Amphibian declines: future directions

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Abstract. The amphibian decline problem is complex, and there is no easy solution. I highlight four major areas of future research that should increase our ability to detect declines, elucidate their underlying mechanisms, and advance our capacity to manage and conserve amphibian populations. First, a statistically sensitive monitoring approach is necessary to determine the distribution and abundance of amphibian populations, to assess whether they are declining, and to quantify the extent of declines. Most amphibian populations characteristically fluctuate, detection probabilities may be low for many species and populations tend to decline in numbers between years more often than they increase. These traits make establishing monitoring programmes difficult and distinguishing declines from natural fluctuations challenging. It is thus necessary to determine the best monitoring techniques based on their statistical power and to use appropriate statistical methods for detecting population trends. Secondly, although amphibian population studies occur most commonly at single or few breeding sites, research should occur often at the landscape level, and conservation efforts should focus on suitable habitat (whether or not it is occupied) and dispersal capabilities of species. Metapopulation dynamics are probably important for many species, but we must be cautious how we define metapopulations. That is, the term ‘metapopulation’ is currently used to define a wide range of demographic situations in amphibian populations, each with different management implications. Thirdly, recent advances in molecular genetic techniques make it possible to infer demographic events such as effects of recent fragmentation, bottleneck or hybridization. Molecular techniques can be used in conjunction with census surveys to bolster knowledge about demographic processes such as declines. Alternatively, in the absence of long-term census data, molecular data can be used to infer population trends. New genomic approaches may make estimating adaptive genetic variation more feasible. Fourthly, multi-factorial studies are needed to disentangle the complexity of the several putative causes that probably interact to cause amphibian declines. Recent studies demonstrate the value of a multi-factorial approach, and more work is needed to elucidate the synergistic effects of multiple environmental factors affecting amphibian populations simultaneously worldwide.

Key words. Amphibian declines, metapopulation dynamics, molecular genetics, monitoring, multi-factorial studies.
climate change (Carey & Alexander, 2003), all of which detrimentally affect other organisms. Furthermore, amphibians are thought to be indicator species, providing an early warning of more general environmental problems. Consequently, the declining amphibian case can serve as a model for understanding the global biodiversity crisis in general.

Science has provided some answers with regard to the role of habitat destruction and non-indigenous species, the best-documented causes of declines (Alford & Richards, 1999). These issues must be dealt with at a policy level (Collins & Storfer, 2003; Kats & Ferrer, 2003). 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 (e.g. disease and climate change) require an interdisciplinary approach to untangle their inherent subtle and synergistic interactions (Collins et al., 2003; Collins & Storfer, 2003). Indeed, more research is needed before we can predict, halt or even reverse declines. I provide recommendations with regard to general approaches to the amphibian decline problem in the following sections. While not intended to be exhaustive, I highlight four pivotal areas of research: monitoring, metapopulation dynamics, molecular population genetics and multi-factorial studies.

MONITORING

A number of important factors should 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.

Despite standardization of survey methods (Heyer et al., 1994), design of appropriate monitoring programmes remains a central issue in the study of amphibians because little is known about sensitivities of most monitoring techniques to amphibian population fluctuations. Because resources are limited, monitoring programmes should entail surveys frequent enough to be statistically sensitive to declines or increases but not so frequent as to be economically infeasible. One way to optimize monitoring programmes is to compare several monitoring techniques at fine-grained temporal scales using time series analyses to determine predictive power. Fine-grained temporal data can be re-sampled at more coarse-grained temporal scales to determine optimal sampling intervals (i.e. when statistical sensitivity to population change is lost).

Direct comparisons of different amphibian monitoring methodologies at the same site should help researchers determine which method may be most sensitive and accurate; however, few studies have taken this approach. One example is a study of amphibian diversity at Jatún Sacha, Ecuador that compared use of artificial habitats for anuran oviposition, artificial cover and diurnal and nocturnal transects, with highest diversity detected using nocturnal transects (Pearman et al., 1995). Using four different salamander monitoring techniques (leaf litter bags, 1 m² quadrats, 50 × 1 m visual encounter transects and electric shocking), Jung et al. (2000) showed similarities in relative bias (defined as variation among sites in proportion of animals detected using mark–recapture methods), and concluded that choice of survey method should be based on logistics and precision (Jung et al., 2000).

Sampling bias of monitoring techniques is an important general consideration (Jung et al., 2000). Little is known about sampling bias of different techniques, but bias can clearly affect our determinations as to the status of populations. For example, some techniques may consistently over- or underestimate numbers of animals. Another possibility is that sampling bias can cause over estimation of numbers at large population sizes and underestimations at small sizes. Monitoring bias of particular methods may be species-specific, but studies of bias could focus on different techniques and representative amphibian life histories (e.g. terrestrial salamanders...
with direct development, aquatic-breeding frogs, etc.). Such studies will be useful for choosing appropriate monitoring techniques for study species.

Despite the fact that few studies directly compare monitoring techniques, some researchers have argued that numerical counts of individuals are preferred to mark–recapture studies because of large standard errors associated commonly with mark–recapture (Alford & Richards, 1999). However, both direct counts and mark–recapture studies often take place only at breeding sites (e.g. via drift fence surveys or call surveys), which can be misleading. Seasonal variation in water availability, variation in behaviour and proximity to other breeding sites can make breeding pond surveys inaccurate (Alford & Richards, 1999, see Fig. 2). Single visits suffer from the same limitations. Thus, repeated visits to breeding and non-breeding sites may be necessary to obtain accurate estimates of amphibian population sizes. In addition, it is crucial to study amphibians at larger spatial scales than a single pond or a single site (Semlitsch, 2000). That is, amphibians are thought commonly to act as metapopulations (see below), with recruitment and breeding occurring at a landscape level (Alford & Richards, 1999; Semlitsch, 2000, 2002).

Once a method has been established to determine how to estimate population sizes, it is then necessary to determine whether they are in decline. Many amphibian species undergo characteristic, wide fluctuations in population numbers (Pechmann et al., 1991). Thus, detecting few animals in a given year or even over several years does not necessarily translate into a decline. Many years of data are typically needed before statistical analyses have sufficient power to detect changes in amphibian population trends. However, a meta-analysis of long-term amphibian population data suggests that coefficients of variation (CV) increased with time in 27 of 29 studies that were greater than 8 years in duration (Marsh, 2001). This observed trend goes against the expectation that CVs will level off through time, suggesting power for even long-term amphibian monitoring datasets may be low (Marsh, 2001).

Before the initiation of a monitoring programme, power analyses should be conducted to resolve issues such as sampling frequency and number of samples needed to detect population trends accurately. General guidelines for monitoring programmes and power analyses of monitoring frequency can be found at http://www.mpl-pwrc.usgs.gov/powcase/index.html provided by the U.S. Geological Survey (USGS) (Gibbs, 1995). Given limited resources, there is a trade-off between the number of sites that can be sampled and the number of visits per season or year. This trade-off between sampling periodicity and number of samples should be considered for the species being studied. A recent study suggests a single annual search at a larger number of sites yields higher statistical power than repeated seasonal searches for some species of terrestrial salamanders (Smith & Petranka, 2000).

Once an appropriate sampling strategy is chosen based on resources, reliability and statistical power, a correct null hypothesis must also be selected to determine statistically whether populations are increasing or declining (Pechmann et al., 1991; Pechmann & Wilbur, 1994; Reed & Blaustein, 1995; Alford & Richards, 1999). Alford & Richards (1999) show, with both a heuristic model and a meta-analysis of long-term amphibian data, amphibian populations tend to decrease in numbers from one year to the next more often than they increase or remain stable. Thus, finding that a population is decreasing (between proximal years) during the majority of years under study does not necessarily mean that it is declining. Correlations of numbers of animals detected with year of the study are probably a better measure (Blaustein et al., 1994; Alford & Richards, 1999). Two recent studies provide such an analysis, based on 936 amphibian population datasets from 1950 to 2000 (Houlahan et al., 2000; Alford et al., 2001). Although these studies disagree as to the timing, they both suggest that amphibians have exhibited periods of global decline. It is worth noting that whereas a negative correlation suggests a decline, the lack of a significant correlation could mean that population size is fluctuating, or that the study lacks sufficient statistical power to determine that a population is declining or increasing.

Recent work suggests that collecting site occupancy (i.e. presence/absence) data may help researchers circumvent these logistical problems (MacKenzie et al., 2002). Estimating proportion of
area occupied by a species, rather than abundance data, is central to the Amphibian Research and Monitoring Initiative established by the USGS (http://edc2.usgs.gov/armi/paoEstimator.asp). Critical to estimating proportion of area occupied is determining the detection probability of species being studied; i.e. because amphibians are often fossorial, cryptic or generally in low numbers, they may be missed due to low detection probabilities, which bias estimates of proportion of area occupied downward. MacKenzie et al. (2002) develop a model whereby proportion of area occupied can be estimated while accounting for detection probability. Declines may then be detected potentially by a decrease in proportion of area occupied. The proportion of area occupied methodology avoids problems with determining decreases in abundances and can be less costly in terms of labour. The application of this approach has received limited testing, and it may have utility for species other than those studied (Pseudacris crucifer and Bufo americanus).

In summary, monitoring amphibians is difficult, particularly if the goal is detecting declines. Some recent studies show promise for amphibian monitoring programmes, but long-term data are necessary to obtain adequate statistical power to detect declines. This may present a logistical problem for species suspected in decline; they may become extinct before declines are detected statistically. Potential molecular genetic solutions to this problem are discussed below. Declines are often noticed when a species disappears from portions of its range, highlighting the potential value of using the proportion of area occupied approach (MacKenzie et al., 2002). None the less, a careful approach should be used when establishing monitoring programmes, which includes using appropriate monitoring techniques, statistical tests of population trends and tests of statistical power before determining population status (i.e. fluctuating, declining or increasing).

METAPOPULATION DYNAMICS

Amphibians are characterized commonly as species with metapopulation structure. As defined originally by Levins (1970), a ‘classical’ metapopulation consists of many connected local populations that each has a substantial probability of extinction. Consequently, extinction–colonization events are common under Levins’s (1970) model, and metapopulation persistence occurs at the regional scale. Considerable theoretical and empirical work has been directed at this question since then, resulting in different definitions of the term because environments are complex and patchy, and species differ in dispersal capabilities (Harrison & Taylor, 1996; Hanski, 1999).

As a result, the term ‘metapopulation’ is currently used more loosely than Levins’s original definition, at least in part because extensive research is necessary to substantiate classical metapopulation structure (Hanski, 1999). A variety of definitions are now used, and while these differences are semantic (Harrison & Taylor, 1996), they may have important management implications. An example is illustrated by comparing two classic amphibian ‘metapopulation’ studies. The pool frog (Rana lessonae) undergoes frequent extinction–recolonization events in Sweden (Sjögren-Gulve, 1994), and resembles Levins’ (1970) original model closely. Extinction probability increases with isolation from nearby sites (Sjögren-Gulve, 1994), and thus corridors to enhance movement among ponds might be an appropriate management strategy. In contrast, a study of red-spotted newts (Notophthalmus viridescens; Gill, 1978) does not satisfy the movement criterion. Of more than 8500 animals studied, adults exhibited extreme site philopatry, with only a single individual that dispersed between ponds (Gill, 1978). Population dynamics were apparently governed by reproductive failure in most ponds in most years, with reproductive success probably occurring at ‘hot spot’ ponds that varied spatiotemporally (Gill, 1978). In this case, corridors would probably be an ineffective management tool. Thus, the role of dispersal in metapopulation persistence is critical in one system, but not in the other. Both studies suggest, however, that because positive recruitment varied among population(s) in time, landscape-level management with equal conservation effort placed on suitable habitats would be appropriate.

In contrast, amphibian metapopulations that follow a source–sink model, whereby a few ponds show consistently high recruitment, while others consistently show little to no recruitment, might be managed differently. For example, Columbia spotted frogs (Rana lutiventris) in central Idaho...
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move among lakes and ponds in a mountain basin, and habitat use shifts seasonally (Pilliod et al., 2002). Some water bodies serve as overwintering habitats, while others are summer habitats, and yet others are breeding sites. A detailed analysis suggests that ponds that serve as both breeding and overwintering sites are rare, but these ponds are critical source populations that sustain frog populations across the basin. Pilliod et al. (2002) found that most breeding sites in the study area were too shallow to provide suitable overwintering habitat and, consequently, metamorphosed animals from shallow breeding sites move to deeper lakes to overwinter. These deeper lakes (= 2 m) most often contain non-native predatory trout (Bahls, 1992), resulting in no positive recruitment to the system despite often high numbers of metamorphs that emerge from associated breeding ponds (Pilliod & Peterson, 2001). In this source–sink system, conservation focus should be placed on the few breeding ponds that do not have associated overwintering sites with fish, rather than equal distribution of conservation effort on the system as a whole (Pilliod & Peterson 2001). Additional effort may focus on eradication of fish, which were introduced by humans, from other overwintering sites.

My purpose here is not to discuss the semantics of what constitutes a metapopulation, but to focus instead on the fact that the term has variable usage, and a detailed understanding of the biology of a system is important for conservation and management. We should be careful what we mean when we call a group of interconnected amphibian sites a metapopulation. As illustrated by the examples above, different types of metapopulations should be managed in different ways. Thus, assuming that a patchily distributed amphibian habitat is a metapopulation can result potentially in ineffective or inefficient management.

It is critical to understand dispersal among sites to determine the extent of a metapopulation. Both direct (mark–recapture) and indirect (gene flow) measures can be used to estimate movement among habitats. Ideally, both should be used because dispersal to a new site does not necessitate successful breeding, and genetic estimates may often reflect past events, rather than contemporary gene flow (Slatkin, 1987). Genetic data may also suggest occasional successful dispersal and breeding among sites that may only otherwise be detectable with long-term demographic studies; a single migrant per generation is theoretically sufficient to counteract genetic drift among sites (Wright, 1931).

Studies of genetic population structure and gene flow among populations (see below) may yield important data as to the extent of a metapopulation. If gene flow is high (e.g. greater than four migrants per generation, see Wright, 1931, 1951), reflecting panmixis, then groups of breeding ponds should be considered a single interbreeding population. In other cases gene flow may be limited, resulting in individual ponds being discrete genetic units. For example, the endangered Sonora tiger salamander (Ambystoma tigrinum stebbinsi) is found in human-made, earthen cattle tanks distributed across the San Rafael Valley in South-central Arizona (Collins et al., 1988). Despite close geographical proximity (< 500 m) of many ponds, gene flow estimates based on microsatellites suggest that each pond is an independent genetic unit and thus constitutes a separate population (Storfer, unpublished). Salamanders generally show high site fidelity via direct (e.g. mark–recapture) estimates of dispersal or indirect (i.e. genetic) estimates of movement (Gill, 1978; Routman, 1993; Larson et al., 1994; Storfer, 1999). Other studies also suggest that amphibian gene flow can be low, despite geographical proximity of sites (Driscoll, 1999; Scribner et al., 2001). Similar trends were observed in a long-term mark–recapture study of 14 species of amphibians among eight geographically proximal ponds in the Ocala National Forest, Florida. Of over 60 000 total animals recaptured over the past 8 years, very few (< < 1%) dispersed among ponds (Storfer & Greenberg, unpublished data).

Conversely, dispersal can be high. In a review by Marsh & Trenham (2001), the highest amphibian dispersal rates ranged from 2.5 km per year in Pacific treefrogs (Hyla regilla) to 15.1 km per year in Cane toads (Bufo marinus). Interpond migration rates varied widely among species and life stages within species, suggesting that the extent of a metapopulation varies spatially among species.

When there is dispersal among ponds, conservation should often occur at the landscape level;
extinction–recolonization dynamics may become an important characteristic of these populations (Sjögren-Gulve, 1994). That is, if a local pond undergoes an extinction event, it may be recolonized by dispersing animals from a nearby pond. Persistence of amphibian populations thus often relies on maintenance of connected sites, whether or not they are currently occupied (Sjögren, 1991; Hanski, 1999; Marsh & Trenham, 2001; Semlitsch, 2002).

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 & 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 (Sjögren, 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 & 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. Although outside the scope of this paper, 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. While these techniques have been reviewed elsewhere (see Avise & Hamrick, 1996; Hedrick, 2001; Frankham et al., 2002; Jehle & Arntzen, 2002), I highlight some areas in which they may be particularly useful for amphibian conservation and management. Major uses of molecular genetic tools for conservation include both 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).

Population genetic structure

Understanding genetic connectivity of populations can yield important information, particularly in the absence of mark–recapture estimates of dispersal. Recent technological advances have made molecular estimates of genetic population structure less costly, and it is now possible to obtain such data in less time than a well-designed mark–recapture study.

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. As discussed above, appropriate conservation and management strategies can be employed based on these data. Most commonly, estimates of migration (i.e. gene flow) among populations are determined by calculating $F_{ST}$ and its analogues, which estimates the distribution of heterozygosity among (sub)populations relative to that as if they were mating randomly (Wright, 1931; Slatkin, 1987). One problem with using gene flow to estimate contemporary dispersal is that estimates of gene flow may not reflect current events and depend on the coalescence time of the molecular markers (Templeton et al., 1995). Recent theory, such as nested clade analysis, provides a statistical means to distinguish between current and historical gene flow (Templeton, 1998). In addition, hypervariable markers such as microsatellites hold promise for contemporary gene flow studies (Goldstein & Schlötterer, 1999; Hedrick, 1999, 2001; Jehle & Arntzen, 2002).

A leading cause of amphibian declines is introduction of non-indigenous species. When introduced species are related closely, they may hybridize with native amphibians and compromise their genetic purity. Genetic markers can help to detect recent hybridization events or interspecific gene flow (reviewed in Allendorf et al., 2001). An example is illustrated by the case of the endangered Sonora tiger salamander, found only in the San Rafael Valley of south-central Arizona. A major concern is that barred tiger salamanders ($A. t. mavortium$), which are
used widely throughout the western United States as fishing bait, may be introduced into San Rafael Valley ponds. A combination of mitochondrial DNA and microsatellite data suggests that there are mixtures of the two parental subspecies and hybrids in some ponds (Storfer unpublished). Statistical methods, such as the assignment test (Paetkau et al., 1995) can assign individuals to source populations, or estimates of gametic disequilibrium (Allendorf et al., 2001), which can indicate recent mixing, can be used to infer recent hybridization events.

Genetic markers also can be used to detect recent fragmentation events, which may lead to declines. A study of bank voles (Clethrionomys glareolus) used microsatellites to compare gene flow across a small country road, a railway and a highway (Gerlach & Musolf, 2000). Significant genetic subdivision was detected between voles on both sides of the highway (built in the last 50 years) but not across the country road or railway (Gerlach & Musolf, 2000). Roads were also shown to increase genetic distance among habitats of European moor frogs (Rana arvalis; Vos et al., 2001). Logging that occurred over the past 20 years had significant effects on genetic distance of red-backed voles (Clethrionomys gapperi); relative to contiguous forest fragments or those that were connected by corridors, clearcut sites showed higher genetic subdivision based on microsatellite data (Mech & Hallett, 2001). Together, these studies suggest that microsatellites are sensitive enough to detect recent anthropogenic fragmentation events.

Detecting genetic isolation among once contiguous populations suggests they may now be isolated units, each with smaller effective population size and more prone to extinction from demographic stochasticity.

Genetic variation, inbreeding and effective population size

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. Inbreeding generally increases with small population sizes, as indicated by deficiency of heterozygotes relative to expected frequencies (Wright, 1931; Hedrick, 2001). Genetic studies can also be used to estimate effective population size, which is usually orders of magnitude smaller than census size (Frankham, 1995). A number of demographic factors can cause this result, such as skewed sex ratios, reproductive failure and assortative mating (reviewed in Lande & Barrowclough, 1987). Consequently, populations can be inbred despite large census sizes, and genetic methods could be used in conjunction with census estimates to infer demographic processes such as reproductive failure (reviewed in Jehle & Arntzen, 2002).

Longitudinal studies of genetic variability and effective population size could be used to infer declines, especially for species prone to fragmentation events. However, in the absence of reference data there are methods to calculate whether populations have been through bottlenecks, caused by several generations of reduced effective population size (Cornuet & Luikart, 1996; Luikart et al., 1998). An excess of heterozygosity relative to predicted levels is expected for a short time following a bottleneck, because rare alleles are lost more easily than common ones due to genetic drift, which has greater effects in smaller populations (Cornuet & Luikart, 1996). A study of natterjack toads (Bufo calamita) in Great Britain supports this theory, because populations thought to have undergone bottlenecks based on census population size had higher estimates of heterozygote excess than those not bottlenecked (Beebee & Rowe, 2001). Allele frequency shifts may also occur, which can be detected graphically (Luikart et al., 1998). These methods may be valuable for determination as to whether a population is declining, particularly in the absence of appropriate (long-term) population census data.

Genomics

One problem with estimates of genetic variation is that they occur most often at selectively neutral loci, and the correlation between neutral genetic variation and adaptive variation is often weak (Storfer, 1996). New molecular techniques, such as genomic or proteomic approaches, show promise for estimating adaptive variation at the population level. Microarrays, which contain thousands of segments of coding DNA printed
on slides or membranes, can be used to survey genomes for differences in gene expression between individuals exposed to different treatments. For example, one can compare up-regulated and down-regulated genes in an individual exposed to pesticides vs. one that is not. These types of comparisons can help to narrow the field of genes that may be affected when amphibians are exposed to particular stressor and suggest which genes are associated with increased resistance. Ideally, then, researchers can look for sequence differences in these genes among individuals that vary in susceptibility. Once identified, variation in key genes can be estimated in natural populations which can help scientists to predict effects of chemicals or pathogens, for example, at the population level. This knowledge can also help to guide translocations to enhance genetic variability of key genetic traits in populations that are depauperate (Dodd & Siegel, 1991). These ideas are highly speculative, because the utility of genomic approaches for predicting population-level effects has not been tested and the theory has not been sufficiently developed. Genomic techniques are also currently costly and time-consuming, which may prevent their use in many situations. However, as we look to the future, just as studies of neutral genetic variation have become used widely for conservation, these approaches will probably become more feasible.

MULTI-FACTORIAL STUDIES

Multi-factorial 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. Pesticides, for example, are often used in conjunction with herbicides and other chemicals such as fertilizers. Non-point source pollution can bring a combination of chemicals, fertilizers, and effluent into amphibian breeding sites, thereby exposing animals to complex mixtures of pollutants. In addition, foreign anthropogenic substances can interact with ‘natural’ stressors, such as predators or habitat ephemerality to affect amphibian populations negatively.

Two recent studies showed that anthropogenically induced stresses interacted synergistically with natural factors to increase amphibian mortality. Relyea & Mills (2001) showed that when grey treefrog tadpoles (Hyla versicolor) were 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. This scenario has been suggested as one potential cause of disease outbreaks and population declines in Central American frogs (Pounds, 2001). Habitat loss could also reduce the number of breeding ponds in an interconnected amphibian population, increasing densities and potential for disease transmission. Increased transmission rate has been shown to select for higher pathogen virulence (Ewald, 1994; Ebert, 1999), which could result in even higher mortality than in less dense populations. Disease transmission can also be augmented via introductions of non-indigenous species that are disease reservoirs; for example, hatchery fish carry Saprolegnia (Daszak et al., 2000, 2003; Kiesecker et al., 2001b).

Other multi-factorial studies showed interactions of acidity (low pH) and environmental factors. Amphibians differ in sensitivity to low pH, and several species show few effects in response to low pH (< 5) alone (Horne & Dunson, 1995); however, studies of pH and aluminium together showed increased larval mortality relative to either treatment alone (Horne & 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). In a study of leopard frogs, UV-B radiation and pH strongly
interacted, resulting in significantly lower survival than either factor alone (Long et al., 1995).

Sublethal effects
Toxicity studies generally do not consider sublethal effects, but rather focus on establishing an LC₅₀ level (i.e. the chemical concentration lethal to 50% of the experimental animals). Recent evidence suggests that ecologically relevant doses, which are often much lower, should be used to make predictions in natural populations. Hayes et al. (2002) showed that remarkably low levels (0.1 p.p.b.) of one of the most commonly used herbicides, atrazine, were sufficient to produce both testes and ovaries in male South African clawed frogs (Xenopus laevis). Ecologically realistic exposures were used (i.e. levels detected in the field), which were much lower than those to elicit 50% mortality. In fact, the levels of atrazine used had no effects on mortality, time to metamorphosis or size at metamorphosis, highlighting the need for studying sublethal physiological effects (Hayes et al., 2002). In another study, African clawed frogs exposed to ecologically relevant concentrations of ammonium perchlorate showed delayed development, inhibited thyroid function, altered gonadal differentiation and strongly female-biased sex ratios (Goleman et al., 2002a,b).

Such sublethal effects could make animals incapable of attracting mates or mating. Male African clawed frogs exposed to atrazine had a significantly smaller larynx than control males (Hayes et al., 2002), suggesting that they may not be able to call as loudly as unexposed males, and thus not attract mates as well. If all males in a breeding population are affected, overall mating success could be reduced.

Sublethal doses may also result in unpredictable community-level effects. For example, in a multi-species study, Woodhouse's toad (Bufo woodhousii) survival increased in treatments containing carbaryl, and more tadpoles survived in higher density environments than lower density treatments (Boone & Semlitsch, 2002). One resulting possibility is that increased larval survival could translate to increased subadult and adult competition for resources and, consequently, to lower adult survivorship. The same study suggested that carbaryl exposed green frog (Rana clamitans) tadpoles developed more slowly than controls (Boone & Semlitsch, 2002), which could translate to reduced survival in ephemeral habitats. In another study, tiger salamanders (Ambystoma tigrinum) had reduced growth rates at the low end of the range of naturally occurring pH levels (~4.5), but a sympatric species and prey of tiger salamanders, chorus frogs (Pseudacris triseriata), did not show lower growth rates (Kiesecker, 1996). The result was that tiger salamanders preyed less effectively on chorus frogs, probably due to gape limitation. Collectively, this work highlights the need for multi-species studies to unravel indirect effects.

Finally, intraspecific genetic variability in susceptibility to environmental chemicals necessitates proper replication before effects of particular chemicals can be discounted. Both leopard frogs (Rana sphenocephala) and grey treefrogs showed significant variation among families in susceptibility to carbaryl (Bridges & Semlitsch, 2000; Semlitsch et al., 2000).

Behaviour
Sublethal effects of chemicals, disease or other environmental factors may include behavioural alterations that could make animals more susceptible to predators, unable to feed or incapable of mating. One study showed that anurans survived a 14-day exposure period to atrazine (Rana pipiens, Rana sylvatica and Bufo americanus), but stopped eating (Allran & Karasov, 2002). Kats et al. (2000) showed that Rana cascadae tadpoles exposed to UV-B radiation spent significantly more time moving in the presence of predatory newts (Taricha granulosa) than controls. In addition, UV-B-exposed boreal toad (Bufo boreas) metamorphs did not avoid chemical cues from injured conspecifics as often as control animals avoided cues. The latter two experiments suggested that UV-B exposure may make amphibian larvae of some species more susceptible to predation via diminished avoidance (Kats et al., 2000).

Sublethal effects of environmental stressors may also inhibit activity level. Eight anuran species exposed to carbaryl showed significantly reduced activity level relative to controls (Bridges & Semlitsch, 2000). Amphibians have been shown to bask to induce ‘behavioural fever’, whereby body temperature is increased above the optimum for pathogen replication (Kluger, 1977).
Reduced activity level could inhibit such behavioural responses, resulting in increased disease susceptibility.

Overall, few studies have examined the effects of various environmental stressors on amphibian behaviour, despite the fact that behavioural studies are a necessary component of understanding sublethal effects. Mate choice experiments between exposed and control animals or direct estimates of breeding success, for example, could be used to translate behavioural effects of various factors on individuals to fecundity estimates. Reduced mating success as a result of exposure can clearly affect fecundity which, in turn, affects population viability.

**CONCLUSIONS**

The global amphibian decline problem is complex, and several factors are probably at work. Habitat loss, introductions of non-indigenous species, over-exploitation, global climate change, chemical contaminants and diseases all affect amphibians negatively. These factors may act alone or synergistically to eliminate populations and species, and the effects of these general environmental problems on amphibians may be viewed in the context of the larger biodiversity crisis. The papers in this volume review several of these factors in an attempt to provide a comprehensive overview of scientific progress in understanding amphibian declines and extinctions.

Here, I have emphasized four critical areas of research to improve our understanding of mechanisms underlying amphibian declines and, in the future, work to mitigate these causes. Monitoring programmes are crucial for establishing baselines and should be statistically sensitive to changes in amphibian population trends. Amphibian population dynamics are often governed by processes at a landscape scale, and our monitoring and management programmes should reflect the spatial scale that is biologically relevant to target species. Recent developments in molecular genetic tools can provide a valuable means to infer whether populations are declining, to estimate the effects of anthropogenic change such as fragmentation, and to aid in predictions as to how species may respond to future environmental change. Finally, multi-factorial studies are needed to unravel the synergistic effects of the several factors probably affecting amphibian populations and species. The worldwide coordination and collaboration among amphibian researchers aimed at comprehending these problems gives us hope for the survival of amphibians in the future.

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