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Differential grazer-mediated effects of high summer temperatures on pico- and nanoplankton communities

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Abstract

We investigated the role of a macrograzer (the filter feeding mussel *Dreissena polymorpha*) in mediating effects of high summer temperatures on the dominant components of natural river plankton (i.e., bacteria, algae, and heterotrophic flagellates) in flow channel experiments. Effects of adaptation (by comparing mussels from a southern and a northern population) and thermal acclimation of the mussels were considered. Both heterotrophic flagellates and algae are released from grazing pressure and increase in abundance at temperatures above 20°C. Bacterial abundance, however, decreased with increasing temperature, suggesting a trophic cascade (mussel–flagellates–bacteria) that is altered by the temperature response of the mussel ingestion rate. Warm acclimation of the mussels did not change the outcome of the experiments. The dreissenids from the southern population showed a significantly higher ingestion rate than those from the northern population only in July. The general pattern (i.e., decreasing ingestion rates at high temperatures) was found in both populations. Microbial communities controlled by macrofauna can experience substantial changes in warm summers because of differential development of direct and indirect grazing effects with increasing temperature.

Currently we are facing a temperature increase caused by anthropogenic emission of greenhouse gases. In the most probable scenarios, the average global surface temperature is projected to increase by between 1.7°C and 4.0°C during the 21st century (IPCC 2007). Temperature changes can even be greater on a local or temporal scale. Examples are European summer heat waves, which are predicted to occur in high frequencies in the near future (Schär et al. 2004).

The temperature increase already affects organisms and ecosystems on different levels, e.g., by influencing the feeding rates of organisms and the strength of species interactions (e.g., Sanford 1999) or by leading to shifts in the geographic ranges of organisms (for review see Parmesan 2006). It is important that ecologists are able to understand and predict the ecological consequences of temperature increases. To do so, it is essential to identify processes that (1) contribute significantly to ecosystem functioning and (2) are sensitive toward small temperature changes (cf. Sanford 1999). The grazing of plankton by benthic filter-feeders, particularly mussels, in rivers, shallow lakes, and coastal areas is such an interaction. It can have a considerable influence on ecosystem functions since it has a strong effect on the composition of the plankton and acts as a link through which a large part of primary and secondary plankton production is imported into the benthos (Welker and Walz 1998; Jack and Thorp 2000; Weitere and Arndt 2002). Both the grazing rates (Walz 1978; Aldridge et al. 1995; Lei et al. 1996) as well as the growth rates of the planktonic organisms (e.g., Montagnes et al. 2003) depend strongly upon temperature. However, the two rates can show different responses toward warming. We have recently shown that the grazing rate of the invasive freshwater mussel *Corbicula fluminea* on planktonic heterotrophic flagellates (HF) decreases with high summer temperatures relative to the growth rate of its prey, leading to a rapid increase in HF abundance at high temperatures due to the grazing release (Viergutz et al. 2007). The differential development of the macrofaunal grazing rates and the growth rates of unicellular organisms is therefore one way through which temperature changes can alter the structure of microbial communities.

Benthic filter-feeding communities among the macrofauna are often dominated by relatively few species; this is especially the case when they are dominated by invasive species and when the increase of the invaders' abundance is correlated with dramatic decreases in the abundance of native competitors, as has been demonstrated for the zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Ricciardi et al. 1998; Schloesser et al. 2006). Indigenous to the Ponto-Caspian area, this dominant and efficient benthic filter-feeder has invaded large parts of Europe and North America, where it is now widespread in various fresh- and brackish water environments (Reid and Orlova 2002). It has been shown that the invasion of *D. polymorpha* can lead to a strong restructuring of aquatic communities (Caraco et al. 1997; Findlay et al. 1998; Caraco et al. 2006). The success of many invasive species such as *D. polymorpha* is at least partly attributed to environmental changes (Dukes and Mooney 1999; Stachowicz et al. 2002). However, the secondary effect of environmental warming on communities dominated by the invader is as yet poorly explored.

Here we analyzed the consequences of temperature-driven changes in the grazing pressure of *D. polymorpha* on the major components of the riverine planktonic food web, i.e., bacteria, algae, and HF. These three groups dominate

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Table 1. Experimental conditions for the four grazing experiments. The lowest temperatures in experiments one and two reflect the long-term mean temperature during the particular time period. The temperature in the Rhine before the start of the experiments reflects the acclimation temperature for experiments one and two, whereas the mussels were acclimated to constant temperatures in experiments three and four.

Experiment	1	2	3	4
Starting date	23 May 2005	17 Jul 2005	10 Jul 2006	19 Sep 2005
Origin of mussels	Rhine/Danube	Rhine/Danube	Danube	Danube
Acclimation temperature (°C)	18.5 (mean Rhine temp.)	23.3 (mean Rhine temp.)	20 and 28	25
Test temperatures (°C)	18.9; 20.9; 22.9; 24.9	22.9; 24.9; 26.9; 28.9	25.0; 28.0; 30.0	20.0; 25.0; 28.0; 30.0
Volume per flow channel (L)	9	9	10	10
Flow rate (mL min^{-1})	14	14	0	0
Number of mussels per flow channel	10	12	5	6
Mussel size (cm)	1.8–2.2	1.8–2.5	2.1–2.5	1.7–2.2
Total mussel AFDW	0.39 (Rhine)	0.21 (Rhine)	0.127 (20°C accl.)	0.076
Per flow channel (g)	0.26 (Danube)	0.25 (Danube)	0.123 (28°C accl.)	
Mean proportion of active filtering mussels (%)	97 (Rhine) 80 (Danube)	89 (Rhine) 83 (Danube)	Not measured	Not measured
Starting algal abundance (cells mL^{-1})	4,497±1,736 SD	1,353±676 SD	5,206±1,184 SD	325±119 SD
Algae size classes (μm) (first, second, and third quartile)	3, 7, 30	5, 11, 30	7, 14, 20	Not measured
Starting bacterial abundance (10^5 cells mL^{-1})	9.23±1.3 SD	6.49±0.44 SD	8.36±1.18 SD	13.14±4.62 SD
Bacteria size classes (μm) (first, second, and third quartile)	0.3, 0.4, 0.6	0.3, 0.4, 0.5	0.4, 0.5, 0.8	0.3, 0.4, 0.7
Starting abundance of heterotrophic flagellates (HF) (cells mL^{-1})	622±102 SD	156±38 SD	144±51 SD	287±91 SD
HF size classes (μm) (first, second, and third quartile)	Not measured	Not measured	Not measured	3, 4, 6

the plankton biomass and production in rivers (Servais et al. 2000; Chetelat et al. 2006; Joaquim-Justo et al. 2006) and contribute to about 99% of the total plankton biomass in our study area, the river Rhine (Weitere et al. 2005). An important focal point of the study was revealing whether the mussel-mediated effects of summer temperature increase appear in the same manner for the three groups or whether indirect effects (particularly trophic cascading, cf. Polis et al. 2000) generate varying effects in the different prey groups. It is likely that algae respond in a similar manner as demonstrated earlier for the HF under the grazing of *C. fluminea* (Viergutz et al. 2007), because both groups belong to the size class preferred by mussels (mainly nanoplankton, Sprung and Rose 1988; Lei et al. 1996) and both groups are poorly controlled by other planktonic consumers in the Rhine food web (Weitere et al. 2005). Bacteria, however, are less efficiently consumed by mussels (Sprung and Rose 1988; Lei et al. 1996; Frischer et al. 2000) and are strongly preyed upon by planktonic HF within riverine food webs (Servais et al. 2000; Weitere et al. 2005; Joaquim-Justo et al. 2006). Studies show that bacterivorous protists act as a trophic link between bacteria and mussels (e.g., Loret et al. 2000) and field observations show a stimulating effect of the presence of *D. polymorpha* on planktonic bacteria, probably due to a negative effect of the grazing on the HF as main planktonic consumers of the bacteria (Findlay et al. 1998). Here we tested first the dependence of the grazing pressure of *D. polymorpha* on algae and bacteria at high temperatures. Acclimation of the mussels to high temperatures was investigated as well as adaptation effects by considering mussels with different invasion histories (see below). In a second step, we analyzed the net effect (as a result of loss and growth processes) of temperature increase on both heterotrophic groups (the bacteria and their main planktonic consumers, the HF) under the effects of mussel grazing.

Materials and methods

General setup and grazers—A total of four experiments using *D. polymorpha* as a grazer was performed. The first two experiments (experiments one and two, Table 1), conducted in May (moderate temperatures) and July (high temperatures) of 2005, were based on the hypothesis that a temperature increase has differing effects on pico- and nanoplankton subjected to mussel grazing. The role of temperature adaptation was also studied. This was followed by a grazing experiment on the role of mussel acclimation to warm temperature (experiment three, Table 1). In a final experiment (experiment four, Table 1), particular attention was paid to the different grazing effects on bacteria and HF (the latter being the main bacterial consumers in the plankton) after different grazing effects on the algae and bacteria due to warming had been

identified in the previous experiments. The experiments were performed at the Ecological Rhine Station of the University of Cologne in Cologne-Bayenthal (Rhine km 685, which refers to the distance from Lake Constance, the source of the nonalpine part of the Rhine). The water used in the experiments was pumped into the experimental channels directly from the river Rhine. In this way the effects of temperature on mussel ingestion could be investigated using a natural plankton community. Samples of the two *D. polymorpha* populations used in the experiments were taken from the Main-Danube canal at Kelheim immediately downstream of the outflow of the Danube and the Lower River Rhine at Rees (km 836). The two populations represent two different and genetically distinguishable invasion lines, i.e., the southern invasion route (up the Danube) and the northern invasion route (across the Dnieper, Prybet, Bug, Vistula, and Midland Canal to the river Rhine and beyond) (Müller et al. 2001).

Forty mussels of each population were weighed for each experiment; they were dried at 60°C for at least 48 h and the dry weights (DW) were measured. The mussels were then combusted at 550°C for 15 h and the ash-free dry weight (AFDW) was calculated as the difference between DW and the ash weight. A length–weight regression was calculated using the AFDW and the shell lengths. This regression was used to calculate the total mussel AFDW from the mussel shell length for each channel (Table 1).

The experimental conditions are summarized in Table 1. Experiments were performed in flow channels as described by Weitere et al. (2003). The channels were equipped with a temperature-controlling system to maintain a constant temperature and with an inflow and an outflow that allowed a constant flow of river water with its natural plankton community. The round channels had an outer diameter of 30 cm and an inner diameter of 10 cm. The water height was 14.3 or 15.9 cm for volumes of 9 and 10 liters, respectively (Table 1). The water surface was partially covered by a rotating disc spiked with combs, which generated a constant water current (20 rotations min^{-1}). In this way the water within the channel was well mixed. There were no differences detectable in the abundances of pico- and nanoplankton in the central part of the channel, in direct proximity to the mussels on the bottom of the channel and in the outflow of the channel in pre-experiments. The experiments were performed in a windowed room, allowing about 1.5% of the natural light intensity to penetrate.

Experiments one and two—The first two experiments (Table 1) were performed under a constant flow of Rhine water. An exchange rate of 2.24 d^{-1} was chosen as a compromise between two competing objectives: The exchange rate had to be high enough to maintain a high degree of similarity to the natural plankton community in the Rhine but low enough to allow the generation of indirect effects within the plankton community in response to the mussel grazing. The mussel number per channel (see below) was chosen in pre-experiments. It reflects the number under which the algal abundance was reduced by an average of one-third in relation to the inflowing water. However, reductions of up to 50% were measured in the main experiments. The experimental setup consisted of control channels, channels with mussels from the Danube, and channels with mussels from the river Rhine for each of the four temperatures (average in situ temperature plus 0°C, 2°C, 4°C, and 6°C). All mussels were acclimated to the main flow of the Rhine at the ambient temperature for the particular season for at least 1 month (Table 1). Three to four replicates were considered for each treatment for all experiments. The number of mussels per channel was 12 and 10 in the experiments performed in May and July, respectively (Table 1). The mussels ranged in length from 1.8 to 2.5 cm with exactly the same sizes of mussels from the Rhine and the Danube for each experiment. Even though their shell sizes were controlled, mussel AFDW differed occasionally between the populations (Table 1). However, the filtration rate depended on the mussel's gill size, which depends on body size rather than on body weight (Lei et al. 1996). The mussels' filtration period (defined as time period of open shells with visible siphons) was recorded over a daily cycle on a half-hour basis for experiments one and two. No further control of filtration activity was undertaken in the later experiments (see below), as no effect of temperature on the filtration period was recorded beforehand.

At the beginning of experiments one and two, the flow channels were filled with 9 liters of Rhine water. Mussels were cleaned carefully with a brush and put into the channels within half an hour after filling. The mussels remained in the channels for 22 h. A stable equilibrium in the abundances of the plankton occurred as a result of the balance between constant water exchange and the filtration activities of the mussels. At that time samples were taken from the inflow and the outflow of the channels.

Mussel-mediated loss rates (LR, cells per vessel d^{-1}) were calculated to show the effect of the mussels on the planktonic algae and bacteria. The LR represents the changes in the cell number directly or indirectly induced by the presence of the mussels. In addition, the ingestion rates (IR, cells per ind. d^{-1}) were calculated for the algae (which are retained by the mussels with a high efficiency, Sprung and Rose 1988; Lei et al. 1996). The LR was calculated using the formula recommended by Filgueira et al. (2006) for clearance rate calculation under conditions of recirculation multiplied by the cell abundance. It is based on the abundances in the in- and outflow (A_i and A_o, cells L^{-1}) and on the flow rate through the experimental channel (f, L d^{-1}):

$$LR = A_i f[(A_i - A_o)/A_o] \quad (1)$$

The rates were corrected for plankton growth by calculating the difference between the rates determined in the grazer treatment and in the mussel-free control. For algae, corrected LRs were divided by the mussel number within the vessel to obtain the ingestion rate.

Experiments three and four—Experiments three and four were performed in stagnant Rhine water for 22 h with mussels from the southern invasion line (Danube), which had been identified in experiment two as being the more

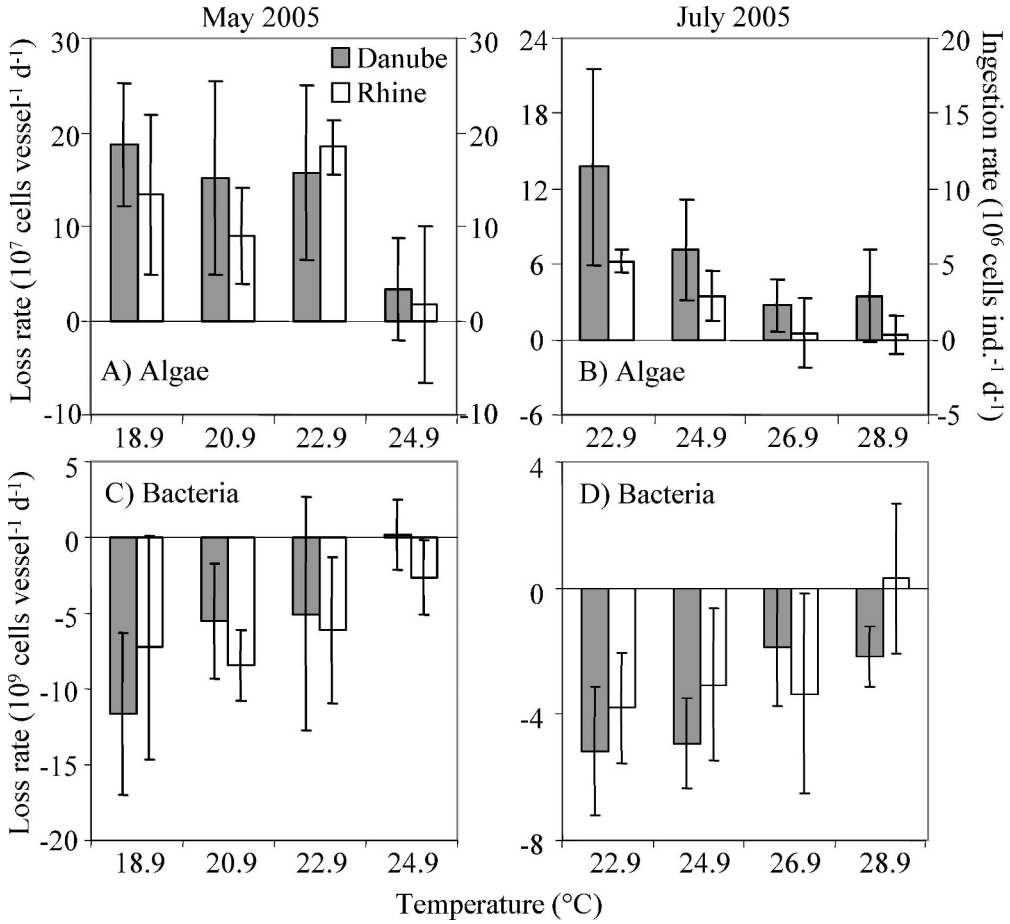

Fig. 1. Temperature and adaptation effects: Mean (± SD) loss rates for *D. polymorpha* from the two populations (Danube and Rhine) with different invasion histories on (A, B) planktonic algae and (C, D) bacteria in the experiments conducted in May and July. Significant temperature effects on the rates were shown for both dates and both groups, whereas significant population effects were found for algae in July only (Table 2). The second *y*-axis gives the corresponding ingestion rates for algae.

efficient grazers at high temperatures. Five and six mussels per channel were used in experiments three and four, respectively. Again, mussels of exactly the same size were used for each treatment and replicate within one experiment. Experiment three, which was focussed on the effect of acclimation on the ingestion rates at high temperatures, was run at temperatures of 25°C, 28°C, and 30°C. The experiment consisted of mussel-free controls, treatments with mussels acclimated for 3 weeks to 20°C, and treatment with mussels acclimated for 3 weeks to 28°C. Experiment four, which was focussed on the net effects on bacteria and HF, was run at temperatures of 20°C, 25°C, 28°C, and 30°C. Treatments containing the grazers as well as grazer-free controls were considered. Here the mussels were acclimated to a constant 25°C for 3 weeks. A small part of the fourth experiment (i.e., the clearance rates on heterotrophic flagellates for 25°C and 30°C) has been published by Viergutz et al. (2007).

For experiment three, mussel-mediated LR (cells per vessel d^{-1}) were calculated for both algae and bacteria, and IR (cells per ind. d^{-1}) were additionally calculated for algae as also done for experiments one and two (see above). The LR was calculated by multiplying the rate of change in the abundance (r, d^{-1}) by the start abundance (A_s, cells L^{-1}) and the total water volume in the vessel (V, liters):

$$\mathrm{LR} = VrA_s \tag{2}$$

The rate of change in abundance r was calculated from the abundances at the start and end of the experiment (A_s and A_e, cells L^{-1}) and of the duration of the experiment (t, d):

$$r = [\ln(A_e) - \ln(A_s)]/t \tag{3}$$

The rates were corrected for plankton growth. For experiment four, we calculated the rate of change in abundance measured in the mussel-free control (r_{co}, termed hereafter "gross growth rate") and the rate of change in abundance measured under the presence of the grazer (r_g, termed hereafter "net growth rate") for both bacteria and HF. For the HF, which fell (as did the algae) into the

Table 2. Results of two-factorial ANOVAs testing the effects of temperature and mussels' origin on the loss rates of algae (10^8 cells per vessel d^{-1}) and bacteria (10^{10} cells per vessel d^{-1}) for experiments one (May 2005) and two (July 2005).

	SS	df	*F*	*P*
May 2005, algae				
Temperature	7.940	3	4.767	0.015
Origin of mussels	0.410	1	0.738	0.403
Temperature × origin	0.740	3	0.444	0.725
Residual	8.884	16		
May 2005, bacteria				
Temperature	2.847	3	4.338	0.020
Origin of mussels	0.107	1	0.488	0.495
Temperature × origin	0.196	3	0.299	0.826
Residual	3.500	16		
Jul 2005, algae				
Temperature	2.734	3	6.895	0.003
Origin of mussels	1.093	1	8.267	0.010
Temperature × origin	0.247	3	0.623	0.610
Residual	2.379	18		
Jul 2005, bacteria				
Temperature	0.476	3	3.645	0.033
Origin of mussels	0.082	1	1.876	0.188
Temperature × origin	0.141	3	1.083	0.381
Residual	0.783	18		

preferred size spectrum of the mussels (Sprung and Rose 1988; Lei et al. 1996), the grazing rates were calculated as the difference of gross and net growth rate.

Analysis of the plankton—The plankton was fixed with ice-cold glutaraldehyde solution (final concentration: 2 %) immediately after sampling. For quantification of algae (mostly autotrophic nanoplankton) and bacteria, 4 mL of the glutaraldehyde-fixed samples were stained with 4′,6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980) within 10 h after sampling, with a final DAPI concentration of 10 μg mL^{-1} for algae and 5 μg mL^{-1} for bacteria. The stained samples were filtered on black polycarbonate-membrane filters (0.2 μm, Whatman Nucleopore, Whatman) and kept frozen at $-20^{\circ}C$ until the algae and bacteria were counted under the epifluorescence microscope. At least 60 algae and 300 bacteria per filter were counted in randomly distributed spots on the filter. The cell dimensions of all algae counted and of 100 bacteria per filter were measured to determine the size spectra. In the fourth experiment (Table 1), we particularly focussed on the HF. Since a definite attribution of stained particles to HF in DAPI-stained fixed samples is not possible in all cases, we used a live-counting technique immediately after sampling, as described by Weitere and Arndt (2002).

Statistical analysis—Statistical analysis was conducted using the software package SPSS 12.0 for Windows. The dependence of the rates on temperature and origin of mussels as well as the dependence of the rates on test temperature and acclimation conditions was tested in two-factorial ANOVA designs. The dependence of the net and gross growth rates on temperature in experiment four was tested with Spearman rank correlations.

Results

Temperature effect on grazing pressure on algae and bacteria—Temperature increase generally resulted in significant decreases of grazing rates on algae for both mussel populations (Fig. 1A,B, Table 2). These decreases were recorded for temperatures above $22.9^{\circ}C$ in both the first and second experiment, whereas no effect of temperature on the loss rates was recorded at temperatures between $18.9^{\circ}C$ and $22.9^{\circ}C$ in experiment one. Experiment one (performed under moderate temperatures in May) revealed no differences in the LRs on algae between the two mussel populations, whereas experiment two (performed at high temperatures in July) revealed significant population effects (Fig. 1A,B, Table 2). At this time mussels from the southern invasion line (Danube) showed higher ingestion rates than mussels from the northern invasion line (Rhine). The bacteria were generally stimulated by the grazing activity of the mussels, as indicated by the negative LRs at moderate temperatures (Fig. 1C,D). These stimulating effects decreased significantly with increasing temperature parallel to the decreasing grazing pressure on the algae (Fig. 1C,D, Table 2). However, effects of mussel origin were found neither for the first nor for the second experiment.

Both the positive effect of increasing temperature on algae and the negative effects on the bacteria were confirmed in the acclimation experiment at test temperatures of between $25^{\circ}C$ and $30^{\circ}C$ (Fig. 2, Table 3). The acclimation temperatures ($20^{\circ}C$ and $28^{\circ}C$) had no significant effect on ingestion rates at the high temperatures (Table 3).

Grazer-mediated opposing effects of warming on bacteria and on HF—After demonstrating the opposite development

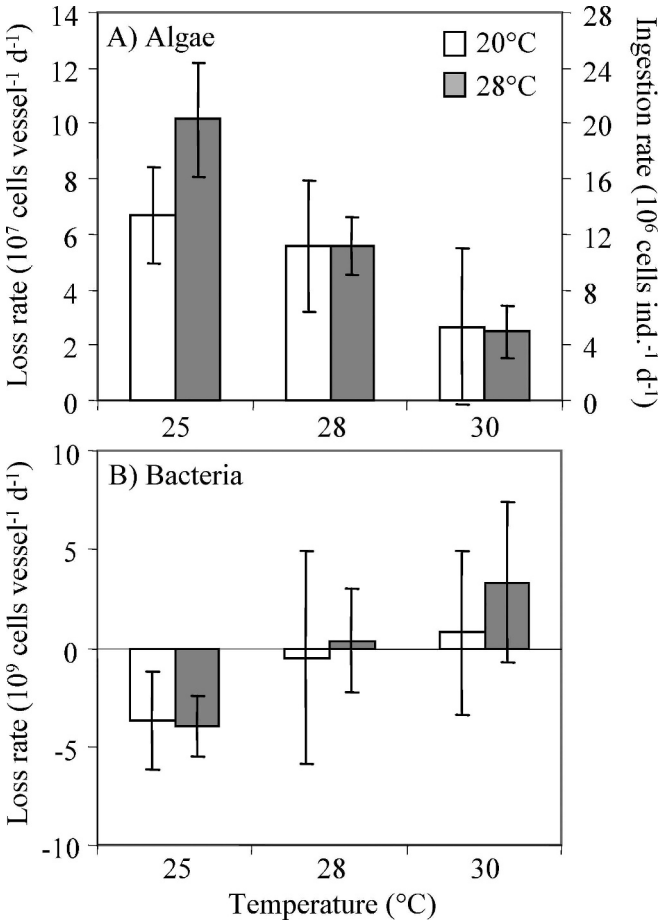

Fig. 2. Test of acclimation effects on the loss rates of (A) algae and (B) bacteria under high temperatures. The second *y*-axis gives the corresponding ingestion rates for algae. No effect of the acclimation temperatures (20°C and 28°C) could be shown, whereas the test temperature effects were significant for both groups (Table 3).

of the grazing pressure on phytoplankton and bacteria with warming, the fourth experiment was focussed on bacteria and HF and on the net effects (integrating grazing and growth effects) on these groups. The gross growth rate of the HF in the natural plankton community did not show a significant temperature response in the absence of mussels (Fig. 3A, Table 4). The grazing rate, however, decreased between 20°C and 30°C by about 75%. Together, these significant effects of warming resulted in a strong positive development of the HF net growth rate with increasing temperature ($R = 0.97$; $p < 0.001$) (Fig. 3A, Table 4). For the bacteria, grazing by the mussels at 20°C and 25°C resulted in enhanced net growth rates under mussel grazing compared with the gross growth rates measured in the controls (Fig. 3B). With rising temperature and the resulting decreasing grazing pressure on the HF, the stimulating effect on the bacteria decreased; the net and gross growth rates of the bacteria were equal at 30°C (Fig. 3B, Table 4). Overall, the net growth rate of the bacteria decreased with increasing temperature ($R = -0.73$; $p = 0.003$), a result that is in contrast to the net growth rate of the HF, whereas the gross growth rate of both bacteria and HF remained unaffected by temperature.

Discussion

Reduction of the grazing pressure on algae and HF with temperature increase—The nanoplankton (to which the majority of algae and HF in running waters belong, Weitere et al. 2005; Chetelat et al. 2006; Table 1) fall into the preferred prey size spectrum of *D. polymorpha* (Sprung and Rose 1988) and is thus efficiently grazed upon by the mussels. The grazing pressure on the nanoplankton by alternative planktonic predators (e.g., ciliates, rotifers, crustaceans) is particularly low in the Rhine, also in comparison with other rivers (Weitere et al. 2005). It was demonstrated in this study that an increase in temperature had a significant negative effect on ingestion rates of *D. polymorpha* on algae and HF at temperatures above 20°C. For *C. fluminea*, a negative effect of temperature increase on the grazing rate occurred above 25°C (Viergutz et al. 2007). Several authors describe the dependence of the filtration rate of *D. polymorpha* on temperature as a normal curve with temperature optima between 10°C and 20°C (Walz 1978; Lei et al. 1996). Thus the decrease in the filtration rate with high summer temperatures is a general pattern in *D. polymorpha*. Temperatures beyond the temperature optimum for the filtration rate of *D. polymorpha* are frequently reached in central European rivers. Maximal temperatures of 29°C have been measured in the lower Rhine in recent years (see temperature data presented by the "Landesumweltamt NRW", http://luadb.lds.nrw.de/LUA/), and even higher temperatures are likely to occur in the future (Schär et al. 2004).

The general trend of decreasing ingestion rates with increasing temperature was confirmed for both the northern and the southern invasion lines. Interestingly, the absolute rates between the two populations differed only in midsummer and not in May. Mussels from the southern invasion line (Danube) displayed higher ingestion

Table 3. Results of two-factorial ANOVAs testing the effects of test temperature (25°C, 28°C, and 30°C) and acclimation temperature (20°C and 28°C) on the loss rates of algae (10^8 cells per vessel d^{-1}) and bacteria (10^{10} cells per vessel d^{-1}).

	SS	df	*F*	*p*
Algae				
Test temperature	1.260	2	15.512	<0.001
Acclimation temperature	0.129	1	3.170	0.095
Test temp. × acclimation temp.	0.117	2	1.441	0.268
Residual	0.609	15		
Bacteria				
Test temperature	1.276	2	5.211	0.019
Acclimation temperature	0.028	1	0.226	0.641
Test temp. × acclimation temp.	0.098	2	0.401	0.677
Residual	1.837	15		

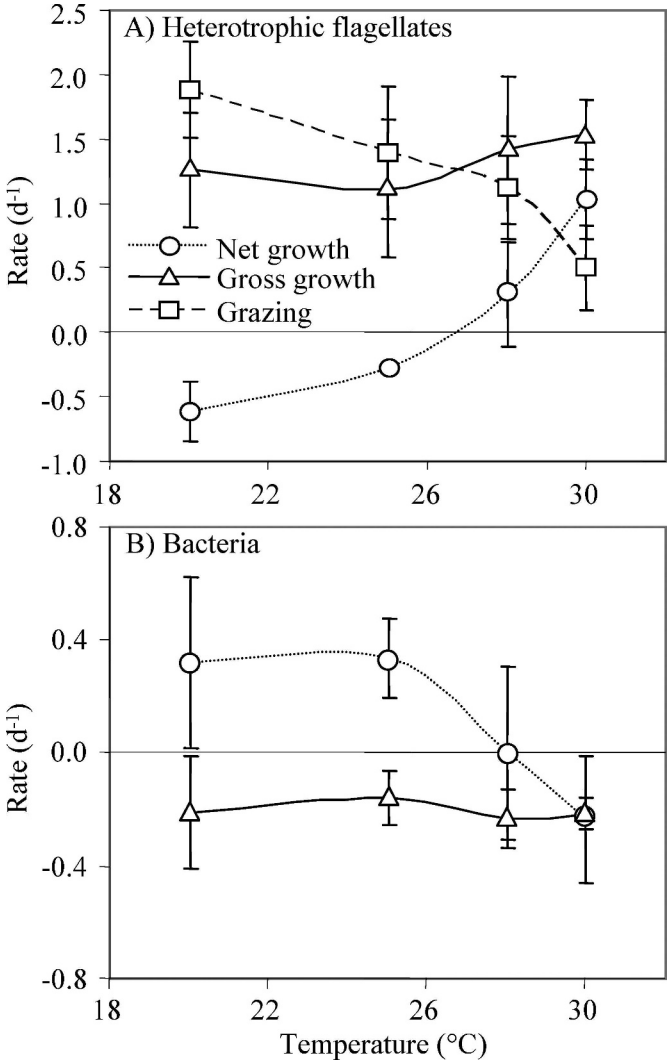

Fig. 3. Results of the grazing experiment performed in September on (A) heterotrophic flagellates and (B) bacteria: Temperature response of mean (± SD) gross growth rate (without mussel effect), net growth rate (under mussel effect), and (for algae) grazing rate. The gross growth rate is dependent on temperature neither for the flagellates nor for the bacteria, whereas the net growth rate is significantly positively related to temperature for the flagellates and significantly and negatively for the bacteria (Table 4).

rates in July than those from the northern invasion line (Rhine), even though the latter population was characterized by larger body mass and longer filtration periods (measured as open shells, see Table 1). Other differences between populations from different latitudes in the performance at high temperatures have been found in growth and survivorship of North American *D. polymorpha* populations (Thorp et al. 1998). The authors found that southern zebra mussel populations were more tolerant toward warm temperatures than northern populations were. Such intraspecific differences might have significant functional consequences when populations mix during the course of changing environmental conditions. Invasions by populations with higher thermal optima might lessen the negative effect of high summer temperatures on the ingestion rates of *D. polymorpha*. However, our data suggest that the general trend of decreasing ingestion rates due to increasing temperature would not be affected by the invasion of dreissenids from the Danube population into the Rhine.

Table 4. Results of Spearman–Rank Correlations for testing the dependencies of gross growth and net growth rates of both HF and bacteria, and the grazing rates of HF on temperature for experiment four (September 2005).

	R	*p*
HF		
Grazing rate	−0.799	<0.001
Gross growth rate	0.347	0.134
Net growth rate	0.972	<0.001
Bacteria		
Gross growth rate	−0.022	0.473
Net growth rate	−0.734	0.003

Acclimation to high summer temperatures seems to have no effect on the ability of the mussels to filter algae (Fig. 2). This finding matches results found by Aldridge et al. (1995), who demonstrated that the algal filtration rates of *D. polymorpha* decreased significantly at high test temperatures for mussels that had been acclimated to the particular temperature for 1 month. Similar results have been found for other freshwater and marine mussels such as *C. fluminea* (Viergutz et al. 2007) and *Mytilus edulis* (Kittner and Riisgard 2005). However, contrasting results can be found in other studies. For example, Lei et al. (1996) found that the filtration rate of *D. polymorpha* was significantly affected by both acclimation temperature and test temperature. Mussels acclimated to 20°C showed higher filtration rates than those acclimated to 8°C at test temperatures of 8°C and 14°C, although the rates do converge at test temperatures of 20°C and higher.

Together, these findings support the conclusion that the grazing pressure of *D. polymorpha* on nanoplankton (algae and HF) decreases at high summer temperatures regardless of the acclimation temperature and population history. Short-term increases in temperature will release the nanoplankton from mussel grazing pressure, even over a larger temperature range. When the ingestion rates of *D. polymorpha* decrease at temperatures above 20°C, HF still display high growth rates up to 30°C (Fig. 3; Viergutz et al. 2007), leading to a net release of the HF under mussel grazing at high temperatures. This combined effect of grazing on and growth rates of algae was not further considered here, and it is unclear whether or not our conclusions gathered from laboratory experiments also apply to the turbid and turbulent conditions found in the field for this light-dependent group. However, algal species can show high growth rates at high temperatures similar to the heterotrophic protists (for review see Montagnes et al. 2003) and thus a positive net effect of summer temperature increase also on the algal abundance under mussel grazing is likely to occur.

Indirect negative effects of temperature increase on bacteria exposed to mussel grazing—A significant finding of the present study is the stimulation of bacteria in the presence of mussels and the contrasting effect of warming on picoplankton (bacteria) and on nanoplankton (algae, HF) under mussel grazing pressure. This finding indicates that processes other than direct grazing of the mussels act on the bacteria. The stimulation of bacteria in the presence of mussels is supported by field observations (Findlay et al. 1998) but stands in contrast to findings from laboratory experiments, which demonstrate the direct grazing of bacteria by *D. polymorpha* (Silverman et al. 1995). Reasons for these contrasting conclusions are probably the often larger sizes of bacteria grown under optimal conditions in the laboratory compared with bacteria from field communities. Although bacteria used by Silverman et al. (1995) measured 1 to 4 μm, the majority of the bacteria in our study was below 0.8 μm (Table 1). The grazing efficiency of *D. polymorpha* was greatly reduced for prey sizes of between 4 and 0.5 μm (Sprung and Rose 1988; Lei et al. 1996), and small natural bacteria were shown to be grazed upon with low efficiencies (Cotner et al. 1995; Frischer et al. 2000). The utilization of natural bacterial biomass by mussels rather takes place via the consumption of HF as trophic link between bacteria and mussels than by bacteria themselves (Loret et al. 2000). Experiments with large laboratory-cultured bacteria might therefore lead to misinterpretations about the grazing effects of mussels on naturally occurring planktonic bacteria.

The stimulation of the bacteria by the presence of mussels found here can be explained either by growth stimulation or by a decreasing grazing pressure. Although growth stimulation of bacteria in the presence of grazers can occur because of resource recycling (Cotner et al. 1995; James et al. 1997), it is unlikely that resource recycling alone explains the phenomenon observed in the present short-term experiments. *D. polymorpha* increases its metabolic activity and excretion of waste products with increasing temperature up to at least 32°C (Aldridge et al. 1995) and thus decouples the development of grazing rate and metabolic rate at high temperatures. The stimulation of the bacteria should be correlated with the excretion of waste products and should therefore increase with temperature, at least within the temperature range applied in the experiments. However, bacterial abundance decreased rather than increased with temperature. It is therefore more likely that the effects in the bacteria community are due to a reduction of the mortality rate of the bacteria with increasing grazing pressure by the mussels. HF are important planktonic consumers of bacteria (e.g., Berninger et al. 1991) and are the only significant planktonic consumers of bacteria in the Rhine because of the extremely low densities of planktonic ciliates and metazoans (Weitere et al. 2005). HF abundance increased when the grazing pressure of the mussels decreased at increasing temperatures. Hence, a trophic cascade (mussel–HF–bacteria) is the most likely explanation for the stimulation of the bacteria (cf. Polis et al. 2000; Shurin and Seabloom 2005). The significant finding here is that the strength of the indirect effects is altered by temperature; warming has an influence not only on the strength of direct predator–prey interactions (here: mussels–flagellates), but also on indirect effects on a third group (here: bacteria), together leading to significant shifts in the plankton structure.

The data show that microbial communities controlled by a macrofaunal component can experience substantial changes at high summer temperatures because of differential development of direct and indirect grazing effects with temperature. Remarkably, such a strong temperature effect is not apparent in many natural microbial communities without the presence of macrofaunal grazers (see also gross growth rates of HF and bacteria in Fig. 3), probably due to resource limitation as well as to the synchronous development of grazing and growth processes with changing temperature (compare Pomeroy and Wiebe 2001; Norf et al. 2007; Viergutz et al. 2007).

The varying reactions of communities toward environmental warming are relevant on different timescales. The effects revealed here, i.e., temperature-dependent grazing effects on microbial communities, are probably significant for short and intermediate timescales, when heat waves occur in increasing frequencies in the context of global warming. Both thermal adaptations and the invasion of grazers with a higher thermal optimum could dampen the effects when stable warm temperatures are reached. However, there are indications that environmental changes currently facilitate such grazer-mediated effects on plankton communities. Environmental changes and particularly the occurrence of heat waves can lead to strong decreases in the species richness of riverine mollusc communities (Mouthon and Daufresne 2006) and to a facilitation of invasive species such as *D. polymorpha*, which often outcompete native species (Ricciardi et al. 1998; Dukes and Mooney 1999; Stachowicz et al. 2002). Such decreases in the consumer diversity probably increase the strength of trophic cascades (cf. Finke and Demno 2004).

Since summer heat waves are predicted to occur in high frequencies in the near future (Schär et al. 2004), it is likely that grazer-controlled riverine plankton communities undergo significant structural changes. Rivers are characterized by short water residence durations, water movement, and by the associated pattern that temporal changes in the plankton community correlate with changes on the spatial scale. On the basis of our results, a possible future scenario is that summer heat waves cause an enhanced import of organic load into the lower stretches of the river because of a stimulation of the algal biomass and a reduction of the bacterial degradation activity. Such functional aspects need further attention in future studies.

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