Emerging Infectious Diseases of Amphibians and Reptiles: Implications for NJ Herpetofauna

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Why Herpetofauna?

- Do not disperse long distances so they cannot migrate to avoid disturbance

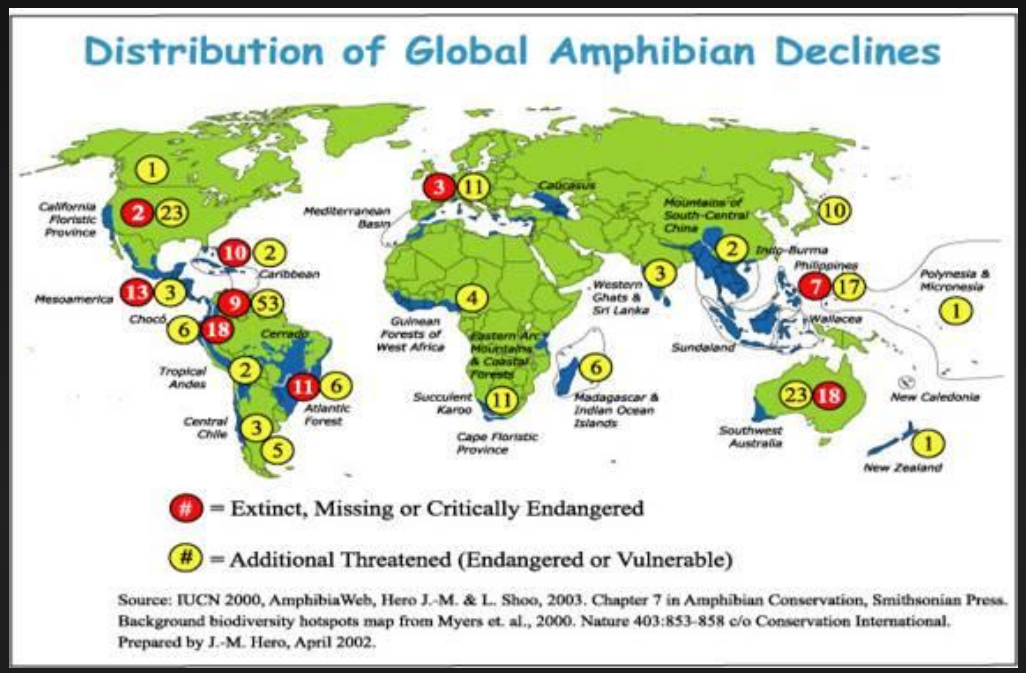
- Life stages require use of different microhabitats, so exposed to multiple types of disturbance

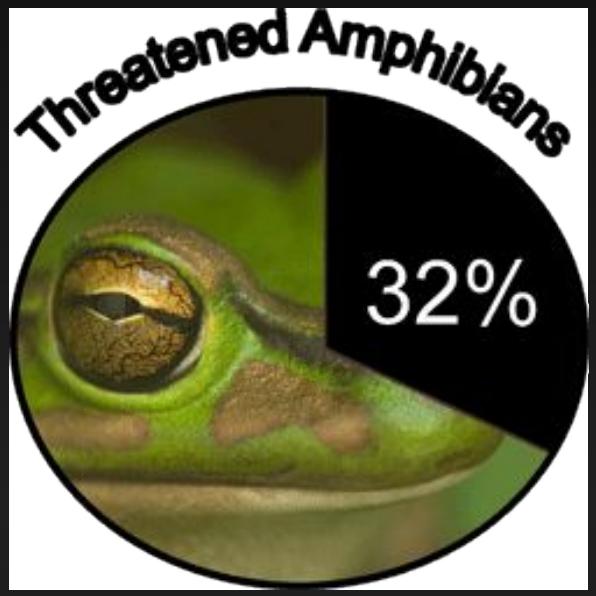
- Collected and smuggled often intensively

- Integral parts of ecosystems

- Coolness factor

Amphibian Declines

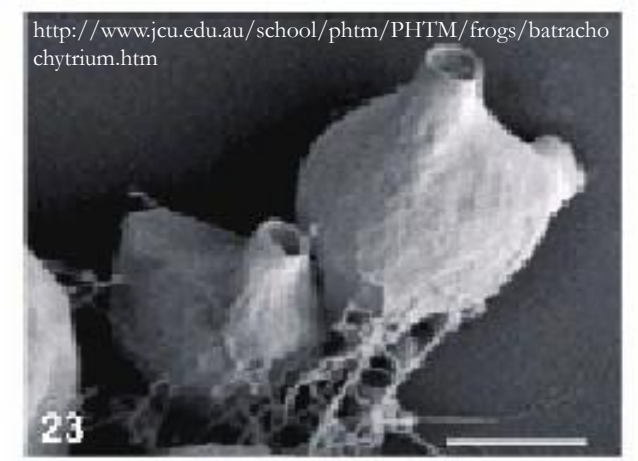




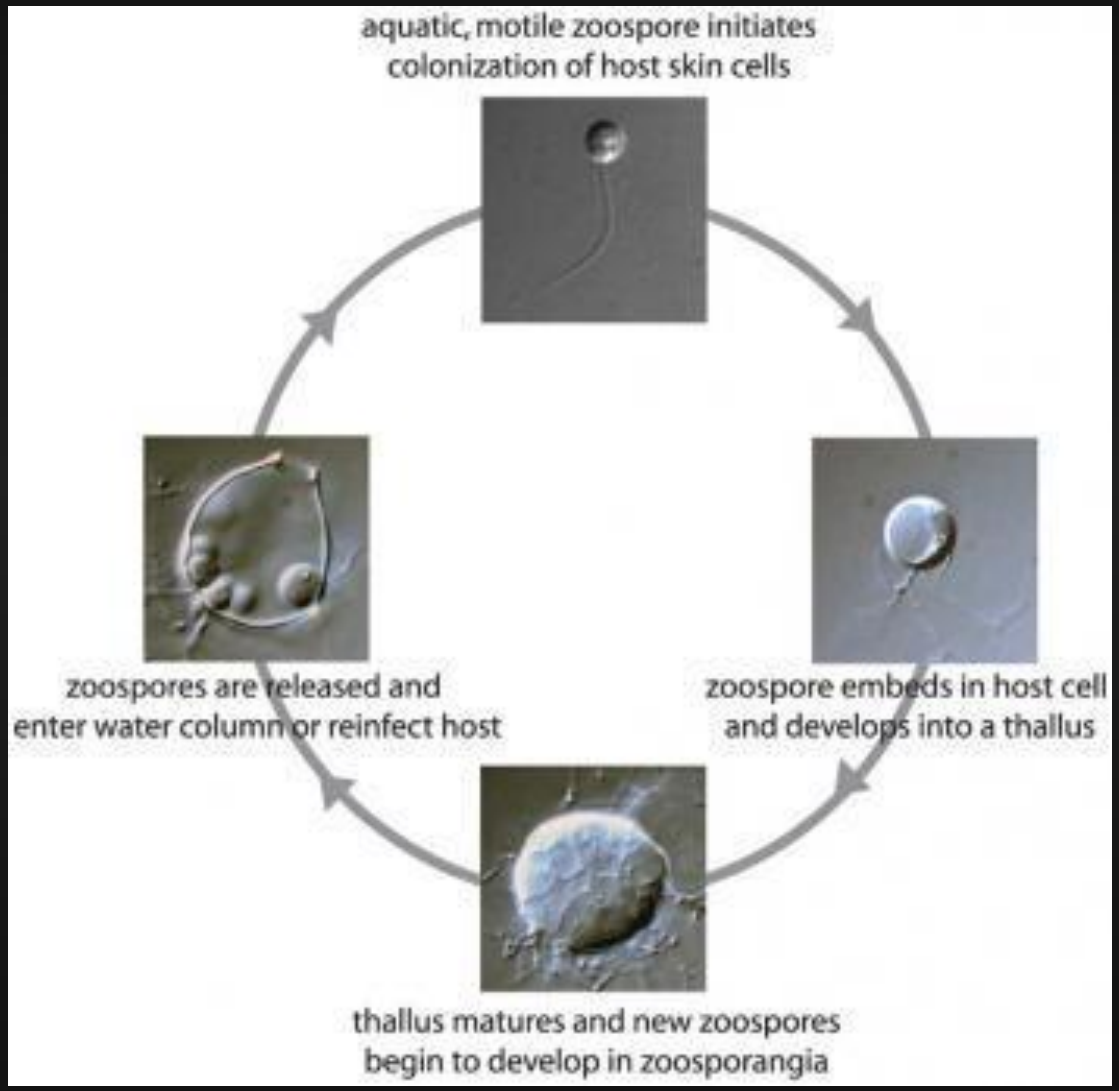
- IUCN data: 42% of all amphibian species show declines in their numbers, so number of threatened species will most likely increase in the future

- Habitat loss and pollution play major roles in declines, HOWEVER emerging infectious diseases are major contributors

Wildlife Disease - Chytridiomycosis



- *Batrachochytrium dendrobatidis* (Bd)
  - Chytrid fungus with motile zoospores
  - Cause of chytridiomycosis

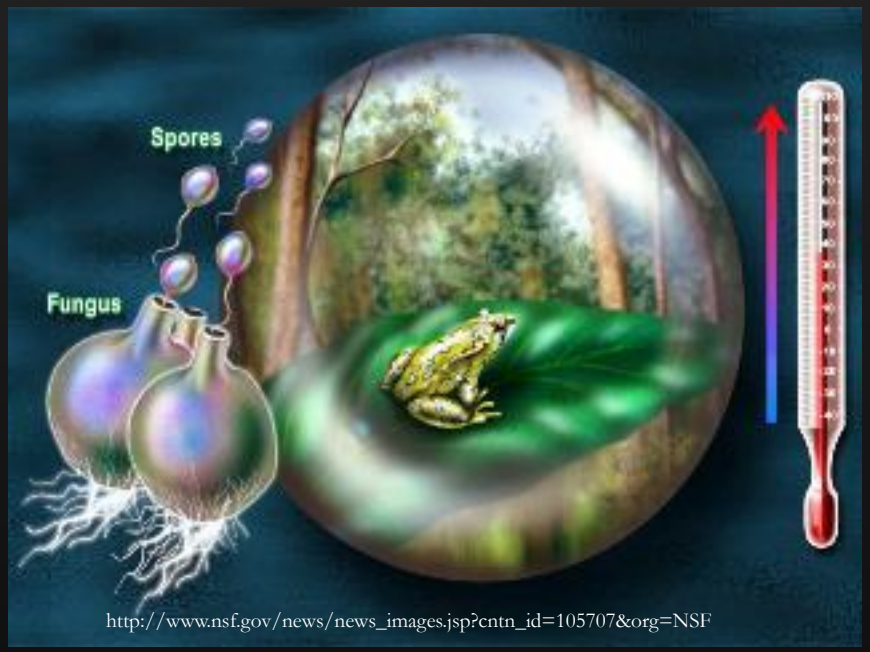


http://www.eol.org/pages/998631

Wildlife Disease - Chytridiomycosis

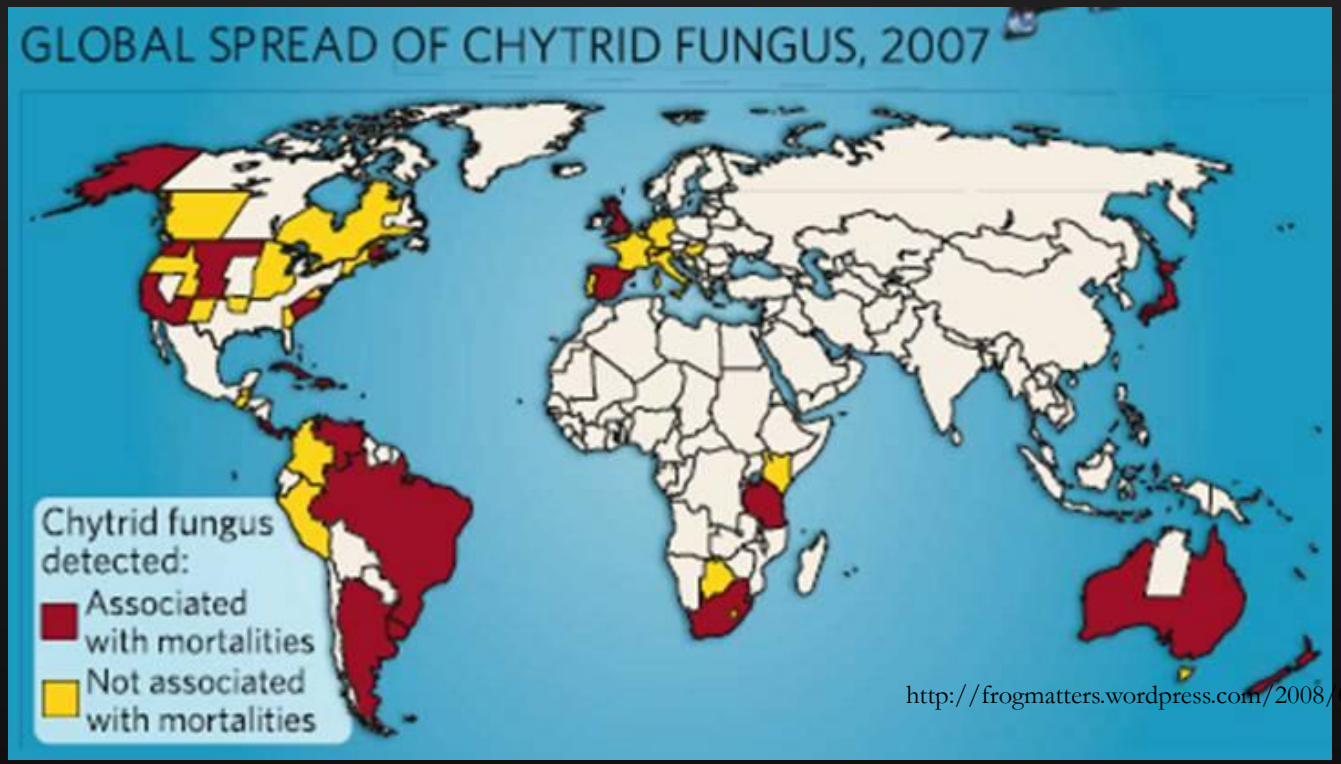
- Preferred substrate is keratin

- Sensitive to temperature and moisture



Wildlife Disease - Chytridiomycosis

- In some cases, massive die-offs, in others frogs persist with endemic Bd

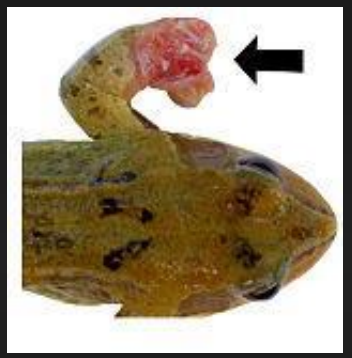


- Die-offs most commonly associated with pristine areas

Wildlife Disease - Ranavirus

- Ranavirus
  - Type species is Frog Virus-3 (FV-3), but found in many different types of species
  - Possibly originated in fish

http://webspace.qmul.ac.uk/ranichols/research.htm

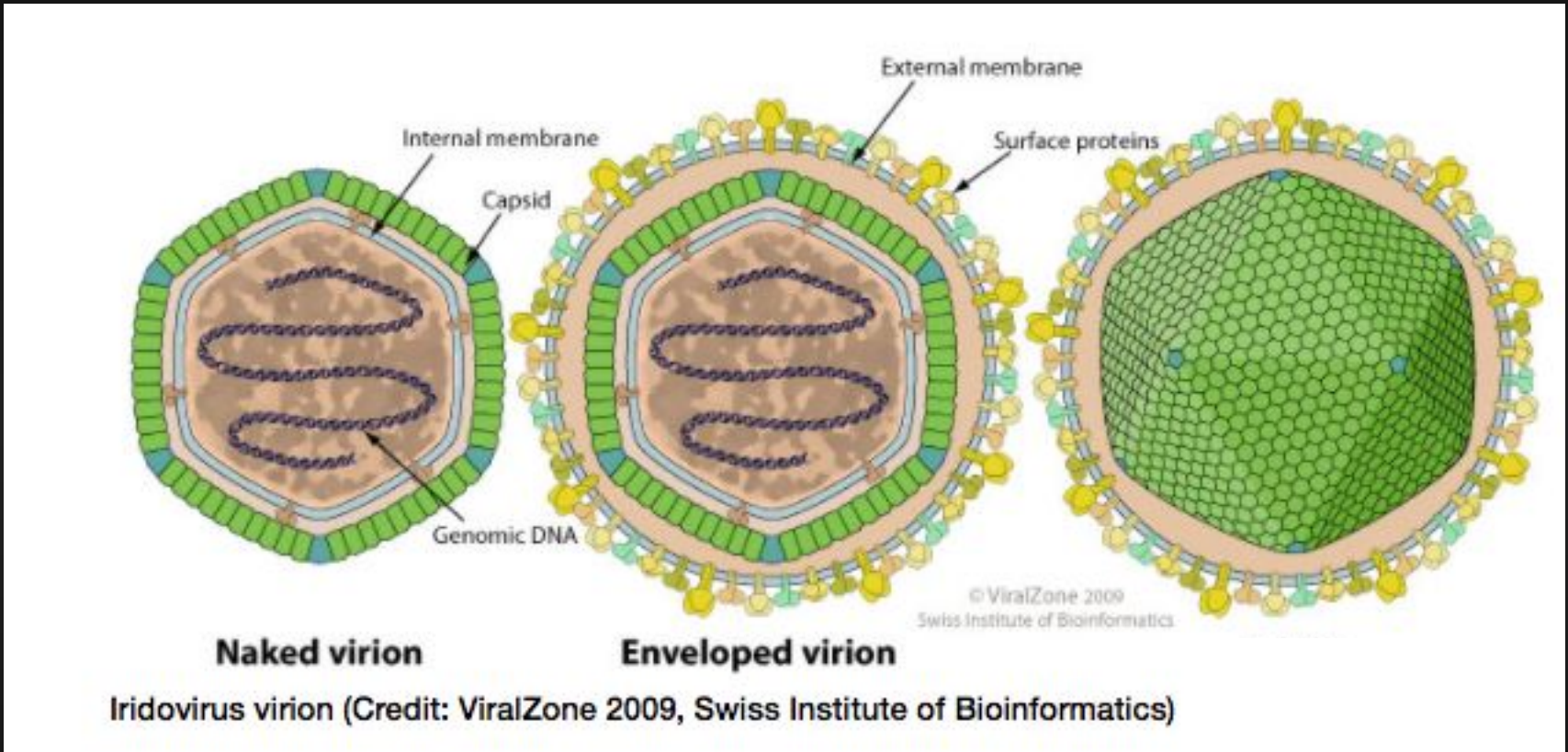


http://www.wildlifeextra.com/go/news/frog-disease278.html#cr

http://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=2657

http://endangerednj.blogspot.com/2009_06_01_archive.html

Wildlife Disease - Ranavirus



Iridovirus virion (Credit: ViralZone 2009, Swiss Institute of Bioinformatics)

- Can infect as enveloped or un-enveloped

Wildlife Disease - Ranavirus

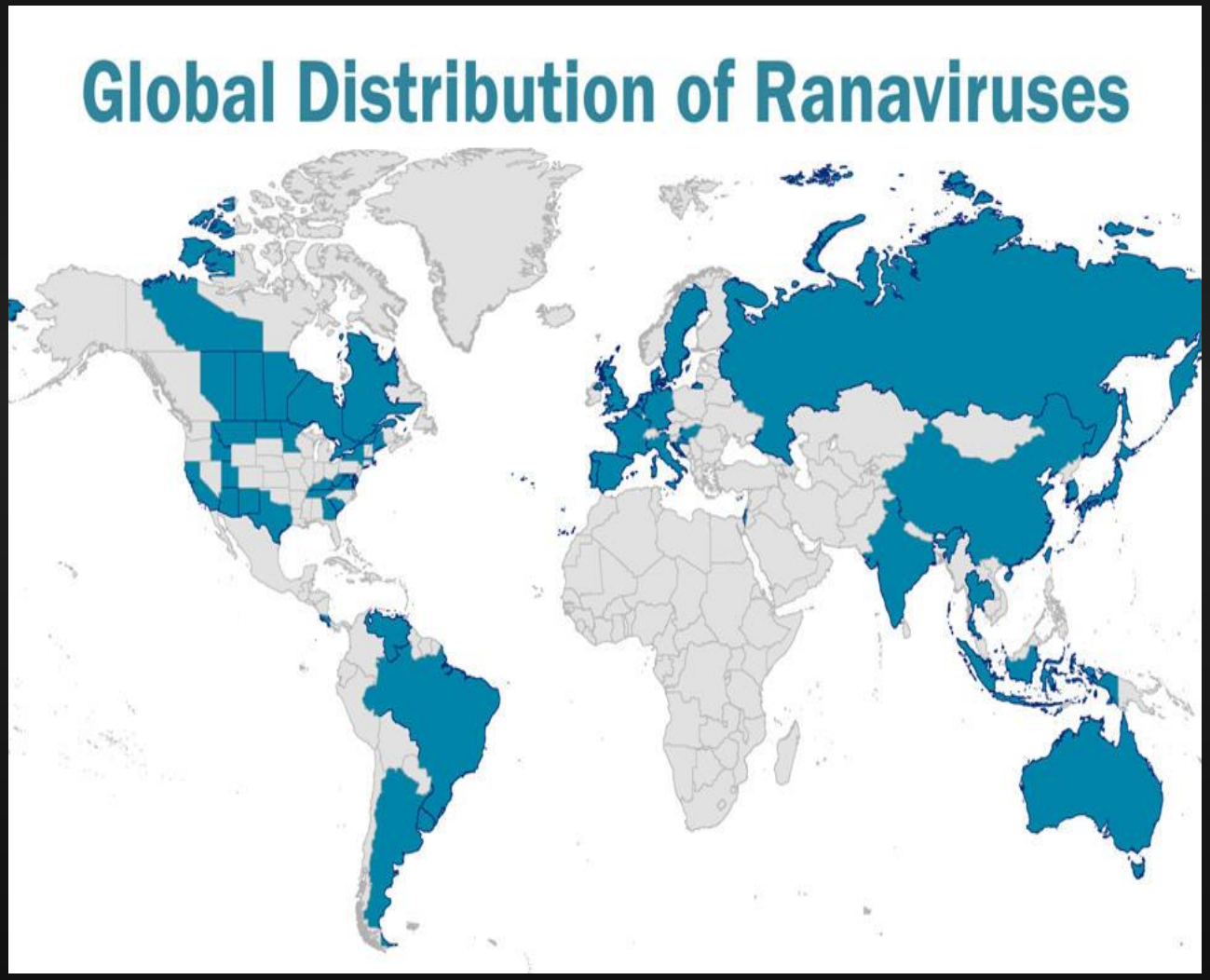
- No clear link between population or environmental characteristics and probability of Ranavirus presence

Pond-breeding species?

Anthropogenic activity?

Warmer or cooler Temperatures?

Wildlife Disease - Ranavirus



- Die-offs not restricted to pristine areas

- Die-offs occur at a much more rapid rate, often with close to 100% mortality after less than 2 weeks

Wildlife Disease - Ranavirus

- Disease spread through a variety of mechanisms, many related to human activities

- Different life stages can serve as reservoirs

- Eating infected tissue drastically speeds up time to death

Objectives

- Monsen-Collar *et al.* (2010) documented the first case of Bd in New Jersey, at the NJ School of Conservation (NJSOC)

Objective 1. To determine the extent of Bd at the NJSOC and throughout the state of NJ

- In 2011, we were alerted to a mass tadpole die-off in Ocean County, NJ, with characteristics similar to Ranavirus outbreaks

Objective 2. To determine whether this die-off was due to Ranavirus and to document the extent of this disease throughout the state of NJ

Materials and Methods

- Amphibians were retrieved by net or by hand in wetlands chosen in collaboration with NJ Division of Fish and Wildlife Endangered and Non-game Species Program

- Wetlands chosen based on accessibility and proximity to NJ Calling Amphibian Monitoring Project (CAMP) sites

- Bd only adults and metamorphs sampled (preferred substrate for Bd is keratin, and keratin only found on mouthparts of tadpoles)

- Ranavirus attempt to sample any life stage, but tadpoles most often sampled (evidence that tadpoles most dramatically affected)

Bd Sampling

- In June and July of 2010, 200 samples were collected from the NJSOC (Sussex County) and a total of 72 from Cape May County

- In 2011, samples were obtained from throughout the state

Bd samples - 2011

County	Month	Number
Cape May	May	20
	April	63
Atlantic	April	19
Burlington	April	17
Ocean	June	40
Salem	May	56
	June	28
Passaic	April	13
	June	6
Sussex	April	19
	May	92
Morris	April	11
	June	43
Middlesex	April	27
Monmouth	April	28
Mercer	April	13
Somerset	April	11

Ranavirus sampling

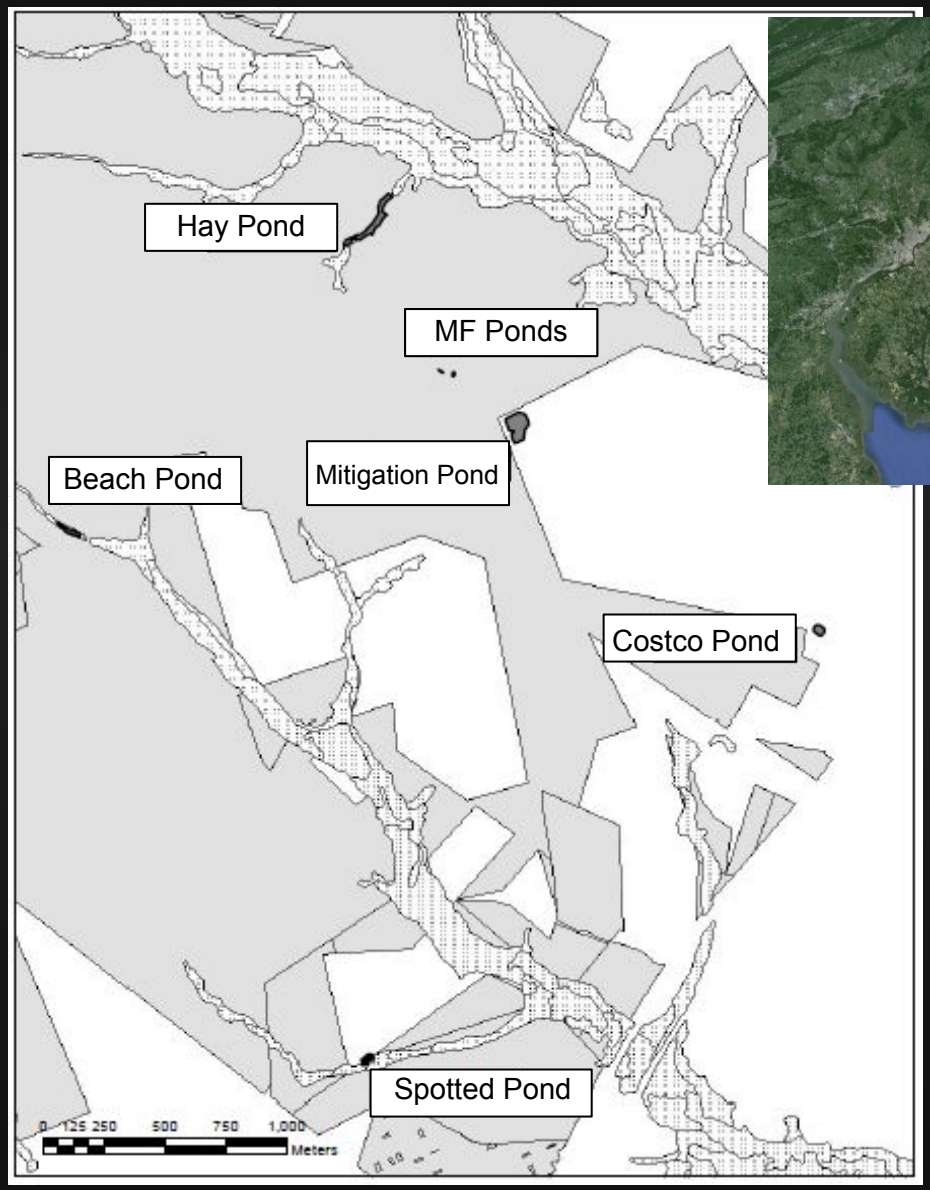
Ranavirus Samples (2011-2013)		
County	**Month**	**Number**
Ocean	May/June 2011	114
	May 2012	24
Camden	June 2012	10
Morris	April 2011	32
	June 2013	30
Sussex	April 2011	140
	June 2013	66
Passaic	June 2011	3
	June 2013	30
Monmouth	April 2011	15
Mercer	April 2011	12
Middlesex	April 2011	7
Cape May	April 2011	12
Warren	June 2013	120

- After the suspected outbreak in Ocean County, sampling for Ranavirus was carried out throughout the state

Disease Sampling

- Swabs for Bd; tissue samples for Ranavirus

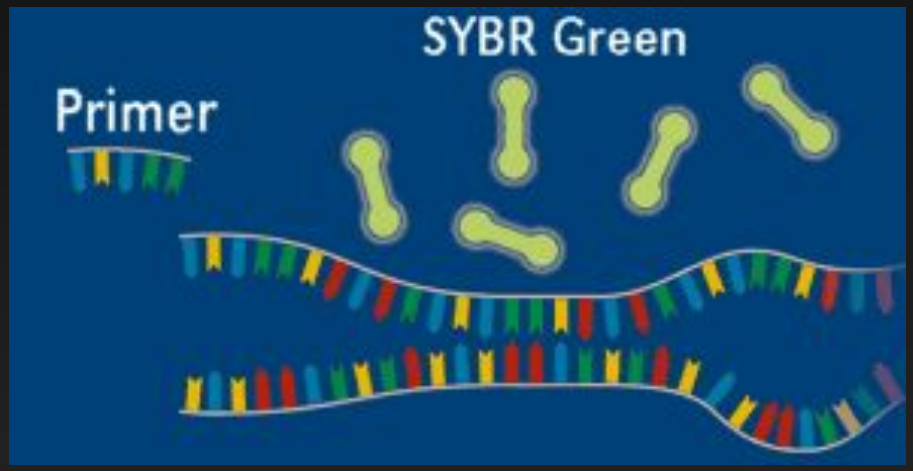
Suspected Ranavirus Outbreaks

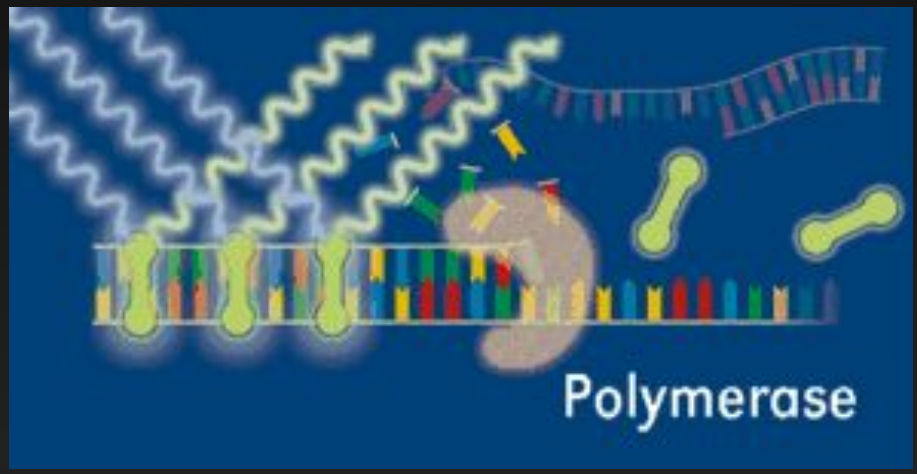


- Particular attention was paid to Stafford Business Park in Ocean County…

….and the NJSOC

Assessment of Disease Presence

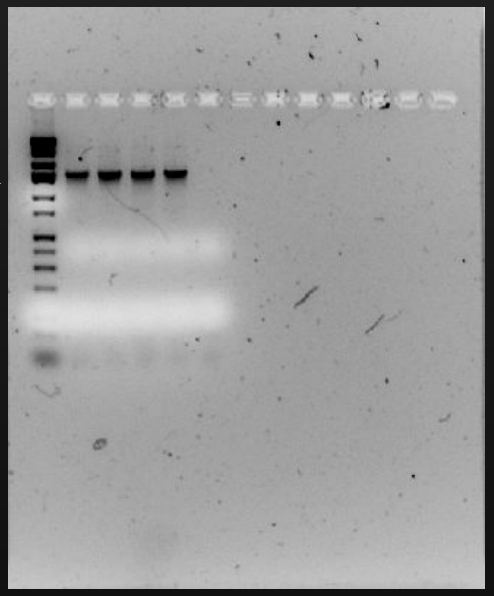




http://homepages.strath.ac.uk/~dfs97113/BB310/lect403.html

- Bd RT-PCR only

- Ranavirus Both RT-PCR and Traditional PCR



Results and Discussion

- Samples from 2010 and 2011 for Bd tested Negative

  - Negative results not so great for a publications, etc….but Yay! Does this mean amphibians in NJ are safe from disease?

Results and Discussion

SBP

- Nope.

- Die-offs at SBP and NJSOC consistent with Ranavirus outbreaks

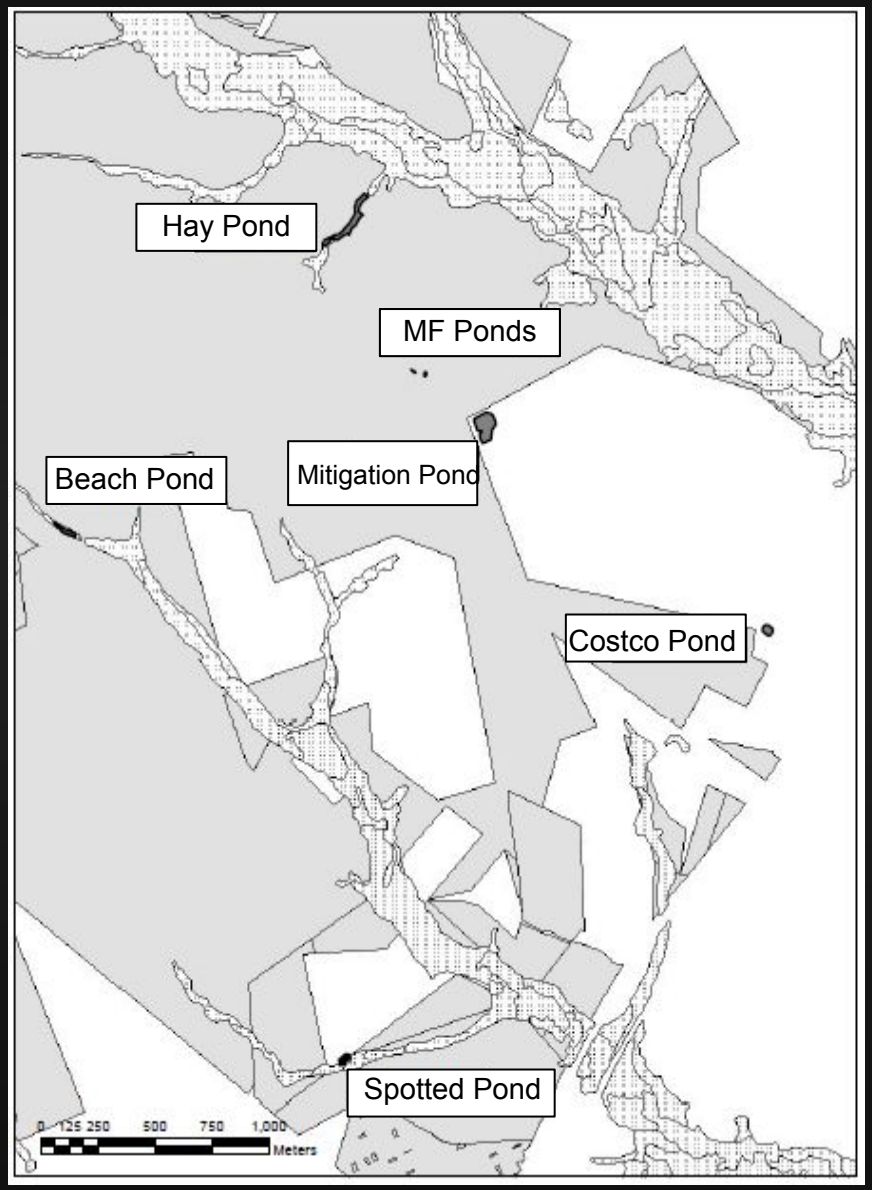
SBP

SBP

NJSOC

SBP

First Documented Case of Ranavirus in NJ



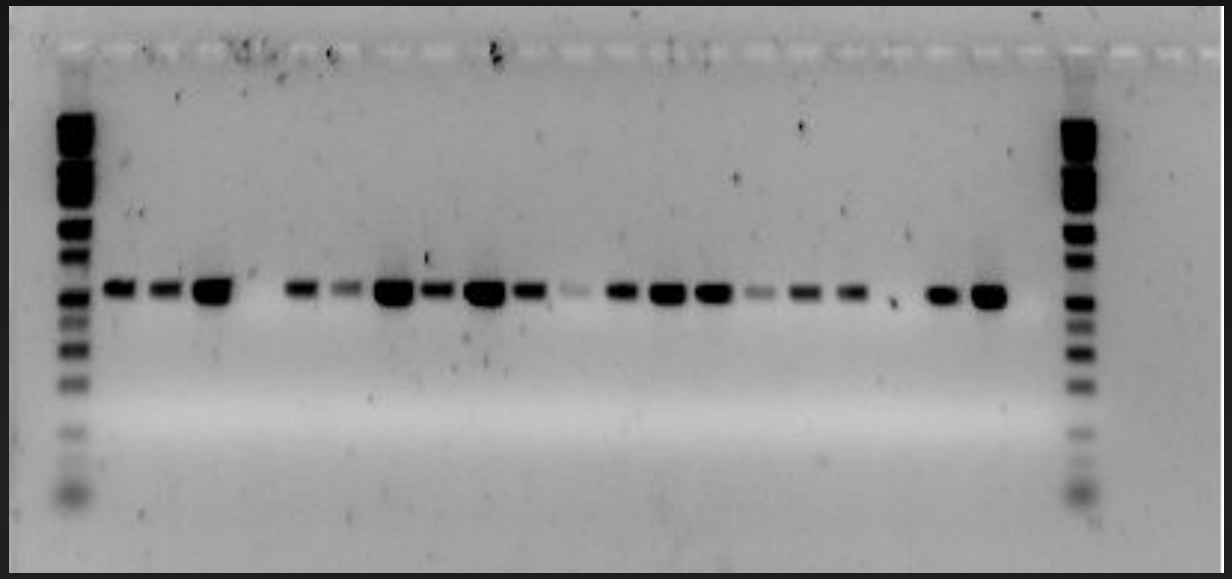
- In 2011 Hay Pond (0/18), Beach Pond (0/2), MF Ponds (4/17 and 0/11), Mitigation Pond (26/48), Costco Pond (2/13), Spotted Pond (0/2)

Dead southern Leopard frog only adult to test positive

On first trip. *A. fowleri* tadpoles were healthy, on second trip, we observed die-offs

- In 2012 Total of 24 samples collected from Hay Pond, Costco Pond and Beach Pond; 16 positive, including Hay Pond and Beach Pond (negative in 2011)

Traditional PCR vs. RT-PCR

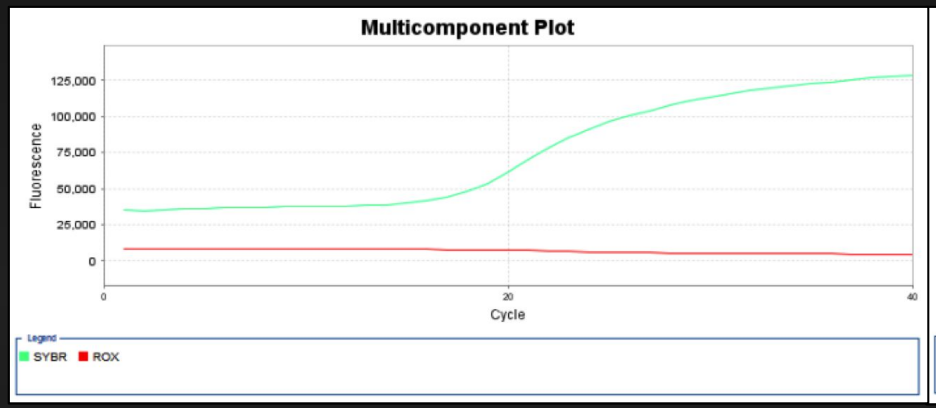


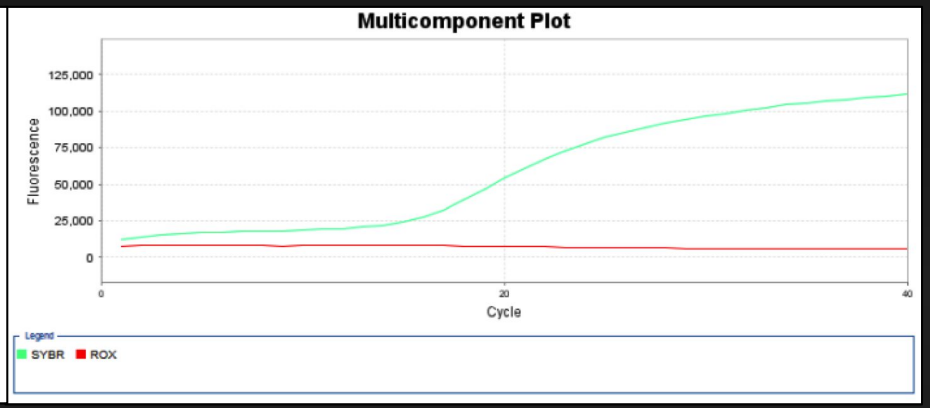
- Collected environmental samples at SBP let Fowler's toads "swim" in water in Eppendorf tube, released tadpole but kept water

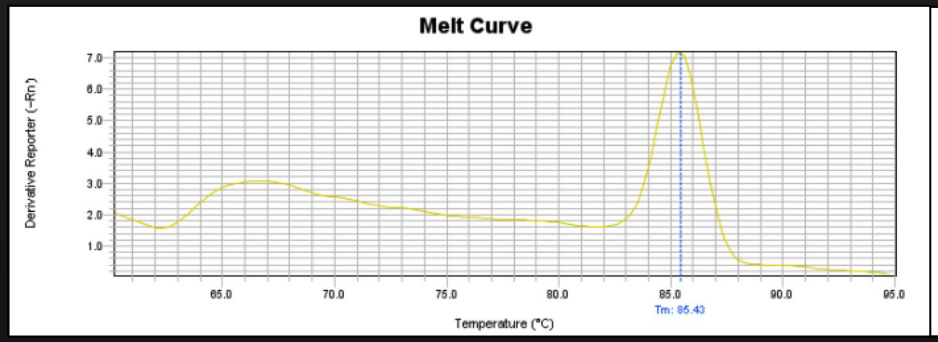
- Out of 14 samples, 0 tested positive with traditional PCR

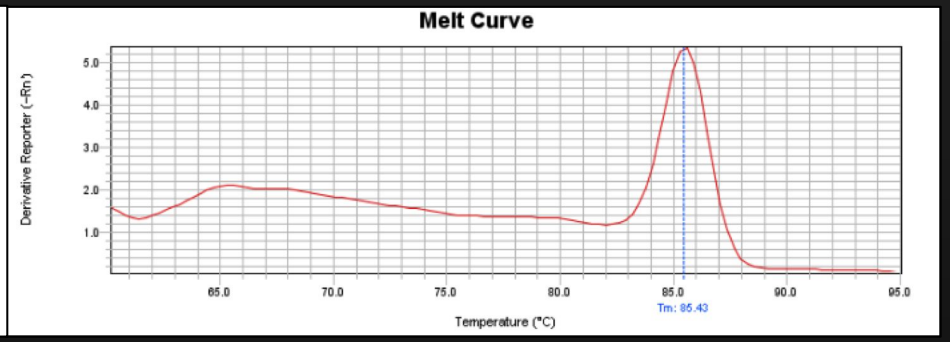
Traditional PCR vs. RT-PCR

- With RT-PCR, 8 of those 14 tested positive for Ranavirus









Positive Control

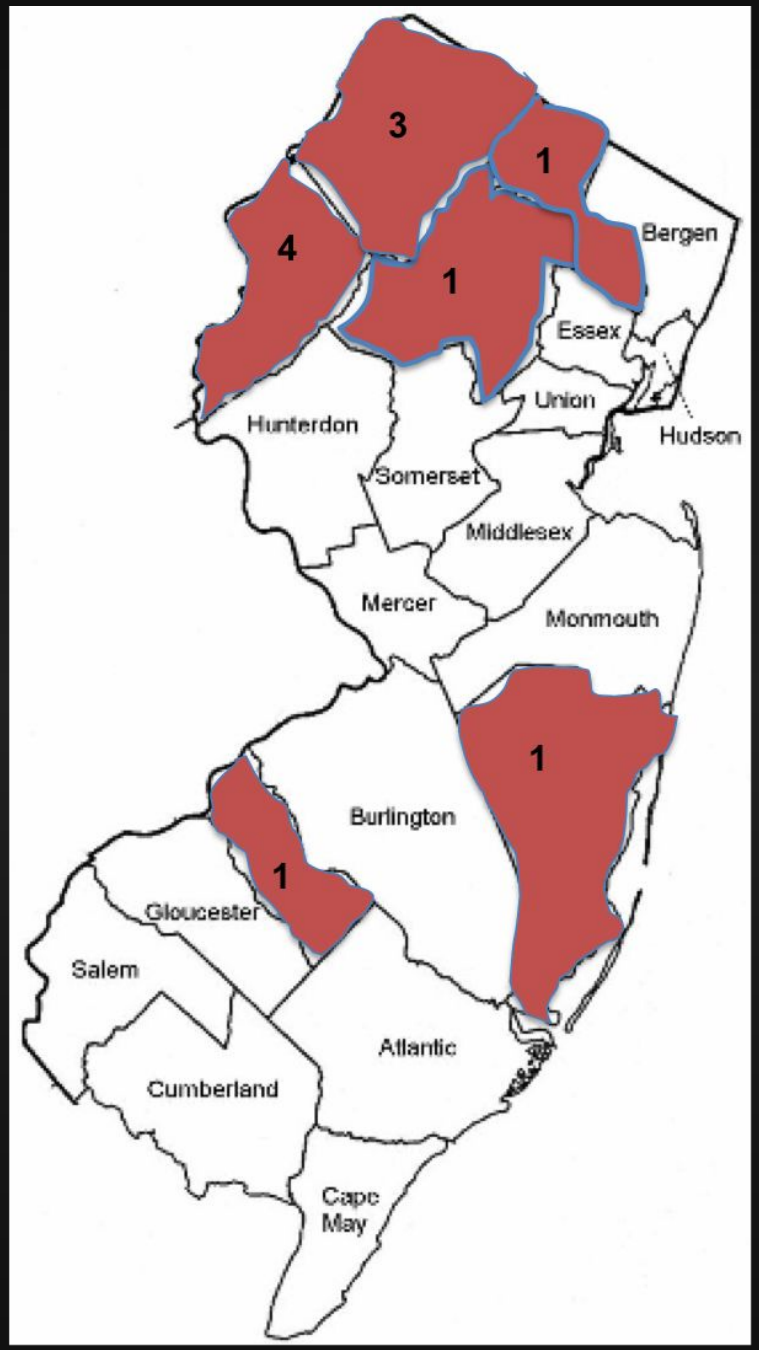
Sample positive from the NJSOC

Traditional PCR vs. RT-PCR

Site	Total samples collected	Postives with traditional PCR	Positives with RT-PCR
Stafford (2011)	114	24	32
NJSOC (2013)	6	1	4

- RT-PCR more sensitive for detecting presence of lower viral loads and in environmental samples (absence of tissue)

- All further samples screened with RT-PCR



Current Extent of Ranavirus in NJ

- Total of 11 sites in the state
  - 3 sites in Sussex County
  - 4 sites in Warren County
  - 1 site in Morris County
  - 1 site in Passaic County
  - 1 site in Camden County
  - 1 site in Ocean County

Management Implications

- SBP used by Pine Barrens Tree frogs state threatened

Disjunct population

- SBP also home to Fowler's toads
species of special concern

- NJSOC site home to obligate vernal pool breeders

- Jefferson's salamander (species of special concern);
within the range of endangered Blue-spotted salamander

Management Implications

- SBP managed for the benefit of threatened northern pine snake

- Ranavirus has been shown to infect reptiles, including snakes

  Local population could be at risk

  Could act as reservoirs for the disease

But wait! There's more…Batrachochytrium salamandrivorans

- First described in 2013

- Originated in Asia, infected Fire salamanders (*Salamandra salamandra*) in Netherlands, Belgium, Germany, UK

- Pet trade

- Population declines of over 90%

Photo : © UGent – Salamandre tachetée infectée par Bsal

New threat – Batrachochytrium salamandrivorans

- Deep ulcerations all along skin; ulcerations often colonized by opportunistic pathogens

- Abnormal body posture & behavior; anorexia, apathy

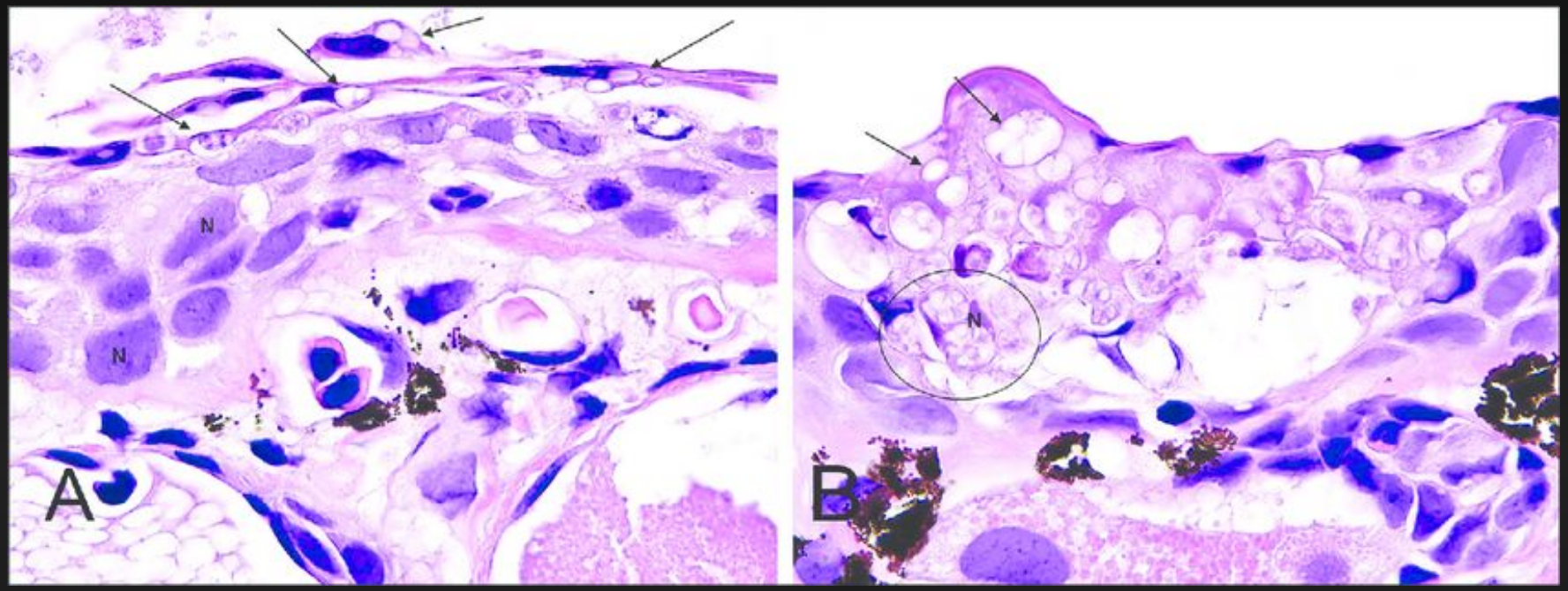
New threat – Batrachochytrium salamandrivorans

- Alpine newts and Smooth newts also infected

- Death has occurred in as little as 3-7 days after infection

- 2 healthy salamanders in tank with an infected salamander one died 22 days, the other 27 days after contact

New threat – Batrachochytrium salamandrivorans



- Lower thermal preference than Bd optimal growth between 10 and 15 degrees C

- Bsal results in ULCERATIONS (necrosis and loss of tissue);

  * Bd results in Hyperplasia and Hyperkeratosis (thickening of tissue)

Management Implications

- World Organization for Animal Health (OIE) has listed Ranavirus and Bd as "notifiable" diseases (mandatory reporting and prevention of the spread of disease)

- DECONTAMINATION is essential – 3% bleach solution for 1 minute

Management Implications - Bsal

- North America is a hotspot for salamander biodiversity (over 50% of world's species);

  * USA = 190 species
  * Mexico = 137 species
  * Canada = 21 species

- As of 2016, no reported cases of Bsal in US ban has been implemented

Conclusions

- Because herpetofauna often have limited dispersal abilities, survival strategy may be to tolerate some level of human disturbance

Proper understanding of the consequences of human activities to appropriately manage wildlife in the presence of disturbance

- Results of amphibian disease study have been shared with state wildlife biologists and environmental educators to stress the importance of decontamination

- Loss of amphibians could lead to compromised ecosystems, which could in turn lead to compromised ecosystem services

Acknowledgements

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NEW JERSEY DIVISION OF Fish and Wildlife

New Jersey Flows

New Jersey Water Resources Research Institute

EPA Announces New Initiative To Conserve Whatever's Left

NEWS · Environment · Government · News · ISSUE 50•13 · Apr 2, 2014

EPA administrator Gina McCarthy says her agency is fully committed to saving all types of flora and fauna, provided any of them still exist.