CARRY-OVER EFFECTS OF RESOURCE POLYMORPHISMS

Sofía María de la Serna Buzón

A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School.

Chapel Hill
2019

Approved by:

Karin S. Pfennig

David W. Pfennig

Ryan A. Martin

Christopher Martin

William Kier

ABSTRACT

Sofia de la Serna Buzon: Carry-over Effects of Resource Polymorphisms
(Under the direction of Karin S. Pfennig and David W. Pfennig)

An individual's early-life environment or phenotype frequently influence its adult traits. Recently, scientists have begun to examine carry-over effects, how factors at one life stage can impact an individual's performance in the following stages, to better understand how factors during development impact adult phenotypes. Carry-over effects are especially important to study in organisms with complex life cycles since these often undergo dramatic tissue reorganization that have the possibility of uncoupling and resetting early life experiences. Here I examine carry-over effects on a resource polymorphic species to better understand how early life phenotype and environment carry over across life stages. Using spadefoot toads as the model system, I examine in Chapter II whether environment- or phenotype-dependent 'carry-over effects', respectively, are associated with alternative, environmentally induced, phenotypes. I ask whether carry-over effects are evident in terms of size and timing to metamorphosis, size and timing to sexual maturity and survival differences. I find that pond environment is the larger predictor of carry-over effects. Larval morphotype has important impacts in terms of timing to metamorphosis and sexual maturity. In Chapter III, I evaluate whether there are behavioral differences carried over post-metamorphosis. I evaluate foraging behavior in particular since, post-metamorphosis, spadefoot toads converge on diet, so I would not expect toads to differ when trying to capture the same food items. Interestingly, I found that toads who were previously the carnivorous morphotype were more efficient foragers and consumed more prey items. Finally, in Chapter IV, I examine whether resource polymorphisms at the larval stage are accompanied by anatomical differences, and if these persist to adulthood. Any anatomical differences could be indicative of physiological differences in how resources are used and stored. I found that carnivore and omnivores differ in liver sizes and these differences are still present at the juvenile stage and in wild caught populations. Together my research shows that there are carry-over effects of early life experiences in spadefoot toads.

To my grandfathers, your curiosity, passion and scholarship inspires me.

ACKNOWLEDGEMENTS

First, I would like to thank my advisors Dr. Karin Pfennig and Dr. David Pfennig, without whom none of this work would have been possible. Through many tireless meetings they have pushed me to become a better thinker, a better planner, and a better writer. Both Karin and David have taught me to focus and to see a project through: from conception to writing. The many roles they have trusted me with have undoubtably prepared me to one day running my own lab. I want to especially thank Karin for encouraging me to apply for the program and her lab. This has been the right path for me without a doubt.

I also want to thank the rest of my committee for all the time and advice they have given me, especially Dr. Ryan Martin whose extensive stats knowledge and advice has pushed me to better understand my own models. I want to thank my awesome lab mates throughout the years: Gina Calabrese, Audrey Kelly, Catherine Chen, Emily Shmidt, Pat Kelly, Nick Levis, Andrew Isdaner, Chris Ackali, Paul Durst, Spencer Ingley, Amanda Pierce, and Becca O'Brien. Thank you for the laughs, the solidarity, and the friendship. Graduate school would have been a lonely journey without you.

My time in this lab has certainly shaped me professionally, but it has also changed me personally. It is here that I most serendipitously met my best friend, staunchest supporter and husband, Antonio Serrato. With you I am complete. Thank you for always pushing me to be better, think harder, and work smarter. Thank you for taking the time to listen to my ideas, read my not-so-awesome first drafts and when necessary, push me out of my comfort zone. I look forward to all the adventures ahead of us, as our family grows and our careers mature. To Matias, thank you for being such an awesome little dude and good baby. You came suddenly into this world and are already forging your own path; I am lucky to get the change to guide you.

A big thank you to my family. My mother and father have always encouraged my curiosity and fomented my passion for science and nature. They have shown tremendous support for my pursuit of a doctorate. They have provided financial support that has eased the stress of graduate school, but most importantly, they have always lent an ear to my many woes, be it dealing with toad mortalities or working on a deadline. Thank you for all your sacrifices that made my career choice even possible. Thank you to my sister and brothers, your love and support always lifts me up, it has been awesome growing up with you guys. Thank you to everyone in ‘Team Matias’ (Vianey, David, Mom, Lupita), your excellent care of my little baby made it so that even when I missed him like crazy, I always knew he was in excellent hands.

TABLE OF CONTENTS

CHAPTER III: A TADPOLE'S DIET PREDICTS ITS FORAGING BEHAVIOR AS JUVENILE TOAD

CHAPTER IV: PHYSIOLOGICAL BASES OF CARRY-OVER EFFECTS ASSOCIATED WITH ALTERNATIVE RESOURCE-USE MORPHS

LIST OF TABLES

LIST OF FIGURES

.

CHAPTER I: GENERAL INTRODUCTION

A central goal in evolutionary biology is to understand the mechanisms that lead to phenotypic change, both within and between generations. Complex and heterogeneous environments constantly influence an organism's phenotype as they grow and develop. Consequently, variation in the quality of natal habitats across landscapes and through time can lead to performance differences of adult phenotypes. Studies focusing on carry-over effects (COE) aim to understand the lingering effects of past environments on future fitness. In an ecological context, carry-over effects arise in scenarios where an individual's previous experiences help explain their current and future performance (O'Connor, Norris, Crossin & Cooke, 2014). To understand the role of natal experiences on adult phenotype, it is necessary to track individuals across life-history stages and through complex spatial and temporal landscapes (Harrison, Blount, Inger, Norris & Bearhop, 2011). Carry-over effects of early life experiences are ubiquitous throughout nature and can significantly alter organismal lifetime fitness, behavior and physiology, including plants (Oksanen & Saleem, 1999), to invertebrates (Ituarte, Vázquez, González-Sagrario & Spivak, 2014), and many vertebrates (Fish: Auer, Lopez-Sepulcre, Heatherly, Kohler, Bassar, Thomas & Reznick, 2012; Reptiles: Ceriani, Roth, Tucker, Evans, Addison, Sasso, Ehrhart & Weishampel, 2015; Birds: Firth & Sheldon, 2016; Mammals: Sanderson, Young, Hodge, Kyabulima, Walker & Cant, 2014; Amphibians: Tarvin, Silva Bermúdez, Briggs & Warkentin, 2015).

In polymorphic organisms, alternative morphs can differ in fitness and survival because of unique life trajectories that originate in differences in morphology, behavior, and physiology during ontogeny. Hence, the magnitude and direction of COEs among polymorphs can vary because of differences in responsiveness to environmental stressors or as a result of variation in the availability and use of resources (Zandonà, Auer, Kilham, Howard, López-Sepulcre, O'Connor, Bassar, Osorio, Pringle & Reznick, 2011). Polymorphs in variable environments can then differ in their long-term ability to grow, reproduce, and survive depending on the developmental context. Exploring carry-over effect differences among polymorphs in detail can help uncover the dynamics that ultimately shape populations.

One of the most drastic manifestations of polymorphisms occurs in organisms that produce resource polyphenic individuals (ecomorphs) (Mayr, 1963). Ecomorphs, in order to alleviate intraspecific competition, often exploit alternative resources and diverge in the phenotype required for the acquisition and assimilation of such resources (Skulason & Smith, 1995). Ecomorphs thus encounter trade-offs whereby one morph might excel on one resource at the cost of being less competitive for the other (Paull, Martin & Pfennig, 2012; Scharnweber, Strandberg, Marklund & Eklöv, 2016). Diet quality changes can concomitantly affect developmental times and morphological traits (Wissinger, Steinmetz, Alexander & Brown, 2004) such that each morph type is favored across different environmental contexts, depending on the availability and reliability of the inducing cue (Martin & Pfennig, 2010; Pfennig, 1992; Pfennig, Mabry & Orange, 1991). Such trade-offs are ubiquitous throughout nature, and often form the basis of compelling research for their role in shaping multi-trait evolution, and population dynamics (Svanback & Persson, 2004); yet, further examination is needed to fully uncover the long-term consequences of those trade-offs across life stages.

Integrating individuals of differing life-history traits may shape population dynamics. Life-history traits include those that affect an individual's timing and condition to specific life stages (Reznick, Bryga & Endler, 1990). These stages include, but are not limited to: birth, sexual maturation and death. Studies of life-history traits often measure how individuals differ, such as in timing to organism specific life stages, size when reaching such stages, investment in offspring size and number, and survival, among other traits (Reznick et al., 1990). Life-history strategy differences originate from variation in competition, predation, and availability of resources (Reznick et al., 1990; Reznick, Butler, Rodd & Ross, 1996). When conditions from previous stages influence life-history traits, the earlier factors are recognized as having 'carry-over effects'. In organisms with complex life cycles, each developmental stage presents new challenges, be it intrinsic or extrinsic, that may exacerbate or ameliorate carried-over differences.

Behavioral carry-over effects also occur when it can be established that an organism's current behavior can be linked to factors experienced at earlier stages. Behaviors can be expected to carry-over if mechanisms underlying behaviors do not change between developmental stages (Bell, 2005; van Oers, de Jong, Drent & van Noordwijk, 2004). However, development is a complex process where individuals not only grow in size, but sometimes undergo drastic somatic reorganizations, as seen in metamorphosing species that undergo permanent changes in morphology and physiology. In these instances, behavioral correlations may decouple if the underlying proximate mechanisms become disrupted (Moran, 1994). Extrinsic factors, like those experienced when there is a shift in occupied ecological niches, may also uncouple correlated behaviors during development, with new selective pressures favoring different behavioral traits from those expressed in early life (Brodin, 2009; Sih, Bell, Johnson & Ziemba, 2004). Resource polyphenic species are ideal for studying behavioral carry-over effects because ecomorphs may use similar strategies for extraction and assimilation of shared resources. Carried-over differences in foraging behavior across seasons or life stages can often have notable influences on the later performance of individuals. Thus, exploring the effects of alternative strategies in detail can help uncover the dynamics that ultimately shape populations.

Resource polyphenisms are generally thought to arise as a consequence of natural selection favoring those individuals that are best able to compete for and acquire resources (Pfennig, Wund, Snell-Rood, Cruickshank, Schlichting & Moczek, 2010; Skulason & Smith, 1995). Yet, specialization on alternative resources can also generate differences in the underlying physiology involved in metabolizing and storing those alternative resources. Moreover, physiological differences at the developmental stage that impact condition and energy use could have effects on fitness and performance later in development (Warne & Crespi, 2015). Indeed, alternative resource polymorphs might require differences in how those resources are used within the organism, which could for the basis for fitness and performance differences in subsequent stages. Yet, despite the potential importance physiological differences between ecomorphs might play in mediating carry-over effects, few studies have examined whether and how exploitation of alternative resources leads to different physiological adaptive responses.

Resource polyphenisms and their carry-over effects provide a unique perspective from which to explore drastic morphological responses within single generations. To investigate the relationship between developmental plasticity and carry-over effects, I focus my dissertation research on the desert dwelling, spadefoot toads, *Spea multiplicata,* which occur in the southwestern US and Mexico. In this demanding habitat, conditions are harsh and reproduction is limited to 1-2 months during the summer. *Spea* reproduce once during the season when monsoon rains fill ephemeral ponds. Females lay their eggs, which develop into one of two discrete morphs: a carnivorous specialist and an omnivorous generalist. Depending on their diet, these desert dwelling tadpoles develop into either an ‘omnivore’ ecomorph, a generalist which consumes organic detritus, and small invertebrates, or a ‘carnivore’ ecomorph, that specializes on fairy shrimp and other tadpoles. Spea tadpoles express a suite of unique, complex morphological traits that distinguish the two ecomorphs: if enough shrimp and tadpoles are ingested, the tadpole develops into a carnivore with large jaw muscles and serrated beaks; if not, the tadpole defaults to the rounder-bodied omnivore ecomorph. Carnivorous tadpoles develop faster while omnivorous tadpoles prolong development in favor of accruing more resources prior to metamorphosis. Since carnivores develop faster, they have a higher emergence and survival rate when ponds are short lasting but, when ponds have a long duration, omnivores are favored. This trade-off most likely exists because as pond longevity increases, carnivorous food items are depleted and diluted without being replenished. Meanwhile, in long-lasting ponds, omnivores are favored.

In Chapter II of this dissertation I evaluate the impact of carry-over effects in spadefoot toads of each resource polyphenism post-metamorphosis. I measure whether toads differ in size and timing to metamorphosis, size and timing to sexual maturity, and finally, survival. Additionally, since developmental background may affect an ecomorphs’ performance, it is important to evaluate whether the patterns of carry-over effects are pervasive throughout all contexts. The two contexts explored are ponds of varying hydroperiod: a short-lasting pond and a long-lasting pond.

In Chapter III, I test whether resource-use behaviors post-metamorphosis consistently differ in individuals who, prior to metamorphosis, expressed different resource polyphenism. Foraging behaviors carried-over from the larval stage may help explain if and why differences in initial size post-metamorphosis could potentially be erased during the juvenile phase and into the sexually mature phase (Chapter II).

Finally, in chapter IV, I evaluate whether during development, resource polyphenic individuals undergo notable somatic changes that could contribute to life-history and behavioral carry-over effects seen later in life (Chapter II and III). I measured organ sizes in tadpoles, metamorphs, and wild caught adults and reasoned that, if ecomorphs use different physiological processes for metabolizing and storing consumed resources, then these may be reflected in the internal morphology.

REFERENCES

Auer SK, Lopez-Sepulcre A, Heatherly T, 2nd, Kohler TJ, Bassar RD, Thomas SA, Reznick DN. 2012. Life histories have a history: effects of past and present conditions on adult somatic growth rates in wild Trinidadian guppies. *J Anim Ecol* **81:** 818-826.

Bell AM. 2005. Behavioural differences between individuals and two populations of stickleback (Gasterosteus aculeatus). *J Evol Biol* **18:** 464-473.

Brodin T. 2009. Behavioral syndrome over the boundaries of life—carryovers from larvae to adult damselfly. *Behavioral Ecology* **20:** 30-37.

Ceriani SA, Roth JD, Tucker AD, Evans DR, Addison DS, Sasso CR, Ehrhart LM, Weishampel JF. 2015. Carry-over effects and foraging ground dynamics of a major loggerhead breeding aggregation. *Mar Biol* **162:** 1955-1168.

Firth JA, Sheldon BC. 2016. Social carry-over effects underpin trans-seasonally linked structure in a wild bird population. *Ecol Lett* **19:** 1324-1332.

Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S. 2011. Carry-over effects as drivers of fitness differences in animals. *J Anim Ecol* **80:** 4-18.

Ituarte RB, Vázquez MG, González-Sagrario MdlÁ, Spivak ED. 2014. Carryover effects of predation risk on postembryonic life-history stages in a freshwater shrimp. *Zoology* **117:** 139-145.

Martin RA, Pfennig DW. 2010. Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. *Biological Journal of the Linnean Society* **100:** 73-88.

Mayr E. 1963. Animal Species and Evolution Cambridge, MA: Harvard University Press.

Moran NA. 1994. Adaptation and Constraint in the Complex Life-Cycles of Animals. *Annual Review of Ecology and Systematics* **25:** 573-600.

O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5:** 1-11.

Oksanen E, Saleem A. 1999. Ozone exposure results in various carry-over effects and prolonged reduction in biomass in birch (Betula pendula Roth) *Plant,Cell and Environment* **22:** 1401-1411.

Paull JS, Martin RA, Pfennig DW. 2012. Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biological Journal of the Linnean Society* **107:** 845-853.

Pfennig DW. 1992. Proximate and Functional Causes of Polyphenism in an Anuran Tadpole. *Functional Ecology* **6:** 167-174.

Pfennig DW, Mabry A, Orange D. 1991. Environmental Causes of Correlations between Age and Size at Metamorphosis in Scaphiopus-Multiplicatus. *Ecology* **72:** 2240-2248.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol Evol* **25:** 459-467.

Reznick DA, Bryga H, Endler JA. 1990. Experimentally Induced Life-History Evolution in a Natural-Population. *Nature* **346:** 357-359.

Reznick DN, Butler MJ, Rodd FH, Ross P. 1996. Life-history evolution in guppies (Poecilia reticulata) .6. Differential mortality as a mechanism for natural selection. *Evolution* **50:** 1651-1660.

Sanderson JL, Young AJ, Hodge SJ, Kyabulima S, Walker SL, Cant MA. 2014. Hormonal mediation of a carry-over effect in a wild cooperative mammal. *Functional Ecology* **28:** 1377-1386.

Scharnweber K, Strandberg U, Marklund MHK, Eklöv P. 2016. Combining resource use assessment techniques reveals trade- offs in trophic specialization of polymorphic perch. *Ecosphere* **7:** 1-19.

Sih A, Bell AM, Johnson JC, Ziemba RE. 2004. Behavioral syndromes: An integrative overview. *Quarterly Review of Biology* **79:** 241-277.

Skulason S, Smith TB. 1995. Resource polymorphisms in vertebrates. *Trends Ecol Evol* **10:** 366-370.

Svanback R, Persson L. 2004. Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. *Journal of Animal Ecology* **73:** 973-982.

Tarvin RD, Silva Bermúdez C, Briggs VS, Warkentin KM. 2015. Carry-over Effects of Size at Metamorphosis in Red-eyed Treefrogs: Higher Survival but Slower Growth of Larger Metamorphs. *Biotropica* **47:** 218-226.

van Oers K, de Jong G, Drent PJ, van Noordwijk AJ. 2004. A genetic analysis of avian personality traits: correlated, response to artificial selection. *Behav Genet* **34:** 611-619.

Warne RW, Crespi EJ. 2015. Larval growth rate and sex determine resource allocation and stress responsiveness across life stages in juvenile frogs. *J Exp Zool A Ecol Genet Physiol* **323:** 191-201.

Wissinger S, Steinmetz J, Alexander JS, Brown W. 2004. Larval cannibalism, time constraints, and adult fitness in caddisflies that inhabit temporary wetlands. *Oecologia* **138:** 39-47.

Zandonà E, Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O'Connor MP, Bassar RD, Osorio A, Pringle CM, Reznick DN. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Functional Ecology* **25:** 964-973.

CHAPTER II

ENVIRONMENTAL VARIATION IMPACTS THE MAGNITUDE OF CARRY-OVER EFFECTS ASSOCIATED WITH ALTERNATIVE PHENOTYPES

Co-authors: Ryan A. Martin, David W. Pfennig

Introduction

Organisms experience their environments in a sequential manner, and the conditions an organism encounters at different stages of its life cycle can potentially have long-lasting fitness consequences. This is likely to be especially true for organisms with complex life cycles that undergo metamorphosis. Although metamorphosis has traditionally been regarded as a disruptive event that uncouples two distinct stages from one another (Moran, 1994; Wilbur, 1980), there is growing awareness of strong connections between larval and post-metamorphic phenotypes with organisms rarely resetting between life stages. Thus, the environment experienced early in life commonly affects performance in parts of the life cycle long after the environment was encountered (O'Connor, Norris, Crossin & Cooke, 2014; Pfennig, Wund, Snell-Rood, Cruickshank, Schlichting & Moczek, 2010; West-Eberhard, 2003). Because these ‘carryover effects’ can strongly influence an organism’s survival and reproduction in new environments and later life stages (Dananay et al. 2015; Moore et al. 2015), they have the potential to limit or intensify the effects of natural selection in previous life stages. Yet, despite this capacity for carryover effects to impact lifetime fitness, their evolutionary consequences remain largely unexplored.

A context in which carryover effects might be especially important is when the environment directly alters an organism's phenotype; i.e., in organisms that display conspicuous phenotypic plasticity. Among the most dramatic examples of plasticity are species that exhibit 'alternative phenotypes'—distinct phenotypes produced in the same sex, life stage, and population (West-Eberhard, 1986; West-Eberhard, 1989; West-Eberhard, 2003). Examples include heterophylly (alternative leaf forms) in plants (Lloyd, 1984; Wells & Pigliucci, 2000), castes in social insects (Wilson, 1971), mating polymorphisms (Gross, 1996; Shuster & Wade, 2003), and resource polymorphisms (Smith & Skúlason, 1996). These phenotypic alternatives have long fascinated evolutionary biologists for many reasons, especially since they have been proposed to represent a key phase in major, lineage-specific innovations (West-Eberhard, 1986; West-Eberhard, 1989; West-Eberhard, 2003). According to this idea, if alternative phenotypes are subject to independent selection and act as separate modules (developmentally and functionally), then their occurrence could permit the elaboration of an entirely novel phenotype without elimination of an established one (West-Eberhard, 1986; West-Eberhard, 1989; West-Eberhard, 2003). However, the degree to which there is such an uncoupling of selection acting between alternative phenotypes is unclear.

Another issue requiring clarification is how alternative phenotypes are maintained in evolution; in other words, how individuals who are distinct in terms of morphology, physiology and behavior remain in a population. Longstanding theory suggests that alternative phenotypes can be maintained evolutionarily if there are functional trade-offs associated with these phenotypic alternatives (Levene, 1953; Levins, 1968; Maynard Smith, 1962). For example, among the most common forms of alternative phenotypes are resource polymorphisms (i.e. ecomorphs), which occur when a single population contains alternative morphs showing differential resource use (sensu Skulason & Smith, 1995; Smith & Skúlason, 1996). Resource polymorphisms are thought to represent adaptations for utilizing alternative resources, because the morphological, physiological, and/or behavioral traits required to utilize one resource often preclude use of different resources (Benkman, 1996; Bolnick, Svanback, Fordyce, Yang, Davis, Hulsey & Forister, 2003; Ellerby & Gerry, 2011; Martin & Pfennig, 2009; Robinson, Wilson & Shea, 1996). Hence, an ecomorph may encounter trade-offs whereby one morph specializes on a narrowing breadth of resources at the cost of being less competitive for other resources (Bolnick, Svanbäck, Yang, Davis, Hulsey & Forister, 2003). Incidentally, depending on the availability and reliability of the inducing cue, changes in diet quality can affect developmental times and morphological traits such that each resource polyphenism is favored across different environmental contexts and dietary resources (Jablonka et al., 1995). Consequently, compared to a generalist ecomorph in the same population, resource specialists may suffer lower fitness if their resource suddenly becomes scarce (Terraube, Arroyo, Madders & Mougeot, 2011). Thus, the interplay between resource polymorphisms and the environments in which they occur is of vital interest when exploring the potential life-long impacts of carry-over effects.

It is not well understood, however, what happens when alternative phenotypes occurring early in life later on converge on a single phenotype. For example, many invertebrates and amphibians have evolved complex life histories, where individuals pass from a juvenile (non-reproductive) stage to an adult (reproductive) stage by way of drastic morphological changes. In some of these cases, juvenile and adult stages are both morphologically distinct and occupy different habitats throughout those different stages. However, even after losing the traits distinguishing resource polymorphic individuals, their ontological past could manifest itself in the adult stage in terms of different survival, growth, and reproductive strategies (Carroll, Marler, Winchell & Dingle, 2006). Elucidating the relationship between ontological resource polymorphisms and adult phenotype is essential for a better understanding of any potential carry-over. I focus on organisms where the inducing cue is naturally removed due to ontogenic niche shits, dispersal or migration.

In *Spea*, alternative carnivore and omnivore morphs are maintained in many populations by frequency-dependent disruptive selection, in which competitively mediated selection favors the rarer morph (Martin & Pfennig, 2009). Depending on their diet, *Spea* tadpoles develop into either an 'omnivore' ecomorph, which eats plants, organic detritus, and small invertebrates, or a 'carnivore' ecomorph, which specializes on fairy shrimp and other tadpoles (Paull, Martin & Pfennig, 2012; Pfennig, 1990; Pomeroy, 1981) and which expresses a suite of unique, complex morphological traits (Pfennig, 1992). Omnivores are the default morph; carnivores are induced when a young omnivore eats shrimp or other tadpoles (Levis, de la Serna Buzon & Pfennig, 2015; Pfennig, 1990; Pomeroy, 1981). Previous work has also suggested that carnivores are favored in rapidly drying ponds because they develop faster, whereas omnivores are favored in more slowly drying ponds because they can access a wider range of resources and thus suffer reduced intra-morph competition (Pfennig, Mabry & Orange, 1991). However, whether these two morphs differ in life history traits as adults, i.e., whether there are any 'carry-over effects' associated with these morphs, is unclear.

I sampled carnivore and omnivore tadpoles from two different ponds, each representing two different selective environments that a single population of *Spea* commonly experience during ontogeny. Specifically, one pond was moderate-sized and filled once, lasting for more than a month. The other pond was initially small (rapidly drying), before subsequently refilling, which greatly increased its volume (and, hence, duration). Thus, the environment selected for differential rates of development in the tadpoles to metamorphose, slowly and rapidly respectively. I reared these field-collected carnivores and omnivores to maturation (i.e., sexual maturity) under uniformly favorable conditions in the lab to determine if any systematic differences between morphs exist in size and age at metamorphosis, size and age at sexual maturation, and survival. Identifying carry-over effects of an individuals' phenotype across distinct environments and physiological demands is important for understanding their influence on life-history traits. This has significant implications for every taxa of life, as environment and resource availability are unlikely to remain homogenous throughout lifetime. Notwithstanding environmental changes/fluctuations, resource polymorphisms on their own can have notable physiological effects on an organism that impact life-history traits. However, little is known about how variable environments affect resource polymorphic individual's life history traits.

Materials and methods

Field collections

In the summer of 2016, I collected from two ponds near the Southwestern Research Station in Portal, Arizona (coordinates Horseshoe: 31.9389, -109.0864, PO2-N: 31.9142, -109.0836; 1.72 miles from each other that generally do not differ ecologically from one another) where *Spea multiplicata* bred on July 2nd after a monsoon rain. Eggs hatched and tadpoles emerged in both ponds on July 4th. Tadpoles were left in their native ponds until close to metamorphosis so they could experience their native tadpole environment throughout larval development and develop as carnivore or omnivore ecomorphs naturally. When they approached the later developmental stages, carnivores and omnivores were collected and moved to 5-foot wading pools in the Southwestern Research Station (SWRS) in Portal, Arizona where they remained for three days before being shipped to the University of North Carolina. Horseshoe tadpoles developed faster (see results) and were thus collected on July 26th, eighteen days after hatching, while PO2-N tadpoles took longer to develop and were collected on July 30th, 26 days after hatching. Tadpoles in wading pools were separated according to ecomorph type, carnivores were fed fairy shrimp and omnivores were fed detritus. It was necessary to collect them as tadpoles since after metamorphosis toads converge on phenotype and diet and are indistinguishable from one another.

As noted above, tadpoles from PO2-N took longer to reach the later stages of development. This was potentially caused by a secondary rain event, which often affects natural developmental patterns of carnivores and omnivores. An increase in pond size can be disadvantageous for the carnivore ecomorph since their food source (shrimp and other smaller tadpoles) becomes diluted, thus cuing carnivores to metamorphose earlier and at smaller sizes to escape an environment of dwindling resources. Omnivores on the other hand, prolong the larval stage since an increase in pond size increases the surface area of the pond, generally expanding resources (detritus) and accrue more resources and achieve larger size prior to metamorphosis. I chose these two ponds for collection in order to compare differences in carry-over effects between the two types of ecomorphs across and among ponds where different ecomorphs were favored.

Lab methods

Tadpole rearing

Though some animals underwent metamorphosis in transit, other animals arrived to the lab still as tadpoles. Upon arrival, these tadpoles were housed in in groups of twelve and fed detritus made up of ground up fish food (Hikari Cichlid Staple Floating Pellets for Pets) and brine shrimp ad libitum. Individuals from each population and ecomorph were maintained separately from one another.

Toad rearing

Upon emergence of front limbs, tadpoles were moved to a ‘beach’ box consisting of sand in an incline on the distal side of the cage. Water was added so metamorphs could be fully submerged but sufficient sand was above water for the metamorph to be able to rest. Animals in the beach boxes were not fed as the reabsorption of their tails provides the necessary energy and nutrients. Once resting on the sand, animals were moved to small cages with moist paper towels in groups of 1 to 3 individuals and fed gut-loaded crickets dusted with vitamins (Herptivite with beta carotene Multivitamin for reptiles and amphibians) and calcium (Flukers) ad libitum every other day. After six weeks, toads were moved to larger plastic terraria (11.9 x 7.8 x 8.1 inches) containing a moistened play sand substrate. After the initial six weeks, toads resting on top of the sand were fed dusted crickets twice a week and every other week they were additionally dug out of the sand and fed dusted crickets. Terraria were assigned to randomized locations on shelving in an ambient temperature and humidity room with lights on in a reversed cycle from 2000h to 0800h. Cages with new sand were changed every two months. Toads were dug out of the sand once a week to feed and every four weeks their mass and snout-vent length (SVL) was recorded. Additionally, I provided dusted crickets ad libitum when toads rested above the sand.

Growth, timing to metamorphosis and sexual maturity and survival

I measured size as the snout-vent-length (SVL) in the two discrete morphs at the time of metamorphosis, again after every three months and at the time of sexual maturity. Here metamorphosis is marked as the moment where an individual's tail is reabsorbed. Sexual maturity was determined by the presence of sexual traits such as nuptial pads in males and presence of eggs in the abdomen of females. Any deaths were recorded as they occurred. I assessed whether body size at metamorphosis is influenced by the larval pond environment of the larval morphotype by using a linear model that included population and morphotype and an interaction for these two as fixed effects. I again looked at body size at the onset of sexual maturity and if there were any carry-over effects from pond environment and larval morphotype and if differences existed among individuals of different sex. I evaluated how time to metamorphosis was influenced by the larval pond environment or by larval morphotype by using a generalized linear model with a negative binomial distribution to account for over dispersion. I next evaluated whether age at sexual maturity was influenced by larval pond environment or by larval morphotype or sex using a linear model. Finally, I looked at how survival throughout the period observed (14-months) was influenced by pond or morphotype. I assessed survivorship by a parametric survival analysis following a Weibull distribution with population of origin and larval morphotype and their interaction as fixed effects.

Results

Is body size at metamorphosis influenced by the larval pond environment or by larval morphotype?

Directly after metamorphosis, body-length was different between pond environment ($F_{3,482} = 49.94$, $P < 0.001$), but not different among ecomorphs ($F_{3,482} = 0.08$, $P = 0.782$). There was a significant interaction between pond environment and larval morphotype ($F_{3.482} = 7.61$, $P = 0.006$; table 2.1), with Horseshoe individuals being larger than PO2-N overall, suggesting that ecomorph types reacted differently depending on pond of origin. I tested the differences between morphs in each pond using a Tukey's HSD post-hoc test which showed each group emerged at different lengths, carnivores were larger compared to omnivores in Horseshoe but in PO-2N carnivores were smaller than omnivores (mean ± se: HS carnivore = 21.12 ± 0.16mm, HS omnivore = 20.66 ± 0.20mm, Tukey HSD HS O – C: t-ratio = 1.964, P = 0.050; PO2-N carnivore = 19.23 ± 0.16mm, PO2N omnivore = 19.79 ± 0.12, PO2-N O – C: t-ratio = -1.956, P = 0.051; figure 2.1a).

Is time to metamorphosis influenced by the larval pond environment or by larval morphotype?

I estimated the age at metamorphosis for each larval morphotype from each pond environment by counting the number of days from pond filling until emergence of a front limb. Age at metamorphosis was significantly different among ecomorphs ($\chi^2_{3,482} = 130.38$, $P < 0.001$) and among populations ($\chi^2_{3,482} = 304.22$, $P < 0.001$) with a significant interaction between population of origin and tadpole ecomorph type ($\chi^2_{3,482} = 61.97$, $P < 0.001$; table 2.2). Within populations, Horseshoe carnivores metamorphosed more quickly than omnivores (mean ± se: HS carnivore = 23.73 ± 0.28 days, HS omnivore = 32.36 ± 0.56 days, Tukey HSD HS C –

O: z-value = -13.51, $P < 0.001$), while in PO2-N individuals did not significantly differ in their timing to metamorphosis (PO2-N carnivore = 36.82 ± 0.64 days, PO2-N omnivore = 38.76 ± 0.46 days, Tukey HSD PO2-N C – O: z-value = -2.17, P = 0.030; figure 2.2).

Is size at sexual maturity influenced by the larval pond environment or by larval morphotype?

When toads from each population reached sexual maturity, they did so at different sizes ($F_{7,72} = 13.86$, $P < 0.001$; table 2.3). Horseshoe individuals were larger. However, larval morphotypes did not differ in size at sexual maturity ($F_{7,72} = 0.01$, P = 0.911; table 2.3). Horseshoe carnivores and omnivores do not differ in size (mean ± se: HS carnivore = 43.69 ± 0.54 mm, HS omnivore = 43.64 ± 0.55 mm, Tukey HSD HS C – O: t-ratio = 0.22, P = 0.826), and in PO2-N individuals did not significantly differ in their size at sexual maturity (PO2-N carnivore = 40.71 ± 0.63 mm, PO2-N omnivore = 41.17 ± 0.82 mm, Tukey HSD PO2-N C – O: t-ratio = -0.331, P = 0.741; figure 2.1b). Individuals of different sexes also did not appear to differ in size reached at the onset of sexual maturity ($F_{7,72} = 0.001$, P = 0.992). Additionally, there are hints of sex by morph interactions but I have very limited power to test this and the effects are not significant ($F_{7,72} = 3.76$, P = 0.056).

Is age at sexual maturity influenced by the larval pond environment or by larval morphotype or sex?

Toads originating from each of the populations reached sexual maturity at different times ($F_{7,72} = 12.48$, $P < 0.001$; table 2.4), with those from Horseshoe maturing an average of 92 days earlier. Overall, toads who developed as different ecomorphs did not differ in time to sexual maturity ($F_{7,72} = 3.15$, P = 0.080; table 2.4). Moreover, the sexes did not differ in timing to sexual maturity ($F_{7,72}$ = 1.59, P = 0.210389; table 2.4). A post-hoc analysis of ecomorphs within each population revealed no significant difference between time to sexual maturity for each morph (mean ± se: HS carnivore = 304.95 ± 16.27 days, HS omnivore = 345.00 ± 17.79 days, Tukey HSD HS O – C: t-ratio = -1.485, P = 0.141; PO2-N carnivore = 389.30 ± 31.71 days, PO2N omnivore = 438.36 ± 29.62 days, PO2-N O – C: t-ratio = 0.733, P = 0.466; table 2.5).

Is survival influenced by pond or morphotype?

Survival rate was best estimated using a Weibull distribution with a constant shape and scale parameter. Survival significantly differed between populations ($\chi^2_{3,492}$ = 25.52, P < 0.001; table 2.6), but not among morphs ($\chi^2_{3,492}$ = 0.82, P = 0.367; table 2.6), but there was a significant interaction between population and larval morphotypes ($\chi^2_{3,492}$ = 7.87, P = 0.005; table 2.6). Within populations, Tukey HSD showed that in Horseshoe, carnivores had a significantly higher survival (t-ratio = 2.55, P = 0.011; figure 2.3), while in PO2-N there was no difference in survivorship between the two morphotypes (t-ratio = -1.38, P = 0.167; figure 2.3).

Discussion

Here, I show how certain life-history traits are carried over from either larval environment and/or larval resource polymorphisms. My study reveals that: (1) immediately after metamorphosis, larval pond environment had a large effect on size at metamorphosis, but larval morphotype had a marginal effect. Both larval pond environment and larval morphotype had an influence on timing to metamorphosis. (2) Upon reaching sexual maturity, larval pond environment had a large effect on size and age, while larval morphotype had no effect. Finally,

(3) both larval environment and larval morphotype affected post-metamorphic survival, though larval morphotype's effect was marginal.

In short duration ponds, recently (1) metamorphosed carnivores were larger and metamorphosed earlier than omnivores, thereby giving them an advantage in a rapidly drying pond. In the slow drying ponds however, the pattern is reversed and omnivores were marginally larger than carnivores. Throughout this study, pond hydroperiod had a notable effect in all variables tested. In ephemeral pond systems, pond drying is not always a static process; secondary rain events or even flooding can refill ponds at any time. Unpredictable changes in environments are important factors to evaluate when exploring carry-over effects of resource polymorphic organisms as sudden and drastic changes can influence resource abundance (Denoel, 2006; Terraube et al., 2011). Lengthening the hydroperiod through refilling events can increase pond volume and surface area, thus extending the time organisms spend in their aquatic stages, allowing for the acquisition of more resources. Nevertheless, pond refilling can also have a negative effect as it can lead to dilution of a non-replenishable food source or even the addition of more or new competitors (Pintar & Resetarits, 2018).

Once individuals reached sexual maturity, (2) I again evaluated whether there were any size and timing carry-over effects from larval experiences. I found that individuals from the rapidly drying pond reached sexual maturity at larger sizes and sooner than those of the slowly drying pond. I found no evidence that these morphs differ intrinsically in adult size or timing to sexual maturity; i.e., there were no long-term size carry-over effects associated with being a carnivore or an omnivore. There were indications that within each morphotype, individuals of different sexes may reach sexual maturity at different sizes, but the sample size is too small to draw any meaningful conclusions. The magnitude and direction of carry-over effects among toads can vary because of differences in responsiveness to environmental stressors or simply as a result of variation in the availability resources within each pond. It is notable that I did not detect any carry-over effects of resource polymorphisms at sexual maturity. This could indicate that there is growth rate plasticity post-metamorphosis that differs among morph types, such that any initial differences in size are attenuated by the time each morphotype reaches reproductive maturity. Such plasticity likely allows organisms to adjust growth rates to maximize future reproductive fitness, especially in species with indeterminant growth, as is the case with *Spea*. Hence, environmental stressors or poor resource quality early in life can suppress growth rates, but if environmental conditions improve, individuals can exhibit compensatory growth rate in which smaller individuals make up for poor conditions during ontogeny by growing faster (Hector & Nakagawa, 2012; Radder, Warner & Shine, 2007; Stoks & Cordoba-Aguilar, 2012; Tarvin, Silva Bermúdez, Briggs & Warkentin, 2015). This is especially important in organisms such as anurans where size at reproduction is correlated with mating success and/or fecundity.

Additionally, toads from the fast-drying pond matured faster than those from the slow drying pond. Individuals who mature faster may have increased fitness from increased opportunities to reproduce and thus have a higher reproductive output than those who take longer to mature. Differences in timing can have important implications for population composition (Rodd & Reznick, 1997).

Survival throughout the study period was mostly influenced by larval pond environment, though larval morphotype had a marginal effect. The rapidly drying pond had higher survival than the slow drying pond. Within the rapidly drying pond, carnivores had a higher survival than omnivores. Finally, although carnivores had higher adult survival than omnivores when derived from the rapidly drying (single-filling) pond, the two morphs did not differ in survival when derived from the slowly drying (double-filling) pond. Results here suggest that both larval pond environment and larval morphotype can influence carry-over effects in *Spea multiplicata* toads. However, more work is required to establish how pervasive these results are across other populations.

Despite there being some intrinsic morph-specific differences in the measured life-history traits, there were consistent—and pronounced—differences in all of the five life-history traits between the two different pond environments. Indeed, overall, individuals derived from the large-sized (double filling) pond had lower fitness than individuals derived from the moderate-sized (single filling) pond. Specifically, individuals derived from the large-sized (double filling) pond, took longer to reach both metamorphosis and maturation, were smaller at both metamorphosis and maturation, and were less likely to survive as adults.

Thus, my results suggest that life-history trade-offs, by themselves, cannot account for the maintenance of alternative phenotypes. Instead, environmental variation was of paramount importance. Generally, environmental variation, especially when unpredictable, may be more critical than life-history trade-offs in the maintenance of alternative phenotypes.

REFERENCES

Benkman CW. 1996. Are the ratios of bill crossing morphs in crossbills the result of frequency-dependent selection? *Evolutionary Ecology* **10:** 119-126.

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003. The ecology of individuals: incidence and implications of individual specialization. *Am Nat* **161:** 1-28.

Bolnick DI, Svanbäck R, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003. The ecology of individuals: incidence and implications of individual specialization. *American Naturalist* **161:** 1–28.

Carroll SP, Marler M, Winchell R, Dingle H. 2006. Evolution of Cryptic Flight Morph and Life History Differences During Host Race Radiation in the Soapberry Bug, Jadera haematoloma Herrich-Schaeffer (Hemiptera: Rhopalidae). *Annals of the Entomological Society of America* **96:** 135-143.

Denoel M. 2006. Seasonal variation of morph ratio in facultatively paedomorphic populations of the palmate newt Triturus helveticus. *Acta Oecologica-International Journal of Ecology* **29:** 165-170.

Ellerby DJ, Gerry SP. 2011. Sympatric Divergence and Performance Trade-Offs of Bluegill Ecomorphs. *Evolutionary Biology* **38:** 422-433.

Gross MR. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution* **11:** 92-98.

Hector KL, Nakagawa S. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. *J Anim Ecol* **81:** 583-593.

Jablonka E, Oborny B, Molnar I, Kisdi E, Hofbauer J, Czaran T. 1995. The adaptive advantage of phenotypic memory in changing environments. *Philos Trans R Soc Lond B Biol Sci* **350:** 133-141.

Levene H. 1953. Genetic Equilibrium When More Than One Ecological Niche Is Available. *American Naturalist* **87:** 331-333.

Levins R. 1968. *Evolution in Changing Environments: Some Theoretical Explorations*. Princeton University Press.

Levis NA, de la Serna Buzon S, Pfennig DW. 2015. An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecol Evol* **5:** 1405-1411.

Lloyd DG. 1984. Variation strategies of plants in heterogenous environments. *Biological Journal of the Linnean Society* **21:** 357–385.

Martin RA, Pfennig DW. 2009. Disruptive selection in natural populations: the roles of ecological specialization and resource competition. *Am Nat* **174:** 268-281.

Maynard Smith J. 1962. Disruptive selection, polymorphism and sympatric speciation. *Nature* **195:** 60-62.

Moran NA. 1994. Adaptation and Constraint in the Complex Life-Cycles of Animals. *Annual Review of Ecology and Systematics* **25:** 573-600.

O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5:** 1-11.

Paull JS, Martin RA, Pfennig DW. 2012. Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biological Journal of the Linnean Society* **107:** 845-853.

Pfennig DW. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85:** 101-107.

Pfennig DW. 1992. Proximate and functional causes of polyphenism in an anuran tadpole. *Functional Ecology* **6:** 167-174.

Pfennig DW, Mabry A, Orange D. 1991. Environmental Causes of Correlations between Age and Size at Metamorphosis in Scaphiopus-Multiplicatus. *Ecology* **72:** 2240-2248.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol Evol* **25:** 459-467.

Pintar MR, Resetarits WJ. 2018. Filling ephemeral ponds affects development and phenotypic expression in Ambystoma talpoideum. *Freshwater Biology* **63:** 1173-1183.

Pomeroy LV. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Ph.D. diss., University of California.

Radder RS, Warner DA, Shine R. 2007. Compensating for a bad start: catch-up growth in juvenile lizards (Amphibolurus muricatus, Agamidae). *J Exp Zool A Ecol Genet Physiol* **307:** 500-508.

Robinson BW, Wilson DS, Shea GO. 1996. Trade-Offs of Ecological Specialization: An Intraspecific Comparison of Pumpkinseed Sunfish Phenotypes. *Ecology* **77:** 170-178.

Rodd FH, Reznick DN. 1997. Variation in the demography of natural populations of guppies: the importance of predation and life histories. *Ecology* **78:** 405-418.

Schlichting C, Pigliucci M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer.

Shuster SM, Wade MJ. 2003. *Mating systems and strategies*. Princeton University Press: Princeton, NJ.

Skulason S, Smith TB. 1995. Resource polymorphisms in vertebrates. *Trends Ecol Evol* **10:** 366-370.

Smith TB, Skúlason S. 1996. Evolutionary Significance of Resource Polymorphisms in Fishes, Amphibians, and Birds. *Annual Review of Ecology and Systematics* **27:** 111-133.

Stoks R, Cordoba-Aguilar A. 2012. Evolutionary ecology of Odonata: a complex life cycle perspective. *Annu Rev Entomol* **57:** 249-265.

Tarvin RD, Silva Bermúdez C, Briggs VS, Warkentin KM. 2015. Carry-over Effects of Size at Metamorphosis in Red-eyed Treefrogs: Higher Survival but Slower Growth of Larger Metamorphs. *Biotropica* **47:** 218-226.

Terraube J, Arroyo B, Madders M, Mougeot F. 2011. Diet specialisation and foraging efficiency under fluctuating vole abundance: A comparison between generalist and specialist avian predators. *Oikos* **120:** 234-244.

Wells CL, Pigliucci M. 2000. Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics* **3:** 1-18.

West-Eberhard MJ. 1986. Alternative adaptations, speciation, and phylogeny (A Review). *Proc Natl Acad Sci U S A* **83:** 1388-1392.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20:** 249-278.

West-Eberhard MJ. 2003. *Developmental plasticity and evolution*. Oxford University Press: New York.

Wilbur HM. 1980. Complex Life-Cycles. *Annual Review of Ecology and Systematics* **11:** 67-93.

Wilson EO. 1971. *The insect societies*. Belknap Press: Cambridge, MA.

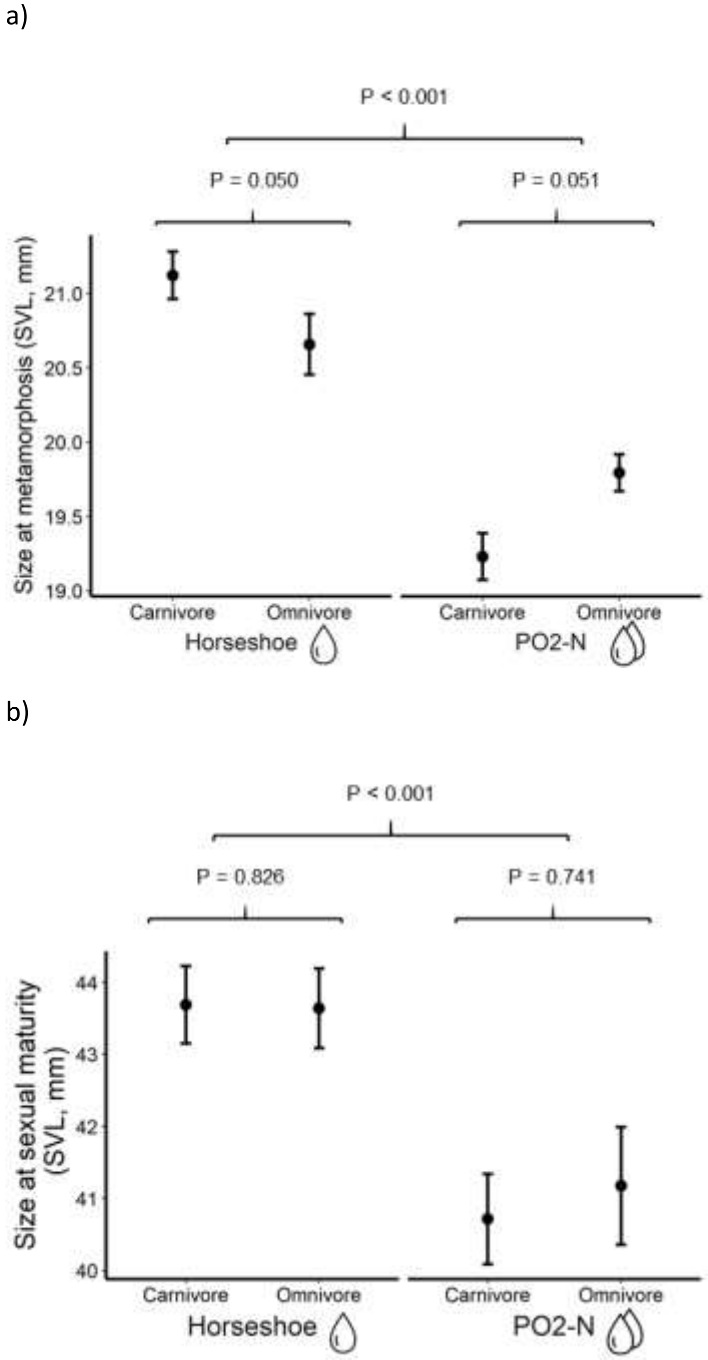

Figure 2.1. Size at (a) metamorphosis and (b) sexual maturity for carnivores and omnivores from Horseshoe and PO2-N ponds.

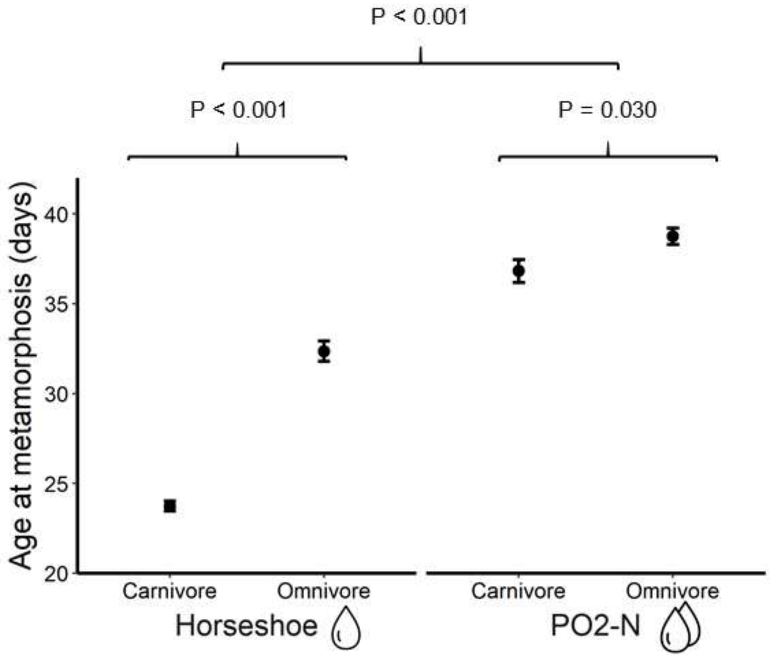

Figure 2.2. Age ftom hatching to metamorphosis for carnivores and omnivores from Horseshoe and PO2-N ponds.

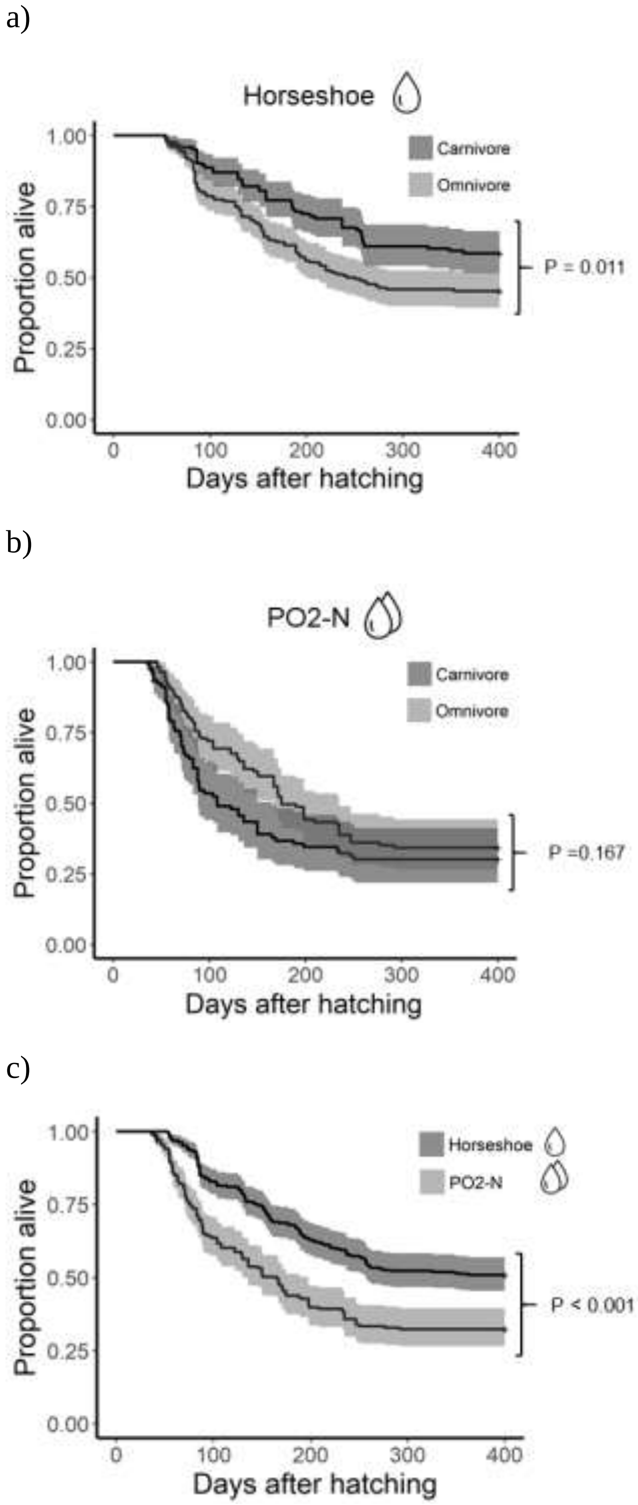

Figure 2.3. Kaplan Meier survival curves with 90% confidence intervals for (a) carnivores and omnivores from Horseshoe pond, (b) carnivores and omnivores from Horseshoe pond PO2-N pond, and (c) both morphs combined from Horseshoe and PO2-N ponds.

Factor	df	F-value	p-value
Population	1,482	49.941	<**0.001**
Larval morphotype	1,482	0.077	0.782
Population*larval morphotype	1,482	7.605	**0.006**

Table 2.1. Results from linear model testing effects of population and larval morphotype on size at metamorphosis in recently metamorphosed *Spea multiplicata* toads. Significance is indicated in bold.

Factor	df	χ^2	P-value
Population	1,484	304.221	<**0.001**
Larval morphotype	1,483	130.379	<**0.001**
Population*larval morphotype	1,482	61.974	<**0.001**

Table 2.2. Results from generalized linear model testing effects of population and larval morphotype on age at metamorphosis in *Spea multiplicata* toads. Significance indicated in bold.

Factor	df	F-value	P-value
Population	1,72	13.859	<**0.001**
Larval morphotype	1,72	0.013	0.911
Sex	1,72	0.001	0.992
Population*larval morphotype	1,72	0.153	0.697
Population*sex	1,72	0.037	0.847
Larval morphotype*sex	1,72	3.762	0.056
Population*larval morphotype*sex	1,72	0.017	0.898

Table 2.3. Results from the linear model testing population, larval morphotype, and sex on size at sexual maturity in *Spea multiplicata.* Significance indicated in bold.

Factor	df	F-value	P-value
Population	1,72	12.748	<**0.001**
Larval morphotype	1,72	3.148	0.080
Sex	1,72	1.595	0.210
Population*larval morphotype	1.72	0.009	0.925
Population*sex	1,72	0.460	0.652
Larval morphotype*sex	1,72	0.688	0.409
Population*larval morphotype*sex	1,72	1.294	0.259

Table 2.4. Results from the linear model testing population, larval morphotype, and sex on size at sexual maturity in *Spea multiplicata.* Significance indicated in bold.

Population	Morph	N	Mean (days)	± SE
Horseshoe	Carnivore	37	304.96	16.262
Horseshoe	Omnivore	29	345.00	17.794
PO-2N	Carnivore	10	392.30	31.707
PO-2N	Omnivore	11	438.36	29.622

Table 2.5. Raw mean and standard errors of Horseshoe and PO-2N carnivore and omnivore linear model of age at sexual maturity.

Factor	df,	χ^2	P-value
Population	1,492	25.516	**<0.001**
Larval morphotype	1,492	0.815	0.367
Population x larval morphotype	1,492	7.868	**0.005**

Table 2.6. Results from survival model with Weibull distribution testing population and larval morphotype in *Spea multiplicata*. Significance indicated in bold.

CHAPTER III

A TADPOLE’S DIET PREDICTS ITS FORAGING BEHAVIOR AS A JUVENILE TOAD

Co-Authors: Spencer J. Ingley, Karin S. Pfennig

Introduction

Foraging involves behaviors that enable organisms to successfully acquire and consume resources. Because resource acquisition has consequences for fitness, behaviors that impact foraging success are potentially under strong selection. Moreover, the expression of those behaviors is expected to vary according to the individual’s state in order to optimize resource acquisition in a given situation or habitat (Macarthur & Pianka, 1966). Indeed, resource use is not always constant throughout an organism’s lifetime and foraging strategy can fluctuate as an organism grows, or as nutritional requirements and resource availability change. For example, as individuals grow, they may have reduced mechanical limitations, and as a result, diet constraints may relax and prey handling improves (Arim, Abades, Laufer, Loureiro & Marquet, 2010; Toscano & Griffen, 2012; Werner & Gilliam, 1984). In other cases, diets may shift altogether as individuals enter new environments or life stages. Environmental and physiological changes occur throughout the life of most organisms. But whether behaviors that facilitate the acquisition or consumption of resources remains constant across life stages is not well studied (Toscano, Gownaris, Heerhartz & Monaco, 2016).

Foraging behavior may be impacted by several factors that vary among individuals such as size, condition, sex, as well as some aspects of behavior, including tendency to explore or be active (Bolnick, Svanback, Fordyce, Yang, Davis, Hulsey & Forister, 2003). Larger individuals require a larger caloric intake to maintain size, and may consume more or larger prey items or may spend more time foraging (Dingeldein & White, 2016; Werner & Anholt, 1993). In bluegill sunfish, both searching ability and prey handling efficiency increase with size (Mittlebach, 1981). Alternatively, smaller individuals may forage more in order to sustain high, early growth rates (Levri & Lively, 1996; Werner & Anholt, 1993). Individual foraging behavior can also be impacted by condition, measured as an individual's mass for a given body size. Individuals who are in greater condition may be more successful foragers as they have more energy available to expend on food search and capture (Dingeldein & White, 2016), as opposed to an individual who is in poor condition, and any failed attempt at capture and consumption could have costly consequences (Booth & Beretta, 2004; Dingeldein & White, 2016). Foraging behavior may also be impacted by other aspects of behavior, such as activity, defined as general activity level in a familiar environment, or exploratory behavior, defined as activity in a novel environment (Reale, Reader, Sol, McDougall & Dingemanse, 2007). Individuals who tend to be more active in non-foraging scenarios can also be better foragers because they may encounter more prey throughout their day (Brodin, 2009; Sweeney, Cusack, Armagost, O'Brien, Keiser & Pruitt, 2013; Werner & Anholt, 1993). Similarly, more exploratory individuals may encounter more (and more novel) prey, as they encounter novel patches with different prey communities (Exnerova, Svadova, Fucikova, Drent & Stys, 2010; Herborn, Macleod, Miles, Schofield, Alexander & Arnold, 2010).

An individual's size, condition, activity, and exploration may lead to differences in foraging behavior and success, and these factors may change throughout development. For instance, size and condition are dependent upon the current state of the individual and can change as an individual grows or develops. On the other hand, activity and exploration are stable over ontogenetic development in some species (Groothuis & Trillmich, 2011; Toscano et al., 2016; Wilson & Krause, 2012b). Because these factors may interact with the more dynamic factors of size and condition, the effects of exploration and activity on foraging behavior throughout development is not altogether clear. Moreover, for species in which some components of foraging behavior are learned or experience-dependent, the outcome of predator-prey interactions depends on both the dynamic behavioral feedback occurring during the encounter and the underlying behavioral type of each participant (McGhee, Pintor & Bell, 2013; Pruitt, Stachowicz & Sih, 2012; Sweeney et al., 2013; Toscano & Griffen, 2014). As an individual grows and changes or expands its diet, foraging behaviors can change in accordance with the novel prey's behavior.

Metamorphosing organisms are well-suited for examining the relationship between foraging behaviors and development as development often changes dietary requirements (Wilbur, 1980; Wilbur, 1997), and therefore, the optimal foraging behaviors suitable for a given stage. Environmentally dependent behavioral adaptations may be decoupled if the original conditions are no longer relevant or have become disrupted, e.g., when moving from an aquatic to a terrestrial setting (Brodin, 2009). To address these concerns, I focused on anurans because they are an ideal taxonomic group in which to study how foraging behavior carries-over through metamorphosis. Generally, throughout anurans, larval tadpoles develop in aquatic environments and are under selection to develop and transition into a terrestrial environment upon metamorphosis. Post-metamorphosis, adults inhabit a variety of terrestrial environments, and their ecology is primarily dedicated to growth, reproduction and dispersal.

In an ecological context, carry-over effects arise in scenarios where an individual's current performance can be explained by the environmental conditions that first induced their phenotype (Burton & Metcalfe, 2014; O'Connor, Norris, Crossin & Cooke, 2014). Behaviors that have been carried-over from one life stage to the next can either be selected for or against, dependent on the how past behaviors assist or hinder organisms across diverse ecological contexts (Brodin, 2009). Resource-use behaviors are expected to remain stable across life stages when the mechanisms underlying such behaviors remain consistent between developmental stages (Groothuis & Trillmich, 2011; Wilson & Krause, 2012a). Thus, carry-over effects can prime individuals for a continuation of past ecological or “life history” trends (Pechenik, Wendt & Jarrett, 1998). Although carry-over effects have been studied across changes in biotic (Firth & Sheldon, 2016) and abiotic (Kristensen, Johansson, Chisholm, Smith & Kokko, 2018) factors, there is much less known regarding the influence of carry-over effects across ontogenetic habitat shifts.

I investigated whether toads who developed as two distinct morphs using different trophic diets (carnivore or omnivore) carry-over differences of their developmental past into the following life stage. I tested whether individuals differ in the following foraging behaviors: (1) foraging trial duration, (2) the amount of time it took individuals to detect and attempt to consume the first prey item, (3) the number of prey items consumed, (4) how many attempts it takes to capture a prey item, and the (5) trial fail rate. Together these measurements provide insight into an individual’s prey handling abilities and quantity of resources consumed. I further examine whether size, condition, activity or exploratory patterns between toads who developed using different trophic diets (carnivore or omnivore) could help explain any differences in foraging behavior. Ultimately, I ask if toads who developed as one of the two morph types are better adapted for foraging demands at the toad stage. Identifying individual differences in foraging behaviors and their correlates (size, condition, activity, exploration) for a species that exhibits resource polyphenisms early in development, but later converges in ecology, diet, and morphology, is an especially powerful approach because it allows me to compare carry-over effects across these contexts.

Methods

Study system

I focused on the Mexican Spadefoot toad, *Spea multiplicata*. Adults live in an arid terrestrial environment where they must spend 9 months of the year underground in order to avoid desiccation. The Mexican Spadefoot toad is capable of producing polyphenic tadpole ecomorphs that can either be generalists (i.e. omnivorous) or specialists (i.e. carnivorous). Each of these tadpole morphs is phenotypically distinct. Carnivore tadpoles are identifiable for their wide jaws, short gut and sharp serrated mouthparts that they use to feed on fairy shrimp and other tadpoles. Omnivorous tadpoles, on the other hand, have a rounder body shape, long gut, and smooth mouthparts, which they use to scrape detritus off the bottom of the temporary ponds (Pomeroy, 1981) . The behavior of these two ecomorphs in the pond is distinct as well. Carnivores are solitary and active on the pond surface, while omnivores tend to aggregate and dwell at the bottom of the pond (Pfennig & Murphy, 2000; Pfennig, Chunco & Lackey, 2007). After maturing to the adult form, these distinct developmental phenotypes disappear, and the toads converge in diet and morphology.

Sample collections

In the summer of 2016, I collected *Spea multiplicata* near Portal, Arizona. Tadpoles remained in their native ponds until close to metamorphosis so they could experience their native tadpole environment throughout larval development and develop as carnivore or omnivore ecomorphs naturally. When they approached later developmental stages, carnivores and omnivores were collected and moved to 5-foot diameter wading pools at the Southwestern Research Station (SWRS) in Portal, Arizona where they remained for three days before being shipped to the University of North Carolina at Chapel Hill. It was necessary to collect them as tadpoles since once they metamorphose toads converge on phenotype and diet and are visually indistinguishable from one another. Tadpoles in wading pools were separated according to ecomorph type, carnivores were fed fairy shrimp exclusively and omnivores were fed detritus.

Upon arrival to the lab, larval tadpoles were housed in plastic aquaria in groups of twelve and fed detritus and brine shrimp ad libitum. Individuals from each population and ecomorph were maintained separately from one another and fed detritus (ground up fish food) and brine shrimp.

Assay of foraging behavior and foraging success

I used wax worms for food since they represent a novel food source. *Spea multiplicata* in the wild feed on a wide breadth of prey items, from ants to grubs (Castaneda-Gaytan, Garcia-De La Pena, Lazcano & Contreras-Balderas, 2006), hence wax worms were deemed an appropriate novel food item. I conducted foraging trials on known carnivore and omnivore juvenile toads where I tested: (1) the duration of the foraging trial, (2) the amount of time it took for a test subject to strike at the first novel food item, (3) the total number of wax worms consumed, (4) the average number of strikes or attempts made to capture wax worms throughout the entirety of the trial; and (5) the failure rate within each group. Before beginning each trial, I measured snout-vent length using Mitutoyo digital calipers and mass for each toad. Next, I placed each toad in an arena with opaque walls and flooring, and allowed it to acclimate for 5 minutes. Toads were allowed to move freely during the acclimation period. After acclimating, wax worms were introduced to the distal side of the arena opposite to the toad. Toads were allowed to hunt freely with no more than three wax worms present at a time. I introduced three wax worms at a time to account for activity differences between worms. Each time a worm was consumed, it was immediately replaced until a toad went longer than three minutes without attempting to capture a worm. At this time, the trial ended and we counted the number of worms consumed and the number of attempts made to capture a worm (successful or not) throughout the trial. Toads who did not attempt to consume any worms during the entirety of the allotted three minutes were deemed a fail.

Assay of activity and exploratory behavior

A subset of juvenile toads of each ecomorph type (omnivores N = 63, carnivores N = 77) was evaluated for activity levels (scored as total proportion of time spent in motion) and exploration of an unfamiliar environment (scored as rate of movement between zones, i.e. the number of zone boundaries crossed during the trial period). I measured activity and exploratory behavior by conducting “open field trials” in which I placed toads individually in a novel environment and tracked their movement. Specifically, I placed each toad in an arena measuring 13 by 8 inches with opaque walls and flooring. The arena was filled with 6mm of water. Each toad was initially placed underneath a cup within the arena and allowed to acclimate. After 5 minutes, a hidden observer released the toad from the cup and the toad was allowed to move freely through the arena.

Once an animal began to move about the arena, I started an automated motion tracking system, the “Tracker” program (Donelson, Kim, Slawson, Vecsey, Huber & Griffith, 2012) to track the movement of the animal throughout the arena for a 10 minute observation period. This program tracks flies in real time and records small movements at any location in the arena. Toad trials were recorded using Logitech C260 webcam that was mounted above the test arena. The digital recordings of each trial were used to quantify toad activity and exploration levels. For each trial, the measures of exploratory behavior were obtained from the motion tracking data to generate measures of exploration and activity behavior in the arena. To do so, I divided the arena into equal-sized rectangular zones and calculated the rate of movement among these zones as the number of zone boundaries crossed per unit time. At the end of the observation period, I removed the toad from the arena and recorded its mass and snout-vent length (SVL; a measure of body size).

Each toad was tested at three times and measurements were averaged across trials to account for any temporal differences in internal state. These three measurements were used to estimate the repeatability of activity and exploration behaviors. The second trial was conducted 24 hours after the first trial, and the third was conducted 72 hours after the first trial. I estimated repeatability of behavior using the R package ICCest and found that exploratory behavior was significantly repeatable within individuals among trials. Therefore, I calculated the average (average of all three trials) and maximum exploratory (max of all three trials) score from the three trials for each toad and used these measures in all subsequent analyses. Results did not differ between average or maximum scores, I report results for the average of the exploratory scores only.

Statistical analysis

Analyses were conducted using R version 3.5.3. All foraging trial analyses included size and condition variables. To perform our analysis, we constructed linear models for the duration of the trial, and time to first worm. For the number of worms consumed I used a generalize linear model with Poisson distribution. For the number of strikes, I fit a negative binomial distribution with an offset for the number of worms consumed. The exploration and the activity models were fit with a generalized linear model with a Poisson distribution.

Results

Foraging trials

I found carnivores had shorter trials (mean ± se: 587.2 ± 79.2 seconds) than omnivore morphs (705.4 ± 116.4 seconds; Morph: $F_{4,51}$ = 8.076, P = 0.006), after accounting for the number of worms consumed (worms: $F_{4,51}$ = 4.269, P = 0.044). It was necessary to control for the number of worms consumed throughout the trial to account for time spent consuming worms (and therefore not actively looking for food). Body size and condition had no effect on trial duration (SVL: $F_{4,51}$ = 3.073, P = 0.086; condition: $F_{4.51}$ = 0.005, P = 0.943; Table 3.1).

I estimated how long it took for a toad to strike at its first prey item. Toads who as tadpoles were carnivores were significantly faster at approaching their first prey, and this was not explained by size or condition differences between the two morphs (C: 31.7 ± 7.7 seconds; O: 68.1 ± 9.8 seconds; Morph: $F_{3,52}$ = 7.052, P = 0.010; SVL: $F_{3,52}$ = 1.141, P = 0.290; condition: $F_{3,52}$ = 2.064, P = 0.157; Table 3.4). These results show that carnivores will detect and approach potential prey items sooner than omnivores.

Next, I counted the number of worms consumed. I included size and condition in this model to account for larger individuals being able to eat more than smaller individuals. Results show that there was no significant difference between the number of worms the two morphs were capable of eating (carnivores: 5.47 ± 0.52 worms vs. omnivores: 3.81 ± 0.27 worms; Morph: $\chi^2_{3,52}$ = 3.428, P = 0.064). Larger individuals did indeed consume more worms, but condition had no effect (SVL: $\chi^2_{3,52}$ = 16.992, P < 0.0001; condition: $\chi^2_{3,52}$ = 0.517, P = 0.472; Table 3.2).

Finally, I looked at the average tries it took an individual to capture a worm. This gave me a measure of foraging efficiency, indicating that individuals who took fewer attempts at capturing prey expended less energy and reduced their risk to predators when foraging. I again included size and condition in the model to account for differences in success between larger and smaller individuals, since larger individuals may have a larger gape that allows them to more efficiently capture prey. Results indicate that carnivores were more efficient at capturing prey (carnivores: 2.42 ± 0.19 strikes/worm vs. omnivores: 3.59 ± 0.43 strikes/worm; Morph: $\chi^2_{3,52}$ = 12.15, P < 0.001, Figure 3.1B; SVL: $\chi^2_{3,52}$ = 1.411, P = 0.235; condition: $\chi^2_{3,52}$ = 2.861, P = 0.091; Table 3.3). Fail rate was determined by toads who did not feed or strike at worms during the duration of the trial was not significant ($\chi^2_{1,65}$:1.379 p=0.2402).

Behavioral assays

Across trials and tested variables, there was no significant difference between toad size and condition, thus, these variables were excluded from all models. Models only included a predictor variable of ecomorph type and the response variable of interest (i.e., average proportion of area explored, max total area explored, and amount of time spend moving). There was no significant difference in max proportion of the arena explored (i.e., average of the three trials conducted measuring the number of zone boundaries crossed throughout the trial) between morph types ($\chi^2_{1,138} = 0.473$, $P = 0.492$). From these trials I was also able to gather information about activity levels of individuals, here measured as the amount of time spent moving throughout the duration of the trial. This is different from exploration since individuals may move often in a small concentrated area, thus exploring little but remaining very active. There were again no significant differences in activity levels between ecomorph types ($\chi^2_{1,138} = 0.001$, $P = 0.982$).

Discussion

I find that foraging behavior is correlated with larval morphotype. Most studies that explore foraging carry-over effects focus solely on effects across environments or life stage effects (Bouchard, O'Leary, Wargelin, Charbonnier, Warkentin & Moore, 2016; Tarvin, Silva Bermúdez, Briggs & Warkentin, 2015). My study specifically finds that (1) foraging behavior carries over to juvenile state from distinct larval morphotypes, (2) differences in juvenile size and condition sometimes accounts for differences in foraging behavior and (3) exploration and activity levels do not differ between juveniles resulting from different larval morphotypes.

The average number of strikes necessary to capture a single prey item, the time spent capturing worms, and how long it took toads to attempt to capture the first food item are best explained by the larval morphotype of the individual. Despite juveniles converging on diet, I find significant differences between the juvenile toads resulting from distinct larval morphotype as tadpoles. This is suggestive of behavioral carry-over between distinct life-stages with stark differences in resources targeted during development. Larval ecomorphs are characterized by behavioral as well as morphological differences, with carnivorous morphs being more active than their omnivorous counterparts. Broadly, juvenile toads that resulted from carnivorous larval ecomorphs seem to be primed for a continuation of that active foraging strategy, despite a variety of extrinsic changes. Therefore, this behavioral carry-over effect appears to be mediated by differences in foraging strategies used at the larval stage, but it is not clear from my data whether these behaviors arose as a result of larval polyphenisms, or if differences in foraging behaviors evolved first, and were later followed by morphological adaptations (Mayr, 1963; Price, Qvarnstrom & Irwin, 2003). Entry into a new niche involves changes in both behavior and morphology and there are many examples in which animals respond to unexploited environments with immediate behavioral changes (Feinsinger & Swarm, 1982; Morse, 1971), followed later by morphological adaptations. Even though I am unable to distinguish which came first, I find that even after the context in which novel foraging behavior was relevant, differences still remain that could form the basis for life long differences in fitness. These differences in foraging behaviors can have important implications for long-term life history traits. For example, foraging inefficiency can result in low condition, which in turn can affect reproductive output and ability to survive to the next breeding season.

Although I find that the size of individuals can influence the number of prey items consumed, with larger individuals consuming more, size and condition did not significantly explain prey handling traits such as: number of attempts made to capture a prey item, average time spent between capturing worms, and how long it took to attempt to capture the first food item. A possible explanation is that larger individuals may be able consume more prey items is because these individuals have larger jaw gapes, making them capable of consuming a greater range of prey size (Arim et al., 2010; Toscano & Griffen, 2012; Werner & Gilliam, 1984).

Interestingly, individual condition, estimated as mass at a given body size did not have any effect on any measured aspects of foraging behavior. This is somewhat surprising as other studies have shown that larger and better condition individuals are more likely to engage in foraging behaviors that are more beneficial to survival (Dingeldein & White, 2016).

Juvenile toads who developed as carnivore tadpoles are not more exploratory or active than juveniles who were omnivorous as tadpoles. Activity level, one of the most well-studied personality traits (Bell, Hankison & Laskowski, 2009), has been shown to vary consistently within populations of amphibians (Urszan, Torok, Hettyey, Garamszegi & Herczeg, 2015) and even across ecological contexts (Sih, Bell & Johnson, 2004). Spadefoot toads may not differ in activity levels as tadpoles since rapid sprint speed and rapid growth are likely to be the main anti-predator mechanisms available for omnivore and carnivore tadpoles alike (Arendt, 2009). More testing is required to evaluate how activity differs at the tadpole stage before establishing whether these would indeed carry-over across life stages. Though other studies have found that activity level did not correlate with average intake (Tarvin et al., 2015), others have found that activity level increased resource consumption (Toscano & Griffen, 2014).

Here I demonstrate the carry-over effects of adaptive morphotypes across life history stages, even after there is convergence in both morphology and general foraging strategy. I find that larval ecomorphs are predictive of future activity levels and foraging efficiency as juveniles, despite drastic changes in physiology and environment between the two phases. Physiological differences that carry-over from larval to juvenile morphs remain to be explored, but may be a mechanism through which the carry-over behaviors are mediated. Studying metamorphizing organisms provides a unique insight into the importance of early behaviors in determining future evolutionary strategies, highlighting the continued effects of early experience throughout the lifetime of an organism.

REFERENCES

Arendt JD. 2009. Influence of sprint speed and body size on predator avoidance in New Mexican spadefoot toads (Spea multiplicata). *Oecologia* **159:** 455-461.

Arim Ma, Abades SnR, Laufer G, Loureiro M, Marquet PA. 2010. Food web structure and body size: trophic position and resource acquisition. *Oikos* **119:** 147-153.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. *Anim Behav* **77:** 771-783.

Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. 2003. The ecology of individuals: incidence and implications of individual specialization. *Am Nat* **161:** 1-28.

Booth DJ, Beretta GA. 2004. Influence of recruit condition on food competition and predation risk in a coral reef fish. *Oecologia* **140:** 289-294.

Bouchard SS, O'Leary CJ, Wargelin LJ, Charbonnier JF, Warkentin KM, Moore I. 2016. Post-metamorphic carry-over effects of larval digestive plasticity. *Functional Ecology* **30:** 379-388.

Brodin T. 2009. Behavioral syndrome over the boundaries of life—carryovers from larvae to adult damselfly. *Behavioral Ecology* **20:** 30-37.

Burton T, Metcalfe NB. 2014. Can environmental conditions experienced in early life influence future generations? *Proc Biol Sci* **281:** 20140311.

Castaneda-Gaytan G, Garcia-De La Pena C, Lazcano D, Contreras-Balderas A. 2006. Dietary composition of the Mexican spadefoot toad (Spea multiplicata) from a sand dune habitat in southwestern Coahuila, Mexico. *Texas Journal of Science* **58:** 55-64.

Dingeldein AL, White JW. 2016. Larval traits carry over to affect post-settlement behaviour in a common coral reef fish. *J Anim Ecol* **85:** 903-914.

Donelson N, Kim EZ, Slawson JB, Vecsey CG, Huber R, Griffith LC. 2012. High-resolution positional tracking for long-term analysis of *Drosophila* sleep and locomotion using the "Tracker" program. *PLoS One* **7:** e37250.

Exnerova A, Svadova KH, Fucikova E, Drent P, Stys P. 2010. Personality matters: individual variation in reactions of naive bird predators to aposematic prey. *Proc Biol Sci* **277:** 723-728.

Feinsinger P, Swarm LA. 1982. "Ecological Release," Seasonal Variation in Food Supply, and the Hummingbird Amazilia Tobaci on Trinidad and Tobago. *Ecology* **63:** 1574-1587.

Firth JA, Sheldon BC. 2016. Social carry-over effects underpin trans-seasonally linked structure in a wild bird population. *Ecol Lett* **19:** 1324-1332.

Groothuis TG, Trillmich F. 2011. Unfolding personalities: the importance of studying ontogeny. *Dev Psychobiol* **53:** 641-655.

Herborn KA, Macleod R, Miles WTS, Schofield ANB, Alexander L, Arnold KE. 2010. Personality in captivity reflects personality in the wild. *Animal Behaviour* **79:** 835-843.

Kristensen NP, Johansson J, Chisholm RA, Smith HG, Kokko H. 2018. Carryover effects from natal habitat type upon competitive ability lead to trait divergence or source-sink dynamics. *Ecol Lett*.

Levri EP, Lively CM. 1996. The effects of size, reproductive condition, and parasitism on foraging behaviour in a freshwater snail, Potamopyrgus antipodarum. *Animal Behaviour* **51:** 891-901.

Macarthur RH, Pianka ER. 1966. On Optimal Use of a Patchy Environment. *American Naturalist* **100:** 603-+.

Mayr E. 1963. Animal Species and Evolution Cambridge, MA: Harvard University Press.

McGhee KE, Pintor LM, Bell AM. 2013. Reciprocal behavioral plasticity and behavioral types during predator-prey interactions. *Am Nat* **182:** 704-717.

Mittlebach GG. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by Bluegills. *Ecology* **62:** 1370-1386.

Morse DH. 1971. The Foraging of Warblers Isolated on Small Island. *Ecology* **52:** 216-228.

O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5:** 1-11.

Pechenik JA, Wendt DE, Jarrett JN. 1998. Metamorphosis Is Not a New Beginning. *Bioscience* **48:** 901-910.

Pfennig DW, Murphy PJ. 2000. Character displacement in polyphenic tadpoles. *Evolution* **54:** 1738-1749.

Pfennig KS, Chunco AJ, Lackey ACR. 2007. Ecological selection and hybrid fitness: hybrids succeed on parental resources. *Evolutionary Ecology Research* **9:** 341-354.

Pomeroy LV. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Ph.D. diss., University of California.

Price TD, Qvarnstrom A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proc Biol Sci* **270:** 1433-1440.

Pruitt JN, Stachowicz JJ, Sih A. 2012. Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. *Am Nat* **179:** 217-227.

Reale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. *Biol Rev Camb Philos Soc* **82:** 291-318.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* **19:** 372-378.

Sweeney K, Cusack B, Armagost F, O'Brien T, Keiser CN, Pruitt JN. 2013. Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. *Behav Ecol* **24:** 1205-1210.

Tarvin RD, Silva Bermúdez C, Briggs VS, Warkentin KM. 2015. Carry-over Effects of Size at Metamorphosis in Red-eyed Treefrogs: Higher Survival but Slower Growth of Larger Metamorphs. *Biotropica* **47:** 218-226.

Toscano BJ, Gownaris NJ, Heerhartz SM, Monaco CJ. 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. *Oecologia* **182:** 55-69.

Toscano BJ, Griffen BD. 2012. Predatory crab size diversity and bivalve consumption in oyster reefs. *Marine Ecology Progress Series* **445:** 65-74.

Toscano BJ, Griffen BD. 2014. Trait-mediated functional responses: predator behavioural type mediates prey consumption. *J Anim Ecol* **83:** 1469-1477.

Urszan TJ, Torok J, Hettyey A, Garamszegi LZ, Herczeg G. 2015. Behavioural consistency and life history of Rana dalmatina tadpoles. *Oecologia* **178:** 129-140.

Werner EE, Anholt BR. 1993. Ecological Consequences of the Trade-Off between Growth and Mortality-Rates Mediated by Foraging Activity. *American Naturalist* **142:** 242-272.

Werner EE, Gilliam JF. 1984. The Ontogenic Niche and Species Interactions in Size-Structured Populations. *Annu. Rev. Evol. Syst.* **15:** 393-425.

Wilbur HM. 1980. Complex Life-Cycles. *Annual Review of Ecology and Systematics* **11:** 67-93.

Wilbur HM. 1997. Experimental ecology of food webs: Complex systems in temporary ponds - The Robert H. MacArthur Award Lecture - Presented 31 July 1995 Snowbird, Utah. *Ecology* **78:** 2279-2302.

Wilson AD, Krause J. 2012a. Metamorphosis and animal personality: a neglected opportunity. *Trends Ecol Evol* **27:** 529-531.

Wilson ADM, Krause J. 2012b. Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? *Behavioral Ecology* **23:** 1316-1323.

Factor	F-value	P-value
Morph	8.076	**0.006**
Worms	4.269	**0.044**
SVL	3.073	0.086
Condition	0.005	0.943

Table 3.1. Results from trial duration linear model. Bold terms indicate significance.

Factor	χ^2	P-value
Morph	3.428	0.064
SVL	16.992	**<0.001**
Condition	0.517	0.472

Table 3.2. Results from generalized linear model for the number of worms consumed. Bold term indicates significance.

Factor	χ^2	P-value
Morph	12.153	<0.001
SVL	1.465	0.226
Condition	2.855	0.091

Table 3.3. Results from generalized linear model for the number of strikes to consume a worm. Bold terms indicate significance.

Factor	F-value	P-value
Morph	7.052	0.01
SVL	1.141	0.29
Condition	2.064	0.157

Table 3.4. Results from linear model for time to first worm. Bold terms indicate significance.

CHAPTER IV

PHYSIOLOGICAL BASES OF CARRY-OVER EFFECTS ASSOCIATED WITH ALTERNATIVE RESOURCE-USE MORPHS

Introduction

Resource polymorphisms—the occurrence of alternative morphs that utilize different resources (‘ecomorphs’) within a population—are among the most striking examples of ecologically relevant phenotypic diversity within species (Liem & Kaufman, 1984; Skúlason, Snorrason & Jónsson, 1999; Smith & Skúlason, 1996; West-Eberhard, 1989; Wimberger, 1994). Among resource-polyphenic organisms, ecomorphs can differ in life-history traits due to unique differences in morphology, behavior, and/or physiology during ontogeny (Burton & Metcalfe, 2014; Crespi & Warne, 2013; Harrison, Blount, Inger, Norris & Bearhop, 2011; Jablonka, Oborny, Molnar, Kisdi, Hofbauer & Czaran, 1995; Tarvin, Silva Bermúdez, Briggs & Warkentin, 2015). Physiological adaptations associated with the utilization of different resources can likewise lead to differences in the ability to metabolize and accrue resources. Moreover, internal energetic resources can impact condition early in development, thus affecting fitness and performance at the adult stage (e.g., see Warne & Crespi, 2015); i.e., they might have “carry-over effects” (sensu O'Connor, Norris, Crossin & Cooke, 2014).

Despite the potential importance that physiological differences between ecomorphs might play in mediating carryover effects, few studies have examined whether ecomorphs differ physiologically. Nevertheless, differences in diet can lead to differences in physiology which become evident in the internal anatomy of an individual. For example, individuals with a carnivorous diet often have shorter guts compared with those with omnivorous diets (Ledon-Rettig, Pfennig & Nascone-Yoder, 2008; Wagner, McIntyre, Buels, Gilbert & Michel, 2009). Such a difference could have downstream consequences for the fitness of individuals that become especially apparent in organisms with complex life cycles consisting of larval and adult stages living in two distinct habitats (Relyea & Auld, 2004). If larvae express alternative ecomorphs with different diets and physiologies, the consequence of these differences could carry over into the adult stage, with possible effects on adult fitness.

The purpose of my study was to investigate the relationship between the larval morphotype and post-metamorphic internal anatomy in the Mexican Spadefoot toad, *Spea multiplicata*, whose tadpoles exhibit a striking resource polymorphism (Bragg, 1965; Martin & Pfennig, 2010; Paull, Martin & Pfennig, 2012; Pfennig, 1990; Pomeroy, 1981). Depending on their diet, *S. multiplicata* tadpoles develop into either an ‘omnivore’ ecomorph, which eats plants, organic detritus, and small invertebrates, and as a result has a long gut, or a ‘carnivore’ ecomorph, specializes on fairy shrimp and other tadpoles (Pomeroy, 1981) and which expresses a suite of unique, complex morphological traits, among which is a short gut (Pfennig, 1992). Omnivores are the default morph; carnivores are induced when a young omnivore eats shrimp or other tadpoles (Levis, de la Serna Buzon & Pfennig, 2015; Pfennig, 1990; Pomeroy, 1981). Given the drastic anatomical differences among the two morphotypes, I set out to evaluate whether liver and fat body size differs between them and whether these effects carry-over to post-metamorphosis. Both livers and fat bodies are important for energetic resource storage in amphibians (e.g. lipids and triacylglycerol in livers: Sheridan & Kao, 1998; fat bodies: Wright, Richardson & Bigos, 2011), and measuring their size provides insight into fitness (Chen, Zhang & Lu, 2011).

To determine how alternative carnivore and omnivore ecomorphs differ in organs important for the storage and regulation of energetic resources, I compared size of: 1) abdominal fat bodies, which are the principle storage site of long-term lipid reserves (Fitzpatrick, 1976); and 2) liver, which is the storage site for readily-available (short-term) lipids and carbohydrates (glycogen; Duellman & Trueb, 1986). For the latter, I specifically calculated *hepatosomatic index* (*HSI*), which corrects for differences among individuals in body size; HSI is a widely used proxy for the amount of lipid (and glycogen) stored in the liver of vertebrates (Ando, Mori, Nakamura & Sugawara, 1993; Chellappa, Huntingford, Strang & Thomson, 1995).

Any morph-specific differences in energetic reserves have the potential to impact *adult* survival and possibly even fecundity and mating success. Abdominal fat bodies (AFB) are fingerlike projections found in the dorsum of amphibians, anterior to the gonads and consist of typical adipose tissue (Fitzpatrick, 1976). They are largest just before hibernation and smallest after breeding (Duellman & Trueb, 1986). This phenomenon has been linked primarily with reproduction (gamete and yolk production, and breeding and brooding activities) (Fitzpatrick, 1976). For example, because the abdominal fat bodies and the gonads of frogs develop from the same tissue (the germinal ridge; Noble, 1931), females with smaller abdominal fat bodies might have smaller ovaries and might therefore have reduced fecundity (indeed, fat bodies tend to be smallest in females that have just finished spawning, and experimental removal of fat bodies results in smaller eggs, indicating that these reserves are essential for egg production; Wells, 2007, p. 226). Similarly, males with smaller lipid reserves might also have reduced fecundity: experimental removal of fat bodies in male frogs results in regression of the testis (Kasinathan, Gunasing & Basu, 1978). Moreover, males with smaller lipid reserves generally are less capable than those with larger reserves to produce the sorts of costly male traits (e.g., more extravagant/vigorous displays) that females prefer in mates (Andersson, 1994) and might therefore have reduced mating success. In toads, calling to attract mates is the most energetically expensive activity performed by male anurans during their lifetimes (Wells, 2007, p. 202). Energetic constraints, such as those wrought by failing to adequately store energetic reserves, can severely limit the ability of individual males to sustain call production for a long period of time, which can, in turn, directly affect a male's mating success (Halliday, 1987; Ryan, 1988). In short, physiological differences early in life could have carry-over effects and thereby form the basis for trade-offs later in life.

Although physiological carry-over effects have been documented in amphibians (Alvarez & Nicieza, 2002; Bouchard, O'Leary, Wargelin, Charbonnier, Warkentin & Moore, 2016; Scott, Casey, Donovan & Lynch, 2007), such effects have not been previously linked to resource polyphenisms. Here, I examined how carnivore and omnivore juvenile ecomorphs differ in liver and fat body sizes, and, if any differences are still present in sexually mature adult populations. I found that ecological specialization leads to differences in organs important for energetic resources, and that such differences have carry-over effects that persist to reproductive maturity. My results suggest that physiological adaptations could constitute the basis for life history differences associated with the utilization of alternative resources.

Materials and methods

I reared known carnivores and omnivores collected at tadpoles (see Chapter II for details) and reared them past metamorphosis. We measured each juvenile's, snout-vent length (SVL), mass, abdominal fat body mass, and liver mass. To measure body, fat body, and liver mass, tissues were blotted and weighed to 0.001 g. I used juvenile toads who died in the lab nine weeks post-metamorphosis and preserved in 95% ethanol. This way I avoided measuring toads during the mass-loss period (Tarvin et al., 2015).

To establish whether the patterns seen in juvenile individuals is maintained to adulthood, I sampled *S. multiplicata* adults from four populations in southeastern Arizona and southwestern New Mexico, USA. These populations consistently differed for at least the past 30 years (i.e., beyond the maximum lifespan of *Spea*) in species composition: some populations contained only *S. multiplicata* (allopatry) and some also contained *S. bombifrons,* a congener of *S. multiplicata* (sympatry). Previous studies had shown that whereas allopatric *S. multiplicata* produce intermediate frequencies of both ecomorphs, sympatric *S. multiplicata* produce mostly omnivores (> 95% of the time) and sympatric *S. bombifrons* produce mostly omnivores (> 95% of the time; this divergence between sympatric species in ecomorph production lessens interspecific resource competition; Pfennig & Murphy, 2000; Pfennig & Murphy, 2002; Pfennig & Murphy, 2003). Because allopatric *S. multiplicata* produce both morphs, these individuals were therefore likely *a mix* of former omnivores and carnivores as tadpoles. Because the sympatric *S. multiplicata* produce mostly omnivores, these individuals were therefore likely *omnivores* as tadpoles. The wild caught adults used died shortly after collection (these individuals died from a disease outbreak that decimated most of the adults collected). Using the same methods as for the tadpoles, I measured mass, and HSI.

Results

In juvenile toads, carnivore and omnivore differed in HSI (carnivore HSI mean ± se: 0.005 ± 0.0005, omnivore HSI mean ± se: 0.004 ± 0.0006; Wilcoxon rank sum test: *P* = 0.022), but not fat bodies (*P* = 0.079; carnivore fat body mean ± se: 0.029 ± 0.0070, omnivore fat body mean ± se: 0.020 ± 0.0053). Individuals who had larger HSI also had larger fat body mass ($F_{1,37}$ = 9.50, P = 0.004).

I also found that HSI differed among population types of *adults* in the manner predicted by these juvenile and tadpole results. Overall, I found significant differences among the two groups of adults in HSI (ANOVA: $F_{1,100}$ = 26.92, $P < 0.001$; allopatric HSI mean ± se: 0.005 ± 0.0003, sympatric HSI mean ± se: 0.003 ± 0.0003; Fig. 4.1). I found that, as predicted, HSI was significantly greater among allopatric *S. multiplicata* (which were likely a mix of carnivores and omnivores as tadpoles) than among sympatric *S. multiplicata* (which were likely omnivores as tadpoles). The two populations types did not differ in fat body size ($F_{1,62}$ = 1.28, P = 0.262).

Discussion

Resource polymorphisms are common across a wide range of animal taxa (Bernays, 1986; Denoël, Whiteman & Wissinger, 2006; Smith & Temple, 1982; Trapani, 2003). More work is needed, however, to understand their physiological basis (Denoël, Joly & Whiteman, 2005). This study sought to determine if resource polyphenisms have an underlying physiological basis, and if any such differences have carry-over effects. Using polymorphic spadefoot toads, *S. multiplicata* as our model system, I found these morph-specific differences in liver size, but not in fat body size were present in juvenile and adult toads.

The larger livers, but not fat bodies of carnivores versus omnivores presumably reflects the former morphs high-nutrient diet. Though fat bodies are the most lipid-rich structure in anurans (Brown, 1964), larger livers are also associated with large energetic reserves in many taxa (Chellappa et al., 1995; Loumbourdis & Kyriakopoulou-Sklavounou, 1991; Penney & Moffitt, 2015; Price, 2017). In many species of fish, liver size and liver lipid content is dependent on trophic diet (Sardenne, Bodin, Chassot, Amiel, Fouché, Degroote, Hollanda, Pethybridge, Lebreton, Guillou & Ménard, 2016), and can be directly related to foraging strategies (Pethybridge, Parrish, Bruce, Young & Nichols, 2014), as well as body and energetic condition (Leonarduzzi, Rodrigues & Macchi, 2014). In the anuran literature, liver size is considered a robust predictor of population dynamics, such as reproductive potential (Loumbourdis & Kyriakopoulou-Sklavounou, 1991), and ability to endure hibernation periods (Chen et al., 2011; Muir, Costanzo & Lee, 2010; Storey & Storey, 1990). To my knowledge, this is the first study to examine differences in liver and fat bodies sizes of carnivore and omnivore resource polyphenic individuals within a single species. Future research should seek to examine if liver size is indicative of actual differences lipid content.

Further, I examined recently metamorphosed juveniles and saw toads, who as tadpoles were carnivores continue to have significantly larger livers, but not fat bodies, compared with toads who once were omnivores. This indicates that differences in the liver initiated at the tadpole stage carry-over through metamorphosis. In juveniles, these differences could have implications for some important aspects of behavior. Bouchard et al. (2016) for example, found that carried over differences of larval liver sizes produced metamorphs that varied widely in body condition, and this variation correlated with feeding behavior after emergence from mesocosms. Low lipid reserves, as indicated by liver and fat body size, were associated with much higher feeding rates during the critical mass loss period (Bouchard et al., 2016; Tarvin et al., 2015). This could indeed be the basis of the behavioral effects carried over that were described in Chapter III.

Next, I sought to examine whether it is possible that these patterns continue once individuals are sexually mature. I did this by examining adults from natural populations. I took advantage of the character displacement taking part in regions of sympatry where *S. multiplicata* co-occurs with congener *S. bombifrons*, since once individuals reach adulthood, it is no longer possible to distinguish which resource polymorphism an individual expressed. In sympatry, *S. multiplicata* primarily produces omnivores while *S. bombifrons* produces carnivores (*S. multiplicata* in allopatry produces both morphs) (Pfennig & Murphy, 2000; Pfennig & Murphy, 2002). I predicted, if the HSI differences exhibited between tadpole morphs are carried-over into adulthood (as seen in the juvenile phase), then the HSI of the sympatric population is expected to be smaller than allopatric populations. I see the expected patterns in liver size hold between sympatric and allopatric *S. multiplicata*, but not in fat body size. These results suggest there are long lasting physiological carry-over effects to larval polymorphisms.

Differences in liver sizes may have important implications at the time of reproduction. For example, liver contents in males increase more with increasing liver mass than in females (Duffitt & Finkler, 2011). Larger males in turn, may be capable of greater levels of activity during the breeding season than smaller males (Finkler, Hayes & Rifai, 2014; Howard, 1988; Sullivan, 1992). In addition to elaborating lipids and storing glycogen, the liver is also responsible for secreting hormones, synthesizing urea, producing bile, among other things. (Duellman & Trueb, 1986). Thus, carried-over differences in liver size may lead to important physiological differences between the two morphs. Sufficient lipid reserves is crucial for long dormancy periods (van Beurden, 1980), but, arguably equally important is the ability to repress metabolic function (Burton, Killen, Armstrong & Metcalfe, 2011).

In summary, these results provide support that resource polyphenisms affect liver and fat body size and further, differences in liver size patterns persist to adulthood. Although the effects of larval environment on post-metamorphic lipid reserves have been previously reported

(Bouchard et al., 2016; Scott et al., 2007), mine is the first study to such polymorphic carry-over effects in a polymorphic species.

REFERENCES

Alvarez D, Nicieza AG. 2002. Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. *Oecologia* **131:** 186-195.

Andersson M. 1994. *Sexual selection*. Princeton University Press: Princeton, NJ.

Ando S, Mori Y, Nakamura K, Sugawara A. 1993. Characteristics of Lipid-Accumulation Types in 5 Species of Fish. *Nippon Suisan Gakkaishi* **59:** 1559-1564.

Bernays EA. 1986. Diet-induced head allometry among foliage-chewing insects and its importance for graminivores. *Science* **231:** 495-497.

Bouchard SS, O'Leary CJ, Wargelin LJ, Charbonnier JF, Warkentin KM, Moore I. 2016. Post-metamorphic carry-over effects of larval digestive plasticity. *Functional Ecology* **30:** 379-388.

Bragg AN. 1965. *Gnomes of the night: the spadefoot toads*. University of Pennsylvania Press: Philadelphia, PA.

Brown GW. 1964. The metabolism of Amphibia. In: Moore JA, ed. *Physiology of Amphibia*. New York: Academic Press.

Burton T, Killen SS, Armstrong JD, Metcalfe NB. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings: Biological Sciences* **278:** 3465-3473.

Burton T, Metcalfe NB. 2014. Can environmental conditions experienced in early life influence future generations? *Proc Biol Sci* **281:** 20140311.

Chellappa S, Huntingford FA, Strang RHC, Thomson RY. 1995. Condition Factor and Hepatosomatic Index as Estimates of Energy Status in Male 3-Spined Stickleback. *Journal of Fish Biology* **47:** 775-787.

Chen W, Zhang LX, Lu X. 2011. Higher pre-hibernation energy storage in anurans from cold environments: a case study on a temperate frog Rana chensinensis along a broad latitudinal and altitudinal gradients. *Annales Zoologici Fennici* **48:** 214-220.

Crespi EJ, Warne RW. 2013. Environmental conditions experienced during the tadpole stage alter post-metamorphic glucocorticoid response to stress in an amphibian. *Integr Comp Biol* **53:** 989-1001.

Denoël M, Joly P, Whiteman HH. 2005. Evolutionary ecology of facultative paedomorphosis in newts and salamanders. *Biological Reviews* **80:** 663-671.

Denoël M, Whiteman HH, Wissinger SA. 2006. Temporal shift of diet in alternative cannibalistic morphs of the tiger salamander. *Biological Journal of the Linnean Society* **89:** 373-382.

Duellman WE, Trueb L. 1986. *Biology of amphibians*. MacGraw Hill: New York.

Duffitt AD, Finkler MS. 2011. Sex-Related Differences in Somatic Stored Energy Reserves of Pseudacris crucifer and Pseudacris triseriata during the Early Breeding Season. *Journal of Herpetology* **45:** 224-229.

Finkler MS, Hayes CJ, Rifai L. 2014. Sexual Dimorphisms in Metabolism, Organ Mass, and Reproductive Energetics in Pre-breeding American Toads (Anaxyrus americanus). *Copeia* **2014:** 447-453.

Fitzpatrick LC. 1976. Life-History Patterns of Storage and Utilization of Lipids for Energy in Amphibians. *American Zoologist* **16:** 725-732.

Halliday TR. 1987. Physiological constraints on sexual selection. In: Bradbury JW and Andersson MB, eds. *Sexual selection: testing the alternatives*. New York: Wiley. 247–264.

Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S. 2011. Carry-over effects as drivers of fitness differences in animals. *J Anim Ecol* **80:** 4-18.

Howard RD. 1988. Sexual Selection on Male Body Size and Mating-Behavior in American Toads, Bufo-Americanus. *Animal Behaviour* **36:** 1796-1808.

Jablonka E, Oborny B, Molnar I, Kisdi E, Hofbauer J, Czaran T. 1995. The adaptive advantage of phenotypic memory in changing environments. *Philos Trans R Soc Lond B Biol Sci* **350:** 133-141.

Kasinathan S, Gunasing A, Basu SL. 1978. Fat bodies and spermatogenesis in south Indian green frog *Rana hexadactyla*. *Bollettino di Zoologia* **45:** 15–22.

Ledon-Rettig CC, Pfennig DW, Nascone-Yoder N. 2008. Ancestral variation and the potential for genetic accommodation in larval amphibians: implications for the evolution of novel feeding strategies. *Evol Dev* **10:** 316-325.

Leonarduzzi E, Rodrigues KA, Macchi GJ. 2014. Proximate composition and energy density in relation to Argentine hake females (Merluccius hubbsi) morphometrics and condition indices. *Fisheries Research* **160:** 33-40.

Levis NA, de la Serna Buzon S, Pfennig DW. 2015. An inducible offense: carnivore morph tadpoles induced by tadpole carnivory. *Ecology and Evolution* **5:** 1405-1411.

Liem KF, Kaufman LS. 1984. Intraspecific macroevolution: functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. In: Echelle AA and Kornfield I, eds. *Evolution of fish species flocks*. Orono, ME: University of Maine Press. 203-215.

Loumbourdis NS, Kyriakopoulou-Sklavounou P. 1991. Reproductive and lipid cycles in the male frog Rana ridibunda in northern Greece. *Comp Biochem Physiol A Comp Physiol* **99:** 577-583.

Martin RA, Pfennig DW. 2010. Field and experimental evidence that competition and ecological opportunity promote resource polymorphism. *Biological Journal of the Linnean Society* **100:** 73-88.

Muir TJ, Costanzo JP, Lee RE, Jr. 2010. Evidence for urea-induced hypometabolism in isolated organs of dormant ectotherms. *J Exp Zool A Ecol Genet Physiol* **313:** 28-34.

Noble GK. 1931. *The biology of the amphibia* McGraw Hill Book Co.: New York.

O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* **5:** 1-11.

Paull JS, Martin RA, Pfennig DW. 2012. Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biological Journal of the Linnean Society* **107:** 845-853.

Penney ZL, Moffitt CM. 2015. Fatty-acid profiles of white muscle and liver in stream-maturing steelhead trout Oncorhynchus mykiss from early migration to kelt emigration. *J Fish Biol* **86:** 105-120.

Pethybridge HR, Parrish CC, Bruce BD, Young JW, Nichols PD. 2014. Lipid, fatty acid and energy density profiles of white sharks: insights into the feeding ecology and ecophysiology of a complex top predator. *PLoS One* **9:** e97877.

Pfennig D. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85:** 101-107.

Pfennig DW. 1992. Proximate and functional causes of polyphenism in an anuran tadpole. *Functional Ecology* **6:** 167-174.

Pfennig DW, Murphy PJ. 2000. Character displacement in polyphenic tadpoles. *Evolution* **54:** 1738-1749.

Pfennig DW, Murphy PJ. 2002. How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution* **56:** 1217-1228.

Pfennig DW, Murphy PJ. 2003. A test of alternative hypotheses for character divergence between coexisting species. *Ecology* **84:** 1288-1297.

Pomeroy LV. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Ph.D. diss., University of California.

Price ER. 2017. The physiology of lipid storage and use in reptiles. *Biol Rev Camb Philos Soc* **92:** 1406-1426.

Relyea RA, Auld JR. 2004. Having the guts to compete: how intestinal plasticity explains costs of inducible defences. *Ecology Letters* **7:** 869-875.

Ryan MJ. 1988. Constraints and patterns in the evolution of anuran acoustic communication. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE and Walkowiak W, eds. *The evolution of the amphibian auditory system*. New York: Wiley. 637–677.

Sardenne F, Bodin N, Chassot E, Amiel A, Fouché E, Degroote M, Hollanda S, Pethybridge H, Lebreton B, Guillou G, Ménard F. 2016. Trophic niches of sympatric tropical tuna in the Western Indian Ocean inferred by stable isotopes and neutral fatty acids. *Progress in Oceanography* **146:** 75-88.

Scott DE, Casey ED, Donovan MF, Lynch TK. 2007. Amphibian lipid levels at metamorphosis correlate to post-metamorphic terrestrial survival. *Oecologia* **153:** 521-532.

Sheridan MA, Kao YH. 1998. Regulation of metamorphosis-associated changes in the lipid metabolism of selected vertebrates. *American Zoologist* **38:** 350-368.

Skúlason S, Snorrason SS, Jónsson B. 1999. Sympatric morphs, populations and speciation in freshwater fish with emphasis on arctic charr. In: Magurran AE and May RM, eds. *Evolution of biological diversity*. Oxford, U. K.: Oxford University Press. 70-92.

Smith T, Temple S. 1982. Feeding Habits and Bill Polymorphism in Hook-Billed Kites. *The Auk* **99:** 197-207.

Smith TB, Skúlason S. 1996. Evolutionary Significance of Resource Polymorphisms in Fishes, Amphibians, and Birds. *Annual Review of Ecology and Systematics* **27:** 111-133.

Storey KB, Storey JM. 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Q Rev Biol* **65:** 145-174.

Sullivan BK. 1992. Sexual Selection and Calling Behavior in the American Toad (Buffo americanus). *American Society of Ichthyologists and Herpetologists* **1:** 1-7.

Tarvin RD, Silva Bermúdez C, Briggs VS, Warkentin KM. 2015. Carry-over Effects of Size at Metamorphosis in Red-eyed Treefrogs: Higher Survival but Slower Growth of Larger Metamorphs. *Biotropica* **47:** 218-226.

Trapani J. 2003. Morphological variability in the Cuatro Cienegas cichlid, Cichlasoma minckleyi. *Journal of Fish Biology* **62:** 276-298.

van Beurden EK. 1980. Energy Metabolism of Dormant Australian Water-Holding Frogs (Cyclorana platycephalus). *Copeia* **1980:** 787-799.

Wagner CE, McIntyre PB, Buels KS, Gilbert DM, Michel E. 2009. Diet predicts intestine length in Lake Tanganyika's cichlid fishes. *Functional Ecology* **23:** 1122-1131.

Warne RW, Crespi EJ. 2015. Larval growth rate and sex determine resource allocation and stress responsiveness across life stages in juvenile frogs. *J Exp Zool A Ecol Genet Physiol* **323:** 191-201.

Wells KD. 2007. *The ecology and behavior of amphibians*. University of Chicago Press: Chicago, IL.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20:** 249-278.

Wimberger PH. 1994. Trophic polymorphisms, plasticity, and speciation in vertebrates. In: Stouder DJ, Fresh KL and Feller RJ, eds. *Theory and application in fish feeding ecology*. Columbia, SC: University of South Carolina Press. 19-43.

Wright ML, Richardson SE, Bigos JM. 2011. The fat body of bullfrog (Lithobates catesbeianus) tadpoles during metamorphosis: changes in mass, histology, and melatonin content and effect of food deprivation. *Comp Biochem Physiol A Mol Integr Physiol* **160:** 498-503.

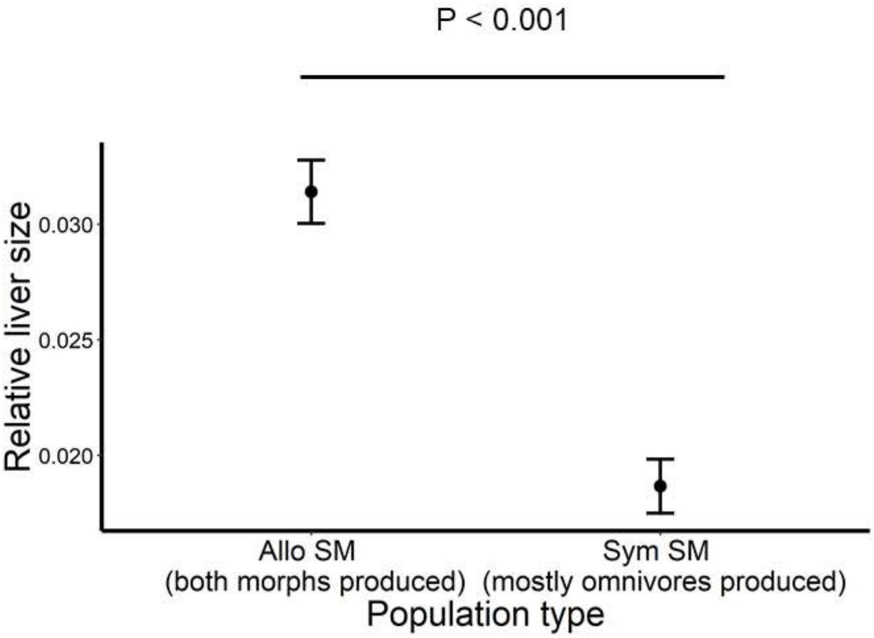

Figure 4.1. Relative liver size as estimated by HSI in wild caught adult populations. Allopatric *S. multiplicata* produce higher numbers of carnivore tadpole morphs than *S. multiplicata* occurring in sympatry with *S. bombifrons.*

CHAPTER V: CONCLUSIONS

In my dissertation, I evaluated whether resource polymorphisms at the tadpole stage have carry-over effects into the following life stages. I focused my studies on the Spadefoot Toad, *Spea multiplicata*, whose tadpoles exhibit striking polymorphisms based on dietary resource early in development (Pfennig, 1990; Pfennig, 1992; Pomeroy, 1981). Interestingly, once these resource polymorphic toads metamorphose, they converge on diet and lose all traits that distinguish identified the two morphs apart. Therefore, in order to successfully study carry-over effects in this organism, longitudinal studies tracking known ecomorphs across life stages are necessary. Previous studies have shown that pond duration and pond dietary contents influence tadpole age and size at metamorphosis (Pfennig, Mabry & Orange, 1991) and smaller individuals are less likely to survive when placed on restricted diets. In Chapter II, I expand on aspects of this work and track known omnivores and carnivores from tadpoles, through post-metamorphosis to sexual maturity. I ask whether recent metamorphs differ in size or timing to metamorphosis and sexual maturity based on morphotype identity during larval development and/or pond condition. The populations sampled differed in hydroperiod. One population experienced a single rain event (i.e. short-lasting) while the other pond experienced a second rain event that caused the re-expansion of the pond (long-lasting). I found that immediately after metamorphosis, toads differed in size and timing. Individuals form the long-lasting pond took longer to reach metamorphosis and produced overall smaller metamorphs. Omnivores of the longer-lasting pond reached marginally larger sizes post-metamorphosis compared to carnivores. In the shorter lasting pond conversely, carnivores reached metamorphosis faster and at larger size. This is consistent with trade-offs associated with diet specialists (Paull, Martin & Pfennig, 2012) as carnivores in the long-lasting pond likely suffered from reduced food sources since pond volume increased during the second rain. I again measured size when individuals reached sexual maturity and this time there were no significant differences in morph type, however, populations continued to differ, with the single-rain pond showing the larger individuals. Individuals who developed as carnivores from short lasting ponds also reached sexual maturity sooner.

I next looked at whether foraging behaviors also carry-over between life stages. Behaviors that have carry-over effects impact the performance of individuals across life stages. In Chapter III I tested how efficient and how many prey items toads were able to consume. Our study specifically finds that toads who belonged to either carnivore or omnivore morphotypes as tadpoles consistently differed in foraging behavior, meaning behavior carries over to juvenile stage. I also find that differences in juvenile size and condition do not account for differences in foraging behavior. Additionally, even though activity levels do not differ between juveniles resulting from larval morphotypes, their exploratory behavior does. These results together indicate that foraging behavior has strong links to larval morphotype.

Finally, in Chapter IV I examine whether there are physiological and anatomical differences between carnivores and omnivores that persist to sexual maturity. I do this by first establishing that carnivore and omnivore tadpoles differ in morphology of their livers and fat bodies. Carnivores had more massive livers and fat bodies. This could potentially result from differences in resource quality during ontogeny. However, differences in the liver but not in the fat bodies set for at the tadpole stage continue to exist past metamorphosis. Once tadpoles metamorphose, individuals converge on diet, thus the persistence of these patterns suggests physiological carry-over effects. In adult wild caught populations, patterns among populations producing primarily omnivores as opposed to both are also consistent with physiological carry over effects. These physiological carry-overs could potentially be the basis for the life-history and behavioral carry-over effects seen in Chapters II and III, but further testing is needed to establish that relationship.

In conclusion, this dissertation aims to demonstrate that environmental and resource polymorphic carry-over effects are acting on spadefoot toads post-metamorphosis and they persist to sexual maturity. These carry-over effects could have important implications for population composition and evolutionary patterns.

REFERENCES

Paull JS, Martin RA, Pfennig DW. 2012. Increased competition as a cost of specialization during the evolution of resource polymorphism. *Biological Journal of the Linnean Society* **107:** 845-853.

Pfennig D. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* **85:** 101-107.

Pfennig DW. 1992. Proximate and Functional Causes of Polyphenism in an Anuran Tadpole. *Functional Ecology* **6:** 167-174.

Pfennig DW, Mabry A, Orange D. 1991. Environmental Causes of Correlations between Age and Size at Metamorphosis in Scaphiopus-Multiplicatus. *Ecology* **72:** 2240-2248.

Pomeroy LV. 1981. Developmental polymorphism in the tadpoles of the spadefoot toad *Scaphiopus multiplicatus*. Ph.D. diss., University of California.