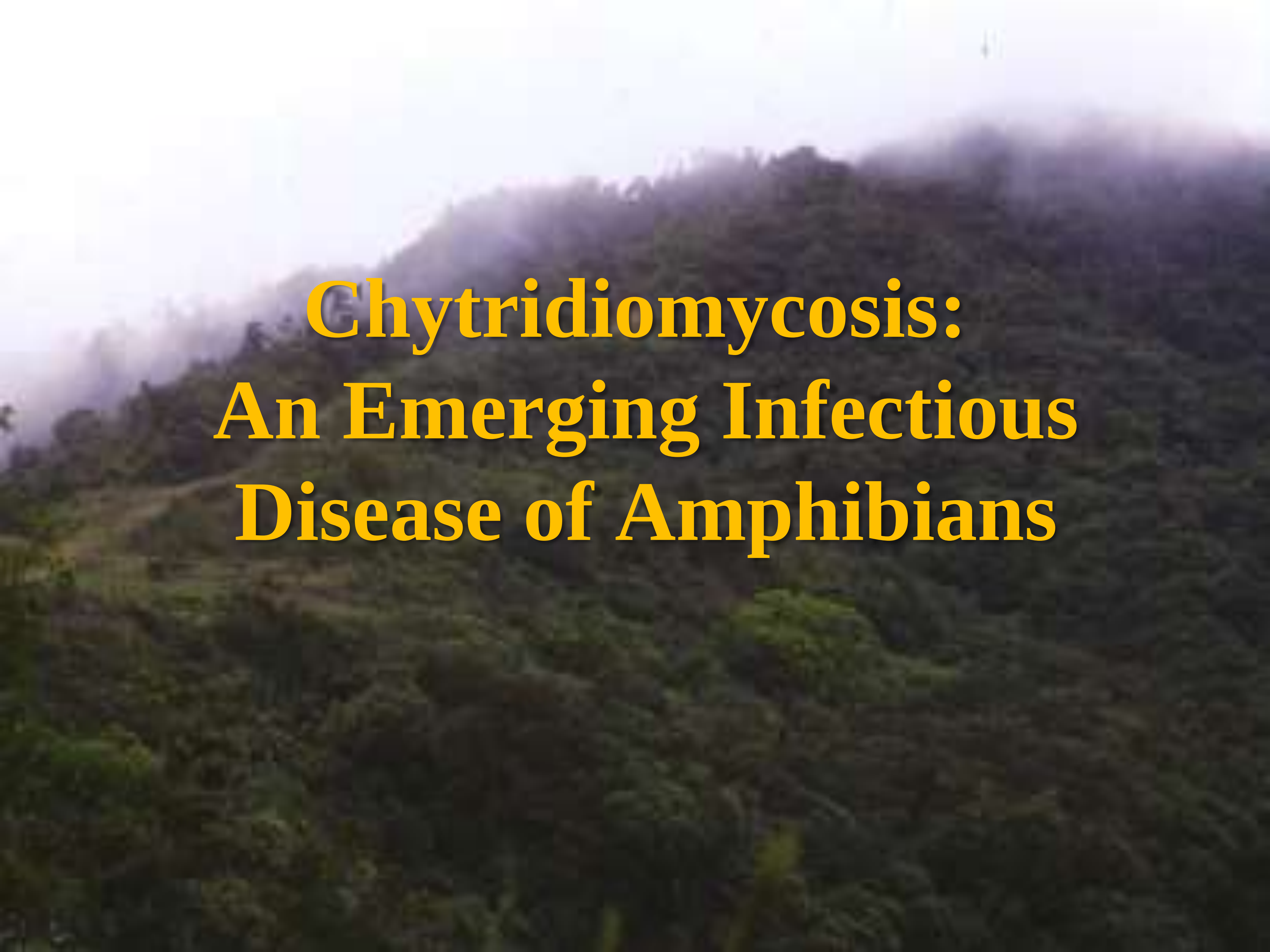


Chytridiomycosis: An Emerging Infectious Disease of Amphibians

Road map

- What is Chytridiomycosis
- Signs of disease
- Origins of pathogen
- Case study “El Cope panama”
- Current efforts
- Future efforts

The problem

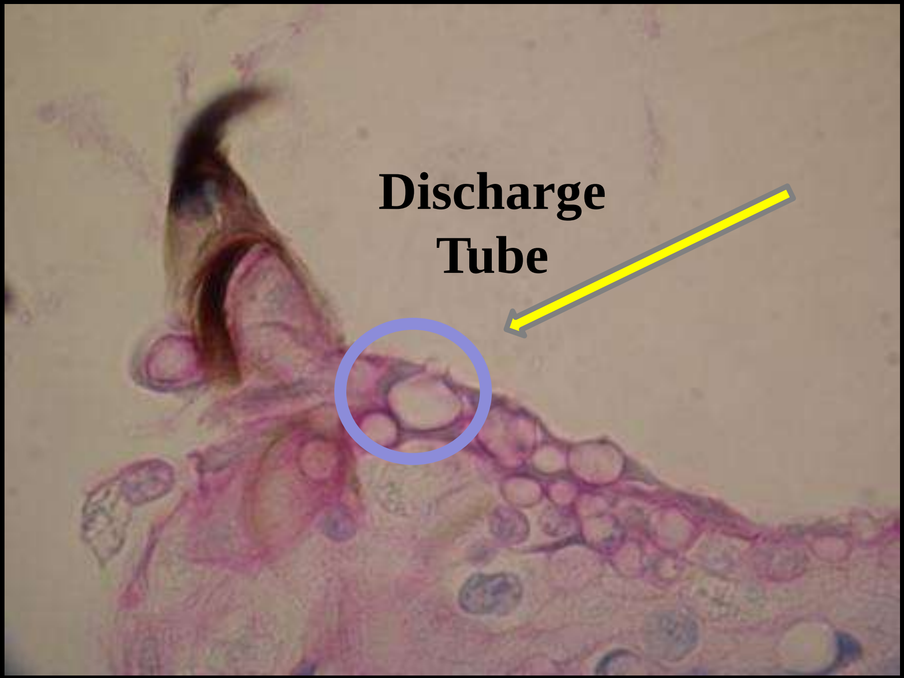
- Amphibian population declines
- Many proposed causes of amphibian declines
- Most noticeable 1980s-present
- Greatest concern = “enigmatic” or mysterious declines
  - Remote, protected areas
  - No obvious causes
  - Sudden massive die-offs, lack of recovery
- Many enigmatic declines attributed to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

The pathogen

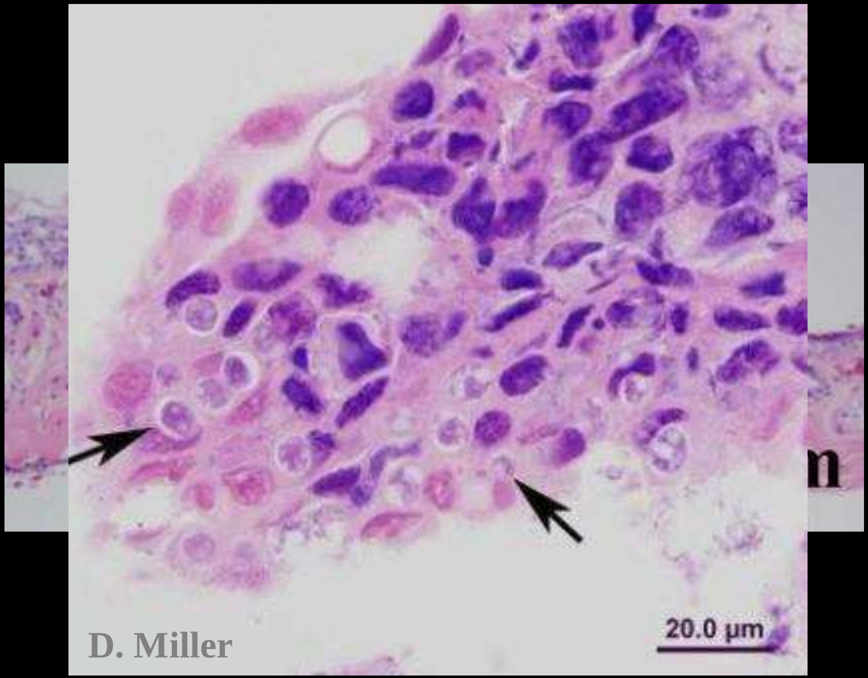
- ***Batrachochytrium dendrobatidis* (*Bd*):**
  - **Emerging infectious disease of amphibians**
  - **First chytrid fungus pathogenic to vertebrates**
- **Infect keratinized tissue**
  - **Mouthparts in larvae**
  - ***Adult skin***
- **3 life stages**
  - **Zoospore – aquatic, flagellated (3-5µm)**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

Histological Signs

Epidermis



Zoosporangia



Proliferation of Epidermal Cells

Stratum Corneum

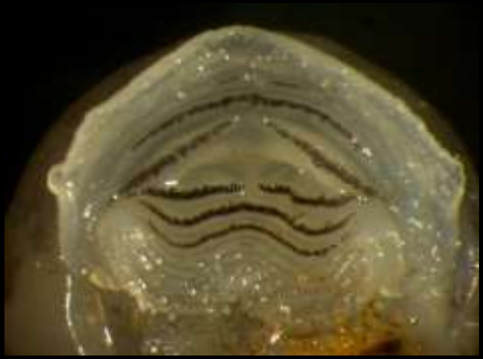
Normal Thickness: 2 – 5 µm
Infected: 60 µm

Epidermal Hyperplasia

→ **Sloughing**

Clinical signs: in field

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



Erythema of legs and ventrum

Petechiation

Cutaneous erosions and ulcerations

Loss of muscle control

Death

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

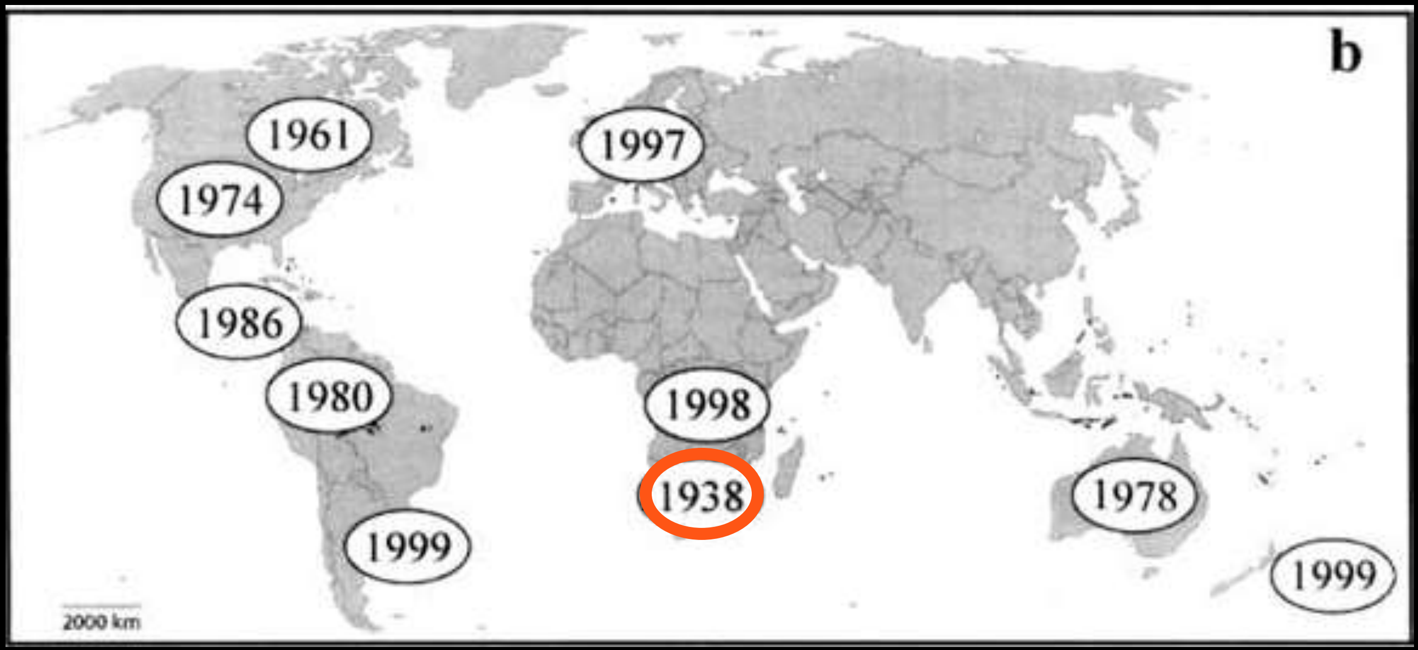
Origins

- 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)

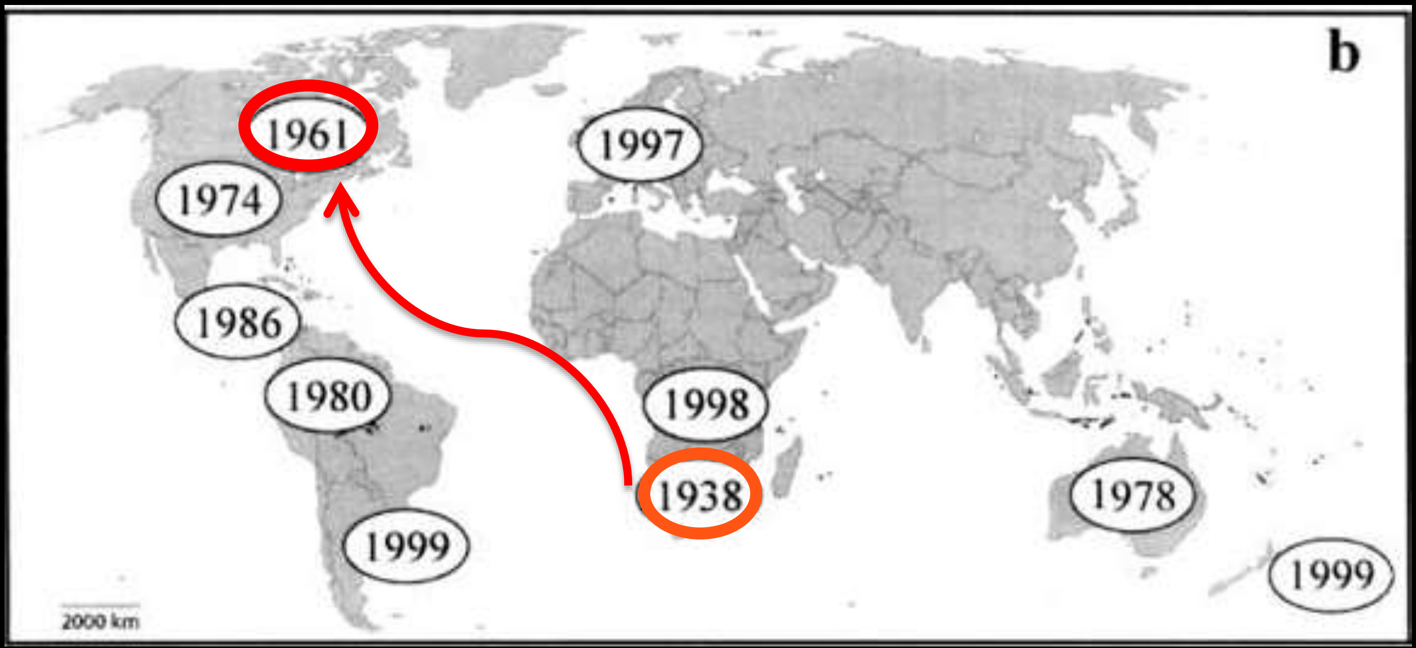
Novel pathogen hypothesis



Rachowicz et al. 2004

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

Novel pathogen hypothesis



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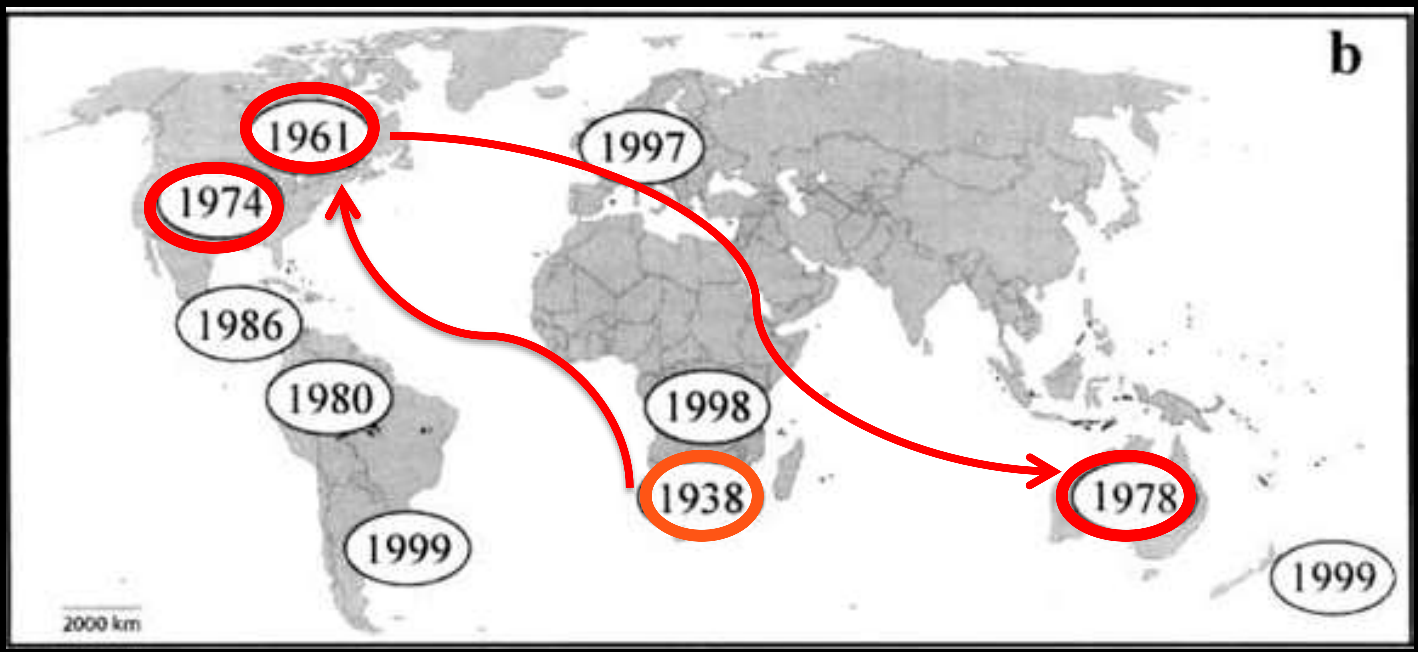
3. Rana Clamitans, Canada (1961) **23** years later

Novel pathogen hypothesis



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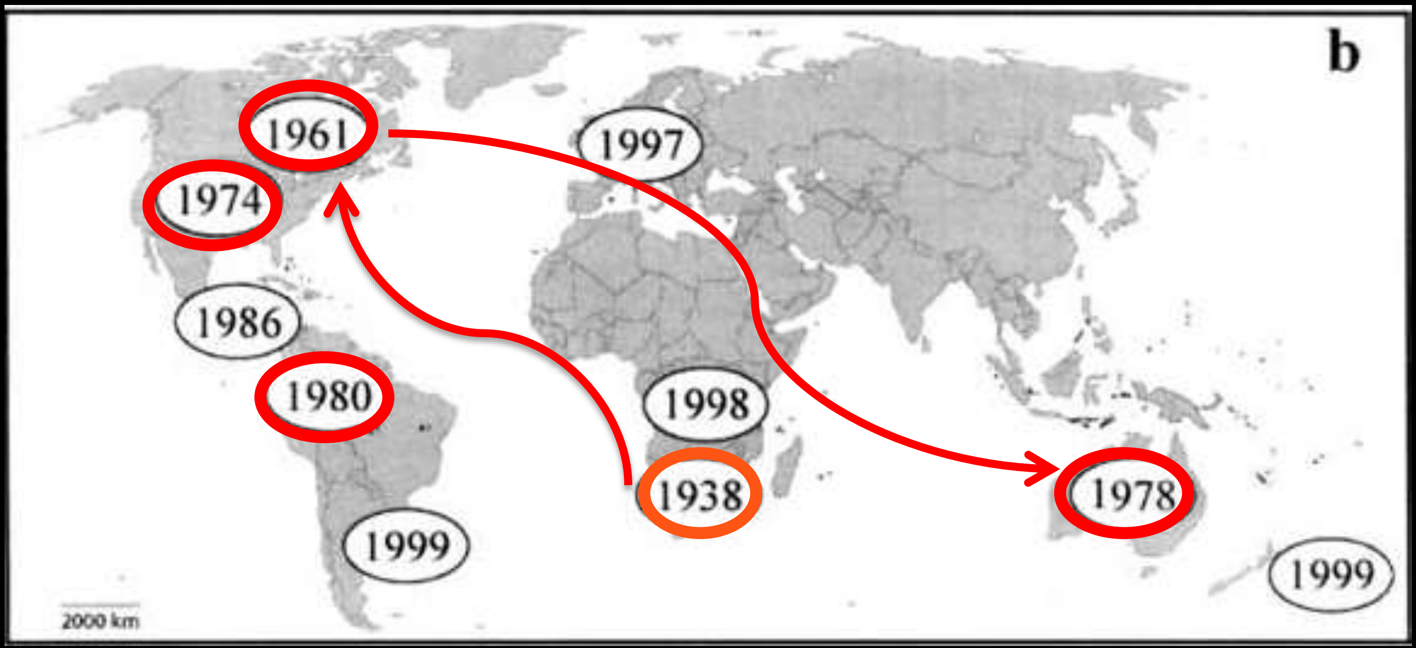
4. 1970's North America and Australia Pregnancy test

Novel pathogen hypothesis



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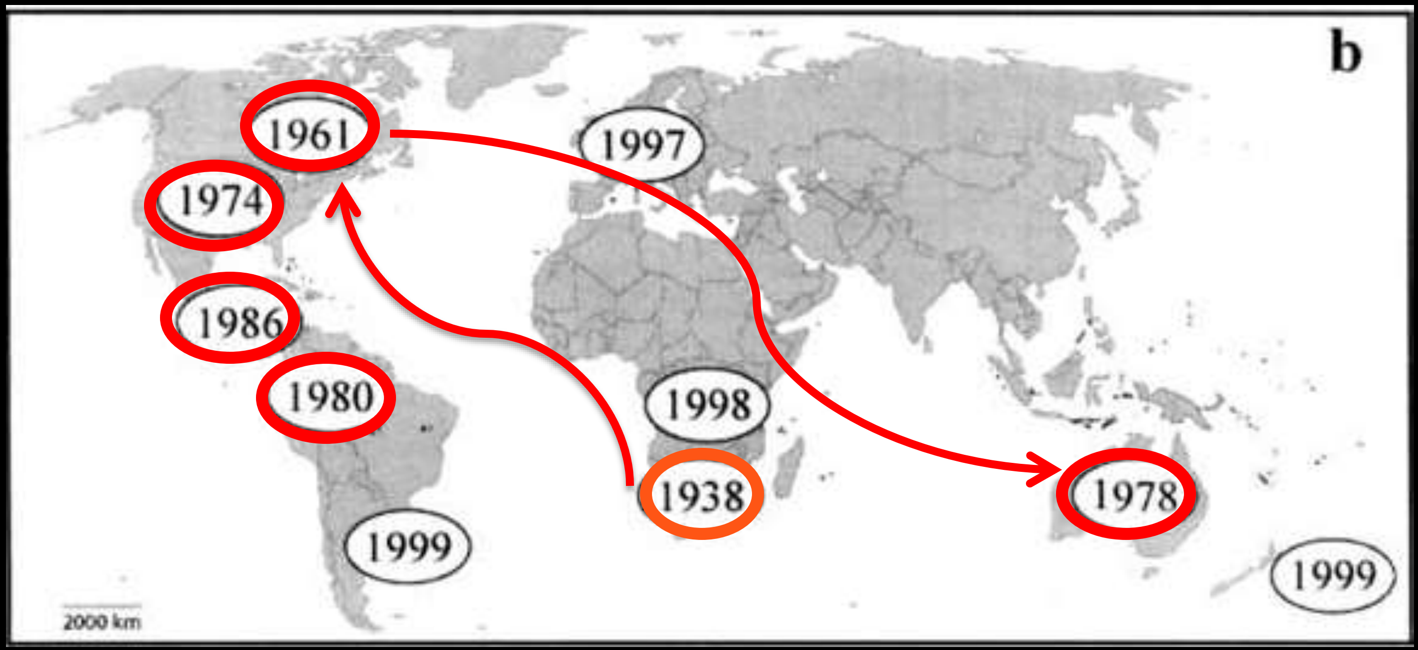
Novel pathogen hypothesis



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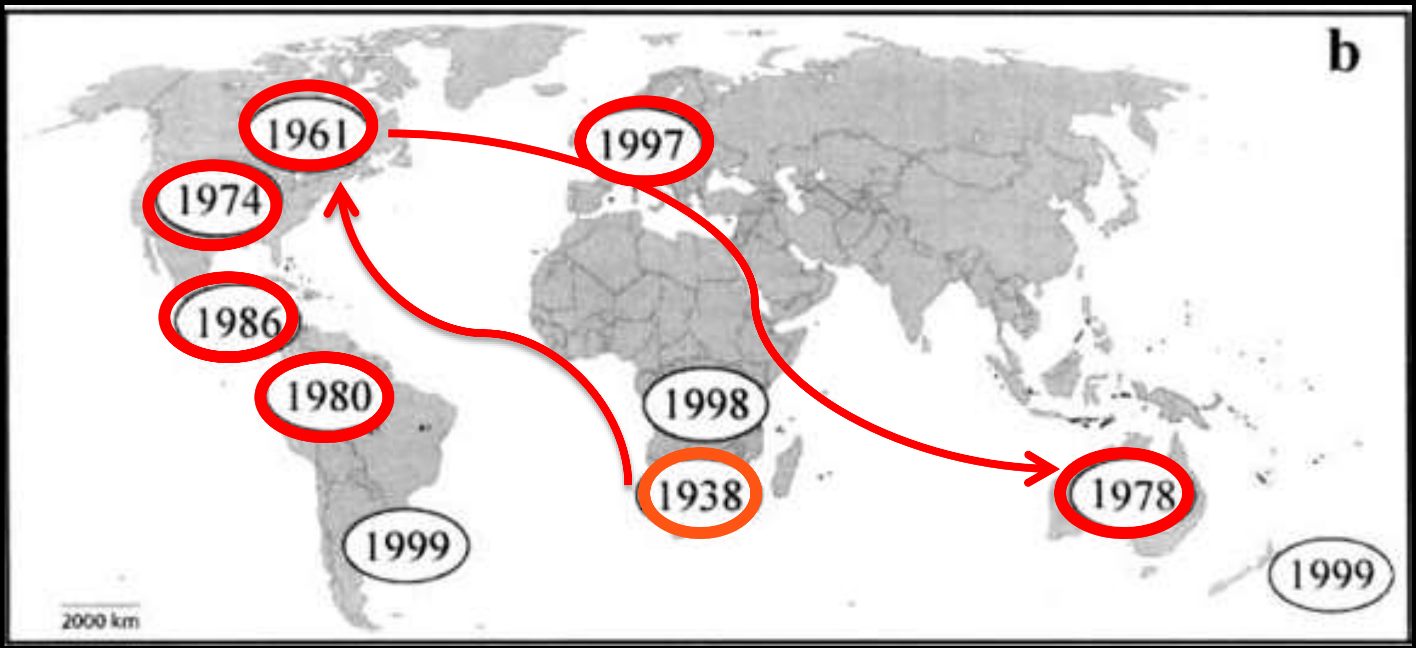

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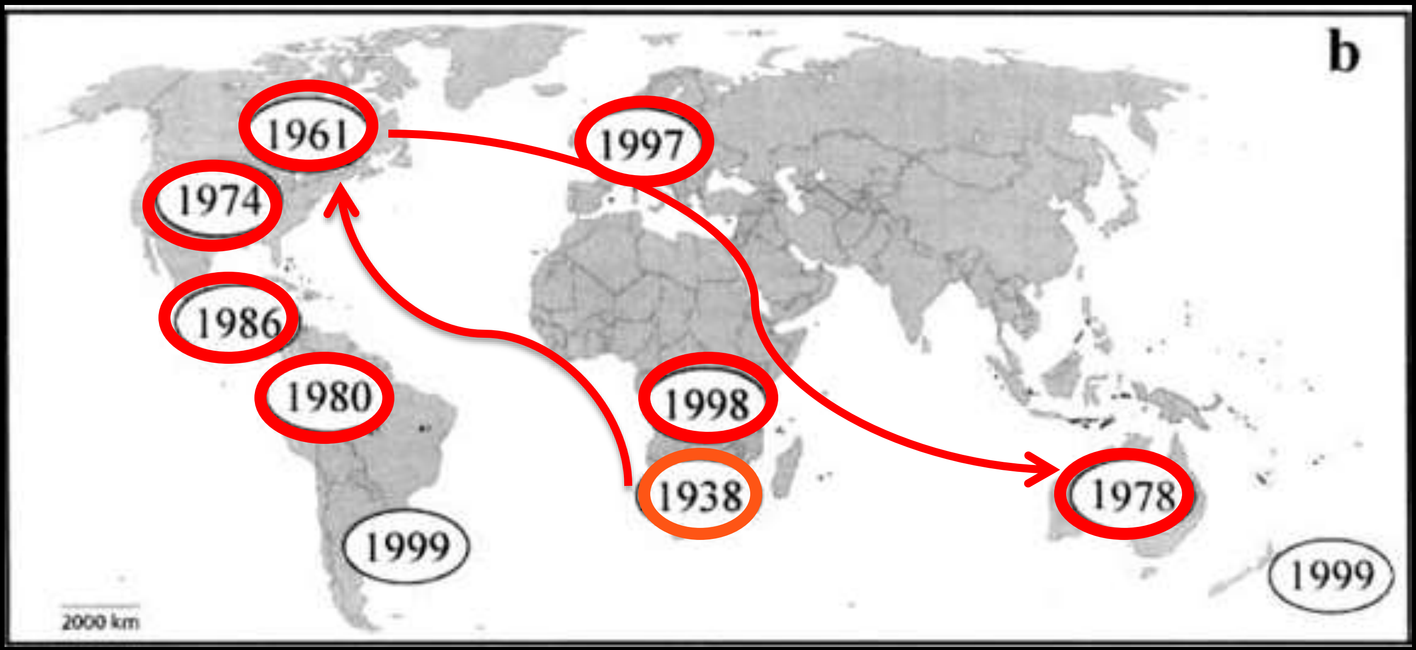

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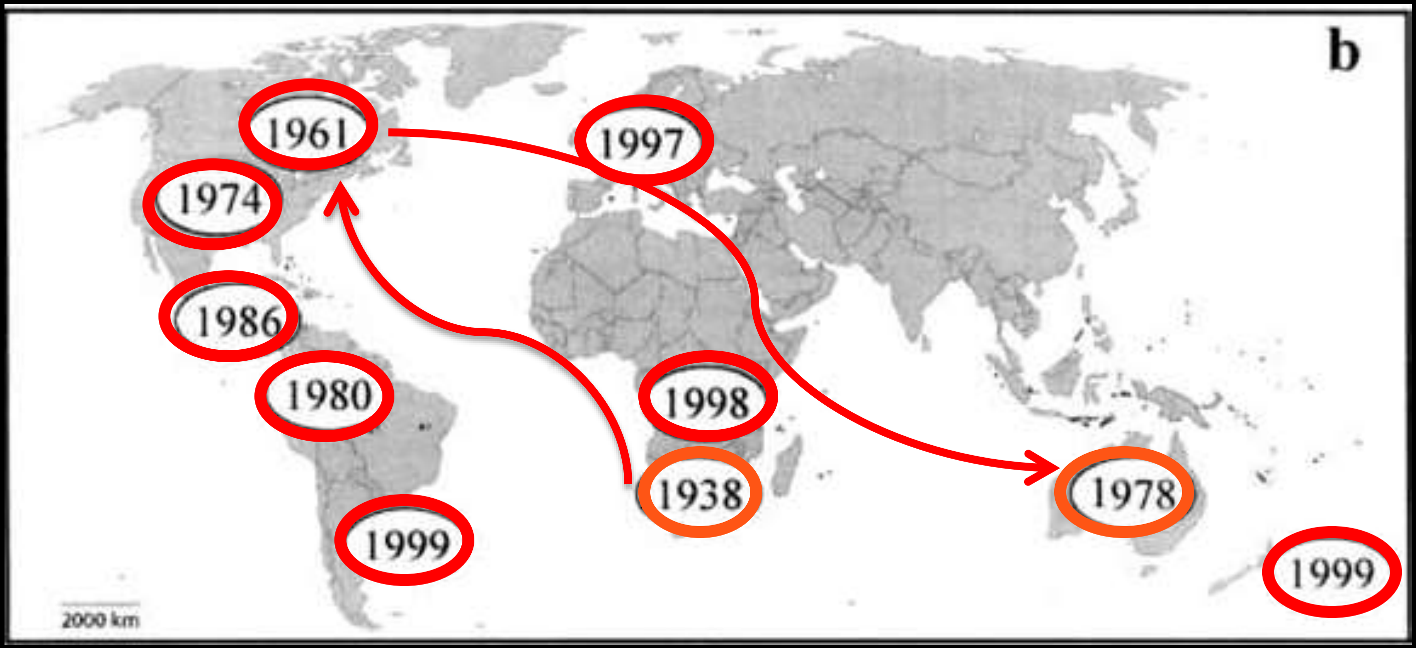

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5. Spread around the world

Endemic pathogen hypothesis

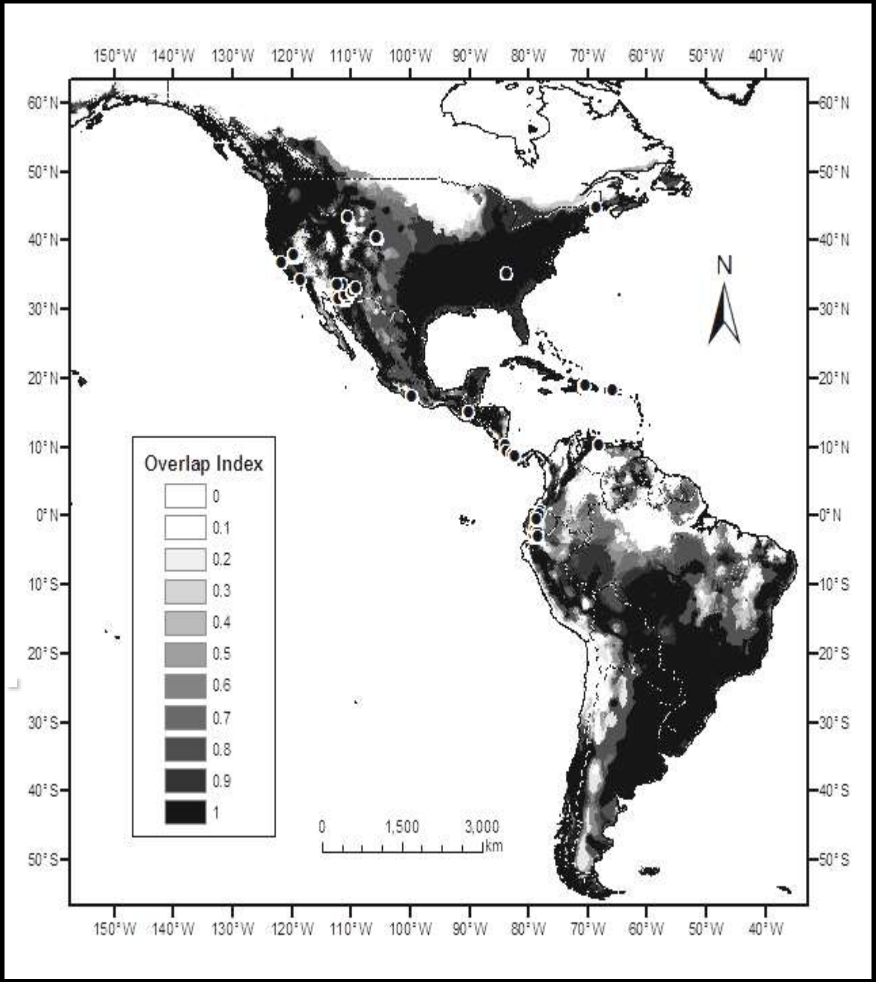
- Susceptibility of host may increase because of environmental changes
  - Inmunosuppresion (Carey 1993)
  - Temperature
  - pH
  - Moisture levels (Pounds 2006)
  - UV-B radiation (Kiesecker and Blaustein 1995)
- Antimicrobial peptides (Rollins-Smith et al., 2002)

Distribution

Bioclimatic modeling of *Bd*:

Q: Where are appropriate habitats?

A: It can live everywhere.



The victims

- <u>Population-level effects:</u>
- 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, Woodhams et al. 2011)
  - Elevate body temperatures clear infection (Woodhams et al. 2003)

Cause of Mortality

- Osmoregulatory Inhibition (suspected #1 cause)
  - **Decreased water uptake & ion exchange; altered electrolyte/solute levels** (decrease Ca ➡ actin & myosin cross-bridge cycle)

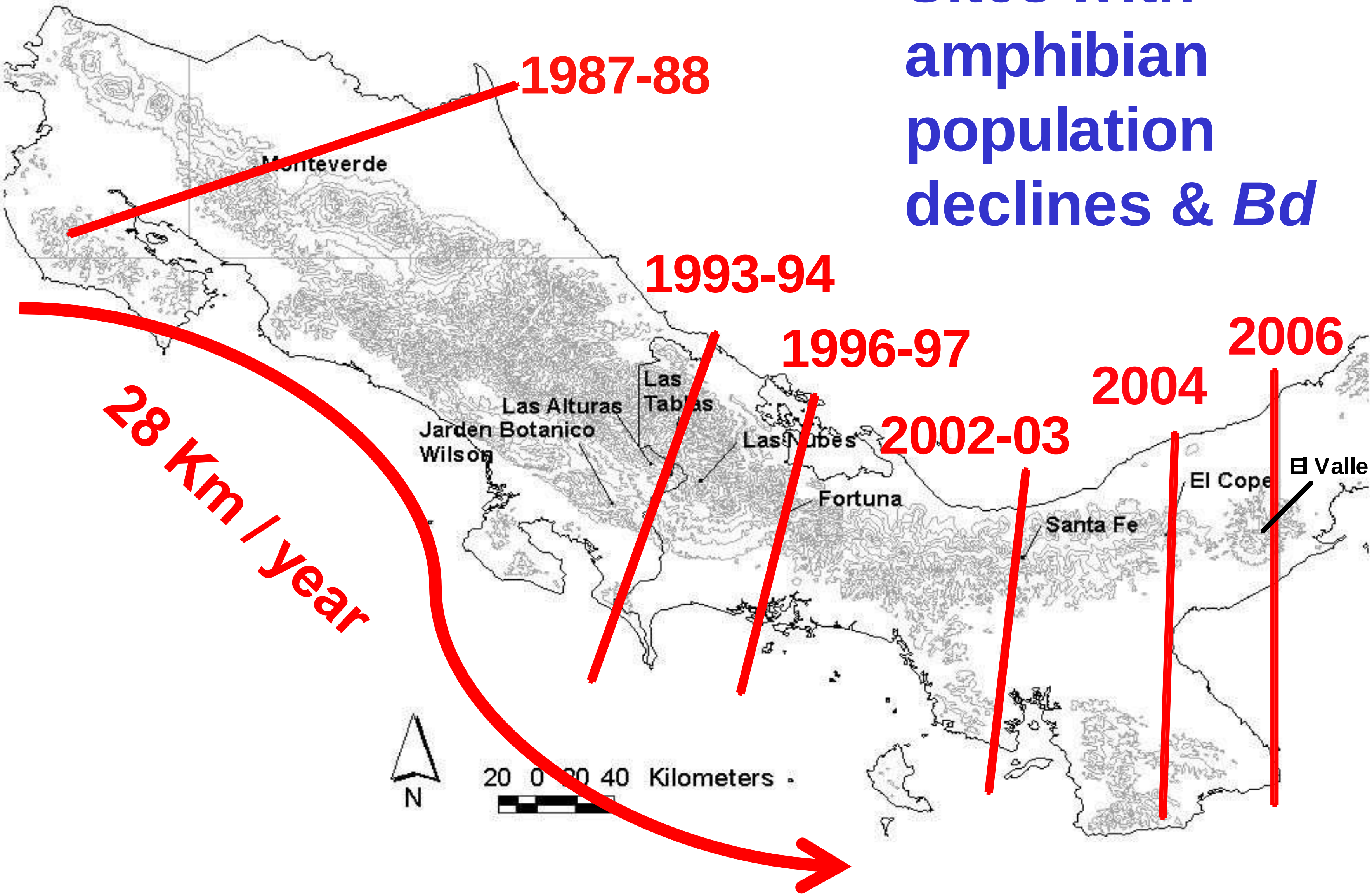
Local Transmission

- **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*

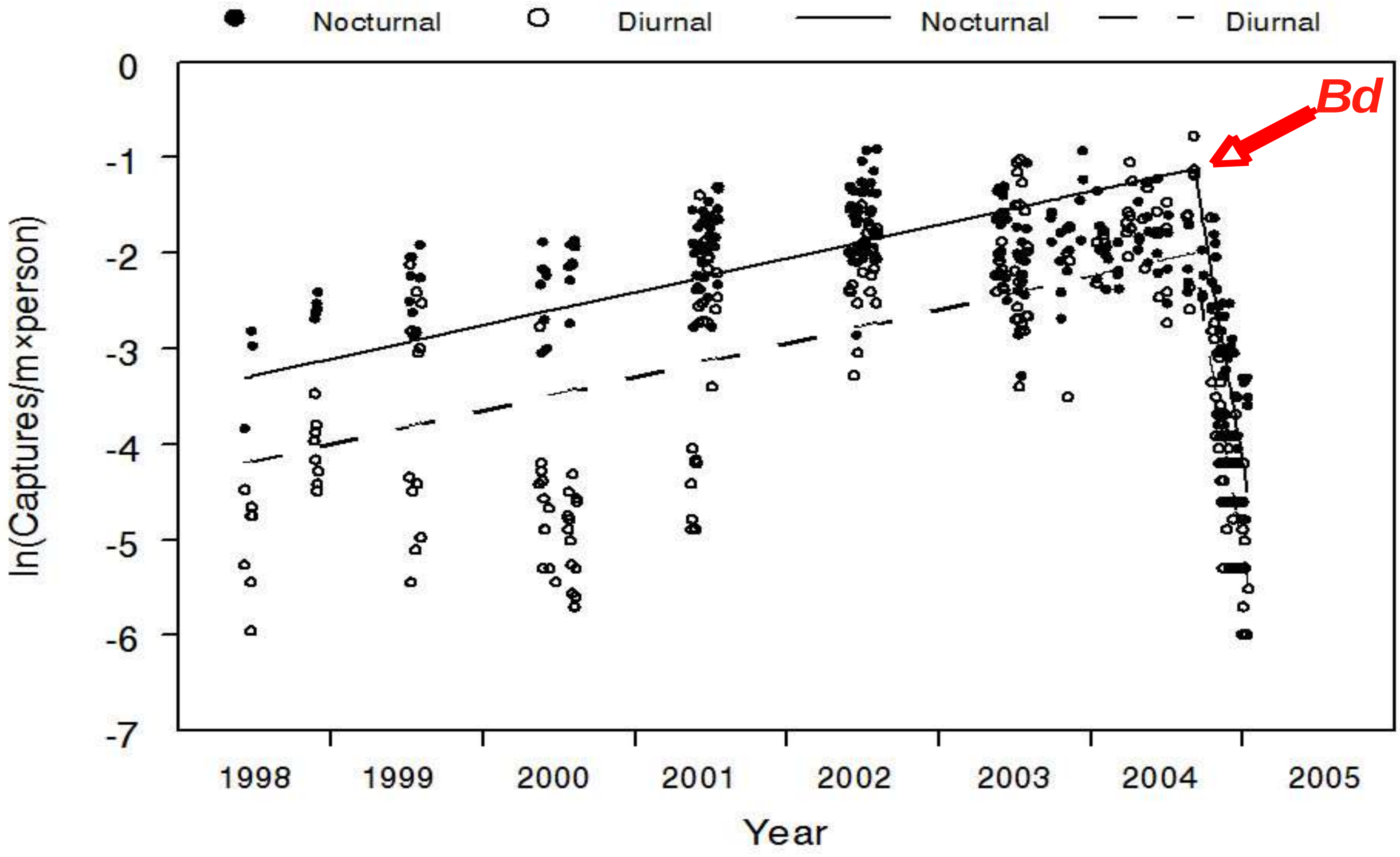


Lips at al. 2006

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**

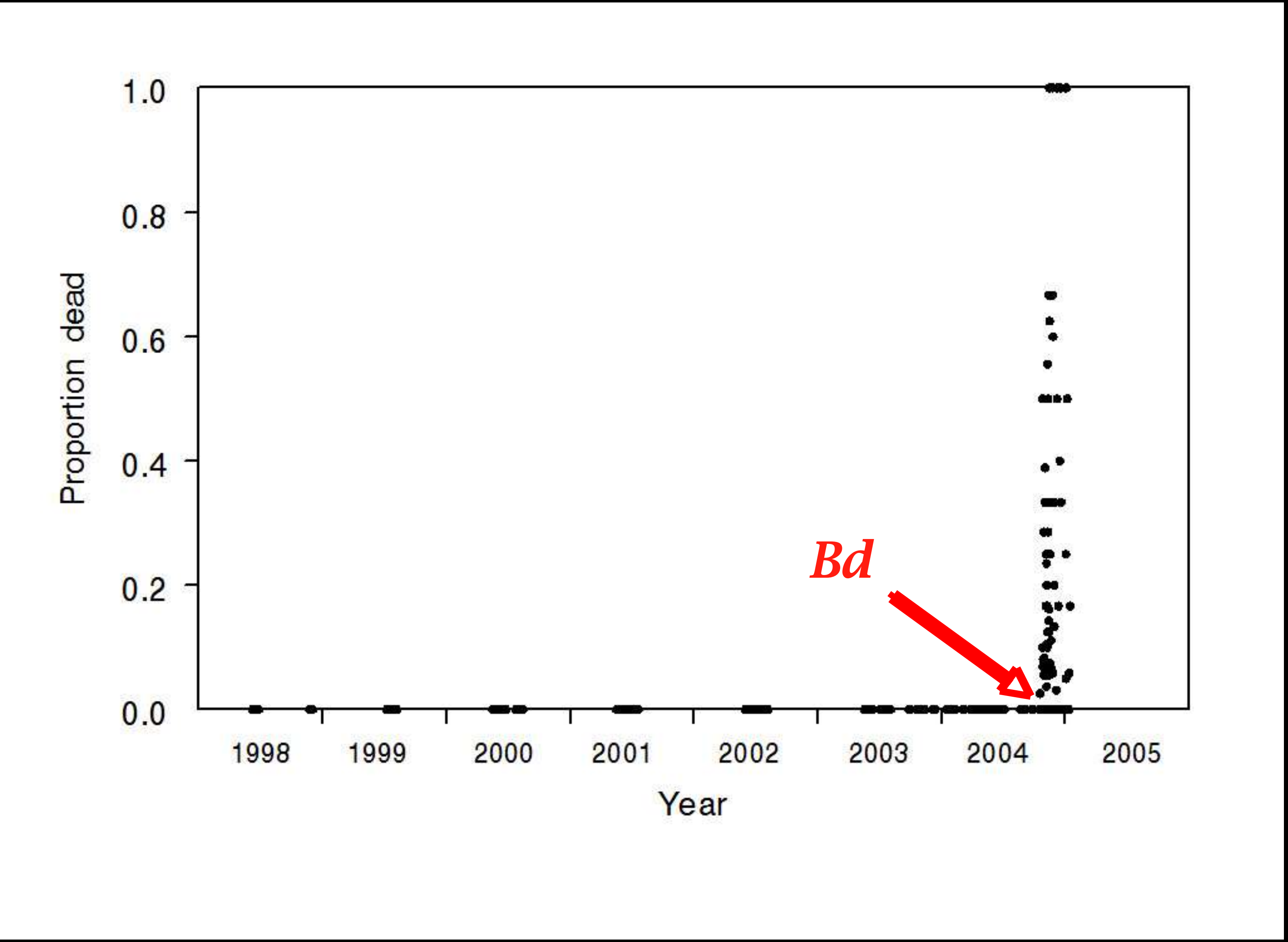
Density decline



***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	Gone by 2005
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	Gone by 2006
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	??	

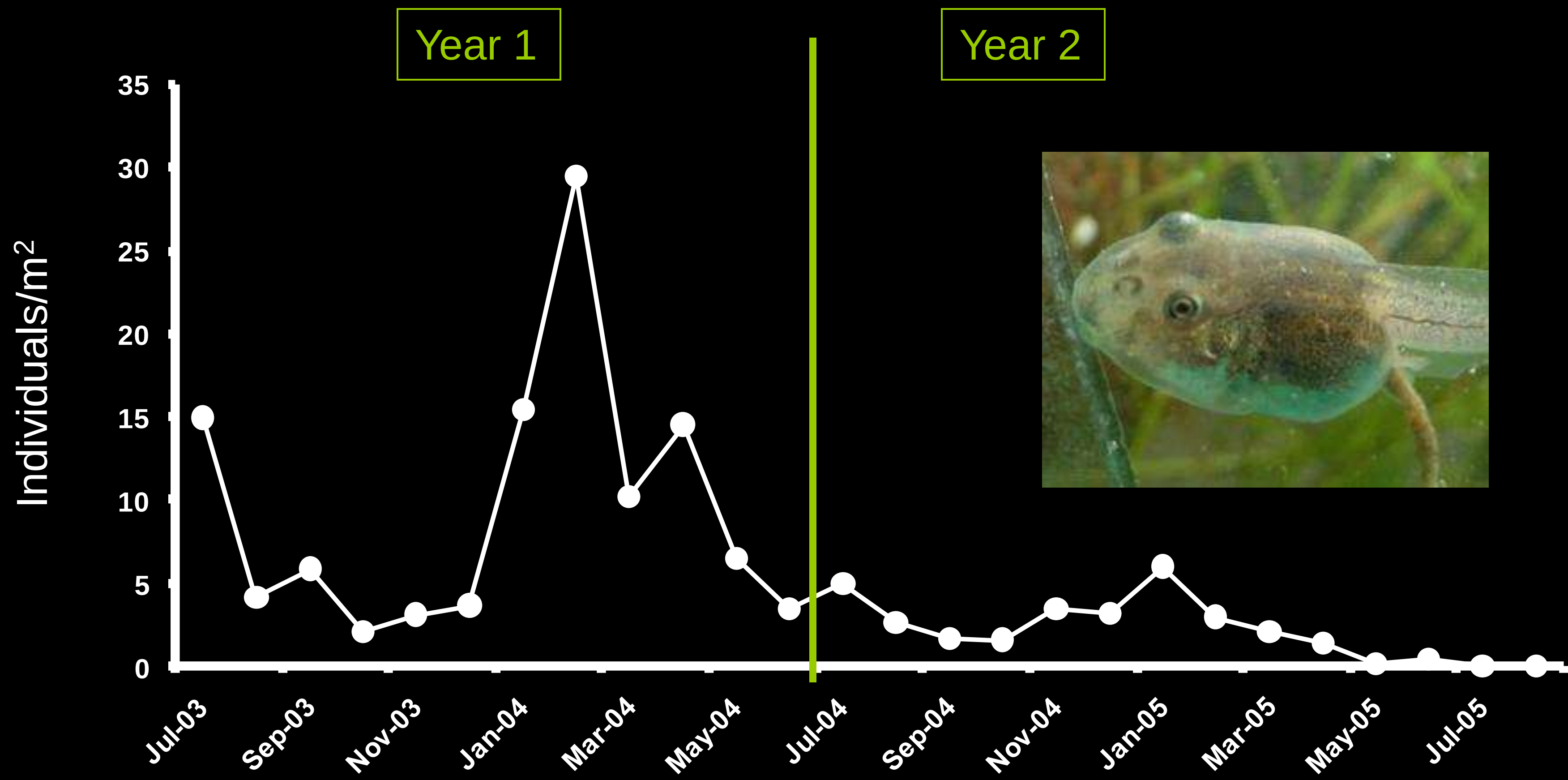
Tadpole decline

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

- Tadpole densities dropp dramatically

- What happened to Ecosystems?

Tadpole declines



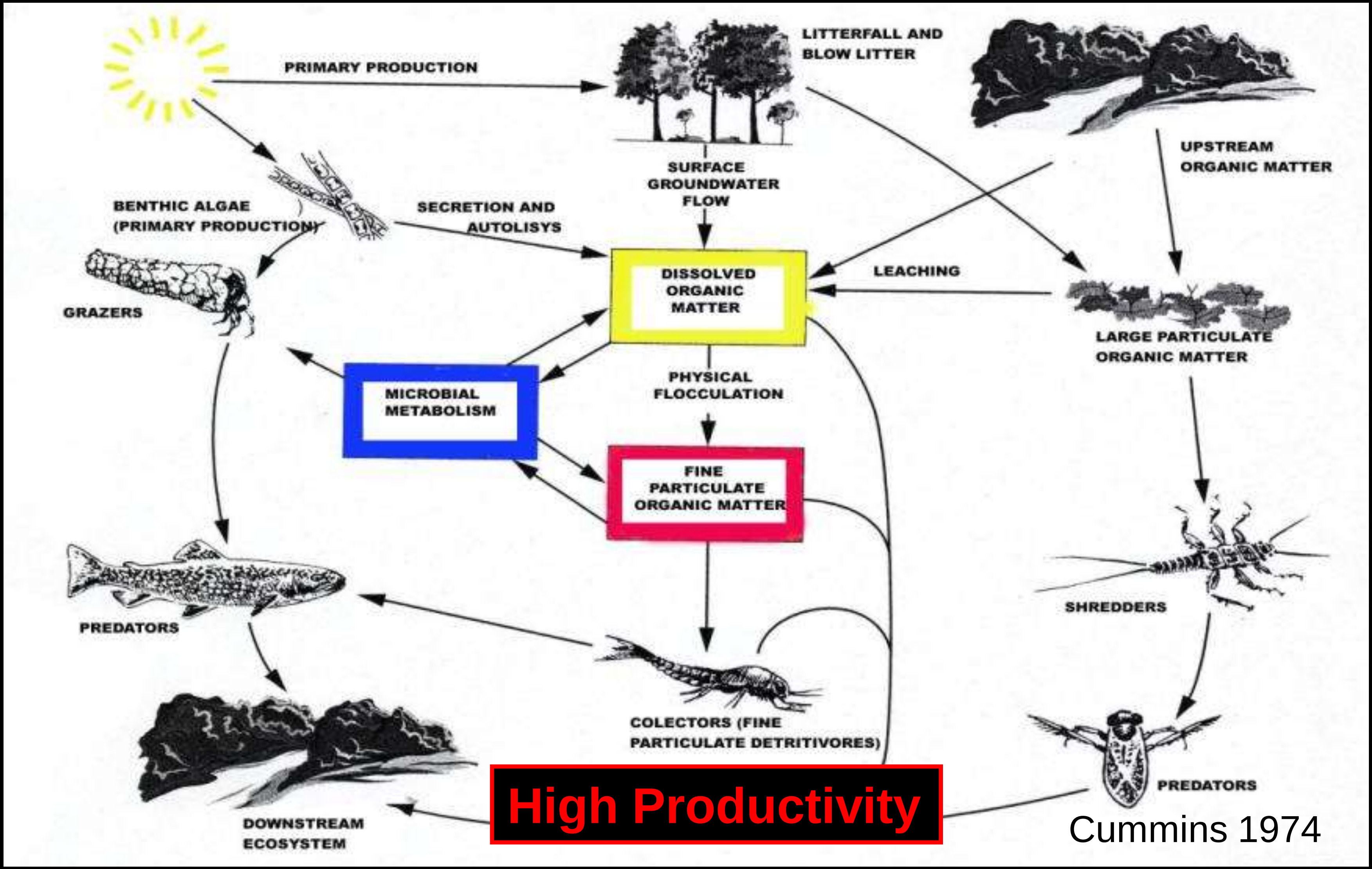
What are the ecological repercussions of amphibian losses?

Potential impacts

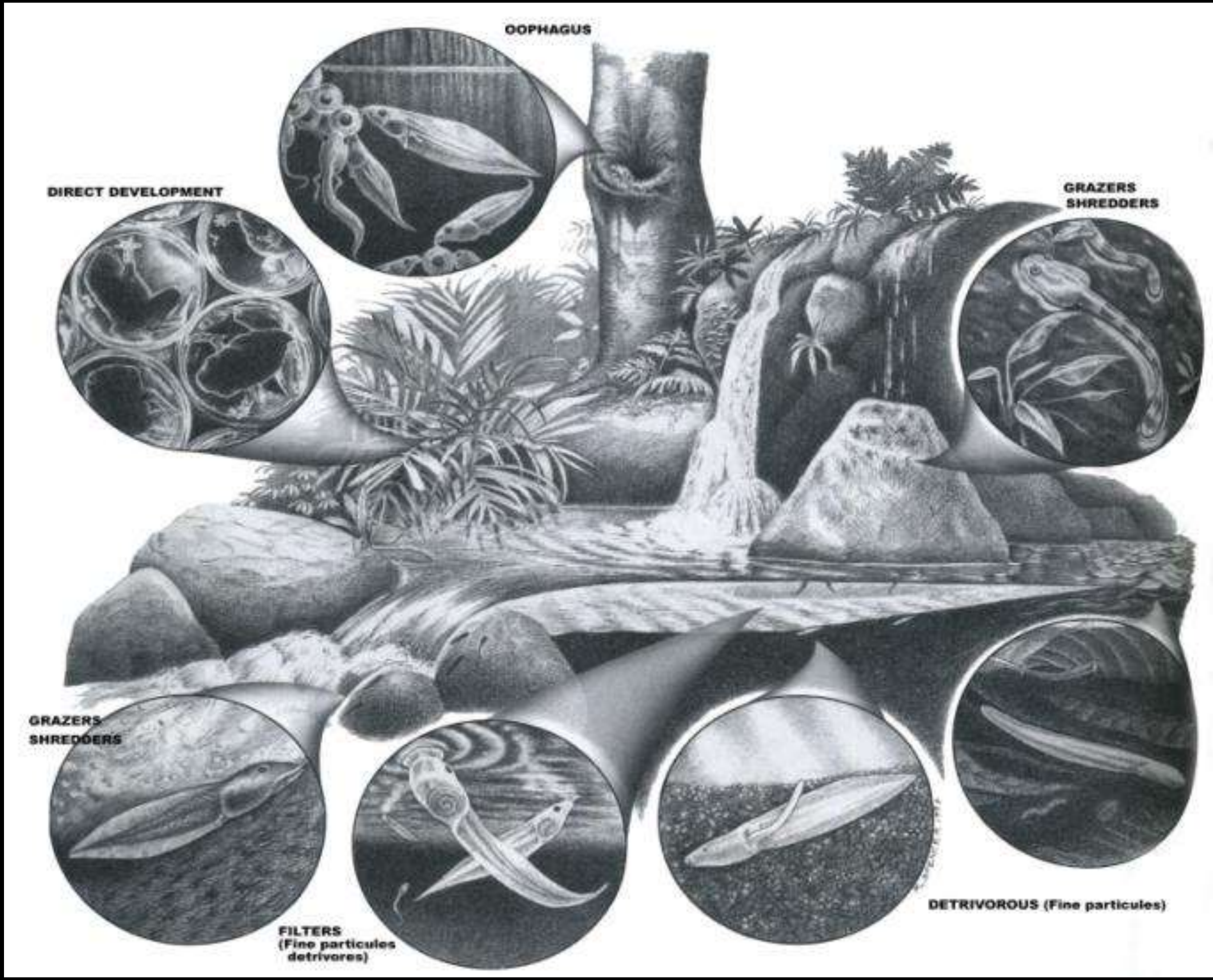
- Energy transfer (Pough ’80, Regester et al. ‘06)
- Loss of biomass (Burton and Likens ‘75; Stewart and Woolbright ‘96)
- Nutrient cycling (Seale ‘80; Beard et al. ‘02)
- Leaf litter decomposition (Wyman ‘98)
- N and P cycling (Seale ‘80)
- Macroinvertebrate communities (Ranvestal et al. ’04)

All studies in adults or temperate zones

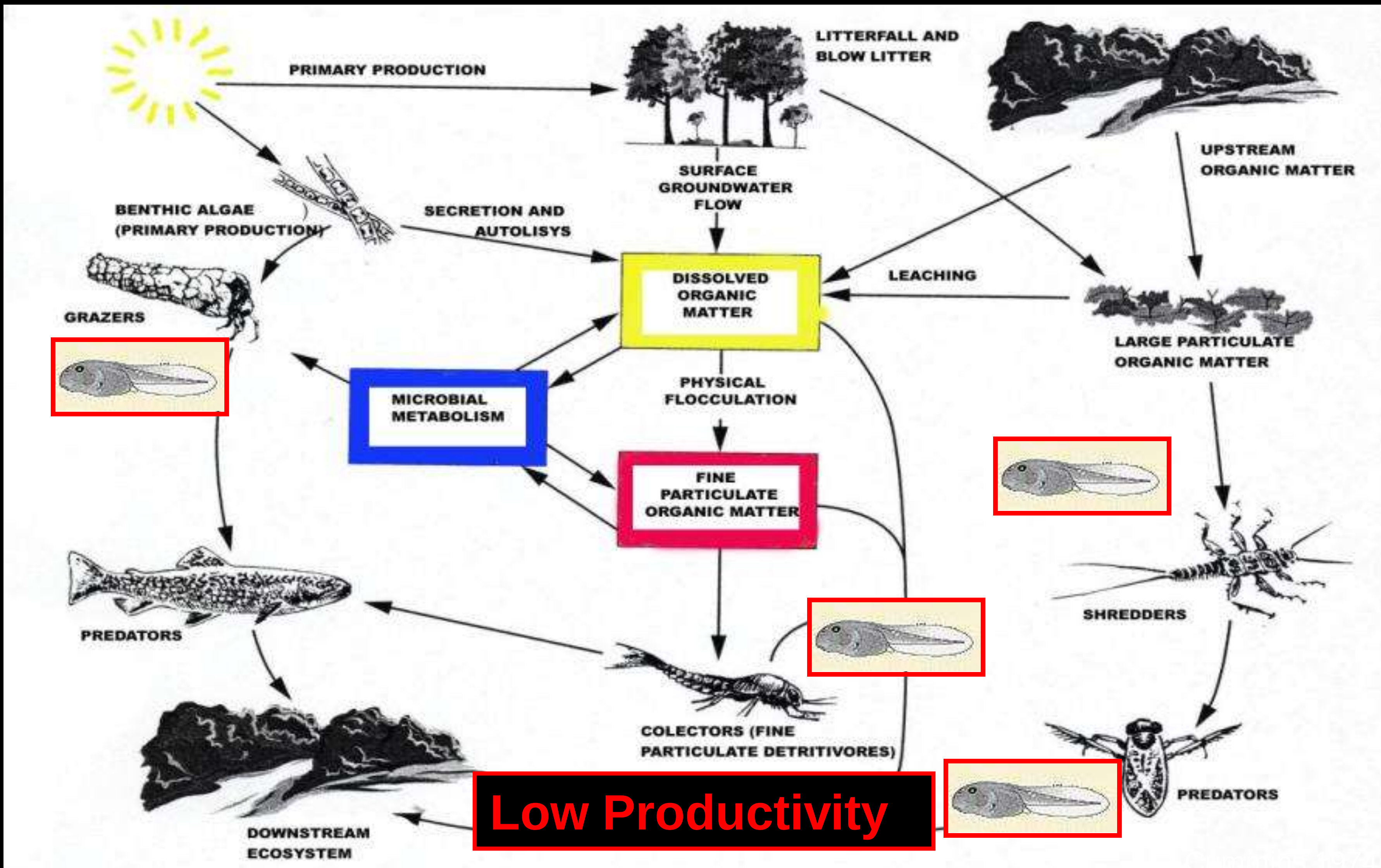
Classical headwater stream ecosystem



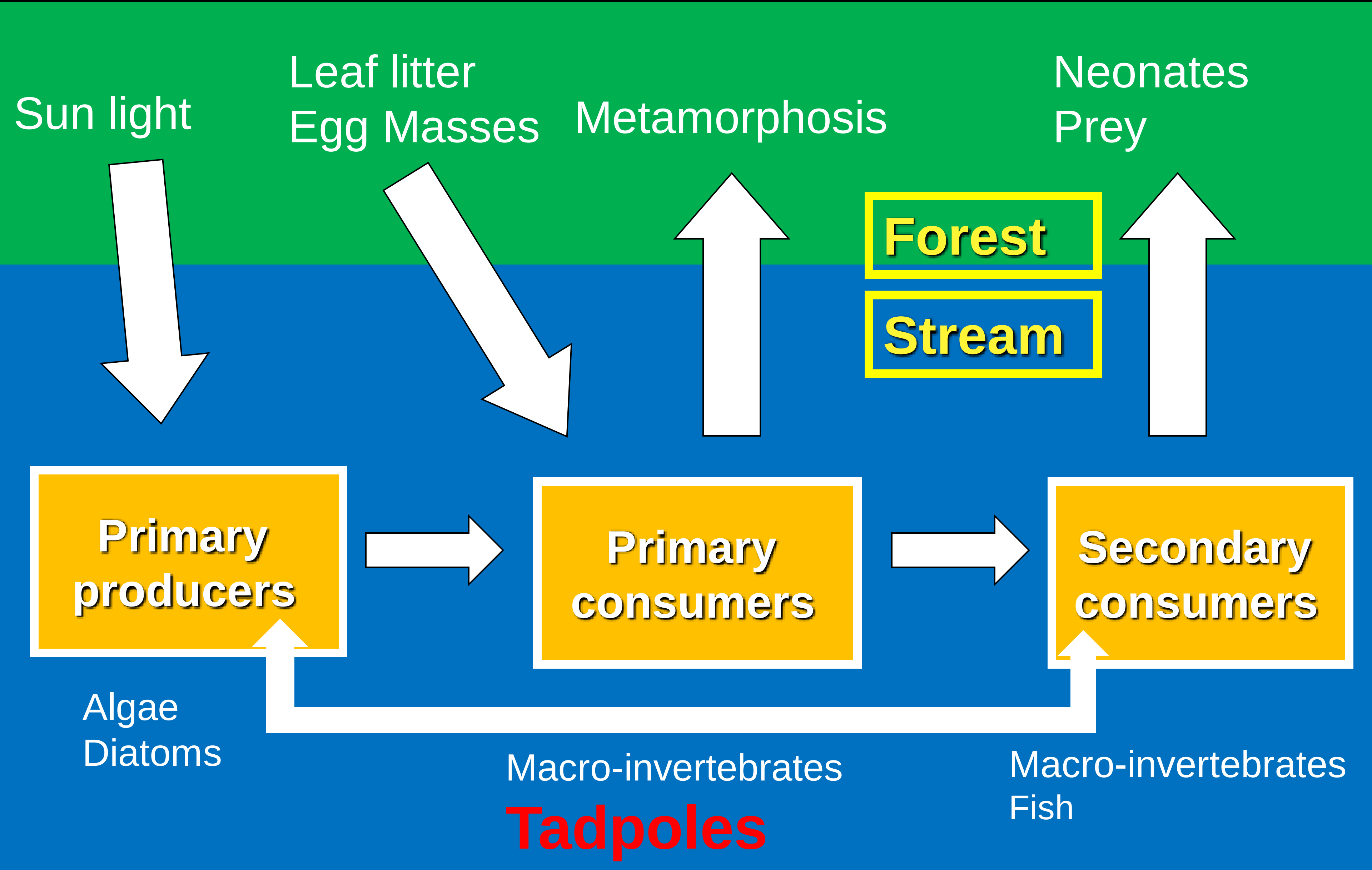
Multiple functional roles of tadpoles



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., *2011*)
- 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

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
Hyla palmeri
} **Filterers**

Hyla colymba
Rana Warszewitschii
Centrolene ilex
} **Grazers**

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

Measuring energy flow

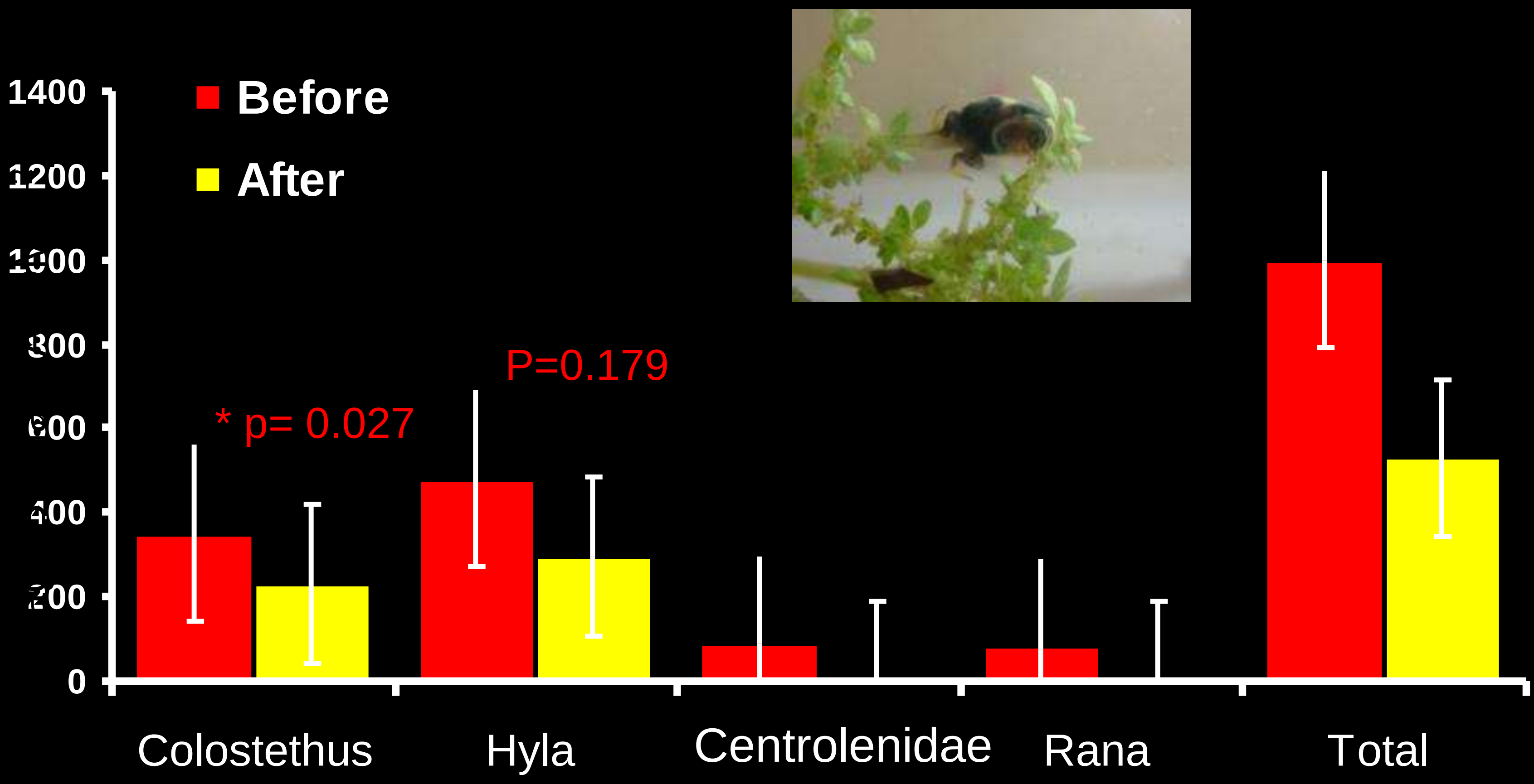
A. Species composition and densities

B. Growth rates

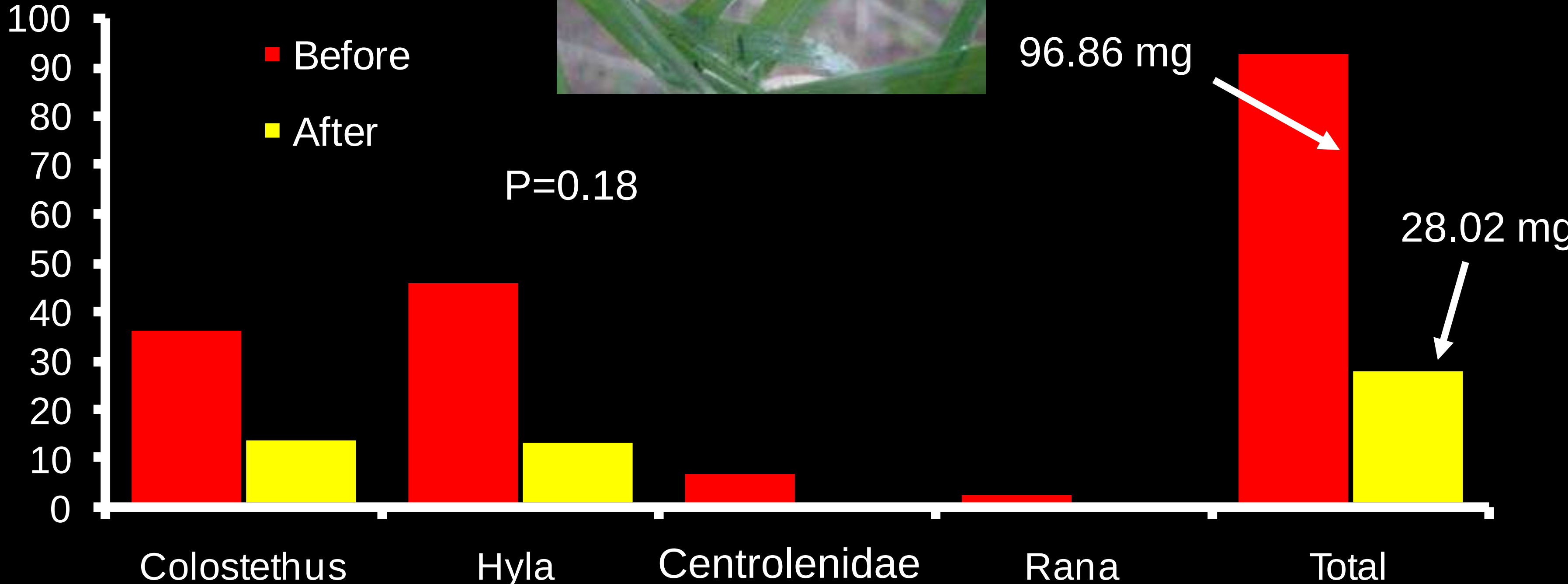
C. Biomass

D. Production

Loss of Biomass



Loss of production

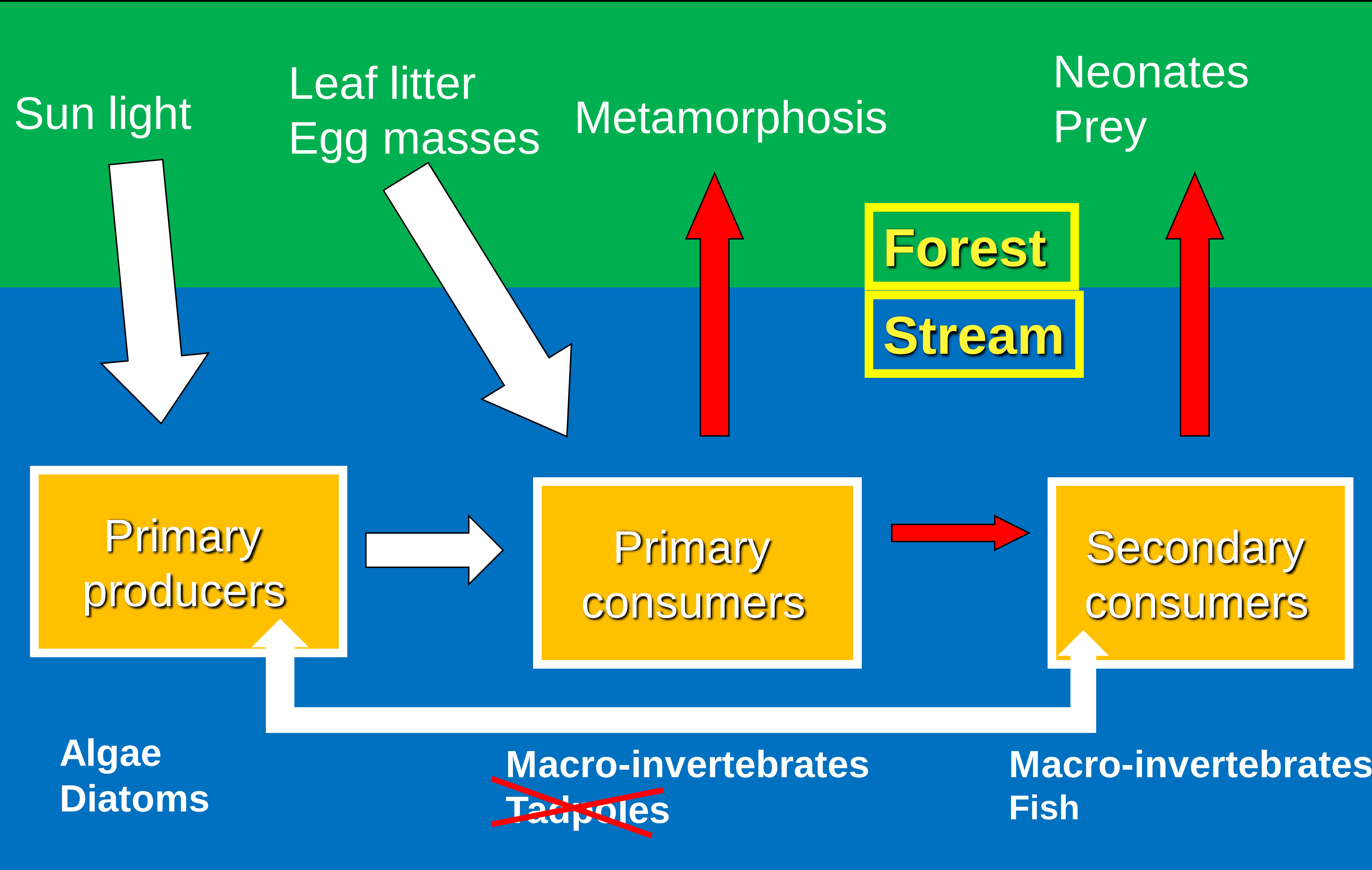


Decline in tadpole populations

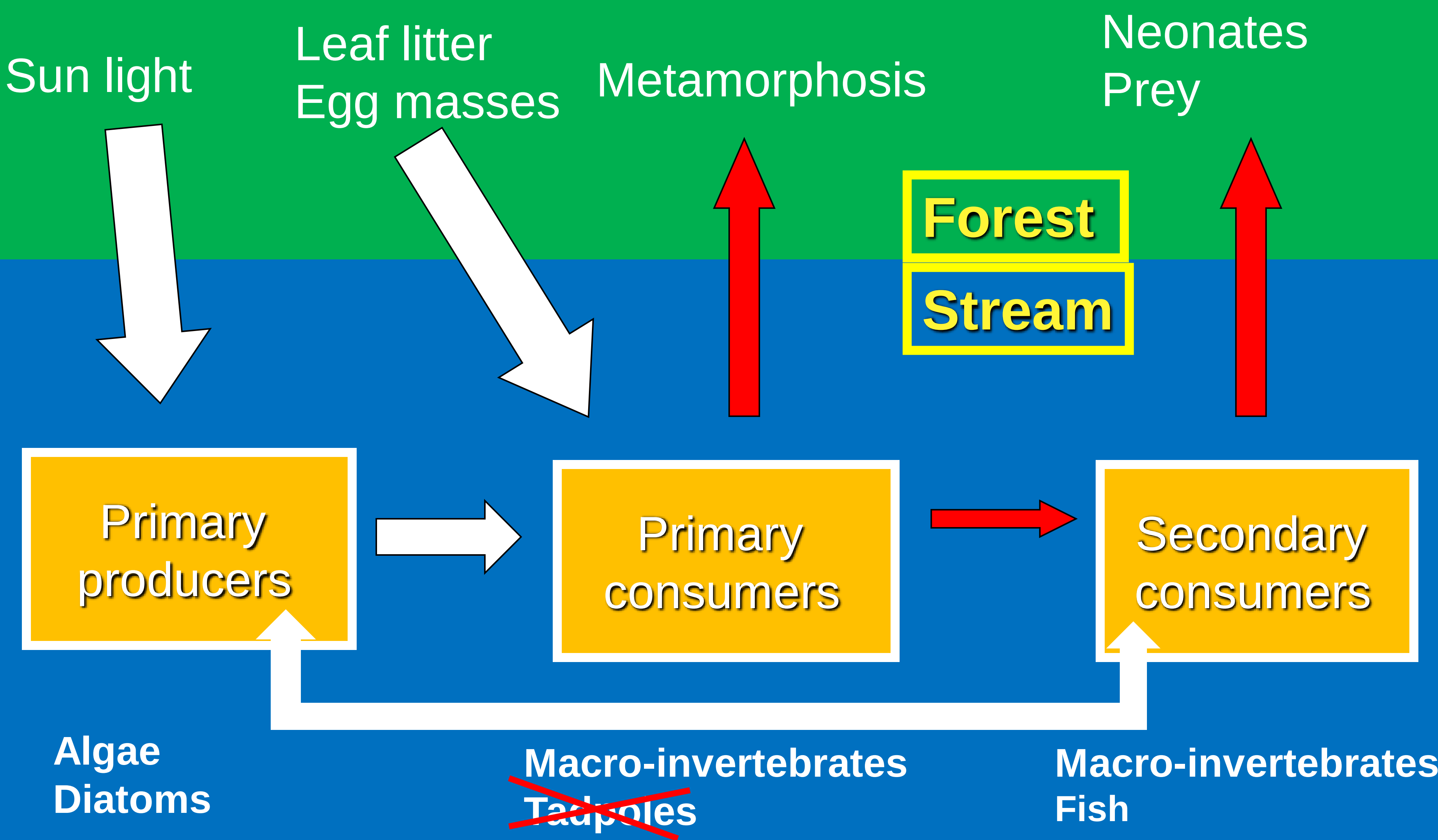
- Density dropped 95%
- Biomass was reduced 47%
- Production reduced 72%

What are the consequences?

How might energy flow change?



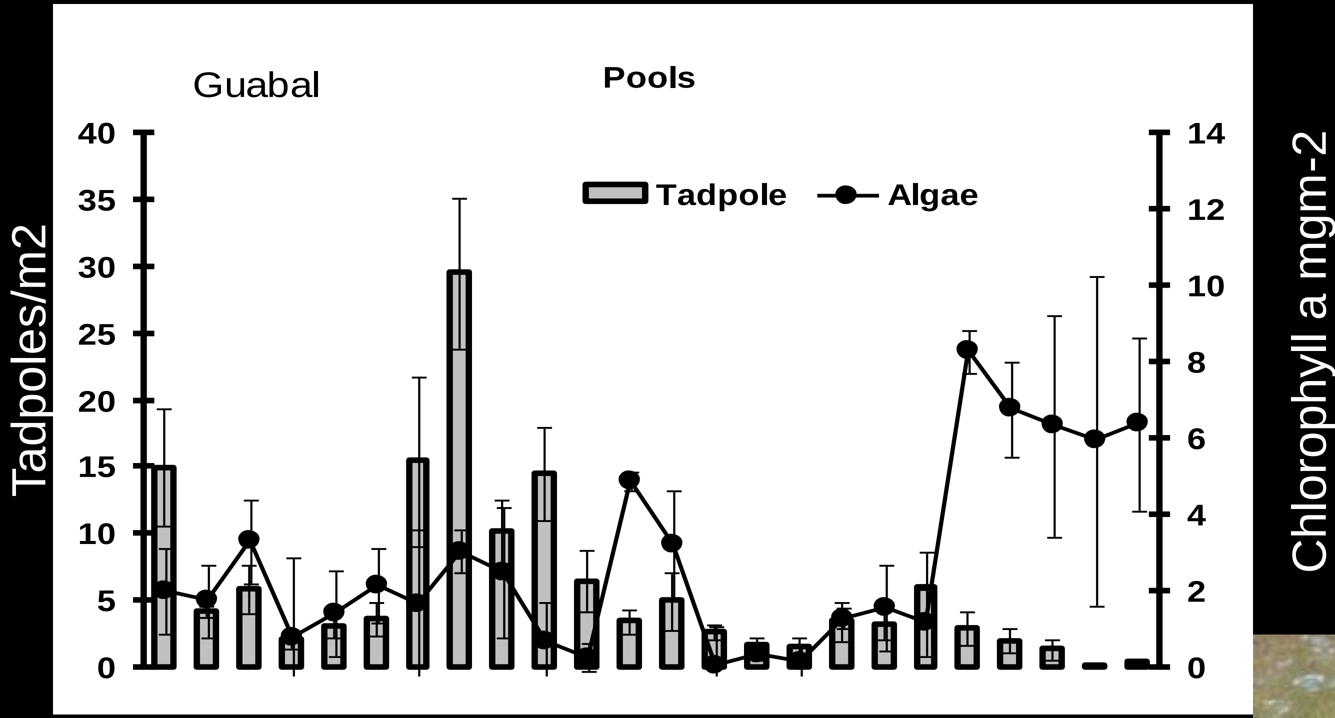
How are other groups affected ?



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?

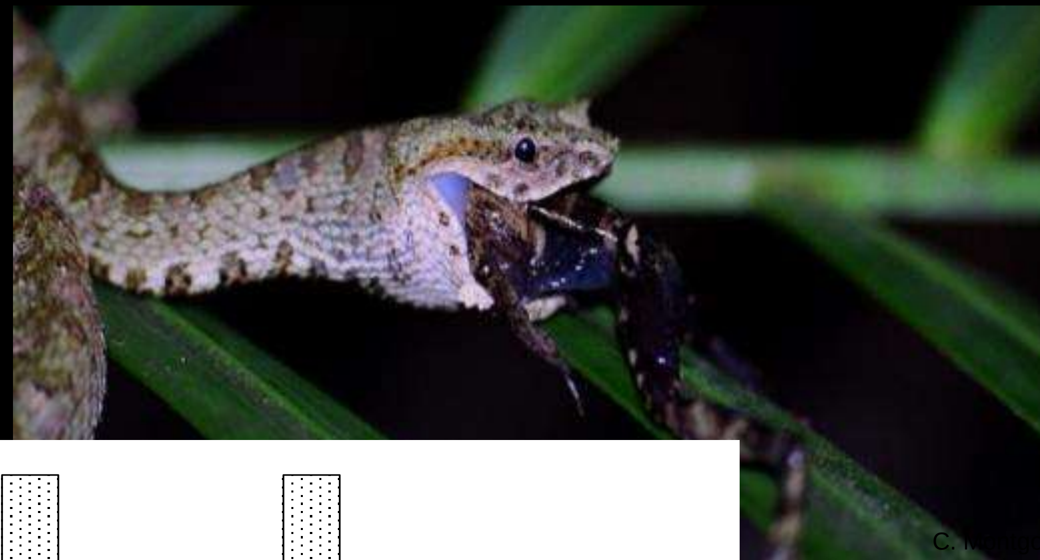
Algae Bloom

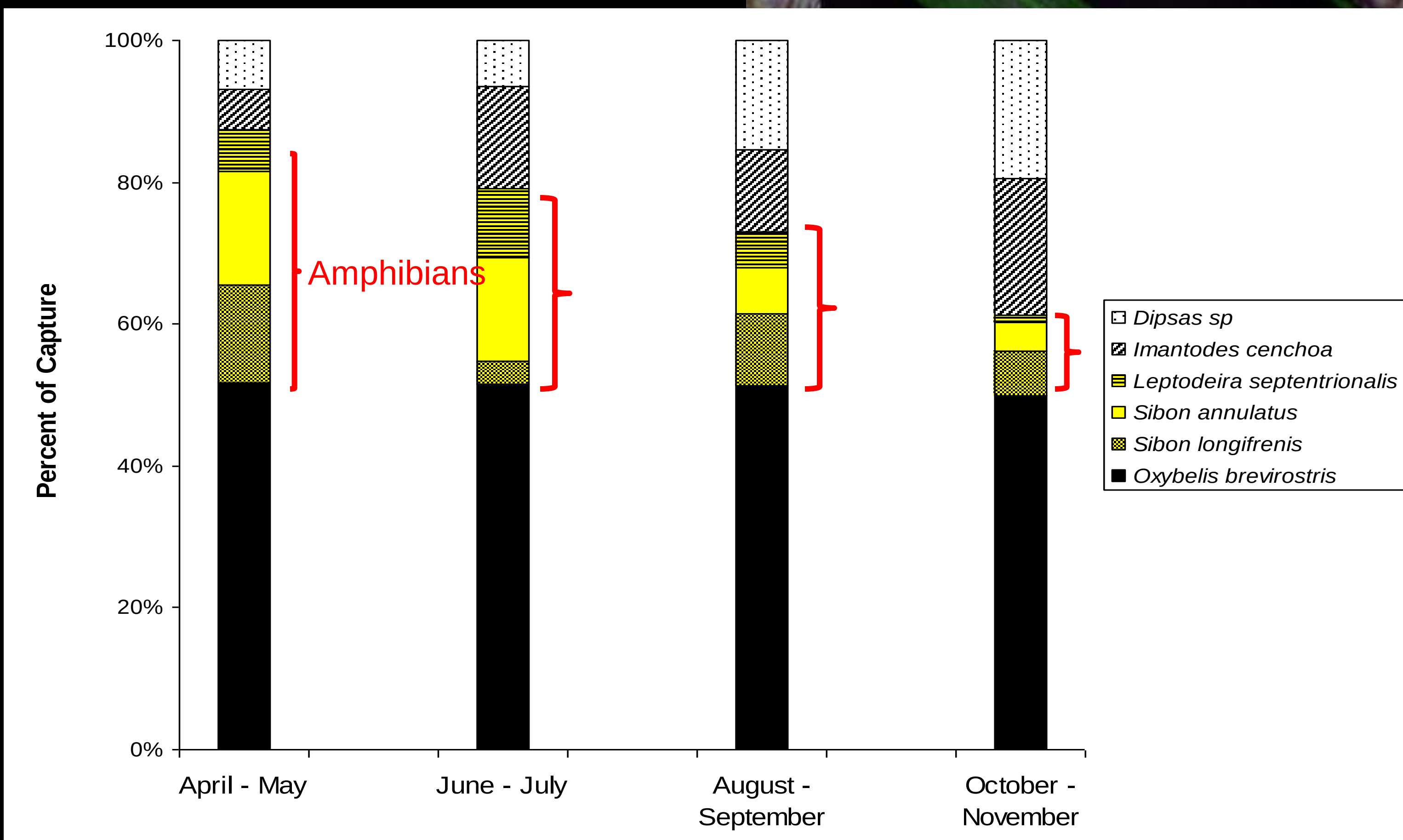


Connelly et al. *2011*

What is going to happened in the Forest

Reduction in amphibian prey






1921
1911

What is Left after the Chytrid

Survivors

- **Some species persist with stable, low level infections of *Bd***
- **<u>No known recovery of populations or sites</u>**
  - **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**

Antimicrobial peptides

- Innate immune response and role of skin antimicrobial peptides (Rolling-Smith 2010)
- Some species seem to be resistant
- Vertical transmission of innate defenses (Woodhams et al. 2011)

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?

Treatments

- **Lamisil AT (1% Terbinafine Hydrochloride) athlete's foot spray pump treatment:** over-the-counter preparation that has been found to be effective
- **Benzalkonium Chloride (alkyl dimethyl benzyl ammonium chloride) Treatment:** readily available disinfectant, antiseptic and spermicide
- **Chloramphenicol Treatment:** cheap antibiotic that has been shown to be effective
- **Formalin/Malachite Green Treatment:** potent mixture sold to treat fish for parasite, fungus and bacterial infections.

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?