

Seasonal feeding patterns, growth dynamics and the impact of warming on the grazing effects of invasive freshwater bivalves

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What if I say I'm not like the others?
What if I say I'm not just another one of your plays?
You're the pretender
What if I say that I will never surrender?

Foo Fighters

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General Introduction and aim of the study

Benthic filter feeders play an important role in the flux of matter of freshwater habitats like rivers and lakes. They can have a large impact on the ecosystems functioning by consuming huge amounts of phytoplankton, as well as bacteria and other particles. They thereby can control both the plankton community and composition. Bivalves often represent a dominant component of the benthic consumers in freshwater habitats. Examples for impacts of freshwater bivalves are strong reductions of phytoplankton (Kryger and Riisgard 1988; Pace et al. 1998), declines in zooplankton (e.g. rotifers and copepods) (Maclsaac 1995), increased water transparency and nutrient concentrations (Effler 1996; Strayer et al. 1999), and therewith increased growth of macrophytes. Additionally, shell deposits can restructure former soft bottoms (sand or silt) into shell gravel, and provide patches of hard substrate for sessile species (Olenin & Leppäkoski 1999). Species that have a disproportionately large effect on structuring the composition and functioning of ecosystems in relation to their biomass are often called keystone species (Paine 1969). Two examples of freshwater bivalves that can be considered to act as keystone species are the invasive species *Dreissena polymorpha* and *Corbicula fluminea*. The Ponto-Caspian species *Dreissena polymorpha* spread across Western Europe in the 19th century (Kinzelbach 1992), while North America was invaded in the 1980s. *Corbicula fluminea* is widely distributed in riverine systems and reservoirs in Africa, Europe, North and South America and in the Pacific islands (Phelps 1994; Rajagopal et al. 2000; Darrigran 2002; Karatyev et al. 2005). Both species have the ability to tolerate a wide range of conditions and are extremely adaptable. Additionally, both bivalves are easily dispersed and highly fecund. Today, both *Corbicula fluminea* as well as *Dreissena polymorpha* belong to the abundant grazers in many freshwater ecosystems, such as the River Rhine, the study site of the present work (Meister 1997; Rajagopal et al. 2000; Karatayev et al. 2003).

The River Rhine is Europe's most important inland waterway. Due to anthropogenic impacts, such as declining water quality and alterations in river morphometry and hydrology, the macroinvertebrate species richness of the River Rhine has been drastically reduced in the course of the last century (Kinzelbach 1983; Ortmann

2003). With improving water quality over the last 30 years the species richness could recover, but the community composition altered substantially with the presence of invasive species like *Corbicula fluminea* and *Dreissena polymorpha* (IKSR 2009). As these bivalves play a key role in aquatic ecosystems, there is large interest in understanding their impact on ecosystem processes. When determining the effects of an invasive bivalve species, two of the most important components are grazing activity and population dynamics, as well as the factors influencing these components. Temperature is considered to be one of the most important factors that not only influences the grazing activity of freshwater bivalves like *D. polymorpha* and *C. fluminea* (Walz 1987; Aldridge et al. 1995; Lei et al. 1996; Schöl et al. 2002; Park et al. 2008), but also the growth dynamics of their planktonic prey community, such as algae and heterotrophic flagellates (Montagnes et al. 2003). High temperatures, for example, can generate significant changes in a freshwater community structure when the effects on the predation rate of the bivalves and the growth rate of the prey develop asynchronously.

Due to the anthropogenic emission of greenhouse gases we are going to face a temperature increase in the next decades. In the most probable scenarios, the average global surface temperature is projected to increase between 1.7°C and 4.0°C during the 21st century (IPCC 2007). For central Europe, the strongest seasonal average temperature increase is predicted for winter times, while extreme summer heat waves are likely to occur in increasing frequencies (Schär et al. 2004; Stott et al 2004).

The first aim of the present study was to examine the effects of temperature increase on the grazing rate of *C. fluminea* and *D. polymorpha* in relation to the effects on their natural prey community. As a prey group natural communities of heterotrophic flagellates (HF) from the River Rhine were used. As main bacterial consumers, they play an important role in the flux of matter in aquatic ecosystems (reviewed in Arndt et al. 2000). The next step was to test the impact of temperature across different trophic levels (bivalves - heterotrophic flagellates - bacteria). The hypothesis was that reduced grazing by *D. polymorpha* at high temperatures, with positive effects on the flagellates abundance, leads to negative effects on the abundance of bacteria which

represent the main prey of the flagellates but are less efficiently grazed by *D. polymorpha*.

After examining the influence of temperature in short-term experiments, seasonal and inter-annual patterns of *C. flumineas* grazing activity were identified. A few studies show that seasonal changes in clearance rates of bivalves cannot be solely explained by temperature (Benjamin 1978; Hornbach et al. 1984), but seem to be connected with seasonal variability associated with life history events (e.g. spawning periods) and factors influencing these life history events (e.g. cold winter periods). These seasonal variations are often overlooked, because studies tend to focus on short-term experiments. However, such seasonal dependent responses to temperature are assumed to be highly relevant to predict temperature effects. In fact, they seem to have the power to surpass direct temperature effects.

Another important factor when trying to understand the ecological role of an organism is the knowledge about the organism's population dynamics. Growth characteristics of individuals are one of the most important components when examining population dynamics. Growth patterns can provide information about reproduction potential, energy metabolism or maximum age. However, individual-based studies focusing on intra-annual growth patterns of bivalves are very rare. Finally, it is necessary to develop local studies, because population dynamics always reflect the environmental conditions and, consequently, transferability is limited.

The thesis is divided into four main chapters:

In **Chapter 1** the hypothesis claiming that extreme summer temperatures can lead to a non-parallel development of grazing rate of the bivalves and growth rate of the unicellular prey community was tested. Therefore experiments in circulating flow chambers using *C. fluminea* and *D. polymorpha* as consumers, and natural communities of heterotrophic flagellates originating from the River Rhine as a prey group, were performed. In a first experimental setup, with addition of an organic carbon source to prevent bottom up limitation of the microbes, the temperature range at which grazing and growth rates developed non-parallel to temperature increase could be identified. The second experimental series was run to test the occurrence of this non-parallel development under natural conditions (untreated river water). Additionally, similar experiments were performed with a benthic microbial predator

community (biofilms dominated by ciliates) to compare the effects of the macrofauna to those of microbial communities. It was shown, that the grazing rate of *C. fluminea* on the flagellates decreases with high temperatures relative to the growth rate of its prey, leading to a rapid increase in the flagellates abundance. This unparallel development could not be found when using microbial communities as predators.

Chapter 2 addresses the question whether the mussel-mediated effects of summer temperature increase appear in the same manner for the different prey groups (algae, heterotrophic flagellates and bacteria), or whether indirect effects (particularly trophic cascading, cf. Polis et al. 2000) generate varying effects in the different prey groups. Therefore, four different experiments were conducted. The first two experiments were based on the hypothesis that a temperature increase has differing effects on pico- and nanoplankton subjected to mussel grazing. In the next experiment the role of mussel acclimation to warm temperature was examined. The final experiment focused on the different grazing effects on both heterotrophic prey groups (the bacteria and their main planktonic consumers, the HF). All experiments were performed in circulating flow chambers 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.

The results of the earlier experiments (chapters 1 and 2) revealed strong temperature effects on the grazing rate of the filter feeders with different effects on the three prey groups in short term experiments. For example it has been shown that even small temperature increases in summer can result in positive effects on the nanoplankton and negative effects on the picoplankton due to the grazing of filter-feeding bivalves. In **Chapter 3**, the natural intra- and inter-annual variability connected to environmental conditions was examined. A few studies show that seasonal changes in clearance rates of filter-feeding bivalves cannot solely be explained by temperature or food level (Benjamin 1978; Benjamin and Burky 1978; Hornbach et al. 1984) and suggest that they must be connected with seasonal variability associated with the life history of the bivalves. The study was based on the hypothesis that seasonal events like spawning periods, inter-annual variations in life history events and environmental factors, such as cold winter periods, have a significant impact on the filtration activity

of *C. fluminea*, and that these factors can surpass direct temperature effects on the clearance rates of *C. fluminea*. To test this hypothesis, long-term grazing experiments were performed to detect seasonal patterns and year-to-year variability over a period of two years. Unlike suggested from the short-term experiments in chapter 1 and 2, the impact of temperature on the feeding activity of bivalves could be surpassed by other factors than actual temperature.

After studying the grazing rates of invasive bivalves in short-term and long-term experiments, the focus was laid on population dynamics of *C. fluminea* in the River Rhine. **Chapter 4** examines the size-dependent, seasonal shell length increase and the growth pattern of *C. fluminea* originating from the River Rhine. This is important, because an understanding of the growth rates can provide a basis for predictions of the capacity for populations to expand and exploit new habitats (Johnson & Carlton 1996). In the case of *C. fluminea*, such information can also be useful when evaluating the influence of the organism on aquatic ecosystems.

In the present study the shell length increase of more than 50 individuals of *C. fluminea* originating from the River Rhine were marked individually and measured repeatedly over a period of more than one year. The shell length of the bivalves ranged from 5 mm up to 34 mm.

The data was then used to develop and validate a growth model using the "von Bertalanffy growth equation". With the help of this model it was possible to generate growth curves of *C. fluminea* spanning over a period of several years. Additionally, the model could provide information such as age at a given length of an individual, maximum age of and maximum shell length of *C. fluminea* in the River Rhine.

The aims of chapter 4 were: (1) to characterize an annual size-dependent shell length increase; (2) to examine size-specific intra-annual growth patterns in relation to water temperature and chlorophyll *a*-concentration; (3) to provide fundamental ecological information on *C. fluminea*.

Taken together the 4 chapters of the present study provide new patterns and mechanics, which are relevant to accurately predict the performance and the effects of invasive bivalves under changing environmental conditions. The data suggests that global warming might lead to a restructuring of predator-prey interactions on different trophic levels. The findings of this work might not only be

limited to *D. polymorpha* and *C. fluminea* and their prey community, but may also be relevant for other aquatic or terrestrial predator-prey interactions.

Chapter 1

Control of microbial communities by the macrofauna: A sensitive interaction in the context of extreme summer temperatures?

Abstract

Climate models predict an increasing frequency of extremely hot summer events in the northern hemisphere for the near future. We hypothesized that microbial grazing by the metazoan macrofauna is an interaction which becomes unbalanced at high temperatures due to the different development of the grazing rates of the metazoans and the growth rates of the microbial community with increasing temperature. In order to test this hypothesis we performed grazing experiments in which we measured the impact of increasing temperatures on the development of the grazing rates of riverine mussels in relation to the growth rates of a unicellular prey community (a natural heterotrophic flagellate community from a large river). In a first experimental series using *Corbicula fluminea* as a grazer and under the addition of a carbon source (yeast extract), the increase of the prey's growth rates was considerably stronger than that of the predator's grazing rates when temperatures were increased from 19°C to over 25°C. This was also the outcome when the mussels had been acclimatized to warm temperatures. Hereafter specific experiments with natural river water at temperatures of 25°C and 30°C were performed. Again a strong decrease of the mussels' grazing rates in relation to the flagellate growth rates with increasing temperature occurred for two mussel species (*Corbicula fluminea* and *Dreissena polymorpha*). When performing the same experiment using a benthic microbial predator community (biofilms dominated by ciliates) instead of the benthic mussels, an increase of the grazing rates relative to the growth rates with temperature could be observed. Our data suggest that predator-prey interactions (between metazoans and microbes) which are balanced at moderate temperatures could become unbalanced at high temperatures. This could have significant effects on the structure and function of microbial communities in light of the predicted increasing frequency of summer heat waves.

Introduction

There is no doubt that we are currently facing a warming of the atmosphere due to the emission of greenhouse gases caused by human activities (IPCC 2001). The climate change already affects organisms on different scales (Walther et al. 2002). For example, studies have demonstrated that global warming could result in changes in the timing of seasonal activities (Both and Visser 2001; Menzel and Fabian 1999; Durant et al. 2005), in the geographic range of species (Parmesan 1996), in interspecific interaction strength (Sanford 1999; Taylor and Collie 2003; Winder and Schindler 2004) and in the reorganization of whole ecosystems (Brown et al. 1997; McGowan et al. 1998).

The intensity of global warming occurs heterogeneously on both temporal and spatial scales. It has been shown recently that the European summer might experience a pronounced increase in year-to-year temperature variability in response to the accumulation of greenhouse gases, and that such an increase would strongly affect the incidence of heat waves (Schär et al. 2004; Stott et al. 2004). It is unclear whether or not communities of the metazoan macrofauna with relatively long generation times (in contrast to microbes) could react to such irregularly occurring heat waves by shifts in their structure towards well-adapted species. It is likely that irregularly occurring extreme temperature events result in immediate effects (i.e. shifts in the metabolic rate of organisms and as a consequence shifts in the interaction strength between individuals) in a given set of organisms rather than in shifts in the community towards well-adapted species, at least on short and medium time scales. One useful way to assess ecosystem consequences of such extreme climate events is therefore to focus on species which contribute disproportionately to maintaining the composition and functioning of ecosystems, so-called keystone species (cf. Sanford 1999). However, the effects of high temperature on the functional response of a keystone species (e.g. an increase in predation rate) might be buffered when the temperature impact on the prey response (e.g. an increase in production) develops in parallel. One way to reveal possible leverage points through which changes in the climate could generate significant changes in the community

structure is therefore to identify interactions among relevant organisms in which both traits develop asynchronously with temperature.

The grazing control of microorganisms (bacteria, fungi, algae and protozoans) by metazoans is an interaction which (i) can have a strong impact on the ecosystem function and (ii) might be sensitive towards extreme temperature. Microorganisms account significantly for the function of both terrestrial and aquatic ecosystems (e.g. Azam et al. 1983; Bonkowski et al. 2000, Gaedke et al. 2002). They are often controlled by metazoans, especially in aquatic systems, where direct grazing of microbes by filter feeders is common (e.g. Silverman et al. 1995; Caraco et al. 1997; Jürgens and Jeppesen 2000). Both the grazing rates of the macrofauna and the growth rates of the microorganisms are affected by temperature. However, while components of the metazoan macrofauna living in moderate climates often decrease their activity at high temperatures (Ansell and Sivadas 1973; Aldridge et al. 1995), communities of microorganisms are often very productive at such temperatures unless they experience resource limitation (Montagnes and Franklin 2001; Weisse et al. 2002; Charlier and Droogmans 2005). A community of microorganisms can change rapidly towards well-adapted species with changing conditions due to the low generation times, while changes in a macrofaunal community occur on a much larger time scale. This combination of a slowly changing set of macrofauna and a rapidly changing microbial community might result in a distinctly slower increase of grazing by the macrofauna than of the growth of its unicellular prey community with extreme summer temperatures. This hypothesis is illustrated in Figure 1. It is based on the assumption that the macrofaunal grazing rate reaches a plateau and then drops off gradually with high temperature after the initial exponential increase (Walz 1978; Reeders and bij de Vaate 1990; Lei et al. 1996) and that the growth rate of the microbial prey community increases linearly with temperature and drops at much higher temperatures than the grazing rate of the macrofauna does (reviewed in Montagnes et al. 2003).

In order to test the hypothesis that extreme summer temperatures can lead to an unparallel development of the growth and consumption rates of the unicellular community, we performed grazing experiments using freshwater mussels (*Corbicula*

fluminea and *Dreissena polymorpha*) as consumers. Both species have invaded Europe and North America and are now common benthic filter feeders in many freshwater systems over the northern hemisphere. As a prey group we used natural communities of heterotrophic flagellates (HF) from the River Rhine. As main bacterial consumers, they play an important role in the matter flux of aquatic ecosystem (reviewed in Arndt et al. 2000). The HF are of a size class which is efficiently filtered by both grazers (Sprung and Rose 1988; Way et al. 1990; Lei et al. 1996). In a first experimental setup we added an organic carbon source in order to accelerate bacterial growth, thereby minimizing flagellate resource limitation and allowing us to focus solely on the grazer-prey interaction. After identifying the temperature range at which the grazing and growth rates became unparallel, we tested the occurrence of this development under natural conditions (untreated river water) in a second experimental series. Here a temperature at which both rates demonstrate a parallel increase from 19°C (25°C) and a temperature at which an unparallel development is observable (30°C, which is only 1°C higher than the maximal summer temperature already occurring in the River Rhine) were considered. As a comparison to the benthic mussels, we additionally tested a benthic biofilm-associated consumer community (predominantly ciliates) in our experiments.

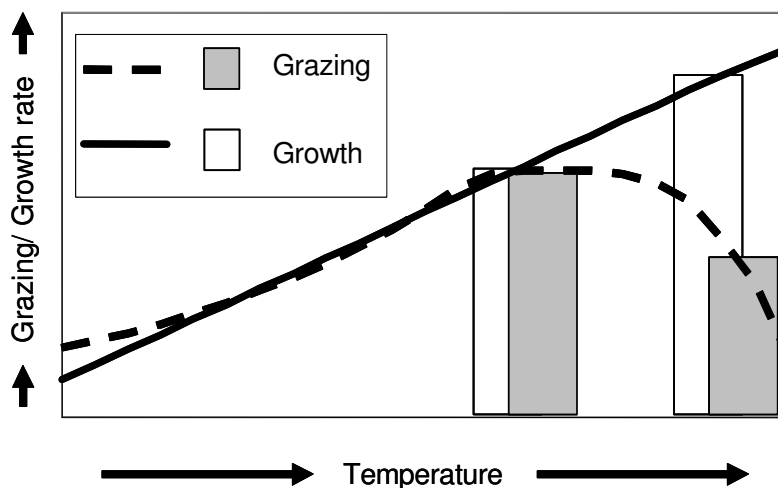


Fig. 1: Descriptive model illustrating the central hypothesis: The development of the gross growth rate of a microbial prey community and the grazing rate of a macrofaunal consumer grazer with temperature. The general mechanism was tested in a first experimental series (“identification of mismatch”) over a larger temperature range (as illustrated by the lines) by reducing resource limitation of the plankton. The occurrence of this mechanism in natural plankton communities without any manipulation was tested in a second experimental series (“test of applicability”). This series was conducted using two temperatures: the upper temperature at which the two rates were in balance (25°C) and a higher temperature within the range of mismatch (30°C).

Materials and Methods

Experimental set-up and organisms

The experiments were conducted in circulating flow channels as used in Weitere et al. (2003). The round vessels had a diameter of 30 cm and were covered with a rotating disk (30 rotations min^{-1}) spiked with combs which provided for a constant water flow over the ground. The vessels were filled with water from the River Rhine (sampled at the Ecological Rhine Station of the University of Cologne in Cologne, Germany: Rhine-km 685) which contained the natural plankton community. No enrichment was conducted before the experiments were started. The zooplankton community in the Rhine is generally dominated by heterotrophic nanoflagellates, while the abundance of planktonic grazers on the nanoplakton (ciliates and metazoans) is generally low (Weitere et al. 2005). Two experimental series were conducted; the setup and conditions are summarised in Table 1. In a first series in July and August 2004 (Table 1A), we performed experiments in which we tested whether or not the HF growth rate and grazing rate of *Corbicula fluminea* as a model grazer develop in parallel at a moderate temperature (reference: 19°C) and at different lower and higher temperatures (12, 25, 28, 30 and 32°C). The vessels were filled with 8 L Rhine water. 20 to 23 small (0.75-1.15 cm) mussels were added to the grazer treatment. Grazer free controls were run in order to measure the HF growth rates. Since we were particularly interested in the grazer-prey interaction, we added 0.5 g of sterile yeast extract to each of the vessels in order to stimulate microbial growth and to minimize resource limitation of the HF. In a second series in August and September 2005 (Table 1B, C, D), we specifically tested whether or not the identified range of unparallel development of grazing and growth rates with temperature also occurred under natural conditions, i.e. in Rhine water without any carbon source supplement. For this purpose we tested at a temperature at which both rates develop in parallel to each other with temperature from 19°C in the first experimental series (25°C), and at a temperature in which the two rates do not develop parallel with temperature (30°C). Three single experiments with different benthic grazers were performed here: One with *Corbicula fluminea*, one with the mussel *Dreissena polymorpha* and one as a reference with a microbial community

(biofilm community) instead of a single macrofaunal species as benthic grazers. 10 L of untreated Rhine water was added to the vessels for these three experiments and grazer free controls were again considered.

In each experiment (for both the first and second series) we considered two temperatures cross-classified with the presence and absence of benthic grazers (Table 1). Each treatment was run in three to four independent replicates; the grazing treatments within one experiment contained exactly the same number and sizes of mussels or were (in the case of the biofilm experiments, see below) pre-treated in exactly the same manner in order to establish a homogenous benthic grazer community. The experiments were performed for 22 to 24 hours in temperature controlled vessels either at a 14:10 light-dark cycle at a controlled light intensity of $10 \mu\text{E m}^{-2} \text{sec}^{-1}$ (first series) or in a windowed room allowing about 1.5% of the natural light intensity to penetrate (second series) The abundance and taxonomic structure of the heterotrophic flagellate community was analysed at the beginning and at the end of the experiments (see below). *Corbicula fluminea* was sampled from the Lower Rhine in the Netherlands in Bimmen (Rhine-km 865).

Table 1: Experimental set-up and conditions during the experiments. The part of the experiments with biofilms resulted from a collaboration with Marcel Kathol and Helge Norf.

Date	Field temp. (°C)	Temperature (°C)	Acclimatization of grazers (°C)	Grazer ash-free dry weight per vessel (g)	Start abund. of HF (ind. ml ⁻¹)	Start abund. of PNAN (ind. ml ⁻¹)
A. <i>Corbicula</i>, addition of carbon source to Rhine water						
07/07/04	21	12 and 19	20	0.63	587±185	1,333±359
14/07/04	19	25 and 19	20	0.62	1,050±202	1,833±275
26/07/04	23	28 and 19	20	1.03	267±153	1,134±702
21/07/04	23	30 and 19	20	0.77	483±104	1,883±275
16/07/04	20	32 and 19	20	0.82	387±140	480±40
02/08/04	24	30 and 19	30	0.75	253±49	740±408
B. <i>Corbicula</i>, untreated Rhine water						
25/08/05	21	25 and 30	25	-	250±74	230±26
C. <i>Dreissena</i>, untreated Rhine water						
19/09/05	18	25 and 30	25	-	300±88	325±119
D. Biofilms, untreated Rhine water						
18/08/05	21	25 and 30	Field temp. (20-22 °C)	-	133±33	1,613±61

HF heterotrophic flagellates; PNAN photoautotrophic nanoplankton

Dreissena polymorpha was sampled in the Lower Rhine in Germany near Rees (Rhine-km 844). Both species were kept at the Ecological Rhine Station of the University of Cologne under constant flow of natural Rhine water. Two weeks before the experiments started, they were transferred to vessels filled with untreated Rhine water. The temperature was adjusted to constantly 20°C (30°C in one experiment for *Corbicula fluminea*) in the first series and 25°C in the second series (Table 1). The water was continuously oxygenated and was changed every two days. The replacement of the water was stopped for five days before experiments started. The biofilm experiment was performed in accordance with the experiments presented in Weitere et al. (2003): The vessels were exposed to a constant flow of Rhine water for two weeks before the experiments started. A natural biofilm community containing consumers of plankton (ciliates and rotifers) established itself on the inner walls of the vessels within the two weeks. The temperature during the two weeks of exposure corresponded to the in situ temperature of the Rhine, which ranged between 20.3 and 22.5°C during this time (August 4th to August 18th 2005). The grazer biomass (ash-free dry weight, AFDW) was calculated for each experiment within the first series separately. We separated the soft body from the shell, dried the soft body for 60 h at 60°C in a drying chamber and then measured the dry weight of the mussels. Thereafter the ash weight (AW) was determined after combustion for 15 h at 550°C. The AFDW was calculated as the difference between DW and AW.

Quantification of plankton and biofilm-dwelling grazers

The experiments were run with untreated Rhine water containing the natural potamoplankton, which is made up not only of our target prey (heterotrophic flagellates), but also of other plankton groups. Since these organisms might influence the outcome of the experiments, we analysed the actual plankton community during the performance of the present experiments. This included counts of the heterotrophic nanoflagellates and the photoautotrophic nanoplankton (which account for the largest part of the phytoplankton abundance in the Rhine) at the start of each experiment (Table 1) and spot analyses of the potential planktonic consumers of

heterotrophic flagellates among the potamoplankton (ciliates and metazoans) on July 6th, 2004 and on August 18th, 2005. The different groups were analysed with the help of routine techniques as described in detail in Weitere and Arndt (2003) for the nanoplankton, Weitere et al. (2003) for the ciliates and Weitere et al. (2005) for the metazoans. This includes live counts for the nanoplankton, DAPI-counts of gutaralehyde-fixed samples for the bacteria, counts of lugol-fixed ciliates in Utermöhl chambers and the count of concentrate (44 µm gauze) and formaldehyde-fixed metazooplankton. Three independent replicates were considered for all plankton counts.

For the biofilm experiment, the abundance and biovolume of the ciliates (which make up the largest proportion of plankton consumers on biofilms) and rotifers were calculated as described by Weitere et al. (2003). The abundance was measured on slides which were exposed to three spots in the channel.

Calculation of grazing and growth rates and statistical analysis

The growth rates of the flagellates (r) were calculated with the help of the flagellate abundances at the beginning (N_0 , ind ml⁻¹) and at the end (N_t , ind ml⁻¹) of the experiments after the time t (d) by assuming exponential growth:

$$r [d^{-1}] = (\ln(N_t) - \ln(N_0)) / t$$

The grazing rate of the benthos (g) was calculated as the difference of the HF gross growth rate measured in the controls (r_{co}) and the HF net growth rates measured in the grazer treatments (r_{gr}):

$$g [d^{-1}] = r_{co} - r_{gr}$$

In order to test the null hypothesis of a parallel development of the growth rate and the grazing rate, we compared the net growth rates (r_{gr}) at the two different temperatures under the presence of the grazers for each experiment separately with the help of a Student's t-test. Significant higher growth rates (r_{gr}) with temperature would indicate a stronger increase in the growth rates compared to the grazing rates.

Results

Identification of the temperature range of unparallel development of grazing and growth rates

The absolute flagellate growth rates (with and without mussel grazing) as measured in the first experimental series are summarized in Table 2. Figure 2 shows the comparison of the flagellate gross growth rates and grazing rates in relation to the growth and grazing rates at 19°C. The increase of the two rates was nearly parallel from 19 to 25°C, but this development was disrupted when temperature increased beyond 25°C. At 28 and 30°C the gross growth rates increased to 2.9 and 2.7 times (respectively) those at 19°C, while the grazing rates only increased to 1.6 and 1.9 times. The flagellate growth rates under mussel grazing (r_{gr}) differed significantly from those at 19°C for both 28 and 30°C, while no significant differences for 32°C were found (Table 2). The asynchronous increase in the gross growth and grazing rates from 19 to 30°C also holds true after the warm acclimatization of the mussels (Fig. 2, Table 2). This data demonstrates a clearly unparallel development of the growth rates and the grazing rates at 28 and 30°C which did not occur at lower and higher temperatures and which is independent from the acclimatization temperature.

Table 2: Summary of the HF increase rates (means \pm SD) for the *Corbicula* experiments with the addition of a carbon source

Temp. (°C)	HF increase rate (d ⁻¹) without mussels	HF increase rate (d ⁻¹) without mussels at 19°C	HF increase rate (d ⁻¹) under mussel grazing	HF increase rate (d ⁻¹) under mussel grazing at 19°C	<i>t</i> -Test results (<i>P</i> -value) for comparison of increase rates under mussel grazing
Mussels acclimatized to 20°C					
12	0.71 \pm 0.16	1.47 \pm 0.23	-0.92 \pm 0.23	-0.99 \pm 0.32	0.782
25	2.64 \pm 0.55	1.31 \pm 0.34	-1.44 \pm 0.57	-0.68 \pm 0.30	0.110
28	4.76 \pm 0.09	1.65 \pm 0.55	-1.69 \pm 0.22	-2.41 \pm 0.23	0.018 *
30	4.24 \pm 0.26	1.55 \pm 0.34	0.25 \pm 0.30	-0.50 \pm 0.08	0.014 *
32	2.69 \pm 0.23	1.47 \pm 0.81	-1.44 \pm 0.45	-1.97 \pm 0.77	0.365
Mussels acclimatized to 30°C					
30	4.57 \pm 0.07	1.91 \pm 0.52	-0.55 \pm 0.29	-1.54 \pm 0.19	0.008 *

The rates refer to three independent replicates of one experiment. Significant differences between the increase rates under mussel grazing (r_{gr}) at a given temperature compared to those at the reference temperature (19°C) are highlighted (*)

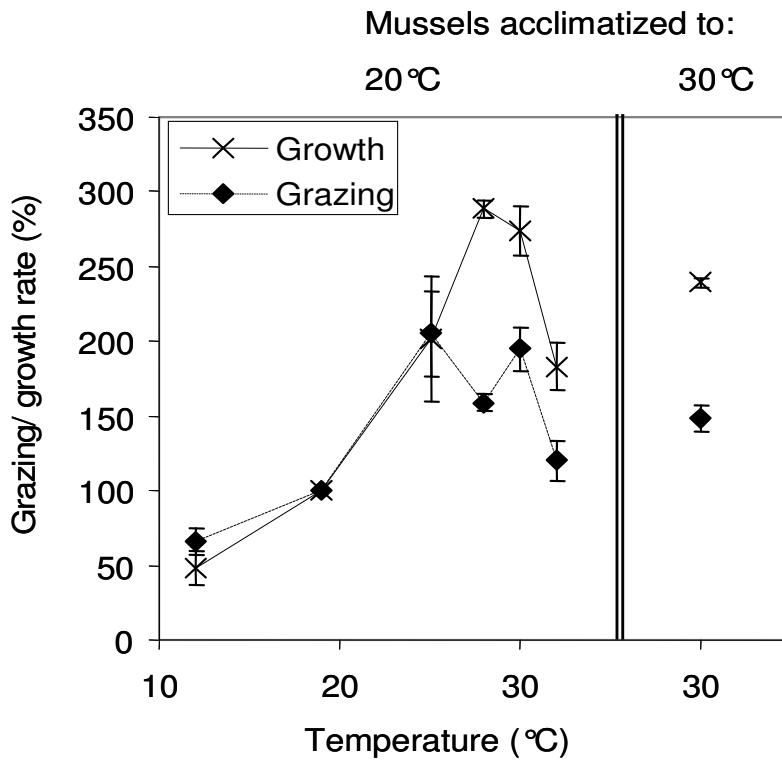


Figure 2: Summary of the results from the first series: Relative changes in the growth rates of the heterotrophic flagellates and the grazing rates by the mussels with temperature in relation to the rates at reference temperature (19°C). Error bars represent SD.

Test of applicability under semi- natural conditions

In contrast to the results of the first experiments with the addition of a carbon source, the HF growth rates in the controls (r_{co}) did not differ between 25 and 30°C for any of the three experiments performed with untreated Rhine water (Fig. 3). However, the HF growth rates under the influence of the grazers (r_{gr}) differed significantly between 25 and 30°C for all of the three grazers, i.e. for the mussels *Corbicula fluminea* and *Dreissena polymorpha* as well as for the biofilm community (Table 3). Interestingly, the differences between macrograzers and biofilms were in direct contrast: For the mussels we found a strong increase in r_{gr} with temperature indicating a decrease in the grazing rates relative to the growth rate. The grazing rates decreased between 25 and 30°C by 37 and 65% in *Corbicula fluminea* and *Dreissena polymorpha*, respectively (Fig. 3). For the biofilms we found in contrast a decrease in r_{gr} with rising temperature, indicating an increase in the grazing rates relative to the gross growth rates. The grazing rates increased between 25 and 30°C by 10% (Fig. 3).

In summary the results confirm the pronounced unparallel development of the growth and grazing rates between 25 and 30 °C for the macrofaunal grazers under semi-natural conditions, even though the growth rates hardly changed with temperature in the natural Rhine water. In contrast to the results found for macrofaunal grazers, an increase of the grazing rates relative to the growth rates was observed for the microbial grazers.

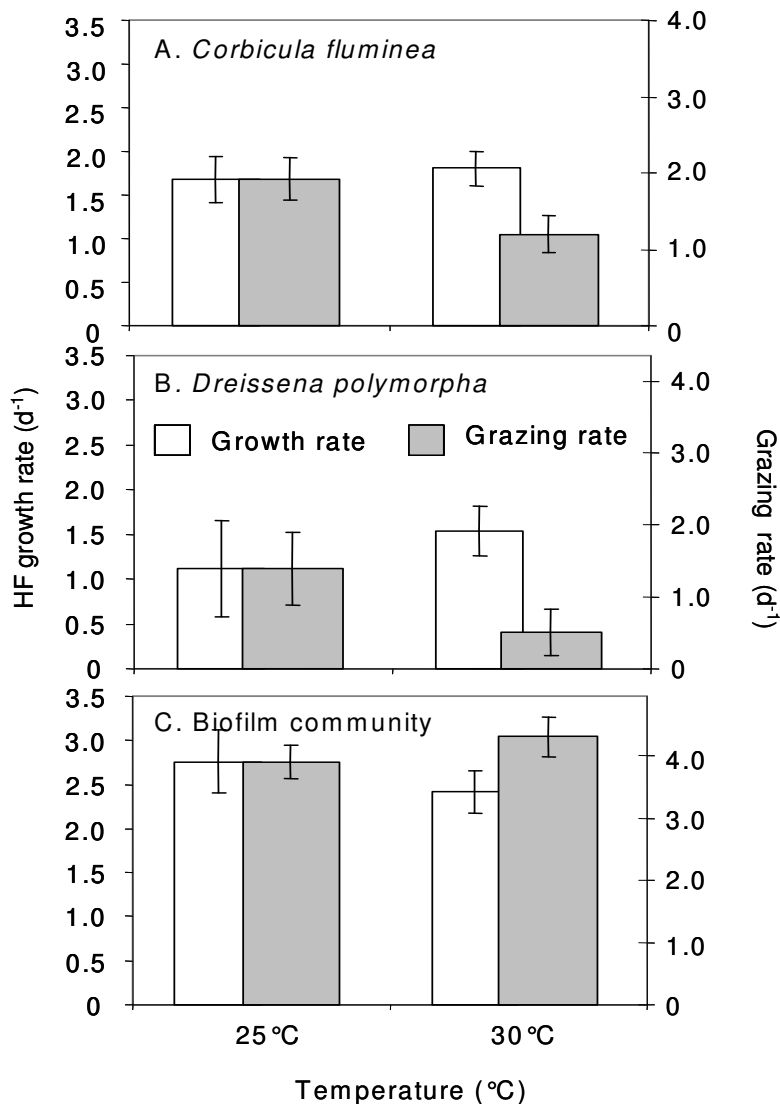


Figure 3: HF growth rates (white bars, means \pm SD) and grazing rates (grey bars, means \pm SD) for the mussels *Corbicula fluminea* (A) and *Dreissena polymorpha* (B) as well as for biofilm communities (C) exposed to 25 and 30 °C under ambient Rhine conditions (no addition of carbon source). The part of the experiment with biofilm communities resulted from a collaboration with Marcel Kathol and Helge Norf.

Background data

The quantification of the abundances of alternative prey (especially photoautotrophic nanoplankton, PNAN, Tab. 1) and potential additional consumers of the HF revealed extremely low densities of both groups in both years: PNAN abundance ranged from 480 to 1,833 ind. ml⁻¹ in 2004 and from 230 to 1,613 ind. ml⁻¹ in 2005, which was far below the incipient limiting level reported for freshwater mussels. The spot tests revealed abundances for planktonic ciliated grazers of 566±208 and 400±115 ind. l⁻¹ in 2004 and 2005, respectively. Abundances of potential planktonic grazers among the metazoans (rotifers and crustaceans together) were with 2.6±0.6 ind. l⁻¹ in 2004 and 4.5±0.6 in 2005 also extremely low. In the biofilm-experiment, the total surface-associated grazer biovolume was (with 267±194 mm³ vessel⁻¹ for ciliates and 4.2 ± 3.2 mm³ vessel⁻¹ for rotifers) clearly dominated by protozoans.

Discussion

Development of grazing and growth rates with high temperatures

The results of the experiments generally confirmed our central hypothesis that extreme summer temperatures can result in a pronounced unparallel development of the growth rate of a microbial prey community and the grazing rate of a macrofaunal consumer. However, the results did not match the initial expectation completely. The HF growth rate increased more exponentially than linearly with increasing temperature

Table 3: Comparison of the HF increase rates (r_{gr} , mean \pm SD) in the presence of different benthic grazers at 25 and 30°C. The part of the experiment with biofilm communities resulted from a collaboration with Marcel Kathol and Helge Norf.

	r_{gr} (25°C)	r_{gr} (30°C)	P
1. <i>Corbicula fluminea</i>	-0.23 \pm 0.28	0.60 \pm 0.24	0.004
2. <i>Dreissena polymorpha</i>	-0.28 \pm 0.04	1.04 \pm 0.31	0.002
3. Biofilm community	-1.14 \pm 0.34	-1.89 \pm 0.24	0.012

and then dropped beyond 30°C when food limitation was excluded. Without the addition of the carbon resource in the second setup, no changes in the HF growth rate between 25 and 30°C were recorded. This indicates that resource availability rather than temperature controls the HF growth under natural conditions here. The resource availability is of crucial importance in influencing the numerical response intensity towards warming in unicellular organisms demonstrated for both populations (Weisse et al. 2002) and communities (Pomeroy and Wiebe 2001; Savage et al. 2004; Staehr and Sand-Jensen 2006; Norf et al. 2007). Nevertheless, even though high temperature did not affect the HF growth in the second setup, it had a strong negative effect on the mussel grazing which still led to an unparallel development of the HF growth rate and mussel grazing rate between 25 and 30°C. The reference experiment with the microbial biofilm community demonstrated a disruption in the opposite direction from that observed in the mussel experiments (significant increase of benthic grazing in relation to growth with temperature). This pattern is basically the

result of a slight increase in the grazing rate parallel to a slight decrease in the growth rate with increasing temperature (Fig. 3C).

The warm acclimatization of *Corbicula fluminea* to 30°C did not change the outcome of the experiments at 30°C (Table 2) compared to the acclimatization to 20°C. Both experiments showed a much stronger increase of the growth rate than the increase of the grazing rate when temperature increased from 19 to 30°C. It is problematic to compare the two grazing rates at 30°C directly, because they were measured in two different experiments which were not designed to allow the direct comparison of the acclimation effect on the grazing rates. Literature data for *Dreissena polymorpha* confirms that strong drops in the grazing rates beyond 24°C occur despite warm acclimatisation. Although Aldridge et al. (1995) acclimatized mussels to 20, 24, 28 and 32°C for longer than a month, they still observed grazing rates which dropped by approximately 50% between 24 and 28°C and even by 73% within the total temperature range (20 to 32°C). Lei et al. (1996) demonstrated that warm acclimation in *Dreissena polymorpha* generally enhances the grazing rate, but this effect declines with increasing temperature. In these experiments, the mussels stopped grazing at 32°C completely, regardless of the acclimatization temperature. Together these studies suggest that acclimation of the macrofaunal grazer only weakly affects grazing rates at extreme summer temperatures (>25°C).

The largest effects occurred at temperatures which are already present in central European rivers or which are slightly beyond (28 and 30°C; compare with data presented by the “Landesumweltamt NRW”, <http://luadb.lids.nrw.de/LUA/gues/welcome.htm>). The Lower Rhine reached 28°C several times in recent years, with a maximal temperature of over 29°C (as daily mean). If the observed effects on the mussel-flagellate interactions occurred to a similar degree in the field, they would cause an enormous effect on the abundance of the microbes. Imagine a flagellate community which is controlled by *Dreissena polymorpha* and which shows no net increase during the river passage at 25°C. If the temperature increased to 30°C, this flagellate community would double every 13 hours (increase rate: 1.3 d⁻¹, which refers to the gap between net growth rate at 30°C to that of 25°C, Table 3).

In the first experimental series, both the HF growth rates and the grazing rates for *Corbicula fluminea* converge again at 32°C due to a sharp drop in the HF growth rate

(Fig. 2, Table 2). It is known that many heterotrophic protists are negatively affected in their growth (Laakso et al. 2003), behaviour (Barbanera et al. 2002) and in their rate of survival (Stauffer and Arndt 2005) above 30°C. The pronounced unparallel development of the growth rates and grazing rates of *Corbicula fluminea* is therefore restricted to a certain temperature range with an upper limit described by the temperature constraints of the prey. Nevertheless, the natural plankton communities used in the experiments were acclimated to field temperatures of between 18 and 24°C (Table 1), which was of the same range as the general acclimatisation temperature of the mussels in the first series (20°C), but lower than the warm acclimatisation temperature for the first series (30°C) and the second series (25°C). It is possible that a warmer acclimatisation of the unicellular plankton could result in a fast development of thermophilic species (e.g. Baumgartner et al. 2003) or even in a rapid evolution (Cooper et al. 2001) towards more thermal tolerant genotypes. Together such processes could result in a plankton community which displays higher growth rates at warm temperatures (as demonstrated here) and thus in an even stronger furcation of grazing and growth with higher temperatures.

The two mussels we used in our study are invasive species originating from the Ponto-Caspian Basin (*Dreissena polymorpha*) and the Asia-Pacific region (*Corbicula fluminea*) and now dominate the filter-feeding macrograzer community in many rivers in the northern hemisphere (e.g. Cohen et al. 1984; Phelps 1994; Strayer et al. 1996; Ricciardi and Maclsaac 2000). Both grazers experienced higher temperatures in their original habitats compared to the conditions in Central Europe and their success is at least to some extent the result of environmental changes (Dukes and Mooney 1999; Stachowicz et al. 2002). The indigenous species among the benthic filter feeders might have a distinctly lower temperature optimum than the two invasive mussels investigated here. We would therefore expect even stronger decreases in the grazing rates with extreme temperatures for these species than demonstrated here. On the other hand, it is likely that warm-adapted species or genotypes of benthic macrograzers could invade natural water bodies of moderate climates over the course of global warming if high temperatures become stable. Unparallel development of the grazing and growth rates with increasing temperature as

demonstrated here might therefore be a phenomenon of either intermediate time scales during the process of warming or of unpredictable conditions such as irregularly occurring heatwaves (Schär et al. 2004; Stott et al. 2004) to which adaptation is difficult.

Background data

The outcome of our experiments could potentially be influenced by a high abundance of alternative prey (especially phytoplankton), which might lead to a reduction of the filtration rates of the mussel, or a high abundance of planktonic predators (planktonic ciliates and metazoans in particular) on the heterotrophic flagellates. In order to minimize such influences, we chose, in accordance with our long term experience in the Rhine (Weitere and Arndt 2002; Scherwass and Arndt 2005; Weitere et al. 2005), a time frame in which the abundances of both the phytoplankton and the planktonic predators were low. Our checks confirmed this annual trend during our experiments in both years. The incipient limiting level for *Dreissena polymorpha* on the green algal *Chlamydomonas* sp. ranged between 16,000 to 81,000 cells ml⁻¹ (Dorgelo and Smeenk 1988; Sprung and Rose 1988). Since the PNAN abundances measured in the Rhine during our experiments were far below these abundances, it is most likely that the mussels showed maximal filtration rates during our experiments which had not been downregulated by high prey abundances. The abundances of additional planktonic consumers on HF (ciliates, rotifers, crustaceans) were also extremely low during our experiments. Taking the clearance rates of the three groups on HF into account (Jürgens et al. 1996), both ciliates and metazoans together caused clearance rates of approximately 1.5 ml h⁻¹ in both years, which is negligible in relation to the grazing activity of the mussels in the experiments. Together, this data suggests that there was no significant impact of alternative prey or additional grazers on the outcome of the grazing experiments.

Conclusion

The results show that one has to consider trophic macrofauna/microbe interactions as being a leverage point through which small changes in the temperature could generate large changes in the composition of important groups of organisms. It is

likely that such effects also occur in other macrofaunal/microbial interactions in both terrestrial and aquatic ecosystems. Nevertheless, the extent of the unparallel development between grazing and growth rates with high temperatures greatly depends on resource availability for the microbes, suggesting that such furcations are stronger in resource-rich than in resource-poor environments. Further research on the temperature impact on such key processes of other relevant macrofaunal species as well as on the temperature response of microorganisms at varying resource quantities is needed in order to more accurately predict consequences for the ecosystem.

Chapter 2

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

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.

Introduction

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 macro-fauna 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 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.

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. The part of the experiment 1 and 2 resulted from a collaboration with Johanna Dahlmann.

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 temperature (°C)	18.9; 29.9; 22.8; 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 per flow channel (g)	0.39 (Rhine) 0.26 (Danube)	0.21 (Rhine) 0.25 (Danube)	0.127/0.123 (20 °C/28 °C accl.)	0.076
Mean prop. 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 class (µm) (first, second and third quartile)	3, 7, 30	5, 11, 30	7, 14, 20	Not measured
Starting bact. abund. (10 ⁵ cells mL ⁻¹)	9.23±1.3 SD	6.49±0.44 SD	8.36±1.18 SD	325±119 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 abund. of heterotrophic flagellates (cells mL ⁻¹)	622±102 SD	156±8 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

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 a 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

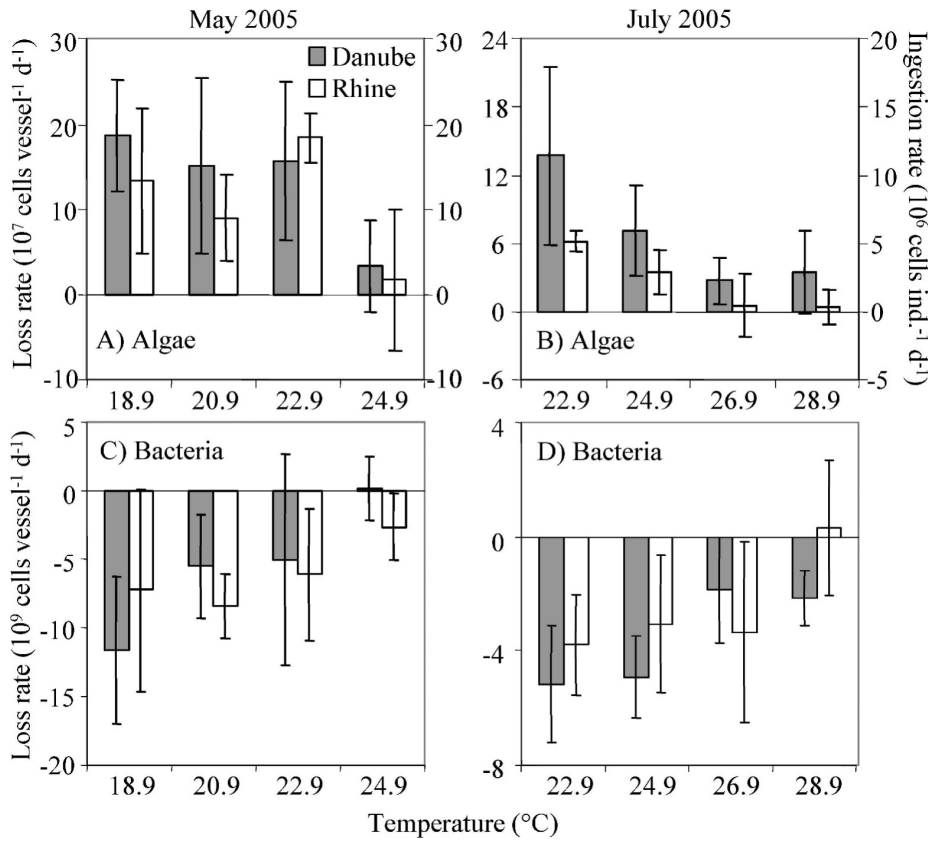


Figure 1: Temperature and adaptation effects: Mean (6 SD) loss rates for *D. polymorpha* from the two populations (Danube and Rhine) with different invasion histories on (A, B) planktonic algae and (C, D) bacteria in the experiments conducted in May and July. Significant temperature effects on the rates 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. The data from this experiments resulted from a collaboration with Johanna Dahlmann.

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 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):

$$LR = VrA_s \quad (2)$$

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):

$$r = [\ln(A_e) - \ln(A_s)] / t \quad (3)$$

Table 2: Results of two-factorial ANOVAs testing the effects of temperature and mussels' origin on the loss rates of algae (10⁸ cells per vessel d⁻¹) and bacteria (10¹⁰ cells per vessel d⁻¹) for experiments one (May 2005) and two (July 2005). The data for the experiments resulted from a collaboration with Johanna Dahlmann.

	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 x 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 x origin	0.196	3	0.299	0.826
Residual	3.500	16		
July 2005, algae				
Temperature	2.734	3	6.895	0.003
Origin of mussels	1.093	1	8.267	0.010
Temperature x origin	0.247	3	0.623	0.610
Residual	2.379	18		
July 2005, bacteria				
Temperature	0.476	3	3.645	0.033
Origin of mussels	0.082	1	1.876	0.188
Temperature x origin	0.141	3	1.083	0.381
Residual	0.783	18		

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 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 $10 \mu\text{g mL}^{-1}$ for algae and $5 \mu\text{g mL}^{-1}$ for bacteria. The stained samples were filtered on black polycarbonate-membrane filters (0.2 mm, Whatman Nucleopore, Whatman) and kept frozen at -220°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°C in both the first and second experiment, whereas no effect of temperature on the loss rates was recorded at temperatures between 18.9°C and 22.9°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°C and 30°C (Fig. 2, Table 3). The acclimation temperatures (20°C and 28°C) had no significant effect on ingestion rates at the high temperatures (Table 3).

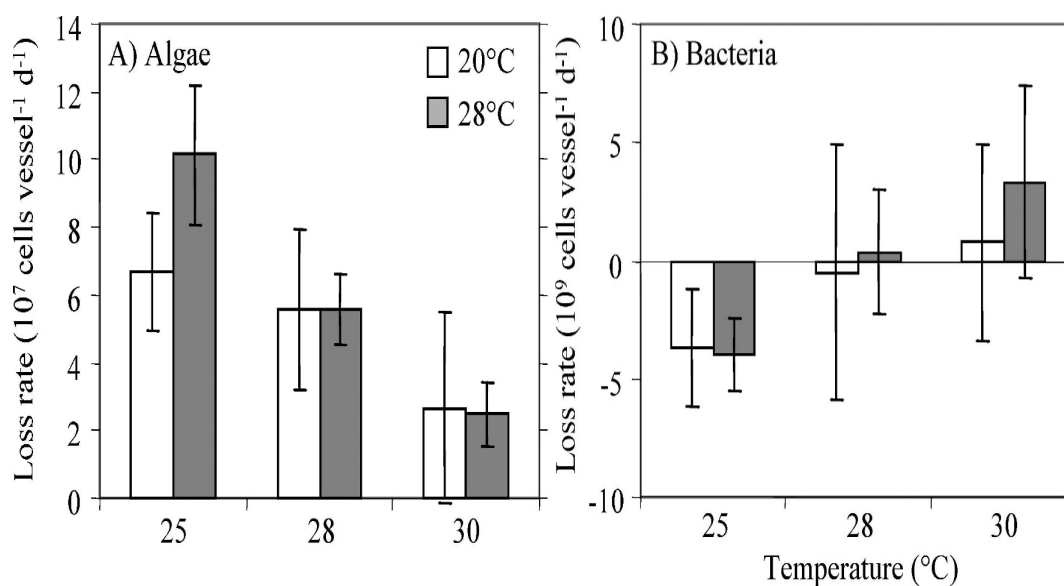


Figure 2: Test of acclimation effects on the loss rates of (A) algae and (B) bacteria under high temperatures. The second y-axis gives the corresponding ingestion rates for algae. No effect of the acclimation temperatures (20°C and 28°C) could be shown, whereas the test temperature effects were significant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

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 (10^9 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	0.129	1	3.170	0.095
Test temp. x	0.117	2	1.441	0.268
Residual	0.609	15		
Bacteria				
Test temperature	1.276	2	5.211	0.019
Acclimation	0.028	1	0.226	0.641
Test temp. x	0.098	2	0.401	0.677
Residual	1.837	15		

Grazer-mediated opposing effects of warming on bacteria and on HF

After demonstrating the opposite development 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

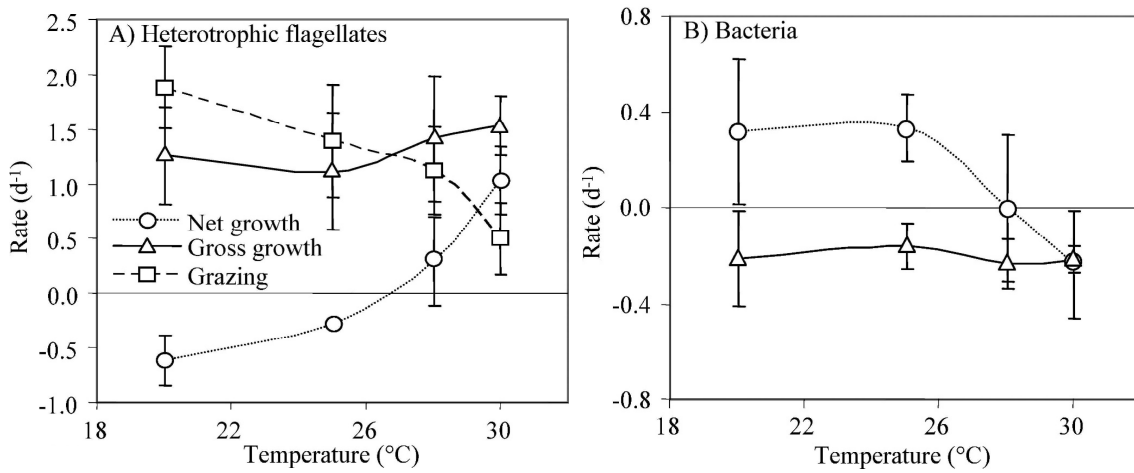


Figure 3: Results of the grazing experiment performed in September on (A) heterotrophic flagellates and (B) bacteria: Temperature response of mean (\pm SD) gross growth rate (without mussel effect), net growth rate (under mussel effect), and (for algae) grazing rate. The gross growth rate is dependent 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).

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 on HF on temperature for experiment four (September 2005).

	<i>R</i>	<i>p</i>
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

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.lids.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 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. 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 mis-interpretations 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 mollusk 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.

Chapter 3

Intra- and interannual variability surpasses direct temperature effects on the clearance rates of the invasive clam *Corbicula fluminea*

Abstract

We measured the clearance rates of the filter-feeding bivalve *Corbicula fluminea* over a period of 2 years. Strong seasonal variations, such as a 50-fold increase from February to July, were observed. These variations were only poorly linked to temperature, as they could be found at both the ambient field temperature and a constant temperature of 15°C. Instead, peaks in the filtration activity corresponded to the spawning activity. Additionally, a strong interannual variability with much lower clearance rates in 2009 than in 2008 was identified. The low clearance rates were linked to a preceding period of low winter temperatures close to the lethal temperature of 2°C and the associated reduced reproduction rates. Our findings demonstrate that other factors besides temperature and body mass can strongly affect clearance rates. These results should be considered when predicting the effects of changing temperatures on the ecosystem impact of filter-feeding bivalves.

Introduction

Filter-feeding bivalves are distributed worldwide in both marine and freshwater habitats. By feeding on phytoplankton, they recycle nutrients and provide a trophic link between planktonic primary production and the consumer food web in the benthos (Lewandowski and Stancykowska 1975; Nalepa et al. 1991; Caracao et al. 2006). This key role of bivalves in aquatic ecosystems becomes particularly clear when invasive species colonize new habitats. Species such as *Dreissena polymorpha* or *Corbicula fluminea* (in freshwater and brackish habitats) as well as *Crassostrea gigas* (in marine habitats) can establish high densities and can dominate the zoobenthos communities in their new habitats (Strayer et al. 1999; Troost 2010). They can thus have a considerable impact on both plankton density and composition (Cohen et al. 1984; Caracao et al. 2006; Weitere et al. 2008). Due to the key role of bivalves in aquatic ecosystems, there is a large interest in modelling ecosystem consequences of the bivalves' grazing activity and predicting their significance under the impact of climate change (e.g. Schöl et al. 2002; Park et al. 2008). Generally, good predictors of feeding rates are food quantity, body mass and temperature; most model approaches and studies focus on these parameters (e.g. Peters and Downing 1984; Sprung and Rose 1988; Aldridge et al. 1995; Lactin et al. 1995; Schöl et al. 2002).

The temperature dependence of feeding rates of key species can cascade to temperature effects on community structures and ecosystem processes (Sanford 1999). For example, it has been demonstrated in short-term experiments that even small temperature increases in summer can result in positive effects on the nanoplankton and negative effects on the picoplankton when the plankton communities are controlled by filter-feeding bivalves (Weitere et al. 2008). These effects were mediated by reduced clearance rates of the bivalves at very warm temperatures and corresponding cascade effects within the planktonic food web. However, predicting such effects remains difficult because literature data on clearance rates of relevant bivalves can show very high interstudy variability, even within the same species and comparable body mass and temperature conditions. As an example, the clearance rate of an average-sized *C. fluminea* specimen (20-25

mm) varies from an average of 26 ml h⁻¹ (Prokopovich 1969) up to an average of more than 900 ml h⁻¹ (Lauritsen 1986) at a temperature of about 20°C. Thus, there might also be other significant factors that affect filtration rates in bivalves besides temperature and body size. Identifying them could potentially reduce unexplained variability in clearance rate estimates and could enable more accurate predictions on the effects of bivalves on ecosystems attributes.

A few studies show that seasonal changes in clearance rates of bivalves cannot solely be explained by temperature or food level (Benjamin 1978; Benjamin and Burky 1978; Hornbach et al. 1984) and suggest that they must be connected with seasonal variability associated with the life history of the bivalves. However, if relevant, such seasonal variations are often overlooked, because most studies on the grazing impact of bivalves centre on short-term experiments within a restricted time period. In the present study, we examined the effects of seasonal and interannual variations on the clearance rates of the clam *C. fluminea* from a large river (Rhine, Germany). *C. fluminea*, which originated from Southeast-Asia, has become one of the most successful freshwater invaders and has spread all over Europe and North America (McMahon 1999). Among several other large rivers, the River Rhine is an example of an ecosystem in which *C. fluminea* has become the dominant benthic filter feeder (Araujo et al. 1993; Kinzelbach 1995). The clam shows distinct seasonal patterns in terms of reproduction and growth (e.g. Meister 1997; Weitere et al. 2009). It typically shows two reproduction periods: one in spring/early summer and a less pronounced one in late summer (e.g. Aldridge and McMahon 1978; Britton and Morton 1986; Meister 1997). However, the extension of the reproduction period and the reproduction success depends on environmental factors. For instance, poor food availability and stress caused by near-lethal winter temperatures can have negative effects on the reproduction. It has been recently demonstrated that the reproduction success of *C. fluminea* in the Rhine was strongly reduced after the clams experienced a cold winter (Weitere et al. 2009). Accompanying warming experiments showed that warming by 3°C above ambient temperature in winter can strongly increase the reproduction success. Such variations in reproduction can potentially affect feeding rates, if reproduction requires a significant amount of energy and a

corresponding amount of food. This is especially true for bivalves like *C. fluminea* whose embryos do not filter on their own but are nourished from secreting cells of the inner demibranch of the adult until they are released (Britton and Morton 1982). The present study is based on the hypothesis that such seasonal as well as interannual variations in life history events and environmental factors such as past cold winter periods have a significant impact on the filtration activity of *C. fluminea*. Unlike suggested from short-term experiments, the function of the clam as filter feeders would thus largely depend on other factors than actual temperature. Over a complete annual cycle, we repeatedly measured the clearance rates of clams from the Rhine both at a standardized constant temperature of 15°C and at the ambient field temperatures in order to disentangle temperature effects from annual variations. We then repeated the measurements the next year after the clams had experienced near-lethal temperatures during a cold winter.

Materials and methods

Filtration experiments

The clams used in the experiments were sampled from the lower Rhine at Cologne (Rhine-km 685) and kept at the Ecological Rhine Station of the University of Cologne under natural temperature and resource conditions with a constant flow of original Rhine water in bypass systems. The experiments were conducted in 200-ml glass beakers with a prepared *Chlorella vulgaris* algal solution of $53 \pm 6 \mu\text{g}$ chlorophyll $a \text{ l}^{-1}$ (which corresponds to an abundance of approximately 20,000 cells l^{-1}). The single cells of *C. vulgaris* had an average diameter of 7 μm (range: 4-10 μm), a size which is considered to be among the optimal food size for bivalves including *C. fluminea* (Sprung and Rose 1988; Way et al. 1990). A single clam was placed in every beaker. The different test temperatures were attained by placing the experimental vessels in temperature-controlled water baths. The water within the beakers was ventilated by an air pump (Nitto LA-80 B) to prevent sedimentation of the algae and to provide the clams with oxygen. Grazer-free controls were run under the same conditions in order to control the algal growth rates. Two experimental series were conducted. In a first series lasting from December 2007 to December 2008, clams of medium size (12-15 mm) were added to the grazer treatment. The clearance rates were measured at a standard temperature of 15°C and temperature steps of 3°C above and below the ambient temperature of the River Rhine. As an example, if ambient temperature was 19.3°C, clearance rates were measured at 18 and 21°C as well as at 15°C. The clearance rate at ambient temperature was then calculated with the help of a linear regression between the clearance rates at the two temperatures surrounding the ambient temperature. Each treatment was run in eight independent replicates twice a month. In a second series lasting from February 2009 to December 2009, mussels of the size ranging from 15 to 20 mm were used, and clearance rates were measured directly at the ambient temperature of the River Rhine. Each treatment of the second series was run in 7-16 independent replicates. The experiments were conducted 4 times a month from March to June and twice a month after that period.

Before the experiments started, the clams were acclimated to the test temperature for 1.5-2 h. All experiments started at the same time of the day, that is, between 10

and 11 Central European Time (CET). The experiments were performed for 2-5 h depending on the level of the clearance rates. Replicates with negative clearance rates or with clearance rates below 15% of the mean clearance rate were removed from further calculation to assure that only filtering individuals were taken into account. At the end of the experiments, samples were taken from each beaker. The controls served to correct for any error that might have resulted from sedimentation, flocculation or reproduction of the micro-algae during the experiments. The chlorophyll *a* concentration ($\mu\text{g l}^{-1}$) as a proxy for the algae density was measured with the help of Aquafluor™ (Turner Designs, USA) in each beaker at the end of each experiment. In addition, the grazer biomass (ash-free dry weight, AFDW) was calculated for each experiment. For this purpose, we separated the soft body from the shell, dried the soft body for 48 h at 60 °C in a drying chamber and then measured the dry weight (DW) of the clams. Thereafter, the ash weight (AW) was determined after combustion for 15 h at 550 °C. The AFDW was calculated as the difference between DW and AW. The AFDW was used to calculate the condition index of the clam as measure for the relative (size-specific) body mass. The condition index was calculated as the ratio of the body mass (AFDW, mg) to the rectangular prism volume of the shells (i.e. shell length x shell width x shell height, cm^3).

Estimation of the effect of a cold winter on the reproduction success in the field

After very low clearance rates were measured in spring and summer 2009 (see "Results" section), we hypothesized that this phenomenon is associated with reduced reproduction success after the clams experienced low winter temperatures (Weitere et al. 2009). In order to estimate the reproduction success of the bivalves, a size class analysis of *C. fluminea* was performed in late summer 2009. The sampling was performed on 22 September at a water depth of 20-50 cm in Cologne at Rhine km 685. PVC pipes with a diameter of 11.5 cm were driven about 10 cm into the sediment and all sediment within the pipes was removed. The clams were collected at seven different sites at Rhine-km 685, covering a total area of 727 cm^2 . All clams present in the samples (833 individuals in total) were measured to the nearest 0.01 mm with the help of a digital slide calliper (TCM 227579). In order to estimate

the age classes of the clams, we used growth rates measurements performed in 2009/2010 (Viergutz, unpublished data). Based on monthly measurements of the shell lengths and calculations of the growth rates, we defined clams up to 11 mm as “young-of-the-year” clams (2009), clams between 11.1 and 19.2 mm as “last years’ clams” (2008) and clams between 19.3 and 24.6 mm as “clams from 2007”. Larger clams were considered to originate from 2006 or earlier. For the calculations, we assumed that the first spawning period took place at the beginning of April and that the juvenile clams spawned with a size of 0.25 mm.

Background data

Data on suspended chlorophyll *a* concentration were provided by the routine weekly measurements of the “Landesumweltamt NRW” (Düsseldorf, Germany) near Cologne at Bad Honnef (km 640). Water temperature was routinely recorded by calibrated data loggers (Ebi-85A, Ebro Electronic, Germany) at 2-h intervals at the Ecological Rhine Station of the University of Cologne.

Calculation of grazing and growth rates and statistical analysis

The rate of change of the algal density (r , d^{-1}) was calculated based on the chlorophyll *a* concentration at the beginning (N_0 , ind. ml^{-1}) and at the end (N_t , ind. ml^{-1}) of the experiments after the time (t , d), by assuming exponential growth:

$$r = (\ln(N_t) - \ln(N_0)) / t$$

The grazing rate of the clams (g) was calculated as the difference between the rate of change measured in the controls (r_{co}) and in the grazer treatments (r_{gr}):

$$g [d^{-1}] = r_{co} - r_{gr}$$

In order to test for differences between the mean clearance rates of 2008 and 2009, we performed Mann-Whitney U tests. For this purpose, clearance rates of March/April, May/June/July, August/September/October and November/December were grouped, and pairwise comparison was performed. The correlations between clearance rates and temperature were tested with the help of Spearman rank correlations separately for each year.

Results

Background data and condition index of the clams

The environmental conditions were similar in both years, with the exception of the winter temperatures. The ambient temperature as measured in the Rhine at the Ecological Rhine Station did not rise above 25°C in the summers of both years (Fig. 1a). Very low temperatures could only be found in January 2009, where temperatures dropped below 2°C for 8 days, whereas the temperature in winter 2007/2008 did not drop below 3°C. The chlorophyll *a* concentration of the River Rhine was generally low in both years (Fig. 1b), not exceeding 10 µg l⁻¹. The chlorophyll *a* concentration peaked in spring 2008 at 8.6 µg l⁻¹ and in spring 2009 at 5.6 µg l⁻¹. The condition index of the clams was higher in 2009 than in 2008 (Fig. 1c). In 2009, the condition index reached values of up to 26 mg AFDW cm⁻³, whereas the highest value reached in 2008 was 15 mg AFDW cm⁻³.

Development of the clearance rates

Figure 2 shows the clearance rates of *C. fluminea* in 2008 and 2009 at the ambient temperature of the River Rhine and, in 2008, also at a constant temperature of 15°C. In 2008, the clearance rates at ambient temperature were below 320 ml ind.⁻¹ d⁻¹ in the beginning of the year when the temperature was below 10°C. The clearance rates increased with rising temperature in the beginning of April and remained at a high level (above 1400 ml ind.⁻¹ d⁻¹) for two and a half months. The maximum clearance rate was measured in July 2008 with 3252±330 ml ind.⁻¹ d⁻¹, which is 50 times higher than the lowest clearance rate (measured in February with 65±35 ml ind.⁻¹ d⁻¹). In August and early September, the clearance rates dropped to a level between 439±168 and 676±226 ml ind.⁻¹ d⁻¹ and increase again to values clearly above 1000 ml ind.⁻¹ d⁻¹ from early September until the end of October. Towards the end of the year, the clearance rate dropped again sharply to below 200 ml ind.⁻¹ d⁻¹ (Fig. 2a).

The strong intra-annual dynamics in the clearance rates, with peaks in early and late summer, did not follow a clear temperature pattern at temperatures above 20°C (Fig. 3). The sharp drop during midsummer, for instance, occurred at a time of constantly

high temperatures. At this time, the clearance rate was $439 \pm 168 \text{ ml ind.}^{-1}$ at a temperature of 21.9°C , while at nearly the same temperature (22.0°C) in the beginning of June, the clearance rate was as high as $3252 \pm 330 \text{ ml ind.}^{-1}$. The effect of temperature on the clearance rate was tested with the help of a Spearman rank correlation. Considering the data for the whole year showed a significant positive

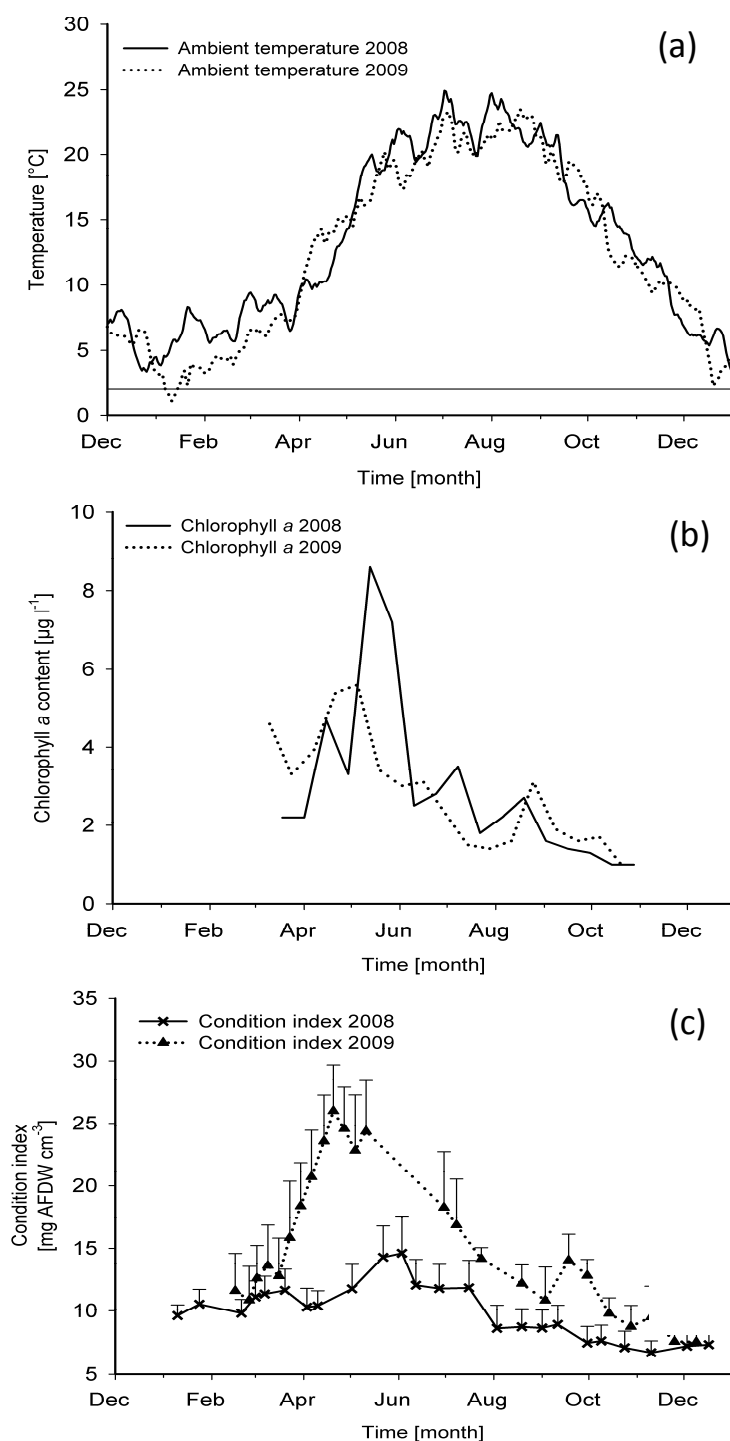


Fig. 1: Development of the environmental conditions (water temperature and chlorophyll *a* concentration) in the Rhine as well as the condition index of the clams during the study period. (a) Temperature in the Rhine at the Ecological Rhine Station of the University of Cologne (Rhine-km 685). (b) Development of the chlorophyll *a* concentration upstream of the study site in Bad Honnef (Rhine-km 640). (c) Condition index of the clams, which represents the ratio of the soft body mass to the rectangular prism volume of the shell. Error bars represent standard deviation. The data from the condition index from January 2009 to June 2009 resulted from a collaboration with Catherin Linn.

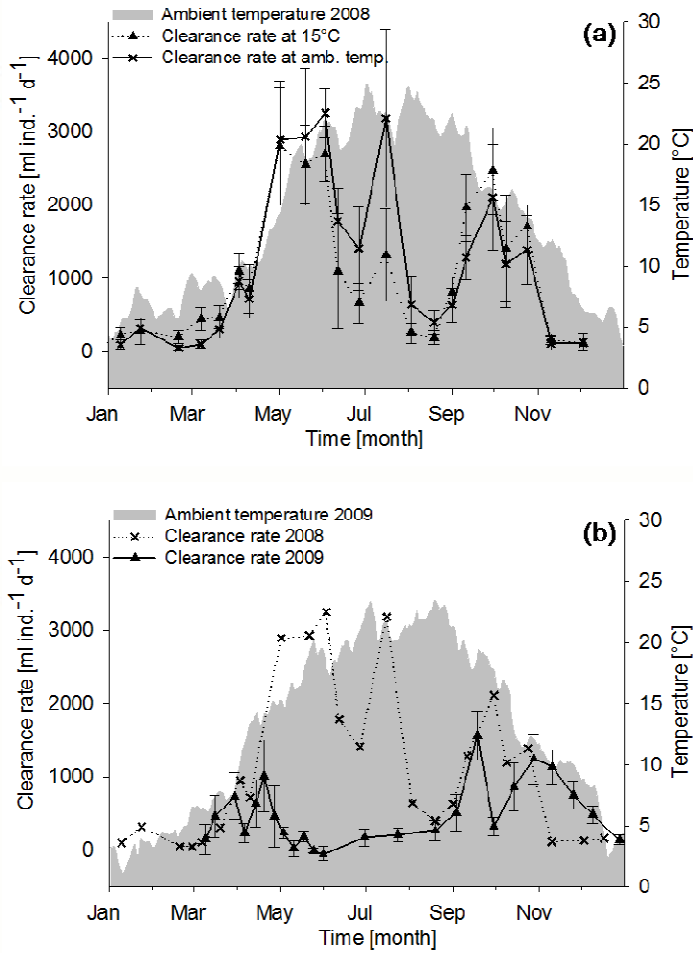


Figure 2: Intra- and inter-annual variability of clearance rates from the feeding experiments in 2008 and 2009: (a) Comparison of the intra-annual pattern of clearance rates measured at ambient temperature and at a standard temperature of 15°C in 2008. The grey area represents the ambient temperature (see Fig. 1a). Error bars represent 95 % confidence interval. (b) Seasonal changes of the clearance rates of *C. fluminea* at ambient temperature in 2009 (in comparison to the dynamics in 2008 taken from panel a, see Table 1 for statistical analyses). The grey area represents the ambient temperature (Fig. 1a). Error bars represent 95 % confidence interval. The data from the condition index from January 2009 to June 2009 resulted from a collaboration with Catherin Linn.

correlation of clearance rate with temperature ($r = 0.696 / p < 0.001$) in 2008 (Fig. 3). However, when only the clearance rates at temperatures above 20 °C were taken into account, significant correlations with temperature could not be demonstrated ($r = -0.048 / p = 0.935$). Furthermore, a similar pattern in clearance rate variations was recorded when measuring the clearance rates at a constant temperature of 15 °C (Fig. 2a). However, when looking at differences between the clearance rates measured at 15 °C and those measured at ambient temperature, clear temperature effects were observed: while clearance rates were higher at summer ambient temperatures of over 15 °C than they were at 15 °C, they were usually lower during winter with ambient temperature below 15 °C.

In 2009, the clearance rates also show intra-annual variability, however, at a lower level than in 2008 (Fig. 2b). The clearance rates ranged from $21 \pm 7 \text{ ml ind.}^{-1} \text{ d}^{-1}$ in late May/early June up to a maximum of $1564 \pm 331 \text{ ml ind.}^{-1} \text{ d}^{-1}$ in September. This clearance rate maximum is about half of the maximal values measured in 2008, even though larger clams (15-20 mm shell length) were used for the experiments in 2009

compared to 2008 (12-15 mm shell length). The most pronounced difference in the clearance rate pattern in 2009 compared to 2008 is the very small peak in spring/early summer 2009, whereas similar late summer/early autumn peaks appeared in both years (Fig. 2b). This pattern is confirmed by the Mann-Whitney U tests, which showed highly significant differences in the clearance rates between the years for May/June/July but not for the other time periods (Table 1). While the Spearman rank correlation revealed a significant positive correlation between clearance rates and temperature throughout the year in 2008 (see above), clearance rates were not significantly related to temperature in 2009 (Spearman: $r = -0.401$, $p = 0.053$, Fig. 3).

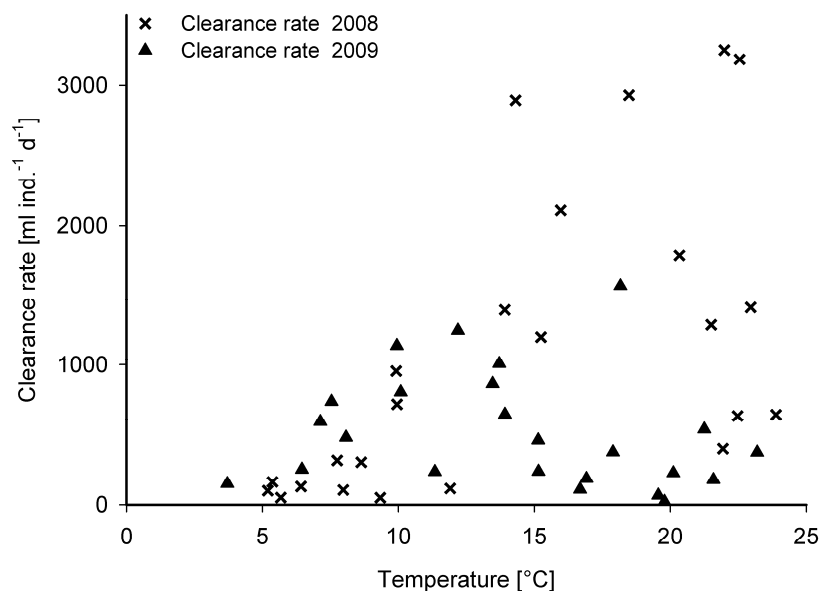


Figure 3: Mean clearance rates of *C. fluminea* measured in 2008 and 2009 in relation to temperature. See text for results of Spearman rank correlations. The data from the condition index from January 2009 to June 2009 resulted from a collaboration with Catherin Linn.

Table 1: Comparison of the clearance rates between 2008 and 2009 at four different time intervals, using Mann-Whitney U-tests. Clearance rates are given as mean and range in brackets over the particular time intervals. The data from the condition index from January 2009 to June 2009 resulted from a collaboration with Catherin Linn.

Period of the year	Clearance rates in 2008 (ml ind ⁻¹ d ⁻¹)	Clearance rates in 2009 (ml ind ⁻¹ d ⁻¹)	Mann-Whitney U test (p)
March, April	520 (105 - 954)	562 (253 - 1007)	0.925
May, June, July	2575 (1408 - 3253)	147 (22 - 238)	0.001
August, September, October	1094 (401 - 2108)	827 (374 - 1564)	0.353
November, December	136 (116 - 163)	643 (151 - 1136)	0.112

Recruitment success

Figure 4 presents the size distribution of *C. fluminea* sampled in the Rhine at Cologne in September 2009, grouped into corresponding year of spawning. The size class analyses of 833 individuals from seven different sites revealed only a very low percentage ($3.2 \pm 4.3\%$) of juvenile young-of-the-year individuals with a shell length of 0.2-11 mm; $19.3 \pm 13.9\%$ of the clams were born in 2008 (11.1-19.2 mm) and $65.8 \pm 23.3\%$ of the individuals in 2007 (19.3-24.5). Only $11.7 \pm 25\%$ of the individuals were larger and had spawned in 2006 or earlier. The very low proportion of small individuals strongly suggests that the recruitment success from the preceding reproduction period in spring/early summer 2009 has been very low and indicates an overall reduced reproduction, although other factors influencing the size distribution of *C. fluminea* cannot be excluded.

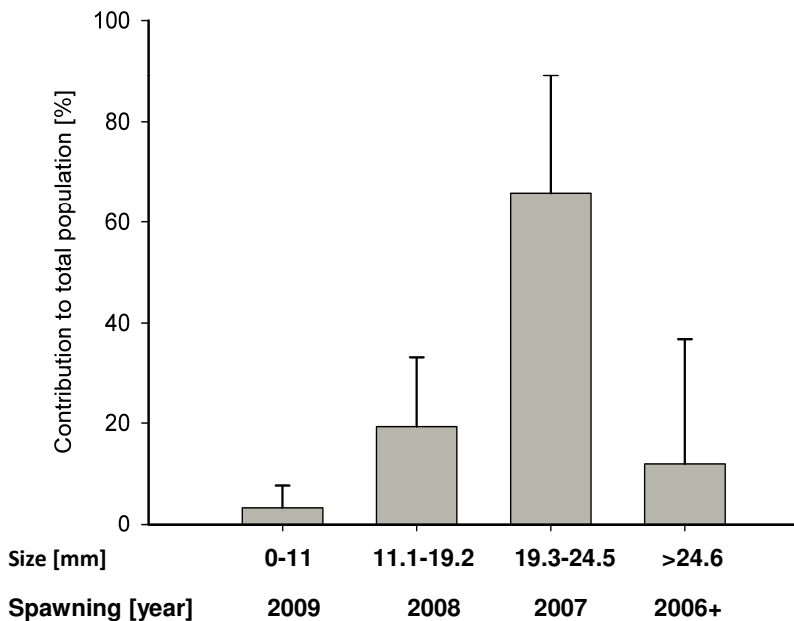


Figure 4: Size class distribution and corresponding age distribution of *C. fluminea* in the Rhine at Cologne in September 2009. Note the low contribution of young-of-the-year clams from the first spawning period 2009 after the cold winter in 2008/09. The age distribution is based on the sampling of 833 individuals from seven different sites. Error bars represent standard deviation.

Annual variability in clearance rates is largely non-temperature driven

The results of our long-term experiments showed a strong seasonal variability in the clearance rates. In 2008, the clearance rates ranged from 65 ± 35 ml ind.⁻¹ d⁻¹ in February up to 3252 ± 330 ml ind.⁻¹ d⁻¹ in July, which is a 50-fold increase. We were able to identify two peaks in that year, of which the first corresponds to the chlorophyll peak in spring. However, the second peak, which lasted from September to the end of October, took place at constantly low chlorophyll levels. Thus, the food level during the acclimation period cannot be used to explain the increase in clearance rates in autumn.

During the period of the second peak, the temperature dropped from 20 to 12°C. In theory, this should have led to a reduction rather than to a peak in the clearance rate, because the optimum temperature for filtration is considered to be between 20 and 25°C (Mattice and Dye 1976; Viergutz et al. 2007). Also during midsummer, temperature cannot be considered to be the factor leading to the reduced feeding activity. In 2008, the temperature during the period with reduced clearance rates was at the same level as the month before, which had high clearance rates. These arguments against a predominantly temperature-driven seasonal variation in clearance rates are supported by our measurements at a constant temperature of 15°C. The clearance rates at constant temperature show the same general pattern as revealed by the experiments at the ambient temperature of the River Rhine. However, the amplitude of seasonal variations is slightly dampened at 15°C, because the clearance rates at that temperature were higher than for those at the ambient temperature in winter (at natural temperatures below 15°C) and lower in summer (at natural temperatures above 15°C). However, this temperature response at a given point in time is relatively small in relation to the large seasonal variations in the clearance rates.

The two peaks of the clearance rate in 2008 correspond to the bimodal pattern of spawning activity (Aldridge and McMahon 1978; Britton and Morton 1986; Meister 1997). The spring peak is usually initiated when temperatures surpass 15°C (Rajagopal et al. 2000), which occurred in May 2008 and April 2009 during the present study. Meister (1997), who examined *C. fluminea*'s reproduction activity in

the River Rhine in 1995 and 1996, described a first spawning period from June to July in 1995 and from April to June in 1996. In both years, this spawning period was followed by a month with almost no spawning activity (August 1995 and July 1996). A second reproduction period was observed in October 1995 and from August to October 1996. The correlation of the clearance rates measured in our experiments with the spawning periods is probably explained by a higher food demand. According to King et al. (1986), the larval stadiums of *C. fluminea* do not filter on their own. Morton (1977) found that *C. fluminea* builds secretory cells during the spawning periods, which might be capable of nourishing the larval stadiums. Our findings are in accordance with the findings from Hornbach et al. (1982, 1984), who examined *Sphaerium striatinum*, another brooding bivalve that belongs to the same superfamily as *C. fluminea*. *S. striatinum* showed the same bimodal pattern in the filtration rate with peaks at the times of intense reproduction.

The large variations in the clearance rates at both constant temperature and at ambient temperature suggest a strong endogenous pattern in the clearance rate regulation and an important role of the energy demand during reproduction. It challenges the present understanding that the predominant role in determining annual clearance rates is played by temperature. Nevertheless, temperature is important in explaining the rates at any given time. By comparing the clearance rates measured at 15°C with those measured at ambient temperatures, temperature responses could be shown. At cold ambient temperatures during winter, clearance rates at ambient temperatures were distinctly smaller than at 15°C, whereas the opposite pattern was shown in summer at high ambient temperatures. This pattern is generally in accordance with common knowledge and the results of earlier studies demonstrating pronounced temperature effects on the clearance rates of *C. fluminea* and other bivalves in short-term experiments (e.g. Aldridge et al. 1995, Sylvester et al. 2005, Viergutz et al. 2007). Nevertheless, our data clearly show that extrapolation of clearance rates determined at a given time to other periods needs to be treated with care even if the temperature ranges are comparable.

Large between-year variability: a possible effect of temperature stress in winter

The differences between the clearance rates measured in 2008 and those measured in 2009 were surprisingly large: particularly from April to July, the clams performed on a much lower level in 2009 than in 2008. This is quite surprising when the different size classes of clams we used in the 2 years are taken into consideration. In 2008, the shell lengths were 12-15 mm; in 2009, we used larger clams with a shell length of 15-20 mm. Both size classes are capable of reproducing (Meister 1997). The fact that larger clams were used in 2009 thus underlines the conclusion of distinctly lower clearance rates in 2009 compared to 2008. Generally, the clams showed a similar pattern of volume-weight dependency, which is used as a condition index of *C. fluminea*, with higher condition indices in spring and lower condition indices in winter. Nevertheless, in spring 2009, the condition index even surpassed the values detected during the same season in 2008, even though clearance rates were distinctly lower in 2009. The food supply (as estimated by the chlorophyll *a* content) was at a low level (below $10 \mu\text{g l}^{-1}$) in both years, but peaked a bit higher in 2008. However, it is unlikely that these small differences in the food supply during the acclimation period can explain the much lower level of clearance rates in 2009.

In the light of the good condition index in 2009 together with the low clearance rates, it appears likely that the energy demand was distinctly smaller in 2009 compared to the high energy demand during the reproduction period in 2008. A high condition index could reflect the mass of a brooding animal. However, our data show that reproduction was low in early summer 2009. Nevertheless, it is possible that the clams were holding the brood until late summer. Studies showed that *C. fluminea* does not spawn if the conditions are poor for the larvae (Cataldo and Boltovskoy 1999; Doherty et al. 1987; Rajagopal et al. 2000). In this case, the brood would account for the higher body mass. Another possible explanation for the differences in the condition index can be found in the cold winter temperatures in 2009 and corresponding effects on the fitness of *C. fluminea*. The water temperature in January 2009 dropped below 2°C , which is considered to be a critical temperature for *C. fluminea* (Mattice and Dye 1976; McMahon 1983). Although Müller and Baur

(2011) showed that *C. fluminea* is able to survive at the temperatures of 2°C for several weeks in laboratory experiments, it is clear that low temperatures can be considered to be a stress factor for *C. fluminea*. Weitere et al. (2009) were able to show that the reproduction rate of *C. fluminea* is strongly reduced in cold compared to warm winters in the Rhine. Furthermore, a slight warming of 3°C above ambient temperature in the winter period had massive positive effects on shell growth, body mass and reproduction success, in both cold and warm winters (Weitere et al. 2009). The size class analysis of the present study in fact supports the conclusion that the recruitment success in spring 2009, after the clams experienced low winter temperatures, was very low. We found only a very low percentage ($3.2\pm 4.3\%$) of clams of the size class 0.2-11 mm in September of 2009. The abundance of clams originating from 2008 was distinctly higher ($19.3\pm 13.9\%$) but still not large compared to the percentage of clams that had spawned in 2007 ($65.8\pm 23.3\%$). Clams that had spawned before 2007 were also rather rare ($11.7\pm 25\%$), which should primarily be connected to natural die-off of older clams (Meister 1997). Our approach to estimate reproduction success from the size distribution leads to an overrepresentation of young age classes, because the older clams are stronger affected by mortality. Thus, the low percentage of young clams from 2009 supports the conclusion of a very low reproduction success in spring 2009. Taking the assumed low reproduction during the first reproduction period in 2009, the strong negative effects of cold temperatures on the reproduction of *C. fluminea* as demonstrated earlier (Weitere et al. 2009) and the finding that highest clearance rates were found during the reproduction period in 2008, our study suggests that cold winter temperatures have long-lasting effects on the feeding of the clams mediated by effects on the reproduction. However, considering the time of the second reproduction period in late summer/early autumn, the clams show a similar increase in the clearance rates during this period in both years. This suggests a recovery in reproduction success towards the second reproduction period.

Conclusion

Our data show a large intra- and interannual variability of the clearance rates that is not primarily explained by temperature. Instead, the annual feeding intensity was also controlled by other factors including endogenous regulation of life history events and probably also by external factors such as past cold winter temperatures that affect life history events. Our data suggest that *C. fluminea* had to recover from the cold winter temperatures in early 2009 and was not able to initiate the reproduction process in spring. The pattern of clearance rates further suggest that they recovered during summer and show regular behaviour in late summer. Bivalves such as *C. fluminea* and *D. polymorpha* act as keystone species in many rivers and other shallow waters. Variations in their feeding behaviour can have direct effects on communities and ecosystem-wide processes (e.g. Strayer et al. 1999; Weitere et al. 2008). When predicting their grazing effects under changing temperature regimes, both long-lasting effects of winter temperature and seasonal variations in their activity need to be considered in addition to short-term temperature responses.

Chapter 4

**Size-dependent seasonal growth pattern and maximum age
of *Corbicula fluminea* in a large European stream**

Abstract

Corbicula fluminea is one of the most important and successful invaders of freshwater habitats in the northern hemisphere. When trying to estimate or to model the ecological impact, knowledge of population dynamics of this organism is essential. In the present study the shell growth of more than 50 individuals under natural conditions over a period of more than one year was examined. The data was used to develop a von Bertalanffy growth model. With the help of the model it was possible to generate shell growth curves of *C. fluminea* over several years, beginning with the freshly released juvenile (0.25 mm). Shell length of 12.0 mm after the first, 19.8 mm after the second and 25.1 mm after the third year were estimated. In accord with previous studies, it was shown that shell growth decreases with increasing shell length. Additionally, a strong positive impact of temperature and food availability on shell growth was detected. With the help of the model we could estimate basic population dynamics, such as maximum age (7.3 years) and maximum size (35.55 mm), for *C. fluminea* in the River Rhine.

Introduction

Corbicula fluminea is among the most successful colonizers of freshwater ecosystems throughout the northern hemisphere and has become very abundant in large parts of Europe and North America (Cataldo and Boltovskoy 1999; McMahon 2000). *C. fluminea* can have a large impact on the function of ecosystems (Cohen et al. 1984; Lauritsen & Mozley 1989, McMahon 1999) by often being the dominant filter feeder in riverine systems and reservoirs and by consuming large amounts of plankton. The high fecundity and the ability to rapidly adapt to different environmental conditions (McMahon 1982; Ortmann and Grieshaber 2003) are the main factors leading to the pervasiveness of *C. fluminea*. Because of its vast distribution and relevance, *C. fluminea* has become the object of intensive research. To understand the ecological role of *C. fluminea*, it is of major interest to gain knowledge of the population dynamics of this bivalve. Knowledge of growth characteristics of individuals is one of the most important components when examining population dynamics. An understanding of the growth rates and patterns of *C. fluminea* can provide a basis for predictions of the capacity for populations to expand and exploit new habitats (Johnson & Carlton 1996). In the case of *C. fluminea*, such information can also be useful when evaluating the influence of the organism on aquatic ecosystems. The growth pattern of *C. fluminea* has been relatively well studied on an annual basis (e.g. Morton 1977; Williams & McMahon 1986; Meister 1997; Schmidlin 2007). Freshwater bivalves typically show a decreasing growth rate with increasing size of individuals (Hanson et al. 1988; Garton & Johnson 2000; Haag and Commens-Carson 2008). *C. fluminea* shows a highly variable growth pattern that does not only depend on the individual's size, but also on the characteristics of habitat types and, therefore, on various environmental conditions (Eng, 1979; Williams & McMahon, 1986; Schmidlin, 2007). These environmental conditions can be substrate type (Kat, 1982; Schmidlin, 2007), mussel density (Bolden and Brown, 2002) or food resource availability (Foe & Knight, 1985). Temperature is considered to be one of the most important factors influencing growth patterns of bivalves (Mattice, 1979; Dettman et al. 1999; Viergutz et al. 2007).

However, individual-based studies focusing on the intra-annual growth pattern are very rare (e.g., Meister 1997). In this study the size-dependent seasonal shell length increase and growth pattern of *C. fluminea* from a large European stream (River Rhine), as well as the influence of environmental factors, such as temperature and food availability was examined to identify general growth patterns. In order to generate growth curves over several years, the shell growth of *C. fluminea* was modelled on a monthly basis using the “Von Bertalanffy growth equation” (von Bertalanffy 1938). With the help of the growth model it was possible to estimate basic population parameters of *C. fluminea*, such as annual shell length increase, age of an individual at a given length, maximum age and maximum shell length.

The aims of the following study were: (1) to characterize an annual size-dependent shell length increase; (2) to examine size-specific intra-annual growth patterns in relation to water temperature and chlorophyll *a*-concentration; (3) to provide fundamental ecological information on *C. fluminea*.

Materials and Methods

Bivalve treatment and measurements

The bivalves were sampled from the River Rhine at Cologne (Rhine-km 685) in June 2009 and were kept at the Ecological Rhine Station of the University of Cologne, a freshwater laboratory next to the navigation channel of the River Rhine (Rhine-km 685). The bivalves were kept in a constant flow of original Rhine water in bypass systems and under natural temperature, substrate (sand) and resource conditions. In order to track individuals, all clams were marked on the shell with individual numbers using white paint. The shell length (from anterior to posterior) of the bivalves ranged from 5-33 mm. Depending on the speed of growth, the shell length was measured with an electronic calliper every 2 to 4 weeks by the nearest of 0.1 mm. Smaller individuals were added regularly to ensure that at least 4 individuals of each size class (5-10 mm, 10-15 mm, 15-20 mm, 20-25 mm, 25-30 mm, 30-35 mm) were present. The measurements were performed from July 2009 to August 2010 (390 days).

Background data

The temperature of the River Rhine, during the experimental period, ranged from 2.1 °C up to 27.3°C (Fig. 1). The temperature was logged directly from the River Rhine near Cologne (Rhine-km 685.5). The data for the chlorophyll *a* content derived from the Ecological Station in Bad Honnef at Rhine-km 640.0 and was generally low during the experimental period (0 – 14 µg l⁻¹) (Fig. 1).

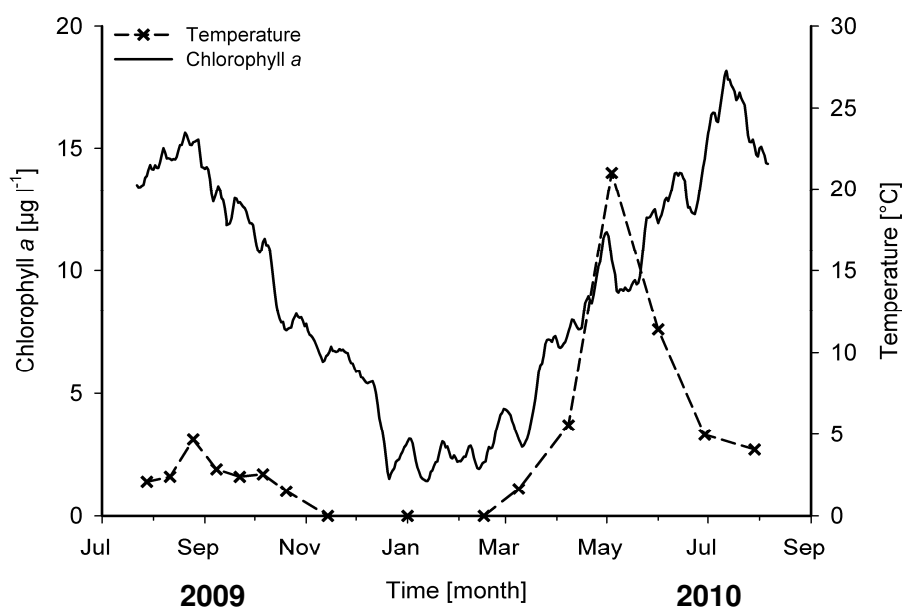


Figure 1: Development of temperature and chlorophyll a concentration during the experimental period from July 2009 to August 2010.

Modelling shell growth (von Bertalanffy growth equation) and statistical analysis

To generate continuous growth curves from the juvenile bivalve (0.25 mm) up to the adult size class (30-35 mm), the shell growth of *C. fluminea* was modelled with the help of the “von Bertalanffy growth equation” (von Bertalanffy 1938). This equation has already been used in several studies to characterize molluscan growth (e.g. Morton 1979; Ituarte 1985; Bauer 1992; Meister 1997). It describes exponential growth that decreases with increasing shell length:

$$L_t = L_{\infty}[1 - e^{-K(t-t_0)}] \quad (1)$$

Where L_t represents the length of the organism at time t (age), L_{∞} represents the theoretical maximum length an organism would reach at infinite age (asymptotic length), K represents the von Bertalanffy growth parameter (calculated to display homogenous time segments for every month) and t_0 represents the theoretical time at which shell length had been zero. Because the growth pattern of *C. fluminea* shows seasonal dynamics besides size dependency, a linear regression between growth

and initial size (at the beginning of the corresponding month) was performed for each month.

The slope (b) and the intercept (a) of the regression lead to the parameters of the following equation:

$$\Delta L/\Delta t = a + bL_t \quad (2)$$

where $\Delta L/\Delta t$ represents the increment in length per unit time (month) and L_t the length of the bivalve at time t (before growth). According to the equations of Ford-Walford (Walford 1946), the length after time (t), dependent on initial size, can be calculated:

$$L_{t+1} = L_t + \Delta L/\Delta t ; b' = b+1 \quad (3)$$

The parameters of the von Bertalanffy equation can now be calculated:

$$L_\infty = a / (1-b') = -a / b \quad (4)$$

$$K = -\ln(b') \quad (5)$$

$$t_0 = \ln[(L_\infty - L_0) / L_\infty] / K \quad (6)$$

Where t_0 is the theoretical age at zero length, L_∞ the asymptotic maximum length and K the von Bertalanffy growth constant. The monthly shell length increase, starting with an initial size of 0.25mm (L_0 = shell size at release from the adult bivalve), was determined on the basis of the monthly constants (K) using the following formulas:

$$t_1 = (-1 / K) \ln[1 - (L_{t_1}) / L_\infty] \quad (7)$$

$$\Delta L = L_\infty [e^{(-K*t_1)} - e^{(-K*t_{1+1})}] \quad (8)$$

Where t_1 represents the point of time before shell length increase, L_{t_1} the initial shell length of the bivalve at time t_1 , ΔL the shell length increase and t_{1+1} the point of time after growth (point of time before growth + 1 month). Following Taylor (1958), the maximum age can be calculated as the age at 95% of L_∞ (A_{95}) using the following formula:

$$A_{95} = t_0 - (1/K) * \ln(1-0.95) \quad (9)$$

The correlations of shell growth with temperature respectively shell growth with chlorophyll *a* content were tested with the help of Spearman rank correlations.

Results

Examining shell growth

Figure 2 shows the total growth in the experimental period (390 d^{-1}) versus the initial shell size. The smallest bivalves showed the largest shell length increase of up to 10 mm while the largest bivalves only grew less than 1 mm during the experimental period. The relationship between initial shell length and total shell growth was a highly significant. The decrease in shell growth with increasing initial size was linear, and 96.5% ($r^2 = 0.965$) of variation in shell growth could be explained by the linear regression model.

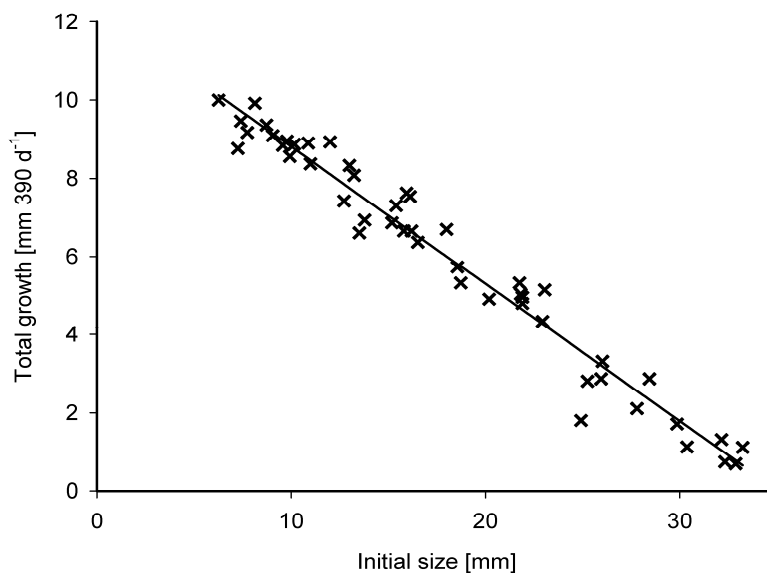


Figure 2: Scatter plot with fitted linear regression of total growth during the experimental period (390 days) versus initial size of *C. fluminea* ($n = 44$). $r^2 = 0.965$; $P < 0.001$.

Figure 3 shows the comparison of growth rates of *C. fluminea* from three different size classes (5-10 mm, 15-20 mm and 25-30 mm) from late summer to winter (A) and spring to early summer (B). Both periods show a clear size-dependency of shell length increase where the smallest bivalves grow at highest and the largest bivalves grow at lowest speed. The largest shell length increase, for every size class, could be found between June and July with $94.5 \pm 9.9 \mu\text{m d}^{-1}$ for the 5-10 mm size class, $75 \pm 7.6 \mu\text{m d}^{-1}$ for the 15-20 mm size class and $44.8 \pm 10.6 \mu\text{m d}^{-1}$ for the 25-30 mm size class. The maximum shell growth of the 5-10 mm size class reaches more than

twice the maximum shell growth of the 25-30 mm size class. No growth was observed from the beginning of 2010 to the end of March. Generally, shell length increase was low at periods with low temperatures and low chlorophyll *a* concentrations (see Figure 1 for temperature and chlorophyll conditions).

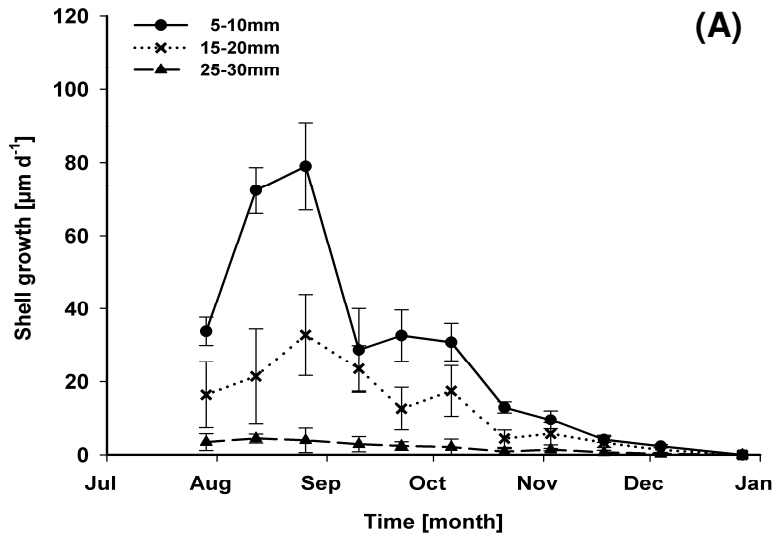
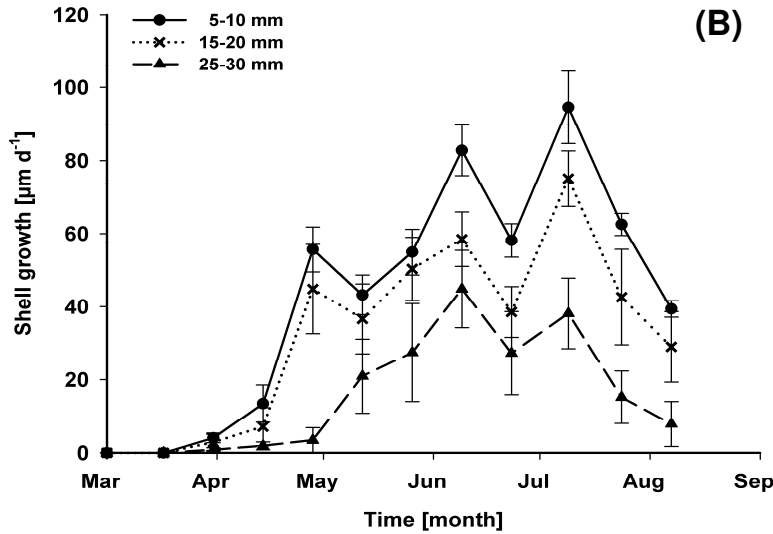


Figure 3: Comparison of shell growth from three different size classes (5-10 mm, 15-20 mm and 25-30 mm) of *C. fluminea* from (A) August to December 2009 and (B) March to August 2010.



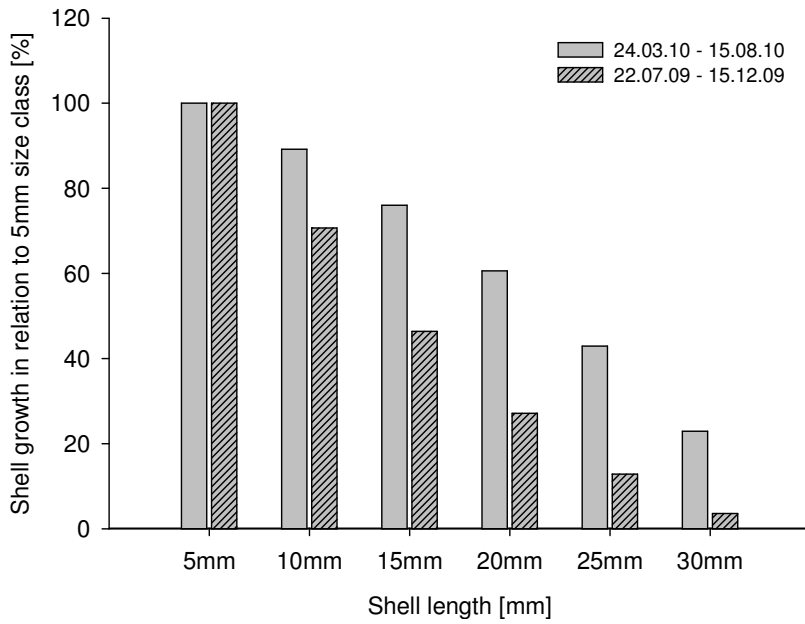


Figure 4: Shell growth of the different size classes in relation to 5 mm size class from July to December 2009 and from March to August 2010. The shell growth at the different shell length was determined by a regression between initial shell length and growth. The shell growth of the 5mm class represents 100% shell growth.

Figure 4 shows a comparison of the percentage of shell growth of the different size classes from July to December 2009 and from March to August 2010 as determined by a regression between initial shell length and growth. The growth of the smallest size class (shell length = 5 mm) was set as standard (100%). Larger bivalves had a much lower shell growth - compared to bivalves with a shell length of 5 mm - in the period from July to December 2009 than in the period from March to August 2010. With increasing bivalves' shell length, the difference in shell growth becomes stronger. For example, the total shell length increase of bivalves with a shell length of 20 mm reached 61 per cent of the shell length increase of bivalves with a shell length of 5 mm in the spring/summer period of 2010, but only 27 per cent in the summer/autumn period of 2009. Very large bivalves (shell length \geq 30 mm) reach 23 per cent of the shell length increase of small bivalves (5 mm) in the spring/summer period of 2010 but only 4 per cent in the summer/autumn period of 2009.

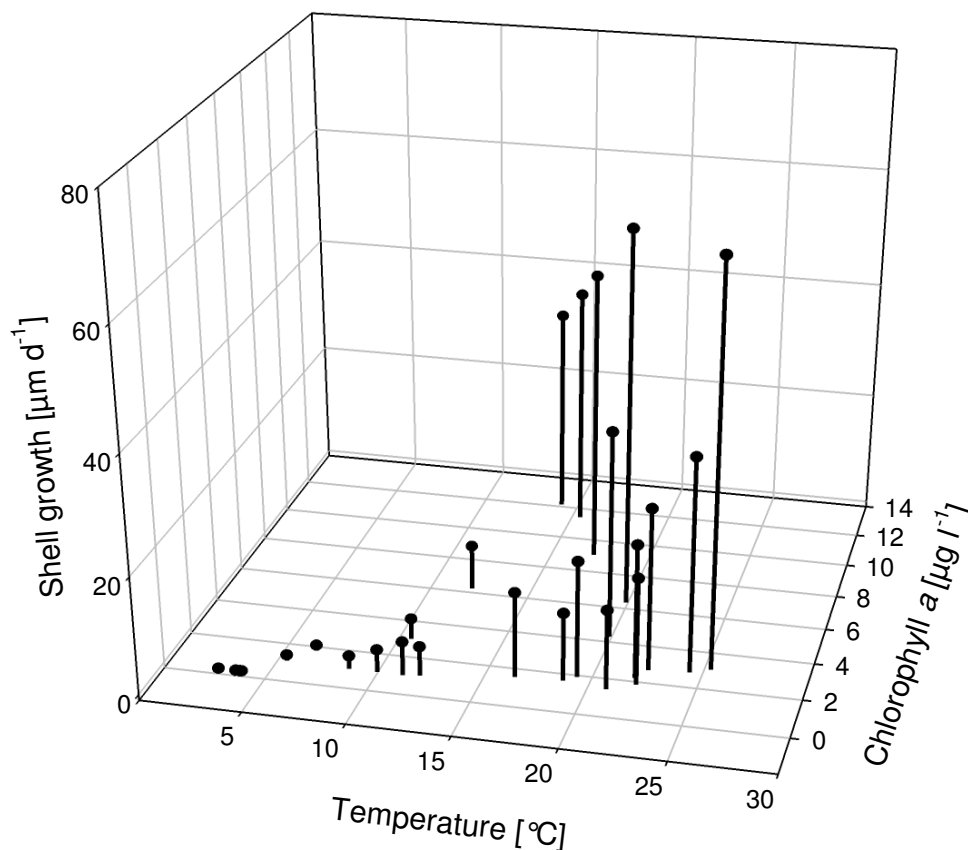


Figure 5: 3D-Plot of shell growth (Z-Axis) of average sized *C. fluminea* (15 - 25mm), Chlorophyll *a* content (X-Axis) and ambient temperature (Y-Axis) in the River Rhine during the experimental period (22/07/09 - 15/08/10).

Figure 5 displays the shell growth of average sized *C. fluminea* (shell length = 15-25 mm), ambient temperature and chlorophyll *a*-concentration in a 3D-plot. The shell growth was positively correlated with both temperature (Spearman: $r = 0.858$, $p < 0.001$) and chlorophyll *a*-concentration (Spearman: $r = 0.756$, $p < 0.001$). Almost no growth could be observed at temperatures below 10°C or at periods with low chlorophyll *a*-content, while the average shell length increase reached its maximum at highest temperatures in June and July 2010 after the chlorophyll *a*-peak of 14 $\mu\text{g l}^{-1}$ in May. Shell growth in August and September 2009 was not as high as in June and July 2010 although the temperatures were at the same range, suggesting that shell growth was limited by the food-concentration which was very low in this period (chlorophyll *a* 1.4 – 3.1 $\mu\text{g l}^{-1}$). In the 2009 period, which does not cover the

spring and early summer, only a small summer peak of $3.1 \mu\text{g l}^{-1}$ chlorophyll *a* could be found. With an average of $1.8 \mu\text{g l}^{-1}$, the chlorophyll *a* concentration in the 2009 period was much lower than in the same season in 2010 (which covers the chlorophyll *a* peak in spring) with $4.8 \mu\text{g l}^{-1}$.

Modelling the shell growth of C. fluminea

Table 1 summarizes the von Bertalanffy growth model parameters which were calculated on a monthly basis. Due to the different growth characteristics in the different months, the values of K_m and t_0 have a high variability. K_m was set to 0, when less than 20 per cent of the mussel experienced growth in the corresponding period.

Table 1: Regression parameters and calculated parameters for the growth modelling (with a = intercept of regression, b = slope of regression, L^∞ = calculated maximum shell length, K_m = calculated monthly growth constant, t_0 = theoretical time at which shell length of the mussel was zero)

Month	a	b	L^∞	K_m	t_0
January	0	0	-	0	-
February	0	0	-	0	-
March	0.033	-0.0008	41.25	0.0008	-7.505
April	0.916	-0.0254	36.06	0.0257	-0.270
May	1.972	-0.0467	42.23	0.0478	-0.124
June	2.658	-0.0636	41.79	0.0657	-0.091
July	3.341	-0.0914	36.55	0.0959	-0.072
August	2.510	-0.0870	28.85	0.0910	-0.096
September	1.482	-0.0487	30.43	0.0499	-0.166
October	0.764	-0.0251	30.44	0.0254	-0.324
November	0.186	-0.0054	34.44	0.0054	-1.347
December	0.040	-0.0012	33.33	0.0012	-6.333

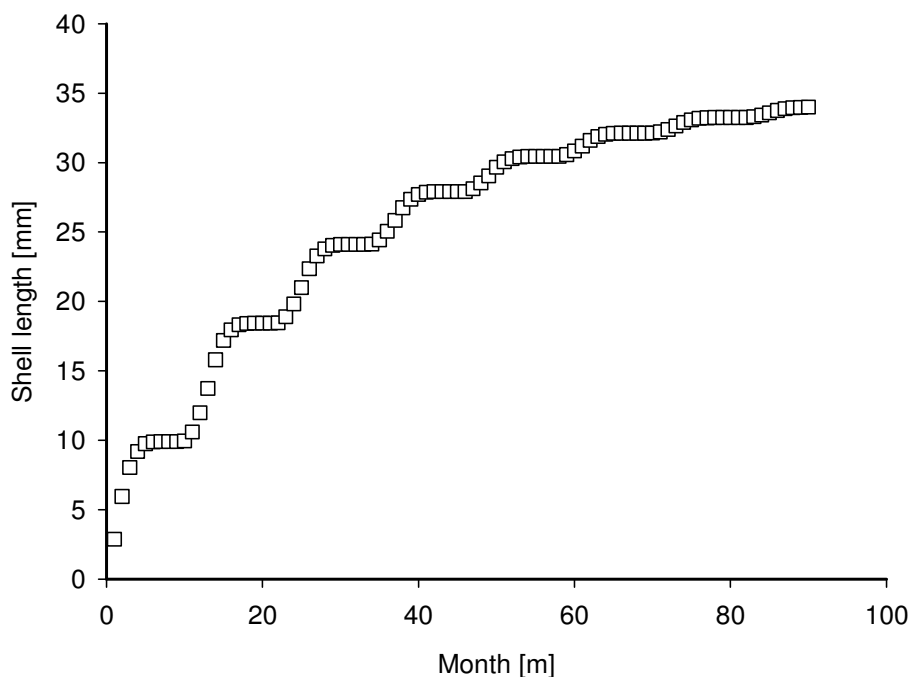


Figure 7: Growth modelling of *C. fluminea* with the determined data using the von Bertalanffy equation. Period = 7.5 years.

The results of growth modelling using the von Bertalanffy growth equation are shown in Figure 7. A period of 7.5 years, beginning with a juvenile *C. fluminea* directly after the release from the adult mussel (Shell-size = 0.25mm), has been modelled, resulting in a shell length of 34 mm (after 90 months of growth). The theoretical average maximum shell-size, as calculated from the monthly values of L^∞ , (see Table 1) was 35.6 mm. Following Taylor (1958), the maximum age can be calculated as the age when 95% of L^∞ is reached. Using formula (9), this results in a maximum age of 7.3 years with a shell length of 33.8 mm.

According to the model, individuals which spawned in May with a shell length of 0.25 mm reached a shell size of 12.0 mm after the first, 19.8 mm after the second and 25.1 mm after the third year. The yearly shell length increase is reduced yearly by 33 per cent.

Discussion

This study clearly demonstrates decreasing shell growth with increasing shell length, as reported for *C. fluminea* and other freshwater bivalves by other authors in past research (Meister 1997, Schmidlin 2007, Haag and Commens-Carson 2008). For both temperature and chlorophyll *a* concentration a significant positive correlation to the seasonal shell length increase could be demonstrated.

Although water temperatures from August to October 2009 were similar to temperatures from May to June 2010 (11.3°C-23.5°C compared to 13.8°C-22.6°C), the shell growth for all size classes was lower. Shell growth was $59.8 \pm 5.8 \mu\text{m d}^{-1}$ for the 5-10 mm size class, $46 \pm 8.1 \mu\text{m d}^{-1}$ for the 15-20 mm size class and $30.1 \pm 11.4 \mu\text{m d}^{-1}$ for the 25-30 mm size class in average from May to June 2010 compared to $42.7 \pm 7.2 \mu\text{m d}^{-1}$ for the 5-10 mm size class, $18.7 \pm 7.6 \mu\text{m d}^{-1}$ for the 15-20mm size class and $2.75 \pm 1.8 \mu\text{m d}^{-1}$ for the 25-30mm size class from August to October 2009. According to Foe & Knight (1985) *C. fluminea* is food-limited at a chlorophyll *a* content of 10-13 $\mu\text{g l}^{-1}$. The chlorophyll *a* content from August to October 2009 peaked at 3 $\mu\text{g l}^{-1}$ (average = 1.5 $\mu\text{g l}^{-1}$), while it peaked at 14 $\mu\text{g l}^{-1}$ (average = 6.1 $\mu\text{g l}^{-1}$) from May to June. The chlorophyll *a* concentration was generally low in the experimental period even when compared to other studies at the same stretch of the River Rhine (Vohmann et al. 2010, Weitere et al. 2009). In both periods, food limitation can be assured. However, in the period from August to October 2009 the limitation was much stronger. Obviously, larger clams suffer stronger from these environmental conditions than smaller ones; the reason for that is the higher food demand which results in a reduced shell length increase compared to large mussels from the period in 2010.

The shell length provides only limited information about the body mass of an individual because these two parameters are controlled differentially (Weitere et al. 2009). Although we did not examine the body mass of the individuals, we know from other studies (Weitere et al. 2009; Vohmann et al. 2010), that the body mass of *C. fluminea* reaches its maximum in early summer and then continuously declines till the beginning of the next growing season of the following year. This might also be an explanation for the higher shell length increase in early summer, since the bivalves

should have more energy to build their shell when they have a higher body mass. However, there might also be other factors that we did not investigate, like spawning activity (and a corresponding higher food demand, compare chapter 3), influencing the shell growth.

According to the literature, the lower threshold temperature for growth is between 9°C and 15°C (Hall, 1984; Mattice & Wright, 1986; McMahon & Williams, 1986a; French & Schloesser, 1991; Morgan et al., 2003; Weitere, 2009). In our study there was almost no growth from December till the end of March, suggesting a threshold temperature of 10°C which corresponds partially with the findings of other European studies. Weitere et al. (2009) for example observed no growth between December and March, but the start of growth correlated with temperatures of approximately 14°C. Meister (1997) observed no growth from January to March and shell growth was initiated at temperatures of approximately 10°C.

The modelling revealed a size of 12.0 mm after the first, 19.8 mm after the second and 25.1 mm after the third year. Literature data shows a broad variability of shell length after the first three years: shell length varies from 8-29 mm after the first, 14 - 36 mm after the second and 19-46 mm after the third year. Studies with comparable temperature and/or chlorophyll *a* conditions (Dubois 1995, Meister 1997, Schmidlin 2007), however, show shell sizes of 8-13 mm after the first, 14-20 mm after the second and 19-24 mm after the third year, thus corresponding to our findings.

The data can be used to estimate the age of an individual at any given shell length. Age determination of *C. fluminea* is a difficult task, because the shell rings do not reflect the age of the individual, in contrast to many other bivalves. Therefore, this approach provides an alternative method to the common cohort, or size/frequency analysis, which is frequently used to determine the age of *C. fluminea* (e.g., Ituarte 1985, Caffrey et al. 2010). However, age determination with the help of the data from the present study is only possible for *C. fluminea* originating from rivers with similar environmental conditions regarding the Chlorophyll *a* content and the temperature. The calculated theoretical maximum age for *C. fluminea* in this study was 7.3 years. North American studies often specify the maximum age to be between 2 and 4 years, thus a much lower figure than our estimates. However, European studies (Dubois

1995, Meister 1997) report maximum ages of 39-60 months. The calculated maximum age is a modelled value, and according to the model the bivalves have to achieve a shell length of 33.8 mm at that age. Individuals that become larger than 33 mm are very rare in the River Rhine. In the present study only one individual with a shell size larger than 33 mm was found at the sample site in the River Rhine. The largest individual found by Meister (1997) in the same river was only 32 mm long. If we assume that the average individual reaches a maximum shell length of 30-32 mm, this figure would correspond to an age of 4-6 years, which is in line with the findings of other European authors, as stated above. Using the von Bertalanffy equation with data derived from one year of observation may be defective, but the environmental conditions during the experimental period did not show exceptional values for temperature and chlorophyll *a*, suggesting a regular growth pattern of *C. fluminea* as well. Nevertheless, there might be additional factors despite temperature and chlorophyll that have not yet been considered, but that might have led to an unusual good or bad period of growth in our studies.

Conclusion

The common view that shell growth is reduced with increasing shell size could be supported by this study. Additionally it could be shown that both temperature and chlorophyll *a*-concentration have a strong impact on shell growth, with larger mussels being much more affected by low food availability than smaller ones.

Our data can serve as a basis that provides fundamental ecological information when modelling the ecological impact of *C. fluminea*. It also enables to perform an age estimation at any given shell length, without having to analyze cohorts or size/frequency distributions of *C. fluminea* in the River Rhine. Further studies are necessary to investigate the growth pattern of *C. fluminea* under different environmental conditions.

Concluding discussion

All parts of this study centered on the invasive bivalves *Corbicula fluminea* and *Dreissena polymorpha*. Both are common and well studied invasive species, that can have a large impact on planktonic community composition and the flux of matter in ecosystems. The Ponto-Caspian species *D. polymorpha* invaded Western Europe in the 19th century (Kinzelbach 1992) and North America in the 1980s. *C. fluminea*, originating from Southeast Asia, is abundant in many riverine systems and reservoirs in Africa, Europe, North and South America and in the Pacific islands (Phelps 1994, Rajagopal et al. 2000; Darrigran 2002; Karatyev et al. 2005). Both species have a disproportionately large effect on structuring the composition and functioning of ecosystems in relation to their biomass and can therefore be called keystone species (Paine 1969). However, their ecological impact under global change scenarios is not well understood. Furthermore, studies considering whole annual cycles are still rare. When determining the effects of an invasive bivalve species, two of the most important components are grazing activity and population dynamics, as well as the factors influencing these components. One of the most important factors, that not only affects the grazing activity of freshwater bivalves but also the growth dynamics of their planktonic prey community, is temperature. In this century we will face an increase of the global surface temperature between 1.7°C and 4.0°C (IPCC 2007). The most likely climate changes in central Europe are milder winters, as well as warmer summers with an increasing frequency and intensity of heat waves like in the summer period of 2003. Because temperature affects nearly all biological processes it is difficult to predict the influence of climate change on biological communities and the functioning of ecosystems.

For example, increasing temperature can generate significant changes in a freshwater community structure, when the effects on the predation rate of freshwater bivalves and the growth rate of their prey community develop asynchronously.

This thesis presents new aspects about factors that control the filtration activity of *D. polymorpha* and *C. fluminea* and contributes to the understanding of the ecological impact of freshwater bivalves in changing environments.

This was done with a combination of laboratory and field related experimental approaches. In a first experimental series the temperature at which an unparallel development of the growth rate of a microbial prey community and the grazing rate of a macrofaunal consumer was identified in short-term experiments. In the second experimental series the effects of high temperatures on different trophic levels (Bivalves - heterotrophic flagellates - bacteria) was investigated.

After examining the influence of temperature in short-term experiments, seasonal and inter-annual patterns of *C. flumineas* grazing activity as well as factors influencing these patterns were identified. Additionally the seasonal population dynamics of *C. fluminea* were investigated to provide a basis for future predictions and modelling approaches.

The first part of the study concentrated on the impact of warming on the grazing rates of *C. fluminea* and *D. polymorpha* and the growth rates of their natural prey organisms such as heterotrophic flagellates. This was done by performing short-term grazing experiments in circulating flow channels to provide a natural current by using untreated water from a large river (River Rhine, NRW, Germany). It was shown that the increase of the prey's growth rates was much stronger than that of the predator's grazing rates when temperatures were increased from 19°C to over 25°C. When performing the same experiments using a benthic microbial predator community (biofilms dominated by suspension-feeding ciliates), an increase of the grazing rates relative to the growth rates with temperature could be observed. This pattern is basically the result of a slight increase in the grazing rate parallel to a slight decrease in the growth rate with increasing temperature.

The data shows, that a predator-prey interaction between the macrofauna (Bivalves) and their unicellular prey community can change significantly under the impact of increasing temperature when consumption and growth rates develop differentially. This can lead to a netto-decrease of the prey abundance. Our central hypothesis that extreme summer temperatures can result in a pronounced unparallel development of the growth rate of a microbial prey community and the grazing rate of a macrofaunal consumer was generally confirmed. It is likely that these effects not only occur in aquatic but also in terrestrial ecosystems. However, the experiments also showed that the extent of the unparallel development between grazing and growth rates with high

temperatures greatly depends on the resource availability for the microbes. The HF growth rate did not change between 25 and 30°C without the addition of a carbon resource. Nevertheless, even though high temperature did not affect the HF growth in the second setup, an unparallel development was still observable because temperature had a strong negative effect on the mussel grazing. Overall the first part of this study provided the basis for the next experiments by identifying the temperature range at which the examined predator-prey interaction becomes unbalanced.

In the second part of this study the effect of increasing temperatures on different trophic levels was examined. Grazing experiments with *D. polymorpha* were performed with algae and heterotrophic flagellates as prey organisms of the bivalves and bacteria as the preferred prey organisms of the heterotrophic flagellates. Again it was demonstrated that an increase in temperature had a significant negative effect on the ingestion rates of *D. polymorpha*. The general trend of decreasing ingestion rate with increasing temperature was confirmed for a northern and a southern invasion line as well as for bivalves that had been acclimatised to high temperatures. Furthermore it could be shown that the bacterial abundance decreases at high temperatures. This finding indicates that processes other than direct grazing of the bivalves act on the bacteria. This result stands in contrast to other findings from laboratory experiments which demonstrate the direct grazing of bacteria by *D. polymorpha* (Silverman et al. 1995). Reasons for these different results are probably the larger sizes of bacteria grown under laboratory conditions compared to the smaller ones in the field. The stimulation of bacteria with increasing temperature can be explained either by growth stimulation or by a decreasing grazing pressure. It has been shown that *D. polymorpha* increases its metabolic activity and excretion of waste products up to a temperature of 32°C (Aldridge et al. 1995). The stimulation of bacteria by the excretion of waste products from *D. polymorpha* therefore should increase with increasing temperature which stands in contrast to our findings. Hence, it is more likely that the stimulation of bacterial abundance is due to a reduction of the mortality rate of the bacteria with increasing grazing pressure by the bivalves. The data suggests a trophic cascade (bivalves - flagellates - bacteria) where the grazing

pressure on the bacteria increases with increasing temperatures because the abundance of the flagellates increases with decreasing grazing activity of the bivalves. The important finding is that temperature not only alters a predator-prey interaction, but also has indirect effects on a third group on a different trophic level. According to IPCC (2007) a possible future scenario is that summer heatwaves are more likely to occur. The data shows that these summer heatwaves can 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. This drift in the matter flux might also affect other organisms on different trophic levels. Such functional aspects need further attention in future studies.

After examining the impact of increased temperature on the grazing activity of freshwater bivalves the next study analyses the intra- and interannual variability of the grazing activity. Although *C. fluminea* is a well analysed organism, only little is known about factors controlling their grazing activity besides direct temperature effects, body mass or food availability. To identify other important factors, long-term grazing experiments with *C. fluminea* were performed over a period of two years. The grazing experiments revealed strong seasonal variations, such as a 50-fold increase from February to July. These variations were only poorly linked to temperature, as they could be found at both the ambient field temperature and a constant temperature of 15°C. Interestingly, the periods of highest grazing activity correlated with the periods of highest reproduction activity. The important finding here is, that endogenous regulation of life history events like reproduction was able to surpass direct temperature effects on the grazing activity of *C. fluminea*. Furthermore it could be shown that external factors like cold winter periods that can restrain *C. fluminea* from initiating the reproduction process in the ongoing reproduction period can lead to a strongly reduced grazing activity. The pattern of clearance rates further suggest that *C. fluminea* recovered during the following spring/summer period and showed regular grazing activity during the second period of reproduction in late summer/autumn. The study demonstrates that other factors besides the direct and indirect temperature effects that were demonstrated in short-term experiments in chapter 1 and 2 can strongly affect the grazing activity of freshwater bivalves. These intra- and interannual variability is often overlooked because the patterns cannot be

found in short-term experiments. However, factors that can surpass direct temperature effects have to be considered to make meaningful and reliable predictions of the ecological impact of changing temperatures on filter-feeding freshwater bivalves like *D. polymorpha* and *C. fluminea*.

After identifying and examining factors controlling the grazing activity of freshwater bivalves, basic population parameters of the most common freshwater bivalve in the River Rhine, *C. fluminea*, were investigated. The shell growth of more than 50 individuals was observed over a period of more than one year. The initial shell length of the bivalves ranged from 5 to 34 mm. It could be demonstrated that the shell growth is reduced with increasing shell size and that shell growth is strongly correlated with both temperature and chlorophyll *a*. The data was used to generate growth curves over a period of several years with the help of a von Bertalanffy growth model. Additionally the model was used to estimate the age at a given length, the maximum age (7.3 years) and the maximum shell length of *C. fluminea* in the River Rhine. The data provides a basis for modeling the ecological impact of *C. fluminea* in the River Rhine. However, further studies under different environmental conditions are necessary because the data cannot be transferred to other ecosystems with different environmental conditions.

Taken together, the different aspects of the present work demonstrate the complexity of an organisms response towards the environmental conditions. In short-term experiments it could be shown that moderate warming can have a large impact on the grazing activity and that direct and indirect effects of this temperature impact can lead to significant changes on interactions on different trophic levels. However, short-term experiments alone do not provide data that is qualified for ecological modeling because there are other factors besides temperature that can have a large impact on clearance rates of freshwater bivalves. This could be demonstrated in chapter 3. Long-term grazing experiments conducted over a period of two years, unveiled that the seasonal context is more important than previous studies suggest. Intra- and interannual variability of grazing activity that was linked to life history events (like spawning periods) and factors influencing this life history events (like cold winter periods) rather than direct temperature effects was identified. The different factors

controlling the grazing activity of freshwater bivalves need to be considered when trying to model their ecological impact under changing environmental conditions. The data shows that different approaches are needed when trying to model the impact of short-term events like extreme summer heatwaves or changing conditions on a larger time scale like gradual climate warming (IPCC 2007). Additionally it is important to develop local studies and growth models to gain knowledge about population dynamics and provide a basis for future modeling approaches. Some past results centering on the impact of global warming on predator-prey dynamics might have to be reconsidered because the seasonal context has often been unattended.

The outcome of the present dissertation is that research is needed on the following points:

1. Local studies have to be performed to investigate population dynamics of aquatic organisms (predator and prey organisms) under different environmental conditions.
2. The temperature response of aquatic organisms and predator-prey dynamics has to be investigated in short-term experiments.
3. Long-term research is needed to identify other factors that surpass or enhance direct temperature effects.

The different research fields have to be connected to build up detailed and reliable ecological models that can be used to predict the impact of changing temperatures on keystone species like the filter-feeding freshwater bivalves *D. polymorpha* and *C. fluminea* and their prey organisms.

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Zusammenfassung

In der vorliegenden Arbeit wurde die Steuerung der Filtrationsaktivität der Süßwassermuscheln *Corbicula fluminea* und *Dreissena polymorpha* sowie deren Konsequenzen auf aquatische Nahrungsnetze unter Klimaveränderungs-Szenarien untersucht. Dazu wurden Untersuchungen unter naturnahen Bedingungen (Rheinwasser-durchfluss) auf der Ökologischen Rheinstation der Universität zu Köln mit Laborversuchen unter kontrollierten Bedingungen kombiniert. Zusätzlich wurden grundlegende Daten zur Populationsdynamik von *C. fluminea* ermittelt.

Der erste Teil dieser Arbeit befasste sich mit dem Einfluss von Erwärmung auf *C. fluminea* und *D. polymorpha* sowie auf deren bevorzugte Nahrung (optimale Größe) in Form von heterotrophen Flagellaten. Hierzu wurden Filtrationsexperimente mit natürlichem Wasser aus einem großen Fluss (Rhein, NRW, Deutschland) in zirkulierenden Fließzylindern durchgeführt. Es zeigte sich, dass bei einer Temperaturerhöhung von 19°C auf über 25°C die Aufnahmeraten der Muscheln weit weniger stark anstiegen als die Wachstumsraten der einzelligen Beuteorganismen.

Eine mikrobielle Prädatoren-gemeinschaft (Biofilm) zeigte im Vergleich zu den Muscheln einen stärkeren Anstieg der Aufnahmeraten im Vergleich zu den Wachstumsraten der einzelligen Beuteorganismen. Die Daten demonstrieren, dass es bei Räuber-Beute-Beziehungen zwischen der Makrofauna (Muscheln) und einzelligen Beutegemeinschaften bei Temperaturänderungen zu einer Verschiebung von Aufnahme- und Wachstumsraten kommen kann. Dies kann schließlich zu einer Netto-Änderung der Beutedichten führen.

Im zweiten Teil dieser Arbeit wurde in Filtrationsexperimenten die Rolle von Makrograzern (*D. polymorpha*) bei der Übertragung von Temperatureffekten auf dominante Komponenten natürlichen Flussplanktons untersucht. Es zeigte sich, dass sowohl heterotrophe Flagellaten als auch Algen von den verringerten Aufnahmeraten der Muscheln bei erhöhten Temperaturen profitieren konnten. Die Abundanz der Bakterien (als bevorzugte Nahrungsquelle der Flagellaten) hingegen, wurde bei zunehmender Temperatur verringert. Dies lässt auf eine trophische Kaskade (Muscheln-Flagellaten-Bakterien) schließen, die durch den Einfluss von Temperatur auf die Aufnahmeraten der Muscheln verändert wird.

Im dritten Teil der vorliegenden Arbeit wurden Langzeitversuche zur Filtrationsleistung von *C. fluminea* durchgeführt. Dabei wurde sowohl die Variabilität der Filtrationsleistung innerhalb eines Jahres als auch die Variabilität zwischen

verschiedenen Jahren untersucht. Es zeigte sich eine starke saisonale Variabilität der Filtrationsleistung, die nicht allein mit dem Einfluss der Temperatur erklärt werden konnte, da sie sowohl bei der normalen Umgebungstemperatur als auch bei einer konstanten Temperatur von 15°C gemessen wurde. Der Zeitpunkt der höchsten Filtrationsleistung korrelierte dabei mit dem Zeitpunkt der höchsten Reproduktionsaktivität. Die Filtrationsleistung war dabei von März bis August 2009, nach einer zweiwöchigen Phase mit Temperaturen um 2°C im Januar 2009, im Vergleich zum selben Zeitraum im Jahr 2008 stark reduziert. Es konnte gezeigt werden, dass es neben direkten Temperatureffekten auch weitere Faktoren gibt, die einen starken Einfluss auf die Filtrationsleistung von Muscheln haben können. Insbesondere Ereignisse wie Reproduktionsphasen der Muscheln bzw. Faktoren die diese Reproduktionsphasen beeinflussen können (niedrige Temperaturen im Winter) zeigten dabei einen großen Einfluß auf die Filtrationsleistung.

Im letzten Teil dieser Arbeit wurde das Schalenwachstum von *C. fluminea* anhand von mehr als 50 Individuen untersucht. Es zeigte sich, dass das Schalenwachstum mit zunehmender Schalenlänge abnimmt. Die erhaltenen Daten wurden verwendet, um mit Hilfe der "von Bertalanffy Wachstums-Funktion" das Wachstumsverhalten von *C. fluminea* über mehrere Jahre zu modellieren. Zusätzlich konnten mit dem Modell Populationsparameter wie z. B. das Alter bei jeder beliebigen Länge, das maximale Alter (7,3 Jahre) und die maximale Schalenlänge (35,6 mm) von *C. fluminea* im Rhein geschätzt werden.

Zusammengefasst zeigen die hier vorgestellten Arbeiten, dass Temperaturerhöhung eine Räuber-Beute Interaktion zwischen Makro- und Mikrofauna verändern kann, indem sich Aufnahmeraten und Wachstumsraten mit steigender Temperatur asynchron entwickeln. Dieser Temperatureffekt kann sich in einer trophischen Kaskade (Muscheln-Flagellaten-Bakterien) auch auf mehrere trophische Ebenen fortsetzen. Zusätzlich konnte gezeigt werden, dass es neben dem Einfluss der Temperatur weitere Faktoren im saisonalen Kontext gibt, die direkte Temperatureffekte überlagern können. Im letzten Teil der vorliegenden Arbeit wurden grundlegende Daten zur Populationsdynamik von *C. fluminea* ermittelt. Die Aspekte dieser Studie können Vorhersagen zum Einfluss von Süßwassermuscheln auf Fließwasserökosysteme sowie zur Entwicklung der Muscheln verbessern.

Summary

In the present study factors affecting the filtration activity and grazing effects of the freshwater bivalves *Corbicula fluminea* and *Dreissena polymorpha* were investigated. This was done with a combination of laboratory and field related experimental approaches. Both bivalves represent keystone species, who can have a large impact on planktonic community composition and the flux of matter in ecosystems. Additionally the whole seasonal population dynamics of *C. fluminea* were investigated to provide a basis for future predictions and modelling approaches.

The first part of the present work concentrated on the impact of warming on the grazing rates of *C. fluminea* and *D. polymorpha* and their natural prey organisms such as heterotrophic flagellates. Therefore filtration experiments were performed in circulating flow channels to provide a natural current by using untreated water from a large river (River Rhine, NRW, Germany). It was shown that the increase of the prey's growth rates was much stronger than that of the predator's grazing rates when temperatures were increased from 19°C to over 25°C. When performing the same experiments using a benthic microbial predator community (biofilms dominated by suspension-feeding ciliates), an increase of the grazing rates relative to the growth rates with temperature could be observed. The data shows, that a predator-prey interaction between the macrofauna (Bivalves) and their unicellular prey community can change significantly under the impact of increasing temperature when consumption and growth rates develop differentially. This can lead to netto-decrease of the prey abundance.

The data suggests that the predator-prey interaction which is balanced at moderate temperatures can become unbalanced with increasing temperature.

In the second part of this work the role of *Dreissena polymorpha* in mediating effects of high summer temperatures on the dominant components of natural river plankton was investigated. It was shown that both heterotrophic flagellates and algae increase in abundance at temperatures above 20°C because of decreasing grazing activity of *D. polymorpha* at such temperatures. Bacteria, as the main prey of the heterotrophic flagellates, decreased in abundance with increasing temperature, suggesting a trophic cascade (mussel - flagellates - bacteria) that is altered by the temperature response of the mussel ingestion. The data thus demonstrates 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.

In the third part of the present work, seasonal and interannual variability of *C. flumineas* grazing activity was investigated. Strong seasonal variations, such as a 50-fold increase from February to July, were observed. These variations were only poorly linked to temperature, as they could be found at both the ambient field temperature and a constant temperature of 15°C. It was shown that highest grazing activity was found at periods of highest reproduction activity. Additionally it was shown that the grazing activity was very low from March to August 2009 compared to the same period in 2008 after the bivalves experienced a period of two weeks with low winter temperatures close to the lethal temperature of 2°C. Such low temperatures lead to reduced reproduction rates as shown in previous studies. It was demonstrated that other factors besides temperature probably associated to life-history can have a large impact on the grazing activity of bivalves.

The last part of this study concentrated on the shell growth of *C. fluminea*. The shell growth of more than 50 individuals was observed over a period of more than one year. It was confirmed, that shell growth decreases with increasing shell size. The data was used to generate growth curves over a period of several years with the help of a von Bertalanffy growth model. Additionally the model was used to estimate the age at a given length, the maximum age (7.3 years) and the maximum shell length of *C. fluminea* in the River Rhine.

Taken together, the present study demonstrated that temperature increase can alter a predator-prey interaction when grazing rates of the predators and growth rates of the prey develop asynchronously with increasing temperature. It was shown that warming can differentially influence organisms on different trophic levels by trophic cascading (mussels-flagellate-bacteria). Additionally, the long-term (whole season) studies showed that there are other factors besides temperature such as cold winter periods or reproduction activity that can surpass direct temperature effects. The last part of the study provided basic data about population dynamics of *C. fluminea*. Both the short-term grazing experiments and the long-term studies provide new patterns and mechanics, which are relevant to accurately predict the performance and the effects of invasive bivalves under changing environmental conditions.

Erklärung zur eigenen Beteiligung

Kapitel 1

Die Eigenleistung bei der Erstellung des zweiten Kapitels bestand in der federführenden Planung, Konzeption, Durchführung und Auswertung der Experimente mit *Corbicula fluminea* und *Dreissena polymorpha* (Experimente A-C, Table 1) sowie in der federführenden Konzeption und Anfertigung des Textes.

Kapitel 2

Die Eigenleistung bei der Erstellung des zweiten Kapitels bestand in der federführenden Planung, Konzeption, Durchführung und Auswertung der Experimente 3 und 4 sowie in der Beteiligung der Planung und Konzeption der Experimente 1 und 2. An der Konzeption und Verfassung des Kapitels war ich maßgeblich zusammen mit Markus Weitere beteiligt.

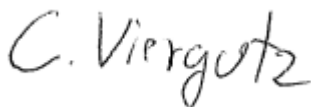
Kapitel 3

Die Eigenleistung bei der Erstellung des dritten Kapitels bestand in der federführenden Planung, Konzeption, und Auswertung aller Versuche. Die Versuche im Jahr 2008 sowie die Versuche von Juli bis Dezember 2009 wurden außerdem von mir durchgeführt. Die Konzeption und Verfassung des Kapitels wurde federführend von mir durchgeführt.

ERKLÄRUNG

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von den unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist, sowie, dass ich eine solche Veröffentlichungen vor Abschluss des Promotionsverfahren nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Hartmut Arndt und Prof. Dr. Markus Weitere betreut worden.

Köln, 15.09.2012



Carsten Viergutz

TEILPUBLIKATIONEN

Viergutz, C.R., Kathol, M., Norf, H., Arndt, H. and Weitere, M. (2007) Control of microbial communities by the macrofauna: a sensitive interaction in the context of extreme summer temperatures? *Oecologia*, **151**, 115-124.

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The final publication is available on link.springer.com.

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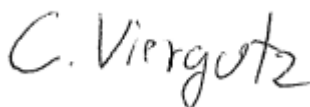
Curriculum Vitae

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