Inducible defenses
and
the dynamics of planktonic food chains

Van der Stap, I. (2007)
Inducible defenses and the dynamics of planktonic food chains
Ph.D Thesis, Radboud Universiteit Nijmegen

Cover design: Egbert van der Gugten
Printed by Gildeprint B.V.

ISBN 978-90-9022209-7
NIOO Thesis 53

Inducible defenses
and
the dynamics of planktonic food chains

Een wetenschappelijke proeve op het gebied van de
Natuurwetenschappen, Wiskunde en Informatica

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op vrijdag 19 oktober 2007
om 13.30 uur precies

door

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This research was financially supported by the Netherlands Organisation for Scientific Research (NWO-ALW).

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Chapter 1

General introduction

Abstract

This thesis presents an analysis of the effects of individual level plasticity on population dynamics and persistence. The main objective of the research was to investigate whether different defense strategies of organisms have contrasting effects on population dynamics. Inducible defenses were expected to stabilize population dynamics as opposed to constitutive defenses or the absence of defenses. This chapter provides a concise introduction to phenotypic plasticity in aquatic organisms, theory on the effects of inducible defenses in two- and three-level food chains and the model system of algae and rotifers.

Parts of this chapter are based on the paper: Van der Stap, I., M. Vos and W.M. Mooij. Inducible defenses and rotifer food chain dynamics. Hydrobiologia in press.

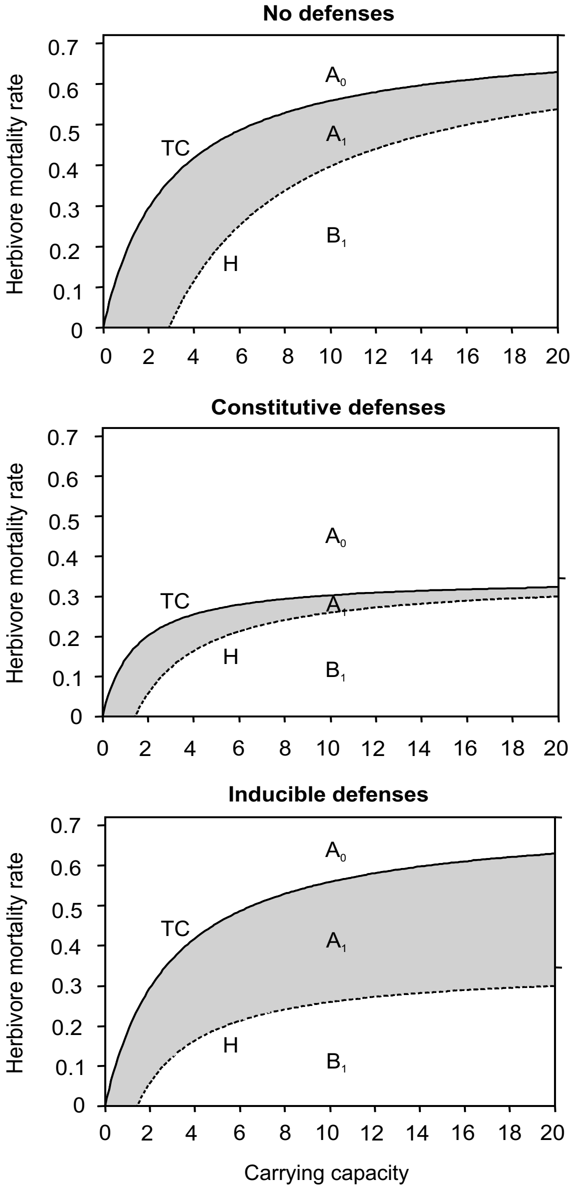


Figure 1.1 The stability boundaries of bitrophic food chains without defenses (top), with constitutive defenses (middle), or with inducible defenses (bottom), for a range of carrying capacities and herbivore mortality rates. The transcritical (TC) bifurcation curve or existence boundary and Hopf (H) bifurcation curve or stability boundary separate areas with qualitatively different dynamics. Area A_0: only algae exist. Grey area A_1: stable coexistence of algae and herbivores. Area B_1: algae and herbivores fluctuate (Vos et al. 2004a).

Phenotypic plasticity

Inducible defenses are phenotypic changes in prey organisms in response to cues that indicate the presence of a predator. They aim at reducing the loss rates in subsequent attack by these predators. They are used by many organisms to adjust their defense level to the current level of predation. In aquatic ecosystems inducible defenses have been found in a wide range of different organisms among which are algae, cyanobacteria, macrophytes ciliates, bryozoans, rotifers, gastropods, insect larvae, amphibians and vertebrate animals (see reviews by Havel 1987, Larsson and Dodson 1993, Wicklow 1997, Snell 1998, Werner and Peacor 2003, Prusak et al. 2005, Van Donk 2006). They include responses in morphology, e.g., increased body depth in crucian carp in response to piscivorous fish such as pike (Brönmark and Miner 1992), in behavior, e.g., diel vertical migration in daphnids in response to visually hunting zooplanktivorous fish (Ringelberg 1991), in life history, e.g., a reduced size at first reproduction in daphnids against fish predation (Stibor and Lüning 1994) and in biochemistry, e.g., increased toxin production in cyanobacteria in response to exposure to herbivorous zooplankton (Jang et al. 2003).

Theoretical background

Inducible defenses modify the interaction strength between predator and prey and are as such individual-level responses that affect the functioning of populations and communities (e.g., Vos et al. 2002, 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). In an unstable system that shows strong population oscillations species might be lost in the troughs of population cycles (McCann et al. 1998, Vos et al. 2004a). Vos et al. (2004a, 2004b) made a theoretical study of the effects of inducible defenses on bi- and tritrophic food chains. The classical formulation of the model, that does not take inducible defenses into account, results in three areas of qualitatively different dynamics. These areas are separated by (1) the existence boundary, indicating that at higher predator mortalities, the predator cannot exist and only prey are present (2) the stability boundary, indicating that at lower predator mortalities, predator-prey cycles would occur with the risk of stochastic extinctions. The existence and stability boundaries converge at high carrying capacities. In the model with inducible defenses a stable coexistence of predator and prey occurs over a wide range of environmental conditions, even at high carrying capacities (Fig. 1.1). To exemplify their results the model was parameterized specifically for algae-rotifer

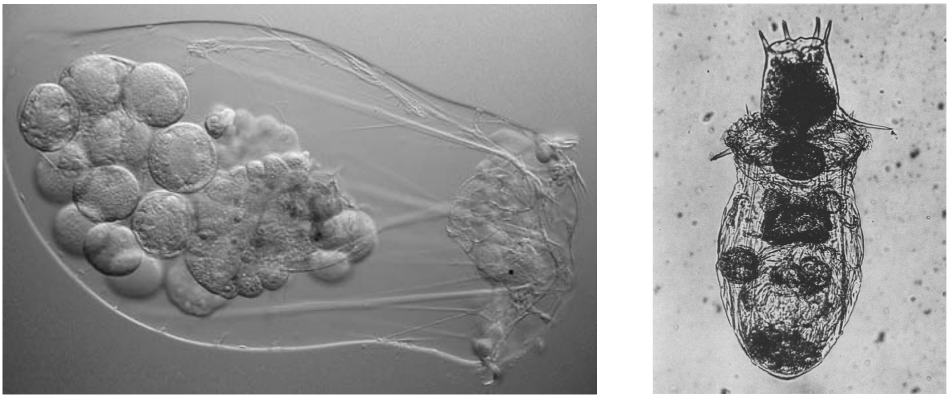


Figure 1.2 To the left: The carnivore *Asplanchna* is a top-predator with a length of approximately 1mm (Photo courtesy Wim van Egmond). To the right: *Asplanchna* captures *Brachionus calyciflorus*, the postero-lateral spines are expanded (Halbach 1971).

food chains using parameter values from the literature and experimental data (Vos et al. 2004a). Theoretical predictions also showed that inducible defenses increased the relative importance of bottom-up control. The variation in consumption rates on defended and undefended prey types caused the biomass of all trophic levels to increase simultaneously in response to enrichment (Vos et al. 2004b).

Model system

The number of experimental studies on the effect of inducible defenses on population level effects, however, is fairly limited (but see Haukioja 1980, Underwood and Rausher 2000). The few that exist originate from terrestrial systems where inducible defenses are present in many plant species (e.g., Agrawal 2001). In my PhD project I investigated the effects of inducible defenses on population dynamics in an experimental planktonic system. In this research I have strived for integration of both theoretical and experimental studies.

A model system of algae (Scenedesmaceae) and rotifers (Brachionidae and *Asplanchna*) was used in a microcosm approach. The model system is ideal for studying the population level effects of inducible defenses given the short generation times of the planktonic organisms. The standard medium used for all cultures and experiments is COMBO medium (Kilham et al. 1998). The exact composition of the medium is described in the Appendix, Table A.1. We recognize that microcosm results may not be directly transferable to the ecosystem scale and can in fact yield erroneous extrapolations. On the other hand, a microcosm approach does increase experimental control and allows for ample replication. Therefore, microcosm experiments were chosen to study selected interactions between zooplankton and their predators and between herbivorous zooplankton and the algae they graze. These included the use of batch, semi-continuous and continuous cultures for bi- and/or tritrophic food chains.

Defenses in herbivorous rotifers

Rotifers are preyed upon by a variety of invertebrate predators including protists, cnidarians, cladocerans, cyclopoid, calanoid and harpacticoid copepods, mysids, insect larvae (species of Chaorboiridae) and other rotifers (Williamson 1983). Williamson's review of invertebrate predation on rotifers was updated by Brandl (2005) who discussed various factors determining the predation rate and impact of freshwater copepods on rotifers. There are numerous examples of defenses in rotifers including changes in morphology, e.g., spine development or elongation (reviewed in Gilbert 1999), a response in behavior, e.g., vertical migration (Gilbert and Hampton 2001) or escape reactions (Gilbert and Kirk 1988), life history changes e.g., high maximum growth rate (Walz 1995) and chemical deterrents (Felix et al. 1995, Walsh et al. 2006). One of the first reports of inducible defenses in rotifers was the induction of spine formation in *B. calyciflorus* by *Asplanchna* (Gilbert 1966, 1967). The observations by Gilbert (1967) suggested that the chemical signal involved in this induction of defenses is a heat-stable protein. More on the chemical ecology of rotifers can be found in Snell (1998).

Juvenile *B. calyciflorus* and adults with no or short postero-lateral spines are highly susceptible to predation by *Asplanchna* (Fig. 1.2), but those with long spines are relatively protected from being captured or ingested (Gilbert 1966, 1967, Halbach 1971). These authors demonstrated that *Asplanchna* has to orient the long-spined prey to allow the passage of the relatively large prey into the small mouth (Fig. 1.2). The time spent on swallowing the prey therefore increases, and ingestion attempts can be unsuccessful. Especially young *Asplanchna* have much difficulty ingesting spined *B. calyciflorus* (Gilbert 1967, 1980). The effects of prey vulnerability on the functional response and growth have been tested for *Asplanchna* preying on *B. calyciflorus* and several other rotifer species (Iyer and Rao 1996, Nandini et al. 2003). The capture success and ingestion of *Asplanchna* on spined *B. calyciflorus* is much lower than on non-spined *B. calyciflorus* (Iyer and Rao 1996). The effectiveness of *Brachionus* spines in preventing ingestion is likely to differ for different *Asplanchna* species.

Response to rotifer predation in the green alga *Scenedesmus*

Inducible defenses are common among herbivorous rotifers that often fall prey to carnivorous zooplankton, but even the prey of herbivorous zooplankton are well-known to exhibit their own form of inducible defense. For example, species of

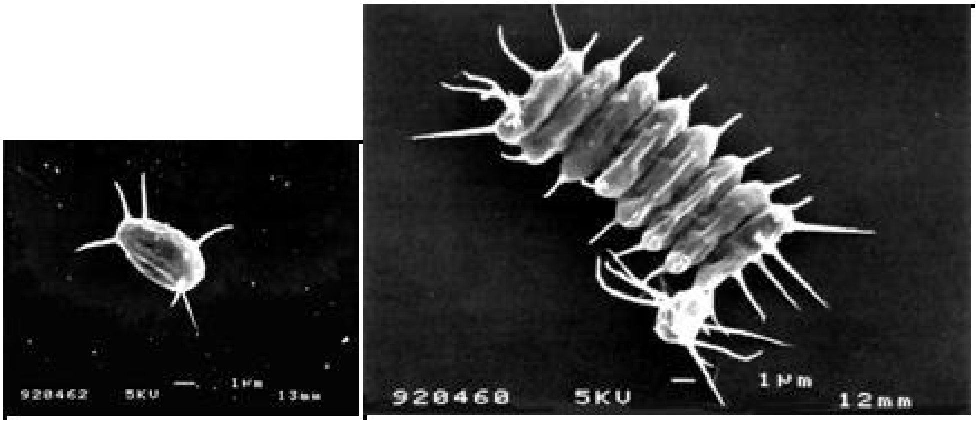


Figure 1.3 Colony formation in *Desmodesmus subspicatus* in response to infochemicals (Hessen and Van Donk 1993).

Scenedesmaceae, a group of green algae, show striking phenotypic plasticity when in the presence of the microcrustacean *Daphnia magna* (Hessen and Van Donk 1993). The authors observed induced colony-formation in an algal species that is now called *Desmodesmus subspicatus* (Fig. 1.3). Shortly thereafter, induced colony-formation was also found in a species formerly known as *Scenedesmus acutus* (Lampert et al. 1994). In response to the presence of grazing-released infochemicals of this herbivore, the alga formed four- to eight celled colonies. This response also occurred when *Scenedesmus* was exposed to grazing-released infochemicals of the rotifers *Brachionus* and *Keratella* (Lürling and Van Donk 1997, Van Donk et al. 1999).

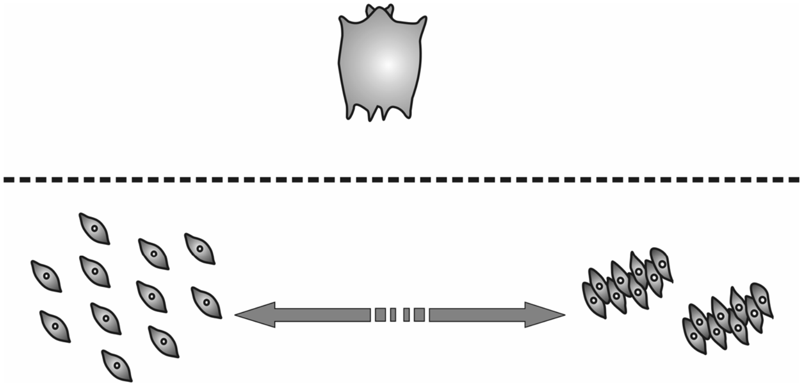
Research has shown that the induction of colony formation in unicellular algae can have a large effect on the feeding efficiency in *B. calyciflorus*. The maximum ingestion rate of this rotifer was lower when feeding on algae with an induced defense than on undefended algae (Verschoor et al. in press). This difference in maximum ingestion rates was due to a difference in handling time rather than a difference in capture efficiency. The algae that were induced or exhibited a constitutive defense did not only reduce the maximum ingestion rates, but also seemed to be assimilated less efficiently by rotifers. Growth experiments provided further support that larger algae caused reduced *Brachionus* growth (Mayeli et al. 2004, Lürling et al. 2005).

Scenedesmaceae and *B. calyciflorus* may co-occur in ponds (Halbach and Halbach-Keup 1974) and they may co-dominate the plankton in shallow lakes (Jeppesen et al. 1990). Many *Brachionus* and *Asplanchna* species co-occur in lakes (Oltra et al. 2001), ponds (Halbach 1972) and rivers (Lair et al. 1998, Kim and Joo 2000). The presence of spines in *Brachionus* in relation to the presence and density of *Asplanchna* has specifically been reported for various water bodies (Gilbert and Waage 1967, Green and Lan 1974, Gilbert 2001).

Thesis outline

This thesis consists of the results gathered during my four years of Ph.D research. Most of the studies have an experimental approach. The chapters are organized according to (1) increasing level of complexity, from individual plasticity to population dynamics and (2) increasing level of dilution rate in the culture system, from batch culture to continuous culture. They were written down to be separately published in international peer-reviewed journals and some chapters may therefore show considerable overlap in the materials and methods sections. Algae or Scenedesmaceae will also be referred to as plants, *Brachionus* as herbivores or grazers, and *Asplanchna* as carnivores or (top-) predators. When mentioning undefended organisms I usually mean organisms with a fixed undefended strategy. The undefended state of organisms that have inducible defenses is typically indicated as an organism with non-induced defenses.

Starting at the plant level my co-workers and I investigated many strains of Scenedesmaceae for their response to infochemicals released by either *Daphnia* or *Brachionus* (see Chapter 2). The effect of defense strategy on population dynamics was tested in batch cultures of *Scenedesmus* and *Brachionus*. In the experiment extinctions of the herbivore were related to prey size (see Chapter 3). In Chapter 4, we further teased apart the mechanism of these extinctions using a modeling approach. Increasing the length of the food chain by adding a top-predator to examine the effects of inducible defenses in two- and three-level food chains resulted in Chapter 5. The results of this chapter led to the hypothesis that herbivore defenses may have prevented an increase in plant biomass in food chains with a top-predator. The hypothesis was tested in Chapter 6, using two- and three level food chains with an undefended herbivore. Aquatic communities are complex and their structure is influenced by both competition and predation. In chapter 7 we increase the complexity of our food chain further by having the inducible defended and undefended herbivores in competition. As a final test of the hypothesis that inducible defenses stabilize dynamics over a wide range of conditions, chemostats were used with various dilution rates imposing different mortality rates on all organisms of the system (see Chapter 8). These chapters are followed by a general discussion (Chapter 9). A concise overview of the main results can be found in the Summary.



Chapter 2

Inducible colony formation within the Scenedesmaceae: Adaptive responses to infochemicals from two different herbivore taxa

Abstract

We studied the occurrence of colony formation within 40 different strains of Scenedesmaceae (Chlorococcales, Chlorophyta) in response to grazing-released infochemicals from the herbivorous zooplankters *Brachionus calyciflorus* Pallas (Rotifera) and *Daphnia magna* Strauss (Cladocera). With the exception of two strains, all strains showed similar responses to both *B. calyciflorus* and *D. magna* infochemicals, either no response or inducible colony formation. Colony size was found to increase with *B. calyciflorus* infochemical concentration and could be described by a sigmoid function. The increase in colony size was more pronounced in the *Scenedesmus* species tested than in *Desmodesmus* species, which was probably due to higher threshold infochemical concentrations for colony induction in *Desmodesmus*. Therefore, the adaptivity of colony formation to the herbivory threat only holds above the threshold concentration for colony induction and as long as maximum colony size has not been attained. Taking this into account, our results suggest that inducible colony formation is a common adaptive response of many Scenedesmaceae to the threat of herbivory.

Verschoor, A.M., I. van der Stap, N.R. Helmsing, M. Lürling and E. van Donk. 2004 Inducible colony formation within the Scenedesmaceae: Adaptive responses to infochemicals from two different herbivore taxa. Journal of Phycology 40(5): 808-814.

Introduction

Herbivory by zooplankton is among the largest selective pressures on the phytoplankton. Inducible defenses are a ubiquitous form of phenotypic plasticity that can be an important adaptive strategy in aquatic environments, especially when herbivory is variable (Tollrian and Harvell 1999). Inducible defenses are expected to be favored especially when the cues of predation are reliable and not fatal (Harvell 1990), and in the pelagic, information chemicals are nonfatal and more reliable and specific than, for example, mechanical cues (Tollrian and Harvell 1999). Since the seminal paper by Hessen and van Donk (1993), herbivore infochemical-induced defenses have been reported in various phytoplankton taxa (raphydophytes, Hansson 1996; cyanobacteria, Jang et al. 2003; prymnesiophytes, Tang 2003; and chlorophytes, Van Donk et al. 1999, Yasumoto et al. 2000).

The family of Scenedesmaceae (Chlorococcales, Chlorophyta) is by far the best studied phytoplankton taxon with respect to grazer-induced colony formation. The recognition of phenotypic plasticity has shed new light on the taxonomy of this family (Trainor 1998). On the basis of morphology, it has been proposed that the genus *Scenedesmus sensu lato* should be split up into a spiny genus, *Desmodesmus*, and a spineless genus, *Scenedesmus* (Trainor et al. 1976). This division has been confirmed by molecular techniques (An et al. 1999, Van Hannen et al. 2002). Induced colony formation in response to *Daphnia* grazing has been found in both genera (e.g., *Desmodesmus subspicatus*, Hessen and Van Donk 1993; *Scenedesmus obliquus* [*sensu* Van Hannen et al. 2000], Lampert et al. 1994). Coenobium formation in these species can be induced by both grazing *Daphnia* and by filtrate of grazing *Daphnia*, indicating that water-borne information chemicals are involved (Hessen and Van Donk 1993, Lampert et al. 1994). Although it has been hypothesized that *Daphnia*-induced colony formation is common in *Scenedesmus sensu lato* (Lampert et al. 1994), not all strains form colonies when exposed to grazing filtrate of this cladoceran (Van Donk et al. 1999). For the most intensively investigated strain, *Scenedesmus obliquus* MPI (Table 2.1), colony formation has been shown to be inducible by various herbivorous zooplankton species (Van Donk et al. 1999, Lürling 2003) but not by carnivorous zooplankton (Lürling 2003). Induced herbivore resistance in higher plants is usually not very specific (Karban and Myers 1989), but it remains debatable whether this can be said for inducible colony formation of Scenedesmaceae with respect to different zooplankton taxa. Therefore, we investigated the distribution of infochemical-induced colony formation within *Scenedesmus* and *Desmodesmus*, in response to grazing-released infochemicals from herbivorous zooplankton species from two different taxa: *Daphnia magna* (Cladocera) and *Brachionus calyciflorus* (Rotifera).

To be adaptive, inducible colony formation should be proportional to the expected fitness costs of herbivory, that is, the grazing risk that the algae are facing. Filtrate from actively feeding *Daphnia* has a stronger colony-inducing effect than filtrate from starved conspecifics, and neither *Daphnia* homogenate nor algal homogenate separately induce a response (Lampert et al. 1994, Von Elert and Franck 1999). There is proportionality between grazing pressure from herbivorous Cladocera and colony induction in *S. obliquus*, regardless of the species (Lürling 2003). If such proportionality could also be demonstrated for taxa other than Cladocera, induced colony formation would be a general response to herbivory, regardless of the herbivore involved. This would show that inducible colony formation is not a rare phenomenon against a few specialized herbivores but that it is a general adaptive response in which algae adjust their size to the risk of being grazed. Therefore, we investigated whether colony size of *S. obliquus* was proportional to the amount of grazing-released infochemicals from *B. calyciflorus*.

Materials and methods

Bioassays on colony formation in different strains to different herbivore infochemicals

This study involves a synthesis of unpublished data from one experiment with the rotifer *B. calyciflorus* Pallas and of both published (Lürling 1999a, Lürling and Beekman 1999) and unpublished data from three experiments using the cladoceran *Daphnia magna* Strauss (Table 2.1). All experiments consisted of standardized bioassays that have been extensively used for the morphological response of Scenedesmaceae to zooplankton filtrate (Lampert et al. 1994, Lürling 2003).

COMBO medium (Kilham et al. 1998) was used as standard medium. Test water was produced by allowing $1{\cdot}10^5$ *B. calyciflorus*/L or 200 *D. magna*/L to graze on at least 10 mg algal C/L, with the algae being the strain of interest. The zooplankton densities were considered to be sufficient to induce colony formation, at least for *S. obliquus* (Van Donk et al. 1999). After 24 h of grazing, algae and zooplankton were removed from the test water by filtration over a zooplankton filter (60 µm) and a precombusted glass fiber filter (Whatman GF/F, Maidstone, UK). Controls consisted of clean glass fiber-filtered medium. Test water (10% v/v) was added to cellulose-plug-stoppered 100 mL Erlenmeyer flasks (Omnilabo International BV, Breda, The Netherlands) containing medium and algal inoculate, making a final volume of 50 mL. Algae were inoculated in densities of $2{\cdot}10^6$ μm^3/mL and were obtained from algae in log-phase (chemostats or

Table 2.1 Algal strains used in the experiments, strain codes used, and results of the bioassays (Mann-Whitney U test).

		Treatment			
		Brachionus		Daphnia	
Strain[a]	Code	MPV	CPC	MPV	CPC
Scenedesmus					
S. acuminatus (Lagerheim) Chodat UTEX 415	S1	*	N.S.	N.S.	-
S. acuminatus (Lagerheim) Chodat V411	S2	-	-	N.S.	-
S. acuminatus (Lagerheim) Chodat V412	S3	-	-	*	-
S. acutiformis Schröder UTEX 416	S4	N.S.	N.S.	-	-
S. acutus f. alterans Hortobagyi UTCC-T7	S5	*	N.S.	-	-
S. acutus f. alterans Hortobagyi UTCC-T10	S6[b]	*	N.S.	N.S.	N.S.
S. ellipticus Corda SAG 64.81	S7	N.S.	N.S.	-	-
S. falcatus Chodat SAG 2.81	S8	N.S.	N.S.	*	*
S. obliquus (Turpin) Kützing MPI	S9	*	*	*	*
S. obliquus (Turpin) Kützing NIVA-CHL6	S10[c]	N.S.	N.S.	*	*
S. obliquus (Turpin) Kützing SAG276/1	S11[c]	*	N.S.	N.S.	*
S. obliquus (Turpin) Kützing SAG276/3a	S12	N.S.	*	*	*
S. obliquus (Turpin) Kützing UTEX 72	S13[b]	*	*	*	*
S. obliquus (Turpin) Kützing UTEX 74	S14	N.S.	N.S.	-	-
S. obliquus (Turpin) Kützing UTEX 78	S15[c]	-	-	*	*
S. obliquus (Turpin) Kützing UTEX 79	S16	N.S.	N.S.	-	-
S. obliquus (Turpin) Kützing UTEX 393	S17	*	*	-	-
S. obliquus (Turpin) Kützing UTEX 417	S18	N.S.	N.S.	-	-
S. obliquus (Turpin) Kützing UTEX 1450	S19[c]	-	-	*	*
S. obliquus (Turpin) Kützing UTEX 2630	S20[c]	N.S.	*	*	*
S. pectinatus Meyen V72	S21	-	-	N.S.	-
S. pectinatus Meyen V99	S22	-	-	N.S.	-
S. platydiscus (Smith) Chodat UTEX 2457	S23	N.S.	N.S.	-	-
S. producto-capitatus Schmula SAG 21.81	S24	*	*	-	-

Table 2.1 continued. Algal strains used in the experiments, strain codes used, and results of the bioassays (Mann-Whitney U test).

		Treatment			
		Brachionus		Daphnia	
Strain[a]	Code	MPV	CPC	MPV	CPC
Desmodesmus					
D. armatus (Chodat) Hegewald MPI	D1	N.S.	N.S.	-	-
D. bicellularis Hegewald CCAP 276/14	D2	N.S.	N.S.	N.S.	-
D. bicellularis Hegewald UTEX LB1359	D3	N.S.	*	*	-
D. communis Hegewald UTEX 76	D4[b]	-	-	N.S.	*
D. maximus Hegewald UTEX 614	D5	N.S.	N.S.	-	-
D. pannonicus (Pringsheim) Hegewald UTEX 77	D6	-	-	*	-
D. quadricauda (Turpin) Hegewald F11	D7[b]	-	-	N.S.	N.S.
D. quadricauda (Turpin) Hegewald NIVA-CHL7	D8[b]	-		N.S.	N.S.
D. sp. Lürling MV3	D9	-	-	N.S.	-
D. sp. Lürling MV5	D10	-	-	N.S.	-
D. sp. Lürling MV7	D11	-	-	N.S.	-
D. subspicatus (Chodat) Hegewald et Schmidt CCAP 276/20	D12	*	N.S.		-
D. subspicatus (Chodat) Hegewald et Schmidt NIVA-CHL55	D13	*	*	*	N.S.
D. subspicatus (Chodat) Hegewald et Schmidt RWTH	D14	-	-	N.S.	N.S.
D. subspicatus (Chodat) Hegewald et Schmidt UTEX 2532	D15	-	-	N.S.	N.S.
D. subspicatus (Chodat) Hegewald et Schmidt UTEX 2594	D16	N.S.	N.S.	N.S.	N.S.

[a] CCAP, Culture Collection of Algae and Protozoa, UK; F, Saskatchewan Research Council, Canada; MPI, Max Planck Institute of Limnology, Germany; MV, NIOO Centre for Limnology, The Netherlands, NIVA, Norwegian Institute for Water Research; RWTH, University of Aachen, Germany; SAG, University of Göttingen, Germany; UTCC, University of Toronto Culture Collection, Canada; UTEX, University of Texas, USA, V, University of Plovdiv, Bulgaria.

[b] *D. magna* bioassay data from Lürling and Beekman, 1999.

[c] *D. magna* bioassay data from Lürling, 1999.

N.S.: treatment not significantly larger than in control; *: treatment significantly larger than control (P<0.05); -: not determined.

semicontinuous batch cultures). Within each experiment, all bioassays ran simultaneously. Flasks were incubated in (at least) triplicates for 48 h in an incubator set at 20°C, 100 rpm, and continuous light (120 µmol photons [PAR]·m^{-2}·s^{-1}, cool white fluorescent tubes). Colony sizes of controls and treatments were measured at t = 48h, using both microscope (counting the number of cells per coenobium of at least 125 algal particles per replicate) and electronic particle counter. Mean particle volumes (MPVs) for *D. magna* bioassays were measured on a Coulter Multisizer II (Beckman Coulter, Inc., Fullerton, CA, USA) and for *B. calyciflorus* bioassays on a CASY cell counter (Schärfe System Gmbh, Reutlingen, Germany).

In Table 2.1, we give the complete list of algal strains that were used for the experiments, including codes that we used in the different figures. For these strains, we compared the relative changes in MPV and mean number of cells per coenobium (CPC) among 16 strains of *Desmodesmus* and 24 strains of *Scenedesmus* (Table 2.1). Data for the algal response after 48 h were first analyzed on homogeneity of variance and normality. Data were heteroscedastic, and transformation of the data did not prove to be sufficient for the use of parametric tests. Therefore, we tested for differences between treatments (test water vs. control) using the Mann-Whitney U test.

Bioassay on colony formation in response to infochemical concentration

To measure the proportionality of induced colony formation with the concentration of test water (containing the infochemical(s) that indicate the herbivore threat), we used the same bioassay as described above. Again, COMBO medium was the standard medium used. To produce test water, 100 *B. calyciflorus*/mL were incubated for 24 h in the dark at 20°C in a food suspension of 10 mg C *S. obliquus* MPI/L. After filtration, this test water was first diluted with clean medium to yield five dilutions: 1x (undiluted), 10x, 100x, 1000x, and 10,000x. Together with the controls (clean COMBO medium), these dilutions were again added in 10% (10 mL v/v) to Erlenmeyer flasks containing 90 mL of medium. Furthermore, an additional treatment of 100 live *B. calyciflorus*/mL in clean medium was added. Treatments were inoculated with $2 \cdot 10^6$ μm^3 *S. obliquus*/mL, and MPV was measured on a Coulter Multisizer II after 48 h.

Further analysis was done for all infochemical dilutions (including controls) but did not include the live *B. calyciflorus* treatment because of unknown infochemical concentration and additional zooplankton effects that could obscure interpretation (e.g., selective grazing). First, these data were tested on homogeneity of variance and normality. Differences between dilutions were tested by one-way analysis of variance, followed by contrast analysis. The dose–response bioassay was further analyzed by nonlinear regression of the general logistic dose– response function:

$$MPV(d) = MPV_{\min} + \frac{a}{1+\left(\frac{h}{d}\right)^{b}} \quad (2.1)$$

representing mean particle volume (MPV) as a function of infochemical dose (*d*), where $\mathrm{MPV}_{\min}$ is MPV at zero dose (minimum response), $\mathrm{MPV}_{\max}$ is MPV at maximum dose (maximum response), *a* is the amplitude ($\mathrm{MPV}_{\max}$ - $\mathrm{MPV}_{\min}$), *h* is the dose at which the half-maximum amplitude is reached (= inflection or transition point), and *b* is a parameter describing the width of the transition zone. All statistical tests were done with STATISTICA for Windows, release 5.1 (Statsoft Inc., Tulsa, OK, USA), and nonlinear regression was done with SigmaPlot 2002 for Windows, version 8.02 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Herbivore-specific differences

Strains that exhibited significant inducible colony formation did this in response to both zooplankton species, except for S6 and S8 (Table 2.1). For the strains that responded significantly, the relative increase in colony size was generally greater in response to *D. magna* infochemicals than to *B. calyciflorus* chemicals (Fig. 2.1). It is not clear whether these differences are due to different chemical properties of the zooplankton infochemicals or to a common infochemical released in different concentrations. For more clarity in interpretation, the structure of the infochemicals involved needs to be resolved. Improved standardization by novel infochemical extraction methods looks promising, and by far the most progress in this area has been made for the *Daphnia* infochemical (Van Holthoon et al. 2003). Herbivore information chemicals are released during the grazing process, probably by digestive processing of the algae or by release of latent alarm substances by the algae (Stabell et al. 2003). Colony formation in various strains of Scenedesmaceae can be induced by infochemicals produced by herbivorous zooplankton that have been grazing on other strains of Scenedesmaceae or even other Chlorophytes (Lürling 1998, Van Donk et al. 1999). Also, filtrate of *D. magna* grazing on *S. obliquus* has been shown to induce toxin formation in the cyanobacterium *Microcystis aeruginosa* (Jang et al. 2003). This suggests that herbivore grazing, at least on Chlorophytes, releases non-specific infochemicals that are capable of inducing defenses in many phytoplankton taxa.

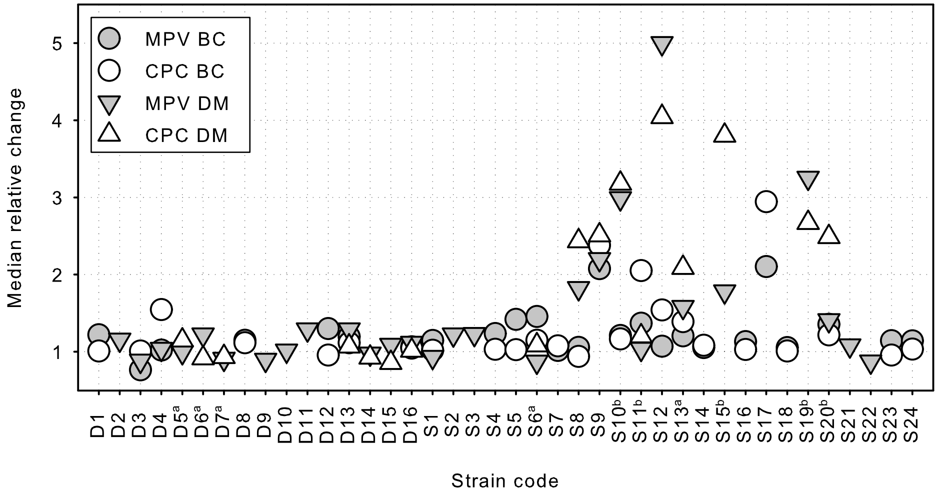


Figure 2.1 Relative increase in colony size of all Scenedesmaceae strains tested, given as median values of the ratios of MPV or CPC between treatments and controls, after exposure to test water (treatment) from *Brachionus calyciflorus* (BC) or *Daphnia magna* (DM). Strain codes and significance of differences are given in Table 2.1. Strain codes supplied with superscripted letters indicate strains for which bioassay results for *D. magna* have been published previously: [a]Lürling and Beekman (1999), [b]Lürling (1999).

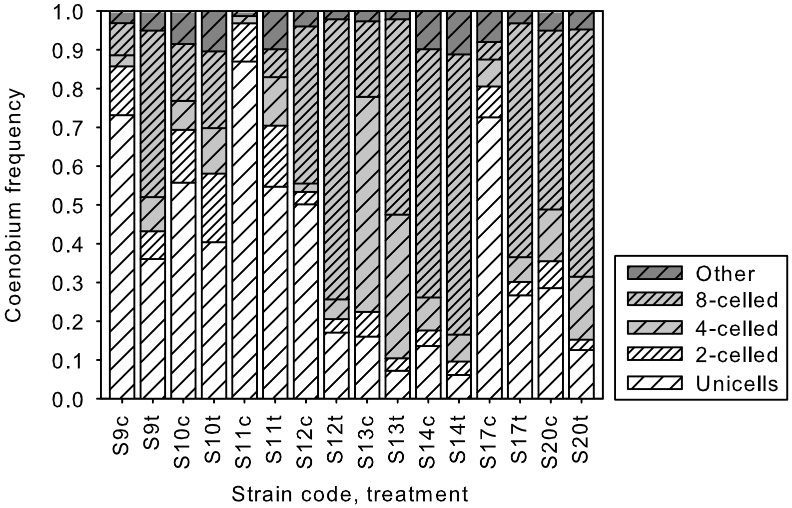


Figure 2.2 Average distribution of phenotypes (unicells, two-, four-, and eight-celled and other colony sizes) among different strains of *Scenedesmus obliquus* after 48-h exposure to test water of grazing *Brachionus calyciflorus*. Strain codes are followed by ''c'' (control) or ''t'' (exposed to test water). Shown are the average distributions from triplicate samples. Strain codes are given in Table 2.1.

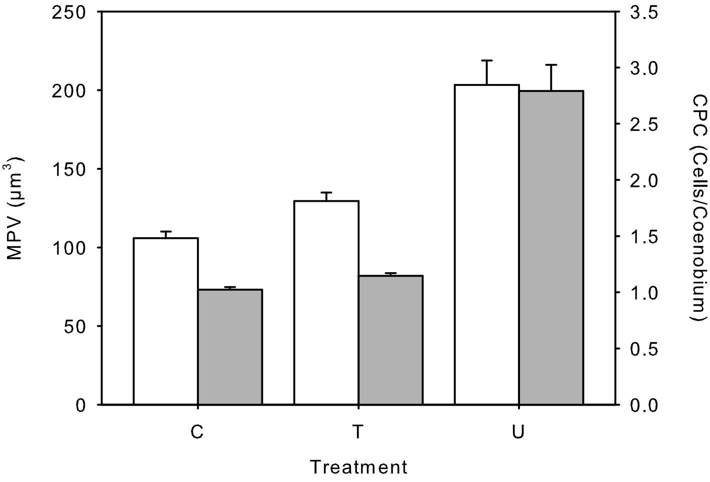


Figure 2.3 Mean particle volume (MPV, open bars) and number of cells per coenobium (CPC, filled bars) after 48 h of incubation of *Desmodesmus subspicatus* NIVA-CHL55 in controls (C), in standard 10 x diluted test water (T) and in undiluted test water (U). Shown are averages, and error bars represent 1 SD (n=3).

Phytoplankton genus-specific differences

Significant colony formation in response to both zooplankton species occurred frequently in *Scenedesmus* strains (48%), especially in *S. obliquus* strains (67% for S9– S20) and less frequently in *Desmodesmus* (24%, Table 2.1). Also, the relative increase in colony size was more pronounced in *Scenedesmus* than in *Desmodesmus* species (Fig. 2.1), which mainly can be attributed to the large representation of *S. obliquus* strains. However, even within *S. obliquus* the relative size increase was highly variable among strains, when exposed to testwater from the same herbivore species. This variability is exemplified by the distribution of the different phenotypes (colony size classes) for different *S. obliquus* strains in response to *B. calyciflorus* test water (Fig. 2.2). Local selection on traits such as threshold concentration for induction, response time, and/or maximum colony size (Adler and Harvell 1990, Larsson and Dodson 1993, Pigliucci et al. 1996) may all lead to different responses in a standardized bioassay.

Most *Desmodesmus* strains did not show significant colony formation; if they did it was not for MPV and CPC simultaneously (Table 2.1). Only *D. subspicatus* NIVA-CHL55 (D13) had weak, but significant and consistent colony formation. If induced colony formation is present within *Desmodesmus*, it is much less pronounced than within *Scenedesmus* (Fig. 2.1), although the latter were overrepresented by *S. obliquus*. We observed the formation of large colonies (up to 16 cells) in *D. subspicatus* after several days of culture in the presence of live

Daphnia or live *Brachionus* (data not shown). This has also been reported by Hessen and Van Donk (1993) on the same strain (D13) but could be the result of selective grazing on unicellular algae. Nonetheless, the concentration of infochemicals could also play an important role here. In the bioassay with D13, we added an additional treatment consisting of undiluted *B. calyciflorus* test water. In this treatment we observed a doubling in final colony size compared with the standard 10% dilution in our bioassays (Fig. 2.3). Apparently *D. subspicatus* requires a higher infochemical threshold concentration for colony formation.

Although *Desmodesmus* are characterized by the possession of spines, and predator-induced spine formation or elongation has been found in many aquatic organisms (Dodson 1989, Harvell 1991), we did not observe this for the *Desmodesmus* strains that we tested, which is corroborated by observations of Lürling and Beekman (1999) on *Desmodesmus* species. However, in mixed chemostats containing *B. calyciflorus* and *D. maximus* UTEX614, we observed an increase in mean cell size and spine length (data not shown), whereas this species did not respond in the bioassay. These effects could be caused directly by the zooplankton (e.g., selective grazing) or a nutrient effect. The possession of spines by *Desmodesmus* species may be a constitutive defense within this genus. Spines hamper ingestion and thus reduce the need for colony formation at low to moderate grazing intensities. On the other hand, the spineless *Scenedesmus* species are more vulnerable to grazing and may rely more on colony formation as inducible defense mechanism. Grazing experiments done with *B. calyciflorus* grazing on *S. obliquus* and *D. quadricauda* confirmed that the latter were less ingested and even less efficiently assimilated than *S. obliquus* (Verschoor et al. in press). Most *Desmodesmus* species predominantly appear as multicellular colonies with long spines and may thus be constitutively defended against grazing zooplankton. Spines should not be regarded as defensive traits only, however, because they may also serve to reduce sedimentation rates of the larger algal species (Conway and Trainor 1972).

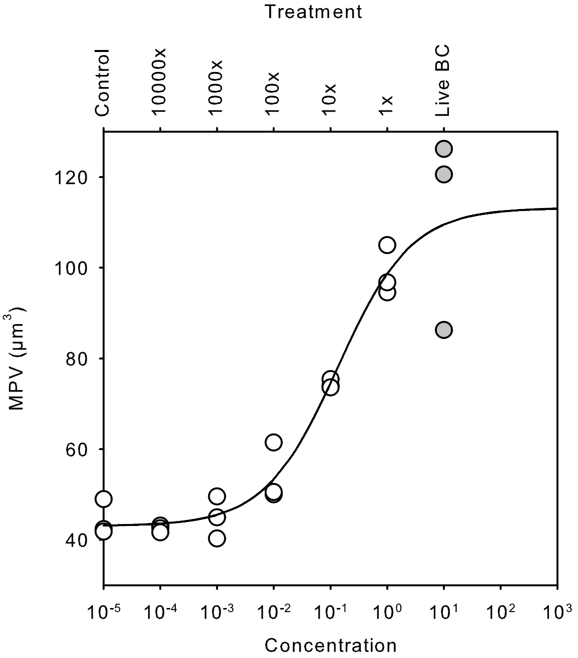


Figure 2.4 Colony size of *Scenedesmus obliquus* in response to *Brachionus calyciflorus* treatments. Open symbols: MPV of *S. obliquus* MPI in response to different dilutions of test water. Test water was produced from different dilutions of filtrate of grazing *B. calyciflorus* on *S. obliquus* (1x =1; 10,000x =10^{-4}) and controls (0). Filled circles: MPV of *S. obliquus* in the presence of live *B. calyciflorus* (Live BC). Densities in the live *B. calyciflorus* treatment were 100 individuals/mL, and the same density as was used in the culture to produce test water. Continuous line: logistic dose-response regression between dilutions of test water and MPV. Treatments are shown on the top axis, and dilutions for the regression on the bottom axis. To match the upper (categorical) scale with the lower (logarithmic) scale, controls (0) were plotted at 10^{-5} and live BC at 10^{1}.

The adaptivity of inducible colony formation

The MPV of *Scenedesmus obliquus* MPI (S9) increased with increasing *B. calyciflorus* infochemical concentration and was greatest in the presence of live *Brachionus* (Fig. 2.4). The analysis of variance yielded significant differences between dilutions ($F_{5,17}$=81.97, $P < 0.001$). A significant size increase only occurred above a certain threshold concentration. Contrast analysis showed that this threshold concentration was "100x" corresponding to a grazing pressure of 0.1 *B. calyciflorus*/mL, which is in agreement with the threshold concentration found by Van Donk et al. (1999) for this strain. All *S. obliquus* strains appear to have significant colony formation, even species that do not have a very large increase in size (e.g., UTEX79, S16 in Fig. 2.1). These species probably have a higher threshold concentration for colony formation. Moreover, both *D. subspicatus* and *S. obliquus* colony sizes increased with test water concentration (Figs. 2.3 and 2.4). This suggests that many other Scenedesmaceae, including the "non-inducible" strains such as *Desmodesmus* spp. discussed previously, may have higher threshold concentrations for colony formation. Below this threshold concentration algae have a canalized (non-inducible) phenotype, whereas above that threshold concentration the inducible phenotype develops. This response type has a polygenic basis, and the frequency of inducible phenotypes depends on selection on threshold dosage (Lively 1986).

In addition to selection on threshold concentration, there are physical, physiological, and ecological constraints to colony size. Colony size for *S. obliquus* never exceeded eight-celled coenobia (Fig. 2.2), and similar observations have been made for the other species. At very high infochemical concentration, the induced colony size will approach the maximum defense level, either as a rectangular hyperbola (Parejko and Dodson 1991) or as a sigmoid curve (Lively et al. 2000). A general mathematical formulation that captures both types of "saturating" curves is the logistic dose–response equation (Eq. 2.1), which becomes a rectangular hyperbola by setting the slope parameter b to 1. Fitting of the logistic dose–response curve (Fig. 2.4, solid line) yielded a significant regression ($F_{3,17} = 151.11$, $P < 0.001$, $r^2_{adj} = 0.97$), with $MPV_{min} = 43.0\ \mu m^3$, $a = 70.1\ \mu m^3$, $h = 0.135$, and $b = 0.674$. By summing MPV_{min} and a, we find that $MPV_{max} = 113.2$, which is within the range found for the "live BC" treatment. The description of bioassay data in logistic response model parameters allows an easier comparison across laboratories, for example by using half-maximum effect concentrations (h), similar to common practice in physiology or ecotoxicology (Colledge et al. 2000).

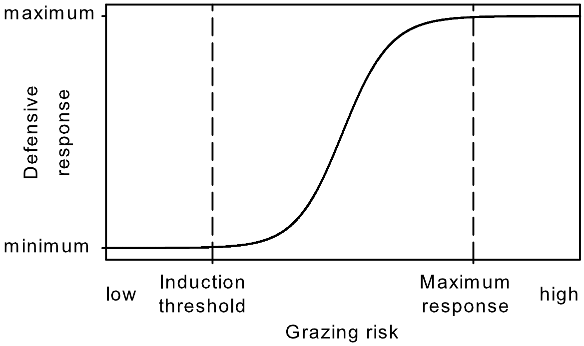
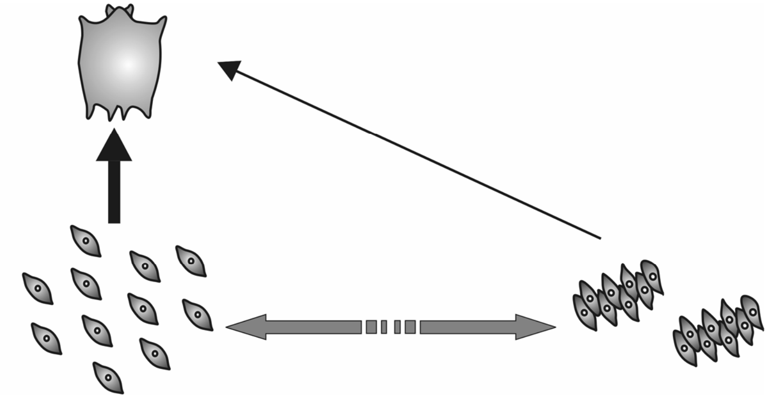


Figure 2.5 Relative strength of the defensive response in response to grazing risk. Going up from low to high grazing threat (infochemical concentration), defenses will only start to be induced at the induction threshold. From there on, the level of defense will increase until the maximum response concentration has been reached. Above that concentration, the defensive response will remain at maximum, regardless of the grazing threat.

The proportionality between infochemical concentration and colony size only applies above the infochemical threshold concentration (minimum colony size) and as long as the induced response is not “saturating” (approaching maximum colony size). The adaptivity of inducible colony formation is thus confined to a range where an increase in size is most beneficial (Fig. 2.5) and depends on the delicate balance between costs of colony formation (Lürling and Van Donk 2000, Verschoor et al. 2005) and costs and predictability of grazing losses. This balance is highly dependent on herbivore preferences. For example, size increase only lowers maximum ingestion rates of *B. calyciflorus* when MPV is above the optimum particle size (Rothhaupt 1990, Verschoor et al. in press). The continuous change in available resources, competitors, and enemies ensures that there is no single best strategy, but that continuous adaptation is essential. The variety of colony formation responses as we observed within the Scenedesmaceae is just one character in this complicated evolutionary play.

Conclusions

In the Scenedesmaceae, we observed similar colony formation responses in the same strains to infochemicals released by herbivores from two different taxa. This suggests that the Scenedesmaceae have strain-specific colony formation to some common cue of herbivory. The size of induced colonies increases with the concentration of grazing-released infochemicals but only within a range between a lower limit, namely the threshold concentration, and an upper limit, the maximum colony size.



Chapter 3

Linking herbivore-induced defenses to population dynamics

Abstract

Theoretical studies have shown that inducible defenses have the potential to affect population stability and persistence in bi- and tritrophic food chains. Experimental studies on such effects of prey defense strategies on the dynamics of predator-prey systems are still rare. We performed replicated population dynamics experiments using the herbivorous rotifer *Brachionus calyciflorus* and four strains of closely related algae that show different defense responses to this herbivore.

We observed herbivore populations to fluctuate at a higher frequency when feeding on small undefended algae. During these fluctuations minimum rotifer densities remained sufficiently high to ensure population persistence in all the replicates. The initial growth of rotifer populations in this treatment coincided with a sharp drop in algal density. Such a suppression of algae by herbivores was not observed in the other treatments, where algae were larger due to induced or permanent defenses. In these treatments we observed rotifer population densities to first rise and then decline. The herbivore went extinct in all replicates with large permanently defended algae. The frequency of herbivore extinctions was intermediate when algae had inducible defenses.

A variety of alternative mechanisms could explain differential herbivore persistence in the different defense treatments. Our analysis showed the density and fraction of highly edible algal particles to better explain herbivore persistence and extinctions than total algal density, the fraction of highly inedible food particles, or the accumulation of herbivore waste products or autotoxins.

We argue that the rotifers require a minimum fraction and density of edible food particles for maintenance and reproduction. We conjecture that induced defenses in algae may thus favor larger zooplankton species such as *Daphnia* spp. that are less sensitive to shifts in their food size spectrum, relative to smaller zooplankton species, such as rotifers and in this way contributes to the structuring of planktonic communities.

Van der Stap, I., M. Vos and W.M. Mooij. 2006 Linking herbivore-induced defences to population dynamics. Freshwater Biology 51(3): 424-434.

Introduction

Many species of plants and herbivores, both in aquatic and terrestrial ecosystems, have inducible defenses against their consumers (Karban and Baldwin 1997, Kats and Dill 1998, Tollrian and Harvell 1999). Such inducible defenses are predicted to have major effects on community dynamics, as has been pointed out in both theoretical (Abrams and Walters 1996, Edelstein-Keshet and Rausher 1989, Lundberg et al. 1994, Ramos-Jiliberto 2003, Underwood 1999, Vos et al. 2001, 2002, 2004a, 2004b) and empirical studies (Peacor and Werner 2000, Raimondi et al. 2000, Turner et al. 2000). Inducible defenses may also affect minimum population densities, and thus the persistence of cycling populations (Verschoor et al 2004b, Vos et al. 2002, 2004a).

Vos et al. (2004a) modeled a bitrophic (algae-rotifer) system and predicted inducible defenses to stabilize the trophic interaction under a wide range of conditions. In general, two different factors are important in determining consumer persistence and extinctions. First, when algal food levels are too low, herbivores will not be able to grow and reproduce sufficiently to maintain population growth. Secondly, when food levels are sufficient, but populations cycle, demographic stochasticity may cause extinctions when population densities are low in the troughs of such cycles. This risk was predicted to be higher when herbivores cycle with algae that have inducible or permanent defenses than with undefended algae (Vos et al. 2004a). This implies that induced defenses may have effects on persistence that work in different directions. On the one hand they promote local stability and thus persistence. On the other hand they may reduce the likelihood of consumer persistence when populations fluctuate, especially in comparison with a no defense scenario (Vos et al. 2004a: Fig. 4, top panel). In addition induced defenses may constrain the possibilities for herbivore persistence by decreasing the availability of highly edible food.

Few experimental studies have been designed to explicitly compare the effects of different defense strategies (inducible, permanent, no defenses) on population dynamics. Here we show the consequences of these different prey defense strategies for predator population dynamics and persistence using a freshwater planktonic system. In such systems particle size is a major determinant of algal edibility for herbivorous zooplankton. Particle size in colonial algae is a combination of single cell size and the number of cells per colony. The closely related genera *Scenedesmus* and *Desmodesmus* show considerable among-strain variation in particle size in the absence of herbivores. In some strains particle size does not respond to the presence of herbivores (e.g., *Daphnia* water fleas, *Brachionus* rotifers), while other strains are phenotypically plastic and respond with an increase in the number of cells per colony (Hessen and Van Donk 1993, Lampert et al. 1994, Lürling 1999b, Verschoor et al. 2004a). Such larger colonies are more difficult to handle for *Brachionus calyciflorus*, leading to reduced ingestion rates at high algal densities (Verschoor et al. in press). We refer to strains with small particle sizes as undefended, to those with fixed large particle sizes as permanently defended and to those that respond to herbivore presence as inducible.

Our main question here concerns the effects of algal defense strategies on herbivore persistence and patterns in population dynamics. The model analysis by Vos et al. (2004a) showed that induced defenses may stabilize population dynamics in a substantial part of parameter space. Under alternative conditions that cause populations to cycle, induced and permanent defenses may increase the probability of herbivore extinctions (Vos et al. 2004a). Interestingly, and in contrast with this latter prediction, one empirical study showed that induced defenses actually prevented large amplitude population fluctuations (Verschoor et al. 2004b). Such fluctuations occurred in the no defense treatment, where they led to the extinction of the top predator. Unfortunately, that study did not include a permanent defense treatment, nor a control to test algal dynamics in the absence of herbivores. Here we do include such treatments in our study of the effects of different algal defense strategies. We performed the experiments in replicated batch cultures, at one nutrient level. Thus our results and discussion are necessarily limited to this particular condition of the environment. However, it does allow us to test whether differences in defense strategies, at this point in parameter space, do lead to qualitatively different patterns in population dynamics.

Methods

Origin and selection of algal strains and rotifers

The various strains tested originated from different locations: *Desmodesmus subspicatus* (Chodat) Hegewald et Schmidt UTEX 2594 and *Scenedesmus obliquus* (Turpin) Kützing UTEX 2630 originate from the Culture Collection of Algae at the University of Texas, USA, *Scenedesmus obliquus* (Turpin) Kützing MPI from the Max Planck Institute for Limnology, Germany, while the *Desmodesmus quadricauda* (Turpin) Hegewald used has an unknown origin. We will refer to *D. subspicatus* as strain subspicatus, to *S. obliquus* MPI as strain obliquus I, to *S. obliquus* UTEX 2630 as strain obliquus II and to *D. quadricauda* as strain quadricauda. Occasionally algae will be referred to as plants.

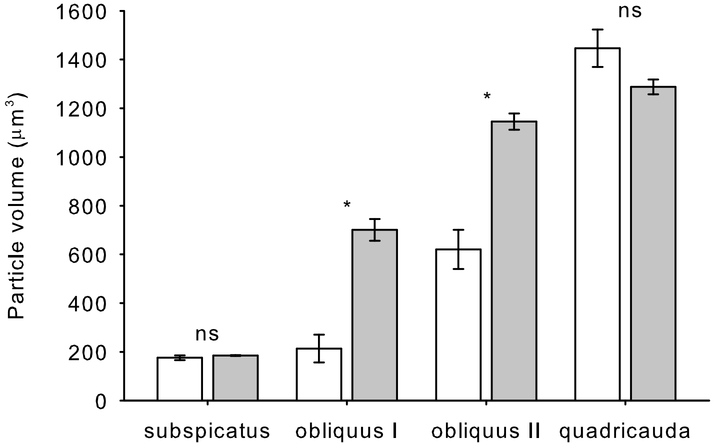


Figure 3.1 Particle volumes of four algal strains after four days in the bio-assay (mean ± SE). The treatment consisted of exposure to medium containing *Brachionus*-released infochemicals (grey bars). Algae in the controls received medium that had not contained this herbivorous rotifer (white bars). ns: no significant difference; * : significant difference ($P < 0.05$) between controls and treatments.

Scenedesmus and *Desmodesmus* species form a morphological diverse group of green algae. *Desmodesmus* used to be a subgenus of *Scenedesmus*, but was only recently placed into its own genus (Hegewald, 2000). The individual cells of these algae are elliptical to spindle-shaped and most *Desmodesmus* species bear spines. Individual strains of *Scenedesmus* can grow as unicells or can form colonies of two, four or eight cells. In cultures without herbivores they usually occur single-celled, but colonies may form under poor nutrient conditions or in the presence of grazers such as *Daphnia* or *Brachionus*. Cells of *Desmodesmus* may also vary with respect to the number and size of spines (for figures of the species used see Hessen and Van Donk 1993: Fig. 1 and Lürling and Beekman 1999: Fig. 4). These species proved to be good food for rotifers in many experimental studies without any signs of toxicity.

The selection of these four strains was based on a prior experiment in which 15 algal strains of the genera *Scenedesmus* and *Desmodesmus* were evaluated. These 15 strains were tested for the effect of *Brachionus* infochemicals on algal morphology in terms of particle volume and the number of cells per colony. Filtered water from a *Brachionus* culture (± 100 animals/mL) (10% v/v) was added to three replicates of 25 mL of algal solution per strain (WC medium, Guillard and Lorenzen 1972) and the cultures were incubated for four days at 20ºC under 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light in 50 mL glass Erlenmeyer flasks. Strain subspicatus remained small and did not show colony formation in response to “rotifer-water” in the bioassay (Fig. 3.1). Two obliquus strains clearly showed an increase in particle volume and the number of cells per colony. Strain obliquus II attained a slightly larger size than strain obliquus I. Strain quadricauda was large and colonial, both in the control and rotifer-water treatment. This difference started to become visible on day two, but was most pronounced on the fourth day of the bioassay. Thus we selected four algal strains that could be labeled as "undefended", "inducible", or "permanently defended", based on the bioassay.

The rotifer *Brachionus calyciflorus* Pallas was obtained from Microbiotests Inc., Belgium and was hatched from cysts. This *B. calyciflorus* strain was also used in the bioassays on basis of which the algal strains were selected. *Brachionus* spp. are cyclical parthenogens, whose populations are dominated by asexual females; males are non-feeding dwarf organisms. Hereafter, rotifer counts and densities refer to females. We will refer to *B. calyciflorus* as *Brachionus*.

Culture of the algal strains and rotifers

The algal strains were kept in batch in 100 mL Erlenmeyer flasks containing 50 mL WC medium. The algal cultures were put on fresh medium twice a week before the population dynamics experiment, to ensure that they were in the exponential growth phase at the beginning of the experiment. They were continuously illuminated from above at an irradiance of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 20ºC and put on a rotating shaking device (100 rpm) in an incubator (Gallenkamp). Rotifers were maintained in continuous culture at 20ºC using *Scenedesmus obliquus* MPI as food algae.

Population dynamics experiment

The experiment was performed using 24 cellulose-plugged 300 mL Erlenmeyer flasks containing 150 mL WC medium with vitamin B_{12} added (50 µg/L). We used a 4x2 factorial design with strain and presence or absence of *Brachionus* as the factors. All treatments were replicated three times. Flasks containing only algae were used as controls of algal growth in absence of the herbivore *Brachionus*. An inoculum of the algae was derived from the exponentially growing cultures. *Brachionus* were harvested from a continuous culture and inoculated at a concentration of 1 animal per mL two days after inoculation of the algae. At that day the total volume of the algae was between 2.1 and $4.1 \cdot 10^7$ μm^3/mL. The experimental cultures were continuously illuminated from above at an irradiance of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 20ºC and put on a shaking device (60 rpm) to reduce sedimentation of the algae. Every day 5 mL samples were taken. After sampling losses were not replenished. The mean particle volume (μm^3), density (number of particles per mL), and biovolume concentration (μm^3/mL) of the algae were determined in the size range of 4.0 to 25.0 µm equivalent spherical diameter (ESD) using a particle counter (CASY 1, Schärfe, Germany) fitted with a 60 µm capillary. Rotifer densities were measured by counting whole samples under a dissecting microscope. If the density of *Brachionus* in the cultures exceeded 100 individuals per sample, a sub-sample of 1 mL was counted. At the last day of the experiment the whole Erlenmeyer flask was checked for surviving rotifers if none were present in the sample.

Data analysis

Analysis of the controls without herbivores was carried out using ANOVA on the total algal biovolume concentration at the end of the experiment (averaged over days 15 to 18 for each replicate) and on the initial exponential growth rate during days 0-4. All of the described analyses were performed using Statistica 6.1.

To test the effect of the different algal strains on the population dynamics of both algae and *Brachionus* a repeated measures ANOVA was performed. To meet the assumptions of ANOVA algal data were log-transformed and *Brachionus* data were square root transformed. The strains were used as a between-samples factor and days in the experiment were used as a within-subject factor. The particle volumes and total volume concentration of the algal strains were averaged over the total duration of the experiment and the effect of the presence of the herbivore was tested using ANOVA. Prior to analysis data were tested for homogeneity of variances using Cochran's C test. Furthermore, data for repeated measures ANOVA were tested for sphericity using the Mauchley Sphericity test.

As a measure of population persistence we checked whether *Brachionus* was still present at the end of the experiment. We related extinction or persistence to the presence of population cycles and four other factors. 1) The cumulative build-up of *Brachionus* densities throughout the experiment as a potential source of autotoxins (Kirk, 1998), measured as the sum of *Brachionus* densities over 18 days, 2) the density of highly edible particles for *Brachionus* (4-7.5 µm ESD, Verschoor et al. in press) at day 18, 3) the fraction of highly edible food particles (4-7.5 / 4-25 µm ESD) at day 18, and 4) the fraction of highly inedible particles (15-25 / 4-25 µm ESD, Rothhaupt, 1990, Verschoor et al. in press) at day 18, as a potential measure for interference with feeding on preferred smaller food particles.

We performed binary logistic regressions with persistence of *Brachionus* (no or yes) as the response variable and different characteristics of the food as the independent variable. The significance of these regressions was evaluated in relation to a null model, using a chi-square test. The null model assumed a probability of persistence of 50%, which is the value we observed in the experiment when combining all treatments.

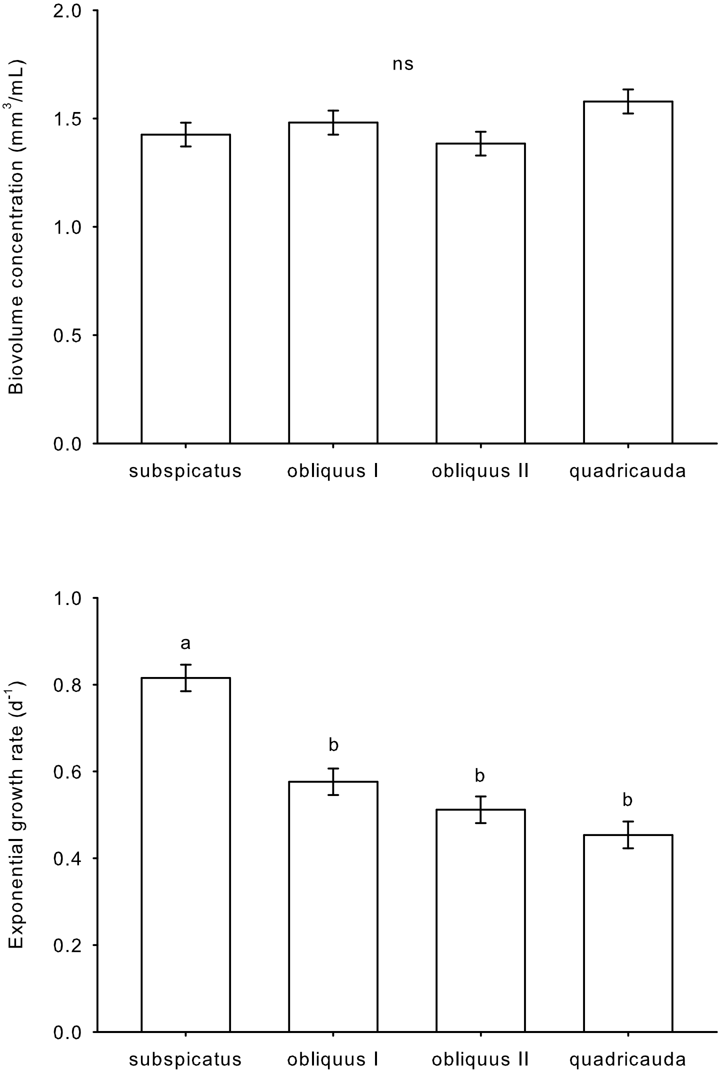


Figure 3.2 Top panel: Total biovolume concentration of the algae in absence of the herbivore (mean ± SE) between 15-18 days. ns: no significant differences among algal strains. Lower panel: The initial biovolume growth rate of the algae in absence of the herbivore (mean ± SE) between 0-4 days. Different letters on bars indicate significant differences among algal strains (Tukey hsd test after ANOVA).

Table 3.1 Results of the analysis of variance for repeated measures showing the effect of time and strain and the interaction between time and strain on both algal and rotifer population dynamics.

	MS	df	Error	df	*F*	*P*
Algal density						
Strain	2.9	3	0.41	8	7.0	<0.05
Time	9.0	18	0.069	144	130.2	<0.001
Time x Strain	0.41	54	0.069	144	6.9	<0.001
Brachionus density						
Strain	70.4	3	8.98	8	7.8	<0.01
Time	184.4	18	1.97	144	93.5	<0.001
Time x Strain	25.6	54	1.97	144	13.0	<0.001

Results

Controls: plants without herbivores

The densities (particle concentrations) reached by algae in the absence of herbivores differed between strains, with subspicatus attaining the highest density and the highest growth rate, followed by the inducible strains obliquus I and obliquus II, while strain quadricauda had the lowest density and growth rate. The algal strains did not differ in biovolume concentration at the end of the experiment (ANOVA for day 15-18, $P = 0.15$, Fig. 3.2). This indicates that all algal strains reached the same carrying capacity. Initial exponential growth rates differed significantly between strains (ANOVA for day 0-4, $P < 0.001$, Fig. 3.2). This was caused by a higher growth rate of the undefended strain subspicatus (Tukey hsd test $P < 0.01$) while no differences were observed among the other three strains (Tukey hsd test $P > 0.05$).

Plant-herbivore dynamics

Patterns in population dynamics were highly replicable within algal strains. A clear contrast in the pattern of population dynamics was observed between the treatments with and without defenses in algae (Fig. 3.3). A repeated measures ANOVA showed a significant effect of time and strain and a significant interaction between time and strain on both total algal biovolume concentration and the densities of *Brachionus* herbivores (Table 3.1). The particle volumes of the algal strains averaged over the total duration of the experiment were larger in strain subspicatus, obliquus I and obliquus II in the presence of the herbivore *Brachionus* than in the absence of this herbivore (ANOVA, $P < 0.001$, $P < 0.01$, and $P < 0.001$ respectively, Fig. 3.4). In strain quadricauda no significant difference was

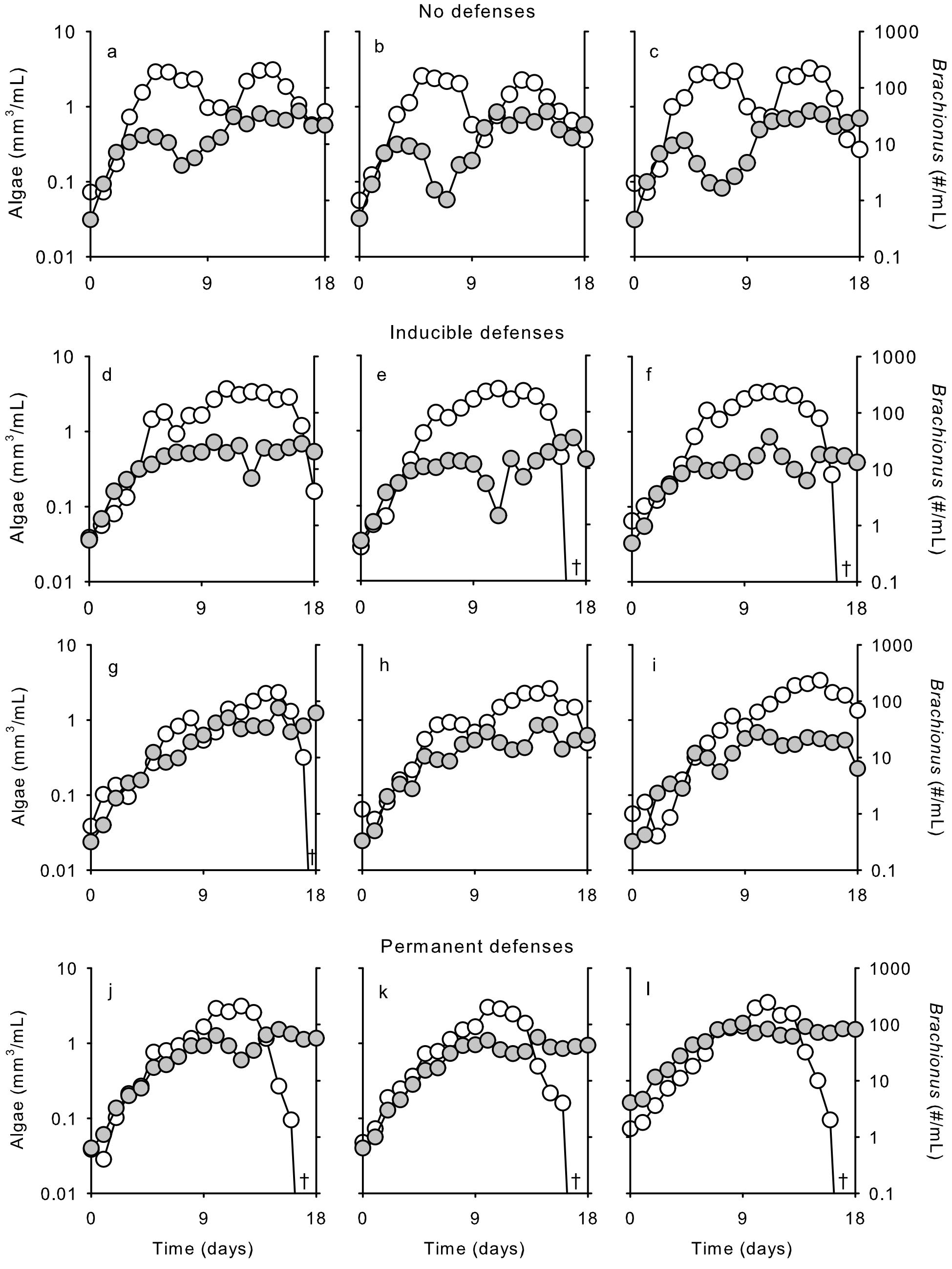


Figure 3.3 Population dynamics of the herbivorous rotifer *Brachionus calyciflorus* and the algae *Desmodesmus subspicatus* (a-c), *Scenedesmus obliquus* I (d-f), *Scenedesmus obliquus* II (g-i), and *Desmodesmus quadricauda* (j-l), showing three replicates. The left axis is used for the density of algal volumes (filled circles), the right axis for the density of herbivores (open circles). The † symbol indicates extinction of the herbivore.

observed between the particle volumes of the algae in the controls and in the presence of *Brachionus* (ANOVA, $P = 0.13$). Maximum *Brachionus* densities, when feeding on strain quadricauda, were about equal to the other treatments. A comparison of average biovolume concentrations between controls without and treatments with *Brachionus* showed a significant grazing effect on all algal strains (Fig. 3.4). The grazing effect was strongest on strains subspicatus and obliquus I (ANOVA, $P < 0.001$) and seemed weaker on strains obliquus II and quadricauda (ANOVA, $P < 0.05$).

Population fluctuations

To gain a more mechanistic insight in these differences in relation to variation in algal defenses, we focused on two aspects of predator-prey dynamics: the occurrence and type of predator population fluctuations, and the presence or absence of *Brachionus* extinctions. We observed distinct predator population fluctuations in the treatment where *Brachionus* was feeding on undefended subspicatus. *Brachionus* initially clearly suppressed algal densities in this undefended strain. Two marked peaks in *Brachionus* densities occurred in all three replicates of this treatment, while minimum rotifer densities remained rather high (Fig. 3.3a-c). Thus *Brachionus* populations faced a minimal risk of extinction under demographic stochasticity in this treatment. This pattern was not observed in the other treatments. *Brachionus* densities in both inducible and permanently defended treatments first increased and then declined towards the end of the experiment.

Persistence of *Brachionus*

The herbivore persisted throughout the experiment in all three replicates on the undefended strain subspicatus (Fig. 3.3a-c). The herbivore went extinct in two out of three replicates with inducible strain obliquus I (Fig. 3.3d-f) and in one out of three replicates with inducible strain obliquus II (Fig. 3.3g-i). The herbivore went extinct in all three replicates of the permanently defended strain quadricauda. Interestingly, the decline leading to the extinction of the herbivore started earlier in this permanently defended strain (Fig. 3.3j-l). Extinctions of *Brachionus* were not related to the occurrence of population fluctuations per se (Fig. 3.3a-c).

Discussion

We conducted this study to experimentally test the effects of prey size and prey defense strategies on the dynamics of predator-prey systems. We showed clear differences in the population dynamics of an herbivorous rotifer when feeding on four strains of algae that showed no defenses, inducible defenses and permanent defenses. Patterns in population dynamical time series were highly replicable within prey strains and significantly different between prey strains. The patterns for the undefended strains were fundamentally different from the patterns for the strains with permanent and inducible defenses. The fact that algal carrying capacities, in terms of biovolume concentration, were equal in the controls, show that we are really dealing with differences due to the quality or size of those algae. Particle densities differed, but biovolume concentrations were equal, implying that the total volume of the algae was packaged in very different ways among algal strains.

In a recent paper, Vos et al. (2004a) made predictions for population level effects of different defense strategies using an extended version of the Rosenzweig-MacArthur model with a focus on stability and persistence. Their model analysis clearly showed that enriched bi- and tritrophic food chains with induced defenses were less likely to show population oscillations than systems with fixed or no defenses. Interestingly, Verschoor et al. (2004b) subsequently showed experimentally that bi- and tritrophic food chains with induced defenses approached a stable equilibrium without any oscillatory tendency, while those without defenses in algae showed high amplitude population fluctuations. The experiments by Verschoor et al. (2004b) were conducted using semi-continuous systems, while those in the present paper were performed using batch systems. This difference in flow-through caused some differences in dynamics between these systems. However, an oscillatory tendency was clearly present in the dynamics of both systems when algae were undefended. A clear advantage of the present study is the inclusion of controls. These provide insight in algal dynamics in the absence of herbivores, showing that the undefended algal strain subspicatus has a higher growth rate than the other strains. This raises an alternative hypothesis for the observed differential dynamics between treatments with and without defenses, i.e. algal growth rate itself. This is possible, but further analysis using the model of Vos et al. (2004a, 2004b) showed that a difference in growth rate between algal strains (of the observed magnitude) is unlikely to cause such a difference in stability in this plant-herbivore system (see Verschoor et al. 2004b).

The particle volumes of the algal strain in the population dynamics experiment (Fig. 3.4) differed from those in the bioassay (Fig. 3.1). Particle volumes in the presence of live *Brachionus* are due to a combined effect of both inducibility and grazing by the herbivore. We observed a doubling of mean particle volume in strain subspicatus (*D. subspicatus* UTEX 2594) in presence of the herbivore *Brachionus* (Fig. 3.4). In previous bioassays no significant responses in this strain were observed, neither in response to *Brachionus* nor *Daphnia* infochemicals (Verschoor et al. 2004a). Another subspicatus strain (*D. subspicatus* NIVA-CHL

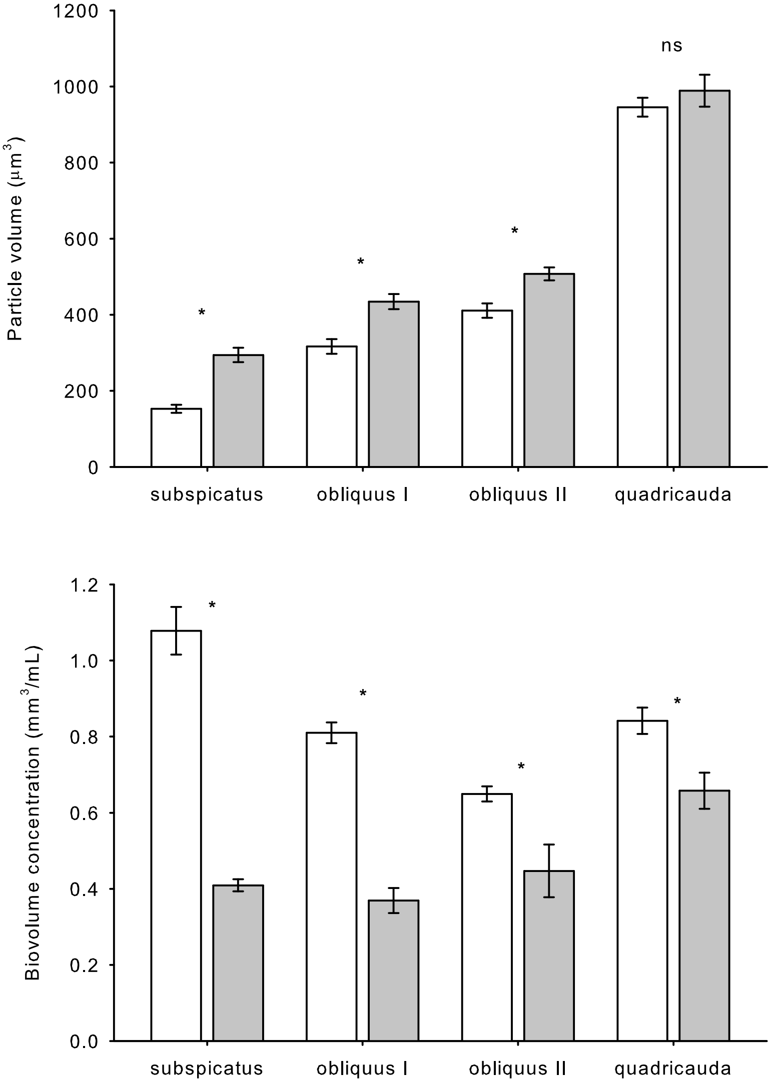


Figure 3.4 Top panel: Particle volumes of four algal strains in the population dynamics experiment. Lower panel: Total biovolume concentration of the algal strains. Shown are means ± SE averaged over the total duration of the population dynamics experiment in the presence of the herbivore *Brachionus* (grey bars) or in the controls in absence of this herbivorous rotifer (white bars). ns: no significant difference; * : significant difference ($P <$ 0.05) between controls and treatments.

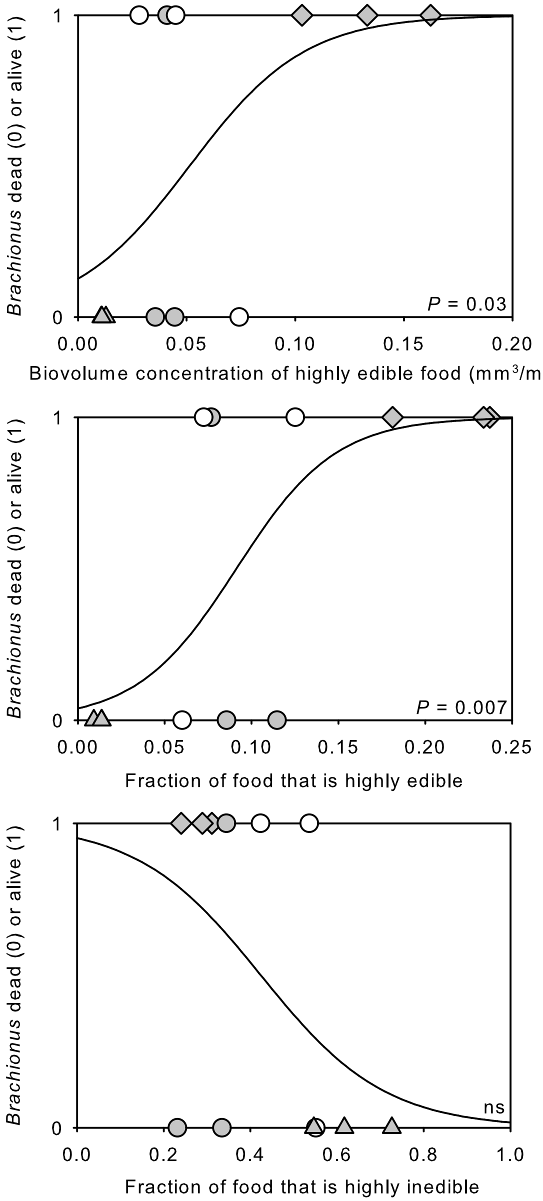


Figure 3.5 Extinction or persistence of the herbivore *Brachionus* at the end of the experiment related to (top panel) the density of food algae that is highly edible, with sizes of 4-7.5 µm ESD, (middle panel) the fraction of food algae that is highly edible, 4-7.5 / 4-25 µm ESD, and (lower panel) the fraction of food algae that is highly inedible, 15-25 / 4-25 µm ESD in the treatments: undefended algae (*Desmodesmus subspicatus*, filled diamonds); inducible algae (*Scenedesmus obliquus* I, filled circles and *Scenedesmus obliquus* II, open circles); permanently defended algae (*Desmodesmus quadricauda*, filled triangles). The line represents the logit regression curve.

55) investigated by Hessen and Van Donk (1993) did show a response to *Daphnia*. Verschoor et al. (2004a) reported a doubling in final colony size in *D. subspicatus* NIVA-CHL 55 if undiluted *Brachionus*-water was added. The increased particle volume of *D. subspicatus* UTEX 2594 in our experiment in the presence of live *Brachionus* fully supports the idea that *D. subspicatus* requires a higher infochemical concentration before colonies are induced. Even in its induced state our strain of subspicatus was still as small as, or smaller than, non-induced obliquus I and II, i.e., than these inducible algae in their undefended state. Thus this strain is still the most edible of the ones studied here.

We were intrigued by the herbivore population crashes in the induced and permanent defense treatments in the batch experiments and evaluated several hypotheses that could explain the observed *Brachionus* extinctions. First of all, we were surprised that herbivores persisted in all the replicates with small undefended algae. The observed population fluctuations did not result in dangerously low herbivore densities. In contrast, herbivore extinctions occurred in all replicates with large permanently defended algae and when algae had inducible defenses. This led us to examine the potential effect of different aspects of food quality (in terms of defenses) on the probability of herbivore extinction. In particular, we related the extinction or persistence of *Brachionus* at the end of the experiment to four factors that are likely to affect the growth of rotifer populations and thus population persistence.

First, the cumulative density of *Brachionus* is a measure for the build-up of herbivore waste products or autotoxins. This cumulative build-up of *Brachionus* densities during the course of the experiment remained low on permanently defended algae and was very high on undefended algae. Since this high cumulative rotifer density in the subspicatus treatment did not co-occur with negative effects on growth rate or persistence, such metabolic waste products or autotoxins are highly unlikely to have been responsible for extinctions in these experiments. Thus we could exclude a density dependent effect of waste products or autotoxins building up during the experiment.

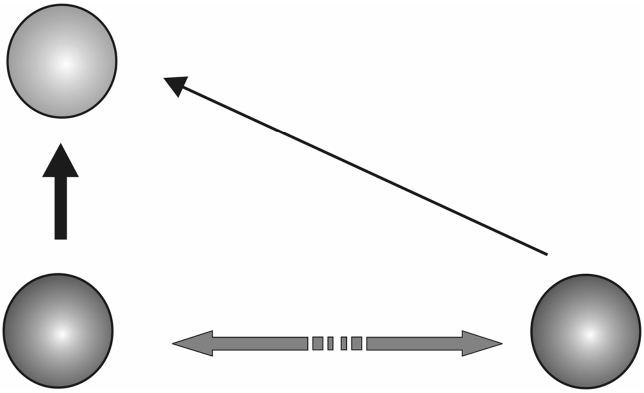
Secondly, total algal densities were high enough to support initial herbivore growth in all defense treatments and did not decrease below the algal densities at inoculation. Therefore it seems unlikely that extinction occurred because of a low food level. The occurrence of herbivore persistence and extinctions was clearly related to the density of highly edible algae, which was high in the no defense treatment and low in the permanent defense treatment (Fig. 3.5, top panel). Algae with inducible defenses took an intermediate position. The density of highly edible small particles was very low in strain quadricauda and *Brachionus* went extinct in all replicates of this treatment. In strain subspicatus this density of highly edible food particles was much larger and the herbivores were alive in all replicates at the end of the experiment. Strains obliquus I and obliquus II were intermediate in the density of small particles and extinctions seemed to be stochastic. We performed a logit regression with persistence of *Brachionus* as the binary response variable (0 for extinction and 1 for persistence, on day 18) and the density of small, highly edible food particles on day 18 as the independent variable. The estimated model showed a significantly better fit to the data than the null model ($\chi^2 = 4.69$, $P = 0.03$).

Thirdly, the algae showed an increase in size as an induced response to the presence of the herbivore and this aspect was reflected in the fraction of small algal particles over the total available algal biovolume concentration. The fraction of highly edible algal particles showed an even more explicit relationship with the probability of persistence than the density of these particles. At the lowest fractions of highly edible algal particles herbivores went extinct in all replicates of quadricauda and one of obliquus, while they persisted at the highest fractions of highly edible algal particles in one replicate of obliquus and all replicates of subspicatus (Fig. 3.5, middle panel). The estimated model yielded a significantly better fit to the data than the null model ($\chi^2 = 7.37$, $P = 0.007$). While the density of these small particles already provided a sufficient explanation for the observed persistence and extinctions, the logit regression of the fraction of highly edible small particles indicated an even better fit to the occurrence of persistence and extinctions. Therefore the chance of persistence at the end of the experiment was positively related to the availability of optimally sized food algae (the larger the density of small, edible particles in relation to total algal availability, the larger the chance of persistence).

Fourthly, the reverse relation was expected for the fraction of very large, inedible particles: a high fraction of these could interfere with feeding of the rotifers on the smaller particles, because of pseudotrochal screening, that reduces ingestion rates (Gilbert and Starkweather 1977, 1978). This fraction of very large particles was small for the permanently undefended algae and large for the permanently defended algae. Extinctions occurred both at high and low fractions of these large particles, especially in the case of inducible algae (Fig. 3.5, lower panel). The estimated model did not yield a significantly better fit to the data than the null model ($\chi^2 = 2.78$, $P = 0.09$). The above implies that the fraction of small, highly edible particles is an important factor for persistence of the herbivore and that if algae attain a size which is slightly larger than the optimal size of 4-7.5 μm ESD this may already have negative effects on herbivore population densities.

Our experimental design did not include a wide range of algal species or strains with a fixed size, nor a range of algae with inducible defenses that may attain different sizes. However, our understanding of the effects of inducible defenses on population dynamics has improved considerably through the experimental results presented here. Incorporating a wider range of algal strains in future experiments, as well as adding combinations of different nutrient levels and herbivore mortality rates (see Vos et al. 2004a: Figs. 2 and 3), could further enhance our understanding of the relative effects of prey size and defense inducibility on the dynamics of such predators and prey.

Our results indicate that algal defenses reduced the fraction of highly edible food particles in these experiments, and thus increased the total algal concentration needed by herbivorous rotifers to maintain constant population densities. From our data we conclude that food particles in the less preferred intermediate size range could interfere with the uptake of highly edible food particles by these rotifers. We conjecture that induced defenses in algae may thus favor larger zooplankton species such as *Daphnia* spp., that are less sensitive to shifts in their food size spectrum, relative to smaller zooplankton species, such as rotifers. This difference between daphnids and rotifers could be one of the ways inducible defenses affect the structure of freshwater communities.



Chapter 4

From inducible defenses to predator-prey dynamics: linking models and experimental data

Abstract

Theory on inducible defenses predicts these to have stabilizing effects. In the present study we link data and theory on inducible defenses by fitting a mechanistic predator-prey model to empirical data. We evaluate predictions about the effects of inducible defenses on stability and on the risk of predator extinctions. The data originated from batch experiments with a rotifer species as the predator and four strains of algae that differed in their defenses as prey. Previous statistical analysis showed that predator persistence was significantly related to the density and fraction of edible algae. The present mechanistic model in which inducible defenses were incorporated accurately described the observed population dynamics, as opposed to a model without defended species. The parameter values we obtained were fed back into a version of the model in which nutrients were not depleted. This allowed a projection of the long-term consequences of induced defenses for predator-prey dynamics. The analysis predicts stability for a broad range of inducible changes in algae. It also shows that quantitative variation in handling times of differently defended prey place predators at distinct locations in the stability area with different risks of stochastic extinction.

Van der Stap, I., M. Vos and W.M. Mooij. From inducible defenses to predator-prey dynamics: linking models and experimental data. Submitted.

Introduction

A central theme in the study of predator-prey systems is the prediction that enrichment will destabilize trophic interactions and lead to high amplitude population cycles that entail a risk of local extinctions for both predator and prey. Several laboratory studies have shown such population oscillations and extinctions (Fussmann et al. 2000, Huffaker et al. 1963, Luckinbill 1973). In contrast, at low carrying capacities or high predator mortalities the predator faces deterministic extinction, because food supplies are not sufficient to allow births to compensate for losses. In between these two extremes of carrying capacity, theory predicts stable coexistence of predator and prey. According to classical models, however, such stable coexistence only occurs for a narrow range of predator mortalities, especially at high carrying capacities. However, communities can be stable for long periods of time in nature (reviewed in DiMichele et al. 2004), and mechanisms that influence community stability have therefore been the subject of intense study (Abrams and Walters 1996, Armstrong 1976, Fussmann et al. 2000, Luckinbill 1973, McCann et al. 1998, McCauley and Murdoch 1987, Rosenzweig 1971, Rosenzweig 1973, Rosenzweig and MacArthur 1963, Schaffer and Rosenzweig 1978, Vos et al. 2001, Yodzis 1981).

A recent model study predicted that inducible defenses can stabilize predator-prey systems (Vos et al. 2004a). Such inducible defenses occur in a wide range of ecosystems and have been shown and studied in hundreds of species of animals and plants (Havel 1987, Karban and Baldwin 1997, Kats and Dill 1998, Tollrian and Harvell 1999). The analysis of Vos et al. (2004a) provided a simple and intuitive explanation as to why inducible defenses should stabilize predator-prey interactions. In an extended version of the Rosenzweig-MacArthur model (1963) two prey types are distinguished: an undefended type and a defended type. At low consumer densities prey will be undefended and edible, thus enhancing predator persistence. At high consumer densities the prey is protected from overexploitation through their defenses. These defenses may result in longer handling times, lower attack rates or both. To see how inducible defenses affect stability Vos et al. (2004a) focused on two boundaries: the transcritical bifurcation or existence boundary, which is the point where the predator is just able to maintain itself at very low densities, and the Hopf bifurcation or stability boundary, the point where exploitation becomes so strong that cycles ensue. In the classical Rosenzweig-MacArthur model the existence and stability boundaries converge at high carrying capacities, leaving little room for stable coexistence. However, the analysis of Vos et al. (2004a) showed that in the case of inducible defenses the two boundaries stay apart, thus allowing coexistence. The model system with inducible defenses has the existence boundary of the undefended prey type, and approximately the stability boundary of the defended prey type. This creates a considerable range of conditions that result in stable predator-prey dynamics, even at high carrying capacities.

To test these model predictions Verschoor et al. (2004a) used semi-continuous cultures in an algae-rotifer system. They showed that the presence of inducible defenses prevented large population fluctuations. Van der Stap et al. (2006) obtained experimental data on the effects of inducible defenses during batch experiments using a comparable algae-rotifer system. This study showed that predator persistence could be related to the defense strategy of the four prey strains used. The statistical analysis of time series by Verschoor et al. (2004) and Van der Stap et al. (2006) provided a first empirical test of the claim that inducible defenses promote stability of these experimental bi- and tritrophic food chains.

In the present study we provide a quantitive link between theory and data in addition to the above mentioned qualitative analyses. Until recently, the integration of quantitative theory and experiments in ecology was often rather weak (Hall 1988, Ives and Agrawal 2005). In the last decade, however, the application of mechanistic models to experimental predator-prey systems has increased (e.g., Kooi and Kooijman 1994, Shertzer et al. 2002, Van Veen et al. 2005). Compared to statistical approaches, the advantage of applying mechanistic models to empirical data is that each of the parameters of the model can be ecologically interpreted in its own right. These parameters include the maximum specific growth rates, attack rates, handling times, conversion efficiencies and mortality rates. If model fits are applied to experimental data that test important ecological hypotheses and concepts, this acts to strengthen the link between theory and data.

In this paper we analyze the batch experiments performed by Van der Stap et al. (2006) on algae-rotifer interactions that tested the effects of algal defenses on population dynamics. In the current analysis we use a mechanistic model suitable for application to the transient dynamics in a batch experiment. Data from these transient dynamics provide more information on the growth, induction and mortality rates than data from equilibrium conditions. Initially, the model did not take inducible defenses into account (Table 4.1, parameter set 0). The model without such shifts in prey size showed a very poor fit (Fig. 4.1). Then, inducible defenses were incorporated and the model was run for a set of a priori defined parameters, followed by a stepwise procedure to replace some of these initial values with best fitting estimates. The significance of the differences of the obtained parameters between algal strains was tested at each step using the replication in the experimental design. Our analysis stresses the importance of explicitly including phenotypic plasticity in prey size for understanding the observed population dynamics. All four algal strains showed such variation in their particle volumes, the factor that affects their handling by predators, but they did so at different levels.

Methods

The experimental data

The experimental data were collected by Van der Stap et al. (2006). The experiments were performed in a batch system using four strains of Scenedesmaceae that differ in their response to herbivore presence. *Desmodesmus subspicatus* UTEX 2594 shows only minor changes in mean particle volume in response to grazing and remains small and edible. Two *Scenedesmus obliquus* strains clearly show an increase in particle volume and the number of cells per colony. *Scenedesmus obliquus* UTEX 2630 attain a slightly larger size than *Scenedesmus obliquus* MPI. The clear response in size in these two strains made us characterize them as inducibly defended. *Desmodesmus quadricauda* is large and colonial, both in the control without predators and in the presence of predators. The experiments were run in a 4x2 factorial design with algal strain and presence or absence of the herbivore *Brachionus calyciflorus* as the factors. Flasks containing only algae were used as controls, showing algal growth in absence of the herbivore. See Van der Stap et al. (2006) for further details. All data were converted to carbon using a carbon to biovolume ratio of 0.32 mg C/mm^3 measured for the algae (Verschoor et al. 2004b) and a conversion of 0.0001 mg C per individual for *B. calyciflorus* (Rothhaupt 1993).

The model

The autonomous prey growth in predator-prey models is often described with a logistic term, which implies a constant tendency of regrowth to a fixed carrying capacity. However, such a formulation is not suitable to model data from experiments in which nutrient depletion progressively limits algal growth during the course of an experiment. We therefore replaced the logistic term with a Monod (1949) type term for nutrient-limited algal growth and included a differential equation for the nutrients. We followed the approach taken by DeAngelis (1992) of modeling nutrient-algae interactions with no particular nutrient in mind. To simplify things further, we expressed the limiting nutrient in the model in units of carbon. Consequently, the initial nutrient concentration in the model directly represents the maximum biomass of algae that can be produced. Nutrients may become available to the algae due to nutrient cycling but we assumed that this process can be ignored at the time scale of the experiments. In a way corresponding with the model of Vos et al. (2004b) inducible defenses were incorporated in the predator-prey equations by allowing a predator density-dependent flow from the undefended part of the prey population to the defended part. Similarly, decay of defenses is included as a predator density-dependent flow from the defended to the undefended part. We could ignore algal losses due to sedimentation because the experiments were done on rotating tables. This results in the following set of differential equations for the hypothetical nutrient N, the undefended fraction of algae A_1, the defended fraction of algae A_2 and the predator B:

$$\frac{dN}{dt} = -\frac{r_A(A_1 + A_2)}{h_N + N}N$$

$$\frac{dA_1}{dt} = \left[c_{NA}\frac{r_A N}{h_N + N} - \frac{a_{A1}B}{1 + a_{A1}h_{A1}A_1 + a_{A2}h_{A2}A_2} - \frac{i_A B^b}{h_I{}^b + B^b}\right]A_1 + \frac{d_A h_I{}^b}{h_I{}^b + B^b}A_2$$

$$\frac{dA_2}{dt} = \left[c_{NA}\frac{r_A N}{h_N + N} - \frac{a_{A2}B}{1 + a_{A1}h_{A1}A_1 + a_{A2}h_{A2}A_2} - \frac{d_A h_I{}^b}{h_I{}^b + B^b}\right]A_2 + \frac{i_A B^b}{h_I{}^b + B^b}A_1$$

$$\frac{dB}{dt} = \left[c_{AB}\frac{a_{A1}A_1 + a_{A2}A_2}{1 + a_{A1}h_{A1}A_1 + a_{A2}h_{A2}A_2} - d_B\right]B$$

The model has 13 parameters (see table 4.1), which is six more than a comparable model without inducible defenses. These include the handling time (h_{A2}) and attack rate (a_{A2}) on the defended form plus the four parameters that govern induction and decay of defenses (i_A, d_A, h_I, b). Note that the model assumes A_1 and A_2 to have the same autonomous growth rate. A notation different from Vos et al. (2004b) for induction and decay functions is used without any consequences for the model.

Linking data and model

We fitted the model by minimizing the percentage of unexplained variance between model and data. To give the algal and *Brachionus* data equal fit we scaled both sets of observations so that the total variance in the scaled data was one for each set. The observed and predicted densities were square root transformed to

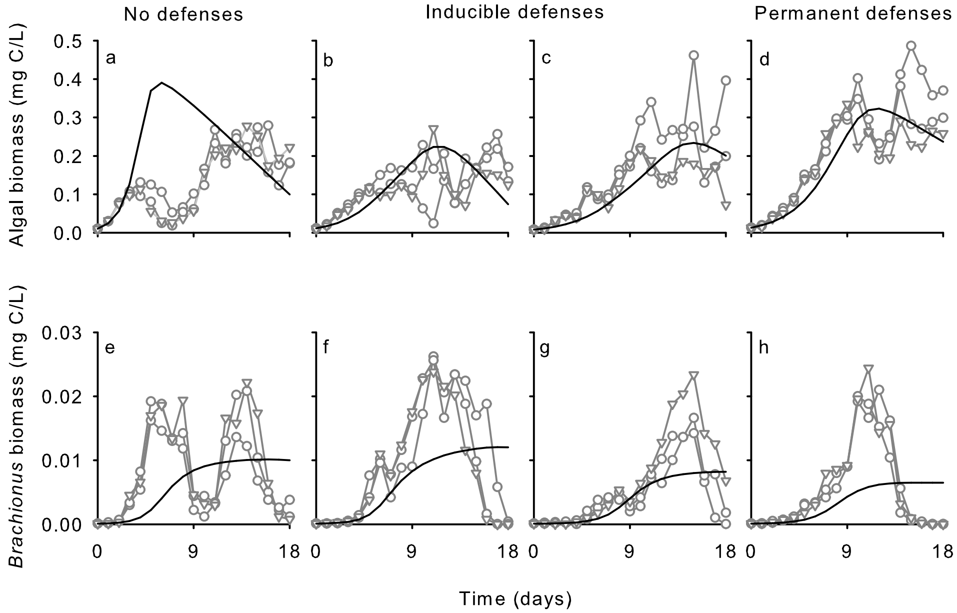


Figure 4.1 Fit of the model in which values specific to the batch culture system were used, but inducible defenses were not incorporated in the model (parameter set 0) to the observed data for algal biomass of *D. subspicatus* (a), *S. obliquus* MPI (b), *S. obliquus* UTEX 2630 (c), *D. quadricauda* (d) and for *Brachionus* biomass (e-h). Shown are each of the three replicates from the experiment (data points connected with lines) and the model mean (line).

make the variances independent of the means. We used the downhill simplex method in multi-dimensions (Press et al. 1992) to estimate the parameters that minimized the squared deviations between model and data assuming measurement errors to be the dominant source of noise in the data. The simplex method is simple in its application because it only requires evaluations of the function that is to be optimized, not derivatives of that function with respect to each of the parameters. As a trade-off it is less efficient in terms of the number of model evaluations that it requires.

Results

The model fit

We estimated an initial nutrient concentration of 0.41 and algal growth rates r_A of the strains in the range of 0.56 to 1.34 (Table 4.1) on basis of the control experiments with only algae. Within a broad range, h_N hardly affected the fit, because this parameter only affects the model results during the short transition period from exponential to nutrient limited growth. Therefore h_N was arbitrarily fixed at half the initial nutrient concentration in all analyses. The total variance in the predator-prey experiment was 0.0197 for the transformed algal data and 0.0230 for the transformed *Brachionus* data. Applying the parameters for the model without inducible defenses (Table 4.1, set 0) to the batch data resulted in a very poor fit (Fig. 4.1). The modeled algal abundances for *D. subspicatus* were higher than observed. The modeled herbivore densities showed a too high maximum for *S. obliquus* MPI and were too low for the other algal strains half-way and too high at the end. Apparently, for these algal strains the handling times of *Brachionus* in the experiment were lower than assumed in the model. The inclusion of inducible defenses in the model resulted in a much better fit (Table 4.1, set 1). However, clear discrepancies remained between model and data (figure not shown). In particular, the model showed a rather poor fit to the fluctuations in the herbivore populations feeding on *D. subspicatus*. This implies that the modeled interaction between *Brachionus* and *D. subspicatus* was not strong enough. Furthermore, the modeled *Brachionus* densities were still too low half-way and too high at the end.

We explored two approaches to capture these dynamics. First, the ratio between the handling time of undefended and defended algae was optimized (Table 4.1, set 2). Second, we optimized the induction and decay rates (Table 4.1, set 3). Both led to slightly improved fits, i.e., lower unexplained variances, but in qualitative terms did not solve the discrepancies mentioned above. When we optimized both the ratio in handling time between undefended and defended prey and the induction rates from the data, however, the modeled patterns showed a

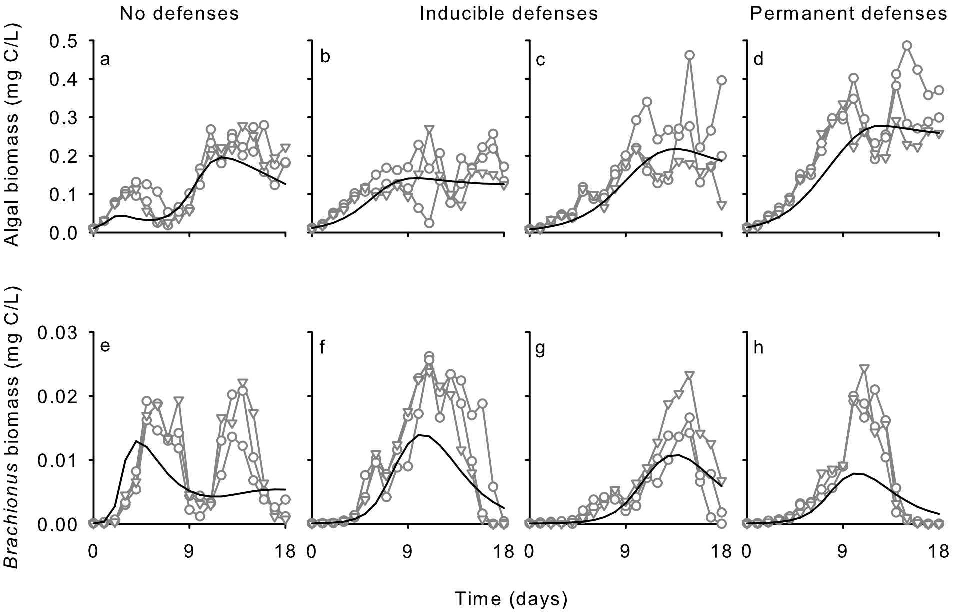


Figure 4.2 Fit of the model in which both the ratio in handling time between undefended and defended prey and the induction rates were estimated from the data (parameter set 5) to the observed data for algal biomass of *D. subspicatus* (a), *S. obliquus* MPI (b), *S. obliquus* UTEX 2630 (c), *D. quadricauda* (d) and for *Brachionus* biomass (e-h). Shown are each of the three replicates from the experiment (data points connected with lines) and the model mean (line).

good fit to the observed data (Fig. 4.2) and the unexplained variance was reduced from 75% to 33% of the total variance (Table 4.1, set 4). Estimating decay rate separately from the induction rate (Table 4.1, set 5) resulted in such a small improvement of the fit that we considered the model in which both the handling time ratio and induction rates were optimized (set 4) the best fitting model.

The resulting parameters

Our analysis resulted in five sets of parameters of the inducible defenses model in addition to the original parameter set used. Because the experiments were replicated, we could perform statistical tests for all parameters that were estimated per replicate: handling time of the edible fraction (sets 1-5), induction rate (sets 1, 2, 4, 5) and decay rate (set 5). Using MANOVA, we found significant differences between the defense treatments for each of the five parameter sets as a whole. Using ANOVA, we zoomed in on each of the parameters per set. Significant differences were found for all parameters per set except for two marginally significant results for handling time in parameter set 2 ($P = 0.075$), and induction rate in parameter set 5 ($P = 0.053$). This indicates that the differences between defense treatments are well reflected in each of the parameter sets and that we can ecologically interpret these differences by inspecting these parameters.

The highest values of handling times were observed for *S. obliquus* UTEX 2630 and *D. quadricauda* (0.198-0.230) and somewhat lower values for *S. obliquus* MPI (0.164-0.201) in all parameter sets. The handling time of *D. subspicatus* varied considerably with increasing fit of the model. In the best fitting parameter sets 4 and 5 the handling time of *D. subspicatus* had a value of 0.073. In the parameter set in which only the induction rate was estimated from the data (set 3), we obtained values of 0.370-0.663 for strains *S. obliquus* MPI, UTEX 2630 and *D. quadricauda* and a higher value of 1.311 for *D. subspicatus*. In parameter sets 4 and 5 the ratio in handling times between undefended and defended prey was much higher than in parameter sets 1-3 and coincided with considerable lower estimates of the induction rate. The pattern of higher induction rates in *D. subspicatus* was even stronger in sets 4-5. We did not further interpret the decay rates, because they contribute minimally to the explained variance.

Table 4.1 Definitions of model parameters (in alphabetical order), their values for each of the parameterization sets and their source; set 0: model without inducible defenses, sets 1-5: model with inducible defenses.

Parameter	Value (set 0)	Value (set 1)	Value (set 2)	Value (set 3)	Value (set 4)	Value (set 5)	Unit	Interpretation
a_{A1}	770	770	770	770	770	770	$mL \cdot d^{-1} \cdot mg\ C^{-1}$	Herbivore attack rate on undefended plants
a_{A2}	-	770	770	770	770	770	$mL \cdot d^{-1} \cdot mg\ C^{-1}$	Herbivore attack rate on defended plants
b	-	2	2	2	2	2		Shape of plant defense functions (induction/decay)
c_{AB}	0.36	0.36	0.36	0.36	0.36	0.36	mg C/mg C	Conversion efficiency from plants to herbivores
c_{NA}	1	1	1	1	1	1	mg C/mg C	Conversion efficiency from nutrients to plants
d_A	-	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	0.426	d^{-1}	Decay rate of defenses of *D. subspicatus*
	-	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$<1 \cdot 10^{-6}$	d^{-1}	Decay rate of defenses of *S. obliquus* MPI
	-	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	0.090	d^{-1}	Decay rate of defenses of *S. obliquus* UTEX2630
	-	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$1 \cdot i_A$	$<1 \cdot 10^{-6}$	d^{-1}	Decay rate of defenses of *D. quadricauda*
d_B	0.93	0.93	0.93	0.93	0.93	0.93	d^{-1}	Death rate of herbivores
h_{A1}	0.285	0.203	0.159	0.201	0.073	0.073	$d \cdot mg\ C^{-1} \cdot mg\ C^{-1}$	Handling time on undefended *D. subspicatus*
	0.228	0.195	0.164	0.201	0.182	0.181	$d \cdot mg\ C^{-1} \cdot mg\ C^{-1}$	Handling time on undefended *S. obliquus* MPI
	0.269	0.213	0.198	0.223	0.206	0.211	$d \cdot mg\ C^{-1} \cdot mg\ C^{-1}$	Handling time on undefended *S. obliquus* UTEX2630
	0.297	0.228	0.209	0.230	0.204	0.201	$d \cdot mg\ C^{-1} \cdot mg\ C^{-1}$	Handling time on undefended *D. quadricauda*
h_{A2}	-	$2.08 \cdot h_{A1}$	$2.90 \cdot h_{A1}$	$2.08 \cdot h_{A1}$	$9.14 \cdot h_{A1}$	$9.11 \cdot h_{A1}$	$d \cdot mg\ C^{-1} \cdot mg\ C^{-1}$	Handling time on defended plants
h_I	-	0.005	0.005	0.005	0.005	0.005	mg C/mL	Herbivore density at which half of the plants is induced
h_N	0.205	0.205	0.205	0.205	0.205	0.205	d^{-1}	Value at which half the maximum growth of plants is reached
i_A	-	1	1	1.311	0.424	0.425	d^{-1}	Induction rate of defenses of *D. subspicatus*
	-	1	1	0.601	0.040	0.039	d^{-1}	Induction rate of defenses of *S. obliquus* MPI
	-	1	1	0.370	0.030	0.031	d^{-1}	Induction rate of defenses of *S. obliquus* UTEX2630
	-	1	1	0.663	0.050	0.049	d^{-1}	Induction rate of defenses of *D. quadricauda*
r_A	1.342	1.342	1.342	1.342	1.342	1.342	d^{-1}	Maximum growth rate of *D. subspicatus*
	0.656	0.656	0.656	0.656	0.656	0.656	d^{-1}	Maximum growth rate of *S. obliquus* MPI
	0.563	0.563	0.563	0.563	0.563	0.563	d^{-1}	Maximum growth rate of *S. obliquus* UTEX2630
	0.614	0.614	0.614	0.614	0.614	0.614	d^{-1}	Maximum growth rate of *D. quadricauda*
Number of parameters	3	9	8	8	7	6		From literature[1]
	2	2	2	2	2	2		Estimated from autonomous algal growth (h_N , r_A)
	1	1	2	1	2	2		Estimated from predator-prey interactions for all (d_B, h_{A2})
	1	1	1	2	2	3		Estimated from predator-prey interactions per strain(d_A, h_{A1}, i_A)
Variance unexplained	142.4	74.8	65.5	73.5	33.4	33.2	%	

[1] h_I: Verschoor et al. 2004a; all other parameters: Vos et al. 2004b

Discussion

Our analysis with a mechanistic predator-prey model showed that including inducible defenses was crucial to understanding the observed population dynamics of predator and differently defended prey. The model results pointed to the importance of the ratio of handling times on defended and undefended prey in combination with the induction rate of defenses. In the experiment we observed predator populations to fluctuate when feeding on small undefended algae (*D. subspicatus*). During these fluctuations minimum rotifer densities remained sufficiently high to ensure population persistence. In the treatments where algae were larger due to induced or permanent defenses (*S. obliquus* MPI, UTEX 2630 and *D. quadricauda*) we observed rotifer population densities to first rise and then decline, with the predator going extinct in several replicates. The mechanistic model accurately described the observed population dynamics, though it did not completely follow the fluctuations of the predator feeding on *D. subspicatus*. We subsequently projected and evaluated the consequences of the above mentioned critical parameters for the long-term dynamics of the predator-prey interaction.

We note that the unexplained variance of the model for the different parameter sets shows three distinct groups: 1) The parameter set 0 of the model without inducible defenses with a very poor fit; 2) the intermediate sets 1, 2 and 3 with an unexplained variance of 65-75% and 3) the best fitting sets 4 and 5 with an unexplained variance of 33%. The most remarkable difference between set 0 and sets 1-3 is the presence of inducible defense parameters (h_{A2}, a_{A2}, i_A, d_A, h_I, b). The inclusion of these extra parameters reduced the unexplained variance to half its original value. The estimate of handling time (0.2 d·mg C^{-1}·mg C^{-1}) in sets 1-3 was lower than the estimate of 0.5 in the model of Vos et al. (2004b). This difference is linked to the difference in the estimate of the *Brachionus* mortality rate of 0.17 d^{-1} (Vos, et al. 2004b) compared to the observed value of 0.93 d^{-1} during the decline of *Brachionus* in the experiment. The estimated handling time of about 0.2 in sets 1-3 leads to an initial population growth rate of *Brachionus* ($\approx c_{AB}/h_{A1} - d_B$) in the order of 0.75 to 1.04 which is higher than the estimate of Vos et al. (2004b) of 0.55 d^{-1}, but are in concordance with the experimental observations. Note that estimating the induction rates from the data in set 4 resulted in induction rates in the same order of magnitude as the estimated values from Vos et al. (2004b). For the undefended *D. subspicatus* we observed a significantly shorter handling time in our best fitting parameter sets 4-5, in concordance with the small particle size of this strain (Van der Stap et al. 2006). Only with a low value of the handling time the model could reproduce the observed population fluctuations for this strain. The handling times

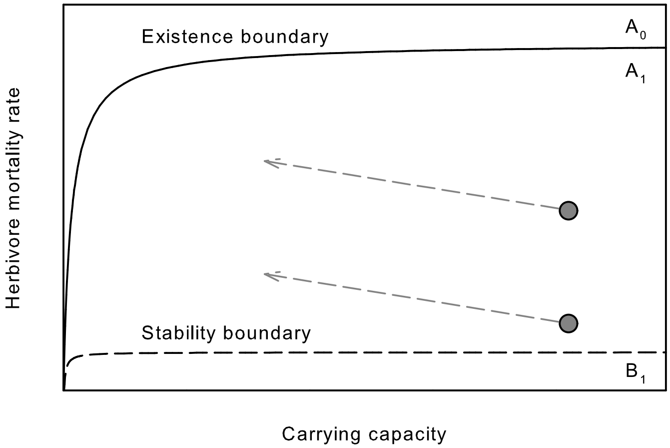


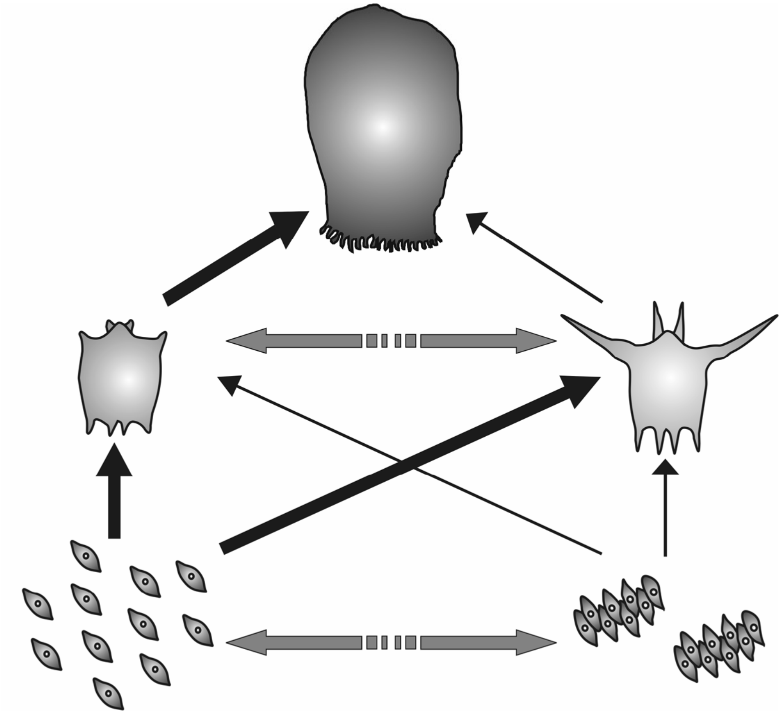
Figure 4.3 The existence and stability boundaries for a range of carrying capacities and herbivore mortality rates in bitrophic food chains. The existence and stability boundaries separate areas with qualitatively different dynamics. Area A_0: only plants exist. Area A_1: stable coexistence of plants and herbivores. Area B_1: plants and herbivores fluctuate. The initial points (filled circles) of the experiment in relation to existence and stability boundaries are shown for prey strains with short (h_{A1}=0.07, lower circle) and long handling time (h_{A1}=0.2, upper circle). Note that the y-position of these points is scaled to their respective existence and stability boundaries. Dashed arrows indicate the movement of the experimental data point during the experiment, in which nutrients were depleted.

longer handling time than the model found. *D. quadricauda* is a permanently large, four-celled algal strain and their large particle size (Van der Stap et al. 2006) together with their long spines would make *D. quadricauda* a hardly edible strain. Inducible defenses in prey, e.g., by an increase in size, increase the handling times and in some species decrease the ingestion rate of their predator, if this predator is gape limited (Verschoor et al. in press). Ingestion rates of the predator can also be decreased by a low food quality of algae that increase the digestion time by nutrient limitation or thickening of their cell wall (Van Donk and Hessen 1993).

To make the final step in linking the experimental data with the theoretical model we interpreted the parameters of set 4 in terms of bifurcation plots. These plots have carrying capacity on the x-axis and predator mortality rate on the y-axis and show the regions of predator extinction, stable coexistence and predator-prey cycles. The start of our experiment can be represented in these plots as a given combination of carrying capacity and predator mortality. Each of the algal strains has its own position between the existence and stability boundaries, depending on the estimates for handling time and attack rate on the undefended and defended algae. The plot for the most edible strain shows that the point representing the initial experimental conditions is just above the stability boundary for this strain and far below the existence boundary (Fig. 4.3). This implies that we should see dampened oscillations for this strain (Vos et al. 2005). These are indeed visible in the model, but less so in the *Brachionus* data (Fig. 4.2e). For the other three prey strains the point representing the realized conditions is half way the existence and stability boundaries (Fig. 4.3). During the course of the batch experiment nutrients were depleted causing the point in the bifurcation plot to move towards a lower carrying capacity and probably towards higher herbivore mortality. Therefore herbivores that have their initial point closer to the existence boundary will go extinct earlier compared with herbivores that start further away from the existence boundary (Fig. 4.3).

During the course of the batch experiment nutrients were depleted and herbivore mortality increased causing the initial point in the bifurcation plot (Fig. 4.3) to move towards a lower carrying capacity and higher herbivore mortality. This will lead to an earlier extinction of *Brachionus* for those strains for which the experimental data point is closest to the existence boundary, due to their longer handling times (Fig. 4.3). This is in agreement with the persistence and extinctions observed in the experiment by Van der Stap et al. (2006). Herbivores always persisted when feeding on undefended algae (*D. subspicatus*), while when feeding on the inducible and permanently defended algal strains (*S. obliquus* MPI, UTEX 2630 and *D. quadricauda*) herbivore extinctions were observed. Future experimental tests of theory on inducible defenses should include a range of carrying capacities (Verschoor et al. 2004) or a range of herbivore mortalities, for example by using chemostats.

Our analysis provides mechanistic insight in the relation of inducible defenses and stability. It shows that a mechanistic model that explicitly takes into account a shift in prey size and handling time can explain the observed population dynamics of both predator and prey. The model analysis explains the experimentally observed pattern of herbivore persistence and extinction in terms of these differences in handling times on differently defended algal prey. At a more general level, we conclude that such a linking of models and experimental data is useful in obtaining a more mechanistic understanding of predator-prey interactions.



Chapter 5

Inducible defenses prevent strong population fluctuations in bi- and tritrophic food chains

Abstract

Recent theoretical work (Vos et al. 2004a) predicts that inducible defenses prevent strong population fluctuations under high levels of nutrient enrichment. Here we evaluate this model prediction and show that inducible defenses in algae stabilize the dynamics of experimentally assembled bi- and tritrophic planktonic food chains. At high phosphorus levels, we observed strong population fluctuations in all food chains with undefended algae. These fluctuations did not occur when algae had inducible defenses. At low phosphorus levels, we observed deterministic consumer extinctions, as predicted by stoichiometric theory. Our study thus shows that both biotically and abiotically induced changes in algal food quality affect the stability and persistence of planktonic food chains.

Verschoor, A.M.., M. Vos and I. van der Stap. 2004. Inducible defences prevent strong population fluctuations in bi- and tritrophic food chains. Ecology Letters 7(12): 1143-1148.

Introduction

Enriched food chains may show extreme population fluctuations that result in stochastic species extinctions, the so-called paradox of enrichment (Rosenzweig 1971). This phenomenon has frequently been observed in laboratory studies (e.g., Luckinbill 1973, McCauley et al. 1999, Fussmann et al. 2000), but rarely in nature (Murdoch et al. 1998).

Inducible defenses are a candidate mechanism for explaining this contrast. They have been predicted to stabilize the dynamics of bi- and tritrophic food chains, even in highly enriched systems (e.g., Abrams and Walters 1996, Vos et al. 2004a). Inducible defenses have been shown to occur in hundreds of species, across a wide range of taxa (Karban and Baldwin 1997, Kats and Dill 1998, Tollrian and Harvell 1999). This indicates their potential importance in natural food webs.

The timing of inducible defenses is of crucial importance for their effect on stability. Inducible defenses may destabilize dynamics when considerable time lags occur between changes in consumer density and the induction or relaxation of defenses (e.g., Underwood 1999). However, even when delays are present, population fluctuations may be damped and thus not sustained indefinitely (Lundberg et al. 1994). Interestingly, inducible defenses may stabilize dynamics when delays are absent or minimal (Abrams and Walters 1996, Ramos-Jiliberto 2003, Vos et al. 2004a). Inducible defenses may thus promote or endanger population persistence (Vos et al. 2002, 2004a) and this effect may differ between bi- and tritrophic food chains (Vos et al. 2004a).

Empirical studies on the effects of inducible defenses on population stability are rare (Haukioja 1980, Fowler and Lawton 1985, Seldal et al. 1994). Rigorous empirical tests of any population level effect of different defense strategies are scarce (Underwood and Rausher 2002). No previous empirical study has addressed the combined effects of contrasting defense strategies on stability and persistence in both bitrophic and tritrophic food chains. Here we experimentally test predictions from the theoretical study by Vos et al. (2004a), who analyzed models of planktonic food chains. Their study explored the effects of enrichment (a bottom-up effect) and consumer mortality rate (a top-down effect) on stability, in scenarios with different defense strategies in algae. This analysis (and additional unpublished analyses) revealed a large area in a realistic part of parameter space in which a bitrophic system monotonically reaches a stable equilibrium when algae have induced defenses. In contrast, when algae have no defenses, the system exhibits strong population fluctuations, either damped or sustained. This area is characterized by intermediate to high values of algal carrying capacity and herbivore mortality (Vos et al. 2004a). Similarly, in an enriched tritrophic food chain a monotonic approach of a stable equilibrium is possible when algae have induced defenses. This occurs at low mortality rates of the carnivore. In this area of parameter space population cycles are expected when algae are undefended, as the possibilities for a stable equilibrium are severely restricted.

These model predictions lead to the following empirical research questions. 1) Do enriched food chains without defenses in algae exhibit strong population fluctuations, either damped or sustained, and are these fluctuations prevented by induced defenses in algae? 2) Are there differential effects of inducible defenses on the stability and persistence of bitrophic and tritrophic food chains?

Vos et al. (2004a) parameterized their food chain models for planktonic organisms: carnivorous and herbivorous rotifers and green algae with inducible defenses or without defenses. We have experimentally assembled these food chains in medium that was either low or high in phosphorus. Here we show that inducible defenses prevented strong population fluctuations under eutrophic conditions and caused consumer densities to stay further away from zero. Low phosphorus levels caused deterministic consumer extinctions.

Methods

Food chains were composed of the following organisms: (1) algae: *Desmodesmus bicellularis* Hegewald (Chlorococcales, Chlorophyta) (hereafter: *Desmodesmus*), UTEX LB1359, or *Scenedesmus obliquus* (Turpin) Kützing (Chlorococcales, Chlorophyta) (hereafter: *Scenedesmus*), UTEX 2630; (2) herbivorous zooplankton: *Brachionus calyciflorus* Pallas (Rotifera) (hereafter: *Brachionus*); and (3) carnivorous zooplankton: *Asplanchna brightwellii* Gosse (Rotifera) (hereafter: *Asplanchna*). COMBO medium (Kilham et al. 1998) was used in all cultures and experiments.

Species from the genera *Scenedesmus* and *Desmodesmus* can be dominant phytoplankton species in rivers (Garnier et al. 1995) and shallow lakes (Burchardt et al. 2003), where they may co-dominate the plankton with *B. calyciflorus* (Jeppesen et al., 1990). Many *Brachionus* and *Asplanchna* species co-occur in rivers (Lair et al. 1998, Kim and Joo, 2000) and shallow lakes (K. Jürgens, pers. comm.), and the particular species *B. calyciflorus* and *A. brightwelli* we used here, may co-occur (Gilbert and Waage, 1967) or co-dominate the zooplankton (Oltra et al., 2001).

Scenedesmus forms colonies when exposed to *Brachionus*-released infochemicals. These colonies disintegrate in the absence of this herbivore (Verschoor et al. 2005). In contrast, our *Desmodesmus* strain, that is similar in size

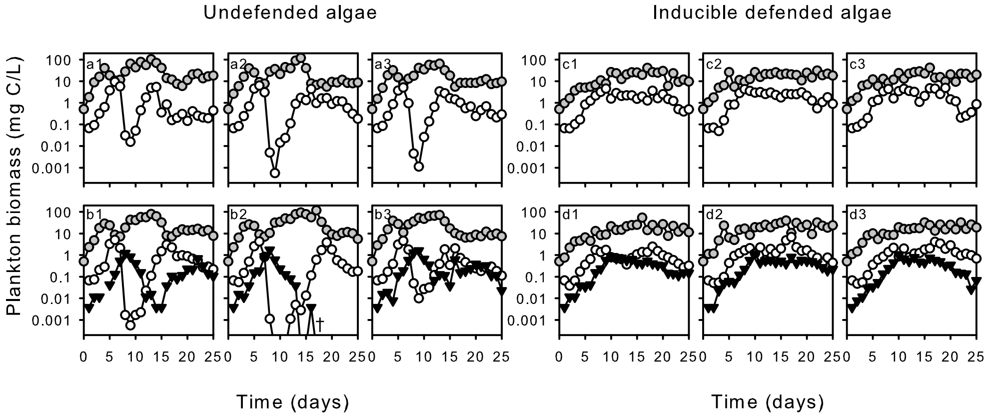


Figure 5.1 Population dynamics of planktonic food chains in the high phosphorus treatments, with densities expressed as mg C/L. Filled circles denote phytoplankton, open circles herbivorous zooplankton (*Brachionus*) and filled triangles carnivorous zooplankton (*Asplanchna*). a, b, food chains with undefended phytoplankton (*Desmodesmus*); c, d, food chains with inducible defenses in phytoplankton (*Scenedesmus*); numbers indicate different replicates. Zooplankton extinctions are marked with †.

and morphology to undefended *Scenedesmus*, does not respond in mean particle volume to herbivore infochemicals, as most other species in this genus (Verschoor et al. 2004a). For Scenedesmaceae between 44 and 757 µm³, the maximum ingestion rate of *Brachionus* decreases with increasing particle volume (Verschoor et al. in press). This algal size effect on *Brachionus* functional responses and the presence or absence of herbivore-induced colony formation were used as criteria to define algae without defenses (*Desmodesmus*) and algae with inducible defenses (*Scenedesmus*).

In the food chain experiments, full medium (50 µmol P/L) and phosphorus-depleted medium (0.5 µmol P/L) were used. This yielded a design consisting of 2 algal defense strategies x 2 food chain lengths (bitrophic and tritrophic) x 2 medium types = 8 treatments. Algae were centrifuged (10 min, 2500 RPM) and resuspended twice in either medium type. Zooplankton was added one day after algal inoculation. Inoculum densities were 0.5 mg C/L for *Scenedesmus* and *Desmodesmus*, 1200 ind/L (0.066 mg C/L) for *Brachionus* and 10 ind/L (0.0039 mg C/L) for *Asplanchna*. Triplicates for all eight treatments ran simultaneously in 1 liter microcosms. Plankton was incubated at 21°±0.5 °C in continuous light (120±10 µmol photons [PAR] m^{-2} s^{-1}) and kept in suspension by gentle shaking (90±5 RPM). Every 24 h, 100 mL (10%) of the plankton suspension was sampled and replaced by fresh medium. Phytoplankton and zooplankton samples were separated by a 30 µm filter and fixed in 1% of Lugol's iodine solution. Algal subsamples were counted on a particle counter (CASY 1, Schärfe System Gmbh, Reutlingen, Germany). Zooplankton samples were counted under a dissecting microscope. Experiments lasted 25 days in the full medium and (due to zooplankton extinctions) 20 days in the P-depleted medium.

Densities of the different populations in time were analyzed for differences in minima, coefficients of variation (CV) and maximum amplitude (^{10}log [maximum/minimum]). These parameters were calculated excluding the first 6 days, to prevent an over-representation of the initial transient phase. Because of heteroscedasticity and non-normality, the Scheirer-Ray-Hare extension of the Kruskall-Wallis test was used (Sokal and Rohlf 1995). For carnivore densities we used the Mann-Whitney U-test to compare food chains with inducible or no defenses in algae.

Results

In the high phosphorus treatment with undefended algae (*Desmodesmus*), we observed maximum amplitudes of two to four orders of magnitude in zooplankton population densities (Fig. 5.1a, b). In contrast, in high phosphorus treatments with inducible defenses (*Scenedesmus*), amplitudes typically spanned less than one order of magnitude (Fig. 5.1c, d). The initially strong fluctuations on undefended algae damped during the course of the experiment. In the inducible defense treatment, a stable equilibrium was approached rapidly and almost monotonically. The observed contrast in dynamics was highly replicable and similar for bitrophic and tritrophic food chains. In the high phosphorus treatment with undefended algae the carnivore *Asplanchna* went extinct in one replicate, after a very low minimum in the herbivore *Brachionus* (Fig. 5.1, b2).

Compared to food chains with inducible defenses in algae, food chains with undefended algae had significantly lower minimum densities of herbivores, (P = 0.0040), but not of carnivores (P = 0.1266). Food chains with undefended algae had higher CVs for the biomass densities of all trophic levels (algae and herbivores P = 0.0040, carnivores P = 0.0495) and higher maximum amplitudes (algae P = 0.0163, herbivores P = 0.0040, carnivores P = 0.0495). Neither food chain length, nor its interaction with defense strategy had a significant effect on any of these variables.

Both undefended *Desmodesmus* and *Scenedesmus* algae with inducible defenses initially showed fluctuations in particle size following their transfer from continuous cultures to the microcosms. On days six and seven of the bitrophic

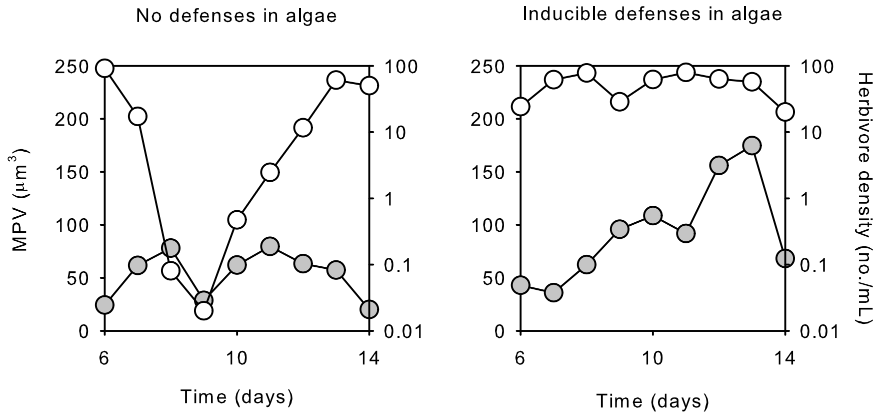


Figure 5.2 An example of algal size dynamics in terms of mean particle volumes in relation to herbivore densities in the bitrophic high phosphorus treatment, with *Scenedesmus* and *Desmodesmus* in the left and right panels respectively. Day 6-14 is the period with most distinctly contrasting herbivore dynamics. Filled circles denote mean particle volumes (MPV, µm^3), open circles represent herbivore densities (no./mL).

experiment both species had reached similar sizes, around 50 µm^3, as can be seen in the example in Fig. 5.2. *Brachionus* in the *Scenedesmus* treatment first increased in numbers to reach a density of 78 rotifers per mL on day 8. Subsequently densities slightly decreased and increased again to reach a regime of densities that varied around a mean of 56 herbivores per mL (Fig. 5.2, left panel). These changes in herbivore densities were followed by similar changes in algal particle size, all with a delay of about two days. *Scenedesmus* size reached a maximum of 175 µm^3 on day 13 (Fig. 5.2), and then decreased to fluctuate around a mean of 104 µm^3 during the rest of the experiment. Sizes of the undefended algae remained similar throughout the experiment, with an average of 59 µm^3 (range 41-88 µm^3; Fig. 5.2, right panel). *Desmodesmus* thus did not show major changes in particle size in response to the wild population fluctuations of its herbivore (Fig. 5.2, right panel).

In the low phosphorus treatments, both herbivorous and carnivorous zooplankton went extinct in all replicates, while the algae grew to carrying capacity (Fig. 5.3). Herbivores in food chains with undefended algae went through a cycle with a very deep trough before going extinct (Fig. 5.3a, b). In the treatment with inducible defenses herbivores went extinct more gradually, without a clear oscillatory tendency (Fig. 5.3c, d).

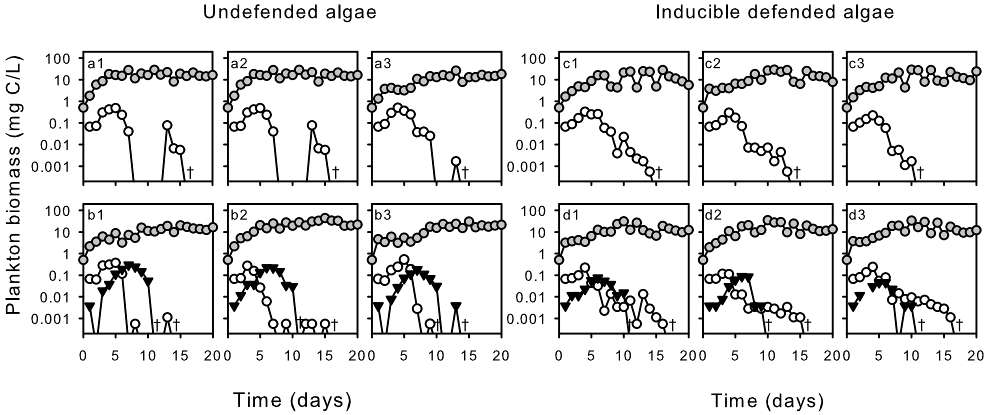


Figure 5.3 Population dynamics of planktonic food chains in the low phosphorus treatments, with densities expressed as mg C/L. Symbols are used as in Fig. 5.1.

Discussion

We showed strong population fluctuations that spanned several orders of magnitude in enriched food chains with undefended algae (Fig. 5.1a, b). In contrast, such strong fluctuations were absent in food chains with inducible defenses in algae (Fig. 5.1c, d). Particle sizes in this treatment were more variable (Fig. 5.2) due to variation in the number of cells per colony. This prey heterogeneity may have contributed to food chain stability in the induced defense treatment, see Abrams and Walters (1996) and Vos et al. (2004a). Similar stabilizing heterogeneities include differential predator swimming speeds (Luckinbill 1973), the presence of prey size refuges (Balčiūnas and Lawler 1995), and differential edibility of different algal prey species (McCauley et al. 1999, Persson et al. 2001).

Our experimental results are qualitatively in concordance with the model prediction that enriched food chains with induced defenses in algae are likely to monotonically approach a stable equilibrium. In the same part of parameter space the model predicts bitrophic food chains with undefended algae to show damped or sustained population cycles (Vos et al. 2004a, Vos unpublished). Indeed, our experimental treatment with undefended algae resulted in initially strong, but damped population fluctuations. Model analysis suggests that this damping occurred intrinsically, because the equilibrium was a stable focus (see Vos et al. 2004a, 2005). Another stabilizing factor, such as the formation of detrital aggregates, could also explain stabilization towards the end of the experiment.

Such a factor is likely to have contributed to the observed damping, as we do not theoretically expect damping towards a stable focus in a highly enriched tritrophic system with undefended algae (Vos et al. 2004a).

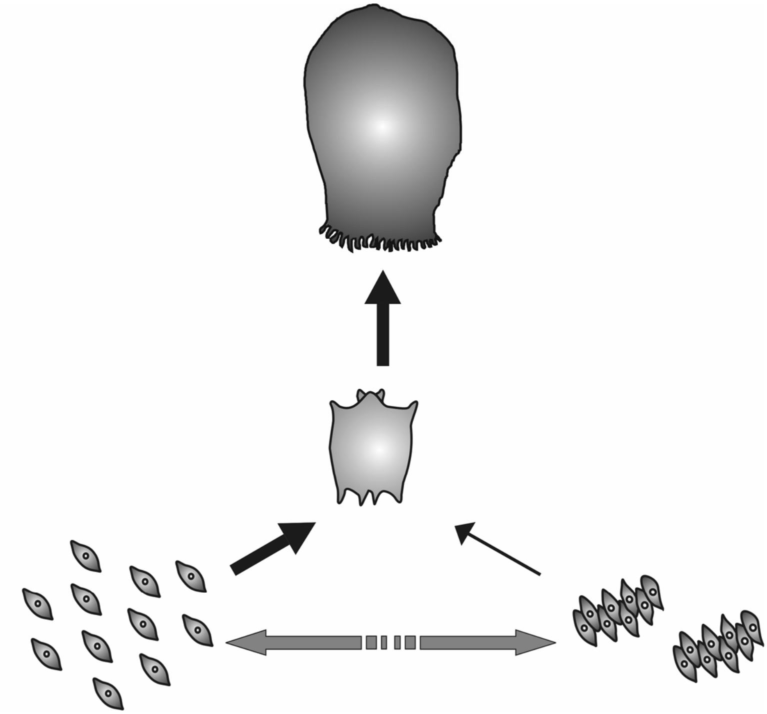
Both in nature and in experiments a variety of mechanisms might contribute to stability and persistence. We used the bitrophic version of the model by Vos et al. (2004a, 2004b), to evaluate four alternative mechanisms. In these model scenarios defenses were fixed, i.e., not inducible. We took undefended algae that cycled with their herbivores as a reference, using the parameter values of Vos et al. (2004a, 2004b). From there we decreased (a) the attack rate on algal prey and (b) algal growth rate, and increased (c) the handling time on algal prey and (d) the conversion efficiency from algal to herbivore biomass. We found that differences in growth rate between algal strains were unlikely to cause differences in stability of the plant-herbivore system. However, the above mentioned changes in handling time, attack rate and conversion efficiency all had a stabilizing effect. It is important to note that these characteristics, that we can consider to be part of fixed defense strategies, did not fundamentally resolve the paradox of enrichment. Their stabilizing effect is restricted in the sense that a higher level of enrichment is required to destabilize the system. In contrast, inducible defenses may prevent a paradox of enrichment altogether (Vos et al. 2004a). However, in experimental terms this evaluation implies that a strain with fixed defenses may be stable, and an undefended strain unstable, at a given level of enrichment. This can also be seen in Vos et al. (2004a): fig. 2 and means that future experimental work should elaborate on the dynamic consequences of fixed as opposed to inducible defenses.

Our main point here is that differences in algal defense strategies could explain the large and highly replicable differences in population dynamics we observed in this experiment. Work by Van der Stap et al. (2006) in batch systems on other algal strains shows that such population level effects of defense strategies are not a mere effect of species or strain identity.

The *Brachionus* strain used here exhibits induced defenses (spine formation) against its carnivore *Asplanchna*. These induced defenses at the herbivore level did not prevent strong population fluctuations, while induced defenses at the plant level did (Fig. 5.1). This is also predicted by the model of Vos et al. (2004a, 2004b). If defense inducibility in herbivores is removed from this model, very little changes in terms of stability occur. However, if defense inducibility in plants is removed from the model, one immediately regains the paradox of enrichment (M. Vos, unpublished analyses).

Both herbivores and carnivores went extinct in all low phosphorus replicates (Fig. 3b, d). At first sight, the strong declines in herbivore densities seem odd, given the abundance of algal food. We attribute herbivore extinctions to a progressively decreasing food quality of the algae, due to phosphorus depletion. With the strains used, food quality effects on *B. calyciflorus* population growth already occur at moderate P-limitation of *Scenedesmus* (atomic C:P ratios 600-650, Jensen and Verschoor 2004), whereas algal C:P ratios in a similar population dynamics experiment under identical abiotic conditions attained values between 1500-2500 (Van der Stap and Verschoor unpublished). The consistent extinctions of herbivores and carnivores at high carbon but low phosphorus levels are in close agreement with predictions from various stoichiometric models (Andersen 1997, Loladze et al. 2000, Grover 2003).

Inducible defenses are one of the individual level mechanisms that are predicted to have major effects at higher levels of ecological organization, in terms of trophic structure (Abrams and Vos 2003, Vos et al. 2004b), persistence (Vos et al. 2002, 2004a), resilience (Vos et al. 2005) and stability (Abrams and Walters 1996, Ramos-Jiliberto 2003, Vos et al. 2004a). Here we show that inducible defenses in algae are indeed capable of preventing strong population fluctuations. This is important because strong population fluctuations with low minimum densities endanger the persistence of species at higher trophic levels. Thus, defense strategies in prey species contribute to the probabilities of persistence and extinctions in higher-level consumers.



Chapter 6

Induced defenses in herbivores and plants differentially modulate a trophic cascade

Abstract

Inducible defenses are dynamic traits that modulate the strength of both plant-herbivore and herbivore-carnivore interactions. Surprisingly few studies have considered the relative contributions of induced plant and herbivore defenses to the overall balance of bottom-up and top-down control. Here we compare trophic cascade strengths using replicated two-level and three-level plankton communities in which we systematically varied the presence or absence of induced defenses at the plant and/or herbivore levels. Our results show that a trophic cascade, i.e., a significantly higher plant biomass in three-level than in two-level food chains, occurred whenever herbivores were undefended against carnivores. Trophic cascades did not occur when herbivores exhibited an induced defense. This pattern was obtained irrespective of the presence or absence of induced defenses at the plant level. We thus found that herbivore defenses, not plant defenses, had an overriding effect on cascade strength. We discuss these results in relation to variation in cascade strengths in natural communities.

Van der Stap, I., M. Vos, A.M. Verschoor, N.R. Helmsing and W.M. Mooij. Induced defenses in herbivores and plants differentially modulate a trophic cascade. Ecology in press.

Introduction

Debates on trophic cascades have moved on from the original question of whether communities are ‘green’ because of complete top-down control (Hairston et al. 1960, Oksanen and Oksanen 2000) to investigations of the balance between bottom-up and top-down control across a variety of ecosystems (Brett and Goldman 1996, Schmitz et al. 2000, Halaj and Wise 2002, Shurin et al. 2002, 2006). Ecologists have also strongly focused on the biotic and abiotic factors that explain variation in cascade strengths within certain types of ecosystems (Power et al. 1992, Polis and Strong 1996, Chase 2003). Potential explanations for variation in the strength of community-wide trophic cascades include predator species diversity (Finke and Denno 2004, Bruno and O'Connor 2005, Byrnes et al. 2006, Snyder et al. 2006), herbivore species diversity (Schmitz et al. 2000), intraguild predation (McCann et al. 1998, Hart 2002), refuges against intraguild predation (Finke and Denno 2002, 2006) and heterogeneity in edibility within trophic levels (Leibold 1989, Power et al. 1992, Bell 2002, Vos et al. 2004b).

The importance of variation in (induced) defenses and thus edibility as modulators of top-down control has been recognized for a long time (Murdoch 1966, Power et al. 1992, Strong 1992, Polis and Strong 1996, Bell 2002, Vos et al. 2002, 2004b, Schmitz et al. 2004). Under a regime of increasing predation, species that differ in their defense levels may change in relative abundance (Abrams and Vos 2003), or species turnover may change community composition altogether (Leibold et al. 1997, Chase et al. 2000). Through these community processes, plant and herbivore levels as a whole may change in terms of their edibility, even when defense levels of each of the comprising species are fixed.

Traits of individuals are rarely entirely fixed in nature. Phenotypic plasticity is in fact ubiquitous (Tollrian and Harvell 1999, Peacor and Werner 2000, Agrawal 2001), and defensive responses in both plants and herbivores often seem tuned to consumer density and thus predation risk (Schmitz et al. 2004, Vos et al. 2004a, 2004b). Consumers often seem to balance foraging activity and food intake against predation risk (e.g., Anholt et al. 2000). Carnivore-mediated reduction in herbivore feeding has two effects that differentially affect cascade strength. It may decrease carnivory, but it also decreases the consumption of plant biomass by “inactive” herbivores (Abrams 1984). In principle, this allows carnivores to have weak or strong, positive or negative effects on plant biomass, depending on the involved trade-offs (Schmitz et al. 2004). Nonetheless, whenever herbivores substantially reduce their feeding activity in the presence of carnivores, this may result in reduced plant damage levels, and even in increased plant growth or biomass. This would entail a behaviorally mediated trophic cascade (Schmitz et al. 2004).

Not all defenses, however, involve a reduced resource intake. Reduced feeding activity is a typical pre-detection or predator avoidance defense of otherwise vulnerable species. Species may also combine several defenses that interfere with different parts of the predation cycle, or may entirely rely on defenses that prevent ingestion (the final stage of the predation cycle). Such defenses should have qualitatively different effects on trophic cascades. For many plant and herbivore species, avoiding encounters with, and detection by, consumers is not an option. This may be the case because of a sedentary or planktonic (drifting) life style or for a suite of other reasons that require a reliance on defenses that interfere with the final stages of the predation cycle. Such defenses may include defensive colony formation in algae and the formation of defensive spines in zooplankton that hinder or prevent their ingestion by herbivores and gape-limited carnivores, respectively. Many defenses, including other changes in morphology, honest signals of strength, toxicity or noxiousness, as well as flight or autotomy and other evasion tactics following encounters with predators are part of this defense category. None of these are likely to involve substantial costs in terms of reduced resource intake. Defenses in this category are not restricted to any particular ecosystem. They affect predation in freshwater, marine, and terrestrial food webs.

Terrestrial plants may also show indirect defenses, as many of these attract the parasitoids and carnivores of their insect herbivores through the emission of volatiles, after herbivore damage has started to occur (Vet and Dicke 1992, Dicke and Vet 1999, Vos et al. 2001, 2006). In the case of terrestrial plants, we can generally state that their defenses are unlikely to involve reduced nutrient uptake as a trade-off.

Post-encounter defenses may occur in plants or herbivores, or in both of these, in any particular food chain within a wider community. These defenses have important implications for the strength of trophic cascades and could entirely block the transmission of carnivore effects to the plant level. For example, when such defenses are strong in herbivores, this would make them independent of the top-down effects of secondary carnivores, whose role has been considered of major importance in differentiating pelagic communities from terrestrial ones (Hairston and Hairston 1997).

Here we evaluate the effects of induced defenses that act during the later stages of the predation cycle, i.e., defenses other than those that reduce activity levels. We focus on the effects of these defenses on the strength of trophic cascades using replicated two- and three-level planktonic food chains. We employ simple communities in order to disentangle the relative contributions of induced defenses

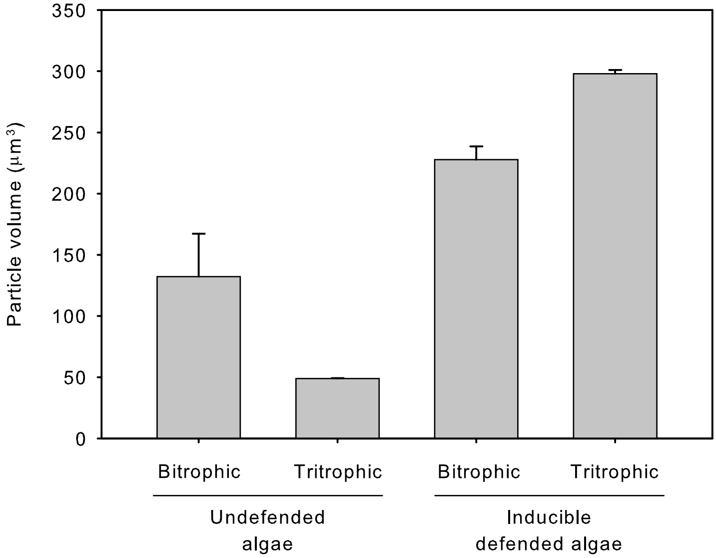


Figure 6.1 A measure for plant defense: particle volumes of undefended algae (*Desmodesmus*) and of algae with inducible defenses (*Scenedesmus*) in two- and three-level food chains (bi- and tritrophic) with undefended herbivores (*Brachionus rubens*). Shown are means (+SE) of three replicates, with each of these means being the particle volume averaged over the total duration of the experiment. Particle volumes differed significantly between algal strains (Mann-Whitney U test, $P < 0.01$).

in plants and herbivores, not because we think that real communities are simple (see *Discussion* in chapter 6). At the plant level, we use green algae from the Scenedesmaceae family, with *Desmodesmus bicellularis* being undefended while *Scenedesmus obliquus* forms defensive colonies in the presence of herbivorous zooplankton (Fig. 6.1; Verschoor et al. 2004a, see also Verschoor et al. 2004b: Fig. 2). At the herbivore level we use *Brachionus* rotifers, with *B. rubens* being undefended while *B. calyciflorus* forms defensive spines in the presence of carnivorous *Asplanchna* rotifers (Fig. 6.2; Gilbert 1966). At the carnivore level we use the gelatinous rotifer *A. brightwellii*. A previous experiment using a subset of these species led to the observation that induced defenses in algae had a major effect in terms of damping population fluctuations in food chains (Verschoor et al. 2004b). Nonetheless, algal defenses seemed to have little effect in terms of affecting algal biomass. This in turn led to our hypothesis that herbivore defenses in this data set had relatively strong effects and thus obscured the effect of plant defenses. We therefore performed a new experiment with an herbivorous rotifer without inducible defenses (*B. rubens*) in the same experimental set-up to investigate how the presence or absence of induced defenses in herbivores and plants affects the strength of trophic cascades.

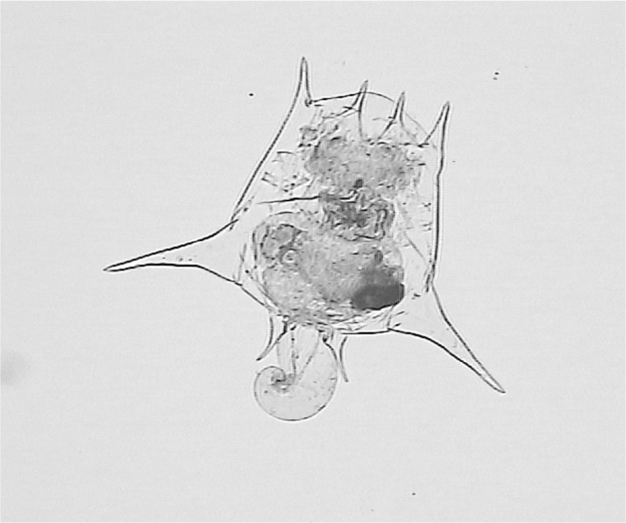


Figure 6.2 Picture of the herbivore *Brachionus calyciflorus* with induced postero-lateral spines that protect the rotifer from ingestion by the carnivorous rotifer *Asplanchna*. The presence of *Asplanchna* caused the percentage of defended *B. calyciflorus* herbivores to rapidly increase to more than 90% in the first three days. This high defense level was maintained during the entire experiment. Photo credit: N.R. Helmsing.

Material and methods

Food chains in the present experiment were composed of the following organisms: (1) algae (often referred to as plants) comprising the undefended species *Desmodesmus bicellularis* Hegewald (Chlorococcales, Chlorophyta) strain UTEX LB1359, or inducible defended *Scenedesmus obliquus* (Turpin) Kützing (Chlorococcales, Chlorophyta) strain UTEX 2630; (2) undefended herbivorous zooplankton *Brachionus rubens* Ehrenberg (Rotifera); and (3) carnivorous zooplankton *Asplanchna brightwellii* Gosse (Rotifera). COMBO medium (Kilham et al. 1998) was used in all cultures and experiments. This system mimics the one studied by Verschoor et al. (2004b), but with the important difference that the herbivore in our study is undefended, whereas Verschoor et al. (2004b) used an inducible defended herbivore (*B. calyciflorus*). The rotifer *B. rubens* contains less carbon per individual than *B. calyciflorus* (Rothhaupt 1993), so we adjusted the initial densities of *B. rubens* in order to have initial carbon concentrations as in the experiment of Verschoor et al. (2004b).

The experiment was set up using a factorial design consisting of two algal defense strategies x two food chain lengths (two- and three-level) making four treatments. Algae were centrifuged for 10 min at 2500 rpm and resuspended twice in full medium containing 50 µmol P/L. Zooplankton was added one day after algal inoculation. Inoculum densities were 0.5 mg C/L for *Scenedesmus* and *Desmodesmus*, 2200 individuals/L (0.121 mg C/L) for *Brachionus* and 10 individuals/L (0.0039 mg C/L) for *Asplanchna*. Three replicates of all treatments ran simultaneously in 1-L microcosms for 14 days. Plankton was incubated at 21° (minimum 20.5°C, maximum 21.5°C) in continuous light (120 µmol photons [PAR]·m^{-2}·s^{-1} [minimum 110, maximum 130 photons·m^{-2}·s^{-1}]) and kept in suspension by gentle shaking (90 rpm [minimum 85 rpm, maximum 95 rpm]). Every 24 hours, 100 mL (10%) of the plankton suspension was sampled and replaced by fresh medium. Phytoplankton and zooplankton samples were separated by a 30-µm filter and fixed in 1% of Lugol's iodine solution. Algal subsamples were measured on a particle counter for the number of particles (unicells or colonies) and particle volume (CASY 1, Schärfe System Gmbh, Reutlingen, Germany). Particle volumes within each replicate were averaged over time and differed significantly between algal strains (Fig. 6.1; Mann-Whitney U, $P<0.01$). Zooplankton samples were counted under a dissecting microscope.

The effect sizes of predator manipulations were measured as (1) the log of the ratio of average plant densities in the presence and absence of carnivores and (2) the log of the ratio of average herbivore densities in the presence and absence of carnivores. These measures are also referred to as cascade strength. Biomass levels of algae and herbivorous rotifers were determined by taking the average biomass over time for each replicate. The effects of algal strain (defenses present or absent) and number of trophic levels on the biomass were analyzed using factorial ANOVA. Prior to analysis, data were tested for homogeneity of variances using Cochran's C test. Biomasses at each of the different trophic levels were analyzed using Tukey hsd post-hoc tests. Statistical analyses were performed using STATISTICA version 7.1. (StatSoft, Tulsa, Oklahoma, USA).

We evaluated our results within this study in relation to those from a previous study, performed in exactly the same set-up and conditions, that used the inducible defended herbivore *B. calyciflorus* (population dynamics data published in Verschoor et al. 2004b; not previously analyzed for effects of defenses on trophic cascade strength). Within three days following inoculation with carnivorous *Asplanchna,* 90-100% of the *B. calyciflorus* population showed induced spines. Herbivore defense levels remained this high throughout the experiment. Undefended individuals had essentially no spines (lengths of 0-4 µm), whereas defended individuals had spines of 13-70 µm, with a mean of about 35 µm (n = 240). Statistical comparisons were only made within each of the two data sets. For the analysis of the *B. calyciflorus* data set we again used cascade strength as defined above and average biomass levels over days 1 to 14.

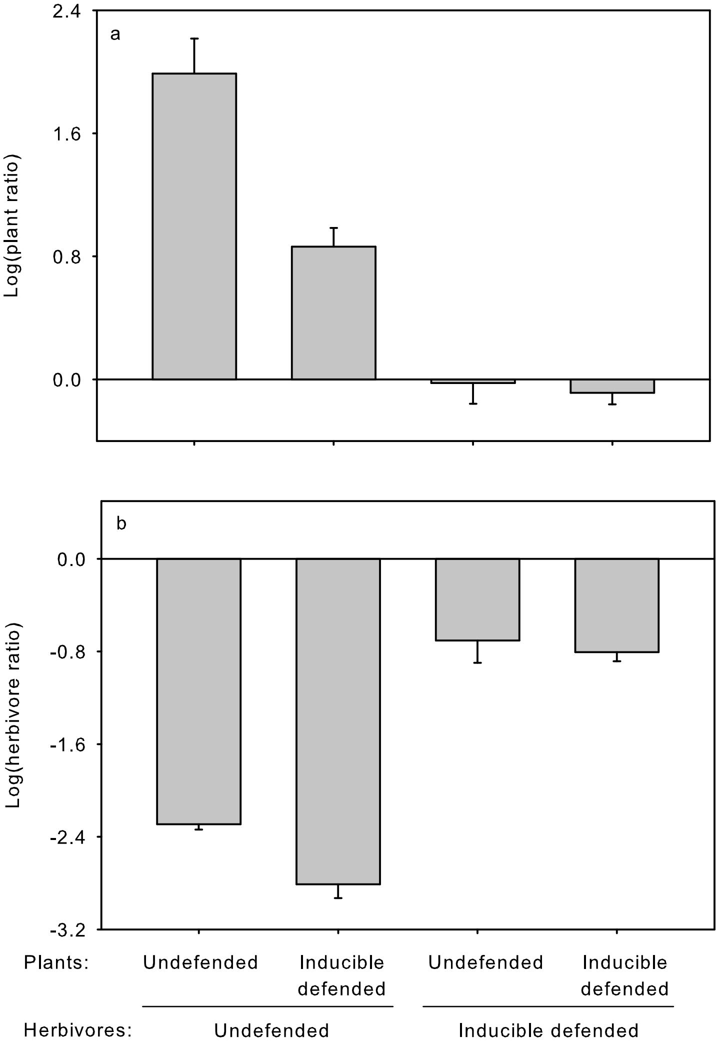


Figure 6.3 Effect of inducible defenses on the strength of trophic cascades in food chains differing in the presence or absence of inducible defenses in (a) plants (algae) and (b) herbivores (zooplankton). Cascade strength was measured as (a) log(plant ratio), the mean plant biomass in the presence of carnivores divided by the mean plant biomass in the absence of carnivores, and (b) log(herbivore ratio), the mean herbivore biomass in the presence of carnivores divided by the mean herbivore biomass in absence of carnivores. Shown are means (with SE) of three replicates.

Table 6.1 Summary of ANOVA testing the differences in biomass of all trophic levels (plants, herbivores and carnivores) among plants (algal strains), food chain length (two- or three-level) and the interactions between algal strain and food chain length.

Effects	df	MS	*F*	*P*
Food chains with undefended herbivores				
Plants (algae)				
Algal strain	1	0.759	0.2382	0.64
Food chain length	1	386.6	121.4	<0.001
Strain x food chain length	1	29.34	9.209	<0.05
Error	8	3.186		
Herbivore (*B. rubens*)				
Algal strain	1	0.5375	77.67	<0.001
Food chain length	1	2.231	322.5	<0.001
Strain x food chain length	1	0.3134	45.29	<0.001
Error	8	0.0069		
Carnivore (*Asplanchna*)				
Algal strain	1	0.0184	59.70	<0.01
Error	4	0.0003		
Food chains with inducible defended herbivores				
Plants (algae)				
Algal strain	1	1414	128.3	<0.001
Food chain length	1	3.257	0.2954	0.60
Strain x food chain length	1	0.006	0.0006	0.98
Error	8	11.02		
Herbivore (*B. calyciflorus*)				
Algal strain	1	0.1861	0.5215	0.49
Food chain length	1	8.401	23.54	<0.01
Strain x food chain length	1	0.0732	0.2050	0.66
Error	8	0.3568		
Carnivore (Asplanchna)				
Algal strain	1	0.0005	0.2427	0.65
Error	4	0.0021		

Results

Presence or absence of a trophic cascade

A strong trophic cascade was present when neither plants nor herbivores were defended, as indicated by the largest difference between plant biomass in absence and presence of carnivores, resulting in the highest positive log plant ratio value (Fig. 6.3a). Cascade strength decreased when plants had an induced defense, but only when herbivores were undefended (Fig. 6.3a). The trophic cascade disappeared altogether when herbivores had an induced defense, irrespective of whether plants were defended or not, as indicated by log plant ratio values close to zero (Fig. 6.3a). As expected, herbivore biomass was strongly negatively affected by carnivores when herbivores were undefended, and only weakly affected when herbivores had an induced defense (Fig. 6.3b).

These results are quantified using ANOVA (Table 6.1). When herbivores were undefended, food chain length significantly affected herbivore biomass (ANOVA, $P < 0.001$) and plant biomass (ANOVA, $P < 0.001$). In contrast, when herbivores had induced defenses, food chain length still affected herbivore biomass (ANOVA, $P < 0.01$), but plant biomass was no longer affected (ANOVA, $P = 0.60$).

The biomass of the different trophic levels is given in Fig. 6.4, left panels, showing a pattern of strong top-down control when herbivores were undefended. In this case we observed that the presence of carnivores decreased herbivore biomass (Tukey's hsd test, all P values ≤ 0.001) and increased plant biomass, both when plants were undefended (Tukey's test, $P < 0.001$) and when they had an induced defense (Tukey's test, $P < 0.01$).

The alternative case, with induced defenses in herbivores, shows a pattern of weaker top-down effects that did not make it to cascade down to the plant level (Fig. 6.4, right panels). Here we observed that carnivores caused a marginally non-significant decrease in herbivore biomass when plants were undefended (Tukey's test, $P = 0.057$) and a marginally significant decrease in herbivore biomass when plants had an induced defense (Tukey's test, $P = 0.023$). However, the addition of carnivores to a two-level food chain had no discernable effect on plant biomass, whether these plants were defended or not (ANOVA, $P = 0.60$, Fig. 6.4f).

Other biomass patterns

We observed three striking patterns in biomass distribution apart from the absence or presence of a trophic cascade. First, carnivore biomass was significantly lower in a food chain based on algae with an induced defense than in a food chain based on undefended algae, but only when herbivores were undefended (ANOVA, $P <$

0.01, Table 6.1, Fig. 6.4a), and not when herbivores were defended (ANOVA, P = 0.65, Table 6.1, Fig. 6.4b). Second, the undefended herbivore had a lower biomass in two-level food chains with inducible defended plants than in two-level food chains with undefended plants (Tukey's test, $P < 0.001$, Fig. 6.4c). This effect did not occur in the defended herbivore (Fig. 6.4d). Third, plant biomass was significantly lower in a food chain based on algae with an induced defense than in a food chain based on undefended algae, but only when herbivores had an induced defense (ANOVA, $P < 0.001$, Table 6.1, Fig. 6.4f), and not when herbivores were undefended (ANOVA, P = 0.64, Table 6.1, Fig. 6.4e).

Discussion

Trophic cascades

Many biotic and abiotic factors cause cascade strengths to vary in nature. Top-down effects may come as trophic trickles or as strong trophic cascades and these top-down effects may be food chain specific or community wide. Nonetheless, in all of these cases we can ask how the traits of plants and herbivores within these communities contribute to overall effect sizes.

In the present study we showed that herbivores with induced defenses caused a complete block of top-down carnivore effects on plants. In contrast, when herbivores were undefended this allowed a strong trophic cascade to exist. In this case the addition of a carnivore to a two-level system resulted in herbivore suppression and increased plant biomass. The fundamental mechanism underlying the results of this study with defended herbivores is that herbivore defenses effectively prevented ingestion by carnivores while not limiting the herbivore's intake of algal food. If, in contrast, a carnivore-induced defense would reduce the herbivore's feeding rate on plants, this could allow plant biomass to increase. In the latter case, a behavior-mediated trophic cascade would occur. We thus have the somewhat counterintuitive situation that herbivore defenses may either prevent or facilitate a trophic cascade, depending on whether there is a trade-off between the defense and the herbivore's food intake. Defenses that act early in the predation usually involve such a trade-off, while many defenses that act later on in the predation cycle hardly affect resource intake rates. The system with *Asplanchna* as a carnivore and spined *B. calyciflorus* as a defended herbivore is an example of the latter situation.

In this model system, herbivore defenses were more efficient in preventing ingestion by carnivores than algal defenses were in preventing ingestion by herbivores. In principle, a highly efficient plant defense could similarly buffer top-down effects on its biomass by species higher up in the food web. Cascade strength

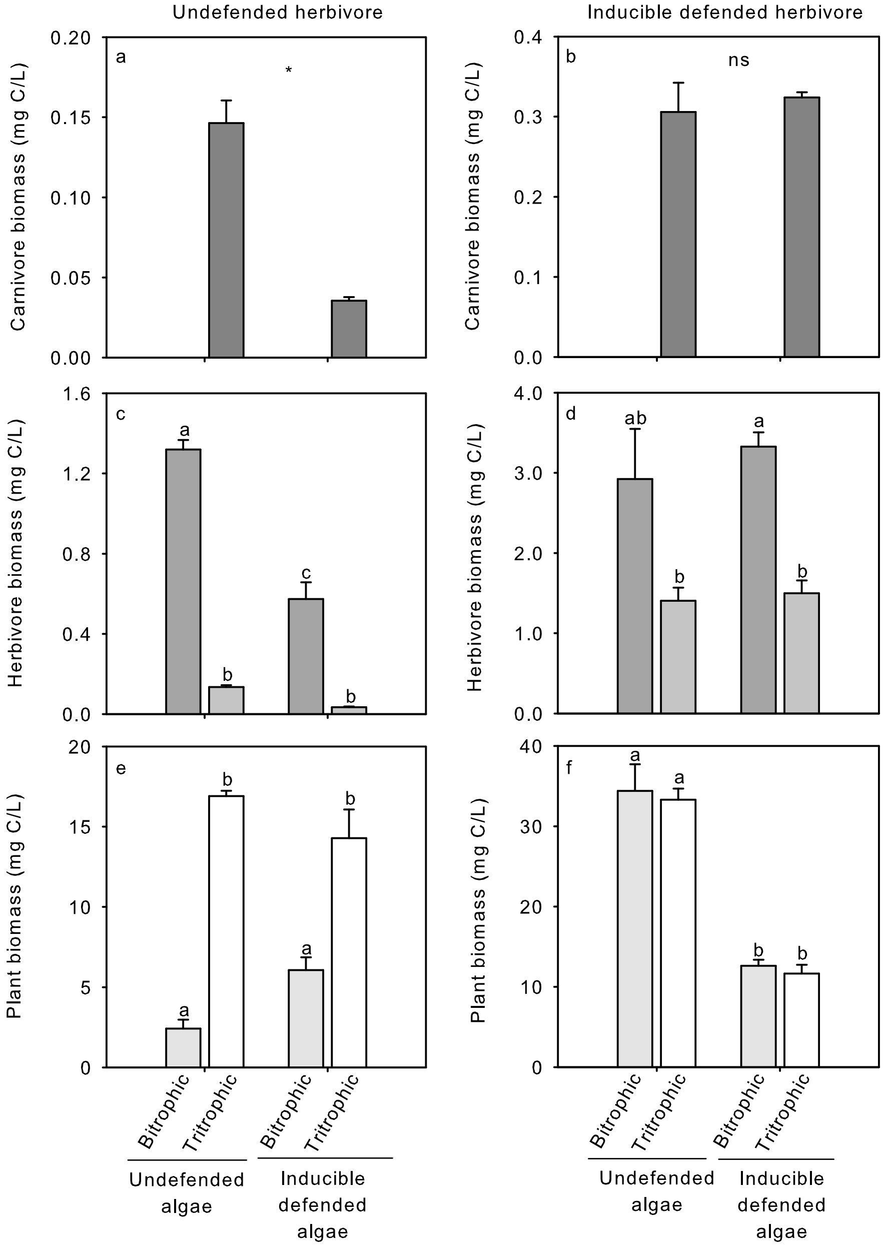


Figure 6.4 Biomass of all trophic levels, with carnivores (top row), herbivores (middle row) and plants (bottom row). The left panels show two- and three-level food chains with either undefended or inducible defended plants and an undefended herbivore, the panels to the right show these food chains with the inducible defended herbivore (based on data from Verschoor et al. [2004], not previously analyzed for cascade strength). Shown are means (+SE) of three replicates. Different letters within panels indicate significant differences among treatments (Tukey's hsd test after ANOVA, $P < 0.05$).

is often observed to attenuate at the plant-herbivore interface, both in aquatic (Brett and Goldman 1996) and in terrestrial (Schmitz et al. 2000) communities. Our results show that induced herbivore defenses alone can cause such an attenuation of cascade strength at the herbivore-carnivore interface. We showed this in a simple model system using food chains that are otherwise highly comparable, e.g., with respect to their stoichiometry.

Natural communities are complex and diverse, and as our study shows, not all herbivore species are equally defended. However, effective induced defenses occurring in a variety of herbivore species within a community could substantially dampen top-down control. In the present study, we worked with a limited number of species, i.e., we analyzed data from a food chain with a single herbivore species that represented the undefended defense type and related these results to data from a food chain with a single defended herbivore species. The extent to which our results can be extended to community-wide trophic cascades depends on the ways in which dominant plant and herbivore species respond to multispecies complexes of their consumers. Many defenses, such as toxins and spines, are likely to be effective against a wide variety of consumer species. This is also the case for reduced activity defenses such as described by Abrams (1984) and Schmitz et al. (2004). Since reduced activity and defenses that act later on in the predation cycle can have opposite effects on cascade strength, the community-wide effects of herbivore defenses will depend on the relative abundance and importance of different herbivore species employing these different kinds of defenses. This implies that analyses of cascade strength need to take into account how herbivores defend themselves against their consumers. It similarly means that “herbivore diversity” cannot be a simple variable in analyses of top-down and bottom-up control.

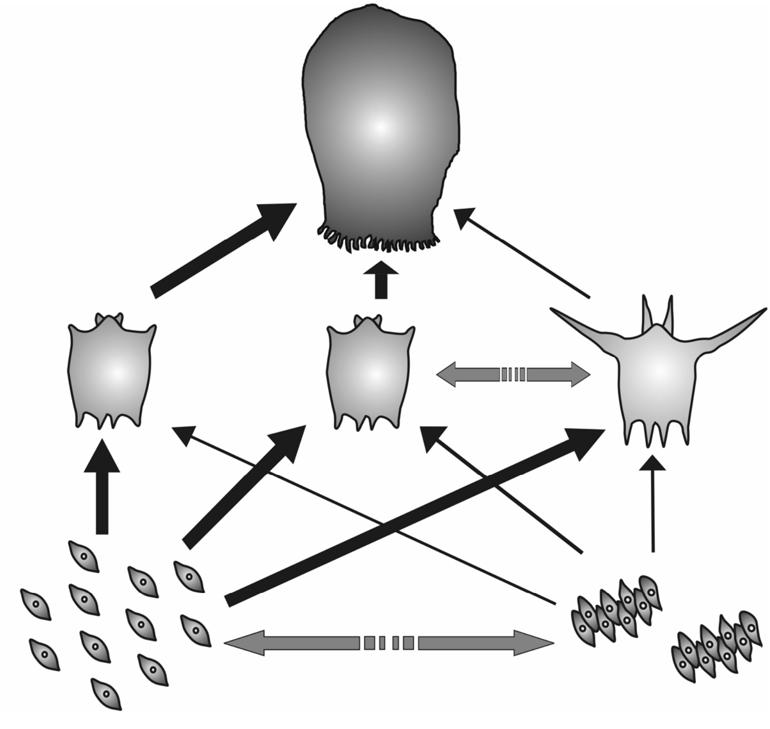
Other biomass patterns

We found that both herbivore biomass in two-level food chains and carnivore biomass in three-level food chains were lower when these food chains were based on inducible defended algae (as compared to chains based on undefended algae, see Fig. 6.4a, c). This effect of plant defenses on the accumulation of biomass higher up in the food chain only occurred when herbivores were undefended. This effect can be understood from the fact that the undefended herbivore *B. rubens* is smaller than the defended herbivore *B. calyciflorus* and is thus more affected by defensive colony formation in algae. *B. rubens* has previously indeed been shown to have a lower ingestion rate than *B. calyciflorus* on large algae (Rothhaupt 1990).

In food chains with defended herbivores, plant biomass was significantly higher when these chains were based on undefended algae. This was the case in both two-level and three-level food chains (Fig. 6.4f). This biomass pattern can be understood from the underlying pattern in population dynamics. Both two-level and three-level food chains showed highly fluctuating population densities when these were based on undefended algae, while those based on inducible defended algae monotonically approached a stable equilibrium (Verschoor et al. 2004b). The high densities of undefended algae we observed are related to low herbivore densities that occurred during these population fluctuations. This makes clear that any analysis of trophic cascade strength has to take into account whether the underlying population dynamics are stable or cyclic, because "measured cascade strength" will be affected by the duration of the experimental period in systems that show cyclic dynamics in nature. Short-term experiments (relative to the organisms' life and population cycles) would be especially vulnerable to sampling either peaks or troughs of population cycles. However, we emphasize here that our main result in this paper, the presence or absence of a trophic cascade depending on the presence or absence of herbivore defenses, was independent of population stability: The effect of herbivore defenses on cascade strength occurred no matter whether the algal population was undefended or inducibly defended, and thus also irrespective of whether population fluctuations occurred or were absent.

Conclusion

We conclude that both the presence and the type of herbivore defenses can be important in determining the relative effects of bottom-up and top-down control, and thus the strength of trophic cascades. Herbivore defenses strongly inhibited top-down control by carnivores in our model system, thereby effectively preventing the trophic cascade that was strongly present when herbivore defenses were absent. Our results make clear how herbivore defenses that prevent ingestion by carnivores (with no trade-off in the herbivore's rate of plant consumption) will effectively attenuate trophic cascades at the herbivore-carnivore interface. This study indicates the importance of discriminating between herbivore defenses that act late in the predation cycle (e.g., by preventing ingestion through morphological changes) and those that act early on in the predation cycle (e.g., by preventing detection through reduced feeding activity), as these may have qualitatively different effects on cascade strengths in natural communities. The predation cycle is a fundamental aspect of feeding in any kind of food web, whether it is part of a freshwater, marine or terrestrial ecosystem. In all of these systems, herbivore defenses may counteract predation during early and/or later stages of this cycle, with or without trade-offs on herbivory rates. The differential effects of these different kinds of defenses should be of importance to any comparison of trophic cascade strengths within and between ecosystems in nature.



Chapter 7

Inducible defenses, competition and shared predation in planktonic food chains

Abstract

Ecologists have debated the role of predation in mediating the coexistence of prey species. Theory has mainly taken a bi-trophic perspective that excludes the effects of inducible defenses at different trophic levels. Our aim was to investigate how inducible defenses at different trophic levels affect the possibilities for predator-mediated coexistence in replicated experimental plankton communities. In particular, we analyzed how the presence or absence of inducible defenses in basal prey affected the outcome of competition between an inducible defended and an undefended herbivore in the presence or absence of carnivory. We found the undefended herbivore to be a superior competitor in the absence of predation. This outcome was reversed in the presence of a shared carnivore: Populations of the undefended herbivore strongly declined. In food chains with undefended algal prey this herbivore became undetectable for most of the duration of the experiment. This as opposed to food chains with inducible defended algae where the undefended herbivore also crashed to low densities, but it could still be detected for most of the time. In this case, the carnivore failed to reach high densities and it had limited its top-down control on the two competing herbivores. We discuss our results in terms of the consequences of inducible defenses for coexistence as opposed to exclusion of one of the herbivores under shared predation.

Van der Stap, I., M. Vos, R. Tollrian and W.M. Mooij. Inducible defenses, competition and shared predation in planktonic food chains. Submitted.

Introduction

Communities are structured by ecological mechanisms such as predation (Holt 1977, Sih et al. 1985, Kerfoot and Sih 1987) and competition for resources (Tilman 1982). Predation and competition may interact in a variety of ways (Chase et al. 2002). A classical view is that predators can allow for the coexistence of prey species that would not be able to coexist under competition alone. This idea of 'keystone predation' derives from seminal work by Paine (1966) who experimentally demonstrated that preferential predation on a dominant competitor mediated its coexistence with several subdominant species. Coexistence may similarly be obtained when parasites preferentially attack host species that are dominant competitors, for example within plant communities (e.g., De Deyn et al. 2003). Keystone predation in the wider sense could thus represent one of the ecological mechanisms that may allow the persistence of diverse species assemblages in nature.

However, several factors complicate the general applicability of predator-mediated coexistence as a principle that promotes diversity. These factors determine whether the effect of predators on the coexistence of competitors is absent, positive or negative (Chase et al. 2002). One of these factors is productivity (Leibold 1996). Using a simple model Leibold (1996) demonstrated that a vulnerable prey will exclude a resistant prey at a low nutrient level that just supports the predator population. At very high productivity the predator drives the less resistant species extinct, allowing the more resistant prey to dominate. In between these two levels of productivity predator-mediated stable coexistence may occur (Leibold 1996). In a single unreplicated trial Halbach (1969) observed reversal of the dominant species when he added a predator to two competing herbivores. The competitively superior, but vulnerable *B. rubens* went extinct shortly after introduction of the predator, and the more resistant *B. calyciflorus* lived together with the predator *Asplanchna* for a longer period. This observed predator-mediated extinction of the vulnerable species matches the model predictions (Leibold 1996) for a system in which nutrient levels are high.

A second factor that may prevent the general applicability of predator-mediated coexistence is the importance of population fluctuations in nature. Such fluctuations may occur as transient dynamics following environmental perturbations; they may occur through external (e.g., seasonal) forcing, or they may exist as limit cycles. Cyclic dynamics have been shown to severely limit the possibilities for predator-mediated coexistence (Abrams 1999). The theoretical studies by Leibold (1996) and Abrams (1999) both bear relevance to the possibilities for predator-mediated coexistence in nature, because both stable and cyclic dynamics are quite common in the field. In an extensive study in which about 700 long-term time series were analyzed, ca. 30 percent of the studied field populations showed cyclic dynamics (Kendall et al. 1998). It is somewhat surprising that the majority of time series did not reveal cyclic dynamics, as we know from a great body of laboratory experiments that many predator-prey interactions are unstable, especially in enriched and simplified laboratory environments (Huffaker et al. 1963, Luckinbill 1973, Fussmann et al. 2000). One of the reasons for such instability is the fact that many consumer species have Type II functional responses that have a destabilizing effect on the predator-prey interaction. Apparently, a variety of ecological mechanisms may act to stabilize predator-prey interactions in nature. Our point here is that such stabilizing mechanisms are not only important for the persistence of specific predator-prey systems, they also have significant implications for the role of keystone predation as a mechanism that promotes diversity. One mechanism that has been predicted to stabilize predator-prey interactions in both two-level and three-level food chains is the presence of inducible defenses in basal prey (Vos et al. 2004a).

Experiments have shown that such defenses may dramatically dampen population fluctuations in planktonic food chains (Verschoor et al. 2004b). This dampening of fluctuations brought minimum population densities further away from zero and thus promoted coexistence of species by preventing stochastic extinctions (Verschoor et al. 2004b). Here we further hypothesize that such induced defenses in basal prey may also affect coexistence by indirectly affecting carnivores and their control of competing herbivores. The greater part of existing theory on the effects of predation on the outcome of competition has focused on simple systems in which only two living trophic levels were considered. Natural communities are multi-trophic, which allows complex feedback loops between carnivores and plants. We reason that ecological theory should not only consider the trade-off between predator resistance and competitive ability in herbivores. This trade-off is similarly present in basal prey and this may have profound dynamical consequences (Yoshida et al. 2004).

To test the effects of shared predation on the outcome of competition between two herbivores (with and without inducible defenses) we used planktonic experimental communities with either undefended or inducible defended algae as basal prey. Carnivores were then either absent or added to our replicated alga-herbivore systems. We incubated these two-level and three-level species assemblages in replicated microcosms and evaluated the fate of each species over the 30-day duration of the experiment. We evaluate the qualitative outcome of competition in each of the four ecological scenarios and interpret the results in terms of the modulating effects of induced defenses in basal prey on the occurrence of very low minimum population densities.

Material and methods

Culture organisms

Food chains were composed of the following organisms: (1) algae: *Desmodesmus bicellularis* Hegewald (Chlorococcales, Chlorophyta) UTEX LB1359, or *Scenedesmus obliquus* (Turpin) Kützing (Chlorococcales, Chlorophyta) UTEX 2630; (2) herbivorous zooplankton: *Brachionus rubens* Ehrenberg (Rotifera) LMU, and *Brachionus calyciflorus* Pallas (Rotifera) Microbiotests Inc.; and (3) carnivorous zooplankton: *Asplanchna brightwellii* Gosse (Rotifera) LMU (hereafter: *Asplanchna*). COMBO medium (Kilham et al. 1998) was used in all cultures and experiments. The herbivorous rotifers *B. rubens* and *B. calyciflorus* were used as competitors.

B. calyciflorus exhibits induced defenses, i.e. spine formation, against its carnivore *Asplanchna* (DeBeauchamp 1952, Gilbert and Waage 1967). The rotifer *B. rubens* does not exhibit these inducible morphological defenses. This rotifer has a lower carbon content than *B. calyciflorus* (Rothhaupt 1993). Therefore initial density of *B. rubens* was twice the number of *B. calyciflorus*.

S. obliquus forms defensive colonies when exposed to *Brachionus*-released infochemicals (Lürling and Van Donk 1997, Van Donk et al. 1999). These colonies disintegrate in the absence of this herbivore (Verschoor et al. 2005). In contrast, our *D. bicellularis* strain, that is similar in size and morphology to non-induced *S. obliquus*, shows no response in particle volume to herbivore infochemicals.

Methods & sampling

The design of the food chain experiments consisted of 2 algal defense strategies x carnivory or no carnivory (*Asplanchna* present or absent) = 4 treatments. Algae were centrifuged (10 min. at 2500 rpm) and resuspended in full COMBO medium (50 µmol P/L). Zooplankton was added one day after algal inoculation. Inoculum densities were 0.5 mg C/L for *D. bicellularis* and *S. obliquus*, 1000 individuals/L (0.055 mg C/L) for *B. rubens*, 500 individuals/L (0.05 mg C/L) for *B. calyciflorus* and 10 individuals/L (0.0039 mg C/L) for *Asplanchna*. Three replicates for all four treatments ran simultaneously in 1-L microcosms. Plankton was incubated at 21°±0.5 °C in continuous light (120±10 µmol photons (PAR) m^{-2} s^{-1}) and kept in suspension by gentle shaking on a rotating table (90±5 RPM) for 30 days. Every 24 h, 100 ml (10%) of the plankton suspension was sampled and replaced by fresh medium. Phytoplankton and zooplankton samples were separated by a 30-µm filter and fixed in 1% of Lugol's iodine solution. Algal subsamples were counted on a particle counter (CASY 1, Schärfe System Gmbh, Reutlingen, Germany) to determine algal concentration and particle volumes. Zooplankton samples were counted under a dissecting microscope. *Asplanchna* were enumerated by counting the whole sample while the more abundant *Brachionus* were counted in smaller subsamples.

Data analysis

The densities of the different rotifer species in terms of biomass (mg carbon per L) were analyzed using repeated measures ANOVA. Algal strain, predation and their interaction were used as between samples factors and days in the experiment were used as a within-subject factor. To meet the assumptions of ANOVA rotifer data were square root transformed. The first 7 days of data were excluded from the rotifer biomass analysis to prevent an over-representation of the initial transient phase. The data for repeated measures ANOVA were tested for sphericity using the Mauchley Sphericity test. Densities of the rotifer populations were further analyzed for amplitude using factorial ANOVA with algal strain and presence or absence of carnivory as the factors. The amplitude of population fluctuations was measured as the average difference between subsequent minimum and maximum densities of the dominant herbivore. Algal particle volumes were averaged over the total duration of the experiment and the effects of algal strain, carnivory and their interaction was tested using factorial ANOVA.

Results

Competition without predation

The undefended herbivore *B. rubens* became the dominant competitor in all two-level food chain replicates, both when the two herbivore species competed for undefended algae (Fig. 7.1a-c) and when competition was for inducible defended algae (Fig. 7.1d-f). The undefended superior competitor *B. rubens* reached a high density of 3.1 ± 0.12 mg C/L (mean ± SE) when feeding on undefended *D. bicellularis* algae (Fig. 1a-c). On inducible defended *S. obliquus* algae this density was 1.0 ± 0.08 mg C/L (mean ± SE; Fig. 7.1d-f). This corresponds to about 56 and 18 *B. rubens* individuals/mL respectively. In contrast, *B. calyciflorus* showed densities of about 2.2 and 1.8 individuals/mL respectively, in the last few days before they became undetectable on day 30 (Fig. 7.1a-f). The amplitude of *B. rubens* population fluctuations was significantly smaller when the basal algal prey had inducible defenses (Tukey's hsd test after ANOVA, $P < 0.01$).

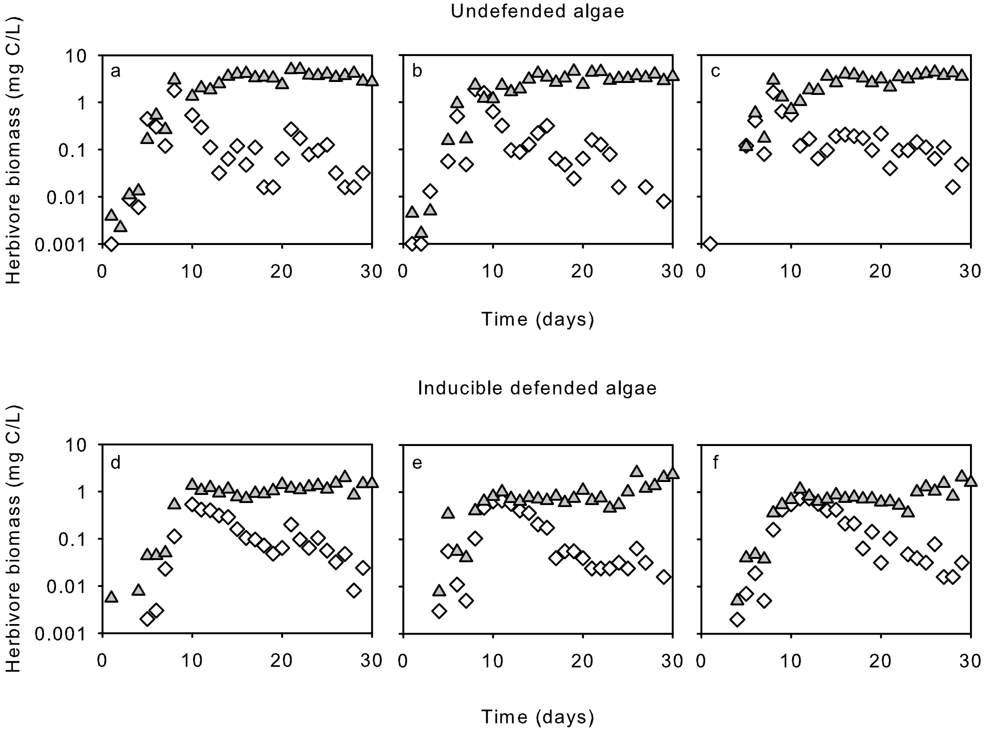


Figure 7.1 Competition between two herbivores, *B. rubens* and *B. calyciflorus* on undefended *D. bicellularis* algae or on inducible defended *S. obliquus* algae, in absence of a carnivore. Filled triangles denote undefended *B. rubens*, open diamonds denote non-induced *B. calyciflorus*, algae are not shown. All three replicates per algal strain are shown; densities are expressed as mg C/L.

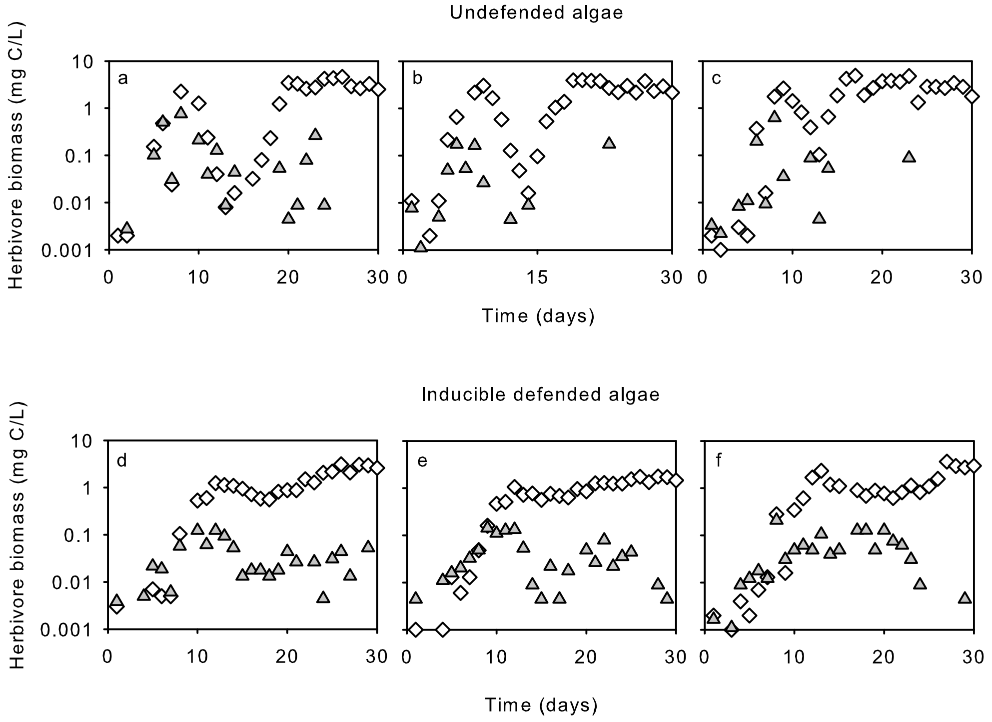


Figure 7.2 Competition between two herbivores, *B. rubens* and *B. calyciflorus* on undefended *D. bicellularis* algae or on inducible defended *S. obliquus* algae, under shared predation by the carnivore *A. brightwellii*. Filled triangles denote undefended *B. rubens*, open diamonds denote induced *B. calyciflorus*, algae and carnivores are not shown. All three replicates per algal strain are shown; densities are expressed as mg C/L.

Competition under shared predation

In contrast, when both herbivores competed in the presence of carnivores, the other herbivore, *B. calyciflorus* that exhibited an inducible defense, became dominant (Fig. 7.2). Irrespective of predation risk, food chains with undefended algae as basal prey had higher amplitudes of the dominant competitor *B. calyciflorus* in comparison with food chains that were based on algae with inducible defenses (Tukey's hsd after ANOVA, $P < 0.01$). The undefended competitor *B. rubens* now declined strongly during the second half of the experiment. The last time *B. rubens* was observed in the experiment occurred significantly later in the experiment when basal algae had an induced defense (T-Test, $P < 0.001$). On inducible defended algae *B. rubens* was last observed on day 29 in all replicates, i.e., almost up to the end of the experiment. In contrast, *B. rubens* was last observed on day 23.3 ± 0.33 in the treatment with undefended algae.

In both three-level food chain treatments population fluctuations with low minimum densities were observed. However, when algae had inducible defenses, the number of days that the population density of *B. rubens* dropped below our detection limit was significantly reduced. *B. rubens* was undetectable in 16.7 ± 1.9 days when algae were undefended and only in 6.3 ± 0.3 days when algae had induced defenses (means ± SE; T-Test, $P < 0.01$). This did not change the outcome of competition under shared predation: *B. calyciflorus* clearly won.

Meanwhile, the inducible defended competitor *B. calyciflorus* reached a high density of on average 1.2 ± 0.13 mg C/L (mean ± SE) on inducible defended *S. obliquus* algae (Fig. 7.2d-f). A high density of 2.2 ± 0.17 mg C/L (mean ± SE) was also reached on undefended *D. bicellularis* algae (Fig. 7.2a-c). This corresponds to about 12 and 22 *B. calyciflorus* individuals/mL respectively.

Algal defenses

Particle volumes differed significantly between algal strains (ANOVA, $P < 0.001$; Fig. 7.3). Undefended algae were smaller (less than 100 μm^3) than inducible defended algae (approx. 200 μm^3). There was a small, but significant indirect effect of the carnivore *Asplanchna* on the particle volumes of the undefended and inducible defended algal strains (ANOVA, $P = 0.045$). The algae were slightly smaller in the presence of their herbivore's carnivore. The interactive effect of algal strain and carnivory was not significant (ANOVA, $P = 0.15$).

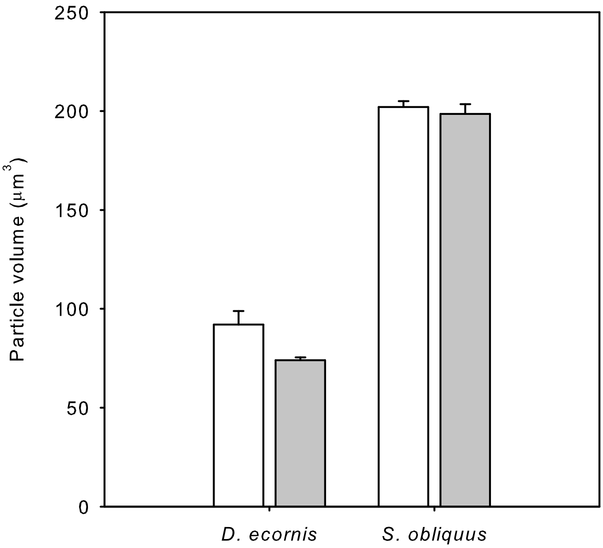


Figure 7.3 Particle sizes of the green algae at the basis of the food web: undefended *D. bicellularis* and inducible defended *S. obliquus*, averaged over the total duration of the experiment. White bars indicate two trophic levels or no carnivory, grey bars are part of food webs with three trophic levels or carnivore presence. Particle volumes shown are means (+SE) of three replicates.

Discussion

We studied how anti-predator defenses at different trophic levels affected the possibilities for predator-mediated coexistence or extinction in replicated experimental plankton communities. In particular, we analyzed how the presence or absence of inducible defenses in basal prey affected the outcome of competition between an inducible defended and an undefended herbivore in the presence or absence of a shared carnivore. We evaluate our results in terms of the consequences of inducible defenses for coexistence as opposed to exclusion of one of the competitors under shared predation.

In our experiment undefended *B. rubens* was the better competitor in the absence of carnivory. In this case the herbivore with a non-induced defense, *B. calyciflorus*, strongly declined. *B. calyciflorus* was not completely outcompeted during the course of the experiment, but its density was marginal in comparison with the superior competitor.

Under the experimental conditions used we found no true keystone predation or predator-mediated coexistence. Instead we found that the presence of the carnivore merely reversed who would outcompete the other, the undefended herbivore or the one with inducible defenses. Our observations are in concordance with an unreplicated pilot experiment by Halbach (1969) who also observed reversal of the dominant species in a competition experiment under predation. We cannot exclude the possibility that we would have observed predator-mediated coexistence at a lower nutrient level, as is suggested in Leibold's model analysis (1996). Future experiments could include an entire range of nutrient levels to test for this possibility.

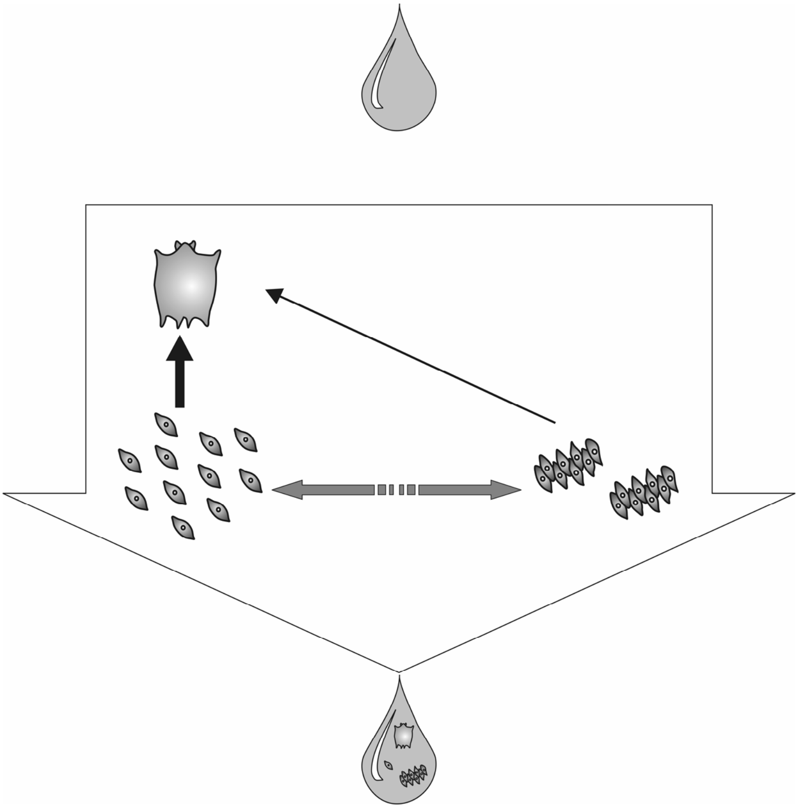
As we hypothesized, the presence of inducible defenses in the algae dampened the fluctuations of the victor in each scenario. Induced defenses in algae also delayed the population decline towards consistently low or undetectable densities of the undefended herbivore species in the shared predation treatments. We therefore observed that induced defenses had several stabilizing effects on the dynamics of the system, although we should note that delaying extinction is more properly referred to as an equalizing effect (Chesson 2000). In this case we observed at least a moderate equalizing effect of induced defenses. It would be necessary to run the experiment for a longer period to precisely quantify if and when the undefended herbivore would go extinct under shared predation in a food chain based on algae with inducible defenses (Fig. 7.2d-f). In any case, induced defenses in the algae did not change the final outcome of the competition in terms of which herbivore species was the victor.

Although carnivores had low densities in this experiment, they were detectable in most samples, and did not appear to decline towards extinction. Carnivores thus seemed to coexist with herbivores that had induced defenses, but without competing undefended herbivores. These were evidently unable to deal with the combined forces of carnivory and competition in the shared predation treatments. When we compare figures 7.1a-c with 7.2a-c, the addition of the carnivore to a two-level system seems to destabilize rather than stabilize community dynamics. In the three-level system the initial population fluctuations seem more pronounced, and the herbivore that is being outcompeted quickly reached densities below our detection limit.

It is hard to infer the exact underlying mechanisms for the outcome of our plankton food chain dynamics based on the population dynamics data themselves. Several factors are likely to have played a role. First it is important to note that the preference of the two herbivores for algal size differs (Rothhaupt 1990): *B. rubens* most efficiently ingests the smaller particles (like our *D. bicellularis*), whereas *B. calyciflorus* has a lower clearance efficiency on these smaller particles than its competitor, even though they represent better food for *B. calyciflorus* than large colonial algae (Van der Stap et al. 2006). The higher efficiency of ingesting smaller particles by *B. rubens* could have caused the pattern observed in the treatment with undefended algae without carnivory, where *B. calyciflorus* was outcompeted on undefended algae (Fig. 7.1a-c). It is important to note that the alga *S. obliquus* is rarely 100% defended: Though the proportion of large colonial algae increases in the presence of herbivores, a certain proportion remains of edible size. The higher efficiency of ingesting the smaller particles by *B. rubens* may have left *B. calyciflorus* with a relatively large proportion of less suitable food in the two-level treatments with inducible defended algae. This could have led to the outcompetition of *B. calyciflorus* on algae with inducible defenses (Fig. 7.1d-f). At the same time *B. rubens* may experience a more pronounced effect of colony formation by the algae with inducible defenses, because of this rotifer's smaller body size. In combination these effects could have been the cause of the relatively low carnivore densities that we observed in the three-level food chains with inducible defended algae. Limited top-down control by the carnivore may have caused the delay in the extinction of *B. rubens* in treatments with inducible defended algae (Fig. 7.2d-f) compared to those with undefended algae (Fig. 7.2a-c). The relatively low carnivore pressure, however, did not prevent reversal of the outcome of competition.

Interestingly, different *Brachionus* and *Asplanchna* species have been found to co-occur in natural communities like lakes (Oltra et al. 2001), ponds (Halbach 1972) and rivers (Lair et al. 1998, Kim and Joo 2000). Even coexistence of rotifer sibling species originating from natural communities that differed in vulnerability to predation has been shown to occur (Lapesa et al. 2002). The mechanism suggested by the authors was that the competitively dominant and less resistant species were preferred as prey (Ciros-Pérez et al. 2004). Of course most explanations in ecology have to be multi-causal (Vos et al. 2004b), and many mechanisms may in fact interact to shape such coexistence in natural communities. Our experiment indicates that it is not self-evident that even less than a handful of species will coexist in a planktonic community.

We chose the nutrient level in our experiment based on the experiments by Verschoor et al. (2004b). These authors showed that too low a nutrient concentration caused zooplankton extinctions due to an insufficient food quality of the algae. Conversely, at a high nutrient concentration three-level food chains mostly showed persistence of all species (Verschoor et al. 2004b). We chose the latter concentration to be used in the present experiment, to prevent extinctions due to insufficient quality of the basal algal prey. However, at the nutrient level used in our system no predator-mediated coexistence was observed. Instead we found predator-mediated extinction of an otherwise superior competitor, and this effect was delayed by induced defenses in algae, the basal prey in this planktonic system. The notion that preferential predation on competitive dominants generally promotes multi-species coexistence is thus an oversimplified idea.



Chapter 8

Do inducible defenses stabilize population dynamics? A chemostat model and experiment

Abstract

The effect of inducible defenses on predator-prey interactions was investigated in a freshwater planktonic system with rotifers as predators and algal strains with different defense strategies as prey. An existing model was modified to make specific predictions for the effect of inducible defenses on the stability and persistence of predators and prey in chemostat experiments. Analysis of this model showed that at high nutrient concentrations in combination with high dilution rates only algae should exist. At intermediate dilution rates, algae and rotifers should show stable coexistence, while at low dilution rates predator-prey cycles should develop with the risk of stochastic extinctions. In the model, the width and location of these areas with different qualitative dynamics critically depends on the defense strategy of the algae. The results of the experiments with inducible defended algae were in accordance with model predictions. At intermediate dilution rates, algae and rotifers coexisted in relatively stable densities. At lower dilution rates, the average algae and rotifer densities increased as well as the variation in abundance. In at least one of the triplicate chemostats this led to a stochastic extinction of the rotifers. In all chemostats with permanently defended algae, rotifers occurred only at low numbers at the brink of extinction due to food limitation. We had expected higher rotifer densities at the lowest tested dilution rates for the treatments with these algae on basis of the model results. This quantitative difference may imply that the defended algae in the experiment were less edible than was assumed in the model. Our results clearly show that qualitative shifts in predator-prey dynamics as a function of dilution rate and algal defense strategy occur.

Van der Stap, I., M. Vos, B.W. Kooi, E. van Donk and W.M. Mooij. Do inducible defenses stabilize population dynamics? A chemostat model and experiment. Submitted.

Introduction

Inducible defenses are individual level responses tuned to the level of predation. Such defenses have been observed in many organisms of both terrestrial and aquatic ecosystems (e.g., Karban and Baldwin 1997, Tollrian and Harvell 1999). This phenotypic plasticity of individuals can have a large effect on the interactions between prey and predators, as indicated in a number of theoretical studies that have incorporated inducible defenses (Edelstein-Keshet and Rausher 1989, Lundberg et al. 1994, Abrams and Walters 1996, Underwood 1999, Ramos-Jiliberto 2003, Vos et al. 2004a, 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). The population level effects of inducible defenses predicted by theoretical models have been confirmed in empirical studies on terrestrial and aquatic food webs (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 and co-workers (Chapter 4) showed that the dynamics observed in an aquatic system consisting of algae and rotifers could only be explained if inducible defenses were taken into account in their model.

Inducible defenses resolved the paradox of enrichment in a theoretical study on the effect of inducible defenses in Rosenzweig-MacArthur predator-prey dynamics (Vos et al. 2004a). Enrichment does not destabilize the system at intermediate mortality levels of the predator. The classical formulation of the model, that does not take inducible defenses into account, results in three areas of qualitatively different dynamics. These areas are separated by (1) the existence boundary, indicating that at higher predator mortalities, the predator cannot exist and only prey are present (2) the stability boundary, indicating that at lower predator mortalities, predator-prey cycles would occur with the risk of stochastic extinctions. The existence and stability boundaries converge at high carrying capacities. In the model with inducible defenses a stable coexistence of predator and prey occurs, even at high carrying capacities. To exemplify their results the 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 in either batch cultures in which nutrients were depleted (Van der Stap et al. 2006) or semi-continuous cultures in which part of the medium was replenished daily (Verschoor et al. 2004b). Inducible defenses prevented large population fluctuations in bi- and tritrophic food chains (Verschoor et al. 2004b, Van der Stap et al. 2006) as predicted by the model. In their semi-continuous cultures two nutrient conditions were used to determine the effect of inducible defenses at high and low productivity, but the low nutrient conditions were too low to maintain zooplankton biomass, due to very high C:P ratios in the algae (Verschoor et al. 2004b). Effectively, these experiments provided information for a single environmental condition. For a more critical test of qualitative model predictions we tested the effect of inducible defenses in continuous cultures (chemostats) with 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). At intermediate dilution rates the populations were oscillating, while at either high or low dilution rates the populations were at equilibrium.

Here we present model predictions on the effect of inducible defenses on population dynamics that would be expected under chemostat conditions, together with the first results of a chemostat experiment with algae with different defense strategies and rotifers. The main research questions that we focused on were 1) whether the three predicted qualitative types of predator-prey dynamics could be observed: only prey, stable equilibrium and predator-prey cycles with possible stochastic extinctions and 2) whether the dilution rates at which these changes occurred varied with the defense strategy of the algae.

Materials & 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 set-up including inducible defenses consists of four differential equations:

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

$$\frac{dP_1}{dt} = \left[\frac{c_N r_1 N}{h_{N1} + N} - \frac{v_1 H}{1 + v_1 h_1 P_1 + v_2 h_2 P_2} - \frac{iH^b}{h^b + H^b} - D\right] P_1 + \frac{dh^b}{h^b + H^b} P_2$$

$$\frac{dP_2}{dt} = \left[\frac{c_N r_2 N}{h_{N2} + N} - \frac{v_2 H}{1 + v_1 h_1 P_1 + v_2 h_2 P_2} - \frac{dh^b}{h^b + H^b} - D\right] P_2 + \frac{iH^b}{h^b + H^b} P_1$$

$$\frac{dH}{dt}=\left[\frac{c_1v_1P_1+c_2v_2P_2}{1+v_1h_1P_1+v_2h_2P_2}-D\right]H$$

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 undefended and defended state of the algae is described by induction and decay term in the algal equations. Notice that the expressions for the induction rates are equivalent to those in Vos et al. (2004a). An overview of the parameter values and their sources is given in Table 8.1. For the sake of simplicity the parameters that describe algae growth and nutrient-algae interaction are equal for all algal species.

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

$$\frac{dN}{dt}=D(N_i-N)-\left[\frac{r_1P}{h_{N1}+N}\right]N$$

$$\frac{dP_1}{dt}=\left[\frac{c_Nr_1N}{h_{N1}+N}-D\right]P_1$$

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

$$N^*=\frac{Dh_{NP1}}{c_Nr_1-D}$$

$$P_1^*=\frac{D(N_i-N^*)(h_N+N^*)}{r_1N^*}$$

In figure 8.1 the algal equilibrium concentration is plotted as a function of the dilution rate D.

Chemostat experiment

The single-stage chemostats were inoculated with the following organisms: (1) algae: undefended *Desmodesmus bicellularis* Hegewald, UTEX LB1359, or 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 form colonies in the presence of herbivores (Hessen and Van Donk 1993). The undefended algae are single-celled independent of grazing pressure, and colonial algae are constitutively defended. The defense strategies of the different algal strains were determined on 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

Table 8.1 Definitions of model parameters and their values.

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

* Parameter values originate from Vos et al. 2004b

' Parameter values originate from Chapter 4

used in all cultures and experiments. Algae were 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 three hours. This, however, did not prevent appearance of *S. obliquus* in cultures with *D. bicellularis*. After three weeks the algae in the *D. bicellularis* cultures were completely taken over by *S. obliquus* and we decided to stop running these chemostats and continue only with *S. obliquus* and *D. quadricauda* treatments.

Initially twelve 1.6L mixed culture chemostats were run. Chemostats were inoculated with $3.25 \cdot 10^8$ μm^3/mL of the respective algal strain and herbivores at 400 individuals/L in triplicate. Cultures were provided with 24h light and kept at a temperature of 20 ± 0.2ºC. Total mixing in the chemostat was ensured by moisturized and filtered air with a flow rate of 0.5 ± 0.1 L/min. Dilution rate of the

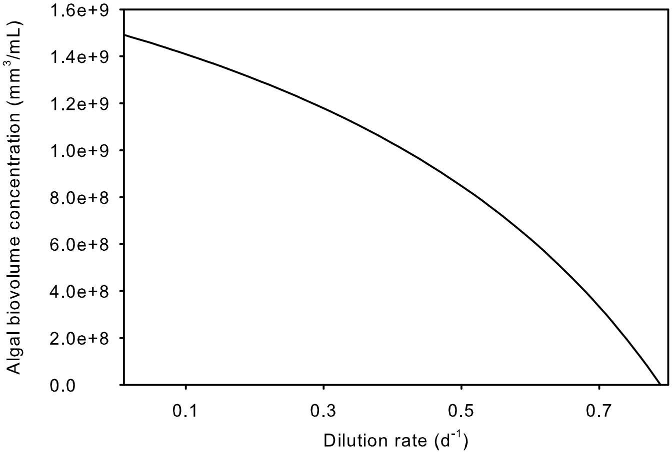


Figure 8.1 Algal densities for the nutrient-algae model as function of dilution rate.

chemostat was changed every three weeks from 0.7 to 0.5 to 0.3 and finally 0.1/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 (#/mL), mean particle volumes (μm^3) and biovolume concentration (μm^3/mL) were measured on a cell counter (CASY, Schärfe, 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. Rotifer densities were estimated every other day by microscopic counts. In the data analysis we focused on average biomass per dilution rate, minima and maxima for both algae and rotifers.

Results

Model

Three qualitatively different types of dynamics occurred 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 (Fig. 8.2). 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 in a stable equilibrium (area A_1). Low dilution rates and high nutrient concentrations lead to instability 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 undefended algae (Fig. 8.2, top), defended algae (Fig. 8.2, middle) and inducibly defended algae (Fig. 8.2, bottom) clearly shows the stabilizing effect of inducible defenses under a wide range of conditions. The presence of the three qualitatively different types of dynamics is fully in line with the predictions of an inducible defenses model with logistically growing algae (Vos et al. 2004a).

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). The dilution rate is comparable to the herbivore mortality rate. This resemblance allows for a quantitative comparison of both models. We observed that the location of the TC curves is almost identical. 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 co-existence between algae and zooplankton is larger in the chemostat model than in the model by Vos et al. (2004a). The model presented in this paper does not allow for the formulation of an analytical expression of the Hopf curves as a function of the nutrient inflow concentration N_i , because the chemostat model for fixed defense strategies is a three-dimensional model due to the explicit formulation of nutrient dynamics. Mathematical analysis shows that the existence and stability boundary for undefended algae at infinite high concentrations of nutrient approaches a dilution rate equal to c_1/h_1 (with parameter values of Table 8.1 equal to 0.72/day). This implies that the model shows the paradox of enrichment when inducible defenses are not present in the algae, as the region of stable co-existence of algae and zooplankton vanishes at high concentrations of the inflowing nutrient.

Chemostats with constitutive defended algae and rotifers

For constitutive defended algae, algal biomass and dilution rate were inversely related in all three replicates (Fig. 8.3a-c). The minimum algal densities were fairly constant and remained far away from zero. These results resemble the pattern for equilibrium population densities in absence of herbivores (Fig. 8.1). *Brachionus* densities were indeed very low in two out of three replicates (Fig. 8.4a-c) and never exceeded the initial density. In one replicate *Brachionus* densities do increase above their initial density (Fig. 8.4b), but the maximum density is still much lower in comparison to the maxima reached on inducible defended algae.

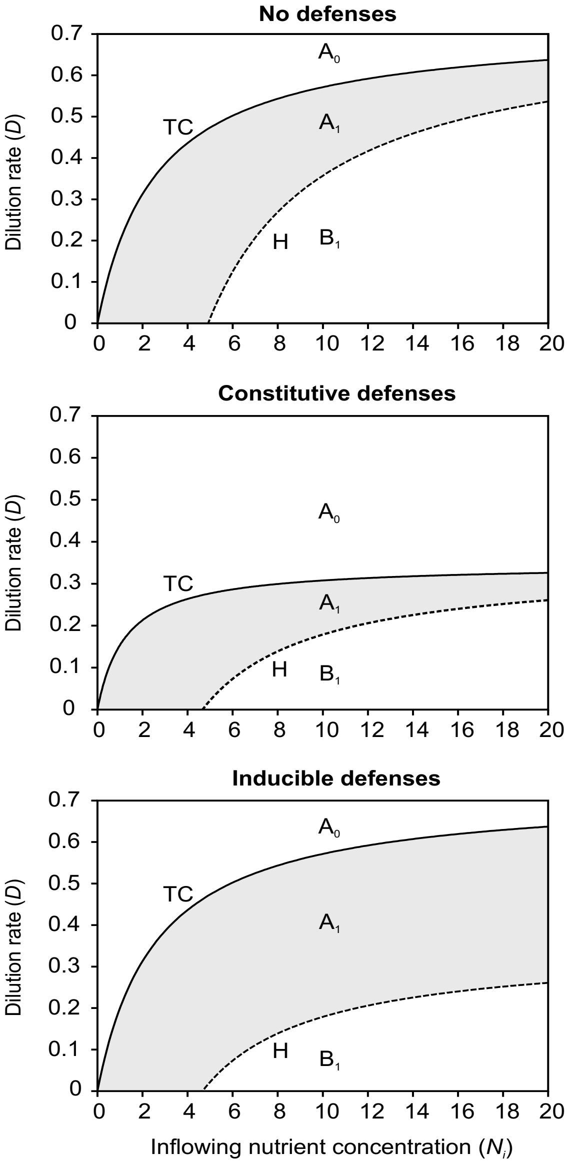


Figure 8.2 The stability boundaries of bitrophic food chains without defenses (top), with constitutive defenses (middle), or with inducible defenses (top), for a range of the inflowing nutrient concentrations (N_i) and dilution rates (D). The transcritical (TC) and Hopf (H) bifurcation curves separate areas with qualitatively different long-term dynamics. Area A_0: only plants exist. Grey area A_1: stable coexistence of plants and herbivores. Area B_1: plants and herbivores fluctuate. Parameter values used as in Table 8.1.

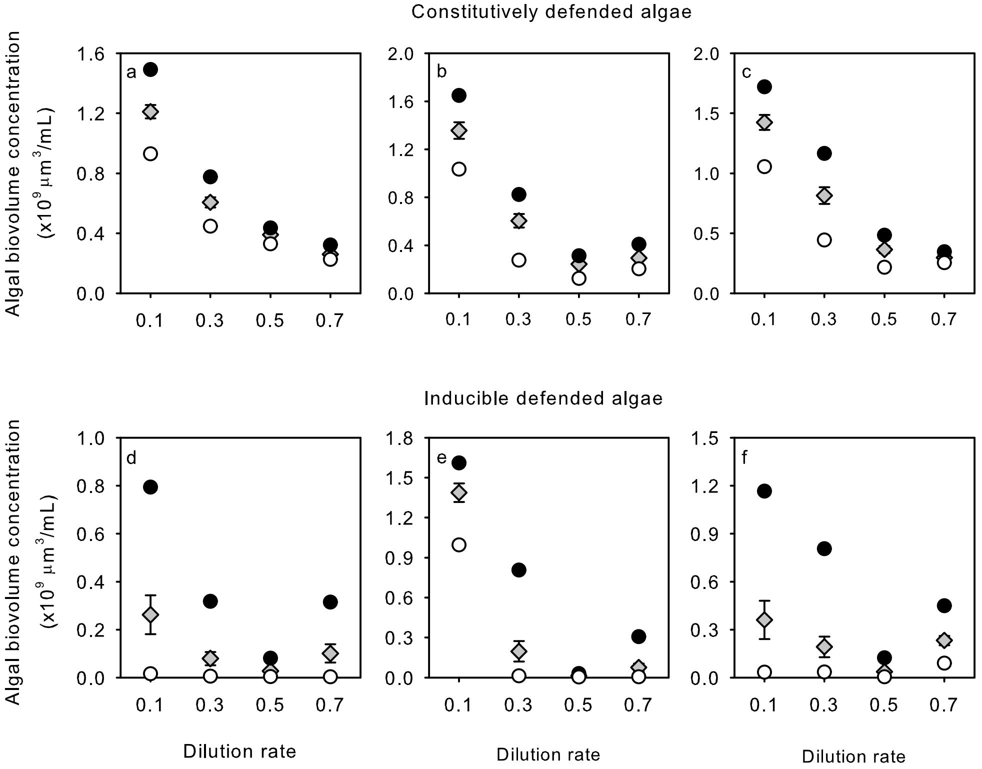


Figure 8.3 Variation in algal densities at each dilution rate in the chemostats. The mean biomass of algae per dilution rate is denoted in filled diamonds (mean±SE), open and closed circles indicate minima and maxima per dilution rate. All three replicates are shown (a-c) constitutively defended algae *Desmodesmus quadricauda*, (d-f) inducible defended algae *Scenedesmus obliquus*.

Chemostats with inducible defended algae and rotifers

At a low dilution rate of 0.1/day algal minimum densities were close to zero in two out of three chemostats while maximum densities were very high indicating population cycles (Fig. 8.3d-f). Increasing the dilution rate to 0.3/day narrowed the difference between the minima and maxima and at the dilution rate of 0.5/day this difference was further minimalized to almost zero indicating a stable population at low abundance of algae. At the highest dilution rate of 0.7/day the difference between minima and maxima increased again. The patterns of algal dynamics were almost identical for the three replicates (Fig. 8.3d-f).

Brachionus densities were low at high dilution rates, and densities increased with decreasing dilution rate. In one replicate *Brachionus* highest population densities were reached at a dilution rate of 0.1/day following the algal pattern (Fig. 8.4f), while in the other two replicates the highest densities were reached at a dilution rate of 0.3 or 0.5/day (Fig. 8.4d, e). Peak herbivore densities coincided with their highest minima in two out of three replicates, indicating that the variation in density is minimal at these dilution rates. In one replicate *Brachionus* actually went extinct at the low dilution rate of 0.1/day (Fig. 8.4e). This immediately released the grazing pressure on the algae resulting in a higher algal minimum for this replicate (Fig. 8.3e).

Discussion

The model results showed that three qualitatively different types of dynamics occurred for bitrophic food chains in continuous cultures, i.e., only prey, stable equilibrium and predator-prey cycles. The model predicted stable equilibrium over a wider range of conditions for inducible defenses than for the other defense strategies. These predictions were tested in chemostat experiments. At intermediate dilution rates we observed low variation in population densities indicating relatively stable population dynamics in the chemostats with inducible defenses. Towards lower dilution rates the variation increased as if crossing the stability boundary and a stochastic extinction of the predator occurred in one replicate with highly variable population densities.

Herbivores did not seem to have a major impact on the algal abundances of constitutively defended *D. quadricauda*. The algal abundances in these chemostats followed the pattern of dynamics that was predicted for a system with only algae, which is coherent to the low *Brachionus* densities throughout the experiment. According to the model results for the permanently defended algae, however, *Brachionus* should be able to build up a population at the lowest dilution rate of 0.1/day (Fig. 8.2a), irrespective of the concentration of inflowing nutrients. We

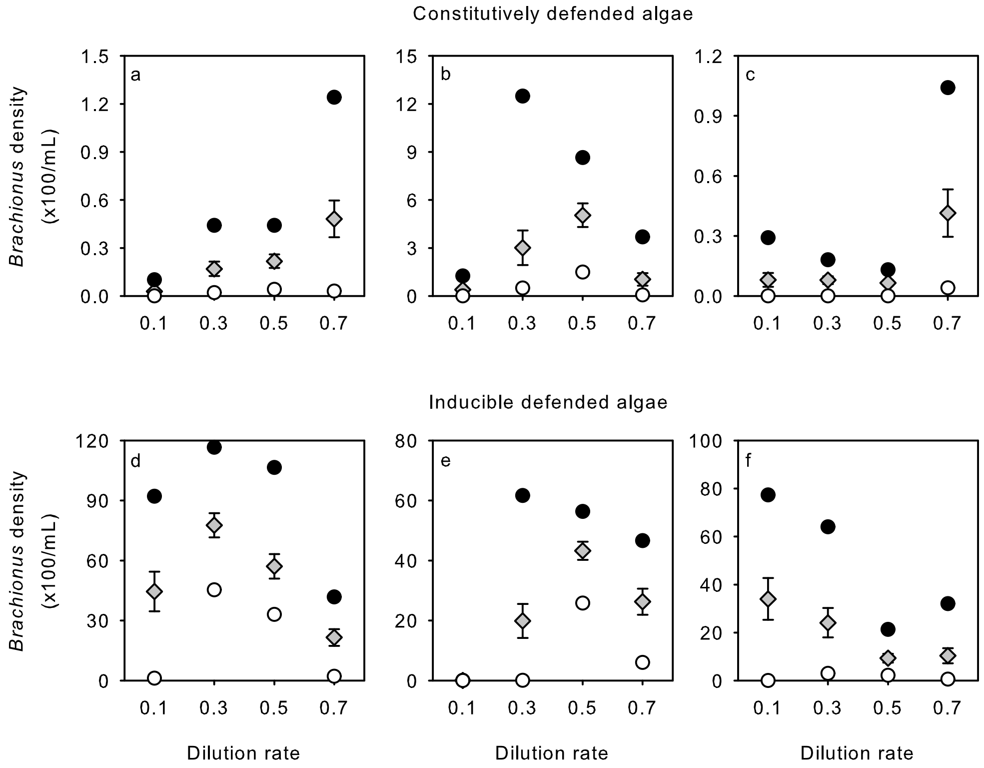


Figure 8.4 Variation in rotifer densities at each dilution rate in the chemostats. The mean biomass of *Brachionus* per dilution rate is denoted in filled diamonds (mean±SE), open and closed circles indicate minima and maxima per dilution rate. All three replicates are shown (a-c) constitutively defended algae *Desmodesmus quadricauda*, (d-f) inducible defended algae *Scenedesmus obliquus*.

assume that *D. quadricauda* has a higher handling time or lower conversion efficiency in the chemostat experiment than was estimated a priori in the model, which may have led to the low herbivore numbers in the experiment.

The results for the experiment with the inducible defended algae *S. obliquus* are in accordance with the expectations on basis of the model. At intermediate dilution rates of 0.3/day and 0.5/day *Brachionus* established a large population with minimal densities away from zero. The decrease in *Brachionus* densities at the highest dilution rate showed that we are moving towards the point where *Brachionus* will be flushed out and we would cross the existence boundary. Remarkably, crossing the transcritical bifurcation does not actually occur, indicating that *S. obliquus* might have a lower handling time or higher conversion efficiency than was assumed in the model. The strong variation in density that was observed at the lowest dilution rates of 0.1 and 0.3/day show that we have crossed the Hopf bifurcation, as predicted by the model. As the amplitude of these cycles increases, stochastic extinction of the herbivore or the algae becomes a threat to their coexistence. We observed one such extinction in our experiments at a dilution rate of 0.1/day (Fig. 8.4b), indicating that there is a considerable chance of losing the herbivore from the system in this way.

There is a clear difference in algal dynamics between the chemostats with *S. obliquus* and the ones containing *D. quadricauda* (Fig. 8.3), while the dynamics of each replicate within treatments were rather similar. This holds to a lesser extent for the observed patterns of *Brachionus* abundance (Fig. 8.4). We can only speculate what is causing this higher variation between replicates in the *Brachionus* data, it could be either due to subtle differences in edibility of algae between replicates or an inherent property of culturing rotifers in chemostats.

The experimental set-up included a treatment with undefended *D. bicellularis* for comparison with the dynamics with the other algal defense strategies. Initially, large fluctuations in algal and rotifer densities were observed at high dilution rates (not shown). These treatments, however, unintentionally became invaded by inducible defended *S. obliquus.* Consequently the undefended algae were outcompeted by inducible defended algae, despite the fact that the undefended algae have a higher growth rate than the inducible defended algae (Van der Stap et al. 2006). This successful invasion can be understood from the fact that it happened under herbivory and the undefended algae are more vulnerable than the inducible defended algae. In Kooi and Kooijman (2000) a bifurcation analysis is presented for the dynamics of a nutrient-two-prey-predator system in a chemostat. A result of the analysis was that the presence of a predator can allow coexistence of two competing prey populations feeding on a single nutrient. The loss of the undefended treatment is unfortunate because in a previous experiment the largest distinction in dynamics was observed between the treatment with undefended algae and treatments with inducible or permanently defended algae (Van der Stap et al. 2006).

The cost of defenses has 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 in higher sinking rates (Lürling and Van Donk 2000). Colonies disintegrated less rapidly with increasing daily light dose which reveals photosynthetic costs associated with colony maintenance (Verschoor et al. 2005). Under natural conditions sedimentation and light cycle are two examples of environmental costs. The used experimental set-up 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.

Our results support previous experiments where it was shown that population fluctuations did not occur with inducible defenses in a batch culture system (Van der Stap et al. 2006) or semi-continuous system (Verschoor et al. 2004b). In a two-stage chemostat system where prey and predators were grown separately (Boraas 1983), the algae did not form defenses and large fluctuations occurred again (Lürling et al. 2005). In the one-stage chemostats used here with inducible defended algae, fluctuations occurred at low dilution rates but were diminished at intermediate dilution rates.

We conclude that our model results clearly show that qualitative shifts in predator-prey dynamics as a function of dilution rate occur and that the dilution rates at which these shifts take place are dependent on the algal defense strategy. The experimental results showed such qualitative differences as a function of dilution rate in the dynamics of food chains with inducible defenses in the algae. In food chains with constitutive defended algae rotifer densities remained low and algal dynamics resembled a model without herbivores.

Chapter 9

General discussion

Introduction

In this thesis the effects of individual level plasticity on population dynamics and persistence were investigated. The specific research question was raised whether various defense strategies of organisms have distinguishable effects on the dynamics of experimental plankton communities. From theoretical studies it was predicted that inducible defenses stabilize population dynamics as opposed to constitutive defenses or the absence of defenses. But do theory and observations match? This topic and a few selected others will be discussed, some future prospects are indicated and the main conclusions are drawn.

Parts of this chapter are based on the paper: Van der Stap, I., M. Vos and W.M. Mooij. Inducible defenses and rotifer food chain dynamics. Hydrobiologia in press.

Individual-level responses

Inducible defenses in aquatic systems occur in a wide array of organisms ranging from algae to vertebrate animals (Tollrian and Harvell 1999). Phenotypically plastic responses in morphology interrupt the predation cycle between the attack and capture, whereas behavioral responses may interrupt the cycle already before the predator encounters the prey (Brönmark and Hansson 2000). Morphological defended individuals are hardly ever completely invulnerable and are therefore still attacked and handled as prey (Jeschke and Tollrian 2000). This results in a longer handling time for gape limited predators. The effectiveness of behavioral responses like refuge use or DVM is usually expressed in a reduced attack rate. Inducible defenses therefore affect the functional response of their consumers (Jeschke and Tollrian 2000, Verschoor et al. in press).

The algae of the Scenedesmaceae family showed either no response or inducible colony formation to released infochemicals by actively feeding herbivores (Chapter 2). The observed response was not always consistent with the response in the live predator-prey experiments. The particle volumes of *D. subspicatus* UTEX 2594 and *D. bicellularis* LB 1359 almost doubled in the presence of a live herbivore (Chapter 3 and 5 respectively), while these strains did not show a response in particle size to herbivore infochemicals (Chapter 2). Therefore we hypothesized that this algal strain had an infochemical threshold that was higher than administered in the bioassays. The initial size of algae in the experiments ranged between smaller than 100 μm^3 for fixed undefended algae, between 100-400 μm^3 for inducible defended algae and larger than 400 μm^3 for constitutive defended algae. This indicates that there is a relation between size and defense strategy. The small algae have a high growth rate and remain small in presence of herbivores, the medium sized algae show an increase in particle volume and cell number in response to herbivores while large algae, have a lower growth rate, but remain large throughout their life cycle. This trade-off suggests that costs are involved in the evolution of inducible defenses. In Scenedesmaceae the costs of colony formation may involve sedimentation out of the euphotic zone (Lürling and Van Donk 2000) or costs to the returning to unicells (Verschoor et al. 2005). The costs of postero-lateral spine formation in *Brachionus* have not been investigated here, but may be in the increased drag when swimming around as suggested by Gilbert (1966).

The induction process is a consumer density-dependent process (Anholt and Werner 1999, Van Donk et al. 1999). In the experiments with organisms with inducible defenses the percentage of induced algae and/or rotifers was very high, indicating a very low threshold for induction of the morphological response. The inducible defended organisms appear as constitutive defended even at very low predator densities. This influences the half saturation constant in the induction and decay function in the inducible defenses model (Vos et al. 2004a, 2004b; Chapters 4 and 8).

Population-level responses

The empirical studies in this thesis have shown that inducible defenses act as an important ecological factor with effects at the population level (Chapters 3, 5, 6, 7 and 8). The experimental observations can be interpreted in the context of the model by Vos et al. (2004a), which predicted stability of predator-prey interactions for only a narrow range of parameter values when food chains were based on undefended algae. In contrast, for food chains with inducible defenses, stable coexistence of predator and prey was predicted for a relatively wide range of conditions. The dynamics in the tritrophic experiments with *Brachionus calyciflorus* were high amplitude fluctuations, which seemed to dampen out in the case of undefended algae at the base of the food chain, while a monotonic approach to a stable equilibrium was seen in the case of algae protected by inducible defenses (Chapter 5). The latter is directly in agreement with our expectation as based on the model: inducible defenses stabilized dynamics.

However, the dampened fluctuations in the food chains with undefended algae can be explained in two alternative ways. The first possibility is that dynamics were truly cyclic, but some factor, such as increased heterogeneity through accumulation of detrital particles, stabilized the system late in the experiment. This explanation is consistent with our observations. However, it is also possible that the dynamics were inherently stable, but approached equilibrium through a damped oscillation, instead of monotonically. In this case, the dynamics of systems with undefended versus algae protected by inducible defenses are not separated by a Hopf bifurcation, but by a stable focus-node boundary, which is situated within the stability area itself (Yodzis and Innes 1992, Vos et al. 2005). On one side of this line a stable equilibrium is approached monotonically, on the other side this occurs through a damped oscillation (Vos et al. 2005). We cannot formally exclude this possibility based on current evidence.

The fit of a standard Rosenzweig-MacArthur predator-prey model to experimental population dynamical data collected in batch cultures (Chapter 4) was greatly improved by the inclusion of inducible defenses into the model. While the width of the stable region is larger when inducible defenses are included, longer handling times of defended prey do increase the risk of extinction by crossing the existence boundary (Area A_0) sooner than undefended prey if nutrients are depleted in the system (Chapter 3 and 4). Inducible defenses have been shown to prevent population fluctuations in both two and three-level food chains and enhance the coexistence of predators and prey (Chapters 3, 5 and 7). However, herbivores may also go extinct below the stability boundary where cycles increase the risk of stochastic extinction (Area B_1). This occurred in one replicate of tritrophic food chains with undefended algae at the basis (Chapter 5) and in one replicate of the chemostat experiment with inducible defenses in the algae at low dilution rates (Chapter 8).

A particularly interesting observation is that inducible defenses at the herbivore level had little or no effect on stability in the model of Vos et al. (2004a, 2004b). It seems paradoxical that some aspects of the food web interactions are determined by plant defenses, such as persistence while others depend fully on the presence of herbivore defenses, such as trophic cascade strength (top-down effects). What is the relative effect of inducible defenses either at plant or herbivore level? In the face of predation the herbivore defenses seemed more important than the plant defenses. Without herbivore defenses there was a clear trophic cascade in three-level food chains (Chapter 6). In this case, the absence of herbivore defenses had an overriding effect on the role of plant inducible defenses.

When induced defenses occur in a variety of species within a community they could make a considerable contribution to a dampening of top-down control and dampening of population fluctuations. The experiments showed that the algae-rotifer model system is very reproducible within batch, semi-continuous and continuous cultures, but caution is needed when making extrapolations to nature based on any of these systems alone. The degree to which our results can be extended to community dynamics depends on the ways in which dominant plant and herbivore species respond to multi-species complexes of their consumers. Many defenses, such as toxins, spines or reduced activity are likely to be effective against a wide variety of consumer species. The overall community-wide effects of inducible defenses will depend on the relative abundance and importance of different species employing these different kinds of defenses. Besides the inducible defense of spines in *B. calyciflorus*, at least two other species in this genus employ predatory defense strategies. *Brachionus rubens* does not exhibit these morphological defenses, rather it has the ability to attach to cladocerans, which may serve as an alternative escape mechanism from predation by *Asplanchna* (Iyer and Rao 1996), although this may actually increase their predation by fish. Rotifers of the *Brachionus plicatilis* complex differ in body size and vulnerability to copepod predation. This differential sensitivity to predation promoted coexistence in a situation where the competitively dominant species were preferred as prey (Ciros-Pérez et al. 2004).

Future research

The majority of studies on chemical communication in aquatic systems including the present study of inducible defenses have been performed under controlled laboratory conditions. In a natural environment toxicants may disrupt information transfer between organisms. The use of rotifers in ecotoxicological studies has substantially increased over the last decade (Snell and Janssen 1995). The algae-herbivorous rotifer-carnivorous rotifer system could perfectly be used to test the effect of toxicants on the higher trophic levels. In this way it can provide a measure of ecological impact that goes beyond the individual level. For ecotoxicological studies it is of great importance to clarify the chemical structure of the infochemical. Progress has been made the last few years in elucidating the infochemical inducing colony formation in the alga *Scenedesmus* by *Daphnia* (Lampert et al. 1994, Von Elert and Franck 1999, Van Holthoon et al. 2003). The most recent report indicates that aliphatic sulfates may be involved (Yasumoto et al. 2006). No recent analyses on the kairomone that induces spine formation in *Brachionus* by *Asplanchna* have been made, but earlier observations suggested that the chemical signal involved in this induction is a heat-stable protein (Gilbert 1967). The use of new techniques offers opportunities to gain more insight into the chemical ecology (Pohnert et al. 2007), as well as the genetic and developmental basis of inducible defenses. These include an ecogenomics (Dicke et al. 2004) and physiological approach.

Another aspect to be considered is that in an environment where predators and prey interact, predators can induce prey to defend, and predators may subsequently respond to morphological prey defenses. This so-called reciprocal phenotypic plasticity occurs for example in the predator-prey interaction of ciliates (Kopp and Tollrian 2003) and of salamanders and tadpoles (Kishida et al. 2006). A reciprocal phenotypic response has not yet been studied or observed for the experimental predator species *A. brightwellii.* However, omnivorous *Asplanchna priodonta* is able to grow larger jaws (Fontaneto and Melone 2005) and this may be one way of how a predator can respond to spines in brachionid prey. For behavioral responses predators may fool their prey by chemical camouflage or suppression of the release of chemical substance as, for instance, in trout (Brown et al 1995).

In natural systems the presence of inducible defenses is not the only mechanism that stabilizes webs. Other mechanisms include, e.g., omnivory (Kooi et al. 2002), intraguild predation (Diehl and Feissel 2001), mutualism (Hay et al.

2004) or resource switching (Van Baalen et al. 2001). Future studies might investigate the interplay between inducible defenses and other stabilizing mechanisms. Theoretical models could be used to generate hypotheses on the interaction between these various mechanisms. The generated hypotheses may subsequently be tested using an experimental approach. The model system of algae and rotifers proved to be the ideal model system to test the higher order effects of inducible defenses on population dynamics (this thesis). The advantage of this system is that it may easily be expanded and the role of inducible defenses in combination with other factors or in multi-species more complex food webs can be investigated. The factors taken into account may be the addition of trophic levels, e.g., fish or carnivorous zooplankton, increased biodiversity at each trophic level, or omnivory, e.g., by omnivorous rotifers *Asplanchna priodonta*. Such an approach might help in disentangling the importance of the various mechanisms that affect the stability and persistence of food webs.

Conclusions

Phenotypic plasticity in individual prey can have profound effects on population dynamics, affecting both consumers at higher trophic levels and the prey populations themselves. Plasticity in defenses creates variation in handling times and/or attack rates of predators on different prey types. This can cause prey with inducible defenses, as opposed to those without defenses, to prevent strong population fluctuations in both bi- and tritrophic food chains. Linking the empirical results with a theoretical model showed that different defense strategies of prey place model predators at distinct locations in the parameter space of the model with associated different risks of extinction. The strength of trophic cascades was diminished by the presence of inducible morphological defenses, especially when present at the middle trophic level of tritrophic food chains. When the simple food web was based on inducible defended algae, differently defended herbivores which were competitors coexisted for a longer period under shared predation. Inducible defenses have an effect on both stability and persistence of experimental food webs, and might have a similar effect in nature.

Summary

Starting at the individual plant level we investigated many strains of Scenedesmaceae for their response to infochemicals released by the herbivores *Daphnia* or *Brachionus* (Chapter 2). The algal strains showed either no response, i.e., were either fixed undefended or constitutively defended, or inducible defense by colony formation. Colony size was found to increase with *Brachionus* infochemical concentration and could be described by a sigmoid function. Inducible colony formation in Scenedesmaceae may be a general defense response to herbivory by zooplankton.

To identify whether phenotypic plasticity at the individual level had consequences for population dynamics the effect of defense strategy was tested in batch cultures of *Scenedesmus* and *Brachionus* (Chapter 3). We observed fluctuations in the herbivore populations when feeding on undefended algae and a rise and decline of population densities when the algae were larger due to inducible or constitutive defenses. Persistence and extinctions of the herbivore were related to the concentration and fraction of small algal particles needed for maintenance and reproduction. Inducible defenses in the algae may favor larger zooplankton species such as *Daphnia* spp. that are less sensitive to shifts in their food size spectrum, relative to smaller zooplankton species, such as rotifers and in this way contribute to the structuring of planktonic communities.

In Chapter 4, we further teased apart the mechanism of persistence and extinctions observed in the bitrophic food chains using a modeling approach. Mechanistic predator-prey models were fitted to the experimental population dynamics data collected in batch cultures. Realizing that the inducible defense model by Vos et al. (2004) assumed constant conditions, the model was modified for application to the transient dynamics in a batch experiment. A good match was obtained between model and data, with 77% of the variance in the data being accounted for by the model. Moreover, results from the model showed that quantitative differences in handling times of prey placed the system at different locations in parameter space, in particular relative to the predator existence boundary. Long handling times on defended prey brought predators closer to the existence boundary, where they go extinct earlier when nutrients are being depleted in the system than predators that are further away from the existence boundary.

The length of the experimental food chain was increased by adding a top-predator to examine the effects of inducible defenses in two- and three-level food chains (Chapter 5). High fluctuations were observed in food chains with undefended algae and these did not occur when algae had inducible defenses. Surprisingly, the addition of a third trophic level did not result in a trophic cascade.

This led to the hypothesis that herbivore defenses may have prevented an increase in plant biomass in food chains with a top-predator. This hypothesis was tested in Chapter 6, where a significantly higher plant biomass in three-level than in two-level food chains occurred whenever herbivores were undefended against carnivores. We compared trophic cascade strengths between the plankton communities in which herbivores were undefended against the results of Chapter 5 where herbivores exhibited inducible defenses. The herbivore defenses, not plant defenses, had an overriding effect on cascade strength.

Aquatic communities are complex and their structure is influenced by both competition and predation. In chapter 7 we increased the complexity of our food chain by having the inducible defended and undefended herbivores in competition. Addition of a predator to these competitors resulted in reversal of the outcome of competition. In absence of predation the undefended herbivore was the superior competitor over the non-induced defended herbivore. Conversely, in the presence of predation the inducible defended herbivore outcompeted the undefended herbivore. The persistence of the undefended herbivore was prolonged by the presence of inducible defenses in the algae.

As a final test of the hypothesis that inducible defenses stabilize dynamics over a wide range of conditions, chemostats were used with various dilution rates imposing different mortality rates on all organisms of the system (Chapter 8). The experimental results showed that qualitative differences occurred in the dynamics of food chains with inducible defenses depending on dilution rate.

Appendix

Table A.1 Composition of the standard medium used in all cultures and experiments. Nutrients were dissolved in de-ionized water.

Compound	mg/L
Major nutrients	
$CaCl_3 \cdot 2\ H_2O$	36.76
$FeCl_3 \cdot 6\ H_2O$	1.00
H_3BO_3	24.00
$K_2HPO_4 \cdot 3\ H_2O$	8.71
$Na_2EDTA \cdot 2\ H_2O$	4.36
$NaHCO_3$	12.60
$NaNO_3$	85.01
$MgSO_4 \cdot 7\ H_2O$	36.97
Trace elements	
$CoCl_2 \cdot 6\ H_2O$	0.01200
$CuSO_4 \cdot 5\ H_2O$	0.00100
H_2SeO_3	0.00016
$MnCl_2 \cdot 4\ H_2O$	0.18000
$NaMoO_4 \cdot 2\ H_2O$	0.02200
Na_3VO_4	0.00180
$ZnSO_4 \cdot 7\ H_2O$	0.02200
Vitamin	
$C_{63}H_{88}CoN_{14}O_{14}P$ (B_{12})	0.00055

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Samenvatting

Een prooi kan zich op drie verschillende manieren verdedigen tegen predatoren (1) niet (onverdedigd), (2) altijd (permanent verdedigd) of (3) alleen wanneer er predatoren in de buurt zijn (induceerbaar verdedigd). In dit proefschrift wordt onderzocht of deze drie verdedigingsstrategieën mogelijk verschillende effecten hebben op de populatiedynamica en het voortbestaan van predatoren en prooien. Dit heb ik gedaan middels experimenten in een modelvoedselketen voor het zoete water, bestaande uit algen, algenetende raderdiertjes, en de predatoren van de algeneters.

De ééncellige groenalg *Scenedesmus obliquus* vormt meercellige kolonies in reactie op informatiestoffen uitgescheiden door het herbivore (algenetende) raderdiertje *Brachionus calyciflorus*. *Brachionus* wordt weer gegeten door carnivore (vleesetende) raderdieren van het geslacht *Asplanchna*, en ontwikkelt lange stekels in reactie op infochemicaliën van *Asplanchna*. De (induceerbare) vorming van kolonies in *Scenedesmus* en van stekels in *Brachionus* wordt gezien als verdedigingsmechanisme tegen hun predatoren. Volgens de theorie zouden voedselketens met induceerbare verdediging zowel meer als minder sterke fluctuaties in aantallen kunnen hebben dan voedselketens met hetzij onverdedigde soorten, hetzij permanent verdedigde soorten. Voor het ophelderen van waar dit verschil van afhangt in meer natuurlijke systemen zijn experimenten een belangrijke bron van verder inzicht.

In hoofdstuk 2 hebben we gekeken naar het voorkomen van verschillende verdedigingsstrategieën in verschillende stammen van de groenalgenfamilie Scenedesmaceae. Deze stammen werden blootgesteld aan infochemicaliën afkomstig van de herbivoren *Daphnia* en *Brachionus*. Soms vonden we hierbij geen respons, dat wil zeggen dat deze stammen ofwel onverdedigd, danwel permanent verdedigd waren. In andere gevallen zagen we een duidelijke toename van koloniegrootte als reactie op de infochemicaliën, wat wijst op induceerbare kolonievorming. De grootte van de kolonies nam toe met een toenemende infochemicaliën concentratie en kon worden beschreven met een sigmoïde functie. Omdat we induceerbare kolonievorming vonden in stammen van twee verschillende geslachten binnen de Scenedesmaceae, en omdat deze respons optrad bij infochemicaliën van twee verschillende soorten herbivoren, kan induceerbare kolonievorming een algemene verdedigingsreactie zijn op algenetend zoöplankton.

In hoofdstuk 3 zijn de effecten van verschillende verdedigingsstrategieën nader onderzocht in kweken met daarin *Scenedesmus* (algen) en *Brachionus* (herbivoren). We vonden fluctuaties in de herbivorendichtheden wanneer ze onverdedigde algen aten, en een éénmalige toe- en afname van de herbivorendichtheden wanneer de algen induceerbaar of permanent verdedigd waren. Het voortbestaan en uitsterven van de herbivoren was gerelateerd aan het aandeel en de dichtheid van kleine algendeeltjes, welke het meest geschikte voedsel bleken te zijn voor de raderdiertjes. Induceerbare verdediging in de algen pakt ongunstiger uit voor kleinere zoöplankton soorten zoals raderdieren dan voor grotere zoöplankton soorten zoals *Daphnia* en kan op deze manier een bijdrage leveren aan het structureren van plankton gemeenschappen.

In hoofdstuk 4 zochten we met behulp van een model verder uit wat de oorzaak van voortbestaan danwel uitsterven was, zoals we gezien hadden in de voedselketens met twee trofische niveaus. Hiervoor werden predator-prooi modellen gefit op experimentele data verkregen uit de bovengenoemde kweken. Bij de beste fit kon 77% van de variantie in de data door het model verklaard worden. Bovendien lieten de modelresultaten zien dat verschillen in prooiverwerkingstijden belangrijk waren voor het voortbestaan van de predator. Langere verwerkingstijden van verdedigde prooien brachten predatoren dichter bij hun bestaansgrens, waardoor ze eerder uitstierven wanneer de draagkracht van het systeem omlaag ging.

We hebben nader onderzoek verricht naar het verschil in populatiedynamiek tussen onverdedigde en verdedigde *Scenedesmus* in systemen waarbij de voedingsstoffen dagelijks aangevuld werden (hoofdstuk 5). Om hierbij de verschillen tussen voedselketens met twee en drie trofische niveaus te bestuderen, werd al dan niet een top-predator (*Asplanchna*) toegevoegd aan het *Scenedesmus*-*Brachionus*-systeem. In voedselketens met onverdedigde algen vonden we grote fluctuaties in dichtheden van alle organismen, welke niet optraden wanneer algen geïnduceerd verdedigd waren. Verrassend genoeg leidde de toevoeging van *Asplanchna* niet tot meer *Scenedesmus*, een zogeheten trofische cascade (de invloed van een top-predator, via de herbivoor, op de algen).

Dit leidde tot de hypothese dat verdedigde herbivoren een algentoename kunnen voorkomen in voedselketens met een top-predator, welke werd getest in een volgend voedselketen-experiment (hoofdstuk 6). In voedselketens met onverdedigde herbivoren vonden we inderdaad significant hogere algendichtheden in voedselketens met drie trofische niveaus ten opzichte van voedselketens met twee niveaus. We bepaalden de sterkte van de trofische cascade in deze voedselketens met onverdedigde herbivoren, en vergeleken deze met de resultaten van hoofdstuk 5 (geïnduceerd verdedigde herbivoren). Hieruit bleek dat het effect van plantenverdediging op de sterkte van de cascade teniet werd gedaan door de aanwezigheid van herbivorenverdediging.

Aquatische gemeenschappen zijn complex en hun structuur wordt beïnvloed door zowel competitie als predatie. In hoofdstuk 7 vergrootten wij de complexiteit van de voedselketen door herbivoren met en zonder induceerbare verdediging met elkaar te laten concurreren. Het toevoegen van een predator aan deze concurrenten resulteerde in omkering van de competitieve verhoudingen. In afwezigheid van predatie was de onverdedigde herbivoor de dominante soort, maar in aanwezigheid van predatie moest deze het onderspit delven tegen de induceerbaar verdedigde herbivoor. Het voortbestaan van de onverdedigde herbivoor werd begunstigd door de aanwezigheid van induceerbare verdediging in de algen.

Als laatste test van de hypothese dat induceerbare verdediging stabiliserend werkt op de dynamiek werden continucultures gebruikt. Verschillende doorstroomsnelheden resulteren in verschillende sterftesnelheden van alle organismen van het systeem (hoofdstuk 8). De experimentele resultaten laten zien dat er kwalitatieve verschillen waren in de dynamiek van voedselketens met induceerbare verdediging, afhankelijk van de doorstroomsnelheid.

Dit proefschrift laat zien dat verschillende verdedigingsstrategieën invloed kunnen hebben op zowel predatoren, concurrerende soorten als op de prooisoort zelf. Soorten met induceerbare verdediging kunnen populatiefluctuaties voorkomen in voedselketens met twee of drie trofische niveaus. Analyse van de experimentele resultaten met een theoretisch model liet zien dat verschillende verdedigingsstrategieën tot andere risico's op uitsterven leidden. Het eten van verdedigde prooisoorten leidde tot eerder uitsterven van de predatoren wanneer de draagkracht van het systeem omlaag ging. De sterkte van trofische cascades werd verminderd door de aanwezigheid van induceerbare verdediging, vooral wanneer deze aanwezig was op het middelste trofische niveau (herbivoren). Met induceerbaar verdedigde algen kunnen concurrerende, maar verschillend verdedigde herbivoren langer met elkaar samenleven in de aanwezigheid van een top-predator. Induceerbare verdediging heeft een effect op zowel de stabiliteit als het voortbestaan van experimentele voedselwebben en kan zo mogelijk een belangrijke rol spelen in de natuur.

Dankwoord

Ik wil graag iedereen bedanken die op welke manier dan ook een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift!

Alhoewel een geschreven dankjewel niet alles kan omvatten, wil ik een aantal personen met name noemen. Ik wil graag bedanken mijn promotor, Ellen, voor je bijzondere bijdrage aan het tot stand komen van dit proefschrift. Onze bijeenkomsten waren altijd goed om weer overzicht te krijgen. Ik wil graag ook bedanken mijn twee co-promotoren Wolf en Matthijs, voor jullie bijdrage in de vorm van overleg over proefopzetten, discussies, commentaar op de verschillende manuscripten en hele schrijfsessies. Jullie waren fantastische dagelijkse begeleiders en ik ben jullie dankbaar dat jullie er van begin tot eind zo ontzettend betrokken bij waren. Hartelijk dank ook aan Nico en Arie, voor jullie tomeloze inzet voor de verschillende experimenten en tellingen van fyto- en zoöplankton, zonder jullie vele liters medium, CASY en microscoop-uren had ik het niet zo ver gebracht.

Al mijn werkzaamheden op het Centrum voor Limnologie heb ik uitgevoerd binnen de groep van Voedselweb Onderzoek, alle collega’s over de jaren heen hartelijk dank voor jullie bijdragen. Dankjewel Gabi, voor je steun, het delen van de goede en minder goede tijden op het CL en daarbuiten, en ook voor de warme kopjes thee, vele autoritjes en fantastische duiken met jou en Fin. Dat we nu geen collega’s meer zijn vind ik jammer, maar zelfs beter dan dat zijn we nu goede vrienden gebleven. Miguel, dankjewel dat je altijd klaar stond om te helpen op het lab en dat je altijd wel zin had in een kopje thee of een rondje lopen door het bos, dat vond ik erg fijn. Arnout, dank je voor het delen van de frustaties rond onderzoek en proefschrift en je geweldige verhalen en video-opnames die m’n hoofd af en toe fijn daarvan afleidde. Anthony, jij bent degene met wie ik vooral in het begin op de werkvloer nauw heb samengewerkt. Dankjewel voor de prettige samenwerking en de verschillende werkbezoeken die we samen hebben afgelegd. Stephan, het lijkt al weer lang geleden dat jij mij ontvangen hebt op mijn eerste werkdag op het CL (16 september 2002), ik vond het altijd heerlijk rustig met jou op de kamer. Ik herinner me vooral je passie voor literatuur, de daarbij behorende bibliotheek met referenties en grote kast met artikelen. De lege plek die je achterliet werd later opgevuld door Lisette. Lisette, jij vulde onze werkkamer altijd met warmte, letterlijk en figuurlijk. Hartelijk dank voor alle adviezen en jouw visie

over onderzoek. Ik vond het fijn om samen met jou in de borrelcie grote NIOO- of CL-evenementen te organiseren, een congres te bezoeken en daarna op stap te gaan. Wees niet verbaasd als ik een volgende keer bij een congres ineens voor je neus sta. Bram, als student van Larenstein heb je je volledig ingezet voor het chemostaten experiment, dankjewel daarvoor en veel succes voor de afronding van je huidige universitaire studie aan de UvA.

Alle medewerkers van het NIOO-CL hartelijk bedankt voor jullie ondersteuning en de geweldige tijd die ik daar heb doorgebracht. Speciaal wil ik al het personeel van de ondersteunende diensten danken voor de zovele handelingen achter de schermen die het hele centrum draaiende houden en daarmee bijgedragen hebben aan de afronding van dit proefschrift. Thijs en Peter nu dat mijn proefschrift af is, is het weer tijd voor een goed feest! Jullie hoeven het dit keer niet te organiseren, de geweldige tijd met onze CL borrelcie is voorbij. Ik hoop dat jullie erbij kunnen zijn, zonder jullie is er geen feest compleet. Alle huidige promovendi wil ik veel succes wensen voor het beginnen danwel afronden van hun proefschrift, het CL is echt een uitstekende plek om onderzoek te doen.

Tijdens mijn onderzoek heb ik twee keer drie maanden doorgebracht aan de Ludwig-Maximillians Universität te München in de groep van Wilfried Gabriel. Ich möchte mich bei Ralph, Christian, Ina, Claudia, Elke, und Andreas für die gute Zeit in München bedanken. Mechtild und Lena sei dafür gedankt, dass sie sich um die Rotatorien gekümmert haben. Ein herzlicher Dank geht an Sebastian Diehl für die Benutzung der CASY. Michael und Frank, zwei Studenten der Statistik, danke ich für ihre Untersuchung.

I would like to thank the International Rotifer Family for their wonderful meetings. Dr. SSS. Sarma and Dr. S. Nandini thank you very much for your visit to the Netherlands and for inviting me for a presentation at the conference in Mexico.

Vrienden door dik en dun en bij koud of warm weer Mandy en Achim, jullie waren er altijd voor mij en Greg. Masha danki pa tur kos. Lieve Tessa, Floor, Maartje en Mirjam bedankt dat jullie zulke goede vriendinnen zijn geweest de afgelopen jaren. Beste Opa, dankuwel voor uw liefde, u bent mijn grote voorbeeld. Uw toespraken inspireren mij altijd en zullen dat ook in de toekomst blijven doen. Papa, Mama, Peter en Ronald ontzettend bedankt voor jullie onvoorwaardelijke steun en liefde. Nu we allemaal verder uitvliegen hoop ik dat we elkaar toch nog veel kunnen blijven zien. Danki Gregory, dushi ami stimabo hopi anto den futuro nos ta bai traha nos kas na Boneiru en een dikke knuffel voor jou om je speciaal te bedanken voor je liefde en steun door de jaren heen.

Irene

Curriculum Vitae

Irene van der Stap was born on April 20, 1978 in New York City, NY, USA. Barely a year old she moved with her parents to the Netherlands and grew up in Papendrecht. She went to the Johan de Witt Gymnasium in Dordrecht in 1990 and obtained her Gymnasium diploma in 1996.

She studied Biology at Leiden University from 1996 to 2002. In her second year she specialized in Organismal Biology. Her first research project was an eco-morphological one at the Department of Integrative Zoology, Leiden University. Here she studied the head-morphology of two closely related cichlid species from Lake Victoria under the supervision of Dr. F. Witte. With much enthusiasm she also was a teaching assistant in the practical of invertebrate zoology for first year students supervised by Dr. R. Kooi. Her second project was carried out at the University of Wales in Bangor with Prof. R.N. Hughes from February till September 2001. The subject this time was the behavioral ecology of three-spined sticklebacks. She observed the possible transfer of learned skills of these sticklebacks, while attacking and consuming various kinds of prey.

After having successfully completed her Biology studies in 2002, she started the Ph.D project: “Inducible defences: from individual plasticity to food web dynamics and persistence” at the Department of Food Web Studies, Netherlands Institute for Ecology (NIOO-KNAW, CL) at Nieuwersluis, the Netherlands. She was advised by Dr. W.M. Mooij and Dr. M. Vos, who were engaged with theoretical models that predicted the effects of inducible defenses on population dynamics. Irene chiefly carried out the experiments to check these model predictions, but also performed some model studies. The organisms that were the subject of observation here were various rotifers and green algae. For this PhD project she also worked at the laboratory of Dr. R. Tollrian in Munich, Germany, twice for a period of three months.

Since February 2007 Irene works as a post-doctoral researcher at the Department of Biology, University of Miami, Coral Gables, FL, USA on the development of a grid-based version of the Everkite model of snail kites in central and southern Florida for use in scenario evaluation for the Comprehensive Everglades Restoration Plan.

List of Publications

Van der Stap, I., M. Vos and W.M. Mooij. 2006. Linking herbivore-induced defences to population dynamics. Freshwater Biology 51(3):424-434.

Van der Stap, I., M. Vos, A.M. Verschoor, N.R. Helmsing and W.M. Mooij. Induced defenses in herbivores and plants differentially modulate a trophic cascade. Ecology in press.

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Van der Stap, I., M. Vos and W.M. Mooij. From inducible defenses to predator-prey dynamics: linking models and experimental data. Submitted.

Van der Stap, I., M. Vos, R. Tollrian and W.M. Mooij. Inducible defenses, competition and shared predation in planktonic food chains. Submitted.

Van der Stap, I., M. Vos, B.W. Kooi, E. van Donk and W.M. Mooij. Do inducible defenses stabilize population dynamics? A chemostat model and experiment. Submitted.