Chytridiomycosis: An Emerging Infectious Disease of Amphibians

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The problem
- Amphibian population declines
- Many proposed causes of amphibian declines
- Most noticeable 1980s-present
- Greatest concern = “enigmatic” or mysterious declines
  - Remote, protected areas
  - No obvious causes
  - Sudden massive die-offs, lack of recovery
- Many enigmatic declines attributed to Chytrid fungus (Bd)
  - most tropical, montane, riparian

The pathogen
- *Batrachochytrium dendrobatidis (Bd)*:
  - Emerging infectious disease of amphibians
  - First chytrid fungus pathogenic to vertebrates
- Infect keratinized tissue
  - Mouthparts in larvae
  - Adult skin
- 3 life stages
  - Zoospore – aquatic, flagellated (3-5µm)
  - Thallus – in epidermis
  - Zoosporangium – zoospores discharged
**Infection in adults**

- Environmentally sensitive
  - Cool temperatures: 17-24°C (killed if >30°C)
  - Moist environments (killed by desiccation)
  - No resting stage

**Zoosporangium**

- Environmental persistence
  - Up to 7 weeks in pond water (Johnson and Speare 2003)
  - Up to 6 weeks in mesocosm
  - At least 3 days in the environment

**Bd Ecology**
**Origins**

- **Novel pathogen hypothesis**
  - Out of Africa (Weldon 2004)

- **Endemic pathogen hypothesis**
  - Environmental changes (Pounds 2006)

**Novel pathogen hypothesis**

- **Out Of Africa** (Weldon 2004)
- **Exotic, introduced pathogen**
  - Low genetic variation globally
  - Recent global spread (Morehouse et al 2003)
  - Broad range of host species
  - No resistant individuals
  - Lack of host immune response
  - Not present prior to dieoffs (no coevolution)

1. *Xenopus laevis*; South Africa (1938)
2. *Xenopus gilli*; South Africa (1943)
Novel pathogen hypothesis

1. Xenopus laevis; South Africa (1938)
2. Xenopus gilli; South Africa (1943)
3. Rana Clamitans, Canada (1961) 23 years later
4. 1970’s North America and Australia
5. Spread around the world

Rachowicz et al. 2004
Novel pathogen hypothesis

1. Xenopus laevis; South Africa (1938)
2. Xenopus gilli; South Africa (1943)
3. Rana Clamitans, Canada (1961) 23 years later
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Rachowicz et al. 2004
**Novel pathogen hypothesis**

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**Endemic pathogen hypothesis**

- Susceptibility of host may increase because of environmental changes
  - Immunosuppression (Carey 1993)
  - Temperature
  - pH
  - Moisture levels (Pounds 2006)
  - UV-B radiation (Kiesecker and Blaustein 1995)
- Antimicrobial peptides (Rollins-Smith et al., 2002)

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**Distribution**

Bioclimatic modeling of *Bd*:

Q: Where are appropriate habitats?

A: It can live everywhere.
The victims

- Population-level effects:
  - 90% decrease in amphibian abundance, 50% decrease in species richness, no recovery
  - Streams faster, greater losses than terrestrial
  - Extirpations of high elevation populations
  - Extinctions of montane endemic species

Survivors

- No immune response
  - Survive in low numbers
- Defenses
  - AMPs = Antimicrobial skin peptides (Rollins-Smith et al. 2003)
  - Elevate body temperatures clear infection (Woodhams et al. 2003)

Cause of death

- Osmoregulatory inhibition (suspected)
  - Decreased water uptake; altered electrolyte/solute levels
- Toxic byproducts: no (?)
**Clinical signs: in field**

- Infected individuals appear healthy
- Lethargic
- Sloughing skin
- Loss & depigmentation in mouthparts of larvae
Local Transmission

- Direct transmission
  - Frog-frog contact (Adults, Larvae)
  - Amplexus
  - Territoriality
- Environmental transmission
  - Adults - Spatial or temporal overlap of species
  - Larvae - shared aquatic environment
  - Water-facilitated transport

Geographic transmission

- Site to site, country to country
- Not known
  - Anthropogenic (pet, food trades)
  - Frog-frog?
  - Other vectors (insects, birds)?
  - Rain, wind, blowing leaves?
  - Streams & rivers?

Sites with amphibian population declines & Bd

- 1987-88
- 1993-94
- 1996-97
- 2002-03
- 2004
- 2006

Lips at al. 2006
Case study: El Cope, Panama

• Die-off October 2004
• 347 individuals
• 40 species, 7 families
• 70% of fauna (47/67 spp)
• All habitats & all communities
  – Terrestrial, arboreal, riparian
• All heavily infected with Bd, but no other disease

Density decline

\[ t = -24.44, \; df = 486, \; P < 0.0001 \; \text{Sept. 4 (1-6)} \]

Lips et al. 2006

Mortality

Lips et al. 2006
### Species capture rates through Dec. 2005

<table>
<thead>
<tr>
<th>Species</th>
<th>min - max loss (%)</th>
<th>Gone by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostethus panamensis</td>
<td>100 - 100</td>
<td>2004</td>
</tr>
<tr>
<td>Eleutherodactylus punctariolus</td>
<td>100 - 100</td>
<td>2004</td>
</tr>
<tr>
<td>Rana warszewitschii</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>Phyllomedusa lemur</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>E. megacephalus</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>Bufo haematiticus</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>E. gollmeri</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>E. bufoniformis</td>
<td>100 - 100</td>
<td>2005</td>
</tr>
<tr>
<td>E. talamancae</td>
<td>99.0 - 99.5</td>
<td>NA</td>
</tr>
<tr>
<td>C. euknemos</td>
<td>94.5 - 99.3</td>
<td>??</td>
</tr>
<tr>
<td>C. prosoblepon</td>
<td>98.8 - 99.2</td>
<td>??</td>
</tr>
<tr>
<td>E. pardiellii</td>
<td>96.9 - 99.1</td>
<td>??</td>
</tr>
<tr>
<td>C. litoralis</td>
<td>96.5 - 98.8</td>
<td>NA</td>
</tr>
<tr>
<td>E. crassidigitus</td>
<td>95.2 - 97.8</td>
<td>??</td>
</tr>
<tr>
<td>E. diastema</td>
<td>90.7 - 97.7</td>
<td>??</td>
</tr>
<tr>
<td>Aboliopus zeteki</td>
<td>88.6 - 96.8</td>
<td>??</td>
</tr>
<tr>
<td>Bolitoglossa colonnea</td>
<td>89.2 - 95.4</td>
<td>??</td>
</tr>
<tr>
<td>B. schizodectyla</td>
<td>70.0 - 90.4</td>
<td>??</td>
</tr>
</tbody>
</table>

### Tadpole decline

- In 2004 adults decline ~80% of abundance and 50% species (Lips et al., '06)

- Tadpole densities dropped dramatically

- What happened to Ecosystems?

### Tadpole declines

![Tadpole declines graph](image)
What are the ecological repercussions of amphibian losses?

Potential impacts

• Energy transfer (Pough '80, Regester et al. '06)
• Loss of biomass (Burton and Likens '75; Stewart and Woolbright '96)
• Nutrient cycling (Seale '80; Beard et al. '02)
• Leaf litter decomposition (Wyman '98)
• N and P cycling (Seale '80)
• Macroinvertebrate communities (Ranvestal et al. '04)

All studies in adults or temperate zones

Classical headwater stream ecosystem

Cummins 1974

High Productivity

Multiple functional roles of tadpoles
Tropical headwater stream ecosystem

Energy flow in tropical systems

Tadpoles role in ecosystems

- Energetic contribution year-round by different functional groups
  - Nutrient cycling (Seale '80)
  - FPOM production (Colon et al., in press)
- Energy transfers to riparian environments
  (Polis et al. '04, Regester '05)
  - Larvae abundant in dry season
  - Adults abundant in wet season (Brenes and Lips unpublished data)
- Contributions to energy flow in streams
Tropical Amphibian Declines in Streams (TADS)

- Post-extirpation effects on:
  - Primary producers (Connelly & Pringle)
  - Amphibian larval and adult biomass (Brenes & Lips)
  - Amphibian predators (Lips & Montgomery)
  - Macroinvertebrate communities (Colon-Gaud & Whiles)
  - Food webs (Hunte & Kilham)

Study site: Omar Torrijos National Park, El Copé, Panamá

Amphibian community

- 76 species of amphibians
  - 3 Cecilians
  - 8 Salamanders
  - 68 Frogs
  - 41 live in riparian habitats
  - Breeding season for most spp during wet season

- 22 species in streams
  - 9 very common spp

Tadpole densities in 2001 (50/m²) (Ravenstel et al. 2004)
Tadpole Community

- 11 species in 3 functional groups:
  - Filterers: Colostethus flotator, Colostethus panamensis, Colostethus nubicola
  - Grazers: Hyla palmeri, Hyla colymba, Rana Warszewitschi
  - Collector-gatherers: Centrolene ilex, Centrolene prosoblepon, Cochranella granulosa, Cochranella albomaculata, Hyalinobatrachium colymbiphyllum

Measuring energy flow

A. Species composition and densities

B. Growth rates

C. Biomass

D. Production

Tadpole density and abundance

- Four 200-m reaches; same watershed
- 4 techniques for different habitats
  - Kick nets (Rifflies)
  - Stovepipe core (Leaf packs)
  - Exhausted removal (Isolated pools)
  - Nocturnal visual encounters (Shallow pools)
- 4 techniques, 3 x per month in all 4 streams for 12 months = 576 samples
Tadpoles present all year

Density (ind/m²)

Aug 03 Sep 03 Oct 03 Nov 03 Dec 04 Jan 04 Feb 04 Mar 04 Apr 04 May 04 Jun 04 Jul 04 Aug 04

Dry season

Seasonal abundance

Number of individuals

Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Aug

Centrolenidae
Rana sp
Colostethus spp
Hyla spp

Instantaneous growth rates

• Tadpoles grown in situ
• 40 plexiglass chambers
• In stream for 4-6 weeks
• Size specific instantaneous growth rates obtained: ln(wf / w₀)
Instantaneous growth rate

\[ y = -0.0168 \ln(x) + 0.057 \]

\[ R^2 = 0.663 \]

<table>
<thead>
<tr>
<th>Group / Taxa</th>
<th>Small</th>
<th>med</th>
<th>large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostethus spp</td>
<td>0.004169</td>
<td>0.00972</td>
<td>0.000215</td>
</tr>
<tr>
<td>Hyla spp</td>
<td>0.01845</td>
<td>0.00728</td>
<td>0.000578</td>
</tr>
<tr>
<td>Rana sp</td>
<td>0.01417</td>
<td>0.00856</td>
<td>0.001079</td>
</tr>
<tr>
<td>Centrolenidae</td>
<td>0.00712</td>
<td>0.00352</td>
<td>0.00237</td>
</tr>
</tbody>
</table>

**Calculate Biomass**

- Animals used in growth experiments
  - Dry  Weighted
  - Burned  re-weighted
- Obtain AFDM
- Length-mass regressions for biomass estimates
- Other individuals collected throughout the year were extrapolated

**Average biomass by taxa**
Calculating secondary production

- Best way to measure energy flow and to determine organism function (Benke '93)

\[ P = \sum (B_i \cdot G_i) \]

where \( G = \ln \left( \frac{W_{t+1}}{W_t} \right) \)

Production (mg AFDM m\(^{-2}\) yr\(^{-1}\))

Production by Species

- Centrolenids
- Colostethus spp
- Rana sp
- Hyla spp

Total Annual Production= 96.86 mg AFDM m\(^{-2}\)yr\(^{-1}\)

Tadpole declines

Year 1

Year 2
Loss of Biomass

- Colostethus
- Hyla
- Centrolenidae
- Rana
- Total

Average Biomass AFDM/m²y

- *p = 0.027
- P = 0.179

Loss of production

- Colostethus
- Hyla
- Centrolenidae
- Rana
- Total

- 96.86 mg
- 28.02 mg

Centrolenidae

- *p = 0.03
- P ≥ 0.5

Decline in tadpole populations

- Density dropped 95%
- Biomass was reduced 47%
- Production reduced 72%

What are the consequences?
**How might energy flow change?**

- Sun light
- Leaf litter
- Egg masses
- Metamorphosis
- Neonates
- Prey
- Forest
- Stream
- Primary producers
- Algae
- Diatoms
- Primary consumers
- Macro-invertebrates
- Tadpoles
- Secondary consumers
- Macro-invertebrates
- Fish

**How are other groups affected?**

**What is going to happened in the stream?**

- Loss of production will be compensated by other groups?
- Increase in densities of macro-invertebrates?
- Excess of primary production will be wasted?
- Overall energy budget of stream will be diminish?
Algae Bloom

What is going to happen in the Forest

Reduction in amphibian prey
What is Left after the Chytrid

Survivors

• Some species persist with stable, low level infections of *Bd*
• No known recovery of populations or sites
  – Environmental persistence of *Bd*
  – Reservoir species & life stages
  – Extirpation of pops at cool, moist sites; survival at low, dry, warm sites
  – Extinction - High endemism and restricted ranges = limited potential for recovery
Solutions?

• *Bd* is unstoppable & untreatable in the wild
• Few unaffected areas or species remain
• *Bd* is moving into new, unaffected sites
  – Massive additional losses expected
• We can predict species & areas affected
• Treatable in captivity
• Time is running out

*So what do we do?*

Is Captive breeding the solution?

Many practical, ethical, & legal complications:

Where do we start?
What species get chosen?
Who gets to decide?
How do we do it?

So many spp, so little space!!
**Major husbandry challenges:**

- **Space** - not enough for 5,800 species
  - Only 37 amphibians in captivity
  - Room for 200 individuals of ~10 species
- **Aesthetic value** - easy for only colorful, unique, educational species
- **Technical know-how**
  - Specialized ecology, habitats, diets
- **Expenses**
  - Staff, infrastructure, maintenance

**New approaches:**

- **Take pre-emptive actions at sites predicted to become infected.**
  - Ex situ ?? Noah’s ark
  - In situ ???
- **Form link between in situ & ex situ programs**
  - Research, treatment, reintroduction

**Conclusion**

Are amphibians in an extinction vortex?

Is there a way to stop Chytrid?

Are ecosystems going to recover?
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