

Viral threats of percid farming

laurent.bigarre@anses.fr

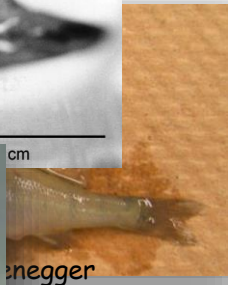
Infectious risks: higher and higher

↗ Increase of farming

- ↗ Trade = worldwide circulation of virus
- ↗ stress = susceptibility of host
- Mix of species = inter-species transmission
- Diagnostic tools poorly developed
- Sanitary measures insufficiently used

Known viruses

- + • Iridovirus (Rana- and Lymphocysti-)
- + • Betanodavirus
- • Retroviruses
- • Herpesvirus 
- Rhabdoviruses
- + • Novirhabdovirus : VHSV (notifiable virus !)
- ± • Perhabdoviruses (previously 'vesiculo')



Diagnostics: the methods

Cell culture



- Screen a wide range of viruses (but not all)
- Sensitive (may take weeks)
- large production of virus, facilitates genetic characterisation (sequencing, etc.), serological reactants
- National reference laboratories in Europe can perform it

- Needs well-preserved samples
- If virus-positive, no or limited identification
- Moderately useful for screening genitors
- Possible toxicity with larvae

Diagnostics: the methods



Molecular methods (cPCR and real-time PCR)

- (very) specific targets
- Rapid (1 day)
- Long time conservation of the sample (-20°C)
- Accurate genetic identification (sequencing)

- insufficiently developed ... yet (in progress)
- to date, not adapted for screening genitors
- will be moderately adapted (real-time PCR)

Diagnostics: an overview



Cell culture

Fresh tissue
(kidney, liver, spleen, brain)

Screen many viruses
Time consuming
No genetic information



genetic test

✓ Fresh tissue
✓ Frozen
✓ RNAlater

Specific of some viruses
Rapid
Molecular identification

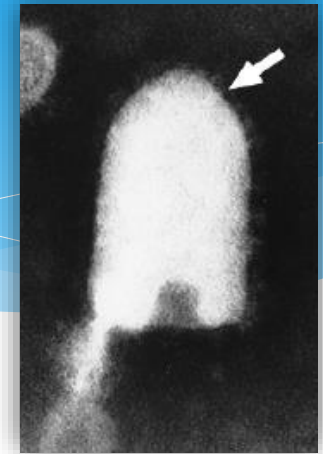
- When possible, **test different complementary methods** CC + PCR !
- Test sick **and healthy** fish

Diagnostics: Take-home messages

- The perfect tool DOES NOT Exist
- Care of the samples for expedition
- For sick fish: Cell culture or PCR, or better: both
- For healthy fish:
 - No serological test !
 - Try cell culture / PCR, but false negative are possible

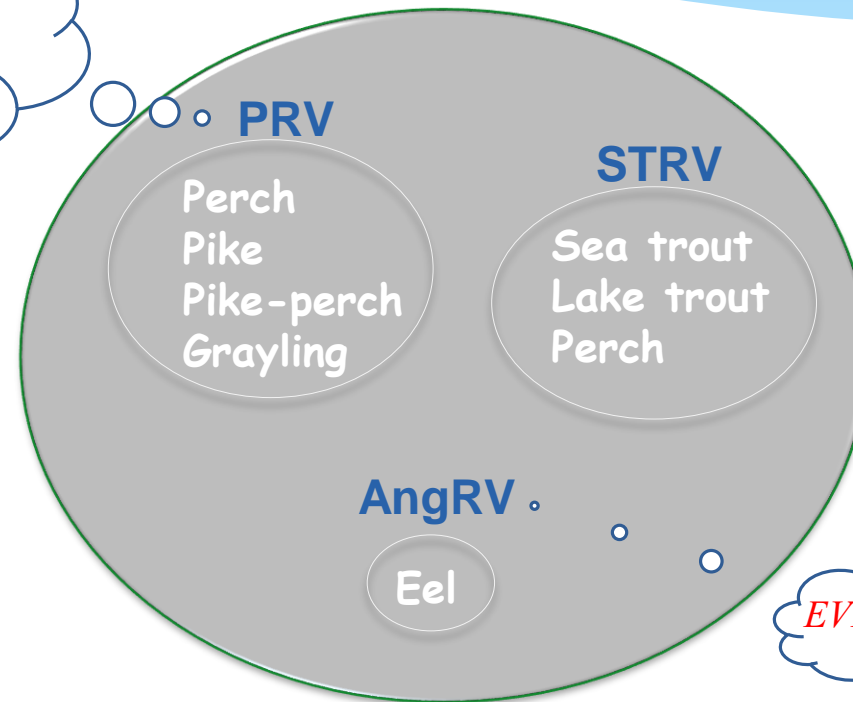
Percid Perhabdoviruses

Classification of the Perhabdoviruses (2018)



Betts 2003

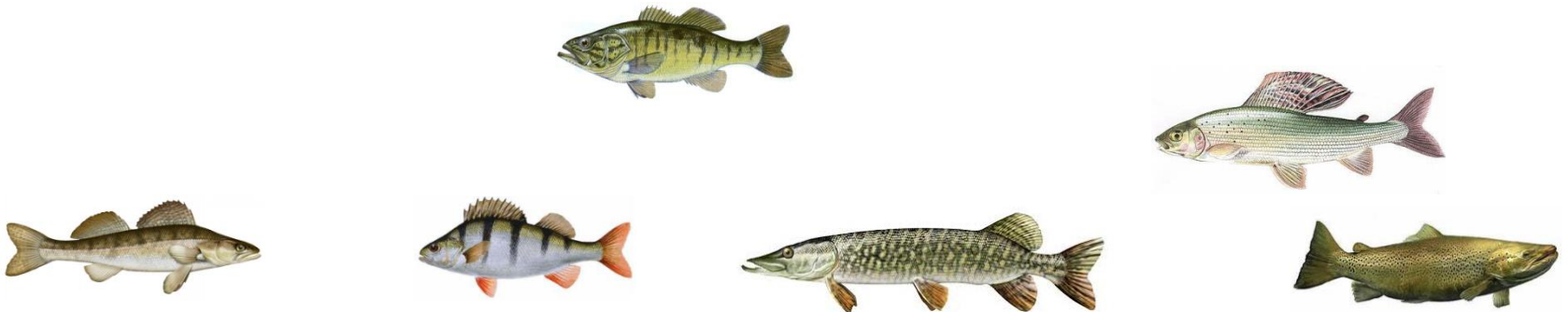
*No confusion with
piscine reovirus*



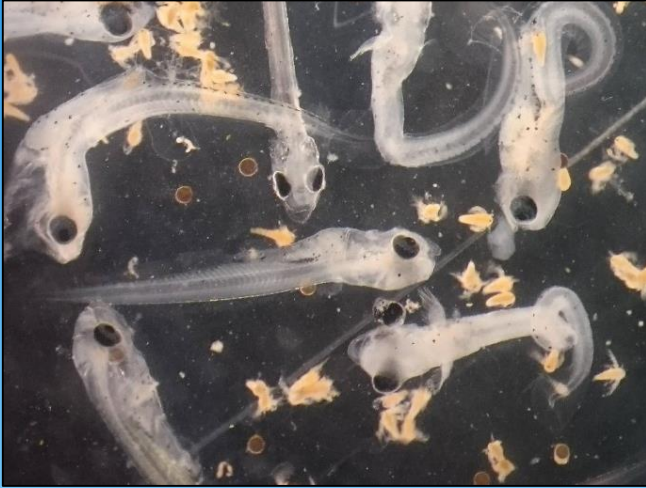
EVEX, EVA

Percid perhabdoviruses (PRV and STRV)

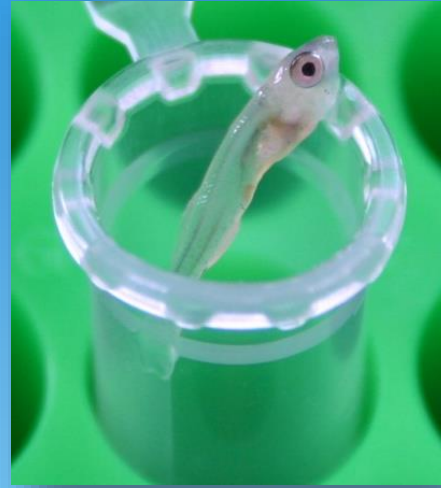
- Discovered in France in 1980 (perch)
- High genetic diversity (more to characterize)
- Host range limited (really ?)



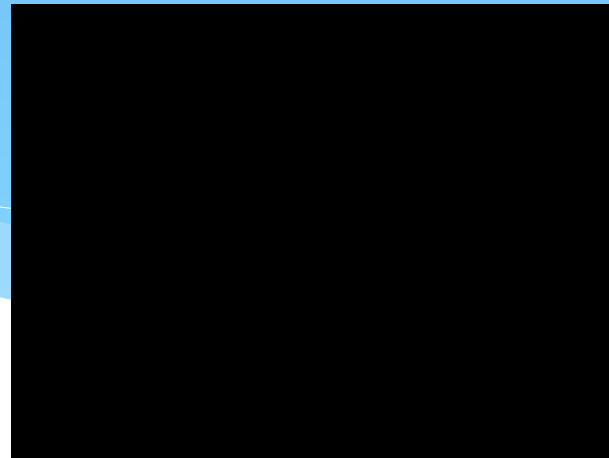
PRV/STRV associated with fish mortalities at various stages



Pike-perch

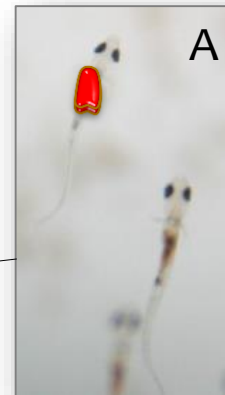
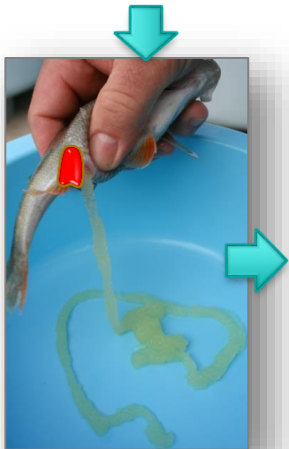


Perch



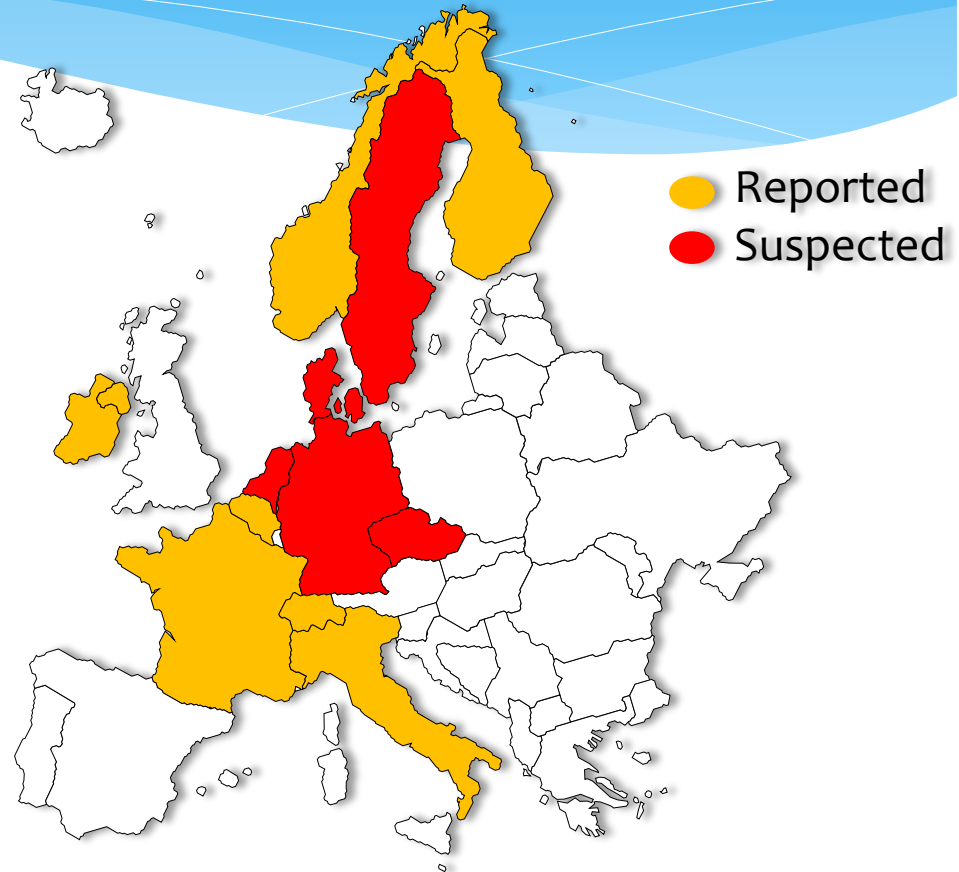
Transmissions

- Pseudo-Vertical (genitors to fry)
- Horizontal (between fish, tanks, farms)



Epidemiology of Percid perhabdoviruses (PRV and STRV)

- **First outbreaks: fish from the wild introduced in facilities**
- **Nowadays:**
 - **farm-to-farm**
 - **wild**



Kinetics of disease

- Variable ! A few days to several weeks
- Depends of age, species, virus, T°C, other factors

Wild perch introduced in facilities

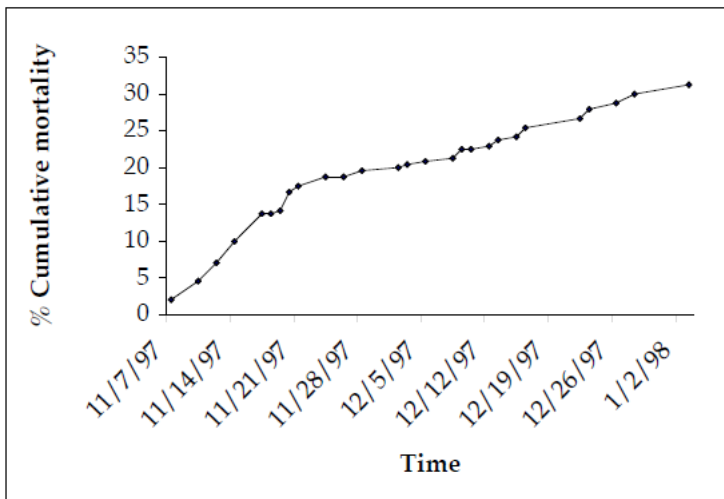


Fig. 1. Cumulative mortality (%) during the growth experiment (data from 3 tanks containing 80 individuals each are included).

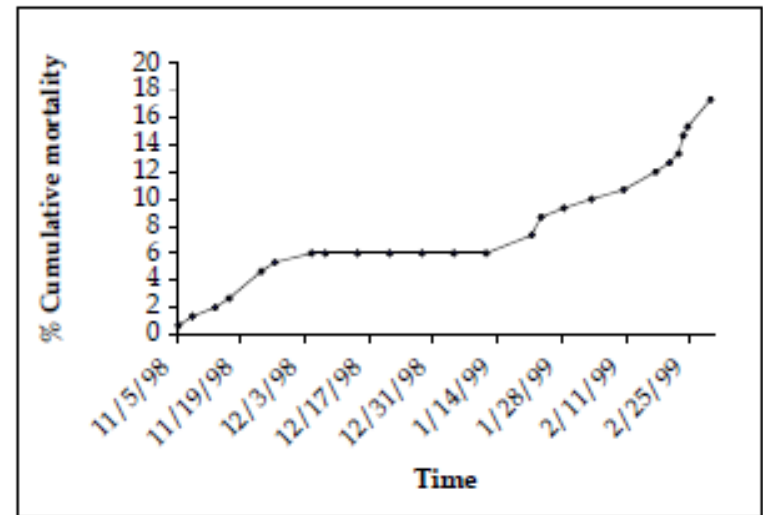


Fig. 2. Cumulative mortality (%) during the reproduction experiment (n=150).

Control of perhabdoviruses


- Prevent introduction
 - Desinfect eggs (as much as possible; tricky for perch)
 - Quarantine
 - Diagnostics (still to improve and disseminate)
- Identify and eliminate infected animals (sick and healthy carriers)
- Elucidate the origin = prevent re-introduction
-> molecular tracing

Tracing perhabdoviruses within Europe



Molecular tracing: why and how ?

- ISSUE: Obtaining the genomic sequence of a virus
- WHY ? To understand its origin : host, geography, provider, etc.
- HOW ? To compare the sequence of a virus to **published** viruses with available epidemiological data (country, host species, year): **in progress**
- To compare the sequences of 2 viruses from 2 outbreaks: are they the same or not ?



Molecular tracing provides only clues. Needs of communication with stakeholders to understand what really happened.

A leaflet to disseminate

Percid perhabdoviruses: a threat for fish farming !

A group of potentially devastating pathogens

- A group of viruses :
 - 1 genus: perhabdoviruses
 - 2 species which infect percids (more to discover...)
 - Perch perhabdovirus (PRV=PRkV)
 - Sea trout perhabdovirus (STRV)
- Various fish hosts (humans are not affected)
- Widespread in Europe (farms and wild)
- Symptoms seen in clinical cases:
 - rapid death of fish larvae (up to 100% < 1 week)
 - chronic mortalities over months for adult fish
 - hemorrhages on the fins
 - desequibrated swimming
- The virus may be transmitted
 - vertically (parent fish to eggs)
 - horizontally (fish to fish, via contact or water)
 - by fomites
- Infected fish may die or survive, and then still carry the virus, with or without symptoms.
- Survivors may transmit the virus to other naive fish.



Disease control

- Disinfect eggs (iodophore, aldehydes): efficient on pike-perch, but take care to avoid toxicity ! Very tricky with perch.
- Only introduce 'certified' virus-free fish !
- Quarantine newly introduced fish !
- Identify causes of disease in production and quarantined fish (diagnostic by vet + lab)
- Rapidly cull all fish (sick and healthy) from infected batches and other batches in contact (water equipment, etc.)
- Disinfect fomites and water (UV, chlorine, etc.)
- Reduce production stress of fish

Laboratory diagnostics

- ✓ Cell culture + Immunofluorescence (better with fresh organs)
- ✓ Cell culture + PCR (better with fresh organs)
- PCR on tissues (brain, kidney), fresh or frozen: methods in progress



Conclusions

- Various viral families are threats. More will emerge !
- Rhabdos are the major risks
- Each fish movement is a risk of virus spread
- Always send samples to labs when sick fish... and test healthy as well **periodically**
- Diagnostics methods exist and are in progress. Contact you national ref. lab. or others