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REVIEW

Complexity in conservation: lessons from the global decline of amphibian populations

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Abstract

As part of an overall "biodiversity crisis" many amphibian populations are in decline throughout the world. Numerous causes have been invoked to explain these declines. These include habitat destruction, climate change, increasing levels of ultraviolet radiation, environmental contamination, disease, and the introduction of non-native species. In this paper, we argue that amphibian population declines are caused by different abiotic and biotic factors acting together in a context-dependent fashion. Moreover, different species and different populations of the same species may react in different ways to the same environmental insult. Thus, the causes of amphibian population declines will vary spatially and temporally. Although some generalizations (e.g. those concerning environmental stress and disease outbreaks) can be made about amphibian population declines, we suggest that these generalizations take into account the context-dependent dynamics of ecological systems.

Keywords

Amphibian population declines, biodiversity, chytridiomycosis, climate change, ozone depletion, pathogens, *Saprolegnia*, UV-B radiation.

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INTRODUCTION

Global biodiversity loss is currently a major international concern. Although the exact number of species being lost is not known, it is estimated that the current rate of extinction is greater than any known in the last 100 000 years (Eldridge 1998). Despite the widespread interest in understanding these losses, ecological theory has been able to provide little predictive insight into these problems. Furthermore, despite the recognition of this complexity by many conservation efforts, research programmes directed at understanding species losses typically focus on the direct effects of single factors. This is illustrated in attempts to understand the global decline of amphibian populations. Yet recent studies directed at this problem suggest that global amphibian losses are the result of interactions between a number of highly context-dependent causal factors. Clearly ecological theory needs to develop ways in which to manage the context dependency that is prevalent in ecological systems.

The global loss of amphibian populations was first recognized in 1989 as a phenomenon that deserved world-wide attention (Wake 1991; Blaustein 1994; Alford & Richards 1999). By 1993, more than 500 populations of frogs and salamanders were reported to be in decline or were listed as of special conservation concern (Alford & Richards 1999). Concern about amphibians is in large part due to their value as indicators of environmental stress (Blaustein 1994; Blaustein & Wake 1995). They are in close contact with water as larvae and most have some contact with land as adults. Therefore, they experience both aquatic and terrestrial stressors. They have moist, permeable skin and unshelled eggs that are directly exposed to soil, water and sunlight. They are important components of many ecosystems where they may comprise the highest fraction of vertebrate biomass (Blaustein *et al.* 1994a). Through their contribution to trophic dynamics in many communities, a world-wide decline in amphibians could have important impact on other organisms (Blaustein *et al.* 1994a). Adult amphibians are important predators as well as prey and larval amphibians may be important herbivores (Blaustein *et al.* 1994a).

Unfortunately, with an increasing literature on the documentation of amphibian declines (e.g. Alford & Richards 1999; Houlahan *et al.* 2000), there has been little consensus on the causes of the declines. Moreover, in the few cases where particular amphibian population declines have been investigated in detail, it seems that the declines are context dependent and the result of ecological dynamics

that are constantly changing, rather than static. Some investigators have certainly recognized that several factors may be involved in amphibian population declines (e.g. see review by Alford & Richards 1999). However, the majority of studies continue to focus on single factors affecting amphibian populations (Alford & Richards 1999 and references cited therein). For example, in a recent debate concerning the role of a pathogen in amphibian declines, one biologist stated "you can rule out any of these cofactors" regarding stressors that may induce a particular disease outbreak (Morell 1999). We believe that complex interactions among multiple factors should be emphasized to more fully understand the amphibian population decline phenomenon. We emphasize that cofactors may be especially important in triggering causes for amphibian population declines.

In this paper, we first briefly review some of the factors that apparently contribute to amphibian population declines. We then provide evidence that many amphibian population declines are probably the result of complex interactions among multiple factors. Thus, we suggest that a single factor for amphibian population declines is highly unlikely. Furthermore, we argue that population declines are caused by different factors in different regions.

CAUSES OF AMPHIBIAN POPULATION DECLINES

Habitat destruction and alteration

The most obvious factors contributing to amphibian population declines are habitat destruction and alteration (reviewed in Alford & Richards 1999). Clearcutting forests, draining wetlands and altering vegetation may directly affect amphibian populations (e.g. Petranka *et al.* 1993; Semlitsch 1998). Petranka *et al.* (1993) provide one example of the impact of habitat destruction on amphibian populations. They compared species richness and abundance of salamanders on six recent clearcuts with salamanders in mature forest stands. Salamander catches were five times higher in mature stands than in clearcuts. They estimated that clearcutting in U.S. national forests results in the loss of nearly 14 million salamanders annually.

Global environmental change

There is evidence that global climate change such as changes in precipitation and temperature has affected the breeding phenology of some (but not all) amphibian populations (discussed recently in Blaustein *et al.* 2001a; in press). For example, Beebee (1995), plotted the start of breeding activities for six amphibian species in southern England over 16 years, and showed that amphibians are responding to climate change by breeding earlier. Gibbs & Breisch (2001) showed that over the last century, daily temperatures increased near Ithaca, New York and several species of anurans vocalized earlier compared with calling dates between 1900 and 1912.

Increasing ultraviolet-B (UV-B) radiation caused by stratospheric ozone depletion and other environmental changes may contribute to amphibian population declines. For example, the hatching success of a number of amphibian species is lower when they are exposed to ambient levels of UV-B radiation compared with eggs that are shielded from UV-B (Blaustein *et al.* 2001b). Moreover, in many cases, exposure to UV-B radiation induces sublethal effects that may affect growth and development, behaviour and the physiology and anatomy of amphibians (Blaustein *et al.* 2001b). Continued mortality in early life stages may ultimately contribute to a population decline.

Diseases

A variety of pathogens affect wild amphibian populations. These include viruses, bacteria, parasites, protozoans, oomycetes, and fungi (e.g. Blaustein *et al.* 1994b; Jancovich *et al.* 1997; Kiesecker & Blaustein 1997a; Longcore *et al.* 1999; Johnson *et al.* 2002). These pathogens can be the proximate causes of mortality or they can cause sublethal damage such as severe developmental and physiological deformities. Pathogens may infect amphibians at various life stages. There are some observations of pathogens causing massive die-offs of amphibians. Of particular concern to a number of investigators is whether the diseases of amphibians are novel or if they are being triggered by an environmental change (see below).

Contaminants

A wide array of contaminants may affect amphibian populations. These include pesticides, herbicides, fungicides, fertilizers and numerous pollutants (Sparling *et al.* 2000; Boone & Bridges in press). Toxic substances can severely affect amphibians in a variety of ways. They can kill amphibians directly, affect their behaviour, reduce their growth rates, act as endocrine disrupters or induce immunosuppression (Alford & Richards 1999).

Introduced species

Introduced species have the potential to affect amphibians in a variety of ways. They may compete with native amphibian species, prey upon them, or introduce diseases that may affect them. For example, bullfrogs (*Rana catesbeiana*) introduced from eastern to western North America may contribute to population losses of some native amphibians (e.g. Kiesecker & Blaustein 1997b, 1998; Kupferberg 1997;

Kiesecker *et al.* 2001a). Mosquitofish (*Gambusia affinis*) and crayfish (*Procambarus clarkii*) introduced to California are effective predators of larval newts (Gamradt & Kats 1996). Hatchery-reared salmonid fishes may eat native amphibians (Bradford 1989) or infect them with pathogens (Blaustein *et al.* 1994b; Kiesecker *et al.* 2001b).

CONTEXT DEPENDENCY

Although several studies have linked amphibian population declines to specific causes (discussed above), studies attempting to examine similar factors in other places or at other times have often failed to duplicate the results. Furthermore, it is becoming increasingly clear that many of the factors proposed to explain amphibian population declines exhibit a high degree of temporal and spatial variation. Thus, the ways in which various agents contribute to population declines will also vary and will probably be seen through a series of complicated local interactions. Below we discuss some of the context dependency that has been observed among several potential factors that have been proposed to explain amphibian population declines.

INTERACTIONS BETWEEN ABIOTIC FACTORS

By themselves, numerous abiotic agents, including pesticides, herbicides, fungicides, fertilizers, pollutants, weather patterns, and UV-B radiation may affect amphibians in a number of ways (Sparling *et al.* 2000; Blaustein *et al.* 2001b; Boone & Bridges in press). As examples of the complexity by which abiotic factors may affect amphibians, we will concentrate on the effects of UV-B radiation and contaminants in this section. Both of these agents have been the subject of a number of recent investigations with regard to amphibians.

UV-B radiation

Over evolutionary time, UV radiation has been a ubiquitous stressor on living organisms and has probably exerted selection pressure resulting in the evolution of defences against its effects (Cockell 2001). Natural events such as impacts from comets and asteroids, volcanic activity, cosmic events such as supernova explosions, and solar flares can cause large-scale ozone depletion with accompanying increases in UV radiation (Cockell & Blaustein 2000; Cockell 2001). However, these natural events are transient, and may only have significant effects for a few years. This is obviously different from human-induced production of chlorofluorocarbons (CFCs) and other chemicals that continuously deplete stratospheric ozone inducing long-term increases in UV-B radiation at the surface. Decreases in stratospheric ozone, climate warming and lake acidification leading to decreases in dissolved organic carbon concentrations (e.g. Schindler *et al.* 1996) all result in increasing levels of UV radiation. In fact, information from several sources (e.g. remote sensing) indicates that levels of UV-B radiation have recently risen significantly (especially since 1979) in both tropical and temperate regions (Kerr & McElroy 1993; Middleton *et al.* 2001). However, as discussed in detail by Middleton *et al.* (2001), data gathered from remote sensing have many limitations. For example, there are a number of shortcomings when using data generated from satellites, including the fact that resolution of the satellite-generated data is not accurate enough to approximate ground-level interpretations (Middleton *et al.* 2001). Local atmospheric conditions such as cloud cover, precipitation patterns and conditions of the water, such as amount of dissolved organic material may influence the amount of UV radiation reaching the surface. Nevertheless, given the limitations, the study by Middleton *et al.* (2001) is consistent with mounting experimental evidence that UV radiation is harmful to amphibians and that increasing UV-B levels may be contributing to amphibian population declines (Blaustein *et al.* 1998, 2001b).

At the terrestrial surface, UV-B (280–315 nm) radiation is extremely important biologically. Critical biomolecules absorb light of higher wavelength (UV-A; 315–400 nm) less efficiently, and stratospheric ozone absorbs most light of lower wavelength (UV-C; 200–280 nm) (Cockell & Blaustein 2001). UV-B radiation can cause mutations and cell death. At the individual level, UV-B radiation can slow growth rates, cause immune dysfunction, and induce sublethal damage (Tevini 1993).

Using field experiments, investigators at various sites around the world have shown that ambient UV-B radiation decreases the hatching success of some amphibian species at natural oviposition sites (Blaustein *et al.* 1994c, 1998, 2001b). These studies have demonstrated that the embryos of some species are more susceptible to UV-B radiation than others (Blaustein *et al.* 1998). For example, in the Pacific Northwest (USA), the hatching success of Cascades frogs (*Rana cascadae*), western toads (*Bufo boreas*), long-toed (*Ambystoma macrodactylum*) and Northwestern (*A. gracile*) salamanders was lower when exposed to ambient UV-B radiation than when eggs were shielded from UV-B (Blaustein *et al.* 1998). However, the hatching success of spotted (*R. pretiosa* and *R. luteiventris*), red-legged (*R. aurora*) and Pacific tree (*Hyla regilla*) frogs was not significantly different between the UV-shielded and UV-exposed treatments (Blaustein *et al.* 1998). In California, the hatching success of Pacific treefrogs was not affected by ambient levels of UV-B radiation but hatching success was lower in California treefrogs (*Hyla cadaverina*) and California newts (*Taricha torosa*) exposed to UV-B (Anzalone *et al.* 1998). The hatching success of Common toads (*Bufo bufo*) in Spain was lower in UV-B-exposed eggs than in those shielded by

UV-B, whereas there was no effect of UV-B on the hatching success of the Natterjack toad (*B. calamita*) (Lizana & Pedraza 1998). In Finland, the hatching success of moor frogs (*Rana arvalis*) increased when embryos were shielded from UV-B, but there was no effect on hatching success when embryos of Common toads (*Bufo bufo*) and common frogs (*Rana temporaria*) were shielded from UV-B (Häkkinen *et al*. 2001).

As the studies described above illustrate, there are differences in how the hatching rates of different amphibian species are affected by UV-B radiation. Like any abiotic factor, sensitivity to UV-B radiation is not always consistent within a given taxon, and detrimental responses in one species does not mean that members of another species will respond in the same way. Moreover, researchers investigating the effects of UV-B radiation on amphibians often use very different methods. Interpreting the results of studies using different techniques and conducted in different regions is difficult (Blaustein *et al*. 1998). We argue strongly that these are all important points to consider when interpreting the results of studies concerning UV-B radiation (and any other abiotic variable).

For example, using field experiments, Blaustein *et al*. (1994c) found that hatching success of western toads (*Bufo boreas*) in Oregon was lower when developing embryos were exposed to ambient UV-B radiation compared with controls shielded from UV-B. Using very different experimental methods, hatching success in toads (*B. boreas*) in Colorado was unaffected by UV-B radiation (Corn 2000). These different results were said to be contradictory by Carey (2000) and Corn (2000). We do not feel that these results are contradictory. Rather, they reflect differences in methodology, the biology of the species being examined, and their ecological characteristics of the regions where studies were conducted.

Toads in Oregon are subjected to a variety of abiotic and biotic components that differ from those in Colorado. Moreover, it is not surprising that different populations or different species of amphibians react differently to UV-B radiation or any environmental variable (in fact the toads studied in Oregon and Colorado are probably different species; see Blaustein *et al*. 1998). Variability in response to UV-B radiation has been found in many organisms, including amphibians (Blaustein *et al*. 1998, 1999, 2001b; Belden *et al*. 2000; Cockell & Blaustein 2001; Belden & Blaustein in press). A number of recent studies have revealed that the effects of UV-B radiation on some species may be subtle and more complex than many of the original studies have shown. Thus, even though hatching rates of some species may appear unaffected by ambient UV radiation in field experiments, an increasing number of studies illustrate a variety of sublethal effects caused by UV exposure (Blaustein *et al*. 2001b). For example, when exposed to UV-B radiation, amphibians may change their behaviour (Nagl & Hofer 1997; Blaustein *et al*. 2000; Kats *et al*. 2000) growth and development may be slowed (e.g. Belden *et al*. 2000; Smith *et al*. 2000; Pahkala *et al*. 2001; Belden & Blaustein 2002), or UV can induce developmental and physiological malformations (e.g. Blaustein *et al*. 1997; Fite *et al*. 1998). Sublethal effects may become evident even in species whose embryos appeared to be resistant in field experiments.

Several experimental studies illustrate that early exposure to UV-B radiation causes delayed effects in later stages. For example, UV-B radiation did not influence hatching success of plains leopard frogs (*Rana blairi*) but growth and development were slower in tadpoles when as embryos they were exposed to high levels of UV-B radiation (Smith *et al*. 2000). Embryos of *Rana temporaria* exposed to UV-B radiation showed no effects on survival rates, frequency of developmental anomalies, or hatching size (Pahkala *et al*. 2001). However, larvae exposed to UV-B radiation as embryos displayed an increased frequency of developmental anomalies, metamorphosed later and were smaller than larvae shielded from UV-B as embryos (Pahkala *et al*. 2001). Ambient levels of UV-B radiation have no effects on hatching success in red-legged (*R. aurora*) frogs (Blaustein *et al*. 1998) but larvae exposed to UV-B radiation as embryos were smaller and less developed than those shielded from UV-B radiation (Belden & Blaustein 2002).

Under "normal" circumstances, amphibians living in ephemeral habitats have a variety of responses to accelerate their growth and development as the habitat dwindles (Wilbur 1997 and references therein). However, delayed growth and development after exposure to UV-B radiation may significantly affect populations of amphibians that live in ephemeral habitats. For example, if growth and development is slowed significantly and amphibians cannot metamorphose and move to land before a pond dries or freezes, significant mortality may occur (e.g. Blaustein *et al*. 2001c).

There is significant cause for concern about the effects of UV-B radiation on amphibians. Yet, the effects of UV-B radiation on amphibians are complex. UV-B radiation can kill amphibians directly, cause sublethal effects or it can work in concert with contaminants, pathogens or with changes in climate to adversely affect amphibians (described in further detail below). Furthermore, the effects of UV-B radiation may vary with species, between populations of the same species, with weather conditions, geography, and water chemistry (Blaustein *et al*. 1998).

Contaminants

Contaminants may be spread globally, or act on a local scale. Contaminants transported atmospherically have the potential to harm amphibians in remote, relatively undisturbed

environments. Although levels of pollutants from atmospheric deposition are typically low, these levels may still significantly impair developing amphibians under complex, ecological conditions. For example, in California, USA, atmospheric deposition of organophosphate pesticides from the highly agricultural Central Valley may have contributed to declines of frogs (Sparling *et al.* 2001). Davidson *et al.* (2001) concluded that patterns of decline in red-legged frogs in California, were most likely caused by pesticides carried upwind from the Central Valley.

Like the effects of UV-B radiation on amphibians, it is becoming increasingly clear that there are interspecific differences in tolerance to various contaminants. The effects on various species may differ and interactions between pesticides and other abiotic agents may be significant.

For example, the insecticide, carbaryl may reduce growth and development, affect larval behaviour and influence species interactions in amphibians (Bridges 1997; Boone & Semlitsch 2001). But these differences may differ among species and in different ecological contexts (Sparling *et al.* 2000).

There are well-documented interspecific differences in susceptibility of amphibian species to nitrogenous fertilizers and acid pollution, both of which may impact amphibian populations significantly (e.g. Harte & Hoffman 1989; Hecnar 1995; Kiesecker 1996; Marco *et al.* 1999). Mortality rates and behaviour of amphibians may be affected in species exposed to nitrogenous fertilizers or low pH alone. For example, recent experimental studies illustrated that in the presence of nitrogenous fertilizers, the larvae of some species reduce feeding activity, swim less vigorously, display disequilibrium, develop malformations of the body and die (e.g. Marco *et al.* 1999; Hatch & Blaustein 2000). The larvae of other species are less affected (Marco *et al.* 1999). Even after metamorphosis, amphibians of some species may alter their feeding behaviour in the presence of nitrogenous fertilizers, whereas others do not (e.g. Hatch *et al.* 2001).

The effects of environmental contamination may be more complex than studies of single contaminants have shown. Thus, even species that are unaffected by exposure to low pH and nitrogenous fertilizers alone may be greatly affected when they are exposed to combinations of these stressors. For example, survival was not affected in *Rana cascadae* tadpoles exposed to ambient levels of UV-B radiation, high nitrate levels and low pH alone (Hatch & Blaustein 2000). However survival and activity levels were greatly reduced when *R. cascadae* tadpoles were exposed to a combination of these factors (Hatch & Blaustein 2000).

Several studies have examined synergistic interactions between UV and chemical contaminants in developing amphibians (e.g. Kagan *et al.* 1984; Hatch & Burton 1998; Zaga *et al.* 1998; Monson *et al.* 1999). Such studies illustrate the importance of understanding how complex interactions affect individual animals, and perhaps ultimately, whole populations. There are different ways by which synergisms may affect amphibians.

Synergism may occur when developing amphibians have reduced ability to respond to one stressor in the presence of another. For example, some species may only be impacted by acid pollution when it is combined with another abiotic environmental insult, such as UV-B radiation (Long *et al.* 1995).

Synergism between UV and environmental pollutants may also occur when one factor enhances the toxicity of the other agent. Thus, chemical contaminants that absorb strongly in some portion of the UV spectrum may be especially phototoxic (Blaustein *et al.* 2001b). When toxicity occurs via bioaccumulated chemical interactions with UV, greater toxicity will be observed when animals are exposed to both UV and the chemical or to the chemical first and then to UV light, as in the case of polycyclic aromatic hydrocarbons (PAHs). Toxicity may also occur when UV directly alters a chemical, making it more toxic, as in the case of some insecticides.

PAHs are pervasive multiple-ringed hydrocarbons that contaminate ponds and streams via road runoff, direct industrial discharge, or atmospheric deposition. PAHs absorb UV-A and are acutely toxic (Blaustein *et al.* 2001b). In the presence of sunlight, some PAHs (such as anthracene, benzo(a)pyrene and fluoranthene) can be extremely toxic to aquatic organisms, such as amphibians, at environmentally realistic levels (Hatch & Burton 1998; Monson *et al.* 1999).

These studies demonstrate important interactions between environmental stressors that may affect amphibians at the population level. In many cases, an individual abiotic stressor alone may not affect an amphibian. But, as these studies illustrate, multiple stressors acting in combination may be especially dangerous. Amphibians are most likely exposed to numerous abiotic agents simultaneously rather than to single stressors. Such interactions may ultimately affect amphibians at the population level.

INTERACTIONS BETWEEN BIOTIC FACTORS

Introduced species present a variety of problems to native organisms. These include the introduction of potential competitors, predators and vectors for disease transmission. There is evidence that all three of these problems are contributing to amphibian population declines. Below, we provide examples of work examining the impacts of introduced species on native amphibians in the western United States (Kiesecker & Blaustein 1997b, 1998; Kiesecker *et al.* 2001a). This research illustrates the complex interrelationships among environmental changes, introduced species and amphibian population declines (Fig. 1).

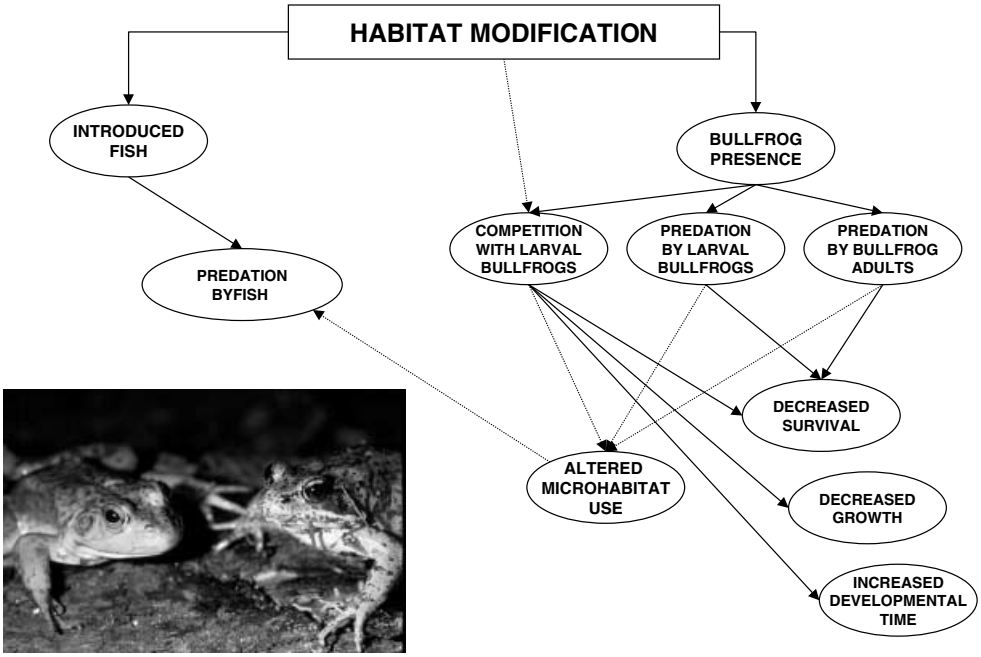

Figure 1 Interaction web for impacts of habitat modification, introduced bullfrogs, *Rana catesbeiana* (left), and predatory fish on red-legged frogs, *Rana aurora* (right), in the western United States. Arrows represent direct (solid) and indirect (dashed) interactions that have been tested in experimental studies in Oregon, Washington, and California. Other direct and indirect interactions are possible but have not been tested experimentally.

Impact of introduced bullfrogs on red-legged frogs

Population declines of ranid frogs native to the western United States have been reported by a number of investigators (e.g. Hayes & Jennings 1986). One hypothesis often invoked to explain these declines is the introduction of non-native bullfrogs (*Rana catesbeiana*) which may compete with and prey upon other frog species and have been introduced into the western U.S. (e.g. Kiesecker & Blaustein 1998). Adult bullfrogs feed on a variety of aquatic prey, including other amphibians (Kiesecker & Blaustein 1998 and references therein). Tadpoles of *R. catesbeiana* also prey on tadpoles of other species (e.g. Kiesecker & Blaustein 1997b). The specific impacts of bullfrogs on native frog populations are often unclear because at many sites their introductions have occurred simultaneously with habitat modifications and/or the introduction of predatory fish (Hayes & Jennings 1986; Adams 1999; Kiesecker *et al.* 2001a). However, several recent studies, primarily experimental in nature, have developed a more mechanistic understanding of the impacts of bullfrogs on native amphibians (Kiesecker & Blaustein 1997a, 1998; Kupferberg 1997; Kiesecker *et al.* 2001a).

For example, in the Willamette Valley of Oregon the impact of bullfrogs on native red-legged frogs (*Rana aurora*) is complex, involving both direct and indirect interactions between these species (Fig. 1). Moreover, these interactions are mediated by habitat modifications that appear to promote the success of introduced species and intensify interactions between bullfrogs and red-legged frogs (Kiesecker *et al.* 2001a). Surveys of red-legged frog breeding sites in the Willamette Valley indicate that red-legged frogs are absent from a large portion of their historical breeding sites (Kiesecker, unpublished data) and breeding bullfrogs are found at most of these sites.

In the presence of bullfrog larvae and adults, red-legged frogs alter their use of microhabitat, making them more susceptible to fish predation (Kiesecker & Blaustein 1998). Laboratory experiments illustrate that red-legged frog tadpoles from populations that are syntopic with bullfrogs display antipredatory behaviours when presented with chemical cues of bullfrogs (Kiesecker & Blaustein 1997b). In contrast, red-legged frog tadpoles from populations that are allotopic to bullfrogs do not. Field and laboratory experiments illustrate that these behavioural differences result in higher rates of predation in tadpoles from allotopic populations (Kiesecker & Blaustein 1997b, 1998). Thus, individuals (e.g. red-legged frogs in Oregon) that are unfamiliar with novel introduced predators (e.g. bullfrogs in Oregon) may not possess adaptations that would prevent a negative encounter. However, even red-legged frogs that are syntopic with bullfrogs accrue costs in their antipredatory behaviour. Thus, in field experiments, *R. aurora* tadpoles that shifted their microhabitat use in response to bullfrog

presence, grew more slowly and were at increased risk of predation by introduced fish. Moreover, survivorship of red-legged frogs was only significantly affected when they were exposed to the combined effects of bullfrog larvae and adults or bullfrog larvae and smallmouth bass (*Micropterus dolomieui*). Thus, the interaction between stages (larval/adult) or species (bullfrog/smallmouth bass) produced indirect effects that were larger than when each factor was considered separately.

Habitat modifications play a major role in the interactions between red-legged frogs and bullfrogs. In western North America, modification of wetlands frequently benefits introduced bullfrogs and introduced fish (Hayes & Jennings 1986; Adams 1999). For example, in the western U.S., large, ephemeral wetlands are commonly converted to smaller permanent ponds that become prime breeding areas for bullfrogs (Adams 1999; Kiesecker *et al.* 2001a). These ponds may contain less shallow water and emergent vegetation, which tends to be clumped along the edges of ponds. Reducing and clumping vegetation increases competition between larval bullfrogs and red-legged frogs (Kiesecker *et al.* 2001a) and may intensify predation of adult bullfrogs on larval and juvenile red-legged frogs (Kiesecker & Blaustein 1998). Thus, although both bullfrogs and introduced predatory fish (e.g. smallmouth bass) have negative impacts on the long-term survival of red-legged frogs, the outcome of these interactions is clearly dependent on the context of the interactions.

Disease

Pathogens are becoming an increasingly important focus with regard to amphibian population declines (e.g. Daszak *et al.* 1999, 2001; Kiesecker *et al.* 2001c). Moreover, recent research suggests that disease outbreaks may often be the result of complex interactions among a number of factors, including those related to environmental change.

Hypotheses related to disease-mediated amphibian population declines have been placed into two broad categories (1) those that suggest that pathogens are novel; and (2) those that have emphasized that other factors, including those related to environmental change may trigger a disease. In either case, according to the broadest definition, the disease may be considered an Emerging Infectious Disease (EID).

Thus, as described by Daszak *et al.* (2001), EIDs are diseases that have recently increased in incidence or geographical range, recently moved into new host populations, recently been discovered or are caused by newly evolved pathogens. Several predictions can be made regarding the disease-related hypotheses.

If the disease is novel, we predict that where diseases are implicated in a population decline (1) records of the disease should not be found in such historical items as museum specimens or field notes in the region of the decline; and (2) there should be some geographical pattern for an outbreak associated with the point of introduction of the novel pathogen.

If cofactors are involved in triggering a disease outbreak, we predict that (1) historical records of the disease would be found in such items as museum specimens and field notes; (2) the pattern of outbreak would be linked to a suspected environmental change; (3) we would observe disease outbreaks in areas experiencing such an environmental change; and (4) we would not observe a disease outbreak in the absence of the environmental change in question.

Three pathogens

Three pathogens that have received recent attention with regard to amphibian population declines are the chytridiomycete, *Batrachochytrium dendrobatidis*, found in several areas where population declines have occurred, a pathogenic oomycete, *Saprolegnia ferax*, contributing to large-scale amphibian embryonic mortality in North America, and an iridovirus (*Ambystoma tigrinum* virus, ATV) isolated from diseased tiger salamanders. All three of these pathogens appear to be involved in complex interactions with biotic and abiotic agents. Furthermore, studies of all three pathogens fulfil Koch's postulates (Jancovich *et al.* 1997; Kiesecker *et al.* 2001b; Nichols *et al.* 2001). That is (1) the pathogen in question was always associated with the disease; (2) the pathogen was isolated and grown in culture; (3) the culture produced the disease in a susceptible amphibian; and (4) the pathogen was recovered from an experimental animal.

Chytridiomycosis is one such disease that may be involved in some of the mortality and die-offs found in Central and South America (Morell 1999; Ron & Merino 2000). A number of investigators have categorized chytridiomycosis as an EID (e.g. Daszak *et al.* 1999, 2001). In fact, several biologists have suggested that it is a novel disease and that cofactors are not involved in its emergence (Morell 1999). Nevertheless, much of the information regarding chytrid infections suggests that cofactors are indeed involved.

Although chytridiomycosis may be a novel disease in some regions, there are no published systematic surveys of museum specimens to see if they were infected with chytrid before widespread declines occurred. Moreover, some regional surveys of museum specimens suggest that amphibians may have carried chytrid before widespread population declines occurred. For example, sampling amphibian museum specimens from Ecuador showed that several species were infected with chytrid as early as 1980 (Ron & Merino 2000). This was before amphibian declines were noted in Ecuador in the late 1980s (Ron & Merino 2000). Ron & Merino (2000) suggest that cofactors were involved and the presence of chytrid in the 1980 specimens

supports that contention. Moreover, "wavelike" patterns of infection, a characteristic predicted for novel diseases, and described by Laurence *et al.* (1996) in tropical Australian frogs have been challenged on statistical grounds and for other reasons (Alford & Richards 1999).

If chytrid infection is indeed responsible for some of the highly publicized and documented population declines of amphibian species in Costa Rica (Pounds *et al.* 1997; Lips 1998) as suggested by Morell (1999), environmental changes, such as changes in precipitation and temperature, are strongly implicated as potential triggers for such outbreaks (Lips 1998; Pounds *et al.* 1999; Pounds 2001). Moreover, it has been suggested that environmental contamination may also play a role in the spread of chytrid, even in the tropics. Pounds & Crump (1994) suggested that atmospheric scavenging of contaminants by clouds might concentrate contaminants and release them in remote areas such as in Monteverde, Costa Rica, where numerous amphibian populations have declined (Pounds *et al.* 1999). This effect may be particularly important under unusually dry conditions (Pounds & Crump 1994; Pounds *et al.* 1999). Along with weather pattern changes, contaminants may exacerbate chytrid outbreaks (see discussion in Pounds 2001). Thus, environmental changes and contamination may act as cofactors influencing chytrid outbreaks in tropical America.

Recent evidence (Middleton *et al.* 2001) that UV-B levels have increased significantly since 1979 in all areas where amphibian populations have declined in Central America adds more complexity to the role of diseases in the region. This is significant if UV-B radiation acts as a stressor on amphibian immune systems (Tevini 1993). Indeed, many (but obviously not all) tropical amphibian species are exposed to sunlight and UV-B radiation. The amount of exposure a particular amphibian is subjected to depends upon the species, the life stage, and where it lives. For example, a number of species lay their eggs in direct sunlight in Central America (L.B. Kats pers. comm.; D. B. Wake pers. comm.; Lips 1998). L.B. Kats has seen eggs of at least one species of frog in Costa Rica die after being laid in direct sunlight (pers. comm.). Lips reported that *Hyla calypsa* clutches in Costa Rica are exposed to sunlight and incidental radiation during their three to eight week developmental period. Many tropical species frequent light gaps (personal observations of ARB and JMK in Costa Rica), including four species whose populations are in decline in Costa Rica (A.J. Pounds pers. comm.). UV-B radiation can compromise amphibian immune systems, making them more susceptible to infection. Exposure to increasing levels of UV-B radiation appears to be coincident with outbreaks of disease in some regions in Central America. Even if just one individual is compromised and becomes infected, it can potentially spread the disease to others, eventually infecting an entire population. Cross-species contamination is also possible.

Cofactors may also be involved in the spread of chytrid in temperate regions. For example, recent evidence implicates changes in water pH triggering chytrid outbreaks in Spain (Bosch *et al.* 2001).

Complex interactions may also to be involved in the spread of the iridovirus (*Ambystoma tigrinum* virus, ATV) isolated from diseased tiger salamanders and determined to be the cause of epizootics. The virus might originate from multiple sources including introduced fish, bullfrogs, or possibly introduced salamanders that are used as fish bait (Jancovich *et al.* 1997). It is unclear whether cofactors, such as contaminants, increasing UV-B radiation, or weather changes are involved in its spread. Given that the spread of other pathogens appears to be influenced by a number of cofactors, we suggest that cofactor involvement is a parsimonious, but untested explanation for the spread of the iridovirus.

Several studies have shown how the spread of *Saprolegnia* is influenced by a variety of cofactors and other variables. These include introduced vectors of transmission, exposure to UV-B radiation, and even the behaviour of host amphibians. These points are reviewed briefly below.

TRANSLATING GLOBAL ENVIRONMENTAL CHANGE INTO LOCAL SPECIES LOSS

Kiesecker *et al.* (2001c) illustrated the complex interrelationships among global environmental changes and amphibian population declines (Fig. 2). Kiesecker *et al.* (2001c) reported that periodic mass mortality of boreal toad (*Bufo boreas*) embryos in Oregon resulted from a synergism between UV-B radiation and the pathogenic oomycete *Saprolegnia ferax*. Previous work had shown that susceptibility to *Saprolegnia* is enhanced when developing eggs are exposed to UV-B radiation (Kiesecker & Blaustein 1995). UV-B exposure was in large part determined by water depth at oviposition sites. Kiesecker *et al.* (2001c) linked El Niño/Southern Oscillation (ENSO) events with decreased winter precipitation in the Oregon Cascade Range. They suggested that less winter snow pack resulted in lower water levels when western toads (*Bufo boreas*) breed in early spring. Toad embryos developing in shallower water are exposed to higher levels of UV-B radiation, which results in increased mortality from *Saprolegnia* infection. Thus, global events clearly affect local populations.

Furthermore, the outcome of interactions between developing western toad embryos and *Saprolegnia* infection may be influenced by a number of other variables. For example, recent evidence suggests that *Saprolegnia* may affect amphibians in several ways and its infection rate and virulence depend upon a number of factors. For example, in

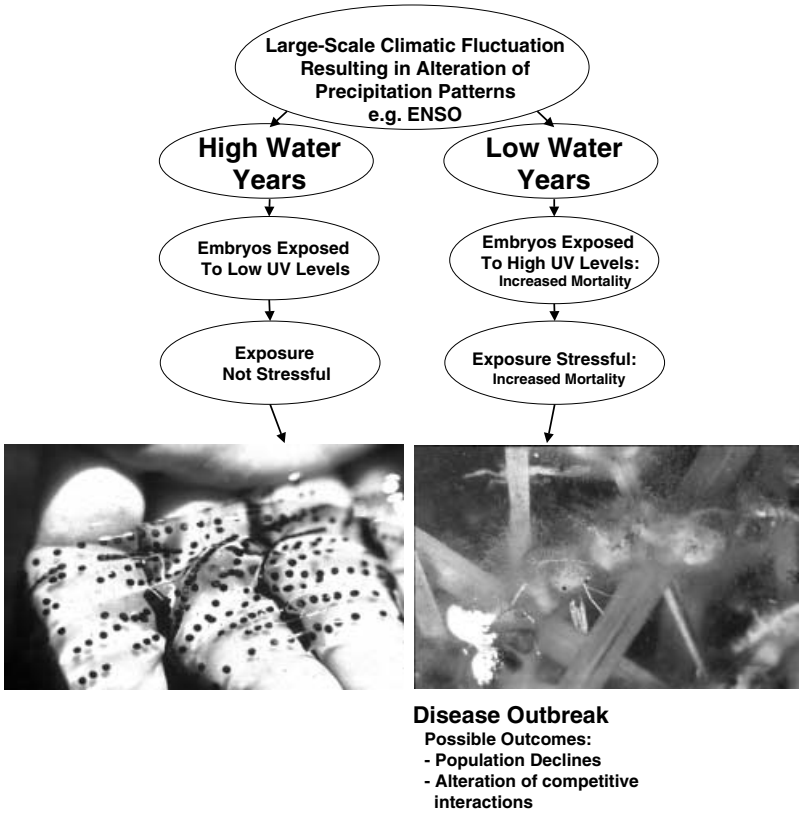

Figure 2 Flow diagram of possible outcomes of climatic fluctuations on amphibian populations in the Pacific Northwest of the United States. This diagram is based on the results of numerous experimental and observational studies (see text for details). Other direct and indirect interactions are possible but have not been tested.

field experiments, where the spatial position and time of egg laying was manipulated, the highest mortality from *Saprolegnia* infection was found in eggs laid later and in closer proximity to communal masses (Kiesecker & Blaustein 1997a). Furthermore one of the main vectors of *Saprolegnia* infection appears to be introduced fishes (Kiesecker *et al.* 2001b). Laboratory experiments have shown that hatchery reared rainbow trout (*Oncorhynchus mykiss*) can transmit *S. ferax* to developing amphibians and to soil substrate (Kiesecker *et al.* 2001b). Amphibian embryos exposed to either infected fish or infected soil were more likely to develop *Saprolegnia* infections and had higher mortality rates compared to embryos exposed to control conditions (Kiesecker *et al.* 2001b). Different strains of *Saprolegnia* may have different virulence (Kiesecker *et al.* 2001b). Thus, introduced fish may transmit strains of *Saprolegnia* that are more virulent to native amphibians.

These studies parallel work by Pounds *et al.* (1999). They found that changes in water availability associated with changes in large-scale climate processes may significantly affect amphibian populations in a Costa Rican cloud forest. They showed that extended dry periods associated with global warming are correlated with amphibian losses. In Costa Rica and potentially in other high altitude tropical sites, global warming appears to have resulted in a decrease in the amount of mist precipitation received in the forest, because of increased altitude of the cloud bank. This decrease in available moisture may weaken amphibians, making them susceptible to a host of other stressors, including disease.

It is clear that the factors involved in disease transmission are complex. In some cases, cofactors, including those associated with environmental change, have been identified as playing a role in disease outbreaks. With regard to climate and disease outbreaks, Pounds (2001) states, "Climate only loads the dice for disease outbreaks; it does not dictate when and where they will occur, and whether or not they will spread".

CONCLUSIONS

It is clear that amphibians are subjected simultaneously to a cocktail of abiotic and biotic stressors that affect them in a variety of ways. Thus, we suggest that amphibian population declines are the result of complex interactions among numerous factors often acting synergistically. Moreover, global changes (including stochastic events) such as changes in temperature, precipitation, UV-B radiation, and global spread of contaminants may affect amphibian populations on a local scale. However, different populations of

amphibians may react differently to the same combination of stressors. Differences in susceptibility to stressors depend on numerous variables including life stage, species, population, geography, weather parameters, water chemistry, history of experiencing particular stressors and numerous other factors. Interpreting the results of various studies and comparing the results of different studies must take these factors into account as well as the type of study a particular investigator has undertaken (e.g. experimental, observational, correlational).

Although it may be difficult to generalize about amphibian population declines, some generalities can be made. One consistent theme appears to be the interactions between environmental change at local (e.g. habitat modifications), regional (e.g. acidification or contaminants) and global scales (e.g. climate change or UV-B radiation) with the modification of local biotic interactions (e.g. disease or introduced species). For example, there appears to be a link between stressors and disease outbreaks (Pounds *et al.* 1999; Kiesecker *et al.* 2001c). However, the stressors that modify local interactions and the interactions that are modified may differ in different regions.

Given the complexity of dynamic ecological systems, we argue that invoking single stressors or risk factors to explain amphibian population declines, may, in general be too simplistic. Furthermore, we argue that field experiments incorporating a multifactorial approach have led to the most unambiguous explanations regarding amphibian population declines. These conclusions highlight two important points: (1) the difficulty that will exist in predicting how global and regional environmental change will be translated into local species loss; and (2) the critical need for ecological theory to address the pervasiveness of such context dependency. Specifically, we believe that it is necessary for those studying amphibian population declines (and other types of species loss) to develop methods that embrace context dependency. This will allow us to generalize more efficiently about the causes and implications of phenomena like amphibian population declines.

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