LIFE-HISTORY STRATEGIES OF ORGANISMS WITH COMPLEX LIFE-CYCLES AND TEMPORARY LARVAL HABITATS:

The Fire Salamander as a Model

ASAF SADEH

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

University of Haifa

Faculty of Natural Sciences

Department of Evolutionary & Environmental Biology

August 2011

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Recommended by: _____ Date: 23.8.2011
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ACKNOWLEDGEMENTS

The completion of this thesis was enabled first and foremost thanks to the dedicated guidance, care and support of my advisor and coauthor, Leon Blaustein. Leon gave me the freedom and support to explore my own way in research, even when it took time or was different than his own way. He pointed out for me many roadblocks ahead of time – in my choice of goals and research questions, in experimental design, and in manuscript writing. I learned a lot from these advices when I heeded them, and even more when I didn't. I hope I also picked some of his remarkable kindness and lighthearted approach to the difficulties and less enjoyable sides of academic life. Hopefully, the full extent of my gratitude will become apparent in our continued friendship and future collaboration.

I was fortunate to spend three months of remarkable intellectual growth at UC Santa Cruz, learning from Marc Mangel the principles of dynamic state modeling and acquiring basic literacy in theoretical biology. While the fruit of these skills have not ripened in time to be included in this thesis, they continue to develop and have been aiding my subsequent work. I am deeply grateful for Marc's generous and patient teaching that opened a new window for me to theoretical analysis and changed the way I see biological problems, and for his support and involvement from afar in my research projects and academic decisions.

It is thanks to Ofer Ovadia that I started my PhD in the first place, and embarked on a scientific career. He is the first to deserve the title of being my "academic father" for two crucial reasons. First, he initiated my irreversible shift of passion from plant physiology to evolutionary and behavioral ecology. More importantly, he guided my metamorphosis from an inquisitive junior undergrad, eager to learn in classes and from textbooks, into a researcher who is able to come up with a question, design experiments to answer it, analyze the results, and finally generalize them, write up the study and publish it.

I owe many thanks to my labmates in Leon's lab: Shai Markman, Shirly Bar-David, Alon Silberbush, Ori Segev, Nir Peleg, Iftach Sinai, Lior Blank, and especially to Arik Kershenbaum. I also thank Tamar Keasar for allowing me to join the interesting multi-lab meetings that she organized, and for letting me play with parasitoid wasps in her lab and bat ideas with her.

I am grateful to the faculty and staff of the Department of Evolutionary & Environmental Biology, and for the Advanced Studies staff at the University of Haifa for

providing me with all the available resources so that I could do my best in my PhD research. In particular, I'd like to thank Moshe Inbar and Ido Izhaki for their quiet support that did not go unnoticed, and Amots Dafni for always keeping his door open and kettle boiling for me to discuss plant ecology, poetry and the challenges of academic life, all spiced with his *bons mots* and humorous anecdotes.

The PhD track can be a test of friendship, and two precious friends, Hagai Shemesh and Noam Elron, withstood it and even helped me in my research – discussing ideas and experimental results and occasionally joining me to the field in rainy nights. More importantly, they kept me company throughout my journey. It is hard to say how much good friends matter.

Finally, my greatest gratitude is due to my family, without which I would not be me. To my father, Amos, who imbibed me with the love of nature and of scientific thinking, and to my uncle, 'Dodyo', who inspired a philosophical inclination in me. It is probably the combination of these influences that led me to the kind of research that I do today. Above all, there is my wife, Noa, and daughter, Rimon, who have given me the greatest gifts one can have of loving and being loved.

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ABSTRACT

Organisms with complex life cycles display dramatic switches in habitat conditions and ecological niches when proceeding from one stage to another. They comprise the majority of multicellular species on Earth, and many of them are important to humankind agriculturally, medically or environmentally. Understanding common mechanisms behind the ecological patterns and evolutionary processes acting on these species will both enhance our basic understanding of life and evolution, and allow us to successfully manage populations of interest. Due to their strict stage-specific habitat separation, organisms with complex life cycles and discrete larval habitats are excellent systems for teasing apart the contributions of migration, survival and reproductive success to the observed distribution and abundance of species. The survival, migration and oviposition habitat selection of the adults influence the patterns and limits of species distributions, while the larval ecology, survival, growth and development strongly influence the adults' reproductive success and determine local patterns of recruitment to adult populations.

The evolution of oviposition habitat selection is shaped in large part by the expected performance of offspring in their larval habitats, and is predicted to be responsive to the ecological factors that determine the quality of a specific larval habitat. Due to the interactive nature of different factors of the larval ecology, the responsiveness of oviposition behavior may be both context-dependent and temporally variable.

Once oviposition habitat selection has been made, larvae are confined to their habitats and have to cope with the conditions therein until they metamorphose. These conditions, including competition for food, predation and cannibalism, social structure, size structure and intercohort priority effects may vary temporally and influence larval individual and social behaviors, developmental strategies and metamorphic success, especially in ephemeral habitats.

I studied life history strategies of the fire salamander (*Salamandra infraimmaculata*) as a model for organisms with complex life cycles and ephemeral larval habitats. I explicitly addressed temporal aspects of the ecology of larval habitats, specifically focusing on habitat ephemerality, repeated colonizations and the time interval between them, and their effects on larval behavior and development patterns as well as on adult oviposition behavior. The specific questions that I asked and the studies designed to answer these questions were:

1. Do gravid fire salamander females select their larviposition site according to the expected trophic function of their progeny, depending on their order of arrival?

   I manipulated rates of larval cannibalism using the degree of habitat structural complexity to determine their differential effects on two larval cohorts. This experiment verified that structural complexity reduces cannibalism rates, and found that this reduction improved the survival of the late cohort but delayed the time to metamorphosis of the first cohort. In an oviposition habitat selection experiment, I allowed gravid females at different positions in the order of colonization to choose the level of structural complexity of larval habitats. Females facing conspecific-free pools preferred structurally simple habitats, while females facing only pools with older conspecific larvae preferred complex habitats.

2. How does kin-selective cannibalistic behavior affect the performance of larval cohorts under time and food limitations?

   I manipulated the genetic heterogeneity of kin-discriminating larval cohorts using single or mixed sibships, grown in mesocosms with poor food resources and short hydroperiod. While rates of cannibalism were higher in mixed cohorts, overall survival was similar between treatments. Furthermore, rates of metamorphosis in the mixed cohorts were higher than those in the sibling cohorts. Correlation analyses show that initial body size variation does not explain these results. Thus, the better overall performance of mixed cohorts compared to sibling cohorts may be due to higher cannibalism rates.

3. How does the time interval between larval cohorts influence the performance of each of the cohorts? Specifically, how do the intensities of intercohort competition and cannibalism vary as a function of intercohort time interval? Does this shape superoviposition decisions (self vs. conspecific) of gravid females as a function of intercohort time interval?

   I manipulated the time interval between two cohorts as well as larval densities in a

factorial mesocosm experiment, and followed the performance of both cohorts. I showed that the strength of competition experienced by the older, dominant cohort reduces with intercohort time interval. Further support is lent to previous findings that rates of cannibalism upon younger cohorts increase with intercohort time interval. In this experiment, the relative strength of density-dependent exploitative competition was greater than that of interference and cannibalism, and lead to considerable metamorphic failures. I also conducted sets of oviposition habitat selection trials at different intercohort time intervals, allowing females to choose between pools with their own previously deposited larvae (self superoviposition) vs. pools with larvae deposited by unrelated females (conspecific superoviposition). Salamander females demonstrated a seemingly maladaptive indifference to the genetic relatedness of prior cohorts. Since previous studies showed that females are capable of detecting the presence of cannibalistic larvae, and that larvae are capable of kin recognition, this may indicate an ontogenetic constraint on kin recognition.

4. Do larvae sense the recent desiccation of conspecifics from previous cohorts as an early cue of their habitat's duration? Do they respond adaptively by altering their developmental rates to increase their probability of metamorphosing before their habitat desiccates? Is developmental plasticity ontogenetically limited, i.e. can the larvae update their rates of development according to later cues that indicate the habitat's actual duration more reliably, to the point of complete compensation? In case compensatory development occurs, does it carry endogenous developmental costs? I allowed newborn larvae to grow in laboratory pools in the presence or absence of the remains of desiccated conspecifics. This manipulation was crossed with a manipulation of actual water level regimes (constant or decreasing) resulting in consistent or contradictory cues. I followed the developmental trajectories of the larvae as well as their patterns of mortality prior to metamorphosis. I found that larvae respond early in ontogeny to dried conspecifics as a cue for future desiccation, but can fully compensate for this response in case more reliable but contradictory cues are later perceived. Patterns of mortality suggested that endogenous costs may depend on instantaneous rates of development, and revealed asymmetrical costs of compensatory development between false positive and false negative early information. I used the empirical results to model the costs of development rates as a fundamental phenotype production cost, and analyze the resulting tradeoff between production costs of developmental plasticity with phenotype-environment mismatch costs. The model

predicts the pattern of ontogenetic loss of plasticity, commonly observed in many organisms.

This thesis demonstrates that an explicit temporal approach to analyzing ecological processes may potentially yield important insights on the biology of the studied organism, including time-dependent asymmetric interactions among individuals in a population, complex oviposition behaviors and mechanisms of larval developmental plasticity.

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GENERAL INTRODUCTION

"All his days he eateth in darkness. For four years he burrowed in the ground, and now he has four weeks to sing in the sweet light of the sun. Is it any wonder that he is so lusty and loud?"
"Does the cicada remember its four years underground? Or the pretty swallowtail – does it recall having been a clumsy caterpillar on leaves of rue?"
"The pupal period is not just a stage of maturation and quiet readying for a new life. It is also one of forgetting and oblivion, an impenetrable screen between the larva and the imago, those two so contradictory life phases of a single creature."

--- Pinness, in *The Blue Mountain*. Meir Shalev (1988)

1.1 ORGANISMS WITH COMPLEX LIFE CYCLES

Organisms with complex life cycles comprise the majority of species on Earth, including insects, amphibians, aquatic and terrestrial vertebrates and invertebrates. Many of these species are important to humankind, acting as potential agricultural pests or biocontrol agents, parasites of humans and livestock, vectors of disease, or endangered species in need of protection. Understanding common mechanisms behind the ecological patterns and evolutionary processes acting on these species will both enhance our basic understanding of life and evolution, and allow us to successfully manage populations of interest, either down-controlling or supporting them.

Organisms with complex life cycles display dramatic switches in habitat conditions and ecological niches when proceeding from one stage to another (Istock 1967). A common characteristic in many of these organisms is that the larval stages inhabit discrete, confined habitats. Often these habitats are limited in duration, either as an intrinsic property (e.g. ephemeral ponds, tidal pools, carrion, etc.) or because of the irreversible exploitation of some resource by the inhabiting larvae (e.g. hosts of parasitoids and phytophagous insects, fallen fruit, etc.). In most of these cases, the main challenges facing the larvae are surviving and growing, and completing the developmental process required for proceeding into the next life history stage before their habitats terminate. Another common characteristic in these organisms is that post-larval stages tend to be relatively

free-moving in continuous habitats. This results in the ability of adults to access multiple potential larval habitats, and choose among them for their progeny (Resetarits 1996).

Three factors – migration, survival and reproductive success – contribute to observed distributions and abundances of a particular species. Organisms with complex life cycles and discrete larval habitats are excellent systems for teasing apart the contributions of these factors due to their strict stage-specific habitat separation. The survival, migration and oviposition habitat selection of the adults influence the patterns and limits of species distributions, while the larval ecology, survival, growth and development strongly influence the adults' reproductive success and determine local patterns of recruitment to adult populations (e.g. Wissinger *et al.* 2010).

1.2 OVIPOSITION HABITAT SELECTION BEHAVIOR

Non-randomly selecting a habitat for offspring, among a choice of available habitats may involve costs to the selecting adult (Weisser *et al.* 1994; Scheirs *et al.* 2000) through missed feeding and mating opportunities, energy invested into orientation and movement to potential habitats, exposure to predation and other risks on the way there, and the development and maintenance of mechanisms for sensing informative environmental variables in potential habitats. These costs compromise the adult's survival and future reproductive success in order to increase the performance of the current clutch of offspring. The trade-off between these components of fitness (see also Einum & Fleming 2000) defines oviposition habitat selection behavior as a form of parental care that is invested prior to birth.

Oviposition habitat selection in organisms with complex life cycles and discrete larval habitats is a single behavioral action that determines the conditions under which the offspring will grow throughout their entire larval stage, since they cannot emigrate. This single action provides more decisive information on the relative importance of ecological factors than short-term patch selection studies, since its fitness consequences are equivalent to those of the sum of numerous habitat or patch choices that freely-moving organisms in continuous habitats make over extended periods of time.

The evolution of oviposition habitat selection is therefore shaped in large part by the expected performance of offspring in their larval habitats (Jaenike 1978), and is predicted to be responsive to the ecological factors that determine the quality of a specific larval habitat, such as predation, competition, habitat duration, pathogens, etc. Due to the interactive nature of different factors of the larval ecology, the responsiveness of

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oviposition behavior may be both context-dependent and temporally variable. Oviposition behavior studies sometimes consider the ecological conditions of the larval habitat to be temporally fixed. In other words, the conditions of each larval habitat at the time of oviposition are assumed to remain fixed throughout the period of larval development, and the conditions in the set of larval habitats available to the ovipositing females are assumed to remain fixed throughout the breeding period.

For example, the presence of conspecifics in the larval habitat may be a particularly important biotic factor influencing oviposition habitat selection. The avoidance of conspecifics is considered to be adaptive in cases of strong density dependence or high rates of cannibalism (Marsh & Borrell 2001; Poelman & Dicke 2007), while the preference of sites containing conspecifics is considered to be adaptive when the presence of conspecifics is less important than other environmental factors, or when there are greater direct benefits to aggregation (Stamps 1988). However, when offspring deposition is limited to a certain season, and the end of that season is approaching a general loss of selectivity is expected in response to conspecifics (Edgerly *et al.* 1998), or the function of oviposition may change (Poelman & Dicke 2007). When the availability of suitable larval habitats is limited in the landscape, avoidance of prior cohorts is possible only before all habitats are occupied. Prior to this point, any oviposition into a vacant larval habitat must account for the expectation of repeated consecutive ovipositions by conspecifics into the same habitat. From that point on, all offspring must be deposited with prior conspecifics and other factors may come into play, such as the densities of conspecifics in each habitat (e.g. Kiflawi *et al.* 2003), the availability of refuge from cannibalism, the genetic relatedness of prior cohorts and the time interval between cohorts.

1.3 LARVAL ECOLOGY AND DEVELOPMENT

Once oviposition habitat selection has been made, larvae are confined to their habitats and have to cope with the conditions therein until they metamorphose. The most obvious component of larval success is surviving to reach metamorphosis. Immediate metamorphosis and emergence from the larval habitat is not always possible when conditions are bad since larvae may be constrained by size thresholds to initiate metamorphosis (Wilbur & Collins 1973; Day & Rowe 2002) and by minimal requirements of resources allocated to differentiation (Smith-Gill & Berven 1979; Harris 1999). Another component of larval success is the size it reaches by the time of metamorphosis, which is often correlated with postmetamorph survival, date of first reproduction and/or size at

maturity (Semlitsch *et al.* 1988; Berven 1990; Altwegg & Reyer 2003). A third component is time to metamorphosis, which has been shown to be negatively correlated with postmetamorph survival and size at reproductive maturity (Semlitsch *et al.* 1988; Berven 1990; Altwegg & Reyer 2003). Additionally, time to metamorphosis may have to fit seasonal and other time-dependent constraints imposed by the environment outside the pool. In order to maximize their success in the pool, larvae respond developmentally and behaviorally to their environment's conditions. Such plasticity allows them to make metamorphic decisions and increase habitat utilization (Wilbur & Collins 1973; Werner 1986; Rowe & Ludwig 1991; Rudolf & Rodel 2007). Below I introduce key aspects of larval ecology and development that this thesis focuses on.

Competition

I focus primarily on larval competition for food resources. Competition for food is particularly critical in ephemeral larval habitats since insufficient larval growth or development rates may result in their failure to emerge in time to the next life stage (Audo *et al.* 1995). Competitive superiority may be defined as the ability of an individual to exploit the limited food resources at a higher rate than, and at the expense of, other individuals, thus out-competing them in the development race to timely emergence. This definition of competition includes both interference and exploitative competition as mechanisms that eventually modify the distribution of food resources within or between species. Thus, the effects of competition may manifest in the delaying of metamorphosis, reduction in body size, and metamorphic failure. The latter effect may potentially lead to mortality rates comparable to those generated by predation, making the food vs. safety tradeoff more balanced than is generally considered (e.g. 'the life-dinner principle', Stephens & Krebs 1986). Levels of competition may vary temporally due to temporal variations in habitat productivity, population dynamics, and shifts in population size structure.

Predation and cannibalism

Predation redistributes energy between individuals located in different trophic levels. In ephemeral larval habitats, surplus energy beyond that required for body maintenance is mostly converted to increase in body mass and to the developmental progress of the predators. Consumption by predators can thin out the prey population, and as a consequence, may relieve prey populations from competition. Prey may also respond

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behaviorally, physiologically and morphologically to the mere risk of predation. These consumptive and nonconsumptive effects of predation can be expected to influence the growth, development and metamorphic success of potential predators and of surviving prey. Intraguild predation and cannibalism are ubiquitous phenomena that lead to more complicated food web models compared to the simple model of primary producer-herbivore-carnivore. In these complex food webs, the processes summarized above occur in predators and victims within the same trophic level or species. Therefore, the strict distinction between trophic levels may fade or co-vary temporally with the stage or size structure of the interacting populations. In these cases, the effects of ecological variables that modify predation rates may be complex.

For example, high structural complexity generally reduces the encounter rate between active predators and their prey because of limited visibility and maneuverability, and it provides prey with protected, enemy-free spaces to escape predation (Huffaker 1958; Smith 1972; Crowder & Cooper 1982; Warfe & Barmuta 2004). Primary consumers and herbivores accumulate in complex habitats largely due to refuge from predation (Heck & Crowder 1991), and many studies support the idea that habitat complexity also provides predators with refuge from intraguild predation and cannibalism (reviewed in Denno *et al.* 2005). Reduced predation rates have different implications for organisms with different trophic functions; Primary consumers, as the victims of predation, benefit from its reduced rates. In contrast, top predators lacking natural enemies suffer from the reduction in predation rates because it reduces their energy intake. However, for species assuming intermediate trophic functions, reduced predation may have both a positive effect on survival (as for primary consumers) and a negative effect on foraging and energy intake (as for top predators). Furthermore, many organisms' trophic functions change throughout their ontogeny or are context-dependent (due to external factors) because of ontogenetic diet changes (e.g. Huss *et al.* 2008), growth to size refuge from predation (Persson & Eklov 1995; Rudolf & Armstrong 2008) and priority effects (Eitam *et al.* 2005). For these animals, the consequences of habitat structural complexity are not straightforward and in need of better understanding.

Kin selective behavior
Groups of larvae densely inhabiting confined habitats may be strongly influenced by social structure and the aggregated effects of social interactions between individuals. Kin selection theory (Hamilton 1964a,b) has been used widely to explain individual behaviors

and social interactions such as interference (Pakkasmaa & Aikio 2003), cooperation (Nowak 2006), cannibalism (Pfennig 1997), coalition (Widdig *et al.* 2006) and aggregation patterns (Kokko & Lindstrom 1996). It predicts that aggressive behavior will be directed preferentially towards unrelated individuals rather than towards siblings. Numerous behavioral studies demonstrated larval kin discrimination, by directing interference, aggression and cannibalism more towards unrelated conspecifics than towards siblings (reviewed in Pfennig 1997). While kin discrimination tends to display context-dependent expression (Hokit *et al.* 1996; Pakkasmaa & Aikio 2003), varying with size differences between individuals, their densities and food availability, it is generally believed that pre-reproductive, cannibalistic cohorts perform better if their members are genetically related, due to reduced aggression (reviewed in Wells 2007). However, alternative ecological outcomes can be hypothesized when considering kin-selective behavior and its effects in a cohort of larvae throughout the entire habitat's duration, since both the cost to the victim and the benefit to the potential cannibal clearly depend on their current states, the state of the environment, and the expected future dynamics in environmental conditions that are relevant to the fitness realization of both individuals. Focusing on the group-level consequences of this behavior may be informative for population-ecological processes (Johansson & Crowley 2008; Wissinger *et al.* 2010), as well as for analyzing female oviposition habitat selection strategies in clutch-depositing organisms.

Intercohort Priority effects

Phenotypic and interaction asymmetries among members of structured populations may affect life-histories (De Block & Stoks 2004) and lead to complex population-level patterns (Claessen *et al.* 2004). Population age-structure and size-structure are major sources of such asymmetries. In discrete larval habitats of organisms with complex life cycles, size structure is often highly linked to the pattern of larval colonization through variation in hatching times (e.g. Hopper *et al.* 1996) or multiple oviposition events (e.g. Anholt 1994) that lead to the co-occurrence of sequential cohorts. Few studies have focused on the interactions between sequential cohorts, known as intercohort priority effects, and their implications (Eitam *et al.* 2005), probably due to methodological difficulties of distinguishing members of different cohorts. Knowledge is particularly limited on priority effects as a function of the time interval between cohort colonizations.

  Intercohort priority effects may include asymmetric interference competition and cannibalism, usually biased in favor of the older cohorts of larger individuals (Anholt

1994; Godfray 1994; Eitam *et al.* 2005; Sadeh *et al.* 2009), although the opposite pattern can sometimes be found (Crump 1986; Chau & Maeto 2008). While older cohorts are usually considered to dominate the larval habitat, they may still be subject to exploitative competition exerted by the younger cohorts (Hjelm & Persson 2001).

Ephemeral larval habitats impose stress and time constraints on larval development (Loman 2002; Sadeh *et al.* 2011), potentially exacerbating the impacts and importance of intercohort priority effects. Exploitative and interference competition as modifiers of feeding and energy acquisition rates may have greater importance under the food-fueled developmental race to timely emergence, compared to continuous habitats. Compromised rates of food intake due to competition and interference may result in greatly increased mortality rates due to habitat termination, in addition to compromised body sizes, while cannibalism may partially alleviate these effects or even reverse them. Priority effects may also exert selective forces on the parental choice of larval habitat (Godfray 1994; Spieler & Linsenmair 1997).

Larval developmental plasticity

Phenotypic plasticity is a widespread phenomenon: individuals alter their phenotypes in response to environmental cues, often as an adaptation to variable environments. This multidisciplinary concept has recently been of increasing interest to biologists as a feature of both normal and abnormal individual development that is not only shaped by evolution, but also one that influences the function of individuals, the structure of ecological communities, and evolutionary trajectories (Schlichting & Pigliucci 1998; West-Eberhard 2003; Gilbert & Epel 2009; Berg & Ellers 2010). To arrive at a better understanding of the evolution of phenotypic plasticity, we must identify its costs and limits, and illuminate the functional relationships between them (DeWitt *et al.* 1998; Schlichting & Pigliucci 1998; Callahan *et al.* 2008).

Explaining patterns of size and age at life-history transitions such as metamorphosis has been an ongoing, central challenge in evolutionary biology. Larval development towards the completion of metamorphosis in amphibians involves two directional processes: growth in body size and the differentiation and remodeling of tissues and organs (Smith-Gill & Berven 1979). The rates of both of these processes generally respond to various environmental factors, ultimately determining size and age at metamorphosis, respectively, and have been the focus of extensive research involving analyses of phenotypic plasticity (e.g. Wilbur & Collins 1973; Smith-Gill & Berven 1979;

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Hensley 1993; Leips & Travis 1994; Denver *et al.* 1998; Harris 1999; Day & Rowe 2002; Rose 2005). Some of this work has focused on developmental responses to the risk of habitat termination, with the overwhelming majority showing that larvae accelerate development and metamorphose earlier (reviewed in Wells 2007). This response tends to result in a smaller size at metamorphosis due to a shortened growth period. However, few studies have explicitly addressed other, endogenous costs (e.g. Gervasi & Foufopoulos 2008; Marquez-Garcia *et al.* 2009), particularly such that lead to increased mortality (Altwegg 2002; Lane & Mahony 2002). Moreover, costs associated with certain phenotypes (e.g. low body size associated with early metamorphosis) are not costs of plasticity *per se*. A developmental approach, i.e. manipulating and following the temporal trajectories of phenotypes during larval ontogeny, may be useful for studying true costs of plasticity rather than costs of phenotypes.

1.4 THE FIRE SALAMANDER

This thesis focuses on the fire salamander (*Salamandra infraimmaculata*; Steinfartz *et al.* 2000) in northern Israel as a model species for studying adult oviposition habitat selection and larval behavior and development. When rains begin in the autumn following a long dry summer, fire salamander females migrate to breeding sites to larviposit and mate. Natural breeding sites include a few to tens of pools of volumes ranging from a few tens of liters (most frequent) to thousands of liters, and of variable hydroperiods. In the studied regions of Mt. Carmel and the Lower Galilee, large adult populations exploit breeding sites containing relatively few, mostly ephemeral pools (Spencer *et al.* 2002; Segev *et al.* 2010), whereas permanent pools are far less abundant. The females spread their larvae both temporally within the breeding season, and spatially among available temporary pools and display sensitive selectivity for pool depth (Segev *et al.* 2010). A preliminary study indicates that gravid females avoid pools containing large conspecific larvae (O. Segev, personal communication). Although *S. infraimmaculata* are ovoviviparous, I use both 'larviposition' and 'oviposition' interchangeably in this thesis, since my questions also apply for the latter and more inclusive term.

The larvae function as top predators within ephemeral pools and fishless permanent pools (Blaustein *et al.* 1996; Eitam *et al.* 2005), and often rely also on cannibalism because of strictly limited food resources (Degani *et al.* 1980; Eitam *et al.* 2005). Cannibalism is largely size-dependent (Degani *et al.* 1980; Reques & Tejedo 1996; Sadeh *et al.* 2009), and

likely constitutes a significant mechanism of intercohort priority effects in this species, along with nonlethal interference and exploitative competition (Eitam *et al.* 2005; Segev 2009). Thus, larval size structure and cannibalism constitute a special case of intraguild predation, where the trophic function of an individual depends on its position in the order of arrival. The larvae are capable of kin discrimination, displaying increased aggressive behavior towards distantly related conspecifics compared to siblings (Markman *et al.* 2009).

1.5 THESIS OBJECTIVES

I used a combination of mesocosm, laboratory experiments and modeling to study life history strategies of the fire salamander as a model for organisms with complex life cycles and ephemeral larval habitats. I explicitly addressed temporal aspects of the ecology of larval habitats, specifically focusing on habitat ephemerality, repeated colonizations and the time interval between them, and their effects on larval behavior and development patterns as well as on adult oviposition behavior.

Specifically, I addressed the following questions:

1. Do gravid fire salamander females select their larviposition site according to the expected trophic function of their progeny, depending on their order of arrival? (Chapter 2).

   To answer this question, I manipulated rates of larval cannibalism using the degree of habitat structural complexity to determine their differential effects on two larval cohorts. In an oviposition habitat selection experiment, I allowed gravid females at different positions in the order of colonization to choose the level of structural complexity of larval habitats.

2. How does kin-selective cannibalistic behavior affect the performance of larval cohorts under time and food limitations? (Chapter 3).

   To answer this question, I manipulated the genetic heterogeneity of kin-discriminating larval cohorts using single or mixed sibships. I followed the development, growth and survival of these cohorts in mesocosms with poor food resources and short hydroperiod.

3. How does the time interval between larval cohorts influence the performance of each of the cohorts? Specifically, how do the intensities of intercohort competition and cannibalism vary as a function of intercohort time interval? Does this shape superoviposition decisions (self vs. conspecific) of gravid females as a function of

intercohort time interval? (Chapter 4).

To answer these questions, I manipulated the time interval between two cohorts as well as larval densities in a factorial mesocosm experiment, and followed the performance of both cohorts. I also conducted sets of oviposition habitat selection trials at different intercohort time intervals, allowing females to choose between pools with their own previously deposited larvae (self superoviposition) vs. pools with larvae deposited by unrelated females (conspecific superoviposition).

4. Do larvae sense the recent desiccation of conspecifics from previous cohorts as an early cue of their habitat's duration? Do they respond adaptively by altering their developmental rates to increase their probability of metamorphosing before their habitat desiccates? Is developmental plasticity ontogenetically limited, i.e. can the larvae update their rates of development according to later cues that indicate the habitat's actual duration more reliably, to the point of complete compensation? In case compensatory development occurs, does it carry endogenous developmental costs? (Chapter 5).

To answer these questions, I allowed newborn larvae to grow in laboratory pools in the presence or absence of the remains of desiccated conspecifics. This manipulation was crossed with a manipulation of actual water level regimes (constant or decreasing) resulting in consistent or contradictory cues. I followed the developmental trajectories of the larvae as well as their patterns of mortality prior to metamorphosis. I used the empirical results to model the costs of development rates as a fundamental phenotype production cost, and analyze the resulting tradeoff between production costs of developmental plasticity with phenotype-environment mismatch costs.

Ethics statement

The fire salamander is a locally endangered species in Israel (Dolev & Perevolotzky 2003), and extra care was taken to minimize harm to individuals and populations in the course of research. Experiments were mostly limited by the number of individuals allowed by the Israel Nature and Parks Authority. Field collection of salamanders, experimentation and return of individuals to their original habitats were conducted according to the Israel Nature and Parks Authority permits 2007/30818, 2008/31998, 2009/36605, 2010/37885 as well as the University of Haifa Animal Experimentation Ethics Committee permits 120/08, 124/08, 190/10, 212/11.

CONTEXT-DEPENDENT REPRODUCTIVE HABITAT SELECTION: THE INTERACTIVE ROLES OF STRUCTURAL COMPLEXITY AND CANNIBALISTIC CONSPECIFICS

2.1 ABSTRACT

Structural complexity generally reduces predation and cannibalism rates. Although the benefits from this effect vary among environmental contexts and through time, it has been the common explanation for high species abundances in complex habitats. I hypothesized that oviposition habitat selection for structural complexity depends on the expected trophic function of the progeny. In *Salamandra infraimmaculata* larvae, expected trophic function is dictated by their sequence of deposition. First cohorts cannibalize later arriving cohorts, while all compete for shared prey resources. In a mesocosm experiment, I show that gravid salamanders facing conspecific-free pools preferred structurally simple habitats (no rocks), while females facing only pools with older conspecific larvae preferred complex habitats (with rocks). Context-dependent preference of habitat complexity for managing food/safety tradeoffs may be extended from classic foraging patch decisions to breeding habitat selection. These tradeoffs vary with dynamic larval processes such as priority effects and ontogenetic diet shifts, potentially leading to complex maternal parturition behaviors.

2.2 INTRODUCTION

Habitat structure can be defined as the arrangement of physical objects in the space in which species interact (McCoy & Bell 1991). An important feature of habitat structure is its degree of complexity, a feature that has been much studied in the context of predation. High structural complexity generally reduces the encounter rate between active predators and their prey because of limited visibility and maneuverability, and it provides prey with protected, enemy-free spaces to escape predation (Huffaker 1958; Smith 1972; Crowder & Cooper 1982; Warfe & Barmuta 2004). Thus, ecologists have historically recognized that structural complexity tends to reduce predation rates, prevent prey extinction and stabilize predator-prey co-existence (MacArthur 1972; Smith 1972; Janssen *et al.* 2007).

Experimental evidence indicates that structurally complex habitats tend to promote higher abundances for many species. While it is acknowledged that primary consumers and herbivores accumulate in complex habitats largely due to refuge from predation (Heck & Crowder 1991), the mechanisms underlying this pattern for predatory species remain understudied (Denno *et al.* 2005). Several hypotheses have been proposed, but most studies support the idea that habitat complexity provides predators with: (1) refuge from intraguild predation and cannibalism, (2) refuge from physical disturbances, and (3) access to alternative resources (reviewed in Denno *et al.* 2005).

Both the availability of alternative food resources and refuge from physical disturbances are qualities of complex habitats that are similarly relevant for both predatory species and herbivores. On the other hand, reduced predation rates have different implications for organisms with different trophic functions. Primary consumers benefit from reduced predation rates due to structural complexity because they are strictly victims of predation. In contrast, top predators lacking natural enemies suffer from the reduction in predation rates because it reduces their energy intake. However, the trophic function of organisms is a continuum along which many species take intermediate positions. For such species, reduced predation may have both a positive effect on survival (as for primary consumers) and a negative effect on foraging and energy intake (as for top predators). Preferable habitat structure, then, is a species- and context-specific tradeoff of foraging rate vs. safety. Complex habitats may be preferred when the cost of predation risk is considerably higher than that of food limitation. On the other hand, simple habitats may be preferred when the opposite cost relations occur. Furthermore, most organisms' trophic functions change throughout their ontogeny or are context-dependent (due to external factors) because of ontogenetic diet changes (e.g. Huss *et al.* 2008), growth to size refuge

from predation (Persson & Eklov 1995; Rudolf & Armstrong 2008) and priority effects (Eitam *et al.* 2005). For these animals, the consequences of habitat structural complexity are not straightforward.

Three factors – migration (including habitat selection), survival and reproductive success – contribute to observed distributions and abundances of a particular species. Organisms with complex life cycles with discrete larval habitats (e.g. amphibians, parasitoids, insects with terrestrial adults and aquatic larval stages, etc.) are excellent systems for teasing apart the contributions of these factors due to their strict stage-specific habitat separation. Furthermore, oviposition habitat selection in such systems is a single behavioral action that determines the conditions under which the offspring will grow throughout their entire larval stage, since they cannot emigrate. This single action provides more decisive information on the relative importance of ecological factors than short-term patch selection studies, since its fitness consequences are equivalent to those of the sum of numerous habitat or patch choices that freely-moving organisms in continuous habitats make over extended periods of time. In addition, the tradeoff between foraging rate and risk of predation is more critical in systems with ephemeral larval habitats since insufficient larval development rates may result in their failure to emerge in time to the next life stage (Audo *et al.* 1995). In these systems, competitive superiority may be defined as the ability of an individual to exploit the limited food resources at a higher rate than, and at the expense of, other individuals, thus out-competing them in the development race to timely emergence. This form of competition may be just as lethal as the effects of predation, making the food vs. safety tradeoff more balanced than is generally considered (e.g. 'the life-dinner principle', Stephens & Krebs 1986).

Previous studies of organisms with complex life cycles have shown that in species whose larvae function as prey, mothers tend to prefer structurally complex habitats for them, in order to provide refugia from their predators and increase the rate of successful emergences (Boecklen *et al.* 1990; Orr & Resh 1992; Meiners & Obermaier 2004; Bond *et al.* 2005). In contrast, I found no literature on oviposition habitat selection into discrete habitats for species whose larvae function as top or intermediate predators in the context of habitat complexity. One would expect that in the absence of predators and strong negative intraspecific interactions such as interference, predatory species would prefer simpler habitats, in order to allow their larvae more effective foraging.

I hypothesized that oviposition habitat selection for structural complexity depends on the expected trophic function of the progeny. To test this hypothesis, I studied the

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ovoviviparous fire salamander (*Salamandra infraimmaculata*; Steinfartz *et al.* 2000) whose larvae are deposited mostly into temporary pools in northern Israel, where they function as top predators (Blaustein *et al.* 1996; Eitam *et al.* 2005), and often rely also on cannibalism because of strictly limited food resources (Degani *et al.* 1980; Eitam *et al.* 2005). The intensity of cannibalism is dictated by size differences between individuals, as bigger larvae deposited earlier into the pool consume the younger, smaller conspecifics (Reques & Tejedo 1996; Eitam *et al.* 2005). Thus, larval size structure and cannibalism constitute a special case of intraguild predation, where the trophic function of an individual depends on its position in the order of arrival. Similarly, strong priority effects are also likely caused by both interference and exploitative competition (Eitam *et al.* 2005). *Salamandra* females are capable of spreading larval clutches among pools and through time, as well as choosing pools to deposit into according to their perceived quality (O. Segev unpublished). A preliminary study indicates that gravid females avoid pools containing large conspecific larvae (O. Segev, personal communication). Pool structural complexity, in the form of rocks or aquatic vegetation, is predicted to reduce the degree of cannibalism and conspecific interference by providing shelter for smaller larvae, and by reducing the encounter rate between individuals.

I report the results of an outdoor larviposition choice experiment designed to test the hypothesis that gravid salamander females select their larviposition site according to the expected trophic function of their progeny, depending on their order of arrival. Thus, I predicted a switch in larviposition preference from low structural complexity when pools are free of conspecific larvae, to high complexity when pools already contain older conspecific larvae. A larval survivorship experiment verified the hypothesized negative effect of structural complexity on intercohort cannibalism rates.

2.3 METHODS

When rains begin in the autumn following a long dry summer, *Salamandra* females migrate to breeding sites to larviposit and mate. Natural breeding sites include a few to tens of pools of volumes ranging from a few liters (most frequent) to thousands of liters. These pools vary greatly not only in size, but also in their structural complexity. Some are completely covered by rocks, a layer of sediment and/or terrestrial vegetation. Others have smooth rocky surfaces and little or no plants. During the first autumn rains of 2007 (November 9-11 and 19-21), I collected 26 gravid females on their way to breeding sites on Mt. Carmel and the Galilee mountains. The number of individuals collected was the

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maximum allowed by the Nature and Parks Authority, since *S. infraimmaculata* is listed as locally endangered. Collection during the very first rains, when temporary pools only start to fill, assured that these females did not have a chance to deposit many, if any, larvae prior to their collection.

In order to compare the larviposition decisions of first-arriving females facing conspecific-free pools with those of later-arriving females facing already colonized pools, I conducted two bouts of larviposition trials in an outdoor site with ten 7.5 m^2 enclosures, each containing four evenly distributed 35 liter plastic tubs (surface area 0.22 m^2) dug into the ground. The tubs were filled with tap and rainwater prior to the trial. For each enclosure, I placed four rocks per tub into two randomly selected tubs. The rocks were fully immersed and displaced ~6 liters of water per tub, covering ~50% of its floor area. The other two tubs contained the same volume of rocks prior to the trial, in order to control for possible chemical effects of the rocks on the water. Control rocks were taken out just before the gravid females were introduced to the enclosures.

Larviposition bout 1: conspecifics absent

During three nights in early December, 2-4 hours after sunset, I introduced 13 gravid females, one in each enclosure. I used sprinklers to simulate rain since larviposition occurs almost exclusively during rainy nights. Once per hour, until all the females had each larviposited once (for a total of 8 hours), I observed the females without illumination and from a distance of ~2 meters. I removed each female from its enclosure after it made its first choice of pool for larviposition, allowing it to finish depositing that first clutch into the pool and climb out without interruption. Similar mesocosm experiments indicate that the first chosen pool is the most preferred pool (O. Segev, unpublished data). My hourly observations revealed that conservatively, at least 80% of the females also visited at least one pool other than the pool that was eventually chosen for larviposition.

Larviposition bout 2: conspecifics present

After the first bout of larviposition, I removed all the larvae, pumped ~75% of the water out of all pools, homogenized the remaining waters between pools within each enclosure, and refilled the tubs with tap and rainwater. This was done to dilute and homogenize any potential signals emitted by the larvae or their mothers into the water. On December 12 and 15, I collected 200 large larvae from pools in the Galilee site and the Carmel site (mean snout-vent length = 2.12 cm, SD = 0.15 cm). Within each enclosure, I placed five larvae in

each of the four tubs, keeping larval biomass uniform across tubs. This density of first cohort larvae is well within the range occurring naturally following the first larviposition into a pool (Blaustein, unpublished data). I left the larvae in the tubs for 24 hours with rocks before taking the rocks out of half of the pools and placing one gravid female per enclosure. The 13 females used for the second bout were not the same individuals used in the previous bout. Gravid females from the Carmel site were placed in enclosures with larvae from the Galilee site, and vice versa. This was done to increase genetic distance and potential cannibalism of the older cohort on the newly deposited larvae (aggression between individual larvae increases with increasing genetic distance: Markman *et al.* 2009). The second bout of larviposition in the presence of an older cohort was conducted as in the first bout.

The two-week time interval between the two larviposition bouts is ecologically relevant, as rain events in Israel are often that long apart, not allowing larviposition in between (mean inter-rain interval times in December during yrs 1976 to 2008 on Mt. Carmel = 5.73 d; SD = 5.44 d; range = 1 to 28 d; Laboratory of Climatology, Dept. of Geography and Environmental Studies, Univ. of Haifa, Israel). This interval served to homogenize the time each individual was retained in the lab prior to experimentation. I did not expect a time-related reduction in pool selectivity by females due to the approaching end of the breeding season (Clark & Mangel 2000) because I conducted the experiment rather early in the 5-6 month long breeding season (October/November to March).

A total of four females in both larviposition bouts were removed from analyses because they began depositing larvae in their containers just before being placed into the experimental enclosures, they deposited in more than one pool between observations, or they did not larviposit at all.

Larval survivorship experiment

The predictions of the larviposition experiment described above were based on the assumption that structural complexity reduces inter-cohort cannibalism. The following season (2008/9), I set up a mesocosm experiment, in part to test this assumption. Here I briefly describe the study and present pertinent results. The full study will be presented elsewhere. In 20 tubs, identical to those used in the larviposition bouts and filled with 35 L aged tap water, I established two structural complexity treatments by introducing rocks to half of them. The rocks covered 60% (SD = 2%) of the tubs' bottom area and displaced 5.8 L (range: 5.25 – 6.5 L) of water. I introduced a first cohort of three newborn larvae into

each tub (mean wet mass: 268 mg, SD = 25 mg; mean snout-vent length: 1.74 cm, SD = 0.06 cm). Twenty three days later, during which I fed the first cohort larvae *ad-libitum*, I added a second cohort of 5 newborn larvae into each pool (mean wet mass: 251 mg, SD = 12 mg; mean snout-vent length: 1.76 cm, SD = 0.05 cm). I recorded larval survivorship to day 80 following the addition of the second cohort. During the experiment, I observed the tubs regularly to distinguish cannibalism from other causes of mortality and emerging metamorphs.

In accordance with local authorities regarding ethical treatment and minimizing mortality to this species, all the field-collected females were safely returned to their capture location as soon as the studies were over, and before the breeding season ended. Similarly, all the remaining larvae were returned to their natal pools or the pools nearest to the capture locations of their mothers.

2.4 RESULTS

Larviposition trials

During the first larviposition bout (Figure 2.1a), in the absence of an earlier cohort, one female chose a rock pool, depositing a single larva, while nine females chose rock-free pools and deposited a total of 109 larvae into them. The difference in pool choice was statistically significant (sign test: p=0.011). During the second larviposition bout (Figure 2.1b), in the presence of an older, unrelated cohort, a preference switch occurred as only two females deposited a total of 13 larvae in rock-free pools, whereas 10 females deposited a total of 89 larvae in 11 rock pools. Here, the preference for rock pools was significant (sign test: p=0.019). The preference switch was also significant (normal approximation test of equality of proportions: p<0.001).

Larval survivorship experiment

First cohort individuals displayed similar survivorship (mean with rocks: 93.3%, SE = 6.7%; mean without rocks: 83.3%, SE = 7.5%; Mann-Whitney: U = 36.50, p=0.178), but more of them metamorphosed in tubs without rocks (mean percent of cohort remaining: 60.0%, SE = 8.3%) than with rocks (mean = 90.0%, SE = 7.1%; Mann-Whitney: U = 20.00, p=0.014). The mean second-cohort survivorship was 42.0% (SE = 13.2%) with rocks, and 4.0% (SE = 2.6%) without rocks (Mann-Whitney: U= 25.00, p=0.032). This significant difference in mortality of the second cohort was entirely due to cannibalism by first cohort individuals, even though tubs with rocks contained on average more first cohort

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larvae over the observed time period. Thus, structural complexity reduces inter-cohort cannibalism, suppressing the predation rate of first cohorts upon later cohorts.
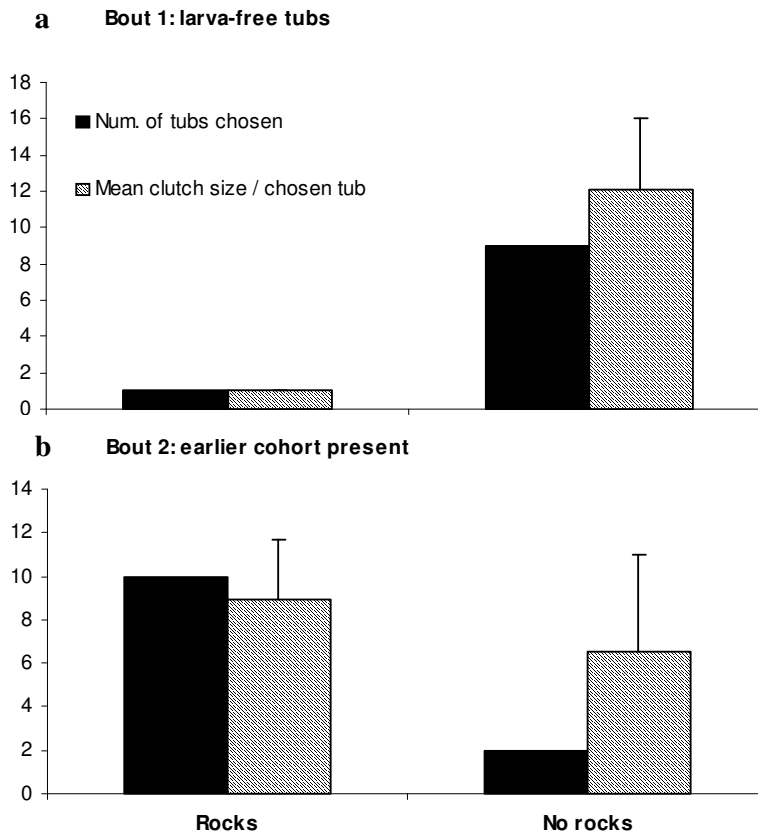
Figure 2.1: First-tub structural complexity preferences of females facing (**a**) vacant tubs, and (**b**) tubs containing older conspecifics. Solid bars present the number of first tubs chosen for larviposition of each type. Hatched bars present the mean deposited clutch size per tub where larviposition occurred. Error bars are 1 SE, where applicable.

2.5 DISCUSSION

I found a strong switch in larviposition pool preferences by gravid salamanders. These results are consistent with my hypothesis that oviposition/larviposition habitat preference in response to structural complexity depends on the expected future trophic function of the progeny, which is determined in this system by the order in which females arrive at a breeding pool. Recently, several hypotheses have been proposed to explain the superiority of complex habitats for predatory species (reviewed in Denno *et al.* 2005). Most of these habitat effects (i.e. more abundant prey, access to alternative resources and more favorable

microclimate) are usually fixed for a given organism. In contrast, the importance of refuge from predation or from cannibalism may depend on the order of colonization.

When a late-arriving female encounters a set of pools that is already populated by older conspecific larvae, her larvae are likely to be preyed upon by the larger conspecifics. My larval survivorship experiment verified that habitat complexity reduces *Salamandra* intercohort cannibalism rates. Moreover, within both relative and absolute refuges that are available in complex habitats, individuals of younger cohorts may have more opportunities to forage compared to simple habitats, where reduced activity as a prey behavioral response to the risk of cannibalism may be stronger (Lima & Dill 1990). Hence, I conclude that late-arriving mothers prefer complex habitats for increased refuge from predation, in accordance with previous studies on other organisms (Boecklen *et al.* 1990; Orr & Resh 1992; Meiners & Obermaier 2004; Bond *et al.* 2005; Denno *et al.* 2005).

In contrast, first-arriving females that confronted vacant pools preferred simple habitats. This behavior may imply that the limited intra-cohort aggression and cannibalism are not as considerable for first cohort larvae as exploitative competition that is exerted by individuals both of the same cohort and later cohorts. First-cohort larvae are generally deposited days after pools fill and have yet to accumulate invertebrate prey resources (generally microcrustaceans and dipteran larvae). As the season progresses temperatures drop, further slowing down the accumulation of basal and prey species. Thus, salamander larvae are confronted with an extended initial period of low food availability, during which insect colonization is negligible and in the vast majority of pools, the larviposition of additional conspecifics increases competition. The members of these later cohorts are the largest food items available for first cohort individuals. In order to reduce the probability of failure to metamorphose and emerge before the pool dries, larvae are predicted to maximize their consumption and development rates. Structurally simple pools increase the encounter rates between first cohort and later cohort larvae, and deprive the smaller larvae of refugia, thus enabling the first cohort larvae to prey on them more effectively as I have demonstrated in the larval survivorship experiment. This increase in food availability for larvae of the first cohort may alleviate competition among members of this cohort. Additionally, each later-cohort individual consumed represents not only a substantial meal for first cohort individuals, but also reduced competition for shared prey resources (Polis *et al.* 1989). Therefore, the preference for structurally simple pools by first arriving mothers can be explained also by the reduction of intercohort competition upon the first cohort.

I cannot infer from my experimental design the mode by which terrestrial females determine either structural complexity or predation risk. Oviposition site selection behavior can be responsive to indirect environmental cues or proxies that are correlated with risk of predation rather than the presence of predators *per se* (Blaustein 1999). The females may have responded to the time that has elapsed after the first rains as a reliable proxy for presence of large conspecifics in the pools rather than detecting the larvae directly. However, in a preliminary study, gravid females avoided larvipositing in pools containing caged and hidden large conspecific larvae (O. Segev, personal communication), implying a capability for direct chemical detection of conspecifics.

The role of refuge from physical disturbances in preference of complex habitats (see Denno *et al.* 2005) was not tested here, but it is likely that this factor acts in combination with the behavioral effects of cannibalism rather than independently. Towards the end of the rainy season, the frequency of rains reduces and pools may dry for a few days or weeks between rains. Incompletely developed larvae in dried pools aggregate underneath rocks where retained moisture may extend their survival by a critical length of time (Sadeh, personal observations). My experiment was conducted well before pools begin to dry, as reflected in the results of the first larviposition bout. However, the presence of cannibalistic first cohorts induces reduced foraging in the later-cohort larvae, and their development rate is retarded until the first cohort metamorphoses (Sadeh, unpublished). Therefore, much of their development is delayed towards the end of the larval season when the risk of desiccation is high. Thus, increased risk of desiccation as a possible cause for preferring complex pools early in the season may also be derived in this system from the order of larviposition and the ensuing risk of cannibalism.

This is the first study suggesting preference of habitat complexity as a maternal management strategy for larval foraging vs. predation risk tradeoffs that produces complex switching behaviors in the selection of discrete larval habitats. The classic food vs. safety tradeoff has been studied extensively in the context of animal foraging for food (Brown & Kotler 2004; Morris *et al.* 2008), and numerous species have been shown to prefer foraging effectively in structurally simple patches, but escape to safer complex patches when faced with a predator (e.g. Persson & Eklov 1995; Stoks 1999; Borcherding 2006), or predator cues (e.g. Rypstra *et al.* 2007). Physiological state was shown to influence foraging patch decisions, as hungry animals venture back to simple patches (Wellborn & Robinson 1987; Pettersson & Bronmark 1993). Oviposition site selection theory has addressed the problem of predation risk upon the forager as a tradeoff between current and future reproduction

and predicts shifts in site selectivity (Godfray 1994; Clark & Mangel 2000). My application of the food/safety tradeoff to discrete breeding habitat selection is not trivial since such decisions are irreversible when the offspring are confined and unable to emigrate until they have completed their larval stage. While foragers choosing patches have the luxury of responding in real time to their instantaneous body state and risk of predation, breeding mothers also make a long-term decision that will affect their offspring's tradeoffs. Thus, breeding behaviors should respond to environmental conditions at the time of delivery but also reflect the expected future dynamics in food and safety. These dynamics may be driven by processes such as ontogenetic diet shifts (e.g. Huss *et al.* 2008), population size structures and growth race to size-refuge from predation (e.g. Rudolf & Armstrong 2008), and, as suggested here, order of habitat colonization and priority effects. Studying oviposition habitat selection with respect to such dynamic processes is expected to reveal more complex habitat-selection patterns than those described so far.

KIN-SELECTIVE CANNIBALISM AND COMPENSATORY MORTALITY IN LARVAL SALAMANDER COHORTS INHABITING TEMPORARY POOLS

This chapter is under review for publication as:
Sadeh A. Kin-selective cannibalistic behavior results in poor performance of sibling larval salamander cohorts.

3.1 ABSTRACT

Question: Related pairs of individuals tend to express reduced aggression. What are the consequences of this behavior on groups of developing larvae under food and time limitation?

Hypothesis: The positive effects of reduced cannibalism among related larvae on their success at reaching metamorphosis may be neutralized or even outweighed by negative density-dependent effects.

Organism: Kin-discriminating, cannibalistic fire salamander (*Salamandra infraimmaculata*) larvae from two populations in northern Israel.

Methods: I Compared sibling vs. genetically mixed larval cohorts throughout their larval period in short-lived, outdoor mesocosms.

Conclusions: The experiment supported the hypothesis. Rates of cannibalism were higher in mixed cohorts. Nevertheless, overall survival prior to habitat loss was similar between treatments. The probability that a larva would attain metamorphosis prior to early habitat loss was higher in the mixed cohorts than those in the sibling cohorts.

3.2 INTRODUCTION

Kin selection theory (Hamilton, 1964a,b) has been used widely to explain individual behaviors and social interactions such as interference (Pakkasmaa & Aikio, 2003), cooperation (Nowak, 2006), cannibalism (Pfennig, 1997; Walls & Roudebush, 1991), coalition (Widdig *et al.*, 2006) and aggregation patterns (Kokko & Lindstrom, 1996). It predicts that aggressive behavior will be directed preferentially towards unrelated individuals rather than siblings. Numerous behavioral studies have demonstrated larval kin discrimination, by directing interference, aggression and cannibalism more towards unrelated conspecifics than towards siblings (reviewed in Pfennig, 1997). Kin discrimination tends to display context-dependent expression (Hokit *et al.*, 1996; Pakkasmaa & Aikio, 2003; Walls & Blaustein, 1995), varying with size differences between individuals, their densities and food availability. However, it is generally assumed that groups of pre-reproductive, potentially cannibalistic individuals perform (i.e. grow and survive to maturity) better if their members are genetically related, due to reduced aggression (reviewed in Wells, 2007). Focusing on the group-level consequences of this behavior over the entire larval period may be informative for recruitment patterns to populations (Johansson & Crowley, 2008; Wissinger *et al.*, 2010), as well as for analyzing female oviposition habitat selection strategies. In clutch-depositing organisms, such as clutch-spreading amphibians (e.g. Segev *et al.*, 2011) and superparasitism decision in gregarious parasitoids (e.g. Segoli *et al.*, 2010), maternal behavior is expected to optimize the female's fitness payoff from her entire clutch.

Intraspecific larval interactions in temporary habitats are typically strongly competitive, and often aggressive, since the exploitation of the limited food resources determines the likelihood of emerging early enough to avoid death when the habitat disappears (Crump, 1983; Dennehy *et al.*, 2001; Wissinger *et al.*, 2004). Generally, a selfish act is predicted to be carried out when its benefit to the actor (b) is greater than its cost to the victim (c), weighted by the relatedness (r) of the victim to the actor (i.e. when $b>rc$; Hamilton, 1964a,b). Specifically, the cost of full cannibalism to the victim is its expected fitness if it were not cannibalized, determined largely by its probability of surviving to emergence from the larval habitat, whereas the benefit to the cannibal is primarily the increase in its probability of survival to emergence (Eickwort, 1973). Several studies have shown reduced aggressive or cannibalistic behavior towards kin in the larval stages of organisms inhabiting ephemeral habitats (Markman *et al.*, 2009; Pfennig, 1997; Segoli *et al.*, 2009; Walls & Roudebush, 1991). Consistently low rates of cannibalism can

be hypothesized to result in lower mortality rates and therefore better performance of groups of relatives, compared to groups of unrelated individuals.

An alternative ecological outcome can be hypothesized when considering kin-selective behavior and its effects in a group of larvae under food and time limitations. Consistently low rates of cannibalism in groups of related individuals may maintain high larval densities and therefore high levels of hunger (Polis, 1981). Under sufficient food limitation, this may result in increased non-cannibalistic, density dependent mortality due to deteriorated physiological conditions, compared to populations that are thinned by cannibalism. Reduced food availability due to competition may also depress development rates, therefore increasing the risk of death when temporary habitats disappear (Wells, 2007). Additionally, Hamilton's rule ($b>rc$) may lead to behaviors that vary temporally. As time runs out before the habitat disappears, cannibalism might be necessary to provide the cannibal with the amount of energy required to emerge in time (i.e. b is very large), whereas the victim might die due to habitat loss even if not cannibalized (i.e. c is very small). Thus, in food-deprived temporary habitats, the tendency of highly related individuals to avoid cannibalizing each other may be lost with time or neutralized by other, density dependent sources of mortality. This may potentially lead to similar or even reduced performance of sibling cohorts compared to cohorts of more distantly related individuals.

This study considers the performance of salamander larval cohorts in temporary pools. A recent study by Markman et al (2009) showed that pairs of same-sized larvae exhibited significant relatedness-biased aggression. However, their laboratory experiment isolated this behavior from its potential interactions with other density-dependent and temporal factors, and kept the participating individuals at uniform feeding levels. Thus, the influence of the demonstrated relatedness-biased aggression on larval cohorts' performance and expected fitness remains unclear. To test this, I set up mesocosms with cohorts of either siblings or genetically-mixed individuals under relatively low food availability and a short hydroperiod, and monitored their performance through most of their larval period.

3.3 METHODS
Study organism
Adult female fire salamanders (*Salamandra infraimmaculata*) in northern Israel are ovoviviparous and larviposit mostly into temporary pools. Most natural rock pools hold a few tens of liters of water (e.g. Spencer *et al.*, 2002), while a few ancient man-made pools

are also utilized and support large populations (Segev *et al.*, 2010). Hydroperiods vary greatly both among pools and among years, ranging from several weeks to a few months and forcing the larvae to complete their development before the pools dry. This developmental race against time is often carried out with high larval densities and poor food resources that are crucial for fueling development. *Salamandra* larvae are top predators in temporary pools and cannibalism and intra-specific interference are frequent (Reques & Tejedo, 1996; Sadeh *et al.*, 2009). The outcomes of these agonistic interactions are largely determined by the relative size of their participants, where large individuals of older cohorts dominate the pool, exerting detrimental priority effects on younger cohorts (Eitam *et al.*, 2005; Sadeh *et al.*, 2009). In highly ephemeral pools, excess energy acquired through cannibalism is allocated to development to accelerate metamorphosis and escape death by desiccation (Sadeh *et al.*, 2009). I used larvae from the Carmel and Galilee, two regions inhabited by genetically distinct populations of fire salamanders (Peleg, 2008; L Blank et al., unpublished). Individuals from these regions exhibit relatedness-biased aggression (Markman *et al.*, 2009).

Experimental design

I placed 20 mesocosms in the field during the winter and let them fill up with rainwater. The mesocosms were 35 L opaque plastic tubs, within the water volume range of natural temporary pools used by salamanders. To each mesocosm I added 3 large limestone rocks for refuge, covering ~25% of the pool bottom area. I inoculated each pool with an equal volume of water from a well-mixed stock containing zooplankton from an outdoor, semi-natural pool. This inoculate was visibly dominated by *Arctodiaptomus similis* (Copepoda) and also included various daphnids. The concentration of invertebrates in the mesocosm following inoculation was similar to that of the pool from which they were collected. The mesocosms were organized spatially in pairs that I matched for similar daily periods of exposure to direct solar radiation. Within pairs, I assigned mesocosms randomly to two treatments; a cohort of siblings and a genetically mixed cohort.

On the first day of the experiment, I introduced six larvae into each of the pools. This density is within the range of larval densities in natural pools (L Blaustein, personal observations). In each "sibling" pool, all six larvae were the offspring of the same mother. These sibships were born in the lab by placing ten gravid females in tubs with aged tap water. Five of these females were collected from the Carmel, and five from the Galilee. In each "mixed" pool, three of the larvae were from the Carmel and the other three from the

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Galilee regions, such that none of the pool mates were siblings. In these mixed cohorts, most of the larvae were born in the lab to 13 females, while some of the larvae (21 individuals, distributed randomly among pools) were field-collected from natural pools, since I was limited by the Israel Nature and Parks Authority in the number of gravid females I could collect and in the duration of their retention in the lab. However, both their sizes and the visible amounts of remaining yolk in their bodies were similar to those of the lab-born larvae, indicating similarly young ages of individuals in both of these groups (estimated as up to 7 days old at the time of collection). The proportions of survival and successful metamorphoses among the field collected larvae (identified individually by the spot patters on their tailfins; Eitam & Blaustein, 2002) by the end of the experiment were similar to those of the lab-born larvae, and the statistical analyses for these response variables for the full data set (see *statistical analysis* below) produced similar results to those conducted for cohort means that excluded the field-collected larvae (not presented).

The larvae grew in the pools under natural weather conditions, under the cover of screening (1cm^2 holes) to exclude potential predation by birds. This screening also reduced oviposition by mosquitoes into the pools, therefore reducing the input of large prey species. I monitored the larvae's developmental progress and survival and weighed them to the nearest mg every two weeks until day 58. In the first three monitoring dates, I also sampled the number of cannibalistic events observed in each treatment, recording the cumulative number of detected events to day 43. After this date, algal concentrations prevented direct observation of larvae in the pools. Instances of cannibalism were determined during survival counts either by direct observation of the act or by observing the typical appearance of the remains of partly cannibalized bodies; necrophagy is not known to occur in this species. This sampling protocol did not allow detection of the full extent of cannibalism that occurred up to day 43, since in each survey I could only observe cannibalistic events that had occurred up to a few days earlier. However, it allowed me to compare the rates of cannibalism between the treatments as a confirmation of the behavior documented by Markman et al. (2009).

Due to a mild winter, temperatures rose abruptly approaching day 58, and pools lost water quickly. Most experimental pools subsequently desiccated within eighteen days, coinciding with the desiccation of many natural pools, and forcing larvae to either metamorphose or die. Such mild winters are a frequent occurrence due to the high among-year variability in temperature and precipitation. A number of individuals were observed on day 58 to have initiated metamorphosis (abrupt darkening of skin color, loss of tailfin

coloration, shrinking of tailfin and external gills, developed walking ability). Since metamorphosis can, once initiated, be completed quickly even after pools dry (Sadeh, personal observations), these individuals were scored as successful metamorphs and their weight was assumed to approximate their size at metamorphosis – a correlate of an individual's expected lifetime reproductive success (Altwegg & Reyer, 2003). I terminated the experiment at day 58 since afterwards it became impossible to distinguish between emerged metamorphs and perished larvae in accounting for missing individuals. However, metamorphosing individuals stop feeding and lose weight (Sadeh, unpublished data). Since the pool-mean weights of the remaining individuals showed no reduction, few additional larvae were likely to have reached metamorphosis during this experiment.

I returned all surviving larvae either to the pools where their mothers were collected, or to large pools in same region for cases in which their natal pools had dried. Whenever possible, approaching the end of the experiment, I collected larvae that had not initiated metamorphosis from recently-dried experimental pools and returned them to the field to reduce unnecessary loss of lives.

Statistical analysis

To account for the spatial pairing of mesocosms while comparing larval performance between "mixed" and "sibling" cohorts, I used paired t-test and Wilcoxon's signed ranks test (when parametric assumptions were not met) on survival proportions and the mean numbers of individuals initiating metamorphosis, respectively, by the time the experiment terminated. I used independent-sample t-test to compare size at the initiation of metamorphosis between treatments, since the number of metamorphosing individuals was small and they had to be pooled in each treatment.

To test for differences in cannibalism rates between treatments, I used Z test for difference between treatments in the proportions of cumulative cannibalistic events detected.

To detect negative density dependent effects on larval performance, I used Pearson's correlation to test for the relationship between larval densities on day 43 and average sizes of survivors per pool at the end of the experiment.

Low genetic relatedness among members of a cohort is often correlated with high variation in initial sizes, potentially contributing to increased levels of aggression (Ziemba & Collins, 1999) compared to sibling cohorts, in addition to the effect of kin-discriminating behavior. Indeed, mean pool coefficients of variation in initial sizes were

higher in mixed cohorts (14.4%) than in sibling cohorts (6.6%; paired t-test *p*=0.002). While kin-discrimination and size variation may be confounded across treatments, they are not confounded within treatments. Specifically, within the mixed cohorts, both cannibalism rates and size variations were high, while the degree of relatedness among members was invariably small. Therefore, I used Pearson's correlations to determine whether the cohorts' coefficients of variation in initial body sizes can predict their performance variables, survival and metamorphic success.

3.4 RESULTS

By day 43, a total of six cases of cannibalism were detected in the mixed cohorts treatment (10% of the initial number of larvae) compared to only one case in the sibling cohorts treatment (1.7% of the larvae; Z= 2.106; p=0.035), confirming previous findings in this species (Markman *et al.*, 2009). My ability to detect cannibalism in surveys that were spaced two weeks apart was partial. Therefore, these numbers of observed cannibalistic events are an underestimate of the magnitude of cannibalism that actually occurred during that period. However, detectability should not differ between treatments, and undetected cases is expected to be distributed among them by a similar ratio.

Despite the significantly higher rates of cannibalism in mixed cohorts, overall mortality by that time was similar in mixed (15 deaths) and sibling (12 deaths) cohorts. Thus, overall mortality during that period compensated for the differences between treatments in cannibalistic mortality. This was also evident by the end of the experiment (day 58); sibling and mixed cohorts did not differ significantly in survival (Figure 3.1a; Table 3.1). During the 15-day time interval between the two final surveys (day 43 and day 58), four individuals died in the mixed cohorts and two in the sibling cohorts, hinting that cannibalism was still stronger in mixed cohorts by the end of the experiment. Larval densities on day 43 negatively correlated to the sizes of surviving larvae by day 58 (Figure 3.2; Pearson's *r* = -0.54; *p*=0.0136).

By the end of the experiment, 5% of the initial number of larvae in the sibling cohorts had initiated metamorphosis, a significantly lower rate than that of the mixed cohorts (15%; Figure 3.1a; Table 3.1). The sizes of individuals at initiation of metamorphosis did not differ between treatments (Figure 3.1b; Table 3.1).
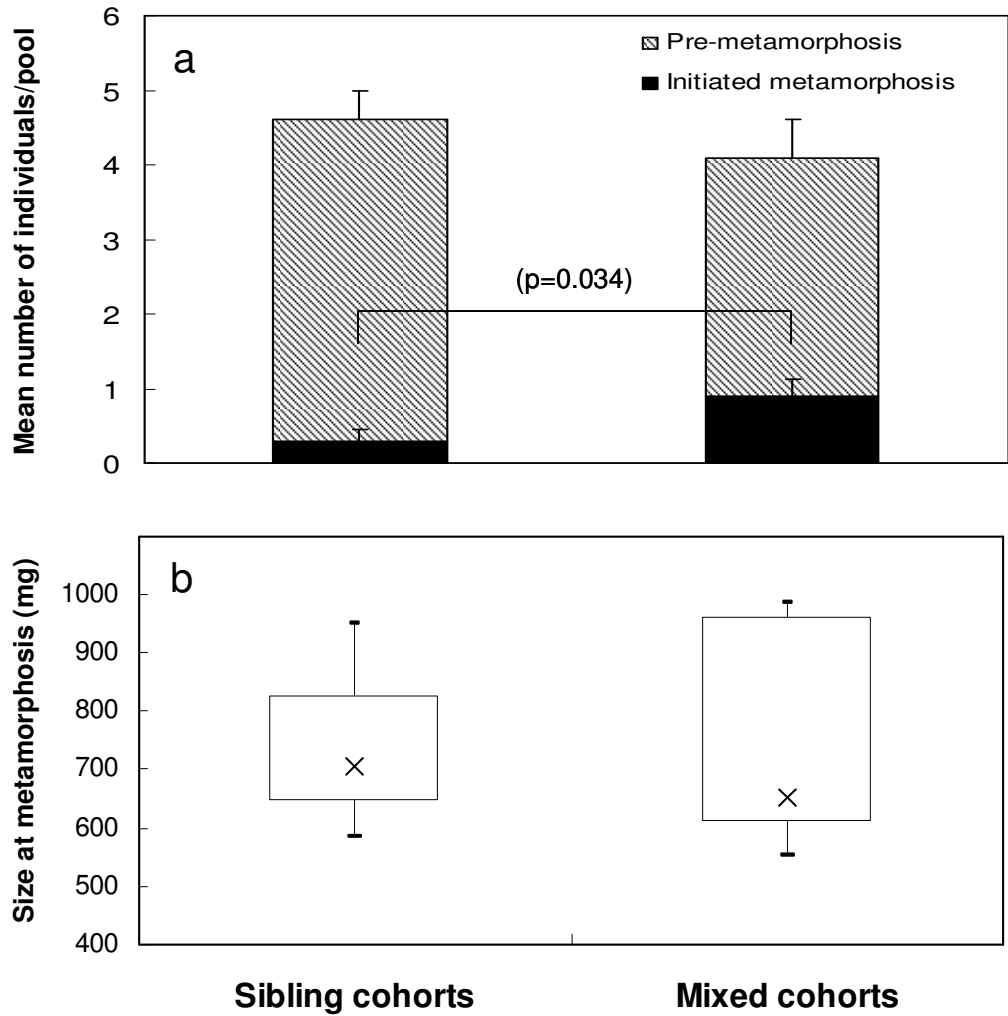
Figure 3.1: (a) Mean number of larvae surviving to the end of the experiment in sibling and mixed cohorts. Hatched bars represent individuals that had not reached metamorphosis. Solid bars represent individuals that initiated metamorphosis. Bottom error bars are 1 SE for larvae that initiated metamorphosis, top error bars are 1 SE for total survival. (b) Distributions of sizes at the initiation of metamorphosis in sibling and mixed cohorts.

Table 3.1: Test statistics for treatment comparisons of larval performance variables. Note that survival and initiation of metamorphosis were compared using paired tests, while size at initiation of metamorphosis was tested using an independent samples t-test.

Variable measured	Cohort composition	Mean	Statistic	*p*-value
Survival	Siblings	76.7%[*]	$t_{(df=9)} = 0.785$	0.453
	Mixed	68.3%[*]		
Initiation of metamorphosis	Siblings	5%[*]	Wilcoxon's $Z = -2.121$	0.034
	Mixed	15%[*]		
Size at initiation of metamorphosis	Siblings	0.746 g	$t_{(df=10)} = 0.018$	0.986
	Mixed	0.744 g		

[*] Percentages are of initial cohort numbers.
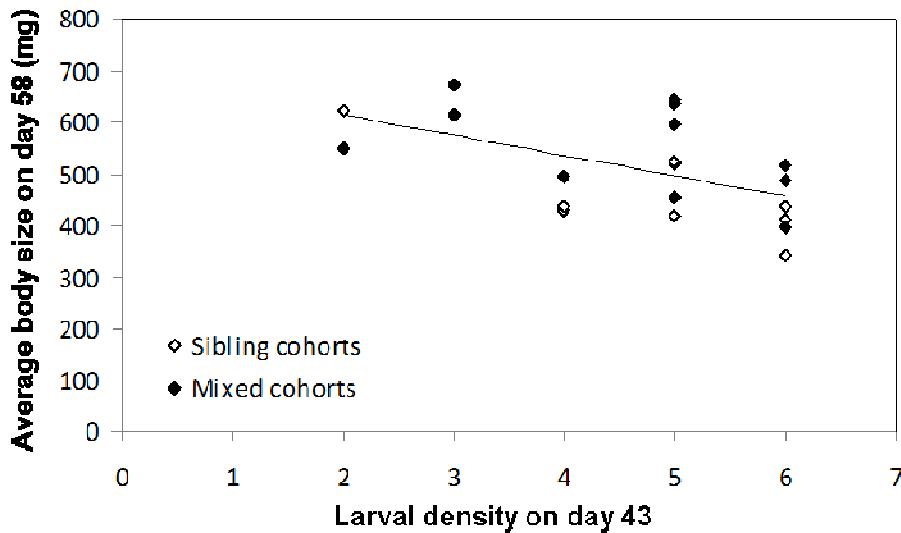
Figure 3.2: Average larval size per pool by the end of the experiment as a function of larval densities observed on day 43 (Pearson's r = -0.54; p=0.0136).

Although the coefficients of variation in initial sizes of individuals were different between treatments, possibly reflecting differences in birth sizes of *S. infraimmaculata* larvae among populations (Blaustein et al, unpublished data), the coefficients of variation in initial larval mass did not correlate significantly with survival rates or with successful initiation rates of metamorphosis in the mixed cohorts (Table 3.2).

Table 3.2: Pearson's correlations of mixed cohorts' coefficients of variation in initial sizes with their larval performance variables.

Response variable	N	Pearson's *r*	*p*-value
Survival	10	- 0.047	0.898
Initiation of metamorphosis	10	0.121	0.739

3.5 DISCUSSION

I found that reduced cannibalistic mortality among sibling cohorts due to kin-selective behavior is continuously compensated for prior to pool drying, resulting in similar total mortality rates to those of more cannibalistic mixed cohorts. In addition, I found that reduced cannibalism can lead to over-compensatory mortality if pools dry early and therefore result in poorer performance of sibling larval cohorts. I suggest that these patterns of mortality may be driven by a density-dependent feedback to cannibalistic mortality as well as by the direct nutritional benefits of cannibalism.

Prior work on fire salamanders that isolated larval behavior from the effects of density and risk of habitat drying showed that larval aggression towards conspecifics is inversely related to the degree of their genetic relatedness (Markman *et al.*, 2009). Such kin-selective behavior was also evident in this study, at least until day 43 of the experiment by which a greater proportion of mortality was the result of cannibalism in the mixed cohorts, compared to sibling cohorts. These results join a line of studies showing patterns of expression of aggressive behaviors that are consistent with kin selection theory (e.g. Pfennig, 1997; Segoli *et al.*, 2009; Walls & Roudebush, 1991). However, overall survival rates to day 43 and to the end of the experiment were similar across treatments, revealing the action of a compensatory process. Plausible mechanisms underlying this compensation may be density-dependent non-cannibalistic mortality that resulted from the low *per capita* resource availability, or the loss of kin-discriminating behavior between day 43 and day 58, shortly before the desiccation of the pools (e.g. Blaustein *et al.*, 1993). The negative relationship observed between larval densities and body sizes at the final part of the experiment supports the former mechanism.

A greater number of early initiations of metamorphosis occurred in the mixed cohorts, lending further support to the idea that overall cannibalism was stronger in these cohorts throughout the larval period. In highly temporary habitats, animals often perceive cues for the approaching loss of their habitats and respond by accelerating their development rates (Johansson *et al.*, 2001; e.g. Laurila & Kujasalo, 1999; Marquez-Garcia *et al.*, 2009; specifically shown in this species by Sadeh *et al.*, 2011). In such cases, trophic energy is mostly allocated to differentiation and metamorphosis (Harris, 1999). Here, hydroperiods were undoubtedly short enough to induce accelerated development in all the larvae (see Sadeh *et al.*, 2011). Thus, the higher rates of initiation of metamorphosis in the mixed cohorts suggest that a greater proportion of individuals, compared to the sibling cohorts, could employ sufficient food resources and utilize them for reaching minimal size

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for metamorphosis and fueling the required developmental changes. I suggest that this increase in food availability was due to greater overall rates of aggressive interference and cannibalism. A similar effect metamorphic success was documented in this species when inter-cohort cannibalism was manipulated through habitat structural complexity (Sadeh *et al.*, 2009; Sadeh unpublished data). In groups expressing high levels of cannibalism, the distribution of food resources (both conspecific victims and shared resources) among members is not uniform (Polis, 1981), and is biased in favor of the cannibals. This implies that if some loss of kin-discrimination in the final days of the experiment had occurred, it was not sufficient to equalize overall cannibalism rates between the treatments.

Greater rates of cannibalism among distantly related individuals can therefore improve cohort performance in short-lived habitats by increasing development rates and metamorphic success. Both the direct nutritional benefit acquired by some larvae through cannibalism, and the indirect benefit of relaxed competition fuel the acceleration of metamorphosis in genetically mixed cohorts. In contrast, siblings refraining from cannibalism may remain uniformly starved and susceptible to death by delayed development and habitat loss. West *et al* (2002) argued that altruistic behavior towards relatives may lead to increased competition between relatives, potentially reducing or even removing kin selection. How might kin discriminative aggressive behavior be maintained in fire salamander populations, then? The effects of kinship demonstrated in this study may weaken or even act in the opposite direction in long-lived habitats with sufficient food availability, where large groups of siblings are neither impeded by densities nor are they in risk metamorphic failure (Pakkasmaa & Laurila, 2004). If so, the spatio-temporal variation of pool hydroperiods may be the key to the retention of kin selection in this system.

The genetic composition of a cohort in nature may affect both kin-selective behaviors that determine the motivation to cannibalize, and differences in body sizes that determine the capacity to cannibalize (Dong & Polis, 1992; Polis, 1981). Sometimes, even small initial size differences that may result from genetically or maternally determined sizes at birth may diverge with larval growth, especially when size differences determine the beneficiaries of interference competition (Ziemba & Collins, 1999). While these two mechanisms may have operated in concert in this experiment to increase cannibalism rates among mixed cohorts and influence metamorphic success and survival, Markman et al (2009) provided evidence that similar-sized individuals of this species do express relatedness-biased aggression. Moreover, coefficients of variation in initial larval sizes did not predict the patterns of mortality or metamorphosis by the end of the experiment within

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the mixed cohort treatment, where initial size variation and kin-selective cannibalism are not confounded. Thus, it can be concluded that behavioral kin discrimination had a significant role in driving the observed interactions.

Predictions for maternal oviposition habitat selection strategies can be derived from these results. The mean performance of a cohort is an estimation of the expected performance of an oviposited clutch. Consider a case where a pregnant female encounters two habitats, in one of which she has recently oviposited, and in the other an unrelated female has recently oviposited. *Salamandra* females, as well as females of other organisms, are known to spread their larval clutches spatially and temporally, allowing such scenarios to occur (e.g. Segev *et al.*, 2011; Spieler & Linsenmair, 1997). Such organisms are often capable of demonstrating sensitivity to multiple ecological variables in their habitat preferences (e.g. Sadeh *et al.*, 2009). In solitary parasitoids where competition is also lethal but not relatedness-biased (Godfray, 1994), conspecific superparasitism generally has a higher pay-off than self-superparasitism (e.g. Visser, 1993). Where groups of larvae demonstrate kin-biased aggression, it might be intuitively expected that maternal behavior would avoid the high levels of aggression in groups of distantly related larvae. However, depending on offspring food and time limitations, their expected performance may be worse if she chooses to deposit twice in the same habitat. Therefore, under such limitations females may prefer sites occupied by unrelated larvae.

Conservation strategies for highly cannibalistic species may require the consideration of the role of cannibalism in larval performance and recruitment patterns to the adult population (e.g. Wissinger *et al.*, 2010). While cannibalism might be perceived merely as detrimental to population growth, the results of this study imply that this behavior may sometimes be important for larval recruitment, and measures to reduce it may not always be desirable. The spatial and temporal spreading of larvae demonstrated in *S. infraimmaculata* (Segev *et al.*, 2011), combined with its long distance movements during the breeding season (Bar-David *et al.*, 2007) imply that the mixing of distantly related sibships may occur in natural populations. The conservation of landscape connectivity between breeding sites may be important to maintain this mixing, which may in turn influence the success of larval recruitment.

LARVAL INTERCOHORT PRIORITY EFFECTS AND MATERNAL SELF/CONSPECIFIC SUPEROVIPOSITION AS A FUNCTION OF INTERCOHORT TIME INTERVAL

4.1 ABSTRACT

The larval stages of many organisms with complex life cycles inhabit discrete habitats, where size and age structure may be important for larval performance and recruitment to the adult population. Few studies have focused on the interactions between sequential cohorts, known as intercohort priority effects, and their implications, particularly as a function of the time interval between cohort colonizations, and the densities of younger cohorts. Using a mesocosm experiment with fire salamander larvae, I show that the strength of competition experienced by the older, dominant cohort reduces with intercohort time interval. Further support is lent to previous findings that rates of cannibalism upon younger cohorts increase with intercohort time interval. In this experiment, the relative strength of density-dependent exploitative competition was greater than that of interference and cannibalism, and lead to considerable metamorphic failures. I also conducted outdoor pool selection trials to test the hypothesis that the above patterns of priority effects will influence maternal conspecific/self superoviposition decisions as a function of intercohort time interval. Salamander females demonstrated a seemingly maladaptive indifference to the genetic relatedness of prior cohorts. Since previous studies showed that females are capable of detecting the presence of cannibalistic larvae, and that larvae are capable of kin recognition, this may indicate an ontogenetic constraint on kin recognition. The effects of intercohort priorities and time intervals on larval survival and ontogeny may influence post-metamorphic life history, recruitment patterns to the adult population and juvenile dispersal.

4.2 INTRODUCTION

Phenotypic and interaction asymmetries among members of structured populations may affect life-histories (De Block & Stoks 2004) and lead to complex population-level patterns (Claessen *et al.* 2004). Population age-structure and size-structure are major sources of such asymmetries.

Organisms with complex life cycles display dramatic switches in ecological niches when proceeding from one stage to another, thereby reducing considerably such asymmetric interactions between larvae and adults (Istock 1967). However, the larval stages of many terrestrial organisms with complex life-cycles inhabit discrete, confined habitats, where size and age structure may be important for larval performance and recruitment to the adult population. In some extreme cases, only up to one of the inhabitants may emerge from the larval habitat (Godfray 1994; Poelman & Dicke 2007). In these habitats, larval size structure is often highly linked to the pattern of larval colonization through variation in hatching times (e.g. Hopper *et al.* 1996) or multiple oviposition events (e.g. Anholt 1994) that lead to the co-occurrence of sequential cohorts. Few studies have focused on the interactions between sequential cohorts, known as intercohort priority effects, and their implications (Eitam *et al.* 2005), probably due to methodological difficulties of distinguishing members of different cohorts. In particular, we know little about priority effects as a function of the time interval between cohort colonizations (hereafter referred to as intercohort time interval, which I denote by Δt).

Longer time intervals between consecutive cohort colonizations into a habitat generally lead to greater differences between their body sizes distributions. These size differences may dictate inter-cohort priority effects via asymmetric interference competition and cannibalism, usually biased in favor of the older cohorts (Anholt 1994; Godfray 1994; Eitam *et al.* 2005; Sadeh *et al.* 2009), although the opposite pattern can sometimes be found (Crump 1986; Chau & Maeto 2008). Rates of cannibalism of the older cohort upon younger cohorts can be hypothesized to increase with Δt (Figure 4.1) since victims are easier to subdue, especially in gape-limited predators (Polis 1981; Wissinger *et al.* 2010). However, this increase may level off due to other limitations on the rate of cannibalism, such as encounter rates between individuals and minimal digestion/handling times (Juanes 2003). In addition, at very long Δt, rates of cannibalism may be limited and even drop due to shorter temporal overlap between the cohorts' occurrence as a result of the approaching metamorphosis and emergence of the older cohort.
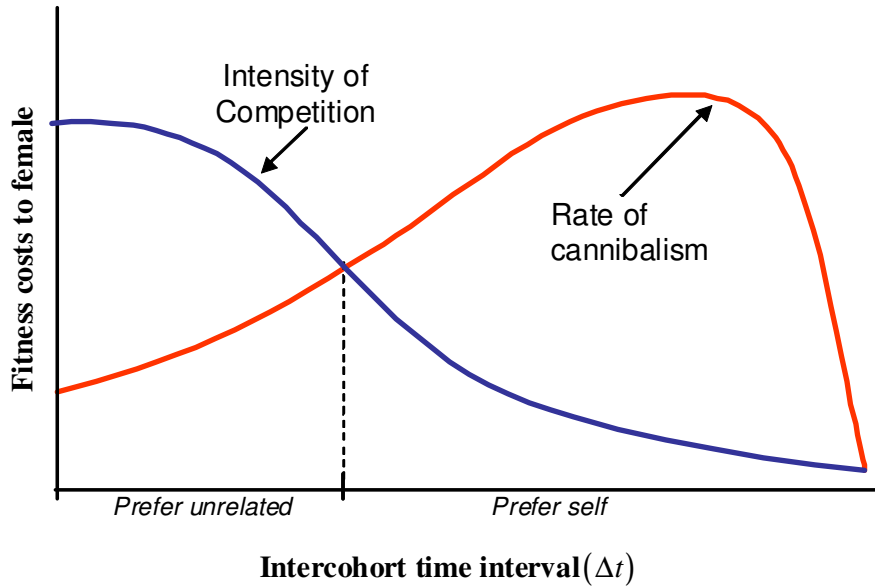
Figure 4.1: Hypothesized relative importance of competition experienced by the early cohort, and of the rate of cannibalism upon the late cohort, as a function of intercohort time interval (Δt). This hypothesis predicts the preference of a superovipositing female for habitats containing her own offspring or unrelated conspecifics, as a function of Δt. The ordinate axis scales the fitness costs of competition and cannibalism to the female depositing the early and the late cohort, respectively.

While older cohorts usually dominate the larval habitat, they may still be subject to exploitative competition exerted by the younger cohorts (Hjelm & Persson 2001). I hypothesized that the severity of such competition experienced by the older cohorts decreases with Δt (Figure 4.1). This decrease may result from (1) a longer growing period before the introduction of competing younger cohorts; (2) higher cannibalism rates eliminate competitor densities, and effectively turn the younger cohorts into an additional source of food (Polis 1981); and (3) reduced foraging activity by the younger cohorts and/or exclusion from rich resource patches by stronger interference and fear of higher cannibalism rates (Lima & Dill 1990; Werner & Peacor 2003; Preisser *et al.* 2005).

Ephemeral larval habitats impose stress and time constraints on larval development (Loman 2002; Sadeh *et al.* 2011), potentially exacerbating the impacts and importance of intercohort priority effects. The food-fueled developmental race to timely emergence also places greater importance on exploitative and interference competition as modifiers of feeding rates, compared to continuous habitats. Cannibalism allows individuals to accelerate their rate of development, and in some cases may be a key source of food

allowing timely emergence and avoidance of death by habitat termination (Wells 2007, Sadeh under review). Compromised rates of food intake due to competition and interference may result in greatly increased mortality rates, in addition to compromised body sizes.

Priority effects may also exert selective forces on the parental choice of larval habitat (Godfray 1994; Spieler & Linsenmair 1997). When suitable larval habitats are limited in the landscape, avoidance of prior cohorts is possible only before all habitats are occupied. From that point on, all offspring must be deposited with prior conspecifics and other factors may come into play, such as the availability of refuge from cannibalism (e.g. Sadeh *et al.* 2009), the densities of conspecifics in each habitat (e.g. Kiflawi *et al.* 2003), the genetic relatedness of prior cohorts and the time interval between cohorts. The hypothesized trends of rates of cannibalism upon younger cohorts and the intensity of competition experienced by the older cohort, both as a function of Δt (Figure 4.1) can be used to predict the preference of the genetic relatedness of prior cohorts during the second oviposition into habitats (superoviposition). At short Δt, the relative importance of competition may exceed that of cannibalism. Thus, the young cohort will compete strongly with the prior cohort. In this case, females are predicted to prefer habitats with prior larvae from unrelated females (unrelated superoviposition), to avoid sibling competition (Hamilton & May 1977). At long Δt, cannibalism becomes more significant, and I predict that the young cohort will suffer high cannibalism rates and in itself accrue low fitness payoffs to its parent. Under these conditions, females are predicted to prefer habitats that contain their own previously-deposited larvae (self superoviposition), and thereby feed their own older offspring rather than those of unrelated females (Perry & Roitberg 2006; Poelman & Dicke 2007). In case either cannibalism or competition scales to greater fitness costs than the other for all Δt (so the curves in Figure 4.1 do not intersect), a constant preference for self or unrelated superoviposition, respectively, is predicted.

Fire salamander (*Salamandra infraimmaculata*; Steinfartz *et al.* 2000) females breed mostly in temporary pools in northern Israel, where large adult populations exploit breeding sites containing relatively few and mostly small pools (Spencer *et al.* 2002; Segev *et al.* 2010), necessitating deposition by multiple mothers into each pool. The females spread their larvae both temporally within the breeding season, and spatially among available temporary pools (Segev *et al.* 2011), and display sensitive selectivity for pool quality, including depth (Segev *et al.* 2011), structural complexity, and the presence of large conspecific larvae (Sadeh *et al.* 2009). The larvae display strong inter-cohort priority

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effects (Eitam *et al.* 2005; Segev 2009) including size-dependent cannibalism (Degani *et al.* 1980; Reques & Tejedo 1996; Sadeh *et al.* 2009). Although *S. infraimmaculata* are ovoviviparous, I use both 'larviposition' and 'oviposition' interchangeably in this paper, since my questions also apply for the latter and more inclusive term.

In this study, I used the fire salamander in a factorial mesocosm experiment to test my hypotheses on the strength of intercohort priority effects as a function of the time interval between consecutive larval colonizations (Figure 4.1), and their dependence on the densities of the younger cohort. Since the size- and age-dependence of cannibalism rates is well established in this species, the experimental design focused more on the competition experienced by the older cohort. I also conducted sets of larviposition pool selection trials to test my prediction regarding self vs. unrelated superoviposition decisions by pregnant females as a function of Δt.

4.3 METHODS

Intercohort priority effects experiment

In this mesocosm experiment, I sought primarily to assess the competitive effects of the second cohort on the first cohort as a function of the time interval between first and second cohort colonizations. A secondary goal was to assess the effects of time of second cohort deposition on the performance of the second cohort, as a result of both cannibalistic and competitive impacts of the first cohort, as well as the shorter time available for development. I crossed three intercohort time intervals ($\Delta t = 0$, 9 and 22 days) between colonizations by the first and the second cohort larvae, with two densities of second cohort larvae (three and six larvae/pool). I replicated each treatment combination nine times and allocated treatments randomly to the 54-mesocosm array. I enclosed the entire outdoor experimental array in chicken wire (mesh size 3 cm) to prevent salamander predation by birds.

I filled the mesocosms with 40 liters aged tap water. Five days before the experiment began, I added to each mesocosm 2,220 g (range 2,210 - 2,230 g) soil from a dried temporary pool containing a natural population of crustacean resting eggs. I placed a plastic net (mesh size 1 mm) over the soil, covering the bottom and walls of the pool to the rims. I placed five rocks (each displacing ~150 ml of water) on the plastic net for structural complexity. The net allowed ease of periodically lifting out the salamander larvae during the experiment for counting and measurement. I attached to the net, on the rims of each

tub, a horizontal plastic ledge (5 cm wide) to prevent climbing metamorphs from escaping the mesocosms.

I collected gravid females on their way to their natural breeding sites on Mt. Carmel during early winter, 2010, and induced larviposition in the lab by placing them in water-filled tubs. Upon births on days 1, 10 and 23 of the experiment, I grouped larvae according to their maternal site of origin and introduced them into the outdoor mesocosms.

On day 1, I placed in each mesocosm three newborn larvae, referred to as the 'first cohort', all from the same natural site, though not necessarily the same female (mean wet mass 246 mg, SD=39 mg, mean snout-vent length [SVL] 1.71 cm, SD=0.07 cm). I added an additional group of either three or six individuals from a different natural site (mean wet mass 206 mg, SD=17 mg, mean SVL 1.72 cm, SD=0.11 cm) to 18 mesocosms, referred to as the 'second cohort' at $\Delta t = 0$ days. I photographed the tailfins of all first cohort larvae for individual identification and for discrimination between members of the first and the second cohorts throughout the experiment (see Eitam & Blaustein 2002).

I added a second cohort of three and six larvae to the rest of the tubs on days 10 and 23, ($\Delta t = 9$ and 22, respectively). On day 10, the second cohort larvae that were added weighed 216 mg (SD=27 mg), while the first cohort in these mesocosms had reached a mean weight of 348 mg (SD=31 mg). On day 23, the second cohort larvae that were added weighed 254 mg (SD=51 mg), while the first cohort in these mesocosms had reached a mean weight of 519 mg (SD=40 mg).

On days 8 and 16, I added 120 and 230 ml water, respectively, from a natural pond to provide zooplankton at availabilities similar to those of natural pools. The first inoculate contained predominantly *Ceriodaphnia* species, while the second contained mostly *Arctodiaptomus similis*, *Ceriodaphnia* and a *Cyzicus sp*. These are common prey of *Salamandra* in the region (Blaustein *et al.* 1996; Eitam *et al.* 2005).

Water levels fluctuated naturally according to weather conditions, except for days 18 and 44, when I added aged tap water to 50% of the capacity of the tubs to prevent the drying of my mesocosms due to an atypically dry early winter. In that season, rains persisted exceptionally late into the spring, resulting in longer than usual hydroperiods both in the field and in my mesocosms. I allowed the tubs to dry completely and consequently, by day 163, the experiment ended. Throughout the experiment, I followed larval mass gain every two or three weeks, and their survival generally every week. I distinguished cases of cannibalism either by direct observation of the act, which may take many hours and up to one day, by finding the typical remains of partly cannibalized larvae,

or by the disappearance of larvae coinciding with the sudden increase in mass of one of its pool mates. This is a reliable estimation of cannibalism since *S. infraimmaculata* larvae usually attack moving prey, and numerous lab observations (Sadeh, personal observations) show that they very rarely, if ever, consume dead conspecifics that have not been recently killed. Metamorphosing individuals climbed on the plastic net on the tubs' walls, and remained beneath the ledges that prevented their escape. Once the first metamorphs were found, I visited the tubs to collect metamorphs every two to three days to collect and remove metamorphs. For each metamorph, I recorded its time, mass and SVL at emergence.

To analyze the treatment effects on the first and the second cohorts' fitness, I used two-way ANOVAs to compare mean mortality, time to- and size at metamorphosis. Sizes at metamorphosis reflect the combined underlying larval patterns of both growth and development. Therefore, I also compared mean larval mass gain rates across treatments for each cohort by the last measurement date prior to first metamorphosis (day 94 for first cohort, day 122 for second cohort). This was calculated as the total mass gained divided by larval age at the last measurement date. Log and rank transformations were used on sizes at metamorphosis of the first and the second cohorts, respectively, to improve homogeneity of variances. The treatment combination of $\Delta t = 9$ and low larval density showed zero variance for first-cohort mortality. Therefore I excluded the intermediate level of $\Delta t = 9$ from the analysis, using a 2x2 factorial ANOVA. Upon graphical inspection (Figure 4.2b), the pattern of the $\Delta t = 9$ treatments does not differ from that of the two tested levels. I present the similar results for tests on both this subset of the dataset and on the full 3x2 dataset.

Larviposition choice trials

During the first winter rains, 2008, I collected 30 gravid females on their way to their natural breeding sites in the Carmel and the Galilee. Since this species is endangered in Israel, I was restricted by the National Parks Authority to this number of individuals. I assumed that these females deposited few, if any, larvae prior to their collection. The Carmel and the Galilee are mountainous regions separated by ~20 Km of lowlands, and the populations of these regions are genetically distant (L Blank, unpublished). Aggression and cannibalism between larvae originating from these two regions is significantly higher than between siblings (Markman *et al.* 2009, Sadeh under review). I kept the females in terraria in the lab at 19C, the natural photoperiod, and were fed with field-collected slugs.

Gravid *S. infraimmaculata* generally begin the season with more than 100 larvae in their uterus. I induced larviposition of only a partial clutch (first-cohort larvae) by all the females by placing them in water-filled tubs until they gave birth to 13-25 larvae. The females were then returned to their terraria to prevent them from further larviposition. All first-cohort larvae were each weighed, photographed for individual identification and documented for their parental identity. I kept the larvae in covered tubs outdoors and fed them frequently with field-collected zooplankton, including crustaceans and mosquito larvae.

I matched groups of 10 first-cohort larvae from each mother of the Carmel region with a group of similar number and mass from mothers of the Galilee region. The average age difference between larvae of matched groups was 0.75 days and did not exceed three days. I used these groups of 20 first-cohort larvae as 'self' and 'unrelated' treatments for their two parents in both early (short Δt) and late (long Δt) larviposition decision bouts, to test the effect of Δt on superoviposition decisions.

For both the early and late larviposition bouts, I introduced the first-cohort larvae into tubs dug into the ground and filled with 35 L aged tap water, in outdoor enclosures. Each enclosure contained four tubs, and I placed five sibling larvae in each tub. This density of larvae is ecologically relevant and was sufficient to induce maternal habitat selection responses in a previous study (Sadeh *et al.* 2009), where a similar experimental procedure was used. Of the four tubs in each enclosure, two contained first-cohort larvae who were born to the focal mother ('self' treatment), and the other two contained first-cohort larvae born to a female from the alternate geographic region ('unrelated' treatment). The first-cohort larvae were kept in these tubs for 24 hours before introducing a pregnant female into each of the enclosures.

Early larviposition bouts (short Δt) – When the first-cohort larvae were 3-11 days old (average Δt= 6 days), and still small (mass = 0.258 g; SD = 0.043 g) I placed one gravid female in each of the enclosures, with a choice of two pools containing her own first-cohort offspring ('self') and two pools containing distantly related first-cohort larvae ('unrelated'). I used water sprinklers in the absence of natural rain to encourage the females to larviposit. Every hour, I observed the pools in enclosures where the females were not in the water to avoid interrupting the larviposition behavior. Any female that finished depositing her first clutch was taken out of the enclosure back to the lab while leaving the larvae in the pools. Females that did not deposit were left in their enclosures for the next inspection after another hour, until either they gave birth, or the night was over.

Observations each hour during this experiment revealed that most females also visited pools other than those that were eventually chosen for larviposition. At the end of the night I counted the numbers of deposited larvae in each pool. After the early larviposition trials, I took all the females and larvae back to their rearing facilities in the lab and in outdoor tubs, as described above.

Late larviposition bouts (long Δt) – I conducted similar larviposition trials 3.5 weeks later. This time interval is within the range of time intervals between rain events in Israel, during which salamander movement and larviposition is naturally restricted. Due to limitations by the Israel Nature and Parks Authority, I was not able to keep the pregnant females in the lab over a longer time interval. At this point, the first-cohort larvae were 23-34 days old (average Δt= 31 days) and larger (mass = 0.536 g; SD = 0.083 g).

I used sign tests to determine whether preference of a particular pool type was significantly different than 0.5 in each larviposition bout. I used normal approximation Z tests for proportions to test for differences in the proportions of preferred pool types between early and late bouts, as well as for differences in the proportions of tubs where cannibalism occurred between pool types and between early and late bouts. Since I was interested mostly in the time-specific comparisons between the treatments, and in the effect of time itself on clutch size decisions, I analyzed mean clutch sizes using three planned orthogonal contrasts: (1) early 'self' vs. early 'unrelated', (2) late 'self' vs. late 'unrelated', and (3) early vs. late.

4.4 RESULTS

Intercohort priority effects on first cohort

I summarize the statistical tests in Table 4.1. Increasing intercohort time intervals generally increased larval mass gain rate of the first cohort measured through day 94 (the last sampling date before the occurrence of any metamorphosis), while higher densities of the second cohort significantly decreased mass gain rate (Figure 4.2a).
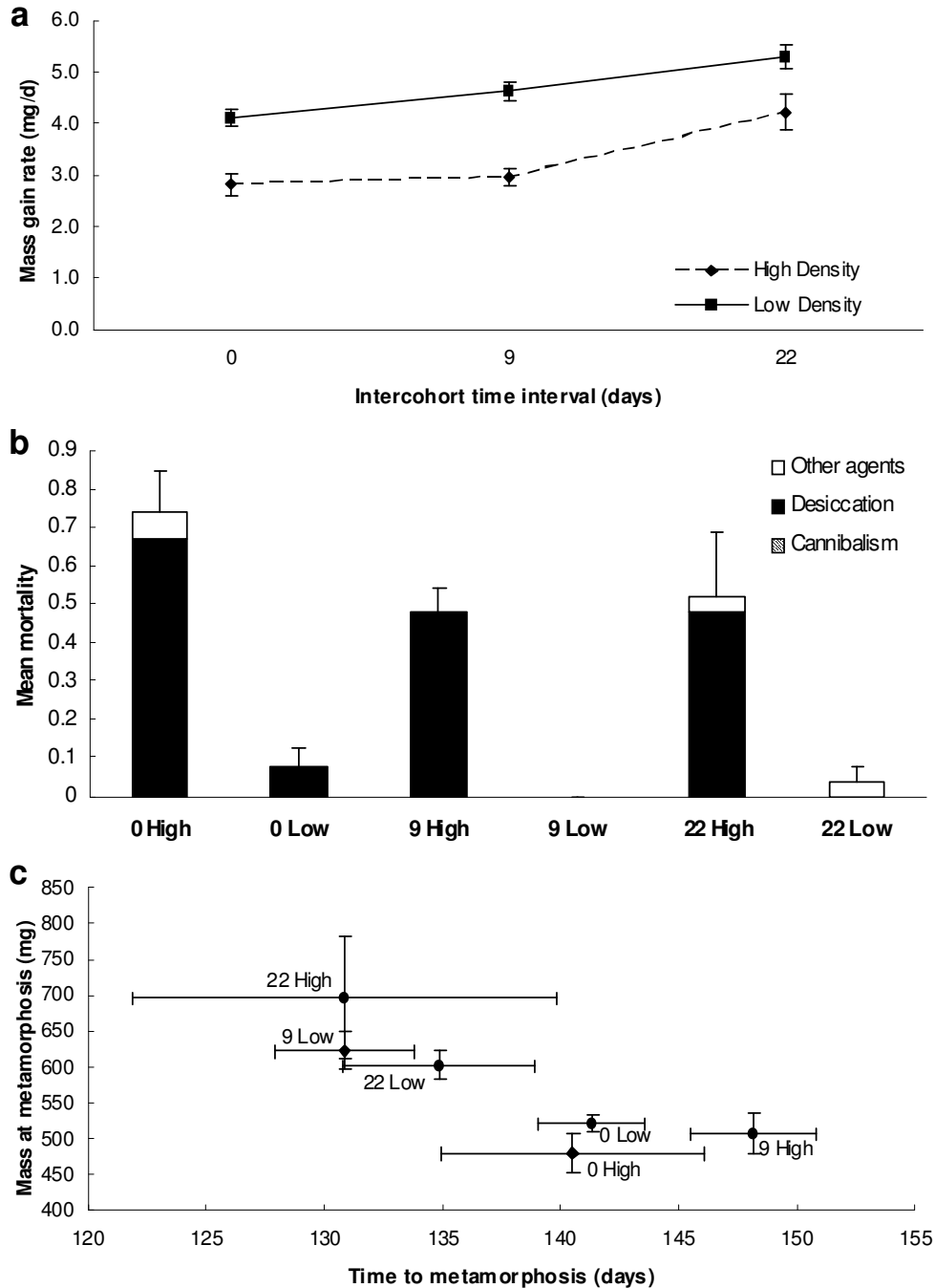
Figure 4.2: Effects of treatment combinations of intercohort time interval ($\Delta t = 0, 9, 22$ days) and second cohort densities (High and Low) on first-cohort larvae: (a) Mean larval daily mass gain rates prior to any metamorphosis of the first cohort. (b) Mean larval mortality by different sources. None of the first cohort larvae were cannibalized. The $\Delta t = 9$ treatments were excluded from the ANOVA due to zero variance in the '9-Low' treatment. (c) Mean time to (horizontal axis) and size at (vertical axis) metamorphosis. All error bars are 1 SE.

Table 4.1: Two-way ANOVA tests for the effects of intercohort time interval (Δt) and second cohort larval density on larval fitness proxies. Results in parentheses under first cohort larval mortality are for the test conducted on the full dataset (see the methods section for details).

Response variable	Factor	df (factor, error)	F	p-value
First cohort				
Larval mass gain rate through day 94	Δt	2,48	16.42	<0.0005
	Density	1,48	49.85	<0.0005
	Δt×Density	2,48	0.75	0.477
Larval mortality	Δt	1,32	1.54	0.223
		(2,48)	(1.96)	(0.153)
	Density	1,32	30.27	<0.0005
		(1,48)	(56.53)	(<0.0005)
	Δt×Density	1,32	0.79	0.382
		(2,48)	(0.73)	(0.487)
Size at metamorphosis	Δt	2,39	7.97	0.001
	Density	1,39	1.56	0.219
	Δt×Density	2,39	4.50	0.017
Time to metamorphosis	Δt	2,39	1.96	0.155
	Density	1,39	1.45	0.235
	Δt×Density	2,39	4.26	0.021
Second cohort				
Larval mass gain rate through day 122	Δt	2,48	0.75	0.477
	Density	1,48	9.10	0.003
	Δt×Density	2,48	0.33	0.724
Larval mortality	Δt	2,48	4.35	0.018
	Density	1,48	49.77	<0.0005
	Δt×Density	2,48	1.28	0.288
Size at metamorphosis	Δt	2,40	3.43	0.042
	Density	1,40	16.66	<0.0005
	Δt×Density	2,40	0.68	0.513
Time to metamorphosis	Δt	2,41	44.44	<0.0005
	Density	1,41	3.78	0.059
	Δt×Density	2,41	1.41	0.255

Intercohort time intervals did not affect mortality in the first cohort, but higher densities of the second cohort significantly increased it. Mortality was almost entirely due to desiccation of the pools prior to metamorphosis, and none of the first-cohort larvae mortalities were assigned to cannibalism (Figure 4.2b).

Mean time to metamorphosis of the first cohort was not significantly affected by either of the main effects. However, the Δt×density interaction was significant. The more favorable treatment combinations ($\Delta t = 22$ days, and low densities at $\Delta t = 9$ days) resulted in shorter larval periods than the more stressful combinations ($\Delta t = 0$ and the high densities treatment at $\Delta t = 9$ days; Figure 4.2c).

Size at metamorphosis of the first cohort (log-transformed) generally increased with Δt, and larval densities did not significantly affect it. However, the $\Delta t \times$density interaction was significant. Thus, it followed a similar pattern as time to metamorphosis, where favorable conditions generally resulted in greater body sizes (Figure 4.2c).

Intercohort priority effects on second cohort

Intercohort time intervals did not significantly affect second cohort average growth rates measured through day 122, before the occurrence of any metamorphosis, but higher densities retarded it significantly (Figure 4.3a).

Both increasing Δt and higher densities significantly increased mortality in the second cohort. Mortality was mostly due to desiccation of the pools prior to metamorphosis, but some cannibalism also occurred (Figure 4.3b).

Times to metamorphosis of the second cohort were significantly shorter with increasing Δt. While larval density had only a marginally significant effect ($p=0.059$) on time to metamorphosis, its pattern was consistent in all three time intervals (Figure 4.3c), showing a slight delay by higher second-cohort densities.

Size at metamorphosis (rank transformed) of the second cohort was significantly reduced by larval densities. While the effect of Δt was significant in an ANOVA, post-hoc Tukey HSD tests between the levels of this factor revealed that sizes under $\Delta t = 0$ were significantly lower than under $\Delta t = 9$ ($p=0.018$), whereas the other comparisons were not significant ($\Delta t=0$ vs. $\Delta t=22$: $p=0.261$; ($\Delta t=9$ vs. $\Delta t=22$: $p=0.465$). Moreover, the graphical pattern of this response variable does not imply a consistent effect of Δt (Figure 4.3c).
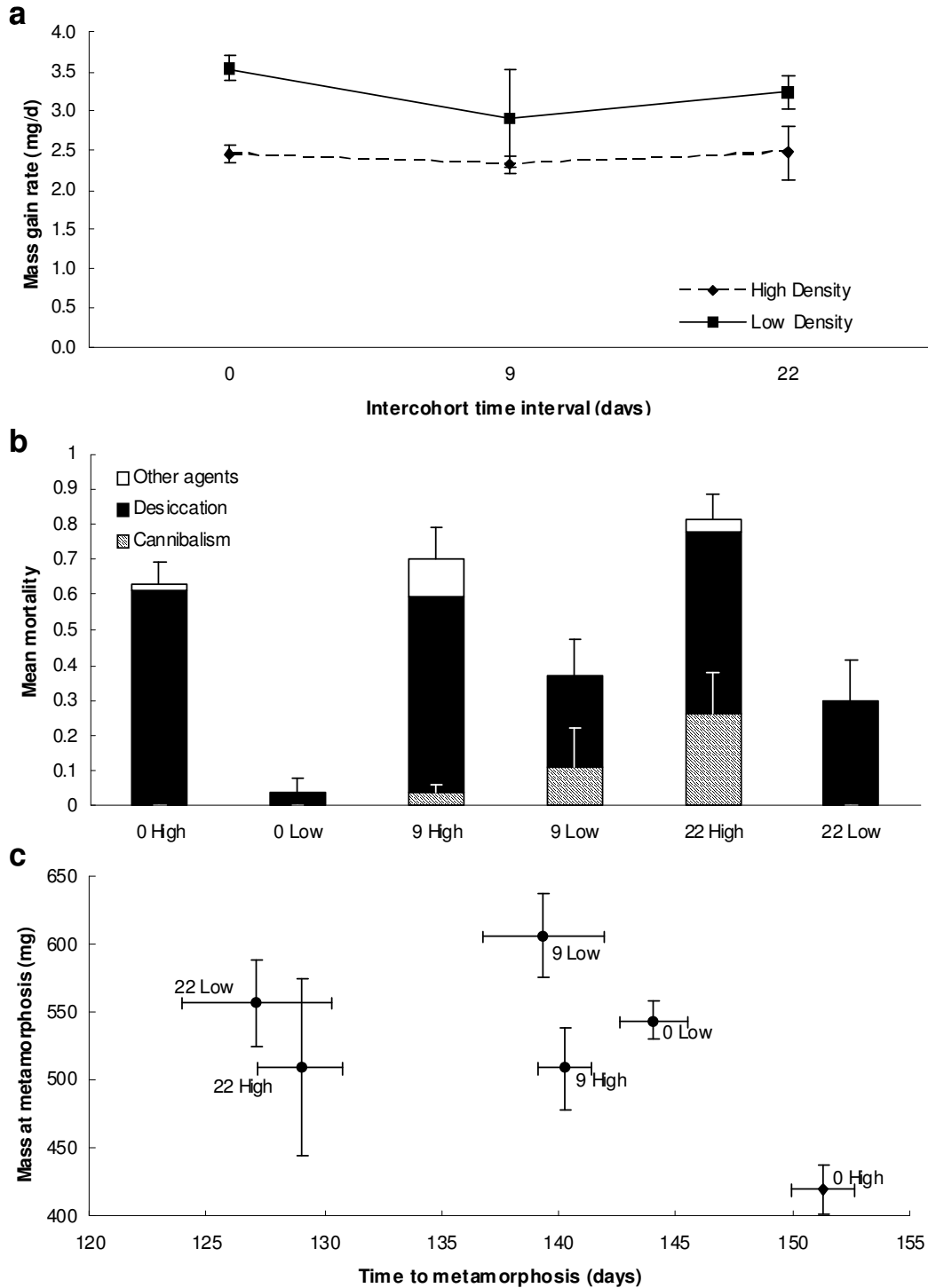
Figure 4.3: Effects of treatment combinations of intercohort time interval (Δt = 0, 9, 22 days) and second cohort densities (High and Low) on second-cohort larvae: (a) Mean larval daily mass gain rates prior to any metamorphosis of the second cohort. (b) Mean larval mortality by different sources. (c) Mean time to (horizontal axis) and size at (vertical axis) metamorphosis. All error bars are 1 SE.

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Larviposition choice trials

Seventeen of the 23 females used in the early trials (short Δt) larviposited. Two of them deposited into more than one tub before the tubs were first inspected, and another began to give birth while she was carried to the experimental site, and then immediately spawned many larvae into the first tub it encountered. These three females were not included in the data set. Of the remaining 14 females, eight selected tubs containing their own previously deposited larvae, and six selected tubs containing unrelated larvae (sign test p=0.791; Figure 4.4a). The mean number of larvae deposited per tub was 4.00 (1.12 SE) into tubs with the females' own offspring, and 2.50 (0.96 SE) into tubs with unrelated larvae (contrast 1: $t_{(df=27)}$=0.242; p=0.811; Figure 4.4b). Through the end of the night in each trial, larvae did not display cannibalism in any of the tubs.
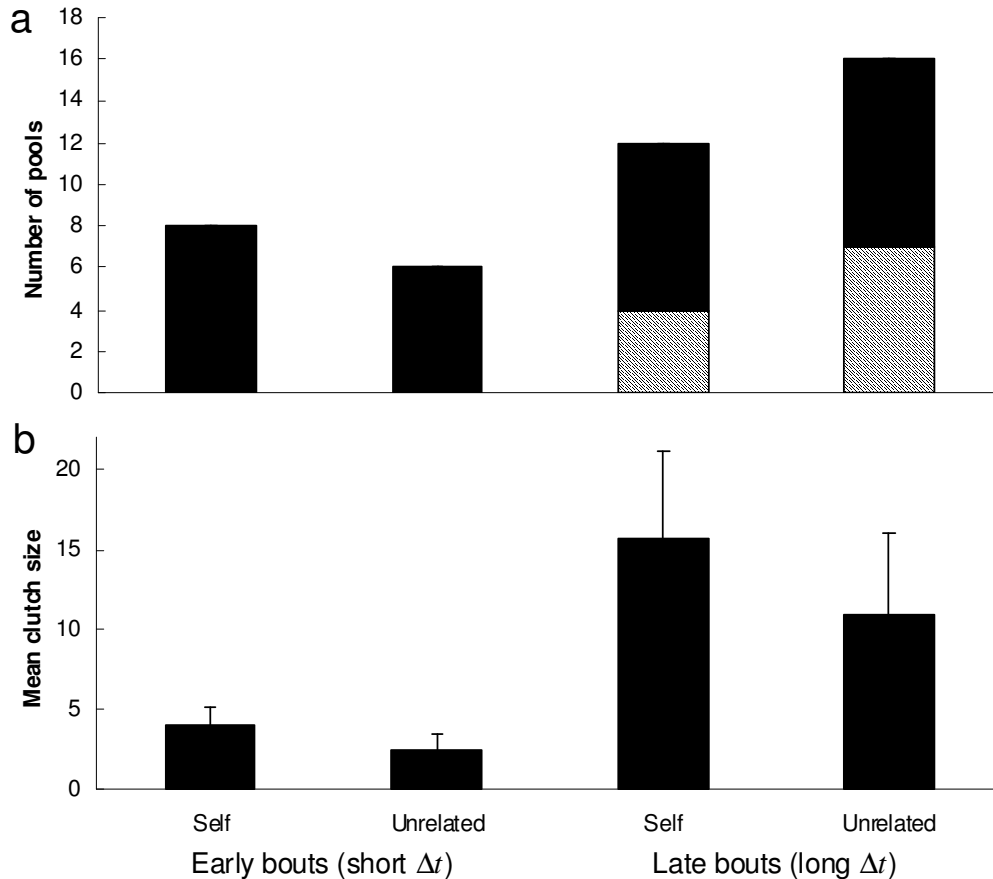
Figure 4.4: Female decisions in early (short Δt) and late (long Δt) larviposition trials. (a) The numbers of 'self' and 'unrelated' pools chosen by the females in each larviposition bout. Hatched bars represent pools where intercohort cannibalism occurred. (b) Mean clutch sizes deposited into 'self' and 'unrelated' pools during each larviposition bout. Error bars are 1 SE.

Similarly, 17 of the 20 females used in the late larviposition trials (long Δt) larviposited. Eight of them selected tubs containing their own previously deposited larvae, and nine selected tubs containing unrelated larvae (sign test $p=1.000$; Figure 4.4a). The mean number of larvae deposited per tub was 15.63 (5.47 SE) into tubs with the females' own offspring, and 11.00 (4.98 SE) into tubs with unrelated larvae (contrast 2: $t_{(df=27)}=0.829$; $p=0.415$; Figure 4.4b). Intercohort cannibalism occurred in a total of 11 tubs by the end of the night; four 'self' and seven 'unrelated' tubs. In all of these cases, first-cohort larvae cannibalized one or two newborns. While the proportions of tubs where cannibalism occurred did not differ significantly between 'self' and 'unrelated' tubs ($Z= -1.212$; $p=0.225$), the occurrence of cannibalism following the late larviposition bout was significantly higher than its occurrence following the early bout ($Z= 5.180$; $p<0.0005$). The proportions of selected 'self' or 'unrelated' pools did not differ significantly between early and late larviposition bouts ($Z= -0.560$; $p=0.575$). Mean clutch sizes were significantly larger in the late (mean = 13.18, SE = 3.61) compared to the early bouts (mean = 3.36, SE = 0.76; contrast 3: $t_{(df=27)}=2.412$; $p=0.023$).

4.5 DISCUSSION

The results of my intercohort priority effects experiment present clear evidence for competitive effects of late cohorts upon early cohorts that are negatively related to intercohort time interval, consistent with my hypothesis (Figure 4.1, blue curve). Growth rates of first-cohort larvae were significantly retarded by higher densities of the second cohort, and this effect reduced with increasing intercohort time intervals (Figure 4.2a). This effect, likely in large part due to exploitative competition, was strong enough at high larval densities to prevent many members of the first cohort from metamorphosing in time and lead to death by desiccation, regardless of intercohort time interval (Figure 4.2b). This effect also shaped the metamorphosis of survivors; favorable conditions (low larval densities and long time intervals) resulted in shorter larval periods and greater sizes at metamorphosis, while detrimental conditions (high larval densities and short time intervals) resulted in longer larval periods and smaller sizes at metamorphosis (Figure 4.2c) which was likely due to resource limitation.

Cannibalism was inflicted strictly by first-cohort upon second-cohort larvae, and occurred, as expected, only in intermediate or long intercohort time intervals (Figure 4.3b), but its general rates in this experiment were exceptionally low (4.7% over the entire

experiment, 25.9% in the 22-day time interval and high larval density treatment) compared to previous studies in the same species (Sadeh *et al.* 2009, Sadeh unpublished data). This may be the result of high structural complexity caused by very thick blooms of filamentous algae, folds in the nets lining the pools' bottoms and walls, and the rocks on the pool bottoms. Increased structural complexity reduces intercohort cannibalism considerably (from 96% to 58% in Sadeh *et al.* 2009), likely due to reduced encounter rates. Also, my permits only allowed collecting salamanders from breeding sites within a limited region. Therefore, the genetic distance between cohorts may have been small, reducing aggression when encounters occurred (see Markman *et al.* 2009, Sadeh under review).

Mortality among second-cohort larvae in this experiment was mostly from failure to metamorphose before the pools dried (Figure 4.3b), and was mostly density dependent. Longer intercohort time intervals also increased mortality, both due to cannibalism and as a result of the shorter time available for second-cohort development. Other than mortality caused by cannibalism and desiccation, longer intercohort time intervals also induced higher development rates in second-cohort larvae (Figure 4.3c) – a plastic developmental response to their perception of the shorter available hydroperiod that may incur further physiological costs (Ficetola & De Bernardi 2006; Sadeh *et al.* 2011), especially under conditions of food limitation. While second-cohort mortality was positively related to intercohort time interval, exerting maternal fitness costs consistent with Figure 4.1 (increasing section of red curve), the data presented here provide only moderate support for my hypothesis that intercohort time interval is positively related to rates of cannibalism upon the late cohort. However, that cannibalism could only be observed in the late bouts, but not in the early bouts in my larviposition trials (Figure 4.4a), further supports this hypothesis. Furthermore, size-dependent cannibalism was shown several other studies in the fire salamander (Degani *et al.* 1980; Reques & Tejedo 1996; Sadeh *et al.* 2009; Sadeh under review), and is the rule in many organisms (Polis 1981; Anholt 1994; Claessen *et al.* 2000; Wissinger *et al.* 2010).

Under field conditions with low rates of cannibalism such as observed here, both early and late cohorts suffer from strong density dependent effects with detrimental consequences to their survival, growth and metamorphosis (Figures 4.2 and 4.3). It can be expected that under field conditions with stronger expressions of cannibalism (see Warburg *et al.* 1979; Degani 1993), these patterns of exploitative competition will be relaxed to some extent. This would likely further improve the performance of early cohorts with increasing intercohort time intervals. Mortality of later cohorts, caused by greater

cannibalism, may be balanced to some extent by a reduction in their mortality by desiccation due to the relaxation of competition, and by larger body sizes at metamorphosis attained by survivors.

Despite the general support for my hypotheses on the strength of priority effects as a function of intercohort time interval, the larviposition trials did not support my predictions for female superoviposition behavior. Females at both short and long intercohort time intervals did not display any preference for the genetic identity of prior larvae, either their own offspring or those of unrelated females (Figure 4.4). This lack of preference appears particularly maladaptive in the long intercohort time interval trials, where cannibalism rates were very high (Figure 4.4a). Moreover, Sadeh (under review) has shown that reduced aggression in groups of highly related larvae of similar sizes leads to reduced metamorphic success, compared to more aggressive groups of distantly related members, providing an additional mechanism for favoring unrelated superoviposition at short intercohort time intervals. Deposited clutch sizes were larger in the late bouts compared to early bouts. This may result from the approaching end of the breeding season that generally tends to increased clutch sizes in sequentially-ovipositing organisms due to diminishing opportunity costs (Mangel 1987; Segev *et al.* 2011). According to the results of my larval priority effects experiment, a less likely interpretation of the increase in clutch size is a cannibal-satiation strategy, potentially employed by the females to increase the probability of survival of some portion of the second cohort, since this behavior would also increase competition considerably and reduce the probability of timely metamorphosis of all the larvae in the pool.

Larval *Salamandra* show kin recognition (Markman *et al.* 2009), with ecological consequences (Sadeh under review). Furthermore, the densities of first-cohort larvae used in this experiment were similar to those used by Sadeh et al (2009), where prior larvae were perceived by pregnant females who in response altered their preferences of larviposition pool characteristics. If detection is based on chemical compounds released by larvae into the water, greater concentrations may be required to assess the genetic relatedness of larvae than to merely detect their presence. Alternatively, this ability may be lost in adulthood, either entirely or only the ability to assess the relatedness of aquatic larvae by the terrestrial adult stage. The ontogenetic loss of kin recognition has been documented in other amphibian species (Walls 1991; Blaustein *et al.* 1993). Constraints on cross-stage kin discrimination may become considerable when a large adult population utilizes few large pools that contain many cohorts of larvae of different sibships. Each of

the specific breeding sites where I collected the females contains one central, large pool surrounded by a varying number of satellite smaller pools. If kin recognition of the majority of the larval population is unachievable due to constraints, selection for any cross-stage kin recognition may be nullified.

The literature on parasitoids presents a few examples where similar questions of host discrimination in superparasitism were studied. Much of this literature considers solitary parasitoids, where two individual larvae, rather than cohorts, compete to death within their mutual host (reviewed in Godfray 1994). Segoli et al (2010) considered this question with the gregarious parasitoid wasp, *Copidosoma koehleri*, where larval cohorts originating from a mated egg develop a soldier morph that cannibalizes the larvae of other cohorts. Their experiment, examining superparasitism decisions at one, short intercohort time interval, showed that females were more inclined to superparasitize hosts containing unrelated conspecifics than hosts containing their own offspring. Tena et al (2008) studied another gregarious parasitoid wasp species, *Metaphycus flavus*, where cannibalistic priority effects favor older cohorts over younger ones. In this species, ovipositing females kill the offspring of the older cohort to avoid this risk while taking advantage of another, positive intercohort priority effect: the first cohort progressively weakens the host's defense response and improves the survival of younger cohorts with intercohort time interval length. These studies, in conjunction with mine, indicate the variability and potential constraints in evolved oviposition habitat selection responses to larval intercohort time intervals, depending on the nature of the intercohort priority effects.

The influence of intercohort time intervals on larval growth, size at and time to metamorphosis through the modification of environmental conditions and stress levels may influence post-metamorphic phenotypes and life-history, including morphology (e.g. Ficetola & De Bernardi 2006), physiology (e.g. Gervasi & Foufopoulos 2008), behavior (e.g. Krause *et al.* 2011), reproduction and senescence (reviewed in Metcalfe & Monaghan 2001). Mass at metamorphosis is considered to be highly related to post-metamorphic survival, age at first reproduction and fecundity (Wells 2007). In addition, while little is known about the fire salamander's post-metamorph juvenile stage, this is believed to be the dispersing stage in herpetofaunal populations (Cushman 2006; Templeton *et al.* 2007). Individuals that metamorphose late and emerge during the hot and dry summer are likely severely constrained in their movement, therefore limiting their dispersal range and foraging opportunities during their first year, and potentially reducing connectivity and

increasing the effects of fragmentation among spatially-structured subpopulations (Cushman 2006).

At the population level, Wissinger et al (2010) have shown that in high altitudes, the co-occurrence of annual tiger salamander larval cohorts and paedomorphs generates a predictable larval size structure in which dominant cohorts regulate recruitment and drive cannibalism-induced population cycles, in agreement with theory (Claessen *et al.* 2004). In my study system, cohorts are intra-annual, and larval size structures are mostly driven by the pattern of dispersed rain events to which oviposition behavior is limited. In years with large intervals between the first winter rains, cannibalism and late cohort mortality is predicted to be high, resulting in high recruitment mostly from the early cohorts, larger metamorph sizes and relatively low genetic heterogeneity or recruits. When initial rains are frequent, larval mortality among the early cohorts will increase, and recruits may be smaller in size and genetically more heterogeneous. In habitats with short hydroperiods relative to larval densities, such condensed oviposition may lead to metamorphic failures of dramatic proportions of the larval population. This study may potentially guide annual observations of colonization patterns and recruitment from ponds of various characteristics to test these predictions, and determine whether cannibalism impedes recruitment or improves it.

COMPENSATORY DEVELOPMENT AND COSTS OF PLASTICITY: LARVAL RESPONSES TO DESICCATED CONSPECIFICS

5.1 ABSTRACT

Understanding constraints on phenotypic plasticity is central to explaining its evolution and the evolution of phenotypes in general, yet there is an ongoing debate on the classification and relationships among types of constraints. Since plasticity is often a developmental process, studies that consider the ontogeny of traits and their developmental mechanisms are beneficial. I manipulated the timing and reliability of cues perceived by fire salamander larvae for the future desiccation of their ephemeral pools to determine whether flexibility in developmental rates is constrained to early ontogeny. I hypothesized that higher rates of development, and particularly compensation for contradictory cues, would incur greater endogenous costs. I found that larvae respond early in ontogeny to dried conspecifics as a cue for future desiccation, but can fully compensate for this response in case more reliable but contradictory cues are later perceived. Patterns of mortality suggested that endogenous costs may depend on instantaneous rates of development, and revealed asymmetrical costs of compensatory development between false positive and false negative early information. Based on the results, I suggest a simple model of costs of development that implies a tradeoff between production costs of plasticity and phenotype-environment mismatch costs, which may potentially underlie the phenomenon of ontogenetic windows constraining plasticity.

5.2 INTRODUCTION

Phenotypic plasticity is a widespread phenomenon: individuals alter their phenotypes in response to environmental cues, often as an adaptation to variable environments. This multidisciplinary concept has recently been of increasing interest to biologists as a feature of both normal and abnormal individual development that is not only shaped by evolution, but also one that influences the function of individuals, the structure of ecological communities, and evolutionary trajectories (Schlichting & Pigliucci 1998; West-Eberhard 2003; Gilbert & Epel 2009; Berg & Ellers 2010). Despite its apparent adaptive superiority, phenotypic plasticity is neither universal nor infinite in expression. To arrive at a better understanding of its evolution, we must identify its costs and limits, and illuminate the functional relationships between them (DeWitt *et al.* 1998; Schlichting & Pigliucci 1998; Callahan *et al.* 2008). Auld et al (2010) suggested that many of these limits and costs may be alternative views of the same constraint, arguing that most of them are merely special cases or consequences of two fundamental costs, phenotype-environment mismatch (costs of phenotypes) and costs of the ability to be plastic (primarily maintenance and production costs).

Phenotype-environment (P-E) mismatch results in an ecological cost and is often caused by imperfect cue reliability and/or developmental lag times in the induced traits. Some phenotypes (e.g. morphological as opposed to behavioral) require substantial lengths of time to be expressed after their induction has been triggered (Padilla & Adolph 1996), and an early warning is necessary well before the anticipated condition occurs to avoid this cost. However, cue reliability tends to decline with the duration between the cue and the environmental condition it predicts. False early information can also induce the wrong phenotype for the eventual environmental condition (Moran 1992). Therefore, organisms are expected to rely on multiple cues (e.g. Bourdeau 2010), that may be available at different times before the anticipated condition and with variable degrees of reliability. Such continuous integration of multiple cues from the environment may be limited by the problem of processing contradictory cues. An additional limitation that has been frequently observed or assumed in models is the restriction of developmental flexibility to certain ontogenetic windows, beyond which developmental trajectories become canalized (e.g. Hensley 1993; Leips & Travis 1994). However, general explanations for the occurrence of such windows are mostly lacking (but see Hoverman & Relyea 2007).

In the absence of ontogenetic windows that limit the expression of plasticity, or within such windows, the contradiction of early information by late but more reliable

information is expected to induce compensatory development, requiring the organism to express an extreme degree of plasticity. Accelerated development, while avoiding the cost of P-E mismatch, may incur greater costs of producing the target phenotype in the form of reduced life expectancy (Inness & Metcalfe 2008), compromised immune system (Gervasi & Foufopoulos 2008), locomotor performance (Ficetola & De Bernardi 2006). However, constraints on plasticity – both the onset of late ontogenetic canalization and the costs of compensatory development – may be asymmetrical for different developmental trajectories induced by false early information. In other words, compensating for a false alarm for a particular stress may be constrained to a different degree than compensating for the unexpected occurrence of the same stress.

Explaining patterns of size and age at life-history transitions such as metamorphosis has been an ongoing, central challenge in evolutionary biology. Larval development towards the completion of metamorphosis in amphibians involves two directional processes: growth in body size and the differentiation and remodeling of tissues and organs (Smith-Gill & Berven 1979). The rates of both of these processes generally respond to various environmental factors, ultimately determining size and age at metamorphosis, respectively, and have been the focus of extensive research involving analyses of phenotypic plasticity (e.g. Wilbur & Collins 1973; Smith-Gill & Berven 1979; Hensley 1993; Leips & Travis 1994; Denver *et al.* 1998; Harris 1999; Day & Rowe 2002; Rose 2005). Some of this work has focused on developmental responses to the risk of habitat termination, with the overwhelming majority showing that larvae accelerate development and metamorphose earlier (reviewed in Wells 2007). This response tends to result in a smaller size at metamorphosis due to a shortened growth period. However, few studies have explicitly addressed other, endogenous costs (e.g. Gervasi & Foufopoulos 2008; Marquez-Garcia *et al.* 2009), particularly such that lead to increased mortality (Altwegg 2002; Lane & Mahony 2002).

I studied the responses of fire salamander larvae to two cues for habitat termination that differ in their timing and reliability, to test the following hypotheses: 1) larvae sense recent desiccation of conspecifics from previous cohorts as an early cue of their habitat's duration, and respond adaptively by altering their developmental rates to increase their probability of metamorphosing before it desiccates; 2) if developmental plasticity is not ontogenetically limited, rates of development will be updated according to later cues that indicate the habitat's actual duration more reliably, to the point of complete compensation; 3) in the latter case, environmental demand for extreme plasticity will carry asymmetric

developmental costs. The acceleration of a biological process that requires energy inputs is intuitively expected to demand increased effort and thus incur greater costs. Therefore, I specifically predicted that compensatory, hyper-accelerated development following a false negative early cue (i.e. an unexpected catastrophe) will be more costly to execute than a compensatory delay in development following a false positive early cue (i.e. a false alarm). Based on the results of my experiment, I suggest a simple model of costs of development rates. I use it to demonstrate how phenotype production costs of plasticity are influenced by the timing of information and its reliability, and how production costs are traded off with P-E mismatch costs to explain the frequently-observed ontogenetic loss of plasticity.

5.3 METHODS

Study organism

I studied the ovoviviparous fire salamander (*Salamandra infraimmaculata*; Steinfartz *et al.* 2000), whose larvae are deposited during the winter into mostly temporary pools in northern Israel. These pools vary greatly in various ecological characteristics, including their water holding capacity. Many temporary pools dry more than once within the same breeding season, particularly during early winter (October – December) and spring (March – April), when rains are infrequent, temperatures are high and the ground water level is low (Spencer *et al.* 1999). Pool desiccation is a very important factor contributing to salamander larval mortality and reproductive failure (Warburg 1992). Many early-born larvae that die in early-winter events of pool desiccation dry in the sun (Sadeh, personal observations), with their flesh likely changing in chemical composition. Most of their decomposition occurs only after the pools are reflooded later in the season, possibly emitting unique chemicals that may be perceived by conspecifics. A pool's water holding ability depends on its floor structure, connectedness to the ground water table and exposure to solar radiation, and often does not change within a single breeding season. Thus, the presence or absence of such chemicals can convey moderately reliable information to the bulk of conspecifics that are deposited during mid-winter regarding the pool's liability to early-spring desiccation. Although previous experiments showed that *S. infraimmaculata* habitat selection behavior is finely tuned to changing ecological conditions (e.g. Sadeh *et al.* 2009), a preliminary mesocosm experiment indicated that gravid females may not be responding to this cue in their choice of larviposition pool (Sadeh, unpublished data). Thus the larvae, deposited indiscriminatingly with regards to formerly desiccated larvae, are predicted to perceive this cue and respond to it by adjusting their development rates.

Animal collection and return

To prepare the cue of recent desiccation, I collected larvae that either died in desiccated natural pools or in other mesocosm experiments during previous years (up to 3 years), dried them by placing under a light bulb until their mass stopped decreasing and stored them in sealed plastic bags at -20˚ C. Since no larva was intentionally killed to prepare this manipulation, the experiment was limited in size by the number of available dried larvae. In order to hasten their physical decomposition, I ground the dried larvae to a heterogeneously fine powder using a manual pestle and mortar after weighing 50 individuals to determine their mean individual dry mass. Thirty six hours before the experiment, I mixed powder quantities of 0.445 g (equivalent to 9 larvae) into outdoor tubs, each filled with 40 L tap water. The 36-hour waiting time allowed chlorine to dissipate from the water, and the powder to partially dissolve and initiate the organic decomposition process. *Salamandra* females often larviposit into pools within the first few days after they fill (Sadeh, personal observations). After this waiting time, I stirred the tubs and took water from them to fill the experiment's "early cue" treatment tubs. I took water for the "no early cue" treatment tubs from similar outdoor tubs that held 36-hour-aged tap water and did not contain any conspecific powder. With this manipulation, I did not control for the possible perception of the dried conspecifics by the focal larvae as a risk of predation, either by sensing their death (but not drying) as an alarm cue, or by sensing their presence (but not death) as older, cannibalistic conspecifics. However, the risk of predation is known in this species to induce reduced foraging and delayed development (Lima & Dill 1990, Sadeh unpublished data; Eitam *et al.* 2005), the opposite pattern than predicted for the perception of risk of desiccation.

During November 2009, I collected gravid females from natural breeding sites and placed them in field enclosures containing mesocosms (identical to those described in Sadeh *et al.* 2009) to larviposit. I weighed and photographed each larva ventrally, and after removing larvae that were at the extremes of the mass distribution, I paired individuals from the same mother and of similar mass. Up to 10 hours after birth, I randomly allocated each pair to an experimental tub. Mean individual mass of the larvae at the beginning of the experiment was 0.243 g (SD=0.041 g), and their mean snout-vent length (SVL) was 1.7 cm (SD=0.1 cm). After giving birth, I returned all the adult females and excess larvae to their sites of collection. After the experiment ended, I returned all the surviving metamorphs to the natural pools nearest to the location of their mother's collection.

Experimental design

I conducted a factorial design experiment combining two levels of early cue of desiccation (presence/absence of dried conspecific powder, hereafter "early cue") with two levels of water regime (constant/reducing water volume according to Figure 5.1). Thus, the experiment tested the developmental responses (growth and larval period) of larvae to the early information embodied in the presence or absence of the cue, both in situations when this early information was either true or false. I replicated each treatment combination 8 times in indoor, cuboid tubs (floor dimensions 36 by 21 cm), initially filled with 12 liters of water and containing 2 sibling larvae. I prepared the early cue as described above and implemented it as a single pulse manipulation before day 1. I removed water from the reducing water regime throughout the experiment by periodically filtering out water through coffee filter paper, and returning the residues from the filter paper back into the tub. This was also done to the constant water tubs but both residues and water were returned. I compensated weekly for water loss from constant water tubs due to evaporation by adding deionized water. The resulting increasing difference in solutes between treatments is a natural effect of pools drying partially due to evaporation. I only decreased the water volume to 2 liters in the reducing water regime because I sought to measure the larval response until metamorphosis without killing them or imposing a limitation on their swimming and feeding behavior. I fed the larvae *ad libitum* throughout the experiment to ensure similar food intake among treatments. This feeding regime also eliminated any potential chemical effects or indirect trophic effects of the introduced dried conspecifics material. I fed the larvae either a mixture of field-collected zooplankton organisms [mainly *Arctodiaptomus similis* (Copepoda), various daphnids and mosquito larvae (*Culiseta longiareolata* and *Culex laticinctus*)], or purchased chironomid (*Chironomus* sp) larvae.
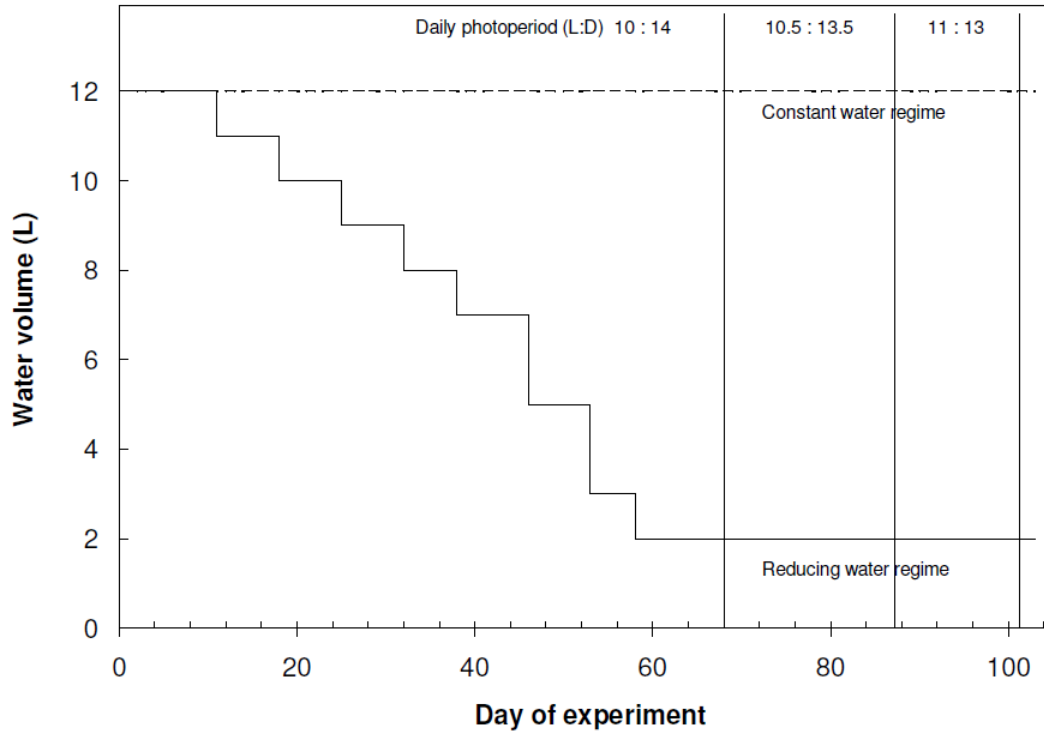
Figure 5.1: Experimental water volume regimes. Constant (dashed) and reducing (solid) water volumes over time. Vertical lines represent times of photoperiod increment by 30 minutes.

I followed natural daily photoperiod in the experimental room, beginning with 10:14 (L:D) on day 1, 10.5:13.5 from day 68, 11:13 from day 87, and 11.5:12.5 from day 101 to the end of the experiment at day 103. Similarly, I kept temperatures at the region's long-term mean daily maximal temperature for each month, according to the Israel Meteorological Service website (http://www.ims.gov.il/IMSEng/CLIMATE). Temperatures were: 16-18 degrees during days 1-76, 17-19 degrees during days 77-99 and 19-21 degrees from day 100. However, on days 76 and 85, due to air conditioning system malfunction, air temperatures rose to 36° C and 26° C, respectively, for 1-2 days before the problem was corrected, equally affecting the water temperatures of all treatments. The first case of air conditioning failure marked the beginning of emergence of metamorphs for this experiment. While these sharp deviations in temperature were not planned, they occurred during springtime when severe heat waves occur naturally in Israel, raising air and water temperatures to yearly extrema of up to 40° C and affecting natural ponds in a similar way when not completely drying them.

Response variables and metamorphosis

I recorded larval mortality and growth prior to metamorphosis, as well as size at and time to metamorphosis. To determine growth, I weighed all the larvae to the nearest mg and photographed them on days 1, 5 (weight only), 22, 42, 58, 76, 81, 87, 95, 101 and 103. Once the first larvae started displaying progressive metamorphic morphology (dark skin color, a reduced tailfin and/or reduced gills), I checked the tubs every one to two days to collect emerging metamorphs. I photographed emerging metamorphs to determine size at metamorphosis, and recorded their times to metamorphosis. The photographs were used to determine SVL to the nearest mm using image processing software (ImageJ 1.40g). Body mass is a good index of an individual's immediate condition and short-term growth as it includes the mass gained by recent meals that may be stored in lipid reserves or quickly used up. Therefore it is quick to respond to environmental conditions and carries relatively high intra-individual variation (Sadeh, personal observations). In contrast, SVL is slower to respond and less sensitive to short-term conditions, but gives a better estimate of long-term growth, as it is the result only of the portion of energy that was allocated into skeletal development and growth in body size.

Statistical analyses

I used repeated measures ANOVA to test the effects of the early cue and water regime on larval growth trajectories during most of their growth period, both in mass (five dates) and in SVL (four dates), using tub means as independent data points. The repeated measures analysis was done up to day 58, before any larva metamorphosed to prevent the distortion of test results by the reductions in mean sizes due to the removal of the usually larger emerged larvae. However, considerable shifts in the response patterns occurred during the metamorphic period. The results of these shifts are captured in a two-way ANOVA used to test the effects of early cue and water regime on time to- and size (SVL) at metamorphosis. I removed from this analysis two tubs in which both larvae died. To test my hypotheses regarding the costs of the expression of plasticity, I used three orthogonal planned contrasts on mean larval mortality data (Quinn & Keough 2002): the main effect of water reduction (the mean of the two reducing water treatments vs. the mean of the two constant water treatments), the effect of a false positive early cue (early cue + reducing water vs. no early cue + reducing water) and the effect of a false negative cue (no early cue + constant water vs. early cue + constant water).

5.4 RESULTS

The overall mass growth during the first 58 days (prior to any metamorphosis) was positively affected by the early cue (p=0.016). There was no significant effect yet of the water regime, during this period, nor a significant early cue × water regime interaction (Table 5.1; Figure 5.2). The mass growth trajectory was positively affected by both water volume regime (time × water regime interaction: p=0.001) and the early cue (time × early cue interaction: p=0.008), but not by their interaction (time × water regime × early cue interaction: p=0.876; Table 5.1). Qualitatively similar results were obtained for the test of these factors' effects on larval SVL growth (Table 5.2).

Table 5.1: Results for a repeated measures ANOVA test on larval mass growth up to day 58.

Between subjects	SS	df	MS	F	P
Early Cue	222494.514	1	222494.514	6.520	0.016
Water regime	99326.139	1	99326.139	2.911	0.099
Early cue × Water regime	31823.702	1	31823.702	0.933	0.342
Error	955471.619	28	34123.986		
Within subjects	Wilks' λ	Hypoth. df	Error df	F	P
Time	0.016	4	25	396.654	<0.0005
time × early cue	0.586	4	25	4.406	0.008
time × water regime	0.465	4	25	7.205	0.001
time × early cue × water regime	0.954	4	25	0.298	0.876

Table 5.2: Results for a repeated measures ANOVA test on larval SVL growth up to day 58.

Between subjects	SS	df	MS	F	P
Early Cue	0.238	1	0.238	7.147	0.012
Water regime	0.061	1	0.061	1.836	0.186
Early cue × Water regime	0.044	1	0.044	1.315	0.261
Error	0.932	28	0.033		
Within subjects	Wilks' λ	Hypoth. df	Error df	F	P
Time	0.006	3	26	1498.046	<0.0005
time × early cue	0.543	3	26	7.300	0.001
time × water regime	0.555	3	26	6.953	0.001
time × early cue × water regime	0.991	3	26	0.076	0.973

The larvae responded quickly to the early cue of desiccation risk by accelerating their mass growth rates (Figure 5.2). An *a posteriori* t-test of larval mass on day 5 of the experiment revealed that the early cue has already produced a significant effect (t=2.219, p=0.034). By day 22, larvae in the treatment combinations that received a positive early cue grew to a larger mean size compared to those of treatments without this cue (a 13% difference). Growth rates considerably slowed under the reducing water level regime after

day 42, and came to almost a complete cessation following day 58 (Figure 5.2) due to the cessation of feeding during metamorphic climax. Under the constant water level regime this growth restriction was evident only after day 76. Thus, the water level regime gained an increasing effect on growth that overwhelmed the effect of the early cue only near metamorphosis, after day 58 (Figure 5.2).
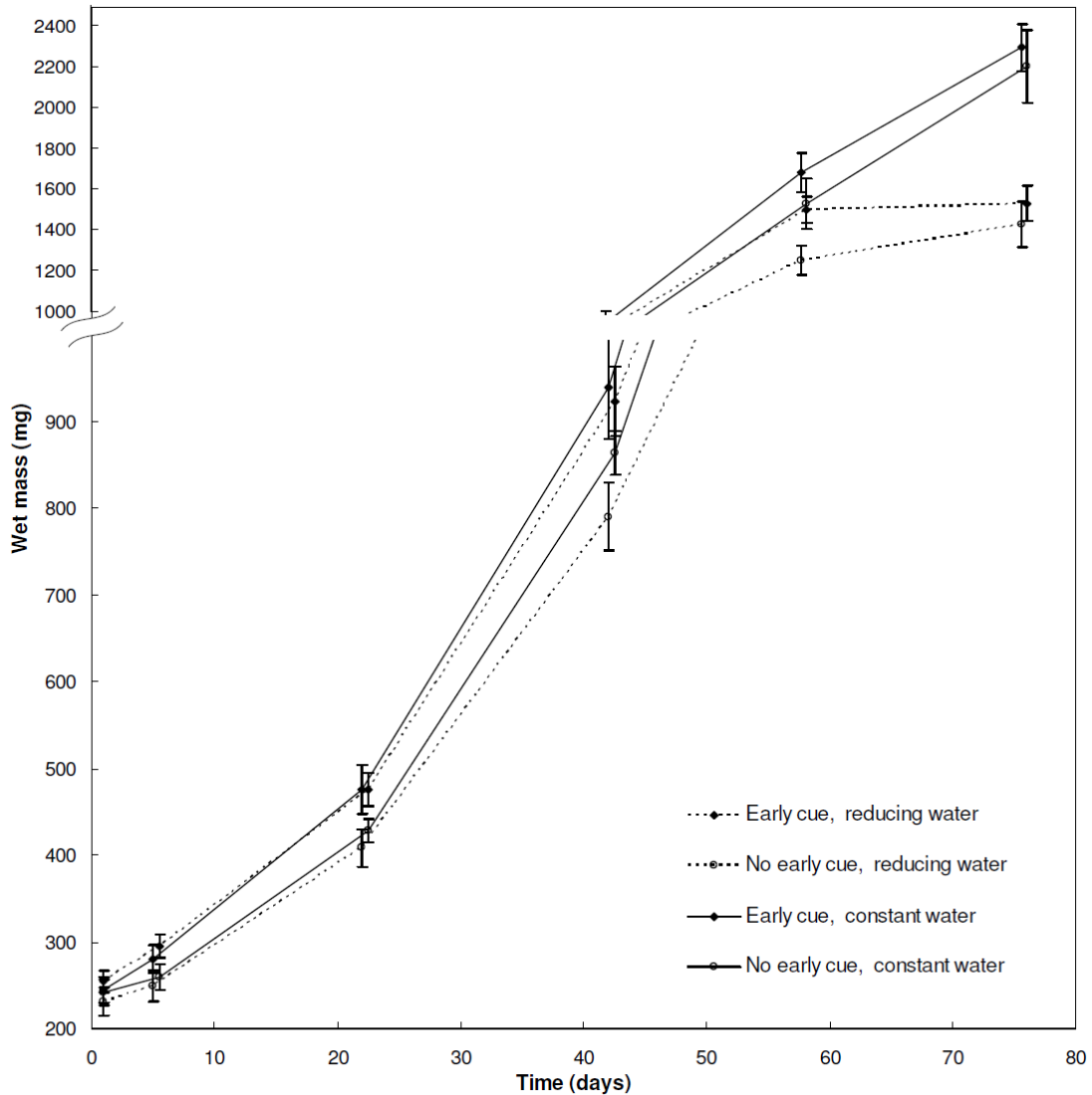
Figure 5.2: Mean larval growth trajectories in wet mass. The ordinate axis was rescaled at 1000 mg to magnify the patterns during the early larval period, where the early cue demonstrated an accelerating effect on development. Error bars are SE.

Time to emergence responded significantly only to the water volume regime (Figure 5.3; two-way ANOVA: $P<0.0005$; Table 5.3), with a LS mean emergence time of 80.4 days for the reducing water volume treatments and 92.4 days for the constant water volume treatments. The early cue effect and the water regime × early cue interaction term

were both non-significant, indicating that the larvae compensated for their initial response to early information according to prevailing hydroperiod conditions. Though the central tendencies of the early cue treatments did not differ significantly from those without the cue, their mean times to metamorphosis were slightly shorter under both water regimes. The temporal distribution of individual emergences revealed an initial surge of metamorphoses in both of the treatment combinations with the early cue compared to treatments without it (Figure 5.4). In both water level regimes, the maximal difference between cumulative metamorph frequencies of the two early cue treatments occurred at the first quartile (day 76 and day 88 in the reducing water and the constant water regimes, respectively). *A posteriori* comparisons between the proportions of metamorphs at these times between early cue treatments showed that this initial surge was significant in both water level regimes (reducing water regime: Z= -1.750, p=0.040; constant water regime: Z= -1.703, p=0.044; one-sided normal approximation tests of equality of proportions). Thus, the initial developmental response to the early cue of desiccation risk may have skewed the distribution of emergences to the left, but was mostly compensated for when eventually contradicted by prevailing conditions.
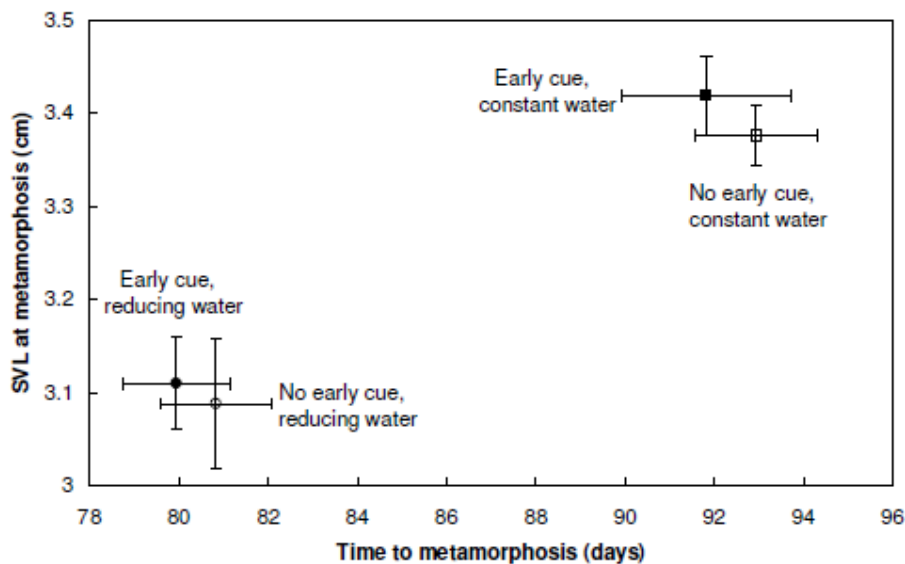
Figure 5.3: Final phenotype reaction norms of mean time to- and size at metamorphosis. Only the water level regime exerted a lasting effect on final phenotypes. Error bars are SE.
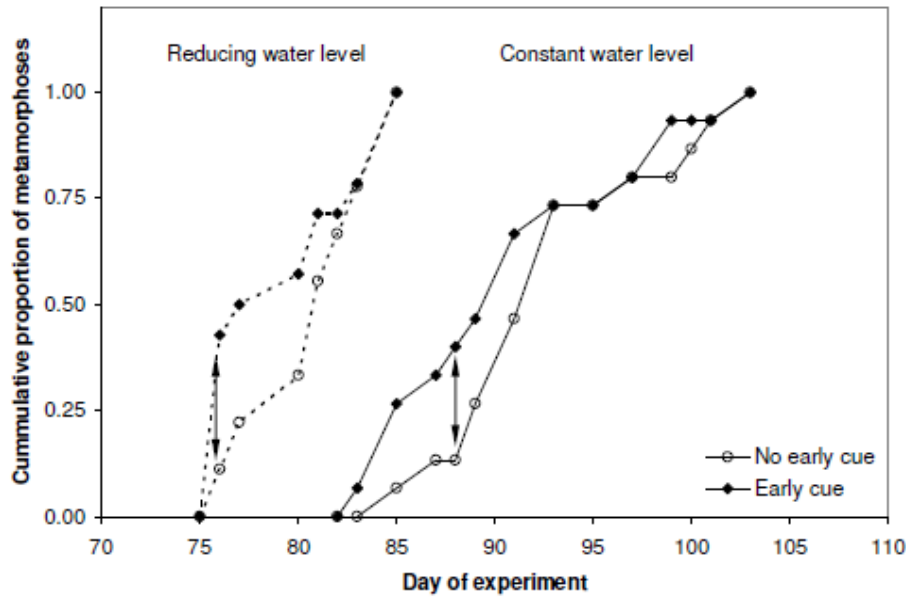
Figure 5.4: Cumulative proportions of individual metamorphoses over time. Metamorphoses in the 'early cue' treatments were skewed to the right compared to the 'no early cue' treatments in both water level regimes. Arrows indicate the times of maximal distances between the cumulative distributions.

Table 5.3: Results for two-way ANOVA tests on SVL at- and time to metamorphosis.

SVL at metamorphosis	Sum-of-Squares	df	Mean-Square	F-ratio	P
Early cue	0.008	1	0.008	0.458	0.504
Water regime	0.659	1	0.659	38.887	0.000
Early cue × water regime	0.001	1	0.001	0.046	0.832
Error	0.440	26	0.017		
Time to metamorphosis	Sum-of-Squares	df	Mean-Square	F-ratio	P
Early cue	7.539	1	7.539	0.451	0.508
Water regime	1061.539	1	1061.539	63.559	0.000
Early cue × water regime	0.097	1	0.097	0.006	0.940
Error	434.240	26	16.702		

Similar to the overall pattern of time to metamorphosis, final larval sizes at metamorphosis only differed significantly between water level regimes (P<0.0005; Figure 5.3; Table 5.3), with a LS mean SVL of 3.1 cm in the reducing water volume treatments and 3.4 cm in the constant water volume treatments. This pattern indicates that increased allocation of energy to hastened differentiation and/or a shorter growth period compromised the total larval growths, whereas initial responses in development rates to false early information on risk of desiccation were compensated for by later responses to actual hydroperiod conditions.

Larval mortality was most pronounced under the reducing water regime without an early cue (43.75%). Under the reducing water regime and with the early cue mortality rate

was 12.5%, whereas in the other treatment combinations, mortality rates were 6.25% (Figure 5.5). The reducing water regime significantly increased mortality compared to the constant water regime, regardless of the early cue (P=0.029). However, when the water loss was unexpected, mortality increased significantly (by 31.25%; P=0.028) compared to the same condition when preceded by the presence of the early cue. In contrast, mortality under the constant water regime was similar following a false early cue or its absence (6.25%; P=1.000). See Table 5.4 for the statistical summary of these contrasts. No larval mortality occurred before day 75.
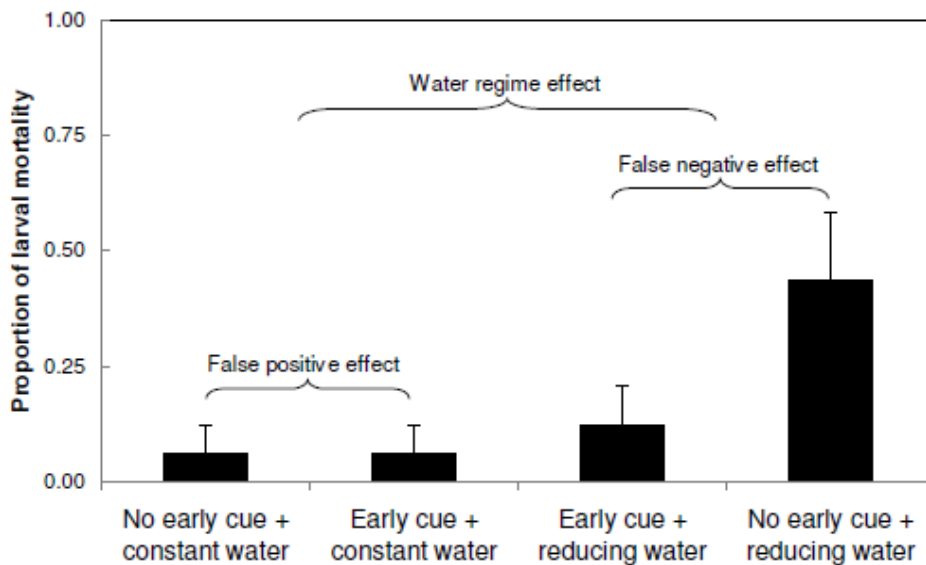
Figure 5.5: Larval mortality prior to metamorphosis. Mortality rates were greater in the reducing, compared to the constant water level regime. Within this regime, the false negative early cue caused considerably greater mortality. No effect was found for the false positive early cue. All larval mortality occurred after day 75 of the experiment.

Table 5.4: Results for planned contrasts on larval mortality.

Contrast	Sum-of-Squares	df	Mean-Square	F-ratio	P
Water regime	0.383	1	0.383	5.277	0.029
False negative early cue	0.391	1	0.391	5.385	0.028
False positive early cue	0.000	1	0.000	0.000	1.000
Error	2.031	28	0.073		

5.5 DISCUSSION

Responses to and compensation for early cues

The results support the hypothesis that the recent desiccation of conspecifics serves as an early cue for risk of pool desiccation, accelerating development at least via early larval growth (Figure 5.2). Since it is generally agreed that size thresholds limit differentiation

rates (Wilbur & Collins 1973; Day & Rowe 2002; Wells 2007), an early cue indicating a potentially severe time constraint on development and requiring its acceleration is predicted to induce accelerated growth so that differentiation remains unconstrained. This response is opposite to that of larvae under risk of predation or cannibalism (Eitam *et al.* 2005, Sadeh unpublished data), and therefore I rule out the larvae's perception of dried-up conspecifics as a cue for predation/cannibalism risks.

No staging system has been developed for this species to indicate its ontogenetic progress. However, gradual morphological changes (in skull shape, skin color and limb usage patterns) occur throughout larval ontogeny before the conspicuous final stage of metamorphosis (Sadeh, personal observations), indicating that differentiation is occurring throughout the larval period, along with growth in body size (consistent with Smith-Gill & Berven 1979; Harris 1999; O'Laughlin & Harris 2000). Therefore, similar to growth rate, differentiation rate is likely also hastened by the early cue for desiccation. My *a posteriori* analysis of the temporal distributions of metamorphoses (Figure 5.4) suggests that the early cue had an accelerating effect on the rates of differentiation under both water regimes, but this effect did not last to significantly affect mean time to metamorphosis as a result of full developmental compensation by most of the individuals for the false early information.

By the end of the larval period, the effects of the early cue had practically vanished, with only the water regime exerting a strong effect. This is not surprising since the dynamics of water depth, water volume and concentration of solutes are far more reliable cues for future desiccation than the scent of recent death by desiccation. Larvae markedly compensated for their initial response to false early information according to prevailing hydroperiod conditions, showing considerable developmental plasticity that is not limited to early phases of the larval period. This was evident by practically identical reaction norms for both size at- and time to metamorphosis for true and false early cues, under both water regimes (Figure 5.3).

Costs of developmental rates
Compensatory development was mostly apparent between days 42 and 76 (Figure 5.2), followed by increased mortality (Figure 5.5), that occurred only after day 76 and to the end of the experiment. The water reduction regime induced a high average rate of development (larval period^{-1}), regardless of the presence of the early cue, associated with increased mortality compared to the constant water regime. However, a significantly greater contribution to this increased mortality was due to the compensation demanded by the

unexpected reduction in water, in the absence of the early cue, where the larvae had to hyper-accelerate their development. In contrast, I found no detectable costs of reducing the developmental rate to compensate for an early false alarm. This pattern of mortality suggests that it was the result of the maximum instantaneous rate of development performed by the larvae.

Recent studies have found that high growth rates and especially compensatory growth are traded-off with other life-history traits and body functions over various time scales (Metcalfe & Monaghan 2001), including lifespan (Inness & Metcalfe 2008), reproductive output (Auer *et al.* 2010) and locomotor performance (Billerbeck *et al.* 2001). This possibly occurs through the accumulation of cellular damage caused by oxidative stress, or through increased allocation of resources to its repair (Sorensen *et al.* 2003; Mangel & Munch 2005; Monaghan *et al.* 2009) at the expense of other functions. For example, a study by Inness & Metcalfe (2008) suggested that three-spined sticklebacks that reproduce only once in their lifetime, cannot afford to divert resources away from reproduction in order to repair damage inflicted by compensatory growth. Therefore, fish under these conditions suffered increased rates of mortality. A similar tradeoff may underlie the pattern of mortality in my experiment; metamorphosis requires high inputs of energy (Sheridan & Kao 1998) and cannot be delayed or compromised to repair the damage caused by compensatory development when the larval habitat approaches termination. In contrast to compensation in growth rates, little work has been done on compensatory differentiation rates, and their costs are less understood. Accelerated differentiation of stem cells may reduce the available pool of undifferentiated cells and limit other functions they may serve (A. Kopp, personal communication). At the tissue level, Arendt & Hoang (2005) suggested that accelerated differentiation of muscle tissue results in numerous but smaller fibers and reduced performance of the tissue. At the whole-organism level, some tradeoffs for accelerated differentiation rates have been identified in amphibians, with the effects sometimes carried beyond metamorphosis (Lane & Mahony 2002), such as decreased immune function (Gervasi & Foufopoulos 2008) and locomotor performance (Ficetola & De Bernardi 2006). These costs may stem from adaptive allocation of limited resources to various body functions, or from compromised whole-organism coordination of different tissues, resulting in disruption of homeostasis and increased vulnerability to environmental stress. Studies on heat shock protein expression also indicate that stress resistance and development appear to be negatively related (reviewed in Sorensen *et al.* 2003). Indeed, mortality in my experiment may have resulted

also from a compromised ability to cope with the accidental heat waves that occurred in the lab, similar to those that frequently occur in nature late in the larval period.

Costs and limits of plastic phenotype development

Callahan et al (2008) emphasized the need to distinguish costs of phenotypes from costs of plasticity *per se*, and to address their potential interactions. I agree with this important distinction, but see no reason why the fundamental production cost of phenotypes should differ between plastic and fixed development. Development is a cumulative process. Therefore, the total phenotype production cost for a certain trait value is the sum of costs incurred during each small time interval throughout its development. I suggest that this cost may *accumulate* differently throughout the development of a trait to incur increased costs for plastic development. Based on my results and the growing body of recent literature on the costs of accelerated, compensatory development, I will show that the instantaneous production cost can be generally characterized as an increasing, strictly convex function of the instantaneous rate of development.

Consider an organism that must develop a certain trait, p, from an initial state of $p=0$ to some required final state, p_{req}, within a limited time interval, $0 \leq t \leq T$, where T is unknown and must be assessed. Furthermore, in order to minimize ecological phenotype-environment mismatch costs, the organism must reach p_{req} at time T exactly. For example, an aquatic larva that is born into an ephemeral pond must progress the differentiation of body systems for terrestrial function while growing, and complete metamorphosis before the pond dries to avoid death by desiccation. However, upon completion of metamorphosis it must emerge from the water and miss further opportunities for larval growth in case this occurs too early. Thus, the organism must continuously assess its time limit and adjust its rate of development, r, accordingly, such that

$$\int_0^T r(t)dt = p_{req} \qquad (1)$$

The following analysis can equivalently correspond to cases where the time for development is fixed with the organism having to assess the expected environmental conditions at that time and match the target trait value accordingly (e.g. produce defenses against an uncertain predation level). Either way, the problem is that of adjusting developmental rates. However, development incurs costs. Defining the development rate-

dependent instantaneous production cost, $f(r)$, the cumulative cost of producing the required trait is

$$C = \int_0^T f\big(r(t)\big)\,dt \qquad (2)$$

The results of my experiment, as well as recent literature on compensatory development imply that

$$C_{\text{low development rate}} < C_{\text{high development rate}} \qquad (3a)$$

$$C_{\text{constant dev. rate}} < C_{\text{changing dev. rate}} \qquad (3b)$$

Equation (3a) clearly implies that $df/dr > 0$ for any value of r. I will now show that Eqn. (3b) implies that $d^2f/dr^2 > 0$ for any value of r.

At $t=0$, the organism perceives mildly reliable information on its future time limitation, and sets an initial rate of development, r_1, accordingly (or this can be a genetically-determined default rate in the absence of any information). Unless additional information is perceived later by the organism, allowing it to reassess the remaining time available for development, this rate will remain constant. Assume that at time t_c ($0<t_c<T$) the organism perceives a perfectly reliable information on the time limitation, allowing it to readjust its developmental rate to r_2, such that Eqn. (1) is satisfied. Thus,

$$\bar{r}T = r_1 t_c + r_2(T - t_c)$$

$$\bar{r} = r_1 \frac{t_c}{T} + r_2\left(1 - \frac{t_c}{T}\right), \text{ or substituting } \frac{t_c}{T} = \lambda,$$

$$\bar{r} = \lambda r_1 + (1-\lambda) r_2 \qquad (4)$$

is the required average rate of development over the entire time interval [0 T] to attain p_{req}, where $0 < \lambda < 1$ is the relative weight of the duration of development at the rate of r_1. In reality, organisms continuously perceive and integrate multiple cues that bear imperfect information and readjust their developmental rates, resulting in more curved developmental trajectories than assumed in my analysis. However, these curved trajectories can be approximated as a sequence of many short linear intervals similar to those considered here.

From Eqn. (3b) we know that

$$f(\bar{r})T < f(r_1)t_c + f(r_2)(T - t_c) \qquad (5)$$

Substituting (4) into (5) yields:

$$f\left(\lambda r_1 + (1-\lambda)r\right)T < f(r_1)t_c + f(r_2)(T - t_c)$$

$$f\left(\lambda r_1 + (1-\lambda)r\right) < \lambda f(r_1) + (1-\lambda)f(r_2) \qquad (6)$$

Equation (6) is the mathematical definition of strict convexity for $f(r)$. In other words, $\frac{d^2f}{dr^2} > 0$ for any value of r.

That $f(r)$ is a strictly increasing and convex function has important implications for life-history tradeoffs of development. Specifically, as reliable information regarding the required developmental rate of a trait is perceived later in that trait's ontogeny, complete compensation demands increasing endogenous costs, to the point that they may exceed the ecological costs of phenotype-environment mismatch that compensation is aimed at minimizing. This would not have been the case if $\frac{d^2f}{dr^2} = 0$, since then (3b) would be an equality, and the cost for any given final phenotype would be constant regardless of the developmental trajectory leading to it. Thus, for any plastic trait my analysis predicts that at some point in its ontogeny, development will become canalized and cease to respond to environmental cues that otherwise induce its acceleration. The specific timing of loss of plasticity depends on the specific forms of $f(r)$ and of the phenotype-environment mismatch cost as a function of the deviation of the realized phenotype from the required phenotype, and can only be considered in a full, system-specific life-history model. Ontogenetic loss of plasticity has been documented frequently in various organisms (e.g. Alford & Harris 1988; Hensley 1993; Leips & Travis 1994; Hoverman & Relyea 2007), and I suggest that my analysis provides a potential general explanation for this phenomenon. In contrast, compensatory deceleration of development demands little costs. In this case, the extra costs are incurred for the needlessly high initial rates of development before the perception of corrective information. Therefore, the deceleration of development is not predicted to be limited by endogenous costs throughout ontogeny.

To graphically illustrate the model's behavior and implications, I arbitrarily chose a function that upholds the general requirements of $f(r)$, i.e. an increasing and convex function of r:

$$f(r) = ar(t)^2 \qquad (7)$$

where a is a scaling coefficient. In Figure 5.6A I simulate the developmental trajectories of a fixed slow developer, a fixed fast developer, as well as plastic developers that accelerate or decelerate their developmental rates following the reception of perfectly reliable cues at some point. Figure 5.6B demonstrates how production costs accumulate for these

developmental trajectories, calculated according to equations (2) and (7), resulting in greater costs for fast developers, and increased costs for the expression of compensatory development. Figure 5.6C shows the increase in the extra production costs incurred by compensatory development (the production cost of plasticity), as reliable information is perceived later. While the extra production cost for accelerating compensation approaches infinity as reliable information is perceived later towards the time limit, it only approaches a finite value in the case of decelerating compensation.

My model predicts a lower cost of compensating for a "false alarm" than for an "unexpected catastrophe". However, in my experiment, I found no evidence for any cost for the former. Detecting endogenous costs empirically is very difficult, since they could manifest in different body functions, through adaptive tradeoffs or physiological outcomes. Mortality is the ultimate cost, but it bears information only on the extreme cases of physiological compromise, below which costs may remain undetected. Thus, sub-lethal costs may have been incurred in my experiment, but remained below my detection threshold.

I suggest that phenotype production costs accumulate to greater costs for individuals expressing plastic development since they do not follow the most efficient trajectory towards their final phenotype. Therefore, early information is extremely valuable for reducing deviations from the most efficient trajectory, but it tends to be less reliable. Complex and more effective information acquisition strategies (e.g. plants extrapolating temporal dynamics into the future: Shemesh *et al.* 2010) are expected to evolve to moderate this tradeoff, but these strategies and the maintenance of their underlying physiological mechanisms may themselves be costly (DeWitt *et al.* 1998). Analysis of constraints on phenotypic plasticity has mostly utilized final-phenotype reaction norms, comparing trait values at the end of their development over different environments, thus capturing the phenotype-environment relationship at the end-point of the underlying developmental process. Clearly, more may be learned about plasticity and its constraints by considering the development of the ultimate phenotype and the role of plasticity in it (Kaplan & Phillips 2006; Hoverman & Relyea 2007; Sultan 2007). My study motivates future studies to manipulate the timing of perceived cues and their reliability throughout the ontogeny of the focal phenotype, as well as to determine the system-specific proximate mechanisms of costs of developmental rates at the cellular, tissue and whole-organism levels. Such combined ecological and developmental approaches, with system-specific life-history modeling, hold a promise for advancing our understanding of

developmental plasticity, its costs and evolution.
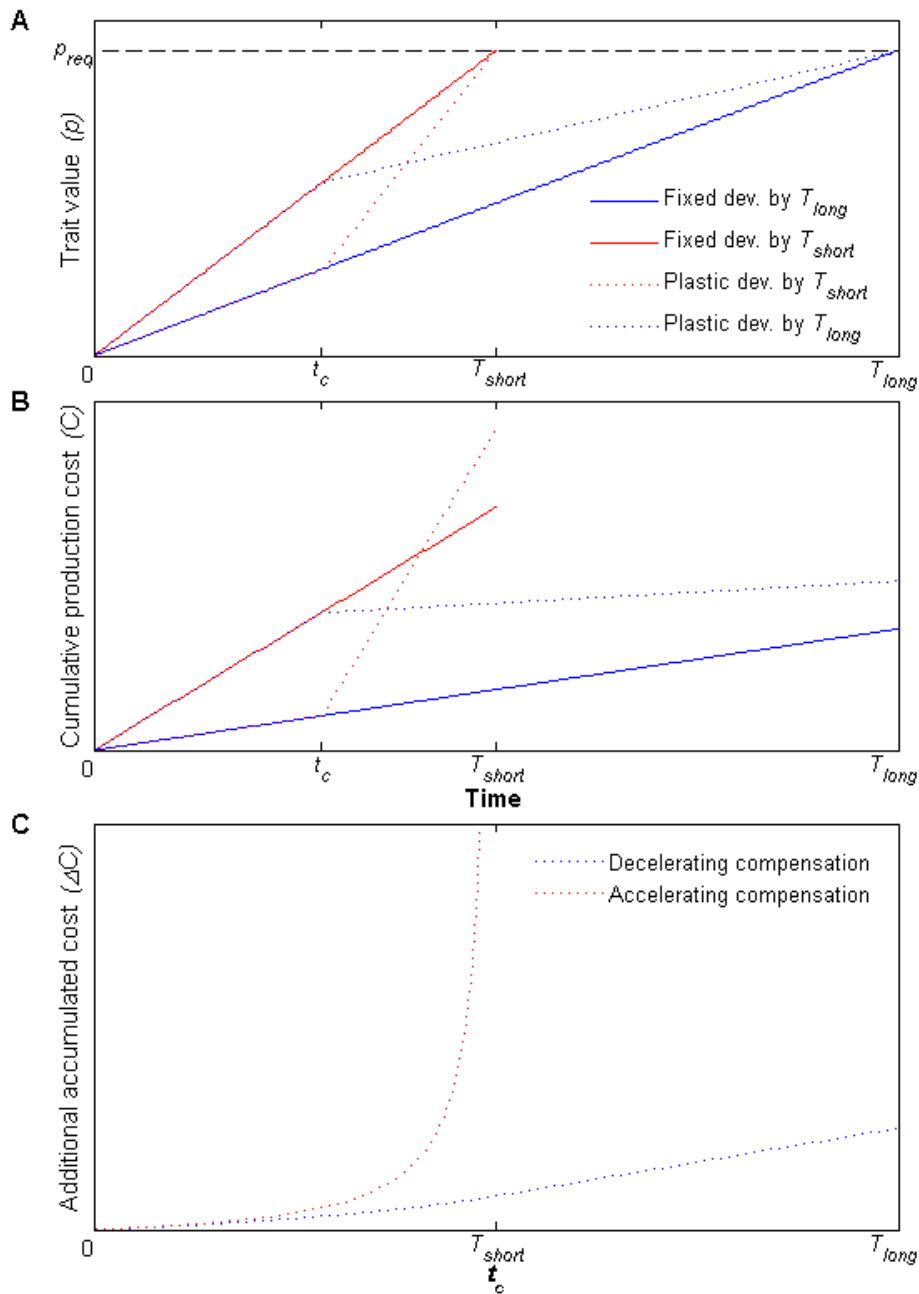
Figure 5.6: Trait developmental trajectories and their production costs. (A) A simulation of trait development trajectories towards a required trait value, p_{req}, under time limitations. Red and blue lines complete development by T_{short} and T_{long}, respectively. Solid lines stand for fixed developers that do not or cannot change their developmental rate. Dotted lines stand for plastic developers that change their developmental rate upon perception of information at t_c. (B) Cumulative production costs for the corresponding developmental trajectories. (C) The additional accumulated costs of compensatory development as a function of the time of perception of reliable information. Red and blue lines accelerate or decelerate their initial rates of development to attain the required trait value at T_{short} or T_{long}, respectively.

GENERAL DISCUSSION

"The most consequential change in man's view of the world, of living nature and of himself came with the introduction, over a period of some 100 years beginning only in the 18th century, of the idea of change itself, of change over periods of time."

--- Ernst Mayr (1978) *Evolution*. Scientific American 239:1, 47.

All four studies in this thesis conceptually address the temporal aspects of various ecological processes. Specifically, they address the changes in ecological conditions within the larval habitats of organisms with complex life cycles and their interactions with organismal life history strategies – larval ontogeny and behavior as well as adult breeding behavior.

The temporal aspect of the larval habitat that stands out most evidently in all the chapters is its ephemerality, which plays a central role in shaping adaptations, particularly those of the larvae. In Chapter 4, the greatest agent of larval mortality was failure to metamorphose before the pools dried. In nature, pool desiccation is a significant source of larval mortality in the fire salamander (Warburg 1992), as well as in other amphibians and pond dwelling species (Blaustein & Schwartz 2001; Williams 2006). Chapter 5 addressed direct adaptations to ephemerality, demonstrating that salamander larvae display responsiveness to multiple cues of pool desiccation; both water levels and the remains of previously desiccated conspecifics. I showed that the acceleration of larval development carries costs that may even result in the death of individuals. Sub-lethal physiological costs that could not be detected in the experimental design that I used are very likely. However, since failure to metamorphose in time leads to certain death, fitness-optimizing individuals may accept these costs and even induce hyper-accelerated and highly costly compensatory development, depending on the reliability and timing of the perceived desiccation cues.

Ovipositing females are not likely to be sufficiently sensitive to differences in rates of change in pool water levels that are meaningful for larval development due to the difference in temporal scales between these functions, although females may visit a set of

pools multiple times after inundation before they first larviposit (personal observations, see also Spieler & Linsenmair 1997). However, one might expect that the presence of dried conspecifics would be perceived by ovipositing females, since the physiological mechanism exists in the larval stage. Interestingly, a preliminary experiment (unpublished) indicated that pregnant salamander females may not respond to this cue, despite the prime importance of hydroperiod for larval success. In another study, salamander females responded to the depths of pools, preferring deeper pools for oviposition allegedly since pool depth is correlated with its hydroperiod length (Segev *et al.* 2011). Possibly, pool depth is a more reliable cue for the duration of the pool than the remains of desiccated conspecifics. An alternative hypothesis is that pool size is strongly correlated with larval densities, in addition to being a predictor of hydroperiod. Pools with greater water volumes may offer more food resources *per capita* and reduced encounters between individuals that may result in agonistic interactions, compared to smaller pools of similar duration, leading to greater rates of metamorphic success. Ideal free distribution of multiple ovipositions into a set of available habitats theoretically leads to uniform fitness values of larvae in all habitats, when assuming symmetrical density-dependent interactions, i.e. that the fitness reduction of prior larvae by late-comers is similar in magnitude as the vice versa fitness reduction (Fretwell & Lucas 1970). However, asymmetrical interactions and priority effects among temporally distributed cohorts (Chapters 2 and 4) result in an advantage to early cohorts and in higher fitness gains from deposition in habitats of superior quality.

In Chapter 5, the larvae were fed *ad libitum* throughout the experiment, and therefore their development rates were not likely limited by energy availability. In nature, however, such resource abundance occurs rarely. Since the risk of lethal habitat termination assigns great value to fast development, it requires large, continuous energetic inputs. In Chapter 2, cannibalistic early cohorts displayed higher rates of successful metamorphosis in structurally simple habitats, where cannibalism was unhindered. Similarly, in Chapter 3, reduced intracohort genetic relatedness increased rates of cannibalism and with it rates of metamorphic success. Under these circumstances, competition for food becomes critical for survival and may sometimes outweigh other risks, such as the risk of aggression and cannibalism. Larval death from desiccation in Chapter 4, where cannibalism was relatively weak, demonstrated a density-dependent pattern both in the early and the late cohorts. The known benefits to cannibals from preying upon conspecifics are the nutritional gain and the elimination of competitors (Polis 1981). Both of these imply greater energetic gains for the cannibalistic individual that can be

allocated to faster development and a reduced risk of death from habitat termination. Thus, natural selection for cannibalistic behavior may be particularly strong in ephemeral larval habitats. This also implies that from the ovipositing females' perspective, cannibalism among larvae should not always be avoided as intuition might lead us to think.

A common feature of cannibalism in many organisms, including the fire salamander, is its size-dependent direction and magnitude (Degani *et al.* 1980; Polis 1981; Hopper *et al.* 1996; Reques & Tejedo 1996; Claessen *et al.* 2000; Wissinger *et al.* 2010). In Chapters 2 and 4, only the smaller larvae of late cohorts suffered from cannibalism that was carried out by the larger early cohorts. While in a laboratory study by Markman et al (Markman *et al.* 2009), intra-cohort aggression between size-matched pairs of larvae never resulted in full blown cannibalism, In Chapter 3 intracohort cannibalism occurred, probably due to the development of size structures within the mesocosms as a result of random variation in feeding success and growth rates.

Intercohort priority effects are asymmetric, temporally-determined interactions between members of different cohorts that mediate both qualitative and quantitative temporal changes in the ecology of larval habitats. These may include interference and cannibalism (Anholt 1994; Godfray 1994; Eitam *et al.* 2005), competition for resources and space (Bremset & Berg 1999; Samhouri *et al.* 2009), and manipulation of the habitat itself (e.g. Tena *et al.* 2008). In the fire salamander, the dependence of effective cannibalism, as well as interference behavior, on size differences between individuals, combined with the temporal pattern of oviposition as the main source of intercohort size differences, is the central cause for intercohort priority effects.

The order of colonization determines the trophic functions and dominance structure of different cohorts and therefore qualitatively influences their interactions and even the roles of other, fixed, habitat characteristics on their performance. Chapter 2 demonstrates this point with respect to structural complexity – a fixed trait of larval habitats. In agreement with previous findings in predator-prey systems (Huffaker 1958; Smith 1972; Crowder & Cooper 1982; Warfe & Barmuta 2004), I found that structural complexity reduces rates of cannibalism by the larger larvae of early cohorts on the smaller individuals of later cohorts. Therefore, structural complexity is beneficial to late cohorts by providing refuge and increasing survival, but it is detrimental to early cohorts since it reduces their foraging efficiency. As discussed earlier, reduced foraging efficiency may be lethal in ephemeral larval habitats. This ambivalent effect of structural complexity on larval

performance reflects in larviposition behavior, as females prefer opposite levels of complexity depending on their position in the queue of colonization.

While the order of colonization affects larval interactions qualitatively, the time interval between colonization events often determines the strength of priority effects, and modifies the interactions between larvae of different cohorts quantitatively. Chapter 4 shows that the strength of cannibalism depends on the time and size differences between cohorts. It also shows that the competitive effect of later cohorts upon the first cohort reduces with intercohort time interval. This may be because with longer time intervals the first cohort has more time to grow with little competition. Another reason may be the growing strength of cannibalism and interference upon the later cohorts. Even without actual cannibalism occurring, or with cannibalism at low actual rates, the presence of large cannibalistic conspecifics may influence later cohorts' habitat use and foraging behavior (Lima & Dill 1990; Werner & Peacor 2003; Preisser *et al.* 2005). Clearly, the effects of competition upon the first cohort in Chapter 4 were very strong, but it is very likely that with lower structural complexity, increasing intercohort time intervals would lead to greater cannibalism and a greater relief of competition from the first cohort. Chapter 4 also presents compelling data to hypothesize that the above dynamics in intercohort cannibalism and competition as a function of time interval may potentially select for complex oviposition behavior in time, similar to that demonstrated in Chapter 2. Fire salamander females did not demonstrate an ability to recognize kin, despite the capability to do so at the larval stage. Therefore, the hypothesis that intercohort time intervals would select for temporally complex oviposition behavior could not be supported in this case. However, the results also do not refute this hypothesis, as would be the case if a time-independent preference had been demonstrated, but indicate a possible ontogenetic constraint (see also Walls 1991; Blaustein *et al.* 1993).

This thesis demonstrates that a temporal approach to analyzing ecological processes may potentially yield important insights on the biology of the studied organism. Chapters 2 and 4 are novel in addressing the change over time of the roles of cohorts and the strengths of their interactions, and their effect on the evolution of oviposition behavior. Chapter 3 showed that initially reduced rates of cannibalism due to kin-selective behavior may be balanced over time by negative density-dependent stress, leading to increased mortality by the loss of kin discrimination, by starvation, or by eventual habitat termination. These chapters motivate further studies that would explicitly analyze the change in organismal behaviors as a function of time or of the temporal changes in biotic and a-biotic

environmental conditions. In Chapter 5, I used a temporally-explicit, developmental approach to studying phenotype induction. In many studies on organismal responses to multiple, simultaneous risk cues, their perception is shown to result in additive, synergistic or antagonistic responses in the expressed final phenotypes, leading to conclusions regarding the reliability, specificity and complementarity of the tested cues (e.g. Bourdeau 2010). A methodology that exploits the significance of the temporal pattern of cue perception allows addressing questions on the very process of phenotype development, its constraints and tradeoffs that underlie and provide alternative explanations to patterns of final phenotype expression. The results motivate experimental methodologies in which the combination of the nature of phenotype-inductive cues and their temporal sequence are manipulated.

While biological processes are composed of underlying physical, chemical and statistical processes, the distinguished role of biological science is to identify and study the set of unique, emergent properties that appear in the realm of living systems – organisms, their populations and communities. In this level of organization, processes are inherently time-dependent and historically-contingent. Early explanations of the diversity of species, adaptations and of the development of adult morphologies from embryos employed static, preformationist concepts such as theories of special creation and the embryonic homunculus. Modern evolutionary biology is a temporally explicit explanatory framework for diversity and adaptation, and is now accepted as the central organizing force in all biological phenomena. Modern developmental biology focuses more on the temporal pattern of gene activation than on static gene composition to explain variation in organismal development. Ecology often employs static concepts (such as the 'ecological niche') in explaining the interactions of individuals with their environments and the emerging population and community patterns (Lewontin 2000). Behaviors and ecological interactions are seen as resulting from certain states of the organism and the environment. Phenotypic plasticity and complexity of interactions are often viewed as 'context-dependence', i.e. that interactions are sensitive to multiple states of the system rather than to a single state. However, sources of complexity arising from the temporal patterns in the states of systems are less often addressed. Roughgarden (Roughgarden 2009), in contemplating a general theory of community ecology, relates this to the structural, rather than formational intellectual history of ecology. She suggests moving towards a general formational theory of community ecology that would be logically parallel to evolutionary theory. In evolutionary biology, the diversity of traits in any population is seen as supplied

by mutations and shaped by interactions that result in natural selection. In parallel, the diversity of species in any community can be seen as formed by the supply of immigrating species and shaped by the interactions such as predation, competition, etc. that result in the exclusion of some species and the dominance of others. Thus, generalities should be sought in the formation of communities – an explicitly temporal process which leads to historically contingent structures of specific communities. Recent empirical studies following this intellectual path include work on historical contingency in community assembly and its effect on community structure (Olito & Fukami 2009; Fukami & Nakajima 2011). This thesis suggests conceptual and experimental approaches for studies in population, developmental and behavioral ecology that are consistent with this trend.

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אסטרטגיות היסטורית חיים של אורגניזמים בעלי מחזור חיים מורכב ובתי גידול לרוואליים זמניים: *הסלמנדרה המצויה כמודל*

אסף שדה

תקציר

אורגניזמים בעלי מחזור חיים מורכב מציגים חילופים דרמטיים בתנאי בית הגידול ובנישות האקולוגיות אותן הם מאכלסים במעבר משלב חיים אחד למשנהו. אורגניזמים אלה מהווים רוב המינים הרב-תאיים על פני כדור הארץ, ורבים מהם חשובים לאדם מבחינה חקלאית, רפואית וסביבתית. השגת מנגנונים המשותפים לדפוסים האקולוגיים ולתהליכים האבולוציוניים שפועלים על מינים אלה הן תשפר את הבנתנו את החיים ואת התהליך האבולוציוני, והן תאפשר לנו לנהל בהצלחה אוכלוסיות על פי הצורך. הודות להפרדה המוחלטת בבתי הגידול המשמשים שלבי חיים שונים, אורגניזמים עם מחזור חיים מורכב ובתי גידול לרוואליים בדידים הם מערכות מחקר מצויינות המאפשרות הפרדה בין השפעות הגירה, הישרדות והצלחה רבייתית על התפוצה ושפע המינים הנצפה. ההישרדות, ההגירה ובחירת אתר ההטלה של הבוגרים משפיעים על הדפוסים ועל גבולות התפוצה של מינים, בעוד שההישרדות, הגדילה, ההתפתחות והאקולוגיה הלרוואלים משפיעים על ההצלחה הרבייתית של הבוגרים וקובעים את דפוסי הגיוס המקומיים לאוכלוסיה הבוגרת.

האבולוציה של בחירת אתר הטלה מעוצבת באופן ניכר על-ידי הביצועים הצפויים של הצאצאים בבית גידולם הלרוואלי, וניתן לצפות שבחירה זאת תהיה רגישה לגורמים האקולוגיים שקובעים את איכותו של בית הגידול הלרוואלי. הודות לטבעם של הגורמים האקולוגיים השונים לקיים יחסי גומלין בינם לבין עצמם, התגובתיות של התנהגות ההטלה לסביבה עשויה להיות הן תלוית-הקשר והן משתנה בזמן.

מהרגע שנבחר אתר הטלה, הלרוות מוכלים בבתי גידולם ונאלצים להתמודד עם התנאים בהם עד לגלגולם לשלב הבוגר (מטמורפוזה). תנאים אלה, הכוללים תחרות על מזון, טריפה וקניבליזם, מבנה חברתי, מבנה גדלי גוף ואפקט הראשונים בין עוקבות, עשויים להשתנות בזמן ולהשפיע על התנהגויות אינדיבידואליות וחברתיות, אסטרטגיות התפתחותיות והצלחה מטמורפית.., בעיקר בבתי גידול זמניים.

אני חוקרת אסטרטגיות היסטורית חיים של הסלמנדרה המצויה (*Salamandra infraimmaculata*) כמודל לאורגניזמים בעלי מחזור חיים מורכב ובתי גידול זמניים. בעבודתי התייחסתי באופן מפורש למימד הזמן של האקולוגיה של בתי הגידול הלרוואליים, ובפרט להתמקדתי בזמניות בית הגידול, באירועי קולוניזציה חוזרים ופערי הזמן ביניהם ובהשפעתם של אלה על דפוסי ההתנהגות וההתפתחות הלרוואלית וכן על התנהגות ההטלה של הבוגרים. השאלות הספציפיות ששאלתי והפרויקטים שעיצבתי כדי לענות על שאלות אלה הם:

1. האם נקבות סלמנדרה הרות בוחרות את אתר ההטלה בהתאם לתפקוד הטרופי הצפוי לצאצאיהם וכתלות בסדר הגעתן לבית הגידול?

ביצעתי מניפולציה על קצבי הקניבליזם בין לארוות באמצעות דרגת המורכבות המבנית של בית הגידול על-מנת לקבוע את ההשפעות השונות של קצבים אלה על ביצועיהן של שתי עוקבות.. ניסוי זה וידא שמורכבות מבנית מפחיתה קצבי קניבליזם, ושהפחתה זאת מועילה להישרדות העוקבה המאוחרת, אך מעכבת את קצב ההתפתחות למטמורפוזה של העוקבה המוקדמת. בניסוי בחירת אתר הטלה, הנחתי לנקבות הרות במיקומים שונים בסדר ההגעה לבית הגידול לבחור בין מידות מורכבות מבנית שונות. נקבות שפגשו לבריכות ריקות מלארוות העדיפו בריכות פשוטות, בעוד שנקבות שפגשו רק בבריכות המאוכלסות על-ידי לארוות העדיפו בריכות מורכבות.

2. כיצד ברירת קרובים בהתנהגות קניבלית משפיעה על הביצועים של עוקבה לרוואלית תחת אילוצי זמן ומחסור במזון?

ביצעתי מניפולציה על ההטרוגניות הגנטית של עוקבות לרוואליות בעלי יכולת זיהוי קרובים על-ידי גידול קבוצות אחים או קבוצות מעורבות בבריכות דלות במזון ובעלות אורך חיים קצר. בעוד קצבי קניבליזם ביו גבוהים בעוקבות המעורבות, רמת התמות הכוללת היתה דומה בין הטיפולים. בנוסף, קצבי המטמורפוזה בעוקבות המעורבות היו גבוהים מאלה של עוקבות האחים. ניתוחי קורלציה מראים שהשונות בגדלי הגוף ההתחלתיים לא מסבירים את התוצאות הללו. לפיכך, יתכן שהביצועים הכלליים הטובים יותר של העוקבות המעורבות נובעים מרמת הקניבליזם המוגברת.

3. כיצד פרק הזמן בין עוקבות לרוואליות משפיע על הביצועים של כל עוקבה? בפרט, האם עוצמת התחרות והקניבליזם בין העוקבות משתנות כפונקציה של פער הזמן בין העוקבות? האם ההשפעות הנ"ל משפיעות בתורן על החלטות הטלה כפולה (self vs. conspecific superoviposition) של נקבות הרות כפונקציה של פער הזמן בין העוקבות?

ביצעתי מניפולציה של פערי הזמן בין שתי עוקבות וכן של צפיפויות הלארוות בניסוי בריכות רב-גורמי, ועקבתי אחר הביצועים של שתי העוקבות. הניסוי מראה שעוצמת התחרות שנחוות על-ידי העוקבה המוקדמת והדומיננטית יורדת עם פער הזמן בין העוקבות. תמיכה נוספת מתקבלת למחקרים קודמים שהראו שקניבליזם כנגד העוקבות הצעירות גובר עם פער הזמן בין העוקבות. בניסוי זה, החשיבות של תחרות ניצול תלוית-צפיפות היתה רבה מזאת של הפרעה וקניבליזם, והביאה לתמותה משמעותית בשתי העוקבות כתוצאה מכשל מטמורפי. ערכתי גם סדרת ניסויי בחירת אתר הטלה כפולה בפערי זמן שונים, בהם הנחתי לנקבות לבחור בין בריכות המכילות לארוות שהן עצמן השריצו (הטלה כפולה עצמית) לבין בריכות המכילות לארוות של נקבות זרות (הטלה כפולה זרה). נקבות הסלמנדרה הציגו אדישות לכאורה בלתי-אדפטיבית לזהות הגנטית של עוקבות קודמות. מכיוון שמחקרים קודמים הראו שנקבות מסוגלות להבחין בנוכחותן של עוקבות קודמות, וכן שהלארוות מסוגלות להבחין בקרבה גנטית, תוצאות אלה עשויות להעיד על מגבלות אונטוגנטיות על יכולת זיהוי קרובים.

4. האם לארוות חשים בהתייבשות של בני מינם מעוקבות קודמות שהתרחשה טרם הולדתם כאות מוקדם לאורך החיים הצפוי של בית הגידול? האם הם מגיבים באופן אדפטיבי על-ידי שינוי קצב ההתפתחות

שלהם על-מנת להגביר את ההסתברות למטמורפוזה לפני שהבריכה תתייבש? האם פלסטיות התפתחותית מוגבלת אונטוגנטית, כלומר, האם הלארוות יכולים לעדכן את קצבי התפתחותם בהתאם לאותות מאוחרים ואמינים יותר לגבי אורך החיים של הבריכה, עד כדי פיצוי מלא? במידה ופיצוי התפתחותי אכן מתקיים, האם הוא גורר מחירים אנדוגניים?

הנחתי ללארוות לגדול במיכלים במעבדה בנוכחות או היעדר שאריות של לארוות שהתייבשו. מניפולציה זאת שולבה במניפולציה של נפחי מים בפועל (נפח קבוע או יורד) כך שנוצרו זוגות של אותות עקביים או סותרים זה את זה. עקבתי אחר מסלולי ההתפתחות של הראשנים ואחרי דפוסי התמותה לפני מטמורפוזה. מצאתי שלארוות מגיבים בשלב מוקדם בחייהם לשאריות ראשנים שהתייבשו כאות לסכנת התייבשות עתידית, ושהם מסוגלים לפצות באופן מלא על תגובה זאת במידה ואות סותר ואמין יותר נקלט מאוחר יותר. דפוסי התמותה מעידים על כך שמחירים אנדוגניים עשויים להיות תלויים בקצבי התפתחות רגעיים, ומציגים מחירים א-סימטריים לפיצוי התפתחותי בין המקרים של אתראה מוקדמת חיובית-שקרית לעומת שלילית-שקרית. השתמשתי בתוצאות האמפיריות כדי למדל את המחיר של קצבי התפתחות כמחיר יסודי של ייצור פנוטיפ, וכדי לנתח את שקלול התמורות הנובע מכך בין מחירי הייצור של פלסטיות התפתחותית לבין מחירי אי-התאמה בין הפנוטיפ לסביבה. המודל חוזה אובדן אונטוגנטי של פלסטיות, דפוס הנצפה בהרבה אורגניזמים.

התיזה הזאת מדגימה שגישה המתייחסת למימד הזמן באופן מפורש בניתוח של תהליכים אקולוגיים עשויה להניב תובנות חשובות אודות הביולוגיה של האורגניזם הנלמד, הכוללות יחסי גומלין דינאמיים א-סימטריים בין פרטים באוכלוסיה, התנהגויות הטלה מורכבות ודינמיות ומנגנוני פלסטיות התפתחותית של לארוות.

אסטרטגיות היסטורית חיים של אורגניזמים בעלי

מחזור חיים מורכב ובתי גידול לרוואליים זמניים:

הסלמנדרה המצויה כמודל

מוגש על-ידי

אסף שדה

בהנחיית

פרופ' ליאון בלאושטיין

חיבור לשם קבלת התואר

דוקטור לפילוסופיה

אוניברסיטת חיפה

הפקולטה למדעי הטבע

החוג לביולוגיה אבולוציונית וסביבתית

אוגוסט 2011

אסטרטגיות היסטורית חיים של אורגניזמים בעלי מחזור חיים מורכב ובתי גידול לרוואליים זמניים: *הסלמנדרה המצויה כמודל*

אסף שדה

חיבור לשם קבלת התואר
דוקטור לפילוסופיה

אוניברסיטת חיפה
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