Ecology and Threat of Batrachochytrium salamandrivorans (Bsal)

Matthew Gray[1], Patrick Cusaac[1], Davis Carter[1], Jennifer Spatz[1], Laura Reinert[2], Louise Rollins-Smith[2], Doug Woodhams[3], and Debra Miller[1,4]

[1]UTIA Center for Wildlife Health
[2]Vanderbilt School of Medicine
[3]Dept of Biology, UMass-Boston
[4]UTIA College of Veterinary 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

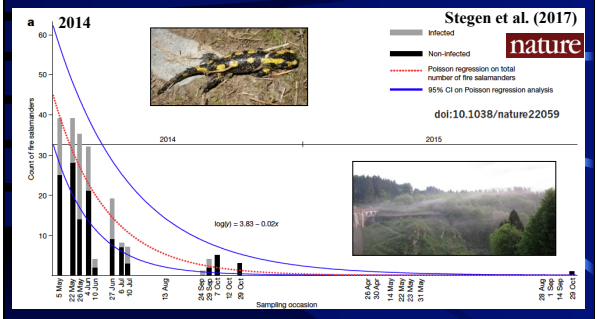
14 of 55 sites: 3 species

Ichthyosaura alpestris
Lissotriton vulgaris

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

Martel et al. 2013, PNAS; Laking et al. 2017. Scientific Reports
Martel et al. 2014. Science;
Cunningham et al. 2015. Veterinary Record;
Sabino-Pinto et al. 2015. Amphibia-Reptilia

Outbreak at Robertville, Belgium



$P[I]_{10\text{-day}} = 0.33$

10% of its original size in 6 months

1% present 1.5 years later

A *Bsal* lesion viewed under the microscope…

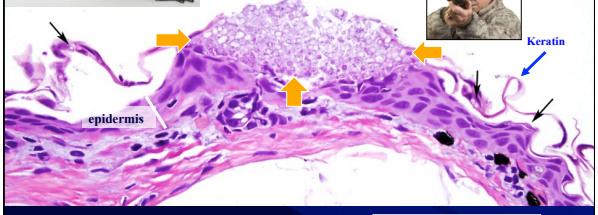
VETERINARY RESEARCH

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

"Death by a thousand holes"

Keratin

epidermis



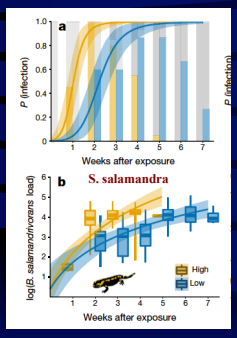
Multifocal erosions and deep ulcerations of the skin throughout the body
Death generally occurs in under 3 weeks

Van Rooij et al. (2015)

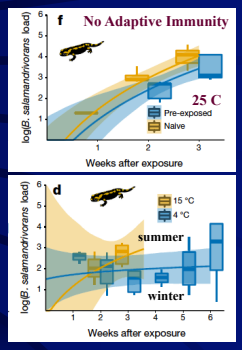
Photomicrograph courtesy Allan Pessier, UC Davis

Amplification Species

Stegen et al. (2017)



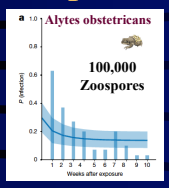
100% Infected Regardless of Dose
(100 vs 10,000)

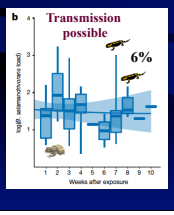


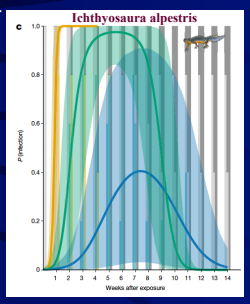
More Pathogenic at 15 C
Persistent Infections at Low Temp

Susceptibility of Syntopic Species

Stegen et al. (2017)





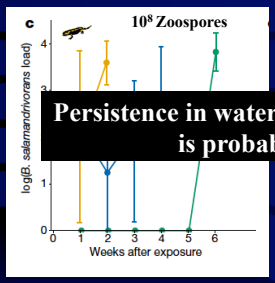


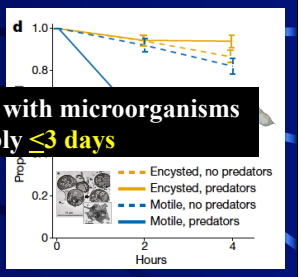
Doses = 100, 1000, 10,000

Reservoirs:
- **Low Susceptible** = midwife toad
- **Mod Susceptible** = alpine newt

Environmental Persistence of *Bsal*: Water

Stegen et al. (2017)





Persistence in water with microorganisms is probably ≤3 days

Zoospore load in infected salamanders at 0, 14, and 31 days PI in Filtered Pond Water

Pond Water with Micropredators

Encysted = 5% in 4 hrs, 100% in 80 hrs

Motile = 50% in 4 hrs, 100% in 8 hrs

Environmental Persistence of *Bsal*: Soil

Stegen et al. (2017)

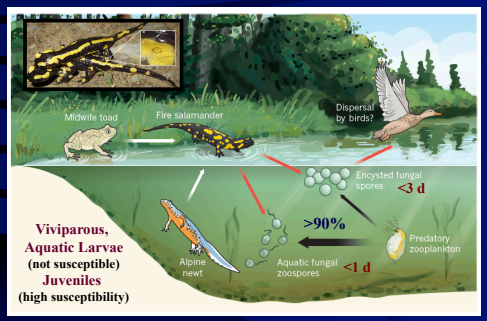
	Infection load (GE) of the source animal	*B. salamandrivorans* transmitted to sentinel animal
Replacement after 24h	168	NO
	8560	YES
	9421	YES
	335	NO
	3329	YES
	625	NO
	245	NO

Infected animals (source animals) were housed in a terrarium at 15 °C with a forest soil substrate and replaced with a healthy individual after 24 h (immediate replacement) or removed after 24 h and replaced by another 24 h later (replacement after 24 h). GE, genomic equivalent.

Most infectious animals shed enough for transmission but zoospores are short-lived in soil (≤2 days)

Ecology of *Bsal*

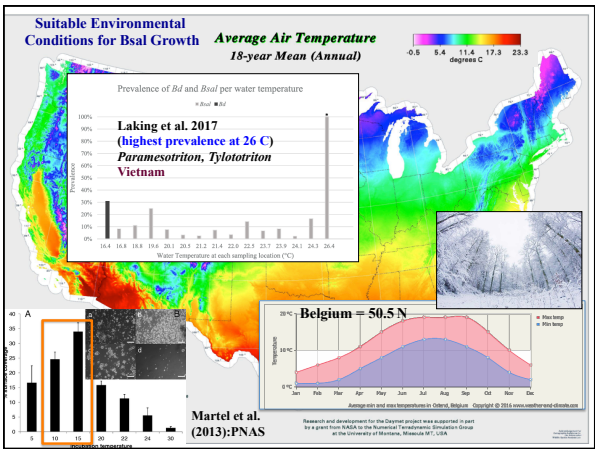
Stegen et al. (2017), Fisher (2017)



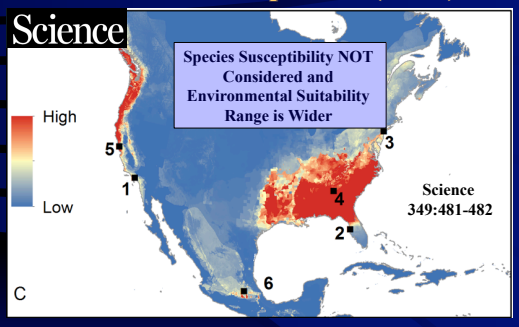
Spillover Reservoir (carriers!)

Frequency Dependent Transmission – Breeding & Hibernation

Two Zoospore Types: Motile, Encysted



Risk Model: Yap et al. (2015)



Final Risk Assessment Model

- Relative Risk = SpRich * Log ClimSuit Bsal

Ecology of *Bsal* Summary

We know that Bsal:

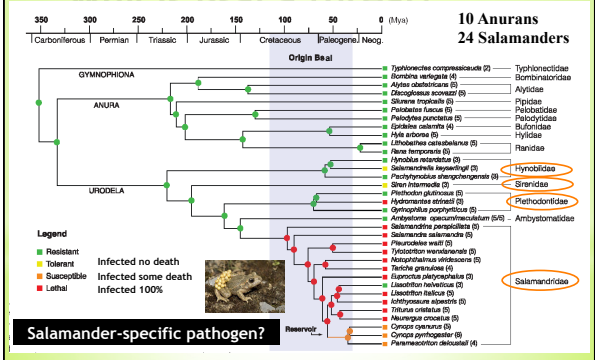
- Wide host range including anurans
- Community composition matters for Bsal outbreaks to occur – Hyper-susceptible spp.
- Bsal can infect hosts: 5 – 26 C
- Environmental persistence of Bsal is probably <1 week – can adhere to waterfowl feet

Host reservoir likely is needed!

1. What is the host range of Bsal in North America?
   i. Amplification, carriers, and dead-end hosts
   ii. Are there SGCN that are highly susceptible?
2. Can Bsal be amplified and maintained in North American amphibian communities?

*Why is Bsal a threat?

Science



Martel et al. 2014

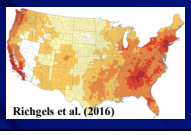
Initial *Bsal* Research in the USA

Test the susceptibility of various North American amphibian species to *Bsal*

- Tested 22 salamander and 6 anuran species
- Susceptibility: infection, mortality, & disease generally across 4 *Bsal* doses (n = 5-10 / dose)

Robustly estimate RISK

(Yap et al. 2015:NA, Richgels et al. 2016: USA, Feldmeier et al. 2016: Europe)



Richgels et al. (2016)

Role: Resistant, Tolerant, Amplification Species

Study Animals: UTIA CWH

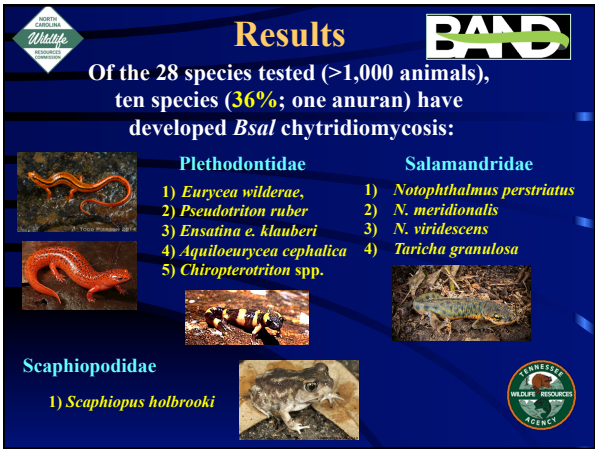
Salamanders (22; 5)

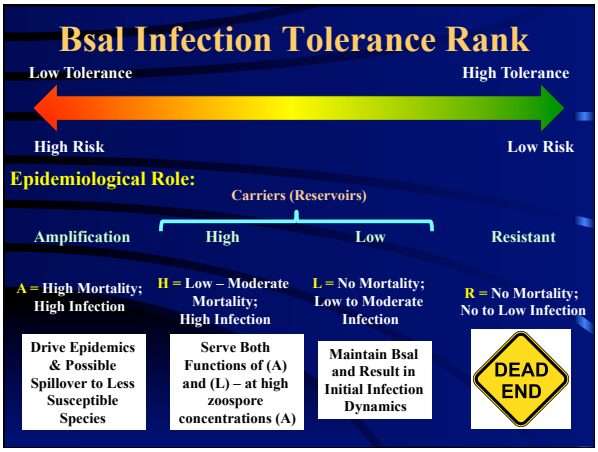
Family	Species
Ambystomatidae	*Ambystoma opacum*
	A. laterale
	A. mexicanum
Proteidae	*Necturus maculosus*
Cryptobranchidae	*Cryptobranchus alleganiensis*
Plethodontidae	*Hemidactylium scutatum*
	Aneides aeneus
	Aquiloeuryea cephalica
	Chiropterotriton spp.
	Desmognathus ocoee
	D. aeneus
	D. monticola
	Ensatina eschscholtzii (2 subspecies)
	Eurycea wilderae (3 populations)
	Eurycea lucifuga
	Plethodon shermani x P. teyahalee
	P. metcalfi
	Pseudotriton ruber
Salamandridae	*Notophthalmus perstriatus*
	N. meridionalis
	N. viridescens (6 populations and efts)
	Taricha granulosa

Frogs (6; 4)

Family	Species
Bufonidae	*Anaxyrus americanus*
Hylidae	*Hyla chrysoscelis*
Ranidae	*Lithobates sylvaticus*
	L. chiricahuensis
	L. catesbeianus
Scaphiopodidae	*Scaphiopus holbrookii*

Methods
Bsal National Task Force:
Research Working Group
https://www.youtube.com/watch?v=IMu8Hq2fvZI
http://www.salamanderfungus.org
Davis Carter
Patrick Cusaac

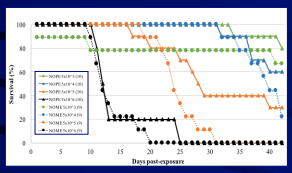

Results
BAD
Of the 28 species tested (>1,000 animals), ten species (36%; one anuran) have developed *Bsal* chytridiomycosis:
Plethodontidae
1) *Eurycea wilderae*,
2) *Pseudotriton ruber*
3) *Ensatina e. klauberi*
4) *Aquiloeurycea cephalica*
5) *Chiropterotriton* spp.
Salamandridae
1) *Notophthalmus perstriatus*
2) *N. meridionalis*
3) *N. viridescens*
4) *Taricha granulosa*
Scaphiopodidae
1) *Scaphiopus holbrooki*


Bsal Infection Tolerance Rank
Low Tolerance
High Tolerance
High Risk
Low Risk
Epidemiological Role:
Carriers (Reservoirs)
Amplification
High
Low
Resistant
A = High Mortality; High Infection
H = Low – Moderate Mortality; High Infection
L = No Mortality; Low to Moderate Infection
R = No Mortality; No to Low Infection
Drive Epidemics & Possible Spillover to Less Susceptible Species
Serve Both Functions of (A) and (L) – at high zoospore concentrations (A)
Maintain Bsal and Result in Initial Infection Dynamics
DEAD END

Results (Newts)

Highly Susceptible: Amplification Species

All (– 1) Became Infected

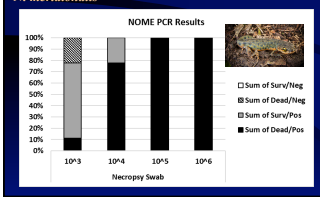


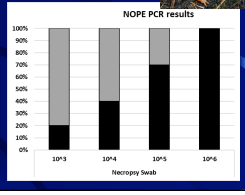
75% Died = NOME

55% Died = NOPE

N. perstriatus

N. meridionalis

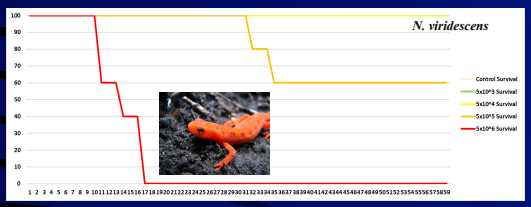


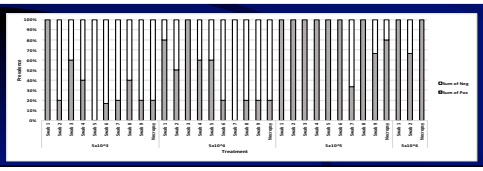


Results (Newts)

Highly Susceptible: Amplification Species (efts only)

N. viridescens

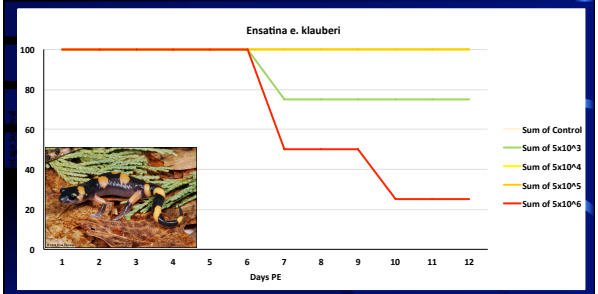




Results

Highly Susceptible: Amplification Species

Ongoing



Results (Mexico)

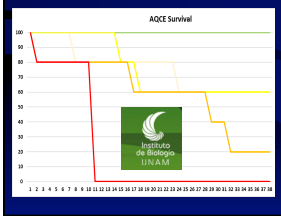
Highly Susceptible: Amplification Species

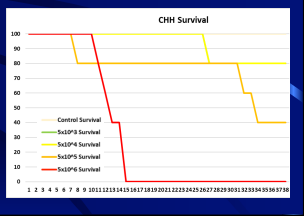
Dr. Becky Hardman

Aquiloeurycea

Dra. Gabriela Parra Olea

Chiropterotriton





Results

Moderately Susceptibility Species

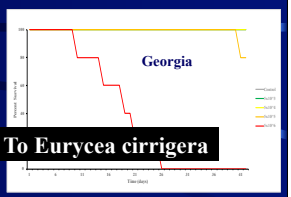
Amplification or Carriers

Moderate Conservation Risk

Results

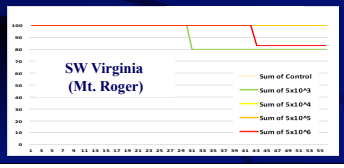
Eurycea wilderae

Moderately Susceptible: Amplification Hosts or Carriers



More Closely Related To Eurycea cirrigera

Variation among Populations (or species complex)

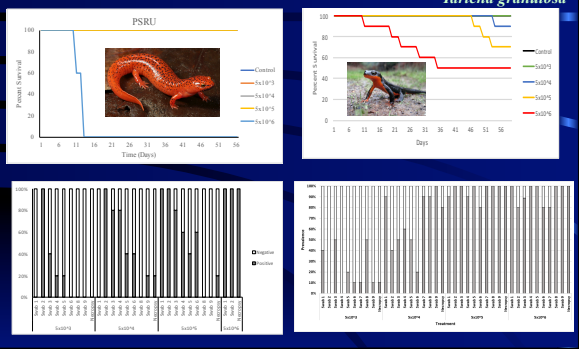


Results

Moderately Susceptible: Amplification Hosts or Carriers

Pseudotriton ruber

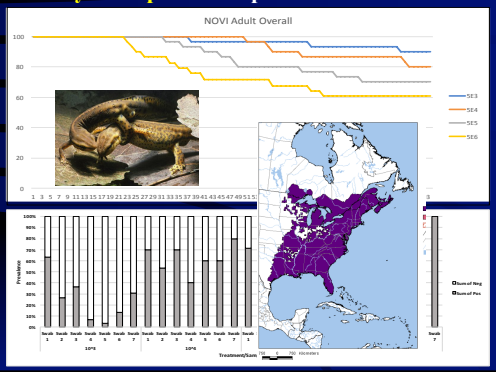
Taricha granulosa



Results

N. viridescens (adults)

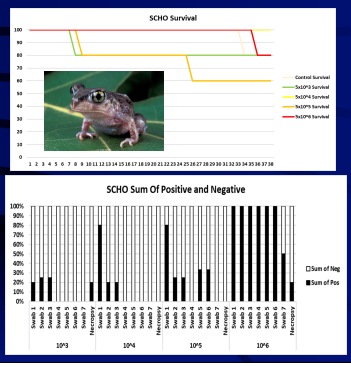
Moderately Susceptible: Amplification Hosts or Carriers



Results

Scaphiopus holbrooki

Moderately Susceptible: Amplification Hosts or Carriers



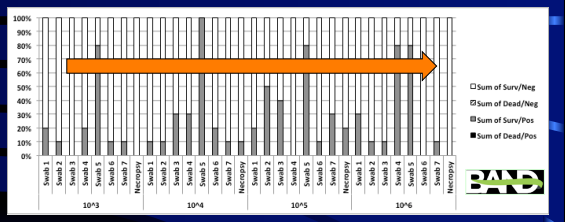
Results

Low Susceptibility Species

Carriers (Reservoirs)

Low Conservation Risk

Mexican Axolotl: Carrier Species



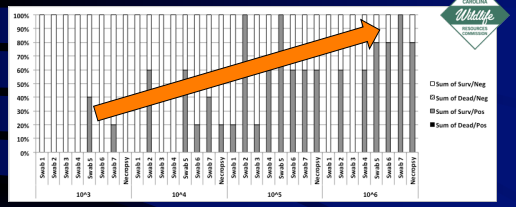
Infected at all doses and maintained low-grade infections throughout duration of experiment (6 weeks).

Biomedical and Pet Trade

AXOLOTL CITY
An online community, marketplace and resources for all things Axolotls.

Pathogen Spillover

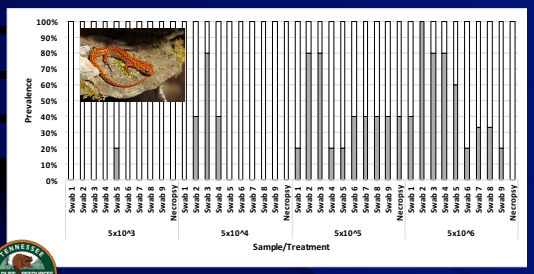
Green Salamanders and Bsal: Possible Conservation Concern



Dose-dependent response with infection prevalence increasing with time.

If >6 weeks, disease might have developed.

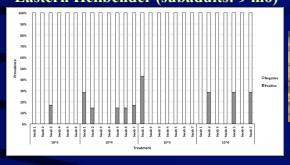
Cave Salamanders and Bsal: Eurycea are not Equally Susceptible



Subclinical Infection: Salamanders

Possible Carrier Species

Eastern Hellbender (subadults: 9 mo)



Low-grade infection for long durations

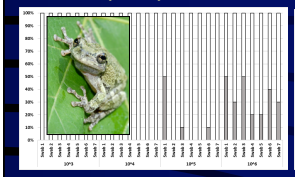
D. ocoee

Higher Doses

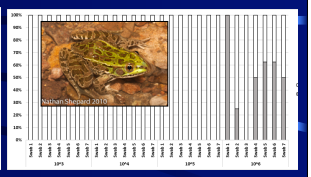
P. metcalfi

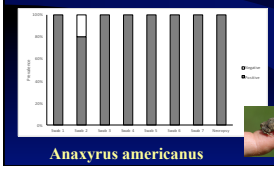
Subclinical Infection: Anurans

Hyla chrysoscelis



Lithobates chiricahuensis





Anaxyrus americanus

American bullfrog (high zoospore doses)

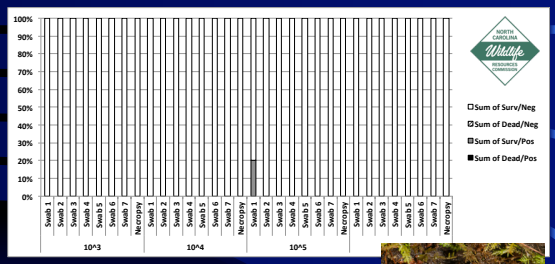
Results

Resistant Species

Dead-end Hosts

No Conservation Risk

Four-toed Salamander: Apparent Resistance

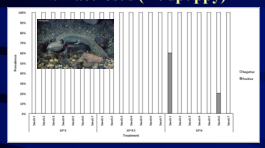


One Detection on Swab 1 (residual pathogen)

Subclinical Infection: Salamanders

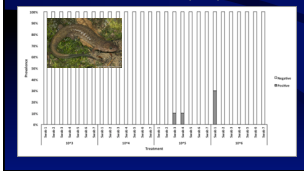
Possible Resistant Species

N. maculosus (mudpuppy)

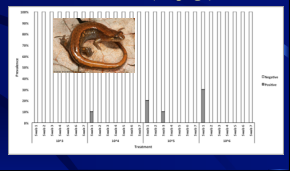


Infrequent detection

D. monticola (seal)



D. aeneus (seepage)

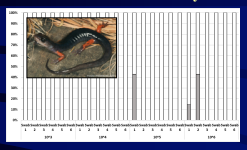


Subclinical Infection: Salamanders

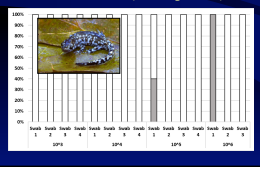
Possible Resistant Species

Plethodon shermani x P. teyahalee

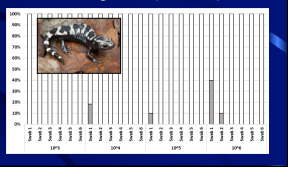
Infrequent detection



A. laterale (blue spotted)



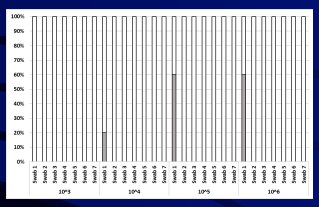
A. opacum (marbled)



Subclinical Infection: Anurans

Possible Resistant Species

Lithobates sylvaticus:



Bsal Infection Tolerance Rank

Low Tolerance — **High Tolerance**

High Risk — **Low Risk**

Epidemiological Role:

Carriers (Reservoirs)

Amplification	High	Low	Resistant
Notophthalmus perstriatus *N. meridionalis* *N. viridescens (Efts)* *Ensatina e. klauberi* *Aquiloeuryea cephalica* *Chiropterotriton spp.*	*N. viridescens (adults)* *Taricha granulosa* *Eurycea wilderae* *Pseudotriton ruber* *Scaphiopus holbrookii**	*Aneides aeneus* *Eurycea lucifuga* *Desmognathus ocoee* *Plethodon metcalfi* *Cyptobranchus alleganiensis* *Ambystoma mexicanum* *Anaxyrus americanus** *Hyla chrysoscelis** *L. chiricahuensis** *L. catesbeianus**	*Hemidactylium scutatum* *D. aeneus* *D. monticola* *P. shermani x P. teyahalee* *Necturus maculosus* *Ambystoma opacum* *A. laterale* *Lithobates sylvaticus**
A = High Mortality; High Infection	**H** = Low – Moderate Mortality; High Infection	**L** = No Mortality; Low to Moderate Infection	**R** = No Mortality; No to Low Infection

* = frog species

Conclusions

- Most North American amphibian assemblages will be composed of suitable hosts with different tolerances to Bsal infection
  - Managing composition of amplification, reservoir, and dead-end hosts is a disease intervention option
- All frog species tested (except one) were suitable hosts
  - Host range of Bsal is greater than expected, which increases the likelihood of entry through trade
- Several SGCN are classified as high risk
  - Bsal represents a significant threat to U.S. biodiversity

The combination of amplification and carrier species and suitable environmental conditions exist in the USA create the "perfect storm" for Bsal emergence.

Questions??

https://ag.tennessee.edu/fwf/bsalproject/

mgray11@utk.edu
dmille42@utk.edu
jcusaac@utk.edu
douglas.woodhams@umb.edu
louise.rollins-smith@Vanderbilt.Edu

The Center for Wildlife Health

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