

Adding Complexity to Predator-Prey Interactions: Feeding with Conspecifics on
Heterogeneous Prey

by

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B.Sc., Palacky University, 1995
M.Sc., Palacky University, 1997

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Abstract

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Natural communities are structured by a complex of direct and indirect species interactions. It is well recognized theoretically that if these interactions are weak, the entire community is more likely to persist. Several mechanisms can weaken a predator-prey interaction. I studied interference among conspecific predators and heterogeneity at the prey level. Incorporating these mechanisms into realistic functional responses is required for accurate model predictions at the community level. However, controversy remains on which dependencies need to be included. Using laboratory microcosms, I was able to demonstrate moderate predator-dependence in my model system. This effect was present even at low predator densities and after accounting for prey depletion. In separate experiments, I experimentally compared the functional responses of a gape-limited predator feeding on its prey in the absence or presence of species outside the predator's diet (non-prey). I demonstrated that both density and diversity of non-prey species can also substantially reduce the strength of predator-prey interactions. I further tested this non-prey effect on a long time-scale, where I compared the population dynamics of

predator and prey alone to the dynamics of predator and prey when a non-prey species was present. Prey and predators had both gone extinct at the end of the experiment for all replicates containing only predator and prey. However, in the treatment that included non-prey, all species persisted in 4 out of the 5 replicates until the last day of the experiment. Prey species also spent significantly more time above the detection limit in the treatment with non-prey. In addition, I studied how inducible anti-predator defenses affect an aquatic food web with intraguild predation. I detected substantial clonal variation in the expression of a morphological inducible defense and the long-term experiment showed that the intraguild prey with greater ability to increase their body width as a response to predation also have longer persistence times. These results show that predator interference and prey heterogeneity may be important factors that increase persistence of predator and prey as predicted by theory. Their incorporation into simple food web models can improve our ability to reliably predict community dynamics.

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Chapter 1: GENERAL INTRODUCTION

The assemblages of coexisting species found in nature are structured by direct and indirect interactions among species. These communities are intrinsically dynamic.

Diversity and composition of species as well as the strength and direction of links among them change continuously over time and space. The question arises: How much detail is required to describe such amazing complexity?

Because most species are involved in several consumer-resource interactions simultaneously, often acting as both consumer and resource in different links, food web models have been developed to describe whole communities. Feeding relationships are often depicted by a diagram connecting consumers to their resources and nonlinear mathematical models are used to generate predictions of food web dynamics (Pimm 1982, Cohen et al. 1990, de Ruiter et al. 2005, Rooney et al. 2007). Considerable progress in the characterization of food webs has been made recently using dynamic non-equilibrium models of predator and prey, or tritrophic food chains. Nonetheless, such models and simplified laboratory systems with only two or three species are often unstable and can result in rapid species extinctions (e.g. Gause 1934, Huffaker 1958, Rosenzweig and MacArthur 1963, Luckinbill 1973, May et al. 1974, Fussman et al. 2000, Bonsall et al. 2002). This is in contrast to the long-term persistence of interacting predators and prey species observed in natural settings. The search for mechanisms that allow such persistence has become a central research area in population and community ecology (e.g. May 1973, Pimm and Lawton 1977, Hassell 1978, 2000, Murdoch et al. 2003). Spatially complex environments, metapopulation dynamics, predator interference, adaptive predation, and heterogeneity at the prey level are all potentially stabilizing

mechanisms. Theoretical work generally suggests that the stability and persistence of predators and prey is enhanced if most of the trophic interactions are moderate or weak and several mechanisms reducing the interaction strength have been proposed (McCann et al. 1998, 2000). This is also supported by empirical studies that suggest most interactions in nature are indeed moderate or weak with a few strong trophic links (Wootton 1997, Berlow 1999, Neutel et al. 2002). It also has been demonstrated that variability in the strength of trophic interactions may result in the higher resilience of marine communities (Navarrete and Berlow 2006).

The main objective of my dissertation is to investigate the ecological mechanisms that have the potential to reduce the strength of interactions between predators and prey and thereby contribute to the stability and persistence of food webs. I focused on two mechanisms: i) interference among conspecific predators and ii) interspecific and intraspecific heterogeneity at the prey level. I assessed the potential effect of these mechanisms on the functional responses of model predators in short-term experiments (chapter 2 and 3) and then tested their consequences for long-term population dynamics (chapter 4 and 5). Generally, I was interested in determining whether including these two factors into predator-prey models would increase their realism and predictive power for empirical studies.

1.1. Model organisms and general methods

My research uses a mechanistic approach and combines methods from mathematics, statistics, ecology, and evolutionary biology. Simplified microcosm experiments have been instrumental in the first tests of mathematical models and in the assessment of whether these models should be explored in more natural settings (Desharnais 2005).

Such experiments allow us to look at both short term interactions and long term dynamics of several interacting species, and specifically test particular ecological mechanisms by excluding the inevitable complexity of natural systems. Within these assembled food webs, environmental conditions can be strictly controlled and the investigations can focus on species of interest and their interactions. This control means that highly reproducible results can be obtained using many treatment replications. Building on the work of Shelly Duquette (Duquette 2005 MSc thesis, Duquette et al. 2005), I developed a model microcosm system composed of a top predator, the turbellarian flatworm *Stenostomum virginianum*, feeding on its prey, ciliates *Paramecium aurelium* and various species and clones of the genus *Euplotes*. Hypotrich ciliates of genus *Euplotes* rapidly respond to predatory *Stenostomum* by increasing their maximum body width (Kusch 1993, Altwegg et al. 2004, Duquette et al. 2005) and this inducible anti-predator defense can substantially reduce predation rates by the gape-limited *Stenostomum* (Altwegg et al. 2006). Algae or bacteria were used as basal resources for my experimental food webs.

I investigated per capita consumption rates of the model predator as a function of prey density under different conditions (predator interference, non-prey species). These functional responses are a major component of many community and food web models and are well known to modify their stability and persistence. I compared the dynamics of food web modules composed of different species and their interactions and included ecological mechanisms that were predicted to have a stabilizing effect (inducible defenses, non-prey species). Persistence of these experimental food webs was then measured. My experimental setup allowed me to investigate predictions of mechanistic

models that are rarely tested in natural systems due to logistical considerations and long generation times of many species.

1.2. Interference among conspecific predators

The first attempt to incorporate mutual interference among predators in order to increase stability of a host-parasitoid model was carried out four decades ago by Hassell and Varley (1969). Despite considerable attention to the area, especially in the realm of theoretical ecology, the relevance of predator interference for modeling real food webs continues to be widely debated (Abrams and Ginzburg 2000, Fussmann et al. 2005, 2007, Jensen et al. 2007). Pilot data and the personal observations from our lab suggested some intraspecific behavioural interactions among *Stenostomum* flatworms exist. I overcame the limitations of many previous studies and tested for the effect of conspecific density on per capita consumption rates in our model system (Chapter 2). The analysis accounted for prey depletion and also was repeated at low predator densities. Furthermore, I conducted behavioural observations of foraging predators in order to estimate the encounter rate among conspecifics.

1.3. Interspecific heterogeneity at the prey level

1.3.1. Functional responses modified by non-prey species

Heterogeneity at the prey level may result from the presence of non-prey species that are permanently inedible for predators. The classical assumption of predator-prey models is that per capita consumption rates depend solely on prey density (Holling 1959).

Functional responses are often investigated in simplified systems in which predators only

encounter a single prey species and other species in the food web are excluded. This assumption is, however, rarely met in natural communities, where the vast majority of species within the food web are non-prey. These often ignored species may modify trophic interactions by diluting prey concentration, providing a masking background for prey and/or by confusing predators through interference mediated by similarity with prey. In Chapter 3, I assessed how density, diversity, and identity of non-prey species (species outside a predator's diet) modify the strength of predator-prey interactions and was able to show that the presence of non-prey species can reduce the strength of predator-prey interactions (Kratina et al. 2007).

1.3. 2. Predator-prey dynamics modified by non-prey species

The long-term effect of non-prey species was tested in Chapter 4. Specifically, I was interested to determine whether the weakening of predator-prey interactions by non-prey can promote the persistence of predator, prey and non-prey species.

In cooperation with my colleagues from the Netherlands Institute of Ecology (NIOO-KNAW), I carried out a series of pilot chemostat experiments. In one experiment, I compared the dynamics of a model predatory flatworm feeding on its ciliate prey in the presence and absence of two non-prey rotifers. I also included a treatment with predator alone feeding on basal resources (algae), in order to assess the level of omnivory of the top predator. Treatments consisting of prey alone versus prey in the presence of the two rotifers, all feeding on algae were also investigated to estimate the competition strength between non-prey and prey. Despite considerable effort I was not able to find an effect of non-prey species on persistence of my experimental food webs as all species went rapidly extinct. I was unable to detect any difference in the population decline of prey (Fig. 1-1.

a) or predator (Fig. 1-1. b) between the treatments with and without the two non-prey species. Non-prey rotifers never increased in density to numbers that may have substantial effect on the process of predation.

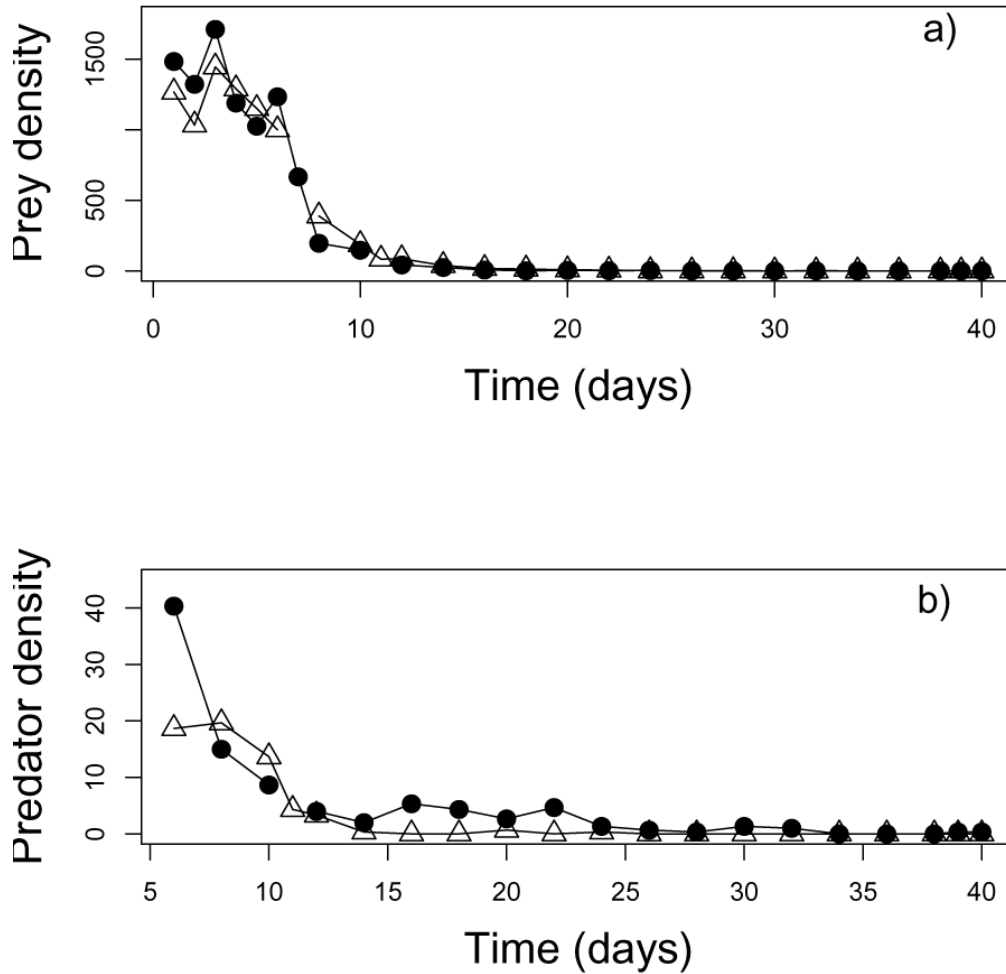


Figure 1-1 The mean ($n = 3$) dynamics of a) prey and b) predator did not differ among the treatments and rapidly declined to very low densities. Solid circles denote the treatment with two non-prey rotifers, open triangles represent the treatment without the two non-prey species. Densities are expressed in number of individuals per 100 mL.

However, results from the chemostat trials helped me design a new long-term microcosm experiment in semicontinuous cultures (Chapter 4). This large scale experiment included six different combinations of predator, prey, and non-prey species, each replicated five times. Using different non-prey species, basal resources, and nutrient replacement, I was able to maintain predators, prey, and non-prey over 250 prey generations. The new design allowed me to separate the effects of predation and competition, and detect the effect of non-prey species on the dynamics of a simple food web.

1.4. Intraspecific prey heterogeneity

In addition to variability in predator vulnerability among prey species, heterogeneity at the prey level also arises from phenotypic plasticity expressed among individuals of a single species. Across many ecosystems, hundreds of species respond to relevant cues from predators, herbivores, parasites, and competitors by expressing inducible defenses (Gilbert 1966, Harvell 1990, Karban and Baldwin 1997, Tollrian and Harvell 1999). Prey species can modify one or more elements of their morphology, physiology, life history, or behaviour in the presence of predators and this change is often reversible when predation risk declines. Although the consequences of inducible anti-predator defenses for individual fitness are relatively well studied, population and community consequences of these changes are much less explored (but see Verschoor et al. 2004, van der Stap et al. 2006, 2007, 2008). Inducible defenses can provide a refuge from predation, thereby reducing the interaction strength between predators and prey. Density-dependent variation in the expression of inducible defenses can result in a negative feedback loop (more predators-more defended prey-fewer predators-fewer defended prey-more predators) and potentially stabilize predator-prey dynamics.

Substantial variability in the expression of inducible defenses among closely related species or even among clones of the same species has been demonstrated by others (Wiackowski et al. 2003, Duquette et al. 2005) as well as by my own early pilot studies. In one experiment where I measured the increase in the maximum body width of *Euplotes* after 24 hour incubation with predatory *Stenostomum*, I was able to identify two highly reactive clones and two less reactive clones of *Euplotes*. I used this material to conduct a long-term chemostat experiment comparing the dynamics of these four clones embedded in intraguild food webs. The experimental design allowed me to control environmental variables, meaning the only systematic variation in the experiment was the magnitude of anti-predator defense expressed by different clones. This inducibility was then related to the persistence time of the intraguild prey in experimental food webs (Chapter 5).

Mechanistic understanding of species interactions has broad application. Natural communities are stressed by accelerating extinctions of native species and invasions of alien species (Loreau et al. 2006). The consequences of such homogenizations are largely unknown. Understanding the role of often ignored species, such as non-prey, and identifying ecological mechanisms that allow systems to persist over long periods of time is especially timely and crucial for the development of conservation strategies.

Chapter 2: FUNCTIONAL RESPONSES MODIFIED BY PREDATOR DENSITY¹

2.1. Introduction

Food webs are descriptive devices defined by the linkages between consumers and their resources. These systems are extraordinarily complex and intrinsically dynamic.

Functional responses are central components of community and food web models and their mathematical forms strongly influence the dynamics and stability of ecological systems (May 1973, Oaten and Murdoch 1975, Vos et al. 2001, Gross et al. 2004, McCann et al. 2005). A consumer's functional response is a function of resource density (Solomon 1949, Holling 1959, 1966). However, it has been shown that other species and other predators can directly or indirectly alter the process of predation. Whether these interactions need to be incorporated into food web models and how exactly they affect the shape and the magnitude of functional responses is still poorly understood.

It has been previously demonstrated that foraging success of consumers declines with increasing diversity of resources (Hillebrand and Cardinale 2004), perhaps because consumers become less efficient when the density and diversity of species outside their diet increases (Vos et al. 2001, Grabowski 2004, Kratina et al. 2007). Functional responses can be further modified by the presence of other consumers. Additional predators can either facilitate predation, as in group hunting (e.g. lions), or more often interfere with the ability of a focal predator to successfully find and capture prey (Salt

¹ Kratina, P., M. Vos, A. Bateman, and B. R. Anholt. 2009. Functional responses modified by predator density. *Oecologia* **159**:425-433.

1967, 1974, Arditi and Akçakaya 1990, Osenberg et al. 1999). Many mechanisms have been proposed that can generate a predator-dependent consumption rate of individual predators. For example, predator-dependence may arise from group formation, spatial distribution (Cosner et al. 1999) or strong social interactions among predators (Abrams and Ginzburg 2000). Other processes may include aggressive behaviour resulting from population size-structure and cannibalistic interactions (Crowley and Martin 1989, Crumrine 2005, Rudolf 2006, 2007).

Logistical considerations have constrained many previous empirical studies of predator-dependence to not vary predator and prey densities together and to do so only over a restricted range of densities (Salt 1974, Crowley et al. 1987, Crowley and Martin 1989, Mills and Laca 2004). Often they used relatively few predator densities and compared the per capita consumption rates of the different densities to each other (but see Fussmann et al. 2005, Schenk et al. 2005). Some studies inferred predator-dependence indirectly from time series data (Jost and Arditi 2000, 2001, Jost and Ellner 2000) or the direct experiments failed to account for the depletion of resources over the course of the experiments (e.g., Salt 1974, Hayward and Gallup 1976, Helgen 1987, Crowley and Martin 1989, Hansson et al. 2001, Lüring et al. 2003). Very few studies measured functional responses directly on a demographic time scale and accounted for prey depletion in their analysis. Among such rare exceptions that investigated predator-dependence in natural populations are Vucetich et al. (2002), Jost et al. (2005), and Griffen and Delaney (2007).

Three distinct classes of functional response models have been traditionally used to analyze predator-prey data. Classical “prey-dependent” models assume that the

predation rate is a function of only prey density (Holling 1959). Because intraspecific interactions between individual predators can affect predator efficiency, “predator-dependent” models estimate the predation rates that depend on densities of both prey and predator (Beddington 1975, DeAngelis et al. 1975). “Ratio-dependent” models are a special case of predator-dependence where predation rate does not depend on absolute numbers of either species but only on the prey to predator ratio (Arditi and Ginzburg 1989). Which models should be used for predicting predator-prey or food web dynamics remains controversial (Abrams and Ginzburg 2000, Fussmann et al. 2005, 2007, Jensen et al. 2007).

There is no general agreement on the appropriate time scale of experimental studies. Feeding experiments that are nearly instantaneous relative to the generation times of model organisms are criticized for detecting only physical interference and failing to capture the interference caused by chemicals or inducible defenses (Arditi and Ginzburg 1989, Jensen et al. 2007). Conversely, longer-term studies without prey replacement will produce the appearance of predator-dependence due to faster resource depletion in treatments with higher predator densities (Arditi and Saïah 1992, Abrams 1994). Although predator-dependence is expected to arise at extremely high densities of predators (Abrams and Ginzburg 2000, Fussmann et al. 2005) it is still unclear whether functional responses remain predator-dependent at low predator densities.

In contrast to most previous empirical studies, we aimed to directly estimate the effect of predator-dependence on the detailed shape and magnitude of the functional response while simultaneously varying both the predator and prey densities over a large range. We investigated the functional response of the flatworm *Stenostomum virginianum*

(hereafter predator or *Stenostomum*) feeding on its prey *Paramecium aurelia* (hereafter prey or *Paramecium*). Our major novel contribution was to determine whether the inclusion of predator-dependence in the functional response model improves our description of predation at low predator densities. Furthermore, using an information theoretic approach, we directly compared the fits of three different model classes (prey-dependent, ratio-dependent, and predator-dependent) to the same data set. We used integrated functional response models to account for the decline in prey density over the course of the four-hour experiment (Royama 1971, Rogers 1972, Juliano 2001).

In addition, we conducted a separate experiment wherein we measured and compared the encounter rate of focal predators with conspecifics over a range of predator densities and estimated the time that predators spend interacting with each other. Based on the results of both experiments and fitting models which either included or excluded predator-dependence, we conclude that predation in this system is predator-dependent even at relatively low predator densities.

2.2. Materials and methods

2.2.1. Model organisms and functional response experiment

We measured the per capita ingestion rate of the benthic flatworm *Stenostomum virginanum* (Rhabdocoela, Turbellaria) feeding on its prey *Paramecium aurelia* (Ciliophora) in experimental microcosms where we manipulated the densities of both predator and prey. Both species were originally isolated from sediments of freshwater ponds on the University of Victoria (UVic) campus and maintained as asexually reproducing cultures since then.

We randomly assigned treatment combinations of predator and prey densities into 24-well tissue culture plates. We set prey densities at 60, 160, and 200 individuals in 900 μL of the protozoan medium. Although we always pipetted low (1) medium (9), and high (19) numbers of *Stenostomum* into each treatment, some individuals were lost during the transfer (due to strong affinity of flatworms to the pipette tips). This resulted in an almost continuous distribution of predator experimental densities between 1 and 19 individuals per 900 μL . Actual numbers of predators were counted at the end of the experimental period. The culture medium was prepared by filtering 1.5 crushed protozoan pellets (~ 0.7 g each; Nr. 13-2360, Carolina Biological Supply Company, NC, USA) through double-layered No.4 coffee filters and dissolved in 2 L of mineral water (NAYA). We measured the number of *Paramecium* eaten per predator in four hours. This is an intermediate time frame between instantaneous predation rate measurements and the animal's generation time (48-72 hours). Predation was terminated by the addition of two drops of 5% acid Lugol's solution and all individuals of both species were counted under a dissecting microscope (Leica MZ8).

2.2.2. Data analyses

We used sigmoid functional response models for our analyses, as we have previously demonstrated that predatory *Stenostomum* forages on its prey with a Holling Type III functional response (Altwegg et al. 2006, Kratina et al. 2007). Because it was logistically impossible to replace each consumed prey during the experiment, we accounted for depletion by integration of the functional response models (Royama 1971, Rogers 1972, Juliano 2001). To discriminate between predator-dependence, ratio-dependence, or prey-dependence and estimate whether an increasing density of predators negatively affects

per capita consumption rate, we fit four functional response models to the data: (i) a modified form of the Beddington-DeAngelis model that incorporates interference as time spent during encounters with other predators, (ii) a modified form of the Arditi-Akçakaya model, (iii) a pure ratio-dependent Arditi-Ginzburg model, and (iv) a pure prey-dependent Holling Type III model. The use of a model selection approach (Burnham and Anderson 2002) allowed comparison of mechanistic (Beddington-DeAngelis and Holling Type III) to phenomenological (Arditi-Akçakaya and Arditi-Ginzburg) functional responses that are not nested and cannot be compared using likelihood ratios tests. The integrated form of the Beddington-DeAngelis Type III model has not been previously used to analyze ecological data.

In order to estimate the influence of predator interference at low predator densities we also performed the fitting procedure for a data subset of only 1-5 *Stenostomum* predators per 900 μL .

a) Arditi-Akçakaya functional response

The original model (Arditi and Akçakaya 1990) that distinguishes between prey- and ratio-dependence was modified to Type III (see Schenk et al. 2005):

$$f(N, P) = \frac{a \left(\frac{N}{P^m} \right)^2}{1 + ah \left(\frac{N}{P^m} \right)^2}$$

where $N = N(t)$ is the prey density as a function of time t , P is the predator density, f is the ingestion rate (number of prey eaten per predator per unit time), m is the interference coefficient (this parameter is 0 for pure prey-dependence and 1 for pure ratio-

dependence), a is the encounter rate, and h is the handling time. For all models f must

satisfy the differential equation: $\frac{dN(t)}{dt} \frac{1}{P} = f(N, P)$.

When integrated over time to allow for prey depletion and solving the differential equation for ΔN we have:

$$\Delta N = \frac{P^{2m} + ahN^2 + PaTN - \sqrt{(P^{2m} + ahN^2 + PaTN)^2 - 4TPha^2N^3}}{2ahN}$$

where $\Delta N = Nt - N0$ is the change in the prey density from the start of the experiment to time t and T is the duration of the experiment (the unit of time here $T = 4$ hours).

b) Beddington-DeAngelis functional response

The original predator-dependent model (Beddington 1975, DeAngelis et al. 1975) was modified to Type III:

$$f(N, P) = \frac{aN^2}{1 + bw(P - 1) + ahN^2}$$

and then integrated and solved to give:

$$\Delta N = \frac{(1 + i(P - 1)) + haN^2 + PTaN - \sqrt{((1 + i(P - 1)) + haN^2 + PaTN)^2 - 4TPha^2N^3}}{2Nha}$$

The parameters are the same as in the previous model, but predator-dependence is modeled as $i = bw$, where b is the rate of encounter with other predators (analogous to the encounter rate with prey a), and w is the time wasted on other predators (analogous to the handling time h spent on each prey item). Because these two parameters are

mathematically non-identifiable (they always appear together in the model) we grouped them into one parameter i .

c) Arditi-Ginzburg functional response

The original ratio-dependent model (Arditi and Ginzburg 1989) was modified to Type III:

$$f(N, P) = \frac{a \left(\frac{N}{P} \right)^2}{1 + ah \left(\frac{N}{P} \right)^2}$$

and integrated and solved to give:

$$\Delta N = \frac{P^2 + ahN^2 + PaTN - \sqrt{(P^2 + ahN^2 + PaTN)^2 - 4TPha^2N^3}}{2ahN}$$

d) Holling Type III functional response

The original prey-dependent model (Holling 1959):

$$f(N) = \frac{aN^2}{1 + ahN^2}$$

was integrated and solved to give:

$$\Delta N = \frac{ahN^2 + PaTN - \sqrt{(ahN^2 + PaTN)^2 - 4TPha^2N^3}}{2ahN}$$

All functional response models were fit to the observed data using nonlinear least-squares regression (Juliano 2001). We used the function *nls* in R software, version 2.6.0 (R Development Core Team 2007) and compared the explanatory power of the models using the sample size adjusted Akaike's information criterion (Burnham and Anderson 2002).

2.2.3. Behavioural experiment

In a separate experiment we examined the number of encounters between the focal predator and its conspecifics. We visually monitored the focal predator in 9-well Pyrex depression plates with 900 μL of the protozoan medium (see above) over 20 minutes. Our treatments consisted of 60 *Paramecium* and 2, 4, 8, or 16 *Stenostomum* per experimental well. All treatments were replicated six times. Stronger illumination from the microscope in the behavioural trials as compared to the functional response trials caused different light condition in the two experiments. We compared the fits of linear, hyperbolic, and sigmoid models and based on the smallest AIC value, we used the hyperbolic function

$y = \frac{a(P-1)}{1+ab(P-1)}$ to analyze the predator encounter data. The number of encounters is

denoted as y , P is the predator density, a and b are constants that can be interpreted as the encounter rate with conspecific predators and the time spent per encounter respectively. The term $(P-1)$ ensures that the focal predator cannot interfere with itself.

2.3. Results

Predator per capita ingestion rate declined with increasing density of conspecifics and this effect was evident especially at high prey densities of 160 and 200 *Paramecium* per experimental well (Fig. 2-1, Fig. 2-2).

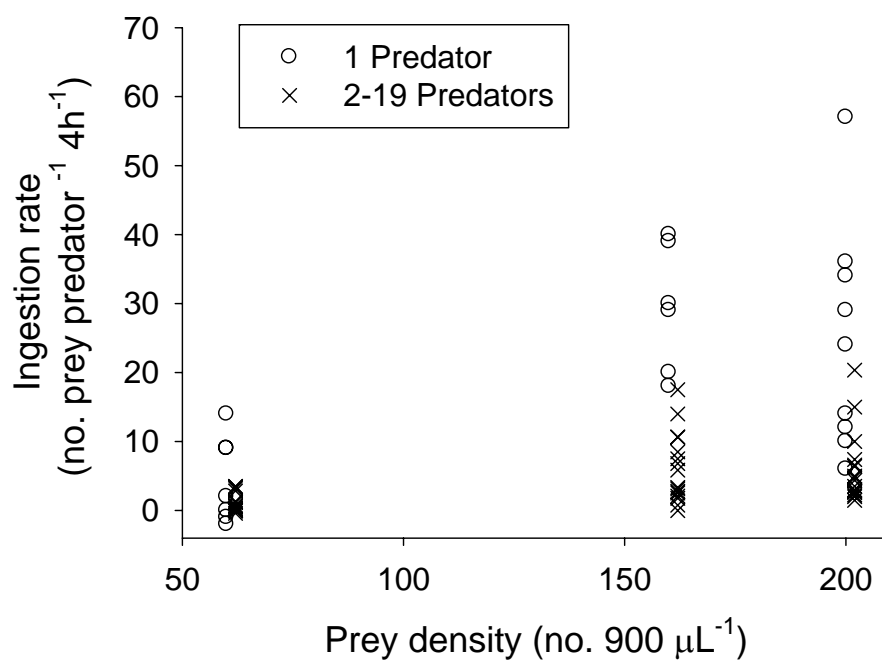


Figure 2-1 Per capita ingestion rate by predatory *Stenostomum* feeding on *Paramecium* for four hours at three prey densities: 60 (23 replicates), 160 (22 replicates), and 200 (26 replicates) per 900 μL of protozoan medium. Empty circles represent the treatment where the predator was feeding without conspecifics (i.e. 1 predator), crosses symbolize the treatment where predator density ranged from 2 to 19 *Stenostomum* in 900 μL of protozoan medium. The three negative values of the predator ingestion rate at the lowest prey density resulted either from inaccurate pipetting or from *Paramecium* fission during the experiment. Individual symbols represent results of all replicates.

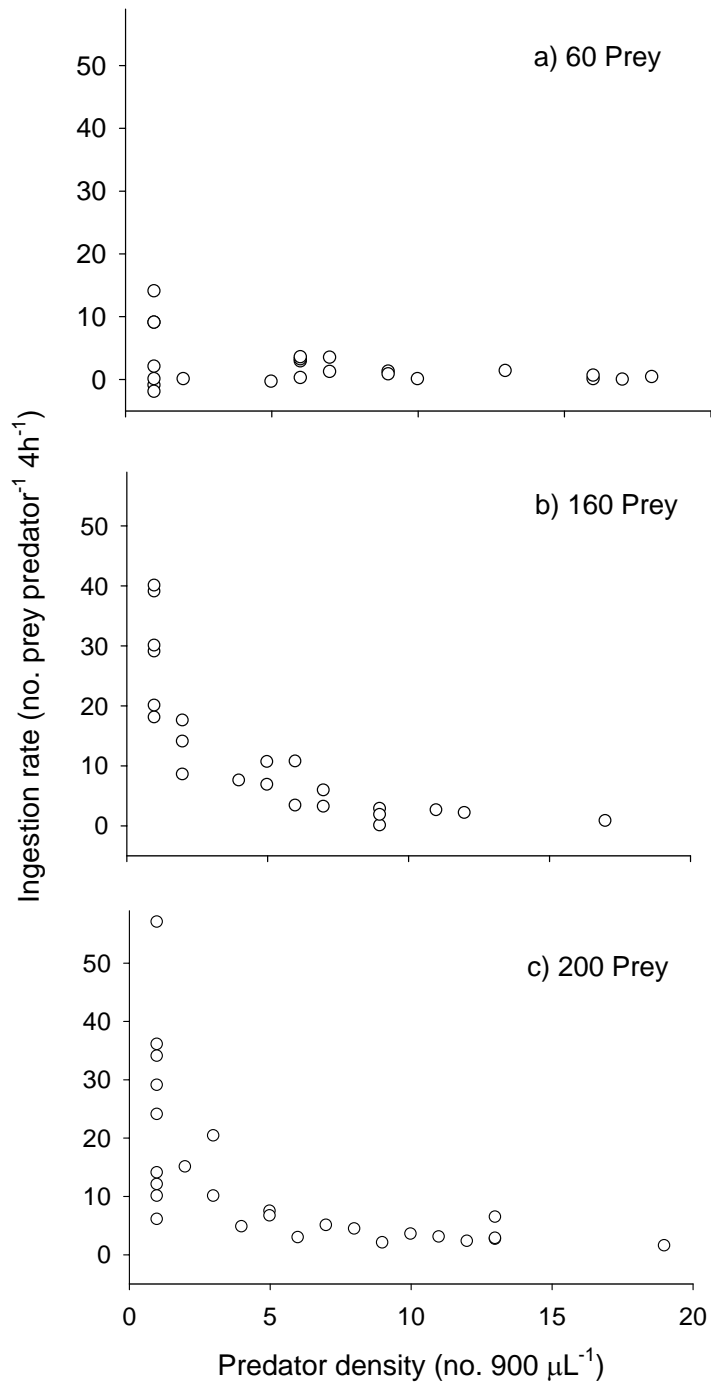


Figure 2-2 Per capita ingestion rate of *Paramecium* by predatory *Stenostomum* over a range of 1-19 predator densities while feeding for four hours on three prey densities a) 60, b) 160 and c) 200 *Paramecium* per 900 μL of protozoan medium.

The Arditi-Akçakaya and Beddington-DeAngelis functional responses produced almost identical fits to our data (Table 2-1, Fig. 2-3a, b). The estimated parameters of both models were also very similar; with encounter rate $a = 6.7 \times 10^{-4}$ and handling time $h = 0.11$ in the Arditi-Akçakaya model and encounter rate $a = 8.4 \times 10^{-4}$ and handling time $h = 0.11$ in the Beddington-DeAngelis model. Both models also detected a substantial level of predator interference; parameter $m = 0.67 \pm 0.11$ (SE) and parameter $i = 2.77 \pm 2.56$ (SE). The fits of the two best functional responses were closely followed by the fit of Arditi-Ginzburg functional response (Table 2-1). The Holling Type III model without the effect of conspecific density produced the worst fit and a negative handling time that is not biologically sensible (Table 2-1).

Table 2-1 The parameter estimates (± 1 SE) and summary of model selection analysis for the fits of four functional response models to the complete data set and to the data for low predator densities (1-5 predators). A lower value in adjusted Akaike's information criterion (AICc) indicates a superior model, Δ AICc shows the difference from the best model (in bold), K represents the number of parameters.

| Model | Encounter rate (a) | Handling time (h) | Interference parameter | K | AICc | Δ AICc |
|------------------------------|---|-------------------|------------------------|---|--------------|---------------|
| <i>Complete data:</i> | | | | | | |
| Beddington-DeAngelis | $8.4 \times 10^{-4} \pm 7.6 \times 10^{-4}$ | 0.11 ± 0.04 | $i = 2.77 \pm 2.56$ | 3 | 576.0 | 0.8 |
| Arditi-Akçakaya | $6.7 \times 10^{-4} \pm 3.4 \times 10^{-4}$ | 0.11 ± 0.04 | $m = 0.67 \pm 0.11$ | 3 | 575.2 | 0.0 |
| Arditi-Ginzburg | $2.9 \times 10^{-3} \pm 0.4 \times 10^{-3}$ | 0.18 ± 0.03 | - | 2 | 581.5 | 6.3 |
| Holling Type III | $7.6 \times 10^{-6} \pm 2.7 \times 10^{-6}$ | -3.46 ± 1.39 | - | 2 | 622.6 | 47.4 |
| <i>Data (1-5 predators):</i> | | | | | | |
| Beddington-DeAngelis | $7.7 \times 10^{-4} \pm 6.6 \times 10^{-4}$ | 0.11 ± 0.04 | $i = 2.06 \pm 1.85$ | 3 | 289.1 | 0.0 |
| Arditi-Akçakaya | $6.6 \times 10^{-4} \pm 4.9 \times 10^{-4}$ | 0.11 ± 0.05 | $m = 0.66 \pm 0.23$ | 3 | 289.3 | 0.2 |
| Arditi-Ginzburg | $1.7 \times 10^{-3} \pm 0.4 \times 10^{-3}$ | 0.14 ± 0.02 | - | 2 | 289.9 | 0.8 |
| Holling Type III | $7.6 \times 10^{-5} \pm 4.5 \times 10^{-5}$ | -0.13 ± 0.26 | - | 2 | 307.8 | 18.7 |

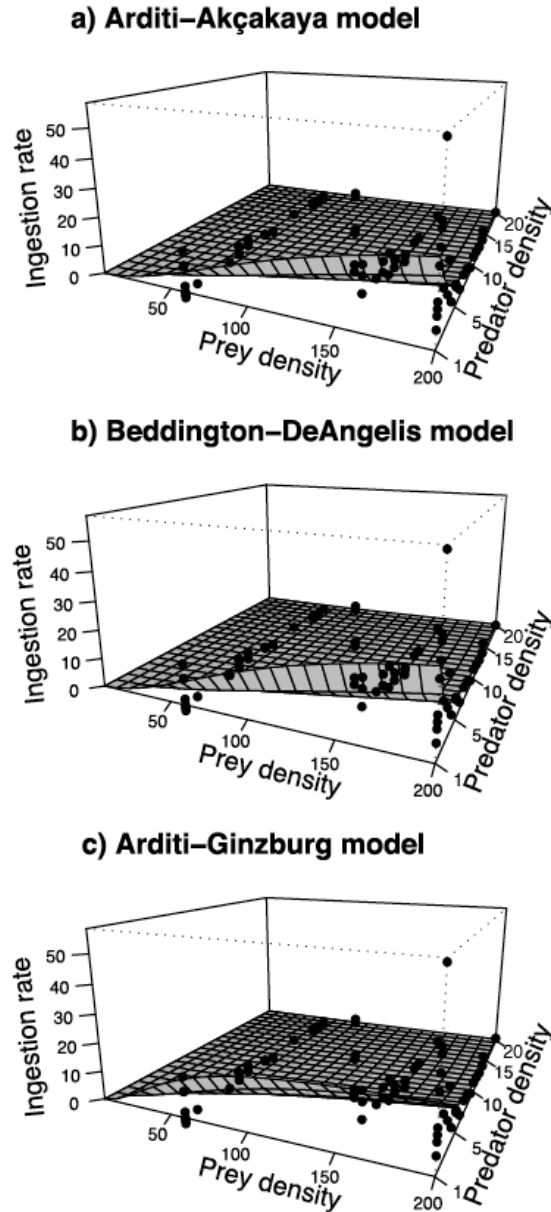


Figure 2-3 The three integrated Type III functional response models, representing the effect of changing predator and prey densities on the per capita ingestion rate. a) Arditi–Akçakaya model ($a = 6.7 \cdot 10^{-4}$, $h = 0.11$, $m = 0.67$), b) Beddington–DeAngelis model ($a = 8.4 \cdot 10^{-4}$, $h = 0.11$, $i = 2.77$), c) Arditi–Ginzburg model ($a = 2.9 \cdot 10^{-3}$, $h = 0.18$). The Holling Type III functional response did not converge to an acceptable fit due to the negative handling time. Ingestion rate was measured as the number of prey consumed per predator per 4 h; predator and prey densities were measured as number of individuals per 900 μL of protozoan medium. The parameter symbols are defined in the Materials and methods section.

To assess whether the functional response is also affected by conspecifics at low densities, we repeated this analysis on a subset of the data with only one to five predators per experimental well. We obtained very similar parameter estimates and model order as for the whole data set. The interference parameters were $m = 0.66 \pm 0.23$ (SE) and $i = 2.06 \pm 1.85$ (SE) for the Arditi-Akçakaya and the Beddington-DeAngelis models respectively (see Table 2-1).

In the behavioural experiment, the number of encounters with conspecific predators increased as a hyperbolic function of predator density (AIC was larger for both linear and sigmoid functions, Fig. 2-4). From this model we estimated the encounter rate ($a = 6.94 \pm 3.53$ SE) and handling time ($b = 0.04 \pm 0.01$ SE) on other predators. Although the functional response and the behavioural experiments were conducted under different conditions (i.e., higher illumination during the behavioural trials), the product of $a*b = 0.29$ falls within the SE of the interference parameter ($i = 2.77 \pm 2.56$ SE) estimated from the Beddington-DeAngelis model.

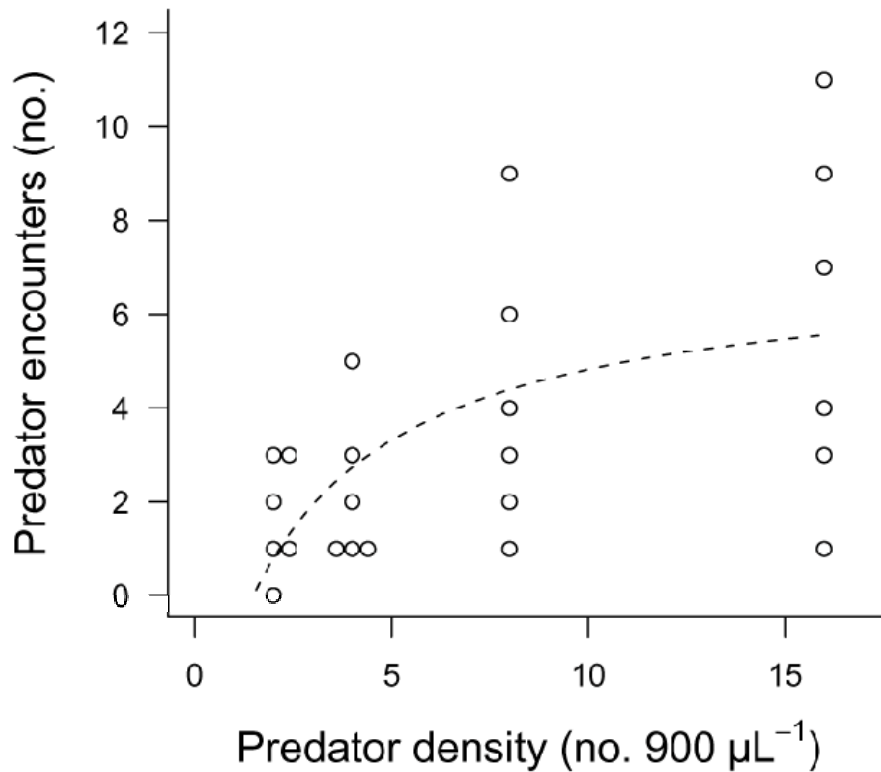


Figure 2-4 Behaviour of the focal *Stenostomum* predator with 60 *Paramecium* prey individuals at four predator densities 2, 4, 8, and 16 per 900 μL protozoan medium (6 replicates at each predator density). The number of encounters with conspecific predators y was described by the hyperbolic function $y = \frac{a(P-1)}{1+ab(P-1)}$, where P is the predator density, a and b are constants.

2.4. Discussion

Recent advances in modeling natural food webs depend on our mechanistic understanding of species interactions. Incorporation of biological realism into simple

consumer-resource models can improve our ability to reliably predict complex food web dynamics. Even though the stability effects of functional responses that depend on both predators and prey have been discussed for forty years, rigorous direct experiments investigating the detailed shape of functional responses over a large gradient of predator and prey densities are still rare.

In our experiments we overcame the limitations of many previous studies and clearly demonstrated predator-dependence in the predator's functional response even at low predator densities and after accounting for prey depletion. We detected that the two structurally different models, mechanistic Beddington-DeAngelis and phenomenological Arditi-Akçakaya, surprisingly resulted in closely similar AICc values and parameter estimates (Fig. 2-3, Table 2-1). All models with the effect of conspecific predators described our data substantially better than the Holling Type III functional response. Analyzing the data with only 1-5 *Stenostomum*/0.9 mL, we confirmed that the functional responses were predator density-dependent also at very low predator densities (Figs. 2-1, 2-2, Table 2-1). Quantitative data on natural densities of microturbellaria are very limited and vary both spatially and seasonally. Natural densities similar to those in our experiment (4 individuals * cm⁻²) have been reported from shallow littoral regions (reviewed in Kolasa 2001).

Our results accord with some previous findings from other predator-prey systems. For example, predator-dependent functional responses described 18 of 19 data sets better than a solely prey-dependent Type II functional response (Skalski and Gilliam 2001). Most empirical studies that measured predator-dependence directly, however, did not consider the depletion of resources, which might have occurred over the course of the

experiments (e.g., Salt 1974, Hayward and Gallup 1976, Helgen 1987, Hansson et al. 2001, Lüring et al. 2003).

Few direct experiments have been conducted on a relatively short time scale to avoid prey depletion, or, alternatively, have accounted for prey depletion in their analyses. A prey-dependent Type III functional response was demonstrated in a rigorous short-term study of the rotifer *Brachionus calyciflorus* foraging on the green alga *Monoraphidium minutum* (Fussmann et al. 2005). Predator-dependence was significant only at unusually high rotifer densities of ~ 125 *Brachionus*/1 mL (Fussmann et al. 2005). Using the random predator equation, the predation rate of the clerid beetle (*Thanasimus dubius*) feeding on the bark beetle (*Dendroctonus frontalis*) was shown to be strongly dependent on predator density (Reeve 1997). Corroborating prior studies (Schenk et al. 2005, Tschanz et al. 2007), we estimated the interference parameter at an intermediate level between the two extreme models ($m = 0.67$), with the value shifting the equation closer to ratio-dependence than to pure prey-dependence. Values much closer to the extreme cases for two species of invasive crabs ($m = 0.9$ for *Carcinus maenas* and $m = 0.1$ for *Hemigrapsus sanguineus*) were also found (Griffen and Delaney 2007). These authors suggest that the strength of predator dependence can be specific to different predator species. The ratio-dependent model lacks a clear mechanistic basis (Abrams 1994), but functional response models can be viewed on a continuous scale for the degree of predator-dependence (Stow et al. 1995). This could be more productive than viewing prey- and predator-dependent models solely as competing alternatives. The magnitude of predator-interference estimated from the mechanistic Beddington-DeAngelis model ($i = 2.77$) also suggested that consumption rates were modified by densities of conspecifics.

Furthermore, we obtained some support for the effect of predator density from the behavioural observations. We found that flatworm predators waste a relatively large amount of time during encounters with each other (Fig. 2-4). Such an increase in the proportion of predator's time spent on interactions with conspecifics inevitably reduces the time available for other activities including feeding.

Many mechanisms have been suggested to generate predator-dependent functional responses (e.g., Crowley and Martin 1989, Cosner et al. 1999, Crumrine 2005, Rudolf 2006, 2007). Although size-related interference probably plays a minimal role in our model system, we have observed a tendency of *Stenostomum* predators to aggregate spatially with many inter-individual contacts (personal observations from culturing *Stenostomum* and other experiments). Time spent in this common behaviour will lead to reduced foraging time. While we have focused on changes in predator behaviour, prey are also known to alter their behaviour in response to predators. Prey are predicted to reduce their vulnerability when predation risk increases (Werner and Anholt 1993). Consequently, increasing predator density may reduce per capita predation rates through modification of prey behaviour (Anholt and Werner 1995). Such anti-predator behaviour has been well documented in ciliates (Kusch 1993). Although we only have evidence for direct interference among predators, it is clear that both direct and indirect effects of predator density can simultaneously result in predator-dependence in many systems. More empirical work is needed in order to determine the reasons for variation in the degree of predator-dependence in functional responses and to identify the underlying mechanisms.

Choosing appropriate functional responses is crucial for adequate predictions of food web dynamics. When predator-dependence is incorporated into predator-prey models their stability is usually enhanced (DeAngelis et al. 1975, Arditi et al. 2004, Rall et al. 2008). There is now evidence to show that predation is modulated by the densities of prey (simple functional responses), by the density and diversity of other non-prey species (Vos et al. 2001, Kratina et al. 2007), and by other predators (this study). Such multiple dependencies of functional responses that include prey, non-prey, and predators are essential for a full understanding of food-web dynamics.

Chapter 3: SPECIES DIVERSITY MODULATES PREDATION²

3.1. Introduction

A predator's ingestion rate as a function of prey density is known as its functional response (Solomon 1949, Holling 1959). This relationship is an important component of community and food web models that are central to theoretical ecology and its applications in conservation biology, fisheries management, and biological control. However, experimental studies of functional responses are usually carried out in simplified systems in which predators only encounter a single prey species (e.g., Hassell 1978, Gross et al. 1993). Much of the structural complexity and diversity of non-prey species in the food web are often excluded from experimental set-ups, even though these may have substantial effects on a predator's ability to locate and pursue prey in nature. Nearly all functional responses published in the literature suffer from this simplification. As a result, much of the current predator-prey theory may contribute more to understanding trophic interactions in relatively contrived laboratory settings than give insight into predation and food web dynamics in realistic natural environments.

Naturally, there is good reason to exclude much of the complexity observed in the real world when modeling or performing experiments. Most of the non-prey species in a food web are irrelevant to particular predator-prey interactions. However, for each specific predator a subset of species will modify its interaction with any given prey. This may occur for a variety of reasons. Some non-prey species provide structural complexity that allows prey to avoid and evade predators more easily (Mayer et al. 2001, Grabowski

² Kratina, P., M. Vos, B. R. Anholt. 2007. Species diversity modulates predation. *Ecology* **88**:1917-1923.

2004). Other species provide a masking background in terms of infochemical cues or a cryptic background in terms of visual cues that make prey less detectable (Wootton 1992, Vos et al. 2001). Some non-prey may be similar to prey in one or more aspects of their shapes, colours, sounds, or odours and these similarities may cause confusion in predators. All of the above effects force predators to spend increasing amounts of time on information processing as the diversity of 'relevant' non-prey and the ratio of such non-prey to prey in the environment increase (Vos et al. 2001).

These kinds of effects are a form of interaction modification (Abrams 1983, Wootton 1993) where one species alters the interaction between individuals of two other species. There is a growing recognition that interaction modifications are likely to be important in nature (e.g. Anholt and Werner 1995, 1998, Peckarsky and McIntosh 1998, Palomo et al. 2003, Cardinale et al. 2003). However, few studies have started to address consequences of interaction modifications in communities caused by species diversity (but see Vos et al. 2001, Thébault and Loreau 2006).

Empirical studies across many taxa (Drutz 1976, Kareiva 1985, Stachowicz and Hay 1999, Mayer et al. 2001, Vos et al. 2001, Grabowski 2004, van Veen et al. 2005) show that a non-prey species can interfere with the foraging behaviour of predators and parasitoids and thus influence rates of predation or parasitism. However, we are not aware of any study that systematically investigates the effects of non-prey density and diversity on functional responses. Many such studies are needed to test whether simple functional responses can be extrapolated to natural settings in which many non-prey species exist.

In this study we experimentally investigated whether (and how) non-prey species modify consumption rates by a predator. We first observed the functional response of the predatory flatworm *Stenostomum virginianum* (hereafter referred to as predator or *Stenostomum*) on its ciliate prey *Paramecium aurelia* (hereafter prey or *Paramecium*) in the absence and presence of a single non-prey species. *Stenostomum* usually detects potential prey from a very short distance and may ‘try’ to test its suitability (personal observation) before ingestion. Prey and non-prey species are distinguished mainly on the basis of their size. This experimental system is thus especially well-suited as a model for predation under gape-limitation, an important factor in many aquatic predator-prey systems. However, it is also a useful model in the wider sense for any system in which the predator or parasitoid needs to spend some time distinguishing between prey and non-prey.

In the second experiment, we measured whether an increasing density and diversity of non-prey reduced predation rates. We also tested whether species identity effects occurred, with some non-prey species having stronger effects on predation than others, or whether diversity itself modified the interaction. Answering these questions is important for obtaining a reference to how the shapes of functional responses could differ in simple versus more diverse communities. This is crucial to our ability to predict the dynamics and functioning of complex ecological systems.

3.2. Materials and methods

3.2.1. The experimental system: Predator, prey, and non-prey

The predatory flatworm *Stenostomum* (Rhabdocoela, Turbellaria) is a benthic omnivore that has been cultured asexually in Pyrex crystallizing dishes with 300 mL of NAYA mineral water (Mirabel, Quebec, Canada) and autoclaved wheat grains, since we isolated it from sediments of a freshwater pond on the University of Victoria campus in 2002. In our cultures, the flatworms feed on bacteria and small flagellates. The prey in our experiments was the holotrich ciliate *Paramecium*, also isolated from a UVic pond. Three other species, having sizes that make them inedible for this gape-limited predator were used in the experiment: the hypotrich ciliate *Euplotes aediculatus* (clone DMP), originally obtained from K. Wiackowski (Jagiellonian University, Krakow, Poland), the ciliate *Spirostomum ambiguum*, and the bdelloid rotifer *Philodina roseola*, both obtained from Sciento (Manchester, U.K.). The non-prey are hereafter referred to as *Euplotes*, *Spirostomum*, and *Philodina*. All prey and non-prey were cultured in 100 – 200 mL of medium, which was prepared by filtering 1.5 crushed protozoan pellet (Nr. 13-2360, Carolina Biological Supply Company, NC, USA) through double-layered No.4 coffee filters and dissolved in 2.0 L of NAYA water. One wheat grain was added to the medium, which was then autoclaved before inoculation. We subcultured all species every 3-5 weeks. All cultures were kept and the experiments were conducted at a laboratory temperature of approximately 21 °C.

We used freezer-killed *Stenostomum* to induce a morphological defense in *Euplotes*, 24 hours prior to each experiment. The procedure involved exposing 4 mL of dense *Euplotes* culture to 600 µL of *Stenostomum* kairomone (250 individuals/mL, stored

in 1.5 mL Eppendorf tubes, at -20°C), which caused *Euplotes* to increase in body width to an inedible size. Before the start of each trial we photographed 20 to 50 *Euplotes* and measured the maximum body width using Image Pro Plus software to check whether they were too large to be ingested, (i.e. $>80\ \mu\text{m}$, Altwegg et al. 2006).

3.2.2. Functional response with one non-prey

We compared functional responses of the predator on *Paramecium* prey in the presence or absence of non-prey *Euplotes*. We set densities of *Paramecium* at 40, 60, 80, 100, 120, 140, 160, and 200 individuals per 900 μL of medium, with or without 60 induced *Euplotes* ($101.5 \pm 11.9\ \mu\text{m}$, mean body width \pm SD, $n = 104$). We randomly assigned treatment combinations of predator density, prey density, and presence or absence of non-prey species to individual wells in 24-well tissue culture plates (Costar, Corning Incorporated, NY, USA). To introduce them into wells, all organisms were individually pipetted and counted under a dissecting microscope (Leica MZ8). We washed and then pipetted 16 predators into each well in order to obtain average predation rates. This was intended to even out variation among predators. As some predators stuck to the pipette tips, the actual number of predators per well in this experiment varied, and predation rates are therefore given as number of prey ingested per predator. The average length of *Stenostomum* was $505.2 \pm 144\ \mu\text{m}$ ($n=79$). The feeding experiment was stopped after four hours by addition of two drops of acid Lugol's solution (5%). For each well the remaining prey, as well as predators and non-prey were counted. Replication resulted in 8 *Paramecium* densities * 2 *Euplotes* treatments (absence / presence) * 6 replicates of each = 96 data points.

In order to determine the shape of the predator's functional response on prey with or without one non-prey species, we performed non-linear curve fitting of Holling Type II and Type III functional response models to our data. We fit separate functional responses in the presence and absence of non-prey, as well as a single functional response which ignored the presence or absence of non-prey. We also examined both model types with the addition of an intercept to account for possible systematic counting error, cell death or reproduction during the experiment.

We fit the following functional response models to the data:

$$\text{Holling Type II: } y(X) = \frac{a \cdot X}{1 + a \cdot b \cdot X} \quad (1a)$$

$$\text{Holling Type II with intercept: } y(X) = c + \frac{a \cdot X}{1 + a \cdot b \cdot X} \quad (1b)$$

$$\text{Holling Type III: } y(X) = \frac{a \cdot X^2}{1 + a \cdot b \cdot X^2} \quad (2a)$$

$$\text{Holling Type III with intercept: } y(X) = c + \frac{a \cdot X^2}{1 + a \cdot b \cdot X^2} \quad (2b)$$

where y represents ingestion rate per predator, X is prey density, a is attack rate, b is handling time, and c is intercept. Because each of the six replicates of all treatments was conducted on a different day, we tested for the effect of blocks by using non-linear mixed effects models (Pinheiro and Bates 2000). Prey density was treated as a fixed effect and block as a random effect. We chose the best model based on the smallest Akaike's Information Criterion (AIC) and log-likelihood values (Burnham and Anderson 2002). Statistical analyses were performed in R software, version 2.2.1. (R Development Core Team 2003). The calculated measure for the functional response's contribution to stability

was the range of prey densities for which the Type III functional response showed positive density-dependent predation. This stabilizing part of the functional response is equivalent to the part of the curve describing the predation risk per prey that has a positive slope, i.e. $[y(X)/X]' > 0$, which requires $y'(X) > y(X)/X$ (Oaten and Murdoch 1975). We only compared the ranges of prey densities for which the curves were stabilizing after having determined by AIC that two separate models for the data with and without non-prey were better supported than a single model for these data combined. If predators spend time on non-prey this could cause estimates of the attack rate to decrease and of the handling time to increase. Such changes would result in a right-down shift of the functional response on prey in the presence of non-prey. In case of Type III functional responses this would entail an increase in the range of prey densities for which the curve is stabilizing.

3.2.3. Non-prey species diversity, density and identity

In the second experiment we used single *Stenostomum* feeding on *Paramecium* in 800 μL of NAYA water in the presence or absence of *Philodina*, *Spirostomum*, induced *Euplotes* ($102.0 \pm 13.1 \mu\text{m}$, mean \pm SD, $n = 358$), and their combinations. Using a single predator per well avoided any potential confounding effect of predator interference. We observed *Stenostomum* feeding on either 60 or 90 *Paramecium* alone, with one non-prey (30 *Euplotes*), with two non-prey (30 *Euplotes* + 30 *Philodina*), and with three non-prey (30 *Euplotes* + 30 *Philodina* + 30 *Spirostomum*). Here the density of non-prey species (0-90) increased together with their diversity.

To separate the effect of density and diversity per se, we included treatments of a single *Stenostomum* feeding on 60 *Paramecium* in the presence of non-prey

monocultures: either 30, 60, or 90 *Euplotes*, 30, 60, or 90 *Philodina* or of 90 individuals of *Spirostomum* (see Fig. 3-3). This allowed us to compare, for example, the effect of 90 individuals of a single non-prey species to the effect of 90 non-prey individuals consisting of a mixture of 3 non-prey species (30 of each). We were also able to compare the relative effect of each non-prey species (species identity).

In order to check whether non-prey species were indeed not consumed by *Stenostomum*, we incubated two densities (30 or 60 individuals per 800 μ L) of each non-prey species with and without one *Stenostomum* predator. We also incubated 60 or 90 *Paramecium* alone to estimate the accuracy of our pipetting and counting, and to control for other factors such as prey reproduction.

All experimental organisms were individually pipetted and counted. Treatments were randomly distributed over 24-well tissue culture plates and replicated in time using an incomplete random block design. The experiment was replicated eight times, except the single *Stenostomum* + 60 *Paramecium* + 90 *Spirostomum* treatment, for which $n = 6$. We stopped the experiment after four hours by adding two drops of acid Lugol's solution (5%) and counted the fixed individuals of all species. The analysis was performed in R software, version 2.2.1., using multiple regression.

We estimated that *Stenostomum* had on average density of about 1 predator per 6 mL in UVic ponds. This estimate for the average density is lower, but not orders of magnitude lower than the density of 1 predator per 0.8 mL we used in experiment 2. In a pond studied by Finlay and Esteban (1998) ciliates ranged in density from 0-3466 individuals per mL. In a lake study, protozoans ranged in density from 7.11 to 97.5 per mL (Gomes and Godinho 2003). In the latter dataset *Paramecium* comprised 7% of the

total density and 39 % of the protozoan community biomass on the bottom of the lake. Based on size, two protozoan species in that lake would have been inedible to a specialist predator of small *P. aurelia*. One of these, *Spirostomum*, can comprise 18% of the total bottom-dwelling protozoan biomass. Rotifers may further contribute to the number of relevant non-prey species (Finlay and Esteban 1998, this study).

3.3. Results

3.3.1. Functional responses in the presence and absence of one non-prey species

The functional response of *Stenostomum* feeding on *Paramecium* is best described by two sigmoid (Type III) curves, in the presence and absence of non-prey *Euplotes* (Fig. 3-1, Table 3-1). The model where both treatments were described by one single Type III functional response was not supported. Type II responses were unsatisfactory because the estimated handling times were all negative. Positive handling times were only possible if we included an intercept into the model. This model was, however, not supported by AIC and Log likelihood, and Type III models always gave a better fit to the data (Table 3-1). Neither the blocks in the non-linear mixed effects models, nor the addition of an intercept for the Type III model improved the fit.

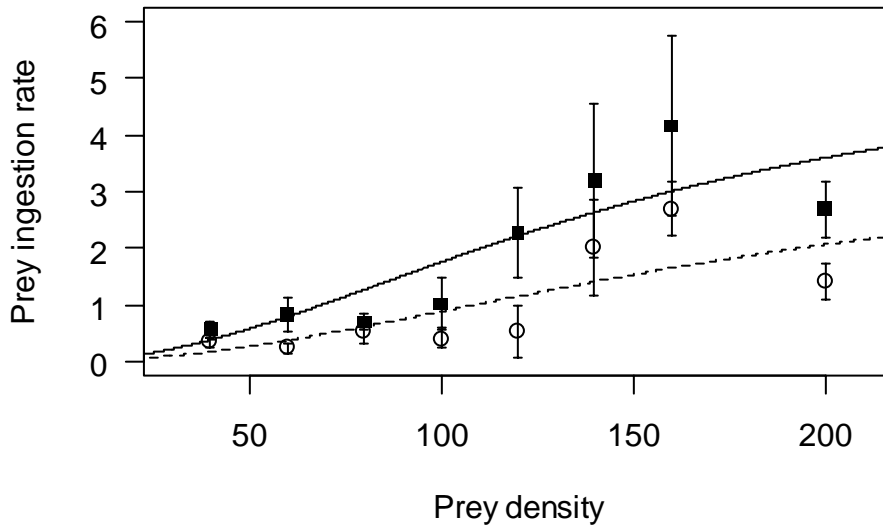


Figure 3-1 Number of *Paramecium* eaten per predator in four hours, in 900 μL . Empty circles and filled squares denote the presence or absence of 60 non-prey *Euplotes* per well. Symbols are means of 6 replicates \pm 1 SE. Solid line (without non-prey) and dashed line (with non-prey) are fitted Type III functional response models.

We observed a consistent effect of the presence of non-prey species on the functional response of the predator. The ingestion rate of prey was lower in the treatments with induced non-prey *Euplotes*, and the effect was more pronounced at high prey densities (Fig. 3-1). Two separate Type III responses were best supported and showed that the stabilizing prey density widened from 0-147 to 0-181 prey per 900 μL in the treatment with non-prey *Euplotes*.

Table 3-1 Information statistic summary for the fit of seven functional response models describing per capita ingestion of *Paramecium* prey by *Stenostomum*, as a function of prey density in 900 μL wells, over four hours. The table shows the maximum log likelihood and the Akaike's information criterion (AIC) values. The non-prey effect refers to the model that discriminates between the treatments with and without non-prey *Euplotes*; K is the number of parameters; the best model is in bold font.

| Model | Mixed | Non-prey | K | Log | | Akaike |
|-----------------|-----------|------------|----------|----------------|---------------|--------------|
| | effects | effect | | likelihood | AIC | weight |
| Type III | No | Yes | 4 | -172.43 | 352.86 | 0.999 |
| Type III | No | No | 2 | -185.70 | 375.40 | < 0.001 |
| Type III | Yes | Yes | 12 | -172.43 | 368.86 | < 0.001 |
| Type III | Yes | No | 6 | -185.70 | 383.40 | < 0.001 |
| Type III + int | Yes | Yes | 14 | -172.33 | 372.66 | < 0.001 |
| Type III + int | Yes | No | 12 | -185.64 | 395.28 | < 0.001 |
| Type II + int | Yes | Yes | 20 | -173.01 | 386.01 | < 0.001 |

3.3.2. Non-prey species diversity, density and identity

The predator had a higher overall ingestion rate at a density of 90 prey per 800 μL than at a density of 60 prey per 800 μL ($P < 0.001$ for *Paramecium* density, Table 3-2, Fig. 3-2). According to the analysis the predator consumed 3.06 more prey individuals in four hours for each 30 *Paramecium* prey added (regression coefficient in Table 3-2) and all other variables are held constant. This effect of prey density gradually disappeared when non-prey density and diversity increased (Fig. 3-2).

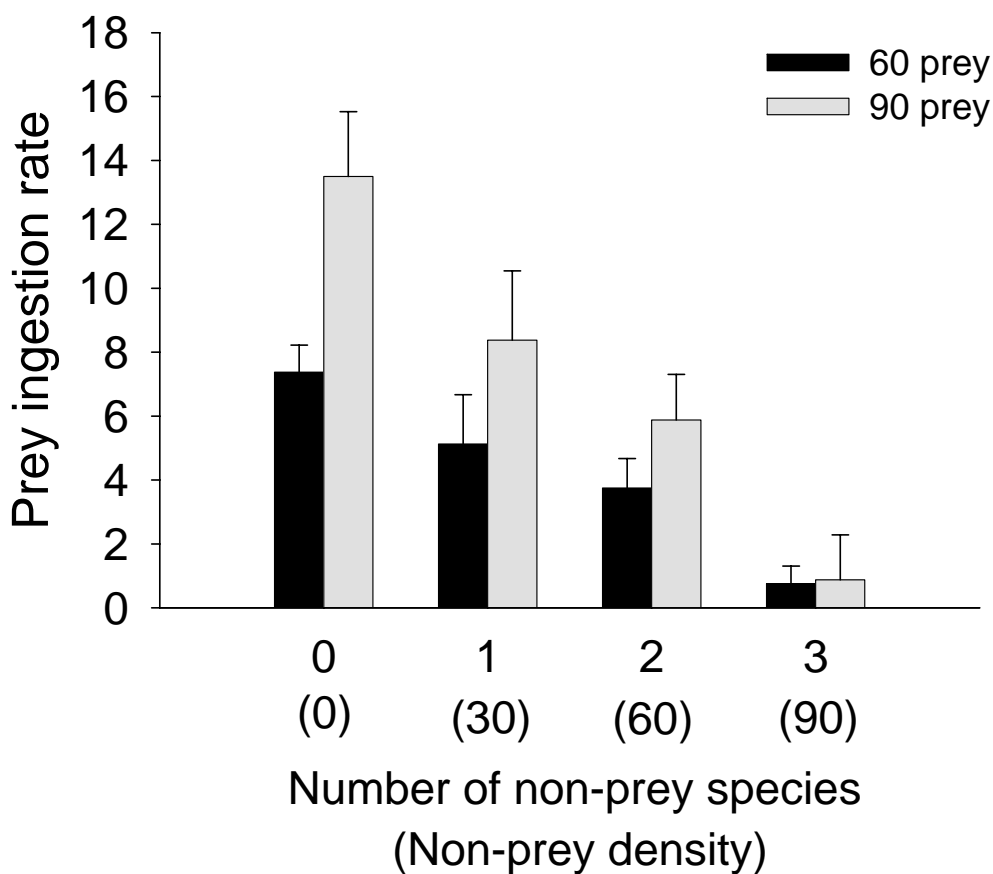


Figure 3-2 The effect of diversity and density of non-prey species on the predator's feeding rate. A single *Stenostomum* was feeding on either 60 or 90 *Paramecium* per 800 μ L of NAYA water in the presence or absence of non-prey species. Combinations were: *Stenostomum* + *Paramecium* alone, with one non-prey (30 *Euplotes*), with two non-prey (30 *Euplotes* + 30 *Philodina*), and with three non-prey (30 *Euplotes* + 30 *Philodina* + 30 *Spirostomum*). Data are means of 8 replicates \pm 1 SE.

Each non-prey species in the experiment caused a significant reduction in the predator's consumption rate, both at 60 and 90 prey per 800 μ L ($P = 0.002$ for *Euplotes* when *Paramecium* prey density in the model, $P < 0.001$ for *Philodina*, when *Paramecium* and *Euplotes* in the model and $P < 0.001$ for *Spirostomum*, when

Paramecium, *Euplotes*, and *Philodina* in the model). The magnitude of reduction in predation rate was similar for all three non-prey. Addition of 30 *Euplotes*, *Philodina* or *Spirostomum* non-prey reduced the predator's consumption by 1.29, 0.99, and 1.17 prey individuals respectively (Table 3-2, Fig. 3). Therefore, non-prey species identity was not an important factor in our experiment. The non-prey diversity had the strongest effect ($P < 0.001$), as an equivalent addition of 30 individuals of different species reduced the predator's consumption by 1.92 prey individuals.

Table 3-2 Results of multiple regression analysis of the effects of non-prey species on the predator's prey consumption rate (n = 8). For treatment with *Spirostomum* (n = 6). Estimated coefficients of prey and non-prey densities were multiplied by 30 individuals.

| Effects | Df | SS | F value | P | Est. consumption rates / 30 additional prey or non-prey |
|-----------------------------------|----|---------|---------|---------|---|
| <i>Paramecium</i> prey density | 1 | 189.96 | 14.839 | < 0.001 | 3.06 |
| <i>Euplotes</i> non-prey density | 1 | 126.11 | 9.851 | 0.002 | - 1.29 |
| <i>Philodina</i> non-prey density | 1 | 228.60 | 17.858 | < 0.001 | - 0.99 |
| <i>Spirostomum</i> non-prey | 1 | 295.05 | 23.049 | < 0.001 | - 1.17 |
| Non-prey diversity | 1 | 152.86 | 11.942 | < 0.001 | - 1.92 |
| Residuals | 11 | 1433.73 | | | |

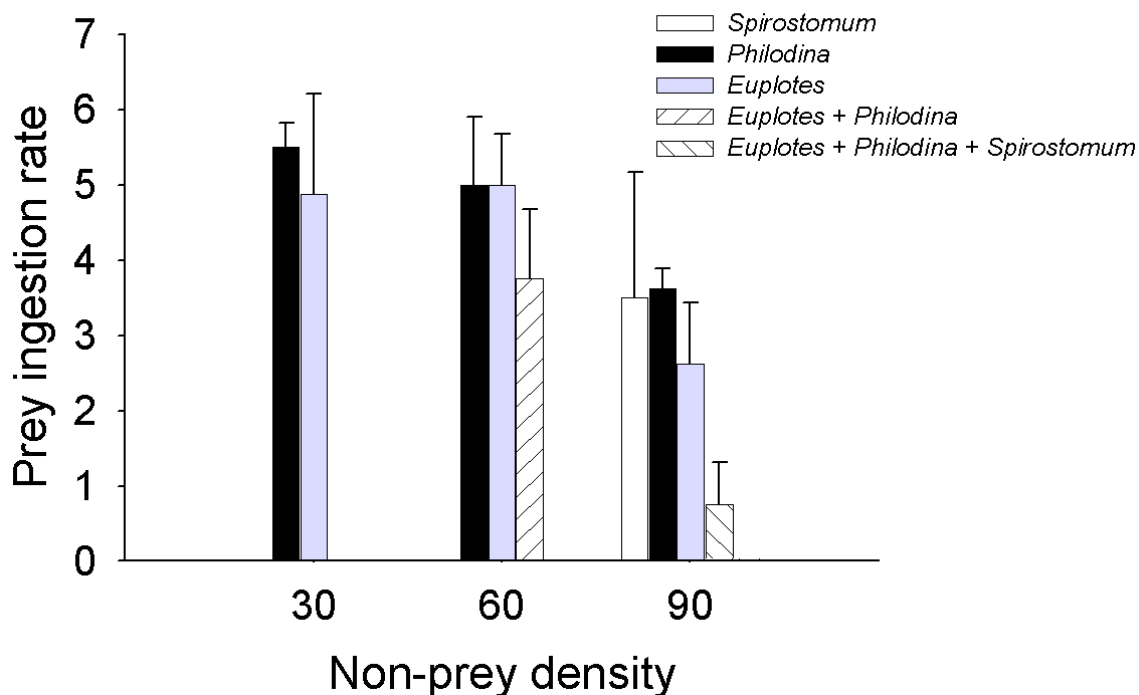


Figure 3-3 The effect of diversity, density and species identity of non-prey species on the predator's feeding rate. A single *Stenostomum* predator was feeding on 60 *Paramecium* prey in 800 μ L of NAYA water. Multiple non-prey bars represent: 30 *Euplotes* + 30 *Philodina* (at non-prey density 60) and 30 *Euplotes* + 30 *Philodina* + 30 *Spirostomum* (at non-prey density 90). Data are means of 8 replicates, *Spirostomum* monoculture data represent 6 replicates \pm 1 SE.

3.3.3. Control treatments

When incubating 30 or 60 *Paramecium* for four hours without predators we lost, on average, 2.13 or 2.75 individuals respectively. In both control treatments this average was strongly affected by two extreme data points. Without these, there was a difference of +0.33 from the original 30, and of -0.17 from the original 60 individuals. We believe the extreme points are true outliers, as we could reliably and consistently measure low

predation rates of about 0.5 prey per predator per well per four hours (see Fig. 3-1). There was no difference in the mean number of non-prey retrieved from wells with or without a predator, which shows that non-prey were indeed not eaten (t-tests, $n = 7$, $P = 0.66$ for *Euplotes*, $P = 0.94$ for *Philodina*, $P = 0.92$ for *Spirostomum*).

3.4. Discussion

Many models in food web and community ecology incorporate ecological realism by using nonlinear functional responses (e.g. Rosenzweig 1973, Williams and Martinez 2000, Brose et al. 2003, 2006, Kondoh 2006 and references therein). An assumption of these models is that per capita predation rates depend only on the densities of prey species. However, this assumption is probably rarely met in natural communities, where predator and prey populations are never isolated from the diversity and complexity of their biotic surroundings. Many other species directly or indirectly interact with predators or prey and these complex linkages may strongly affect the process of predation (Peacor and Werner 2004).

We observed *Stenostomum* preying on *Paramecium* with a Type III functional response (see also Altwegg et al. 2006). We showed that a single non-prey species can substantially modify this interaction. The presence of a relatively low density of *Euplotes* non-prey decreased the predator's functional response. Non-prey species additionally caused the Type III functional response to be density-dependent over a wider range of prey densities. Through this effect the presence of non-prey may contribute to the stability of the predator-prey interaction. The observed change in the shape of the functional response was most likely caused by predators spending some time trying to

capture non-prey individuals. Consequently, they would have had less time to search for and handle their prey. The results lend support to the ‘wasted-time’ hypothesis for predation in more diverse communities (Vos et al. 2001). Those data, however, still indicate that the basic shape is a sigmoid Type III functional response.

The results from our second experiment establish that interaction modification is not necessarily a matter of only one species affecting the interaction between two others. These results clearly show that several non-prey species, or such non-prey diversity, may modify the interaction between a predator and its prey. The non-prey diversity range over which strong effects of diversity itself occur may differ greatly among systems, with some species or taxonomic groups being more sensitive than others. Differential sensitivities to diversity can be important within the context of both conservation and invasion ecology. Spending considerable time on non-prey can weaken and stabilize predator-prey interactions that would otherwise be unstable. The effect may either be complete stabilization or a damping of predator-prey population cycles (McCann et al. 1998, Vos et al. 2001, van Veen et al. 2005). The results presented here pertain to a specific system in the laboratory. If our results are general, as suggested by laboratory as well as field data on bacteria, leaf beetles, parasitoids and fish, that have all been shown to waste time on non-prey (Drutz 1976, Kareiva 1985, Stachowicz and Hay 1999, Mayer et al. 2001, Vos et al. 2001, Grabowski 2004, van Veen et al. 2005), this could have far-reaching consequences for ecological theory on the functioning of complex ecological systems.

We found that non-prey diversity and non-prey density both reduced the predator’s feeding rate in our experiments. Our analysis allowed us to tease apart their relative

contributions and showed a strong effect of diversity itself. This result indicates that a predator needs to spend more time to discriminate between prey and non-prey items when non-prey are more diverse. In our experiment, no single non-prey species was particularly hard to deal with (Fig. 3-3, Table 3-2). We can therefore rule out the possibility that the very strong effect on predation rate in the high diversity treatment was caused by non-prey species identity. Although the three non-prey species were very different in shape, they were similar in terms of being too large to be ingested by the gape-limited predator. When a predator has different recognition or handling times on different non-prey, species identity effects would be expected.

Non-prey species can modify predator-prey interactions by masking prey, diluting prey concentrations, or confusing predators (Vos et al. 2001). Our results suggest how species diversity itself could contribute to the stability of diverse communities. Diversity-modified predation is an ecological mechanism that could cause a prevalence of weak and moderate interaction strengths in natural systems (see McCann et al. 1998). Ecological mechanisms that explain how diversity itself affects coexistence in natural communities have largely been lacking in the long-standing debate about diversity-stability relations (McCann 2000, Vos et al. 2001, Brose et al. 2003, Kondoh 2006). Recognizing that the diversity of species that seem to be unconnected to certain predator and prey species, could promote the persistence of the community as a whole, has important implications for the conservation of diverse ecosystems and for the development of strategies to effectively manage biodiversity. We further conclude that one of the new challenges emerging from our study is to pay more attention to which

seemingly irrelevant species are in fact part of the community context that determines predation rates and the strength of food web links in nature.

Chapter 4: PRESENCE OF NON-PREY SPECIES ALLOWS LONG-TERM COEXISTENCE OF PREDATOR AND PREY

4.1. Introduction

Community dynamics are often modeled using systems of difference or differential equations connecting predators and their prey (de Ruiter et al. 2005, Rooney et al. 2007). The interplay between such models and experiments helps us to understand and investigate the specific ecological mechanisms that shape food webs over many generations (Desharnais 2005). Contrary to the way models and simplified experiments tend to be constructed, in natural settings predators consume their prey while surrounded by many species that are not included in their diet. These non-prey species can modify trophic interactions in which they are not directly involved as consumer, resource, or competitor (interaction modification *sensu* Wootton 1993, 1994).

It has been demonstrated that both diversity and density of seemingly unimportant non-prey species can reduce the strength of trophic interactions (Vos et al. 2001, Kratina et al. 2007, Jactel and Brockerhoff 2007) and consequently cause a prevalence of weak or moderate interaction strengths in nature. Interaction strength is a key parameter in food web models and theory shows that such prevalence of weak trophic links increases persistence and stability of complex food webs (McCann et al. 1998, McCann 2000, Neutel et al. 2002). However, it remains to be answered experimentally, whether this reduction in interaction strength caused by non-prey in the short-term, translates into long-term modification of predator-prey population dynamics. The long-term consequences of non-prey species for predator-prey dynamics are poorly studied

empirically (but see van Veen et al. 2005) and non-trophic links are rarely included in food web models (for exceptions see Arditi et al. 2005, Goudard and Loreau 2008, Vos et al. unpublished manuscript).

Many study systems have been used to investigate predator-prey dynamics in laboratory settings and the difficulty of maintaining predators and prey together over many generations has been a recurring issue (e.g. Gause 1934, Huffaker 1958, Luckinbill 1973, May et al. 1974, Fussman et al. 2000, Bonsall et al. 2002). The failure of simple predator-prey systems to persist is typically caused by the over-exploitation of prey species by predators (Nicholson and Bailey 1935, Huffaker 1958, Bonsall et al. 2002). Following the collapse of prey populations, predators succumb to starvation. Contrary to this, in natural settings predators and prey coexist for long periods in the same ecosystem and this difference between laboratory and natural systems may be linked to the strength of predator-prey interactions. In simple systems, interactions between predators and prey tend to be strong, as a consequence of environmental homogeneity and few species. In natural ecosystems, predator-prey interactions are arranged in complex networks and heterogeneous environments where the majority of species interactions are weak. Areas of refuge caused by abiotic heterogeneity (Maynard-Smith 1974) and the presence of non-prey species that can interfere with the predator-prey interactions (Wootton 1993, Vos et al. 2001, Grabowski 2004, van Veen et al. 2005, Kratina et al. 2007, Jactel and Brockerhoff 2007) can both contribute to reduced interaction strength and prolong species persistence. Here, we investigate whether a model predator-prey system can coexist for a longer period of time when a non-prey species is included.

It has been recognized that natural communities are structured by predation and competition simultaneously and that both processes therefore need to be explored together (Holt et al. 1994, Gurevitch et al. 2000, Chase et al. 2002, Haag et al. 2004, Chesson and Kuang 2008). Non-prey species not only reduce the strength of predation but can also negatively affect prey density by competition for shared resources. These two mechanisms can antagonistically affect predator-prey persistence. We studied tri-trophic microcosm food web modules composed of predator, prey, non-prey, and basal resources. We investigated different species combinations in order to separate and estimate the effects of non-prey species on trophic interaction between predator and prey and assess the strength of competition between the prey and non-prey species. Our study aims to separate the effects of predation, competition, and presence of non-prey species to better understand how different ecological mechanisms combine to shape coexistence in simple model food webs and elucidate the discrepancy between model predictions, laboratory experiments, and complex natural communities.

4.1. Materials and methods

Based on pilot studies, we set up a long-term microcosm experiment in semi-continuous cultures. In order to investigate the effect of non-prey species on predator-prey dynamics, we incubated i) predator + prey alone, ii) predator + prey + non-prey, iii) predator + non-prey. We also included treatments with iv) prey alone, v) non-prey alone, and vi) prey + non-prey to assess the strength of competition between prey and non-prey species. All six treatments (species combinations, Fig. 4-1) were replicated five times, resulting in 30 microcosms in total. We used turbellarian flatworms, *Stenostomum virginianum*, (hereafter referred to as model predator or *Stenostomum*), its ciliate prey *Paramecium*

aurelia (hereafter prey or *Paramecium*), and hypotrich ciliates *Euplotes patella* (hereafter non-prey or *Euplotes*). *Stenostomum* is a benthic omnivore that has been isolated from a freshwater pond on the University of Victoria campus and that feeds on many species of ciliates, flagellates, and algae.

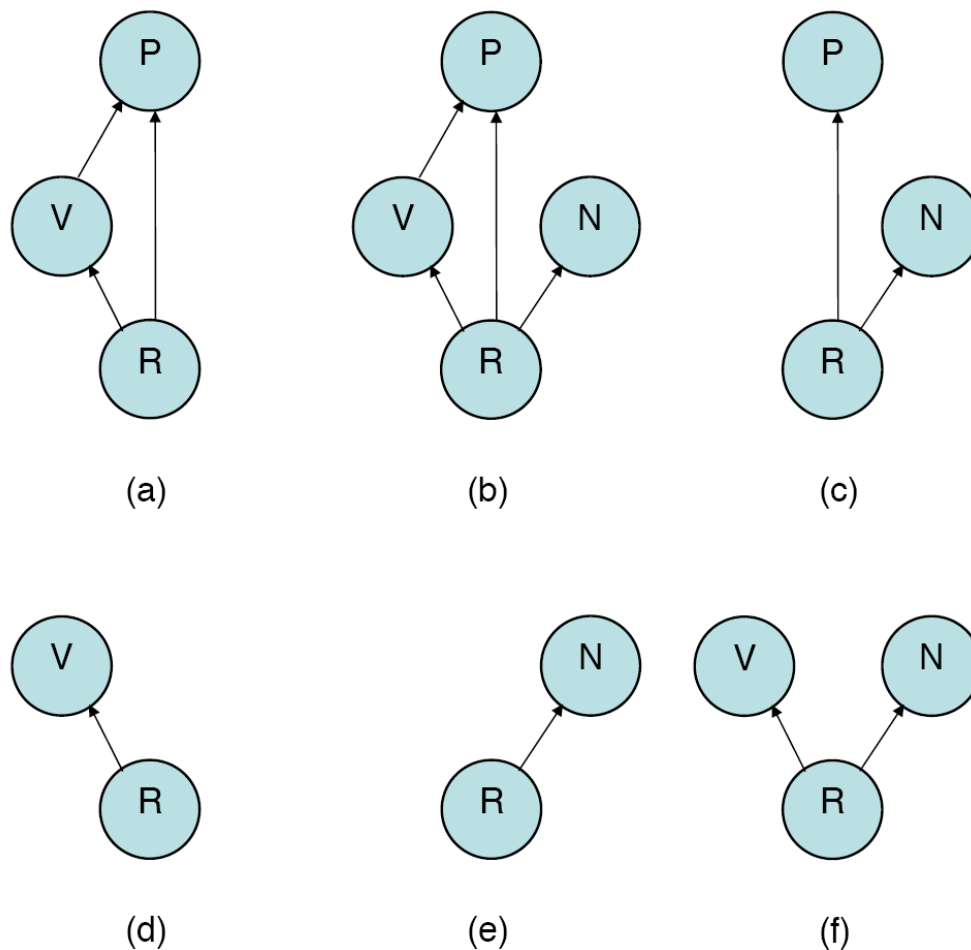


Figure 4-1 Six different food web configurations that were investigated over 250 prey generations in order to separate the effects of predation, competition, and non-trophic interactions by non-prey. (a) predator + prey alone, (b) predator + prey + non-prey, (c) predator + non-prey, (d) prey alone, (e) non-prey alone, and (f) prey + non-prey. Predator is denoted as P, prey as V, non-prey as N, and resource as R.

Although our model predator can probably consume some smaller individuals of *Euplotes* (especially soon after cell division), *Euplotes* is considerably less vulnerable to predation than *Paramecium* due to its large maximum body width. The body width of the non-prey *Euplotes* is $74.4 \pm 1 \mu\text{m}$ (mean \pm SE, $n = 3$ cultures) in its uninduced state and when exposed to predatory *Stenostomum* it increases its body width up to $93.7 \pm 4.3 \mu\text{m}$ (mean \pm SE, $n = 3$ cultures). It is due to this size that we consider *Euplotes patella* to be non-prey in our model system, as it has been shown that predation by gape-limited *Stenostomum* on individuals larger than $80 \mu\text{m}$ is minimal (Altwegg et al. 2006). The maximum body width of *Paramecium*, however, is $42.25 \pm 1.64 \mu\text{m}$ (mean \pm SE, $n = 4$ cultures), leaving it well below the gape size of *Stenostomum*.

We incubated all species in 250 mL Erlenmeyer vessels holding 100 mL of medium consisting of crushed protozoan pellets (Nr. 13-2360, Carolina Biological Supply Company, Burlington, North Carolina, USA) dissolved in NAYATM water (Mirabel, Québec, Canada) at a concentration of $0.6 \text{ g}\cdot\text{L}^{-1}$. The medium was filtered through double-layered coffee filters and autoclaved before use. In all microcosms 100 individuals of the appropriate prey and/or non-prey species were inoculated after the cultures had been left to stand for 24 hours. This 24-hour delay allowed bacterial growth that served as a basal resource for the organisms. Within two hours of adding prey and non-prey, 20 *Stenostomum* predators were added in the predator treatments and this was taken as day one of the experiment. Microcosms were sampled every three days by taking a 10 mL sample from each experimental vessel. Predators were counted live in the whole sample and then 1 mL sub-samples were fixed with acid 5 % Lugol's solution. Prey and non-prey densities were counted together in this 1 mL sub-sample; if less than 5 ciliates

of either species were detected, the entire 10 mL sample was used to estimate their densities within the microcosms. All counts were carried out in a counting chamber under a dissecting microscope (Leica MZ8). Microcosms were shaken prior to sampling to ensure homogenous mixture. Samples were discarded after counting and 10 mL of fresh media was added to the microcosms. This replacement of media (10% of total) prevented nutrient depletion in the microcosms over the course of the experiment and imposed density independent mortality of the model organisms.

The experiment was terminated after 120 days, representing approximately 250 prey and 80 non-prey generations. In one of the five replicates in the predator + prey alone treatment, prey were not detected between days 21 and 63 and increased to extremely high densities after re-appearance. This phenomenon was only observed in this one microcosm, and it is unlikely that not a single individual would be observed for 42 days. Hence, we believe this replicate was contaminated by prey from a different vessel and removed it from the subsequent analysis. We used Fisher's Exact Test to assess whether persistence of predators and prey was associated with the presence/absence of non-prey species. We also compared the number of samples in which predators and prey were not detected in microcosms with and without non-prey species using a two sample t-test. Analyses were performed in R software, version 2.6.0 (R Development Core Team 2007).

4.3. Results

We found substantially different population dynamics among the six different food web configurations (Appendix A). Mean *Paramecium* prey density in the treatment without non-prey *Euplotes* declined to very low densities rapidly and prey were not detected in

any replicate after day 84. In contrast, when non-prey *Euplotes* were present, prey persisted until the last day of the experiment (120) in four out of five replicates, although numbers fluctuated over time (Fig. 4-2a). *Stenostomum* predators were present in four out of five replicates at the end of the experiment (day 120) when non-prey were present, but were below detection limit in all replicates by day 105 when non-prey species were absent (Fig. 4-2b). Fisher's Exact Tests on the proportion of replicates with surviving predators and prey showed that extinctions were not independent of the non-prey treatment ($P = 0.047$). Both predators and prey were significantly more likely to persist until the end of the experiment in the treatment where non-prey were present.

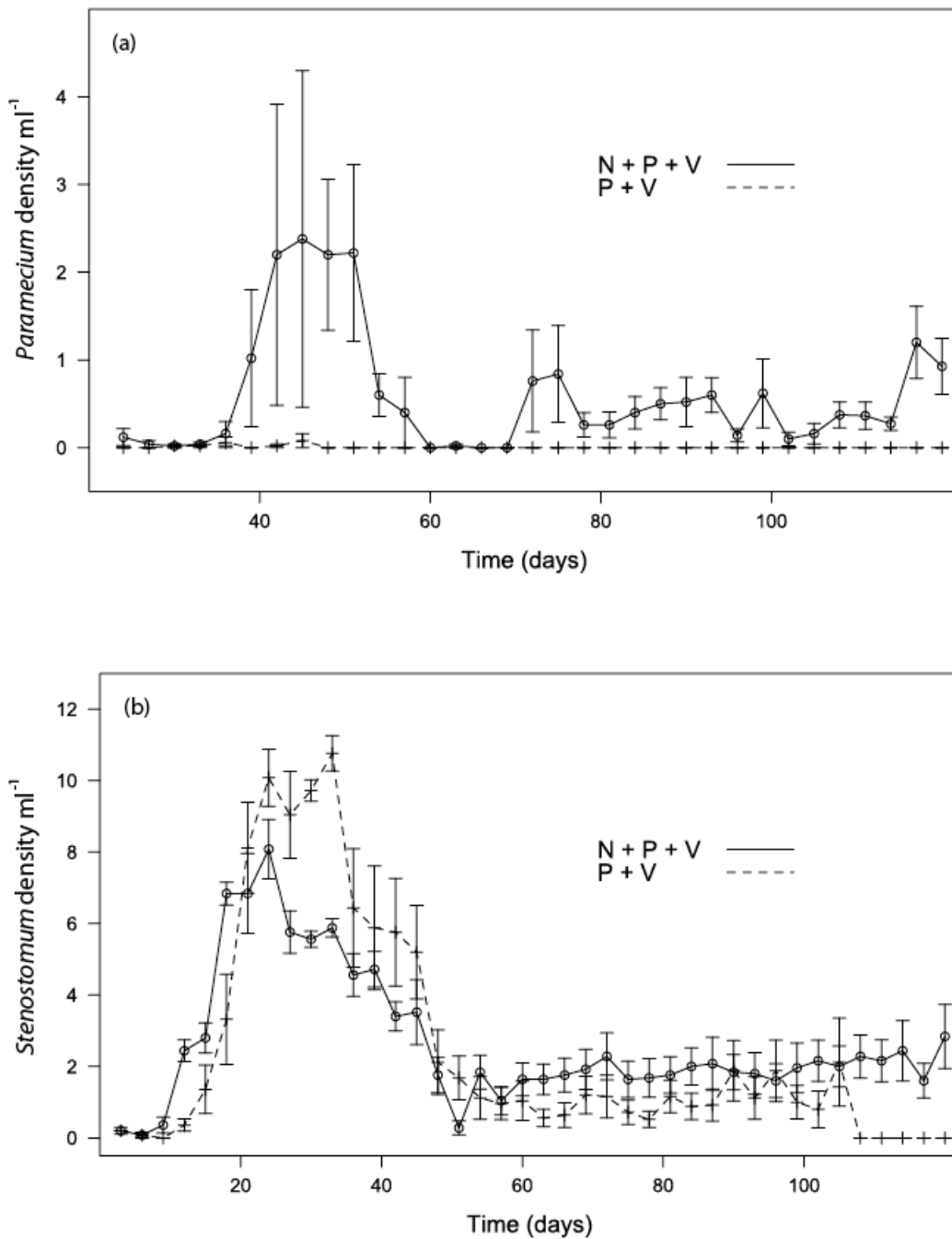


Figure 4-2 Mean population dynamics of (a) *Paramecium* prey and (b) *Stenostomum* predator in two treatments. Predator (P) and prey (V) alone is denoted as hypens and dashed lines, predator (P) and prey (V) incubated with non-prey (N) is denoted as open circles and solid lines. Data represent means of 5 replicates for the N + P + V treatment, 4 replicates for the P + V treatment, whiskers represent ± 1 standard error. The first 20 days for prey is truncated.

Mean prey density at the end of the experiment in the treatment with non-prey was $0.93 \text{ individuals} \cdot \text{mL}^{-1}$ and the 95% confidence interval ($0.04 - 1.81$) did not overlap 0. In contrast, mean prey density was 0 in the treatment without non-prey. Mean predator density at the end of the experiment in the treatment with non-prey was $1.42 \text{ individuals} \cdot \text{mL}^{-1}$ and the 95% confidence interval ($0.18 - 2.66$) did not overlap 0. In contrast, mean predator density was 0 in the treatment without non-prey.

All replicates of prey and predators appeared to go through transient dynamics up to day 60 (Fig. 4-2). Mean prey and predator densities for the last 60 experimental days were consistently higher in the treatment with non-prey species as compared to the treatment without non-prey, although the overall effect was not significant. Mean prey density was $0.14 \pm 0.15 \text{ mL}^{-1}$ (mean \pm SE, $n = 4$) in the treatment without non-prey, and $0.40 \pm 0.13 \text{ mL}^{-1}$ (mean \pm SE, $n = 5$) in the treatment with non-prey (t-test, $P = 0.20$). Predator density in last 60 days was $0.521 \pm 0.197 \text{ mL}^{-1}$ (mean \pm SE, $n = 4$) in the treatment without non-prey, and $0.973 \pm 0.250 \text{ mL}^{-1}$ (mean \pm SE, $n = 5$) in the treatment with non-prey (t-test, $P = 0.18$).

Prey spent significantly less time below detection in the treatment with predator and non-prey than in the treatment with predator only (t-test, $P = 0.03$, Fig. 4-3a). There were no significant differences in log-transformed time spent below detection limit for predators over the course of the experiment (t-test, $P = 0.10$, Fig. 4-3b).

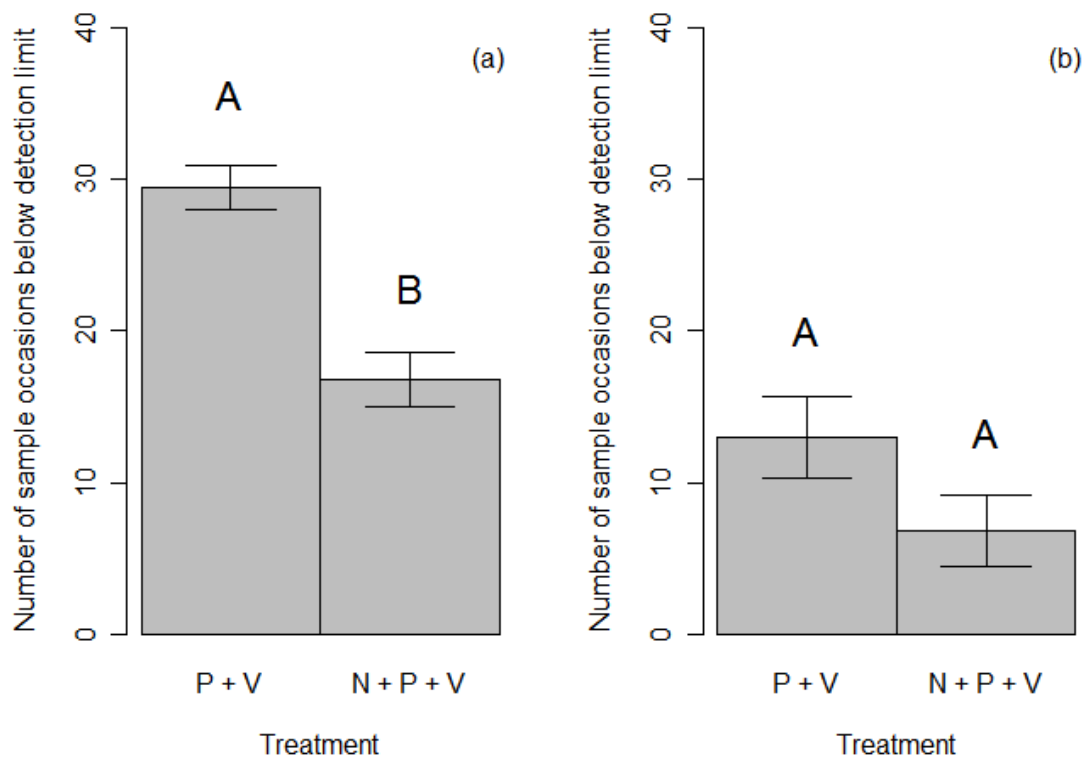


Figure 4-3 Total number of sampling occasions over the experimental duration where no individual was detected for (a) prey and (b) predators. Significant differences were observed between predator (P) + prey (V) and non-prey (N) + predator (P) + prey (V) treatments in the mean number of sampling occasions in which prey densities were below the detection limit (t-test, $P = 0.03$). The mean differences between P + V and N + P + V treatments in the number of sampling occasions in which predators were below the detection limit were not significant (t-test, $P = 0.10$ for log-transformed data). Data represent means of 5 replicates for all the treatments (4 replicates for the P + V treatment), whiskers represent ± 1 standard error. The different capital letters denote the significantly different treatments in each figure ($P < 0.05$).

We also investigated the strength of competition between the prey and non-prey species in the presence and absence of predators (Fig. 4-4). Although prey population

density was not substantially affected by the presence of non-prey, non-prey were able to reach higher densities when incubated alone when compared to the treatment with prey (Appendix A). This shows that the smaller, less defended prey species is a better competitor than larger non-prey in the absence of predation. Rapid increase of prey density to very high numbers followed by a decline in population densities was observed in all replicates when prey were incubated alone. However, in the treatment with non-prey species, prey reached a lower peak density and the decline was followed by second population increase (Figs. 4-4 a, b). In this treatment, prey species always maintained a larger equilibrium population size than non-prey. In contrast, when predators were present, non-prey had a larger population size (Fig. 4-4c). All three species were, however, able to coexist in this treatment, except for one replicate.

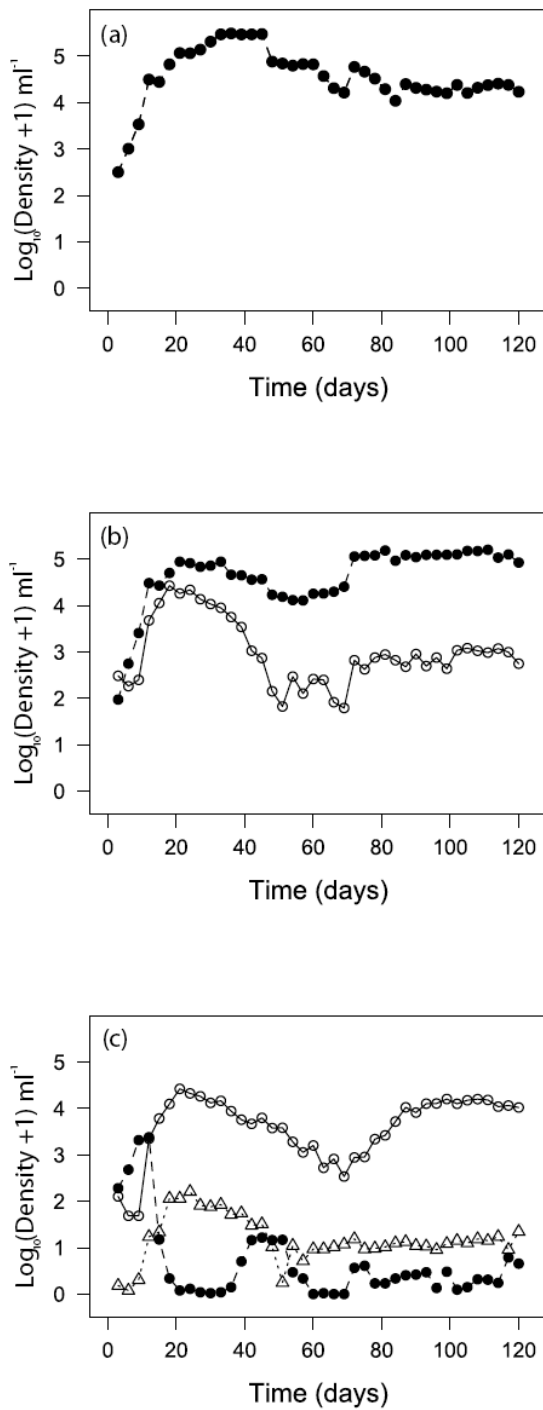


Figure 4-4 Mean ($n = 5$) dynamics of $\log_{10}(x+1)$ transformed densities for (a) prey alone, (b) prey and non-prey, and (c) predator, prey and non-prey. Filled circles \bullet denote prey (*Paramecium*), open circles \circ non-prey (*Euplotes*), and open triangles Δ are predators (*Stenostomum*).

4.4. Discussion

In natural ecosystems, predators and prey exist alongside many other species that do not form part of the predator's diet, do not feed on the focal prey, and therefore do not compete with the predator. As these species outside of the focal predator-prey relationship are not directly involved in a particular trophic interaction, they have often been overlooked in food web theory (but see Arditi et al. 2005, Goudard and Loreau 2008, Vos et al. unpublished manuscript). These non-prey species could, however, affect the process of predation through non-trophic interactions. Non-prey can for example dilute prey density or provide a masking background for prey, reducing searching efficiency as predators have to waste time differentiating their prey from non-prey. Non-prey may also interfere with predators during the process of predation. Each of these mechanisms would reduce the strength of the interaction between the focal predator and prey. Theoretical studies suggest that when interactions are weaker, predators and prey are more likely to persist and this is observed in natural settings where predators and prey both coexist in the same ecosystems for many generations (McCann et al. 1998, McCann 2000). This is in contrast to many ecological models that consider only trophic interactions, and laboratory studies that house predators and prey in isolation in homogeneous environments. In both cases, strong interactions can lead to extinctions. Our results suggest that the ratio of non-prey to prey species in complex natural systems can explain why predator-prey interactions are highly susceptible to extinctions in laboratory condition, whereas they appear ubiquitous in nature and persist over very long time scales.

As predicted by theoretical models, and previously observed in simple microcosms, prey populations were soon exploited to extinction in all of our microcosms containing just predators and prey, and predator populations followed suit. In the microcosms that housed predator, prey, and non-prey, all model species remained present until the end of the experiment (~ 250 prey generations) in all but one replicate. As prey populations were not exploited to extinction in these microcosms, it appears that the strength of the predator prey interaction has been weakened by non-prey, thus promoting coexistence. Previous short term experiments measuring predator functional responses have shown that the presence of non-prey species reduces predator success in this model system (Kratina et al. 2007).

Modifying the strength of the trophic interaction is not the only way a non-prey species can affect predator-prey dynamics. Non-prey can also negatively affect prey density by competition for shared resources. When housed alone, prey populations quickly grew to high densities, exploited their resources, and then declined. Non-prey species reduced the initial maximum prey density but allowed recovery and a second increase in prey population dynamics. In this treatment (V + N), non-prey always reached much lower densities than when incubated alone and was outcompeted by prey. This suggests that prey is the superior competitor in the absence of predation than the morphologically better defended non-prey species. However, in the microcosms containing all three species, the densities of non-prey tended to be much higher than prey. Prey population growth was substantially reduced by the presence of predators, releasing non-prey from the strong competition. In the presence of predators, the ability to acquire resources may be less important than the ability to avoid predation, leading to the

completely defended species dominating the better competitor. Such interspecific trade offs between the ability to effectively acquire resources and susceptibility to predation may reverse the outcome of competition after the addition of species at higher trophic levels (Brooks and Dodson 1965, Persson 1991, Ciroso-Pérez et al. 2004). Preferential feeding on a superior competitor may allow coexistence with an inferior competitor that is more defended against predation (Holt et al. 1994, Ciroso-Pérez et al. 2004). It is less recognized that this mechanism may be often accompanied by the non-trophic interactions between the predator and inferior competitor (non-prey) and that both mechanisms promote the coexistence simultaneously (this study).

Stenostomum populations were maintained for a period after the prey, *Paramecium*, populations had dropped below the detection limit and also in the treatment with only the non-prey *Euplotes*. This suggests *Stenostomum* predators were able to exploit some other nutrient source. As the population size of non-prey was larger when they were housed with *Stenostomum* than in isolation, this would suggest that if *Stenostomum* were consuming *Euplotes* then predation rates were minimal. As well as *Paramecium*, *Euplotes*, and *Stenostomum*, other smaller species of flagellates, such as *Cryptomonas*, and ciliates were present and formed part of the basal resource within the microcosms. *Stenostomum* has been previously observed to consume these smaller species and this could explain how they are able to survive in the absence of *Paramecium*.

Similarly to our study, coexistence of insect parasitoid and host when a single non-host species was added also has been demonstrated from a different model system (van Veen et al. 2005). Here, the braconid parasitoid *Aphidius ervi* was prevented from

overexploiting its host aphid *Acyrosiphon pisum* by non-host *Megoura viciae*. It has been hypothesized that coexistence was achieved by the non-host reducing the searching efficiency of the parasitoid feeding on host and the parasitoid reducing the competition strength between the two aphid species by preferentially feeding on the superior competitor. These results are qualitatively similar to our findings and show that despite the slight differences in the food web configuration (our model predator is an omnivore able to exploit resources at basal trophic level) the coexistence-promoting effect of non-prey species may be a general phenomenon.

These findings can have crucial implications for conservation and management strategies and the generality of the potential non-prey effect demands further investigations. Loss of the non-prey species from complex communities can result in an overall increase in strengths of trophic interactions and consequent destabilization of the whole system. Our data show that even one non-prey species can substantially enhance the persistence of predators and prey. New insight into our understanding of community dynamics and food web ecology will be gained by investigating the degree to which species persistence is facilitated by increasing diversity of non-prey species (Kratina et al. 2007).

Chapter 5: INDUCIBLE DEFENSES ENHANCE PERSISTENCE OF INTRAGUILD PREY

5.1. Introduction

Intraguild predation is a form of omnivory in which a predator consumes an intermediate prey, as well as the resource of this prey (Polis et al. 1989, Holt and Polis 1997). Although food webs with intraguild predation are considered inherently unstable, tending to rapid species extinctions (Pimm and Lawton 1977, Holt and Polis 1997, but see also Krivan 2000), intraguild predation is common in natural communities (Polis et al. 1989, Arim and Marquet 2004), and most species above the herbivore trophic level are omnivorous (Thompson et al. 2007). Several factors promoting stability and persistence of such systems have been proposed. For example, persistence of food web structure is enhanced when an intraguild predator facilitates the growth of the intermediate prey, the intermediate prey is an essential food source for the intraguild predator, or refuges against predators are included (HilleRisLambers et al. 2006). Because anti-predator inducible defenses also protect prey and could be considered a form of prey refuge, they may be an important ecological mechanism promoting the persistence of food webs with intraguild predation (see Holt and Polis 1997, Kimbrell et al. 2007).

Strong selection pressure applied by consumers on their resources has driven the evolution of a vast array of defense mechanisms, which can be broadly grouped into two core categories, constitutive and inducible. As opposed to permanently expressed constitutive defenses, inducible defenses are only expressed following cues from consumers, competitors, or parasites (Harvell 1990, Harvell and Tollrian 1999). Such

defenses occur across a wide variety of taxa including plants (Karban and Baldwin 1997), unicellular organisms (Kuhlmann and Heckmann 1985), invertebrates (Gilbert 1966), and vertebrates (Brönmark and Miner 1992, Werner and Anholt 1996), and induce shifts in the morphology, physiology, life history, and/or behaviour of prey species. Inducible anti-predator defenses ensure greater flexibility in biotic environments where the impact of natural consumers varies spatially and temporally. Although the consequences of this type of phenotypic plasticity for individual fitness are relatively well understood, the effects of inducible defenses on long-term population and food web dynamics have been rarely studied experimentally until recently and many questions remain unresolved (Verschoor et al. 2004, van der Stap et al. 2007).

Theoretical studies suggest that inducible defenses have the potential to increase the persistence of simple systems. Inducible defenses have been shown to stabilize one predator – one prey models (Abrams 1982, 1984, Ives and Dobson 1987, Abrams and Walters 1996, Ramos-Jiliberto 2003, Vos et al. 2004a, Kopp and Gabriel 2006, but see Abrams and Matsuda 1997). Increasing complexity slightly, predator-induced defenses increased population persistence in a tritrophic food chain model parameterized to a rotifer-algae system (Vos et al. 2004a). However, no differences between constitutive and inducible defenses were observed in a bitrophic model when minimum population density was used as a measure of persistence. In contrast to constitutive defenses, inducible defenses eliminate the paradox of enrichment (*sensu* Rosenzweig 1971) in both bitrophic and tritrophic model food chains (Vos et al. 2004a). More complex results were obtained from an alternative formulation of a tritrophic food chain that, like the Vos et al. (2004a, b) models, discriminates between inducible defenses affecting either attack rate or handling

time of predators (Ramos-Jiliberto et al. 2008a). This model system was also generally stabilized by inducible defenses. Furthermore, increased persistence and equilibrium coexistence of two predators on a single prey were demonstrated in models that incorporated induced defenses (Ramos-Jiliberto et al. 2008b). Conversely, a destabilizing effect is predicted by models which incorporate inducible defenses but have considerable time-lags between the onset of predation risk and induction or relaxation of defenses (Underwood et al. 1999, Luttbeg and Schmitz 2000). Time-lags were also incorporated into the models that considered inducible behavioural defenses (Abrams 1982, 1984). System stability was not influenced by the time delays in these model formulations (Abrams 1992).

Despite their crucial importance for the understanding of food web dynamics and the propagation of trait-mediated indirect effects throughout communities (Agrawal 2001), the stabilizing effect of inducible defenses has been rarely tested empirically. Empirical examples demonstrating the importance of inducible defense for long-term dynamics of linear aquatic food chains include Verschoor et al. 2004, van der Stap et al. 2006, 2007, 2008. These empirical examples demonstrate how induced defensive spines in herbivorous rotifers reduced the strength of trophic cascades (van der Stap et al. 2007). Morphological inducible defenses in the basal trophic level (algae) removed strong population fluctuations and increased species persistence (Verschoor et al. 2004, van der Stap et al. 2008). However, defenses at the middle trophic level (herbivores) did not affect stability of food chains (Verschoor et al. 2004), but did change the outcome of competition between herbivorous rotifers with and without induced defenses (van der Stap et al. 2008). Food webs with intraguild predation and different levels of inducible defenses have not been the subject of either theoretical or empirical studies even though both mechanisms are

predicted to modulate species persistence. Although many studies focus on a single ecological mechanism, it is still unclear how different mechanisms combine to affect the persistence of natural communities. There is a clear need for more multi-causal approaches in ecology (Vos et al. 2004b, Amarasekare 2007).

In this study we investigate the dynamics of food webs that incorporate both intraguild predation and inducible defenses. Our experimental food webs consist of the turbellarian flatworm, *Stenostomum virginianum*, (intraguild/top predator) feeding on hypotrich ciliates of the genus *Euplotes* spp. (intraguild prey) and the unicellular algae *Rhodomonas minuta* (basal resource). Ciliates of the genus *Euplotes* quickly reorganize their cytoskeleton and induce anti-predator morphology in the presence of predatory *Stenostomum*. They change their morphology from an ovoid, undefended morph to a circular, defended morph characterized by the development of extended lateral “wings” (Kuhlmann and Heckmann 1985). Winged morphs have a considerably greater body width than undefended individuals and are less likely to be swallowed by gape-limited predators (Kuhlmann and Heckmann, 1994, Altwegg et al. 2006). Morphological changes are initiated within 2-4 hours and can increase for up to 24-36 hours (Kusch 1993, Kuhlmann et al. 1999). Substantial variability in the expression of morphological inducible defenses have been demonstrated among closely related species or even among clones of the same species (Wiackowski et al. 2003, Duquette et al. 2005, present study). We compared the population persistence of two highly inducible and two less inducible clones of *Euplotes* spp. and quantified the relationship between inducibility and persistence time. Our objective was to assess whether clones with a higher ability to induce defensive anti-predator morphology (more plastic clones) also have longer

persistence times in the replicated food webs with intraguild predation. This work was carried out with a view to provide insights into how phenotypic responses at the individual level affect the persistence of populations and account for the widespread occurrence of theoretically unstable intraguild predation in nature.

5.2. Materials and methods

5.2.1. Interclonal variation in inducibility

In the first experiment we measured variation in inducibility of our four different *Euplotes* spp. clones. Pilot studies had shown that two of our clones (AED33 and SC8) induced small morphological changes and two (B8 and S5-1) induced large morphological changes. To quantify this difference we exposed *Euplotes* to *Stenostomum* cue for 24 hours and measured inducibility as an increase in the maximum body width – the difference between the body widths of individuals incubated with and without predator cue. First, we introduced 100 *Euplotes* in 400 μ L of protozoan media to 24-well tissue culture plates (Costar, Corning, Corning, New York, USA). The media consisted of 1.5 crushed protozoan pellets (~0.7 g each; Nr. 13-2360, Carolina Biological Supply Company, NC) dissolved in 2L NAYA mineral water (Mirabel, Québec, Canada). We then added 400 μ L of predator cue consisting of freeze-killed *Stenostomum* at a density of 250 per 1 mL. We systematically scanned the bottom of each experimental well and photographed the first twelve *Euplotes* cells encountered using a CoHU CCD camera (San Diego, USA) connected to an inverted microscope (Leica DM IRB). We measured the maximum cell width using Image Pro Plus 4.5 image analysis software (Media Cybernetics, Silver Spring, USA). To avoid pseudoreplication, the median of twelve

measurements within each well was treated as an independent data point rather than individual *Euplotes*. Each clone was replicated three times, except clone AED33, which was replicated two times. An ANOVA and post-hoc Tukey's tests for multiple comparisons were performed in R software, version 2.6.0 (R Development Core Team 2007).

5.2.2. Population persistence of clones varying in inducibility

Here, we established experimental intraguild food webs using one of the four *Euplotes* clones that differed in their ability to induce morphological defenses after exposure to predator cue (Fig. 5-1). *Stenostomum* is a benthic omnivore that was isolated from a freshwater pond on the University of Victoria campus and feeds on many species of ciliates, flagellates, and algae. It has been previously shown in pilot studies that this turbellarian can feed on algae exclusively and survive for many generations without *Euplotes* prey. We monitored the densities of all three species (*Rhodomonas*, *Euplotes*, and *Stenostomum*) over 40 *Euplotes* generations and compared the persistence times of *Euplotes* clones with low inducibility (AED33, SC8) to the persistence times of clones with high inducibility (S5-1, B8). We used a continuous flow-through system (one stage chemostat) composed of three 15 L carboys as source of sterile Bold's Basal Medium (BBM, Stein 1973). Culture vessels containing the experimental food webs held a constant 200 mL with 14.2 mL of growth medium continuously added by peristaltic pumps and an identical amount overflowing into waste collection vessels every day. The waste was collected and measured every second day to estimate the dilution rate ($\delta = 0.071 \pm 0.001$ SE). Such a low dilution rate was used as the previous pilot chemostat studies with high dilution rates failed to sustain our model organisms for many generations. The culture

vessels were submerged in a water bath to ensure a constant temperature (21 ± 0.5 °C), and permanently illuminated to facilitate algal growth. There were a total of 12 culture vessels representing three replicates of each of the four different *Euplotes* clones. To establish experimental food webs in the chemostats we introduced 19.3×10^4 *Rhodomonas* algae cells to each vessel. The following day we added 4.5×10^3 *Euplotes* and 9 days after adding the algae we added 100 *Stenostomum* predators to each vessel. We used sterile techniques throughout to prevent bacterial contamination.

We sampled 10 mL (5%) of well-mixed culture directly from the culture vessels every second day at the same time (1400 hrs) and determined the densities of all three species. Algae were counted immediately using an Elzone® II5390 Particle Size analyzer (Micromeritics, Norcross, GA, USA). Prior to the experiment the Particle Size analyzer was calibrated by comparing the counts of algae to the numbers obtained from an improved Neubauer hemocytometer. Herbivores and predators were fixed with Lugol's solution and counted in counting chambers under a dissecting microscope (Leica MZ8). The experiment was terminated after 58 days of incubation, representing approximately 40 *Euplotes* or 20 *Stenostomum* generations. At the end of the experiment the entire contents of each culture vessel was examined and all remaining *Euplotes* and *Stenostomum* were counted.

To quantify the relationship between the inducibility of clones and their persistence times during the course of experiment we calculated the mean density for each clone at each sampling time and compared when the different clones reached the mean density of one individual per sample. As a second measure, we recorded the time when each individual population first dropped below a detectable limit and calculated the mean time for the three replicates. Subsequently, we tested for significant differences in persistence

time, measured as the day on which the population was first recorded as zero, using parametric survival analysis. This method accounts for censored data (microcosms that survived until the end of the experiment) and does not assume a constant variance. As variance in time to first recorded zero density is expected to increase with the mean (Flemming and Harrington 1991), survival analysis is superior to using a simple ANOVA. We used the functions *surv()* and *psm()* in the R programming language and followed the methods outlined in Crawley (2007).

We also recorded the total time each *Euplotes* population density was below the detection limit (0 individuals in a 10 mL sample) during the course of the experiment, and used ANOVA and post-hoc Tukey's test for multiple comparisons to test for significance. These persistence measures largely follow those used by van der Stap et al. (2008) for the alga-rotifer inducible defense system, allowing broad comparisons across systems. All analyses were performed in R software, version 2.6.0 (R Development Core Team 2007).

5.3. Results

5.3.1. Morphology

We detected substantial variability among *Euplotes* clones in their ability to increase maximum body width after 24-hr exposure to the predator cue (inducibility). Two clones with high inducibility, B8 and S5-1 increased their mean body widths from 49.93 μm (± 1.66 SE) by 26.24 μm (± 1.09 SE) and from 66.41 μm (± 2.06 SE) by 23.67 μm (± 1.67 SE) respectively. The two clones with low inducibility, SC8 and AED33 increased their mean body widths of 51.85 μm (± 1.94 SE) by 18.12 μm (± 0.74 SE) and 68.53 μm (± 0.89 SE) by 17.77 μm (± 1.77 SE) respectively (Fig. 5-1). These differences were shown to be

significant by a one-way ANOVA ($F_{(3, 7)} = 9.96$, $P < 0.01$, $r^2 = 0.81$), and Tukey's HSD ($P = 0.01$, Fig. 5-1). The maximum body width of clones without predator exposure did not have a significant effect on their inducibility (ANCOVA, $P = 0.76$). Selection of these four clones allowed us to measure population dynamics of one large and one small clone with high inducibility and a large and a small clone with low inducibility.

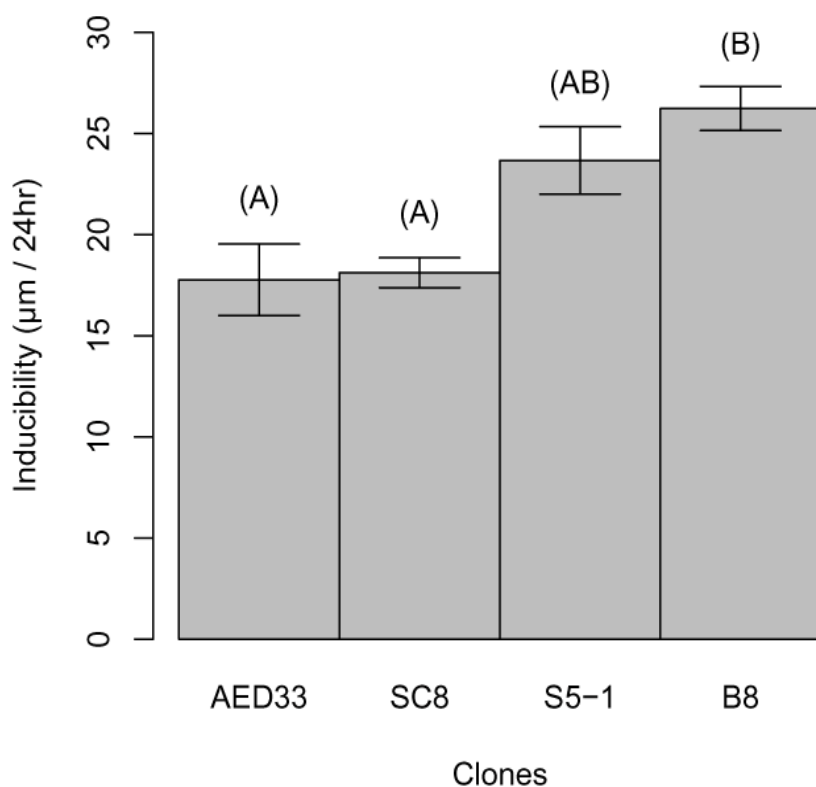


Figure 5-1 Inducibility measured as an increase in the maximum body width of four *Euplotes* clones after 24-hr exposure to the predator cue. Error bars are ± 1 standard error from the mean of three replicates ($n=2$ for the clone AED33). The same capital letters above the bars denote treatments not significantly different from each other ($P < 0.05$).

5.3.2. Persistence time

Striking and repeatable differences in population densities through time were detected among the four *Euplotes* clones (Fig. 5-2, 5-3). The order in which the four clones dropped to the mean density of 1 individual per sample closely followed their inducibility (compare Fig. 5-1 and Fig. 5-3).

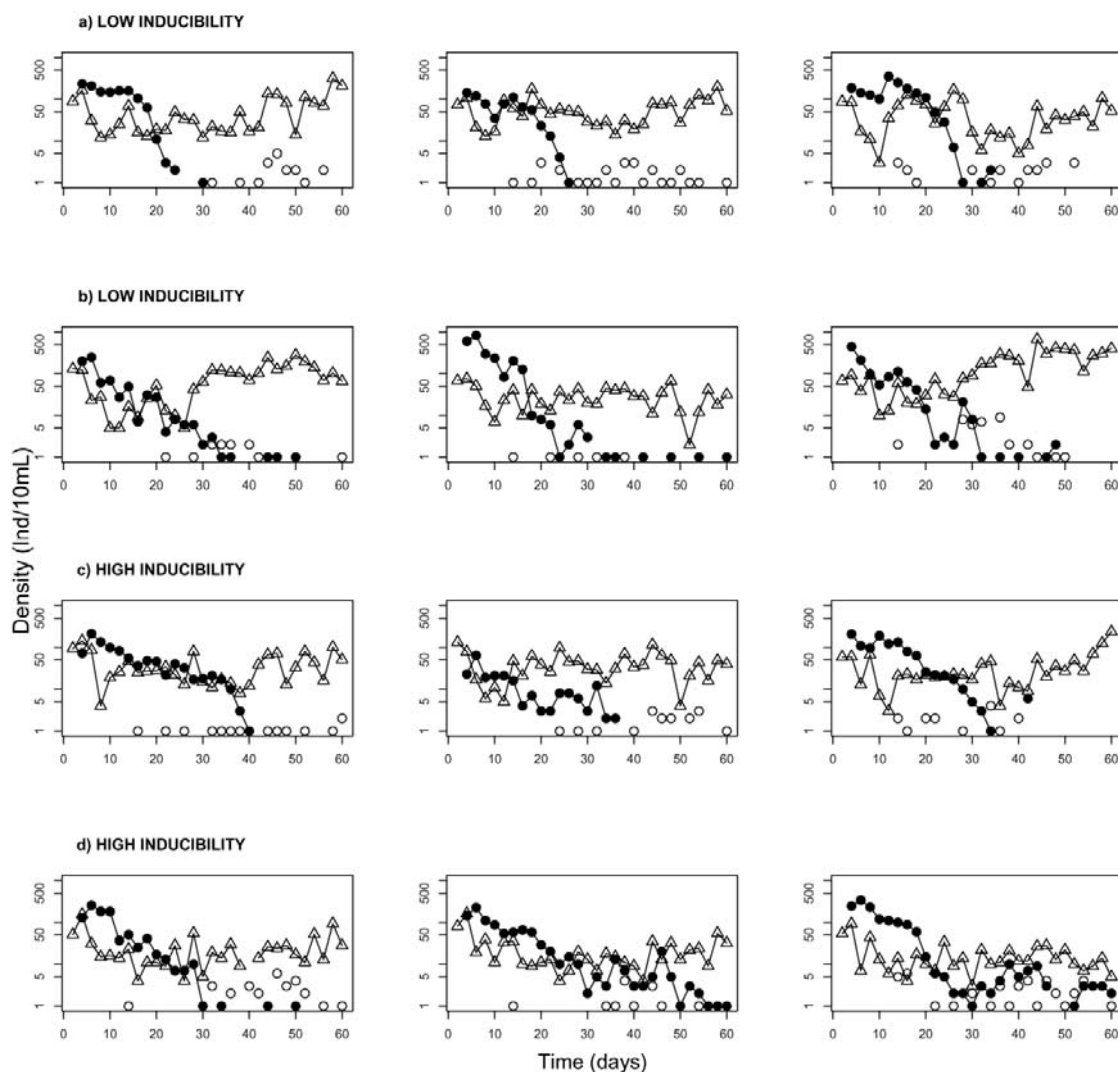


Figure 5-2 Dynamics of twelve intraguild food webs incubated in continuous chemostats; filled circles represent hypotrich ciliates of the genus *Euplotes*, triangles algae *Rhodomonas*

minuta, and open circles denote predatory turbellarians *Stenostomum virginianum*. Algal densities are expressed in hundreds of individuals per 10 mL. Different *Euplotes* clones a) AED33, b) SC8, c) S5-1, and d) B8, vary in their ability to induce morphological defense in the presence of the predators. Samples were taken and all three species were counted every two days. *Stenostomum* were introduced 8 days after the introduction of *Euplotes* and the first predator counts started next day (absence of the symbols reflect 0 individuals detected in a 10 mL sample on that particular day).

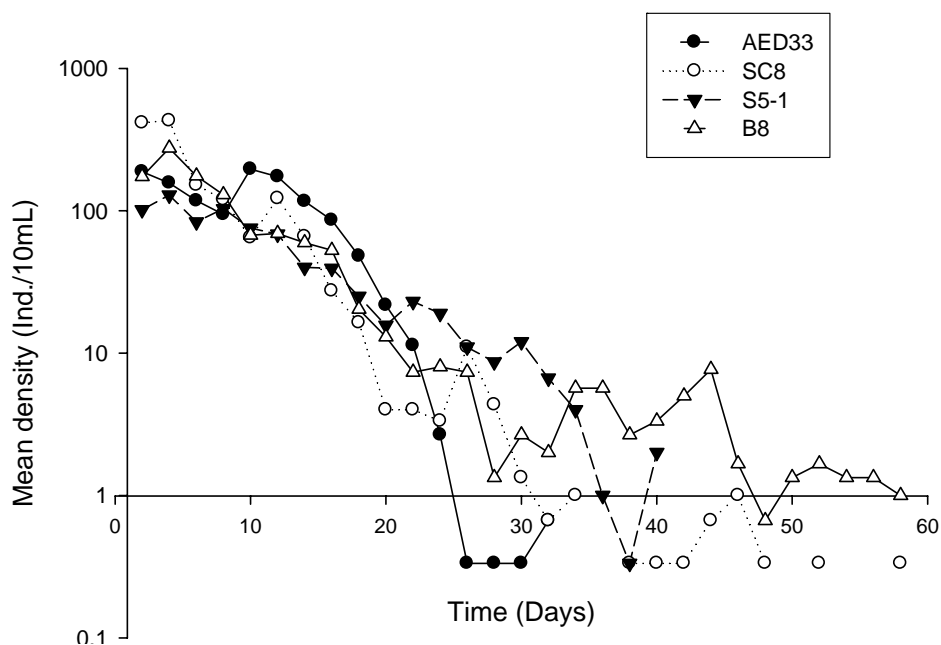


Figure 5-3 Mean (n=3) population dynamics of four *Euplotes* clones. Note the time at which clones with different inducibility dropped below a mean density of one individual per sample (i.e. when they crossed the x-axis). *Euplotes* clone with low inducibility declined faster than the clones with high inducibility (compare to Fig 5-1).

Comparing the clones, we found that the two with low inducibility (AED33, SC8) dropped below detection limit faster than the clones with high inducibility (B8, S5-1) and the order in which different clones declined below detectable levels matched their relative

inducibility (Fig. 5-4). The differences in persistence times among *Euplotes* clones were statistically significant (parametric survival analysis, $\chi^2 = 10.47$, d.f. = 3, $P = 0.015$).

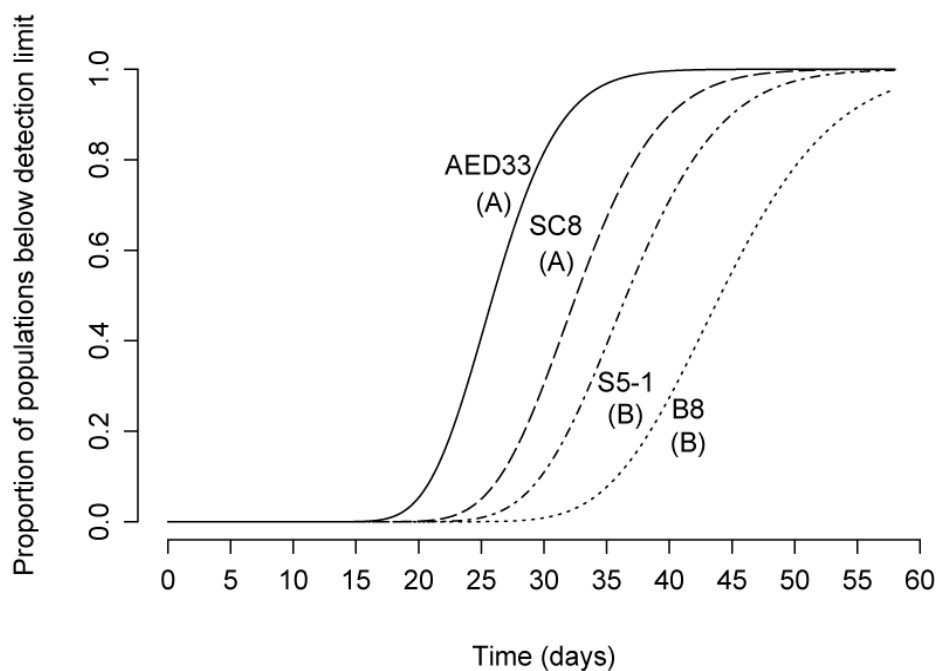


Figure 5-4 Time to the first drop below detection limit for the four *Euplotes* clones with different ability to modify their morphology in the presence of predators. Survival analysis represents rate and order in which different clones dropped below detection limit (response variable). Different letters denote the clones that were significantly different ($P < 0.05$). Although this figure was produced using individual clones as a predictor, consequent survival analyses that used different combinations of inducibility, initial and final sizes as explanatory variables confirmed that the persistence time is prolonged by inducibility alone (see Table 5-1).

In order to assess whether longer persistence is indeed caused by larger inducibility and not by other sources of variation among clones, we constructed a series of survival analysis models incorporating inducibility, and initial and final body sizes as explanatory

variables (Table 5-1). In all analyses the response variable was the day when zero *Euplotes* were recorded (whether the microcosm survived to the end or not). This response variable was then compared to the explanatory variables using parametric analysis (Crawley 2007). Neither initial body size nor final body size had a significant effect on persistence time in any model (Table 5-1). The effect of inducibility was, however, significant, with clones expressing higher levels of inducibility surviving longer (Table 5-1, parametric survival analysis, $\chi^2 = 7.96$, d.f. = 1, $P = 0.0048$). The best model predicting persistence, selected using the sample size-adjusted Akaike's information criterion, included only inducibility (AICc = 77.93, Table 5-1). This model was supported 2.3 times more than the models that also included initial or final *Euplotes* body width (Table 5-1).

Table 5-1 Model parameters and their significance for different survival analysis models of persistence time in the *Euplotes* clones. Initial body size, inducibility and final size all treated as linear variables. All models were fitted to the data by maximum likelihood and compared by the sample size-adjusted Akaike's information criterion (AICc). The Akaike weights give the relative strength of support for one model over another within the model set; the best model is shown in italics.

| Model | Parameter significance | z-score | Model χ^2 | df | Model P-value | AICc | Akaike weight |
|-----------------------------|--|----------------|----------------------------------|-----------|----------------------|-------------|----------------------|
| <i>Inducibility only</i> | <i>Inducibility (P<0.001)</i> | 3.34 | 7.96 | 1 | 0.0048 | 77.93 | 0.526 |
| Initial size only | Initial size (P=0.066) | -1.84 | 3.01 | 1 | 0.0830 | 86.93 | 0.006 |
| Final size only | Final size (P=0.497) | -0.68 | 0.45 | 1 | 0.5000 | 85.53 | 0.012 |
| Initial size + inducibility | Initial size (P=0.141) Inducibility (P=0.003) | -1.47 3.00 | 9.97 | 2 | 0.0069 | 79.60 | 0.228 |
| Final size + inducibility | Final size (P=0.141) Inducibility (P<0.001) | -1.47 3.77 | 9.97 | 2 | 0.0069 | 79.60 | 0.228 |

We also detected significant differences in the amount of time that clones with different inducibilities spent below the detection limit (one-way ANOVA, $F_{(3, 8)} = 5.88$, $P = 0.02$, $r^2 = 0.69$). The clone with the strongest ability to induce defenses, B8, spent significantly less time below the detection limit than the clone with the lowest inducibility AED33 (post hoc Tukey's Test, $P = 0.01$). The differences between the clones SC8 and S5-1 were not significant (post hoc Tukey's Test, $P = 0.88$). Although SC8 dropped very quickly to low densities, we were able to detect one individual per sample in one replicate for a long period of time, reducing the mean time spent below the detection limit. In contrast, clone S5-1 declined to low densities more slowly than clone SC8 but no individuals were detected after day 42 of the experiment.

5.4. Discussion

Natural food webs are assemblages of species structured by complex interactions between predators and their prey. It is recognized theoretically that if these interactions are moderate or weak, systems are more likely to be stable (McCann et al. 1998). The magnitude of predation is often reduced by interspecific and/or intraspecific heterogeneity at the prey level (Vos et al. 2004b). Interspecific heterogeneity may be caused by increasing the ratio of non-prey to prey species in high diversity systems (Vos et al. 2001, Kratina et al. 2007) and intraspecific prey heterogeneity by an expression of anti-predator inducible defenses in prey species (van Donk et al. 1999, Vos et al. 2004a, b). Due to the strong theoretical interest in the potentially stabilizing consequences of inducible defenses for predator-prey dynamics, several recent studies investigated the long-term effect of this type of phenotypic plasticity in replicated experiments (Verschoor et al. 2004, van der Stap et al. 2006, 2007, 2008). The relative importance of different ecological mechanisms acting simultaneously to

affect population persistence, however, remains largely unexplored. Our investigation combines intraguild predation and inducible defenses of different strengths. We focused on the persistence of aquatic experimental food webs composed of intraguild predators, intraguild prey with an inducible defense, and a basal resource over 40 *Euplotes* or 20 *Stenostomum* generations. As in van der Stap et al. (2008), we defined persistence as the time during which all species in the food web remained at detectable densities.

Theoretically, intraguild food webs have been shown to be unstable and have specific requirements for species coexistence (Pimm and Lawton 1977, Holt and Polis 1997). Within the intraguild systems, the combined pressures of competition and predation on intraguild prey put this species at a high risk of extinction (Holt and Polis 1997). However, food webs with intraguild predation are common in nature, raising questions about what mechanisms may compensate for their detrimental effect on persistence. Although extinction of the intraguild prey eventually did occur in most of the experimental vessels (except for two replicates of B8 clone, where 37 and 71 *Euplotes* per vessel were found after the termination of the experiment, see Fig. 5-4), increased ability to induce morphological defenses significantly prolonged prey persistence times. Interestingly, neither the initial nor the final size of *Euplotes* clones had a significant effect on persistence time, suggesting that the ability to modify body size has a greater effect on clone persistence than body size per se. This is further supported by the fact that the clone surviving the longest (B8), and showing the greatest inducibility, had a smaller post-induction size than post-induction AED33, the clone inducing the smallest change and persisting for the shortest time. The results imply that clones or species with a larger ability to change their defensive phenotype have higher survival time in a system with intraguild

predation than the less flexible clones or species. In such systems, an intraguild prey faces a risk of extinction not only through predation, but also competition. When competition is high, the ability to survive on fewer resources may be important in preventing extinction through starvation. As a consequence, the clone with the greatest ability to be large and inedible in times of high predation risk, but also be small in times of limited food availability should be the most likely to persist, as was observed in this study. Therefore in highly dynamic systems with intraguild predation, we would expect the most plastic intraguild prey species to be the most successful. As coexistence of all three species was achieved only in two replicates of the most reactive clone (B8), the question arises, how much flexibility is needed in order to achieve stable coexistence. Multiple mechanisms delaying extinctions and allowing many species to coexist are likely to work simultaneously in natural settings and the anti-predator inducible defense may be one of them. It is also important to notice that the differences in the mean population trajectories were detected especially at lower *Euplotes* densities (Fig. 5-3), where the dynamics may have been also affected by stochastic factors.

The population dynamics of either top predator or resources did not differ among treatments. The intraguild predators *Stenostomum* were detected in all microcosms at density of 35 ± 11 (mean \pm SE, $n = 12$) predators per chemostat after the termination of the experiment, surviving exclusively on the algal diet. In a previous study of rotifers feeding on algae (Verschoor et al. 2004), inducible defenses at the basal trophic level of linear aquatic food chains increased the food web persistence by moving minimum population densities further away from zero. It has been suggested that inducible defenses at the middle trophic level have substantially weaker stabilizing effects (Verschoor et al. 2004,

Vos et al. 2004a) than those at basal trophic levels. Here we show that inducible defenses at the middle trophic level enhance persistence of intraguild prey, in particular when these defenses are stronger. Our findings thus contribute to more complete understanding the interactive effects of intraguild predation and inducible defenses in modules of real food webs.

Adaptive intraguild predation where the top predator optimally forages on intraguild prey or basal resource stabilizes mathematical models (Krivan 2000). In this model, a predator adaptively switches between food sources in a way that maximizes its intrinsic population growth rate (Krivan 2000). Because inducible defenses in intraguild prey often depend on density of their predators, they are considered to be adaptive and may impose similar stabilizing effect in intraguild food webs (Holyoak and Lawler 2005). Density-dependent variation in prey vulnerability to predation brought on by inducible defenses weakens the trophic interactions between predator and intraguild prey. Lower reproduction and increased investment in prey defenses often follow increases in predator population size. This consequently leads to a drop in the predator's population density and creates a negative feedback loop that may stabilize predator-prey dynamics. Alternatively, inducible defenses in prey species may be viewed as a form of refuge as they have been widely shown to reduce predation risk (Jeschke and Tollrian 2000, Relyea 2001, Vos et al. 2002, Altwegg et al. 2006). The presence of refugia has been widely recognized as a stabilizing factor in predator prey interactions (Maynard-Smith 1974, Vos et al. 2002, Gonzalez-Olivares and Ramos-Jiliberto 2003), and in intraguild food webs specifically (HilleRisLambers et al. 2006, Amarasekare 2007, see also Janssen et al. 2007).

Phenotypic plasticity in intraguild prey not only changes the interaction with intraguild predators but it may also influence the interaction with basal resources. The extent of morphological inducible defenses seems to affect foraging efficiency in *Euplotes* (Duquette et al. 2007). The feeding rate was similar for induced and uninduced forms in two low reactive *Euplotes* clones. However, the clone with the largest morphological defense showed reduced foraging efficiency after induction of its anti-predator morphology (Duquette et al. 2007). Despite such possible fitness cost, the two clones with the largest ability to modify their defensive morphology after exposure to predators (B8 and S5-1) persisted significantly longer in our experiment than the two clones with a minor inducible defenses (SC8 and AED33). This implies that the relative advantage of inducible defenses is possibly stronger than the disadvantage of reduced foraging in the food webs with intraguild predators.

Recent studies show that increased persistence of intraguild predation in real food webs take place by processes that either enhance the resources available to intraguild prey or reduce the strength of interaction between the predator and intraguild prey (Amarasekare 2007, Daugherty et al. 2007, Janssen et al. 2007, Kondoh 2008). We propose that inducible defenses are one of the ecological mechanisms that promote persistence of intraguild predation systems by reducing the interaction strength between the predator and intraguild prey. Given that inducible defenses are widespread, the results of our study may have equally broad implications for species persistence and biodiversity conservation. As nutrient levels are also known to affect persistence of food webs with intraguild predation, a valuable insight would be gained from future research

investigating intraguild predation with different magnitudes of inducible defenses across a productivity gradient.

Chapter 6: SUMMARIZING DISCUSSION

6.1. Historical view of communities

The simplest concept used to describe community structure was developed in the early 20th century (Elton 1927, Lindeman 1942), where communities were split into three to five trophic levels (e.g. primary producers, herbivores, and predators) connected by energy flow. The formulation of the “green world” hypothesis (Hairston et al. 1960) made an important contribution to the understanding of trophic interactions. This hypothesis assumes that a whole trophic level is dynamically equivalent to a single species and that predators regulate densities of herbivores, thereby releasing primary producers from depletion. The hypothesis was later extended to more than three trophic levels by Fretwell (1977, 1987) and formally analyzed by Oksanen et al. (1981).

Increased plant productivity was expected to translate into an increased equilibrium density of top predators and all even-numbered trophic levels below them. Equilibrium densities of odd trophic levels were expected to remain constant. The crucial achievement of this simple theory was the dynamical coupling of several trophic levels. However, the application of such simple models to real ecosystems is often limited by heterogeneity within a trophic level and prevalence of reticulate (as opposed to linear food chains) species interactions (Cousins 1987, Walters et al. 1987, Leibold 1989, Kretzschmar et al. 1993, Polis 1999, Polis and Strong 1996, Persson 1999). Empirical work has shown that trophic levels in natural systems are never homogeneous and rarely behave as a single species (e.g. Brooks and Dodson 1965, Cousins 1987, Leibold 1989). Food web models allow this heterogeneity to be included.

6.2. Community consequences of heterogeneity within a trophic level

Simple linear food chains with strongly coupled species are inherently unstable (McCann et al. 1998). Introducing heterogeneity at the prey level can reduce the interaction strengths between predator and prey, and increase their stability and persistence. Prey heterogeneity may result from the presence of non-prey species that are permanently inedible for predators (Kretzschmar et al. 1993, Vos et al. 2001, Vos et al. unpublished manuscript) or from the expression of inducible anti-predator defenses by some species (Abrams and Walters 1996, Vos et al. 2004, Verschoor et al. 2004). A similar stabilizing effect is predicted as a result of interference among conspecific predators (DeAngelis et al. 1975, Arditi et al. 2004, Rall et al. 2008). In my thesis, I combined laboratory experiments with mechanistic ecological models in order to assess interference among conspecific predators, the effects of non-prey species and inducible anti-predator defenses on short-term predation rates and long-term predator-prey dynamics.

Since I had observed higher predation rates at lower predator densities in my pilot study, I decided to perform a direct and highly controlled experimental test of predator-dependence (Chapter 2). Even though the stabilizing effects of predator-dependence have been discussed in the theoretical literature for forty years, rigorous direct experiments investigating the detailed shape of functional responses over a large gradient of predator and prey densities remain rare. The predation experiment I conducted clearly showed a substantial level of predator-dependence in our model system and, crucially, the effect could be detected at naturally plausible predator densities and after accounting for prey depletion. Model selection methods demonstrated very similar fit of the two best models, the mechanistic predator-dependent Beddington-DeAngelis model and phenomenological

predator-dependent Arditi-Akçakaya model. Fit of the ratio-dependent Arditi-Ginzburg model followed closely, but a Holling Type III functional response that does not account for predator interference did not converge to a reasonable fit. A behavioural experiment supported my results as I was able to observe substantial numbers of encounters among conspecific predators. These findings illustrate that the classic assumptions of prey-dependent functional responses should be approached with caution, as some level of predator-dependence needs to be considered to accurately predict predator-prey dynamics (Kratina et al. 2009, this thesis).

In Chapter 3, I demonstrate that density and diversity of often ignored non-prey species (species outside a predator's diet) can substantially weaken the strength of trophic interactions. My analyses allowed me to tease apart the relative contributions of density and diversity and showed a surprisingly strong effect of diversity itself. Such fundamental findings can contribute to the long-standing diversity-stability debate. Although early mathematical models predicted lower stability in more diverse food webs (May 1972, 1973), a positive relationship between diversity and stability has been shown when interactions among species are mostly moderate or weak (McCann et al. 1998). For a focal predator, the vast majority of species in food webs are non-prey and their ratio to prey increases with increasing diversity. In these diverse food webs, predators must spend considerable time on non-prey species which reduces their foraging efficiency. My results suggest that non-prey diversity can cause a prevalence of weak interaction strengths in such food webs and thus contribute to their stability and persistence.

Given this observed influence of non-prey in short-term experiments, a logical extension was to investigate how this effect translated into long-term predator-prey

dynamics. To understand the long-term consequences of the effects uncovered in Chapter 3, I set up an experiment that spanned more than 250 prey generations (Chapter 4). I found that predator and prey went extinct from all replicates when incubated alone but persisted until the end of my experiment in four out of five replicates when non-prey were present. Two main mechanisms were responsible for the coexistence of predator, prey, and non-prey. Suppression of an otherwise superior competitor (prey) by the predator enhanced the growth of non-prey, and interference between non-prey and predator prevented prey from being completely overexploited. Although the effect of non-prey is rarely accounted for in ecological studies, both of these mechanisms potentially interact in enhancing coexistence of multispecies assemblages in nature.

I also investigated food webs with intraguild predation, such as tri-trophic food webs in which a top predator consumes an intermediate prey, as well as the resource of this prey (Chapter 5). Although such food webs are considered inherently unstable, tending to rapid extinctions of intraguild prey, intraguild predation is common in natural communities. Due to the potentially stabilizing consequences of inducible defenses for predator-prey dynamics, several recent studies have investigated the long-term effect of this type of phenotypic plasticity in linear food chain experiments (Verschoor et al. 2004, van der Stap et al. 2006, 2007, 2008). Although many studies focus on a single ecological mechanism, this study takes our knowledge a step forward by assessing the persistence of food webs in which two major ecological mechanisms, intraguild predation and inducible defenses, combine to govern population dynamics.

Striking and repeatable differences in the shape of population curves of intraguild prey with different inducibilities were detected. I demonstrated a strong positive

relationship between the ability of an organism to defensively change its morphology and its persistence time. Consequently, statistical models that included initial prey size, final prey size, and prey ability to change its morphology as a response to predation were compared. Both model simplification through the removal of non-significant terms and Akaike's Information Criterion (AIC) showed that a model containing only inducibility regardless of initial or final size best explained intraguild prey persistence. These results suggest that inducible defenses are an important ecological mechanism promoting persistence of natural food webs with intraguild predation.

6.3. Use of laboratory experiments and future directions

Laboratory microcosm experiments are often instrumental in the first tests of mathematical models and evaluating whether these models should be further explored under more natural settings (Desharnais 2005). Such experiments allow us to assess the long-term dynamics of several interacting species, and specifically test particular ecological mechanisms by excluding the inevitable complexity of natural systems. Environmental conditions are strictly controlled and only the species of interest and their interactions are investigated. This approach means that very reproducible results can be obtained. Despite such benefits, an extrapolation of the results to natural food webs should be undertaken with caution. Natural communities are much more complex than laboratory set ups and the focal species often react to complexes of multiple consumers, competitors, and are simultaneously exposed to several ecological mechanisms. Studies that investigate the stabilizing effect of more than one mechanism at the same time (e.g. intraguild predation and inducible defenses) are urgently needed to contribute to our understanding of their relative strengths.

It has been increasingly recognized that in addition to the prevalence of weak interactions, system persistence is also affected by the distribution (i.e. configuration) of weak and strong species interactions in a particular food web (McCann 2007, Ings et al. 2009). Such a structural property of food webs can be also investigated in laboratory microcosms. Species assemblages of increasing complexity, with inclusion of cannibalism, omnivory, or loops can be easily constructed and their persistence measured. This effect can be also investigated across a productivity gradient.

Furthermore, including non-trophic links, such as mutualisms or the wasted time of non-prey species, into the food web models is a promising venue for the future research. My results (Kratina et al. 2007, this thesis) and a recent theoretical study (Vos et al. unpublished manuscript) show that new insight may be gained by investigating the stabilizing influence of non-prey diversity. How many non-prey species are necessary to sustain a stable food web over a long period of time? Are some non-prey species more important than others? What are the relative strengths of these versus other mechanisms that can increase or reduce community stability and persistence? These are just a few questions crucial for our understanding of community dynamics and their answering may improve our decisions in conservation management.

Ecological models derived from first principles can provide a mechanistic, and thus more predictable, explanation of predator-prey dynamics. Incorporating predator interference and prey heterogeneity can increase the realism of such models. Addition of extra mechanisms or parameters usually results in better fit of the particular model but including too many mechanisms or parameters can produce parameter estimates that lack precision. Complex models with excessive numbers of parameters are also challenging to

use by a broad audience. Maximum likelihood estimation of parameters in these mechanistic models and an information theoretic approach, such as AIC, to choose among models is a general approach that allows comparison of multiple models and has proven useful in my research. This approach of comparing multiple mechanistic models with experimental data represents a step forward from using phenomenological models. Crucial findings in the laboratory can highlight topics that deserve further investigation on a large scale and ecological research can benefit from using a combination of mechanistic models, laboratory experiments, large-scale manipulations and surveys in natural settings.

A suitable mechanistic model for the dynamics in Chapter 4 has so far resisted development. We combine continuous time population dynamics with regular bouts of density independent mortality. Estimating the parameters of such a model can tell us much more than whether the system is more or less stable. For example, changes in parameter values between treatments can tell us why these dynamics are observed and direct the focus of future research. Building a mechanistic model remains a priority.

6.4. Implications

Global biota is becoming homogenized owing to the accelerating loss of biodiversity and invasions of alien species to new regions. Community consequences of such homogenizations are still poorly understood. Crucial to the understanding of the consequences of homogenization and its role in biodiversity loss are the identification of ecological mechanisms that allow ecosystems to persist over long periods. Knowing what factors are important to maintain ecosystems is the first critical step in developing effective conservation and management strategies. My research has centered around

uncovering what these important ecological mechanisms are and showed that predator interference, density, and diversity of non-prey species, and inducible anti-predator defenses may be fundamental for other species and links to persist. Species outside the predator's diet (non-prey) that have often been treated as redundant can actually play important roles in enhanced persistence and stability of whole ecosystems. Reducing non-prey diversity may be followed by secondary extinctions of other focal species. Species that are flexible in changing their phenotype as a response to predation can enhance food web stability and persistence and deserves the attention of conservation practitioners. On the other hand, when a novel invasive predator is introduced to the system, native prey do not have the mechanisms to recognize or estimate predation risk and therefore do not induce their anti-predator phenotype. The absence of inducible defences may then promote strong interactions between predator and prey which can lead to rapid prey overexploitation. The ineffectiveness of mechanisms that otherwise weaken trophic interactions may cause destabilization and restructuring of natural communities. Incorporating this biological realism into simple food web models can improve our predictions about consequences of species invasions, biodiversity loss, and global changes.

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Appendix A

Population dynamics of predator, prey, and non-prey in six different food web configurations from Chapter 4 (all replicates shown). Filled circles ● denote predators (*Stenostomum*), open triangles Δ prey (*Paramecium*), and open circles ○ are non-prey (*Euplotes*).

