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Comment on "Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity"

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Scheele *et al.* (Reports, 29 March 2019, p. 1459) bring needed attention to the effects of amphibian infectious disease. However, the data and methods implicating the disease chytridiomycosis in 501 amphibian species declines are deficient. Which species are affected, and how many, remains a critical unanswered question. Amphibians are imperiled; protective actions require public support and robust science.

Empirical data on imperiled populations and species are difficult to gather but are essential for effective conservation planning (*1*). It is estimated that more than 40% of amphibian species face extinction (*2*). Many threats, including infectious diseases, confront amphibians (*3*, *4*). Chytridiomycosis, the disease caused by the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*), is unambiguously implicated in amphibian declines. But how much amphibian diversity—and which species—has chytridiomycosis affected?

Scheele *et al.* (*5*) implicated chytridiomycosis in 501 amphibian species declines and extinctions. We reexamined their study and found insufficient evidence implicating chytridiomycosis in most declines. These inaccuracies can impede effective conservation. We outline issues in Scheele *et al.* and highlight best practices to rigorously research chytridiomycosis' role in global amphibian declines.

Scheele *et al.*'s analyses linking chytridiomycosis to amphibian traits (e.g., taxonomy, elevation, life history) hinge on which species were included in their analysis. The authors generated "an expert-curated list" of 501 amphibian species (50% of which occur in only four countries) and then assigned categories of evidence linking chytridiomycosis to each decline (presented as a numerical category in column I of Scheele *et al.*'s data table). For 11.6% of included species, chytridiomycosis' role in a decline was based solely on expert opinion (Scheele *et al.*'s evidence category 1). However, 83.8% of species reportedly had additional lines of correlative evidence linking chytridiomycosis to their declines (categories 2 and 3), and another 4.6% reportedly had "robust before-after decline sampling" (category 4). Scheele *et al.*'s reported approach and methodology for evidence categories are illustrated in Fig. 1, A and B.

We used Scheele *et al.*'s data table ("data S1") to recreate evidence categories linking chytridiomycosis to declines and could not replicate their results, leaving chytridiomycosis' role in many declines questionable. Our reanalysis is shown in Fig. 1C. In Scheele *et al.*'s data table, lines of correlative evidence are presented in columns J to M. However, 451 data cells (22%) are blank or contain statements deviating from yes/no/no-data responses [e.g., "likely (no data)"]. Even when data responses were clear, the evidence category assigned to a species often did not match the lines of correlative evidence reported. We tried replicating Scheele *et al.*'s evidence categories on the basis of their published data, but found that more than half of the species' assignments changed, many dropping from category 3 (multiple lines of evidence) to category 1 (expert opinion only; Fig. 1C). Thus, evidence for decline categories is not reproducible based on the data presented.

Beyond missing correlative evidence in their data table, cited references were also often insufficient to recreate evidence categories. For example, one study (*6*) is a survey of expert opinions and is the sole reference for 21 category 2

and category 3 species assignments, despite containing no evidence relevant to these categories. Using references provided, we attempted to recreate evidence categories for species with the highest reported level of evidence (category 4, n = 23), as well as species with the next highest reported level of evidence (category 3) but for which no data were supplied (n = 62). We could not unambiguously do so for >75% of the category 4 species and encountered numerous problems with most category 3 species, including references devoid of relevant data and ambiguity in defining what scale (e.g., transect, range overlap, country) of sympatry was used. Inaccurate referencing makes it difficult to impossible to reproduce reported evidence categories, making the inclusion of most species in this analysis data-deficient.

Expert opinion is critical to conservation efforts, and local expertise is essential for understanding and combating amphibian declines. However, to be effective, expert opinion studies must use best practices, including training assessors by providing feedback on judgments, thoroughly documenting unpublished information, and detailing methods for evaluating resources (e.g., how were non–peer-reviewed sources collected and assessed?) (*7*, *8*). Assessor opinions—the foundation of Scheele *et al.*'s analysis—were not documented or validated, leaving the dataset largely irreproducible. Following best practices also reduces the potential for motivational bias (experts have a stake in their study system being taken seriously) and accessibility bias (experts know more about their system, possibly focusing on a subset of evidence) (*7*). We are not critiquing the importance of expert opinion, but failing to clearly report how and when expert opinion is used impedes conservation efforts.

When evaluating threats to biodiversity, including chytridiomycosis, we need studies that systematically and transparently identify and assess at-risk species and weigh evidence for multiple threats. In Scheele *et al.*'s main text, the evidence linking chytridiomycosis to declines appears equal for all 501 assessed species. Neglecting distinctions among species (e.g., those included solely on the basis of expert opinion, those with correlative evidence such as sympatric species declines, and those with robust before/after decline sampling) is misleading and influences interpretation of downstream analyses. In addition, although other threats (e.g., habitat loss, climate change, or overharvesting) are beyond the scope of Scheele *et al.*'s analysis, they should be mentioned, given that Scheele *et al.*'s own references often attribute declines in particular species to other threats. We are in no way diminishing the role of chytridiomycosis in amphibian declines, but when reporting quantitative data—empirical or otherwise—on population (let alone species) declines (and recoveries), transparency in how evidence is collected and assessed is critical.

We applaud Scheele *et al.* for bringing together a collaborative, international team to investigate a key threat to amphibian biodiversity, but we call for a more comprehensive approach and outline best practices for investigating the causes of species' declines (Fig. 2). Chytridiomycosis has irrefutably harmed amphibians. Existing evidence already warrants actions to mitigate chytridiomycosis. However, methodological and transparency issues leave Scheele *et al.*'s conclusions largely unsubstantiated. Collecting empirical data for declining and endangered species is difficult (*1*) but must be prioritized to identify specific species affected by chytridiomycosis. Biodiversity is in crisis and needs defensible narratives based on the most accurate evidence possible to strengthen public support and enact appropriate management (*9*–*11*).

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A **Within the main text, Scheele et al. briefly describe methods used to assess chytridiomycosis' role in species declines:**

"*Species declines were attributed to chytridiomycosis on the basis of diagnosis of infection causing mortalities in the wild or, if this was unavailable, evidence consistent with key epidemiological characteristics of this disease.*"

Despite stating assessments of chytridiomycosis were based on direct empirical evidence, methods outlined within their Supplemental Materials show that category-1 species (11.6% of total) had declines linked to chytridiomycosis solely by expert opinion and category-2 and -3 species (83.8% of total) had declines linked to chytridiomycosis by correlative evidence.

B **Within Supplemental Methods, Scheele et al. detail how evidence was ranked for chytridiomycosis' role in declines:**

"*The strength of evidence linking B. dendrobatidis to each species decline was then scored from one to four, with four being the strongest evidence of B. dendrobatidis-associated declines.*
One = expert opinion of the assessor only.
Two = single line of correlative evidence.
Three = multiples lines of correlative evidence.
Four = robust before-after decline sampling demonstrating declines were caused by B. dendrobatidis."

Lines of correlative evidence used to elevate evidence from category 1 (solely expert opinion) to category 2 or 3

- Was *Bd* detected using histopathology and/or PCR to confirm infection during mass die-offs or sudden declines?
- Did declines coincide with documented emergence and spread of *Bd* in the region?
- Did sympatric species decline simultaneously due to *Bd*?
- Is the declined species highly susceptible to *Bd* in lab experiments or field studies?

Based on methods reported within their Supplemental Materials, it appears that evidence categories 2–4 should be reproducible based on provided empirical evidence.

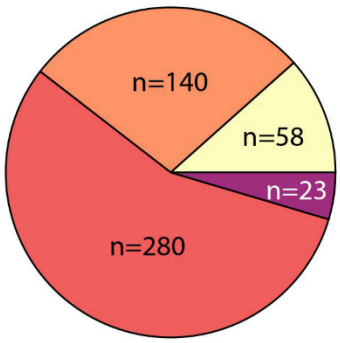
However, we find a lack of empirical evidence within Scheele et al.'s data table to support the evidence categories linking chytridiomycosis to many species' declines.

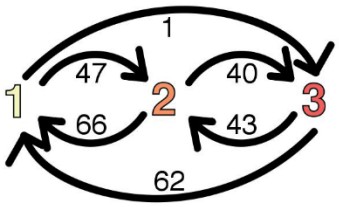
The link between chytridiomycosis and species' declines relies substantially more on undocumented expert opinion than Scheele et al. reported in their main text.

C **We attempted to recreate species evidence categories using Scheele et al.'s supplemental data table.**

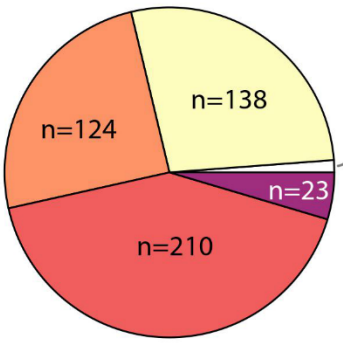
This reassessment is conservative because it assumes accuracy in Scheele et al.'s data table, but our main text demonstrates the provided references often do not support what is reported in the data table.

# of species in evidence categories presented in Scheele et al.





# of species in evidence categories based on correlative evidence provided within Scheele et al. data table



n = 6 could not be interpreted from Scheele et al.'s data table

Fig. 1 (preceding page). Scheele *et al.*'s reported methodology for linking chytridiomycosis to species declines and our attempt to recreate reported categories of evidence based on Scheele *et al.*'s data table. (A) Excerpt from Scheele *et al*. suggesting that empirical data were used to implicate chytridiomycosis in species declines. (B) Text from supplementary materials of Scheele *et al*. describing evidence used to link chytridiomycosis to species declines and specifying the lines of correlative evidence used for categories 2 and 3. (C) Frequency of evidence categories linking chytridiomycosis to declines as stated by Scheele *et al*. in column I of their data table (left pie chart) and based on our attempt to recreate those categories using the underlying evidence provided by Scheele *et al*. in columns J to M of their data table (right pie chart). Transition states among evidence categories are presented between the pie charts, indicating the number of species up- or down-categorized.

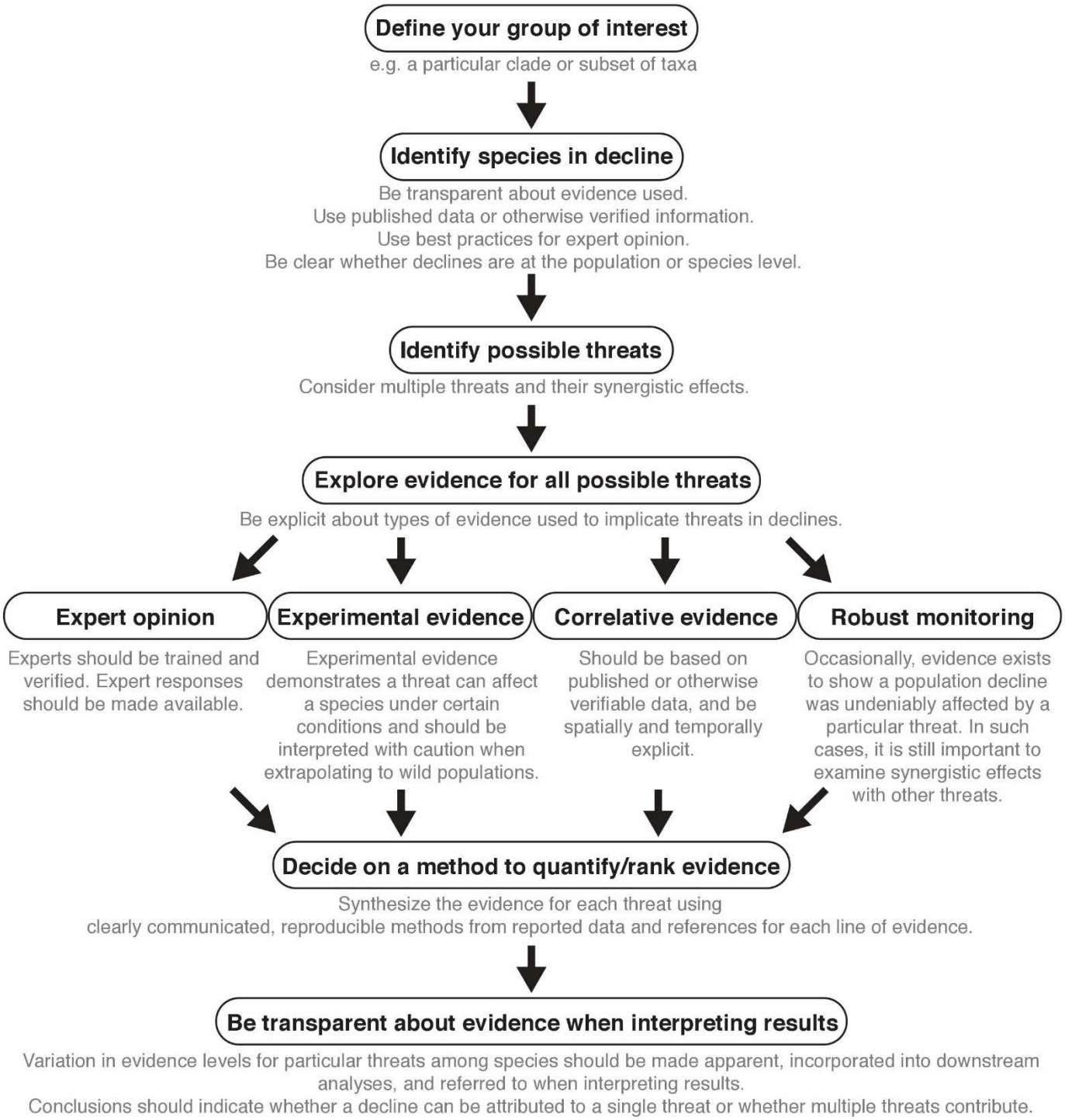


Fig. 2. Flow diagram illustrating best practices for determining causes underlying a focal taxon's decline.

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