

Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis

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We document major declines of many species of salamanders at several sites in Central America and Mexico, with emphasis on the San Marcos region of Guatemala, one of the best studied and most diverse salamander communities in the Neotropics. Profound declines of several formerly abundant species, including 2 apparent extinctions, are revealed. Terrestrial microhabitat specialists at mid- to high elevations have declined more than microhabitat generalists. These terrestrial microhabitat specialists have largely disappeared from multiple sites in western Guatemala, including in well-protected areas, suggesting that the phenomenon cannot be explained solely by localized habitat destruction. Major declines in southern Mexican plethodontid salamanders occurred in the late 1970s to early 1980s, concurrent with or preceding many reported frog declines. The species in decline comprise several major evolutionary lineages of tropical salamanders, underscoring that significant portions of the phylogenetic diversity of Neotropical salamanders are at risk. Our results highlight the urgent need to document and understand Neotropical salamander declines as part of the larger effort to conserve global amphibian diversity.

climate change | elevational transect | Guatemala | Mexico | Plethodontidae

It is estimated that one-third of all amphibian species worldwide are endangered or threatened with extinction (1). Efforts to understand the causes of this alarming decline, known as the global amphibian crisis, have focused primarily on frogs; comparatively little attention has been paid to salamanders (2–5). Reasons for this bias include the fact that most salamanders are secretive in nature, so population trends may not be apparent as in frogs. At well-studied sites in Central America where salamanders are present, they are often relatively uncommon compared with frogs (6). Nonetheless, given the precipitous declines and recently documented extinctions (2, 6, 7) in an array of tropical frog species, tropical salamanders demand attention as well.

The lungless salamanders (family Plethodontidae) include the only tropical salamanders, which have radiated dramatically in the Neotropics and now account for 40% of all salamanders (www.amphibiaweb.org). Nuclear Central America (between the Isthmus of Tehuantepec and the Nicaraguan lowlands) and southern Mexico contain a high diversity of plethodontid species (8). The salamander fauna has long been of interest to herpetologists; past studies and associated museum collections provide a rich historical database of abundance and distribution. One of the richest datasets available for tropical salamanders comes from the studies of Wake and colleagues (9, 10), who sampled along an elevation gradient on the southern slopes of Volcán Tajumulco in the Department of San Marcos, Guatemala. This dataset provides the best opportunity to examine changes in salamander populations over time. Here, we assess salamander population status by using historical (1970s) and recent (2005–2007) survey data from sites in Guatemala and Mexico across

multiple elevations. Our sampling focuses on high elevation and cloud forest communities, which harbor the highest diversity of salamander species (9, 10). Our data include a broad phylogenetic sample of the Middle American salamanders across a wide geographic area (Fig. 1). According to this dataset, salamander species exhibit differences in elevational distribution, habitat, and microhabitat preference, allowing us to identify factors associated with declines or changes in population status and differentiate among hypotheses to explain these changes.

Results

Dramatic declines in abundance were seen for most species from the upper cloud forest and high elevation salamander assemblages on the San Marcos transect (Table 1). Two of the most common salamander species in the 1970s, *Pseudoeurycea brunnata* and *Pseudoeurycea goebeli* (10), were not found on any of our recent visits (see *Methods*) at either of the 2 sites where they previously occurred in great abundance (Fig. 2). The third, undescribed species of *Pseudoeurycea* (*P. sp. nov.* “San Marcos”) was also not found on any of our recent surveys, despite being found on 15 of 25 visits to the El Rincon site in the 1970s. At El Rincon, *Bolitoglossa rostrata* was found on 22 of 25 visits in the past, generally in high abundance (mean = 64.4 salamanders per visit, $n = 22$ visits), but not a single specimen was seen on our recent surveys. This site represents the lower elevational limit of *B. rostrata*, and its absence provides evidence of an elevational range contraction on the transect. The species found at the highest elevations on the transect, *Pseudoeurycea rex*, was only found on 2 of our 5 visits, at a significantly lower encounter rate [0.58 salamanders found per person-hour vs. 8.92 in the 1970s (Student’s *t* test: $t = -8.338$, $P = 0.023$)] and lower abundance (mean = 1.8 individuals per visit vs. 77.7 in the past). The species was absent on our 2 surveys at a site in the high elevation zone where 174 specimens were found on a single visit in 1972. Three of the 4 species of *Pseudoeurycea* found on the upper part of the San Marcos transect (*P. rex*, *P. brunnata*, and *P. goebeli*) were also known from nearby Volcán Chicabal. Despite 2 intensive searches in 2006 and 2007, we found only a single specimen of *P. goebeli* and no *P. rex* or *P. brunnata*. This finding represents a precipitous decline in encounter rate for all 3 of these species (Table 1 and Fig. 2), and raises the possibility that *P. brunnata* may be extinct.

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The results of the studies cited above indicate that lowland and premontane deforestation could have had a substantial impact on moisture conditions in the areas where the greatest declines occurred. Pounds *et al.* (25) used modeling to show that large-scale warming led to a greater decrease in relative humidity at Monteverde compared to that caused by deforestation. Species of cloud forest salamanders that can still be found rely at least in part on bromeliads. Bromeliads depend on cloud water deposition and are predicted to be particularly vulnerable to climate change (26, 27). Therefore, if climate change is in part responsible for the declines we observed, arboreal salamander species that are presently not in decline may soon suffer the same fate as the fully terrestrial species.

The results of this study point to widespread and severe declines of upland salamanders at multiple sites in Guatemala and Mexico, including the most intensively-studied salamander transect in the neotropics. Although the causes of these declines are not yet well understood, the drastic reductions in salamander numbers and changes in community composition in this region indicate that the salamander populations of many upland species are in need of protection. Until the forces causing these declines are identified, however, an effective conservation strategy cannot be devised. Protecting habitat, although important, is insufficient to conserve populations of many of these species. Furthermore, other recent studies have also provided evidence of declining salamander populations in the neotropics (4, 5). The global amphibian crisis, usually discussed in terms of frogs, clearly involves Middle American salamanders as well.

Methods

Most salamanders analyzed in this study were collected as voucher specimens and deposited in the Museum of Vertebrate Zoology (MVZ). Detailed field notes and specimen catalogues were recorded and are stored at the MVZ, including data on numbers of salamanders seen but not collected. We made 3 visits to the San Marcos transect in November 2005, September 2006, and August 2007 during the wet season when salamanders, especially terrestrial species, were found in large numbers in the past. We revisited 5 of the same sites along the San Marcos transect that were worked by D. Wake and colleagues in the 1970s, summarized in ref. 10 (Fig. 1 and Table S4). In addition to the San Marcos sites, we surveyed a nearby Volcán with primary upper cloud forest in a protected reserve, Volcán Chicabal, in an attempt to distinguish between habitat alteration and other potential reasons for decline. Several of the same collectors who worked in the 1970s (D.B.W., T.J.P., locals) were involved in the more recent surveys, allowing us to visit the exact sites that were surveyed in the past and to use similar methods.

We collected all salamanders seen and quantified collecting effort by recording the number of collectors and search time. Each of the 5 sites was visited at least twice, and efforts were made to search at each site under favorable weather conditions to maximize the chance of finding salamanders. We attempted to survey the best habitat available, which included areas of primary forest, well-established secondary forest with extensive epiphyte growth, and more disturbed areas such as road banks and cleared areas.

We used the field notes of the collectors who worked most extensively on the San Marcos transect in the 1970s (D.B.W., J. Lynch, T.J.P., and L. Houck) and several other MVZ collectors to quantify the encounter rate of salamanders at the 5 focal sites on the transect (see *SI Text* for a detailed description of methods for field note data). Catalogues and field journal entries were used

to record the number of salamanders of each species collected or observed during each collecting event. The search time and number of collectors were also recorded in the journal entries in most cases. Collecting events were assigned to the 5 focal sites (listed in Table S4), and only species known to be present at a site based on the detailed surveys of the 1970s were considered at each site (Table S4). For each collecting event, the number of individuals found was divided by the number of people searching and the number of hours spent searching to give a measure of salamanders encountered per person per hour for each species. The mean encounter rate and proportion of visits on which a species was found were calculated for each one of the sites. The same procedure was repeated for the 2005–2007 visits.

Although our systematic survey efforts concentrated on the San Marcos area, records were also kept of species detection in other areas of western Guatemala and Chiapas, Mexico that share species in common with San Marcos (Fig. 1 and Table S1). *P. rex*, *B. rostrata*, and *P. goebeli* were found at these sites in the 1970s. Adult salamanders collected in 2006 and 2007 were swabbed and tested for *B. dendrobatidis* by using the real-time PCR assay described by Boyle *et al.* (28).

To compare population trends in a broader geographic and phylogenetic scope, data from 4 sites in Mexico north of the Isthmus of Tehuantepec were analyzed: Cerro San Felipe in Oaxaca, Parque Nacional El Chico in Hidalgo, Cerro Chicahuaxtla in Veracruz, and Puerto del Aire on the Puebla/Veracruz border (Fig. 1). These sites, similar in elevation, habitat type, species diversity, and patterns of elevational zonation (10) to the Guatemalan sites, provide independent communities in which to analyze salamander population trends. In addition to *Pseudoeurycea*, they contain salamanders of the genera *Thorius* and *Chiropterotriton* not found in Guatemala. The 4 sites were visited on multiple occasions by researchers (D.B.W., T.J.P., J. Lynch, and G.P.-O.) who kept detailed field notes. For the first 3 sites, the number of salamanders of each species found was recorded and totaled by year, and the number of collectors and field days were also recorded. Encounter rate (salamanders per person per day) for each species was estimated by dividing the number of salamanders found each year by the number of people searching and the number of days spent searching. For the Puerto del Aire site, the number of *Thorius* found per visit was obtained from 5 museums (American Museum of Natural History, Natural History Museum of Los Angeles County, MVZ, National Museum of Natural History, University of Michigan Museum of Zoology) dating back to 1970, with no quantification of collection effort. Although 3 species are present at that site (*Thorius dubitus*, *Thorius magnipes*, *Thorius troglodytes*), identification to species level was not always possible, so only the total number was used. All 3 of these species use terrestrial microhabitats, and 2 (*T. dubitus* and *T. troglodytes*) are exclusively terrestrial (29).

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Supporting Information

Rovito *et al.* 10.1073/pnas.0813051106

SI Text

RT-PCR *Batrachochytrium dendrobatidis* Assay. This assay uses species-specific primers ITS1–3 Chytr and 5.8S Chytr and the probe ChytrMGB2 to amplify ITS-1 and 5.8S region (1). DNA was extracted by using the PrepMan Ultra protocol for DNA extraction (Applied Biosystems). DNA standards (provided by A. D. Hyatt, Commonwealth Scientific and Industrial Research Organisation, Geelong, Australia) were diluted to give 100, 10, and 1 genome equivalents for use in *Taqman* assay (1). Results from the assay are presented quantitatively as the number of genomic equivalents or zoospore equivalents recovered from tissue from each specimen.

Analysis of Historical Field Note Data. The quantity and identification of the specimens were confirmed by checking the MVZ online database (mvzarcots.berkeley.edu) and, when necessary, by examining specimens in the collection. In cases where only a minimum number of salamanders found was given, this number was used to provide a conservative estimate of encounter rate. When only approximate search times were given, the following classification was used to quantify search time: brief stop = 1 h, a couple of hours = 2 h, a few hours = 3 h, morning or afternoon = 3 h, most of a day = 4 h, full day = 6 h. In cases where the exact number of collectors was not recorded, the following numbers were assigned: several = 5 collectors, many = 10 collectors. These approximations were necessary because the original data were not collected for analysis in this framework. In most cases, only a portion of this time was actually spent

actively searching, and fewer collectors were probably involved. The high estimates of search time and number of collectors were chosen to provide an extremely conservative estimate of salamander encounter rate, to avoid reporting an artifact as a decline in encounter rate. Mean encounter rate per species in the 1970s and from 2005 to 2007 was compared by using a *t* test (in cases of similar sample size) or a Wilcoxon test (in cases of unequal sample sizes) for the highest site on the gradient and at Buena Vista, where sample sizes from 2005 to 2007 were sufficiently large for statistical analyses.

Collections from the Buena Vista site were made primarily by local collectors in the past who may have brought salamanders from both the upper and lower cloud forest zones. These collectors consistently worked in the same areas, however, justifying the inclusion of these collecting events in a single site. At this site, *P. brunnata*, *P. goebeli*, and *P. sp. nov.* San Marcos were only ever found in a very limited elevational range by MVZ researchers. For this reason, only collecting events between 2,350- and 2,400-m elevation were counted when calculating encounter rates for the three species of *Pseudoeurycea*. Although *Bolitoglossa rostrata* occurs at high elevations on the upper slopes of Volcan Tajumulco, many of the sites visited on this part of the transect in the past were above its upper elevational limit. For this reason, *B. rostrata* was analyzed only for the El Rincon site. Two seldom-encountered species from Finca Santa Julia, *Oedipina stenopodia* and *Bolitoglossa salvinii*, were excluded from the analysis because of a lack of data from the 1970s (19 *O. stenopodia* and 0 *B. salvinii* collected from 1970–1979) and a lack of recent search effort.

1. Boyle DG, Boyle DB, Olsen V, Morgan JA, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time *Taqman* PCR assay. *Dis Aquat Organ* 60:141–148.

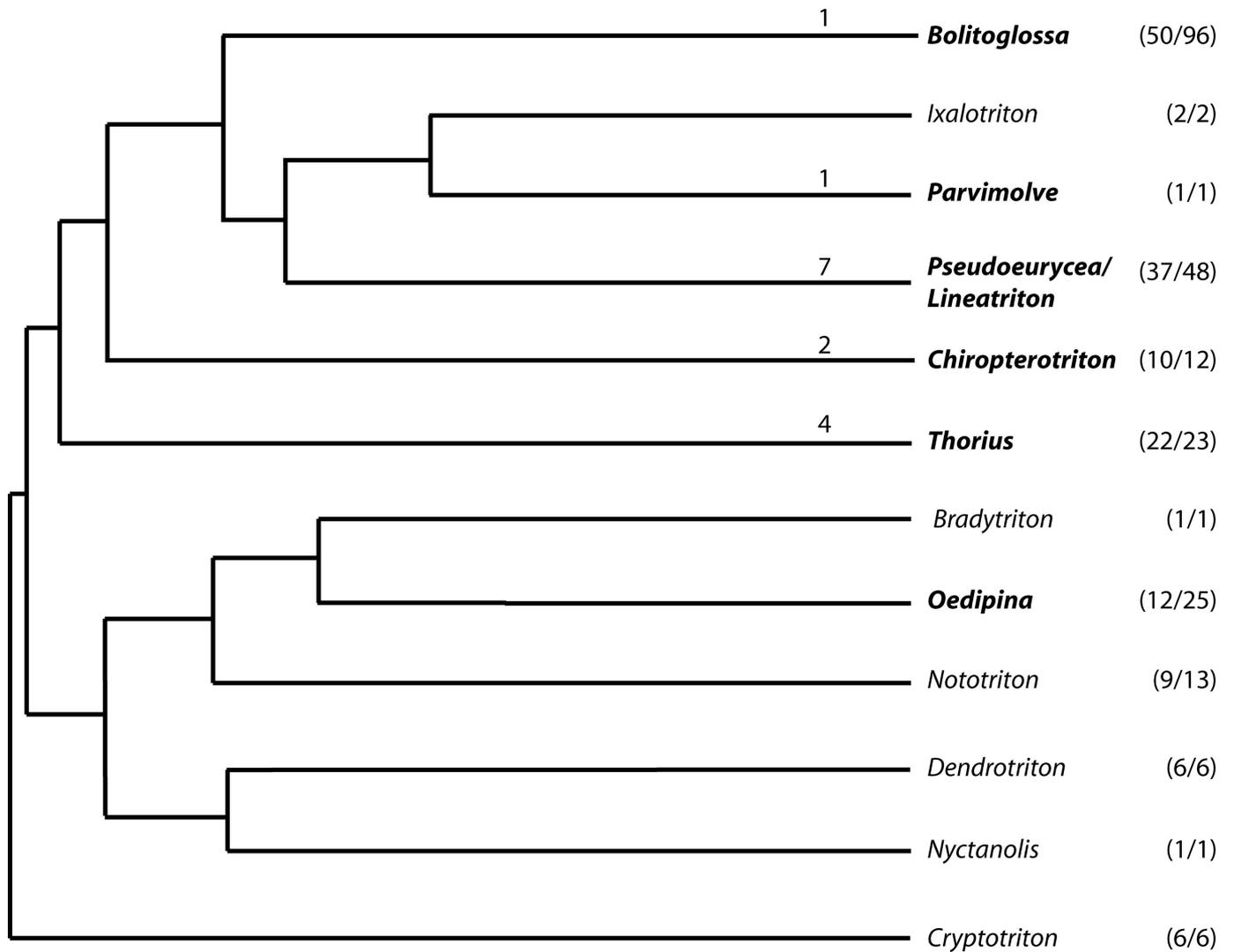


Fig. S1. Phylogeny of Neotropical plethodontid genera (redrawn from ref. 1), with branch lengths proportional to time. Genera containing species with reported declines in the literature (2, 3) are shown in bold; numbers on branches indicate number of species shown to be declining in this study. At right, number of threatened species based on 2008 global amphibian assessment (IUCN categories CR, EN, VU)/total number of species in genus. *Pseudoeurycea*, shown to be paraphyletic in Wiens *et al.* (1), is shown as a single lineage (including *Lineatriton*) for simplicity.

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Table S1. Total collection numbers for *B. rostrata*, *B. morio*, *P. rex* at localities in western Guatemala and Chiapas, 2005–2007

Site	Department/State	Visits	<i>B. rostrata</i>	<i>B. morio</i>	<i>P. rex</i>	Search time in person × hours
Sierra de los Cuchumatanes	Huehuetenango	2 (Oct. 2005, Aug. 2007)	56, >20	**	1, 0	19.35, 23
Rancho de Tejo	Totonicapan	1 (Nov. 2005)	39	**	**	3.75
High point on Panamerican Highway	Totonicapan/Solola	2 (Nov. 2005, Aug. 2007)	0, 0	0, 5	0, 1	3.75, 10
Cerro Tecpan	Chimaltenango	2 (Oct. 2005, Aug. 2006)	0, 0	1, 0	0, 0	10, 5.4
Cerro Tzontehuitz	Chiapas	1 (Aug. 2006)	2	**	**	4
Cerro Mozotal	Chiapas	1 (Aug. 2006)	**	**	0	8

Double asterisk indicates that site is outside the range of a species. Sierra de los Cuchumatanes totals do not include sites below lower elevational limit of *P. rex*.

Table S2. Collection totals for *Thorius* at Puerto del Aire, Veracruz/Puebla, Mexico

Month	Total <i>Thorius</i> collected	Museum
April, 1970	2	MVZ
September, 1971	10	LACM
September, 1972	82	LACM
January, 1973	9	MVZ
January, 1974	55	MVZ
January, 1974	14	MVZ
January, 1974	6	MVZ
January, 1974	74	MVZ
April, 1974	148	LACM
July, 1974	286	LACM
July, 1974	49	LACM
September, 1975	60	NMNH
February, 1976	1	MVZ
February, 1976	8	MVZ
February, 1976	29	MVZ
July, 1976	12	NMNH
July, 1976	2	MVZ
July, 1976	17	MVZ
July, 1976	12	MVZ
July, 1977	27	MVZ
July, 1977	3	MVZ
July, 1977	61	MVZ
January, 1982	1	MVZ
October, 1997	4	MVZ
April, 1999	2	MVZ
August, 1999	0	MVZ
January, 2000	1	MVZ
September, 2000	1	MVZ
January, 2001	3	MVZ

MVZ, Museum of Vertebrate Zoology; LACM, Natural History Museum of Los Angeles County; NMNH, Smithsonian Institution National Museum of Natural History.

Table S3. Results of RT-PCR assay for *Batrachochytrium dendrobatidis* from salamanders on the San Marcos transect and other sites in western Guatemala and Chiapas

Site	No. tested	Positives	Species testing positive	Zoospore equivalent
San Marcos: Tajumulco	6	0		
San Marcos: El Rincon	35	1	<i>D. bromeliacius</i>	20.5
San Marcos: Buena Vista	5	1	<i>B. lincolni</i>	475.2
San Marcos: "South transect"	8	1	<i>B. franklini</i> × <i>lincolni</i> hybrid	9894.8
Lowland sites (<1,500 m elevation)	8	4	<i>B. occidentalis</i>	0.74, 2.1, 83.2, 356.8
San Marcos Total	62	7		
Sierra de los Cuchumatanes	17	0		
High point on Panamerican Highway	6	0		
San Cristobal de las Casas area	2	0		
Volcan Chicabal	2	0		
Western Guatemala and Chiapas Total	27	0		

Table S4. Species present within each elevational zone on the San Marcos transect, elevation ranges of these zones, and survey sites

Elevational zone	Species present	Elevational range, m	Survey sites
High-elevation grassland and woodland	<i>Pseudoeurycea rex</i> , <i>Bolitoglossa rostrata</i>	2,800–4,200	Upper slopes of Volcán Tajumulco (>3,000 m)
Upper cloud forest	<i>Pseudoeurycea goebeli</i> , <i>Pseudoeurycea brunnata</i> , <i>Pseudoeurycea sp. nov. "San Marcos"</i> , <i>Bolitoglossa rostrata</i> , <i>Bolitoglossa lincolni</i> , <i>Bolitoglossa morio</i> , <i>Dendrotriton bromeliacius</i>	2,400–2,800	El Rincon (2,700 m), Buena Vista (2,400 m)
Lower cloud forest	<i>Bolitoglossa engelhardti</i> , <i>Bolitoglossa franklini</i> , <i>Bolitoglossa flavimembris</i> , <i>Dendrotriton bromeliacius</i>	1,600–2,400	Finca Insula (2,200 m)
Lower wet forest	<i>Bolitoglossa occidentalis</i> , <i>Bolitoglossa flaviventris</i> , <i>Bolitoglossa salvinii</i> , <i>Oedipina stenopodia</i>	0–1,600	Finca Santa Julia (1,100 m)