Infectious Disease and Worldwide Declines of Amphibian Populations, with Comments on Emerging Diseases in Coral Reef Organisms and in Humans

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Many populations of amphibians are declining on all six continents on which they occur. Some causes of amphibian declines, such as habitat destruction, direct application of xenobiotics, and introduction of predators or competitors, are clearly attributable to human activities. Infectious disease appears to be the direct cause of mass amphibian die-offs in relatively undisturbed areas of the world where anthropomorphic environmental disruption is minimal. In these cases, it is not yet clear whether these epizootics result from the natural evolution of new pathogens or from environmental changes that promote the emergence of pathogenic forms and/or that weaken the immune defenses of amphibians. Because some aspects of pathogen-related amphibian mass mortalities are similar to outbreaks of new diseases in humans and coral reef organisms, amphibian declines may be part of a much larger pattern than previously appreciated. *Key words*: amphibians, amphibian declines, amphibian immune system, antimicrobial peptides, chytrid fungi, climate change, coral reefs, emerging diseases, ranaviruses, ultraviolet light. — *Environ Health Perspect* 108(suppl 1):143–150 (2000).
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Amphibians are represented today by more than 3,900 species, grouped in three orders: Anura (toads and frogs), Caudata (salamanders), and Gymnophonia (caecilians) (*1*). The class Amphibia has persisted, with extinctions of some groups and emergence of new forms, for about 250 million years. Many environmental changes, such as the waxing and waning of ice ages, have occurred during that time (*2*). However, historically unprecedented changes in amphibian populations have been observed over the past 25 years. Rapid declines in the sizes of many amphibian populations and even extinctions of several species have been noted on all six continents on which they live (*3*). These mass mortalities have been portrayed in the scientific literature and mass media as indicators of worldwide ecosystem degradation that could negatively impact other organisms, including humans (*4–6*). The goals of this review are *a*) to present the patterns of amphibian declines, *b*) to evaluate what is known about the causes of these declines, and *c*) to review the evidence concerning whether anthropomorphic environmental degradation is a contributing factor. The final section of this review evaluates similarities and differences among recent outbreaks of infectious disease in amphibians, coral reef organisms, and humans. It is hoped that these comparisons will stimulate communication, cooperation, and interdisciplinary research on causes of these outbreaks in diverse groups of animals and plants.

The occurrence of numerous amphibian deformities in wild amphibians is another recent phenomenon that has attracted considerable attention. Although some deformities result from natural causes such as parasites (*7*), impairment of normal developmental processes by xenobiotics is likely in other cases (*8,9*). Although deformities result in decreased survivorship of tadpoles in the laboratory (*7*), conclusive evidence is not yet available that wild populations exhibiting high levels of deformities are declining in overall size. Furthermore, geographic localities, types of habitats, and taxonomic status of many groups showing deformities differ in many respects from those species experiencing mass mortalities and extinctions. Most important, populations experiencing rapid declines do not exhibit deformities of the type and prevalence noted in populations with deformities. Although future research may uncover common mechanisms causing deformities and major population declines, at this time they appear to be two distinct phenomena. Therefore, the deformity issue will not be treated further in this review.

History and Patterns of Declines in Amphibian Populations

Although a few localized die-offs of amphibians occurring prior to the 1970s are documented in the literature (*10*), large-scale mass mortalities of toads (*Bufo*) and frogs (*Rana*) started in the 1970s in mountainous areas of the western United States (*11–15*) and Brazil (*16,17*) and on the plains of southern Canada (*18,19*). The magnitude and timing of many of these die-offs can be pinpointed, since populations were monitored in preceding months or years (*11–14*). Although small populations of each species have survived to the present, these groups have never recovered their former geographic distribution (*14,20*). Mass die-offs in tropical areas began in the late 1980s and continue to the present (*21–26*). A few species have apparently become extinct (*21*). A new database containing species and habitat descriptions at locations at which die-offs have been documented and also where amphibians have not declined has been created and is now available for public use (*27*).

Some population declines are clearly caused by human activities, such as habitat destruction (*28*), direct application of xenobiotics such as pesticides (*29,30*), or introduction of predators (*31*). Even when environmental disruptions like these are not sufficiently severe to kill the amphibians directly, they can negatively impact populations by disrupting gene flow (*32*) or affecting food supplies, breeding success, availability of hibernation sites, migration routes, etc. Amphibian declines due to these types of environmental manipulations could be avoided if human societies chose to stop these activities.

However, a perplexing number of amphibian populations have declined in relatively undisturbed areas of the world such as national parks and wilderness areas of the American West (*12–15,20*) and tropical rainforests of Central America and Australia (*21–26*). In these cases, human manipulation of the environment is not obvious. Most of these die-offs share the following characteristics: infectious disease appears to be the direct cause of death, mass mortalities are geographically widespread, metamorphosed individuals are the primary target of the pathogen, the population experiences 50–100% mortality, the die-offs occur principally at relatively higher altitudes or in cold climates, and some, but not all, of the total amphibian species at a given site are affected (*10,22–24,33*).

The recent discovery of skin infections caused by a chytrid fungus (*Batrachochytrium*

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dendrobatidis) provided a major breakthrough in the search for the cause of amphibian declines in relatively undisturbed areas of Central America and Australia (*24,34*). Fungi of the phylum Chytridiomycota are ubiquitous in fresh water and soil; many forms in this group are saprobic decomposers of organic matter, whereas others are parasites on other organisms, including insects. Involvement of *Batrachochytrium dendrobatidis* in amphibian mortalities, confirmed by fulfillment of Koch's postulates, represents the first known infection of a vertebrate by a chytrid (*34,35*). Ultrastructural morphology and 18s DNA sequences differ from those of other chytrid fungi (*24,34*). *Batrachochytrium* preferentially attacks metamorphosed amphibians because of its affinity for keratin, a protein produced in the epidermis after metamorphosis. Amphibian larvae possess keratin only in their mouth parts and do not appear to be negatively affected by the fungus, even if fungal infection causes abnormal development of these structures (*36*). Because skin lesions were the only consistent finding upon necropsy, the method by which the fungus kills amphibians is unknown (*35*). Two hypotheses have been advanced: *a*) the fungus releases proteolytic enzymes or toxic compounds that are absorbed through the skin of the amphibian, or *b*) the fungus disrupts the ability of the skin to regulate water and electrolyte balance (*36*).

Concurrent with this discovery, mycotic dermatitis, sometimes associated with secondary bacterial infections, was found to be associated with the demise of wild Wyoming toads (*Bufo baxteri*) (*37*). *Basidiobolus ranarum*, a known pathogen of fish, amphibians, reptiles, and humans (*38,39*), was isolated from the toads. Because *Basidiobolus* is frequently carried in the guts of amphibians without necessarily causing infection, the causative agent of the deaths of Wyoming toads is currently under study.

The mass die-offs of toads and frogs in the mountains of the western United States during the 1970s were thought at the time to result from bacterial infections (*13,14*) or from disease along with other factors (*12*). New evidence suggests that fungal skin diseases also caused these mass mortalities. Histologic analyses have identified fungi resembling chytrids in the skin of museum specimens of *Bufo canorus* collected in the Sierra Nevada of California in the 1970s (*12,40*) and in *Rana pipiens* collected in the Colorado Rockies in 1974 (*41*). Since fungi cannot be cultured from preserved specimens, it will be difficult to verify their taxonomic identity and their relatedness to *Batrachochytrium*. Unfortunately, specimens of other species (*Bufo boreas boreas* and *Rana muscosa*) that also experienced mass mortalities in the 1970s in the western United States were not preserved. Although fungal skin infections will never be proven conclusively as the direct cause of death in the die-offs of these species, the patterns of mortality of toads and frogs in the western United States were very similar to the mass mortalities of a number of anuran species in Central America and Australia that were associated with the chytrid fungus. Although many populations of montane species of anurans were eradicated by these mass die-offs, no species extinctions have occurred (*14,20*). Other species that appear to have become extinct in the last few years, such as the golden toad (*Bufo periglenes*) in Costa Rica (*21*), disappeared before specimens could be analyzed for the presence of chytrid fungi.

Although the few remaining populations of toads and frogs at high altitudes in the western United States have been relatively stable since the catastrophic die-offs in the 1970s, recent outbreaks of chytrids have now been identified histologically in populations of *Rana muscosa* and *Bufo boreas boreas* in the Sierras and Rockies, respectively (*42,43*), in *Rana yavapiensis, Rana chirachahuensis,* and *Hyla arenicolor* in the deserts of Arizona (*44*), and in *Acris crepitans* in Illinois (*35*). These observations indicate that the fungus, apparently either absent or quiescent in the western United States since the 1970s, has returned or has regained virulence. In this current outbreak, the fungus also appears to have broadened its range of host species and habitats.

Other recent mass mortalities of salamanders and frogs have been associated with outbreaks of infectious disease caused by iridoviruses, sometimes associated with secondary bacterial infections (*45–47*). The iridoviruses pathogenic for fish and amphibians are all grouped in the genus *Ranavirus* (*48*). However, DNA analysis of the virus responsible for the mass mortality of tiger salamanders (*Ambystoma tigrinum*) in Manitoba, Canada, has confirmed the existence of a novel, highly infectious ranavirus (*47*). The pattern of infection linked to this virus differs considerably from that noted above as characteristic of the fungal diseases: mortalities are usually restricted to small geographical areas, even a single pond; larval, metamorphosing, or postmetamorphic stages of amphibians may succumb; outbreaks occur frequently, but not exclusively, in areas disturbed by human activities; and infections occur especially at high population densities (*10*).

Amphibian Immune Systems

The observations that amphibians are dying in large numbers due to infectious disease raise the critical question concerning why amphibian immune systems are not successful in combating these pathogens. Amphibian immune defenses involve both innate and adaptive components that together lack only a few elements of mammalian immune systems (*10,49*). The immune systems of a few species are well described and some information exists on the influence of environmental factors on the effectiveness of these defenses (*10*). However, the interaction of amphibian immune systems with viruses and the defenses against fungal skin infections have not been examined in detail.

Components of the innate immune system, such as macrophages, neutrophils, and antimicrobial peptides, provide the primary protection against fungal skin infections (*10*). Histologic examinations of the epidermis of amphibians suffering from fungal skin infections reveal relatively few immune responses. Fungal penetrations into the outer layer of epidermis caused little or no inflammation of the epidermis, and few neutrophils, lymphocytes, and macrophages were observed in infected skin (*35,37*). Inflammation is caused by cytokines produced by macrophages and neutrophils in response to a foreign invader (*50*). Inflammation is an important component of immune defense because it results in increased permeability of blood vessels. The change in permeability fosters the release of soluble mediators, such as immunoglobulins and complement, and also assists in recruitment of circulating leucocytes to the site of infection. The lack of an inflammatory response could result from a number of factors: *a*) these fungi could produce compounds that inhibit the inflammatory response, *b*) macrophages and neutrophils may not recognize these fungi as pathogens, and *c*) the fungi cause insufficient tissue necrosis to stimulate the inflammatory response, or other factors. The relatively low involvement of macrophages in mycotic epidermal infections suggests that formation of memory T and B cells and antibodies would be limited. Few amphibians appear to survive chytrid infections (*36*), but if some do, they would likely face a repeat attack with little improvement in their resistance.

Since it appears that the response of neutrophils and macrophages to these fungal skin infections is weak, antimicrobial peptides may be the primary, and possibly the only, immunologic defense against these pathogens. Potent antimicrobial peptides in dermal glands of amphibian skin were first demonstrated by Csordas and Michl (*51*). The potential for pharmaceutical use of these peptides has led to the isolation and chemical characterization of several of these peptides. Because they are species specific, they have usually been named after the species from which they are isolated (e.g., "esculentin" after *Rana esculenta*, "brevenin" after *Rana*

brevipoda, etc.). These compounds differ considerably in length, amino acid composition, and in the pathogens against which they are effective (*52*). Although the chemistry of a number of antimicrobial peptides has been characterized, little is known about the effects of various environmental factors (temperature, skin hydration, water pH, trace metals, xenobiotics, ultraviolet light, etc.) on the secretion of and activity of these peptides against various pathogens. These peptides may be unable to defend against fungal skin infections because the fungi have some effective means of defending themselves against the peptides or because environmental factors inhibit the secretion of or activity of these peptides.

Why Are Amphibians Succumbing to Infectious Disease?

Although amphibians, like all animals, suffer from a variety of infectious diseases in natural conditions (*53*), the rapidity with which *Batrachochytrium* and ranaviruses have apparently caused amphibian populations in relatively undisturbed habitats to decline or to become extinct is unprecedented in the scientific literature. The frequency of similar epizootics in the past is unknown. A number of hypotheses have been formulated to explain why amphibians are susceptible to these pathogens (*10*). These hypotheses focus on factors that could increase the virulence of the pathogens and/or diminish the effectiveness of immune defenses. Unfortunately, such little information is currently available about these pathogens and their interactions with immune defenses of amphibians that it is not known whether these pathogens have evolved through natural processes or whether environmental change has served as an ultimate or indirect cause of the disease outbreaks. In particular, no evidence yet supports or refutes the hypothesis that environmental change(s) cause immunosuppression leading to increased susceptibility of amphibians to pathogenic attack. Immunologists do not fully agree on what changes in immune function constitute immunosuppression, even in humans, and essential studies on characteristics of immune function of healthy populations of amphibians in the field have not been conducted. Furthermore, although the possibility that stress generated by multiple, sublethal environmental factors causes immunosuppression in amphibians has been proposed (*14*), no evidence for this possibility yet exists. In the next two sections, we examine the evidence for two scenarios, with emphasis on the chytrid fungus: *a*) the fungus evolves from a nonpathogenic into a pathogenic form by natural processes and overwhelms the defenses of amphibians without the necessity of immunosuppressive factors, and *b*) environmental change, man-made or not, has promoted an increase in the virulence of the fungus and/or suppression of immune defenses.

Possible Natural Occurrence and Spread of Pathogens

New pathogens have repeatedly evolved throughout the history of life and have periodically decimated animal and plant populations (*54*). The transformation of chytrid fungi from nonpathogenic to pathogenic forms for amphibians could have resulted through natural processes such as mutation, recombination, or genetic drift. These pathogenic fungi also could have emerged through natural processes from preexisting pathogens that shift hosts, from a change in the vulnerability of host populations, or from transport into a new population of hosts (*55*). In these situations, amphibian declines would not result from anthropogenic alteration of the environment.

Certainly, humans could transport the fungi by moving diseased amphibians or contaminated soil or water from one locality to another. However, if DNA analyses prove that the chytrid fungi infecting amphibians in Central America, North America, and Australia are relatively indistinguishable, the mechanism by which they move from place to place could occur by natural means. Although certain other types of chytrid fungi produce a resting spore (*56*), it is unknown whether *Batrachochytrium* produces resting spores that can survive prolonged desiccated conditions (*36*). However, the direction of prevailing winds at certain times of the year matches the direction of the apparent movement of outbreaks of fungal infections in Central America (north to south) and Australia (south to north) (*57*). Therefore, an object that can be blown over distance, such as an insect carrying the fungus in a moist environment like the gut, could be a natural cofactor. The fact that insects can be carried by air currents for thousands of miles (*58*) could account for possible movement of the fungus between mountain ranges in the American West and between continents.

If the chytrids infecting amphibian populations on various continents prove to be of different genetic stock, outbreaks of infectious chytrids could occur in various locations as a natural part of their biology. Some parasitic chytrids are known for the evanescent nature of their occurrence; they suddenly appear, rapidly multiply, decline, and disappear, possibly into a resting spore stage (*56*). If chytrids were responsible for the mass mortalities of toads and frogs in the Sierras and Rockies in the 1970s, this type of phenomenon could account for the apparent recent reappearance of the fungus in the same geographic areas. The environmental factors that could induce chytrid epidemics are thought to be seasonal temperature variation, water pH, light, nutrition, and oxygen concentrations (*59*).

The chytrid fungi and ranaviruses of amphibians may be able to kill amphibians without the involvement of any man-made environmental change that either suppresses the immune system or enhances pathogenic virulence. These pathogens may inherently possess sufficient virulence to kill the amphibians quickly before a complete immune defense can be mounted. Ranaviruses cause death of tiger salamanders within about 14 days after exposure of seemingly healthy animals (*46,47*), probably an insufficient amount of time for production of antiviral defenses even at optimal body temperatures. Metamorphosed anurans experimentally exposed to *Batrachochytrium* fungi died within 10–47 days, depending on dose and temperature (*36*). As mentioned above, insufficient information exists about defenses in the skin against fungal infections to determine how quickly they can be activated and conduct an effective defense.

Possible Environmental Correlates

If infectious disease is the direct or proximate cause of population declines of many amphibians in relatively undisturbed habitats, it is essential to learn whether environmental factors are serving as indirect or ultimate causes. Attempts to determine if environmental factors serve as indirect causal mechanisms for any phenomenon are fraught with difficulties because the environment is comprised of many variables, none of which can be experimentally separated from the rest in the outdoor environment. Correlations between environmental change and outbreaks of infectious disease suggest the existence of a causal relation but do not constitute proof. Caveats that must be considered when looking for causal relations between environmental change and amphibian declines have recently been reviewed (*27*). Large gaps in our knowledge exist about how environmental change might affect the transition from nonpathogenic to pathogenic forms, but some evidence indicates that a variety of environmental stresses can increase genetic alteration of microorganisms by mutation (*60*). Similarly, little information exists concerning how environmental change might affect amphibian immune defenses (*10*).

However, some important similarities exist among the environments in which mass mortalities, most of which are thought to be due to chytrid outbreaks, have occurred. Almost all these deaths have occurred in relatively undisturbed areas of the world at

relatively high altitudes (*12–15,18–20, 22–24,61*). Montane environments are colder, receive higher levels of ultraviolet light, and receive greater deposition of airborne contaminants than adjacent lowlands (*27*). Furthermore, windblown insects, possibly carrying fungi in their guts, are deposited on mountain slopes as air rises to pass over mountain ranges. Global environmental changes are likely to impact montane environments differently than those in the lowlands (*62*). The next several sections will evaluate various types of environmental changes and how they might impact both pathogens and amphibian defenses. Although they are presented here as discreet variables, it is certain that they, and probably other variables, interact together in a complex manner with amphibians in the field.

Temperature. The climate of the earth has been in continual flux throughout millions of years in which amphibians have existed. The persistence of amphibians as a group throughout major climatic cataclysms is a testament to the fact that they have considerable tolerance of climate change. Additionally, most amphibians can withstand wide variations in body water content and body temperature (*27*). However, the possibility that current global climate change is causing worldwide disruption in many ecosystems has concerned many scientists. Even small temperature changes have important impacts on community ecology. For instance, decreases in water temperature of 3–5°C during the upwelling of cold water during a recent El Niño-Southern Oscillation (ENSO) event caused a decrease in both the local density of sea stars (*Pisaster ochraceus*) and the consumption of their prey (*Mytilus*) (*63*). Because many, but not all, abrupt climatic changes in the geologic record have been correlated with extinctions of numerous groups of plants and animals (*64*), global climate change merits a full evaluation as a potential factor in the decline of amphibian populations in relatively undisturbed habitats.

The ENSO phenomenon has attracted considerable attention as a possible causative factor in emerging diseases (see below). This climatic event has been associated with a number of alterations in patterns of temperature and rainfall (*65*). The pattern of ENSO events changed dramatically in 1976 from that observed over the last 120 years (*66*), with the result that El Niños now are more frequent, last longer, and are more intense than those prior to 1976 (*67*). Both temperatures and rainfall at the sites at which amphibians have suffered chytrid outbreaks in Australia and Central America have been affected by the change in ENSO patterns since 1976 (*65,68,69*). Although one might hypothesize that this climatic change could have played a causative role in amphibian declines in the 1970s, most of these die-offs started between 1973 and 1975 (*10*).

Temperature appears to be associated with outbreaks of fungal skin infections in amphibians. The chytrid fungus *Batrachochytrium* grows best at cooler temperatures (*34*). Outbreaks of fungal skin diseases have generally been centered in mountainous areas where temperatures are cooler than in adjacent lowlands. Additionally, epidemics have been correlated with seasonal changes in temperature: outbreaks occurred in winter in *Litorea caerulea* in the mountains of eastern Australia (*36*), in winter in boreal toads hibernating in the Colorado Rocky Mountains (*14*), and after cold snaps in several species of *Rana* wintering in Arizona deserts (*44*). Additionally, Wyoming toads experienced outbreaks of mycotic skin infections after the first cold weather in the fall (*37*). The only boreal toads that survived a mass mortality episode in Yellowstone National Park were those that hibernated in water warmed by hot thermal springs (*70*). Although cooler temperatures may be optimal for chytrid growth, the effectiveness of amphibian immune defenses is likely to be impaired. Amphibian immune systems, like those of fish and most reptiles, are temperature dependent (*71*). In general, higher body temperatures enhance the immune response of amphibians, whereas colder temperatures diminish or abolish activity (*72*). Even a 3°C increase in body temperature improves the immunologic ability of newts (*Notophthalmus viridescens*) to reject a skin graft (*73*). In certain fish, certain nonspecific (innate) components of the immune system may compensate for lower adaptive immune function in the cold (*74*). The relation between fungal growth and immune function, particularly antimicrobial peptide activity, needs to be evaluated at a variety of temperatures.

If amphibian immune systems function most effectively at warmer temperatures and infectious chytrids prefer cooler temperatures, one might predict that outbreaks of chytrid infections should occur during cold periods. A recent study used two types of weather models to examine patterns of air temperatures at the dates and localities of amphibian mass mortalities (*57*). These models can be used to determine temperatures retroactively in remote parts of the earth where accurate weather measurements have not been made. Both models estimate temperatures over fairly large geographic grids or pixels. One model indicated that temperatures just prior to and during those declines in the tropical mountains of eastern Australia and Central America in the 1990s were warmer than normal but not abnormally so. In the Colorado Rocky Mountains, where mass die-offs occurred in the 1970s, temperatures were slightly colder than normal. The other model found that temperatures in these locations did not deviate noticeably from the average. Therefore, the association between air temperatures and amphibian declines in relatively undisturbed areas of the world is inconclusive. This relationship, however, could be strengthened if similar thermal conditions in the past were also associated with amphibian die-offs. However, the lack of historical records of amphibian mass mortalities prevents a search for such correlations.

These weather models are one of several means of estimating air temperatures in a particular locality. Data accumulated by other methods suggest that a warming trend exists in both temperate and tropical mountains (*68,75*). Average temperatures of temperate mountain ranges have increased, due largely to increases in daily minimum temperatures (*75*). The altitude at which freezing occurs (zero degree isotherm) in tropical mountains has risen about 100 m per decade for the last 30 years (*68*). The observations that chytrid outbreaks occur in areas in which temperatures appear to be rising, rather than falling, conflict with predictions that chytrid outbreaks should occur during cool or cold periods. The conflict between observation and prediction suggests that we need a great deal more research about the effects of temperature on chytrid growth and virulence and on amphibian immune defenses before we can fully understand this phenomenon.

Moisture. Both the chytrid fungi pathogenic to amphibians and their amphibian hosts are found in or near streams (*10,22–26*). Fully terrestrial amphibians living in the same localities as the stream-dwelling amphibians have not experienced population declines (*22,23*). Evaluation of moisture patterns by the same weather models described above indicates that conditions were drier than normal, but not abnormally so, around the times of amphibian declines in northeastern Australia, Central America, Puerto Rico, and the Colorado Rockies (*57*). A change in dry-season mist frequency in the mountains of Costa Rica associated with an ENSO event in 1987 was correlated with population crashes in a number of amphibian species (*76*). Because a slightly weaker 1982 ENSO was not correlated with mass amphibian mortalities (*76*), this phenomenon merits further research.

Although the correlation between moisture patterns and amphibian declines is not particularly strong and cannot be verified with correlations of historical die-offs with similar moisture patterns, the effects of water availability and variation in water chemistry on virulence of chytrid fungi need further study. Similarly, the effects of variations in skin

hydration on immune defenses of amphibians also need to be investigated.

Ultraviolet radiation. The potentially harmful effects of increasing ultraviolet-B (UV-B) exposure resulting from depletion of stratospheric ozone has caused considerable concern. Controversy has revolved around the role, if any, of UV-B in amphibian declines. Some studies have described significant mortality of amphibian eggs exposed to UV-B (*77–81*), whereas others have failed to find an effect (*82–85*) or have demonstrated an effect only when UV-B exposure was coupled with low pH (*86*). The methods employed by these studies have been criticized (*87,88*). None of these studies evaluated UV-B effects on metamorphosed amphibians, the life stage that is attacked by chytrid fungi.

A recent analysis of satellite data indicates that UV-B has significantly increased since 1979 at all sites in Central America at which amphibians have declined (*89*). However, because tropical amphibians are largely nocturnal or active only in shade, it is difficult to estimate actual exposures. Exposures to sublethal levels of UV-B can cause immunosuppression in mammals, including humans [see citations in (*10*)], but no data are yet available on effects of ecologically relevant UV-B exposures on immune function in amphibians, particularly antimicrobial peptides in the skin.

Xenobiotics. Although it seems unlikely that man-made chemicals could foster the conversion of a nonpathogenic chytrid fungus to a pathogenic form, little is known about the factors that foster pathogenicity of this chytrid and such a scenario cannot be ruled out at this time. The possibility that toxicants could diminish or abolish skin immune defenses, however, is plausible. Amphibians are exposed to a variety of man-made chemicals wherever they live, even in relatively undisturbed environments. A recent investigation found a surprising number of airborne contaminants in the air upwind from the mountains of Costa Rica and Panama (*90*) where amphibian mass mortalities associated with the chytrid fungus have occurred (*22–24*). Some of these contaminants, such as hydrogen peroxide, carbon monoxide, and a variety of short-chain organic molecules (ethane, ethene, etc.), probably originate from fires in Africa and South America. The rates of deposition of these and other airborne contaminants in montane environments of Central America and the effects of exposures to these contaminants on amphibian immune defenses and chytrid fungi deserve further research.

A large number of existing studies have measured tolerance levels of amphibians to xenobiotics (*91,92*), but most of these studies evaluated lethal effects of toxicants on amphibian eggs or early larvae. Few studies have examined tolerances of metamorphosed individuals to single chemicals or mixtures of these chemicals. Furthermore, little information exists on effects of sublethal doses on amphibian immune function. More experiments, such as those on Woodhouse's toads (*Bufo woodhousii*) exposed to various doses of malathion and then challenged with a typical bacterial pathogen (*Aeromonas hydrophila*) (*93*), are needed to link field exposures to various toxicants with susceptibility to disease.

Environmental endocrine disruptors. A wide variety of man-made chemicals are known to disrupt the synthesis, release, transport, binding, activity, and/or degradation of natural hormones in animal systems (*94*). These chemicals can exert effects at tissue concentrations well below levels that are detectable with current technology (*95*). Because animal immune systems regularly interact directly with their endocrine systems, disruption of normal endocrine function could affect susceptibility to pathogens (*94*). The possibility that endocrine-disrupting chemicals might be contributing to recent outbreaks of infectious disease in humans and other animals has been hypothesized (*96*). Although significant relationships exist between concentrations of endocrine-disrupting chemicals and the prevalence of intersexuality in at least one species of amphibian (*Acris crepitans*) (*97*), no evidence exists to date concerning whether amphibian declines due to infectious disease are caused by environmental chemicals that disrupt endocrine function. If suppression of amphibian immune systems is ultimately found to underlie their susceptibility to chytrid fungi and ranaviruses, it would be useful from the mechanistic standpoint to understand whether the suppression is caused indirectly by endocrine-disrupting chemicals or by an environmental factor, such as temperature, UV-B, or toxicants acting directly on the immune system. However, the net outcome of immunosuppression, whether caused by direct or indirect effects of one or more environmental variables, would probably be the same for the amphibian.

Summary. As the above sections indicate, very little is known about how pathogenic chytrid fungi and ranaviruses interact with amphibian immune systems and how (or if) environmental factors tip the balance in favor of the pathogens. No conclusive evidence yet exists that man-made environmental disruption has played a role either in the emergence of these pathogens or in suppression of amphibian immune responses. As research continues on these issues, however, one can hope that whatever factors have caused these unprecedented declines can be identified and reversed before major amphibian taxa become extinct.

Parallels between Infectious Disease Outbreaks in Amphibians and in Other Animals

Outbreaks of infectious disease appear to be increasing in a number of organisms other than amphibians. Emerging diseases in coral reef organisms and humans have been particularly well reviewed. In this section, a brief introduction to the patterns of outbreaks in these groups is provided as a means by which similarities and differences among the phenomena in amphibians, coral reef organisms, and humans might be presented. It is hoped that readers familiar with outbreaks of infectious disease in these and other organisms, including plants, will find other useful parallels and patterns. To date, relatively little interdisciplinary communication has occurred among those working on outbreaks of infectious disease in various organisms. In their respective isolation from other groups, all those concerned about emerging diseases might be in a situation analogous to that of the proverbial blind man who tried to describe an elephant by feeling the trunk. It is essential that we learn as quickly as possible whether the apparent increase in emerging diseases in a variety of organisms merely signals improvements in observation and detection of disease or whether current research efforts are discovering different parts of the same elephant.

Infectious Diseases of Coral Reef Organisms

Although several outbreaks of infectious disease have been noted historically in marine organisms, the number of reports of disease in marine plants, invertebrates, and vertebrates has increased dramatically since 1980 (*98*). The epizootics in coral reef organisms have received particular attention. Although human activities, such as fishing, diving, pollution, reef mining, and dredging have destroyed some coral reefs and natural disasters such as hurricanes have also taken their toll (*99*), disease outbreaks have been particularly apparent in scleractinian and gorgonian corals, sea urchins, and coralline algae (*100*). Perhaps the most severe epidemic recorded in the Caribbean was the mass mortality of a sea urchin (*Diadema antillarum*) population between 1983 and 1984 (*101*). Although the usual incidence of disease in coral reef organisms is unknown, many of these diseases fit the criteria established to designate new, emerging diseases (*98*).

Although the pathogen of *Diadema* was never identified (*102*), progress has been made in the characterization of coral pathogens. Of the 13 types of coral diseases that have been described, a characteristic pathogen or microbial consortium has been

identified in only three cases, Koch's postulates have been verified in only two instances, and the means by which the pathogen causes death is known for only one (*103*). The pathogens seem to be specific for one or only a few species (*104*). Some of the pathogens are quite infectious; *Diadema* was reduced from a very common reef organism to near extinction within a year of the first outbreak of the disease (*102*). Certain coral pathogens have caused death of 95% of the colonies on a particular study reef within a 5-year period and some stands of coral have disappeared (*103*). The pathogens are likely spread, in part, by ocean currents; the patterns of mortalities of *Diadema* followed the movement of currents from Panama to the northwest Caribbean and Gulf of Mexico (*105*). However, the spread of the pathogen from Panama along the coast of South America toward Barbados was in the opposite direction of the current flow. Possibly the pathogen was transported in ship ballasts (*105*).

Some of the coral diseases that have been described are, in fact, not diseases. For instance, coral bleaching results not from a pathogen but from a general stress response that can be triggered by elevated temperature, salinity, light, excessive turbidity, or pollution (*106*). Recovery from bleaching is possible if the stress is not prolonged. Onset of widespread coral bleaching coincided with high temperatures during the 1997–1998 ENSO in the Caribbean (*98*).

Immune defenses of coral and other coral reef invertebrates are not well characterized. Defenses of corals against pathogens include mucocytes that release a mucous secretion that protects the epithelium (*107*). This secretion forms a barrier that should protect the coral tissue from pathogens and toxins and may carry immunoglobulin A and other antimicrobial agents. During the inflammatory response to the detection of a pathogen, mucous secretion increases to provide a thick barrier. Mesenterial filaments on the epithelial surfaces of corals release digestive enzymes that can retard the colonization of microbial pathogens and prevent overgrowth by sponges, calcareous algae, or other organisms. Epidermal cells on the coral also possess cilia that generate currents that prevent small particles, such as pathogens, from approaching the surface. A breakdown of the mucous barrier, mesenterial filaments, or cilia leaves the coral open to microbial attack (*107*).

Much controversy exists about whether these outbreaks of coral diseases result from man-made modification to the environment. The well-defined coral diseases show little spatial or temporal relation to obvious pollution, except black band disease, which has occurred in nutrient-rich water (*100*). However, changes in temperature, pH, oxygen concentration, water clarity, sedimentation, eutrophication, or grazing can increase the metabolic costs of excessive mucous production and may debilitate coral organisms (107). The possibility has been raised that multiple stressors, both natural and man-made, are acting in concert on coral reefs, with the result that the ability to cope with continued or additional stressors is diminished (*108,109*).

Many similarities exist between recent outbreaks of infectious disease in amphibians and coral reef invertebrates: *a*) Observations of mass mortalities began in 1970s to 1980s. *b*) The origin of these pathogens, either from natural processes or from man-made environmental change, is unclear. *c*) The vulnerability of the corals and amphibians to these pathogens might result from exposure to new, virulent pathogens or from diminished defense mechanisms of the hosts, or both. *d*) Outbreaks of infectious disease are transmissible and spread over broad geographic areas. *e*) The pathogens cause 95–100% mortality in some cases and extinction of some populations. However, few extinctions of species have occurred. *f*) These newly emerging pathogens strike a variety of age classes, including older individuals. *g*) Most outbreaks have no clear spatial or temporal correlation with obvious pollutant sources. *h*) Global climate changes may foster the spread or abundance of pathogens. *i*) Stress caused by multiple, sublethal environmental factors might lead to increased susceptibility to stress. *j*) Populations of both amphibians and corals that survived these epizootics have not regained their former sizes or geographic distibutions. The study of amphibian declines has been tremendously enhanced by a global monitoring network. As monitoring of coral reefs improves, more similarities may emerge.

Infectious Diseases of Humans

Throughout recorded history, epidemics of infectious disease such as bubonic plague, influenza, and smallpox have decimated human populations. By the mid-1970s, improvements in public hygiene, availability of antibiotics, and other medical advances appeared to have infectious disease in industrialized societies nearly under control (*110*). Compared to the study of outbreaks of infectious disease in amphibians and coral reefs, emerging human diseases have received considerable attention (*54,110,111*). Criteria have been formulated by which the symptoms, incidence, and prevalence can be compared against baseline data to determine whether a disease is new or not (*110*). By these criteria, a number of new pathogens have emerged since the 1970s: Lyme disease (1975), Legionnaires disease (1978), AIDS (1981), chronic fatigue syndrome (1985), hantavirus (1993), a new variant of cholera (1992), and others (*110,112*).

Human behavior and technology are clearly factors in the emergence and spread of certain new diseases: improved reporting of infectious outbreaks, development of new techniques for diagnosing illness, travel, poverty, crowding, aging of industrialized societies, population growth and associated environmental disruption (*54,110,112*). For example, Valley Fever is a pulmonary infection caused by inhalation of arthroconidia of *Coccidioides immitis*, a fungus that occurs in the desert soil of the southwestern United States (*113*). Growing human populations in these areas have disrupted the soil layer through landscaping, construction, and agricultural activities and have released the arthroconidia into the air, making them available for humans to inhale. As a result, incidence of the disease has risen dramatically in the last 10 years (*113*).

In other cases, the possibility that the emergence of one or more of these pathogens is due to natural factors cannot be ruled out. The possible roles of human activities or environmental disruption in promoting emergence of some pathogens need further research. Environmental correlates such as climate change (whether man-made or not) are thought to play an important role in the incidence, distribution, and emergence of infectious disease in humans. As noted by Colwell and Patz (*114*), however, the strength of the postulated causal relationships must be verified. Global warming may allow mosquito vectors to spread into areas in which they do not now occur and to put many different human populations at risk (*112*). Changes in temperature and moisture patterns associated with ENSO events are thought to have caused an increase in cholera cases in South America and India (*102*) and hantavirus outbreaks in the American Southwest (*114*). However, few attempts have been made to rule out other possible environmental factors or to examine the interactions of environmental factors.

The impacts of other potential environmental factors (e.g., UV-B and endocrine-disrupting chemicals) as ultimate or indirect causes of the emergence of these pathogens need evaluation. An example from marine mammals underscores the importance of determining the relation between chemical contaminants and human disease: massive die-offs of seals in the North Sea in 1988 and dolphins in the Mediterranean in 1990–1992 have been associated with several strains of phocine distemper viruses (*115*). Increased tissue levels of pollutants (i.e., polychlorinated biphenyls) are correlated with decreased lymphocyte responses in bottlenose dolphins (*Tursiops truncatus*) and may have been a possible indirect cause of these die-offs (*116*).

The relation between tissue accumulations of pollutants and human susceptibility to disease needs further research.

As noted by Hayes and Goreau (*107*), emergence of infectious diseases in humans and coral reefs differs in a number of respects that also apply to the situation in amphibians: *a*) Humans are the sole species of concern in medical approaches to these issues, whereas many species in coral reefs and amphibians are targets of emerging pathogens. *b*) The multiplicity of species in coral reefs leads to the problem that infection by the same pathogen can appear in many forms, whereas infected humans share similar signs and symptoms. *c*) Whereas coral reefs and humans can repair themselves over time, the loss of many coral reef species and amphibians during an epizootic results in disruption of the entire ecosystem. Human societies are less likely to be impacted by widespread mortality. *d*) Medical science has a broad base of expertise from which to obtain help when a new disease emerges, but knowledge of the pathology of coral reefs and amphibians is restricted to a few trained individuals. *e*) Human epidemics may be controlled through quarantine, but in the ocean and in amphibian habitats, containment is difficult or impossible. *f*) The human immune system is well understood, but immune defenses of amphibians are well documented for only a few species. Those of coral reef organisms are only partially characterized.

The main similarity among the outbreaks of infectious diseases in humans, coral reef organisms, and amphibians is that the number of disease outbreaks caused by new pathogens appears to be increasing since the mid-1970s. Human activities may foster the emergence of these diseases, but the possibility that some of these pathogens have emerged due to natural causes cannot yet be ruled out. Certainly human activities have caused major environmental disruptions, such as increased UV-B and increased exposure to man-made chemicals in air, food, and water, fostering an increase in virulence of pathogens, ability of pathogens to switch hosts, suppression of immune function, etc. The increased incidence of outbreaks of new diseases in these three different groups of organisms may be coincidental; however, similarities among emerging and new pathogens of humans, coral reef organisms, and amphibians suggest that these phenomena may be part of a much larger problem than has been formerly appreciated.

Communication among groups working on outbreaks of infectious disease and immune defenses of plants and animals must be fostered and multidisciplinary research must be encouraged to determine how widespread these problems are and the nature of the causal mechanisms. Because correlations are frequently designated in the literature as causes or links, consistent and rigorous standards must be developed through which causes can legitimately be determined and public policy can be accurately formulated.

References and Notes

1. Duellman WE, Trueb L. Biology of Amphibians. New York:McGraw Hill, 1986.
2. Rage JC. Palaeobiological and palaeogeograghical background of the European herpetofauna. In: Atlas of Amphibians and Reptiles in Europe (Gasc JP, Cabela A, Crnobrnja-Isailovic J, Dolmen D, Grossenbacher K, Haffner P, Lescure J, Martens H, Martinez Rica JP, Maurin H, et al. eds). Paris:Museum National D'Histoire Naturelle, 1997;23–28.
3. Vial JL, Saylor L. The Status of Amphibian Populations. IUCN/SSC Declining Amphibians Populations Task Force, Working Document No. 1. Gland, Switzerland:The World Conservation Union, 1993.
4. Barinaga M. Where have all the froggies gone? Science 247:1033–1034 (1990).
5. Phillips K. Where have all the frogs and toads gone? BioScience 40:422–424 (1990).
6. Vitt LJ, Caldwell JP, Wilbur HM, Smith DC. Amphibians as harbingers of decay [Editorial]. BioScience 40:418 (1990).
7. Johnson PEJ, Lunde KB, Ritchie EG, Launer AE. The effect of trematode infection on amphibian limb development and survivorship. Science 284:802–804 (1999).
8. Ouellet M, Bonin J, Rodrigue J, Des Granges JL, Lair S. Hindlimb deformities (ectomelia, ectodactyly) in free-living anurans from agricultural habitats. J Wildl Dis 33:95–104 (1997).
9. Bishop CA, Gendron AD. Reptiles and amphibians: shy and sensitive vertebrates of the Great Lakes basin and St. Lawrence River. Environ Monitor Assess 53:225–244 (1998).
10. Carey C, Cohen N, Rollins-Smith L. Amphibian declines: an immunological perspective. Devel Comp Immunol 23:459–472 (1999).
11. Baxter GT, Stromberg MR, Dodd CK. The status of the Wyoming toad (*Bufo hemiophyrs baxteri*). Environ Cons 9:348 (1982).
12. Kagarise Sherman C, Morton ML. Population declines of Yosemite toads in the eastern Sierra Nevada of California. J Herpetol 27:186–198 (1993).
13. Bradford DF, Graber DM, Tabatabai F. Population declines of the native frog, *Rana muscosa*, in Sequoia and Kings Canyon National Parks, California. Southwestern Nat 39:323–327 (1994).
14. Carey C. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Cons Biol 7:355–362 (1993).
15. Corn PS, Fogleman JC. Extinction of montane populations of the northern leopard frog (*Rana pipiens*) in Colorado. J Herpetol 18:147–152 (1984).
16. Heyer WR, Rand AS, Cruz CAG, Peixoto OL. Decimations, extinctions, and colonizations of frog populations in southeast Brazil and their evolutionary implications. Biotropica 20:230–235 (1988).
17. Weygoldt P. Changes in the composition of mountain stream frog communities in the Atlantic mountains of Brazil: frogs as indicators of environmental deterioration? Stud Neotrop Fauna Envir 243:249–255 (1989).
18. Koonz W. Amphibians in Manitoba. In: Declines in Canadian Amphibian Populations: Designing a National Monitoring Strategy (Bishop CA, Pettit KE, eds). Ottawa, Ontario:Canadian Wildlife Service, 1992;19–20.
19. Roberts WE. What happened to the leopard frogs? Alberta Nat 11:1–4 (1981).
20. Drost CA, Fellers GM. Collapse of regional frog fauna in the Yosemite area of the California Sierra Nevada. Cons Biol 10:414–425 (1996).
21. Pounds JA, Crump ML. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Cons Biol 8:72–85 (1994).
22. Lips KR. Decline of a tropical montane amphibian fauna. Cons Biol 12:106–117 (1998).
23. Lips KR. Mass mortality and population declines of anurans at an upland site in western Panama. Cons Biol 13:117–125 (1999).
24. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, et al. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci 95:9031–9036 (1998).
25. Richards SJ, McDonald KR, Alford RA. Declines in populations of Australia's endemic tropical rainforest frogs. Pac Cons Biol 1:66–77 (1993).
26. Trenerry MP, Laurance WF, McDonald KR. Further evidence for the precipitous decline of endemic rainforest frogs in tropical Australia. Pac Cons Biol 1:150–153 (1994).
27. Carey C, Heyer WR, Wilkinson J, Alford RA, Arntzen JW, Halliday T, Hungerford L, Lips KR, Middleton EM, Orchard SA, et al. Unpublished data.
28. Welsh HH, Ollivier LM. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. Ecol Appl 8:1118–1132 (1999).
29. Kirk JJ. Western spotted frog (*Rana pretiosa*) mortality following forest spraying of DDT. Herpetol Rev 19:51–53 (1988).
30. Matson TO. Estimation of numbers for a riverine *Necturus* populations before and after TFM lampricide exposure. Kirtlandia 45:33–38 (1990).
31. Knapp RA, Matthews KR. Nonnative fish introductions and the decline of the mountain yellow-legged frog (*Rana muscosa*) from within protected areas. Cons Biol (in press).
32. Hitchings SP, Beebee TJC. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. Heredity 79:117–127 (1997).
33. Scott NJ. Postmetamorphic death syndrome. Froglog 7:1–2 (1993).
34. Longcore JE, Pessier AP, Nichols DK. *Batrachochytrium dendrobatidis* gen. and sp. nov., a chytrid pathogenic to amphibians. Mycologia 91: 219–227 (1999).
35. Pessier AP, Nichols DK, Longcore JE, Fuller MS. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J Vet Diagn Invest 11:194–199 (1999).
36. Berger L, Speare R, Hyatt A. Chytrid fungi and amphibian declines: overview, implications and future directions. In: Declines and Disappearances of Australian Frogs (Campbell A, ed). Canberra:Environment Australia, 1999;21–31.
37. Taylor SK,Williams ES, Thorne ET, Mills KW, Withers DI, Pier AC. Causes of mortality of the Wyoming toad (*Bufo baxteri*). J Wildl Dis 35:49–57 (1999).
38. Nickerson MA, Hutchison JA. The distribution of the fungus, *Basidiobolus ranarum* Eidam, in fish, amphibians and reptiles. Am Midl Nat 86:500–502 (1971).
39. Davis SR, Ellis DH, Goldwater P, Dimitriou S, Byard R. First human culture-proven Australian case of entomophthoromycosis caused by *Basiodiobolus ranarum*. J Med Vet Mycol 31:225–230 (1994).
40. Green D, Kagarise Sherman C. Unpublished data.
41. Green D, Carey C, Corn SC. Unpublished data.
42. Green D, Fellers G. Unpublished data.
43. Green D, Jones M, Longcore J, Loeffler C. Unpublished data.
44. Sredl M. Unpublished data.
45. Cunningham A A, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, MacGregor SK. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). Phil Trans Roy Soc Lond B 351:1539–1557 (1996).
46. Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis Aquat Org 31:161–167 (1997).
47. Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG. Pathology, isolation and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. J Wildl Dis 35:413–429 (1999).
48. Hyatt AD, Gould AR, Zuanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, Coupar BEH. Comparative studies of piscine and amphibian iridoviruses. Arch Virol 144:1–31 (1999).
49. Du Pasquier L, Schwager J, Flajnik MF. The immune system of *Xenopus*. Ann Rev Immunol 7:251–275 (1989).
50. Janeway CA, Travers P. Immunobiology. London:Current Biology Limited, 1996.
51. Csordas A, Michl H. Primary structure of two oligopeptides of the toxin of *Bombina variegata*. Toxicon 7:103–108 (1968).
52. Nicolas P, Mor A. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu Rev Microbiol 49:277–304 (1995).
53. Bibliography of Amphibian Diseases. Available: *http://www.jcu.au/school/phtm/PHTM/frogs/bibliog.htm* [cited 15 September 1999].
54. Wilson ME, Levins R, Spielman A, eds. Disease in Evolution: Global Changes and Emergence of Infectious Disease. Ann NY Acad Sci 740:1–503 (1994).
55. Wilson ME. Disease in Evolution. Ann NY Acad Sci 740:1–12 (1994).

56. Sparrow FK. Aquatic Phycomycetes, 2nd Ed. Ann Arbor, MI:University of Michigan Press, 1960.
57. Alexander MA, Eischeid JK. Unpublished data.
58. Johnson CG. Migration and Dispersal of Insects by Flight. London:Methuen, 1969.
59. Sparrow FK. Ecology of freshwater fungi. In: The Fungi (Gainsworth G, Sussman AS, eds). New York:Academic Press, 1968;41–93.
60. Miller RV. Genetic stability of genetically engineered microorganisms in the aquatic environment. In: Aquatic Microbiology: An Ecological Approach (Ford TE, ed). Boston:Blackwell, 1993;483–511.
61. Joglar RL, Burrowes PA. Declining amphibian populations in Puerto Rico. In: Contributions to West Indian Herpetology: A Tribute to Albert Schwartz (Powerll R, Henderson RW, eds). Ithaca, New York:Society for the Study of Amphibians and Reptiles, 1996;371–380.
62. Beniston M. Mountain Environments in Changing Climates. London:Routledge, 1994.
63. Sanford E. Regulation of keystone predation by small changes in ocean temperature. Science 283:2095–2097 (1999).
64. Crowley TJ, North GR. Abrupt climate change and extinction events in earth history. Science 240:996–1002 (1988).
65. Kiladis GN, Manabe S. Model assessment of decadal variability and trends in the tropical Pacific Ocean. J Climate 11:2273–2296.
66. Ebbesmeyer CC, Cayan DR, McClain DR, Nichols FH, Peterson DH, Redmond KT. 1976 step in Pacific climate: forty environmental changes between 1968-1975 and 1977-1984. In: Proceedings of the 7th Annual Pacific Climate (PACLIM) Workshop, April 1990 (Betancourt JL, Tharp VL eds). California Department of Water Resources. Interagency Ecolog Study Prog Tech Rep 26:115–126 (1991).
67. Trenberth KE, Hoar TJ. The 1990–1995 El Niño-Southern Oscillation event: longest on record. Geophys Res Lett 23:57–69 (1996).
68. Diaz HF, Graham NE. Recent changes in tropical freezing heights and the role of sea surface temperatures. Nature 383:152–155 (1996).
69. Nicholls N, Lavery B, Frederiksen C, Drosdowsky W. Recent apparent changes in relationships between El Niño-Southern Oscillation and Australian rainfall and temperature. Geophys Res Lett 23:3357–3360 (1996).
70. Peterson C. Unpublished data.
71. Clem LS, Miller NW, Bly JE. Evolution of lymphocyte subpopulations, their interactions, and temperature sensitivities. In: Phylogenesis of Immune Functions (Warr GW, Cohen N, eds). Boca Raton, FL:CRC Press, 1991;191–210.
72. Maniero GD, Carey C. Changes in selected aspects of immune function in the leopard frog, *Rana pipiens*, associated with exposure to cold. J Comp Physiol B 167:256–263 (1997).
73. Cohen N. Tissue transplantation immunity in the adult newt, *Diemictylus viridescens*. III: The effects of x-irradiation and temperature on the allograft reaction. J Exp Zool 163:231–240 (1966).
74. Le Morvan-Rocher C, Troutaud D, Deschaux P. Effects of temperature on carp leucocyte mitogen-induced proliferation and nonspecific cytotoxic activity. Devel Comp Immunol 19:87–95 (1995).
75. Diaz HF, Bradley RS. Temperature variations during the last century at high elevation sites. Climatic Change 36:253–279 (1997).
76. Pounds JA, Fogden MPL, Campbell JH. Biological response to climate change on a tropical mountain. Nature 398:611–615 (1999).
77. Blaustein AR, Hoffman PD, Hokit DG, Kiesecker JM, Walls SC, Hays JB. UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? Proc Natl Acad Sci USA 91:1791–1795 (1994).
78. Blaustein AR, Edmond B, Kiesecker JM, Beatty JJ, Hokit DG. Ambient ultraviolet-radiation causes mortality in salamander eggs. Ecol Appl 5:740–743 (1995).
79. Blaustein AR, Hoffman PD, Kiesecker JM, Hays JB. DNA repair activity and resistance to solar UV-B radiation in eggs of the red-legged frog. Cons Biol 10:1398–1402 (1996).
80. Anazalone CR, Kats LB, Gordon MS. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina*, *Hyla regilla*, and *Taricha torosa*. Cons Biol 12:646–653 (1998).
81. Lizana M, Padraza EM. The effects of UV-B radiation on toad mortality in mountainous areas of central Spain. Cons Biol 12:703–707 (1998).
82. Grant KP, Licht LE. Effects of ultraviolet radiation on life-history stages of anurans from Ontario, Canada. Can J Zool 73: 2292–2301 (1995).
83. van de Mortel TF, Buttemer WA. Are *Litoria aurea* eggs more sensitive to ultraviolet-B radiation than eggs of sympatric *L. peronii* or *L. dentata*? Aust Zool 30:150–157 (1996).
84. Nagl AM, Hofer R. Effects of ultraviolet radiation on early larval stages of the alpine newt, *Triturus alpestris*, under natural and laboratory conditions. Oecologia 110: 514–519 (1997).
85. Corn PS. Effects of ultraviolet radiation on boreal toads in Colorado. Ecol Appl 8:18–26 (1998).
86. Long LE, Saylor LS, Soule ME. A pH/UV-B synergism in amphibians. Cons Biol 9:1301–1303 (1995).
87. Licht LE. Disappearing amphibians [Editorial]. BioScience 45:307 (1995).
88. Licht LE, Grant KP. The effects of ultraviolet radiation on the biology of amphibians. Am Zool 37:137–145 (1997).
89. Middleton EM, Herman JR, Celarier EA, Rusin RJ, Wilkinson JW. Unpublished data.
90. Stallard RF. Unpublished data.
91. Devillers J, Extrayat JM (eds). Ecotoxicity of Chemicals to Amphibians. Philadelphia:Gordon and Breach Science Publishers, 1992.
92. Amphibian and Reptile Contamination and Toxicology Bibliography. Available: *http://cs715.cciw.ca/green-lane/herptox/reference-list/html* [cited 15 September 1999].
93. Taylor SK, Williams ES, Mills KW. Effects of malathion on disease susceptibility in Woodhouse's toads. J Wildl Dis 35:436–541 (1999).
94. Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, Hoffmann JL, Morrow MS, Rodier DJ, Schaeffer JE, et al. Environmental endocrine disruption: an effects assessment and analysis. Environ Health Perspect 106 (suppl 1):11–56 (1998).
95. Korach KS. Surprising places of estrogenic activity. Endocrinology 132:2277–2278 (1993).
96. Cheek AO, Vonier PM, Oberdörster E, Burow BC, McLachlan JA. Environmental signaling: a biological context for endocrine disruption. Environ Health Perspect 106(suppl 1):5–10 (1998).
97. Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, Eisold J, Wheeler MB, Warner R, Murphy JE, et al. Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (*Acris crepitans*). Environ Health Perspect 106:261–266 (1998).
98. Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DH, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM, et al. Emerging marine diseases—climate links and anthropogenic factors. Science 285:1505–1510 (1999).
99. Hughes TP. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science 265:1547–1551.
100. Goreau TJ, Cervino J, Goreau M, Hayes R, Hayes M, Richardson L, Smith G, DeMeyer K, Nagelkerken I, Garzon-Ferrera J, et al. Rapid spread of diseases in Caribbean coral reefs. Rev Biol Trop 46(suppl 5):157–171 (1998).
101. Lessios HA, Robertson DR, Cubit JD. Spread of *Diadema* mass mortality through the Caribbean. Science 226:335–337 (1984).
102. Lessios HA. *Diadema antillarum* ten years after mass mortality, still rare, despite help from a competitor. Proc Roy Soc B 259:331–337 (1995).
103. Richardson LL. Coral diseases: what is really known? TREE 13:438–443 (1998).
104. Peters E, Oprandy J, Yevich P. Possible cause of "white band disease" in Caribbean corals. J Invert Pathol 41:394–396 (1983).
105. Phinney JT, Muller-Karger F, Dustan P. Unpublished data.
106. Williams EH, Williams LB. The world-wide coral reef bleaching cycle and related sources of mortality. Atoll Res Bull 335:1–71 (1990).
107. Hayes RL, Goreau NI. The significance of emerging diseases in the tropical coral reef ecosystem. Rev Biol Trop 46(suppl 5):173–185 (1998).
108. Hughes TP, Connell JH. Multiple stressors on coral reefs: a long-term perspective. Limnol Oceanogr 44:932–940 (1999).
109. Porter JW, Lewis SK, Porter KG. The effect of multiple stressors on the Florida keys coral reef ecosystem: a landscape hypothesis and a physiological test. Limnol Oceanogr 44:941–949 (1999).
110. Levins R, Awerbuch T, Brinkmann U, Eckardt I, Epstein P, Makhoul N, Albuquerque de Possas C, Puccia C, Speilman A, Wilson ME. The emergence of new diseases. Am Sci 82:52–60 (1994).
111. Horsburgh CR, Nelson AM. Pathology of Emerging Infections. Washington, DC:American Society of Microbiology, 1997.
112. Colwell RR. Global climate and infectious disease: the cholera paradigm. Science 274:2025–2031 (1996).
113. Kirkland TN, Fierer J. Coccidioidomycosis: a reemerging infectious disease. Emerg Infect Dis 3:192–199 (1996).
114. Colwell RR, Patz JA. Climate, Infectious Disease, and Health. An Interdisciplinary Perspective. Washington, DC:American Academy of Microbiology, 1998.
115. Heide-Jorgensen MP, Harkonen T, Dietz R, Thompson PM. Retrospective of the 1988 European seal epizootic. Dis Aquat Org 13:37–62 (1992).
116. Lahvis GP, Wells RS, Kuehl DW, Stewart JL, Rhinehart HL, Via CS. Decreased lymphocyte responses in free-ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. Environ Health Perspect 103(suppl 4):67–72 (1995).