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Dynamics of cyanobacteria–ciliate grazer activity in bitrophic and tritrophic microcosms

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ABSTRACT: Direct and indirect interactions among components of bitrophic and tritrophic communities were studied using laboratory microcosms. The filamentous cyanobacterium *Phormidium* sp., capable of inducible defence, was used as the primary producer. *Phormidium* reacts to ciliate attacks by withdrawing inside a polysaccharide envelope, overproducing exopolysaccharide material and remaining in dense and compact clumps. All of these defences are induced by the ciliate *Pseudomicrothorax dubius,* a specialised grazer of filamentous cyanobacteria, representing the second trophic level in our system. The gymnostomatid ciliate *Homalozoon vermiculare*, which preys on *Pseudomicrothorax* but does not directly affect cyanobacteria, was the top predator within the microcosm community. The experiment showed that *Homalozoon*, very effective in a simple bitrophic cascade, had little effect on its prey when *Phormidium* was introduced into the system. Under grazer pressure, the cyanobacterium defended itself against the grazer by creating clumps of entangled filaments that also served as refuges for *Pseudomicrothorax* from *Homalozoon*. The prey ciliate *Pseudomicrothorax* reacts to cyanobacterial defence by increasing its encystation rate. Gradually decreasing grazing pressure resulted in a diminished *Phormidium* defence reaction, which enabled *Pseudomicrothorax* to resume feeding on filaments. Changing the extent of induced defence in *Phormidium* thus resulted in the stabilisation of the microcosm community.

KEY WORDS: Ciliated protozoa · *Homalozoon* · Induced defence · *Phormidium* · Predator–prey interactions · *Pseudomicrothorax*

INTRODUCTION

In temporary freshwater ecosystems, microorganisms such as cyanobacteria and ciliated protozoa are the main components of the aquatic microbial community. Their ecological functions, along with predator-prey interactions, have been well studied (e.g. Gasol & Duarte 2000, Wiąckowski et al. 2001, Ventelä et al. 2002, Pinckney et al. 2003, Samuelsson & Andersson 2003); however, the detailed links among different components of trophic cascades remain for the most part unclear. In both laboratory microcosms and natural ecosystems, direct predator–prey interactions evoke oscillations in predator and prey abundances. These predator–prey abundances, which depend on experimental design or ecosystem properties, usually lead to prey population decrements or prey extinction (e.g. Gause 1934, Salt 1967, Luckinbill 1973, Veilleux 1979). Under conditions of food shortage, the predator population may decrease or even completely disappear. Most ecologists agree that the trophic cascade in a variety of ecosystems is a balance between bottom-up and top-down control (Brett & Goldman 1996, Halaj & Wise 2002, Shurin et al. 2002, 2006). The role of the predator in a food web can be complicated by prey heterogeneity (Bohannan & Lenski 2000, Steiner 2001) or by the availability of prey representing alternative trophic levels (Balčiūnas & Lawler 1995, Kołaczyk & Wiąckowski 1997). Bohannan and Lenski (1997) observed that the invasion of a model laboratory community by bacteriophage-resistant mutants of *Escherichia coli* subjected to bacteriophage T4 had a strong effect on the subsequent population dynamics of both predator and prey. Both the equilibrium density

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and stability of the *E. coli* population increased following the invasion. Kołaczyk and Wiąckowski (1997) showed reduced predation on ciliate *Euplotes octocarinatus* by *Stylonychia mytilus* in the presence of an abundant green flagellate population, which served as alternative food for the predator. Trophic cascade interactions are also modified when the prey has access to refuges (Křivan 1998) or activates predator-induced defences (Fiałkowska & Pajdak-Stós 1997, Fyda 1998, Tollrian & Harvell 1999, Pajdak-Stós et al. 2001). These predator-induced defences include adaptive prey behaviours, such as predator avoidance reactions or escape responses, as well as the use of refuges that make prey less susceptible to predator attacks and consequently, reduce predator–prey oscillations. In addition, low refuge carrying capacity leads to stability of predator–prey dynamics, while stability is lost when the carrying capacity of the refuge is high (Křivan 1998). In the presence of their predators, some ciliates reveal induced defences consisting of cell shape changes, as in the case of *Colpidium* (Fyda 1998), or develop additional spines, as in *Onychodromus quadricornutus* and *Aspidisca turrita* (Wicklow 1997). Likewise, in the presence of their predators, several *Euplotes* species enlarge cell width and develop dorsal spines. The induced morphotype stands a better chance of avoiding the predator attack and a higher probability of survival in the predator's presence (Kuhlman & Heckmann 1994, Kusch 1995, Fyda & Wiąckowski 1998, Altwegg et al. 2006). An interesting example of induced defence in filamentous cyanobacteria was discovered by Fiałkowska and Pajdak-Stós (1997). In the presence of the ciliate *Pseudomicrothorax dubius*, which is specialised for ingesting filaments, cyanobacterium *Phormidium* sp. reacts to a ciliate attack by withdrawing inside a polysaccharide sheath where the filament is inaccessible to grazers. Moreover, instead of dispersing as in ciliate-free controls, in the presence of *Pseudomicrothorax*, cyanobacteria filaments entangle in dense and compact clumps, surrounded by a layer of exopolysaccharides, which further protects them from attack (Fiałkowska & Pajdak-Stós 1997, 2002, Pajdak-Stós et al. 2001).

Induced defence, widespread in both terrestrial and aquatic ecosystems, has broader impacts on the community than mere prey survival (Tollrian & Harvell 1999, Gomez & Zamora 2002). As discovered recently, it also affects other direct and indirect interactions among community components (Van der Stap et al. 2006, 2007a,b, 2008). Induced defences decrease per capita consumption rates of predators and increase the relative importance of bottom-up control (Vos et al. 2004a). These defences also promote population persistence and stability in simple bitrophic and tritrophic food chains (Verschoor et al. 2004, Vos et al. 2004b, Fyda et al. 2009). Moreover, inducible defence causes an absence of the 'paradox of enrichment', which in simple food chains leads to destabilisation of the predator population (Rosenzweig 1971, Vos et al. 2004b).

The present study examined the changes in tritrophic levels of microcosm communities composed of an autotrophic producer, its ciliate consumer and a top ciliate predator. Our aim was to study how the predacious ciliate affects prey ciliate abundance and activity, and how *Phormidium* predator-induced defences affect predator-grazer mediated coexistence.

MATERIALS AND METHODS

Cultures. All microorganisms used in this study are common species in freshwater puddles and ponds and coexist in nature. As a primary producer, we used the filamentous cyanobacterium (C) *Phormidium* sp. (described by Fiałkowska & Pajdak-Stós 1997). The second trophic level (P1) was represented by *Pseudomicrothorax dubius*, which is a specialised grazer of filamentous cyanobacteria. The top predator (P2) used in some treatments was the gymnostomatid ciliate *Homalozoon vermiculare*. Both ciliate species used in the experiment were taken from clone populations maintained at the Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland. Hereafter, we refer to the species by their generic names. The strain of *Phormidium* and the clone of *Pseudomicrothorax* were obtained from single filaments/cells isolated from a freshwater aquarium filled with pond water, and *Homalozoon* was isolated from a puddle. The pond and the puddle were located near the university campus on wetlands (50° 01′ 34″ N, 19° 54′ 05″ E).

Prior to the experiment, clones of the ciliates and *Phormidium* were cultivated in 5 cm diameter Petri dishes in Sanyo MLR-350 versatile environmental test chambers at a constant temperature of 20°C. The dishes contained BG11 medium (Stanier et al. 1971) prepared according to a formula obtained from the Culture Collection of Algae and Protozoa (CCAP, Ambleside, UK). The cultures of *Phormidium* were kept at a light intensity of 70 µmol photon m^{-2} s^{-1} under a 12:12 h light:dark cycle at 20°C. The ciliate cultures were kept in constant darkness. Twice a week, the clone of *Pseudomicrothorax* was fed *Phormidium* filaments, whereas the clone of *Homalozoon* was fed ciliate *Colpidium colpoda*. About 100 µl of dense *Colpidium* culture was added by micropipette to the Petri dishes containing *Homalozoon* every 2 d.

Experimental design and sampling regime. A microcosm experiment was carried out in cross-combinations of the presence or absence of the ciliate predator *Homalozoon.* The microcosms were estab-

lished in 24-well polystyrene tissue culture test plates (Renner GmbH). Each well contained 1 ml of BG11 medium. Approximately equal-sized (1 × 1 mm) pieces cut from the *Phormidium* mat were added to 12 wells. The presence of *Phormidium* in the treatments is marked by the symbol C. The wells were divided into 4 groups: to the first (C+P1+P2), 100 *Pseudomicrothorax* individuals and 5 *Homalozoon* individuals were added to each well; to the second (C+P1), 100 *Pseudomicrothorax* individuals were added; the third (C+P2) contained 5 *Homalozoon* cells per well, and the fourth (P1 + P2) contained 100 *Pseudomicrothorax* and 5 *Homalozoon* cells. As a control (C), we used wells containing only *Phormidium* mat. A second control (P1), containing only 100 *Pseudomicrothorax* individuals in 1 ml of BG11 medium, was also established. All ciliates were individually transferred with a pipette from their culture dishes to the experimental wells. Four replicates of every experimental treatment and control were made. Experimental plates were placed in a climate chamber and kept at 20°C at a light intensity of 70 µmol photon m^{-2} s^{-1} under a 12:12 h light:dark cycle. The experiment lasted 7 d. During this period, all experimental wells were checked under an inverted Olympus IX 71 microscope at 40× after 2 h (Day 0), then after 2, 6 and 7 d. During those surveys, the bottom of each well was scanned systematically and *Pseudomicrothorax* were counted using the following categories: well-fed with green vacuoles, active and dead cells. During the microscope surveys, ciliate cysts were also counted using 2 categories: full cysts with a ciliate inside and empty cyst walls remaining after excystment, referred to as empty cysts. Neither dead/paralyzed (by predator) *Pseudomicrothorax* individuals nor their cysts were removed from the wells. After 2 h and after 3 and 6 d, the percentage of *Phormidium* filaments ending with polysaccharide sheaths was calculated from 100 randomly chosen filament endings along the edge of the mat. At the end of the experiment, a 10× reference image of every treatment well was recorded with a DP70 microscope digital camera attached to the microscope in order to evaluate the condition of the cyanobacteria mat.

Statistical analysis. We used repeated-measures analysis of variance (ANOVA) to estimate the effects of *Homalozoon* presence on *Pseudomicrothorax* abundance and activity during the experiment. The presence of the top predator (P2) and cyanobacteria (C) and the interactions between the 2 were used as between-treatment factors and consecutive days in the experiment were used as a within-subject factor. To avoid non-linearity of percentage values, the arcsine transformation was used (Sokal & Rohlf 1981). All statistical analyses were performed using the data analysis software system STATISTICA StatSoft (2007), version 8.0.

RESULTS

Effects of *Homalozoon* on *Pseudomicrothorax*

On Day 0, the mean number of active *Pseudomicrothorax* per well ranged from 18 ± 5 (mean ± SD here and elsewhere) in pure BG11 medium (P1 treatment) to 23 ± 5 on average in all other treatments that day (Fig. 1). The number of active *Pseudomicrothorax* individuals varied significantly between treatments on consecutive days (Fig. 1). In C+P1+P2, the number increased significantly on the second day of the experiment, and remained at a similar level until the end of experiment. A similar effect was observed in C+P1 where *Homalozoon* was absent. The ciliate predator significantly affected the abundance of *Pseudomicrothorax* only in P1+P2 where no cyanobacteria were present. In this treatment, the number of active *Pseudomicrothorax* rapidly decreased, and no active ciliates were observed after the second day. This predation effect was significant (repeated-measures ANOVA, $p < 0.003$), and almost all prey ciliates were paralysed or ingested by *Homalozoon*. Similar results, though much later in the experiment, were observed in the P1 treatment with only *Pseudomicrothorax* in BG11 medium: after a small increase in the number of active cells on the second day, the number of *Pseudomicrothorax* decreased and no active cells were observed at the end of experiment (Fig. 1). The statistical significance of the results is shown in Table 1.

The mean percentage of well-fed *Pseudomicrothorax* per experimental well remained at a similar level throughout the experiment in the C+P1+P2 treatment, ranging from 74 ± 13% at the beginning to 95 ± 4.6% after 1 wk. In the C+P1 treatment, well-fed *Pseudomi-*

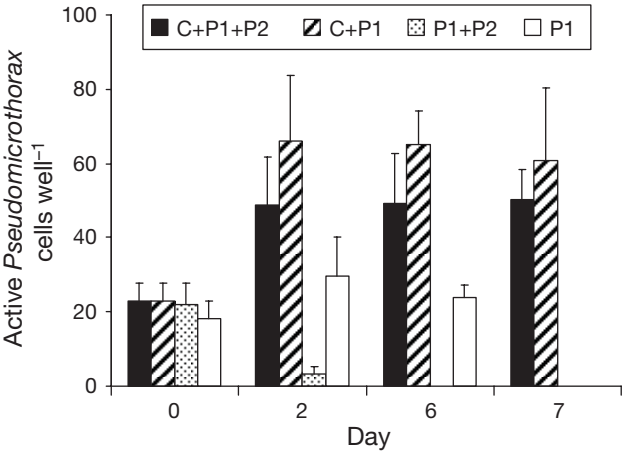

Fig. 1. *Pseudomicrothorax dubius*. Changes in the mean (+SD) number of active cells during the experiment. Treatments: P1: *Pseudomicrothorax*, P2: *Homalozoon*, C: *Phormidium*

Table 1. The results of repeated-measures ANOVA showing effects of *Phormidium* (C) and *Homalozoon* (P2) absence/presence on the abundance of the cyanobacteria grazer *Pseudomicrothorax* in microcosms during the time (T) of the experiment. Significant results are given in bold

State	Source of variation	df	SS	*F*	p
Pseudomicrothorax well fed cells	Between-treatments				
	Homalozoon	1	109.0	14.38	**<0.01**
	Error	6	45.5		
	Within-treatments				
	Time (T)	5	2105.6	8.16	**<0.001**
	T × P2	5	277.8	1.08	0.393
	Error (T)	30	1548.7		
Pseudomicrothorax active cells	Between-treatments				
	Phormidium	1	20 988.77	152.54	**<0.001**
	Homalozoon	1	2013.77	14.64	**<0.003**
	C × P2	1	1.89	0.0137	0.909
	Error	12	1651.19		
	Within-treatments				
	Time (T)	3	2333.05	10.77	**<0.001**
	T × C	3	6418.67	29.62	**<0.001**
	T × P2	3	1525.17	7.04	**<0.001**
	T × C × P2	3	283.30	1.31	0.287
	Error (T)	36	2600.56		
Pseudomicrothorax dead cells	Between-treatments				
	Phormidium	1	73.50	10.08	**<0.01**
	Homalozoon	1	4.17	0.57	<0.464
	C × P2	1	10.67	1.46	<0.250
	Error	12	87.50		
	Within-treatments				
	Time (T)	5	201.33	14.64	**<0.001**
	T × C	5	159.00	11.56	**<0.001**
	T × P2	5	60.83	4.24	**<0.002**
	T × C × P2	5	79.83	5.81	**<0.001**
	Error (T)	60	165.00		
Pseudomicrothorax cysts	Between-treatments				
	Phormidium	1	135 792.3	214.99	**<0.001**
	Homalozoon	1	950.7	1.505	<0.243
	C × P2	1	1600.0	2.533	<0.137
	Error	12	7579.6		
	Within-treatments				
	Time (T)	8	245 128.0	183.052	**<0.001**
	T × C	8	256 393.1	191.465	**<0.001**
	T × P2	8	2769.9	2.069	**<0.05**
	T × C × P2	8	2612.9	1.951	0.06
	Error (T)	96	16 069.4		

crothorax comprised 85 ± 7 and 93 ± 7% of total numbers on Days 0 and 7, respectively, which was not significantly different from that of treatments with *Homalozoon*.

The mean number of *Pseudomicrothorax* cysts per well was low in the C+P1+P2 treatment during the first 2 d of the experiment, then increased to 165 ± 24.9 on Day 6, and did not change significantly on the last day (Fig. 2a). A similar situation was observed in the treatment without the predator (C+P1), where the total number of cysts reached 198.8 ± 35.7 and remained at a similar level until the end of the experiment (Fig. 2a). *Phormidium* significantly affected cyst formation during the entire experiment (repeated-measures ANOVA, $p < 0.001$), whereas *Homalozoon* did not (repeated-measures ANOVA, $p = 0.243$). The number of empty cysts remaining after excystment — noticed for the first time on Day 2 — was low in all treatments and differences were not significant (Fig. 2b).

The mean number of dead *Pseudomicrothorax* cells varied during the experiment depending on the treatment. A few dead cells of *Pseudomicrothorax* were observed on the second day in the P1+P2 treatment where no *Phormidium* was added. Over the next few days, the number of dead cells increased significantly in both the C+P1+P2 and C+P1 treatments, whereas they were no longer observed in P1+P2 wells (Fig. 2c). The presence of the cyanobacterium affected the number of dead *Pseudomicrothorax*, whereas the presence of *Homalozoon* had no significant effect (repeated-measures ANOVA, $p < 0.01$, $p = 0.464$, respectively).

Effects of *Pseudomicrothorax* on *Phormidium*

The effect of *Pseudomicrothorax* on cyanobacterial filaments is shown in Fig. 3. In the absence of the grazer, the filaments began to disperse over the bottom of the experimental wells (Fig. 3a). Similar results were observed in wells where only *Homalozoon* was present (Fig. 3b). The appearance of mats was different in the C+P1 and C+P1+P2 treatments (Fig. 3c,d). Filament dispersion was clearly limited, the effect being more pronounced when there was no predator in the wells (Fig. 3c). In addition, it was clear that the mats under strong *Pseudomicrothorax* pressure created extremely dense, compact clumps (Fig. 3c), whereas those in the C+P1+P2 treatment resembled a nest with filaments loosely packed in the centre where active *Pseudomicrothorax* gathered, and much more densely packed at the edge (Fig. 3d). The impact of *Pseudomicrothorax* on *Phormidium* was also reflected in an increasing proportion of filaments ending with empty polysaccharide sheaths (Fig. 4). The highest proportion

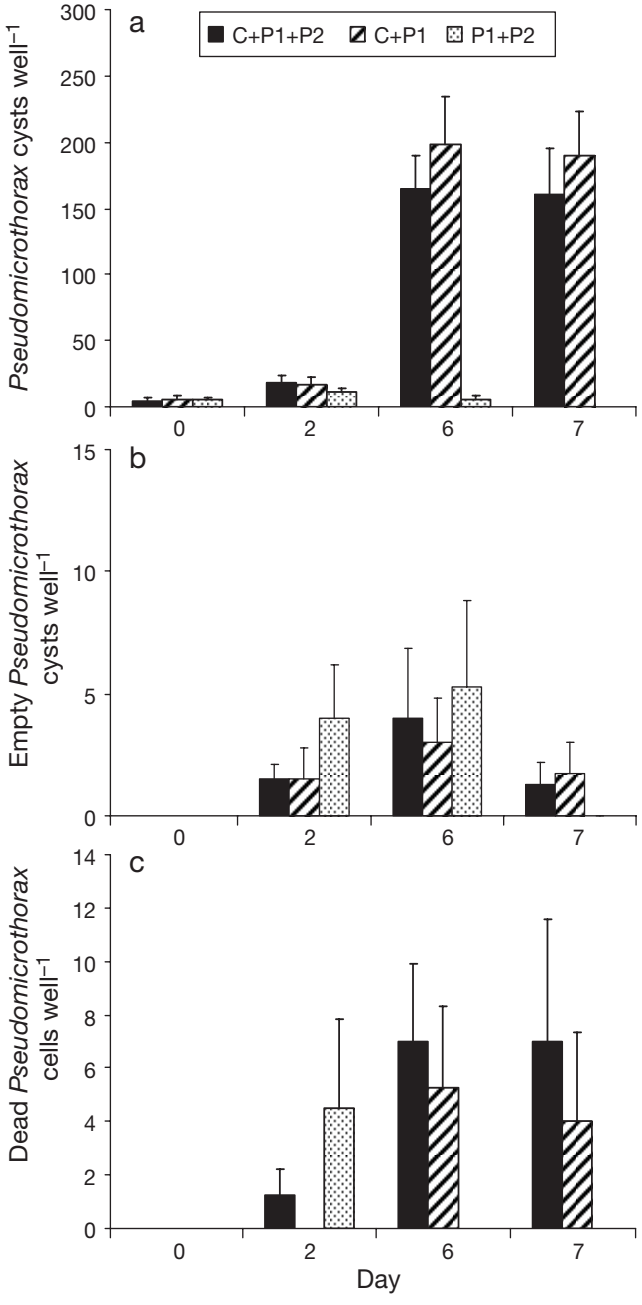

Fig. 2. *Pseudomicrothorax dubius.* Changes in the mean (+SD) number of (a) all cysts, (b) empty cysts and (c) dead *Pseudomicrothorax* cells in all treatments during the experiment. Treatments: P1: *Pseudomicrothorax*, P2: *Homalozoon*, C: *Phormidium*

of these ends was observed in the wells containing *Pseudomicrothorax* (C+P1+P2 and C+P1 treatments), and was significantly higher (repeated-measures ANOVA, $p < 0.001$) than in the C+P2 and C treatments (Table 2). Again, the effect was slightly more pronounced in the C+P1 treatment where the cyanobacterial grazer was not controlled by *Homalozoon*.

DISCUSSION

Effects of *Homalozoon* on *Pseudomicrothorax* activity in bitrophic and tritrophic microcosms

Predatory pressure plays an important role in ecosystem formation, and predator–prey oscillation patterns depend on ecosystem complexity and functionality (Chase et al. 2002, Strom 2002). In simple bitrophic microcosms, strong predatory pressure often leads to prey extinction in a short period. This is the case when prey is devoid of any kind of refuge or has insufficient time or nutrition resources to defend itself by inducible defences (Tollrian & Harvell 1999). In our experimental bitrophic microcosms where only *Homalozoon* and *Pseudomicrothorax* were present, we observed predatory pressure strong enough to completely eliminate prey, much stronger than in the tritrophic system where the cyanobacterial mat was present. This effect was strengthened by the fact that both predator and prey are bottom-dwelling ciliates and their encounter probability in the experimental wells was very high (Baumberg & Hausmann 2007). In addition, *Homalozoon* is a very effective raptorial ciliate feeder, with a peristome armed with toxicysts that paralyze the prey (Foissner et al. 1995).

Our experiments showed how the introduction of another trophic level — *Phormidium* mats — complicated the system. In the presence of *Pseudomicrothorax*, *Phormidium* defends itself against grazing by staying inside dense clumps (Pajdak-Stós et al. 2001). In the wells with *Homalozoon*, the *Phormidium* formed 'nest-like' clumps with the central space occupied by *Pseudomicrothorax* (Fig. 3d). We observed that *Pseudomicrothorax* took advantage of refuges offered by bundled filaments of cyanobacteria clumps. The cyanobacteria apparently gave *Pseudomicrothorax* a better chance to survive and even thrive. The lower density of filaments inside the clumps creating the refuge could be the effect of constant grazing pressure by *Pseudomicrothorax* or a rapid behavioural 'escape' reaction of filaments after ciliate attack (Fiałkowska & Pajdak-Stós 1997). Contrary to our bitrophic system results, in the tritrophic microcosms, *Pseudomicrothorax* was able to survive up to the end of the experiment (Fig. 3d). We expected that the effect of predation would be, to some extent, compensated by the fact that *Pseudomicrothorax* was able to feed on the cyanobacteria and therefore to proliferate (Fig. 1), but its usage of cyanobacteria clumps as refuges was a surprise.

Many small aquatic organisms are known for their encystment ability, regarded as a way to survive unfavourable environmental conditions, such as starvation, dryness or changes in environment chemistry (Gutiérrez et al. 2001, Müller 2007). In some cases,

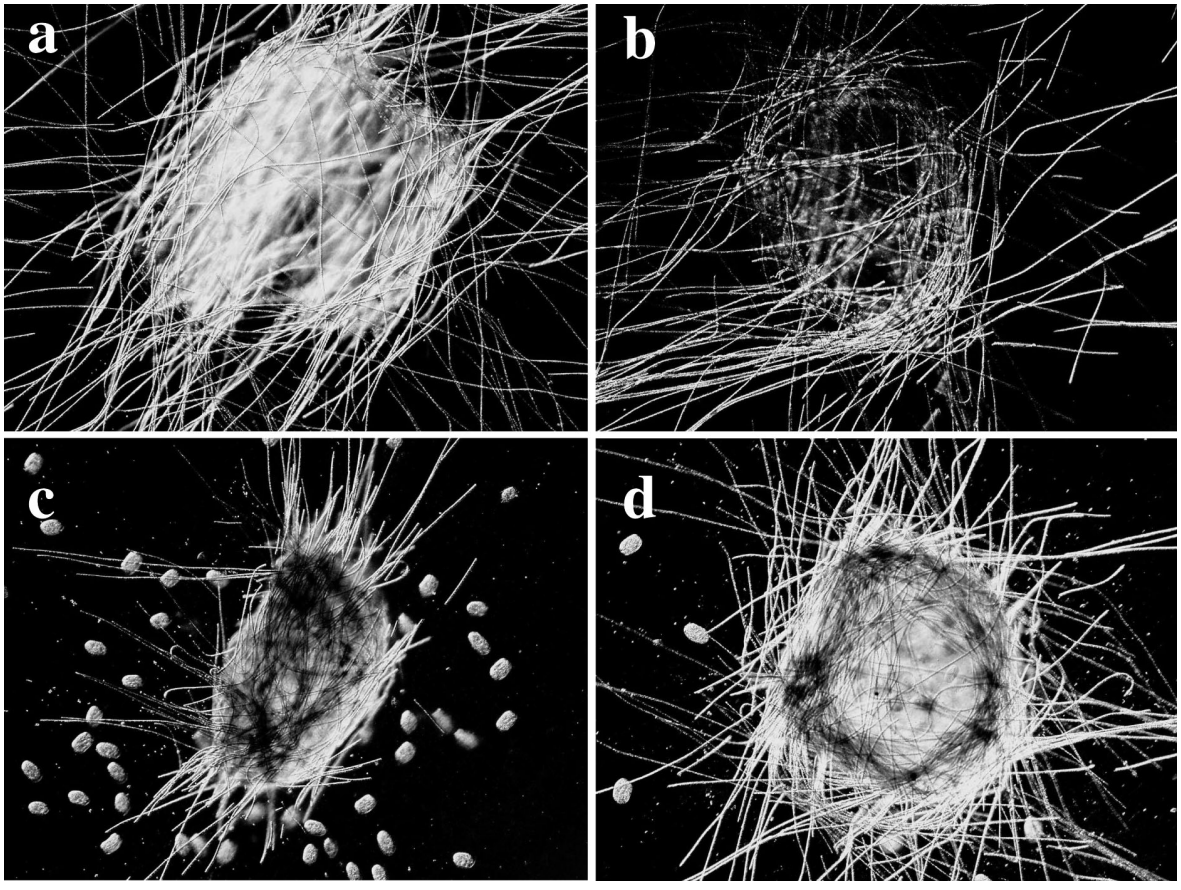

Fig. 3. *Phormidium* mat after Day 7 of the experiment in (a) the control, and under pressure from (b) *Homalozoon*, (c) *Pseudomicrothorax* and (d) both ciliates. Note numerous *Pseudomicrothorax* in refuges created by the cyanobacteria filaments (d)

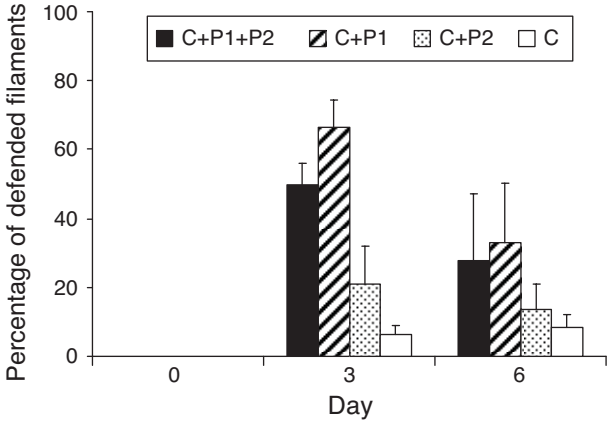

Fig. 4. *Phormidium* sp. Mean (+SD) percentage of defended cyanobacterial filaments observed in all treatments during the experiment. Treatments: P1: *Pseudomicrothorax*, P2: *Homalozoon*, C: *Phormidium*

encystment was reported to be a predator-mediated defence (Rengefors et al. 1998, Fyda et al. 2005). Fyda et al. (2005) noted that the ciliate *Euplotes muscorum* showed a higher rate of encystation in the presence of its predators; however, our results did not indicate predation as a factor responsible for *Pseudomicrothorax* encystment. Apparently, even if *Pseudomicrothorax* encystment was strengthened by predatory pressure, it was insufficient as a defence against *Homalozoon*. In the bitrophic (P1+P2) microcosms, the number of full cysts was extremely low during the experiment (Fig. 2a), and the majority of prey was eaten by predators. However, as shown in Fig. 2b, a few empty cysts were noticed on the second and sixth days in this treatment, which means that ciliate prey left the cysts in spite of the predators' presence. The numbers of cysts in the tritrophic treatment and in the treatment where only *Pseudomicrothorax* and *Phormidium* were present (C+P1) remained at the same level throughout the experiment. Therefore, it seems that *Pseudomicrothorax* encystment was caused by the lack of edible cyanobacterial filaments, the result of the *Phormidium* induced defence, rather than by the presence of predators. What is interesting is that almost all active ciliates remained satiated while the number of cysts was increasing. This could be explained as a control of active ciliate density by encystment. As a consequence of decreased density of active ciliates and thus grazer

Table 2. The results of repeated-measures ANOVA showing the effects of *Homalozoon* (P2) and *Pseudomicrothorax* (P1) absence/presence on the number of *Phormidium* (C) filaments defended by a polysaccharide envelope during the time (T) of the experiment. Significant results are given in bold

	Source of variation	df	SS	*F*	p
Phormidium	Between-treatments				
defended	*Homalozoon*	1	13.80	0.0253	0.876
filaments	*Pseudomicrothorax*	1	10616.59	19.459	**<0.001**
	C × P2	1	881.85	1.616	0.228
	Error	12	6547.14		
	Within-treatments				
	Time (T)	2	3011.19	10.499	**<0.001**
	T × P2	2	6.25	0.022	0.098
	T × C	2	1350.60	4.709	**<0.02**
	T × P2 × P1	2	307.43	1.072	0.358
	Error (T)	24	3441.78		

pressure, cyanobacterium trichomes leave their shelter (Fiałkowska & Pajdak-Stós 2002, Fyda et al. 2009). The fact that empty cysts were noticeable in all treatments with *Pseudomicrothorax*, starting on the second day, indicates that excystment takes place regardless of predator presence (Fig. 2b). In treatments with *Phormidium*, it might be the reaction of ciliates to the slowly increasing accessibility of food. However, why the prey ciliates leave the cysts when there is a predator in the habitat and no food is available remains unexplained. Possibly, ciliates in cysts are unable to monitor predator presence in the environment and leave cysts at random.

Effects of *Pseudomicrothorax* on induced defence in *Phormidium*

In the aquatic environment, predator-induced defence can be a factor that strongly influences predator-prey oscillations (Kusch 1998, Tollrian & Harvell 1999, Vos et al. 2004a, Van der Stap et al. 2007a, 2008). Prey that are able to undergo predator-induced morphological changes in unpredictable aquatic environments have a better opportunity to survive in the presence of the predator than an undefended morphotype (Wicklow 1997, Fyda & Wiąckowski 1998, Van der Stap et al. 2007b). Although the induced defence is energetically more costly than the constitutive form, it is very effective, especially when prey can adjust the extent of their reaction to the real predatory threat (Tollrian & Harvell 1999). This has been thoroughly studied in several ciliates from the genera *Euplotes*, *Colpidium* and *Coleps* (Kuhlmann & Heckmann 1985, Fyda & Wiąckowski 1998, Wickham & Gugenberger 2008) as well as in green algae (Lürling & Van Donk 1996) and cyanobacteria (Fiałkowska & Pajdak-Stós 2002). Our observations strongly support the results obtained by Fiałkowska & Pajdak-Stós (2002), in that *Phormidium* adapts its induced defence to grazer pressure and density. During the first 3 d of the experiment, strong pressure from *Pseudomicrothorax* induced a strong defence reaction in *Phormidium* filaments. Induced prey defence can stabilise and reduce the oscillations of predator–prey populations, and this effect was reinforced in the case of *Phormidium* by the refuges created by cyanobacterial filaments. Recent studies have indicated that predator-induced defence promotes population persistence in tritrophic food chains (Vos et al. 2004a, 2004b), prevents high amplitude predator–prey fluctuations and stabilises community dynamics (Verschoor et al. 2004, Vos et al. 2004a, Altwegg et al. 2006, Van der Stap et al. 2007a,b), as well as increasing the relative importance of bottom-up control (Vos et al. 2004b). The results of our work support these hypotheses.

It is worth stressing that our experiment showed that bottom-up control can strongly override top-down control in some cases. *Homalozoon* quickly eliminates *Pseudomicrothorax* in a bitrophic system, but in the presence of *Phormidium*, the influence of the top predator on the prey population is very weak.

The number of dead *Pseudomicrothorax* cells, very similar in treatments with and without the predator, indicated low *Homalozoon* pressure. *Homalozoon* survived to the end of the experiment in numbers similar to its initial abundance. The results described by Fyda et al. (2009) showed that a top predator (*Chaetogaster*) is able to strongly control an undefended cyanobacteria grazer, whereas defended *Euplotes* survive under predatory pressure. This might have resulted from the reaction of *Phormidium* — in Fyda et al. (2009), the grazers were eliminated quickly enough to let the filaments disperse on the well bottoms. In the present study, the induced defence of *Phormidium* offered good shelter for ciliates grazing on *Phormidium*.

The observed equilibrium between cyanobacteria and active ciliates was possible because of grazer density-dependent inducible defence in cyanobacteria on the one hand, and the ability of *Pseudomicrothorax* to react to this form of defence by means of rapid encystment on the other. The top predator effect on this equilibrium turned out to be negligible because of the ability of *Pseudomicrothorax* to use clumps of cyanobacterial mats as refuges.

Our simple microcosm experiment shows how sophisticated mechanisms can be involved in the interaction between trophic levels. However, we should be

very careful when extrapolating the results from bitrophic experiments to the natural biocenosis. Additional laboratory experiments and data from field studies are needed to reveal and explain the links among different components of microbial food webs.

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