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

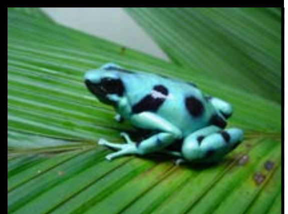
Infection in adults

Zoosporangium

Bd Ecology

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

Origins



- Novel pathogen hypothesis
  - Out of Africa (Weldon 2004)
- Endemic pathogen hypothesis
  - Environmental changes (Pounds 2006)

Novel pathogen hypothesis

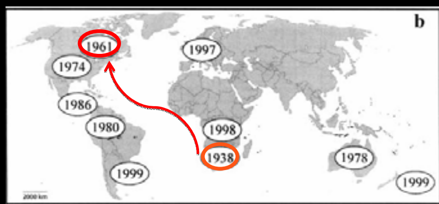
- **Out Of Africa (Weldon 2004)**
- **Exotic, introduced pathogen**
  - **Low genetic variation globally**
  - **Recent global spread (Morehouse et al 2003)**
  - **Broad range of host species**
  - **No resistant individuals**
  - **Lack of host immune response**
  - **Not present prior to dieoffs (no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

Novel pathogen hypothesis

Rachowicz et al. 2004

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

Novel pathogen hypothesis



Rachowicz et al. 2004

1. *Xenopus laevis ;* South Africa *(*1938)
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3. Rana Clamitans, Canada (1961) **23** years later

Novel pathogen hypothesis

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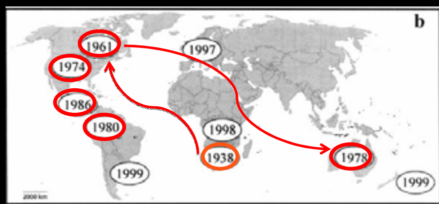

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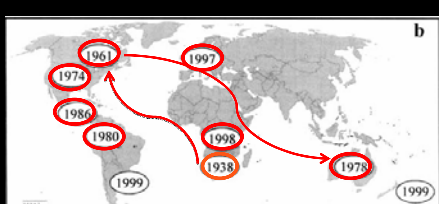

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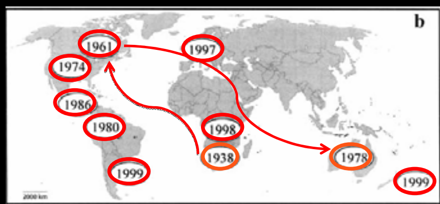

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Endemic pathogen hypothesis

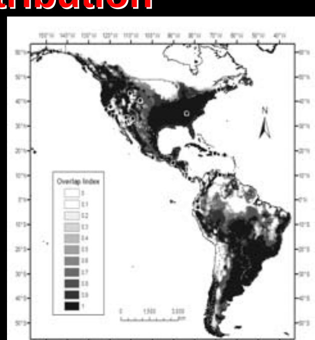
- Susceptibility of host may increase because of environmental changes
  - Inmunosuppresion (Carey 1993)
  - Temperature
  - pH
  - Moisture levels (Pounds 2006)
  - UV-B radiation (Kiesecker and Blaustein 1995)
- Antimicrobial peptides (Rollins-Smith et al., 2002)

Distribution

Bioclimatic modeling of *Bd*:

Q: Where are appropriate habitats?

A: It can live everywhere.



(Galiardo et al. 2004)

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

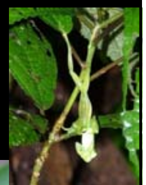
- Osmoregulatory inhibition (suspected)
  - Decreased water uptake; altered electrolyte/solute levels
- Toxic byproducts no (?)

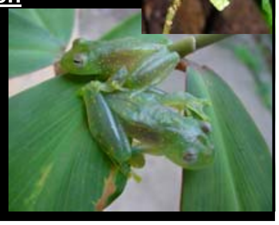
Clinical signs: in field

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

Local Transmission

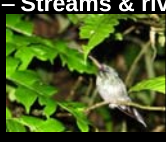
- Direct transmission
  - Frog-frog contact (Adults, Larvae)
  - Amplexus
  - Territoriality
- Environmental transmission
  - Adults - Spatial or temporal overlap of species
  - Larvae – shared aquatic environment
  - Water-facilitated transport



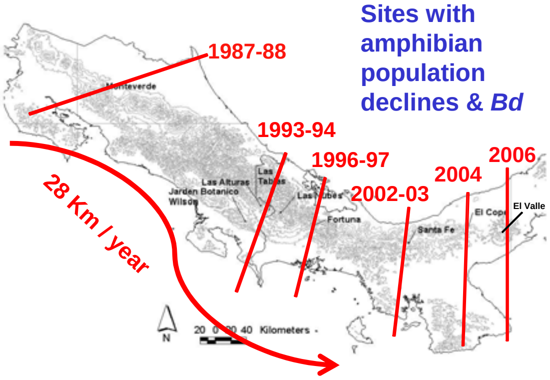


Geographic transmission

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

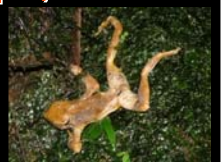


Sites with amphibian population declines & *Bd*



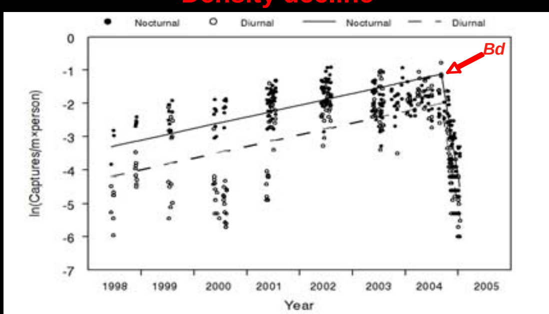
Lips at al. 2006

Case study: El Cope, Panama



- <u>Die-off October 2004</u>
- 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

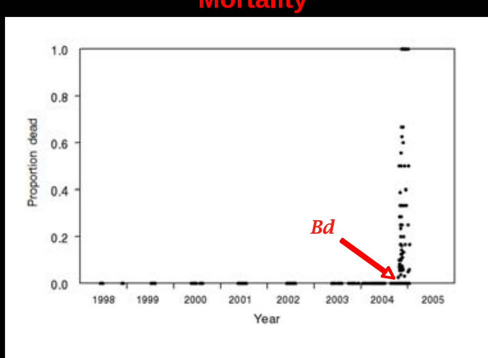
Density decline



t=-24.44, df=486, *P*<0.0001 Sept. 4 (1-6)

Lips *et al.* 2006

Mortality



Lips *et al.* 2006

Species capture rates through Dec. 2005

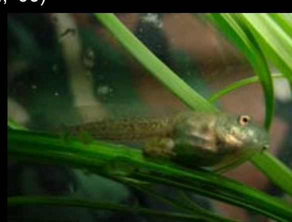
Species	min - max loss (%)	Gone by
Colostethus panamensis	100 - 100	2004
Eleuth. punctariolus	100 - 100	2004
Rana warszewitschii	100 - 100	2005
Phyllomedusa lemur	100 - 100	2005
E. megacephalus	100 - 100	2005
Bufo haematiticus	100 - 100	2005
E. gollmeri	100 - 100	2005
E. bufoniformis	100 - 100	2005
E. talamancae	99.0 - 99.5	NA
C. euknemos	94.5 - 99.3	??
C. prosoblepon	98.8 - 99.2	??
E. pardalis	96.9 - 99.1	??
C. ilex	96.5 - 98.0	NA
E. crassidigitus	95.2 - 97.8	??
E. diastema	90.7 - 97.7	??
Atelopus zeteki	88.6 - 96.8	??
Bolitoglossa colonnea	89.2 - 95.4	??
B. schizodactyla	70.0 - 90.4	??

Gone by 2005

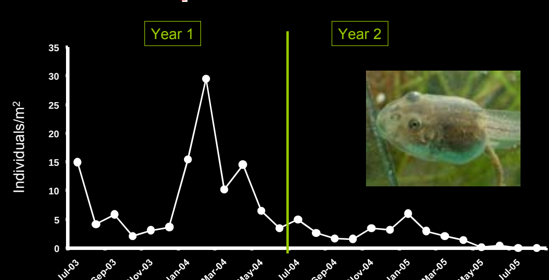
Gone by 2006

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



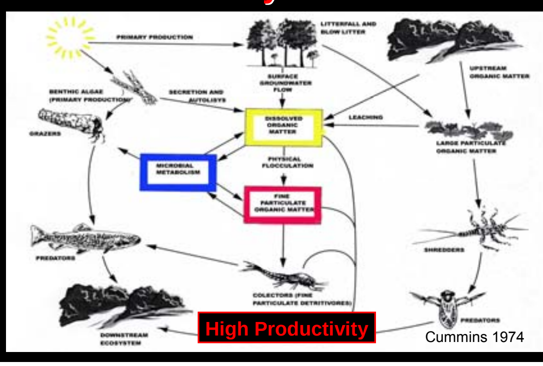
What are the ecological repercussions of amphibian losses?

Potential impacts

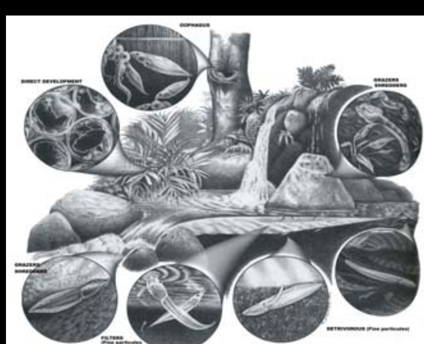
- Energy transfer (Pough '80, Regester et al. '06)
- Loss of biomass (Burton and Likens '75; Stewart and Woolbright '96)
- Nutrient cycling (Seale '80; Beard et al. '02)
- Leaf litter decomposition (Wyman '98)
- N and P cycling (Seale '80)
- Macroinvertebrate communities (Ranvestal et al. '04)

All studies in adults or temperate zones

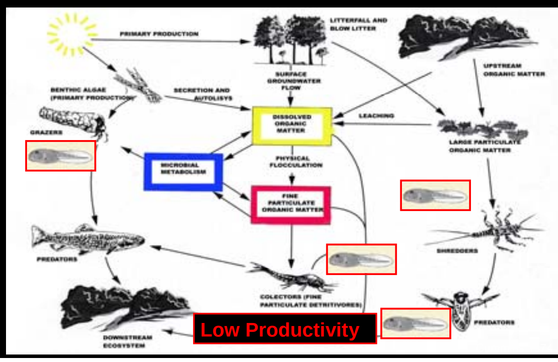
Classical headwater stream ecosystem



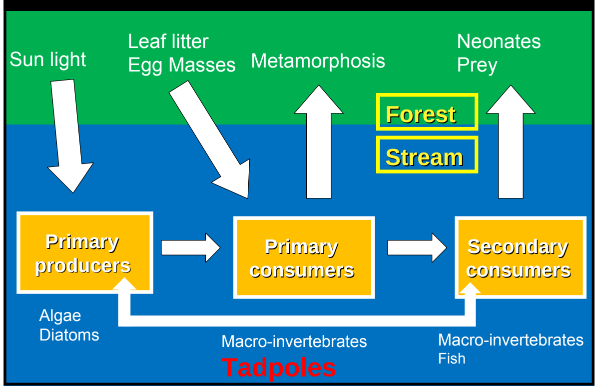
Multiple functional roles of tadpoles



Tropical headwater stream ecosystem



Energy flow in tropical systems



Tadpoles role in ecosystems

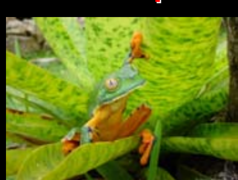
- Energetic contribution year-round by different functional groups
  - Nutrient cycling (Seale '80)
  - FPOM production (Colon et al., *in press*)
- Energy transfers to riparian environments (Polis et al. '04, Regester '05)
  - Larvae abundant in dry season
  - Adults abundant in wet season (Brenes and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

Tropical Amphibian Declines in Streams (TADS)

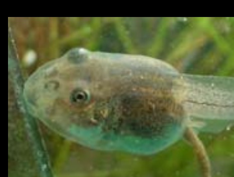
- Post-extirpation effects on:
  - Primary producers (Connelly & Pringle)
  - Amphibian larval and adult biomass (Brenes & Lips)
  - Amphibian predators (Lips & Montgomery)
  - Macroinvertebrate communities (Colon-Gaud & Whiles)
  - Food webs (Hunte & Kilham))

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

Amphibian community



76 species of amphibians
3 Cecilians
6 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/m^2) (Ravenstel et al. 2004)

Tadpole Community

Colostethus flotator

Colostethus panamensis
Colostethus nubicola } Filterers
Hyla palmeri

Hyla colymba
Rana Warszewitschii } Grazers
Centrolene ilex

Centrolene prosoblepon
Cochranella granulosa
Cochranella albomaculata
Hyalinobatrachium colymbiphyllum } Collector-gatherers

Measuring energy flow

A. Species composition and densities

B. Growth rates

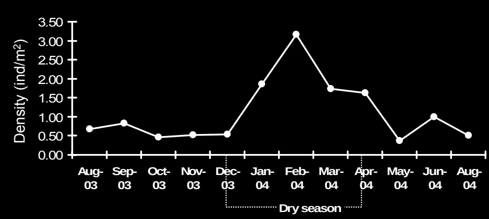
C. Biomass

D. Production

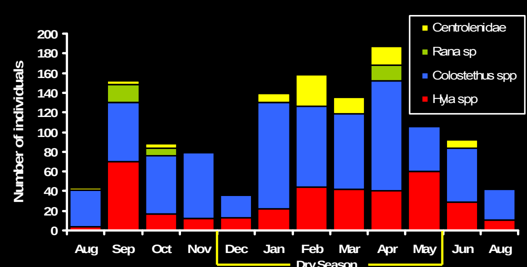
Tadpole density and abundance

- Four 200-m reaches; same watershed
- 4 techniques for different habitats
  - Kick nets (Riffles)
  - 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



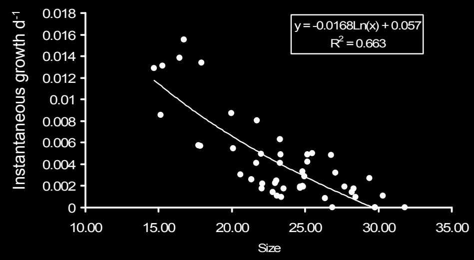
Seasonal abundance



Instantaneous growth rates

- Tadpoles grown *in situ*
- 40 plexiglass chambers
- In stream for 4-6 weeks
- Size specific instantaneous growth rates obtained: $\ln(w_f / w_i)$

Instantaneous growth rate

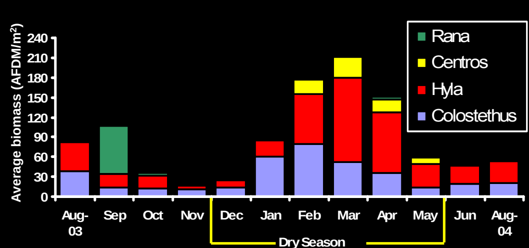


Group / Taxa	Small	med	large	
Colostethus spp	0.004169	0.005972	0.000215	← Fast
Hyla spp	0.018845	0.005728	0.000578	← Slow
Rana sp	0.01417	0.008196	0.001079	
Centrolenidae	0.00712	0.00352	0.00237	

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



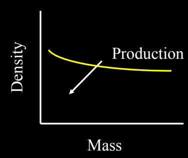
Calculating secondary production

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

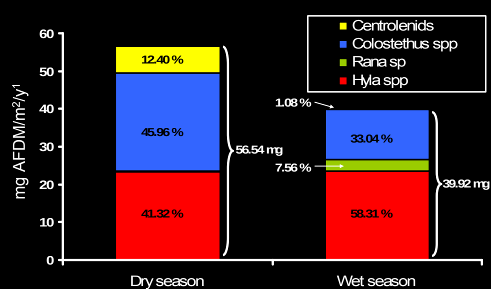
Biomass   Growth rate

$$P = \Sigma(\bar{B})(G) \quad \text{where } G = \ln \frac{W_{t+1}}{W_t}$$

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

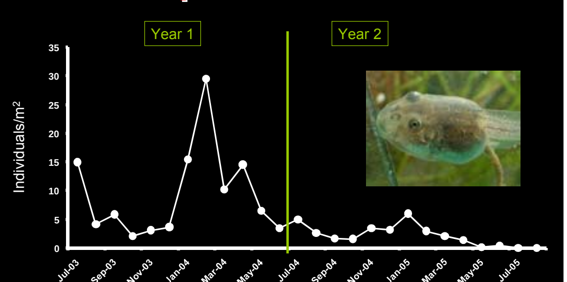


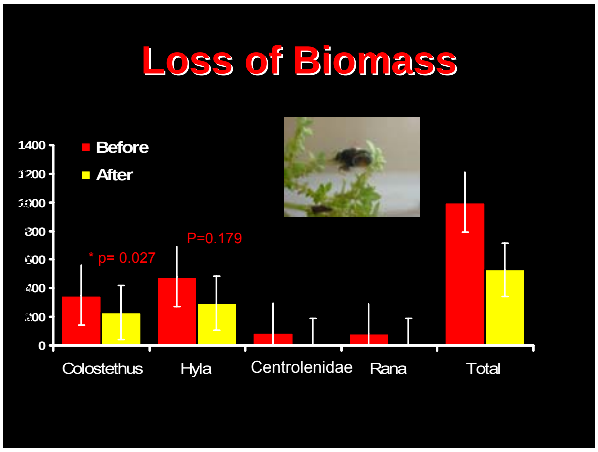
Production by Species

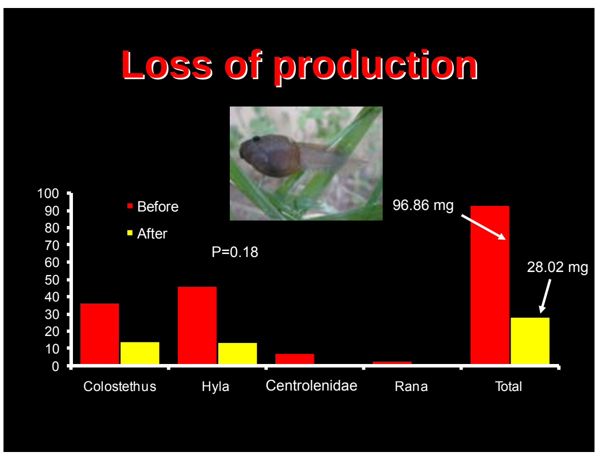


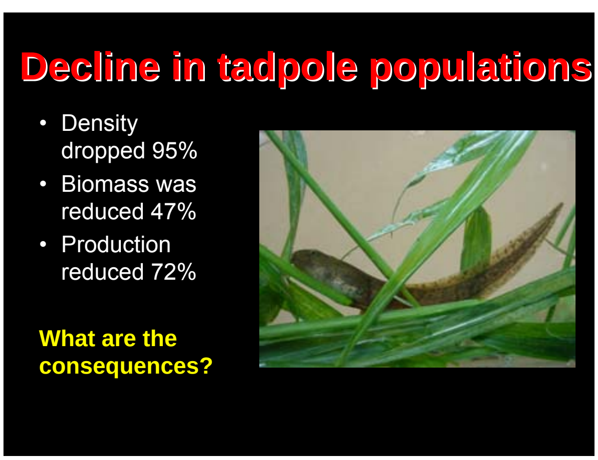
Total Annual Production= 96.86 mg AFDM m^{-2}y^{-1}

Tadpole declines

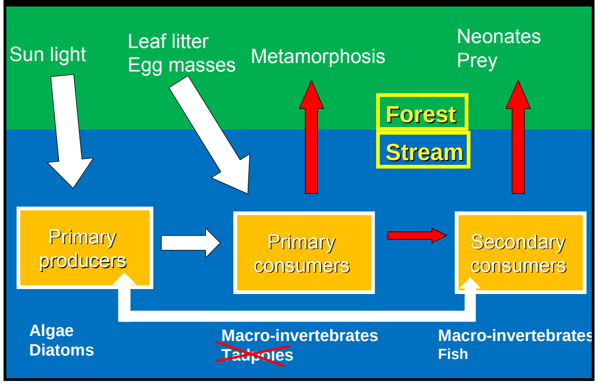



Loss of Biomass
1400
0
Before
After
* p= 0.027
P=0.179
Colostethus
Hyla
Centrolenidae
Rana
Total

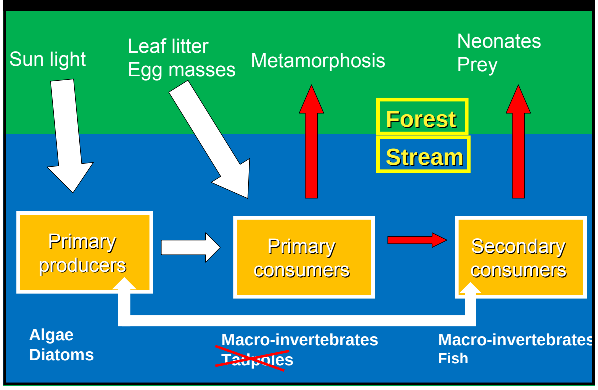

Loss of production
100
90
80
70
60
50
40
30
20
10
0
Before
After
P=0.18
96.86 mg
28.02 mg
Colostethus
Hyla
Centrolenidae
Rana
Total


Decline in tadpole populations
• Density dropped 95%
• Biomass was reduced 47%
• Production reduced 72%
What are the consequences?

How might energy flow change?



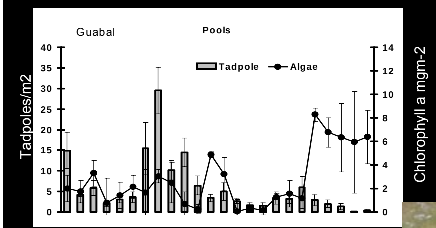
How are other groups affected ?



What is going to happened in the stream

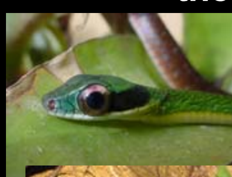
- Loss of production will be compensated by other groups?
- Increase in densities of macro-invertebrates?
- Excess of primary production will be wasted?
- Overall energy budget of stream will be diminish?

Algae Bloom

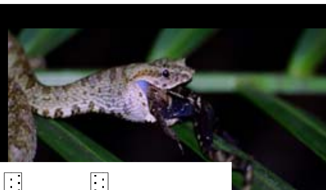


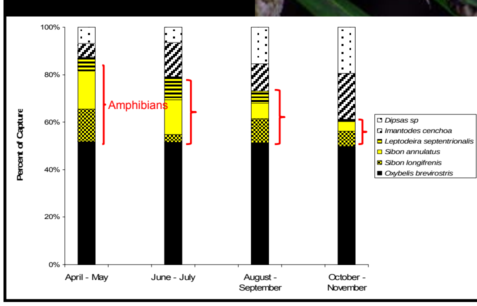
Connelly et al. *in press*

What is going to happened 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 ?

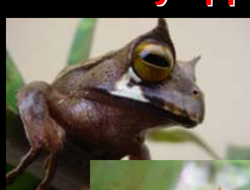
<u>**Many practical, ethical, & legal complications:**</u>

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?

Acknowledgements

- **Funding and Logistical**
  - National Science Foundation (DEB #0001615, 0234386)
  - Smithsonian Tropical Research Institute, Republic of Panama
  - Autoridad Nacional del Ambiente (ANAM)
  - Parque Nacional Omar Torrijos Herrera
- **Data collection and comments**
  - Sofia Rodríguez (University of Costa Rica)
  - “Tony” Frazier (SIU)
  - Scott Connelly (University of Georgia)
  - Matt Whiles (SIU)
  - Jose “Checo” Colon-Gaud (SIU)
  - SIU herpetology laboratory
  - Cy Mott (SIU)
  - Mason Ryan