Management of Amphibian Chytridiomycosis

Panama rocket frog

Colostethus panamensis
Why care about frogs?

http://www.toptenz.net/10-things/happen-without-frogs.php

Panama Epizootic

What happens next?

After the epizootic

Voyles, Woodhams, et al. 2018 Science
Reduced $Bd$ prevalence

Voyles et al. 2018 Science, Woodhams et al. 2010 EcoHealth

Rediscovered species with low $Bd$

Dr. Andreas Hertz

Hertz et al. 2018

Guatemala

Jackson's climbing salamander ($Bolitoglossa jacksoni$) disappeared in 1975, rediscovered after 42 years!
In the early 1990s, Bd extirpated populations of the endangered common mistfrog, *Litoria rheocola*, at high-elevation sites, while populations of the species persisted at low-elevation sites. Today, populations have reappeared at many high-elevation sites where they presently co-exist with the fungus.

**Note:** Phillott et al. 2013. *Chytridiomycosis and Seasonal Mortality of Tropical Stream-Associated Frogs 15 Years after Introduction of Batrachochytrium dendrobatidis*
Northern leopard frogs

The Live Frog Is Almost Dead 1971, BioScience
Erich L. Gibbs, George W. Nare, and Marvin B. Ermans

Lineages of Batrachochytrium dendrobatidis (Bd)

Global Panzootic: Lineage (BdCAPE)
South Africa (BdCAPE)
Switzerland (BdCH)

Farrer...Woodhams, PLoS Genetics 2013

Essen, Germany: February 2018
THE COMING SALAMANDER PLAGUE

Batrachochytrium salamandrivorous (Bsal)

Martel et al. 2014 Science

A Coordinated, Proactive Response

► Bsal Task Force: http://www.salamanderfungus.org/
  (Grant et al. 2017 Front Ecol Env, Gray et al. 2017 Herp Rev)
► Surveillance - USGS
► Policy – USFW international trade ban
► Risk assessment – species susceptibility, disease modeling
► Management tools
  ● Disease phase and scale specific
    (Wobeser 2005, Langwig et al. 2015)
  ● Tested by evolution of natural populations

Can we design Disease Management based on mechanisms of naturally persistent or recovering amphibian populations?

► Why are certain species resistant to disease?
► How do some populations recover?
Why are certain species resistant to disease?

Woodhams et al., 2018
Encyclopedia of Life

Amphibian Immunity

Life-history adjustments
Behavioral defenses
Behavioral fever
Microhabitat choice
Classic immune defenses

Innate
- Complement and Lysozyme
- Phagocytic cells and Natural Killer cells
- Toll-like receptors
- Antimicrobial peptides (AMPs)
- Microbiota, microbial products in mucus

Acquired/Adaptive
- Lymphocytes: B and T cells
- Major histocompatibility complex (MHC)
- Antibodies (IgM, IgY, IgX, IgD)
- Cytokines and chemokines
- T-cell mediated immunity

Reviewed in Rollins-Smith et al. 2009 Frontiers in Bioscience

Immune organs

Planes
Mesonephros
Thymus
Liver
Spleen
Kidney
Bone marrow

Artwork by Robert M. Brucker

Rollins-Smith & Woodhams 2011 In: Eco-Immunology
Amphibian skin

Rollins-Smith et al. 2011 Integrative and Comparative Biology

Mucosal microbes

Barthart et al. 2017 Microbial Ecology
Microbiome

Acquired immune system regulates hosts, secretes, activates, limits infection, and alters directional selection on MHC II. It recognizes and responds, induces production of antimicrobial compounds, competes for resources.

Amphibian skin mucosome

Skin defense compounds

Amphibian skin mucosome

corticosterone

Amphibian skin mucosome

Skin defense compounds
Mucosome function predicts risk of infection & disease

- 3,100 field-sampled amphibians
- logistic regression, $P < 0.0001$

Woodhams et al., 2014 PLoS ONE

Mucosome vs. Bsal

$P < 0.01$

Amphibian skin mucosome
Skin defense peptides kill fungal zoospores

Woodhams et al., 2012 Fungal Biology

Skin defense peptides predict Panama epizootic outcome

Woodhams et al. 2006 J. Wildlife Diseases, Woodhams et al. 2007 Animal Conservation

Only 1 species persisted into enzootic phase

Woodhams et al. 2006 J. Wildlife Diseases, Woodhams et al. 2007 Animal Conservation
How do some populations recover?

No reduction in *Bd* virulence

- Compared 3 historic and 3 contemporary *Bd* isolates from Panama
  - Equally virulent in exposure challenges
  - Similar capacities to inhibit lymphocyte proliferation
  - Similar responses to symbiotic bacteria and host defense peptides
  - All global panzootic lineage and no evidence for genetic shifts

Host adaptations
Host adaptations

► Changes in host assemblages
► Changes in skin peptide defenses
► Changes in skin microbiota

Host defense peptides

Skin defense peptides more effective in enzootic phase
Antifungal isolates database of amphibian skin-associated bacteria and function against emerging fungal pathogens. 2015. Ecology

Amphibian microbiota

Anti-Bd skin bacterium (*Pseudomonas fluorescens*) is more prevalent after *Bd* enters the ecosystem.
**Proportion of amphibians with at least one anti-Bd isolate**

![Graph showing proportion of hosts with anti-Bd bacteria for Emerging and Enzootic populations.](image)

*Fisher’s Exact Test, p = 0.0001*

**Herd Immunity threshold (p)**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Approximate value of p (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox</td>
<td>70-80</td>
</tr>
<tr>
<td>Measles</td>
<td>92</td>
</tr>
<tr>
<td>German measles</td>
<td>83</td>
</tr>
<tr>
<td>Whooping cough</td>
<td>94</td>
</tr>
<tr>
<td>Chicken pox</td>
<td>90</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>80</td>
</tr>
<tr>
<td>Scarlet fever</td>
<td>80</td>
</tr>
<tr>
<td>Mumps</td>
<td>80</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>83</td>
</tr>
</tbody>
</table>

Based on specific locations and times

**Herd immunity with microbiota**

![Graph showing predicted protected and unprotected populations.](image)

*Dr. Molly Bletz*
How should we manage disease?

Wildlife Disease Management
- Management methods for wildlife disease
- Chytridiomycosis management

Principles of Disease Management
- Is management desirable?
- Methods: A management matrix:

<table>
<thead>
<tr>
<th>Target:</th>
<th>Goal</th>
<th>Agent</th>
<th>Host</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eliminate</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

- Assessment: Monitoring effectiveness and adaptive management
Eradication or Elimination

► Not often possible
  ● Examples:
    ● Smallpox (eradicated = global prevalence of 0)
    ● Rhinderpest
    ● Human Hookworm (eliminated in the USA)

Management methods

► Transmission / Vector control
  ● Zooprophylaxis
  ● Mosquito nets, draining wetlands, DDT, etc.
  ● Killing fleas
  ● Sterilizing vectors
  ● Others

‘Zooprophylaxis’

► Divert mosquitoes to surrounding cattle to reduce malaria
Ditching

- During the 1930’s, 90% of the Atlantic coastal marshes were grid-ditched to drain surface water where mosquitos breed.
Mosquito control

Management methods

► Transmission / Vector control
► Host management
  ● Immunization
  ● Treatment
  ● Captive breeding and reintroduction
  ● Culling
  ● Quarantine
  ● Others

Sylvatic Plague management

Successful Immunization of Black-Footed Ferrets

Scientists at NPFRC have demonstrated in the laboratory that vaccination can protect black-footed ferrets from plague. Collaborative field studies with the FWS and the USGS Fort Collins Science Center have verified that vaccination can improve ferret survival in the wild. The plague vaccine used for ferrets is an injectable protein, developed for humans by the U.S. Army Medical Research.

USDA scientist injects a vaccine into a black-footed ferret.

Vaccine-Laden Bait: A New Method for Field Application

FWS scientist sprays pesticide on a prairie dog burrow to kill plague-carrying fleas.

USDA Fort Collins, CO
Sylvatic Plague management

Black-footed ferrets crouched by male that died 5 years ago.

Related images:

- Black-footed ferret
- Man holding ferret

Black-footed ferrets are a very rare species.

Ethical Issues for Host Management

► Examples:

- Culling hosts to reduce density-dependent transmission,
- or altering interactions with reservoir hosts

- Reintroduction

- Treatment of wildlife

“Leaky” vaccines

Here we show experimentally that immunization of chickens against Marek’s disease virus enhances the fitness of more virulent strains, making it possible for hyperpathogenic strains to transmit.
Vaccination strategy
► Live vaccine - Bsal followed by clearing
► Dead vaccine – Bsal
  ● Zoospores only?
  ● How killed – heat, freezing, or formalin-fixation to preserve epitopes
► Anti-toxin vaccine
  ● Methylthioadenosine (MTA) inhibits lymphocytes
  ● Is synthesis pathway in Bsal distinct from amphibian cells?
► Adjuvants (to stimulate immune responses)
  ● Freund’s complete adjuvant
  ● Closely related chytrid fungus found on amphibians

Vaccination against Bd
Repeated exposures to Bd, followed by clearing of the infection, led to an increase in survival
McMahon et al. 2014. Nature
Management methods

► Transmission / Vector control
► Host management
► Habitat management
  • Change habitat area or connectivity
  • Managing disease reservoirs
    • Vegetation, prey, predators
  • Wetland drainage / creation / decontamination
  • Soil cultivation, de-acidification
  • Others

► Modifying human activities
  • Quarantine
  • Limiting movement of wildlife and pathogens
  • Legislation
  • Education
  • Others
**Field Quarantine**

Hess 1996 Ecology

- Separate facility for new animals
- No contact with animals to be released
- Optimal health conditions
- Defined time period
- Veterinary examination and diagnostic procedures
- Treatment
  - Prophylactic treatment of at-risk animals
- Decontaminate all waste water

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**Reducing field transmission**

Field Hygiene

► Single use gloves, or plastic bags
► Define separate sites in field
► Clean and disinfect between sites
  ● equipment
  ● clothing
  ● footwear
  ● vehicles

U.S. Fish and Wildlife Service
International Trade Moratorium

Salamander Species Listed as Injurious Wildlife
Under 50 CFR 16.14
Due to Risk of Salamander Chytrid Fungus
Effective January 28, 2016

Unprecedented Action:
• Used to Lacey Act to list a species as Injurious due to the possibility of being a suitable host for a microorganism.
• Listed 29 genera of salamanders
• Native species (32%) were listed
• Stopped international and inter-state movement

US Court of Appeals decision came April 7 in the US Association of Reptile Keepers v. [Ryan Zinke as] Secretary of Interior,
Appeal = Only International Trade

Chytridiomycosis Management
Woodhams et al. 2018.
Encyclopedia of Life

Disease Triangle
Management Scale

Management and Invasion Phase

Context-dependent conservation responses to emerging wildlife diseases

- Identify the invasion stage - monitoring
- Stage-specific management
  - Pre-arrival
  - Invasion Front
  - Epidemic
  - Established
  - Learn from populations recovering naturally
Pre-arrival example

► First report of Batrachochytrium salamandrivorans in Germany

► Captive S. salamandra from this collection showed a 100% prevalence of infection with B. salamandrivorans.

► Mortality also affected captive S. algira, S. corsica, and S. infraimmaculata, and individuals with confirmed Bs infection belonged to 7 S. salamandra subspecies

Pre-arrival: captive outbreak

► What to do in a captive/quarantine facility outbreak?
  ● Tracing origin of infection
  ● Decontamination
    ● Bleach waste water, sterilize waste
  ● Disease Treatments
    ● 1. Heat (Blooi et al. 2015 Scientific Reports: 25°C for 10 days)
    ● Amphotericin B, Nikkomycin Z, others…
Pre-arrival: research
► As part of risk assessment
  ● Targeted monitoring of pet trade
  ● BsAl exposure trials on US amphibians
  ● Use of mucosome assays to predict susceptibility and risk to US species
  ● Test suitability of habitats across landscape

Pre-arrival: collections
► As part of risk assessment
  ● Probiotic culture collection and testing

Pre-arrival: planning
► Depending on risk to US salamanders
  ● Build capacity for captive breeding
  ● Plan ahead for field-based management

Bletz et al. 2015, Scientific Reports
Bletz et al. 2013, Ecology Letters:
Sampling microbiota in Madagascar before Bd epidemic (invasion front)
Invasion front

- Fungicide treatments on habitat
- Removing and treating amphibians
- Eradication may be possible in isolated invasion locality
- Possibly more risky interventions warranted at this stage?

Invasion Front / Epidemic

- Eradication of Bd has not been effective
- Slowing invasion progress
  - Prophylactic treatment to boost immunity
    - Probiotics
    - Vaccination
  - Habitat modification
    - Salt
    - Canopy gaps
    - Biotic communities
  - Augmenting demographic rates
    - Head starting, reducing predation/other threats

Probiotics in the lab

Woodhams et al. 2007 Biological Conservation; Harris et al. 2009 ISME Journal
Probiotics in mesocosms

Disease Treatment

Prophylactic Treatment

Promising field results

Vredenburg et al.

Rescuing the Yellow-legged Frog - New York Times
Probiotic therapy

► Best practices
  ● Choose native bacteria or fungi
  ● Choose “core microbes” through development
  ● Probiotic should increase upon chytrid exposure
  ● Test for safety (non-target effects) and efficacy
  ● Apply early in development
  ● Volatile compound activity

  ● Aim to permanently shift microbiomes to be more anti-chytrid

Upcoming probiotic trials
Salt

NaCl > 2 ppt reduces infection loads
► Stockwell et al. 2015, Oekologia
► Karraker et al. 2008, Ecol. Appl.: potential negative effects of road salt on vernal pool breeders

Canopy Gaps
► Raffel et al. 2010, EcoHealth
► Rowley & Allford 2013, Sci. Reports
► Daskin et al. 2011, Plos One

Biotic communities

A. Lauer – in Woodhams et al. 2011, FIZ
Biotic communities


Augmenting populations

- Head-starting populations

Scheele et al. 2014, Conservation Biology

Established

- Protecting disease refuge habitats
- Translocations to promote genetic diversity of remnant populations
- Augment populations / protect from further threats: land-use change, climate, etc.
- Habitat modification
- Vaccination
- Probiotic therapy
- Facilitated evolution
Bd Established in Panama

► Chytrid fungus remains virulent in Panama
► Selection on hosts leading to population recovery from Bd
  • Changes in amphibian assemblages – population sizes and species composition
  • Changes in skin defenses
    • Peptides
    • Microbiota
  • Changes in host behavior
  • Changes in MHC gene frequencies

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Damage-Response Framework

Casadevall and Pirofski 2003 Nat Rev Microbiol

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Damage-Response Framework
"The pathogen is nothing. The terrain is everything."

-Louis Pasteur
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