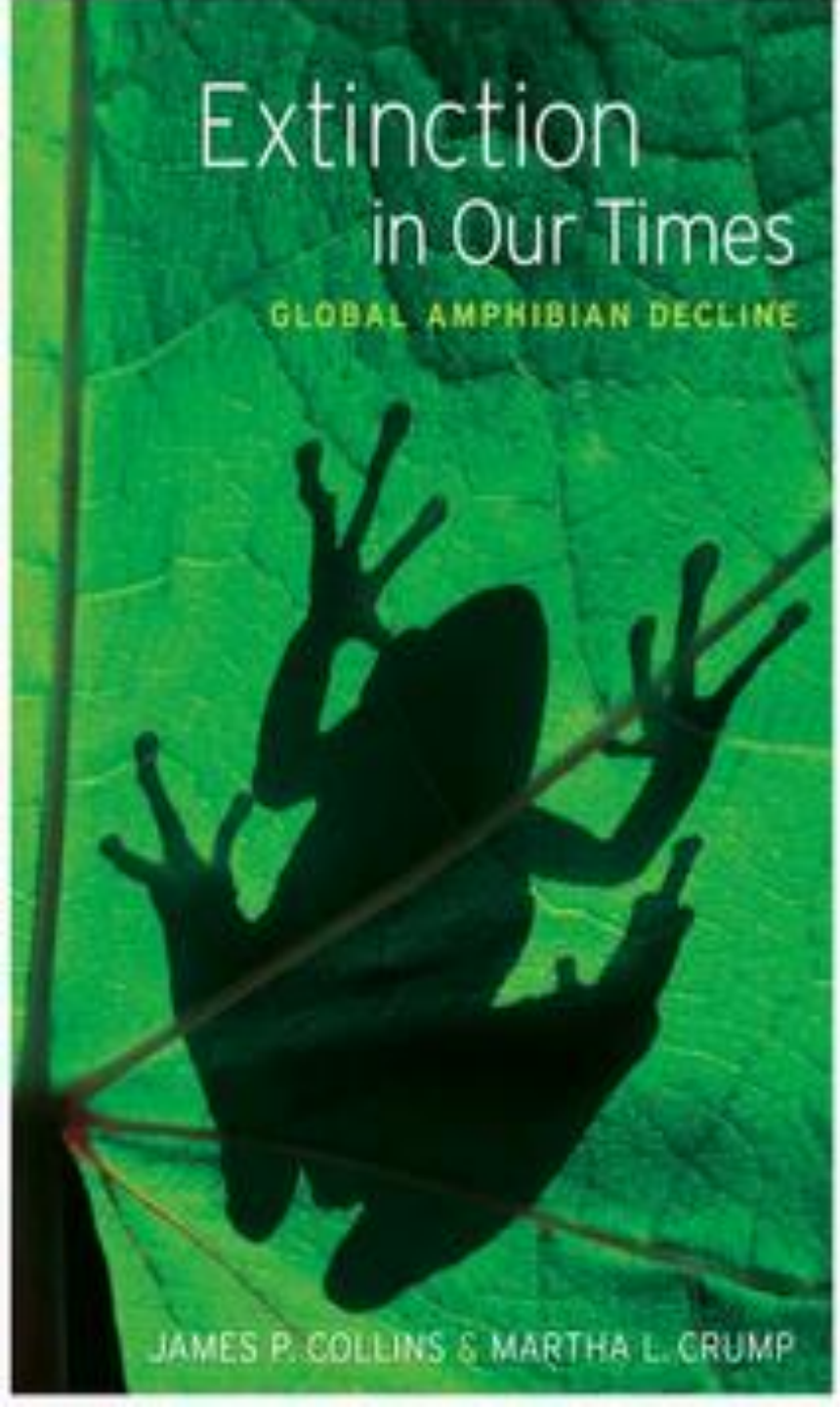
Can we control a deadly infectious amphibian disease before it is too late?

Vance T. Vredenburg
San Francisco State University

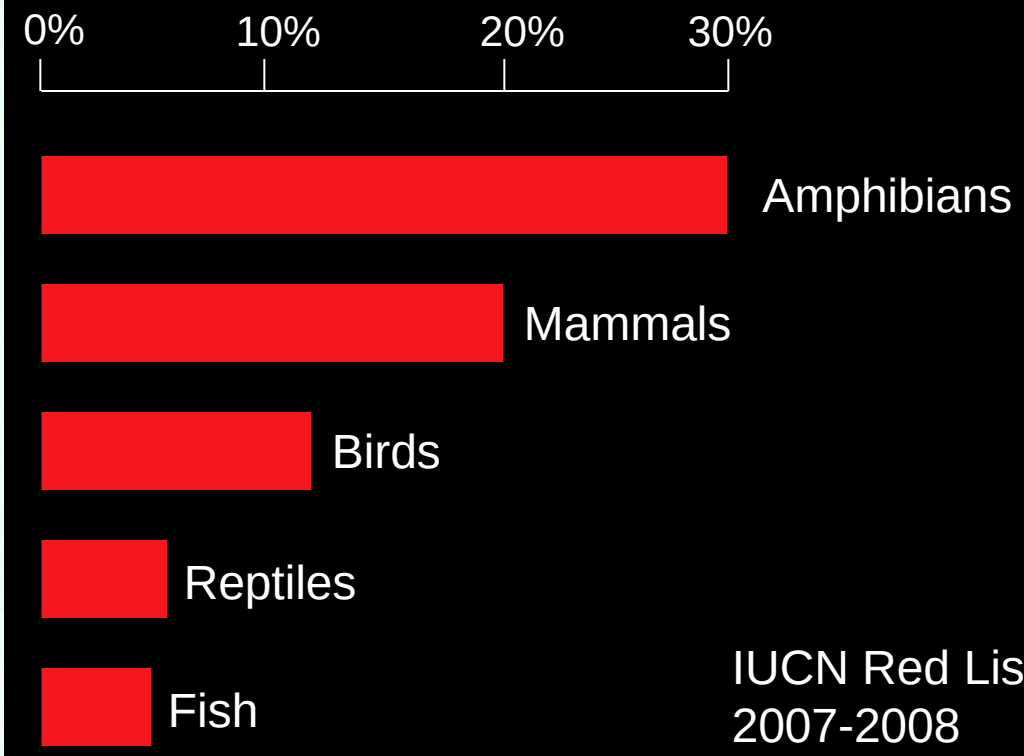
Photo by: Anand Varma

Joel Sartore



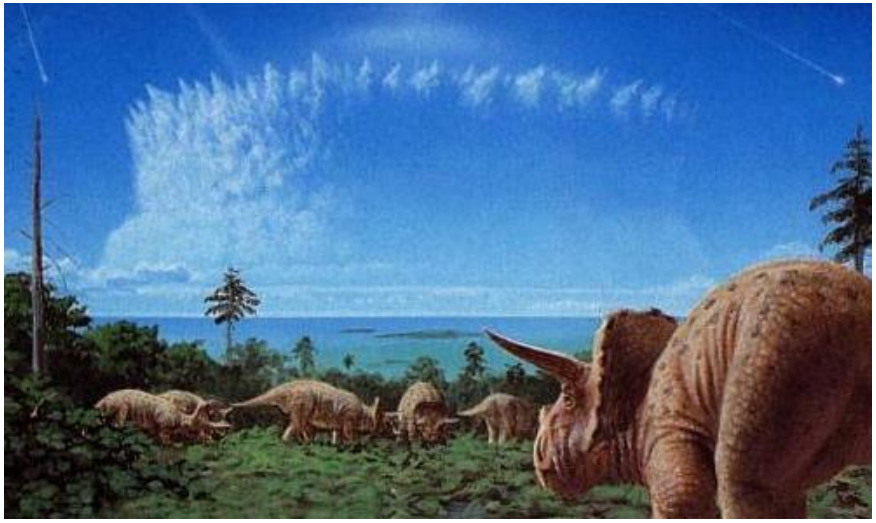
7,121 described Amphibian Species

- 41% declining
- 30% threatened with extinction



IUCN Red List 2007-2008

~5 mass extinction events in earth's history



Amphibians have survived the last 4

PNAS

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

David B. Wake*† and Vance T. Vredenburg*‡

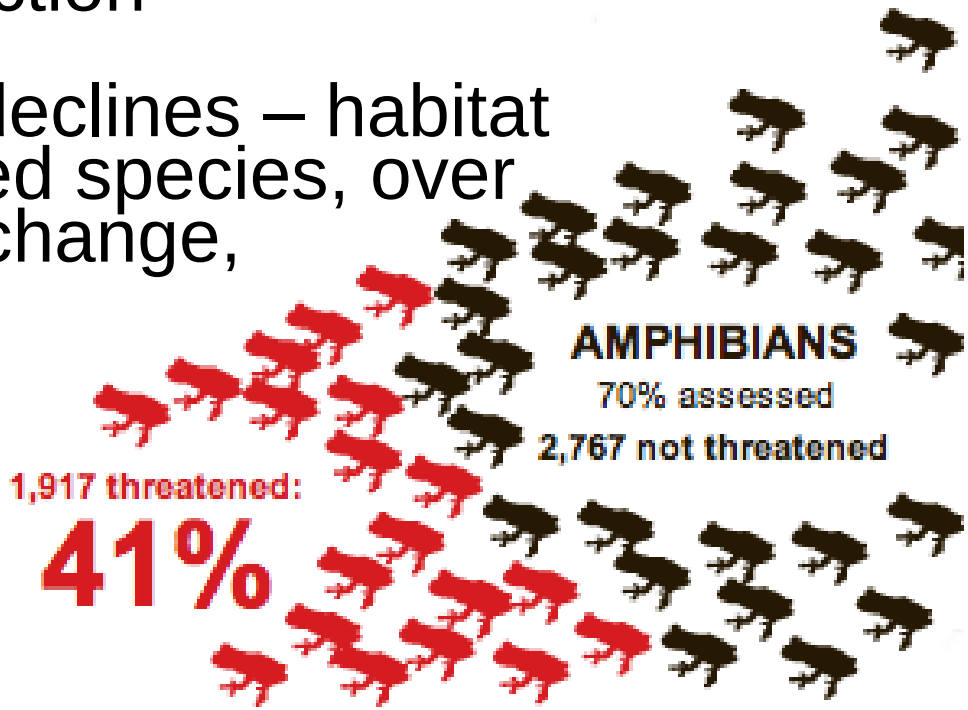
*Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720-3160; and ‡Department of Biology, San Francisco State University, San Francisco, CA 94132-1722

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 environ-

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

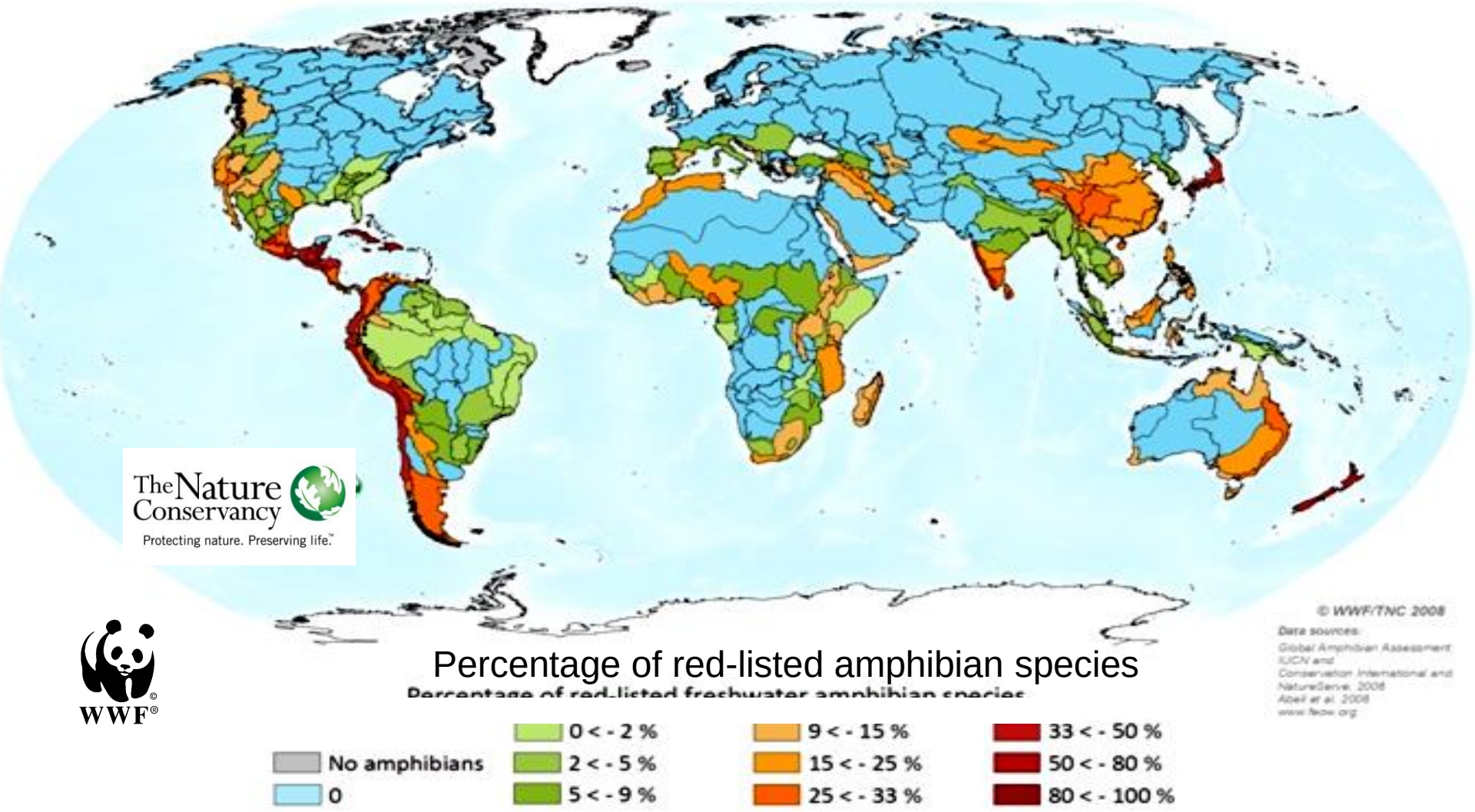
Sierra Nevada
California
Joel Sartore

Possible Causes Amphibian Declines

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

Joel Sartore

Global Amphibian Declines



Costa Rican Golden Toad
Last seen 1989

Journal of Herpetology, Vol. 25, No. 2, pp. 174–177, 1991
Copyright 1991 Society for the Study of Amphibians and Reptiles

Mass Mortality and Extinction in a High-elevation Population of *Rana muscosa*

DAVID F. BRADFORD

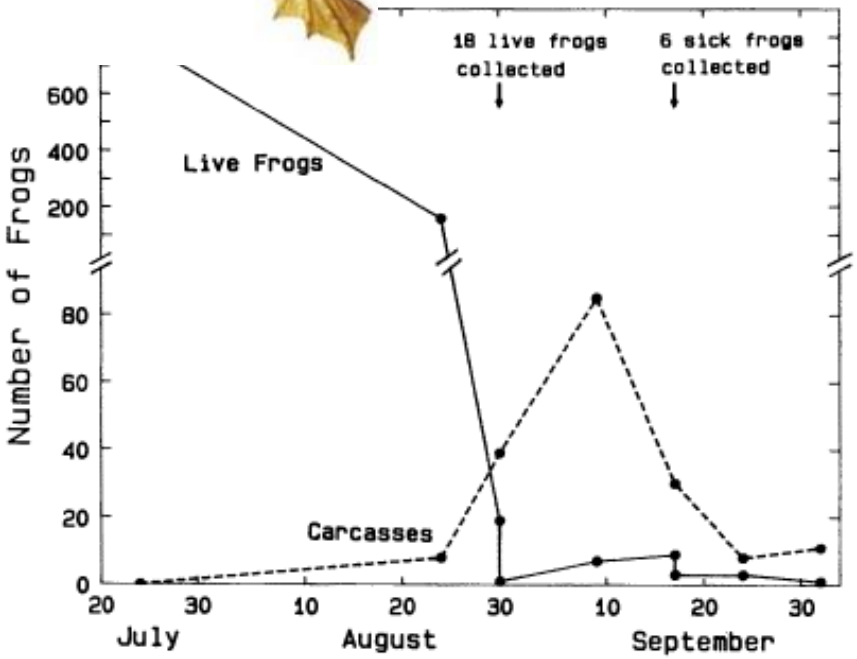


FIG. 1. Numbers of live frogs present and frog carcasses collected in Ridge Lake during summer, 1979.

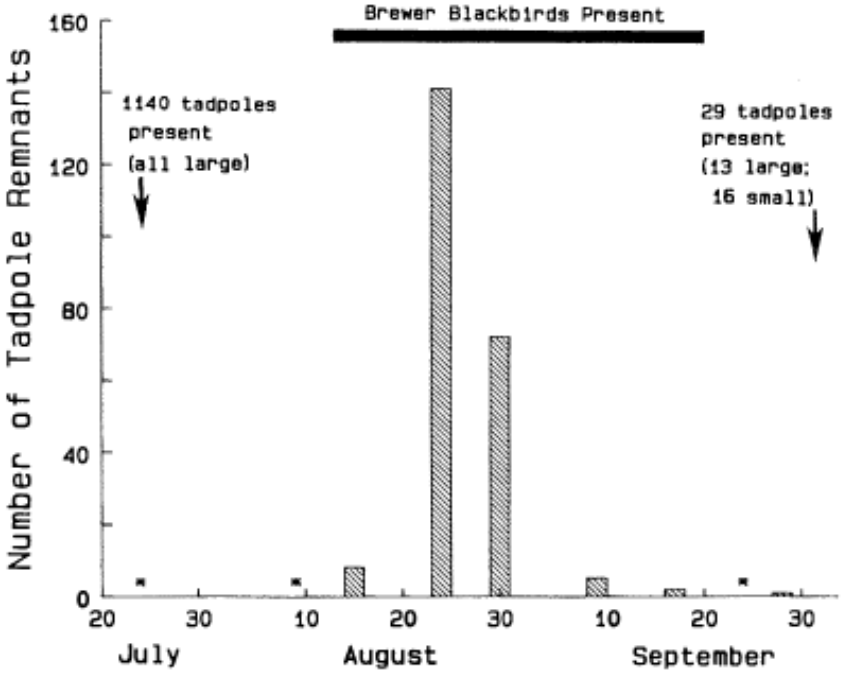
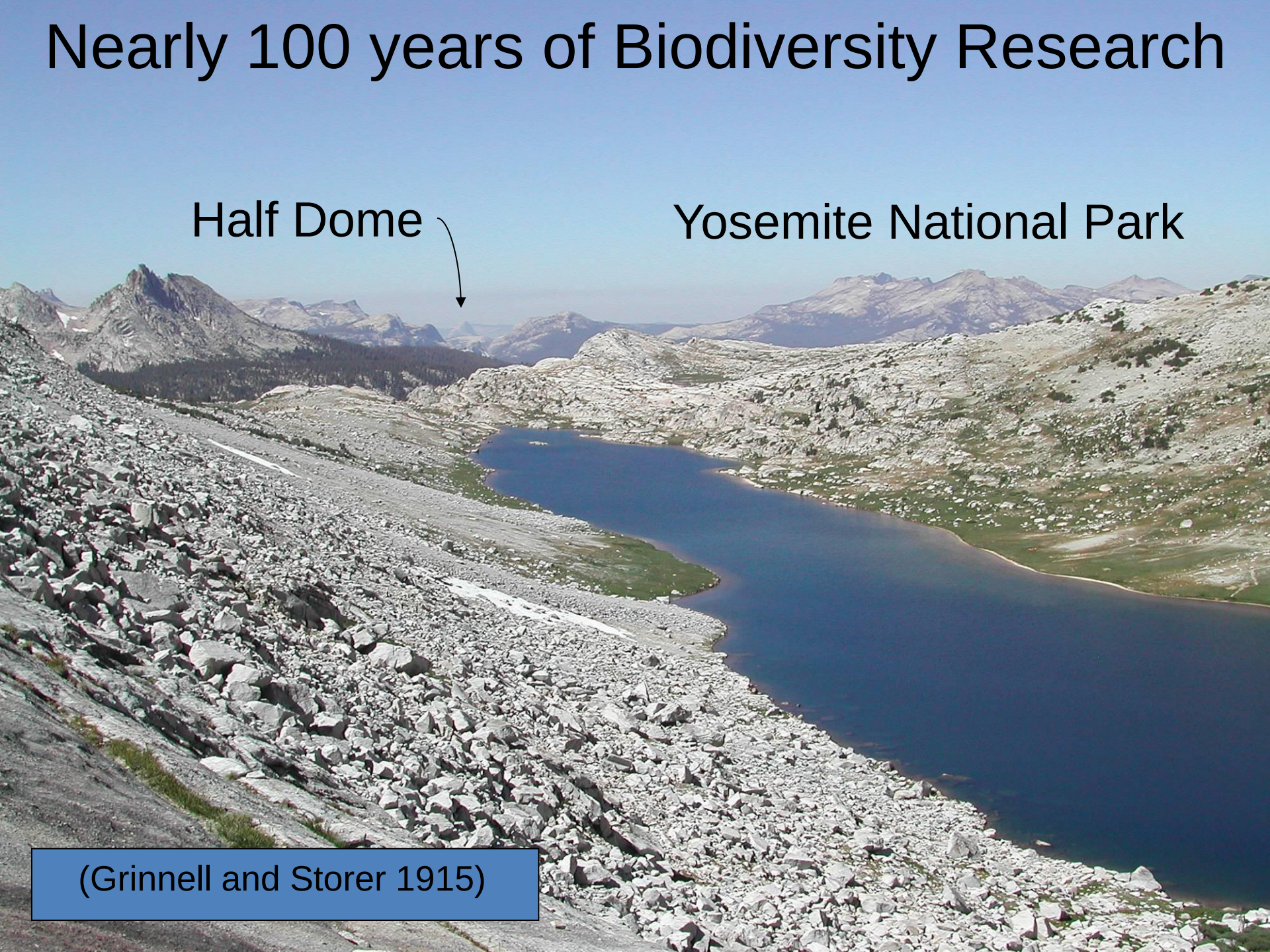


FIG. 2. Numbers of tadpole carcasses collected at Ridge Lake during summer, 1979. Asterisks indicate that no tadpole remains were found on indicated dates.

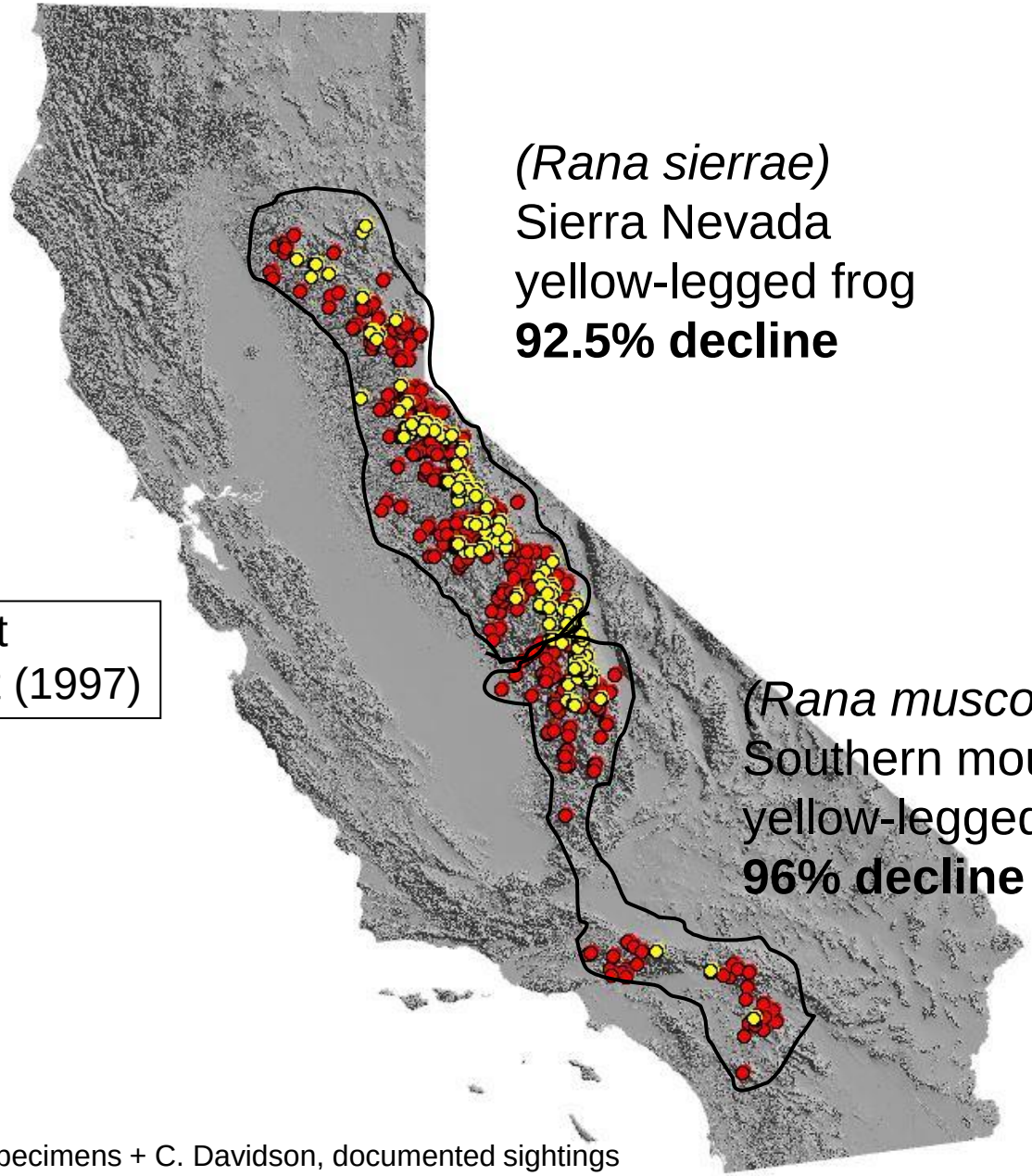
Sierra Nevada: a protected area

Nearly 100 years of Biodiversity Research



(Grinnell and Storer 1915)

Major declines despite protected habitat



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

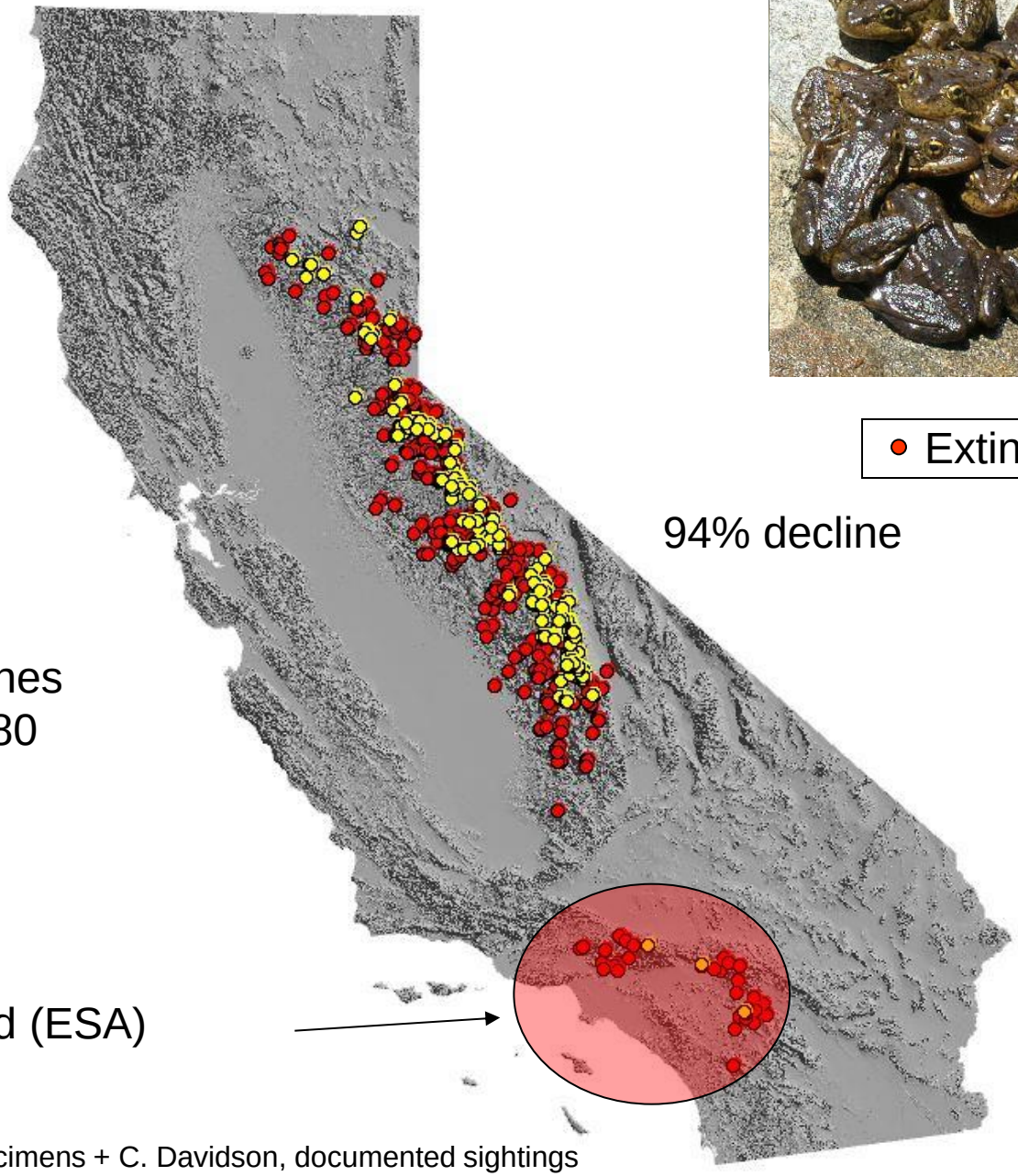
- Extinct
- Extant (1997)

(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



Extinctions

94% decline

Major declines
began ~1980

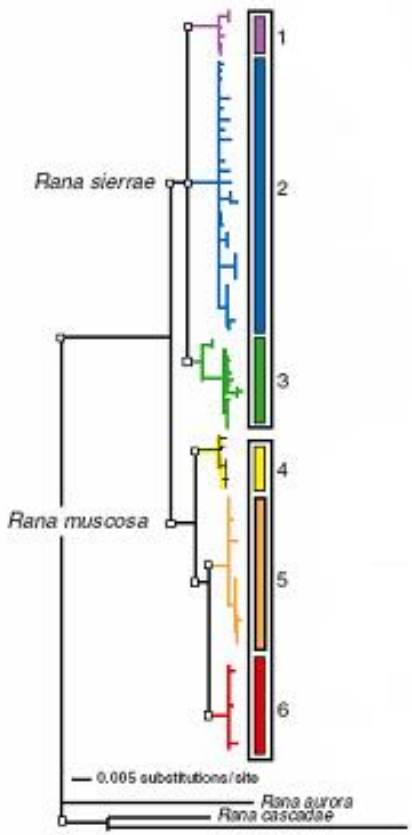
Endangered (ESA)

99% decline

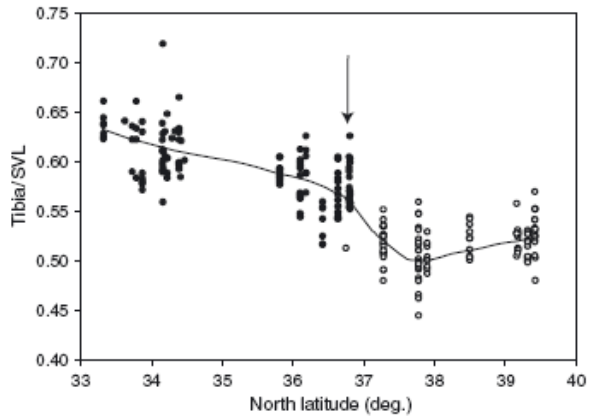
Data: MVZ, CAS specimens + C. Davidson, documented sightings
Data: Knapp, Briggs et al., CAS, CDFG, USFS, USGS recent surveys

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

mtDNA

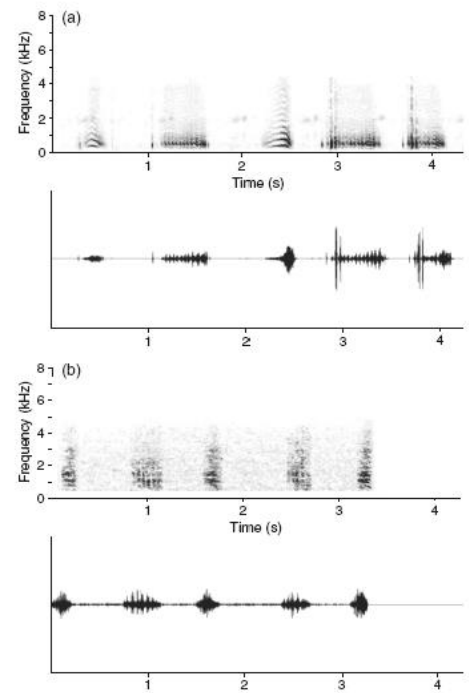


Morphology



Lake adapted ⟺ Stream adapted

Frog vocalizations

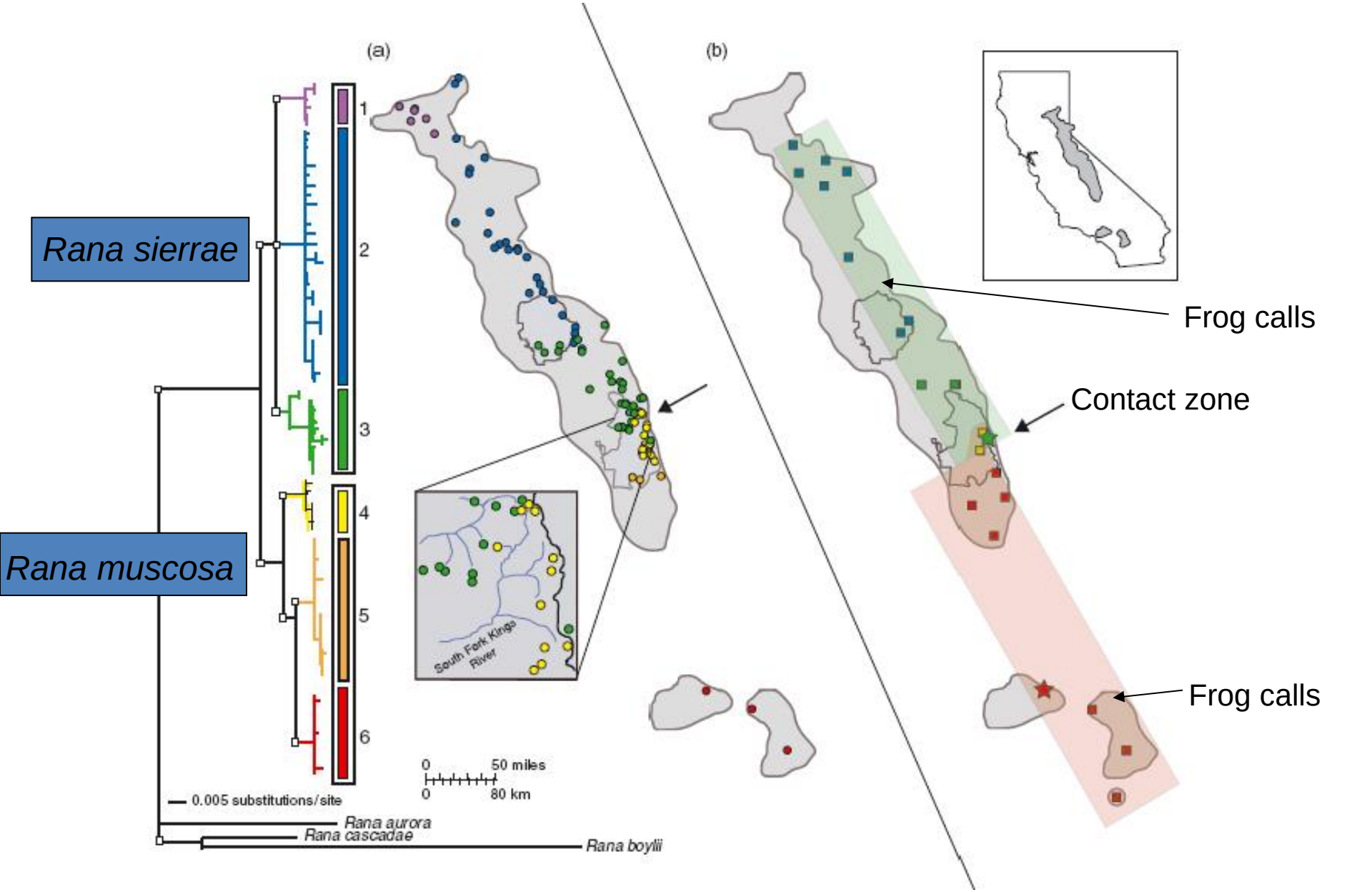


Northern

Southern

(Vredenburg et al 2007; J Zool)

Concordant data delineate new taxonomy– two species

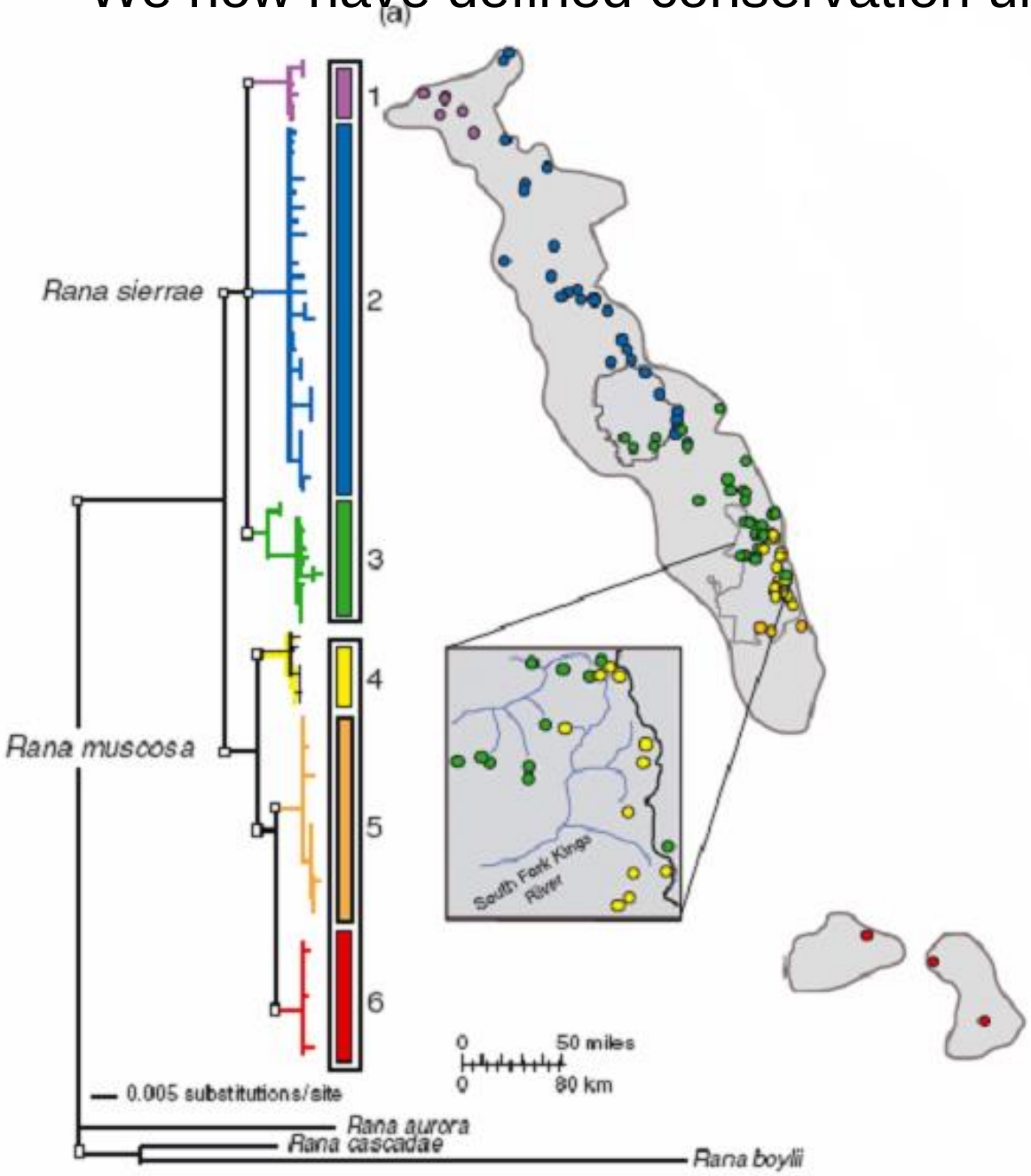


mtDNA

morphology

(Vredenburg et al 2007; J Zool)

We now have defined conservation units: Now what?



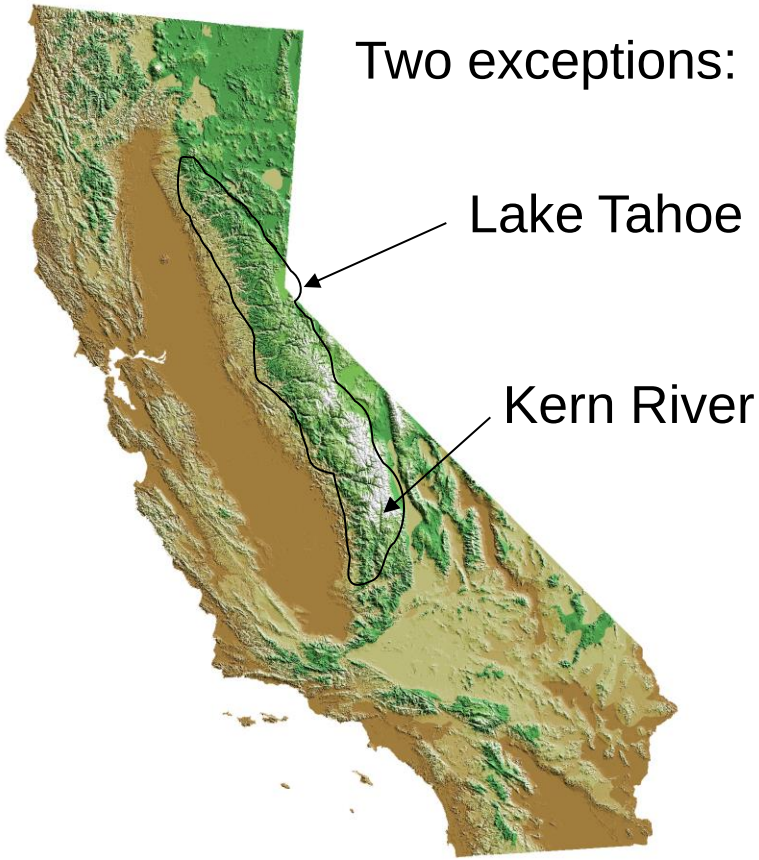
Conclusion:

2 species
-6 ESU's

ESU	% extinction
1	93
2	92
3	95
4	96
5	93
6	98

(Vredenburg et al 2007; J Zool)

Introduced species: No native fishes above 1,500 m



(reviewed in: Vredenburg et al 2005)

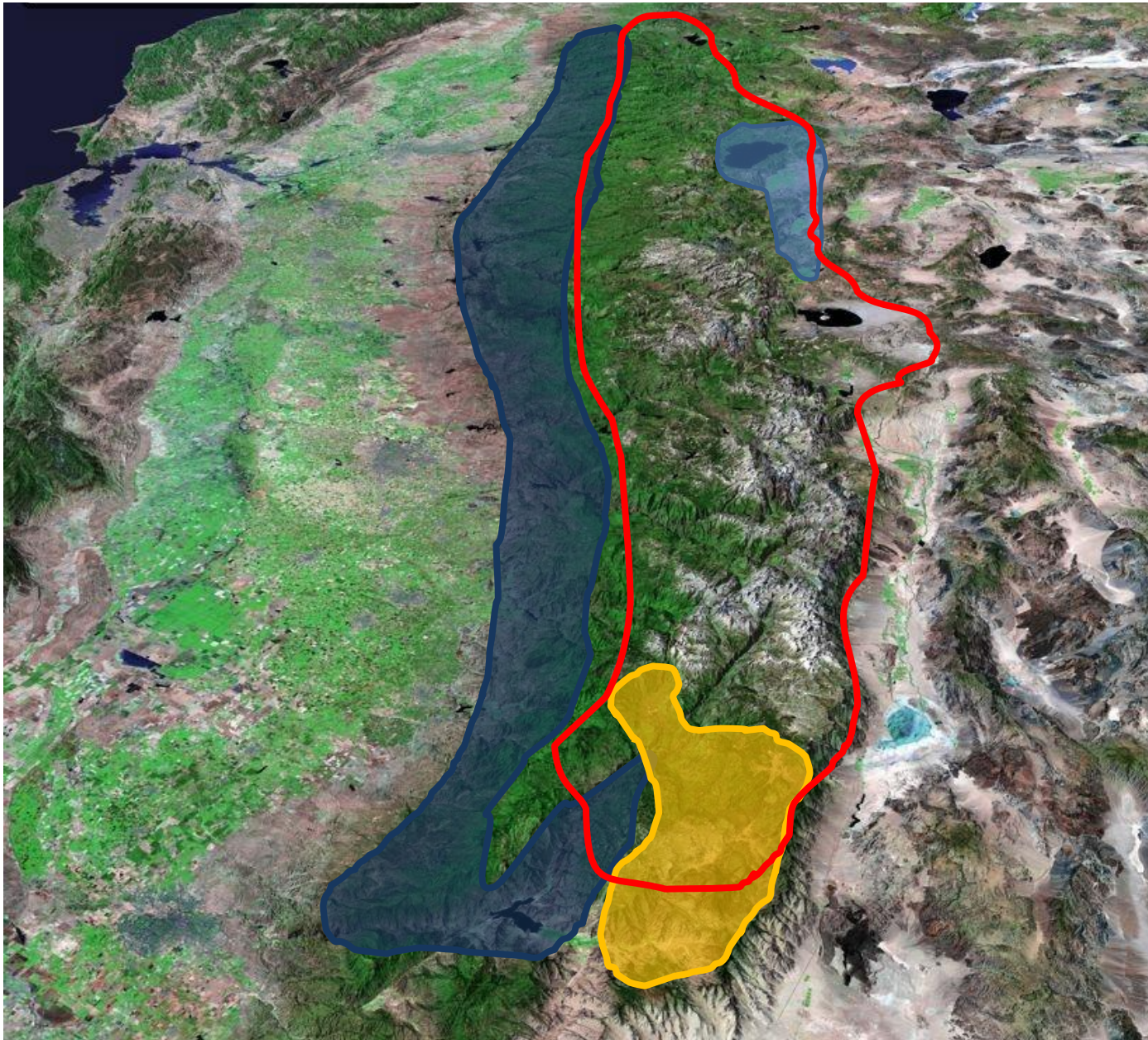
THREATS – Introduced Fish

BACKGROUND

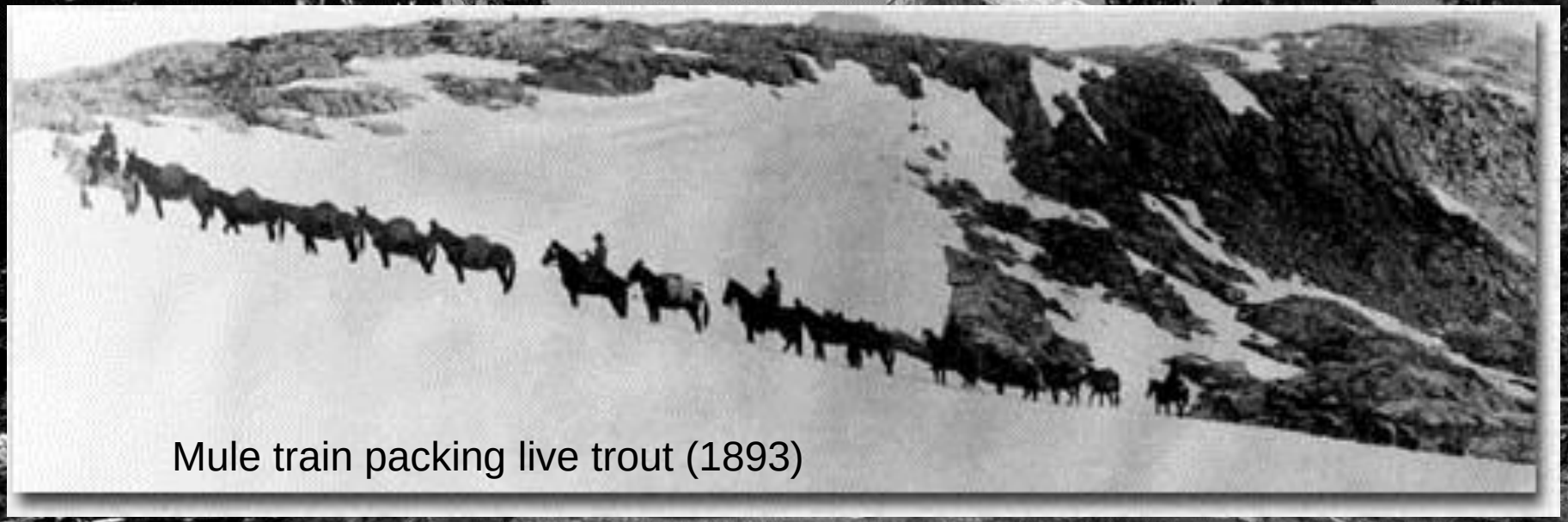
•Historically fish found only at lower elevations of Sierra Nevada.

•Filled colored areas are native fish ranges

•Red outline is the range of the two species of mountain frog



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



Mule train packing live trout (1893)

1. Trout native in some areas

2. Historical introductions of trout began in 1890s, but frog declines in late 1970s

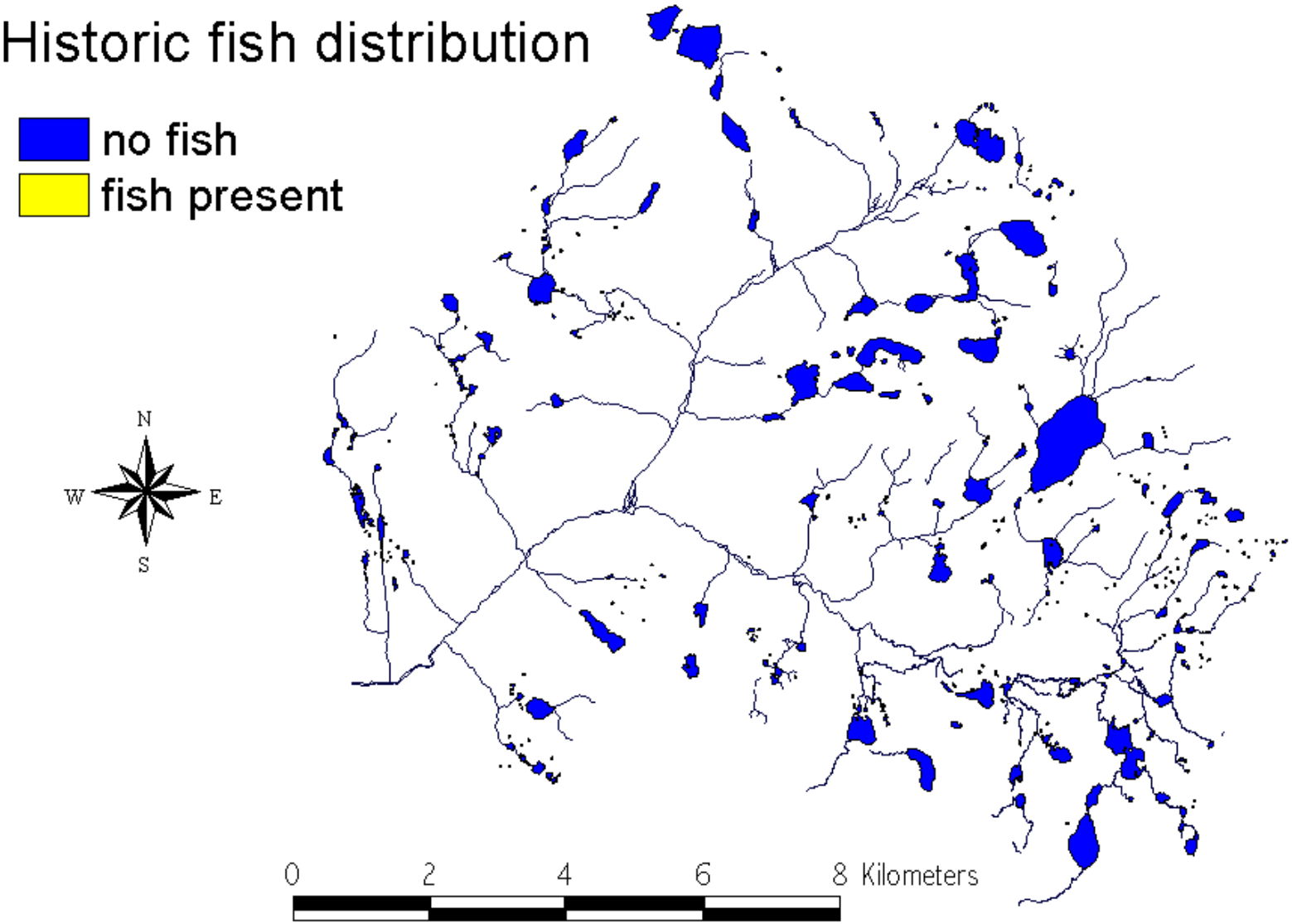
…therefore trout not responsible!

In the 1950-60's, industrial trout introductions began

Flying fish can climb mountains

Humphreys Basin & French Canyon

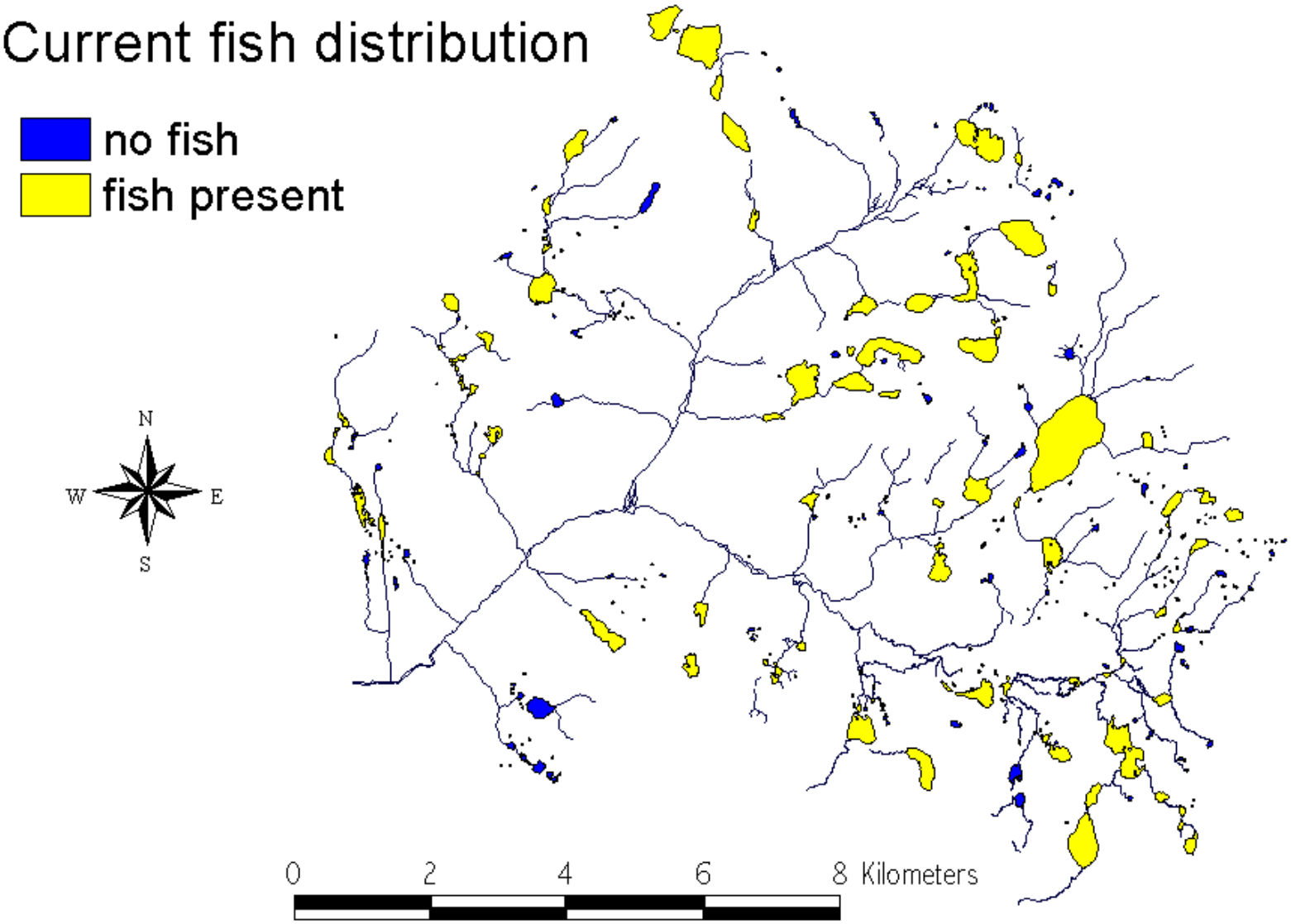
Historic fish distribution



(R. Knapp unpublished)

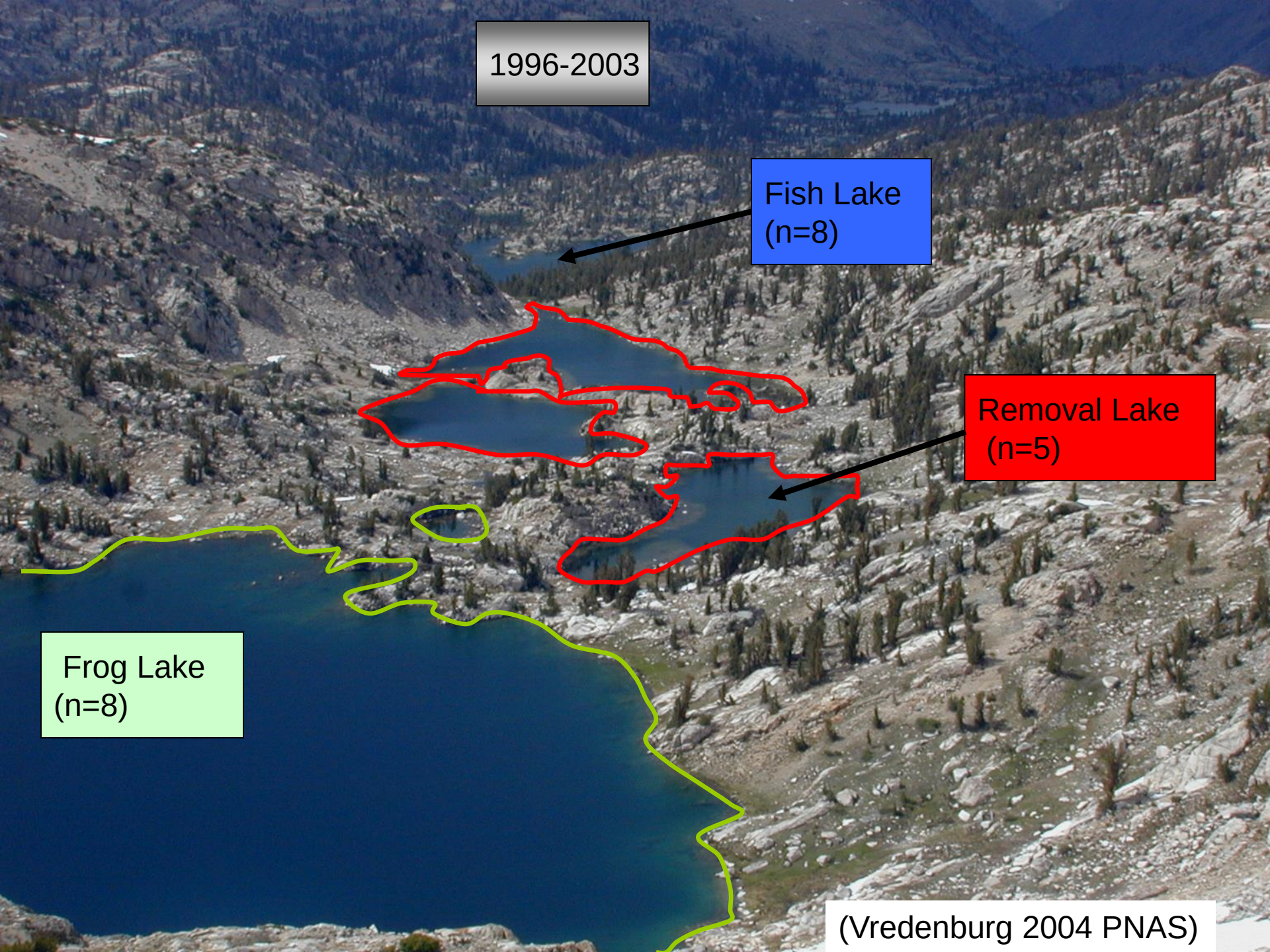
Humphreys Basin & French Canyon

Current fish distribution



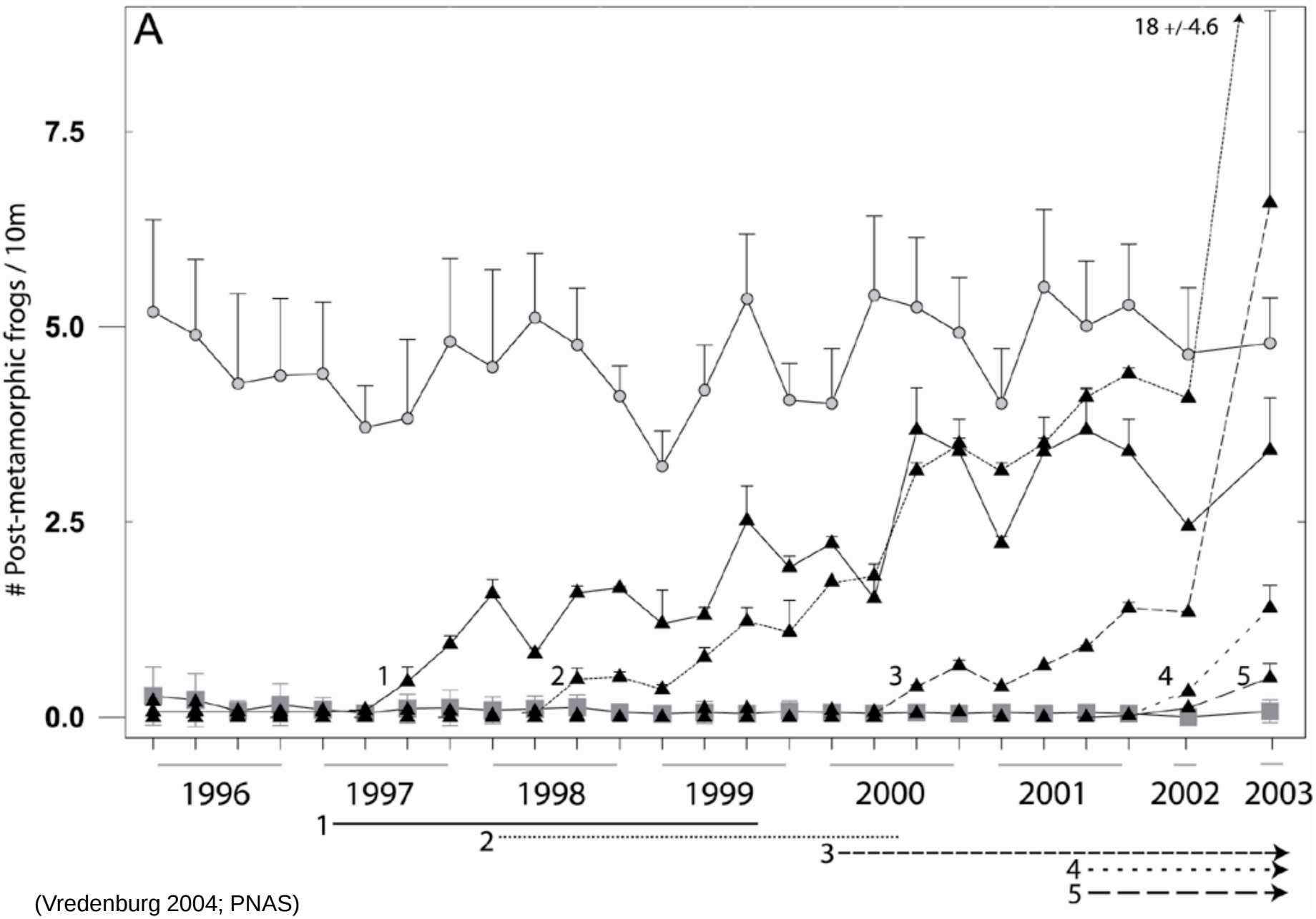
(R. Knapp unpublished)

Key life cycle of the Mountain Yellow-legged Frog



(Vredenburg 2004 PNAS)

Frog populations can quickly recover

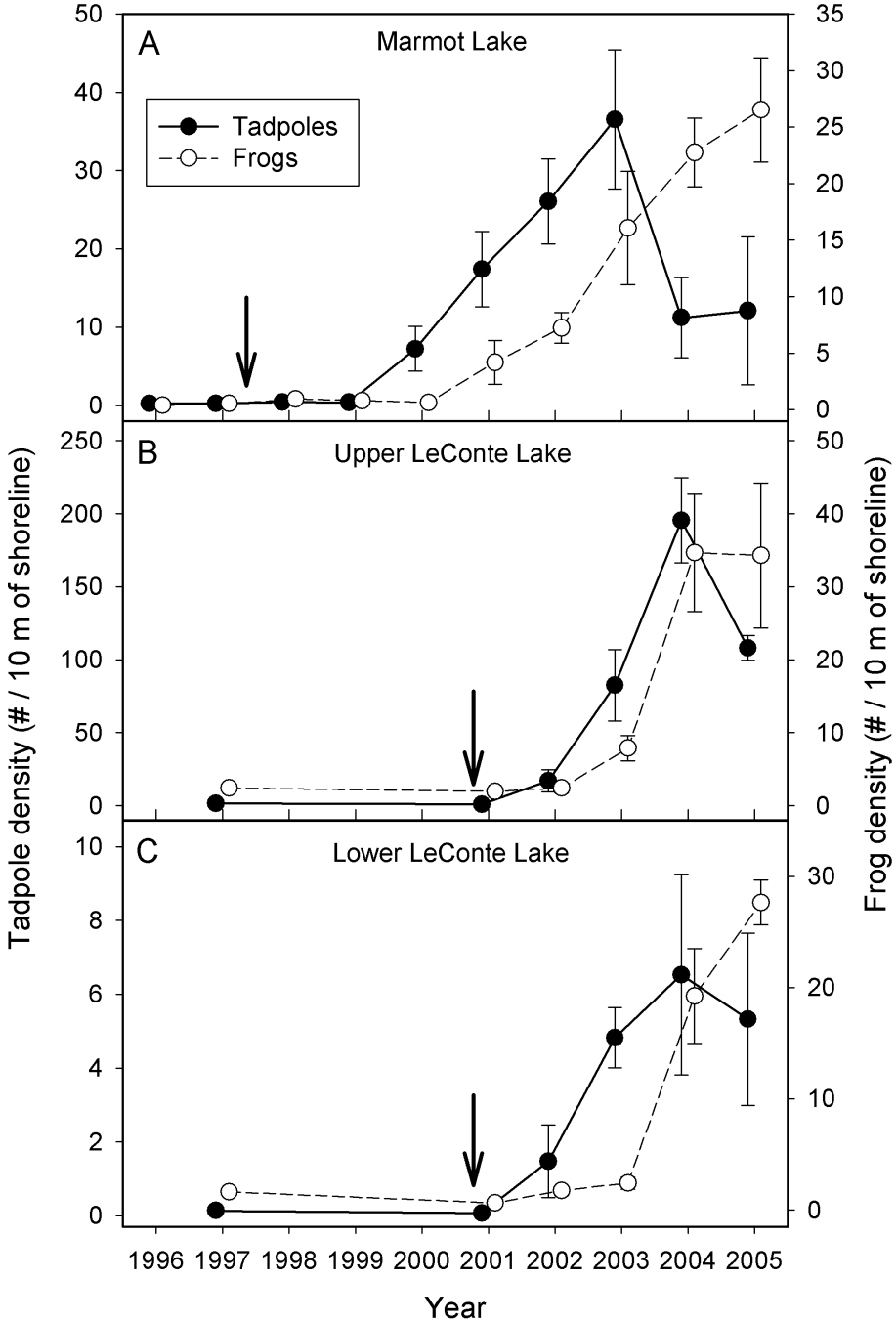


(Vredenburg 2004; PNAS)

Conservation Biology:
A long hard road.

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

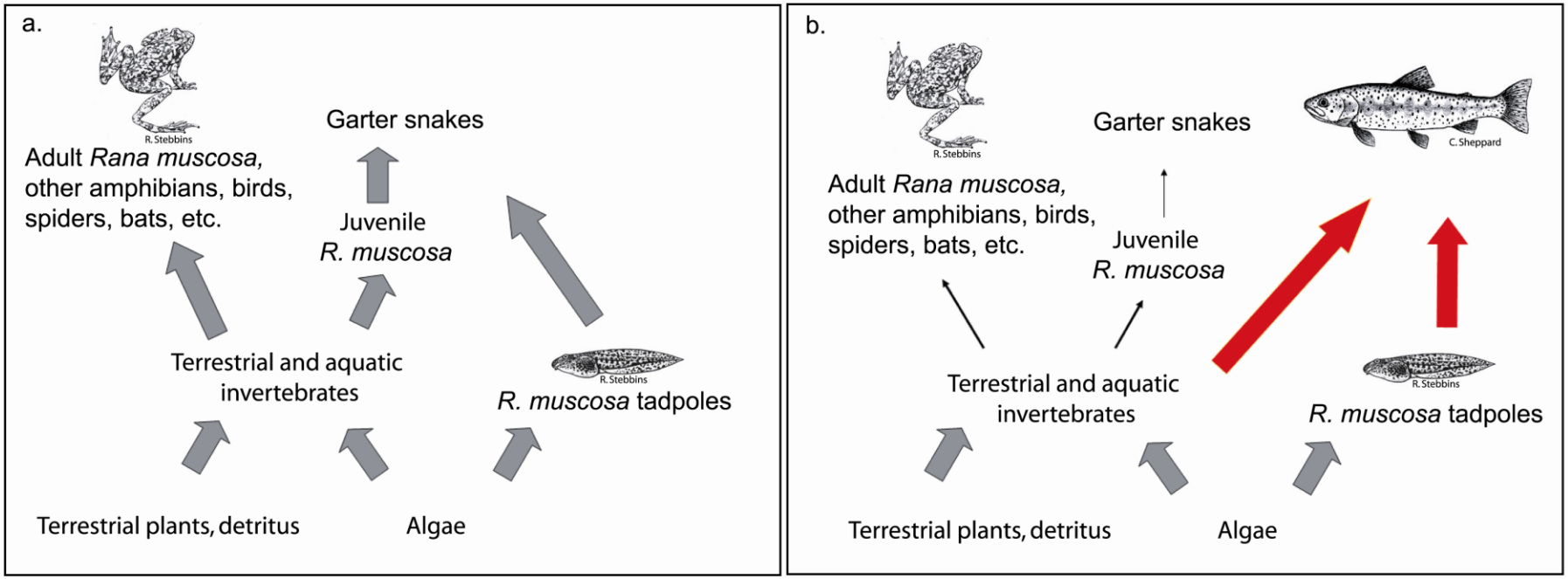
Same result:
Removal introduced trout leads to rapid recovery of threatened frog populations.



(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!

Journal of Herpetology, Vol. 25, No. 2, pp. 174–177, 1991
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Mass Mortality and Extinction in a High-elevation Population of *Rana muscosa*

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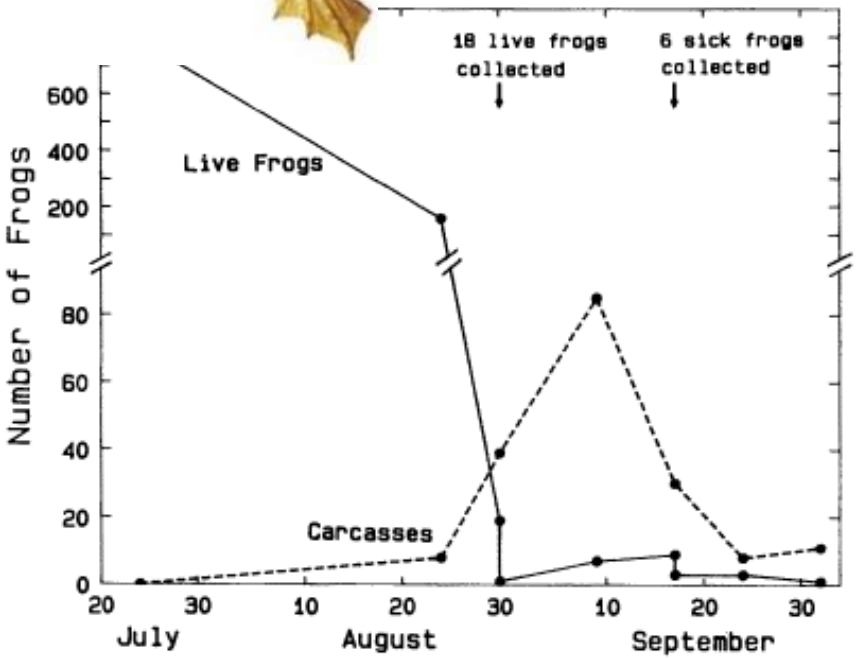

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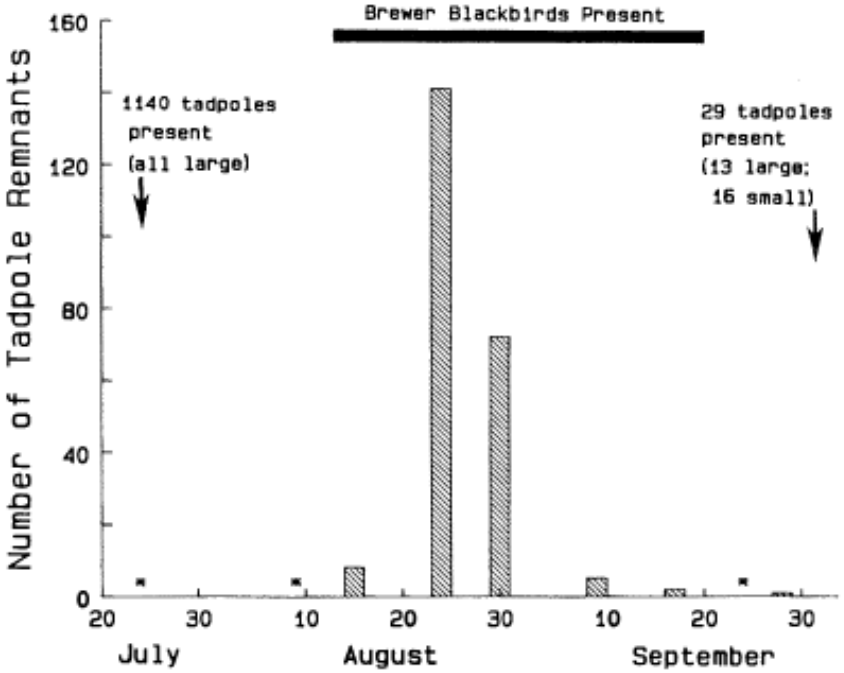
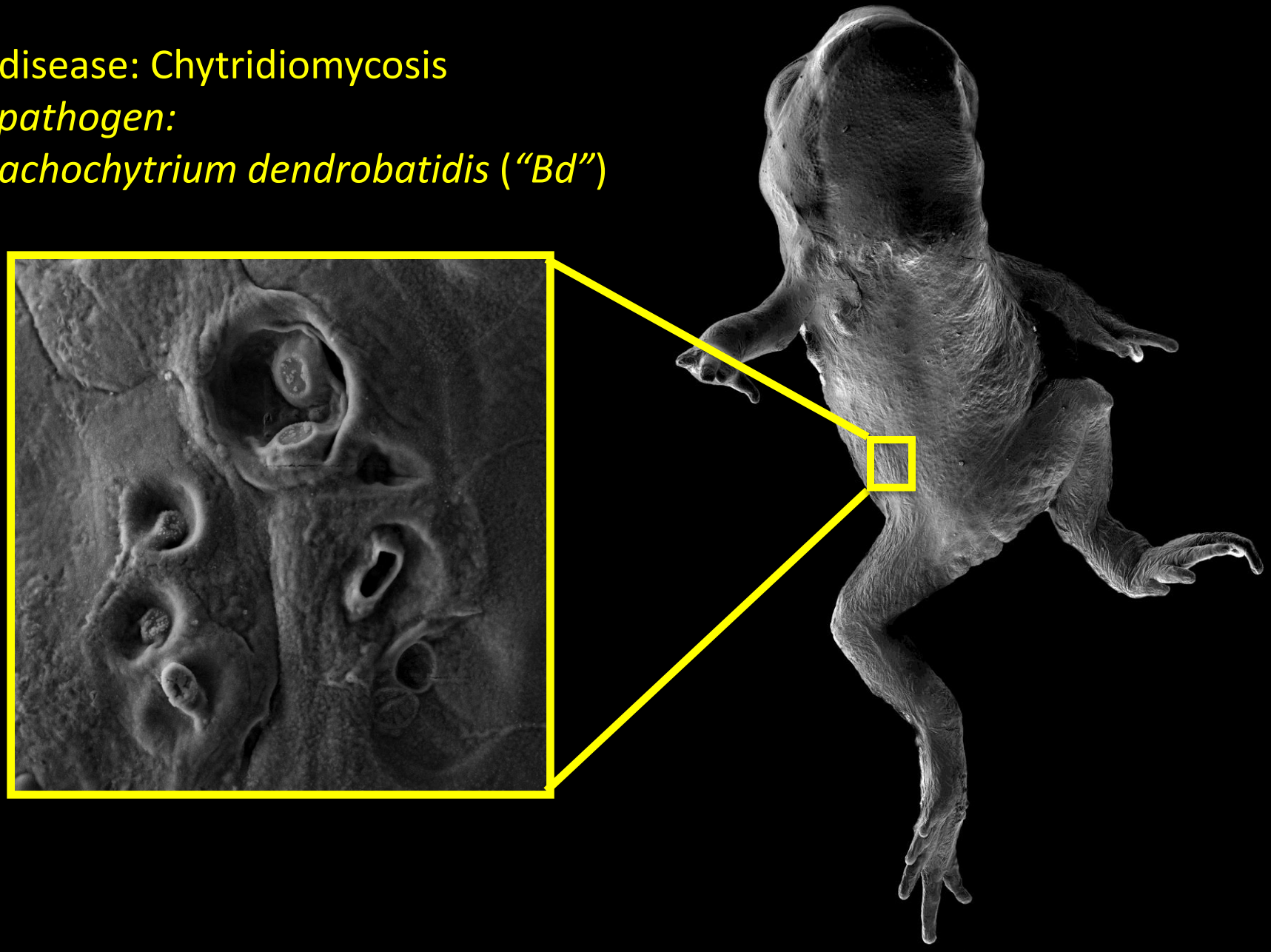


FIG. 2. Numbers of tadpole carcasses collected at Ridge Lake during summer, 1979. Asterisks indicate that no tadpole remains were found on indicated dates.

The disease: Chytridiomycosis

The pathogen:

Batrachochytrium dendrobatidis (*"Bd"*)



Fungal Pathogen:

Batrachochytrium dendrobatidis (Bd)

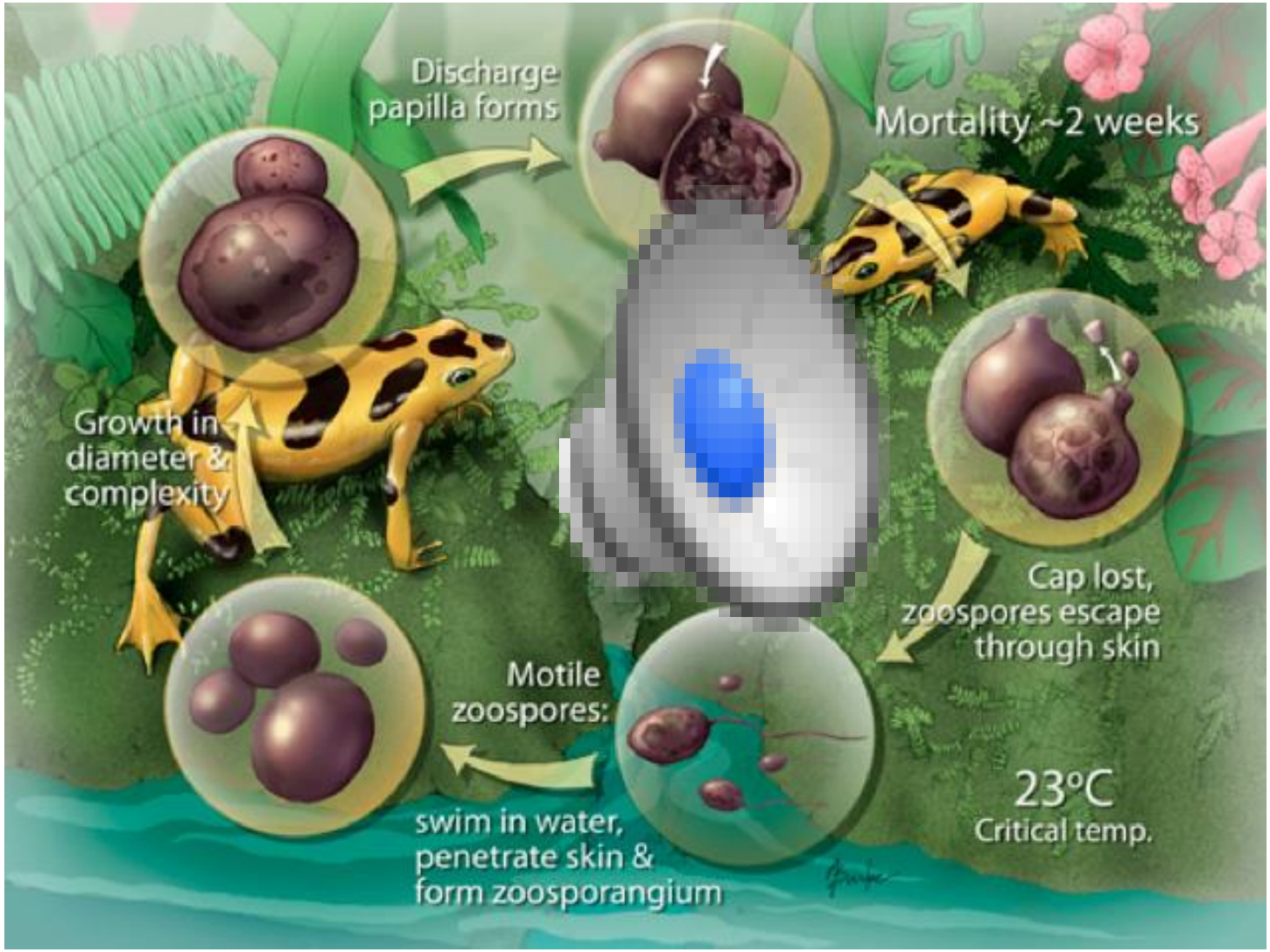
Frog

Chytridiomycete fungus

First described in a dendrobatid frog

Disease: chytridiomycosis

Chytridiomycosis (chytrid)



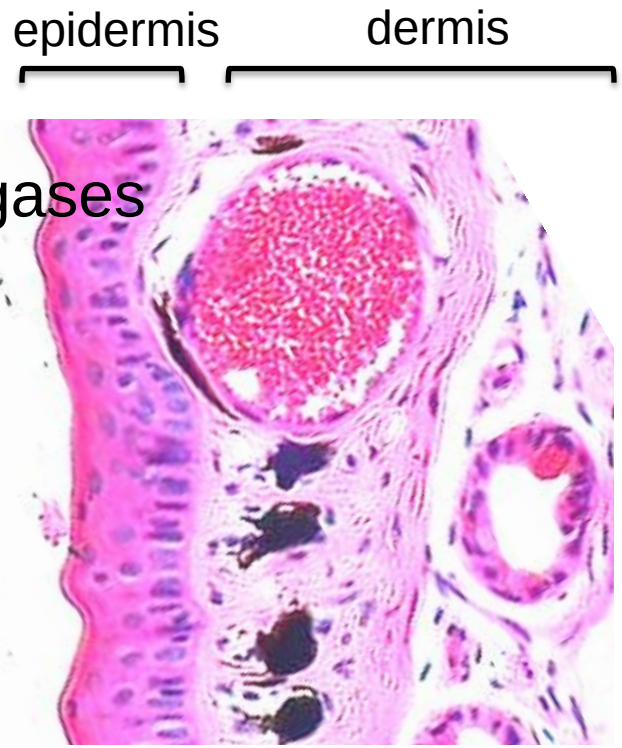
M.Forzan

Detection methods:

- Real time PCR assay
  - Non-destructive
  - Quantitaive 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

REVIEW

Emerging fungal t
and ecosystem hea

Matthew C. Fisher[1], Daniel. A. Henk[1], Cheryl J. Briggs[2],
& Sarah J. Gurr[5]

**The past two decades have seen an increasing numbe
landscapes. In both animals and plants, an unpreco
caused some of the most severe die-offs and extin
security. Human activity is intensifying fungal dise
new opportunities for evolution. We argue that nasc
with wider implications for human and ecosystem h**

Emerging infectious diseases (EIDs) caused by fungi are incre
recognized as presenting a worldwide threat to food se
(Table 1 and Supplementary Table 1). This is not a new p
and fungi have long been known to constitute a widespread threat
species. Plant disease epidemics caused by fungi and the fun
oomycetes have altered the course of human history. In the nin
century, late blight led to starvation, economic ruin and the dow
the English government during the Irish potato famine and,
twentieth century, Dutch elm blight and chestnut blight laid bar
and forest landscapes. The threat of plant disease has not abated,
is heightened by resource-rich farming practices and exaggerate
landscape by microbial adaptation to new ecosystems, brought a
trade and transportation[3], and by climate fluctuations[4,5].

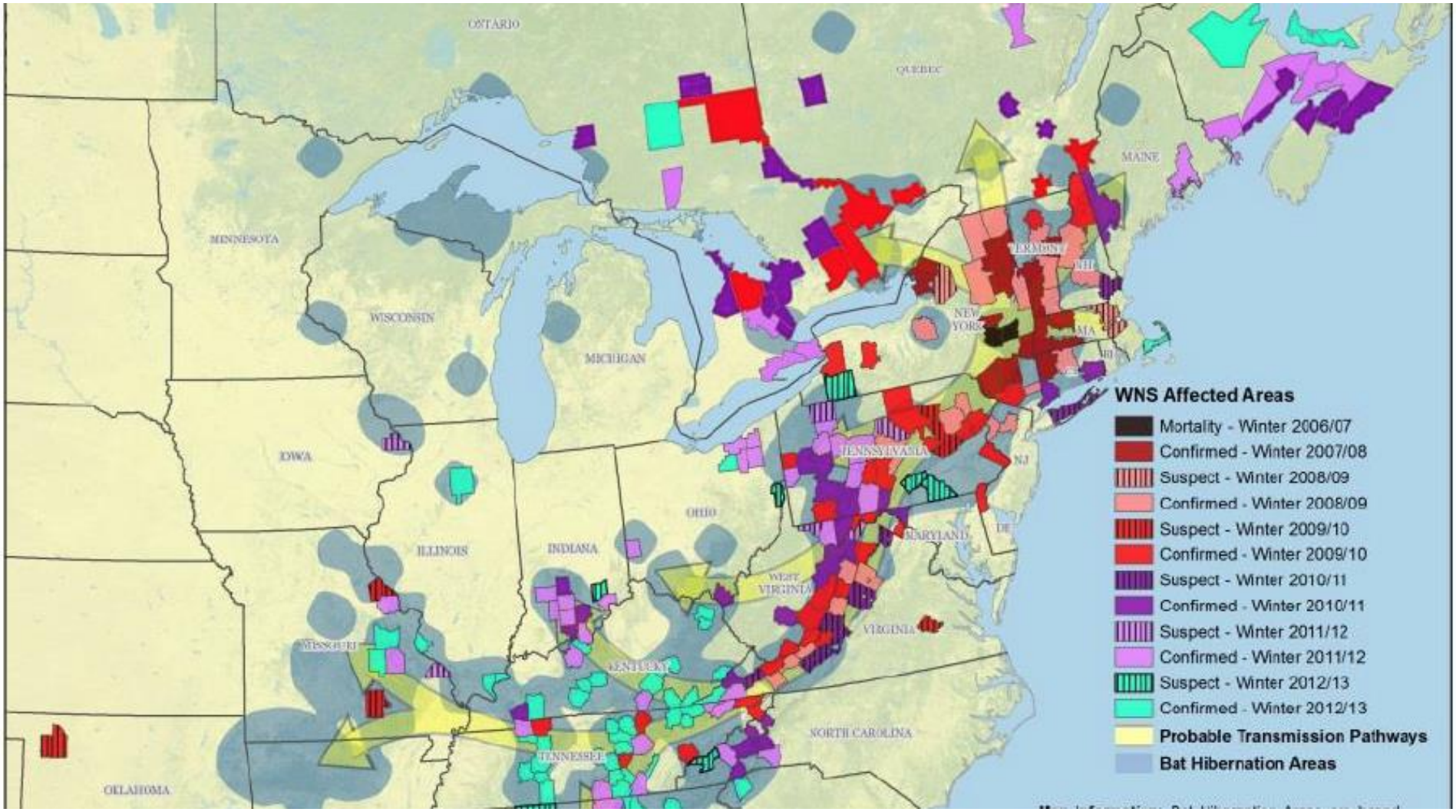
However, pathogenic fungi (also known as mycoses) have n
widely recognized as posing major threats to animal health. T
ception is changing rapidly owing to the recent occurrence of
high-profile declines in wildlife caused by the emergence of pre
unknown fungi[6,7]. For example, during March 2007, a routine ce
bats hibernating in New York State revealed mass mortalities[8]. W
group of closely clustered caves, four species of bats were mark
striking fungus growing on their muzzles and wing membranes,
name 'white nose syndrome' (WNS) was coined. After the init
break, the ascomycete fungus *Geomyces destructans* was shown
Koch's postulates and was described as the cause of WNS in A
bat species[9,10]. Mortalities exhibiting WNS have subsequentl
found in an increasing number of bat overwintering sites a
2010, the infection was confirmed to have emerged in at least 11
across the United States and Canada, spanning over 1,200 km (
Bat numbers across affected sites have declined by over 70% a
lyses have shown that at least one affected species, the little bro
Myotis lucifugus, has a greater than 99% chance of becoming
extinct within the next 16 years (ref. 11). Other species of bats

Institute of Medicine, Board on Global Health
National Academy of Sciences

Fungal Diseases: An emerging challenge to human, animal, and plant health.

Human	Animal	Plant
Cryptococcal disease *Cryptococcus gattii*	**Colony Collapse Syndrome (bees)** *Nosema apis*	**Sudden Oak Death** *Phytophthora ramorum*
Coccidioidomycosis (Valley Fever) *Coccidioides immitis*	**Aspergillosis (birds)** *Aspergillus fumigatus*	**Fusarium wilt (tomatoes etc.)** *Fusarium oxysporum*
Aspergillosis *Aspergillus fumigatus*	**White-nose Syndrome (bats)** *Geomyces destructans*	**Stripe rust (wheat)** *Puccinia striiformis*
Candida *Candida albicans*	**Chytridiomycosis (amphibians)** *Batrachochytrium dendrobatidis*	**Chestnut blight** *Cryphonectria parasitica*

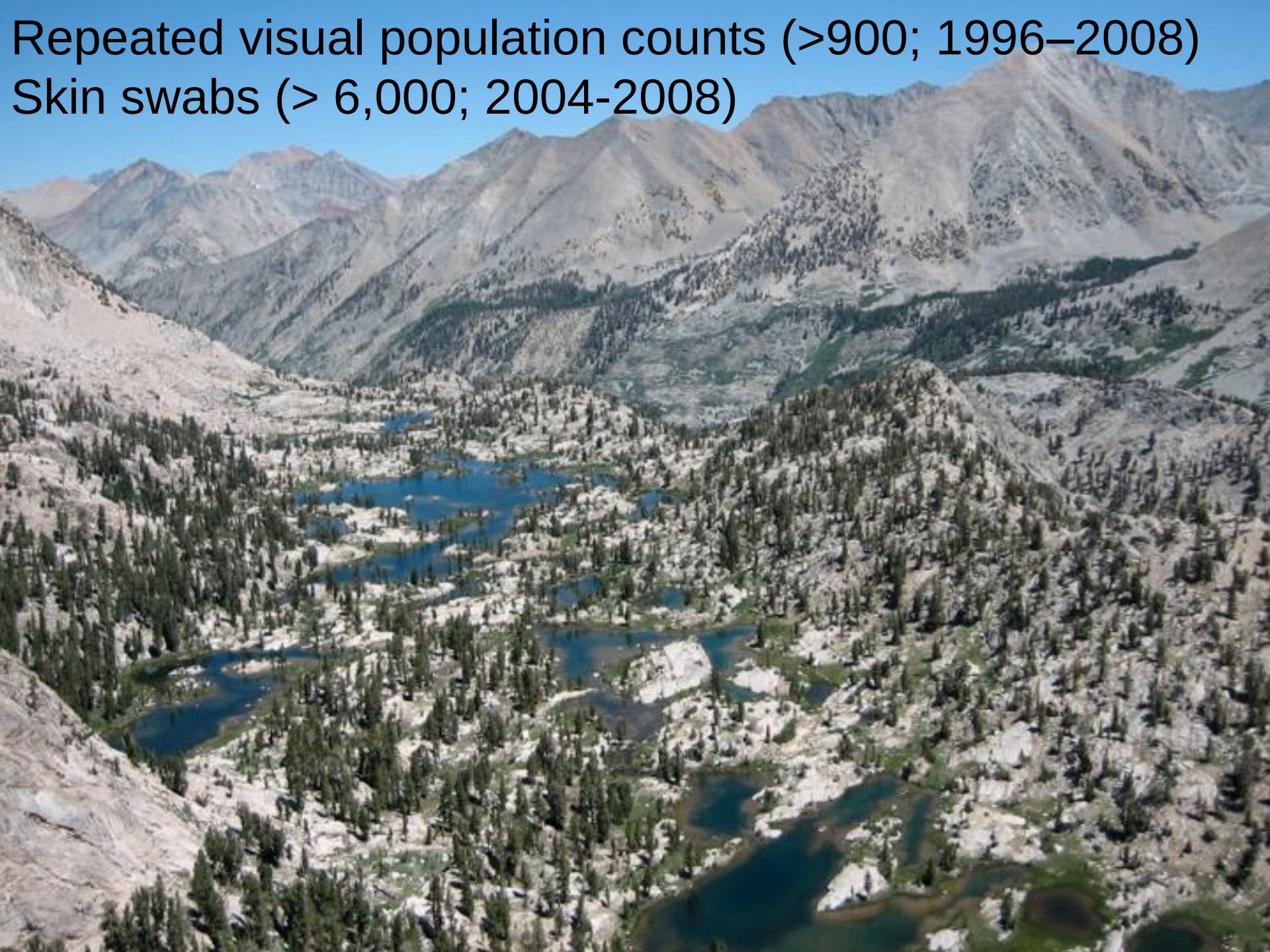
batcon.org
BAT CONSERVATION
INTERNATIONAL



Sierra Nevada, California

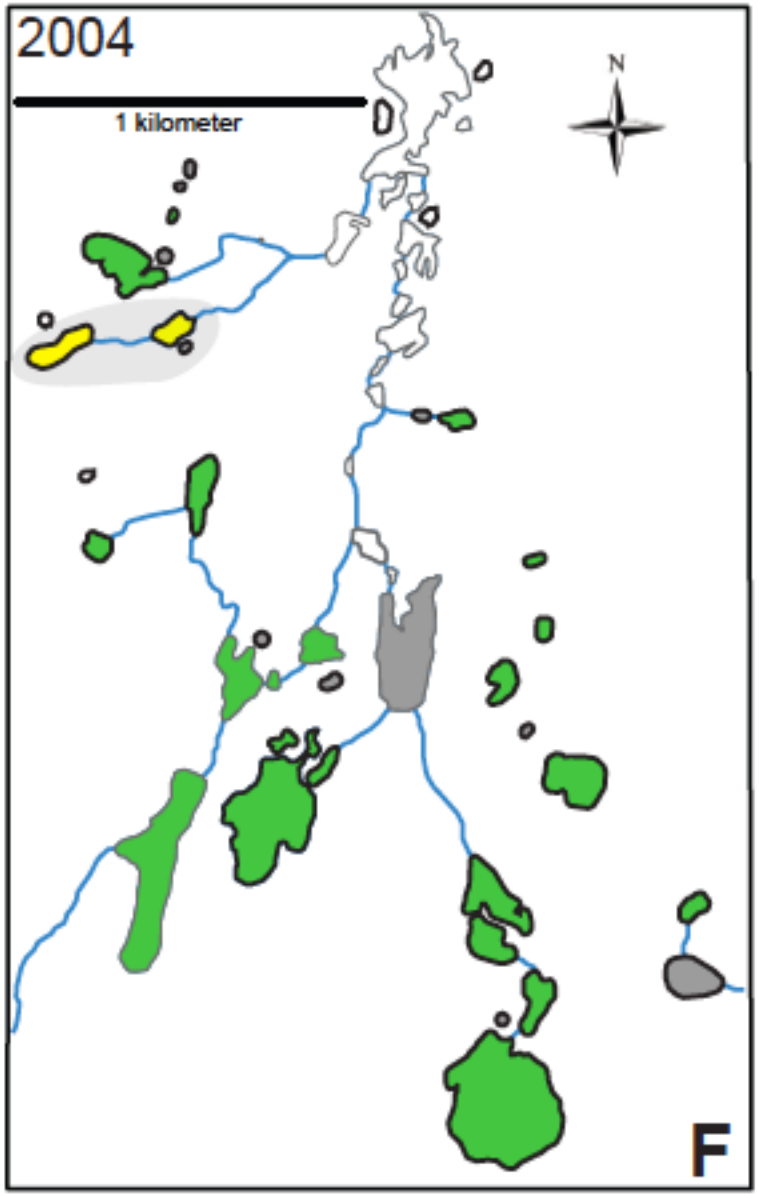
How does this pathogen work???

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



Source: National Park Service THE NEW YORK TIMES

(Vredenburg, et al. 2010; *PNAS*)

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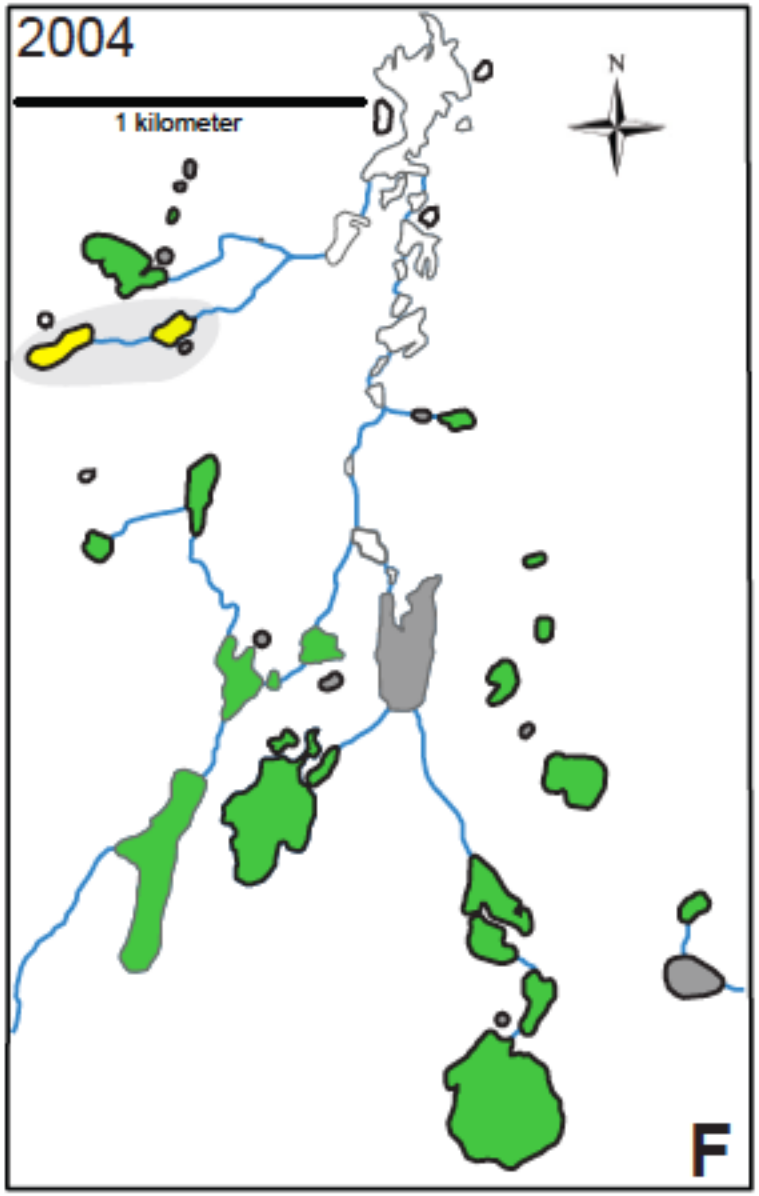

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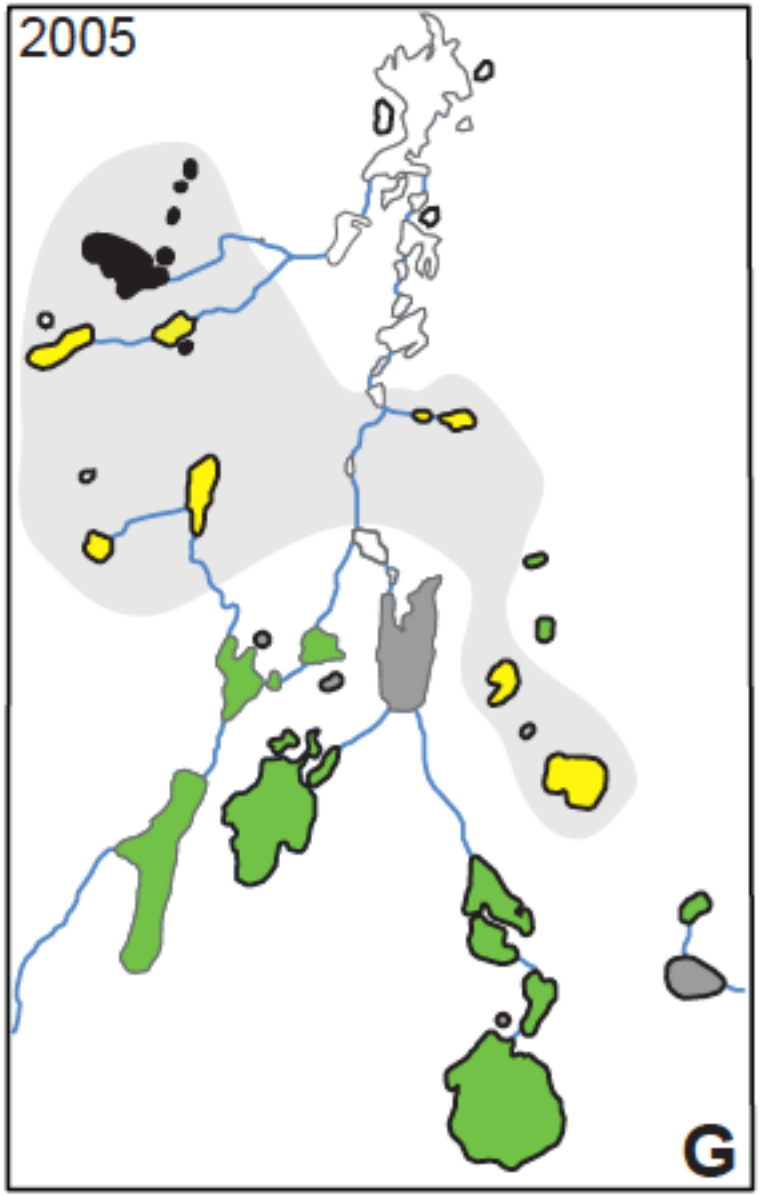

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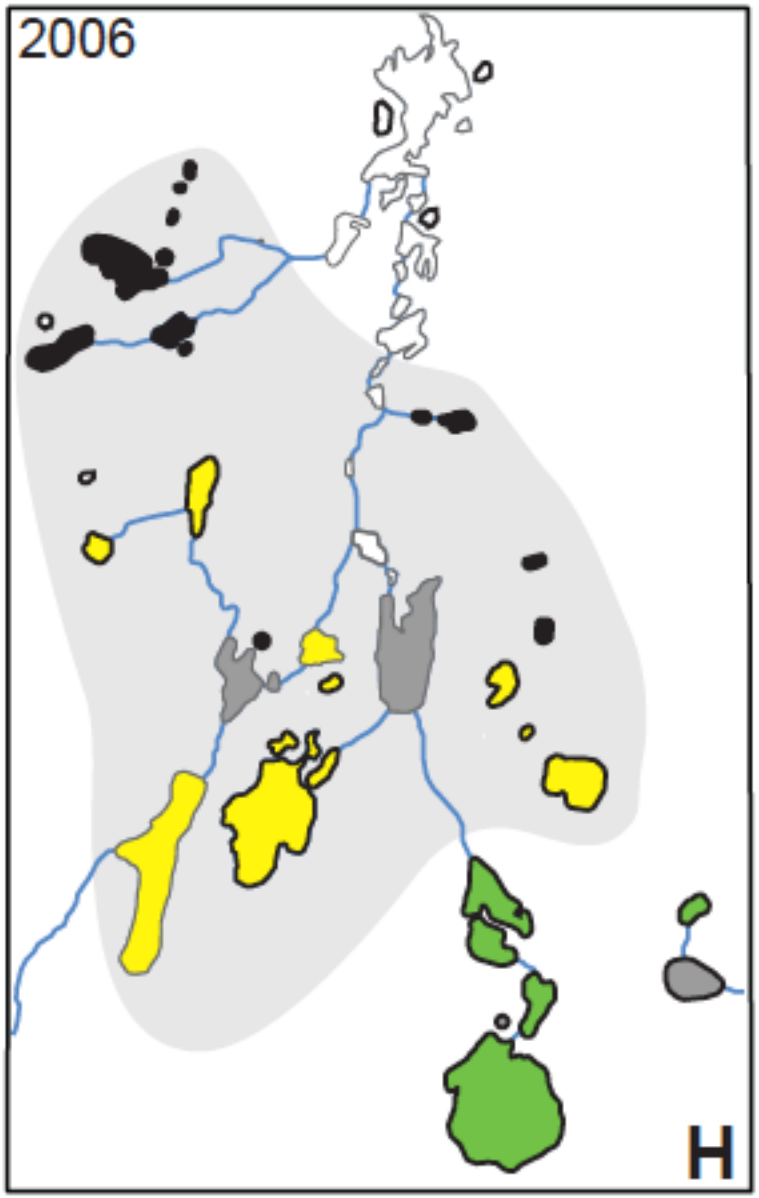

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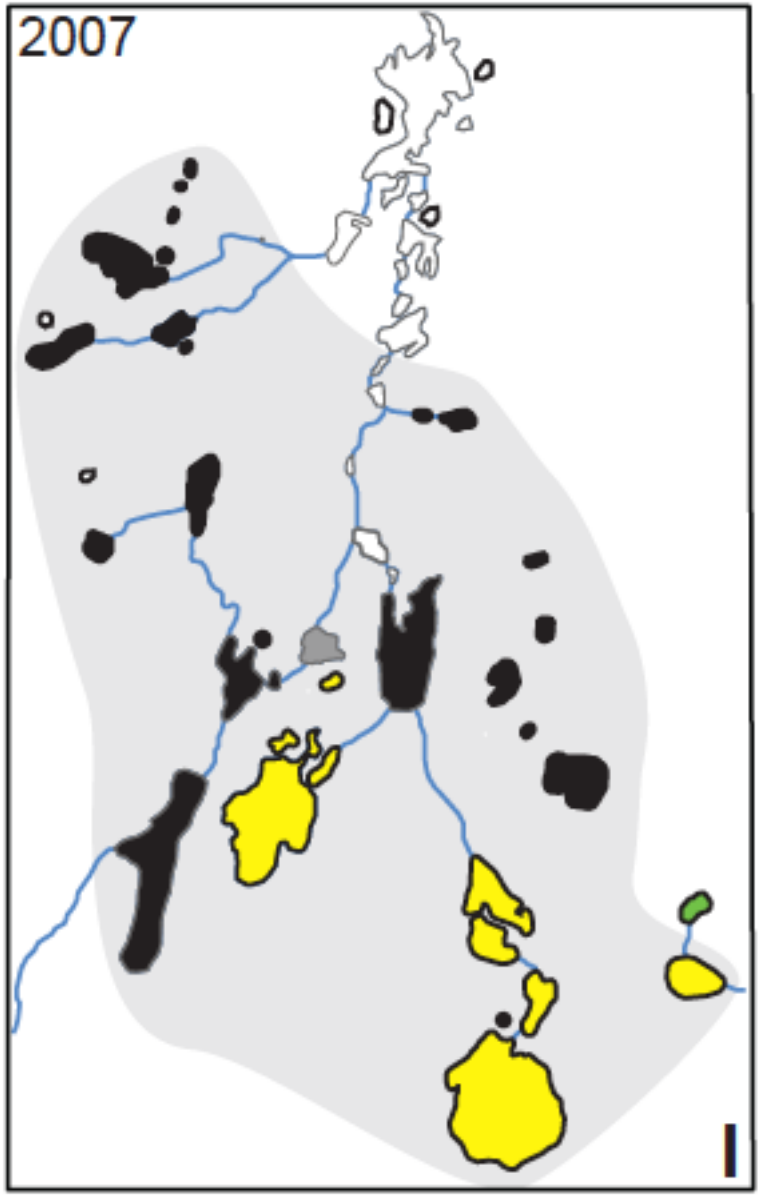

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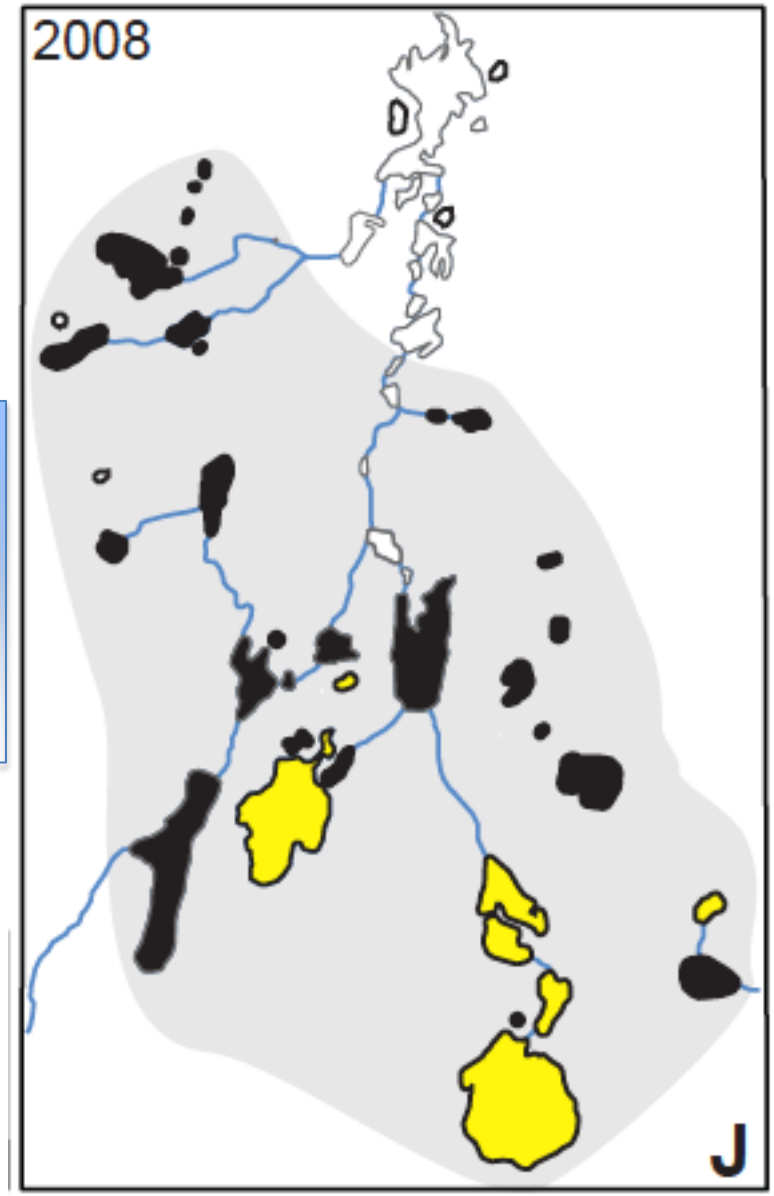

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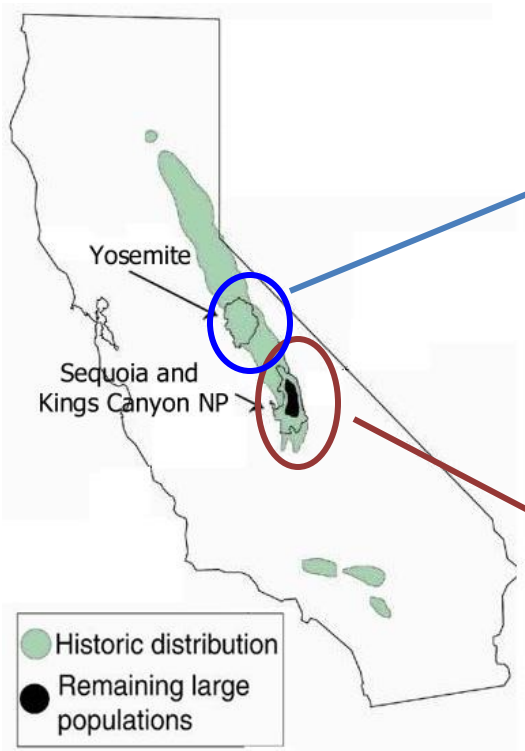
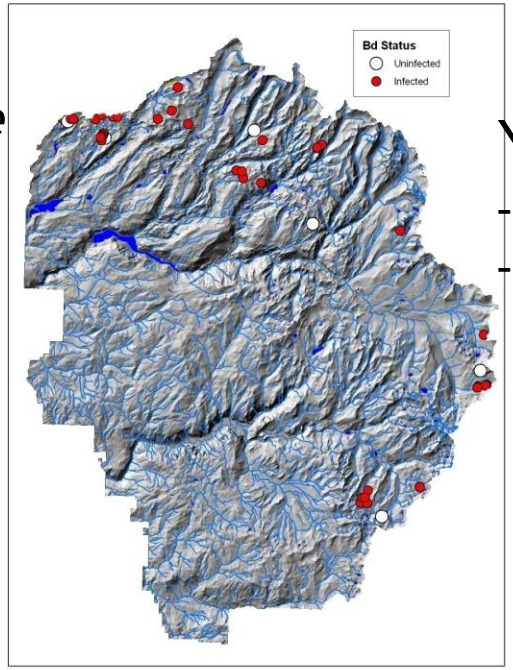
Mountain Yellow-legged Frogs
Rana muscosa & Rana sierrae

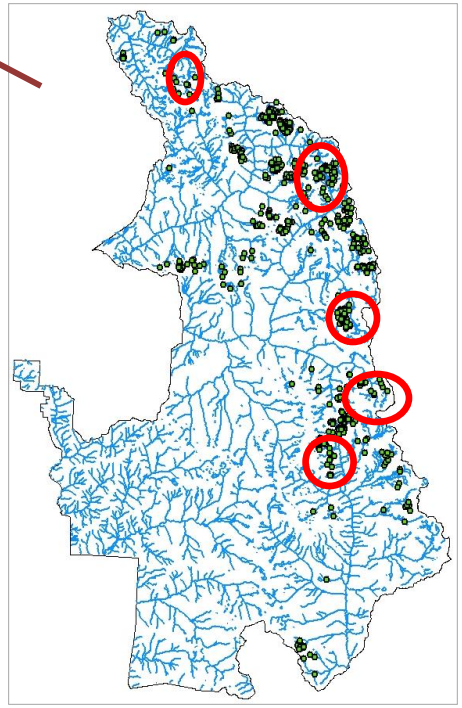




Yosemite National Park
- Infected since 1970's or 80's?
- Small infected populations of frogs are persisting with Bd.

"persistence"

endemic



Sequoia & Kings Canyon National Parks
- Bd currently spreading, leading to 100's of local extinctions.

rapid population decline/ local extinctions

epidemic

Different Outcomes in Different Species

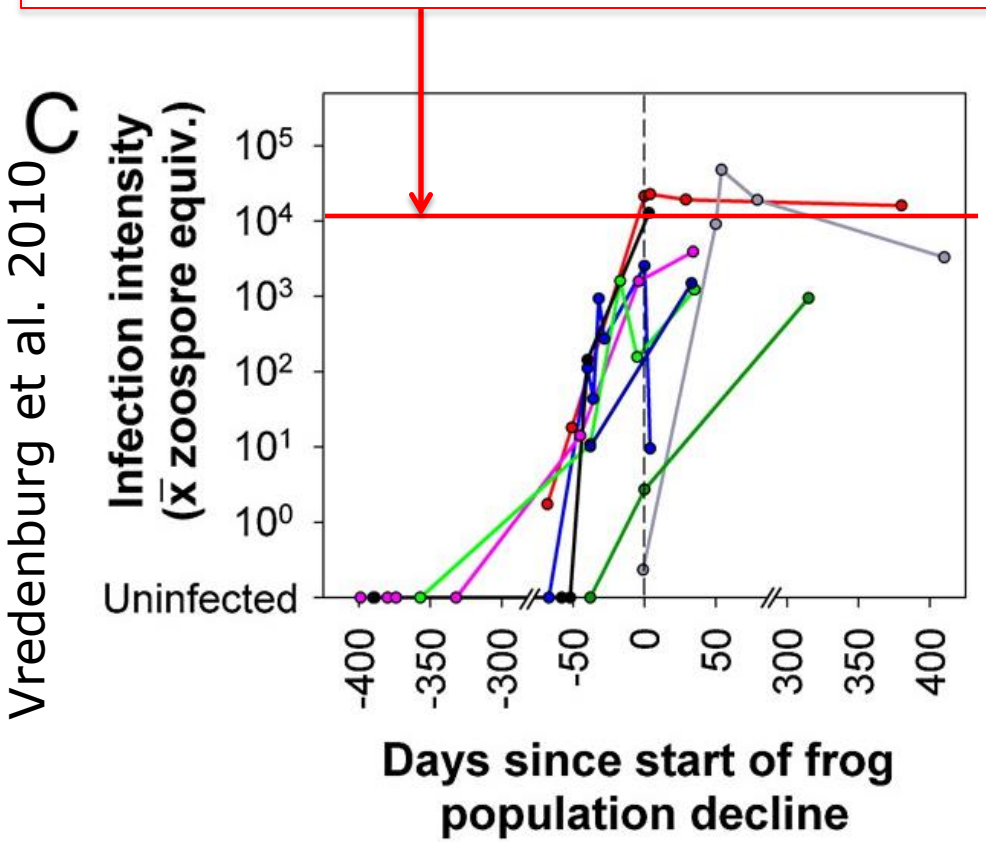
Lithobates catesbeiana

Litoria moorei

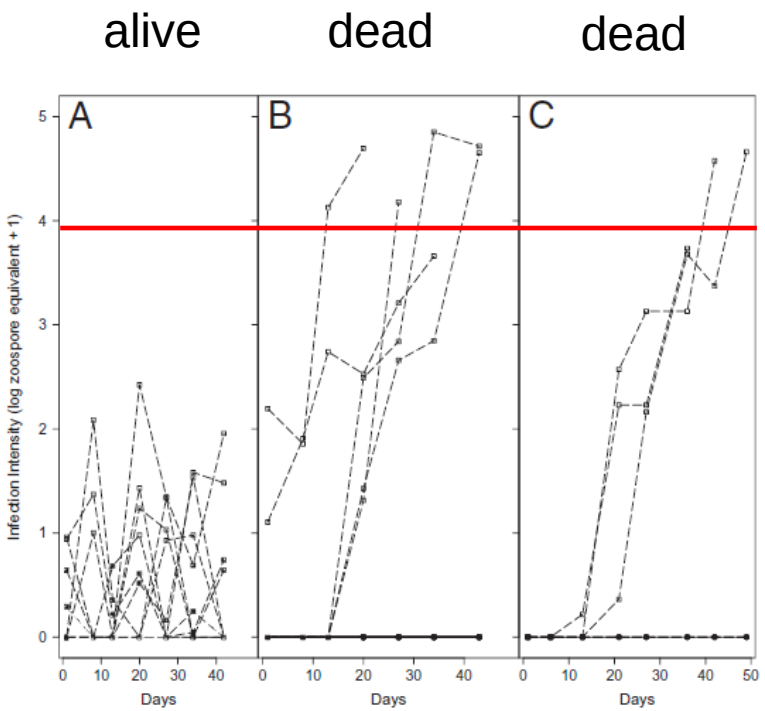
Xenopus laevis

Xenopus tropicalis

We discovered a *Mortality Threshold*



Vredenburg et al. 2010



Cheng et al. 2011

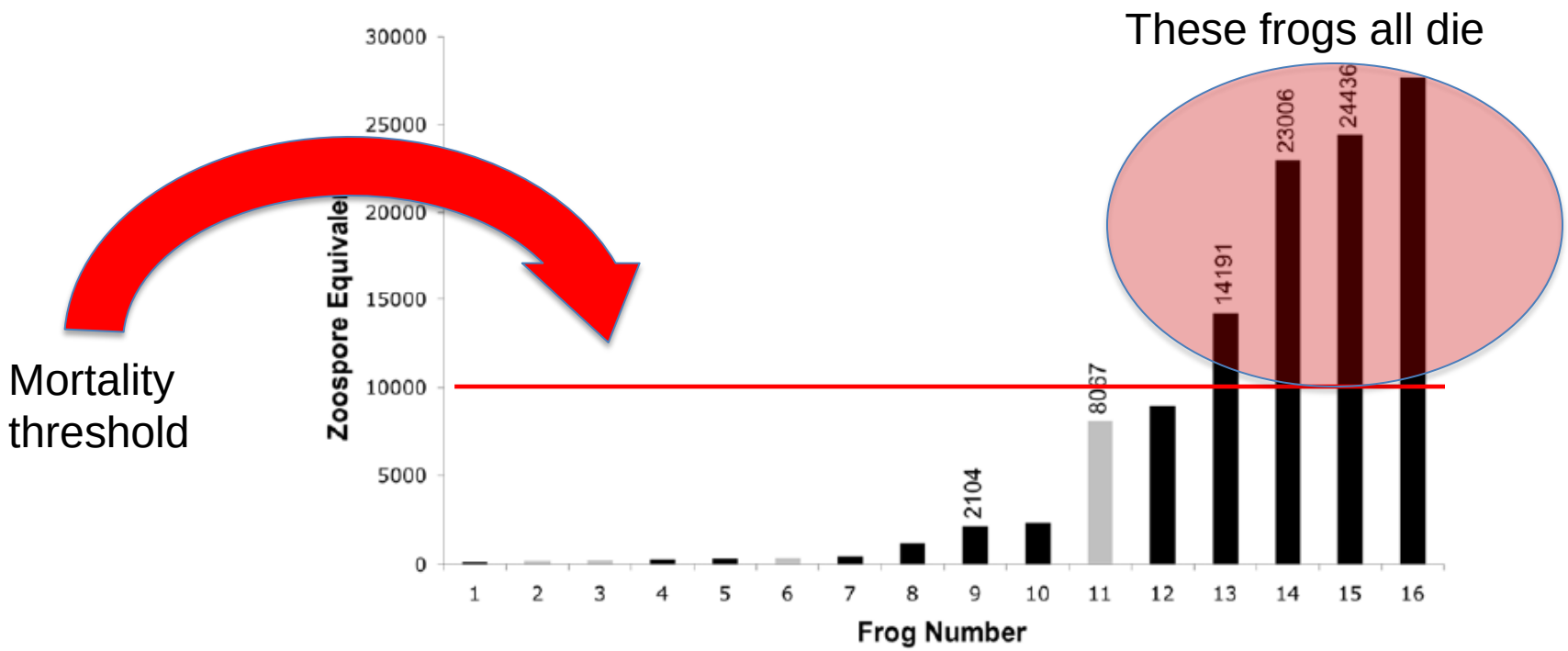
How does Bd work?

OPEN ACCESS Freely available online

PLoS one

Seasonal Pattern of *Batrachochytrium dendrobatidis* Infection and Mortality in *Lithobates areolatus*: Affirmation of Vredenburg's "10,000 Zoospore Rule"

Vanessa C. Kinney[1], Jennifer L. Heemeyer[1], Allan P. Pessier[2], Michael J. Lannoo[3]*



OPEN ACCESS Freely available online

PLoS one

A Reservoir Species for the Emerging Amphibian Pathogen *Batrachochytrium dendrobatidis* Thrives in a Landscape Decimated by Disease

Natalie M. M. Reeder[1]*, Allan P. Pessier[2], Vance T. Vredenburg[1]

1 Department of Biology, San Francisco State University, San Francisco, California, United States of America, 2 Wildlife Disease Laboratories, Institute for Conservation Research, San Diego Zoo Global, San Diego, California, United States of America

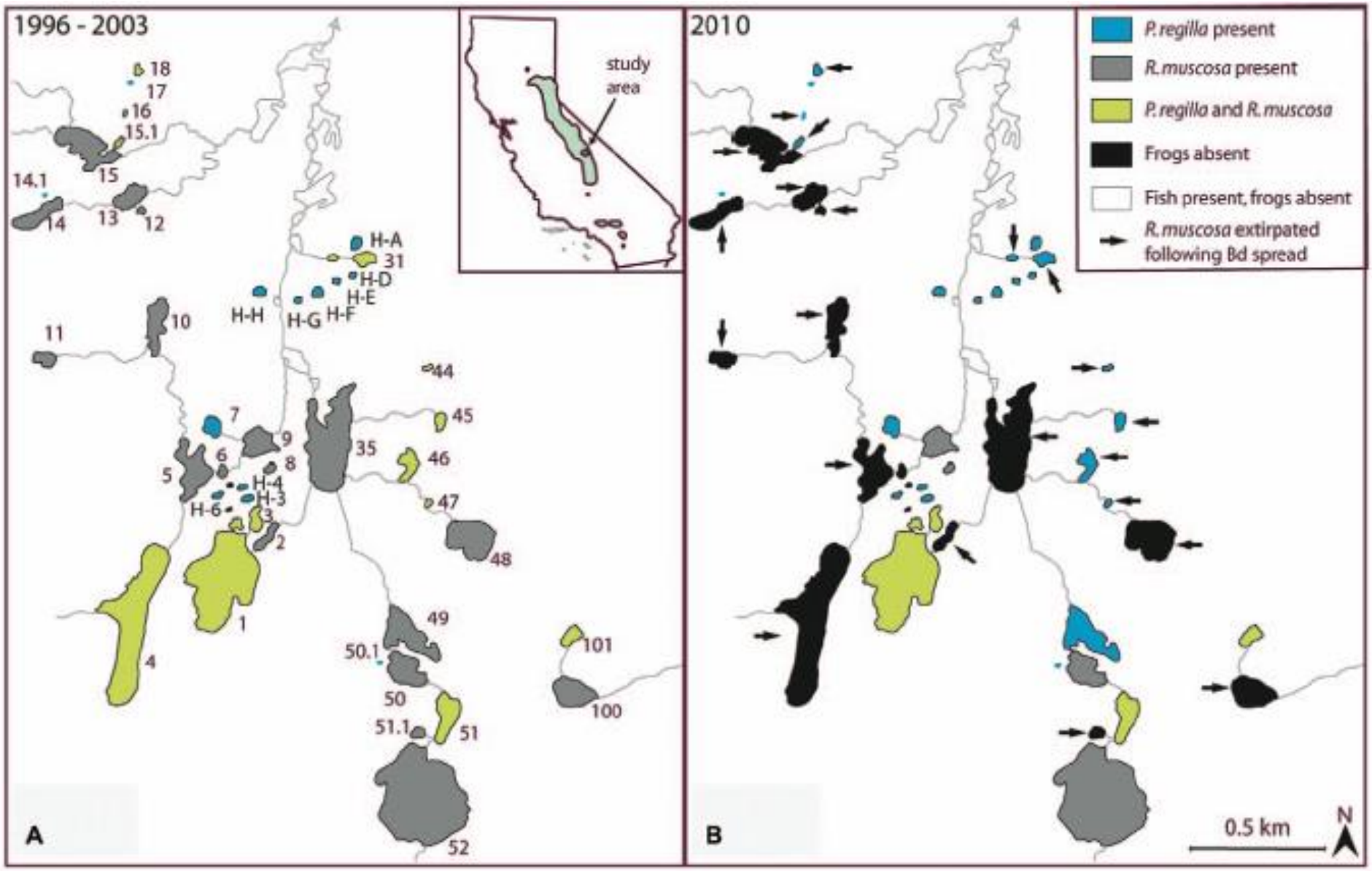
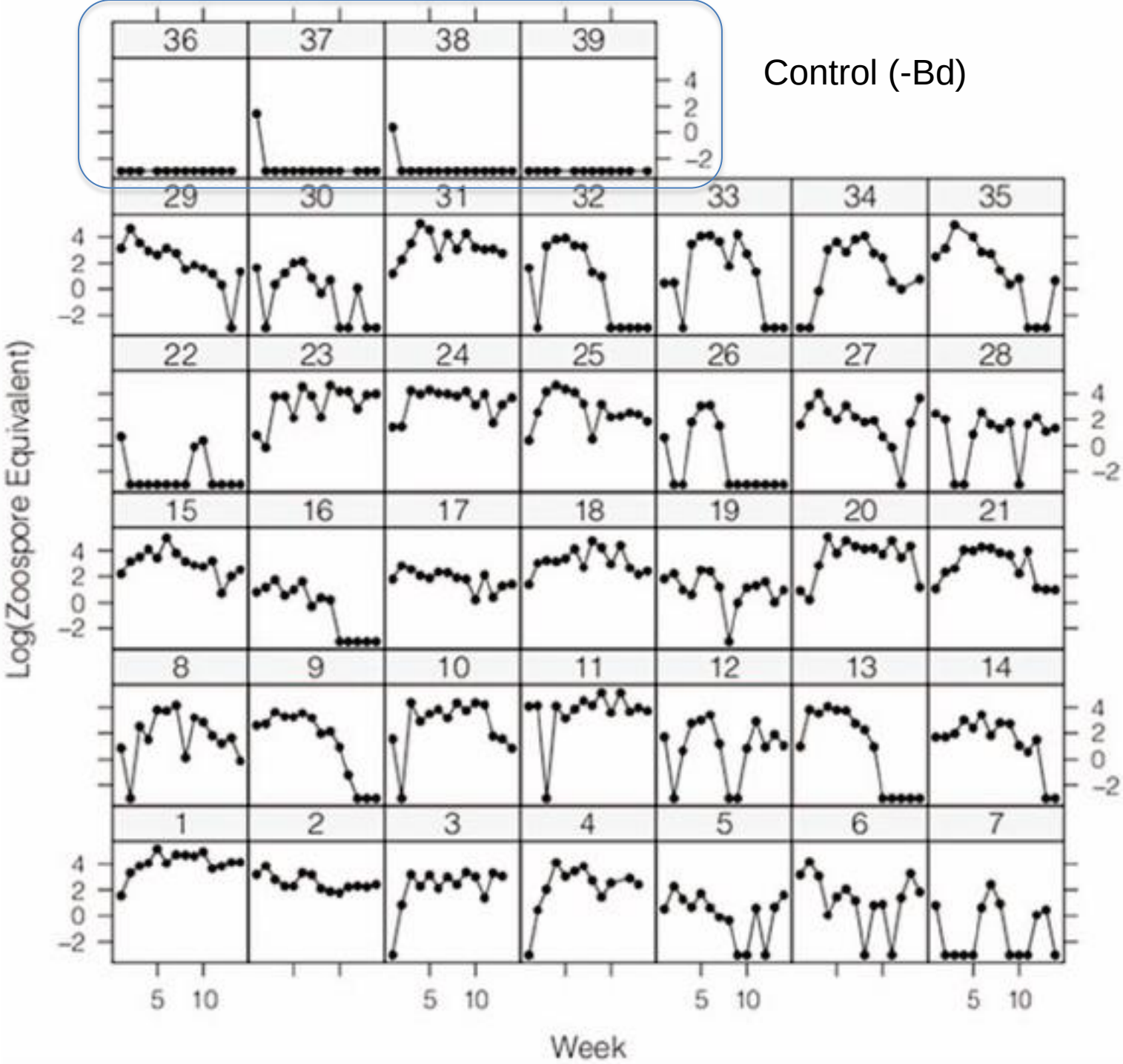


Figure 1. Habitat occupancy before and after disease spread. Range of *P. regilla* and *R. muscosa* in 60 Lake Basin (a) before and (b) after the spread of *Bd* through the basin.
doi:10.1371/journal.pone.0033567.g001


Control (-Bd)
36
37
38
39
29
30
31
32
33
34
35
22
23
24
25
26
27
28
15
16
17
18
19
20
21
8
9
10
11
12
13
14
1
2
3
4
5
6
7
Log(Zoospore Equivalent)
Week

Bd infection is localized on the skin – most of the skin continues to function

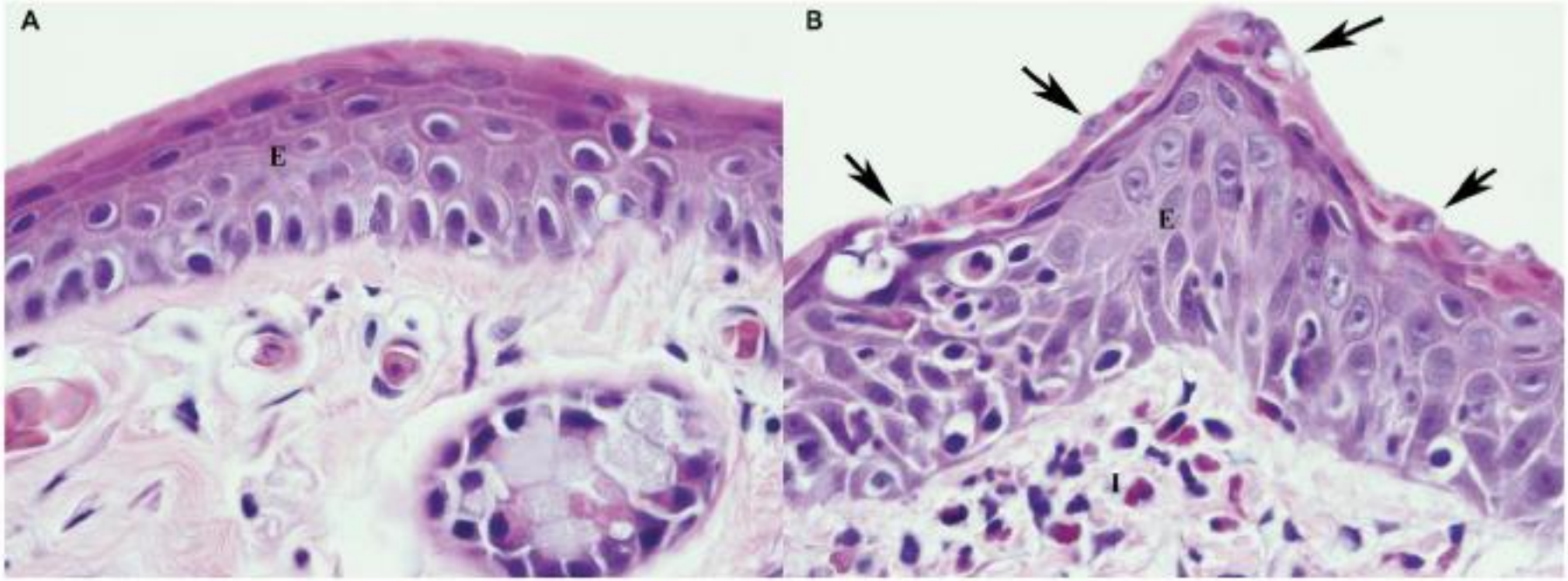
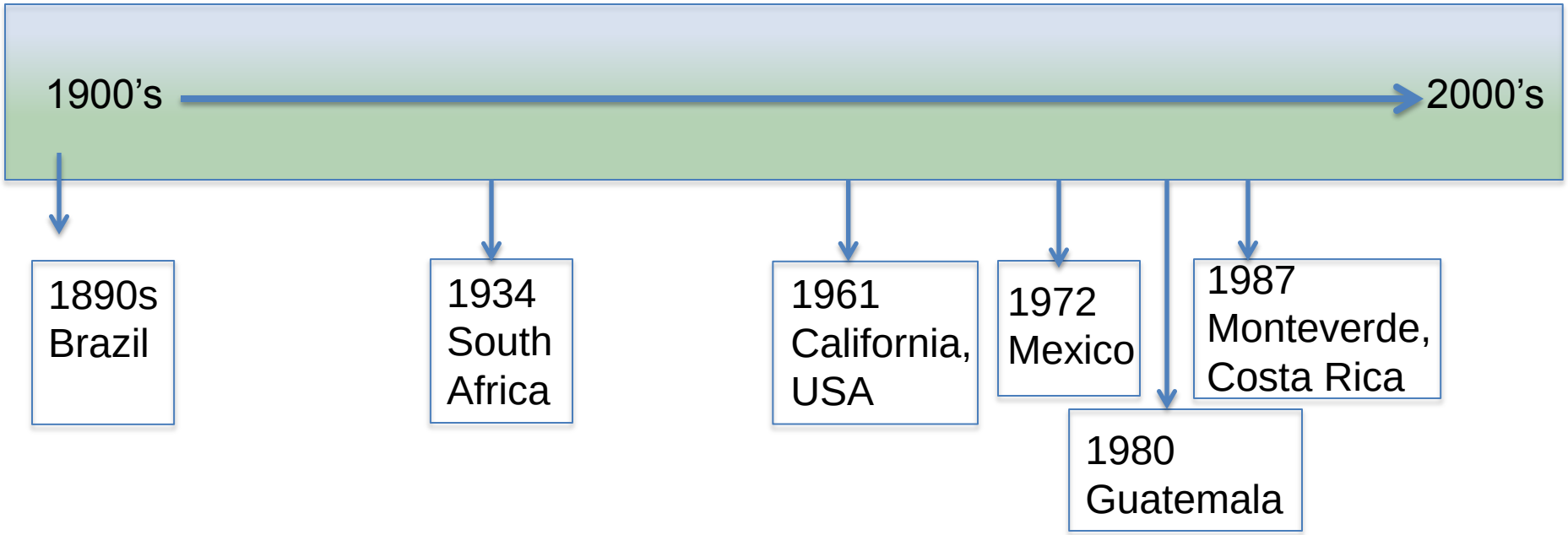


Figure 5. Histological views of skin from an infected but asymptomatic individual. Photomicrographs of histologic sections from the foot webbing of *P. regilla* # 32. In (a) the epidermis is well organized with minimal variation in nuclear size and a single keratinized layer consistent with normal foot 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.

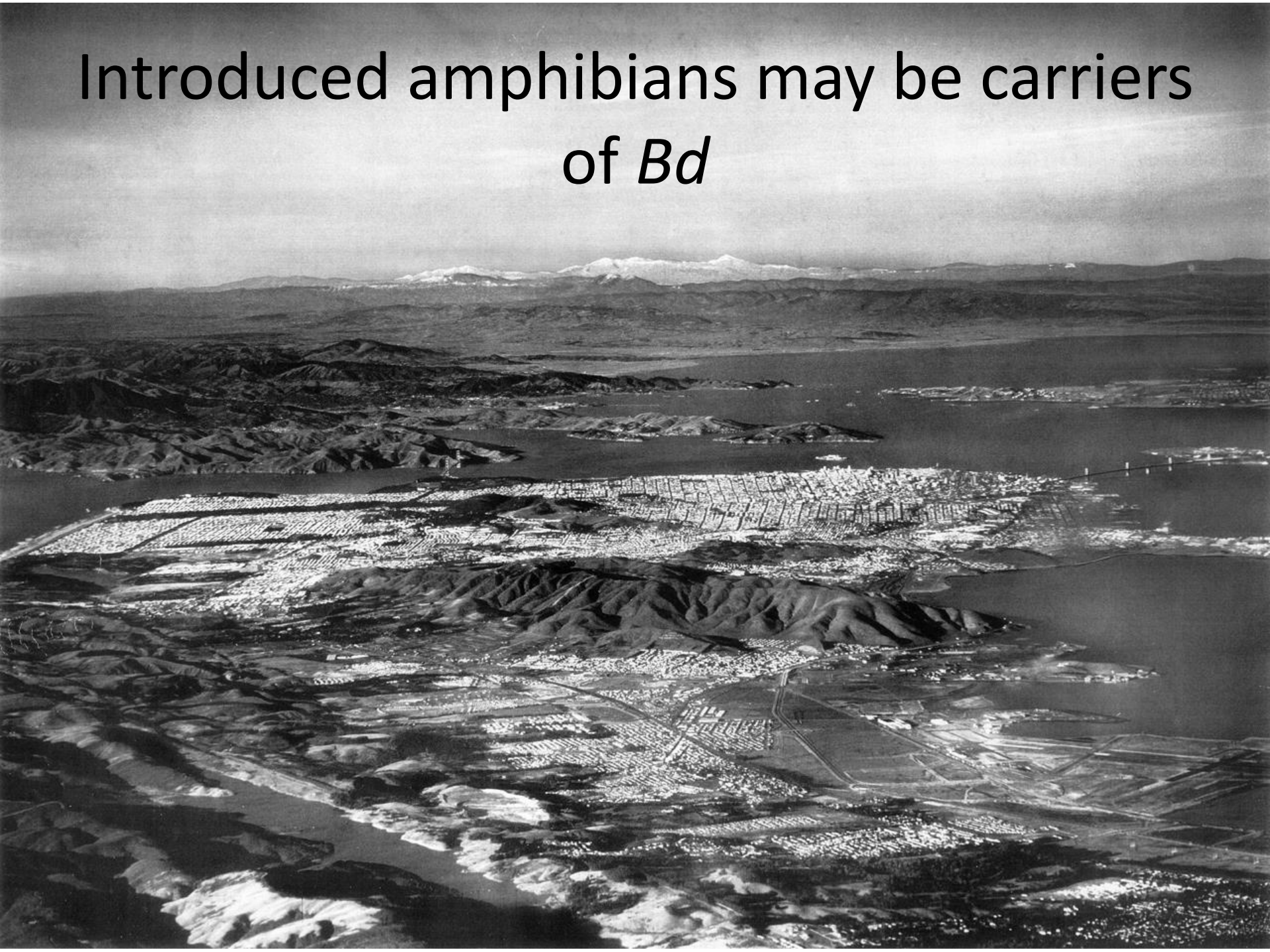
Introduced frogs spread Bd

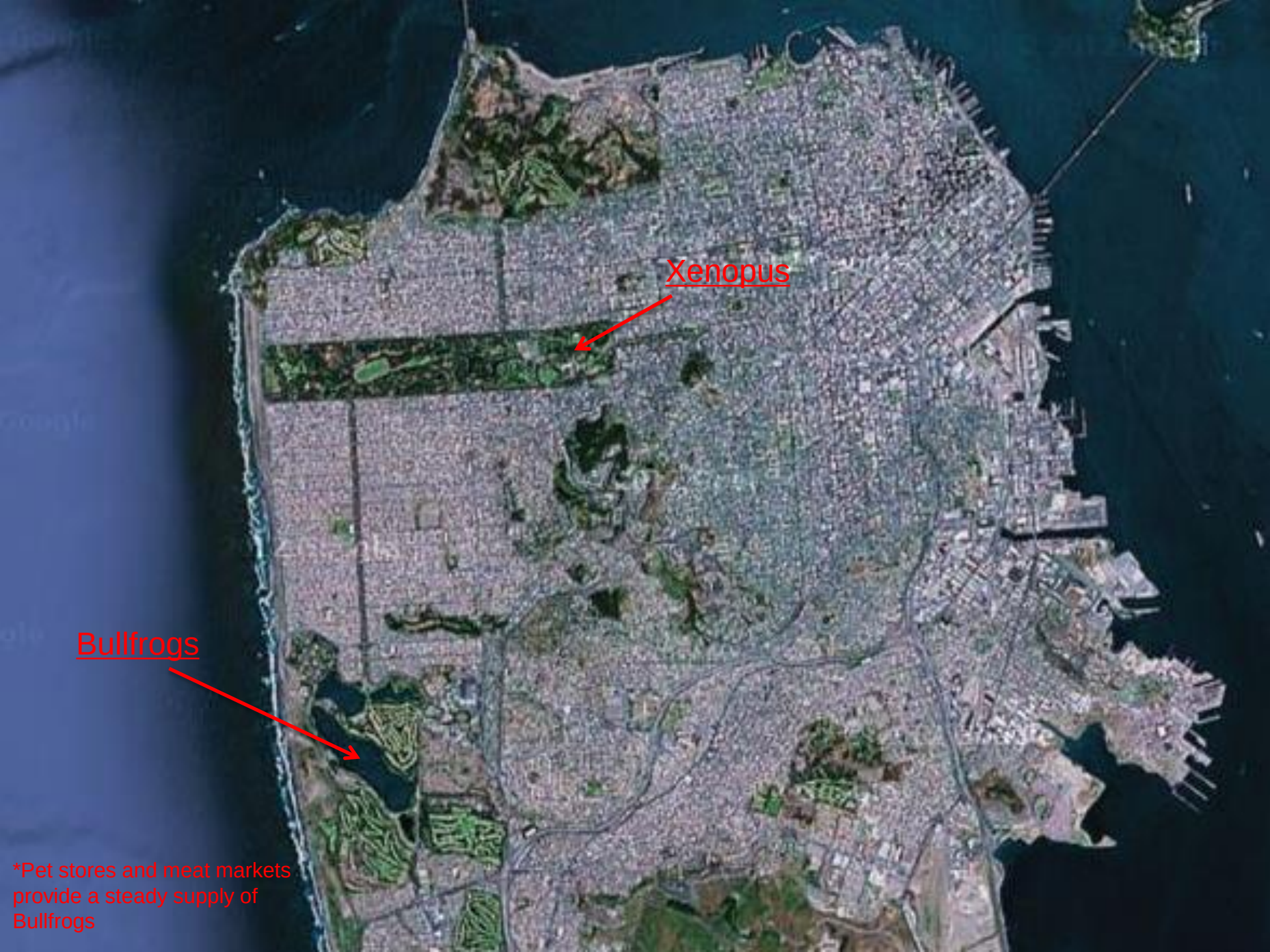
The transport and release of *Xenopus laevis* or other non-native carrier species followed by spread of *Batrachochytrium dendrobatidis* in native amphibians.

Vredenburg et al 2013 PLOS ONE

Frog legs anyone?
American Bullfrog
(*Rana catesbeiana*)

Introduced amphibians may be carriers of *Bd*

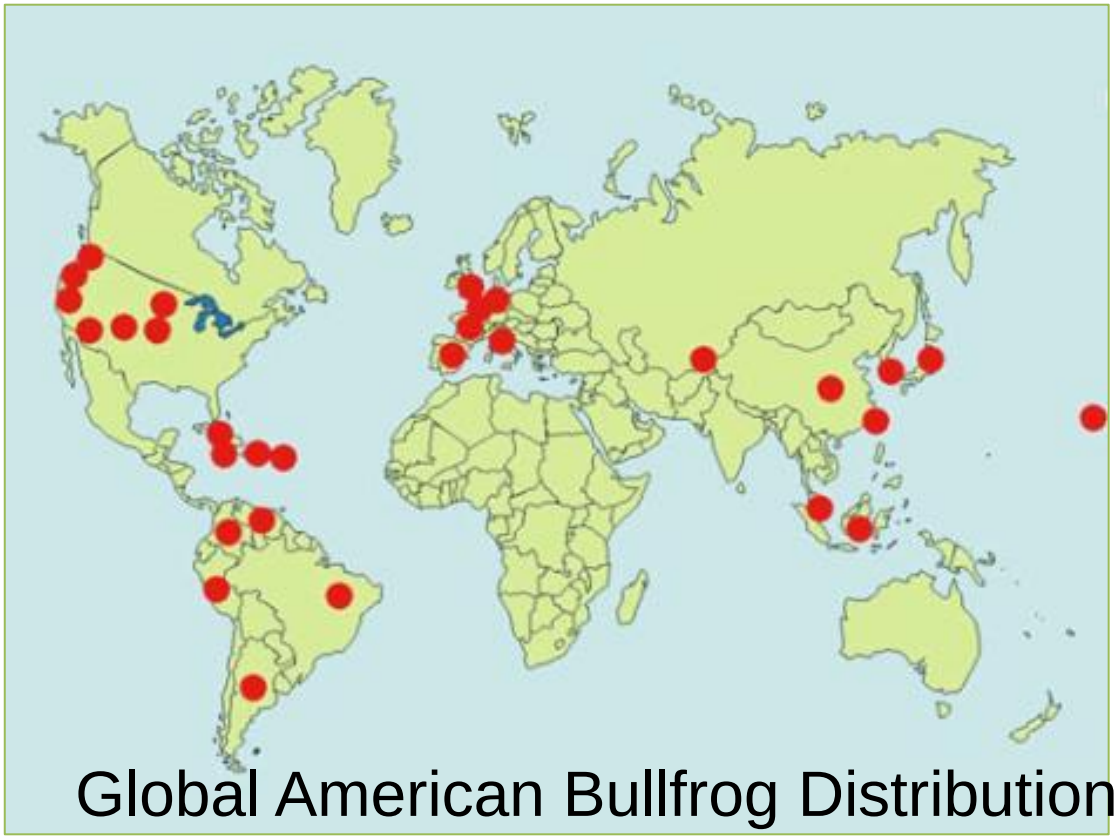




*Pet stores and meat markets provide a steady supply of Bullfrogs

How did Bd spread?

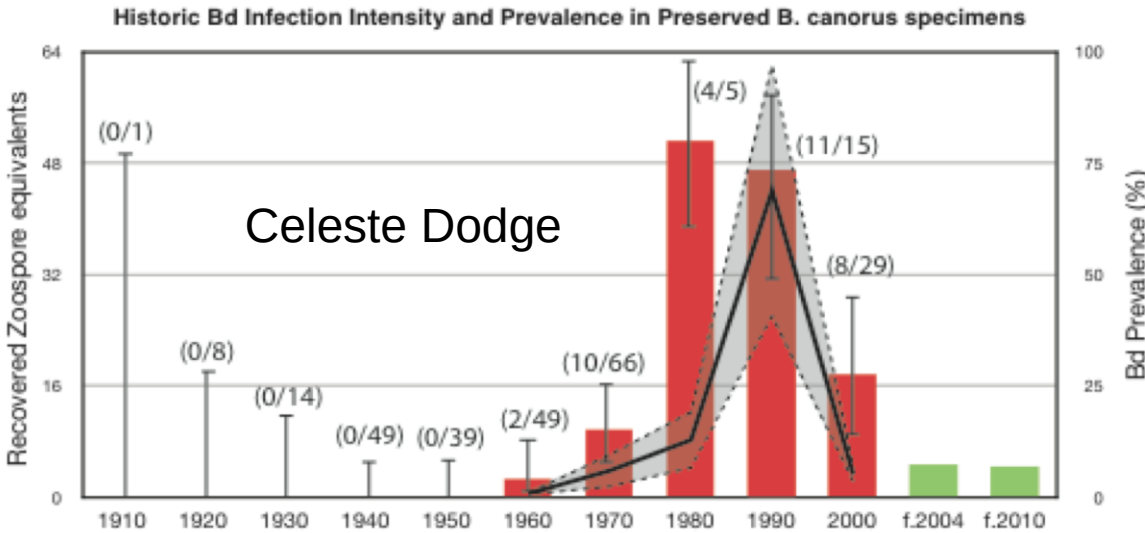
- Not all species are susceptible
- Some act as disease reservoirs or vectors

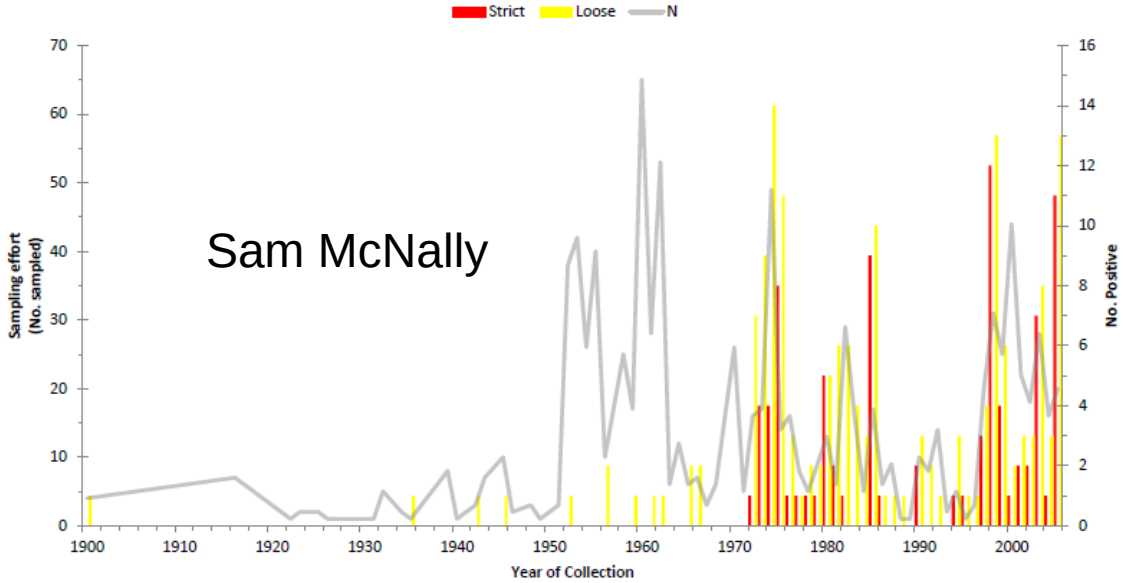


Lannoo 1993

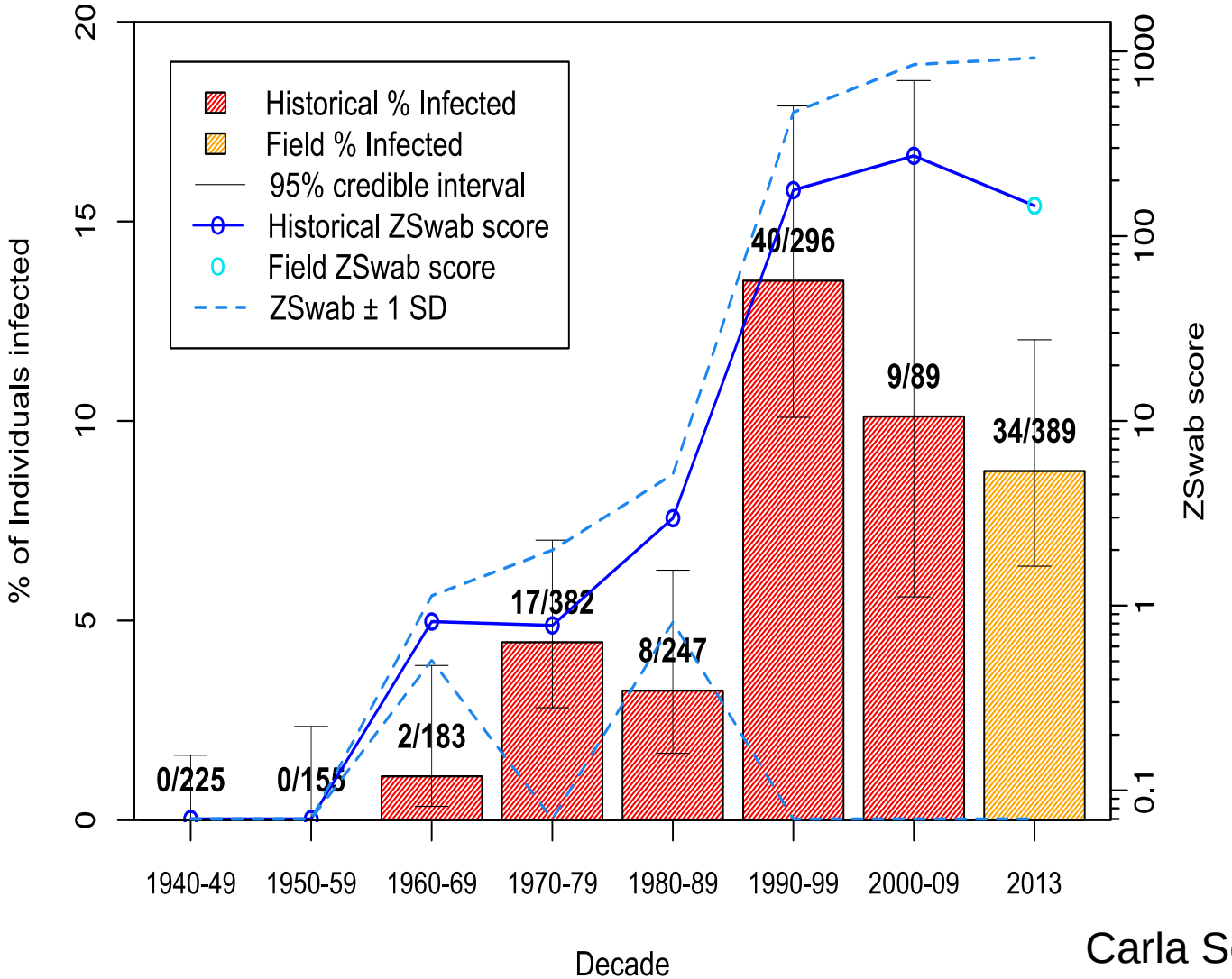
Farms, Pet and Food Trade in Asia (Yunnan, China)

When did *Bd* invade California?



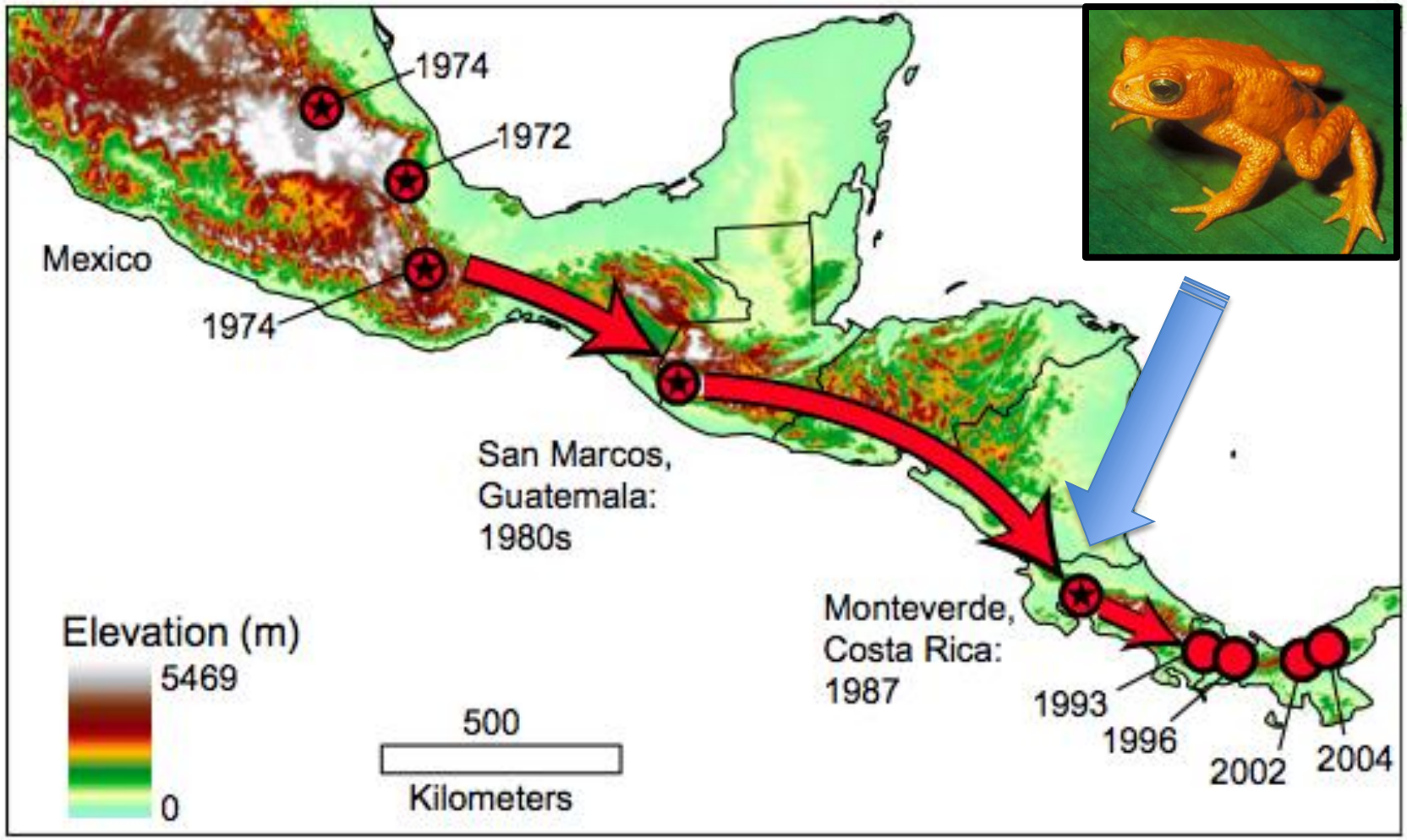


Bd emergence in *Batrachoseps attenuatus*



Carla Sette

Museum specimens reveal how fungal epidemic spread in amphibians

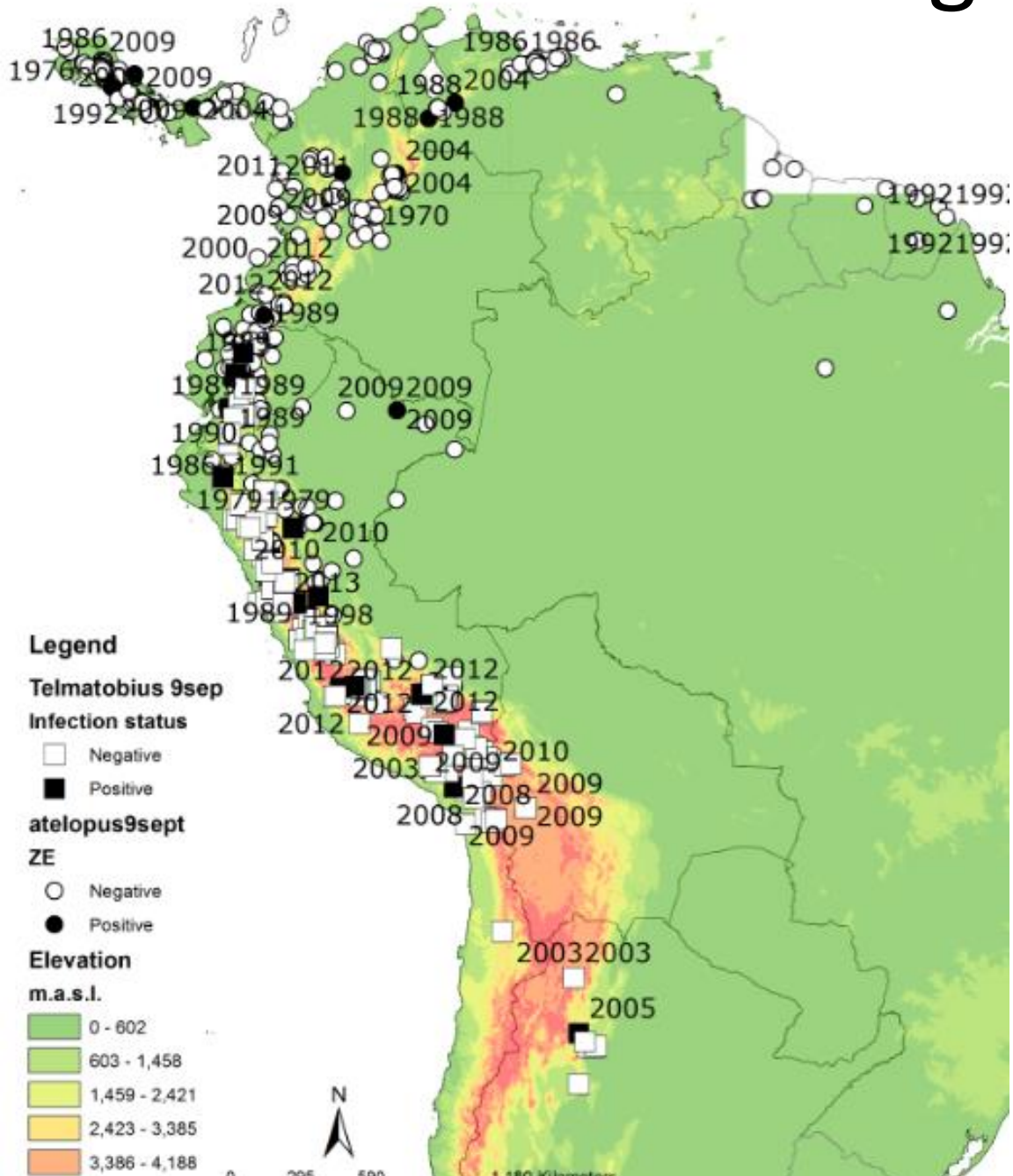


Cheng, T. L., S. M. Rovito, D. B. Wake, and V. T. Vredenburg. 2011. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen Batrachochytrium dendrobatidis. *Proceedings of the National Academy of Sciences*.

Timeline of proposed Bd epidemics in the Andes

Lips *et al.* 2008, *PLoS Biology*

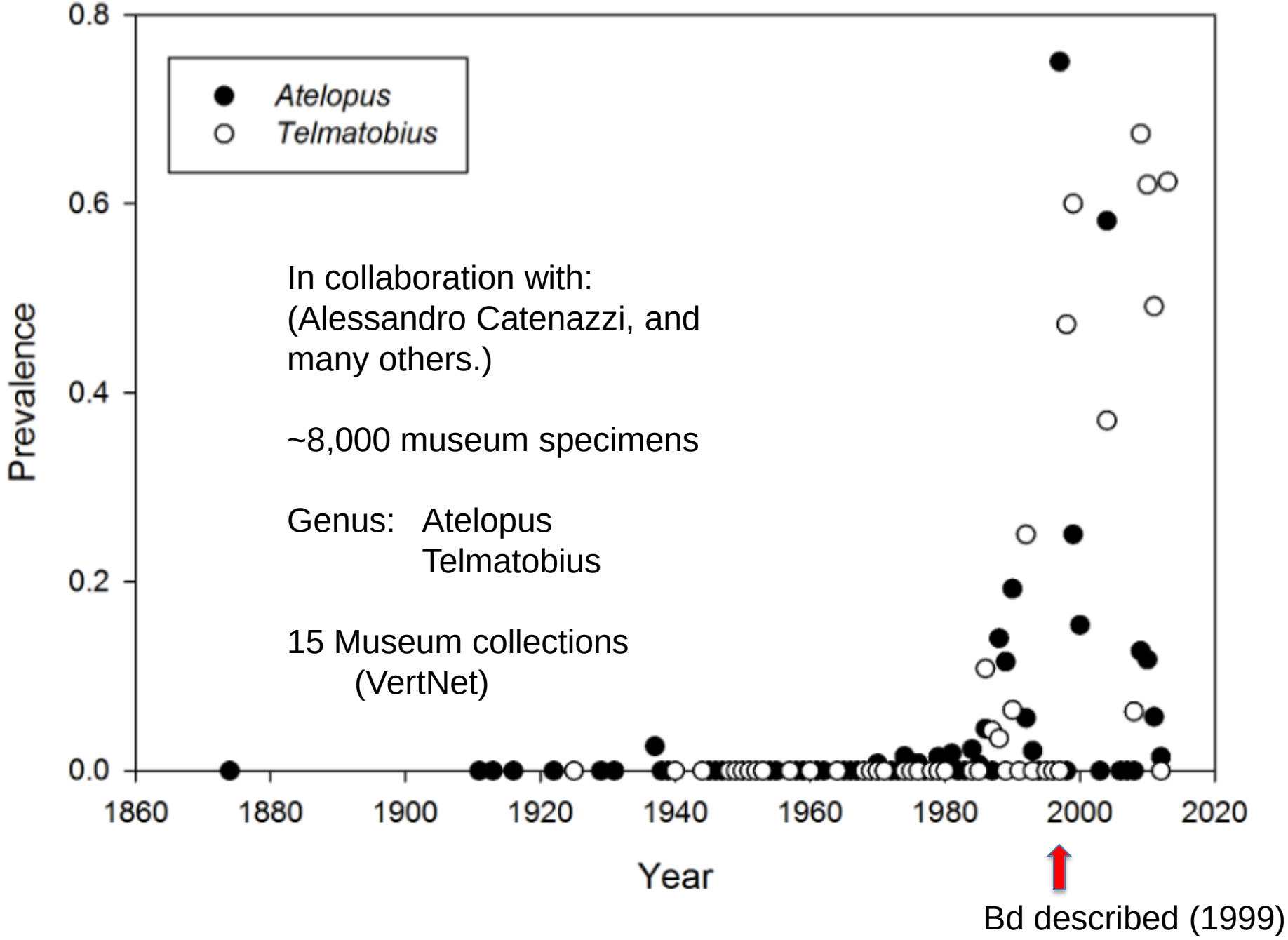
When did Bd emerge in S. America?



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

~8,000 museum specimens

Genus: Atelopus
Telmatobius


Atelopus
Telmatobius
Prevalence
0.0
0.2
0.4
0.6
0.8
1860
1880
1900
1920
1940
1960
1980
2000
2020
Year
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/

hot water & internet

socializing education

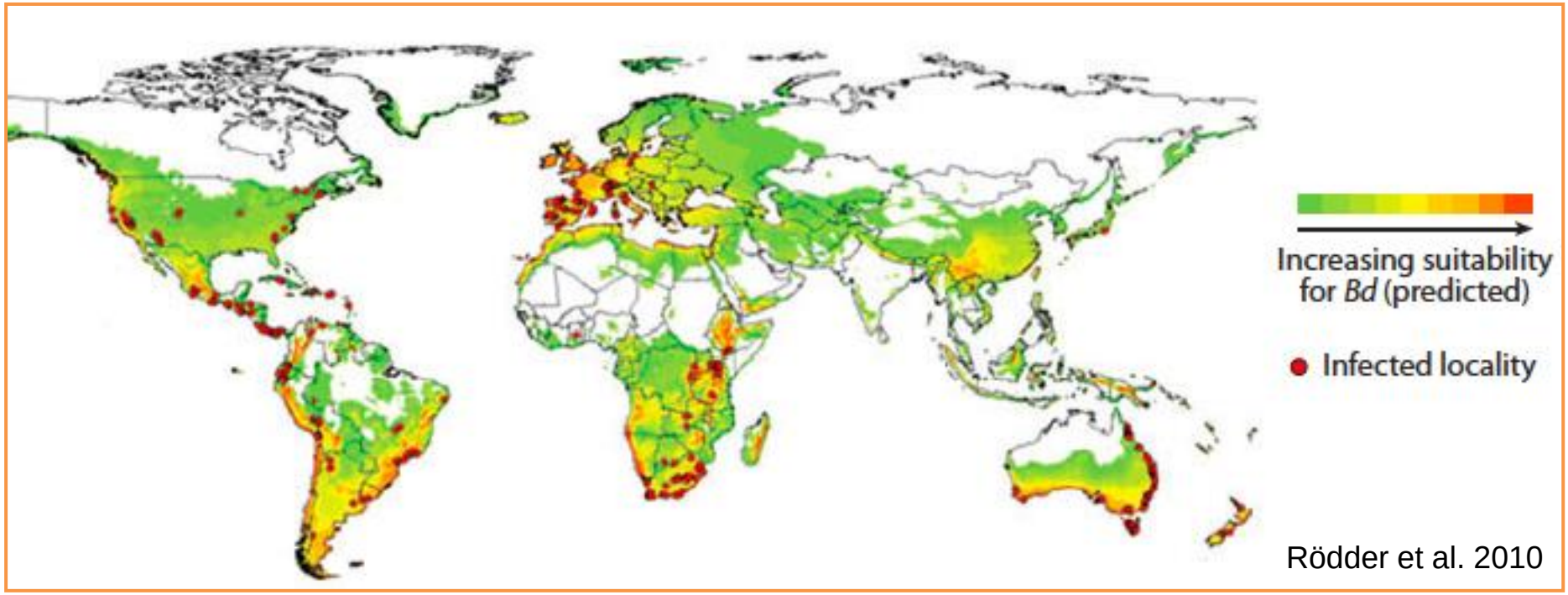
makeshift lab

spectacular views

earthy food

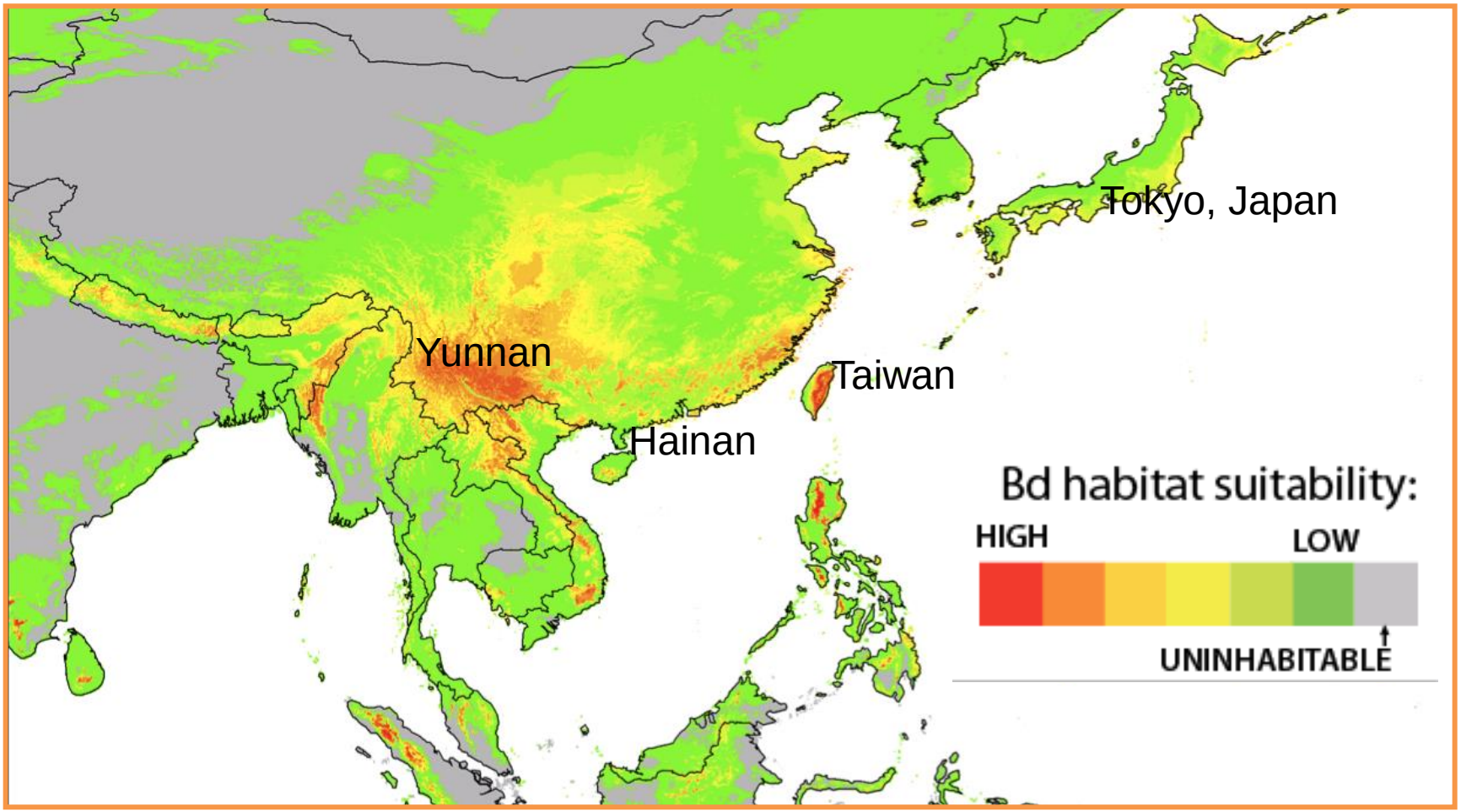
Susceptibility trials: Peru highlands

Predicted suitable habitat for Bd



Rödder et al. 2010

Asia is Vulnerable



Yunnan Province, China (Sept 15, 2013)

Raul Figueroa

Retrospective survey suggests recent emergence of Chytridiomycosis in Japanese amphibians

Gabriela Rios-Sotelo

Vance Vredenburg PhD

San Francisco State University

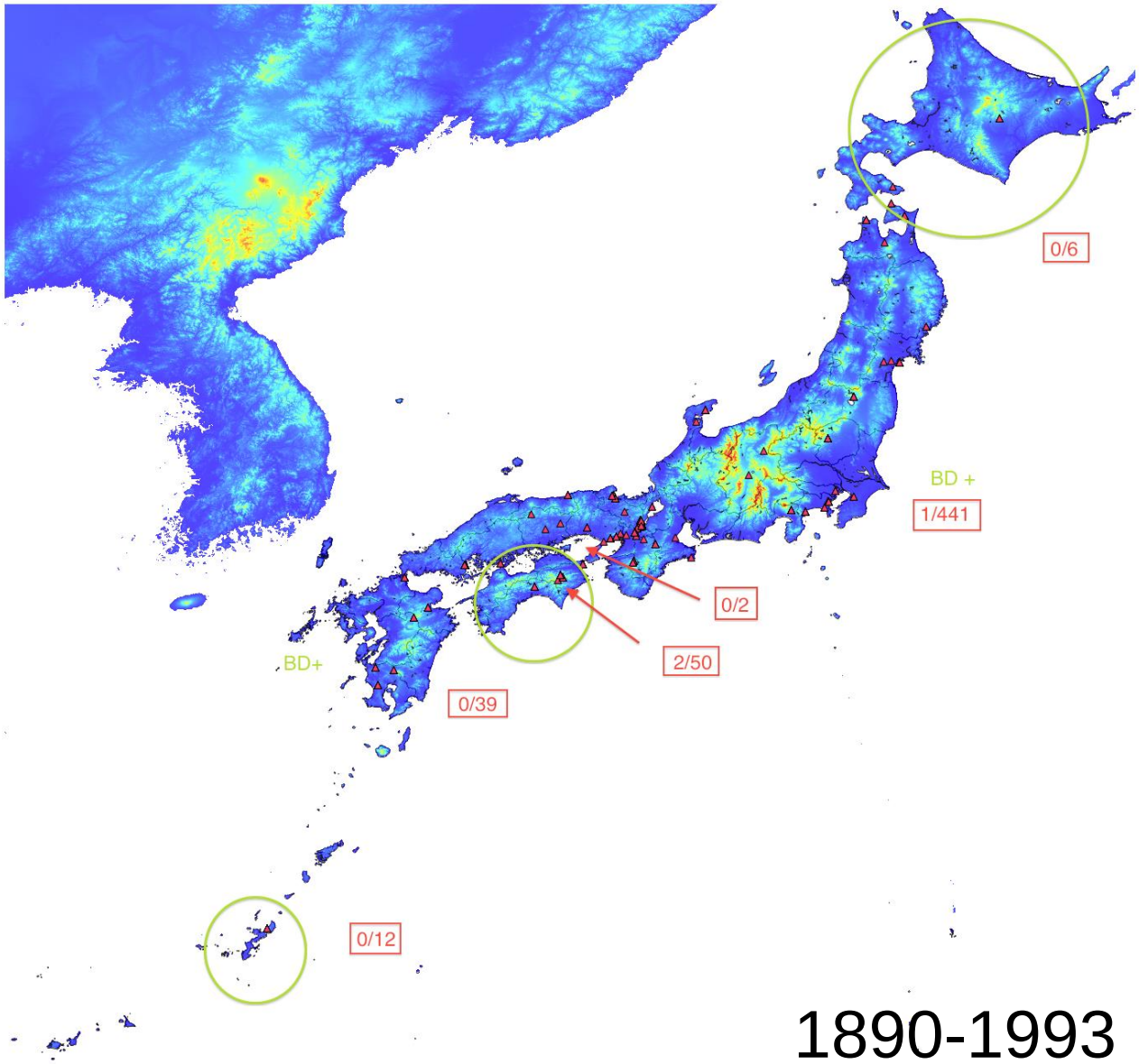
First report of Bd in Asia

- 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

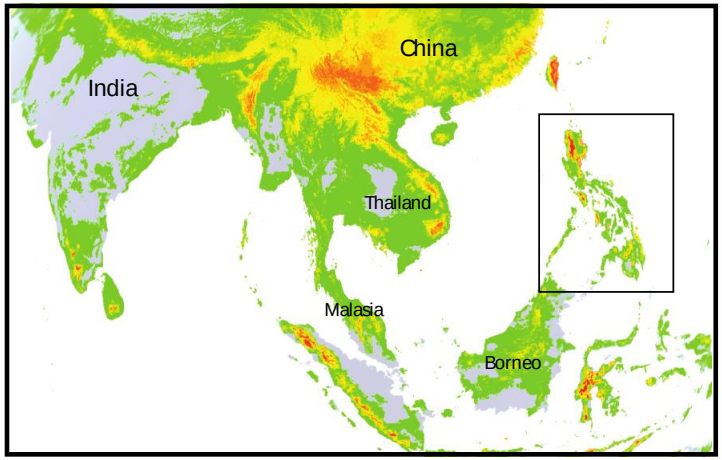


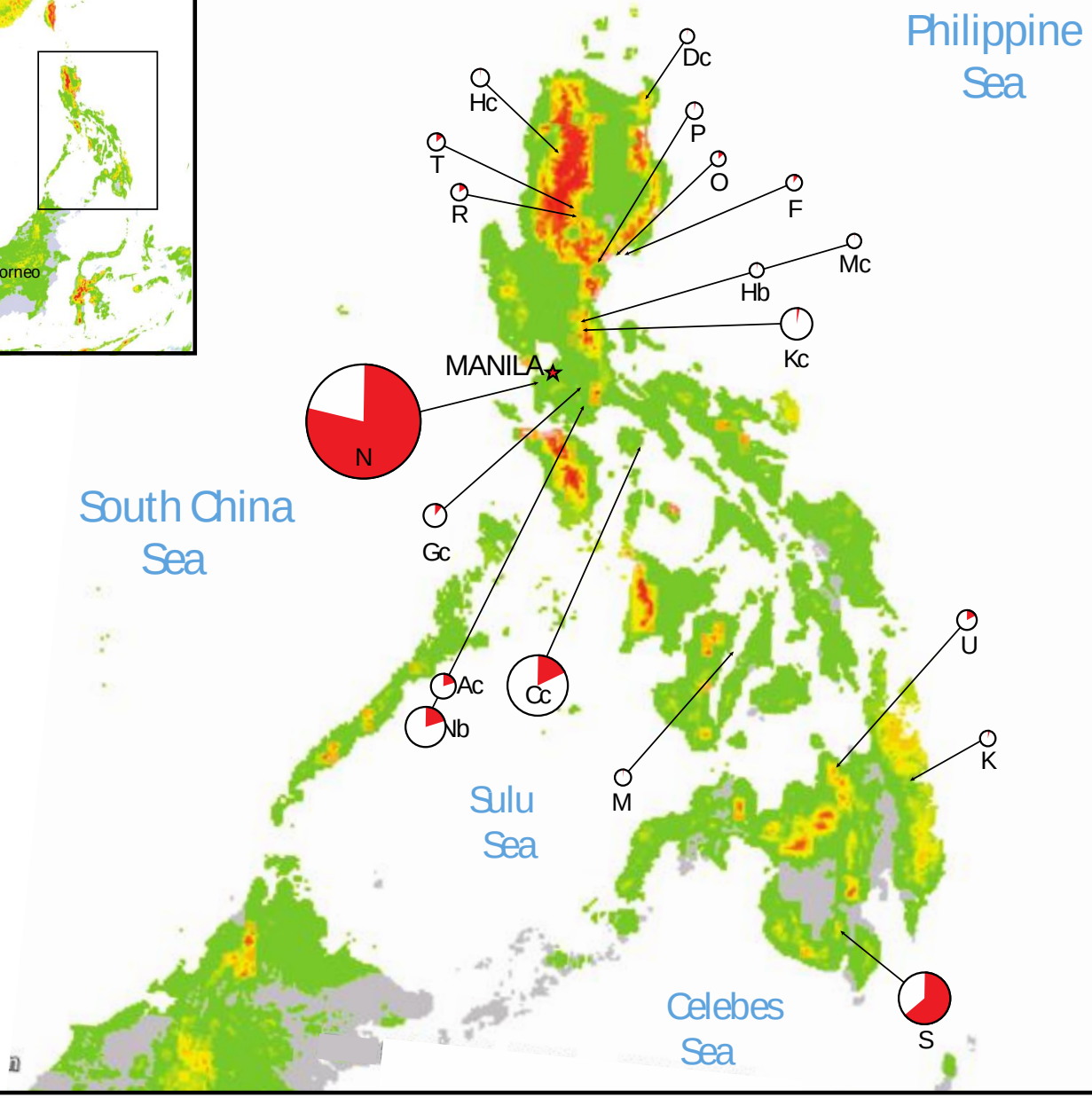
1890-1993

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

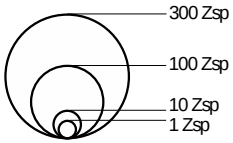
in the Philippines 2003-2011





Percent Bd+

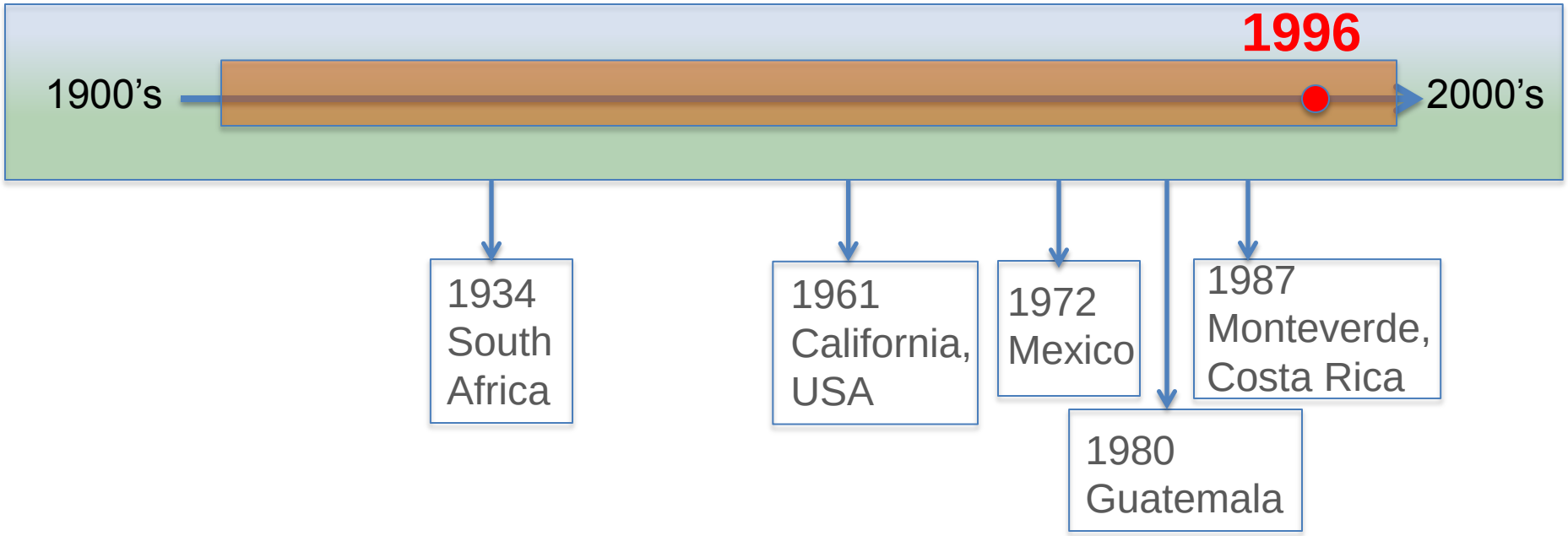
Infection Intensity (Zoospore equivalents):



*Scale dictated by square root of Zsp

locality = approximate

Bd Infection Timeline: Philippines



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 ibrought forth the spread of *Batrachochytrium dendrobatidis* in native amphibians.

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

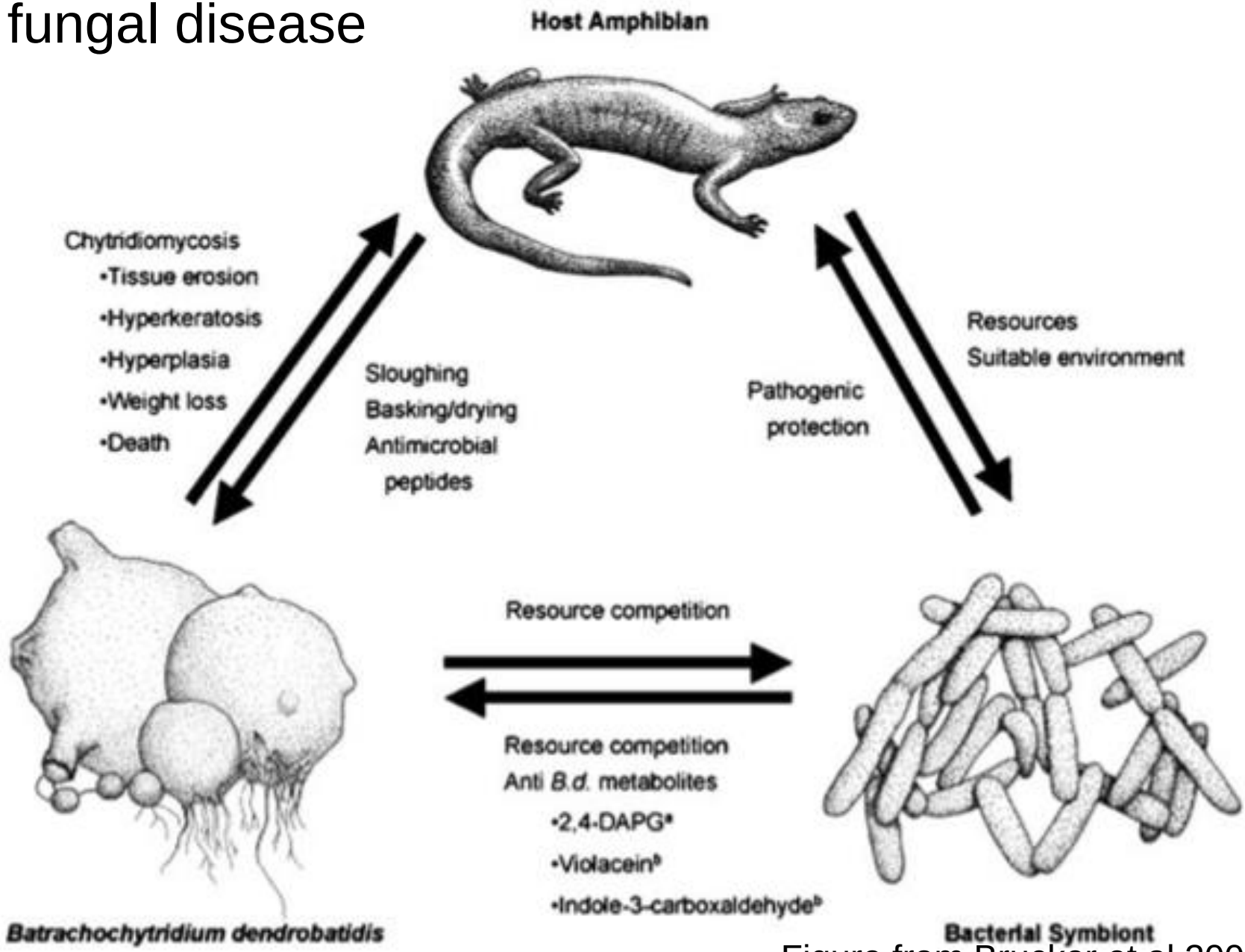


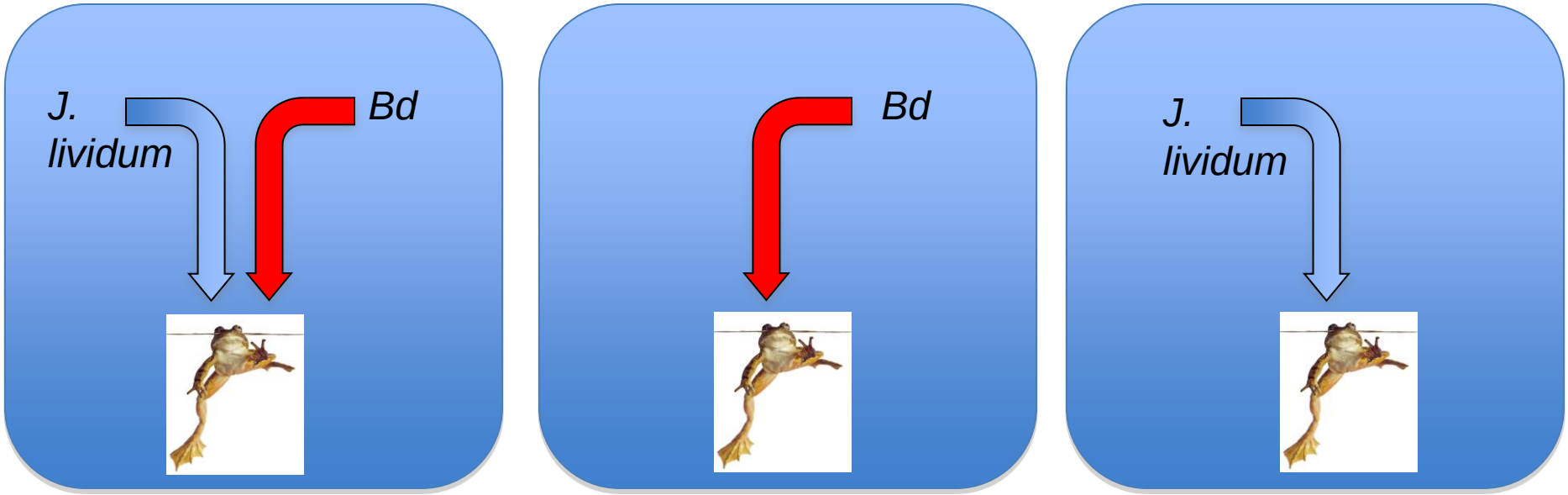
Figure from Brucker et al 2008

Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus

Reid N Harris[1], Robert M Brucker[2], Jenifer B Walke[3], Matthew H Becker[1], Christian R Schwantes[4], Devon C Flaherty[4], Brianna A Lam[1], Douglas C Woodhams[5], Cheryl J Briggs[6], Vance T Vredenburg[7] and Kevin PC Minbiole[4]

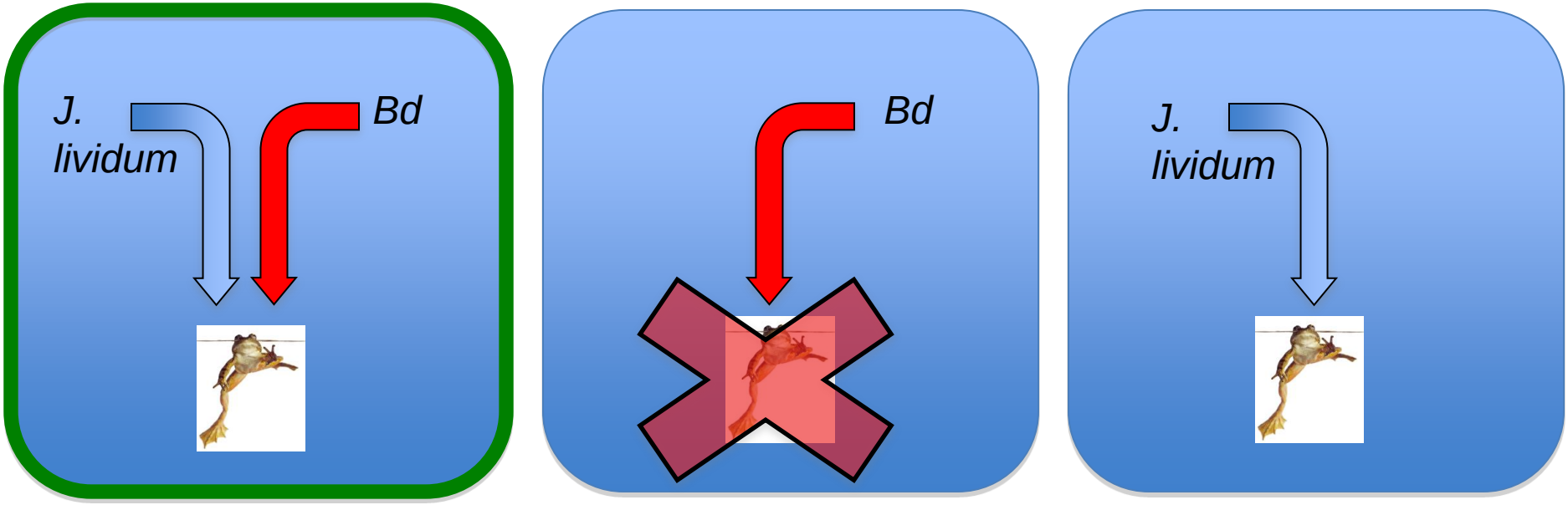
In the Lab, we measured:

1. Survival
2. Weight gain or loss
3. Bd load (infection intensity)



Harris et al. 2009.*The ISME Journal* 3:818-824

Bioaugmentation works in the Lab!



Can we protect susceptible frogs in the wild?

Harris et al. 2009.*The ISME Journal* 3:818-824

Bioaugmentation of skin microbiome may save amphibians in the wild

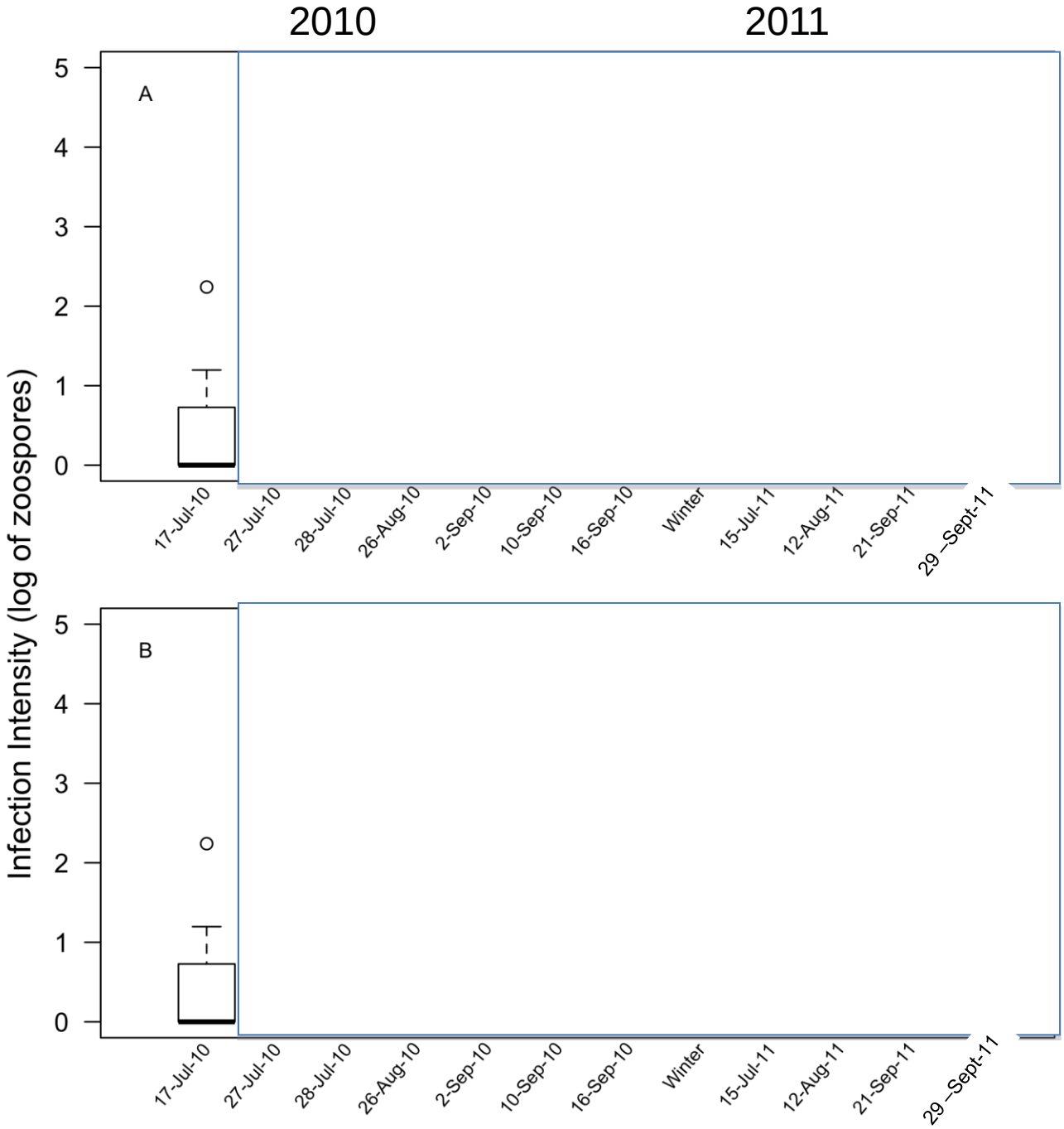
Holding cages:
+Bacteria
Control

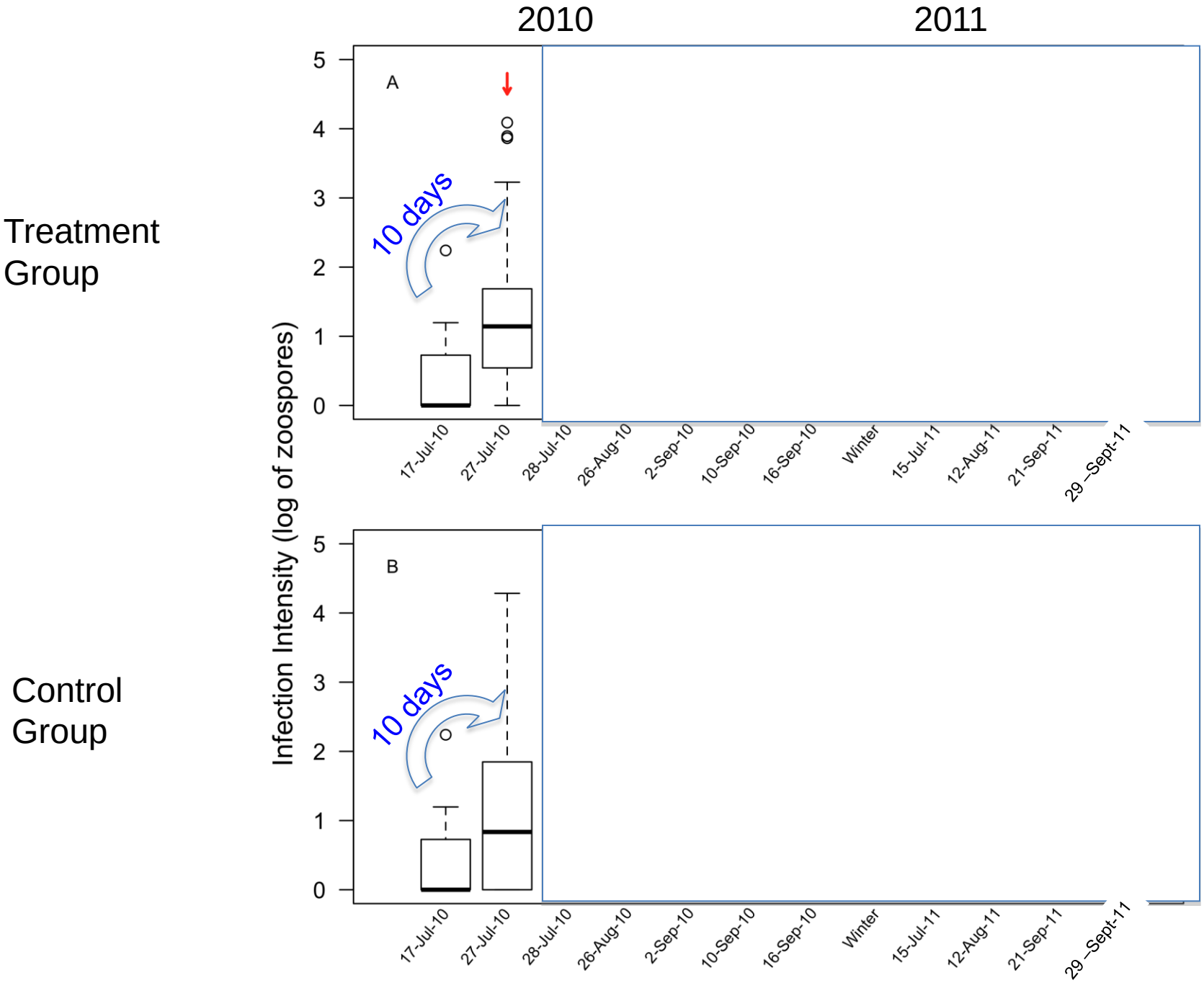
1_6_SEP3

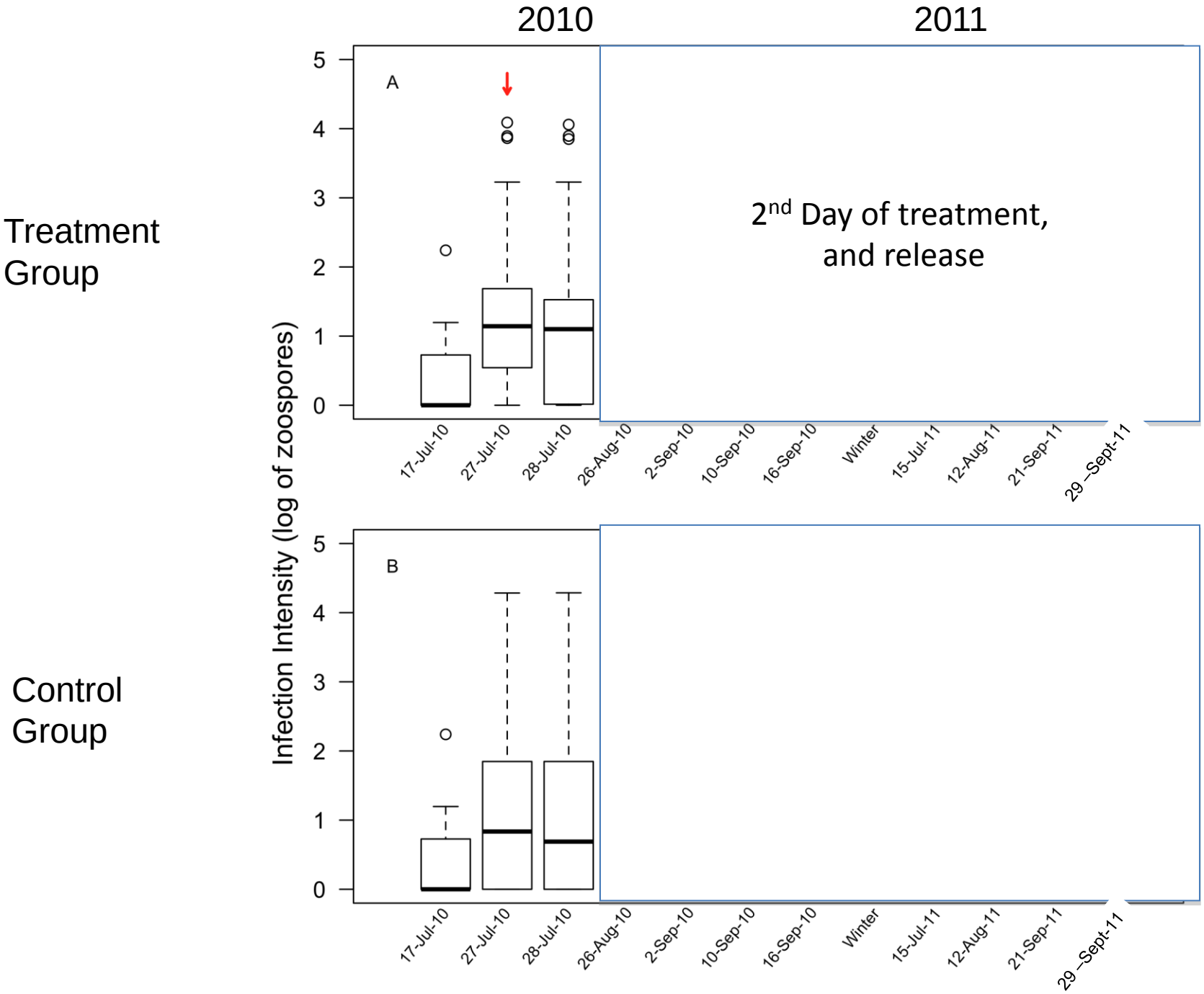
Bacterial
Cultures collected

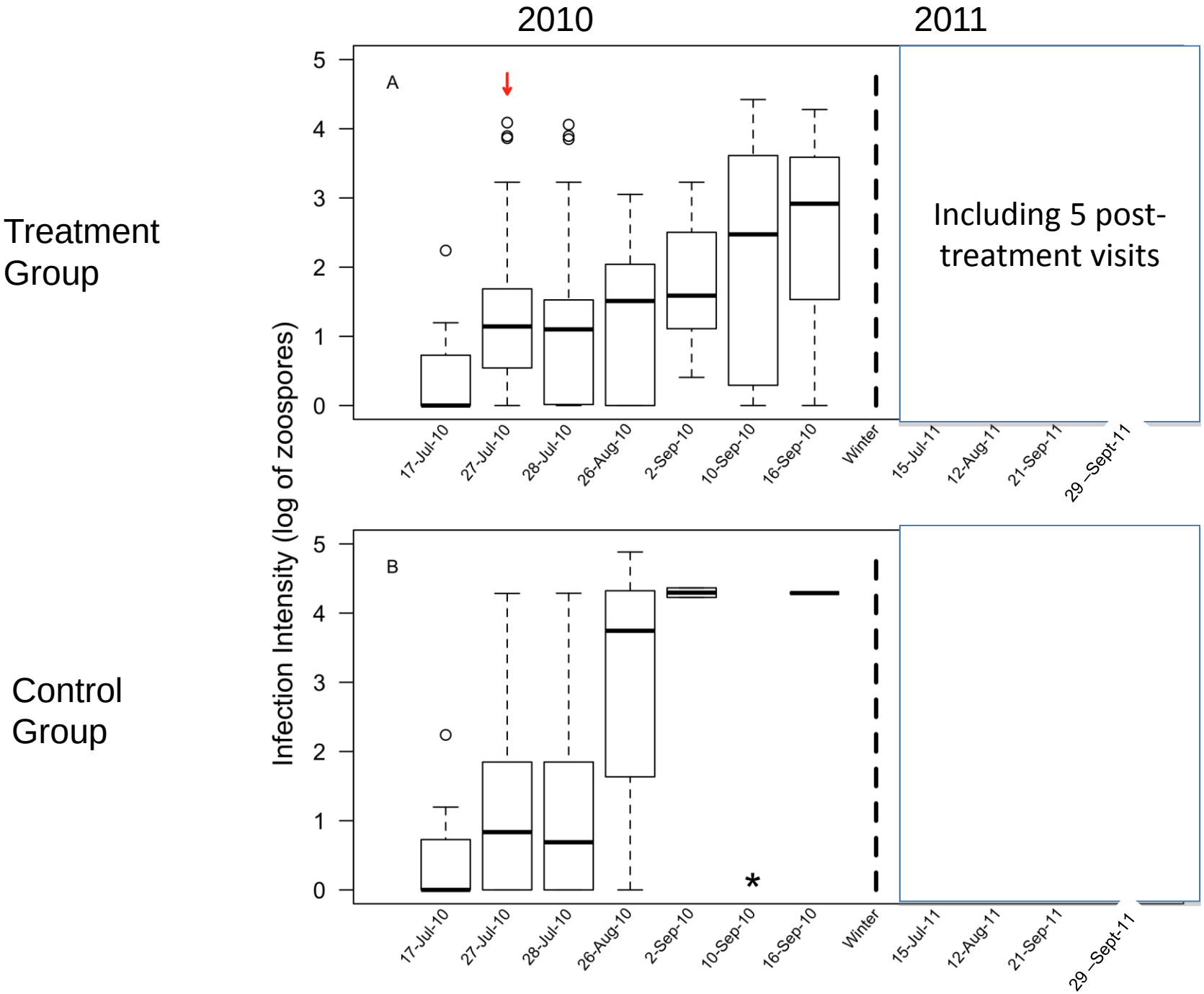
Treatment
Group

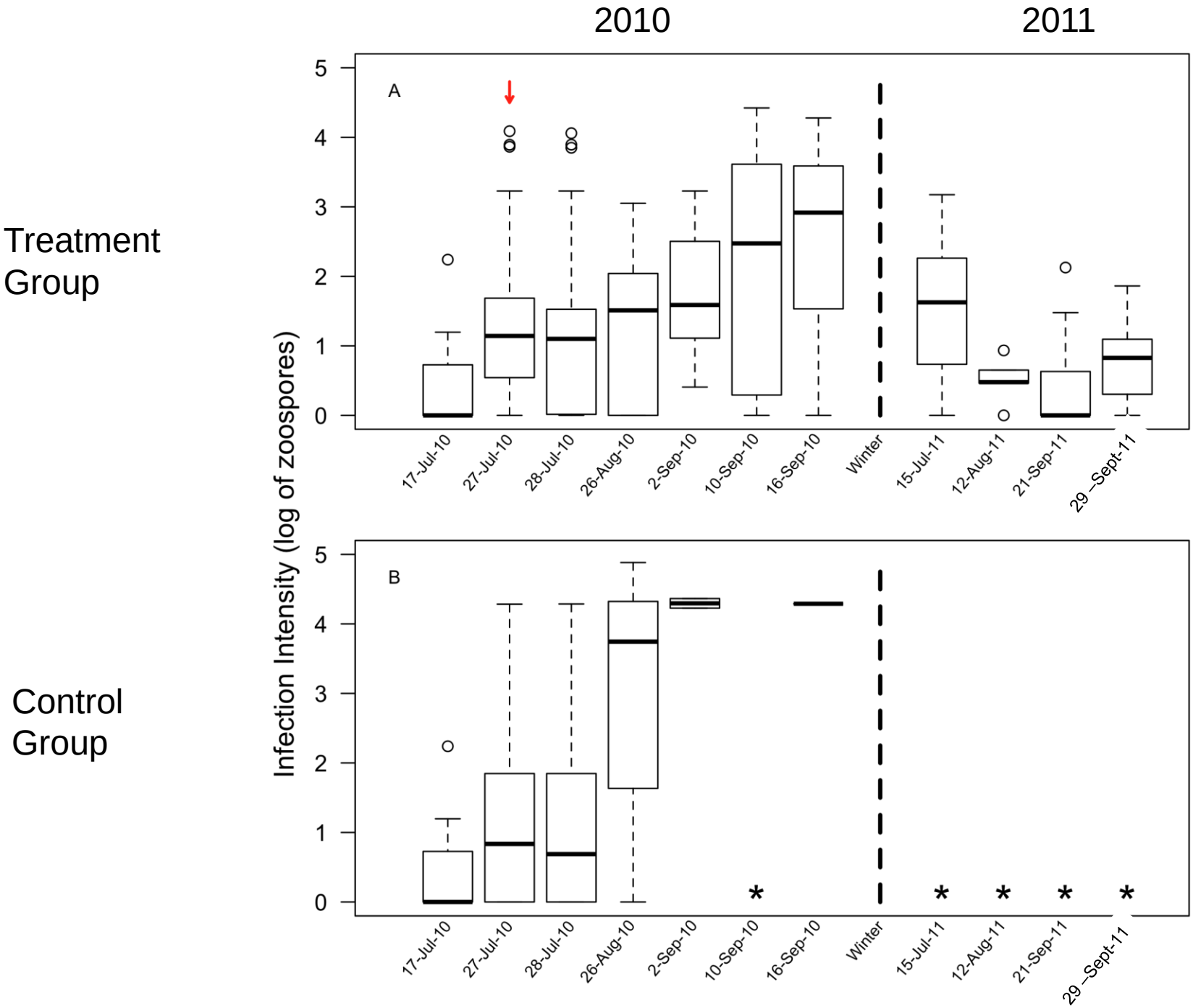
Control
Group




2010
2011
Treatment Group
Control Group
Infection Intensity (log of zoospores)
A
B
10 days
10 days
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 –Sept-11


2010
2011
Treatment Group
Control Group
Infection Intensity (log of zoospores)
A
B
2nd Day of treatment, and release
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 –Sept-11


2010
2011
Treatment Group
Control Group
Infection Intensity (log of zoospores)
A
B
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 –Sept-11
Including 5 post-treatment visits
*


2010
2011
Treatment Group
Control Group
Infection Intensity (log of zoospores)
A
B
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 –Sept-11

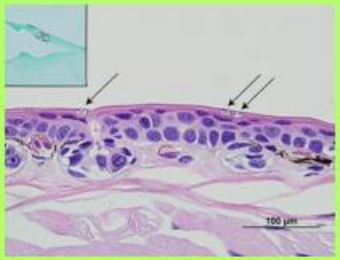
Dusy Basin
July 16, 2010

Dusy Basin
July 14, 2011

Dusy Basin
July 17, 2012

Vredenburg Lab

Amphibian Biodiversity and Conservation



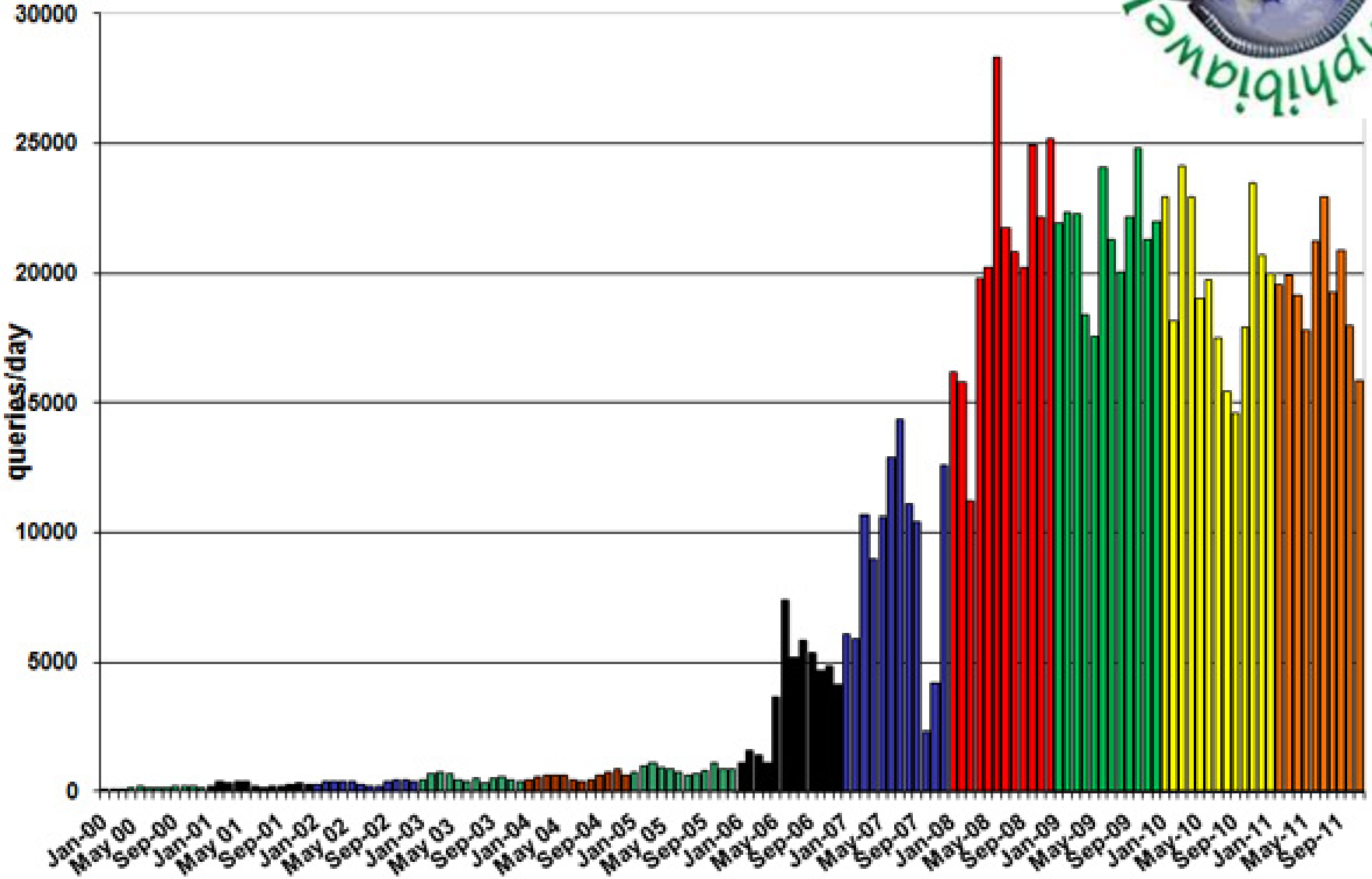
Graduate students:

Celeste Dodge, Silas Ellison, Gabriela Rios-Sotello, Cory Singer, Raul Figueroa, Sam McNally, Danqing Shao, Stephanie Hyland,, Andrea Manzano, Jacobo Conde, Jonathan Young (rencetly finished: Tina Cheng, Natalie Reeder, Meghan Bishop)

Undergraduates:

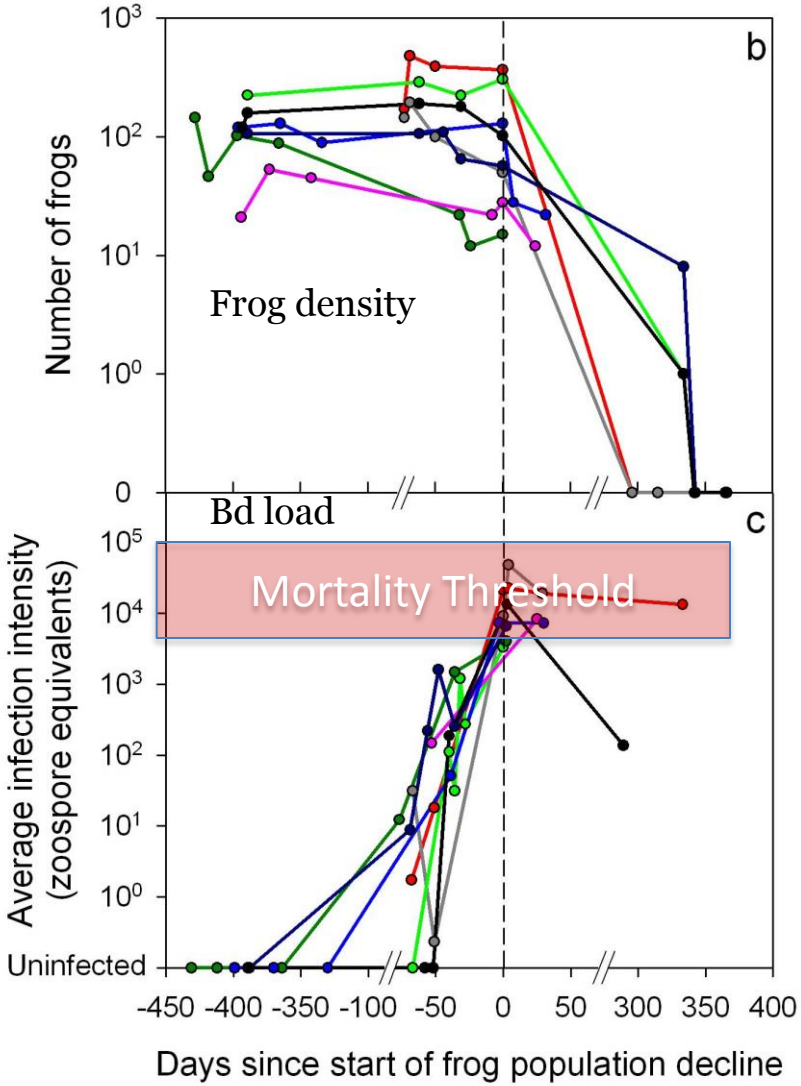
Mark Russell, Nina Hang, Alex Harencar, Amanda Carbajal, Bo Heinz, Corinna Inmann, Hahn Pham, Hannah Durbin, Ivet Lolham,Jason Anders, Jina Kim, Jourdan McPhetridge, Karl Alicando, Kirsten Liaz, Laurece Henson, Mackenzie Beaschler, Robert Tom

AmphibiaWeb an online Conservation Resource



At “die-off” sites:

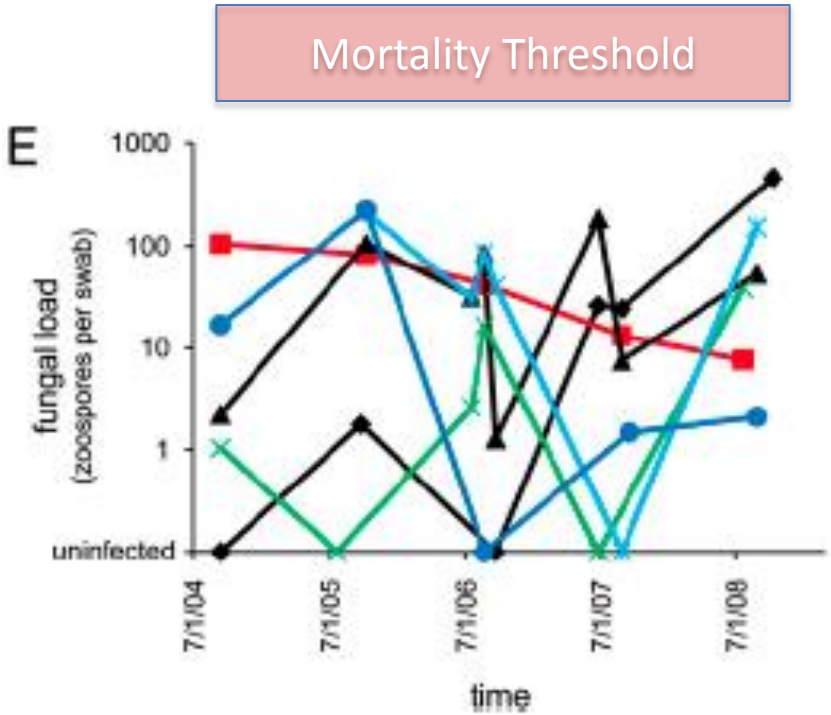
Bd load increases rapidly to high levels



(Vredenburg, et al. 2010; *PNAS*)

At “persistent” sites:

Bd load is low and does not reach critical threshold



(Briggs, Vredenburg et al 2010; *PNAS*)

ADDRESSING FUNGAL DISEASES

"Fatal frog fungal disease figured out." Nature News October 27, 2009

"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

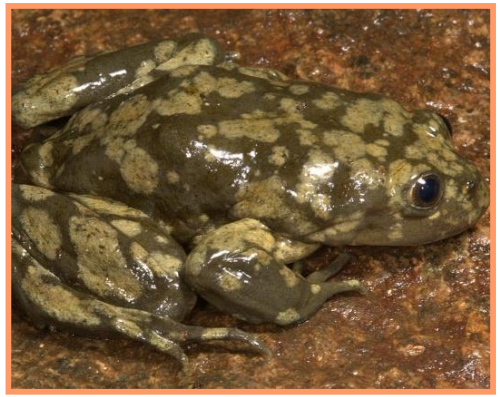
"Stopping a frog killer.... a tool for saving species in the wild
BBC, June 6 2008

"Amphibians afloat and fighting"
BBC October 13, 2008

"Biologists solve a great mystery of modern ecology."
USA Today October 22, 2009

Telmatobius *frogs*

Telmatobius lady



5 control: 7-day itraconazol

15-20 infected: no itraconazol

Experimentally infected frogs

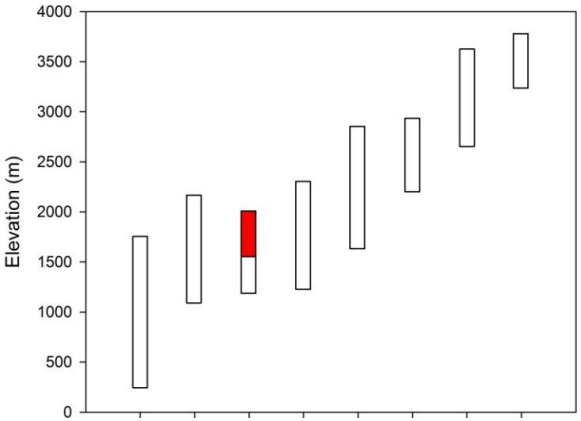
Night surveys

8-25 frogs/species

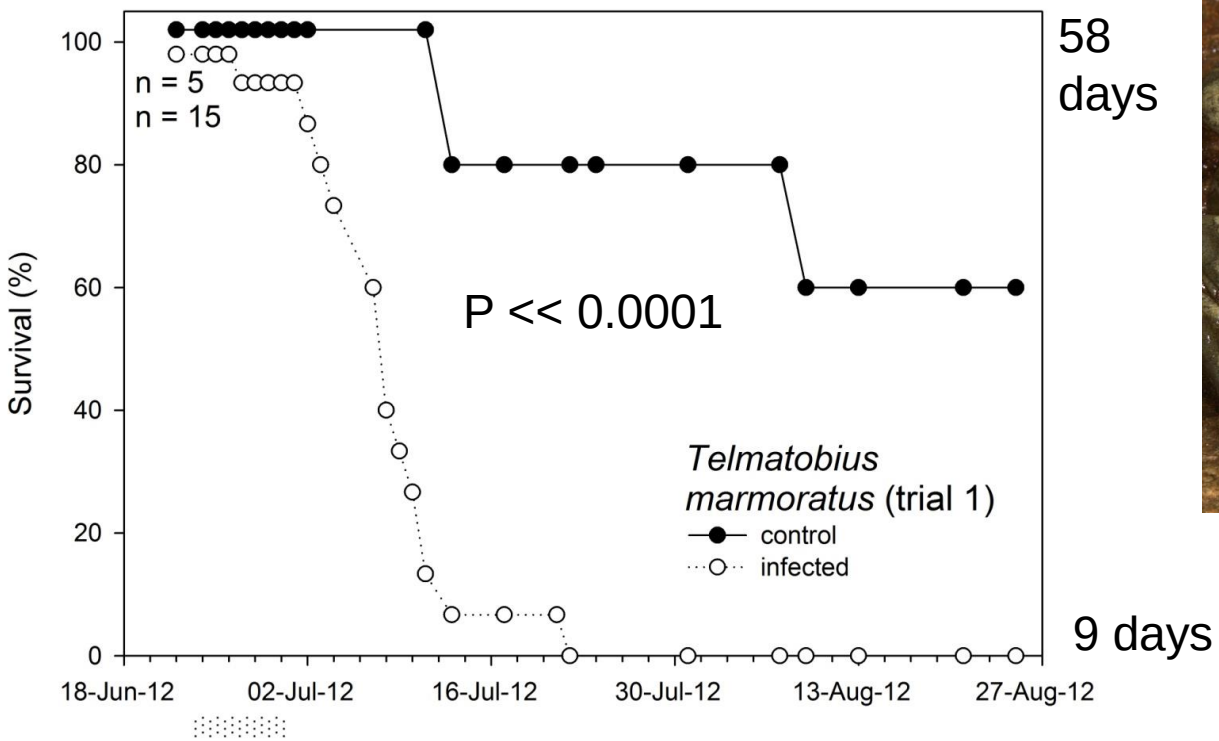
all frogs:
7-day itraconazol

4-5 <u>control</u>:
no exposure

4-20 <u>infected</u>:
Telmatobius exposure



Peru Bd susceptibility trials: experimentally infected frogs

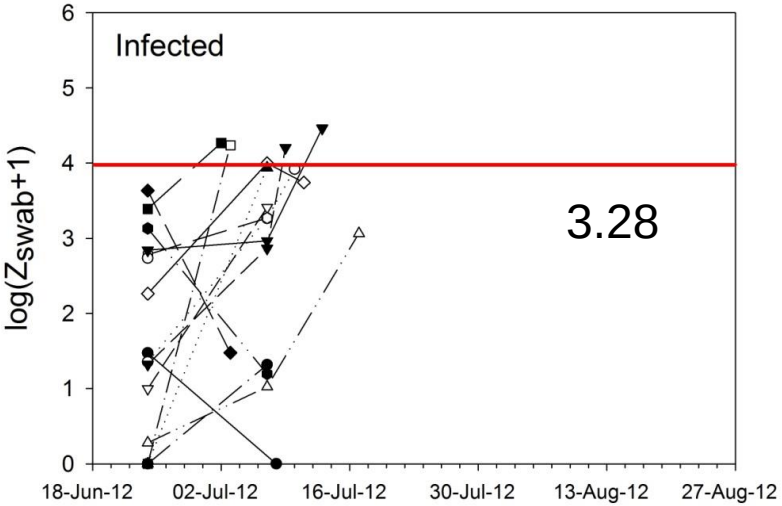


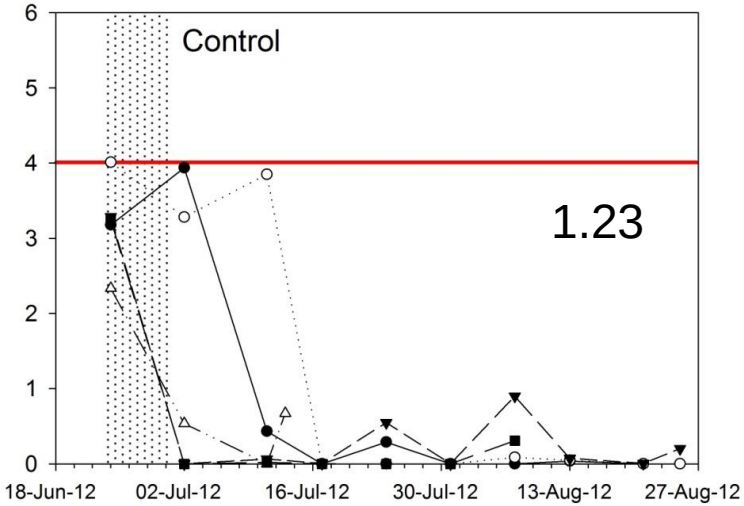
58 days

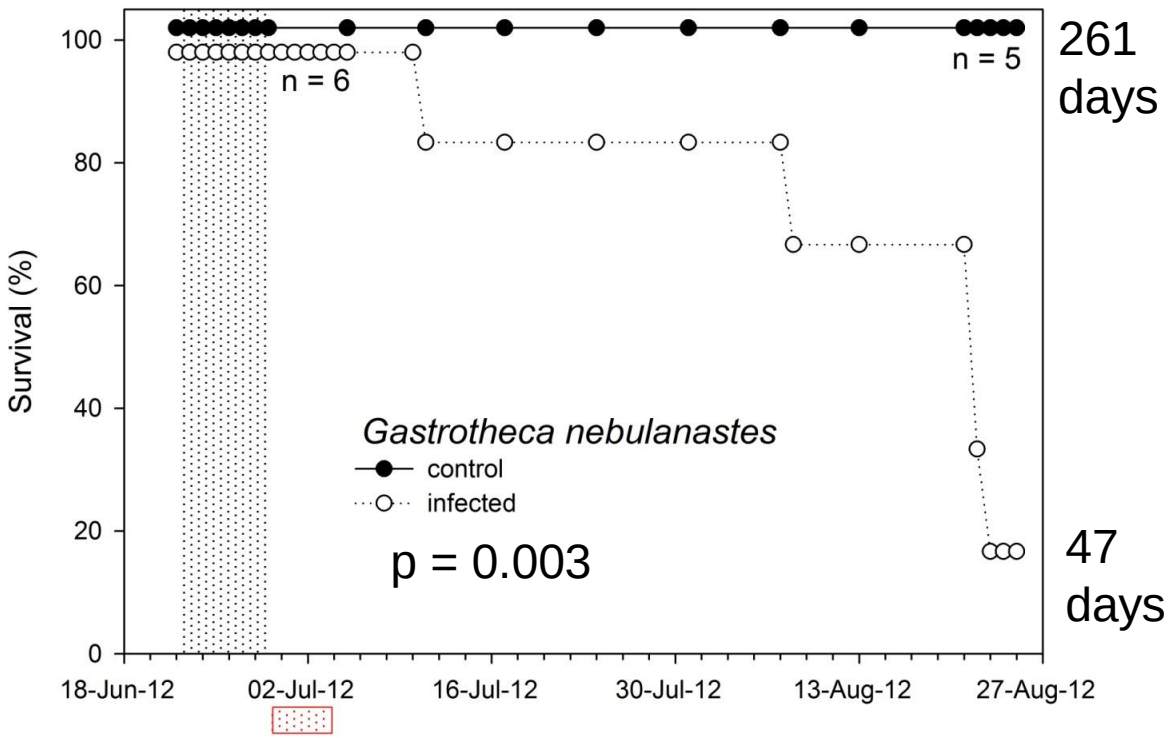
55 days

9 days

p = 0.02





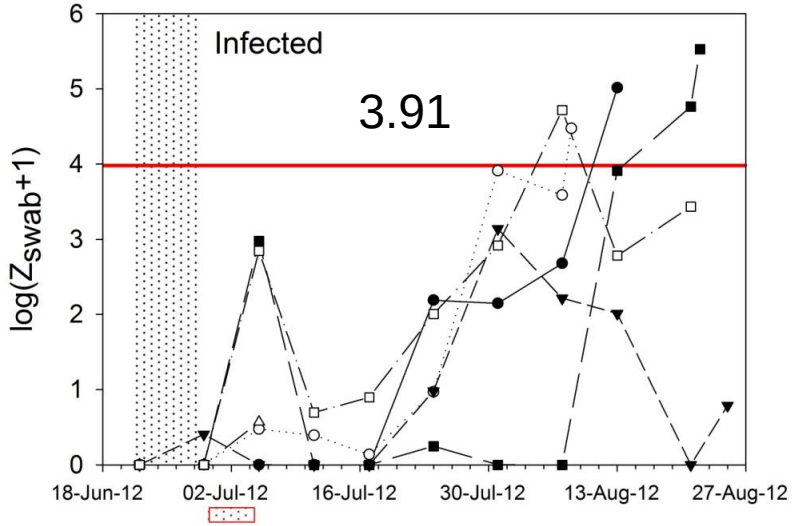


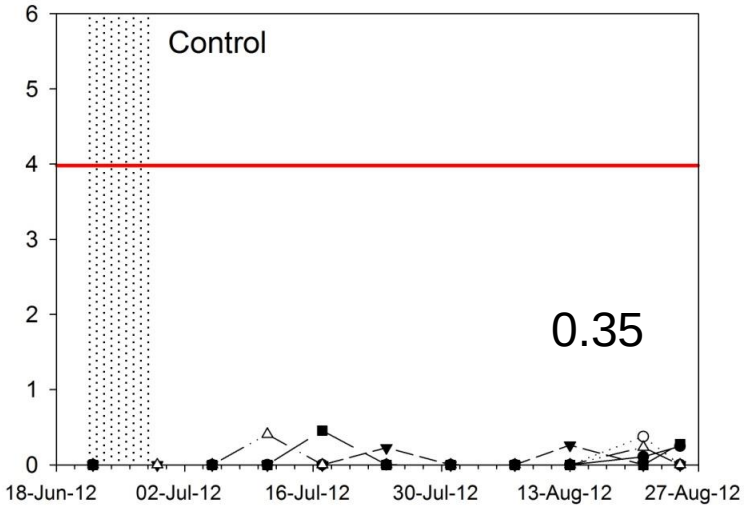
261 days

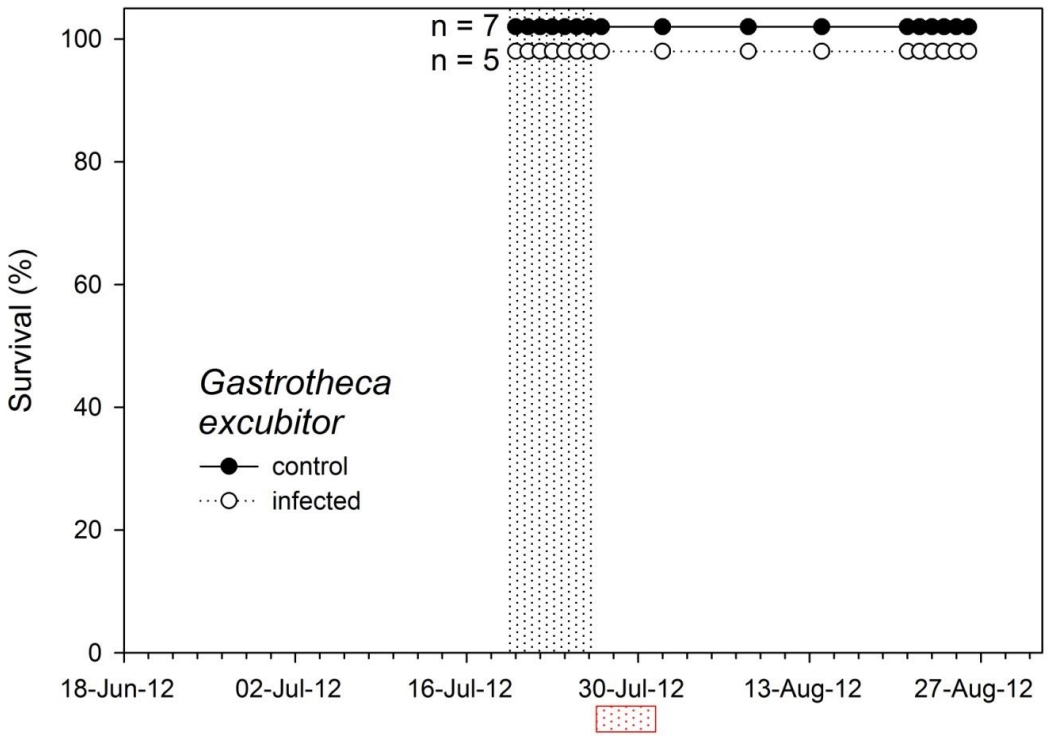
47 days

51 days

p = 0.002

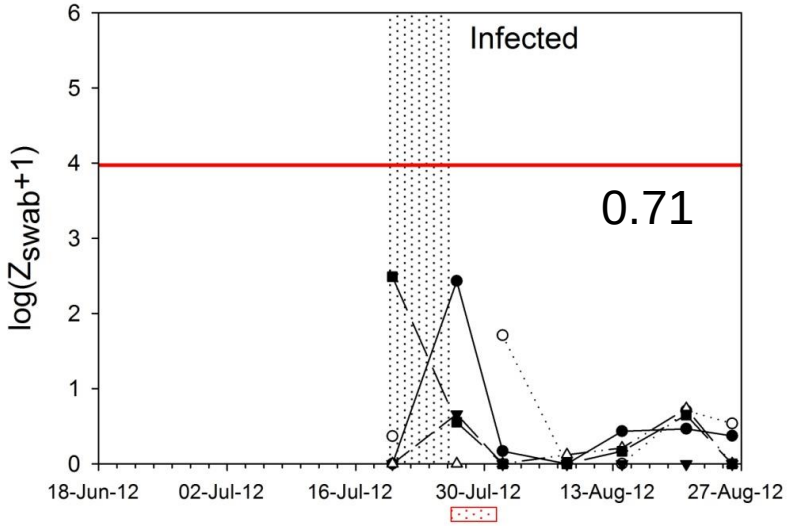


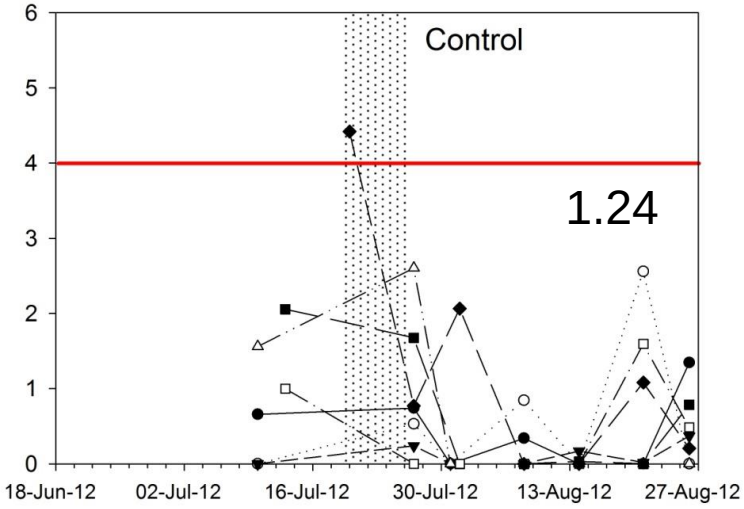



n = 7
n = 5
Survival (%)
100
80
60
40
20
0
Gastrotheca excubitor
control
infected
18-Jun-12
02-Jul-12
16-Jul-12
30-Jul-12
13-Aug-12
27-Aug-12

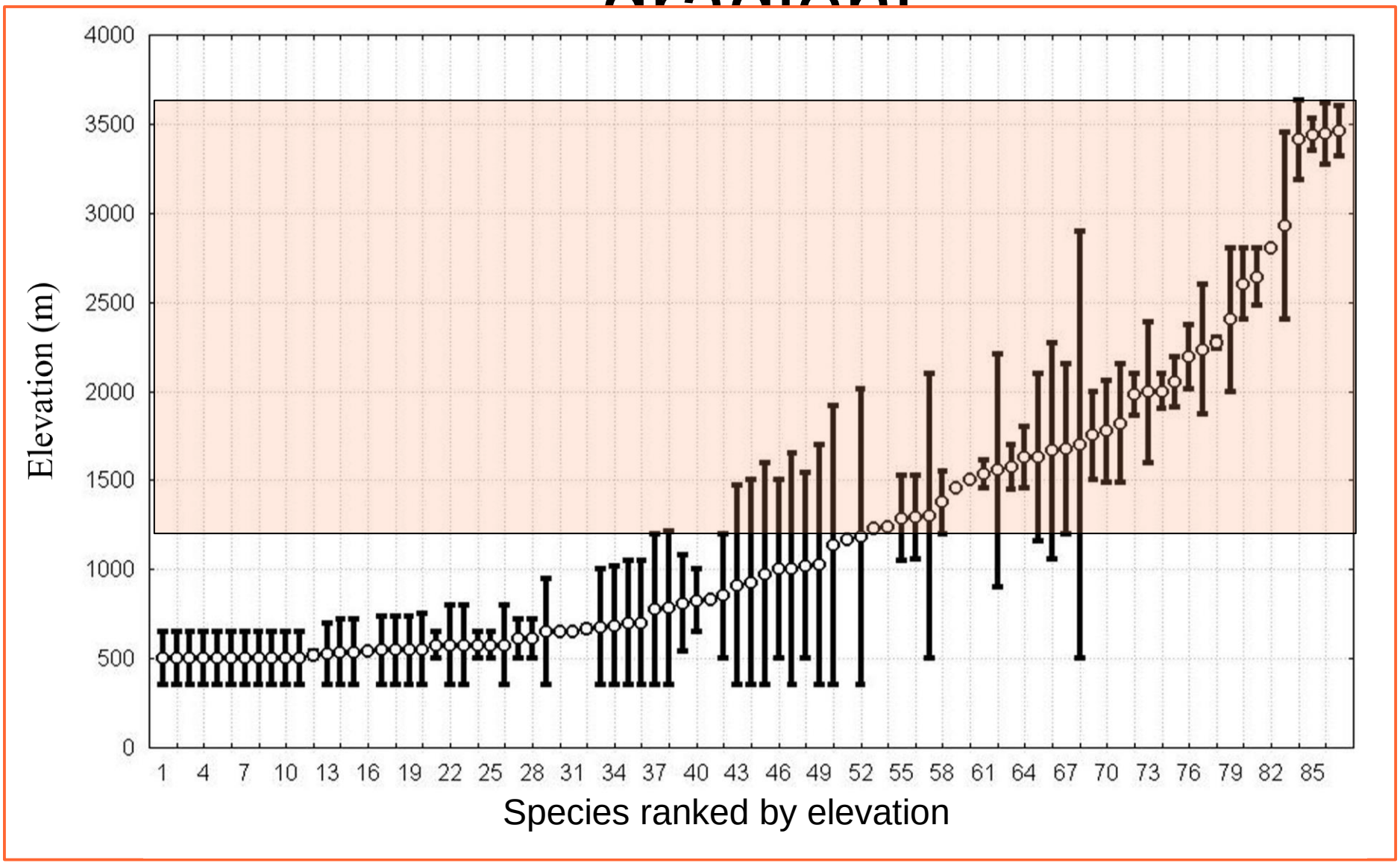
26 days

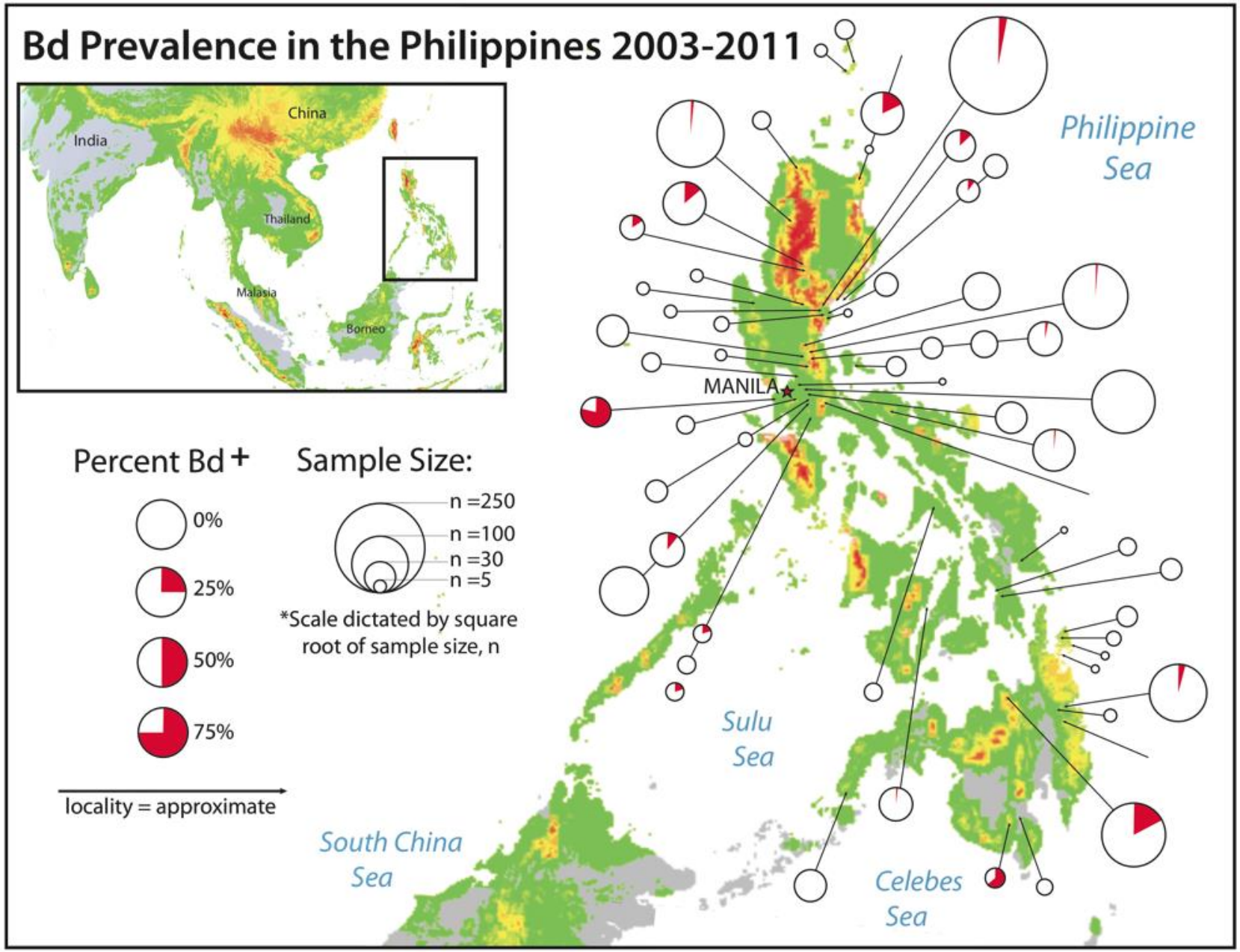
p = 0.13

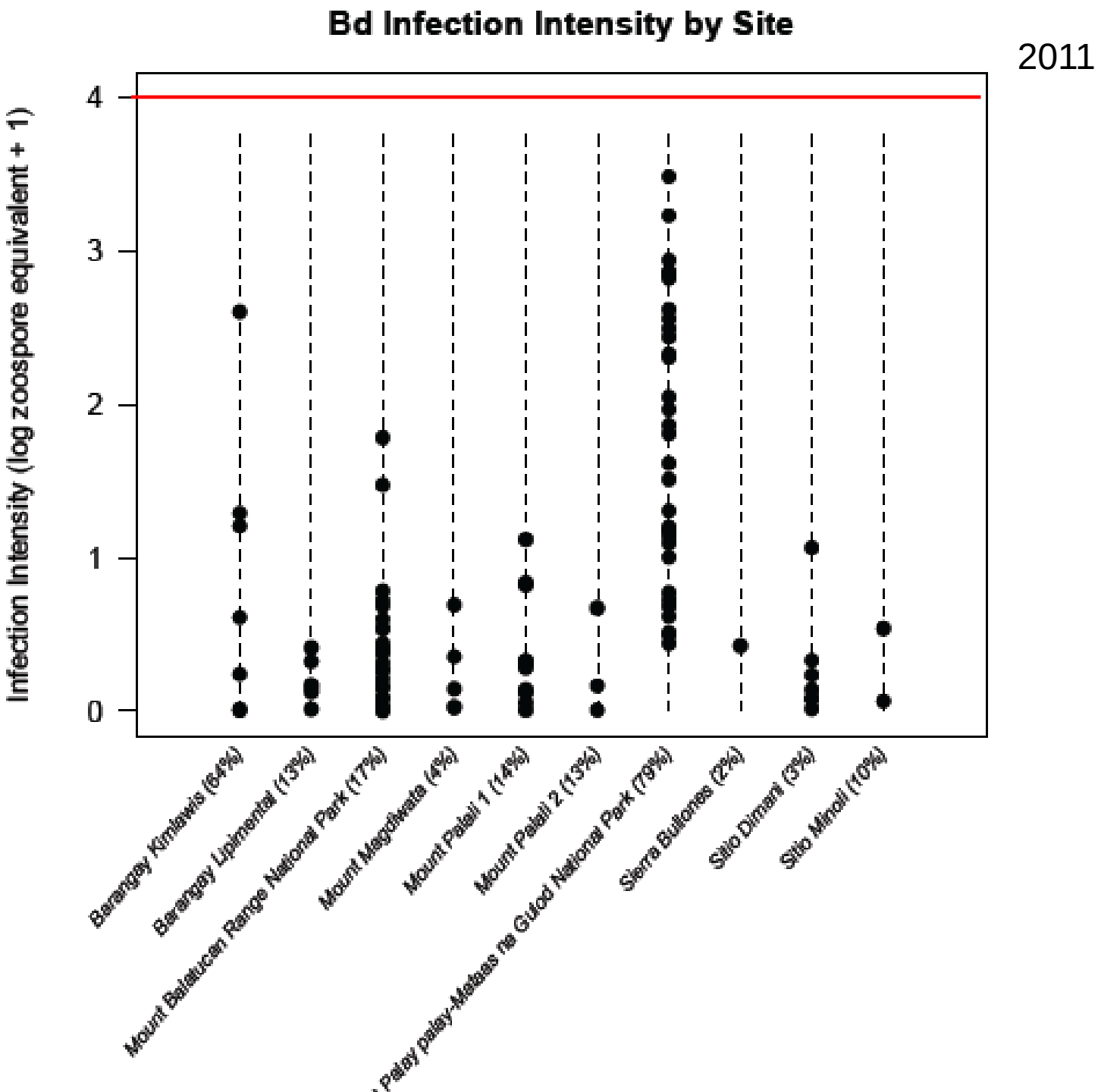

Infected
0.71
log(Zswab+1)
6
5
4
3
2
1
0
18-Jun-12
02-Jul-12
16-Jul-12
30-Jul-12
13-Aug-12
27-Aug-12


Control
1.24
6
5
4
3
2
1
0
18-Jun-12
02-Jul-12
16-Jul-12
30-Jul-12
13-Aug-12
27-Aug-12

Frogs along the elevational gradient

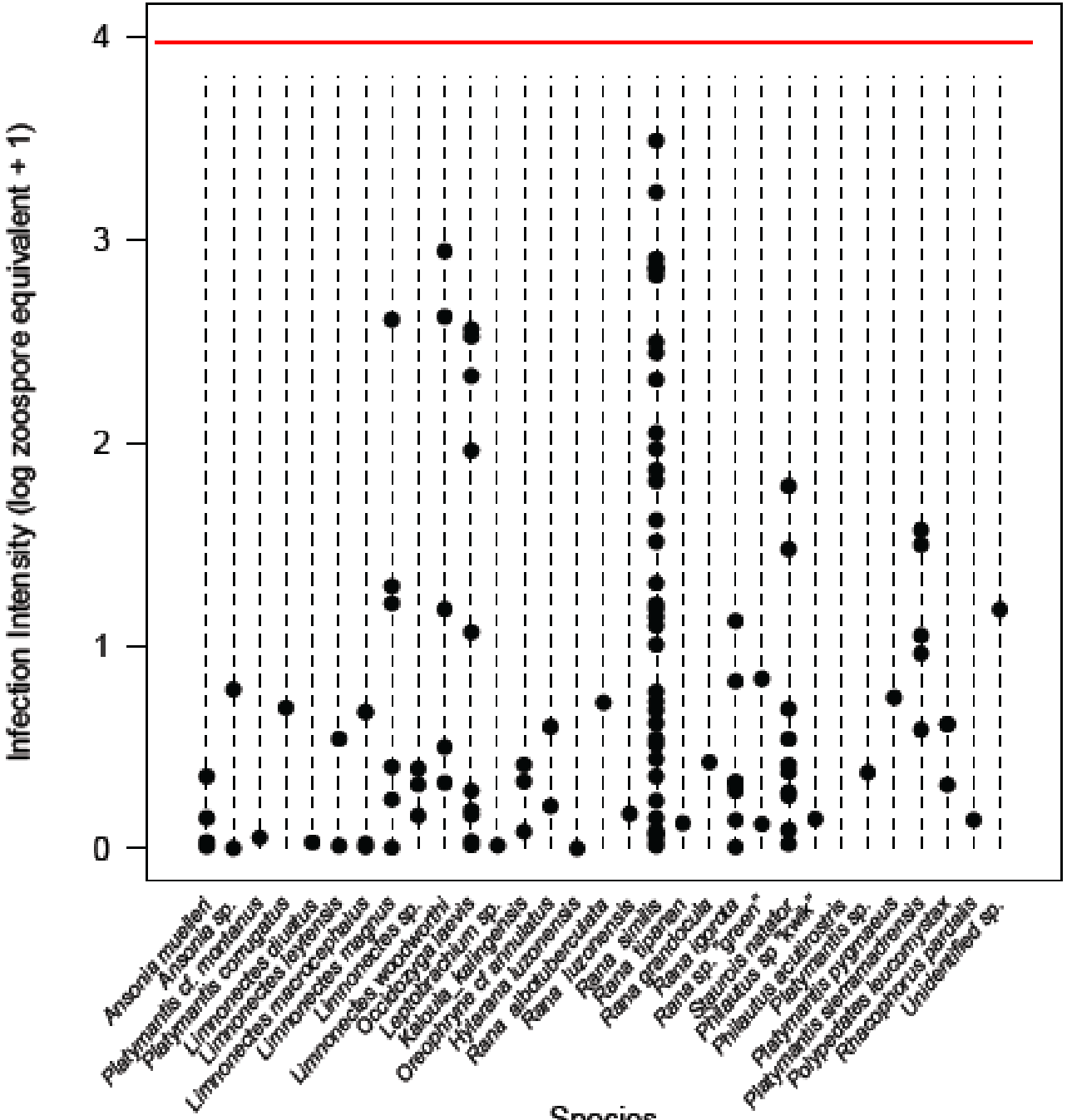



Bd Prevalence in the Philippines 2003-2011
China
India
Thailand
Malasia
Borneo
Philippine Sea
MANILA
Sulu Sea
South China Sea
Celebes Sea
Percent Bd +
0%
25%
50%
75%
Sample Size:
n =250
n =100
n =30
n =5
*Scale dictated by square root of sample size, n
locality = approximate


Bd Infection Intensity by Site
2011
Infection Intensity (log zoospore equivalent + 1)
0
1
2
3
4
Barangay Kimlawis (64%)
Barangay Lipimental (13%)
Mount Balatucan Range National Park (17%)
Mount Magdiwata (4%)
Mount Palali 1 (14%)
Mount Palali 2 (13%)
Palay palay-Mabas na Gutod National Park (79%)
Sierra Bullones (2%)
Sitio Dimari (3%)
Sitio Minoli (10%)

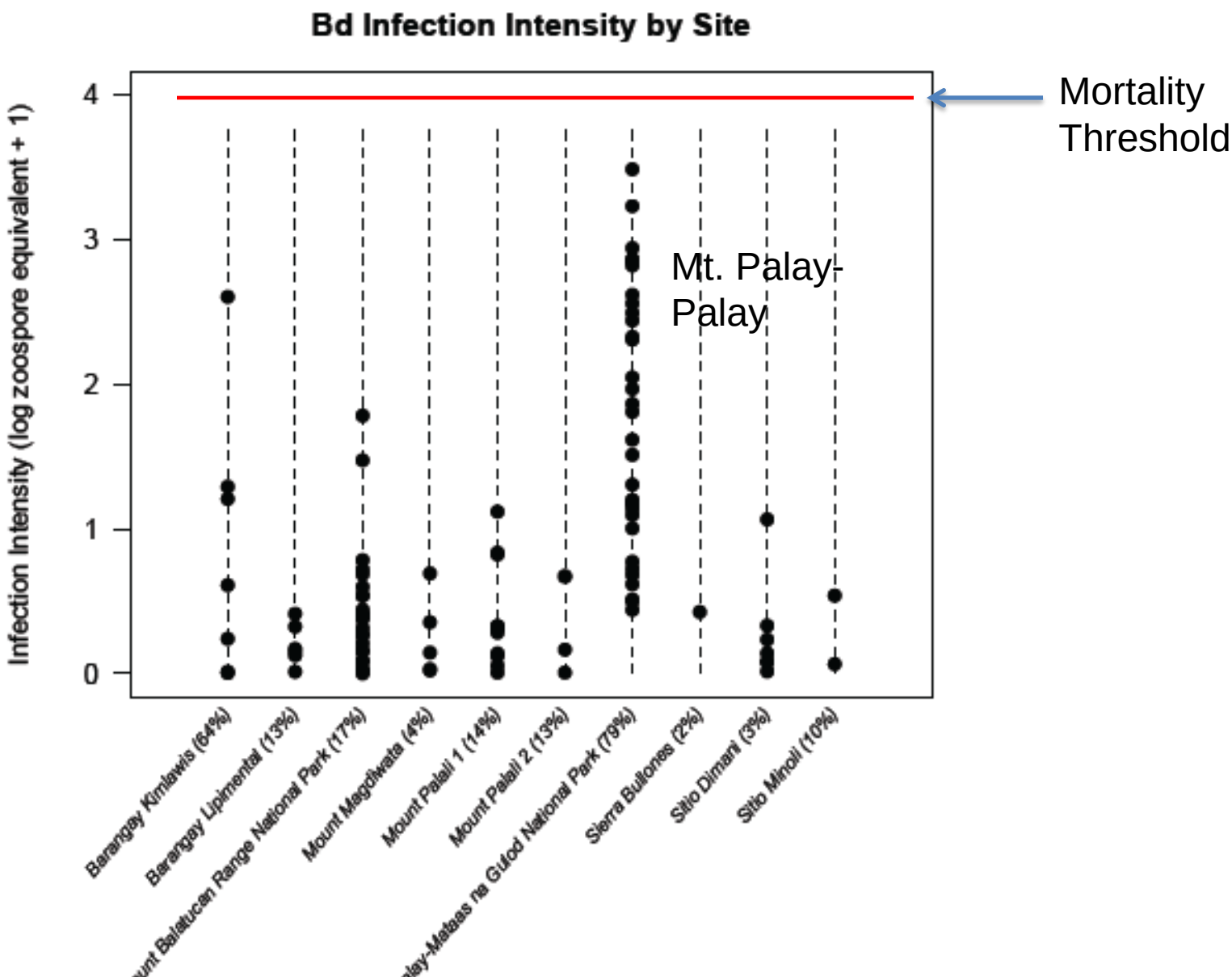
Bd Infection Intensity by Species

2011



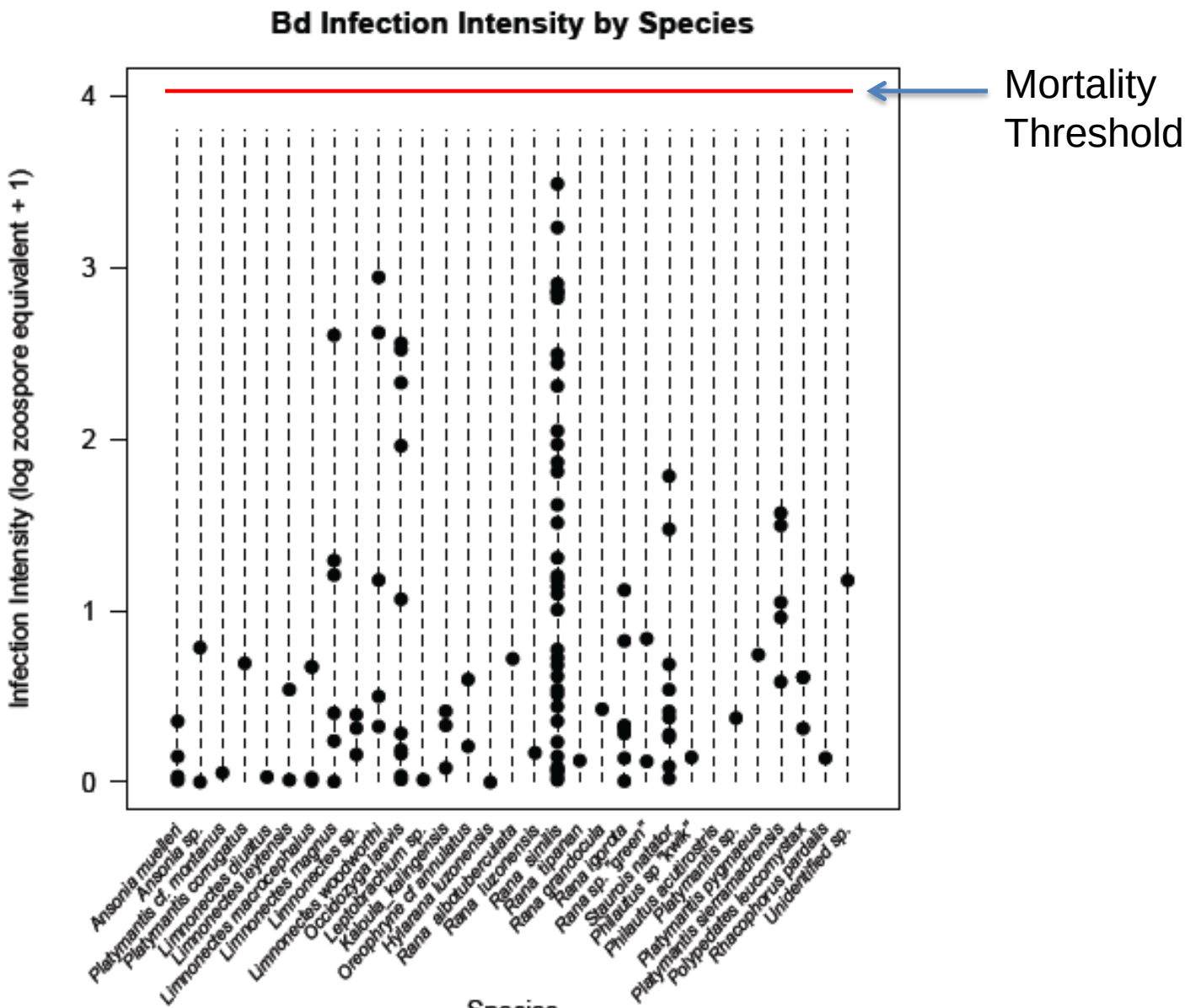
2012

300,000 zoospores

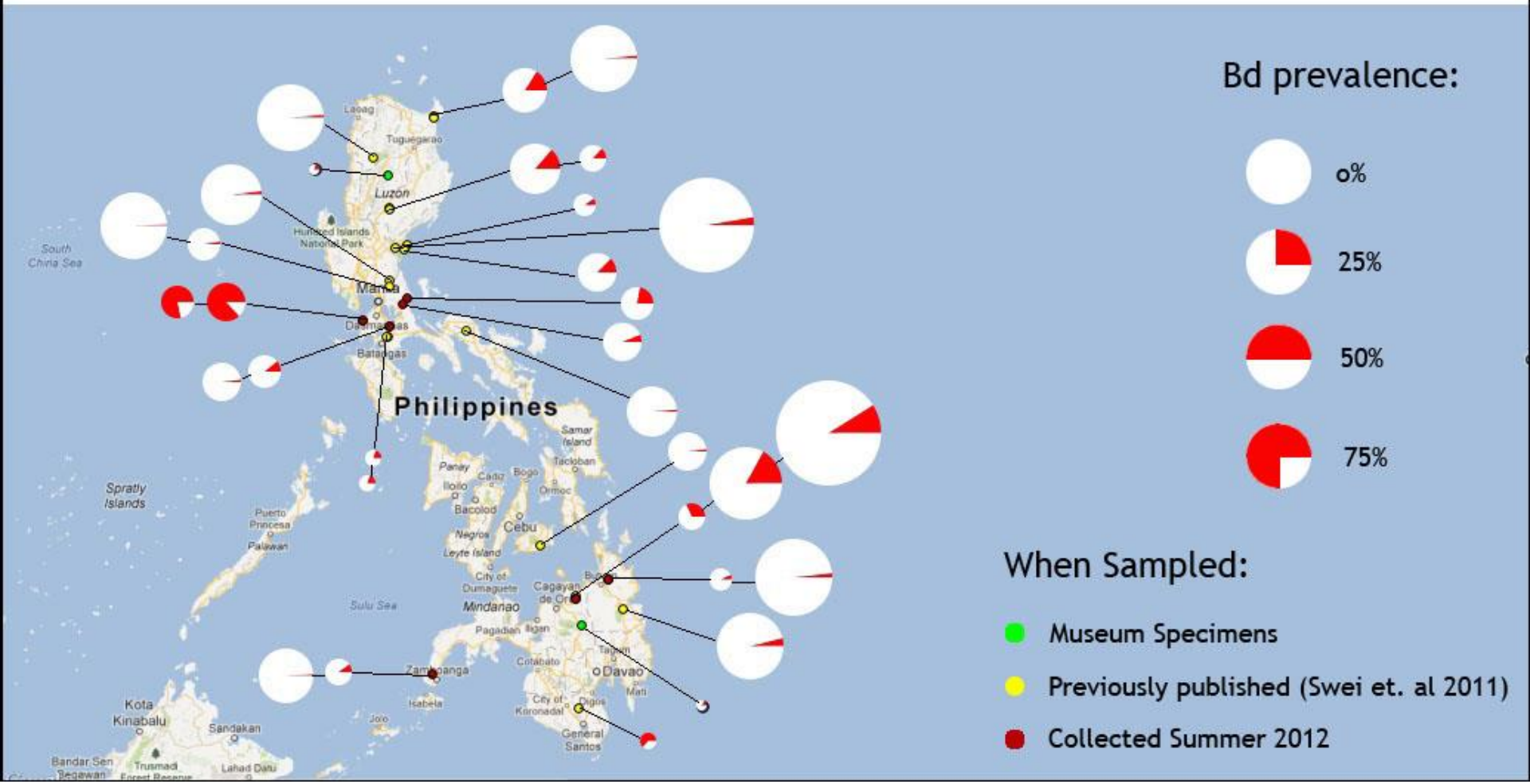


2012

300,000 zoospores



Bd Positive sites in the Philippines 1996 - 2012



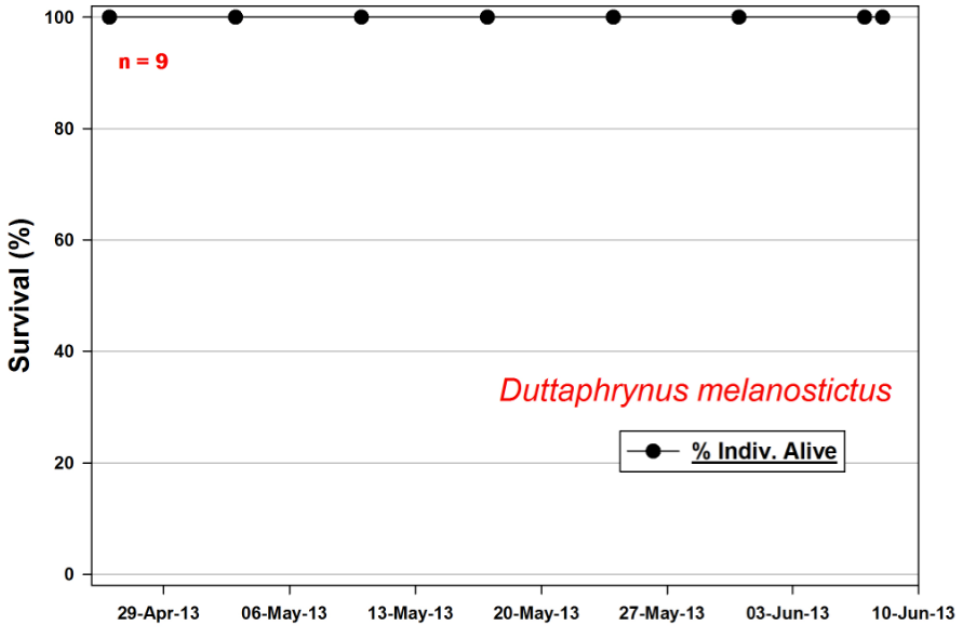
First Bd outbreak in Asia: Mt Palay-Palay

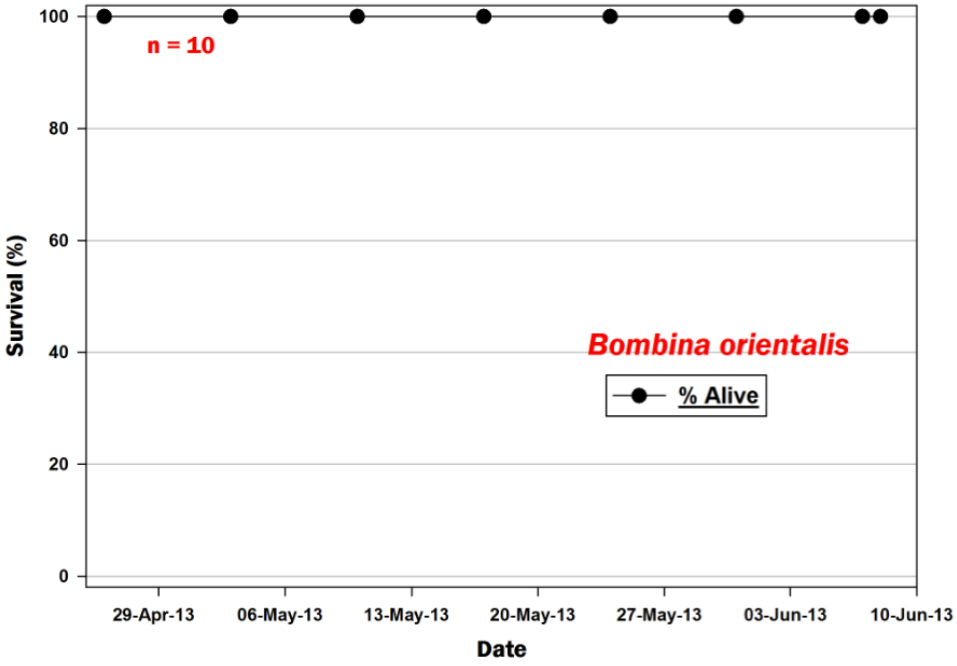
Bd Susceptibility Lab Trials
SF State University

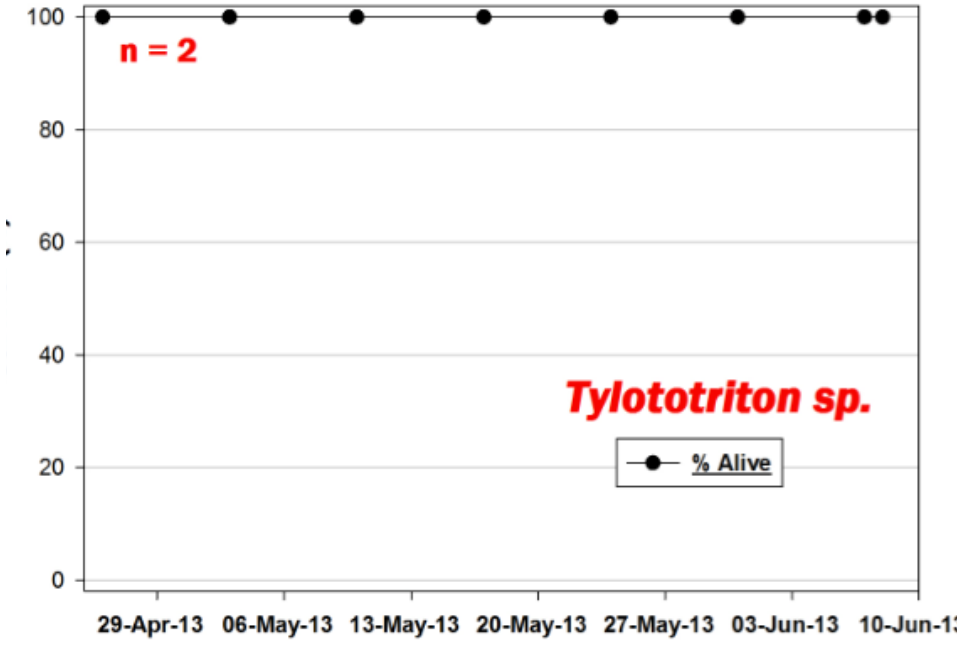
Animals purchased from pet trade (Petco, San Francisco, CA)

All tested Bd+ on arrival at SFSU

Raul Figueroa (SFSU master's)







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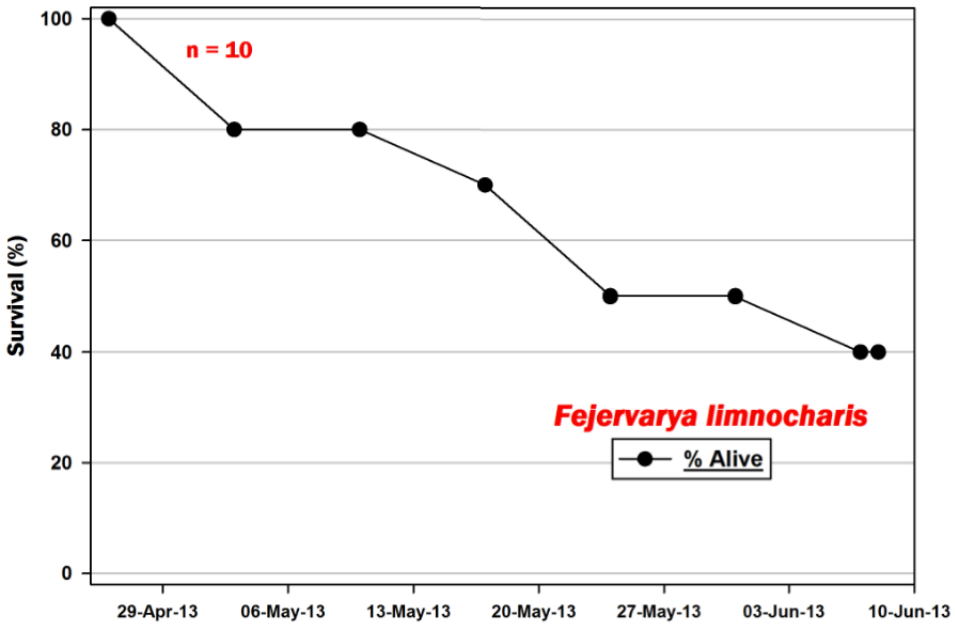
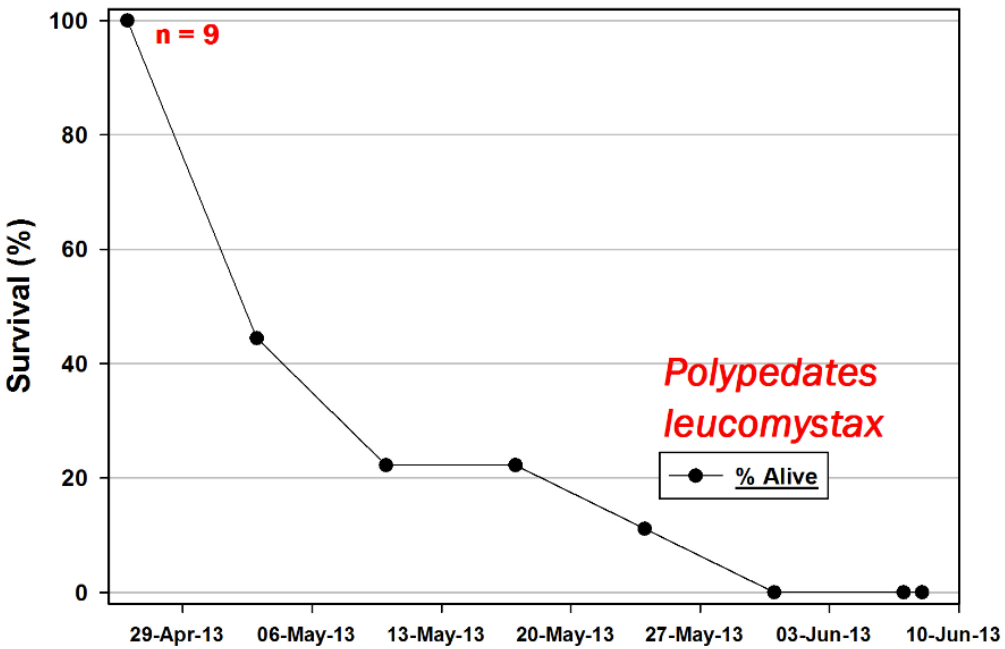
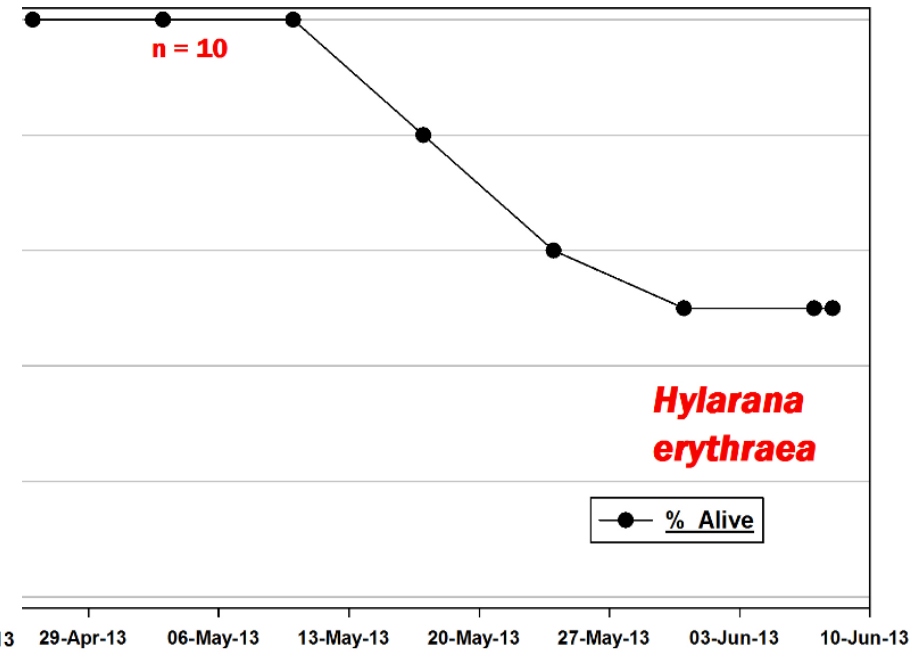
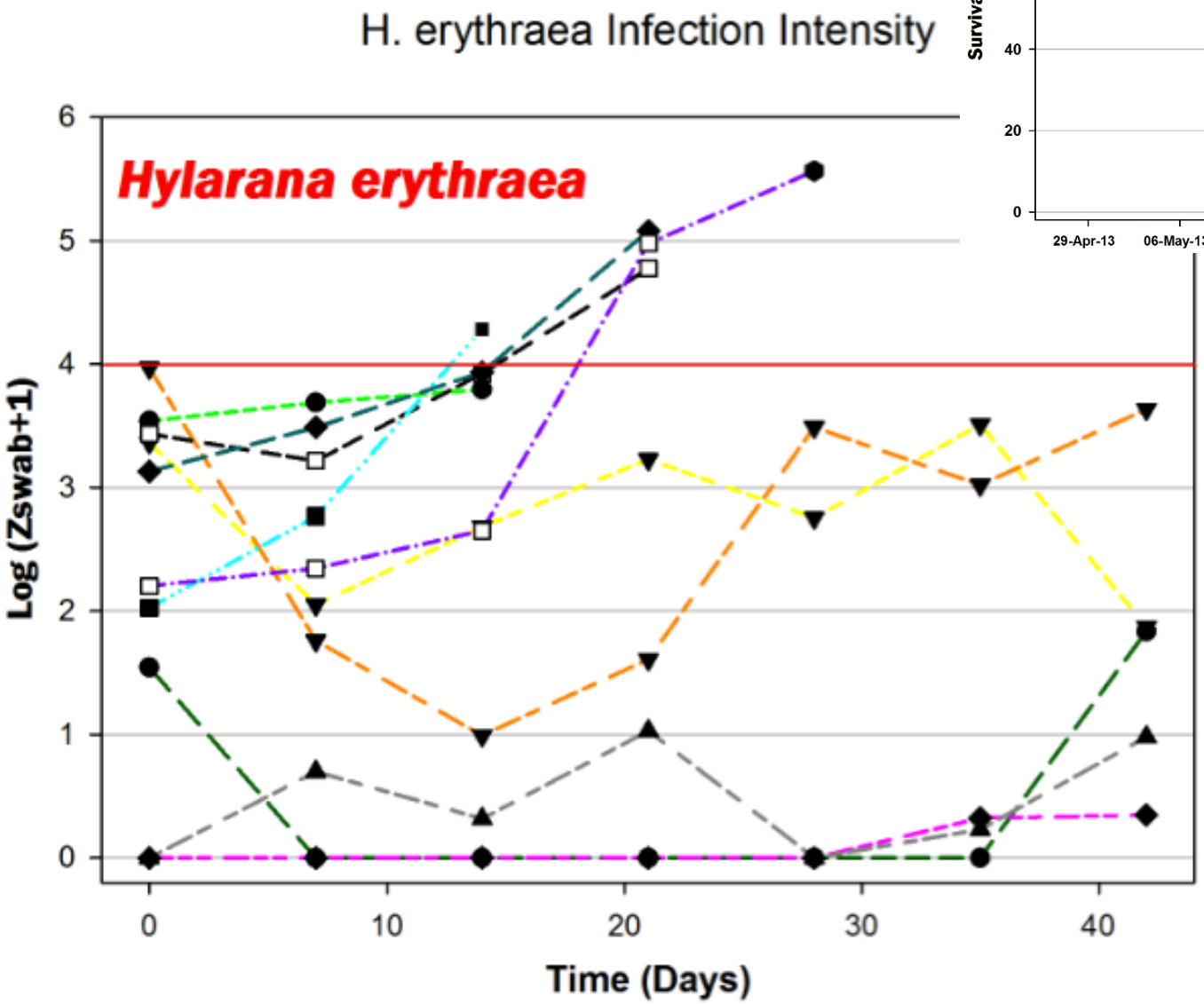
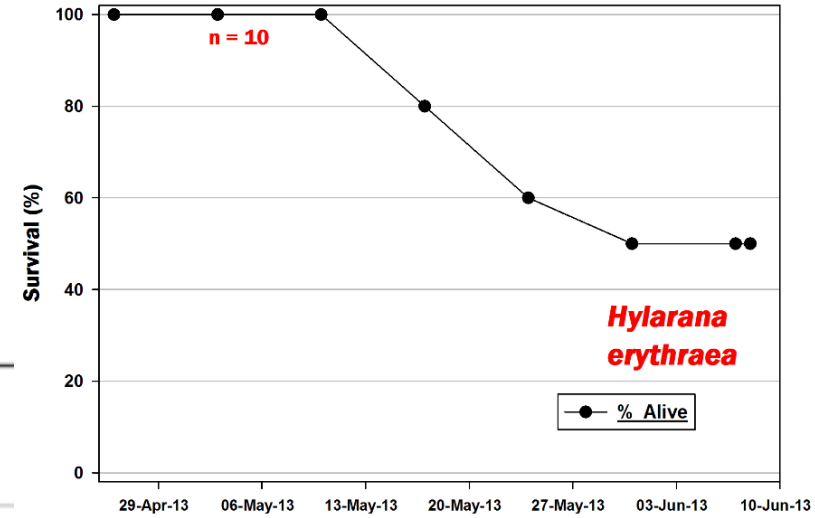







H. erythraea Infection Intensity
Hylarana erythraea
Log (Zswab+1)
Time (Days)
0
1
2
3
4
5
6
0
10
20
30
40


n = 10
Hylarana erythraea
% Alive
Survival (%)
0
20
40
60
80
100
29-Apr-13
06-May-13
13-May-13
20-May-13
27-May-13
03-Jun-13
10-Jun-13