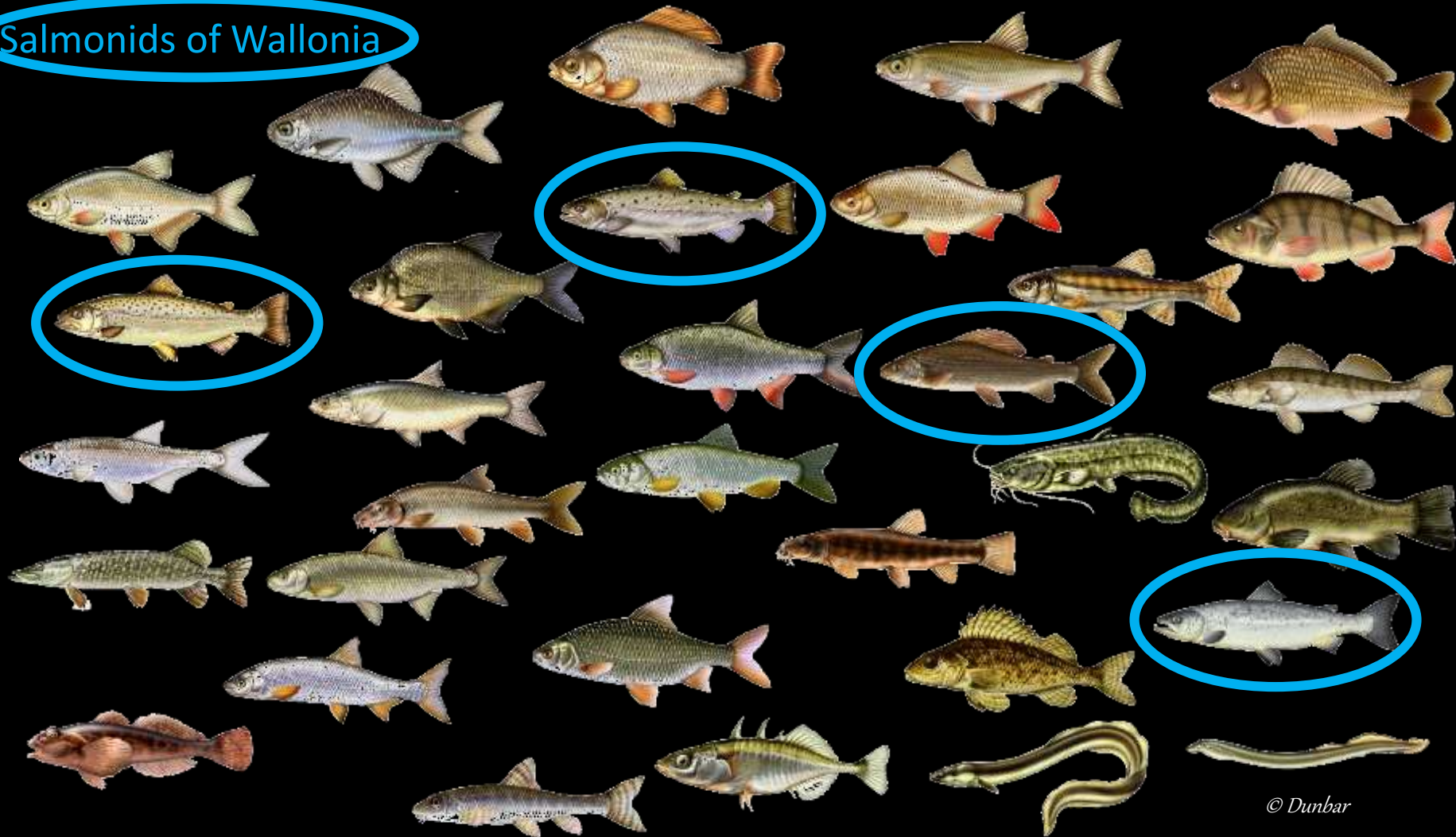


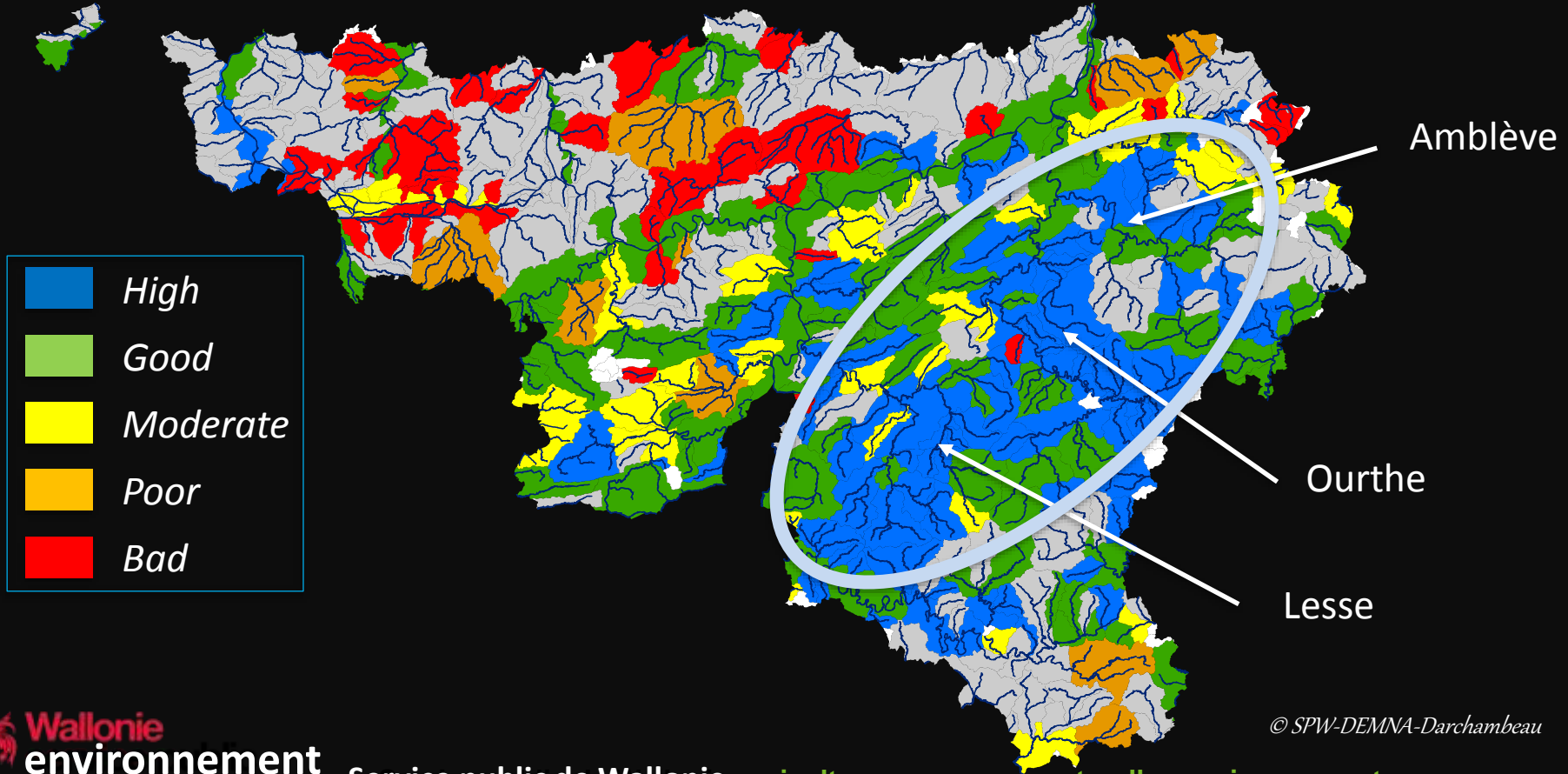
Meuse District



Salmonids of Wallonia



Quality of fish populations in Wallonia (IBIP)





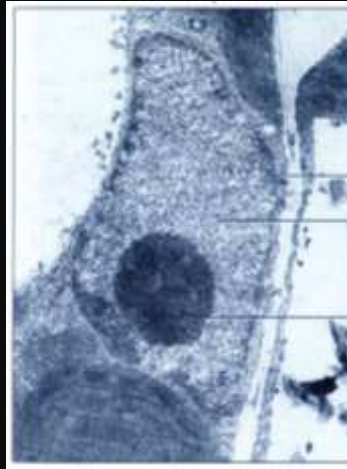
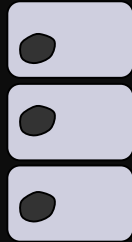
Using genetic tools to inform management in Salmonids in the river Meuse basin: the example of Wallonia

Xavier ROLLIN¹ & Marie-Christine Flamand²

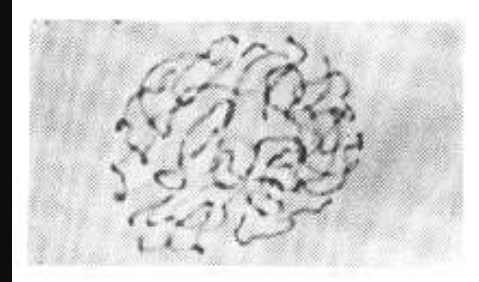
1. Public Service of Wallonia – DGARNE – Nature & Forest Department – Fisheries Service
2. UCLouvain – Louvain Institute of Biomolecular Science and Technology

From salmonid fish to DNA...

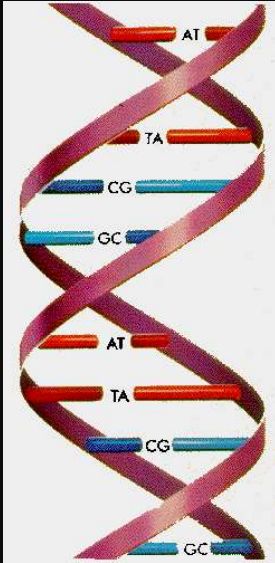
Adipose
fin



Chromosomes



From DNA to microsatellite markers...

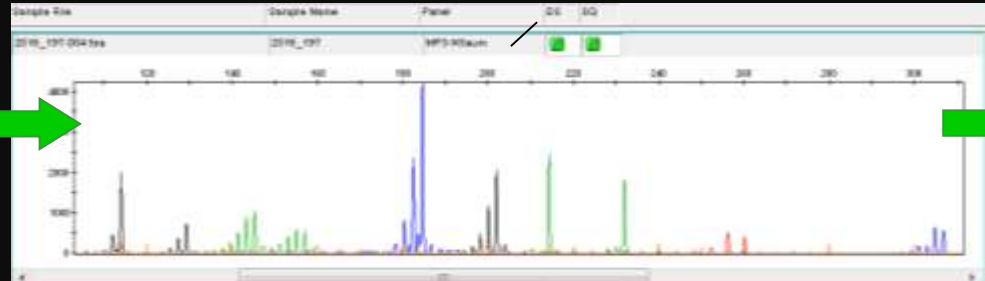


Specific DNA regions: short repeated sequences **of variable length**



Analyse about 20 DNA regions using specific markers:
& measure the size of each allele

Genetic imprinting:
one single bar code per individual fish

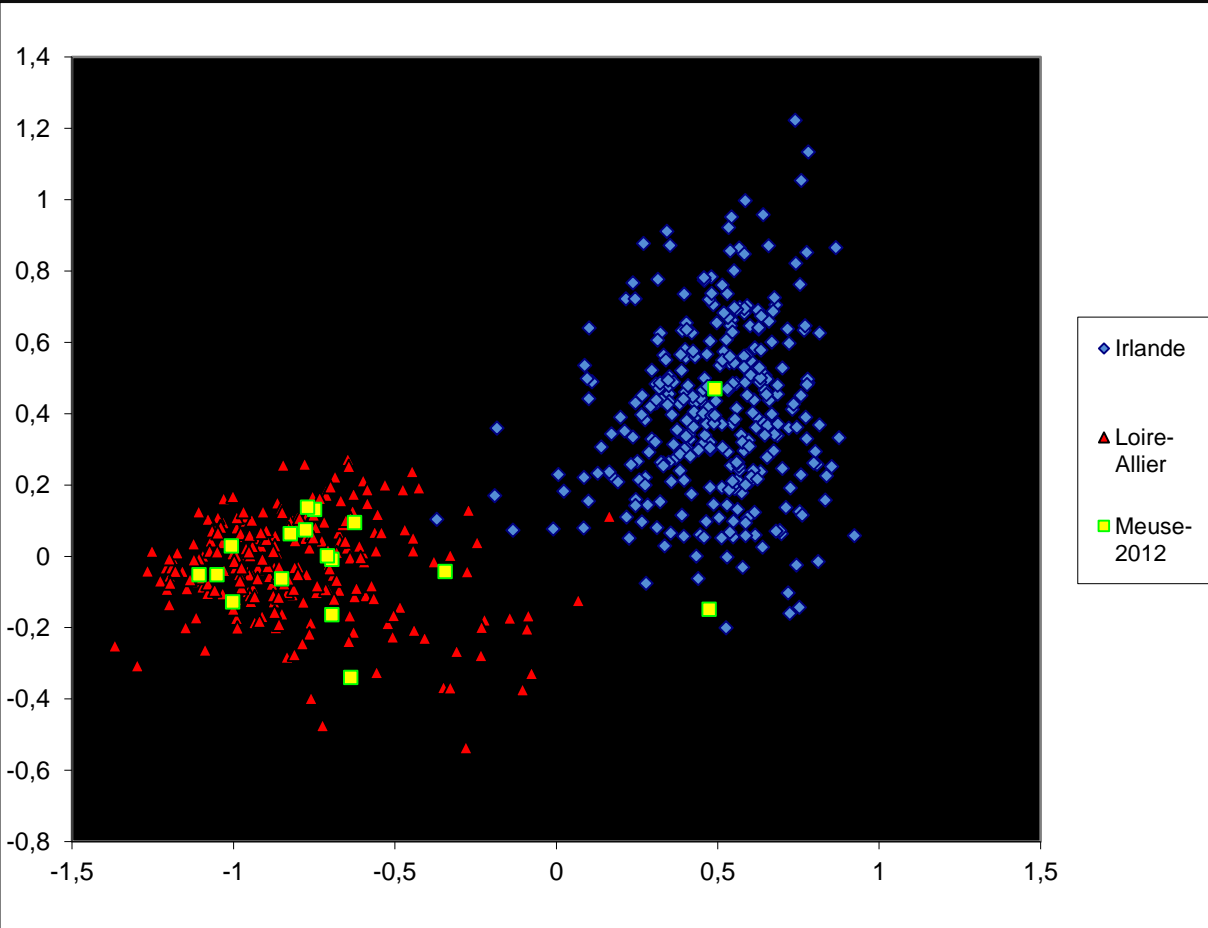


Some examples of genetic tool utilisation in Wallonia

I. ATLANTIC SALMON

A. Group assignation

1. *Atlantic salmon* – A. **Group** assignation



Different strains of Atlantic salmon are restocked in the Meuse river system since 1988

With genetic imprinting, each salmon returnee can be assigned to its most probable stock of origin:

- Irish stock (in blue)
- Loire-Allier (in red)

With this tool you can see that the best strain in terms of returning success is the Loire-Allier strain (here for 2012)

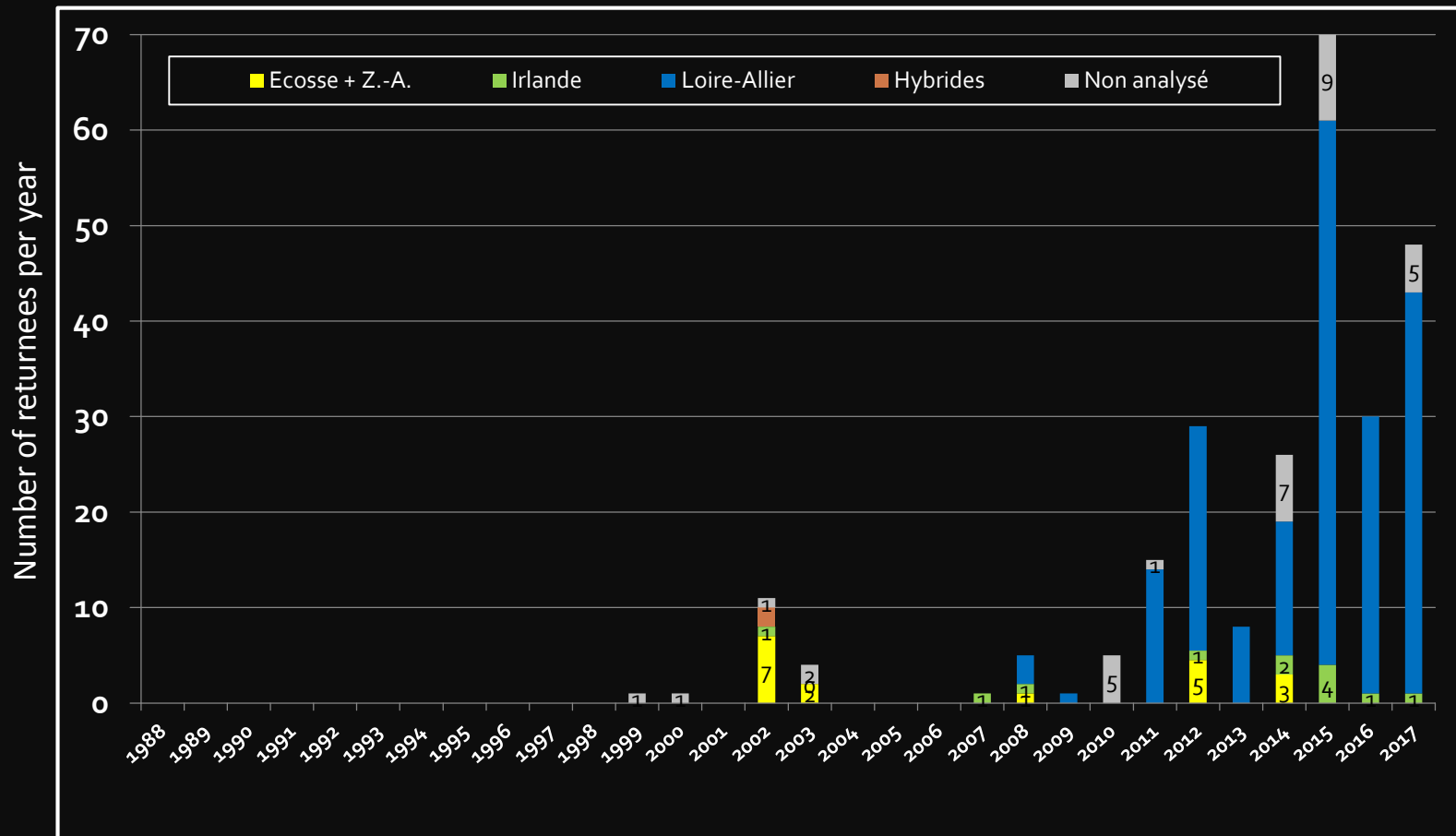
1. *Atlantic salmon* – B. **Group** assignment

Salmon Number	Catch date	Length	Weight	Sex	% Stock Assignment		
	2018	(cm)	(kg)		Loire-Allier	Irland	Scotland + Zieg-Atran
SM201	4-mai	97	8.1	F	98		
SM202	4-mai	70	2.7	M	98		
SM203	4-mai	78	3.9	F	93		
SM204	6-mai	66	2.0	M	91		
SM205	13-mai	92	6.0	F	95		
SM206	20-mai	83	4.5	M	96		
SM207	21-mai	79	4.5	M	98		
SM208	26-mai	79	4.1	F	20	61	15
SM209	30-mai	95	7.2	M	97		
SM210	22-juin	71	2.5	M	91		
SM211	22-juin	72	2.9	M	98		
SM212	5-oct	92	5.2	F	99		
SM213	11-oct	81	3.5	M	95		
SR57	25-avr	83	4.8	F	80	14	
SR58	12-mai	93	?	?	97		
SR59	11-juin	71	3.1	F	98		

Most of salmon caught in the Meuse river system in 2018 are of Loire-Allier genetic origin (SM206):

- Mostly related to stocking (only Loire-Allier strain is stocked since 2015)

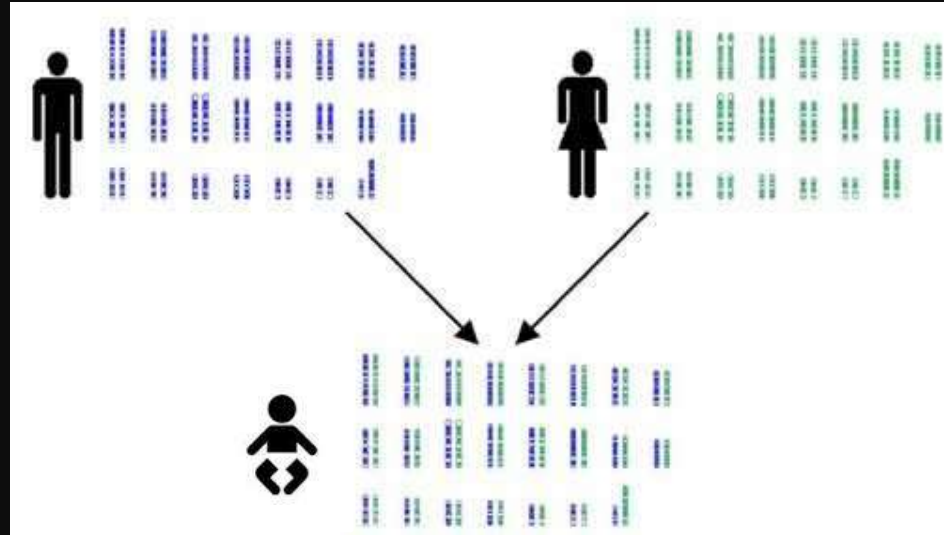
Strain of **adult salmon** caught in the Meuse basin from **1988 to 2017**



I. ATLANTIC SALMON

B. Parental assignation

From genetic imprinting to parental assignment...



Wallonie
environnement
SPW

Service public de Wallonie **agriculture ressources naturelles environnement**

1. Atlantic salmon – B. Parental assignment

Father

Son

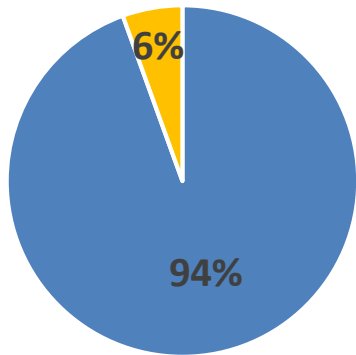
Mother

M (2014) = SM62			SM206 (2018)			F (2012) = SM44		
Markers	Alleles		Markers	Alleles		Markers	Alleles	
SSsp2201	278	290	SSsp2201	290	306	SSsp2201	294	306
SSsp2210	144	169	SSsp2210	148	169	SSsp2210	136	148
SSsp3016	152	152	SSsp3016	152	156	SSsp3016	156	156
SSspG7	128	140	SSspG7	140	144	SSspG7	140	144
Ssa197	193	193	Ssa197	193	193	Ssa197	193	197
Ssa202	261	261	Ssa202	261	269	Ssa202	253	269
Ssa410	263	267	Ssa410	267	267	Ssa410	207	267
SsaD157	359	367	SsaD157	355	359	SsaD157	355	359
SSsp1605	230	230	SSsp1605	230	230	SSsp1605	230	230
SSsp2216	235	235	SSsp2216	235	235	SSsp2216	235	243
Ssa14	144	144	Ssa14	144	144	Ssa14	144	144
Ssa289	121	123	Ssa289	121	121	Ssa289	121	121
SsaD144	183	183	SsaD144	139	183	SsaD144	139	187
SsaD486	175	175	SsaD486	175	175	SsaD486	175	175
SsaF43	113	113	SsaF43	113	113	SsaF43	113	113
Ssa85	134	146	Ssa85	130	134	Ssa85	130	132
Ssa171	230	236	Ssa171	230	236	Ssa171	218	236
Ssa408	300	300	Ssa408	276	300	Ssa408	260	276
SSL85	192	206	SSL85	192	206	SSL85	190	192
SSL417	186	198	SSL417	186	186	SSL417	186	200
SSL438	114	118	SSL438	114	114	SSL438	114	114

Example:

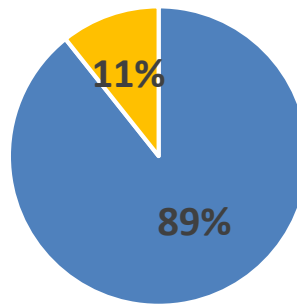
- Atlantic salmon
- 21 markers
- Reproduction 2014 in CoSMos - Erezée
- The son « SM206 » has inherited one allele of each of his parents
- The combination of the different alleles is specific to each individual = genetic imprinting

Eggs produced in winter 2014
by semi-wild salmon returnees (%)



- Eggs/parrs of captive salmon stocks - Erezée & Chanteuges
- Eggs/parrs of semi-wild caught Meuse/Roer salmon

% of juveniles stocked in 2015-2016 that
came out of semi-wild parents reproduced in
2014 (%)

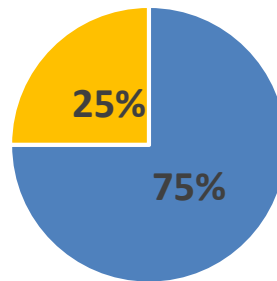


- Eggs/parrs of captive salmon stocks - Erezée & Chanteuges
- Eggs/parrs of semi-wild caught Meuse/Roer salmon

We observed a much higher % of salmon returnees in 2018 that came out of semi-wild parents (25%) than the contribution of these parents to the 2014 reproduction (6% of eggs):

- Better gamete quality of semi-wild salmon compared to captive stocks!
- Better fitness of semi-wild salmon in the Meuse river system? → to be verified at larger scale!
- Powerful tool to measure part life-cycle fitness or stocking strategy (stage, size, place...)

% of salmon returnees in 2018 that came
out of semi-wild parents reproduced in
2014



- Eggs/parrs of captive salmon stocks - Erezée & Chanteuges
- Eggs/parrs of semi-wild caught Meuse/Roer salmon

1. Atlantic salmon – B. Parental assignment

Salmon Number	Catch date	Length	Weight	Sex	% Assignment			Parental Assignment	
	2018	(cm)	(g)		Loire-Allier	Irland	Scotland + Zieg-Atran	♀	♂
SM201	4-mai	97,1	8100	F	98,1				
SM202	4-mai	70,4	2697	M	97,6			SM44 (2012)	SM70 (2014)
SM203	4-mai	78,3	3898	F	93,2				
SM204	6-mai	66,5	2018	M	90,7				
SM205	13-mai	92,5	6050	F	95,4				
SM206	20-mai	83,7	4541	M	95,8			SM44 (2012)	SM62 (2014)
SM207	21-mai	79,7	4464	M	98,1				
SM208	26-mai	79,3	4105	F	19,8	60,9	14,6		
SM209	30-mai	95,5	7250	M	97,4				
SM210	22-juin	71,2	2534	M	90,6				
SM211	22-juin	72,7	2887	M	97,6			SM57 (2014)	SM70 (2014)
SM212	5-oct	92	5226	F	98,7			SM35(2012)	?
SM213	11-oct	81	3495	M	95,2				
SR57	25-avr	83,5	4803	F	80,2	13,8			
SR58	12-mai	93		?	96,6				
SR59	11-juin	71,5	3069	F	98,4			?	SM70 (2014)

Some Loire-Allier parents seem better perform than others in terms of offspring return rate in the Meuse river system:

- By chance? → incomplete data set! → should be generalised to all known parents at the basin scale
- Is there a genetic basis explaining the better fitness?
- Sex related?
- A way for artificial selection?
- Interest for sperm/milt cryopreservation for an *a posteriori* selection of best males (collaboration with University of Namur, Belgium)

II. BROWN TROUT

A. Group assignation

II. Brown trout – **A. Group** assignation

Objectives:

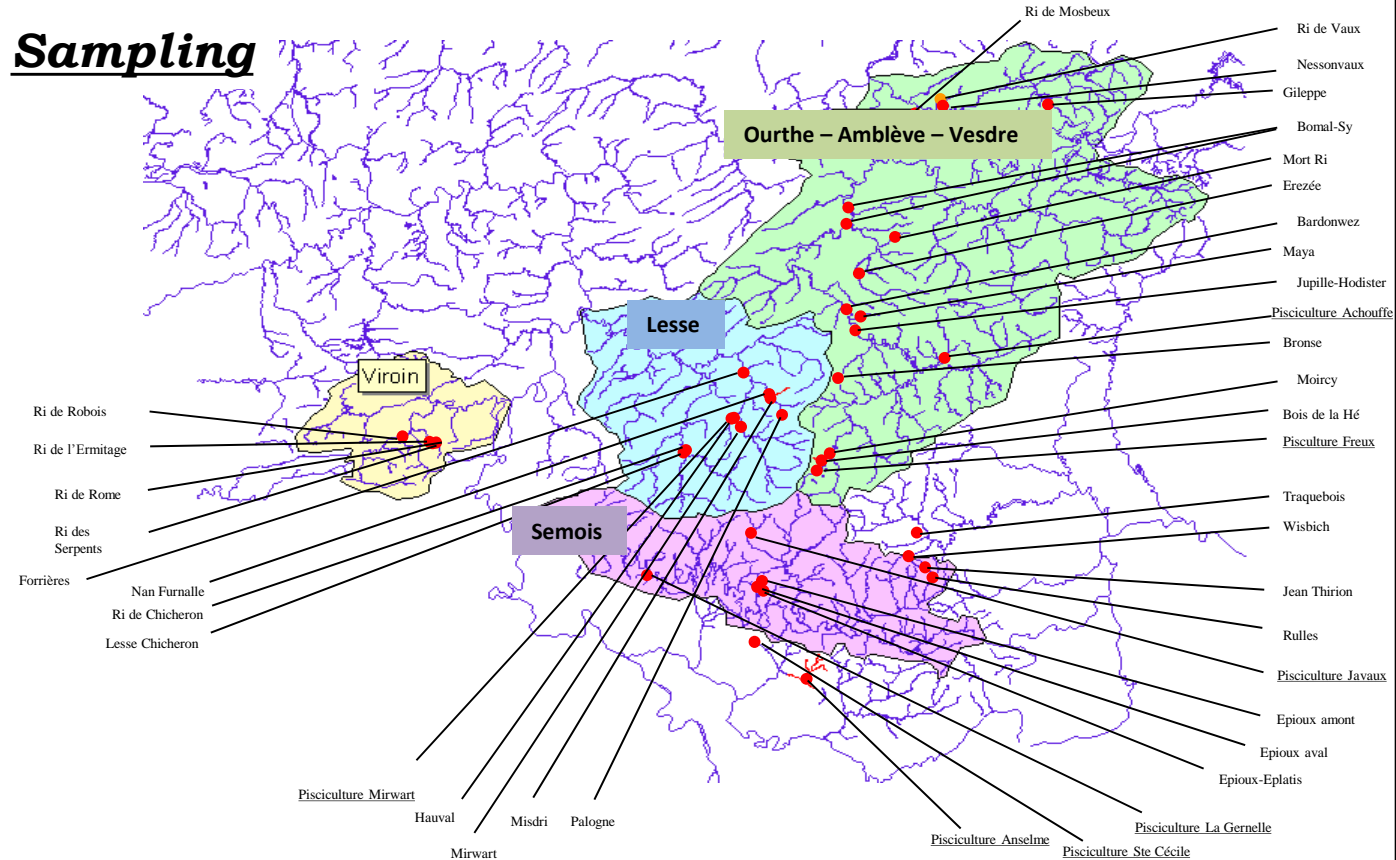
- Inventory of the genetic diversity & heritage of brown trout in Wallonia
- Identification of autochthonous populations (preserved of introgression by domesticated brown trout)
- Better fisheries management: conservation of native populations and supportive breeding in introgressed populations

Sampling of some brown trout populations in Wallonia

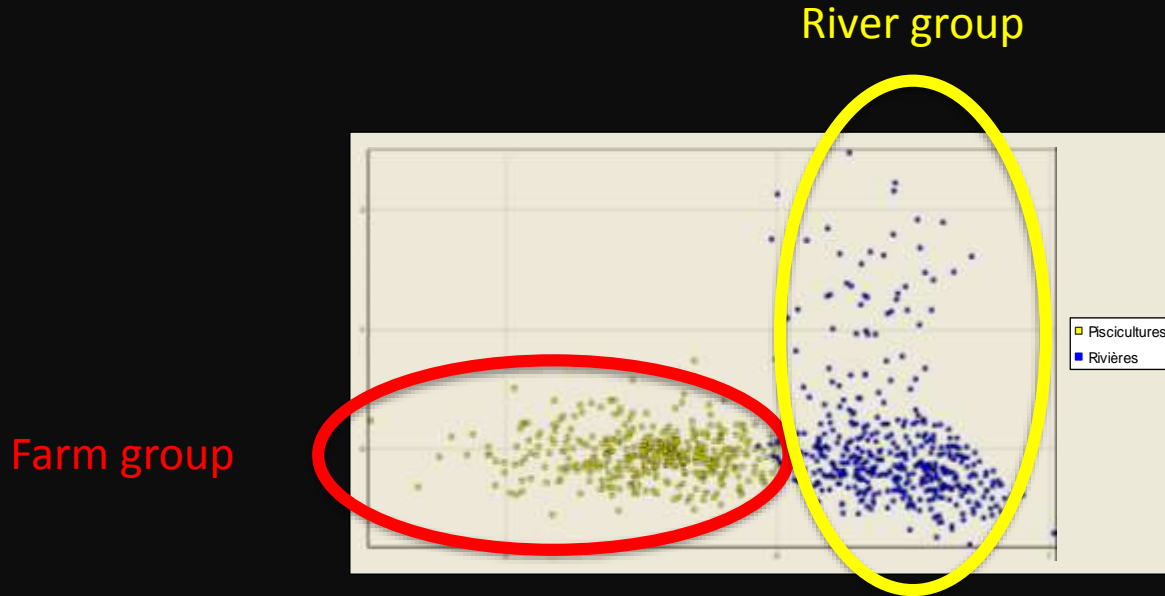
Adipose Fin



Sampling

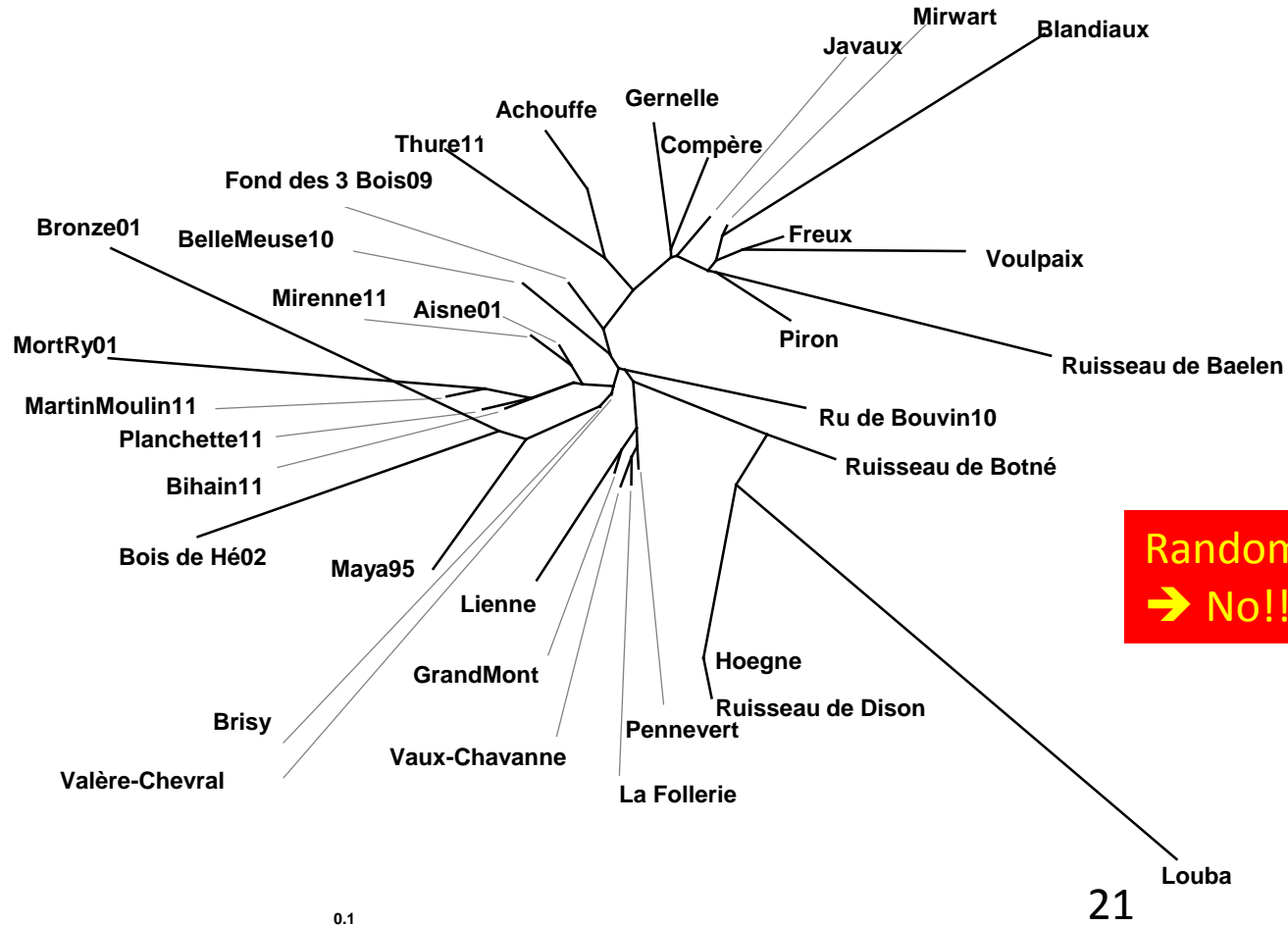


II. Brown trout – A. Group assignation

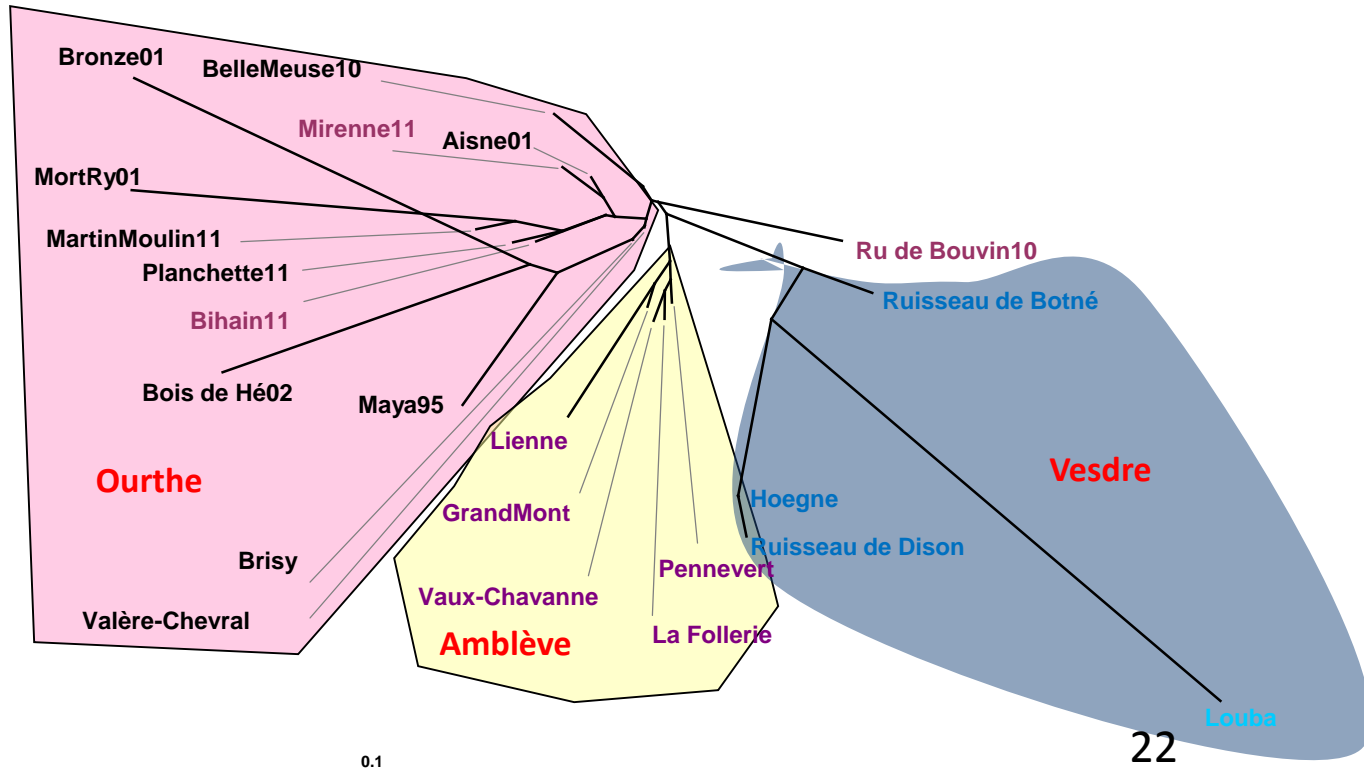


- Two groups can be easily distinguished :
 - River type
 - Farm type
- Since both groups can be differentiated, it is possible to calculate an introgression coefficient for each individual brown trout or groups of individuals and a genetic distance between two groups

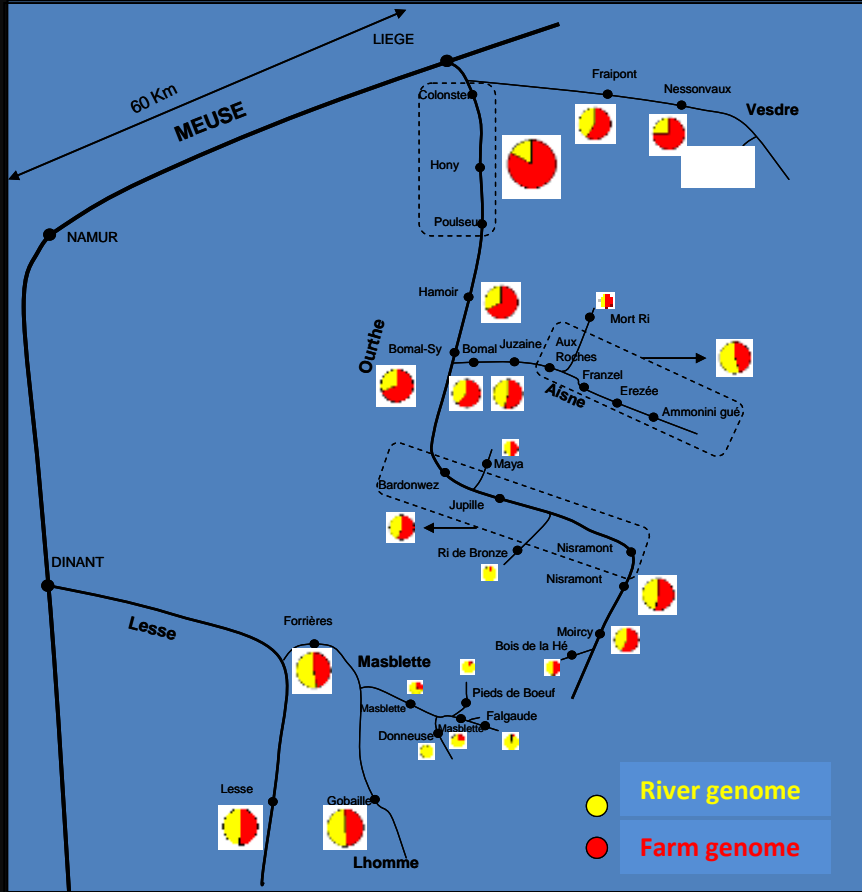
Observed genetic diversity & distance



Each river its own brown trout population...



Effect of repeated stocking with farm brown trout



- Ourthe & Lesse river systems
- In red, introgressed individuals
- In yellow, river type individuals
- The bigger the circle the higher the stocking intensity
- Introgression increases with stocking intensity
- Regulate trout stocking and preserve native stocks

II. BROWN TROUT

B. Genetic tagging

II. Brown trout – B. Genetic tagging – a study of stocking impact on fisheries and wild trout population



- What is the efficiency of stocking brown trout at a young stage (0+) in a brook in the Ardenne containing wild BT?
- 0+ pit-tagging is difficult or a source of experimental error
→ genetic tagging preferred !
- Two experimental stockings in late summer 2014 & 2015 in a 2 km brook isolated downstream by a pipe (no upstream migration possible)
- Experimental stocking (about 300 0+ juveniles/year) were done with a domestic strain that showed:
 - a specific phenotype « Fine Spot brown trout » or « Fine Mouchetée » « FM », different of the wild type or « Sauvage » (S)
 - a specific genotype with one homozygous rare allele « 178-178 », not present in the wild type brown trout (genetic tagging).

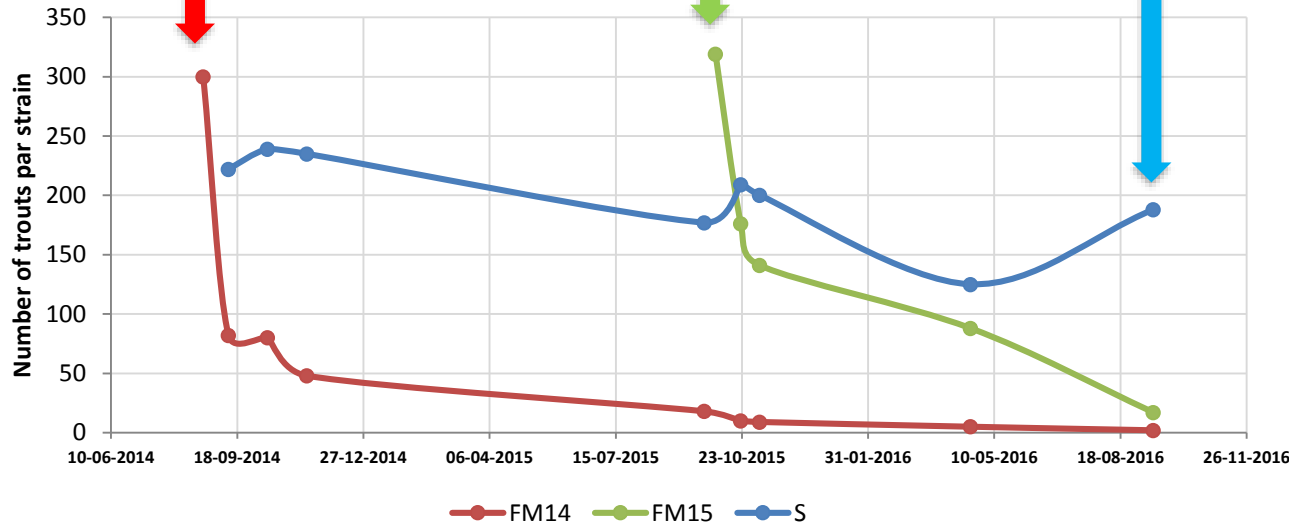
Results (electrofishing)

FM 178-178 0⁺ 2015 stocking

FM 178-178 0⁺ 2014 stocking

Wild S

Evolution of the number of trouts with time after late summer stocking (2014-2016)



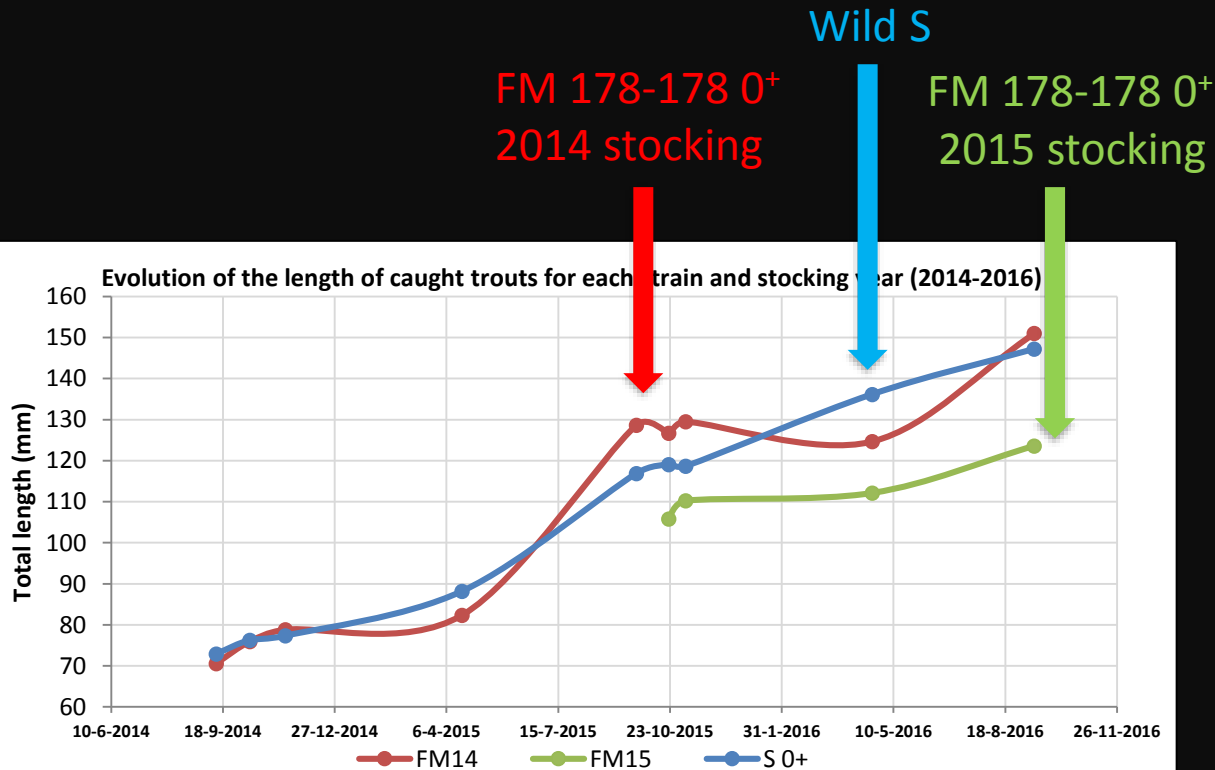
Quick reduction of introduced FM 178-178 juveniles to low level:

- in both 2014 & 2015
- by contrast to wild type S (+/- constant)
- No positive effect on recreational fisheries!
- No complete disappearing → FM reproduction?

Source : Segers et al. 2017

Results (electrofishing)

Source : Segers et al. 2017



- Remaining stocked FM trouts do grow at similar rate than wild-type S brown trouts:

- in 2014, less clear in 2015
 - no lag-phase
 - lower growth of FM during winter-early spring compared to wild-type S brown trouts, but not during late summer & autumn
- Some FM trouts stocked in 2014 were ready to spawn in autumn 2016 → efficiently?

Results (rare allele tracking)

FM178 FM	
Homozygous 178-178	Heterozygous 178-S
TMR_017	TMR_001
TMR_019	TMR_032
	TMR_039
	TMR_045
	TMR_122
	TMR_232
	TMR_247
	TMR_256
	TMR_262
	TMR_268
	TMR_270

A few remaining stocked FM trouts did reproduce efficiently and introgressed the wild brown trout local population (FM x S):

- Introgression Coefficient (IC) of the whole brook population increased from 2.6 to 7.5%
- Mean number of alleles increased from 3.6 to 5.5 (+53%)
- Introgression negative impact on local adaptation?

Population	Introgression coef.	Hexp	Hn.b.	Hobs	Mean number of alleles	Fis
Brook -2014	0,026	0,46	0,46	0,47	3,6	-0,012 ^{NS}
Brook -2017	0,075	0,51	0,51	0,50	5,5	0,018*

Source : Flamand et al. 2019

Conclusions

- Genetic imprinting and tagging are wonderful tools for marking fish at any stage or size of their life cycle and only slightly invasive (10 mg of fin per fish is fine).
- They can give precious information about diverse fisheries or nature conservation practices.
- For salmon, salmon parental assignation should be extended to the whole Meuse basin scale and all known potential parents should be genotyped.
- For brown trout, it is not too late to protect the last native populations but it is time to act and to regulate brown trout stocking for fisheries purpose and their potential negative effect on the remaining natural populations.

*Thank you to the numerous people that
contributed to these results
& thank you for your attention*