Review

Global amphibian declines: A review

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Global biodiversity losses have assumed the status of a crisis and as a part of it, many amphibian populations are declining. It is estimated that the current rate of extinction is greater than any known in the last 100,000 years. Recent studies have shown that nearly one-third of amphibian species are threatened. Amphibians are thought to be indicator species of overall environmental health, and understanding their declines may serve as a model for understanding species declines in general. The present review discusses the present state of knowledge about the complex problem of amphibian declines: the hypotheses which have been propounded, the remedial measures which have been proposed and the future directions for research in this important area.

Key words: Biodiversity, amphibian declines, extinction, threatened, indicator species, environmental health, hypotheses.

INTRODUCTION

Global biodiversity loss is currently a major international concern (Stuart et al., 2004; Beebee and Griffiths, 2005; Mandal, 2011; Mfunda and Roskaft, 2011). Although the exact number of species being lost is not known, it is estimated that the current rate of extinction is greater than any known in the last 100,000 years (Eldridge, 1998). As part of the global loss of species, amphibian populations are declining throughout world (Wake, 1991; Alford and Richards, 1999; Houlan, et al., 2000; Blaustein and Kiesecker, 2002; Stuart et al, 2004; Monney et al., 2011). Concern about amphibians is due in part to their value as indicators of environmental stress (Blaustein and 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. Because of their contribution to trophic dynamics, loss of amphibians will probably affect other organisms (Blaustein et al., 1994; Blaustein and Kiesecker, 2002).

The current wave of interest in amphibian biology and in the possibility that there is a global pattern of decline and loss began in 1989 at the First World Congress of Herpetology (Barinaga, 1990). Historical data indicate that decline began as early as the 1970s in the Western United States (Drost and Fellers, 1996) and northeastern Australia (Collins and Storfer, 2003). Sudden disappearances of montane species were noted simultaneously in Costa Rica, Ecuador and Venezuela (Pounds and Crump, 1994; Pounds et al., 1997; Young et al., 2001). In some regions, many declines took place in seemingly pristine habitats (Pounds and Crump, 1994; Drost and Fellers, 1996; Pounds et al., 1997; Young et al., 2001). A number of reports mainly in 1990s convinced herpetologists that amphibian declines are non-random unidirectional events.

Amphibians have been found to be far more threatened than either birds (BirdLife International, 2004) or mammals (IUCN, 2003), with 1896 species (32%) being globally threatened, as compared with 12% of birds and 23% of mammals. Many amphibian species are on the brink of extinction, with 436 species (7.37%) listed as critically endangered, as compared with 179 birds (1.8%) and 184 mammals (3.8%). The level of threat to amphibians is underestimated because 1401 species

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(23.67%) are Data Deficient, as compared with only 78 birds (0.8%) and 256 mammals (5.3%). 34 species of amphibians have become extinct since 1500 AD, and nine of these extinctions have taken place since 1980 (Stuart et al., 2004). Species described since the Global Amphibian Assessment completed its first round of evaluating the status of all then-recognized species in 2004 are mostly too poorly known to be assessed with more than 20% of analyzed species in the top three categories of threat, that is, Vulnerable, Endangered, or Critically Endangered (Wake and Vredenburg, 2008). With the inclusion of species which are "Possibly Extinct", the IUCN-Global Amphibian Assessment (GAA) estimates that 168 amphibian species have become extinct and at least 2,469 (43%) are declining in population. The rate of extinction of amphibians is alarming. In a recent study, it has been estimated that the current rates of extinction are 211 times the background extinction rate for amphibians and it has been projected that rates as high as 25,000 to 45,000 times greater would be there if all of the currently threatened species go extinct (McCallum, 2007).

Understanding the cause of declines is important in order to explain why amphibians are at risk. Amphibians are indicators of general environmental health, and thus causes of declines might also threaten other species. A key step in understanding and perhaps slowing amphibian declines will be sorting among the potential causes and clarifying the circumstances when each is operating alone or in combination.

HYPOTHESES PROPOUNDED TO EXPLAIN AMPHIBIAN DECLINES

Six leading hypotheses have been sorted into two classes by Collins and Storfer (2003). For Class I hypotheses, alien species, over-exploitation and land-use change have been included. For these, we have a good understanding of the basic ecological mechanisms underlying declines. Included in the Class II hypotheses are global change (including climate change and UV radiation), contaminants and emerging infectious diseases. For these, we have a poor but improving understanding of how each might cause declines.

Class I hypotheses

**Alien species**

Alien species often cause declines and even extinctions of native amphibian populations. Multiple mechanisms may act alone or together, including predation by alien species on natives, competition between one or more life stages, introduction of pathogens by non-natives and hybridization (Collins and Storfer, 2003). An example of this phenomenon is provided by effects of non-native fish on yellow-legged frogs (Rana muscosa). In the early 1900s, R. muscosa was common and widespread above 1500 m in the Sierra Nevada Mountains of California and Nevada (USA). By 1910, these populations declined or disappeared from lakes where non-native trout had been stocked since the 1800s. Predation by trout proved to be the direct mechanism causing frog declines; although hatchery reared fish may also transmit disease (Knapp et al., 2001). Disruption of trophic connections between aquatic and terrestrial food webs is an important but poorly understood consequence of fish introduction in montane lakes and streams. The same mechanism has been suggested to be responsible for population decline of R. muscosa in some of the lakes in Sierra Nevada (Finlay and Vredenburg, 2007).

Although the mechanism by which aliens can cause amphibian declines or extinctions is relatively straightforward, dealing with aliens once they are introduced is difficult. In addition, aliens can interact with other factors, such as acting as vectors for emerging infectious diseases, resulting in complex indirect effects. Introduced predators may also have more subtle effects. Some R. muscosa populations persisting in fish-free environments have become isolated from other populations by surrounding aquatic habitats containing introduced fishes. This may lead to regional extinction by preventing migration among local populations, thus disrupting the metapopulation structure (Pilliod and Peterson, 2001). Removal of introduced fish from some lakes in Sierra Nevada have shown an increase in the population of R. muscosa thus suggesting that at least in part, the removal of non native fish could help in reversing the trend of population decline of native amphibian species (Knapp et al., 2007).

**Over-exploitation**

The effects of harvesting on amphibian populations are poorly known, but there is evidence that it can be significant. Humans have devastated frog populations in several countries for the frog-leg trade. Before 1995, about two hundred million frogs were exported annually from Asia. By 1990, India was still illegally exporting approximately seventy million frogs each year, resulting in serious population declines (Oza, 1990). Lannoo et al. (1994) estimated that between 1920 and 1992 amphibian populations in one Iowa County declined from at least 20 million frogs to 50,000; one-third of this decline was attributed to harvesting with the rest due to wetland drainage.

**Land use/land cover change (habitat reduction)**

Land use change can facilitate local and eventually
regional extinction of populations and species by killing organisms, removing habitat or preventing access of animals to breeding sites. Land use change involves altering the way the humans use land, while land cover change is alteration of the physical or biotic nature of a site e.g. converting a forest to grassland (Meyer and Turner, 1992). They occur together or separately and both have generally been referred to as the term ‘land use change’ (Vitousek, 1994).

Land use change is the best documented cause of amphibian population declines. It reduces amphibian abundance and diversity in the areas directly affected (Heenan and M’Closkey, 1996; Heenan, 1997). Logging exposes terrestrial amphibians to drastically altered microclimatic regimes (Ash, 1997), soil compaction and desiccation, and reduction in habitat complexity (Welsh, 1990). It exposes aquatic amphibians to stream environments with increased siltation (Corn and Bury, 1989) and reduced woody debris (Bury and Corn, 1988).

Draining wetlands directly affects frog populations by removing breeding sites (Johnson, 1992), and by fragmenting populations (Elmberg, 1993; Semlitsch and Bodie, 1998), increasing the regional probability of extinction (Corn and Fogleman, 1984). Modification of terrestrial and aquatic habitats for urban development can reduce or eliminate amphibian populations. Populations of some amphibians in urban Florida declined after degradation of upland, dry season refuges and modification of wetlands used for breeding (Delis et al., 1996). Protection of aquatic breeding sites may be of little value if adjacent terrestrial habitats used by amphibians for feeding and shelter are destroyed (Semlitsch, 1998).

**Class II hypotheses**

Some amphibian species are declining, even under suitable habitat conditions, for reasons that are not fully understood. This has been referred to as “enigmatic decline” (Stuart et al., 2004). There are complex and often subtle interactions that connect global change or emerging infectious diseases with amphibian population trends. Population changes depend on many locally interacting variables (Blaustein and Kiesecker, 2002; Blaustein et al., 2003), but can be influenced by changes in other variables far from the target population (Kiesecker et al., 2001a).

**Global change (global environmental change)**

Global change is exemplified by increasing concentrations of carbon dioxide in the atmosphere, increased nitrogen fixation, increased atmospheric concentrations of gaseous nitrous oxide, widespread concentrations of synthetic organic compounds, altered biogeochemistry of global element cycles, harvesting of natural populations by humans, land use/land cover change and biological invasions by non-native species (Vitousek, 1994). Among the various factors proposed as causes for amphibian declines, global climate change has received relatively little attention (Linder et al., 2003).

Throughout the 350 million years of amphibian evolution (Duellman and Trueb, 1985), climate change has been the rule rather than the exception. Periods of relative stability have been punctuated with drastic, and sometimes quite abrupt, change (Alley et al., 1997; Taylor, 1997; Lang et al., 1999; Severinghaus and Brook, 1999; Birks and Ammann, 2000). For climatic factors to serve as a direct cause of amphibian declines, temperature and/or moisture patterns should be shown to have exceeded the lethal limits of a given population. As yet, no compelling evidence exists that these variables, acting singly or synergistically, have reached lethal levels for any amphibian population. A number of studies have found a correlation between climatic factors and amphibian declines. Few studies have made the effort to determine whether the observed climatic factors might have reached lethal levels—direct cause or facilitated some other factor that caused amphibian deaths—indirect cause (Carey and Alexander, 2003).

Indirect effects of climate change on amphibian population include factors that cause amphibian mortalities by affecting some factor that causes mortality. It is vital for a species to breed successfully in order to persist over time. Global climate change could potentially disrupt amphibian breeding if global warming caused amphibians to breed earlier in the spring than in previous years, and population recruitment suffered as a consequence (Carey and Alexander, 2003). Several studies have shown that the initiation of breeding (arrival at breeding ponds and/or initiation of calling) of at least some amphibian species track variation in ambient temperature (Reading, 1998; Blaustein et al., 2001a). For instance, breeding activity of the common toad, Bufo bufo, as judged by the first date of the annual arrival at the breeding ponds over a period of 19 years, occurred up to 7 weeks earlier in milder winters than in average or cold winters (Reading, 1998). The limited evidence available currently indicates that both benefits and problems may result from early breeding. In years in which B. bufo breed early in the spring, the larval stage lasted 30 days longer than in late spawning years (Reading and Clarke, 1999). Even so, the tadpoles still metamorphosed an average of 36 days earlier than in late spawning years. The advantage of early metamorphosis is that the toadlets have increased time to grow and store energy prior to hibernation in the autumn. The disadvantage is that tadpole mortality is greater in early years because the cold temperatures associated with early breeding cause increased mortality (Reading and Clarke, 1999).

**UV radiation**

Depletion of stratospheric ozone and resultant seasonal
increases in ultraviolet B (UV-B) radiation at the earth's surface (Kerr and McElroy, 1993) have stimulated interest in the possible relationship between resistance of amphibian embryos to UV-B damage and declines. Data from several sources indicate that levels of UV-B radiation have risen significantly in the tropics and in temperate regions (Kerr and McElroy, 1993; Herman et al., 1996; Middleton et al., 2001). UV-B (280 to 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 sub lethal damage (Tevini, 1993). UV-B radiation can kill amphibians directly, cause sub lethal effects or act in concert with contaminants, pathogens or with changes in climate to adversely affect amphibians (Blaustein et al., 2003).

A comparison of the hatching rates between groups of embryos exposed to ambient UV-B radiation with those shielded from UV-B in field experiments showed that the embryos of many species displayed greater hatching rates in shielded regimes (Blaustein et al., 1998, 2001a). A number of studies suggest that the embryos of certain species use various defenses against the harmful effects of UV-B radiation better than others. Even within a species, there may be variation between populations in their ability to cope with UV-B radiation (Belden and Blaustein, 2002a, b).

A variety of sub lethal effects are induced in amphibians after exposure to UV-B radiation. It can alter their behaviour (Nagl and Hofer, 1997; Blaustein et al., 2000; Kats et al., 2000), slow growth and development (Belden et al., 2000; Pahkala et al., 2000, 2001; Smith et al., 2000; Belden and Blaustein, 2002a), or induce developmental and physiological malformations (Worrrest and 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 caused a number of developmental and physiological deformities in frogs and toads (Worrrest and Kimeldorf, 1976; Hays et al., 1996). These included 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. It needs to be emphasized that 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 and Belden, 2003). Thus, amphibians may avoid sunlight, possess pigmentation 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 (Blaustein and Belden, 2003).

**Contaminants**

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

Relatively, a large literature on the effects of contaminants on amphibians is available, yet little is known about how contaminants affect amphibians at the population level (Alford and Richards, 1999). Laboratory studies have demonstrated the lethal effects of many pesticides including hampering of growth and development, and developmental and behavioural abnormalities (Blaustein et al., 1997b; Bridges and Semlitsch, 2000; Sparling et al., 2000; Boone and 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 and Seiber, 1997; Sparling et al., 2001). Several pesticides and their degradation products acting as endocrine disruptors cause altered reproductive systems in amphibians (Colborn and Clement, 1992; Stebbins and Cohen, 1995; Hayes, 2000). Hayes et al. (2002) showed that the herbicide atrazine induced hermaphroditism and demasculanized the larynges of exposed African clawed frog (*Xenopus laevis*) males. They hypothesized that atrazine disrupted steroidogenesis resulting in demasculinization and hermaphroditism. 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).

Intensive agricultural and industrial production and pollution from mines has increased the prevalence of heavy metals in surface waters that may ultimately affect amphibian populations. Metals such as aluminium, lead, zinc, cadmium, mercury, silver, copper, arsenic, manganese, molybdenum and antimony have a number of effects on amphibians. They can be lethal or induce sub lethal effects such as slowing growth and development and altering behaviour (Blaustein et al., 1997b; Lefcort et al., 1998, 1999; Raimondo et al., 1998;
Blaustein et al., 2003). In many cases, the effects of heavy metals on amphibian survival have been observed to be linked closely to acidification, because heavy metals leach from soils in contact with acidic water (Blaustein et al., 2003). Laboratory experiments have shown that aluminium levels as low as 10 to 20 ppb at pH 4.7 cause reduced hatching success of B. americanus and R. sylvatica eggs (Clark and LaZerte, 1987).

Amphibian species have been found to be susceptible to nitrogenous fertilizers (Heen, 1995; Marco et al., 1999; Rouse et al., 1999). Experimental studies have 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 (Marco et al., 1999).

**Emerging infectious diseases**

Emerging infectious diseases are diseases that are newly recognized, newly appeared in a population or rapidly increasing in incidence, virulence or geographical range (Daszak et al., 2000, 2003). It has been hypothesized that emerging pathogens can cause the decline and even extinction of amphibian populations. Three pathogens that have received recent attention with regard to amphibian population declines are the chytridiomycete, *Batrachochytrium dendrobatidis*, found in several areas where population declines have occurred, a pathogenic oomycete - *Saprolegnia ferax*, contributing to large scale amphibian embryonic mortality in North America, and an iridovirus--*Ambystoma tigrinum* virus (ATV) isolated from diseased tiger salamanders. All three of these pathogens appear to be involved in complex interactions with biotic and abiotic agents (Jancovich et al., 1997; Kiesecker et al., 2001b; Nichols et al., 2001). The chytrid fungus has been found to be associated with anuran declines and extinctions in Australia, Central America and North America, but it also co-exists with non-declining species in the same area. This is important for two reasons: all wild amphibians are potential pathogen reservoirs, and species specific differences in susceptibility and life history affect host survivorship, and thus reservoir size (Collins and Storfer, 2003). Declines due to chytrid epizootics are most common at higher elevations in the tropics (Alford et al., 2001; Young et al., 2001). The most susceptible species have restricted elevational ranges, large body sizes and breed in streams. Ranaviruses are important pathogens of amphibians worldwide (Collins et al., 2003b). Two ranaviruses were isolated independently from tiger salamander epizootics - *Ambystoma tigrinum* virus from Arizona (Jancovich et al., 1997) and ranavirus from Saskatchewan, Canada (Bollinger et al., 1999). In contrast to chytrids that characteristically cause declines, long-term studies of Arizona tiger salamander populations suggest that iridoviruses cause population fluctuations (Collins et al., 1988). Recently an important finding has been that populations of *Rana muscosa* having high proportion of anti *Batrachochytrium dendrobatidis* bacterial populations survive even after the emergence of *B. dendrobatidis* infection in the population (Lam et al., 2010). In another study, it has been found that the presence of symbiotic bacteria on the skin of *R. muscosa* may inhibit infection and colonization of the skin by *B. dendrobatidis* and thus increase the effectiveness of innate defence mechanisms in the skin (Woodhams et al., 2007).

**REMEDIAL MEASURES FOR AMPHIBIAN DECLINES**

To cope with the problem of amphibian declines, a mix of short-term and long-term actions is needed and the challenge requires the participation of scientists, conservationists, policy makers and general public at large. The remedial measures which have been suggested include:

**Habitat protection**

For nine out of every ten amphibian species that are threatened by extinction, habitat loss is a risk factor. Therefore strengthening and expanding systems of public parks and private reserves must be the top priority for conservationists. Indeed, for many species, the existence of a protected natural area means the difference between survival and extinction (Young et al., 2004). In many cases in the developing world, “paper parks” provide little on-the-ground protection due to the lack of funding and parks staff (Young et al., 2004). In these cases, support is urgently needed to convert good intentions into functioning protected areas.

**Legal protection and public policy**

Legislation creating lists of endangered species is an important start in protecting these species from extinction. These lists need to be revised and made up to date to reflect current knowledge of taxonomy and threat status. Endangered species legislation needs to provide provisions for protection from habitat destruction as well as direct exploitation (Young et al., 2004). Import of exotic species needs to be severely restricted or banned, and also the trade and unrestricted exploitation of native species needs to be regulated based on ongoing population monitoring. Trade in species known to transmit diseases needs to be banned or severely restricted (IUCN, 2003).

**Captive breeding**

Species which are vulnerable to disease need to be bred...
in captivity, by making use of the best husbandry techniques for the target species. Breeding facilities with sustainable funding sources need to be established in developing countries with threatened fauna. Research efforts are needed for improving reintroduction success (Young et al., 2004).

Education and awareness

The role of media is tremendous in the arena of highlighting the plight of local threatened species. People need to be made aware of the problem. Topics related to amphibian declines should be part of the curriculum for school children. People need to be encouraged to create artificial fish free ponds to provide habitats for amphibians (Young et al., 2004).

Additional research

We have come a long way toward understanding amphibian declines since the first reports of population declines in the late 1980s. Despite major advances in recent years, amphibians remain poorly known creatures. For one-fifth of the species evaluated, scientists do not know enough to assign a threat category. Reasons cited include doubts about taxonomic status, incomplete range information, or lack of recent abundance and population trend data. Future research should focus on filling in the gaps for these data-deficient species (Young et al., 2004). There is an urgent need for population monitoring. Properly designed monitoring programs should encompass not only amphibian populations but also environmental variables if we are to understand the causes of population changes. Research about prevention of diseases and the effects of contaminants are thrust areas.

Understanding disease

Understanding amphibian diseases better and learning how to manage them in wild populations are the priority areas for preventing future amphibian extinctions. It is essential to understand the relationship between climatic events such as droughts and disease outbreaks.

FUTURE DIRECTIONS OF RESEARCH

Research has provided some answers with regard to the role of habitat destruction and non-indigenous species, the best-documented causes of amphibian declines (Alford and Richards, 1999). However, amphibian declines and extinctions often have been documented in areas not obviously affected by habitat destruction or non-indigenous species (Laurance et al., 1996; Pounds et al., 1997; Lips, 1998, 1999; Wake, 1998; Pounds, 2001). The complex relationships between putative causes underlying these examples require an interdisciplinary approach to untangle their inherent subtle and synergetic interactions (Collins et al., 2003a; Collins and Storfer, 2003). Indeed more research is needed before we can predict, halt or even reverse declines. Storfer (2003) has highlighted four areas of research: monitoring, metapopulation dynamics, molecular population genetics and multifactorial studies.

Monitoring

A number of important factors need to be considered when initiating or continuing a monitoring programme. First, appropriate monitoring methodologies must be used, which are often species-specific e.g., terrestrial salamanders vs. aquatic breeding anurans (Heyer et al., 1994). Choosing a correct sampling strategy also depends on resources (e.g., money, accessibility of field sites, personnel) and statistical sensitivities and potential sampling biases of monitoring methods to fluctuations of amphibian communities or target species. Secondly, an appropriate null hypothesis or baseline should be used, and finally appropriate statistical tests should be conducted to determine whether populations are declining, increasing or stable.

Metapopulation dynamics

Amphibians are characterized commonly as species with metapopulation structure. As defined originally by Levins (1970), a metapopulation consists of many connected local populations that each has a substantial probability of extinction. The term ‘metapopulation’ is currently used more loosely than Levins’ original definition, at least in part because extensive research is necessary to substantiate classical metapopulation structure (Hanski, 1999).

A conservative approach entails studying amphibians at the landscape level because regional persistence is probably governed at a spatial scale much larger than that of a single site (Semlitsch and Bodie, 1998; Semlitsch, 2000, 2002). Amphibian population dynamics (for aquatic breeding species) are controlled by the density distribution and quality of wetlands across the landscape and by the dispersal of amphibians among individual wetlands (Sjogren, 1991; Semlitsch, 2002). At a minimum, buffer zones are needed around amphibian breeding ponds, because individuals frequently travel at least hundreds of metres from a breeding pond (Semlitsch and Bodie, 1998; Semlitsch, 2000). Terrestrial species with direct development, such as some plethodontid salamanders, may not be studied as easily
because they do not aggregate at breeding sites. In these cases, appropriate monitoring techniques should be used to determine their distribution and density across the landscape. Applications of geographical information systems and gap analysis techniques are becoming increasingly accurate for identifying suitable habitats, and when combined with dispersal data of target species can provide valuable information as to the scope of areas that should be protected (Peterson et al., 2003).

Molecular genetic studies

Rapid advances in technology have made molecular genetic approaches to ecology and conservation more cost-effective, less time-consuming and more powerful. Major uses of molecular genetic tools for conservation include studies of genetic population structure and estimates of genetic variation, inbreeding and effective population size. New genomic and proteomic approaches also hold promise for conservation genetic studies (Hedrick, 2001). Molecular genetic estimates of gene flow can be used to infer dispersal patterns among populations across a landscape and, thus, the spatial extent of a metapopulation (Storfer, 2003). Genetic markers can help to detect recent hybridization events or interspecific gene flow (Allendorf et al., 2001), thus helping detection of presence of non-indigenous species. Genetic markers also can be used to detect recent fragmentation events, which may lead to declines.

Molecular genetic techniques allow estimation of genetic variability in populations which can have a variety of important implications, including inferring evolutionary potential of species. Genetic variability can be compared against reference populations (e.g., those of the same species not in decline) to determine whether populations are in decline (Storfer, 2003). Inbreeding generally increases with small population sizes, as indicated by deficiency of heterozygotes relative to expected frequencies (Wright, 1931; Hedrick, 2001).

Multifactorial studies

Multifactorial studies are critical for understanding and predicting amphibian population trends. The majority of studies to date have investigated effects of single factors on amphibian mortality, such as individual chemical contaminants, UV-B radiation or heavy metals. However, amphibians are probably exposed to several such environmental 'stressors' at once. Relyea and Mills (2001) showed that when grey tree frog (Hyla versicolor) was exposed to the pesticide carbaryl and predator chemical cues, they had significantly higher mortality than those exposed to carbaryl alone. Kiesecker et al. (2001a) showed that, via a complex interaction, exposure to UV-B radiation made western toad (Bufo boreas) eggs more susceptible to infection by a natural oomycete fungus, Saprolegnia ferax.

Various environmental factors could interact to increase amphibian susceptibility to disease. Chemical contaminants can affect amphibian immune systems negatively via, for example, increased serum cortisone levels, which are well known to suppress immune function (Carey, 1999). Climate change could constrict typically prolonged amphibian breeding seasons in the tropics, resulting in higher breeding densities and increased disease transmission (Pounds, 2001). Studies of pH and aluminium together showed increased larval mortality relative to either treatment alone (Horne and Dunson, 1995). Low pH and increased metal concentrations in Appalachian streams caused extreme larval salamander mortality, which led to population declines (Kucken et al., 1994).

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