Impact of warming on fitness, phenology and feeding activity of riverine benthic filter feeders

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General Introduction

The grazing of benthic filter feeders on planktonic organisms is an important interaction in freshwater ecosystems in that it has consequences on ecosystem function by controlling the plankton communities and affecting plankton composition. It can have ecosystem-wide consequences by influencing the water transparency and therewith the growth of macrophytes. Furthermore, benthic filter feeders import the planktonic primary and secondary production into the benthic food web. (Welker & Walz, 1998; Strayer et al., 1999; Jack & Thorp, 2000; Weitere & Arndt 2002; Ricciardi, 2003).

The benthic grazer community is diverse. Filter-feeding insect larvae, biofilm communities, sponges, bryozoans and mussels can be abundant in freshwater ecosystems. Their composition depends on the ecosystem and the prevalent conditions. Community composition, function and activity is very sensitive towards changes of the environmental factors. Temperature is one such important factor in that it influences the filtration activity of benthic filter feeders (Walz, 1987; Aldridge et al., 1995; Lei et al., 1996); the growth of planktonic organisms is also mainly controlled by temperature (Montagnes et al., 2003). A change in the temperature regime might thus lead to pronounced changes in plankton communities due to the differential development of grazing and growth with warming (cf. Viergutz et al., 2007; Weitere et al., 2008).

We are currently facing a global climate change due to the emission of greenhouse gases (IPCC, 2007). For central Europe, the strongest seasonal temperature increase is predicted for winter (IPCC, 2007), while the summers will probably experience irregularly occurring heat waves with increasing frequencies (Schär et al., 2004; Stott et al., 2004). An increase in the average global temperature of 0.7°C already occurred in the second half of the 19th century. Suppositions regarding the temperature increase scenarios for the 21st century are based on the assumption of an average global temperature increase of between 1.7°C and 4°C (IPCC, 2007).

Temperature has the a very strong influence on the metabolic rates of organisms (Gillooly et al., 2002; Savage et al., 2004). An increase in temperature, up to a certain limit, has consequences on the food uptake, and in the case of benthic consumers, leads to an increase in the grazing pressure on the plankton. Other possible responses of temperature changes may include changes in the timing of seasonal activities (Both & Visser 2001; Menzel et al., 2001) which may result in an unparallel shift of interspecific interactions between grazers and the plankton communities. In addition, warming can affect the fitness of the organisms. In this context it is important to understand the impact of temperature on benthic filter feeders to be able to draw conclusions regarding the consequences of increasing temperatures to aquatic ecosystem. For such studies it is logical to choose organisms which have been investigated well on the one hand and are important for the respective ecosystem on the other hand.

Mussels are efficient filter feeders and 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). The River Rhine is the largest river in central Europe and one of its most important waterways (Tittzer & Krebs, 1996). The canalization and the industrial use of rivers in the last century has caused drastic changes in the ecosystem structure and has resulted in consequences such as the destruction of natural habitats, higher flow velocities, organic and inorganic pollution as well as pollution in terms of heat emissions. Especially the pollution with industrial chemicals has affected the ecosystem in the second half of the last century and has considerably depleted the native fauna of the river (Ortmann, 2003).

In the last decades, a continuous improvement in water quality has stimulated faunal recovery. But the debilitated fauna has become sensitive towards invasion. The freshwater clam *Corbicula fluminea*, native to Southeast Asia, has successfully invaded freshwater habitats in Europe, Africa, North and South America and the Pacific islands (Phelps, 1994; Darrigran, 2002; Karatayev et al., 2005). The Rhine was invaded by *Corbicula fluminea* in the 1980s, probably by man-made vectors such as the ballast water of ships (Bij de Vaate & Greijdanus-Klaas, 1990; Bij de Vaate, 1991; Karatayev, 2007). Its ability to tolerate a wide range of environmental conditions, its high fecundity, brood care and young age of maturity are considered to be reasons for the successful spreading of *Corbicula fluminea*. Today, it is the most common mussel in the River Rhine (Rajagopal et al. 2000; McMahon 2002; Karatayev et al., 2003). Impacts on filter-feeding clams include strong decreases in phyto- and zooplankton abundances, nutrient releases and increases in bacterial abundances (Kryger & Riisgard, 1988; Strayer et al., 1999; Vaughn & Hakenkamp, 2001).

Corbicula fluminea has been well studied over the last decades, and some of these studies focussed on the impact that temperature has on the mussels' feeding behaviour (Viergutz et al., 2007). These authors found an increase in feeding rate with temperature increases of up to 25°C, but decreasing feeding rates above this temperature. We know from other studies on freshwater mussels that warm temperatures result in an increase of the metabolic rate, whereas the feeding rate cannot cope with the demand (Aldridge et al., 1995; Lei et al., 1996) which may alter the fitness of the mussels (Ortmann & Grieshaber, 2003).

The present study was stimulated by the observation that *Corbicula fluminea* can experience mass mortalities affecting all size classes during warm summers (for the Rhine see: Westermann & Wendling, 2003; Demuth et al., 2004; Kroner et al., 2004; own observations, for other rivers see: Cooper et al., 2005). Other mussels such as *Dreissena polymorpha* and *Corbicula fluminalis* as well as other species of macrozoobenthos did not show a corresponding mortality in the River Rhine. Temperature as the only reason for the mass mortality was ruled out, since *Corbicula fluminea* originates from warmer areas and tolerates temperatures of up to 37°C (Mattice & Dye, 1976). We wanted to find out whether or not high energetic costs at high temperatures and simultaneous low plankton concentrations in the River Rhine during summer (Weitere et al., 2005) lead to a negative energetic balance of *Corbicula fluminea.* In winter, an increase in temperature seems to have the opposite impact on *Corbicula fluminea*; this observation was supported by the finding that an exposure to temperatures of between 1°C and 2°C correlates with increased mortality (Mattice & Dye, 1976; Rodgers et al., 1977; Morgan et al., 2003). These findings led to the hypothesis that summer versus winter warming results in contrasting effects on fitness parameters of *Corbicula fluminea.*

As mentioned above, mussels were often chosen as model organisms for analysing the impact of benthic filter feeders and their responses to changing environmental conditions. However, mussels are only one (albeit important) representative of the benthic filter-feeder community (Monakov, 2003). Another aim of the present study was to introduce two new species into the debate on the influence of warming on benthic filter feeders. The focus was to determine whether or not the results found for *Corbicula fluminea* can be applied to other groups of filter feeders to form the basis of prediction models for forecasting the consequences of global change. Sponges and bryozoans are widely distributed, common filter feeders (Okamura & Doolan, 1993; De Santo & Fell, 1996; Pile et al., 1997) which have often been overlooked when analysing the filtration impact of benthic grazers, so there are relatively few studies available on the grazing behaviour of these two freshwater taxa. We used *Epydatia muelleri* and *Plumatella emarginata* as common representatives of freshwater sponges and bryozoans respectively (Ricciardi & Reiswig, 1994; Økland & Økland, 1996; Wood et al., 1998; Gugel, 2000; Økland & Økland, 2001; Fesl et al., 2005; Dröscher & Waringer, 2007), and analysed their grazing rates under experimental warming to determine in what way temperature increase affects their feeding.

Both taxa form resting stages and spend the winter months in diapauses (Pronzato et al., 1993; Wood & Okamura, 2005); both are seasonal activities which may be sensitive to temperature changes (Black & Proud, 1976; Weissenfels, 1989; Gugel, 2000). We observed the phasing out of trophic stages in the fall and the winter months until the germination of the sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* in spring under experimental warming by 3°C above the ambient Rhine temperature. The hypothesis was that temperature affects the timing of these seasonal activities, resulting in a later phasing out in autumn or/and an earlier germination in spring.

Chapter 1 deals with the hypothesis that the food quantity (under natural conditions) affects the body mass and the shell length increase of *Corbicula fluminea* during summer. The mussels were kept in glass tanks which were filled with a 3 cm sand and were connected to a river bypass. In one experiment, the food concentration was manipulated by using different flow velocities of the fresh Rhine water. The hypothesis of a negative energetic balance of *Corbicula fluminea* in summer due to high energetic costs and low plankton concentrations was tested by measuring the body mass and the shell length increase.

Chapter 2 focussed on the contrasting effects of summer versus winter warming, which included experimental temperature elevations. In a summer experiment, the temperature was elevated to 3°C above ambient Rhine temperature, and a food supplementation was achieved by using an additional food source. This experiment was designed to reveal the influence of food and temperature levels on *Corbicula fluminea*.

In a winter experiment, the mussels from the summer with their different summer temperature histories were used and divided crosswise into ambient and warmed temperature treatments. For both experiments the body mass as well as the shell length development was analysed as a measure of the fitness, and the fecundity at the end of the experiment in spring 2006 was detected by analysing the incubation rate (number of mussels with incubated larvae as a percentage of all mussels) and the absolute number of larvae. The influence of winter warming and the role of the temperature history of the previous summer on the fitness and fecundity of *Corbicula fluminea* in spring was considered when planning this experiment.

Chapter 3: The results of the earlier experiments on *Corbicula fluminea* (chapters 1, 2) revealed contrasting effects of temperature between summer and winter. Summer warming resulted in a decrease of the body mass and an increase of the mortality rate, whereas winter warming led to a body mass increase and a positive impact on fecundity in spring. The experiment described in chapter 3 was arranged to (i) detect the time at which the temperature impact on *Corbicula fluminea* became negative towards the summer and (ii) to better understand the connection between the roles of temperature and food quantity.

We thus lengthened the experimental period and started the experiment in April 2007, just at that time of year when the winter experiment described in chapter 2 was terminated and the positive impact of temperature was still present. A high frequency of shell length measurements gave a detailed picture of growth of *Corbicula fluminea* during summer. Five measurements of body mass during summer revealed the development of body mass between April and October and enabled the determination of periods with increasing and decreasing body mass values. The analysis of respiration rates enabled the interpretation of the fitness data sampled by this study in comparison to the results on feeding rate reassessments of *Corbicula fluminea* reported by Viergutz et al. (2007).

In order to see if the mussels' response to warming is representative for all filter feeders, other benthic filter feeders of which little is known regarding their interactions with plankton communities as well as their response to temperature changes were studied and the results given in chapter 4. Suitable set ups were required to permit the study the sponge *Ephydatia muelleri* and the bryozoan *Plumatella emarginata*. Compared to mussels, it is more difficult to divide these taxa into defined units. Furthermore, the determination of the species is only possible by taking the resting stages into account. Different types of flow cells were chosen to measure clearance rates of the bryozoan and the sponge. Feeding rates were determined by connecting the flow cells to the river bypass and exposing them in temperature-controlled water tanks. Measurements of the food concentration from the inflow and the outflow formed the bases for the calculation of clearance rates. The timing of phonology events (e.g. forming of resting stages in autumn and re-colonisation in spring) was analysed with help of winter experiments in bypass systems under temperature manipulation by 3°C above the ambient river temperature.

Chapter 1

Strong body mass decrease of the invasive clam *Corbicula fluminea* during summer

Abstract

The filter-feeding clam *Corbicula fluminea* has widely spread from its Asiatic origin into freshwater habitats on several continents, where it often has a considerable impact on ecosystem processes. The present study was stimulated by the observation that *Corbicula fluminea* can experience mass mortality during warm summers, even when temperatures are still far below the lethal level. We hypothesised that starvation due to low food quantities during summer is a main factor in this context. In order to test nutritional conditions in an environment where summer mortality occurred, the clams´ body mass was tracked in river bypass systems installed at the Lower River Rhine (Germany and The Netherlands). Two food levels were adjusted in the bypass channels: one corresponding to the original chlorophyll level in the river (ambient food) and one with a chlorophyll *a* level reduced by about 50% (low food). The clams kept at the ambient food level increased their shell length during summer, although growth rates decreased at low food levels in the River Rhine in late summer. In comparison to shell length, body mass decrease in late summer cumulated in 94% reduction from August until October. This trend was enhanced by the experimental food reduction, i.e. clams kept in the low food level treatments weighed 60% less than the clams in the ambient food treatment at the end of summer. However, mortality was low in both treatments. The data demonstrate a high plasticity in the body mass of *Corbicula fluminea*, which helps to survive during starvation periods. Nevertheless, the extremely low body masses at the end of summer also suggest that further increases in the starvation stress in years with lower phytoplankton biomasses or higher energetic demands at high temperatures could lead to mass mortality events.

Introduction

The bivalve *Corbicula fluminea*, native to Southeast Asia, has successfully invaded freshwater habitats in Europe, Africa, North and South America and the Pacific islands (Phelps, 1994; Darrigran, 2002; Karatayev et al., 2005). The River Rhine in central Europe, the study area of the present investigation, was invaded by *Corbicula fluminea* in the 1980s, probably via ballast water (Bij de Vaate & Greijdanus-Klaas 1990; Bij de Vaate, 1991; Karatayev, 2007).

Properties such as the ability to tolerate a wide range of environmental conditions, a high fecundity, brood care and a young maturity age are considered to be important reasons for the successful spread of the species (Rajagopal et al., 2000; McMahon, 2002; Karatayev et al., 2003).

Once local populations are established, *Corbicula fluminea* can attain high densities (Cataldo & Boltovskoy, 1998; Schöll, 2000; Morgan et al., 2003). Dense populations of filter-feeding clams can have a considerable impact on the structure of planktonic communities and correspondingly on ecosystem processes (Strayer et al. 1999; Ricciardi 2003). Impacts include strong decreases in phyto- and zooplankton abundances, nutrient releases and increases in bacterial abundances (Kryger & Riisgard, 1988; Strayer et al., 1999; Vaughn & Hakenkamp, 2001). For *Corbicula fluminea* in particular, considerable decreases in the phytoplankton densities have been observed in response to its invasion, resulting in secondary and tertiary effects, e.g. increase of water transparency, followed by the establishment of macrophytes (Cohen et al., 1984; Phelps, 1994; Caraco et al., 2006).

Given the wide distribution of *Corbicula fluminea* as well as its potentially strong impact on ecosystem processes, there is great interest in factors which hinder the spread of the clam. In the past years, a high mortality of *Corbicula fluminea* has occurred in the summer months in the River Rhine affecting all size classes (Westermann & Wendling, 2003; own observations). Such rapids die-offs under conditions of warm summer temperatures were also observed in other rivers (Cooper et al., 2005). Other mussels such as *Dreissena polymorpha* and *C. fluminalis* as well as other species of macrozoobenthos did not show a corresponding mortality in the River Rhine. Reasons for the mass mortality in *Corbicula fluminea* are unknown. High water temperature alone does not seem to be a plausible explanation for this phenomenon; the clam originates from much warmer areas and can tolerate temperatures of up to 37°C (Mattice & Dye, 1976; McMahon & Williams, 1986), while maximum temperature in the Rhine did not exceed 28°C (http://www.lanuv.nrw.de/aktuelles/umwdat.htm). Lack of oxygen could be excluded as a reason for mortality (Johnson & McMahon, 1998), as oxygen concentrations at the time when most clams died were sufficiently high with values of 6-7 mg l^{-1} (Ortmann & Grieshaber, 2003; Westermann & Wendling, 2003).

Another reason for stress in the clams, which might cumulate in mortality, is low food quantity during summer. A positive correlation between chlorophyll *a* concentration and the growth rate of *Corbicula fluminea* was demonstrated in field studies (Cohen et al., 1984; Foe & Knight, 1985). In contrast to native inhabitants of the northern hemisphere, *Corbicula fluminea* is characterized by high growth rates and fecundity for rapid population recovery; the disadvantages for the species are high metabolic rates and correspondingly high food demands (McMahon, 2002). According to Foe & Knight (1985), food limitation for *Corbicula fluminea* occurs at a chlorophyll *a* concentration of below 20 µg l^{-1} in spring and below 47.3 µg l^{-1} in summer.

In the River Rhine, algae abundance decreased strongly after a spring peak, as expressed by low chlorophyll *a* concentrations below 10 µg l^{-1} in late summer (Weitere et al., 2005, see also Fig. 1). This low food level coincided with a high temperature and a correspondingly high energy demand of the clams (McMahon, 2002; Ortmann & Grieshaber, 2003). Mouthon (2001) reported similarly low chlorophyll concentrations in the Saone River (France) between 1987 and 1999. The author found clear indications for starvation in *Corbicula fluminea* such as reduced growth and only a single instead of two annual reproductive periods.

The present case study for the Rhine is thus based on the hypothesis that *Corbicula fluminea* faces longer starvation periods during the summer, leading to stress as expressed by body mass decrease and mortality. To test this hypothesis, we constructed river bypass systems to study growth, condition and survival of *Corbicula fluminea* under both *in situ* and reduced food levels. The experimental systems were run at two stations situated in the lower Rhine.

Material and Methods

Study sites and general experimental set-up

In order to test the impact of food availability on growth and survival of *Corbicula fluminea* under natural conditions, we performed two experiments in a river bypass system. A similar bypass system had been used to test the effect of food limitation and food competition on filter feeding mayfly larvae at the Ecological Rhine Station of the University of Cologne (Kureck & Bieg, 2001), where the main experiment of this study was conducted. The laboratories of the station are located on a boat anchored in the vicinity of Cologne-Marienburg (Germany, Rhine-km 684.5, which refers to the distance from Lake Constance, the source of the non-alpine Rhine). This experiment lasted the entire summer; it was started on May 12, 2004 and terminated on October 13, 2004. A second experiment with the same set-up was run in the lower Rhine in a floating measuring station of the Institute for Inland Water Management and Waste Water Treatment (RIZA) in Lobith (The Netherlands, Rhine-km 863.0). This experiment started on May 19, 2004 and terminated on July 31, 2004 after it was confirmed that the same trends were observed as in the main experiment in Cologne. Both stations were permanently supplied with fresh Rhine water. Flat glass tanks (49.5cm x 35.5cm x 10cm) were used in the experiments as river bypass systems. Two different food conditions were established at both locations by adjusting different flow rates of Rhine water. In one treatment, a rate of 600 l h^{-1} (high flow rate) was used; this flow rate is high enough to maintain an almost equal chlorophyll *a* concentration in the tanks as found in the Rhine (Fig. 1). A lower flow rate of 60 l h^{-1} was used to maintain a chlorophyll *a* concentration of approximately 50% less than in the first treatment. The two food conditions are termed hereafter “high food level” and “low food level” respectively. Experimental tanks were randomly chosen for an exposure to high or low food levels. The tanks contained a sandy sediment layer of about 3 cm. Small aquarium air pumps (SERA UW-pump P400 reg.) were used to aerate the tanks. Thus, constant oxygen saturation and a constant current independent form the experimentally manipulated flow rates were provided. Each treatment was run in three replicates at each station. One extra tank per treatment (“replacement tanks”) was used to restock clams to maintain a density of 150 clams in each tank after sampling for body mass determinations (and in the case of mortality). At the start of the experiment, the shell length of the clams was 10-11 mm, with exactly the same size distribution in the different treatments. The density of 150 clams per vessel corresponded to

860 individuals per m^2, which is in accordance with observed densities in the Rhine (Schöll, 2000).

Organisms

Corbicula fluminea used in the experiment originated from a flow-channel, filled with a sand layer, in the Ecological Rhine Station in Cologne-Marienburg. The channel was suspended in the Rhine one year before the experiment started. Drifting larvae were able to colonize the channel during the main larval settlement in April/ May 2003 due to the constant water flow through the channel. Hence all clams used in the experiments were definitely from both the same origin and age cohort. In addition, they were kept under ambient Rhine water conditions before the experiment was started.

Measurement of clam growth

Measurements of the clam's shell length and body mass were conducted on May 25 (only shell length measurements on this date), June 22, July 20, August 17, September 13 and October 13, 2004. The shell lengths of all clams were measured with a digital calliper. For body mass determination (ash free dry weight, AFDW), between five and eight clams were randomly taken from each of the experimental tanks. The soft body of each clam was dried at 60°C for 48 hours. The dried soft body was weighed (dry weight) and then combusted for 15 hours at 500°C. The AFDW was calculated as the difference between the dry weight and the weight of the incombustible component of the soft body. A length-weight ratio was used as a proxy for the nutritional conditions (Sprung & Borcherding, 1991).

Background data

The chlorophyll *a* content, a proxy for the food concentration (Jantz & Neumann, 1998), was measured at two-week intervals at both locations in the inflowing Rhine water as well as in the outflow of the experimental tanks. Chlorophyll *a* content was measured by in-situ fluorescence using an Aquafluor^{TM} –fluorimeter (Turner Designs, USA). Clearance rates (CR, $l\ ind.^{-1}\ h^{-1}$) of the clams in the low food tanks were calculated from the flow rate (*f*) and the chlorophyll *a* concentrations in both the in- (C_i, $\mu g\ l^{-1}$) and outflow (C_o, $\mu g\ l^{-1}$)

(Hildreth & Crisp, 1976): $CR = f \times ((C_i - C_o) / C_o)$. Extremely low differences between in- and outflows did not allow CR calculations for the high food level tanks. Tanks without clams exposed under the same conditions as the experimental tanks in Cologne-Marienburg were used as control tanks in order to check whether differences of chlorophyll *a* content of the in- and outflowing water were affected by the experimental set-up. Two tailed *t*-test revealed significant differences between in- and outflowing water neither for the high flow rate ($p=0.76$) nor for the low flow rate tanks ($p=0.41$). This shows that no other significant losses of planktonic chlorophyll *a* were detectable apart from the clam filtration. The calculated clearance rates were used to estimate the ingestion rate (IR, µg Chl. ind^{-1} h^{-1}) of the clams. Ingestion rates were calculated by multiplying the CR with the chlorophyll *a* concentration in the experimental tanks (which corresponded to the concentrations in the outflow due to the well-mixed condition). Water temperature was recorded by calibrated data loggers (Ebi-85A, Ebro Electronic, Germany) at two hour intervals in the tanks at Cologne- Marienburg and was checked daily at Lobith.

Statistical analyses

Statistical analyses were performed using SPSS for Windows 15.0 software. Length-weight regressions were calculated in order to analyse the relative body mass of the clams. The relationship between body mass (AFDW) and shell length (SL) followed a power function of the general formula $AFDW = a \times SL^b$. This relationship was used to calculate the average AFDW of a standardized 17 mm clam for each sampling date, as well as the body mass of each clam. As a test for a potential dependence of the size-specific body mass on sampling date and food quantity, an analysis of covariance (ANCOVA) was performed with AFDW as the dependent variable, sampling date and food quantity as independent variables, and shell length as the covariate. The AFDW was log-transformed in order to achieve a linear relationship between AFDW and shell length.

Impacts of the sampling date (independent variable) and food quantity (independent variable) on the average clam biomass (dependent variable) within the experiment as well as their impact on the average shell length (dependent variable) were tested in two-factorial analyses of variance (ANOVAs).

Results

Conditions during the experiments and food uptake

The general situation at both locations was similar, but not identical. The water temperature was slightly lower at Cologne than at Lobith at the beginning of the experiment; the situation was reversed towards the end of the experiments (Fig. 1). The chlorophyll *a* content of the Rhine water was slightly higher at Lobith during the whole study. Nevertheless, the chlorophyll *a* concentration decreased strongly after the spring bloom in May at both locations (Fig. 1).

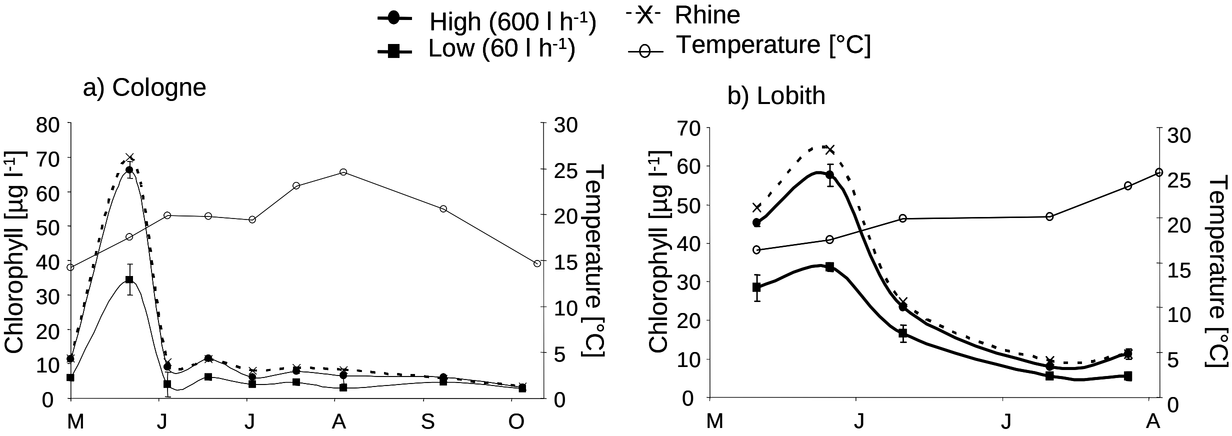

Fig. 1: Development of temperature and chlorophyll *a* concentration for both locations: (a) Cologne-Marienburg and (b) Lobith. The chlorophyll *a* concentrations are given for the outflow of both low and high food level treatments as well as for the corresponding Rhine concentrations (which is the same as the concentration in the inflow).

The chlorophyll *a* concentration in the mussel tanks was at the same level as the chlorophyll *a* concentration in the Rhine water for the high food level treatments. Since the mussels could not significantly reduce the food concentration, no clearance rates were calculated under these conditions. Food concentration (chlorophyll *a*) in the low food level treatment tanks was about 50% of that in the Rhine between May and August, i.e. between 2.9 and 34.4 µg l^{-1} compared to concentrations between 6.1 and 66.3 µg l^{-1} in the high food level treatments (Fig. 1). The relative differences between inflow and outflow were distinctly smaller in September and October. On the basis of the differences in the food concentrations between inflow and outflow, the clearance rates and ingestion rates were calculated for the low food treatments in Cologne (Fig. 2). The average clearance rate ranged between 364 and 745 ml ind^{-1} h^{-1} from May until August, and then decreased strongly to 94 and 111 ml ind^{-1} h^{-1} in September and October, respectively. The ingestion rate peaked at the end of May during the highest chlorophyll *a* concentration and decreased strongly afterwards (Fig. 2). The ingestion rates were lowest in September and October (at low chlorophyll *a* concentrations and low clearance rates).

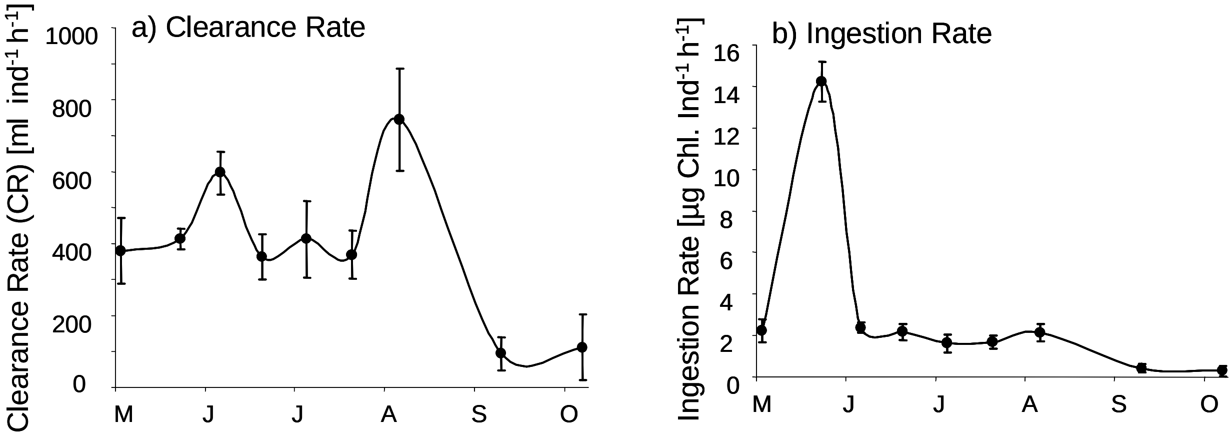

Fig. 2: Food uptake of *Corbicula fluminea* estimated in Cologne-Marienburg for the low food level treatment: (a) clearance rate, (b) ingestion rate.

Impact of food concentration on shell-growth

Shell length increase was high at the beginning of the experiment, i.e. between 0.089 and 0.115 mm d^{-1} in all treatments (Fig. 3 a, b). At that time the clams in the low food supply treatment grew as fast as the clams with a high food supply. From July to August, however, the shell length increase of the clams kept at low food concentrations decreased (to 0.007 mm d^{-1} in August), whereas the growth at high food levels was equal in July and August (approximately 0.07 mm d^{-1}). From August to October, the growth of the clams decreased gradually to levels below 0.01 mm d^{-1} in both treatments (Fig. 3 a, b). The ANOVA revealed highly significant effects of both the food concentration and sampling date on the shell length for both locations (Table 1). The significant interaction term (food x date) further supports the finding that the reduced food supply caused temporarily varying effects.

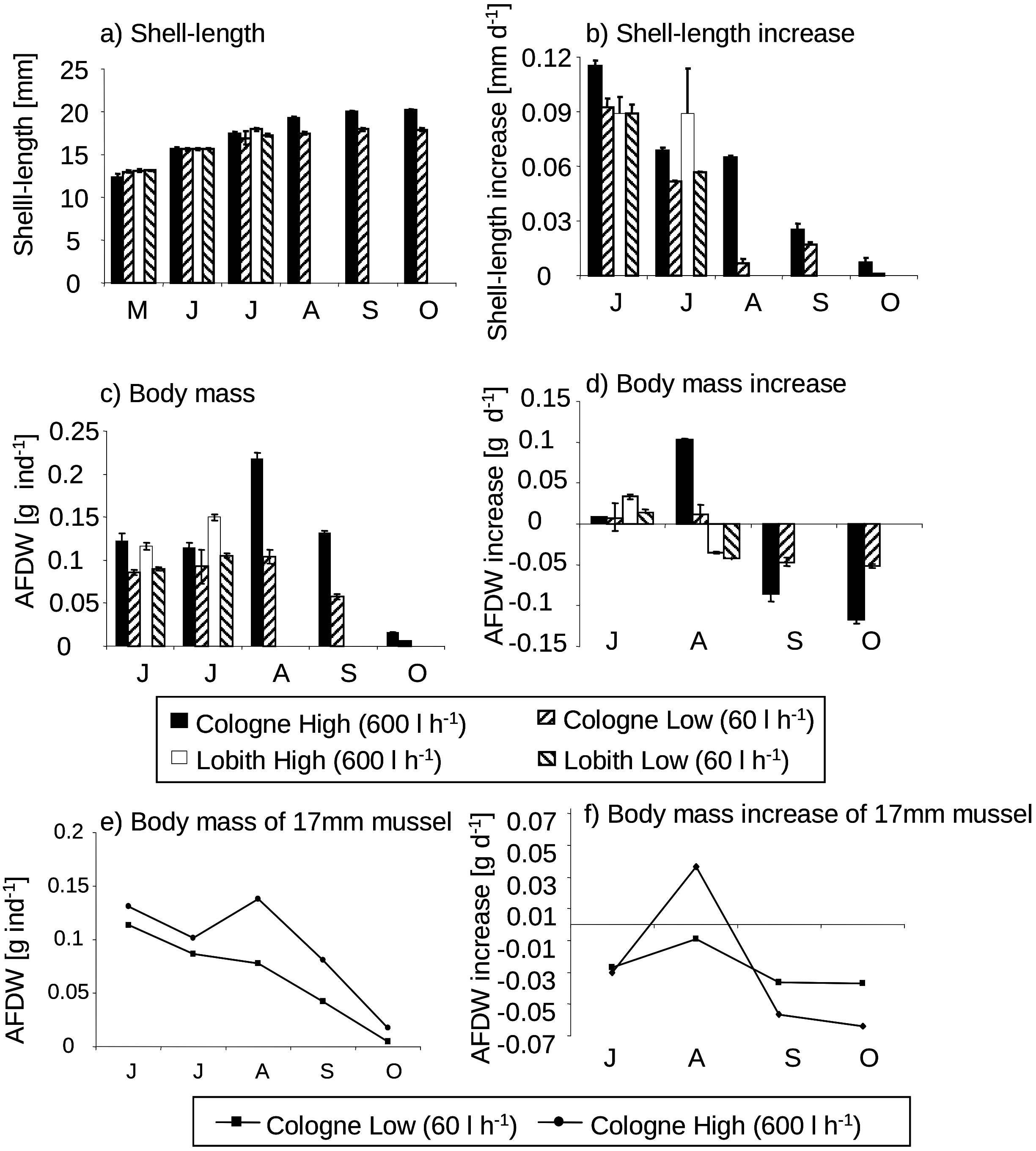

Fig. 3: Development of the shell length and body mass of *Corbicula fluminea* based on the monthly measurements for both locations: (a) average shell length, (b) shell length increase, calculated as differences between two samplings, (c) average body mass, (d) body mass increase calculated as differences between the two samplings, (e) calculated body mass of a standardized clam with 17mm shell length, (f) calculated body mass increase of a standardized clam with 17mm shell length.

Table 1: Analysis of variance (ANOVA) results for testing the effect of food quantity and time on the shell-length (SL) and body mass (BM) of *Corbicula fluminea* at Cologne-Marienburg and Lobith during the study period.

	SS	df	F	p
a) Cologne, dependent variable: SL				
Date	50.853	4	147.409	<0.001
Food	13.178	1	152.804	<0.001
date x food	6.629	4	19.215	<0.001
Error	1.725	20		
b) Cologne, dependent variable: BM				
Date	0.070	4	288.532	<0.001
Food	0.020	1	332.295	<0.001
date x food	0.011	4	44.741	<0.001
Error	0.001	20		
c) Lobith, dependent variable: SL				
Date	11.746	1	968.258	<0.001
Food	0.351	1	28.942	<0.001
date x food	0.540	1	44.473	<0.001
Error	0.097	8		
b) Lobith, dependent variable: BM				
Date	0.002	1	187.307	<0.001
Food	0.004	1	387.475	<0.001
date x food	0.0002	1	25.293	=0.001
Error	7.8×10^{-5}	8		

Impact of food concentration on body mass

The body mass (AFDW) of the clams which were kept at high food levels exceeded the weight of clams kept at low food concentrations on all dates and at both locations (Fig 3 c, d, Table 1). The clams lost soft body weight at Cologne during the whole study period except between July and August in the high food level treatment (Fig. 3 c-f, Table 1). The AFDW in October was only about 7% of the AFDW in August. In the low food level treatment, the AFDW in October was even 60% less than the AFDW in the high food level treatment. However, the clams showed significant shell length increases in both treatments (Fig. 3b). The size-specific body mass as assessed by the shell length – body mass regression of the clams is illustrated in Fig. 4. There was a clear distinction between the two food levels (flow rates) for all sampling dates and locations as statistically supported by highly significant effects of food on the relative body mass for all dates and for both locations in the ANCOVAs (shell length as covariate, Table 2). The data for Cologne show the strongest decrease in relative body mass. This is also illustrated by the body mass calculation for the standardized 17 mm clam (Fig. 3 e, f). The relative body mass per unit of shell length decreased distinctly stronger than the absolute body mass, particularly in the treatment with the lower amount of food between June and September. The similarly low body mass of clams from the experiment and those taken directly from the Rhine at Lobith in October 2004 (Fig. 4 h) showed that the experimental food manipulation mimicked the field conditions quite well.

Despite the strong body mass loss during summer in both the field and the experimental tanks, the mortality rate over the total time of the experiment did not exceed 12% in any replicate of the two treatments in Cologne (with the exception of one replicate of the high food level treatment which had a 19% mortality rate). There was no significant difference in mortality between the two food level treatments.

Table 2: Analysis of covariance (ANCOVA) results for testing the effect of food quantity on the shell-length specific (shell length (SL) as covariate) body mass (log(AFDW), dependent variable) of *Corbicula fluminea* for each month during the experiment and for both locations.

	SS	df	F	p
a) Cologne June				
SL	0.555	1	374.610	<0.001
Food	0.031	1	21.053	<0.001
Error	0.053	36		
b) Cologne July				
SL	1.125	1	321.562	<0.001
Food	0.073	1	20.965	<0.001
Error	0.164	47		
c) Cologne August				
SL	0.559	1	51.460	<0.001
Food	1.636	1	150.576	<0.001
Error	0.369	34		
d) Cologne September				
SL	0.610	1	39.057	<0.001
Food	0.268	1	17.189	<0.001
Error	0.578	37		
e) Cologne October				
SL	0.278	1	8.080	0.007
Food	0.340	1	11.351	0.002
Error	0.034	43		
e) Lobith June				
SL	0.319	1	238.468	<0.001
Food	0.133	1	99.579	<0.001
Error	0.045	34		
f) Lobith July				
SL	0.671	1	64.554	<0.001
Food	0.067	1	6.448	0.015
Error	0.436	42		

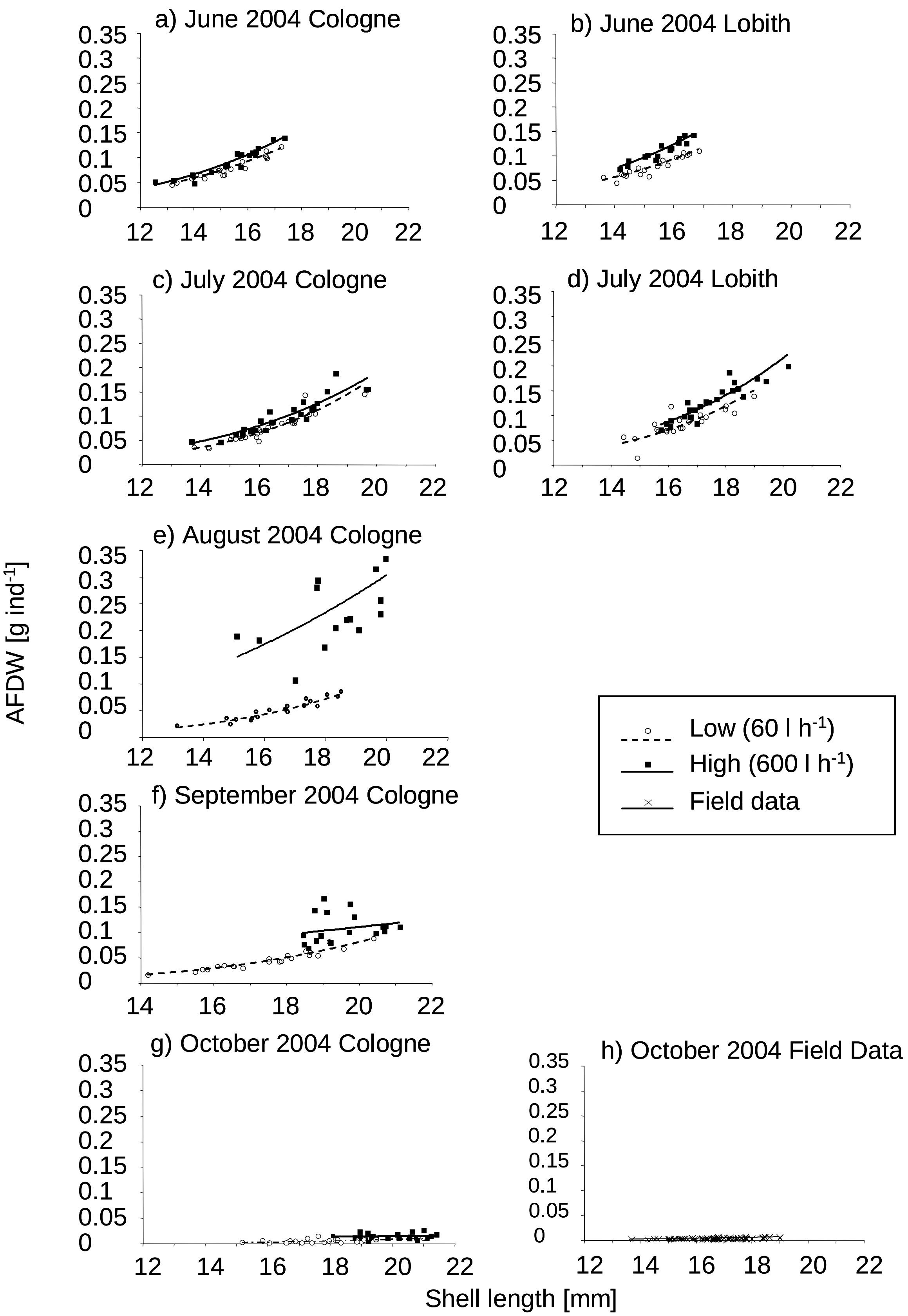

Fig. 4: Regression of body mass to shell length: (a-g) data of each of the monthly body mass measurement of the clams at both locations, (h) regression of body mass to shell length of clams taken from the River Rhine at Lobith in October 2004.

Discussion

The results revealed a high body mass plasticity of *Corbicula fluminea*, with a maximum average body mass decrease of 94% recorded from August (maximum body mass) to October in the high food level treatment. In October, the clams in the low food level treatment weighed distinctly (60%) less than the clams in the high food level treatment. Due to positive shell growth during the course of the summer, this decrease was even stronger regarding the relative body mass.

No mass mortality was observed in any of the treatments in 2004, a finding which was in contrast to the remarkably high mortality in the preceding summers. The large body mass plasticity seems to be one strategy for surviving starvation periods. In the following discussion, we first discuss the conditions during the investigation period 2004 (when no mass mortality in the Rhine occurred) in comparison to the conditions in 2003 (with mass mortality during summer) followed by a consideration of starvation effects.

Conditions during the investigation period

One hypothesis of the present study was that low food concentrations during summer causes starvation in *Corbicula fluminea*, resulting in increased mortality as observed in the Rhine in summers of 2002 (personal observations) and 2003 (Westermann & Wendling, 2003, personal observations). During the summers of 2002 and 2003 we also observed a considerable mortality in the Ecological Rhine Station at Cologne-Marienburg. In flumes with running Rhine water, 34% of the 1494 *Corbicula fluminea* collected on July 18th 2002 were dead, and all size classes were affected in equal measure. *C. fluminalis*, which had settled in the same flume, showed a mortality rate of only 1.9% (n=209).

The chlorophyll *a* concentrations in the Rhine during the present investigation in 2004 (Fig. 5) showed the typical pattern for the Rhine as found for the last years, with a pronounced spring peak and subsequently very low values (<10 µg l^{-1} chlorophyll *a* starting in June) over the course of the summer (Weitere et al. 2005). This concentration is expected to be too low to support optimal growth and reproduction of *Corbicula fluminea* (Foe & Knight, 1985; Rajagopal et al., 2000; Mouthon, 2001; Mouthon & Parghentanian, 2004). However, no mass mortality occurred in the Rhine in 2004. It is also remarkable that the experimentally reduced food concentration in the bypass systems did not lead to mass mortality. A comparison of the chlorophyll *a* concentrations in the Rhine during the investigation period in 2004 with values for the same months in 2003 revealed a higher rather than a lower chlorophyll *a* concentration during this year (Fig. 5). It is thus unlikely that food concentration is the only reason for the mass mortality observed in 2003.

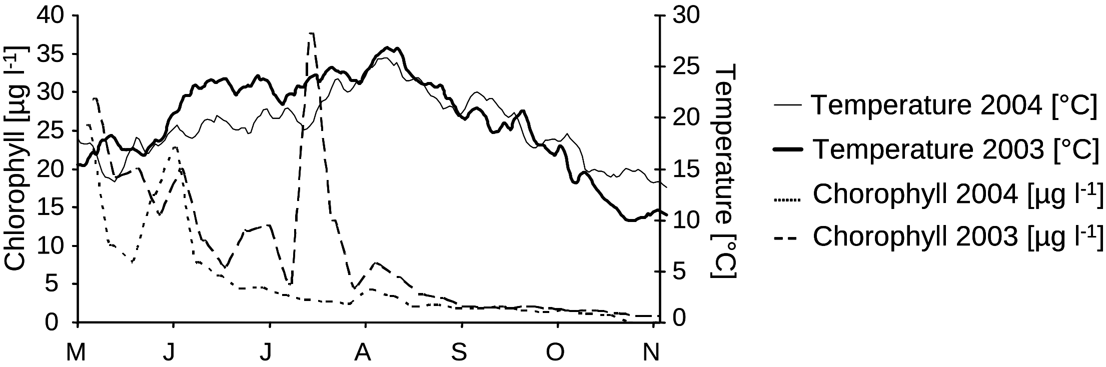

Fig. 5: Comparison of chlorophyll a concentrations in the Rhine at Bad Honnef (measurement station of the "Landesumweltamt NRW" near Cologne) and water temperature at Cologne-Marienburg in summer of the years 2003 and 2004.

There was a considerable difference in ambient water temperature in the Rhine between 2004 and 2003 (Fig. 5). Particularly in the early summer, i.e. before the onset of mass mortality in 2003, the temperature was distinctly higher in 2003 than in 2004. Water temperature in the Rhine exceeded 23°C for 1468 hours in 2003, but only for 638 hours in 2004. Mouthon and Daufresne (2006) reported that the high temperatures in 2003 led to a strong restructuring of the freshwater mollusc community in the River Saône. At least for the River Rhine, however, we reject the hypothesis that temperature was solely responsible for summer mortality in *Corbicula fluminea*. The lethal temperature for *Corbicula fluminea* is about 37°C (Mattice & Dye, 1976; McMahon & Williams, 1986), which is still about 10°C higher than the maximal temperature in the Rhine in 2003. Nevertheless, temperature enhances the metabolic rate and thus the food demand of ectotherms such as *Corbicula fluminea.* Possible interactions between low food level and high temperature are discussed below.

Shell length increase versus body mass decrease during starvation

A remarkable result of the present study was the dramatic reduction of body mass without mass mortality. Also remarkable was the long period over which a continuous decrease of the body mass occurred. After finishing the present study, we left *Corbicula fluminea* in the flumes with Rhine water discharge over winter and could confirm the survival of the clams without any significant mortality. Thus the large plasticity in the body mass and the apparent possibility to respire body mass can be seen as one strategy to survive longer starvation periods.

Both food demand and food supply have to be taken into account when evaluating nutritional conditions. Food supply was measured based on (i) the chlorophyll *a* concentration as well as on (ii) the clearance and (iii) ingestion rates, as estimated for the low food level treatment (Fig. 2). The clearance rate was relatively high between May and August and low in September and October. This drop cannot be solely explained by decreasing temperature during the end of summer (compare Viergutz et al., 2007), because the temperatures at that time were similar to those recorded in May and June, during which high clearance rates were measured. Thus the decreasing clearance rates during the end of summer are mainly due to other factors and possibly represent a result of the strong starvation. In fact, the decrease in the clearance rate (by 85% between August and October) occurred parallel to an even stronger decreased in the body mass (by 94% during the same time interval), which resulted in an increase in the body mass specific clearance rate. The ingestion rates (and thus the actual amount of food consumed by the clams) showed a three-step development during the investigation: (i) relatively high rates at high clearance rates and chlorophyll *a* concentrations in early summer (end of May and beginning of June), (ii) lower rates due to strongly decreasing chlorophyll *a* concentrations at stable clearance rates from June until August and (iii) extremely low rates at both low chlorophyll *a* concentrations and low clearance rates in September and October.

It is vital to take temperature into account when discussing food demand, because the metabolic rate and thus the requirement for food generally increases with rising temperature in ectotherms (e.g. Chomsky et al., 2004;

Savage et al., 2004; Gillooly et al., 2001). An increase of 5°C results in an approximately two-fold increase in energy demand in *Corbicula fluminea*. (Ortmann & Grieshaber, 2003) Increasing temperature under limited food conditions in summer can lead to a negative energy balance and thus to a reduction of body mass due to respiration of storage tissue (Bielefeld, 1991; Borcherding, 1995; Chomsky et al., 2004). Nevertheless, such a potentially negative temperature effect can be compensated by increasing clearance rates with increasing temperature, which has been demonstrated for *Corbicula fluminae* from the Rhine population at temperatures between 12 and 25°C (Viergutz et al., 2007) and which was also observed in this study in August. However, an increase of clearance rates in *Corbicula fluminea* only occurs for temperature up to 25°C; higher temperatures lead to a decrease (Viergutz et al., 2007). It is possible that water temperatures higher than 25°C in combination with decreasing algal concentrations and decreasing filtration rates over the course of the summer resulted in a negative energetic balance sufficiently high to cause mass mortality in 2003. Compared to many other mussels, *Corbicula fluminea* is less able to compensate energy losses at high temperatures by decreasing its metabolic activity (McMahon, 2002). Our results suggest, however, that compensation up to a certain point is achieved by the strong respiration of storage tissue.

One additional reason for body mass decrease besides starvation is reproduction, i.e. the release of offspring (cf. Bagatini et al., 2007). The first reproductive period in the River Rhine occurs when the water temperature reaches 15°C, which usually occurs in April or May (Meister, 1997). A second, much less pronounced reproduction period occurs in autumn (Meister, 1997). In this study, most of the body mass variation occurred between these two potential reproduction periods, i.e. is not related to the release of offspring. If reproduction occurred in autumn (although starvation usually leads to a single reproduction period in spring only; Mouthon, 2001), this event did not account for the major part of the body mass variation. The body mass variation thus mainly reflects the nutritional conditions.

In contrast to body mass, shell length increased during the whole study period in both treatments. The maximum growth rate was 0.115 mm day^{-1} (measured in Cologne in June) This value lies in the upper range of data reported in literature: 0.068-0.094 mm day^{-1} in Kanawha River, USA (Joy 1985), 0.025-0.181 mm day^{-1} in Trinity River, USA (McMahon and Williams 1986), and 0.003-0.038 mm day^{-1} in the River Rhine (Meister, 1997). However, the increase rate decreased strongly in response to food level reduction as observed in both the low and high food level treatments during the course of the summer. The clams in the low food level treatment showed a much earlier reduction of shell length increase rate, while clams in the high food level treatment showed relatively strong reduction rate after August. However, it is remarkable that the clams' shells did not stop growing, even at times of the greatest body mass decrease. Together, this led to even stronger decreases in the size-specific body mass than observed in the absolute body mass.

Conclusions

Our results from 2004 show that the food concentration in the Rhine during summer causes drastic losses in the body mass of *Corbicula fluminea* above the lethal level both directly in the Rhine and in the bypass systems fed with Rhine water. Although this body mass decrease was significantly enhanced due to a 50% lower food concentration, no mass mortality occurred. This strong plasticity and the corresponding respiration of storage tissue can be seen as a strategy for surviving longer starvation periods up to a certain level. The positive shell growth at times of decreasing body mass shows the largely independent control of shell length and body mass. This contrasting development leads, however, to an enhanced loss of size-specific body mass.

Despite the lack of mass mortality observed in the present study, the extremely low body mass of the clams at the end of the summer 2004 suggests that further starvation stress can surpass a lethal level, as observed in summer 2003. Reduced eutrophication (i.e. a reduction of phytoplankton), as has been achieved in many areas through improved wastewater treatment, is thus a promising way of controlling *Corbicula fluminea*.

Chapter 2

The impact of summer versus winter warming on the fitness of the invasive clam *Corbicula fluminea*

Abstract

Climate warming is discussed as a factor which can favour the invasion success of neozoans. In the present study we analysed potential fitness gains of moderate warming (3°C above field temperature) on the invasive clam *Corbicula fluminea* and whether or not these fitness gains occur consistently for both summer and winter warming. The experiments were performed under semi-natural conditions in a bypass-system of a large river (Rhine, Germany). In a first set performed from July until October, we showed that warming on top of late summer field temperatures results in significant decreases of the clams' growth and survival rate. The addition of planktonic food dampens the negative effect of warming on the growth rates. This suggests that the reason for the negative growth effect of temperature increase in summer is a negative energetic balance due to an enhanced metabolic rate at limited food levels. In contrast, warming in winter (November until April) had a positive effect on the clams' growth rate. Furthermore, the reproduction success during the main breeding period (April) was strongly enhanced by winter warming, as expressed in (1) a two-fold increase of the incubation rate and (2) a more than five-fold increase in the larval number produced per clam. This strong, positive effect of moderate winter warming on the clams' fitness is probably one reason for the recent invasion success of *Corbicula fluminea* in the northern hemisphere. Nevertheless, warm summer events might counteract the positive winter warming effect, which could trade-off the fitness gains.

Introduction

There is broad consensus among scientists that the global temperature will increase significantly during the next decades due to the mainly man-made emission of greenhouse gases (IPCC, 2007). The expected local temperature increase can vary strongly form the expected global average temperature increase. For central Europe, the strongest seasonal average temperature increase is predicted for winter times (IPCC, 2007), while the summers will probably experience irregularly occurring heat waves in increasing frequencies (Schär et al., 2004; Stott et al., 2004).

Such temperature changes will broadly affect the composition of ecosystems by different mechanisms (e.g. Walther et al., 2002; Parmesan & Yohe, 2003; Graham & Rehfisch, 2005; Lehikoinen et al., 2006; Parmesan, 2006). While some of such changes occur continuously, others occur rapidly as has been shown for changes in a freshwater mollusc community during the central European heat wave in 2003 (Mouthon & Daufresne, 2006). Another rapid change in local community structure can be caused by the settlement of invasive species (Suarez et al., 2001; Ricciardi, 2007). Whereas their introduction is usually caused by man, their ability to establish local communities in their new environment is often supported by environmental changes (Dukes & Mooney, 1999; Stachowicz et al., 2002; Holzapfel & Vinebrooke, 2005). The successful establishment of invasive species can have particularly serious ecological consequences if they displace native organisms and function as keystone species which have a broad impact on ecosystem structure and function. In European and North American freshwater ecosystems, such an invasion has occurred by bivalves, particularly *Corbicula fluminea* and *Dreissena polymorpha* (Ricciardi et al., 1997; Karatayev et al., 2003; Strayer & Malcom, 2007). In their function as filter feeders, they can have tremendous impact on plankton communities and thereby on ecosystem-wide processes (Cohen et al., 1984; Hakenkamp & Palmer, 1999; Caraco et al., 2006). It is important to understand mechanisms which both support and constrain the success of such species in the context of changing environments.

The focus species of the present study, *Corbicula fluminea*, originates from Asian habitats and invaded the study area of the present investigation, the River Rhine, in 1980s, probably via ballast water of ships (Bij de Vaate, 1991; Ricciardi, 2003). Today, *Corbicula fluminea* is the most common bivalve in several rivers of the northern hemisphere, including the River Rhine (Karatayev et al., 2005). One of the suppositions for the establishment of *Corbicula fluminea* in its new environments besides structural changes (Bij de Vaate, 1991) is seen in the temperature increase caused by both the heat emission of power plants and climate warming. This conclusion is mainly drawn from the finding that (i) exposure time to temperatures below 1 to 2°C correlates with mortality rate (Mattice & Dye, 1976; Rodgers et al., 1977; Morgan et al., 2003) and (ii) the occurrence of *Corbicula fluminea* in rivers such as the St. Clair River (Michigan, USA) and the lower Connecticut River (New England, USA) is restricted to the thermal refuges provided by power plant discharges in the cold winter months (French & Schlösser, 1991; Morgan et al., 2003). However, these apparently positive effects of winter temperature increase on the survival of *Corbicula fluminea* in cold environments is in contrast to the observation of a mass mortality of *Corbicula fluminea* under conditions of warm summer temperatures (Westermann & Wendling, 2003; Cooper et al., 2005). In the Rhine, such a massive die-off occurred during the hot summer of 2003, which had peak temperatures of 28°C (Westermann & Wendling, 2003). However, it is not likely that this phenomenon is due to the approach of lethal temperatures, which are around 37°C for *Corbicula fluminea* (Morgan et al., 2003) and therefore far above the peak field temperatures in the Rhine. It seems more likely that the clams experienced a negative energetic balance at high temperatures due to an enhanced metabolism (Gillooly et al., 2001; Enquist et al., 2003). This is known for ectotherms which cannot compensate the energy loss of the heightened metabolisms either because they live under limited food conditions (Chomsky et al., 2004; Weitere et al., 2004) and/ or because they are unable to increase their feeding rates proportionally to their metabolic rate at high temperatures (Aldridge et al., 1995). This hypothesis is supported by the recent finding that *Corbicula fluminea* experiences a drastic loss of body mass in the Rhine during the summer, particularly starting in July, probably due to resource limitation (Vohmann et al., subm.). Temperature increases might thus enhance the body mass decrease in extreme cases to a lethal stage.

The present study focuses on the question as to whether or not warming in summer versus winter can lead to contrasting effects on fitness parameters of the invasive clam *Corbicula fluminea* under natural conditions. The experiments were carried out in bypass systems fed with river water carrying the ambient food composition. Temperature conditions in the experiments were (1) ambient river temperatures and (2) 3°C above the ambient temperature. For summer, we hypothesised a negative effect of warming on fitness due to a negative energetic balance with high temperatures. If this mechanism matches, the negative temperature effect should be compensated by increased food supply.

For winter, we hypothesised a positive effect of warming on the fitness, because the ambient temperature conditions are below the species-specific optimum. The clams which experienced the two different summer temperature histories were apportioned into the two winter temperature conditions. In that way it was possible to separately measure the effect of both summer and winter warming on the reproduction success in April, the main breeding season of *Corbicula fluminea* in the Rhine (Meister, 1997).

Material and Methods

Overview of study site and experiments

The experiments were performed in a river bypass system fed with natural Rhine water at the Ecological Rhine Station of the University for Cologne (Cologne: Rhine-km 685, which refers to the distance from Lake Constance, the source of the non-alpine Rhine). Two main experiments were performed, one during late summer (July 20, 2005 until October 20, 2005) and one during winter (October 20, 2005 until April 18, 2006). During the summer experiment we tested the hypothesis that summer temperature increase has a negative effect on the body mass of the clam under naturally limited food conditions. For this purpose, two temperature treatments (ambient Rhine temperature [Δ0] and ambient Rhine temperature plus 3°C [Δ3]) were combined with two plankton levels (ambient plankton and addition of an enriched natural algal assemblage). The start of the summer experiment was set at a time when strongest decreases in the body mass of *Corbicula fluminea* started to occur in the Rhine in a previous study in 2004 (Vohmann et al., subm.) and was terminated when the temperature dropped below 20°C in the warmed treatments. The focus of the winter experiment was on the role of both summer and winter temperature increase on the reproduction success. Therefore, clams from the summer experiment which were grown under ambient food conditions at both Δ0 and Δ3 were apportioned into the winter temperatures of Δ0 and Δ3 and ambient food conditions. Thereby each winter temperature arrangement contained one treatment with clams from the Δ0 and one with clams from the Δ3 summer experiment. The winter experiment was started after the termination of the summer experiment and was run until the main breeding season in *Corbicula* *fluminea* in the Rhine in April (Meister, 1997). Clams were therefore either held constantly (summer and winter) at ambient temperature (Δ0), at three degrees enhance temperature (Δ3), or at mixed temperatures (Δ0 in summer to Δ3 in winter and vice versa).

Experimental set-up

Glass tanks (25.0cm x 17.5cm x 10.0cm) were used for the river bypass experiments as done by Vohmann et al. (subm.). Sixteen tanks were used for the summer experiment, divided among the four treatments (Δ0 and Δ3, with ambient and enhanced food levels each), with three parallel tanks per treatment plus one parallel each for the determination of the body mass within the running time of the experiment (see below). For the winter experiment, three replicates per treatment were considered and the body mass determination was performed at the end of the experiment for each replicate separately. The tanks were filled with a sand layer of three centimetres. The flow of fresh Rhine water (containing its natural plankton community) through each tank was adjusted to 30 L h-1. The flow was high enough to maintain the natural Rhine temperature (Δ0) without further temperature manipulation. The 3°C temperature increase (Δ3) was achieved by an aquarium heater. With an average of 3.3°C (summer) and 3.4°C (winter) above ambient, the altered temperature was quite close to the desired 3.0°C difference (see Fig. 1 for temperature development).

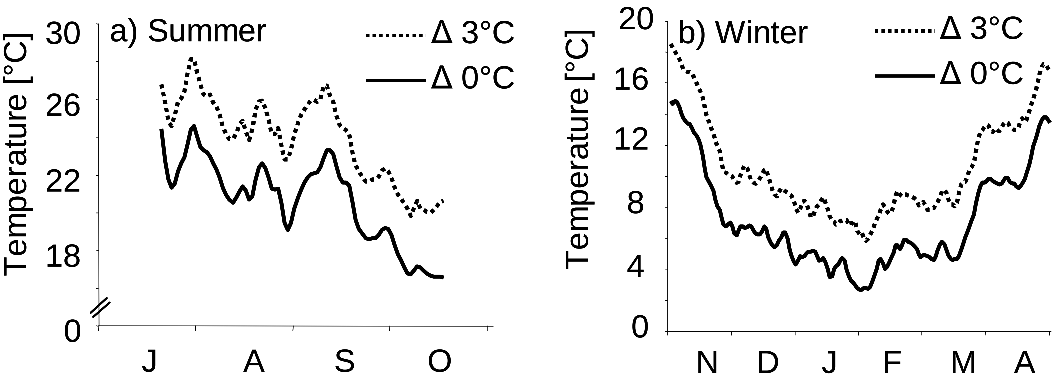

Fig. 1: Temperature conditions during the two experimental periods (a) summer/ early autumn and (b) winter. Δ0°C reflects the ambient temperature in the River Rhine and in the control experiments, and Δ3°C the temperature in the warmed experiments, respectively.

The clams fed on the natural plankton community of the Rhine (which was supplemented with enriched natural plankton as one manipulation in summer). Due to the grazing activity of the clams, the realized food level in the tanks in summer was an average of 26% lower than the food level in the Rhine. This was determined by regularly measuring the chlorophyll a concentration, which provides a good proxy for the actual food level for mussels in rivers (Jantz & Neumann, 1998). In winter, the grazing activity of the clams was distinctly lower, leading to no measurable differences in the abundance of planktonic algae and protozoans during the cold months (December, January and February). The reduced food level during the summer experiment was restored by adding a suspension containing enriched natural phytoplankton from the Rhine. For this purpose, stock solutions based on those used for producing WC-medium (Guillard & Lorenzen, 1972) were added to fresh Rhine water filled into transparent tanks. After light exposure under turbulent conditions in a batch system for three days, a dense algal suspension with a chlorophyll concentration of about 1000 µg l-1 was established. This algal suspension contained a diverse planktonic community, including all algal groups which occur in the Rhine (dominated by centric diatoms). This suspension was continuously added to the tanks, resulting in an approximately 40% enhancement of the chlorophyll concentration. The chlorophyll concentration within the tanks was regularly checked in summer with the help of the AquafluorTM (Turner Designs, USA). The temperature within the tanks was recorded continuously at two hour intervals with the help of data loggers during summer and winter.

Origin of clams and measurement of their shell sizes and body mass

To ensure that experimental organisms consists of individuals with a known life history, experimental clams were collected from a cohort settled in flow channels of the Ecological Rhine Station which had been filled with natural sediments and fed continuously with natural Rhine water. The mussels originated from the larvae deposition in spring 2005. As preparation for the fist experiment performed in late summer 2005, the clams were classified into shell-size classes. A total number of seventy clams were added to each tank, corresponding to 860 individuals per square meter, which is close to the field density (Schöll, 2000). The shell sizes for the summer experiment were 13 mm (25 clams), 14 mm (29 clams) and 15 mm (16 clams). The residual clams from the ambient food treatment of the summer experiment were used for the winter experiment. At the end of the summer experiments, we could gather 30 clams per replicate which had experienced the adjusted temperature conditions over the total summer experiments. Those clams were randomly divided into 15 individuals per replicate which were placed at elevated winter temperature (Δ3) and 15 individuals per replicate which were placed at ambient winter temperatures (Δ0). In order to maintain a constant density of clams, the tanks were, in addition to the target clams (with defined summer temperature history), stocked with colour-marked clams from the Rhine.

The shell-size of all clams was measured every second week for the summer experiment as well as at the start and end of the winter experiment with help of a digital calliper square. The ash-free dry weight (AFDW) was analysed for each treatment on August 23 (summer experiment), October 20 (end of summer/ start of winter experiment) and April 18 (end of winter experiment). For this purpose, 25 clams per treatment were taken for each of the two measurements during the summer experiment. Because the clams were killed during the dry weight determination, they were taken from the fourth replicate (see above); the three other replicates were not influenced by the dry weight determination. In order to maintain a constant density in the fourth replicate during the summer experiment, the clams which were taken to analyse the AFDW were replaced by same-sized clams from the river. The shells of those replaced clams were colour-marked so they could be distinguished from the experimental clams, and they were not further considered in the analyses. After the final termination of the winter experiment, 15 clams from each of the three replicates per treatment were taken for the dry weight determination after the number of larvae per clam was counted (see below). The AFDW was determined after drying the soft body (for 30 h at 60°C) and combusting for 15 h at 550°C. The AFDW is the difference between the dry weight and the weight of the incombustible components.

Determination of reproductive success

One important goal of the winter experiment was the determination of the reproduction success in spring for the different temperature histories. The early development of Corbiclua fluminea larvae takes place between the gills of the mother before they are released into the surrounding water during the main breeding season in late April. Water temperatures in the Rhine are usually above 15°C by this point (Meister, 1997). The hatching of the larvae within the gill structure takes place about two weeks earlier (Meister, 1997). Because the experimental warming might influence the larval releasing time in our experiment, different approaches were used in order to accurately determine the larval number per clam. In March (before 15°C were reached in the warmed treatments), a gauze strip of 80 µm mesh size was placed at the efflux of the tanks and the filtrate was checked for larvae in order to ensure that no larvae had already been released. At the same time, clams were regularly vivisected and the developmental stage of the larvae was determined. Clams were sampled for the larval analyses on April 18th. At this time all larvae were present in the gill structure in all temperature treatments and had not been released into the surrounding water yet. From the sampling time onwards, we further analysed clams every week for a possible appearance of new larvae. This was done until mid-May. These controls revealed that all larvae were released into the water by April 28th and that no new larvae had developed since April 18th, confirming that this time point was appropriate for the analysis of the offspring number. For the sampling on April 18th, 15 clams per replicate were wrapped up into damp tissues and stored at 4°C. Within five days, all clams were dissected and the number of larvae per clam was counted. The tissues of the clams were than used for the AFDW-determination (see above). Two measures for the reproduction success were calculated: the incubation rate (percentage of clams carrying larvae) and the total number of larvae per clam.

Statistical analyses

Statistical analyses were performed with the help of SPSS 15.0 software. The dependence of shell length on the summer temperature and the food conditions (summer experiment) as well as the dependence of the shell length and body mass on the winter and summer temperature conditions (winter experiment) was tested in two-factorial ANOVAs (analysis of variance). The difference in mortality between the treatments during the summer experiment was tested with the help of a non-parametric Kruskal-Wallis H-test because of the lack of normal distribution. After the H-test had revealed significant treatment effects, we tested the dependence of mortality separately on temperature and on food conditions using U-tests (Bonferroni-corrected for multiple comparisons).

Results

Impact of summer warming on growth and survival

Temperature increase had a significant negative effect on the shell length of the clams in October (Table 1, Fig. 2a). This pattern was already seen as non-significant trend in August (four weeks after start of experiment). Furthermore, temperature increase resulted in negative effects on the body mass (Fig. 2b). The effects cumulated in a 13.1 and 13.8% lower average body mass in the warmed treatments in August and October (respectively) than in the ambient temperature treatments for the same dates (Fig. 2b). Between August and October, a slight positive increase in the average shell length of the clams was recorded, while the absolute body masses decreased slightly for all treatments (Fig. 2). The temperature effects were compensated by the addition of food. Clams in the warmed food-supplement treatment reached a similar average size and body mass to those in the non-supplemented treatment at ambient temperature. Food addition resulted in a significantly higher shell-length and a higher body mass in October for both temperature regimes (Fig. 2, Table 1). This pattern was also seen as non-significant trend in August.

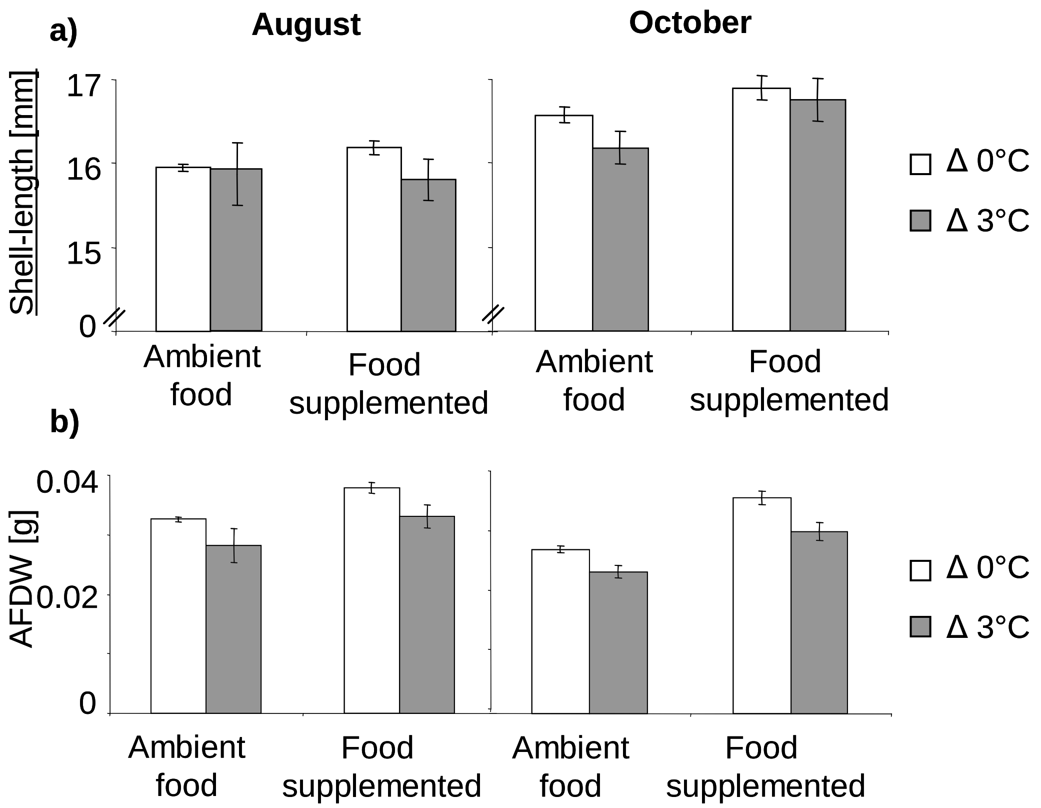

Fig. 2: Shell length and body mass (AFDW) of *Corbicula fluminea* during the summer-experiment in dependence on temperature and food level measured on August 2 (four weeks after start of experiment) and October 15. (a) Mean (± SD) shell lengths. See Table 1 for ANOVA results. (b) Estimate of the mean (± SD) AFDWs based on the shell length and length-weight regression.

Table 1 ANOVA-results of tests on the effect of temperature and food level on the shell-length of *Corbicula fluminea* for the sampling in (A) August and (B) October during the summer experiment. Bold p-values indicate significances.

	SS	*df*	F	p
A. August				
Food level	$1610\text{x}10^{-4}$	1	2.718	0.138
Temperature	$1490\text{x}10^{-4}$	1	2.513	0.152
Temperature x food level	$1.34\text{x}10^{-4}$	1	0.002	0.963
Error	$4730\text{x}10^{-4}$	8		
B. October				
Food level	$54.7\text{x}10^{-2}$	1	18.289	**0.003**
Temperature	$18.9\text{x}10^{-2}$	1	6.311	**0.036**
Temperature x food level	$4.14\text{x}10^{-2}$	1	1.385	0.273
Error	$23.9\text{x}10^{-2}$	8		

The negative effect of the summer temperature increase on the clam growth coincided with a distinct increase in the mortality rate in the warmed treatments (Fig. 3). Mortality rates of between 1.4 and 20% were recorded in the single replicates of both Δ3°C treatments, whereas no mortality occurred in the Δ0°C treatments. No impact of the food supplement could be shown. The Kruskal-Wallis H-test revealed significant (p=0.021) differences in the mortality rates between the different treatments. A posteriori U-tests confirmed the significant impact of the temperature (p=0.002) and the lack of significant food effects (p=0.732).

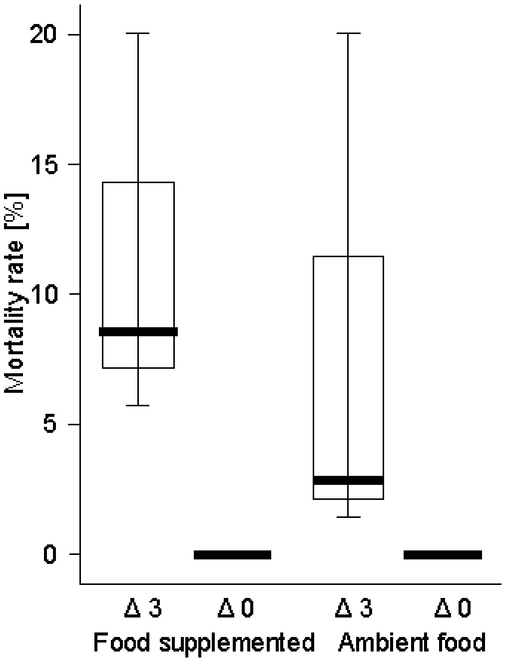

Fig. 3: Mortality rate (cumulative percentage of dead clams over the course of the summer-experiment in relation to total population) in dependence on the temperature and food conditions during the summer-experiment. Box-Whisker-Plots display first, second (median) and third quartiles as well as extremes.

Impact of winter warming on growth

The results of the winter experiment stand in direct contrast to the results of the summer experiment. Winter warming resulted in a clear, positive effect on the clams´ body mass. While the clams in the Δ0°C-treatments hardly increased their mean body mass between end of October and April, the clams in the Δ3°C treatment showed a nearly twofold body mass increase (compare Figs. 2b and 4b). The mean AFDW of the ambient winter temperature treatments were 0.041g (± 0.001g) (for both, the ambient summer temperature and the warmed summer temperature history) while they were 0.086g (± 0.001g) and 0.084g (± 0.003g) for the warmed winter temperature (for the ambient summer temperature and the warmed summer temperature history, respectively). The two-factorial ANOVA revealed a highly significant impact of the winter temperature but no effect of the temperature history during summer on the body mass in April (Table 2).

Despite the compensation of the summer temperature effect on the body mass during winter, the shell length in April showed a significant dependence on the summer temperature history (Fig. 4a, Table 2). In April, clams which had experienced warming during summer were still significantly smaller than clams which had been grown under ambient summer temperature, particularly after the exposure to warm winter temperatures.

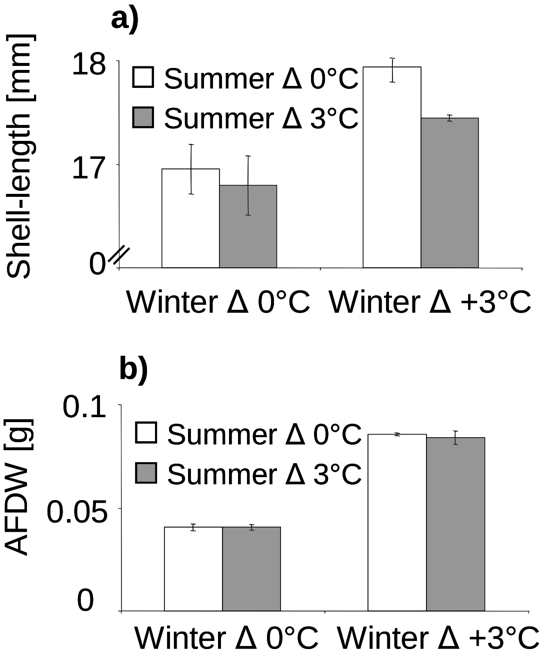

Fig. 4: Shell length and body mass (ash free dry weight; AFDW) of *Corbicula fluminea* at the end of the winter-experiment in dependence on winter and summer temperature conditions. (a) Mean (± SD) shell lengths. (b) Mean (± SD) AFDWs.

Table 2 ANOVA-results of tests on the effect of winter temperature and temperature history (from the summer experiment) on (A) the shell-length and (B) the body mass (AFDW) of *Corbicula fluminea* in April 2006 as well as (C) the incubation rate and (D) the number of larvae per individual. Bold p-values indicate significances.

	SS	*df*	F	p
A. Shell-length				
Summer temperature	0.319	1	7.881	**0.023**
Winter temperature	2.032	1	50.176	**<0.001**
Summer x Winter temp.	0.089	1	2.194	0.177
Error	0.324	8		
B. Body mass (AFDW)				
Summer temperature	$1.37x10^{-6}$	1	0.373	0.558
Winter temperature	$6000x10^{-6}$	1	1601.3	**<0.001**
Summer x Winter temp.	$1.9x10^{-6}$	1	0.518	0.492
Error	$29.37x10^{-6}$	8		
C. Incubation rate				
Summer temperature	$0.753x10^{2}$	1	1.015	0.343
Winter temperature	$5.184x10^{2}$	1	69.784	**<0.001**
Interaction	$2.543x10^{2}$	1	3.423	0.101
Error	$5.943x10^{2}$	8		
D. Number of larvae per calm				
Summer temperature	$1.138x10^{-3}$	1	0.021	0.827
Winter temperature	$161.3x10^{-2}$	1	30.234	**<0.001**
Interaction	$4.476x10^{-2}$	1	0.839	0.386
Error	$42.68x10^{-2}$	8		

Reproduction success

Warming of 3°C in winter had a large impact on the reproduction success of *Corbicula fluminea.* The incubation rate (percentage of clams which reproduced, i.e. which carried offspring) in the warmed treatments was (at 82 ± 10%) distinctly higher than the incubation rate at ambient temperature (41 ± 6%, Fig. 5a, Table 2). Furthermore, the total number of larvae per clam (related to those which carried larvae) in the warmed treatments (358 ± 168 larvae ind.$^{-1}$) was distinctly higher than the number of larvae per clam in the ambient temperature treatments (64 ± 27 larvae ind.$^{-1}$, Fig. 5b, Table 2). When the incubation rate and the average larval number per clam are considered together, winter warming resulted in a more than tenfold increase in the larval number of the total population. As shown for the body mass, an impact of the summer temperature history could neither been shown for the incubation rate nor for the larval number per clam (Fig. 5, Table 2).

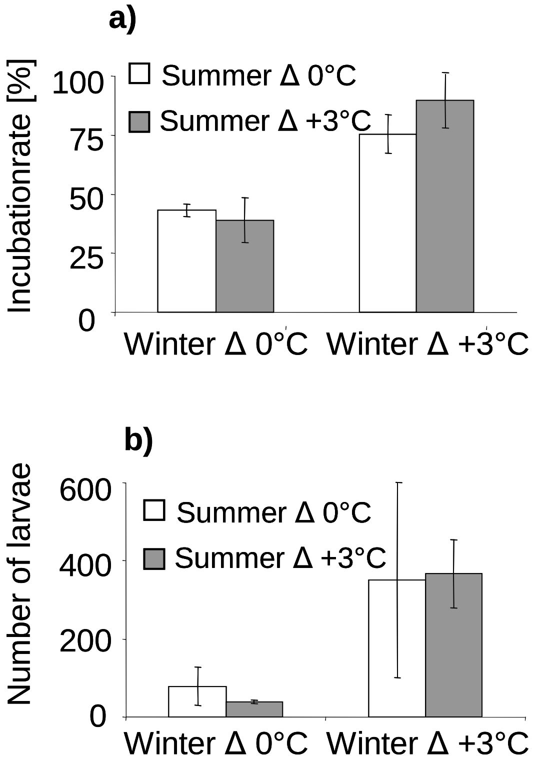

Fig. 5 Reproduction success of *Corbicula fluminea* after the termination of the winter-experiment in April 2006 in dependence on winter and summer temperature conditions: (a) Incubation-rate (percentage of clams which contributed to reproduction, i.e. which carried offspring). (b) Number of larvae per clam (related to those clams, which contributed to reproduction).

Discussion

A temperature increase of only 3.3 to 3.4°C resulted in strong effects on fitness parameters such as body mass, length of shell, morality rate and reproduction rate. The effects, however, differed considerably between summer and winter warming.

Negative effects of summer warming

The results show a decrease in the body mass of *Corbicula fluminea* between August and October, even at the natural Rhine temperature. This body mass loss during summer was distinctly stronger in similar bypass-experiments at the same station in 2004, when we measured a 93% body mass decrease during the same period of time (Vohmann et al., subm.). Together with the findings of the present study, the results show that *Corbicula* experienced food limitation in the Rhine during summer, however at different intensities in different years. This conclusion is supported by low algal biomasses in the Rhine during summer (Weitere et al., 2005). Our experiments demonstrated that this body mass decrease during summer was significantly enhanced by warming and that the negative warming effect could be restored by the addition of planktonic food. These findings strongly suggest that the reason for the negative effect of temperature increase on both body mass and shell length increase lies in an enhanced food demand (due to increasing metabolic rate with a rise in temperature), which could not be compensated for at limited food quantities. Such a negative impact of temperature elevation on body mass increase at limited food conditions has been reported for different ectotherms (Chomsky et al., 2004; Weitere et al., 2004). This conclusion is also supported by model calculations revealing a negative relationship between the carrying capacity of a population and temperature because each individual uses resources at a higher rate (due to elevated metabolic rate) when operating at a higher temperature (Savage et al., 2004).

It is likely that a strong reduction in body mass during summer could at some stage lead to mortality. In the present study, we found a significantly enhanced mortality rate due to warming in summer; however, this effect could not be compensated by additional food supply. Nevertheless, it is unlikely that the temperatures (maximum in the warmed treatment: 28.6°C, recorded on July 29th) in our experiment were directly lethal for *Corcicula fluminea*. The clam faces much higher temperatures in their original Asian habitats than in the River Rhine (Johnson & McMahon, 1998). Morgan et al. (2003) reported that temperatures above 37°C are lethal for *Corbicula fluminea*, which is far above the temperatures in the present experiments. It is thus more likely that the (food-quantity independent) temperature effects on the mortality rate are due to further factors which were not tested in our experiments, such as food quality (Wacker & von Elert, 2003). Nevertheless, the mortality rate in the warmed treatments observed here was still moderate, and there was no mass mortality during our investigation in the Rhine as observed in other years and also for other systems (Westermann & Wendling, 2003; Cooper et al., 2005).

Positive effects of winter warming

In contrast to the summer warming, winter warming results in positive effects on the clams' fitness. Besides the temperature-stimulated increases of both body mass and shell length, the strong effect of the moderate warming on the reproduction rate was remarkable. When the incubation rate and the average larval number per clam are considered together, the winter temperature increase of 3.4°C resulted in a more than tenfold increase in the larval number of the total population. This indicates a massive fitness gain through warming, even though the survival rates of the larvae released under the different temperature conditions in the field were not tested here. However, there is no convincing argument that the much higher number of larvae produced under better body mass conditions of the parents in the warmed treatment should not be able to establish a more successful clam generation than those from the treatment running at ambient temperature.

During winter, the coldest temperature in the $\Delta 0$ treatment was 2.5°C, recorded on January 29th. Some authors report temperatures of ≤2°C as lethal for *Corbicula fluminea* (Mattice & Dye, 1976; Rodgers et al., 1977); in another study, a correlation between temperatures of ≤1°C and mortality was found (Morgan et al., 2003). The lowest temperature reached during the present study was above any of the lethal levels given in such studies, and winter mortality did not occur here. Nevertheless, negative fitness effects of cold winter temperatures occurred already.

The effects of winter warming on the body mass increase seems to overwhelm the negative warming effects in summer, as the body mass at the end of winter was independent from the summer temperature history. Nevertheless, the effect of summer warming on the shell length was still significant at the end of winter, showing enduring effects of summer warming. Shell size usually correlates with larval number and thus with fitness in clams (Haag & Staton, 2003). However, this effect was not significant here, in the light of overall slight effects of summer warming on the shell length.

Conclusions

The common view that the invasion success of neozoans such as *Corbicula fluminea* is favoured by environmental warming (Stachowicz et al., 2002; Morgan et al., 2003; Holzapfel & Vinebrooke, 2005) could partly be supported by our study, while negative effects of warming also occurred. It is difficult to evaluate the negative summer effects versus the positive winter effects. In terms of the warming effect on the body mass, it seems that the summer effects were completely compensated by the winter effects, while significant summer effects on the shell length last over winter. Nevertheless, the mortality rate was significantly enhanced by summer warming, suggesting that the stress is already severe enough to cause death. It is likely that an additional increase in stress (e.g. due to higher summer temperatures, earlier onset of temperature increase in summer and/or lower food levels) causes mass mortalities in *Corbicula fluminea* (as reported by Westermann & Wendling, 2003; Cooper et al., 2005). In light of the predictions of increasing frequencies of summer heat waves in central Europe (Schär et al., 2004; Stott et al., 2004), one possible scenario is that the fitness gain of *Corbicula fluminea* caused by winter warming will be increasingly constrained due to summer warming. Together, the result highlight the importance of the temporal pattern of warming and of the annual life cycle of the organisms (compare Adahl et al., 2007), which needs to be considered under the light of temporarily varying effects of global warming (IPCC, 2007).

Chapter 3

The impact of temperature increase and food quantity on the body mass, growth and respiration of *Corbicula fluminea*

Abstract

High summer mortalities of *Corbicula fluminea* have been observed in recent years. The probable cause is a negative energetic balance due to a higher food demand at high temperatures in combination with low food concentrations in the River Rhine in summer. Based on the previous findings (chapters 1, 2), this study further analysed the impact of temperature and food quantity on growth and body mass of the clam. Frequent measurements of shell length increase and the ash-free dry weight (AFDW) were performed. Because of the knowledge of an alteration between the temperature impacts on *Corbicula fluminea* during the seasons (positive in winter and negative in summer), this study started in April and thus filled an important time gap which had not been considered before. Furthermore, the respiration rates of the clams were analysed in order to test the hypotheses that metabolic demands increase with high summer temperatures.

The results showed an initial body mass increase between April and June. The body mass decreased in all treatments between June and October, but more strongly in the unfed treatments; the body mass fell below the starting values in October. The elevated food concentration balanced the body mass decrease, verifying the result of the previous study (chapter 2).

Shell length increase was shown to be stimulated by temperature elevation, especially in early summer, whereas the body mass was mainly controlled by the food concentration (especially between August and October). This differential control of body mass and shell length resulted in higher shell length to body mass ratio under warming. Measurements of the metabolic rates (oxygen consumption) revealed higher metabolic rates of the mussels in the warmed treatments. Temperatures between 19 and 30°C resulted in an exponential increase of the oxygen consumption in contrast to decreasing grazing rates above 25°C as shown by other authors, and therewith a disproportion between energy demand and feeding activity became obvious. Together with the previous experiments (chapter 1, 2), the body mass decrease during summer was also seen in this study and is thus seen as a general response of *Corbicula fluminea* to the conditions in the River Rhine during summer. However, the absolute body mass in October varies between the years.

Introduction

Corbicula fluminea is an important filter feeder in many freshwater ecosystems (e.g. Ricciardi et al., 1997; Karatayev et al., 2003; Strayer & Malcom, 2007), and it is a challenge to understand the factors controlling their abundance. The interactive impact of temperature and food quantity has been studied in this context (Chapters 1, 2; Mouthon & Daufresne, 2006; Viergutz et al., 2007). The temperature impact became important because of the findings of mass mortalities and strong body mass decrease of *Corbicula fluminea* during summer in the River Rhine (Chapters 1, 2; Westermann & Wendling, 2003), as well as the finding of massive die-offs in combination with warm summer temperatures in other rivers (Cooper et al., 2005). The predicted development of global warming (IPCC, 2001, IPCC, 2007), e.g. the increasing occurrence of extreme weather such as summer heat waves, necessitated the ongoing research of the impact of temperature.

We have shown earlier that the natural conditions in the River Rhine in summer repeatedly led to a strong body mass decrease of *Corbicula fluminea* between June and October, which was significantly amplified by a temperature elevation (chapter 1, 2). Low algal concentrations in summer (Weitere et al., 2005) and the increase in energetic demand with increasing temperatures (as suggested by general temperature – metabolic rate relationships, Gillooly et al., 2001; Enquist et al., 2003) led to the assumption that limited food concentrations in combination with high temperatures lead to body mass decrease. Chapter 2 showed the negative temperature impact on the body mass of *Corbicula fluminea* and the possiblility of counteracting the temperature effect by food supplementation. The contrasting effects of temperature between summer and winter were also clearly demonstrated: In contrast to the negative impact of temperature in summer, winter warming stimulated fecundity and body mass increase.

The motivation of the present study was (i) to analyse at which time the temperature impact switches from positive (winter) to negative (summer) and (ii) to reveal mechanistic explanations for the summer effects by frequent measurements of shell length and body mass as well as respiration measurements. For this purpose, the present experiment started in spring (April 16), the time when the experiment from chapter 2 was terminated and the stimulation of body mass and shell length increase by temperature was still persistent. From previous data we knew about effects of temperature and food. We used the rate of oxygen consumption as a measure of the respiration rate at different temperatures to compare it to the grazing rate data reported in literature (Viergutz et al., 2007).

Material and Methods

The experiment was performed under almost natural conditions at the Ecological Rhine Station of the University of Cologne, a freshwater laboratory next to the navigation channel of the River Rhine (Rhine km 685.5). For a more detailed description on the facility see chapter 1. The experiments until July were the result of a collaboration with Catherine Linn.

Experimental set-up

The same general set-up as used in chapter 2 was applied. Glass tanks (10 × 23 × 35 cm) with a volume of 8 L were used for the experiment, and both manipulations were performed on the basis of the natural Rhine water (a river by-pass with a constant flow of 75 L h^{-1}), which fed the experimental vessels. Four replicates were considered per treatment. The detailed assembling of the tanks is given in Fig.1.

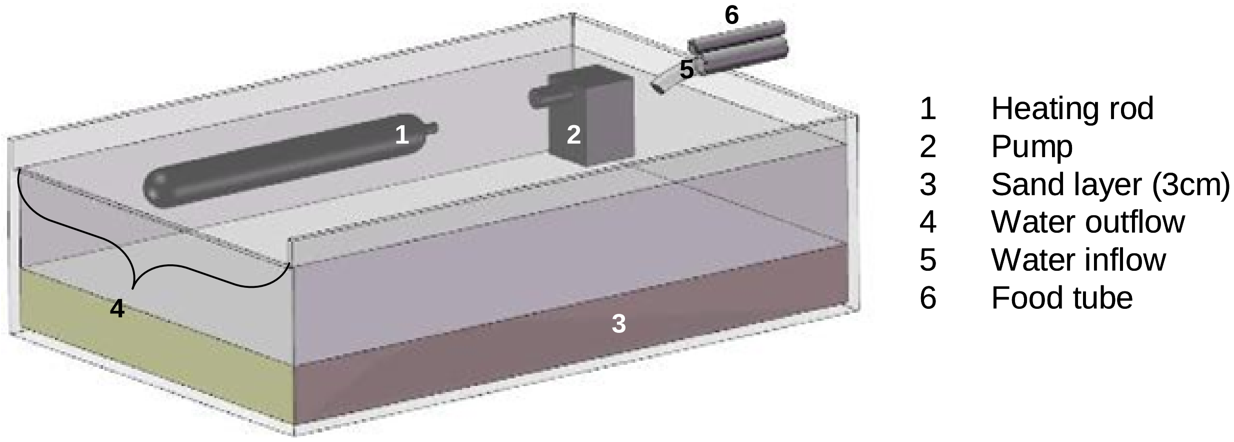

Fig. 1: Schematic illustration of one tank of the experiment. The figure contains both the heater (Δ +3°C) and the food tube (food supplementation). Depending on the treatments the tanks contained either the heater or the food tube or both. The control tanks (Δ 0°C, unfed) contained neither the heater nor the food tube.

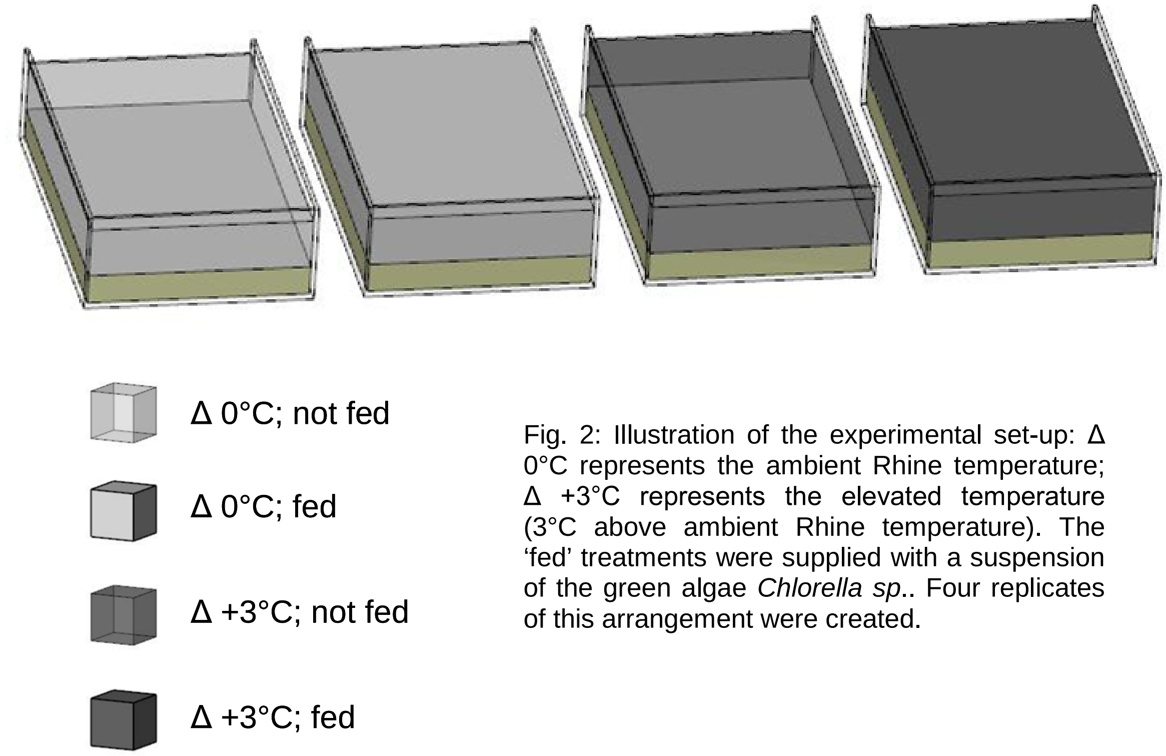

Fig. 2: Illustration of the experimental set-up: Δ 0°C represents the ambient Rhine temperature; Δ +3°C represents the elevated temperature (3°C above ambient Rhine temperature). The 'fed' treatments were supplied with a suspension of the green algae *Chlorella sp.*. Four replicates of this arrangement were created.

Temperature in the Δ0°C treatments was indistinguishable from that of the Rhine due to the high rate of water exchange in the tanks. The Δ3°C treatments were continuously warmed using aquarium heaters on the side walls of the tanks. The bottom was covered with about 3 cm of sand to imitate the natural habitat of *Corbicula fluminea* (Meister, 1997). Small aquarium pumps (Eheim type 1000 220; maximum flow 300 L h^{-1}) were installed inside the tanks to ensure a constant current. The tanks were cleaned and the detritus was removed twice a week.

In contrast to the experiments of chapter 2, the mussels were fed using a suspension of the green algae *Chlorella sp*. (MS-Aquaristikshop, Glinde, Germany). 60 g were suspended in 20 L of water and were supplemented within 24 h to the tanks with the help of a tube pump (Watson Marlow 2058). Thus the amount of 7.5 g powder (dry weight) was supplemented to each tank per day, resulting in a final concentration of 4 mg *Chlorella*-dry weight L^{-1}. The mean algal biomass in the River Rhine in spring/ early summer is 0.8 mg dry weight L^{-1} (Weitere et al., 2005; it is assumed that the dry weight of the algae is two times higher than its carbon content). Hence, a five time increase of the mean algal biomass was reached in the + algae treatments. Fluorescent measurements of the chlorophyll *a* concentration were generally performed twice a week with the help of Aquafluor^{TM} (Turner Designs, USA).

Organisms and measurements of growth parameters

The mussels which were used in the experiment originated from the River Rhine. The larvae reached the flow channels of the Ecological Rhine Station which were permanently fed with Rhine water and which contained soft bottom during the larvae fall in spring 2006. 70 mussels between 15 and 21 mm were placed in each tank, corresponding to 860 mussels per square meter, which is in accordance with the field density (Schöll, 2000). The size selection of each tank was exactly the same at the beginning of the experiment.

The size of the mussels was measured weekly with the help of a digital calliper. Dead mussels were replaced by marked mussels to maintain a constant density in the tanks. However, the mussels were not used for further experimentation. The ash-free dry weight (AFDW) of 48 mussels was measured at the beginning of the experiment on April 20. Seven to eight mussels (which corresponds to 28 to 32 mussels per treatment) were taken for AFDW determination on June 01, July 03, August 08 and on October 14 (the end of the experiment).

Because of an error in October, the AFDW measurements of the C+ treatments (ambient temperature and food supplementation) and the W- treatments (warmed and ambient food) were performed with only three replicates. The clams taken for the measurements were replaced by marked ones (see above). The soft body was separated from the shell and dried for 48 hours at 60°C, after which the dry weight was measured. After combustion for 15 hours at 550°C, the AFDW was calculated as the difference of the dry weight and the weight of the incombustible components.

Estimate of metabolic rate by oxygen consumption measurements

The oxygen consumption of *Corbicula fluminea* was measured as a measure of the temperature impact on the relative metabolic demand. Oxygen consumption rates were measured with a temperature-compensated oxygen meter for fibre-optic oxygen mini-sensors (FIBOX 3, 505 nm, PreSens, combined with FIBOX 5.20 software for data logging). The vessels had a volume of 62 ml and were kept in a temperature-controlled water bath. The oxygen concentration was measured in 30 second intervals; the total measurement per clam lasted about 20 to 40 minutes, i.e. as long as a linear decrease in oxygen consumption of at least 0.5 mg O_2 L^{-1} was recorded. By comparing with a grazer-free control, this oxygen consumption was corrected for the oxygen consumption of pure Rhine water. The absolute oxygen consumption of the individuals was calculated with the corrected oxygen concentration decrease per time and the total water volume within the vessels.

The oxygen consumption rates of the mussels at a temperature of 3°C over ambient. For this purpose, seven mussels per tank (which corresponds to 28 mussels per treatment) were randomly taken from the unfed replicates of the two temperature treatments. oxygen consumption was measured twice, once from May 29 to June 01 (at the time of the highest body mass) and than again from 26 October to 08 November (at the time of the lowest body mass of the mussels). At both times the mussels' oxygen consumption was measured at 18°C (ambient Rhine temperature) and at 21°C.

The main experiment was terminated on October 15. At this time the Rhine temperature was 17.3°C in the non-warmed and 20.1°C in the warmed treatments. Between that date and the measurement of the respiration rate, the mussels were kept at 18°C (ambient treatment) and 21°C (warmed treatment) in flowing Rhine water. Shell length and AFDW were determined after the measurement of the respiration rates (see above).

For analysing the oxygen consumption over a larger temperature range, oxygen consumption was measured between May 29 and June 01 at temperatures of 19°, 25°, 28°, 30° and 32°C. These temperatures correspond to those at which Viergutz et al. (2007) measured grazing rates for *Corbicula fluminea*. For this experiment, one mussel per replicate from the unfed treatments at either 18°C (ambient) or 21°C (warmed) was used. The mussels were acclimatised for one hour under the experimental conditions at the respective base temperature before the experiment was started. Again a correction of the values with help of control measurements using Rhine water took place.

Statistical analyses

Statistical analyses were performed using SPSS 15.0 for Windows. Length-weight regressions were calculated in order to analyse the relative body mass of the clams. The relationship between body mass (AFDW) and shell length (SL) followed a power function of the general formula AFDW = a x SL^b. As a test for a potential dependence of the size-specific body mass temperature and food quantity, an analysis of covariance (ANCOVA) was performed with AFDW as the dependent variable, temperature and food quantity as independent variables, and shell length as the covariate. Both AFDW and SL were log-transformed in order to achieve a linear relationship between AFDW and shell length. Impact of temperature and food quantity on the average mortality within the experiment was tested in an analysis of variance (ANOVA). Using a Pearson-correlation, the dependence of mussel AFDW and SL on the oxygen consumption was tested.

Results

Background data (temperature and food concentration)

The temperature development along the experimental period was measured frequently, generally every second day. Numerous checks in all replicates of both the ambient and the warmed treatments revealed only small variations (max. 0.3°C) between the replicates of the same treatments. The general temperatures of the set-up of the ambient and the warmed treatments are shown in Figure 3. The average realized temperature increase in the warmed treatments was 3.4°C

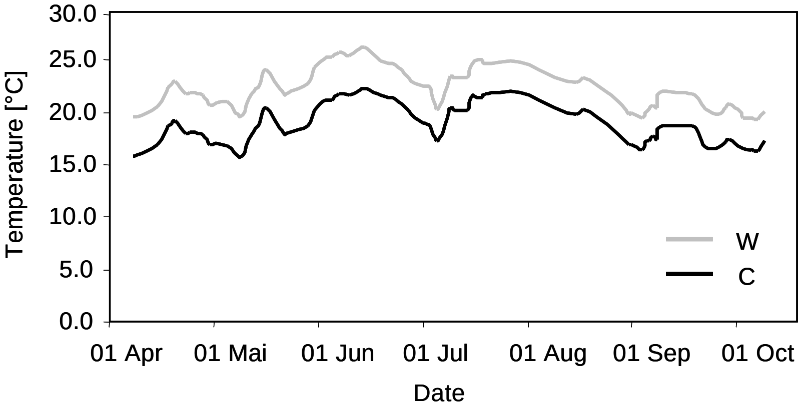

Fig. 3: Temperature develop-ment during the experimental period in the warmed treatment (W = warm) and the ambient treatment (C = cold).

The amount of chlorophyll *a* can be used to determine the difference in food concentrations between the different treatments. Figure 4 gives the results of all chlorophyll measurements from all experimental treatments as well as the Rhine chlorophyll content (inflow) over the experimental period. However, because the supplemented algae were dried, some chlorophyll *a* may have been destroyed. Thus chlorophyll concentration is only a very rough allocation of the biomass.

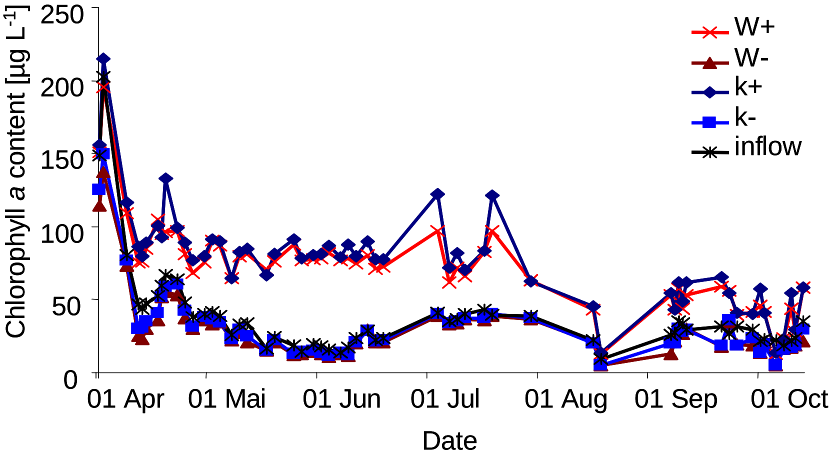

Fig. 4: Development of chlorophyll *a* content in the warmed (W) and the ambient (C) temperature treatments with (+) and without (-) additional food as well as the chlorophyll content of the Rhine water (inflow). The data until July resulted from a collaboration with Catherine Linn.

Development of shells size and bodymass

The impact of warming and increased food supply on the growth of shell length and the body mass of *Corbicula fluminea* was measured. Figure 5 shows the AFDW over four months in comparison to the starting value, given as a potential regression of AFDW versus shell length. There is a strong increase in body mass between April and June (Fig. 5a). From June to August the body mass decreases back to the starting values in October in spite of a continued growth of the shell length. At that time the impact of temperature and food gains in importance. In July and August the body mass in the fed treatments was significantly higher than in the unfed treatments, and the mussels in the warmed treatments had a lower body mass than those in the Δ0°C treatments (Fig. 5 b, c, Tab. 1). In October the AFDW fell drastically below the starting values, especially in the unfed treatments. Nevertheless the mussels increased their shell length since the beginning in April by an average of 3.4 mm in the unfed Δ0°C treatment (C-). The impact of temperature in the treatments without additional food is no longer detectable in October, whereas the food effect is still significant (Fig. 5d, Tab. 1).

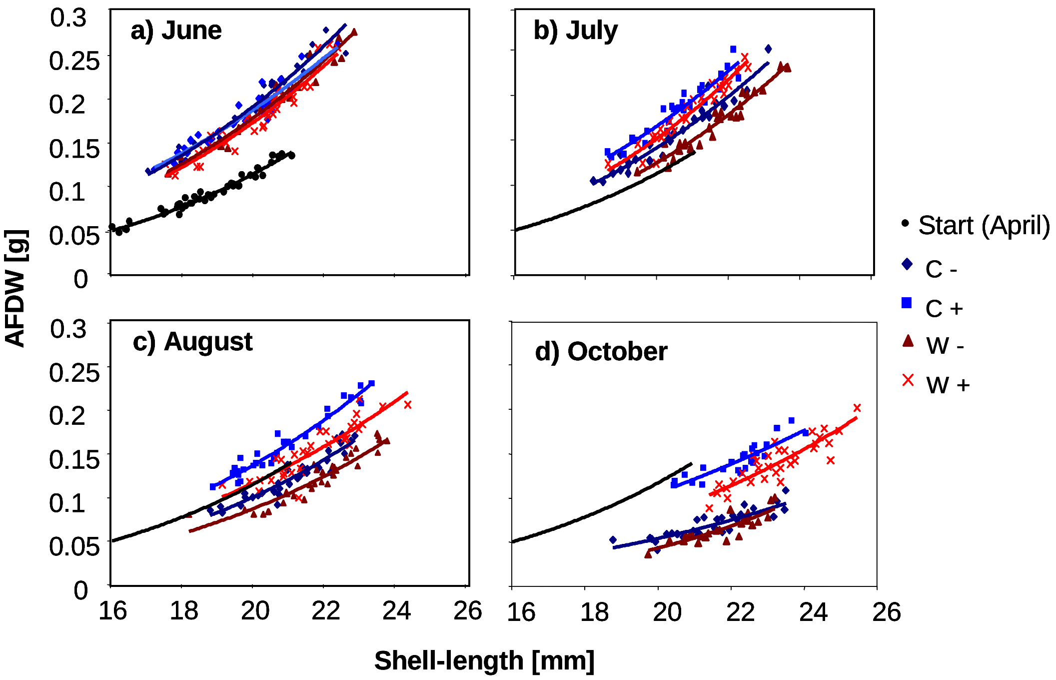

Fig. 5: Regressions of the ash-free dry weight (AFDW) versus shell length of *Corbicula fluminea* in dependence on the four experimental treatments: Ambient (C) and warmed (W) temperature with (+) and without (-) additional food on four dates of measurement (a-d). The black marks represent the starting values from April. For simplicity, only trend lines are shown in b-d. Because of an error, the AFDW measurements of the W- and the C+ treatments were performed with only three replicates in October. The data until July resulted from a collaboration with Catherine Linn.

Tab. 1: ANCOVA (analysis of covariance) results for the effect of food quantity and temperature on the shell length specific (log(SL) as covariate) body mass (log(AFDW), dependent variable) of *Corbicula fluminea* for each month. The data until July resulted from a collaboration with Catherine Linn.

a) June	SS	*df*	F	p
SL	1.018	1	1669.828	<0.001
Temperature	0.032	1	51.779	<0.001
Food	0.003	1	4.514	<0.001
Error	0.069	113		
b) July				
SL	0.7099	1	1254.389	<0.001
Temperature	0.037	1	65.193	<0.001
Food	0.147	1	259.607	<0.001
Error	0.058	106		
c) August				
SL	0.954	1	772.317	<0.001
Temperature	0.138	1	353.842	<0.001
Food	0.437	1	111.359	<0.001
Error	0.151	122		
e) October				
SL	0.582	1	297.008	<0.001
Temperature	0.107	1	54.641	<0.001
Food	1.244	1	634.546	<0.001
Error	0.208	106		

All treatments started in April with the same average shell length of 18.1 mm. Both food and temperature had positive effects on shell length growth. At the end of the experiment the mussels in the warmed treatments with additional food (W+) were the largest, whereas the smallest mussels were found in the ambient tempered treatments without additional food (C-). The treatments with only one manipulated factor (either food or temperature) were very similar in terms of shell length at the end of the experiment (Fig. 6). The impact of food level on shell growth was not positive in the two temperature treatments until June. The increased temperature in combination with the increased food concentration benefitted the growth of the mussels particularly between July and October, i.e. at the time of the greatest body mass decrease (see below).

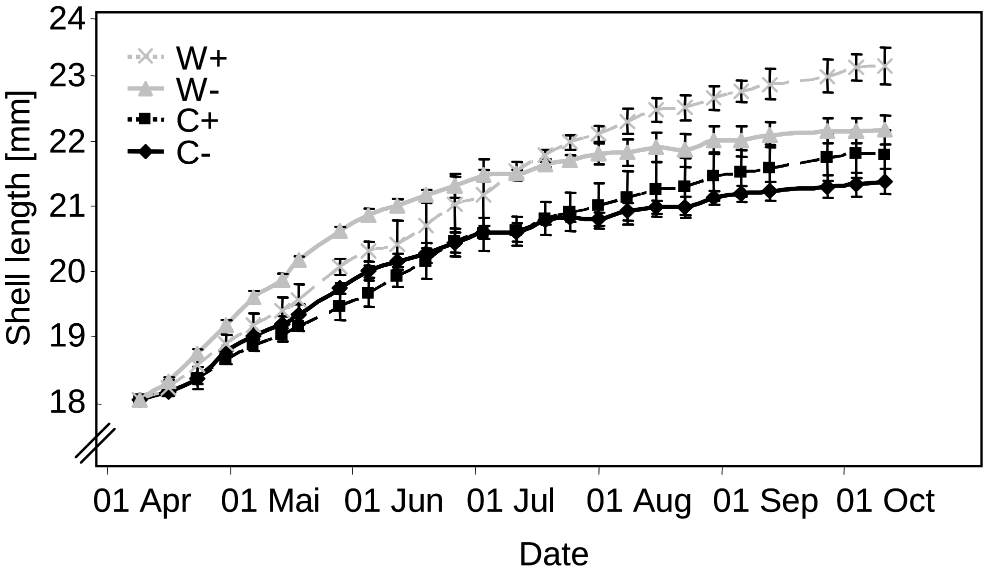

Fig. 6: Shell lengths of *Corbicula fluminea* given as the average of the four replicates for each treatment. Average starting length (April) was 18.1 mm for all treatments and replicates. The temperatures are symbolized with W (warm) for the warmed and C for the non-warmed (cold) treatments, + symbolized the food supplementation and – the natural food level. The data until July resulted from a collaboration with Catherine Linn.

Tab. 2: ANOVA (analysis of variance) results for testing the effect of temperature and food quantity on the shell-length (SL) and ash-free dry weight (AFDW) of *Corbicula fluminea*. Bold values indicates significances. The data until July resulted from a collaboration with Catherine Linn.

	SS	**df**	**F**	**p**
June, dependent variable: SL				
Temp.	1.329	1	9.341	**0.001**
Food	1.187	1	8.342	**0.014**
Temp. x Food	0.0003	1	0.002	0.963
Error	1.707	12		
June, dependent variable: AFDW				
Temp.	1.8×10^{-5}	1	0.089	0.770
Food	0.002	1	8.285	**0.014**
Temp. x Food	5.884	1	0.003	0.958
Error	0.002	12		
July, dependent variable: SL				
Temp.	0.1891	1	10.106	**0.008**
Food	0.221	1	1.183	0.298
Temp. x Food	0.788	1	4.212	0.062
Error	2.245	12		
July, dependent variable: AFDW				
Temp.	5.3×10^{-5}	1	0.266	0.615
Food	0.002	1	9.257	**0.010**
Temp. x Food	0.0002	1	1.327	0.272
Error	0.002	12		
August, dependent variable: SL				
Temp.	2.360	1	14.291	**0.002**
Food	0.030	1	0.180	0.679
Temp. x Food	0.007	1	0.042	0.841
Error	1.982	12		
August, dependent variable: AFDW				
Temp.	7.4×10^{-5}	1	1.238	0.288
Food	0.005	1	80.776	**<0.001**
Temp. x Food	2.9×10^{-5}	1	0.490	0.497
Error	0.0007	12		
October, dependent variable: SL				
Temp.	1.638	1	5.965	**0.035**
Food	4.505	1	16.402	**0.002**
Temp. x Food	0.525	1	1.912	0.197
Error	2.746	10		
October, dependent variable: SL				
Temp.	2.9×10^{-5}	1	0.392	0.545
Food	0.019	1	249.017	**<0.001**
Temp. x Food	6.2×10^{-6}	1	0.083	0.779
Error	0.0007	10		

The two contrasting results of the experiment, i.e. the continuous growth of the mussels in all treatments and the decrease of the body mass in all treatments can be seen in Figure 7. The data used for the figure originated only from those mussels which were randomly sampled for the AFDW analysis. This enables a direct comparison of the absolute body mass with absolute shell length. The impact of food quantity becomes important towards the end of the experiment.

The strong body mass decrease is compensated to a great extent by the additional food source, irrespective of the temperature.

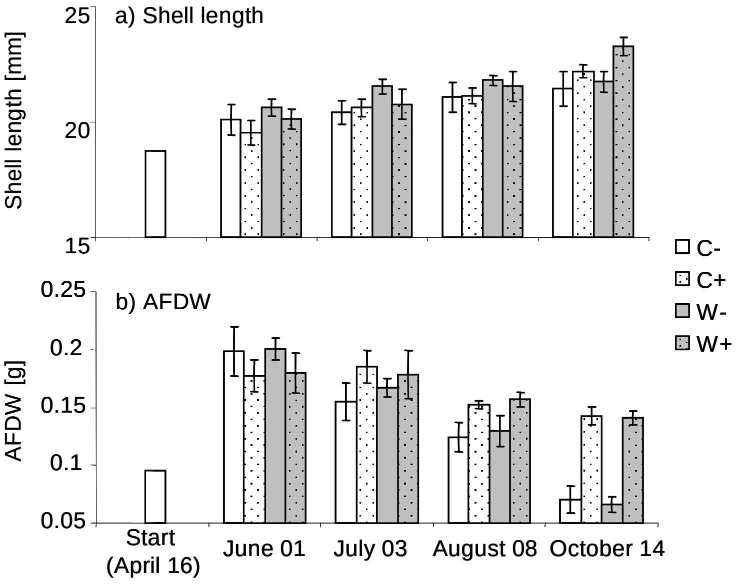

Fig. 7: Average shell length (a) and body mass (b) of the randomly chosen mussels from the AFDW measurement. The temperatures are symbolized with W for the warmed and C for the non-warmed treatments, + symbolized the food supplementation and – the natural food level treatment. The data until July resulted from a collaboration with Catherine Linn.

Impact of temperature on metabolic rate

Oxygen consumption was analysed as a measure of the metabolic activity; only the unfed mussels (at increased or ambient temperature) were taken for these measurements. The results of the first measurement in June, at the time of the highest body mass of the calms during the experiment, are given in Figure 8 as a regression of O_2-consumption versus AFDW (Fig. 8a) and versus shell length (Fig. 8b). Even though the spread of the single values is relatively high, there is a significant increase of O_2-consumption with increasing body mass and shell length, with the exception of a non-significant effect of the AFDW on O_2-consumption in the warmed treatment (Tab. 3). The respiration of the mussels for the warmed treatment was generally higher than the respiration for the mussels of the non-warmed treatment. The second measurement in October, at the time of the lowest body mass, revealed that the O_2-consumption was independent from both body mass and shell length for both temperature treatments (Fig. 8c, d; Tab. 3).

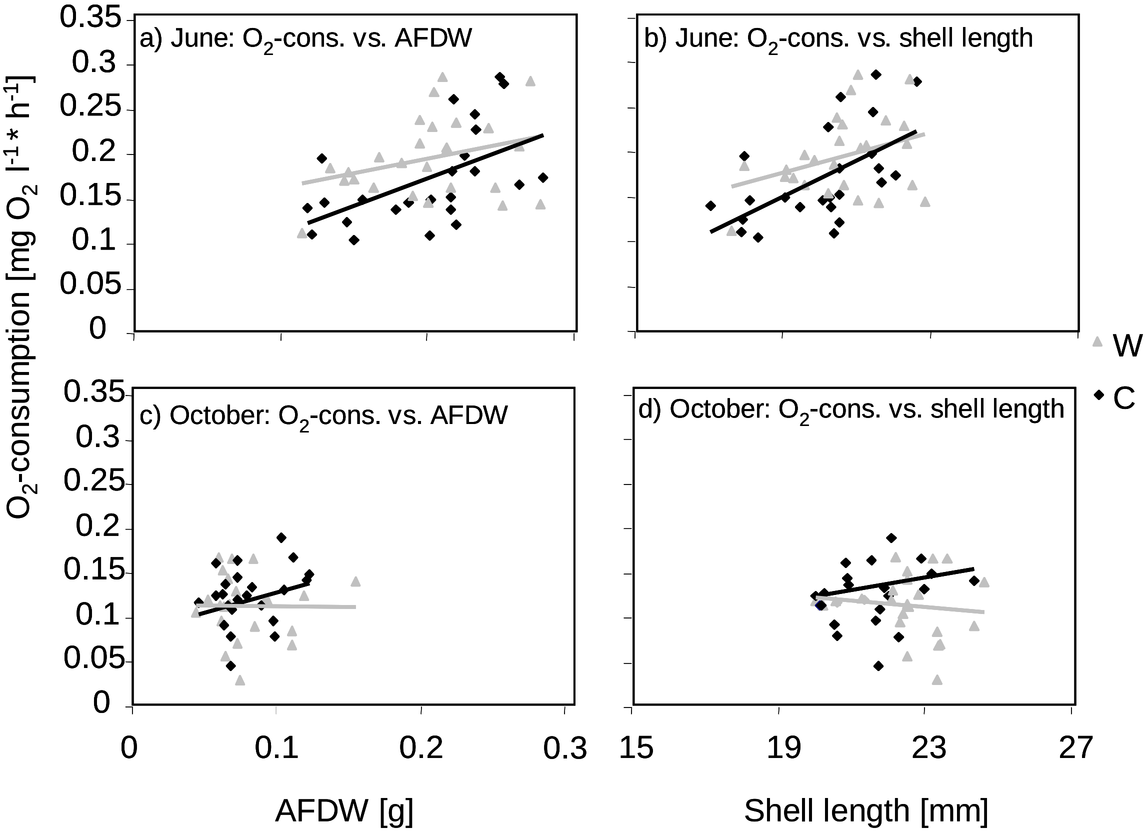

Fig. 8: O_2-consumption of *Corbicula fluminea* given as linear regressions versus AFDW (a+c) and versus shell lenth (b+d). The measurements were performed in June (a+b) and in October (c+d) at 18°C (C) and 21°C (W). The part of the experiment in June resulted from a collaboration with Catherine Linn.

Tab. 3: Results of the linear regressions and correlation analyses regarding the dependence of oxygen consumption on (a) AFDW and (b) shell length. Bold values indicate significant relationships. The part of the experiment in June resulted from a collaboration with Catherine Linn.

a) Linear regression and correlation of oxygen consumption versus AFDW

Date	Temperature	Formula	R²	p
June	C	y=9.614x+0.05	0.297	**0.003**
	W	y=0.319x+0.132	0.095	0.063
October	C	y=0.446x+0.089	0.086	0.087
	W	y=-0.013x+0.115	0.0001	0.433

b) Linear regression and correlation of oxygen consumption versus shell length

June	C	y=0.020x-0.231	0.329	**0.001**
	W	y=0.011x-0.035	0.118	**0.043**
October	C	y=0.007x-0.031	0.062	0.126
	W	y=-0.004+0.197	0.014	0.293

The linear regressions revealed the best fit (with respect to the coefficient of determination compared to exponential and logistic models) in displaying the results of the oxygen consumption experiment in spite of the high variances. Specific parameters for the regressions are given in Tab. 3.

The results regarding the dependence of respiration rate on the test temperature (19-32°C) revealed an exponential increase of O_2-consumption with the temperature up to 30°C (Fig. 9). Within that temperature range, the average Q_{10} values were 2.77 for the Δ0°C treatment and 3.40 for the Δ+3°C (Table 4).

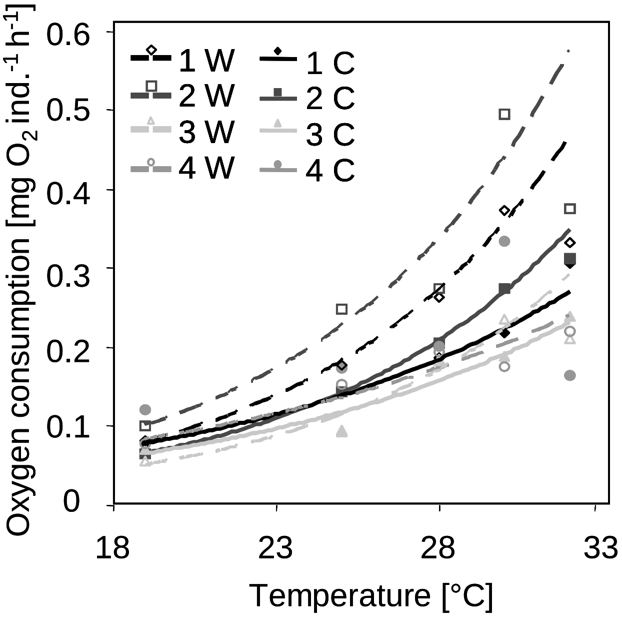

Fig. 9: Oxygen consumption of unfed mussels of the Δ+3°C treatment (W) and the Δ0°C treatment at 19°C, 25°C, 28°C, 30°C and 32°C. The regressions were calculated on the basis of the measurements between 19°C and 30°C. This part of the experiment resulted from a collaboration with Catherine Linn. See Fig. 11 for a comparison of respiration rate and feeding rate with temperature.

Tab. 4: Results of the exponential regression analyses for the dependence of oxygen consumption rate on temperature (19-30°C) following the formula $y = A*e^{(B*X)}$ with y = oxygen consumption rate at temperature X. The measurements were performed at 19°C, 25°C, 28°C, 30°C and 32°C. C and W represent mussels taken from the ambient or the warmed temperatures, respectively.

Mussel		AFDW [g]	Shell length [mm]	A	B	r^2	Q_{10}	Mean Q_{10}	SD of Q_{10}
C	1	0.177	20.68	0.013	0.095	0.997	2.58	2.77	0.58
	2	0.107	17.84	0.006	0.128	1.000	3.60		
	3	0.157	20.13	0.011	0.096	0.888	2.62		
	4	0.123	18.87	0.024	0.082	0.875	2.26		
W	1	0.171	21.67	0.006	0.134	0.997	3.83	3.40	0.78
	2	0.193	22.12	0.008	0.133	0.950	3.78		
	3	0.114	19.20	0.004	0.132	0.955	3.75		
	4	0.123	19.04	0.019	0.080	0.889	2.23		

The temperatures which were chosen for this experiment were the same as those used by Viergutz et al. (2007) for measurements of the grazing rate of

Corbicula fluminea. This now enables a discussion of the decreasing grazing rate data in context to the respiration rate (see Fig. 11 for the comparison of grazing and respiration rates).

Mortality rates

The mortality of *Corbicula fluminea* was recorded during the weekly shell length measurements. Dead mussels were replaced by colour marked mussels. The replaced mussels were not considered for the mortality recording at later measurements.

The impact of food and warming on the mortality of *Corbicula fluminea* is shown in Fig. 10. Temperature had a significant impact on the mortality of *Corbicula fluminea* in both warmed treatments (Table 4). An average of 11 (±6 SD) mussels (out of a total number of 70) died in the warmed and unfed treatments and 14 (±6 SD) in the warmed and fed treatments, whereas only 4 (±2 SD) mussels died in average in the non-warmed and unfed treatments and 5 (±2 SD) in the non-warmed and fed treatments (Fig. 10a). In the Δ 0°C treatments mortality occurred more or less continuously over the course of the experiment (Fig. 10b). The impact of food on the mortality rate was not significant regarding the total number of dead mussels. However, the development of mortality events was different between the fed and the unfed mussels especially in the warmed treatments. Here the main period of mortality was in the warmed tanks without food supplementation between September and October (Fig. 10b).

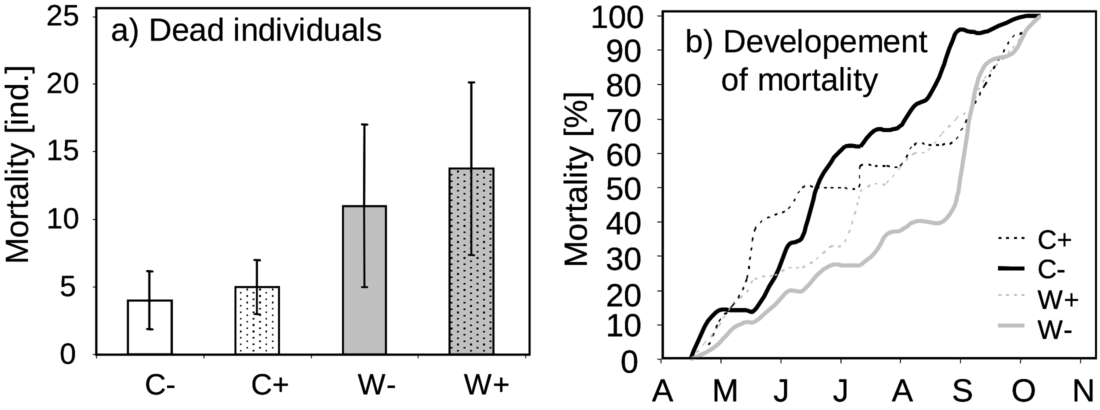

Fig. 10: Mortality of *Corbicula fluminea*. (a) Average number of dead mussels for the time period from the start in April (with 70 mussels per tank) until the end of the experiment in October. Replaced mussels or those which were taken for the AFDW analysis were not considered for this figure. (b) Temporal development of mortality (as given in percentage to total mortality). The data until July resulted of a collaboration with Catherine Linn.

Tab. 4: ANOVA results for testing the impact of food and temperature on total mortality rate of *Corbicula fluminea* during the total experimental period.

	SS	*df*	F	p
Temperature	127.973	1	4.969	0.046
Food	0.473	1	0.018	0.894
Temperature x food	38.285	1	1.487	0.246
Error	309.047	12		

Discussion

Temperature elevation by 3.4°C and a constant food elevation over the whole experiment affected body mass, growth and respiration rate of *Corbicula fluminea*. Temperature influences different aspects of the biological activity of ectotherms, such as the metabolic rate, feeding activity and growth (Gillooly et al., 2001; Enquist et al., 2003; Savage et al., 2004). Thus temperature increase stimulates growth rates within an optimal temperature range and under optimal nutritional conditions. However, warming can also decrease the standing crop (population and individual body mass) at times of limited resources to balance enhanced metabolic costs (Savage et al., 2004).

The body mass decrease over the summer as well as the continuous loss of the shell length specific body mass was reproducible in the experiments performed in the summers of 2004, 2005 and 2007 (chapters 1, 2, 3) as well as the influence of temperature on body mass (chapters 2, 3). The effect of supplemented food was already discussed in chapter 2. The present study was characterized by a long period of investigation, very frequent shell length measurements and a high number of body mass analyses. Together with measurements of respiration rates, this data gives evidence that shell growth and body mass of *Corbicula fluminea* are semi-independently controlled by temperature and food, respectively.

Different mechanisms of controlling shell length and body mass increase

The results show a loss of body mass between June and October which was significantly enhanced by temperature elevation in July and August (Table 1). The mussels in the River Rhine face low plankton concentrations during summer (Weitere et al., 2005) which result in food-limited conditions (chapters 1, 2), even under ambient Rhine temperatures. This was also the case in the present study (Fig. 4). The temperature elevation increased the metabolic rate and therewith the nutritional requirements of *Corbicula fluminea*, an effect which is also known from other ectotherms (Chomsky et al., 2004; Weitere et al., 2004). An additional food source resulted in a distinctly higher body mass and an enhanced shell growth.

Remarkable is the shell length increase in all treatments over the whole experiment, even at those times of the highest body mass decrease. Whereas body mass is controlled by the amount of food and the ability of *Corbicula fluminea* to accommodate for starving periods by adjusting their body mass, the growth of the shell length is controlled mainly by temperature. However, food also affected shell length increase, particularly during periods of low food concentration in summer, resulting in the highest shell length increase in the warmed and fed treatments.

Temperature impact on respiration rate

As one would expect, higher oxygen consumption rates were recorded at higher temperatures. The idea that metabolic rate depends on body mass (Gillooly et al., 2001) was found only in the measurements from June. In October, when the lowest body mass was reached, the oxygen consumption was clearly reduced and the values from the warmed treatment seem to be independent from body mass or shell length (Fig. 7).

An exponential increase of the respiration rate was found between 19 and 30°C. By comparing the respiration rates to the filtration rates measured by Viergutz et al., (2007) (Fig. 9), a mismatch between food uptake and food demand becomes visible (Fig. 11).

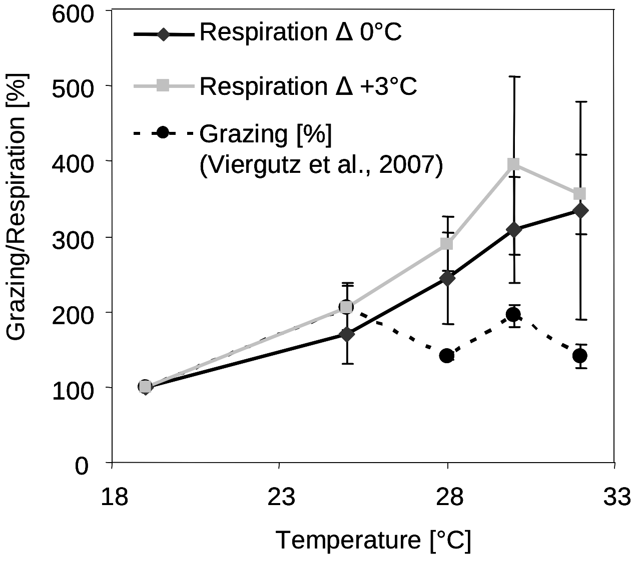

Fig. 11: Relative respiration rates of the Δ 0°C and the Δ +3°C treatments given as averages (single values see Fig. 9) versus grazing rates of *Corbicula fluminea* (after Viergutz et al., 2007). Respiration rates as well as grazing rates at 19°C were set to 100%. Measurements of respiration rates resulted from a collaboration with Catherine Linn.

Respiration rate and grazing rate increased in parallel up to 25°C, after which point the grazing rate decreases whereas the oxygen consumption still increases up to 30°C. Thus, there is a clear gap between the increasing food demand and the constant or even decreasing food uptake beyond 25°C. There are two factors which might influence the body mass of the mussels. At temperatures below 25°C the limiting factor for the mussels' nutrition is food content of the surrounding water.

Temperatures between 25°C and 30°C might be the range in which the highest body mass decrease takes place, because this seems to be the beginning of an increasing gap between food uptake rate and food demand. In 2004 (chapter 1), the Rhine temperature exceeded 25°C for 204 hours. The duration in which the temperature in the Rhine was above 25°C in 2005 (chapter 2) was only 12 hours (just one day on July 29), but was 764 hours in the warmed treatments. The ambient temperature in the present (2007) study never exceeded 25°C during the whole experiment and only a short period of eight days in the warmed treatments. The body mass decreased in all the three experiments but the intensity was different between the years. The average AFDW of the mussels in October 2004, the year with the highest summer temperatures, was 0.016 g. The other experiments were terminated with average AFDWs of 0.032 g and 0.071 g in 2005 and 2007, respectively. These differences can be explained by the different sizes of the mussels. A comparison of the body mass situation in all the three experiments of the non-manipulated mussels (which experienced non-manipulated Rhine conditions during the whole experiment) in October demonstrates that the size-specific body mass in 2004 is clearly different from the other years (Fig. 12). When comparing the mussel sizes in 2005 and 2007, the mussels in 2007 were larger (due to larger starting sizes), but they displayed similar relative body masses according to the length-weight regression (Fig. 12). The duration and intensity of the starving situation is essential for the manifestation of body mass decrease in the respective years, which gives a perspective to the scenarios of the predicted increasing frequency of summer heat waves. In summary, the comparison between the temperature response of the respiration and ingestion rate suggests that temperatures above 25°C are critical for *Corbicula fluminea*, a range which was rarely reached in the present experiments.

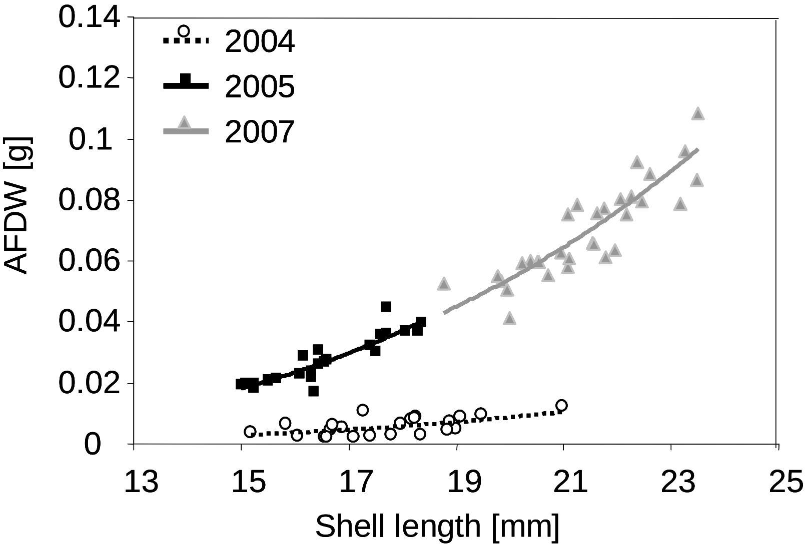

Fig. 12: Comparison of size-specific body mass (AFDW) of *Corbicula fluminea* in October between the three experiments (chapters 1, 2, 3). Only the non-manipulated treatments were used for this comparison. Different mussel sizes resulted from different starting values.

Mortality rates

The initial assumption was that summer mass mortality in Corbicula fluminea is due to a negative energetic balance because of high metabolic rates at low food uptake levels (low algal abundances in summer). This low food uptake is even enforced at high temperatures because of the differential development of grazing (decrease above 25°C) and respiration (increase up to 30°C) (Fig. 11). In light of these energetic constraints, our hypothesis was that increased mortality can be negated by food supplement, which would not be the case when otherwise lethal temperatures are reached. However, this hypothesis had to be rejected; mortality was independent of food quantity. The reasons for the warming-related mortality are as yet unclear; it is still unlikely that temperatures lethal for *Corbicula fluminea* were reached in the Rhine and in our experiments, as the clam originates from warmer areas and tolerates temperatures up to 37°C (Mattice & Dye, 1976). However, one has to keep in mind that we did not face mass mortalities in the experiments; mortality rates were still below 20% in the warmed treatments. Furthermore, the temperature was always relatively low during the experiments presented here (see Fig. 3). Thus, future studies should be aimed at determining whether or not mass mortalities at high temperatures (as observed in warm years) can be compensated by high food levels.

Conclusions

The study revealed new details about the response of *Corbicula fluminea* to environmental changes such as temperature and food quantity in terms of the fitness factors body mass, growth and respiration. The influence of temperature on the body mass decrease during summer and the direct relationship between body mass, temperature and food quantity was shown in this study. However, the impact of temperature differs with the seasons. In spring and early summer, temperature elevation stimulated shell length as well as body mass increase. But this situation changed towards summer and autumn when temperature elevation had a negative impact on the fitness of *Corbicula fluminea.* However, the mussels grew over the whole experiment, stimulated by temperature increase even during periods of body mass decrease, which was likewise enhanced by temperature elevation. Such differential control of shell length (by temperature) and body mass (by resource level) is one mechanism how warming generates mussels with low size-specific body mass.

Together with the results of Viergutz et al. (2007), the experiments showed a gap between grazing rate and respiration rate at temperatures above 25°C. This can result in a negative energetic balance because of a mismatch between food uptake rate and food demand when the food concentration of the river water is low. We found decreasing body mass values during summer in all experiments, even when temperature did not exceed 25°C. In consequence, a disproportion between respiration and food uptake might occur already below 25°C if the food concentration is too low. However, the present data suggests more rapid effects (including possibly mass mortality) above 25°C, a possibility which needs to be tested in the future.

Chapter 4

Grazing impact and phenology of the freshwater sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* under experimental warming

Abstract

Both the clearance rates and the autumn to spring occurrence during of the freshwater sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* were tested under different temperature regimes. The experiments were performed in bypass systems of the River Rhine (Cologne, Germany) in which the temperature could be manipulated. The impact of temperature increase on the grazing rates depends upon the grazer. *Ephydatia muelleri* showed a clear increase in the clearance rates with increasing temperature. *Plumatella emarginata* was not significantly affected by the experimental warming.

Distinct differences in the food preference were found for the sponge (which in an efficient grazer on bacteria and small algae) and for the bryozoan *Plumatella emarginata* (which feeds primarily on large algae; no significant grazing on bacteria). In contrast to the temperature response of the clearance rates, the respiration of both *Plumatella emarginata* and *Ephydatia muelleri* increased with temperature between 19 and 32°C, suggesting that the risk of experiencing energy deficiency at high temperatures (due to a greater increase of energetic demand than ingestion rate) is particularly high for the bryozoan. A temperature elevation of 3°C above the natural Rhine temperature resulted in a delay of the disappearance of active tissue and formation of resting stages for the *Ephydatia muelleri* in autumn. This delay was of between eight days (beginning of gemmulation) and 22 days (termination of gemmulation). In contrast, there was no distinct effect of warming on the disappearance of active zooids of the two bryozoan species in autumn. However, warming can positively affect the maintenance of active zooids during winter in *Fredericella sultana*. In spring, the appearance of active zooids of *Plumatella emarginata* was clearly stimulated by temperature elevations, whereas the hatching of both *Fredericella sultana* and *Ephydatia muelleri* was hardly affected by warming. The study demonstrated some different patterns in the thermal ecology of both freshwater sponges and bryozoans in comparison to other filter feeders, particularly mussels. Such patterns need to be considered when predicting the impact of temperature on the pelagic-benthic coupling in aquatic habitats.

Introduction

Benthic filter feeders can play an important role in the matterflux of shallow aquatic ecosystems. Particularly in rivers, they can control the abundance and composition of plankton communities via their grazing activity and thus act as trophic link which imports planktonic production into the benthic food web (Welker & Walz, 1998; Jack & Thorp, 2000; Descy et al., 2003). The increasing awareness of the importance of this biological process in recent years has changed our earlier understanding which was that predominantly physical factors control the plankton in lotic ecosystems (Thorp & Casper, 2003; Strayer et al., 2008).

Most previous studies dealing on the benthic control of plankton in freshwater systems concentrated so far on bivalves, particularly on the invasive species *Dreissena polymorpha* and *Corbicula flumniea* (Cohen et al,. 1984; Jack & Thorp, 2000; Descy et al., 2003; Strayer et al., 2008). This focus stands in contrast to the large variety of potential consumers of plankton in the benthos including protozoans, sponges, bryozoans and several different insect larvae (e.g. Weitere et al., 2003; Brown et al., 2005; Dröscher & Waringer, 2007). Among the macrofauna, particularly sponges and bryozoans are potential consumers of pico- and nanoplankton (Okamura & Doolan, 1993; De Santo & Fell, 1996; Pile et al., 1997), which generally dominates the plankton biomass in rivers (Reynolds & Descy, 1996; Weitere et al., 2005). Several studies have shown that both sponges and bryozoans can reach high densities in rivers and other shallow freshwater systems (e.g. Ricciardi & Reiswig, 1994; Økland & Økland, 1996; Wood et al., 1998; Gugel, 2000; Økland & Økland, 2001; Feslet al., 2005; Dröscher & Waringer, 2007). Both taxa are often considered as important filter feeders, even though their grazing behaviour has barely been measured in freshwater systems.

The importance of the bentho-pelagic coupling in many river ecosystems has raised questions as to which factors influences the strength of the interactions between benthic filter feeders and their planktonic prey (e.g. Ackerman, 1999; Weitere & Arndt, 2002, Strayer, 2008). Especially temperature has a great impact on biological rates and thus on both the clearance rates of the filter feeders (Reeders & bij de Vaate 1990; Aldridge et al., 1995; Lei et al., 1996) as well as on the growth rates of their planktonic prey (Montagnes et al., 2003) Temperature might thus be an important predictor for variations in the grazing impact of benthic filter feeders under both natural temperature variations as well as under man-made temperature changes due to heat emissions and global warming (IPCC, 2007). By using the mussels *Corbicula fluminea* and *Dreissena polymorpha* as model grazers, we have recently shown that warming can strongly alter the abundance of natural nanoplankton communities under the control of the mussels due to a differential development of grazing and growth rate with temperature (Viergutz et al., 2007; Weitere et al., 2008). Due to size-selective grazing by the mussels, this warming effect on the nanoplankton (the predominant prey of mussels) can cause cascading effects on the picoplankton (weakly preyed upon by the mussels but predominant prey of heterotrophic nanoflagellates) (Weitere et al., 2008). Whether or not such mechanisms revealed from experiments are applicable for the field depends largely on the grazing behaviour of concurrent dominant filter feeders. Important steps towards an understanding of such processes are determing of both the temperature dependence of the grazing activity as well as the pray size selection of other relevant filter feeders besides mussels.

In contrast to mussels, both freshwater sponges and bryozoans form non-trophic resting stages during winter and also during starvation and drought periods (Pronzato et al., 1993; Wood & Okamura, 2005). The resulting large inter-annual variation in the abundance of these grazers, particularly the disappearance of trophic stages during winter, is another important factor (besides feeding rates) which determines their grazing impact. Both the formation of resting stages in autumn as well as their hatching in spring can correlate with certain temperatures (Black & Proud, 1976; Weissenfels, 1989; Gugel, 2000). Nevertheless experimental on the temperature-dependence of the timing of these events are lacking.

In the present study we performed comprehensive measurements on the feeding behaviour and the timing of dormant phases in a freshwater sponge (*Ephydatia muelleri*) and two freshwater bryozoans (*Plumatella emarginata* and *Fredericella sultana*) under increasing temperatures. The measurements were accompanied by measurements on the temperature dependence of the respiration rate in order to gain a proxy for the relative energy demand in relation to the feeding rates. The following null-hypotheses were tested for both taxa:

(1) The clearance rates depend on temperature in a similar pattern as observed for filter-feeding mussels.
(2) With increasing temperature, the feeding rates develop in parallel to the respiration rates, thus balancing metabolic losses at high temperatures.
(3) The formation of resting stages in autumn as well as their hatching in spring is temperature-controlled. Warming thus reduces the non-trophic time period during winter.

Material and Methods

Study site

The experiments were performed at the Ecological Rhine Station of the University of Cologne, a laboratory on a ship situated in the navigation channel of the River Rhine in Cologne, Germany (Rhine km 685.5). By using pumps which are installed at the bottom of the ship and which inject the river water into a pipe system, the laboratories are served with fresh Rhine water. Such river bypasses were used for the experiments described below.

Origin and growing conditions for **Plumatella emarginata** *and* **Fredericella sultana**

The two bryozoan species *Plumatella emarginata* and *Fredericella sultana* form dense populations in the Rhine and accordingly within the Rhine-water supported flow channel systems of the Ecological Rhine Station of the University of Cologne. The bryozoans started growing (hatching of statoblasts) in March and the zooids usually disappeared in November. The filtration experiments for *Plumatella emerginata* took place between May 16 and October 31 and the organisms for the experiments were taken from the stocks within the flow channels, which were thus adapted to natural Rhine conditions (including ambient temperatures and ambient concentrations of suspended food particles).

For the experiments on the winter phenology, bryozoan-covered object slides were used. The object slides were exposed in the flow channels in spring 2006 and 2007. During the time between exposure and start of the experiments in October (see below) both *Plumatella emarginata* and *Fredericella sultana* covered the surface of the object slides.

Origin and growing conditions for **Ephydatia muelleri**

As a typical freshwater sponges *Ephydatia muelleri* was used for the experiments. In the case of sponges it is difficult to sample defined units (e.g. defined number of zooids as done for the bryozoans) for the performance of grazing experiments. Furthermore, a species determination is only possible by considering the gemmules. In order to have homogenous units within each experiment and to ensure the existence of single species assemblages, sponges were grown according to a standardized protocol. For this purpose, gemmules of *Ephydatia muelleri* were sampled at the banks of the River Rhine next to the Ecological Rhine Station (see above) in autumn 2006. The gemmules were kept at a constant 4°C until February 2007. At that time the gemmules were put on object slides (one per slide) and were incubated at 25°C inside a Petri dish for 48 hours. The object slides were moistened with one drop of water to stimulate the hatching of the sponge in the middle of the object slide and without washing the gemmules away during the time of incubation. A wetted tissue next to the object slides ensured a constantly high humidity within the Petri dishes to prevent the withering. After 48 hours the germination had started and was controlled with the help of a binocular microscope. At this time of hatching the object slides with the small sponges attached were vertically fixed inside a 120 l aquarium filled with Rhine water. The water was aerated and a quarter of the volume was changed weekly. The temperature was adjusted to a constant 17°C, and the sponges were fed with Liquifry No.1 (Liquifry Co. Ltd.,Dorking, GB) according to a protocol recommended by Weissenfels (1985). The sponges reached a size of about 5mm in diameter within four weeks. At that time the object slides were transferred into a channel exposed to the direct flow of the Rhine at the Ecological Rhine station. In this position the sponges grew with the natural food particles of the river at ambient temperatures. From this stock the sponge-covered object slides were chosen randomly for each filtration and respiration experiments, as well as for the phenology experiment (Table 1).

Tabel 1: Overview and conditions for the filtration experiments. The Rhine temperature as well as the plankton abundances gives the actual conditions in the river water at each experiment. The lowest experiment temperature is the half-monthly average since 1989. All experiments were performed in 2007.

	Plumatella emarginata			***Ephydatia muelleri***		
Date	**May 16**	**Jun 29**	**Aug 31**	**Jun 01**	**Jul 06**	**Oct 05**
Age [weeks]	-	-	-	11	16	29
Rhine temp. [°C]	16.2	21.5	18.9	18.1	20.2	17.4
Experimental temp. [°C]	19, 22, 25	22, 25, 28	24, 27, 30	20, 23, 26	22, 25, 28	17, 20, 23
Chamber vol. [ml]	16	16	16	15	15	15
Retention time [h]	0.05	0.22	0.14	0.08	0.10	0.10
Zooid number	263 ±27SD	218 ±50SD	269 ±35SD	-	-	-
AFDW [g]	-	-	-	0.0085 ±0.002SD	0.012 ±0.003SD	0.04 ±0.011SD
Colonized surface area [mm²]	-	-	-	397 ±78SD	530 ±91SD	864 ±158SD
Start abundace nanopl. [cells ml^{-1}]	6412 ±955SD	950 ±215SD	1000 ±47SD	1350 ±370SD	750 ±272SD	530 ±38SD
Start abundace picopl. [cells ml^{-1}]	2x10^6 ±2x10^5SD	1.7x10^6 ±1.4x10^5SD	2.6x10^6 ±3.7x10^5SD	5.2x10^6 ±1.8x10^5SD	3x10^6 ±6.3x10^4SD	1.8x10^6 ±3x10^5SD
Bacteria size classes (first, second and third quartile) [µm]	0.3, 0.4, 0.8	0.3, 0.4, 0.7	0.4, 0.6, 1.0	0.3, 0.4, 0.7	0.4, 0.5, 1.0	0.4, 0.7, 1.0

Filtration experiments

The filtration experiments were performed with the bryozoan *Plumatella emarginata* and the sponge *Ephydatia muelleri* in flow-though systems perfused with natural Rhine water. The natural plankton organisms (containing both pico- and nanoplankton) served as food items. The clearance rates were calculated based on the abundances of the food items in the in- and outflow of the flow chambers and were corrected for passive losses by considering flow chambers with the same setup with heat-killed grazers as controls.

For the sponge experiments flat flow chambers which resembled the flow cells as used by Norf (2008) were used. In the present study the chambers were modified so that they were large enough to place sponge-covered object slides into them. The cells had the inner dimensions of 7.75 x 2.75 x 0.70 cm, which corresponds to a volume of 15ml. The cells were equipped with three inflows and three outflows to ensure a regular flow over the whole width of the cells.

For the bryozoan experiments small tubes of 17 mm in diameter, 120 mm in length and with a total volume of 16 ml were used. During the experiments the tubes were vertically adjusted and equipped with an inflow at the tapered bottom and an outflow at the screw top.

For both taxa (Porifera and Bryozoa) three filtration experiments were performed between May and October 2007. The dates and conditions during the experiments, including the grazer densities are summarized in Table 1. The flow chambers were placed into temperature-controlled water tanks (three different temperatures for each experiment simultaneously) for all experiments. The lowest temperature (base temperature) at each experiment was calculated as the half-monthly average of the Rhine at Cologne since 1989 and the other two temperatures represented enhancements of 3°C and 6°C above the half-monthly averages (Table 1). Three to four replicates were considered for each temperature treatment.

A bypass with natural river water was installed to each of the flow chambers. The setup was prepared on the day before the experiment started in order to allow an acclimation time of 15 hours to the experimental conditions before the clearance rates were measured. Pre-experiments at the base temperature but with different flow velocities were performed in preparation to the experiments. These pre-experiments were used to adjust the flow rate. The flow rate for the main experiments was adjusted to allow reduction by ⅓ of the preferred planktonic food (nanoplankton for bryozoans; bacteria for sponges) due to the grazing activity within the chamber. However, the flow velocities in all experiments were generally low and laminar (see Table 1).

Quantification of plankton organisms and analysis of clearance rates

Grazing rates were determined for different size classes of the plankton organisms. Planktonic algae and heterotrophic flagellates (mostly nanoplankton, 2-20 µm) were live-counted using phase-contrast microscopy as described by Weitere and Arndt (2002). The size of each individual was measured with the help of a calibrated eye-piece micrometer. Bacteria (exclusively picoplankton, 0.2-2 µm) were fixed in glutaraldehyde, stained with 50 µg ml^{-1} DAPI (4',6-Diamidino-2-phenylindol) (Porter & Feig, 1980) and filtered onto black polycarbonated membrane filters (0.2 µm Whatman-Nucleopore). Bacteria were quantified with help of fluorescence microscopy and their size was estimated by dividing them into size classes.

The clearance rates were determined by considering the abundances of the planktonic organism in the inflow (N_0, ind ml^{-1}) and the outflow (N_1 ind ml^{-1}) of the chambers as well as the retention time of the water within the chambers (Table 1). The values were corrected with help of control treatments using dead organisms (heat-killed by exposure for ten seconds to hot [100°C] water), in order to check for passive losses such as by sedimentation. Changing rates (r, h^{-1}) were calculated by assuming exponential changes:

$$r = (\ln N_1 - \ln N_0) / t \qquad (1)$$

The differences of the changing rates of the experimental treatments containing the active organisms (r_a) and those of the control treatments (r_c) were considered as grazing rates (r_{gr}).

$$r_{gr} = (r_a) - (r_c) \qquad (2)$$

The grazing rate multiplied by the volume of the flow cell (V) (see Table 1) and divided by the number of zooids (for bryozoans) or the ash-free dry weight

(AFDW, mg) (for sponges), gives the clearance rate (CR, ml ind^{-1} d^{-1}, respectively ml mg^{-1} d^{-1}). The measurement of the AFDW is described below.

Experiments on winter phenology

Experiments on the winter phenology were performed during two consecutive years, in winter 2006/2007 and in winter 2007/2008. In the first experiment, the two bryozoan species (*Plumatella emarginata* and *Fredericella sultana*) were compared. *Ephydatia muelleri* was additionally considered in the second experiment in 2007/2008. The experiments generally focussed on the impact of warming on both the formation of resting stages in autumn and the hatching from resting stages in spring.

In order to test the hypothesis that the formation of non-trophic resting stages depends on temperature, eight flow channels (flow rate 120 l h^{-1}, which corresponds to a flow velocity of 0.004 m s^{-1}) were connected to the river bypass system and divided in two temperature treatments (four replicates each). Thereby the temperature of the channels was, by default, that of the bypass, and was as high as the actual temperature of the River Rhine water. The temperature was increased in the other four channels by 3°C with help of aquarium heaters. The surface area of the sponge growing on object slides was adjusted to exactly 1.5 cm² by cleaning the remaining slide area and afterwards one slide was exposed vertical in each channel. The procedure for the bryozoans was similar. The object slides which were exposed to the River Rhine in spring (see above) were cleaned on one side, the surface on the other side was cleaned, leaving 2.5 cm² covered with bryozoans. The object slides were randomly distributed among the different treatments. Each replicate of the two temperature treatments contained active zooids of both bryozoan species at the start of the experiments in autumn.

The phenology experiment started in winter 2006/2007 on October 11 and in winter 2007/2008 on October 26. None of the sponges in winter 2007/2008 contained gemmules at the beginning of the experiment. The development of gemmules was regularly controlled and the abundance of gemmules within the sample square was quantified until no further changes were detectable. At that time, gemmules were closely packed on the 1.5 cm² and the sponge tissue was degenerated and just the macroscleres (large silicate nails) remained. This area was colour marked on the reverse side of the object slide using a waterproofed marker. When the sponges hatched from the gemmules in spring, the marked area was covered by active sponge tissue within a few days due to the closly packed gemmules. Growth of the sponges was quantified by measuring the area colonized beyond the primary colour marked border.

The two bryozoan species grew on the object slides during the summer season and the total number of the active zooids of each colony within the sample square was counted at the beginning of the experiment. Subsequently, the active zooids within this square were quantified regularly. The frequency of quantification was adapted to the respective situations. At the beginning of the experiment in autumn as well as in spring quantification was performed 2-3 times a week. Almost no changes occurred in December and January. During this period the treatments were checked at intervals of two weeks at which time the water tubes were cleaned and detritus was removed from the channels.

Measurement of oxygen consumption

To determine the impact of temperature on the relative energetic demand, we measured the oxygen consumption in addition to the clearance rates. For this purpose a fibre-optic oxygen mini-sensor (FIBOX 3, 505 nm, PreSens, combined with FIBOX 5.20 Software for data logging) was used to measure the oxygen concentration in a closed glass vessel with a volume of 62 ml. The animals originated from the stock flow channel with Rhine water discharge and were exposed to the test temperatures for two hours before the measurement of the oxygen consumption started. The measurements were performed at 19°C, 25°C, 28°C, 30°C and 32°C. The oxygen consumption per unit (bryozoans or sponge) was calculated from the linear decrease of the oxygen content within the experimental vessel. This oxygen consumption was corrected for the oxygen consumption of the Rhine water (grazer free control). The absolute oxygen consumption due to the individuals was calculated with the corrected oxygen concentration decrease per time and the total water volume within the vessels. Measurement were taken as long as the oxygen concentration within the vessels continued to decreased by at least 0.5 mg L^{-1} which was between

13 and 64 minutes, depending on the temperature. The total number of bryozoan zooids per measurement varied between 207 and 329. The volume of the sponge tissue varied between 808 and 1170 mm^3. The sponge tissue was dried at 60°C for 48 hour in aluminium foil, after which the dry weight was measured. After combustion for 15 hours at 550°C the AFDW was measured as the difference between the dry weight and the incombustible components. The AFDW varied between 0.0107 and 0.0151 g. The oxygen consumption was related to the number bryozoan zooids and to the ash-free dry weight (AFDW) of the sponges within the vessel.

Statistical analyses

The statistical analyses were performed with the software SPSS 15.0 for Windows. To test for significant effects of the temperature on the clearance rates, Spearman rank correlations were performed. The effect of food size class of the nanoplankton on the clearance rates was tested with help of a one-factorial ANOVA. If the ANOVA revealed significant effects, pair wise comparisons per performed with the help of the REGW post-hoc test.

Results

Impact of warming on grazing rates

In both summer experiments (June and July), the clearance rates of *Ephydatia muelleri* feeding on nanoplankton increased significantly under experimental warming up to 470 ml $mg(AFDW)^{-1}$ d^{-1} at 26°C in June and up to 520 ml $mg(AFDW)^{-1}$ d^{-1} at 29°C in July (Fig. 1a). For the picoplankton, clearance rates of up to 600 ml $mg(AFDW)^{-1}$ d^{-1} (June) and 310 ml $mg(AFDW)^{-1}$ d^{-1} (July) were measured (Fig. 1b). The grazing rate of *Ephydatia muelleri* was very low in October (between 62 and 69 ml $mg(AFDW)^{-1}$ d^{-1} on nanoplankton), and there was no significant temperature-induced stimulation measurable on either nano- or picoplankton. For June and July, the positive effect of temperature increase on the clearance rate was significant for both plankton size classes (see Table 2 for results of Spearman rank correlations).

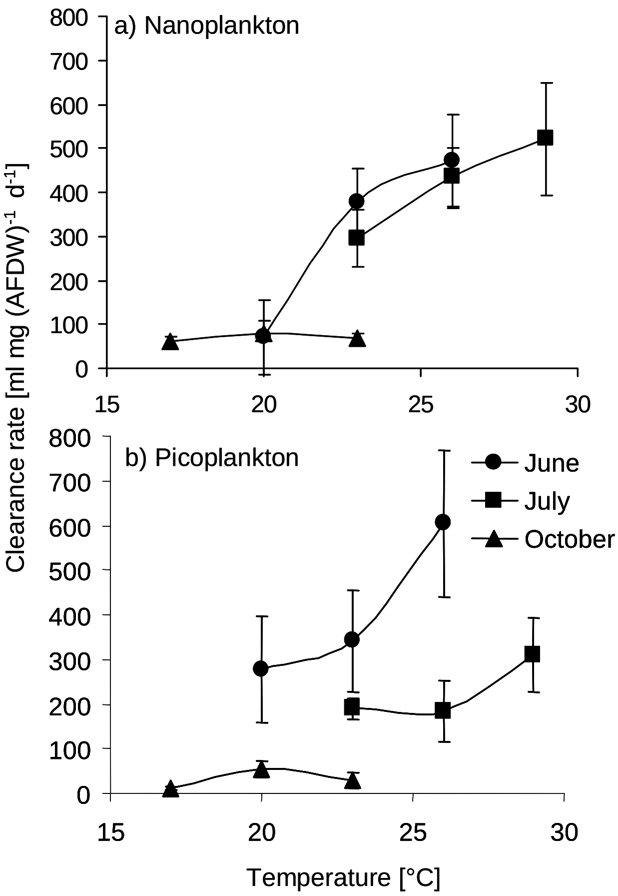

Fig. 1: Temperature dependence of the clearance rates of *Ephydatia muelleri* on (a) nanoplankton and (b) picoplankton for the experiments performed on June 1, July 6 and October 5, 2007.

Table 2: Spearman rank correlations for testing the influence of temperature on the clearance rates of the bryozoan *Plumatella ermarginata* and the sponges *Ephydatia muelleri*. Bold values indicate significances.

Plumatella ermarginata

Date	Temp. range [°C]	Plankton size class	R	p
16 May	19-25°C	Nanoplankton	0.248	0.244
16 May	19-25°C	Picoplankton	-0.321	0.183
29 Jun	22-28°C	Nanoplankton	-0.296	0.175
29 Jun	22-28°C	Picoplankton	0.005	0.494
31 Aug	24-30°C	Nanoplankton	-0.089	0.392
31 Aug	24-30°C	Picoplankton	-0.316	0.204

Ephydatia muelleri

Date	Temp. range [°C]	Plankton size class	R	p
01 Jun	20-26°C	Nanoplankton	0.809	**0.002**
01 Jun	20-26°C	Picoplankton	0.719	**0.009**
06 Jul	23-29°C	Nanoplankton	0.843	**0.002**
06 Jul	23-29°C	Picoplankton	0.726	**0.008**
05 Oct	17-23°C	Nanoplankton	0.270	0.225
05 Oct	17-23°C	Picoplankton	0.424	0.111

The clearance rates of *Plumatella emarginata* showed no significant response to experimental warming in any of the grazing experiments (Fig. 2a, Table 2). The highest grazing activity was detected in May (clearance rates of up to 30 ml ind^{-1} d^{-1} on nanoplankton) at highest abundances of planktonic algae, whereas the two later dated experiments (June 29 and August 31) revealed distinctly lower grazing rates (Fig. 2a). No significant grazing of *Plumatella emarginata* on bacteria (Fig. 2b) was detected. The bacteria in the Rhine were generally small (median between 0.4 and 0.7 µm, see Table 1).

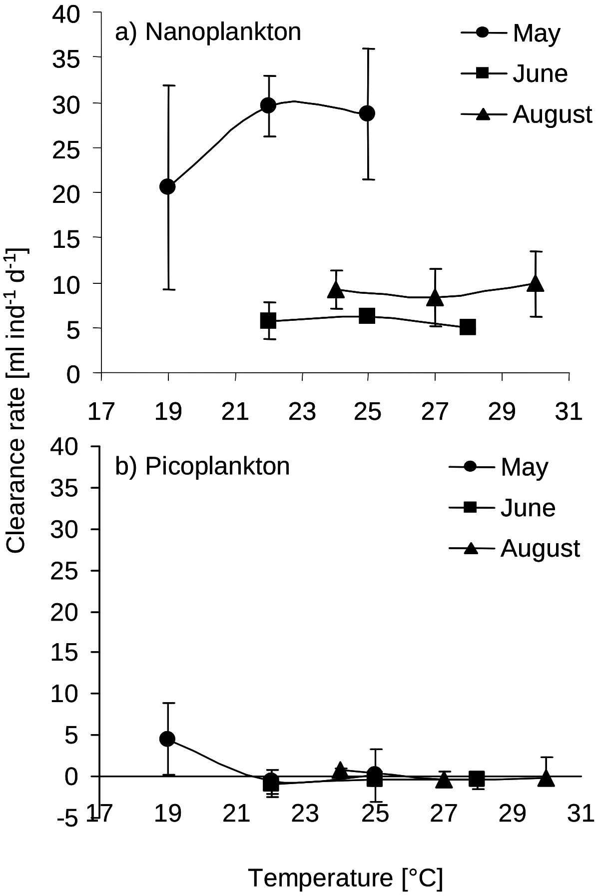

Fig. 2: Temperature dependence of the clearance rates of *Plumatella emarginata* on a) nanoplankton and b) picoplankton for the experiments performed on May 15, June 29 and August 31, 2007. The lowest temperature represents the long-term average temperature at the particular time of the year.

Regarding the size preference in the nanoplankton, we found a significant, stronger grazing impact of *Plumatella emarginata* on the larger size classes (see Table 3 for ANOVA-results and Fig. 3 a-c for post-hoc results). There were significantly higher clearance rates for the smallest size class (2-5 µm) compared to the intermediate size class (5-15 µm) for all three experiments. The intermediate size class had a higher clearance rate than the large size class (>15µm) in the June-experiment only. *Ephydatia muelleri*, which was shown to feed on pico- and nanoplankton organisms at similar rates, showed no significant preference to the nanoplankton size classes in July and a preference for the 5 – 15 µm size class in comparison to the >15 µm size class in June (Fig. 3d, e, Table 3).

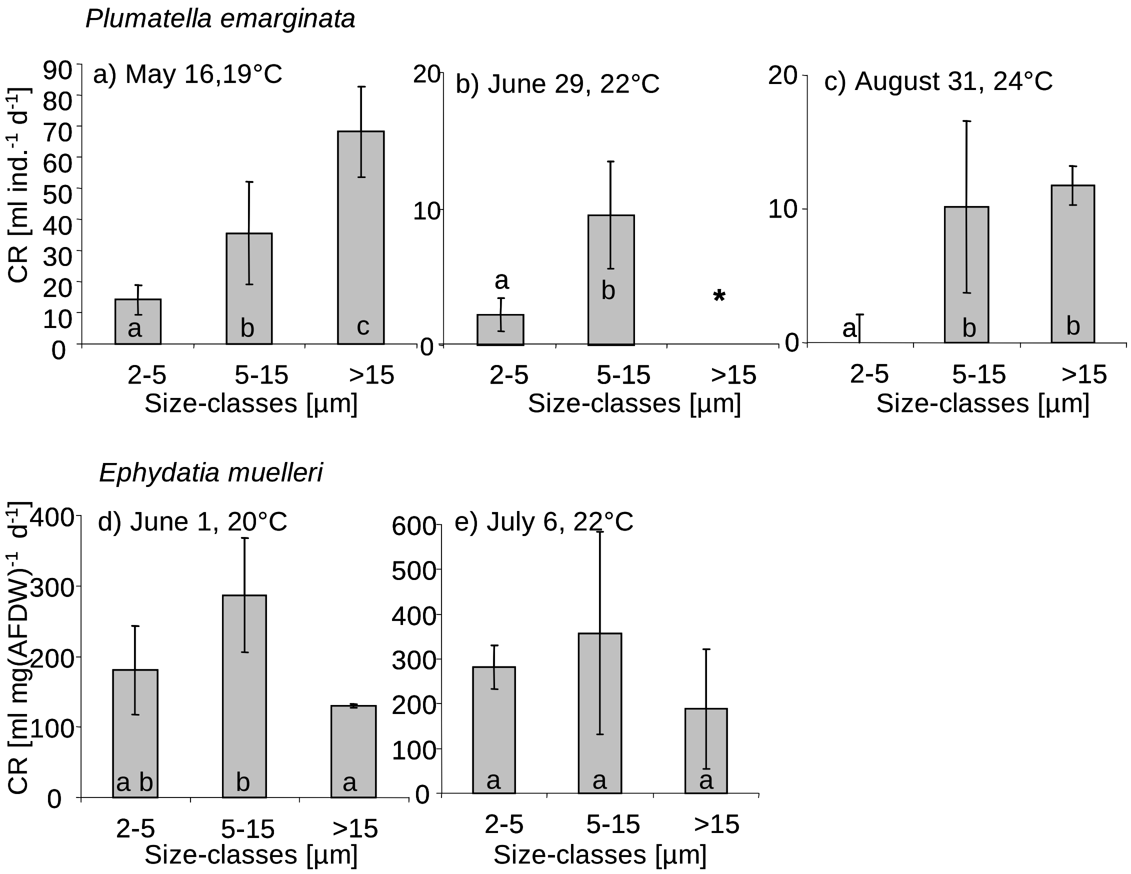

Fig. 3: Size selection in the bryozoan *Plumatella emarginata* (a, b, c) and the sponge *Ephydatia muelleri* (d, e) on three nanoplankton size classes (2-5µm, 5-15µm and >15µm). The data refer to the base temperature of each experiment. Because of extremely low abundances of the size class '>15µm' in the River Rhine on June 29, only the clearance rates (CR) of the first two size classes is given in the figure at this date. The last experiment on *Ephydatia muelleri* is not considered because of extremely low total clearance rates (see Fig. 2). Different letters indicate significant differences in REGW post-hoc test. See Table 3 for ANOVA results.

Table 3: ANOVA results for testing the influence of the nanoplankton size classes (2-5 µm) on the grazing efficiency of the bryozoan *Plumatella ermarginata* and the sponges *Ephydatia muelleri*. Because of low abundances of plankton organisms larger than 15µm on June 29, the ANOVA was performed with only 2 size classes at this date.

Experiment (date)	SS	*df*	F	p
Plumatella ermarginata				
Size (May 16)	5937	2	17.684	**0.001**
Error	1510	9		
Size (Jun 29)	80	1	9.430	**0.037**
Error	34	4		
Size (Aug 31)	261	2	7.934	**0.021**
Error	99	6		
Ephydatia muelleri				
Size (Jun 01)	45498	2	5.814	**0.033**
Error	27392	7		
Size (Jul 06)	49831	2	1.088	0.388
Error	160370	7		

Impact of warming on respiration rate

The oxygen consumption increased exponentially with increasing temperature for both the sponge and bryozoan. For *Ephydatia muelleri,* the response of the respiration rates (RR, mg O_2 g(AFDW)$^{-1}$ h^{-1}) on temperature (t, °C) followed the formula RR = $0.097e^{0.501t}$ (r^2=0.955) (Fig. 4a). For *Plumatella emarginata* the response of the respiration rates (RR, mg O_2 g(AFDW)$^{-1}$ h^{-1}) to temperature changes followed the formula RR = 10^{-06} $e^{0.079t}$ (r^2= 0.864). This corresponds to Q_{10} values of 1.67 and 2.20 for the sponge *Ephydatia muelleri* and the bryozoan *Plumatella emarginata*, respectively. The respiration rates increased with increasing temperature up to the highest considered temperature (32°C, Fig. 4), which stands in direct contrast to the results of the grazing experiments for *Plumatella emarginata*, in that the food uptake was not affected by warming (Fig. 2).

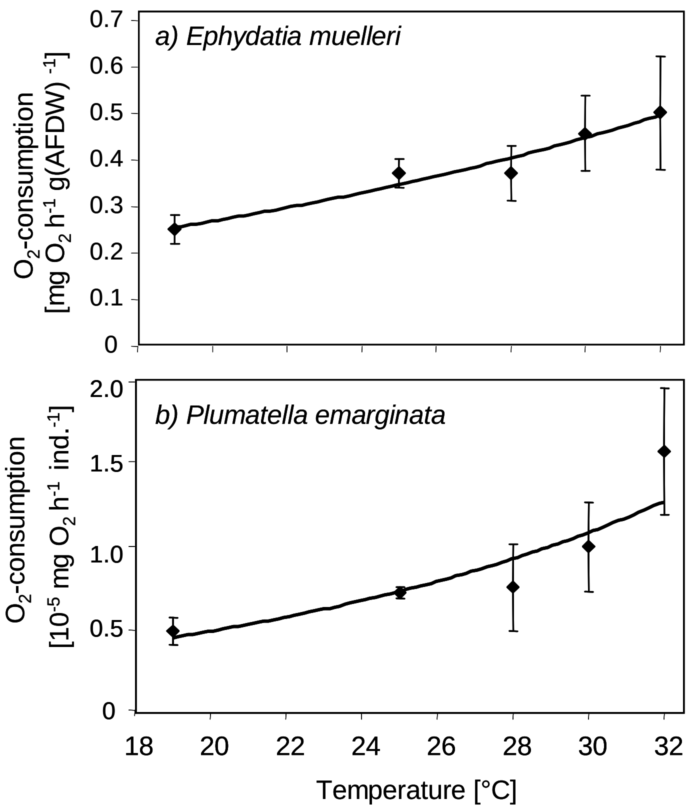

Fig. 4: : Effect of temperature on the respiration rate (O_2-consumption) of (a) the sponge *Ephydatia muelleri* and (b) the bryozoan *Plumatella emarginata*. The trend lines represent the exponential regression lines.

Impact of warming on phenology during winter

The effects of warming of 3°C rise in temperature above the ambient Rhine temperature differed between the bryozoan species *Plumatella emarginata* and *Fredericella sultana* as well as the sponge *Ephydatia muelleri* with respect to the phasing out of trophic stages in autumn, the overwintering of trophic stages (in one case) and the hatching from resting stages in spring. The phasing out of *Plumatella emarginata* was not affected by temperature increase in either winter (2006/07 and 2007/08). In this species, no active zooids were encountered in winter after November 28 in 2006/07 and after November 22 in 2007/08 (Figs. 5b, 6b). The abundance of *Fredericella sultana* also decreased strongly in October of both years (Figs. 5c, 6c). However, active zooids of this specie remained during the warm winter of 2006/07, but not during the colder winter of 2007/08 (see Figs 5a, 6a for temperature records). In contrast to the phasing-out in autumn, the hibernation of active zooids was stimulated by warming. Between November 2006 and January 2007, the abundance of *Fredericella sultana* was distinctly higher in the warmed treatment (maximal abundances within this time period: 2.5 ± 0.5 ind. cm^{-2} in the warmed treatment and 1.0 ± 0.7 ind. cm^{-2} in the ambient treatment). Hatching and re-colonisation of *Plumatella* *emarginata* started at the beginning of March 2007. In this year, the temperatures in April were high (up to 20°C on April 29). Correspondingly, a strong increase of the abundance of *Plumatella emarginata* took place at the time of the strongest temperature increase (i.e. from March 25 onwards) and resulted in a more than five times higher abundance in the warmed treatments on April 25. In 2008, we found similar temperature effects on the hatching of *Plumatella emarginata* (Fig. 6b), which hatched in the ambient temperature treatment on April 02 and in the warmed treatments on March 03, initially with low abundances (<30 ind. cm^{-2}). Here, however, the ambient temperature in early spring was distinctly lower than in spring 2007. Correspondingly, significant increases in the abundance of active zooids were found from April 12 (at 12.8°C) onwards in the warmed treatments and twelve days later (from April 24 [at 12.2°C] onwards) in the cold treatments. Subsequent mass developments were found in the warm treatments in the period from May 01 to May 09, when temperatures increased from 13.6 to 18°C. In *Fredericella sultana*, distinct increases in the zooid abundance appeared earlier than in *Plumetella emarginata* and no distinct effects of the experimental temperature manipulation occurred, whereas the pattern differed between the years (start of rapid increase on March 06 in 2007 and in April 01 in 2008). The quantification of the bryozoans was terminated in both yeas when very high abundances of *Plumatella emarginata* were reached, making any further accurate quantification impossible.

Gemmulation of the sponge *Ephydatia muelleri* in autumn started at the end of October 2007 at temperatures of 10°C. The start of gemmulation was distinctly earlier in the ambient temperature treatments, in the warmed treatment gemmulation started with a delay of eight days. The counting was continued until no further changes in the amount of gemmules were detectable and all living sponge tissue was phased out. This stable phase (no further change in the abundance of gemmules) was reached on November 21 for the ambient temperature and 21 days later for the warmed treatment. Gemmulation started with several single gemmules within the living sponge tissue which were in the colour of amber. At later stages of gemmulation the colour of the gemmules turned to brown, the sponge tissue disappeared and only the silicate nails rested among the gemmules. In contrast to the clear temperature impact on the gemulation in autumn, no temperature effect was found on the hatching of *Ephydatia muelleri* in spring. Hatching from the closed packed gemmules started at the end of February in both treatments. Furthermore, the subsequent growing of the sponges tissue beyond the area of former gemmules was not stimulated by experimental warming (Fig. 6d).

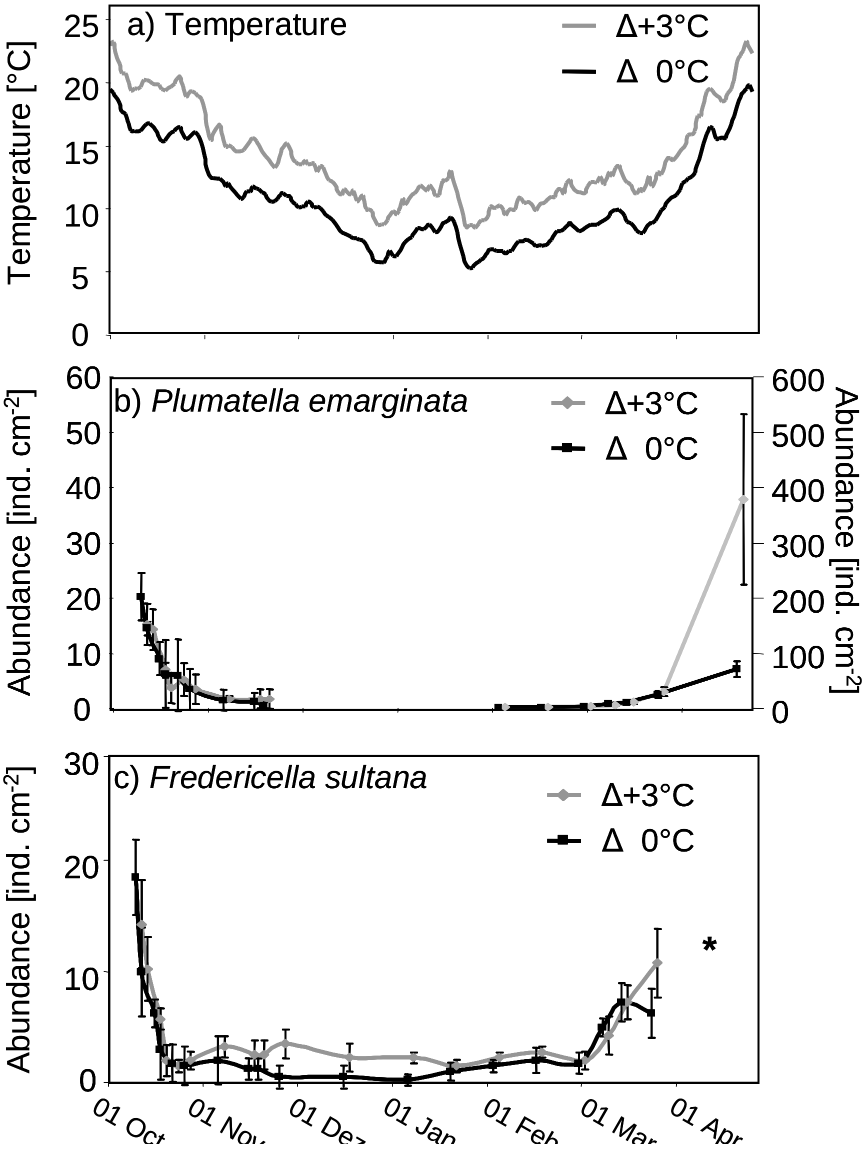

Fig. 5: Hibernation of the bryozoans *Plumatella emarginata* and *Fredericella sultana* under two different temperature regimes (ambient Rhine temperature and elevation by 3°C) during winter 2006/2007. a) Temperature development. b) Development of the abundance of active zooids of *Plumatella emarginata*. The left y-axis refers to the abundances before the hibernation, while the right y-axis refers to the abundance after the hibernation. c) Development of the abundance of active zooids of *Fredericella sultana.*
* *Fredericella sultana* disappeared at the time of mass development of *Plumatella emarginata.*

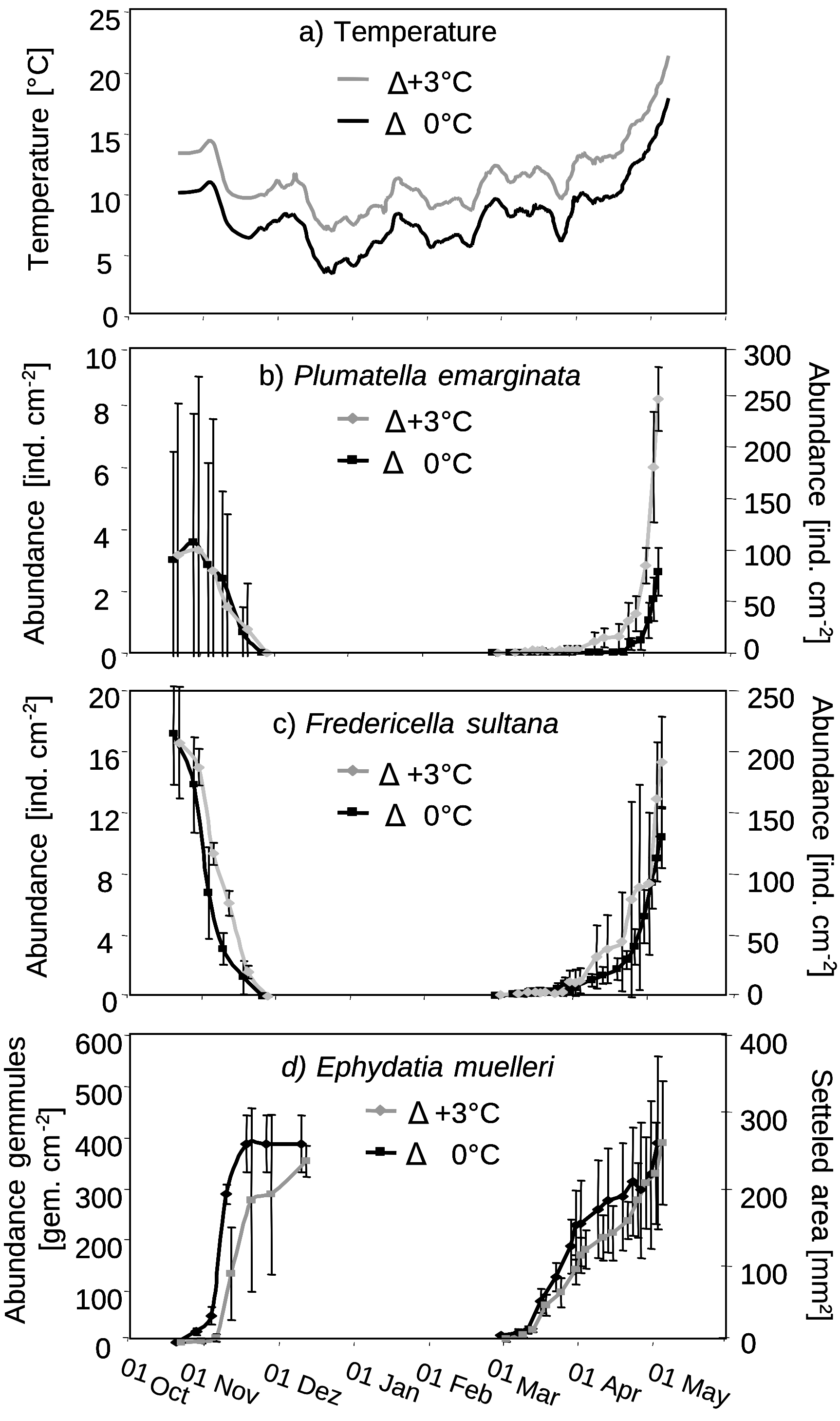

Figure 6: Hibernation of the sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana* under two different temperature regimes (ambient Rhine temperature and elevation by 3°C) during winter 2007/2008. a) Temperature development. b) Development of the abundance of active zooids of *Plumatella emarginata*. The left y-axis refers to the abundances before the hibernation, while the right y-axis refers to the abundance after the hibernation. c) Development of the abundance of active zooids of *Fredericella sulta*na. The left y-axis refers to the abundances before the hibernation, while the right y-axis refers to the abundance after the hibernation. d) Development of the abundance of gemmules (October until December, left y-axis), respectively area covered by active sponge tissue beyond the area covered by gemmules (from February onwards, right y-axis). The development of gemmules during autumn correlated with the decrease of active tissue. No active sponge tissue was found between December and February.

Discussion

Different size selective filtration in sponges and bryozoans

We detected different size preferences in the food spectrum of the freshwater bryozoan (*Plumatella emarginata*) and sponge (*Ephydatia muelleri*). While *Ephydatia muelleri* utilized nano- and picoplankton at similar clearance rates, *Plumatella emarginata* showed no significant grazing on bacteria at all. These preferences were also detected within the nanoplankton, for which the sponge showed no consistent correlation with nanoplankon size (with a slightly lower retention efficiency of the >15 µm size fraction), whereas the bryozoan species showed increasing efficiencies with increasing food size.

These different preferences of the two taxa can be explained by the use of different filtration mechanisms. Bryozoans possess ciliated tentacular crowns, the so-called lophophores, which create a current directed towards the crown and guide the cached food particles towards the mouth (Wood & Okamura, 2005). In sponges, the filtration system is internal (Weissenfels, 1992); water flows in through a series of tiny pores (ostia), which are located between flattened cells of the epiderimis, the so called pinacocytes. The water current is generated by choanocytes which line the inner cavity of the sponge. Those cells also ingest the food items by phagocytosis. The water is finally discharged through larger opening tube called the osculum.

These different filtration strategies of these two taxa results in different constrains regarding the food size. The spaces between the tentacles of the bryozoanas determine the minimal retention size. In the marine bryozoan *Celleporella hyalina*, Rijsgard & Manriquez (1997) reported no retention of particles smaller 4 µm, increasing retention efficiencies between 4 and 6 µm and maximal retention efficiency for particles sizes larger than 6 µm. In general the lophophores acting as mechanical sieves and allow for the passage of small particles (Rijsgard et al., 2004). This size spectrum for the marine species correlates with the results found in this study for the freshwater bryozoans, during which we observed no grazing on bacteria, small (if any) retention efficiencies for particles between 2 and 5 µm and increasing efficiencies for larger particles.

Considering the morphology of the filtration apparatus of sponges, the retention has a distinct upper size limit due to the size of the ostia and the maximal handling size for phagocytosis, rather than a lower size limit as found for the bryozoans. In three coral reef sponges, decreasing retention efficiency was reported between 0.7 and 18 µm (Duckworth et al., 2006). Efficient grazing on particle within this size range has been reported by other studies on both freshwater and marine sponges as well (e.g. Pile et al., 1997; Peterson et al., 2006; Wehrl et al., 2007). Our study covered this range and found also similar efficiencies. However, the bacteria in the Rhine are very small; about 75% are smaller than 1 µm (Weitere & Arndt, 2002, see Table 1) and the efficiency on the total bacterial abundance was still large here.

Together, the study shows an overlap in the food size classes of the sponge and bryozoan between 5 and 15µm. However, food competition between these two taxa is limited due to their specific ranges. These specific food spectra need to be considered when predicting the grazing effects of benthic filter feeders in freshwaters. Up to now, mostly mussels were considered in models on the grazing impact of filter feeders on the plankton (e.g. Schöl et al., 2002; Descy et al., 2003). They prefer nano-sized food items (e.g. Sprung & Rose, 1988; Lei et al., 1996).

Response of clearance rates to experimental warming

Biological rates in ectotherms, including respiration and ingestion rates, are generally temperature dependent (e.g. Gillooly et al., 2001). The general pattern in the feeding rates is of increasing rates with warming at low temperatures, a range of more or less constant feeding rates at intermediate temperatures and a range of decreasing rates with warming when the optimal temperature range is exceeded. In dominant filter-feeding mussels such as *Dreissena polymorpha* and *Corbicula fluminea*, this latter range of decreasing rates is found beyond approximately 20°C (*Dreissena polymorpha*, Lei et al., 1996; Weitere et al., 2008) or 25°C (*Corbicula fluminea*, Viergutz et al., 2007). In the filter-feeding freshwater sponges and bryozoans studied here, the appearance of trophic stages is oftentimes restricted to warm temperatures during summer, while both taxa form resting stages during winter (Weissenfels, 1989; Wood & Okamura, 2005; see sections on phenology). Grazing responses are thus only relevant at higher temperatures, i.e. ranges which are typical for the range of stable or decreasing feeding rates with temperature increase in mussels. Nevertheless, the present study revealed distinct differences in temperature response of the feeding rate between the sponge and the bryozoan. Furthermore, the patterns of the two taxa differed from that of the mussels.

One difference to the temperature-response pattern found in mussels such as *Dreissena polymorpha* and *Corbicula fluminea* is the grazing behaviour at high temperatures: Neither the sponge nor the bryozoan reduced their grazing rate at high temperatures (of up to 29°C for *Eyphydatia muelleri* and up to 30°C for *Plumatella emarginata* as measured here). However, while the sponge showed increasing grazing rates at high temperatures, the bryozoan showed almost constant grazing rates over a larger temperature range. However, there was a high seasonal variability visible, with high grazing rates in May (20-25 ml ind.$^{-1}$ d^{-1}) and much lower grazing rates in June and August (5-10 ml ind.$^{-1}$ d^{-1}). These findings for the bryozoans might be explained by food-concentration dependent grazing. Riisgard & Goldson (1997) demonstrated that the feeding activity of a marine bryozoan colony (*Electra pilosa*) depended on the algae concentration of the surrounding water. The authors found a lower ‘trigger level’ of algae concentration and a higher ‘satiation level’, between which the bryozoans zooids continuously utilise their clearance capacity. There was no physiological regulation of the filter rate on the zooid-level. Differences in the colony clearance rate were achieved by alterations in the proportion of active (filter-feeding) versus non-active zooids, which depended on the food concentration. In our experiment we found the highest clearance rates in May at the time of the highest food particle concentration (Table 1). The clearance rates in June and August were much lower with slightly higher rates at higher algal concentrations in August. In relation to temperature, the present study further shows for the freshwater specie that temperature within the applied ranges seems to be irrelevant in controlling clearance rates for freshwater species. Regarding the function of the bryozoans, it seems that the variations in their clearance rates during the active phase in summer are due to changes in the plankton concentrations (e.g. de Ruyter van Steveninck et al., 1992; Weitere et al., 2005) rather than to temperature fluctuations. This is a fundamental difference from other filter feeders, which usually follow a type I functional response of the clearance rates (i.e. they display maximal clearance rates at low food concentrations and reduce the clearance and stabilize ingestions rates once the incipient limiting level is exceeded, cf. Jeschke et al., 2004).

The temperature responses of the bryozoans probably have functional consequences. In mussels, it was demonstrated that the decreasing clearance rates with warming during summer can strongly alter the structure of the planktonic food webs due to direct and indirect grazing effects (Viergutz et al., 2007; Weitere et al., 2008). Such patterns are probably not transferable to bryozoan grazers. Predictions on the effects of warming in plankton-filter feeder systems thus need to critically consider the group-specific temperature responses of the grazers.

We also found season-specific clearance rates in *Ephydatia muelleri*. The rates measured here were similar for the two summer experiments (performed in June and July) but were much lower (and without significant temperature response) for the October-experiment. To our knowledge there is no data on other freshwater sponges available for the comparison (see also Monakov, 2003). The weak grazing in October might be related to the expiring active summer season, .i.e. that active tissue is gradually reduced (compare Fig. 6d). Together with the phenology-experiments, the result demonstrates the restricted time period of the year in which the sponge has an active grazing impact on the plankton (see also Weissenfels, 1989; Pronzato & Manconi, 1995).

Differential response of respiration and clearance rates towards warming

The measurements of the respiration rates showed increasing rates with temperature up to 32°C in both taxa with Q_{10} values of 1.67 and 2.20 for the *Ephydatia muelleri* and *Plumatella emarginata*, respectively. Regarding the bryozoan, this pattern stands in direct contrast to the temperature-response of the clearance rates. Whereas the metabolic rate (and thus energetic requirements) increased by 2.20 times at a temperature increase of 10°C, the clearance rates (and thus the energy uptake) remained at a constant level. This implies that the growth conditions for the species decreased with high temperatures in summer due to energetic constraints (increasing energetic costs at constant food uptake). However, we could not find results from long-term experiments or field surveys which demonstrate a negative effect of high summer temperature on the density of freshwater bryozoans. The high temperature performance might become relevant in the near future as summer heat waves are predicted to occur with increasing frequencies (Schär et al., 2004; Stott et al., 2004). Further studies need to show whether or not the different relationships between metabolic and feeding rate in the sponge and the bryozoan lead to different performance under high summer temperatures.

Phenology

In contrast to filter-feeding mussels, bryozoans and sponges form resting stages during the cold season (Weissenfels, 1989; Wood & Okamura, 2005). In order to evaluate the impact of temperature on the grazing activity of the two taxa, it is thus important to understand the effects temperature has on the occurrence of trophic stages. In this study we concentrated on both the disappearance of trophic stages in autumn and the occurrence of trophic stages in spring.

The phenology, i.e. the time of recurring natural phenomena such as hatching or germination is controlled by the reaction norm of the species and modified by environmental factors such as temperature as well as seasonal marker (such as photoperiod) (Benfey & Reiswig, 1982). An important question in the context of climate change is whether or not the seasonal events are basically controlled by intrinsic rhythms or by temperature (Gerten & Adrian, 2002; Adrian et al., 2006; Tobin et al., 2008).

It was demonstrated that the timing of the formation of resting stages during winter in the sponge *Ephydatia fluviatilis* depended on the geographic latitude (Pronzato et al., 1993; Gaino et al., 2003). In northern Italy, the sponge forms resting stages during winter as found here. In southern Italy, however, the sponge remained active during winter, whereas it formed resting stages during summer (when water bodies often dried up). This implies a fixed intrinsic rhythm. However, the role of external triggers was not tested. There is (to our knowledge) only very limited data available on the role of temperature in this context for both freshwater bryozoans and sponges. Some authors describe that the exposure to low temperatures during winter is an important conditions for the successful hatching of freshwater sponges in spring (Befey & Reiswig, 1982; Wessenfels 1989; Fell, 1995). These authors report that hatching at temperatures above 20°C could be induced during winter in laboratory experiments only after exposure to low temperatures (3-5°C). However, our data for *Ephydatia muelleri* stands in contrast to these findings; there were no temperature effects detectable for the timing of hatching for this species. The sponges hatched in all treatments even when the temperature in the warmed treatments was distinctly higher than 5°C during the whole winter (Fig. 6). In contrast, the hatching of *Plumatella emarginata* (but not *Fredericella sultana*) showed distinct stimulations with warming in spring, which was reproducible for both years. Hatching started at water temperatures above 10°C, which is in accordance with hatching temperatures of 9°C reported for other *Plumatella* species by Smith (2005). Our data together with the findings reported by Benfey & Reiswig (1982), Weissenfels (1989) and Smith (2005), suggest that the effect of warming on the hatching behaviour is species-specific in both sponges and bryozoans.

These different temperature effects on the timing of hatching stand in contrast to the temperature effect on the phasing out of active stages in autumn; only *Ephydatia muelleri* showed a clear delay of gemmulation with warming. Gemmulation started roughly at temperature of 10°C, which lead to a distinct time delay in the warmed treatments. This temperature response of the phasing out in autumn was not found for both bryozoans.

Conclusion

The study showed that the grazing impact of both the sponge and the bryozoan differed considerably from those of mussels such as *Dreissena polymopha* and *Corbicula fluminea* with respect to temperature response, size preference and seasonal occurrence. Both mussels are oftentimes considered to be the dominant benthic filter feeders in large rivers, even though the abundances of other grazers such as bryozoans and sponges have barely been analysed. In previous studies with these two mussels we found that the decreasing grazing rates with warm summer temperature can have positive effects on the nanoplankton (preferred prey of the mussels) and negative effects on the picoplankton (Viergutz et al., 2007; Weitere et al., 2008). As demonstrated here, the effects are different for both sponges and for bryozoans than for mussels, because of both different temperature responses and different food size preferences. Any predictions of the grazer impact on plankton communities under natural conditions thus needs to consider the composition of the specific grazer community.

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General Conclusions

The first part of the study dealt with *Corbicula fluminea,* one of the most common and well analysed freshwater filter feeders of the northern hemisphere. Thus factors which constraints the occurrence of *Corbicula fluminea* in the context of changing environments are of particular interest and were in the focus of the present study. The experiments showed that the natural conditions for *Corbicula fluminea* in the River Rhine leading generally to a body mass decrease during summer. It was detected that this effect is enhanced by warming but on the other hand a higher food concentration damps the body mass decrease. In this point the hypothesis was verified that a higher food demand at high temperatures due to higher metabolic rates and low plankton concentrations of the River Rhine in summer, are responsible for the body mass decrease. Another impact of warming was the stimulation of the shell length increase. The oppositional controlling mechanisms of shell length and body mass resulted in an additional negative impact on the shell length specific body mass. Furthermore, measurements of the respiration rate revealed an exponentially increase of the oxygen consumption between 19 and 30°C. In contrast to the effect of warming on the respiration rate, Viergutz et al. (2007) analysed grazing rates of *Corbicula fluminea* and found a decrease of the grazing rate above 25°C.

Concluding the impacts of temperature on *Corbicula fluminea* in summer we have to distinguish two possible negative temperature situations. The first is at temperatures below 25°C when the grazing rate increases parallel to the respiration rate, but the low food concentrations of the River Rhine during summer are not sufficient for the mussels to satisfy the demand. In consequence the mussels face a starvation period which leads to a body mass decrease and which depends in its intensity on the food concentration, as it was shown in this study. The second and additional negative situation arises when temperature exceeds 25°C and the decrease of the grazing rate takes place. This enlarges the gap between food demand and food uptake once again. However, the mass mortalities of *Corbicula fluminea* during summer which were the initial suggestions for this study were not found within the period of investigations. We found a significant increase of mortality under experimental warming in this study, which was, however, independent from the food concentration. At this point we have to reject our initial hypothesis, that mortality can be averted by food supplementation. One may assume that the increasing respiration rate beyond 25°C (which was rarely reached in the present study in contrast to past years) plays an important role in questions of the reasons of mortality rather than the food concentration, which has to be tested in the future. The overall negative impacts of temperature elevation in summer stands in contrast to the positive impacts of temperature in winter and spring. Exposure to low temperatures (between 1°C and 2°C) correlates with mortality (Mattice & Dye, 1976; Rodgers et al., 1977). The temperature elevation of large rivers such as the River Rhine by power plants and climate change are is seen as an important factor for successful invasion *Corbicula fluminea*, since the winter temperatures in winter are almost above the critical border. This is supported by the finding in the St. Clair River (Michigan, USA) and the lower Connecticut River (New England, USA) (French & Schlösser, 1991; Morgan et al., 2003) where the mussel is restricted to the thermal refuges provided by power plant discharges in the cold winter months. However, the present study presents the first experimental tests on the effect of winter warming under natural conditions. The comparatively cold winter 2005/2006, when the winter experiment of this study was performed, an experimental temperature elevation by 3°C led to a strong stimulation of the body mass and the fecundity in spring 2006. Obviously the temperature conditions in cold winters are far below the optimum of *Corbicula fluminea* and warming improved this situation. Interestingly, this leads to a trade-off between (positive) winter warming effects and (negative) summer warming effects within one species.

Another part of the study contains analyses of other filter feeders to detect whether or not general mechanisms which were found here and in the grazing study of Viergutz et al. (2007) in the model organism *Corbicula fluminea* are transferable to other benthic grazers. The freshwater sponge (*Ephydatia muelleri*) and two freshwater bryozoan species (*Plumatella emarginata* and *Fredericella sultana*) were used for this purpose. These species are very abundant in most freshwater ecosystems including the River Rhine (Okamura & Doolan, 1993; De Santo & Fell, 1996; Pile et al., 1997) but rarely considered in analysis regarding their influence on the ecosystem processes. Feeding experiments as well as studies regarding the temperature impact on the seasonal occurrence of trophic stages of these organisms were performed in the present study.

The feeding experiments with the sponge *Ephydatia muelleri* and the bryozoan *Plumatella emarginata* revealed species specific responses to experimental warming. *Ephydatia muelleri* showed an increasing grazing rate with the temperature up to 29°C. *Plumatella emarginata* did not respond towards warming. The grazing rates differed during the season which correlated positive with the abundance of phytoplankton in the River Rhine rather than responses to experimental warming. Due to their very different grazing mechanisms, there were strong differences in the preferred food size spectrum detected between *Plumatella emarginata* and *Ephydatia muelleri*. Small organisms like bacteria and small algae may pass the tentacles of the bryozoan lophophores. There was no grazing of bryozoans on bacteria detected while the retention efficiency increased with the food size as from 5 µm. The filtration mechanism of the sponges enables efficient grazing on bacteria as well as on algae. We found efficient grazing rates of all size classes with a slight decrease on larger plankton organisms (>15 µm). These size selective feeding in both the sponge and the bryozoans thus revealed distinct separations of the food spectra with some overlap. Negative impacts of high temperatures on the grazing rates like it is reported for *Corbicula fluminea* (Viergutz et al., 2007) were neither detected for the sponge nor for the bryozoan.

Sponges as well as bryozoans form resting stages to outlast the winter months (Pronzato et al., 1993; Wood & Okamura, 2005). In the River Rhine the statoblasts of the bryozoans and the gemmules of the sponges are non-trophic stages which remain over winter. The timing of seasonal activities is oftentimes sensitive towards temperature chances and thus the influence of warming on the phasing out of trophic stages in autumn as well as on the germination in spring was tested. For *Ephydatia muelleri* the temperature increase resulted in a time delay of the gemmulation. However, the germination in spring was not affected by temperature. This implies that the control mechanisms of *Ephydatia muelleri* for building resting stages are temperature sensitive in autumn but the germination in spring is controlled by other factors, possibly by a genetically fixed intrinsic rhythm. The bryozoan species *Plumatella emarginata* and

Fredericella sultana showed no response to warming in autumn regarding the timing of resting stage formation. However, in the comparatively warm winter 2006/2007 low abundances of *Fredericella sultana* appeared during the whole winter which were higher in the warmed treatments. In 2007/2008 both species disappeared in autumn. For *Plumatella emarginata* warming stimulated the appearance of active zooids in spring consistently in both years and resulted in an earlier mass development.

Together the study revealed different mechanisms how the occurrence and activity of benthic filter feeders can be altered by warming. We could show that temperature is one very important factor which is, however, closely connected to other factors (e.g. food conditions or season). Very sensitive responses could be initiated by moderate alterations in temperature conditions. Depending on the season, temperature affected the fitness of *Corbicula fluminea* positive (winter) or negative (summer). The food conditions emerged as important factor for the intensity of the negative summer temperature impact. The study assessed a presumed gap between grazing and respiration by comparing the decreasing grazing rates (Viergutz et al., 2007) to the increasing respiration rates above 25°C. As mentioned above the intensity of starvation depends on the food conditions, but the role of the food quantity in questions of mortality is not clear yet. The different control mechanisms of body mass and shell length by either food quantity or temperature result in autumn in an additional negative impact on the shell length specific body mass.

When comparing different benthic filter feeders such as the sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana,* and the mussel *Corbicula fluminea* it becomes clear that the temperature changes lead to varying responses which depend on a multitude of factors, especially on the grazer species and the season.

season.

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Zusammenfassung

Das Ausmaß des Klimawandels sowie seine ökologischen Folgen rückt verstärkt in wissenschaftliches Interesse. Bislang ist bekannt, dass sich das globale Klima in der zweite Hälfte des 19. Jahrhunderts um 0,7°C erhöht hat. Klimamodelle für das 21. Jahrhundert lassen eine Erhöhung von 1,7 bis 4°C vermuten (IPCC, 2007). Die Erwartungen möglicher Szenarios gehen von einer eher kontinuierlichen Erwärmung in den Wintermonaten aus, während für die warme Jahreszeit mit immer häufiger auftretenden Extremtemperaturereignissen (wie bei der europäischen Hitzewelle 2003) gerechnet wird.

Der Einfluss von Temperaturänderungen kann sich sowohl direkt auf einzelne Organismengruppen auswirken, als auch durch zeitliche oder quantitative Verschiebungen trophischer Interaktionen, indirekte Effekte erzeugen. Ein Beispiel dazu ist die bentho-pellaische Kopplung. Benthische Filtrierer üben einen Fraßdruck auf planktische Organismen aus und kontrollieren damit die Planktongemeinschaft. Die Filtrations- und Respirationsrate, der Filtrierer sind dabei ebenso stark durch die Temperatur beeinflusst, wie auch die Wachstumsraten des Planktons einer Temperatursteuerung unterliegen. Klimaveränderungen können sich unterschiedlich auf die einzelnen Arten dieser Gemeinschaften auswirken und damit unparallele Verschiebungen hervorrufen. Um den Einfluss der Temperatur auf benthische Filtrierer zu untersuchen, ist es daher wichtig die Diversität dieser Organismengruppe zu beachten. Muscheln, Schwämme, Moostiere, filtrierende Insektenlarven und Biofilmgemeinschaften zählen dabei zu den wichtigsten Gruppen. Muscheln, insbesondere die eingewanderten Arten *Dreissena polymorpha* und *Corbicula fluminea* dienten bereits in vielen Arbeiten, die den Einfluss der Filtrierer auf das Plankton untersuchten, als Modellorganismen. Auch der Einfluss der Temperatur auf das Filtrationsverhalten dieser Muschelarten ist bereits untersucht worden.

Die vorliegende Arbeit knüpft zunächst an die Untersuchungen des Modellorganismus *Corbicula fluminea* an. In den Sommermonaten vergangener Jahre wurde immer wieder ein Massensterben der Körbchenmuschel *Corbicula fluminea* beobachtet. Mittels Rheinwasser durchströmter Aquarien mit natürlichem Sediment wurde die Hypothese untersucht, ob hohe Sommertemperaturen (wegen des gesteigerten Metabolismus der Muscheln, bei gleichzeitig niedrigem Futterangebot im Sommer) zu einer

Nahrungslimitation führen und für die Mortalität verantwortlich sind. In einem der Ansätze wurde durch hohen Rheinwasserdurchfluss (600 L h^{-1}) eine natürliche Nahrungssituation hergestellt. Der reduzierte Durchfluss im zweiten Ansatz (60 l h^{-1}) führte zu einer Futterreduktion von etwa 50%, gemessen am Chlorophyll *a* Gehalt des Wassers, die durch die Filtration der Muscheln hervorgerufen wurde. Das wichtigste Ergebnis dieses Experimentes war die Beobachtung einer starken Gewichtsreduktion von *Corbicula fluminea* im Verlauf des Sommers, verstärkt durch die Nahrungslimitation. Aber auch unter natürlichen Bedingungen nahm die Körpermasse der Muscheln stark ab, in der Zeit von August bis Oktober um 84%. Als Gründe für die Gewichtsabnahme wurden die sommerlichen Bedingungen im Rhein diskutiert. Hohe Wassertemperaturen in Kombination mit geringer Planktondichte könnten erklären, weswegen die Gewichtsabnahme auch in den Aquarien auftrat, in denen natürliche Bedingungen herrschten. Die Analyse der Körpermasse von Freilandmuscheln im Oktober desselben Jahres, zeigte vergleichbar geringe Werte.

Im darauf folgenden Jahr sollte ein weiteres Experiment zu diesem Thema zeigen, ob der Hypothese folgend, eine experimentelle Erhöhung der Temperatur um 3°C, zu einer Verstärkung der Gewichtsabnahme führt und dadurch möglicherweise eine Massenmortalität erzeugt werden kann, wie sie in warmen Jahren beobachtet wurde. Zusätzlich wurde eine Manipulation der Futtermenge dadurch vorgenommen, dass ein Gemisch natürlicher Algen den Becken kontinuierlich zugegeben wurde. Mit Nährstoffen angereichertes Rheinwasser wurde dazu 3 Tage lang belichtet, was eine Vermehrung der Algen hervorrief und zu einem Chlorophyll *a* Gehalt der Futtersuspension von durchschnittlich 1000 µg l^{-1} führte. Der Chlorophyll *a* Gehalt in den gefütterten Aquarien konnte mittels dieser Methode um ca. 50% erhöht werden. Es wurde erwartet, dass die Zufütterung zu einer Verringerung der Mortalität führt, die durch die Temperaturerhöhung hervorgerufen werden sollte.

Die Ergebnisse bestätigten die Hypothesen weitgehend. Eine Temperaturerhöhung um 3°C hatte signifikant negative Effekte auf die Körpermasse der Muscheln. Gemessen an der Körpermasse vermochte die Zugabe der zusätzlichen Futterquelle den negativen Temperatureffekt zu kompensieren. Es kam in diesem Experiment zu einer signifikanten Steigerung der Mortalität durch die Temperaturerhöhung, auf die die Futterzugabe aber keinen Einfluss hatte. Wie schon im Vorjahr war auch in diesem Experiment die allgemeine Abnahme der Körpermasse von *Corbicula fluminea* im Verlauf des Sommers zu beobachten. Insgesamt fiel diese aber nicht so stark aus wie im Vorjahr, was an der vergleichsweise geringeren Durchschnittstemperatur im zweiten Versuchsjahr gelegen haben kann.

Corbicula fluminea stammt ursprünglich aus Asien und ist dort im Sommer deutlich höheren Temperaturen ausgesetzt. Die letale obere Temperaturgrenze ist mit 37°C beschrieben, wohingegen die untere Temperaturgrenze unter 1°C liegt, allerdings wird auch eine positive Korrelation der Mortalitätsrate und der Zeit festgestellt, in der die Temperatur 2°C unterschreitet (Mattice & Dye, 1976; Rodgers et al., 1977; Morgan et al., 2003). Im St Claire River (Michigan, USA) und im Connecticut River (New England, USA) wird das Phänomen beobachtet, dass *Corbicula fluminea* im Winter ausschließlich im Bereich warmer Kraftwerkseinleitungen vorkommt (French & Schlösser, 1991; Morgan et al., 2003). Auch der die Rheintemperatur ist durch warme Industrieabwässer beeinflusst, was als wichtige Voraussetzung für die erfolgreiche Einwanderung der *Corbicula fluminea* angesehen werden kann.

Vor dem Hintergrund prognostizierter Temperaturerhöhung in den Wintermonaten wurde der Temperatureinfluss auf die Fitness der *Corbicula fluminea* auch in einem Winterexperiment getestet. Diesem Experiment lag u.a. die Frage zugrunde, welchen Einfluss der negative Temperatureffekt des vergangenen Sommers auf die Fitness der Muscheln im Winter hat und ob es nachweisbare Effekte auf die Fekundität von *Corbicula fluminea* in der für die Muscheln wichtigsten Reproduktionszeit im April gibt. Das Winterexperiment schloss sich daher direkt an das Sommerexperiment an und es wurden sowohl solche Muscheln verwendet, die im Sommer der Erwärmung um 3°C ausgesetzt waren, als auch solche, die aus den natürlichen Temperaturbedingungen stammten. Muscheln aus beiden Fraktionen wurden im Winter jeweils auf die beiden Temperaturbedingungen (experimentelle Temperaturerhöhung und natürliche Rheintemperatur) aufgeteilt. Aus den beiden unterschiedlichen Vorbehandlungen und den beiden Wintertemperaturen ergaben sich somit vier verschiedene Versuchsbereiche. Auch für das Winterexperiment wurde der Temperatureinfluss anhand der

Werte für Körpermasse und Wachstum der Schalenlänge ermittelt. Die Fekundität wurde im April unmittelbar nach dem Experiment, der wichtigsten Fortpflanzungsperiode für *Corbicula fluminea* im Rhein, anhand der Inkubationsraten (prozentualer Anteil Larven tragender Muscheln an der Gesamtanzahl der Muscheln) und der absoluten Lavenzahl ermittelt. Im Ergebnis ergab sich ein sehr deutlicher Temperatureffekt, der die Körpermasse, wie auch die Fekundität der Muscheln positiv beeinflusste. Die Inkubationsrate war um das doppelte, die absoluten Larvenzahlen waren in den erwärmten Versuchsbereichen sogar um ein Vielfaches höher verglichen mit der natürlicher Rheintemperatur und auch die Körpermasse war durch die Erwärmung etwa um den Faktor 2 erhöht.

Der betreffende Winter war verhältnismäßig kalt, verglichen mit Vor- und Folgejahren. Die Rheintemperatur war mit Minimalwerten von 2,5°C sehr nahe an der Temperaturgrenze (2°C), unterhalb derer es zur Mortalität kommen kann (s.o.). Speziell wegen der wärmeren Herkunftsregion der *Corbicula fluminea* ist anzunehmen, dass Temperaturbedingungen, auch wenn sie nicht direkt letal sind, wohl aber der letalen Grenze sehr nahe kommen, unterhalb des optimalen Temperaturbereiches liegen. Eine Erwärmung stellt somit eine Verbesserung dar, die sich auch in den gemessenen Fitnessparametern niederschlug.

Der aus den ersten Experimenten bekannte negative Temperatureffekt auf die Körpermasse der Körbchenmuschel steht im Gegensatz zu der im Winter ermittelten Stimulation durch Temperatur. Eine der Fragen, die daher dem dritten Experiment zugrunde lagen, war der Zeitpunkt des Umschlages von positiven zu negativen Effekten durch Temperatur. Der Start eines weiteren Experimentes wurde daher in den April gelegt und damit deutlich früher als der, der ersten beiden Experimente. Wie schon im zweiten Experiment wurde sowohl die Temperatur, als auch die Nahrung manipuliert. Ein Pulver, bestehend aus Zellen der Alge *Chlorella sp.,* ermöglichte eine noch genauere Einstellung der Futtermenge. 7,5g suspendiert in 2,5 Liter Wasser pro gefüttertem Becken wurden über 24 Stunden kontinuierlich zugetropft. Wöchentliche Messungen der Schalenlänge und insgesamt 5 Analysen der Körpermasse zwischen April und Oktober ergaben ein hoch aufgelöstes Bild der Entwicklung der entsprechenden Faktoren und der Beeinflussung durch die jeweiligen Manipulationen.

Außerdem wurden, parallel zu diesem Experiment, Messungen der Respiration durchgeführt. So wurde der Einfluss der Temperatur auf den Sauerstoffverbrauch von *Corbicula fluminea* genau bei den Temperaturen ermittelt, die von Viergutz et al. (2007) zur Analyse der Filtrationsraten verwendet wurden. Bei den Filtrationsraten ergab sich eine Zunahme der Filtrationsrate bis 25°C während darüber hinaus die Filtrationsrate wieder abnahm. Die Respirationsmessungen ergaben eine exponentielle Steigerung des Sauerstoffverbrauches bis zu Temperaturen von 30°C. Diese Daten verdeutlichen, dass es bei hohen Temperaturen über 25°C zu einer negativen Stoffwechselbilanz kommen kann. Geringe Planktonkonzentrationen des Rheinwassers im Sommer, steigende Respiration mit der Temperatur bis 30°C und eine abnehmende Filtrationsrate bereits ab 25°C sind die wahrscheinlichsten Ursachen für die abnehmende Körpermasse im Verlauf des Sommers. In solchen Jahren, in denen die nahrungslimitierende Situation besonders intensiv oder lange andauert, könnte Mortalität auftreten.

Die Abnahme der Körpermasse wurde auch in diesem Experiment wieder bestätigt und kann somit als generelles Muster von *Corbicula fluminea* unter den im Sommer im Rhein vorherrschenden Bedingungen angesehen werden. Die Intensität dieses Gewichtsverlustes war in den jeweiligen Experimenten verschieden und korreliert positiv mit der durchschnittlichen Sommertemperatur. Im Sommer des ersten Experimentes überschritt die Rheintemperatur die 25°C Marke für 204 Stunden und im Oktober dieses Jahres wurden die niedrigsten Asche freien Trockengewichte (AFTG) ermittelt. Nur 12 Stunden waren es im Sommer des zweiten Experimentes in den natürlichen Versuchsansätzen. Im Sommer des dritten Versuches erreichte die Rheintemperatur und somit auch die Temperatur in die nicht erwärmten Ansätzen die 25°C Marke nie und in diesem Jahr war auch der höchste AFTG Wert im Oktober mit 0,071g zu verzeichnen. Abbildung 1 zeigt, dass die Jahre 2005 und 2007 hinsichtlich der AFTG Werte, ebenso wie die Temperaturen der beiden Jahre, sehr ähnlich waren und sich nur in der Schalenlänge unterschieden.

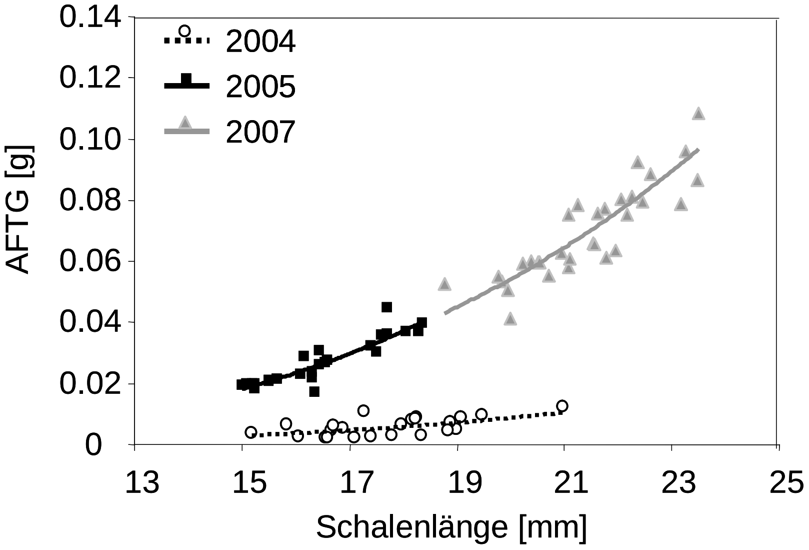

Abbildung 1: Vergleich der Größenspezifischen Körpermasse von *Corbicula fluminea* im Oktober der Jahre 2004, 2005 und 2007. Nur Werte der nicht manipulierten Versuchsbereiche sind angegeben.

Durch die dicht gelegenen Messungstermine der Schalenlängenmessungen, konnten die Wachstumsentwicklung und der Einfluss von Futter und Temperatur im Verlaufe des Sommers gut aufgelöst werden. Bemerkenswert ist die Stimulation des Schalenwachstums durch die Temperaturerhöhung speziell im Frühsommer, die durch den frühen Start des Experimentes hier erstmals, im Vergleich zu den anderen Experimenten, beobachtet wurde. Auch das höhere Futterangebot wirkte sich auf die Schalenlänge positiv aus und in Kombination mit der Erwärmung wurden hier die höchsten Wachstumswerte erzielt. Zwischen Juni und Oktober nahm die Körpermasse in allen Versuchsbereichen ab, zwischen Juni und August signifikant stärker in den erwärmten Ansätzen, wurde dann aber zwischen September und Oktober nur noch durch die Nahrungsmenge beeinflusst. Zeitgleich war eine kontinuierliche Zunahme der Schalenlänge zu beobachten, wenn auch langsamer als im Frühjahr, und weiterhin stimuliert durch die Temperatur. Temperaturerhöhung bewirkte hier einerseits, besonders zwischen Juni und August, eine Abnahme der Körpermasse, andererseits aber gleichzeitig eine Stimulation des Schalenlängenwachstums. Zusammen führte diese unterschiedliche Kontrolle von Schalenwachstum und Gewichtszunahme zu einer deutlichen Abnahme des relativen Gewichtes mit Erwärmung. Abbildung 2 fasst die Ergebnisse der Muschelexperimente zusammen.

		Körpermasse	**Schalenlänge**	**Respiration**
Nahrung	**Sommer**	↑	↑	—
Temperatur	**Sommer**	↓	↑	↑
	Winter	↑	→	—

Abbildung 2: Zusammenfassung der Ergebnisse dreier Experimente zur *Corbicula fluminea*. Berücksichtigt sind 3 Faktoren: Nahrung, Temperatur und Jahreszeit (Sommer, Winter) und deren Einfluss auf die Körpermasse, Schalenlänge und die Respiration (wurde nur im Sommer gemessen). Die Pfeile symbolisieren die Effekte: aufwärts = positiver Effekt; abwärts = negativer Effekt; horizontal = ohne Effekt. Ein Strich bedeutet, das der betreffende Parameter zu dieser Zeit nicht gemessen wurde.

Neben den Untersuchungen zum Modellorganismus *Corbicula fluminea* sollte in weiteren Experimenten mit anderen benthischen Filtrierern die Hypothese untersucht werden, dass es artspezifische Reaktionen auf Temperaturveränderungen gibt. Die wichtigen Erkenntnisse aus den Experimenten mit *Corbicula fluminea,* gelten dieser Hypothese folgend nicht generell für die Filtrierer des Benthos, weswegen es jeweils einzelner Fallstudien bedarf.

Mittels kleiner, Rheinwasser durchströmter Fließkammern, wurden Filtrationsexperimente mit dem Süßwasserschwamm *Ephydatia muelleri* und dem Moostierchen *Plumatella emarginata* durchgeführt. Als Basistemperatur der Experimente wurde das langjährige Monatsmittel seit 1989 des jeweiligen Versuchstermins gewählt und eine Manipulation der Temperatur in Form einer Erwärmung um 3°C und um 6°C durchgeführt. Anders als Muscheln bilden beide Taxa zur Überdauerung der kalten Jahreszeit Dauerstadien. Die Filtrationsexperimente fanden daher in der trophisch aktiven Phase (Sommerhalbjahr), zu jeweils 3 Terminen statt.

Deutliche Unterschiede ergaben sich hinsichtlich der Filrationsreaktion der Organismen auf die Temperaturmanipulation. Beide Organismen zeigten im Frühjahr und im Sommer (Mai bis Juli) deutlich höhere Filtrationsraten als im Spätsommer oder Herbst (August bis Oktober). Nur die Filtrationsleistung des Süßwasserschwamms *Ephydatia muelleri* wurde durch die Temperaturerhöhung stimuliert, während *Plumatella emarginata* innerhalb eines Versuchstermins bei allen Temperaturen nahezu gleiche Filtrationsleistungen zeigte, wohl aber jahreszeitliche Schwankungen aufwies. Die Filtrationsraten von *Plumatella emarginata* korrelierten positiv mit den Planktonkonzentrationen des Rheins. Bei keinem der beiden Taxa war die Abnahme der Filtration bei hohen Temperaturen festzustellen, wie es für *Corbicula fluminea* beschrieben ist. Die Filtration von *Ephydatia muelleri* stieg mit der Temperatur bis 29°C an, *Plumatella emarginata* zeigte bis 30°C konstante Filtrationsleistungen, ohne aber durch die experimentelle Temperaturerhöhung beeinflusst zu sein. Die Futterspektren der beiden Filtrierer überlappten nur wenig. *Ephydatia muelleri* filtrierte sehr effektiv Bakterien und kleine Algen, während die Filtrationseffezienz von *Plumatella emarginata* mit der Planktongröße, vom Picoplankton zum Nanoplankton stieg. Je nach Dichte der jeweiligen Filtrierer sind daher indirekte Effekte auf die Planktongemeinschaften, wegen ungleichmäßiger Entwicklung des Fraßdrucks auf die Plankton-Größenklassen, zu erwarten.

Bekannt ist, dass Temperatur, wie eingangs beschrieben, auch phenologische Ereignisse beeinflussen kann. In diesem Zusammenhang wurde der Temperatureinfluss auf die Winterruhe untersucht. Dabei gab es drei Phasen, die als besonders temperatursensitiv betrachtet wurden und daher im Fokus der Untersuchungen standen. Einerseits der Rückzug trophisch aktiver Stadien, an dessen Ende die Dauerstadien zur Überwinterung zurückblieben. Die Winterruhe ihrerseits wurde durch regelmäßige Kontrollen überwacht und schließlich die Auskeimung neuer trophisch aktiver Organismen aus den Dauerstadien im Frühjahr.

Rheinwasser durchströmte Rinnen mit natürlicher Temperatur und solche, deren kontinuierlicher Durchfluss um 3°C erwärmt wurde, dienten als Versuchsanordnung. Die stärkste Reaktion auf die Temperatur zeigte *Plumatella emarginata*. Eine Massenentwicklung fand in zwei aufeinander folgenden Jahren immer dann statt, wenn die Rheintemperatur im Frühling binnen kurzer Zeit mehrere Grad Celsius anstieg, so zum Beispiel zwischen dem 01. und 09. Mai 2008 von 13.6°C auf 20°C, mit sehr viel stärkerem Ausmaß in den erwärmten Ansätzen. *Fredericella sultana*, eine zweite Bryozoen Art, zeigte im Frühling kaum Reaktionen auf die

Temperaturmanipulation und schien in der Konkurrenz um Raum, gegen *Plumatella emarginata,* zum Zeitpunkt der Massenentwicklung, unterlegen zu sein. Im Herbst allerdings wurde bei keiner der beiden Arten ein Temperatureinfluss auf den Beginn der Winterruhe festgestellt. Der Winter der ersten Untersuchungsperiode 2006/2007 war verhältnismäßig warm. In den Ansätzen, die um weitere 3°C erwärmt waren, kam es bei *Fredericella sultana* zu keinem vollkommenen Rückzug der trophischen Stadien. Zwar ging die Abundanz stark zurück, aber in den erwärmten Ansätzen waren zu jeder Zeit aktive Zooide anzutreffen.

Genau entgegengesetzt stellte sich die Situation bei *Ephydatia muelleri* dar. Im Herbst war die Rückbildung des Schwammgewebes und die Bildung der Gemmulae durch die Temperaturerhöhung deutlich verzögert und auch die Zeit bis zur vollständigen Gemmulation war verlängert. So begann die Gemmulation in den erwärmten Rinnen 8 Tage später und der völlige Rückzug des Schwammgewebes und der Zeitpunkt an dem keine weiteren Gemmulae hinzukamen, war 3 Wochen später erreicht.

Abbildung 3 fasst die Ergebnisse der Experimente zusammen.

	Ephydatia muelleri	***Plumatella emarginata***	***Fredericella sultana***
<u>Temperatureinfluss</u>			
Filtrationsrate	↑	→	—
Eintritt der Winterruhe	↑	→	↑
Keimung im Frühjahr	→	↑	⇗

Abbildung 3: Zusammenfassung der Experimente mit Süßwasserschwämmen- und moostieren. Abgebildet ist der Temperatureinfluss auf die Filtrationsrate, die Winterruhe und die Keimung aus den Dauerstadien im Frühjahr. Die Pfeile symbolisieren die Effekte: aufwärts = positiver Effekt; abwärts = negativer Effekt; horizontal = ohne Effekt; grauer, gestrichelter Pfeil = schwacher Effekt, der wegen der Verdrängung von *Fredericella sultana* durch *Plumatella emarginata* nicht deutlicher wurde. Ein Strich bedeutet, das der betreffende Parameter bei dieser Art nicht gemessen wurde.

Die hier dargestellten Arbeiten zeigen zusammengefasst, dass eine moderate Erwärmung von 3°C erhebliche Auswirkungen auf benthische Filtrierer hat. Diese Effekte variieren stark und sind vom jeweiligen Organismus und den Umweltbedingungen abhängig. Die Abschätzung möglicher Folgen einer globalen Erwärmung bleiben weiterhin schwierig, besonders wegen der Vielfältigkeit der einzelnen Effekte und möglicher indirekter Temperatureffekte, die sich aus dem Einfluss der Filtrierer auf das Plankton ergeben.

Studien an Modellorganismen können daher immer nur der erste Schritt der Klimafolgenforschung sein. Vorhersagen über die Effekte der Klimaerwärmung auf Interaktionen wie die bentho-pellagische Kopplung, müssen stets eine Vielzahl von Fallstudien zu den einzelnen Filtrierern enthalten, wie auch jahreszeitliche und indirekte Effekte berücksichtigen.

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Kurzzusammenfassung

Aufgrund des aktuellen, anthropogen verursachten Klimawandels erlangt ökologische Klimafolgenforschung zunehmende Wichtigkeit. In den hier vorgestellten Untersuchungen wurde der Einfluss von Erwärmung auf das Fraßverhalten, die Phenologie und die Fitness benthischer Filtrierer untersucht. Diese steuern durch ihren Planktonkonsum maßgeblich Stoffumsatzprozesse besonders in großen Fließgewässern. Zudem ist ihre Aktivität in hohem Maße sensitiv gegenüber Temperaturänderungen. Die hier durchgeführten Untersuchungen wurden unter naturnahen Bedingungen (Durchfluss natürlichen Rheinwassers) auf der Ökologischen Rheinstation der Universität zu Köln durchgeführt.

Die Körbchenmuschel *Corbicula fluminea* diente zunächst als Modellorganismus. Sie ist eine neuzeitlich eingewanderte Art, die in hohen Abundanzen in Fließgewässern der nördlichen Hemisphäre vorkommt und bezüglich ihrer Filtrationseffekte häufig untersucht wurde. Hier wurde der Effekt von Erwärmung auf Fitnessparameter in temperaturmanipulierten Bypasssystemen (natürliche Temperatur sowie moderate Erhöhung um 3°C) zum Rhein untersucht. Es konnte gezeigt werden, dass es bereits unter natürlichen Bedingungen (unmanipuliertes Rheinwasser) im Verlauf des Sommers zu einer Gewichtsreduktion der Körbchenmuschel kommt. Dieser Effekt wurde durch die experimentelle Erwärmung signifikant verstärkt. Eine experimentelle Erhöhung der Futterkonzentration dämpfte den negativen Temperatureinfluss und führte zu einem deutlichen Anstieg der Körpermasse. Dies zeigt zum einen, dass *Corbicula fluminea* in den Sommermonaten im Rhein nahrungslimitiert ist. Zum anderen stützt das Ergebnis die Hypothese, dass erhöhte energetische Kosten mit steigender Temperatur und bei limitierter Nahrung als wichtige Ursache für die beobachteten Gewichtsverluste zu sehen sind. In Respirationsmessungen konnte dazu gezeigt werden, dass Erwärmung bis 30°C zu einem exponentiellen Anstieg des Grundstoffwechsels (und damit des Energiebedarfs) führte, während Literaturdaten zeigen, dass Erwärmung über 25°C bereits zu einer Abnahme der Filtrierrate (und damit der Energieaufnahme) führen.

Im Gegensatz zur Kontrolle der Körpermasse wurde das Schalenwachstum durch Erwärmung stimuliert. Diese unterschiedliche Steuerung von Körpermasse und Schalenlänge führte zu einem zusätzlichen negativen Effekt

von Erwärmung auf die relative Körpermasse. Die insgesamt negativen Effekte durch sommerliche Erwärmung stehen im Gegensatz zu positiven Effekten von winterlicher Erwärmung auf die Körpermasse sowie die Fekundität zur Hauptreproduktionszeit der Muscheln im April.

In einem weiteren Teil der Arbeit wurden der Süßwasserschwamm *Ephydatia muelleri* und die Moostierchen *Fredericella sultana* und *Plumatella emarginata* betrachtet. Beide Taxa kommen in hohen Dichen in Süßgewässern und besonders in großen Fließgewässern vor, fanden bislang aber wenig Beachtung in Untersuchungen zu deren Funktion im Ökosystem. In Fließkammern wurde hier erstmalig die Filtrierrate der Organismen auf verschiedene planktische Größenklassen quantifiziert und der Temperatureinfluss auf die Filtration getestet. Die Filtrationsrate von *Ephydatia muelleri* stieg mit steigender Temperatur an. *Plumatella emarginata* zeigte zwar eine hohe saisonale Variabilität der Filtrationsrate, allerdings keine Reaktion auf die Erwärmung innerhalb eines Versuchstermins. Sowohl Schwämme, als auch Moostierchen bilden im Winter Dauerstadien. Eine Erwärmung um 3°C hatte dabei eine Verzögerung der Dauerstadienbildung bei *Ephydatia muelleri* zur Folge. Ähnliches konnte für keine der beiden Moostierchenarten gezeigt werden. Der verhältnismäßig milde Winter 2006/2007 führte bei *Fredericella sultana* über den ganzen Winter zu geringen Abundanzen aktiver Zooide, die in den warmen Versuchsrinnen deutlich höher waren. Die Erwärmung führte bei *Plumatella emarginata* zu einer deutlich stärkeren Entwicklung aktiver Zooide im Frühjahr. Ein vergleichbarer Temperatureinfluss im Frühjahr konnte weder für *Ephydatia muelleri*, noch für *Fredericella sultana* eindeutig gezeigt werden.

Zusammenfassend zeigen die Resultate unterschiedliche Einflüsse von Temperaturerhöhung. Im Vergleich der Bryozoen und Schwämme wurden dabei artspezifische Unterschiede sowohl in der Reaktion der Filtrierraten als auch des Timings der Winterruhe auf Erwärmung gemessen. Für *Corbiula fluminea* wurde gezeigt, dass sich Klimaerwärmung jahreszeitlich unterschiedlich auf die Fitness der Art auswirken kann. Die daraus resultierende Vielseitigkeit der Temperatureffekte muss in Modellen zur Abschätzung von Klimaänderungen auf Fließwasserökosysteme berücksichtigt werden.

Abstract

The current global warming challenge ecologists to asses consequences on ecosystem processes. The present study focussed the impact of warming on the feeding behaviour, the phenology and the fitness of benthic filter feeders. Benthic filter feeders can have a considerable impact on shallow freshwater ecosystems, especially large rivers, by controlling plankton communities,. The activity of benthic filter feeder is sensitive towards temperature changes. The experiments of this study were performed under natural conditions at the Ecological Rhine Station at the University of Cologne by using river water in bypass systems.

In the first parts of this thesis, *Corbicula fluminea* was used as model organism. The invasive mussel is abundant in many freshwater ecosystems of the northern hemisphere and well studied regarding their filtration impact. It has colonized the Rhine in the 1980s. Here the focus was on the impact of warming on the fitness of the mussel using experimentally warmed bypasses to the River Rhine. It was shown that the body mass decreases strongly during summer even under natural conditions (non-manipulated treatments), which was significantly enhanced under experimental warming of 3°C above the natural Rhine temperature. An experimental food supplementation compensated the negative warming effect on the body mass. This demonstrates on the one hand that *Corbicula fluminea* is food limited during summer in the Rhine. On the other hand it supports the hypothesis that higher energetic costs with increasing temperatures at limited food conditions during summer is an important reason for the observed body mass decrease. On this account, measurements of the oxygen consumption showed an increase of the respiration rate until 30°C, whereas data reported in literature showed a decrease of the filtration rate with warming already at 25°C. This differential development of food uptake and food demand can thus result in a further increase the starvation stress at warm temperatures.

In contrast to the body mass, the increase of the shell length was stimulated by temperature. This different control of body mass and shell length resulted in an additional negative impact of the relative (size-specific) body mass. The overall negative impacts of summer warming stands in contrast to a positive response to winter warming. The moderate experimental warming of 3°C above the river temperature resulted in strong and significant increases of the body mass as well as of the fecundity in spring, the main reproductive period of the mussels.

Another part of the study dealt on other potentially important freshwater filter feeders, i.e. the sponge *Ephydatia muelleri* and the bryozoans *Plumatella emarginata* and *Fredericella sultana.* Both taxa can reach high abundances in freshwater ecosystems, especially in large rivers, but were often overlooked regarding their function within the ecosystem. The size-selective feeding rate as well as its impact on temperature was quantified in this study for the first time. The feeding rate of *Ephydatia muelleri* was stimulated by temperature. *Plumatella emarginata* showed a high seasonal variability in their feeding activity, which was, however, not affected by warming within one experiment. Both, the sponges as well as the bryozoans forming resting stages during winter. Warming of 3°C above river temperature resulted in a delay of the diapause of *Ephydatia muelleri*. No such results regarding the formation of resting states during fall could not be found for the bryozoans *Plumatella emarginata* or *Fredericella sultana.* During the relatively warm winter 2006/2007, low abundances of active zooids of *Fredericella sultana* remained during the whole winter, a phenomenon which was stimulated by experimental warming. Furthermore, experimental warming led to an earlier and stronger development of active zooids of *Plumatella emraginata* in spring. Similar effects were neither found for *Fredericella sultana* nor for *Ephydatia muelleri*.

Altogether we found different impacts of warming on the seasonal occurrence, fitness and feeding rates of freshwater filter feeders. Comparing the sponge and the bryozoans we found species specific responses to warming regarding the feeding activity as well as regarding the timing of the diapauses. For *Corbicula fluminea* we found seasonal contrasting impact of warming on the fitness, showing a trade-off between summer and winter warming. These different types of responses towards warming must be considered when modelling the consequences of climate change on the bentho-pelagic coupling in freshwater ecosystems.

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 Abbildungen und Tabellen - die anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, 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 auf der folgenden Seite angegebenen Teilpublikationen, noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Hartmut Arndt betreut worden.

Köln, 03. 07. 2008

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[1] Innhaltlich Kapitel 1 der Dissertation

[2] Innhaltlich Kapitel 2 der Dissertation

[3] Innhaltlich Kapitel 4 der Dissertation

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