Effects of resource supplements on mature ciliate biofilms: an empirical test using a new type of flow cell

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Biofilm-dwelling consumer communities play an important role in the matter flux of many aquatic ecosystems. Due to their poor accessibility, little is as yet known about the regulation of natural biofilms. Here, a new type of flow cell is presented which facilitates both experimental manipulation and live observation of natural, pre-grown biofilms. These flow cells were used to study the dynamics of mature ciliate biofilms in response to supplementation of planktonic bacteria. The results suggest that enhanced ciliate productivity could be quickly transferred to micrometazoans (ciliate grazers), making the effects on the standing stock of the ciliates detectable only for a short time. Likewise, no effect on ciliates appeared when micrometazoan consumers were ab initio abundant. This indicates the importance of ‘top-down’ control of natural ciliate biofilms. The flow cells used here offer great potential for experimentally testing such control mechanisms within naturally cultivated biofilms.

Keywords: epibenthic ciliates; field communities; trophic transfer; periphyton; predator–prey dynamics; running waters

Introduction

Microbial assemblages on surfaces play an important role in the matter flux of many aquatic ecosystems, especially in shallow and running waters (Bryers and Characklis 1982; Fischer et al. 2002). These so-called biofilms (Wetzel 2001) are composed from a variety of prokaryotic and eukaryotic organisms, of which ciliates often dominate in terms of biomass (Finlay and Esteban 1998; Gong et al. 2005). Depending on the community structure, ciliate communities can play a major role in importing organic particles (eg bacteria and microalgae) from the plankton into the benthos (Eisenmann et al. 2001; Weitere et al. 2003; Kathol et al. 2009). The resource level in return is a factor influencing both the abundance and the structure of the ciliate communities (Norf et al. 2009).

Due to their relatively short generation time (ca 6–80 h at 20°C; cf Finlay 1977), ciliates can respond to environmental changes quickly (Müller and Geller 1993). Consequently, increased resource availability can enhance ciliate growth until carrying capacity is reached (Orland and Lawler 2004). This attribute accounts for the strong bottom-up control of ciliate communities as reported for both planktonic (Wiackowski et al. 2001; Andrushchyshyn et al. 2003; Scherwass and Arndt 2005; Tiros and Gaedke 2007) and epibenthic ciliate communities (Wickham et al. 2004; Gong et al. 2005; Primc-Habdija et al. 2005).

A second factor influencing both ciliate abundance and community structure is the activity of grazers. Laboratory studies have shown that micrometazoans such as cladocerans, copepods, nematodes, oligochaetes and rotifers can significantly reduce ciliate abundance and biomass (reviewed in Gifford 1991). Similar observations made in field studies demonstrated a negative relationship between ciliates and micrometazoans (Hillebrand et al. 2002; Domenech et al. 2006). After measuring stable isotopes of 2-week-old and 6-week-old biofilms, Augspurger et al. (2008) reported that ciliate field biofilms can also experience strong grazing pressure by micrometazoans. Although the distinct impact of top-down mechanisms on biofilm-dwelling ciliate communities (hereafter: ciliate biofilms) is not completely understood (Schmid-Araya et al. 2002), there are strong indications that particularly monogonont rotifers could play a key role in controlling ciliate biofilms analogue to their reported impact on planktonic ciliate communities (see above).

Despite the importance of both bottom-up (resource availability) and top-down (grazer activity) factors, little is as yet known about the interplay between these factors in controlling ciliate biofilms. This is primarily due to the poor accessibility of field biofilms; many techniques known for studying trophic interactions in planktonic systems (eg size fractionation experiments or dilution experiments) cannot be
adapted for biofilm research, making it difficult to experimentally test community responses to changes in specific environmental factors. Furthermore, short-term community dynamics, which are common among microbial communities (Nisbet et al. 1997; Montagnes and Berges 2004), increase the probability of missing key events in the succession of complex field biofilms when making only a few surveys within a relatively long time span. These aspects pose a challenge for the development of adequate experimental and sampling techniques for field studies.

One approach that combines the advantages of laboratory analyses with those of field experiments was presented by Norf et al. (2007). In this approach, ciliate communities are cultivated in miniature flow cells used as river bypass systems, facilitating both the continuous observation and the controlled manipulation of developing consumer biofilms. With the use of such flow cells, increased planktonic bacterial density was shown to significantly affect the early development of ciliate biofilms (starting from blank surfaces; Norf et al. 2009). The experiments further demonstrated the importance of environmental conditions when addressing community responses towards resource supplements. Important factors were the ambient resource load (planktonic bacterial density) and the activity of grazers of the ciliates (micrometazoans). This finding indicates that mature biofilms, which harbour a different set of organisms than early biofilms (including distinctly higher metazoan abundances), are likely to respond differently to resource supplements. Further differences between young and mature biofilms arise from the importance of species migration in controlling community dynamics. While immigration from the plankton can particularly influence early stages in biofilm succession, mature biofilms are comparably resistant against immigration (Hunt and Parry 1998; Wey et al. 2009). Therefore, mature ciliate biofilms could be controlled in particular by intrinsic growth conditions, e.g. resource availability and grazing.

Due to the enormous sediment load in running waters, the longevity of common flow cell systems used in experiments with field water bypasses is limited (cf Norf et al. 2007). Thus, it is very difficult to study community dynamics of mature biofilms running at carrying capacity. For this reason, a new flow cell was designed to take advantage of common flow cells, i.e. in situ observations of undisturbed consumer biofilms, in experiments with natural, mature biofilms. In the following, they were used to study the effect of increased planktonic bacterial density on consumer biofilms that had matured in the field. The planktonic bacterial supplements were adjusted to the same cell density as in earlier studies on the control of young biofilms, in which the standing stock of ciliates was shown to be significantly increased by such resource supplements (see Norf et al. 2009). Three experiments were performed during different seasons, incorporating different ciliate and micrometazoan abundances.

**Material and methods**

**Flow cells and experimental design**

Three experiments (March, May and August 2007, see Table 1 for exact time periods) were performed to test the impact of increased resource availability on mature consumer biofilms. The experiments were performed aboard the Ecological Rhine Station of the University of Cologne, Germany (50° 54′ 25″ N, 6° 58′ 43″ E). This former boat tender harbours several laboratories equipped with bypasses to untreated field water. The biofilms for the experiments were pre-cultivated on microscope slides which had been exposed in flumes (L: 90 cm, W: 9 cm, H: 15 cm) fed with untreated field water (flow rate: 0.15 m s⁻¹). The flumes were set up in a windowed room which allowed about 1.5% of natural light intensity to penetrate. After pre-cultivation for 8 weeks, one microscope slide each was transferred to a new type of flow cell (Figure 1; see supplementary material [Supplementary material is available via a multimedia link on the online article webpage] for more detailed figures). The slide remained submerged during the transfer in order to prevent mechanical disturbance of the biofilms. These flow cells are suitable for both direct manipulation (of, e.g. resource availability, temperature, and hydrochemical parameters; see Norf et al. 2007, 2009) and live observation of the same undisturbed mature consumer biofilms over a longer time span. The flow cells principally work like those utilized in earlier studies for which ciliate communities were cultivated starting from blank surfaces (Norf et al. 2007, 2009; Wey et al. 2008). They consist of a 82 × 37 × 8 mm plastic frame with an internal volume of 8.4 ml. Three miniature pipes on two sides of the flow cells serve as...
the inflows and the outflows for the field water bypass. A combined sediment and bubble trap (as described by Norf et al. 2007) was installed in front of the inflows to remove fine-grained sediment from the water flow and to keep otherwise destructive air bubbles from the biofilms. The mature biofilms within the flow cells were then supplied with a permanent flow of untreated river water via peristaltic pumps (one volume exchange per minute) and were kept in temperature-controlled water baths at ambient field water temperature and unchanged illumination (see above) during the experiments (see Table 1). The experimental manipulation was started after an acclimatization period of 24 h. Four flow cells were maintained without any manipulation; they only received the suspended resources from the field water flow (ambient conditions; hereafter: AMB). A suspension of a planktonic bacterium (*Pseudomonas putida* Migula 1895 MM1; Duetz et al. 1994) was put into the field water bypass of four other flow cells (bacteria supplemented treatment; hereafter: BAC). The pre-cultured biofilms were randomly assigned to the experimental treatments (AMB; BAC). For preparation, cryo-preserved *P. putida* cells were cultured in Erlenmeyer flasks containing 100 ml of 50% M9 culture medium (Hahm et al. 1994) + 0.04 g 1⁻¹ glucose at room temperature (20°C). The cultures were harvested by centrifugation (3400g; 15 min.) after cultivation for 2 days. This treatment has been shown to reduce the ability of *P. putida* to form biofilms (Bell et al. 2005). The pellet thus obtained was resuspended in Pratt minimal medium (Pratt and Salomon 1980) in order to wash the residual glucose from the cultures. The bacterial cells were then harvested again as described above. After determination of the cell density with a Helber counting chamber (W. Schreck, Hofheim, Germany), a suspension of *10^8* cells ml⁻¹ was prepared in standard reagent bottles. Using peristaltic pumps, this suspension was added to the inflowing water before it entered the flow cells. The bacterial solution was diluted 1:100 with field water in the flow cells, so that the final concentration of supplemented *P. putida* was *10^6* cells ml⁻¹. This bacterial concentration has been shown to distinctly enhance ciliate density within early biofilms (Norf et al. 2009). Data on the ambient load of planktonic bacteria (DAPI counts) and algae (live counts) in the River Rhine during the experiments were collected twice (once at the beginning and once at the end of the experiments) and are given in Table 1. Even if the cell density of supplemented bacteria was geared to the ambient load of planktonic bacteria, the supplemented bacterial biovolume was much higher due to the generally larger cell dimensions of *P. putida* compared to those bacteria naturally occurring in the River Rhine. The bacterial suspension was stored in a water bath (at a constant 6°C) during the experiments and was renewed every 2 days.

### Data collection and statistical analyses

The consumer biofilms were analysed by putting the flow cells directly under the microscope. The first survey was performed after the 24 h acclimatization period and immediately before the resource supplement was applied (day zero), providing the base line for the further observations. A minimum of 60 ciliates per flow cell was determined in defined areas (usually 0.2–0.5 cm² total area size) which were randomly distributed over the biofilms at 100 × magnification using a microscopic grid on each sample date. Due to the strong variation in the zooid abundance of colonial ciliates (up to several hundred zooids per colony) and to the often patchy distribution on biofilms, their abundance was recorded separately over a larger area at 50 × magnification (usually 1.0–2.0 cm²) in order to reduce artefacts from the observation data. Ciliate identification was performed according to the identification keys of Foissner and Berger (1996) and Lynn and Small (2002). In addition to the ciliate communities, micrometazoan abundances were recorded. They were either determined in the same areas as the colonial ciliates (applied for gastrotrichs, insects and rotifers) or for the complete biofilm area (applied for oligochaetes and platyhelminths) of 14 cm². The abundances of bdelloid and monogonont rotifers

| Experiment  | Date            | Temperature (°C) | Planktonic bacteria (10⁶ cells ml⁻¹) | Planktonic algae (cells ml⁻¹) |
|-------------|-----------------|-----------------|-------------------------------------|------------------------------|
| March 2007  | 10–22 March 2007| 9.4 ± 0.6       | 1.6 ± 0.2                           | 718 ± 176                    |
| May 2007    | 7–19 May 2007   | 17.5 ± 0.7      | 2.6 ± 0.6                           | 1750 ± 232                   |
| August 2007 | 10–19 August 2007| 19.8 ± 0.7     | 1.6 ± 0.3                           | 417 ± 111                    |

The abundance of planktonic bacteria (DAPI counts) and algae (live counts) was determined at the beginning and at the end of the experiments. The values given for bacteria and algae are means (± SD). Water temperature was logged daily with a digital thermometer. The given value is the mean temperature (± SD) over the entire experimental runtime.
were recorded separately. The following community analyses were started 1 day after the initiation of the resource supplement and were repeated every 1–3 days.

The statistical analyses were concentrated on investigating the effects of bacteria supplements on the ciliate as well as micrometazoan abundances. Repeated-measures ANOVAs (hereafter: rmANOVA) were calculated with time as the inner-subject factor and treatment (AMB vs BAC) as the between-subject factor. In addition, significant interactions between the two factors (time × treatment) were calculated; thus, the effects of increased resource availability on the total abundance of ciliates as well as on the abundance of specific taxonomic units (the five dominant classes or sub-subclasses) as the dependent variables were analysed. For micrometazoans, the rmANOVA were performed for likely grazers of ciliates (oligochaetes, platyhelminths and rotifers, cf Gifford 1991) as well as for the analyses of sum effects on the total micro-metazoan abundance. The significance levels were adjusted by the Bonferroni procedure in order to account for the six comparisons for the ciliates and for the four comparisons for the micrometazoans (three comparisons for the March experiment due to the absence of Platyhelminthes). Data discussion will be restricted to significant differences between the experimental treatments in the factor ‘treatment’ (for general effects) and to significant interactions ‘time × treatment’ (for temporary effects) on the consumer communities. The complete results from the rmANOVAs are given in Tables 2 and 3.

Results

Starting conditions of the consumer biofilms

Although the consumer biofilms were similar in terms of abundance at the beginning of the different experiments, distinct seasonal variations in organism densities were detected. The starting ciliate abundances were similar in March and May 2007, despite the different ambient resource loads (Table 1) during the two experiments. The highest ciliate abundances were recorded in August 2007. Regarding the ciliate community structure, peritrichs were dominant in all experiments and generally accounted for 460% of the total ciliate abundance (Figures 2–4). The highest micrometazoan densities were observed in May 2007, whereas generally lower abundances were observed in March and in August 2007 (Figure 5). All values given hereafter refer to the mean organism abundances based on four replicates ± SD.

Effects of bacterial supplement on ciliate biofilms

In March 2007, ciliate abundance in the AMB flow cells ranged between 280 ± 50 (day zero) and

| Time          | Treatment | Time × treatment |
|---------------|-----------|------------------|
| March 2007    | ss        | F                | ss        | F                | ss        | F                |
|               | Total     | 647,657          | 6.18***    | 468,520    | 15.88*            | 789,053   | 7.53***           |
| dftime (5, 20)|           |                  |            |            |                  |            |                  |
| Chor          | 13,465    | 2.80             | 900        | 0.56       | 6,025              | 1.25       |
| Het           | 1,166     | 2.64             | 491        | 2.73       | 874                | 1.98       |
| Lit           | 855       | 4.15*            | 187        | 0.67       | 574                | 2.79       |
| Per           | 808,488   | 8.68***          | 380,009    | 13.12      | 737,163            | 7.91***    |
| Scut          | 599       | 1.06             | 1,481      | 4.08       | 842                | 1.48       |
|               | ss        | F                | ss        | F                | ss        | F                |
| May 2007      | Total     | 59,255           | 2.23       | 5,783      | 0.18              | 52,406    | 1.97              |
| dftime (6, 24)|           |                  |            |            |                  |            |                  |
| Chor          | 537       | 4.35**           | 0.05       | 0.00       | 68                 | 0.55       |
| Het           | 6,598     | 24.18***         | 144        | 0.81       | 2,090              | 7.66***    |
| Lit           | 162       | 1.01             | 136        | 1.91       | 30                 | 0.19       |
| Per           | 25,718    | 0.70             | 4,293      | 0.10       | 54,676             | 1.49       |
| Scut          | 961       | 2.10             | 434        | 1.64       | 365                | 0.80       |
|               | ss        | F                | ss        | F                | ss        | F                |
| August 2007   | Total     | 1,213,108        | 6.62***    | 10,867     | 0.13              | 481,183   | 2.63              |
| dftime (5, 20)|           |                  |            |            |                  |            |                  |
| Chor          | 29        | 0.28             | 151        | 12.57      | 45                 | 0.44       |
| Het           | 1,554     | 0.98             | 2,688      | 0.12       | 578                | 0.37       |
| Lit           | 63        | 3.19             | 0.96       | 0.09       | 83                 | 4.21**     |
| Per           | 1,468,975 | 7.73***          | 4,484      | 0.05       | 553,889            | 2.91       |
| Scut          | 3,184     | 5.30***          | 274        | 1.04       | 707                | 1.18       |

ss = sum of squares.

The rmANOVAs were calculated for the total abundance of ciliates (Total) and for the abundant taxonomic groups as indicated in the corresponding Figures 2–4. Degrees of freedom (df) for the tested factors of each experiment are given in the left hand column below the corresponding sample date. * = significant differences between the treatments after sequential Bonferroni correction for multiple comparisons (*p < 0.1, ** p < 0.05, *** p < 0.01). Chor = Choreotrichia; Het = Heterotrichia; Lit = Litostomatea; Per = Peritrichia; Scut = Scuticocilia.
530 ± 140 individuals (ind.) cm⁻² at the end of the experiment (Figure 2a); peritrichs were generally dominant. Although choreotrichs also contributed significantly (35%) to the total ciliate abundance on day zero, they were replaced by peritrichs after cultivation for one further day. The BAC supplement resulted in an increase in ciliates from 320 ± 40 ind. cm⁻² (day zero) to a maximum of 1050 ± 360 ind. cm⁻² on day 6, before decreasing to a final abundance of 440 ± 40 ind. cm⁻² (Figure 2a). The rmANOVAs revealed significant effects of the BAC supplement on total ciliate abundance (treatment: \( p < 0.1 \); time × treatment, \( p < 0.01 \)). The effects were strongest for peritrichs, as reflected by the significant interactions time × treatment (\( p < 0.01 \); Table 2). This was due to the decline of peritrichs in the BAC flow cells from day 6 onwards to a final abundance of 280 ± 120 ind. cm⁻² (Figure 2c), whereas they maintained rather constant abundances of ca 400 ind. cm⁻² in the AMB treatment until the end of the experiment (Figure 2b).

Different results were obtained in May 2007. Ciliate abundances were similar in both treatments throughout the experimental period, ranging between 320 ± 40 ind. cm⁻² (day zero) to a maximum of 1050 ± 360 ind. cm⁻² in the AMB and between 230 ± 70 and 380 ± 60 ind. cm⁻² in the BAC flow cells (Figure 3a). No significant effect of the BAC supplement on the total ciliate abundance was detected (Table 2). The only significant group effect appeared among large heterotrich ciliates, as reflected by significant interactions time × treatment (\( p < 0.01 \); Table 2).

In August 2007, starting ciliate abundances were 530 ± 120 ind. cm⁻² in the AMB and 440 ± 120 ind. cm⁻² in the BAC flow cells (Figure 4a). After a short increase on the first day of the experiment, the ciliate abundance remained constant (ca 800 ind. cm⁻²) in the AMB flow cells. In contrast, ciliate abundance increased to a maximum of 1150 ± 400 ind. cm⁻² on day 5 in the BAC treatment, before declining to 780 ± 150 ind. cm⁻² at the end of the experiment (Figure 4a). However, the only significant effect appeared among litostomes (time × treatment, \( p < 0.05 \)) which were enhanced (max. 2.5 ± 2.5 ind. cm⁻²) in the AMB and 6.5 ± 2.5 ind. cm⁻² in the BAC treatment; Figure 4b,c) in response to the bacteria supplement.

### Table 3. Results from the repeated-measures ANOVAs (rmANOVAs) testing the effects of bacterial supplement (10⁶ cells ml⁻¹) on micrometazoans.

| Time          | Treatment | ss    | F     | ss    | F     | ss    | F     |
|---------------|-----------|-------|-------|-------|-------|-------|-------|
| March 2007    | Total     | 63.53 | 0.69  | 75.90 | 3.19  | 120.15| 1.31  |
| dftime (5, 20) | Oligochaeta| 1.42 | 1.00  | 0.284 | 1.00  | 1.42  | 1.00  |
| dftime (5, 20) | Platyhelminthes| –   | –     | –     | –     | –     | –     |
| dftime × treatment (5, 20) | Rotifera| 17.18 | 0.30  | 98.74 | 10.27*| 80.98 | 1.44  |
| May 2007      | Total     | 4,130.16| 2.66  | 154.22| 1.53  | 2,756.76| 1.78  |
| dftime (6, 24) | Oligochaeta| 4.88 | 0.26  | 11.20 | 1.34  | 28.35 | 1.54  |
| dftime (6, 24) | Platyhelminthes| 135.05| 3.26* | 0.50  | 0.01  | 156.57| 3.79**|
| dftime × treatment (6, 24) | Rotifera| 4,331.04| 5.64***| 394.45| 5.67  | 2,593.88| 3.38**|
| August 2007   | Total     | 110.65| 0.41  | 0.55  | 0.01  | 159.54| 0.60  |
| dftime (5, 20) | Oligochaeta| 10.46| 0.87  | 0.02  | 0.01  | 22.12 | 1.84  |
| dftime (5, 20) | Platyhelminthes| 9.18 | 2.15  | 0.13  | 0.07  | 6.44  | 1.51  |
| dftime × treatment (5, 20) | Rotifera| 70.48 | 1.36  | 6.60  | 0.64  | 33.01 | 0.64  |

ss = sum of squares.

The rmANOVAs were calculated for the total abundance of micrometazoans (Total) and for those grazers which were likely consumers of the ciliates. Platyhelminthes were not present in March 2007. Degrees of freedom (df) for the tested factors of each experiment are given in the left hand column below the corresponding sample date. * = significant differences between the treatments after sequential Bonferroni correction for multiple comparisons. (*\( p < 0.1 \), **\( p < 0.05 \), ***\( p < 0.01 \)).
Effects of bacterial supplement on micrometazoans

As observed for the ciliate communities, the micrometazoan communities exhibited distinct variations in their base settings as well as in their responses to the resource manipulations in the different experiments (Figure 5). The description hereafter concentrates on resource effects on micrometazoans which could have benefited from increased ciliate abundances due to their reported ability to graze on ciliates. Hence, oligochaetes, platyhelminths and rotifers were included in the statistical analyses (Table 3). Because the proportion of bdelloid rotifers to total rotifer abundance was generally low (maximum 15%) in all experiments, total rotifer abundances were used for the statistical analysis.

In March 2007, micrometazoan abundance ranged between $3.5 \pm 3.5$ and $6.5 \pm 6.0$ ind. cm$^{-2}$ in the AMB flow cells (Figure 5a). In the BAC treatment, the starting micrometazoan abundance was $5.0 \pm 3.5$ ind. cm$^{-2}$; it then increased until day 3 to a maximum of $11.5 \pm 6.5$ ind. cm$^{-2}$. These values were due to a stimulation of rotifers which reached their maximum on day 12 in the AMB (5.0 $\pm$ 4.0 ind. cm$^{-2}$) and on day 6 in the BAC flow cells (9.5 $\pm$ 6.0 ind. cm$^{-2}$). This observation was supported by a significant treatment effect ($p < 0.1$) on the total rotifer abundance in the rmANOVA (Table 3). The rotifer community in the BAC treatment was clearly dominated by monogononts, which accounted for $\approx 90\%$ of the rotifer community throughout the experiment.

Micrometazoan abundances of a whole magnitude greater were observed in May 2007 (Figure 5c,d), with
49.5 ± 10.5 ind. cm⁻² in the AMB and 31.5 ± 10.5 ind. cm⁻² in the BAC flow cells on day zero. After the experiments were started, micrometazoans gradually decreased to 27.5 ± 4.5 ind. cm⁻² (AMB) and to 21.0 ± 4.5 ind. cm⁻² (BAC) on day 12. Overall, no significant treatment effects (rmANOVA: p > 0.1 for both treatment and time × treatment) on the total abundance of micrometazoans were recorded in May (Table 3). However, significant temporary responses to the BAC supplement were detected for rotifers (time × treatment, p < 0.1), the abundance of which was temporarily higher on day 3 (AMB: 13.0 ± 9.0; BAC: 57.5 ± 34.9 ind. cm⁻²), and for platyhelminths (time × treatment, p < 0.05) which were stimulated by the BAC supplement towards the end of the experiment (Figure 5c,d). As observed in the previous experiment, the contribution of monogononts to the total rotifer abundance was >90% in the AMB and >80% in the BAC flow cells.

In the last experiment (August 2007), total micrometazoan abundances were rather low, similar to the observations made in March 2007 (Figure 5e,f). Their abundances ranged between 5.5 ± 2.5 and 14.0 ± 11.5 ind. cm⁻² in the AMB flow cells and 7.0 ± 2.0 and 14.0 ± 14.5 ind. cm⁻² in the BAC flow cells. Furthermore, they were not significantly affected by the BAC treatment, neither in terms of the total micrometazoan abundance nor of the distinct taxa. The only notable (though non-significant) observation was an increase in monogonont rotifers in the BAC flow cells (6.5 ± 6.0 ind. cm⁻²; AMB: 1.5 ± 1.5 ind. cm⁻²) on the last day of this experiment (day 9).

Discussion

Application of the new flow cell method

The present work is the first successful application of a new type of flow cell which facilitates experimental manipulation and in situ observation of mature consumer biofilms. The biofilms were previously cultivated in the field so that the direct effect of increased resource availability (here: planktonic bacteria) on ciliate biofilms previously matured under environmental conditions could be tested. Unlike common sampling techniques used to survey eukaryotic biofilms, the new flow cell technique allows the effects of controlled manipulations on the same (undisturbed) mature consumer communities to be investigated over an extended time span, ie over several successive generations. This in turn allows the disentangling of short-term community dynamics (eg predator–prey dynamics) which could be easily overlooked on a broader time scale. Investigating such short term dynamics has thus far been restricted to young biofilms developed within flow cells (Norf et al. 2007, 2009). Higher trophic levels such as micrometazoans are not properly represented in such young biofilms, leading to an under-representation of top-down effects (and other properties of mature biofilms) in the earlier biofilm experiments. This drawback has been solved by the successful introduction of the new flow cells.

Resource effects on the standing stock of mature ciliate biofilms are probably masked by top-down control

Regarding the supplemented resource, previous experiments (Norf et al. 2009) have demonstrated that the same bacterial supplement as applied here (P. putida, 10⁶ cells ml⁻¹) can generate strong and sustained effects on the quantity of early ciliate biofilms which showed similar final ciliate densities as the mature ciliate communities considered here. However, early consumer biofilms incorporate much lower (if any) abundances of micrometazoans than mature biofilms. This is probably the reason why the responses of mature biofilms to the bacterial supplement as observed here differed strongly from those observed among early communities. In all three experiments, the final ciliate abundances did not differ between the control and the bacteria-supplemented flow cells, although the higher resource level was expected to affect the ciliate quantity.

Thus, one likely explanation for the lack of sustained community responses is that elevated ciliate productivity could be ‘hidden’ behind the micrometazoan consumer activity. Clear indications supporting this assumption are given by the community dynamics observed in March 2007, in which the initial stimulation of ciliates was followed by an increase in micrometazoans (particularly rotifers) and a subsequent drop in ciliate abundance. Similarly, no effect of the resource supplement on the ciliate quantity was detected when micrometazoan consumers were abundant from the beginning, as was the case in May 2007. Both experiments suggest that increased ciliate productivity could be immediately transferred to a higher trophic level (here: micrometazoan consumers). As a consequence, no quantitative effect of the bacterial supplement on the standing stock of ciliates would be obvious, although ciliate sum productivity was enhanced. Such an alternative was also discussed by Hillebrand et al. (2002), who found no effects of field fertilization on ciliates, but rather on micrometazoans. In contrast, Domenech et al. (2006) found strong effects of stream fertilizations on heterotrophic protists, but not on micrometazoans. Such a discrepancy could possibly be explained by the occurrence of short-term dynamics among biofilm-dwelling consumer communities as observed in March 2007. With only
two surveys at the beginning and at the end of the experiment, neither the temporary stimulation of ciliates nor the temporary increase of micrometazoans would have been detected. On this account, Wickham et al. (2004) suggested that results drawn from experimental observations can crucially depend on the particular dates of community analyses. This demonstrates the strength of the new flow cell method. After the consumer biofilms have been transferred to the flow cells, they can be tracked over a longer time span without additional disturbance.

**Monogonont rotifers – key players in controlling ciliate biofilms?**

A key component of the micrometazoan community was rotifers. In March, increased ciliate abundances were followed by an increase in the rotifer abundance. In May, rotifers showed a temporary treatment effect. In August, rotifers also peaked (though not significantly) in the BAC treatment on the last day of the experiment, whereas other micrometazoans (dominated by chironomid larvae) showed no response at all. In response, ciliate abundance decreased as soon as rotifers became abundant. Likewise, rotifers were not able to maintain a higher standing stock when ciliate abundance was low, although the supplementation with planktonic bacteria was maintained, as observed in March 2007. The latter finding argues against a strong direct transfer from the supplemented bacteria to the rotifers. It is therefore suggested that a close predator–prey relationship was responsible for these findings rather than competitive interactions between ciliates and rotifers. This assumption is stressed by the numerous live observations made within this study of monogonont rotifers (e.g., Brachionus sp. Wenroenberg-Lund 1899) preferentially populating colonial, nonloricated peritrichs (e.g., Carchesium polypinum Linnaeus 1758, Campanella umbellaria Linnaeus 1758) instead of the biofilm surfaces. Similarly, they do not populate naked peritrich stalks, i.e., stalks that are lacking zooids. However, when the same peritrich colony is observed over a longer period of time, it can be seen that the number of zooids decreases with time when rotifers are present. Gilbert (1980) and Gilbert and Jack (1993) reported grazing by the monogonont rotifer Asplanchna girodi de Guerne 1888 upon the zooids of the stalked ciliate Campanella umbellaria. Combined with the results from this study, this indicates the likely importance of interactions particularly between monogonont rotifers and ciliates in biofilm communities, as have already been demonstrated for planktonic food webs (e.g., Gilbert and Jack 1993; Mohr and Adrian 2002; Weisse and Frahm 2002; Joaquim-Justo et al. 2004) and has previously been suggested also for early ciliate biofilms (Norf et al. 2009). Based on stable-isotope measurements, Augspurger et al. (2008) reported that rotifers can be an important energy sink in mature (> 6 weeks) consumer biofilms, whereas they are virtually absent in early, 2-week-old biofilms. These authors further reported a positive correlation between rotifer and ciliate abundance in mature biofilms but no correlation between rotifer abundance and the planktonic resource load. It is suggested that combining two different experimental approaches (in situ analysis of the community dynamics followed by energy flux measurements via e.g., stable isotopes) could improve the understanding of the interplay between rotifers and ciliates in consumer biofilms. The flow cells used here offer the opportunity to conduct such combined studies.

It should be noted that some rotifers can also consume planktonic bacteria. This applies especially to bdelloid rotifers (Ricci 1984). Thus, the possibility that some rotifers compete with the ciliates for the resource manipulated in the present study cannot be fully excluded. However, the contribution of bdelloids to the total rotifer abundance was low in the present study (and did not increase with bacterial supplement), and both the live observations as well as the ciliate–rotifer dynamics suggest a greater importance of predator–prey relationships.

**In situ monitoring of community dynamics using flow cells**

In addition to the quantification of the in situ community dynamics, flow cells offer the opportunity to directly detect interactions (such as grazing) and spatial dynamics by live observations. As discussed above, qualitative live observations were used to strengthen the conclusions on the trophic transfer between ciliates and rotifers. Besides the top-down control of the ciliate biofilms by micrometazoans, feedback was also detected between ciliates and micrometazoans by live observations (data not shown). The litostome Loxophyllum meleagris Dujardin 1841, which was the dominant litostome in all experiments, was observed to prey on smaller micrometazoans such as gastrotrichs and rotifers as well as on vagile ciliates, but was never observed to prey on stalked ciliates such as peritrichs (being the most abundant ciliates in all experiments). For this reason, the possibility that Loxophyllum contributed to the observed ciliate community effects in response to the resource supplements was dismissed. However, Loxophyllum did not generally consume its prey; especially when preying on rotifers, the ciliate often only narcotized its prey with the toxocysts and was not able to ingest the large particle; after several unsuccessful ingestion attempts,
Loxophyllum generally abandoned its designated prey. This observation demonstrates that some effects of resource supplements on mature consumer biofilms can be indirect, as has already been reported for early biofilms (Norf et al. 2009). Such indirect effects were made detectable by in situ observations of complex biofilm communities in an undisturbed environment as facilitated by the use of flow cells.

In addition to the quantitative aspects as discussed above, distinct adjustments in the taxonomic structure of the ciliate communities were also detected. In May 2007, for example, smaller peritrich ciliates were replaced by larger (probably resistant against micro-metazoan grazing) heterotrichs towards the end of the experiment in both resource treatments. However, generally the strongest responses of ciliate taxa to the resource supplements were detected among the smaller peritrichs, which presumably show higher growth rates than larger ciliates (Müller and Geller 1993). It is thereby notable that the strongest short-term response of the ciliate communities to the resource supplement was detected in March 2007, incorporating low ambient water temperatures (<10°C). This suggests that temperature was not a limiting factor in determining the magnitude of community responses to increased bacterial densities, and showed that ciliate communities can rapidly respond to enhanced densities of planktonic resources with enhanced productivity, even at relatively low temperatures.

Conclusion

The application of the new flow cell method presented here allowed insights into the short-term dynamics of mature consumer biofilms in response to resource increases. The results obtained add important aspects to the current knowledge on the control of ciliate biofilms, i.e. the predominant top-down control of mature ciliate biofilms by micrometazoans. This work demonstrates both the complexity of biofilm food webs (incorporating several trophic levels) and that resource effects on the standing stock of ciliates might be masked by a rapid transfer of enhanced productivity to higher trophic levels. However, bottom-up effects may persist and alter the community composition, even if the organism density remains unchanged (due to a strong simultaneous top-down control of the total ciliate abundance).

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