Amphibian ranaviruses in Canada historical, current and future research directions

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Thanks

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THE ESSENTIAL ELEMENT

Environment Canada Environnement Canada

Ranaviruses:
an emerging threat to ectothermic vertebrates

1) host range and geographic range

2) ecological & environmental correlates with disease

3) validating & improving non-lethal diagnostic tests

4) viral biology including phylogenetics and annotating genomes

5) future research directions / wish list

1) host range and geographic range

FV3-like viruses detected in:

Lithobates sylvaticus

L. pipiens

L. clamitans

Ranavirus assumed to be FV3-like

(unsequenced PCR products using MCP 4/5 primers):

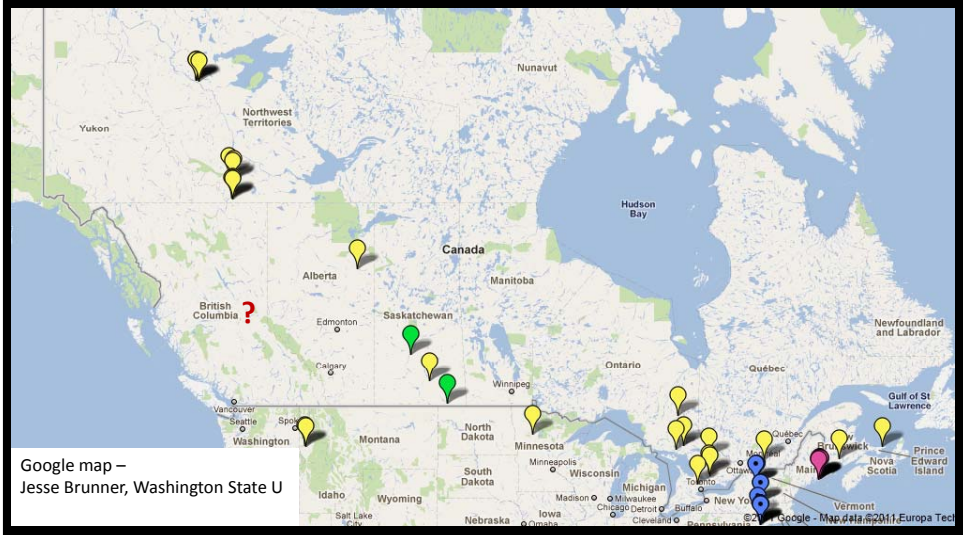
Pseudacris sp

Hyla sp

Ambystoma sp

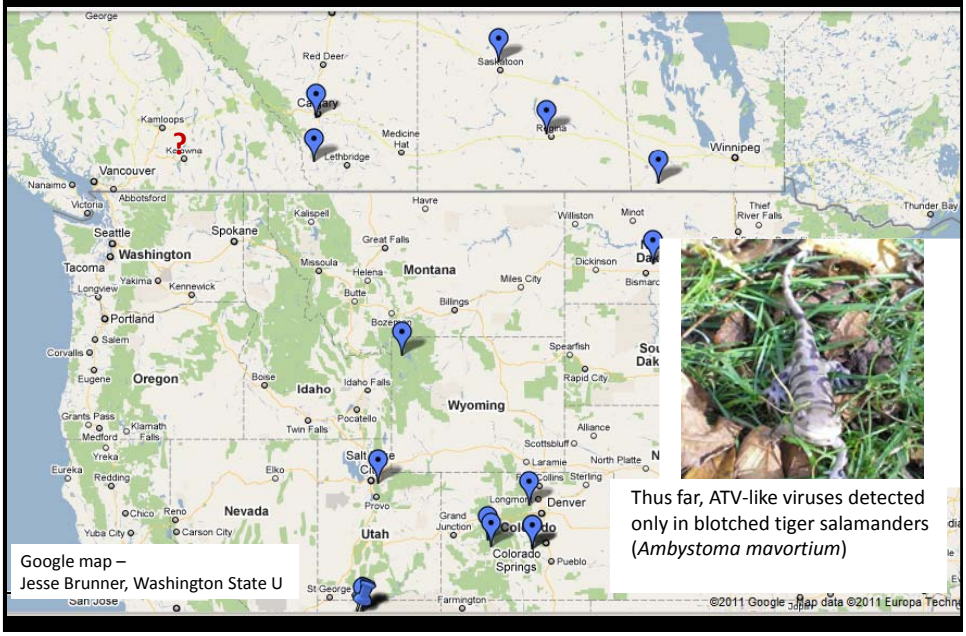
Notophthalmus viridescens

FV3-like viruses in Canada



Google map –
Jesse Brunner, Washington State U

ATV-like viruses in Canada

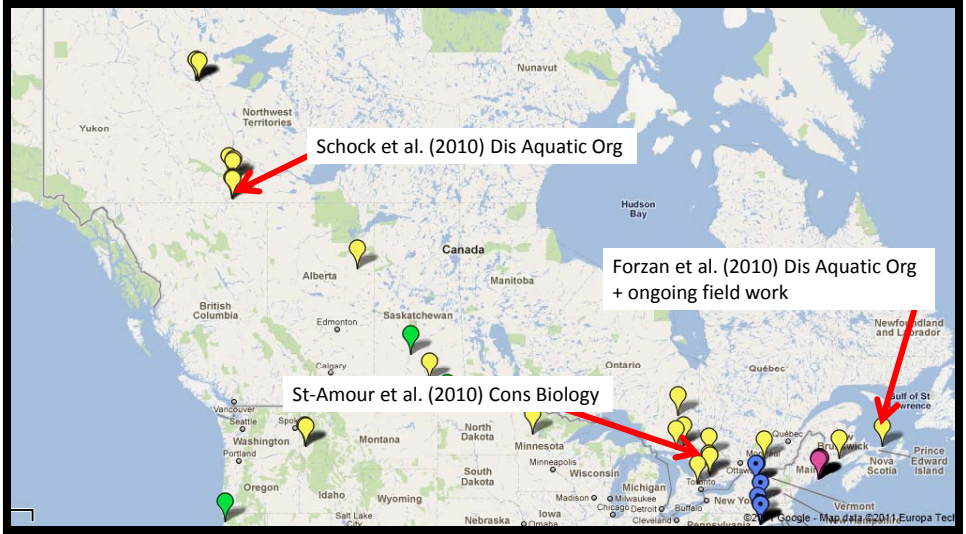


Thus far, ATV-like viruses detected only in blotched tiger salamanders (*Ambystoma mavortium*)

Google map –
Jesse Brunner, Washington State U

FV3 & ATV in the same pond at the same time

FV3 & *Bd* in the same pond at the same time



2) ecological & environmental correlates with disease

David Lesbarreres Lab, Laurentian University

St-Amour et al. (2010) Cons Biology

Fluctuating asymmetry and ranavirus infection are positively correlated *(L. clamitans)*

St-Amour et al. (2008) Emerg Infect Dis

Proximity to human disturbance and prevalence are positively correlated *(L. clamitans)*

Echaubard et al. (2011) PLoS One

Host density is a pivotal determinant of the effects of ranavirus infections *(L. pipiens)*

David Lesbarreres Lab & collaborators, this symposium

2) ecological & environmental correlates with disease

Cam Goater, U Lethbridge

ATV & blotched tiger salamanders
Livingstone Lake area in south-western Alberta

Annual variation in the prevalence of ATV & potential environmental covariates including:

- host density
- host size at metamorphosis
- trout stock rate
- paedomorph density
- precipitation

2) ecological & environmental correlates with disease

*Intra*specific genetic variation in hosts and viruses in Canada:

ATV

Schock et al. (2009) EcoHealth
- ATV & blotched tiger salamanders

Underlying causes of intraspecific variation

- current Canadian studies?

- US studies:
Andrew Storfer Lab – evidence of co-evolution (& lack of co-evolution) in ATV & blotched tiger salamanders, *this symposium*

2) ecological & environmental correlates with disease

*Intra*specific genetic variation in hosts and viruses in Canada:

FV3
Canada?

US studies:
- ***Jason Hoverman**, this symposium*

European studies:
- Pearman & Garner (2005) Ecology Letters
- ***Amanda Duffus**, this symposium*

2) ecological & environmental correlates with disease

Danna Schock, Keyano College
2 current research projects examining amphibian health in relation to industrial activity in the boreal forest s of Alberta & NWT

2) ecological & environmental correlates with disease

Project 1: Testing for correlations between proximity to oil sands mining activities and:

1) prevalence of ranavirus & *Bd* infections

2) rates & kinds of physical abnormalities

3) residues of organics and heavy metals in frog tissues & water from breeding ponds

Danna Schock (PI)

Catherine Soos & Bruce Pauli – Envi Canada,
Maria Forzan - UPEI, Allan Pessier – San Diego Zoo,

Canada
Northwest Territories
Alberta Conservation Association
Environment Canada
KEYANO COLLEGE
CANADIAN WILDLIFE FEDERATION / FÉDÉRATION CANADIENNE DE LA FAUNE

2) ecological & environmental correlates with disease

Project 2: Evaluating wetlands constructed for reclamation of oil sands mining sites for their suitability for amphibians

Danna Schock – Keyano College, Judit Smits – U Calgary
James Jancovich – Cal State San Marcos
Jan Ciborowski – U Windsor

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Syncrude

2) ecological & environmental correlates with disease

Source: national geographic online images

Schock – Project 2:

Upon closure, mine sites must be ecologically sustainable and capable of supporting endemic species.

One reclamation strategy involves the creation of wetlands to age and detoxify oil sands process-affected materials

2) ecological & environmental correlates with disease

Source: national geographic online images

An active area of research is examining to what extent these engineered wetlands are capable of sustaining endemic flora and fauna.

2) ecological & environmental correlates with disease

Schock – Project 2:

Several ponds - high survival of tadpoles.

What happens when amphibians reared in water from these engineered wetlands are exposed to their natural pathogens?

wood frog tadpoles + FV3 originally isolated from wood frogs

5 water sources x 3 virus doses; 40-45 tadpoles/treatment

Response variables include:

- mortality rates
- sublethal infection rates
- growth & developmental rates

3) validating & improving non-lethal diagnostic tests

Long-term monitoring of infection status in wild populations requires a reliable, non-lethal way to collect samples.

Having the ability to sample the same individuals multiple times is ideal (arguably, even absolutely necessary)

3) validating & improving non-lethal diagnostic tests

Swabs are one option that researchers have tried:

No/minimal harm to animal

Possibility of collecting a single sample and doing a single DNA extraction if also screening for *Bd*

However, questionable relevance & utility
- Concerns about environmental contamination
- Concerns about virus detection only in viremic animals

First report of amphibian ranaviruses in Quebec detected by swabs of *Lithobates pipiens*
Paetow et al. (2011) Herp Review

3) validating & improving non-lethal diagnostic tests

Toe clips and **tail clips** are another option but not ideal:

Possibility of collecting a single sample and doing a single DNA extraction if also screening for other pathogens and/or doing amphibian genetics

Questions about negative effects on the animals

** much of the published discussion about the impacts of toe clips on amphibians refer to studies where no attempt was made to prevent transmission of pathogens among individuals*

3) validating & improving non-lethal diagnostic tests

Toe clips and **tail clips** are another option but not ideal:

Questions about detection ability:

The biology of latent & low-level ranavirus infections suggests that clips probably underestimate prevalence

- clips don't assay liver, spleen etc
- (- neither do swabs)

So, just how badly are we under-estimating prevalence?

- *outside of epidemic peaks?*
- *in non-clinical animals?*

3) validating & improving non-lethal diagnostic tests

Toe clips and **tail clips** are another option but not ideal:

St-Amour & Lesbarreres (2007) Cons Genetics

- found good agreement between toe-clips & liver samples (*Lithobates clamitans* & FV3)

Greer & Collins (2007) J Wildl Dis

- only strong agreement between tail-clips & liver samples when infection & disease are advanced (*A. mavortium* & ATV)

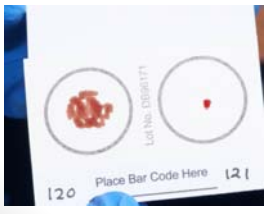
Additional information is being sought, new techniques examined

3) validating & improving non-lethal diagnostic tests

Maria Forzan and colleagues

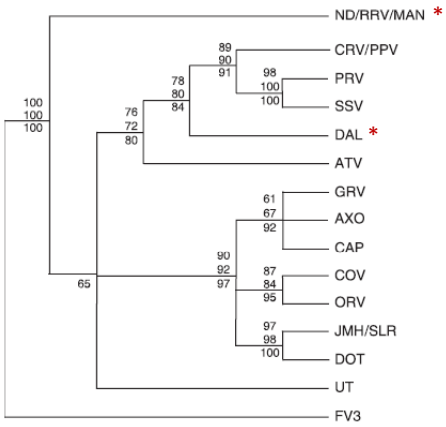
Cndn Cooperative Wildlife Health Centre, U Prince Edward Island node

Evaluation of liver fine-needle aspirate, toe-clip, and blood to determine the most suitable sample for the detection of ranavirus infection in frogs through PCR



4) viral biology including phylogenetics, gene expression, and annotating genomes

Canadian isolates are often included in larger analyses by **James Jancovich** and **Andrew Storfer**



From: Jancovich et al. (2005) Molec Ecology

4) viral biology including phylogenetics, gene expression, and annotating genomes

Craig Brunetti Lab, Trent University

Eaton et al. (2010) Viruses

Genomic Diversity and Phylogenetic Relationship in the Family Iridoviridae

Eaton et al. (2007) Virology Journal

Comparative genomic analysis of the family Iridoviridae: re-annotating and defining the core set of iridovirus genes

Eaton et al. (2008) Virology Journal

Expression of frog virus 3 genes is impaired in mammalian cell lines

4) viral biology including phylogenetics, gene expression, and annotating genomes

5) future research directions / wish list

At least some of the positives detected in surveys of wild amphibian populations should be sequenced.

Guidance on which segments of the genome to use in surveys?
- standardization/recommendations?

FV3 genome = ~ 106,000 bp; MCP gene = 1,500 bp

Most studies sequence about 500 bp of the MCP,
with different studies targeting different regions of the MCP

This impedes comparing across studies.

5) Future research directions / wish List

"Amphibian" ranaviruses and reptiles? fish?

Long-term studies that can speak to the role of ranaviruses in amphibian population stability, persistence, demographics

Validated diagnostic tools necessary to conduct those long-term studies

Ecological correlates with outbreaks / long-term effects
- use of amphibians and their ranaviruses as model systems for understanding wildlife disease dynamics in general

5) Future research directions / wish list

Studies of wild amphibian populations (e.g., monitoring) that explicitly recognize the multi-host nature of ranaviruses

Causes and consequences of intraspecific variation of hosts and viruses

Sublethal effects of infection – e.g., slowed growth rates
Ramifications in wild populations?

5) Future research directions / wish list

Interactions between FV3 strains (species?) within hosts and within populations

Interactions between FV3 and ATV
– within hosts? Populations? Amphibian communities?

Interactions between ranaviruses and other pathogens (e.g. *Bd*, *Saprolegnia*)

Interactions with other environmental stressors?

Wood frog tadpole being scavenged by water striders.
Die-off characteristics and tadpole lesions consistent with FV3; molecular & histological confirmation pending.
Photo by Dawn Hall
Fort McMurray, AB, 2 July 2011