Global Increases in Ultraviolet B Radiation: Potential Impacts on Amphibian Development and Metamorphosis

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ABSTRACT

Levels of ultraviolet B radiation (UVBR) reaching the Earth's surface have increased since the 1970s as a result of stratospheric ozone depletion caused by the emission of ozone-depleting substances (ODSs) such as chlorofluorocarbons. Despite international agreements to phase out harmful ODSs, these substances are persistent, and even under the most optimistic scenarios, stratospheric ozone levels will not return to pre-1980 levels for several decades. Furthermore, climate change may enhance chemical stratospheric ozone depletion. Global phenomena such as climate change, ozone depletion, and acidification of aquatic ecosystems interact to modify dissolved organic carbon levels in aquatic systems, thereby increasing the penetration of UVBR. Since amphibians inhabit both aquatic and terrestrial habitats and have unshelled eggs and permeable skin, they are vulnerable to changes in environmental conditions and habitat quality. Increased exposure of amphibians to UVBR can produce lethal and sublethal effects, especially in individuals that do not possess adequate defense mechanisms to protect themselves. In this article, we discuss worldwide increases in UVBR and the adverse effects of UVBR exposure on amphibians. Specifically, studies on the effects of UVBR on amphibian development and metamorphosis are summarized, and possible mechanisms of thyroid system disruption caused by UVBR exposure are considered.

Introduction

Disturbances in global environmental conditions such as climate change have received increased attention from the media and the scientific community, but our knowledge of detailed mechanisms remains incomplete. Consequently, no clear plan for international cooperation has been developed. Climate change is a factor that plays an important role in recent ozone depletion (Perin and Lean 2004; Aucamp 2007; McKenzie et al. 2007). In addition, ozone depletion, climate change, and acid rain are large-scale atmospheric stressors that interact to increase the amount of ultraviolet B radiation (UVBR) reaching aquatic organisms and to disrupt essential biogeochemical processes on which the aquatic ecosystem depends.

In light of our growing awareness of anthropogenic contributions to atmospheric disturbances, it is important to investigate how our actions affect the health and survival of amphibian populations. This is especially essential when one considers the importance of amphibians in the food chains of various ecosystem communities and as biological indicators of environmental health. Several studies have shown that increased exposure of amphibians to UVBR can negatively affect their survival and produce a variety of sublethal effects. For example, developing amphibians subjected to UVBR often exhibit developmental anomalies including spinal curvature, edema, and eye and limb malformations (e.g., Hays et al. 1996; Blaustein et al. 1997; Ankley et al. 2000; Croteau et al. 2008). Disruptions in the development and metamorphosis of amphibians subjected to UVBR have been reported in many studies and require careful consideration; however, the literature on this issue has not yet been reviewed in a comprehensive way.

Here we discuss global increases in UVBR with regard to amphibian exposure, and we review existing data on the influence of UVBR on the rate of amphibian development and metamorphosis. Although several studies have demonstrated that UVBR affects amphibian development, none have considered or discussed the possible mechanism(s) of action behind this UVBR-mediated effect. Thyroid hormones (THs) stimulate developmental and metamorphic transformations in amphibians (Shi 2000). Therefore, we consider the small number of studies that have demonstrated impacts of ultraviolet radiation (UVR) on the thyroid system of organisms and discuss these findings in terms of possible mechanisms of action of UVBR on the thyroid system of affected amphibians.

Global Amphibian Population Declines

Amphibian populations have been declining on a global scale since the 1960s (Houlahan et al. 2000) and are more threatened and in faster decline than populations of birds or mammals (Stuart et al. 2004). For example, 32.5% of all amphibian species are threatened worldwide (i.e., listed in the International Union

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for Conservation of Nature and Natural Resources [IUCN] Red List categories of vulnerable, endangered, or critically endangered), and 7.4% are listed as critically endangered (IUCN category of highest threat), compared with 12% and 1.8% of bird species and 23% and 3.8% of mammalian species, respectively. These percentages are likely to be underestimates because there is an inadequate amount of information to determine the status of approximately one-quarter of all amphibian species (Stuart et al. 2004).

Since the 1990s, several comprehensive reviews on amphibian population declines have been published (e.g., Stebbins and Cohen 1995; Alford and Richards 1999; Corn 2000; Blaustein and Kiesecker 2002; Blaustein et al. 2003, 2004; Collins and Storfer 2003; Lannoo 2005), and there has been a dramatic increase in research on the effects and mechanisms behind the different anthropogenic factors that may be negatively affecting individuals in the wild. However, the causes of population declines in several regions of the world remain to be elucidated. The main stressors that have been postulated as possible contributors to reductions in amphibian populations include (1) introduced/invasive species, (2) overexploitation, (3) habitat modification/loss, (4) global environmental change (including increasing UVBR and global climate change), (5) environmental contamination, (6) disease, and (7) interactions among various factors (see reviews listed above). Because it is likely that a number of stressors are interacting in the environment to affect amphibian populations, it has been difficult for researchers to establish what specifically is causing the observed declines. Stuart et al. (2004) recently stated that habitat loss and overexploitation have caused the loss of many of the 435 rapidly declining amphibian species identified using the IUCN Red List; however, 48% of these are enigmatic decline species, which are declining quickly where suitable habitat remains, for unknown reasons.

Over the past 3 decades, there have been concerns regarding the biological consequences of enhanced UVBR levels reaching the Earth's surface. Many scientists believe that increased exposure of amphibians to UVBR may have contributed to the decline of several populations worldwide. It is very difficult to confirm that UVBR has affected wild populations because it is impossible to assess the harm caused to populations that have already declined or that no longer exist. Nevertheless, results have demonstrated that UVBR exposure can be detrimental to amphibian health (discussed in further detail below) and may play an important role in the decline of some amphibian populations. Studies have shown that there is a strong correlation between the activity of photolyase (an enzyme that repairs DNA damage), exposure of eggs to solar radiation, and resistance to UVBR (Blaustein et al. 1994, 1998, 2001). The hatching success of *Bufo boreas* (western toad) and *Rana cascadae* (cascade frog) embryos was negatively affected by ambient UVBR in their natural oviposition site. It was found that these animals had lower photolyase levels and that their populations were experiencing significant declines when compared with those of *Hyla regilla* (Pacific tree frog) embryos, which had higher hatching success and whose populations were not known to be in decline (Blaustein et al. 1994). However, other defense mechanisms and the depth of oviposition can also affect the extent of UVBR-induced damage in amphibians and should be considered when making such correlations (Smith et al. 2002). In addition, remote sensing data from 20 sites indicates that regions in Central and South America where UVBR levels have increased since 1979 overlap with areas where many amphibian population declines have been reported (Middleton et al. 2001).

Despite mounting evidence from laboratory and field experiments that UVBR exposure is harmful to amphibians, researchers are required to extrapolate experimental results to natural systems and have yet to establish that amphibian population reductions have actually been caused by UVBR. Importantly, however, a recent meta-analysis using a substantial body of literature on the effects of UVBR in aquatic ecosystems has clearly demonstrated that UVBR has a large negative impact on the growth and survival of both marine and freshwater organisms (including amphibians), an effect that crosses life-history stages, habitats, trophic groups, and experimental venues (Bancroft et al. 2007). Numerous studies have shown that UVBR is detrimental to the health of various aquatic species—such as phytoplankton, zooplankton, and fish (reviewed in Perin and Lean 2004)—and has the potential to affect species at the population level. For example, wild fish populations of *Oncorhynchus mykiss* (steelhead trout), *Oncorhynchus kisutch* (coho salmon), *Oncorhynchus tschwytscha* (chinook salmon), and *Salmo salar* (Atlantic salmon) have been declining in the Northern Hemisphere since the mid-1980s, particularly in sunny regions. Exposure to UVBR in freshwater has been suggested as a contributing factor to these declines that may cause metabolic damage that can affect the survival of organisms during subsequent stressful events such as ocean entry and smolting (Walters and Ward 1998). While UVBR may not contribute to the population declines of all amphibian species, it may be involved in the declines of some species, particularly those that oviposit in open, clear, and shallow water and are more susceptible to UVBR-induced DNA damage (Blaustein et al. 1998). A variety of agents may be acting alone or in combination to cause current amphibian losses; therefore, future research programs must consider the potential of combined effects of multiple factors polluting the aquatic environment.

Global Increases in UVBR Exposure

Stratospheric Ozone Depletion

The anthropogenic destruction of stratospheric ozone has been caused by the emission of industrial chemicals such as chlorofluorocarbons (CFCs) and other gases containing chlorine and bromine. The first quantitative evidence for a downward trend in stratospheric ozone was reported in 1985. Results demonstrated that springtime ozone levels between 1975 and 1984 had dropped by approximately 40% over Antarctica (Farman et al. 1985), forming a massive hole in the ozone layer. The Antarctic ozone hole has been occurring every spring since the

mid-1970s, and since 1985 it has been generally growing larger, forming earlier, lasting longer, and showing greater depletion, with more than 60% depletion compared with pre-1980 values (Jones and Shanklin 1995).

It is believed that climate change has played an important role in recent ozone depletion (for further details on the link between climate change and ozone depletion, see Perin and Lean 2004; Aucamp 2007; McKenzie et al. 2007). Ozone depletion has typically been worse in Antarctica than in the Arctic. Strong stratospheric winds that flow in a circular motion and extremely low temperatures create a polar vortex that allows the formation of polar stratospheric clouds (PSCs) in the ozone layer. PSCs are formed in the stratosphere at and near both poles during the winter when temperatures are below −78°C and enhance the destruction of ozone by providing a reaction site at which to chemically convert halogen source gases such as CFCs into reactive free-radical forms (Perin and Lean 2004; references therein). The Antarctic ozone hole of 2006 was the largest ever recorded, with an average area of 10.6 million square miles and was the result of very high levels of ozone-depleting substances (ODSs) and record cold conditions found in the stratosphere (NASA 2006). Since minimum winter temperatures in the Arctic stratosphere are approximately 10°C warmer than those of the Antarctic stratosphere, the Arctic polar vortex is not as strong and PSCs do not always form. However, ozone depletion over sub-Arctic and Arctic regions has recently become comparable to the loss of ozone over Antarctica. Greenhouse gases trap heat in the troposphere and radiate energy and heat away from the stratosphere. Therefore, the temperature at the Earth's surface is expected to continue increasing as a result of climate change, whereas the temperature of the stratosphere is expected to cool (i.e., radiative cooling). In fact, a small cooling of the lower stratosphere has been taking place since the 1970s, and several Arctic winters in the 1990s were characterized by record-low stratospheric temperatures and a stronger polar vortex. Studies suggest that climate warming may be responsible for the formation and persistence of PSCs, leading to the increased ozone loss observed over the Arctic in the 1990s, and that it may cause more severe ozone loss in the Arctic and increased severity and duration of the Antarctic ozone hole in the future. Because the atmosphere naturally produces the same amount of new stratospheric ozone each year (although the total amount of ozone destroyed is growing), the ozone-depleted air from polar regions mixes with the ozone-rich air from outside the poles, leaving the entire (average) ozone supply of the Earth slightly more diminished every year. Stratospheric ozone depletion also takes place directly at latitudes between the equator and the polar regions, but to a lesser degree (Perin and Lean 2004; references therein).

Several studies have demonstrated that UVBR levels have increased in regions where stratospheric ozone has decreased (e.g., Blumthaler and Ambach 1990; Kerr and McElroy 1993; Herman et al. 1996; McKenzie et al. 1999). For example, Kerr and McElroy (1993) measured daily ozone levels and the intensity of UVBR near 300 nm from 1989 to 1993 in Toronto, Canada, and found evidence that the observed increase in UVBR (35% and 7% per year for winter and summer, respectively) coincided with the decreasing ozone levels measured within this same time period. It is estimated that for each 1% decrease in stratospheric ozone, there is a 1%–2% increase in UVBR transmitted to the Earth (Kerr and McElroy 1993; Lubin and Jensen 1995; Hanelt et al. 2001). Therefore, even slight decreases in stratospheric ozone can have considerable impacts on living organisms and ecosystems through increased exposure to UVBR. Although the Montreal protocol and its amendments have required that industrialized countries phase out harmful ODSs, these substances are persistent, and it will take several decades for ozone levels to return to pre-1980 levels (McKenzie et al. 2007).

Underwater UVR Levels

The depth of penetration of solar radiation in freshwater systems is dependent on the concentration of dissolved organic carbon (DOC; see Scully and Lean 1994; Lean 1998*a*; references therein). DOC is the organic residue of decaying organic matter and contains chromophores, chemical structures that absorb light and UVR (Xenopoulos and Schindler 2001). Shorter wavelengths of solar irradiance are attenuated more rapidly by DOC than are longer wavelengths (Lean 1998*a*). Consequently, UVBR is highly attenuated in aquatic environments with high concentrations of DOC. For example, measurements taken under midday sun in June at a pond with high DOC content in Ontario indicated a UVBR intensity of 2.22 W/m^2 above the water surface and 0.046 W/m^2 at a depth of 5 cm inside the water column (Crump et al. 1999*a*). On the other hand, UVBR intensities can reach depths of several meters in clear (low DOC) freshwater ecosystems (Lean 1998*b*).

Interactions among global atmospheric stressors such as stratospheric ozone depletion, climate change, and acidification can modify DOC levels in aquatic environments, thereby increasing underwater UVBR levels (e.g., Schindler et al. 1996; Yan et al. 1996; Lean 1998*a*, 1998*b*) and increasing the risks associated with UVBR exposure. Figure 1 illustrates these interactions, which are briefly described below.

Increasing levels of UVBR penetrating the water column as a result of ozone depletion can lead to the enhanced photobleaching of light-absorbing chromophores of DOC, making them less efficient in absorbing UVBR. Changes in global precipitation patterns are expected to result from climate change, and as a consequence, some regions will receive significantly more or less precipitation than they have in the past (Caldwell et al. 2007). With less rain, water is retained in lakes for longer periods, and there is increased photodegradation of DOC (Lean 1998*a*, 1998*b*). Warmer, drier conditions may reduce the contribution of DOC from terrestrial runoff into aquatic ecosystems, also leading to greater penetration of UVBR (Lean 1998*b*; Aucamp 2007). Acidification of lakes and rivers caused by anthropogenic emissions of sulfur and nitrogen oxides lowers the

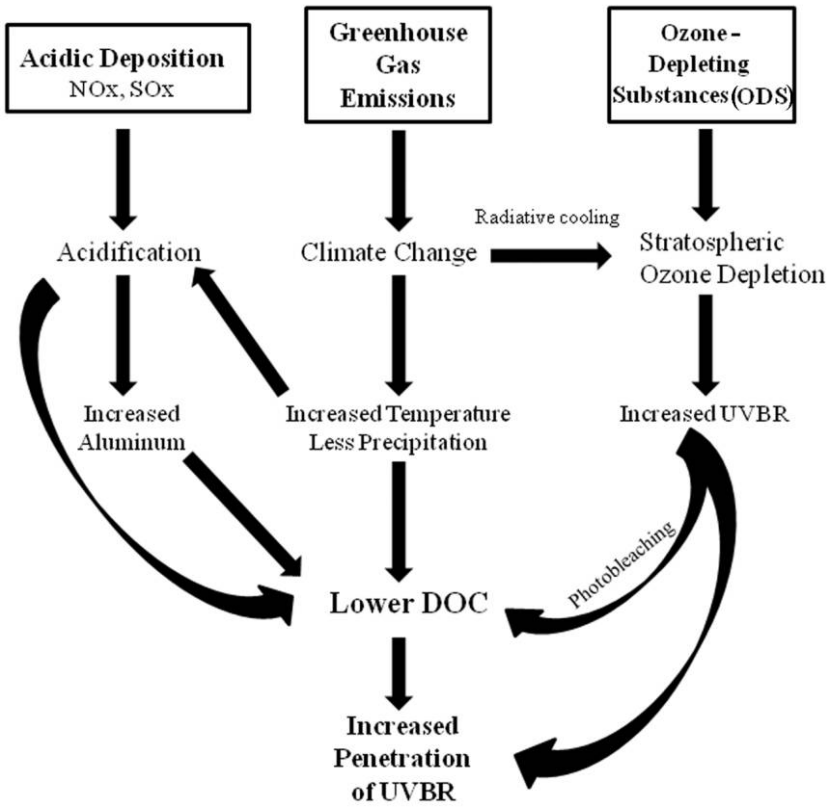

Figure 1. Schematic model describing the overall impact of the interaction of acidic deposition, greenhouse gas emissions, and ozone-depleting substances on levels of dissolved organic carbon (*DOC*) and thus the penetration of ultraviolet B radiation (*UVBR*) in aquatic ecosystems. Adapted from Lean (1998*a*).

pH in precipitation and in poorly buffered aquatic systems. This results in increased DOC aggregation and sedimentation, thus removing DOC from the water column. Increased acidity also results in the mobilization of metals (e.g., aluminum), which also leads to increased aggregation and sedimentation of DOC (Lean 1998*b*). Climate warming may cause further acidification of aquatic habitats by decreasing water levels. The exposed littoral sediments that contain reduced sulfur can be reoxidized and can remobilize acid into the water, thus further diminishing DOC levels (Yan et al. 1996).

Twenty years of data collected at the Experimental Lakes Area in northwestern Ontario (Canada) demonstrated that in clear, shallow aquatic ecosystems, decreases in DOC caused by climate warming and/or acidification can be more effective in increasing the exposure of aquatic organisms to UVBR than changes to incident UVBR as a result of stratospheric ozone depletion (Schindler et al. 1996). Although the amount of UVBR reaching terrestrial and aquatic ecosystems can be attenuated by several other factors, such as plant canopy, surface albedo, cloud cover, atmospheric pollutants, aerosols (Xenopoulos and Schindler 2001), and water turbidity (Ovaska et al. 1997; Aucamp 2007), even small increases in UVBR exposure may be harmful to organisms at sensitive stages of development or to organisms that do not possess adequate defense mechanisms in preventing UVBR-induced damage.

UVBR and Amphibians

Exposure and Defense Mechanisms

A variety of behaviors exhibited by different amphibian species can contribute to increased exposure to UVBR. Since many amphibian species oviposit at or near the surface of the water, proximity to the surface may increase harm to eggs and embryos because of greater UVBR exposure (Murphy et al. 2000). Species that breed in the spring, when UVBR levels are high, are especially at risk because this coincides with early and sensitive stages of embryo and tadpole development (Kerr and McElroy 1993). Furthermore, amphibians inhabiting shallow or clear aquatic environments are also susceptible to increased UVBR exposure because of the deep penetration of sunlight, which can sometimes reach the bottom of the water column, depending on the water's depth and DOC level (Morris et al. 1995).

While eggs remain relatively stationary once they are laid, larvae and adults can move freely within their habitat and may be able to seek areas that reduce their exposure to solar radi-

ation (Blaustein and Belden 2003). However, studies have demonstrated that some amphibian species do not actively avoid UVBR exposure. Belden et al. (2003) performed outdoor and laboratory UVBR avoidance tests with *Rana cascadae* tadpoles by recording the number of animals found on the UVBR-filtered side versus those found on the UVBR-exposed side of containers in which they were housed for a given time period. Tadpoles did not avoid environmental levels of UVBR in outdoor and laboratory avoidance tests. Results of field exposures to ambient UVBR suggest that *R. cascadae* tadpoles may not perceive light in the UVBR range, since survival was significantly lower in tadpoles subjected to UVBR for 42 d, but appeared not to respond physiologically to exposure. UVBR exposure did not affect whole-body levels of corticosterone (a stress hormone), although *R. cascadae* tadpoles increased corticosterone in response to confinement stress (Belden et al. 2003). Similar lack of UVBR avoidance or perception has been reported for *Rana arvalis* (moor frog), *Rana temporaria* (common frog), and *Bufo bufo* (common toad; Pahkala et al. 2003*a*).

Basking in the sun to thermoregulate is also a behavior that can contribute to increased exposure of tadpoles and adults to UVBR (Stebbins and Cohen 1995), especially for species that may not perceive or avoid UVBR. Exposure to enhanced levels of UVBR as a result of (1) ozone depletion, (2) modifications of DOC levels in aquatic systems (Schindler et al. 1996), and (3) clear cutting of vegetation or habitat destruction (eliminating shelter from solar radiation) make basking in sunlight a greater threat to amphibians than in the recent past because of the many detrimental effects of current UVBR levels (see below for details on lethal and sublethal effects; Murphy et al. 2000).

Amphibians have several defense mechanisms that allow them to cope with the harmful effects of UVBR by either limiting UVBR-induced damage or repairing it after it has occurred (Blaustein and Belden 2003). Shade provided by flora, organic debris, and other substrates found in the environment can minimize exposure of individuals to UVBR. Size and shape of egg masses are also factors that influence the amount of UVBR reaching eggs and embryos. Different amphibian species oviposit large masses or chains across the surface of the water, small clumps of eggs, or single eggs attached to aquatic vegetation (Murphy et al. 2000). Although eggs on the surface of spherical clutches may be exposed and adversely affected by UVBR, surrounding eggs may shield those in the middle of the egg mass. Consequently, species that lay their eggs in large globular masses (e.g., *Rana pipiens*, northern leopard frog) are likely to be better protected from UVBR than those that lay them in thin sheets or strings (e.g., *Bufo* spp.; Ovaska 1997). The jelly layer surrounding eggs can be very efficient at filtering UVBR, depending on its UVBR absorbance properties (Smith et al. 2002). Melanin layers found in amphibian eggs and larvae that develop in sunlight can also help protect tissues from normal levels of UVBR (reviewed in Blaustein and Belden 2003). Furthermore, photolyase is a photoreactivating enzyme responsible for the repair of damaged DNA (cyclobutane pyrimidine dimers) after UVBR exposure (reviewed in Blaustein and Belden 2003). Photolyase levels found in amphibian eggs have been positively correlated with the survival of UVBR-exposed embryos and their expected exposure to UVBR in the wild (Blaustein et al. 1994, 1998).

Considering recent and future increases in UVBR, it is possible that these protective mechanisms will not be adequate in preventing UVBR-induced damage to many amphibian species. Stratospheric ozone depletion has considerably skewed the ratio of UVBR to ultraviolet A radiation (UVAR) and visible light reaching the surface of the Earth, in that UVBR levels have increased without a concurrent increase in UVAR/visible light. It is unknown how or if this shift affects the balance between UVBR-induced DNA damage and UVAR/visible light–activated repair in organisms.

Several factors influence the exposure and susceptibility of amphibians to solar radiation as well as the effectiveness of defense mechanisms that help mitigate its damaging effects. It is therefore very difficult to assess or predict the actual exposure of amphibians to UVBR and the resulting consequences for different populations and species. Despite the various coping mechanisms that can help protect amphibians against UVBR, many studies have determined that present-day environmental levels of UVBR can have harmful effects on development and health.

Lethal and Sublethal Effects

Here we provide a brief overview of the lethal and sublethal effects often exhibited in amphibians exposed to UVBR (also see reviews in Crump 2001; Blaustein et al. 2001, 2003). Numerous laboratory and field experiments have shown that amphibian embryos and/or larvae can experience reduced survivorship when exposed to ambient or enhanced levels of UVBR (e.g., Worrest and Kimeldorf 1975, 1976; Blaustein et al. 1994, 1997; Ovaska et al. 1997; Anzalone et al. 1998; Ankley et al. 2000, 2002; Belden et al. 2000; Flamarique et al. 2000; Häkkinen et al. 2001; Tietge et al. 2001; Belden and Blaustein 2002*b*; Weyrauch and Grubb 2006; Croteau et al. 2008). However, some studies have found that exposure of amphibian embryos and/or larvae to UVBR had no effect on survival (e.g., Pahkala et al. 2000, 2003*b*; Smith et al. 2000). Häkkinen et al. (2001) examined the effects of solar UVBR on *R. temporaria*, *B. bufo*, and *R. arvalis* and found that UVBR decreased the survival of *R. arvalis* embryos and *B. bufo* tadpoles but not *R. temporaria* embryos and tadpoles. Variations in the sensitivity of different life stages, species, and populations to UVBR exposure have been observed for many end points in many studies and will be discussed in further detail later in this article.

Sublethal effects such as disruptions of growth (e.g., Belden et al. 2000; Pahkala et al. 2000, 2001; Belden and Blaustein 2002*a*, 2002*b*), developmental rate (e.g., Smith et al. 2000; Pahkala et al. 2002*a*, 2002*b*; Croteau et al. 2008), metamorphosis (e.g., Pahkala et al. 2001, 2003*b*; Croteau et al. 2008), and behavior (e.g., antipredator behavior, activity level; e.g., Blau-

Table 1: Examples of studies demonstrating adverse effects of interactions between UVBR and various stressors on amphibians

Species	Stressor and Effect of Interaction	Reference
Pseudacris crucifer	Copper chloride; reduces survival	Baud and Beck 2005
Rana perezi, *Bufo bufo*	Sodium nitrite; reduces survival	Macías et al. 2007
Rana pipiens	4-octylphenol; increases weight, accelerates hindlimb emergence, and affects hypothalamic gene expression	Crump et al. 2002
R. pipiens	4-tert-octylphenol; increases mortality, produces developmental anomalies, delays development, and decreases the percentage of tadpoles reaching metamorphosis[a]	Croteau et al. 2008
Rana sphenocephala	Certain fire-retardant chemicals with yellow prussiate of soda; reduces survival[b]	Calfee and Little 2003
Rana sylvatica	Low genetic diversity; reduces survival	Weyrauch and Grubb 2006
Rana temporaria	Bisphenol A; produces developmental anomalies and reduces survival	Koponen and Kukkonen 2002

[a] The interaction term was not statistically significant in this study, but the effect of the combination treatments was most often worse than that of either factor alone.

[b] Yellow prussiate of soda (sodium ferrocyanide) is a corrosion inhibitor that is added to firefighting chemicals.

stein et al. 2000; Kats et al. 2000) can occur as a result of UVBR exposure. Although UVBR is probably not the cause of the high incidence of gross limb malformations recently observed in amphibians in nature (Blaustein and Johnson 2003; Ankley et al. 2004), several other types of developmental malformations and abnormalities have been observed in experiments exposing embryos and tadpoles to UVBR. Spinal curvature has been observed in several indoor and outdoor UVBR exposure experiments (e.g., Worrest and Kimeldorf 1975, 1976; Hays et al. 1996; Blaustein et al. 1997; Bruggeman et al. 1998; Pahkala et al. 2001; Weyrauch and Grubb 2006; Croteau et al. 2008) and can be fatal to animals, depending on the severity of the affliction. *Rana pipiens* tadpoles chronically exposed to environmentally relevant levels of UVBR in a laboratory experiment exhibited this malformation and did not survive; they swam in circles and could not forage for food (Croteau et al. 2008). Additional types of developmental malformations and abnormalities produced by UVBR may include edema or bloating (e.g., Hays et al. 1996; Blaustein et al. 1997), eye abnormalities (e.g., Worrest and Kimeldorf 1975, 1976; Bruggeman et al. 1998; Fite et al. 1998; Ankley et al. 2000, 2002; Flamarique et al. 2000), and hindlimb malformations (e.g., Ankley et al. 1998, 2000, 2002; Pahkala et al. 2001). Such developmental anomalies may cause direct mortality or may significantly disrupt the normal activities of individuals in nature by preventing animals from effectively foraging for food or evading predators.

Interactions between UVBR and Other Environmental Stressors

Although many studies have demonstrated the negative effects of UVBR on amphibians, studies examining single stressors may not provide adequate information on how amphibians are coping with exposure to several stressors in the wild. Blaustein et al. (2003) reviewed the effects of interactions between UVBR and numerous stressors present in the aquatic environment on amphibian health. The stressors include (1) the pathogenic fungus *Saprolegnia ferax*, (2) low pH, (3) nitrates, (4) pesticides (e.g., carbaryl), and (5) polycyclic aromatic hydrocarbons. Interactions between two stressors may occur when animals have a reduced capacity to respond to one agent in the presence of another (Blaustein et al. 2001). Exposure to UVBR can compromise the immune system of amphibians, making them more vulnerable to the effects of pathogens (reviewed in Blaustein and Kiesecker 2002). Interactions may also occur between UVBR and contaminants, when one factor enhances the toxicity of the other factor (Blaustein et al. 2001). Other recent studies on amphibians have demonstrated that interactions between UVBR and various stressors—including (1) low genetic diversity (Weyrauch and Grubb 2006), (2) sodium nitrite (Macías et al. 2007), (3) bisphenol A (Koponen and Kukkonen 2002), (4) fire-retardant chemicals (Calfee and Little 2003), (5) octylphenol (Crump et al. 2002; Croteau et al. 2008), and (6) copper chloride (Baud and Beck 2005)—produce adverse effects in exposed amphibians (Table 1).

In recent years, UVBR levels have increased dramatically in some aquatic environments, whereas levels in other water systems have moderately or scarcely changed because of high DOC levels. Even if underwater UVBR levels remain the same or increase only slightly, interactive effects of UVBR and other stressors could significantly harm amphibians (Lannoo 2005). Because UVBR levels are predicted to increase in the future, the risk for certain species of amphibians could increase considerably, as demonstrated in studies reporting detrimental effects in amphibians exposed to enhanced levels of UVBR (e.g., Ovaska et al. 1997; Pahkala et al. 2001). Regardless of the situation, the lethal and sublethal effects described above have the potential to significantly affect amphibian health, and more research is needed to determine whether UVBR alone or combined with multiple stressors in the environment can have a direct effect on populations.

Effects of UVBR Exposure on the Rate of Amphibian Development and Metamorphosis

Numerous experiments conducted outdoors or in the laboratory have shown that UVBR emitted from sunlight or artificial lights can influence the rate of amphibian development and metamorphosis. Summarized in Table 2 are the results of 15 published studies demonstrating that amphibians subjected to UVBR can exhibit delays, accelerations, or no change in developmental and/or metamorphic rate.

Nine of the studies presented in Table 2 reported a delay in the development and/or metamorphosis of embryos or tadpoles exposed to UVBR for either short or long time periods (e.g., from a few days to several months) in at least one test species examined. Higgins and Sheard (1926) published the first article to our knowledge that reported a delay in the development of amphibians exposed to UVBR for short time periods and kept in either constant daylight or constant darkness thereafter. They showed that *Rana pipiens* embryos were developmentally delayed after 3 d of exposure to a quartz mercury lamp (emits both UVBR and ultraviolet C radiation [UVCR]) for 1 min/d in the daylight treatment. After exposure for 2 min/d for 3 d (and a subsequent 4-d postexposure period), embryos in both the daylight and darkness treatments were also developmentally delayed compared with respective controls. Even though animals were exposed to very high levels of both UVBR and UVCR (UVCR is not environmentally relevant) and kept in constant daylight or darkness, this study provided useful information because it demonstrated that UVR can not only produce developmental anomalies and outright mortality but also cause sublethal effects. Many studies presented in Table 2 demonstrated that environmentally relevant UVBR levels can also impede the development of exposed animals (e.g., Blaustein et al. 1997; Croteau et al. 2008), and one study demonstrated that exposure to UVBR can result in inhibited or blocked development and metamorphosis (Grant and Licht 1995). *Rana clamitans* (green frog) tadpoles chronically exposed to UVBR in the laboratory did not develop past Gosner stage 34 (early digit development) and only reached early stages of paddle foot and digit formation (Gosner stages 31–33) by 22 mo. Exposure to UVBR not only delayed their development but also completely inhibited or blocked the metamorphosis of these animals. However, animals were exposed for short time intervals (minutes per day) to very high irradiances in relation to environmental UVBR levels. Croteau et al. (2008) observed similar responses in *R. pipiens* tadpoles exposed to subambient levels of UVBR in the lab. Tadpoles were significantly delayed in development, with a small percentage of animals developing past Gosner stage 34, and the percentage of animals to reach metamorphosis was also significantly lower in UVBR-treated groups. These findings corroborate a preliminary study conducted in the same lab, which demonstrated a delay in development, inhibition of metamorphosis (none metamorphosed in UVBR treatments after 6 mo of exposure), and the result that none of the animals exposed to UVBR developed past stage 34 (Croteau et al. 2001; not included in Table 2).

Although most studies reporting delayed development and/or metamorphosis consisted of lab exposures (six out of nine studies; Table 2), three studies consisted of outdoor exposures of amphibians to sunlight in natural aquatic habitats (Blaustein et al. 1997) or in containers/enclosures filled with pond or lake water (Smith et al. 2000; Belden and Blaustein 2002*a*). This demonstrates that the developmental rate of amphibians subjected to present-day UVBR levels can be negatively affected and that some populations or species may not be able to cope with exposure to rising UVBR levels expected in the future. It should be noted that all outdoor studies reporting developmental delays focused on embryonic development and exposed animals to UVBR for short time periods. Future outdoor studies should consider exposing animals to UVBR throughout all life stages, including metamorphosis, in order to assess delays in development in the wild and to better reflect natural exposure conditions. Of six studies performed outdoors in ambient or filtered sunlight, three showed no effect of UVBR on development and/or metamorphosis (Anzalone et al. 1998; Merilä et al. 2000; Ankley et al. 2002). This may indicate a difference in species/population sensitivity to UVBR exposure or may be a consequence of differences in experimental approaches and design (discussed below).

Delayed or arrested development could be fatal to animals that have not metamorphosed before their habitat freezes or dries, and this is especially important for amphibian species living in temporary habitats (e.g., *Ambystoma macrodactylum*, long-toed salamander). Since these species must develop quickly before their habitat disappears, they seek sunlight to enhance their developmental rate (Blaustein and Belden 2003) and thus are likely to be exposed to more damaging UVBR levels. Since amphibian eggs, larvae, and newly metamorphosed tadpoles are typically the life stages that are most vulnerable to predation (with the egg stage being most vulnerable of all; Pough et al. 2001), the delayed development or metamorphosis of embryos and tadpoles subjected to UVBR could result in increased predation at these life stages. Two short-term laboratory exposure experiments conducted by Pahkala et al. (2002*a*, 2002*b*) reported an acceleration in development after exposure to UVBR in at least one of several *Rana temporaria* populations studied (Table 2). Although all four studies conducted by this group used the same species and UVBR levels (normal and enhanced), each study produced a different outcome. For example, Pahkala et al. (2002*a*) examined the effects of UVBR on eight different populations of *R. temporaria* in Sweden and found that UVBR did not affect the developmental rate of animals in two of the populations examined. However, they did observe that one population exhibited accelerated development in the normal and enhanced UVBR treatments and that five populations exhibited developmental delays only in the enhanced treatment. This underscores the importance of taking into consideration population sensitivity differences when conducting or interpreting the results of UVBR exposure

Table 2: Summary of studies on the effects of UVBR on amphibian development and metamorphosis

Species, Type of Exposure, and UVBR Level[a]	Conversion to W/m^2	Filters	Developmental Stage Exposed	Length of Exposure	Effect on Developmental Rate	Effect on Metamorphosis	Reference
Ambystoma macrodactylum:							
Outdoor:							
94% ambient UVBR removed (ambient UVBR range 4.77–25.5 μW/cm^2)[b]	NA	Mylar	Embryo (<24 h old)	Until embryos hatched or died			Blaustein et al. 1997
90% ambient UVBR transmitted	NA	Acetate	Embryo (<24 h old)	Until embryos hatched or died	Delayed	NA	Blaustein et al. 1997
Hyla cadaverina, *Hyla regilla*, *Taricha torosa*:							
Outdoor:							
<10% UVBR transmittance[b]	NA	Mylar	Embryo	Until embryos hatched or died			Anzalone et al. 1998
Ambient sunlight	NA	None	Embryo	Until embryos hatched or died	No effect	NA	Anzalone et al. 1998
~80% UVBR transmittance	NA	Acrylic	Embryo	Until embryos hatched or died	No effect	NA	Anzalone et al. 1998
Hyla versicolor:							
Laboratory:							
Daylight fluorescent lights[b]	NA	None	Tadpole (Gosner stage 21)	Until metamorphosis			Grant and Licht 1995
.113 W/cm^2	1,130	None	Tadpole (Gosner stage 21)	Until metamorphosis; 2 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.170 W/cm^2	1,700	None	Tadpole (Gosner stage 21)	Until metamorphosis; 3 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.113 W/cm^2	1,130	Acetate (varying layers)	Tadpole (Gosner stage 21)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.961 W/cm^2	9,610	Acetate (varying layers)	Tadpole (Gosner stage 21)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
Bufo americanus:							
Laboratory:							
Daylight fluorescent lights[b]	NA	None	Tadpole (newly hatched)	Until metamorphosis			Grant and Licht 1995
.113 W/cm^2	1,130	None	Tadpole (newly hatched)	Until metamorphosis; 2 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.170 W/cm^2	1,700	None	Tadpole (newly hatched)	Until metamorphosis; 3 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.961 W/cm^2	9,610	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
1.152 W/cm^2	11,520	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
1.440 W/cm^2	14,400	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.961 W/cm^2	9,610	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d for 5 consecutive days weekly	No effect	No effect	Grant and Licht 1995

Rana clamitans:							
Laboratory:							
Daylight fluorescent lights[b]	NA	None	Tadpole (newly hatched)	Until metamorphosis			Grant and Licht 1995
.113 W/cm^2	1,130	None	Tadpole (newly hatched)	Until metamorphosis; 2 min/d, 3 times weekly on alternate days	Delayed	Blocked	Grant and Licht 1995
.170 W/cm^2	1,700	None	Tadpole (newly hatched)	Until metamorphosis; 3 min/d, 3 times weekly on alternate days	Delayed	Blocked	Grant and Licht 1995
Rana sylvatica:							
Laboratory:							
Daylight fluorescent lights[b]	NA	None	Tadpole (newly hatched)	Until metamorphosis			Grant and Licht 1995
.113 W/cm^2	1,130	None	Tadpole (newly hatched)	Until metamorphosis; 2 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.17 W/cm^2	17,00	None	Tadpole (newly hatched)	Until metamorphosis; 3 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.113 W/cm^2	11,30	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.961 W/cm^2	9,610	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
1.056 W/cm^2	10,560	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
1.152 W/cm^2	11,520	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
1.440 W/cm^2	14,400	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d, 3 times weekly on alternate days	No effect	No effect	Grant and Licht 1995
.961 W/cm^2	9,610	Acetate (varying layers)	Tadpole (newly hatched)	Until metamorphosis; 240 min/d for five consecutive days weekly	No effect	No effect	Grant and Licht 1995
Rana aurora:							
Outdoor:							
UVBR blocked[b]	NA	Mylar	Embryo (Gosner stages 2–6)	6 wk; until Gosner stages 19–21			Belden and Blaustein 2002*a*
~80% UVBR from ambient sunlight	NA	Acetate	Embryo (Gosner stages 2–6)	6 wk; until Gosner stages 19–21	Delayed	NA	Belden and Blaustein 2002*a*
Rana blairi:							
Outdoor:							
Filtered sunlight (~58% UVBR transmittance)	NA	Petri dish cover	Embryo (~3 d old; ≤Gosner stage 9)	8 d; until embryos hatched	No effect	NA	Smith et al. 2000
Filtered sunlight (~84% UVBR transmittance)	NA	Saran Wrap	Embryo (~3 d old; ≤Gosner stage 9)	8 d; until embryos hatched	Delayed	NA	Smith et al. 2000

Table 2 (*Continued*)

Species, Type of Exposure, and UVBR Level[a]	Conversion to W/m^2	Filters	Developmental Stage Exposed	Length of Exposure	Effect on Developmental Rate	Effect on Metamorphosis	Reference
Rana pipiens:							
Laboratory:							
Constant daylight or darkness[b]	NA	NA	Embryo	3 d			Higgins and Sheard 1926
Quartz mercury lamp + constant daylight or darkness[c]	NA	NA	Embryo	3 d; 1 min/d	Delayed (daylight treatment)	NA	Higgins and Sheard 1926
Quartz mercury lamp + constant daylight or darkness[c]	NA	NA	Embryo	3 d; 2 min/d	Delayed (daylight and darkness treatments)	NA	Higgins and Sheard 1926
Normal laboratory fluorescent light[b]	Not detected	None	Embryo (2–3 h postfertilization)	113 d; UVBR/VIS provided for 12 h of 16-h light period			Ankley et al. 1998
UVBR at air water interface (44 μW/cm^2) attenuated by ~76% at 3 cm water depth	NA	None	Embryo (2–3 h postfertilization)	113 d; UVBR/VIS provided for 12 h of 16-h light period	No effect	No effect	Ankley et al. 1998
Outdoor:							
Ambient sunlight	NA	None	Embryo (Gosner stages 5–7)	Until metamorphosis	NA	No effect	Ankley et al. 2002
Filtered sunlight (various UVR levels)	NA	Nitex and stainless steel screen[d]	Embryo (Gosner stages 5–7)	Until metamorphosis	NA	No effect	Ankley et al. 2002
Laboratory:							
Full-spectrum laboratory lighting[b]	NA	None	Tadpole (Gosner stage 21)	10 d; 12L : 12D photoperiod			Crump et al. 2002
7 μW/cm^2	.07	None	Tadpole (Gosner stage 21)	10 d; 360 min/d	No effect	NA	Crump et al. 2002
Fluorescent/incandescent lighting[b]	Negligible	Mylar	Tadpole (Gosner stage 25)	8 mo; 12 h/d			Croteau et al. 2008
.22 W/m^2	.22	None	Tadpole (Gosner stage 25)	8 mo; 12 h/d	Delayed	Delayed	Croteau et al. 2008
Rana temporaria:							
Outdoor:							
UVBR and shorter wavelengths removed[b]	NA	Mylar	Embryo (~1 d old)	12 d; until majority of embryos hatched (Gosner stage 25)			Merilä et al. 2000
Ambient sunlight	NA	None	Embryo (~1 d old)	12 d; until majority of embryos hatched (Gosner stage 25)	No effect	NA	Merilä et al. 2000
Wavelengths shorter than UVBR removed	NA	Preburned acetate	Embryo (~1 d old)	12 d; until majority of embryos hatched (Gosner stage 25)	No effect	NA	Merilä et al. 2000
Laboratory:							
UVBR blocked[b]	NA	Mylar	Embryo (<2 h old)	Until majority of larvae reached Gosner stage 25; 137 min/d			Pahkala et al. 2001
1.254 kJ/m^2	.1526	Acetate	Embryo (<2 h old)	Until majority of larvae reached Gosner stage 25; 137 min/d	NA	No effect	Pahkala et al. 2001
1.584 kJ/m^2 (enhanced by 26%)[e]	.1927	Acetate	Embryo (<2 h old)	Until majority of larvae reached Gosner stage 25; 173 min/d	NA	Delayed	Pahkala et al. 2001
UVBR blocked[b]	NA	Mylar	Embryo (<2 h old)	Until larvae reached Gosner stage 25; 137 min/d			Pahkala et al. 2002*a*

1.254 kJ/m^2		.1526	Acetate	Embryo (<2 h old)	Until larvae reached Gosner stage 25; 137 min/d	No effect/ accelerated[f]	NA	Pahkala et al. 2002*a*
1.584 kJ/m^2 (enhanced by 26%)		.1927	Acetate	Embryo (<2 h old)	Until larvae reached Gosner stage 25; 173 min/d	Delayed/no effect/ accelerated[f]	NA	Pahkala et al. 2002*a*
UVBR blocked[b]	NA		Mylar	Embryo (<2 h old)	~12 d; 137 min/d			Pahkala et al. 2002*b*
1.254 kJ/m^2		.1526	Acetate	Embryo (<2 h old)	~12 d; 137 min/d	Accelerated	NA	Pahkala et al. 2002*b*
1.584 kJ/m^2 (enhanced by 26%)		.1927	Acetate	Embryo (<2 h old)	~12 d; 173 min/d	No effect	NA	Pahkala et al. 2002*b*
UVBR blocked[b]	NA		Mylar	Embryo (<2 h old)	Until metamorphosis; 137 min/d			Pahkala et al. 2003*b*
1.254 kJ/m^2		.1526	Acetate	Embryo (<2 h old)	Until metamorphosis; 137 min/d	NA	Delayed	Pahkala et al. 2003*b*
1.584 kJ/m^2 (enhanced by 26%)		.1927		Embryo (<2 h old)	Until metamorphosis; 173 min/d	NA	Delayed	Pahkala et al. 2003*b*

Note. UVR, ultraviolet radiation; UVBR, ultraviolet B radiation; NA, not applicable; VIS, visible light.

[a] UVBR levels have been shown to range between 1 and 2.5 W/m^2 in ambient sunlight above water in Ontario (Crump et al. 1999*a*, 1999*b*; Croteau et al. 2008) and between 0.0042 and 0.72 W/m^2 in various types of aquatic environments in North America (at a depth of 10 cm; Barron et al. 2000; Calfee and Little 2003), depending on environmental factors.

[b] Control treatment for the indicated experiment.

[c] Quartz mercury lamps emit both UVBR and ultraviolet C radiation.

[d] With or without glass or acrylamide.

[e] The 26%-enhanced UVBR level was obtained using a computer model that calculated the daily increase in UVBR that would follow from 15% ozone depletion under clear sky conditions, which is within the observed daily variation in ozone in central Sweden in April.

[f] This study examined the effects of UVBR on eight different populations in Sweden. UVBR did not affect development in two populations. One population exhibited accelerated development in the normal and enhanced UVBR treatments. Five populations exhibited developmental delays in the enhanced treatment only.

experiments. Acceleration in development may also point to a physiological disruption, and it should be acknowledged that subjecting the same species to similar exposure regimes can produce different sublethal responses.

Conversely, some studies described in Table 2 either observed no effect of UVBR on developmental and metamorphic rates or found both effects and no effects when using different amphibian species or UVBR levels. Species or population sensitivity differences to UVBR exposure are not unusual, and they may explain why conflicting data exist in the literature on the effects of UVBR on amphibian health. For example, various studies have found that exposure of amphibian embryos or larvae to UVBR had no effect on mortality (e.g., Pahkala et al. 2000, 2003*a*; Smith et al. 2000) or the occurrence of developmental anomalies (e.g., Pahkala et al. 2000, 2003*b*), even though several other studies have reported the opposite (see "UVBR and Amphibians"). It has been demonstrated that the effectiveness of the different defense mechanisms employed by amphibians to cope with UVBR exposure can vary greatly between species and populations, which could influence the extent of UVBR to which they are subjected as well as subsequent detrimental effects. Smith et al. (2002) examined photolyase levels and UVBR absorbance properties of embryo jelly of seven amphibian species from south-central Ontario. They found that significant differences in the absorbance of the protective jelly at 320 nm exist between these species and that jelly absorbance at this wavelength increases as the activity of photolyase decreases. On the other hand, Little et al. (2003) found that UVBR exposure affected the survival of *Bufo woodhousii* (Woodhouse's toad) but not of *Bufo boreas* tadpoles, and this was not attributed to differences in the UVBR-absorbing substances of the egg jelly or nonmelanin photoprotective substances in the skin but potentially to a greater amount of melanin in the skin of *B. boreas*. However, other studies have found that melanin does not protect exposed animals from the damaging effects of UVBR (e.g., Belden and Blaustein 2002*c*). Hansen et al. (2002) have shown that the egg mass jelly of *Hyla regilla* embryos displayed no absorption in the UVR range. Removal of the jelly coat of both *H. regilla* and *Bufo canorus* (Yosemite toad) embryos and subsequent exposure to UVBR in a solar simulator also demonstrated that the jelly played no apparent role in protecting embryos; hatching success was similar in eggs with and without the egg jelly (see also Rasanen et al. 2003).

The ecological characteristics of natural aquatic habitats can also influence the degree of exposure and susceptibility of different amphibian species and populations to environmental UVBR levels. These can include water DOC levels (Palen et al. 2005), turbidity of water (Ovaska 1997), type of vegetation, and geographical location of the site as well as its altitude (Xenopoulos and Schindler 2001). For example, populations that are naturally exposed to elevated levels of UVBR, such as those living in elevated regions (e.g., mountain populations), may be more adapted to coping with the effects of radiation and have higher survival rates (Belden et al. 2000; Belden and Blaustein 2002*b*). On the basis of these findings, it is difficult to extrapolate results from one study or situation to another, even when using the same species or population, because differences in sensitivity to UVBR can exist as a result of differential exposure and differences in the physiological capacity of animals to withstand it.

Differences in experimental approaches and procedures carried out for a UVBR exposure experiment can influence the outcome or interpretation of results. Currently, there seems to be no consensus among UVBR researchers on experimental design. There are inconsistencies in measuring and reporting UVBR levels in outdoor studies. In order to ensure that reported UVBR levels represent what animals would be exposed to in natural aquatic environments, direct measurements of underwater UVBR or DOC levels (to estimate UVBR levels in water) should be reported. Some studies do not directly measure UVBR levels in the air or water and simply indicate that animals were exposed to ambient sunlight or give the percent transmission of the filter used without directly measuring UVBR underneath. This makes it difficult or impossible to determine the levels to which the animals were exposed, especially if the meteorological conditions (i.e., sunny vs. cloudy) during the exposure period were not reported. In addition, UVBR exposure levels in laboratory experiments should be consistent with levels to which a specific population would be exposed at their natural oviposition site. The quantity and quality of artificial lighting must also be considered. The enzyme photolyase uses light energy mostly between 350 and 450 nm as an energy source to repair damaged DNA (Banaszak 2003; Sancar 2003); therefore, wavelengths within this range should be available to exposed animals in order to provide the opportunity for adequate repair.

Sublethal effects such as disruptions in the rate of development often express themselves only in later stages of post-exposure development (e.g., Smith et al. 2000; Pahkala et al. 2001; Belden and Blaustein 2002*a*). Consequently, experiments that measure end points after a short exposure period, early in development, may underestimate the actual effects of UVBR. This highlights the importance of observing UVBR-exposed animals until later stages or until metamorphosis if possible.

The use of different species or populations, stages of development, and/or research methodologies (e.g., different equipment, laboratory vs. field exposures) may complicate the interpretation of results; however, it is clear from the studies summarized in Table 2 that UVBR has the potential to disrupt developmental and metamorphic rates of exposed animals. The actual mechanism by which UVBR disrupts amphibian development remains to be elucidated. Crump et al. (2002) hypothesized that the developing hypothalamus might be a potential environmental sensor for neurotoxicologic studies because of its role in the endocrine control of metamorphosis. They demonstrated that a 10-d exposure of *R. pipiens* tadpoles to subambient levels of UVBR (7 μW/cm^2, with or without the chemical 4-octylphenol) altered the expression of important hypothalamic genes (e.g., glutamate decarboxylase 67) at metamorphic climax as well as genes in the tadpole diencephalon

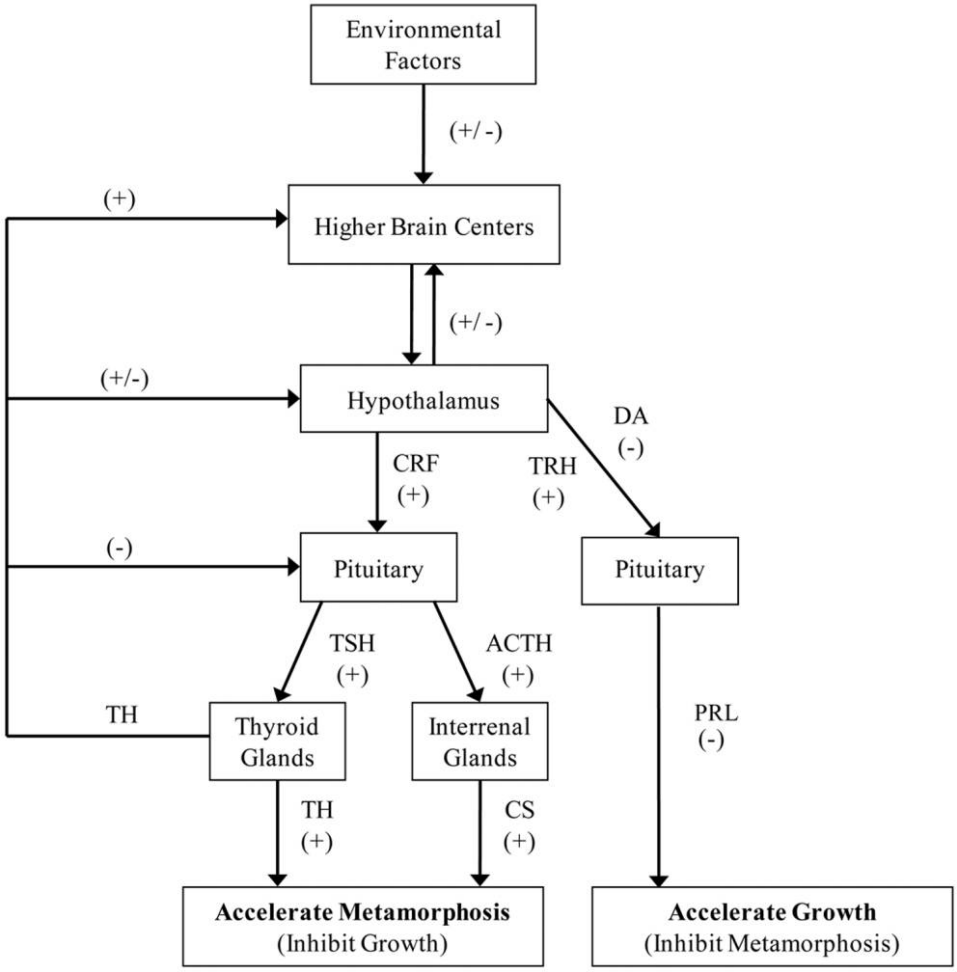

Figure 2. Schematic overview of the endocrine systems controlling amphibian metamorphosis. A plus sign indicates a positive, or stimulatory, action, and a minus sign indicates a negative, or inhibitory, action. Thyroid hormone and corticosteroids stimulate metamorphosis, whereas prolactin inhibits metamorphosis. Hormonal pathways are under complex neuroendocrine regulation involving both positive and negative feedback depending on developmental stage or physiological state (feedback loops for corticosteroids are not shown). *ACTH*, adrenocorticotropin; *CRF*, corticotropin-releasing factor; *CS*, corticosteroids; *DA*, dopamine; *PRL*, prolactin; *TH*, thyroid hormone; *TRH*, thyrotropin-releasing hormone; *TSH*, thyroid-stimulating hormone. Adapted from Denver (1997, 1998) and Shi (2000).

(e.g., brain-specific angiogenesis inhibitor 3). However, that study did not examine the effects of UVBR on the expression of specific genes that regulate amphibian development and metamorphosis. Basic information regarding the endocrine and molecular effects of UVBR exposure is lacking and is needed because amphibian development and metamorphosis make up a highly coordinated process controlled by the thyroid system. Therefore, it is important that we assess the thyroidal status of UVBR-exposed animals to understand the possible mechanisms by which UVBR disrupts developmental and metamorphic rates in some amphibians.

Potential Disruption of the Endocrine Control of Amphibian Development and Metamorphosis

Although THs influence many physiological processes in amphibians, such as reproduction, metabolism, growth, and molting, the induction of metamorphosis by THs is by far the most dramatic and well-studied endocrine event in these animals. The hormonal regulation of amphibian development and metamorphosis is very complex and perhaps part of the basis for their extreme sensitivity to exogenous factors that disrupt the endocrine system. To assess how environmental stressors such as UVBR affect amphibian development, it is important to understand the endocrine axis controlling amphibian metamorphosis. We provide a brief overview of the hormonal pathways involved, which have been extensively described by several authors (e.g., Dodd and Dodd 1976; Denver 1996; Tata 1996, 2006; Shi 2000; Shi and Ishizuya-Oka 2001; Denver et al. 2002; Brown 2005; Brown and Cai 2007; Fort et al. 2007).

Amphibian metamorphosis is under neuroendocrine control via the hypothalamus-pituitary-thyroid axis (Fig. 2; based on Denver 1997, 1998; Shi 2000). The hypothalamus is central in

regulating hormone secretion from the pituitary gland during metamorphosis (reviewed in Shi 2000). Corticotropin-releasing factor is a neuropeptide that is released from the hypothalamus and induces metamorphosis by stimulating the pituitary gland, which in turn releases the thyroid-stimulating hormone (TSH; Denver 1988; Denver and Licht 1989). On stimulation, the thyroid gland secretes TH, the primary hormone that controls metamorphosis and regulates the various developmental changes that occur in several different tadpole tissues. The conversion of TH to active and inactive forms by deiodinase enzymes plays an important role in regulating TH levels in various tissues at different stages of tadpole development. Deiodinase enzymes D1 and D2 convert T4 (thyroxine) to the biologically active T3 (triiodothryonine) in the thyroid gland or within target tissues (St. Germain 1994; Denver et al. 2002; Dubois et al. 2006; Kuiper et al. 2006). This conversion is imperative for amphibian development and metamorphosis because T3 is the active hormone and has an ~10 times higher affinity for the thyroid receptors (TRs) than does T4 (Jorgensen 1978). On the other hand, the conversions of T4 to reverse T3 and of T3 to T2 are carried out by deiodinase enzymes D1 and D3 (St. Germain 1994; Denver et al. 2002; Dubois et al. 2006; Kuiper et al. 2006). Because reverse T3 and T2 have a very low affinity for the TRs, these conversions inactivate T4 and T3 (St. Germain 1994; Denver et al. 2002). TH levels are relatively low during early larval development; they increase during metamorphic climax and then drop dramatically after tail resorption, remaining low in the adult amphibian (Burggren and Just 1992). TH binds to its TR, which forms a heterodimer with liganded 9-*cis*-retinoic acid receptors (RXR) and binds the thyroid response element to activate the expression of genes involved in development and metamorphosis (Shi 2000).

Although amphibian metamorphosis is primarily dependent on TH (Denver et al. 2002; Brown 2005), corticosteroid levels released from the interrenal glands rise concurrently with plasma TH levels during late prometamorphosis and metamorphic climax and may synergize with TH to accelerate metamorphosis (Kikuyama et al. 1993; for further details, see Shi 2000; Fig. 2). On the other hand, prolactin (PRL), a pituitary hormone controlled by the hypothalamic neurosecretion of thyrotropin-releasing hormone (TRH), is implicated in the inhibition of amphibian development and in the acceleration of growth (Fig. 2). The antimetamorphic action of PRL may be mediated through the blockage of the autoinduction of TR gene expression (Baker and Tata 1992; Tata et al. 1993).

UVBR levels have increased globally over the past few decades, yet very few studies have examined mechanisms whereby UVBR may alter endocrine function in vertebrates. There exist very limited data on the impacts of UVBR exposure on the TH system in general. Since it has been shown that UVBR can induce disruptions in developmental and metamorphic rates of amphibians and because developmental changes are correlated with distinct and specific hormonal cues, the investigation of UVBR-mediated effects on the TH system of amphibians is warranted. Belden et al. (2003) conducted the only study to our knowledge on the effect of UVBR exposure on the part of the endocrine axis controlling amphibian development. They found that *Rana cascadae* tadpoles did not avoid low levels of UVBR and that whole-body corticosterone levels were unaffected by exposure. However, rates of development were not measured in these animals because the focus of the study was to investigate UVBR avoidance behavior and survival in exposed tadpoles. Because effects of UVBR on the thyroid system in amphibians remain to be fully explored and because the brain-pituitary-thyroid axis is well conserved among vertebrates (Crespi and Denver 2005; Fort et al. 2007), examining previous findings on the effects of UVBR on the thyroidal axis in other organisms may provide insight into potential impacts on amphibians.

Some studies in mammals have shown that UVBR exposure can affect key aspects of the thyroid system, while others have not. Exposure of calves to artificial UVBR increased the activity of the thyroid gland; the circulating concentration of T4 in exposed animals was significantly increased, but no significant change in T3 was observed (Broucek et al. 1987). Exposure of mouse skin cells (keratinocytes) to UVBR in vitro reduced the levels of retinoid receptor proteins (RXR α and RAR α; Darwiche et al. 2005). While it is not known whether this would occur in UVBR-exposed tadpoles, RXR is critical for metamorphosis because it heterodimerizes with TRs to initiate TH-mediated transcription of key genes in the metamorphic cascade. Thus, it is plausible that UVBR could upset these signaling pathways to affect some aspects of metamorphosis. Studies in humans have also demonstrated that various hormones may be affected by exposure to artificial sources of UVBR. For example, a group of 24 healthy men exposed to UVBR did not exhibit significant changes in serum levels of several hormones, including TSH, PRL, T4, and T3 (Falkenbach et al. 1997). On the other hand, Altmeyer et al. (1983) found a significant decrease in the average cortisol plasma level measured in 49 healthy test persons after 60 min of whole-body UV irradiation. Irradiation also caused a decrease in the concentrations of T4 and T3 (13.1% and 22.1%, respectively; statistically significant only for T3), but levels of adrenocorticotropin and TSH remained unchanged.

Decreased rates of development and metamorphosis in amphibians exposed to UVBR suggest a disruption of the TH system. The examples described above demonstrate that UVBR has the potential to affect this endocrine system in some vertebrates. Though most of these studies were not conducted using amphibians, the results may be useful in choosing end points in experiments that aim to investigate similar effects of UVBR in amphibians. The thyroid axis is very complex. Several possible mechanisms of action whereby UVBR may suppress development and metamorphosis, either directly or indirectly, may exist. With several gene sequences currently available for various amphibian species and with amphibian genome projects completed or in progress (e.g., *Xenopus tropicalis*), the study of the molecular mechanisms behind physiological disruptions of animals exposed to environmental stressors has been facil-

itated. Potential mechanisms that have not been investigated in any amphibian species exposed to UVBR include decreased production of TH or disruption of the expression of genes coding for key proteins regulating TH action (e.g., TH receptors, deiodinases).

Conclusions and Perspectives

While it is clear that there exist regions where UVBR is increasing, much remains to be learned about the extent to which this will affect amphibian populations. Key questions remain regarding, for example, the minimum change in UVBR that would impact a given population or species. There are certainly major differences in species and population sensitivity to UVBR that remain to be elucidated not only by identification of the most sensitive species but also by determining which life-history stages are most susceptible to UVBR. There is also a need to include a greater variety of species in the study of UVBR effects on amphibians. Nishikawa and Wassersug (1988) reported that approximately one-third of citations on anuran biology and three-quarters of citations on their neurobiology are based on just two genera, *Rana* and *Xenopus*—*Rana* because they inhabit regions where most herpetologists live (North America and temperate Europe) and *Xenopus* because they are easily reared in captivity (Burggren and Just 1992). The data presented in Tables 1 and 2 corroborate this observation, since most of the species used in the studies mentioned are from the genus *Rana*. However, several other genera of amphibians are impacted by environmental stressors and are currently in decline, such as those inhabiting tropical ecosystems, and these should be equally represented in studies to reflect the actual effects of UVBR and other stressors to amphibians in general.

The mechanisms and physiological basis for the observed sublethal effects of UVBR are poorly understood. Multidisciplinary approaches must be used to fully understand how organisms respond to environmental stressors. We must move beyond solely examining overt death of amphibians and begin to understand that subtle effects of UVBR alone and in combination with other stressors can have profound effects on development, metamorphosis, and other physiological processes (e.g., sexual development, reproduction, somatic growth). A disruption in development caused by UVBR either directly or indirectly ultimately leads to a disruption at the level of the endocrine system that regulates amphibian development and metamorphosis, as discussed in this article. Delays in development may be occurring in nature and could negatively impact amphibian populations. However, there is no one cause for amphibian declines. Multiple stressors are likely working in concert, contributing to the problem. It is therefore imperative that the impacts of multiple stressors be considered in future experimental approaches.

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