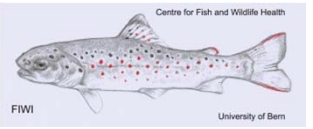
UNIVERSITÄT BERN

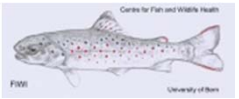


Factors influencing the fish health: The example of temperature and Proliferative Kidney Disease

Heike Schmidt-Posthaus, Thomas Wahli, Nicole Strepparava

Centre for Fish and Wildlife Health

Fish health and influencing factors



Proliferative Kidney Disease

Infectious agents

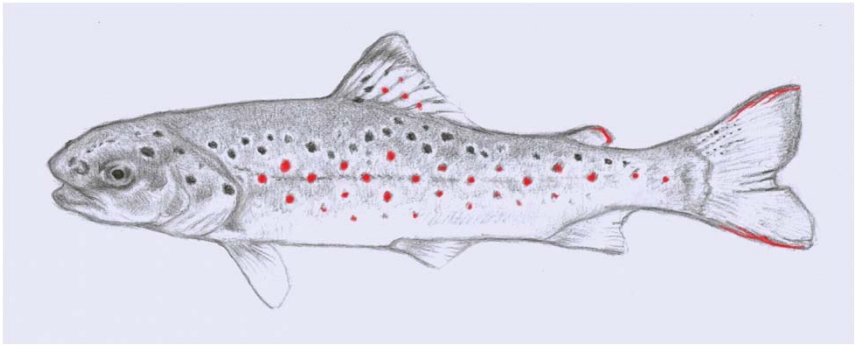
Parasites

Bacteria

Viruses

Temperature

Water quality



Competitors

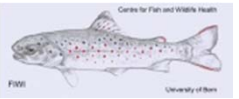
Predators

Hosts

Nutrition

Habitat

Proliferative Kidney Disease (PKD)



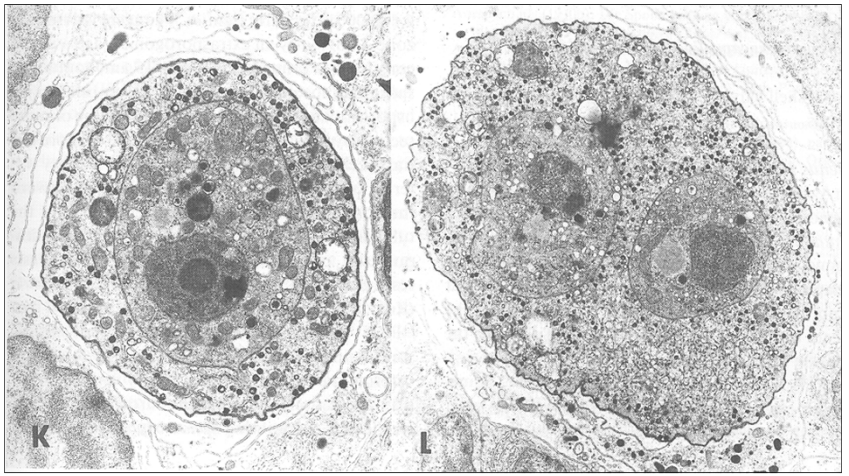
Etiology*: Tetracapsuloides bryo-salmonae,* Malacospora (myxozoa)

Multicellular obligate endoparasite (metazoa)

Now classified as Cnideria

Life-cycle includes vertebrate and invertebrate hosts

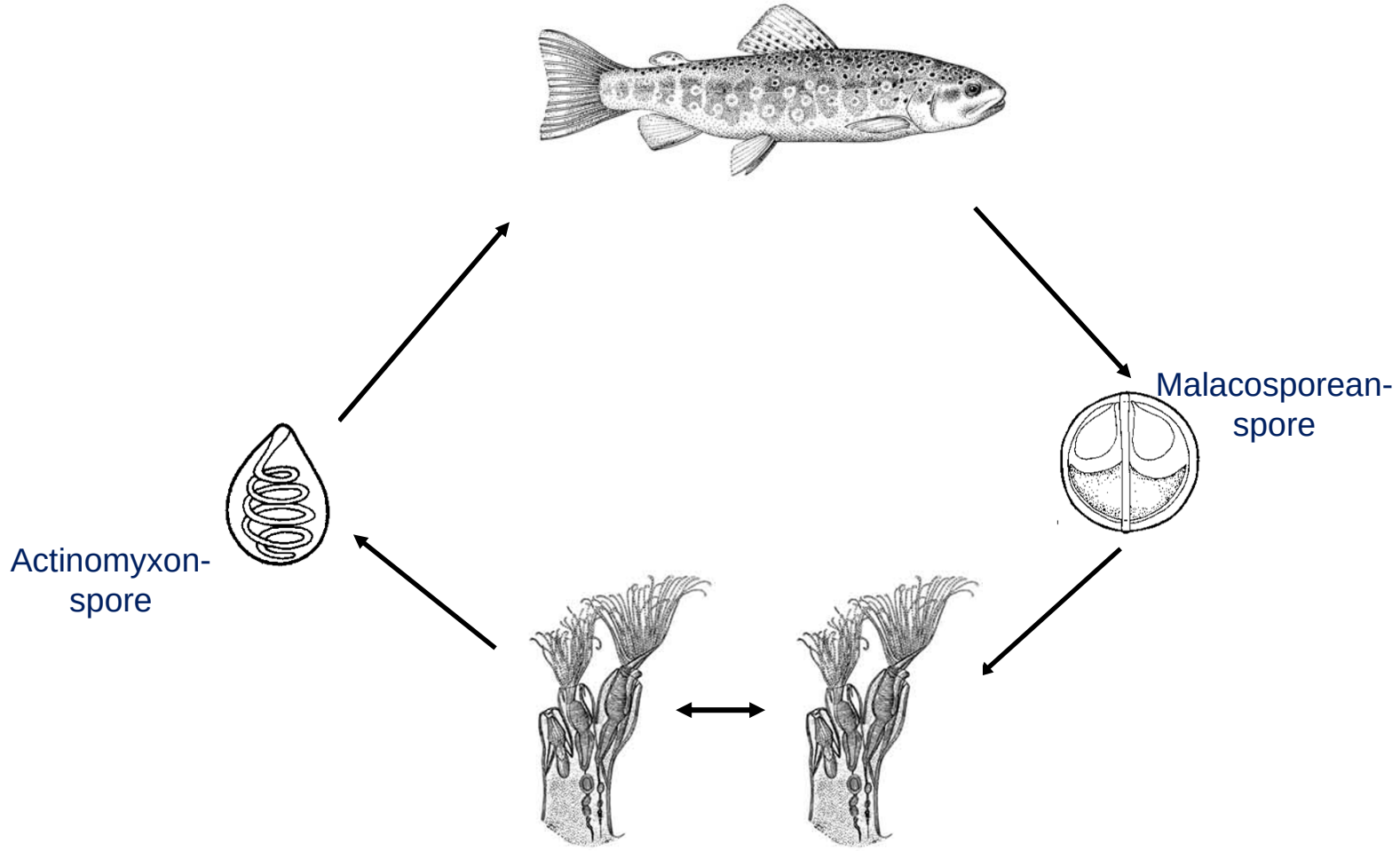
Mortality in fish host up to 100%



From: Lom & Dykova, Protozoen parasites of fishes

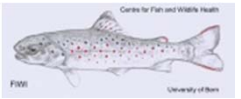
Okamura et al. (2011)

Parasite cycle



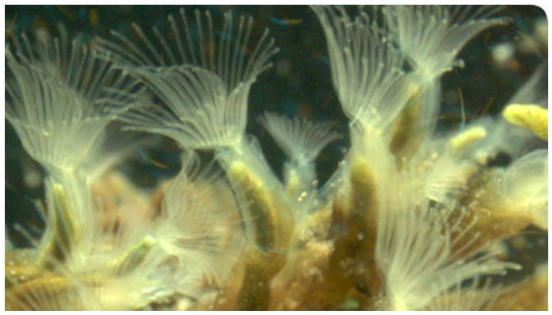
Okamura et al. (2011)

Vertebrate and invertebrate hosts

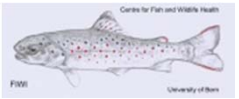


Salmonids (brown trout, rainbow trout, Atlantic salmon, charr, grayling)

Bryozoa, different species

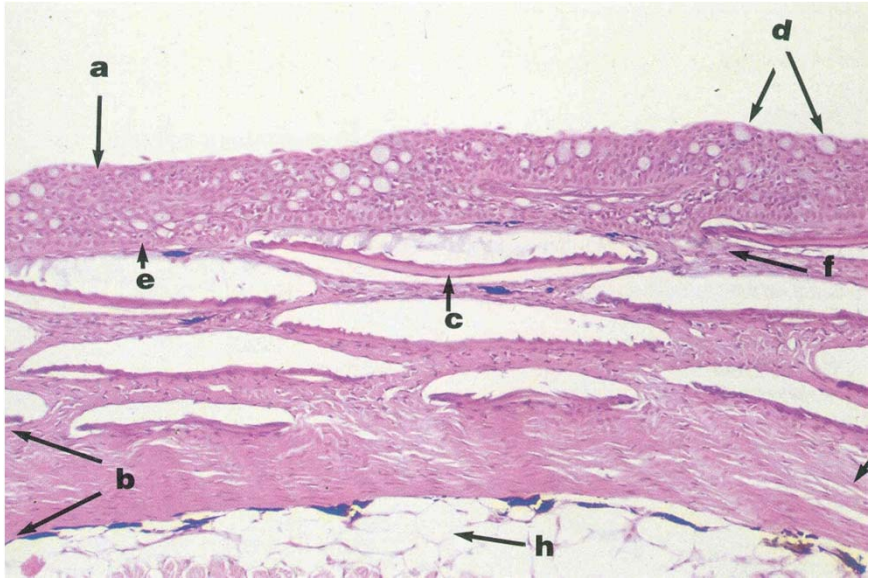


Infection pathway



Possible entry ports:

→ Gills are a major pathogen entry site



Skin:

Multiple cell layers

Barrier composed of mucus, mucopoly-sacharides, immunoglobulins, etc

Goblet cells, eosinophilic granular cells, lymphocytes, macrophages

Gills:

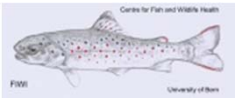
Single epithelial cell-layer

Mucus/immune barrier less prominent

High ventilation rate

Large surface (up to 60x body surface)

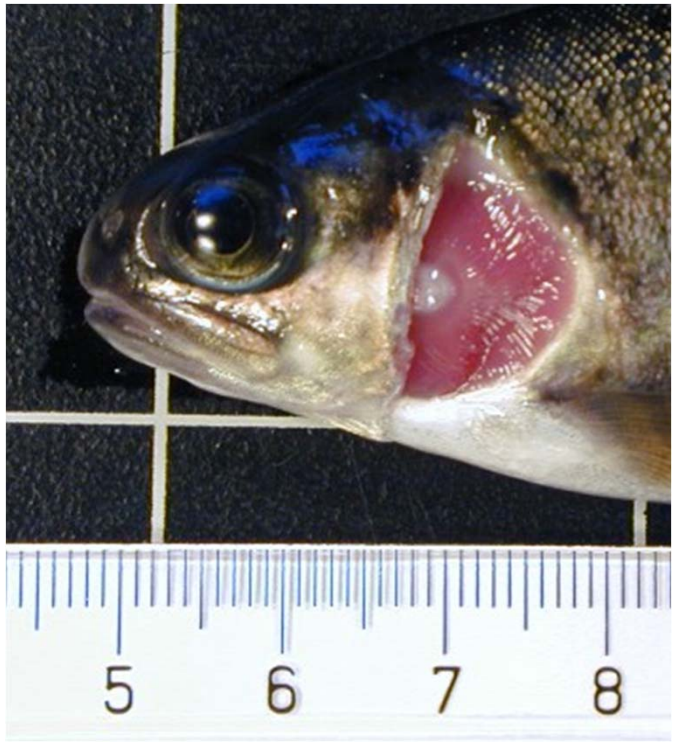
Anatomy of organs - Gills

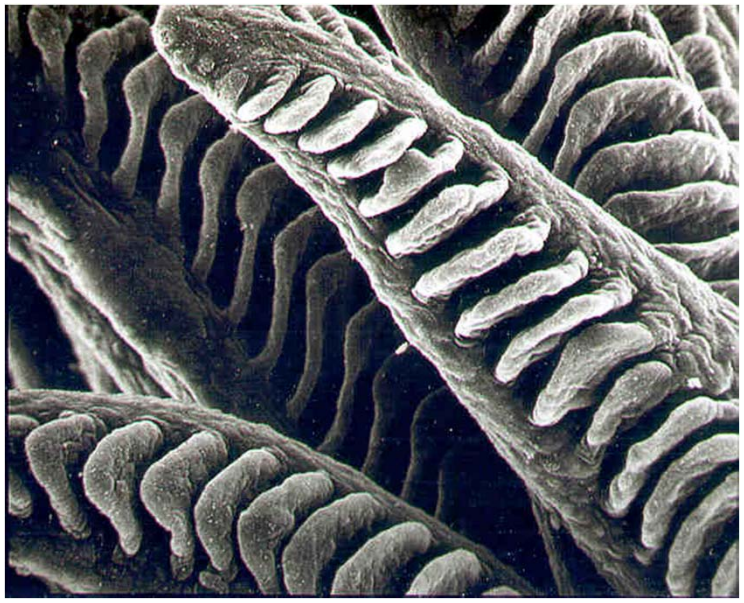


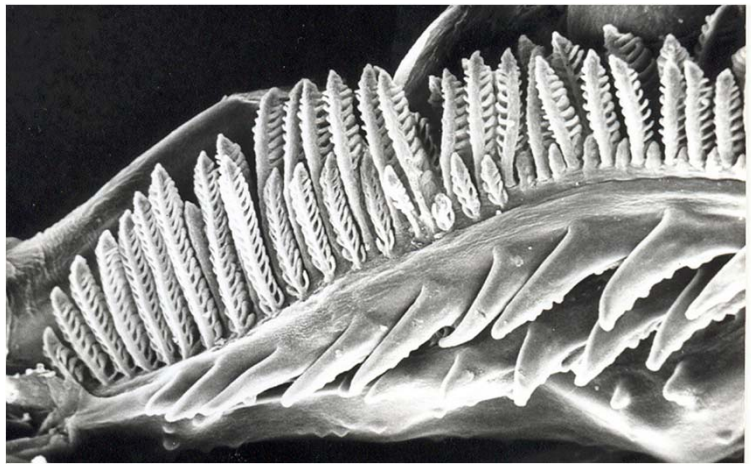
4 holobranches at each side of pharynx

Each holobranch consists of 2 hemibranches

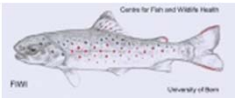
Rows of filaments with two semilunar folds of lamellae







Respiration in fish compared to mammals



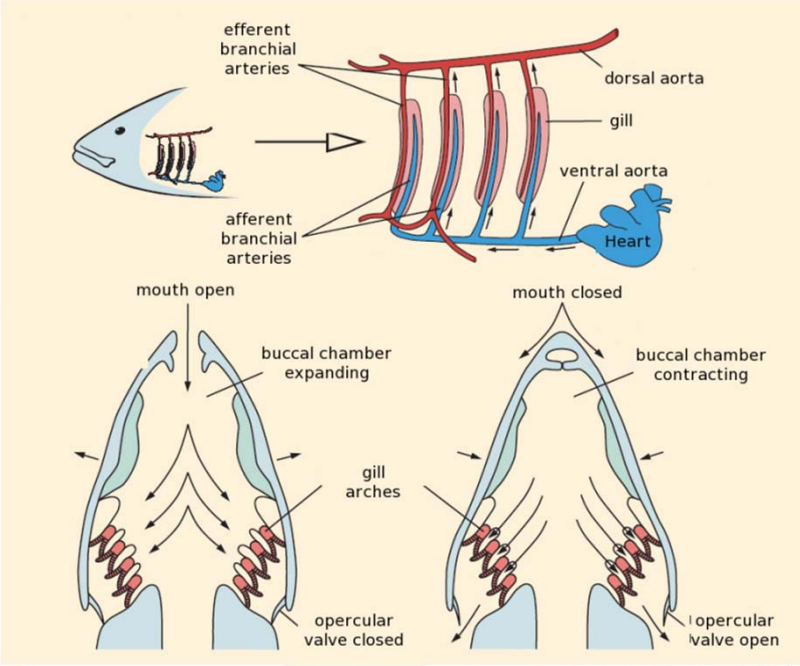
Water compared to air

lower O_2 partial pressure

(30x less compared to air)

Dependent on water temperature

Respiratory system is very efficient

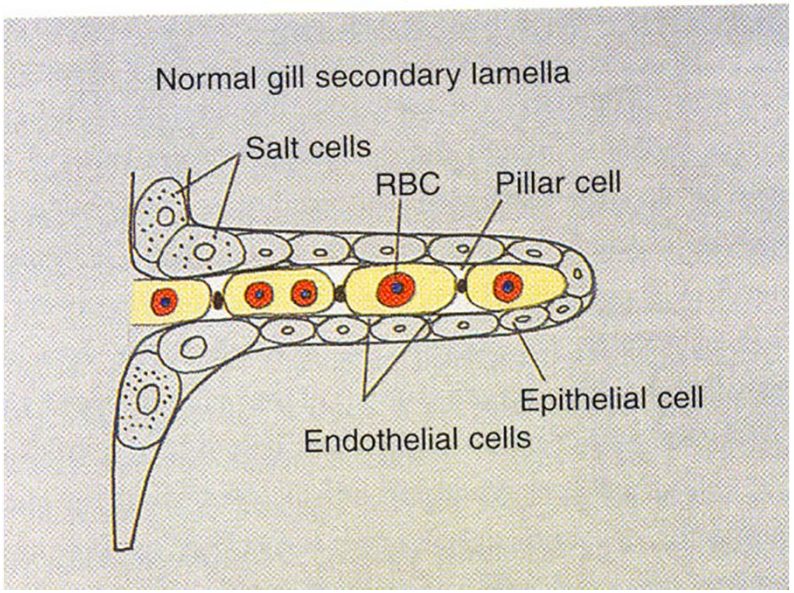


Respiration in fish is based on diffusion

Function

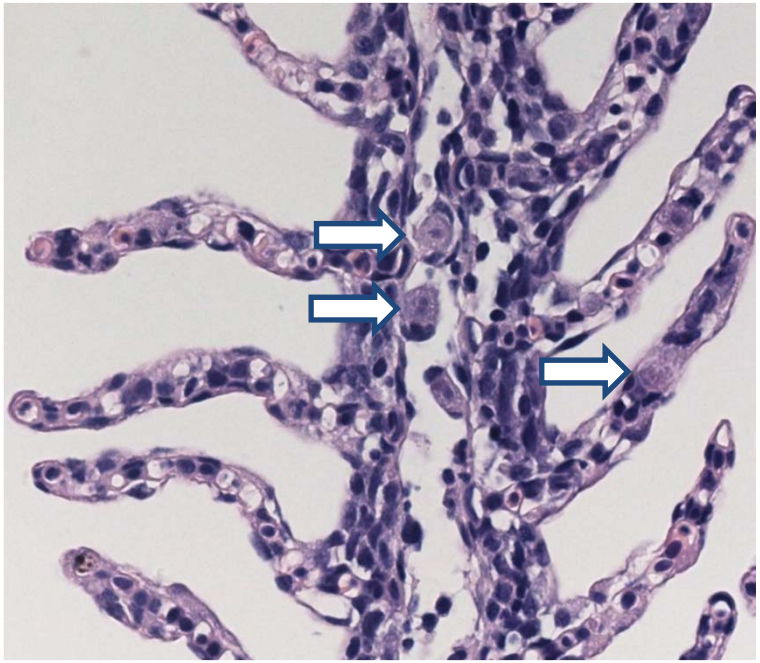
Uptake of O_2 only in dissolved form

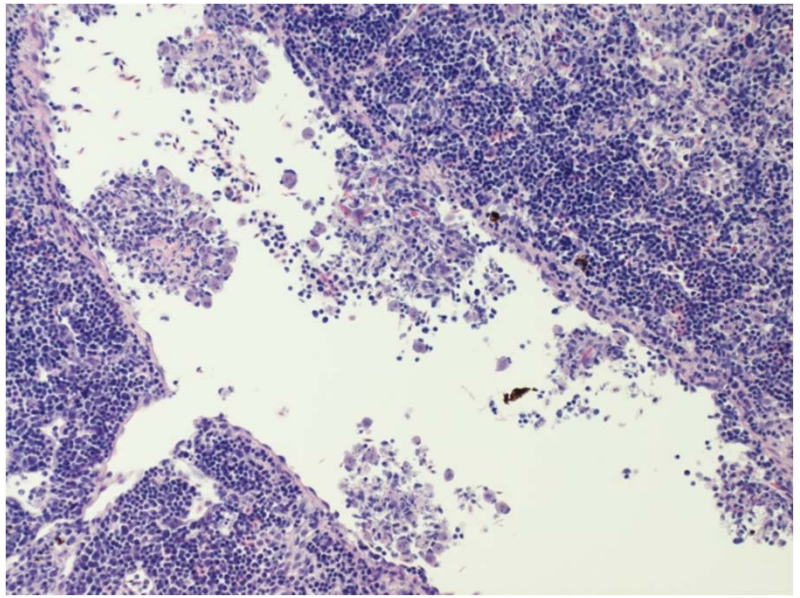
Gas exchange, removal of nitrogenous waste, electrolyt exchange (Cl^-/Ca^{2+} via chloride cells, Na^+ via pavement cell)



Infection pathway

Entering through the gills → into the blood system → and then ?

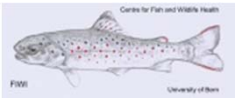


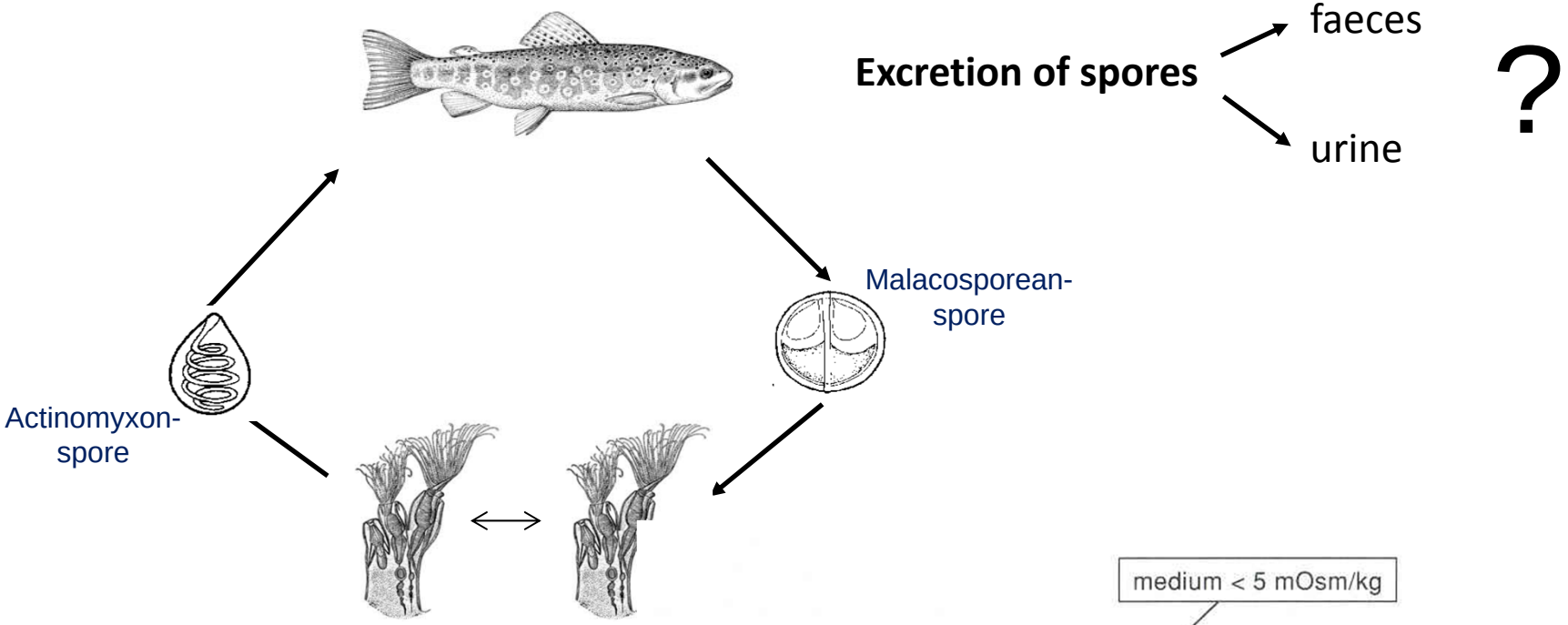


?

Bettge et al. (2009)

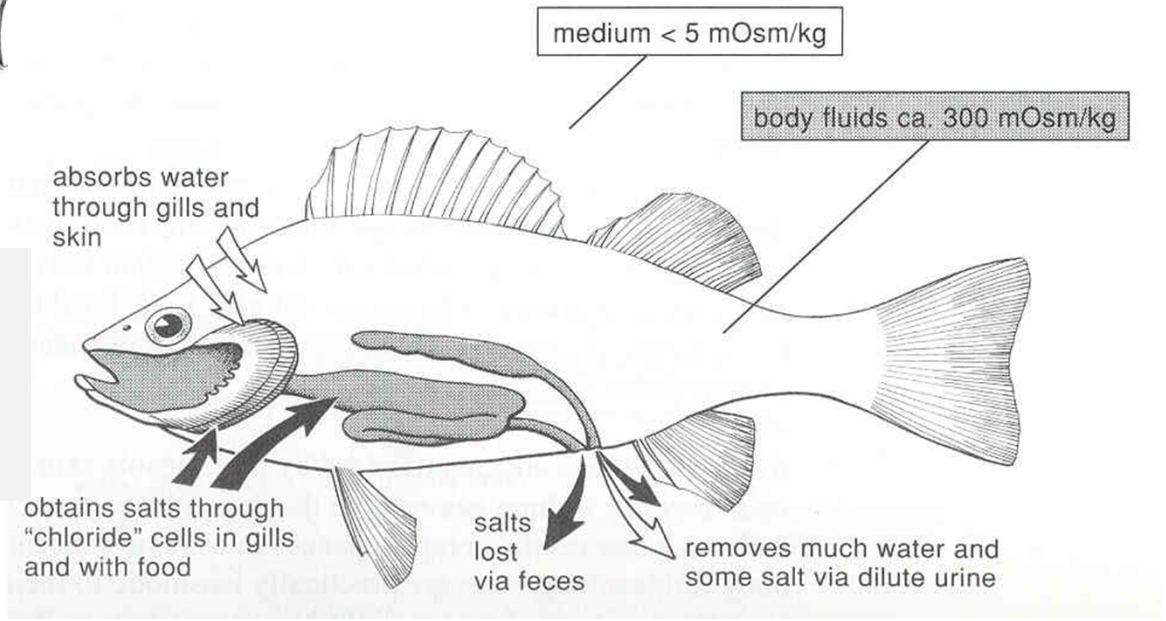
Which organ may be a good target organ ?





Passive (osmotic) inflow of water

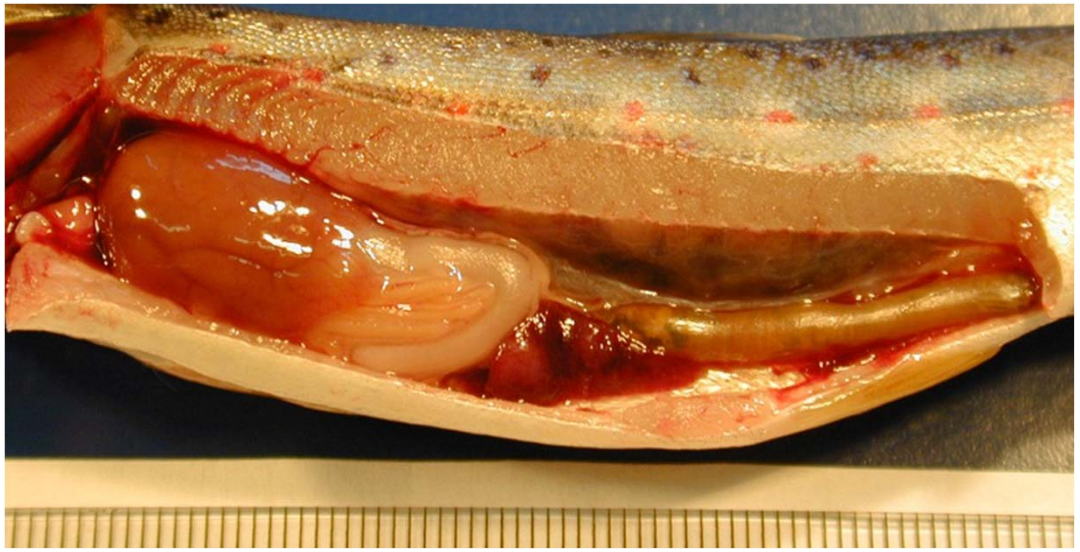
⇒ High rate excretion of dilute urine through the kidneys



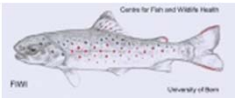
Infection pathway

Once the parasite has entered the fish through the gills, it heads for the kidneys, as this is a perfect side for spore release

Main internal target organ = kidney



Anatomy of the trout kidney



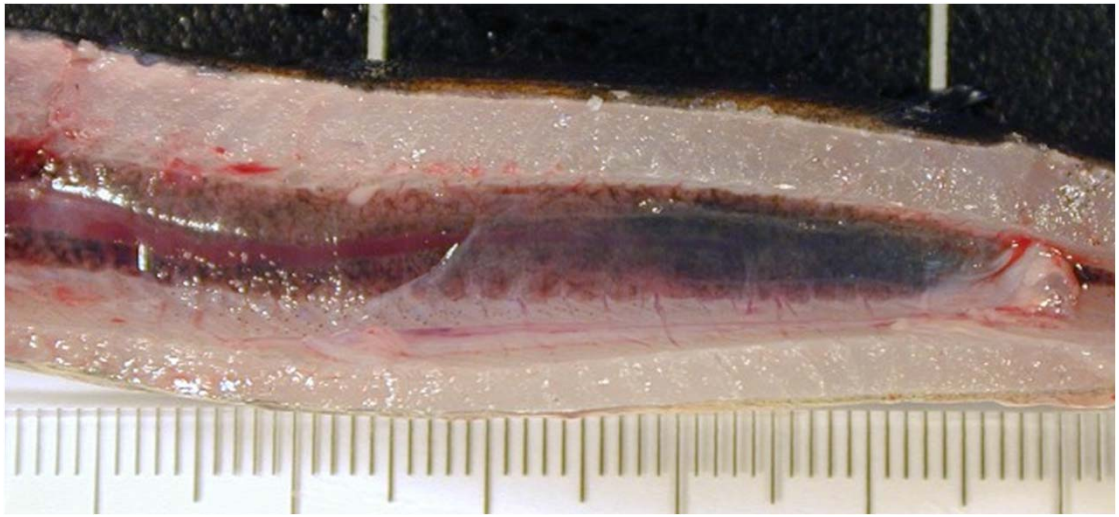
Location: Retroperitoneally ventral of spine

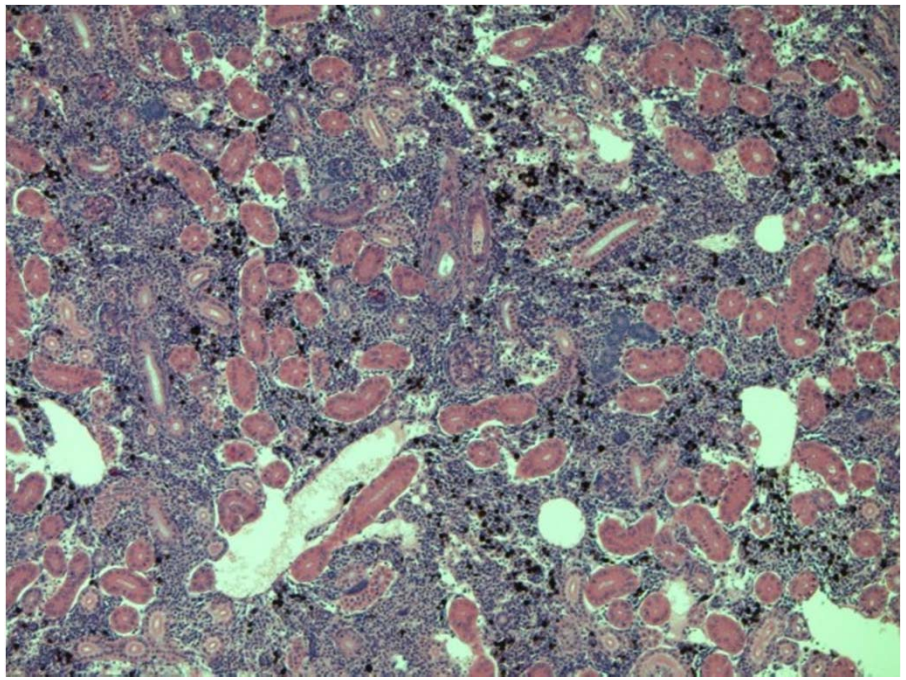
Maybe divided transversely (head and trunk kidney): 2 functions:

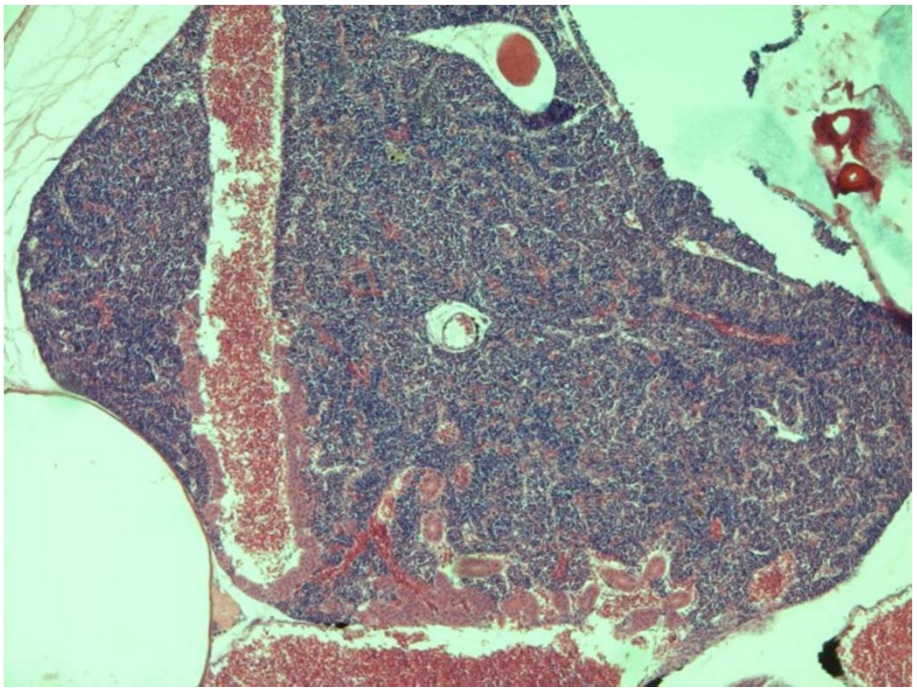
Head kidney: haematopoietic tissue (pronephros)

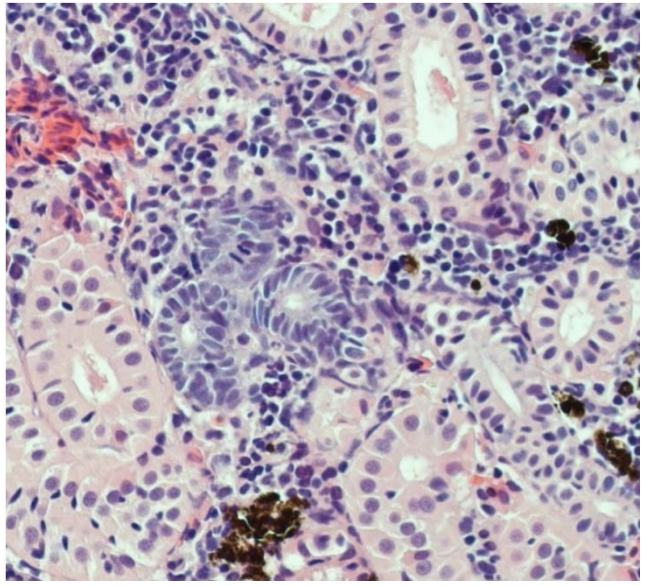
Trunk kidney: haematopoietic tissue and excretory elements (metanephros)

Function of excretory part: primarily water regulation (nitrogenous waste mainly excreted through gills)

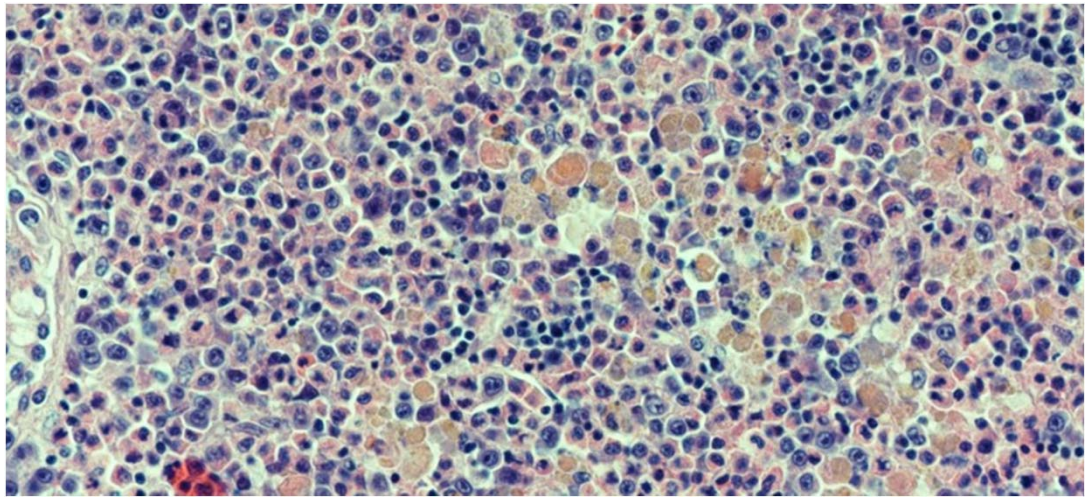








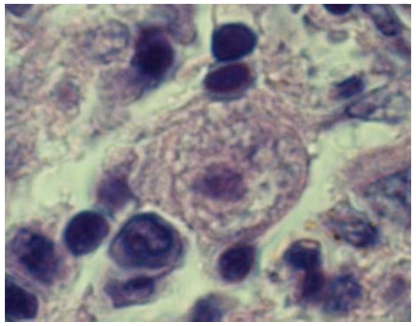
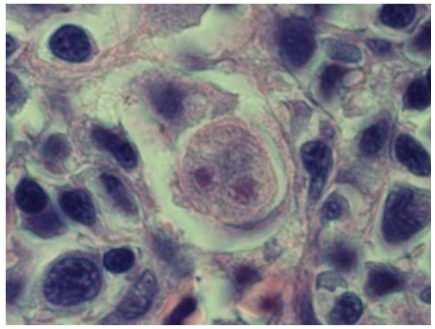
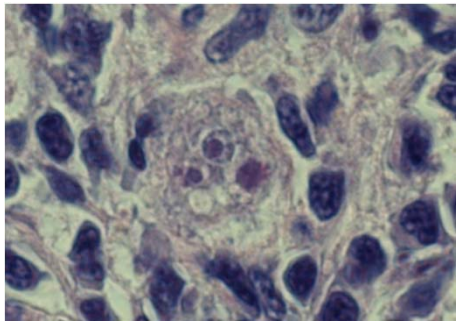
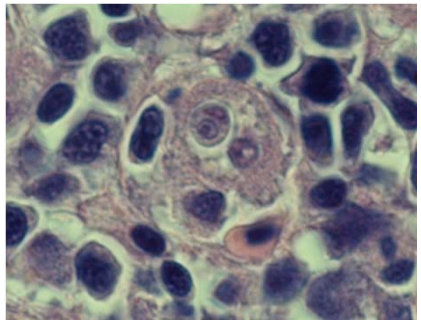
Tubuloneogenesis



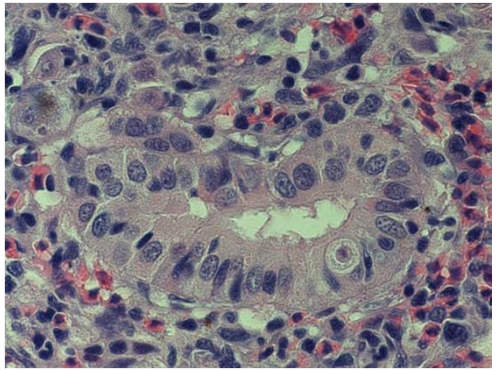
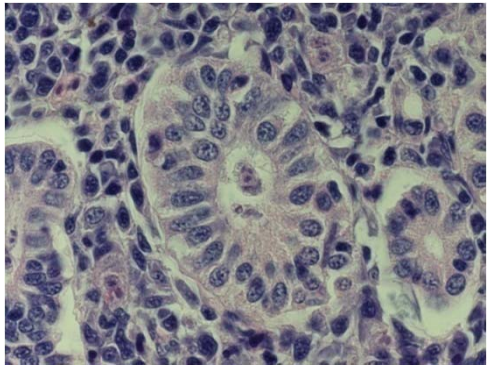
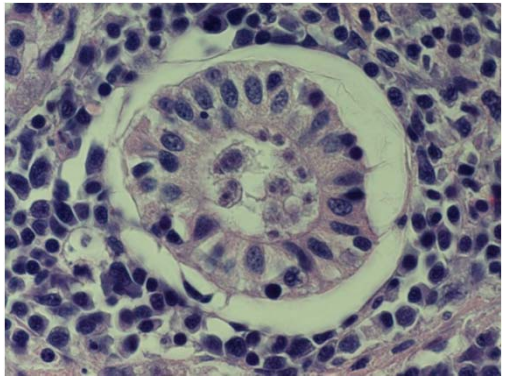
Melanomacrophage centres in different organs containing melanin, haemosiderin, ceroid, lipofuscin

Parasite development in the kidney

Exosporogonic form (15 μm in diameter) from unicellular to multicellular stage
Development in the haematopoietic tissue

Sporogonic form, production of soft-shelled spores and excretion → hyperregulation!

Clinical findings

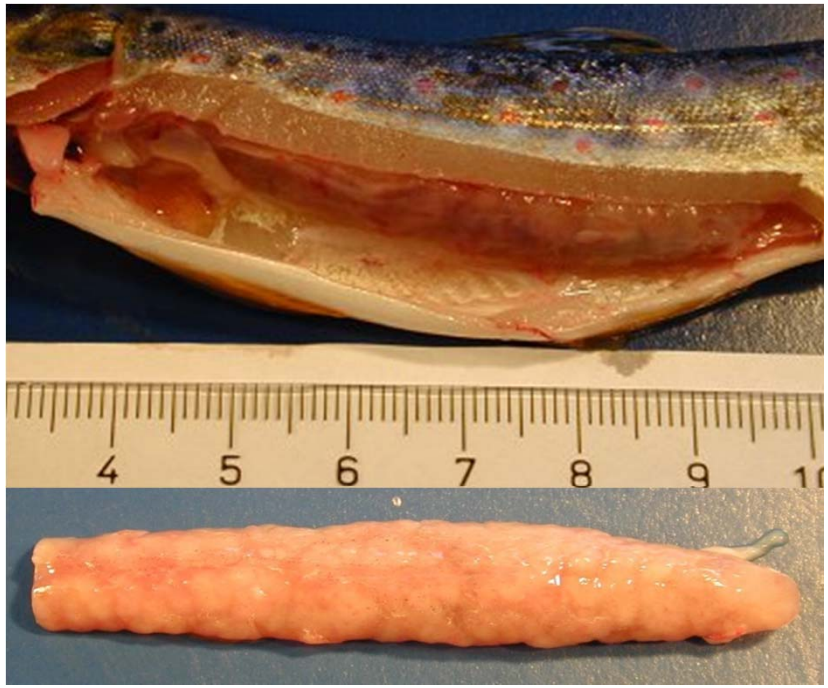
Dark coloration, exophthalmia

Ascites, pale gills

Proliferative and granulomatous nephritis,

numerous greyish areas, splenomegaly,

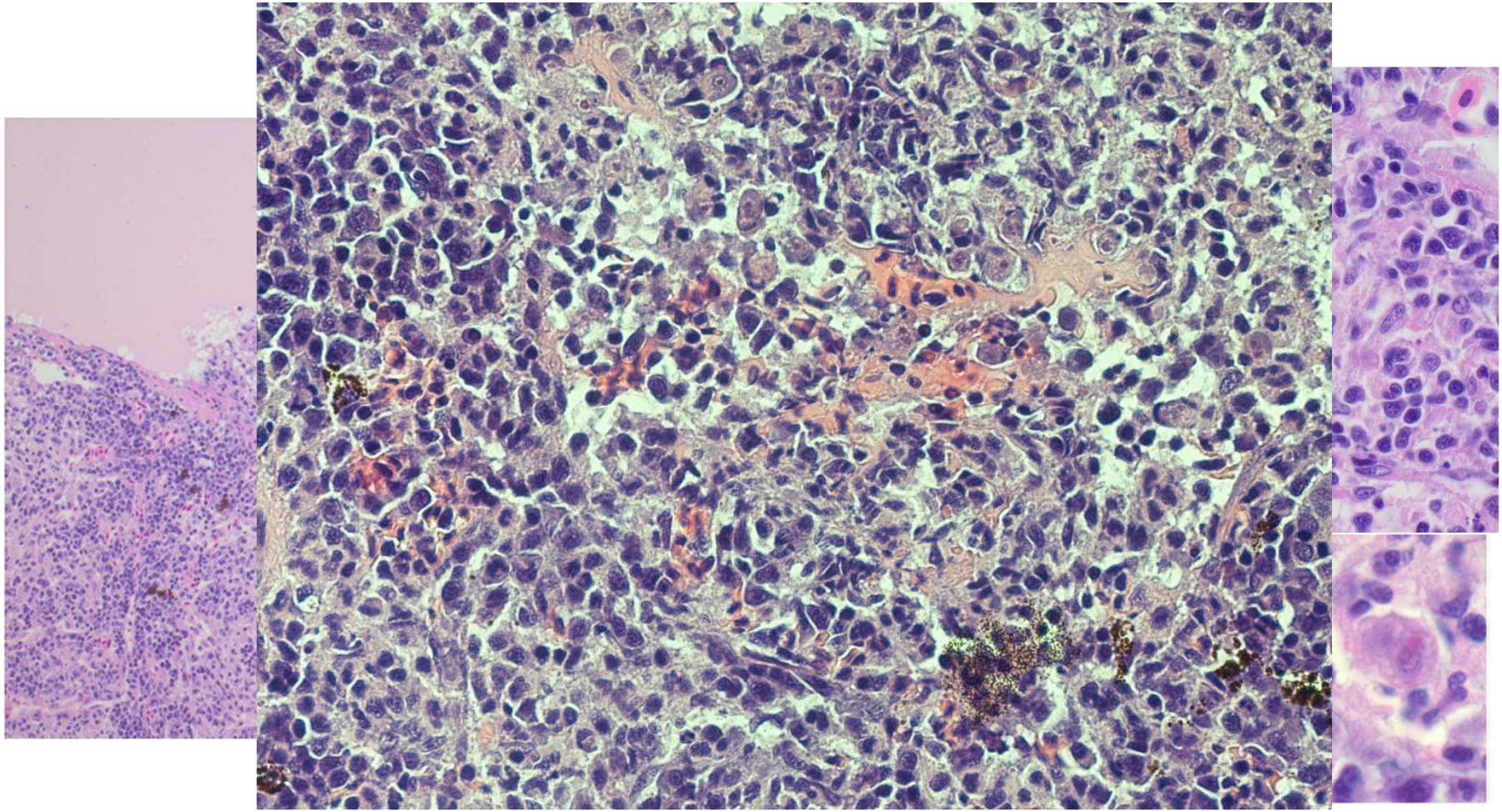
granulomatous splenitis and hepatitis



Histological kidney lesions

Vasculitis, widespread necrosis

Proliferative and granulomatous interstitial nephritis

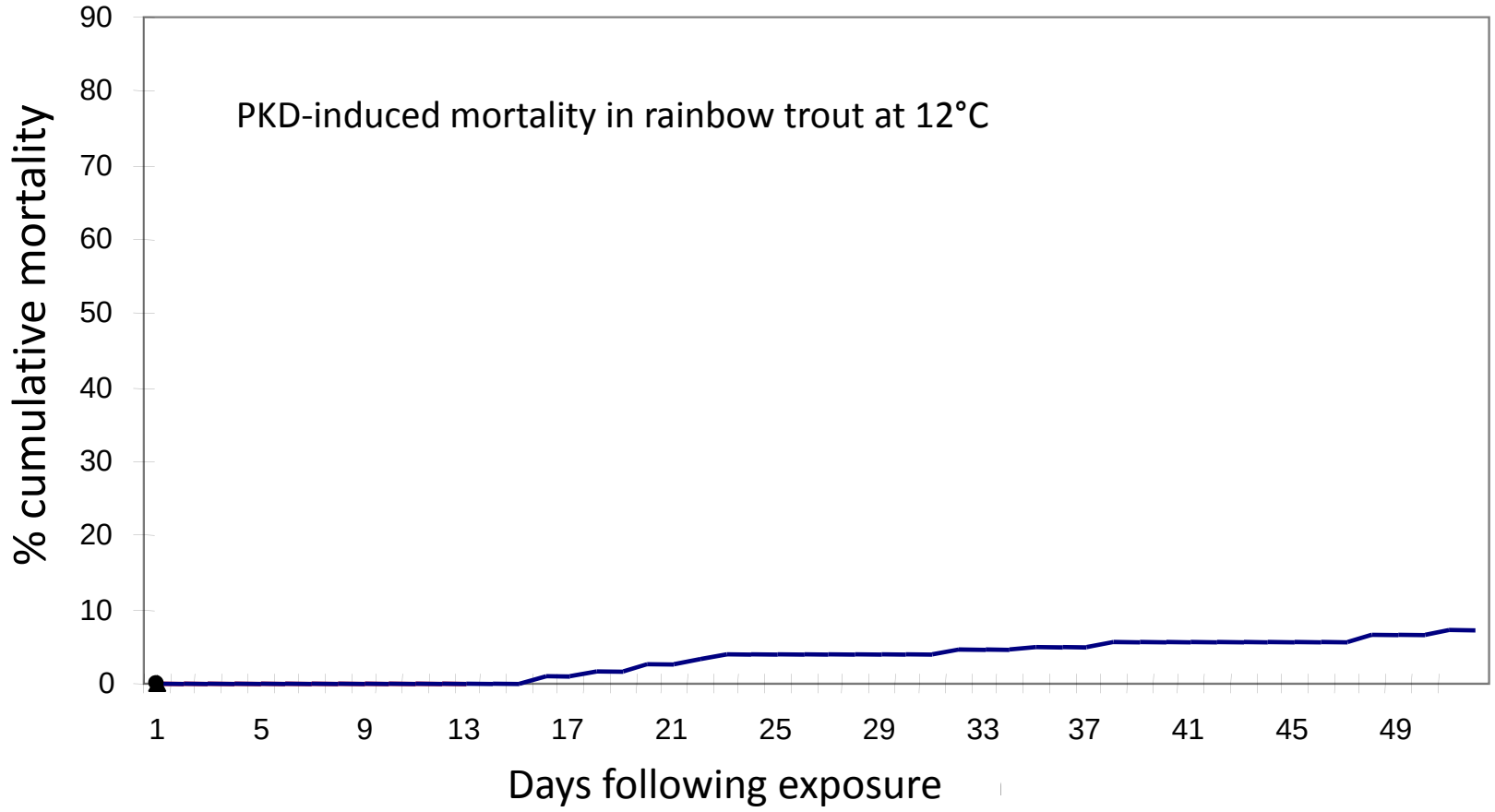


Is PKD causing massive fish mortalities?

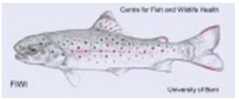
NO

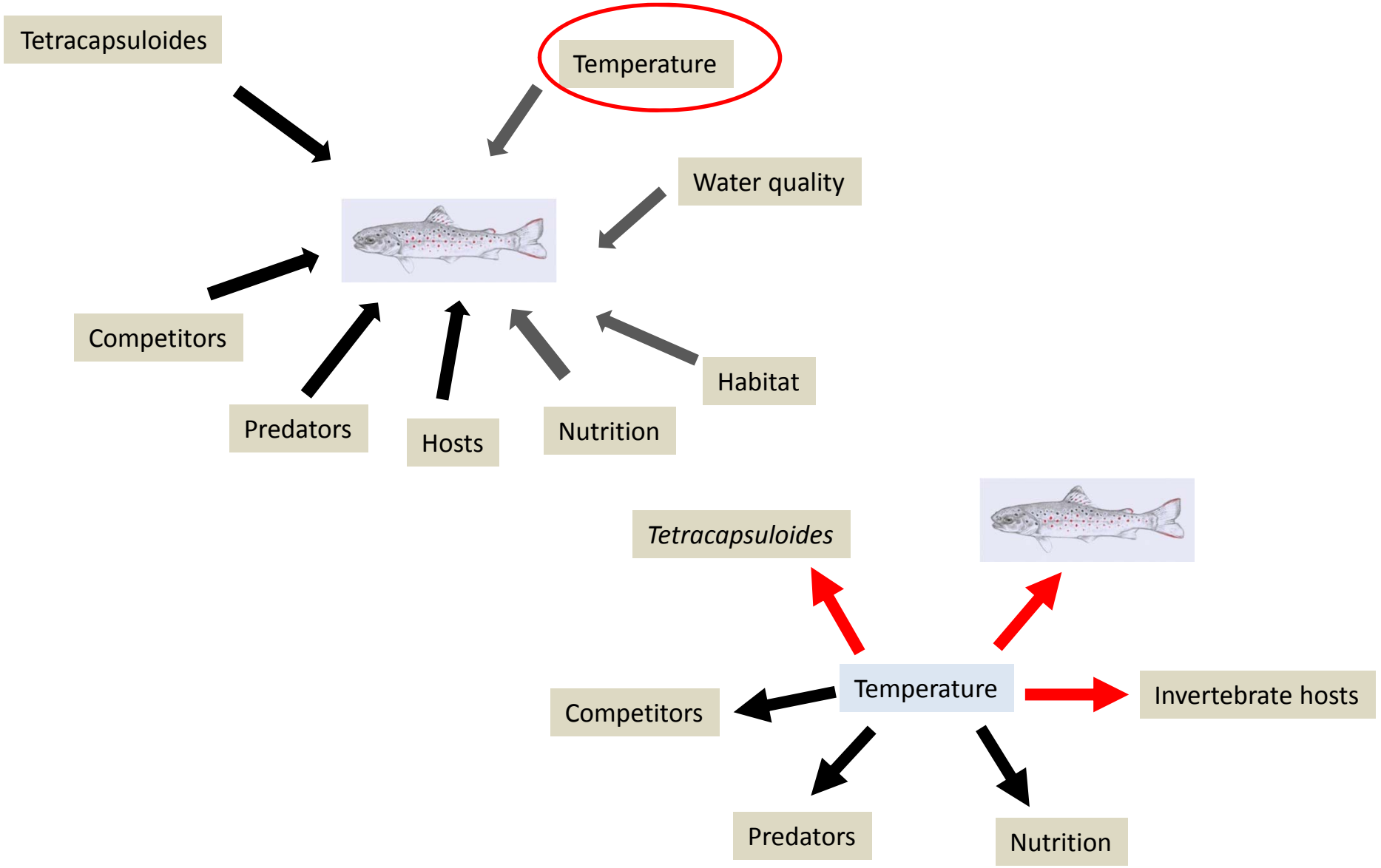
Why»no»?

Co-Evolution can lead to stable host-parasite systems

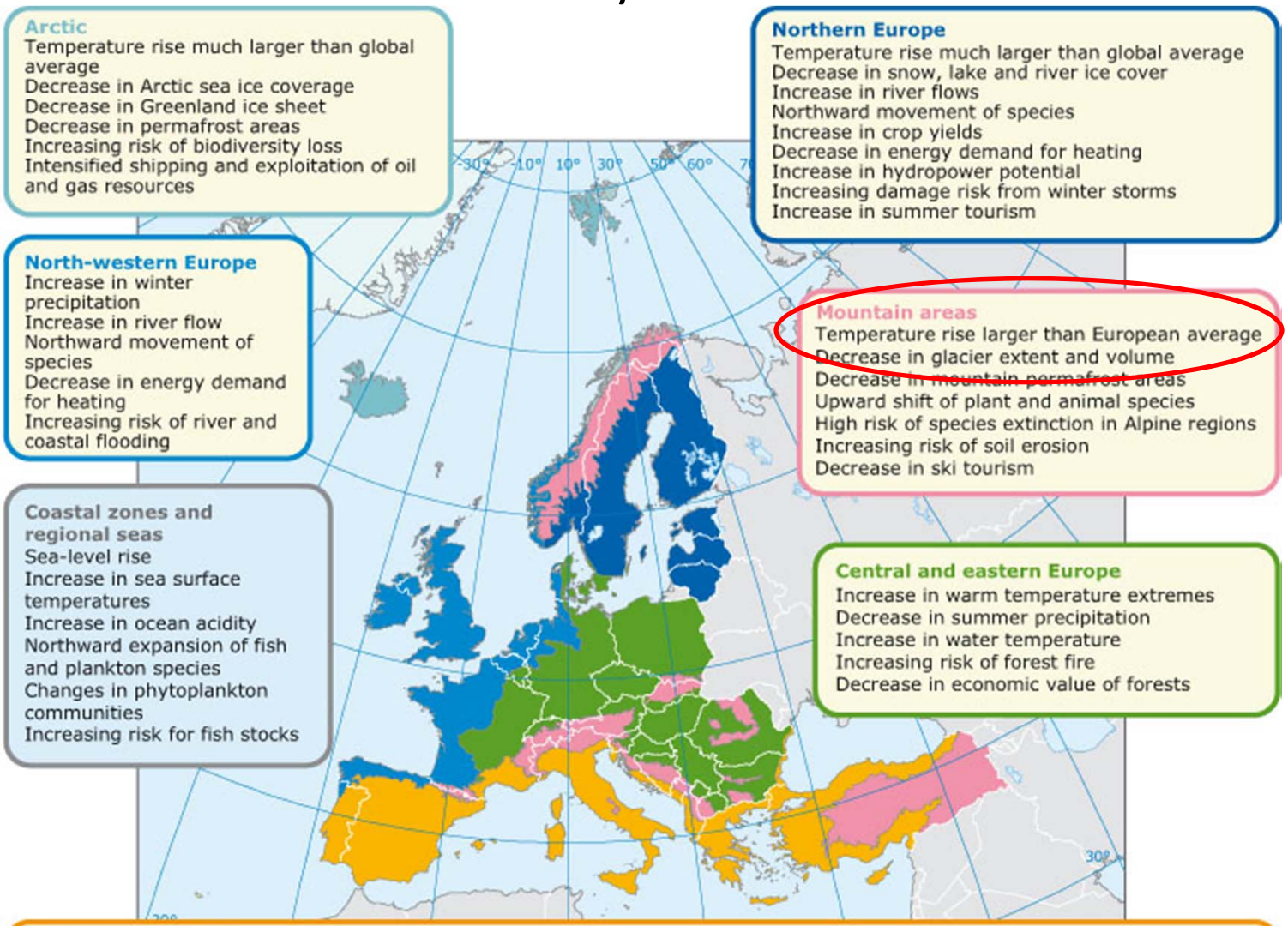


Which other factors may influence PKD?





Climate change as a major threat to freshwater ecosystems



Arctic
Temperature rise much larger than global average
Decrease in Arctic sea ice coverage
Decrease in Greenland ice sheet
Decrease in permafrost areas
Increasing risk of biodiversity loss
Intensified shipping and exploitation of oil and gas resources

Northern Europe
Temperature rise much larger than global average
Decrease in snow, lake and river ice cover
Increase in river flows
Northward movement of species
Increase in crop yields
Decrease in energy demand for heating
Increase in hydropower potential
Increasing damage risk from winter storms
Increase in summer tourism

North-western Europe
Increase in winter precipitation
Increase in river flow
Northward movement of species
Decrease in energy demand for heating
Increasing risk of river and coastal flooding

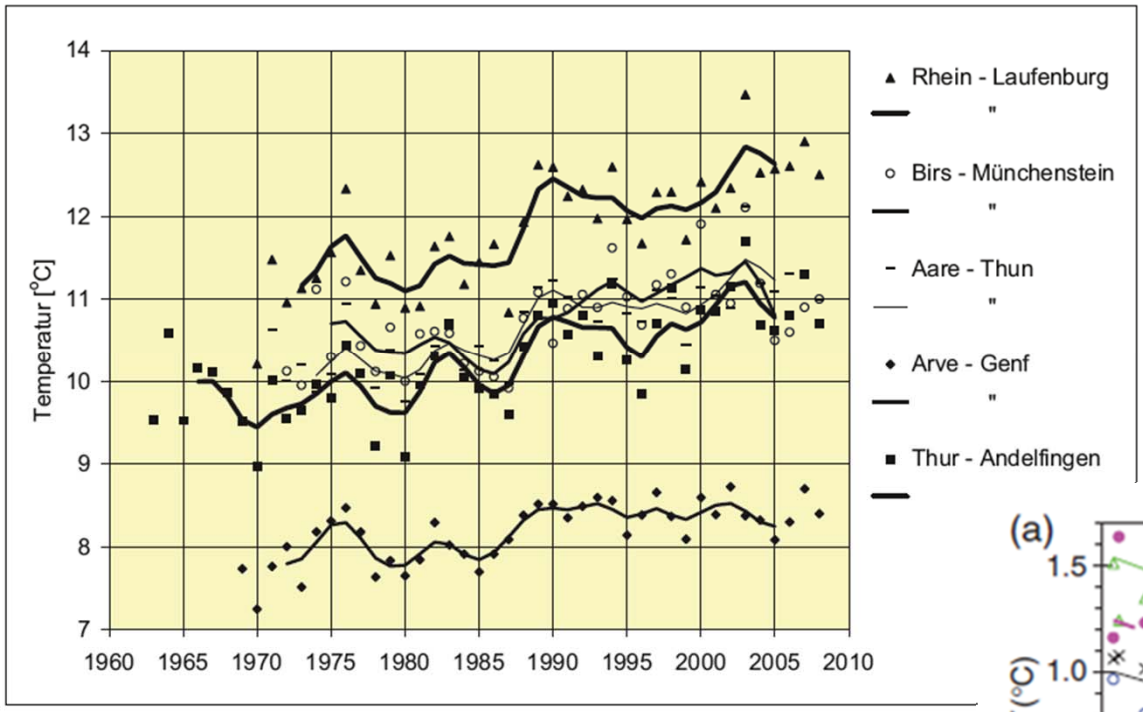
Mountain areas
Temperature rise larger than European average
Decrease in glacier extent and volume
Decrease in mountain permafrost areas
Upward shift of plant and animal species
High risk of species extinction in Alpine regions
Increasing risk of soil erosion
Decrease in ski tourism

Coastal zones and regional seas
Sea-level rise
Increase in sea surface temperatures
Increase in ocean acidity
Northward expansion of fish and plankton species
Changes in phytoplankton communities
Increasing risk for fish stocks

Central and eastern Europe
Increase in warm temperature extremes
Decrease in summer precipitation
Increase in water temperature
Increasing risk of forest fire
Decrease in economic value of forests

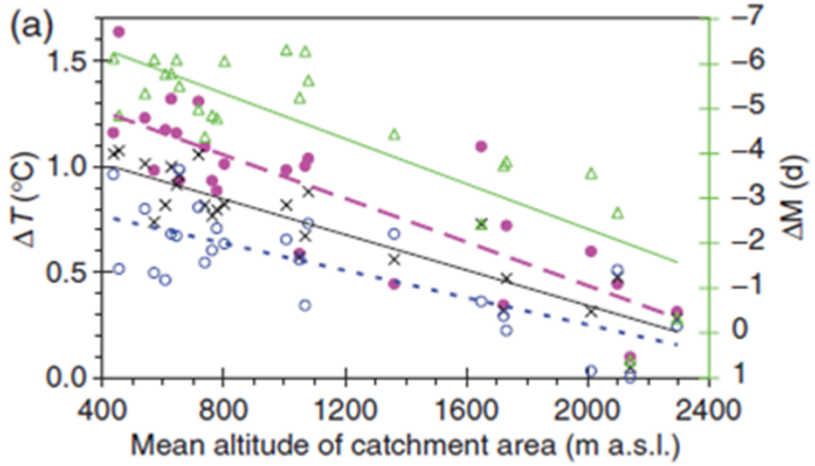
Mediterranean region
Temperature rise larger than European average
Decrease in annual precipitation
Decrease in annual river flow
Increasing risk of biodiversity loss
Increasing risk of desertification
Increasing water demand for agriculture
Decrease in crop yields
Increasing risk of forest fire
Increase in mortality from heat waves
Expansion of habitats for southern disease vectors
Decrease in hydropower potential
Decrease in summer tourism and potential increase in other seasons

Are water temperatures of Swiss rivers rising?



Mean annual temperatures

⇒ More extreme changes in min-max temperatures

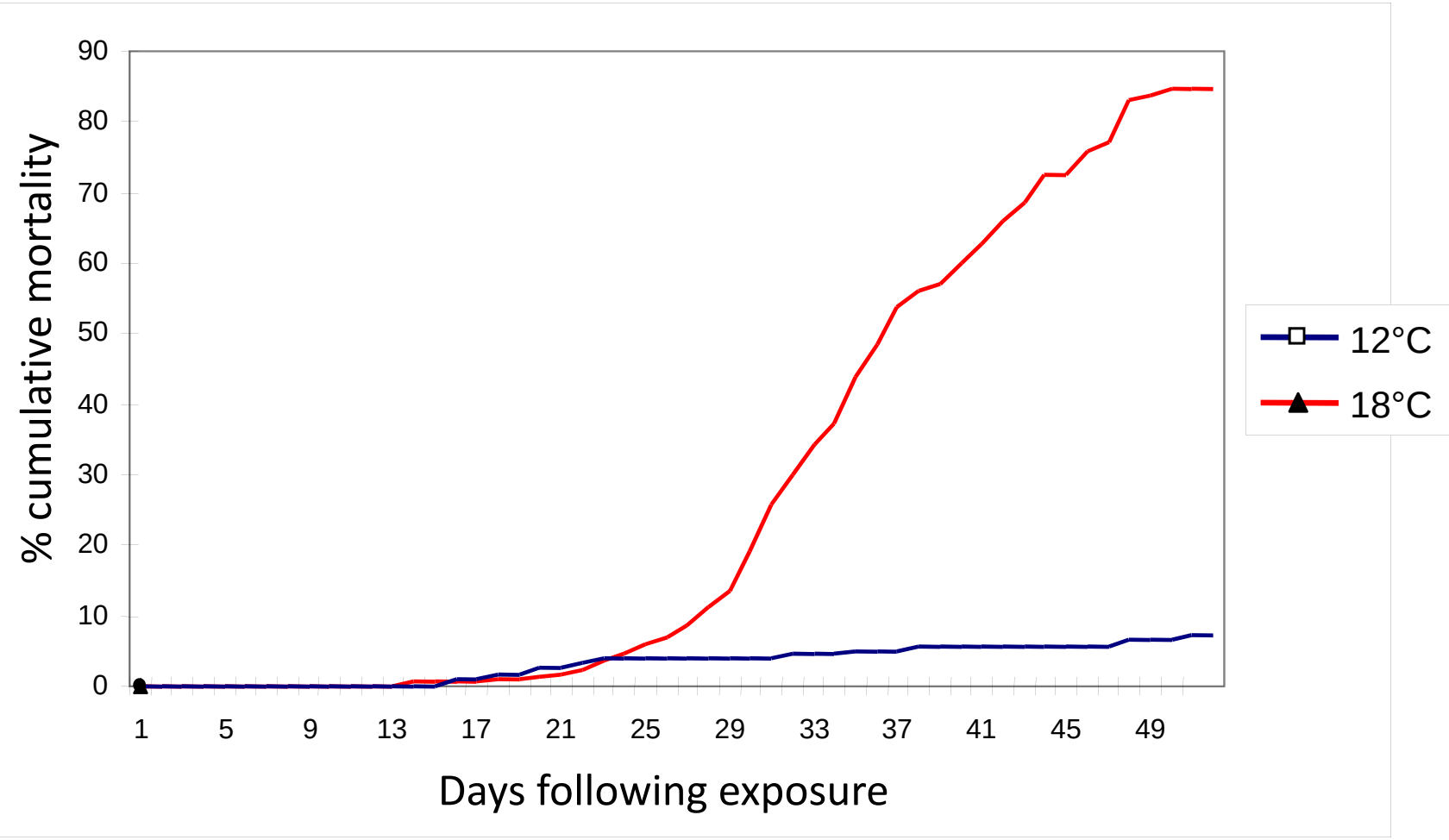


Hari et al. 2006

Yes, there is a significant temperature increase over the last decades

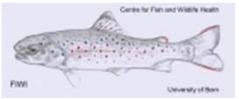
Water temperature effects on PKD

If water temperature increases, what does this mean for PKD-induced fish mortality?



Bettge et al. (2009)

Causes for differences in mortality



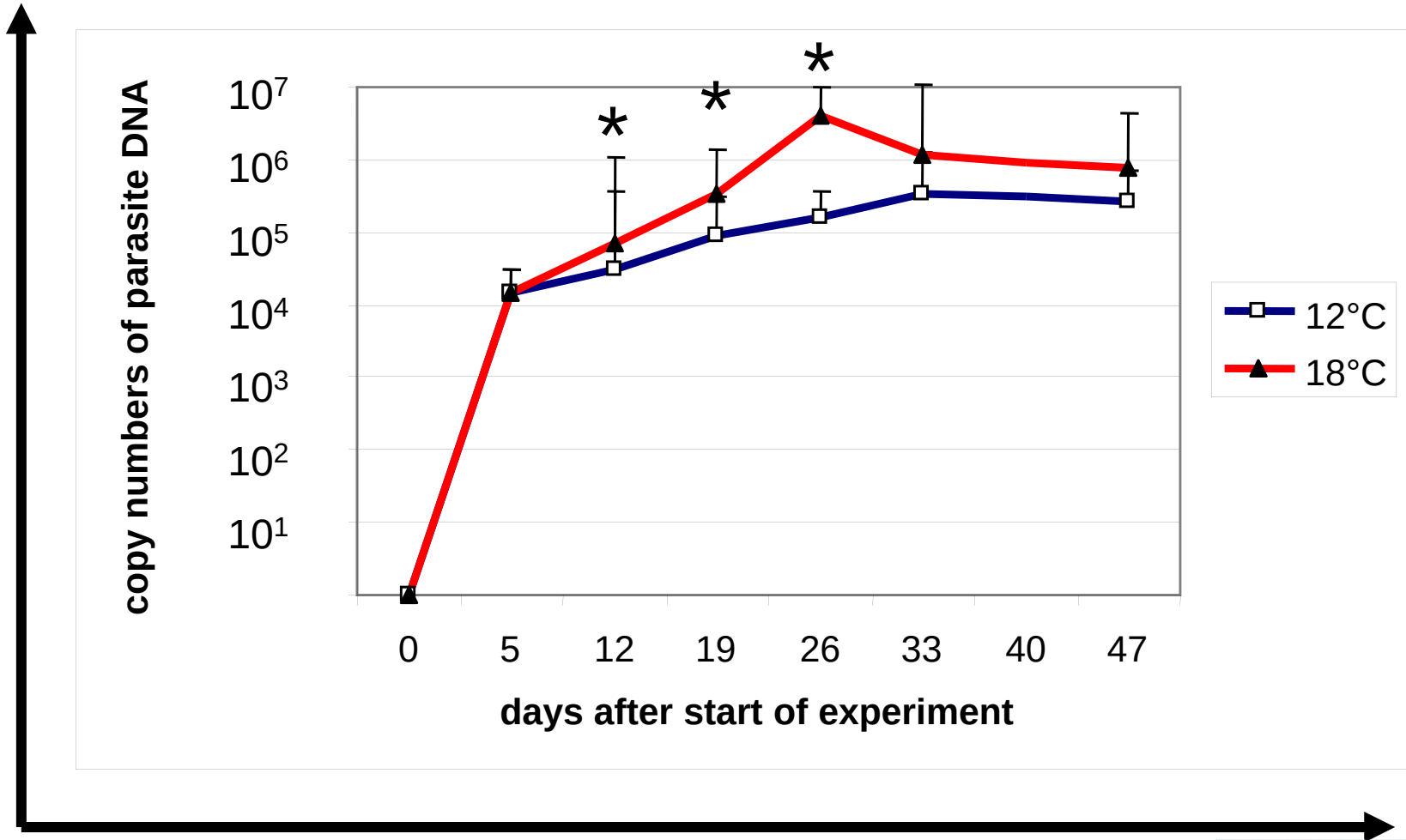
Why higher mortality at higher water temperature?

Hypotheses

- Temperature promotes parasite proliferation?
- Temperature enhances tissue pathology
- Temperature impairs immunocapacity of fish host?

Influence of temperature on parasite proliferation

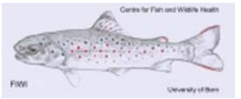
After infection until clinical phase

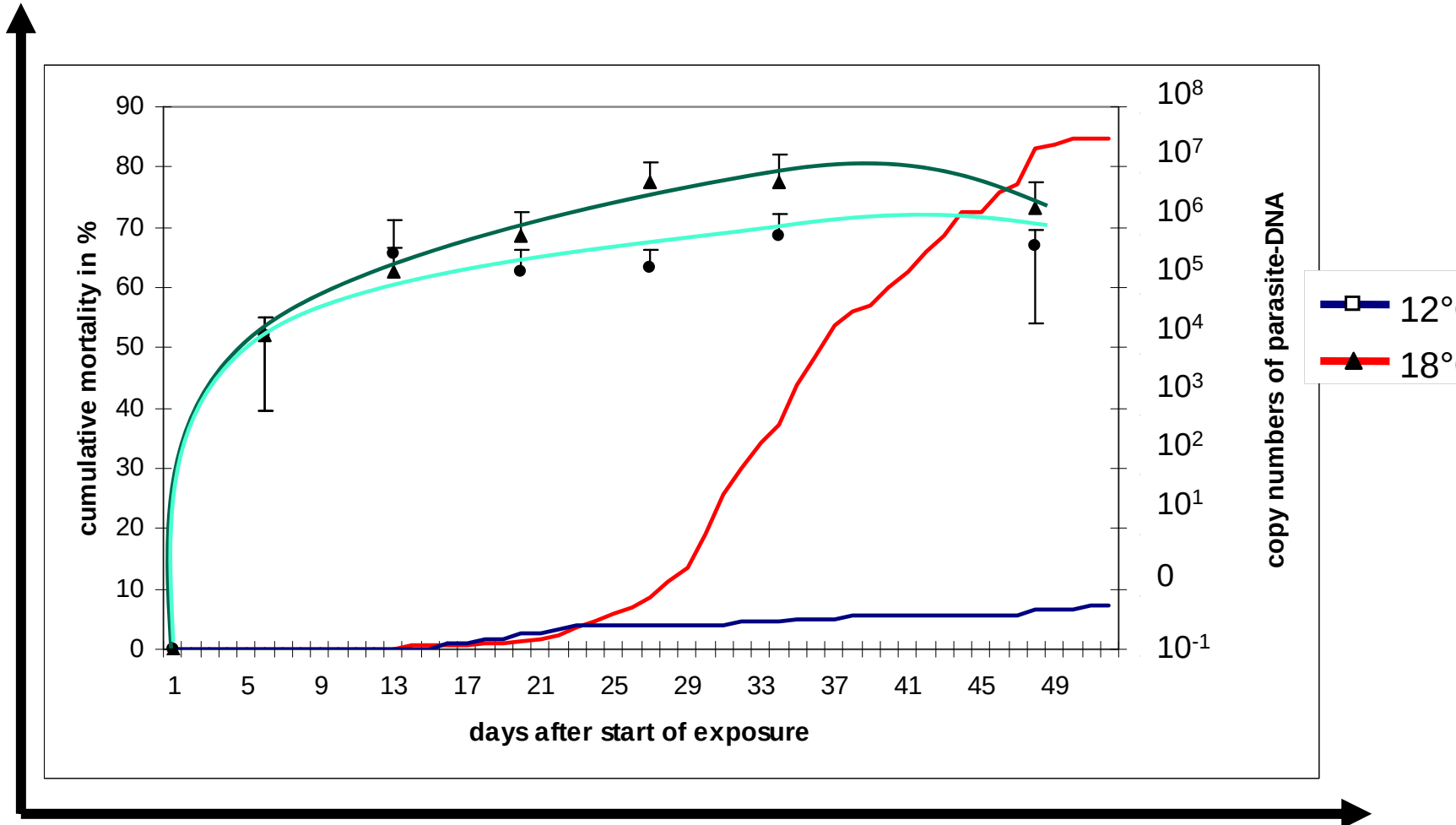


preclinical phase

clinical phase

Influence of temperature on parasite proliferation





preclinical phase

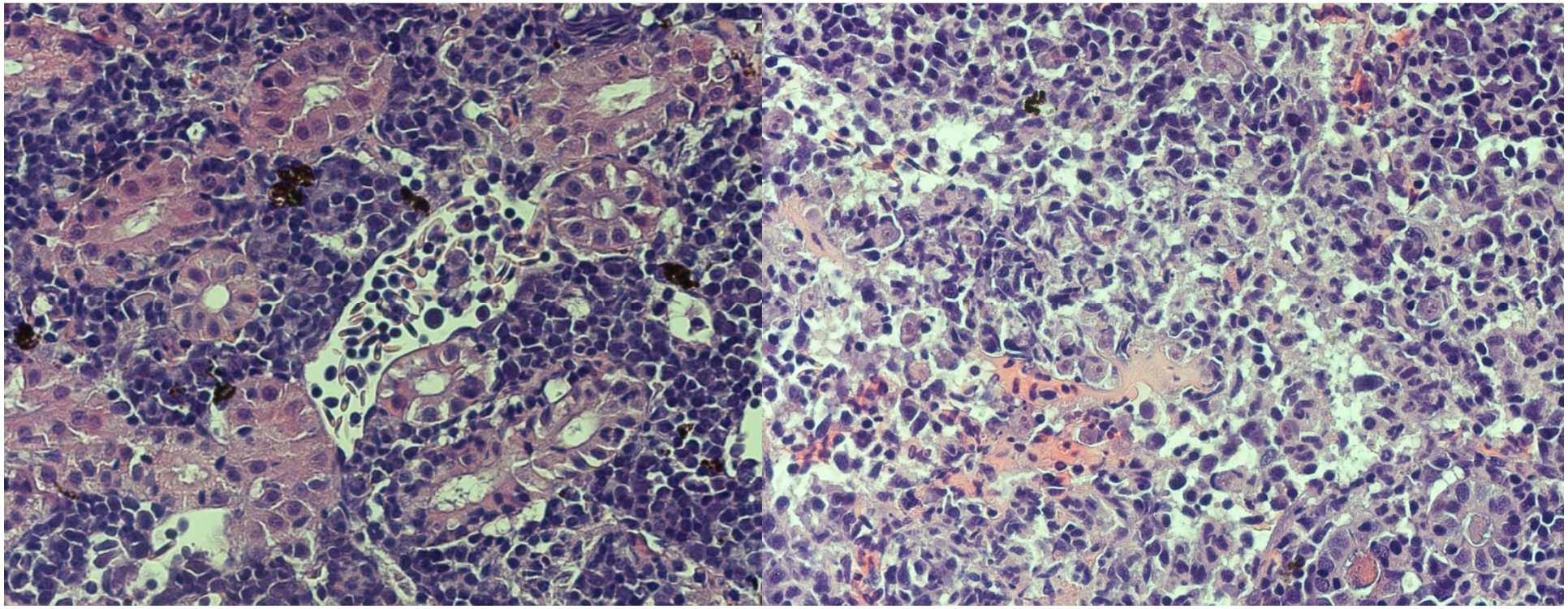
clinical phase

Minor difference in parasite proliferation not explaining difference in mortality

Influence of temperature on pathology

12°C

18°C

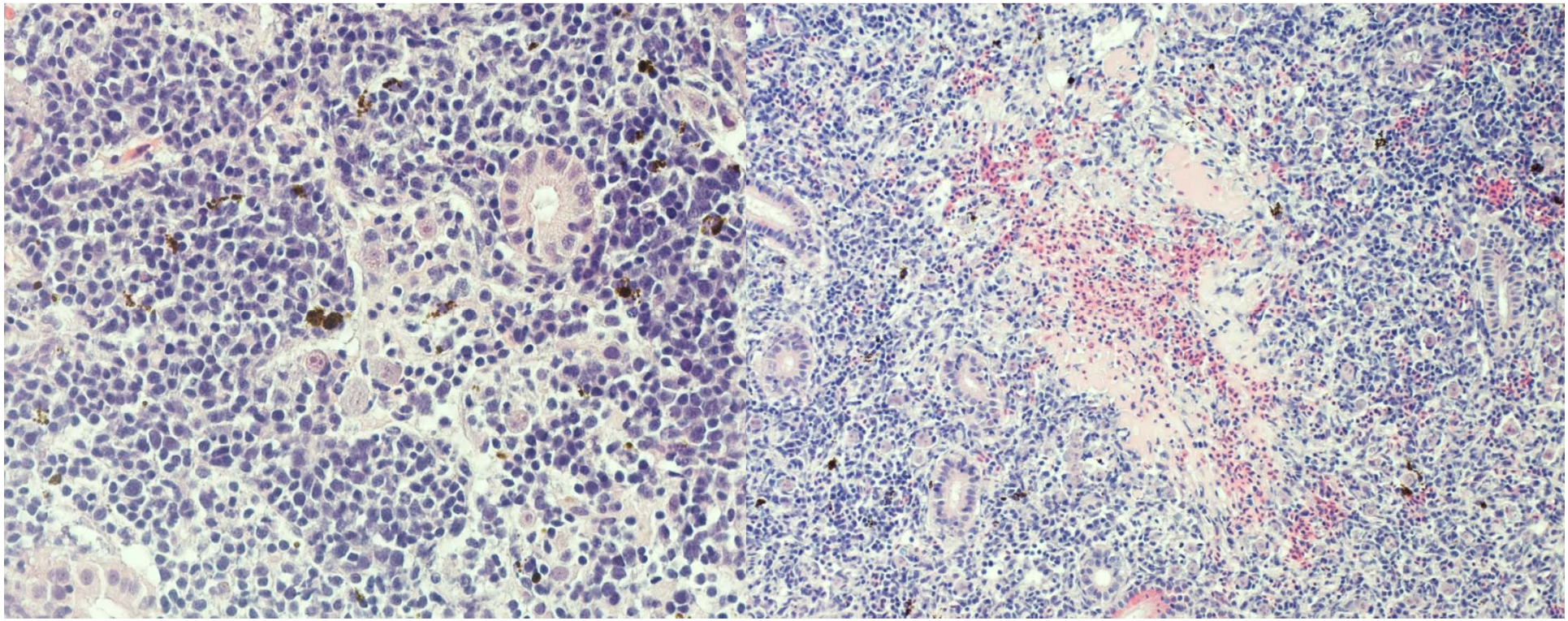


Preclinical phase

Influence of temperature on pathology

12°C

18°C

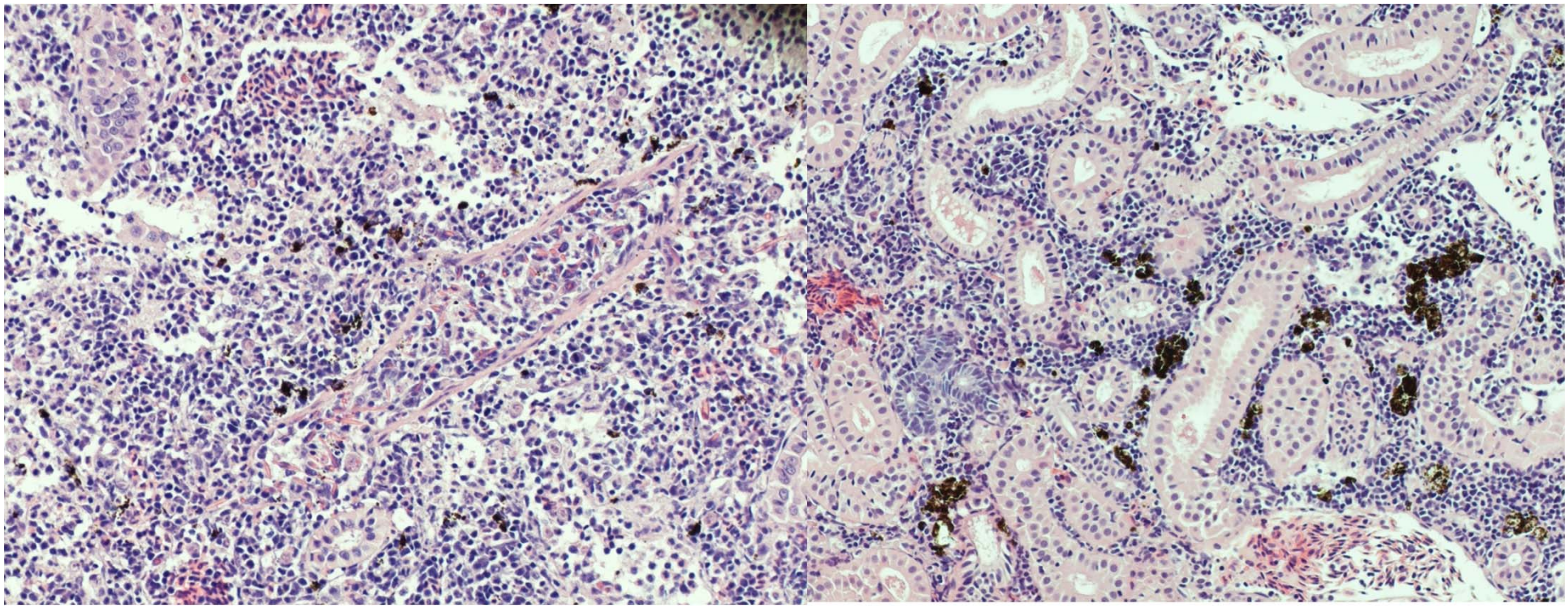


Clinical phase

Influence of temperature on pathology

12°C

18°C



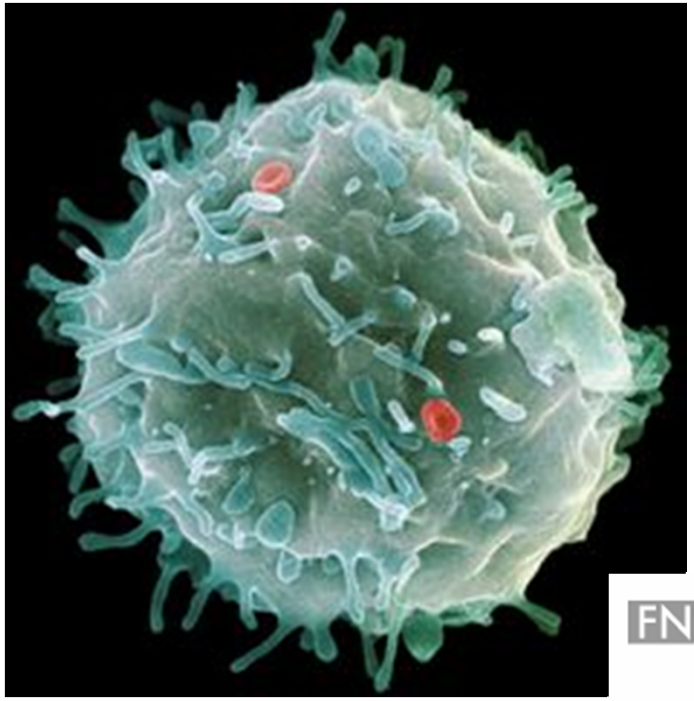
Postclinical phase

Minor differences in pathology not explaining difference in mortality

Influence of temperature on immune response

The influence of temperature on immune response: Does temperature modulate the immune response of trout to PKD?

Christyn Bailey

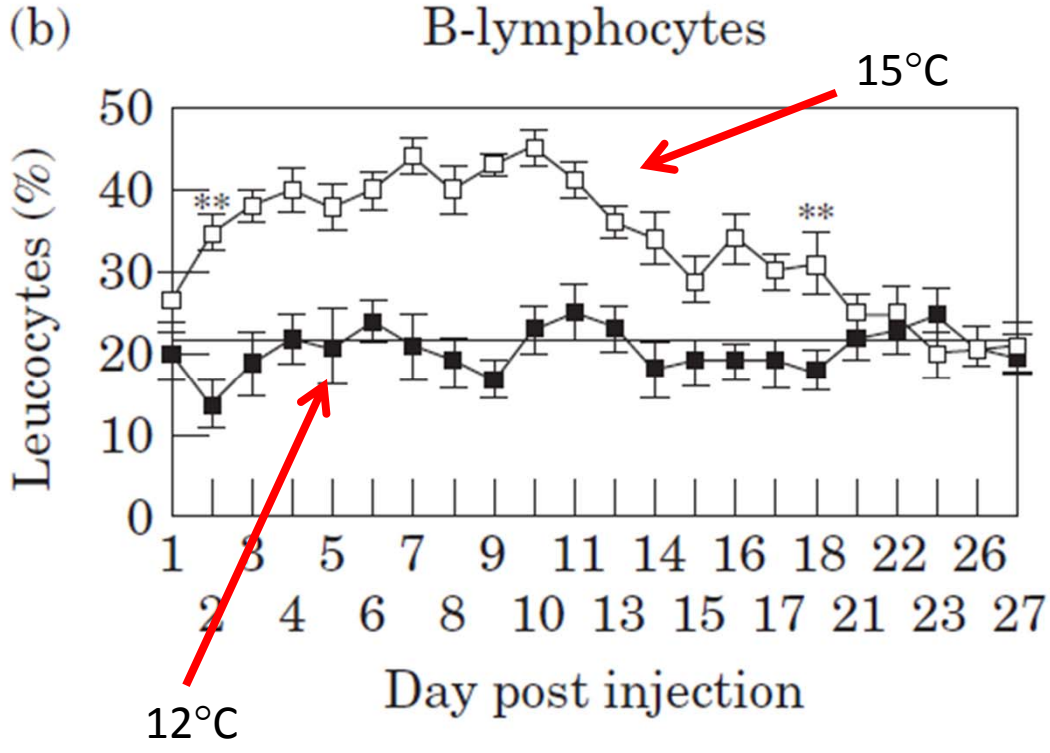


https://s-media-cache-ak0.pinimg.c

SCHWEIZERISCHER NATIONALFONDS
ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

Why temperature?

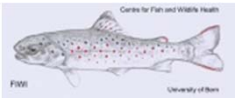
- The immune system of fish is influenced by temperature owing to the poikilothermic nature of fish (mechanisms include temperature effect on biochemical rates, or on energy metabolism)
- In many cases, low environmental temperatures are immunosuppressive for teleosts especially on the humoral responses



Rainbow trout

Köllner and Kotterba 2002

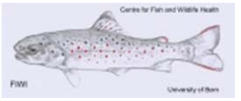
Why temperature?



- Further evidence for an influence of temperature on the immune system of fish

Fish	Response to high temperature	Response to low temperature	Reference
Oncorhynchus mykiss	At 15-17°C prominent activation of blood and spleen leukocytes in fish infected with *Aeromonas salmonicida*	At 10-12°C weak to no reaction in blood and spleen in fish infected with *Aeromonas salmonicida*	Köllner & Kotterba 2002
Oncorhynchus mykiss	At 15-20°C lytic activity of both total and alternative pathways increased	At 5-10°C respiratory burst opsonization capacity of plasma delayed	Nikosske-lainen et al 2004
Oncorhynchus Mykiss	Production of macrophage activating factor (MAF) secretion and respiratory burst activity increased	Inhibition of macrophage activating factor (MAF) secretion	Hardie et al 1994

Does temperature modulate the immune response of trout to PKD?



To explore the (cellular) immune response of trout towards *T. bryosalmonae* at different temperatures, the following hypothesis was formulated

1. Temperature modulates immune response
2. The altered immune response reduces resistance towards the parasite

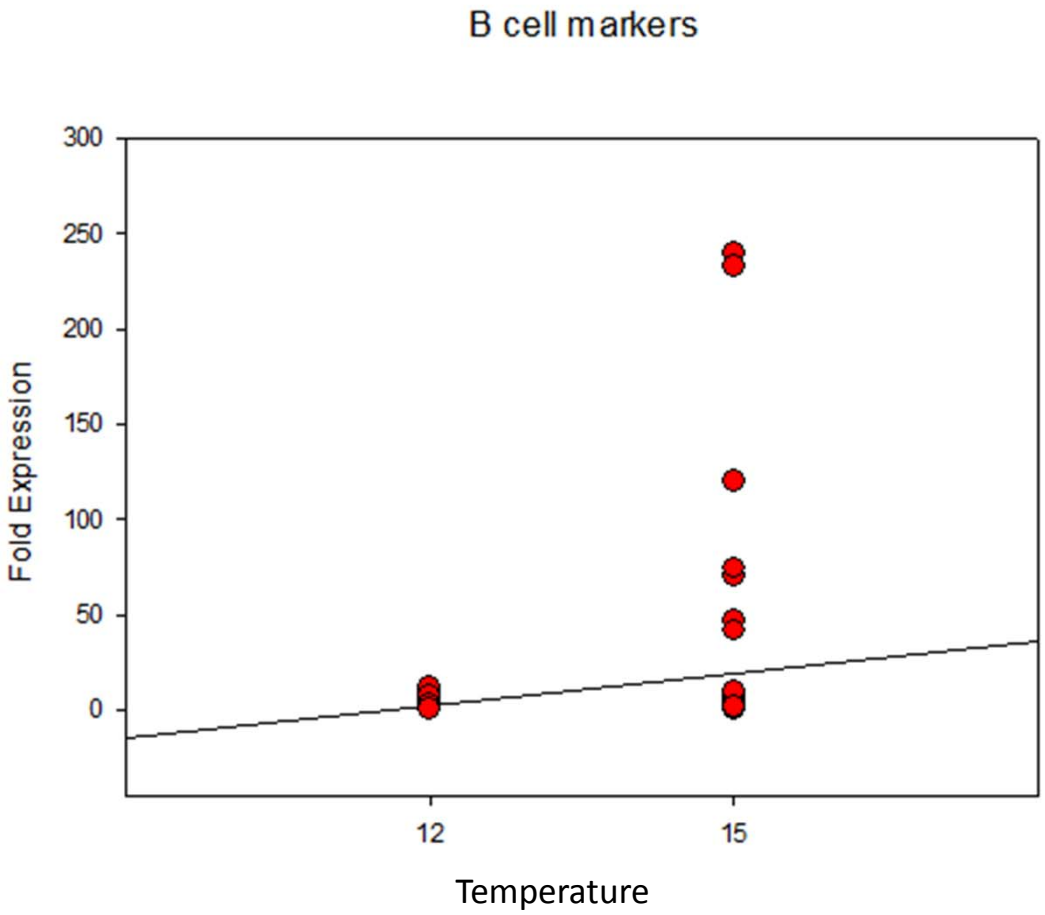
Experiment design

- All experiments carried out at 12°C and 15°C analysing head kidney (HK) and trunk kidney (TK) over the infection cycle

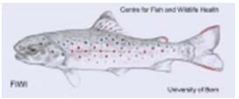
- qRT-PCR of immune genes attributed to functional pathway(s) or cell lineage(s) and FACS analysis of immune cells

Temperature effect on immune response

Measurement of B cell markers in *T. bryosalmonae* infected fish at different temperatures



Temperature effect on immune response

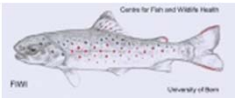


Conclusion

- At higher temperatures immune response not inhibited but in contrary increased

- Temperature related differences in immune response not sufficient to explain differences in mortality

Influence of temperature on physiology



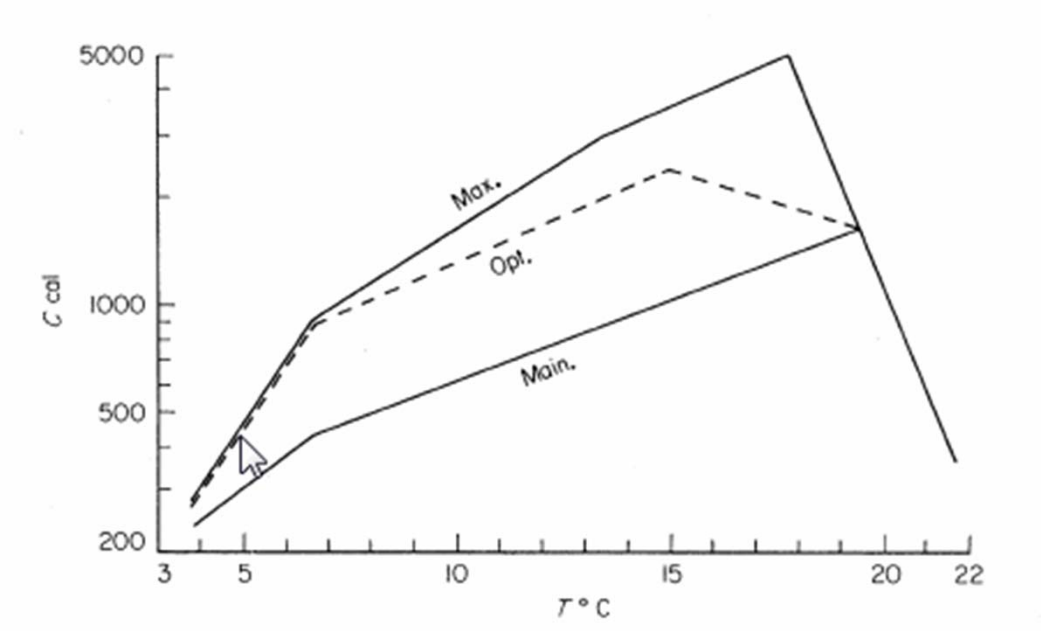
What else?

Higher water temperature affects fish physiology

- Increased metabolism
- Increased osmotic demand → increased water excretion
- Increased oxygen demand
- Stress by suboptimal temperature range

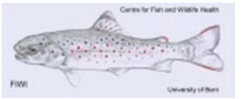
Metabolism

- Metabolism influenced by water temperature



Relationship between the maximum, optimum and maintenance energy intake (C cal day- 1) for trout of 50 g initial weight at different temperatures (Elliot 1976)

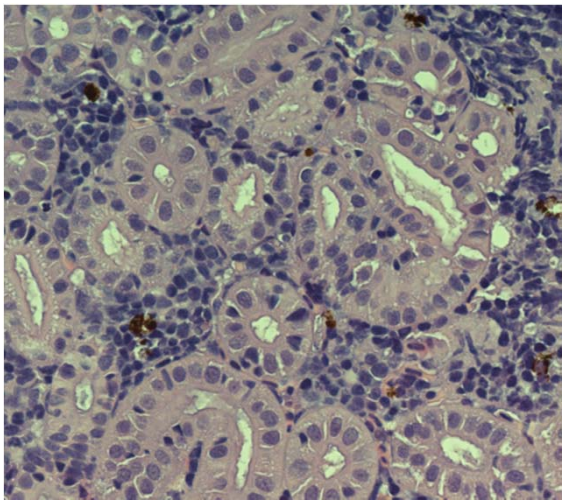
Different functions of the kidney in fish



Osmoregulatory function

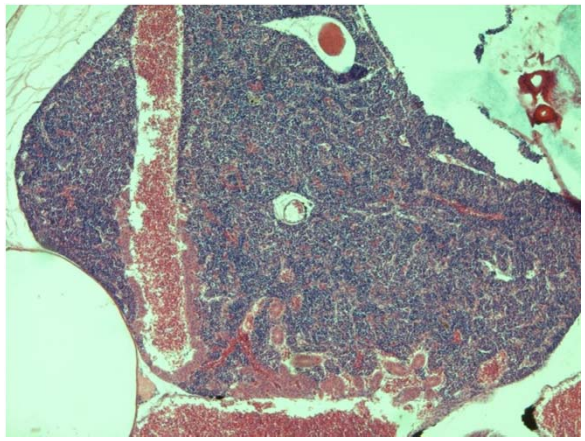
enhanced demand at higher temperatures

due to elevated need for urine excretion

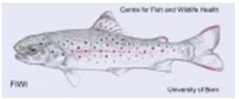


Haematopoietic function

enhanced need for erythropoiesis at higher water temperatures



Excretion function



- Excretion function influenced by water temperature

Urine flow rates (UFR) in rainbow trout

Temperature (°C)	UFR (ml/kg per day)	Reference
7	33.6	Hoffmann and Butler 1979
10	52.8	Lloyd and Orr 1969
20	100.8	Lloyd and Orr 1969

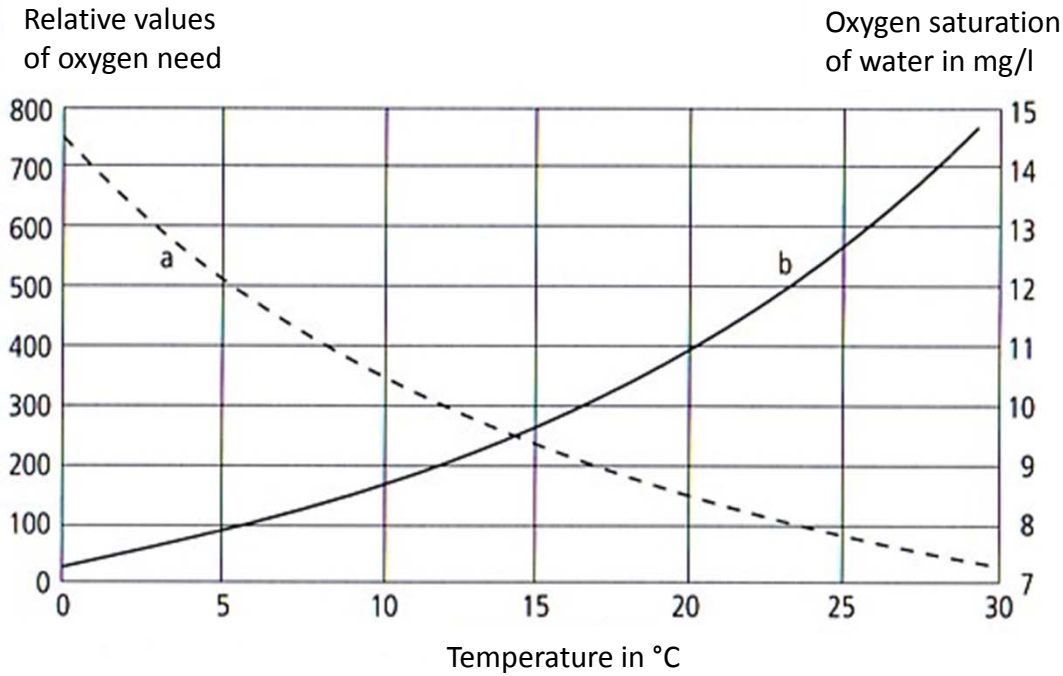
Calculation of Q_{10} from these data: approx. **2** (Hunn 1982)

Oxygen demand

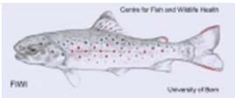
- Oxygen consumption of salmonids affected by temperature, fish size, physical and physiological activity
- Increase in water temperature results in increase in oxygen demand and decrease in oxygen carrying capacity of water
- Oxygen partial pressure (saturation level) determines diffusion through gills and saturation level of hemoglobin

Saturation level of water:

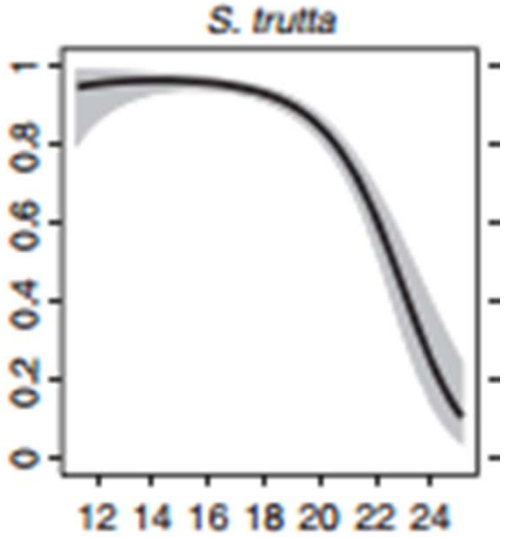
0°C = 14.5 mg/l
10°C = 11.1 mg/l
20°C = 8.9 mg/l
30°C = 7.2 mg/l



Species specific temperature tolerance



Fish are poikilothermic animals, with a particular range of suitable temperatures



Temperature range of of brown trout (*Salmon trutta*)

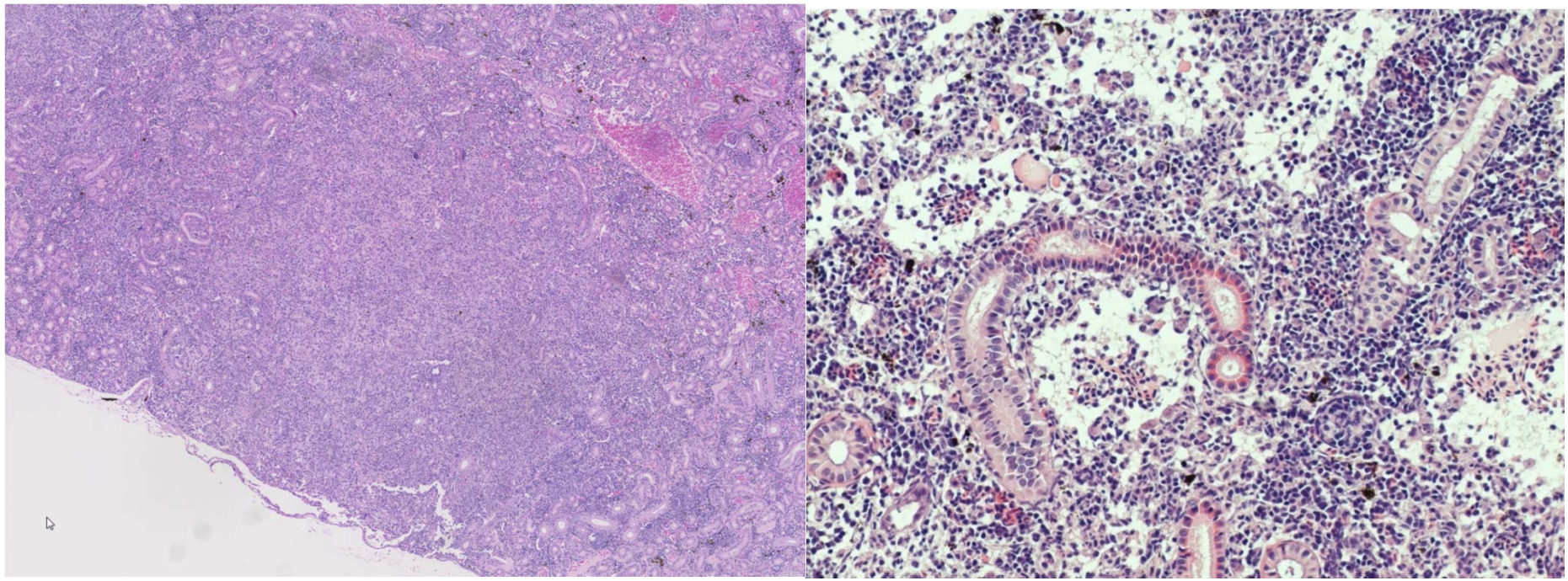
Species	Optimal temp.
Brown trout	4-19
Rainbow trout	10-22

Elliot 1981

Increased demand on kidney functions

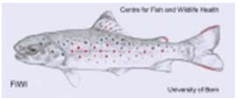
Effect of infection:

- Effect on excretory compartments
- Replacement and necrosis of haematopoietic tissue



Imbalance between increased demand and altered kidney structure and function + general stress

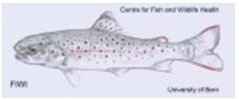
Influence of temperature on physiology



Conclusion

→ Temperature related alteration of physiology appear to be the main factor for increased mortality of PKD affected trout at higher water temperatures

Epidemiology of PKD



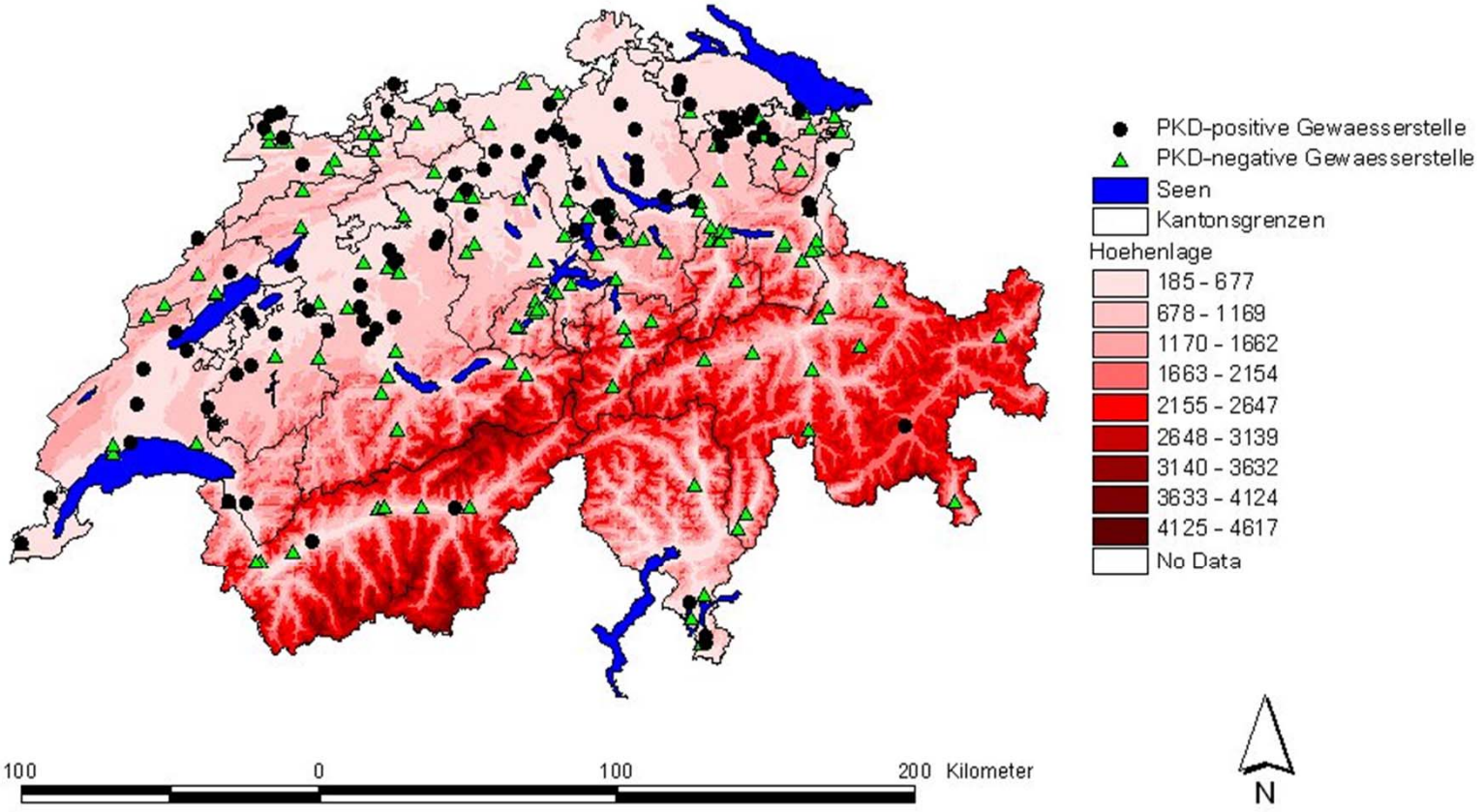
Geographic distribution of PKD:

Which factors have to be considered?

- Catchment (catchment-specific?)
- Habitat structures
- Ecological requirements of host
- Ecological requirements of invertebrate host

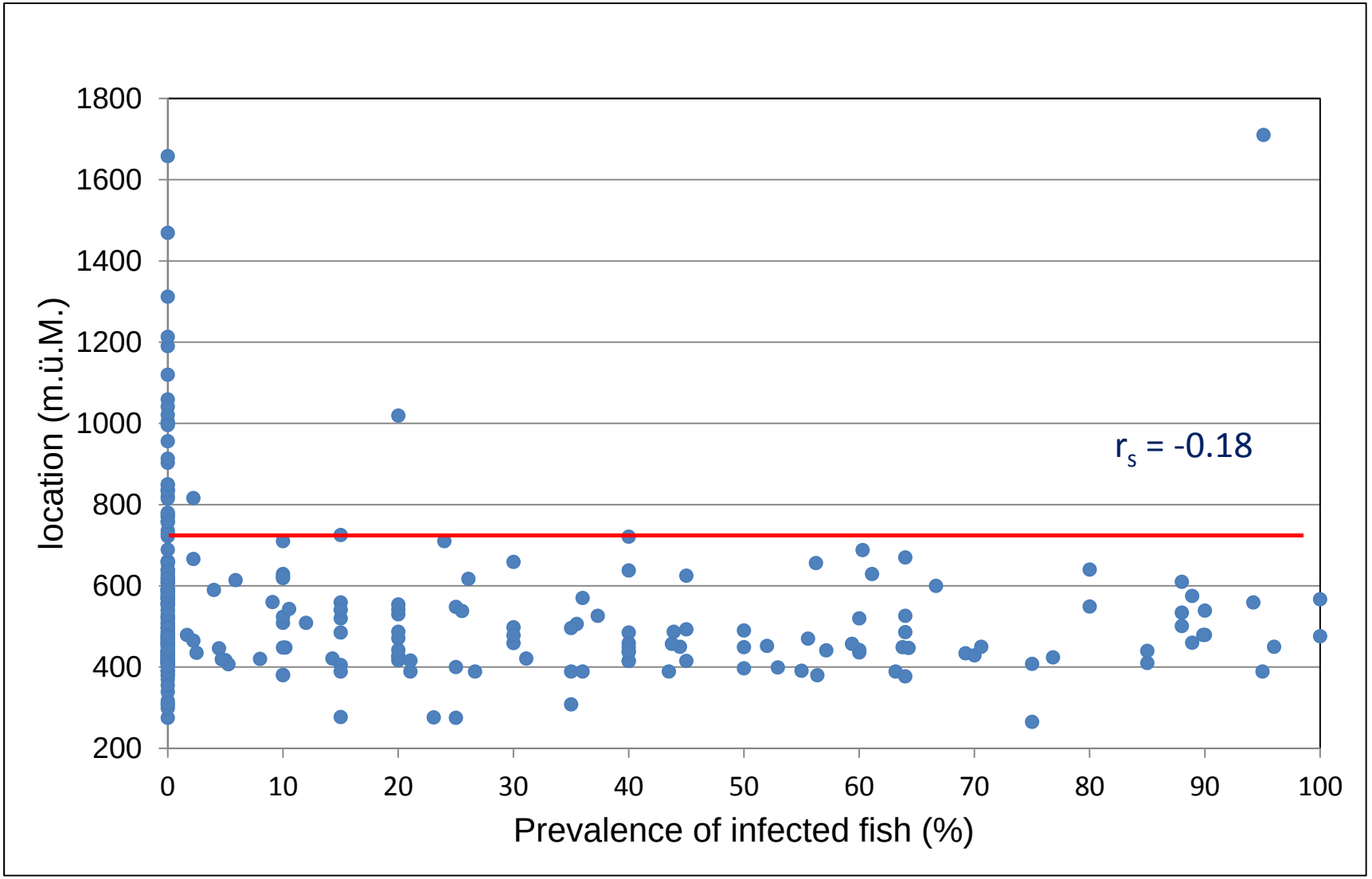
Disease distribution

Mainly in Swiss midlands and in larger valley areas



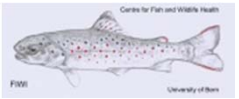
Wahli et al. (2008) Role of altitude and water temperature as regulating factors for the geographical distribution of *Tetracapsuloides bryosalmonae* infected fish in Switzerland. *Journal of Fish Biology*, 73: 2184-2197

Temperature influence on disease distribution



Wahli et al. (2008) Role of altitude and water temperature as regulating factors for the geographical distribution of *Tetracapsuloides bryosalmonae* infected fish in Switzerland. *Journal of Fish Biology*, 73: 2184-2197

Temperature influence on disease distribution



Conclusions:

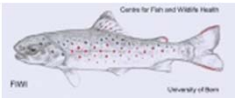
- Almost all PKD-positive fish below 800 m.a.s.l.
- Above 800 m.a.s.l. only 3 sites with infected fish

- Evidence for “ON / OFF” effect driven by altitude and accordingly temperature
- Effect possible on both, parasites in bryozoa and parasites in fish

Effect of PKD in the future

No more fishing for brown trout in future?

Temperature influence on disease



Temperature impacts disease distribution, how?

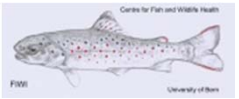
- Affecting the trout
- Affecting the parasite
- Affecting the bryozoan
- By changing the surrounding environment

Temperature driven emergence of Proliferative Kidney Disease in salmonid fish – role of ecology, evolution and immunology for aquatic diseases in riverine landscapes

(Sinergia-Project CRSII3_147649_1)

FNSNF
SCHWEIZERISCHER NATIONALFONDS
ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

Project Aims



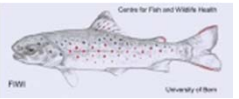
Understand maintenance and transmission dynamics of *Tetracapsuloides bryosalmonae* causing PKD with a particular focus on temperature changing environments (global warming)

- Disease maintenance
  parasite transmission rate, factors involved in spore production modulation

- Disease spread
  modeling and evaluation of infection timing, seasonal variability, genetics affecting disease susceptibility

- Disease Emergence
  how future rise of temperature will impact PKD emergence

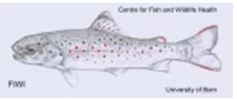
What's a Sinergia Project?



Sinergia …

is a project where different Universities with different approaches to the same topic working together, sharing material and information to gather the most accurate scenario on that topic.

Sinergia Project Actors



Vertebrate host:
Brown Trout

eawag
aquatic research

Invertebrate host:
Bryozoan

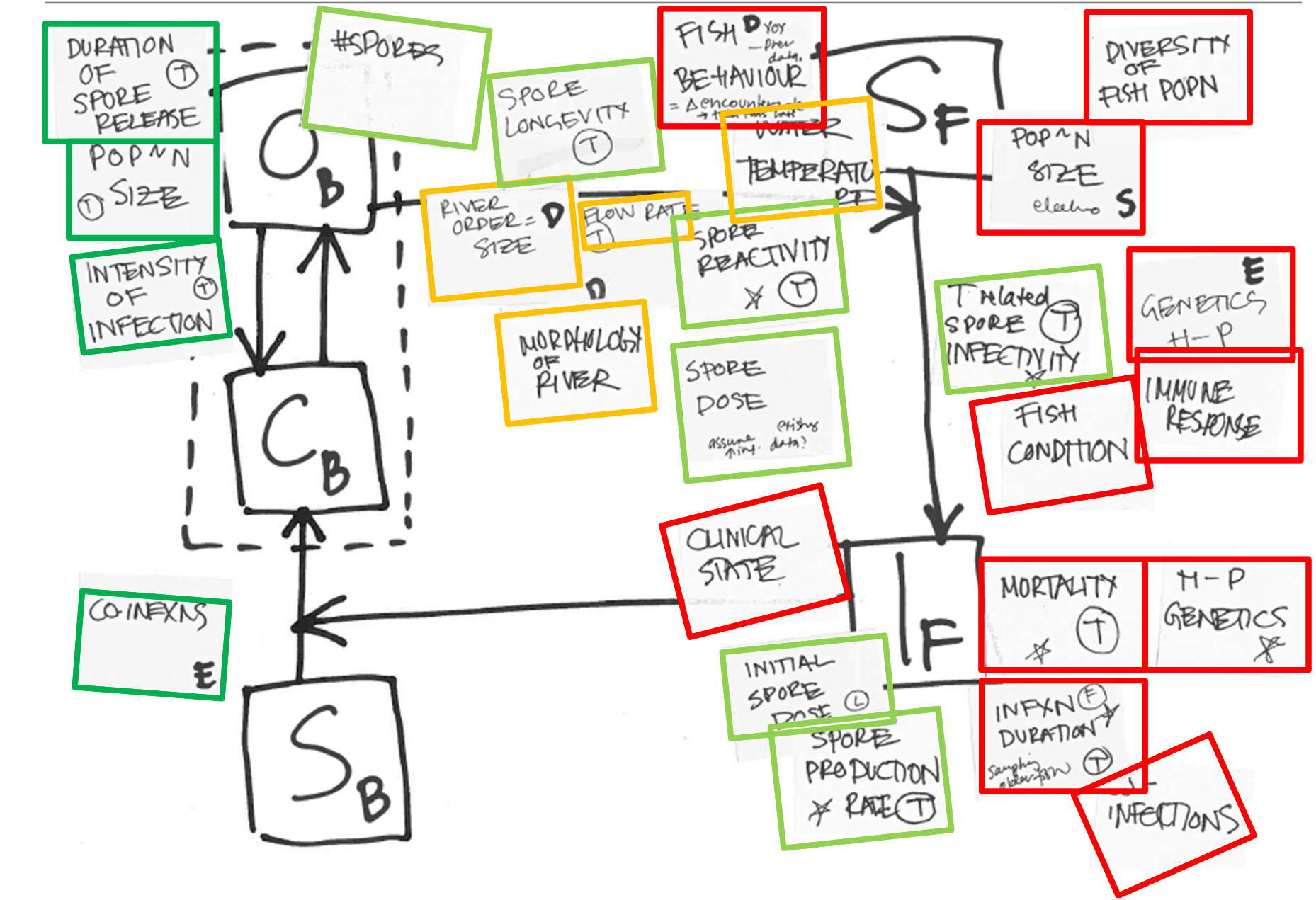
EPFL
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Disease modeling in river systems

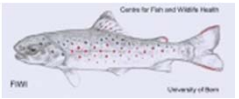
1495
UNIVERSITY
OF ABERDEEN

Host-parasite immunological interaction

Life cycle influencing factors

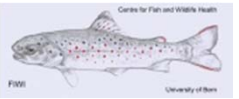


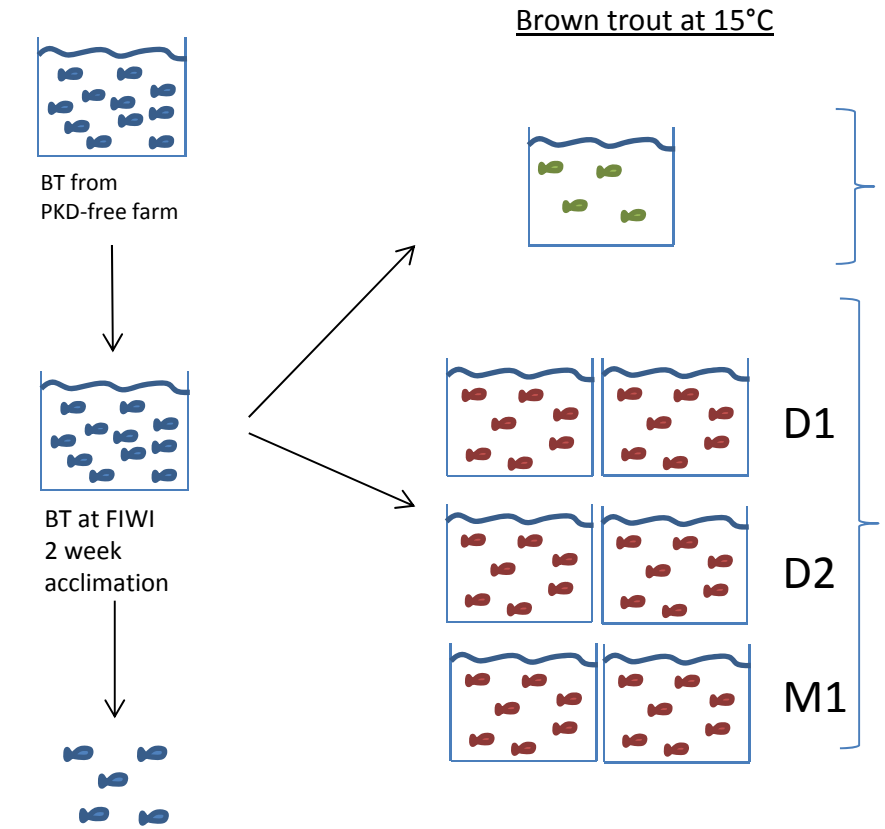
Laboratory experiment



1. Temperature experiment:
    i. Is there any infection prevalence difference between temperatures?
    ii. Which factors trigger the spore release?
    iii. Is there any difference among release of the parasite at different temperatures?
2. Density experiment
    i. How infection develops in the fish exposed to different parasite spores densities?
    ii. Is there any difference in the number of spores produced/released by the fish with respect to initial parasite density infection?

Experimental Design





CONTROL GROUPS:

Sample 10 at each sampling
Histology, Samples for PKD status (Histology and qPCR from gills, blood, kidney)

Dead fish => Parasitology, Bacteriology, Histology

PARASITE EXPOSED GROUPS:

Trace of both infection development and prevalence
Sample 10 fish per replicate at different time-point

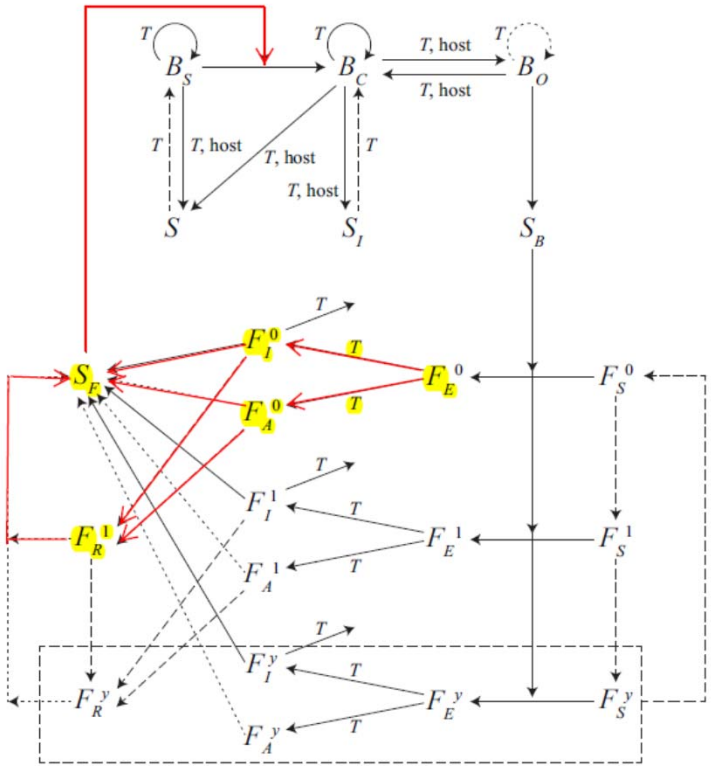
Starting D10: each 5 days sampling water

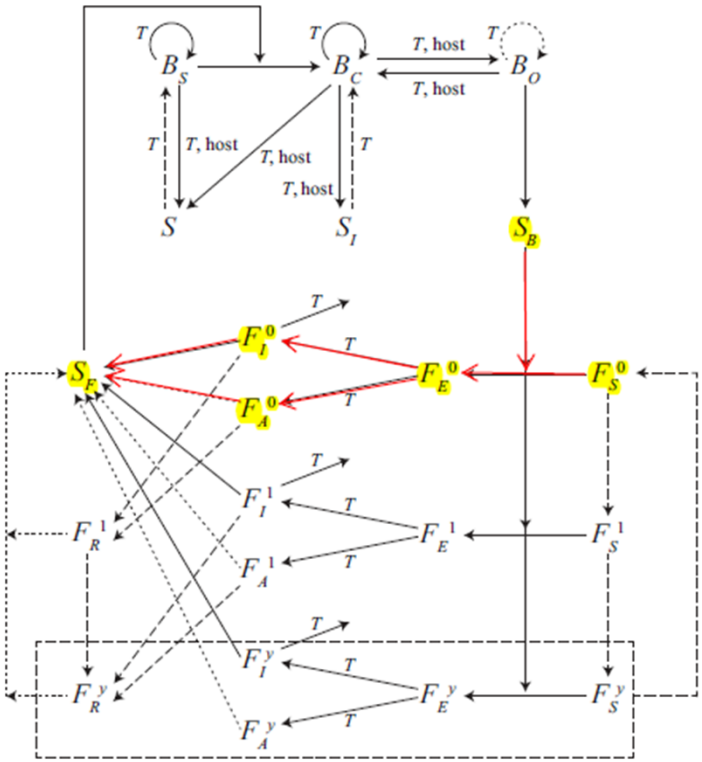
Collect spores to
quantify the release

10 BT screened for presence of PKD and other diseases (Parasitology, Bacteriology)

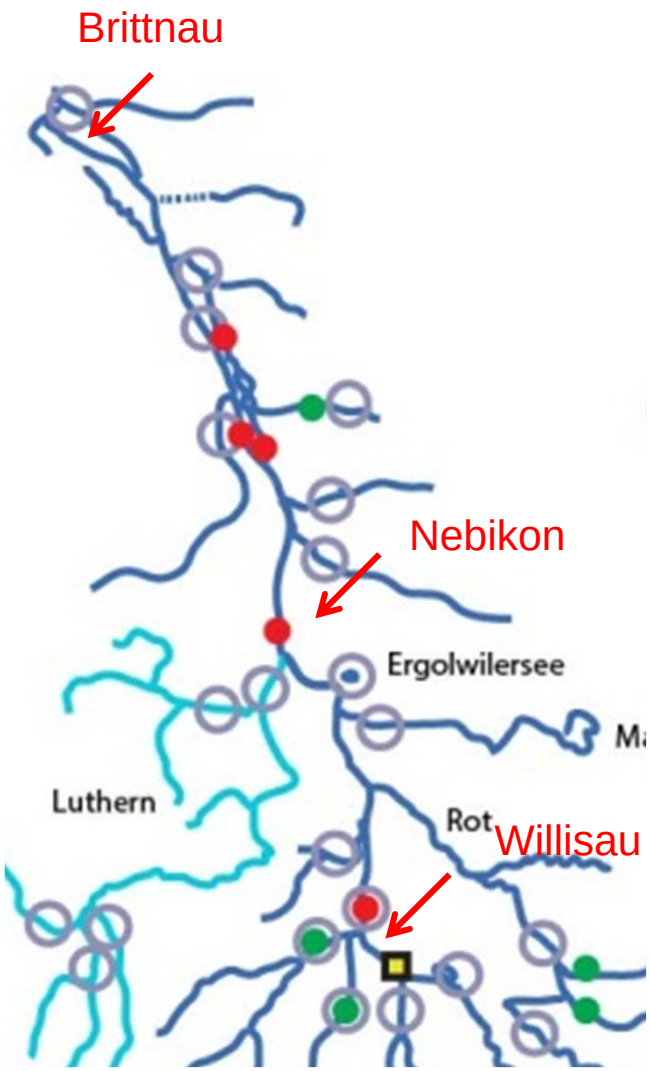
D1 = 1x dose parasite density
D2 = 100x dose parasite density
M1 = multiple times 1x dose parasite density

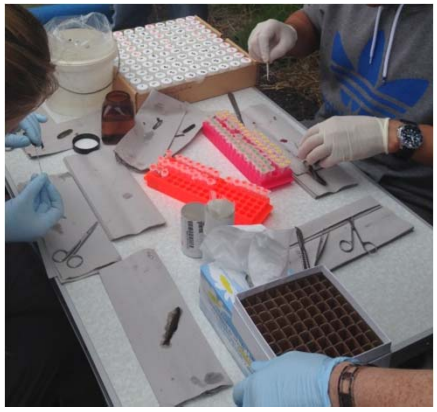
Building the model





Field PKD monitoring





Questions?