DYNAMICS AND ADAPTIVE SIGNIFICANCE OF THE INDUCIBLE TROPHIC POLYMORPHISM OF TETRAHYMENA VORAX

By

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A Dissertation Submitted to:

Graduate School – New Brunswick
Rutgers, The State University of New Jersey

in partial fulfillment of the requirements for the degree of:

Doctor of Philosophy
Graduate Program in Ecology & Evolution

Written under the direction of:

Dr. Peter J. Morin

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New Brunswick, New Jersey
October, 2011
ABSTRACT OF THE DISSERTATION:

Dynamics and Adaptive Significance of the Inducible Trophic Polymorphism of

*Tetrahymena vorax*

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This dissertation provides a four-part synopsis of the methodology, results, and conclusions of an integrated set of experiments designed to explore the ecology of inducible trophic polymorphisms (ITPs). These experiments were conducted using isogenic populations of the polymorphic freshwater hymenostome ciliate, *Tetrahymena vorax* Kidder, in combination with other protozoa, bacteria, algae, and micro-invertebrates. Chapter 1 addresses the autecological significance of ITPs, revealing some of their potential costs and benefits to individuals in the context of competition under varying resource regimes. Chapter 2 demonstrates how ITPs can give rise to novel trait-mediated indirect interactions that affect community structure and population dynamics. Chapter 3 elucidates the extent to which the population dynamics of species exhibiting ITPs are affected by the trophic complexity of their habitat. Chapter 4 presents a detailed review of indirect offenses, a versatile adaptive strategy that ITPs can give rise to where inducing agents are symbionts. The contents of these chapters provide unique insights into the link between ITPs and related phenomena – such as cannibalism, intraguild predation, omnivory, and expanded diet breadth – and open new avenues of inquiry regarding the importance of the genetic and functional diversity of natural communities.
ACKNOWLEDGEMENTS

First and foremost, I must thank Peter Morin, my academic advisor. I cannot begin to describe the extent to which I am indebted to him for the countless ways in which he has nurtured my personal growth and professional development since the day I came to Rutgers. Rather than try to mold me into a re-creation of himself or persuade me to become a mindless automaton to be exploited for his own agendas, Peter chose to show respect for me both as his junior colleague and as an individual, which, I must add, he does with all of his students, irrespective of where they are on the academic totem pole. He did as much as he could to look out for me and was always willing to not only hear me out, but also draw upon his vast, encyclopedic knowledge and years of experience to help me achieve my goals. Time and again, I would come to him with what I thought was an insurmountable obstacle, muttering profusely in my frustration with it, only to have him take a quick look at it and systematically turn it into something that I knew I could handle. Peter encouraged me when I needed it, straightened me out when I needed it, left me to my own devices when I needed it, and did everything else that was required to ensure that I would become a competent, self-respecting scientist who was capable of finding his own way in the world. I could never have asked for a better mentor, role-model, or friend than what Peter has been to me these past several years.

Next, I must thank Marsha Morin, without whose intelligence, resourcefulness, integrity, diligence, awareness, reliability, compassion, cat-herding ability, and sheer tolerance for pain, my dissertation (along with our entire department) would have most certainly burst into flames and/or collapsed into some kind of gravitational point
singularity long ago. Her warmth and supportiveness have been a great source of strength and joy for me from the very first day we met. To my gratitude for all of this, I must also add my gratitude for the fact that she at some point forgave me for having addressed her as “ma’am” for the first year or so of our acquaintance. Especially in light of the fact that my parents had given both her and Peter express permission to beat me, I consider this to have been most kind.

I thank my committee members (Tim Casey, Rebecca Jordan, Mike Pace, and Cesar Rodriguez-Saona) for their always meaningful and prompt feedback on my various drafts and progress reports, their willingness to work around their respective schedules to accommodate my needs, their support of my research by way of resources and ideas, and their having made every major milestone toward my degree fun and fulfilling for me.

I thank Martha Haviland, Doreen Glodowski, and Jana Curry (along with Jana’s predecessor, Barbara Sena and the Busch MSLC’s administrative coordinator, Margie Eickhorst) for having provided me with the opportunity and the means to take up teaching, for having stood by me and come through for me whenever I needed help solving problems and making things happen, for tolerating (and often even encouraging) my various teaching-related shenanigans, and for being down-right awesome in general.

I thank the following people for helping me get started on the path to becoming a full-fledged ecologist: Jim “Doc” Applegate, Barbara Goff, Colleen Hatfield, Bruce Hamilton, Ted Stiles, Charles Harman, Fred Westcott, Ralph Caiazzo, Carol VanDalen, Joyce Hartmann, and Kathy Parsell. Each of them in their own unique ways gave me guidance, encouraged me to follow my heart, and enabled me to catch a glimpse of my true potential. Their wisdom and their friendship will forever be a part of who I am.
I thank the following fellow participants in the 2005 Community Ecology course (S211) at the Research School for Socio-Economic and Natural Sciences of the Environment for the stimulating discussions and fun we had back then and the stimulating discussions and fun we have had since: Anja Rubach, Karin Nilsson, Irene van der Stap, Wade Ryberg, Tim Engelkes, Björn Christian Rall, Magnus Huss, Órla McLaughlin, Euridice Leyequien, Geraldine Thiere, Sonia Kéfi, Martin Pedersen, Elly Morrien, Jordie Netten, Maarten Eppinga, Roy van Grunsven, Mariska te Beest, Cleo Gosling, Yuki Fujita, Ciska Veen, Marleen Riemens, Marleen Pierik, Tessa van der Hammen, Charlotte Borvall, Johan Ahnström, Frans Kuenen, Ellen Weerman, and Matthijs Schouten. Being together and working together with all of these fellow hard-core community ecologists / citizens of the world will remain one of my fondest memories in the years to come. Soon it will be our turn to do what our supervisors did for us.

I thank my former lab mates (Lin Jiang, Christina Kaunzinger, Zac Long, Timon McPhearson, Jen Price, Jenn Adams Krumins, Henry Stevens, Irene Karel, and Alex Kulczycki) for showing me the ropes, helping me to imbibe the principle of K.I.S.S. (Keep It Simple, Stupid!), and being a pleasure to hang out with.

I thank my current lab mates (Kevin Aagaard, Laura Chen, Jean Deo, Sean Hayes, Ruchi Patel, Jack Siegrist, Maria Stanko, Holly Vuong, and Talia Young) for suffering through my roughest drafts; putting up with my various quirks and ineptitudes within our common, confined space; and sharing with me their ideas, enthusiasm, and friendship.

I thank the following faculty members and loose affiliates for profound, timely advice and countless fascinating discussions (including everything from the need for

While I owe every single one of my friends from Rutgers my gratitude and acknowledgement for their empathy, encouragement, and collaboration, the following deserve special recognition for having gone above and beyond the call of duty for me on numerous occasions: Carrie Norin, Elena Tartaglia, Blake Mathys (and his lovely wife, Dimitria), Wayne Rossiter (and his lovely wife, Melissa), Ai Wen (and her lovely husband, Kenneth Elgersma), Dave Smith, Andrea Egizi, Ari Novy, Charlie Kontos, Aspa Chatzieftimiou, Diana Johnson, Tricia Ramey-Balci, Kerri-Ann Norton, Rob Hamilton IV, Kristen Ross, Em Stander, Jenni Momsen, Sharron Crane, Alison Seigel, Alex Hernandez, Faye Benjamin, Wes Brooks, Brian Clough, Jeremy Feinberg, Zac Freedman, Sona Mason, Christine Minerowicz, Mat Pompliano, and Stephanie Baccarella.

Most of all, I thank my family. They have understood the formula.
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INTRODUCTION

Phenotypic plasticity is the ability of an organism to alter its morphology or physiology in response to environmental cues. It is distinct from evolutionary adaptation in that it occurs at the individual level, rather than at the population level, and generally involves no change in the organism’s genetic material (DeWitt and Scheiner 2004). Many examples of phenotypic plasticity are conspicuous and familiar, such as the color changes of chameleons (Stuart-Fox et al. 2008) and the phototropism of plants (Correll and Kiss 2002). Others, including the various internal responses that allow for immunity (Frost 1999) and homeostasis (Woods 2009), are more subtle and easy to overlook. In population studies, phenotypic plasticity is fascinating not only as a versatile adaptive trait (Via et al. 1995), but also as a potential driver of diversification and speciation (Adams and Huntingford 2004, Pfennig et al. 2010).

Phenotypic plasticity has been shown to influence the dynamics and stability of ecological communities (Agrawal 2001, Garay-Narváez and Ramos-Jiliberto 2009). A particularly intriguing way that it may do so is by generating inducible trophic polymorphisms. Inducible trophic polymorphisms (hereafter abbreviated as ITPs) occur when some individuals in a population alter their morphology or physiology to expand the number of trophic levels on which they feed. Induction of alternative morphs occurs in response to environmental factors, such as specific chemical cues released by competitors and/or potential prey. ITPs are functionally complementary to inducible defenses among prey (Tollrian and Harvell 1999). As is true for inducible defenses, ITPs may initially seem like unusual adaptations that occur infrequently, but are in reality widely distributed across taxa.
The consequences of ITPs for long-term population and community dynamics are likely significant, but difficult to assess. For most long-lived organisms, the amount of time and effort required to monitor populations through two or more generations is substantial, and opportunities for genetic recombination can make it nearly impossible to distinguish induced variation from constitutive variation. To approach the challenge of addressing these consequences, I have conducted my research using protists in experimental microcosms. This approach has been shown to be valuable in testing ecological and evolutionary theory and unveiling the mechanisms that underlie general patterns (Cadotte et al. 2005, Holyoak and Lawler 2005). Experimental microcosms make for highly controlled and replicable systems. The short generation times of protists, together with their large population sizes, facilitate the study of population dynamics (Morin and Lawler 1996, Price and Morin 2004). In the microcosms I made use of in my research, the focal species was the freshwater hymenostome ciliate, *Tetrahymena vorax* Kidder.

The natural history of *T. vorax* makes this a fascinating and powerful model system for the study of ITPs. *T. vorax* exhibits a conspicuous ITP. Rapid morphological differentiation allows different individuals in a single isogenic population to feed lower (on bacteria) or higher (on ciliates that consume bacteria) in the same food chain (Kidder et al. 1940, Ryals et al. 2002). The different morphs of *T. vorax* are easily distinguished and counted via microscopy. Populations in the early logarithmic stage of growth typically consist entirely of small (~ 45-75 μm long), pear-shaped morphs, called “microstomes,” which feed on bacteria. In response to known chemical cues that are released by competing bacterivores, microstomes may transform into larger (~ 250 μm
long), oval-shaped morphs, called “macrostomes,” which feed on other ciliates (Smith-Somerville et al. 2000, Ryals et al. 2002). Once a microstome has transformed into a macrostome, it can give rise to additional macrostomes, or it can revert into a microstome at the time of cell division (~ every 6 hours).

My use of *T. vorax* for my experimental investigations has enabled me to explore the autecological significance of ITPs, evaluate the potential of ITPs to generate complex trait-mediated indirect effects, and elucidate the extent to which population and community dynamics associated with ITPs are affected by trophic complexity. In so doing, I have: (1) uncovered potential costs and benefits of different inducible morphologies in the context of competition, (2) demonstrated how ITPs can potentially mediate interactions such as “apparent competition” (Holt 1977), and (3) identified possible roles of major groups of aquatic consumers in limiting the distribution of polymorphic ciliates in nature. A portion of my work – Chapter 1 of this dissertation – has already been published in the journal *Functional Ecology* (Banerji and Morin 2009).

References


CHAPTER 1

Phenotypic Plasticity, Intraguild Predation, and Anti-Cannibal Defenses in an Enigmatic Polymorphic Ciliate

Summary

1. Inducible trophic polymorphisms are greatly underappreciated forms of phenotypic plasticity that allow organisms to respond dynamically to environmental variation by enabling them to change the trophic level upon which they feed. Although inducible trophic polymorphisms occur in a diverse array of organisms, their costs and benefits and their consequences for long-term population and community dynamics are poorly understood.

2. I studied the inducible trophic polymorphism of the freshwater hymenostome ciliate Tetrahymena vorax, whose isogenic populations can contain three distinct morphs: pyriform, bacterivorous microstomes; larger, carnivorous macrostomes; and elongate, “tailed” microstomes. I tested whether: (1) the tailed microstome constitutes an inducible defense against macrostomes and (2) the transformation of microstomes into macrostomes is size-dependent. I also recorded the dynamics of the three morphs in the presence and absence of an intraguild prey (Colpidium) across a gradient of growth medium concentrations to infer potential tradeoffs in the success of different morphs at different productivity levels.

3. Macrostomes do not discriminate between pyriform microstomes and readily-consumed heterospecific prey (Colpidium) when provided only one of these
alternative prey items. Tailed microstomes display greatly reduced susceptibility to consumption by macrostomes as compared to undefended, pyriform microstomes. Morph dynamics are consistent with the hypothesis that tailed microstomes serve as an inducible defense against cannibalism; tailed microstomes and macrostomes appear simultaneously, in both the presence and absence of *Colpidium*. At low productivity, *T. vorax* achieves higher rates of growth when able to feed on *Colpidium* than when feeding on bacteria alone. At higher productivity, this pattern is reversed, with growth rates maximized in the absence of *Colpidium*.

4. The reduced consumption rate of tailed microstomes by cannibalistic macrostomes, together with the simultaneous induction of tailed microstomes and macrostomes, suggests that both morphs comprise a coordinated adaptive response to the presence of intraguild prey.

**Key-words:** Induced offenses, *Tetrahymena vorax*, inducible trophic polymorphism, omnivory
Introduction

Phenotypic plasticity can potentially have profound effects on the population dynamics of interacting species. For instance, numerous examples of inducible defenses have been shown to completely alter the predicted outcome of predator-prey interactions (Tollrian and Harvell 1999). These inducible defenses can involve an array of behavioral, physiological, metabolic, or life-history traits and can be direct or indirect (Porter and Porter 1979; Abrahams 1993; Tollrian and Harvell 1999; Altwegg 2002). While inducible defenses are the subject of extensive study (Tollrian and Harvell 1999), their logical counterpart, inducible offenses (Padilla 2001), remain little-studied and greatly underappreciated. Inducible offenses often involve inducible trophic polymorphisms. In these situations, certain organisms alter the trophic level upon which they feed via changes in morphology, physiology, or behavior, modifying the number and types of interactions in their food web.

Inducible trophic polymorphisms take many forms. In some organisms, reversible, morphological transformations provide flexible responses to environmental variation within a single generation. In others, transformations constitute irreversible, developmental trajectories that commit individuals to particular trophic roles. Some inducible trophic polymorphisms produce changes in diet without changing the mode of acquisition, such as where a predator turns into a higher-level predator or a cannibal (Gilbert 1973, Wainwright et al. 1991). Others represent both a change in diet and in the mode of acquisition, such as where an herbivore turns into a carnivore (Pfennig 1992, Afik and Karasov 1995) or where an autotroph becomes a parasite (Baird and Riopel 1984).
Inducible trophic polymorphisms occur within a wide range of taxa that includes birds (Afik and Karasov 1995), amphibians (Pfennig 1992), fish (Wainwright et al. 1991), gastropods (Padilla 2001), plants (Baird and Riopel 1984), fungi (Barron 1977, Barron 1992, Kerry and Jaffee 1997), rotifers (Gilbert 1973), protists (Washburn 1988), and bacteria (Berleman and Kirby 2007). The diversity and prevalence of inducible trophic polymorphisms provide a compelling motivation to investigate what effects inducible trophic polymorphisms have on long-term population dynamics. The study of inducible trophic polymorphisms can also provide insights into related phenomena, such as cannibalism (Fox 1975, Polis 1981), intraguild predation (Polis et al. 1989, Holt and Polis 1997), omnivory (Polis 1991, Morin 1999, Diehl and Feissel 2000), variation in diet breadth (Wainwright et al. 1991), and phenotypic plasticity in general (DeWitt and Scheiner 2004). Inducible trophic polymorphisms may even shed light on certain evolutionary processes, due to the fact that they potentially allow reciprocal induced offenses and defenses to occur rapidly over short ecological time frames, changes that are comparable to the shifting phenotypes seen over much longer time frames as a consequence of reciprocal genetic changes in co-evolving species (Agrawal 2001).

The inducible trophic polymorphism of the freshwater protozoan Tetrahymena vorax Kidder (Ciliatea: Hymenostomatida) offers five main advantages as a model system for the experimental study of inducible offenses: (1) its small size (60-250 µm) and ease of culture makes experimental studies relatively convenient; (2) its short generation time (~ 6 hours) makes possible the collection of long-term population dynamics data in a matter of days or weeks; (3) its clonal mode of reproduction (the cells are amicronucleate and unable to conjugate) ensures that any short-term phenotypic
variation does not reflect underlying genetic variation; (4) its discrete polymorphism facilitates distinguishing between morphs and quantifying the transformation; and (5) the proximal cues triggering transformations are known and are easily manipulated (Ryals et al. 2002).

Isogenic populations of *T. vorax* contain three distinct morphs: microstomes, macrostomes, and tailed microstomes. Microstomes (~ 60 µm in length) are pyriform, with a small cytostome that restricts their diet to bacteria. Macrostomes (~ 200 µm) are ovoid, with a larger cytostome that allows them to prey on other smaller ciliates. Tailed microstomes (~ 90 µm) also consume bacteria, but unlike pyriform microstomes, are narrow, elongate cells with the posterior drawn out into a stiff, tail-like structure (Kidder et al. 1941, Williams 1961). New *T. vorax* populations (at low density and high resource levels) initially consist entirely of pyriform microstomes. As population density increases, some of the pyriform microstomes transform into macrostomes and tailed microstomes. Each of the three morphs can transform into any of the others (Fig. 1).

Microstomes transform into macrostomes when exposed to known cues, the nucleic acid catabolites uracil and hypoxanthine in the presence of ferrous iron (Smith-Somerville et al. 2000). The cues are released by other ciliates that are competitors for bacteria, such as *Colpidium striatum* Stokes (Ciliatea: Hymenostomatida) and also by conspecifics. In the absence of predators, *Colpidium* can reduce bacterial densities to a greater extent than *T. vorax* (Kaunzinger and Morin 1998, Price and Morin 2004), which suggests that microstomes might be competitively excluded by *Colpidium*. The ability of *T. vorax* to mount an inducible offense against its competitors may provide a potentially significant fitness advantage (Thingstad et al. 1996). However, as with any inducible
response, there may be constraints and/or costs that prevent this response from being constitutive (Reluye 2002, DeWitt and Scheiner 2004). If, for example, the induced macrostomes cannot discriminate between conspecifics and heterospecifics, the potential advantage of being able to eat interspecific competitors could be offset by the costs of cannibalism (Polis 1981).

Cannibalism can be adaptive if it allows a lineage to survive and reproduce during severe resource limitation (Crump 1983, Van den Bosch et al. 1988, Hoffman and Pfennig 1999). However, the observed rarity of cannibalism under less severe conditions and among taxa in general suggests that it exacts significant fitness costs (Pfennig and Collins 1993, Pfennig and Frankino 1997, Kusch 1999, Pfennig 1999, Pfennig 2000, Michimae and Wakahara 2002). Therefore, where inducible trophic polymorphisms create the potential for cannibalism, there is likely to be strong selection for mechanisms that minimize cannibalism on close relatives, or that divert attacks to non-kin (Gilbert 1973, Pfennig and Collins 1993, Pfennig and Frankino 1997, Kusch 1999, Pfennig 1999).

Grønlien et al. (2002) have demonstrated that macrostomes capture heterospecific prey in preference to conspecific microstomes and will engulf available microstomes only after exhausting the supply of heterospecific prey. These results suggest that T. vorax may indeed use kairomones or other cues to discriminate between kin and non-kin, but they also indicate that this ability does not actually prevent macrostomes from ultimately consuming smaller conspecifics. Nevertheless, T. vorax may still minimize the costs of cannibalism through reciprocal, coordinated, morphological changes among macrostomes and microstomes (Agrawal 2001, Kopp and Tollrian 2003a,b). My preliminary observations indicated that tailed microstomes may be less susceptible to
cannibalism by macrostomes because elongate tailed microstomes cannot be completely
engulfed. If so, tailed microstomes may constitute an inducible defense against
cannibalism that would allow for a highly adaptive mixed foraging strategy, assuming
they are consistently present when macrostomes are present.

I speculated that the polymorphism of *T. vorax* could be the result of size-
dependent differentiation. Microstomes vary somewhat in size because of recent feeding
history, position in the cell cycle, and chance events. The successful transformation from
a microstome into a macrostome could depend on cell size at the time of induction, where
larger microstomes transform into macrostomes upon induction, while smaller ones
transform into tailed microstomes. Presumably, the proportion of larger microstomes in
the population is determined by the overall abundance of energy and nutrients (in the
form of bacteria) within the system. If macrostome production is energetically costly, or
depends on higher levels of productivity, then decreased bacterial abundances should lead
to a decreased frequency of macrostomes within the population. If resource limitation is
not a significant constraint on macrostome production, however, intraguild predation
theory predicts that *T. vorax* should switch from feeding on bacteria, the shared resource,
when bacteria are abundant, to feeding on *Colpidium*, the intraguild prey, when
*Colpidium* is abundant relative to bacteria. Increasing bacterial abundance by increasing
productivity would, in this case, reduce the relative frequency of macrostomes in the *T.
vorax* population and decrease intraguild predation of *Colpidium* by *T. vorax* (Křivan

This paper describes a series of studies designed to elucidate the costs and
benefits associated with each morph of *T. vorax*. I explore three questions arising from
the inducible trophic polymorphism. (1) Do tailed microstomes serve as an inducible defense against cannibalism by conspecific macrostomes? (2) Is size-dependent differentiation responsible for the mix of macrostomes and microstomes seen in isogenic *T. vorax* populations? (3) Is the proportion of macrostomes in *T. vorax* populations more indicative of the energetic and physiological requirements of transformation or of the relative advantage to being a predator?

**Methods**

I obtained *T. vorax* from the American Type Culture Collection (strain 30421). Isogenic populations of *T. vorax* were cultured in 240-ml glass jars containing 100 ml of complex organic medium. Medium consisted of 0.37 g of Carolina Biological Supply protozoan pellets (Carolina Biological Supply Company, Burlington, NC 27215, USA) per L of filtered well water. Autoclaved medium was inoculated after cooling with three species of bacteria (prey for the ciliates – *Serratia marcescens*, *Bacillus subtilis*, and *Bacillus cereus*). I autoclaved all culture vessels before use. Screw caps on the jars permitted some air circulation, while restricting evaporation and limiting contamination.

The experimental units used in this study contained detritus-based food-chains, consisting of organic matter, bacteria, and ciliates. The organic matter in the growth medium provided a conveniently manipulated energy source. Differences in the concentration of the medium create differences in bacterial densities, which simulate changes in the productivity of the system (Morin 1999).

**Prey Selection by Macrostomes**

To determine the potential effectiveness of the tailed microstome as an inducible defense against cannibalism, I evaluated rates of prey consumption by macrostomes
feeding on different densities of pyriform microstomes, tailed microstomes, and *Colpidium*. Feeding took place in 15-mm Petri dishes in 0.2 ml of medium, to confine all cells (macrostomes and their prey) within the field of view of the microscope.

Each trial initially contained 30 macrostomes exposed to one of nine combinations of prey type (pyriform microstomes, tailed microstomes, *Colpidium*) and prey density (low ~ 201 cells / mL, medium ~ 403 cells / mL, and high ~ 599 cells / mL). Prey densities varied to ensure that any differences found among the capture rates for different prey items would be due to prey morphology, rather than to density-dependent encounter rates. The response was the number of successful captures observed within a 15-minute interval. I removed macrostomes that engulfed prey items using a micropipette, to keep track of captures by macrostomes. Individual macrostomes were, therefore, limited to completing a single capture per the 15-minute observation period. There were three replicates for each combination of prey type and prey density.

I compared the number of prey captured per unit time with an analysis of covariance (ANCOVA), focusing on differences among the three prey types (*Colpidium*, microstomes, and tailed microstomes) using prey density as a covariate. Differences in the slope of the prey-captured-vs.-prey-density relation among prey types would indicate differences in susceptibility to macrostomes.

**Phenology of the Tailed Microstome**

I grew isogenic populations of *T. vorax* with and without *Colpidium* across a gradient of 5 different nutrient concentrations to explore whether the phenology of tailed microstomes is consistent with the possibility of their serving as an inducible defense against cannibalism by macrostomes. The nutrient concentrations used were 50% (0.19
g/L), 75% (0.28 g/L), 100% (standard, 0.37 g/L), 125% (0.46 g/L), and 150% (0.56 g/L). I recorded the population density for each morph and for *Colpidium* on 6 days (days 1, 2, 3, 4, 5, and 7) over a 7 day interval, sampling approximately 0.3 ml from each jar without replacement. I first combined these observations of the densities of tailed microstomes and macrostomes in a single correlation analysis to evaluate whether the changing population density (cells/ml) of tailed microstomes was consistently correlated with the changing population density of macrostomes over time. One microcosm became contaminated with flagellates on Day 4 of the experiment. The data from this replicate was subsequently discarded for Days 4, 5, and 7, yielding a total of 177 data points for the analysis. This analysis assumes that population dynamics are sufficiently rapid that observations made on different days in the same microcosm are effectively independent. Given that *T. vorax* has a generation time of about 6 hours, this is a reasonable assumption. A more conservative analysis evaluated the correlation between densities of tailed microstomes and macrostomes separately for each of the sampling days (30 replicates on days 1, 2, 3; 29 replicates on days 4, 5, 7), treating each microcosm as an independent observation on a given day. A positive correlation between the densities of tailed microstomes and macrostomes would be consistent with the hypothesis that tailed microstomes are more abundant when macrostomes are present, though it would not indicate whether they arise in response to cues released by macrostomes or as a size-dependent response to a common cue released by prey. Inclusion of populations grown in the absence of *Colpidium* in this experiment ensured that the presence or absence of *Colpidium* had no bearing on whether or not tailed microstomes are more abundant when macrostomes are more abundant.
**Effect of Microstome Cell Size on Transformation**

Effects of cell size at the time of induction on macrostome formation were determined by sorting microstomes into two size classes, small (< 60 µm) and large (≥ 60 µm), and culturing them within 15-mm Petri dishes containing 2 ml of medium (standard concentration) with or without *Colpidium*. I then monitored microstomes every 12 hr, over a 48-hr period, and recorded the number of each morph within experimental groups. Each experimental group consisted initially of 5 pyriform microstomal cells, and there were 3 replicates for each group. I first compared differences in macrostome frequency using a pooled two-way analysis of variance (ANOVA), after confirming that the effects of time were not significant. I then conducted a more conservative analysis comparing differences in macrostome frequency for each sampling time individually.

**Population Dynamics in the Presence and Absence of an Interspecific Competitor across a Productivity Gradient**

Using the data collected from the phenology experiment, I compared the individual morph population densities of each treatment to determine the effect of productivity on the frequency of macrostome formation. In addition, I measured the population growth rates of *T. vorax* as a whole, calculated as the natural log of the mean number of cells per ml over time, with and without *Colpidium* across the productivity gradient, to measure fitness.

**Results**

**Prey Selection by Macrostomes**

Capture rates by macrostomes were influenced by both prey type (ANCOVA, \(F_{2,21} = 6.84, p = 0.0052\)) and prey density (ANCOVA, \(F_{1,21} = 106.44, p < 0.0001\)). Below
approximately 200 prey individuals / ml, prey capture rate by macrostomes was near 0, irrespective of prey type (Fig. 2). There was a significant interaction between prey type and prey density (ANCOVA, $F_{2,21} = 23.61, p < 0.0001$), which indicates that the slope of the relation between prey density and the number of prey captured differed among prey types. Macrostomes captured *Colpidium* and pyriform microstomes at approximately the same rate, while few tailed microstomes were captured even at the highest prey density.

**Phenology of the Tailed Microstome**

The correlation analysis for which I had combined population densities across time revealed that the population density of tailed microstomes was significantly correlated with that of macrostomes ($r = 0.687, p < 0.0001, n = 177$). The more conservative analysis yielded a significant positive correlation between the densities on Day 2 ($r = 0.770, p < 0.0001, n = 30$), Day 3 ($r = 0.764, p < 0.0001, n = 30$), Day 4 ($r = 0.739, p < 0.0001, n = 29$), and Day 7 ($r = 0.502, p = 0.0055, n = 29$). On Day 1, as one might expect, given that the cells would not have had time to differentiate, the correlation between the densities tailed microstomes and macrostomes was not significant ($r = 0.054, p = 0.7763, n = 30$). The correlation was also not significant on Day 5 ($r = 0.251, p = 0.189, n = 29$). Since, even in the conservative analysis, the correlation is positive on four of the six days, the pattern appears robust. At each simulated productivity, in both the presence and absence of *Colpidium*, macrostomes and tailed microstomes appeared simultaneously. At standard productivity, with *Colpidium* present, pyriform microstomes disappeared by the second day of population growth and did not reappear until after *Colpidium* disappeared (Fig. 3).

**Effect of Microstome Cell Size on Transformation**
ANOVA revealed a significant interaction between microstome cell size and *Colpidium* presence/absence on the induction of macrostomes (F\(_{1,56} = 29.57, p < 0.0001\)). This interaction was still observed when the analysis was conducted on each sampling time separately (F\(_{1,8} = 15.21, p = 0.0045\) for Hour 12; F\(_{1,8} = 147.05, p < 0.0001\) for Hour 24; F\(_{1,8} = 201.79, p < 0.0001\) for Hour 36; and F\(_{1,8} = 387.68, p < 0.0001\) for Hour 48). Microstomes less than 60 μm long did not transform into macrostomes in the presence of *Colpidium*. Although some pyriform cells transformed into tailed microstomes, the majority remained microstomes. The majority of microstomes greater than 60 μm transformed into macrostomes when induced by the presence of *Colpidium* (Fig. 4).

**Effects of Productivity and Intraguild Prey on Population Dynamics**

Although the population density of macrostomes was significantly correlated with both productivity (r = 0.296, p < .0001, n = 177) and the total population density of *T. vorax* (r = 0.795, p < .0001, n = 177), the proportion of macrostomes within the *T. vorax* population was not significantly correlated with these variables (r = 0.314, p = 0.0748, n = 177 for productivity and r = 0.048, p = 0.5229, n = 177 for total population density). Pyriform microstomes, which, at low productivity and in the presence of *Colpidium* achieved the lowest densities of all the morphs, increased and achieved a higher density than macrostomes at high productivity, in the absence of *Colpidium*. Tailed microstomes achieved the highest densities of all the morphs in each treatment (Fig. 5). The overall population growth rate of *T. vorax* was significantly influenced by productivity and the presence or absence of *Colpidium* (ANCOVA, F\(_{4,28} = 3.40, p = 0.0295\)). At higher levels
of productivity, *Colpidium* decreased the growth rate of *T. vorax*. At low productivity, the pattern was reversed, with *Colpidium* increasing *T. vorax* growth rates (Fig. 6).

**Discussion**

The results of my investigation strongly support the hypothesis that tailed microstomes constitute a defense by the smaller size fraction of microstomes to potential cannibalism by macrostomes. I recognize that I cannot separate whether the appearance of the tailed microstome is directly induced by the appearance of macrostomes, or whether tailed microstomes and macrostomes constitute different size-dependent developmental responses to the same cue released by high densities of prey or by conspecifics. Tailed microstomes clearly exhibit greatly reduced susceptibility to consumption by macrostomes, relative to pyriform microstomes. Macrostomes do not appear to discriminate between consuming conspecific and heterospecific cells, as shown by the observation that the capture rates of pyriform microstomes and *Colpidium* by macrostomes did not differ. Furthermore, tailed microstomes appeared simultaneously with macrostomes, even in the absence of *Colpidium*. The very low consumption rates of tailed microstomes by macrostomes, together with the phenology of tailed microstome morphs, enables the tailed microstome to serve as a coordinated, induced defense against cannibalism when macrostomes appear in the population. Synchronous inductions of offensive and defensive phenotypes such as this are unlikely to have evolved under conditions favoring cannibalism, as such a defense would negate any selective advantage of cannibalism. It is instead conceivable that the simultaneous induction may be part of a mixed strategy to reduce intraspecific competition and to capitalize on multiple types of available resources (Fisher 1958, Ehlinger 1990, Dudgeon and Buss 1996). In the case of
*T. vorax*, as the macrostomes feed on available ciliates, the tailed microstomes continue to graze on bacteria, while remaining immune to the predation pressure that their interspecific competitors face from macrostomes.

Differences in cell size at the time of induction provide one explanation for how the polymorphism allowing for this potential mixed strategy arises. The significant effect of cell size on the proportion of macrostomes induced within *T. vorax* populations suggests that a size threshold exists for the successful transformation from microstome to macrostome. Given that well-fed protozoa are larger than starved individuals (Kidder et al. 1941), high productivities, where bacteria are abundant, should yield larger proportions of macrostomes than low productivities, where bacteria are scarce. However, increasing nutrient concentration did not significantly increase the proportion of macrostomes, but instead merely increased the overall population density of *T. vorax*. The fact that cell division was favored over transformation in a situation where *Colpidium* was present at high density, but was neither as available nor as abundant as bacteria, indicates that macrostome induction is not as much determined by the energetic costs of transformation as it is by the relative advantage to being a predator.

The dominance of tailed microstomes over the other morphs at both high and low productivity and in both the presence and absence of *Colpidium* may indicate that tailed microstomes have a greater conversion efficiency of prey than either macrostomes or pyriform microstomes. This, in combination with the resistance of the tailed microstome to predation by cannibalistic macrostomes (and perhaps other gape-limited predators), begs the question of why *T. vorax* populations contain pyriform microstomes at all. The
fact that the tailed microstome does not appear to be constitutive implies the existence of either an unknown trade-off or a physiological constraint.

The reduced growth rate of T. vorax in the presence of Colpidium at the higher productivity levels can be attributed to the inefficiency of energy transfer between trophic levels. All else being equal, T. vorax, has less energy available to it when feeding on Colpidium than it does when feeding directly upon the basal resources (the bacteria). However, at low productivity, T. vorax had a higher growth rate in the presence of Colpidium than it did when feeding on bacteria alone. This could suggest that, at higher productivities, Colpidium is more difficult to capture and, therefore, yields a lower energy return. The cell size of Colpidium increases with productivity or nutrient concentration (Balciunas and Lawler 1995), and it is possible that Colpidium becomes too large for macrostomes to consume at higher productivities.

Alternatively, this might indicate that intraguild predation can be particularly advantageous at low resource levels. I can infer that Colpidium, in the absence of predation, is a superior competitor to T. vorax microstomes, knowing that, in monoculture, Colpidium reduces bacterial densities to a greater extent than T. vorax (Kaunzinger and Morin 1998, Price and Morin 2004). At low productivity, where bacteria are relatively sparse, T. vorax may gain greater net benefits from feeding on Colpidium than from feeding on the bacteria. In addition, cells that do not transform into macrostomes may still benefit from the presence of macrostomes as a result of macrostomes reducing the competitive ability of Colpidium (Tilman 1982, Thingstad 1997, Fox 2002). Measuring the impact of the presence of Colpidium on microstome growth rates to test this hypothesis would be virtually impossible because Colpidium
induces transformation in microstomes. However, future studies might examine the impact of *Colpidium* on closely related *Tetrahymena* species that do not exhibit inducible trophic polymorphism, such as an amicronucleate strain of *T. pyriformis* (Nanney et al. 1998). By comparing the population dynamics of *T. pyriformis* with and without *Colpidium* over a range of productivity, I could indirectly evaluate the potential costs of interactions with *Colpidium* to *T. vorax* microstomes and directly test the predictions of competitive outcomes based on resource use.

Inducible trophic polymorphisms as spectacular as those exhibited by *T. vorax*

may be unusual. Even among protozoa, where the rapid reversibility of transformations that take place within a single generation is far more commonplace than in larger, more complex organisms with developmentally stable exo- or endoskeletons, the changes in size that allow for shifts in trophic position are very seldom accompanied by drastic reconstructions of morphology (Kidder et al. 1941, Agrawal 2001). However, functionally similar trophic polymorphisms do occur in a diverse array of organisms and habitats, and not necessarily through changes in morphology. Behavior can be an extremely plastic trait in many higher-level organisms (DeWitt and Scheiner 2004) and can greatly influence the diet and diet breadth of those organisms (Schluter and Grant 1984, Packer and Ruttan 1988, Dean et al. 1990). Investigating the mechanisms of how inducible trophic polymorphisms occur may shed new light on our understanding of how phenomena such as diet breadth (Polis 1991, Morin 1999, Diehl and Feissel 2000, Jiang and Morin 2005), cannibalism (Polis 1981), and intraguild predation (Polis et al. 1989) impact community structure and dynamics, and even long-term evolutionary processes.

**Acknowledgements**
I would like to thank Timothy Casey, Lin Jiang, Ben Baiser, Andrea Kornbluh, Jean Deo, Jack Siegrist, Maria Stanko, Holly Vuong, and Jennifer Adams Krumins for their helpful comments and invaluable insights. Support for this research came from the Rutgers School of Environmental and Biological Sciences and the New Jersey Agricultural Experiment Station.

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Fig. 1. Diagram of the inducible trophic polymorphism of *T. vorax*. Gray arrows represent developmental pathways. Black arrows represent trophic links. Arrow size represents relative interaction strength. Transformation of microstomes into macrostomes is triggered by chemical cues released by conspecifics and by interspecific competitors for bacteria, such as *Colpidium* (Kidder et al. 1941; Smith-Somerville 2000; Ryals et al. 2002).
Fig. 2. Comparison of the differences in susceptibility of microstomes, tailed microstomes, and *Colpidium* to consumption by macrostomes. Black squares mark the regression line for microstomes; black triangles mark that of tailed microstomes; and gray circles mark that of *Colpidium*. 
**Fig. 3.** Morph dynamics of *T. vorax* at standard medium concentration in the presence and absence of *Colpidium* during a 7-day period. Large unfilled black squares represent macrostomes. Black triangles represent tailed microstomes. Small black squares represent microstomes. Gray circles represent *Colpidium.*
Fig. 4. Effect of microstome cell size (small < 60 µm; large ≥ 60 µm) on the outcome of induction. Symbols are as in Fig. 3.
Fig. 5. Morph dynamics in the presence and absence of *Colpidium* at low (50% of standard) and high (150% of standard) medium concentrations. Symbols are as in Fig. 3.
Fig. 6. Interaction between the effects of productivity and the presence or absence of *Colpidium* on the rate of growth of *T. vorax*, calculated as the natural log of the mean cell density of *T. vorax* over time. Black diamonds represent growth rates with *Colpidium* present. White diamonds represent growth rates with *Colpidium* absent.
CHAPTER 2
Prey-Induced Gape Expansion Mediates Apparent Competition

Summary

1. Phenotypic plasticity allows certain predators to increase their gape by feeding on increasingly large prey. Where predators increase their gape enough to feed on initially inedible species, a novel, trait-mediated indirect interaction may occur – one that is akin to apparent competition.

2. I tested the hypothesis that feeding on *Colpidium kleini*, a large intraguild prey, enables macrostomes of *Tetrahymena vorax* to increase their gape enough to engulf *Paramecium aurelia*, a species too large for macrostomes to engulf prior to such feeding.

3. My results were consistent with the hypothesis. Macrostomes allowed to feed on *C. kleini* showed a significant increase in their mean cell diameter over time. When exposed to *P. aurelia*, these enlarged macrostomes were able to engulf *P. aurelia*, whereas macrostomes that had been cultured in the absence of *C. kleini* were unable to engulf *P. aurelia*. The population dynamics of *T. vorax*, *C. kleini*, and *P. aurelia* during a 22-day period revealed that the occurrence and outcome of expanded diet breadth resulting from increased gape in *T. vorax* depended on the recent history of community assembly.

**Key-words:** Chasmatectasis, phenotypic plasticity, priority effect, inducible trophic polymorphism (ITP), *Tetrahymena vorax*, trait-mediated indirect interaction (TMII)
 Introduction

Phenotypic plasticity is the ability of a single genotype to produce two or more alternative phenotypes in response to differing environmental conditions (DeWitt and Scheiner 2004). One intriguing consequence of phenotypic plasticity is the generation of inducible trophic polymorphisms, wherein morphologically or physiologically different individuals within a population come to occupy different trophic positions (Kopp and Tollrian 2003a, Banerji and Morin 2009). Inducible trophic polymorphisms can result in the utilization of a broader range of available resources than would be used by monomorphic populations. In addition, induced individuals may be capable of consuming some of their natural enemies (Washburn et al. 1988, Padilla 2001, Price and Morin 2004). Since a major determinant of whether or not a predator is able to capture and consume its prey is the disparity in size between the two (Maret and Collins 1996, Urban 2007), it is not surprising that, in inducible trophic polymorphisms, a shift from a lower trophic position to a higher one often simply entails an increase in size – either of the body in its entirety or of specific internal and external feeding structures (Polis 1981, Ricci et al. 1989, Wainwright et al. 1991, Maerz et al. 2006, Olsson et al. 2007).

More surprising is that certain species with inducible predatory morphs can continue to increase their gape after the initial induction of their predatory morphology by ingesting increasingly large prey. As I have not been able to find a precise term for this phenomenon anywhere in the ecological literature, I have borrowed the pseudo-clinical term used jokingly by a handful of medical students with whom I am acquainted to describe the preferred training method of professional gurgitators (Levine et al. 2007, O’Connor 2007): chasmatectasis (from the Greek words khasma, “gape,” and ektasis,
“stretching”). Chasmatectasis is an inducible offense (Padilla 2001) that has the potential to significantly alter the shape and outcome of ecological dynamics (Miner et al. 2005, Jeschke 2006, Mougi et al. 2010). It may, for example, provide a counter-measure against the inducible defenses of prey (Kopp and Tollrian 2003b, Kishida et al. 2006) or enhance the ability of predators to survive and recover from periods of starvation (Hewett 1987). There is anecdotal evidence of the occurrence of chasmatectasis in rotifers (Gilbert 1973), cladocerans (Abrusán 2003), amphibians (Collins and Cheek 1983, Pfennig 1990, Walls et al. 1993), fish (Wainwright et al. 1991, Persson et al. 2004), and reptiles (Queral-Regil and King 1998). Among protists, and particularly among ciliates, definite examples of chasmatectasis have been observed relatively frequently (Giese and Alden 1938, Rickards and Lynn 1985, Hewett 1987, Ricci et al. 1989, Kopp and Tollrian 2003a).

An ecological aspect of chasmatectasis that has not previously been explored is its potential to give rise to novel indirect interactions (Wootton 1994). In particular, chasmatectasis might enable an induced predator to increase its gape enough to consume a species that would have been too large for it to consume at the time of its initial induction. The resulting exchange of negative indirect effects between the smaller and larger prey species via their now shared predator would constitute apparent competition (Holt 1977, Bonsall and Hassell 1997, Chaneton and Bonsall 2000). However, the effects would be mediated via the predator’s gape rather than its population size, making this a “trait-mediated” indirect interaction, instead of a “density-mediated” indirect interaction (Peacor and Werner 2001). To explore this possibility, I made use of the
inducible trophic polymorphism of the freshwater protozoan *Tetrahymena vorax* Kidder (Ciliatea: Hymenostomatida).

Isogenic populations of *T. vorax* may contain both “microstomes” and “macrostomes.” Microstomes are small, pyriform individuals that feed on bacteria. Macrostomes are larger, ovoid individuals that consume other ciliates. Microstomes can reversibly transform into macrostomes in the presence of intraguild prey, which may include conspecifics (Kidder et al. 1941, Williams 1961, Banerji and Morin 2009). My preliminary observations of macrostomes exposed to varying sizes of intraguild prey indicate that macrostomes are capable of some degree of chasmatectasis. Here I describe my experimental approach to quantifying these observations and testing the hypothesis that feeding on accessible prey, such as *Colpidium kleini* Foissner (Ciliatea: Hymenostomatida), enables macrostomes to attain sizes large enough to engulf species such as *Paramecium aurelia* Müller (Ciliatea: Hymenostomatida), which, prior to such feeding, would exceed the limit of their gape (Fig. 1).

**Methods**

**Culturing of Ciliates**

The distribution of *T. vorax* in nature is poorly known, a situation that is typical for protists and other microbes (Fenchel et al. 1997, Finlay 2002, Foissner 2009). Fortuitously, many of the protists known to occur with *T. vorax* have cosmopolitan distributions (Finlay 2002). Among these are species of the genus *Colpidium*, which Kidder et al. (1941) were able to identify as intraguild prey of *T. vorax* almost immediately upon its discovery. Members of the widely distributed *Paramecium aurelia* complex are also likely to be found where *T. vorax* is present (Sonneborn 1975, Finlay...
I chose *C. kleini* and *P. aurelia* for use in my investigation for the ease with which they could be obtained and cultured.

I purchased *T. vorax* (strain 30421) and *P. aurelia* (Item # 131546) from the American Type Culture Collection and the Carolina Biological Supply Company, respectively, and isolated *C. kleini* from the Adelphia Plant Science Research and Extension Center (Freehold, NJ 07728, USA). I grew separate, isogenic lines of each of these three ciliates in 240-mL glass jars containing 100 mL of complex organic growth medium. The medium I used consisted of 0.37 g of Carolina Biological Supply protozoan pellets per L of filtered well water. I sterilized and cooled the medium to room temperature and then inoculated it with three different species of bacteria – *Serratia marcescens*, *Bacillus subtilis*, and *Bacillus cereus* – to serve as a basal resource for the ciliates. Loosely tightened screw caps on the jars permitted air circulation, while limiting evaporation and contamination.

**Measuring Chasmatectasis in *T. vorax***

To verify that macrostomes are able to increase their size and consume larger prey, I performed an induction experiment. I inoculated six 15-mL Petri dishes, containing 7 mL of growth medium, with *T. vorax*. I allowed three of these *T. vorax* cultures to feed solely on bacteria (control replicates), while immediately inoculating the other three with *C. kleini* (treatment replicates). After *C. kleini* had been extirpated from the treatment replicates and all six replicates had entered into logarithmic growth (~ 4.5 days), I inoculated all six cultures with *P. aurelia*. I sampled approximately 0.3 mL from the cultures every 12 hours over the course of 10 days to record average macrostome cell size and the frequency of predation.
I measured average macrostome cell size as the mean cell diameter, in micrometers, of macrostomes that were present within the sample. I made individual cell diameter measurements by taking digital photomicrographs of the macrostomes in my samples at 400X magnification. I used a Nikon Eclipse 80i microscope equipped with a Nikon DXM1200C camera and ACT-1C imaging software. I analyzed the photos using the NIH program, ImageJ (see Rasband 2007). I measured frequency of predation on *P. aurelia* as the total number of observed incidences of a macrostome having engulfed *P. aurelia* during my sampling interval (~ 5 minutes per dish).

**Determining the Effect of Community Assembly on the Occurrence and Outcome of Chasmatectasis in *T. vorax***

I performed a community assembly experiment to address: (1) whether feeding on *C. kleini* prior to being exposed to *P. aurelia* is required for chasmatectasis in *T. vorax* and (2) how chasmatectasis in *T. vorax* affects the relative fitness of all three species. My experimental design involved four treatments, with five replicates per treatment. Each replicate consisted of 100 ml of growth medium in a covered 240-mL glass jar that was initially inoculated with *T. vorax*. *C. kleini* and *P. aurelia* were later added to the replicates in different temporal sequences (Table 1).

I monitored the populations of all of the species present in each jar approximately every 24 hours, over the course of 22 days, sampling approximately 0.3 mL without replacement from each jar. To ensure that effects of the treatments were not confounded by the build-up of toxins and metabolic wastes in the system, I removed and discarded approximately 10% of medium by volume from each jar and replaced it with an equivalent volume of fresh medium on Day 16. No measurements were recorded on this
day. Adhering to the same procedure, I constructed and monitored a pair of controls (5 replicates per control), each containing both *C. kleini* and *P. aurelia*, but without *T. vorax*. This provided a baseline to assess any indirect effects among *C. kleini* and *P. aurelia* mediated by *T. vorax* (Table 1).

**Results**

*Measurement of Chasmatectasis in T. vorax*

Macrostomes fed *C. kleini* showed a significant increase in size (ANOVA, $F_{1,277} = 87.41, p < 0.0001$) over the 10-day period of study (Fig. 2, Fig. 3). On Days 6-8, Day 9, and Day 10, I recorded no average cell diameter of macrostomes in the control replicates because no macrostomes were present in the control replicates during this period. Macrostomes in the control replicates that had been cultured in the absence of *C. kleini* appeared to have reverted to microstomes in the presence of *P. aurelia*. While I observed 8 separate incidences of macrostomes engulfing *P. aurelia* in the treatment replicates, I did not observe any incidence of macrostomes doing so in the control replicates.

*Effect of Community Assembly on the Occurrence and Outcome of Chasmatectasis in T. vorax*

The order in which *T. vorax* was exposed to *C. kleini* and *P. aurelia* had a significant effect on the mean population densities of both *C. kleini* (ANOVA, $F_{1,8} = 407.57, p < 0.0001$) and *P. aurelia* (ANOVA, $F_{1,8} = 3162.48, p < 0.0001$) across time. It also had a significant effect on the mean densities of macrostomes (ANOVA, $F_{1,8} = 144.2, p < 0.0001$) and microstomes (ANOVA, $F_{1,8} = 179.98, p < 0.0001$) in the *T. vorax* population. In replicates where *T. vorax* was exposed to *C. kleini* first and to *P. aurelia* second, *P. aurelia* was ultimately extirpated. In replicates where *T. vorax* was exposed to
*P. aurelia* before being exposed to *C. kleini*, however, *P. aurelia* was able to persist throughout the experiment. In the controls, where *C. kleini* and *P. aurelia* were paired in the absence of *T. vorax*, both species persisted throughout the experiment (Fig. 4). *C. kleini* and *P. aurelia* were both able to persist at relatively high population density when paired alone with *T. vorax* (Fig. 5). *T. vorax* macrostomes, in the presence of both prey, attained higher densities and persisted longer when exposed to *C. kleini* first. In the presence of *C. kleini* alone, *T. vorax* macrostomes attained higher population densities and persisted longer than in the presence of *P. aurelia* alone (Fig. 6).

**Discussion**

My results strongly support the hypothesis that chasmatectasis can enable a predator to expand its diet breadth to include species that were previously inaccessible due to their large size relative to the predator’s initial gape. By feeding on *C. kleini*, a relatively large but still initially consumable prey, *T. vorax* was able to expand its gape enough to consume *P. aurelia*, a prey that *T. vorax* could not consume upon initial transformation. Furthermore, I have demonstrated that the occurrence and ecological consequences of chasmatectasis can be influenced by the history of community assembly. Where *T. vorax* was exposed to *C. kleini* before being exposed to *P. aurelia*, *T. vorax* underwent chasmatectasis and became able to feed on *P. aurelia*. Yet, where *T. vorax* had been exposed to *P. aurelia* first, *T. vorax* not only failed to undergo chasmatectasis, but also maintained a lower population density of macrostomes throughout the experiment. It is conceivable that competition with *P. aurelia* for bacterial resources might have prevented *T. vorax* from crossing the minimum size threshold for transformation into macrostomes (Banerji and Morin 2009) upon the arrival of *C. kleini*
and that maintenance of the macrostome morphology requires ingestion of prey. In the replicates in which *T. vorax* was able to undergo chasmatectasis and feed on *P. aurelia*, *P. aurelia* was extirpated. Given the low rate of consumption of *P. aurelia* by *T. vorax* indicated by the results of the induction experiment, the extirpation of *P. aurelia* in these replicates cannot be attributed to predation alone. However, given the persistence of *P. aurelia* in the replicates in which *T. vorax* had been unable to undergo chasmatectasis, it appears that predation must still have played a significant role.

Chasmatectasis is an intriguing manifestation of phenotypic plasticity that warrants further investigation in other taxa. The underlying genetic and physiological basis for chasmatectasis has yet to be revealed, and its environmental basis in natural systems remains uncertain. In the case of my system, for example, it is unclear whether the ability of *C. kleini* and other large, but accessible prey to trigger chasmatectasis in *T. vorax* is a function of the size of the prey, in and of itself, or of the composition – i.e., nutritional or energetic content – of the prey. Still to be determined, also, is the upper size limit of prey that can be consumed by *T. vorax* via chasmatectasis. Future studies might readily address these questions by repeating my experimental design using different prey species of varying size and composition.

Chasmatectasis is not limited to ciliates. Though they differ from the examples seen in ciliates in that they are generally irreversible, examples of chasmatectasis, as previously mentioned, can be found in fish, amphibians, reptiles, and a range of invertebrates. Dynamics similar to those I have uncovered in this investigation may very well occur in these other systems. Such dynamics could have repercussions for the study of colonization and succession. For example, given that overall body size has been
shown to play a significant role in determining the invasion success of predators (Persson et al. 2004, Schröder et al. 2009), diversity of body sizes among incumbent prey may facilitate invasion by predators capable of undergoing chasmatectasis.

Dynamics resulting from chasmatectasis might also have implications for management practice, as shown by my demonstration of chasmatectasis mediating apparent competition. Apparent competition has been shown to be an important determinant of community structure (Jeffries and Lawton 1984, Settle and Wilson 1990, Bonsall and Hassell 1997, Juliano 1998, Orrock et al. 2008), and classical apparent competition theory has been applied in the contexts of biological control (Karban et al. 1994, van Veen et al. 2006) and restoration (Norbury 2001, Hoddle 2004). Understanding the workings of chasmatectasis could enable us to determine if apparent competition that is mediated by chasmatectasis has the same significance in ecological communities as classical apparent competition and enable us to avoid the potential pitfalls (Howarth 1991, Paynter et al. 2010) associated with its use in biological control and restoration.

Acknowledgements

I gratefully acknowledge Rebecca Jordan, Mike Pace, Kevin Aagaard, Laura Chen, Jean Deo, Sean Hayes, Ruchi Patel, Jack Siegrist, Maria Stanko, Holly Vuong, and Talia Young for their worthwhile comments and suggestions. Support for this research came from the School of Environmental and Biological Sciences and the New Jersey Agricultural Experiment Station.

References


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**Table 1.** Inoculation sequence for community assembly experiment. Treatment replicates were initially inoculated with *T. vorax* and later inoculated with *C. kleini*, *P. aurelia*, or both in different orders. Control replicates consisted of *C. kleini* and *P. aurelia* without *T. vorax*. 
Fig. 1. Chasmatectasis in *T. vorax* may create negative trait-mediated indirect effects between *C. kleini* and *P. aurelia*. Black arrows represent trophic links, while gray arrows represent developmental pathways.
**Fig. 2.** Mean macrostome cell diameter over a 10-day period. White diamonds represent diameters observed in the presence of *C. kleini*; black diamonds represent diameters in the absence of *C. kleini*. On Days 6-8, Day 9, and Day 10, no macrostomes were observed in the absence of *C. kleini*. On Day 8.5, only 3 macrostomes were observed in the absence of *C. kleini*, one of which being a low-end outlier in terms of its cell diameter.
Fig. 3. Representative micrographs (differential image contrast scans) of *T. vorax* macrostomes observed on the last day of my induction experiment (Day 10). Shown on the left is a macrostome from a control replicate (monoculture with bacteria as the only available prey). Shown on the right is a macrostome from a treatment replicate (initially containing *C. kleini* as well as bacteria).
**Fig. 4.** Population dynamics of *C. kleini* and *P. aurelia* in replicates where they were paired in the presence (top) and absence (bottom) of *T. vorax*. Gray circles represent the abundance of *C. kleini*, and black crossed diamonds represent that of *P. aurelia*. Note the log scales of the Y-axes.
**Fig. 5.** Population dynamics of *C. kleini* (left) and *P. aurelia* (right) alone with *T. vorax*. Symbols are as in Fig. 4.
Fig. 6. Morph dynamics of *T. vorax* in the replicates shown at the top of Fig. 4 (top) and shown in Fig. 5 (bottom). Large unfilled squares represent the abundance of macrostomes. Small black squares represent that of microstomes. Note the log scales of the Y-axes.
CHAPTER 3

Dynamics between Protists and Crustacean Zooplankton: Do Copepods Show Preference for the Predatory Morph of *Tetrahymena vorax*?

**Summary**

1. Studies of protists in microcosms have made important contributions to ecology, but some critics argue that the small spatial scale and trophic simplicity of microcosms make them inappropriate as models of complex natural systems. This concern is especially important to address in cases where both the nature and strength of trophic links within the community are density-dependent.

2. I used outdoor experimental mesocosms to assess whether increased trophic complexity and variable abiotic conditions would alter the dynamics of a simple freshwater protist community module that I had previously examined in laboratory settings. This community contained the polymorphic ciliate *Tetrahymena vorax* Kidder. I tested the following hypotheses: (1) that the volume and ambient abiotic conditions of the mesocosm habitat would inhibit the induction of predatory morphs of *T. vorax*, and (2) that predation by crustacean zooplankton would result in the exclusion of *T. vorax*.

3. Differentiation did occur in the *T. vorax* population over the course of the experiment. The proportion of *T. vorax* macrostomes was unaffected by the presence or absence of crustacean zooplankton. *T. vorax* was not extirpated in the presence of the zooplankton. However, the population densities of each of
the morphs of *T. vorax*, as well as that of *Colpidium kleini* (a competing ciliate), were significantly reduced in the presence of zooplankton, relative to what they were in the absence of zooplankton.

4. These results show that community patterns may change radically with the scale and complexity of experimental systems, demonstrate the utility of microcosms as a baseline for studies of larger systems, and offer insight into possible reasons for the limited distribution of *T. vorax* in natural aquatic systems.

**Key-words:** density-dependence, induced offenses, induced polymorphism, microcosm experiments, predation, spatial scaling, *Tetrahymena vorax*, zooplankton
Introduction

The experimental tractability of protists in laboratory microcosms has made them valuable tools for testing ecological and evolutionary principles and for uncovering the mechanisms that underlie general patterns (Drake et al. 1996; Fraser and Keddy 1997; Cadotte et al. 2005; Holyoak and Lawler 2005). The small size and self-contained structure of microcosms allow for highly controlled experiments with adequate replication (Jessup et al. 2004; Cadotte et al. 2005) – a feature that is sometimes lacking and occasionally impossible in field experiments (Hurlbert 1984; Hargrove and Pickering 1992). These same convenient attributes can potentially make microcosms limited models of larger, more complex systems (Carpenter 1996, 1999; Srivastava et al. 2004). As noted by Schindler (1998) and Petersen and Hastings (2001), careful scaling corrections must often be made even when the attempted extrapolation is simply from a smaller habitat to a larger one of the same kind, located in the same geographic region, and containing the same species. Seemingly contradictory results have been reported even for investigations of community patterns thought to be scale-independent. For instance, while productivity has been shown to be an important determinant of food chain length in aquatic microbial communities (Kaunzinger and Morin 1998), it appears to be unrelated to food chain length in lake systems (Post et al. 2000).

Recently, microcosm experiments with protists have been used to investigate the phenomenon of inducible trophic polymorphisms, wherein phenotypic plasticity enables organisms to alter their position in the food web and respond dynamically to changes in resource availability and competitors (Kopp and Tolrian 2003; Price and Morin 2004; Banerji and Morin 2009). In the case of the freshwater hymenostome ciliate
*Tetrahymena vorax* Kidder, exposure to chemical cues released by competing ciliates, which include conspecifics, triggers a transformation from small (~ 60 µm), pyriform, bacterivorous morphs, termed “microstomes,” to larger (~ 200 µm), ovoid, predatory morphs, termed “macrostomes,” which can consume other ciliates (Kidder et al. 1941; Smith-Somerville et al. 2000; Ryals et al. 2002). This ability to transform and subsequently feed on competitors might not only enhance the fitness of *T. vorax* under a variety of circumstances, but also give rise to trait-mediated indirect effects that would influence the composition and stability of the community as a whole (Banerji and Morin 2009; Banerji and Morin in preparation). The fact that isogenic lines of *T. vorax* differentiate into different morphs in response to high population densities of conspecifics and/or heterospecifics indicates that transformation of microstomes into macrostomes is a density-dependent response (Fig. 1).

The life-history of *T. vorax* in natural systems remains essentially unknown. All lineages of *T. vorax* available in culture collections are descended from the collection made by Kidder et al. (1940, see also Corliss 1952, 1953) from a small pond in the vicinity of Providence, Rhode Island. *T. vorax* was also collected once from a stream in Pennsylvania (Nanney et al. 1998). Other polymorphic *Tetrahymena* may enjoy less restricted distributions (Ryals et al. 2002), but none appear to be as common or as widely distributed as their smaller monomorphic congeners (Elliot 1973). Polymorphic *Tetrahymena* species lack a drought-resistant resting stage and are consequently restricted to permanent waters. Beyond this constraint, the reasons for the apparently limited distribution of natural populations of *T. vorax* are unclear and raise fundamental questions about the factors limiting the distribution of trophically plastic species.
Here I describe a study of *T. vorax* dynamics in outdoor experimental mesocosms designed to uncover some of the possible causes of its limited distribution in nature. In so doing, I also provide some insight as to whether or not the kinds of dynamics observed in laboratory microcosms can be scaled up to larger, more complex, and more realistically variable systems in the field.

**Methods**

*Preparation of Protozoan and Bacterial Inoculants*

I obtained *T. vorax* from the American Type Culture Collection (strain 30421) and *Colpidium kleini* Foissner (Ciliatea: Hymenostomatida) from the Adelphia Plant Science Research and Extension Center, Freehold, NJ 07728, USA. Species of the genus *Colpidium* appear to have cosmopolitan distributions (Finlay 2002) and were found to be naturally occurring prey/competitors of *T. vorax* in the original collections made by Kidder et al. (1940). I cultured isogenic populations of *T. vorax* and *C. kleini* in 240-ml glass jars containing 100 ml of complex organic medium. Loosely fastened screw caps on the jars permitted some air circulation, while minimizing evaporation and contamination. The complex organic culture medium used consisted of 0.37 g of Carolina Biological Supply protozoan pellets (Carolina Biological Supply Company, Burlington, NC 27215, USA) per L of filtered well water. After autoclaving and cooling, I inoculated the medium with three species of bacteria (*Serratia marcescens*, *Bacillus subtilis*, and *Bacillus cereus*) to standardize initial conditions and provide food for the protozoa.

*Construction of Outdoor Experimental Mesocosms*
I modified twelve 19-L plastic buckets (Homer’s All-Purpose Bucket, The Home Depot ®) to serve as my experimental units. I added 2 g of autoclaved dried alfalfa leaf, 14 L of well water, and 100 mL of bacterized alfalfa medium to each bucket. I covered the buckets with 5-µm Nitex ® mesh glued to a removable plastic lid to prevent contamination by larger aquatic organisms. The mesh size was small enough to exclude the nauplii of cyclopoid copepods and enable me to maintain initial control over ciliate species composition. The well water I used came from the same source used for the medium of my laboratory cultures of *T. vorax* and *C. kleini*. The total volume in these experimental units constituted a >140-fold increase from that of the jars used in my microcosm experiments. The dilute alfalfa medium provided a nutrient source for the bacteria, as well as some structural heterogeneity for the protozoa, that could be adjusted to simulate the contributions of plant litter to natural pond systems. I placed the buckets in a designated clearing located at the Hutcheson Memorial Forest of Rutgers University (East Millstone, NJ, USA), arranging them with approximately 1 m of space between them, with control and treatment replicates alternated to compensate for any potential environmental gradients within the site (see Hurlbert 1984). Two days after filling and positioning the buckets, I added a 2-day-old, 100-mL culture of *T. vorax* and a 2-day-old, 100-mL culture of *C. kleini* to each bucket.

**Crustacean Zooplankton Treatment**

There is an abundance of experimental and observational evidence implicating cladocerans and copepods as important predators of the ciliates that persist in permanent waters (Pace and Funke 1991; Burns and Gilbert 1993; Wickham 1995; Burns and Schalenberg 1996; Adrian and Schneider-Olt 1999; Gaedke and Wickham 2004).
However, none of these studies address the very different problem of whether these crustacean zooplankton actively exclude some ciliate taxa from otherwise favorable sites. *T. vorax* in its macrostome state, for instance, could be more susceptible to grazing by crustacean zooplankton due to its being more conspicuous than other, smaller co-occurring ciliates. In addition, crustacean zooplankton could decrease the relative abundance of macrostomes indirectly by reducing densities of all ciliates, and thereby reducing the concentration of cues that induce macrostome formation in *T. vorax*.

After sampling to obtain baseline counts of the densities of *T. vorax* and *C. kleini* in each bucket, I added 50 mL of unfiltered pond water from a local source to 6 of the 12 buckets (treatment replicates). The local source was a small permanent pond I had chosen for the convenience of its proximity, containing cyclopoid copepods, cladocerans, and other small aquatic organisms. To the remaining half of the buckets (controls), I added pond water from the same source that had been filtered through 5-µm mesh to remove the crustacean zooplankton, rotifers, and any large protozoa.

**Sampling of Outdoor Experimental Mesocosms**

I collected 7-mL samples from the surface and lower depths of each bucket (for a combined total of 14 mL) in sterile Falcon® centrifuge tubes using a pair glass tubes that I rinsed with hot water before and after each sampling interval. I counted copepods by eye in the entire 14 mL sample, after confirming their identity via microscopy. I then sub-sampled approximately 0.3 mL (measured by weight) from each of the 14 mL samples using sterile glass pipettes to quantify the population densities of the protozoa. I enumerated both macrostomes and microstomes of *T. vorax* and *C. kleini*. I also recorded
the presence (and, in some cases, density) of the other protozoa, algae, and macroscopic zooplankton.

Results

Species Composition of Replicates after Initial Setup

The unfiltered pond water used for my treatment replicates introduced a number of taxa to the treatment replicates. In addition, despite my use of the 5-µm mesh, all replicates, including the control replicates, as of Day 26, were contaminated with large densities of an alga of the genus *Chlorella* (mean average of 140.34 cells / mL) and a species of microflagellate that I was unable to identify (mean average of 45.73 cells / mL). As of Day 24, in treatment replicates Trt 1 and Trt 5 and in control replicate Con 4, I observed hypotrichs of the genus *Oxytricha* (mean average of 10.84 cells / mL). During the same period, in treatment replicate Trt 1 and control replicate Con 3, I observed peritrichids of the genus *Vorticella* (mean average of 14.99 cells / mL).

Although I had observed species of *Daphnia* and other cladocerans in my source pond, I did not observe any of these in my treatment replicates. The crustacean zooplankton present in my treatment replicates consisted entirely of cyclopoid copepods, the majority of which I could identify as being of the genus *Acanthocyclops* (either *A. vernalis* or *A. robustus*), with two individuals (seen on Day 10 in Trt 6) possibly being of the genus *Diacyclops*. One of the replicates with zooplankton added (Trt 1) also contained eggs of the mosquito species *Aedes albopictus* (= *Stegomyia albopicta* Skuse), which had presumably entered through my unfiltered pond water addition. These emerged as larvae, developed into pupae, completed their metamorphosis into adults, and then died between Days 17 and 26.
**Effects of Zooplankton on Protist Community Dynamics**

The population densities of *T. vorax* and *C. kleini* in treatment replicates were significantly lower than in controls, which lacked large zooplankton (MANOVA, $F_{3,8} = 10.83$, $p = 0.0034$; Fig. 3). The appearance of the mosquitoes in Trt 1 did not appear to change the overall patterns of abundance of *T. vorax* and *C. kleini* in that replicate, as compared to the other treatment replicates. By Day 19, however, copepods were no longer present in the replicate. In all of the treatment replicates, including Trt 1, protozoa declined upon the addition of the unfiltered pond water containing macroscopic zooplankton (Fig. 4). By contrast, without the zooplankton, *T. vorax* microstomes and *C. kleini* maintained relatively constant population densities until the last three days of the experiment (Fig. 5). Differences between the proportion of *T. vorax* macrostomes in the presence and absence of crustacean zooplankton were not significant. In both cases, the proportion of *T. vorax* macrostomes was significantly lower than what I had observed in laboratory settings, across a range of productivities (ANOVA, $F_{5,15} = 23.87$, $p < 0.0001$; see Banerji and Morin 2009). Follow-up contrast analysis of the means showed that these differences were significant for each productivity-level compared to the outdoor mesocosm settings.

**Discussion**

I am skeptical of there being a causal relationship between the presence of mosquito larvae in Trt1 and the extirpation of copepods in that replicate. It is, for example, highly unlikely that the copepods were consumed by the larvae. Apart from the fact that I did not observe any such predation event in the course of my sampling, copepods such as those I had observed prior to the emergence of the mosquito larvae
have been used as biological agents to kill mosquito larvae (Marten 1989, Marten and Reid 2007). I can only speculate that other factors were responsible for the loss of copepods in this replicate and that the timing was mainly coincidental. The steep decline of the population densities of each of the protozoa in the control replicates during the last three days of the experiment might be attributed to the fact that the water temperatures of all of the replicates consistently exceeded 25 °C during this period.

Although *T. vorax* was not entirely extirpated from mesocosms in the presence of crustacean zooplankton, the decline of *T. vorax* and *C. kleini* population densities indicates that predation by crustacean zooplankton is still likely to be a factor in limiting the distribution and abundance of *T. vorax* and other ciliates among natural aquatic systems. This is not unexpected, as cladocerans and copepods have been shown to be capable of affecting the abundance of protists in permanent ponds (Adrian and Schneider-Olt 1999; Gaedke and Wickham 2004). Since the proportion of macrostomes in the *T. vorax* population did not appear to differ between control and treatment replicates, it is unlikely that crustacean zooplankton preferentially feed on macrostomes. It is uncertain why the proportion of macrostomes in my outdoor mesocosms was smaller than what I have observed in laboratory settings. The low density of ciliates and cues is a likely cause, but I cannot rule out the possibility of the contents of the medium also having an effect. It has been demonstrated, for example, that populations of *T. vorax* grown in proteose peptone medium are unable to differentiate. Given that the dilute alfalfa medium I used in this experiment resembled the environment of natural ponds more closely than the protist pellet medium used for my laboratory cultures, it could be that the
expression of ITPs among protists raised in standard growth media is exaggerated, relative to what it is in natural settings.

The results of my experiment show that community patterns may change radically with the scale and complexity of experimental systems, but simultaneously demonstrate the value of combining microcosm studies with field studies to uncover the mechanisms underlying the dynamics of large, complex systems.

**Acknowledgements**

I thank Brian and Amy Clough for their aid in the setup of this experiment. I also thank Kevin Aagaard, Jean Deo, Jack Siegrist, Maria Stanko, and Holly Vuong for their helpful feedback. Support for this research came from the Rutgers School of Environmental and Biological Sciences and the New Jersey Agricultural Experiment Station, in the form of the Hutcheson Memorial Forest Summer Research Grant.
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Fig. 1. Diagram of the inducible trophic polymorphism of *T. vorax* (reproduced from Banerji and Morin 2009). Gray arrows represent developmental pathways. Black arrows represent trophic links. Arrow size represents relative interaction strength. Transformation of microstomes into macrostomes occurs in the presence of chemical cues released by competitors for bacteria.
Fig. 2. Semi-quantitative measurements of the abundances of macroscopic zooplankton in treatment replicates. Black hexagons represent the abundance of copepods (*Acanthocyclops* spp.). Dark gray triangles represent that of mosquitoes (*Aedes albopictus*). Note that mosquitoes were detected as larvae on Days 19 and 21, as pupae on Day 24, and as (dead) adults on Day 26.
**Fig. 3.** Effects of copepods on protozoa. Black columns represent the mean population density of macrostomes of *T. vorax*. Light gray columns represent the mean population density of microstomes of *T. vorax*. Dark gray columns represent the mean population density of *C. kleini*. 
Fig. 4. Population dynamics of protozoa in treatment replicates (replicates containing crustacean zooplankton). Large unfilled black squares represent abundances of macrostomes of *T. vorax*. Small black squares represent abundances of microstomes of *T. vorax*. Gray circles represent abundances of *C. kleini*. 
Fig. 5. Population dynamics of protozoa in control replicates (replicates lacking crustacean zooplankton). Symbols are as in Fig. 4.
CHAPTER 4

On the Strategy of Mounting an Indirect Offense: What If My Enemy Has Allies, Too?

Summary

An indirect offense is the use of symbionts or recruited associates by consumers to increase their rate of acquisition of prey or hosts. This versatile adaptive strategy is employed by a wide array of consumers in various aquatic and terrestrial habitats. Here I present a review of indirect offenses, the purpose of which being to draw attention to the phenomenon and elucidate its significance within ecology and evolution. I explain the utility of recognizing indirect offenses as a unique category of species interaction, present examples to illustrate and clarify the modes and diversity of indirect offenses, and highlight promising directions for future research.

**Key-words:** Functional response; indirect defense; indirect offense; parasite; parasitoid; predator; symbiosis.
Introduction

In recent years, the phenomenon of species relying on symbionts or recruited associates for an “indirect defense” against their natural enemies has been of growing interest to ecologists (Poelman et al. 2008, Degenhardt 2009). Best-known are the cases of terrestrial plants deterring herbivores using fungus-derived compounds (Huitu et al. 2008, Afkhami and Rudgers 2009, Hartley and Gange 2009) or higher-level consumers (Dicke and Sabelis 1988, Turlings et al. 1995, Thaler 2002, Hountondji et al. 2006), but indirect defenses are also employed by a variety of other taxa (McAtee 1944, Owens and Goss-Custard 1976, Wheelwright et al. 1997, Machado and Vital 2001, Oliver et al. 2003, Hay et al. 2004, Damiani 2005, Fenton et al. 2011). Studies have shown that this use of symbionts or recruited associates can drastically reduce the rate of acquisition of prey or hosts by consumers (Damiani 2000, Kessler and Baldwin 2001, Sabelis et al. 2001, Turlings and Wäckers 2004) and significantly influence the composition of ecological communities (Poelman et al. 2008, Utsumi et al. 2010). What these studies have generally failed to take into account, however, is that the use of symbionts and recruited associates is not unique to species under threat. In the same way that a species might make use of another species to protect itself from natural enemies, a species might also make use of another species to enhance its ability to feed on prey or hosts. In other words, not only are there indirect defenses in ecological communities, but also “indirect offenses.”

The Utility of the Concept of an Indirect Offense

Indirect offenses occur when consumers make use of symbionts or recruited associates to increase their rate of acquisition of prey or hosts. Many kinds of symbiosis
and recruited association can give rise to indirect offenses, including both resource-
exchange mutualisms (Hoeksema and Schwartz 2003, Holland et al. 2005) and parasite-
host interactions (Poulin 1994, Lafferty 1999). Since the term “indirect offenses” has
never before been used to categorize these interactions, it seems appropriate to briefly
address the question of how it would be meaningful to apply the term currently. Simply
stated, the term provides us with a conceptual framework. It implies, as it is meant to,
that these interactions are the logical counterpart to indirect defenses. Recognizing the
existence and common features of these interactions enables us to generalize across taxa
and make predictions regarding their consequences and dynamics. In turn, our ability to
generalize and make predictions allows for new avenues of academic and applied
research, such as the development of integrated pest management strategies that target the
symbionts and recruited associates of invasive species or that manipulate the symbionts
and recruited associates of biological control agents.

**Examples of Indirect Offenses**

Theory predicts that, while foraging, a consumer’s rate of acquisition of prey or
hosts – also known as its “functional response” – is constrained by two separate factors:
“search time” and “handling time” (Abrams 1982, Jeschke et al. 2002). Search time is
the amount of time that the consumer spends locating its prey or hosts. Handling time is
the amount of time that the consumer spends subduing and assimilating its prey or hosts.
In an indirect offense, symbionts or recruited associates may benefit the consumer by
reducing one or the other of these limiting factors. In this section, I present some of the
ways in which they go about this.

**Indirect Offenses That Reduce Search Time**
… By Revealing the Presence of Prey or Hosts

Many prey species rely on the cover of darkness to avoid detection by visual predators (Nelson and Vance 1979, Rydell and Speakman 1995, Wcislo et al. 2004). In response, many nocturnal and deep-sea visual predators have evolved specialized adaptations for foraging under low-light conditions (Healy and Guilford 1990, Collin and Partridge 1996). The **loose-jawed dragonfish**, *Malacosteus niger*, has evolved an especially unique adaptation. Using the green-sulfur bacteria residing in its suborbital photophores, the dragonfish is able to project far-red light. All other known animals of the deep sea are blind to this wavelength, which enables the dragonfish to illuminate its prey without giving its own presence away (Douglas et al. 2000, Herring and Cope 2005).

… By Locating Prey or Hosts

Typically, if two species rely on the same food source, one would expect their interaction with each another to be mostly competitive. Interspecific cooperative foraging associations that involve guiding behavior allow for a fascinating exception to this rule (Morse 1977, Giraldeau 1984, Berner and Grubb 1985). One of the most striking examples is the guiding behavior of the **coral grouper**, *Plectropomus pessuliferus*, which often leads its fellow predator, the **giant moray eel**, *Gymnothorax javanicus*, to the location of prey that have taken refuge in narrow crevices within a reef. The eel, due to the shape of its body, is able to enter these crevices, but the grouper is not.

It is the grouper that recruits the eel in this association. The grouper signals the eel to follow it by approaching the eel at the eel’s den and performing a ritualized set of visual displays. This entails the grouper rapidly shaking its head directly in front of the
eel, a few centimeters away from the eel’s head. While doing so, the grouper keeps the soft part of its dorsal fin erect and the bony part flat. Once the grouper has persuaded the eel to leave the den and has led the grouper to the hiding place of the prey, the grouper positions itself where it is best able to capture whatever prey the eel happens to flush out without catching (Diamant and Shpigel 1985, Bshary et al. 2006).

... By Luring Prey or Hosts

For some consumers, the simplest way to reduce search time is to have their prey or hosts come to them. The classic example of this kind of consumer is the bathypelagic predatory female anglerfish, *Melanocetus murrayi*. The anglerfish uses the bioluminescent, intracellular bacteria (Vibrionaceae) that reside in its light organ to lure in its prey. It remains motionless with the exception of its light organ, which it wiggles in order to mimic the movements of much smaller, bioluminescent organisms. Able to discern only the wiggling light organ through the darkness, the anglerfish’s prey come within range to investigate what they perceive as a potential meal. The moment they do, the anglerfish is able to quickly swallow them whole (Hulet and Musil 1968).

Surprisingly, predators do not have to be larger than their prey to employ the strategy of the anglerfish. In shallower waters, the squaloid shark, *Isistius brasiliensis*, known also as the “cookiecutter shark,” uses its endosymbiotic bioluminescent bacteria to camouflage all but a small portion of its body. Seeing it from below, larger pelagic predators, such as tuna, mistake the shark for a very small, harmless prey. When these larger predators attempt to consume the shark, the shark ambushes them and extracts plugs of flesh from their bodies (Jones 1971, Widder 1998).
Another manifestation of the use of symbionts to lure prey or hosts is the attraction of definitive hosts by parasites with complex life-cycles. This phenomenon has been termed “parasite-increased trophic transmission” (Lafferty 1999) and involves parasites modifying the behavior or physiology of their intermediate hosts in such a way as to make them more vulnerable to capture and consumption. In many cases, it can be readily distinguished from mere artifacts of nutrient and energy loss because of the nature of the changes in the host (Poulin 2000, Brown et al. 2001). A wide range of parasites make use of this strategy, including the tapeworm, *Schistocephalus solidus*. This tapeworm manipulates its intermediate host, the threespine stickleback, *Gasterosteus aculeatus*, so as to be able to infect its definitive host, the piscivorous common kingfisher, *Alcedo atthis*. It dramatically reduces the melanin content of the stickleback’s scales and darkens the stickleback’s eyes, while causing the stickleback to move slowly and be less responsive to danger. The stickleback thereby becomes significantly more noticeable from above to its visual predator and significantly easier to catch (Ness and Foster 1999, Milinski 2006).

**Indirect Offenses That Reduce Handling Time**

*… By Concealing the Presence of the Consumer*

Just as prey species can use symbionts to avoid detection by their predators (Gradstein and Equihua 1995), predators can use symbionts to avoid detection by their prey. The Hawaiian bobtail squid, *Euprymna scolopes*, for example, uses the bioluminescent bacteria in its mantle to create disruptive color patterns that break up its silhouette from beneath and eliminate its shadow. This enables the squid to approach
prey such as red shrimp, *Halocaridina rubra*, without being seen (Travis 1996, Jones and Nishiguchi 2004).

**… By Enabling the Consumer to Metabolize Prey or Host Content**

The presence of structural and chemical defenses can make feeding on prey or hosts anything from unappealing to lethal for consumers (Simms and Fritz 1990, Jeschke 2006). Plants are especially well-equipped in terms of such defenses (Coley and Barone 1996). One of the ways in which herbivores and other consumers may respond to this is by relying on specialized gut endosymbionts (Dyer 1989, Karban and Agrawal 2002). The *cigarette beetle*, *Lasioderma serricorne*, for example, achieves this result using the hydrolytic detoxifying enzymes of its symbiotic yeast (Dowd 1989, Dowd and Shen 1990, Dowd 1991).

It should be noted that a consumer’s inability to digest prey or host content is not always a matter of the content being defensive in nature. In some cases, it is a matter of the constraints set by the consumer’s own evolutionary history. The *carnivorous plants*, *Roridula gorgonias* and *Roridula dentata*, for example, despite being fully capable of trapping insects on their sticky leaves, lack the digestive enzymes necessary to metabolize the insects. In lieu of the enzymes, these plants have developed a mutualistic symbiosis with the predatory hemipteran, *Pameridea roridulae*. The hemipteran, which is able to move freely across the plants’ sticky leaves, gains easy access to the already-captured insects and enables the plants to assimilate nutrients from the insects via direct absorption of its excrement (Anderson and Midgley 2003, Anderson 2005).

**… By Poisoning Prey or Hosts**
Many consumers use venom to immobilize and/or kill their prey or hosts (Whittaker and Feeny 1971). Some, however, are able to use pathogens or symbiont-derived toxins to envenomate their prey or hosts, in lieu of endogenously produced toxins. Soil-dwelling, predatory nematodes of the genus Heterorhabditis, for example, make use of Photorhabdus luminescens, a bioluminescent bacterial symbiont they carry within their guts. The tiny nematodes burrow inside of their larval insect prey and release P. luminescens into the insects’ circulatory system, causing a “raging septicemia” which kills the larvae within 24 hours. As P. luminescens begins to decompose the dead larvae and rapidly proliferate, the nematodes complete their life cycle by laying eggs. Newly hatched nematodes of the next generation will later feed on a portion of the P. luminescens colony for 10 to 14 days and then assimilate the remainder as endosymbionts (Mlot 1997, Fenton et al. 2000).

Larger-scale predators can also rely on microbial pathogens for venom. On land, the Komodo dragon, Varanus komodoensis, compounds the lethal effects of its venomous saliva with the use of up to 57 different species of virulent bacteria which it obtains by feeding on carrion and houses in its gingival tissue (Montgomery et al. 2002, Fry et al. 2006). In rocky intertidal zones, the blue-ringed octopus, Octopus maculosus, paralyzes and kills its prey using the neurotoxins it obtains from 16 of the 22 bacterial strains that reside in its posterior salivary glands and intestines (Sheumack et al. 1978, Hwang et al. 1989, White 1998, Bonnet 1999).

Certain parasitoids use a similar strategy to subdue their hosts. The wasp, Campoletis sonorensis (Hymenoptera: Ichneumonidae), for example, makes use of its co-evolved endosymbiotic virus (Polydnaviridae). The virus replicates in the ovary of the
wasp and is injected along with the wasp’s egg into a host caterpillar during oviposition. There, it acts to suppress the caterpillar’s growth and encapsulating immune response to the wasp’s egg, allowing the egg to successfully develop inside the caterpillar (Edson et al. 1981, Theilmann and Summers 1988, Whitfield 1990, Shiga 2005).

... **By Transporting the Consumers to Prey or Hosts**

Phoresy is the symbiotic transportation of one organism by another (Binns 1982). Consumers may make use of phoretic partners to acquire prey and hosts that are more mobile than they are or are otherwise difficult to reach. One such consumer is the mesostigmatid **mite, Poecilochirus necrophori**, which uses burying beetles of the genus *Necrophorus* for transportation to the location of fly maggots and carrion on which to feed. In return, the burying beetles may obtain the benefit of increased reproductive success from the mite’s removal of their competitors (Springett 1968, Wilson 1983).

Pathogens commonly make use of phoretic partners, as well. In their case, however, we refer to the partners as “vectors.” The mechanisms of recruitment of vectors by pathogens are very similar to the attraction of primary hosts by parasites, in that they often involve dramatic examples of host-manipulation. The **fungus** responsible for Mummy-Berry Disease in blueberries and huckleberries, *Monilinia vaccini-corymbosi*, relies on pollinators for transportation from the leaves of its hosts to the flowers, where it can sexually reproduce to make spores, which the pollinators then spread to other host plants. To recruit the pollinators, the fungus causes the wilting leaves of its host to secrete sugars, exude sweet odors, and reflect ultraviolet light in a manner similar to that of nectar-rich flowers. While consuming the sugars, pollinators that have been attracted
to these imitation flowers take on some of the fungus and later deposit it into real flowers as they make their normal feeding rounds (Ingvarsson and Lundberg 1993, Kaiser 2006).

**Indirect Offenses versus Alternative Strategies for the Acquisition of Exogenous Offensive Traits**

Symbioses and recruited associations are favored where the benefits of association outweigh the costs. Often included among the costs of association are significant investments of nutrients, energy, and time (Dicke and Sabelis 1988, Thomas et al. 1998, Siemens et al. 2002, van Rijn et al. 2002, Huntzinger et al. 2004, Wäckers and Bonifay 2004). In some cases, the costs of association may also include conflicts between partner-provided services and endogenous adaptations (Eisner et al. 1998) or increases in the risk of attracting natural enemies and partners that are unsuitable (Anderson and Midgley 2002, van Rijn et al. 2002, Horiuchi et al. 2003, Mouritsen and Poulin 2003, Shenoy and Borges 2010). Given such costs, it would be reasonable to assume that indirect offenses evolve when the alternative of developing and maintaining endogenous offensive traits is still more costly. However, even in a situation where there is strong selection for these traits and developing these traits endogenously is a physiological impossibility, forming an indirect offense association is not the only recourse. Besides indirect offenses, there are at least two other strategies for the acquisition of exogenous offensive traits: opportunistic association and annexation. By “opportunistic association,” I mean interspecific relationships such as scavengry (Tenney 1877, Farner and Kezer 1953), kleptoparasitism (Hamilton 2002), and aggressive mimicry (Sazima 2002). By “annexation,” I mean the assimilation of exogenous traits
through collection (Levey et al. 2004) or consumption (Rothschild and Edgar 1978). In this section, I expand on and clarify the distinctions between these alternative strategies.

**Acquiring Exogenous Offensive Traits via Opportunistic Association**

The ocellated antbird, *Phaenostictus mcleannani*, is an obligate associate of army ants. It monitors the ants’ activity and follows them as they perform their raids, so that it can capitalize on the swarms of hidden insect prey that the ants tend to flush out (Willis and Oniki 1978, Swartz 2001, Chaves-Campos 2010). The antbird benefits from its association with the ants in much the same way that other consumers benefit from mounting an indirect offense. Yet, to obtain this benefit, the antbird neither has to recruit the ants nor be recruited by the ants. It simply takes advantage of what the ants must do irrespective of its presence or absence, scavenging off of them in a way that may actually reduce their success rate in capturing prey (Wrege et al. 2005).

If being an opportunist is inherently less costly than initiating and maintaining an indirect offense association, why, then, do species such as the coral grouper, *Plectropomus pessuliferus*, invest in the latter (Diamant and Shpigel 1985, Bshary et al. 2006)? The likely scenario is that species such as the grouper, in reality, have more to gain from recruiting and more to lose from scavenging. Unlike army ants, the moray eels that the groupers rely on to flush out their hidden prey do not perform raids during the grouper’s period of activity. Furthermore, if properly positioned, the eels are just as capable as the grouper of chasing after the prey that flee vertically when flushed. As a result, if the grouper were to simply follow the eel and scavenge off of it, rather than recruit it, the grouper would be more likely to lose hidden prey to the eel when foraging.
with the eel, as well as miss the opportunity to capture prey during its own optimal foraging period.

**Acquiring Exogenous Offensive Traits via Annexation**

To infiltrate a colony of aphids and avoid detection by the ants protecting this colony, antlions, the predatory larvae of the green lacewing, *Chrysopa slossonae*, camouflage themselves using the waxy remains of aphids they have already captured (Caltagirone 1999). Similarly, predatory nudibranchs, such as *Aeolidia papillosa*, can assimilate the stinging cells of their sea anemone prey and use them not only as a defense against their own predators, but also as a tool for capturing and killing additional prey (Greenwood and Mariscal 1984, Östman 1997, Edmunds 2009). Considering the previously mentioned potential costs of association, what incentive would a species capable of annexation have for mounting an indirect offense? There are at least two that are apparent: (1) reduction of the handling time necessary to obtain the traits of another species; and (2) renewability of the traits upon assimilation. Species that acquire exogenous traits via annexation may be unable to replenish their supply of the traits when necessary (Williams 2010), due to the absence or inaccessibility of their suppliers, for which they themselves may be responsible. By contrast, species that form indirect offense associations have better assurance of the presence of their suppliers, due to their symbiosis and/or recruiting investments. In addition, species that form indirect offense associations can, in some cases, also manipulate the level of production of the traits they obtain from their associates to suit their short-term needs.

**Future Research Questions**
The paucity of attention given to indirect offenses thus far leaves many openings for potential exploration and research. There is still much to be learned, for instance, with regard to how indirect offenses have evolved and might continue to evolve, what consequences indirect offenses give rise to, and what undocumented forms of indirect offenses may be present in ecological communities. In this section, I highlight a few key examples of these promising future directions.

**Determining How Indirect Offenses That Involve Symbiosis Differ in Terms of Their Evolution from Indirect Offenses That Involve Recruited Association**

Interspecific associations that do not involve one partner living within, on top of, or beside the other can often be so tightly co-evolved that researchers might still deem it appropriate to refer to them as symbioses (Sapp 2004, Cimino et al. 2010). This is certainly true for many of the cases of recruited association that give rise to indirect offenses. Yet, differences between these types of associations and ones in which the participants function all together as a single biological unit (Dyer 1989) can have meaningful effects on how the associations persist. The former may be more easily disrupted, for example, by cascading trait-mediation (Liere and Larson 2010) and fluctuating abiotic conditions (Peterson 1995, Kersch and Fonseca 2005) than the latter, even when the latter is highly exploitative rather than reciprocally beneficial (Law and Dieckmann 1998, Kiers and van der Heijden 2006). Several explanations have been put forth regarding why certain taxa have evolved intricate – and perhaps even obligate – symbioses, while other taxa – even when closely related – have not (Keeler 1981, Daida et al. 1996, Stadler and Dixon 1999, Kramer et al. 2009, Schemske et al. 2009). How well any of these explanations apply to indirect offense associations remains to be seen.
Exploring the Overlap between Indirect Offenses and Indirect Defenses

I refer earlier to indirect offenses as being the logical counterpart to indirect defenses and depict them throughout this manuscript as essentially being analogous in form, but diametrically opposed in purpose. This portrayal, though somewhat expressive of how indirect offenses and defenses factor into consumer-resource dynamics, provides little insight into the actual biological relatedness of these strategies. The relationship between indirect offenses and defenses in general is often complex and can have intriguing ecological and evolutionary significance. Indirect offenses can, for instance, often be coupled with indirect defenses. Consider, for example, the case of the cassava plant, *Manihot esculenta*. In response to herbivory by the green mite, *Mononychellus tanajoa*, the plant releases a specific blend of volatile emissions that triggers infective sporulation in the mite-pathogenic fungus, *Neozygites tanajoae* (Hountondji et al. 2006). In this situation, the plant relies on the fungus for protection from the mite – meaning that it is employing an indirect defense. Meanwhile, it simultaneously enhances the fungus’ ability to acquire new hosts – meaning that it is providing an indirect offense. Such potential overlap between indirect offenses and indirect defenses leads to some interesting questions. For example, how might we assess the relative importance of one or the other in a situation like this?

Consider, also, the association between the red-billed oxpecker, *Buphagus erythrorhynchus*, and the large mammals upon which it forages. This relationship is obligate for the oxpecker and has, in classical literature, been cited as a mutualism – the oxpecker reducing the mammals’ parasite loads by feeding. Tolerance of the plucking, pecking, and scissoring behavior of the oxpecker foraging on their backs (Samish and
Rehacek 1999, Plantan et al. 2009), in other words, allows the mammals to obtain an indirect defense against their ectoparasites. However, it has been demonstrated more recently that the relationship between the oxpecker and the large mammals may be more of a parasitism than a mutualism. Weeks (2000), for instance, reports that the exclusion of oxpeckers from cattle in Zimbabwe has no significant effect on the adult tick load of the cattle and that oxpeckers significantly prolong the healing time of wounds in cattle. If open wounds in large mammals are attractive to other ectoparasites besides ticks, such as flesh flies (Stevens et al. 2006), prolonging the healing time of wounds – and perhaps even creating fresh wounds – could be an effective way for the oxpecker to acquire a larger or steadier food supply. Employing this strategy would amount to exploiting the traits of its mammalian hosts for an indirect offense. What factors might cause the association to shift from being an indirect defense to being an indirect offense, or vice versa?

**Examining the Use of Symbionts and Recruited Associates in Interference Competition**

Species in competition can make use of symbionts and recruited associates to reduce the foraging ability, survival, or reproduction of their competitors. This phenomenon constitutes a novel form of interference (Park 1962) and could also be considered a “competitor” indirect offense. It has been observed in **plants** (Janzen 1966, Janzen 1969, Schupp 1986, Agrawal 1998, Mattner and Parbery 2001, Mangla et al. 2008), **insects** (Yan and Stevens 1995, Yan et al. 1998, Currie et al. 2003, Little and Currie 2007), **free-living ciliates** (Preer et al. 1953, Preer et al. 1974), and food-borne microbial **pathogens** (Stecher et al. 2007, Brown et al. 2008). There is even some evidence of its occurrence in **mammals** (Schmitz and Nudds 1994). What are the
consequences of this phenomenon in terms of population dynamics and community structure? Do they fit the patterns we would expect to see with other trait-mediated indirect interactions between competitors (Wootton 1994, Peacor and Werner 2001), such as apparent competition (Holt 1977, Bonsall and Hassell 1997, Chaneton and Bonsall 2000)?

**Conclusion**

Indirect offenses are widespread, captivating, and diverse. Their occurrence provides unique insights into the roles of symbiosis and solicited association in shaping community structure and dynamics. There is still much to be learned with regard to how indirect offenses have evolved and might continue to evolve, what consequences indirect offenses give rise to, and what undiscovered forms of indirect offenses may occur in natural systems. Although indirect offenses offer an enormous potential for promoting interdisciplinary collaboration and fostering new ideas, their significance as a versatile and general adaptive strategy has gone virtually unnoticed in the field of biology. It is my hope that the contents of this review will draw attention to the phenomenon of indirect offenses and stimulate further investigation into its mechanisms and potential broader impacts in ecology and evolution.

**Acknowledgements**

I gratefully acknowledge Peter Morin, Jennifer Rudgers, Jeremy Fox, Nina Fefferman, Michael Sukhdeo, Marilyn Scott, Robert Paine, Paul Craze, Mathew Leibold, John Maron, Mark McPeek, Rebecca Jordan, Cesar Rodriguez-Saona, Michael Pace, Timothy Casey, Peter Smouse, Jason Grabowsky, Deborah Goldberg, Robert Raguso, James D. Thomson, John J. Wiens, Bradley Hillman, Claus Holzapfel, Michael May,
Zsofia Szendrei, Carolyn Norin, Ari Novy, Kristen Ross, my labmates (Kevin Aagaard, Jean Deo, Jack Siegrist, Maria Stanko, and Holly Vuong), and a very long list of other friends and colleagues for their helpful comments and invaluable insights. Support for this review was provided by the Rutgers School of Environmental and Biological Sciences and the New Jersey Agricultural Experiment Station.

References


CONCLUSION

The research presented in this dissertation has uncovered many of the ecological and evolutionary facets of inducible trophic polymorphisms (ITPs), as well as a good deal of the fascinating life history of Tetrahymena vorax. My investigation of the potential costs and benefits of ITPs allowed me to document the significance of the “tailed microstome” morph of T. vorax as a reciprocal induced anti-cannibal defense. I was able to demonstrate that the co-occurrence of the tailed microstome and the predatory “macrostome” morph of T. vorax allows for a coordinated adaptive response to the presence of intraguild prey. In exploring the potential of ITPs to give rise to novel trait-mediated indirect interactions, I was able to draw attention to “chasmatectasis,” the phenomenon of induced predators increasing their gape via the ingestion of increasingly large prey. I demonstrated that chasmatectasis can mediate a previously undescribed form of apparent competition. In assessing the differences between the expression of ITPs in artificial and natural settings, I was able to show how community patterns may change with the scale and trophic complexity of experimental systems, demonstrate the utility of microcosms as a baseline for studies of larger systems, and offer insight into possible reasons for the limited distribution of T. vorax in natural aquatic systems. Finally, I also introduced the concept of an “indirect offense,” the use of symbionts or recruited associates to increase the rate of acquisition of prey or hosts.
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