REVIEW ARTICLE

Disease and the Drying Pond: Examining Possible Links among Drought, Immune Function, and Disease Development in Amphibians*

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Accepted 1/26/2019; Electronically Published 4/16/2019

ABSTRACT

Drought can heavily impact aquatic ecosystems. For amphibian species that rely on water availability for larval development, drought can have direct and indirect effects on larval survival and postmetamorphic fitness. Some amphibian species can accelerate the timing of metamorphosis to escape drying habitats through developmental plasticity. However, trade-offs associated with premature metamorphosis, such as reduced body size and altered immune function in the recently metamorphosed individual, may have downstream effects on susceptibility to disease. Here, we review the physiological mechanisms driving patterns in larval amphibian development under low water conditions. Specifically, we discuss drought-induced accelerated metamorphosis and how it may alter immune function, predisposing juvenile amphibians to infectious disease. In addition, we consider how these physiological and immunological adjustments could play out in a lethal disease system, amphibian chytridiomycosis. Last, we propose avenues for future research that adopt an ecoimmunological approach to evaluate the combined threats of drought and disease for amphibian populations.

Keywords: amphibian metamorphosis, *Batrachochytrium dendrobatidis*, chytridiomycosis, drought, immunosuppression, ecoimmunology.

Introduction

Drought is a climate extreme that is expected to increase in frequency in some parts of the world under anthropogenic climate change scenarios (Milly et al. 2005; Seager et al. 2007, 2013). Drought can reduce water runoff, surface-water volumes, and soil moisture, all of which may alter the hydroperiod (i.e., duration of water availability) of aquatic systems (Milly et al. 2005; Seager et al. 2013). In extreme and prolonged drought conditions, wetlands and ephemeral pools may be severely affected or completely lost (Brooks 2004, 2009; Lee et al. 2015), which is problematic for animals, such as amphibians, whose life history and physiology are dependent on water (Corn 2005; Green et al. 2014; Ryan et al. 2014; fig. 1).

Drought can directly and indirectly affect all amphibian life stages due to amphibian physiology and reliance on water bodies for breeding and tadpole development (Duellman and Trueb 1986; Berven 1990; Carey and Alexander 2003). Many amphibian species have a biphasic life cycle and undergo metamorphosis, a transition from an aquatic larval (tadpole) stage to a terrestrial adult stage (fig. 2). For these species, both larval development and the process of metamorphosis require water (Araújo et al. 2006; reviewed in Walls et al. 2013). Severe drought conditions can directly cause mortality in all life stages due to dehydration and desiccation (Pounds and Crump 1994; Kiesecker and Skelly 2001; Carey and Alexander 2003; Daszak et al. 2005; reviewed in Li et al. 2013). As such, it has been proposed that drying conditions may play an important role in worldwide amphibian declines, an idea that has been explored in several studies (reviewed in Li et al. 2013).

Drought can also indirectly affect amphibians at the individual, population, and community levels. Many ecological factors associated with drying habitats indirectly affect amphibian behavior, physiology, and fitness (reviewed in Blaustein et al. 2010; Walls et al. 2013; Edge et al. 2016). At the landscape level, drought

*This paper was submitted in response to a call for papers for a Focused Issue on "Ecoimmunology."
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Physiological and Biochemical Zoology 92(3):339–348. 2019. 1522-2152/2019/9203-8058$15.00. DOI: 10.1086/703137

Figure 1. Couch's spadefoot toad (*Scaphiopus couchii*) at a drying pond in southwest New Mexico. Drought conditions, which lead to drying of ponds, may accelerate amphibian metamorphosis and compromise normal development of the immune system. A color version of this figure is available online.

can alter amphibian ranges and breeding phenology with consequences for reproductive success (reviewed in Blaustein et al. 2010; Li et al. 2013; Walls et al. 2013). Within an individual water body, changes in hydroperiod can lead to changes in water temperature, larval densities, food availability, host-pathogen interactions, and other ecological factors with implications for amphibian survival and reproduction (Kiesecker and Skelly 2001; Morey and Reznick 2001; Altwegg and Reyer 2003; Bustamante et al. 2010; Murphy et al. 2011; reviewed in Blaustein et al. 2010; Walls et al. 2013; Edge et al. 2016). Tadpoles developing under drying conditions, with the associated pressures from a changing pool ecosystem, often metamorphose at smaller body sizes (Wilbur and Collins 1973; Crump 1989; Newman 1989; Denver et al. 1998; Morey and Reznick 2004; Koprivnikar et al. 2014). A smaller body size at metamorphosis may impose long-term costs on an individual's fitness because smaller individuals take longer to reach reproductive maturity and have lower fecundity than individuals that are larger at metamorphosis (Semlitsch et al. 1988; Berven 1990). These examples suggest that indirect effects of drought can create a wide range of challenges for amphibians that are less evident than direct mortality from desiccation.

Although drought poses direct and indirect threats to amphibian survival, many amphibian species exhibit plasticity in their larval development time in response to a drying habitat (Wilbur and Collins 1973; Denver et al. 1998; Laurila and Kujasalo 1999; Loman 1999; Bagwill et al. 2016). While developmental plasticity allows larval amphibians to escape a drying environment and avoid desiccation, there are trade-offs associated with this plasticity, including reductions in body condition, immune function, and postmetamorphic survival (Newman 1992; Denver 1997; Daszak et al. 2005; Gervasi and Foufopoulos 2008; Denver and Middlemis-Maher 2010; Richter-Boix et al. 2011; Burraco et al. 2017). With more frequent and sustained droughts, many amphibian species (even those with high plasticity in the timing of metamorphosis) are likely to experience adverse effects of reduced water availability.

The direct and indirect effects of a decreased hydroperiod may be compounded in the presence of additional stressors,

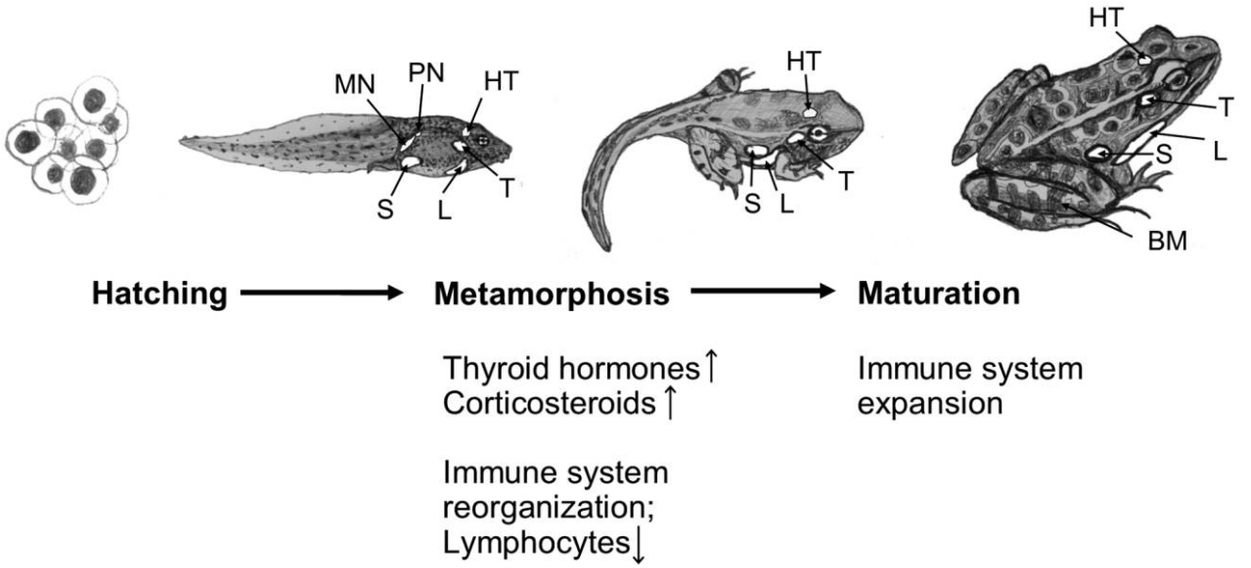

Figure 2. Changes in morphology, hormone production, and immune function across amphibian development. During the tadpole life stage, lymphocytes are produced in the thymus (T), spleen (S), liver (L), pronephros (PN), and mesonephros (MN). During the adult stage, lymphocytes are also produced in bone marrow (BM) and in mature kidneys. Amphibian metamorphosis is governed by increases in thyroid and corticosteroid hormones, induced by a hormonal cascade initiated in the hypothalamus (HT). Rearrangement and development of lymphoid tissues occur during metamorphosis and are accompanied by a brief period of immunosuppression, caused by increases in circulating corticosterone. Metamorphs may be particularly vulnerable to pathogen infection during this period of immunosuppression. Environmental factors that further increase corticosterone levels or induce physiological costs before and during metamorphosis may exacerbate vulnerability to disease.

such as pathogens (Kiesecker and Skelly 2001). For amphibians, one particularly devastating pathogen is *Batrachochytrium dendrobatidis* (*Bd*), which causes the disease chytridiomycosis (Longcore et al. 1999; Skerratt et al. 2007; James et al. 2015). The pathogenesis of chytridiomycosis differs across amphibian life stages (Voyles et al. 2011). Due to a variety of physiological and immunological changes, it is thought that metamorphosis is a time of high vulnerability to chytridiomycosis (Rachowicz and Vredenburg 2004; Briggs et al. 2010; Rollins-Smith 2017).

Many studies have focused on understanding the physiology of amphibian larval development and metamorphosis in response to environmental variation (reviewed in Edge et al. 2016) and the possibility that this process predisposes amphibians to disease (Rollins-Smith 1998; Gervasi and Foufopoulos 2008). In this review, we examine one potential interaction in a lethal disease system that could result in greater amphibian mortality: the effects of reduced hydroperiod and accelerated metamorphosis on susceptibility to *Bd*. We begin by discussing the endocrinology of amphibian metamorphosis and the development of the amphibian immune system. Then we examine the developmental and physiological changes that take place in larvae under low water conditions and how it may alter immune function and predispose amphibians to disease. We present a case study of how this may play out in the disease chytridiomycosis. Because amphibians are experiencing dramatic declines worldwide, we discuss future directions for the study of multiple synergistic threats from an eco-immunological perspective.

Amphibian Metamorphosis and Development of the Immune System

Amphibian metamorphosis—the process of transformation from a tadpole to a juvenile amphibian—involves a series of complex internal and external changes (Lynn 1961; fig. 2). The onset of this life-stage transition is triggered by intrinsic development as well as changes in the external environment (Lynn 1961). Environmental cues, such as changing water levels and temperatures, may accelerate the onset of a hormone cascade that orchestrates dramatic changes in amphibian physiology, morphology, and ecology (e.g., a shift in ecological niche from the aquatic to terrestrial habitat (Lynn 1961; Denver 1998; Rollins-Smith 1998, 2017).

The major hormones involved in metamorphosis are similar to those involved in the stress response and include the thyroid hormones triiodothyronine (T3) and thyroxine (T4) and the corticosteroid hormones corticosterone (CORT) and aldosterone (Denver 2009; reviewed in Kikuyama et al. 1986). The larval hypothalamus produces corticotropin-releasing hormone (CRH), which in turn induces production of pituitary thyroid-stimulating hormone and adrenocorticotropic hormone (Denver 2009). During tadpole development, thyroid hormones are maintained at low levels (Kikuyama et al. 1993). However, on metamorphosis, a rise in the concentrations of thyroid-stimulating hormone and adrenocorticotropic hormone triggers an increase in the circulating concentrations of thyroid and corticosteroid hormones

(Kikuyama et al. 1993; Tata 2006; Chambers et al. 2011; Crespi and Warne 2013; fig. 2). Thyroid and corticosteroid hormones act in concert to induce morphological changes (Kikuyama et al. 1993; Denver 2009), including hind-limb growth and tail absorption (Kikuyama et al. 1993; Brown and Cai 2007). The timing of metamorphosis is dependent on these increases in hormone levels (Bonett et al. 2010; Gomez-Mestre et al. 2013).

During metamorphosis, endocrine changes alter the development of the immune system (Rollins-Smith et al. 1997; Rollins-Smith 1998). Metamorphosis is accompanied by a brief period of immunosuppression when lymphocyte numbers are reduced (Rollins-Smith 1998). Over the course of tadpole development, lymphocyte numbers in the thymus, spleen, liver, pronephros, and mesonephros of tadpoles steadily rise until immediately before metamorphosis, when they decrease markedly (reviewed in Rollins-Smith 1998). At the climax of metamorphosis, circulating CORT reduces net lymphocyte counts as it binds to lymphocytes and induces cell apoptosis (Du Pasquier and Weiss 1973; Rollins-Smith et al. 1988, 1997; Rollins-Smith and Blair 1993; Rollins-Smith 1998; Forson and Storfer 2006; Davis et al. 2008). It is thought that reduced lymphocyte counts in response to increased CORT levels compromise the ability of new metamorphs to generate robust immune responses (Rollins-Smith et al. 1997).

The immune system that exists in a newly metamorphosed frog is very different from that in the premetamorphic animal (Rollins-Smith et al. 1997). The brief period of immunosuppression during metamorphosis is characterized by involution of the thymus and spleen, followed by a rapid expansion of lymphocyte populations after metamorphosis (Rollins-Smith 1998). As a result, the lymphocyte populations at these two life stages have different characteristics. For example, while juvenile amphibians exhibit complete expression of major histocompatibility complex (MHC) I and II antigens (reviewed in Rollins-Smith 1998), tadpoles lack MHC class I expression until just before metamorphosis (reviewed in Rollins-Smith 1998). In juvenile frogs, there is initiation of natural killer (NK) cell-like activity in splenocytes (reviewed in Rollins-Smith 1998) and production of antimicrobial peptides (AMPs) in the skin (Bovbjerg 1963). Last, while some lymphocytes from the tadpole stage may persist throughout the process of metamorphosis, most do not (reviewed in Rollins-Smith 1998). Thus, there are important differences between tadpole and adult amphibian immune repertoires. The intermediate state of the juvenile (i.e., postmetamorphic) immune system may leave a recently metamorphosed amphibian more vulnerable to infectious pathogens (Rollins-Smith et al. 1997; reviewed in Rollins-Smith 1998, 2017).

Accelerated Metamorphosis, Immune Function, and Disease Susceptibility

Environmental stressors (e.g., decreases in water availability) may increase circulating CORT above the already-elevated levels associated with the initiation of amphibian metamorphosis (Denver 1998; Crespi and Warne 2013). This elevation in CORT concentrations above levels expected during this life-stage transition may truncate the developmental period and suggest a mechanism for accelerated metamorphosis (Denver 2009). Tadpoles that develop under stress may exhibit exceedingly elevated CORT levels due to the increased production of ACTH (Denver 1997, 1998, 2009; Rollins-Smith et al. 1997; Gervasi and Foufopoulos 2008; Amburgey et al. 2012; Rollins-Smith 2017). This physiological response may help an amphibian survive by facilitating escape from unfavorable conditions in a drying water body. Tadpoles may directly sense the rate of decrease in water level or may be responding to environmental changes associated with decreasing water volume, such as increased larval densities, a depletion of food resources, and/or an increase in water temperature (Denver 1997; Denver et al. 1998; Loman 1999; Glennemeier and Denver 2002; Altwegg and Reyer 2003; reviewed in Edge et al. 2016). These cues could provide stimuli that initiate the physiological cascade of development and accelerate the process of metamorphosis (Loman 1999). However, accelerated metamorphosis under low water conditions is associated with developmental costs such as reduced body size, lower fat reserves, and altered immune system development, which may have downstream consequences on disease susceptibility (Newman 1989; Gervasi and Foufopoulos 2008; Denver 2009; Kulkarni et al. 2011; Gomez-Mestre et al. 2013).

In amphibians, the activation of the hypothalamic-pituitary-interrenal (HPI) axis by environmental stress can positively and negatively affect immune function (Denver 2009; Warne et al. 2011). Acute stressors can result in a heightened immune response, but chronic stressors are often immunosuppressive (Dhabhar 2009). In addition, environmental stressors that cue accelerated metamorphosis may negatively affect immune function though a trade-off in energy allocation between normal developmental growth and immune system development (Warne et al. 2011; reviewed in Carey et al. 1999).

Environmental stressors can alter both acquired and innate immune function due to elevated CORT (reviewed in Rollins-Smith 1998, 2017). For acquired immunity, elevated CORT can cause significant apoptosis of lymphocytes and granulocytes during metamorphosis (Barker et al. 1997; Rollins-Smith et al. 1997; Rollins-Smith 2017; Belden and Kiesecker 2005; reviewed in Rollins-Smith 1998). For example, while there is normally a ~40% decrease in the number of lymphocytes during metamorphosis under favorable conditions, hormonally accelerated metamorphosis can result in an ~80% loss of lymphocytes (Rollins-Smith et al. 1988; reviewed in Rollins-Smith 1998). Elevated CORT can also increase neutrophil-to-lymphocyte ratios in adult amphibians (Davis and Maerz 2008; Falso et al. 2015), a common stress response observed across vertebrate taxa (reviewed in Davis et al. 2008). Increases in neutrophil-to-lymphocyte ratios in response to stress have been associated with increased susceptibility to disease in birds (Al-Murrani et al. 2002), but it is unclear how this elevated ratio affects disease susceptibility in amphibians (reviewed in Rollins-Smith 2017).

For innate immunity, it is thought that pharmacologically elevated CORT inhibits the production of AMPs in the skin (Rollins-Smith et al. 2011). However, a recent study using

mRNA to investigate AMP precursors suggested that CORT may enhance the production of AMPs (Tatiersky et al. 2015). Thus, it is currently unclear how physiologically relevant levels of stress hormones affect the synthesis of AMPs, and this is an area that needs further research. Nonetheless, the available data suggest that juvenile amphibians may experience changes in acquired and innate immune defenses as a result of accelerated metamorphosis induced by environmental stressors, which may increase susceptibility to infectious pathogens (Rollins-Smith 1998, 2017; Bagwill et al. 2016).

A Case Study: Chytridiomycosis

The disease chytridiomycosis has caused global amphibian population declines (Daszak et al. 1999, 2003). *Bd* has the ability to emerge, spread rapidly, and cause high levels of mortality in phylogenetically distant taxa (Skerratt et al. 2007; reviewed in Kilpatrick et al. 2010). The impacts of *Bd* infection on health and fitness differ between amphibian life stages (reviewed in Kilpatrick et al. 2010). In tadpoles, *Bd* infects the keratinized mouthparts and can reduce body condition, but *Bd* pathogenesis does not appear to lead to direct mortality at the larval stage (Marantelli et al. 2004; Rachowicz and Vredenburg 2004; Garner et al. 2009). In adult amphibians, *Bd* colonizes the skin, resulting in an osmoregulatory imbalance that can lead to death (Voyles et al. 2009). The pathophysiology of chytridiomycosis is assumed to be similar in recent metamorphs, although infection intensities and mortality rates are frequently much higher in juveniles compared to adults (Briggs et al. 2010). Multiple exposure studies have noted rapid mortality when infecting metamorphs with *Bd*, prompting speculation that the immunosuppression associated with metamorphosis renders juveniles more susceptible to chytridiomycosis and death (Rachowicz et al. 2006; Garner et al. 2009).

Environmental factors are known to influence chytridiomycosis infection dynamics in natural populations (Woodhams and Alford 2005; Phillott et al. 2013). Some of these factors, such as temperature and humidity, limit *Bd* growth (Woodhams et al. 2008; Voyles et al. 2017). Based on this information, some investigators have proposed that increases in the frequency and severity of drought conditions might reduce the risk of chytridiomycosis by decreasing the prevalence, severity, and spread of *Bd* (Kriger 2009). In addition, environmental factors also influence the host's susceptibility to infection in adult life stages in ways that are not yet well understood (Richards-Zawacki 2009; Raffel et al. 2013; Rowley and Alford 2013; Cohen et al. 2017). To date, few studies have taken an integrative approach to understanding the impact of environmental stressors and disease across amphibian life stages.

Drying conditions and disease could interact in a variety of different ways, with mechanisms involving ecological and physiological factors. For example, low water availability may increase the densities of hosts and the concentration of *Bd* in water bodies, thereby increasing the exposure to and transmission of *Bd* (Kiesecker and Skelly 2001). In this scenario, developmental plasticity and accelerated metamorphosis would reduce the risk of infection by allowing amphibians to escape *Bd* exposure (Raffel et al. 2010). Alternatively, accelerated metamorphosis and premature development may negatively affect amphibian immune system development and increase susceptibility to chytridiomycosis in postmetamorphic individuals (Gervasi and Foufopoulos 2008). The immunosuppressive effects of accelerated metamorphosis may provide an opportunity for *Bd* to establish an infection in postmetamorphic individuals and cause disease (Rollins-Smith et al. 2011). The increased opportunity for infection just after metamorphosis may explain why researchers have observed higher rates of mortality in newly metamorphosed individuals (Bosch et al. 2001; Muths et al. 2003; Rachowicz et al. 2006; Briggs et al. 2010). Thus, the confluence of accelerated metamorphosis and immunosuppression may increase susceptibility to infection and compromise survival under the combined threats of drought and *Bd* (fig. 3).

Future Directions

Researchers have undertaken considerable efforts to study how drought and disease can impact amphibians as disassociated risks, but less is known about how these threats may interact to result in amphibian declines. Drought may severely alter aquatic habitats, leading to changes in amphibian survival, reproduction, and phenology (Beebee 1995; Gibbs and Breisch 2001; Carey and Alexander 2003; Daszak et al. 2005; Reading 2007; Whitfield et al. 2007; McMenamin et al. 2008). Diseases, caused by a wide range of infectious agents (*Batrachochytrium* fungi, ranaviruses, and protist parasites), also threaten amphibians around the world (Skerratt et al. 2007; Wake and Vredenburg 2008; Gray et al. 2009; Chambouvet et al. 2015; Isidoro-Ayza et al. 2017). Some correlative approaches have been used to investigate the synergistic effects of climate on disease susceptibility, suggesting that changes in temperature and weather variability may predispose amphibians to infection and disease (Alexander and Eischeid 2001; Pounds et al. 2006; Alford et al. 2007; Rohr and Raffel 2010; Hof et al. 2011; Puschendorf et al. 2011; Rohr et al. 2011; Cohen et al. 2017). However, far more research is needed to investigate these complex interactions (Rohr et al. 2008; Burrowes 2009). Here, we suggest some potential avenues for future research.

Taking ecoimmunological approaches will help resolve the complex interactions between drought and disease, improving understanding of how reduced water availability alters stress hormones and the physiology and development of the amphibian immune system. We suggest conducting controlled step-wise experimental studies that investigate the changes in different immune system components of amphibians at their various life stages and under drought-like conditions. This experimental approach would help assess immune defenses before and following metamorphosis. Building from previous work (Rollins-Smith 1998; Gervasi and Foufopoulos 2008; Rollins-Smith et al. 2011; Crespi and Warne 2013; Bagwill et al. 2016; Rollins-Smith 2017), we suggest tracking changes in hormone levels, immune function, and morphology in individuals from early development to adulthood, both before and after pathogen exposure. This integrative approach could provide insights into mechanisms and trade-offs govern-

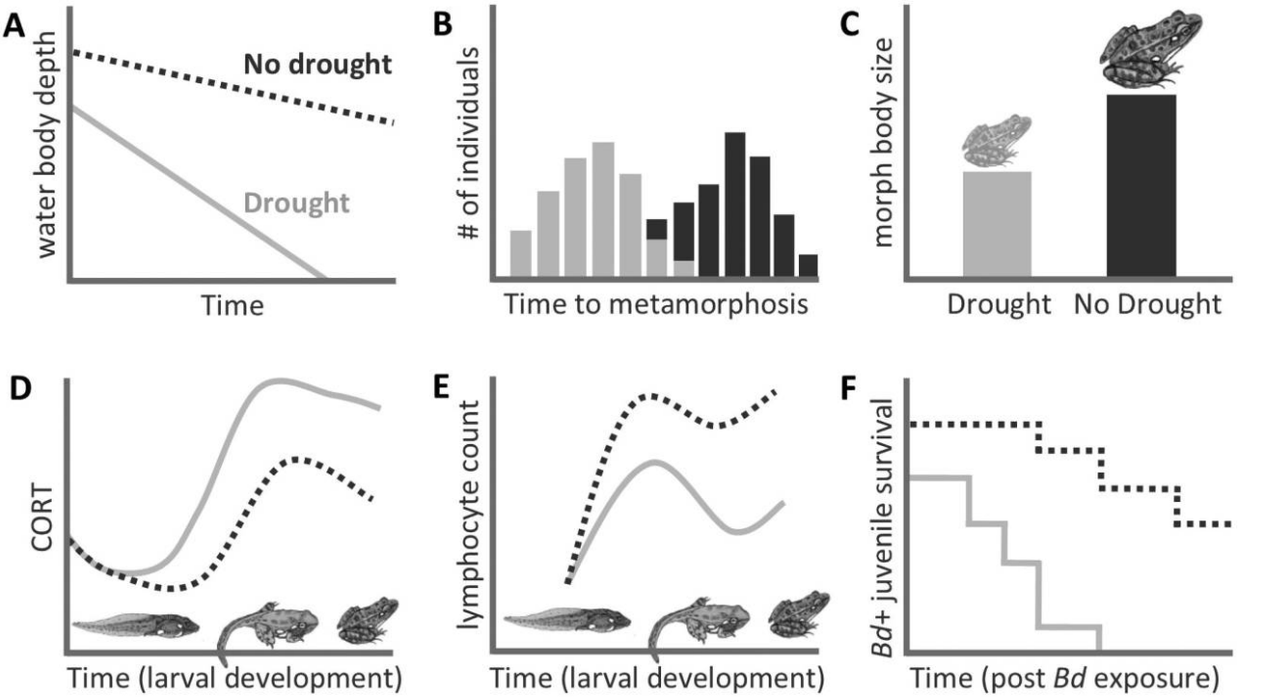

Figure 3. Model of the predicted physiological responses of amphibians to larval development under drying conditions and infection with *Batrachochytrium dendrobatidis* (*Bd*). Amphibian larvae that develop under a reduced hydroperiod (*A*) may accelerate metamorphosis to escape mortality from desiccation (*B*). Trade-offs associated with accelerated metamorphosis—such as smaller body size (*C*), elevated stress (i.e., corticosterone levels; *D*), and reduced immune function (e.g., lymphocyte count; *E*)—may increase risk of disease postmetamorphosis (*F*). A color version of this figure is available online.

ing the effects of changes in hydroperiod on disease susceptibility across life stages.

To provide an ecological context to controlled studies, we recommend pairing laboratory investigations with mesocosm experiments and field studies. Using noninvasive sampling procedures, researchers can assess glucocorticoid levels, immune function, body size, and pathogen infections. These methods can be applied to wild populations in variable habitats with different hydroperiods and allows for repeated sampling over time without sacrificing individuals. Following individuals or populations in the wild would also allow for measurements of other important environmental factors, such as air and water temperatures, tadpole densities, and predation risk. This approach may resolve which environmental parameters have the greatest effect on host physiological processes and, in turn, disease outcomes.

Investigations that focus on the interactions among multiple stressors, such as drought and disease, are important for conservation efforts. Changes in hydroperiod, as experienced in the case of a drying pond, can have varying negative effects on individuals, but we know far less about the impacts of these factors on populations. Cumulative research thus far indicates that population health can be compromised when larvae experience stress during development (Acevedo-Whitehouse and Duffus 2009). In addition, some studies that measured plasma CORT levels following *Bd* exposure indicate that a stress response may increase vulnerability to chytridiomycosis (Gabor et al. 2015, 2018; Fonner et al. 2017). Deleterious effects due to stress may accumulate over time, leading to declines in population health, even if large-scale mortality is not observed. There is also the risk that disease dynamics may shift in response to altered environmental conditions. For example, host populations that are currently coexisting with pathogens could begin to experience disease-related declines as climate and environment change. Therefore, a better understanding of how the development of the immune system in amphibians is affected by environmental changes, such as pond drying, may help direct conservation efforts and guide intervention strategies that aim to prevent further amphibian declines.

Acknowledgments

This work was funded by the US National Science Foundation (IOS: 1649443,1121758, 1557634, 1603808; DEB: 1551488) and the US Department of Defense (SERDP: RC-2638).

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