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Review

The amphibian decline crisis: A watershed for conservation biology?

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Abstract

Amphibians have declined dramatically in many areas of the world. These declines seem to have worsened over the past 25 years and amphibians are now more threatened than either mammals or birds, though comparisons with other taxa are confounded by a shortage of reliable data. Research into amphibian declines has focused on: (1) documentation at the landscape or population level; and (2) observational and experimental work on potential causes of declines. Although loss of habitat is known to have impacted amphibians for decades, recent research has focused on the effects of environmental contaminants, UV-B irradiation, emerging diseases, the introduction of alien species, direct exploitation, and climate change. Such factors may interact with each other, but high levels of mortality do not necessarily lead to population declines. Major challenges remain in extrapolating from experimental data to population level effects, and in developing methodologies that will provide unbiased descriptions of amphibian population dynamics. Although amphibians have been widely advocated as good biological indicators, there is little evidence to suggest that they are more effective than other taxa as surrogate measures of biodiversity or habitat quality. As many of the threats facing amphibians are extremely difficult to neutralize in the short- to medium-term, the chances of ameliorating – let alone reversing – amphibian declines seem very poor.
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1. Introduction

The discipline of conservation biology emerged specifically to understand, quantify and ameliorate the current biodiversity crisis (Soulé, 1986). Amphibians are playing an increasingly prominent role in this issue. The current era of research into amphibian declines followed the First World Congress of Herpetology in 1989 (Wake, 1991, 1998), though reports of declines go back at least 25 years before then (Pechmann and Wilbur, 1994). However, the Congress certainly precipitated an upsurge of interest as within a few years declines were reported for more than 500 amphibian species out of an estimated global total (at that time) of over 4000. A recent report from the IUCN's Global Amphibian Assessment indicates that as many as a third of amphibian species, now estimated at over 5700, have undergone severe declines or extinction (Stuart et al., 2004) and that neotropical montane, stream-associated species are at particular risk. The declines have various likely causes, but there has been widespread controversy about their significance (Pechmann et al., 1991; Pechmann and Wilbur, 1994; Alford and Richards, 1999; Blaustein and Keisecker, 2002). In this review we: (1) briefly summarize research on causative factors (more comprehensive reviews on this aspect have been published elsewhere); (2) discuss the difficulties of extrapolation from experiments revealing possible causes of decline to demonstrating effects at the levels of populations and geographic ranges; and (3) consider how the study of amphibian declines relates to wider biodiversity issues, and the prospects for preventing continued amphibian declines. It is, however, important to be clear about how amphibian declines are defined (Green, 1997). In particular, we can identify declines in numbers of populations at the relatively large scale of biogeographic range, and fine-scale declines of specific populations in terms of overall size. Both are important but, as we shall see, the methods appropriate for investigating them can differ substantially.

2. Possible causes of amphibian declines

2.1. Habitat destruction, agrochemicals and chemical pollution

Some reasons why amphibians decline have been understood for decades (Collins and Storfer, 2003). Temporal patterns of amphibian decreases reflect the major impact of habitat destruction in Europe during the mid-twentieth century, in contrast with more recent declines elsewhere (Houlahan et al., 2000). This conclusion has been contested, with the suggestion that global declines only began in the 1990s (Alford et al., 2001), but intensive arable farming starting in the mid-20th century has destroyed much habitat in the northern hemisphere and led to general reductions of many species (Kolozsvary and Swihart, 1999; Ray et al., 2002). Road traffic also impacts significantly on amphibians, particularly during migrations to breeding sites (Hels and Buchwald, 2001). A general difficulty with assessing the effects of habitat destruction is measuring the full consequences over time and space. Findlay and Bourdages (2000) demonstrated that although negative consequences of road construction on amphibian populations were typically manifest within eight years, the full effects could take several decades to develop. Similarly, amphibian species richness in Ontario (Canada) ponds was heavily influenced by forest cover and road density, the effects of which were felt up to thousands of metres from the ponds (Houlahan and Findlay, 2003). "Long-range" consequences of habitat alteration will probably often lead to an underestimation of the significance of habitat destruction.

Concordant with habitat destruction has been the widespread use of fertilisers and pesticides that have also had deleterious consequences. Unlike the effects of habitat change that have been known for at least 100 years, our understanding of the effects of chemicals on amphibians is comparatively recent (Collins and Storfer, 2003). Atrazine, a widely applied herbicide, may cause feminisation of frogs at extremely low concentrations (<1 part per billion in pond water; Hayes et al., 2002a,b). If confirmed in other species and systems, these results could have serious ecological and economic consequences. Proving deleterious effects of pesticides at the population (as opposed to the laboratory, field enclosure or mesocosm) level is a difficult problem, but increasingly sophisticated analyses taking account of historical pesticide application data have strongly linked organophosphates and carbamates with the declines of four Californian anurans (Davidson, 2004). Another potential hazard to amphibians is acid precipitation. Amphibian embryos and larvae are vulnerable to both direct and indirect effects of low pH, but although local effects have been reported there is no evidence to link acidification with widespread amphibian declines (Vertucci and Corn, 1997). Indeed, none of these factors

readily account for the recent declines that have often been in areas apparently remote from human influence. In recent years, attention has therefore shifted to five other possible culprits: increased exposure to ultraviolet radiation, emerging diseases, the spread of alien species, direct exploitation and climate change.

2.2. UV-B irradiation

Anthropogenic ozone depletion has significantly increased UV-B (280–315 nm wavelength) radiation at ground level in high latitudes. UV-B has multiple effects on ecosystems, some harmful and some beneficial to individual organisms (Paul and Gwynn-Jones, 2003). Experiments in natural ponds, mostly in mountainous regions of North America, implicated increases in UV-B radiation as a possible cause of amphibian declines. Embryos of some species survived much better when shielded from UV-B than when exposed to current ambient levels (e.g., Blaustein et al., 1994). Similar damaging effects of elevated UV-B have also been observed in the laboratory, including with European species (e.g., Pahkala et al., 2003a). Furthermore, satellite-based measures of UV radiation levels at 20 sites in Central and South America recorded increases between 1979 and 1998 that were greatest in areas where amphibian declines have been most severe (Middleton et al., 2001). However, embryos and larvae of many declining species in tropical rainforests are not exposed to UV-B in the same way as those reproducing in the open mountain pools and lakes of temperate countries. Another problem with attributing declines to increases in UV-B radiation is that moderate concentrations of dissolved organic matter (DOM) ameliorate the effects of UV-B below the water surface. One recent study suggests that eggs and larvae in the majority of breeding sites used by amphibians in North American mountain regions are well protected by the DOM (Palen et al., 2002). Nevertheless, the significance of increased UV-B radiation in amphibian declines remains uncertain, and ongoing conflicts over methodologies, analysis and interpretation of available data show no sign of waning (e.g., Kats et al., 2000, 2002; Cummins, 2002; Blaustein et al., 2003; Heyer, 2003; Licht, 2003). As is often the case with amphibian responses to other anthropogenic factors, it seems likely that responses to UV-B – as well as interactions between UV-B and other agents of decline – vary considerably between species, regions and microclimates.

2.3. Diseases

Dramatic mass mortalities observed in some declining amphibian populations have provided evidence that diseases may play a significant role in some species and regions (e.g., Cunningham et al., 1996; Berger et al., 1998; Lips, 1999; Daszak et al., 2003). *Ranaviruses* (Iridoviridae) cause high levels of mortality in tiger salamanders (*Ambystoma tigrinum*) but populations usually recover afterwards (Jancovich et al., 1997, 2001). The genome of this virus is now completely sequenced, and there is no evidence of long-term persistence of viral particles outside salamanders (Collins et al., 2004). *Ribeiroia ondatraeis* is a trematode worm that causes leg deformities in frogs. Increased eutrophication favours the snails that provide a secondary host for this parasite (Johnson et al., 2002; Johnson and Chase, 2004). In this situation, changes in water quality caused by human activities have altered community structures, and thus predation patterns, to favour snails (*Planorbella* species) exclusively used as first intermediate hosts by *R. ondatraeis*. In this case, there is no evidence that amphibian populations with high incidences of parasite-caused deformities are undergoing declines, but such subtle effects might well be important in other host–pathogen and host–parasite systems. Complex interactions such as this might explain why outbreaks of disease are often not simply relatable to local presence or absence of pathogens. Saprolegniaceous fungi can cause high levels of egg mortality in amphibians and in this case there may be synergistic effects between fungal infection and UV-B (Kiesecker and Blaustein, 1995). However, the taxonomy of these fungi is confused, and different amphibian species may be vulnerable to different strains of *Saprolegnia* (Robinson et al., 2003). Undoubtedly the most worrying pathogen so far discovered, however, is a chytrid fungus *Batrachochytrium dendrobatidis*. This has been implicated in mass mortalities and population declines of amphibians in the Americas, Europe, Australia and New Zealand (Berger et al., 1998). It attacks the skin of post-metamorphic amphibians and causes death by impairing cutaneous respiration and osmoregulation. Disease outbreaks may follow either: (1) a weakened immune response in the amphibians caused, perhaps, by another stressor; or (2) an increased virulence of the pathogen. A molecular study on the phylogeography of the chytrid fungus indicated that the recent spread of a new, virulent clone may be responsible for its dramatic effects (Morehouse et al., 2003). Nevertheless, there are some perplexing aspects of chytrid infection that do not sit easily with a universal destructor of amphibians. In Australia, chytrid infection rate during 1994–1998 was higher in a species that has not declined (*Litoria wilcoxii*) than in a sympatric species (*Taudactylus eungellensis*) that had declined dramatically, purportedly due to chytridomycosis, during the 1980s (Retallick et al., 2004). Furthermore, infected specimens of *T. eungellensis* survived as well as uninfected specimens in the field. *Rana catesbeiana* may also carry the pathogen without showing clinical signs of chytridiomycosis (Daszak et al., 2004). Evidently there is substantial interspecific and intraspecific variation in

susceptibility to this pathogen, and interaction with other factors may be critical to its epidemiology. Chytrid fungi might therefore be responsible for some amphibian population declines, but it remains uncertain as to whether they are primary or secondary causes.

2.4. Introduced species

Evidence for alien species impacting on amphibian populations is based on two types of study: (1) negative correlations between alien predators and amphibians; and (2) experiments on the mechanisms by which aliens affect amphibians. Declines and local extinctions have been variously ascribed to introduced fish, other amphibians such as bullfrogs *R. catesbeiana* and cane toads *Bufo marinus*, and crayfish (Kats and Ferrer, 2003). Competition and predation generate the most obvious effects, causing reduced growth or survivorship, or alterations in behaviour or habitat use. Eggs and larvae are usually the most vulnerable stages, but even with very high levels of mortality, density dependence may still permit coexistence (Vonesh and De la Cruz, 2002). However, a well-studied example with clear population effects concerns the introduction of trout, for sporting purposes, into mountain lakes in the Californian Sierra Nevada range. These fish precipitated a major decline in mountain yellow legged frogs *R. muscosa*, which also use permanent water bodies, by predation of their larvae (Knapp and Matthews, 2000). By disrupting metapopulation structure, it is likely that the fish introductions ultimately also caused the demise of frog populations even in sites lacking the new predators. This also provides one of the few examples where a decline hypothesis has been tested empirically, with considerable success. Removal of trout from some lakes has resulted in rapid recovery of *R. muscosa* populations (Vredenberg, 2004).

Another danger of introduced species concerns the pathogens they can bring with them. It has been disconcerting to discover that the North American bullfrog, *R. catesbeiana*, is an effective carrier of chytridomycosis (Daszak et al., 2004). This large frog has become established in many parts of the world well outside its natural range.

Hybridization is another problem. In Switzerland and in southern England, introduced Italian crested newts (*Triturus carnifex*) have hybridized with native northern crested newts (*T. cristatus*), but the impacts on populations appear to be local rather than regional (Arntzen and Thorpe, 1999; Brede et al., 2000). On the other hand, introduced marsh frogs *R. ridibunda* have replaced the related water frog *R. lessonae* in several areas of western and central Europe, and probably this is at least partly a result of complex genetic consequences of hybridisation (Vorburger and Reyer, 2003). Due to hybridogenesis, in which the *lessonae* genome is lost in hybrid frog germ lines prior to meiosis, hybridisation favours increasing numbers of *R. ridibunda* in situations where the incoming *ridibunda* genomes are novel (i.e., have not been clonally cycled many times through the fertile hybrid *R. esculenta*, with consequent loss of viability). This is exactly the situation in places where *R. ridibunda* has been introduced.

2.5. Exploitation

Humans have long exploited amphibians, particularly the larger species, as a food resource in many parts of the world. Although this is undoubtedly the most substantial direct impact of human predation, others include collection for the pet trade, education and medical research, use as bait by anglers, and even conversion into leather as fashion accessories (Jensen and Camp, 2003). Tens of millions of amphibians, mostly large frog species, are sacrificed for food each year. Only a tiny proportion of this consumption is supported by captive breeding or farming enterprises. The bulk is a result of collection, much of it illegal, from wild populations in Asia. Although local population declines have been documented in areas of intense harvesting, there is little information about long-term or large-scale consequences.

2.6. Climate change

Recent changes in the global climate might impact adversely on amphibian populations. Global mean temperature rose by about 0.6 °C over the past 100 years with an accelerating trend since the 1970s, and there is increasing evidence for multiple effects of climate change on wildlife and ecosystems (e.g., Walther et al., 2002; Parmesan and Yohe, 2003). There is currently no evidence that climate change has led to tolerance limits in temperature or moisture being exceeded in amphibians (Carey and Alexander, 2003). There have, however, been detectable effects of climate change on breeding phenology although the extent of this varies between studies (Table 1). These differences may represent real variation between species and regions, or may be a function of different methodologies or study timeframes. Asynchronous changes in phenology might alter predation rates and thus disadvantage particular species within communities. The golden toad (*B. periglenes*) of the Costa Rican rainforest disappeared completely at the end of the 1980s and has not been seen since (Pounds et al., 1997). Many species of this rainforest biota declined over the past 20 years and several taxa previously restricted to lower altitudes have ascended higher into the mountains. It seems that warmer sea surface temperatures in the Pacific have caused thermal uplift in the atmosphere, such that cloud formation over the Costa Rican mountains is higher than in earlier decades. The forest has consequently become drier, and amphibian

Table 1
Summary of studies on long-term changes in breeding phenology in amphibians

Species	Time frame	Location	Breeding
Triturus vulgaris[1]	1978–1994	England	Earlier
Triturus helveticus[1]	1978–1994	England	Earlier
Triturus cristatus[1]	1978–1994	England	Earlier
Rana esculenta[1]	1978–1994	England	Earlier
Bufo calamita[1]	1978–1994	England	Earlier
Rana temporaria[1]	1978–1994	England	No change
Bufo bufo[2]	1980–1998	England	No change
Bufo boreas[3]	1980–2000	USA	No change
Rana cascadae[3]	1980–2000	USA	No change
Bufo fowleri[3]	1980–2000	Canada	No change
Pseudacris crucifer[3]	1980–2000	USA	No change
Pseudacris crucifer[3]	1900–1999	USA	Earlier
Rana sylvatica[4]	1900–1999	USA	Earlier
Rana catesbeiana[4]	1900–1999	USA	No change
Hyla versicolor[4]	1900–1999	USA	Earlier
Bufo americanus[4]	1900–1999	USA	No change
Rana clamitans[4]	1900–1999	USA	No change

Data from Beebee (1995)[1], Reading (1998)[2], Blaustein et al. (2001)[3], Gibbs and Breisch (2001)[4].

breeding less successful (Pounds et al., 1999). However, the recent climate patterns are not unprecedented and there is no evidence that similar conditions within the past 50 years led to amphibian declines (Alexander and Eischeid, 2001). It is therefore uncertain as to whether recent climate change is a significant cause of amphibian declines. Carey and Alexander (2003) make recommendations to address this issue, by suggesting improved methodologies to investigate climate change as an agent of amphibian declines.

2.7. Complex causes

The "context dependency" (Blaustein and Keisecker, 2002) of factors involved in amphibian declines is currently receiving increasing attention. A combination of reduced precipitation, consequent increased exposure to UV-B in shallow pools and high infestation with pathogenic fungus has been suggested as a complex cause of *B. boreas* declines (Kiesecker et al., 2001). Habitat destruction and pesticide drift from nearby agricultural areas were implicated as the main causes of amphibian declines in California, with weak evidence of UV-B and no evidence of climate change effects (Davidson et al., 2001, 2002). Developmental abnormalities in wood frog (*R. sylvatica*) tadpoles, caused primarily by trematode infections, were strongly enhanced by pesticides in agricultural run-off (Kiesecker, 2002). Low pH (4.5) acting synergistically with UV-B sharply reduced the survival of common frog (*R. temporaria*) tadpoles in a north Swedish population, but there were no significant effects on a southerly population with a history of exposure to low pH (Pahkala et al., 2003b). We should not be surprised that these complex studies are more realistic than those focusing on single putative agents of decline, and they certainly give a good idea of the difficulties faced when dealing with wild populations. Clearly, multifactorial approaches to testing agents of decline will continue to play a prominent role in future studies (Storfer, 2003).

3. Outstanding problems in the study of amphibian declines

3.1. Measuring effects at the distributional level

The overall significance of amphibian declines is manifest at the geographical range scale. Determining trends across a range requires an estimation of numbers of occupied sites at the landscape level (Green, 1997), usually with substantial resource implications due to the amount of effort involved. It is nevertheless essential because instability – or even declines – of local populations may not be reflected in overall changes at larger geographic scales. Green frog *R. clamitans* populations, for example, showed local instabilities but no overall trends across a larger spatial scale (Hecnar and M'Closkey, 1997). Estimates of overall distributional changes are very sensitive to survey effort and require multiple-year resurveys for high confidence (Skelly et al., 2003). For relatively rare species it may be possible to assess range scale effects directly, by intensive survey over many years (e.g., Buckley and Beebee, 2004). For more widespread species, methods have been developed to estimate site occupancy using techniques analogous to mark-recapture for estimating individual population sizes (MacKenzie et al., 2002). This approach has been developed by Royle (2004) to allow for varying abundance levels (not just presence or absence) when recording anuran calls. In a recent application of the MacKenzie et al. (2002) method with call surveys for four species of European anurans, Pellet and Schmidt (2005) showed that multiple (up to six) visits to each site were needed to establish presence or absence of three species with high confidence. The fourth species was too rare to make a robust assessment. This gives some idea of the high workload needed to assess potential declines at the range scale.

3.2. Measuring effects at the population level

A second aspect of the assessment of amphibian declines is the accurate measurement of changes in the sizes of individual populations. Obtaining quantitative data for this more finely scaled type of analysis has proved difficult in amphibians for several reasons (Green, 1997). A non-trivial issue is how to define a population in the first place. For pond-breeding species it has been common practice to treat breeding assemblages

as populations (i.e., pond = population), and that has been the case in many of the studies discussed below. However, this is obviously unsafe in many situations, for example in habitats where multiple temporary ponds occur in close proximity. Petranka et al. (2004) showed that for two North American species (*Ambystoma maculatum* and *R. sylvatica*) there was often a lack of demographic independence among clusters of ponds a few hundred metres apart, and genetic studies are arriving at similar conclusions. Gene flow among bullfrog (*R. catesbeiana*) breeding ponds in Canada, and common frog (*R. temporaria*) breeding ponds in Britain, is so high over distances of several kilometres (Austin et al., 2004; Brede and Beebee, 2004) that individual pond assemblages cannot meaningfully be treated as discrete populations. This problem is of course even more difficult with respect to stream breeding and fully terrestrial species, and these constitute the majority of amphibians globally. Amphibian populations may behave as metapopulations with boundaries that are difficult to delimit. Of course, breeding aggregations will remain valuable as sampling sites, but assessing demographic trends will need to take account of population structure in ways that will vary substantially among species.

A second major problem arises because many amphibians display wide natural fluctuations in population size over time. Frogs and salamanders in South Carolina, for example, showed substantial variations in both numbers of breeding adults and annual recruitment between 1979 and 1990 (Pechmann et al., 1991), suggesting that short-term 'snap-shot' surveys may be misleading. If amphibians breed repeatedly but have intermittent reproductive success, then populations may display short periods of rapid recruitment followed by longer periods of gradual decline. This suggests that a population can be declining for most of the time, yet remain stable in the long-term (Pechmann and Wilbur, 1994; Alford and Richards, 1999). Three common frog (*R. temporaria*) populations in Switzerland all followed this model over a 23–28 year period, and only one population – which was suffering predation from introduced fish – really declined over the same timeframe (Meyer et al., 1998). However, a meta-analysis of amphibian population fluctuations showed that life history mode, family and latitude were all predictors of variation in population size (Marsh, 2001). Variation in the fluctuation of population size over time decreased in the order: pondbreeding species, stream-breeding species, terrestrial species, suggesting (for example) that land-dwelling salamanders display more stable populations than aquatic frogs (Green, 2003). Contrary to earlier work, this study indicated that relatively long periods of decline compensated for by short periods of rapid recruitment are not universal properties of amphibian populations. Indeed, most species do not show this pattern. It follows that the ease of interpreting declines in population size with respect to long-term threat varies among species. Certainly for most pond-breeding amphibians, however, populations need to be monitored for multiple generations before trends can be reliably detected (Blaustein et al., 1994).

The practicalities of monitoring amphibian populations over time also constitutes a substantive problem, yet adequate monitoring is vital both for detecting declines and measuring any recoveries subsequent to conservation efforts. Amphibian populations are usually censused at one of three levels: presence–absence, relative abundance or estimates of actual population size. As discussed above (*measuring effects at the distributional level*), presence–absence surveys are useful for determining changes in distribution patterns and numbers of populations (Green, 1997), but – by definition – yield no information about trends within individual populations. Measuring relative abundance of amphibians involves counting individuals or scoring populations on an ordinal scale. Search effort may be standardized by using time-constrained or area-constrained protocols (e.g., transects or quadrats) and population densities may be obtained (Heyer et al., 1994; Griffiths et al., 1996). In Britain, for example, a night count by torchlight is the most widely used general survey method for newts (e.g., Griffiths and Raper, 1994; Cooke, 1995). Surveying for male advertisement calls (e.g., Bridges and Dorcas, 2000; Crouch and Paton, 2002) and counting spawn clumps or strings (e.g., Crouch and Paton, 2000) also fall into this category. However, all methods that involve making simple counts of amphibians have limitations in terms of how the resulting data may be interpreted (e.g., Buckley and Beebee, 2004). The main problem lies with the lack of control over detection probabilities that is inherent in such studies. The probability of detection of an amphibian varies between recorders, between habitats and between time periods. Unless this can be quantified, extrapolating from simple counts even to relative population size estimates is highly problematical, and this may confound the analysis of population trends even within long-term data sets that have been collected in a standardized way (Schmidt, 2003, 2004a). In addition to this general issue, there may also be problems with specific methods that use indices of population size. For example, it is not straightforward to relate calling males to population size (Shirose et al., 1997), and spawn counts may vary according to the proportion of females choosing to breed in any one season as well as to changes in the actual population size (Denton and Beebee, 1993).

When it is combined with demographics, estimating actual population size is the highest level of census and the one that provides the most useful data for analysing long-term population trends. In some cases it may be possible to achieve this using a complete census in which every individual in the population is identified or counted (e.g., in very small pools). More often,

however, an incomplete census is undertaken that requires extrapolation from a sample. Such a sample may be obtained from a series of quadrats or transects placed randomly within a habitat, and the actual population size estimated by extrapolating up to the total area of habitat available. However, amphibians often have contagious distributions even within superficially homogenous areas of habitat that may make such extrapolations tenuous, and this method still suffers from the detection probability issues described above. Incomplete censuses that utilize mark–release–recapture (MRR) methods, distance sampling or Bayesian approaches provide the most informative data on population size (Schmidt, 2003, 2004a). Such methods can control for variation in detection probabilities and allow the attachment of confidence limits to population estimates. Although MRR models make explicit assumptions about the properties of the data, it is possible to test these assumptions using goodness-of-fit tests, and arrive at a model that provides an optimum fit to the data. This is a major advantage of MRR over census methods based on simple counts, which make implicit assumptions that cannot be tested. On the down side, it will often be impractical to carry out a detailed mark–recapture analysis at large spatial scales for logistical reasons, and where the data that informs conservation decision-making is collected largely by teams of volunteers (e.g., Britain), the methodologies may be too unwieldy to implement.

An altogether different approach to estimating species abundance is based on the combined use of resource selection functions (RSFs) and geographical information systems (GIS). This method is habitat-based, and relies on a thorough knowledge of habitat requirements to estimate the probability of species presence and thus population density (Boyce and McDonald, 1999). However, this technique has substantial difficulties for amphibians because they often require complex combinations of aquatic and terrestrial habitats. It also assumes equilibrium population dynamics, and the method may be difficult to apply where declines are occurring rapidly, especially where these are disease-mediated with no concurrent change in habitat.

3.3. Effective population size

Assuming it is possible to obtain a meaningful estimate of actual (census) population size (N_c) for an amphibian species, a further issue arises concerning the value of such data for conservation planning (Green, 1997). A measure of the genetically effective population size (N_e) may be much more useful for analysing the viability of a population than either breeding numbers or census population size estimates, because N_e more accurately reflects the evolutionary potential of a population. Unfortunately, it is even more difficult to estimate N_e than it is to estimate N_c, but it is well known that N_e tends to be much smaller than N_c (Frankham, 1995) and this is certainly true for most amphibians (Table 2). This means that many amphibian populations have very small effective population sizes, commonly less than 100 (Funk et al., 1999). This in turn makes amphibian populations especially susceptible to loss of genetic diversity by random drift, and ultimately to the effects of inbreeding depression and high genetic load (Frankham et al., 2002; Rowe and Beebee, 2003). Conservation measures for declining amphibian populations will need to take account of this vulnerable type of population structure, especially in fragmented landscapes where the risks of population isolation are tending to increase.

Table 2
Amphibian N_e/N_c ratios

Species	N_e/N_c	Reference
Bufo marinus	0.016–0.088	Esteal and Floyd (1986)
Bufo bufo	0.007–0.012	Scribner et al. (1997)
Bufo calamita	0.02–0.19	Rowe and Beebee (2004)
Rana pipiens	0.01–0.67	Merrell (1968)
Rana sylvatica	0.21–0.81	Berven and Grudzien (1990)
Geocrina alba/*G. vitellina*	c.1.0	Driscoll (1999)
Triturus cristatus/*T. marmoratus*	0.09–0.16	Jehle et al. (2001)

Molecular genetic methods are increasingly used for the estimation of N_e, though the best ones have the disadvantage of requiring two population samplings taken at least one generation apart (e.g., Waples, 1989; Wang and Whitlock, 2003). However, genetic analyses with highly polymorphic markers such as microsatellites can provide valuable information about recent population trends based on single population samplings. Changes in N_e generate transient increases or decreases in expected heterozygosity, relative to allele numbers, during population bottlenecks or increases, respectively (Cornuet and Luikart, 1996). Empirical tests comparing levels of heterozygosity excess and known bottleneck history in British natterjack toad populations demonstrated a high level of congruence (Beebee and Rowe, 2001). The heterozygosity excess persists until a new equilibrium is reached and thus can only detect bottlenecks over a relatively short time period (0.2–4.0 N_e generations), though this should make it particularly useful in the context of recent and severe population declines.

3.4. Relating cause and effect in population declines

Despite the results of elaborate field and laboratory experiments, and correlative work between amphibian abundances and environmental characteristics, relating this information to effects at the population level remains a major challenge and a source of ongoing

debate (Sih et al., 2004a,b; Schmidt, 2004b). For example, does the fact that elevated UV-B significantly reduces embryonic survival of some amphibians mean that populations will decline as a result? Or might mechanisms such as reduced density-dependent competition among larvae compensate for high embryonic mortality (Vonesh and De la Cruz, 2002)? Experimental and observational approaches alone are unlikely to answer this question, which is central to understanding (and potentially reversing) the declines of wild populations. As better empirical data accumulate they should be used to develop and refine models of amphibian population dynamics that might help reveal the potential of regulatory processes. Norris (2004) reviewed the methods available to conservation biologists for predictions of future trends. These fall into three main classes, notably statistical, demographic and behavioural modelling. All require substantive data on environmental factors, demographic parameters or individual behaviour patterns relevant to the species in question. Demographic modelling has been the basis of much work on population viability analysis, but behavioural modelling may offer the best prospects of predicting population changes under unprecedented circumstances. All three modelling approaches have their place in the investigation of amphibian declines, but all require detailed information that will often not be available except for the relatively few well-studied species. Consequently, the species that are easiest to model are likely to be those of least conservation concern. It will nevertheless be interesting to see how well modelling methods predict susceptibility to declines – as well as the impact of ameliorative management – in those situations where they can be applied with robust data. In particular, modelling population dynamics and life history effects on population growth will no doubt provide increasingly useful guidance for population management (Griffiths and Williams, 2000; Biek et al., 2002; Sjögren-Gulve, 2004).

Life history studies have shed light on some of the more puzzling aspects of amphibian declines that have been occurring in relatively pristine montane areas that are remote from direct human disturbance. Amphibians from high altitudes or high latitudes tend to have short breeding seasons, resulting in few clutches of eggs. Cool temperatures in these regions also mean that larval development is slow, size at metamorphosis is large, and it takes a long time to reach sexual maturity. The cumulative effect of these life history attributes is a long generation time coupled with low fecundity. This in turn makes the populations unable to recover quickly from disturbance, and thus particularly vulnerable to extinction (Morrison and Hero, 2003). In Central America, declining amphibian species have certain features in common (use of aquatic habitats, restricted elevational ranges and large body size) that set them apart from non-declining species (Lips et al., 2003). While not always revealing the causes of decline, analyses of this type at least permit testable predictions of which other species are likely to decline in future.

4. Amphibians and the wider biodiversity crisis

Within the context of the wider biodiversity crisis, two fundamental questions about amphibian declines arise: (1) Are amphibians particularly good indicators of a wider biodiversity malaise? (2) Are amphibians declining faster than other taxa? Since 1989 amphibians have been widely advocated as excellent 'biological indicators' or sensitive indicators of environmental health (e.g., Blaustein and Wake, 1990; Vitt et al., 1990; Wyman, 1990; Wake, 1991). These claims have been reiterated in more recent literature (e.g., Collins and Storfer, 2003; Storfer, 2003; Blaustein et al., 2003). Despite some confusion in the literature over the precise definition of 'biological indicator' and associated terminology, there is a consensus that a good biological indicator serves as a surrogate measure of: (1) environmental contamination and/or habitat quality; or (2) population trends in other species. (e.g., Landres et al., 1988; Noss, 1990; Simberloff, 1998; Caro and O'Doherty, 1999). To be useful as such a surrogate, then, covariation between the abundance of the indicator and the parameter of interest must be strong and well understood, and the indicator must be relatively easy to measure (Table 3). Probably because of the problems described above in quantifying amphibian populations, few studies provide compelling data for strong covariation between amphibian abundance and contaminants or the abundance of other taxa. Some of the early amphibian decline literature therefore represents a paradox – amphibians were flagged as good biological indicators despite the fact that the authors concerned had little idea at the time what they were indicating! Although our understanding of the relationships between amphibian abundance and various types of environmental degradation has improved immeasurably over the past decade or so, there have still been no explicit tests of amphibians as biological indicators and there are few data to suggest that they are any better than other taxa in this respect. Lawler et al. (2003), for example, compared the ability of seven potential indicator groups to provide protection for other species in the Middle Atlantic region of the USA. Although amphibians performed better than mammals and birds, this was because the latter taxa did not embrace aquatic habitats. Moreover, using amphibians as indicators resulted in fewer 'at risk' species being embraced than when fish were used. Kati et al. (2004) compared six groups of taxa to determine their efficiency as biodiversity indicators in northern Greece. Although amphibians were lumped with freshwater turtles as 'aquatic herpetofauna', the best correlations between different taxa were

Table 3
Amphibians as potential biological indicators

Biological indicator properties	Present in amphibians?
Covariation of population size with other taxa	Not known
Covariation of population size with environmental contaminants	Some species
Covariation of population size with habitat change	Some species
Well-known biology	Some species
Easily sampled	Some species
Low variability in response to environmental stressors	No
Easy to distinguish natural population changes from those induced by environmental stressors	No

The list of biological indicator properties has been distilled from the reviews by Landres et al. (1988), Noss (1990) and Caro and O'Doherty (1999).

between woody plants and small terrestrial birds. 'Aquatic herpetofauna' correlated with woody plants and birds, but overall, woody plants and small terrestrial birds were the best indicators of other taxa.

Two features of amphibians – their use of both aquatic and terrestrial habitats and their sensitive skin – are often quoted as making them particularly vulnerable to environmental change and therefore good indicators (e.g., Blaustein and Wake, 1990; Wyman, 1990; Lips, 1998). This assumption may be over-simplistic. Very few amphibians divide their time equally between water and land, either within or between life-history stages. Moreover, a large number of species lead either entirely terrestrial or entirely aquatic existences through direct development or paedogenesis. Consequently, there is likely to be a considerable imbalance between the relative impacts of aquatic and terrestrial stressors for most species, and where both types of stressor are important, the complexity involved with disentangling their respective impacts may mitigate against using amphibians as surrogates for measuring those stressors. Equally, there are many other physiological parameters apart from skin sensitivity that may affect vulnerability to contaminants. Indeed, what little comparative literature exists suggests that amphibians vary considerably in their sensitivity to chemicals, and the notion that they are more sensitive than other taxa is not supported (e.g., Hall and Henry, 1992; Pechmann and Wilbur, 1994; Zhang, 1999). Of course, it is important to recognise that amphibians are a diverse group that may contain some as yet undiscovered and highly effective biological indicators. Nevertheless, as a class, they do seem to lack some of the fundamental properties of good indicators (Table 3). As previous authors have pointed out, all too often the advocacy of indicator groups is more related to the interests of their main proponents than to evidence that they are effective in this regard (Williams and Gaston, 1994; Stork and Samways, 1995; McGeoch and Chown, 1998). At present then, it may be easier to measure environmental stressors directly rather than attempting to use amphibians as surrogates.

Rather more progress has been made on the second issue of whether amphibians are declining faster than many other taxa. Reviewing evidence of status changes between 1600 and the 1970s, Honegger (1981) discovered only two amphibian extinctions compared with 28 for reptiles. Similarly, Smith et al. (1993) concluded that only around 0.07% of amphibian species were lost since 1600 and about 2% were under threat, in both cases the lowest figures for any vertebrate group. By striking contrast, Stuart et al. (2004) concluded that some 1856 amphibian species (32.5% of the total) are currently under threat, with 427 species (7.4%) critically endangered, according to IUCN categorisation. This compares with 12% of birds and 23% of mammals in the IUCN "under threat" designations. The situation for amphibians therefore seems to have worsened dramatically over the past 25 years. The evidence upon which these conclusions are based is rather scanty for many species, but there seems little doubt that the problem is now both real and serious. Nevertheless, even in relatively recent times the evidence suggests that in Europe reptiles have declined at least as much as amphibians (Beebee, 1992). It is also important to note that not all amphibians are declining. Many species remain apparently stable, including some that breed in the same ponds as declining species. A few, such as introduced cane toads *B. marinus*, are expanding their ranges to the extent of becoming pests (Lever, 2001).

Although, we might hope to learn some general principles about threats to species from the study of amphibian declines, the converse may be at least equally important. There is widespread evidence of long-term bird declines around the world, including studies that cover multi-decade time series (e.g., Pain et al., 1997; Holmes and Sherry, 2001; Lane and Alonso, 2001). As with amphibians, situations in which declines of some species are occurring commensurate with stability or increases of others are not uncommon (e.g., Fuller et al., 1995; Coppedge et al., 2001). Unlike amphibians, however, most recent bird declines are readily explained by habitat change or destruction, or direct persecution. However, half a century ago many raptor species declined dramatically across much of Europe and North America. Most other birds were much less affected by what at the time was a completely mysterious problem. The cause turned out to be the widespread post-war application of organochlorine insecticides, residues of which accumulated in predatory birds, causing the

thinning of egg shells and thus dramatically increasing embryonic mortality. The discovery of this complex consequence of pesticide applications was a dramatic piece of detective work including both field observations and laboratory experiments (e.g., Moore, 1967; Ratcliffe, 1970). Proof of its importance was provided by the subsequent recovery of many raptor populations following the gross reductions in application rates of the offending chemicals that began during the 1970s.

Earlier work on pesticides and birds could therefore provide a valuable paradigm for current concerns about amphibians. Arguably the only meaningful test of whether a cause of decline has been discovered is the subsequent recovery of natural populations when the cause is removed (Caughley, 1994). For raptors this has taken decades and remains incomplete. If ozone holes or global warming really are major agents underpinning amphibian declines (and probably many other ecosystem effects) it will probably be at least as long before we know for sure. Even this presupposes that political and legal instruments designed to address global threats to biodiversity – such as climate change – are implemented at the international level and work effectively. This will be a much more daunting task than the control of pesticides or removal of predators. At present the prospects for reversing anthropogenic climate change look bleak, though efforts to this end will no doubt continue. So far, and aside from habitat restoration, only in the specific and localised case of predatory fish introductions has a cause of amphibian declines been confirmed by removal (Vredenberg, 2004). Perhaps most difficult of all to address will be the consequences of emerging diseases, if these are confirmed as major causes of amphibian declines.

5. Conclusions

What, then, should be the priorities for research into the amphibian decline phenomenon? In a thought-provoking essay, Caughley (1994) opined that conservation biologists would be unable to realise their goals of saving species from extinction unless empirical case studies of declining populations were underpinned by more theory. He argued that conservation biology is therefore progressing in two directions – enshrined within the 'declining population paradigm' and the 'small population paradigm', respectively, – and that these need to be better integrated in conservation programmes. The problem of amphibian declines is firmly rooted in the 'declining population paradigm'. Research has gene-rally focused on documenting empirical case studies of particular species or habitats, with

Table 4
Possible causes of amphibian decline and prospects of amelioration

Cause of decline	Major life stages affected	Possible amelioration	Limits to amelioration
Habitat destruction	All	Protection of natural sites Creation of amenity habitats (e.g., garden ponds)	Habitat still fragmented Not all species benefit
Road traffic	Adults and juveniles	Under-road tunnels	Expensive; may not be adequately used by many species
Pollution	All	Control of pollutant emissions Development of natural resistance	Economic pressures Probably too slow in most situations
Pathogens	All	Reduce transmission by stringent hygiene rules Selection for resistance	Will not control spread by non-human agents Probably not possible in small populations or too slow for novel, virulent pathogens
Increased UV irradiation	Eggs, embryos and larvae	Control of ozone-depleting chemical emissions Dissolved organic matter in breeding ponds Density dependent effects on survival	Long time-frame, may be too late Not easily manipulated and may have other unpredictable effects Only helps if larval survival initially regulated by density
Alien species	Eggs, embryos and larvae	Extirpation of aliens Persistence of habitats inaccessible to aliens	Often impracticable or impossible Often few or nonexistent
Climate change	Eggs, embryos and larvae	Reduce emission of greenhouse gases Facilitate change of biological range to new, suitable areas	Long time-frame, may be too late Difficult in developed landscapes and for endemics with no alternative sites
Collection by humans	Adults	Laws to control exploitation (e.g., for food)	Difficult to enforce; probably not a major cause of declines

little underpinning by the more theoretical approaches enshrined within the 'small population paradigm'. Revisiting Caughley's (1994) declining population paradigm, Norris (2004) points out that much of the theory that it requires is, in fact, well established but has just not been put into practice. As alluded to by Biek et al. (2002), what is therefore missing from amphibian decline research are explicit applications of the theoretical approaches embraced within the small population paradigm.

In practical terms, there is the crucial question of population monitoring. This is important both to detect declines and to determine responses to conservation work. In general, it will be more practicable to concentrate on methods for recording presence/absence (i.e., large scale changes) than to measure changes in individual population sizes. Exceptions to this could include species with relatively small numbers of well-characterised populations, such as *B. calamita* in Britain (Buckley and Beebee, 2004).

Although plausible causes of amphibian declines have been identified, we still do not fully understand how they operate at the population level. The effects of direct exploitation and habitat loss are often self-evident. More problematic are the consequences of climate change, pollutants and disease. Density-dependence of larval survival may dampen the effects of high early mortality (e.g., from UV-B) on adult population sizes (Kiesecker et al., 2001; Vonesh and De la Cruz, 2002; Schmidt, 2004b). High levels of mortality therefore do not necessarily translate into population declines. Of the various pathogens that have been investigated, chytrid fungi provide the most compelling link between amphibian die-offs and population declines (Daszak et al., 2003), though even this organism is evidently tolerated in some situations (Daszak et al., 2004; Retallick et al., 2004). Clearly, bridging the gap between studies of the effects of agents of decline on individuals and landscape-level impacts on populations will require a multidisciplinary approach including experimental and modelling methods, and thus a much closer integration of 'declining population paradigm' and 'small population paradigm' approaches.

Finally, there should be increased efforts to test decline hypotheses at the population level. Successful re-establishment of amphibian populations by reintroductions to sites where the species recently became extinct is a potentially powerful way of demonstrating that the original causes of decline were correctly identified and remedied (Denton et al., 1997).

It seems likely to be some time before we can be sure that the main causes of amphibian declines have been accurately identified, by which point it will probably be too late to save many species. For reasons summarised in Table 4, it will be exceedingly difficult to ameliorate any of the likely major causes of amphibian declines sufficiently to prevent many more losses over the coming decades. Habitat destruction, road traffic and direct exploitation are likely to accelerate as the human population continues to increase. Global pollution levels will be difficult to control for the same reason, though tighter regulations have led to local improvements in some instances. The ever-increasing mobility of humans makes controlling the spread of aliens and pathogens less and less feasible. UV irradiation levels will take decades to fall back to pre-21st century levels, assuming high rates of compliance with the international treaties. Amphibians often have rather long generation times and small effective population sizes, features that mitigate against adaptive responses to pathogens or pollutants within short time frames. Production of corridors or other movements of species in response to climate change are likely to be impractical in most situations, especially for rare endemics. We therefore take a pessimistic view of the future prospects of amphibian biodiversity on earth, while of course supporting further research on causes of the problem and advocating urgent remedial action wherever possible in the hope of making a dire situation slightly less dreadful.

Although biological research has led to great strides in our understanding of amphibian declines, apart from continuing to inform the issues with rigorous scientific data, it can do little about reversing global threats. Addressing this problem lies firmly within the disciplines of politics, legislation and socioeconomics. A major challenge for the future therefore lies in breaching traditional boundaries between scientific and social scientific approaches to the biodiversity crisis.

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References

Alexander, M.A., Eischeid, J.K., 2001. Climate variability in regions of amphibian declines. Conservation Biology 15, 930–942.

Alford, R.A., Richards, S.J., 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30, 133–165.

Alford, R.A., Dixon, P.M., Pechmann, J.H.K., 2001. Global amphibian population declines. Nature 412, 499–500.

Arntzen, J.W., Thorpe, R.S., 1999. Italian crested newts (*Triturus carnifex*) in the basin of Geneva: distribution and genetic interactions with autochthonous species. Herpetologica 55, 423–433.

Austin, J.D., Lougheed, S.C., Boag, B.T., 2004. Controlling for the effects of history and non-equilibrium conditions in gene flow estimates in northern bullfrog (*Rana catesbeiana*) populations. Genetics 168, 1491–1506.

Beebee, T.J.C., 1992. Amphibian decline? Nature 355, 120.

Beebee, T.J.C., 1995. Amphibian breeding and climate. Nature 374, 219–220.

Beebee, T.J.C., Rowe, G., 2001. Application of genetic bottleneck testing to the investigation of amphibian declines: a case study with natterjack toads. Conservation Biology 15, 266–270.

Berger, L., Speare, R., Daszak, P., Green, E., Cunningham, A.A., Goggin, C.L., Slocombe, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences USA 95, 9031–9036.

Berven, K.A., Grudzien, T.A., 1990. Dispersal in the wood frog (*Rana sylvatica*): implications for genetic population structure. Evolution 44, 2047–2056.

Biek, R., Funk, C.W., Maxell, B.A., Mills, S.L., 2002. What is missing in amphibian decline research: insights from ecological sensitivity analysis. Conservation Biology 16, 728–734.

Blaustein, A.R., Keisecker, J.M., 2002. Complexity in conservation: lessons from the global decline of amphibian populations. Ecology Letters 5, 597–608.

Blaustein, A.R., Wake, D.B., 1990. Declining amphibian populations: a global phenomenon. Trends in Ecology and Evolution 5, 203–204.

Blaustein, A.R., Wake, D.B., Sousa, W.P., 1994a. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8, 60–71.

Blaustein, A.R., Romansic, J.M., Kiesecker, J.M., Hatch, A.C., 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. Diversity and Distributions 9, 123–140.

Blaustein, A.R., Belden, D.H., Olson, D.M., Green, D.M., Root, T.L., Kiesecker, J.M., 2001. Amphibian breeding and climate change. Conservation Biology 15, 1804–1809.

Blaustein, A.R., Hoffman, P.D., Hokit, D.G., Kiesecker, J.M., Walls, S.C., Hays, J.B., 1994b. UV repair and resistance to solar UV-B in amphibian eggs; A link to population declines? Proceedings of the National Academy of Sciences USA 91, 1791–1795.

Boyce, M.S., McDonald, L.L., 1999. Relating populations to habitats using resource selection functions. Trends in Ecology and Evolution 14, 268–272.

Brede, E.G., Beebee, T.J.C., 2004. Contrasting population structure in two sympatric anurans: implications for species conservation. Heredity 92, 110–117.

Brede, E.G., Thorpe, R.S., Arntzen, J.W., Langton, T.E.S., 2000. A morphometric study of a hybrid newt population (*Triturus cristatus*/*T. carnifex*): Beam Brook Nurseries, Surrey, UK. Biological Journal of the Linnean Society 70, 685–695.

Bridges, A.S., Dorcas, M.E., 2000. Temporal variation in anuran calling behaviour: implications for surveys and monitoring programs. Copeia 2000, 587–592.

Buckley, J., Beebee, T.J.C., 2004. Monitoring the conservation status of an endangered amphibian: the natterjack toad *Bufo calamita* in Britain. Animal Conservation 7, 221–228.

Caro, T.M., O'Doherty, G., 1999. On the use of surrogate species in conservation biology. Conservation Biology 13, 805–814.

Carey, C., Alexander, M.A., 2003. Climate change and amphibian declines: is there a link? Diversity and Distributions 9, 111–121.

Caughley, G., 1994. Directions in conservation biology. Journal of Animal Ecology 63, 215–244.

Collins, J.P., Storfer, A., 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distributions 9, 89–98.

Collins, J.P., Brunner, J.L., Jancovich, J.K., Schock, D.M., 2004. A model host–pathogen system for studying infectious disease dynamics in amphibians: tiger salamanders (*Ambystoma tigrinum*) and *Ambystoma tigrinum* virus. Herpetological Journal 14, 195–200.

Cooke, A.S., 1995. A comparison of survey methods for crested newts (*Triturus cristatus*) and night counts at a secure site, 1983–1993. Herpetological Journal 5, 221–228.

Coppedge, B.R., Engle, D.M., Masters, R.E., Gregory, M.S., 2001. Avian response to landscape change in fragmented southern Great Plains grasslands. Ecological Applications 11, 47–59.

Cornuet, J.M., Luikart, G., 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Crouch, W.B., Paton, P.W.C., 2000. Using egg-mass counts to monitor wood frog populations. Wildlife Society Bulletin 28, 895–901.

Crouch, W.B., Paton, P.W.C., 2002. Assessing the use of call surveys to monitor breeding anurans in Rhode Island. Journal of Herpetology 36, 185–192.

Cummins, C.P., 2002. Testing for the effects of UV-B radiation on anti-predator behavior in amphibians: a critique and some suggestions. Ethology 108, 643–648.

Cunningham, A.A., Langton, T.E.S., Bennett, P.M., Lewin, J-F., Drury, S.E.N., Gough, R.E., Macgregor, S.K., 1996. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). Philosophical Transactions of the Royal Society of London B 351, 1539–1557.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9, 141–150.

Daszak, P., Strieby, A., Cunningham, A.A., Longcore, J.E., Brown, C.C., Porter, D., 2004. Experimental evidence that the bullfrog (*Rana catesbeiana*) is a potential carrier of chytridomycosis, an emerging fungal disease of amphibians. Herpetological Journal 14, 201–207.

Davidson, C., 2004. Declining downwind: amphibian population declines in California and historical pesticide use. Ecological Applications 14, 1892–1902.

Davidson, C., Shaffer, H.B., Jennings, M.R., 2001. Decline of the California redlegged frog: climate, UV-B, habitat and pesticide hypotheses. Ecological Applications 11, 464–479.

Davidson, C., Shaffer, H.B., Jennings, M.R., 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate change hypotheses for California amphibian declines. Conservation Biology 16, 1588–1601.

Denton, J.S., Beebee, T.J.C., 1993. Reproductive strategies in a female-biased population of natterjack toads (*Bufo calamita*). Animal Behaviour 46, 1169–11175.

Denton, J.S., Hitchings, S.P., Beebee, T.J.C., Gent, A., 1997. A recovery program for the natterjack toad (*Bufo calamita*) in Britain. Conservation Biology 11, 1329–1338.

Driscoll, D.A., 1999. Genetic neighbourhood and effective population size for two endangered frogs. Biological Conservation 88, 221–229.

Esteal, S., Floyd, R.B., 1986. The ecological genetics of introduced populations of the giant toad, *Bufo marinus* (Amphibia: Anura): dispersal and neighbourhood size. Biological Journal of the Linnean Society 27, 17–45.

Findlay, C.S., Bourdages, J., 2000. Response time of wetland biodiversity to road construction on adjacent lands. Conservation Biology 14, 86–94.

Frankham, R., 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66, 95–107.

Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Fuller, R.J., Gregory, R.D., Gibbons, D.W., Marchant, J.H., Wilson, J.D., Baillie, S.R., Carter, N., 1995. Population declines and range contractions among lowland farmland birds in Britain. Conservation Biology 9, 1425–1441.

Funk, W.C., Tallmon, D.A., Allendorf, F.W., 1999. Small effective population size in the long-toed salamander. Molecular Ecology 8, 1633–1640.

Gibbs, J.P., Breisch, A.R., 2001. Climate warming and calling phenology of frogs near Ithaca, New York, 1900–1999. Conservation Biology 15, 1175–1178.

Green, D.M., 1997. Perspectives on amphibian declines: defining the problem and searching for answers. In: Green, D.M. (Ed.), Amphibians in Decline: Canadian Studies of a Global Problem. Society for the Study of Amphibians and Reptiles, USA, pp. 291–308.

Green, D.M., 2003. The ecology of extinction: population fluctuation and decline in amphibians. Biological Conservation 111, 331–343.

Griffiths, R.A., Raper, S.J., 1994. A review of current techniques for sampling amphibian communities. JNCC Report No. 210. Joint Nature Conservation Committee, Peterborough, UK.

Griffiths, R.A., Raper, S.J., Brady, L.D., 1996. Evaluation of a standard method for surveying common frogs (*Rana temporaria*) and newts (*Triturus cristatus*, *T. helveticus* and *T. vulgaris*). JNCC Report No. 259. Joint Nature Conservation Committee, Peterborough, UK.

Griffiths, R.A., Williams, C., 2000. Modelling population dynamics of great crested newts (*Triturus cristatus*): a population viability analysis. Herpetological Journal 10, 157–163.

Hall, R.J., Henry, P.F.P., 1992. Assessing the effects of pesticides on amphibians and reptiles: status and needs. Herpetological Journal 2, 65–71.

Hayes, T.B., Collins, A., Lee, M., Mendoza, M., Noriega, N., Stuart, A.A., Vonk, A., 2002a. Hermaphroditic, demasculinised frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences USA 99, 5476–5480.

Hayes, T.B., Haston, K., Tsui, M., Hoang, A., Haeffele, C., Vonk, A., 2002b. Feminization of male frogs in the wild. Nature 419, 895–896.

Hecnar, S.J., M'Closkey, R.T., 1997. Spatial scale and determination of species status of the green frog. Conservation Biology 11, 670–682.

Hels, T., Buchwald, E., 2001. The effect of road kills on amphibian populations. Biological Conservation 99, 331–340.

Heyer, W.R., 2003. Ultraviolet-B and amphibia. Bioscience 53, 540–541.

Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., hayek, L.-A., Foster, M.S., 1994. Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Smithsonian Institution Press, Washington.

Holmes, R.T., Sherry, T.W., 2001. Thirty-year bird population trends in an unfragmented temperate deciduous forest: importance of habitat change. Auk 118, 589–609.

Honegger, R.E., 1981. List of amphibians and reptiles known or thought to have become extinct since 1600. Biological Conservation 19, 141–158.

Houlahan, J.E., Findlay, C.S., 2003. The effects of adjacent land use on wetland amphibian species richness and community composition. Canadian Journal of Fisheries and Aquatic Sciences 60, 1078–1094.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyer, A.H., Kuzmin, S.L., 2000. Quantitative evidence for global amphibian population declines. Nature 404, 752–755.

Jancovich, J.K., Davidson, E.W., Morado, J.F., Jacobs, B.L., Collins, J.P., 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Diseases of Aquatic Organisms 31, 161–167.

Jancovich, J.K., Davidson, E.W., Seiler, A., Jacobs, B.L., Collins, J.P., 2001. Transmission of the *Ambystoma tigrinum* virus to alternate hosts. Diseases of Aquatic Organisms 46, 159–163.

Jehle, R., Arntzen, J.W, Burke, T., Krupa, A.P., Hödl, W., 2001. The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus*, *T. marmoratus*). Molecular Ecology 10, 839–850.

Jensen, J.B., Camp, C.D., 2003. Human exploitation of amphibians. In: Semlitsch, R.D. (Ed.), Amphibian Conservation. Smithsonian Books, Washington.

Johnson, P.T.J., Chase, J.M., 2004. Parasites in the food web: linking amphibian malformations and aquatic eutrophication. Ecology Letters 7, 521–526.

Johnson, P.T.J., Lunde, J.K.B., Thurman, E.M., Ritchie, E.G., Wray, S.N., Sutherland, D.R., Kapfer, J.M., Frest, T.J., Bowerman, J., Blaustein, A.R., 2002. Parasite (*Riberiroia ondatrae*) infection linked to amphibian malformations in the western United States. Ecological Monographs 72, 151–168.

Kati, V., Devillers, P., Dufrene, M., Legakis, A., Vokou, D., Lebrun, P., 2004. Testing the value of six taxonomic groups as biodiversity indicators at a local scale. Conservation Biology 18, 667–675.

Kats, L.B., Ferrer, R.P., 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9, 99–110.

Kats, L.B., Kiesecker, J.M., Chivers, D.P., Blaustein, A.R., 2000. Effects of UV-B radiation on anti-predator behavior in three species of amphibians. Ethology 106, 921–931.

Kats, L.B., Kiesecker, J.M., Chivers, D.P., Blaustein, A.R., 2002. Effects of UV-B radiation on anti-predator behaviour in amphibians: reply to Cummins. Ethology 108, 649–654.

Kiesecker, J.M., 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proceedings of the National Academy of Sciences USA 99, 9900–9904.

Kiesecker, J.M., Blaustein, A.R., 1995. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. Proceedings of the National Academy of Sciences USA 92, 11049–11052.

Kiesecker, J.M., Blaustein, A.R., Belden, L.K., 2001. Complex causes of amphibian decline. Nature 410, 681–684.

Knapp, R.A., Matthews, K.R., 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14, 428–438.

Kolozsvary, M.B., Swihart, R.K., 1999. Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. Canadian Journal of Zoology 77, 1288–1299.

Landres, P.B., Verner, J., Thomas, J.W., 1988. Ecological uses of vertebrate indicator species: a critique. Conservation Biology 2, 316–328.

Lane, S.J., Alonso, J.C., 2001. Status and extinction probabilities of great bustard (*Otis tarda*) leks in Andalucia, southern Spain. Biodiversity and Conservation 10, 893–910.

Lawler, J.J., White, D., Sifneos, J.C., Master, L.L., 2003. Rare species and the use of indicator groups for conservation planning. Conservation biology 17, 875–882.

Lever, C., 2001. The Cane Toad. The History and Ecology of a Successful Colonist. Westbury Publishing, West Yorkshire, UK.

Licht, L.E., 2003. Shedding light on ultraviolet radiation and amphibian embryos. Bioscience 53, 551–561.

Lips, K.R., 1998. Decline of a tropical montane amphibian fauna. Conservation Biology 12, 106–117.

Lips, K.R., 1999. Mass mortality and population declines of anurans at an upland site in western Panama. Conservation Biology 13, 117–125.

Lips, K.R., Reeve, J.D., Witters, L.R., 2003. Ecological traits predicting amphibian population declines in Central America. Conservation Biology 17, 1078–1088.

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Royle, J.A., Langtimm, C.A., 2002. Estimating site occupancy when detection probabilities are less than one. Ecology 83, 2248–2255.

Marsh, D.M., 2001. Fluctuations in amphibian populations: a meta-analysis. Biological Conservation 101, 327–335.

McGeoch, M.A., Chown, S.L., 1998. Scaling up the value of bioindicators. Trends in Ecology and Evolution 13, 46–47.

Merrell, D.J., 1968. A comparison of the estimated and the "effective" size of breeding populations of the leopard frog *Rana pipiens*. Evolution 22, 274–283.

Meyer, A.H., Schmidt, B.R., Grossenbacher, K., 1998. Analysis of three amphibian populations with quarter-century long time-series. Proceedings of the Royal Society of London B 265, 523–528.

Middleton, E.M., Herman, J.R., Celarier, E.A., Wilkinson, J.W., Carey, C., Rusin, R.J., 2001. Evaluating ultraviolet radiation exposure with satellite data at sites of amphibian declines in Central and South America. Conservation Biology 15, 914–929.

Moore, N.W., 1967. Pesticides and birds: a review of the situation in Great Britain to 1965. Bird Study 12, 222–252.

Morehouse, E.A., James, T.Y., Ganley, A.R.D., Vilgalys, R., Berger, L., Murphy, P.J., Longcore, J.E., 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Molecular Ecology 12, 395–403.

Morrison, C., Hero, J.-M., 2003. Geographical variation in life-history characteristics of amphibians: a review. Journal of Animal Ecology 72, 270–279.

Norris, K., 2004. Managing threatened species: the ecological toolbox, evolutionary theory and declining-population paradigm. Journal of Applied Ecology 41, 413–426.

Noss, R.F., 1990. Indicators for monitoring biodiversity: a hierarchal approach. Conservation Biology 4, 355–364.

Pain, D.J., Hill, D., McCracken, D.I., 1997. Impact of agricultural intensification of pastoral systems on bird distributions in Britain 1970–1990. Agriculture Ecosystems and Environment 64, 19–32.

Pahkala, M., Merila, J., Ots, I., Laurila, A., 2003a. Effects of ultraviolet-B radiation on metamorphic traits in the common frog *Rana temporaria*. Journal of Zoology London 259, 57–62.

Pahkala, M., Laurila, A., Merila, J., 2003b. Effects of ultraviolet-B radiation on common frog *Rana temporaria* embryos along a latitudinal gradient. Oecologia 133, 458–465.

Palen, W.J., Schindler, D.E., Adams, M.J., Pearl, C.A., Bury, R.B., Diamond, S.A., 2002. Optical characteristics of natural waters protect amphibians from UV-B in the US Pacific northwest. Ecology 83, 2951–2957.

Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421, 37–42.

Paul, N.D., Gwynn-Jones, D., 2003. Ecological roles of solar UV radiation: towards an integrated approach. Trends in Ecology and Evolution 18, 48–55.

Pechmann, J.H.K., Wilbur, H.M., 1994. Putting declining amphibian populations in perspective: natural fluctuations and human impacts. Herpetologica 50, 65–84.

Pechmann, J.H.K., Scott, D.E., Semlitsch, R.D., Caldwell, J.P., Vitt, L.J., Gibbons, J.W., 1991. Declining amphibian populations: the problem of separating human impacts from natural fluctuations. Science 253, 892–895.

Pellet, J., Schmidt, B.R., 2005. Monitoring distribution using call surveys: estimating site occupancy, detection probabilities and inferring absence. Biological Conservation 123, 27–35.

Petranka, J.W., Smith, C.K., Scott, A.F., 2004. Identifying the minimal demographic unit for monitoring pond-breeding amphibians. Ecological Applications 14, 1065–1078.

Pounds, J.A., Fogden, M.P.L., Savage, J.M., Gorman, G.C., 1997. Tests of null models for amphibian declines on a tropical mountain. Conservation Biology 11, 1307–1322.

Pounds, J.A., Fogden, M.P.L., Campbell, J.H., 1999. Biological response to climate change on a tropical mountain. Nature 398, 611–615.

Ratcliffe, D.A., 1970. Changes attributable to pesticides in egg breaking and eggshell thickness in some British birds. Journal of Applied Ecology 7, 67–68.

Ray, N., Lehmann, A., Joly, R., 2002. Modelling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. Biodiversity and Conservation 11, 2143–2165.

Reading, C.J., 1998. The effect of winter temperatures on the timing of breeding activity in the common toad. Oecologia 117, 469–475.

Retallick, P.W.R., McCallum, H., Speare, R., 2004. Endemic infection of the amphibian chytrid fungus in a frog community post-decline. PLOS Biology 2, 1965–1971.

Robinson, J., Griffiths, R.A., Jeffreys, P., 2003. Susceptibility of frog (*Rana temporaria*) and toad (*Bufo bufo*) eggs to invasion by Saprolegnia. Amphibia–Reptilia 24, 261–268.

Rowe, G., Beebee, T.J.C., 2003. Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild? Evolution 57, 177–181.

Rowe, G., Beebee, T.J.C., 2004. Reconciling genetic and demographic estimates of effective population size in the anuran amphibian *Bufo calamita*. Conservation Genetics 5, 287–298.

Royle, J.A., 2004. Modelling abundance index data from anuran calling surveys. Conservation Biology 18, 1378–1385.

Schmidt, B.R., 2003. Count data, detection probabilities, and the demography, dynamics, distribution, and decline of amphibians. Comptes Rendues Biologie 326, 119–124.

Schmidt, B.R., 2004a. Declining amphibian populations: the pitfalls of count data in the study of diversity, distributions, dynamics and demography. Herpetological Journal 14, 167–174.

Schmidt, B., 2004b. Pesticides, mortality and population growth rate. Trends in Ecology and Evolution 19, 459–460.

Scribner, K.T., Arntzen, J.W., Burke, T., 1997. Effective numbers of breeding adults in *Bufo bufo* estimated from age-specific variation at minisatellite loci. Molecular Ecology 6, 701–712.

Shirose, L.J., Bishop, C.A., Green, D.M., MacDonald, C.J., Brooks, R.J., Helferty, N.J., 1997. Validation tests of an amphibian call count survey technique in Ontario, Canada. Herpetologica 53, 312–320.

Sih, A., Bell, A.M., Kerby, J.L., 2004a. Two stressors are far deadlier than one. Trends in Ecology and Evolution 19, 274–276.

Sih, A., Kerby, J., Bell, A., Relyea, R., 2004b. Response to Schmidt. Pesticides, mortality and population growth rate. Trends in Ecology and Evolution 19, 460–461.

Simberloff, D., 1998. Flagships, umbrellas, and keystones: is single-species management passé in the landscape era. Biological Conservation 83, 247–257.

Sjögren-Gulve, P., 2004. Modeling amphibians and reptiles: an overview. In: Acakaya, H. et al. (Eds.), Species Management: Case Studies. Oxford University Press, New York, pp. 273–280.

Skelly, D.K., Yurewicz, K.L., Werner, E.E., Relyea, R.A., 2003. Estimating decline and distributional change in amphibians. Conservation Biology 17, 744–751.

Smith, F.D.M., May, R.M., Pellew, R., Johnson, T.H., Walter, K.R., 1993. How much do we know about the current extinction rate? Trends in Ecology and Evolution 8, 375–378.

Soulé, M.E., 1986. Conservation Biology. In: The Science of Scarcity and Diversity. Sinauer, New York, USA.

Storfer, A., 2003. Amphibian declines: future directions. Diversity and Distributions 9, 151–163.

Stork, N.E., Samways, M.J., 1995. Inventorying and monitoring of biodiversity. In: Heywood, V.H. (Ed.), Global Biodiversity Assessment. Cambridge University Press, Berlin, pp. 453–543.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischmann, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783–1786.

Vertucci, F.A., Corn, P.S., 1997. Evaluation of episodic acidification and amphibian declines in the Rocky Mountains. Ecological Applications 6, 449–457.

Vitt, L.J., Caldwell, J.P., Wilbur, H.M., Smith, D.C., 1990. Amphibians as harbingers of decay. BioScience 40, 418.

Vonesh, J.R., De la Cruz, O., 2002. Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. Oecologia 133, 325–333.

Vorburger, C., Reyer, H-U., 2003. A genetic mechanism of species replacement in European water frogs. Conservation Genetics 4, 141–155.

Vredenberg, V.T., 2004. Reversing introduced species effects: experimental removal of introduced fish leads to rapid recovery of a declining frog. Proceedings of the National Academy of Sciences USA 101, 7646–7650.

Wake, D.B., 1991. Declining amphibian populations. Science 253, 860.

Wake, D.B., 1998. Action on amphibians. Trends in Ecology and Evolution 13, 379–380.

Walther, G.-R., Post, E., Parmesan, C., Convey, P., Menzel, A., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature 416, 389–395.

Wang, J., Whitlock, M.C., 2003. Estimating effective population size and migration rate from genetic samples over space and time. Genetics 163, 429–446.

Waples, R.S., 1989. A generalised approach for estimating effective population size from temporal changes in allele frequency. Genetics 121, 379–391.

Williams, P.H., Gaston, K.J., 1994. Measuring more of biodiversity: can higher-taxon richness predict wholesale species richness. Biological Conservation 67, 211–217.

Wyman, R.L., 1990. What's happening to the amphibians? Conservation Biology 4, 350–352.

Zhang, T., 1999. Study on fish and amphibian embryo–larval toxicity test. Environmental Monitoring and Assessment 55, 363–369.