Can we control a deadly infectious amphibian disease before it is too late?

Vance T. Vredenburg
San Francisco State University
7,121 described Amphibian Species

- 41% declining
- 30% threatened with extinction

Amphibians

Mammals

Birds

Reptiles

Fish

IUCN Red List 2007-2008
Amphibians have survived the last 4 mass extinction events in earth’s history.

Are we in the midst of the sixth mass extinction? A view from the world of amphibians

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*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720-3160; and †Department of Biology, San Francisco State University, San Francisco, CA 94132-1722

Many scientists argue that we are either entering or in the midst of the sixth great mass extinction. Intense human pressure, both direct and indirect, is having profound effects on natural environments and species. This mass extinction is characterized by the loss of families and nearly 60% of the genera of marine organisms were lost (1, 2). Contributing factors were great fluctuations in sea level, which resulted from extensive glaciations, followed by a...
Global Amphibian Declines

- Most threatened group of vertebrates on the planet
- Over 1/3 (41%) of amphibians are now threatened with extinction
- Several reasons for declines – habitat destruction, introduced species, over exploitation, climate change, environmental toxins

IUCN 2012; Bill Marsh for NY Times
Sierra Nevada
California
Possible Causes Amphibian Declines

- Habitat destruction
- Over exploitation
- Invasive species
- UV-B radiation
- Climate change
- Infectious disease
Global Amphibian Declines

Percentage of red-listed amphibian species

Percentage of red-listed freshwater amphibian species:
- 0 - 2%
- 2 - 5%
- 5 - 9%
- 9 - 15%
- 15 - 25%
- 25 - 33%
- 33 - 50%
- 50 - 80%
- 80 - 100%

The Nature Conservancy
Protecting nature, Preserving life

© WWF/TNC 2008
Data sources:
Global Amphibian Assessment
Abell et al. 2008
www.few.org
Costa Rican Golden Toad

Last seen 1989
Mass Mortality and Extinction in a High-elevation Population of Rana muscosa

DAVID F. BRADFORD

MASS MORTALITY OF RANA MUSCOSA

Fig. 1. Numbers of live frogs present and frog carcasses collected in Ridge Lake during summer, 1979.

Fig. 2. Numbers of tadpole carcasses collected at Ridge Lake during summer, 1979. Asterisks indicate that no tadpole remains were found on indicated dates.
Sierra Nevada: a protected area
Nearly 100 years of Biodiversity Research

Half Dome

Yosemite National Park

(Grinnell and Storer 1915)
Major declines despite protected habitat

(Rana sierrae)
Sierra Nevada yellow-legged frog
92.5% decline

(Rana muscosa)
Southern mountain yellow-legged frog
96% decline

Data: MVZ, CAS specimens + C. Davidson, documented sightings
Data: Vredenburg, Knapp, Briggs et al., CAS, CDFG, USFS, USGS recent surveys

(Vredenburg et al 2007; J Zool)
Defining Conservation Units

Data: MVZ, CAS specimens + C. Davidson, documented sightings
Data: Knapp, Briggs et al., CAS, CDFG, USFS, USGS recent surveys

Major declines began ~1980

Endangered (ESA)

94% decline

99% decline

Extinctions
Defining conservation units:
Comparison of different types of data collected throughout entire range of the frog

(Vredenburg et al 2007; J Zool)
Concordant data delineate new taxonomy—two species

**Rana sierrae**

**Rana muscosa**

Contact zone

Frog calls

mtDNA

morphology

(Vredenburg et al 2007; J Zool)
We now have defined conservation units: Now what?

Conclusion:

2 species - 6 ESU’s

<table>
<thead>
<tr>
<th>ESU</th>
<th>% extinction</th>
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<tr>
<td>1</td>
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<td>93</td>
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(Vredenburg et al 2007; J Zool)
Introduced species:
No native fishes above 1,500 m

Two exceptions:
- Lake Tahoe
- Kern River

(reviewed in: Vredenburg et al 2005)
THREATS – Introduced Fish

BACKGROUND
• Historically fish found only at lower elevations of Sierra Nevada.
• Filled colored areas are native fish ranges
• Red outline is the range of the two species of mountain frog
The historical introductions of trout began in the 1890s, but there were declines in frog populations in the late 1970s. Therefore, trout were not responsible for the frog declines.

Reasons why trout were originally not seen as a big factor in the frog declines:

1. Trout native in some areas
2. Historical introductions of trout began in the 1890s, but frog declines in the late 1970s

...therefore trout not responsible!
In the 1950-60’s, industrial trout introductions began.
Flying fish can climb mountains
Humphreys Basin & French Canyon

Historic fish distribution

- **Blue**: no fish
- **Yellow**: fish present

(R. Knapp unpublished)
Humphreys Basin & French Canyon

Current fish distribution

- **Blue**: no fish
- **Yellow**: fish present

(R. Knapp unpublished)
Key life cycle of the Mountain Yellow-legged Frog

1-4 years

2 weeks

10+ years
1996-2003

Fish Lake (n=8)

Removal Lake (n=5)

Frog Lake (n=8)
Frog populations can quickly recover (Vredenburg 2004; PNAS)
Conservation Biology: A long hard road.

Repeated same experiment in 3 different study areas in the Sierra Nevada.

Same result:
Removal introduced trout leads to rapid recovery of threatened frog populations.

(Knapp, Boiano and Vredenburg 2007; Biological Conservation)
Does non-native fish removal benefit other native species?

Stable isotope food web study (C, N)

(Finlay and Vredenburg 2007; Ecology)
Science-informed Conservation Recommendations:

1. Remove non-native fishes
2. Restrict planting non-native fish

Conservation Successes:

We are just beginning!
Mass Mortality and Extinction in a High-elevation Population of *Rana muscosa*

DAVID F. BRADFORD

FIG. 1. Numbers of live frogs present and frog carcasses collected in Ridge Lake during summer, 1979.

FIG. 2. Numbers of tadpole carcasses collected at Ridge Lake during summer, 1979. Asterisks indicate that no tadpole remains were found on indicated dates.
The disease: Chytridiomycosis

*The pathogen:*

*Batrachochytrium dendrobatidis* ("Bd")
Fungal Pathogen: 
*Batrachochytrium dendrobatidis* (Bd)

Disease: chytridiomycosis
Chytridioomycosis (chytrid)

Emerging disease in Asia

M. Forzan
Detection methods:

- **Real time PCR assay**
  - Non-destructive
  - Quantitative estimate
    - Comparison to standards
- **Histology**
  - Time consuming
- **Culture**

(Boyle, et. al 2004)
Amphibian skin is a physiologically active organ:

- Regulates exchange of respiratory gases: oxygen, carbon dioxide.
- Maintains osmotic balance: water, electrolytes.
- Involved in amphibian immunity.

Epidermal Dysfunction Hypothesis: *Bd* disrupts cutaneous osmoregulatory function, leading to osmotic imbalance and death.

Voyles et al. *Diseases of Aquatic Organisms* 2007
emerging infectious diseases (EIDs) caused by fungi are increasingly recognized as presenting a worldwide threat to food security (Table 1 and Supplementary Table 1). This is not a new problem as fungi have long been known to constitute a widespread threat to species. Plant disease epidemics caused by fungi and the fungal oomycetes have altered the course of human history. In the nineteenth century, late blight led to starvation, economic ruin and the downfall of the English government during the Irish potato famine and, in the twentieth century, Dutch elm blight and chestnut blight laid bare our urban and forest landscapes. The threat of plant disease has not abated, it is heightened by resource-rich farming practices and exacerbated by landscape by microbial adaptation to new ecosystems, brought about by trade and transportation, and by climate fluctuations.

However, pathogenic fungi (also known as mycoses) have not been widely recognized as posing major threats to animal health. Their detection is changing rapidly owing to the recent occurrence of high-profile declines in wildlife caused by the emergence of previously unknown fungi. For example, during March 2007, a routine check on bats hibernating in New York revealed mass mortalities. When a group of closely clustered caves, four species of bats were marked with striking fungus growing on their muzzles and wing membranes, a name—white nose syndrome (WNS)—was coined. After the initial outbreak, the ascomycete fungus Geomyces destructans was shown to have Koch's postulates and was described as the cause of WNS in American bat species. Mortalities exhibiting WNS have subsequently been found in an increasing number of bat overwintering sites across the United States and Canada, spanning over 2,800 km. In 2010, the infection was confirmed to have emerged in at least 115 species across the United States and Canada, spanning over 2,800 km. Bat numbers across affected sites have declined by over 70% and analyses have shown that at least one affected species, the little brown Myotis lucifugus, has a greater than 99% chance of becoming extinct within the next 16 years. Other species of bats...
Fungal Diseases: An emerging challenge to human, animal, and plant health.

### Human
- Cryptococcal disease
  - *Cryptococcus gattii*
- Coccidioidomycosis (Valley Fever)
  - *Coccidioides immitis*
- Aspergillosis
  - *Aspergillus fumigatus*
- Candida
  - *Candida albicans*

### Animal
- Colony Collapse Syndrome (bees)
  - *Nosema apis*
- Aspergillosis (birds)
  - *Aspergillus fumigatus*
- White-nose Syndrome (bats)
  - *Geomyces destructans*
- Chytridiomycosis (amphibians)
  - *Batrachochytrium dendrobatidis*

### Plant
- Sudden Oak Death
  - *Phytophthora ramorum*
- Fusarium wilt (tomatoes etc.)
  - *Fusarium oxysporum*
- Stripe rust (wheat)
  - *Puccinia striiformis*
- Chestnut blight
  - *Cryphonectria parasitica*
Sierra Nevada, California

How does this pathogen work???
Repeated visual population counts (>900; 1996–2008)
Skin swabs (> 6,000; 2004-2008)
Key:
- Uninfected frog population
- Infected frog population
- Extirpated frog population
- No data

(Vredenburg, et al. 2010; PNAS)
Uninfected frog population
Infected frog population
Extirpated frog population
No data

Key:

(Vredenburg, et al. 2010; PNAS)
Uninfected frog population
Infected frog population
Extirpated frog population
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Infected frog population
Extirpated frog population
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Key:

(Vredenburg, et al. 2010; PNAS)
Key:
- Uninfected frog population
- Infected frog population
- Extirpated frog population
- No data

(Vredenburg, et al. 2010; PNAS)
Key:
- Green circle: Uninfected frog population
- Yellow: Infected frog population
- Black circle: Extirpated frog population
- Gray circle: No data

(Vredenburg, et al. 2010; PNAS)
Mountain Yellow-legged Frogs
*Rana muscosa* & *Rana sierrae*

Yosemite National Park
- Infected since 1970’s or 80’s?
- Small infected populations of frogs are persisting with Bd.

"persistence"

Sequoia & Kings Canyon National Parks
- Bd currently spreading, leading to 100’s of local extinctions.
- Few frog populations remain.
- In time, these populations may resemble "persistent" sites.

"endemic"
Different Outcomes in Different Species

*Lithobates catesbeiana*

**Carrier**

*Xenopus laevis*

**Carrier**

*Xenopus tropicalis*

**Death**

*Litoria moorei*

**Death**

http://birdingwa.iinet.net.au

We discovered a Mortality Threshold

Vredenburg et al. 2010

C

Days since start of frog population decline

Infection intensity (x zoospore equiv.)

alive    dead    dead

Cheng et al. 2011
Seasonal Pattern of *Batrachochytrium dendrobatidis* Infection and Mortality in *Lithobates areolatus*: Affirmation of Vredenburg’s “10,000 Zoospore Rule”

Vanessa C. Kinney¹, Jennifer L. Heemeyer¹, Allan P. Pessier², Michael J. Lannoo³*
A Reservoir Species for the Emerging Amphibian Pathogen *Batrachochytrium dendrobatidis* Thrives in a Landscape Decimated by Disease

Natalie M. M. Reeder¹*, Allan P. Pessier², Vance T. Vredenburg¹

¹Department of Biology, San Francisco State University, San Francisco, California, United States of America, ²Wildlife Disease Laboratories, Institute for Conservation Research, San Diego Zoo Global, San Diego, California, United States of America
Figure 1. Habitat occupancy before and after disease spread. Range of *P. regilla* and *R. muscosa* in 60 Lake Basin (a) before and (b) after the spread of *Bd* through the basin.
doi:10.1371/journal.pone.0033567.g001
Control (-Bd)
Bd infection is localized on the skin – most of the skin continues to function.

**Figure 5. Histological views of skin from an infected but asymptomatic individual.** Photomicrographs of histologic sections from the foot webbing of *P. regilla* # 32. In (a) the epidermis is well organized with minimal variation in nuclear size and a single keratinized layer consistent with normal foot skin. However, (b) shows an adjacent area of skin with disorganization of the epidermis (hyperplasia), hyperkeratosis and numerous *Bd* thalli (arrows). There are infiltrates of inflammatory cells in the epidermis and dermis. E = epidermis; I = inflammatory cells.

doi:10.1371/journal.pone.0033567.g005
**Bd Timeline:**

Where did it come from?
When and how did it emerge?

**Major Worldwide Amphibian Declines Began in the 1980’s.**
Introduced frogs spread Bd

The transport and release of *Xenopus laevis* or other non-native carrier species followed by spread of *Batrachochytrium dendrobatidis* in native amphibians.

Vredenburg et al 2013 PLOS ONE
Frog legs anyone?

American Bullfrog
(Rana catesbeiana)
Introduced amphibians may be carriers of $Bd$
Lots of issues to consider.

Can a federally threatened species persist in this city?

Bullfrogs

*Pet stores and meat markets provide a steady supply of Bullfrogs*
How did Bd spread?

- Not all species are susceptible
- Some act as disease reservoirs or vectors
Farms, Pet and Food Trade in Asia (Yunnan, China)
When did \( Bd \) invade California?

Celeste Dodge

Sam McNally
Bd emergence in *Batrachoseps attenuatus*

Carla Sette
Museum specimens reveal how fungal epidemic spread in amphibians

Timeline of proposed Bd epidemics in the Andes

Atelopus database

Lips et al. 2008, PLoS Biology
When did Bd emerge in S. America?

In collaboration with: (Alessandro Catenazzi, and many others.)

~8,000 museum specimens

Genus: Atelopus
Telmatobius
In collaboration with:
(Alessandro Catenazzi, and many others.)

~8,000 museum specimens

Genus: Atelopus
Telmatobius

15 Museum collections (VertNet)

Bd described (1999)
Susceptibility trials

- Which of the surviving species are most at risk from chytridiomycosis?
- Experimentally expose frogs to Bd infection
- Compare survivorship between infected and treated frogs
- 9 species of 4 families
- 213 frogs
- 3-6 weeks in June-August 2012
Wayqecha Biological Station  http://www.acca.org.pe/

- spectacular views
- earthy food
- socializing
- education
- hot water & internet
- makeshift lab
Susceptibility trials: Peru highlands
Predicted suitable habitat for Bd
Emerging disease in Asia

Asia is Vulnerable

- Yunnan
- Hainan
- Taiwan
- Tokyo, Japan

Bd habitat suitability:
- HIGH
- LOW
- UNINHABITABLE
Retrospective survey suggests recent emergence of Chytridiomycosis in Japanese amphibians

Gabriela Rios-Sotelo
Vance Vredenburg PhD
San Francisco State University
First report of Bd in Asia

- Outbreak in Japan, 2006, Tokyo
- Suspected disease imported by trade

“There is an urgent need to monitor Bd in amphibians in Asia…”

Une et al 2008
Results

Island | 1890-1993
--- | ---
Hokkaido | 0/6
Honshu | 1/441
Shikoku | 2/50
Kyushu | 0/39
Oge-Shima | 0/2
Okinawa | 0/12
Bd infection intensity and prevalence at positive sites in the Philippines 2003-2011

Infection Intensity (Zoospore equivalents):
- 300 Zsp
- 100 Zsp
- 10 Zsp
- 1 Zsp

Percent Bd:
- 25%
- 50%
- 75%

*Scale dictated by square root of Zsp
We tested 1200 samples collected in PH from 1906-2009

Conclusion:
Bd has recently emerged in the Philippines
Introduced frogs spread Bd hypothesis

The transport and release of *Xenopus laevis* or other Non-native species brought forth the spread of *Batrachochytrium dendrobatidis* in native amphibia

[Image](http://www.freshwatermadness.com/t1287-species-profile-african-clawed-frog-xenopus-laevis)
What can we do to save the amphibians?

Bioaugmentation of skin microbiome may save amphibians in the wild.
Mutualistic bacteria play a role in amphibian resistance to fungal disease.
Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus

Reid N Harris¹, Robert M Brucker², Jenifer B Walke³, Matthew H Becker¹, Christian R Schwantes⁴, Devon C Flaherty⁴, Brianna A Lam¹, Douglas C Woodhams⁵, Cheryl J Briggs⁶, Vance T Vredenburg⁷ and Kevin PC Minbiole⁸

In the Lab, we measured:
1. Survival
2. Weight gain or loss
3. Bd load (infection intensity)

Bioaugmentation works in the Lab!

Can we protect susceptible frogs in the wild?

Bioaugmentation of skin microbiome may save amphibians in the wild.
Hold

Holding cages:

+ Bacteria

Control
Bacterial Cultures collected

Treatment Group

Control Group
2010

Treatment Group

Control Group

2011

Infection Intensity (log of zoospores)

17-Jul-10  27-Jul-10  28-Jul-10  26-Aug-10  2-Sep-10  10-Sep-10  16-Sep-10  Winter  15-Jul-11  12-Aug-11  21-Sep-11  29-Sep-11

10 days

10 days
2010

2011

2nd Day of treatment, and release

Treatment Group

Control Group

Infection Intensity (log of zoospores)

0

1

2

3

4

5

17-Jul-10
27-Jul-10
28-Jul-10
26-Aug-10
2-Sep-10
10-Sep-10
16-Sep-10
Winter
15-Jul-11
12-Aug-11
21-Sep-11
29-Sep-11
Graduate students:
Celeste Dodge, Silas Ellison, Gabriela Rios-Sotello, Cory Singer, Raul Figueroa, Sam McNally, Danqing Shao, Stephanie Hyland, Andrea Manzano, Jacobo Conde, Jonathan Young (recently finished: Tina Cheng, Natalie Reeder, Meghan Bishop)
Undergraduates:
Mark Russell, Nina Hang, Alex Harencar, Amanda Carbajal, Bo Heinz, Corinna Inmann, Hahn Pham, Hannah Durbin, Ivet Lolham, Jason Anders, Jina Kim, Jourdan McPhetridge, Karl Alicando, Kirsten Liaz, Laurece Henson, Mackenzie Beaschler, Robert Tom
AmphibiaWeb an online Conservation Resource
At “die-off” sites:

Bd load increases rapidly to high levels

At “persistent” sites:

Bd load is low and does not reach critical threshold

(Vredenburg, et al. 2010; PNAS)
ADDRESSING FUNGAL DISEASES


“Bats and frogs share a common plight: New disease paradigms for wildlife.”
Live Science January 18 2011

“Secrets of a frog killer laid bare.”
BBC October 22, 2009

“Could Bacteria Save Frogs From Extinction?”
Scientific American July 10, 2010

“Stopping a frog killer.... a tool for saving species in the wild”
BBC, June 6 2008

“Amphibians afloat and fighting”
BBC October 13, 2008

“Biologists solve a great mystery of modern ecology.”
USA Today October 22, 2009
Telmatobius frogs

5 control: 7-day itraconazol

15-20 infected: no itraconazol
Experimentally infected frogs

Night surveys

8-25 frogs/species

all frogs: 7-day itraconazol

4-5 control: no exposure

4-20 infected: *Telmatobius* exposure
Peru Bd susceptibility trials: experimentally infected frogs
Telmatobius marmoratus (trial 1)

Survival (%)

P << 0.0001

n = 5
n = 15

58 days

55 days

9 days

p = 0.02

Infected

Control

3.28

1.23
Gastrotheca nebulanastes

- Control
- Infected

$p = 0.003$

Survival (%)

- 261 days
- 47 days
- 51 days

$p = 0.002$

Infected

- 3.91

Control

- 0.35
Survival (%)

Gastrotheca excubitor

- ● control
- ○ infected

n = 7
n = 5

26 days

p = 0.13

log(Z_swab+1)

Infected

0.71

Control

1.24
Frogs along the elevational gradient
Mortality
Threshold

300,000 zoospores

Mt. Palay
Palay

Bd Infection Intensity by Site

Infection Intensity (log zoospore equivalent + 1)
300,000 zoospores

Mortality Threshold

Bd Infection Intensity by Species

Infection Intensity (log zoospore equivalent + 1)

Species
First Bd outbreak in Asia: Mt Palay-Palay
Bd Susceptibility Lab Trials
SF State University

Animals purchased from pet trade
(Petco, San Francisco, CA)

All tested Bd+ on arrival at SFSU

Raul Figueroa (SFSU master’s)
Bd Susceptibility Lab Trials
SF State University

Animals purchased from pet trade (Petco, San Francisco, CA)

All tested Bd+ on arrival at SFSU

Raul Figueroa (SFSU master’s)