Top-down effects on pelagic heterotrophic nanoflagellates (HNF) in a large river (River Rhine): do losses to the benthos play a role?

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SUMMARY

1. The abundance, production and control of pelagic heterotrophic nanoflagellates (HNF) in the Lower River Rhine (Germany) were investigated. Field samples (live-counting technique) were taken at least every 2 weeks at Cologne (km 685) over a period of 20 months. In addition, Lagrangian sampling was carried out 160 km downstream of Cologne (Kalkar–Grieth, km 845) over a period of 12 months. Potential HNF growth rates and loss rates caused by planktonic predators were estimated in the laboratory (size fractionation experiments) and compared with the changes in HNF-density in a water parcel flowing downstream.

2. Mean abundance ($\pm$SD) ranged from 7 ± 6 to 4890 ± 560 individuals mL^{-1} and was positively correlated with discharge. Heterotrophic nanoflagellates abundance increased up to 30-fold during flood events, whereas there were only minor changes in the taxonomic composition.

3. HNF growth rate ranged from 0.16 ± 0.12 to 1.98 ± 0.10 day^{-1}. Between 0 and 95% (mean: 32%) of the gross production was lost to planktonic predators; a larger portion between 0 and 195% (mean: 64%) of the HNF gross production was lost by other means.

4. There may be an important role for benthic predators in the control of pelagic HNF. First, production of HNF was high and grazing losses to planktonic predators low at times when HNF abundance was low. Secondly, high *in situ* loss rates (not explicable by planktonic predators), which were positively related to temperature, indicate the importance of biotic interactions. Thirdly, the dependence of HNF abundance on discharge indicated a decrease grazing intensity with rising water levels (increase in water volume/ colonised river bed ratio).

5. The impact of discharge on planktonic HNF mediated by the grazing impact of benthic predators was modelled, showing a good fit with the field data.

Keywords: large river, protozoans, pelagic flagellates, potamoplankton, water discharge

Introduction

Planktonic organisms are generally less numerous in rivers than in lakes (e.g. Phillips, 1995). This has been attributed mainly to the short residence time of the water (Allan, 1995). However, recent studies have demonstrated that the grazing impact of benthic filter feeders, in particular of mussels, can be responsible for low phyto- and zooplankton densities (e.g. Caraco *et al.*, 1997; Pace, Findlay & Fischer, 1998; Welker & Walz, 1998).

In terms of their size, heterotrophic nanoflagellates (HNF) can also be consumed efficiently by several benthic filter feeders (e.g. Sprung & Rose, 1988; Way *et al.*, 1990) and the importance of a trophic transfer

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from protozoans to macroinvertebrates in rivers has been suggested (Carlough & Meyer, 1989, 1991). Pelagic HNF have a higher potential growth rate than those of ciliates, algae and metazooplankton, with doubling times of a few hours (Hansen, Bjørnsen & Hansen, 1998). Thus HNF should be able to colonise the water column rapidly, effectively replacing losses to benthic predators. It is not known, however, whether such fast growing HNF can be affected significantly by benthic predators. Nevertheless, hints of an impact of benthic predators on pelagic HNF were shown for the Hudson River, where Findlay, Pace & Fischer (1998) observed an increase in the abundance of pelagic bacteria after the invasion by *Dreissena polymorpha* (Pallas, 1771). The authors explained this by a postulated decrease in the abundance of flagellated protozoa, the major consumers of bacteria, due to predation by *D. polymorpha*.

Besides a possible impact of benthic predators, one might expect high numbers of HNF in the River Rhine as a result of the inflow from Lake Constance (Cleven, 1995; Weisse & Müller, 1998) and a high growth rate in the river because of high bacterial and DOC load (de Ruyter van Steveninck *et al.*, 1992; Admiraal *et al.*, 1994; ARW, 1998). In addition, the metazooplankton occurs in low abundance in the River Rhine (de Ruyter van Steveninck *et al.*, 1992; IKSR, 1997; Ietswaart *et al.*, 1999) and should not be able to cause high HNF losses. However, routine studies revealed a low summer abundance of HNF in the River Rhine, when maximal growth rates were expected. Therefore, we hypothesised that the pelagic HNF were controlled mainly by benthic predators. To test this hypothesis, two predictions were made: (1) there should be a loss of HNF-production during downstream transport which cannot be explained by pelagic predators and (2) the grazing intensity should depend on river discharge (which influences the ratio of water volume to the colonised river bed), resulting in higher HNF abundance at high water levels. Therefore, special attention was drawn to short-term dynamics, especially flood events, and to HNF dynamics during downstream transport.

Methods

Study site

The River Rhine is one of the largest mid-European river systems. The non-alpine part of the River Rhine

originates from Lake Constance and reaches its outflow at the Dutch coast of the North Sea after about 1100 km (Fig. 1). The regulation of the river banks to create shipping lanes has led to a turbid and fast-flowing river channel with few backwaters (Tittizer & Krebs, 1996). Owing to high turbulence, the water is well mixed without any stratification regarding abiotic parameters and planktonic organisms. Using a water quality model, there is a total vertical circulation time for particles in the water column of about 37 min (V. Kirchesch, personal communication). This circulation time is only slightly affected by discharge.

The investigations concentrated on the Lower River Rhine (Germany). Surface water samples (three to six independent replicates) were taken in Cologne–Bayenthal (km 685) and 160 km downstream in Kalkar–Grieth (km 845) from the main flow at distances of 30 (Ecological Rhine Laboratory of the Univer-
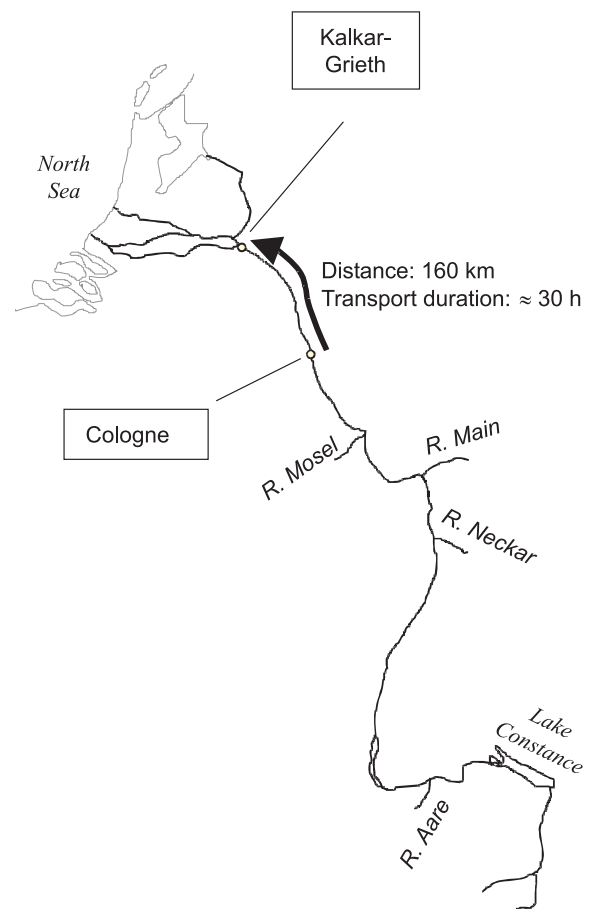

Fig. 1 Location of the two sampling sites at the Lower River Rhine in Cologne (km 685) and Kalkar–Grieth (km 845) (Germany).

sity in Cologne-Bayenthal) and 20 m (Kalkar–Grieth) from the river bank, respectively. Between the two sampling sites there are only a few tributaries, together increasing the mean discharge from about 2000 to 2300 $m^3 s^{-1}$ (Tittizer & Krebs, 1996). The annual range of the water level in Cologne is very high and ranges between about 2.5 and 10 m.

Analyses of HNF community structure

To avoid artefacts caused by fixation (Pfister, Sonntag & Posch, 1999; Sonntag, Posch & Psenner, 2000), and to allow taxonomic differentiation of the HNF, a live-counting technique was employed (Arndt *et al.*, 2000). Counting began within 1 h of sampling in the laboratory. Aliquots of 5 μL were placed in a miniaturised version of a Sedgewick-Rafter chamber. Within one replicate, 15–150 μL were analysed, amounting to a count of 20–100 flagellates per replicate. There were only five dates in the summer of 1998 on which fewer than 20 flagellates could be registered per parallel, because of extremely low HNF abundance at these times. Counting took place under a phase contrast microscope (Axioskop, Fa. Zeiss, Jena, Germany) at 200× magnification and for the taxonomic determination a ≥ 400× magnification and the help of video recording were employed.

The HNF abundance was determined in Cologne from May 1998 to January 2000 weekly or every 2 weeks. The sampling was intensified during flood events. In Kalkar–Grieth samples were taken once at the beginning of February 1999 and, during the period from the end of March 1999 to January 2000, every 2–3 weeks. Samples in Kalkar–Grieth were taken from the same parcel of water that had been sampled previously in Cologne (Lagrangian sampling). Flow duration between Cologne and Kalkar–Grieth was calculated by a PC-program (Spreafico & van Mazijk, 1993), taking the actual water level into consideration.

Water discharge (daily mean values) was taken from the regular recording of the Bundesschiffahrtsamt (Duisburg, Germany). The instantaneous discharge needed for calculating flow duration was taken from the Bundesanstalt für Gewässerkunde (Koblenz, Germany). Water temperature was measured during sampling.

Measurement of HNF growth and loss

Size fractionation experiments (Landry, 1994) were used to measure HNF growth rate and the grazing impact of planktonic predators at the sampling site in Cologne (see above). Experiments were performed from August 1998 to January 2000. In the period from August 1998 to May 1999 experiments were conducted every 1–2 months. From May 1999 to January 2000 experiments were conducted every 1–3 weeks. Two treatments were considered, using (1) water passed through a 10-μm gauze (removing larger predators) immediately after sampling of the River Rhine and (2) untreated water, which included all the planktonic predators found in the field. Three to four independent replicates were made for each of the two treatments. For each replicate, glass bottles were filled with 2 L of water. Incubation was conducted under field conditions (temperature and photoperiod) in the laboratory. Heterotrophic nanoflagellates were quantified at the beginning and after 24 h using the live-counting technique (see above). The growth rate (r, day^{-1}) in the two treatments was calculated assuming exponential growth:

$$r = (\ln N_1 - \ln N_0)/t \tag{1}$$

where N (individuals mL^{-1}) is the HNF abundance at the beginning of the experiment (N_0) and at the end of the experiment (N_1) after 1 day (t). In the following, the growth rate determined from the treatment in which planktonic predators were excluded is called 'gross growth rate' (r_g, day^{-1}) and the growth rate determined from the treatment with planktonic predators is termed 'planktonic net growth rate' (r_{np}, day^{-1}). In addition to those growth rates measured in the laboratory, an *in situ* net growth rate (r_{is}, day^{-1}) was calculated with the help of the HNF abundances measured for Cologne (N_0) and Kalkar–Grieth (N_1) and the flow duration (t, d) between both sampling locations.

The HNF *in situ* net growth rate (r_{is}) considers all losses in the river. It is the difference between the HNF gross growth rate (r_g, day^{-1}), the loss rate to planktonic predators (p, day^{-1}) and the loss rate in the field which cannot be explained by planktonic predators (f, day^{-1}):

$$r_{is} = r_g - p - f \tag{2}$$

The loss rate due to planktonic predators (p) is the difference between the gross growth rate (r_g) and the planktonic net growth rate (r_{np}):

$$p = r_g - r_{np} \tag{3}$$

The loss rate in the field which cannot be explained by planktonic predators (f) was calculated as the difference between the planktonic net growth rate (r_{np}) and the net growth rate in the field (r_{is}):

$$f = r_{np} - r_{is} \tag{4}$$

From May 1999 to January 2000, all necessary data (laboratory experiments and downstream field samples) used for the calculation of the losses were determined simultaneously. From February to May 1999, there was a time lag of up to 1 week between laboratory experiments and field sampling.

Laboratory experiments were not conducted with turbulent water, as in the river. To test whether there were differences in the planktonic net growth rate (r_{np}) between standing and turbulent waters, the planktonic net growth rate was measured on three occasions in flow channels under turbulent conditions, parallel to the routine measurement in the experimental vessels. The flow channels were round vessels (outer diameter 30 cm, inner diameter 10 cm) with a rotating disk placed at the water surface (see Weitere, 2001). The disk was spiked with three combs that penetrated 10 cm into the water to keep the water moving and turbulent. The rotation speed was 30 rotations per minute. The flow channels were filled with 10 L of river water (corresponding to a depth of 16 cm) during the three investigations in May and June 1999.

Modelling the impact of water discharge on HNF abundance

A simple model was designed to reflect the impact of water discharge on the HNF abundance by taking the HNF growth rate, the discharge-dependent predation pressure by benthic predators and the discharge-dependent flow duration over a 685-km river section of the River Rhine into account. The model was based on the exponential growth function for HNF:

$$N_1 = N_0\, e^{r \times t} \tag{5}$$

where the growth rate (r) is the *in situ* net growth rate (r_{is}, day^{-1}, see above). The time (t, day) is the flow duration over a distance of a 685 km river section. This corresponds to the distance between the outflow of Lake Constance and Cologne. Its dependence on the water discharge (W, m^3 s^{-1}) can be given by the following equation:

$$t = 88\ W^{-0.2926} \tag{6}$$

Equation 6 is the result of a regression between the flow times and the respective water discharges (PC-program: Spreafico & van Mazijk, 1993). Combining equation 2 and 3, the *in situ* net growth rate of HNF (r_{is}) was calculated as the difference between the gross growth rate (r_g), the predation rate due to planktonic predators (p) and the loss rate in the field which cannot be explained by planktonic predators (f):

$$r_{is} = r_g - p - f = r_{np} - f \tag{7}$$

The gross growth rate minus the loss rate to planktonic predators ($r_g - p$) was determined in the laboratory (r_{np}, see above). As an assumption of the model, the field losses which cannot be explained by planktonic predators were attributed to benthic predators. Therefore, the loss rate in the field not attributable to planktonic predators (f) was assumed to be the grazing rate of benthic predators (see Discussion). The planktonic net growth rate (r_{np}) was assumed to be independent of the discharge. Assuming a constant maximum filtration rate by the benthos, the clearance rate of pelagic prey should depend on the amount of water passing the benthic community in a certain area. Therefore, the loss rate of HNF caused by benthic predators (f) should depend on discharge and can be described by the following equation:

$$f = f_{2000}\, 2000\, \text{m}^3\, \text{s}^{-1}/W \tag{8}$$

where f_{2000} (day^{-1}) is the loss rate to the benthic community at medium water discharge (2000 m^3 s^{-1}). If the actual water discharge (W) is higher than 2000 m^3 s^{-1}, the loss rate to benthic predators should be smaller than f_{2000}; if the actual water discharge is lower, the loss rate to benthic predators should be higher than f_{2000}. Combining equations 7 and 8, the *in situ* net growth rate (r_{is}) is given by the following equation:

$$r_{is} = r_{np} - f_{2000}\, 2000\, \text{m}^3\, \text{s}^{-1}/W \tag{9}$$

General features of the dependence of HNF abundance on water discharge were calculated on the basis of this model. In addition, the field data gathered

during the two largest flood events were compared with the model by assuming planktonic net growth rates (r_{np}) as measured in the field during the flood events and initial HNF abundances (N_0) of 800 mL^{-1} for autumn and 150 mL^{-1} for late winter. The abundances were in accordance with HNF abundances found for the respective seasons in lakes (cf. Mathes & Arndt, 1995).

Statistical analyses

Statistical analyses were run using the PC-program SPSS 9.0 for Windows. The dependence between temperature and field loss rate (f), as well as the dependence between water discharge and HNF-abundance, were tested using Pearson correlation. Double logarithmic transformation was conducted (one negative value excluded) in the case where the dependence of field loss rate on temperature was analysed. To test whether the water discharge had an impact on the HNF gross growth rate (r_g), multiple regressions of temperature and water discharge (independent variables) with gross growth rate (dependent variable) were used. Linearisation was achieved by the log-transformation of water discharge and gross growth rate. Growth rates with and without the presence of planktonic predators (r_{np} and r_g) were compared for the different dates using a two-way ANOVA. Planktonic net growth rate (r_{np}) and *in situ* net growth rate (r_{is}) were considered to be significantly different when r_{is} lay outside the 95% confidence interval of r_{np}. For comparative investigations of the planktonic net growth rate (r_{np}) in stagnant and turbulent waters, the growth rates were considered equal when the growth rates measured in the turbulent treatment lay inside the 90% confidence interval of the growth rates measured in the standing water. The 90% confidence interval was chosen to minimise the risk that possible differences were not detected.

Results

HNF growth rates and downstream changes in HNF abundance

Mean (±SD) gross growth rates (r_g) determined in the laboratory ranged from 0.16 ± 0.12 to 1.98 ± 0.10 day^{-1}.

Mean planktonic net growth rates ranged from 0.03 ± 0.44 to 1.94 ± 0.05 day^{-1}. There were significant differences between the two treatments (two-way ANOVA: $P < 0.001$) and between the dates ($P < 0.001$), indicating an impact of planktonic predators and seasons on the HNF growth rate. Highest growth rates were measured in the late summer of both years 1998 and 1999. No significant interactions between date and treatment were found ($P = 0.071$ for treatment × date). The highest grazing impact of planktonic predators was found in the summer of 1999 (Fig. 2).

Multiple regressions between HNF gross growth rate (dependent, log-transformed), temperature (independent) and water discharge (independent, log-transformed) showed a significant positive correlation with temperature ($b = 0.636$, $P = 0.001$). A slightly negative, but not significant, correlation could be found between gross growth rate and discharge ($b = -0.279$, $P = 0.094$).

From February 1999 to January 2000, the relative grazing loss due to planktonic predators ranged from 0 to 93% (mean 32%) of the HNF gross production (Fig. 3). Planktonic predators were never able to consume the entire HNF production. Only during summer, and once in November, did their consumption exceed 30% of the HNF gross production. Losses in the field that could not be explained by the impact of planktonic predators ranged from 0 to 195% (mean 64%) of the gross production at the same time (Fig. 3). Values larger than 100% indicate that the total gross production and a part of the standing stock were lost, resulting in a decrease of the standing stock during the downstream transport. From May until the beginning of November, significant differences between the *in situ* net growth rate (r_{is}) and the planktonic net growth rate measured in the laboratory (r_{np}) were demonstrated, whereas there were no significant differences during winter (from the end of November until April, with one exception in February 1999, see Table 1). The HNF loss rate (f) not due to planktonic predators was positively correlated with temperature ($r^2 = 0.596$, $P < 0.001$, see Fig. 4).

Comparing the planktonic net growth rate (r_{np}) in stagnant and turbulent waters, no significant differences could be found during the three comparative measurements in May and June 1999 (Fig. 5).
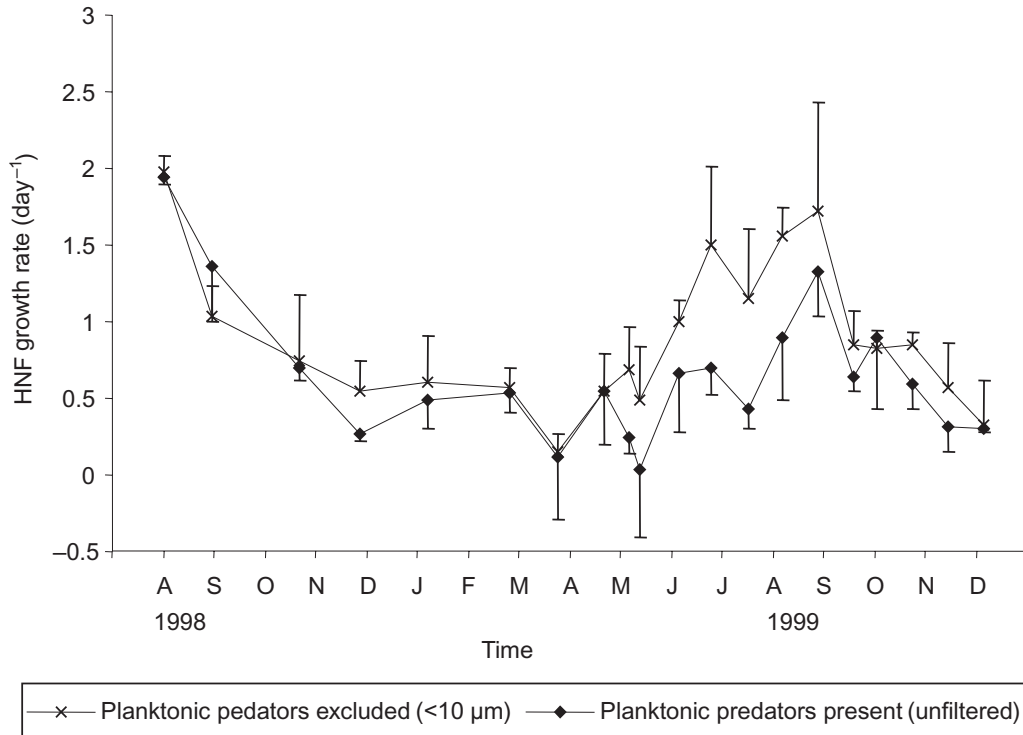
Fig. 2 Growth rate (mean and SD) of HNF with and without the presence of planktonic predators determined in laboratory experiments.
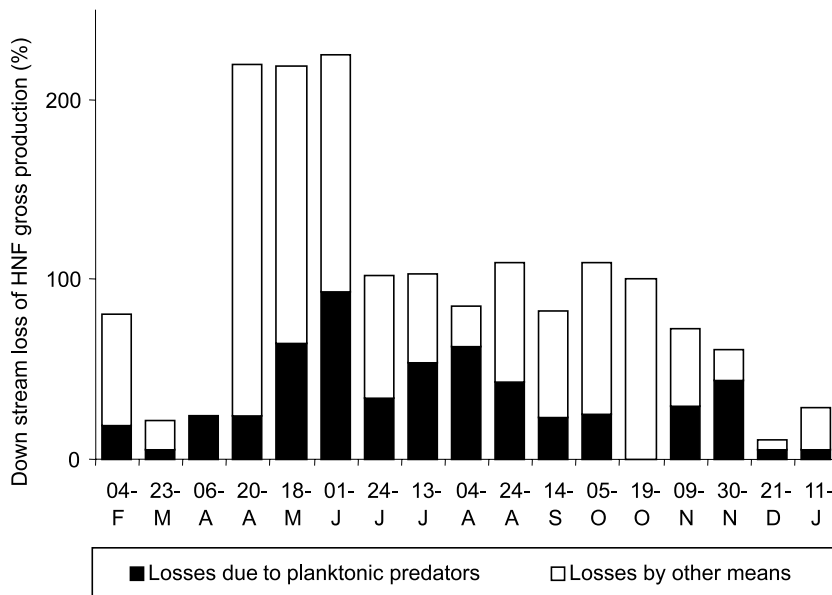

Fig. 3 Loss of HNF gross production in the field from February 1999 to January 2000. Total bar (black plus white section) gives the total loss. Black section indicates losses explained by planktonic predators (size fractionation experiments). White section gives the loss of HNF gross production not due to planktonic predators.

Dependence on water discharge

The HNF abundance in the Rhine at Cologne was very variable, with values ranging from 7 ± 6 to 4889 ± 559 mL^{-1} during the study period (Fig. 6).

Abundance was positively correlated with discharge over the whole study period ($r^2 = 0.687$, $P < 0.001$, Fig. 7). Peaks in abundance corresponded to peaks in discharge. The strongest dependence of HNF abundance on discharge was in the autumn and early

Table 1 Comparison of planktonic net growth rates measured in the laboratory (r_{np}, mean and 95% confidence intervals) and in situ growth rate (r_{is})

| Date | Growth rate (r_{np}, day^{-1}, planktonic predators included) | | *In situ* growth rate (r_{is}, day^{-1}) |
	Mean	95% Confidence interval	
4 February	0.49	0.28–0.70	0.12*
23 March	0.54	0.40–0.68	0.45
6 April	0.12	−0.34–0.58	0.22
20 April	0.12	−0.34–0.58	−0.19
18 May	0.24	0.13–0.35	−0.82*
1 June	0.03	−0.38–0.44	−0.61*
24 June	0.66	0.23–1.09	−0.02*
13 July	0.69	0.49–0.89	−0.04*
4 August	0.42	0.28–0.56	0.17*
24 August	0.89	0.43–1.35	−0.15*
14 September	1.33	1.00–1.66	0.31*
5 October	0.64	0.54–0.74	−0.08*
19 October	0.90	0.37–1.43	0.07*
9 November	0.60	0.41–0.79	0.23*
30 November	0.32	0.12–0.52	0.22
21 December	0.31	0.28–0.34	0.29
11 January	0.29	0.19–0.39	0.22

Significant differences between growth rates are given (*) if the r_{is} lies outside the confidence interval of r_{np}.
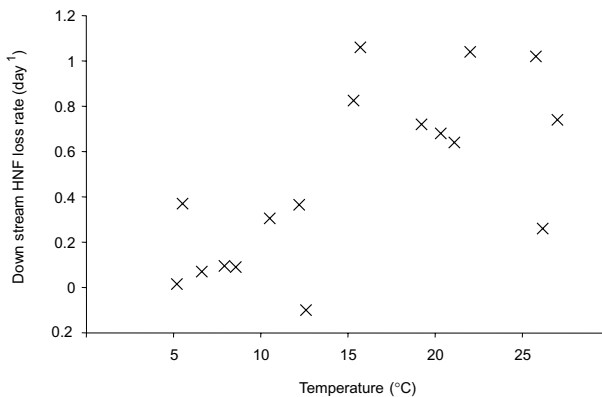

Fig. 4 Temperature dependence of *in situ* HNF loss rate not explained by planktonic predators (f, day^{-1}). Pearson Correlation coefficient is significantly positive ($P < 0.001$, $r^2 = 0.596$).

Fig. 5 Comparison of planktonic net growth rates (r_{np}) measured in stagnant water (mean and 90% confidence) and in flow channels.

winter of 1998 and 1999, whereas the relationship was weak or even absent in late winter (Figs 6 & 8). Changes in taxonomic composition during short-term flood events were small (Fig. 8). Only a slight increase in the percentage of chrysomonads occurred during the flood event in the autumn of 1998. The relative importance of typical benthic HNF groups, such as kinetoplastids and euglenids, that could originate from benthic drift, did not increase during spates (Fig. 8).
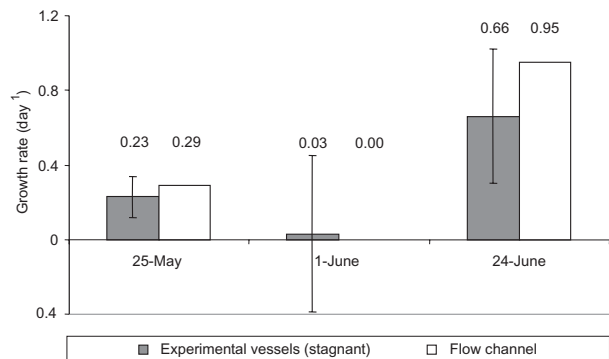
The modelled dependence of HNF abundance on water discharges from 1000 to 10 000 m^3 s^{-1} (Fig. 9) showed the following features: assuming no or little grazing impact of benthic predators, there was a decrease in HNF abundance with discharge. With medium grazing impact relative to HNF production, there was an increase in HNF abundance at low discharge and, after reaching a peak, a slight decrease. At grazing pressures as high or higher than HNF production there was an increase in HNF abundance up to a discharge of 10 000 m^3 s^{-1}. The model was
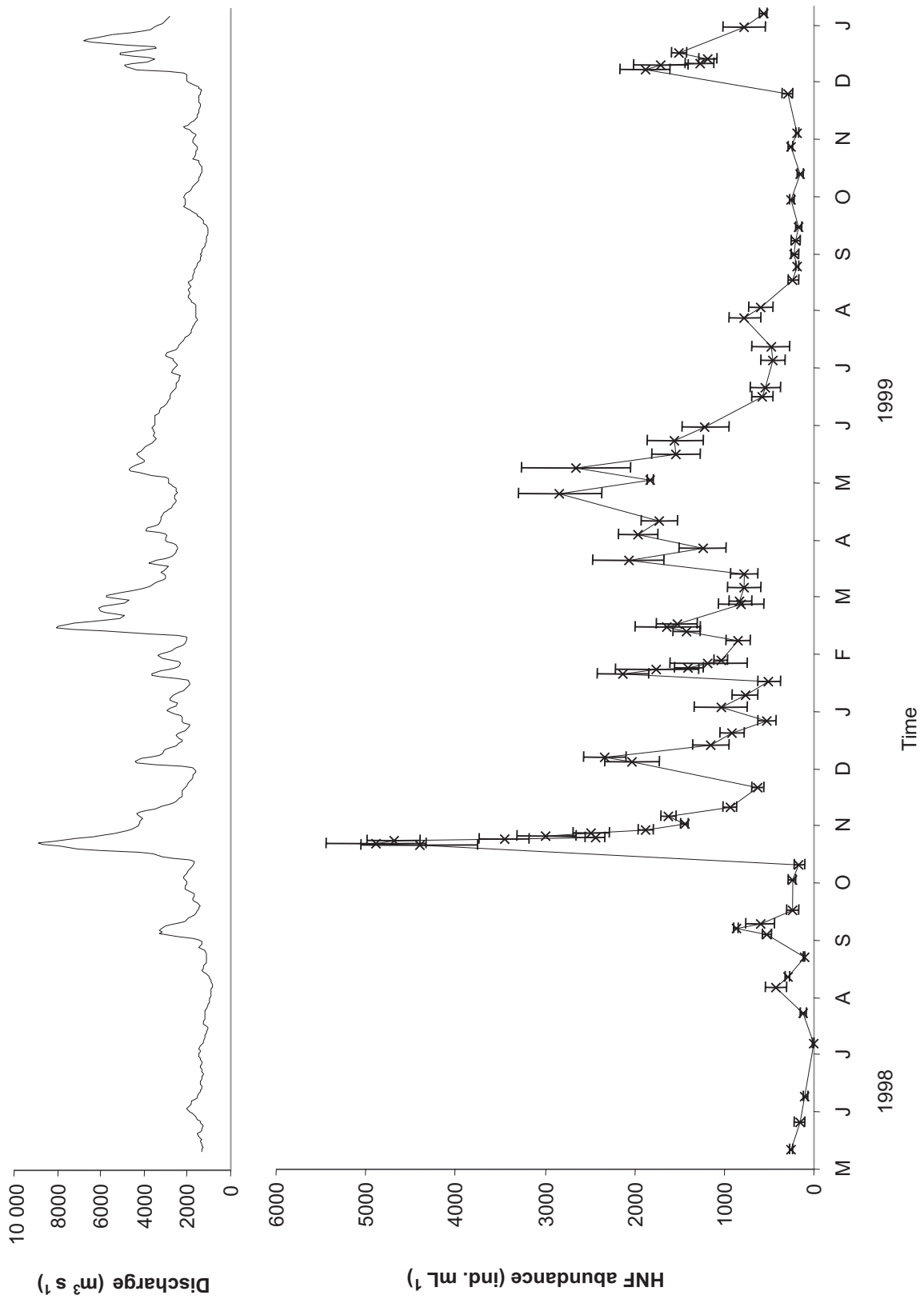
Fig. 6 River discharge (upper panel) and HNF abundance (mean and SD, lower panel) in the River Rhine at Cologne (km 685).
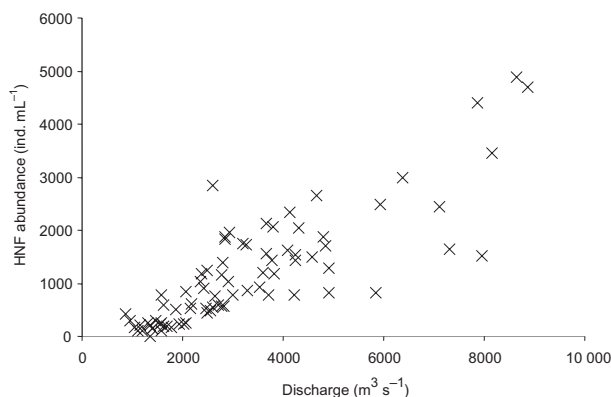
Fig. 7 Relationship between HNF abundance and discharge at Cologne over the whole sampling period (May 1998 to January 2000). Pearson Correlation coefficient is significantly positive ($P < 0.001$; $r^2 = 0.687$).

fitted with the field data during flood events, using measured planktonic net growth rate (r_{np}) of 0.7 day^{-1} for autumn (measured on 12 November, compare with Fig. 2) and 0.5 day^{-1} for late winter (measured on 16 March, compare with Fig. 2). An initial HNF input (N_0) of 800 individuals mL^{-1} for autumn and 150 individuals mL^{-1} for late winter was assumed (cf. Mathes & Arndt, 1995). The initial input influenced the amplitude of the curve, but not the general shape. There was a good fit between HNF abundance and water discharge for grazing rates by benthic predators (f_{2000}) of 1.0 day^{-1} for autumn and 0.4 day^{-1} for late winter (Fig. 8).

Discussion

Downstream losses of HNF production

There are several possible reasons for losses of planktonic organisms in rivers: direct mechanical forces (e.g. Horvath & Lamberti, 1999), sedimentation (e.g. Vyhnálek *et al.*, 1994), sediment filtration and predation by planktonic organisms (e.g. Gosselain, Viroux & Descy, 1998), benthic organisms (e.g. Welker & Walz, 1998) and (in the case of crustaceans) by fish (e.g. Akopian, Garnier & Pourriot, 1999).

In terms of the downstream losses of HNF in the Lower River Rhine, the results of our study demonstrated a significant impact of planktonic predators, especially in late spring and summer. However, the losses to planktonic predators could generally explain only about one third of the total HNF losses in the

field (Fig. 3). Potential predators on HNF in the water column were generally scarce. Ciliate abundance was between 100 and 1200 L^{-1} during the study (mean: 500 L^{-1}; Scherwass, 2001). Rotifer abundances between 3 and 73 L^{-1} (annual mean: 27 L^{-1}) were found in the River Rhine at Cologne during the study period (K. T. Sieben, personal communication). Therefore, the generally low grazing impact of planktonic predators found here is plausible when published filtration rates of metazoans and ciliates on HNF are considered (Jürgens *et al.*, 1996). The abundance of potential planktonic predators in the River Rhine has recently been very low compared with other river systems, where rotifers and ciliates, in particular, can be abundant (e.g. Viroux, 1997; Lair, Jacquet & Reyes-Marchant, 1999). There has been a pronounced decrease in plankton density in the River Rhine over the last two decades, documented for both the phyto- and metazooplankton (compare data in Friedrich & Viehweg, 1984; van Zanten & van Dijk, 1994; IKSR, 1997; LWA, 1992; LUA, 1996, 2000).

For the interpretation of downstream losses in HNF gross production not attributable to planktonic predators, direct mechanical forces, sedimentation, sediment filtration and consumption by benthic predators must be considered. Protists, and particularly small HNF, have much lower Reynolds numbers than metazoans and are unlikely to be damaged mechanically in their relative laminar surroundings (cf. Vogel, 1993). A recent study by Monaghan *et al.* (2001) demonstrates that there can be a gap between the total deposition of particulate organic matter (POM) and diatoms during the downstream passage in two streams and the losses induced by filter-feeding benthic macroinvertebrates. They highlighted the causes of loss other than those caused by benthic macroinvertebrates. However, our data on the temperature dependence of the downstream losses from the large River Rhine indicates biotic interactions. The recorded downstream losses (f) were low in winter (about 0.1 day^{-1} at a temperature below 10 °C; Fig. 4) and high during summer (about 0.8 day^{-1} at a temperature above 20 °C). This seasonal pattern was also present at comparable discharges in different seasons (compare the situations in May, June and July 1999 with the winter situation at similar discharge). Passive losses (as a result of sedimentation, sediment filtration, mechanical forces, etc.) should occur in the same intensity in winter as in
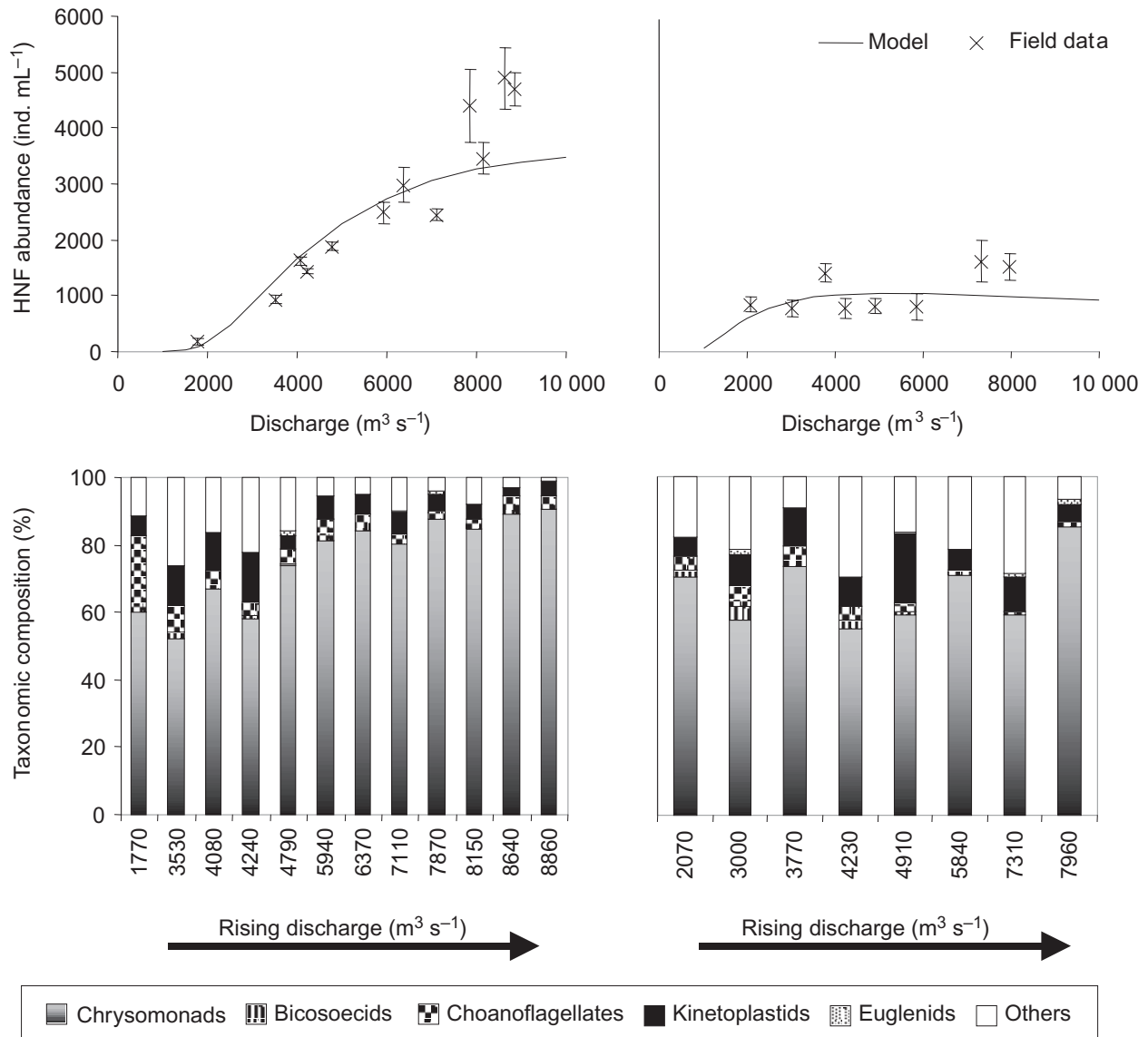
Fig. 8 Changes in the HNF community during two flow events in the autumn of 1998 (22 October to 21 November 1998, left panels) and late winter of 1999 (16 February to 23 March 1999, right panels). Upper panels show the dependence of HNF abundance (mean and SD) on the water discharge and the expected theoretical abundance from the model (assumptions autumn: $N_0 = 800$ individuals $mL^{-1}/r_{np} = 0.7$ $day^{-1}/f_{2000} = 1.0$ day^{-1}; assumptions winter: $N_0 = 150$ individuals $mL^{-1}/r_{np} = 0.5$ $day^{-1}/f_{2000} = 0.4$ day^{-1}). Lower panels show the taxonomic composition of the HNF community in relation to rising water discharge.

summer. The huge difference in losses depending on temperature rules out passive loss as the main factor and points to biotic interactions (i.e. benthic predation) in this highly turbulent river. The filtration activity of benthic filter feeders depends on temperature (e.g. Sprung, 1995; Haure *et al.*, 1998). In addition, the abundance of most taxa is much lower in winter than in summer (Rajagopal *et al.*, 1998 for the macrofauna; Weitere, 2001 for the micro- and meiofauna).

Both the temperature dependence of the filtration rate, and the low abundance of benthic filter feeders in winter, could cause the observed temperature dependence of HNF losses (Fig. 4).

The efficient benthic filter feeders *D. polymorpha*, *Corbicula* spp. and *Corophium curvispinum* (Sars 1895) are abundant in the Lower River Rhine (e.g. Rajagopal, Paffen & van der Velde, 1995; Rajagopal *et al.*, 1998). *Dreissena polymorpha* and *Corbicula* spp. can capture
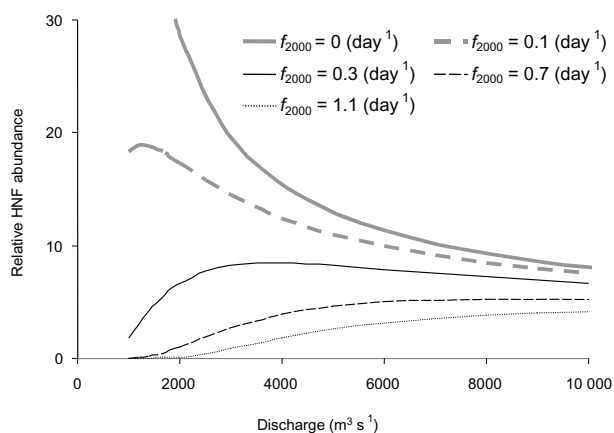
Fig. 9 Modelled dependence of HNF abundance on the river discharge for different grazing rates of benthic predators ($f_{2000} = 0/0.1/0.3/0.7/1.1$ day^{-1}) for a HNF growth rate (r_{np}) of 0.7 day^{-1} as an example. Absolute HNF-abundance = relative abundance × initial abundance (individuals mL^{-1}).

particles of a wide size range, including HNF (2–20 μm). *Dreissena polymorpha* has its optimal capture capacity at a particle size of 5 μm (Sprung & Rose, 1988). *Corbicula fluminea* feeds efficiently on natural particles of 3 × 5 μm (Way *et al.*, 1990) and on algae, ciliates and HNF (Scherwass, Eimer & Arndt, 2001). Ietswaart *et al.* (1999) calculated a filtration rate for the benthic community (including *D. polymorpha*, *Corbicula* spp. and *C. curvispinum*) in the Lower River Rhine of 0.38 day^{-1} for the warm seasons, which could explain about half of the losses observed in this study (Fig. 4). Besides the grazing loss caused by benthic macrofauna, the loss to benthic meiofauna and benthic protozoans should also be considered. Biofilm-dwelling ciliates and rotifers are abundant on hard substrata in the River Rhine and they can significantly affect nano- and microplankton in laboratory experiments (Weitere, 2001; K. Schmidt-Denter, personal communication).

Influence of water discharge on HNF abundance

The dependence of HNF abundance on discharge gives another indication of losses to the benthic environment in that the grazing pressure of benthic predators should decrease at high flow as a result of an increase of the ratio of water volume to the area of river bed colonised by predators. River discharge could vary strongly even within a period of a few days. The area of the river bottom colonised by predators is constant with rising discharge as long as

the river does not exceed its banks. When it does, colonisation of flooded ground takes some time, at least in the case of metazoans, and therefore plays no role in short-term events as observed in the present study. Therefore, there must be a significant decrease in the grazing pressure by benthic predators exerted on HNF at high flow (and particularly floods). On the other hand, there is a decrease in water residence time, resulting in a lower number of HNF generations. The impact of the discharge on both the grazing intensity and the water residence time were reflected in the model. The model shows that there should be a dependency of HNF abundance on the discharge, and that this dependency should vary depending on the ratio of HNF growth rate to the loss rate caused by benthic predators. At a high loss rate relative to the grazing rate, a strong increase in HNF abundance is expected, whereas the HNF abundance can be more or less constant over a huge range of discharge at a lower loss rate relative to HNF growth rate. Both patterns were found during the two high flood events and could be explained by the potential grazing of benthic predators, as demonstrated by the good fit of the field data to the model.

As demonstrated, a release from grazing pressure with increasing discharge could explain the general increase in HNF abundance, as well as seasonal differences in the dependence on the discharge. Nevertheless, an increase in HNF abundance might not be owing only to decreasing predation but also to an increase in gross growth, an increase in benthic drift as shown for ciliates (e.g. Schmitz, 1986; Primc-Habdija *et al.*, 1996; Scherwass, 2001), or to a possible washing in from backwaters or terrestrial environments. The data presented show no increase in gross growth rate with rising water level (see Results). An increase in benthic drift can be excluded because the contribution of benthic taxa (compare Arndt *et al.*, 2000; Dietrich & Arndt, 2000) to the total HNF did not increase at high flow. In addition, wash-in from the terrestrial environment cannot explain the increase in HNF abundance, as we observed no increase in typical soil flagellates (cf. Ekelund & Patterson, 1997) in plankton samples during spates. Also the effects of an input of flagellates from the few backwaters of the River Rhine cannot explain the sharp increase of HNF in the water column. The volume of these backwaters is negligible compared with the discharge. One must consider that the HNF abundance in the river at high flow was as high or even higher than

those known from lakes (compare Sanders, Caron & Berninger, 1992; Arndt *et al.*, 2000).

As an input of HNF and an increase in HNF gross growth rate are unlikely to be the reasons for the increase in HNF abundance with discharge, a decrease of losses to the benthos with rising discharge might be the major cause. Again, seasonally varying patterns in the dependence of HNF abundance on discharge (Figs 6 & 8) can be explained only by seasonality of the losses to benthic organisms and not by passive processes.

Methodological considerations

The general comparability between the HNF growth rates measured in the experimental vessels and those measured under turbulent and turbid conditions in the flow channels was demonstrated in the laboratory experiments (Fig. 5). Nevertheless, bottle effects such as a lower activity of planktonic predators or a depletion of food particles (bacteria) might also influence the determination of the planktonic net growth rate. A lower activity of planktonic predators is unlikely. Although planktonic predators were extremely scarce (see above), they had a significant effect on the HNF, which indicates a high feeding activity. A depletion of food particles seems unlikely as the bacterial number (Weitere & Arndt, in press) as well as the growth rates measured for the HNF were high.

An important result, the variation of the *in situ* losses during different seasons, still holds strong without the results of the laboratory experiments. The *in situ* net growth rate (r_{is}) which was determined from the field data from Cologne and Kalkar–Grieth, was higher during winter and early spring than during late spring and summer (Table 1). Considering potentially high and temperature-dependent growth rate of HNF (Fenchel, 1986), sufficient food availability for the HNF (Weitere, 2001) and generally low density of planktonic predators, the opposite findings should be expected. Therefore, even field data alone leads to the same conclusions: there was a high riverine loss of HNF production which cannot be explained by planktonic predators alone and which shows seasonality (implicating biotic interactions).

The model was designed to show whether loss to the benthic environment could explain (1) the strong dependence of HNF abundance on discharge and (2) the different seasonal features in the dependence of HNF abundance on discharge. The model was simple and does not consider all possible impacts on HNF abundance, such as storage zones that can be important in rivers (e.g. Reynolds, Carling & Beven, 1991; Reynolds & Descy, 1996; Reckendorfer *et al.*, 1999). The River Rhine is mostly regulated and very few backwaters are left (Tittizer & Krebs, 1996), while its water level can fluctuate by 2.5–9.5 m without leaving its canalised river bed. The high degree of regulation is one possible reason why such good fits between model and field data were possible with such simple assumptions.

The approach used to show the impact of benthic predators was indirect. Our conclusions are derived from two lines of evidence, thus minimising possible errors. Compared with direct approaches, by which the filtration rate of potential benthic predators is calculated, this indirect approach has some advantages. It does not depend on accurate population estimates of all potential predators and the calculation of their filtration rate. Population estimates in large rivers such as the River Rhine are problematic, because of the heterogeneity of the substratum as well as problems of sampling. There are also problems in calculating the *in situ* filtration rates, as there is different filtration behaviour under varying flow conditions (e.g. Ackerman, 1999). In addition, direct calculations usually concentrate on single species of macrofauna and do not include all potential predators (such as biofilm communities).

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