Applying Critical Thinking to the Amphibian Decline Problem

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Applying Critical Thinking to the Amphibian Decline Problem

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ABSTRACT
This exercise is designed to foster the practice of critical thinking—a habit of mind characterized by the comprehensive exploration of issues and evidence before accepting or formulating an opinion or conclusion—in the context of a complex and real conservation problem: amphibian declines. The exercise has four parts: a case study, ten questions related to the reading, an in-class discussion in groups, and finally a set of questions for reflection and research, either individually or in groups, to understand how the issue has continued to evolve over the last decade.

1. PART 1: WHY ARE AMPHIBIANS DECLINING?

Nora’s heart was racing when the plane landed. Finally, back in Costa Rica!
She cherished the memories of her research years in Costa Rica, back in the early 1990s. Living and working right near the forest, she had never felt so alive. She had told her students so many stories from that time—especially the incredible feeling of hearing the forest come alive at dusk with the sound of hundreds of calling frogs.

Later that day after settling in at the field station and an early dinner, Nora and her students made their way to the nearest forest trail and started hiking. As dusk approached, her expectation grew, but an eerie feeling came over her as she began to notice that something was different. The forest was definitely turning dark, but it was strangely silent. As night enveloped them and they came to a clearing, the light from twelve headlamps converged on her.

- Ms. Torres, shouldn’t the frogs be calling by now?
- I’m afraid it’s much worse than I ever imagined—she had to admit to them—I just cannot believe that so many species have been lost in such a short time!

Then they all started speaking at once:

- Are they really gone, Ms. Torres?
- How come?
- What happened?

She would have to brush up on the science to answer all their questions by tomorrow.

1.1. Introduction and Instructions

This case study and exercise will illustrate the challenge of understanding and mitigating threats to biodiversity, through the case of worldwide amphibian declines. In our attempts to understand this phenomenon, two main hypotheses have been proposed to explain it. After you’ve read about each hypothesis and its supporting evidence, you will be asked to carefully and critically use the information presented to answer a series of questions.

As you read, keep in mind that you will be asked to answer ten questions afterwards. These questions will
include providing a summary of the problem amphibians experiencing, what you think is the best supported hypothesis to explain them, and why.

### 1.1.1. Global Amphibian Declines

#### 1.1.1.1. Amphibian Diversity

There are 7,704 known species of amphibians (Frost 2017). More than half of amphibian species have been discovered in the last 50 years. These new species are discovered both by explorations of under-surveyed areas or by re-evaluation of species using DNA analysis techniques that can reveal multiple species that previously were assumed to represent a single species.

There are three Orders within the Class Amphibia: Anura (frogs and toads; 6,785 species), Caudata (salamanders and newts, sometimes referred to as Urodela; 713 species), and Gymnophiona (caecilians; 206 species) (Frost 2017; Figure 1). Amphibians occur worldwide, with the exception of the Polar regions. They arose about 400 million years ago, with the major groups (the Orders mentioned above) being well differentiated by the Jurassic (approx. 200 million years ago). Fossils of amphibians since the Jurassic are essentially similar in all respects to modern amphibians, meaning that amphibians “as we know them” in terms of anatomy and natural history co-occurred with the famously extinct dinosaurs (Roelants et al. 2007). Whatever caused the extinction of the dinosaurs— or the mega-mammals of the Pleistocene, for that matter— had no evident effect on amphibians.

A salient characteristic of amphibians is their complex skin, which accomplishes the majority of the gas exchange with the environment. Many amphibians lack lungs (or gills) altogether, and those that do have them appear to actually use them only rarely. The skin is the primary physiological interface with the environment. Gas exchange must take place in an aqueous solution (e.g., in vertebrates, it occurs in the moist tissues inside the lungs) and thus amphibians must maintain a moist skin, which in turn requires them to be associated with moist or humid or aquatic environments. Among vertebrates, amphibians show a spectacularly diverse range of diversity of reproductive strategies and adaptations (Crump 2009).

#### 1.1.1.2. Amphibian Declines

Amphibians are experiencing unprecedented rates of population declines and species extinction. The IUCN Red List reveals the alarming reality that nearly one-third (32.4%) of the world’s species of amphibians are threatened with extinction (IUCN 2017). The contemporaneous decline of roughly 2,000 species constitutes a mass extinction event on par with those famously known only from the geological record. What’s worse is that these terrifying numbers are a certain underestimate because the IUCN committee struggled to decide how to formally list the status of many species.

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2 The Amphibian Species of the World is a database hosted by the American Museum of Natural History. It is updated in real time and can even vary from hour to hour, so check often for the most current numbers.

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Figure 1. Representatives of the Order a) Anoura (Atelopus certus); b) Caudata (Oedipina uniformis); and c) Gymnophiona (Demorphis mexicanus).

Images: a) Brian Gratwike/Flickr [CC BY 2.0], b) Sean Michael Rovito/CalPhotos [CC BY-NC-SA 3.0], c) Sean Michael Rovito/CalPhotos [CC BY-NC-SA 3.0]
that have been seen very infrequently by scientists, and now cannot be located in the wild. There was no option other than to classify those ~1,500 species (about 24.5 percent of all amphibians) into the category of “Data Deficient.” But it is likely that many of them are also experiencing declines.

The five major threats to biodiversity include habitat fragmentation, invasive species, pollution, unsustainable use, and global climate change. More than one factor seems to be responsible for amphibian declines. The Global Amphibian Assessment (GAA) revealed that many declines are due to anthropogenic causes, such as habitat loss and overexploitation. But what was most striking is that 48 percent of the declining amphibian species were initially identified as threatened with extinction by unidentified or enigmatic causes (Stuart et al. 2004). The GAA also found global geographic patterns associated with the declines and their causes. Species declines caused by habitat loss are predominantly found in Southeast Asia, West Africa, and the Caribbean; declines attributed to overexploitation are mostly reported from East and Southeast Asia; declines attributed to enigmatic causes are mainly restricted to South America, Mesoamerica, Puerto Rico, and Australia. Furthermore, the rapid enigmatic declines have particularly affected species that occur at mid- and high elevations and in association with streams.

The fungus *Batrachochytrium dendrobatidis*, also referred to as “Bd” or chytrid fungus, has been proposed as being responsible for the enigmatic amphibian declines. It is known to have caused rapid declines or extinctions of about 200 frog species, many of which were found in remote undisturbed areas (Skerratt et al. 2007). Bd is a pathogenic fungus that causes chytridiomycosis, a skin infection that thickens superficial skin layers compromising a frog’s osmotic regulation and leading to death by cardiac arrest (Figure 2; Berger et al. 1998). The optimal temperature range for Bd growth in the laboratory is between 17–25 °C. The fungus dies at temperatures above 29–30 °C and below 0 °C (Piotrowski et al. 2004).

Figure 2. A chytrid-infected frog. Image: Forrest Brem [CC BY 2.5] via Wikimedia Commons.

Although Bd is now widely recognized as the leading cause of the enigmatic amphibian declines, the underlying mechanisms or processes involved in the declines are still controversial. Why has Bd become so lethal to amphibians now? There are two leading hypotheses that attempt to answer this question: the climate-linked epidemic hypothesis (Pounds et al. 2006) and the spatio-temporal spread hypothesis (Lips et al. 2008).

1.1.1.3. The Climate-Linked Epidemic Hypothesis

The climate-linked hypothesis (Pounds et al. 2006) predicts that climatic changes, such as increases in temperature or related variables, will trigger the growth of pathogens, causing outbreaks of disease. While Bd could be the leading pathogen, the hypothesis is not restricted to this particular species of fungus. Climate change, or global warming, is proposed to be the primary driver of the amphibian declines observed.

Is there a correlation between climate and amphibian decline? In the early 1990s, Pounds et al. investigated the timeline of extinction of two species considered extinct at the time: the Jambato toad (*Atelopus ignescens*) of Ecuador and the Monteverde harlequin frog (*Atelopus varius*) of Costa Rica (Figure 3) and found that the last time these species were observed in the field (last year observed, or LYO) was in 1988³ (Pounds et al. 1994, As of 2017, *A. ignescens* is still considered extinct with LYO in 1988, but in 2005, two *A. varius* frogs were spotted in the central Pacific town of Quepos, Costa Rica (but were not seen again the following year). Then, in 2008, a small population of *A. varius* was found in Talamanca, in south-eastern Costa Rica. The population has fluctuated from five to 40 since 2011.

³
Pounds et al. 1999, Ron et al. 2003), a year after an unusual warmer year 1987. These species went from being common species to being considered extinct in a short period of time.

How widespread was this pattern? The decline pattern was also found for other frog species. After investigating patterns of decline for 100 Atelopus species, Pounds et al. found that of the 51 species that went extinct, about 80 percent disappeared a year after an unusual warmer year. Using simulations, they concluded that these extinction patterns were not random and that they were strongly associated with large-scale warming events (measured as air temperature, or AT, see Figure 4). Furthermore, they reported that the association between extinction and warm temperatures was strong regardless of altitude, latitude, or species range size. Based on this evidence, the authors argue that global warming is a key factor to explain the decline of frog species.

Could warmer years actually affect local frog habitats? Pounds et al. investigated if climate changes at a regional scale were correlated with changes at local scales. The authors modeled climate at a regional scale (the tropics) using data on sea surface temperature (SST) and air temperature (AT). Then, they determined the correlation between the climate trends observed for the tropics and local trends observed for Monteverde cloud forest (at 1,400 m of elevation) in Costa Rica. The authors found that AT and SST for the tropics correlated with the number of dry days in Monteverde, and the daily minimum AT in Monteverde (Figure 5a–c). The data showed that temperatures increased in the tropics between 1975 and 2000 (Figure 5a), which at a local scale in Monteverde caused a reduction in mist frequencies and in relative humidity. Based on these correlations, Pounds et al. concluded that changes in AT for the tropics could predict changes in temperature at a local scale, and in turn affect local ecological processes.

Why would this increase the incidence of Bd infections? These changes in climate are thought to be beneficial to the chytrid fungus. Pounds et al. reported a decrease in the daily maximum temperature and an increase in the minimum daily temperature in Monteverde, Costa Rica and in 11 other locations in Colombia and Venezuela. Recall that the optimal temperature range for the chytrid fungus is 17–25 °C and the optimum temperature is 23 °C (Piotrowski et al. 2004). In Monteverde, the daily temperature is chytrid friendly, but in the microhabitats such as moss mats, bromeliads, or leaf litter, temperatures are higher than 30 °C. Thus, the chytrid fungus does not survive, but an increase in cloudiness due to higher air temperatures blocks the direct sunlight from reaching those habitats, which become cooler and therefore optimal for the chytrid.
Can this explain the vulnerability of frogs living at mid- and high elevations? The authors found that the probability of species disappearance varied across the altitudinal gradient (Figure 6a). The elevation gradient was used as a proxy for a temperature gradient (the higher the elevation, the lower the temperature) and the LYO as a proxy for extinction date. The probability of disappearance was zero at low elevations (0–100 m) but it increased dramatically between 200–1,000 m (63.3%) and even more between 1000 and 2399 m (90.2%). However, this probability dropped between 2,400–4,000 m (65.7%; Figure 5a). Based on these results, Pounds et al. suggest that the lethal effect of the chytrid fungus on amphibians may be restricted to mid-elevations. The authors argue that recent increases in minimum daily temperatures at mid-elevations may allow the survival of the chytrid and are driving the observed declines (Figure 5b).

1.1.1.4. The Spatio-Temporal Spread Hypothesis

The spatio-temporal spread hypothesis (Lips et al. 2008) predicts declines in amphibian populations after the new arrival of a pathogen, and particularly Bd, to a location with optimal environmental conditions. According to this hypothesis, the advancing spread of the pathogen in space (geographically) is the main factor explaining the pattern of declines. An assumption of this hypothesis is that Bd is an exotic species to the Neotropics.

To examine the spatio-temporal patterns of Bd appearance, Lips et al. estimated the date of actual decline (DOD; date of first detection of mortality due to Bd), whenever data was available. They estimated DOD for the same Atelopus species used by Pounds et al. (2006) and other species from Central and South America. Lips et al. argue that “date of actual decline” is a better variable than the “last year observed” (LYO;
used by Pounds et al. 2006) to determine the causes of amphibian decline because of the potential error associated to the LYO records. For instance, detection of the last individual of a population may change with population size, survey frequency, time for populations to go extinct after the decline begins, or rediscovery of the species. On the other hand, considering that populations once hit by Bd usually go extinct relatively fast (for *Atelopus* 3.6 +/- 2.6 years; La Marca et al. 2005), DOD provides a better estimate of the date of the population decline.

Next, they plotted those geographically to see whether amphibian declines followed a pattern consistent with spatial spread. They did: the authors identified one wave of Bd expansion in Central America (Figure 7a) and four for South America (Figure 7b).

The authors suggest that two independent introductions of the chytrid fungus occurred in South America. First, it was introduced to Venezuela in 1977 and then to Ecuador in 1980 (see Figure 7b). From these locations, the fungus spread to the whole Andean region. From Venezuela, it spread southeastern towards Colombia; and from Ecuador it spread in three waves, two to the northern region of Colombia and eastern Venezuela, respectively, and a third one to the south (Peru and Bolivia).

For all but one of the Central and South American waves, the authors found significant correlations between the earliest DOD and the distance and rate of spread of Bd (Figure 8). Thus, they concluded that a spread of the chytridimycosis disease is the lead cause of amphibian decline.

Lips et al. also investigated altitudinal patterns of amphibian decline. They found significant high proportions of declines of *Atelopus* frog species at elevation higher than 200 m (Figure 9). Contrary to the pattern reported by Pounds et al. (2006), Lips et al. found no evidence to suggest that declines are more prevalent at middle elevations. Thus, authors concluded that all *Atelopus* species are critically threatened at middle and high elevations. This is consistent with their hypothesis, which focuses on presence of the fungus, and acknowledges that the fungus and changes in temperature may interact to create different conditions in different habitats.

Finally, to confirm that the amphibian declines were caused by the arrival of Bd, Lips et al. examined tissues from frog museum specimens collected in Ecuador (89 specimens) and Monteverde, Costa Rica (64 specimens) prior to the dates when declines were observed. All specimens examined were negative for Bd in tests with a
Figure 7. a) Central American and b) South American spreading waves of amphibian declines. Years indicate the date of decline (DOD) and rates indicate the rate of spread of the chytrid fungus (in kilometers per year) (adapted from Lips et al. 2008).

Figure 8. Relationship between time since the earliest date of decline (DOD) and the distance of spread for the waves a) in Central America ($p<0.01$, $R^2 = 0.97$); b) from Venezuela towards Colombia ($p = 0.32$); c) from Ecuador to northeastern Colombia ($p<0.01$, $R^2 = 0.47$); d) from Ecuador towards Venezuela ($p<0.01$, $R^2 = 0.4$); and e) from Ecuador towards Peru and Bolivia ($p<0.05$, $R^2 = 0.49$) (adapted from Lips et al. 2008).
95 percent confidence level. Based on this evidence, the authors suggest that Bd was not associated with frogs in Ecuador or Costa Rica prior to their observed declines.

In summary, although Lips et al. recognize climate change as a threat to biodiversity, they do not think this is the main driver of the declines observed. They conclude that chytrid fungus or Bd is an introduced pathogen to the Neotropics and that its spread is the main driver of the unprecedented amphibian declines observed in the last decades.

2. PART 2: EXERCISE QUESTIONS

In this part of the exercise, we ask you to answer the following questions using the information provided above.

1. What problem are amphibians experiencing worldwide? Please explain it as clearly and completely as you can in the space provided (approximately 150 words).

2. What does the “climate-linked epidemic” hypothesis of Pounds et al. (2006) propose? Summarize it in 1–2 sentences, using your own words.

3. List two of the lines of evidence used to support the “climate-linked epidemic” hypothesis.


5. List two lines of evidence used to support the “spatio-temporal spread” hypothesis.

6. How are these two hypotheses different in terms of their predictions? Explain in 2–4 sentences, using your own words.

7. Please list and explain one strength and one weakness of each hypothesis in Table 1.

8. If you were Ms. Torres, and you had to briefly describe to your students why the frogs have disappeared in this forest and what you think is the most likely explanation, what would you say? Please explain it as clearly and completely as you can (~100–150 words).

9. In 2011, Cheng et al. described a new molecular technique by which amphibian museum specimens can be tested for the presence of chytrid fungus through a simple swab sample. This DNA-based

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**Table 1. The strengths and weaknesses of amphibian decline hypotheses.**

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<thead>
<tr>
<th>Hypothesis</th>
<th>Weaknesses</th>
<th>Strengths</th>
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<tbody>
<tr>
<td>Climate-Linked Epidemic</td>
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<tr>
<td>Spatio-Temporal Spread</td>
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Figure 9. Total number of Atelopus species per altitudinal range and the percentage of them that went lost or that showed population declines (in gray). For the analysis, they used the same species dataset used by Pounds et al. (2006) but instead of using the LYO to estimate the declines, they used DOD. In addition, they excluded species classified as Data Deficient (adapted from Lips et al. 2008).
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EXERCISE

technique uses the polymerase chain reaction (PCR)⁴ to detect the presence of Bd DNA in specimens’ skins, and can be used for specimens collected up to 50 years ago.

a. How would you use genetic tools to help understand the causes of enigmatic amphibian declines, knowing that many museums have been collecting specimens from Central and South America for decades? Provide a specific example or scenario of where, and for what purpose, you could use this technique.

b. How might this new kind of evidence strengthen the hypotheses above? Explain.

3. PART 3: GROUP DISCUSSION

Once you have completed the exercise questions above, your instructor will provide guidelines for an in-class discussion. Be ready to share, justify, and discuss your answers with your classmates.

4. PART 4: CHANGING CONTEXTS, CHANGING MINDS?

The knowledge we use rely on to explain the patterns we see in the natural world constantly evolves (or becomes refined), depending on the tools at our disposal and the evidence available. The last part⁵ of this critical thinking exercise will challenge you to research the current literature around the topic of amphibian declines, and to reach an up-to-date understanding of the global amphibian crisis.

Here we have outlined three focal questions to guide you as you research further, individually or in groups.

Your instructor will give you specific instructions for this activity.

Since the original proposals of the “climate-linked epidemic” hypothesis of Pounds et al. (2006) and the “spatio-temporal spread” hypothesis of Lips et al. (2008):

What have we learned about the chytrid fungus?

What have we learned about amphibian declines?

How are conservation biologists protecting amphibians in the face of chytrid fungus?

After you have completed Part 4, you may wish to review your previous answer to Part 2, Question 8: If you were Ms. Torres, and you had to briefly describe to your students why the frogs have disappeared in this forest and what you think is the most likely explanation, what would you say? Did your understanding of the original hypotheses and the global amphibian crisis change?

Complex and dynamic issues such as amphibian declines require ongoing evaluation and critical thinking, such as the skills you have practiced here.

REFERENCES


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⁴ PCR is a molecular technique used to amplify specific fragments of DNA. If the chytrid fungus is present on a frog specimen, chytrid DNA fragments will be amplified with the PCR.

⁵ Part 4 of this exercise was not part of the original Critical Thinking unit of the research study: NSF DUE-0942789, Developing and assessing process skills in Conservation Biology and other integrative fields. For more information about the research study, see What Can Your Students Do? The Importance of Assessing and Developing 21st Century Skills in Conservation Students in Lessons in Conservation 8:5–10 and references cited therein, available from ncep.amnh.org/linc.


