Multi-trait selection and the evolution of the integrated phenotype

by

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For Sam, Cole, and Isabel.

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CHAPTER I

Introduction

An organizing theme in ecology and evolutionary biology is the concept of trade-offs. Trade-offs play a central role in some of the "big questions" for both ecology and evolutionary biology. How do so many species coexist in nature? Why are there so many species? To understand the role trade-offs play in helping to answer both of these questions, it is useful to invert the questions. In doing so, you arrive at a single question: Why doesn't one species dominate in all environments?

This may seem like an absurd question, but it follows logically from the tenets of natural selection. If you take the view of natural selection as an optimizing process, moving organisms towards ever-increasing adaptation, then evolution should produce a super-species capable of dominating all environments. This is the so-called "Darwinian demon" that maximizes all aspects of its fitness simultaneously (Law, 1979). The consensus view is that such Darwinian demons do not exist because organismal traits cannot be optimized individually; rather, there exist trade-offs operating at many different scales, from trade-offs at the level of individual physiology to trade-offs mediated by the environment. These trade-offs prevent any species from coming to dominate in all environments. An example will help to make this point clear.

A generally important trade-off in ecology, and one that has specific implications

for patterns of ecological succession (the change in community composition following a disturbance), is the trade-off between competitive and colonization abilities (Levins and Culver, 1971). Species that have traits that make them good at competing for limited resources do not have traits that make them good at colonizing new environments (Cadotte et al., 2006). An exemplar of this trade-off comes from the plant literature. Plants face a general physiological trade-off in the allocation of resources toward seed production: plants can create many small seeds, or a few large seeds. Producing many small seeds increases colonization ability, whereas producing larger seeds gives a competitive advantage to offspring (Rees, 1995; Turnbull et al., 1999). Theoretical studies have shown that such a trade-off can lead to the evolution of species with different seed size strategies (Geritz, 1995; Rees and Westoby, 1997). Furthermore, competition-colonization trade-offs are thought to be an important mechanism promoting species coexistence (Amarasekare et al., 2004). This simple example illustrates how a trade-off at the level of individual physiology can have both evolutionary and ecological consequences, and can help answer both the question of why there are so many species and how they all coexist.

In this dissertation, I will examine the consequences of trade-offs for the evolution of body size and foraging behavior in consumer populations. The consumer-resource interaction is the fundamental link in natural food webs, and most animal species act in both roles. Evolutionarily, the need to avoid starvation, on the one hand, and to avoid predation, on the other, are two of the strongest selective forces acting on animal populations. In most cases, and for most traits, the consumer's interactions with both resources and predators will play a role in phenotypic evolution. In this dissertation, I focus on understanding the role of trade-offs and environmental dynamics in shaping the evolution of consumer body size and foraging behavior. These traits are fundamental to the interaction of consumers with both resources and predators, and both are subject to important and well-studied trade-offs, as I

will detail in subsequent chapters.

Body size influences a consumer's interaction with its resources, as resource ingestion rates often scale with body size, with larger individuals having higher ingestion rates (Kooijman, 2000). Body size also often affects predation risk, as many predators are size-selective (Taylor and Gabriel, 1992). Foraging behavior influences the interaction between a consumer and its resources. This behavior also influences predation risk, as active foraging can increase exposure to predators (Lima and Dill, 1990). Therefore, both consumer-resource and predator-prey interactions must play a role in the evolution of body size and foraging behavior. Moreover, because both traits affect similar processes, trade-offs associated with one trait can affect the evolution of the other.

An example will help to illustrate these issues. Size at first reproduction is an important and often-studied trait in much of life history theory (Roff, 1992). This size is determined by an number of factors, including (1) allocation of energy between sexual maturation and growth, (2) individual foraging behavior (i.e. energy ingestion), and (3) the abundance of food in the environment. Given that increasing size at maturity typically increases reproductive output, one might expect natural selection to favor an increase in size at maturity. How might such an increase occur?

For a fixed food level, size at first reproduction can be increased by prolonged allocation towards growth or by increased energy ingestion. However, increased allocation to growth necessarily reduces energy allocation towards other processes, such as maturation, leading to an increased age at first reproduction. This trade-off between size and age at maturity is an important constraint on the evolution of body size (Roff, 2001). On the other hand, size at first reproduction could be increased by increasing energy ingestion. This could achieved by increased foraging, but increased foraging will reduce resource abundance and may lead to increased mortality from predators. The trade-off between foraging gain and predation risk is one of the best-

studied trade-offs in behavioral ecology (Abrams, 1984; Lima and Dill, 1990; Werner and Anholt, 1993).

This simple example illustrates a number of points that are important to the rest of this dissertation. First, selection often has multiple pathways along which traits can be modified, as in the example here where either changed energy allocation or foraging behavior can alter body size. Second, because most traits affect multiple interactions, integration among those traits should be common. Here I define integration as a pattern of correlation between traits (Pigliucci, 2003). Third, and most important, trade-offs that involve one trait will have consequences for the evolution of the other trait. For example, because of its central role in energy acquisition, foraging behavior directly affects individual growth, and therefore body size. On the other hand, body size affects foraging behavior, for example through size-dependent habitat shifts (Werner and Gilliam, 1984). The implication of the interrelation of body size and behavior is that size-dependent trade-offs, such as the trade-off between age and size at maturity (Roff, 2001), will affect behavioral evolution, and behavior-dependent trade-offs, such as the trade-off between foraging gain and predation risk (Werner and Anholt, 1993), will affect body size evolution. This interaction between trade-offs emerges a direct consequence of the first two points.

Despite the implicit recognition in the literature that trade-offs cannot be truly independent of one another, there has been almost no theory exploring the consequences of interacting trade-offs for the evolution of species traits (Steiner and Pfeiffer, 2007). Moreover, despite the recognition that species traits display high levels of integration (Pigliucci, 2003), there has been almost no theory exploring the simultaneous evolution of multiple phenotypic traits, and how this depends upon the shapes of the underlying trade-offs. It is this gap that this dissertation is looking to fill.

Chapter II will apply the insights above to the evolution of phenotypic plasticity

in traits that affect predation risk, known as inducible defense. Inducible defenses are modifications of an organism's behavior, life history (e.g., body size), or morphology in order to reduce the risk of predation (Tollrian and Harvell, 1999). Such defenses have been shown, both theoretically and empirically, to have important ecological consequences (Bolker et al., 2003; Werner and Peacor, 2003). The ability to modify some aspect of phenotype in response to predators is widespread in nature, and recent research has suggested that many species are capable of adjusting multiple traits simultaneously (Boersma et al., 1998; Relyea, 2004a). Despite this, it remains unclear what factors promote the evolution of qualitatively different defense strategies, including strategies that involve modification of multiple traits.

Previous theory has shown that individual level trade-offs between the benefits of defense (reduced predation risk) and its costs (which vary depending on the defense under consideration) are key to understanding the evolution of inducible defense. However, this work has generally not considered that trade-offs may not be independent (Steiner and Pfeiffer, 2007). Here I show that changing the shape of the trade-off between foraging gain and predation risk trade-off modifies the interaction between this trade-off and the trade-off between growth and reproduction. I use a novel application of genetic algorithms to "evolve" optimal allocation and behavioral strategies, and show that the shape of the foraging–predation risk trade-off has important qualitative effects on the optimal defense strategy. Highly nonlinear foraging–predation risk trade-offs favor the evolution of behavioral defenses, while linear trade-offs favor life history defenses. Between these extremes, integrated defense responses are optimal, with defense expression depending strongly on ontogeny. These predictions are likely to be general across different defenses and have important implications for theory on the ecological effects of inducible defense, which has not considered qualitatively different defenses might alter ecological interactions.

In Chapter III, I study the evolution of polymorphism in body size and behavior.

This study addresses the question of how evolution has produced the diversity of life. As I noted in the opening paragraph, understanding the process of speciation is of fundamental importance in evolutionary biology. In the past two decades, a growing body of literature has focused on the ability of ecological dynamics to induce evolutionary branching by generating disruptive selection, natural selection favoring extreme values of a trait over intermediate ones (Geritz et al., 1998). A number of recent studies have demonstrated the potential for disruptive selection in standard ecological models for mutualism, competition, exploitation, and parasitism (Doebeli and Dieckmann, 2000; Hoyle et al., 2008). These studies have suggested that knowledge of the shapes of the trade-offs underlying trait expression can be used to predict whether selection will lead to polymorphism in that trait.

However, as was the case in the previous chapter, these studies have all focused on the evolution of a single trait. Because of this, the generality of the predictions is unclear. Here, using one of the first applications of multidimensional adaptive dynamics (Leimar, 2009), I show that selection acting on multiple traits can lead to qualitatively different predictions from those that would be reached from studying evolution of single traits. In particular, I show that it is possible for multi-trait selection to lead to phenotypic polymorphism under circumstances were a single optimum phenotype is predicted by single-trait selection, as well as the converse. These results suggest that explanations for the generation of diversity must consider how selection acts on the entire phenotype, and not only on single traits. This will require consideration of the interaction between trade-offs.

Chapter IV studies the evolution of body size, morphology, and behavior in response to environmental variation from a data-driven perspective. Using experimental measurements of tadpole morphology and behavior both with and without predators for 17 different species of amphibian tadpoles (Van Buskirk, 2002), I used phylogenetic comparative hypothesis testing to test whether differences in

amphibian traits and trait plasticities reflect adaptation to different pond environments (Van Buskirk, 2002). This required inferring the phylogeny for these species, which I accomplished using published gene sequences. Josh Van Buskirk and Earl Werner generously shared data from long-term surveys of amphibian distribution and abundance that allowed me to characterize the ponds each species inhabits across a number of ecologically-relevant variables. With the trait measurements, phylogeny, and habitat data, I performed two sets of phylogenetic analyses. In the first, used standard comparative methodologies (Garland et al., 1992) to test for correlated evolution among habitat variables and phenotypic traits. In the second, I used recently developed phylogenetic tools (Butler and King, 2004) to directly test whether differences in species traits were adaptive. Both analyses arrived at similar conclusions. Amphibian body size and behavioral response to predators are adaptively evolving towards different selective optima based on the types of pond habitats each species prefers. The evolution of size-corrected morphological traits appears to be highly constrained to maintain a functional morphology, while the morphological response to predators seems to be under strong stabilizing selection. These results both confirm previous thinking in this system, as well as suggesting novel hypotheses to explain the distribution of amphibian species in pond communities.

Each of these chapters takes a the view that, in order to understand phenotypic evolution, we must consider how selection acts on multiple traits simultaneously. This requires an understanding both of the trade-offs underlying the expression of these traits, and an awareness that these trade-offs will not be independent drivers of trait evolution. I return to these points in the concluding chapter to suggest profitable future directions for research.

CHAPTER II

Interactions among multiple trade-offs and the evolution of integrated predator-defense plasticity

Introduction

The risk of predation is a powerful force in the evolution of species traits, and many behavioral, morphological and life historical traits confer defense against predators. However, many of these traits are not fixed, but are phenotypically plastic, varying based on environmental context (Tollrian and Harvell, 1999). A large body of empirical work documents both patterns of defense expression and their efficacy in reducing the risk of predation. Recent research has also explored the consequences of these defenses to interactions with other species in the food web (Werner and Peacor, 2003; Relyea, 2004a). Moreover, empirical studies have documented that closely-related species often employ qualitatively different defense strategies (De Meester et al., 1995; Rundle and Brönmark, 2001; Mikolajewski and Johansson, 2004), or that species are capable of expressing multiple defenses, either simultaneously or across ontogeny (Relyea, 2004a; Hoverman et al., 2005; Boeing et al., 2006b; Steiner and Pfeiffer, 2007). Different clones of *Daphnia*, for example, are capable of expressing up to eight different inducible defenses simultaneously, including modifications in life history, behavior, and morphology (Boeing et al., 2006b).

Given that different defenses employed alone, simultaneously, or serially during ontogeny are evolutionary solutions to the risk of predation, the question arises as to what factors favor the evolution of one defensive strategy over another or how are they jointly employed. Understanding how these traits are integrated is central to understanding the evolution of the phenotype, as well as how these traits influence population dynamics, interactions with other species and patterns of species coexistence (Miner et al., 2005).

Theory elucidating the ecology and evolution of inducible defenses has largely focused on single traits (reviewed in Bolker et al. (2003)). Comparatively little theory has been developed to explain the evolution of qualitatively different defense strategies (e.g., behavioral versus morphological defenses), or the manner in which multiple-defense strategies are integrated in the phenotype. At the core of this problem is the issue of how tradeoffs associated with expression of defenses interact and thereby influence the evolution and expression of these defenses.

For example, modification of behavior or life history involves fundamental tradeoffs for the organism. For behavioral defense, the tradeoff is often between foraging gain and predation risk, as a ubiquitous behavioral response to predation is reduction of foraging activity or movement to a suboptimal foraging habitat (Lima and Dill, 1990). For life history defenses, there is often a tradeoff between the allocation of energy to growth or reproduction, leading to a tradeoff between age versus size at maturity (Roff, 2001). Moreover, these tradeoffs are not independent of one another: any modification of behavior may affect the pattern of energy allocation, e.g., if an individual reduces activity level in response to predation risk, growing to the same size at maturity will require either a prolonged growth phase or an increase in the amount of energy allocated per unit time. There has been some empirical work that has recognized that tradeoffs may be interacting, although the end goal of these projects was to attribute patterns of behavior and life history to only one trade-

off, rather than any interaction between tradeoffs (Ball and Baker, 1996; Beckerman et al., 2007). Recent theory has also begun to explore the implications of interacting tradeoffs (Steiner and Pfeiffer, 2007). This work is central to developing a more complete understanding of life history evolution, since tradeoffs form the foundation of life history theory. However, most models treat defense investment as a constant parameter (Abrams and Rowe, 1996; Day et al., 2002; Steiner and Pfeiffer, 2007), rather than a function of individual physiology. This omission effectively ignores the reality that defense investment incurs tradeoffs arising at the level of individual physiology, and that the relative costs and benefits of defense investment will change as individuals age and grow (Clark and Harvell, 1992).

Here, I explore how variation in the shape of the ecological tradeoff between foraging gain and predation risk affects the interaction between this tradeoff and the physiological tradeoff between growth and reproduction, which in turn determines the optimal expression of behavioral and life history (body size) defenses under negative size-dependent predation. I do this both to elucidate the nature of multiple defense expression and to make explicit predictions for this common scenario. My choice of these defensive traits is motivated by the generality of their effects across organisms. First, body size and behavior are traits critical to species fitness; many ecological interactions are size-dependent and body size influences nearly all physiological processes, including resource ingestion, growth, reproduction, and mortality (Werner and Gilliam, 1984; Kooijman, 2000; de Roos et al., 2003). Thus any modification of body size in response to predation risk will have important ecological consequences. Behavior is similarly fundamental; because of its role in resource acquisition, behavior affects many of the same physiological processes as body size and has been shown to strongly impact ecological processes (Bolker et al., 2003; Werner and Peacor, 2003). Furthermore, the relationship between body size and behavior is complicated by their joint dependency on ontogeny. Changes in body size through

ontogeny can affect predation risk and therefore the efficacy of different behaviors, and behavior can influence body size through its effect on foraging gain and growth rates. Finally, the shape of this tradeoff is predicted to have strong impacts on the ecological consequences of behavior (Abrams, 1992; Bolker et al., 2003), but there has been no systematic exploration of its consequences for defense expression.

Using an individual-based physiological model, I investigate how the optimal investment into life history and behavioral defenses varies under an activity-mediated tradeoff between foraging gain and predation risk. This extends previous theory (Abrams and Rowe, 1996; Day et al., 2002; Steiner and Pfeiffer, 2007; Urban, 2007a) in a number of ways. Most importantly, by treating behavior and energy allocation separately, and allowing both to vary with age, I am able to achieve a more complete understanding of how organisms balance competing tradeoffs and how this interaction can give rise to complex, multivariate responses. I discuss the implications of this result for the evolution of trait integration and for the study of the ecological consequences of inducible defense.

Methods

Model description

To investigate the interaction between tradeoffs, I employ an individual-based physiological model where growth, reproduction, and death depend on the current state of the individual. The model is based on the physiologically-structured model developed by Kooijman and Metz (1984). The structure and parameterization of the original model were modified to allow for flexibility in life history and behavior. These changes are discussed in more detail in Appendix A, which also contains the derivation of the growth equation. Basic description of the equations and key parameters follows below. Parameter values have been taken from de Roos et al.

(1990), except as noted in the Appendix A. The energetic assumptions underlying these equations are very general (Kooijman, 2000), so this model represents a general conceptual model for investigating how organisms balance competing life history tradeoffs. Table 1 presents the variables and parameters used in the model, and gives default parameter values.

Characterizing investment in defense

Behavioral defenses are often characterized by changes in activity level or habitat that reduce the encounter rate with predators (Lima and Dill, 1990; Tollrian and Harvell, 1999). In my model, behavioral defense investment will be determined by $\alpha(t)$, the fraction of total available foraging time that is spent in active foraging, as opposed to engaging in defensive behaviors. Life history defenses are typically thought to result from changes in energy investment between growth and reproduction, often in response to size-dependent predation (Taylor and Gabriel, 1992; Ernande et al., 2004; Gårdmark and Dieckmann, 2006). I define $\kappa(t)$ as the fraction of net production allocated towards growth versus reproduction.

The tradeoffs involved in defense expression are mechanistically built into the model by considering how $\alpha(t)$ and $\kappa(t)$ affect physiological processes. Increasing activity level increases foraging gain, but also predation risk, so $\alpha(t)$ will directly affect both energy acquisition and mortality. Prolonged allocation to growth will reduce predation risk from negative size-selective predators by increasing size, but will delay reproductive maturity, so $\kappa(t)$ will directly affect both growth and reproduction, and indirectly affect mortality. The time dependence of $\alpha(t)$ and $\kappa(t)$ reflects the fact that investment may change as a function of an individual's age, size, and physiological state (Clark and Harvell, 1992).

Table 2.1: Model variables and parameters with default parameter values.

Symbol	Description	Units	Default value
t	Age	d	
$\ell(t)$	Length	mm	
$\alpha(t)$	Fraction of time spent foraging		
$\kappa(t)$	Fraction of energy allocated to growth		
$b(t)$	Birth rate	eggs d^{-1}	
$p(t)$	Survivorship of an individual		
R	Resource density	cells ml^{-1}	10^6
v_x	Maximum resource intake rate per unit surface area	cells mm^{-2} d^{-1}	1.8 x 10^6
ξ	Functional response shape parameter	ml $cell^{-1}$	7.0 x 10^{-6}
$\hat{\ell}$	Maximum attainable length under unlimited resources	mm	6.0
$\hat{g}$	Rate constant of growth	d^{-1}	0.5
$\hat{r}$	Rate of offspring production per unit surface area	mm^{-2} d^{-1}	0.14
μ	Background mortality rate	d^{-1}	0.01
ω	Slope of mortality-size curve	mm^{-1}	0.5
ℓ_{mid}	Median size preference of predator	mm	3.5
P_h	Half of maximum predation rate	d^{-1}	0 - 0.2
s	Shape parameter for activity–mortality relation		1 - 10

Foraging gain and maintenance costs

Acquisition of energy from the environment is the key process underlying growth and reproduction. The rate of energy ingestion is modeled as:

$$I(R, \ell, \alpha, t) = v_x \alpha(t) \frac{\xi R}{1 + \xi R} \ell^2, \tag{2.1}$$

where v_x is the maximum rate of resource consumption, R is the constant resource abundance, and $\frac{\xi R}{1+\xi R}$ is a Type II functional response.

Two features of this formulation are noteworthy. First, ingestion rate depends linearly on activity level, $\alpha(t)$, so total resource ingestion is proportional to the fraction of time actively foraging. Second, energy ingestion depends upon the surface area of the individual, which is proportional to ℓ^2. The dependence of feeding rate on surface area is quite general, applying to many different feeding modes (see the discussion in Kooijman (2000), pp. 66-71). This dependence implies that energy ingestion will increase as individuals increase in size; this is the mechanism by which increased size increases reproductive potential. Some energy must be utilized for maintenance, with maintenance costs scaling with body volume (see Kooijman (2000), pp. 89-94).

Growth and reproduction

I assume that maintenance costs are taken directly from ingested resources. Individuals then allocate surplus energy between growth and reproduction. This assumption makes my model a net production model (*sensu* Noonburg et al. (1998)), rather than a net assimilation model, in which energy is allocated to cover both growth and maintenance (de Roos, 1997). Both net assimilation and net production models can be justified on biological grounds; my choice results from my desire that $\kappa(t)$ be free from any constraints. By contrast, net assimilation models require a rule that specifies how energy is to be reallocated when maintenance costs are high.

The fraction of net production allocated to growth is controlled by the time-varying function $\kappa(t)$, with $1-\kappa(t)$ being allocated to maturation and reproduction. This reflects the inherent tradeoff between current growth and future reproduction. When resources are constant, growth follows a modified von Bertalanffy growth equation (Kooijman and Metz, 1984; de Roos, 1997; Kooijman, 2000), which predicts growth in size to approach $\hat{\ell}$ at a rate determined by the parameter $\hat{g}$, assuming that growth allocation $\kappa(t)$ and activity level $\alpha(t)$ are both constant.

$$\frac{d\ell}{dt} = \hat{g}\kappa(t)(\hat{\ell}f(R,\alpha) - \ell(t)), \tag{2.2}$$

where

$$f(R,\alpha) = \alpha(t)\frac{\xi R}{1+\xi R}. \tag{2.3}$$

The remaining energy is allocated towards maturation or reproduction. Before reaching sexual maturity, individuals are assumed to allocate energy towards the development of reproductive tissue: sexual maturity is reached upon investment of a fixed amount of energy into maturation (see Appendix A). The birth rate $b(t)$ is then determined by energy allotment towards reproduction $1-\kappa(t)$ and the rate of offspring production per unit surface area $\hat{r}$.

$$b(t) = \hat{r}(1-\kappa(t))\left(f(R,\alpha)\ell(t)^2 - \frac{\ell(t)^3}{\hat{\ell}}\right). \tag{2.4}$$

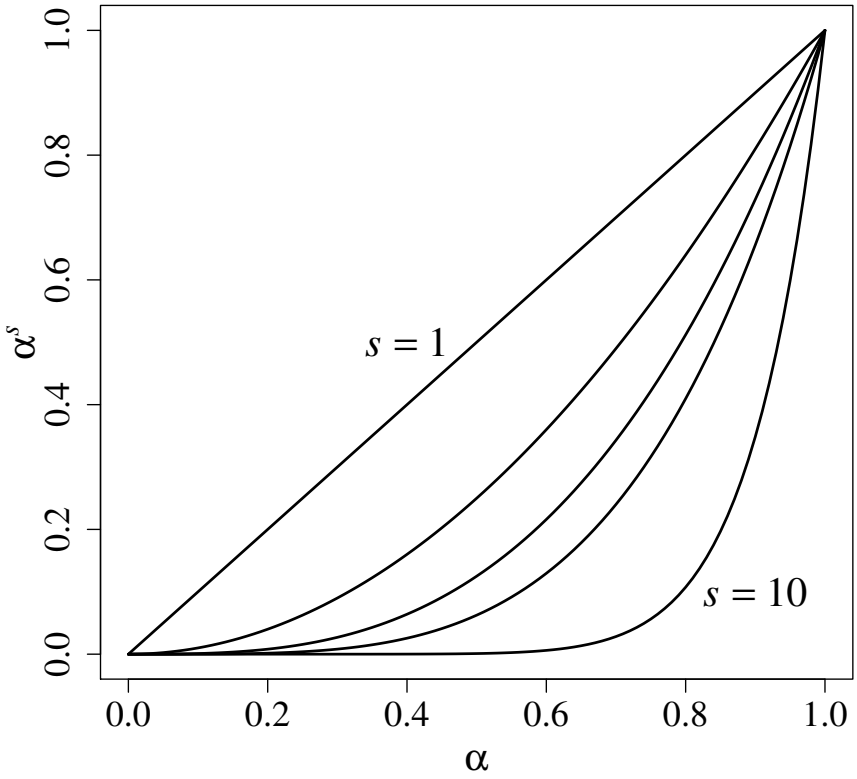

Figure 2.1: The relationship between activity level α and predation rate for different values of s. See the text for the interpretation of s.

Mortality

I consider mortality risk that depends on both behavior and size. A large body of literature has demonstrated increases in predation risk with increases in activity (Lima and Dill, 1990; Werner and Anholt, 1993; Werner and Peacor, 2003). While this pattern is clear, I am unaware of any studies that have directly measured the relationship between foraging activity and predation risk, despite theory indicating that this shape is critical in determining the ecological consequences of behavioral defense (Abrams, 1992). I follow Noonburg and Nisbet (2005) and assume that predation risk scales with $\alpha(t)^s$, a simple function that is flexible enough to take a variety of shapes from concave to convex. Furthermore, an examination of the relationship between activity level and predation rate for different values of s suggests a possible biological interpretation of s as an indicator of the foraging behavior of the predator.

Depending on the value of s, $\alpha(t)^s$ can take three basic shapes (Figure 2.1). If $s = 1$, the relationship between activity and predation risk is linear. This assumption is the default expectation (Gerritsen and Strickler, 1977; Werner and Anholt, 1993). It will hold, for example, when predators move at constant speed and demonstrate no behavioral response to prey movement. If $s > 1$, the curve is convex, suggesting that the per capita predation rate increases with increases in activity level. This can be interpreted as indicating a preference among predators for more active prey. Such preferential foraging has been demonstrated in a number of cases (Furnass, 1979; Wright and O' Brien, 1982; Peterson and Ausubel, 1984; O'Keefe et al., 1998; Utne-Palm, 2000). The biological mechanism behind this preference is likely predators cueing on prey movement. If $s < 1$, the curve is concave, and predation rate asymptotes at high activity levels. The effect of a concave relationship between activity level and mortality on the evolution of defenses was investigated, but the results were identical with the $s = 1$ case; further discussion of the $s < 1$ case will be omitted.

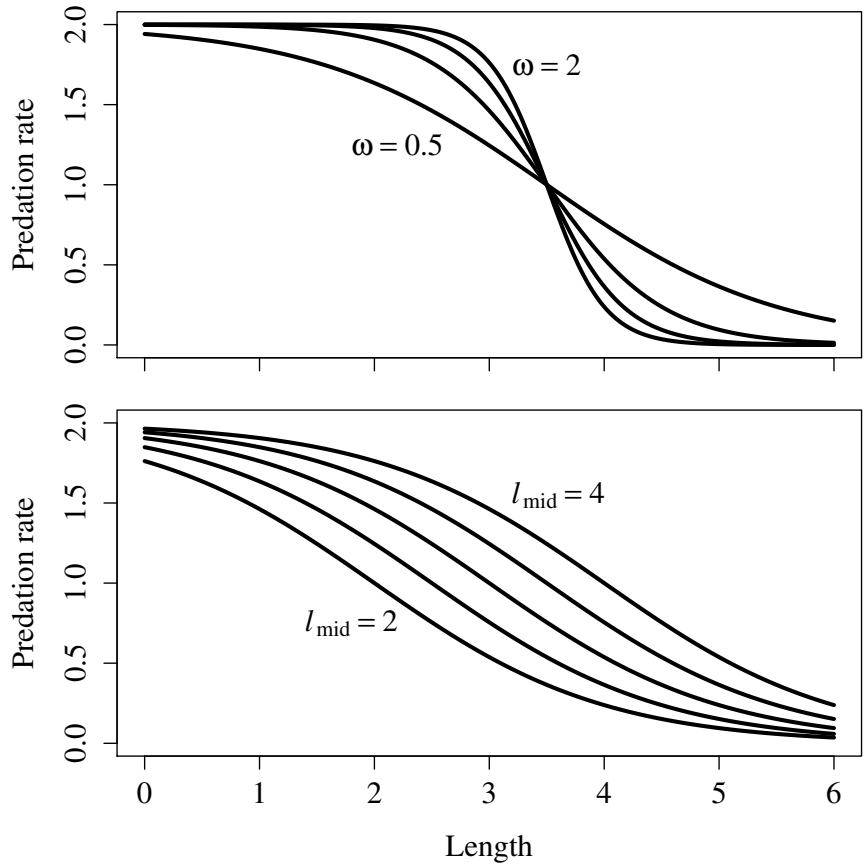

Figure 2.2: These graphs show how mortality changes with size, assuming different values for ω and ℓ_{mid}. For the simulations in the paper, $\omega = 0.5$ and $\ell_{\text{mid}} = 3.5$.

This formulation makes possible two *a priori* predictions about the effect of s on optimal defense expression. As s approaches infinity, even a slight reduction in activity level will reduce predation rate to almost zero, whereas as s approaches zero, reduction in activity level will have no effect on predation risk. From this observation I predict that high values of s will favor behavioral defenses, whereas low values of s will favor either life history defenses or a strategy that forgoes any investment in defense.

Predation rate is also dependent on size. Here I assume that predation risk decreases with length, as happens when predator and prey are similar in size or

predators are gape-constrained. This creates a size refuge against predation, so that individuals exposed to predators may increase growth at the cost of delayed maturation. A reasonable functional form for the relationship between size and predation rate is

$$P_h(1 - \tanh(\omega(\ell - \ell_{\text{mid}})), \tag{2.5}$$

where P_h is half the maximum predation rate. The parameters ω and ℓ_{mid} characterize the size selectivity of the predator (Figure 2.2): ℓ_{mid} gives the inflection point where predation rate is equal to P_h and ω is the slope of the predation rate-size curve. ℓ_{mid} characterizes the size preference of the predator, while ω characterizes how size-limited the predator is: larger values suggest that the predator is very limited by prey size; smaller values indicate a predator that is less size-limited (Rinke et al., 2008).

Considering both the size- and behavior-dependence of predation rate, along with a non-predation mortality term μ, the dynamics of survivorship $p(t)$ are described by:

$$\frac{dp}{dt} = -(\mu + \alpha(t)^s(1 - \tanh(\omega(\ell - \ell_{\text{mid}})))P_h)p. \tag{2.6}$$

Calculating the fitness of an individual

Equations 2.2-2.4 and 2.6 can be used to determine the fitness of an individual. Fitness was measured by the net reproductive rate R_0:

$$R_0 = \int_0^\infty b(t)p(t)dt. \tag{2.7}$$

Net reproductive rate weights birth rate $b(t)$ by survivorship $p(t)$ to determine the expected number of offspring produced by an individual over the course of its life.

This fitness metric is appropriate whenever population size and the environment are constant between generations, as I assume (Benton and Grant, 2000).

Selection experiments

To find the optimal defense investment, one needs to determine the shapes of activity level and growth allocation functions that maximize individual fitness. However, the shapes of these functions are unknown, and might well be quite complex. I used genetic algorithms to determine the optimal shapes $\alpha(t)$ and $\kappa(t)$ (Holland, 1975), an approach that has been successfully applied to other questions in evolutionary ecology (Shertzer and Ellner, 2002; Strand et al., 2002). Details regarding function specification and genetic algorithm implementation can be found in Appendix A.

For any given environment, I characterize behavioral defense investment by the average activity level across an individual's lifespan, while life history defenses are characterized by size at maturity. I report the age at maturity to complete the depiction of the life history strategy. Size and age at maturity are determined by the interaction between $\alpha(t)$ and $\kappa(t)$. In this study, the predation rate P_h was varied between 0 and 0.2; over this range, individual fitness is always greater than replacement level ($R_0 > 1$). Plasticity in defenses is seen by a reduction in average activity level or an increase in size at maturity with changes in P_h. The value of s was varied between 1 and 10 to determine the effect of the shape of the foraging–predation risk tradeoff on the evolution of behavior and life history.

Three different selection scenarios were performed. In the first, only growth allocation was under selection; behavior was assumed constant at the optimal behavior in the absence of predators, $\alpha(t) = 1$. In the second, only behavior was under selection; growth allocation was fixed at the optimal allocation pattern in the absence of predators. In the third, both growth allocation ($\kappa(t)$) and activity level ($\alpha(t)$)

were under selection. These experiments will be abbreviated as the L (life history only), B (behavior only), and LB (life history and behavior) selection experiments, respectively. By fixing either growth allocation or activity level, I fix either the growth–reproduction or foraging–predation risk tradeoffs. Comparing the results when both growth allocation and activity level are flexible to these cases allows us to investigate how the tradeoffs interact to determine optimal behavior and life history.

Results

A short guide to interpreting the results

To facilitate the presentation of the results, Figure 2.3 shows how how the shapes of the growth allocation and behavior functions independently affect maturation size and age. Fig. 2.3B,C shows the maturation size and age for different $\kappa(t)$ functions (Fig. 2.3A), assuming behavior is constant at $\alpha = 1$. In all of the results that follow, the optimal growth allocation function has this same basic shape. Note that age at maturity does not correspond to the age at which energy allocation switches to reproduction. This is because energy is allocated towards maturation first; only after the fixed maturation requirement is met does reproduction begin. The duration of the delay between switching allocation towards maturation and the onset of reproduction is determined by the size at maturity (see Appendix A for more details). The black line in Fig. 2.3A is the optimal predator-free growth allocation. Fig. 2.3E,F show the maturation size and age for different $\alpha(t)$ functions (Fig. 2.3D); the black line in Fig. 2.3D is the optimal predator-free behavior.

As the growth phase is prolonged, size at maturity increases, as does age at maturity. This is entirely straightforward, since more energy is allocated towards growth, and the switch to allocating energy towards maturation and reproduction occurs at later ages. Decreasing activity level causes size at maturity to decrease, because less

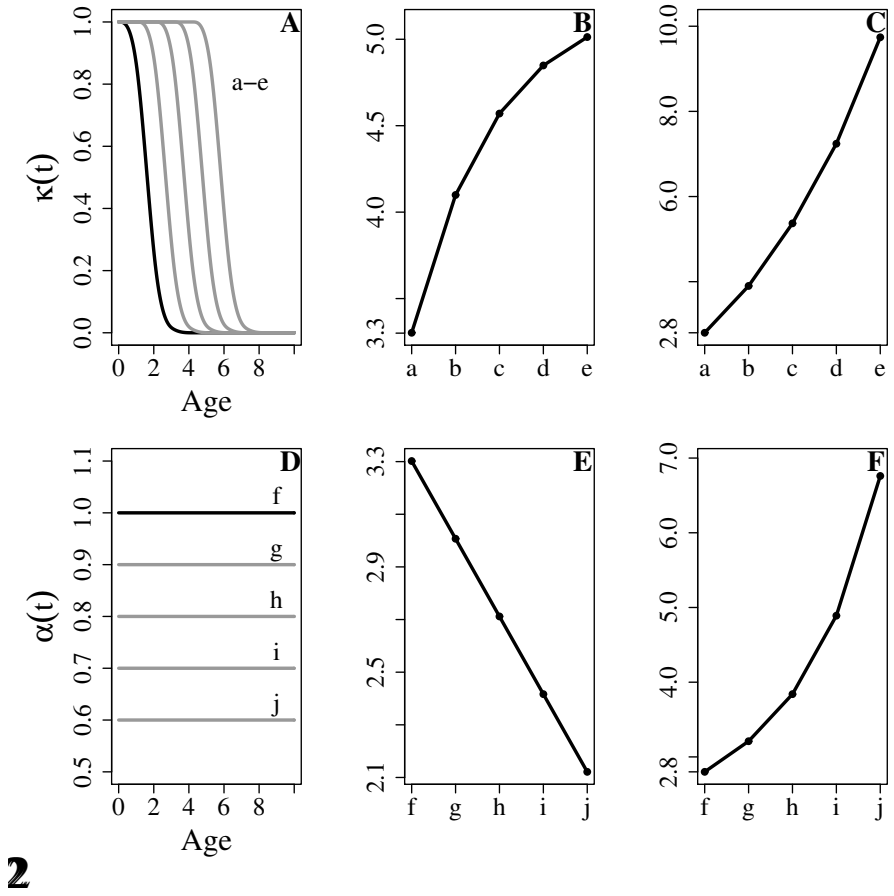

Figure 2.3: The affects of different allocation and behavior functions on size and age at maturity. The affect of different allocation functions on size and age at maturity can be seen in panels A-C. The affect of different behavior functions can be seen in panels D-F. Here I assume constant behavior; observed behavior $\alpha(t)$ was more complex (see Figure 2.5 for an example). The bold lines (a) and (f) in panels A and D show the optimal growth allocation pattern and activity level in the absence of predation. For panels A-C, activity level is held at the optimal predator-free $\alpha(t)$, while for D-F, growth allocation is determined by the optimal predator-free $\kappa(t)$.

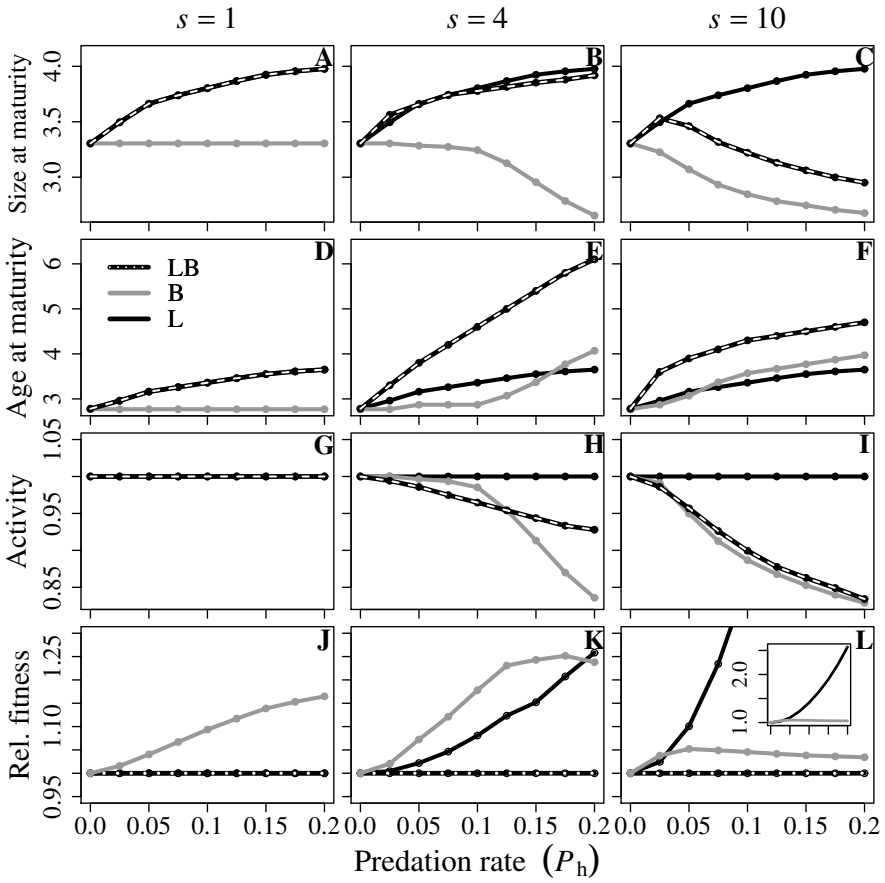

Figure 2.4: The optimal defense investments for s=1, 4, and 10. For each s value, the metrics for life history and behavioral defenses are plotted for each of the three selection scenarios. The LB strategy is shown by the black-and-white line, the B strategy is shown by the gray line, and the L strategy by the black line. Note that for $s = 1$, the LB and L strategies are identical - only the LB strategy can be seen in these plots. The final row shows the relative fitness advantage of the LB strategy. The black-and-white line is LB relative to itself (fixed at 1), the gray line is LB relative to B, and the black line is LB relative to L.

energy has been ingested by the age when allocation switches to maturation and reproduction. Age at maturity increases because it takes longer to meet the maturation requirement as size at maturation decreases, because reduced size reduces energy intake (Appendix A).

Linear tradeoffs favor life history defenses

Above I predicted that low s values would lead to life history defenses. It turns out that even a linear tradeoff, the expected tradeoff shape for a predator with no foraging preference, is enough to produce this result (see Figure 2.4 for the results when $s = 1$). In Fig. 2.4A, D, G, and J, the LB and L strategies are exactly identical. No behavioral defense is being used (Fig. 2.4G). The optimal strategy is to suffer the high mortality risk resulting from high activity levels and "sprint" for the life history refuge by not investing in behavioral defense. The main costs of behavioral defense is delayed maturation. This delay has a direct negative impact on fitness; this cost must be offset by benefit of increased survivorship for investment in behavioral defense to be worthwhile. In such an environment, behavioral defense is not effective enough to offset the delay.

Highly nonlinear tradeoffs favor behavioral defenses

For high values of s, defense expression is dominated by behavioral defenses, as I predicted. The mean activity level for the LB and B strategies are nearly identical across P_h values (Fig. 2.4I). However, the LB strategy is able to compensate for one of the major costs of behavioral defenses (reduced size) by prolonging allocation to growth. This can be seen in the increased size at maturity for the LB compared to the B strategy (Fig. 2.4C). In fact, for $P_h < 0.075$, the LB strategy actually invests in both defenses simultaneously as seen in the increase in size from $P_h = 0$. The cost of prolonged allocation to growth is delayed maturation, but the increase in birth

rate for maturing at a larger size compensates for this delay, giving the LB strategy a significant fitness advantage over both the L and B strategies. I note that for higher values of P_h (not shown), the LB and B strategies converge, though R_0 drops below replacement.

Moderately nonlinear tradeoffs favor multi-defense strategies

With moderate values of s, there is investment in both defenses simultaneously (Fig. 2.4B, H). This is achieved through a specific interaction between the different defense investment functions. Figure 2.5 shows a characteristic case. The LB activity level α is less than the B α early in life, indicating that the LB strategy increases expression of behavioral defenses early in ontogeny (Fig. 2.5B). Later in life, however, the LB curve lies above the B curve, suggesting that behavioral defenses are relatively underexpressed. Simultaneously, the LB strategy prolongs the allocation to growth $\kappa(t)$ (Fig. 2.5A). This allows it to compensate for the decrease in size at maturity caused by reducing activity level. The early reduction in activity level, coupled with prolonged allocation to growth, allows the individual to invest in both defenses. This suggests that the optimal strategy uses $\kappa(t)$ and $\alpha(t)$ to compensate for the costs of the defenses through ontogeny.

Discussion

The shape of the foraging–predation risk tradeoff determines the optimal defense strategy

Physiological and ecological tradeoffs provide a framework for understanding life history evolution. However, most life history theory has assumed that these tradeoffs operate independently of one another (Steiner and Pfeiffer, 2007). This is unrealistic, as multiple tradeoffs can play a role in determining a single trait; optimal trait

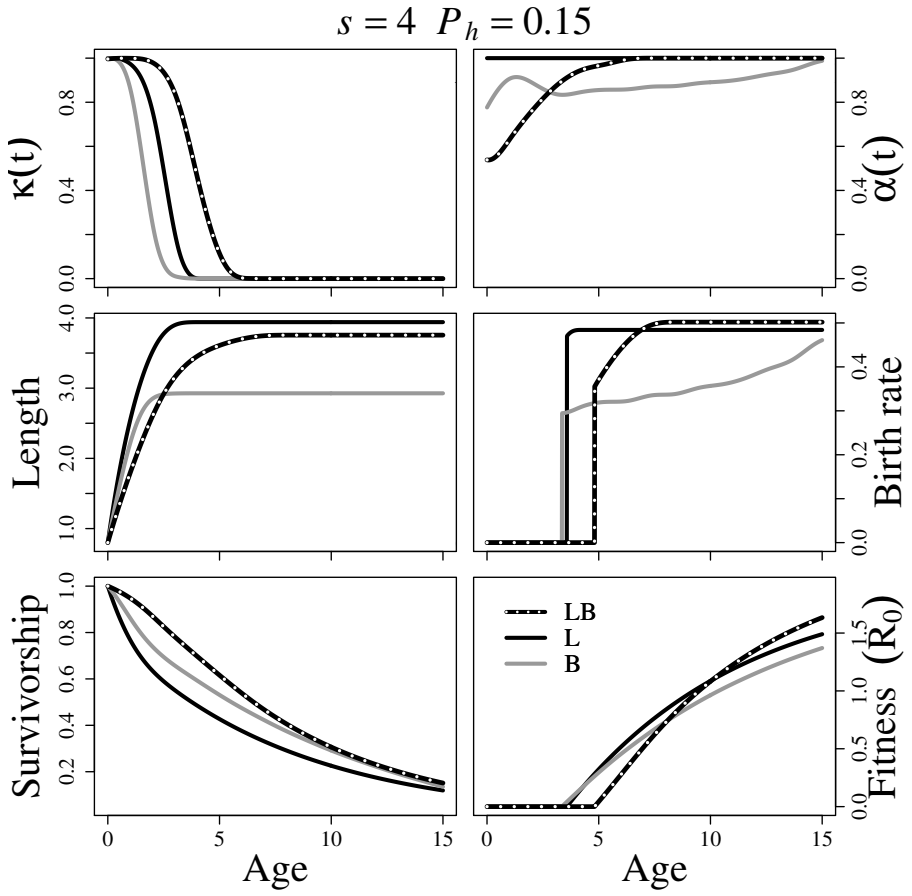

Figure 2.5: The behavioral and life history responses and pattern of growth, reproduction, and mortality that results from each strategy for $s = 4$ and $P_h = 0.15$. The multiple-response experiment invests in both defenses across ontogeny by using each response to compensate for the cost of the other defense. This produces the integrated multiple-defense strategy shown in Fig. 2.4.

expression will then be determined by balancing the costs of interacting tradeoffs. In this study, I consider two fundamental tradeoffs: the physiological tradeoff between growth and reproduction, and the ecological tradeoff between foraging gain and predation risk. The first tradeoff is mediated by the pattern of energy allocation. Increased allocation to growth reduces size-dependent predation risk, but carries the cost of delayed reproductive maturity. The second tradeoff is mediated by activity level. Reducing activity level reduces behavior-dependent predation risk, but at the cost of reduced growth and delayed maturity. That both tradeoffs share a benefit, but differ in costs, suggests that the optimal pattern of behavior and energy allocation will depend on the shapes of these underlying tradeoffs.

I show that by varying the shape of the foraging–predation risk tradeoff, I can arrive at very different patterns of covariation between life history and behavior. When the foraging–predation risk tradeoff is highly nonlinear and accelerating ($s >> 1$), behavioral defenses are highly effective and I predict that behavior and life history will be dominated by this tradeoff. On the other hand, when the tradeoff is linear or decelerating ($s \leq 1$), I predict that behavior and life history are determined by the tradeoff between growth and reproduction. However, for moderately accelerating tradeoff shape, behavior and life history are determined by both tradeoffs. The interaction between the two tradeoffs leads to a multiple-defense strategy, with behavior and life history integrated across ontogeny (Pigliucci, 2003).

A number of models have considered how predation jointly modifies behavior and life history (Abrams, 1991; Abrams and Rowe, 1996; Urban, 2007a; Steiner and Pfeiffer, 2007). Abrams and Rowe (1996) considered how size-independent predation, assuming a concave-up relationship between behavior and predation risk (analogous to $s > 1$), affected optimal age and size at maturity and behavior. In the case most similar to my model where both traits are flexible and non-predation mortality does not depend on behavior (Table 1 of Abrams and Rowe (1996)), the direct response to

predator density was to reduce size at maturity, decrease growth effort, and decrease growth rate. Age at maturity could increase or decrease, depending on the predator density. The model assumptions and predictions agree with the predictions from the $s = 10$ case for my model.

My predictions for the linear ($s = 1$) case match those of Urban (2007a). This model showed that the optimal prey defense strategy could be to forgo a behavioral defense in favor of reaching a size refuge as quickly as possible. This result was much more likely when the relationship between growth and predation risk was linear or decelerating, in analogy with my results. This result was motivated by empirical data (Urban, 2007b), and provides additional evidence for the importance of the foraging–predation risk tradeoff in determining defense expression in nature.

However, neither of the two models above, nor any of the other models that have considered the effect of predation on both life history and behavior, have allowed responses to vary with age. As such, they miss the interactions between the responses that allow for multiple-defense strategies to evolve. Intuitively, I expect that investment in behavioral and life history defense will be negatively correlated. Increasing investment in behavioral defense by reducing activity will reduce size at maturity, and hence, investment in life history defense. However, this intuition ignores how the functions underlying defense investment (i.e., growth allocation $\kappa(t)$ and activity level $\alpha(t)$) interact with one another through ontogeny. For moderate s values, the pattern in these underlying traits that emerges is to reduce activity level early in life, when predation risk is highest, while simultaneously keeping growth allocation high. As size increases, activity level is increased until the individual reaches the size refuge. It is the compensatory dynamics between these responses that gives rise to the integrated multiple-defense strategy.

This compensation suggests the importance of timescale in defense expression. Behavioral defenses are effective immediately, and can be modified quickly and re-

versibly. Life history defenses, on the other hand, are only effective at reducing predation risk after a threshold of energy investment has been made. Nor are life history defenses reversible - individuals of most species are typically not capable of shrinking. This difference in timescale affects the interaction between the two defenses, and highlights the importance of considering defense investment across an individual's lifetime (Clark and Harvell, 1992; Relyea, 2004b; Hammill et al., 2010).

Empirical evidence for the theoretical predictions

There are a number of different mechanisms that could lead to a concave-up nonlinear relationship between activity and predation risk. This nonlinearity is generated whenever predator encounter rates increase at an increasing rate as prey individuals become more active. Simple mechanisms that could produce such a result are active prey selection, where predators choose more active over less active prey (Snyder, 1975; Sarno and Gubanich, 1995), or increased perception of more active prey (O'Keefe et al., 1998; Utne-Palm, 2000). Additionally, predators could increase their own activity levels or foraging speeds in response to more active prey. The prey-detection method of the predator (e.g., visual hunting versus mechanoreception) is probably less important than the hunting mode (e.g., active versus passive predators). Predators that feed via sit-and-wait or filter feeding are less likely to demonstrate preference and are likely to impose a linear foraging–predation risk tradeoff.

Empirical work provides evidence for many of my predictions. Many species are known to exhibit behavioral defenses against negative size-specific predation risk from active predators, including amphibians (Anholt et al., 2000; Urban, 2007b), *Daphnia* (Pangle and Peacor, 2006), fish (Abrahams and Healey, 1993), and snails (Hoverman et al., 2005). Studies also observe that predators that prefer small prey and forage passively select for life history defenses (Crowl and Covich, 1990; Tollrian and Harvell, 1999; Chase, 1999).

However, most of the foregoing studies measured only one phenotypic trait. When multiple defensive traits are measured within the same study, it is almost always found that individuals express multiple defenses, either simultaneously or across ontogeny (Relyea, 2001; Hoverman et al., 2005; Boeing et al., 2006b). Existing theory, which has largely focused on single traits, does not predict how complex multivariate defense strategies may evolve, or how defensive traits may covary with one another. My model shows that, for behavior and life history, the pattern of covariation depends on the interaction between physiological and behavioral tradeoffs underlying these defenses. This suggests a general framework for understanding patterns of covariation between other defensive traits. Furthermore, this recent empirical work suggests that integration of multiple defenses may be the rule, rather than the exception. In particular, a number of studies have shown exactly the pattern observed here: high investment in behavioral defense early in life, but reduced investment through ontogeny (Pettersson et al., 2000; Relyea, 2003b; Brodin et al., 2006; Hammill et al., 2010). Additionally, work in positive size-dependent predation systems have shown a similar effect, albeit operating in the opposite direction. For example, *Daphnia* often do not engage in behavioral defense (diel vertical migration) until they reach large size, because at small sizes they are protected against predation (Leibold et al., 1994). A recent review of integrated defense responses to predation highlighted the importance of studying trait expression through ontogeny to uncover the important interaction between development and defense expression (Relyea, 2004b).

Applicability of these results to other systems

The assumptions of my model were chosen to maximize the potential for interaction between the ecological and life history tradeoffs. That is, I focused on defenses that, all else being equal, are negatively related to one another: reduced activity reduces growth rate and thus, size at maturity. However, it is worth asking how

the insights gained from these results could be used to predict defense expression in situations where the size dependence of predation took a different form and defensive traits other than behavior and growth allocation were modified. I focus here on the cases of positive size-dependent predation and morphological defenses.

Theoretical and empirical research have shown that a common response to positive size-dependent predation is to reduce the allocation to growth and increase the allocation to reproduction, leading to early maturation at reduced size (Taylor and Gabriel, 1992; Tollrian and Harvell, 1999; Ernande et al., 2004). Behavioral defenses, on the other hand, tend to lead to late maturation at reduced size (Beckerman et al., 2007). Since both behavioral and life history defenses against positive size-dependent predation would lead to reduced size at maturity, investment in both defenses simultaneously would seem to be easier. I have investigated how variation in the shape of the foraging–predation risk tradeoff affects behavior and life history under positive size-dependent predation (Appendix A). My results show that multiple-defense strategies are common (present even for $s = 1$), but that a switch to behavior-only defenses occurs for lower values of s. This provides an alternative explanation for life history patterns attributed to either altered energy allocation or behavior in previous studies (Ball and Baker, 1996; Beckerman et al., 2007).

Morphological defenses are ubiquitous in natural systems (Tollrian and Dodson, 1999). Development of morphological defenses is typically thought to require reallocation of energy from growth and reproduction (Steiner and Pfeiffer, 2007). Additionally, life history and morphological defenses both have indirect positive effects on fitness through reduced predation rate. Clark and Harvell (1992) predicts that investment in such traits is best done early in life, with allocation to reproduction only late in life. These similarities suggest that morphological and life historical defenses may show similar patterns of covariance with behavioral defenses. Empirical work supports this supposition. For example, Hammill et al. (2010) has shown that the

ciliate *Euplotes* expresses behavioral defenses initially in response to predators, with morphological defenses expressed later. This temporal separation of defense expression is exactly analogous to the predictions made by this model. However, theoretical work by Steiner and Pfeiffer (2007), however, predict that integration may be common between behavior and morphology. The authors found that increasing predator density increased investment in both morphological and behavioral defenses. Investment in single defense was only found when morphological defense effectiveness was increased until the behavioral defense was no longer necessary. This would be similar to decreasing the value of ℓ_{mid} here.

Implications for ecological theory

Understanding how tradeoffs interact with one another is essential to understanding phenotypic evolution. Two of the best-studied tradeoffs involve the behaviorally-mediated tradeoff between foraging and predation mortality and the physiologically-mediated tradeoff between growth and reproduction. Both of these tradeoffs involve fundamental traits that have wide-ranging impacts on individual-, population-, and community-level processes (Werner and Gilliam, 1984; de Roos et al., 2003). Here we show that varying the shape of the foraging–predation risk tradeoff modifies the interaction between the tradeoffs, leading to the evolution of qualitatively different defense strategies.

The foraging–predation risk tradeoff has been widely cited as important to understanding ecological dynamics (Abrams, 1992; Werner and Peacor, 2003). My results suggest that this tradeoff has important implications for optimal investment in different defenses as well and predicts that the differences in defense strategy between closely related species or clones of single species may be explainable in terms of differences in the shapes of the foraging–predation risk tradeoff. This result has important implications for ecological theory, as it suggests that previous work that

has considered only how behavior modifies ecological interactions is limited. Integrated multiple-defense strategies are common in nature, and my model predicts they should arise under common conditions. However, no theory has yet been developed that explores the ecological consequences of integrated defense strategies. Furthermore, consideration of the ecological dynamics that result from different defense strategies opens up the question of the role of feedback between ecological and evolutionary dynamics in driving selection on behavior and life history. Such feedback can generate diversifying selection, permitting the coexistence of multiple defense strategies (Abrams et al., 1993; Day et al., 2002).

Trait expression is an area of research that has received renewed interest recently (Abrams, 2001). Because of the important effects of dynamic traits on ecological interactions (Abrams, 1995; Werner and Peacor, 2003), understanding how ecological factors promote the expression of different characters becomes crucially important for understanding ecological communities. However, this understanding requires that empiricists and theoreticians move beyond thinking about single traits to a fuller consideration of how the expression of multiple traits varies across ontogeny and across different environments.

CHAPTER III

Multiple traits, multiple trade-offs, and the evolution of phenotypic polymorphism

Introduction

Understanding the processes responsible for the tremendous diversity of life remains one of the fundamental goals of evolutionary biology (Dieckmann et al., 2004). This diversity seems to run counter to the view of natural selection as an optimizing force, and so considerable ink has been spilled attempting to explain this apparent paradox. One mechanism for the generation of adaptive diversity is disruptive selection, wherein selection favors extreme phenotypes. Disruptive selection is considered a necessary prerequisite for sympatric speciation, and there is considerable debate about how common such selection is likely to be in nature. In recent years, a large and impressive body of literature has documented how ecological dynamics can give rise to disruptive selection (Geritz et al., 1998; Doebeli and Dieckmann, 2000; Dieckmann et al., 2004; Hoyle et al., 2008; Boots et al., 2009).

This literature has shown that evolutionary branching in phenotypic evolution is a robust outcome in a wide range of ecological models, including models of competition, mutualism, predator-prey interaction, and host-parasite interaction (Doebeli and Dieckmann, 2000; Boots et al., 2009). Of particular importance to the predicted

evolutionary outcome is the shape of trade-offs underlying the expression of the evolving trait, and certain trade-off shapes are predicted to more robustly give rise to disruptive selection (Hoyle et al., 2008). Thus, we seem to have a fairly clear picture of how ecological dynamics can produce disruptive selection in the evolution of single traits.

However, organisms are not "grab-bags" of independently evolving traits, but instead show considerable integration between phenotypic traits (Pigliucci, 2003). One way in which integration may arise is through multiple traits affecting the same process, and thereby both being under selection simultaneously. One immediate consequence of selection acting on multiple traits simultaneously is that the trade-offs underlying each individual trait will affect the evolution of both traits, that is, the trade-offs themselves are no longer independent. Consider the evolution of body size and foraging behavior in response to size-dependent predation risk. If increasing body size reduces predation risk, then there will be selection towards increased body size that is opposed by the well-studied energetic trade-off between size and age at maturity (Roff, 1992; Taylor and Gabriel, 1992; Gårdmark and Dieckmann, 2006). If reducing time spent foraging also reduces predation risk, then there will be selection towards reduced activity that will be opposed by the need to acquire resources for life processes, including growth (Lima and Dill, 1990; Werner and Anholt, 1993). Thus, selection on behavior and body is integrated through the effect of each on predation, and also through the effect of behavior on body size, making the outcome of selection on both traits simultaneously difficult to predict (Cressler et al., 2010).

Here, I study the joint evolution of behavior and body size in response to both starvation and predation risk using multidimensional adaptive dynamics (Geritz et al., 1998; Leimar, 2005, 2009). I focus on behavior and body size because of the fundamental role each trait plays in affecting individual-, population-, and community-level processes (Bolker et al., 2003; de Roos et al., 2003; Werner and Peacor, 2003).

Furthermore, a number of studies have honed in on the role of predation and predator-mediated fitness trade-offs in generating disruptive selection and polymorphism in organismal life history (Day et al., 2002; Gårdmark and Dieckmann, 2006). Here, I focus on how variation in underlying trade-offs alters evolutionary outcomes, demonstrating that selection acting on multiple traits simultaneously can show very different evolutionary outcomes compared to evolution acting on a single trait. In particular, disruptive selection can arise under circumstances where stabilizing selection would be predicted by single trait evolution, whereas stabilizing selection can arise when disruptive selection would be predicted. These results suggest that explanations for the generation of diversity through ecological dynamics must consider how selection acts on the integrated phenotype, rather than focusing on single traits.

Methods

The model

I consider the evolution of a stage-structured consumer population in response to selection arising from both resource and predator population dynamics. The three populations are dynamically linked, with resource ingestion and predation rates that depend on the behavior and size of the consumer. I consider the evolution of two traits: juvenile activity level (α_J) and consumer length at maturity (L_A). For simplicity, I assume that adults are fully active ($\alpha_A = 1$), and that there is a straightforward relationship between adult length, L_A, and juvenile length, L_J ($L_J = (L_A + L_b)/2$, where L_b is the length at birth).

The consumer population is divided into juvenile and adult stages. Juveniles mature into adults upon reaching a fixed size L_A. Importantly, the amount of time required to reach size at maturity is dependent upon the dynamics of the system. I model the consumer population using a stage-structured delay-differential equation

Table 3.1: Model variables and parameters with default parameter values.

Symbol	Description	Units	Default value
t	Time	d	
$R(t)$	Resource concentration	cell ml^{-1}	
$J(t)$	Juvenile abundance	prey ind.	
$A(t)$	Adult abundance	prey ind.	
$P(t)$	Predator abundance	predator ind.	
$\tau(t)$	Juvenile stage duration	day	
r	Resource growth rate	d^{-1}	0.2
K	Resource carrying capacity	cells ml^{-1}	300,000
v_R	Max. intake rate per prey, per unit SA	cells prey^{-1} mm^{-2} d^{-1}	1.8 x 10^6
ξ	Functional response shape parameter	ml cell^{-1}	7.0 x 10^{-6}
$\hat{r}$	Birth rate per unit SA	mm^{-2} d^{-1}	0.14
L_b	Consumer size at birth	mm	0.8
L_{max}	Max. length under unlimited resources	mm	6.0
$\hat{g}$	Rate constant of growth	d^{-1}	0.15
μ	Resource-dependent mortality rate	d^{-1}	0.02
ω	Slope of size-mortality curve	mm^{-1}	2
b	Predator birth rate	prey^{-1} d^{-1}	1
δ	Predator death rate	d^{-1}	0.001
ρ	Per-predator attack rate	predator^{-1} d^{-1}	4
η	Activity-mortality shape parameter		Varied 0.5-4
Ł$_{mid}$	Median size preference of predator	mm	Varied 2-4
α_J	Juvenile activity level		Optimized
L_A	Size at maturity (adult size)	mm	Optimized
L_J	Size of juveniles	mm	$(L_A + L_b)/2$

with a flexible delay (Nisbet, 1997). With a delay-differential equation, the duration of the juvenile period depends on the environment throughout the juvenile stage and allows for the direct modeling of juvenile growth. This formulation permits an explicit consideration of size while maintaining a distinct separation of juvenile and adult classes. This is especially helpful since I am going to consider the evolution of multiple traits simultaneously, a problem that would be considerably more difficult if I were to use the more explicit formulation of physiologically-structured population model (de Roos, 1997; de Roos et al., 2003; Durinx et al., 2008).

Model variables and parameters are described in Table 3.1. For the consumer population, the default values of the physiological parameters were taken from (de Roos

et al., 1990); these values are specific to the zooplankter *Daphnia magna*, but are assumed here only to locate the model in a biologically reasonable region of parameter space, as the underlying model formulation is quite general (Kooijman, 2000; de Roos et al., 2003). The full model specification is given in Table 3.2.

Resource dynamics are determined by the processes of resource growth and consumer ingestion. These dynamics are described by

$$\frac{dR}{dt} = rR(t)\left(1 - \frac{R(t)}{K}\right) - v_R\left(f_A(t)L_A^2A(t) + f_J(t)L_J^2J(t)\right). \tag{3.1}$$

In the absence of consumption, the resource population $R(t)$ grows to a carrying capacity, K, at a maximum rate, r. Ingestion depends on the maximum ingestion rate v_R and stage-specific functional responses ($f_J(t)$ and $f_A(t)$) and surface areas (assumed proportional to length Kooijman (2000); L_J^2 and L_A^2). Functional responses are given by:

$$f_J(t) = \frac{\alpha_J\xi R(t)}{1 + \alpha_J\xi R(t)} \tag{3.2}$$

$$f_A(t) = \frac{\xi R(t)}{1 + \xi R(t)} \tag{3.3}$$

These functional responses depend a shape parameter ξ and behavior. Juvenile activity level is given by α_J; adult activity level is assumed constant at 1. I conceptualize α_J as the fraction of time spent actively foraging, so it can take values between 0 and 1. From the consumer's perspective, reducing activity level has the same effect as reducing the amount of resources in the environment (but the consequences for the resource dynamics are quite different, obviously).

Juvenile dynamics depend upon three processes: recruitment of new juveniles (births), recruitment of adults (maturation), and behavior- and size-dependent mor-

Table 3.2: Model equations

Dynamical equations:

$$\frac{dR}{dt} = rR(t)\left(1 - \frac{R(t)}{K}\right) - v_R\left(f_A(t)L_A^2A(t) + f_J(t)L_J^2J(t)\right)$$

$$\frac{dJ}{dt} = R_J(t)A(t) - R_J(t-\tau(t))A(t-\tau(t))S(t)\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_J(t)J(t)$$

$$\frac{dA}{dt} = R_J(t-\tau(t))A(t-\tau(t))S(t)\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_A(t)A(t)$$

$$\frac{dP}{dt} = b\frac{\rho}{2}(\alpha_J^\eta(1-\tanh(\omega(L_J - L_{mid})))J(t) + (1-\tanh(\omega(L_A - L_{mid})))A(t))P(t) - \delta P(t)$$

Juvenile and adult functional responses:

$$f_J(t) = \frac{\alpha_J\xi R(t)}{1+\alpha_J\xi R(t)}$$

$$f_A(t) = \frac{\xi R(t)}{1+\xi R(t)}$$

Juvenile recruitment and growth rates:

$$R_J(t) = \hat{r}\left(f_A(t)L_A^2 - \frac{L_A^3}{L_{max}}\right)$$

$$g_J(t) = \hat{g}\left(f_J(t)L_{max} - L_J\right)$$

Integral equations for juvenile survivorship and stage duration:

$$S(t) = \exp\left(-\int_{t-\tau(t)}^{t}\mu_J(\hat{t})d\hat{t}\right)$$

$$L_A - L_b = \int_{t-\tau(t)}^{t} g_J(\hat{t})d\hat{t}$$

Juvenile and adult mortality rates:

$$\mu_J(t) = \frac{\mu}{f_J(t)L_J^2 - \frac{L_J^3}{L_{max}}} + \rho\alpha_J^\eta\frac{(1-\tanh(\omega(L_J-L_{mid})))}{2}P(t)J(t)$$

$$\mu_A(t) = \left(\frac{\mu}{f_A(t)L_A^2 - \frac{L_A^3}{L_{max}}} + \rho(1-\tanh(\omega(L_A - L_{mid})))P(t)\right)$$

tality. The overall dynamics of the juvenile class are described by

$$\frac{dJ}{dt} = R_J(t)A(t) - R_J(t-\tau(t))A(t-\tau(t))S(t)\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_J(t)J(t). \tag{3.4}$$

$R_J(t)$ is the juvenile recruitment rate (the birth of new juveniles):

$$R_J(t) = \hat{r}\left(f_A(t)L_A^2 - \frac{L_A^3}{L_{max}}\right). \tag{3.5}$$

The recruitment of new juveniles depends upon the maximum reproductive rate $\hat{r}$ and net production. Net production is given by subtracting maintenance costs (L_A^3/L_{max}) from net ingestion $(f_A(t)L_A^2)$. Maintenance costs are assumed to be proportional to volume (L_A^3) divided by the maximum length attainable under unlimited resource (L_{max}) (Kooijman and Metz, 1984; de Roos et al., 1990; Kooijman, 2000).

Individuals leave the juvenile class through maturation and death. Recruitment into the adult stage depends on juvenile recruitment at time $t-\tau(t)$, where $\tau(t)$ is the duration of the juvenile stage, juvenile survivorship $S(t)$, and juvenile growth rate at time t relative to growth rate at time $t-\tau(t)$ (Nisbet and Gurney, 1983). An intuitive feel for adult recruitment can be gained using an analogy with a conveyor belt due to G. F. Oster (Nisbet, 1997). Consider grains of sand piling onto a moving conveyor belt of fixed length. The grains of sand move along the conveyor belt, spilling over the sides, before dropping off the end of the belt. The grains of sand represent the juvenile population. The movement of the conveyor belt corresponds to growth of these juvenile individuals. The speed of this movement represents the juvenile growth rate, which is captured by the quantity $g_J(t)/g_J(t-\tau(t))$, where $g_J(t)$ gives the growth rate at time t. If $g_J(t) > g_J(t-\tau(t))$, juvenile growth rate has increased over the juvenile period; in our analogy, the conveyor belt is speeding

up, meaning that maturation rate is increasing. The length of the conveyor belt corresponds to the size at maturity. As the conveyor belt moves, grains spill off the sides, corresponding to the death of juveniles (survivorship). Let me unpack the processes of growth and survivorship a bit more.

Juveniles are assumed to grow according to the von Bertalanffy growth equation, which is a general model for size-dependent growth (Kooijman and Metz, 1984; Kooijman, 2000). This growth rate depends upon juvenile resource ingestion and maintenance costs - the derivation of the equation from first principles can be found in de Roos et al. (1990). The equation for juvenile growth rate is

$$g_J(t) = \hat{g}\left(f_J(t)L_{max} - L_J\right), \tag{3.6}$$

where $\hat{g}$ gives the maximum growth rate per unit size, $f_J(t)$ is the juvenile functional response, L_{max} is the maximum possible length, and L_J is juvenile length. The growth rate equation is then used to determine the length of the juvenile period, $\tau(t)$. The duration of the juvenile stage is the amount of time it takes to grow from length at birth L_b to length at maturity L_A. This is determined by the numerically solving the integral equation

$$L_A - L_b = \int_{t-\tau(t)}^{t} g_J(\hat{t})d\hat{t}. \tag{3.7}$$

Survivorship of juveniles is affected both by starvation and predation (that is, by resource and predator abundances). Because resource ingestion depends on both size and behavior, so too will starvation risk. Predation risk is also dependent on both size and behavior. Juvenile survivorship is given by the integral

$$S(t) = \exp\left(-\int_{t-\tau(t)}^{t} \mu_J(\hat{t})d\hat{t}\right), \text{ where} \tag{3.8}$$

$$\mu_J(t) = \frac{\mu}{f_J(t)L_J^2 - \frac{L_J^3}{L_{max}}} + \rho\alpha_J^{\eta}\frac{(1-\tanh(\omega(L_J - L_{mid})))}{2}P(t)J(t). \tag{3.9}$$

Here, $\mu_J(t)$ is the juvenile mortality rate. The first component of this function addresses resource-dependent mortality. As juvenile net production (resource ingestion ($f_J(t)L_J^2$) minus maintenance costs (L_J^3/L_{max})) approaches zero, the mortality rate increases rapidly from its baseline of μ, reflecting increased starvation mortality (de Roos and Persson, 2003; McCauley et al., 2008). The second component reflects predator-dependent mortality. The predator attack rate is given by ρ. This rate is scaled by both juvenile activity level α_J and juvenile length L_J. The relationship between juvenile activity level α_J and predation risk depends upon the exponent η, which can be interpreted as measure of predator foraging mode (Cressler et al., 2010). Figure 3.1A shows the relationship between activity level and predation risk for different values of the shape parameter η. The relationship between length and predation risk is specified by $(1 - \tanh(\omega(L_J - L_{mid})))/2$ (Rinke et al., 2008). (Division by two is necessary so that behavior- and size-dependent predation risk scale equally.) The shape of this function is varied by changing the parameters ω and L_{mid}. ω determines the slope of the size-risk relationship, characterizing the size limitation of predation. L_{mid} is the length where predation rate drops to half its maximum, capturing the predator's size preference. Figure 3.1B shows the relationship between length and predation risk for different values of L_{mid}. As is evident from Figure 3.1B, the predator is assumed to prefer small prey, but this function is general enough to capture many possible size-dependent predation shapes (Rinke et al., 2008).

Adult dynamics depend upon juvenile maturation rate $(R_J(t-\tau(t))A(t-\tau(t))S(t)g_J(t)/g_J(t-\tau(t)))$ and adult mortality $\mu_A(t)$. As with juveniles, adult mortality has both resource-

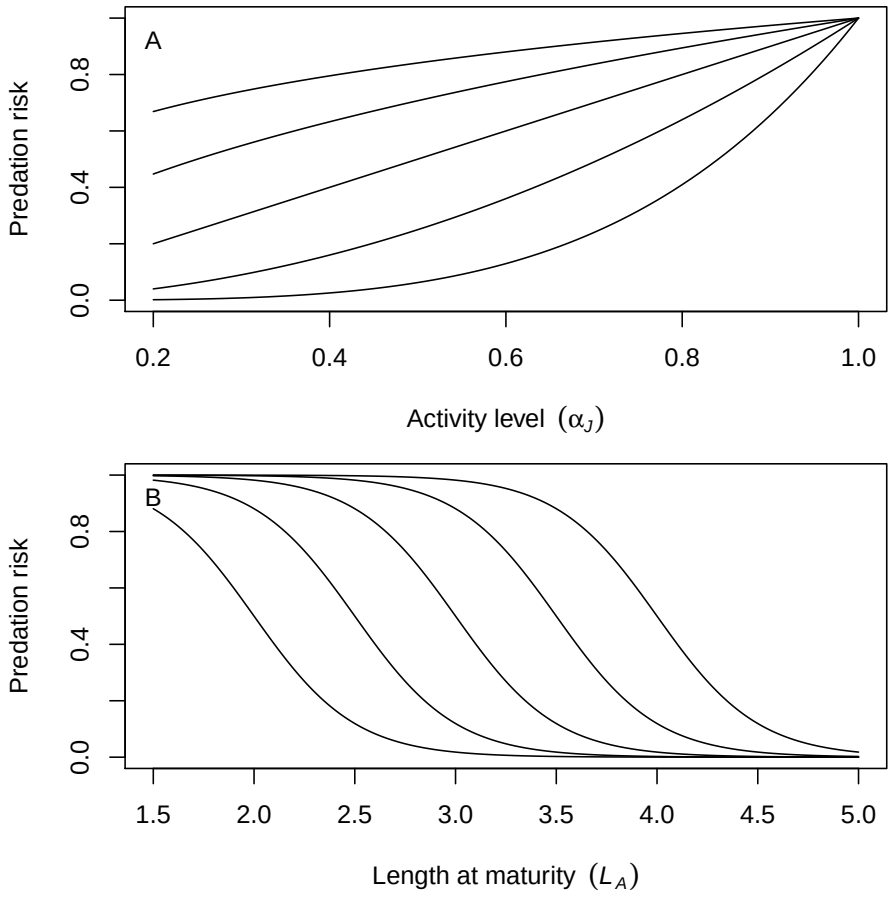

Figure 3.1: Behavior- and size-dependent predation risk. In panel A, the value of η is 0.25, 0.5, 1, 2, and 4, moving from the top line to the bottom. In panel B, $\omega = 2$ and L_{mid} varies from 2 to 4 in steps of 0.5.

and predator-dependent components. However, both resource- and predator-dependent mortality depends only on adult size, as behavior is assumed fixed. Adult dynamics are given by

$$\frac{dA}{dt} = R_J(t-\tau(t))A(t-\tau(t))S(t)\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_A(t)A(t), \text{where} \tag{3.10}$$

$$\mu_A(t) = \left(\frac{\mu}{f_A(t)L_A^2 - \frac{L_A^3}{L_{max}}} + \rho(1-\tanh(\omega(L_A - L_{mid})))P(t)\right). \tag{3.11}$$

The dynamics of the predator population are assumed to depend linearly on the predator's consumption of both juvenile and adult consumers, with b representing the predator birth rate. In the absence of consumers, predators are assumed to die off exponentially.

$$\frac{dP}{dt} = b\frac{\rho}{2}(\alpha_J^{\eta}(1-\tanh(\omega(L_J-L_{mid})))J(t)+(1-\tanh(\omega(L_A-L_{mid})))A(t))P(t)-\delta P(t). \tag{3.12}$$

In practice, it is very difficult to numerically solve the integrals for juvenile survivorship and stage duration. Appendix B gives a formulation of these equations as a system of differential equations (Nisbet and Gurney, 1983; Nisbet, 1997).

Ecological and physiological trade-offs

Since I am going to consider the evolution of juvenile activity level α_J and size at maturity L_A, it is useful to account for the potential costs and benefits of adjustment of either trait. These costs and benefits arise because of ecological and physiological processes.

Activity level α_J affects both juvenile foraging and juvenile predation risk. The shape of this trade-off between foraging gain and predation risk specified by the

shape parameter η (Figure 3.1A). Reducing α_J reduces both net ingestion and predation risk. By reducing net ingestion, reducing juvenile activity level will reduce juvenile growth, thereby delaying maturation. Delayed maturation has two costs: it reduces the reproductive lifespan and, since size-dependent predation risk is higher for juveniles (Figure 3.1B), it also lengthens the period of higher mortality. Thus, delayed maturation is the primary fitness cost of reduced activity level. Reduction in predation risk is the primary fitness benefit of reduced activity level. Previous work has shown that this benefit may offset the costs, leading to the evolution of reduced activity, but that this depends critically on the shape of the foraging–predation risk trade-off, η (Cressler et al., 2010).

Size at maturity L_A affects consumer ingestion rates, juvenile growth rate and stage duration, adult fecundity, and size-dependent predation risk. Increasing L_A (which also increases L_J) will increase juvenile and adult ingestion rates. However, because growth is modeled using the von Bertalanffy growth equation, increasing L_A lowers the juvenile growth rate. Increasing size at maturity therefore greatly increases age at maturity. How increasing L_A affects adult fecundity depends on the value of L_A, since maintenance costs scale with body volume (e.g., L_A^3) whereas ingestion scales only with surface area (e.g., L_A^2) (see Figure 3.2). Therefore, increasing size at maturity will carry the cost of increased age at maturity, and may also lower reproductive rate. The major benefit of increased size at maturity is reduced size-dependent predation risk, both for juveniles and adults. The relationship between length at maturity and predation risk is shown in Figure 3.1B. I will vary the fitness benefit of changing size at maturity by varying the predator's size preference, given by L_{mid}.

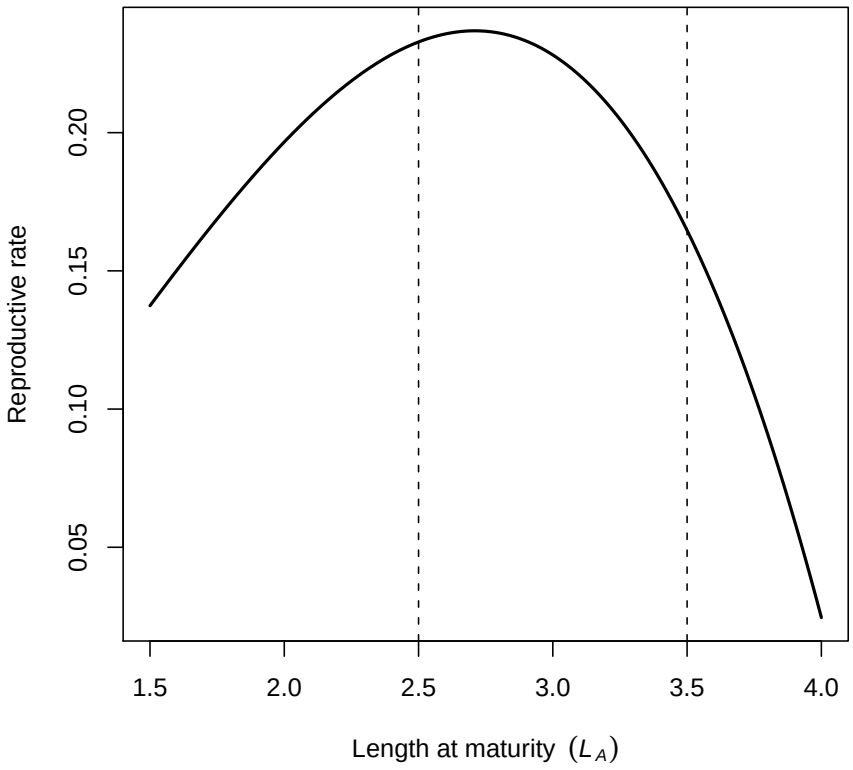

Figure 3.2: The reproductive rate of adults as a function of size at maturity. As size at maturity increases initially, reproductive rate increases. However, because maintenance rates are proportional to body volume, whereas foraging gain is proportional to surface area, at high size at maturity, reproductive rate declines. The dashed lines indicate the size range observed in this study. All parameters are assumed at their default values (Table 3.1).

Multidimensional adaptive dynamics in a nutshell

I use the framework of adaptive dynamics to determine the optimal juvenile activity level and size at maturity (Metz et al., 1992; Geritz et al., 1998). Adaptive dynamics considers trait evolution resulting from a sequential process of mutation and invasion. Its great strength, and one of the primary reasons it has become a useful tool in evolutionary biology, is its ability to model density- and frequency-dependent selection, revealing how disruptive selection is generated through feedback between ecological and evolutionary processes (Waxman and Gavrilets, 2005).

I imagine beginning with a monomorphic population (all individuals have identical juvenile activity level and size at maturity). I allow the populations to approach their limiting attractor; here I will limit the investigation to equilibrium attractors. For convenience, I will follow the notation of Geritz et al. (1998), and use E_x to denote the environment (resource and predator abundances) set by the "resident" population using strategy x, where the strategy is the two-dimensional vector of traits (juvenile activity level and size at maturity), $x = \alpha_J, L_A$. At equilibrium, by definition, the resident's population growth rate, denoted $r(x, E_x)$, will be 0. I then consider the fate of a new mutant "invader" with strategy $y = \alpha'_J, L'_A$. Note that the invader's strategy need differ in only one of the two traits. I assume that the mutant is rare and does not affect the environmental dynamics. This assumption greatly simplifies calculation of the mutant population growth rate. Let $s_x(y) = r(y, E_x)$ be the growth rate of the mutant in the environment set by the resident population; I will refer $s_x(y)$ as the invasion fitness. If $s_x(y) > 0$, then the invader's population can increase; if in addition $s_y(x) < 0$ (a population with strategy x cannot invade the environment set by a population with strategy y), then the invader displaces the resident. Assuming that mutations are small, then the invasion fitness can be approximated by

$$s_x(y) = s_x(x) + S(x)(y - x), \tag{3.13}$$

where $S(x)$ is the fitness gradient; in the multidimensional case, this has components

$$S_i(x) = \left[\frac{\partial s_x(y)}{\partial y_i}\right]_{y=x}, \tag{3.14}$$

where y_i is the ith component of the trait vector $y = \alpha'_J, L'_A$ (Leimar, 2005). Since, by definition, the population growth rate of the resident ($s_x(x)$) is zero, then the sign of the selection gradient determines what strategies can invade. If $S_i(x) > 0$, then only mutants with trait $y_i > x_i$ can invade, whereas the opposite is true for $S_i(x) < 0$.

Potential endpoints of evolution occur for strategies $\hat{x} = (\hat{\alpha_J}, \hat{L_A})$ that satisfy $S_i(\hat{x}) = 0$ for all i (that is, the selection gradient vanishes). Such strategies are termed evolutionarily singular strategies (Geritz et al., 1998). Whether such a strategy actually represents an endpoint of evolution depends on whether the strategy represents a fitness maximum or minimum, and whether the strategy can actually be approached by gradual evolution. In the one-dimensional case, these questions can be answered graphically using "pairwise invasion plots", which show the sign of the invasion fitness for mutants (Geritz et al., 1998). However, such plots are only practical for consideration of the evolution of single traits; even a two-dimensional trait vector would require a four-dimensional pairwise invasion plot! Fortunately, analytic classification of evolutionary singular points is possible. Whether a strategy is a fitness maximum or minimum can be straightforwardly addressed by considering the derivative of the selection gradient with respect to the mutant trait. For a single

evolving trait, this is simply

$$\left[\frac{\partial^2 s_x(y)}{\partial y^2}\right]_{y=x=\hat{x}}. \tag{3.15}$$

If this value is negative, the trait is at a fitness maximum, and often called an evolutionarily stable strategy (ESS). Conversely, if the value is positive, the strategy represents a fitness minimum, and may be a point where evolutionary branching can occur (because a resident population with this strategy can be invaded by invaders with trait values either larger or smaller than the resident). However, whether evolution will eventually lead the population to either an ESS or a branching point depends on whether these points can be approached by gradual evolution; that is, can a resident with strategy near, but not at, the singular strategy be invaded by mutants with strategies closer to the singular strategy? The answer to this question can be assessed by considering the second derivative of the invasion fitness with respect to the resident strategy (Eshel, 1983; Geritz et al., 1998):

$$\left[\frac{\partial^2 s_x(y)}{\partial x^2}\right]_{y=x=\hat{x}}. \tag{3.16}$$

If this quantity is positive, a population that starts near the singular strategy will evolve towards the singular strategy, and we call the singular strategy $\hat{x}$ a convergence stable strategy (CSS). If not, the strategy is a repeller, and a population near the singular strategy will evolve away from it. Thus, a singular strategy that is both an ESS and a CSS represents an endpoint of evolution. A singular strategy that is not an ESS, but is a CSS represents a branching point that can give rise to a polymorphic population. A singular strategy that is neither a CSS nor an ESS is an evolutionary repeller and the direction of evolution will be dictated by which side of

the repeller the trait is on initially. Singular strategies that are ESSs, but not CSSs, should be exceedingly rare in nature, as these strategies are evolutionarily stable but impossible to approach by gradual mutation. Further classification of singular points is possible in the one-dimensional case (Geritz et al., 1998), but this is not possible or necessary for the following analysis.

Similar analyses of evolutionary and convergence stability can be extended to the multidimensional case (Leimar, 2005, 2009). The multidimensional equivalent to the ESS criterion is given by the Hessian matrix of invasion fitness (Leimar, 2005):

$$\mathbf{H}_{jk} = \left[\frac{\partial^2 s_x(y)}{\partial y_j \partial y_k}\right]_{y=x=\hat{x}}, \tag{3.17}$$

where $\hat{x} = \hat{\alpha_J}, \hat{L_A}$ represents a singular strategy (both components of $S(x)$ have vanished). For $\hat{x}$ to be an ESS, the Hessian matrix must be negative definite (both eigenvalues are negative). Positive definite or indefinite Hessians imply there is evolutionary instability in at least one direction in trait space.

Convergence stability is more challenging to address (Leimar, 2009). The multidimensional equivalent to the CSS criterion is given by the Jacobian matrix of the selection gradient:

$$\mathbf{J}_{jk} = \left[\frac{\partial S_j(x)}{\partial x_k}\right]_x = \hat{x} = \left[\frac{\partial^2 s_x(y)}{\partial y_j \partial y_k}\right]_{y=x=\hat{x}} + \left[\frac{\partial^2 s_x(y)}{\partial y_j \partial x_k}\right]_{y=x=\hat{x}}. \tag{3.18}$$

In full generality, whether or not the singular strategy $\hat{x}$ will be a CSS will depend upon the nature of genetic correlations between the two traits, given by the two-dimensional matrix $\mathbf{G}$ (Leimar, 2009). However, in this study, I do not assume any particular covariance structure, so $\mathbf{G}$ is just the two-by-two identity matrix. Leimar (2009) showed then the strategy $\hat{x}$ is a CSS if the Jacobian matrix is negative definite.

Using these results, I can classify singular strategies for the multidimensional case. How then do I calculate the invasion fitness for my model?

Calculating invasion fitness

For the calculation of the mutant's population growth rate, I can ignore the equations for resource and predator dynamics, as these are fixed at equilibria R^* and P^* determined by the resident population. Analytical calculation of these equilibria is not possible for this system of equations because the resource equilibrium involves solving a transcendental equation. This also complicates any attempt at stability analysis. For this study, I calculated stability boundaries numerically, and verified that analytical results and numerical results were in agreement.

Since $R(t)$ and $P(t)$ are fixed at equilibria R^* and P^*, this greatly simplifies the dynamics for a mutant population of juveniles and adults, $J'(t)$ and $A'(t)$:

$$\frac{dJ'(t)}{dt} = R_J^* A'(t) - R_J^* A'(t-\tau^*)S^* - \mu_J^* J'(t) \tag{3.19}$$

$$\frac{dA'(t)}{dt} = R_J^* A'(t-\tau^*) - \mu_A^* A'(t) \tag{3.20}$$

R_J^* gives the juvenile recruitment rate at equilibrium. The juvenile and adult mortality rates at equilibrium are given by μ_J^* and μ_A^*, respectively. The juvenile survivorship S^* and stage duration τ^* at equilibrium can be calculated from integration of equations (4) and (7), respectively. To determine the population growth rate of the mutant (equivalently, the invasion fitness, $s = s_x(y)$), consider exponential trial solutions $J_0 \exp(st)$ and $A_0 \exp(st)$. Substituting these trial solutions into the above equations yields

$$s = R_J^*(1 - S^* \exp(-s\tau^*))\frac{A_0}{J_0} - \mu_J^* \tag{3.21}$$

$$s = R_J^* S^* \exp(-s\tau^*) - \mu_A^*. \tag{3.22}$$

Equation (3.22) can be used to determine the invasion fitness. This transcendental equation has a single real root that can be found numerically. Equation (3.21) can be used to determine the relative abundances of juveniles and adults that give rise to this growth rate. Here, I am only concerned with the sign of the invasion fitness s, and so I focus on equation (3.22). In practice, rather than using the invasion fitness equations directly, I instead found singular strategies by searching for strategies $\hat{\alpha_J}, \hat{L_A}$ where the selection gradient vanished. An explicit equation for the selection gradient with respect to each trait was determined by implicitly differentiating equation (3.22) with respect to juvenile activity level and size at maturity. Note that the selection gradient equations will involve the invasion fitness s, but since this gradient is only evaluated when resident and mutation strategies are identical ($y = x = \hat{x}$), the invasion fitness is by definition zero. Singular strategies for a given parameter set were found using Newton's method.

I am interested in how variation in the shape and magnitude of various trade-offs affects the optimal behavior and life history, as these shapes have been shown in previous studies to have important consequences for the interaction between behavior and life history. As such, I vary the shape of the foraging–predation risk trade-off by varying the value of the shape exponent η. I also vary the size–predation risk trade-off by varying the size selectivity of the predator, L_{mid}. To illustrate how consideration of multidimensional trait evolution can qualitatively alter predictions based only on single trait evolution, I perform both multidimensional and single trait adaptive dynamics for both sets of experiments. That is, for every value of η and

L_{mid}, I find singular strategies for size and behavior simultaneously, as well as for size and behavior independently. When analyzing size at maturity alone, juvenile activity level is assumed fixed at one. This acts to remove the influence of behavior on the dynamics. When analyzing juvenile activity level alone, size at maturity is fixed at 2.8. This value was chosen because it represents a moderate size at maturity; other values were explored and did not qualitatively affect the results.

Results

As the size preference of the predator is varied, there is considerable variation in both size at maturity and juvenile activity level. The predicted size at maturity for both single- and multidimensional adaptive dynamics are strikingly similar. However, the predicted juvenile activity levels are very different between the two.

First, consider the patterns in size and behavior exhibited in the results of the multidimensional optimization. Size at maturity for $L_{mid} = 2$ is very high; the optimal strategy clearly makes use of the size refuge from predation. As L_{mid} increases, there is first a slight drop in size at maturity due to a reduction in activity level, and then an increase in both size at maturity and activity level as L_{mid} climbs. At approximately $L_{mid} = 3.1$, a second singular strategy appears at an evolutionary branching point (that is, it is convergence stable, but not evolutionarily stable). As L_{mid} is increased, this branching point strategy disappears, and there exist three distinct strategies, two of which are CSS and ESS, and an intermediate which is an evolutionary repeller. The two CSS and ESS strategies correspond to a strategy that matures at large size, with relatively high activity level, and a strategy that matures at small size, with low activity level. The emergence of this second, small strategy has to do with development times. The primary cost of growth to large size is the extended juvenile period. For intermediate L_{mid} values, either a fast-developing, small-bodied strategy or a slow-developing, large-bodied strategy may be optimal.

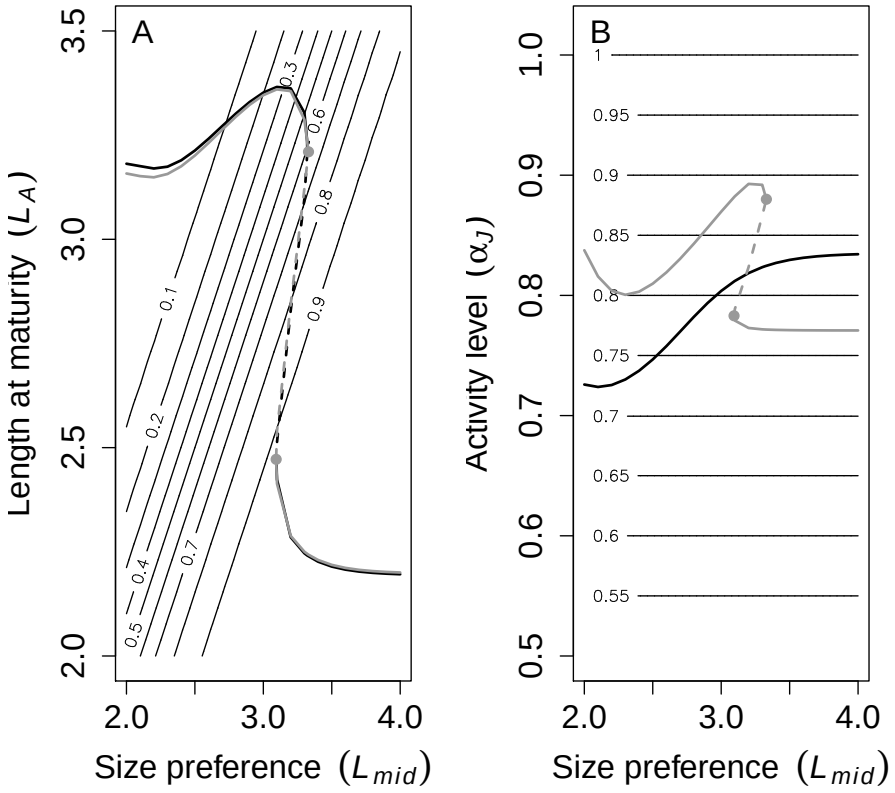

Figure 3.3: Results varying the shape of the size–predation risk trade-off (Figure 3.1). Panel A shows the singular sizes at maturity (L_A) for the both size-only (black lines) and multidimensional adaptive dynamics (gray lines). For the size-only case, activity level is held constant at one. Panel B shows the singular juvenile activity (α_J) levels for both behavior-only (black lines) and multidimensional adaptive dynamics (gray lines). For the behavior-only case, size at maturity is held constant at 2.8. The solid lines depict singular strategies that are both evolutionarily and convergence stable. The dashed lines depict strategies which are evolutionary repellers (neither convergence or evolutionarily stable). The gray points show evolutionary branching points (CSS, but not ESS). The contours in each plot show the size- and activity-dependent predation risk for each strategy. The shape of the behavior–predation risk trade-off is held constant at $\eta = 1$.

Similar polymorphism has been observed in other systems that have studied the evolution of size at maturity only (Day et al., 2002; Gårdmark and Dieckmann, 2006). As predator size preference L_{mid} is increased still further, the evolutionary repeller converges on the large-bodied strategy at an evolutionary branching point. For very high values of L_{mid}, the fitness cost of delayed maturation is too high, and the only evolutionary optimum is a small-bodied strategy with low juvenile activity and rapid development.

The results for size at maturity from the single-trait optimization are both qualitatively and quantitatively similar to the multidimensional case. This indicates that it is the loss of evolutionary stability in size that gives rise to the multidimensional branching point at $L_{mid} = 3.1$, which in turn produces the branching point in juvenile activity level. In the absence of evolution of size at maturity, there is no branching point observed in juvenile activity level. Instead, only a single optimal activity level is predicted for any value of L_{mid}. Juvenile activity level is predicted to increase as L_{mid} increases to allow the population to reach size at maturity more quickly.

To see how variation in the shape of the foraging–predation risk trade-off could alter the results shown in Figure 3.3, I then held L_{mid} constant at a value that produces multiple evolutionary endpoints and varied the value of η. Here, I see large differences between the multidimensional optimization and both single trait optimization.

In the multidimensional adaptive dynamics results, for very low values of η, there is no benefit to reducing activity level, so all strategies remain fully active. This means that the results for the multidimensional and size-only optimizations are identical. A pairwise invasion plot can be used to visualize the results for this case. Figure 3.5 shows this plot for $\eta = 0.5$. The small- and large-bodied strategies are both convergence and evolutionarily stable. Convergence stability can be see by the fact that residents who are near, but not at, the singular strategy can be invaded by mutants

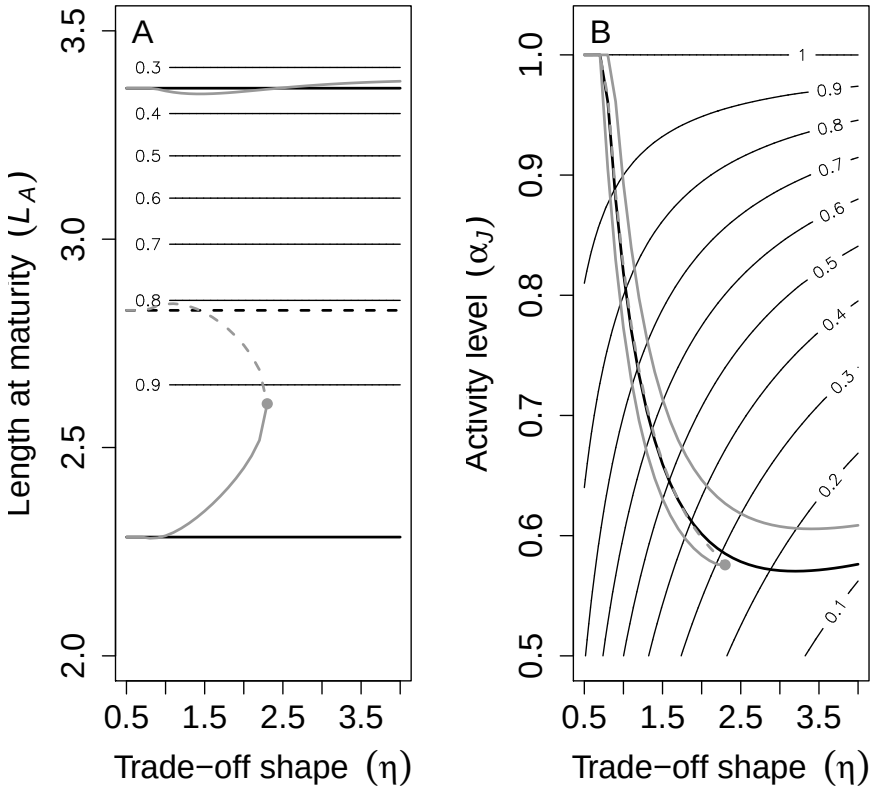

Figure 3.4: Results varying the shape of the foraging–predation risk trade-off (Figure 3.1). See the caption of Figure 3.3 for details. Here, predator size preference and attack rate were held constant at $L_{mid} = 3.2$ and $\rho = 4$.

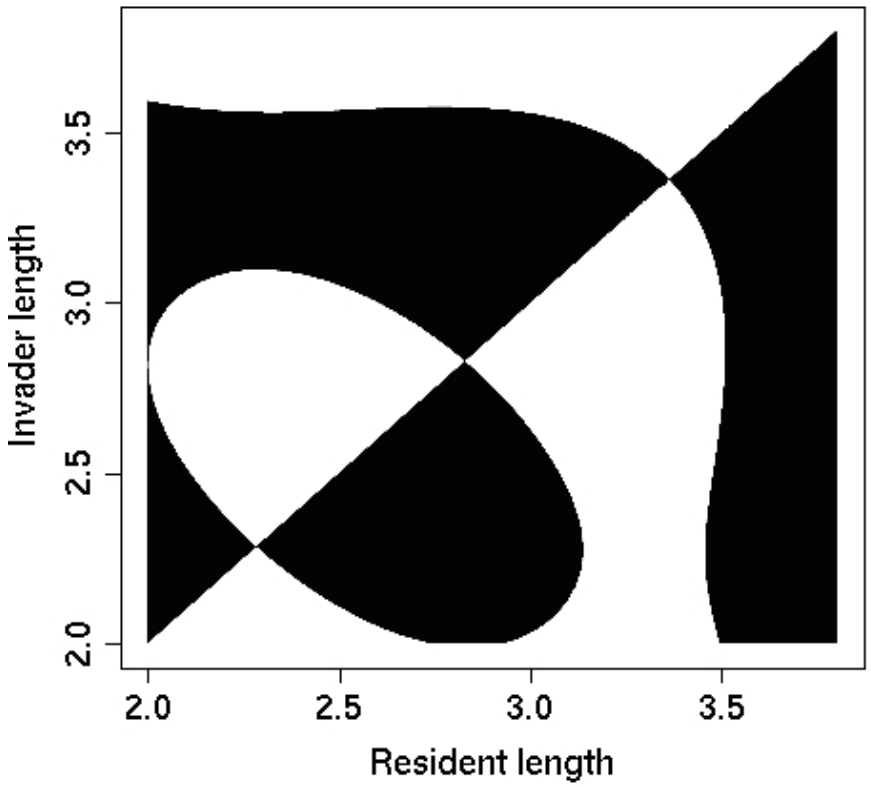

Figure 3.5: Pairwise invasion plot for $\eta = 0.5$. For this value of η, all strategies adopt a constant activity level, so it is possible to perform a standard pairwise invasibility experiment to determine singular strategies and their stability. The small- and large-bodied strategies are both evolutionarily and convergence stable. This can be see by the fact that mutants with strategies closer to these singular strategies can invade, but the singular strategy itself is uninvadable. The intermediate strategy is an evolutionary repeller: it cannot be approached in small mutational steps, and can be invaded by strategies both smaller and larger than itself.

whose strategy is closer to the singular strategy. Evolutionary stability can be seen by the fact that, once the population reaches the singular strategy, it cannot be invaded. The intermediate-sized strategy is an evolutionary repeller. It is not convergence stable because mutants with strategies closer to the singular point cannot invade residents further from the strategy. It is not evolutionarily stable because the singular strategy can be invaded by mutants with both smaller- and larger-bodied strategies.

As η is increased initially, we see that activity level drops very quickly, because the fitness benefit of reduced activity level increases. However, as η continues to increase, there is less need for as severe a reduction in activity level. This can be seen in Figure 3.4 by moving from high to low values of η with activity level fixed; note that the predation risk contours spread out, showing that the benefit of reducing activity increases, but at a decreasing rate. For example, with an activity level of 0.6, activity-dependent predation risk drops by 30% as η is increased from 0.5 to 1.5. However, from $\eta = 2.5$ to $\eta = 3.5$, there is only a 10% drop in predation risk. Because of these diminishing returns, I see a loss of one evolutionary equilibrium as η is increased.

These results differ sharply from those of the results of adaptive dynamics on either size at maturity or juvenile activity alone. In particular, size-only adaptive dynamics predicts the coexistence of two size strategies across the entire range of η values, as modifying the shape of the foraging gain–predation risk trade-off has no affect when activity level α_J is fixed at one.

Discussion

Here I consider the evolution of two phenotypic traits, behavior and life history, that are integrated through their joint influence on physiological processes and ecological interaction, and show that variation in the shapes of the underlying ecological

trade-offs can strongly impact selection. In particular, I show that selection acting on multiple traits simultaneously can both quantitatively and qualitatively alter the predicted outcomes of selection acting on single traits. In particular, multidimensional selection can generate phenotypic polymorphism where a single optimum would be predicted by single trait evolution. Conversely, multidimensional selection can lead to a global optimum where polymorphism would be predicted by single trait evolution. These results echo and reinforce the message of recent work showing the importance of consideration of multiple traits and multiple trade-offs in the evolution of behavior and life history (Steiner and Pfeiffer, 2007; Cressler et al., 2010; Doebeli and Ispolatov, 2010). Further, these results have important implications for the study of the conditions under which disruptive selection can lead to phenotypic polymorphism.

Emergence and maintenance of polymorphism

Early studies examining the effect of size-dependent predation on life history evolution suggested that predation would have straightforward effects on size and age at maturity (Taylor and Gabriel, 1992; Ernande et al., 2004). In particular, increased mortality for small individuals was thought to select for increased growth at the cost of delayed maturation, leading to large size and age at maturity (Taylor and Gabriel, 1992). However, a later study by Gårdmark and Dieckmann (2006) showed that predation on small individuals could lead to (1) maturation at large size, (2) maturation at small size, or (3) bistability, depending on the predation rates on small individuals. This result is very similar to the result I observe as predator size preference is varied (compare Figure 2 of Gårdmark and Dieckmann (2006) with my Figure 3.3). However, Gårdmark and Dieckmann (2006) focused only on evolutionary change in size at maturity, without considering that size at maturity is a complex trait, affected by many physiological processes (Ball and Baker, 1996;

Noonburg and Nisbet, 2005; Beckerman et al., 2007). In particular, the effects of behavioral response on the part of the prey was not considered.

Assuming that activity level α_J is fixed at one, as is done in most studies of the evolution of size at maturity, amounts to taking slices through the multidimensional trait space. In some cases, this slice will give a true picture of the evolutionary scene. For example, the results for size at maturity in both the size-only and multidimensional cases were very similar as L_{mid} are very similar (Figure 3.3A). Additionally, the results are identical when the shape of the trade-off constraining behavioral change (the trade-off between foraging gain and predation risk) is concave down ($\eta < 1$ in this study; see Figures 3.4 and 3.5).

However, as the shape of the trade-off becomes increasingly nonlinear and concave up, ignoring behavior leads to misleading predictions. In particular, for highly nonlinear trade-offs, selection on behavior causes the loss of evolutionary bistability and the existence of only a single optimal strategy (Figure 3.4A). This result is highly non-intuitive, as changing the shape of the foraging–predation risk trade-off would seem to have no effect on size at maturity. However, as η increases, the benefit of reduced activity begins to outweigh the benefit of large size. This result shows how selection acting on multiple traits can lead to interaction between trade-offs (Cressler et al., 2010).

Studies that examine only behavioral change in response to predation risk without considering how behavior affects life history may be equally misleading. In particular, evolutionary branching points or bistability are not possible in this model if size at maturity is held fixed (Figure 3.3B and 3.4B). When selection acts on both size and behavior simultaneously, loss of evolutionary stability in size causes the loss of stability in behavior as well.

A previous study by Cressler et al. (2010) also showed that the optimal behavior and life history of prey under size-dependent predation depended very strongly

on the shape of the trade-off between foraging and predation risk (Lima and Dill, 1990; Werner and Anholt, 1993). This study assumed constant resource and predator densities, and predicted a single optimal strategy for any trade-off shape (η in the current study). In particular, when the trade-off was linear, the optimal strategy was to increase size at maturity, holding behavior constant. As the trade-off became increasingly nonlinear and concave up, the optimal strategy became to mature small, with very low activity level. Here, I see evolutionary bistability across a range of foraging–predation risk trade-off shapes. This bistability only occurs if both resources and predators are dynamic (Day et al., 2002). I do see, however, a similar pattern of no reduction in activity level for low values of the shape parameter η, similar to Cressler et al. (2010). The differing predictions at high values of the shape parameter (that is, highly nonlinear and concave up) are due to the inclusion of resource dynamics. If resources are constant, any reduction in activity level necessarily reduces individual growth rate, potentially creating selection pressure against maturation at large size. With dynamic resources, however, a reduction in activity level increases resource abundance, and this increase can offset the reduction in individual growth rate, allowing for large maturation and reduced activity simultaneously.

Evidence from nature

The results of this study find support in patterns of covariation in size and behavior found in nature. Furthermore, there are several possible empirical tests of the theory's predictions that follow naturally from the theoretical explorations above.

The most notable result above is the change in behavior and especially life history that occurs as the predator's prey size preference is increased. The pattern shown in Figure 3.3 suggests that predators that are less gape-constrained (higher L_{mid}) should select for small maturation size and low foraging activity, whereas highly gape-constrained predators (low L_{mid}) should select for large maturation and high

activity levels. This prediction was confirmed across a naturally-occurring gradient in gape-constrained/gape-unconstrained predation risk (Urban, 2007b). Prey individuals found in habitats with high levels of gape-constrained predation risk were found to mature at larger size, with higher rates of foraging, than prey found in habitats with high levels of gape-unconstrained predation (Urban, 2007a). Indirect evidence for this prediction can be found in systems where species distribution and life history are highly correlated with occurrence of predators. For example, large-bodied zooplankton species, such as *Daphnia magna* and *D. pulex*, are seldom found in lakes with high fish predation, whereas small-bodied zooplankton species, such as *D. galeata*, *D. cucullata*, and *D. hyalina*, are seldom found in lakes lacking fish (Ebert, 2005).

The results shown in Figure 3.3 also provide a explanation for the evolution of predator-induced phenotypic plasticity. Many predators experience significant growth in size during a growing season, and this growth changes their prey size preference (Kolar and Wahl, 1998; Urban, 2007a; Anto et al., 2009). Therefore, for prey species who undergo multiple generations during a single growing season, there is significant temporal heterogeneity in size-specific predation risk. Such temporal heterogeneity could promote selection for phenotypic plasticity (Moran, 1992). An interesting test of this prediction would be to compare the plastic responses of prey taken at different points in the growing season to the same predator cue. For example, the size preference of juvenile fish changes considerably over the first few months of life, changing prey preference (Kolar and Wahl, 1998). Species of *Daphnia* are known to possess considerable predator-induced phenotypic plasticity in both life history and behavior (De Meester et al., 1995; Tollrian and Dodson, 1999). This theory would then predict that an optimal response to predation for individuals born early in the season would be mature at large size, taking advantage of the size refuge, whereas individuals born late in the season would mature at small size. To my knowledge,

there have not been any studies that have compared plastic responses to predators across predator ontogeny.

Conclusion

There is great interest in evolutionary biology regarding the emergence of evolutionary emergence of diversity (Kisdi and Geritz, 2010). The standard view of evolutionary biology is that phenotypic and species diversity arises primarily through geographic separation of populations and subsequent adaptation to differing ecological conditions. In addition to the biogeographical evidence for this hypothesis, its predominance is due in some part to the view of natural selection as an optimizing process, generating a single, most-fit phenotype for any environment. This view received support from population genetics and life history theory (Roff, 1992; Day and Taylor, 1996; Dieckmann et al., 2004). However, in recent years, there has been a growing interest in the conditions under disruptive selection might be generated.

Adaptive dynamics has highlighted the importance of ecological interactions, and the feedback between ecological and evolutionary processes, in generating disruptive selection. A growing adaptive dynamics literature has demonstrated that disruptive selection is a common outcome in a wide variety of ecological interactions, including competition, mutualism, exploitation, and parasitism (Doebeli and Dieckmann, 2000; Hoyle et al., 2008; Kisdi and Geritz, 2010). However, this body of work has almost exclusively considered the evolution of single traits in isolation (Leimar, 2005, 2009; Kisdi and Geritz, 2010), despite the reality that phenotypic space is very high-dimensional and interactions between traits are the rule, rather than the exception (Pigliucci, 2003). Recent work has shown that consideration of multidimensional phenotypes can greatly increase the potential for adaptive diversification (Doebeli and Ispolatov, 2010). The important message of this study is that the study of single traits in isolation may lead to misleading predictions. Theory on the evolution of

organismal phenotypes should consider that traits are often affected by multiple processes, each constrained by its own physiological and environmental trade-offs. The interaction between these trade-offs has important consequences for the evolution of phenotypic integration and diversity (Pigliucci, 2003; Steiner and Pfeiffer, 2007; Cressler et al., 2010; Doebeli and Ispolatov, 2010).

CHAPTER IV

A comparative study of phenotypic evolution in amphibian tadpoles

Introduction

Many species are capable of modifying their phenotype in order to reduce the risk of predation (Agrawal, 2001; Miner et al., 2005). Such phenotypic plasticity, known as inducible defense, is now a widely studied phenomenon, both experimentally and theoretically. Studies have laid out four theoretical prerequisites for the evolution of inducible defense (Harvell, 1990; Clark and Harvell, 1992; DeWitt et al., 1998). First, predation risk must be variable (either in space or time) and must occasionally be low. If predation risk is not variable, or is always high, the expectation is that selection will favor constitutive defenses. Second, there must be reliable cues of predation risk. These cues provide the information necessary to adaptively modify phenotype (Getty, 1996). Absence of reliable cues may lead to the evolution of bet hedging strategies, but not plasticity (Slatkin, 1974; Beaumont et al., 2009). Third, the defense must have a benefit, typically reduced predation due to an effective defense. Finally, defenses should carry a fitness cost, either due to changes in energy allocation or other trade-offs (DeWitt et al., 1998). Such costs favor the evolution of inducibility by allowing organisms to "save" the cost when the defense is not

required.

These prerequisites have all been studied extensively (see Tollrian and Harvell (1999) for examples). It is possible, based on these studies, to make a number of additional predictions regarding the evolution of inducible defense. First, if the evolution of phenotypic plasticity requires environmental variability, then organisms that live in more variable environments may be capable of greater expressions of plasticity (Bradshaw, 1965; Moran, 1992). Second, if different predators select for different defensive strategies, then organisms who are primarily exposed to only one type of predator should have plasticity only in the traits that most mediate the risk from that predator (Cressler et al., 2010). A logical extension of this is that species found in multipredator environments should have more, or different, plasticity than individuals exposed to single predator environments (Relyea, 2003a). Finally, other factors, such as temporal constraints, may affect the evolution of plasticity so that individuals living in different environments may have different adaptive responses to the same predator cue (Rowe and Ludwig, 1991).

Many of these predictions have been tested among populations of a single species (Boersma et al., 1998; Tollrian and Harvell, 1999; Benard, 2006; Boeing et al., 2006a; Kishida et al., 2007; Urban, 2007b), but have seldom been tested at higher levels of biological organization (Richardson, 2001b; Richter-Boix et al., 2007; Van Buskirk, 2002). Here, I test these predictions through a comparative analysis of predator-induced plasticity in behavior and morphology in anuran tadpoles.

Previous studies have shown that anuran tadpoles are capable of dramatic changes in behavior and morphology in response to predation risk (Skelly and Werner, 1990; McCollum and VanBuskirk, 1996; Relyea, 2001, 2003a, 2004a). Indirect evidence that these changes are adaptive comes from the result that individuals expressing these changes have higher survivorship or growth rates than individuals that do not respond (McCollum and VanBuskirk, 1996). However, different anurans inhabit very different

environments in nature (Van Buskirk, 2005; Werner et al., 2007a). This includes variability in the magnitude of predation risk (e.g., biomass or density of predators), in the identity of predators (e.g., some anuran species are never found with fish), and in pond characteristics (e.g., hydroperiod and canopy cover). Therefore, it is likely that species will have different expressions of phenotypic plasticity based on this variability.

In this study, I test whether differences in morphology and behavior among species of amphibian tadpoles inhabiting different environments are adaptive. In particular, I perform two related phylogenetic analyses. In the first analysis, I test for correlated evolution between morphological and behavioral traits and habitat characteristics using phylogenetic regression (Garland et al., 1992; Garland and Ives, 2000). Measurements of morphological and behavioral traits and the plasticities in these traits come from a previous comparative analysis of tadpole morphology and plasticity in response to predators (Van Buskirk, 2002). Pond characteristics come from long-term surveys of amphibian abundance and distribution (Van Buskirk, 2005; Werner et al., 2007a). Phylogenetic regression uses information on the relatedness of species to test whether correlated evolution has occurred between habitat characteristics and species traits. This analysis revealed a number of strong correlations, suggesting that variation in habitats leads to predictable, adaptive variation in morphology and behavior in amphibian tadpoles.

In the second analysis, I test for the signal of adaptation in amphibian traits in response to different environments directly, using recently developed tools for phylogenetic comparative hypothesis testing (Butler and King, 2004). Using the characteristics of ponds inhabited by each amphibian species, I classify species as primarily inhabiting distinct types of ponds (Wellborn et al., 1996; Richardson, 2001b,a), and test whether species in different types of ponds have different morphology, behavior, and plasticity. This analysis revealed that body size and the behavioral response

to predators are evolving towards different optima in different environments. Morphological traits appear to be highly correlated to one another, but not particular environmental factors, while morphological response to predation risk appears to be under strong stabilizing selection. These two analyses yield highly complementary results, confirming and extending previous findings in the amphibian system (Van Buskirk, 2002; Richter-Boix et al., 2007; Van Buskirk, 2009), and suggesting novel explanations for patterns of species distributions.

Methods

Behavior and morphological data

The phenotypic data for this study consist of measurements of behavior and morphology of 17 different anuran tadpoles under two experimental treatments: the presence or absence of predation cues from dragonfly larvae (Odonata: Aeshnidae). The study species span four different amphibian families across two continents (Table 4.1). The measurements of behavior and morphology were collected from a number of different experiments, carried out between 1992 and 2000, as described in (Van Buskirk, 2002). For each species, experiments were conducted in outdoor mesocosms, following a protocol designed to establish seminatural pond ecosystems within the tanks (Wilbur, 1997; Van Buskirk, 2002).

Behavior was measured by observing tadpoles and recording the activity of focal individuals. Activity was defined as the proportion of time spent feeding and swimming, as opposed to hiding from predators. Reduced activity is the most common behavioral defense against predation by dragonfly larvae, which are sit-and-wait predators. Data was available for all species except *R. pipiens* and *H. arborea*.

Morphological measurements were made by collecting individual tadpoles from each treatment and measuring five traits: maximum length and depth of the head,

Table 4.1: Study species with family name and location where each species was sampled. Abbreviations given in parentheses are used throughout to simplify presentation of results.

Species name	Family	Location
Bufo bufo (bbuf)	Bufonidae	Switzerland
B. calamita (bcal)	Bufonidae	Switzerland
Alytes obstetricans (aobs)	Discoglossidae	Switzerland
Bombina variegata (bvar)	Discoglossidae	Switzerland
Hyla arborea (harb)	Hylidae	Switzerland
H. chrysocelis (hchr)	Hylidae	Michigan, U.S.A.
H. versicolor (hver)	Hylidae	Michigan, U.S.A.
Pseudacris crucifer (pcru)	Hylidae	Michigan, U.S.A.
P. triseriata (ptri)	Hylidae	Michigan, U.S.A.
Rana dalmatina (rdal)	Ranidae	Switzerland
R. esculenta (resc)	Ranidae	Switzerland
R. latastei (rlat)	Ranidae	Switzerland
R. lessonae (rles)	Ranidae	Switzerland
R. pipiens (rpip)	Ranidae	Michigan, U.S.A.
R. ridibunda (rrid)	Ranidae	Switzerland
R. sylvatica (rsyl)	Ranidae	Michigan, U.S.A.

length and maximum depth of the tail fin, and total length. This was done for both predator and no-predator treatments.

Since mass was not measured, body size was quantified using principal components analysis of the five measured traits. The first component of this PCA explained 93% of the variance, and weights all measurements approximately equally (coefficients between 0.44 and 0.46; see Van Buskirk (2002) for more detail). Because morphological traits will be highly correlated with body size, size-corrected morphology was determined by regressing the individual head and tail measurements against body size (PC-1) and taking the residuals as size-corrected tail and head measurements.

Phenotypic plasticity was quantified as the change in activity or size-corrected morphology between predator and no-predator treatments divided by the trait value in the no-predator treatment. Therefore, positive values indicate an increase in the value of the trait in response to predators, whereas negative values indicate a

decrease. Since the plasticity measurements are dimensionless, the magnitude of plasticity can be easily quantified.

In addition to quantifying plasticities, I also calculated mean trait values across predator and no-predator treatments. Since the experimental conditions are likely not completely representative of natural conditions, I used the mean value in an attempt to capture information from both treatments simultaneously. Hereafter, I will refer to "means" and "plasticities" when discussing results pertaining to responses observed in mean trait values and trait plasticities, respectively.

Phylogeny

An essential component of this study is an accurate phylogeny for the species. Because these species are sampled from a broad range of amphibian families (Bufonidae, Discoglossidae, Hylidae, and Ranidae), there are no existing phylogenies that could be used. However, a number of detailed phylogenies are available for bufonid (Stock et al., 2008; Van Bocxlaer et al., 2009), hylid (Smith et al., 2007; Hua et al., 2009), and ranid (Wiens et al., 2009) frogs, as well as for the entire amphibian tree of life (Frost et al., 2006). These published phylogenies were used to verify the topology and dating of internal nodes of the phylogeny generated here.

To create the phylogeny for these species, I used sequences found on GenBank for three mitochondrial genes: ribosomal small subunit (12S) and large subunit (16S) and cytochrome *b*. GenBank accession numbers for both ingroup and outgroup species can be found in Appendix C. Sequences for each gene were independently aligned using MUSCLE (Edgar, 2004). Alignments were checked by eye using ClustalX (Thompson et al., 1994).

The full phylogeny was constructed using the maximum likelihood approach implemented in RAxML 6.0.0 (Stamatakis, 2006). This uses the GTR (general time reversible) model. I allowed for variation in the rates of evolution of different genes.

Furthermore, the data supported partitioning the cytochrome *b* gene into three sets of characters based on codon positions (allowing each position of the codons to evolve at a different rate). To find the overall best tree, I performed 100 independent RAxML searches. Individual branch support was quantified from 500 nonparametric bootstraps of the original alignment, mapped onto the most likely tree.

The resulting phylogeny was then converted into an ultrametric tree using the program PATHd8 (Britton et al., 2007). This required specifying an absolute date for at least some of the internal nodes of the tree. The date of divergence between the two hylid clades (species in the genus *Pseudacris* and *Hyla*) was estimated at 45.7 million years ago (Smith et al., 2007). The date of divergence between *Rana temporaria* and *R. lessonae* was estimated at 46.6 mya (Wiens et al., 2009).

The phylogeny used in this study can be seen in Figure 4.1. The topology of this phylogeny agrees exactly with that of published phylogenies. Furthermore, the dates of internal nodes (other than the two specified above) agree with those of published studies. This agreement suggests that this phylogeny is reasonable for this group of species.

Habitat data

For the phylogenetic analyses, I characterized the ponds each species inhabits using long-term survey data on pond characteristics. For the Michigan species, this data came from a yearly survey of 37 ponds on the University of Michigan's E.S. George Reserve in Southeast Michigan that has been carried out every year from 1997 to 2009 (see Werner et al. (2007a,b) for more details). For the European species, the data came from a yearly survey of 78 ponds in central Switzerland carried out from 1997 to 2003 (not every pond was sampled in every year; see Van Buskirk (2005, 2009) for more details.) For each species, I characterized habitat in terms of three broad characteristics that have been shown in previous studies to be important

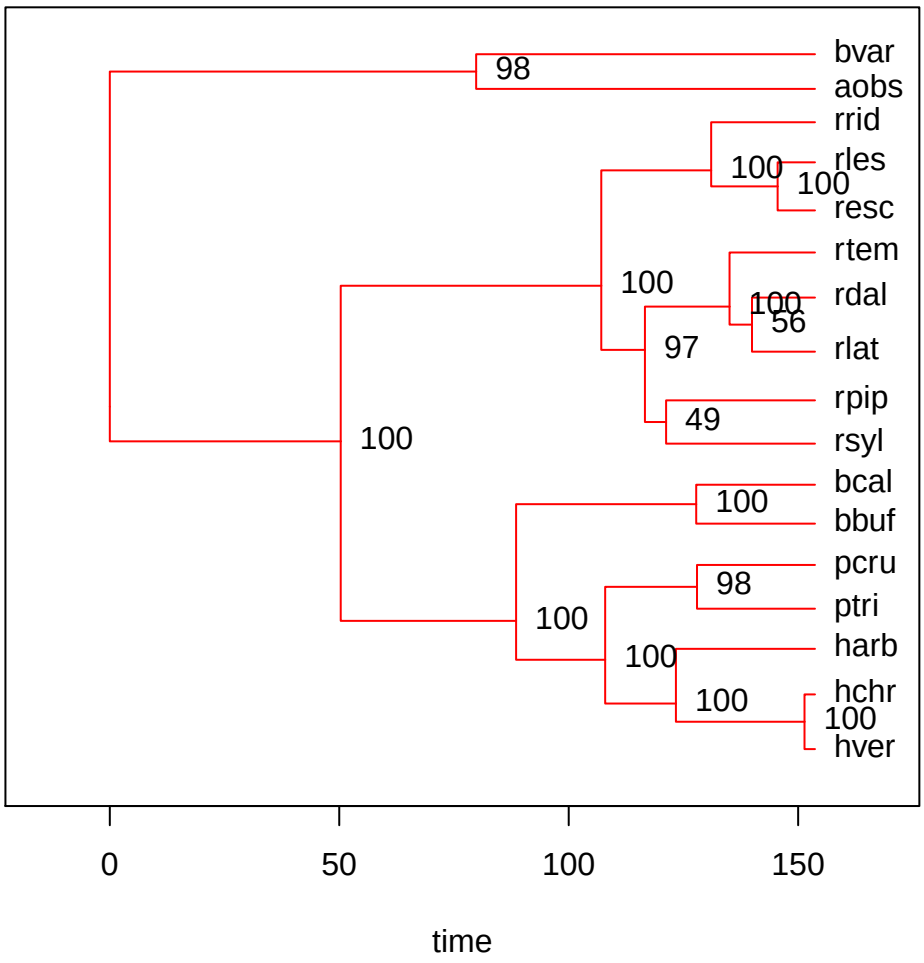

Figure 4.1: The best-supported phylogeny for the species found in this study. The topology of this phylogeny agrees exactly with the topology inferred from previously phylogenies (Frost et al., 2006; Smith et al., 2007; Van Bocxlaer et al., 2009; Wiens et al., 2009). The labels of internal nodes give the number of bootstraps (of 100) that contained the particular branching point.

determinants of larval anuran distribution, abundance, and morphology: habitat permanence (hydroperiod), canopy cover, and predation risk (Werner et al., 2007a; Van Buskirk, 2005, 2009).

Habitat permanence was assessed using three different variables, including pond area, pond depth, and proportion of years the pond dried. Previous studies were able to use principal components analysis to capture the variation in all three of these measures, as they are correlated (Table 4.2; Werner et al. (2007a); Van Buskirk (2005, 2009). This was not possible in this case because the data are taken from separate geographic regions. Table 4.3 gives the May and July average pond characteristics for each region. Separating out each month is useful, as the amphibians in this study have characteristic breeding seasons, and are typically found in the ponds only during one sampling period. Since the average pond characteristics were very different for each region, it does not make sense to perform a single principal components analysis on both sets of data together. Separate principal component analyses were able to capture much of the variation within each region (51% for Switzerland and 81% for Michigan), but the loadings on the variables were very different, suggesting that the two measures are not capturing the variation in the same way. Because of this, I chose to analyze each measurement separately, despite the fact that they are highly correlated. Additional support for this decision comes from Werner et al. (2007a), who showed that pond area effects on tadpole abundance are largely independent of hydroperiod effects.

Proportion of canopy cover was measured as the proportion of the pond surface overhung by forest. Canopy cover has been shown in previous studies to affect the distribution and performance of amphibian species (Werner and Glennemeier, 1999; Skelly et al., 2002; Werner et al., 2007a). These effects have been attributed to the affect of canopy cover on abiotic variables such as temperature, dissolved oxygen, and resource levels. These variables are known to affect growth rates and behavior

	Pond area (m^2)	Pond depth (m)	Prop. of years dry	Canopy cover (%)	Predation risk	Fish
Pond area		0.6 (3.3e-08)	-0.39 (0.0013)	0 (0.98)	-0.03 (0.61)	0.3 (0.027)
Pond depth	0.6 (8.5e-05)		-0.67 (9.2e-09)	-0.04 (0.53)	-0.08 (0.12)	0.41 (0.0038)
Prop. of years dry	-0.74 (1.8e-07)	-0.55 (0.001)		-0.1 (0.23)	0.08 (0.16)	-0.78 (1.1e-08)
Canopy cover	-0.81 (9.2e-10)	-0.51 (0.0011)	0.58 (0.00024)		-0.01 (0.89)	-0.17 (0.14)
Predation risk	0.22 (1.6e-10)	0.34 (9.6e-23)	-0.28 (1.6e-14)	-0.2 (2.7e-08)		-0.13 (0.019)
Fish	0.66 (5e-23)	0.64 (8.9e-20)	-0.65 (1.5e-18)	-0.57 (4.8e-15)	0.08 (0.032)	

Table 4.2: Correlations between habitat variables in the Old World (above the diagonal) and New World (below the diagonal) ponds. Correlation coefficients are Spearman rank correlation coefficients. The values given in parentheses are p-values. For Old World ponds, predation risk is quantified by the weighted sum of invertebrate predator densities whereas for New World ponds risk is the total biomass of invertebrate predators. For Old World ponds, fish are measured as the density of fish, whereas New World ponds measure the biomass of fish.

Location	Month	Pond area (m^2)	Pond depth (m)	Canopy cover (%)	Prop. of years dry	Invertebrates	Fish
Michigan	May	6900	0.50	0.68	0.58	46	190
Switzerland	May	1030	0.49	0.24	0.19	7.0	0.088
Michigan	July	8970	0.54	0.61	0.54	140	330
Switzerland	July	1030	0.50	0.15	0.16	7.7	0.039

Table 4.3: Mean pond characteristics in the two study regions. Note that in Michigan, invertebrate predation risk is quantified as the mean invertebrate biomass across all ponds containing water during the survey period, whereas in Switzerland, this risk is the average of weighted sums of invertebrate density. In Michigan the measure of fish is the biomass, whereas in Switzerland, it is the density. All other measures are equivalent between the two locations.

in amphibians, and have been shown to be higher in open- than closed-canopy ponds (Werner and Glennemeier, 1999; Skelly et al., 2002).

Predation risk was quantified differently in the two study areas. For Michigan ponds, the biomass of different predator taxa was estimated for each pond using estimated species densities and length-weight regressions for each species or broader taxonomic group (Werner et al., 2007a). To have consistency across the different study sites, here I focus only on the biomasses of invertebrate and fish predators. For Swiss ponds, invertebrate predation risk was estimated for each survey date as a weighted sum of the densities of all potential invertebrate predators Van Buskirk (2005). The weights came from previous experiments quantifying mortality rates of *Rana temporaria* exposed to different predator species (Van Buskirk and Arioli, 2005). This approach has the advantage of considering that different species of predators are not present equal risk to tadpoles. The weakness is that this approach implicitly assumes that the weights would be equal for each tadpole species, which is not likely considering the variation in behavior and body size observed between tadpole species. Predation risk from fish was quantified as the density, rather than biomass, of fish. Importantly for this study, despite differences in how predation risk was quantified, both study sites contain many of the same taxonomic groups of invertebrate and vertebrate predators, including aeshnid, libellulid, and corduliid dragonfly larvae, larval dytiscid beetles, and adult backswimmers (Werner et al., 2007a; Van Buskirk, 2005). Furthermore, the amount of variability in each measurement of risk across all sampling sites and dates is approximately the same between locations (CV of invertebrate predation risk: Michigan - 1.39, Switzerland - 1.16; CV of fish predation risk: Michigan - 1.01, Switzerland - 1.11). To be able to compare measurements of predation between sites, I standardized each species-specific measurement using the standard score. For example, the magnitude of invertebrate predation risk experienced by *R. sylvatica* was quantified by the mean invertebrate

biomass in ponds containing *R. sylvatica*, across study years, minus the mean May invertebrate biomass across all ponds and years (I used only May measurements since *R. sylvatica* is a spring breeder), divided by the standard deviation of invertebrate biomass. Similar standardizations were performed for predation risk from fish.

Table 4.4 presents the mean pond characteristics for each species. Since I am also interested in the variability experienced by each species, in addition to the mean pond characteristics, I also calculated the coefficient of variation (species-specific measurement standard deviation divided by species-specific mean). These results are presented in Table 4.5.

Note that I do not have habitat data for three of species in this study (*H. chrysocelis*, *R. latastei*, and *R. ridibunda*). These species will be excluded from some of the phylogenetic analyses.

Phylogenetic analyses

I wanted to test whether there was evidence for correlated evolution between mean and variability in habitat characteristics and trait means and plasticities. However, traditional statistical approaches to correlation are confounded in comparative evolutionary studies because species cannot be considered as independent samples because of shared evolutionary history (Felsenstein, 1985). In general, there are at least three potential explanations for observed patterns of correlation (or lack thereof) between habitat characteristics and organismal traits.

First, a significant correlation may suggest that the mean trait or trait plasticity is adaptive. For example, reduced activity in the presence of predators has been observed in hundreds of studies, and has been shown to increase fitness covariates, such as survivorship (Lima and Dill, 1990; McCollum and VanBuskirk, 1996), so increased plasticity in species that inhabit high risk ponds may be reflective of adaptation.

Second, a correlation (or lack thereof) may be the result of evolutionary history

	Breeding	Pond area (m^2)	Pond depth (m)	Pond drying (%)	Canopy cover (%)	Invertebrates (dim.)	Fish (dim.)
Michigan							
H. versicolor	July	9810	0.60	0.55	0.37	-0.070-	-0.31
P. crucifer	May	8250	0.52	0.62	0.47	0.45	-0.14
P. triseriata	May	6220	0.46	0.76	0.50	-0.026	-0.24
R. pipiens	May	34500	0.81	0.20	0.17	1.1	0.88
R. sylvatica	May	3970	0.45	0.79	0.62	-0.11	-0.24
Switzerland							
A. obstetricans	May	300	0.36	0.044	0.12	-0.22	-0.33
B. variegata	July	180	0.21	0.51	0.0083	-0.72	-0.34
B. bufo	May	2200	0.62	0.07	0.17	0.051	0.18
B. calamita	May	410	0.29	0.075	0.00	-0.56	-0.33
H. arborea	May	1600	0.47	0.17	0.16	-0.086	-0.32
R. dalmatina	May	1300	0.58	0.14	0.51	-0.29	-0.33
R. esculenta	July	1200	0.53	0.12	0.15	0.074	-0.013
R. lessonae	July	880	0.52	0.11	0.15	0.025	0.017
R. temporaria	May	1400	0.48	0.15	0.24	0.036	0.13

Table 4.4: Mean species-specific pond characteristics. Note that predation risk from invertebrate and fish predators was standardized using normal scores, so the measurements in those columns are the number of standard deviations above or below the location- and month-specific means.

	Breeding	Pond area (m^2)	Pond depth (m)	Pond drying (%)	Canopy cover (%)	Invertebrates (dim.)	Fish (dim.)
Michigan							
H. versicolor	July	1.4	0.33	0.61	0.55	0.95	4.8
P. crucifer	May	1.6	0.42	0.52	0.56	1.3	5.0
P. triseriata	May	1.8	0.38	0.25	0.55	1.1	6.7
R. pipiens	May	0.48	0.23	1.1	0.31	0.54	1.6
R. sylvatica	May	1.8	0.31	0.18	0.44	1.1	6.0
Switzerland							
A. obstetricans	May	0.62	0.33	1.6	0.99	0.69	0.00
B. variegata	July	1.4	0.39	0.87	3.5	1.1	0.00
B. bufo	May	1.5	0.55	1.7	1.0	1.1	2.6
B. calamita	May	1.2	0.02	0.47	0.00	0.67	0.00
H. arborea	M-J	1.7	0.43	1.1	1.1	1.1	0.00
R. dalmatina	May	0.43	0.07	1.3	0.48	0.61	0.00
R. esculenta	July	1.9	0.50	1.4	0.99	1.1	3.3
R. lessonae	July	1.9	0.49	1.4	0.97	0.94	3.3
R. temporaria	May	1.8	0.67	1.5	1.1	1.2	2.6

Table 4.5: Variability in species-specific pond characteristics. Variability was quantified using the coefficient of variation.

(Blomberg and Garland, 2002). That is, a recently diverged species that inhabits a novel environment may not have had enough time to adapt itself perfectly to its environment, and may therefore express ancestral traits which are not adaptive in the current environment. For example, previous studies of amphibian tadpoles have shown that a common morphological response to invertebrate predation risk is development of a deeper tail, which improves burst swimming speed, whereas predation risk from fish selects for the development of a narrower tail, improving overall swimming speed (Richardson, 2002; Benard, 2006). An amphibian species currently found in ponds with fish that recently diverged from an ancestral lineage that inhabited only fishless ponds may still express the non-adaptive response of a deeper tail morphology. Methods that explicitly consider the relatedness can account for the effect of shared evolutionary history on trait expression, and so significant correlations may emerge that are not observed by standard correlation approaches.

Third, correlations between traits may influence the correlation between habitat variables and traits. If traits are correlated due to functional or physiological constraints, then an adaptive response in one trait may cause a corresponding change in another trait. For example, since activity level has a direct effect on foraging gain, and subsequently growth, an adaptive reduction in activity level in response to predators may cause a reduction in the size of a morphological trait such as head length. This might suggest that reduced head length is an adaptive response to increased predation risk, where in fact it is simply due to correlations among traits.

It is worth noting that these explanations are not mutually exclusive. For example, correlations may reflect both adaptation and phylogenetic relatedness (explanations one and two). Furthermore, there may be strong correlations among traits that are due to adaptation favoring certain patterns of trait integration (Pigliucci, 2003; Cressler et al., 2010).

To address all of these questions, I made use of a recently developed frame-

work for phylogenetic comparative hypothesis testing, the OUCH project (Butler and King, 2004). This project essential presents a unified framework for performing phylogenetic generalized least squares (PGLS) regression (Martins and Hansen, 1997; Garland and Ives, 2000; Butler and King, 2004). Rather than assuming that species are independent samples, PGLS methods use a model of trait evolution and information contained within the phylogenetic tree to specify the expectations and covariances in trait values between taxa. The OUCH project allows for two different models of trait evolution: Brownian motion or an Ornstein-Uhlenbeck process. Brownian motion is a neutral model of evolution, and underlies the most commonly used comparative method, independent contrasts (Felsenstein, 1985; Garland et al., 1992). Mathematically, trait evolution according to Brownian motion can be modeled by the stochastic differential equation

$$dX(t) = \sigma dB(t), \tag{4.1}$$

where $dX(t)$ is the infinitesimal change in the trait X over the infinitesimal time interval t to $t + dt$. $dB(t)$ is "white noise", independent and identically distributed random variables with mean zero and variance dt and σ is the intensity of random fluctuations. The Ornstein-Uhlenbeck model can be thought of as a model of stabilizing selection (Hansen, 1997; Butler and King, 2004), containing components representing both adaptive and neutral processes. Mathematically, this model

$$dX(t) = \alpha[\theta - X(t)]dt + \sigma dB(t). \tag{4.2}$$

Here, α measures the strength of selection towards an optimum θ. Note that if $\alpha = 0$, you recover the model of Brownian motion. In particular, it is possible to specify different values of θ for taxa thought to be evolving under different selection pressures. For the regressions of habitat characteristics against trait means and

plasticities, I will be assuming a global optimum. Below I describe an approach that specifies different selective optima for different taxa.

The first set of analyses I performed quantified the correlation between the habitat characteristics shown in Tables 4 and 5 and mean traits and trait plasticities. I quantified these correlations assuming either a neutral model of trait evolution (Brownian motion), or an adaptive model of trait evolution (Ornstein-Uhlenbeck). The Brownian motion model assumes that habitat characteristics and phenotype are changing randomly along the phylogeny, whereas the Ornstein-Uhlenbeck model assumes that both are evolving towards a single optimum. These two models are implemented in the R package `ouch` by the functions **brown** and **hansen**, respectively. I performed two phylogenetic comparisons. First, I evaluated whether there has been correlated evolution between mean habitat characteristics and mean trait values, which would be expected if different habitat types select for different phenotypes. Second, I evaluated whether there has been correlated evolution between habitat variability and trait plasticities, which would be expected if habitat variability contributes to selection for phenotypic plasticity (Van Buskirk, 2002). I also tested for correlations between habitat variability and mean trait values and between mean habitat characteristics and trait plasticity. These analyses can be found in Tables C.5 and C.6 in Appendix C.

Additionally, I wanted to directly test the hypothesis that species in different habitats are under different selection pressures. The preceding analyses get at this question, but only indirectly. The R package `ouch` is a likelihood-based framework for performing such analyses. In particular, for each trait mean and plasticity, I can use `ouch` to ask whether the data support the hypothesis that traits are evolving neutrally along the phylogeny, whether they are adaptively evolving towards a global optimum, or whether there are different selective optima operating on different branches of the phylogeny. The latter hypothesis requires specifying a "painting" of

the branches of the phylogenetic tree, where each color of the painting corresponds to a different selective optima (Butler and King, 2004). In this way, different evolutionary hypotheses correspond to different paintings of the phylogenetic tree. `ouch` quantifies the evidence (in terms of likelihood) for different evolutionary hypotheses, allowing for a direct comparison of competing evolutionary stories. Given the importance of pond characteristics to species distributions and prior work documenting their effects on species morphology, I am interested in rigorously testing the hypothesis that different trait expression and/or different plastic responses to predators are optimal in different environments (McCollum and VanBuskirk, 1996; Van Buskirk et al., 1997; Richardson, 2001a,b, 2002; Van Buskirk, 2002; Richter-Boix et al., 2007; Van Buskirk, 2009).

To characterize the environments inhabited by each species, I followed the suggestion in the literature that amphibians can be classified into discrete categories on the basis of pond permanency (Wellborn et al., 1996; Richardson, 2001b,a, 2002). Wellborn et al. (1996) noted two primary transitions in habitat type as you move along a gradient from temporary to permanent ponds. The first they termed the permanence transition, that is, a transition from ponds that dry most years to ponds that remain at least partially full most years. The second transition was termed the predator transition, that is, a transition from permanent ponds where invertebrates are the dominant predator to ponds containing fish. In a series of comparative studies, Richardson expanded this definition to classify species into four discrete categories: "(V)ernal" species are found almost exclusively in ponds that dry every year; "(D)ragonfly" species are found in more permanent habitats, but seldom in ponds containing fish; "(F)ish" species are species that often coexist with fish; finally "(M)ultiple" species are cosmopolitan species that occur in variety of habitats. I used the species-specific habitat characteristics shown in Tables 4 and 5 to bin each of the study species into one of these four categories; for those species for whom

I did not have habitat data (*R. ridibunda*, *R. latastei*, and *H. chrysocelis*), assignments were based on expert opinion. Species within each category were assumed to be evolving towards similar selective optima. To reconstruct the evolutionary history of selective regimes (that is, which selective regime was operating on each internal branch), parsimony was used. When multiple explanations were equally parsimonious, I analyzed each hypothesis separately. Figure 4.2 shows one of these phylogenetic hypotheses.

For this set of analyses, I quantified the support for three different models of trait evolution: neutral, an Ornstein-Uhlenbeck model with a single optimum, and the Ornstein-Uhlenbeck model with separate optima for each habitat type. I analyzed each trait and trait plasticity separately, and then tested for correlated evolution of combinations of traits and plasticities.

Results

Correlated evolution between habitat variables and traits

In all cases, the best supported model was Brownian motion (see AICc scores in Tables C.1 and C.2). Correlations predicted by this model of trait evolution can be understood in the following way: under Brownian motion, habitat characteristics and traits are changing neutrally along the branches of the phylogeny, but changes in, for example, habitat characteristics are associated with changes in mean trait expression. Assuming that changes in habitat characteristics through time lack directionality is reasonable considering all of the factors that would affect these habitat characteristics along the phylogeny (Hansen et al., 2008). However, these changes in habitat characteristics could still select for different phenotypes; evidence for such selection would appear as significant correlations between habitat characteristics and traits.

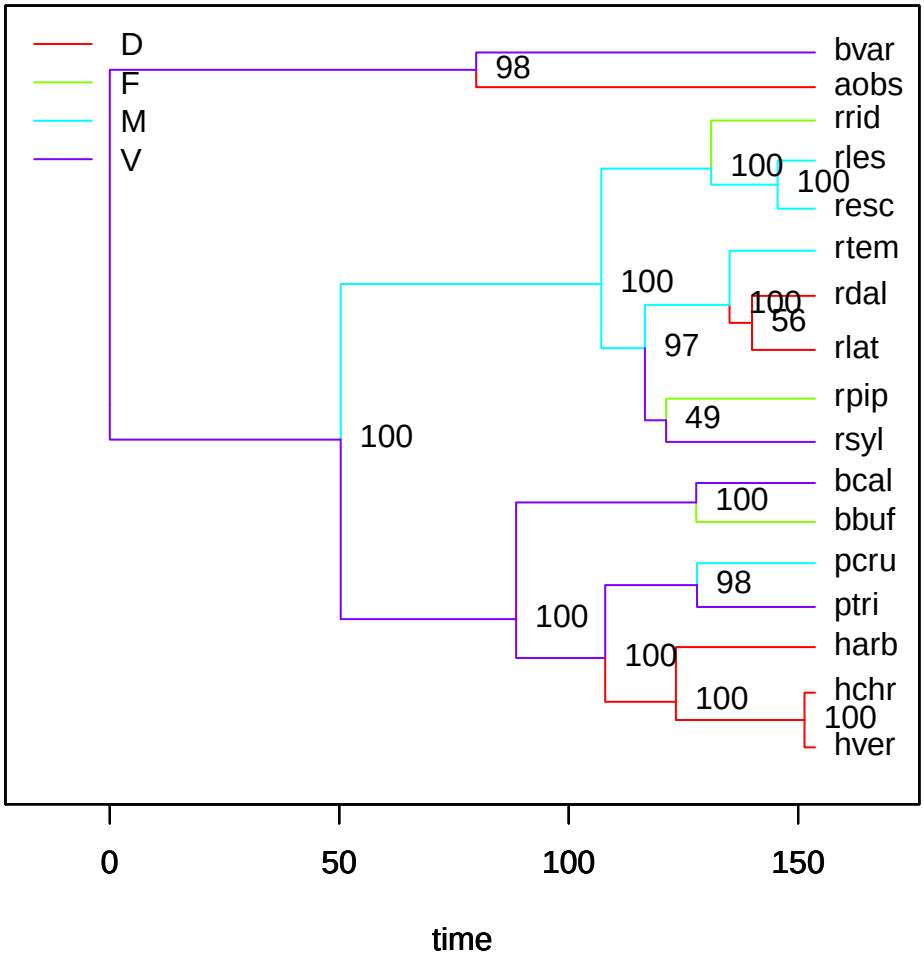

Figure 4.2: The phylogeny with best-supported selective regime painting (Butler and King, 2004). The different colors correspond to different selective regimes. Each extant species was classified into a particular habitat type based on the information in Tables 4 and 5. Ancestral regimes were reconstructed using parsimony.

	Mean area (m^2)	Mean depth (m)	Mean drying (%)	Mean canopy (%)	Mean inverts (dim.)	Mean fish (dim.)
Body size	0.14 (-0.39, 0.59)	0.3 (-0.08, 0.64)	**-0.58 (-0.84, -0.21)**	-0.23 (-0.6, 0.27)	-0.01 (-0.5, 0.49)	0.02 (-0.4, 0.46)
Head length	-0.09 (-0.5, 0.29)	-0.04 (-0.42, 0.44)	-0.35 (-0.7, 0.15)	-0.43 (-0.74, 0.05)	-0.03 (-0.46, 0.39)	0.15 (-0.35, 0.58)
Head depth	**0.6 (0.16, 0.84)**	0.34 (-0.23, 0.65)	0.35 (-0.13, 0.73)	0.07 (-0.39, 0.5)	0.37 (-0.09, 0.75)	**0.42 (0.03, 0.71)**
Tail length	-0.46 (-0.79, 0.08)	-0.23 (-0.64, 0.15)	-0.03 (-0.53, 0.44)	0.31 (-0.2, 0.67)	**-0.46 (-0.74, -0.05)**	**-0.51 (-0.76, -0.07)**
Tail depth	-0.02 (-0.37, 0.48)	-0.05 (-0.46, 0.39)	-0.15 (-0.67, 0.37)	-0.11 (-0.52, 0.37)	0.08 (-0.36, 0.54)	-0.01 (-0.47, 0.41)
Activity	-0.04 (-0.62, 0.49)	-0.32 (-0.68, 0.19)	0.17 (-0.29, 0.57)	-0.16 (-0.66, 0.27)	-0.39 (-0.74, 0.08)	-0.08 (-0.57, 0.4)

Table 4.6: Correlation between mean trait values and mean habitat characteristics.Recall that the measure of invertebrate and fish predation risk are standardized using the normal score to facilitate comparison across Old and New World ponds. Values in the parentheses give the 95 percent bootstrap confidence interval. Correlation coefficients that are significantly different from zero are shown in bold.

	CV area	CV depth	CV drying	CV canopy	CV inverts	CV fish
Head length	**-0.45 (-0.78, -0.06)**	-0.33 (-0.68, 0.19)	0.16 (-0.25, 0.57)	-0.17 (-0.58, 0.26)	-0.36 (-0.71, 0.15)	-0.4 (-0.71, 0.06)
Head depth	-0.28 (-0.68, 0.22)	-0.15 (-0.59, 0.29)	0.19 (-0.27, 0.59)	-0.15 (-0.57, 0.36)	-0.41 (-0.71, 0.05)	-0.14 (-0.52, 0.4)
Tail length	0.23 (-0.31, 0.68)	-0.04 (-0.44, 0.4)	**-0.6 (-0.85, -0.28)**	0 (-0.48, 0.4)	0.02 (-0.42, 0.45)	**0.64 (0.27, 0.86)**
Tail depth	0.42 (-0.05, 0.73)	0.35 (-0.17, 0.72)	0.21 (-0.26, 0.58)	0.32 (-0.14, 0.59)	0.31 (-0.1, 0.63)	-0.21 (-0.57, 0.27)
Activity	**0.46 (0.07, 0.77)**	0.16 (-0.38, 0.6)	-0.42 (-0.79, -0.06)	0.03 (-0.43, 0.5)	0.33 (-0.19, 0.71)	**0.54 (0.11, 0.84)**

Table 4.7: Correlation between trait plasticities and the variability in habitat variables. Values in the parentheses give the 95 percent bootstrap confidence interval. Correlation coefficients that are significantly different from zero are shown in bold.

Table 4.6 presents the correlations between mean habitat characteristics and mean traits. There is a significant negative correlation between a species' body size and the mean frequency of drying in the ponds it inhabits, suggesting that smaller-bodied species are found in less permanent habitats. Additional evidence for this suggestion comes from a number of significant negative correlations between body size and measures of habitat variability (Table C.5), suggesting that more variable habitats select for smaller bodies. Within Table 4.6, there are also significant positive correlations between head depth and mean pond area and mean predation risk from fish. Given the strong positive correlation between pond area and fish predation (Table 4.2), both correlations are suggestive that species with larger heads are more commonly found in large, permanent ponds with fish. There are significant negative correlations between tail length and both invertebrate and fish predation risk, suggesting that increased density of predators tends to select for shorter tails.

Table 4.7 presents the correlations between variability in habitat characteristics (measured by the coefficient of variation) and trait plasticities. There is a significant negative correlation between plasticity in head length and variability in pond area. This suggests that species inhabit ponds that vary considerably in area tend to reduce head length in response to predators. There are two significant correlations for tail length: a negative correlation between plasticity in tail length and variability in pond drying, and a positive correlation between tail length plasticity and variability in fish predation risk. Again, these two correlations are probably reflective of the same constraint, given the strong negative correlation between pond drying and fish predation (Table 4.2). In other words, variability in drying suggests that species are primarily inhabiting temporary ponds, which selects for a reduction in tail length in response to predators. Variability in fish predation suggests that species are inhabiting more permanent ponds, which selects for an increase in tail length in response to predators (Benard, 2006). Finally, plasticity in activity level shows a

positive correlation with variability in pond area and variability in fish predation. The first correlation suggests that species found in more temporary habitats are under selection to respond to predators less than species occurring in more permanent habitats. The second correlation suggests there is a limit to this selection pressure, however: species found in habitats that contain fish are also under selection pressure to reduce activity level less in response to predators.

Explicit testing of phylogenetic hypotheses

I analyzed each trait mean and plasticity individually to determine which model of trait evolution was best supported by the data. The models tested were Brownian motion, an Ornstein-Uhlenbeck (OU) model with a global optimum, and an OU model with separate optima for each of the habitat categories (Figure 4.2). The neutral model was the best supported for mean head length, mean tail depth, mean activity level, plasticity in tail length, and plasticity in tail depth (Table C.3). The single optimum OU model was favored for mean head depth, mean tail length, plasticity in head length, and plasticity in head depth. The multiple-optima OU model was favored for body size and plasticity in activity level. Model AICc scores can be found in Table C.3 in the Appendix.

These results suggest that body size and behavioral plasticity are adaptively evolving towards habitat-type specific values. The function **hansen** returns estimates of the optima for each regime, as well as estimates for selection strength and intensity of random fluctuations. However, a previous simulation study of `ouch` revealed that while the selective optima tend to be well-estimated, the estimates of selection strength and noise intensity are less reliable (C. Cressler, *unpublished manuscript*). The selective optima for each habitat category are shown for body size and activity level in Table 4.8. These optima make ecological sense and accord well with the results presented in the preceding section, providing strong evidence

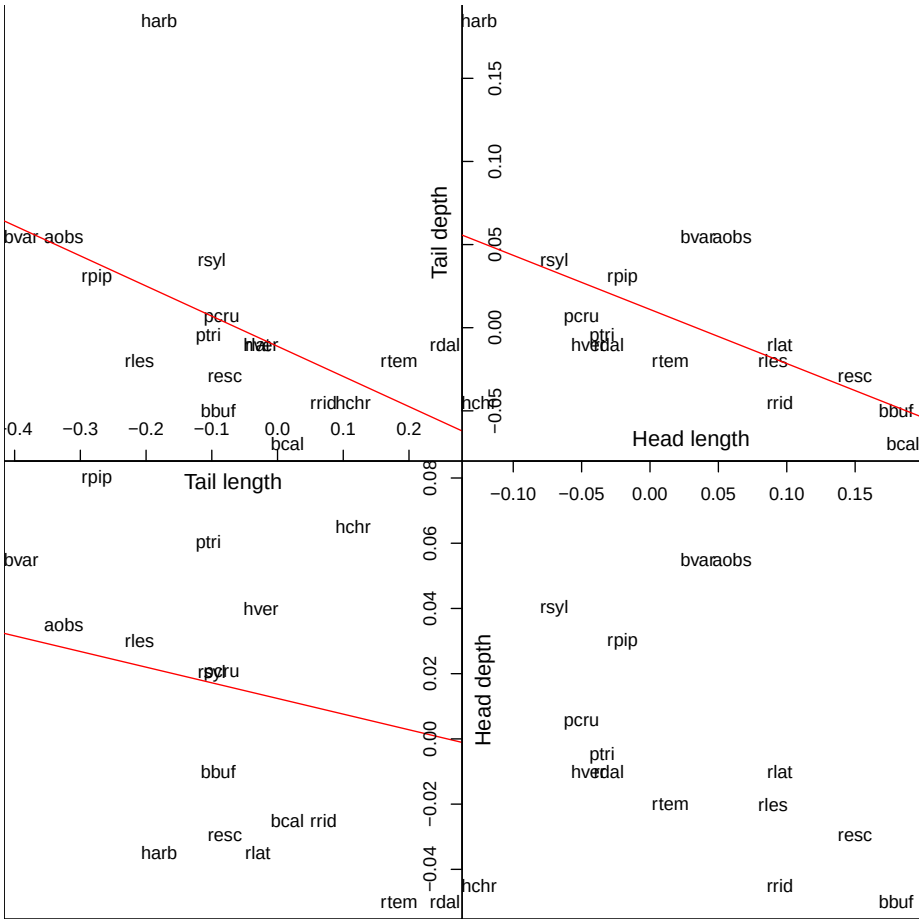

Figure 4.3: The correlations between mean trait values. Red lines show the significant trends in phylogenetic generalized least squares regression.

for adaptive evolution.

All of the other traits, when analyzed individually, appear to be evolving either neutrally or towards a global optimum, though the evidence for stabilizing selection is weak in all cases (see Table C.3 in Appendix C). I also examined whether there was correlated evolution among pairs of traits and pairs of trait plasticities. I tested the same three evolutionary models as in the single trait case, but in this analysis, I looked for correlated evolution in pairs of traits and pairs of trait plasticities. The results of this analysis are presented in Table 4.9.

There is evidence for correlated evolution between mean traits. The best-supported model in all cases was the neutral model, suggesting that these traits are not evolving

	D	F	M	V
Body size (PC-1)	1.53 (0.41, 4.29)	1.08 (0.20, 2.36)	-0.83 (-3.22, 0.33)	-0.39 (-0.89, 0.04)
Behavioral plasticity	-0.71 (-0.84, -0.55)	-0.29 (-0.58, -0.11)	-0.72 (-0.91, 0.53)	-0.25 (-0.41, -0.06)

Table 4.8: Habitat-specific optima for body size and plasticity in activity level. Confidence intervals, obtained by nonparametric bootstrapping, are given in parentheses.

	Head length	Head depth	Tail length	Tail depth	Activity
Head length		-0.24 (-0.63, 0.2)	0.2 (-0.19, 0.61)	**-0.65 (-0.85, -0.29)**	0.15 (-0.29, 0.63)
Head depth	*0.12 (-0.46, 0.48)*		**-0.68 (-0.87, -0.34)**	-0.13 (-0.54, 0.36)	0.03 (-0.42, 0.57)
Tail length	*-0.07 (-1, 0.89)*	0.03 (-0.47, 0.51)		**-0.43 (-0.76, -0.03)**	0 (-0.5, 0.52)
Tail depth	***-0.69 (-0.88, -0.46)***	-0.03 (-0.51, 0.36)	-0.33 (-0.67, 0.03)		-0.11 (-0.57, 0.39)
Activity	*0.13 (-0.36, 0.46)*	*-0.01 (-0.45, 0.46)*	**0.66 (0.25, 0.86)**	0.31 (-0.22, 0.7)	

Table 4.9: Correlations between traits and between trait plasticities. Correlations between mean trait values are shown above the diagonal. Correlations between trait plasticities are shown below the diagonal. Significant correlations are shown in bold. In most cases, the best supported model was neutral evolution - cases for which the global optimum OU model was best supported are shown in italics.

in response to environmental characteristics. Rather traits appear to be evolving in response to one another, as there are significant negative correlations between head length and tail depth, between head depth and tail length, and between tail length and tail depth.

The strongest correlation here is also evidence for correlated evolution among phenotypic plasticity. In particular, there is a significant negative correlation between plasticity in head length and plasticity in tail depth. This correlation suggests that species respond to predation risk by developing shorter heads and deeper tails (Figure 4.4). Previous studies indicate that this is an effective defense against invertebrate predation (Van Buskirk et al., 1997; Relyea, 2004a). There is also a negative correlation between plasticity in tail length and behavioral plasticity.

Discussion

Phenotypic evolution in amphibian tadpoles

These results provide evidence that tadpoles inhabiting different pond types are under different selection pressures, and these different pressures result in interspecific differences in body size and behavioral response to predation risk, rather than in other traits. In fact, after correcting for body size, the most common morphological defense against predation appear to be under strong stabilizing selection.

The results from the analysis of the correlation between habitat characteristics and mean traits and trait plasticities support the conclusions drawn from the second analysis of mean trait and trait plasticity evolution. In particular, the first set of analyses strongly suggested that species that were found in more variable habitats were under selection for reduced body size, as evidenced by the negative correlation between pond drying and body size (Table 4.6) and between several habitat variability measures (Table C.5). Behavioral plasticity is also strongly positively correlated

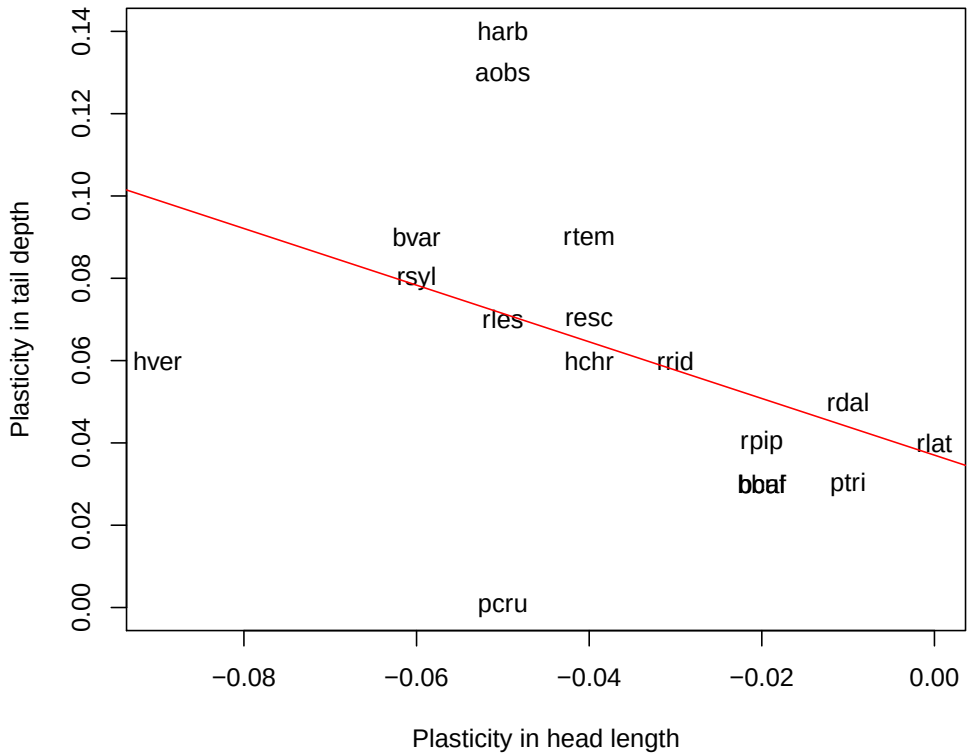

Figure 4.4: The correlation between plasticity in head length and plasticity in tail depth. Species respond to predators by developing shorter heads and deeper tails. This relationship appears to be under strong stabilizing selection.

with both variability in pond area (Table 4.7) and mean drying (Table C.6), reflecting that species inhabiting in less permanent habitats should reduce activity less in the presence of predators.

It is worth comparing these results to other studies that have investigated similar questions. In particular, Van Buskirk (2002) used the same morphological and behavioral data to investigate the hypothesis that species that inhabit more variable environments will be more phenotypically plastic. It is worth noting that it has been argued that it is not meaningful to say that one species is "more plastic" than another. A particular genotype might be more plastic in a given trait, but less so in a different trait (Pigliucci, 2001). Environmental variability is a prerequisite for the evolution of phenotypic plasticity, but beyond this generalization, there is no empirical basis for saying that one species is more plastic than another. Nonetheless, there are interesting comparisons that can be drawn between the two studies.

Most significantly, Van Buskirk (2002) did not find any evidence for correlation between habitat variability and behavioral plasticity, one of the most clear responses in my study. He attributed this weak correlation to not having considered an appropriate scale of habitat heterogeneity. That is, the scale of habitat variation that promotes the evolution of behavioral plasticity was finer than the scales considered in his study. Here I showed significant correlations between behavioral plasticity and pond drying. This emerged from the correlation analysis between habitat characteristics and plasticity, as well as from the multiple-optimum Ornstein-Uhlenbeck model, suggesting that variability in pond hydroperiod promotes the evolution of different behavioral plasticities. This hypothesis has support from other comparative studies, who also showed that species that inhabit temporary ponds tend to respond to predators primarily through morphological, rather than behavioral, plasticity (Anholt et al., 2000; Relyea and Werner, 2000; Richardson, 2001b; Richter-Boix et al., 2007).

This study uncovered a number of interesting evolutionary relationships among morphological traits. The strong negative correlations between mean traits shown in Figure 4.3 suggest that there is evolutionary pressure on maintaining a functional morphology. That is, despite the fact that all of these traits appear to be evolving neutrally along the phylogeny, I do not see random assemblages of traits, but rather strong correlations between morphological traits. It appears that selection may be acting on the integrated phenotype, rather than selection acting on individual traits. For example, species with shallow heads tended to have long, shallow tails (e.g, *R. ridibunda*), whereas species with deep heads tended to have short, deep tails (e.g., *R. pipiens*). However, both of these morphologies must be effective, since *R. ridibunda* and *R. pipiens* were both found in similar habitat types (ponds with fish).

This study also found that mophological plasticity appears to be under selection towards a global size-corrected optimum. The observed morphological responses are consistent with responses observed in other studies; in particular, when exposed to predation risk, species tended to respond by developing shorter heads and deeper tails (Table 9 and Figure 4.4; Van Buskirk et al. (1997); Relyea (2004a)). Van Buskirk (2002) suggested that there was evidence for a negative correlation between morphological and behavioral plasticity, but I do not find evidence for that hypothesis in this analysis - the only significant negative correlation was between behavioral plasticity and plasticity in tail length. Changes in tail length in response to predators may actually be a cost of reduced activity level, rather than an adaptive response.

This result also provides indirect evidence for the hypothesis that morphological defenses do not have significant fitness costs (Tollrian and Harvell, 1999). Many studies that have attempted to measure these costs have not been able to find any (Relyea, 2002; Callahan et al., 2008; Auld et al., 2010). One interpretation of the prediction that, regardless of body size, behavior, or habitat, all amphibian species respond similarly to predators is that these defenses carry very low fitness cost. An

alternative is that dragonfly risk imposes a strong enough selection that all species have evolved the same strategy. This seems less likely, however, given the considerable variation in predation risk observed among species.

The most interesting hypotheses that emerge from this analysis are the predicted evolutionary optima for body size and behavioral plasticity (Table 4.8). In three of the cases, these optima make ecological sense and conform to intuition.

Vernal habitats select for small body size and low reduction in activity level in response to predators. This is because the rapid drying of these habitats necessitates rapid maturation, which is facilitated by small size and rapid growth. The need for rapid growth suggests that species inhabiting these ponds cannot afford to reduce activity level too much (Richardson, 2001b; Richter-Boix et al., 2007).

Dragonfly habitats select for large body size and large reduction in activity level in response to predators. Large body size reduces the risk of predation from gape-limited predators, such as dragonflies, as does reduced activity level. Furthermore, since these habitats tend to be more permanent, there is not the constraint on metamorphosing early, which allows for more dramatic decreases in activity level.

Fish habitats select for moderately large body size and low reduction in activity level. This can be explained by reference to predator foraging ecology. Because fish predators tend to be less gape-limited than invertebrate predators, very large body size is less likely to be adaptive. If it is not pssible to outgrow the gape limitation of one's predators, metamorphosing quickly to escape risk through a habitat shift can become the most adaptive strategy (Werner and Gilliam, 1984; Cressler et al., 2010). Rapid growth would be facilitated by not responding as strongly to predators behaviorally.

Interpreting the adaptive value of the predicted selective optima for species inhabiting many different habitat types is more difficult. These species are predicted to be under selection for very small body size and very strong reduction in activity level

in response to predators. It is possible that these phenotypic characters represent an adaptive compromise among all of the competing selection pressures of the different environmental types. Most of the species that occur in multiple habitat types tend to occur in both vernal and dragonfly habitats. Taking the selective optima of dragonfly and vernal habitats as givens, species that occur in multiple habitats would be under selection both for large (in dragonfly habitats) and small (in vernal habitats) body size, and for both large (in dragonfly habitats) and small (in vernal habitats) reduction in activity level in response to predators. If selection for sharply reducing activity level was stronger than selection for large body size, this might result in species being very small, in order to achieve metamorphosis quickly when inhabiting vernal ponds. However, as far as I am aware the explanation advanced above lacks an empirical or theoretical basis, and so may be an evolutionary "just-so" story (Gould and Lewontin, 1979).

Phylogenetic analysis in perspective

Performing comparative analysis within the `ouch` framework is appealing because it offers the opportunity of quantifying the evidence for competing evolutionary hypotheses, as well as providing a means for direct testing of adaptive hypotheses. However, as with any method, it is necessary to acknowledge both the strengths and weaknesses of the method. Here I outline some of the challenges encountered in this study, as well as suggesting how they could be addressed in future studies.

One challenge in performing phylogenetic inference is that it can be difficult to distinguish between strong stabilizing selection and very weak neutral evolution. That is, if a trait does not appear to be evolving, this may be because the trait had reached an optimum at the root of the tree and is held at there by strong stabilizing selection, or it may be because the intensity of random fluctuations is very weak, so the trait does not drift far away from its value at the root of the tree. The tension

between these two possibilities can be observed in some of the analyses performed here. For example, Appendix Table C.3 shows the AICc scores for the fitting of mean trait and trait plasticity data to each model of evolution. In only a few cases is there a clear best model; often the AICc scores are within two AIC units of one another. Bearing in mind that estimates of selection strength and drift intensity are often biased (Cressler et al., *unpublished manuscript*), if you investigate the selection strength and drift intensities estimated by each model fitting, you find that **hansen** estimates selection strength as being several orders of magnitude stronger than drift intensity, suggesting the trait is under strong stabilizing selection. On the other hand, **brown** estimates drift intensity as being very low, suggesting that the trait is evolving under very weak neutral drift. Table 4.10 shows evolutionary parameter estimates for Brownian motion and a global optimum OU model fit the morphological and behavioral plasticity data. Notice that for all of the morphological plasticity measurements, the Brownian motion model estimates drift as being very weak, whereas the OU model estimates selection as being orders of magnitude stronger than drift. Thus, it is very difficult to conclude whether morphological plasticities are being held at an optimum by strong stabilizing selection, or whether they are evolving very slowly through weak random drift.

Another difficulty arises when working with a phylogeny as deep as the one in this study. As noted above, this phylogeny reaches back into the Jurassic period, pre-dating, for example, the separation of the Indian subcontinent from Africa. This makes inference of the selective regimes operating on these branches challenging. Furthermore, the taxon sampling on the tree is rather sparse. For example, recently published phylogenies for the amphibian families Hylidae (Smith et al., 2007; Hua et al., 2009) and Ranidae (Wiens et al., 2009) contain dozens of species. The sparsity of the taxon sampling also complicates reconstructing ancestral selective regimes. For example, *Hyla versicolor* (hver) and *H. chrysocelis* (hchr) are both classified

	brown σ^2	**hansen** σ^2	**hansen** α
Head length	4.1e-05	6.1e-04	0.62
Head depth	1.8e-05	8.8e-05	0.08
Tail length	2.1e-05	8.1e-05	0.05
Tail depth	2.2e-05	4.0e-05	0.01
Activity	3.4e-03	0.49	3.00

Table 4.10: Squared estimate of drift intensity (σ^2) and strength of selection (α) for two models of trait evolution. Squaring drift intensity is necessary so that the units are equivalent. The evolving traits are the morphological and behavioral plasticities. Notice the the neutral model best-fit estimate of drift intensity is very weak drift, while the single optimum Ornstein-Uhlenbeck model estimates selection strength as being much stronger than drift intensity. This is because of difficulty distinguishing strong stabilizing selection from weak random drift.

as inhabiting dragonfly habitats. These two species are sister species, and so it is reasonable to assume that their ancestor probably also inhabited dragonfly ponds. The most closely related species in this study is *H. arborea*, which also inhabits dragonfly ponds. However, in the phylogeny published by Smith et al. (2007), there are over a dozen species of Hylidae that are more closely related to *H. versicolor* and *H. chrysocelis* than is *H. arborea*. This weakens our confidence that the ancestor of all three species would also have inhabited dragonfly ponds. To take the most extreme case, it could be that these three species inhabit dragonfly ponds, but that every other species within their monophyletic clade inhabits vernal ponds. Under this extreme case, it is certainly inappropriate to assume that the switch to dragonfly habitats happened at the divergence the genus *Hyla* from the genus *Pseudacris*.

Finally, over the depth and width of the phylogeny of this study, there is likely to be considerable variation in the tempo and mode of evolution. That is, over the entire phylogeny, a trait might be evolving neutrally, but within a particular clade, that trait might be under strong selection. Such variation can also contribute to difficulties in resolving the correct model of evolution for a particular trait.

Previously, `ouch` has been used to study character evolution in phylogenies that are more limited in scope; that is, phylogenies for a particular clade of closely related organisms, such as Caribbean anoles (Butler and King, 2004). This permits better taxonomic resolution, and a more careful and complete characterization of regimes. However, even with all of these considerations, it was still possible to make novel predictions regarding phenotypic evolution in amphibians. These predictions are worth testing within specific amphibian families, to see if the predictions are borne out. Furthermore, it might be possible, with better resolution, to get a clearer sense of how different habitats select for different phenotypes and expressions of phenotypic plasticity.

CHAPTER V

Conclusion

In the previous chapters, I have shown that understanding and predicting phenotypic evolution requires considering how selection acts on the entire organism. This need arises out of a number of observations. First, selection arises primarily through an individual's interaction with its environment. For any given interaction (e.g., predator-prey interactions), many traits will be involved in determining the nature and strength of interaction. Second, organisms display a high degree of phenotypic integration, that is, correlation between different traits (Pigliucci, 2003). Finally, because multiple traits are involved in most interactions, and these traits will tend to be highly integrated, an organism's response to selection may proceed along multiple pathways and any trade-offs underlying the expression of one trait will have implications for other traits as well. In the preceding chapters, I have shown that a consideration of these insights can lead to non-intuitive conclusions.

In Chapter II, I showed that variation in the shape of the trade-off between foraging gain and predation risk could lead to qualitatively different predator-defense strategies, including strategies that modified multiple traits simultaneously. Previously, multivariate defense strategies have been explained as an evolutionary consequence of environmental complexity, especially exposure to risk from multiple predators (DeWitt and Langerhans, 2003; Relyea, 2004b). This study proposes an alterna-

tive explanation based on selection acting on multiple traits simultaneously, allowing for organisms to optimize expression of each trait in a compensatory fashion. This result has important implications for the study of phenotypic evolution more generally. Naïvely, one would have expected activity level and size at maturity to be positively correlated, so that reduction in activity level would lead to reduced size at maturity (Abrams and Rowe, 1996). This would be seen as a direct result of reduced foraging gain reducing growth rate. Most studies of phenotypic evolution make similar assumptions about the relationship between traits. For example, many studies of host evolution in response to parasitism posit a negative relationship between host immunity and host reproduction, despite the limited evidence for such a trade-off in nature (Boots et al., 2009; Duffy and Forde, 2009). The result presented here suggests that such a trade-off need not exist, and consequently that studies of phenotypic evolution need to consider how selection acts on e.g. host immune function and host reproductive strategy simultaneously.

Chapter III extends some of the ideas from Chapter II to the case of dynamic environments. By allowing both resources and predators to be dynamically linked to the prey population, I showed that it is possible to generate phenotypic polymorphism in both body size and behavior (Geritz et al., 1998; Day et al., 2002). This chapter also challenges the standard approach of atomizing traits to understand how they are expected to evolve under different selection pressures. In particular, I show that the emerging understanding of how the shape of underlying trade-offs influences evolutionary outcomes may have to be revised (Hoyle et al., 2008). Multivariate selection often leads to opposite predictions from univariate selection. Furthermore, under multivariate selection, changing the shape of the trade-off underlying one trait can affect the evolution the second trait as well, similar to what was observed in Chapter II. Given that evolutionary biologists are becoming increasingly interested in the potential for ecological dynamics to generate disruptive selection, a necessary

prerequisite for sympatric speciation (Dieckmann et al., 2004), these results suggest that future work would do well to consider multivariate evolution.

Chapter IV takes a more data-driven approach to the study of phenotypic evolution, but reaches conclusions that validate the focus on body size and behavior taken in the first two chapters. In particular, I show that tadpole body size and behavioral plasticity show the strongest responses to changes in pond type along the phylogeny. This provides additional justification for the focus on body size and behavior in the first two chapters, which had been based on first principle considerations. Furthermore, the predicted optima for body size and behavioral plasticity demonstrate how selection acting on multiple traits simultaneously can yield non-intuitive results, as the four different environments selected all possible combinations of size and behavioral reduction. Morphology, on the other hand, showed high levels of integration across evolutionary time, perhaps to maintain functional coordination. The morphological response to predators was under strong stabilizing selection. This provides a counterexample to Chapters II and III, as it suggests that there are cases where considering evolution of a single trait will lead to evolutionarily meaningful predictions.

Here, I suggest four future directions for the study of phenotypic evolution: evolution of phenotypic integration, phenotypic evolution in complex food webs, non-optimality approaches, and combining experimental and theoretical approaches in model systems. Continuing the theme developed in this dissertation, these future directions are not independent of one another, and likely must all be integrated in order to come to a more complete understanding of how evolution has shaped organismal phenotypes.

Evolution of phenotypic integration

To some extent, the suggestion that evolutionary biologists, and theoreticians in particular, must consider how evolution acts on multiple traits simultaneously is neither insightful nor particularly helpful. It is not particularly insightful because implicitly all evolutionary biologists realize that selection acts on the entire organism, and not on individual traits (Gould and Lewontin, 1979). It is not particularly helpful because this suggestion could be viewed as simply adding yet another consideration to a problem that is already challenging to study. However, I believe that there are ways forward that acknowledge and account for the reality of phenotypic integration without simply adding complexity (DeWitt and Langerhans, 2003).

In particular, throughout this dissertation I have chosen to focus on body size and behavior. This decision was based on three considerations. First, these traits play fundamental roles in determining an individual's interaction with its environment (Werner and Anholt, 1993; de Roos et al., 2003). Second, both traits are implicated in many of the same interactions, including consumer-resource, predator-prey, and host-parasite interactions (Abrams and Rowe, 1996; Hall et al., 2009; Cressler et al., 2010). Third, although it has been demonstrated that behavior could affect size, and size could affect behavior, there was no mechanistic reason to assume that these traits were functionally correlated in such a way that would constrain them to maintain any particular relationship (DeWitt et al., 1999).

I believe that these considerations simplify considerably the problem of phenotypic integration. For example, the field of allometric scaling has determined that a number of traits are tightly, mechanistically correlated with body size (West et al., 2003). This includes basal metabolism, which itself is thought to control many aspects of an individual's interaction with its environment (Brown et al., 2004). Therefore, by studying the evolution of body size and making use of allometries between body size and other traits, we may be able to gain important insights into the evo-

lution of many other other traits, without having to study the evolutionary response of all of these traits simultaneously. The key, therefore, is to isolate traits that will affect similar processes but do not share a mechanistic basis. For such traits, it is necessary to consider multivariate selection, but it is reasonable to expect the evolution of many traits can be predicted based on allometric or other physiological considerations.

Phenotypic evolution in a food web context

One of the results of the analysis in Chapter III was that, in order to produce disruptive selection and the evolution of phenotypic polymorphism, it was necessary for resource, consumer, and predator populations to be dynamically linked to one another (Day et al., 2002). This result indicates that phenotypic evolution becomes more complicated as the environmental context becomes more complex. Since organisms are embedded in complex food webs, it becomes necessary to understand how evolutionary response is shaped by food web context.

One area of research in this vein is the study of trait-mediated indirect effects. These are indirect interactions between two species that arise from trait, rather than density, changes in an intervening species. For example, if a predator induces a change in consumer foraging behavior, this has effects on the resource population, even if there is no change in the consumer population size (Abrams, 1984; Abrams and Rowe, 1996; Bolker et al., 2003). These studies are considering phenotypic plasticity, and so are focused on the dynamical interaction between traits and populations. However, phenotypic plasticity can be thought of as evolution happening on rapid timescales (Abrams, 2005; Hairston et al., 2005; Fussmann et al., 2007). Therefore, in order to understand the evolution of species traits, we must consider not only their direct interactions with predators and resources, but also their indirect interactions with other species.

Consider, for example, the evolution of a trait affecting both resource acquisition and predation risk as the complexity of the food web context increases. As you move from a predator-prey system to a one-predator, two-prey system, the indirect effect of adding a second prey item (an increase in the abundance of predators) causes a parallel shift in traits towards greater defense in both species (Abrams, 2000). If you additionally add a resource that both prey can consume, then the prey species are likely to diverge in trait expression (Abrams and Chen, 2002). Therefore, predicting the response of any particular species depends on an understanding of the broader food web context.

So far, work in this direction has been mainly limited to exploring the evolution of behavioral traits (Bolker et al., 2003), and there has been nothing that exploring multivariate evolution in a food web context. Again, there are ways forward that do not simply add complexity. Studies have shown that many natural food webs are highly modular, comprised of small networks of strongly interacting species (trophic modules; Holt and Polis (1997)) that are weakly coupled to other such modules. Therefore, studying trait evolution within these modules may explain evolution within the larger food web as well (Stouffer, 2010).

Non-optimality approaches

All of the work I have presented in this dissertation, and the vast majority of studies of trait evolution, make predictions regarding optimal trait evolution. This is reasonable from the perspective of natural selection as an optimizing process. Furthermore, the purpose of optimality is not necessarily to predict the outcome of evolution, but rather to understand how selection leads to adaptation (Parker and Smith, 1990; Bull and Wang, 2010). Despite these considerations, with which I agree, it is still the case that natural populations display considerable variation in most traits, and that this variation often does not appear to be simply stochastic

variability around one or a few selective optima (Auld et al., 2010). It is therefore worthwhile to imagine what a non-optimality approach to trait evolution might look like.

One promising research avenue is this direction is the development of individual-based models (Grimm and Railsback, 2005). In such models, it is possible to take explicit account of genetics, sexual reproduction, mutation, and selection. The absence of the first three of these is one of the most common criticisms leveled against optimality models (Orzack and Sober, 2001). However, a problem with individual-based models could be summarized by the idiom "everything but the kitchen sink." That is, individual-based models can quickly become over-specified, including mechanisms for which limited data or understanding exists. Because of this, individual-based models have been most useful in studies of systems that are well-understood empirically (Schmitz, 2000; Ovadia and Schmitz, 2004).

Combining experiment and theory in the study of phenotypic evolution

By combining studies of experimental evolution in well-studied model systems with theoretical modeling, it should be possible to test the predictions of optimality models, in particular the ubiquitous conclusion that the evolutionary outcome hinges on the shapes of the underlying trade-offs, and determine the circumstances under which more complicated models are needed.

Despite the understanding that the shapes of trade-offs are crucial to predicting evolutionary outcomes, there have been very few studies that have attempted to measure these shapes, a situation that has been repeatedly lamented (Abrams, 1984, 1995; Duffy and Forde, 2009; Peacor and Cressler, 2011). By working with well-studied experimental systems, it is possible to directly measure the shapes of these

trade-offs (Hall et al., 2010). Experimental studies of evolution can then be used to test whether the predicted evolutionary outcomes are realized. This would serve as a powerful test of the entire optimality paradigm.

Furthermore, once the mechanisms of evolution are understood for these model systems, it is possible to construct and parameterize both optimality and individual-based models for the system. The concordance of evolutionary predictions could then be quantified and directly tested to identify under what circumstances the two will produce qualitatively different predictions (Bull and Wang, 2010). Integration of experimental work, genetics, and selection is likely to be a prominent research direction in evolutionary biology as the number of experimental evolution systems increases.

Final thoughts

Each of these future directions will provide new and fascinating insights into the process of evolution. To me, the most compelling reason to pursue the study of multivariate evolution and phenotypic integration is the opportunity it provides to bridge research boundaries. The work presented here, for example, serves as a bridge between behavioral ecology and life history theory. The foraging–predation risk trade-off is an idea that has been developed most fully by behavioral ecology, but this work had not considered the consequences of behavioral adaptation for life history evolution. Similarly, life history theory, which is primarily focused on trade-offs, had largely ignored the work coming from behavioral ecology (but see Werner and Gilliam (1984); Skelly and Werner (1990); Ball and Baker (1996) for some early work in this direction). Here I showed that work emerging from each of these fields of investigation has important implications for the other. By demonstrating the conceptual unity of different disciplines, it becomes more likely that we will be able to achieve a more complete understanding of the origin and maintenance of diversity,

from genetics, phenotypes, and species.

APPENDICES

APPENDIX A

Chapter II

Derivation of the growth equation

This derivation follows the derivation of de Roos et al. (1990) very closely; differences between the two derivations are noted. Table A.1 gives the units of the variables and parameters in the derivation.

Energy ingestion is assumed to be proportional to the surface area (which is itself proportional to the square of length, ℓ^2) of an individual. This assumption is well-justified for many species (Kooijman, 2000, pp. 66-71). For an individual of a given size, energy ingestion is further assumed to depend upon the resource density and also on the behavior of the individual according to a modified version of a Type II functional response. Letting $f(R, \alpha)$ be the functional response, the rate of energy ingestion is

$$I(R, \ell, \alpha) = f(R, \alpha)\ell^2. \tag{A.1}$$

The functional response $f(R, \alpha)$ can be derived following the logic developed by Holling (1959). $\alpha(t)$ represents the fraction of total possible foraging time that is

Table A.1: Derivation variables and parameters.

Symbol	Description	Units
Variables		
t	Age	d(ays)
ℓ	Length	mm
α	Fraction of time spent foraging	
κ	Fraction of energy allocated to growth	
Parameters		
R	Resource density	cells ml^{-1}
v_x	Maximum resource intake rate per unit surface area	cells mm^{-2} d^{-1}
v_e	Maximum energy assimilation rate per unit surface area	kcal mm^{-2} d^{-1}
ξ	Functional response shape parameter	ml $cell^{-1}$
ζ	Maintenance requirements per unit volume	kcal mm^{-3} d−1
η	Energy requirement for growth per unit volume	kcal mm^{-3}
ℓ_{max}	Maximum attainable length under unlimited resources	mm
γ	Rate constant of growth	d^{-1}

actually spent foraging. Note that α is not the fraction of *total* time; the underlying assumption is that a fixed amount of an individual's time budget is spent on other processes (mating, brooding, territory defense, etc.), and that this amount does not change. The dependence of α on t reflects the fact that the amount of time spent foraging may change with the age (and physiological state) of an individual.

Let T be the total time possible for foraging; αT is the actual amount of time spent foraging. The remainder of the total possible time $(1 - \alpha)T$ is assumed to be spent engaging in defensive behaviors. Dividing foraging time into its component processes, $\alpha T = T_h + T_s$, where T_h is total time spent handling food items and T_s is the total time spent searching for food. Total time handling food should be $r * h$, where r is the total number of calories ingested and h is the handling time for each calorie. Following Holling, $r = k * R * T_s$, where k is the search efficiency (volume searched per unit time) and R is the resource density (calories per unit area or volume). h and k are also dependent upon the surface area of an individual, as larger individuals handle more food and search a greater area per unit time. Thus,

the number of food items taken per unit of time spent foraging is then

$$\frac{r}{\alpha T} = \frac{kRT_s}{T_h + T_s} = \frac{kRT_s}{kRT_s h + T_s} = \frac{kR}{1 + khR}, \tag{A.2}$$

and the functional response (items taken per unit of time allocated to foraging) is

$$f(R, \alpha) = \frac{r}{T} = \frac{\alpha kR}{1 + khR}. \tag{A.3}$$

To make this formulation equivalent to that of de Roos et al. (1990), make the following substitutions: $k = v_x \xi$ and $h = \frac{1}{v_x}$. v_x and ξ are defined in Table 2.1. Simplifying, the rate of energy ingestion is

$$I(R, \ell, \alpha, t) = v_x \alpha(t) \frac{\xi R}{1 + \xi R} \ell^2. \tag{A.4}$$

Following de Roos et al. (1990), we can express resource ingestion in terms of energy gain by replacing v_x with its analog v_e. v_e/v_x then determines the conversion of ingested algal cells into assimilated energy. Some of the resource ingested is not usuable for growth or reproduction, but must be allocated towards basic metabolic processes (respiration, tissue repair, etc.). These maintenance costs are taken directly out of ingested energy, and are assumed proportional the volume, V, of an individual. ζ is the energy required for maintenance per unit of volume. Once maintenance requirements have been subtracted off, a fraction κ of the remaining energy is allocated towards growth, where η is the energy requirement for growth per unit volume. Then growth in volume can be modelled as:

$$\frac{dv}{dt} = \frac{\kappa}{\eta} \left(v_e f(R, \alpha) V^{2/3} - \zeta V \right), \text{ where} \tag{A.5}$$

$$f(R, \alpha) = \alpha \frac{\xi R}{1 + \xi R}. \tag{A.6}$$

The key difference between this formulation and the formulation of de Roos et al. (1990) is in the placement and interpretation of the parameter κ. The de Roos et al. formulation had κ inside the parentheses, so that maintenance requirements were subtracted only out of the energy allocated towards growth, making their model a net production model (*sensu* Noonburg et al. (1998)). Because of this assumption, if the energy allocated towards growth was not enough to meet basic maintenance requirements, energy had to be reallocated from reproduction towards growth, necessitating a more complicated formulation. With our formulation, maintenance costs are "taken off the top," (a net assimilation model Noonburg et al. (1998)) so that there is no need for energy rechannelling between different life processes. In the event that total energy ingestion is not enough to meet basic maintenance requirements, an individual is assumed to die from starvation. Biologically, this is not completely realistic, as most organisms maintain an energy reserve that can sustain them during periods of low resources, but since resources are constant in these experiments, this simplification doesn't really have any effect.

This equation for growth in volume can be reformulated as an equation for growth in length following the proportionality assumptions $V = \ell^3$ and $V^{2/3} = \ell^2$:

$$\frac{d\ell}{dt} = \frac{\kappa}{3\eta}(v_e f(R,\alpha) - \zeta\ell). \tag{A.7}$$

Factoring out ζ leaves

$$\frac{d\ell}{dt} = \kappa\frac{\zeta}{3\eta}\left(\frac{v_e}{\zeta}f(R,\alpha) - \ell\right). \tag{A.8}$$

Based on the units given in Table 2.1, $\frac{\zeta}{3\eta}$ has units of days^{-1}, so it is a rate, while $\frac{v_e}{\zeta}$ has units of millimeters, so it is a length. We can make the following substitutions: let $\gamma = \frac{\zeta}{3\eta}$ be the rate constant of growth and let $\ell_{max} = \frac{v_e}{\zeta}$ be the maximum possible

size. That leaves us with the following equation for growth:

$$\frac{d\ell}{dt} = \kappa\gamma(\ell_{max} f(R, \alpha) - \ell), \tag{A.9}$$

which is analogous to the von Bertalanffy growth model, a phenomenological model for growth in length to an asymptote Kooijman (2000).

Modeling sexual maturation

The original model of de Roos et al. (1990) assumed that maturation occurred at a fixed size (2.5mm). This was reasonable because the authors also assumed that κ was fixed at 0.3. Since we are allowing $\kappa(t)$ to vary in time, it is inappropriate to think of maturation as occurring at a fixed size. Instead, it is more reasonable to think of maturation as occuring once some investment into germ tissue was met. In de Roos et al. (1990), the authors noted that there must be energy allocated towards maturation; prior to reaching size at maturity, the 0.7 fraction of ingested energy was implicitly assumed to be going towards maturation. Thus, using the original de Roos et al. model, with its constant $\kappa = 0.3$, it is possible to analytically determine the amount of energy allocated towards sexual maturity when maturation occurs at a fixed size. This amount was then set as a threshold in our model; maturation occurred once enough energy had been allocated towards reproduction to meet this threshold. Thereby the timing and size at maturity were free to change with the allocation strategy employed by an individual.

Genetic algorithm details

Genetic algorithms are a computational tool useful for performing optimization (Holland, 1975). Conceptually, genetic algorithms are an approximation of natural

selection; natual selection operates via the biased reproduction of individuals with higher fitness with random variation acting to maintain diversity for selection to act upon. In order for the analogy to hold up and genetic algorithms to work properly, the following components are needed (Mitchell and Taylor, 1999):

1. A population of candidate solutions for the problem to be solved. The solutions are encoded according to a representation scheme. Extending the analogy of natural selection, these solutions represent chromosomes and the units of encoding are genes.

2. A fitness criterion that assigns a numerical value to each chromosome, determining its quality as a solution to the problem at hand.

3. A way of "mating" different candidate solutions to create a new population of solutions, once the current population has been assigned fitnesses. This reproduction scheme typically incorporates elements of selection, mutation, and crossover.

For the given problem of finding the optimal defense strategy, the analogy with natural selection is very natural; the candidate solutions are the $\alpha(t)$ and $\kappa(t)$ functions and the fitness function is R_0, defined in the text and determined by solving the energetics equations. Because of this, it becomes tempting to view the genetic algorithm as modelling evolution. However, these algorithms are designed only as optimization tools, and do not attempt to model the actual process of evolution via natural selection.

Since we are attempting to determine the optimal shapes of the $\alpha(t)$ and $\kappa(t)$ functions, we need a way of encoding these functions. B-splines are particularly useful, as they are flexible enough to take any shape along a given interval of interest and can be encoded as a sequence of points. To define a B-spline, we must first specify a vector known as a *knot vector*:

$$\mathbf{T} = t_0, t_1, ..., t_m$$

These knots span the interval of interest; in this case, the interval is the life span of an individual. A B-spline is a linear combination of *basis functions*, polynomials of degree n that are defined between each knot. The B-spline also has degree n; here $n = 3$.

A B-spline is also characterized by a sequence of points, $\mathbf{P}_0, \mathbf{P}_1, ..., \mathbf{P}_p$, known as *control points*. The control points are the encoding scheme; an individual's $\alpha(t)$ and $\kappa(t)$ functions are specified by the particular sequence of control points. Thus each individual has a unique set of control points. These control points determine the value of the function at any point in the interval specified by the knot vector. Since $\alpha(t)$ and $\kappa(t)$ are proportions between 0 and 1, as long as each control point is between 0 and 1, the B-spline will also only take values between 0 and 1. The number of control points specified depends upon the length of the knot vector and the degree of the B-spline, according to the relation $p = m - n - 1$. The "wiggliness" of the B-spline is controlled by the number of knots and control points. For these experiments, the number of knots was specified to be 28, so each individual was characterized by two vectors of 24 control points determining the $\alpha(t)$ and $\kappa(t)$ functions.

To calculate the value of the B-spline for any point in the interval $[t_0, t_m]$, we must first calculate the value of the basis functions:

$$\begin{aligned} B_{i,0}(t) &= 1 \text{if } t_i \leq t < t_{i+1} \text{ and } t_i < t_{i+1} \\ &= 0 \text{otherwise} \end{aligned} \tag{A.10}$$

$$B_{i,n}(t) = \frac{t - t_i}{t_{i+n} - t_i} N_{i,p-1}(t) + \frac{t_{i+n+1} - t}{t_{i+p+1} - t_{i+1}} N_{i+1,p-1}(t) \tag{A.11}$$

Then the value of the spline at the point t within the interval $[t_0, t_m]$ is (De Boor, 1978):

$$\mathbf{B}(t) = \sum_{i=0}^{p} \mathbf{P}_i B_{i,p}(t) \tag{A.12}$$

In this way, the value of $\alpha(t)$ and $\kappa(t)$ can be determined for any t.

Using this encoding scheme and the fitness function defined by R_0, the genetic algorithm operates in the following way:

1. Upon initialization, the algorithm randomly generates 800 candidate solutions (individuals) who are defined by two vectors of 24 control points specifying the $\alpha(t)$ and $\kappa(t)$ functions, with each control point generated as a random draw from the distribution $U[0, 1]$. The vectors are the "chromosomes" and the control points are the "genes."

2. The energetics equations are solved for each individual and a fitness is assigned to each individual.

3. Select parents to produce the next generation of solutions (described below). The next generation will be of the same size as the current generation.

4. Crossover occurs between the chromosomes of the two parents.

5. Mutation occurs with a fixed probability for each gene in the chromosome.

6. Return to step two for the next generation. This process is repeated for 1000 generations, which was long enough for convergence to occur in all cases. Convergence was determined by calculating the standard deviation of fitness across

all individuals in the population; the standard deviation was less than 0.005 for all runs, and was typically less than 0.0005.

There are many different approaches to selection (outlined in Mitchell (1998)). A good selection method should have a strength of selection that is relatively constant throughout the run of the genetic algorithm. Some methods have the problem that selection is very strong early in the run, and weak late in the run as each individual is more similar. This can lead to premature convergence of the algorithm. To circumvent this problem we used sigma scaling of raw R_0 values (Mitchell, 1998). The expected number of reproductive events for an individual, i, was calculated as

$$\begin{aligned} \mathbf{E}(R_0(i)) &= 1 + \frac{R_0(i) - \bar{R}_0}{2 * \sigma} \text{for } \sigma \neq 0 \\ &= 1 \text{for } \sigma = 0 \end{aligned} \tag{A.13}$$

where $\bar{R}_0$ is the average fitness of all individuals in the population, and σ is the standard deviation of fitness. The scaling works by smoothing out fitness differences when there is a lot of variation, but emphasizing fitness differences when the population is homogeneous. Early in the algorithm, when σ is large, the most fit individuals are not allocated the majority of the reproductive events. When σ is small late in the run, individuals with higher than average fitness stand out more, allowing evolution to continue.

Once individual fitnesses have been scaled to an expected value, individuals are paired as parents according to stochastic universal sampling, which has zeros bias and minimum spread (Mitchell, 1998). This sampling algorithm ensures that every individual will be chosen as a parent no more than $\mathbf{E}(R_0(i)) + 1$ times, and no fewer than $\mathbf{E}(R_0(i))$ times. This also ensures that the size of the population remains constant at 800 each generation. Once paired, each reproductive event creates two new offspring whose chromosomes are generated by crossover between the two parents,

and mutation of the parental genes. For each offspring, a crossover point is chosen along the length of the chromosome; the offspring inherits the control points of opposite parents on opposite sides of the crossover point. Crossover acts to create variation while preserving combinations of control points that yield high fitness. Mutation occurs with fixed probability (0.01); mutations alter the value of the gene by drawing a new gene value from a normal distribution with mean equal to the current gene value and a standard deviation of 0.1.

This entire process is repeated for 1000 generations to determine the optimal solution. For each parameter combination, the algorithm was run 10 times. The results presented in the text show the $\kappa(t)$ and $\alpha(t)$ functions generated by averaging across all individuals and across the replicate runs (800 individuals x 10 runs).

Results for positive size-dependent predation

We also investigated the consequences of positive size-dependent predation on the interaction between tradeoffs. Positive size dependence in predation risk (that is, predators that prefer large prey items) is common in many of the systems referenced in the main text (e.g., *Daphnia* (De Meester et al., 1995), damselflies (McPeek and Peckarsky, 1998), dragonflies (Mikolajewski and Johansson, 2004), and snails (Rundle and Brönmark, 2001)). Previous theory suggests that the life history response to positive size-dependent predation is to reduce allocation to growth versus reproduction, leading to the defense of early maturation at reduced size (Taylor and Gabriel, 1992; Ernande et al., 2004). However, as we show in the main text, a consequence of behavioral defense investment is reduced energy gain, which can lead to reduced size at maturity. Some experimental work has also recognized that predator-induced life history could be a result of changes in physiology or behavior (Ball and Baker, 1996; Beckerman et al., 2007). Thus, understanding how life history and behavior might covary under positive size-dependent predation is also a compelling

question.

To modify the main text equations to consider positive size-dependence, only one change was necessary: instead of size-dependent mortality taking the form

$$1 - \tanh(\omega(\ell - \ell_{\text{mid}})), \tag{A.14}$$

it takes the form

$$1 + \tanh(\omega(\ell - \ell_{\text{mid}})). \tag{A.15}$$

The effect of this change can be seen in Appendix Figure A.1.

To determine the optimal behavior and life history, we followed the same protocol as in the main text. We found the optimal behavior and life history using genetic algorithms, for three experiments: when both activity level and growth allocation were flexible (**LB** experiment), when activity level was flexible but growth allocation was fixed at the optimal predator-free level (**B** experiment), and when growth allocation was flexible but activity level was fixed at the optimal predator-free level (**L** experiment). Again, s was varied between 1 and 10 and P_{max} was varied between 0 and 0.2. Life history defenses were employed whenever size at maturity is smaller than the predator-free value *and* age at maturity is earlier. Behavioral defenses were employed whenever average activity level was less than 1.

Results for $s = 1$

For $s = 1$, both defenses were employed (Figure A.2), although the investment in behavioral defense is relatively weak. This can be seen by the fact that maturation occurs earlier, at smaller size, and activity level is slightly reduced. Recall that for this s value under positive size-dependent predation, only the life history defense was observed. This suggests that life history-only strategies are present only for values of

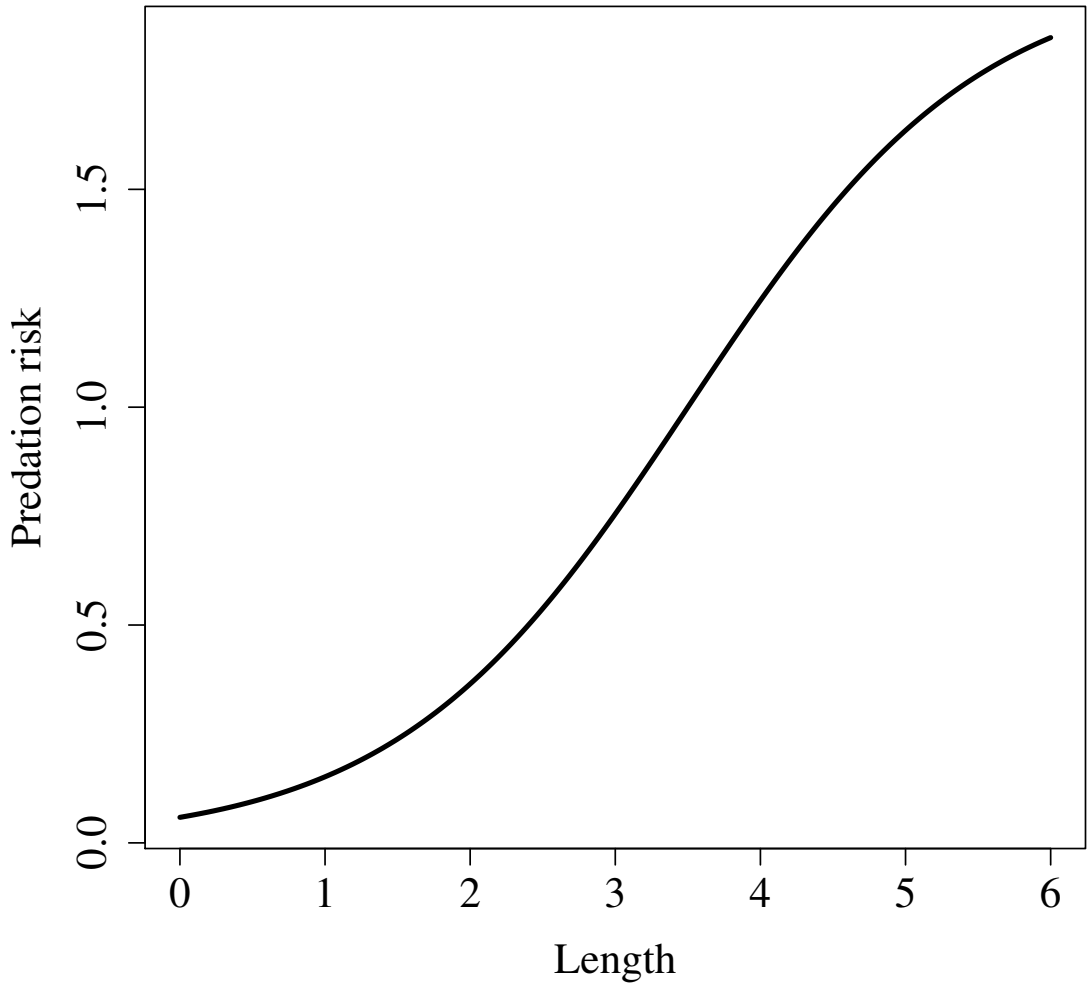

Figure A.1: The scaling of predation rate by length under positive size-dependent mortality risk. $\omega = 0.5$ and $\ell_{\text{mid}} = 3.5$, as in the main text.

s less than 1, that is, where predation rate saturates with increases in activity level.

Results for $s = 2$

For $s = 2$ (a slightly nonlinear relationship between behavior and predation rate), again we see that both defenses are employed, to slighly larger extent.

Results for $s = 3$

Here we see the results starting to change depending upon the maximum predation rate (Figure A.4. At low levels of predation, both defenses are employed. However, as predation rate increases, there is a switch to using only behavioral defenses. This is manifested in the increase in age at maturity as P_{max} increases. Size at maturity is still small, as a consequence of both reduced growth allocation and reduced energy gain.

Results for $s = 10$

For $s = 10$, only the behavioral defense is used (Figure A.5. Predator-induced changes in size at maturity and age at maturity are caused by the reduced activity level and energy intake of the organism, rather than by an altered growth allocation.

Conclusions

Under positive size-dependent predation risk, we still observe both pure behavioral and integrated multi-defense strategies. Pure life history strategies presumably are found in as s is further reduced. The switch to pure behavioral strategies is predicted to happen at lower values of s. The reason for the earlier switch is fairly intuitive. Reduced activity level reduces energy intake; this tends to caused delayed maturation at smaller size. Under negative size-dependent predation, this has multiple costs to the organism: (1) reproduction starts later in life; (2) birth rate is reduced

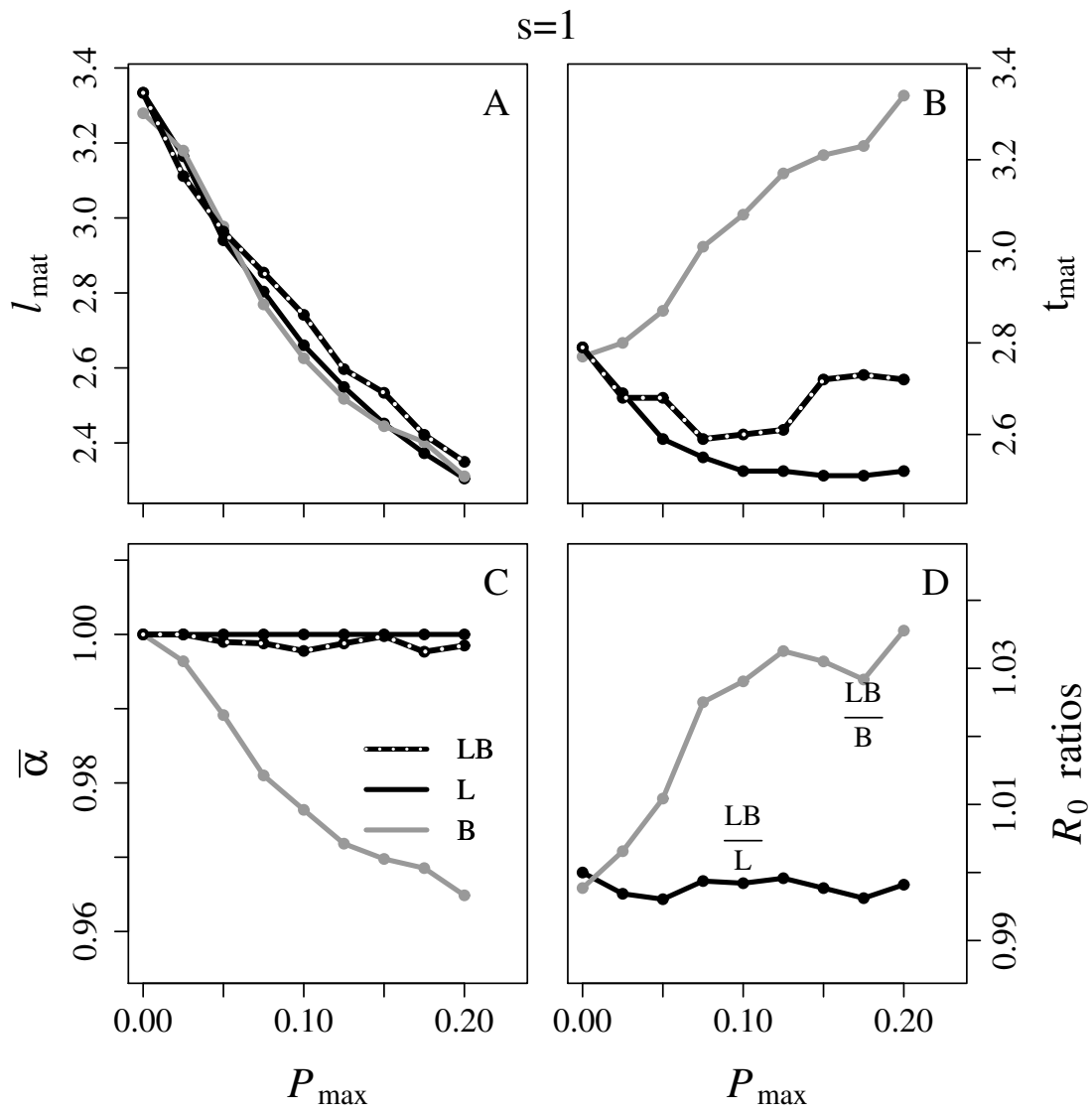

Figure A.2: The optimal behavior and life history for $s = 1$. The optimal multi-defense strategy is shown by the black and white line, the optimal behavior-only strategy is shown by the gray line, and the optimal life history-only strategy is shown by the black line.

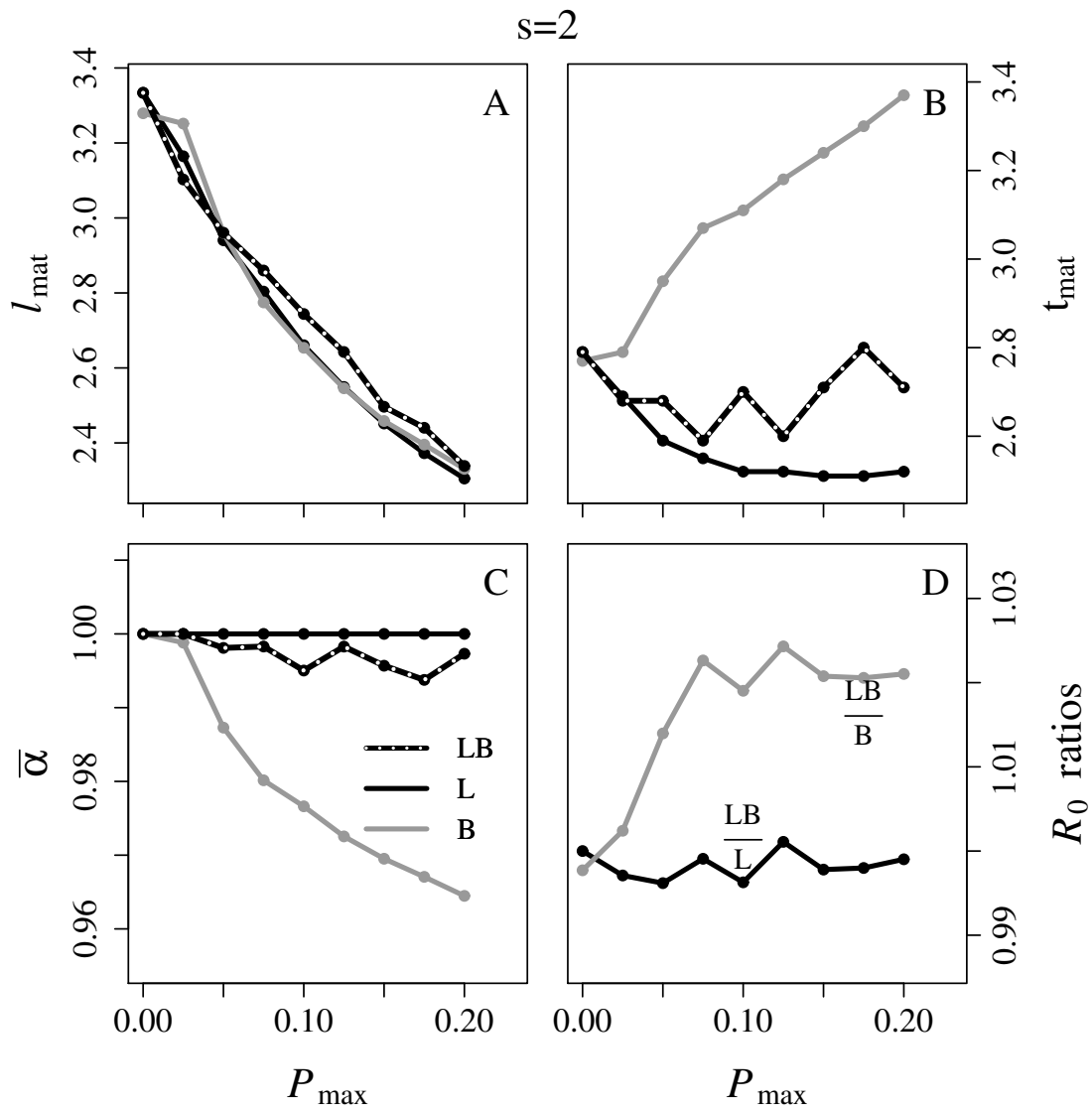

Figure A.3: The optimal behavior and life history for $s = 2$. The optimal multi-defense strategy is shown by the black and white line, the optimal behavior-only strategy is shown by the gray line, and the optimal life history-only strategy is shown by the black line.

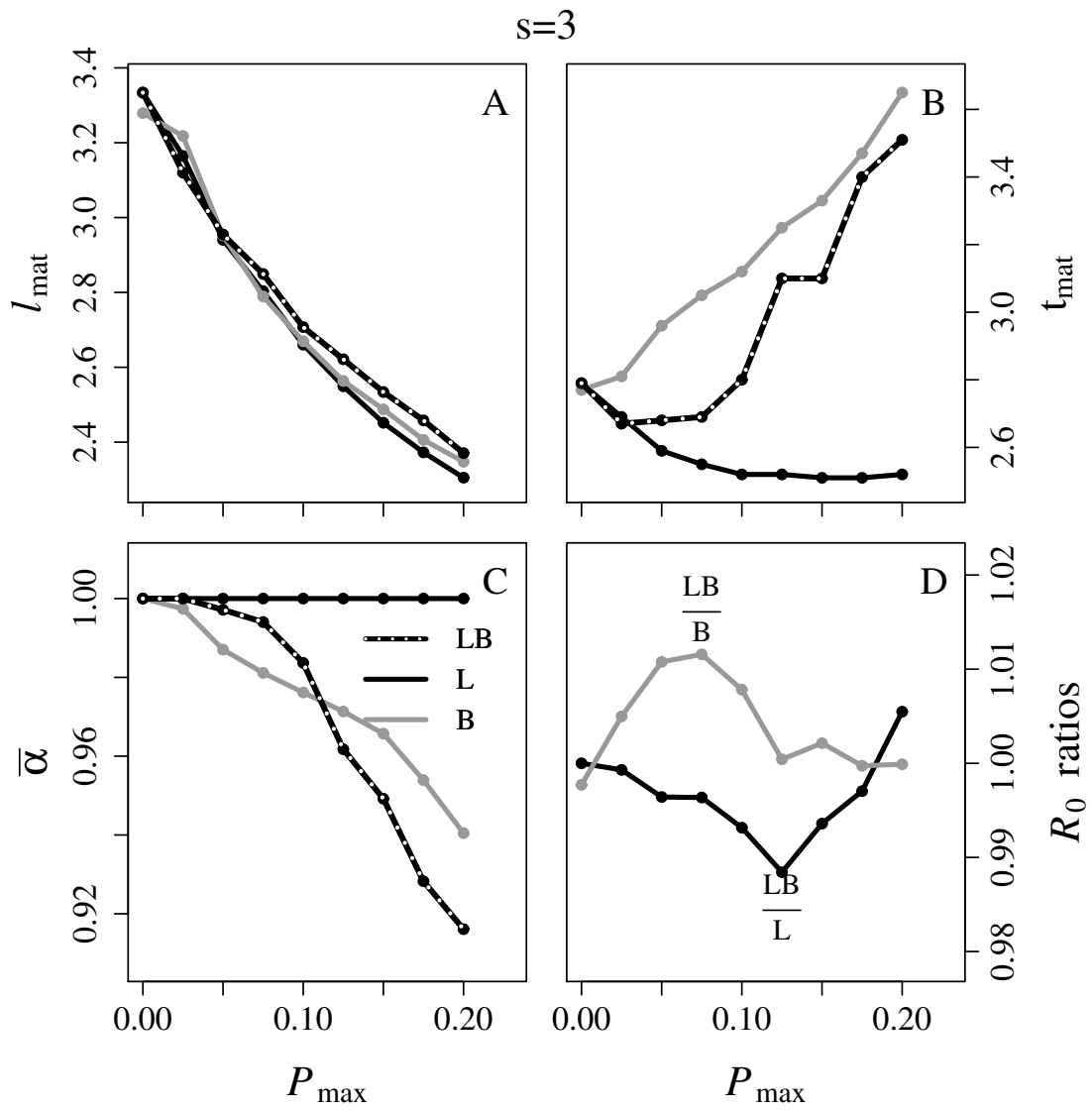

Figure A.4: The optimal behavior and life history for $s = 3$. The optimal multi-defense strategy is shown by the black and white line, the optimal behavior-only strategy is shown by the gray line, and the optimal life history-only strategy is shown by the black line.

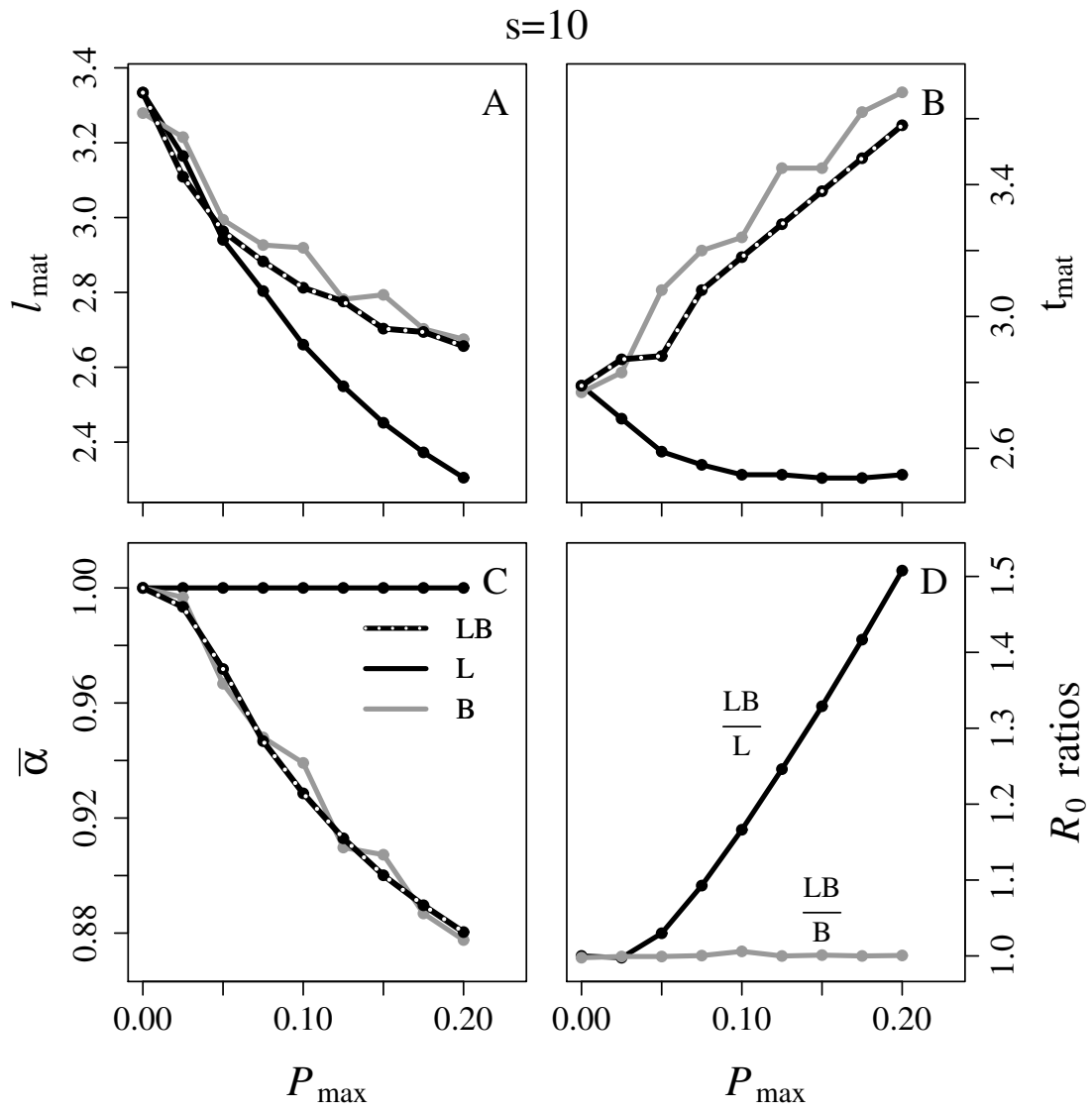

Figure A.5: The optimal behavior and life history for $s = 10$. The optimal multi-defense strategy is shown by the black and white line, the optimal behavior-only strategy is shown by the gray line, and the optimal life history-only strategy is shown by the black line.

because of reduced size; (3) mortality risk is higher because of reduced size. The benefit is, of course, reduced mortality. In order for behavioral defenses to be optimal, this benefit must outweigh all of these costs. However, under positive size-dependent predation, the third cost is not present, and the benefits of activity level reduction need only outweigh the first two costs. The result that integrated multiple-defense strategies are possible can provide alternative explanations for previous experimental work, which has attempted to attribute predator-induced life history patterns to either behavioral or life history defenses, but not both. In particular, Beckerman et al. (2007) found evidence for both defenses contributing to observed life history, but interpreted the results as more strongly supporting the hypothesis that life history was the result of changes in energy allocation.

APPENDIX B

Chapter III

The model as a system of differential equations

Here I show the derivation of a system of ODEs that is equivalent to the equations presented in the text (Nisbet and Gurney, 1983; Nisbet, 1997). To do this, I must define two new state variables that are used to calculate juvenile survivorship and juvenile development and I must derive an equation for the juvenile stage duration.

I will first deal with juvenile development and stage duration, $\tau(t)$. Since $g_J(t)$ is the growth rate of a juvenile, and maturation occurs once an individual has grown from length at birth L_b to length at maturity L_A, it is intuitive that equation (3.7) in the text will hold:

$$\int_{t-\tau(t)}^{t} g_J(\hat{t})d\hat{t} = L_A - L_b. \tag{B.1}$$

I will define a new state variable $\Theta(t)$ such that

$$\Theta(t) = \int_0^t g_J(\hat{t})d\hat{t}. \tag{B.2}$$

Given this definition, $\Theta(t)$ serves as an index of juvenile development (growth). Furthermore, the dynamics of $\Theta(t)$ are described by the differential equation

$$\frac{d\Theta}{dt} = g_J(t). \tag{B.3}$$

From equation (B.1), the following also holds:

$$\Theta(t) - \Theta(t - \tau(t)) = L_A - L_b. \tag{B.4}$$

To arrive at an equation for juvenile stage duration, differentiate this equation with respect to t and use equation (B.3):

$$\frac{d}{dt}\left(\Theta(t) - \Theta(t - \tau(t))\right) = \frac{d}{dt}\left(L_A - L_b\right), \tag{B.5}$$

$$\frac{d\Theta}{dt}(t) + \frac{d\Theta}{dt}(t - \tau(t))\frac{d\tau}{dt} = 1, \tag{B.6}$$

$$\frac{d\tau}{dt} = 1 - \frac{g_J(t)}{g_J(t - \tau(t))}. \tag{B.7}$$

This equation is not sufficient, because it does not describe the dynamics of $\tau(t)$ when $t < \tau(t)$ (that is, when no maturation is occuring, during the first several timesteps; $t < \tau(t)$ is equivalent to $\Theta(t) < L_A - L_b$). If this is the case, $\tau(t)$ grows as t:

$$\frac{d\tau}{dt} = \begin{cases} 1 & \text{if } \Theta(t) < L_A - L_b, \\ 1 - \frac{g_J(t)}{g_J(t-\tau(t))} & \text{otherwise.} \end{cases} \tag{B.8}$$

I must also address juvenile survivorship. For simplicity, define μ_J to be the juvenile mortality rate (equation (3.9) in the main text). Then define the new state variable,

$$\Phi(t) \equiv \int_0^t \mu_J(\hat{t})d\hat{t}. \tag{B.9}$$

Analogous to the variable for development index, this new variable obeys the differential equation

$$\frac{d\Phi}{dt} = \mu_J(t) \tag{B.10}$$

with the initial condition $\Phi(0) = 0$. Then juvenile survivorship, $S(t)$, will be given by $S(t) = \exp(\Phi(t - \tau(t)) - \Phi(t))$.

Therefore, the full system of differential equations is:

(B.11)

$$\frac{dR}{dt} = rR(t)\left(1 - \frac{R(t)}{K}\right) - v_R\left(\frac{\xi R(t)}{1+\xi R(t)}L_A^2 A(t) + \frac{\alpha_J \xi R(t)}{1+\alpha_J \xi R(t)}L_J^2 J(t)\right),$$

(B.12)

$$\frac{dJ}{dt} = R_J(t)A(t) - R_J(t-\tau(t))A(t-\tau(t))\exp(\Phi(t-\tau(t)) - \Phi(t))\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_J(t)J(t),$$

(B.13)

$$\frac{dA}{dt} = R_J(t-\tau(t))A(t-\tau(t))\exp(\Phi(t-\tau(t)) - \Phi(t))\frac{g_J(t)}{g_J(t-\tau(t))} - \mu_A(t)A(t),$$

(B.14)

$$\frac{d\Phi}{dt} = \mu_J(t),$$

(B.15)

$$\frac{d\Theta}{t} = g_J(t),$$

(B.16)

$$\frac{d\tau}{dt} = \begin{cases} 1 & \text{if } \Theta(t) < L_A - L_b, \\ 1 - \frac{g_J(t)}{g_J(t-\tau(t))} & \text{otherwise.} \end{cases}$$

I analyzed this system of differential equations using the function `dde` inside the package **PBSddesolve**. For simplicity, I never initialize the system with juveniles, as this complicates the calculation of adult recruitment $R_A(t)$ considerably (see (Nisbet, 1997)).

APPENDIX C

Chapter IV

Model AIC scores

Model AICc for correlations between habitat characteristics and traits and trait plasticities

Here I present the AIC scores (corrected for small sample size) for the models testing for correlations between habitat characteristics and traits and trait plasticities. I assume either trait evolution according to Brownian motion or Ornstein-Uhlenbeck using the `ouch` functions **brown** or **hansen**.

Table C.1 presents the AICc scores for both models for the correlation between mean habitat characteristics and mean traits. The neutral model is always better supported by AICc (lower AICc scores are "better").

Table C.2 presents the AICc scores for both models for the correlation between variability in habitat characteristics and trait plasticities. Again, the neutral model has lower AICc across all tests.

AICc scores for the three models of trait evolution

Here I report the AICc scores for the three models of evolution for trait means and plasticities. I also report the AICc scores for the studies of correlated evolution

Model	Mean area	Mean depth	Mean drying	Mean canopy	Mean inverts	Mean fish
brown	335.78	23.81	34.97	31.58	56.06	51.22
hansen	1771.27	43.63	55.12	50.77	73.86	66.55
brown	282.23	-28.62	-14.73	-24.29	2.34	-2.83
hansen	2081.17	-9.21	3.25	-6.93	20.26	14.11
brown	264.36	-42.1	-26.58	-33.24	-11.55	-17.01
hansen	1602.39	-31.6	-17.93	-23.25	-3.07	-9.33
brown	299.08	-9.23	7.17	-2.7	19.06	13.39
hansen	861.73	7.68	25.52	13.53	37.97	31.15
brown	276.12	-34.85	-19.45	-27.76	-3.96	-8.72
hansen	1154.56	-21.29	-3.91	-12.85	8.69	1.61
brown	233.37	-3.43	14.85	7.11	17.99	14.13
hansen	397.77	18.35	38.92	30.59	38.07	28.87

Table C.1: AICc scores for each model, testing for correlations between the mean habitat characteristics (along the columns) and mean trait values (along the rows).

Model	CV area	CV depth	CV drying	CV canopy	CV inverts	CV fish
brown	-24.16	-46.98	-25.47	-20.82	-42.24	15.48
hansen	-15.72	-45.53	-15.12	-2.97	-36.9	28.21
brown	-23.17	-46.83	-26.72	-21.84	-44	16.48
hansen	-16.34	-44.62	-18.73	-1.33	-40.66	26.13
brown	-14.02	-37.73	-23.55	-12.7	-32.56	18.32
hansen	-6.6	-35.22	-13.15	7.79	-26.72	32.34
brown	-12.63	-36.27	-14.75	-10.9	-30.64	28.22
hansen	1.24	-29.8	-1.24	11.35	-20.19	41.14
brown	38.51	23.39	38.42	44.28	24.37	69.16
hansen	56.23	33.87	56.86	70.79	38.1	95.29

Table C.2: AICc scores for each model, testing for correlations between the variability in habitat characteristics (along the columns) and trait plasticities (along the rows).

in trait means and plasticities. The three models are Brownian motion, an Ornstein-Uhlenbeck model with a global optimum, and an Ornstein-Uhlenbeck model with four optima corresponding to the four different characterizations of species pond preference (vernal, dragonfly, fish, and multiple).

Additional phylogenetic correlations

Correlation between mean traits and habitat variability

Table C.5 presents the correlation coefficients between mean traits and the variability in habitat characteristics (measured as coefficient of variation). The only significant correlations that emerge from this analysis are significant negative correlations between body size and variability in several habitat measures (pond area, pond depth, invertebrate and fish predation risk). These negative correlations suggest that smaller species are found in more variable habitats, an interpretation that is strengthened by analysis presented in the Results section.

	Brown	OU(1)	OU(4)
Body size	30.78	35.42	28.94
hlen	-24.86	-23.47	-15.98
hdep	-48.85	-50.02	-43.09
tlen	-1.28	-1.96	5.83
tdep	-40.71	-38.97	-33.38
act	6.06	6.80	11.83
tlen plast	-52.28	-70.05	-62.83
tdep plast	-66.15	-69.15	-60.85
hlen plast	-63.24	-62.48	-62.42
hdep plast	-62.87	-57.41	-52.83
act plast	20.95	16.89	13.53

Table C.3: The AICc scores for each model of evolution of trait means and plasticities. Models tested were Brownian motion, a single optimum OU, and a multiple-optimum OU (Figure 4.2 in the main text).

Correlation between trait plasticities and mean habitat characteristics

Table C.6 presents the correlation coefficients between trait plasticities and the mean habitat characteristics. There are three significant correlations in this analysis: positive correlations between both plasticity in tail length and plasticity in behavior and mean proportion of years dry and a negative correlation between tail depth plasticity and pond area. Given that mean proportion of years dry is might better be interpreted as a measure habitat variability, these correlations are meaningful in what they indicate about plasticity. In particular, the positive correlation between tail length plasticity and mean proportion of years dry suggests that species that inhabit ponds that dry more frequently tend to increase the length of their tails in response to predators. I do not know of a biological interpretation for this result. The postive correlation between behavioral plasticity and mean proportion of years dry, on the other hand, is highly interpretable. It suggests that species that inhabit less permanent habitats reduce activity less in the presence of predators that species found in more permanent habitats. This has been observed before, and has been suggested to be an adaptive response to the risk of dessication (Richardson, 2001b).

	Model	Head length	Head depth	Tail length	Tail depth	Activity
Head length	Brown	1	-74.7	-26.83	-63.18	-13.97
	OU(1)	1	-63.87	-14.69	-58.27	-4.81
	OU(4)	1	-50.12	0.73	-40.59	8.96
Head depth	Brown	-116	1	-48.36	-88.74	-40.12
	OU(1)	-131.79	1	-50	-78.83	-26.42
	OU(4)	-113.93	1	-30.28	-62.17	-11.44
Tail length	Brown	-114.04	-128.15	1	-46.48	6.67
	OU(1)	-124.54	-123.46	1	-37.04	17.07
	OU(4)	-113.51	-111.52	1	-24.36	32.29
Tail depth	Brown	-113.23	-127.13	-126.15	1	-44.54
	OU(1)	-121.19	-116.78	-113.5	1	-30.04
	OU(4)	-104.99	-100.36	-102.72	1	-17.14
Activity	Brown	-32.29	-33.4	-35.67	-42.13	1
	OU(1)	-32.69	-33.9	-33.9	-26.1	1
	OU(4)	-28.63	-23.41	-25.33	-16.09	1

Table C.4: Testing for correlated evolution between traits. Above the diagonal are the model AICc scores for each model, looking for correlations between mean trait values. Below the diagonal are the same for correlations between trait plasticities. The neutral model is the best supported for all of the mean trait values, while the adaptive model is best supported for several of the correlations between plasticities.

	CV area	CV depth	CV drying	CV canopy	CV inverts	CV fish
Body size	-0.56 (-0.82, -0.13)	-0.38 (-0.7, 0.01)	0.37 (-0.25, 0.71)	-0.11 (-0.56, 0.35)	-0.57 (-0.84, -0.25)	-0.67 (-0.84, -0.37)
Head length	0.08 (-0.42, 0.45)	0.14 (-0.37, 0.55)	0.3 (-0.16, 0.67)	0.01 (-0.48, 0.39)	0.08 (-0.35, 0.43)	0 (-0.4, 0.45)
Head depth	-0.14 (-0.58, 0.31)	-0.08 (-0.58, 0.39)	-0.27 (-0.68, 0.14)	-0.12 (-0.52, 0.38)	-0.31 (-0.69, 0.05)	0.37 (-0.12, 0.68)
Tail length	-0.1 (-0.56, 0.43)	-0.17 (-0.55, 0.28)	-0.02 (-0.44, 0.44)	-0.21 (-0.62, 0.29)	0.04 (-0.43, 0.49)	-0.06 (-0.48, 0.42)
Tail depth	0.07 (-0.41, 0.47)	0.07 (-0.38, 0.49)	0.1 (-0.35, 0.5)	0.26 (-0.19, 0.62)	0.07 (-0.44, 0.56)	-0.38 (-0.74, 0.07)
Activity	0.19 (-0.41, 0.6)	-0.03 (-0.49, 0.44)	-0.33 (-0.75, 0.06)	-0.17 (-0.62, 0.31)	-0.02 (-0.48, 0.48)	0.16 (-0.31, 0.58)

Table C.5: Correlation between mean trait values and the variability in habitat variables. Values in the parentheses give the 95 percent bootstrap confidence interval.

The tail depth plasticity correlation is also understandable in terms of dessication risk. Assuming that larger ponds are less prone to drying (a reasonable assumption, based on the correlations presented in Table 4.2), this correlation suggests that species found in more permanent habitats tend to have greater decreases in tail depth.

	Mean area	Mean depth	Mean drying	Mean canopy	Mean inverts	Mean fish
Head length	0.13 (-0.32, 0.56)	0.15 (-0.31, 0.57)	-0.34 (-0.68, 0.21)	-0.06 (-0.56, 0.36)	0.04 (-0.36, 0.53)	0.12 (-0.4, 0.55)
Head depth	0.14 (-0.41, 0.57)	0.11 (-0.4, 0.54)	-0.34 (-0.68, 0.14)	-0.34 (-0.72, 0.06)	-0.14 (-0.53, 0.36)	0.15 (-0.34, 0.51)
Tail length	0.23 (-0.23, 0.67)	-0.03 (-0.47, 0.5)	0.71 (0.41, 0.89)	0.24 (-0.26, 0.61)	-0.02 (-0.46, 0.46)	0.09 (-0.32, 0.56)
Tail depth	-0.4 (-0.73, -0.04)	-0.36 (-0.69, 0.11)	-0.24 (-0.66, 0.25)	-0.29 (-0.7, 0.15)	-0.26 (-0.69, 0.19)	-0.18 (-0.58, 0.24)
Activity	0.11 (-0.42, 0.61)	-0.27 (-0.68, 0.19)	0.51 (0, 0.84)	0.05 (-0.48, 0.5)	-0.12 (-0.63, 0.32)	0.07 (-0.49, 0.47)

Table C.6: Correlation between trait plasticities and the magnitude of habitat variables. Values in the parentheses give the 95 percent bootstrap confidence interval.

BIBLIOGRAPHY

Bibliography

Abrahams, M. and Healey, M. A comparison of the willingness of four species of pacific salmon to risk exposure to a predator. *Oikos*, 66:439–446, 1993.

Abrams, P., Matsuda, H. and Harada, Y. Evolutionary unstable fitness maxima and stable fitness minima. *Evolutionary Ecology*, 4:166–175, 1993.

Abrams, P. A. Foraging time optimization and interactions in food webs. *American Naturalist*, 124:80–96, 1984.

Abrams, P. A. Life-history and the relationship between food availability and foraging effort. *Ecology*, 72(4):1242–1252, 1991.

Abrams, P. A. Predators that benefit prey and prey that harm predators - unusual effects of interacting foraging adaptations. *American Naturalist*, 140(4):573–600, 1992.

Abrams, P. A. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *American Naturalist*, 146(1):112–134, 1995.

Abrams, P. A. The evolution of predator-prey interactions: Theory and evidence. *Annual Review Of Ecology And Systematics*, 31:79–105, 2000.

Abrams, P. A. Describing and quantifying interspecific interactions: a commentary on recent approaches. *Oikos*, 94(2):209–218, 2001.

Abrams, P. A. 'adaptive dynamics' vs. 'adaptive dynamics'. *Journal Of Evolutionary Biology*, 18(5):1162–1165, 2005.

Abrams, P. A. and Chen, X. The evolution of traits affecting resource acquisition and predator vulnerability: Character displacement under real and apparent competition. *American Naturalist*, 160(5):692–704, 2002.

Abrams, P. A. and Rowe, L. The effects of predation on the age and size of maturity of prey. *Evolution*, 50(3):1052–1061, 1996.

Agrawal, A. A. Phenotypic plasticity in the interactions and evolution of species. *Science*, 294(5541):321–326, 2001.

Amarasekare, P., Hoopes, M., Mouquet, N. and Holyoak, M. Mechanisms of coexistence in competitive metacommunities. *American Naturalist*, 164(3):310–326, 2004.

Anholt, B. R., Werner, E. E. and Skelly, D. K. Effect of food and predators on the activity of four larval ranid frogs. *Ecology*, 81:3509–3521, 2000.

Anto, J., Majoris, J. and Turingan, R. Prey selection and functional morphology through ontogeny of *Amphipirion clarkii* with a congeneric comparison. *Journal of Fish Biology*, 75:575–590, 2009.

Auld, J. R., Agrawal, A. A. and Relyea, R. A. Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, 277(1681):503–511, 2010.

Ball, S. L. and Baker, R. L. Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? *Ecology*, 77(4):1116–1124, 1996.

Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C. and Rainey, P. B. Experimental evolution of bet hedging. *Nature*, 462(7269):90–U97, 2009.

Beckerman, A. P., Wieski, K. and Baird, D. J. Behavioural versus physiological mediation of life history under predation risk. *Oecologia*, 152(2):335–343, 2007.

Benard, M. F. Survival trade-offs between two predator-induced phenotypes in pacific treefrogs (*Pseudacris regilla*). *Ecology*, 87(2):340–346, 2006.

Benton, T. G. and Grant, A. Evolutionary fitness in ecology: comparing measures of fitness in stochastic, density-dependent environments. *Evolutionary Ecology Research*, 2:769–789, 2000.

Blomberg, S. P. and Garland, T. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology*, 15(6):899–910, 2002.

Boeing, W. J., Ramcharan, C. W. and Riessen, H. P. Clonal variation in depth distribution of daphnia pulex in response to predator kairomones. *Archiv fur Hydrobiologie*, 166(2):241–260, 2006a.

Boeing, W. J., Ramcharan, C. W. and Riessen, H. P. Multiple predator defence strategies in daphnia pulex and their relation to native habitat. *Journal of Plankton Research*, 28(6):571–584, 2006b.

Boersma, M., Spaak, P. and De Meester, L. Predator-mediated plasticity in morphology, life history, and behavior of daphnia: The uncoupling of responses. *American Naturalist*, 152(2):237–248, 1998.

Bolker, B., Holyoak, M., Krivan, V., Rowe, L. and Schmitz, O. Connecting theoretical and empirical studies of trait-mediated interactions. *Ecology*, 84(5):1101–1114, 2003.

Boots, M., Best, A., Miller, M. R. and White, A. The role of ecological feedbacks in the evolution of host defence: what does theory tell us? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1513):27–36, 2009.

Bradshaw, A. D. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13:115–155, 1965.

Britton, T., Anderson, C. L., Jacquet, D., Lundqvist, S. and Bremer, K. Estimating divergence times in large phylogenetic trees. *Systematic Biology*, 56(5):741–752, 2007.

Brodin, T., Mikolajewski, D. J. and Johansson, F. Behavioural and life history effects of predator diet cues during ontogeny in damselfly larvae. *Oecologia*, 148:162–169, 2006.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B. Toward a metabolic theory of ecology. *Ecology*, 85(7):1771–1789, 2004.

Bull, J. J. and Wang, I. N. Optimality models in the age of experimental evolution and genomics. *Journal of Evolutionary Biology*, 23(9):1820–1838, 2010.

Butler, M. and King, A. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *American Naturalist*, 164(6):683–695, 2004.

Cadotte, M. W., Mai, D. V., Jantz, S., Collins, M. D., Keele, M. and Drake, J. A. On testing the competition-colonization tradeoff in a multispecies assemblage. *American Naturalist*, 168(5):704–709, 2006.

Callahan, H. S., Maughan, H. and Steiner, U. K. Phenotypic plasticity, costs of phenotypes, and costs of plasticity: toward an integrative view. *Year In Evolutionary Biology 2008*, 1133:44–66, 2008.

Chase, J. M. To grow or to reproduce? the role of life-history plasticity in food web dynamics. *American Naturalist*, 154(5):571–586, 1999.

Clark, C. W. and Harvell, C. D. Inducible defenses and the allocation of resources - a minimal model. *American Naturalist*, 139(3):521–539, 1992.

Cressler, C. E., King, A. A. and Werner, E. E. Interactions between behavioral and life-history trade-offs in the evolution of integrated predator-defense plasticity. *American Naturalist*, 176:276–288, 2010.

Crowl, T. and Covich, A. Predator-induced life-history shifts in a freshwater snail. *Science*, 247(4945):949–951, 1990.

Day, T., Abrams, P. A. and Chase, J. M. The role of size-specific predation in the evolution and diversification of prey life histories. *Evolution*, 56(5):877–887, 2002.

Day, T. and Taylor, P. D. Evolutionarily stable versus fitness maximizing life histories under frequency-dependent selection. *Proceedings of the Royal Society B: Biological Sciences*, 263(1368):333–338, 1996.

De Boor, C. *A Practical Guide to Splines*. Springer-Verlag, 1978.

De Meester, L., Weider, L. J. and Tollrian, R. Alternative antipredator defenses and genetic-polymorphism in a pelagic predator-prey system. *Nature*, 378(6556):483–485, 1995.

de Roos, A. A gentle introduction to models of physiologically structured populations. In Tuljapurkar, S. and Caswell, H., editors, *Structured Population Models in Marine, Terrestrial and Freshwater Systems*, pages 119–204. Chapman and Hall, New York, 1997.

de Roos, A. and Persson, L. Competition in size-structured populations: mechanisms inducing cohort formation and population cycles. *Theoretical Population Biology*, 63(1):1–16, 2003.

de Roos, A. M., Metz, J. A. J., Evers, E. and Leipoldt, A. A size dependent predator-prey interaction: who pursues whom? *Journal of Mathematical Biology*, 28(6):609–643, 1990.

de Roos, A. M., Persson, L. and McCauley, E. The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. *Ecology Letters*, 6(5):473–487, 2003.

DeWitt, T. J. and Langerhans, R. B. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. *Journal Of Sea Research*, 49(2):143–155, 2003.

DeWitt, T. J., Sih, A. and Hucko, J. A. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Animal Behaviour*, 58:397–407, 1999.

DeWitt, T. J., Sih, A. and Wilson, D. S. Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2):77–81, 1998.

Dieckmann, U., Doebeli, M., Metz, J. A. J. and Tautz, D., editors. *Adaptive Speciation*. Cambridge University Press, 2004.

Doebeli, M. and Dieckmann, U. Evolutionary branching and sympatric speciation caused by different types of ecological interactions. *American Naturalist*, 156:S77–S101, 2000.

Doebeli, M. and Ispolatov, I. Complexity and diversity. *Science*, 328:494–497, 2010.

Duffy, M. A. and Forde, S. E. Ecological feedbacks and the evolution of resistance. *Journal of Animal Ecology*, 78(6):1106–1112, 2009.

Durinx, M., Metz, J. A. J. H. and Meszena, G. Adaptive dynamics for physiologically structured population models. *Journal of Mathematical Biology*, 56(5):673–742, 2008.

Ebert, D. *Ecology, Epidemiology, and Evolution of Parasitism in* Daphnia. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information, 2005. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books.

Edgar, R. C. Muscle: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics*, 5:113, 2004.

Ernande, B., Dieckmann, U. and Heino, M. Adaptive changes in harvested populations: plasticity and evolution of age and size at maturation. *Proceedings of the Royal Society B: Biological Sciences*, 271(1537):415–423, 2004.

Eshel, I. Evolutionary and continuous stability. *Journal of Theoretical Biology*, 103(1):99–111, 1983.

Felsenstein, J. Phylogenies and the comparative method. *American Naturalist*, 125(1):1–15, 1985.

Frost, D. R., Grant, T., Faivovich, J., Bain, R. H., Haas, A., Haddad, C. F. B., De Sa, R. O., Channing, A., Wilkinson, M., Donnellan, S. C., Raxworthy, C. J., Campbell, J. A., Blotto, B. L., Moler, P., Drewes, R. C., Nussbaum, R. A., Lynch, J. D., Green, D. M. and Wheeler, W. C. The amphibian tree of life. *Bulletin of the American Museum of Natural History*, (297):8–370, 2006.

Furnass, T. I. Laboratory experiments on prey selection by perch fry (*Perca fluviatilis*). *Freshwater Biology*, 9(1):33–43, 1979.

Fussmann, G. F., Loreau, M. and Abrams, P. A. Eco-evolutionary dynamics of communities and ecosystems. *Functional Ecology*, 21(3):465–477, 2007.

Gårdmark, A. and Dieckmann, U. Disparate maturation adaptations to size-dependent mortality. *Proceedings of the Royal Society B: Biological Sciences*, 273(1598):2185–2192, 2006.

Garland, T., Harvey, P. H. and Ives, A. R. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology*, 41(1):18–32, 1992.

Garland, T. and Ives, A. R. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist*, 155(3):346–364, 2000.

Geritz, S., Kisdi, E. and Metz, J. Evolutionarily singular strategies and the adaptive growth and branching of the evolutionary tree. *Evolutionary Ecology*, 12:35–57, 1998.

Geritz, S. A. H. Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. *American Naturalist*, 146(5):685–707, 1995.

Gerritsen, J. and Strickler, J. R. Encounter probabilities and community structure in zooplankton: a mathematical model. *Journal of the Fisheries Research Board of Canada*, 34:73–82, 1977.

Getty, T. The maintenance of phenotypic plasticity as a signal detection problem. *American Naturalist*, 148(2):378–385, 1996.

Gould, S. J. and Lewontin, R. C. Spandrels of san marco and the panglossian paradigm: a critique of the adaptationist program. *Proceedings of the Royal Society of London B: Biological Sciences*, 205(1161):581–598, 1979.

Grimm, V. and Railsback, S. F. *Individual-based Modeling and Ecology*. Princeton University Press, 2005.

Hairston, N., Ellner, S., Geber, M., Yoshida, T. and Fox, J. Rapid evolution and the convergence of ecological and evolutionary time. *Ecology Letters*, 8(10):1114–1127, 2005.

Hall, S., Simonis, J., Nisbet, R., Tessier, A. and Cceres, C. Resource ecology of virulence in a planktonic host-parasite system: an explanation using dynamic energy budgets. *American Naturalist*, 174(2):149–162, 2009. PMID: 19527119.

Hall, S. R., Becker, C. R., Duffy, M. A. and C/'aceres, C. E. Variation in resource acquisition and use among host clones creates key epidemiological trade-offs. *American Naturalist*, 176(5):557–565, 2010.

Hammill, E., Kratina, P., Beckerman, A. P. and Anholt, B. R. Precise time interactions between behavioral and morphological defences. *Oikos*, *In press*, 2010.

Hansen, T. Stabilizing selection and the comparative analysis of adaptation. *Evolution*, 51(5):1341–1351, 1997.

Hansen, T. F., Pienaar, J. and Orzack, S. H. A comparative method for study adaptation to a randomly evolving environment. *Evolution*, 62:1965–1977, 2008.

Harvell, C. D. The ecology and evolution of inducible defenses. *Quarterly Review of Biology*, 65(3):323–340, 1990.

Holland, J. H. *Adaptation in Natural and Artificial Systems*. University of Michigan Press, 1975.

Holling, C. S. Some characteristics of simple types of predation and parasitism. *Canadian Entomologist*, 91:385–398, 1959.

Holt, R. and Polis, G. A theoretical framework for intraguild predation. *American Naturalist*, 149(4):745–764, 1997.

Hoverman, J. T., Auld, J. R. and Relyea, R. A. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. *Oecologia*, 144:481–491, 2005.

Hoyle, A., Bowers, R. G., White, A. and Boots, M. The influence of trade-off shape on evolutionary behaviour in classical ecological scenarios. *Journal of Theoretical Biology*, 250(3):498–511, 2008. doi:10.1016/j.jtbi.2007.10.009.

Hua, X., Fu, C. Z., Li, J. T., de Oca, A. N. M. and Wiens, J. J. A revised phylogeny of holarctic treefrogs (genus hyla) based on nuclear and mitochondrial dna sequences. *Herpetologica*, 65(3):246–259, 2009.

Kisdi, E. and Geritz, S. Adaptive dynamics: a framework to model evolution in the ecological theatre. *Journal of Mathematical Biology*, 61:165–169, 2010.

Kishida, O., Trussell, G. C. and Nishimura, K. Geographic variation in a predator-induced defense and its genetic basis. *Ecology*, 88:1948–1954, 2007.

Kolar, C. S. and Wahl, D. H. Daphnid morphology deters fish predators. *Oecologia*, 116(4):556–564, 1998.

Kooijman, S. A. L. M. *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge University Press, 2000.

Kooijman, S. A. L. M. and Metz, J. A. J. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. *Ecotoxicology and Environmental Safety*, 8(3):254–274, 1984.

Law, R. Optimal life histories under age-specific predation. *American Naturalist*, 114(3):399–417, 1979.

Leibold, M. A., Tessier, A. J. and West, C. T. Genetic, acclimatization, and ontogenic effects on habitat selection behavior in daphnia-pulicaria. *Evolution*, 48(4):1324–1332, 1994.

Leimar, O. The evolution of phenotypic polymorphism: Randomized strategies versus evolutionary branching. *American Naturalist*, 165(6):669–681, 2005.

Leimar, O. Multidimensional convergence stability. *Evolutionary Ecology Research*, 11(2):191–208, 2009.

Levins, R. and Culver, D. Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences of the United States of America*, 68(6):1246–1248, 1971.

Lima, S. L. and Dill, L. M. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, 68(4):619–640, 1990.

Martins, E. P. and Hansen, T. F. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist*, 149(4):646–667, 1997.

McCauley, E., Nelson, W. and Nisbet, R. Small-amplitude cycles emerge from stage-structured interactions in daphnia-algal systems. *Nature*, 455:1240–1243, 2008.

McCollum, S. A. and VanBuskirk, J. Costs and benefits of a predator-induced polyphenism in the gray treefrog hyla chrysoscelis. *Evolution*, 50(2):583–593, 1996.

McPeek, M. A. and Peckarsky, B. L. Life histories and the strengths of species interactions: Combining mortality, growth, and fecundity effects. *Ecology*, 79(3):867–879, 1998.

Metz, J. A. J., Nisbet, R. M. and Geritz, S. A. H. How should we define 'fitness' for general ecological scenarios? *Trends in Ecology & Evolution*, 7:198–202, 1992.

Mikolajewski, D. J. and Johansson, F. Morphological and behavioral defenses in dragonfly larvae: trait compensation and cospecialization. *Behavioral Ecology*, 15(4):614–620, 2004.

Miner, B. G., Sultan, S. E., Morgan, S. G., Padilla, D. K. and Relyea, R. A. Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, 20(12):685–692, 2005.

Mitchell, M. *An Introduction to Genetic Algorithms*. MIT Press, 1998.

Mitchell, M. and Taylor, C. E. Evolutionary computation: An overview. *Annual Review of Ecology and Systematics*, 30:593–616, 1999.

Moran, N. A. The evolutionary maintenance of alternative phenotypes. *American Naturalist*, 139(5):971–989, 1992.

Nisbet, R. Delay-differential equations for structured populations. In Tuljapurkar, S. and Caswell, H., editors, *Structured Population Models in Marine, Terrestrial and Freshwater Systems*, pages 89–118. Chapman and Hall, New York, 1997.

Nisbet, R. M. and Gurney, W. S. C. The systematic formulation of population-models for insects with dynamically varying instar duration. *Theoretical Population Biology*, 23:114–135, 1983.

Noonburg, E. G. and Nisbet, R. M. Behavioural and physiological responses to food availability and predation risk. *Evolutionary Ecology Research*, 7(1):89–104, 2005.

Noonburg, E. G., Nisbet, R. M., McCauley, E., Gurney, W. S. C., Murdoch, W. W. and de Roos, A. M. Experimental testing of dynamic energy budget models. *Functional Ecology*, 12:211–222, 1998.

O'Keefe, T. C., Brewer, M. C. and Dodson, S. I. Swimming behavior of *Daphnia*: its role in determining predation risk. *Journal of Plankton Research*, 20(5):973–984, 1998.

Orzack, S. H. and Sober, E., editors. *Adaptationism and Optimality*. Cambridge University Press, 2001.

Ovadia, O. and Schmitz, O. Scaling from individuals to food webs: The role of size-dependent responses of prey to predation risk. *Israel Journal of Zoology*, 50(2-3):273–297, 2004.

Pangle, K. L. and Peacor, S. D. Non-lethal effect of the invasive predator bythotrephes longimanus on daphnia mendotae. *Freshwater Biology*, 51(6):1070–1078, 2006.

Parker, G. A. and Smith, J. M. Optimality theory in evolutionary biology. *Nature*, 348(6296):27–33, 1990.

Peacor, S. D. and Cressler, C. E. The implications of adaptive prey behavior for ecological communities: a review of current theory. In Ohgushi, T., Schmitz, O. and Holt, R. D., editors, *Evolution and Ecology of Trait-Mediated Indirect Interactions: Linking Evolution, Commmunity, and Ecosystem*. Cambridge University Press, 2011.

Peterson, W. T. and Ausubel, S. J. Diets and selective feeding by larvae of atlantic mackerel *Scomber scombrus* on zooplankton. *Marine Ecology Progress Series*, 17(1):65–75, 1984.

Pettersson, L. B., Nilsson, P. A. and Brönmark, C. Predator recognition and defence strategies in crucian carp, *Carassius carassius*. *Oikos*, 88(1):200–212, 2000.

Pigliucci, M. *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, 2001.

Pigliucci, M. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters*, 6(3):265–272, 2003.

Rees, M. Community structure in sand dune annuals - is seed weight a key quantity? *Journal of Ecology*, 83(5):857–863, 1995.

Rees, M. and Westoby, M. Game-theoretical evolution of seed mass in multi-species ecological models. *Oikos*, 78(1):116–126, 1997.

Relyea, R. A. Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology*, 82(2):523–540, 2001.

Relyea, R. A. Costs of phenotypic plasticity. *American Naturalist*, 159(3):272–282, 2002.

Relyea, R. A. How prey respond to combined predators: a review and an empirical test. *Ecology*, 84:1827–1839, 2003a.

Relyea, R. A. Predators come and predators go: the reversibility of predator-induced traits. *Ecology*, 84:1840–1848, 2003b.

Relyea, R. A. Fine-tuned phenotypes: Tadpole plasticity under 16 combinations of predators and competitors. *Ecology*, 85(1):172–179, 2004a.

Relyea, R. A. Integrating phenotypic plasticity when death is on the line. In Pigliucci, M. and Preston, K., editors, *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes*. Oxford University Press, 2004b.

Relyea, R. A. and Werner, E. E. Morphological plasticity in four larval anurans distributed along an environmental gradient. *Copeia*, 2000(1):178–190, 2000.

Richardson, J. M. L. A comparative study of activity levels in larval anurans and response to the presence of different predators. *Behavioral Ecology*, 12(1):51–58, 2001a.

Richardson, J. M. L. The relative roles of adaptation and phylogeny in determination of larval traits in diversifying anuran lineages. *American Naturalist*, 157(3):282–299, 2001b.

Richardson, J. M. L. A comparative study of phenotypic traits related to resource utilization in anuran communities. *Evolutionary Ecology*, 16(2):101–122, 2002.

Richter-Boix, A., Llorente, G. A. and Montori, A. A comparative study of predator-induced phenotype in tadpoles across a pond permanency gradient. *Hydrobiologia*, 583:43–56, 2007.

Rinke, K., Hulsmann, S. and Mooij, W. M. Energetic costs, underlying resource allocation patterns, and adaptive value of predator-induced life-history shifts. *Oikos*, 117(2):273–285, 2008.

Roff, D. A. *The Evolution of Life Histories: Theory and Analysis*. Chapman and Hall, New York, 1992.

Roff, D. A. Age and size at maturity. In *Evolutionary Ecology: Concepts and Case Studies*. Oxford University Press, 2001.

Rowe, L. and Ludwig, D. Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology*, 72:413–427, 1991.

Rundle, S. D. and Brönmark, C. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. *Proceedings of the Royal Society B: Biological Sciences*, 268(1475):1463–1468, 2001.

Sarno, R. J. and Gubanich, A. A. Prey selection by wild american kestrels: the influence of prey size and activity. *Journal of Raptor Research*, 29(2):123–126, 1995.

Schmitz, O. J. Combining field experiments and individual-based modeling to identify the dynamically relevant organizational scale in a field system. *Oikos*, 89(3):471–484, 2000.

Shertzer, K. W. and Ellner, S. P. State-dependent energy allocation in variable environments: Life history evolution of a rotifer. *Ecology*, 83(8):2181–2193, 2002.

Skelly, D. K., Freidenburg, L. K. and Kiesecker, J. M. Forest canopy and the performance of larval amphibians. *Ecology*, 83(4):983–992, 2002.

Skelly, D. K. and Werner, E. E. Behavioral and life-historical responses of larval american toads to an odonate predator. *Ecology*, 71(6):2313–2322, 1990.

Slatkin, M. Hedging one's evolutionary bets. *Nature*, 250(5469):704–705, 1974.

Smith, S. A., de Oca, A. N. M., Reeder, T. W. and Wiens, J. J. A phylogenetic perspective on elevational species richness patterns in middle american treefrogs: Why so few species in lowland tropical rainforests? *Evolution*, 61(5):1188–1207, 2007.

Snyder, R. L. Some prey preference factors for a red-tailed hawk. *Auk*, 92(3):547–552, 1975.

Stamatakis, A. Raxml-vi-hpc: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21):2688–2690, 2006.

Steiner, U. K. and Pfeiffer, T. Optimizing time and resource allocation trade-offs for investment into morphological and behavioral defense. *American Naturalist*, 169(1):118–129, 2007.

Stock, M., Sicilia, A., Belfiore, N. M., Buckley, D., Lo Brutto, S., Lo Valvo, M. and Arculeo, M. Post-messinian evolutionary relationships across the sicilian channel: Mitochondrial and nuclear markers link a new green toad from sicily to african relatives. *BMC Evolutionary Biology*, 8:56, 2008. doi:10.1186/1471-2148-8-56.

Stouffer, D. B. Scaling from individuals to networks in food webs. *Functional Ecology*, 24(1):44–51, 2010.

Strand, E., Huse, G. and Giske, J. Artificial evolution of life history and behavior. *American Naturalist*, 159(6):624–644, 2002.

Taylor, B. E. and Gabriel, W. To grow or not to grow: optimal resource allocation for *Daphnia*. *American Naturalist*, 139(2):248–266, 1992.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. Clustal-w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22):4673–4680, 1994.

Tollrian, R. and Dodson, S. I. Inducible defenses in cladocera: Constrainst, costs, and multipredator environments. In Tollrian, R. and Harvell, C., editors, *The Ecology and Evolution of Inducible Defenses*, pages 177–202. Princeton University Press, 1999.

Tollrian, R. and Harvell, C., editors. *The Ecology and Evolution of Inducible Defenses*. Princeton University Press, 1999.

Turnbull, L. A., Rees, M. and Crawley, M. J. Seed mass and the competition/colonization trade-off: a sowing experiment. *Journal of Ecology*, 87(5):899–912, 1999.

Urban, M. The growth-predation risk trade-off under a growing gape-limited predation threat. *Ecology*, 88:2587–2597, 2007a.

Urban, M. C. Risky prey behavior evolves in risky habitats. *Proceedings of the National Academy of Sciences of the United States of America*, 104(36):14377–14382, 2007b.

Utne-Palm, A. C. Prey visibility, activity, size and catchability's (evasiveness) influence on *Gobiusculus flavescens* prey choice. *Sarsia*, 85(2):157–165, 2000.

Van Bocxlaer, I., Biju, S. D., Loader, S. P. and Bossuyt, F. Toad radiation reveals into-india dispersal as a source of endemism in the western ghats-sri lanka biodiversity hotspot. *BMC Evolutionary Biology*, 9:131, 2009.

Van Buskirk, J. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *American Naturalist*, 160:87–102, 2002. doi:10.1086/340599.

Van Buskirk, J. Local and landscape influence on amphibian occurrence and abundance. *Ecology*, 86(7):1936–1947, 2005.

Van Buskirk, J. Natural variation in morphology of larval amphibians: Phenotypic plasticity in nature? *Ecological Monographs*, 79(4):681–705, 2009.

Van Buskirk, J. and Arioli, M. Habitat specialization and adaptive phenotypic divergence of anuran populations. *Journal of Evolutionary Biology*, 18(3):596–608, 2005. doi:10.1111/j.1420-9101.2004.00869.x.

Van Buskirk, J., McCollum, S. A. and Werner, E. E. Natural selection for environmentally induced phenotypes in tadpoles. *Evolution*, 51(6):1983–1992, 1997.

Waxman, D. and Gavrilets, S. 20 questions on adaptive dynamics. *Journal of Evolutionary Biology*, 18(5):1139–1154, 2005.

Wellborn, G. A., Skelly, D. K. and Werner, E. E. Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, 27:337–363, 1996.

Werner, E. and Peacor, S. A review of trait-mediated indirect interactions in ecological communities. *Ecology*, 84(5):1083–1100, 2003.

Werner, E. E. and Anholt, B. R. Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *American Naturalist*, 142(2):242–272, 1993.

Werner, E. E. and Gilliam, J. F. The ontogenetic niche and species interactions in size structured populations. *Annual Review of Ecology and Systematics*, 15:393–425, 1984.

Werner, E. E. and Glennemeier, K. S. Influence of forest canopy cover on the breeding pond distributions of several amphibian species. *Copeia*, 1999(1):1–12, 1999.

Werner, E. E., Skelly, D. K., Relyea, R. A. and Yurewicz, K. L. Amphibian species richness across environmental gradients. *Oikos*, 116(10):1697–1712, 2007a.

Werner, E. E., Yurewicz, K. L., Skelly, D. K. and Relyea, R. A. Turnover in an amphibian metacommunity: the role of local and regional factors. *Oikos*, 116(10):1713–1725, 2007b.

West, G. B., Savage, V. M., Gillooly, J., Enquist, B. J., Woodruff, W. H. and Brown, J. H. Why does metabolic rate scale with body size? *Nature*, 421(6924):713–713, 2003.

Wiens, J. J., Sukumaran, J., Pyron, R. A. and Brown, R. M. Evolutionary and biogeographic origins of high tropical diversity in old world frogs (ranidae). *Evolution*, 63(5):1217–1231, 2009.

Wilbur, H. M. Experimental ecology of food webs: complex systems in temporary ponds. *Ecology*, 78(8):2279–2302, 1997.

Wright, D. I. and O' Brien, W. J. Differential location of *Chaoborus* larvae and *Daphnia* by fish: the importance of motion and visible size. *American Midland Naturalist*, 108(1):68–73, 1982.