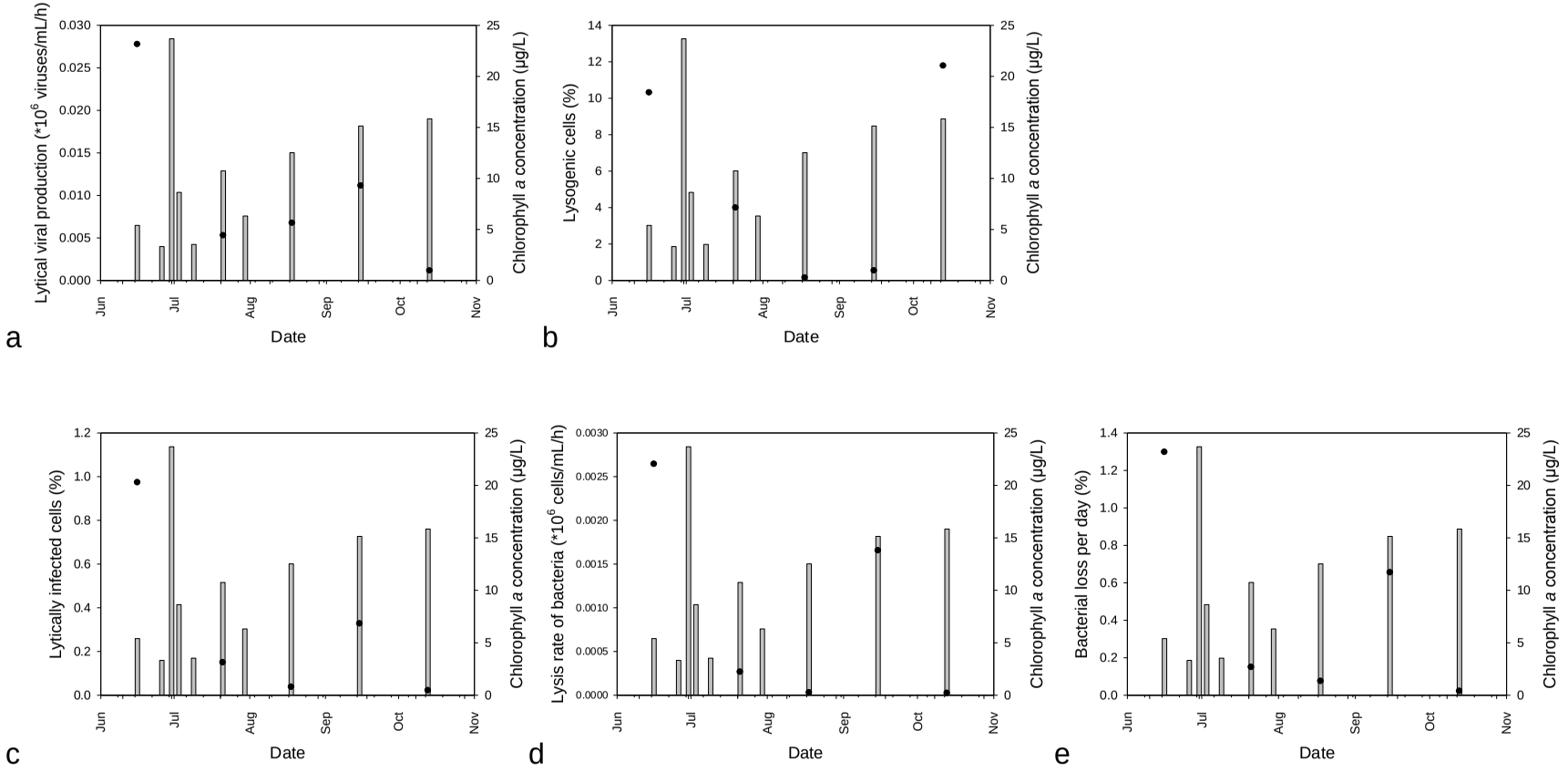


Figure 18. The biotic parameter chlorophyll *a* concentration is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

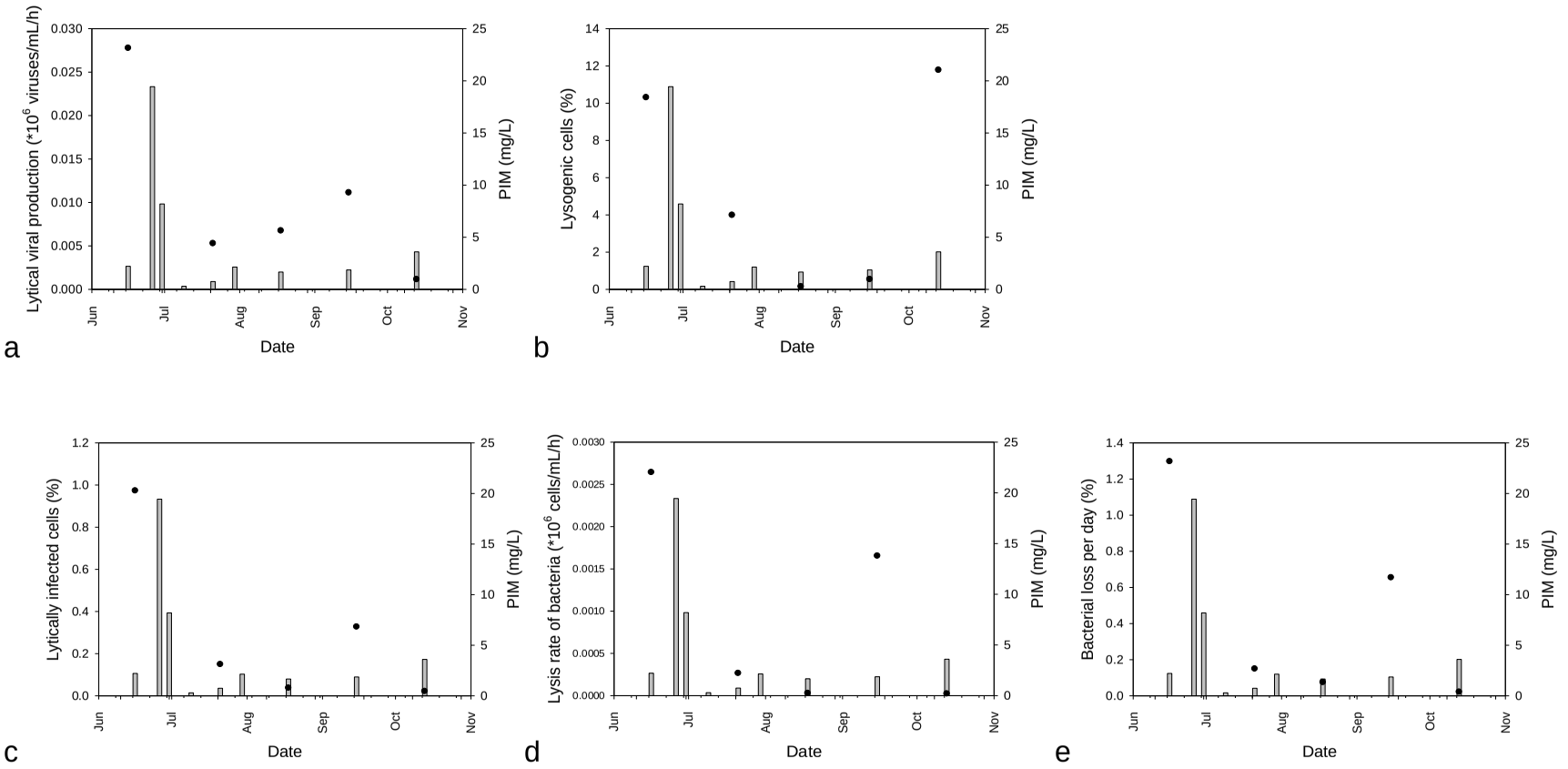


Figure 19. The abiotic parameter particulate inorganic matter (PIM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

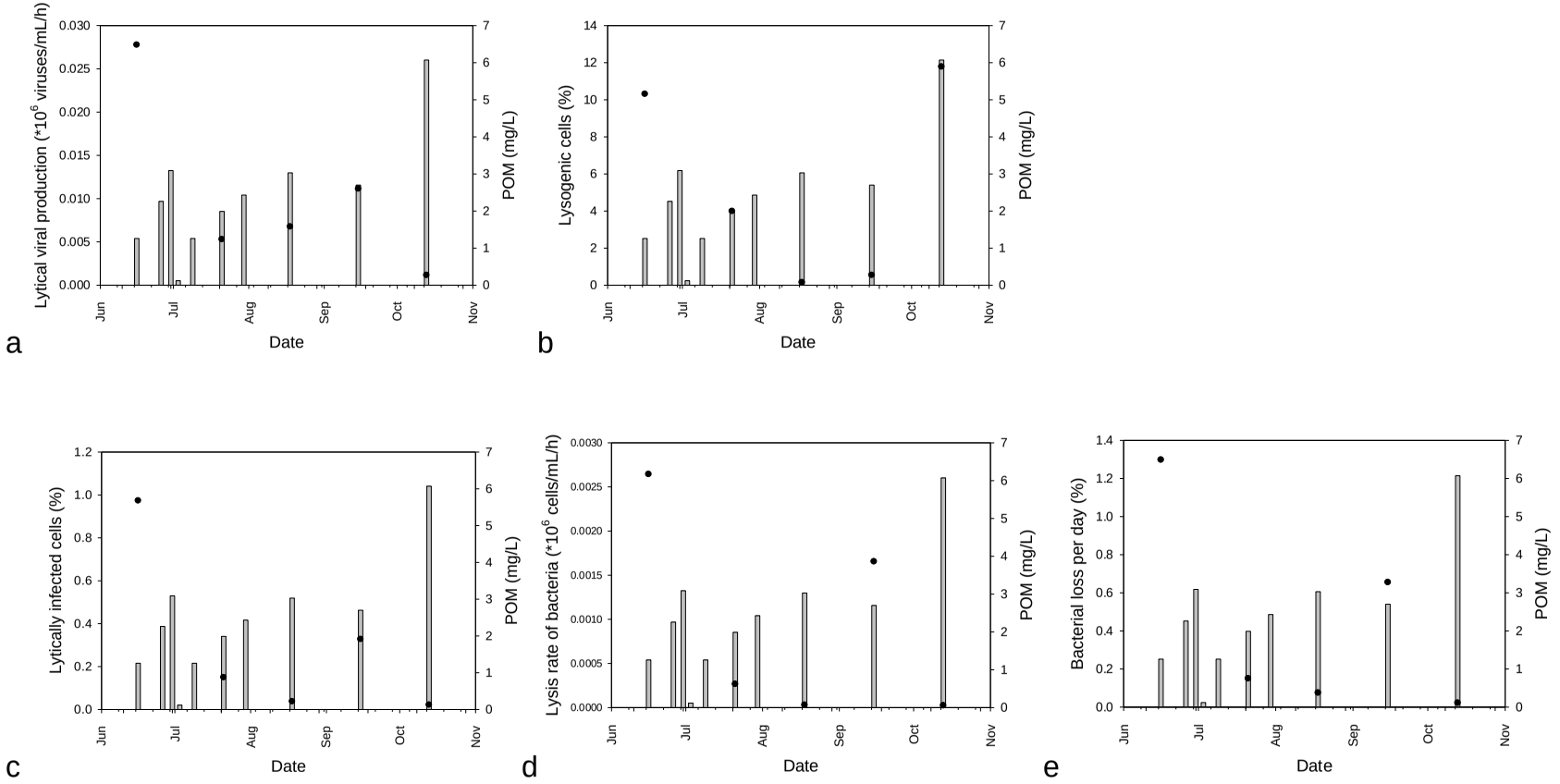


Figure 20. The biotic parameter particulate organic matter (POM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station 2007/Lobau in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

station Main Channel/River Danube											
days	**T [C°]**	**pH**	**O_2 [mg/L]**	**DOC [mg C/L]**	**BSP [µg C/L/h]**	**Chl *a* [µg/L]**	**PIM [mg/L]**	**POM [mg/L]**	**TSS [mg/L]**	**AGA [nm/h]**	**BGA [nm/h]**
16062009	18.6	8.54	11.7	1.38	0.987	9.97	4.7	16.65	21.35	4.55	7.36
*** 26062009**	13.3	7.91	10.1	2.94	nd	3.69	212.4	11.1	223.50	nd	nd
*** 30062009**	16.0	7.97	8.7	3.16	0.598	nd	38.8	3.75	42.55	27.45	35.77
*** 03072009**	16.6	8.0	10.9	3.27	0.409	6.33	115.75	7.9	123.65	22.87	28.59
*** 09072009**	17.3	8.11	11.3	2.83	0.413	4.73	81.28	5.35	86.63	25.19	26.67
21072009	17.5	8.18	10.3	1.88	0.513	4.99	120.84	6.31	127.15	14.98	22.61
30072009	19.6	8.35	9.3	2.33	0.766	6.99	33.77	2.7	36.47	5.20	7.22
18082009	19.2	8.1	9.4	1.80	0.518	8.97	20.3	4.6	24.90	3.30	4.36
15092009	15.7	8.13	9.7	1.84	0.781	2.49	28.15	2.12	30.27	2.94	4.33
13102009	12.3	8.11	9.9	1.43	0.562	9.42	2.45	3.78	6.23	2.65	3.70

Table 5. Measured parameters of the station Main Channel/River Danube; T = Temperature; DOC = Dissolved organic matter; pH; O_2 = Oxygen concentration; BSP = Bacterial secondary production; Chl *a* = Chlorophyll *a* concentration; PIM = Particulate inorganic matter; POM = Particulate organic matter, TSS = Total suspendid solids, AGA = Alpha-glucosidase activity and BGA = Beta-glucosidase activity, nd= no data; on the with * marked days the water column was measured on other stations because of high water level on 26062009 the water body was measured in Orth Damm; on 30062009 in Orth Fadenbach and on the days 03072009 and 09072009 in Danube Orth

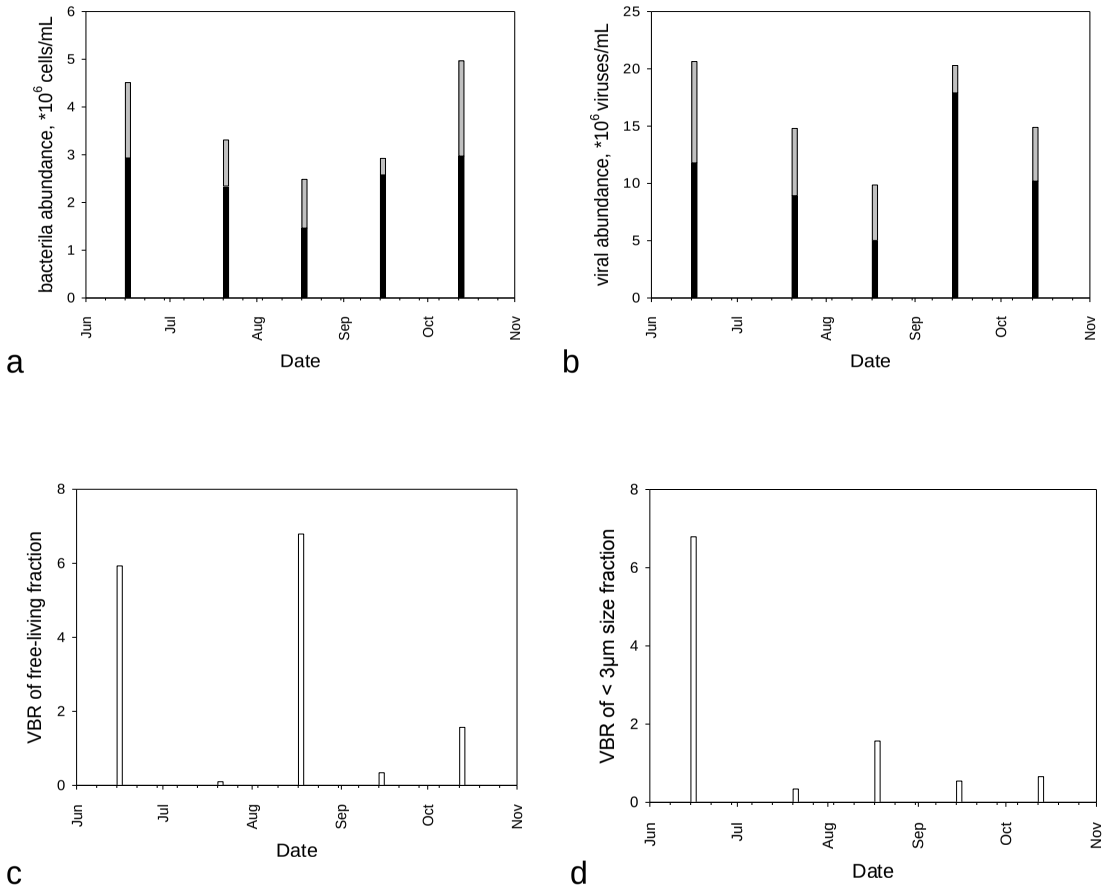


Figure 21. Free-living and attached bacterial (a) and viral abundance (b), Virus to Bacterium Ratio (VBR) of free-living (c) and attached fraction (d) of the station Main Channel/River Danube in the interval from June to October 2009 (grey bar = free-living bacterial and viral abundance; black bar = attached bacterial and viral abundance; white bar = VBR of free-living and > 3µm size fraction; sampling dates are shown in Tab.1).

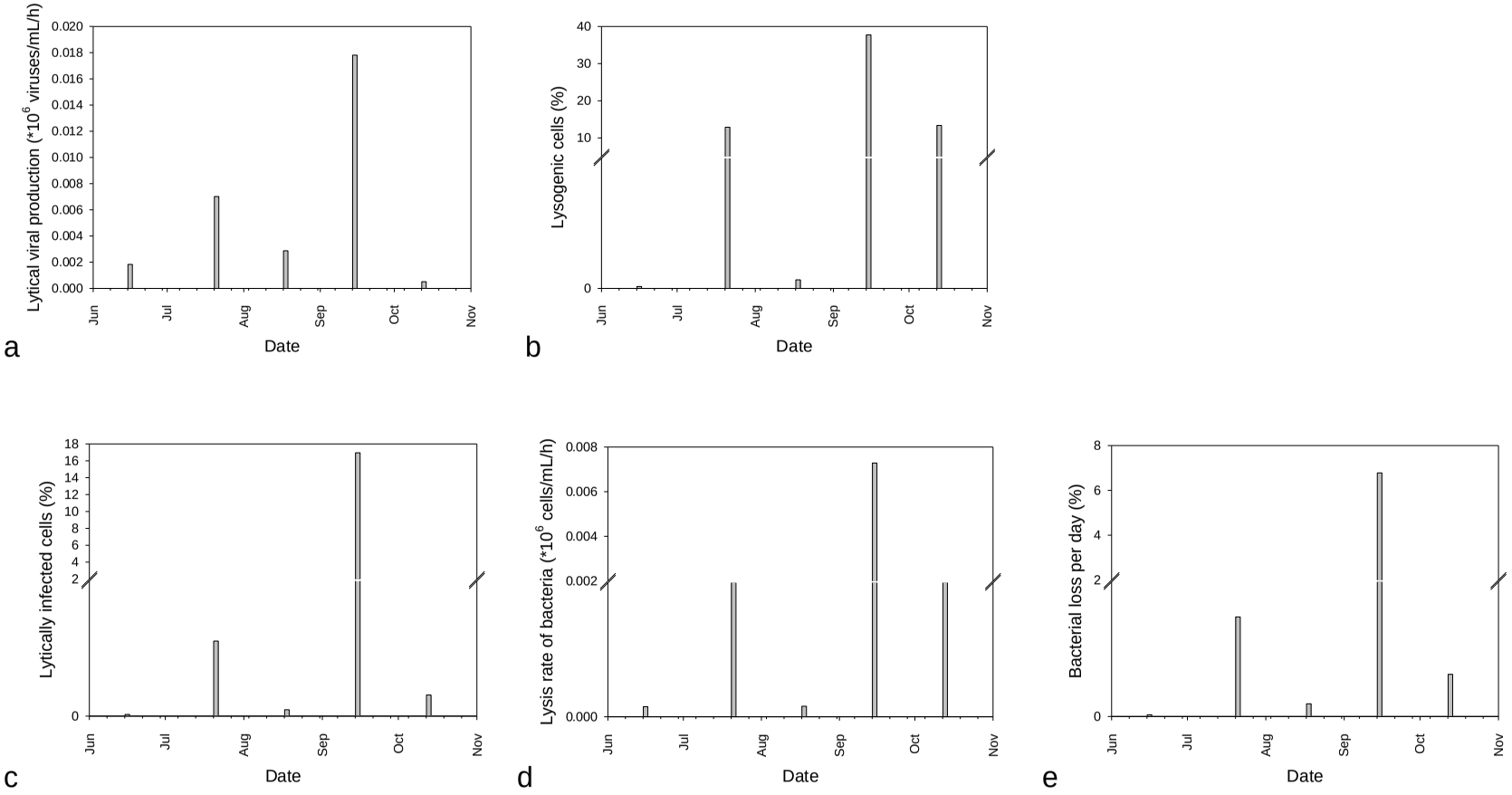


Figure 22. Lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments at the station Main Channel/River Danube in the interval from June to October 2009 (grey bar = viral parameters from VRA; sampling date are shown in Tab.1).

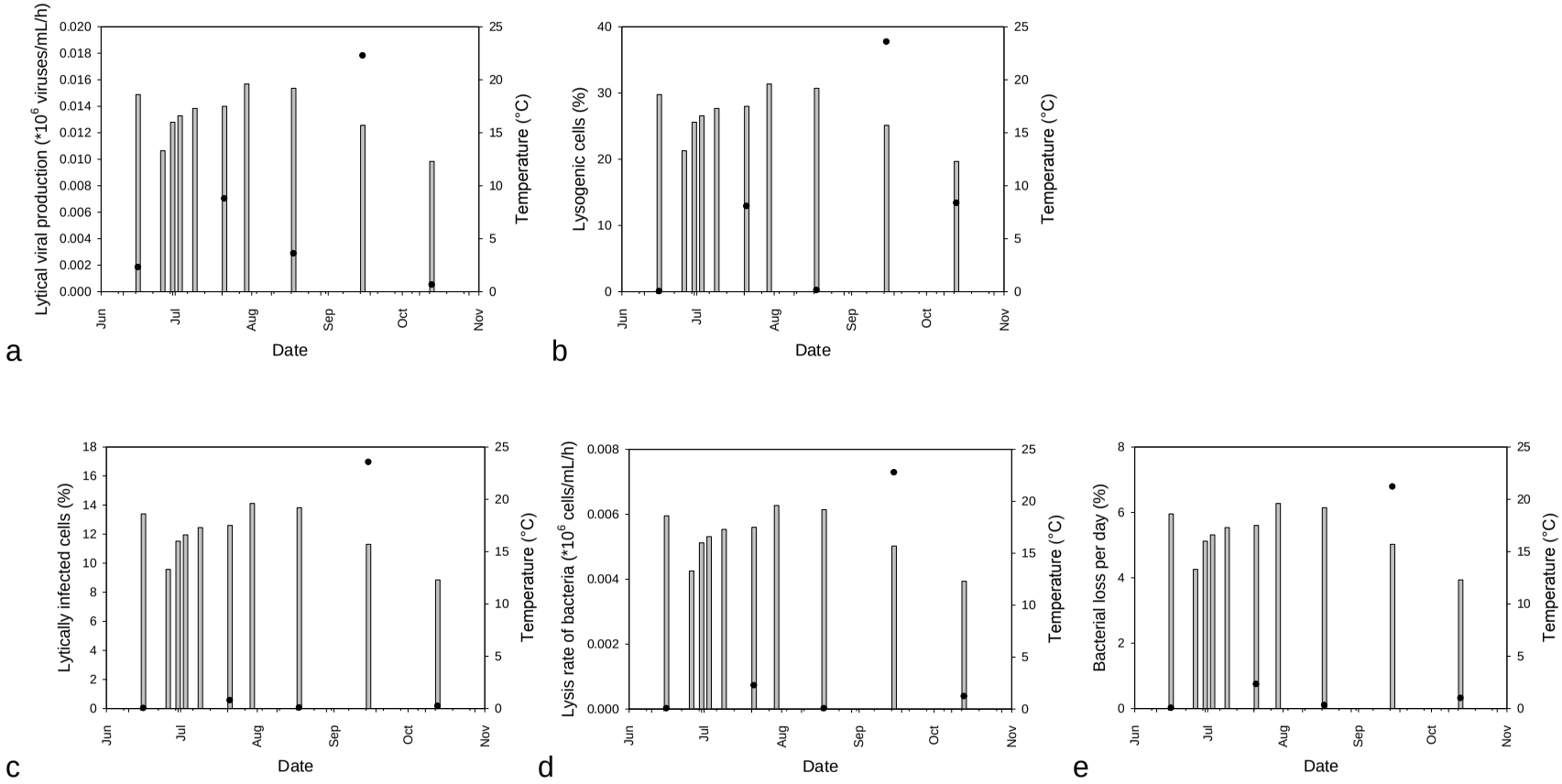


Figure 23. The abiotic parameter temperature is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

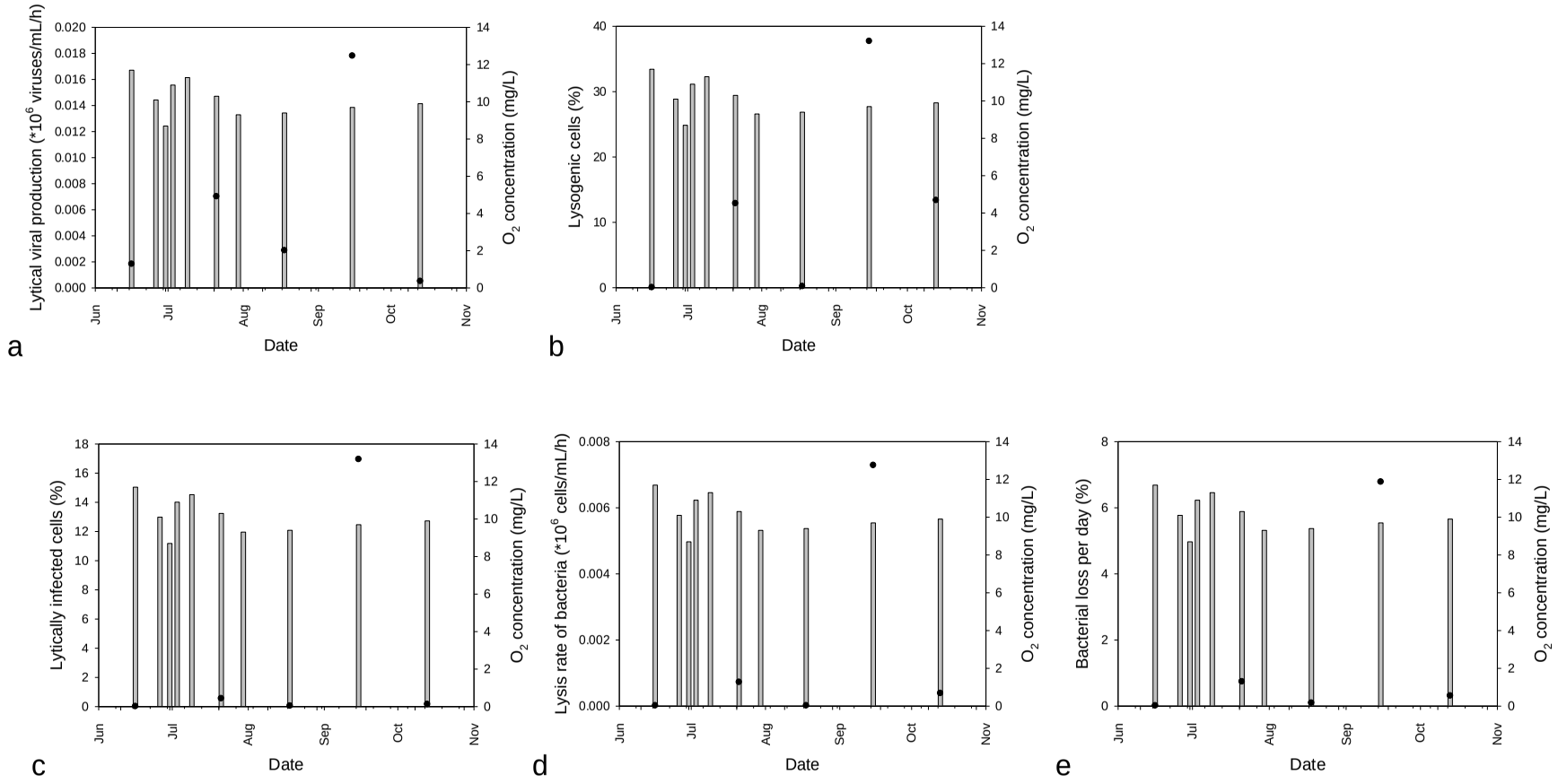


Figure 24. The abiotic parameter oxygen is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

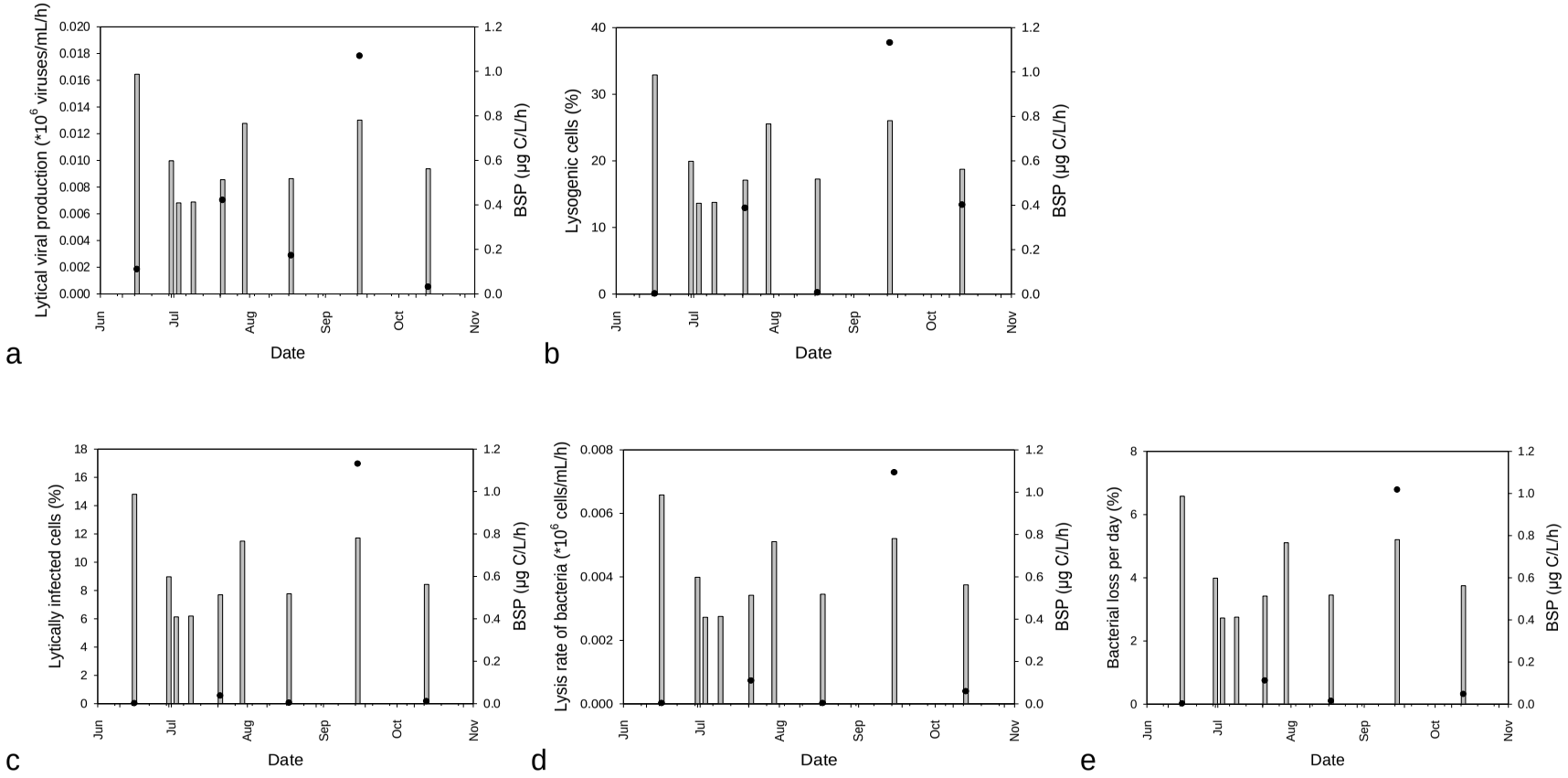


Figure 25. The biotic parameter bacterial secondary production (BSP) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

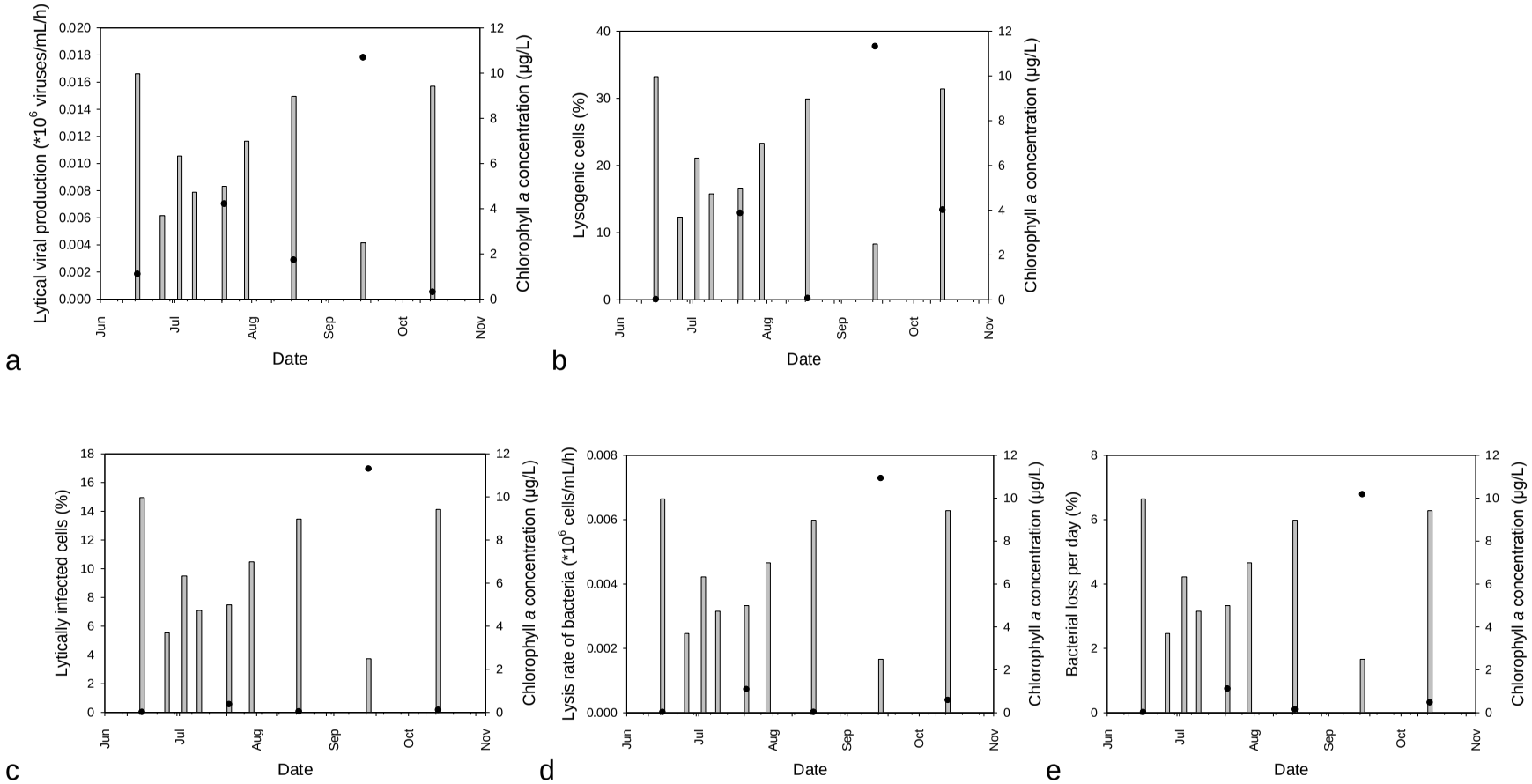


Figure 26. The biotic parameter chlorophyll *a* concentration is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

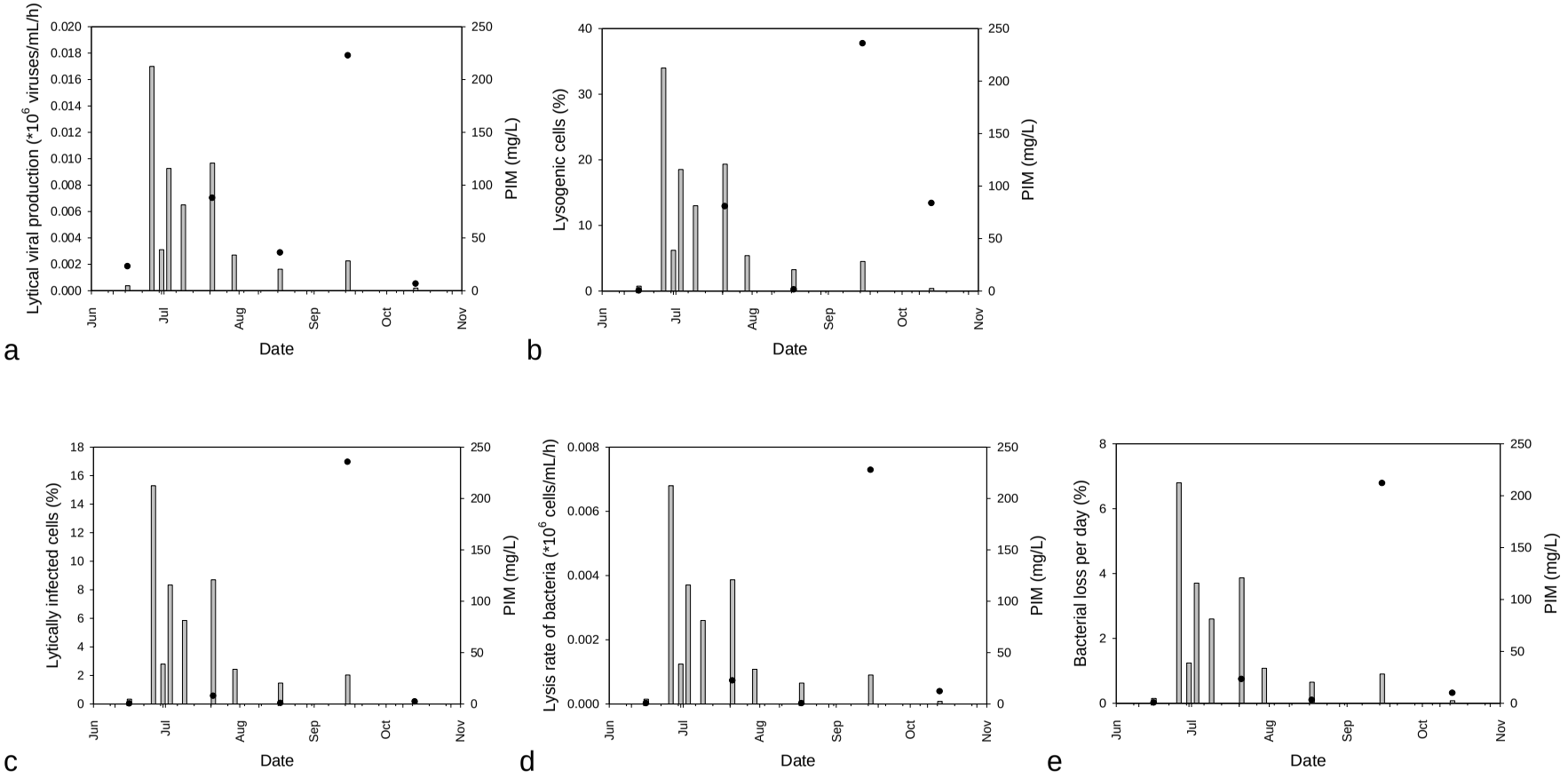


Figure 27. The abiotic parameter particulate inorganic matter (PIM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

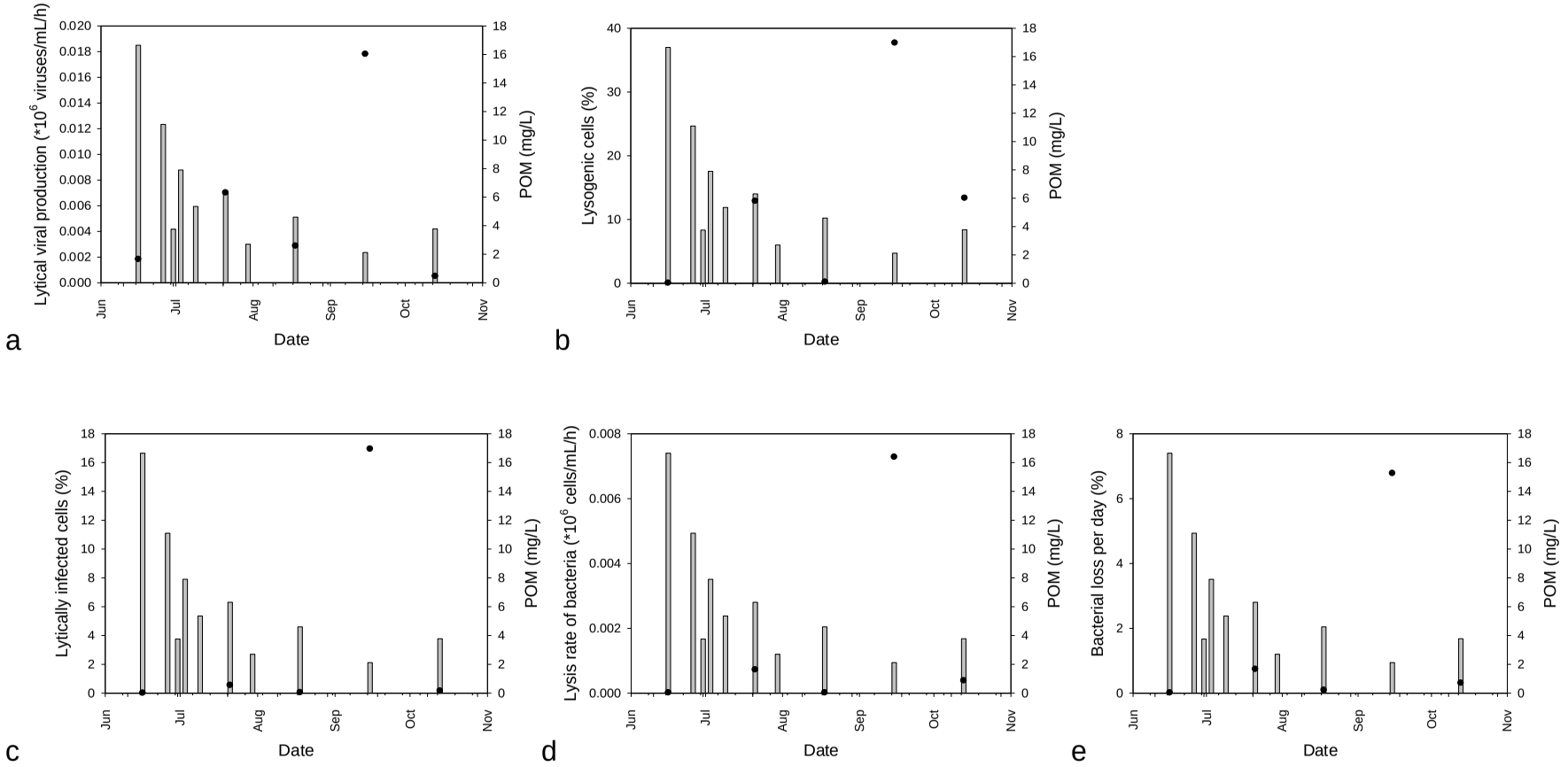


Figure 28. The biotic parameter particulate organic matter (POM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Main Channel/River Danube in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

station Regelsbrunn											
days	**T [C°]**	**pH**	**O_2 [mg/L]**	**DOC [mg C/L]**	**BSP [µg C/L/h]**	**Chl *a* [µg/L]**	**PIM [mg/L]**	**POM [mg/L]**	**TSS [mg/L]**	**AGA [nm/h]**	**BGA [nm/h]**
16062009	19.7	8.1	11.8	1.66	1.824	29.18	5.59	39.82	45.41	24.76	33.85
26062009	15.6	8.01	10.2	3.24	nd	2.85	nd	nd	nd	nd	nd
30062009	16.4	8.15	11.7	3.37	0.897	2.85	57.28	4.0	61.28	20.51	26.47
03072009	16.6	8.23	10.0	nd	0.502	2.96	94.26	5.64	99.9	15.16	19.28
09072009	16.8	8.29	10.3	2.61	0.521	2.74	62.02	5.04	67.06	13.99	16.29
21072009	17.3	8.03	10.2	1.82	0.546	4.72	40.98	2.75	43.73	7.89	11.36
30072009	19.4	8.4	11.07	2.20	1.027	18.14	13.17	3.81	16.98	9.37	12.58
18082009	22.4	8.55	15.2	1.68	1.866	19.1	54.75	10.63	65.38	15.20	20.81
15092009	18.1	8.4	10.5	1.43	1.615	20.62	52.58	5.4	57.98	9.51	12.39
13102009	10.1	8.5	10.5	1.95	1.015	13.03	8.35	98.44	106.79	4.88	7.90

Table 6. Measured parameters of the station Regelsbrunn; T = Temperature; DOC = Dissolved organic matter; pH; O_2 = Oxygen concentration; BSP = Bacterial secondary production; Chl *a* = Chlorophyll *a* concentration; PIM = Particulate inorganic matter; POM = Particulate organic matter, TSS = Total suspenid solids; AGA = Alpha-glucosidase activity and BGA = Beta-glucosidase activity and nd= no data

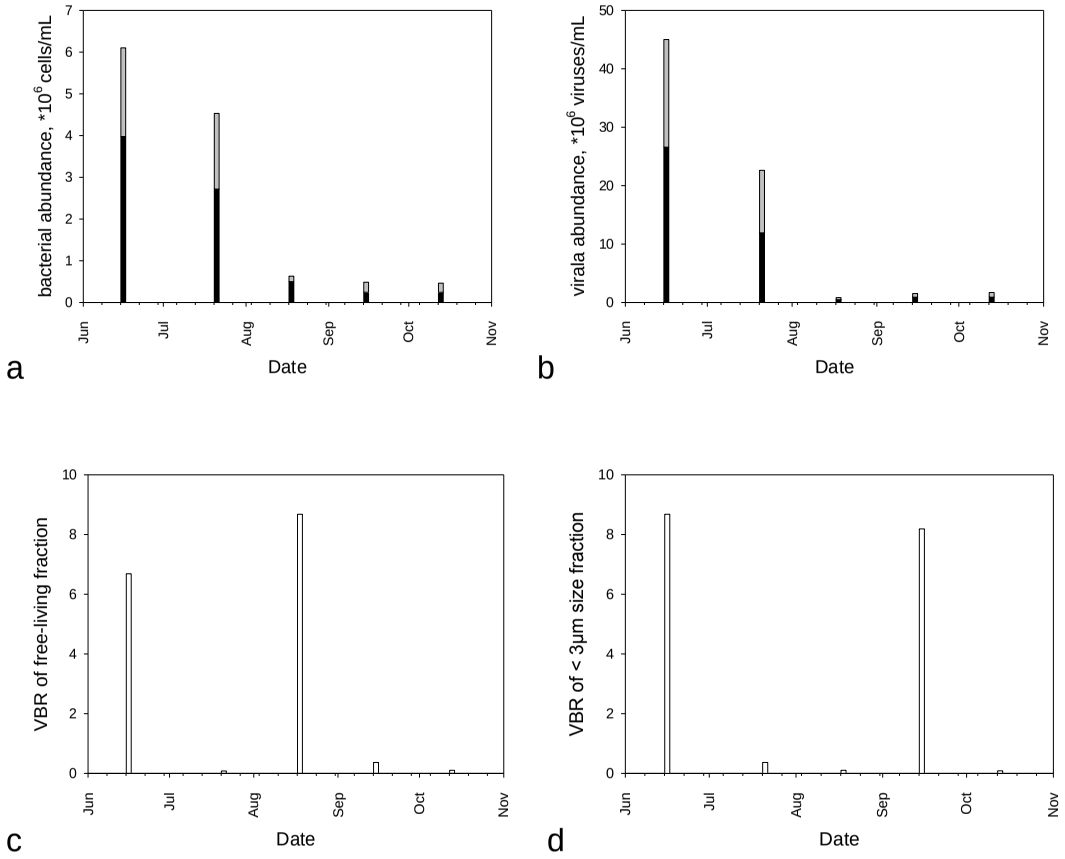


Figure 29. Free-living and attached bacterial (a) and viral abundance (b), Virus to Bacterium Ratio (VBR) of free-living (c) and attached fraction (d) of the station Regelsbrunn in the interval from June to October 2009 (grey bar = free-living bacterial and viral abundance; black bar = attached bacterial and viral abundance; white bar = VBR of free-living and > 3µm size fraction; sampling dates are shown in Tab.1).

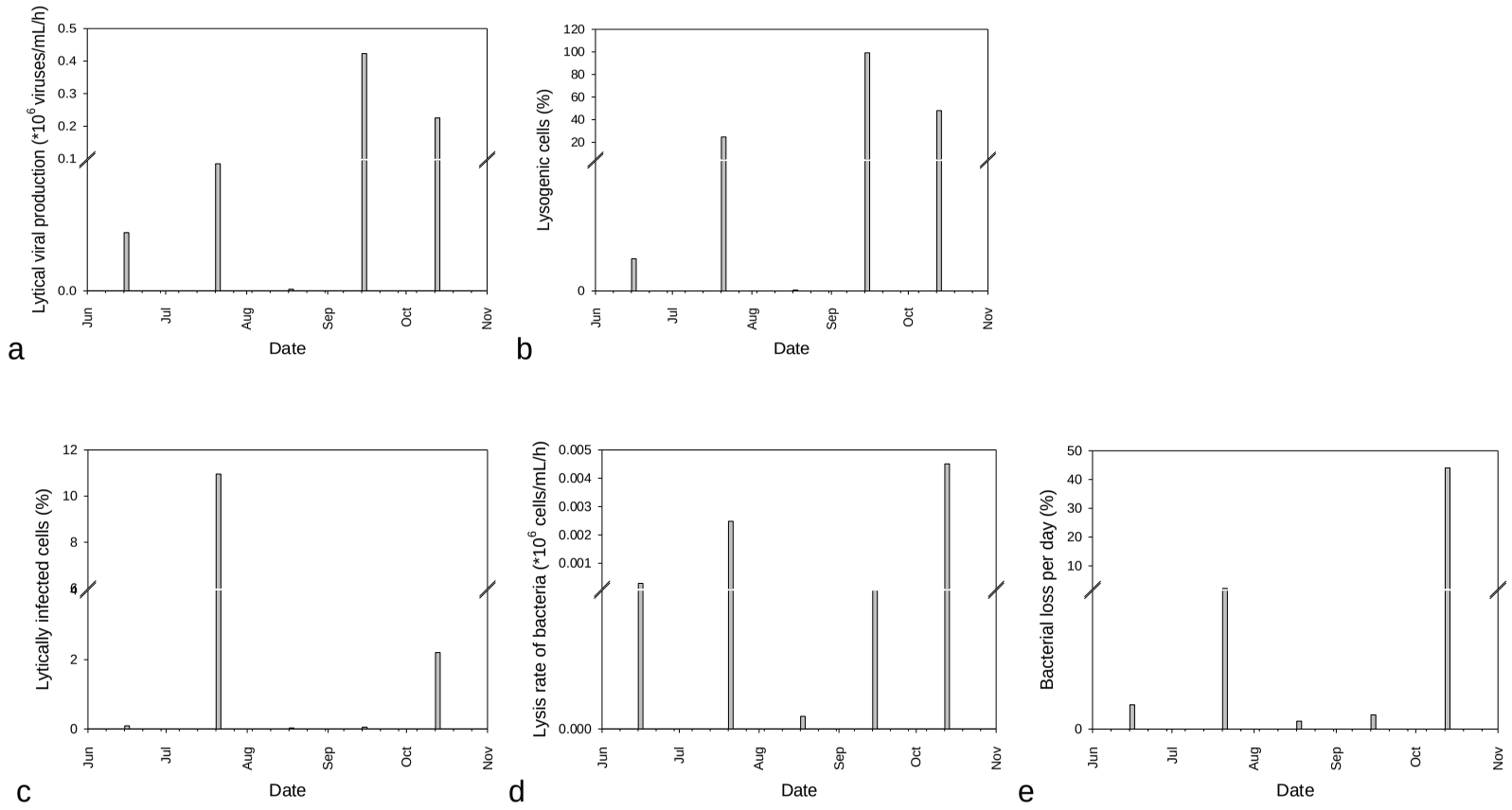


Figure 30. Lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments at the station Regelsbrunn in the interval from June to October 2009 (grey bar = viral parameters from VRA; sampling dates shown in Tab.1).

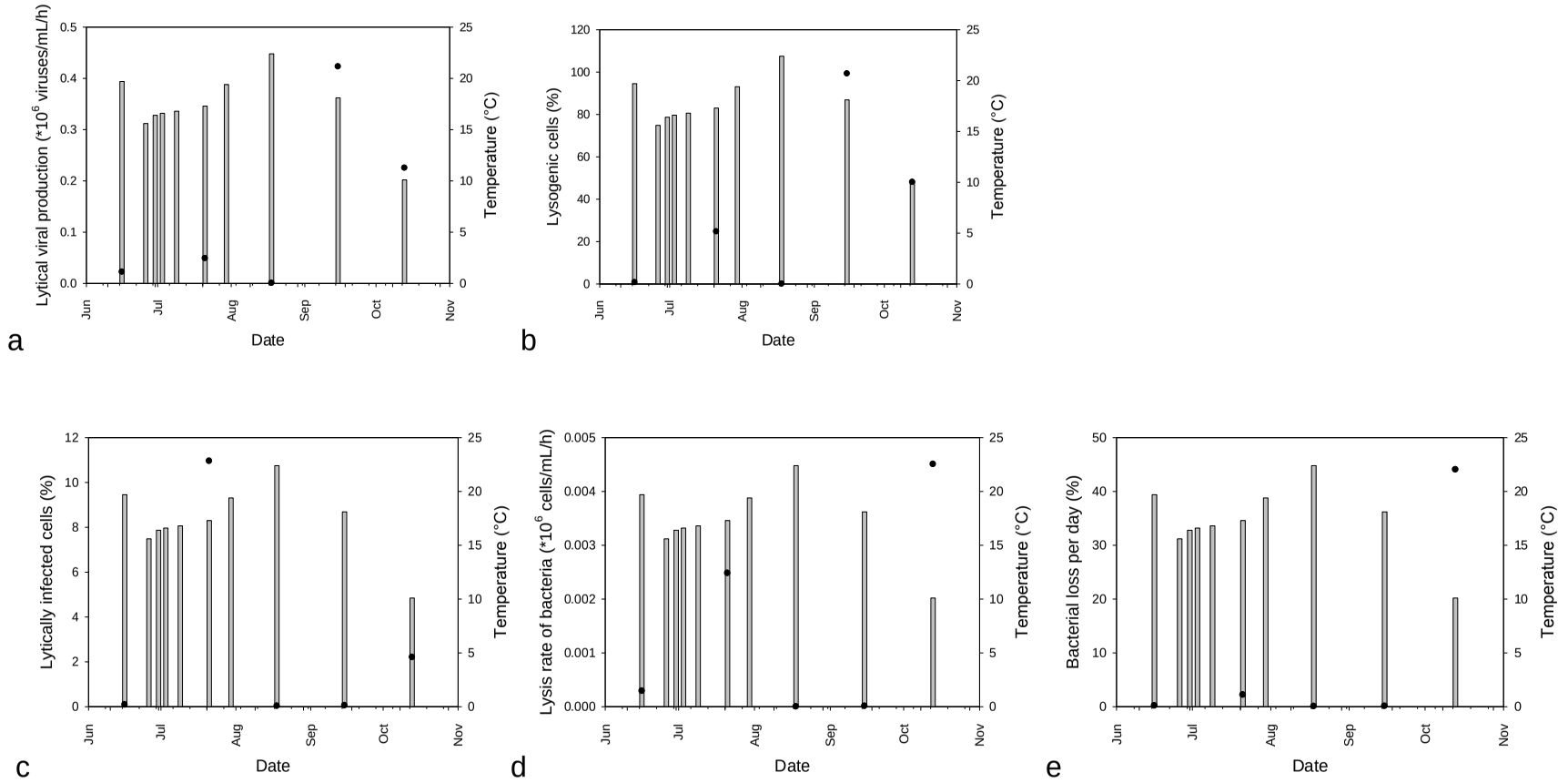


Figure 31. The abiotic parameter temperature is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

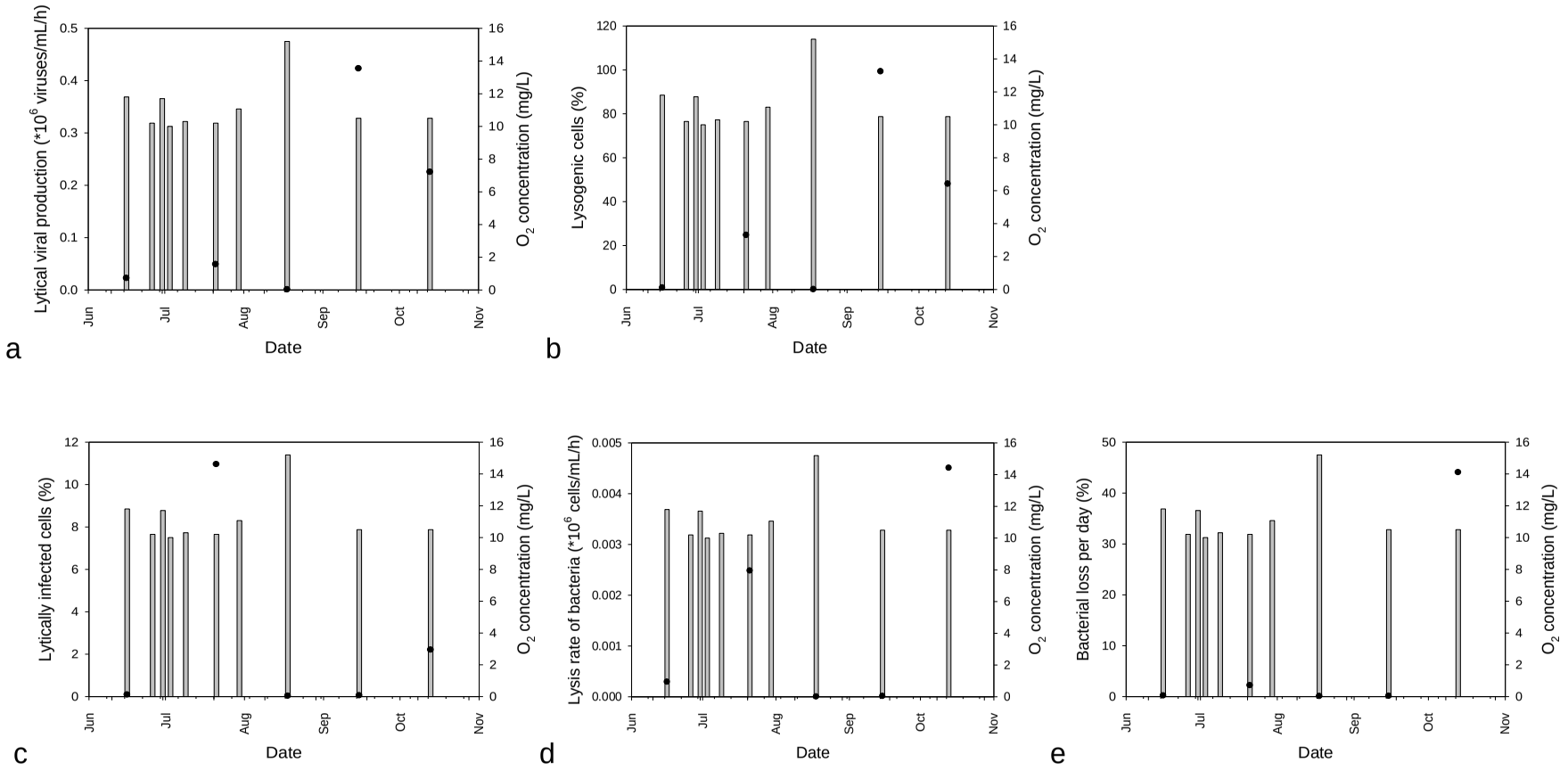


Figure 32. The abiotic parameter oxygen is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

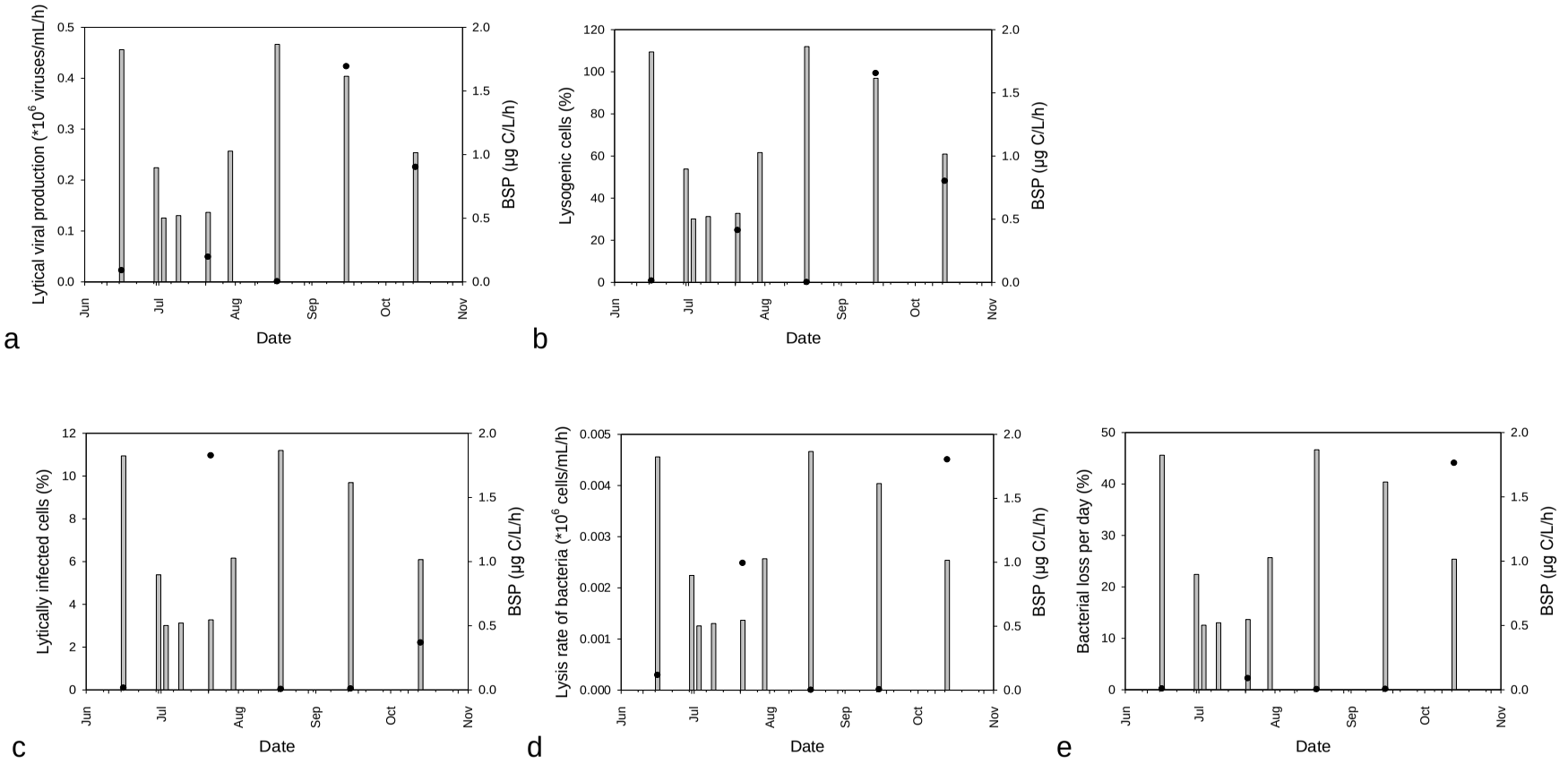


Figure 33. The biotic parameter bacterial secondary production (BSP) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

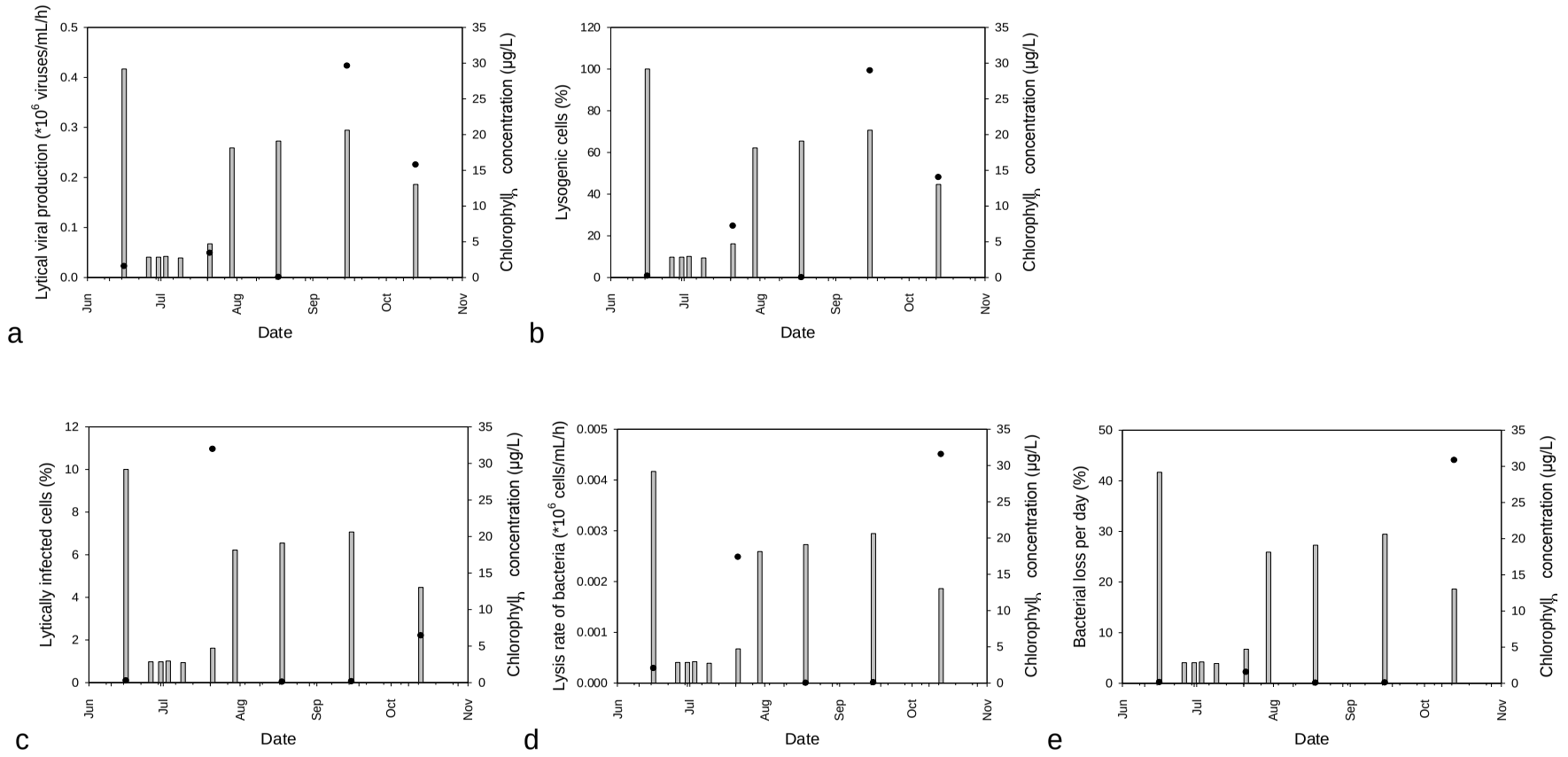


Figure 34. The biotic parameter chlorophyll *a* concentration is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

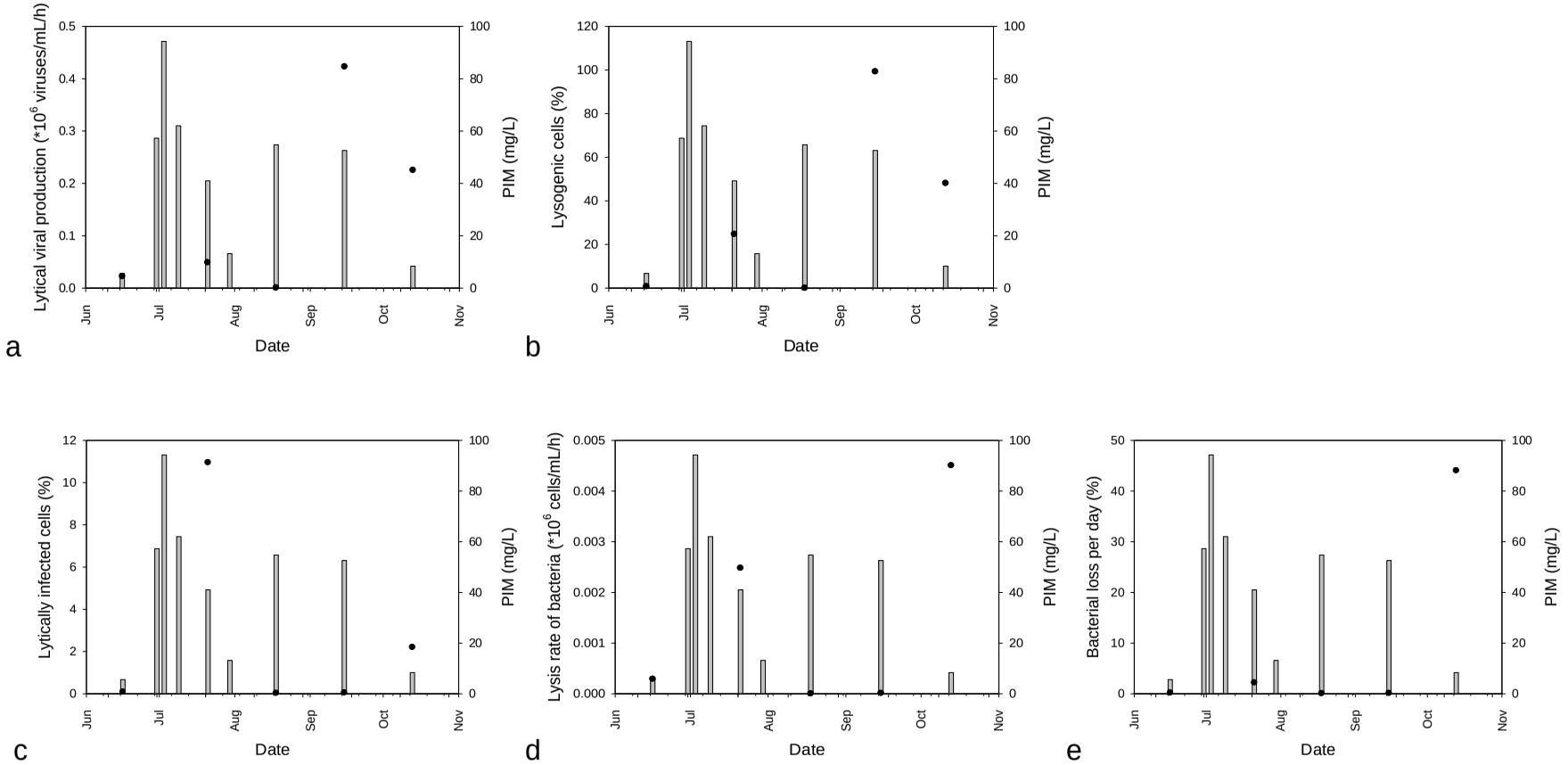


Figure 35. The abiotic parameter particulate inorganic matter (PIM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = abiotic parameters; sampling dates are shown in Tab.1).

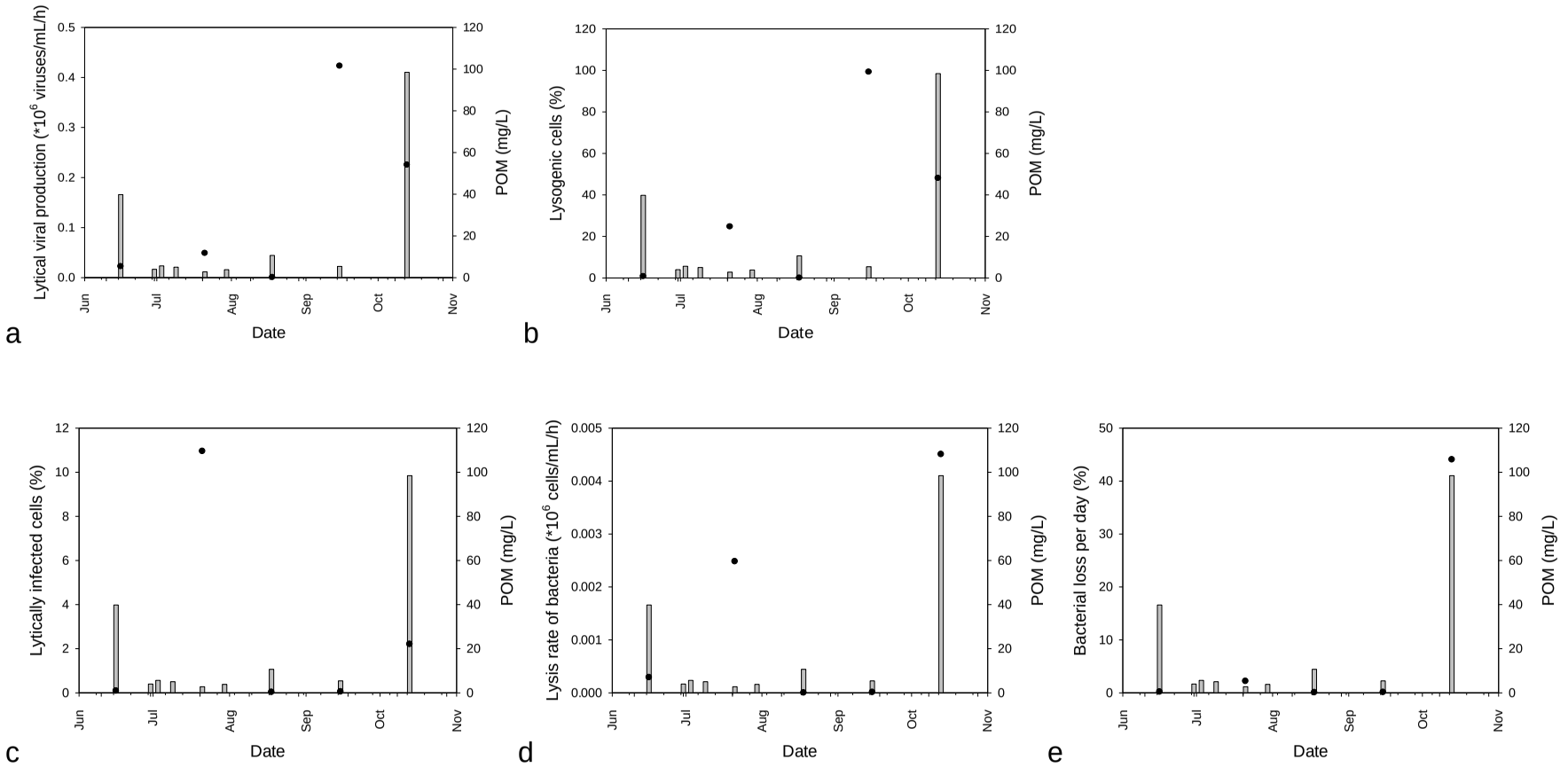


Figure 36. The biotic parameter particulate organic matter (POM) is compared with the calculated lytical viral production (a), % of lysogenic cells (b), % of lytically infected cells (c), lysis rate of bacteria (d) and % of bacterial loss per day (e) using the online tool program VIPCAL at the station Regelsbrunn in the interval from June to October 2009 (black point = viral parameters from Virus Reduction Approach (VRA); grey bar = biotic parameters; sampling dates are shown in Tab.1).

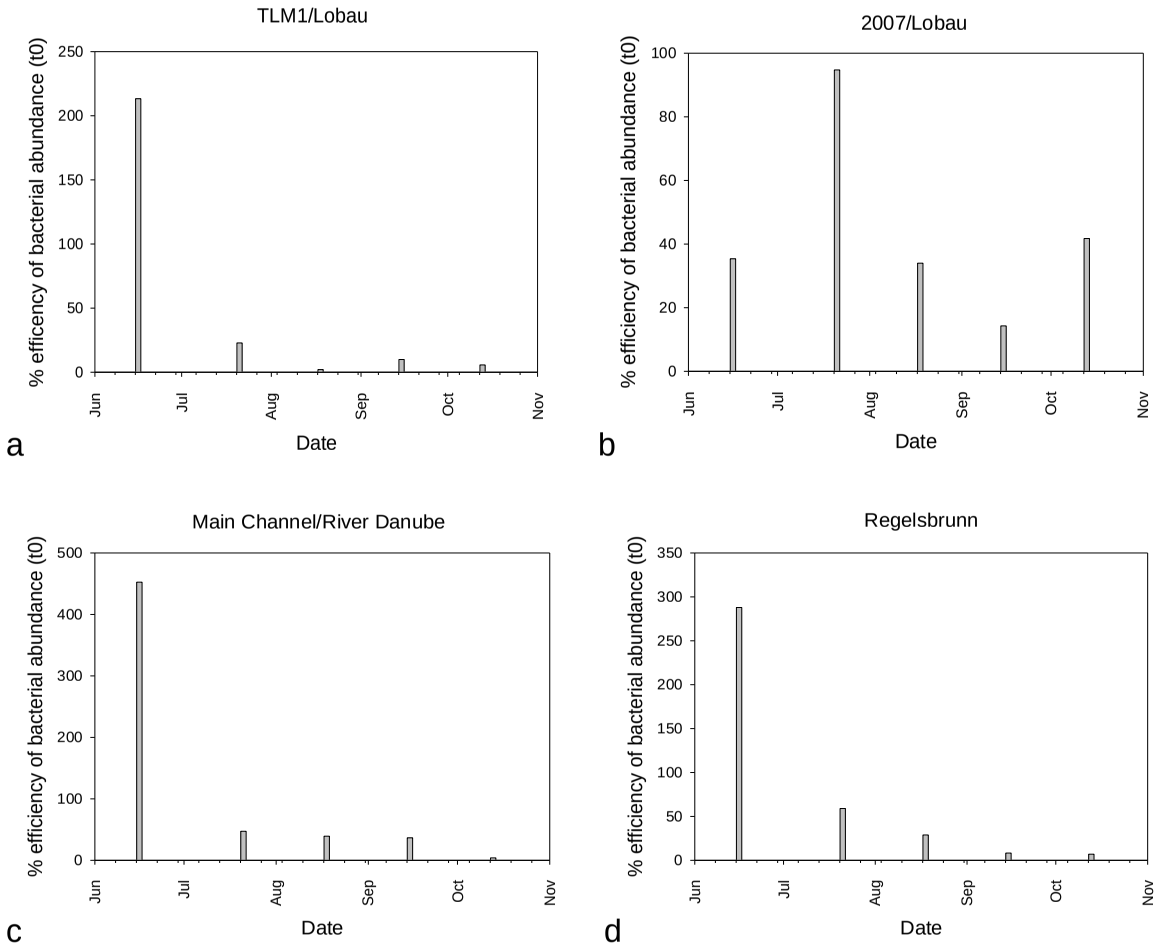


Figure 37. Percentaged recover-efficiency of bacterial abundances at the beginning of Virus Reduction Approach (VRA) experiments at the stations TLM1/Lobau (a), 2007/Lobau (b), Main Channel/ River Danube (c) and Regelsbrunn (d) in the interval from June to October 2009 (grey bar = % efficiency of bacterial abundance at time 0 from Virus Reduction Approach (VRA); sampling dates are shown in Tab.1).

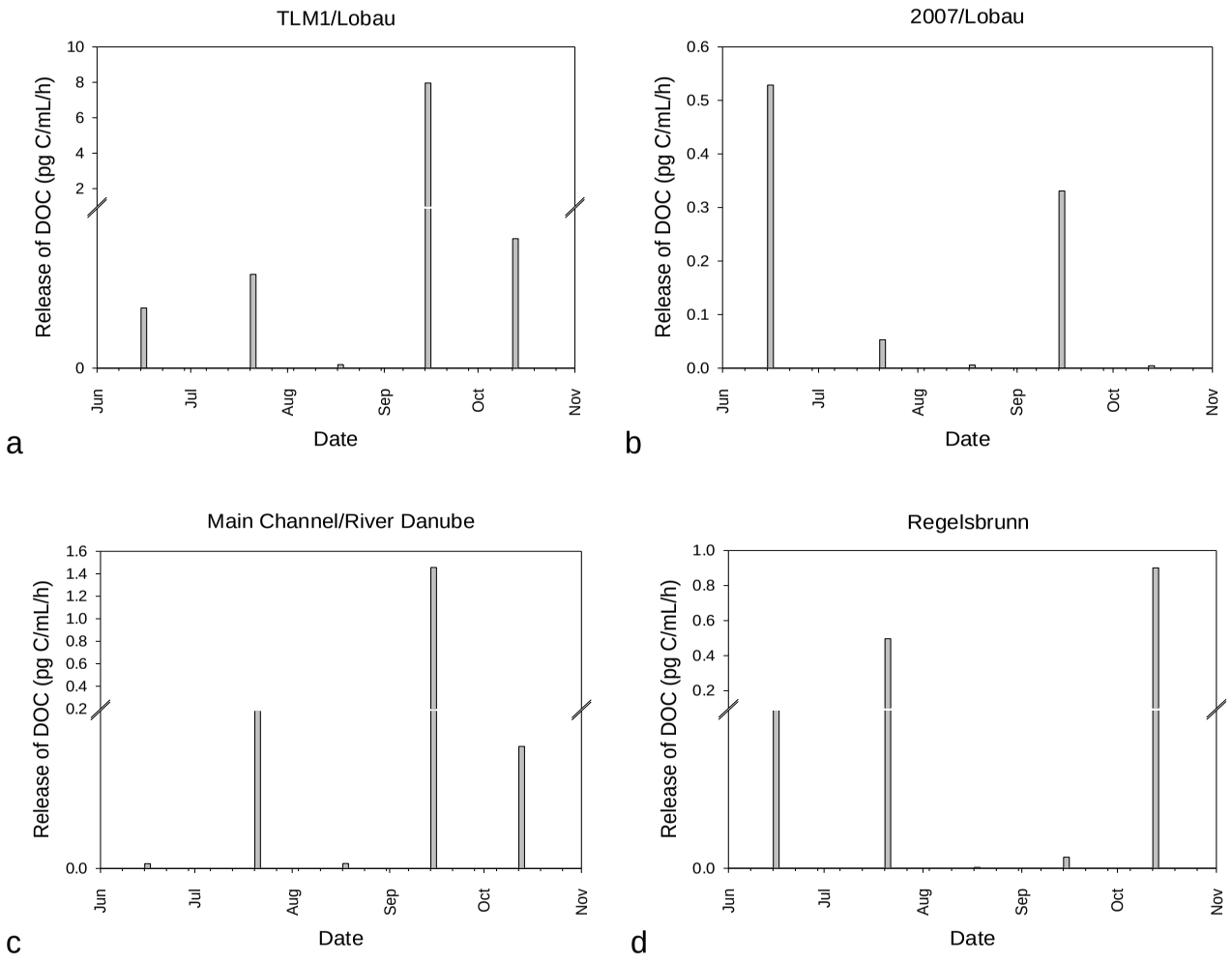


Figure 38. Release of DOC using the online tool program VIPCAL at the stations TLM1/Lobau (a), 2007/Lobau (b), Main Channel/ River Danube (c) and Regelsbrunn (d) from Virus Reduction Approach (VRA) experiments (grey bar = viral parameters from VRA; sampling dates are shown in Tab.1).

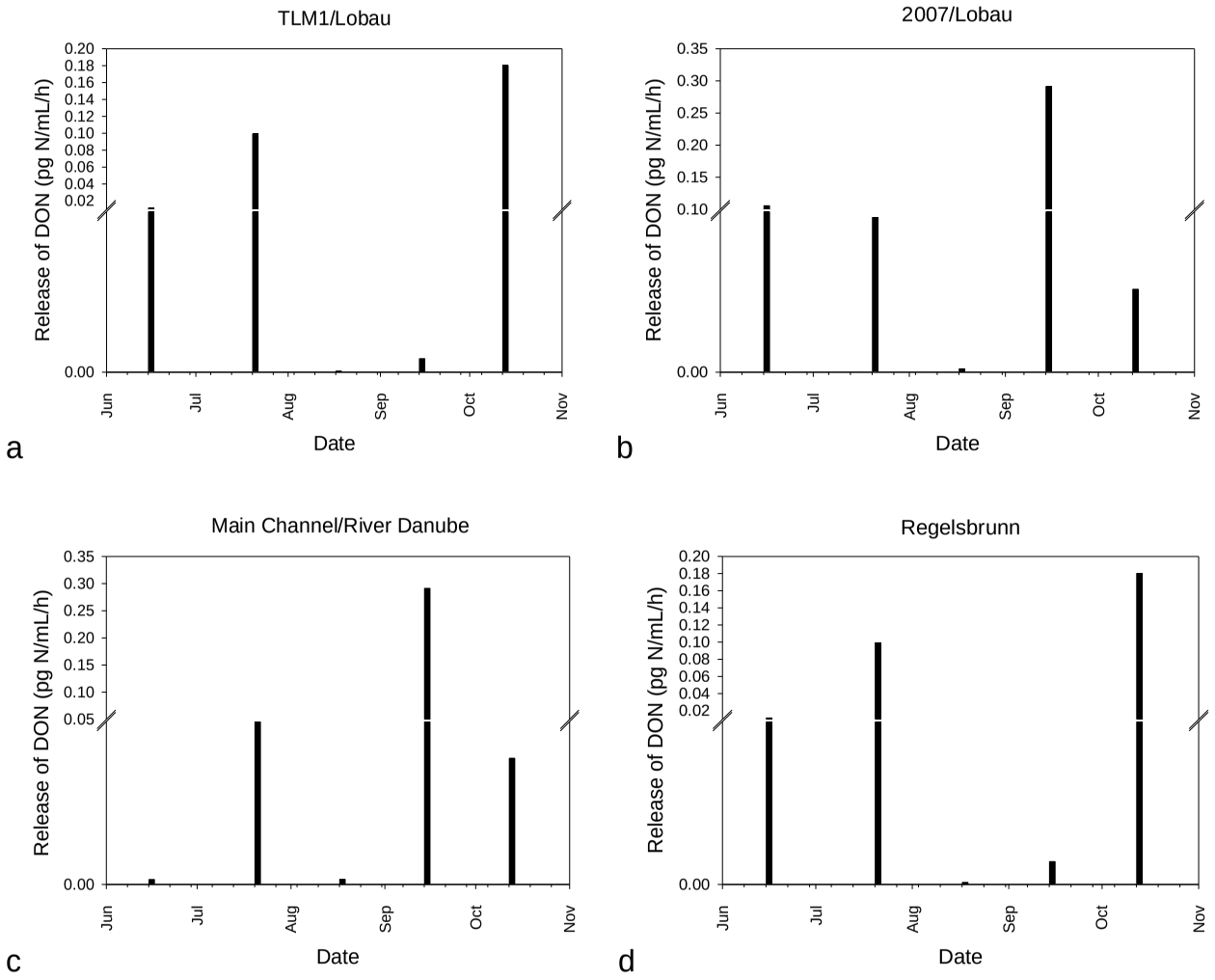


Figure 39. Release of DON using the online tool program VIPCAL of the stations TLM1/Lobau (a), 2007/Lobau (b), Main Channel/ River Danube (c) and Regelsbrunn (d) from Virus Reduction Approach (VRA) experiments (black bar = viral parameters from VRA; sampling dates are shown in Tab.1).

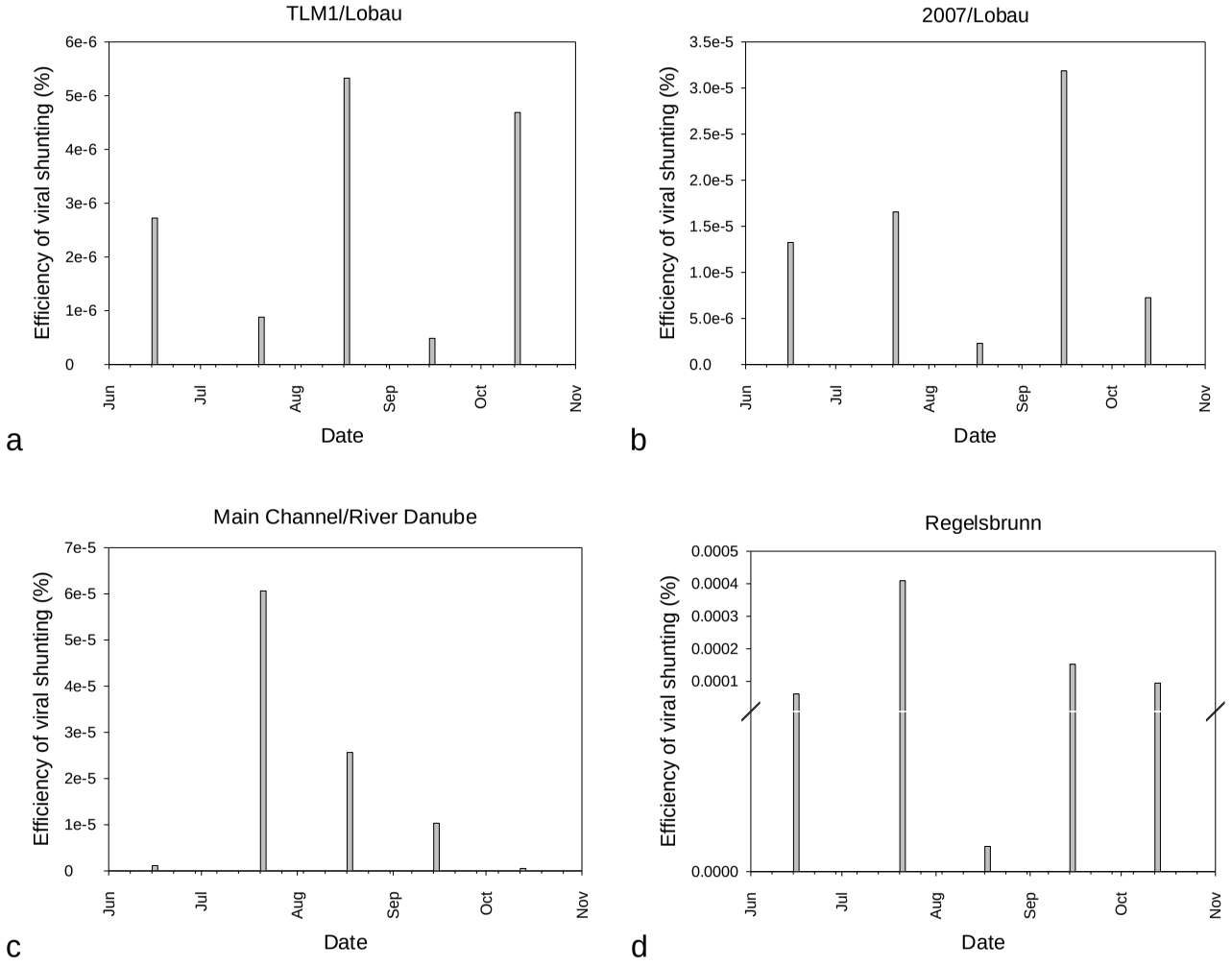


Figure 40. Efficiency of viral shunting (%) using the online tool program VIPCAL of the stations TLM1/Lobau (a), 2007/Lobau (b), Main Channel/ River Danube (c) and Regelsbrunn (d) from Virus Reduction Approach (VRA) experiments (grey bar = viral parameters from VRA; sampling dates are shown in Tab.1).

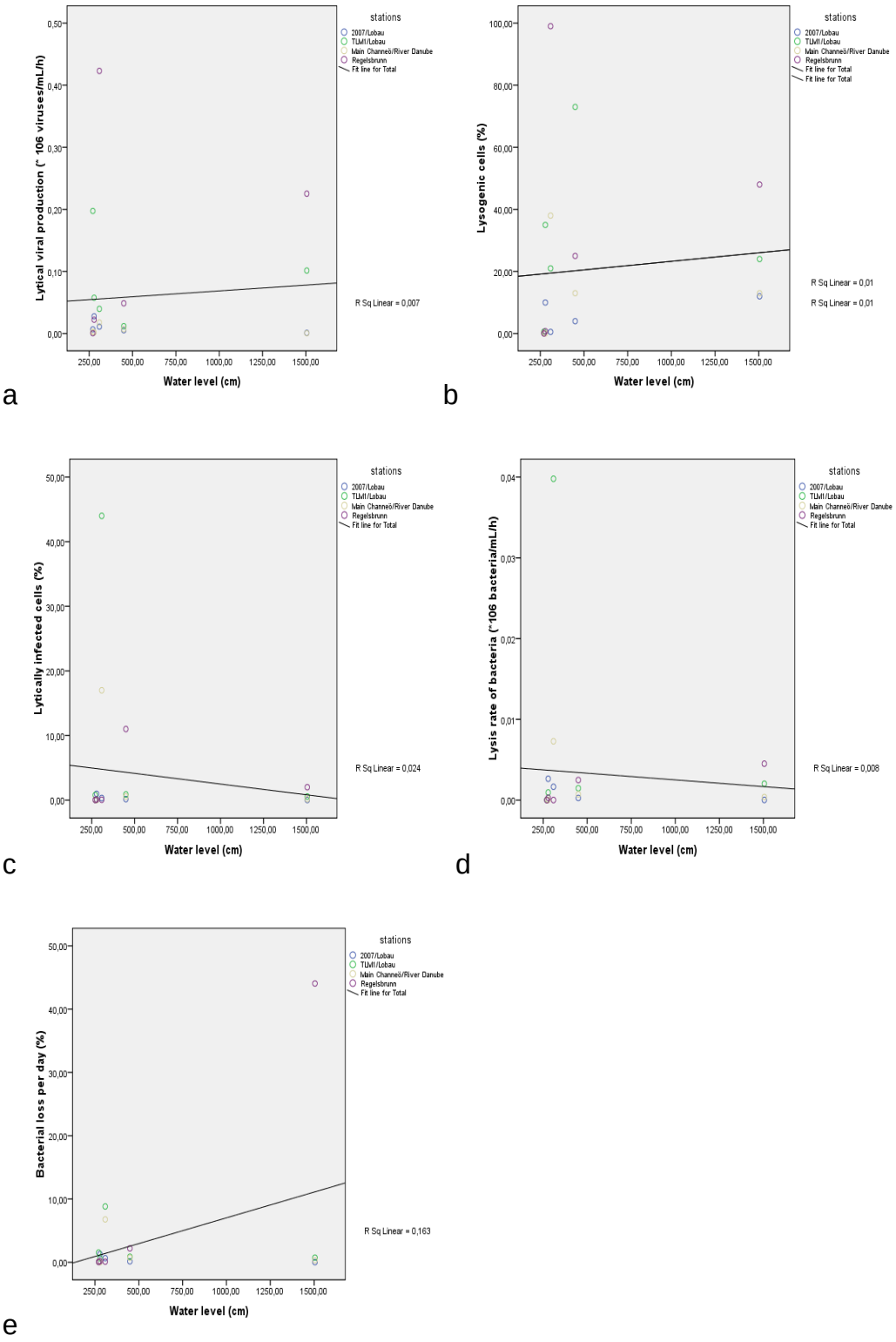
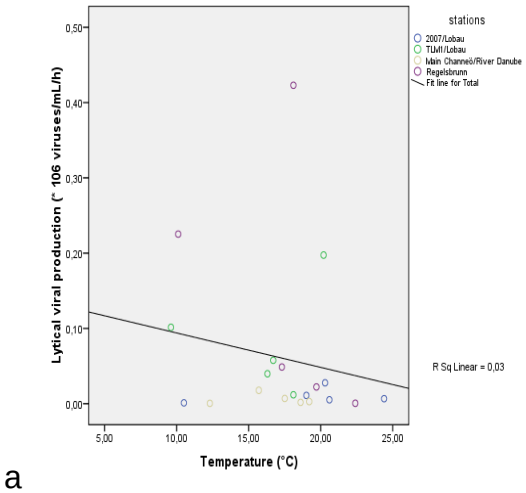
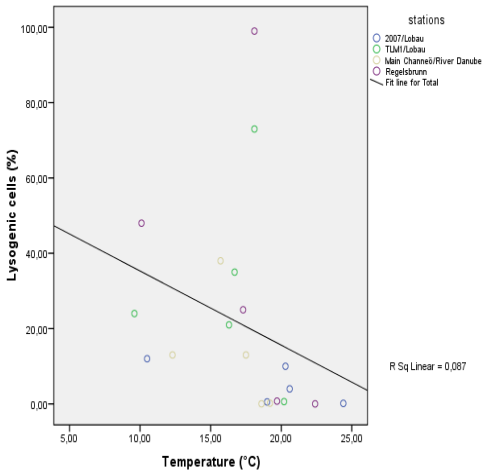


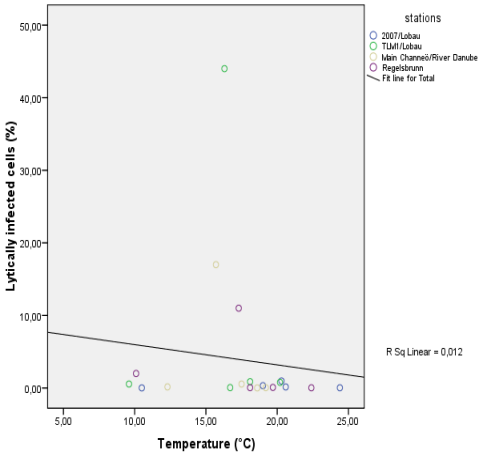
Figure 41. Regression analysis; comparison of the water level of the River Danube with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.



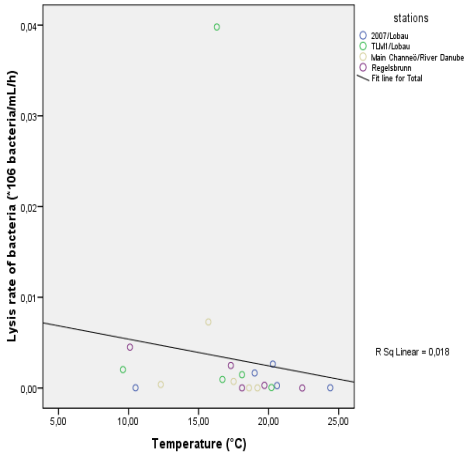
a



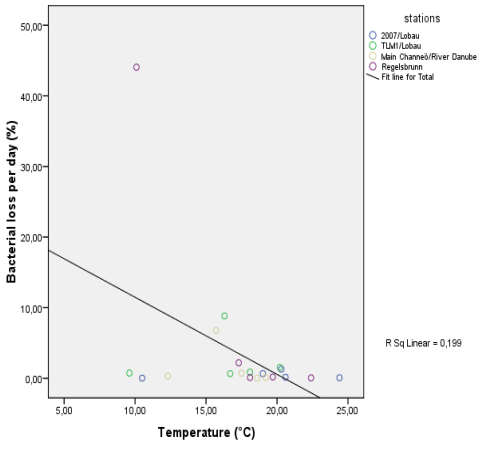
b



c



d



e

Figure 42. Regression analysis; comparison of the abiotic parameter temperature with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

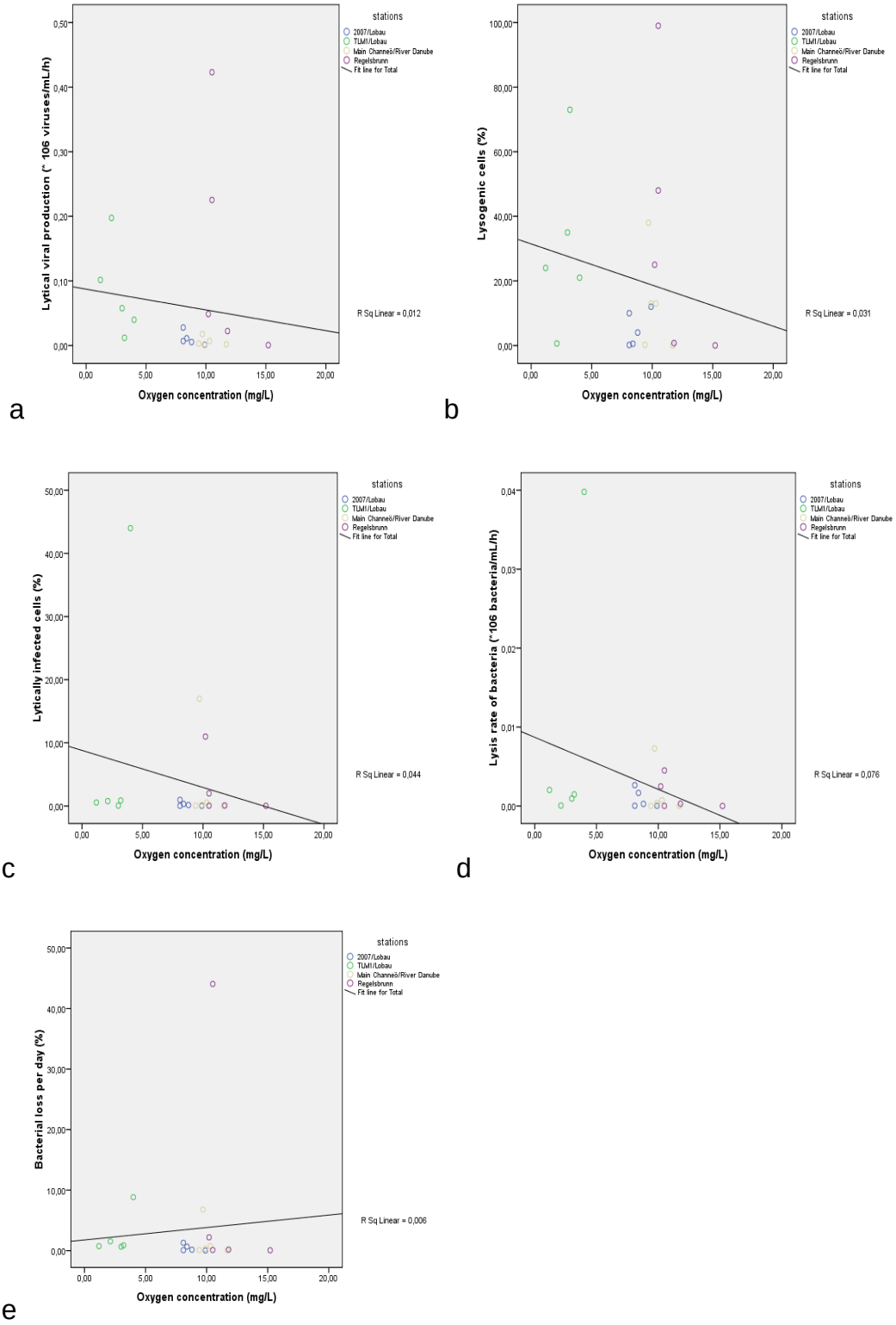


Figure 43. Regression analysis; comparison of the abiotic parameter oxygen with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

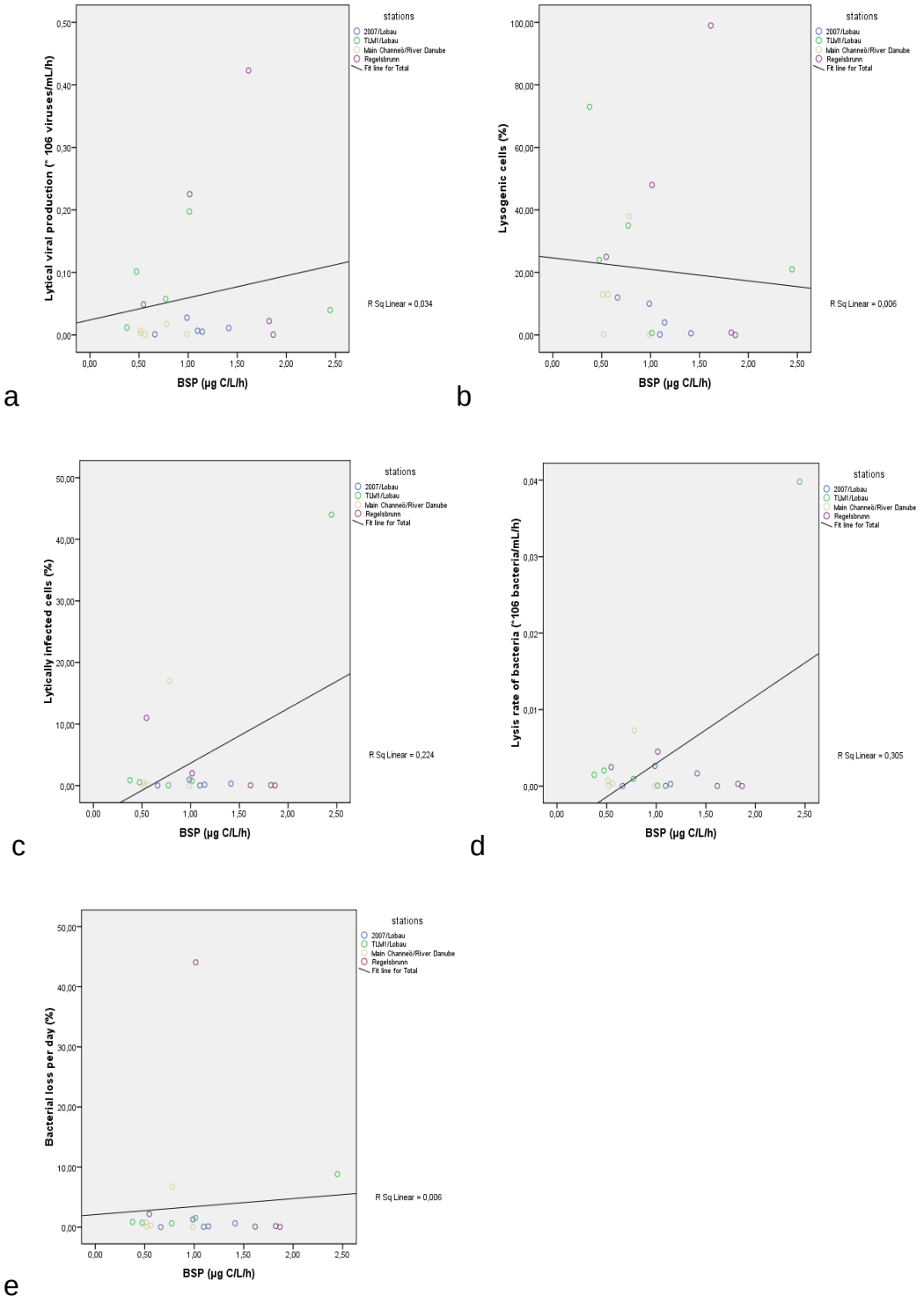


Figure 44. Regression analysis; comparison of the biotic parameter bacterial secondary production (BSP) with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

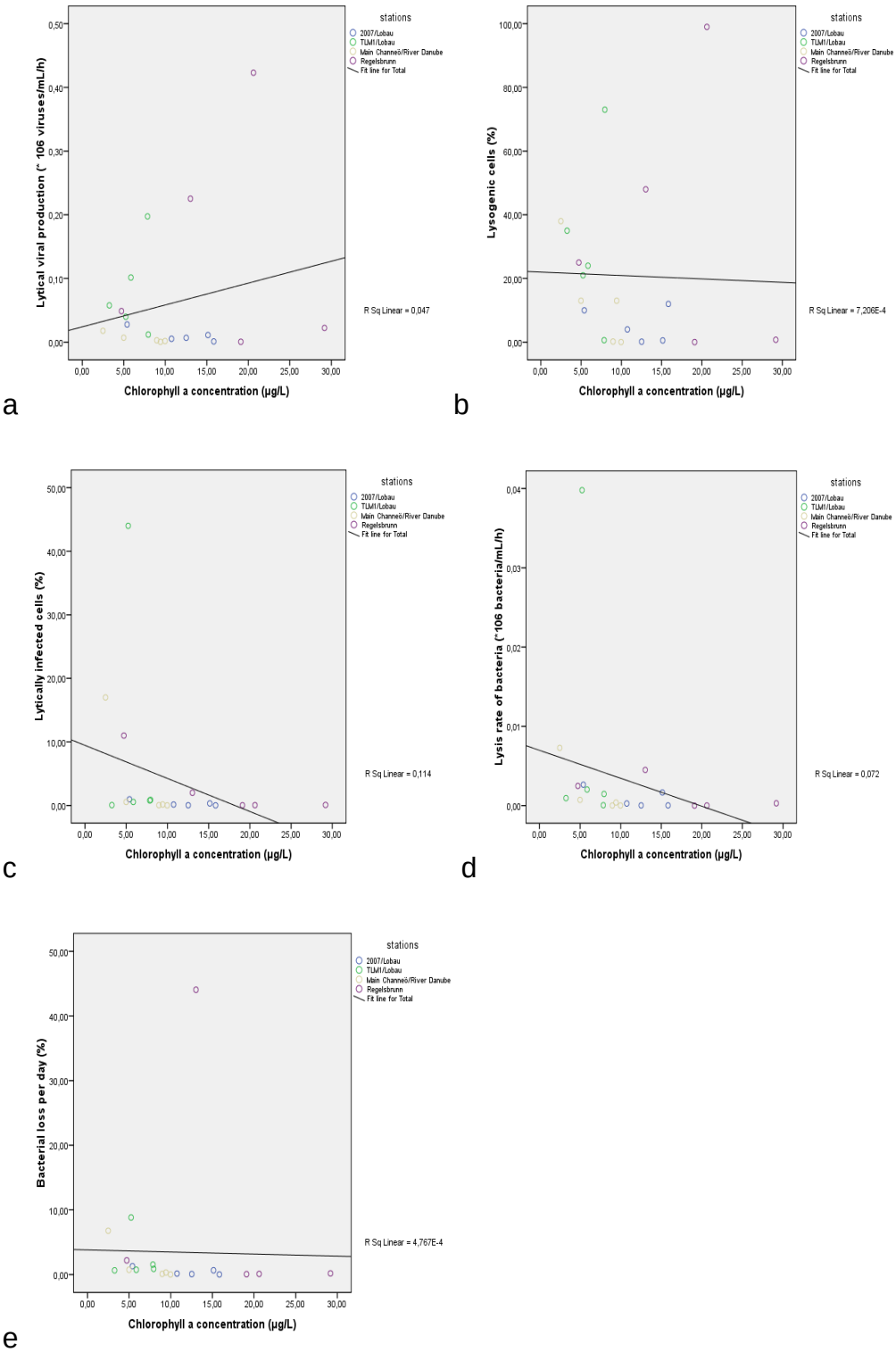


Figure 45. Regression analysis; comparison of the biotic parameter chlorophyll *a* concentration with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

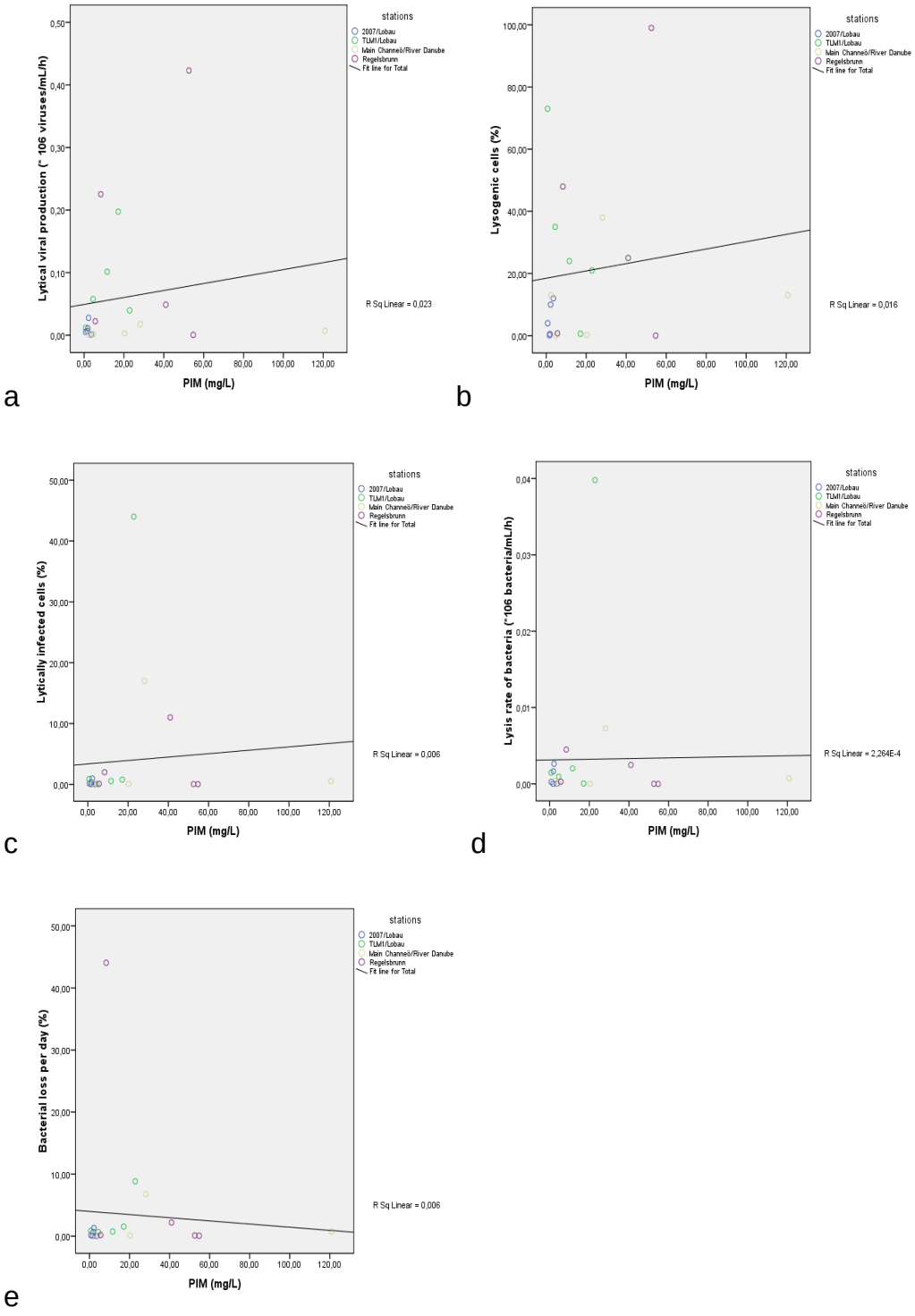


Figure 46. Regression analysis; comparison of the abiotic parameter particulate inorganic matter (PIM) with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL: Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

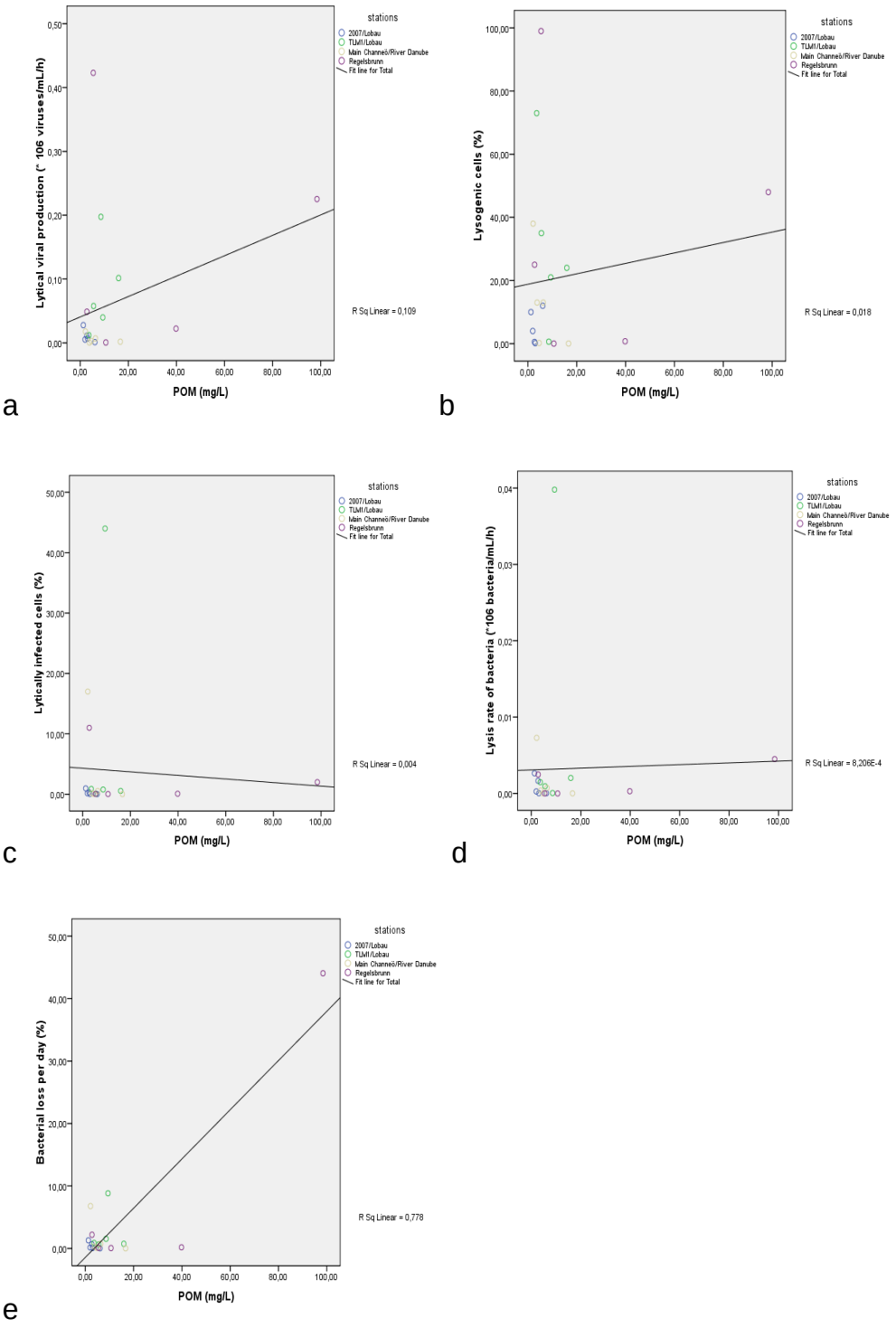
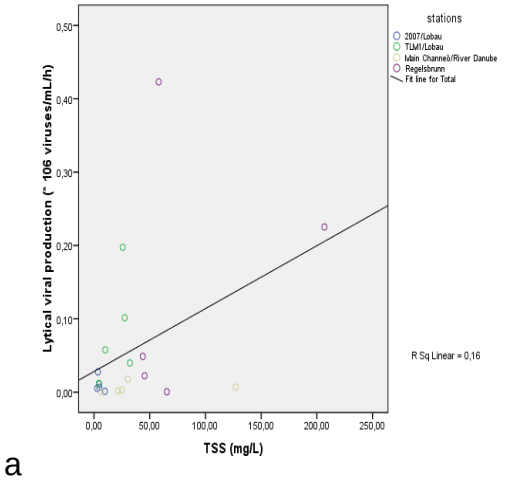
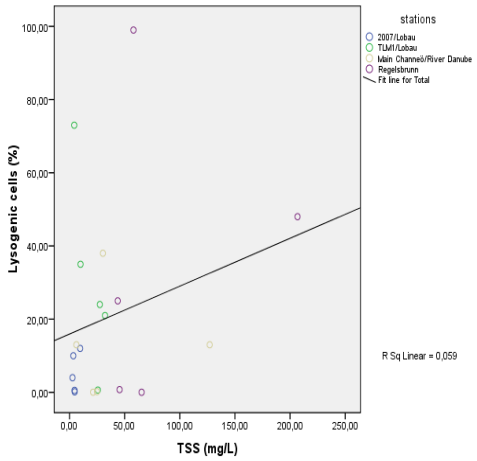


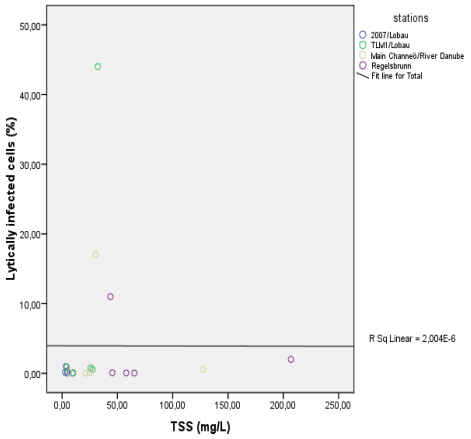
Figure 47. Regression analysis; comparison of the biotic parameter particulate organic matter (POM) with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.



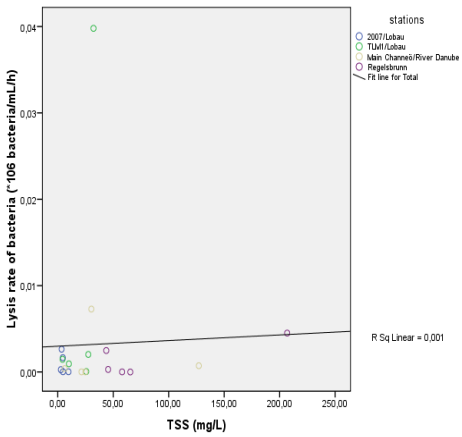
a



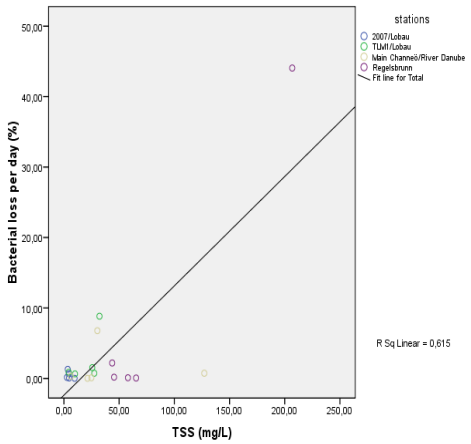
b



c



d



e

Figure 48. Regression analysis; comparison of the biotic parameter total suspended solids (TSS) with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

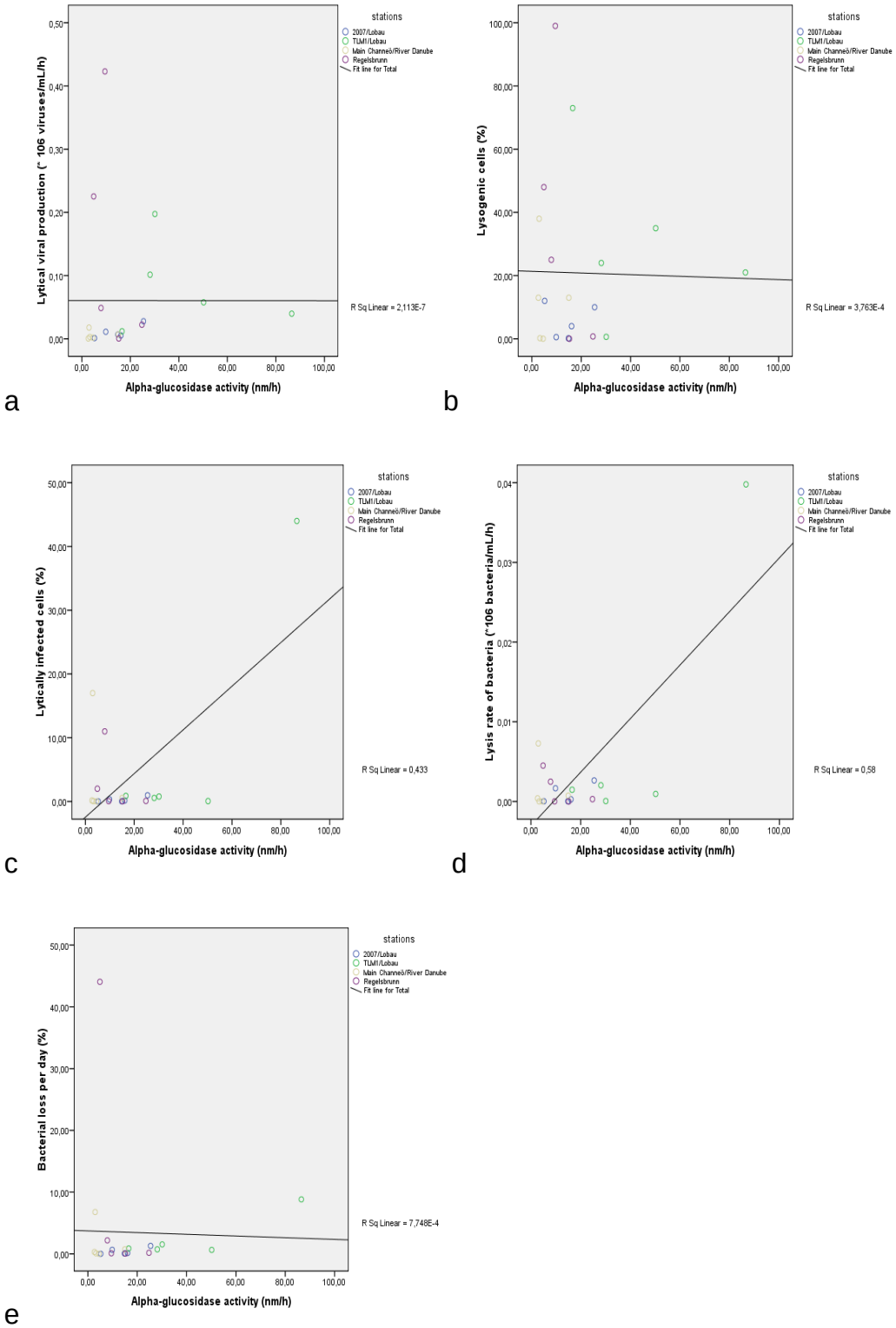


Figure 49. Regression analysis; comparison of the aplha-glucosidase activity with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

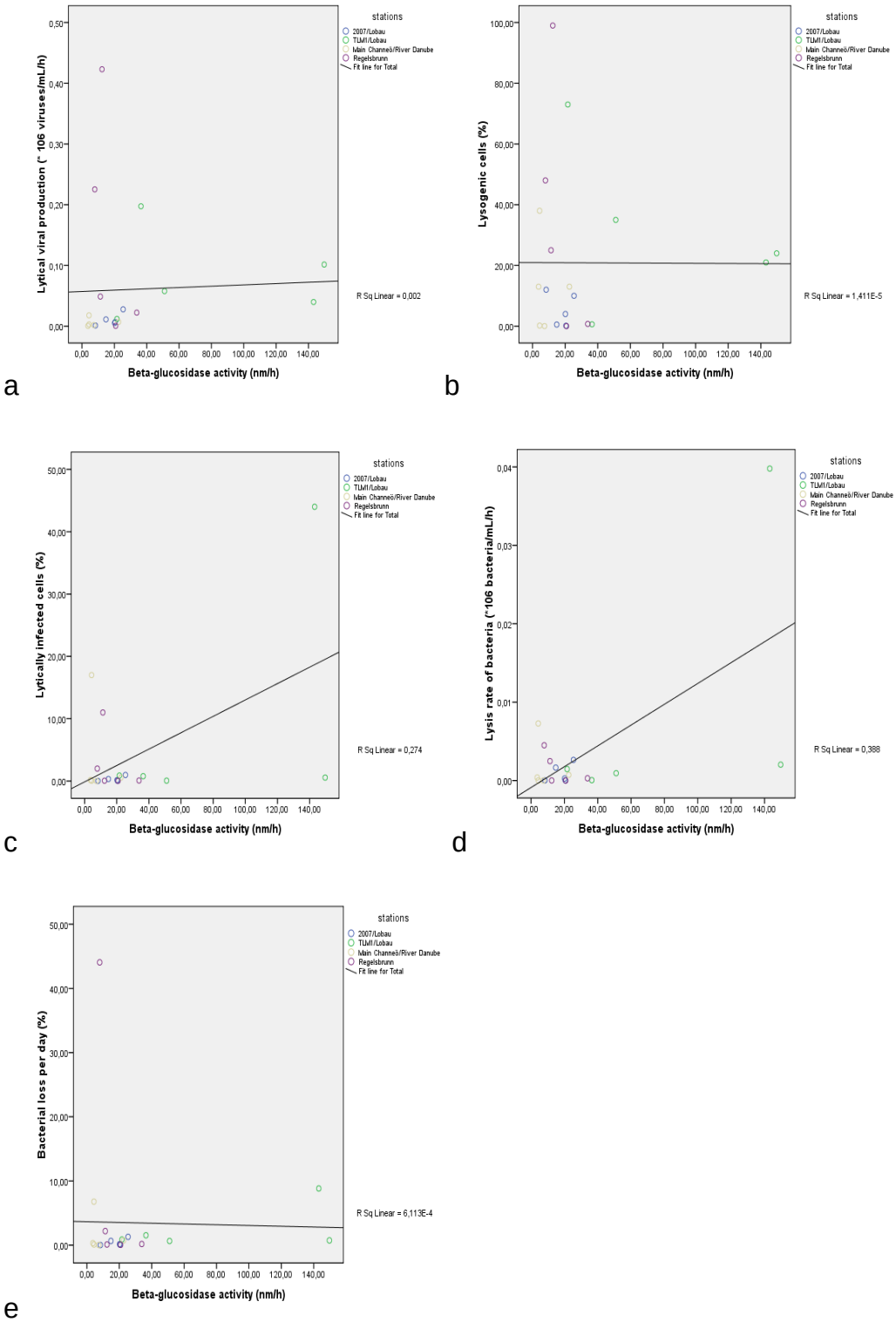


Figure 50. Regression analysis; comparison of the beta-glucosidase activity with the viral parameters lytical viral production (a), lysogenic cells (b), lytically infected cells (c), lysis rate of bacteria (d) and bacterial loss per day (e) from Virus Reduction Approach (VRA) experiments using the online tool program VIPCAL. Pooled data are from stations TLM1/Lobau, 2007/Lobau, Main Channel/ River Danube and Regelsbrunn.

Curriculum Vitae von Jana Tvarogova

Persönliche Daten

Familienname: Tvarogová

Vorname: Jana

E-mail Adresse: tvarogova@gmx.at

Persönliche Fachkenntnisse und Erwerbsfähigkeiten

Muttersprache:	Slowakisch: Ausgezeichnet in Wort und Schrift
Andere Sprache(n):	Deutsch: Ausgezeichnet in Wort und Schrift
	Englisch: Ausgezeichnet in Wort und Schrift
	Tschechisch: Gut in Wort und Schrift
	Französisch: Gut in Wort und Schrift

2006 - : Mitarbeiterin im Besucherservice (verantwortlich für die Betreuung von Führungen, Seminaren und Workshops für Privatgruppen sowie für die Betreuung von Führungen und Seminaren für Schulklassen) im Zoo Wien Schönbrunn, Österreich

2009 - : Übersetzerin für die Firma TWG.sro, Slowakei (Energetische Zertifikate)

Technische Fachkenntnisse und Kompetenzen

Erfahrungen in der Arbeit mit:	ClustalX2
	Excel
	FinchTV
	ForCon
	GeneDoc
	MrBayes
	PAUP 4.0
	Power Point
	SigmaPlot
	SPSS
	TreeView
	Word

Formale Schulbildung

1991-1997: Unterstufe in der Hauptschule Sv.Gorazda, Zilina, Slowakei

1997-2000: Mittelstufe in der Hauptschule Ost, Stockerau, Österreich

2000-2004: Erzbischöfliches Aufbau- und Realgymnasium mit Schwerpunkt in Humanbiologie, Psychologie und Philosophie, Hollabrunn, Österreich

Höhere Schulbildung

2004 - 2010: Universität Wien, Wien, Österreich

2007-2009: Spezieller Schwerpunkt im Bereich Limnologie und mikrobieller Ökologie

Graduierte Praktika und Kurse:

- Aquatische Mikrobielle Ökologie
- Fluorescence-In-Situ-Hybridisierung (FISH) Kurs
- Licht- und Videomikroskopie in der Praxis
- Methoden der Ökogenetik
- Methoden der molekularen Phylogenetik – Von der DNA Extraktion bis Stammbaumrekonstruktion
- Moderne EDV-unterstützte Auswertungsmethoden
- Phylogenie der Prokaryoten
- Schwermetallstress: Ökologie von Organismen
- Submikroskopische Anatomie und Präparationstechnik
- Übungen zur Wasserchemie
- Virale und bakterielle Diversität in antrophogen beeinflussten aquatischen Systemen

2009 - Juli 2010: Diplomarbeit am Institut der Limnologie

Titel der Diplomarbeit: Virale Abundanz, lytische Lebenszyklen und Lysogenie im Fluss-Ausystem (Donau, Österreich)