THE COMPLEX ISSUE OF CRAYFISH PLAGUE IN FINLAND

Satu Viljamaa-Dirks, DVM, OIE expert for crayfish plague

Fish and Wildlife Health Research Unit

Evira Kuopio

OIE reference laboratory for crayfish plague

From

One native species of crayfish

To

Two crayfish species, two types of crayfish plague

How to manage?

Crayfish in Finland

Crayfish plague

Crayfish plague diagnosis

Management crayfish plague

Crayfish in Finland

Crayfish fisheries in Finland

Two crayfish species:
Native noble crayfish (*Astacus astacus*)

Signal crayfish (*Pacifastacus leniusculus*) was introduced 1967

Rapid growth of signal crayfish catches

Noble crayfish is wanted and has high market prices

Crayfish in Finland

Price of individual signal crayfish: 2,00-5,55 €,
noble crayfish 4,95-12,37 €

Crayfisheries' economy

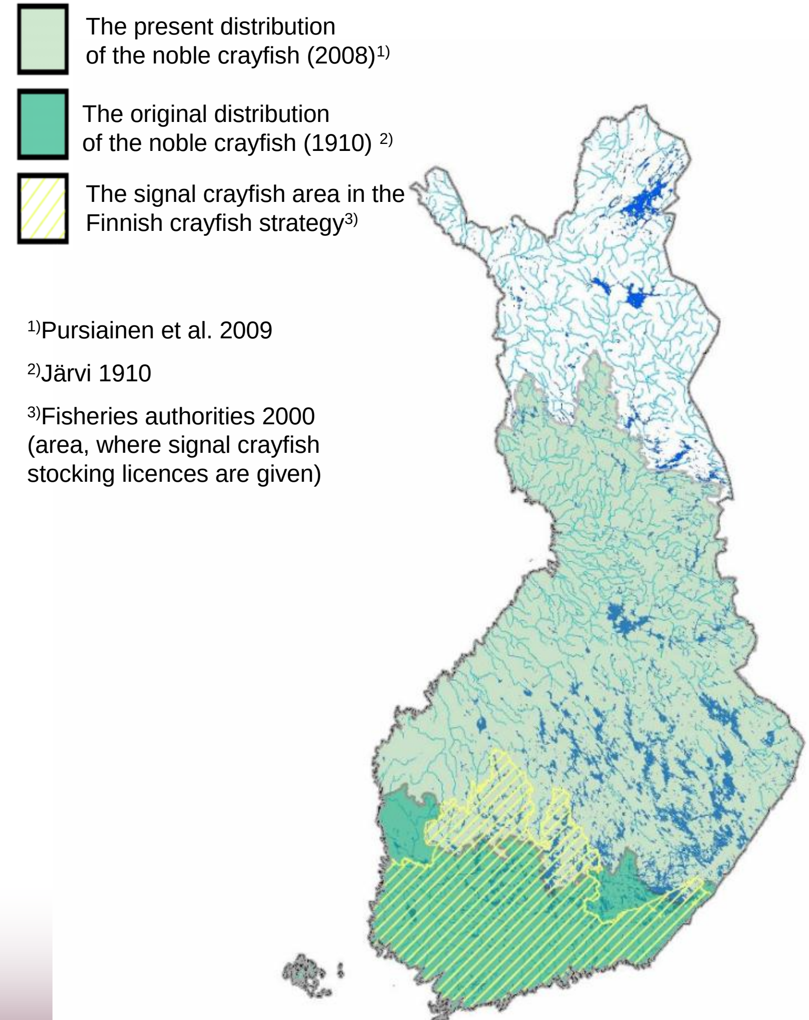
(Slide Markku Pursiainen)

- 2006 production 6,8 mill. ind. (1,6 A.a, 5,2 P.l)
  - Official statistics use the producer's price 2,83 €/ind.
  - Value of the catch >19 M€
- More realistic prices may be 1,5 €/Pl and 2,5 €/Aa
  - Rough estimate of the value ~**12 M€**
  - Signal's share of the value 70 %
- Professional + recreational inland fisheries
  - Vendace 4,4+1,6 = 6,0 M€
  - Pikeperch 0,4+7,4 = 7,8 M€
- Aquaculture production for consumption: 42 M€

Management of crayfish stocks

Any stocking of crayfish needs a permission of fisheries authorities

Crayfish strategy 2000 by the fisheries authorities restricted signal crayfish stocking to southern Finland.

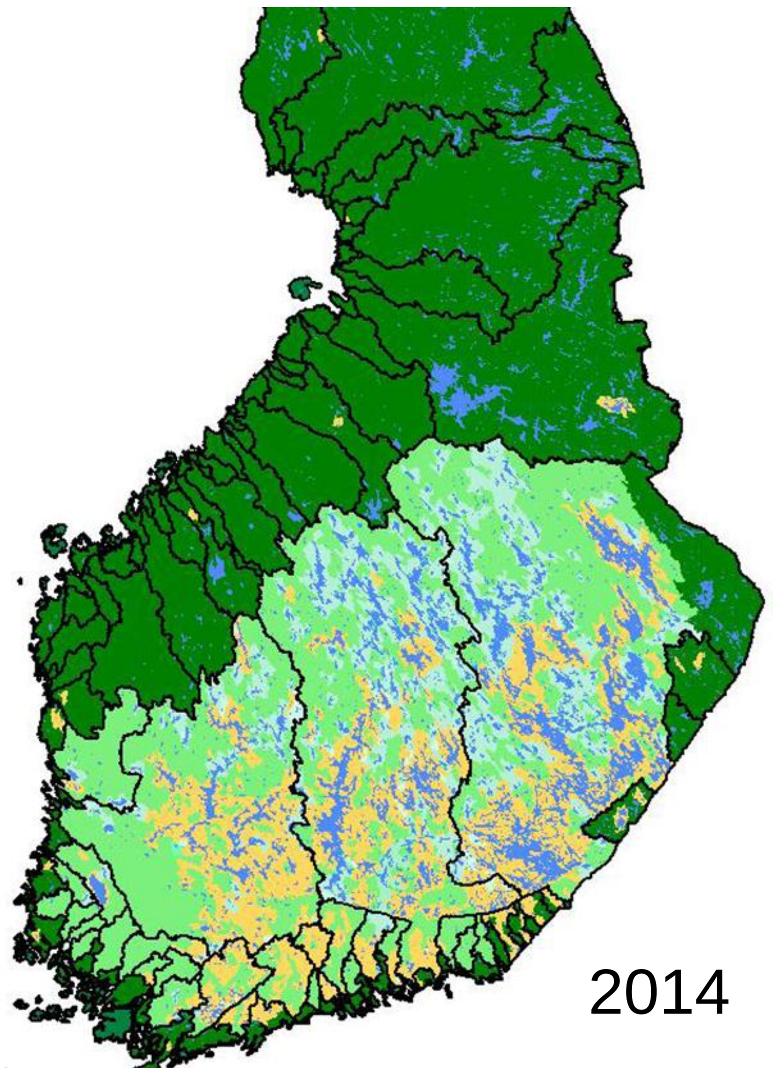


Map: Finnish fisheries institute

Management strategies

Many illegal introductions of signal crayfish to other areas

Strategy was renewed 2014 to allow a much wider area for signal crayfish



Map: Kansallinen Rapustrategia, MMM 2012

Crayfish plague

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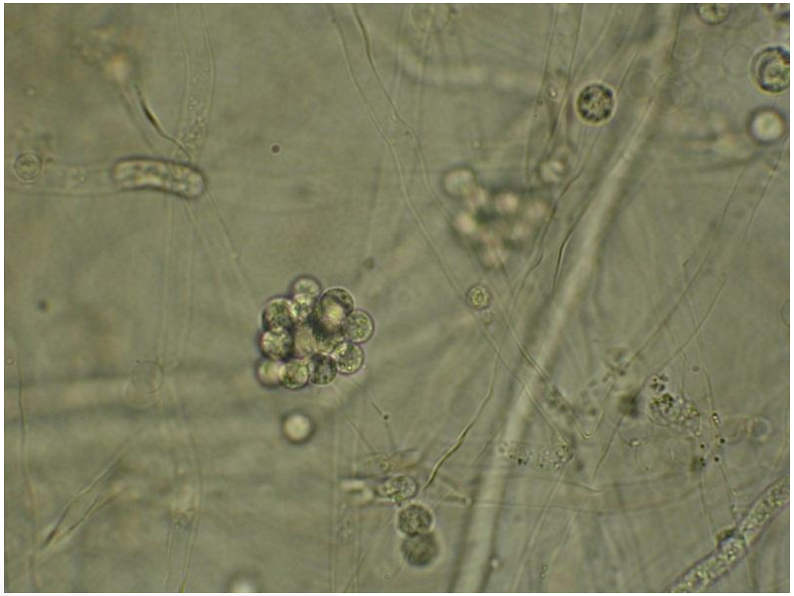
Crayfish plague

- parasite *Aphanomyces astaci* (Schikora)
- Oomycetes-Saprolegniales-Aphanomyces
- Fungal-like growth in the exosceleton of freshwater crayfish
- no sexual propagation found
- growth in 4-24 ºC

The life cycle of *Aphanomyces astaci*

mycelium- sporangium- primary spores-primary cysts-secondary spores (zoospores)-secondary cysts



Majority of spores are formed during molting or death, but considerable amount is released also from intermolt animals (Strand et al. 2012)

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Host-parasite relationship

- *A. astaci* originates from North-America: North-American crayfish species carriers, acute disease only exceptionally

    Pacifastacus leniusculus
    Procambarus clarkii
    Orconectes limosus
    and about 350 other species?

- All European species highly susceptible, as well as Asian and Australian species

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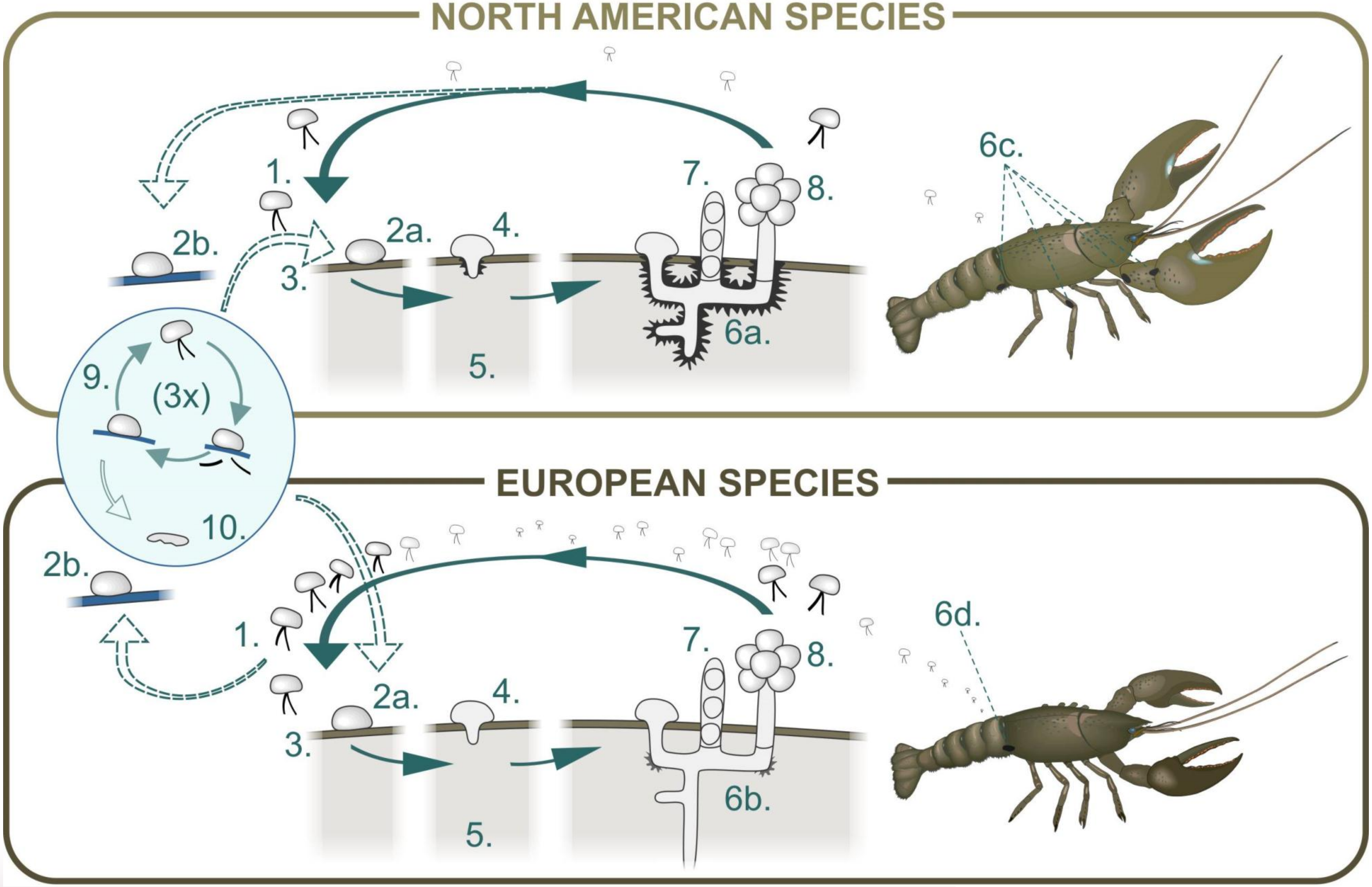
Other possible hosts or vectors:

- Chinese mitten crab *Eriocheir sinensis* can transmit crayfish plague to susceptible species (Schrimpf et al. 2014).Incidentally found in Finland.
- Freshwater crab *Potamon potamios* (Svoboda et al. 2014)
- Freshwater shrimp *Macrobrachium dayanum* (?) (Svoboda et al. 2014)

Crayfish plague



(Imitating the illustration by Iñaki Diéguez-Uribeondo (Souty-Crosset et al. 2006 "Atlas of crayfish in Europe"), © RKTL)

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Symptoms in carrier species

- Alert defensive mechanism prevents the plague agent from effective growth
- Low level infection without visible symptoms
- Melanised areas, missing limbs
- Acute phase and death in stress situations or when infection pressure is high

Symptoms in highly susceptible species

Crayfish plague

- often death without visible symptoms- slow reaction towards the invading parasite
- Scratching, paralysis, daytime activity
- Necrotic (whitish) areas in cuticle
- Occasionally melanised areas, missing limbs

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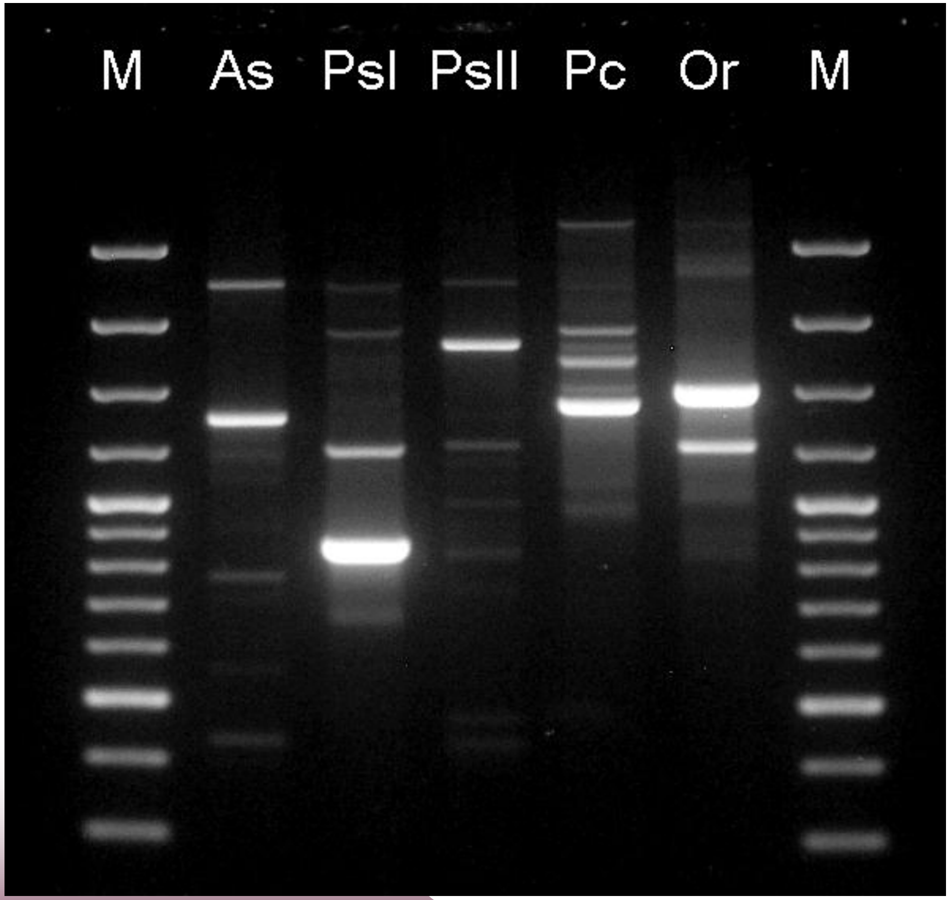
Genotypes of *A. astaci*

- Five different genotypes recognised today:

As (group A): Astacus
Ps1 (group B): Pacifastacus, Astacus, Austropotamobius
Ps2 (group C): Pacifastacus
Pc (group D): Procambarus
Or (group E): Orconectes

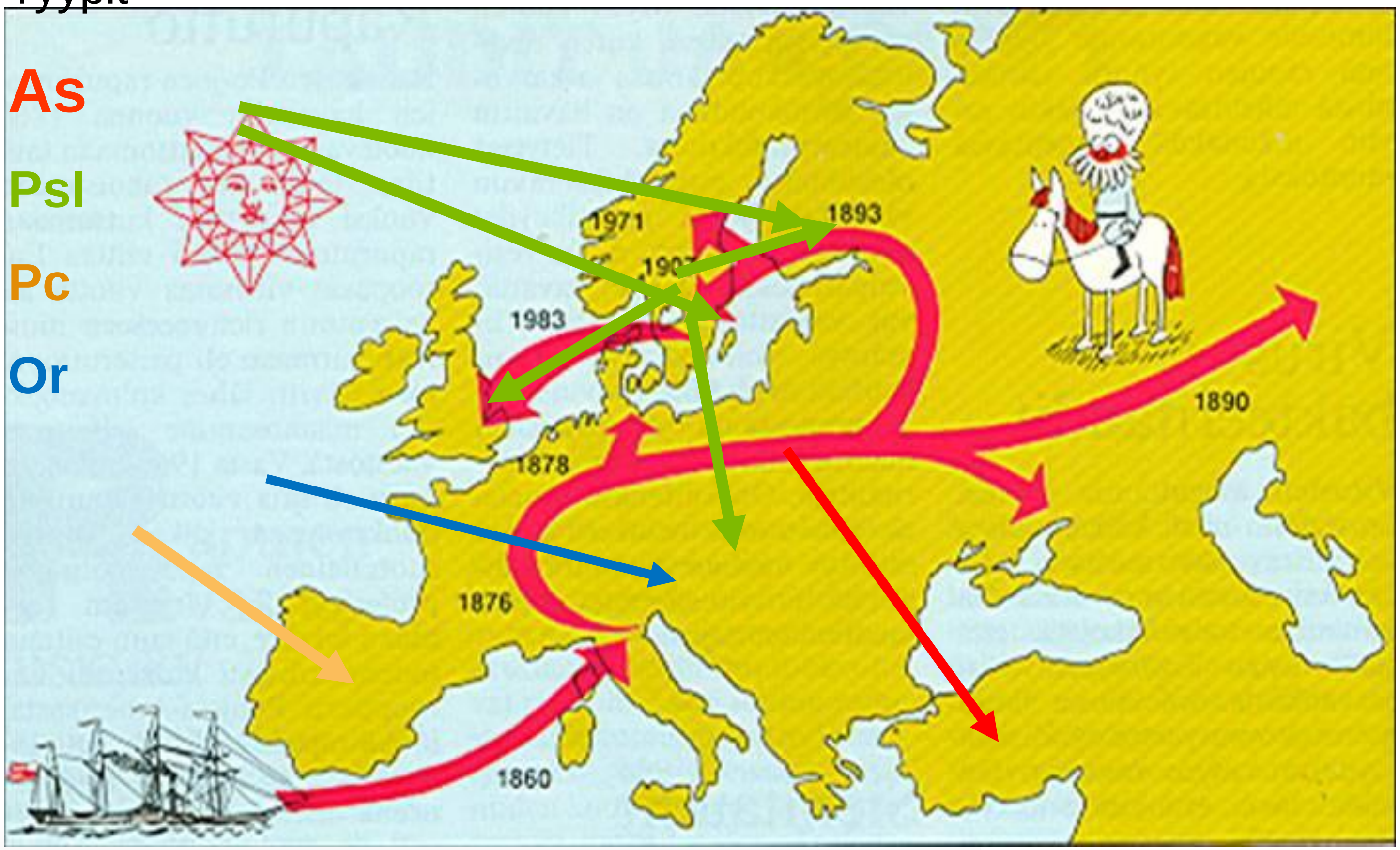
(Huang et al. 1994, Dieguez-Uribeondo et al. 1995, Kozubikova et al. 2011)

- Genotyping based on RAPD-PCR

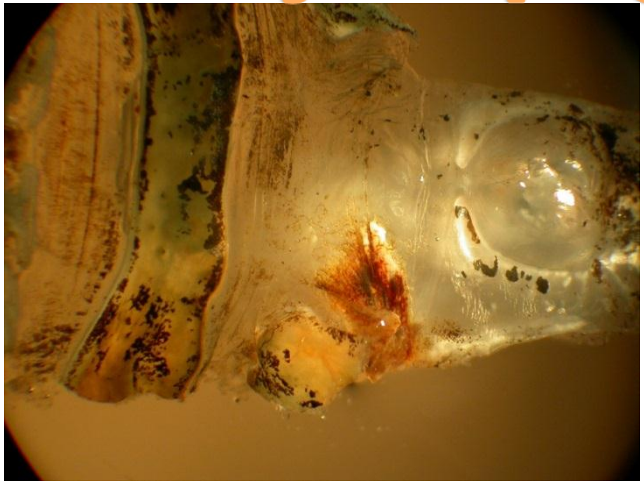


Crayfish plague introduction to Europe

Tyypit



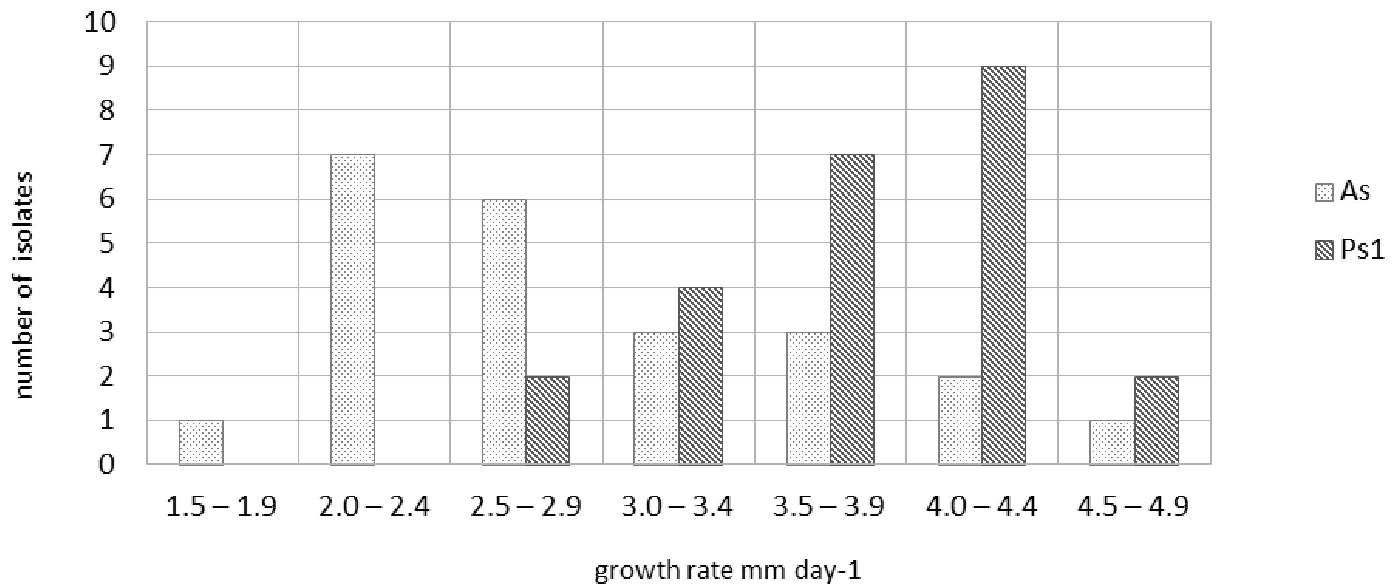
(original drawing Martti Utriainen)



Differences in virulence between the genotypes

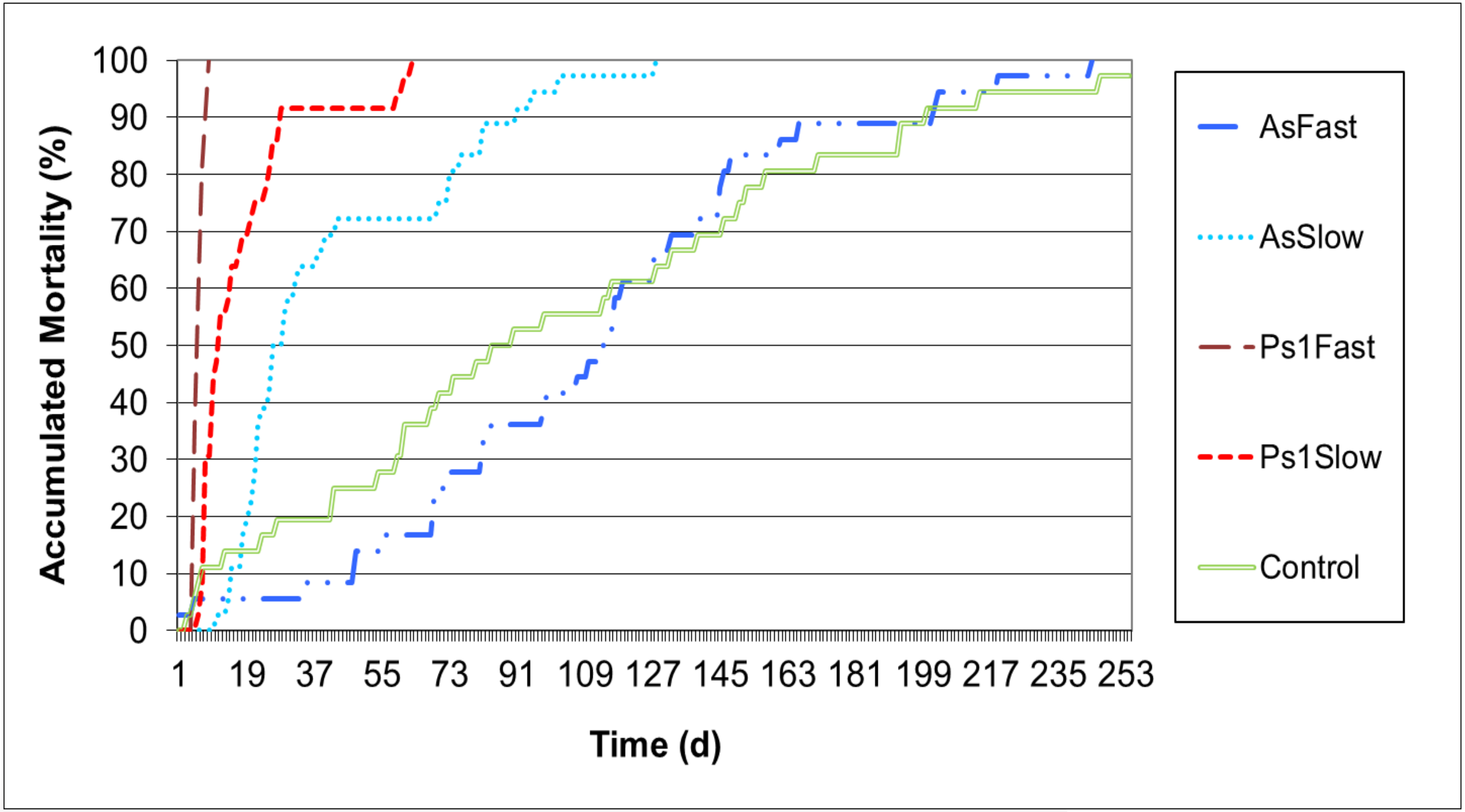
- **Ps1** involved in acute mortality events in noble crayfish
- **As** not found in signal crayfish so far
- **As** recognised as the cause of acute mortalities, but also found in weak populations of noble crayfish that remained after an acute episode
- Difference in virulence detected also in several laboratory trials

Experimental differences between crayfish plague strains



- Does the ability to grow on an artificial medium reflect the ability to grow in crayfish tissue?
- Differences between the genotypes As and Ps also in the chitinase genes (Makkonen et al. 2012)

Variable virulence between the strains



- Ps1 is an efficient killer!

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Evidence about susceptible species coping with plague:

- Narrow clawed crayfish *Astacus leptodactylus* in Turkey, but recently also in Romania
- Noble crayfish in Finland (**As**)
- Whiteclawed crayfish *Austropotamobius* pallipes in Italy, self-limiting outbreak
- **As**-genotype as a cause of plague in farmed crayfish in Italy: wild stock as origin
- Stone crayfish *Austropotamobius torrentium* in Slovenia and the Czech Republic

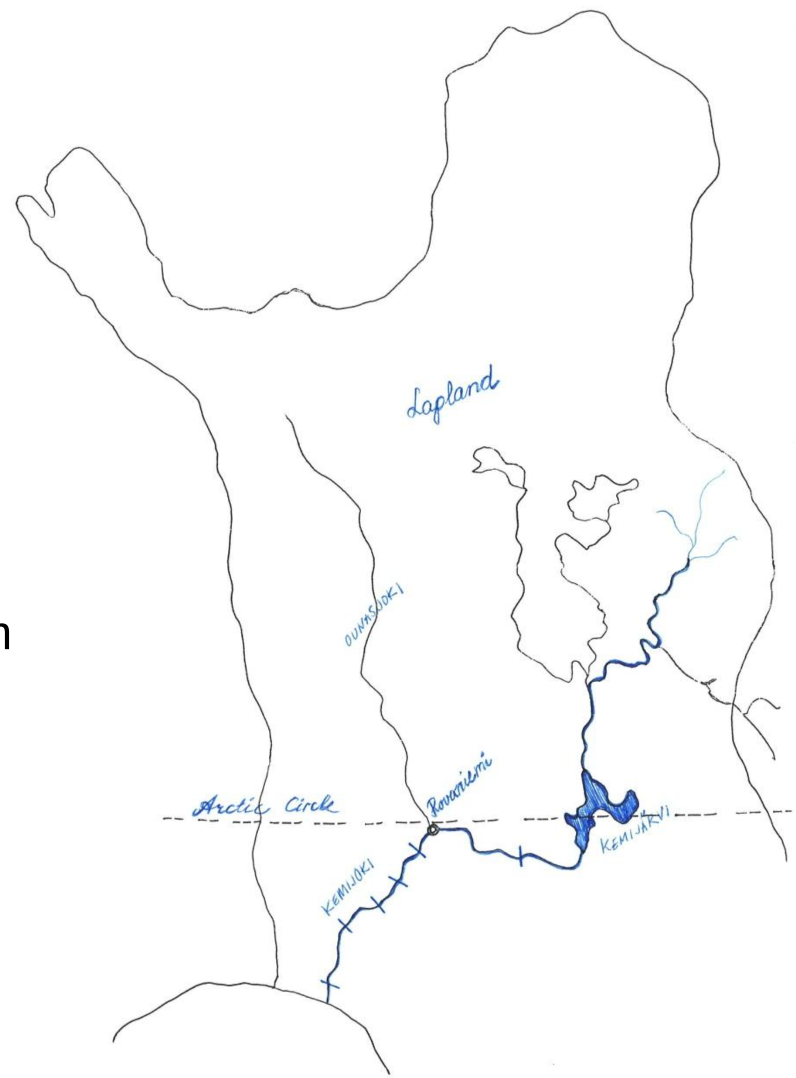
(Svoboda et al. 2012,Harlioglu 2008,Schrimpf 2012,Kokko et al. 2012, Viljamaa-Dirks et al. 2011, Caprioli et al. 2013, Pretto et al. 2014,Kusar et al. 2013, Kozubikova et al. 2014)

Environmental effect?: Slowly progressing infection in the river Kemijoki

- Infection in the end of the year 2005?
- As-type crayfish plague verified in the end of the summer 2006
- Follow up in 2007: infected crayfish to be found from Rovaniemi downwards,diminished population levels but still fishing in some points
- Follow-up in 2008: three infected parts of the river studied with qPCR-method:
  Rovaniemi 11 of 29 positive
  Petäjäkoski 1 of 4 positive
  Taivalkoski 5 of 26 positive
  Situation in 2009-2014: downwards stocks collapsed, crayfish and plague from Rovaniemi upwards

Large river in the north: low spore amounts, low water temperature affecting growth rate of the plague?

Adaptation of the plague agent, diminished virulence?

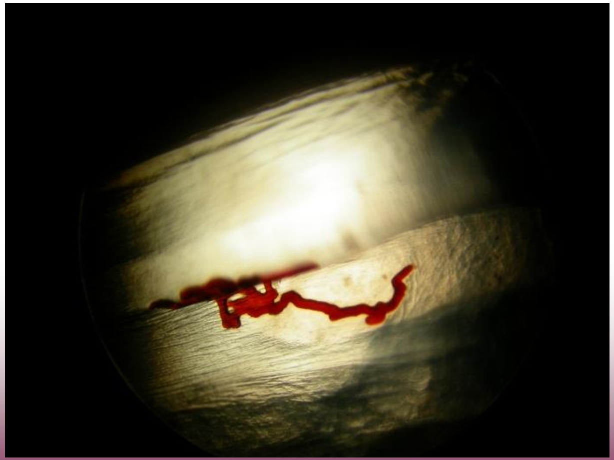


Host immune response

- Differences also between highly susceptible species: narrow clawed crayfish reported as more resistant than noble crayfish

- Can be different also in subpopulations

(Makkonen et al. Differing virulence of *Aphanomyces astaci* isolates and elevated resistance of noble crayfish *Astacus astacus* against crayfish plague. DAO 2012)

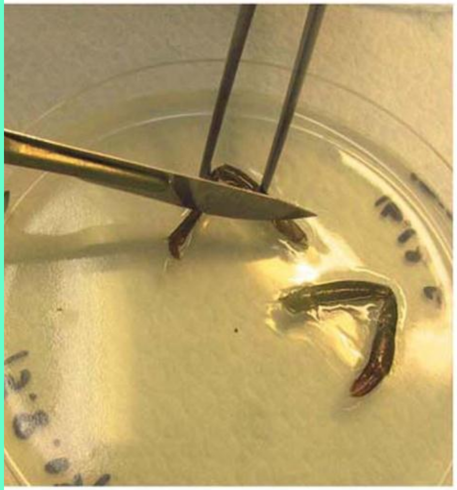


As genotype in the cuticle about 5 months after infection of noble crayfish

Diagnosis

Diagnosis

Diagnosis:
Macroscopic investigation
Microscopic investigation
Culture
Identification (PCR) (Oidtmann et al. 2006)
OR
real time PCR (qPCR) (Vrålstad et al. 2009)
Microsatellite markers (Grandjean et al. 2014)



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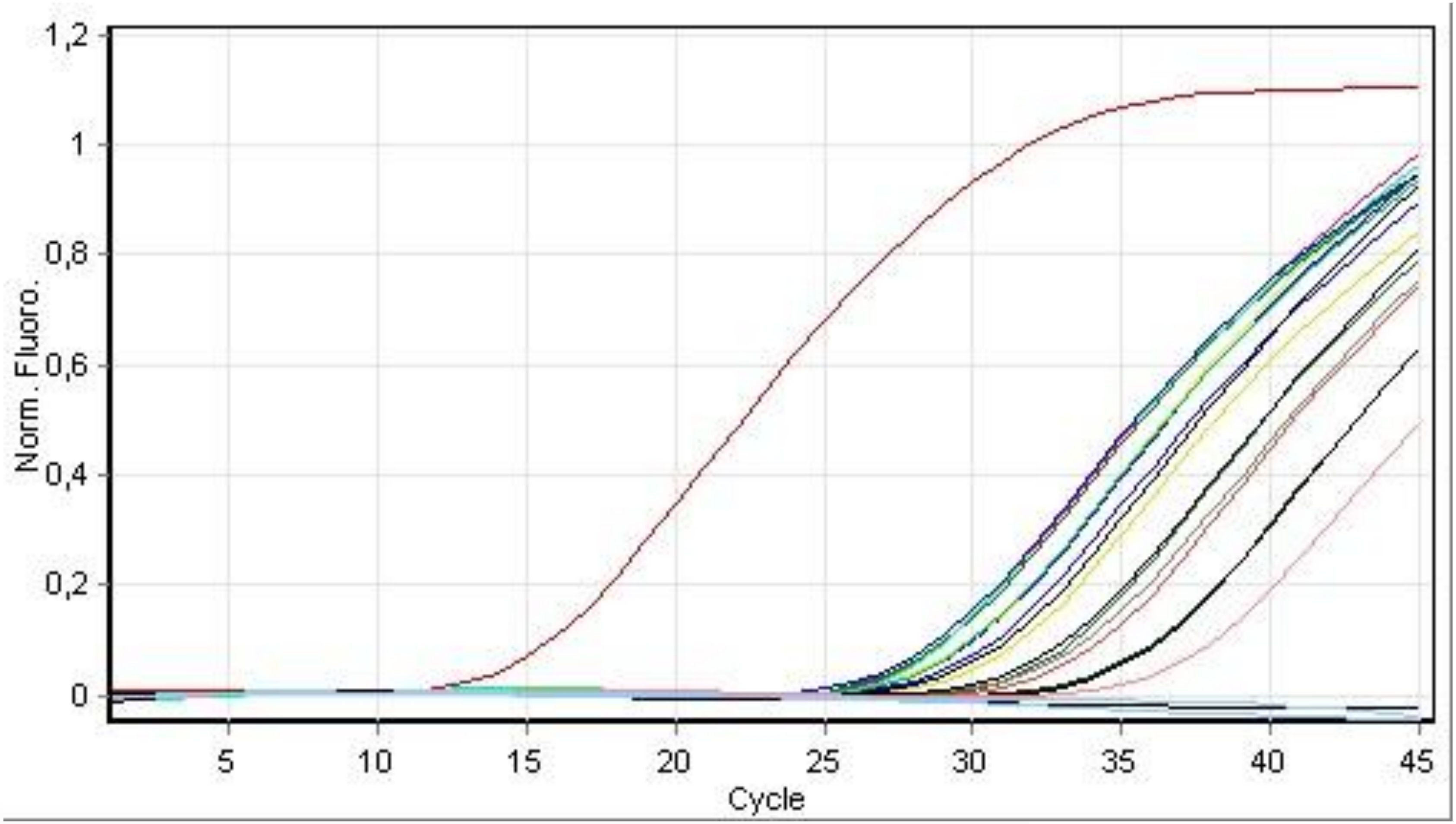
Real time PCR



New methodology for genotyping

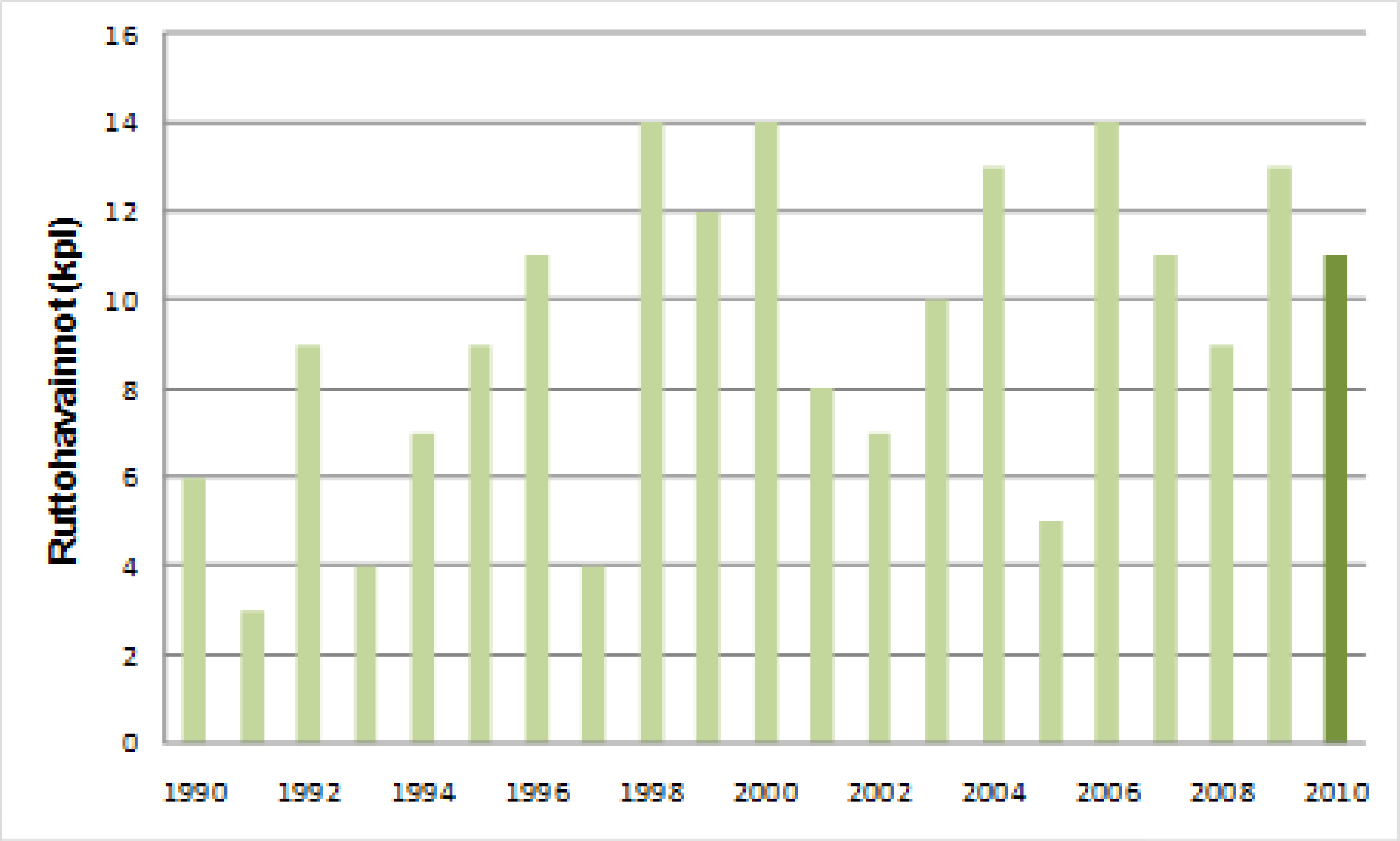
- RAPD-PCR demands a pure culture, which is often difficult or impossible to obtain
- Microsatellite markers can differentiate the known genotypes and reveal new ones (Grandjean et al. 2014)
- Can detect new subtypes inside the genotype groups (Viljamaa-Dirks et al., unpublished)
- Analysis directly from the crayfish cuticle
- Needs moderate to high level of plague agent
- Even successfully employed for historic samples (Vrålstad et al. 2014)
- Other characterization with amplified fragment lenght polymorphism (AFLP)(Rezinciuc et al. 2013)

Detecting spores directly from water

- Environmental DNA detection by filtering water followed by qPCR
- Has been successfully tested in signal crayfish ponds and populations with high infection level, as well as acute cases of noble crayfish plague
- Needs still improvement for more sensitivity
- Large amounts of water:ultrafiltration, specific method

Strand et al: Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. 2011 DAO

Diagnosed cases of crayfish plague in Finland



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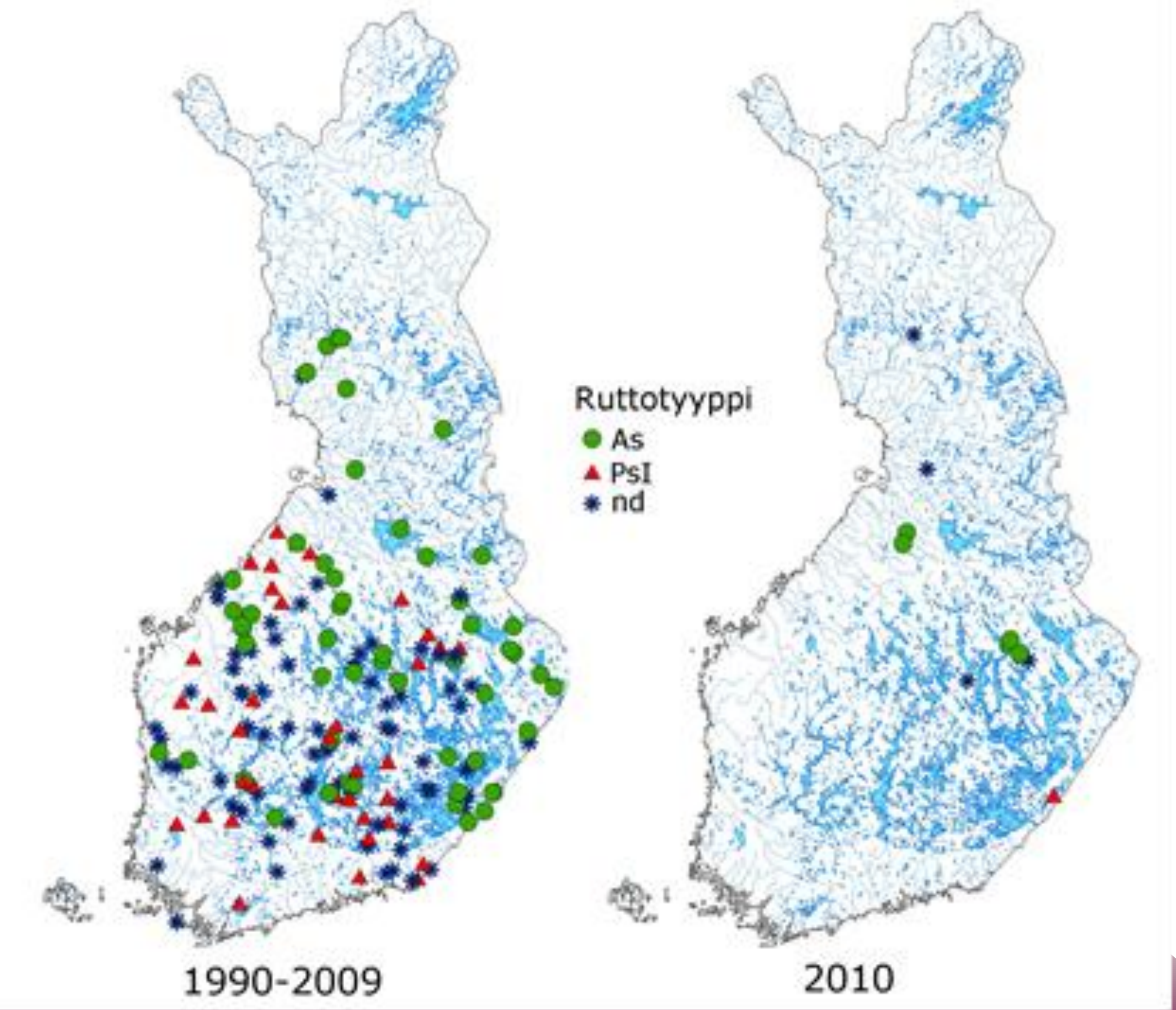
Distribution of the different genotypes

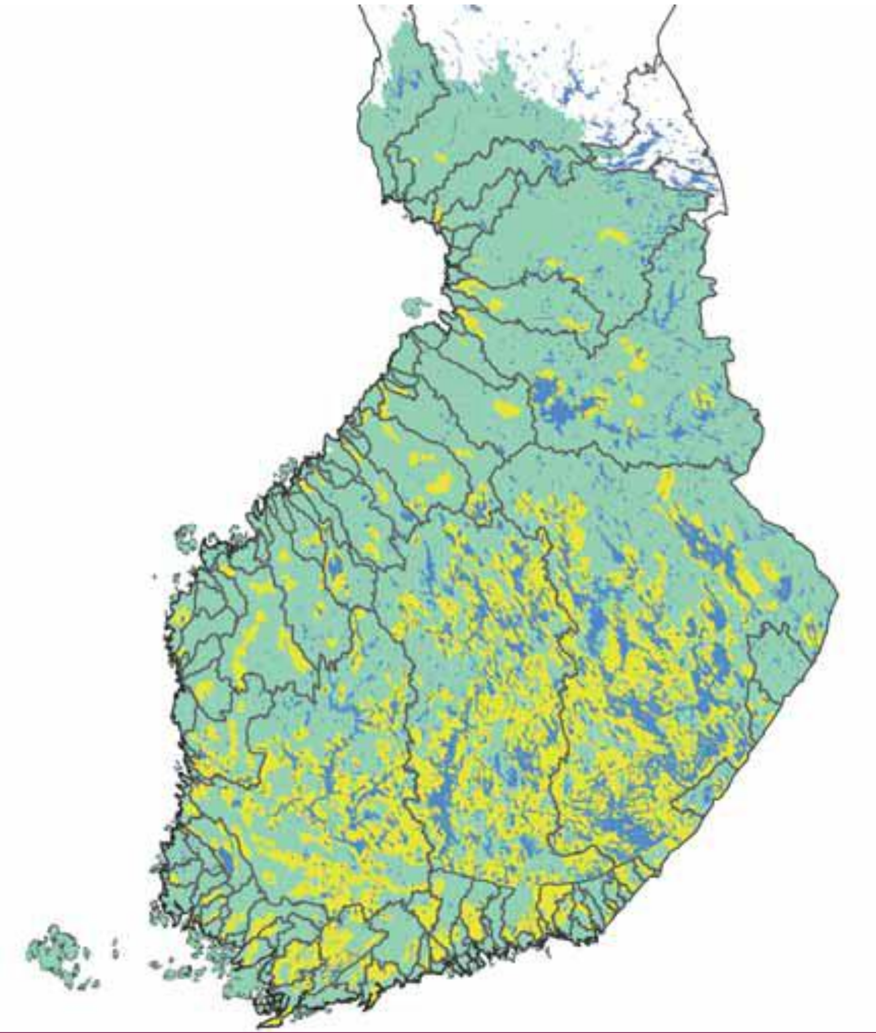


Maps: Finnish Fisheries Institute

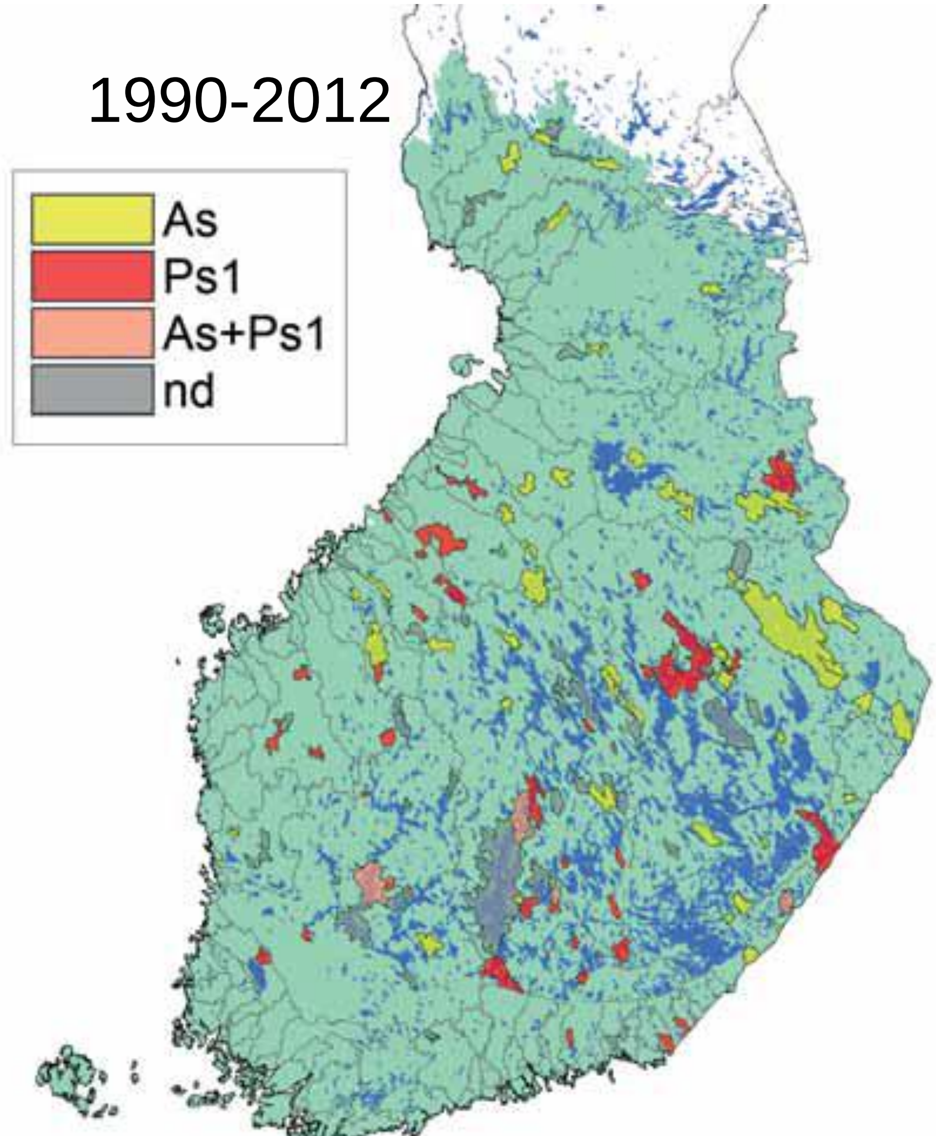
Prevalence of crayfish plague in noble crayfish in Finland

Diagnosis

1893-2000



1990-2012



Maps: Finnish Fisheries Institute

Surveying stocks and water bodies

- To make a successful restocking, the water body has to be controlled for remaining carriers, but also the donating population has to be certified plague-free
- Host-parasite relationship is not sufficiently known concerning the latent/chronic plague infection in noble crayfish
- Sample size is difficult to determine without that knowledge
- Remaining carriers difficult to find, very weak populations
- Presence of a few, unknown signal crayfish always possible

A study to explore possibilities for carrier detection

- qPCR (Vrålstad et al. 2009) chosen for screening as the most sensitive method available, ten populations and nearly one thousand crayfish screened

- Detection of infection in latent phase of infection: 4 % of individuals, in chronic infection: 22 %.

- Expected level of infection 20%, 100% specificity of the method: only 13 targeted samples- 99% specificity: 20 samples

Acute infection:
Lamujoki 2010 14/15 (93%)
Slowly progressing infections:
Simojoki: 2010 7/52 (13%)
2011 0/9 (0%)
Kemijoki: 2010 2/55 (4%)
2011,June 3/67 (4%)
2011,August 12/93 (13%)
Chronic infection:
Pyhäjärvi 2011 13/58 (22%)

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Comparison of symptomfree individuals and crayfish with melanised areas:

population	Symptom-free, pos/total	%	With spots, Pos/total	%
Simojoki	2/41	5%	5/11	45%
Kemijoki 2010	0/49	0%	2/6	33%
Kemijoki 2011, June	2/53	4%	1/14	7%
Kemijoki 2011, August	5/60	8%	7/33	21%
Pyhäjärvi	4/48	8%	9/10	90%

Comparison of the *A. astaci* DNA-levels in abdominal cuticle (A) and melanised areas (B)

sample	Number of pos	Ct, mean
A without melanisation	24	36,55
B	17	28,72
A with melanisation	6	28,21

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From theory to practise

- chosen survey method in Evira: 60 crayfish, that are kept in laboratory at least two months or until death. Ten crayfish is selected for PCR on judgement of macroscopic and microscopic melanisation.
- When studying the suitability of a water body, cage experiments are a traditional and still valid method, nowadays combined with the PCR detection.
- In case of uncertain positives, repeating the sampling the following year is recommended

Management

Classic approach to crayfish plague management

- Expectation was a rapid 100% mortality in noble crayfish
- Restocking from any wild or farmed population
- In some cases cage experiment to ensure survival, especially if diagnosis of plague was lacking
- Restocking as soon as possible, preferably the next season

HOWEVER

recurrent episodes of crayfish plague common, and only 10-15% restockings successful in the long run

Reoccurrence of plague in Finland

- Plague epidemic reoccurring in the same water body than reported earlier
- 1893-2012 951 reported cases (less than 5 years excluded)
- Mean interval 26.6 years (5-90)
- 4th reoccurrence diagnosed in 2014

	water bodies +	1. reoccurrence		2.		3.	
		number	interval				
01-14	493	78	23,5	15	28,7	3	12,7
15-36	325	63	32,2	18	26,7	3	27,3
37-58	59	13	28,1	3	13,7		
59-73	42	4	18,8	2	9,5		
81-99	32	3	19,5				
	951	**161**	**27,1**	**38**	**25,6**	**6**	**20,0**

Cage experiments

The rivers on the West Coast

- Have supported very strong (although introduced) populations of noble crayfish in the past

- Now practically no exploitable stocks- due to environmental changes, worsening water quality- or the plague?

- Survey through cage experiments: Ähtävänjoki.

  32 and 29 individuals kept in two cages from September to begin November: analysis negative

  25 and 26 individuals kept until half December: 2+2 positive. Time in the cages has to be long enough!

Cage experiment project 2011-2013

- targeted two river systems in the western Finland, Kyrönjoki and Karvianjoki

- Each year 12 to 20 cages were placed in different parts of the main rivers or tributaries and followed for at least four months

- Four cages were placed in each locality under study and each cage housed 15 noble crayfish *Astacus astacus*.

- Dead crayfish found in the cages during the experiment as well as remaining individuals at the end of the caging period were subjected to laboratory examination and targeted real time PCR analysis.

Results and conclusions cage experiment

- Both rivers: In 2011 all negative, in 2012 weak positives (inconclusive), in 2013 mostly positive, acute cases of Ps1 type crayfish plague
- Both rivers not suitable for restocking with noble crayfish
- 2013 results point to signal crayfish presence in the river systems
- 2012 weak positives: not clear if due to latent phase of Ps1 infection or presence of noble crayfish that carry As
- Cage period should be even longer than 4 months?
- Follow up for two to three years necessary

Is there future for the noble crayfish exploitation?

- Repeating failures of restockings discourage stockholders to keep the noble crayfish, and encourage the illegal stocking of signal crayfish
- Better understanding of the situation will give enhanced chance for restocking success
- Categorisation of the wild populations will help in management decisions
- Category 1 populations can form the future ark sites for noble crayfish
- The noble crayfish is still valued higher than the signal crayfish and will be more attractive species for stockholders
- BUT:

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What to do about the chronic infections?

- If the plague infection does not destroy the whole population:
- Replacement with signal crayfish?
- In smaller water bodies: possibility to use a more virulent plague strain to get rid of the carriers?

Signal crayfish and the plague infection

- Part of the signal crayfish stocking made with artificially incubated eggs- presumably plague free fry
- The first infection of plague in naif population leads often to a sudden crash of the population, with severe plague marks on the crayfish. Example lake Saimaa: catch diminished locally 80-90%, melanised spots on 70% (affects commercial value). In a few years the situation in normalising in most cases
- Mixed population: also diminishing population or even acute plague through high infection pressure (Lake Puujärvi)
- Effect of environmental stress in the north: colder waters, less molt cycles-more severe plague?

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Problems with signal crayfish stocks

Eroded swimmeret syndrome (ESS) in heavily plague infected individuals reason for poor egg numbers?

Many of the presented studies have been realized together with The Finnish Fisheries Institute (Nowadays LUKE). Markku Pursiainen and all the others that have been involved have made it possible to improve our understanding about the Finnish situation with crayfish and crayfish plague. I also thank the laboratory staff and my colleagues in Evira, especially Sirpa Heinikainen for the molecular work.

Thank you for your attention!