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Status and Trends of Amphibian Declines and Extinctions Worldwide

Simon N. Stuart,^{1*} Janice S. Chanson,¹ Neil A. Cox,¹
 Bruce E. Young,² Ana S. L. Rodrigues,³ Debra L. Fischman,³
 Robert W. Waller³

The first global assessment of amphibians provides new context for the well-publicized phenomenon of amphibian declines. Amphibians are more threatened and are declining more rapidly than either birds or mammals. Although many declines are due to habitat loss and overutilization, other, unidentified processes threaten 48% of rapidly declining species and are driving species most quickly to extinction. Declines are nonrandom in terms of species' ecological preferences, geographic ranges, and taxonomic associations and are most prevalent among Neotropical montane, stream-associated species. The lack of conservation remedies for these poorly understood declines means that hundreds of amphibian species now face extinction.

Scientists first became concerned about widespread amphibian population declines when they met in 1989 at the First World Congress of Herpetology. Historical data indicate that declines began as early as the 1970s in the western United States (1, 2), Puerto Rico (3), and northeastern Australia (4). Subsequent reports revealed the severity of the declines. At one site in Costa Rica, 40% of the amphibian fauna disappeared over a short period in the late 1980s (5). Sudden disappearances of montane species were noted simultaneously in Costa Rica, Ecuador, and Venezuela (5–8). In some regions, many declines took place in seemingly pristine habitats (1–8). These reports were initially received with some skepticism because amphibian populations often fluctuate widely (9), but tests of probabilistic null models showed that the declines were far more widespread and severe than would be expected under normal conditions of demographic variation (5). This finding, in addition to many further reports of declines in the 1990s (8, 10–13), was pivotal in convincing most herpetologists that amphibian declines are nonrandom unidirectional events.

The lack of a comprehensive picture of the extent and severity of amphibian declines prompted us to conduct the IUCN–The World Conservation Union Global Amphibian Assessment (GAA) to gather data on the distribution, abundance, population trends, habitat associations, and threats for all 5743 described species of amphibians (14, 15). From this information, we used the IUCN

Red List Criteria (16) to determine the level of threat to every species. The raw GAA data are publicly available (14). The results demonstrate that amphibians are far more threatened than either birds (17) or mammals (18), with 1856 species (32.5%) being globally threatened [that is, listed in the IUCN Red List Categories (16) of Vulnerable, Endangered, or Critically Endangered], as compared with 12% of birds (1211 species) (17) and 23% of mammals (1130 species) (18). At least 2468 amphibian species (43.2%) are experiencing some form of population decrease, whereas only 28 (0.5%) are increasing and 1552 (27.2%) are stable; 1661 (29.1%) species have an unknown trend.

Many amphibian species are on the brink of extinction, with 427 species (7.4%) listed as Critically Endangered (CR) (the IUCN category of highest threat), as compared with 179 birds (1.8%) (17) and 184 mammals (3.8%) (18). The level of threat to amphibians is undoubtedly underestimated because 1294 species (22.5%) are too poorly known to assess [Data Deficient (DD)], as compared with only 78 birds (0.8%) (17) and 256 mammals (5.3%) (18). A significant proportion of DD amphibians is likely to be globally threatened. Analysis of trends in population and habitat availability indicates

Table 1. Habitat preferences and biogeographic affinities of rapidly declining and enigmatic-decline amphibians in relation to all amphibian species (15). Rapidly declining species are those that now qualify for listing in a IUCN Red List Category of higher threat than they would have had in 1980. Enigmatic-decline species are rapidly declining species that have shown dramatic declines, even where suitable habitat remains, for reasons that are not fully explained.

Habitat preferences	Total number of species (%)	Number of rapidly declining species (%)	Number of enigmatic-decline species (%)
Forest	4699 (81.8)	365 (82.6)	187 (90.3)***↑
Savanna	487 (8.5)	7 (1.6)***↓	0 (0.0)***↓
Shrubland	814 (14.2)	47 (10.6)*↓	14 (6.8)***↓
Grassland	953 (16.6)	81 (18.3)	39 (18.8)
Flowing water	2650 (46.1)	277 (62.7)***↑	164 (79.2)***↑
Marshes/swamps	760 (13.2)	43 (9.7)*↓	14 (6.8)**↓
Still water bodies	2030 (35.3)	107 (24.2)***↓	28 (13.5)***↓
Artificial terrestrial habitats	1304 (22.7)	40 (9.0)***↓	22 (10.6)***↓
Tropical lowland habitats	3392 (59.1)	212 (48.0)**↓	79 (38.2)***↓
Tropical montane habitats	2714 (47.3)	251 (56.8)***↑	155 (74.9)***↑
Biogeographic realms			
Afrotropical	951 (16.6)	28 (6.3)***↓	1 (0.5)***↓
Australasian/Oceanic	561 (9.8)	36 (8.1)	23 (11.1)
Australia and New Zealand	219 (3.8)	32 (7.2)***↑	23 (11.1)***↑
Indomalayan	938 (16.3)	59 (13.3)	1 (0.5)***↓
Nearctic	331 (5.8)	24 (5.4)	9 (4.3)
Neotropical	2,825 (49.2)	279 (63.1)***↑	174 (84.1)***↑
Palaearctic	451 (7.9)	34 (7.7)	2 (1.0)***↓

¹IUCN Species Survival Commission/Conservation International Center for Applied Biodiversity Science Biodiversity Assessment Unit, 1919 M Street N.W., Washington, DC 20036, USA. ²NatureServe, Apartado 75-5655, Monteverde, Puntarenas, Costa Rica. ³Center for Applied Biodiversity Science, Conservation International, 1919 M Street N.W., Suite 600, Washington, DC 20036, USA.

*To whom correspondence should be addressed. E-mail: s.stuart@conservation.org

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (27). ↑Significantly higher than average; ↓significantly lower than average.

a deterioration in the status of amphibians since 1980 (15), when 1772 species (31.0%) would have been globally threatened, including 231 species (4.0%) in the CR category. Thus, in 1980, the percentage of CR amphibians would have been similar to that of mammals today, but the number of CR amphibians has almost doubled since then.

Only 34 species of amphibian are reported to have become extinct since 1500, compared with 129 birds (17) and 74 mammals (18), but there is strong evidence that this situation is worsening, because nine of these extinctions have taken place since 1980 [compared with five birds (17) and no mammals (18)]. Of greater concern is the number of species that can no longer be found (“Possibly Extinct,” not formally “Extinct” until exhaustive surveys to establish their disappearance are completed). The GAA lists 122 such species, and it appears that up to 113 of these have almost or completely disappeared since 1980. Proving extinction beyond reasonable doubt is often very difficult, because many of these declines have been very rapid and have happened only recently, and a few species that were thought to be extinct have been rediscovered in recent years (19). The GAA estimates that between 9 and 122 amphibian species have therefore become extinct since 1980, and extensive fieldwork is needed to produce a more precise number.

There are 435 species that qualify for listing in IUCN categories of higher threat than they would have in 1980 (15). We define these as “rapidly declining” species, and divide them into three groups based on the causes of their decline (15): “overexploited”—declining be-

cause of heavy extraction (50 species); “reduced-habitat”—suffering significant habitat loss (183 species); and “enigmatic-decline”—declining, even where suitable habitat remains, for reasons that are not fully understood, although disease and climate change are emerging as the most commonly cited causes (207 species) (3, 7, 13, 20–24). Five species fall into both the overexploited and enigmatic-decline groupings. Overexploited and reduced-habitat species are widely recorded in many taxonomic groups, such as birds and mammals (17, 18), and are the traditional focus of conservation efforts. However, enigmatic-decline species have never previously been recorded at a level comparable to that currently observed in amphibians. The percentage of enigmatic-decline species increases with increasing extinction risk, from 9.7% of the rapidly declining species in the IUCN Category Near Threatened, to 25.0% in Vulnerable, 47.3% in Endangered, 57.1% in Critically Endangered, and 92.4% in Critically Endangered (Possibly Extinct). This observation suggests that the factors causing “enigmatic” declines are driving species toward extinction particularly rapidly.

The geographic distribution of rapidly declining species is nonrandom (Table 1) (15). Neotropical species are much more affected than, for example, those in the Afrotropical and Indomalayan realms. Species from the Australasian-Oceanic realm show average numbers of rapidly declining species, but if Australia and New Zealand are considered as a separate group, they have significantly more enigmatic-decline species than the average for amphibians as a whole. The geographic distribution of rapidly de-

clining species (Fig. 1) shows that overexploited species are concentrated in East and Southeast Asia; reduced-habitat species occur more widely, but especially in Southeast Asia, West Africa, and the Caribbean; and enigmatic-decline species are restricted mostly to South America, Mesoamerica, Puerto Rico, and Australia. There is remarkably little geographic overlap between concentrations of species in the three groupings.

Enigmatic-decline species present the greatest challenge for conservation, because there are currently no known techniques for ensuring their survival in the wild. Such declines have taken place even within well-protected areas, such as Yosemite National Park (California) (1), Monteverde Cloud Forest Preserve (Costa Rica) (5, 6), and Eungella National Park (Australia) (14). Enigmatic-decline species are positively associated with streams at high elevations in the tropics and negatively associated with still water and low elevations (Table 1). Several studies indicate that the virulence of the fungal disease chytridiomycosis, one of the most commonly cited causes of enigmatic declines (22–24), is greater at higher elevations and among streamside species (3, 7, 25). Most enigmatic declines have been recorded from the Americas south to Ecuador and Brazil, Australia, and New Zealand, but they are spreading, for instance to Peru, Chile, Dominica, Spain, and Tanzania (14, 26, 27). It is likely that the GAA underestimates the number and geographical extent of enigmatic declines, particularly in the tropics, where amphibians have been insufficiently monitored. Indeed, these declines tend to be very rapid, and few of them have actually been observed taking place. More commonly, researchers return to a site to find that several species have disappeared since the last visit. For instance, scientists only recently documented disappearances of frogs in southern Mexico, although some of

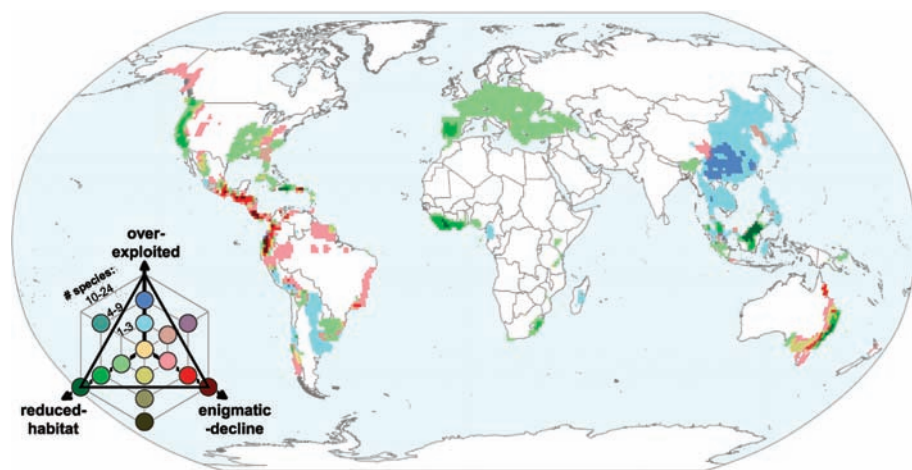


Fig. 1. Geographical pattern of the dominant causes of rapid decline (15) in amphibian species: overexploited (shades of blue); reduced-habitat (shades of green); and enigmatic-decline (shades of red). Where two threat types overlap in the same 1° cell, the color referring to the threat type with the larger number of rapidly declining species in that cell is indicated on the map. Intermediate colors are shown in cases of equal numbers of species experiencing two types of decline in the same cell, as shown in the key. Darker colors correspond to larger numbers of rapidly declining species of any type (not just of the dominant type in the cell in question).

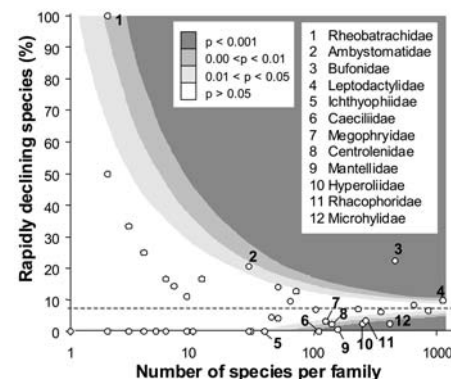


Fig. 2. Percentage of rapidly declining species (15) per amphibian family in relation to the average across all amphibians. The horizontal dashed line represents the overall mean percentage of rapidly declining species (7.6%).

these declines probably took place in the early 1980s (28). Accordingly, well-sampled countries tend to have a higher incidence of enigmatic declines: for example, 12.9% in Costa Rica, compared to 6.0% in the entire geographic region where most enigmatic declines have taken place. It is also possible that some species are not experiencing enigmatic declines yet but are susceptible to doing so, particularly if these are the results of factors such as the spread of a contagious disease (13, 22–24) or increasing severity in environmental conditions due to climate change (20, 21).

Rapid amphibian declines exhibit important taxonomic as well as regional patterns. Four amphibian families have significantly more rapidly declining species than the average for all amphibians: Rheobatrachidae (gastric-brooding frogs), Leptodactylidae (typical Neotropical frogs), Bufonidae (true toads), and the Ambystomatidae (mole salamanders) (Fig. 2) (15). Both known species of Rheobatrachidae are now extinct. Eight families have significantly fewer than the average percentage of rapidly declining species (Fig. 2), but for the two caecilian families, Caeciliidae and Ichthyophidae, this result might be an artifact caused by the large percentages of DD species (61 and 82%, respectively). The analysis depicted in Fig. 2 compares the percentage of rapidly declining species per family with the average for amphibians as a whole. However, taxa such as birds and mammals have few rapidly declining species, suggesting that rapid de-

clines are usually rare occurrences. From this perspective, most amphibian families have significantly more rapidly declining species than should be expected.

Four families contribute overwhelmingly to the total number of rapidly declining species: Bufonidae, Leptodactylidae, Hylidae (treefrogs), and Ranidae (true frogs) (Fig. 3). The three kinds of decline vary in their importance to each family. Overexploitation is much more important in the Ranidae than in the other large families, reflecting the extensive harvest of these species for human consumption, especially in Asia. Declines caused by habitat loss are important in most families, and enigmatic declines have had a particularly major impact in the Bufonidae (29). Some very small families, such as Rheobatrachidae, Rhinodermatidae (Darwin's frogs), and Cryptobranchidae (giant salamanders) also have high proportions of rapidly declining species.

The wide variation between families in the number and proportion of rapidly declining species is confounded by the nonrandom geographic pattern of declines (Table 1 and Fig. 1). Families that are endemic to regions where enigmatic declines have taken place tend to be more susceptible to serious declines. If enigmatic declines spread to other regions, especially in Africa and Asia, then it is likely that some other families will prove to be susceptible to declining rapidly (29).

The findings of the GAA confirm earlier suspicions that rapid and poorly explained declines in amphibian populations are taking place in addition to the typical causes of

biodiversity loss, including habitat loss and overexploitation (which are also serious for amphibians) (5, 10). Most extinction rate models are based on predicted habitat loss, either as a result of direct human activity (30, 31) or climate change (32). Because these models do not take account of enigmatic declines of the type affecting amphibian species, they underestimate the current extinction rate in amphibians. For a species facing an enigmatic decline, the only conservation option currently available is captive breeding, but many of the species affected are hard to maintain in ex situ conditions. Unless these declines are quickly understood and reversed, hundreds of species of amphibian can be expected to become extinct over the next few decades.

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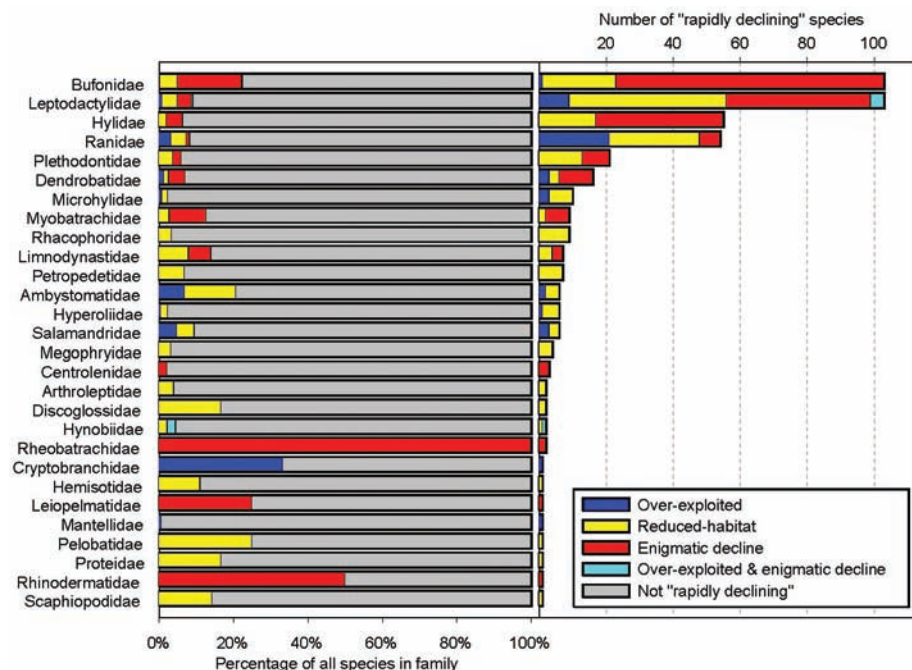


Fig. 3. Percentages and numbers of rapidly declining species (15) in amphibian families (with at least one rapidly declining species), broken into groups reflecting the dominant cause of rapid decline: overexploitation; habitat loss; or enigmatic decline.

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Lysosomal Glycosphingolipid Recognition by NKT Cells

Dapeng Zhou,^{1*} Jochen Mattner,¹ Carlos Cantu III,² Nicolas Schrantz,² Ning Yin,³ Ying Gao,³ Yuval Sagiv,¹ Kelly Hudspeth,¹ Yun-Ping Wu,⁴ Tadashi Yamashita,⁴ Susann Teneberg,⁵ Dacheng Wang,⁶ Richard L. Proia,⁴ Steven B Levery,⁷ Paul B. Savage,³ Luc Teyton,² Albert Bendelac^{1*}

NKT cells represent a distinct lineage of T cells that coexpress a conserved $\alpha\beta$ T cell receptor (TCR) and natural killer (NK) receptors. Although the TCR of NKT cells is characteristically autoreactive to CD1d, a lipid-presenting molecule, endogenous ligands for these cells have not been identified. We show that a lysosomal glycosphingolipid of previously unknown function, isoglobotrihexosylceramide (iGb3), is recognized both by mouse and human NKT cells. Impaired generation of lysosomal iGb3 in mice lacking β -hexosaminidase b results in severe NKT cell deficiency, suggesting that this lipid also mediates development of NKT cells in the mouse. We suggest that expression of iGb3 in peripheral tissues may be involved in controlling NKT cell responses to infections and malignancy and in autoimmunity.

As with protein-derived antigens, lipids, glycolipids, and lipopeptides (either of microbial or self origin) can be recognized by TCR $\alpha\beta$ -expressing T lymphocytes (1). Of these, NKT cells represent an unusual population that recognizes lipids presented by the MHC class I-like CD1d protein and displays characteristics of innate rather than adaptive lymphocytes (2). The TCR of NKT cells is limited mainly to a single invariant α chain (mouse $V\alpha 14$ -J $\alpha 18$ and the homologous human $V\alpha 24$ -J $\alpha 18$) combined with variable mouse $V\beta 8$ and human $V\beta 11$ TCR β . These cells express a phenotype of effector

or memory lymphocytes before encounter with any foreign antigen and display a panoply of inhibitory receptors also expressed on NK cells. Such features suggest that they may respond to conserved endogenous ligands, as well as foreign microbial antigens (3).

Mouse (m) $V\alpha 14$ and human (h) $V\alpha 24$ NKT cells appear to regulate a number of conditions in vivo, including malignancy and infection, as well as autoimmune diseases, through the rapid secretion of T helper 1 ($T_H 1$) and $T_H 2$ cytokines and chemokines (4). Without knowledge of the natural antigens recognized by these cells, it has been difficult to explore the mechanisms that govern their recruitment, activation, and development. Previous work has established the requirement for lysosomal trafficking of CD1d molecules (5) and the role of lysosomal proteases in presenting endogenous lipid antigens (6); the essential function of lysosomal lipid transfer proteins, known as sphingolipid activator proteins, or saposins, is now also established (7–9). These findings, and the recent report that a β -glucosylceramide synthase mutant cell line was defective in $V\alpha 14$ NKT cell stimulation (10), have indicated that the natural

ligands of NKT cells might be lysosomal glycosphingolipids.

We found that mice genetically deficient in the lysosomal glycosphingolipid degrading enzyme β -hexosaminidase b subunit ($Hexb^{-/-}$) (11–13) exhibited a severe reduction in the number of $V\alpha 14$ NKT cells (Fig. 1, A and B). Thus, staining for NKT cells in these mice using tetramers of CD1d complexed with the artificial lipid α GalCer (CD1d- α GalCer) was reduced by 95% on average. All subsets of NKT cells, including the earliest CD44^{low}NK1.1⁻ precursor and the CD4 and CD4-8⁻ cells, were equally affected as early as these cells could be detected in young 2.5-week-old mice [fig. S1 and (14)]. In contrast, the development of classical, naive, and memory CD4 and CD8 T cells, as well as B cells, $\gamma\delta$ T cells, and NK cells were not affected by $Hexb$ deficiency [Fig. 1D and (14)].

Although CD1d surface expression was unaltered in $Hexb^{-/-}$ mice (Fig. 1C), thymocytes from these animals failed to elicit a response from a $V\alpha 14^+$ NKT cell hybridoma (DN32.D3) (Fig. 2A). In contrast, they normally stimulated a $V\alpha 14^-$, CD1d-reactive NKT hybridoma (TCB11) (Fig. 2A). Presentation of the ligand of DN32.D3, but not that of TCB11, is dependent on lysosomal function (7); these results suggested defects in presentation of lysosomal ligands. To rule out general, nonspecific lysosomal defects, resulting, for example, from lysosomal lipid storage in these mutant mice, we tested the lysosomal functions of $Hexb^{-/-}$ cells using a panel of diglycosylated α GalCer derivatives that require lysosomal processing before recognition by $V\alpha 14$ NKT cells (15). The presentation of *N*-acetylgalactosamine (GalNAc) $\beta 1,4$ Gal α Cer by cells deficient in β -hexosaminidase b was selectively defective (Fig. 2B, upper panel), as expected from the specificity of this enzyme (11–13). In contrast, α -galactosidase A (α GalA)-deficient cells were selectively defective in processing Gal $\alpha 1,4$ Gal α Cer and Gal $\alpha 1,2$ Gal α Cer (Fig. 2B, lower panels), as expected from the specificity of α GalA (15). In contrast to these specific processing defects, control cells expressing a truncated form of CD1d missing the cytoplasmic endosomal targeting [CD1-TD “knock-in” (5)] were impaired in the presenta-

¹University of Chicago, Department of Pathology, Chicago, IL 60637, USA. ²The Scripps Research Institute, Department of Immunology, La Jolla, CA 92037, USA. ³Brigham Young University, Department of Chemistry and Biochemistry, Provo, UT 84602–5700, USA. ⁴Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA. ⁵Institute of Medical Biochemistry, Göteborg University, SE 405 30 Göteborg, Sweden. ⁶Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China. ⁷Department of Chemistry, University of New Hampshire, Durham, NH 03824–3598, USA.

*To whom correspondence should be addressed. E-mail: dzhou@midway.uchicago.edu (D.Z.) and abendela@bsd.uchicago.edu (A.B.)