CMTV

"how frogs (and other stuff) sometimes get hammered in Europe"

What is CMTV?

What is CMTV?

Vol. 84: 95–104, 2009
doi: 10.3354/dao02032

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published April 6

Pathology, isolation and molecular characterisation of a ranavirus from the common midwife toad *Alytes obstetricans* on the Iberian Peninsula

Ana Balseiro[1,*], Kevin P. Dalton[2], Ana del Cerro[1], Isabel Marquez[1], Andrew A. Cunningham[3], Francisco Parra[2], José M. Prieto[1], R. Casais[1]

[1]SERIDA, Servicio Regional de Investigación y Desarrollo Agroalimentario, Laboratorio de Sanidad Animal, 33299 Jove, Gijón, Spain

[2]Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, 33006 Oviedo, Spain

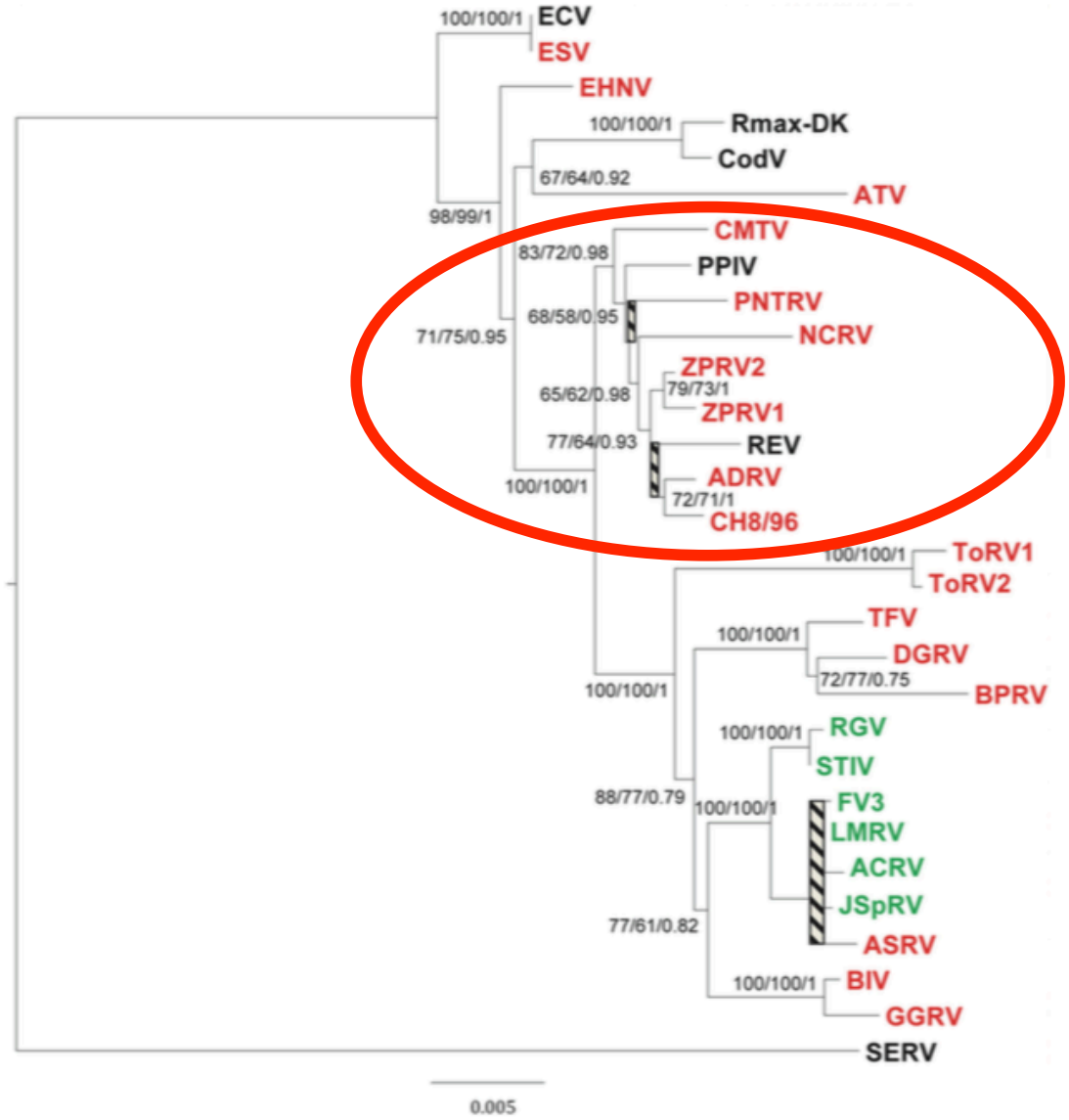
[3]Institute of Zoology, Regent's Park, London NW1 4RY, UK

ABSTRACT: We describe the pathology, isolation and characterisation of a virus responsible for an outbreak of a systemic haemorrhagic disease causing high mortality in tadpoles of the common midwife toad *Alytes obstetricans* in the 'Picos de Europa' National Park in northern Spain. The virus, provisionally designated as the common midwife toad virus (CMTV), was isolated from homogenates of visceral tissue from diseased toad tadpoles following inoculation on epithelioma papulosum cyprini (EPC) cells. Molecular characterisation of the virus, including sequence analysis of the DNA polymerase and major capsid protein genes, showed that the isolated virus was a ranavirus with marked sequence identity to other members of the genus *Ranavirus*. A rabbit antiserum raised against purified virions was prepared and used to definitively demonstrate systemic distribution of the virus in diseased tadpoles, indicating that the isolated virus was the primary pathogen.

KEY WORDS: Common midwife toad · *Alytes obstetricans* · *Ranavirus* · Pathology · Virology · Immunohistochemistry

- Taxon…
- Isolate…
- Strain…
- Species..?

What is CMTV?



- Clade
- "CMTV-like"
- "intermediate"

- Stohr et al., (2015) PlosONE

Is CMTV special?

- Well, in at least one way, yes!
- …and that's in the type of data we've already gathered on host-pathogen dynamics.

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midwife toad · *Alytes obstetricans* · *Ranavirus* · Pathology · Virology ·

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Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Short Communication

Outbreak of common midwife toad virus in alpine newts (*Mesotriton alpestris cyreni*) and common midwife toads (*Alytes obstetricans*) in Northern Spain: A comparative pathological study of an emerging ranavirus

Ana Balseiro [a,*], Kevin P. Dalton [b], Ana del Cerro [a], Isabel Márquez [a], Francisco Parra [b], José M. Prieto [a], R. Casais [a]

Is CMTV special?

Epizootiology of Sixty-Four Amphibian Morbidity and Mortality Events in the USA, 1996–2001

D. EARL GREEN, KATHRYN A. CONVERSE, AND AUDRA K. SCHRADER

United States Geological Survey, National Wildlife Health Center, Madison, Wisconsin 53711 USA

ABSTRACT: A total of 44 amphibian mortality events and 20 morbidity events were reviewed retrospectively. The most common cause of amphibian mortality events was infection by ranaviruses (Family: Iridoviridae). Ranavirus epizootics have abrupt onset and affect late-stage larvae and recent metamorphs. Mortality events due to ranavirus infections affected only widespread and abundant amphibian species, and there was a clear association with high population densities. Chytrid fungal infections accounted for seven mortality events in postmetamorphic anurans only. Chytrid epizootics are insidious and easily overlooked in the field. While both ranavirus and chytrid fungal epizootics were associated with >90% mortality rates at affected sites, only the chytrid fungal infections were linked to multiple amphibian population declines. Three primitive fungal organisms in the newly erected clade, Mesomycetozoa, caused morbidities and mortalities in anurans and salamanders.

KEYWORDS: amphibians; ranavirus; "red leg" disease; iridovirus infections; frogs; toads

-104, 2009
/dao02032

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midwife toad · *Alytes obstetricans* · *Ranavirus* · Pathology · Virology ·

(*Mesotriton alpestris cyreni*) and common midwife toads (*Alytes obstetricans*) in Northern Spain: A comparative pathological study of an emerging ranavirus

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J. Vet. Med. B 45, 373–383 (1998)

ISSN 0931–1793

[1]*Institute of Zoology, Fishery Biology and Fish Diseases, University of Munich, München, Germany;* [2]*Institut National de la Recherche Agromique (INRA), Unite de Virologie et Immunologie Moleculaires, Jouy-en-Josas France*

Comparison of European Systemic Piscine and Amphibian Iridoviruses with Epizootic Haematopoietic Necrosis Virus and Frog Virus 3

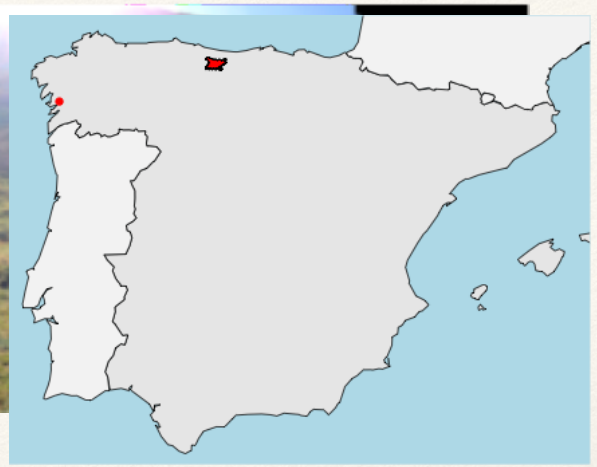
W. AHNE[1,3], M. BEARZOTTI[2], M. BREMONT[2] and S. ESSBAUER[1]

Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*)

A. A. CUNNINGHAM[1], T. E. S. LANGTON[2], P. M. BENNETT[1], J. F. LEWIN[3], S. E. N. DRURY[4], R. E. GOUGH[4] AND S. K. MACGREGOR[1]

[1] *Veterinary Science Group, Institute of Zoology, Regent's Park, London NW1 4RY, U.K.*
[2] *Herpetofauna Consultants International, Triton House, Bramfield, Halesworth, Suffolk IP19 9AE, U.K.*
[3] *The Royal Free Hospital School of Medicine, Pond Street, Hampstead, London NW3 2QG, U.K.*
[4] *Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, U.K.*

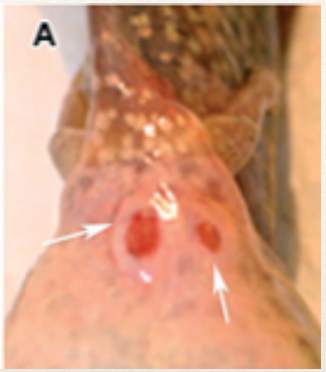
wife toad · *Alytes obstetricans* · *Ranavirus* · Pathology · Virology ·

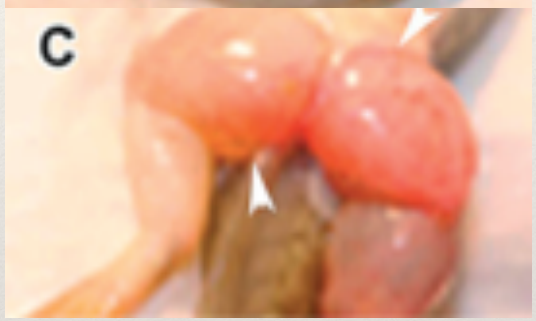


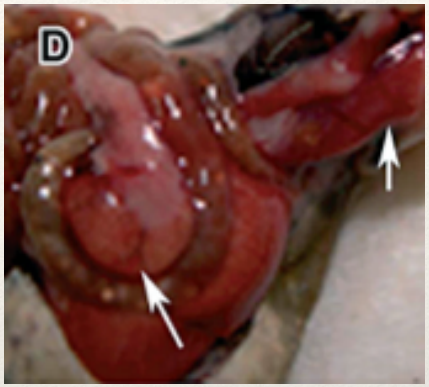
Severe mass-mortality events in Spain

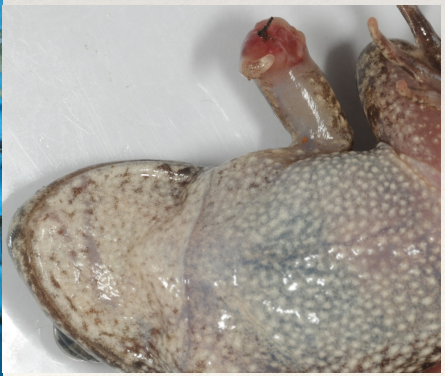
- simultaneous emergence and impacts across host community in protected mountainous region of Spain (Picos de Europa [PNPE])
- mass die-offs and disease

Pathology and clinical signs



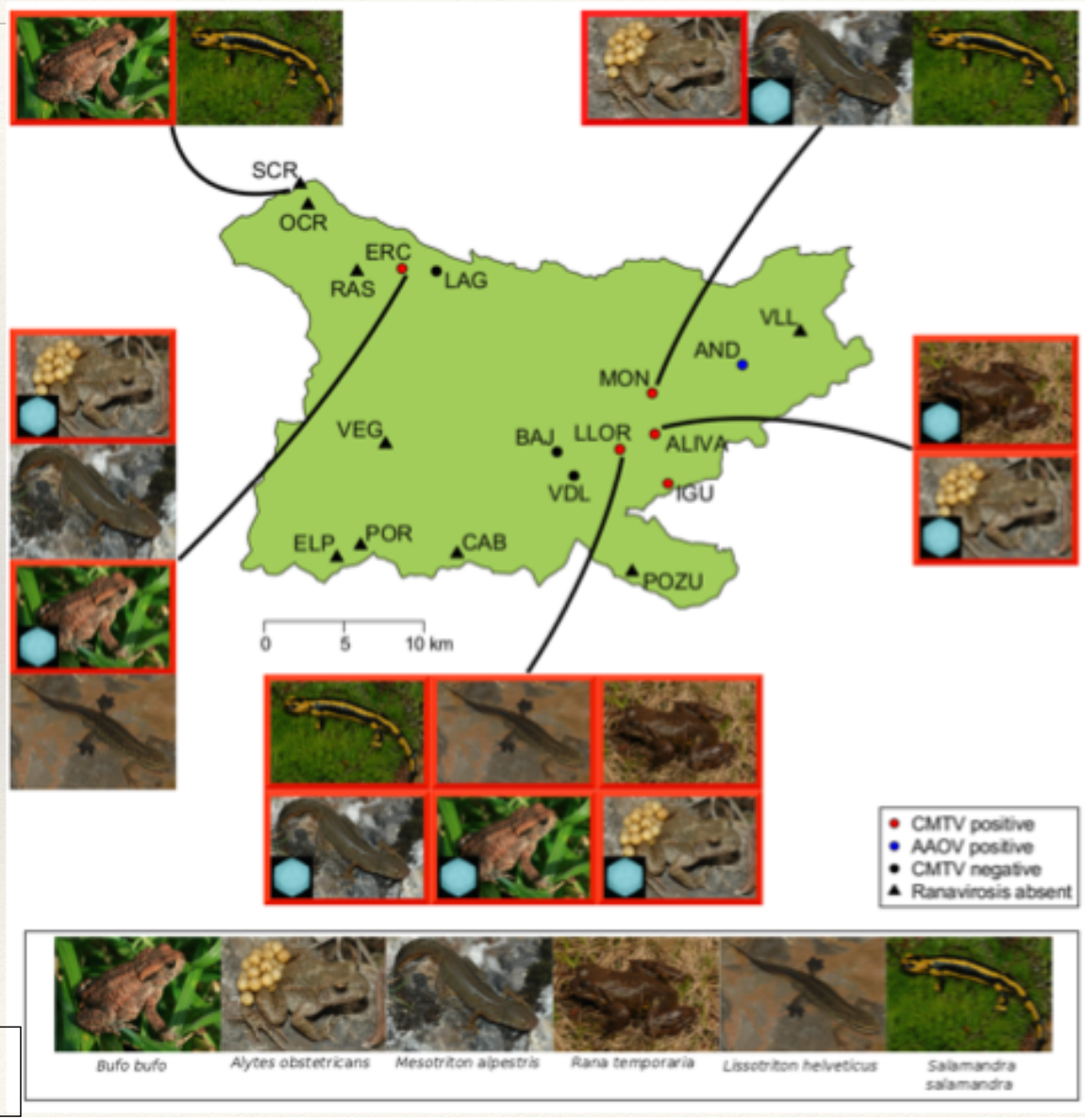






Population impacts: community effects

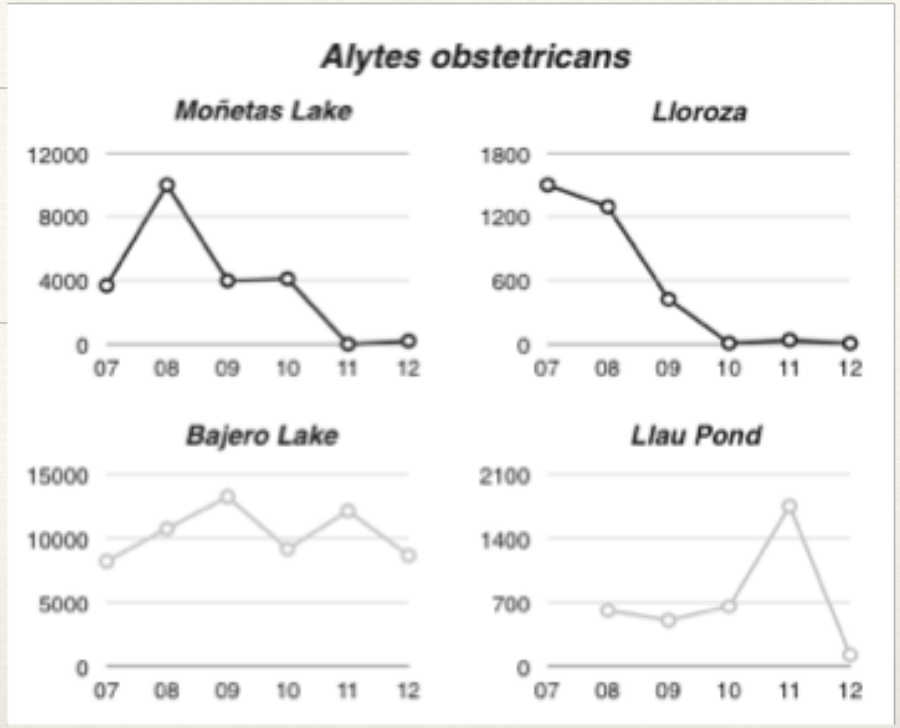
- long-term population monitoring of amphibian communities across the park
- including sites with observed mass die-offs and those without
- all six amphibians present at one site experiencing mortality

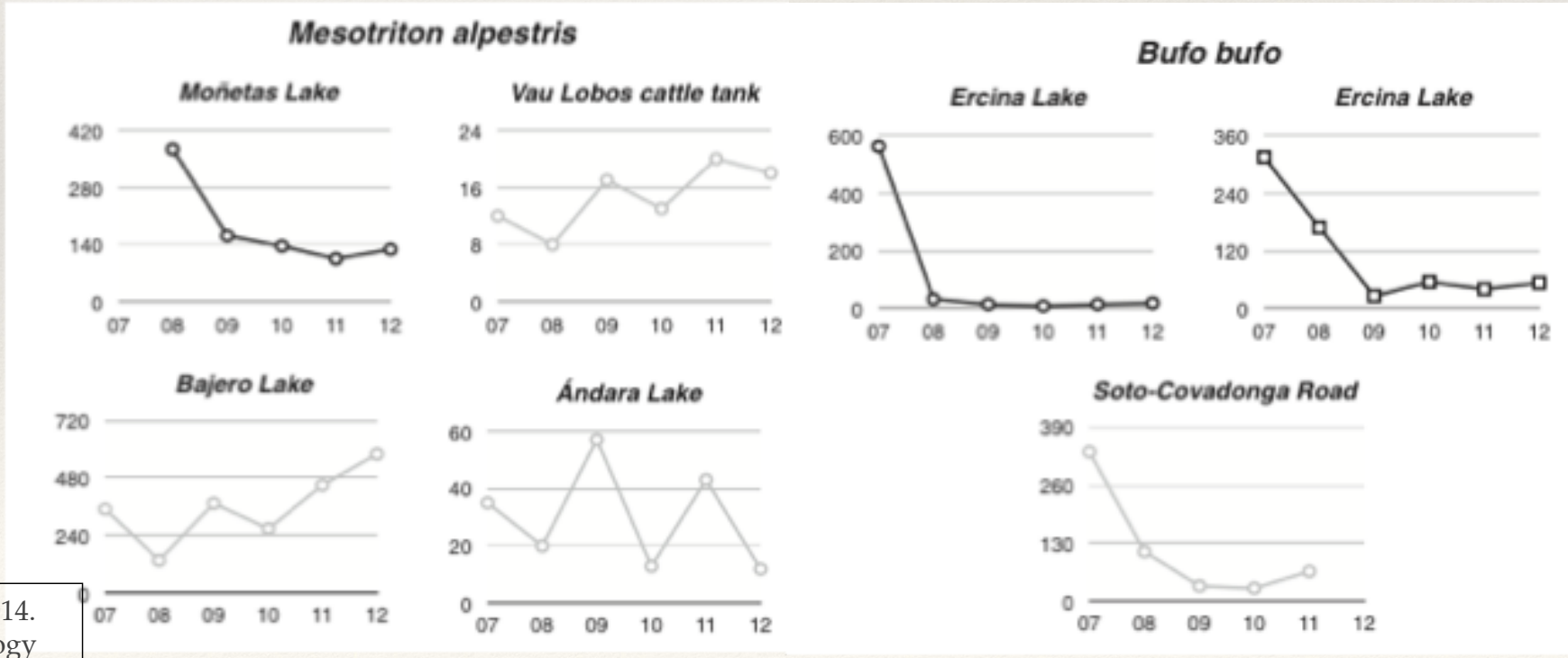


Price et al., 2014. Current Biology

Population impacts: community effects

- severe population impacts for multiple species of host
- CMTV-like viruses appear to be highly virulent generalist pathogens that should be considered a conservation threat

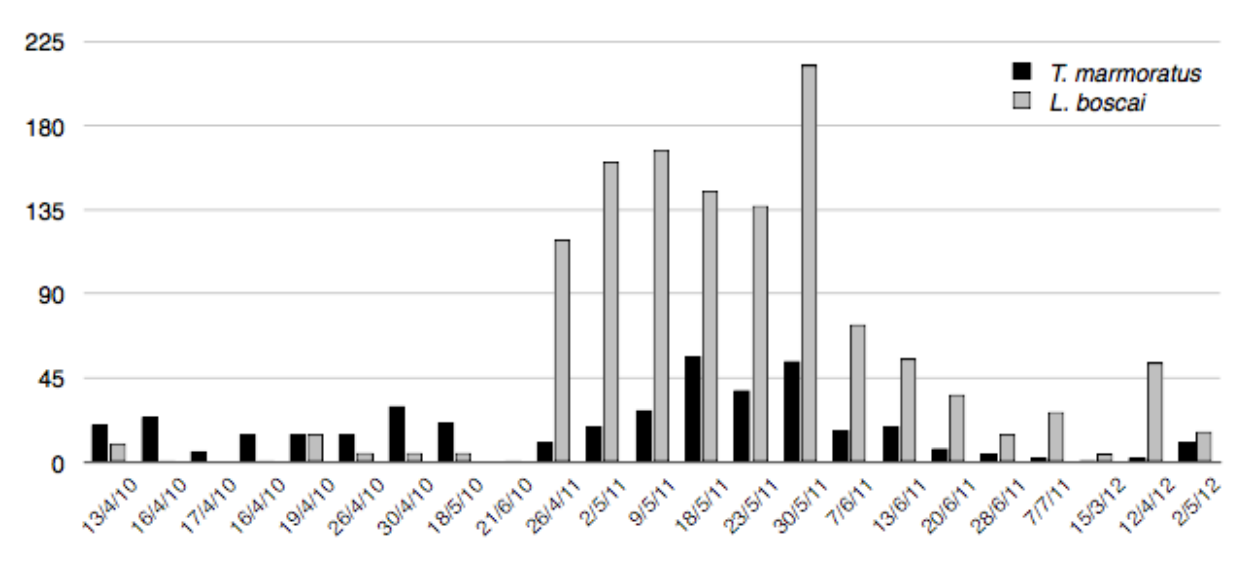




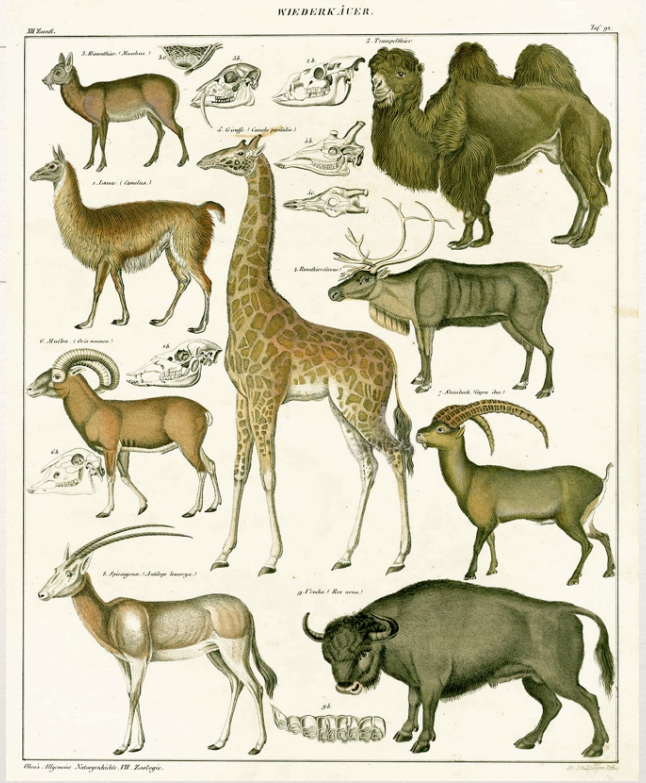
Price et al., 2014. Current Biology

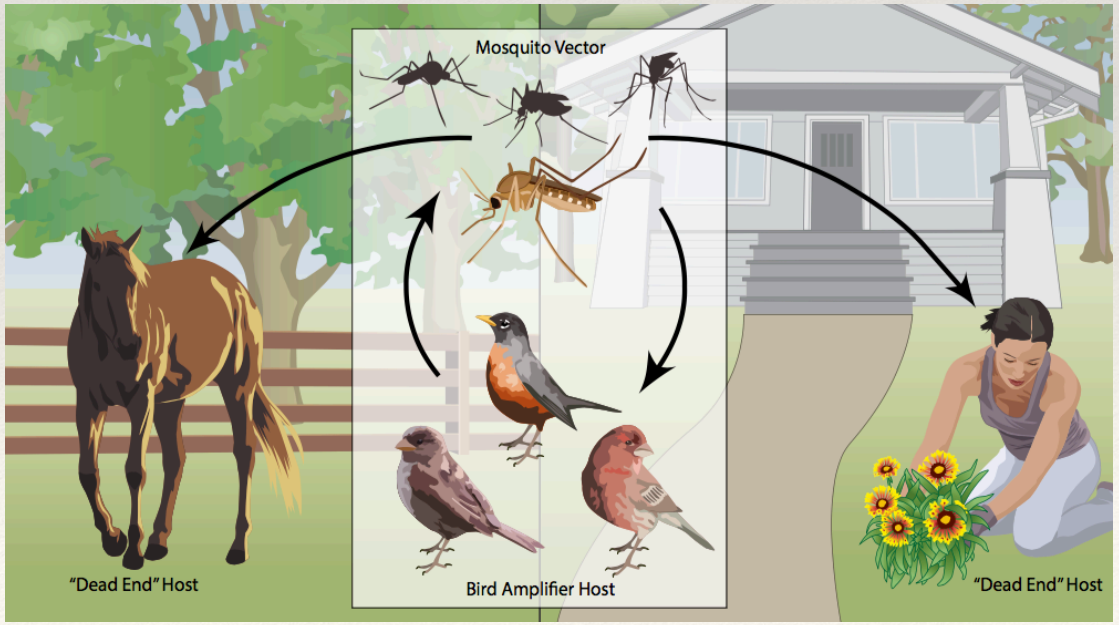
CMTV (Bosca's newt virus) in Galicia

- No population monitoring allowing us to measure declines
- But major annual mass mortality events affecting newts
- Occasional records of infection/disease in snakes



Extraordinary host range





CMTV Hosts in Spain



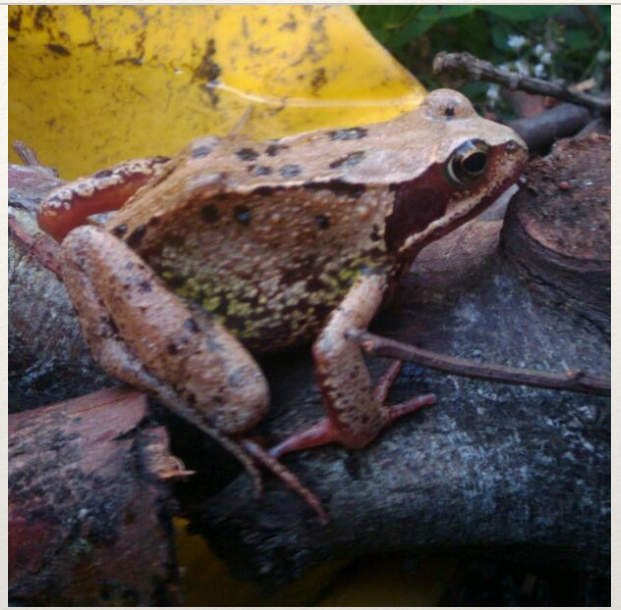






Hosts: Generalism not the rule

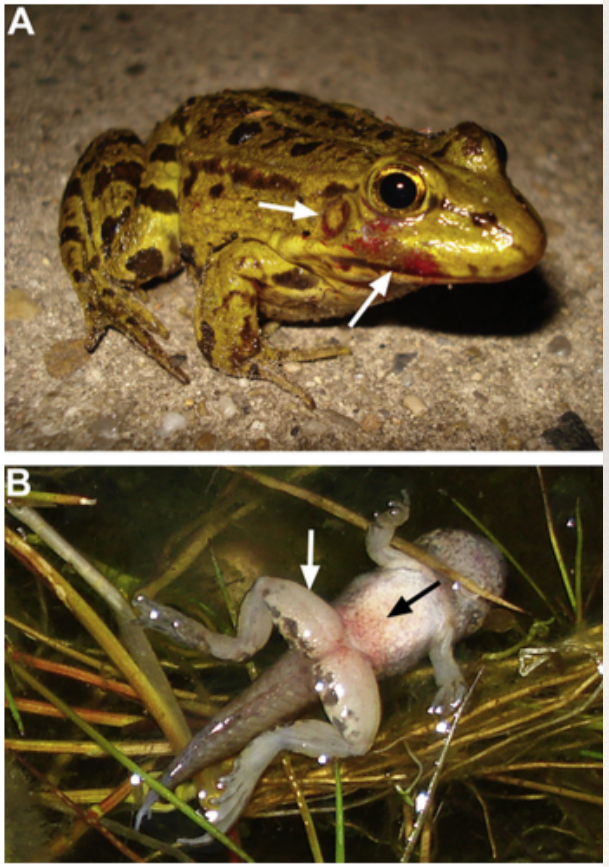
- Duffus et al. (2014) present experimental evidence that common frogs are the primary host for UK rvs with common toads (Bufo bufo) an alternative but lower quality host
- This fits with anecdotal evidence from submissions of dead amphibians from mortality events which test positive for ranavirus infection. Infected toads and smooth newts are received but most carcasses are common frogs (GB Wildlife Disease Surveillance Partnership)



CMTV outbreaks in Europe

Ranavirus-associated mass mortality in wild amphibians, The Netherlands, 2010: A first report

Marja Kik [a,*], An Martel [b], Annemarieke Spitzen-van der Sluijs [c], Frank Pasmans [b], Peter Wohlsein [d], Andrea Gröne [a], Jolianne M. Rijks [a]

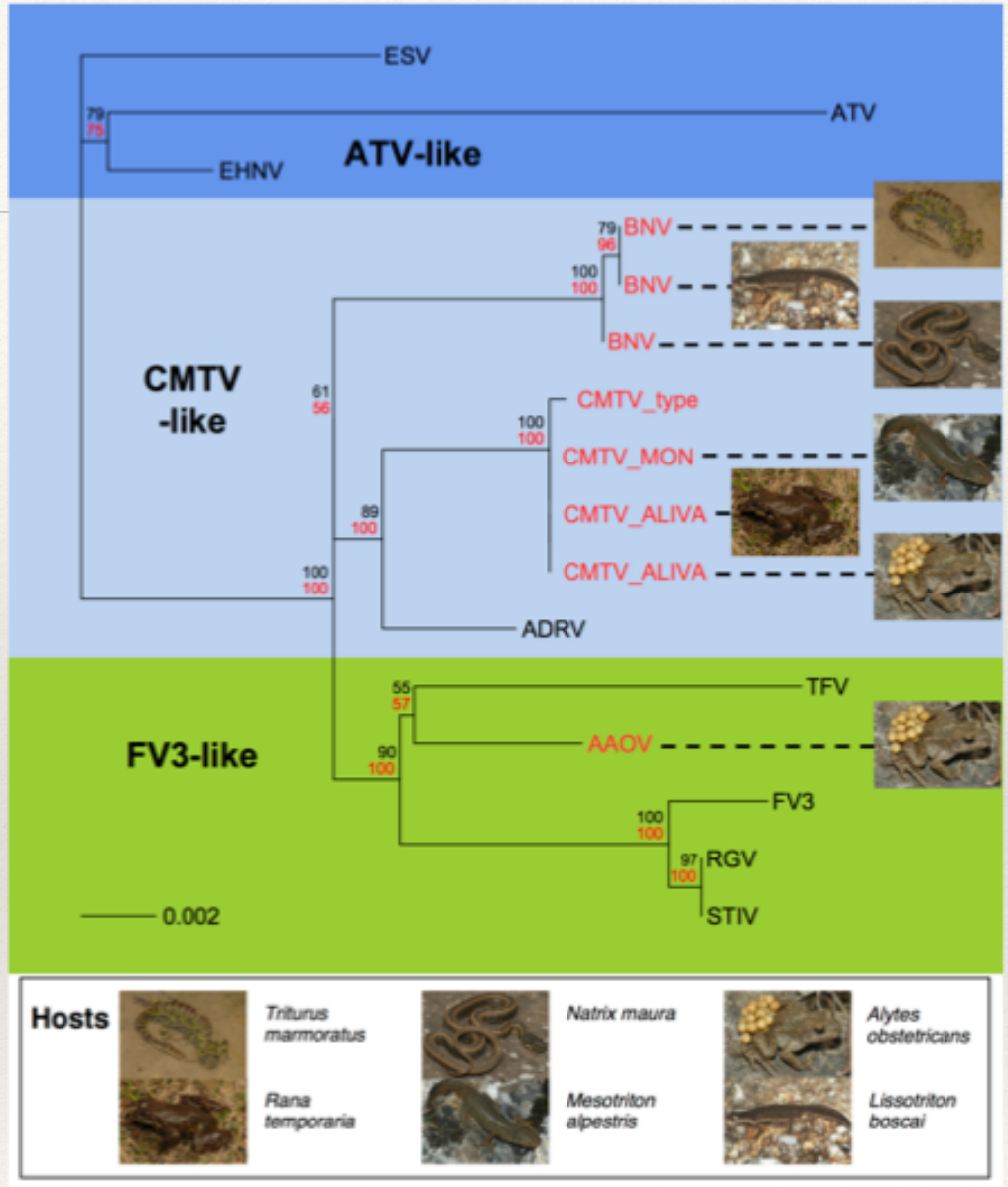


- Focused on Spain as the poster boy of CMTV research!
- But not to overlook other regions
- Disease outbreaks due to CMTV-like viruses appear to be emerging at broad scale in Europe
- France, Netherlands, Portugal are other examples of regions that are home to multihost mass mortality events

What do we know about the evolutionary history of CMTV?

Virus diversity

- significant virus diversity
- 3 distinct viruses found: 2 CMTV-like viruses and an FV3-like
- apparent variation in virulence



Price et al., 2014.
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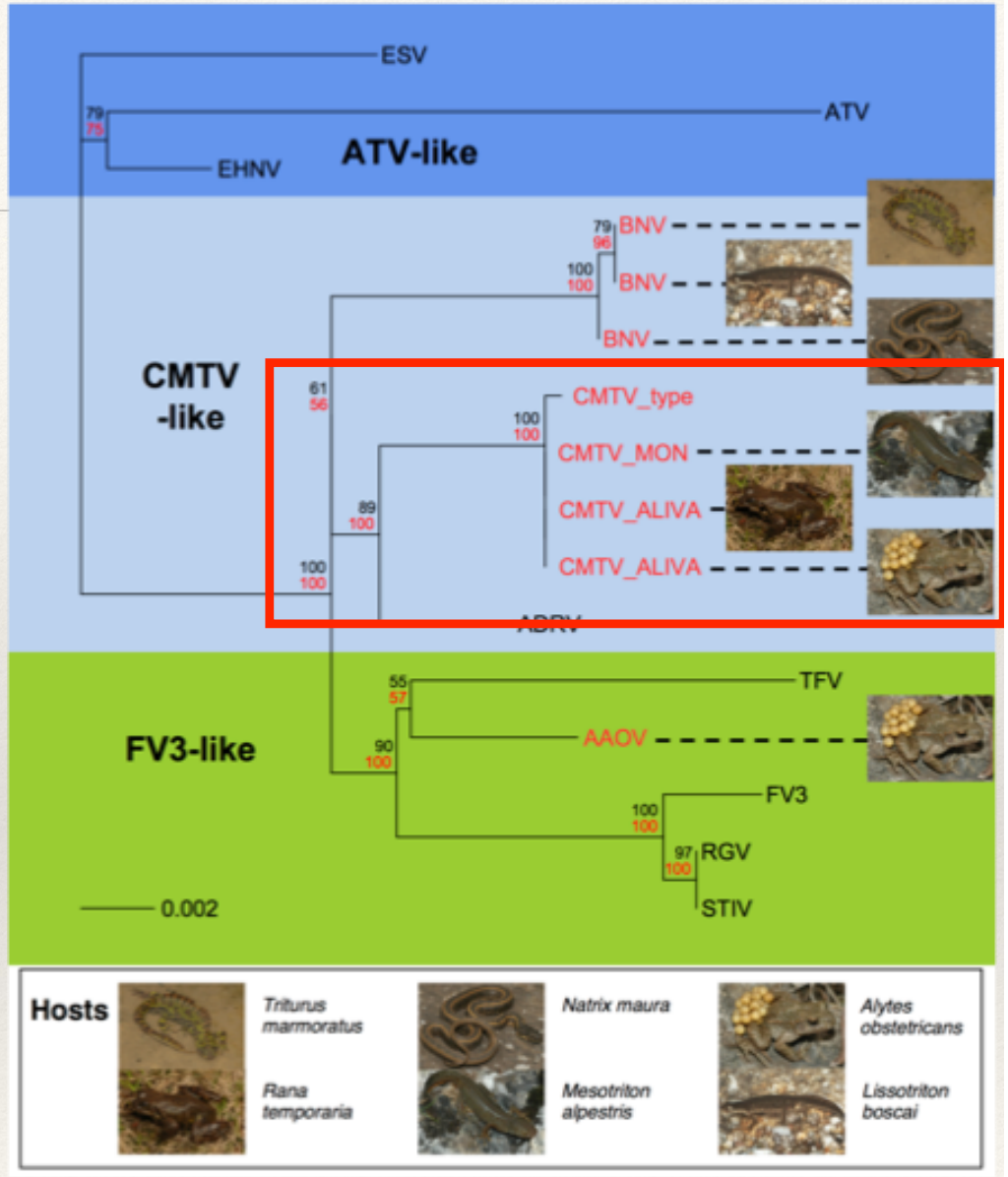

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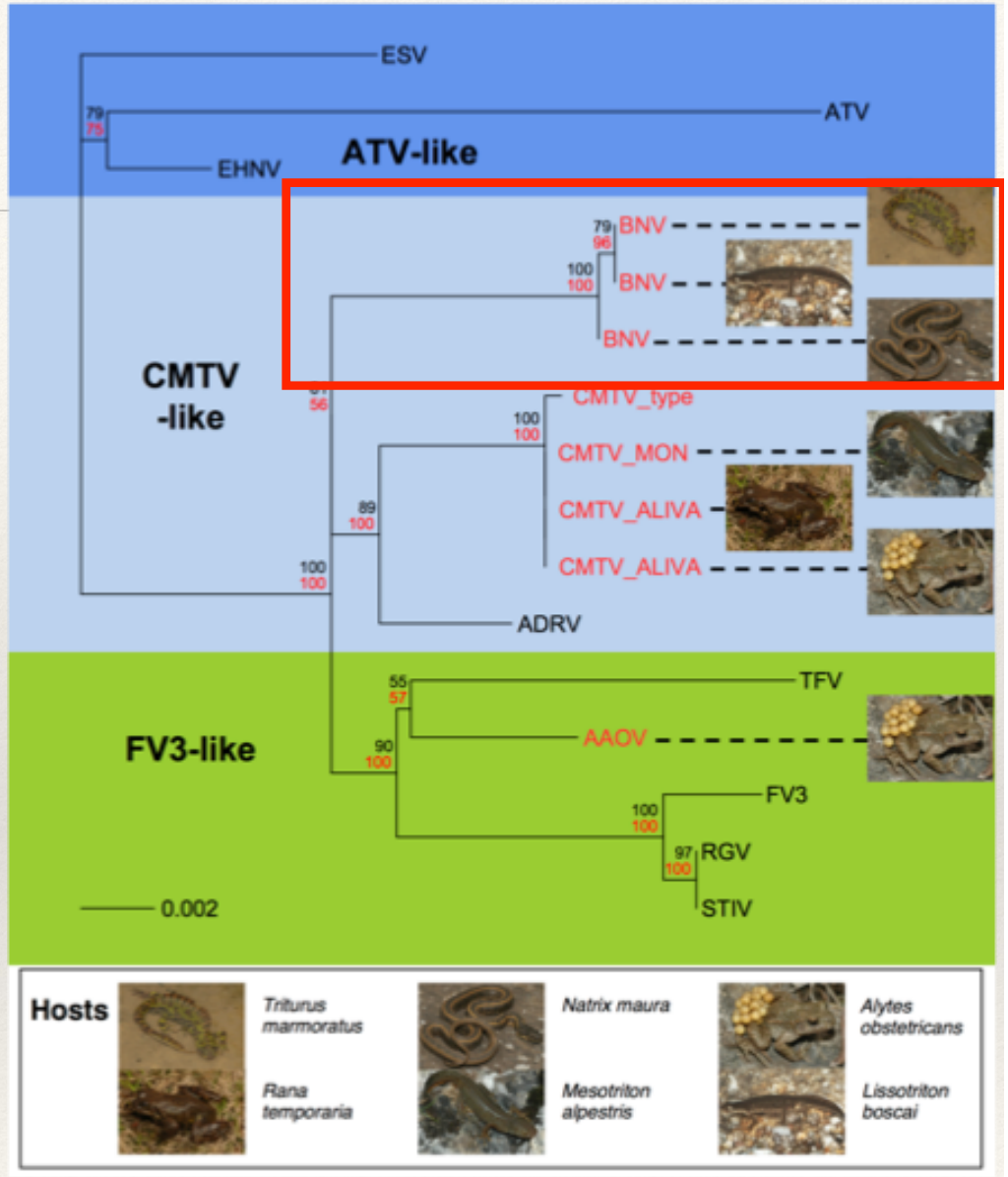

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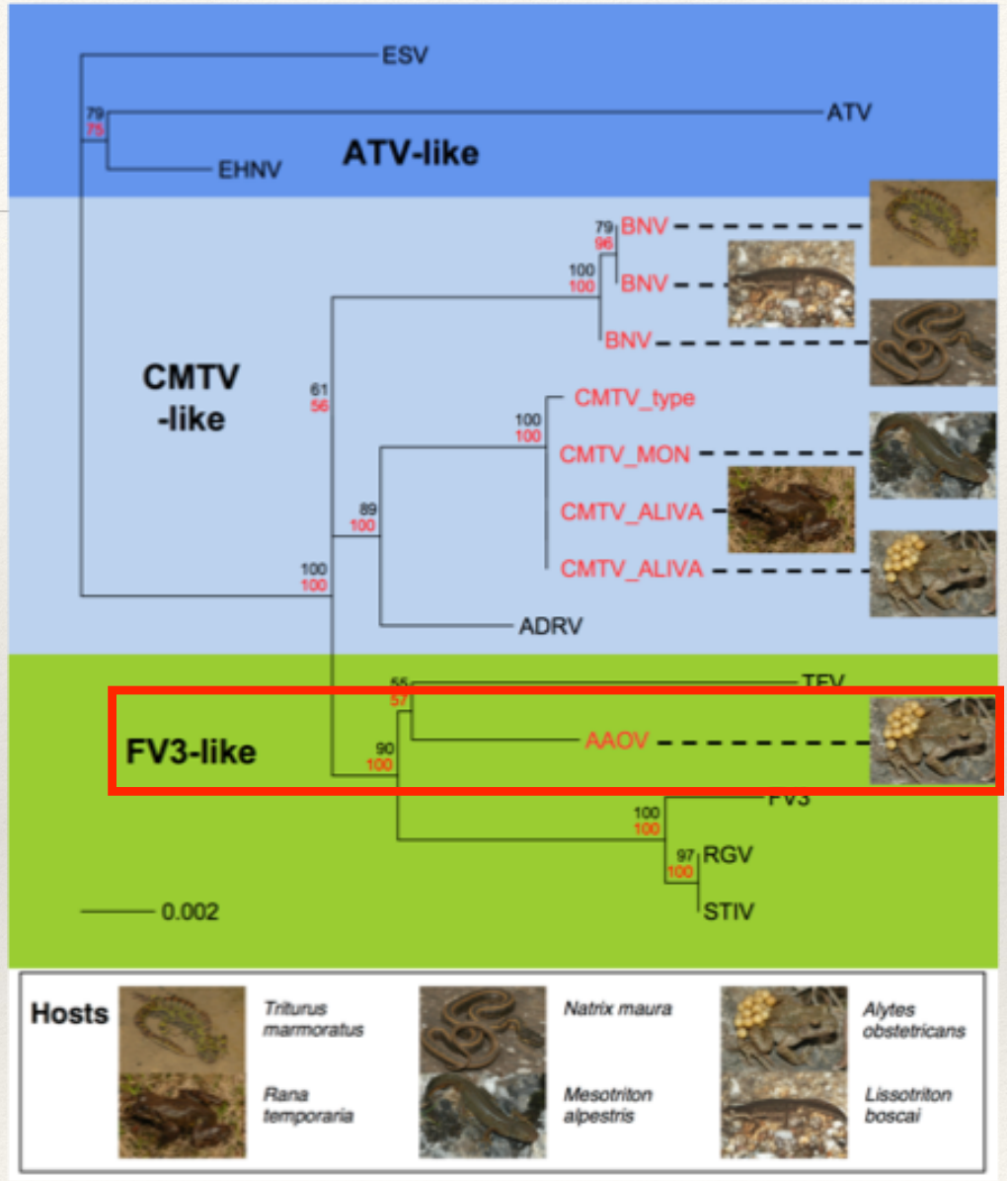

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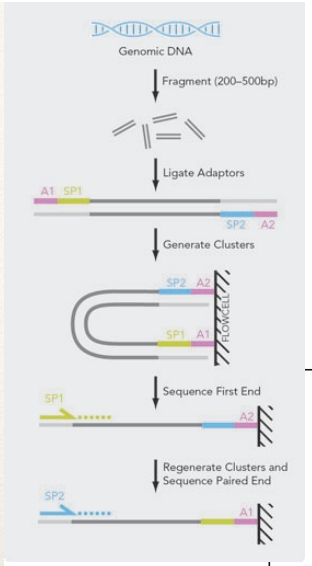
Current Biology

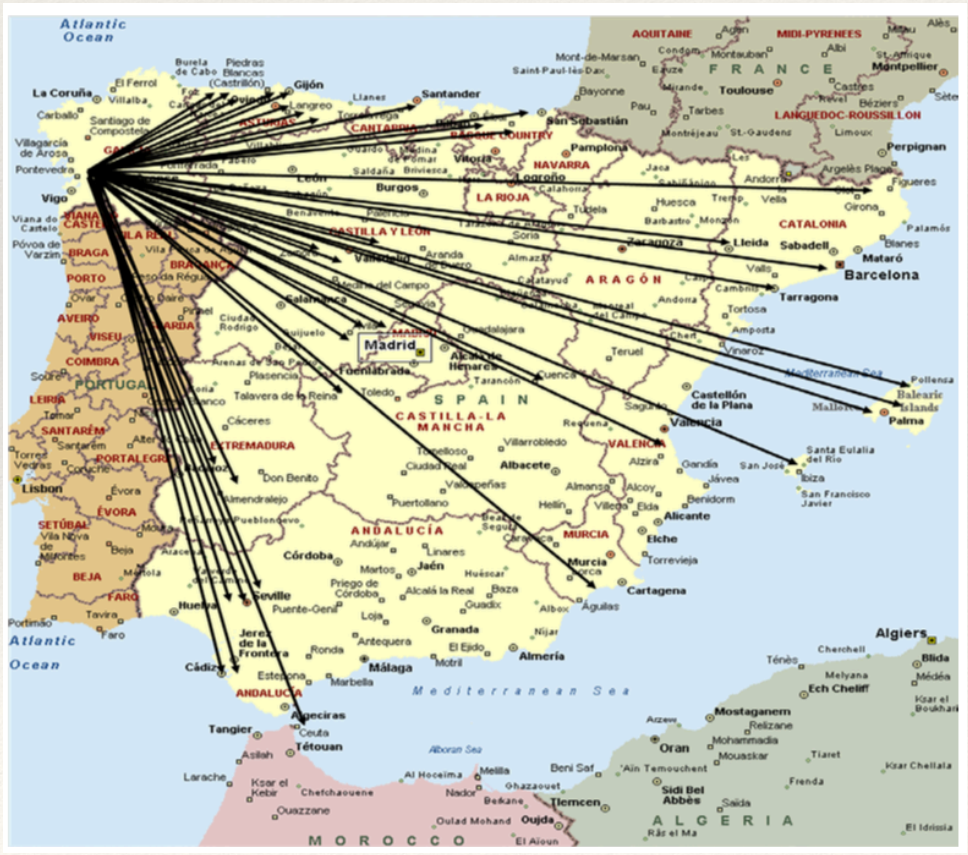


Reconstructing emergence in genomics age

- Next Generation Sequencing technologies (e.g. Illumina MiSeq & HiSeq) mean whole viral genomes now readily available
- Large amounts of data enable more accurate/sensitive phylogenetics, reconstruction of pathogen spread, study of local/host adaptation, and so on.
- Also can start to address questions about gene loss and gain, host range, recombination, etc.
- Difficult at the moment given the amount of data available but the inclusion of chinese giant salamander viruses (ADRV) in the CMTV clade is intriguing given the current reliance on farmed animals for sampling of these viruses.

Smoking guns…

- We've been looking for smoking guns in terms of vectors/routes facilitating global patterns of ranavirus infection for more than 20 years.



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Monday, 28 January, 2002, 14:04 GMT

Mystery virus ravages UK frogs

A common symptom in frogs affected by the virus is ulceration

By Alex Kirby
BBC News Online environment correspondent

Scientists say they are now certain that the disease which has killed millions of British frogs is caused by a virus.

The disease, which began in south-east England, has crossed the Scottish border, hundreds of kilometres to the north.

The animals' paws and feet drop off and they die a protracted and painful death. But money to research the outbreak and possible remedies has run out.

Tom Langton, the director of the Froglife Trust, told BBC Radio 4's Today programme: "It's worse than myxomatosis, the plague that devastated rabbit populations.

WATCH/LISTEN ON THIS STORY
Tom Langton
The virus is spreading north and has reached southern Scotland

See also:
- 31 Aug 01 | Sci/Tech
  Lizards and frogs enter Eden
- 02 Feb 01 | Health
  Frog spawn provides cancer clue
- 24 Jul 00 | Sci/Tech
  Amphibian decline 'has many causes'

Internet links:
- Frogs.Org
- Froglife
- Declining Amphibian Populations Task Force
- AmphibiaWeb
- Taskforce on Amphibian Declines and Deformities
- Institute of Zoology, London

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Humans: Bullfrogs & Ornamental fish

Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*)

Lisa M. Schloegel [a,d,*], Angela M. Picco [b], A. Marm Kilpatrick [a,c], Angela J. Davies [d], Alex D. Hyatt [e], Peter Daszak [a,d,*]

Journal of Fish Diseases 2011, 34, 159–166 doi:10.1111/j.1365-2761.2010.01224.x

Investigation of ornamental fish entering the EU for the presence of ranaviruses

T Vesely[1], K Cinkova[1], S Reschova[1], F Gobbo[2], E Ariel[3], M Vicenova[1], D Pokorova[1], P Kulich[1] and G Bovo[2]

Bull. Eur. Ass. Fish Pathol., 31(3) 2011, 112

NOTE

Common carp (*Cyprinus carpio)* and goldfish (*Carassius auratus*) were not susceptible to challenge with ranavirus under certain challenge conditions

B. Bang Jensen[1,2,4*], S. Reschova[3], K. Cinkova[3], E. Ariel[1,5] and T. Vesely[3]

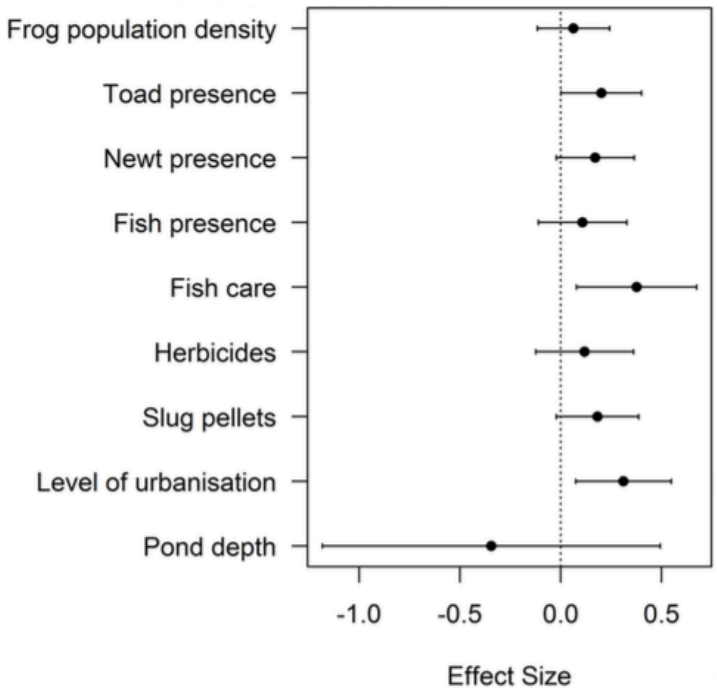


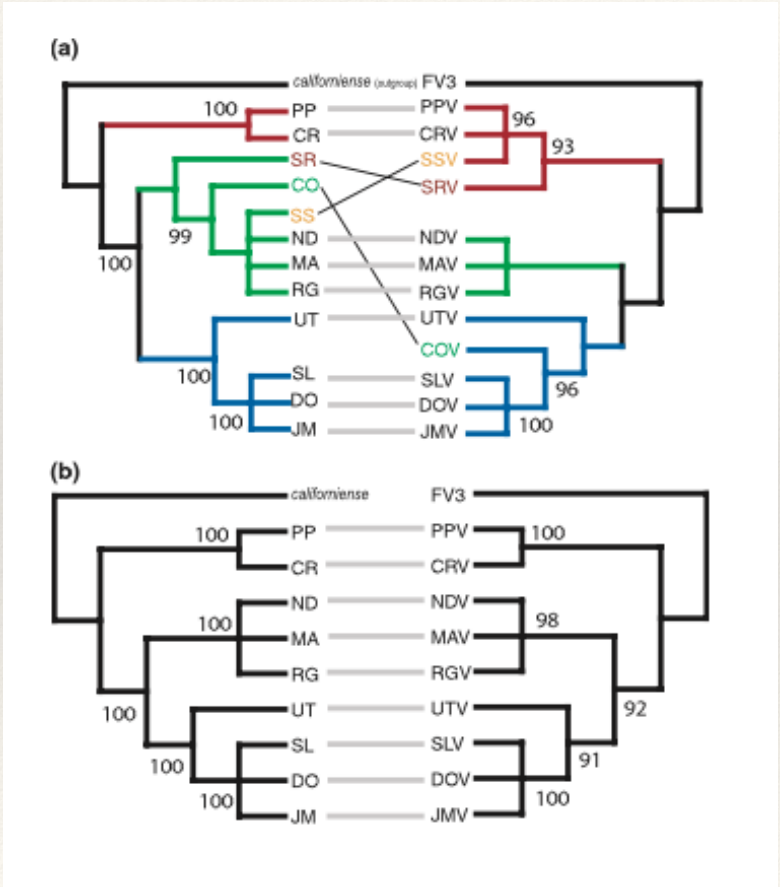
Fig 2. Abiotic and Biotic Variables Influencing Ranavirosis Occurrence. Model estimates and 95% confidence intervals for top ranking parameters ΔAIC <6 for ranavirosis occurrence (Criteria 1; [9]). Zero is indicated with a dotted line to demonstrate the importance of parameters in which confidence intervals do not overlap zero. Effect sizes above zero denote a positive relationship between each variable and ranavirosis occurrence.

Human links

Table. General linear models for the relationships of amphibian emerging infectious disease prevalence and anthropogenic variables

		Ranavirus		*Batrachochytrium dendrobatidis*	
Data point	Degrees of freedom	Mean squares	F value	Mean squares	F value
Intercept	1	0.06	8.47 ($p<0.05$)	0.05	4.61 ($p<0.1$)
Human disturbance	1	0.24	35.35 ($p<0.01$)	0.0009	0.08
Distance to road	1	0.03	4.11 ($p<0.1$)	0.03	2.82
Distance to industry	1	0.06	8.82 ($p<0.05$)	0.07	5.89 ($p<0.1$)
Distance to housing	1	0.06	8.08 ($p<0.05$)	0.06	4.87 ($p<0.1$)
Error	5	0.01		0.01	

Long evolutionary history with hosts?



Ecology Letters, (2007) **10**: 1075–1083 doi: 10.1111/j.1461-0248.2007.01102.x

LETTER

Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic

Andrew Storfer,[1]* Michael E. Alfaro,[1] Benjamin J. Ridenhour,[1] James K. Jancovich,[2] Stephen G. Mech,[1,3] Matthew J. Parris[4] and James P. Collins[5]

[1]School of Biological Sciences, Washington State University, Pullman, WA 99164-4236, USA

[2]Molecular and Cellular Biology Program, School of Life Sciences, The BioDesign Institute, Arizona State University, Tempe, AZ 85287-4601, USA

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Abstract

Distinguishing whether pathogens are novel or endemic is critical for controlling emerging infectious diseases, an increasing threat to wildlife and human health. To test the endemic vs. novel pathogen hypothesis, we present a unique analysis of intraspecific host-pathogen phylogenetic concordance of tiger salamanders and an emerging *Ranavirus* throughout Western North America. There is significant non-concordance of host and virus gene trees, suggesting pathogen novelty. However, non-concordance has likely resulted from virus introductions by human movement of infected salamanders. When human-associated viral introductions are excluded, host and virus gene trees are identical, strongly supporting coevolution and endemism. A laboratory experiment showed an introduced virus strain is significantly more virulent than endemic strains, likely due to artificial selection for high virulence. Thus, our analysis of intra-specific phylogenetic concordance revealed that human introduction of viruses is the mechanism underlying tree non-concordance and possibly disease emergence via artificial selection.

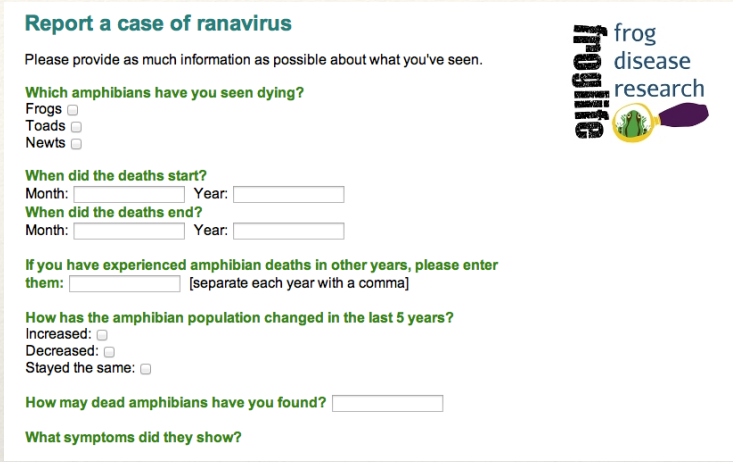
- Traditional view of dsDNA viruses is that they evolve slowly (for viruses) and speciate alongside hosts
- Support for this scenario in ranavirus evolution - phylogenetic concordance of Ambystomid salamanders and ATV
- But this pattern could be explained in other ways and at the least there appears to be a more modern layer of host-pathogen mixing

Are we doing enough?

The Frog Mortality Project

- flagship Citizen Science project now consumed by Garden Wildlife Health
- public participation in disease surveillance and data collection
- > 5000 reports
- > 2 decades of data with collection starting in 1992
- Includes georeference data and information about the mortality event but also contains masses of covariate data including info on pond, management and setting.

http://www.froglife.org/disease/fmpform.aspx



Report a case of ranavirus

froglife frog disease research

Please provide as much information as possible about what you've seen.

Which amphibians have you seen dying?
Frogs ☐
Toads ☐
Newts ☐

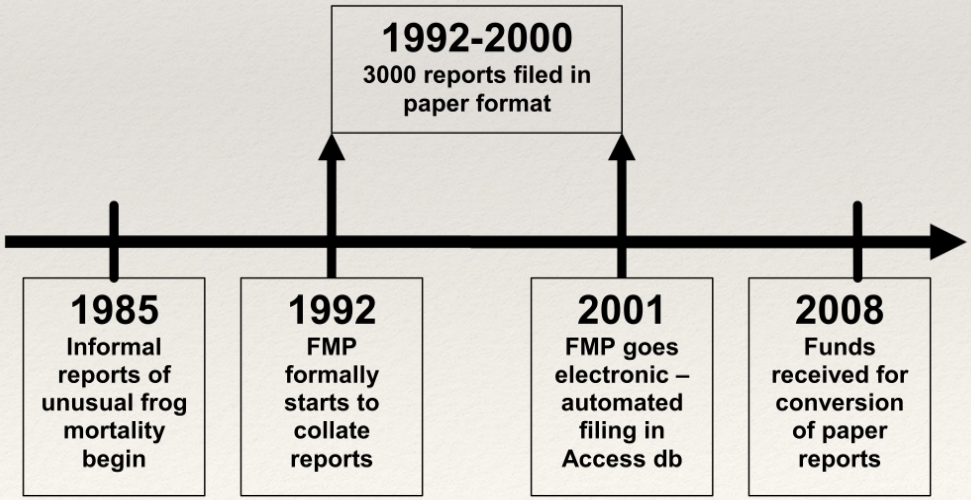
When did the deaths start?
Month: _____ Year: _____
When did the deaths end?
Month: _____ Year: _____

If you have experienced amphibian deaths in other years, please enter them: _____ [separate each year with a comma]

How has the amphibian population changed in the last 5 years?
Increased: ☐
Decreased: ☐
Stayed the same: ☐

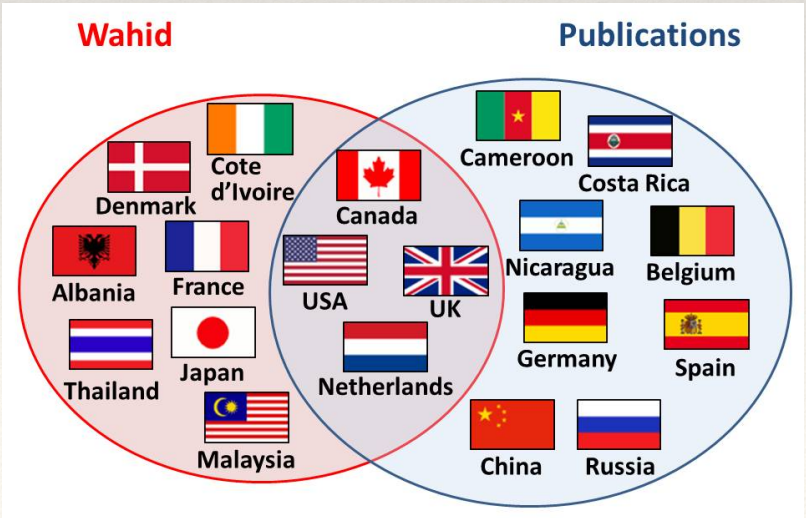
How may dead amphibians have you found? _____

What symptoms did they show?

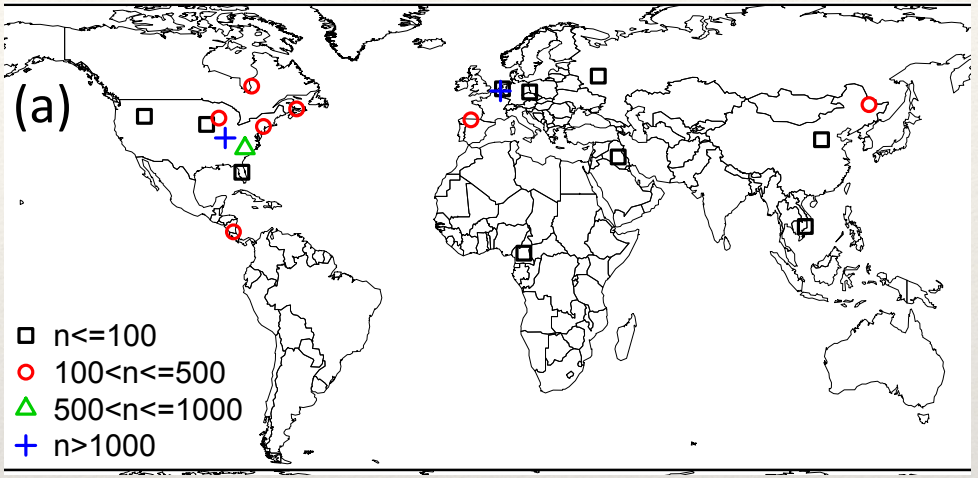


Global surveillance

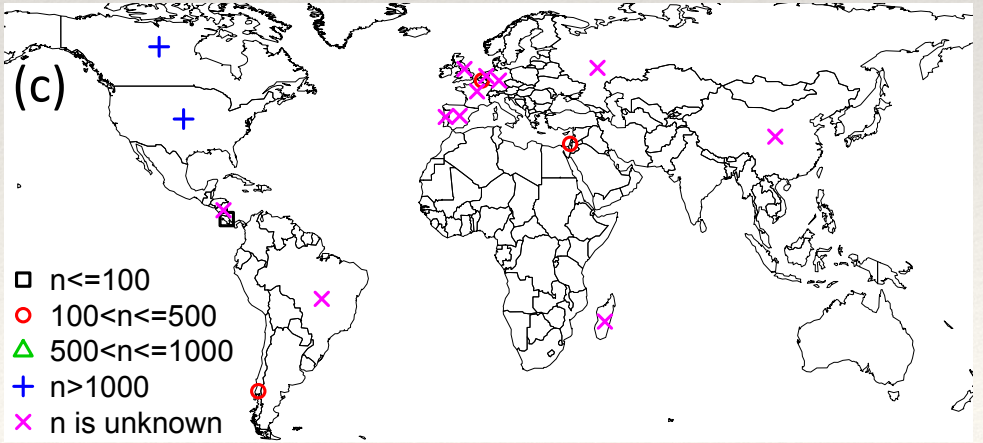
- At both global and local scales surveillance for ranaviruses is extremely patchy and resultant view of rv distribution is highly skewed by sampling bias
- Current reporting mechanisms (OIE + academic literature) are also inadequate.



Black et al., in prep



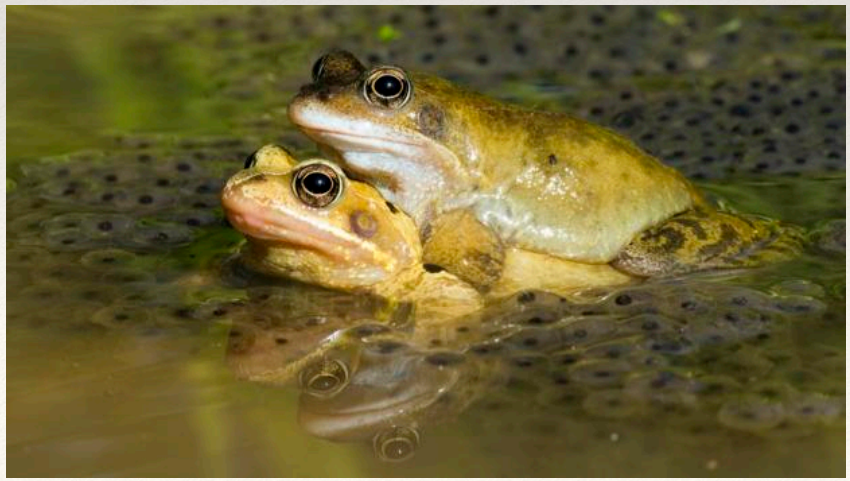
Sampling of amphibians for Ranaviruses based on data from a) published literature c) survey of labs (2012-2014)



Something to take home!

How do hosts respond?
Through genetic and behavioural adaptation…

- Teacher et al. (2009) looked at MHC types (and microsatellites as a neutral control marker) in both 'diseased' and 'uninfected' populations of common frogs. They found evidence that the presence of ranavirus disease may select for less susceptible MHC haplotypes, suggesting that frogs may be adapting to ranavirus infection.



- Using microsatellite markers, the same authors also found evidence for assortative mating in common frog populations faced with ranavirus; uninfected frogs are more likely to mate with other uninfected frogs. Ways that this could occur are if infected (and diseased) animals were less able to compete for mates or if frogs were engaging in active mate choice.

Links & resources

- OIE on ranavirus
  - http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/Ranavirus_card_final.pdf
- Garden Wildlife Health
  - http://www.gardenwildlifehealth.org
- Froglife
  - http://www.froglife.org/disease/ranavirus/
- ARC
  - http://www.arc-trust.org
- Stephen Price; UCL/QMUL/ZSL
  - 2infectious.wordpress.com

Collaborators & Funders

- Francois Balloux
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- Amparo Mora-Cabello de Alba
- Amber Teacher
- Alexandra North
- Dave Hodgson
- Chris Ruis
- PNPE park rangers

NERC, SynTax, DEFRA, ERC

References

Thanks for listening!

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https://2infectious.wordpress.com

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