Arch. Hydrobiol. **160** 4 431–446 Stuttgart, August 2004

Dynamics of an inducible defence in the protist *Euplotes*

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With 3 figures and 2 tables

Abstract: Inducible antipredator defences are expressed according to current predation risk. They enhance survival in the presence of predators but have associated fitness costs. Theory predicts that food webs in which members express inducible defences can be either more or less stable than food webs in which inducible defences are absent. This effect depends, to a large degree, on the ability of the prey defence to track predation risk. We investigated the dynamics of an inducible defence in hypotrich ciliates of the genus *Euplotes* in replicated experimental microcosms. In response to predators or their cues (live or frozen *Stenostomum* sp.), *Euplotes* develop lateral projections that significantly increase their width and effectively protect them from being eaten by this gape limited predator. *Paramecium aurelia* shares resources with *Euplotes*, but does not alter its morphology in response to predators. We exposed *Euplotes*, alone or together with *Paramecium*, to either live, frozen or no predators and followed the dynamics over thirty generations (cell cycles). The morphological defence of *Euplotes* varied over time and loosely tracked predation risk. The dynamics of the defence were affected by predator densities and conspecific densities to a similar degree. Loose coupling between defence level and predation risk is expected to lead to an overall weak impact of the defence on community dynamics. The dynamics of our experimental *Euplotes* populations, characterised by the coefficient of variation in population size, were not noticeably affected by how closely the defence matched risk.

Key words: ciliates, community dynamics, inducible defences, phenotypic plasticity, predator-prey dynamics, protozoa.

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DOI: 10.1127/0003-9136/2004/0160-0431 0003-9136/04/0160-0431 $ 4.00

Introduction

Many organisms react to variable and unpredictable predation risk by employing inducible defences (recently reviewed by Tollrian & Harvell 1999). In the defended state predation is reduced, however, prey with induced defence structures usually grow more slowly than those undefended. As predator densities increase, more prey individuals are expected to be in the defended state. Predator feeding success is expected to decrease, and so is predator population growth. As prey density increases and resources become scarce, prey individuals are expected to invest less in costly defences, become more vulnerable, and enable a fast increase in predator population size (Werner & Anholt 1993). Thus, inducible defences may generate density dependence in both halves of the predator/prey relationship (Anholt & Werner 1999), and possibly stabilise dynamics (Ives & Dobson 1987). However, inducible defences may also destabilise predator/prey dynamics (Abrams & Matsuda 1997), through time delays initiated by the reaction time of prey to changes in predation risk (Luttbeg & Schmitz 2000). Thus, the influence of inducible defences on predator-prey dynamics depends on the ability of prey defences to track predation risk.

The ability of the prey defence to track predation risk depends on three conditions: 1) how quickly defensive traits change in relation to changes in predation risk, 2) reliability and transmission distance of the cue relative to locomotory speed of prey and predators, and 3) the cost-benefit trade-off of the defence, which can be affected by additional factors such as resource levels. These three conditions may cause fluctuations in an inducible defence that cannot be anticipated by short term experiments alone.

We studied the dynamics of an inducible defence in the ciliate protist *Euplotes. Euplotes* change their cell shape by forming wing-like lateral projections that effectively protect them from being eaten by gape limited predators, such as the turbellarian *Stenostomum* or predatory ciliates (Kuhlmann & Heckmann 1994, Kuhlmann et al. 1999). Expressing this defence results in longer cell cycles and thus slower population growth (Kusch & Kuhlmann 1994). The defence is gradually expressed within hours (Kuhlmann & Heckmann 1985), and can be reversed within a single cell cycle. Both the costs and benefits of defence expression in *Euplotes* appear to scale approximately linearly with the level of expression (Wiackowski et al. 2003).

We address two questions in this study by focusing on *Euplotes* population dynamics. 1) How much do predation, predation risk, and competition depress initial population growth, and how do these factors affect mean, maximum, and variation in population numbers over time? 2) How closely does morphological defence track predation risk, as measured by the density of predators and conspecifics?

Methods

Experimental procedures

We examined the consequences of flexible body shape to population dynamics using three cultures of *Euplotes*, which had been initiated from a single cell each and kept as separate clonal lines thereafter. We used one clone of each of three species, *Euplotes aediculatus*, Aed 33, *E. octocarinatus*, Octo C1, and *E. plumipes*, Plu (kindly supplied by K. Wiackowski at Jagiellonian University, Krakow, Poland).

All experiments were conducted in six-well tissue culture plates (Nr. 353046, MULTIWELL™, Becton Dickinson Labware, NJ, USA). Each well held 10 ml of sterile medium, consisting of one protozoa food pellet (Nr. 13-2360, Carolina Biological Supply Company, NC, USA) dissolved in 2 litres of mineral water (NAYA, Mirabel, Québec, Canada). An autoclaved wheat grain was added to each well to provide nutrients. We initially stocked each well with 50 *Euplotes* cells to begin the experiment at low densities. Each clone was subjected to six experimental treatments, randomly assigned to a well within a given plate. The treatments were: i) *Euplotes* alone, ii) with competitors (50 *Paramecium aurelia* were added at the beginning of the experiment), iii) with predatory cue (~ 50 individuals of the predatory *Stenostomum* sp. which had been freezer-killed at – 5 °C), iv) with predatory cue and competitors, v) with live predators (20 predators were added once at the beginning of the experiment), and vi) with live predators and competitors. Dead predators induce the defended phenotype in *Euplotes* (acting as a predatory cue), allowing us to separate the effects of increased defence level from effects of predation. Dead predator treatments had to be replenished every two days to maintain the predation cue at a constant high level. During the freezing process, predators disintegrated, and we do not know how quickly they degraded. By adding dead predators, we added nutrients that potentially increased bacterial growth. However, the volume of predators added was small compared to the constant supply from the wheat grain and the medium, which was replenished every two days. We never observed bacterial overgrowth in any of the wells associated with dead predators. The *Paramecium* cultures unintentionally contained a dicranophorid rotifer, which subsequently reached significant population densities in the competition treatments.

We replicated each treatment five times using a randomised block design, and started the experiment on 23 January 2003. Every two days, we removed 10 % (five samples of 200 µl) of the content of each well and replaced it with sterile medium. All protists, rotifers and *Stenostomum* were counted under a dissecting microscope. Every four days, we photographed one to 33 *Euplotes* cells (median 10) directly through the flat bottom of the experimental containers using an inverted microscope (Leica DmIRB) with an attached CCD camera (COHU). The cell width (measured using the software package ImagePro) obtained from these photographs served as our measure of the level of induction. Constrained by the available workforce, we ended the *E. octocarinatus* treatments after 12 days, *E. plumipes* after 30 days, and *E. aediculatus* after 50 days, except one block which was terminated after 30 days.

Statistical analyses

We divided the time series into a phase of initial population increase (phase 1: the first eight days), and remaining time (phase 2: day 8 and onwards). We estimated the population growth rate during phase 1 by fitting linear regression models to the logarithms of the mean counts per day for each well. The slope provides an estimate of the population growth rate at low population densities. This estimate is lower than the true value because it ignores the 10 % density independent mortality that we imposed every two days through our sampling procedure. Nevertheless, sampling mortality was constant across all treatments and thus we do not further correct for this. We applied analysis of variance (ANOVA) to test for the effects of competition and predator treatment on population growth rate. We used the coefficient of variation of mean population counts (McArdle et al. 1990), and the mean and maximum of each population to characterise population dynamics during phase 2. The effects of competition and predation on these characteristics were then evaluated by multivariate analysis of variance (MANOVA).

If the level of defence closely tracks predation risk, it should respond to changes in predator density and conspecific density. Specifically, we evaluated the following hypotheses: First, predator density alone should explain variation in the level of defence in *Euplotes* if they react only to cues emitted by the predators (Wiackowski et al. 2003). Second, *Euplotes* may base their reaction solely on the number of conspecifics, due either to reliance on the herd effect or changes in resource allocation with conspecific density. Third, a simultaneous response to both predator and conspecific density is possible as predicted by optimality considerations (Wiackowski & Staronska 1999, Peacor 2003). Fourth, there may be an interaction between the reaction to predators and the reaction to conspecific density. For example at low predator densities, prey may not react to changes in conspecific density. Finally, the variation in prey defence level may not directly be related to either predator or conspecific density, for example if the defence strongly depends on the current resource level (Werner & Anholt 1993). We fitted five linear regression models corresponding to these alternative hypotheses to the time series produced by each experimental unit, and assessed the support for each model using the sample-size-adjusted Akaike's Information Criterion (AICc; Burnham & Anderson 2002). Akaike weights assess the relative support a given model has from the data relative to the other models in the set, and summing the Akaike weights for all models containing a particular factor (e. g. predator density) gives a measure of importance of that factor (Burnham & Anderson 2002). The regression analyses related the mean width of all measured cells to the count of predators or conspecifics of the previous day, and we weighted each observation by the square root of the number of measured cells. This procedure assigns a higher weight to those observations for which we have more reliable data. Reduced major axis regression is preferable to ordinary (least-squares) regression in our case because both independent and dependent variables were affected by measurement error (McArdle 1988). Thus, we report the reduced major axis regression slope of cell width on number of predators or conspecifics in the sample. All statistical analyses were calculated using the R software version 1.7.1 (Ihaka & Gentleman 1996; available at: http://www.r-project.org/).

Results

Euplotes population dynamics

The *Euplotes* population failed to establish in two experimental units, which consequently were eliminated from all analyses. The other experimental populations grew exponentially (approximately linear on the log scale, Fig. 1) over the first eight days, and were not significantly affected by their competitors (all $P > 0.5$). Population growth of *E. aediculatus*, but not the other two clones, was reduced by the predator treatments (predator treatment entered the analysis as a factor with two degrees of freedom: $F_{2,20} = 10.06$, $P < 0.001$; *plumipes* and *octocarinatus*: $P > 0.1$), with live predators having a larger negative effect than dead predators. Population growth was not significantly related to the level of defence on day 2, when densities were still consistently low in all experimental units (linear regression of population growth on mean cell width: $P > 0.5$ for all clones).

MANOVA showed that population dynamics after day eight of both *E. aediculatus* and *E. plumipes* (no data were collected for *E. octocarinatus*) was affected by competitors, but not by predator treatment (Fig. 1, Table 1). Subsequent univariate analyses showed that competitors depressed mean and maximum population numbers in both clones, and increased the coefficient of variation in population numbers in *E. aediculatus* (all $P < 0.02$).

Predation risk and level of defence

Euplotes strongly reacted to both live and dead predators by increasing their cell width (Fig. 2). The variation in the level of this defence was larger in predator treatments than predator-free replicates (standard deviation of body width over time: *E. aediculatus*: 5.4, 12.4, and 13.7 in control, dead predator, and live predator treatments; $F_{2,24} = 12.7$, $P < 0.001$; *E. plumipes*: sd = 4.8, 6.0, and 10.2; $F_{2,23} = 13.9$, $P < 0.001$). In *E. aediculatus* but not *E. plumipes*, the varia-

Table 1. Summary of MANOVA results on the effects of competition and predator treatment on *Euplotes* population dynamics after day eight. Population dynamics were characterised by the coefficient of variation in population size, log(mean population size), and log(maximum population size).

	Euplotes aediculatus					*E. plumipes*				
	Wilks λ	F	Num DF	Den Df	P	Wilks λ	F	Num DF	Den Df	P
Competition (C)	0.431	7.923	3	18	0.001	0.686	2.592	3	17	0.087
Predation (P)	0.791	0.748	6	36	0.615	0.830	0.555	6	34	0.763
Block	0.484	1.262	12	47.9	0.272	0.248	2.620	12	45.3	0.010
C × P	0.676	1.300	6	36	0.282	0.629	1.481	6	34	0.214

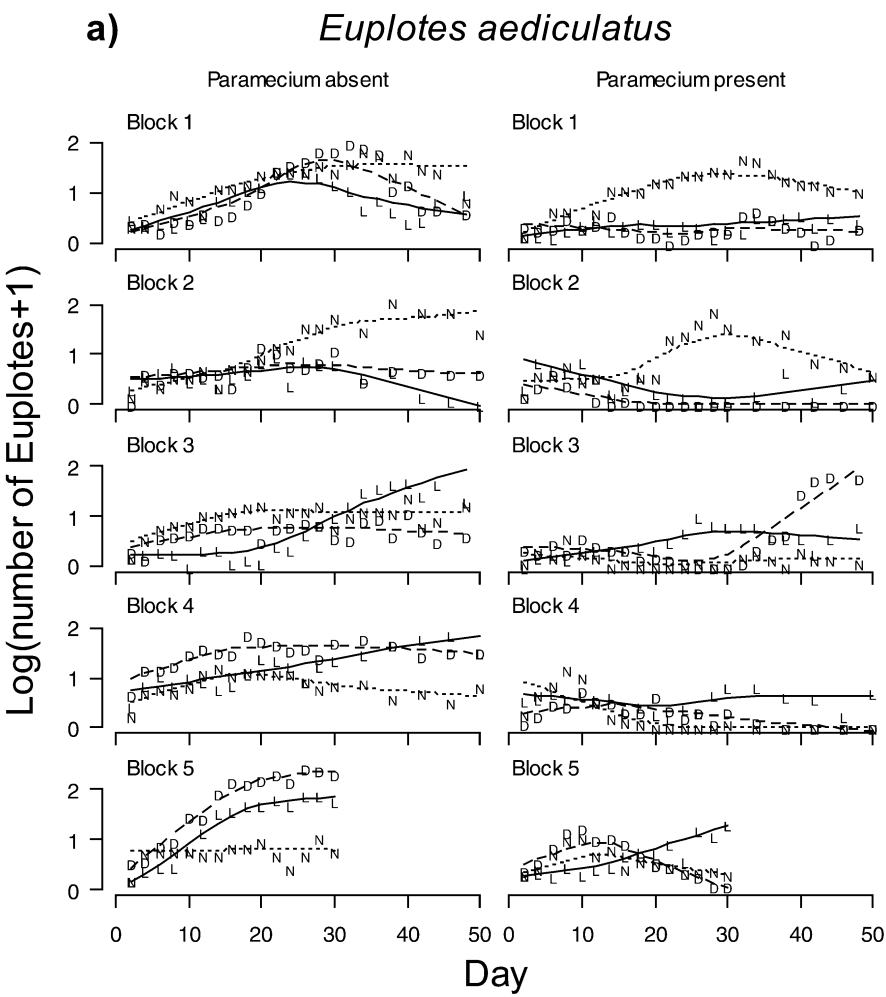

Fig. 1. a.

tion in defence was also larger in treatments with competitors compared to treatments without competitors (*E. aediculatus* 13.0 vs. 7.7; $F_{1,24} = 14.7$, $P < 0.001$; *E. plumipes*: $P > 0.2$).

We further investigated whether variation in defence level in live-predator treatments was caused by fluctuations in predator numbers, conspecific density, or both. Model selection favoured the constant model in 6 out of 10 replicates for *E. aediculatus* and in 9 out of 10 replicates for *E. plumipes* (Table 2). This result reflects a generally loose relationship between risk and the level of defence, and a tendency for the model selection criterion (AICc) to select simple models at the available low sample sizes. Summing the Akaike weights

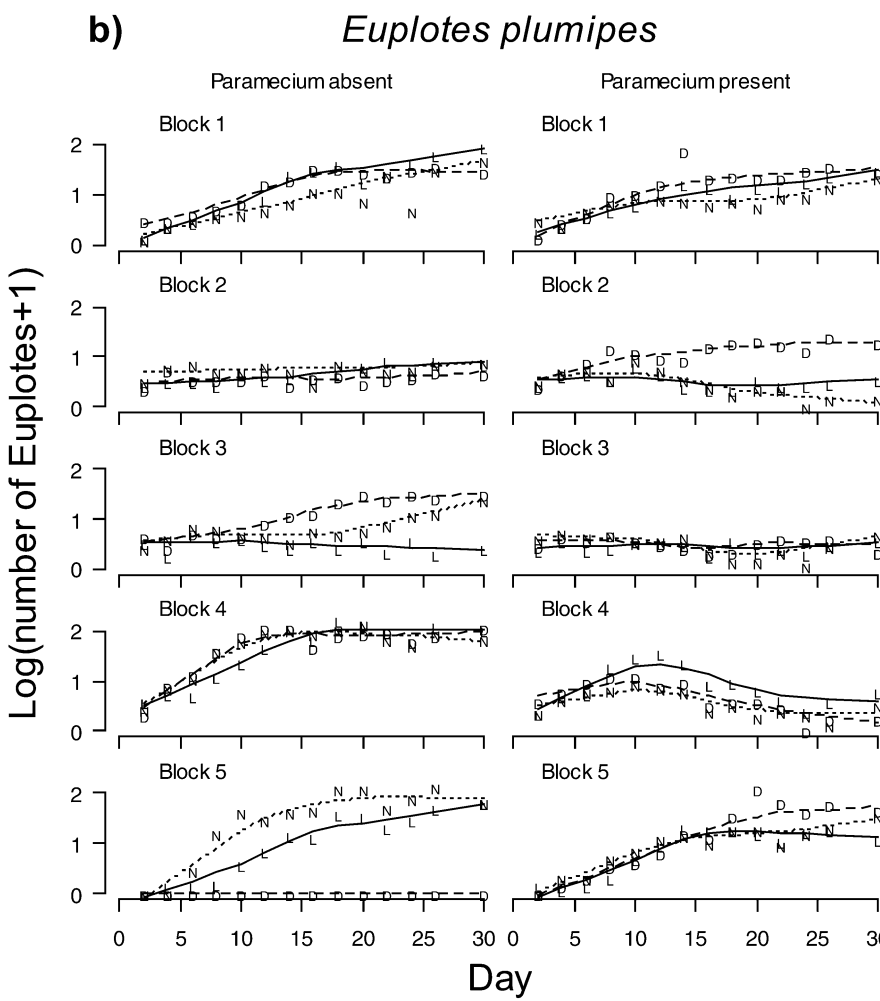

Fig. 1. Changes in population density of *Euplotes aediculatus* **(a)** and *Euplotes plumipes* **(b)** over time. The counts are average number of cells in five samples of 200 µl, on a logarithmic scale. Each block (two panels) represents one replicate of each of six treatments, where the left panel shows treatments without competing *Paramecium* and rotifers, and the right panel shows treatments with competition. The time series correspond to the treatments live predators (L), dead predators (D), and no predators (N). The lines are non-parametric smoother curves produced by the lowess procedure in the program R. Solid lines correspond to treatments with live predators, broken lines with dead predators, and dotted lines are controls.

of all models containing the factors predation and conspecific density respectively shows that these two factors were on average equally important determinants of the defence level [*E. aediculatus*: predation: 0.39 (sd = 0.29), conspecifics: 0.27 (sd = 0.22), compared to constant model: 0.44 (sd = 0.26); *E.*

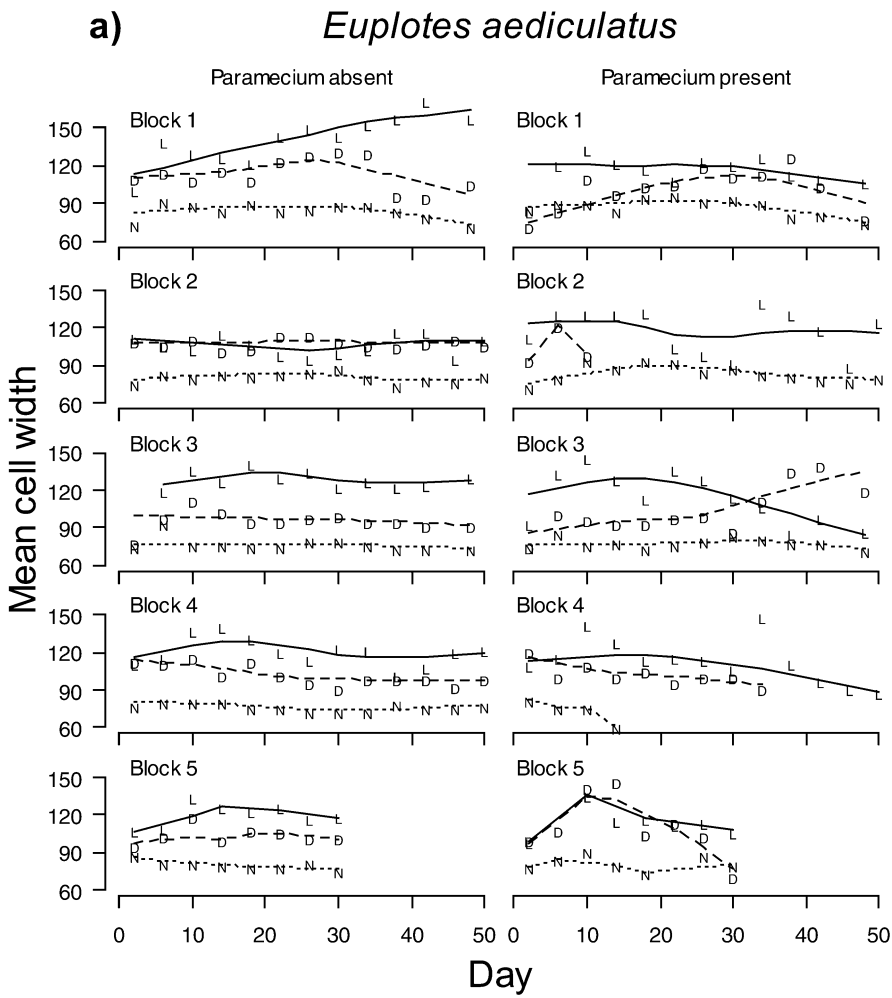

Fig. 2. a.

plumipes: predation: 0.11 (sd = 0.11), conspecifics: 0.16 (sd = 0.20), compared to constant model: 0.74 (sd = 0.27)].

The relationships of cell width (level of defence) to both predator and conspecific densities were quite variable. The slope of the reduced major axis (RMA) regression of cell width on number of predators in the sample varied from −23.81 to 52.65 (mean = 12.57, sd = 26.23) in *E. aediculatus* and from −4.82 to 44.59 (mean = 17.04, sd = 14.52) in *E. plumipes* (Fig. 3). Variation in this relationship is unlikely to be affected greatly by variation in predator size, which was small compared to the variation in number of predators. The RMA slope of cell width on number of conspecifics varied from −6.37 to 12.69

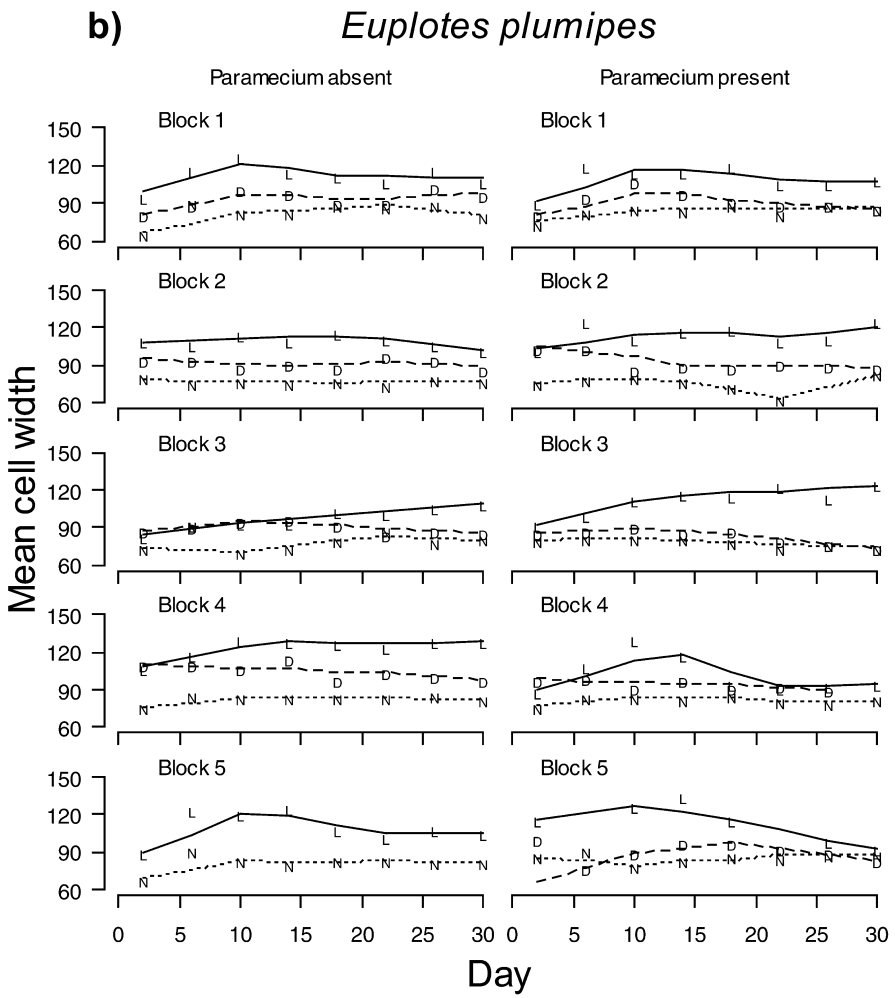

Fig. 2. Mean cell width [µm] of a sample of *Euplotes aediculatus* **(a)** and *E. plumipes* **(b)** cells observed over time. See legend of Fig. 1 for details.

(mean = 1.89, sd = 6.46) in *E. aediculatus* and from −6.47 to 15.30 (mean = 0.46, sd = 5.80) in *E. plumipes*. Differences in how closely the defence level tracked predation risk, as indicated by the slopes of the regressions of defence level on predator and conspecific densities (Fig. 3), did not explain a significant proportion of the variation in the coefficient of variance of *Euplotes* population size over time (multiple regression of RMA slopes on CV population size: *E. aediculatus* $R^2 = 0.342$; *E. plumipes* $R^2 = 0.039$, all coefficients $P > 0.15$). These results did not change if we considered longer time lags by relating defence level to predator densities observed five days earlier.

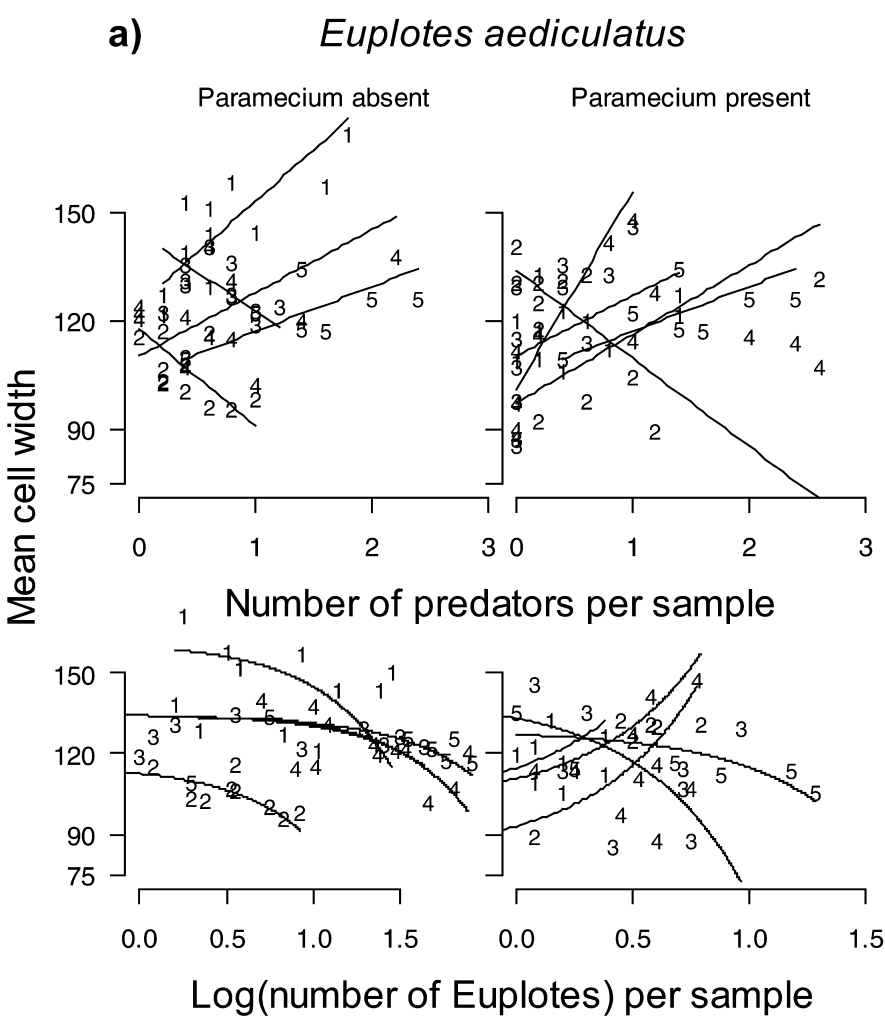

Fig. 3. a.

Discussion

In replicated laboratory communities, we investigated the dynamics of a morphological defence, cell width of the ciliate *Euplotes*, over thirty generations. Wider cells are less likely to be eaten by gape limited predators (KUHLMANN & HECKMANN 1985), but they have a longer cell cycle and thus reduced population growth compared to narrower cells (KUSCH & KUHLMANN 1994). Although we expected defence level (cell width) in *Euplotes* to closely track predation risk, our study revealed that cell width was only loosely related to predation risk. *Euplotes* did increase cell width when exposed to live or dead

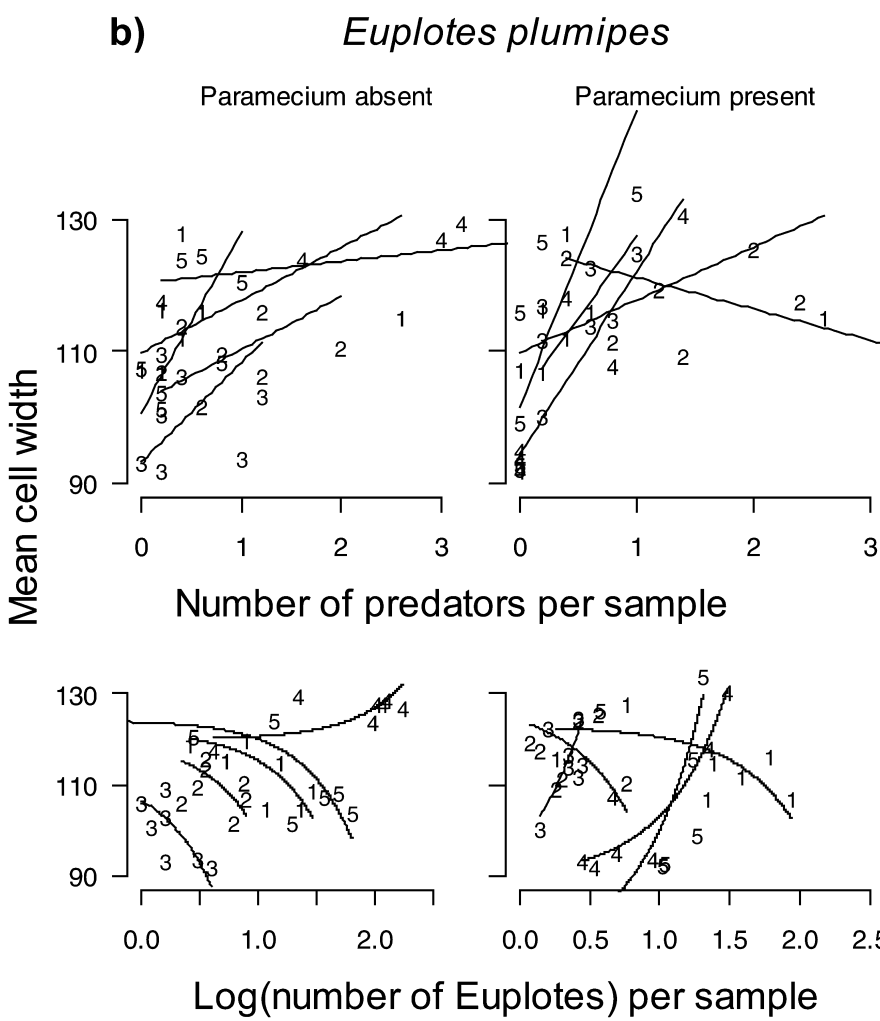

Fig. 3. Mean cell width [µm] of *Euplotes aediculatus* **(a)** and *E. plumipes* **(b)** cells plotted against the number of predators (upper panels) and the number of *Euplotes* (lower panels) per 200 µl sample. Only treatments with live predators are included. Each symbol type and line corresponds to one experimental unit measured repeatedly over time, and the lines are the best fitting reduced major axis (RMA) regression lines. The symbol-numbers correspond to the replicate number ("Block" in Figs. 1 and 2). Predator only treatments are shown in the left panels, whereas treatments with predators and competing *Paramecium* and rotifers are shown in the right panels. If the defence levels match predation risk, we expect cell width to increase with number of predators and decrease with number of conspecifics. Note that the logarithmic scale was used to facilitate the presentation in the lower two graphs. The regression lines are linear on the arithmetic scale.

Table 2. Akaike weights, based on sample-size-adjusted Akaike's Information Criterion (AICc), for five models fitted to each of ten replicate time series of the level of inducible defence in *Euplotes aediculatus* (a), and *E. plumipes* (b). The column 'Model' describes factors included in the model. These factors were either none (Intercept), number of predatory *Stenostomum* (Predation), number of conspecifics (*Euplotes*), and the combination of both factors (P + *E*), including their interaction (P × *E*). K is the number of parameters in the model, including one parameter for the variance. Numbers in parentheses indicate the length of the time series for each replicate. Not all replicates were terminated at the same time, resulting in different lengths. In two cases, we had six measurements only, and the most complex model (P X *E*) could not be fitted. The Akaike weights give relative support for a particular model compared to the other models and add up to one within each replicate.

a) Akaike weights for *E. aediculatus*

Model	K	Rep 1 (11)	Rep 2 (11)	Rep 3 (12)	Rep 4 (12)	Rep 5 (11)	Rep 6 (11)	Rep 7 (12)	Rep 8 (12)	Rep 9 (6)	Rep 10 (7)
Intercept	2	0.025	0.708	0.349	0.264	0.190	0.479	0.310	0.577	0.672	0.866
Predation (P)	3	0.902	0.178	0.061	0.449	0.514	0.126	0.073	0.171	0.294	0.106
Euplotes (E)	3	0.004	0.100	0.207	0.220	0.027	0.366	0.425	0.215	0.034	0.028
P + *E*	4	0.067	0.014	0.157	0.060	0.262	0.029	0.040	0.034	0.000	0.001
P × *E*	5	0.003	0.000	0.227	0.007	0.007	0.001	0.151	0.004	n. a.	0.000

b) Akaike weights for *E. plumipes*

Model	K	Rep 1 (7)	Rep 2 (7)	Rep 3 (7)	Rep 4 (7)	Rep 5 (7)	Rep 6 (7)	Rep 7 (7)	Rep 8 (7)	Rep 9 (7)	Rep 10 (6)
Intercept	2	0.798	0.884	0.928	0.924	0.766	0.779	0.020	0.736	0.693	0.893
Predation (P)	3	0.028	0.030	0.043	0.035	0.023	0.175	0.291	0.225	0.073	0.099
Euplotes (E)	3	0.174	0.086	0.029	0.042	0.210	0.046	0.625	0.038	0.233	0.007
P + *E*	4	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.000	0.001	0.000
P × *E*	5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	n. a.

predators, and cell width varied over time in the predator treatments (see Fig. 2). Earlier short-term studies have shown that *Euplotes* can closely adjust their reaction to different predator densities, and that this reaction happens within hours under highly controlled experimental conditions (Kuhlmann & Heckmann 1985, Kusch 1993). Despite possessing the ability to rapidly and accurately alter their level of defence, our long-term study showed that average defence of a population of *Euplotes* may often not match predation risk closely. Our experimental conditions mimic natural systems by allowing for dynamic changes in food and predator numbers, and our results are consistent with data from natural ponds, where a correlation between defence level and predator density could not be established (Kusch 1995).

Predation risk for a single organism may depend on the density of both predators and conspecifics (Wiackowski & Staronska 1999, Peacor 2003). While defence level was closely related to predator or conspecific densities in

some replicates, as expected, we found no apparent connection between predation risk and defence level in others (see Table 2, Fig. 3). On average, predator and conspecific densities were about equally important across the whole experiment, as predicted by recent theory (Peacor 2003). We observed substantial variation in the slope of cell width regressed on predator and competitor densities across replicates (see Fig. 3), and model selection often favoured the constant model over more complicated ones (see Table 2). This indicates that other factors, such as fluctuating resource levels, may have modified the defensive response of *Euplotes* in our experiment. At low resource levels, organisms are predicted to invest less in costly defences than at high resource levels (Werner & Anholt 1993, Wiackowski & Szkarlat 1996). Our experiment minimised the problem of fluctuating resources by supplementing the semi-continuous batch cultures with a single wheat grain for a constant influx of nutrients (Lawler & Morin 1993, Holyoak et al. 2000). Our procedure also minimised the accumulation of waste products, as we replaced 10 % of the culture content with fresh medium every two days. Available resources may, however, have varied due to changes in food quality as the bacteria community could have responded to selection imposed by the ciliates (Yoshida et al. 2003). A poor correspondence between risk and defence level may have been caused by a poor reliability of the chemical cues, which may have been degraded by enzymes of bacterial origin. In our experiment, however, cue degradation was not fast enough to prevent a strong reaction to added dead predators.

Euplotes showed different population dynamics depending on the type of food web they were embedded in. In the presence of competitors (*Paramecium* and rotifers) the mean, but not the variance in *Euplotes* population density was depressed. *Euplotes* population density consequently showed a higher coefficient of variation in the presence of competitors than in their absence, as has been found in other microcosm studies (Bengtsson & Milbrink 1995). *Euplotes aediculatus* populations increased slowly during the initial phase of the experiment when predators were present. With dead predators, the rates of increase were intermediate between live and no predator treatments, suggesting that part of the effect was caused by non-lethal effects of predation, mediated by the defensive trait of *Euplotes*. Even though we could not establish a significant direct connection between defence level and population growth, this result is consistent with earlier studies showing that inducible defences generally lead to reduced population growth and reduced competitive ability (Kusch & Kuhlmann 1994, McPeek & Peckarsky 1998, Lima 1998). If the competitive ability of a prey species depends on the level of defence, predators can cause indirect effects in food webs without killing prey (trait-mediated indirect effects, reviewed by Werner & Peacor 2003, Bolker et al. 2003). The defensive response in *Euplotes* was induced by dead predator treatments

allowing us to separate trait-mediated effects from effects mediated by direct predation. However, competitors had similar effects on *Euplotes* in the presence and absence of dead predators, suggesting that trait-mediated indirect effects on competitive ability were too weak to be detectable with our experimental design.

Inducible defences and other types of phenotypic plasticity have typically been studied over short time scales compared to the organism's generation time. While earlier studies demonstrated that *Euplotes* reacts within hours, we here also find considerable variation in defence level on the scale of days to weeks. The novel time scale we used (30 generations) therefore revealed dynamics of the inducible defence in *Euplotes* that were on a similar time scale to population dynamics. For example, mean cell width in one replicate holding live predators (*E. aediculatus*, Block 1, Fig. 2) continued to increase over the whole period. Such phenotypic responses occurring over several generations may be difficult to anticipate from short term experiments only. Yet available theory suggests that the time scale of response is important to community dynamics. Rapid and accurate responses tend to stabilise community dynamics, whereas delayed responses are destabilising (Ives & Dobson 1987, Luttbeg & Schmitz 2000). Our experiment yields promising first insights into the dynamics of inducible defence in *Euplotes*, however, the detailed data necessary to gain a thorough understanding of the effects of inducible defences on the dynamics of this community await further study.

Acknowledgements

We thank Jürgen Kusch, Krzysztof Wiackowski and an anonymous reviewer for helpful comments on an earlier version of the manuscript. The research was funded by a NSERC of Canada research grant to BRA and Swiss Nationalfonds grant no 81ZH-68483 to RA.

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Submitted: 8 December 2003; accepted: 16 February 2004.