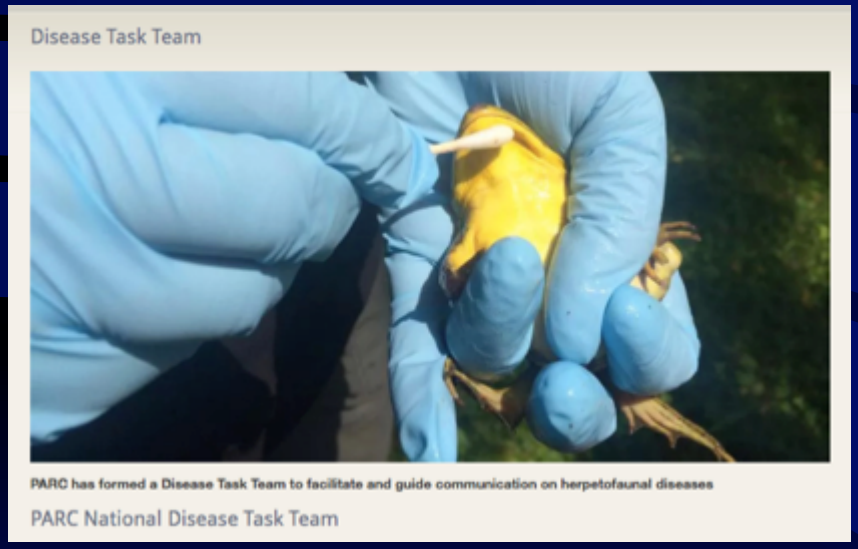
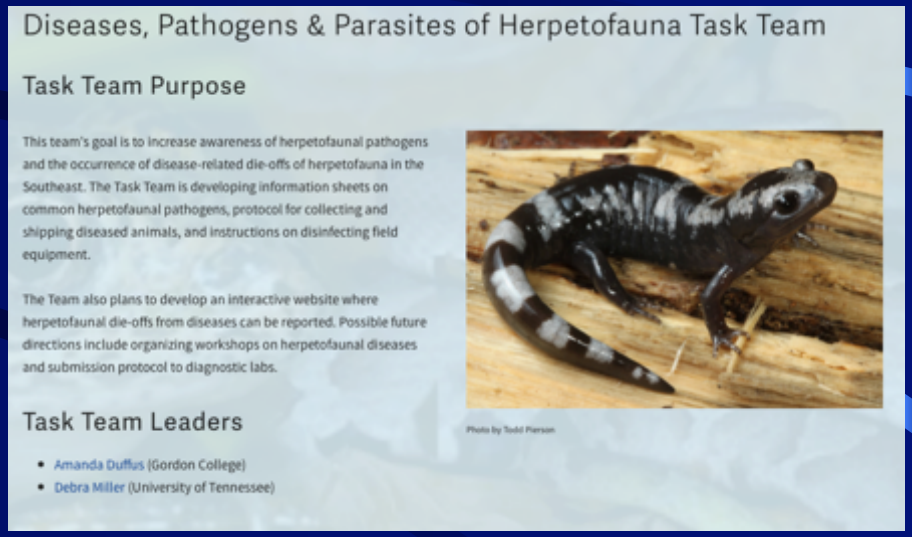
Bsal is NOT Bs – an emerging pathogen threatening global salamander diversity!

PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

SOUTHEAST PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION





http://www.parcplace.org/parcplace/resources/disease-task-team.html

http://separc.org/task-teams/#/disease-team/

Annual Meeting of Southeast PARC, 18 Feb 2017, Little Rock, AR

Bsal is NOT Bs – an emerging pathogen threatening global salamander diversity!

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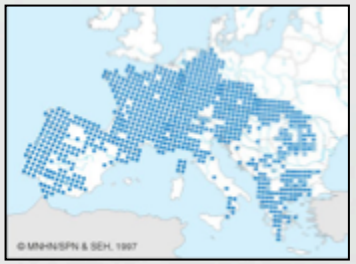
NORTH CAROLINA Wildlife RESOURCES COMMISSION

TENNESSEE WILDLIFE RESOURCES AGENCY

VANDERBILT School of Medicine

*What do we know?



Salamandra salamandra

- *2010: 96% wild mortality in Netherlands
- *2013 & 2014: wild mortality in Belgium
- *2015: UK (trade) and Germany (captivity)
- *2016: Netherlands, Belgium, Germany (wild)
- *Present in: **(Vietnam, Thailand, Japan)**
  - *wild salamanders in Asia
  - *museum records in Asia >150 yrs

Unknown to occur in North America

Martel et al. 2013, PNAS;
Martel et al. 2014. Science;
Cunningham et al. 2015. Veterinary Record;
Sabino-Pinto et al. 2015. Amphibia-Reptilia

14 of 55 sites: 3 species

Spitzen-van der Sluijs et al. (2016); EID

A lesion viewed under the microscope…

Dead cells (orange arrows)
Bsal thalli (black arrows)

"Death by a thousand holes"

Keratin

epidermis

Multifocal erosions and deep ulcerations of the skin throughout the body

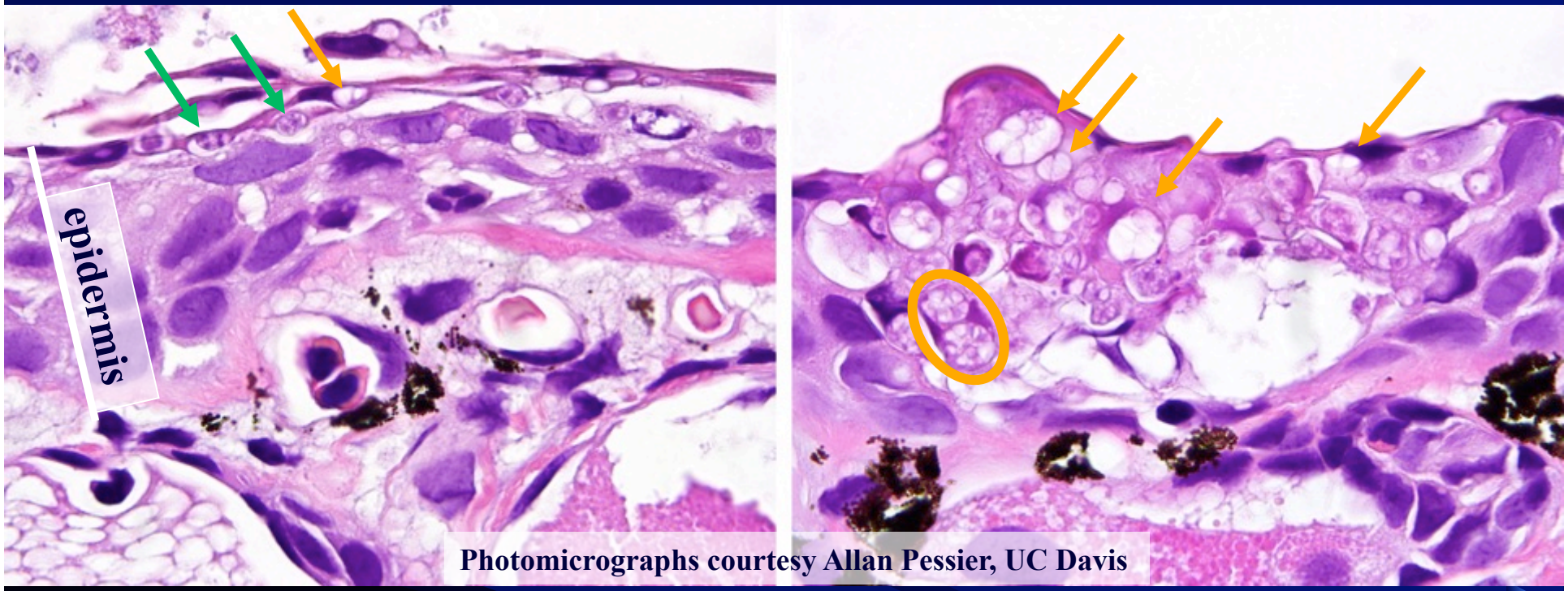
Death generally occurs in under 2 weeks

Van Rooij et al. (2015)

Photomicrograph courtesy
Allan Pessier, UC Davis

How does Bsal chytridiomycosis differ from Bd chytridiomycosis?

Bd ***Bsal***

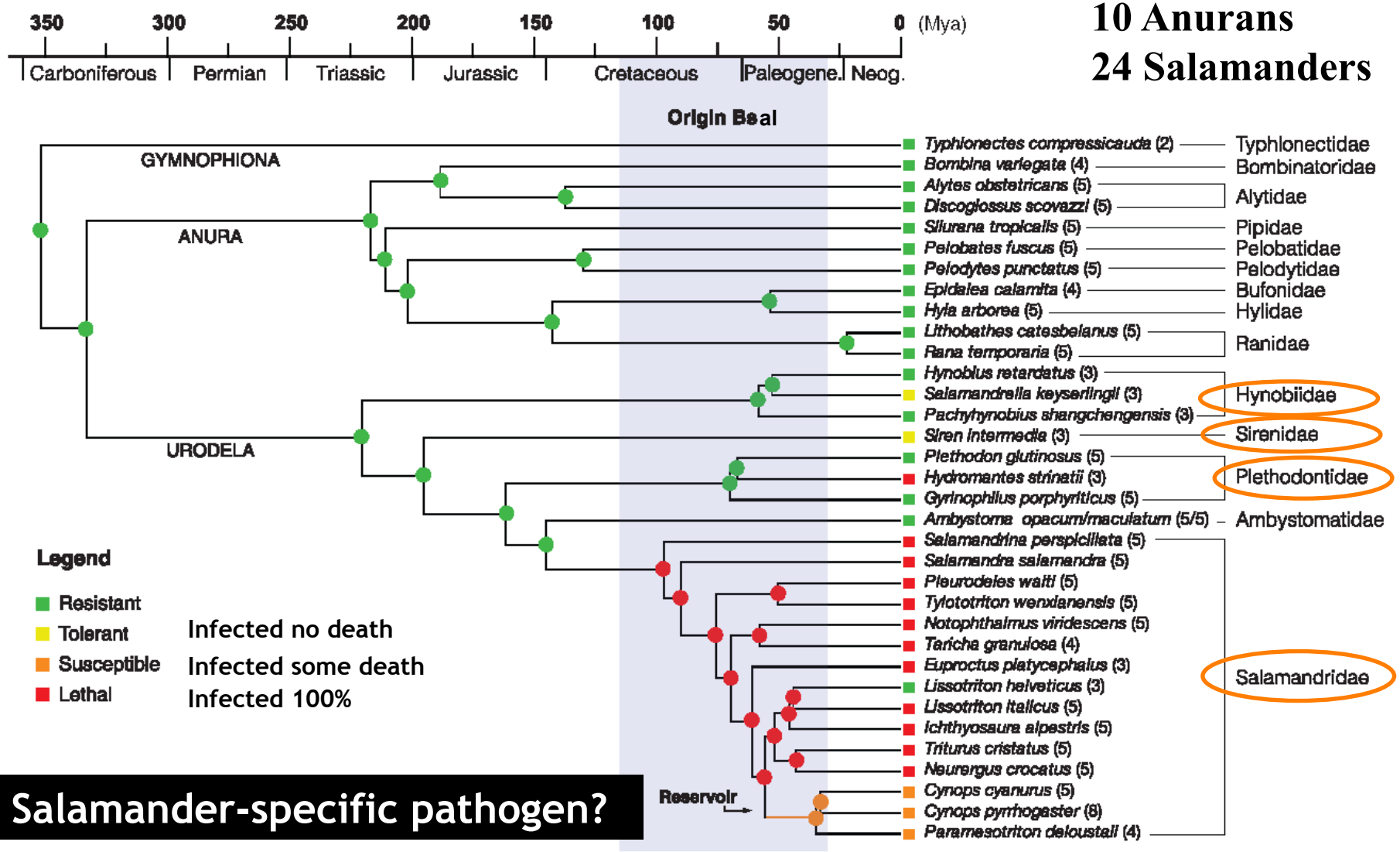


Photomicrographs courtesy Allan Pessier, UC Davis

Thickening of the skin (epidermis) and outer keratin layer with numerous thalli in superficial keratinocytes (note various stages; some with zoospores, green arrows; some empty, orange arrows). The cells (keratinocytes) within the epidermis are still distinct and somewhat in layers.

Near full-thickness necrosis (loss) of epidermis with numerous chytrid thalli (mostly empty) that frequently show internal septa (colonial thalli; arrows). Orange circle shows an intact cell (keratinocyte) with 2 chytrid thalli in its cytoplasm.

*Why is Bsal a threat?



Salamander-specific pathogen?

Martel et al. 2014. Science

North American Species Tested

Martel et al. (2014)

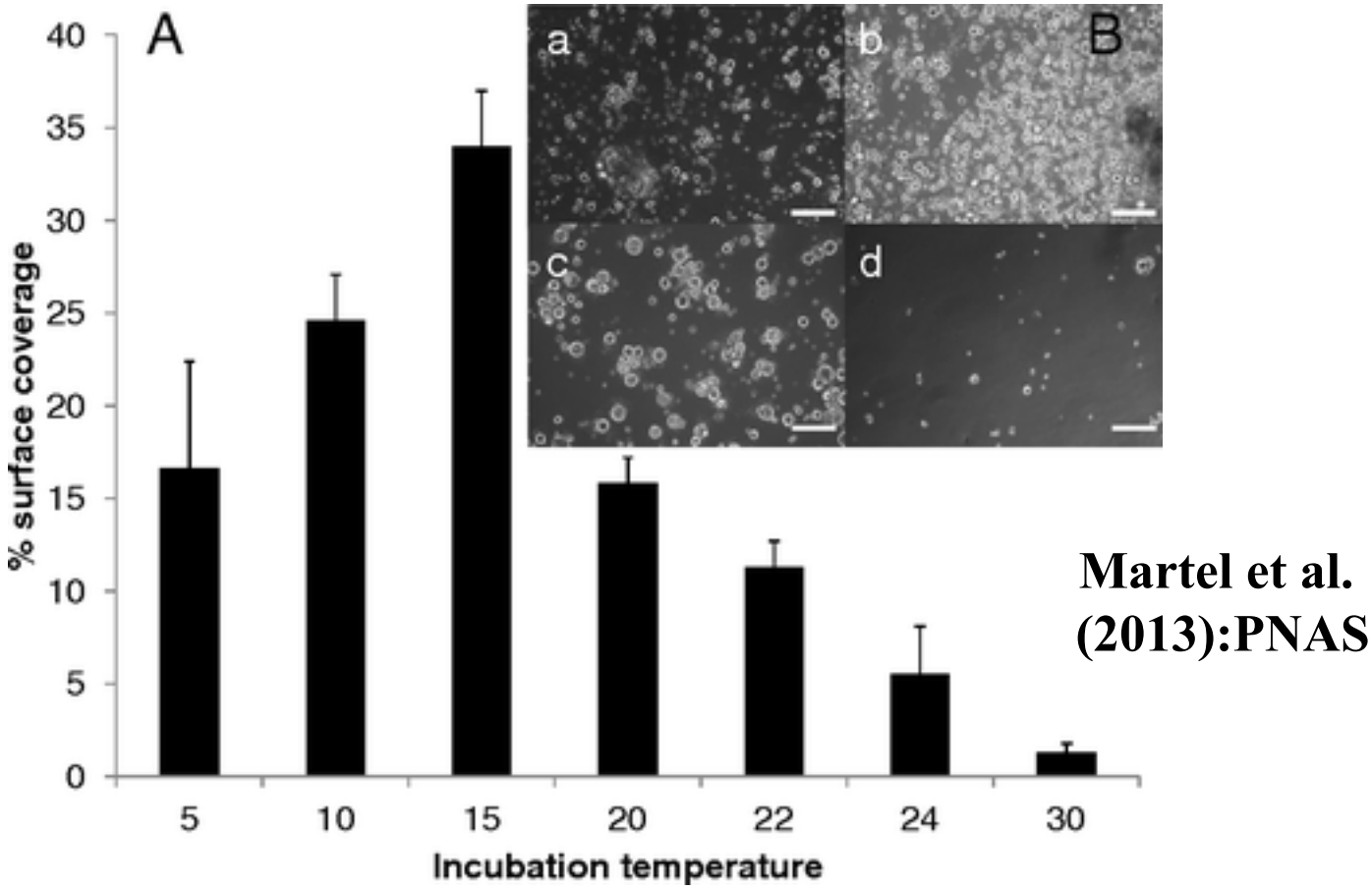
Clinical Disease

Subclinical Disease (Tolerant)

Small *n* and one dose (5×10^3 zoospores)

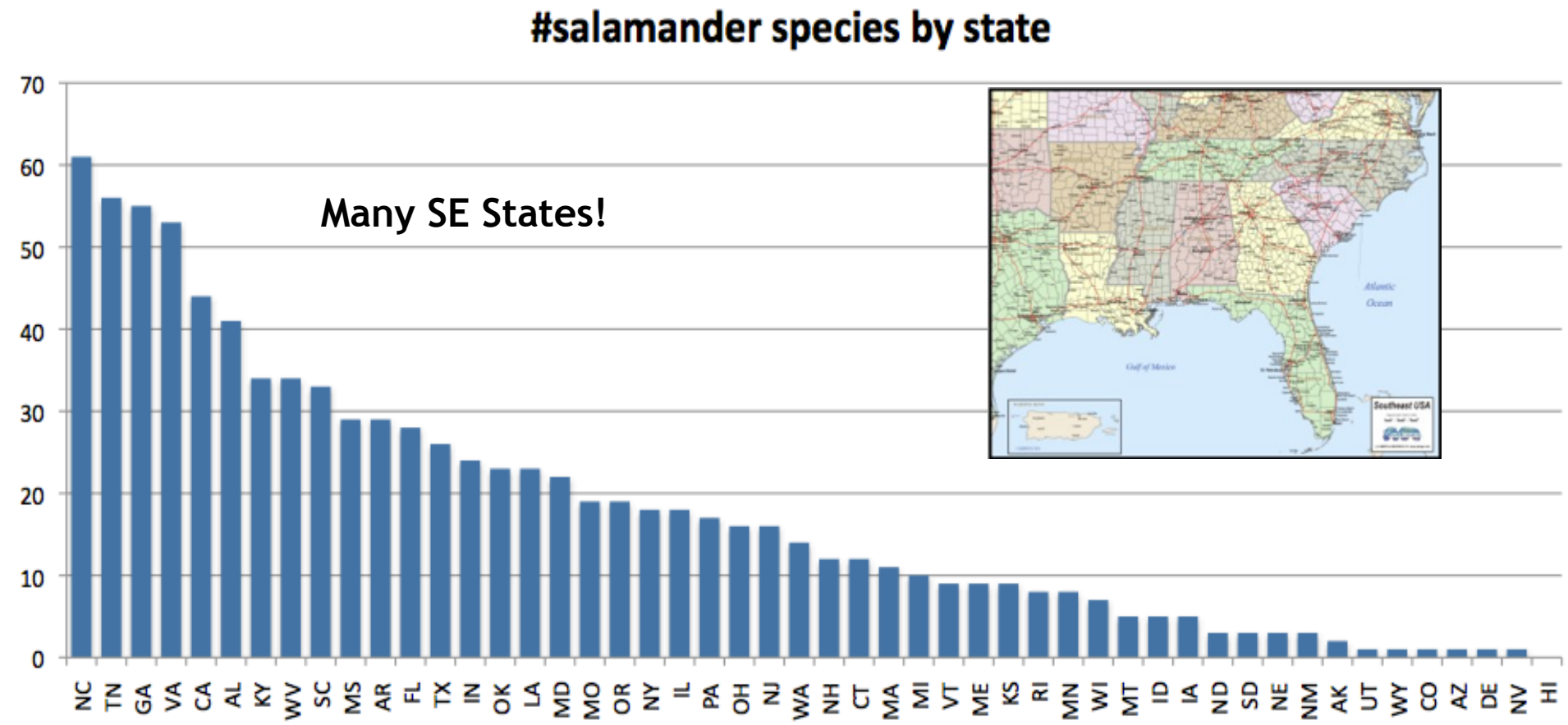
Not infected (or cleared it):

Thermal preference

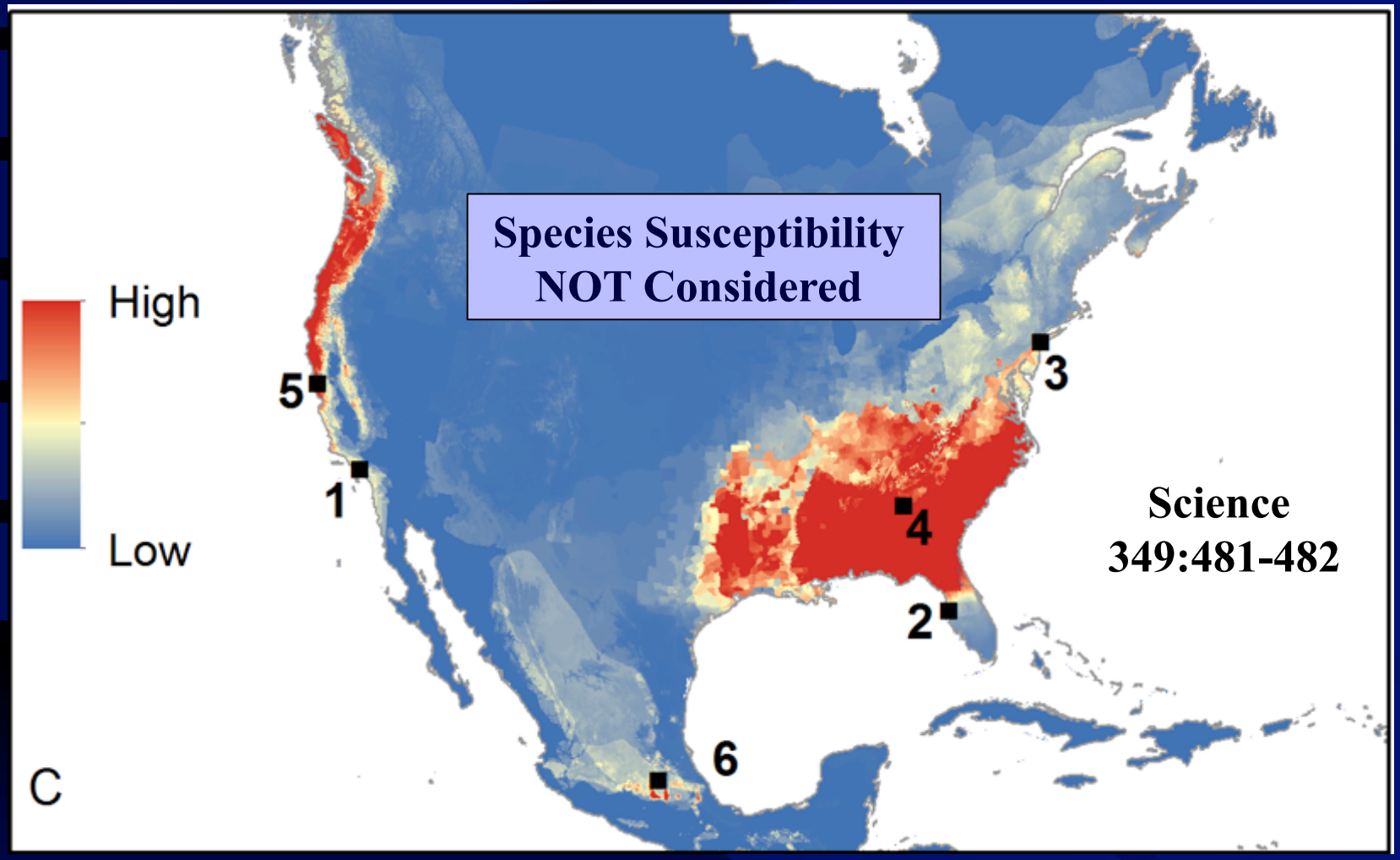


Martel et al. (2013):PNAS

*Why is Bsal a threat?



Risk Model: Yap et al. (2015)



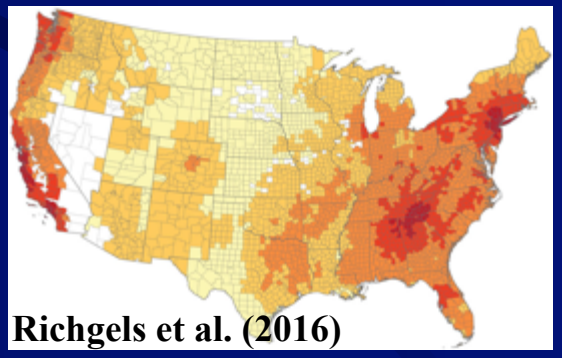
Final Risk Assessment Model

- Relative Risk = SpRich * Log ClimSuit Bsal

Research Objectives

1. Test the susceptibility of various North American amphibian species to *Bsal*
    - Tested 10 salamander and 4 anuran species
    - Susceptibility: infection, mortality, & disease generally across 4 *Bsal* doses (n = 10 / dose)
2. Test if *Bsal* exposure altered behavior of North American amphibian species
    - Locomotion and use of cover objects among *Bsal* doses

Robustly estimate RISK



Richgels et al. (2016)

Study Animals

Salamanders (10; 4)








Frogs (4; 2)




Lithobates sylvaticus, L. chiricahuensis, L. catesbeianus, Hyla chrysoscelis

Ambystoma opacum, A. laterale, Desmognathus ocoee, D. aeneus, D. monticola, Plethodon shermani x P. teyahalee, P. metcalfi, Necturus maculosus, Cryptobranchus alleganiensis, and Eurycea wilderae

Doses & Sample Sizes:

Doses:
5 x $10^{3\text{-}6}$ Zoospores

Target *n*:
10 per dose, 5 controls

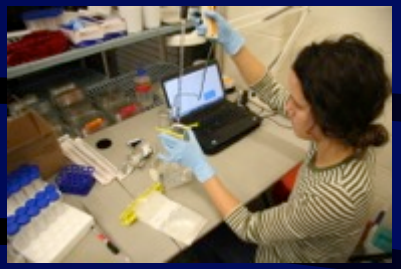
BLUE = captive; juv.

Species	Treatments	n/treatment	Controls	Total Animals
Ambystoma opacum	Control, 10^3, 10^4, 10^5, 10^6	10	10	50
Plethodon shermani/teyahalee	Control, 10^3, 10^4, 10^5, 10^6	7	6	34
Lithobates sylvaticus	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	5	5	25
Lithobates chiricahuensis	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	8	8	40
Lithobates catesbeianus	Control, 5*10^6	4	1	5
Hyla chrysoscelis	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	10	10	50
Desmognathus ocoee	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	10	5	45
Ambystoma laterale	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	5	4	24
Necturus maculosus	Control, 5*10^3, 5*10^4.5, 5*10^6	4 or 5	2	16
Plethodon metcalfi	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	10	5	45
Desmognathus aeneus	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	10	5	45
Desmognathus monticola	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	10	8	48
Cryptobranchus alleganiensis	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	6 or 7	3	30
Eurycea wilderae	Control, 5*10^3, 5*10^4, 5*10^5, 5*10^6	5	5	25

Just Finished

Methods

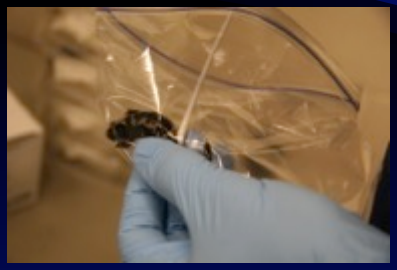
Mucosome



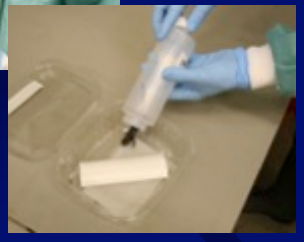
Culture & Enumeration

Chambers: 15 C

Wild: Bd swab



Exposure



10 mL in 100 mL container

24 hour

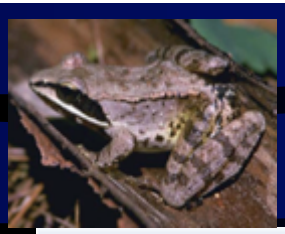
Methods

Daily Checks: 6 weeks

Swabs: 4 days PE, every 6 days

Necropsy

qPCR (Blooi et al. 2016)



Results: Mortality

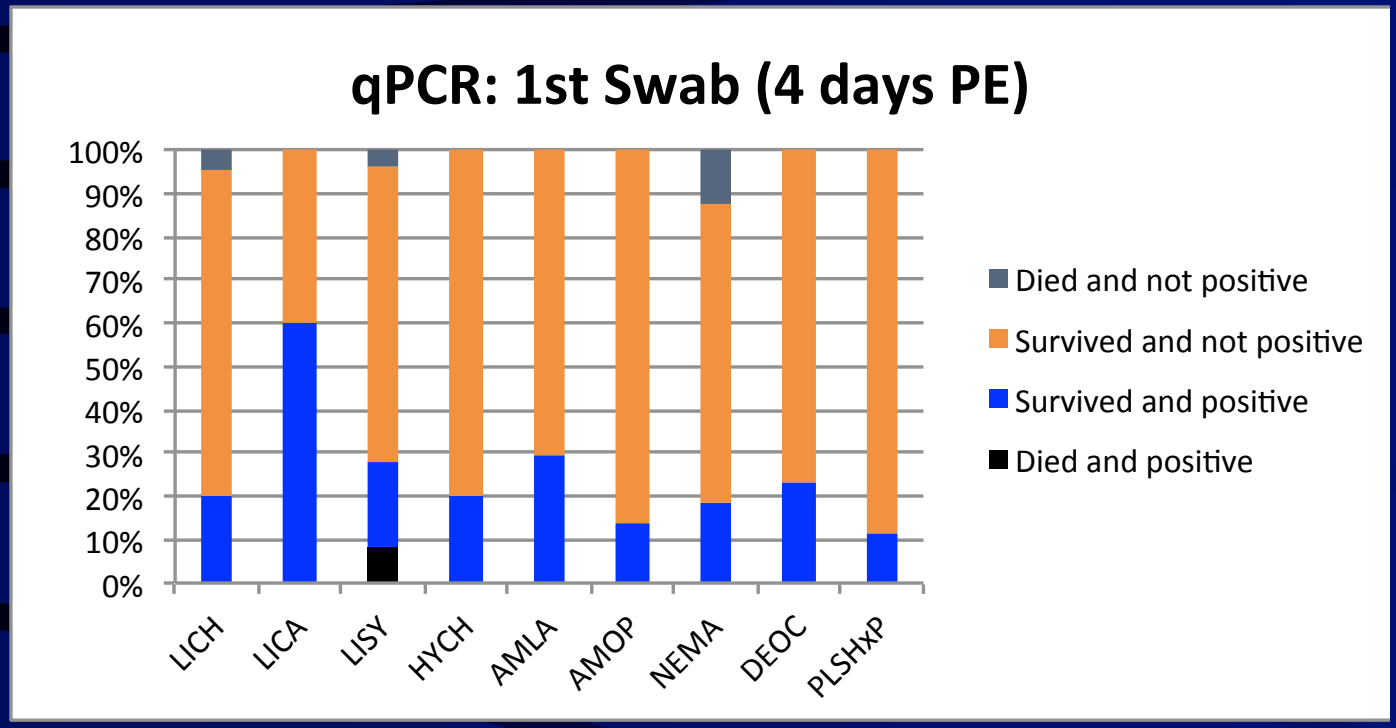
Species	# controls	Control		#/treatment	10^3		10^4		10^5		10^6	
***n* = 437**		Deaths	Percent dead		Deaths	Percent dead	Deaths	Percent dead	Deaths	Percent dead	Deaths	Percent dead
Bsal-Ambystoma opacum	10	0	0	10	0	0	0	0	0	0	0	0
Bsal-Plethodon shermani/ teyahalee	7	0	0	7	0	0	0	0	0	0	0	0
Bsal-Lithobates sylvaticus	5	0	0	5	0	0	**2**	**40**	0	0	**1**	**20**
Bsal-Lithobates chiricahuensis	7	1	14.28	8	0	0	**1**	**12.5**	0	0	0	0
Bsal- Lithobates catesbeianus	1	0	0	4	0	0	0	0	0	0	0	0
Bsal-Hyla chrysoscelis												0
Bsal- Desmognathus ocoee												0
Bsal- Ambystoma laterale	4	0	0	5	0	0	0	0	0	0	0	0

- qPCR of Skin, Toes at death: negative
- No histological evidence of Bsal chytridiomycosis

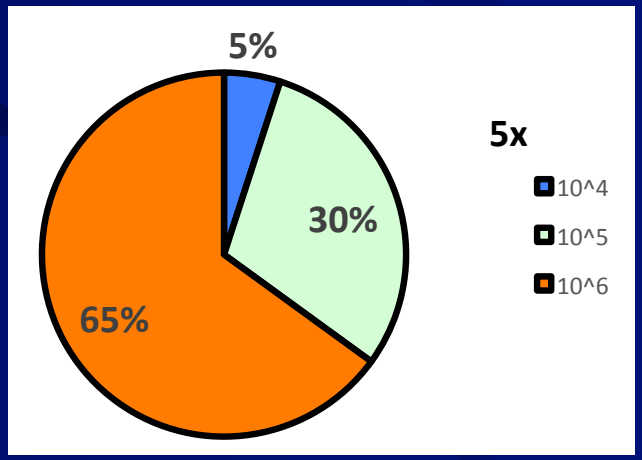
	# controls	Control		#/treatment	10^3 (n=5)		10^4.5 (n=5)		10^6 (n=5)	
		Deaths	Percent dead		Deaths	Percent dead	Deaths	Percent dead	Deaths	Percent dead
Bsal- Necturus maculosus	2	0	0	4 or 5	**1**	**20**	**1**	**25**	0	0

LISY = 24, 31, 34 PE **LICH = 25 PE** **NEMA= 4 and 24 PE**

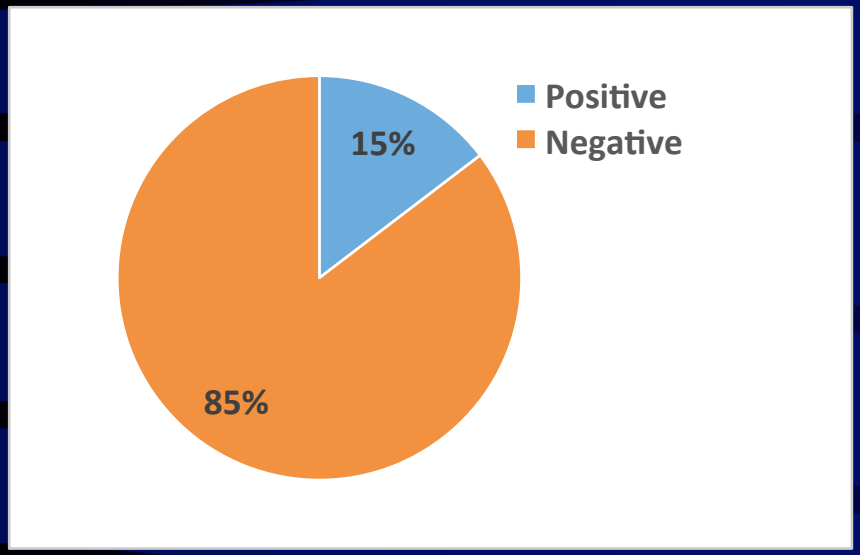
Results: Infection



- **10-60% sub-lethal infection including anuran species!**
- **Greatest infection at high doses**



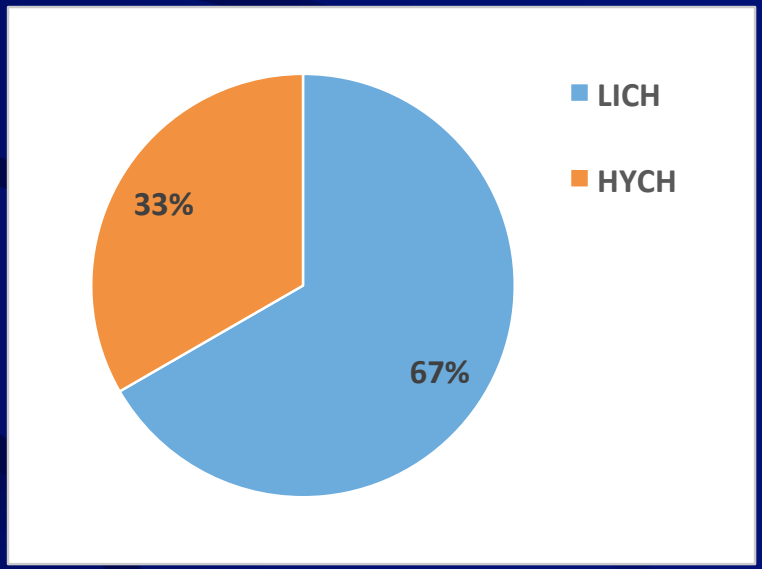
Infection at 22 PE (4th Swab)



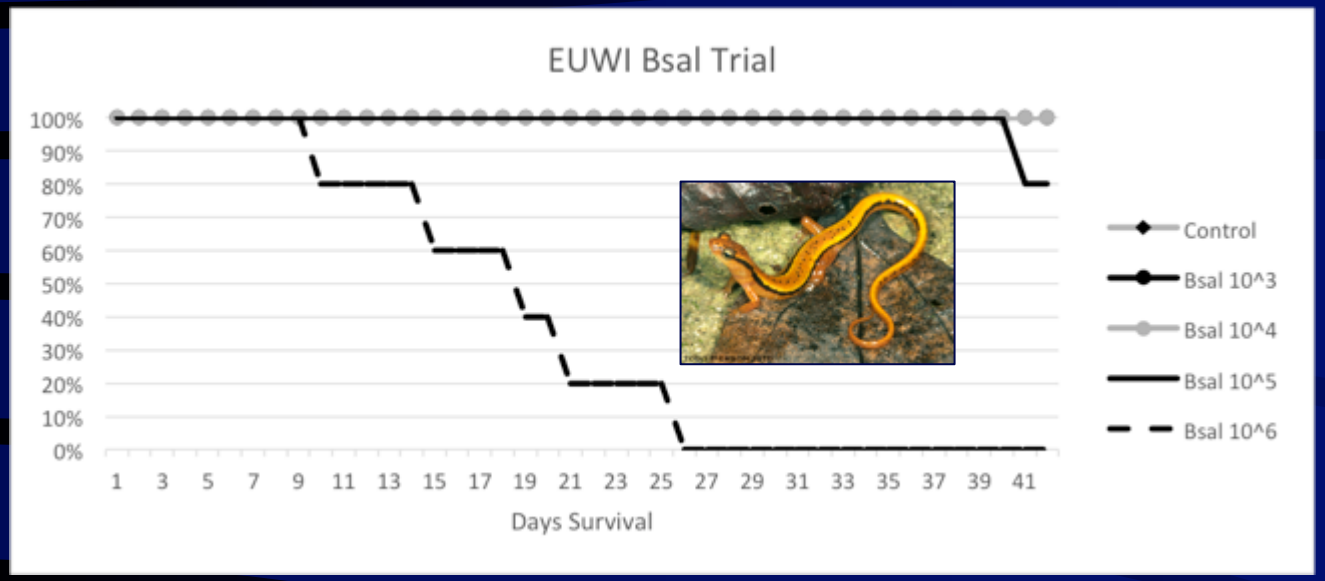
Of those infected at 4 days PE, only 15% were infected at 22 days PE

Clearing the Pathogen

Persistent subclinical infections occurred at 22 PE for Chiricahua leopard frog and Cope's gray tree frog



Survival and Time to Death : EUWI



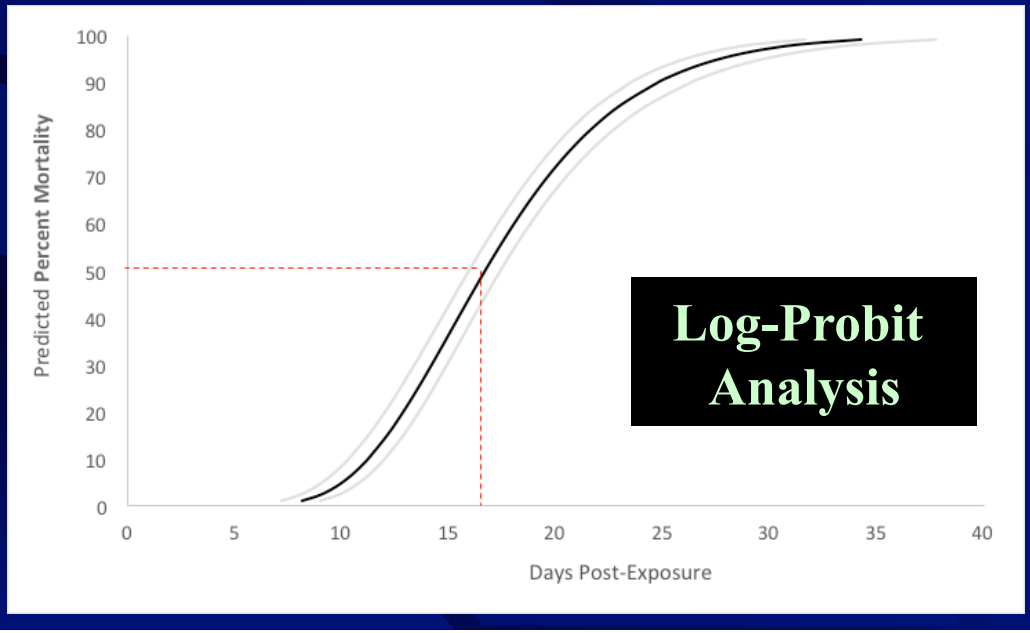
5×10^6

10-27 days

5×10^5

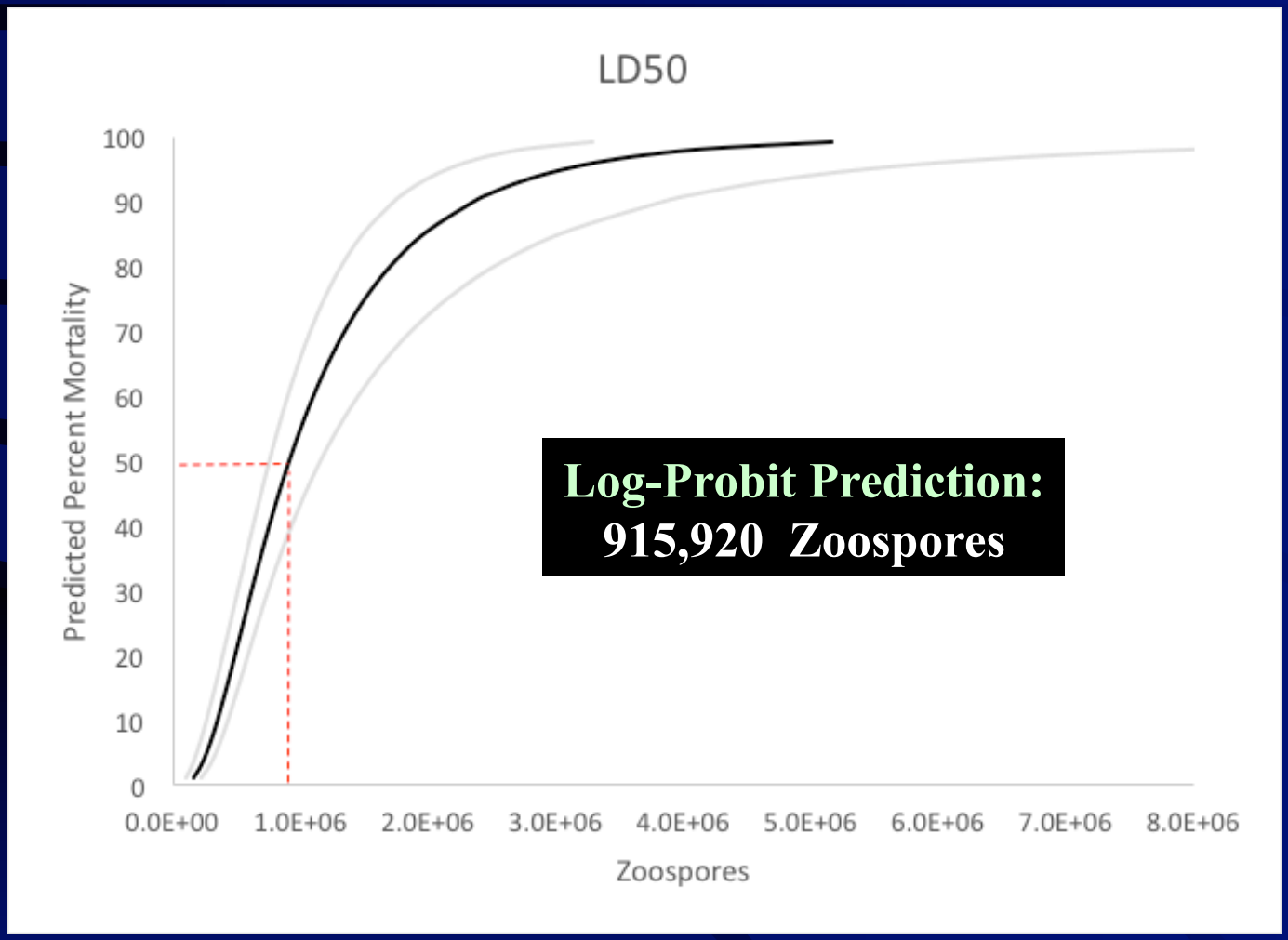
41 days

Of those that died,
Median time to death
= 16.7 days



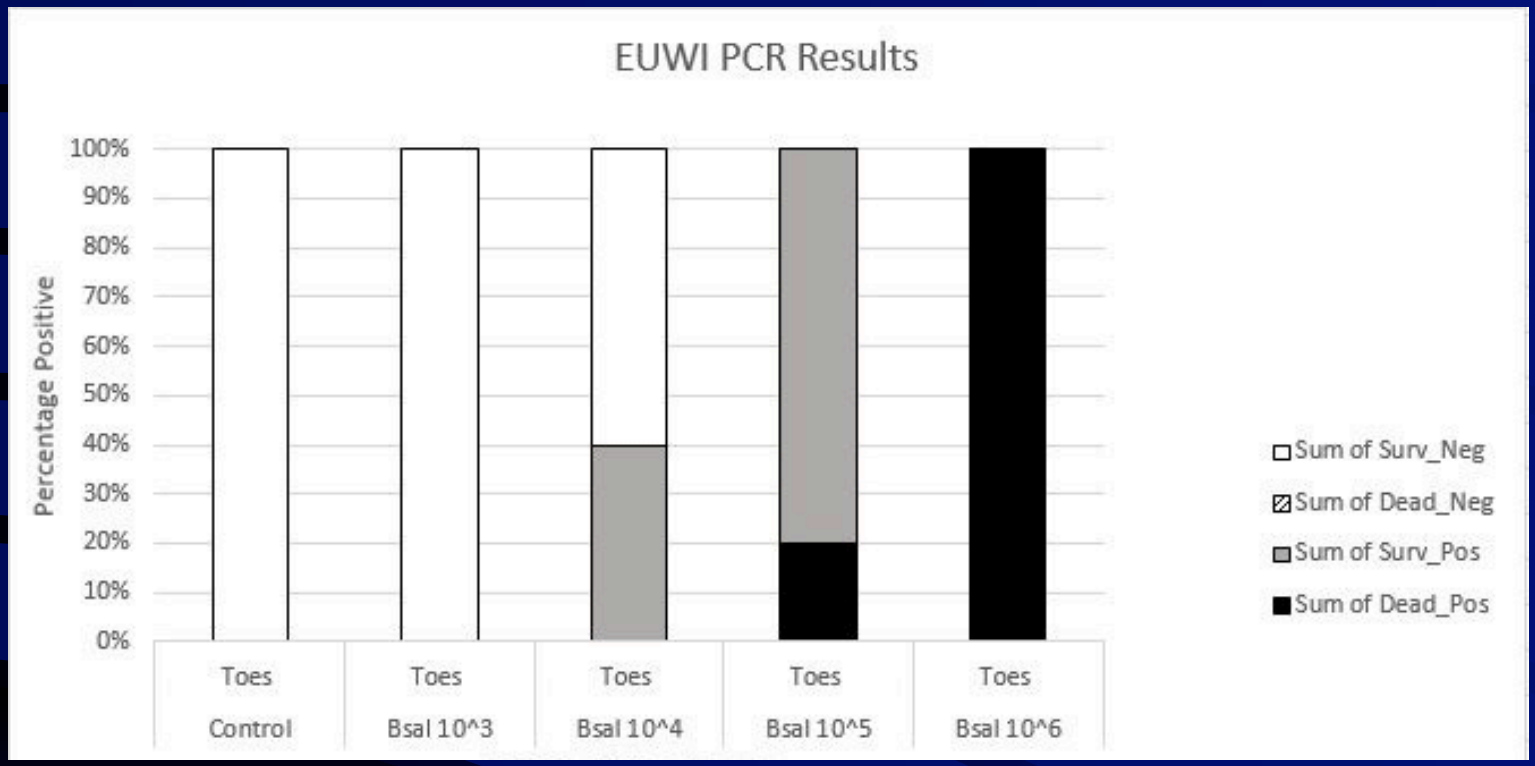
LD-50: EUWI

Zoospores per 10 mL



Log-Probit Prediction:
915,920 Zoospores

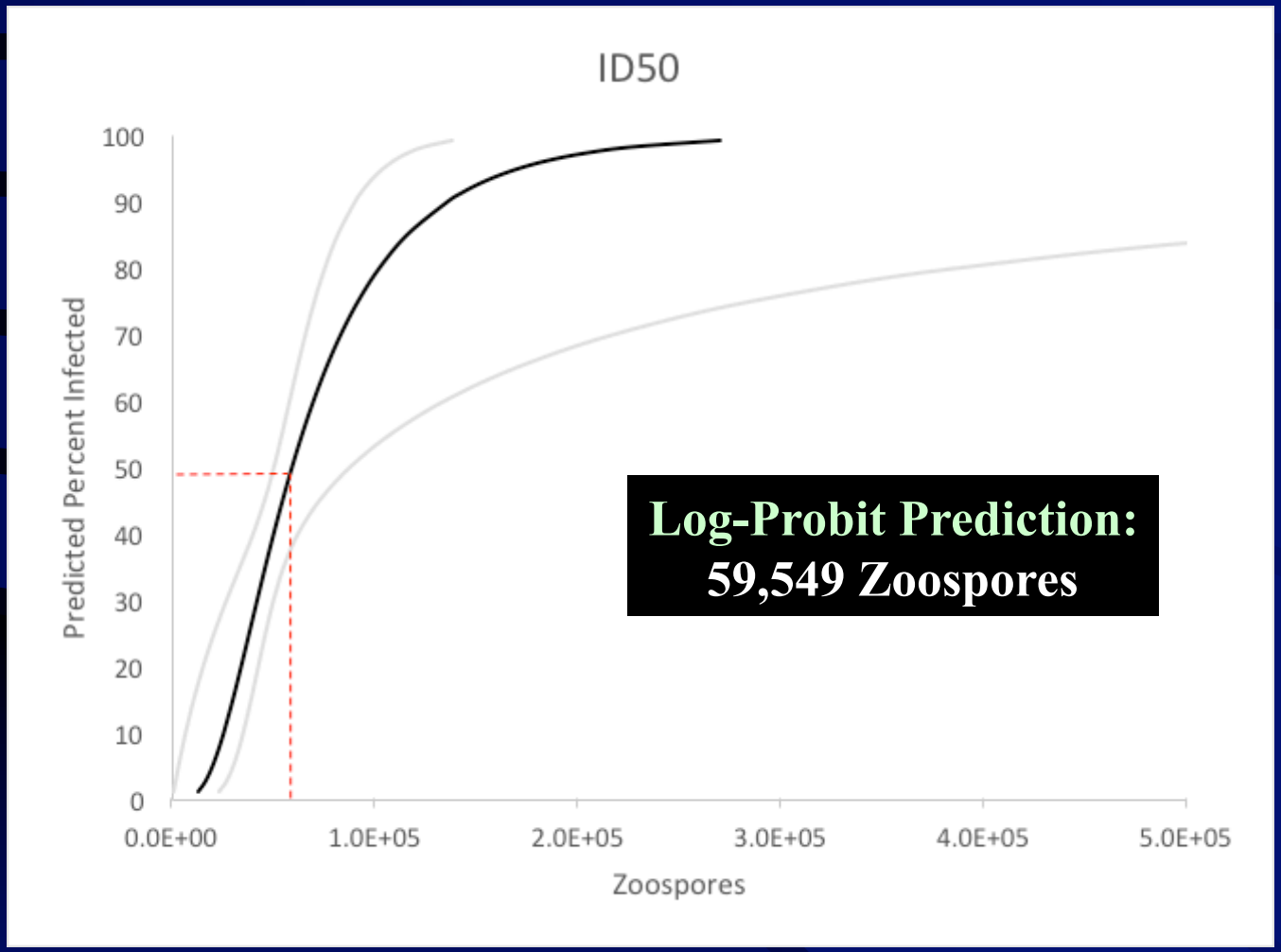
Final Pathogen Prevalence: EUWI



Of those infected at the endpoint of the experiment, 50% died, 50% survived (dose-dep response)

ID-50: EUWI

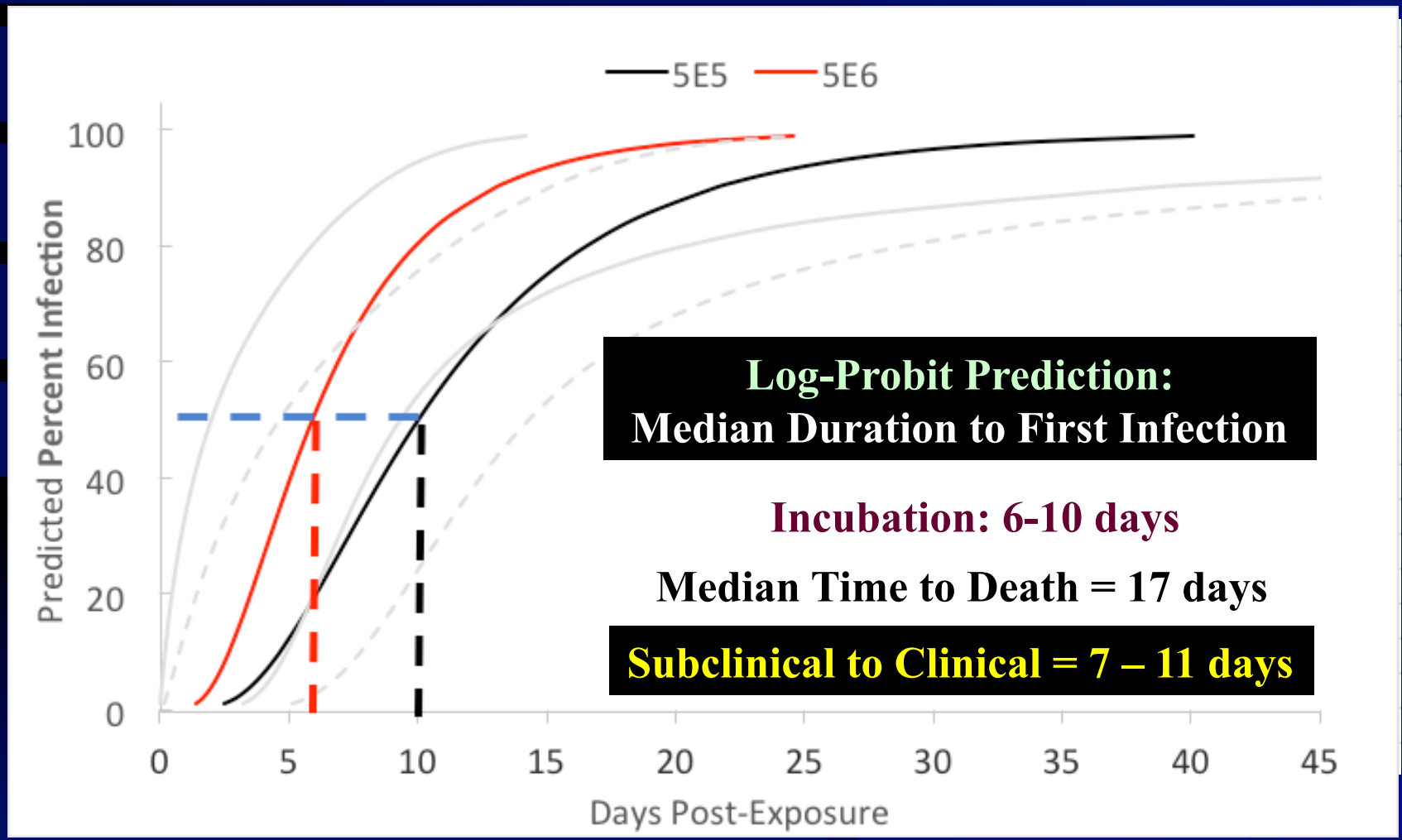
Zoospores per 10 mL



Log-Probit Prediction:
59,549 Zoospores

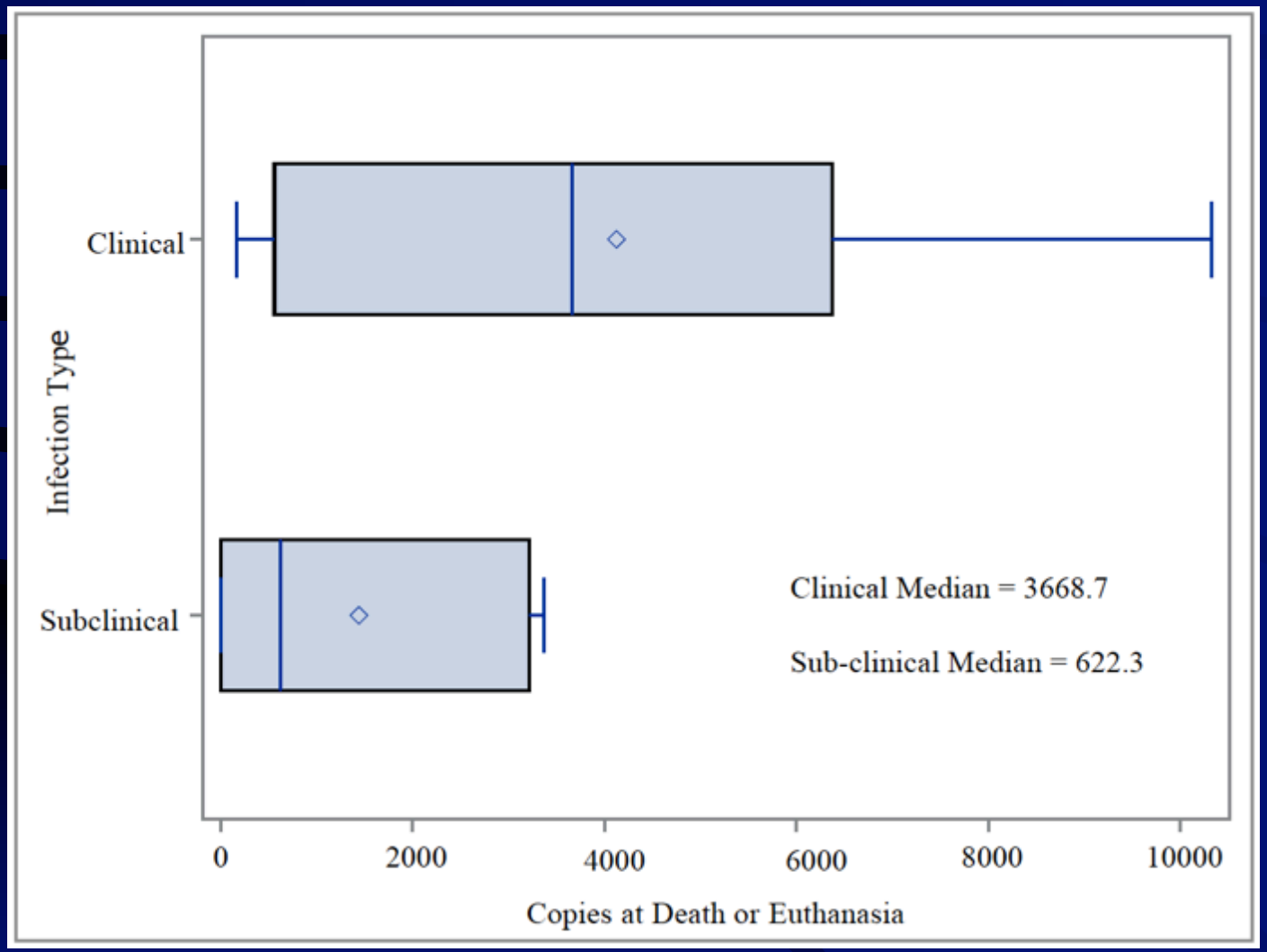
Infection Dynamics: EUWI

Prevalence: PE Duration and Dose



Pathogen Load: EUWI

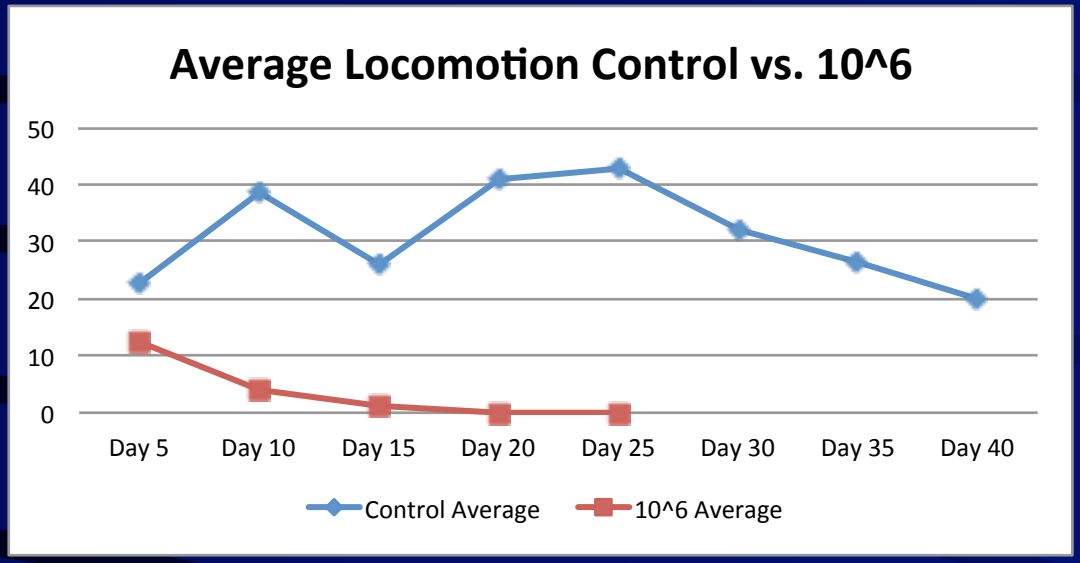
Subclinical vs Clinical Infection

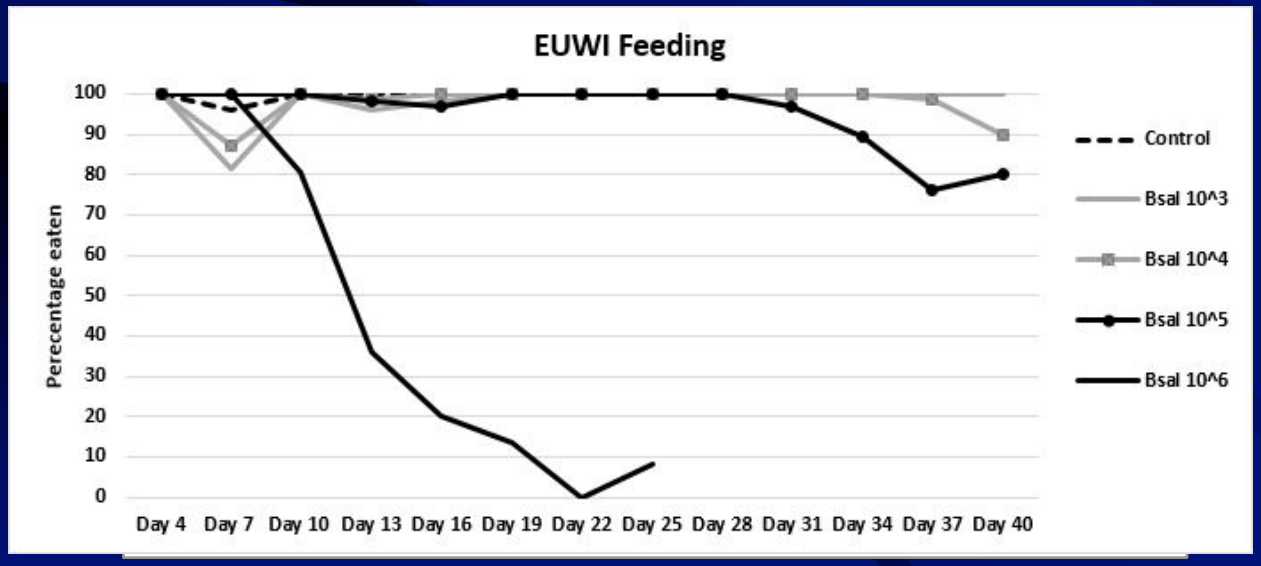


Bsal and Behavior: EUWI

5 x 10^6 vs. Controls

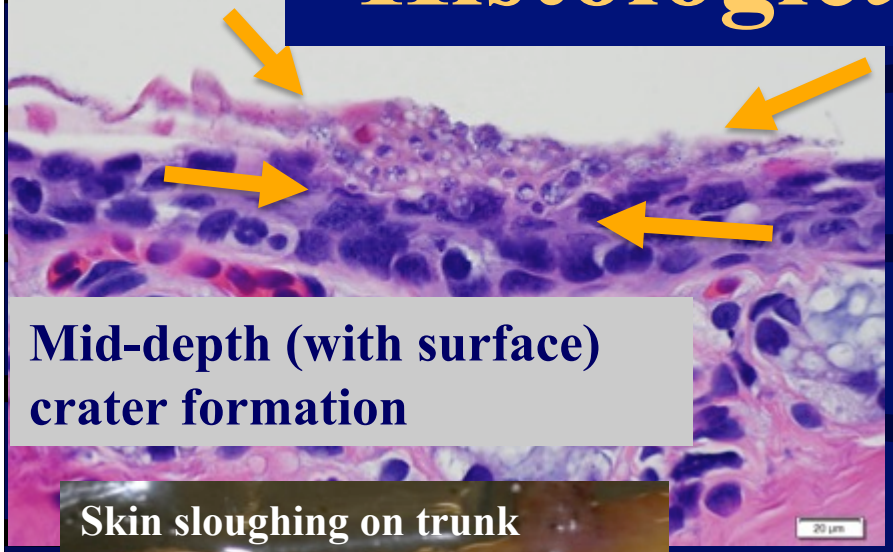
Dr. Gordon Burghardt and Students



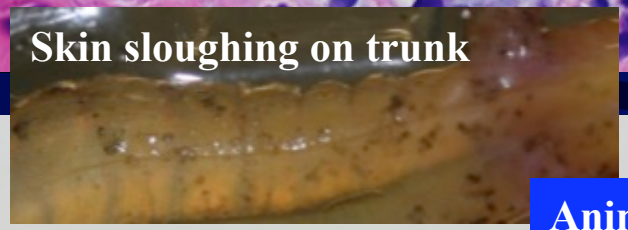


Gross Signs: EUWI

Histological Signs: EUWI

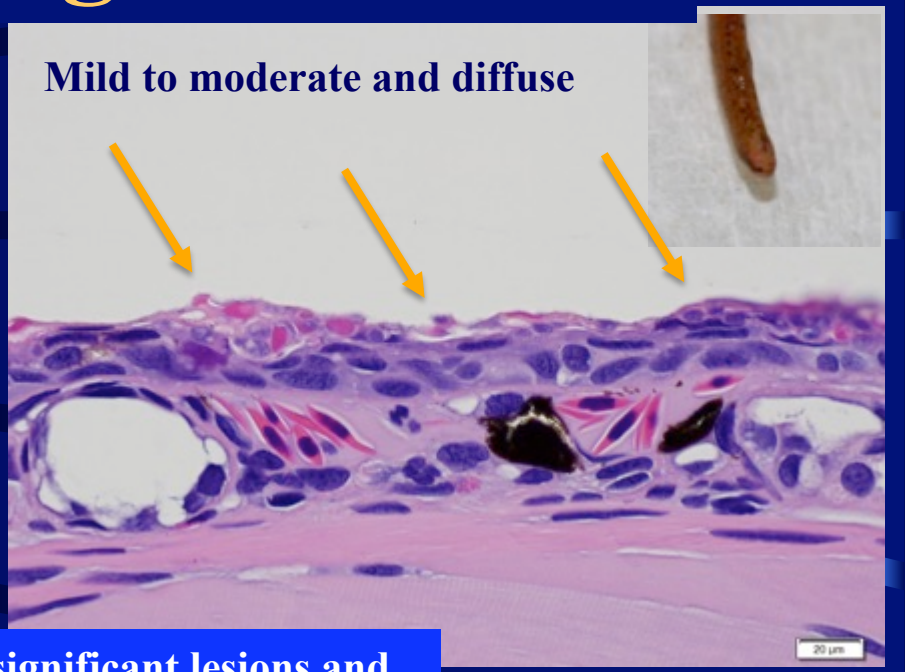


Mid-depth (with surface) crater formation



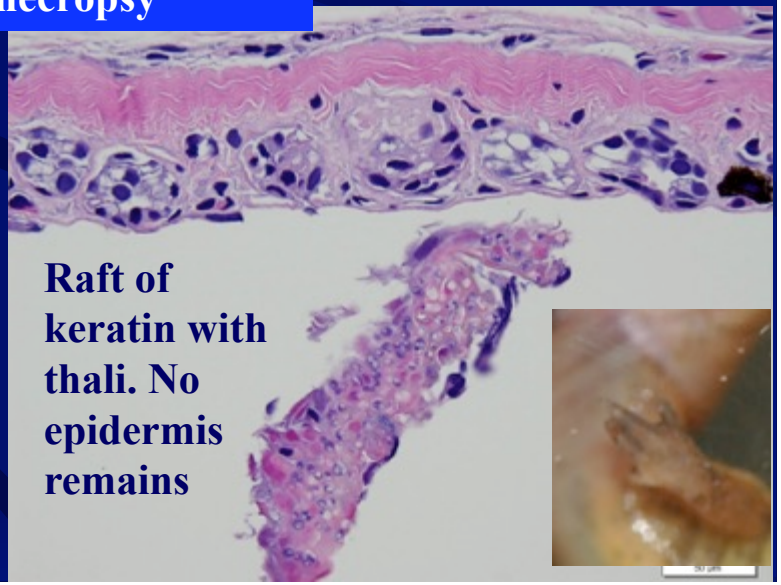
Skin sloughing on trunk

Mild to moderate and diffuse



Animal that died with significant lesions and Ct of 26 (skin) and 27 (toe) at necropsy

More superficial and somewhat diffuse

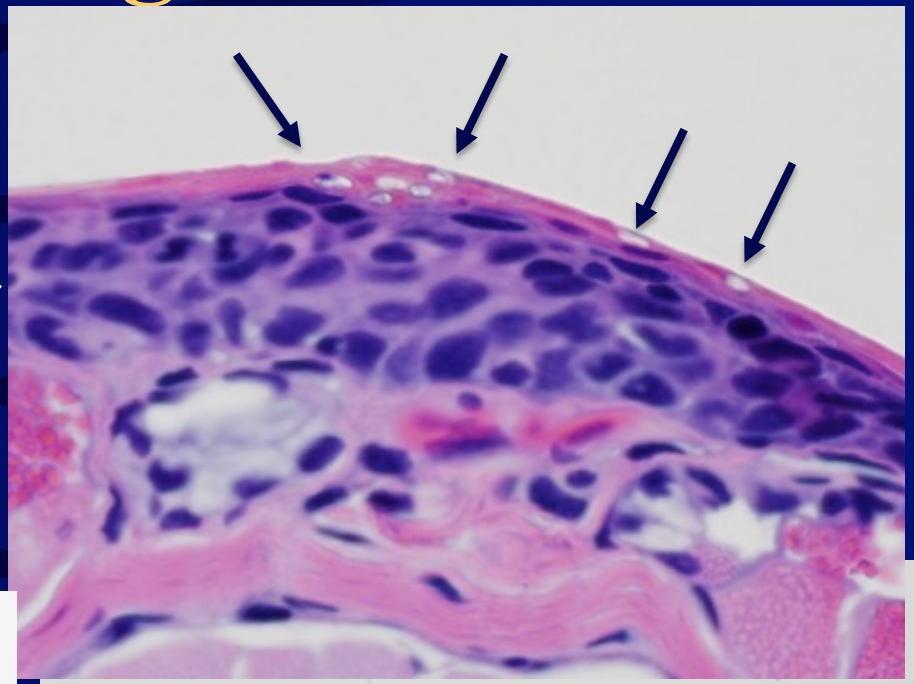


Raft of keratin with thali. No epidermis remains

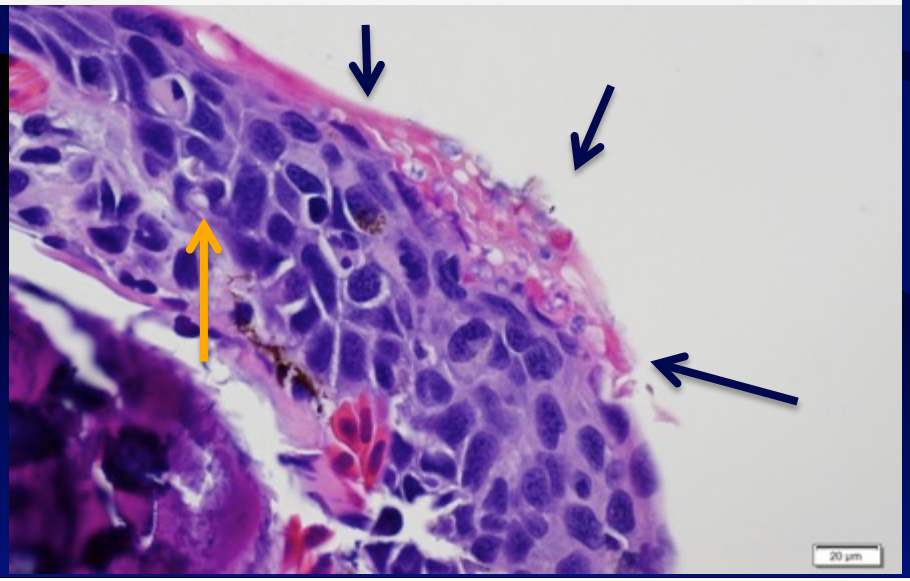
Histological Signs: EUWI

Polyp

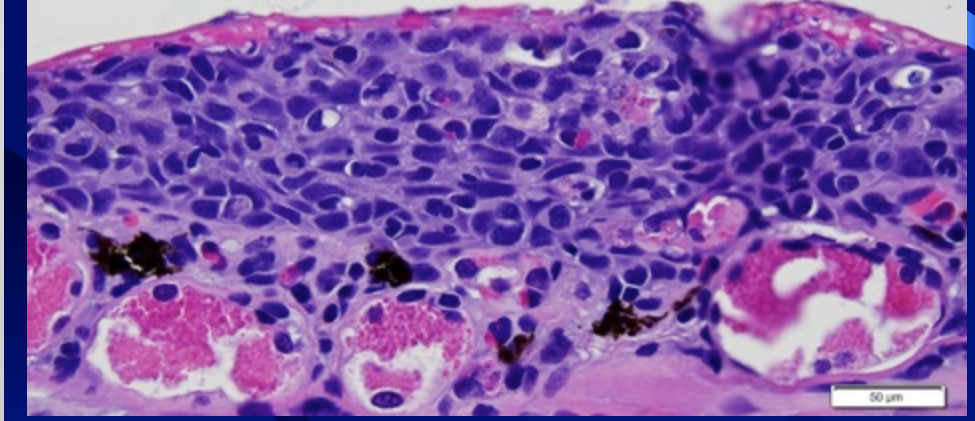
Animal with minimal lesions and Ct of 34 (skin) and 39 (toe) at necropsy



Toe with beginning crater formation (black arrows) & epidermal necrosis (orange arrow)



Tail with thick epidermis, extensive necrosis and numerous thali



Conclusions

- **No significant mortality was observed for 13 North American amphibian species (6 families) exposed to up to 4 doses of *Bsal***
- **Infection occurred in all (9 tested) species 4 days PE to *Bsal*, including the globally traded American bullfrog**
  - **Host range may be wider than expected at higher doses**
- **However, infection in most species tested was short duration (<2 weeks)**

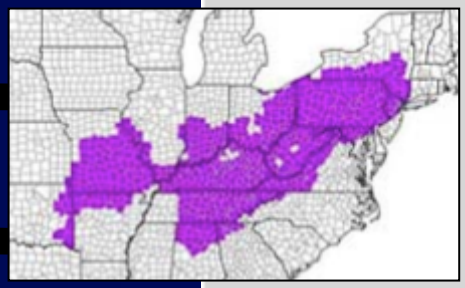
Conclusions

- *Eurycea wilderae* was susceptible at 3 of the 4 doses
  - ID 50 = 60,000 zoospores
  - LD 50 = 900,000 zoospores
  - Become Infectious = 6 – 10 days PE
  - Clinical Disease = 17 days PE

- *Bsal* may represent a significant conservation risk to EUWI and perhaps other *Eurycea* spp.
  - In addition to *Notophthalamus* and *Taricha*, Bsal surveillance should focus on *Eurycea*
  - Additional Bsal challenges with *Eurycea* is warranted
  - 28 *Eurycea* spp in North America
    (43% are listed as VU or EN by IUCN)

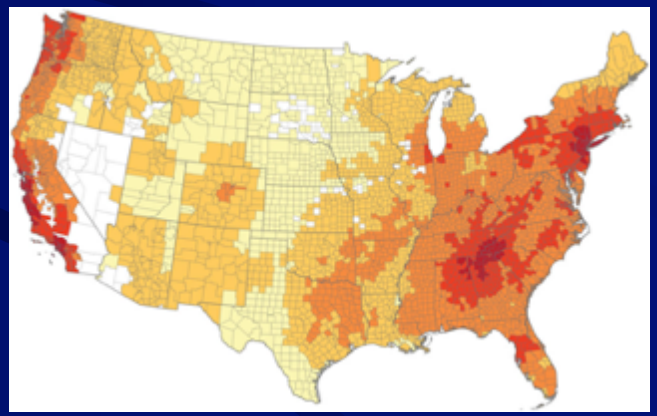
Eurycea Diversity

Endangered	Vunerable	Least Concern	Data Deficient	Region
			Eurycea aquatica	AL, GA, TN
		Eurycea bislineata		Eastern US
			Eurycea chamberlaini	NC, SC
	Eurycea chisholmensis			TX
		Eurycea cirrigera		Eastern US
		Eurycea guttolineata		Southeastern US
	Eurycea junaluska			TN, NC
	Eurycea latitans			TX
		Eurycea longicauda		Eastern US
		Eurycea lucifuga		South-central US
		Eurycea multiplicata		MO, AR, OK
	Eurycea nana			TX
Eurycea naufragia				TX
	Eurycea neotenes			TX
			Eurycea pterophila	TX
		Eurycea quadridigitata		Southeastern US
	Eurycea rathbuni			TX
			Eurycea robusta	TX
	Eurycea sosorum			TX
		Eurycea spelaea		MO, AR, OK
			Eurycea subfluvicola	AR
Eurycea tonkawae				TX
	Eurycea tridentifera			TX
			Eurycea troglodytes	TX
	Eurycea tynerensis			OK, AR, MO
	Eurycea wallacei			FL
	Eurycea waterlooensis			TX
		Eurycea wilderae		Southern Appl



Future Directions

- **Test additional species**
  - **Unique genera (*Aneides, Hemidactylium, Anaxyrus*) – First two ONGOING.**
  - **Mexico (*Pseudoeurycea, Chiropterotriton, and Bolitoglossa*) – Gabriela Parra Olea**
  - **Newts: *Notophthalmus perstriatus, N. meridionalis, and N. viridescens* (6 populations)**
  - **Axolotl: *A. mexicanum***



- **Use information to inform risk models (Yap et al. 2015:NA, Richgels et al. 2016: USA, Feldmeier et al. 2016: Europe)**
- **Identify amphibian attributes (e.g., mucosome properties) that contributes to immunity**


Questions??
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