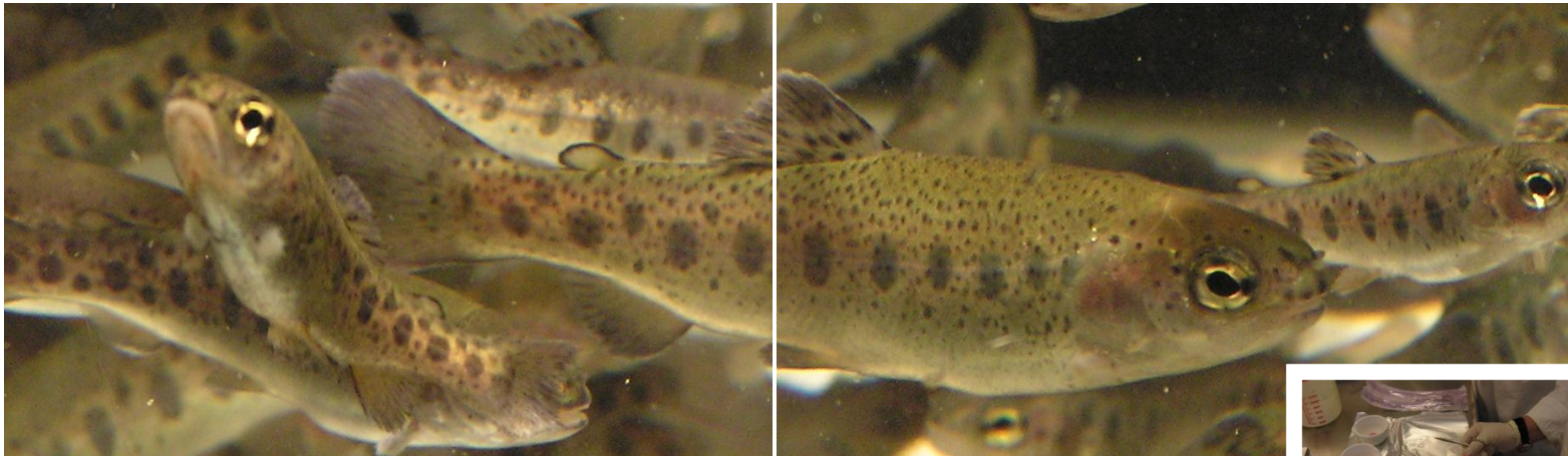


# Viral Haemorrhagic Septicaemia (VHS): clinic, virus and distribution,



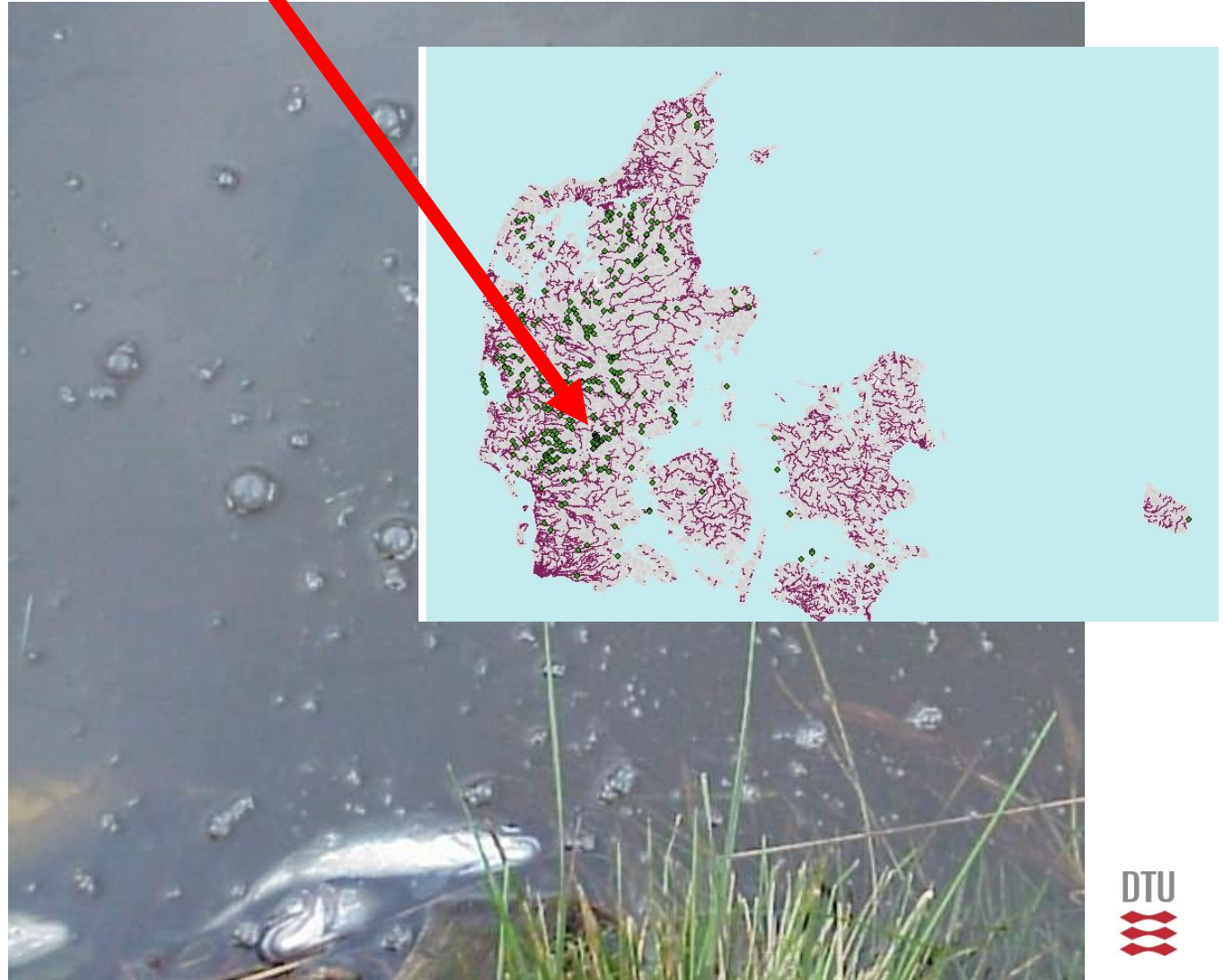
Niels Jørgen Olesen

Exercise Trout  
Bergen 4.12.2013



# VHS - Egtved Disease - first isolation in 1962

VHS outbreak



**Clinical signs:** lethargy  
exophthalmia  
erratic swimming



**VHS infected rainbow trout**

**Notice pale gills and pettechial haemorrhages**



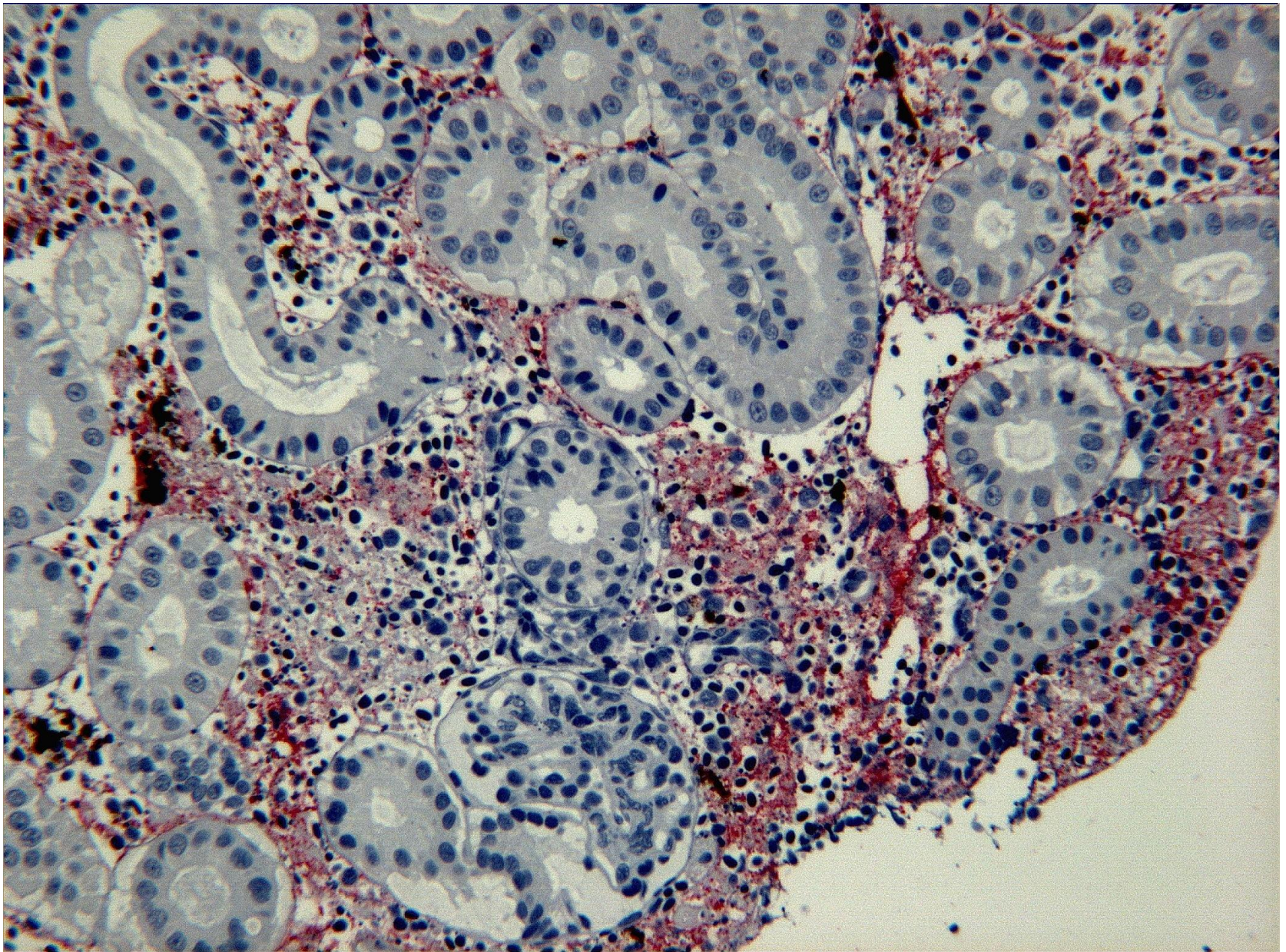
- Swollen spleen and kidney
- Pale gut
- Haemorrhages in adipose fat



Pettechial  
haemorrhages in  
muscle

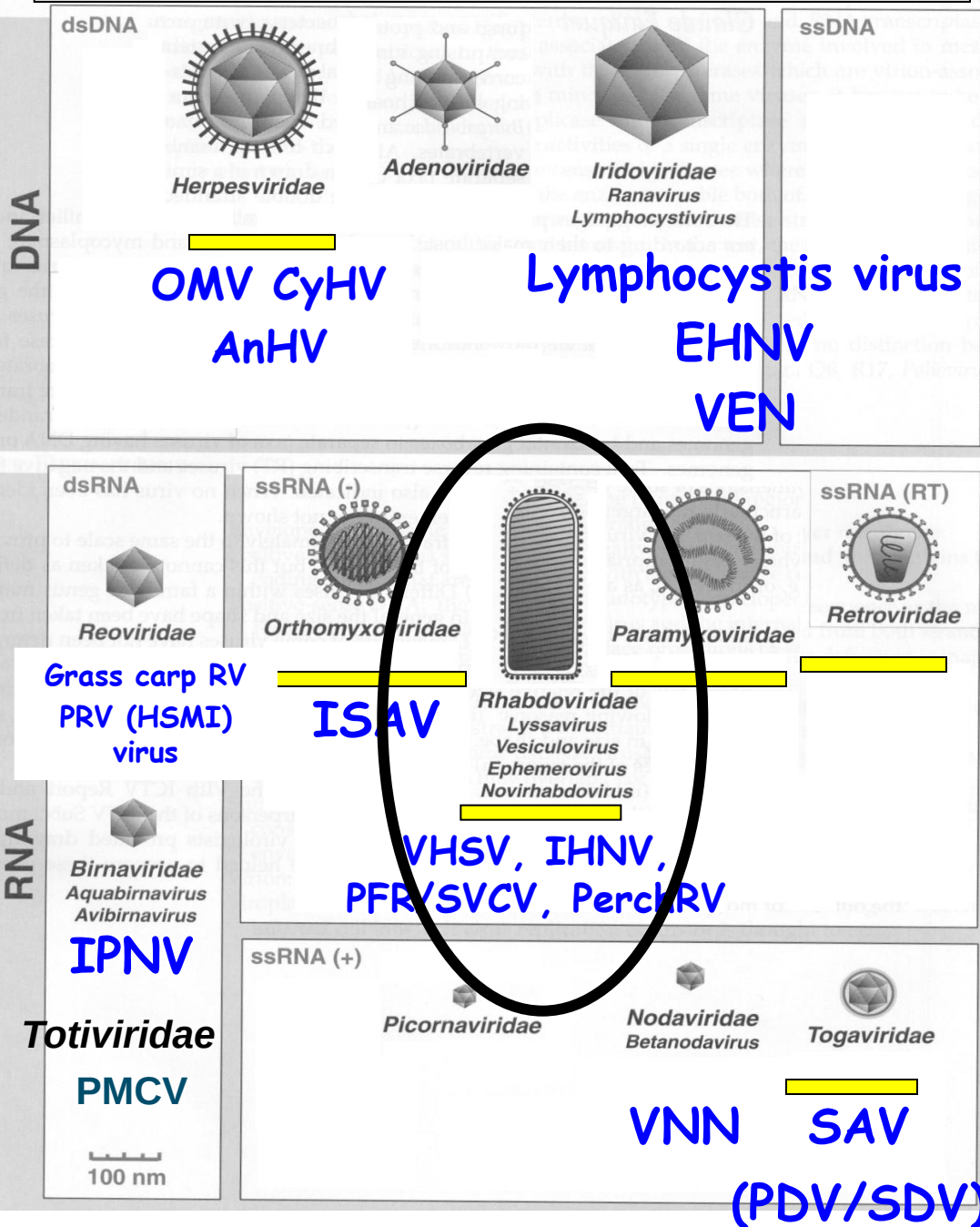


VHS in rainbow trout



**VHS infected kidney with endothelial cell staining  
(Photo: Ellen Lorenzen)**

# Families and Genera of Viruses infecting fish



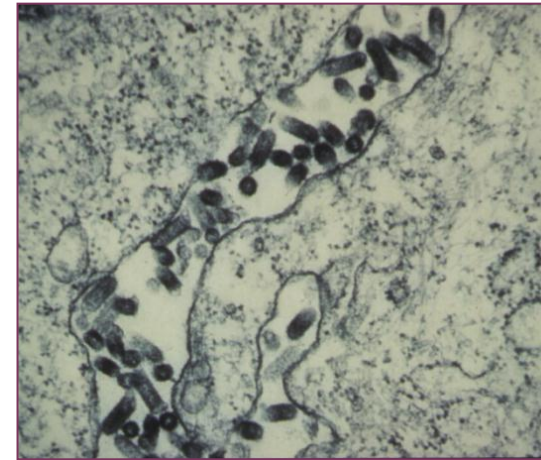
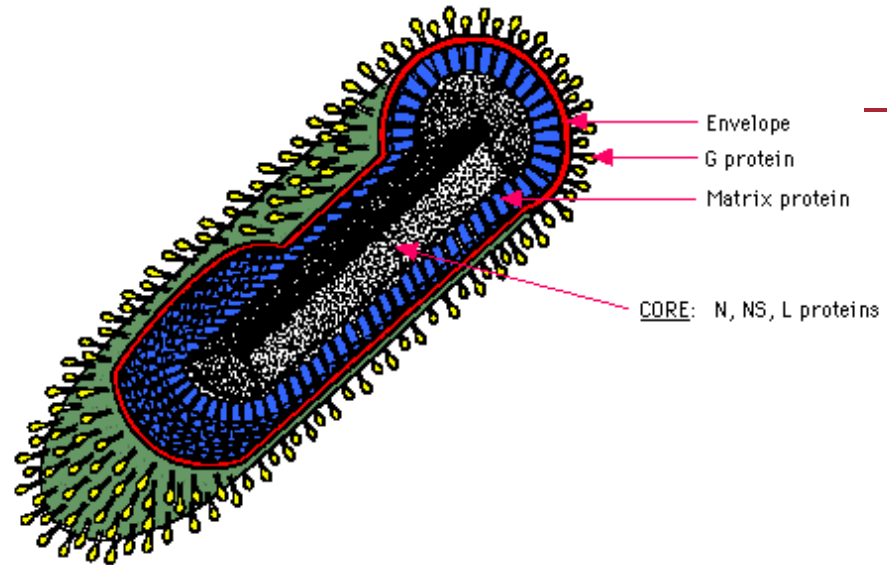
## Rhabdoviridae



# VHS virus

9

Family: Rhabdovirus, Genus: *Novirhabdoviridae*



- Single stranded, negative-sense RNA genome - 11,1 kb

3' | **N** | **P** | **M** | **G** | **NV** | **L** | 5'

Nucleocapsid      Glycoprotein

Phosphoprotein      Non-virion protein

Matrix Protein      (L) Polymerase protein



# Diagnostic method

Journal of Fish Diseases 2012

doi:10.1111/j.1365-2761.2012.01416.x

## **Development and validation of a novel Taqman-based real-time RT-PCR assay suitable for demonstrating freedom from viral haemorrhagic septicaemia virus**

**S P Jonstrup, S Kahns, H F Skall, T S Boutrup and N J Olesen**

Section for Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Technical University of Denmark, Århus N, Denmark

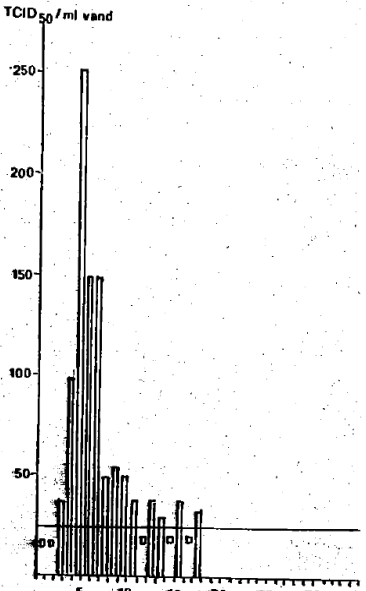
# **VHS virus stability**

**Disinfection.**

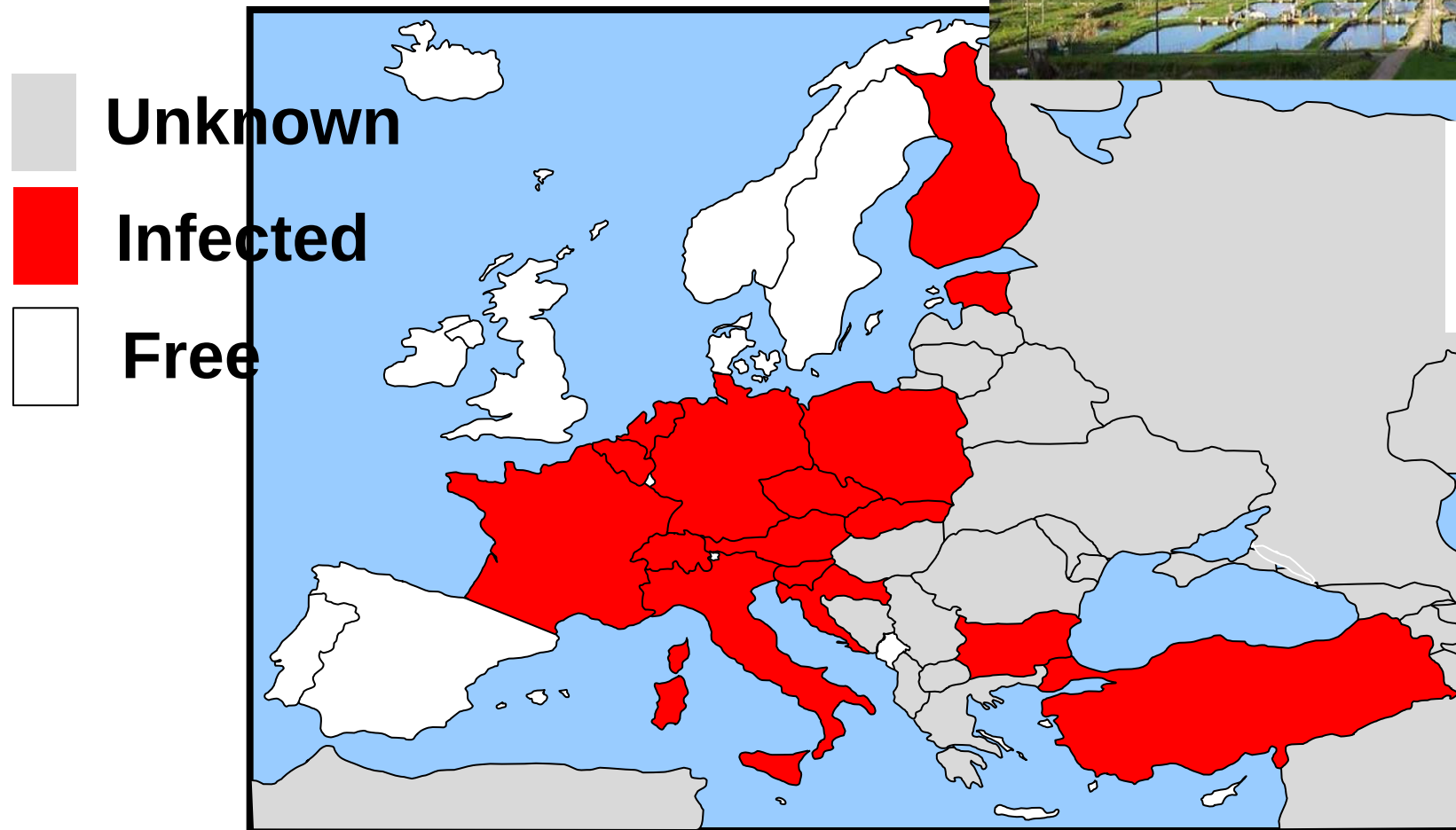
**Iodine, formalin, VirkonS, UV, Ozone  
Temperature**

**Horizontal transmission**

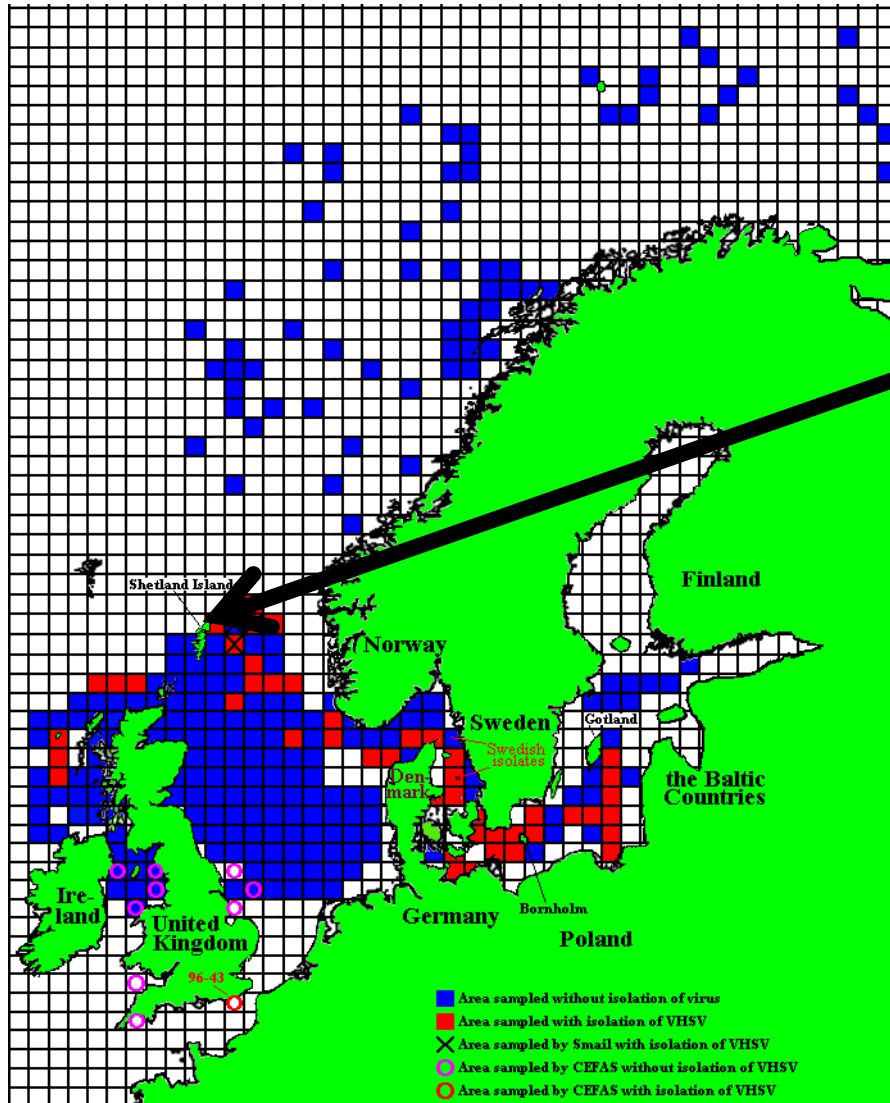
# Lapse of time for VHS infection

|                              | Incubation phase              | Disease                                                                             | Reconvalescence phase |   |
|------------------------------|-------------------------------|-------------------------------------------------------------------------------------|-----------------------|---|
| Virus detection              | Virus propagation<br>— + + +  |  | elimination<br>+ —    |   |
| Danger of infection          | Incubation phase<br>— + + + + |                                                                                     | Carriers<br>+ + (+)   |   |
| Duration<br>Infection trials | 5 - 42 days                   |                                                                                     | days                  | U |
| Duration<br>Fish farms       | 5 - 180 days                  |                                                                                     | 14 - 105 days?        | U |

# VHS in Europe



# From local to global discovery of VHS: VHS in the marine environment





# H/S Dana

**FP5 EU project**

**Collaboration between  
Scotland, Norway and  
Denmark**

**National Veterinary Institute**

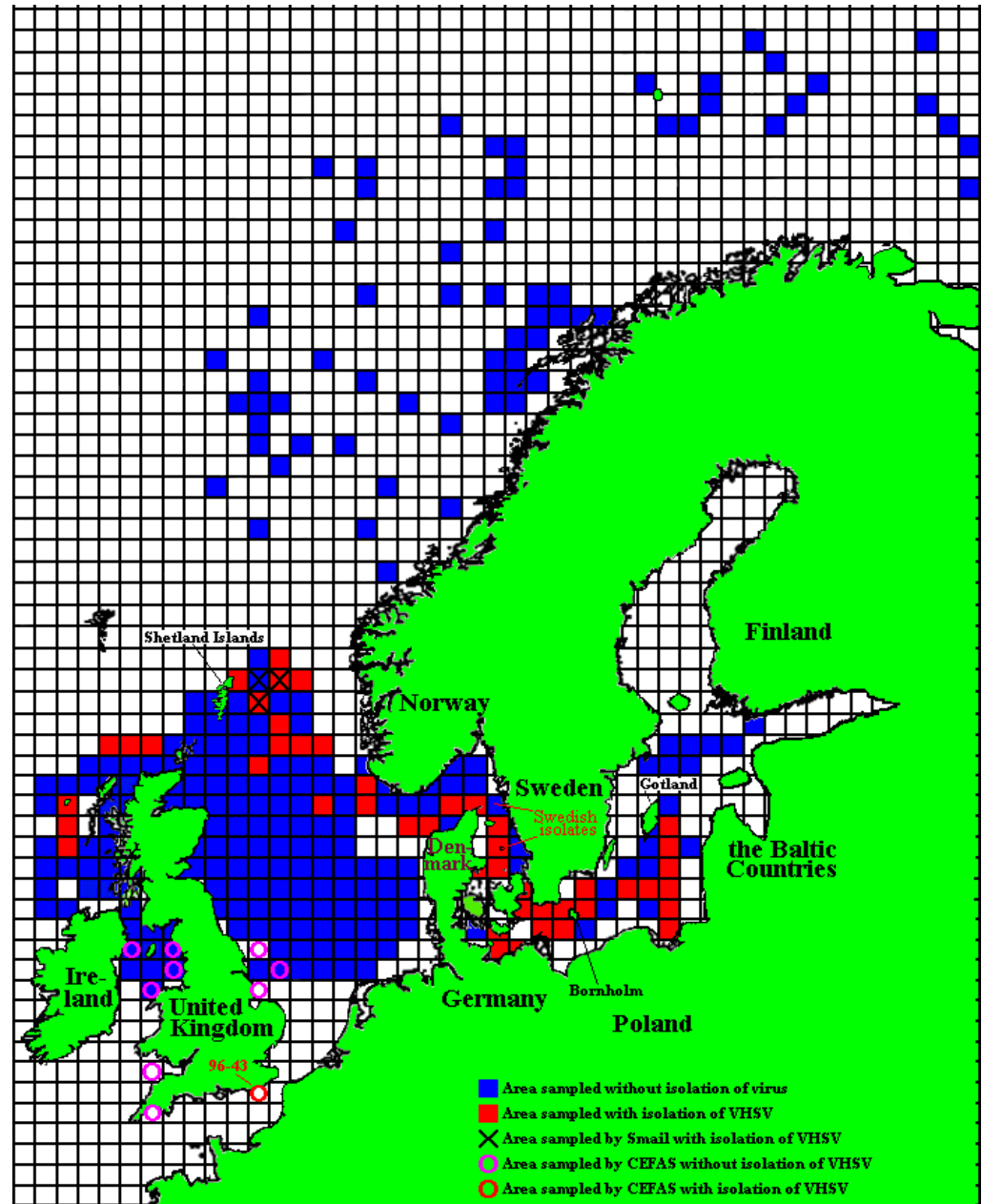


# Map showing the ICES squares sampled

**VHS virus  
isolation**

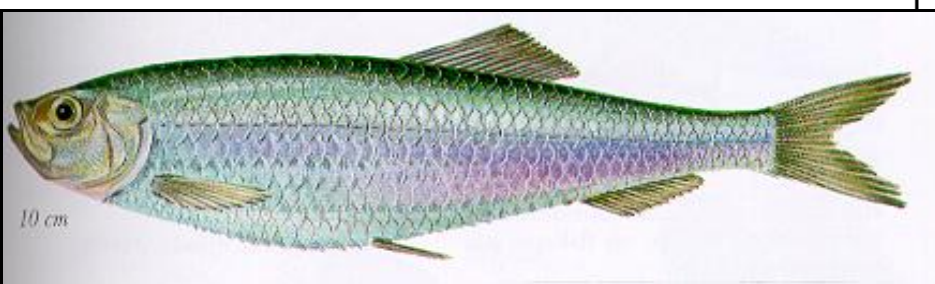
**No VHS virus  
isolation**

National Veterinary Institute

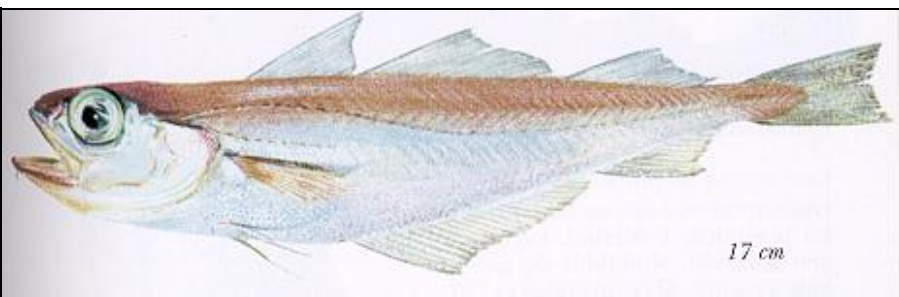




**Herring (sild)**

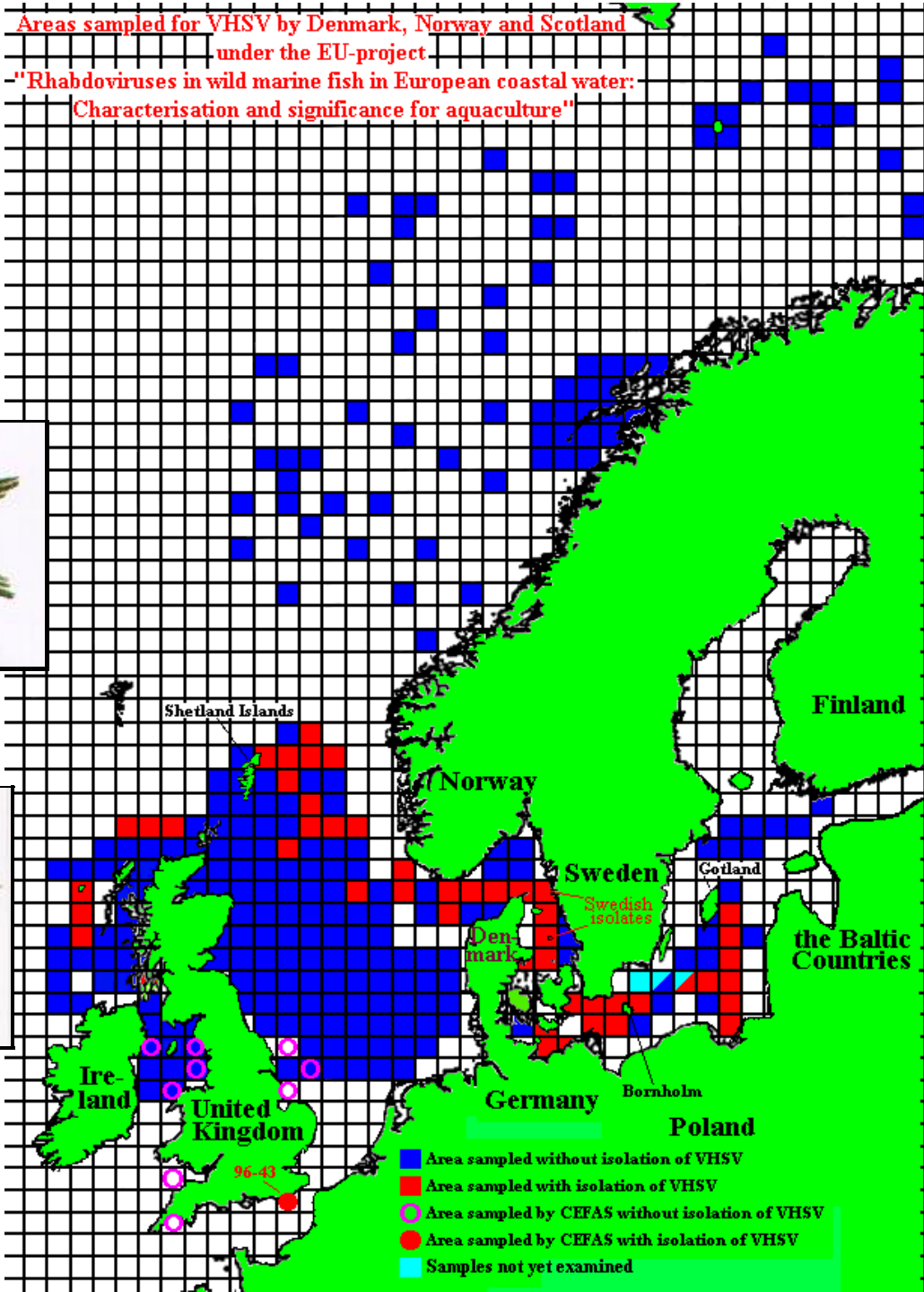


**Sprat (brisling)**

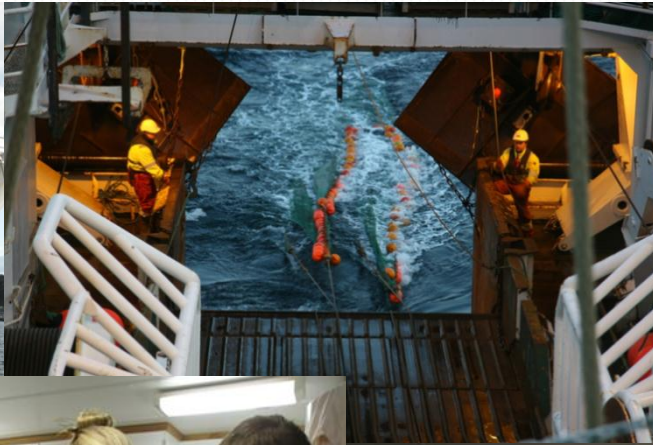


**Norway pout (sperling)**

National Veterinary Institute

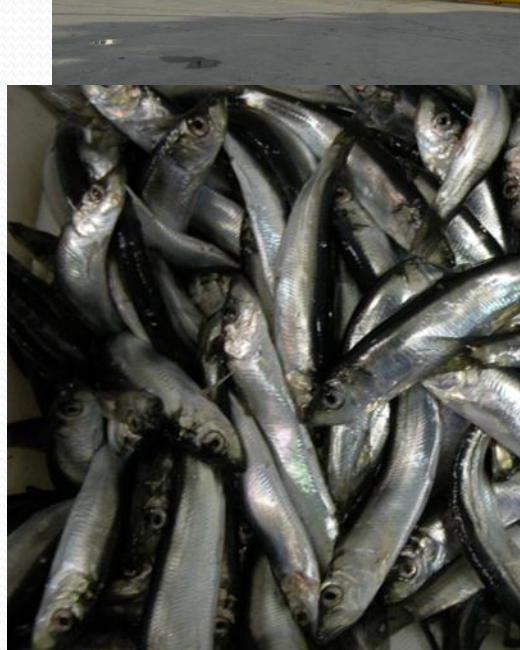


# Sampling 2700 fish of 43 species



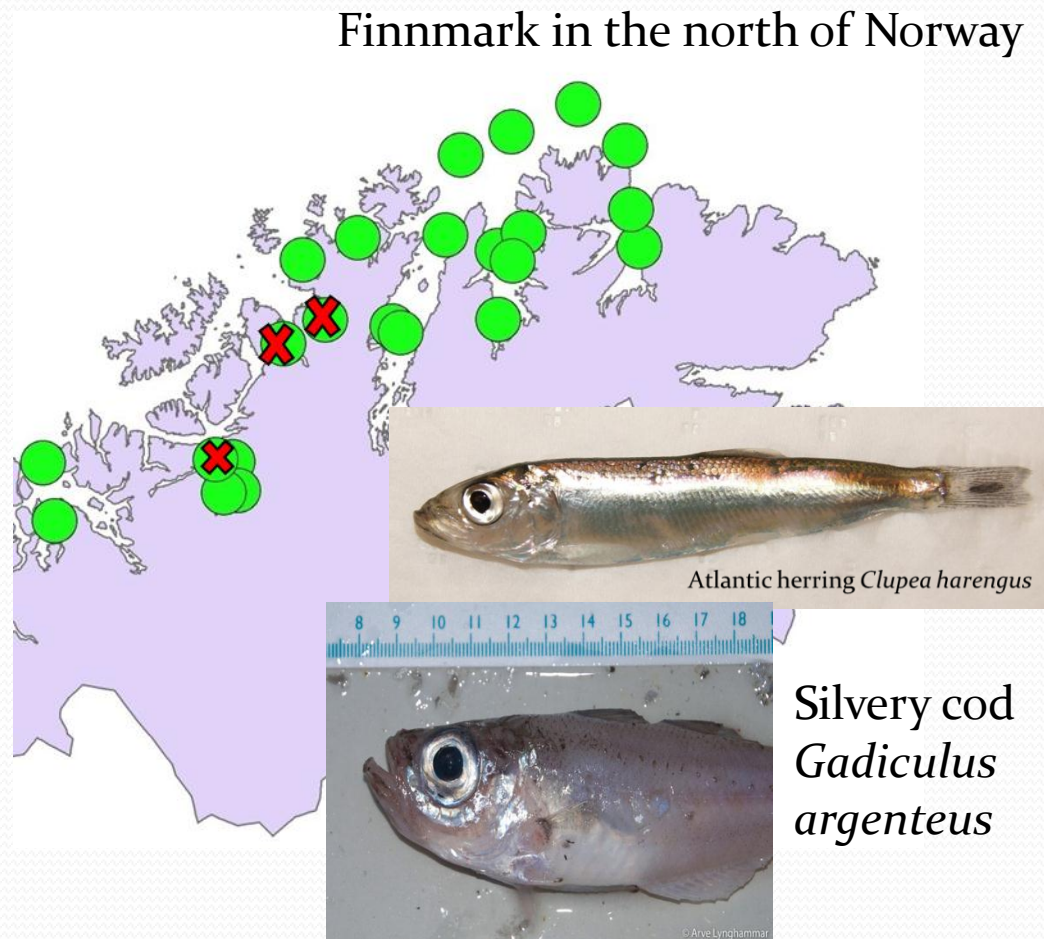
5 trawling cruises  
Some additional fishing

Brain, heart, spleen,  
kidney and gills

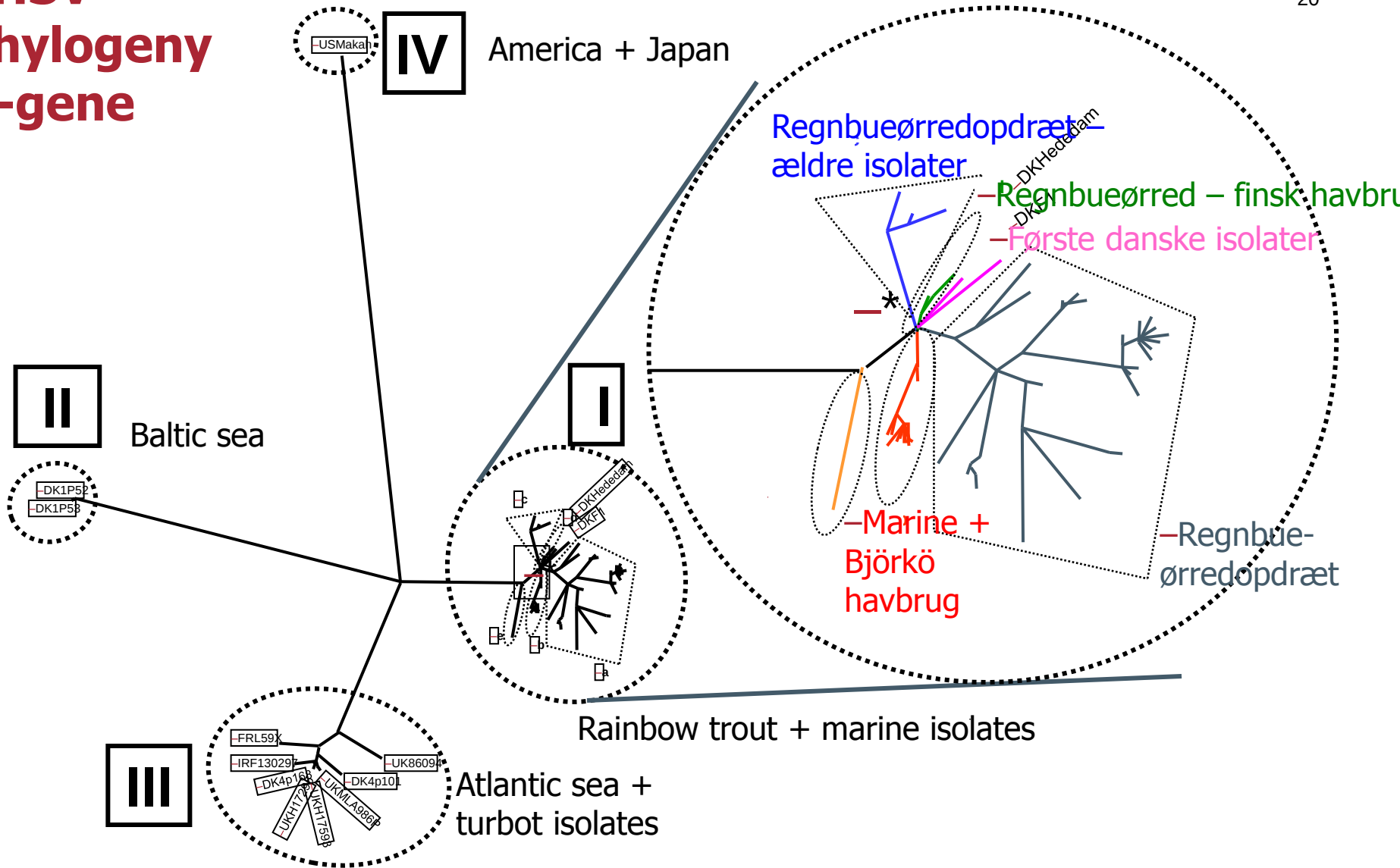


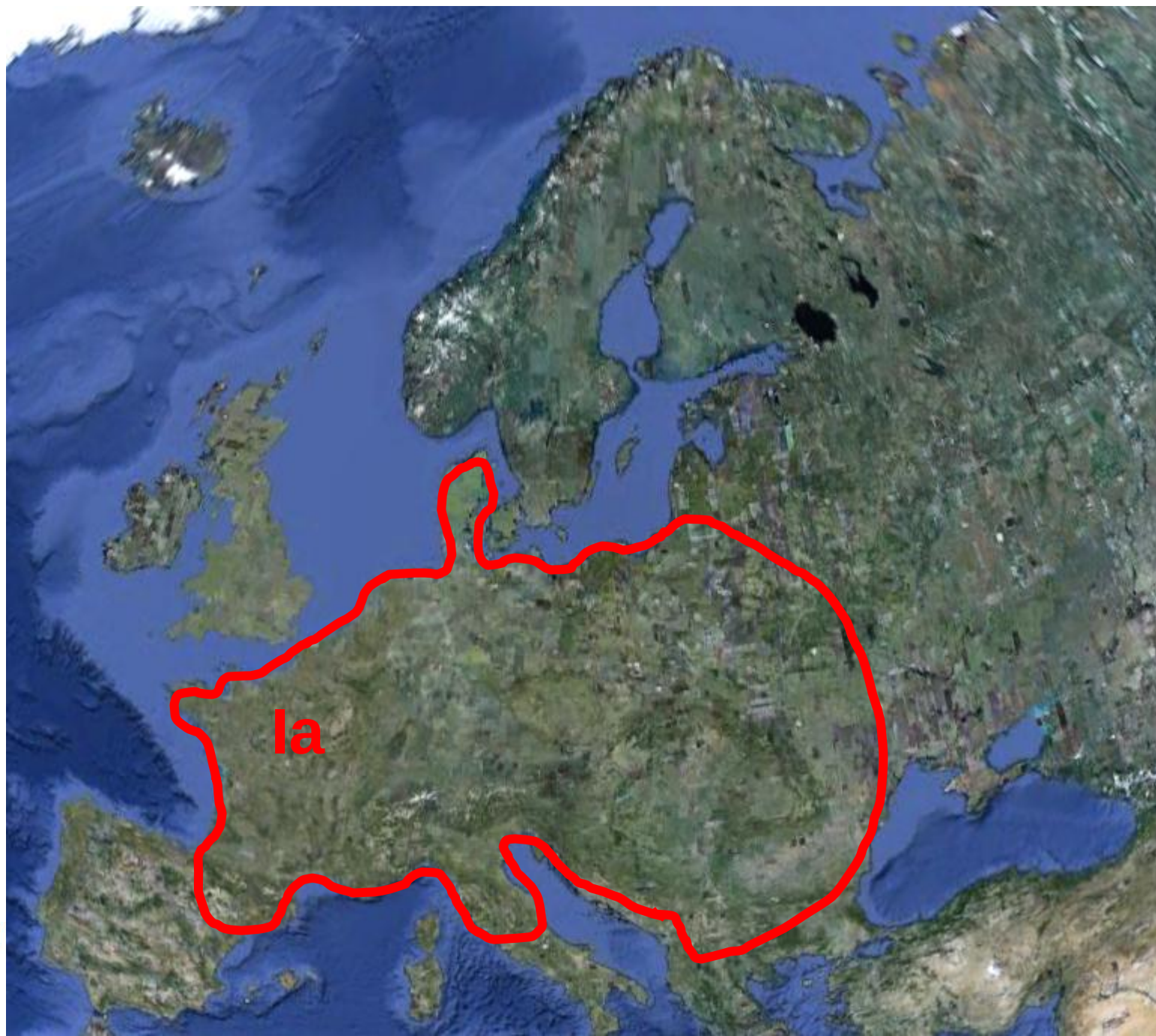
# Most Northern VHSV detection

- Herring isolates
  - Genotype Ib
  - All 4 isolates are identical
  - Identical to VHSV from herring from the west coast
- Silvery cod isolate
  - Genotype Ib
  - Differs from the herring isolates



# VHSV Phylogeny G-gene

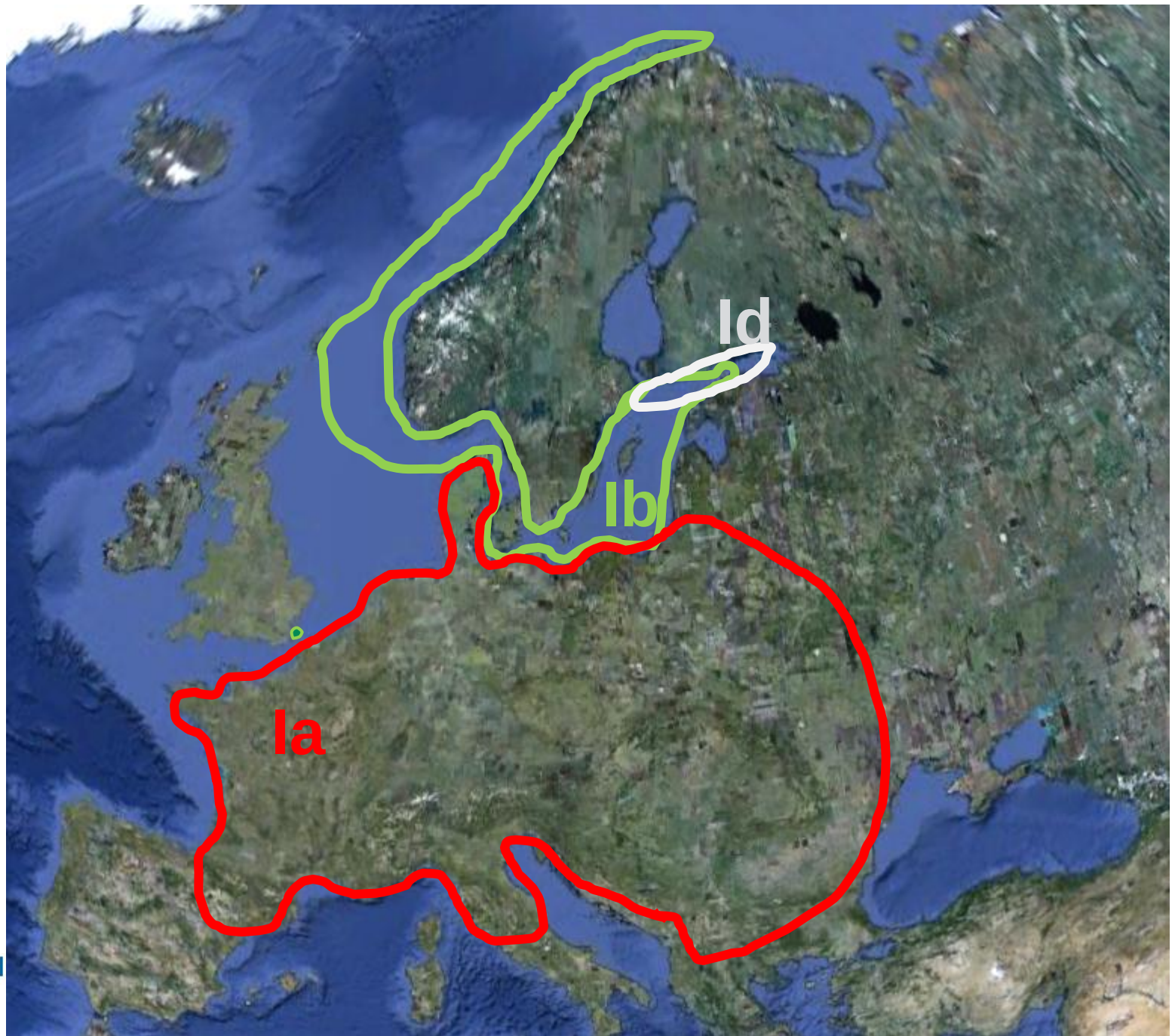




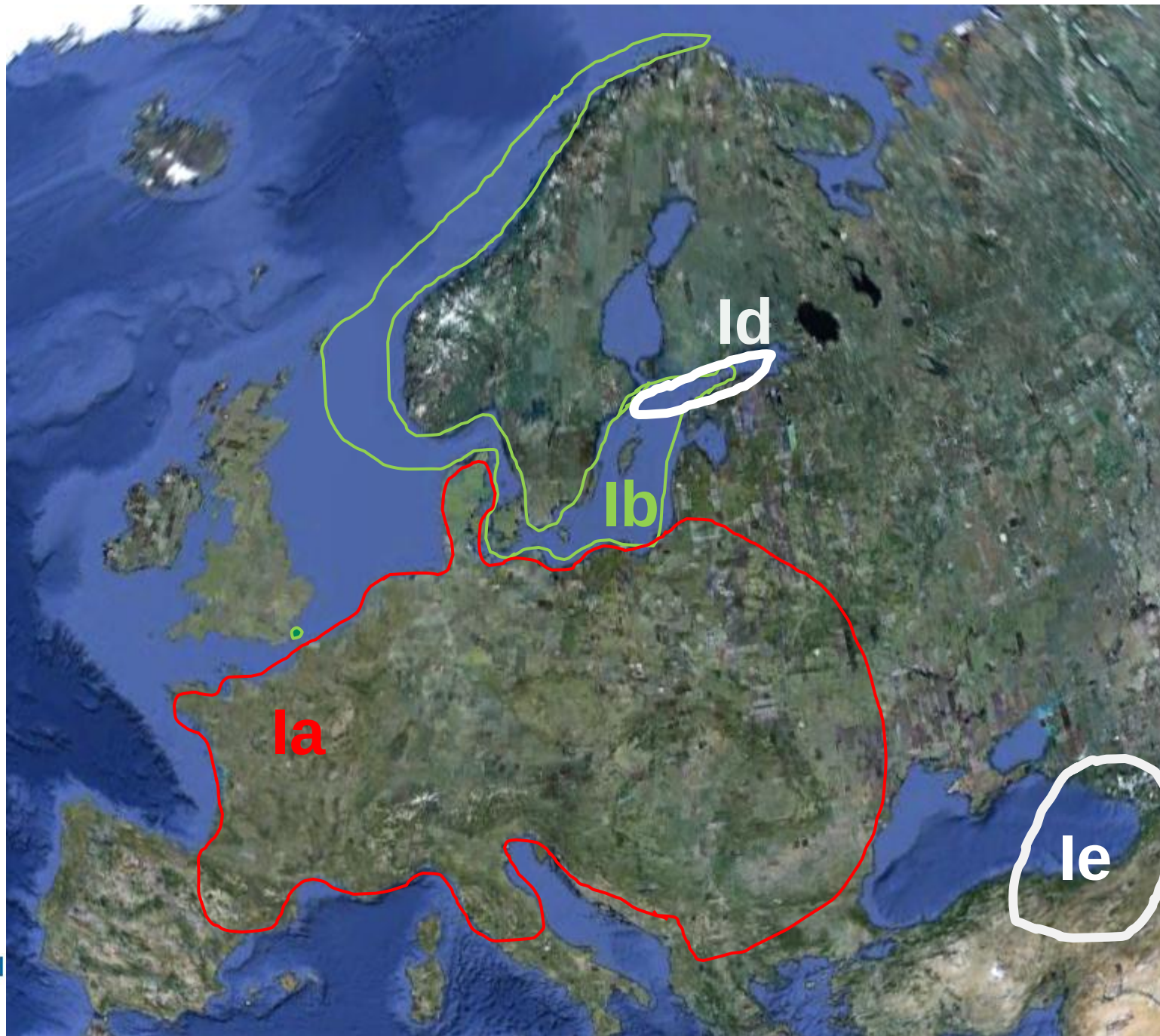
National



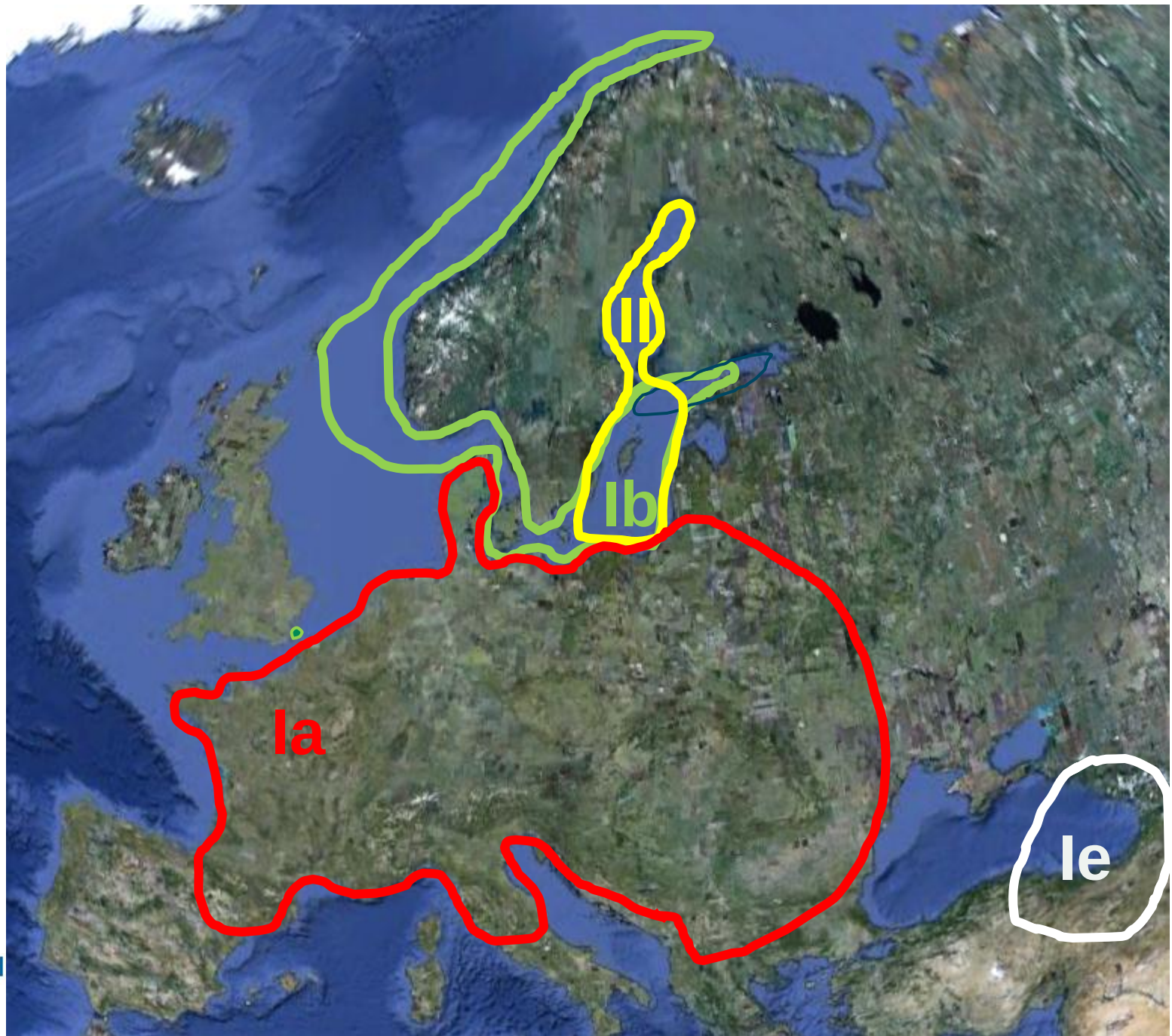
National



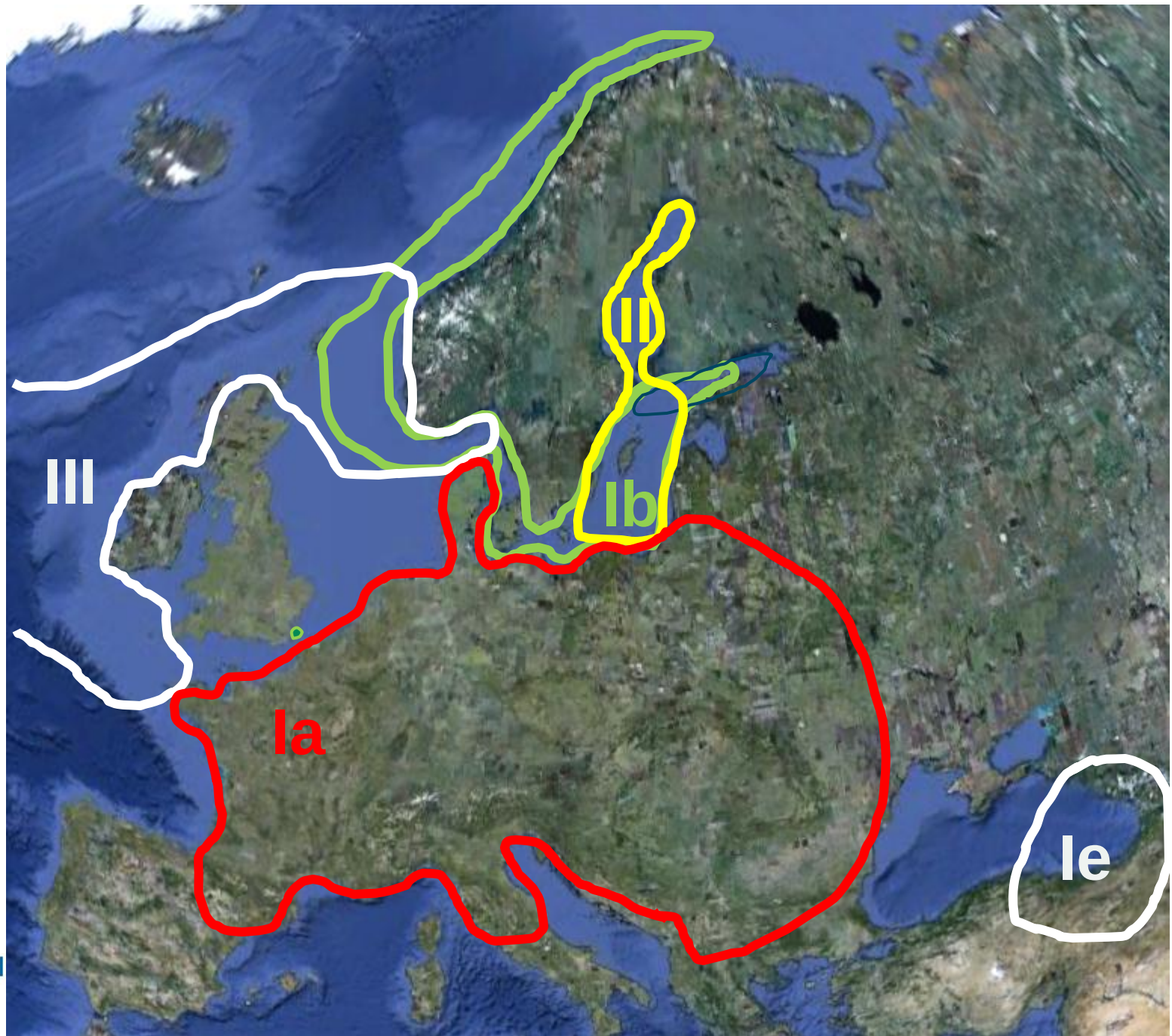
National



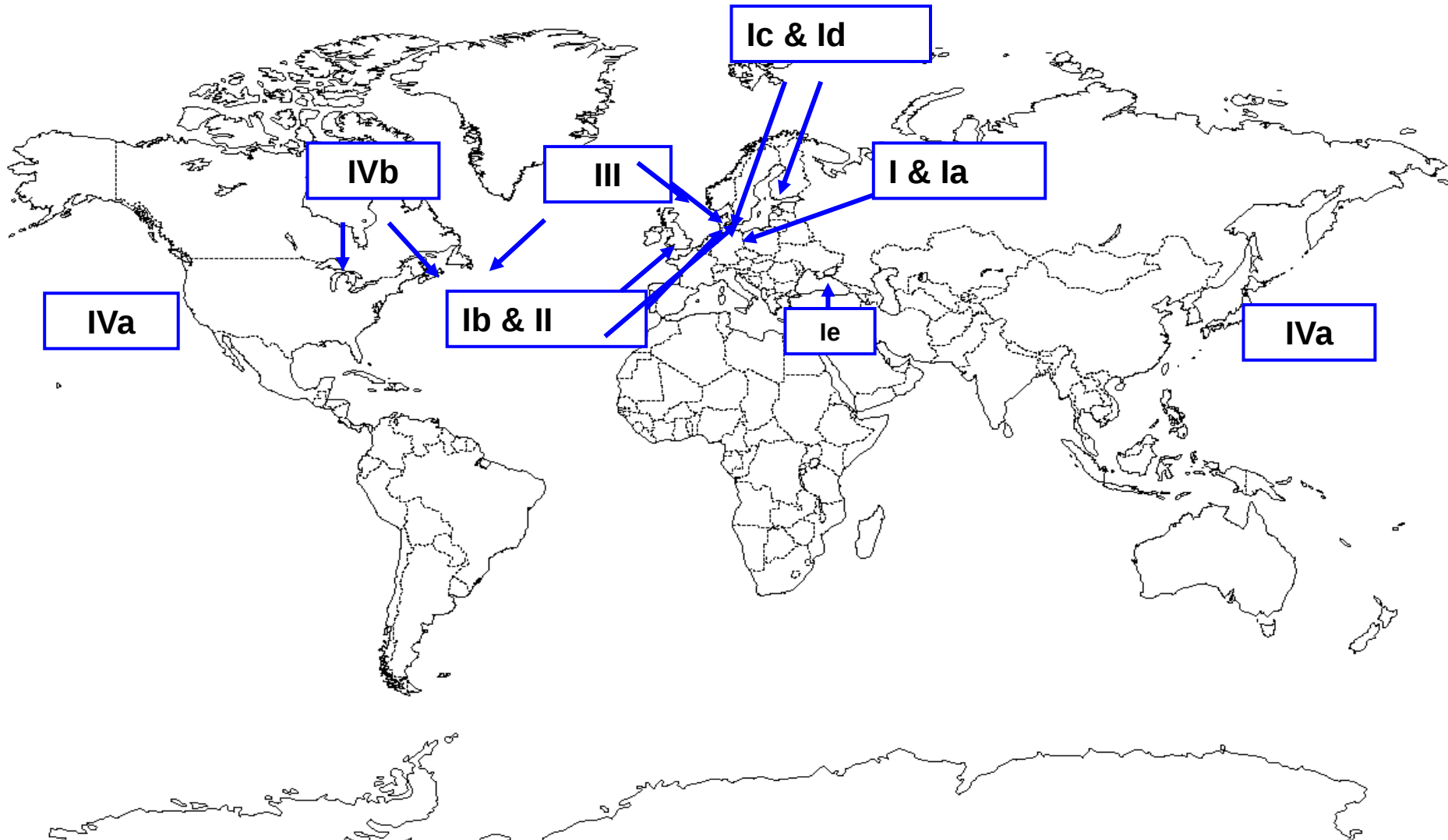
National



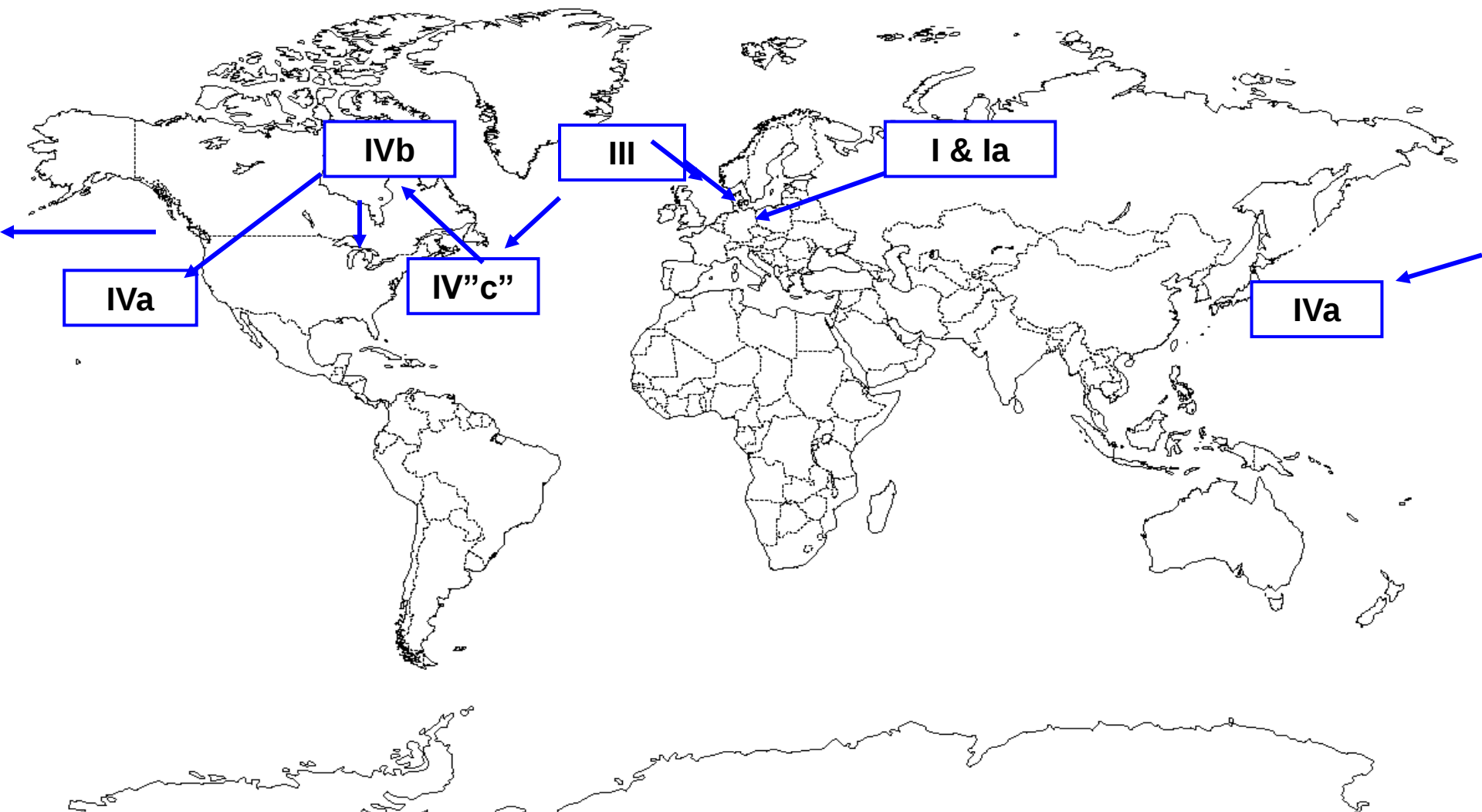
National



# Phylogeographic distribution



# Genotype III is apparently the link between American/Asian isolates and European



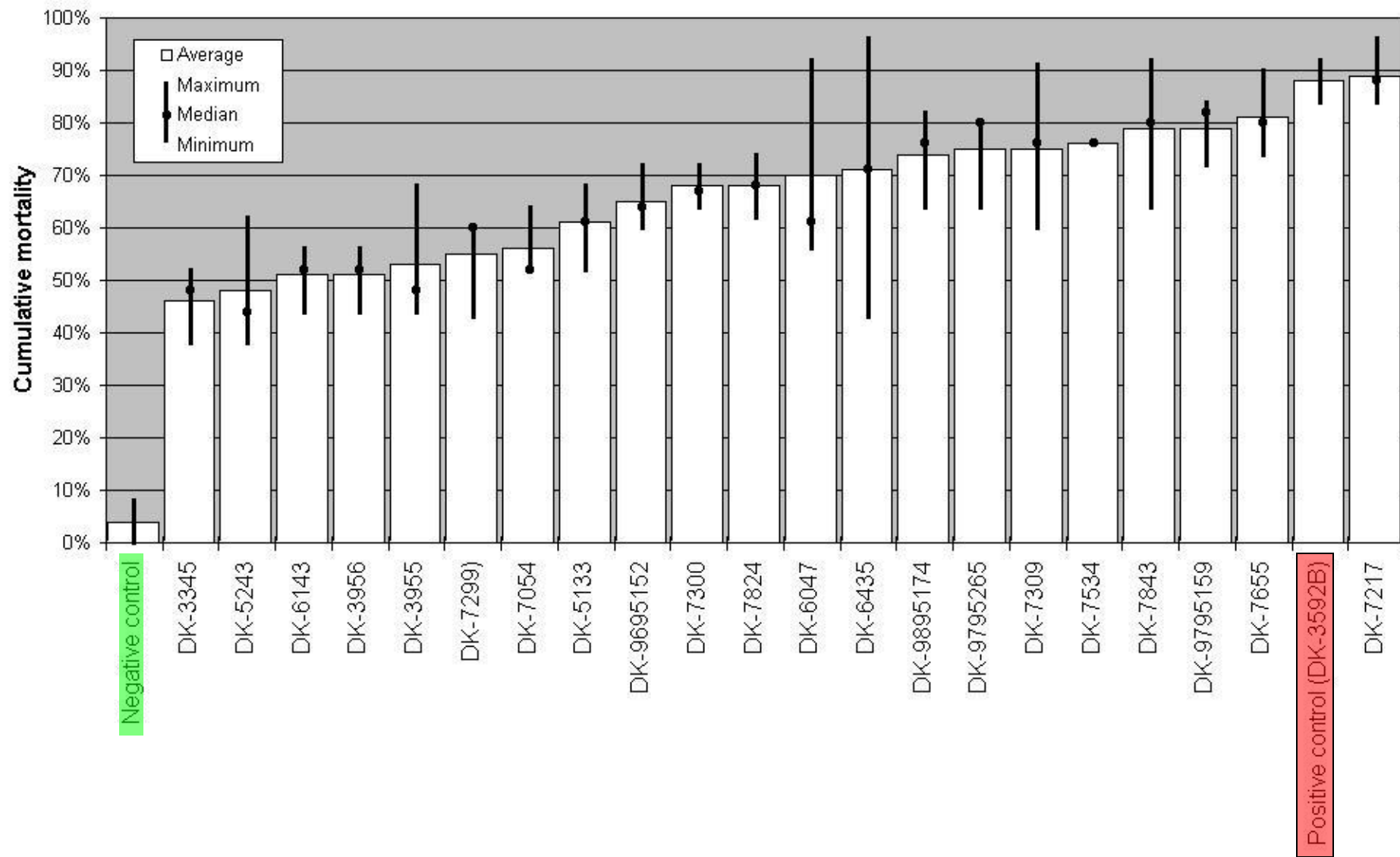
# VHS in rainbow trout

Infection by immersion

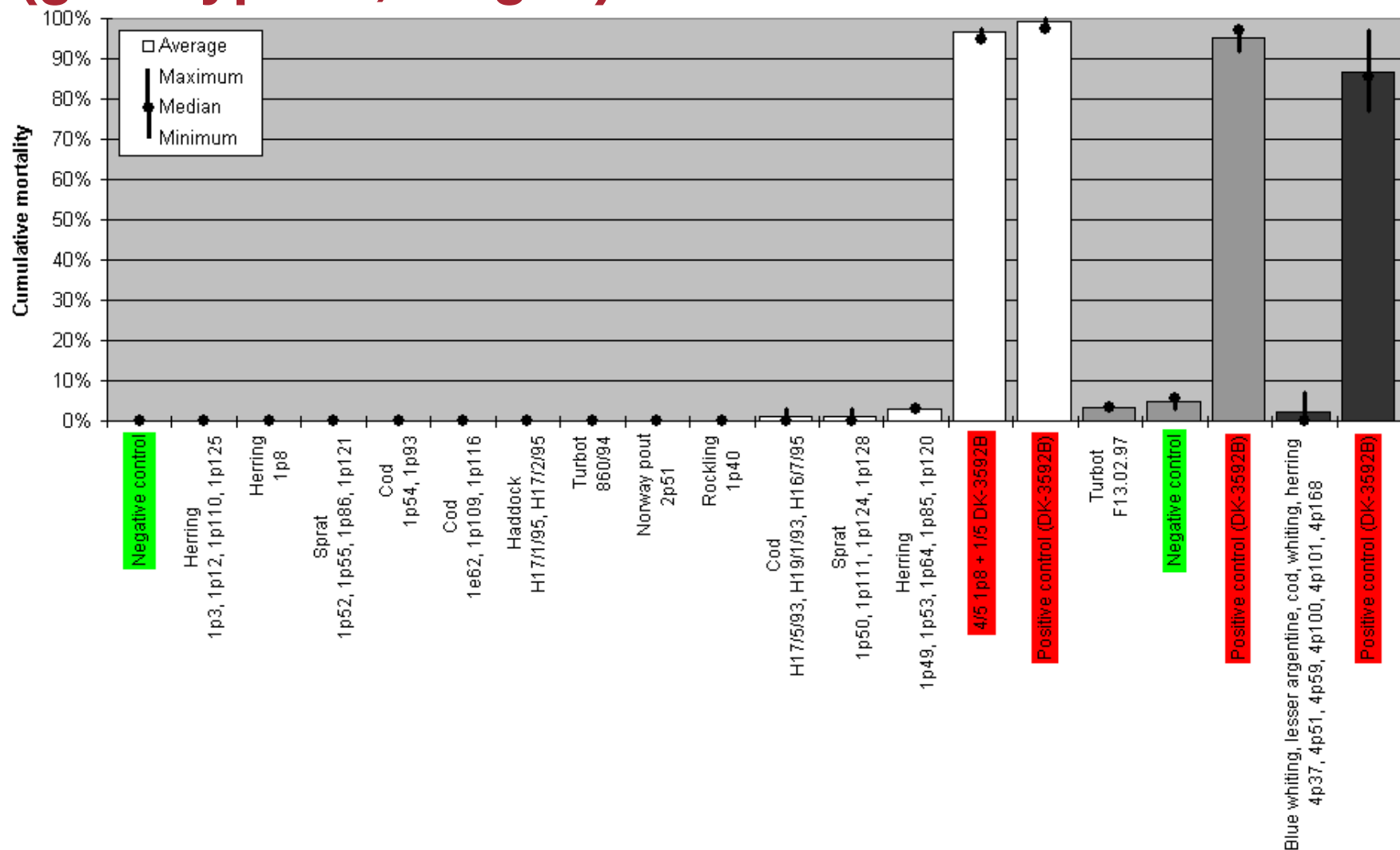


# Infection trials by immersion of rainbow trout with 22 VHSV from farmed rainbow trout (genotype Ia).

30



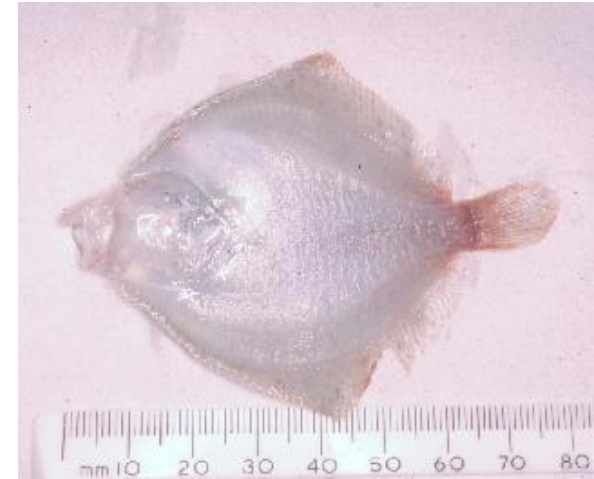
# Infection trials by immersion of rainbow trout with VHSV from marine fish species (genotype Ib, II og III).



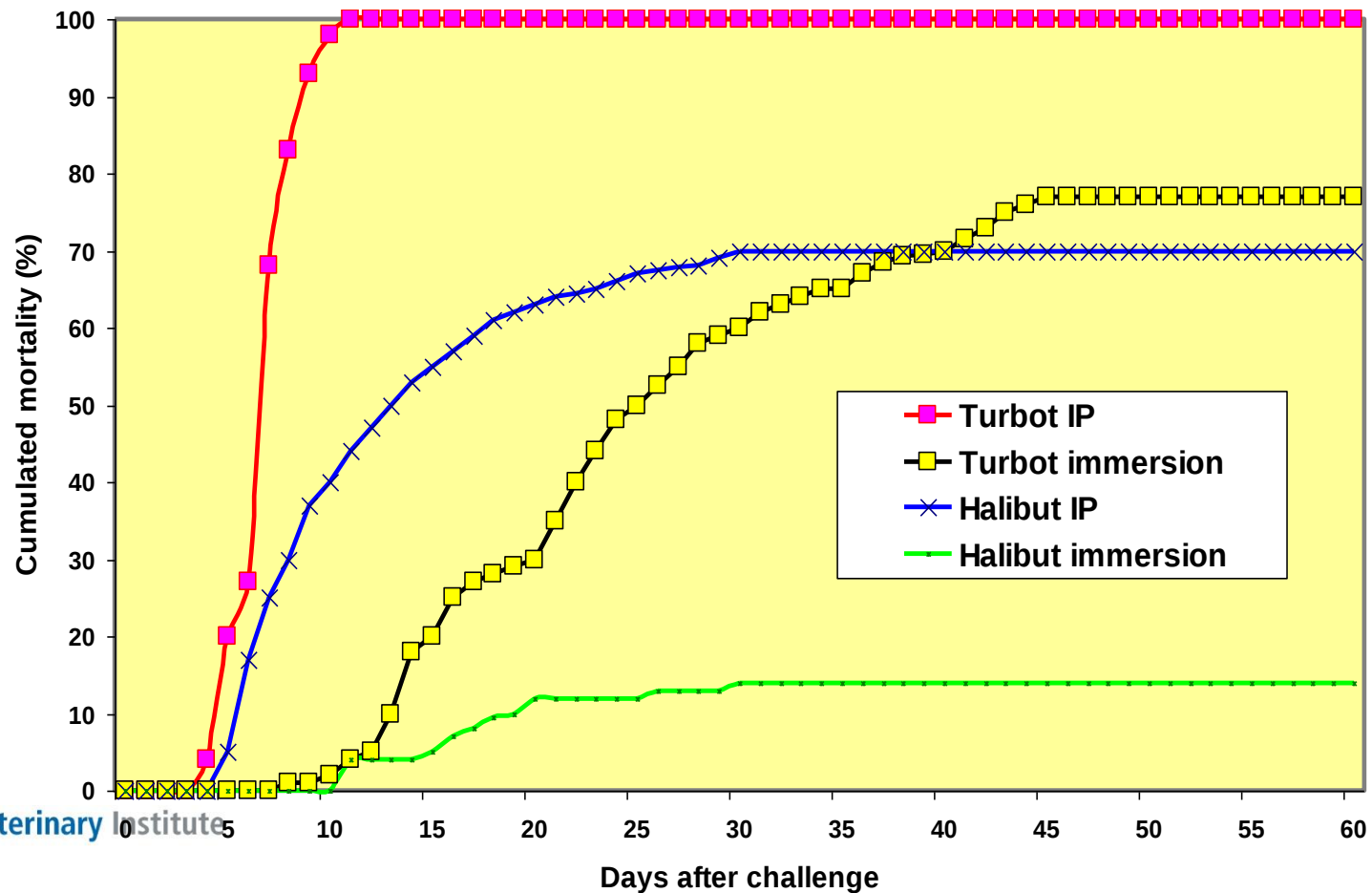
# VHS in other species in Europe:

## Turbot (*Psetta maxima*)

- Outbreaks in UK & Ireland: Type III
- 36 marine VHSV isolates tested
- Immersion
- Mortalitet > 90% for some isolates
- Not high mortality for all isolates



# Halibut and Turbot



## **Atlantic salmon - (*Salmo salar*)**

- **34 marine isolates tested**
- **Immersion: No increased mortality**
- **IP injection: significant mortality**

## Cod

- **1 marine VHSV isolate tested (III)**
- **Immersion: No increased mortality**
- **IP injection: cumulative mortality d.19: 85%**

# High prevalence of VHSV genotype Ib detected in Atlantic herring.

## Importance for herring?

## Importance for farmed fish?

|                                                   |                                                     |                    |
|---------------------------------------------------|-----------------------------------------------------|--------------------|
| Vol. 478: 223–230, 2013<br>doi: 10.3354/meps10208 | MARINE ECOLOGY PROGRESS SERIES<br>Mar Ecol Prog Ser | Published March 25 |
|---------------------------------------------------|-----------------------------------------------------|--------------------|

## High prevalence of viral haemorrhagic septicaemia virus (VHSV) in Norwegian spring-spawning herring

Renate Johansen<sup>1,\*</sup>, Øivind Bergh<sup>2</sup>, Ingebjørg Modahl<sup>1</sup>, Geir Dahle<sup>2</sup>, Britt Gjerset<sup>1</sup>,  
Jens Christian Holst<sup>2</sup>, Nina Sandlund<sup>2</sup>

<sup>1</sup>Norwegian Veterinary Institute, PO Box 750 Sentrum, 0106 Oslo, Norway

<sup>2</sup>Institute of Marine Research, PO Box 1870 Nordnes, 5817 Bergen, Norway

# Summary of paper:

Table 1. *Clupea harengus*. Pooled samples and individual organs from Atlantic herring found viral haemorrhagic septicaemia virus (VHSV)-positive by cell culture and real-time RT-PCR (rRT-PCR). Parentheses: prevalence of positive samples; NS: not sampled; \*: specific sequence data (n = 16) obtained from the indicated VHSV-positive sample types for verification

| Sampling date 2010 | No. of fish sampled | No. of pools <sup>a</sup> | No. of positive pools <sup>a</sup> |          | No. of positive organs from individual fish by rRT-PCR |           |           |            |         |
|--------------------|---------------------|---------------------------|------------------------------------|----------|--------------------------------------------------------|-----------|-----------|------------|---------|
|                    |                     |                           | Cell culture                       | rRT-PCR  | Brain                                                  | Spleen    | Kidney    | Gills      | Gonads  |
| 25 Feb             | 40                  | 8                         | 0                                  | 2        | 1                                                      | 1         | 0         | 12 (30 %)  | 0 / 40  |
| 19 Mar             | 20                  | 4                         | 1                                  | 2*       | 4                                                      | 3*        | 5         | 19* (95 %) | 2 / 20  |
| 12 Apr             | 15                  | 3                         | 0                                  | 0        | 1                                                      | 0         | 2         | 14* (93 %) | NS      |
| 16 Apr             | 15                  | 3                         | 0                                  | 0        | 1                                                      | 2         | 1*        | 13* (87 %) | NS      |
| 22 Apr             | 15                  | 3                         | 3                                  | 3*       | 6                                                      | 7*        | 6         | 14* (93 %) | NS      |
| Total              | 105                 | 21                        | 4 (19 %)                           | 7 (33 %) | 13 (12 %)                                              | 13 (12 %) | 14 (13 %) | 72 (69 %)  | 2 (3 %) |

<sup>a</sup>Brain, spleen and kidney tissues pooled from 5 fish

**Gills only tested by rRT-PCR and had a prevalence of 69%.**

**Exogenous in the mucosa?**

**High prevalence related to spawning?**

## Challenge trial

### **NO-F/2009, genotype Ib**

- 30 fish
- 30 fish
- 50 fish



### **4p168, genotype III**

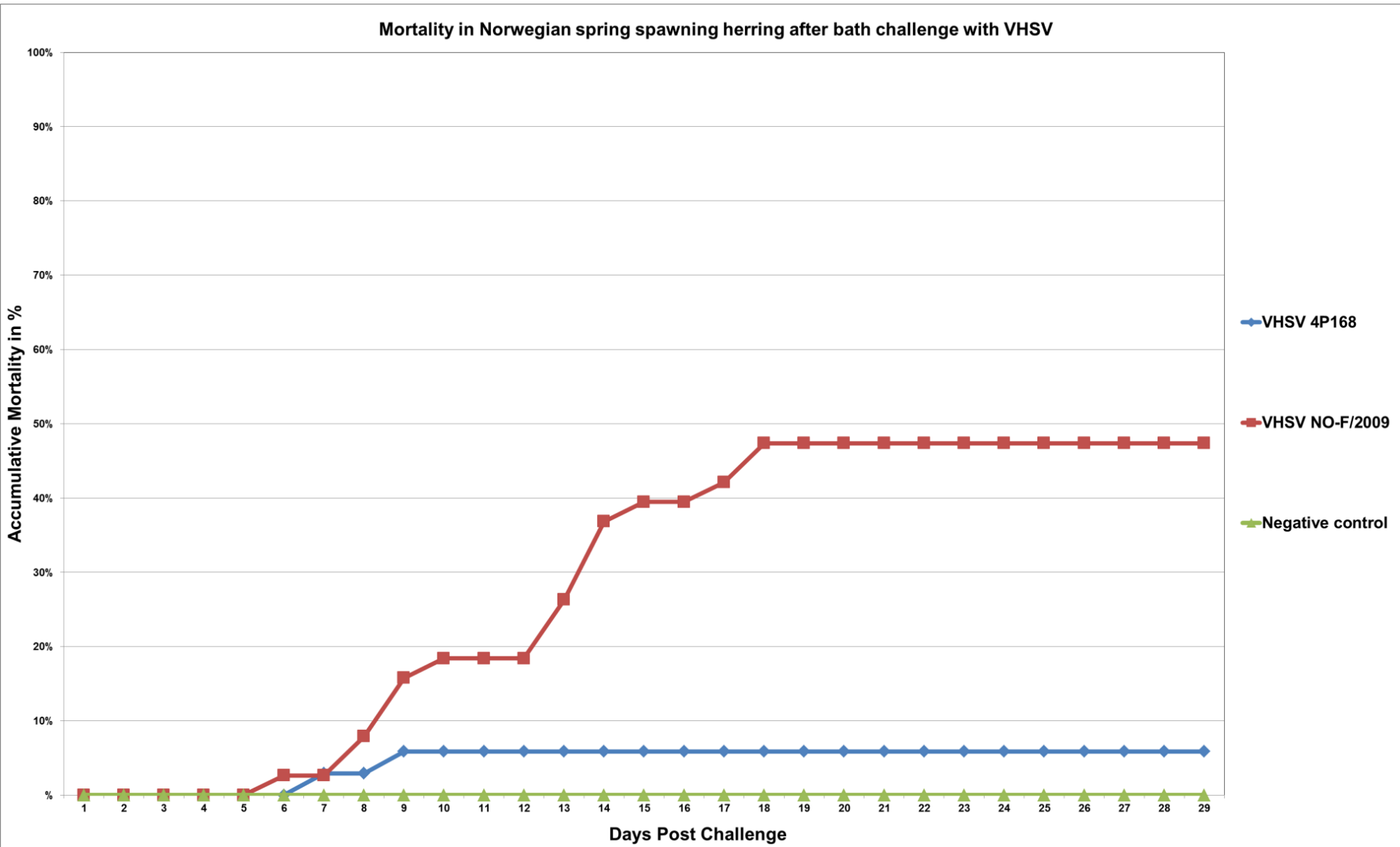
- 30 fish
- 30 fish
- 50 fish

## Negative control

30 fish

**$10^5$  TCID/ml for 2 hours  
followed by partial  
water change**

# Mortality rate in herring fry



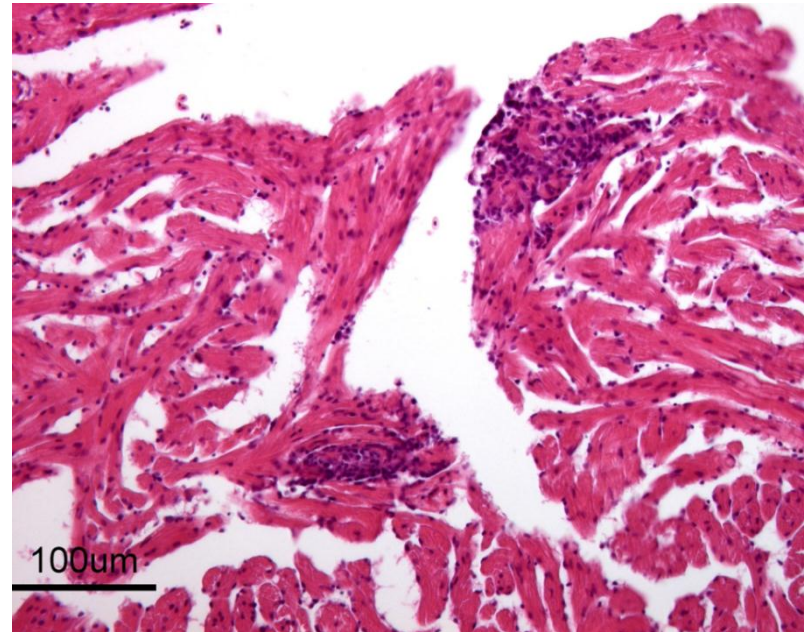
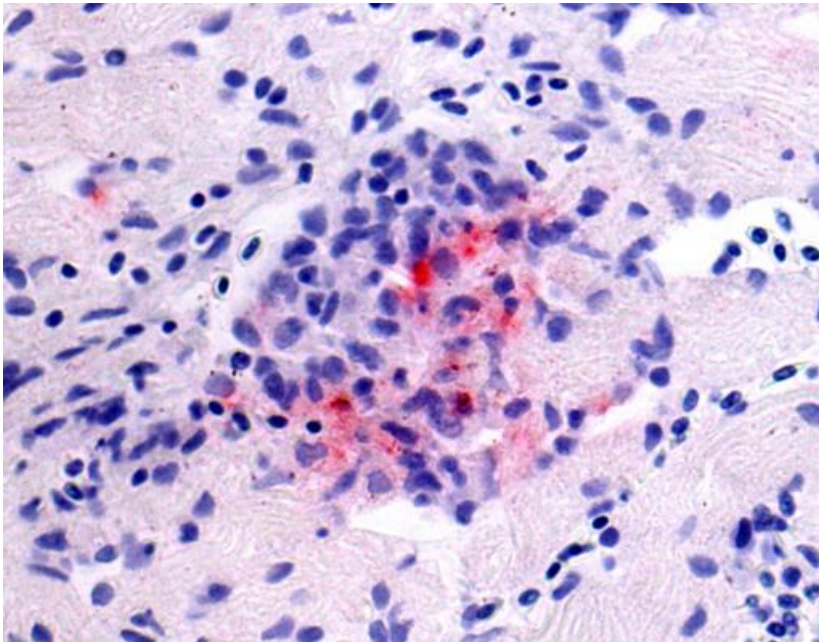
# Clinics



# Gross pathology



# Histopathology and immunohistochemistry



**Multifocal myocarditis with positive immunohistochemical staining of VHSV in both challenge groups**

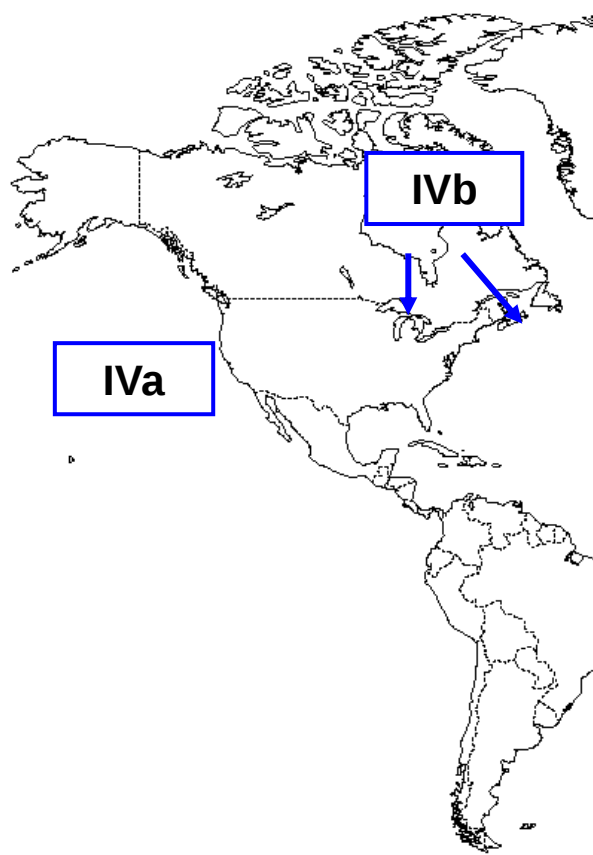
# VHS on the US West Coast

- 1993: VHSV genotype IVa isolated from chinook salmon and Pacific herring
- VHSV enzootic in some populations of Pacific herring (*Clupea harengus*) and pilchard (sardines) (*Sardinops sagax*)



# VHS on the US East Coast and Great Lakes

## >28 fish species affected

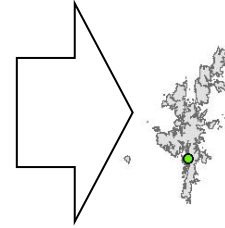


From an outbreak in the St. Lawrence River, 2007

# VHS – Wrasse by Eann Munro, MarinScotland

December 2012 suspicion of VHS at a SW hatchery site in Shetland

● Suspect Site



Mortality in wrasse population (5 species).  
~ 10 000 wrasse on site.

Movement restrictions (IDN)  
and statutory testing:

- a) suspect site
- b) supplying site
- c) sites with cohorts to those at the suspect site



Ballan



Corkwing



Cuckoo



Goldsinny



Rockcook



—Photographs – Paul McKay and Andrea Warwick, FHI



# Epidemiological Investigation

Supplying site – wrasse originate from wild caught populations, some cultured stocks

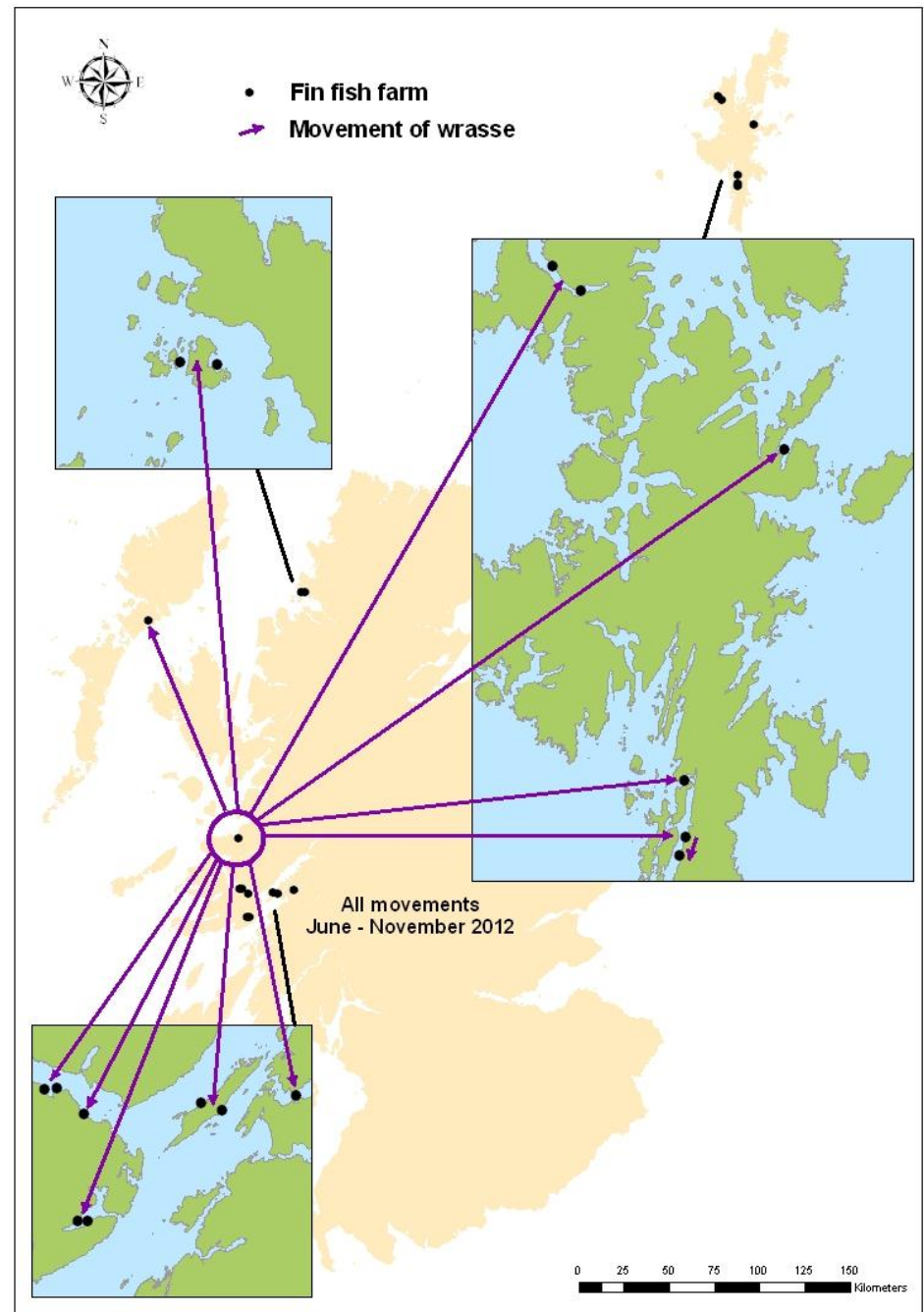
150 fish statutory sample susceptible species

No clinical signs of disease

16 SW cage sites also had wrasse populations (cohorts to the suspect site) from the supplier

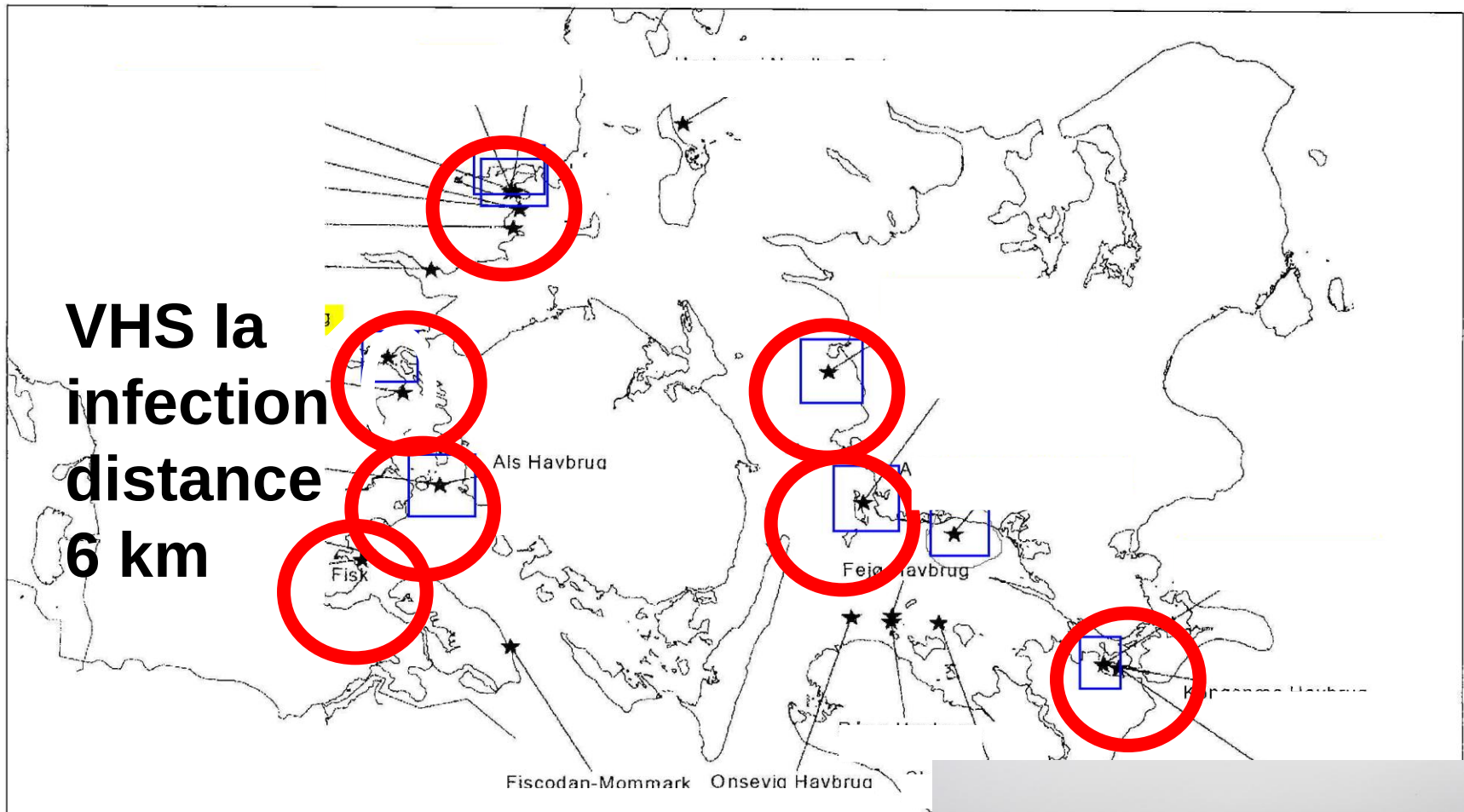
Statutory sampling of 15 fish per site – 16 sites considered as one population

National Veterinary Institute



—Figure courtesy of Stuart Wallace, Epidemiology

# VHS outbreaks in sea farmed rainbow trout in Denmark

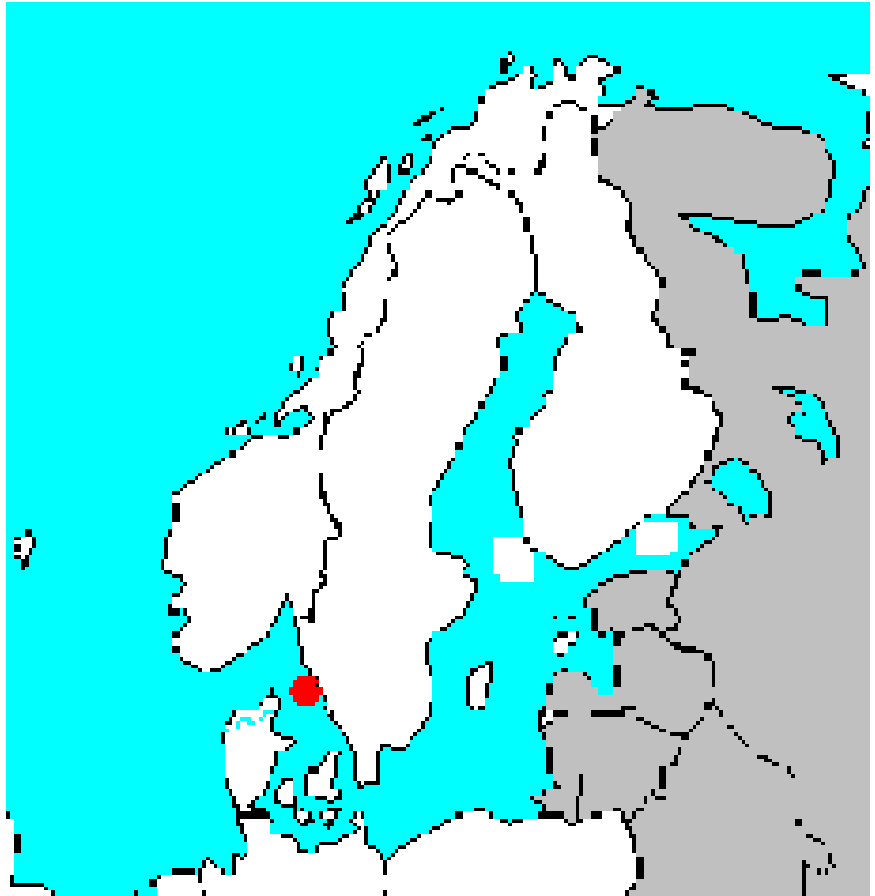


All VHSV typed from outbreaks:  
Genotype Ia



# VHS in sea farmed RT in Sweden

- Sweden
  - VHS outbreak in rainbow trout farm in Kattegat close to Gøteborg in 1998 og 2000
  - Genotype Ib
  - First finding of Ib (marin subtype) in R.T.

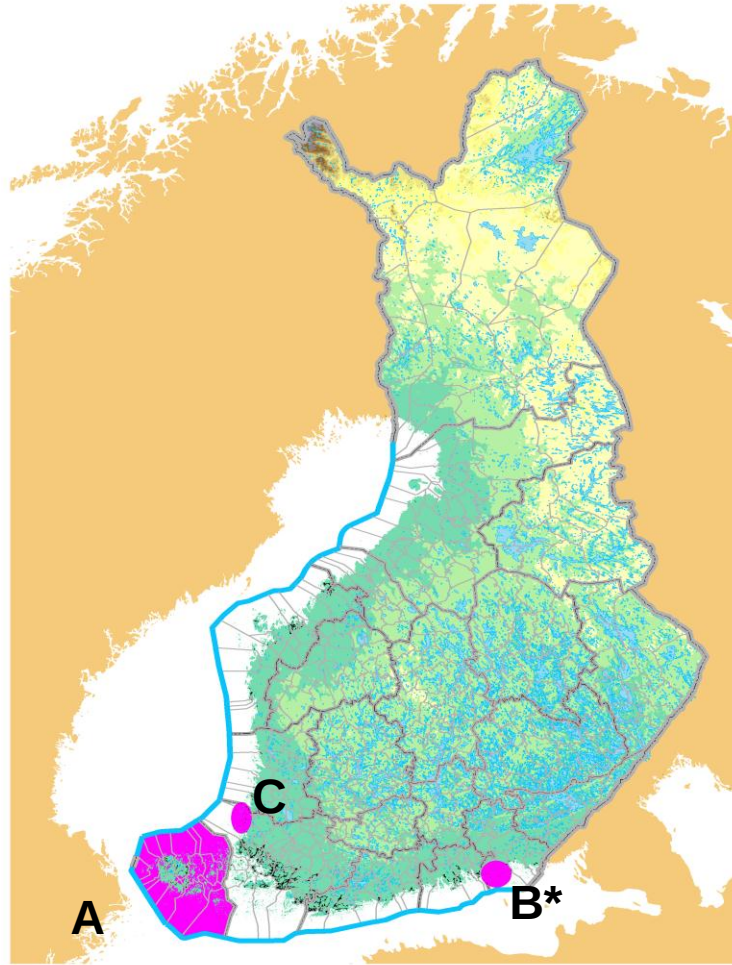


# VHS in Finland

- ☐ Genotype Id (Katja Einer-Jensen et al. 2004) in rainbow trout
- ☐ Resembles old Danish isolates
- ☐ Infection trials 38% mortality (DFVF)
- ☐ Mortalities 1-50%
- ☐ Stress related
- ☐ (Genotype II in wild herring and lamprey)



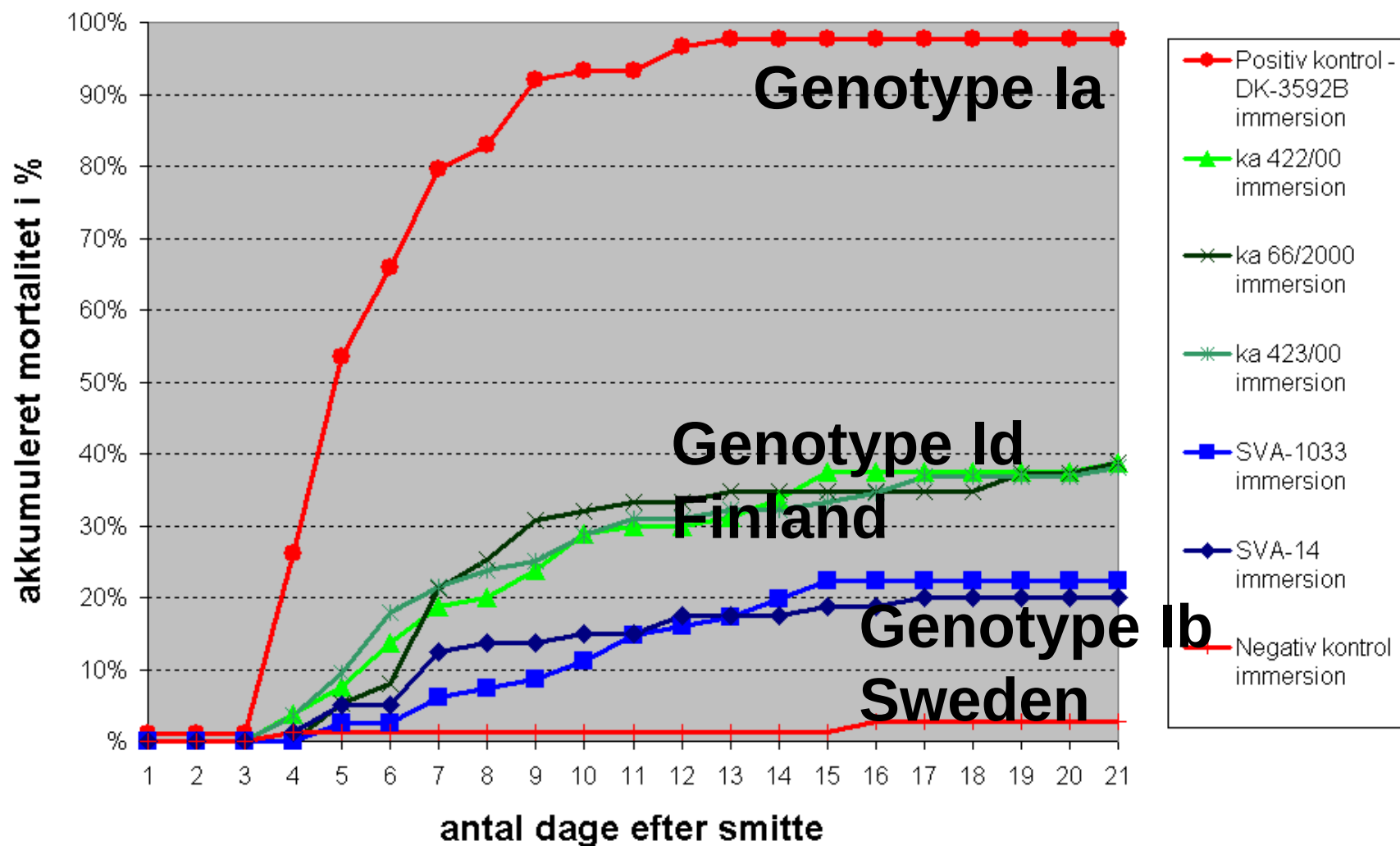
# VHS in Finland



| Year | outbreaks/area |
|------|----------------|
| 2000 | 5/ A+B*        |
| 2001 | 6/ A+B*        |
| 2002 | 14/ A          |
| 2003 | 3/ A+C         |
| 2004 | 2/ A           |
| 2005 | 9/ A           |
| 2006 | 10/ A          |
| 2007 | 2/ A           |
| 2008 | 4/ A+C         |
| 2009 | 6/ A           |

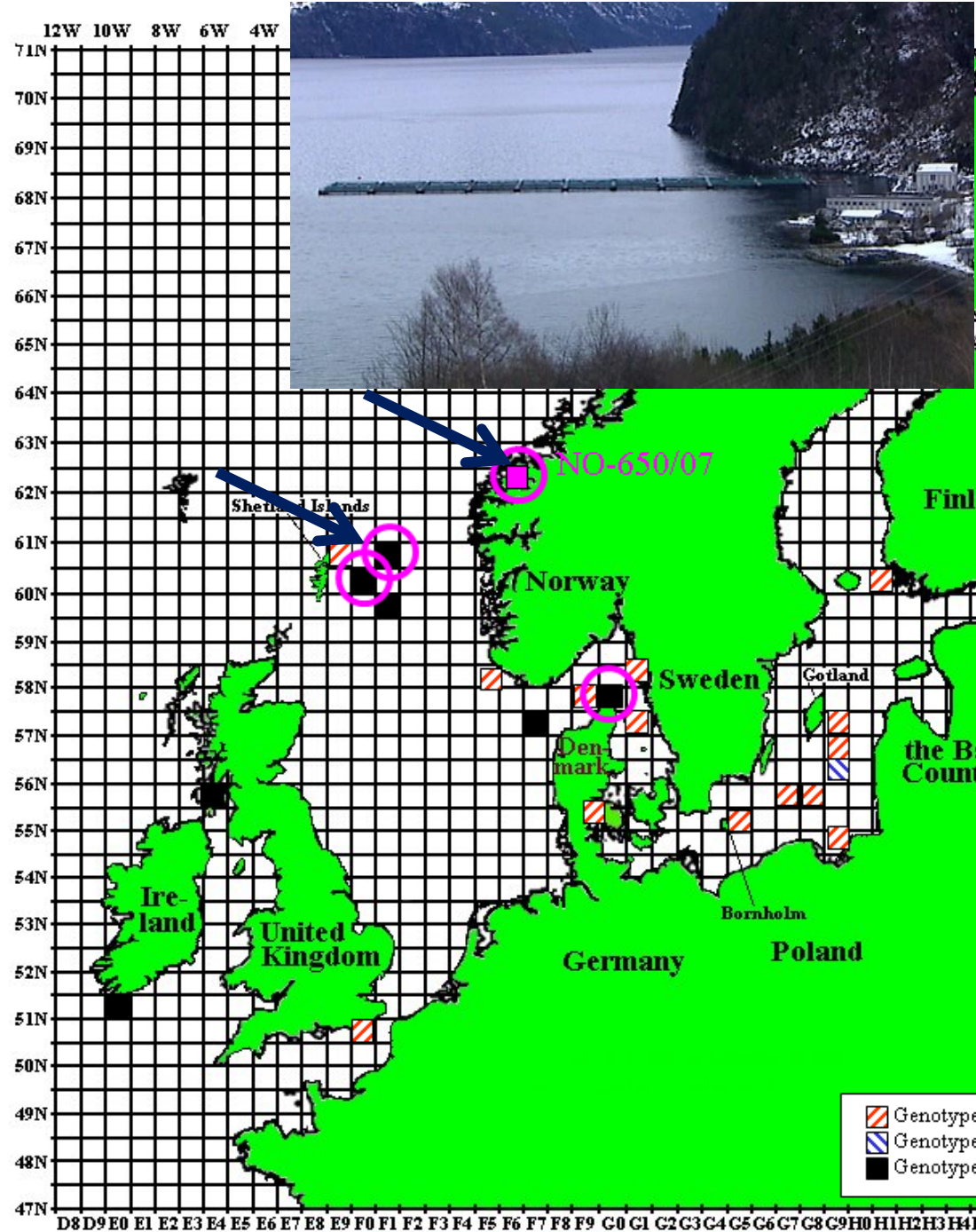
**\*VHS free since 2008**

# Infection trials in rainbow trout with the Swedish and Finnish isolates, respectively

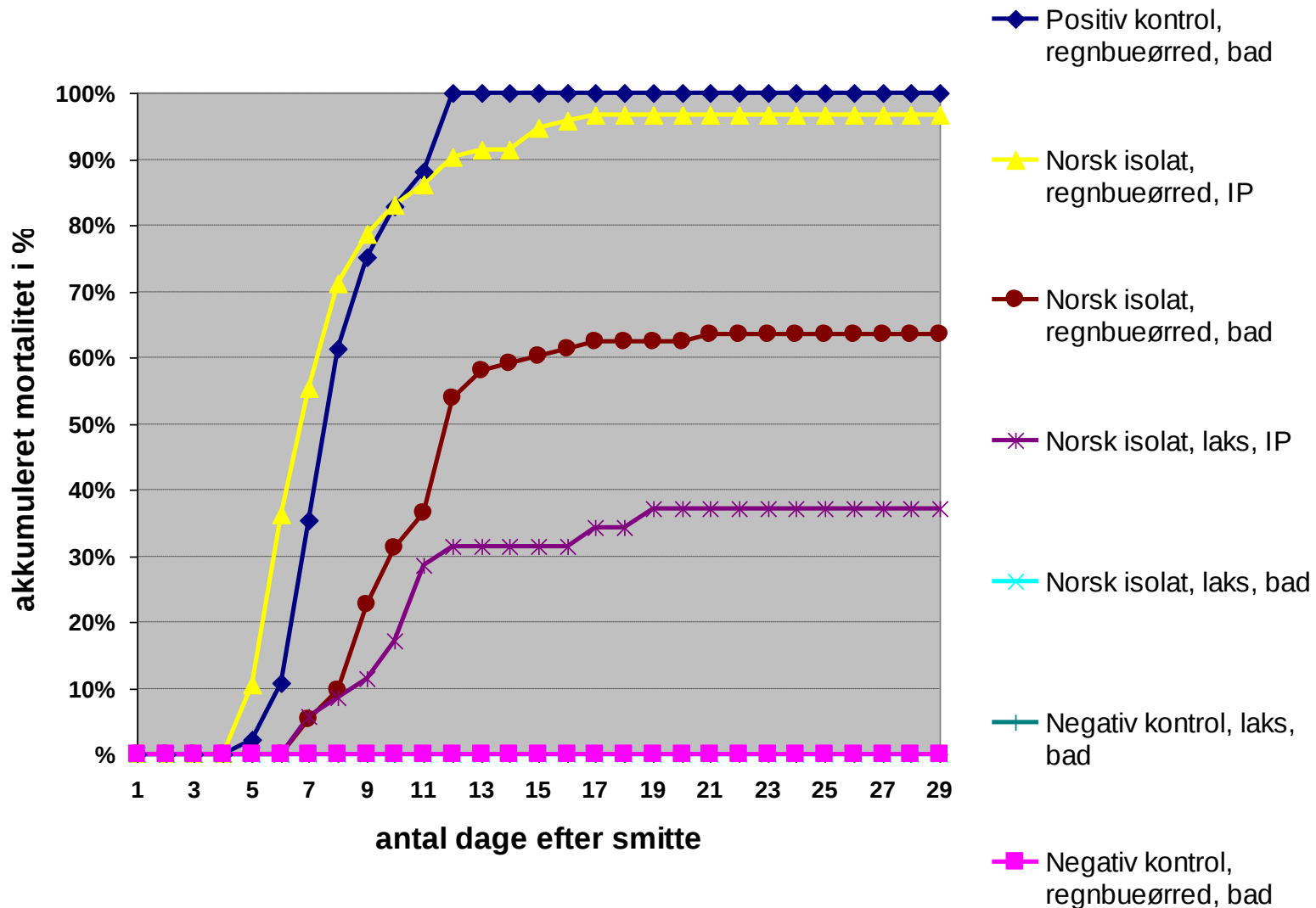


# VHS in sea farmed rainbow trout in Norway

- VHS outbreak autumn 2007
- Genotype III
- First genotype III in rainbow trout (marine genotype)



# Infection trials in rainbow trout with the Norwegian type III VHSV 53



# VHSV in wild marine fish is a risk for farmed fish!

- Proper management can reduce the risk of rainbow trout contracting VHS in sea water.

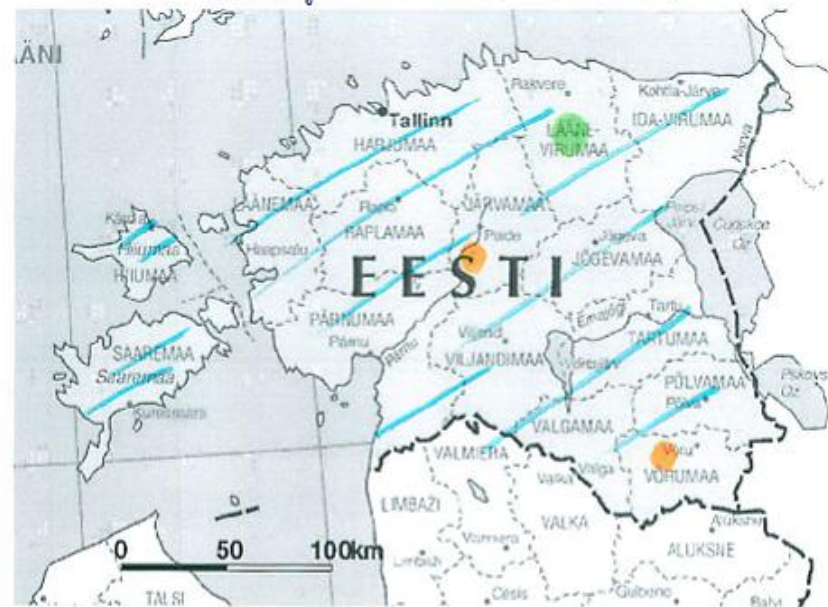
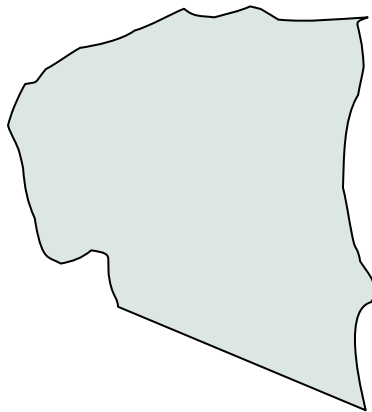
# VHS outbreaks in rainbow trout in fresh water

In **Estonia** 2 outbreaks of VHS in rainbow trout farms year 2011

First isolate VHSV Ia that far north.

*VHS categories in 2011, Estonia*

Page 1 of 1



- - Category II (VHS/IHN)
- /// - Category III
- - Category IV

# VHS outbreak in UK - 2006

Journal of Fish Diseases 2008, 31, 775–784

doi:10.1111/j.1365-2761.2008.00951.x

## **The first report of viral haemorrhagic septicaemia in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum), in the United Kingdom**

D M Stone<sup>1</sup>, H W Ferguson<sup>2</sup>, P A Tyson<sup>1</sup>, J Savage<sup>1</sup>, G Wood<sup>1</sup>, M J Dodge<sup>1</sup>, G Woolford<sup>1</sup>, P F Dixon<sup>1</sup>, S W Feist<sup>1</sup> and K Way<sup>1</sup>



# VHS outbreak in UK - 2006

2006 Classical VHS outbreak in Rainbow trout farm on River Nidd. Only 1 farm affected

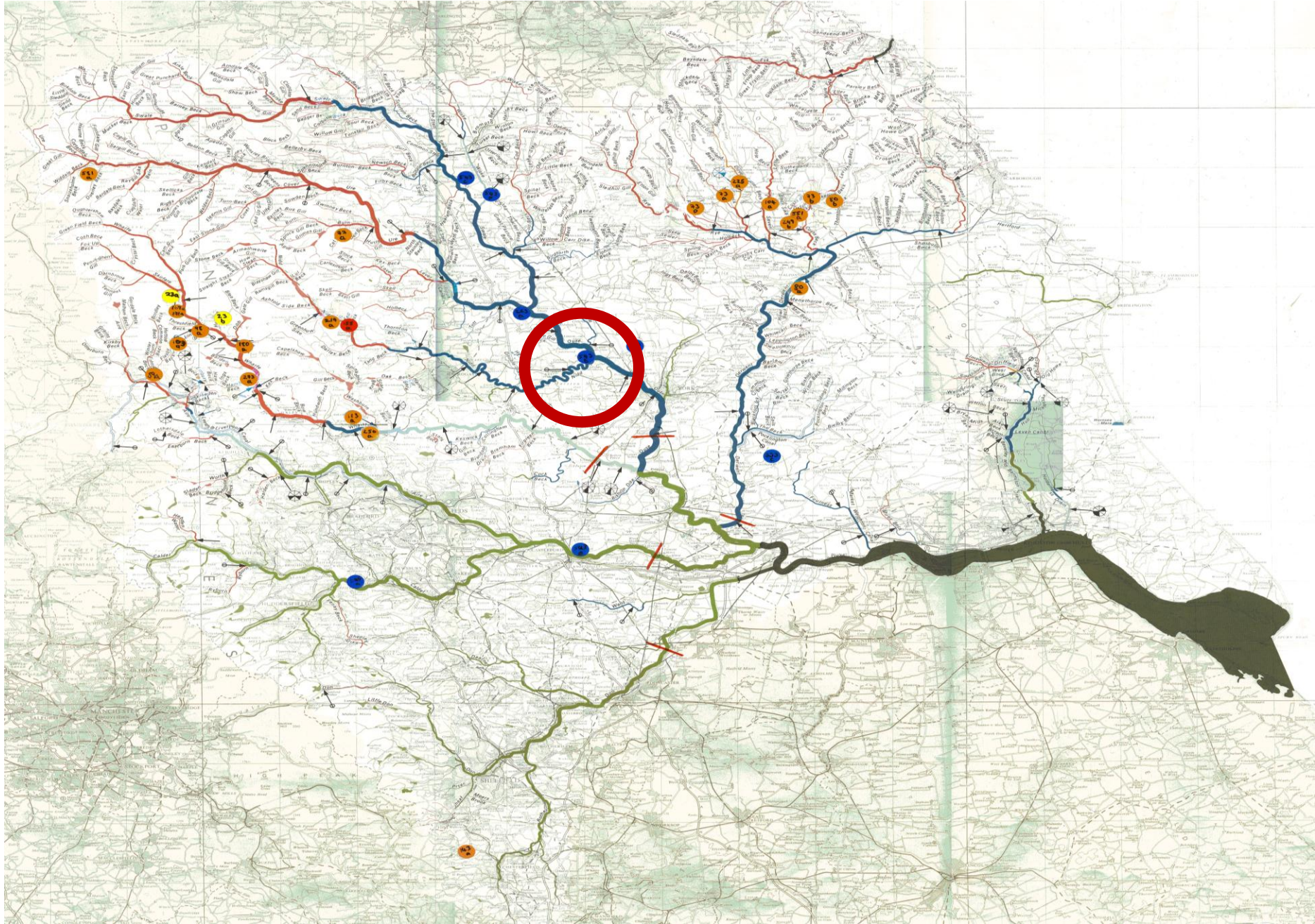
Highly pathogen isolate Genotype Ia (Continental VHSV type).

Immediate zoning and stamping-out, fallowing. Intensive survey.

Disease free status re-achieved 2009



# Controlled area showing rivers and farms



# Lessons learned UK exercise

Improved contingency planning

1 farm  1/3 of RT farming in England affected

Capacity enough if more farms infected??

VHS Risk for wildlife is low



# Conclusion

- The origin of VHS is VHSV from the marine environment
- There is a constant risk for re-introduction from infected zones/areas and from the marine environment
- Many species affected
- Rainbow trout still major target in Europe
- Sensitive specific and rapid diagnosis
- Serology?

*Only dead fish always follow the stream*

**Thank you for  
your attention!**

National Veterinary Institute

