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**UNIVERSITÄT  
BERN**

# Monitoring and surveying the health of wild fish populations in Switzerland

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# Outline

- Fish Health in Switzerland
- General health investigations
- Proliferative kidney disease
- Viral diseases
- Summary and Conclusions



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# Fish health in Switzerland

- No fish disease service
- No regular monitoring of farmed or wild fish
- Farmers, veterinarians and laboratories kept to inform authorities in case of notifiable diseases
- Authorities: federal and cantonal
- Data on fish health situation from:
  - Routine diagnostic by national fish disease laboratory
  - Monitoring projects



# Outline

- Fish health in Switzerland
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- Summary and Conclusions

# River system in eastern Switzerland



## Problem and questions

Decreasing brown trout but increasing rainbow trout populations



Brown trout



Rainbow trout

Competition?  
Affected health?  
Causing factors?

# River system in eastern Switzerland



## Methods

- Competition: laboratory experiments

- Affected health: sampling and analysing wild fish from different locations within system

- Causing factors: analyses of water parameters and morphological features of ecosystem



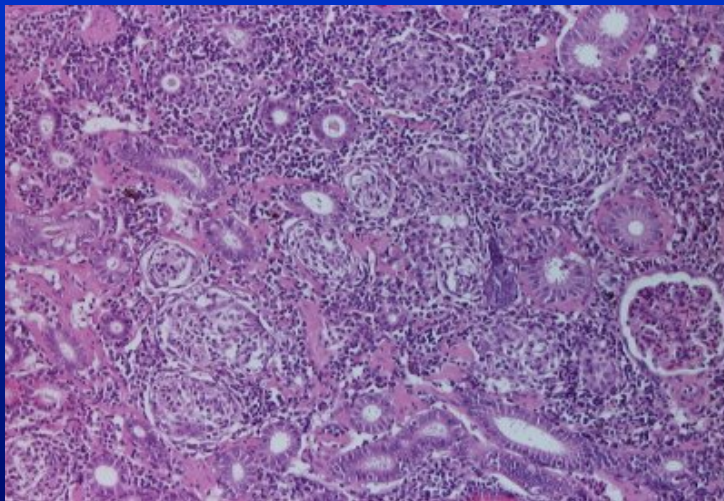
Rhein river valley above Lake of Constance

# River system in eastern Switzerland

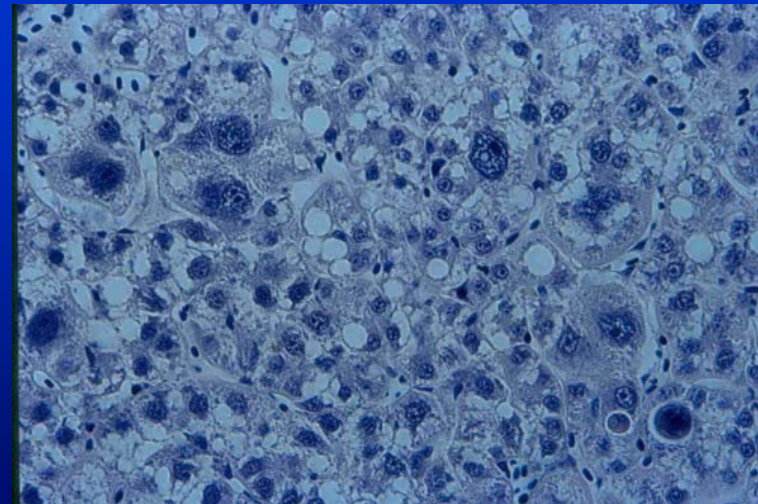


## Health condition

- Pathogens -> no particular pathogen found (virus, bacterium, parasite) except *Tetracapsuloides* in some sites
- Histology of liver and kidney -> alterations present including elevated frequency of tumours in some sites



Necrotic foci in interstitial tissue of kidney



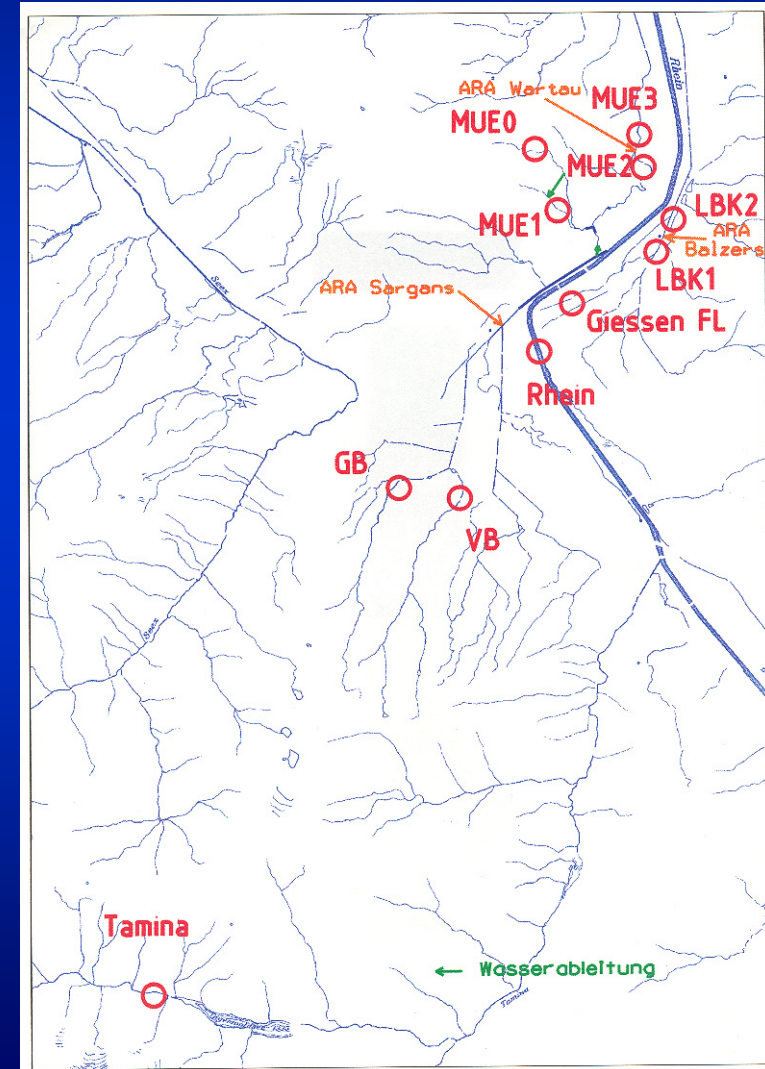
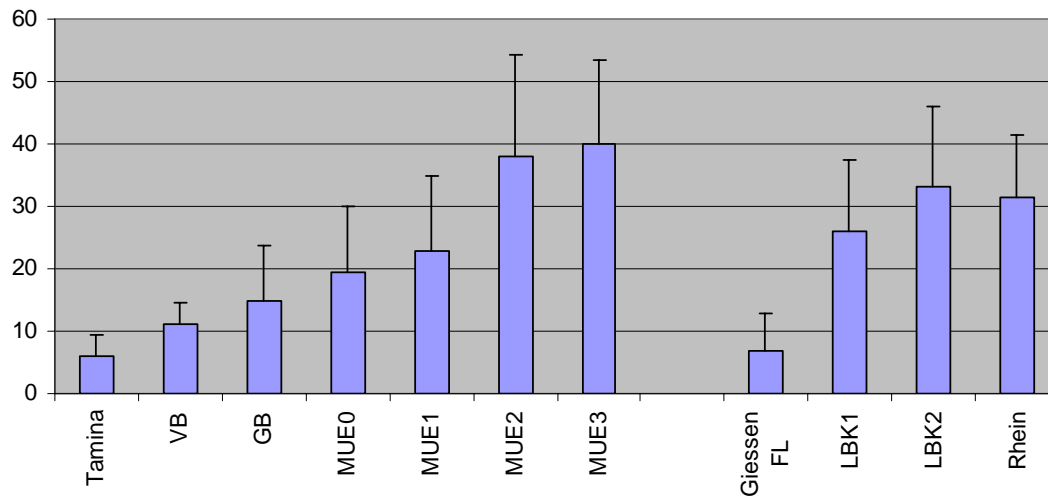
Tumour like alterations in liver

# River system in eastern Switzerland

## Histology of liver and kidney



Health status of fish in Alpenrhein-System  
Histology index of liver and kidney



# River system in eastern Switzerland

## Conclusions I



- No single factor responsible for decrease
- No pathogens found
- Organ alterations increasing with distance from source of rivers
- No particular chemical substance found as causing agent of alterations
- Hypothesis: effects of sewage treatment works

# River system in eastern Switzerland

## Conclusion II



### Evaluation of histology results

- Development of protocol to assess alterations -> comparisons of results from fish of different locations possible

Journal of Fish Diseases 1999, 22, 25-34

#### **Histopathology in fish: proposal for a protocol to assess aquatic pollution**

**D Bernet<sup>1,2</sup>, H Schmidt<sup>1</sup>, W Meier<sup>1</sup>, P Burkhardt-Holm<sup>1,2</sup> and T Wahli<sup>1</sup>**

<sup>1</sup> Centre for Fish and Wildlife Health, Institute of Veterinary Pathology, University of Berne, Berne, Switzerland

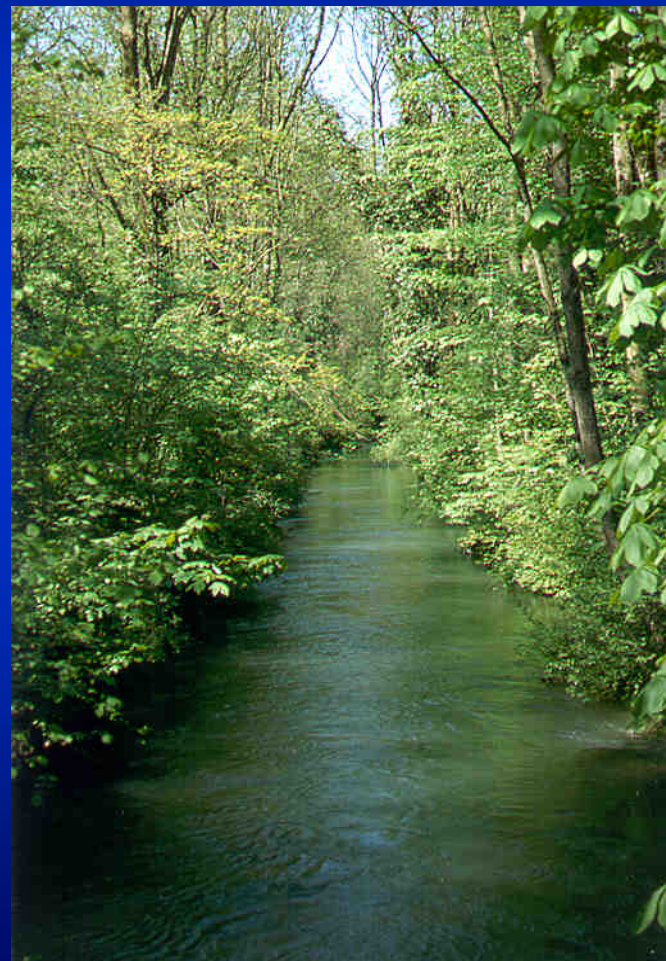
<sup>2</sup> Interdisciplinary Centre for General Ecology, University of Berne, Berne, Switzerland

# Impact of sewage treatment works



## Exposure experiments

- Exposure of fish in tanks or cages to different water qualities including diluted treated waste water
- Evaluation of health status after different exposure periods

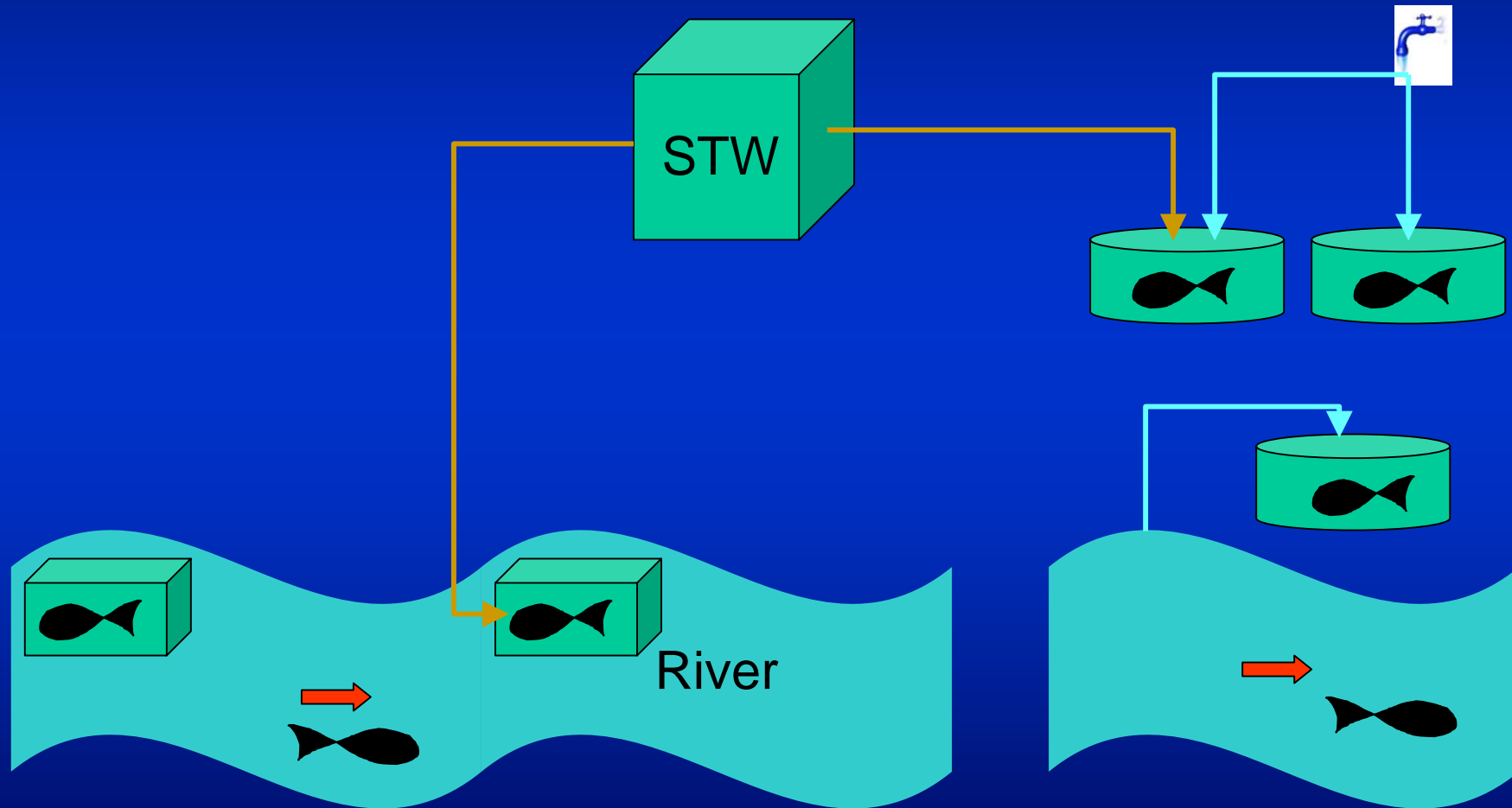


River Alte Aare near Lyss

# Impact of sewage treatment works



## Exposure experiments: designs

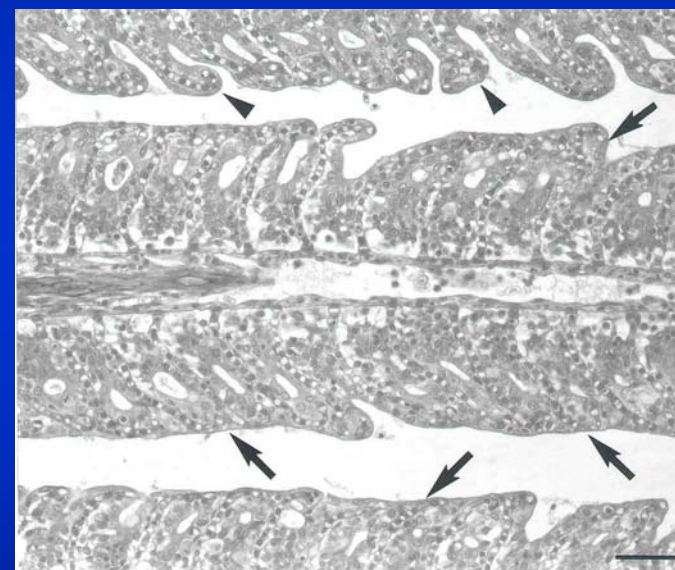


# Impact of sewage treatment works



## Results of exposure experiments

- Fish exposed to treated waste water developed mainly liver but also gill and kidney alterations
- Alterations less pronounced than in fish from river
- Part of alterations of fish kept in river water result of PKD
- Mortalities in exposed fish mainly attributed to furunculosis and PKD
- Some fish exposed to mixture of river and treated waste water infected by *Aeromonas sobria / hydrophila* and *Pseudomonas putida*



Fusion of secondary gill lamellae

Bernet et al. 2001a, b, 2004  
Schmidt-Posthaus et al. 1999, 2001

# Impact of sewage treatment works



## Conclusions from exposure experiments

- Treated diluted waste water adds to organ alterations but for itself causes no mortalities
- Fish exposed to river water in small containments are likely to be infected by different disease agents
- Pathogenic agents in fish kept in containments fed with river water :
  - *Aeromonas salmonicida*
  - *Aeromonas sobria / hydrophila*
  - *Pseudomonas putida*
  - *Tetracapsuloides bryosalmonae*
- No viral diseases detected!

# General Health status



## Monitoring of brown trout in the canton of Berne

- Sampling of fish from different river locations within canton in spring and autumn (20 fish/site)
- Analyses for infectious diseases and organ alterations (liver, kidney)

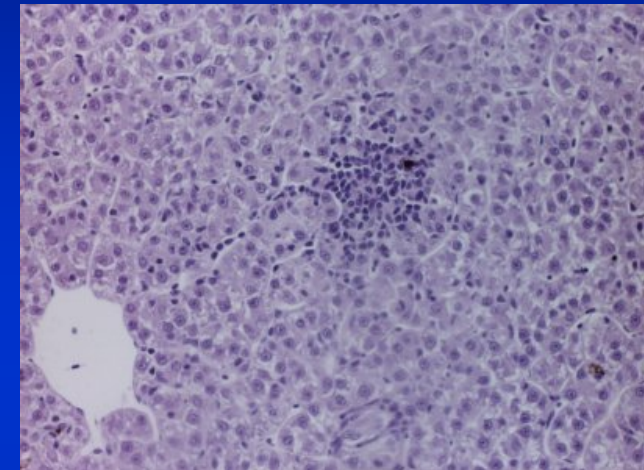


# Monitoring of brown trout in the canton of Berne

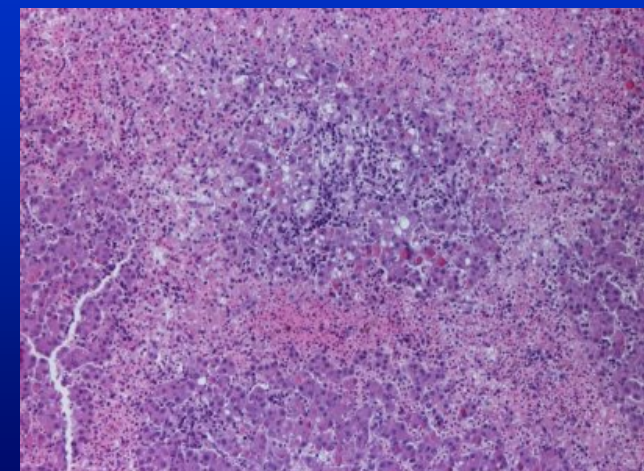


## Results

- No bacterial nor viral infections
- Parasites present but infection rates low
- PKD in several rivers
- Organ alterations:
  - Increasing with distance from river-source
  - In several locations more pronounced below than above STW effluents
  - More pronounced in autumn than in spring



Infiltration in liver tissue



Necrosis and haemorrhages in liver tissue



## Conclusions

- Clear seasonal differences
- In some locations health of fish impaired by STW-effluents
- PKD = only important infectious disease found
- No evidence for viral nor bacterial diseases



# Outline

- Fish health in Switzerland
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- Proliferative kidney disease
- Viral diseases
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# Monitoring project for PKD



## Background and aim

- In several previous projects PKD diagnosed
- In many cases PKD only infectious disease determined
- Overall situation in Switzerland not known
- Hypothesis: PKD possible cofactor for decrease of brown trout catches
- Project aimed at establishing data on presence of PKD in Swiss rivers and fish farms



# Monitoring project for PKD

## Material and Methods

- Sampling of fish from 139 rivers and 66 farms in summer / autumn 2000/2001
- 20 fish per site
- Histological evaluation of fixed kidney samples



# Monitoring project for PKD

## Results

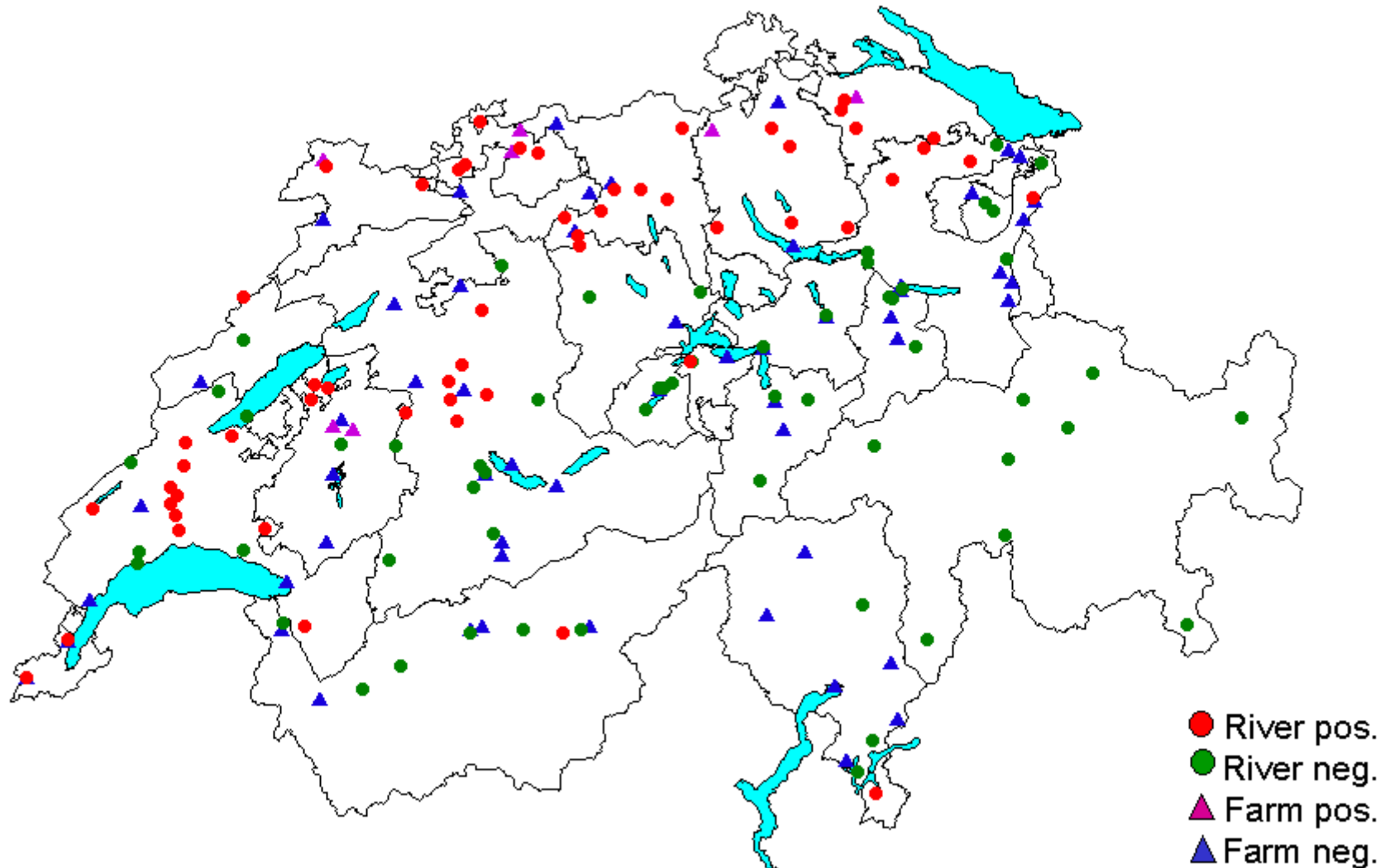
|       | Positive   | Negative    | Total |
|-------|------------|-------------|-------|
| River | 56 (40%)   | 83 (60%)    | 139   |
| Farm  | 7 (10.5%)  | 59 (89.5%)  | 66    |
| Total | 63 (30.7%) | 142 (69.3%) | 205   |

Wahli et al. 2002  
Schubiger et al. 2003

# Monitoring project for PKD



## Distribution of +/- sites

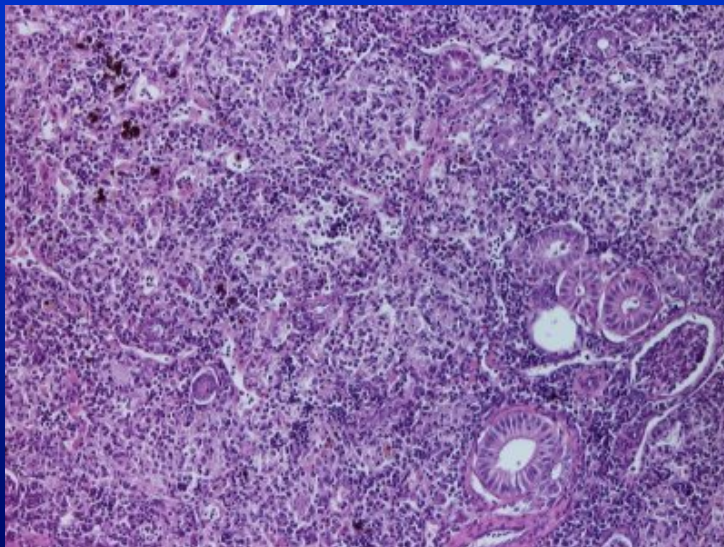




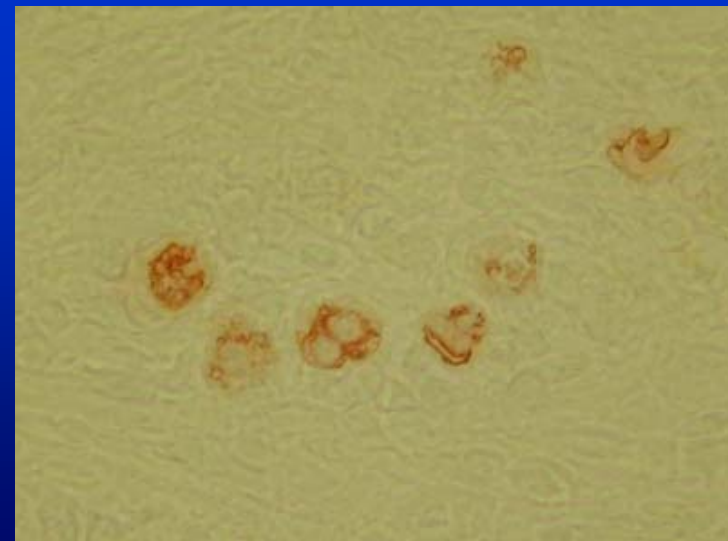
# Monitoring project for PKD

## Results

- In most sites only part of sampled fish infected
- Not in all infected fish macroscopic alterations visible  
-> pathologic changes more pronounced in lower reaches of rivers



Kidney infected by *Tetracapsuloides bryosalmonae*



Immunohistochemistry for *T. bryosalmonae*

# Monitoring project for PKD



## Conclusions

- Percentage of positive sites higher in rivers than in farms
- Overall percentage of positive sites higher than expected from data of routine diagnostic material
- Positive river sites mainly in Swiss midlands
- Hypothesis: influence of water temperature and water quality
- PKD has possible effect on wild fish populations



# Outline

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- Proliferative kidney disease
- **Viral diseases**
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# Viral diseases



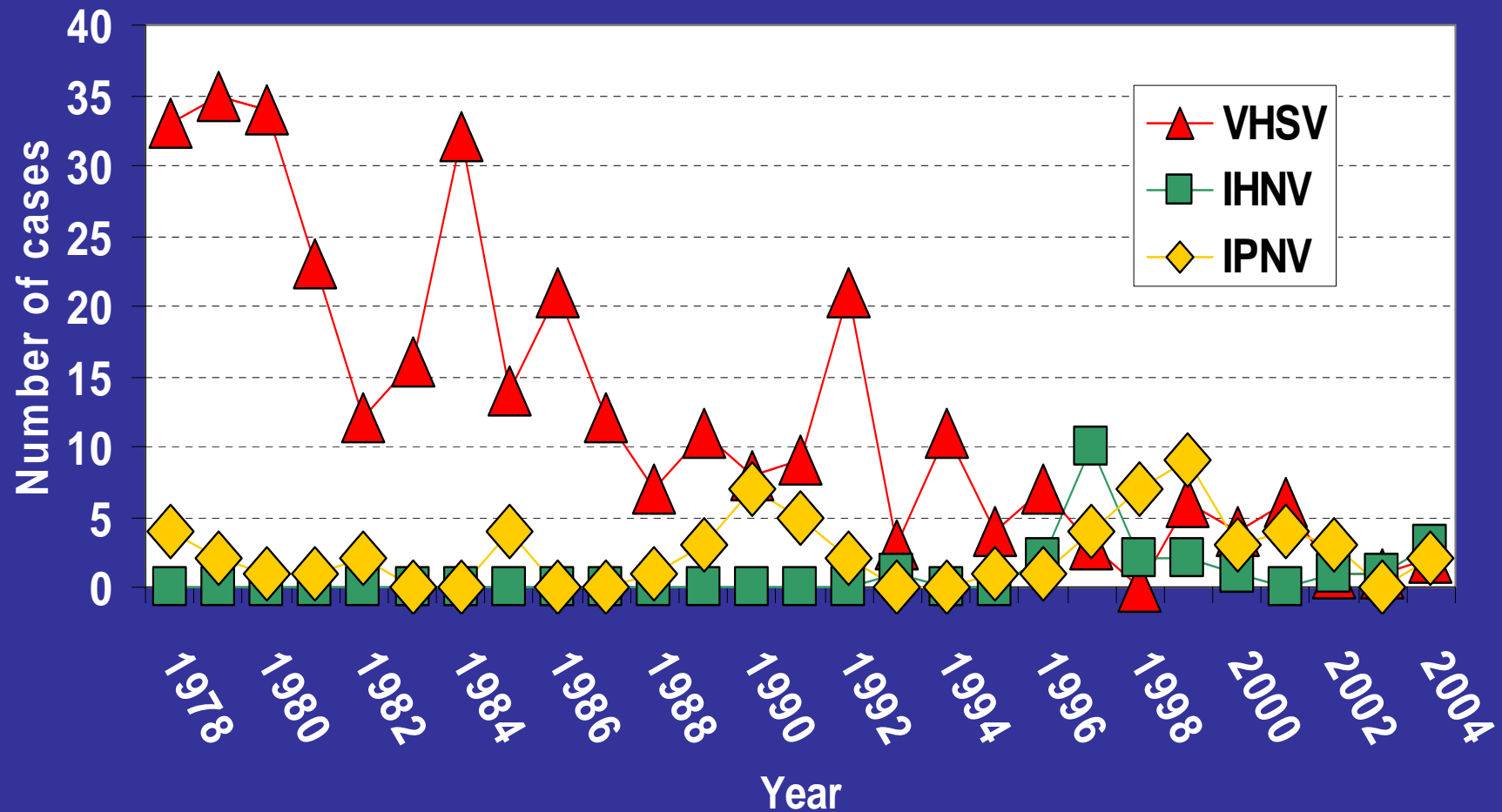
## Background

- In different monitoring programs never evidence for viral diseases
- But: no regular surveillance program
- Last nation wide monitoring in 1984/1985
- Current overall situation unknown

# Viral diseases



## Data from routine diagnostics



Knüsel et al. 2003

# Viral diseases



## Monitoring project

### Diagnostic data

- Selected material -> limited value for a general statement on situation in country -> monitoring needed

### Aims

- Refresh knowledge on situation of viral diseases in Switzerland
- Compare different diagnostic methods (cell culture versus PCR)

# Monitoring project for viral diseases



## Methods

### Sampling

- Sampling of fish from farm and river sites
- 1-5 pools of 5 fish each per site

### Virus-Isolation:

- Cultivation of pooled material on BF- and EPC cell-lines
- IFAT in case of virus growth

### Analyses for anti-VHSV-antibodies

- Single sera for serum-plaque neutralisation test

### PCR

- Selected samples for IHNV and VHSV



# Monitoring project for viral diseases



## Samples

### Fish from:

141 farms (57 governmental, 84 private)

300 pooled samples for virology

870 sera for SPNT

Species: **rainbow and brown trout**,  
brook trout, grayling, pike, whitefish

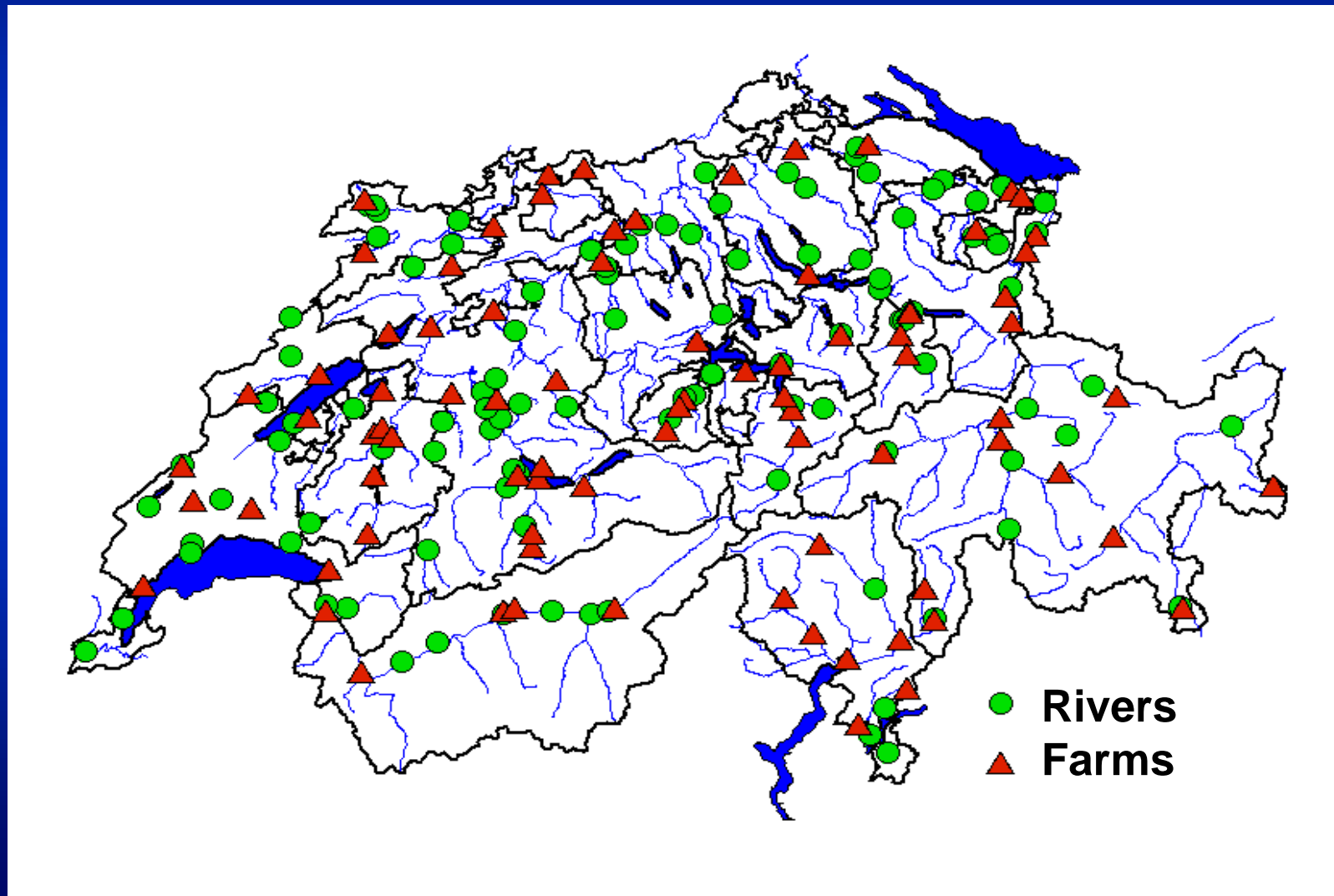
113 rivers

Species: brown trout  
222 pooled samples for virology  
1040 sera for SPNT

# Monitoring project for viral diseases



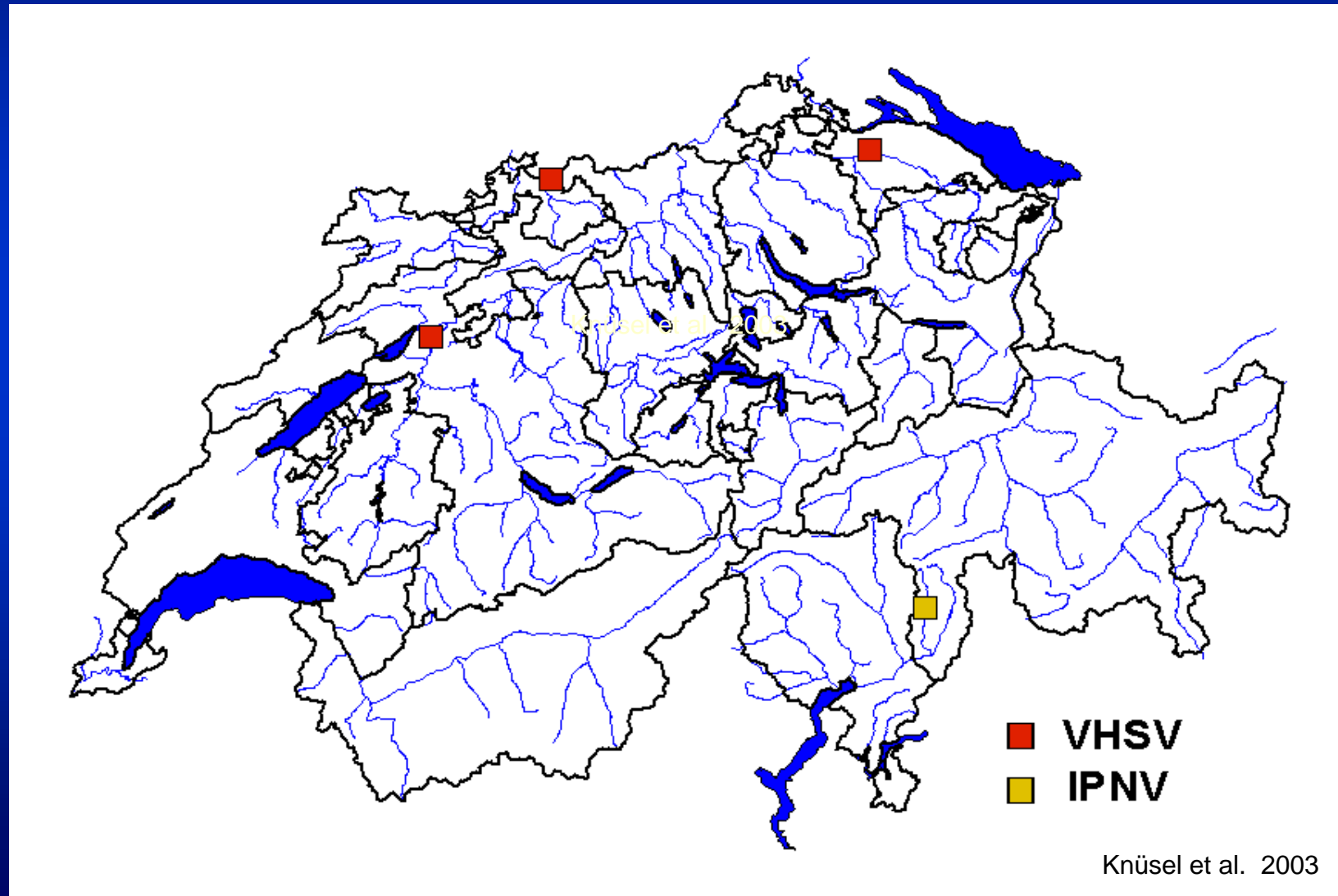
## Sampling sites



# Monitoring project for viral diseases



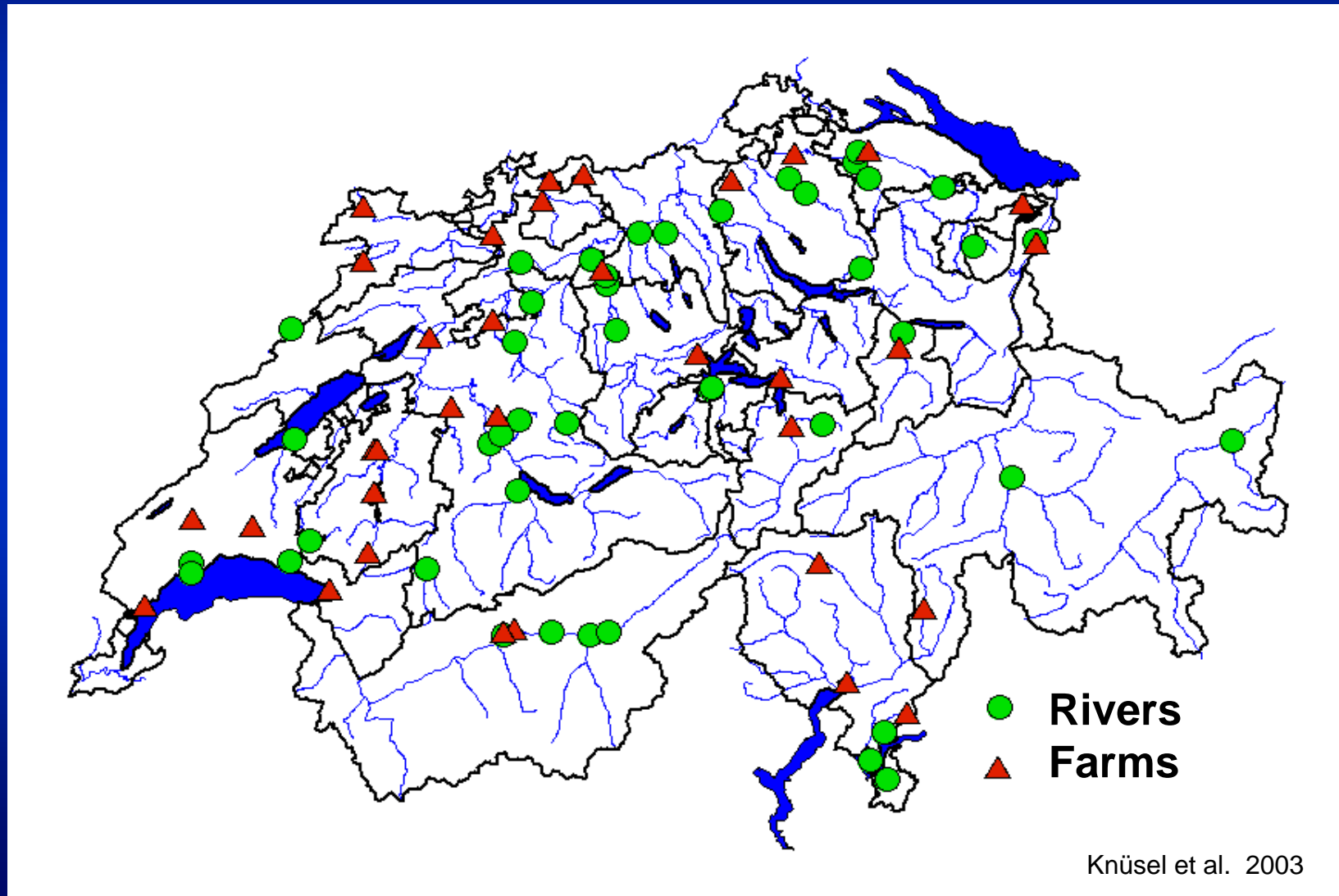
## Results virus isolation



# Monitoring project for viral diseases



## Results SPNT



# Monitoring project for viral diseases



## Summary project part 1

### Virusisolation (cell-culture):

|                     |               |
|---------------------|---------------|
| VHSV pos.           | 3 / 254 sites |
| IHNV pos.           | 0 / 254 sites |
| IPNV pos.           | 1 / 254 sites |
| Type of pos. sites: | Farms only    |

### Serology (SNPT) :

|         |                    |
|---------|--------------------|
| VHSV-Ab | 84 / 229 sites     |
|         | -> 44 / 112 rivers |
|         | -> 40 / 117 farms  |

# Monitoring project for viral diseases



## Questions

Reasons for difference between results of virus isolation and demonstration of antibodies?

Due to low sensitivity of cell culture method?

⇒ Project part II: RT-PCR on part of samples to test sensitivity of cell-culture method

# Monitoring project for viral diseases

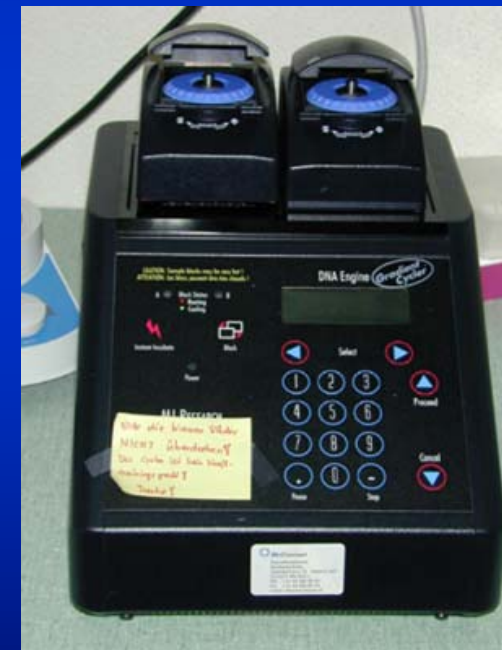


## RT-PCR: samples

Samples from all sites with:

- Virus isolation in part I
- Proof of VHSV-Ab in part I

172 pools from 84 sites



# Monitoring project for viral diseases



## RT-PCR: results

| Virus | Site-type | Virus isolation | RT-PCR | Difference |
|-------|-----------|-----------------|--------|------------|
| VHSV  | River     | 0/44            | 0/44   | 0          |
|       | Farm      | 3/40            | 4/40   | 1          |
| IHNV  | River     | 0/44            | 1/44   | 1          |
|       | Farm      | 0/40            | 1/40   | 1          |

# Monitoring project for viral diseases



## Conclusions from project

- Agreement between project results and data from routine diagnostic
- No major change of situation since surveillance of 84/85
- No major difference between results of virus isolation and RT-PCR
- Difference between virus isolation / RT-PCR and SPNT
- Reason for difference not yet clear
- First detection of IHNV-gene in wild fish in Switzerland (brown trout)



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# Summary

- Most important disease in wild salmonids in Switzerland = PKD
- Low frequency of viral diseases
- Pathogenic bacteria present in rivers but most often not detectable
- Organ alterations frequently found in fish from Swiss rivers but no correlation to infectious diseases (except PKD)

# Conclusions

## (with respect to DIPNET)



- IHN assumed to be introduced to Europe with imported fish -> transmission from farmed to wild fish probable
- Possible spread of PKD in Switzerland by stocking of infected fish
- Surveillance of wild fish difficult due to number of fish needed



# Acknowledgements

- Heike Schmidt-Posthaus, Daniel Bernet, Ralph Knüsel, Carla Schubiger and Helmut Segner
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- Swiss Federal Veterinary Office
- Swiss Agency for the environment forests and landscape