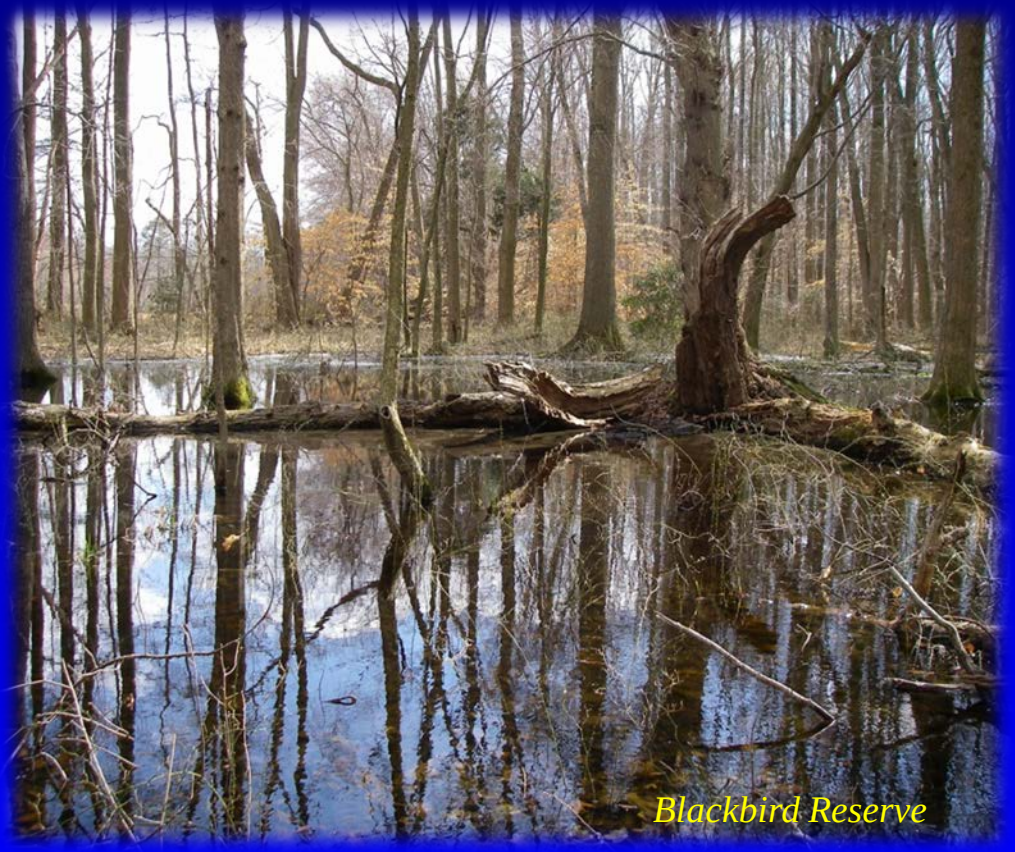
How to Help Prevent Amphibian & Reptile Diseases in Four Simple Steps



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How to Help Prevent Amphibian & Reptile Diseases in Four Simple Steps

- Plight of herps (amphibians and reptiles)
- Describe diseases in our area
- Where they have been found
- How to help prevent spreading disease

Taxa in Trouble

Habitat loss, climate change, contamination, invasive species, disease, over-collection, consumption, direct persecution

- Amphibians declining on large tracts of protected lands
- 32% of amphibian species are threatened with extinction worldwide (compared to 12% of birds and 23% of mammals)
- 43% of all turtle species are listed on the IUCN's red list.

NJ Chorus Frog

Malformed Gray Tree Frog

Bog Turtle

Amphibian & Reptile Diseases

- Chytridiomycosis (Chytrid Fungus, *Bd*)
- Ranavirus (Frog Virus 3)
- Upper Respiratory Tract Disease (URTD; *Mycoplasma* bacteria)
- Snake Fungal Disease
- Salamander Chytrid Fungus (Bsal)
- Others (Herpes Virus, etc.)

Orange = documented in DE

Ranavirus

- Disease of fish, amphibians & reptiles (has jumped taxa groups)
- Likely greatest pathogen threat to amphibians and reptiles in North America (3-4X more common than *Bd*).
- Kills quickly (tadpole die-offs 5-50 days)
- As of 2013, known to affect > 104 species

- Increasingly responsible for turtle die-offs (7 species with box turtles most commonly reported)
- Spreads easily - through water, human handling, introduction of animals and contaminated gear.

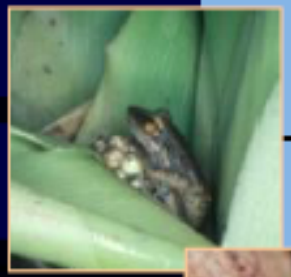
Note: This is one reason why we do not recommend moving turtles from roads to “safer habitats”

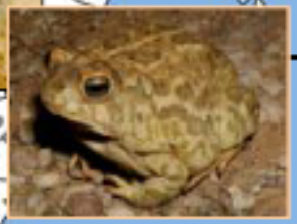
(Gray 2011)

Global Distribution of Ranavirus Die-offs










5 Continents: 1992

All Latitudes, All Elevations

12 Families: Ranidae, Hylidae, Bufonidae, Leptodactylidae, Dendrobatidae, Discoglossidae, Rhacophoridae, Myobatrachidae, Ambystomatidae, Salamandridae, Hynobiidae, Cryptobranchidae

Ranavirus – Five State Study

- MD, DE, PA, NJ and VA
- Standard samples (wood frog tadpoles)
- Die-off samples (any species)

Goals:

- To better understand local prevalence/distribution
- To develop standard testing protocol

Scott Farnsworth

Holly Niederriter

Holly Niederriter

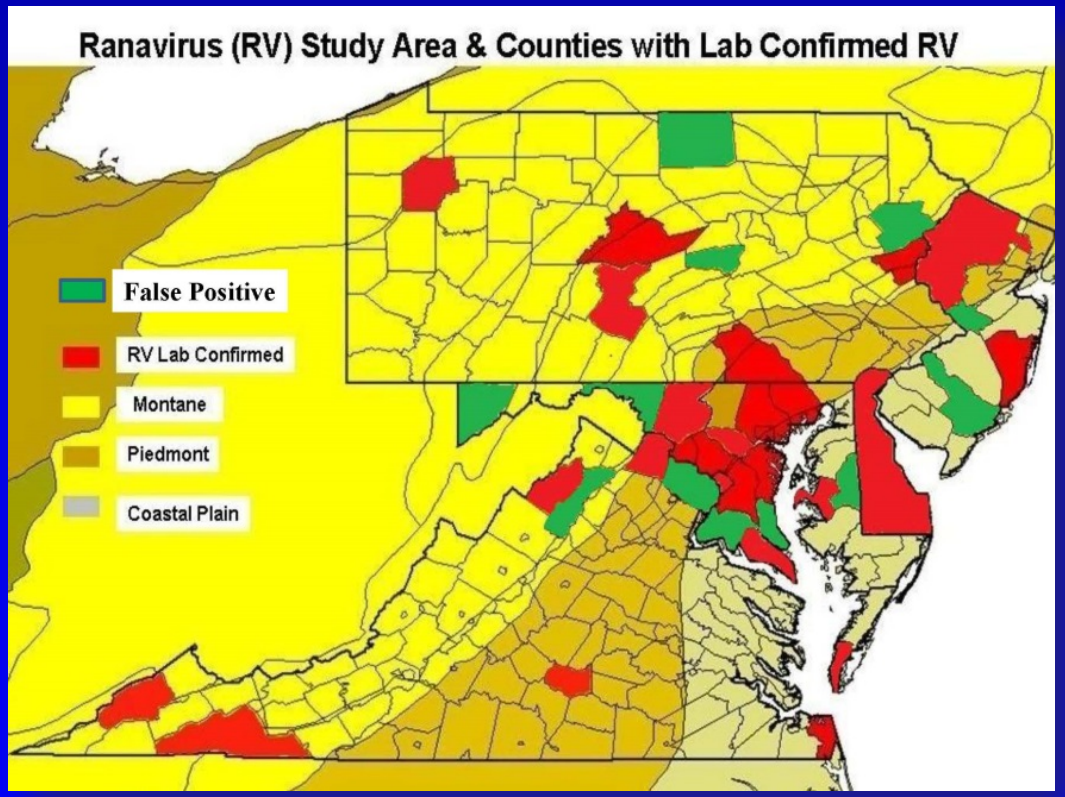
Ranavirus In the Region

Results

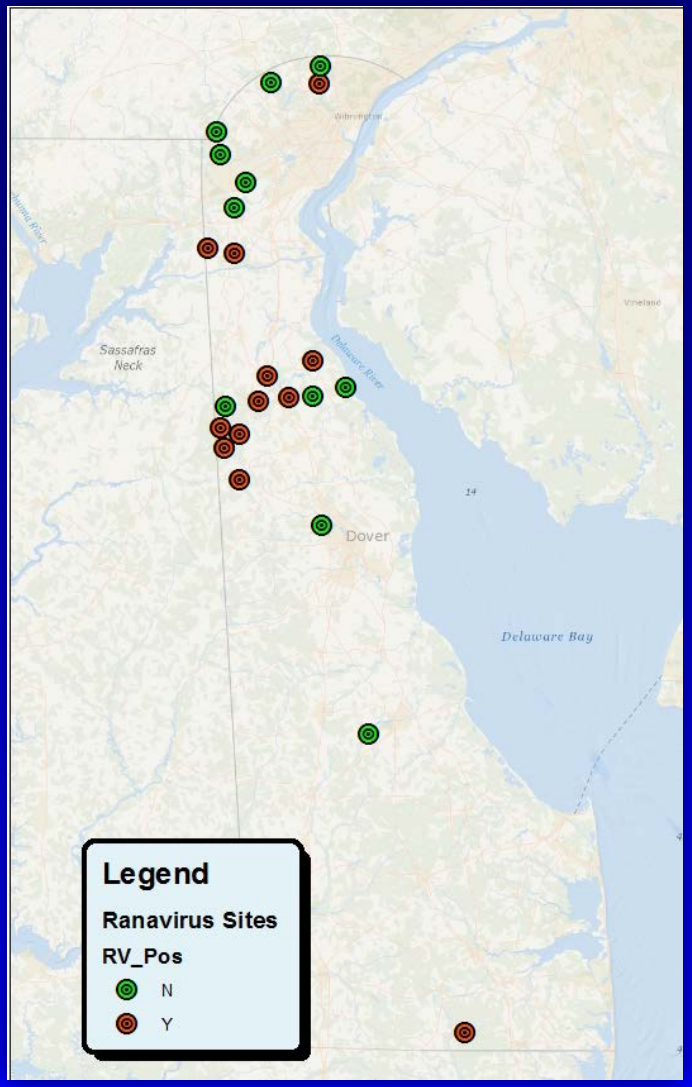
- All States: 34% RV-Positive
- DE: 45% in 2013 and 38% in 2014
- DE: RV die-offs in every county in both years. Twice as many die-offs in half as many ponds in 2014.

RV-Positive Species in our region 2013/14

- Wood frog
- Spring peeper
- Spadefoot toad
- Spotted salamander
- Southern leopard frog
- Box turtle
- Painted turtle
- Bog turtle
- Snapping turtle



Ranavirus Distribution in DE 2013/14



More Diseases

- Upper Respiratory Tract Disease (URTD)
  - Mycoplasma spp. – only turtles affected
  - Symptoms similar to Ranavirus
  - Can only be Lab Confirmed (PCR)
  - Delaware bog turtle in 2012
- Snake Fungal Disease (*Chrysosporium ophiodiicola*)


Holly Niederriter

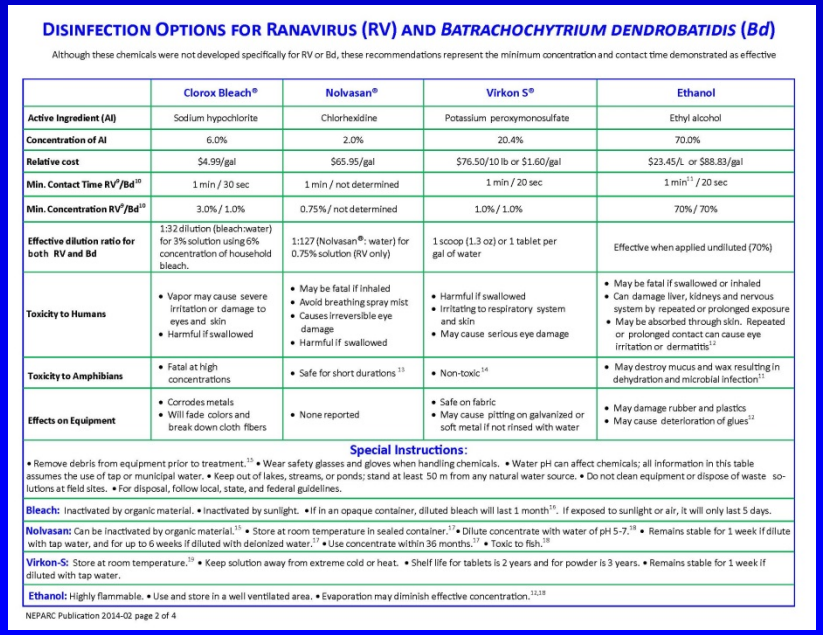

Tom Friedel


Donald McKnight


Ellen Bronson

Need to Disinfect

- Humans can transmit diseases from one place to another and from one organism to another in a short amount of time and over distances the organisms cannot traverse.
- Important for us to prevent the spread of pathogens.
- And pretty easy to do.
- NEPARC did literature review and produced an easy to follow protocol.
  - NEPARC recommends bleach because it degrades quickly in the environment and is accessible to everyone but also provides other options.



DISINFECTION OPTIONS FOR RANAVIRUS (RV) AND *BATRACHOCHYTRIUM DENDROBATIDIS* (*Bd*)

Although these chemicals were not developed specifically for RV or Bd, these recommendations represent the minimum concentration and contact time demonstrated as effective

	Clorox Bleach®	Nolvasan®	Virkon S®	Ethanol
Active Ingredient (AI)	Sodium hypochlorite	Chlorhexidine	Potassium peroxymonosulfate	Ethyl alcohol
Concentration of AI	6.0%	2.0%	20.4%	70.0%
Relative cost	$4.99/gal	$65.95/gal	$76.50/10 lb or $1.60/gal	$23.45/L or $88.83/gal
Min. Contact Time RV[9]/Bd[10]	1 min / 30 sec	1 min / not determined	1 min / 20 sec	1 min[11] / 20 sec
Min. Concentration RV[9]/Bd[10]	3.0% / 1.0%	0.75% / not determined	1.0% / 1.0%	70% / 70%
Effective dilution ratio for both RV and Bd	1:32 dilution (bleach:water) for 3% solution using 6% concentration of household bleach.	1:127 (Nolvasan®: water) for 0.75% solution (RV only)	1 scoop (1.3 oz) or 1 tablet per gal of water	Effective when applied undiluted (70%)
Toxicity to Humans	• Vapor may cause severe irritation or damage to eyes and skin • Harmful if swallowed	• May be fatal if inhaled • Avoid breathing spray mist • Causes irreversible eye damage • Harmful if swallowed	• Harmful if swallowed • Irritating to respiratory system and skin • May cause serious eye damage	• May be fatal if swallowed or inhaled • Can damage liver, kidneys and nervous system by repeated or prolonged exposure • May be absorbed through skin. Repeated or prolonged contact can cause eye irritation or dermatitis[12]
Toxicity to Amphibians	• Fatal at high concentrations	• Safe for short durations[13]	• Non-toxic[14]	• May destroy mucus and wax resulting in dehydration and microbial infection[11]
Effects on Equipment	• Corrodes metals • Will fade colors and break down cloth fibers	• None reported	• Safe on fabric • May cause pitting on galvanized or soft metal if not rinsed with water	• May damage rubber and plastics • May cause deterioration of glues[12]

Special Instructions:

• Remove debris from equipment prior to treatment.[15] • Wear safety glasses and gloves when handling chemicals. • Water pH can affect chemicals; all information in this table assumes the use of tap or municipal water. • Keep out of lakes, streams, or ponds; stand at least 50 m from any natural water source. • Do not clean equipment or dispose of waste solutions at field sites. • For disposal, follow local, state, and federal guidelines.

Bleach: Inactivated by organic material. • Inactivated by sunlight. • If in an opaque container, diluted bleach will last 1 month[15]. If exposed to sunlight or air, it will only last 5 days.

Nolvasan: Can be inactivated by organic material.[15] • Store at room temperature in sealed container.[17] • Dilute concentrate with water of pH 5-7.[18] • Remains stable for 1 week if dilute with tap water, and for up to 6 weeks if diluted with deionized water.[17] • Use concentrate within 36 months.[17] • Toxic to fish.[18]

Virkon-S: Store at room temperature.[12] • Keep solution away from extreme cold or heat. • Shelf life for tablets is 2 years and for powder is 3 years. • Remains stable for 1 week if diluted with tap water.

Ethanol: Highly flammable. • Use and store in a well ventilated area. • Evaporation may diminish effective concentration.[12,18]

Step One

Brush or scrub off mud and vegetation from field equipment (e.g., nets, buckets, boots)

Soil or mud can reduce the effectiveness of the disinfection process

Step Two

Rinse

Step Three

Generously spray or immerse all items in 3-5% bleach solution

- Bleach is toxic to aquatic organisms; stand at least 50 m from any natural water source.
- Lab studies indicate 1 minute contact time to be sufficient to inactivate pathogens but NEPARC recommends 5 minutes in field situations.
- Best to take boots off prior to disinfecting

Step Four

Rinse Again

Rinse bleached items with water to minimize damage to the equipment and to prevent exposing the next wetland to residual bleach.

Use alcohol wipes to disinfect calipers, measuring boards, and other sensitive equipment.

Disinfection Procedures

http://northeastparc.org/disinfection-protocol/

Disinfection of Field Equipment to Minimize Risk of Spread of Chytridiomycosis and Ranavirus[1]

NORTHEAST PARC PARTNERS IN AMPHIBIAN AND REPTILE CONSERVATION

Importance of Disinfection

The spread of pathogens is a major threat to amphibians and reptiles worldwide.[2-5] This is particularly true for Ranavirus (RV) and *Batrachochytrium dendrobatidis* (Bd) responsible for chytridiomycosis. Humans can transmit diseases from one place to another and from one organism to another in a short amount of time and over distances the organisms cannot traverse. With the increasing spread of pathogens and reports of die-offs among amphibians and select reptiles worldwide, it is imperative that field biologists, researchers, hobbyists, and anyone interested in recreational herpetology-related field activities employ basic disinfecting procedures to prevent the spread of pathogens.

Before Leaving for the Field

Although other chemicals are effective (see table), NEPARC recommends a 3% bleach solution to inactivate Bd and most RV's.[3-7] Concentrated bleach is inexpensive and readily available. However, diluted bleach solutions lose their potency if exposed to air, sunlight, or organic material, and should be discarded after 5 days if exposed.[8] To ensure maximum efficacy, prepare only as much solution as you will need for the sampling event.

Suggested equipment:

- Brushes for scrubbing and/or removing mud and vegetation from equipment.
- Hand sanitizers and antiseptic alcohol wipes.
- Handheld bottles and/or pump sprayers for applying bleach and water. Bring clean rinse water.

- Gloves for handling animals. These should be disinfected or discarded between animals.
- Plastic bags of different sizes: examining animals in bag minimizes contact.
- Prepare additional sets of equipment if sampling at multiple locations.
- Trash bags.

Instructions for Large Equipment

Brush off mud, wash with biodegradable soap, disinfect with bleach and rinse all exterior surfaces of boats, canoes, vehicles or trailers and their tires that may have come in contact with potentially affected water (e.g. stream or wetland).

After Each Sampling Event and Before Moving to the Next Site

1. Brush off mud and vegetation from field equipment (e.g., nets, buckets, boots). Soil or mud can reduce the effectiveness of the disinfection process.
2. Generously spray or immerse all items in bleach solution.
   - Bleach is highly toxic to aquatic organisms; stand at least 50 m from any natural water source.
   - Lab studies indicate 1 minute contact time to be sufficient to inactivate pathogens but NEPARC recommends 5 minutes in field situations.
3. Rinse bleached items with water to minimize damage to the equipment and to prevent exposing the next wetland to residual bleach.
4. Use alcohol wipes to disinfect calipers, measuring boards, and other sensitive equipment.

End of the Day

After returning from the field, all equipment should be washed and thoroughly disinfected. If available, set up 2 buckets or large tubs: one with soapy water and one with 3% bleach solution.

Brush or scrub off any soil or vegetation. Immerse into soap, wash then rinse.

- Immerse in bleach and leave for 5 minutes. Rinse thoroughly with water.
- Hang equipment and gear, and allow them to air dry completely.

Other Considerations

- Wear gloves when sampling.
- Some people have two sets of field equipment with them so they can visit two sites and not disinfect between sites
- If measuring herps, keep in individual bags.
- Felt-soled waders are not easy to disinfect and should not be used in wetlands (they are banned in Maryland)
- Disinfect again at office and lay out to dry (ranavirus hates that).

Matt Gray

Other Considerations (Cont.)

Do not move amphibians and reptiles

- Many people want to move animals to “safer” locations.
- In addition to potentially spreading disease, they are also taking the animal to a hostile location; they don’t know where to find safe hiding places, food or mates.
- Turtles will often set out to find their way home.

Thank You