



Ultraviolet radiation, toxic chemicals and amphibian population declines

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Abstract. As part of an overall ‘biodiversity crisis’, many amphibian populations are in decline throughout the world. Numerous factors have contributed to these declines, including habitat destruction, pathogens, increasing ultraviolet (UV) radiation, introduced non-native species and contaminants. In this paper we review the contribution of increasing UV radiation and environmental contamination to the global decline of amphibian populations. Both UV radiation and environmental contaminants can affect amphibians at all life stages. Exposure to UV radiation and to certain contaminants can

kill amphibians and induce sublethal effects in embryos, larvae and adults. Moreover, UV radiation and contaminants may interact with one another synergistically. Synergistic interactions of UV radiation with contaminants can enhance the detrimental effects of the contaminant and UV radiation.

Key words. Amphibian population declines, biodiversity, chytridiomycosis ozone depletion, contaminants, fertilizers, heavy metals, PAHs, pathogens, pesticides *Saprolegnia*, toxic chemicals, UV-B radiation.

INTRODUCTION

Global biodiversity loss is currently a major international concern, with some estimates suggesting that the current rate of extinction is greater than any known in the last 100 000 years (Eldridge, 1998). As part of the global loss of species, amphibian populations are declining throughout the world (Wake, 1991; Alford & Richards, 1999; Houlahan *et al.*, 2000; Blaustein & Kiesecker, 2002). More than 500 populations of frogs and salamanders are in decline and many are listed as of special conservation concern (Alford & Richards, 1999). In some regions, declines of amphibian populations appear to be greater than declines in other taxonomic groups (e.g. Pounds *et al.*, 1999). Numerous factors, including pathogens, introduced non-native

species, contaminants, habitat destruction and global environmental changes are contributing to population declines in amphibians (recently reviewed in Alford & Richards, 1999; Blaustein & Kiesecker, 2002).

Concern about amphibians is due in part to their value as indicators of environmental stress (Blaustein & Wake, 1995). Many 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. Amphibians have moist, permeable skin and unshelled eggs that are directly exposed to soil, water and sunlight and that can readily absorb toxic substances. Moreover, amphibians are important components of many ecosystems, acting as prey, predators or herbivores (Blaustein *et al.*, 1994a). Because of their contribution to trophic dynamics, loss of amphibian populations will probably affect other organisms (Blaustein *et al.*, 1994a).

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Numerous recent studies have been conducted to document causes of amphibian population losses and declines. These studies reveal that mechanisms underlying amphibian population declines are complex, involving interactions among abiotic and biotic components (Blaustein & Kiesecker, 2002). In this paper we review briefly effects and implications of two contributing factors: increasing ultraviolet radiation (primarily UV-B radiation) and environmental contamination. We emphasize on research on UV radiation and interactions between UV radiation and contaminants, because there have been several recent extensive reviews on effects of contaminants on amphibians (Sparling *et al.*, 2000; Boone & Bridges, 2003).

UV-B RADIATION

UV-B (280–315 nm) radiation is the most significant biologically damaging radiation at the terrestrial surface. At the organismal level, UV-B radiation can slow growth rates, cause immune dysfunction and result in sublethal damage (Tevini, 1993). Living organisms absorb UV-B radiation efficiently. Critical biomolecules do not absorb light of higher wavelength (UV-A; 315–400 nm) as efficiently, and stratospheric ozone absorbs most light of lower wavelengths (UV-C; 200–280 nm) (Cockell & Blaustein, 2001). UV-B radiation can lead to mutations and cell death. However, increasing evidence suggests that UV-A radiation, especially in combination with other stressors, is also damaging to living organisms (see below).

Over evolutionary time, UV radiation has been an important stressor on living organisms (Cockell, 2001). Natural events, including impacts from comets and asteroids, volcanic activity, 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 cause significant ozone damage for a few years (Cockell & Blaustein, 2000). Moreover, the amount of ozone damage due to natural events depends upon a number of variables, including the magnitude of the event (e.g. size of the asteroid making impact; Cockell & Blaustein, 2000). This is different from modern-day human-induced production of chlorofluorocarbons (CFCs) and other chemicals

that deplete stratospheric ozone continuously, resulting in long-term increases in UV-B radiation at the surface of the earth. 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. Data from several sources indicate that levels of UV-B radiation recently have risen significantly (especially since 1979) in the tropics and in temperate regions (Kerr & McElroy, 1993; Herman *et al.*, 1996; Middleton *et al.*, 2001).

UV-B radiation can kill amphibians directly, cause sublethal effects or act in concert with contaminants, pathogens or with changes in climate to adversely affect amphibians, as we describe in more detail below. 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; Blaustein & Kiesecker, 2002).

FIELD EXPERIMENTS ON HATCHING SUCCESS

When hatching rates are compared between groups of embryos exposed to ambient UV-B radiation with those shielded from UV-B in field experiments, the embryos of many species displayed greater hatching rates in shielded regimes (reviewed recently in Blaustein *et al.*, 1998; Blaustein *et al.*, 2001; Table 1). This is true for frogs, toads and salamanders from a wide variety of sites, elevations and habitats in North America, Europe and Australia (Blaustein *et al.*, 1998, 2001; Table 1). However, hatching success of other amphibian species was not affected by UV-B radiation (Blaustein *et al.*, 1998). Even in the same experiments, conducted at the same time and at the same sites, hatching success of some amphibians is greatly reduced by UV-B radiation, whereas hatching success of other species is not affected. For example, in Oregon hatching success of Cascades frogs (*Rana cascadae*) and western toads (*Bufo boreas*) was lower when exposed to ambient UV-B radiation than when eggs were shielded from UV-B (Blaustein *et al.*, 1994b). However, hatching success of Pacific tree frogs (*Hyla regilla*) was not significantly different between UV shielded and UV exposed treatments (Blaustein *et al.*, 1994b). Similarly, in Spain

Table 1 Examples of studies showing lethal and sublethal effects of ultraviolet radiation alone or in synergism with other factors on amphibians

Species	Effect of UV	Synergistic effects with UV	References
Frogs and toads			
<i>Bufo boreas</i>	Increases embryo mortality; developmental abnormalities; hampers antipredator behaviour	With <i>Saprolegnia</i> (fungus) increases embryo mortality	Worrest & Kimeldorf (1976); Blaustein <i>et al.</i> (1994a); Kats <i>et al.</i> (2000); Kiesecker & Blaustein (1995); Kiesecker <i>et al.</i> (2001)
<i>Bufo bufo</i>	Increases embryo mortality; reduces tadpole survival		Lizana & Pedraza (1998); Häkkinen <i>et al.</i> (2001)
<i>Crinia signifera</i>	Increases embryo mortality		Broomhall <i>et al.</i> (2000)
<i>Hyla arborea</i>	Causes skin darkening		Langhelle <i>et al.</i> (1999)
<i>Hyla cadaverina</i>	Increases embryo mortality		Anzalone <i>et al.</i> (1998)
<i>Hyla chrysoscelis</i>	Embryonic deformities		Starnes <i>et al.</i> (2000)
<i>Hyla regilla</i>	Developmental and physiological abnormalities; reduces larval survival	With nitrates reduces survival	Hays <i>et al.</i> (1996); Ovaska <i>et al.</i> (1997); Hatch & Blaustein (2003)
<i>Hyla versicolor</i>	Causes skin darkening; decreases swimming activity	With carbaryl decreases swimming activity	Zaga <i>et al.</i> (1998)
<i>Litoria aurea</i>	Behavioural avoidance of UV		van de Mortel & Buttemer (1998)
<i>Littoria peronii</i>	Behavioural avoidance of UV		van de Mortel & Buttemer (1998)
<i>Litoria verreauxii</i>	Increases embryo mortality		Broomhall <i>et al.</i> (2000)
<i>Pseudacris triseriata</i>	Embryonic deformities		Starnes <i>et al.</i> (2000)
<i>Rana aurora</i>	Inhibits growth		Belden & Blaustein (2002a)
<i>Rana arvalis</i>	Increases embryo mortality		Häkkinen <i>et al.</i> (2001)
<i>Rana catesbeiana</i>		With fluoranthene causes skin damage and hyperactivity	Walker <i>et al.</i> (1998)
<i>Rana clamitans</i>	Increases larval mortality; delayed development; morphological abnormalities		Grant & Licht (1997); Tietge <i>et al.</i> (2001)
<i>Rana cascadae</i>	Increases embryo mortality; retinal damage; developmental and physiological abnormalities; hampers antipredator behaviour	With nitrate and low pH, reduces survival and alters behaviour	Blaustein <i>et al.</i> (1994a); Hays <i>et al.</i> (1996); Fite <i>et al.</i> (1998); Kats <i>et al.</i> (2000); Hatch & Blaustein (2000)

Table 1 continued

Species	Effect of UV	Synergistic effects with UV	References
<i>Rana pipiens</i>	Increases larval mortality; causes deformities in larvae and juveniles; slows growth and development	With low pH reduces hatching success; with fluoranthene causes deformities	Long <i>et al.</i> (1995); Ankley <i>et al.</i> (1998); Hatch & Burton (1998); Monson <i>et al.</i> (1999); Smith <i>et al.</i> (2000); Tietge <i>et al.</i> (2001)
<i>Rana sylvatica</i>	Morphological and behavioural abnormalities		Grant & Licht (1997)
<i>Rana septentrionalis</i>	Increases larval mortality		Tietge <i>et al.</i> (2001)
<i>Rana temporaria</i>	Delayed growth		Pahkala <i>et al.</i> (2001)
<i>Xenopus laevis</i>	Causes skin darkening; decreases swimming activity; reduces growth	With fluoranthene causes deformities; with carbaryl changes swimming behaviour	Bruggeman <i>et al.</i> (1998); Hatch & Burton (1998); Zaga <i>et al.</i> (1998)
Salamanders			
<i>Ambystoma gracile</i>	Increases embryo mortality		Blaustein <i>et al.</i> (1995)
<i>Ambystoma macrodactylum</i>	Increases embryo mortality; causes deformities; slows growth; skin darkening	With nitrates affects growth	Blaustein <i>et al.</i> (1997a); Belden <i>et al.</i> (2000); Belden & Blaustein (2002b); Hatch & Blaustein (2003)
<i>Pleurodeles waltii</i>	UV-A enhances toxicity of PAHs		Fernandez & L'Haridon (1992, 1994)
<i>Taricha granulosa</i>	Increases activity; alters antipredator behaviour; skin darkening		Blaustein <i>et al.</i> (2000); Kats <i>et al.</i> (2000); Belden & Blaustein (2002c)
<i>Taricha torosa</i>	Increases embryo mortality		Anzalone <i>et al.</i> (1998)
<i>Triturus alpestris</i>	Skin damage; causes erratic swimming behaviour		Nagl & Hofer (1997)
<i>Triturus cristatus</i>	Skin damage; causes erratic swimming behaviour		Langhelle <i>et al.</i> (1999)

hatching success of common toads (*Bufo bufo*) was lower in UV-B exposed eggs than in those shielded from UV-B, whereas there was no effect of UV-B on the hatching success of the natterjack toad (*B. calamita*) (Lizana & Pedraza, 1998). These studies and others suggest that the embryos of certain species use various defences against the harmful effects of UV-B radiation better than others (discussed below). Even within a species, there may be variation between populations in their ability to cope with UV-B radiation (e.g. Belden & Blaustein, 2002a,b).

Sublethal effects

Mortality, as measured by hatching success, is the most extreme effect of UV-B radiation observed in field experiments such as those described above. However, even though hatching rates of some species are unaffected by ambient UV radiation, an increasing number of studies illustrate a variety of sublethal effects that are induced after exposure to UV-B radiation. These effects may appear in embryos, larvae or in adults. For example, exposure to UV-B radiation can alter the behaviour of amphibians (Nagl & Hofer, 1997; Blaustein *et al.*, 2000; Kats *et al.*, 2000) slow growth and development (e.g. Belden *et al.*, 2000; Pahkala *et al.*, 2000, 2001; Smith *et al.*, 2000; Belden & Blaustein, 2002a), or induce developmental and physiological malformations (e.g. Worrest & Kimeldorf, 1976; Hays *et al.*, 1996; Blaustein *et al.*, 1997a; Fite *et al.*, 1998; Ankley *et al.*, 2002).

Low-level exposure to simulated ambient UV-B radiation in the laboratory causes a number of developmental and physiological deformities in frogs and toads (Worrest & Kimeldorf, 1976; Hays *et al.*, 1996) (Table 1). These include oedema, skeletal anomalies and eye damage. In field experiments, long-toed salamander (*Ambystoma macrodactylum*) embryos exposed to ambient levels of UV-B radiation not only hatched at a significantly much lower frequency (14.5%) than those shielded from UV-B (95%) but also displayed a much higher proportion of deformities (Blaustein *et al.*, 1997a). Moreover, more than 90% of the survivors exposed to UV-B radiation were deformed, compared with only 0.5% that were deformed under UV blocking shields. Adult Cascades frogs from Oregon had

distinctive outer retinal abnormalities in the inferior retina that include the abnormal distribution of retinal pigment, damaged photoreceptors and the presence of large pigment filled macrophages. These abnormalities were consistent with damage due to basking in sunlight (Fite *et al.*, 1998) and may impair vision significantly.

Several experimental studies illustrated that early exposure to UV-B radiation causes delayed effects in later stages. For example, UV-B radiation did not alter the hatching success of plains leopard frogs (*Rana blairi*). However, growth and development were slower in tadpoles when they were exposed to UV-B radiation only as embryos (Smith *et al.*, 2000). Hatching success in the common frog (*Rana temporaria*) was unaffected when embryos were exposed to UV-B radiation (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 at similar stages than larvae shielded from UV-B as embryos (Pahkala *et al.*, 2001). Similarly, UV-B radiation had no effects on hatching success in red-legged frogs (*R. aurora*) (Blaustein *et al.*, 1996; Ovaska *et al.*, 1997). However, red-legged frog tadpoles exposed to UV-B radiation as embryos were smaller and less developed than tadpoles shielded from UV-B radiation as embryos (Belden & Blaustein, 2002a).

Changes in behaviour after exposure to UV-B radiation have been reported in a number of studies. For example, exposure of roughskin newts (*Taricha granulosa*) to UV-B radiation in the laboratory caused them to increase activity (Blaustein *et al.*, 2000). Changes in activity levels after exposure to UV-B radiation have been observed in a number of other species as well (e.g. Nagl & Hofer, 1997; Zaga *et al.*, 1998; Table 1). Antipredator behaviours may also be affected by exposure to UV-B radiation. For example, Cascades frog tadpoles and juvenile western toads (*Bufo boreas*) exposed to low levels of UV radiation did not respond to the chemical cues of predators as quickly as those that were not exposed (Kats *et al.*, 2000).

Defences against UV-B exposure

Selection pressures associated with exposure to solar radiation over evolutionary time have

resulted in molecular, physiological and behavioural mechanisms that limit amphibian exposure to UV-B radiation or help repair UV-B-induced damage after exposure (Epel *et al.*, 1999; Hofer, 2000; Cockell, 2001; Blaustein & Belden, 2003). Thus amphibians may avoid sunlight, possess pigmentations that absorb UV light, surround their eggs in UV-protective jelly, wrap their eggs in leaves or use a variety of molecular mechanisms to repair UV damage (discussed in detail in Blaustein & Belden, 2003; see also Belden & Blaustein, 2002c).

Repair of UV-induced DNA damage seems to be especially important in amphibians exposed to solar radiation (Blaustein *et al.*, 1994b). Exposure to UV can induce formation of photoproducts such as cyclobutane pyrimidine dimers (CPDs) that damage DNA. Formation of CPDs inhibits proper transcription and translation, and can lead to mutation and cell death (Sancar & Tang, 1993; Hearst, 1995). Amphibian species may be relatively resistant to UV-B if they can remove or repair UV damaged DNA efficiently (Blaustein *et al.*, 1994b). The major process by which this is performed in amphibians is through enzymatic photoreactivation, using the enzyme CPD-photolyase (Friedberg *et al.*, 1995). A second related enzyme, [6-4]-photolyase, similarly uses light energy to reverse pyrimidine-[6-4]-pyrimidone photoproducts ([6-4] photoproducts). Moreover, multiprotein broad specificity excision repair processes can remove CPDs and [6-4] photoproducts (discussed in Blaustein *et al.*, 2001). Both mechanisms may be used simultaneously but CPD-photolyase appears to be the first level of defence against CPDs for many organisms exposed to solar radiation (Pang & Hays, 1991; Blaustein *et al.*, 1994b; Friedberg *et al.*, 1995).

Research on embryonic amphibians in the Pacific Northwest, United States demonstrated a strong correlation between photolyase activity and resistance to UV-B exposure (e.g. Hays *et al.*, 1996; Blaustein *et al.*, 1994a; Blaustein *et al.*, 1996; Blaustein *et al.*, 1999). For example, eggs of the most UV-resistant species in field experiments (e.g. *H. regilla*, *R. aurora*, *R. pretiosa* and *R. luteiventris*) have higher CPD-photolyase activity than eggs of more susceptible species (e.g. *R. cascadae*, *B. boreas*, *A. macrodactylum*, *A. gracile*) (Table 1; Blaustein *et al.*, 1998). A similar trend exists for Australian tree frogs, although it is not

as strong as in the Northwest studies (van de Mortel *et al.*, 1998). There is also a general correlation between the amount of photolyase activity, exposure of eggs to solar radiation in nature and, based on limited data, population status (Blaustein *et al.*, 1998). The embryos of amphibian species whose populations are in decline have less photolyase activity than embryos of species whose populations are not declining (Blaustein *et al.*, 2001).

Within a species, individuals from one population may differ from members of another population in their sensitivity to UV-B radiation. This has recently been shown experimentally in long-toed salamanders (*A. macrodactylum*) (Belden & Blaustein, 2002b). The larvae of long-toed salamanders living at low elevations were more sensitive to UV-B radiation than larvae from higher elevation sites. Inter-population differences in sensitivity to UV-B radiation may be due to differences in the ability to repair DNA damage, pigmentations, jelly coats or in behaviour and may reflect local adaptations.

CONTAMINANTS

A wide array of contaminants affects amphibians, including pesticides, herbicides, fungicides, fertilizers and numerous other pollutants (Blaustein *et al.*, 1997b; Bonin *et al.*, 1997; Sparling *et al.*, 2000; Boone & Bridges, 2003). Contaminants may be spread globally or act on a local scale. Contaminants transported atmospherically have the potential to affect amphibians in remote, relatively undisturbed environments. Even low levels of contaminants from atmospheric deposition are potentially harmful to amphibians.

There is a relatively large literature on the effects of contaminants on amphibians (reviewed in Sparling *et al.*, 2000; Boone & Bridges, 2003), yet little is known about how contaminants affect amphibians at the population level (Alford & Richards, 1999). In this section, we provide some examples of how contaminants affect amphibians.

Pesticides

Numerous pesticides have permeated the landscape, including many 'new' postchlorinated hydrocarbons such as organophosphates, carbamates, pyrethroids, herbicides and fungicides.

According to Cowman & Mazanti (2000), more than 100 types of carbamates and organophosphates alone are applied at a rate of about 200 million acre treatments per year in the United States. In combination, the effects herbicides, fungicides and chlorinated hydrocarbons have on amphibians are probably dramatic. Although we do not have information on how all pesticides affect amphibians, accumulating evidence from laboratory studies have demonstrated the lethal effects of many of them (e.g. reviews by Cooke, 1981; Blaustein *et al.*, 1997b; Bridges & Semlitsch, 2000; Sparling *et al.*, 2000; Boone & Bridges, 2003; and references therein). Sublethal effects after exposure to pesticides are also common. For example, pesticides may hamper growth and development, and they may cause developmental and behavioural abnormalities in amphibians (see References in Blaustein *et al.*, 1997b; Bridges & Semlitsch, 2000; Sparling *et al.*, 2000; Boone & Bridges, 2003).

Although few studies have documented effects of pesticide exposure under natural conditions, DDT spraying in Oregon forests may have caused mortality in a population of western spotted frogs, *Rana pretiosa* (Kirk, 1988). In California atmospheric deposition of organophosphate pesticides from the highly agricultural Central Valley may have contributed to declines of frogs (Aston & Seiber, 1997; Sparling *et al.*, 2001). Davidson *et al.* (2001) concluded that patterns of decline in red-legged frogs in California were caused most probably by pesticides carried upwind from the Central Valley. Russell *et al.* (1995) found residues of several pesticides in tissue of spring peepers, *Pseudacris crucifer* from a Canadian National Park 26 years after the application of pesticides had ceased. This suggests that pesticides can have long-term effects on amphibians. The effects of pesticides and their interactions with other contaminants on amphibians are discussed in more detail in sections below.

Heavy metals and acidification

Intensive agricultural and industrial production and pollution from mines has increased the prevalence of heavy metals (and other pollutants) in surface waters that may ultimately affect amphibian populations. Metals such as aluminium (Al), lead (Pb), zinc (Zn), cadmium (Cd), mercury

(Hg), silver (Ag), copper (Cu), arsenic (As), manganese (Mn), molybdenum (Mo) and antimony (Sb) have a number of effects on amphibians. They can be lethal or induce sublethal effects such as slowing growth and development and altering behaviour (e.g. Blaustein *et al.*, 1997b and references therein; Lefcort *et al.*, 1998, 1999; Raimondo *et al.*, 1998).

Several studies have shown damaging effects of heavy metals on amphibians from coal combustion and other sources which contain heavy metals. For example, Rowe *et al.* (1996) found a higher incidence of oral deformities in bullfrog (*Rana catesbeiana*) larvae at a site polluted with coal ash compared with larvae from sites without coal ash. Rowe *et al.* (1998a) introduced *R. catesbeiana* embryos to a site polluted by coal combustion waste and an unpolluted site. Larval survival was lower and the incidence of oral deformities in larvae was higher at the polluted site compared with larvae at the unpolluted site. Moreover, larvae collected from a polluted site had higher metabolic rates than those collected at an unpolluted site. Larvae introduced to a polluted site had higher metabolic rates than those introduced to another unpolluted site (Rowe *et al.*, 1998b). Higher metabolic rates could indicate a physiological cost of exposure to contaminants.

Rowe *et al.* (2001) transplanted larvae of southern toads (*Bufo terrestris*) collected from a clean site to one polluted with heavy metals from coal combustion waste and to a reference site. Throughout the early larval period, survival of southern toads transplanted to polluted sites was lower than those at unpolluted sites. Midway through the larval period, surviving larvae from the polluted site were transplanted to the reference site, or replaced at the polluted site. All those reintroduced to the polluted site died before metamorphosis, while survival to metamorphosis was significantly greater for those transplanted from the polluted to the reference site.

Acidification may contribute to local extinctions of amphibians. For example, Harte & Hoffman (1989) suggested that episodic acidification might have contributed to declines in tiger salamander (*Ambystoma tigrinum*) populations in Colorado. Acid pollution seems to have played a role in the decline of natterjack toads (*Bufo*

calamita) in Britain (Beebee *et al.*, 1990). Acidification alone, or in combination with other factors can have a number of adverse effects on amphibians (Dunson *et al.*, 1992).

In many cases the effects that heavy metals have on amphibian survival are linked closely to acidification, because heavy metals may leach from soils in contact with acidic water. For example, the solubility of aluminium, an abundant element in soils, increases as water pH drops. Inorganic monomeric aluminium often acts synergistically with pH to cause embryo mortality in amphibians (Clark & Hall, 1985; Clark & LaZerte, 1985; Freda & McDonald, 1990; Freda *et al.*, 1990). Laboratory experiments have shown that aluminium levels as low as 10–20 µg per litre at pH 4.7 can cause reduced hatching success of *B. americanus* and *R. sylvatica* eggs (Clark & LaZerte, 1987). Experiments with embryos and larvae of the leopard frog, *Rana pipiens* demonstrated that aluminium alleviated the toxic effects of low pH (4.2–4.4), while becoming toxic at higher pH levels (4.6–4.8) (Freda & McDonald, 1990). Exposure of amphibian embryos and larvae to heavy metals also can have sublethal effects such as reduced growth and development and changes in behaviour (Clark & Hall, 1985; Clark & LaZerte, 1985; Clark & LaZerte, 1987; Perez-Coll *et al.*, 1988; Freda & McDonald, 1990; Freda *et al.*, 1990; Herkovits & Perez-Coll, 1990; Herkovits & Perez-Coll, 1993; Jung & Jago, 1995).

NITROGEN-BASED FERTILIZER POLLUTION

Nitrogen pollution from anthropogenic sources enters aquatic systems via agricultural runoff or percolation associated with nitrogen fertilization, livestock, precipitation and effluents from industrial and human wastes (Rouse *et al.*, 1999). There are well-documented effects on susceptibility of amphibian species to nitrogenous fertilizers (e.g. Hecnar, 1995; Marco *et al.*, 1999; Rouse *et al.*, 1999). For example, recent experimental studies demonstrated 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). Even after metamorphosis, amphibians of some species

may alter their feeding behaviour in the presence of nitrogenous fertilizers (e.g. Hatch *et al.*, 2001).

CONTAMINANTS AS ENDOCRINE DISRUPTORS

One hypothesis for the decline of amphibian populations is that endocrine disruptors, including several pesticides and their degradation products, have altered reproductive systems (Colborn & Clement, 1992; Stebbins & Cohen, 1995; Hayes, 2000). Indeed, evidence is accumulating showing that certain contaminants do affect reproductive and endocrine systems in amphibians.

Recently, Hayes *et al.* (2002) showed that the herbicide atrazine, used commonly in the United States, induced hermaphroditism and demasculinized the larynges of exposed African clawed frog (*Xenopus laevis*) males. Hayes *et al.* (2002) hypothesized that atrazine disrupted steroidogenesis resulting in demasculinization and hermaphroditism.

Contaminants may provoke a stress response resulting in increased production of corticosterone, which can interfere with reproductive–hormonal systems (see Hayes, 2000 for details). For example, Hopkins *et al.* (1997) found that adult southern toads (*Bufo terrestris*) at a site contaminated with coal ash displayed higher corticosterone and testosterone levels than those at a non-polluted site, suggesting that coal ash stimulated production of corticosterone and testosterone. In a field experiment they transplanted toads to both sites, and toads at the polluted site had higher corticosterone levels after both 10 days and after 12 weeks than toads at the unpolluted site (Hopkins *et al.*, 1997). Hopkins *et al.* (1999) injected toads with adrenocorticotrophic hormone (ACTH), and measured the increase in circulating corticosterone levels. Toads from a reference site exhibited an increase in corticosterone levels, while toads from a polluted site showed no increase. Because toads from a polluted site with coal ash already have elevated corticosterone levels, they may be unable to exhibit a normal stress response.

Gendron *et al.* (1997) found evidence for hormonal disruption in the mudpuppy (*Necturus maculosus*) exposed to chlorinated hydrocarbons in the St Lawrence and Ottawa rivers (Canada). At sites contaminated with polychlorinated biphenyls

and organochlorine pesticides, changes in the secretion of corticosterone were observed. Gendron *et al.* (1997) suggested that these hormonal changes could hinder reproductive performance.

Park *et al.* (2001) studied effects of endosulfan, a cyclodiene organochlorine insecticide, on reproductive biology of red-spotted newt (*Notophthalmus viridescens*). In laboratory tests male newts preferred to associate with females that were unexposed to endosulfan in preference to females that had been exposed to low levels of endosulfan. In addition, endosulfan altered the morphology of pheromonal glands in females and interfered with hormonal signalling and mating success (Park *et al.*, 2001).

SYNERGISM BETWEEN CONTAMINANTS AND UV RADIATION

Contaminants including pesticides, polycyclic aromatic hydrocarbons (PAHs; multiple-ringed hydrocarbons that contaminate ponds or streams via road runoff, direct industrial discharge, or atmospheric deposition), fertilizers and low pH all interact with UV-B in a manner that damages developing amphibians. Contaminants transported atmospherically are probably exposed to UV radiation, which can increase their toxicity. Furthermore, atmospheric processes could alter nutrient dynamics or increase water clarity, allowing for greater penetration of ultraviolet radiation (Wright & Schindler, 1995). Some studies have concluded that UV-B is an additional stressor that overwhelms an animal's defences and makes it more susceptible to other anthropogenic stressors (Tevini, 1993).

Long *et al.* (1995) found reduced larval survival when leopard frogs were exposed to both UV-B and low pH, when UV intensity was increased to levels expected at high elevations and pH lowered to 5.0 or 4.5. No significant effects were attributed to either pH or UV-B alone. Hatch & Blaustein (2000) showed that survival and activity levels of Cascades frog larvae were significantly reduced when exposed to low pH, high nitrate levels and UV-B radiation together.

Moreover, Hatch & Blaustein (2003) tested the combined effects of UV-B and nitrate on larval survival, mass and length in Pacific treefrogs and

long-toed salamanders in both high and low elevation populations for each species. They found that UV-B and nitrate together reduced the mass of larval *H. regilla*. In the high elevation experiment, they found that UV-B and nitrate together reduced the survival of larval *H. regilla*. In both the low-elevation and the high-elevation experiment, nitrate increased the mass of larval *A. macrodactylum*. However, in the high elevation experiment, this result occurred only when UV-B was blocked. This result indicates that the effects of nitrate addition could depend upon the presence of other factors such as UV-B.

A synergistic toxic effect may occur when a contaminant (or one of its breakdown products or metabolites) absorbs UV radiation and the contaminant is altered to a more toxic form. For example, some PAHs are phototoxic when UV-A (320–400 nm) radiation is present. In the presence of UV-A, PAHs can be highly toxic to amphibians (Hatch & Burton, 1998; Walker *et al.*, 1998; Monson *et al.*, 1999).

UV-A enhanced the toxicity of the PAHs anthracene, benzo(a)pyrene and 1,12-benz(a)-anthraquinone to larval newts (*Pleurodeles waltl*) exposed in the laboratory for 6 days (Fernandez & L'Haridon, 1992). Concurrent exposure to UV-A and sunlight enhanced the toxicity of benzo(a)pyrene to newts (Fernandez & L'Haridon, 1994). Similarly, exposure to UV-A after newts were exposed to benzo(a)pyrene resulted in greater toxicity than did exposure to benzo(a)pyrene alone without UV-A; presumably, these animals had accumulated benzo(a)pyrene during exposure (Fernandez & L'Haridon, 1994). In another study, northern leopard frog embryos were exposed to the anthracene and either 30 min or 5 h of sunlight, resulting in an LC50 of 65 or 25 µg/L, respectively, with the end point being mortality (Kagan *et al.*, 1984).

Hatch & Burton (1998) exposed three amphibian species, *Xenopus laevis*, *Rana pipiens* and the spotted salamander *Ambystoma maculatum*, to the PAH fluoranthene and UV in the laboratory and in controlled outdoor exposures. In the laboratory, *X. laevis* exhibited the most deformities due to the combination of UV and fluoranthene, while *A. maculatum* exhibited no deformities due to the treatments. Outdoor experiments demonstrated that newly hatched larvae were more sensitive than embryos to phototoxic fluoranthene.

In a similar study, Monson *et al.* (1999) investigated the response of larval *R. pipiens* to fluoranthene with and without a constant (24 h) UV (298–370 nm) exposure. Animals were exposed to fluoranthene for 48 h and then either analysed for fluoranthene bioaccumulation, placed in clean water without UV or placed in clean water with UV (wavelengths stated above). Within 2 days, 80% of the chemical was metabolized by larvae; animals exposed to higher levels of fluoranthene were able to metabolize the compound more rapidly. Another study found that fluoranthene in the presence of UV causes behavioural and histological effects in bullfrogs over a 4-day exposure (Walker *et al.*, 1998). Two-day exposure to 10 µg/L fluoranthene in the presence of UV caused microscopic alterations in the skin. Hyperactivity was noted at an exposure concentration of 60 µg fluoranthene/L after 2 and 4 days, and at 40 µg/L after 4 days of exposure. Overall swimming speed did not change; however, the distance travelled and activity levels were significantly increased in these treatments.

Under certain circumstances UV-B radiation and exogenous chemicals, acting alone or together, may cause limb malformations in amphibians that have been the subject of numerous recent reports (e.g. Ouellet *et al.*, 1997; Gardiner & Hoppe, 1999; Bidges, 2000; Ankley *et al.*, 2002; Johnson *et al.*, 2002). Investigating causes for limb deformities, LaClair *et al.* (1998) studied the breakdown products of the insect growth inhibitor methoprene in combination with sunlight, water and microorganisms in a 4-day exposure in the laboratory. They found that deformities of the body (e.g. curvature, oedema) were caused by some of the purified, extracted and sunlight-exposed breakdown products of methoprene. Ankley *et al.* (1998) exposed eggs of *Rana pipiens* to UV-A and UV-B alone and in combination with methoprene concentrations ranging from 2 to 500 µg/L. They concluded that methoprene exposure did not cause limb deformities, but UV alone caused delayed development, lower weight and deformities at metamorphosis.

Carbaryl is an insecticide with a peak UV absorbance at approximately 300 nm. It has widespread use and is applied to croplands, rangelands, forests, wetlands, oceans and sewage treatment plants to control animal pests (Relyea & Mills, 2001 and references therein). Because of

its wide use in a variety of systems carbaryl can enter amphibian breeding habitats, such as ponds or wetlands, via aerial spraying of nearby fields, runoff from local application, or potentially via atmospheric deposition (Boone & Semlitsch, 2001; Davidson *et al.*, 2001; Relyea & Mills, 2001).

Carbaryl alone can be lethal to amphibians or induce a variety of sublethal effects (e.g. Bridges, 1997; Bridges & Semlitsch, 2000). Carbaryl can also interact with UV radiation. For example, Zaga *et al.* (1998) investigated the interactions between carbaryl and UV-B on *X. laevis* and *Hyla versicolor* embryos and larvae. Approximately 1.5% of ambient UV-B intensity was sufficient to photoactivate carbaryl and result in a 10-fold increase in toxicity. Swimming activity was decreased by UV-B alone and by UV-B in combination with carbaryl. Additionally, *X. laevis* demonstrated increased swimming distance with increasing carbaryl concentration; this response was reversed with concurrent exposure to UV-B, whereby larvae reduced their activity. The photosensitization experiment exposed *X. laevis* to non-irradiated carbaryl without UV and then transferred animals to clean water with UV exposure. Greater mortality was observed at the various carbaryl concentrations tested with increasing levels of UV-B. Zaga *et al.* (1998) investigated further the photoactivation of carbaryl. Mortality increased significantly when the chemical was irradiated, suggesting that the mechanism of toxicity involved photoproducts of carbaryl.

ENVIRONMENTAL STRESSORS AND INCREASED SUSCEPTIBILITY TO DISEASE

Diseases probably play an important role in amphibian population declines (discussed in Alford & Richards, 1999; Daszak *et al.*, 1999 and references therein; Kiesecker *et al.*, 2001; Blaustein & Kiesecker, 2002). It is possible that diseases, such as 'emerging infectious diseases' (EIDs as defined in Daszak *et al.*, 1999) are the result of environmental changes and could be triggered by various co-factors. For example, a complex combination of El Niño/Southern Oscillation (ENSO) events with accompanying changes in precipitation, reduced water levels and

increased exposure to UV-B radiation appears to be responsible for outbreaks of the pathogenic oomycete, *Saprolegnia*, killing amphibian embryos in Oregon (Kiesecker *et al.*, 2001). Environmental changes and contaminants may also play a role in outbreaks of chytridiomycosis (chytrid; discussed in detail in Blaustein & Kiesecker, 2002), a fungal disease found on amphibians from several continents (Daszak *et al.*, 1999; Longcore *et al.*, 1999). For example, chytrid outbreaks in Costa Rica may be triggered not only by environmental changes, such as changes in precipitation and temperature (Lips, 1998; Pounds *et al.*, 1999; Pounds, 2001), but also by environmental contamination. Pounds & Crump (1994) suggests 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). Moreover, increasing levels of UV-B radiation in regions where amphibian populations have declined in tropical America may also play an important role in triggering disease outbreaks (Blaustein & Kiesecker, 2002). Recent evidence implicates changes in water pH triggering chytrid outbreaks in Spain (Bosch *et al.*, 2001).

Although few studies have shown direct effects of contaminants increasing susceptibility of amphibians to disease, Taylor *et al.* (1999) found that low doses (0.011 and 0.0011 mg/g body weight) of the pesticide malathion were high enough to increase the susceptibility of Woodhouse toads (*Bufo woodhouseii*) to lethal infections from injections of the bacterium, *Aeromonas hydrophila*, that causes 'red-leg' disease. Used crankcase oil in combination with silt may make amphibians more susceptible to *Saprolegnia* infection (Lefcort *et al.*, 1997).

COMMUNITY-LEVEL EFFECTS

UV radiation and contaminants may affect community structure by altering competition and predation. Thus, UV-sensitive amphibian species may disappear in certain communities and UV tolerant species may increase in numbers (Blaustein & Belden, 2003). Such changes in species composition may result in drastic changes within ecological communities because different species of amphibians have different predators,

feed on different foods and react differently to abiotic components of the environment.

Low pH can change the strength of intra- and interspecific competition among anuran larvae (Warner *et al.*, 1991, 1993; Pehek, 1995) and can influence predatory interactions between salamander larvae and anuran prey (e.g. Sadinski & Dunson, 1992; Kiesecker, 1996).

Kiesecker (1996) raised western chorus frog (*Pseudacris triseriata*) and *A. tigrinum* larvae in the laboratory at a range of naturally occurring pH and found that low pH decreased growth and increased time to metamorphosis in *A. tigrinum* but did not affect growth and development in *P. triseriata*. In a second laboratory experiment, low pH decreased *A. tigrinum* predation on *P. triseriata*, possibly by reducing growth and therefore gape in *Ambystoma*, thereby reducing their ability to capture prey (Kiesecker, 1996).

Boone & Semlitsch (2001) exposed amphibians to carbaryl in a multi-factorial design incorporating three species of competing anuran larvae, at high and low densities, and predators, *Notopthalmus viridescens*. Carbaryl levels were low, high and absent. Species examined were *Bufo woodhouseii*, *Hyla versicolor* and *Rana clamitans* (green frog). Survival to metamorphosis of *B. woodhouseii* larvae increased under low carbaryl levels, when total anuran density was low. Carbaryl interacted with predators to affect *H. versicolor* larvae. In carbaryl-absent and low carbaryl treatments, predators reduced *H. versicolor* mass at metamorphosis. However, in high carbaryl treatments, *H. versicolor* had greater mass at metamorphosis in predator treatments compared to predator-absent treatments, possibly because of reduced predator activity or effectiveness. In addition, an interaction between carbaryl and anuran density affected *H. versicolor*. Under conditions of low or high carbaryl levels, survival to metamorphosis of *H. versicolor* was greater in high-density treatments compared to low-density treatments. The authors suggest this was caused by an indirect effect of carbaryl on food web dynamics. They noted that carbaryl-treated ponds experienced zooplankton die-offs and algal blooms, and hypothesized that increased algal food resources released *H. versicolor* from competition with other amphibians.

Relyea & Mills (2001) showed that in the presence of predator chemical cues, low levels of

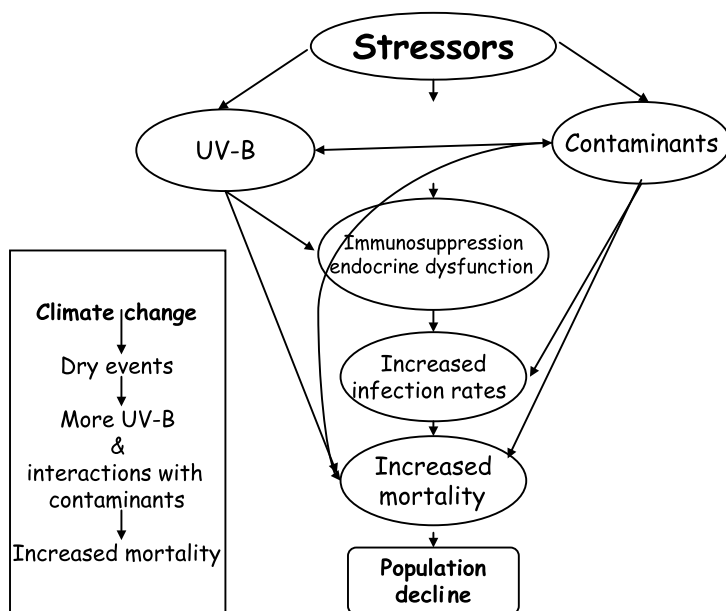


Fig. 1 Complex interactions among abiotic and biotic factors affect amphibian populations. Amphibian populations are subjected simultaneously to numerous stressors that interact in a dynamic fashion. For example, contaminants and ultraviolet-B (UV-B) radiation may kill amphibians or induce sublethal effects. Moreover, these factors may interact with one another and with other agents. Certain abiotic stressors may hamper the immune system of amphibians, making them more susceptible to pathogenic infection. Climate change (left box) also plays a role in amphibian population declines. For example, climate-driven dry events increases the amount of exposure to UV-B radiation and lower water levels may lead to a greater concentration of contaminants. Amphibian eggs and larvae may be especially vulnerable to such events.

carbaryl become highly toxic to larval amphibians. This study is unique in that synergism occurred between predator cues, a common biotic factor and the pesticide. Relyea & Mills (2001) suggest that, in general, predator-induced stress in animals may induce similar interactions among stressed animals and other pesticides. The mechanism underlying the pesticide–predator interaction is unknown.

Alterations of nutrient input could have significant consequences for amphibians. Nitrate concentrations in some North American watersheds are probably high enough to cause death and developmental anomalies in amphibians and impact other animals in aquatic ecosystems (Rouse *et al.*, 1999), which could significantly affect overall community structure. Moreover, nitrogen-based fertilizer pollution affects amphibian prey, such as insects, and their predators such as fish (Rouse *et al.*, 1999).

CONCLUSIONS

Amphibians, like other organisms, encounter a mixture of contaminants and increasing levels of UV radiation, with resulting effects that are often complex (Fig. 1). Both abiotic and biotic factors can interact with one another to impact amphibians severely at various stages of development. Although some populations of amphibians are undoubtedly affected by such interactions, there are no clearly documented cases of how these interactions affect long-term population dynamics. In fact, UV-B radiation and contaminants may have different effects on populations as they impact different life stages (Biek *et al.*, 2002; Vonesh & De la Cruz, 2002). This depends upon the life history characteristics of the species. For example, species especially prone to damage at the egg stage may be more affected at the population level if they lay relatively few eggs compared with

species that lay more eggs. What is clear though, is that studies of single stressors alone or studies of a single life history stage may not provide an overall understanding of how amphibians are impacted by environmental stressors in complex environments.

Besides differential effects on different life stages, effects of UV radiation and chemical contamination on amphibian populations depend upon a number of other variables. This includes the length and levels of exposure, sensitivity and numerous ecological factors, all of which vary between populations, species and region. Moreover, effects may be specific to the field context in question, and should be evaluated using *in situ* assays at specific sites. Continued exposure to increasing levels of UV radiation and contaminants, either alone or in combination, are probably taking a heavy toll on amphibian populations. However, there are few studies documenting population changes due to exposure to UV radiation and contaminants. Long-term studies, preferably with experimental components, on the effects of multiple stressors are necessary to understand more fully how amphibian populations are affected so that conservation efforts can be implemented to ameliorate the problem.

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