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Algal defenses, population stability, and the risk of herbivore extinctions: a chemostat model and experiment

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Abstract The effects of inducible defenses and constitutive defenses on population dynamics were investigated in a freshwater plankton system with rotifers as predators and different algal strains as prey. We made predictions for these systems using a chemostat predator–prey model and focused on population stability and predator persistence as a function of flow-through rate. The model exhibits three major types of behavior at a high nutrient concentration: (1) at high dilution rates, only algae exist; (2) at intermediate dilution rates, algae and rotifers show stable coexistence; (3) at low dilution rates, large population fluctuations occur, with low minimum densities entailing a risk of stochastic rotifer extinctions. The size and location of the corresponding areas in parameter space critically depend on the type of algal defense strategy. In an 83-day high-nutrient chemostat experiment we changed the dilution rate every 3 weeks, from 0.7 to 0.5 to 0.3 to 0.1 per day. Within this range of dilution rates, rotifers and algae coexisted, and population fluctuations of algae clearly increased as dilution rates decreased. The CV of herbivore densities

was highest at the end of the experiment, when the dilution rate was low. On day 80, herbivorous rotifers had become undetectable in all three chemostats with permanently defended algae (where rotifer densities had already been low) and in two out of three chemostats where rotifers had been feeding on algae with inducible defenses (that represented more edible food). We interpret our results in relation to the paradox of enrichment.

Keywords Phenotypic plasticity · Plant defenses · Herbivory · *Scenedesmus* · *Brachionus*

Introduction

Induced defenses have been observed in many plant and animal species in both terrestrial and aquatic ecosystems (e.g., Karban and Baldwin 1997; Tollrian and Harvell 1999). Phenotypic plasticity in prey can have large effects on their interaction with predators, as indicated by a number of theoretical studies of inducible defenses (Edelstein-Keshet and Rausher 1989; Lundberg et al. 1994; Abrams and Walters 1996; Underwood 1999; Ramos-Jiliberto 2003; Vos et al. 2002; Vos et al. 2004a, 2004b; Gabriel et al. 2005; Kopp and Gabriel 2006). Inducible defenses have the potential to either stabilize or destabilize predator–prey interactions (Vos et al. 2005) and strong population level effects of inducible defenses have been confirmed by empirical studies in both terrestrial and aquatic communities (Haukioja 1980; Peacor and Werner 2000; Raimondi et al. 2000; Turner et al. 2000; Underwood and Rausher 2002; Verschoor et al. 2004b; van der Stap et al. 2006; van der Stap et al. 2007a, 2007b).

Inducible defenses were shown to resolve the paradox of enrichment in a theoretical study on the effects of inducible defenses on population dynamics in a Rosenzweig–MacArthur predator–prey model (Vos et al. 2004a). The classical formulation of the RM model, that does not take inducible defenses into account, results in three areas of qualitatively different dynamics. These

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areas are separated by (1) the existence boundary, indicating that at higher predator mortality rates, the predator cannot exist and only prey are present (2) the stability boundary, indicating that at lower predator mortality rates, predator–prey cycles would occur, with a risk of stochastic extinctions. The area between these two boundaries is the area of stable coexistence between predator and prey. The existence and stability boundaries converge at high carrying capacities (under enrichment), making coexistence close to impossible. In the model with inducible defenses, stable coexistence of predator and prey may occur, even at high carrying capacities: the two boundaries do not converge with enrichment, allowing stable coexistence for a range of predator mortality rates, even at very high carrying capacities (Vos et al. 2004a). This model was specifically parameterized for an algae–rotifer system (Vos et al. 2004a, 2004b).

Until recently, empirical work on this algae–rotifer system was performed either in batch cultures in which nutrients were depleted (van der Stap et al. 2006) or in semi-continuous cultures in which part of the medium was replenished daily (Verschoor et al. 2004b). Inducible defenses were shown to prevent large population fluctuations in bi- and tritrophic food chains (Verschoor et al. 2004b). In their semi-continuous cultures, two nutrient conditions were used to determine the effects of inducible defenses at high and low productivity, but the low-nutrient (phosphorus) condition decreased the food quality (C:P ratio) of algae to such an extent that rotifers could not exist (Verschoor et al. 2004b). Effectively, these experiments provided information for a very limited set of environmental conditions. For a more critical test of model predictions, we tested the effects of inducible and constitutive defenses in continuous cultures (chemostats) with a series of different dilution rates. In a chemostat, the dilution rate is the continuous flow of medium through the vessel that determines the equilibrium growth rate of the cultured species (Walz 1993). It has been shown that qualitatively different dynamics can occur in single-stage chemostats in which rotifers and algal prey without inducible defenses are grown together (Kooi et al. 1998; Fussmann et al. 2000; Yoshida et al. 2007).

Here we present model predictions on the effects of different types of algal defenses on population dynamics that would be expected under chemostat conditions together with the results of a chemostat experiment using rotifers and algae with either constitutive or inducible defenses. The main research questions that we focused on were (1) whether the predicted qualitative types of predator–prey dynamics could be observed (only prey, stable predator–prey equilibrium and predator–prey population cycles, possibly with stochastic extinctions), and (2) whether the dilution rates at which these changes occurred varied with the algal defense strategy.

Materials and methods

Model

To model the effect of inducible defenses on population dynamics under chemostat conditions, we changed the formulations of algal dynamics in the model of Vos et al. (2004a, 2004b) from a logistic term to one with explicit nutrient dynamics. The resulting model for the chemostat setup including inducible defenses consists of four differential equations:

$$\frac{dN}{dt} = D(N_i - N) - \left[\frac{r_1 P_1}{h_{N1} + N} + \frac{r_2 P_2}{h_{N2} + N} \right] N \quad (1)$$

$$\frac{dP_1}{dt} = \left[\frac{c_{NR1}N}{h_{N1} + N} - \frac{v_1 H}{1 + v_1 h_1 P_1 + v_2 h_2 P_2} - \frac{iH^b}{h^b + H^b} - D \right] P_1 + \frac{dh^b}{h^b + H^b} P_2 \quad (2)$$

$$\frac{dP_2}{dt} = \left[\frac{c_{NR2}N}{h_{N2} + N} - \frac{v_2 H}{1 + v_1 h_1 P_1 + v_2 h_2 P_2} - \frac{dh^b}{h^b + H^b} - D \right] P_2 + \frac{iH^b}{h^b + H^b} P_1 \quad (3)$$

$$\frac{dH}{dt} = \left[\frac{c_1 v_1 P_1 + c_2 v_2 P_2}{1 + v_1 h_1 P_1 + v_2 h_2 P_2} - D \right] H \quad (4)$$

In this model, N is the nutrient concentration, P_1 the concentration of undefended algae, P_2 the concentration of defended algae, and H the concentration of herbivores. The transition between the undefended and defended state of the algae is described by induction and decay terms in the algal equations. Note that the expressions for induction rates are equivalent to those in Vos et al. (2004a). An overview of the parameter values and their sources is given in Table 1. For the sake of simplicity, the parameters that describe algal growth and the nutrient–algal interaction are taken to be the same for both algal species.

In absence of herbivores, the system reduces to the nutrient N and algae P_1 :

$$\frac{dN}{dt} = D(N_i - N) - \left[\frac{r_1 P}{h_{N1} + N} \right] N \quad (5)$$

$$\frac{dP_1}{dt} = \left[\frac{c_{NR1}N}{h_{N1} + N} - D \right] P_1 \quad (6)$$

For this simplified system the equilibrium P_1^* for nutrients and undefended algae are:

$$N^* = \frac{D h_{NP1}}{c_{NR1} - D} \quad (7)$$

$$P_1^* = \frac{D(N_i - N^*)(h_N + N^*)}{r_1 N^*} \quad (8)$$

Table 1 Definitions of model parameters and their values

Parameter	Value	Unit	Interpretation
D	Variable	day^{-1}	Dilution rate
N_i	Variable	mg nutrient/ml	Inflowing nutrient concentration
r_1	1.42 ^a	day^{-1}	Maximum growth rate undefended plants
r_2	1.42 ^a	day^{-1}	Maximum growth rate defended plants
h_{N1}	0.02 ^b	mg nutrient/ml	Value at which half the maximum growth of undefended plants is reached
h_{N2}	0.02 ^b	mg nutrient/ml	Value at which half the maximum growth of defended plants is reached
c_N	1 ^b	mg C plant/mg nutrient	Conversion efficiency from nutrients to plants
v_1	0.77 ^a	day^{-1}	Herbivore attack rate on undefended plants
v_2	0.77 ^a	day^{-1}	Herbivore attack rate on defended plants
h_1	0.5 ^a	d	Handling time on undefended plants
h_2	1.04 ^a	d	Handling time on defended plants
c_1	0.36 ^a	mg C herbivore/mg C plant	Conversion efficiency from undefended plants to herbivores
c_2	0.36 ^a	mg C herbivore/mg C plant	Conversion efficiency from defended plants to herbivores
i	1 ^a	day^{-1}	Induction rate of defenses
d	1 ^a	day^{-1}	Decay rate of defenses
h	0.06 ^a	mg C herbivore/ml	Herbivore density at which half of the plants is induced
b	2.05 ^a	–	Shape of plant defense functions (induction/decay)

^aParameter values originate from Vos et al. (2004a, b)

^bParameter values originate from van der Stap (2007)

Chemostat experiment

The single-stage chemostats were inoculated with the following organisms (1) algae: inducible defended *Scenedesmus obliquus* (Turpin) Kützing, UTEX 2630 or constitutively defended *Desmodesmus quadricauda* (Turpin) Hegewald of unknown origin; and (2) herbivorous zooplankton: *Brachionus calyciflorus* Pallas (Rotifera). Algae with inducible defenses are small and highly edible in the absence of herbivores but form colonies (that increase mean algal particle volume) in the presence of herbivores (Hessen and Van Donk 1993). The constitutively defended algae are large and colonial, both in presence and absence of herbivores. The particle size of these permanently defended algae may also vary, but most of this size range is much larger than the preferred particle size of the herbivorous rotifer. Defense strategies of the different algal strains were determined on the basis of previously performed bioassays and population dynamics experiments (Verschoor et al. 2004a; van der Stap et al. 2006). COMBO medium (Kilham et al. 1998) was used in all cultures and experiments. This medium contains about 1.55 mg P/L. At a C:P ratio of 100, this implies a resource level in terms of carbon of about 155 mg C/L in the chemostats, which corresponds to an extremely eutrophic system.

Algae were first grown in batch cultures and refreshed twice a week before inoculation of the chemostats, to ensure a high algal growth rate. *Brachionus* were hatched from cysts (Microbiotests Inc., Nazareth, Belgium), grown in batch cultures and raised on *S. obliquus*. Rotifers were rinsed thoroughly and put in clean medium for 3 h. Six 1.6 L chemostats were run. These were inoculated with $3.25 \times 10^8 \mu\text{m}^3 \text{mL}^{-1}$ of the respective algal strain and herbivores at 400 individuals L^{-1} in triplicate. Cultures were kept at a 24 h light regime, at a temperature of $20 \pm 0.2^\circ\text{C}$. Total mixing in the chemostat was ensured by moisturized and filtered air with

a flow rate of $0.5 \pm 0.1 \text{ L min}^{-1}$. Dilution rate of the chemostats was changed every 3 weeks from 0.7 to 0.5 to 0.3 and finally to 0.1 per day; 100-mL samples were taken daily, herbivores and algae were separated using 33- μm filters. Total duration of the experiment was 83 days. Algal particle densities ($\# \text{mL}^{-1}$), mean particle volumes (μm^3) and biovolume concentration ($\mu\text{m}^3 \text{mL}^{-1}$) were measured on a cell counter (CASY, Scharfe, Germany) fitted with a 60- μm capillary. Mean particle volume is correlated to the number of cells per colony as a measure of algal defense (see Verschoor et al. 2004a). Rotifer densities were estimated every other day by microscopic counts. In the data analysis we focused on average biomass over time per dilution rate, and on population minima and maxima for both algae and rotifers. CVs (coefficients of variation) of population densities were calculated for each dilution rate, but by excluding the first period after each change in dilution rate, as these changes resulted in short-term irregular behavior. CVs for dilution rates 0.7, 0.5, 0.3, 0.1 are thus represented by the periods of days 8–18, 28–38, 50–60, and 72–82.

Results

Model

Three qualitatively different types of dynamics occur in the model with inducible defenses in continuous culture. Changes in the stability properties of the food chain are shown across a range of nutrient concentrations and dilution rates (c.q. herbivore death rates) (see Fig. 1). In case of low inflowing nutrient concentrations and high dilution rates, only algae can exist (area A_0). For intermediate values of both factors, algae and herbivores coexist at a stable equilibrium (area A_1). Low dilution rates and high nutrient concentrations lead to instability

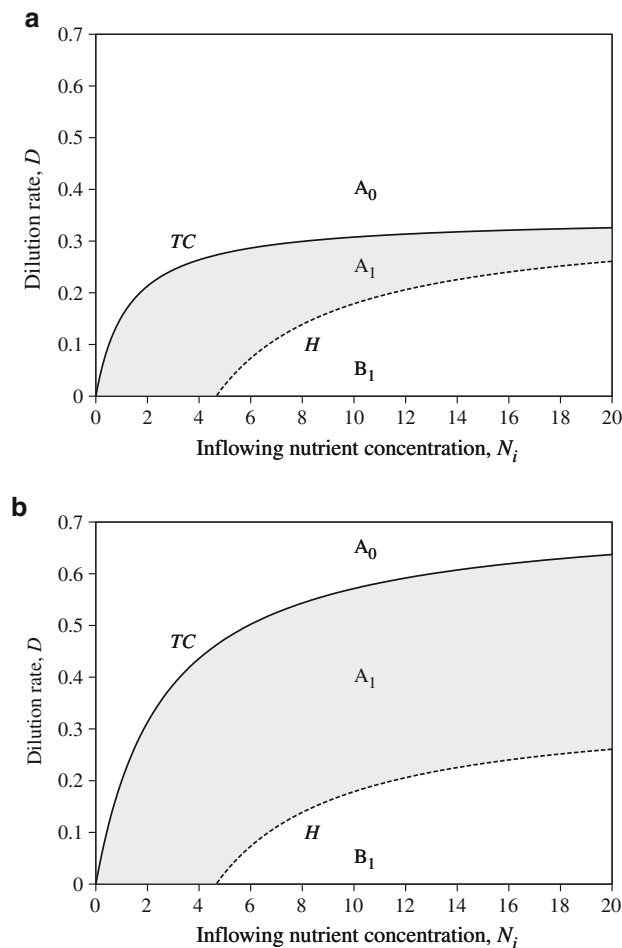


Fig. 1 The stability boundaries of bitrophic food chains with **a** permanent defenses, or **b** inducible defenses, for a range of the inflowing nutrient concentrations N_i and dilution rates D . The transcritical bifurcation curve (TC), which is also called herbivore existence boundary, and the Hopf (H) bifurcation curve separate areas with qualitatively different long-term dynamics. Area A_0 : only plants (algae) exist. Grey area: A_1 stable coexistence of plants and herbivores. Area B_1 : plants and herbivores fluctuate. Parameter values used as in Table 1

of the system (area B_1). The existence boundary or transcritical (TC) bifurcation curve separates areas A_0 and A_1 marking the point where herbivores can enter the system. The stability boundary or Hopf bifurcation curve marks the point where predator–prey cycles start. For combinations of inflowing nutrient concentrations and dilution rates that are between the existence and stability boundary the model predicts a stable coexistence between predator and prey. Comparison of the width of this region for constitutively defended algae (Fig. 1a) and inducibly defended algae (Fig. 1b) clearly shows the stabilizing effect of inducible defenses under a range of dilution rates. Figure 1 zooms in on a natural range of nutrient concentrations (N_i in C), that, when translated to the logistically growing algae in the model of Vos et al. (2004a, 2004b) corresponds to basal carrying capacities of 0–20 mg C/L. As explained in the

“Materials and methods” section, the situation in our chemostats with COMBO medium represents a much more eutrophic situation, corresponding to about 155 mg C/L. Under these conditions, we would strongly expect to see a crossing of the Hopf bifurcation when dilution rate is reduced, as is part of the model’s prediction of the paradox of enrichment (destabilization and extinctions of cycling herbivores). If the paradox of enrichment exists in real experimental systems, it should occur under the conditions of our experiment, when the dilution rate is decreased. The presence of the three qualitatively different types of dynamics is, again, fully in line with the predictions of the alga–rotifer model with logistically growing algae (Vos et al. 2004a, 2004b).

In the chemostat model the inflowing nutrient concentration N_i determines the maximum density of algae. Given the specific parameterization of $c_N = 1$ we can directly compare this concentration with the carrying capacity in the model of Vos et al. (2004a) (see also Kooi et al. 1998). This makes clear that the effects of a decrease in dilution rate in the chemostat model studied here are comparable to the effects of a decrease in herbivore mortality rate in the equivalent model with logistic growth by Vos et al. (2004a, 2004b). This resemblance allows for a quantitative comparison of both models. We observed that the location of the TC curves is almost identical between the two models. The Hopf curves, however, are always lower for the chemostat model at low concentrations of the inflowing nutrient compared with the model with logistically growing algae. As a result, the region with stable coexistence between algae and zooplankton is somewhat larger in the chemostat model than in the model by Vos et al. (2004a), when nutrient levels are low. Mathematical analysis of the chemostat model shows that the existence and stability boundary both approach a dilution rate equal to c_2/h_2 at an infinitely high concentration of nutrients, for constitutively defended algae, as was found by Vos et al. (2004a) in their defense model. With the parameter values of Table 1 this is equal to a D of about 0.346 day^{-1} . Numerical analysis of the chemostat model for a nutrient concentration $N_i = 155$ (our experimental conditions), indicates that both the TC and Hopf bifurcations occur for $0.34 < D < 0.35$. This implies that the model clearly shows the paradox of enrichment when inducible defenses are not present in the algae: Only a very narrow range of dilution rates allows stable coexistence at such a high nutrient level. This narrow region of stable co-existence of algae and zooplankton vanishes at infinitely high concentrations of the inflowing nutrient, similar to the findings of Vos et al. (2004a). One then only expects either deterministic herbivore extinction or cyclic dynamics with (possibly) stochastic herbivore extinctions, as the dilution rate is changed in an experimental chemostat system.

In contrast to the constitutive defense case, a range of dilution rates allows stable coexistence in the alga–rotifer chemostat model, when algae have an inducible defense. At infinitely high nutrient levels, this coexistence

region is demarcated by dilution rate ranging from $D = c_1/h_1$ (about 0.72) for the TC bifurcation or herbivore existence boundary, to approximately $D = c_2/h_2$ (about 0.346) for the Hopf bifurcation or stability boundary. The latter is based on the fact that close to 100% of individuals in the prey population is defended at very high nutrient concentrations, because herbivore densities are very high at these nutrient levels, and defense induction rates are consequently very high as well (Vos et al. 2004a, 2004b). Numerical analysis for $N_i = 155$ shows that the herbivore can exist in a system where the alga has an inducible defense at dilution rates below a D of between 0.71 and 0.72. Such an alga–rotifer system is destabilized (turns cyclic) at a D of between 0.34 and 0.35 per day. The stability boundary is thus predicted to be nearly identical for systems with inducible and constitutive defenses at a high nutrient level (Fig. 1b, c), as in our experiment.

Population densities in chemostats with constitutive algal defenses against rotifers

For constitutively defended algae, algal biomass clearly increased as the dilution rate was decreased, in all three replicates (Figs. 2a–c, 4a). Minimum algal densities remained far away from zero. *Brachionus* densities were very low in two out of three replicates (Figs. 3a–c, 4c) and never exceeded the initial density. In one replicate, *Brachionus* densities did increase above their initial density (Figs. 3b, 4c), but the maximum density was still an order of magnitude lower than the rotifer population

maxima reached on inducible defended algae (Figs. 3d–f, 4d). The difference between population minima and maxima of constitutively defended algae was larger at low dilution rates (0.1 and 0.3 per day) than at high dilution rates (0.5 and 0.7 per day, Figs. 3a–c, 4a). *Brachionus* population densities were also most variable at low dilution rates, as reflected in mean CVs of 1.84, 0.81, 0.67, and 0.51, respectively, for dilution rates 0.1, 0.3, 0.5, and 0.7. *Brachionus* densities dropped below our detection limit (and herbivores were not observed since) in all three constitutive defense replicates, at the end of the experiment, on days 74, 76, and 82, when the dilution rate was low (0.1 per day).

Population densities in chemostats with inducible algal defenses against rotifers

At the start of the experiment, the algae were suppressed by a growing population of rotifers (Fig. 4b, d). This initial increase in the rotifer population and decrease in the algal population is visible as population variability in Figs. 2d–f and 3d–f, at a dilution rate of 0.7 per day. After this, the algal population reached a rather stable density at a dilution rate of 0.5 per day (Figs. 3d–f, 4b). Rotifer population variability was also relatively modest at a dilution rate of 0.5 per day (Fig. 4d). Herbivore population variability increased substantially when the dilution rate was further decreased to 0.3 per day. This led to near-extinction of the herbivore in one chemostat (Fig. 3e) and its final extinction at the subsequent dilution rate of 0.1 per day (Fig. 4d), resulting in a very high

Fig. 2 Variation in algal densities at each dilution rate in the chemostats. The average biomass over time of algae, per dilution rate, is denoted by grey diamonds (mean \pm SE), whereas closed circles indicate minima and maxima per dilution rate (with the unit of D being fraction per day). All three replicates are shown (a–c) constitutively defended algae *Desmodesmus quadricauda* (d–f) inducible defended algae *Scenedesmus obliquus*

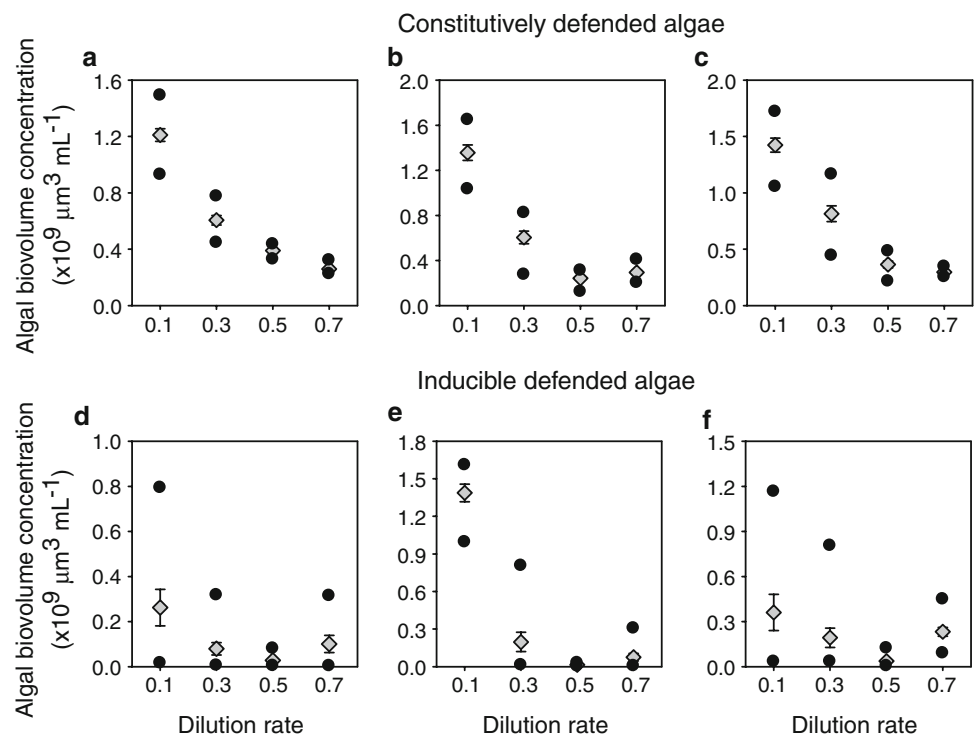


Fig. 3 Variation in rotifer densities at each dilution rate in the chemostats. The average biomass of herbivorous *Brachionus* per dilution rate is denoted by grey diamonds (mean \pm SE), whereas closed circles indicate minima and maxima per dilution rate. All three replicates are shown (a–c) herbivores in the treatment with constitutively defended algae *Desmodesmus quadricauda* (d–f) herbivores in the treatment with inducible defended algae *Scenedesmus obliquus*

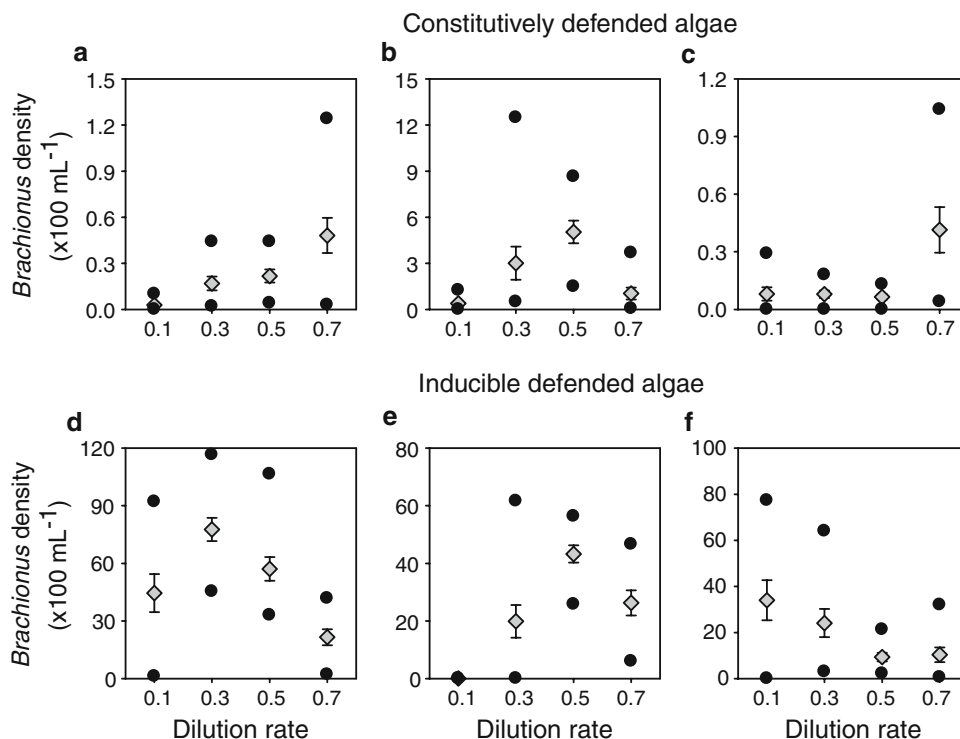
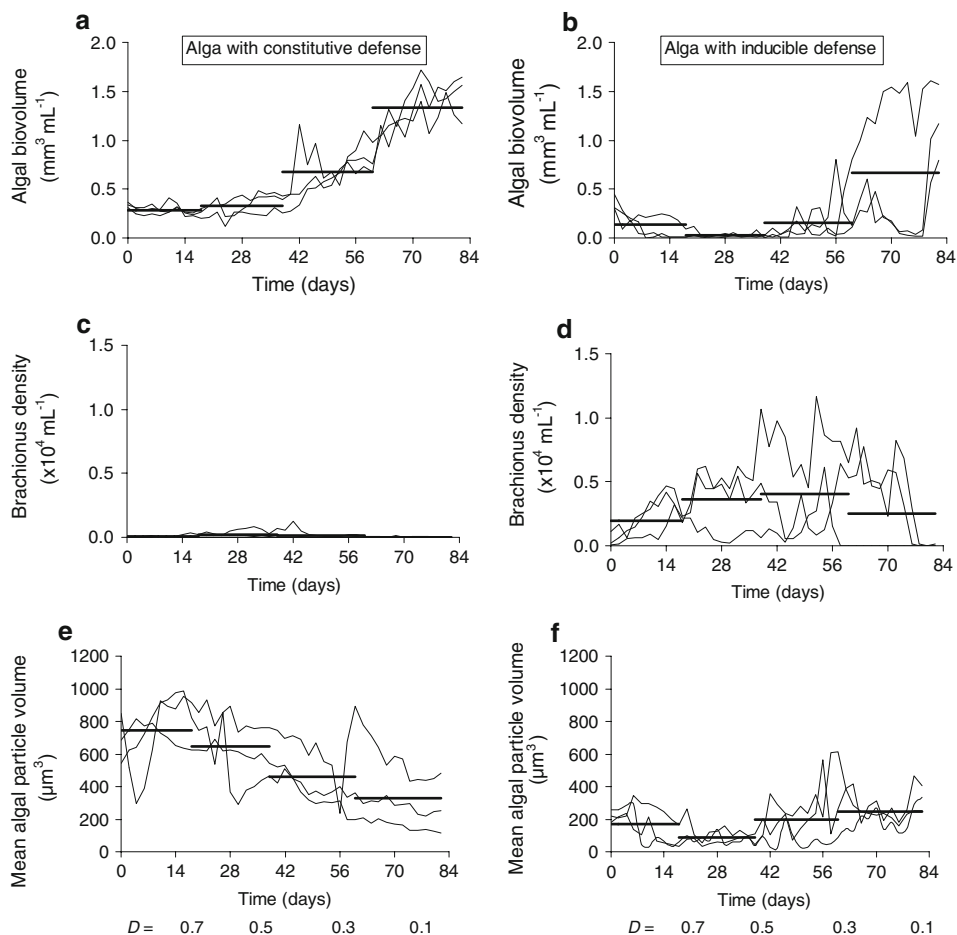


Fig. 4 Time-series data for alga–rotifer dynamics in replicate chemostats, for different subsequent dilution rates (D). D was 0.7, 0.5, 0.3, and finally 0.1 per day for the periods of days 0–18, 19–38, 39–60, and 61–82, respectively. All three replicates are shown in each panel. Horizontal lines denote the grand mean of the three replicates for the period of each separate dilution rate. (a, c, e) data for chemostats with the constitutively defended alga *Desmodesmus quadricauda* (b, d, f) data for chemostats with the inducible defended alga *Scenedesmus obliquus*. (a, b) algal biovolume (c, d) density of the herbivorous rotifer *Brachionus* (e, f) Mean algal particle volume, a measure for algal defense



algal population density (Figs. 2e, 4b). Herbivore population densities in the two remaining chemostats were most variable at a low dilution rate of 0.1 per day (Fig. 3d, f) and the same can be said about algal variability in these chemostats (Fig. 2d, f). In these two chemostats, herbivore populations crashed to extinction following very low algal population densities between days 72 and 78 (Fig. 4b, d). This variation in *Brachionus* population densities at the lowest dilution rate was reflected in a high mean CV. CVs were 1.57, 0.66, 0.35, and 0.38, respectively, for dilution rates 0.1, 0.3, 0.5, and 0.7. On day 82, the herbivore density had dropped to a low level in one of the three chemostats (1.1 individuals per mL) and had become undetectable in the other two (Fig. 4d).

Defense levels in algae with constitutive and inducible defenses

Single-celled algae with an equivalent spherical diameter (ESD) of about 4–7.5 μm are highly edible to *Brachionus calyciflorus* rotifers (van der Stap et al. 2006). Undefended single-celled *Scenedesmus* algae typically have a mean particle volume (MPV) in the order of 50–100 μm^3 . Larger MPVs, especially in the range 250–1,500 μm^3 significantly affect the functional response of this herbivore (Verschoor et al. 2007; also see Rothhaupt 1990a, 1990b). It is clear from the MPVs in Fig. 4e, f that the algae with a constitutive defense tended to be considerably larger than the algae with an inducible defense. MPV varied with dilution rate in the alga with a constitutive defense (Fig. 4e), but was almost exclusively considerably higher than that of an undefended alga. MPV also varied with dilution rate in the alga with inducible defenses (Fig. 4f). MPV was low and relatively stable at a dilution rate of 0.5. At lower dilution rates of 0.3 and 0.1, MPVs moved in and out of the zones that can be considered as undefended and defended (Fig. 4f), along with strong population fluctuations in the algae and their herbivorous rotifers (Fig. 4f). The algae were still growing rapidly towards their carrying capacity in the two chemostats where rotifers had recently gone extinct, and had not yet lost their defense at the end of the experiment (Fig. 4f).

Discussion

As predicted by our model, and in agreement with the paradox of enrichment, we observed a clear increase in population variability and herbivore extinction risk when we decreased the flow-through rate in our highly eutrophic experimental alga–rotifer systems. Below we discuss the qualitative match between the model predictions in terms of dilution rates for which we predicted existence and stability boundaries to occur and our experimental data, and we discuss the quantitative results of our numerical analyses. In the experiment we

imposed rather wide-spaced jumps in dilution rate of 0.2 per day per dilution-step, and thus we cannot pinpoint the exact locations of bifurcations for the experimental systems. We did not consider it useful for the purposes of the present paper to attempt to ‘tweak’ the model or parameter estimates to obtain a better fit. We restrict this discussion to the general similarities and dissimilarities between model predictions and the obtained data.

At the initially high dilution rate of 0.7 per day, herbivores were never washed out in our experiment. Given that both rotifers and algae are known to easily reach growth rates that are substantially above 0.7 per day, it is quite understandable that algae and rotifers could exist together under the studied range of dilution rates below 0.7 per day. Our numerical analysis showed that we should expect such persistence of both algae and rotifers at the high nutrient level ($N_i = 155$) in our chemostat systems, for dilution rates below about 0.71–0.72, in the inducible defense case, which is consistent with the data. We did not expect persistence of herbivores for the constitutive defense case at dilution rates of 0.7 and 0.5. Low densities of rotifers did persist under these dilution rates, possibly initially on basis of reserves, and later on most likely on basis of a small fraction of particles of edible size within the chemostats (see van der Stap et al. 2006).

As qualitatively predicted, CVs of herbivore densities were relatively low when dilution rates were high (0.7 and 0.5 per day). CVs tended to be higher at a dilution rate of 0.3 and they were definitely highest at the lowest dilution rate of 0.1 per day. It should be noted that the observed very high CVs at $D = 0.1$ were influenced by the herbivore population declines at the end of the experiment. Also, when looking at the algal densities, population variability tended to be highest at lower dilution rates (0.1 and 0.3 per day, Fig. 3a–f). Again, we should note that the very high CV at $D = 0.1$ was influenced by the rapid algal population increases that followed herbivore population declines.

Although it is hard to exactly pinpoint the location of the stability boundary for these experimental chemostat communities, it is clear that a marked increase in the difference between algal population minima and maxima occurred between dilution rates of 0.3 and 0.5 per day (Fig. 2a–f). This also appears to be a major transition in the time series data (Fig. 4a–f), for both defense strategies. Our numerical analysis showed that we should expect the Hopf bifurcation to occur, at the high experimental nutrient level ($N_i = 155$) in our chemostat systems, for dilution rates below about 0.34–0.35. The simple model thus predicts limit cycles for dilution rates of 0.3 and 0.1 per day. In terms of population fluctuations, this is consistent with the data: population densities show higher amplitude fluctuations at low dilution rates of 0.3 and 0.1 per day. Fussmann et al. (2000) were also able to show qualitative changes in system behavior using rotifers and algae in chemostats. These authors explained this result in terms of crossing the Hopf

bifurcation, which is the stability boundary H in Fig. 1a, b, that separates areas A_1 and B_1 . Large population fluctuations at low dilution rates coincided with herbivore extinctions in our experiment, as expected on basis of the chemostat model (Fig. 2). This can be seen as the ‘dilution rate aspect’ of the paradox of enrichment: One decreases the mortality rate of zooplankton through a decrease in dilution rate, but promotes their risk of stochastic extinction through increased population fluctuations. Interestingly, *Brachionus* population declines followed when the alga with inducible defenses reached low densities (as expected), but herbivores went extinct in ‘constitutive defense’ chemostats when these algae actually had very high densities. Earlier work by van der Stap et al. (2006) in batch systems helps to explain this: that work showed that *Brachionus* extinction risk is not related to total algal biovolume, but instead responds strongly to the fraction of particles that is of a highly edible size. In the treatment with constitutively defended algae this fraction is exceedingly low (see van der Stap et al. 2006), making it very difficult for *Brachionus* to maintain high population densities, and simultaneously making it relatively vulnerable to fluctuations in its food source. In the treatment with induced defenses the fraction of edible size particles is likely to be higher, at least part of the time (see fluctuations in MPV in Fig. 4f; see also van der Stap et al. 2006), but apparently the rotifers are able to overexploit these within the chemostats when dilution rates are low, leading to the decline of their populations.

Large population fluctuations and herbivore extinctions (the latter measured as declines to below our detection limit) occurred both in the inducible defense and constitutive defense treatments. This is expected on basis of the alga–rotifer chemostat model, as the stability boundary is nearly identical for both types of defenses, at a high nutrient level. It is noteworthy that the model analysis predicts inducible defenses to resolve the paradox of enrichment at intermediate dilution rates (an increase in nutrient concentration does not destabilize the system), but it also predicts that inducible defenses do not prevent destabilization through a decrease in flow-through rate of the system: a ‘cyclic’ area B_1 still exists at low dilution rates. According to the numerical analysis, we expect stable coexistence of inducible defended algae and herbivores at dilution rates between about 0.71 and 0.35. In our chemostats we did observe very high, but relatively stable, densities of both algae and herbivores at dilution rates of 0.7 and 0.5 despite an exceedingly high nutrient level ($N_i = 155$), much higher than in most natural eutrophic systems. There were no herbivore extinctions at these dilution rates. However, we should note the absence of such extinctions also in the constitutive defense case, although herbivore populations were marginal in comparison.

We thus observed a clear difference in herbivore dynamics between the chemostats with *S. obliquus* and the ones containing *D. quadricauda* (Fig. 4c, d),

mainly in terms of densities. Variability among replicates was higher in the *Brachionus* data (Fig. 3) than in the algal data (Fig. 2). This could reflect differences in edibility of the algae and/or minor differences in dilution rate between replicates. Rotifer data are not ‘inherently variable’ or divergent, as replicates were remarkably similar in a previous experiment that made use of semi-continuous cultures (Verschoor et al. 2004b).

The costs of defenses have been mentioned as one of the prerequisites for the evolution of inducible defenses, as opposed to constitutive defenses (Harvell 1990; Tollrian and Harvell 1999). For colonial algae, these costs may manifest themselves as higher sinking rates (Lüring and Van Donk 2000). Colonies disintegrated less rapidly with an increasing daily light dose, which reveals photosynthetic costs associated with colony maintenance (Verschoor 2005). Under natural conditions, sedimentation and the light cycle are thus relevant in terms of environmental costs. The experimental setup used may have decreased these costs, as continuous bubbling of the chemostats did not allow for sedimentation of the algae and continuous light conditions may have slowed the decay or relaxation of defenses.

In a two-stage chemostat system where prey and predators were grown separately (Boraas 1983), the algae did not form defenses and large population fluctuations occurred (Lüring et al. 2005). In the one-stage chemostats used here with inducible defended algae, strong changes in algal population densities occurred at low dilution rates but population fluctuations were relatively small at higher dilution rates.

We conclude that our model clearly predicts qualitative shifts in predator–prey dynamics as a function of dilution rate, and that our experimental data roughly follow these predictions: populations were most variable at low dilution rates and this variability coincided with herbivore extinctions, both when algae had constitutive or induced defenses. Such experimental system behavior is in agreement with the paradox of enrichment. However, alga–rotifer dynamics did not follow ‘clean’ limit cycles, for instance in terms of the expected phase shift between predator and prey. Dynamics also did not appear to be characterized by small-amplitude cohort cycles or the different types of compensatory dynamics as have been studied by Yoshida et al. (2007 and references therein). We conclude that the observed dynamics in our replicate chemostat communities largely followed the predictions of our simple chemostat model, but that several aspects of these dynamics still require further analysis.

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