Trends in Ranavirus Prevalence among Plethodontid Salamanders in the Great Smoky Mountains National Park

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* Photographs by W. Sutton unless noted otherwise

Dorsey Branch, Fall 2012 Sampling

Abrams Creek, GSMNP, 2012

Introduction:

Global scale declines have been noted for nearly 33% of extant amphibians. A variety of causes have been identified, including habitat destruction and global climate change. Emerging infectious diseases have also been identified as a major driver behind these declines.

In addition to the chytrid fungus (*Batrachochytrium dendrobatidis;* Bd), ranaviruses are another important amphibian pathogen that has been implicated in worldwide die-offs. To date, most ranavirus research has focused primarily on single survey events of anuran populations. It is important to expand ranavirus surveillance to include salamander populations, as many salamanders (primarily plethodontid) contribute significantly to ecological function in forested ecosystems. The **primary objective** of this study was to examine trends in ranavirus infection within a southern Appalachian plethodontid salamander assemblage over a **six-year period**. We aimed to investigate the importance of environmental and species-specific factors that may influence infection prevalence.

Methods:

- We surveyed eight study streams in the Great Smoky Mountains National Park (Figure 1) over six-year period (2007 – 2012).
- Streams were distributed at a range of elevations (426 – 1605 m). Five streams were located at low elevations, one stream was located at mid elevation, and two streams were classified as high elevation.
- We generally sampled in spring months and captured salamanders by turning rocks and logs and sorting leaf packs.
- We searched sites for at least one hour or until a maximum of 40 salamanders were captured at each site.
- We rinsed each salamander with distilled water and measured snout-vent length (mm) and mass (g).

- We collected a small (<5 mm) tail clip and swabbed ventral and inguinal areas and toe-tips for ranavirus and Bd testing, respectively.
- We extracted genomic DNA from individual tail tissue and used quantitative PCR (qPCR) to detect ranavirus in each tissue sample.
- We declared infection if the qPCR threshold was < 30.
- We ran each qPCR sample in duplicate along with two positive controls and two negative controls.

- We used Generalized Linear Mixed Models to test the effects of salamander genus, body condition, elevation, year, aquatic dependency on ranavirus prevalence.

Results:

- We captured 691 salamanders of 16 species (Table 1)
- Five species (*D. conanti, D. imitator, D. santeetlah, D. quadramaculatus, E. wilderae, and P. jordani*) accounted for 81% of total captures
- *D. monticola* (45.0%), *D. conanti* (21.0%), and *D. imitator* (20.5%) had greatest infection prevalence
- *D. wrighti* (3.6%) and *G. porphyriticus danielsi* (3.7%) had lowest infection prevalence
- Infection prevalence ($F_{2,\,660}$ = 2.87; P = 0.057) was greatest among *Desmognathus* (17.3%) and lowest among *Plethodon* species (7.9%; Figure 2)
- Infection prevalence differed by year (P < 0.001) for individual species with ≥ 10 positive cases of ranavirus
- Infection prevalence was greater ($F_{1,\,70}$ = 11.47; P < 0.01) for *D. imitator* at mid-elevation sites (26.8%) than high elevation sites (4.5%)
- Ranavirus prevalence differed by elevation and year ($F_{10,\,639}$ = 5.82; P < 0.001) with greater infection at low elevation sites in 2007 and 2008.

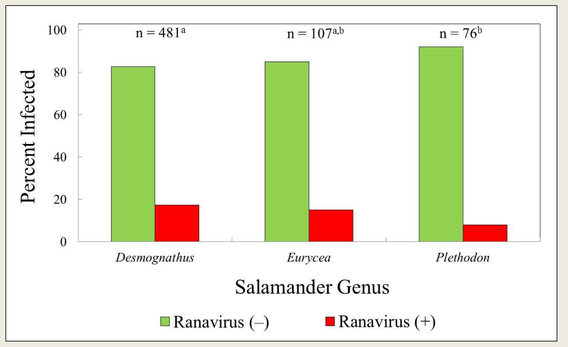

Figure 2: Ranavirus infection prevalence of salamanders in the genus *Desmognathus*, *Eurycea*, and *Plethodon* in the GSMNP. Total sample sizes and Post-hoc differences are listed above the bars.

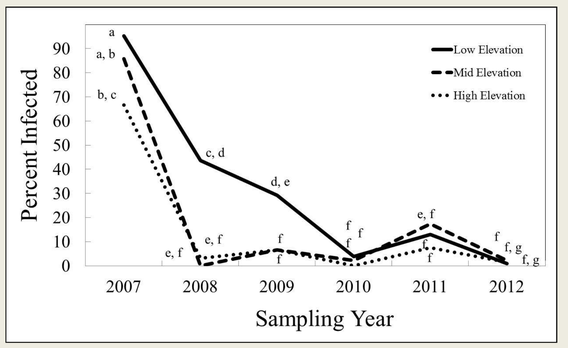

Figure 3: Ranavirus prevalence of salamanders from 2007 – 2012 at low-, mid-, and high-elevation sites in the GSMNP. Post-hoc differences are listed above graph lines.

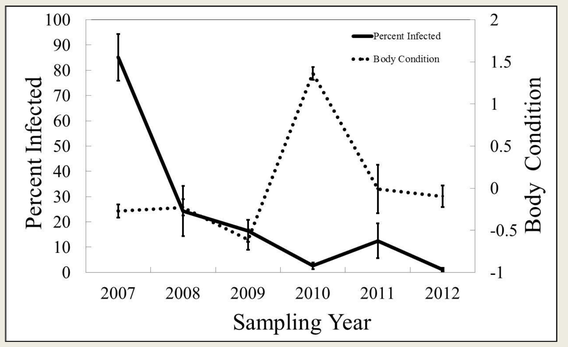

Figure 4: Relationship between percent infection and average body condition across 8 sites from 2007 – 2012 for salamander species with ≥ 10 cases of ranavirus. Please refer to Table 1 for species data.

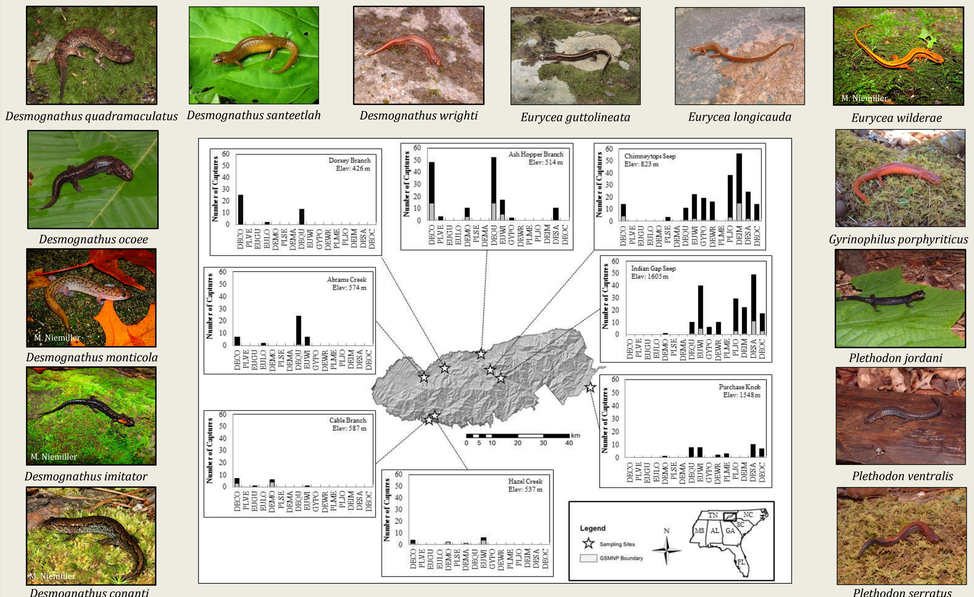

Figure 1: Location of salamander sampling sites in the GSMNP, USA. Histogram black bars represent the number of non-infected individuals and gray bars represent the number of infected individuals. Four-letter codes represent scientific names of sampled salamander species.

Scientific Name	Common Name	Sites Detected	Number Infected	Number Sampled	Percent Infected
Desmognathus conanti	Spotted Dusky Salamander	AC, AH, CB, CH, HC	22	105	21.0
Desmognathus imitator	Imitator Salamander	CT, IG	16	78	20.5
Desmognathus marmoratus	Shovel-nosed Salamander	HC	1	1	100
Desmognathus monticola	Seal Salamander	AH, CB, HC, IG, PK	9	20	45.0
Desmognathus ocoee	Ocoee Salamander	CT, IG, PK	5	38	13.2
Desmognathus quadramaculatus	Black-bellied Salamander	AC, AH, CT, DB, IG, PK	17	118	14.4
Desmognathus santeetlah	Santeetlah Dusky Salamander	AH, CT, IG, PK	13	93	14.0
Desmognathus wrighti	Pygmy Salamander	CT, IG, PK	1	28	3.6
Eurycea guttolineata	Three-lined Salamander	CB	0	1	0
Eurycea longicauda	Long-tailed Salamander	AC, DB	0	4	0
Eurycea wilderae	Blue Ridge Two-lined Salamander	AH, CT, CB, HC, IG, PK	16	102	15.7
Gyrinophilus porphyriticus danielsi	Blue Ridge Spring Salamander	AH, CT, IG	1	27	3.7
Plethodon jordani	Red-cheeked Salamander	CT, IG	6	67	9.0
Plethodon metcalfi	Southern Gray-cheeked Salamander	PK	0	3	0
Plethodon serratus	Southern Red-backed Salamander	CT	0	3	0
Plethodon ventralis	Southern Zigzag Salamander	AH	0	3	0
Total			**107**	**691**	**NA**

Table 1. Species and number of plethodontid salamanders detected during terrestrial and aquatic stream surveys in the Great Smoky Mountains National Park 2007 – 2012. Site abbreviations are as follows: Abrams Creek – AC, Ash Hopper Branch – AH, Cable Branch – CB, Chimney Tops – CH, Dorsey Branch – DB, Hazel Creek – HC, Indian Gap – IG, and Purchase Knob – PK

Discussion:

- Yearly fluctuations and site elevation were important for explaining ranavirus prevalence
- Drought during 2007 (59.10 mm below average) was a potential stressor influencing infection prevalence
- Greater virion concentration may occur at lower elevation sites; ranaviruses may persist in aquatic environments for > 1 month (Nazir et al. 2012)
- *Desmognathus* species had greater prevalence than *Plethodon* species
- *Desmognathus* species are strongly associated with aquatic habitats and ranavirus transmission is likely enhanced in aquatic environments
- We documented an inverse trend between average body condition and infection prevalence for the five species with ≥10 cases of ranavirus infection
- Similar body condition trends have been observed in other amphibians (i.e., Green Frog [*Lithobates clamitans*], St. Armour et al. 2010); however, the link between body condition and infection prevalence is not well understood in plethodontid salamanders