



Analysis of genetic variability of wild and domesticated pikeperch populations: basis for further breeding programs

**Tsigenopoulos C.S.^a, Tsaparis D.^a, Ekonomaki K.^a,
Kyriakis D., Fontaine P.^b,**

^a *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, 71003, Greece*

^b *Animal and Functionality of Animal Products (UR AFPA), University of Lorraine – INRA, Vandoeuvre-lès-Nancy, 54505, France*

Molecular tools...why?

✓ Resolving taxonomic uncertainties, and phylogenetic relationships

traceability

✓ Assisting management practices in aquaculture operations, especially broodstock management

paternity assignment

inbreeding control/monitoring

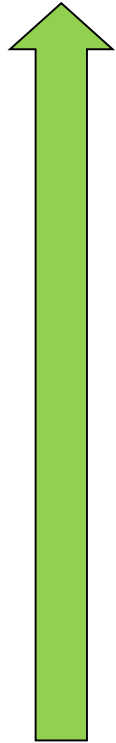
✓ Genetic improvement of important cultured species

marker assisted selection (MAS)

Genomic selection

Molecular markers

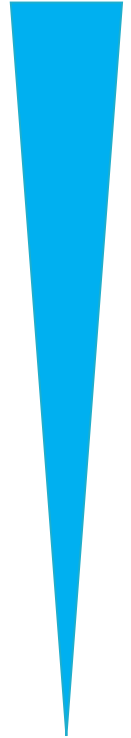
'Genomic' era



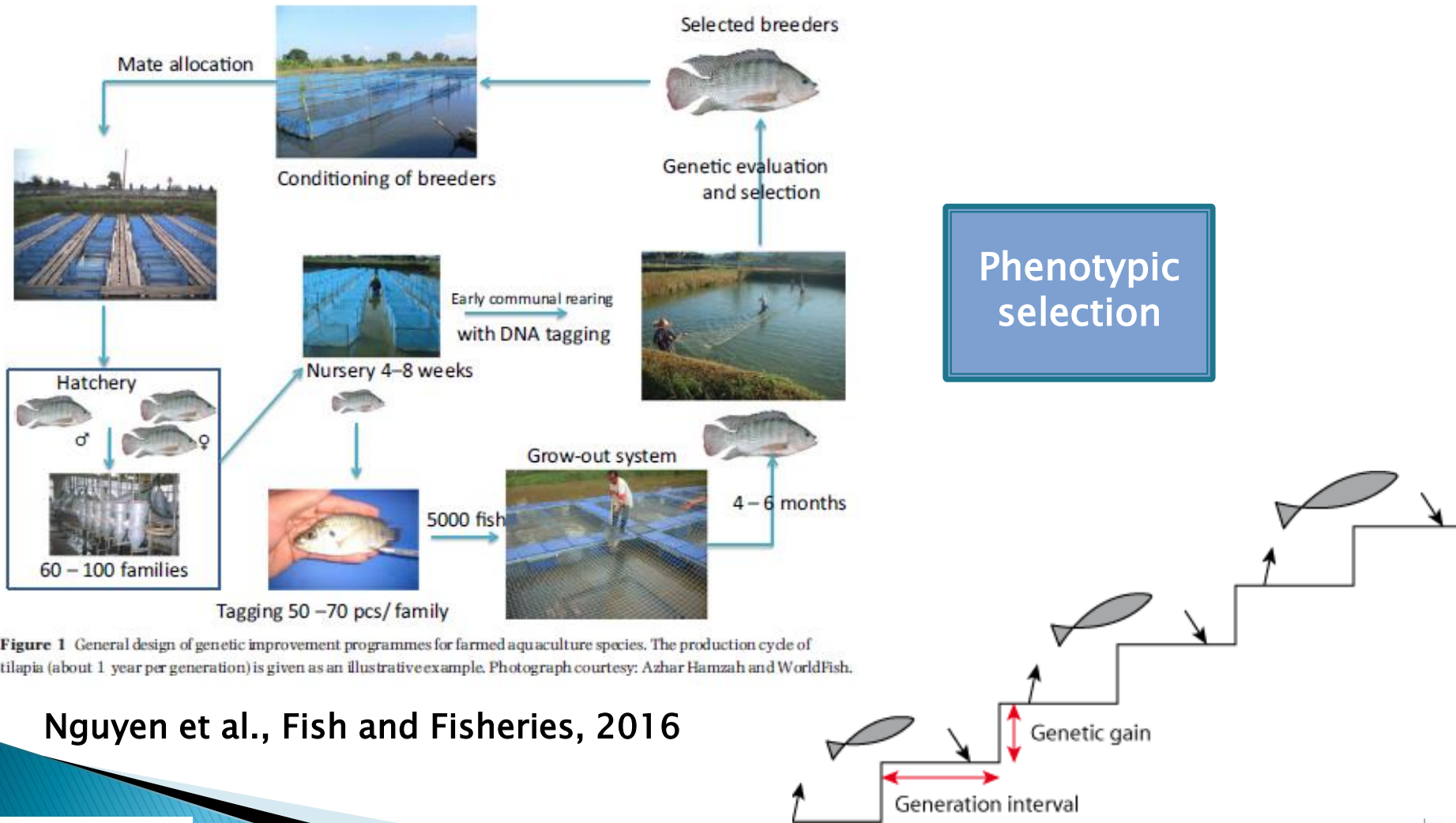
- 🐟 Sequencing (SNPs)
- 🐟 Microsatellites (SSRs)
- 🐟 Multi-locus fingerprints (RFLP)
- 🐟 AFLP (Amplified Fragment Length Polymorphism)
- 🐟 RAPD (Random Amplified Polymorphic DNA)
- 🐟 Allozymes (protein-electrophoresis)

'Classic' era

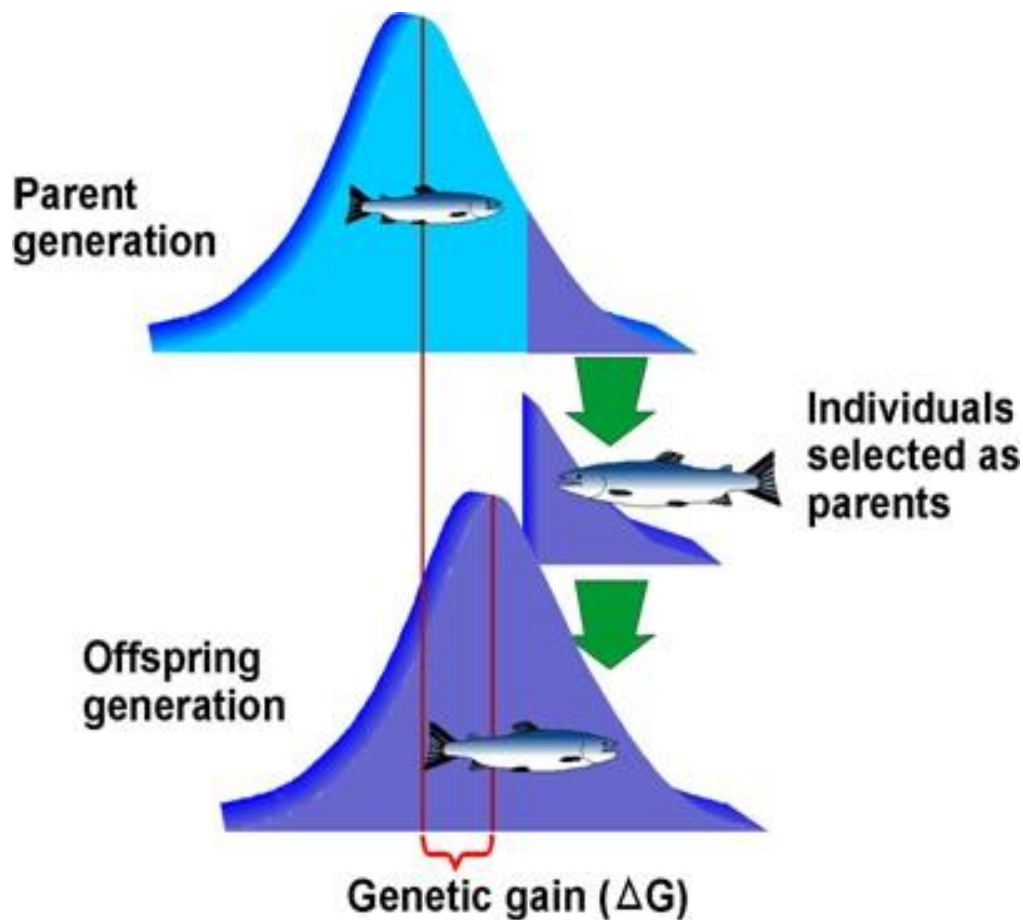
Resolution
power



Classical breeding programs (i.e. selective breeding) remain the mainstream of finfish genetic improvement



The goal of most breeding programs is to predict the genetic merit of an individual and thus allow targeted combinations of desired alleles to improve the performance of the next generation(s).



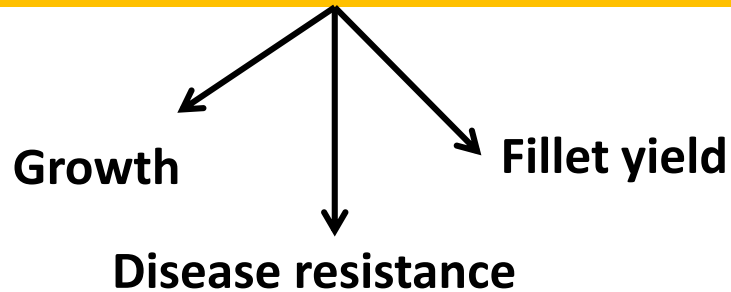
The phenotype of an individual is only in rare cases a good indicator for allelic differences.

Inbreeding?

The use of genetic markers allows tracing detailed information on the inherited part of the genome, other than such observed by the phenotype.

Marker assisted selection (MAS)

Phenotypic variation of economically important traits



Genes + Environmental (G x E) influences



Regions of the genome that contribute to variation in expression of a quantitative character



quantitative trait loci (QTL)

Molecular genetic markers flanking the QTL region can be used in conjunction with traditional selective breeding programs to *improve the selection response and accuracy*



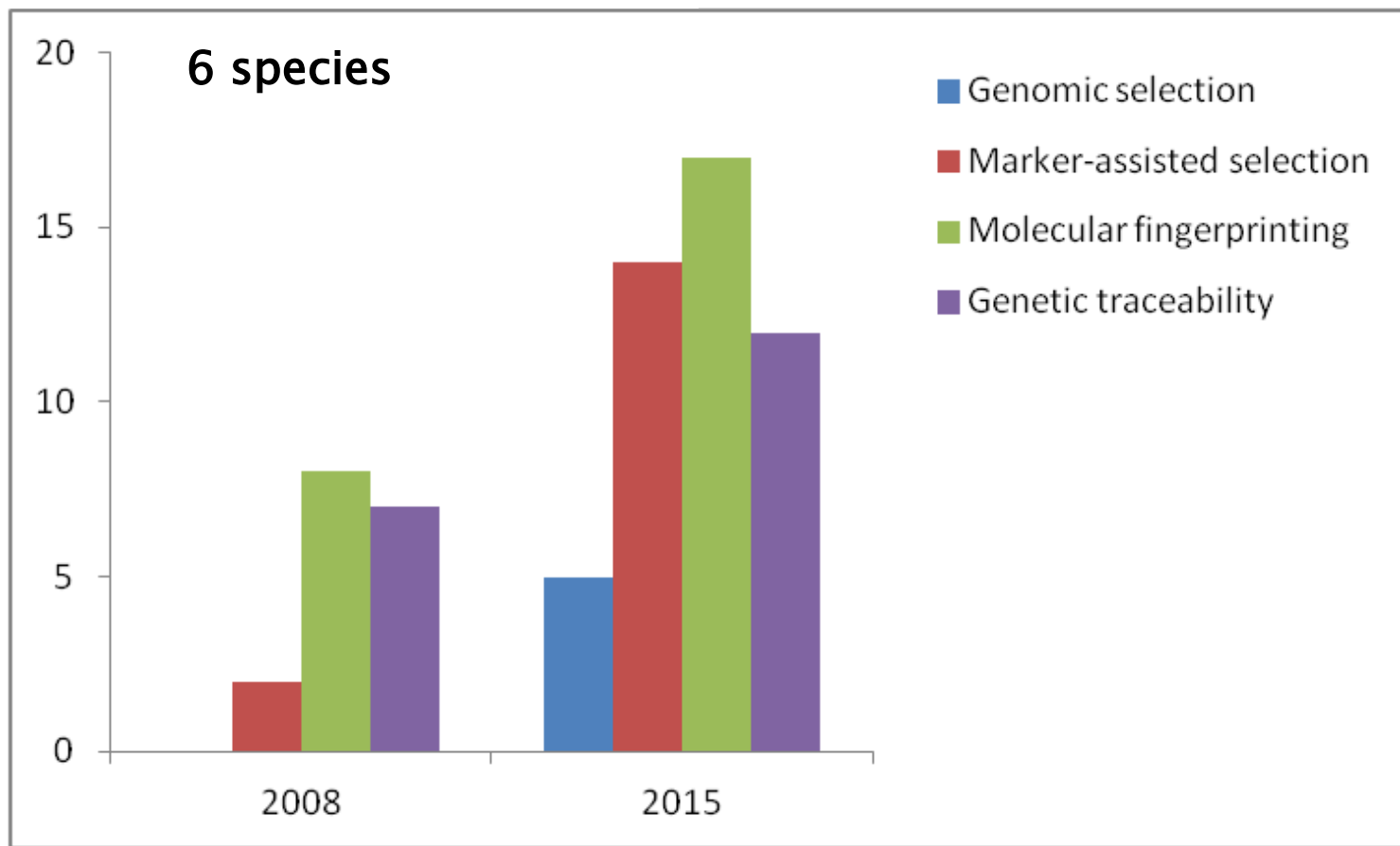
Marker Assisted Selection (MAS)

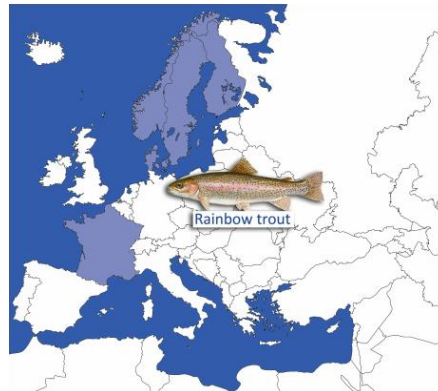
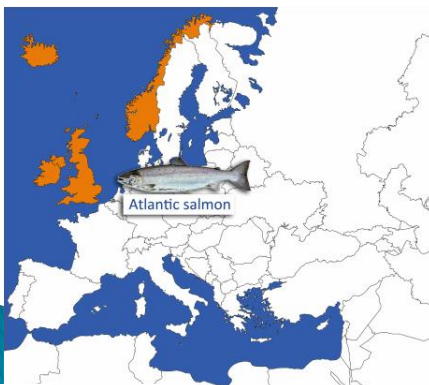
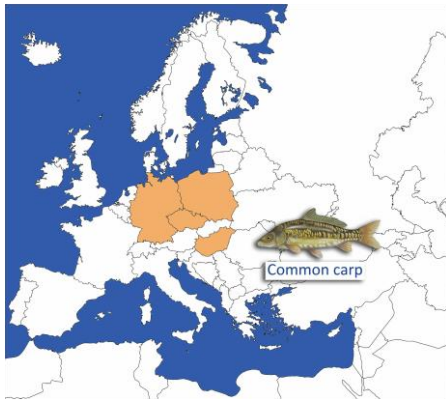
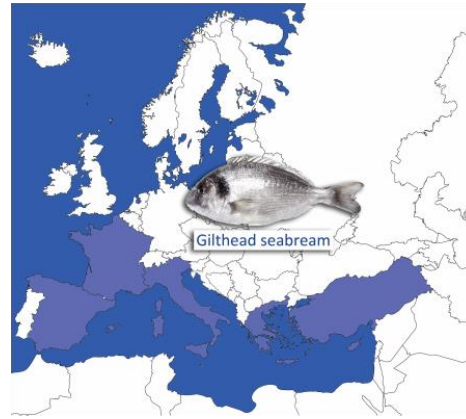
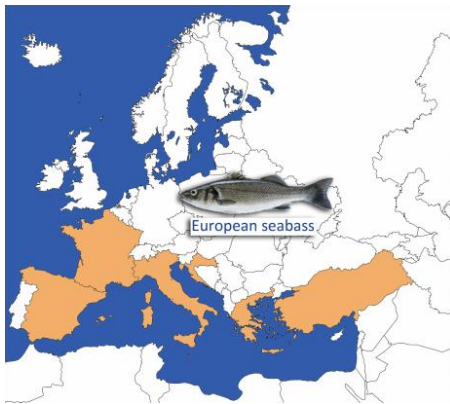


A comprehensive survey on selective breeding programs and seed market in the European aquaculture fish industry

Hervé Chavanne¹ · Kasper Janssen² · Johann Hofherr³ · Franca Contini³ · Pierrick Haffray⁴ · Aquatrace Consortium · Hans Komen² · Einar Eg Nielsen⁵ · Luca Bargelloni¹

Received: 21 September 2015 / Accepted: 16 February 2016
© Springer International Publishing Switzerland 2016





Market share of genetically improved stocks (%) for the six main finfish species in Europe

| Species | Market share (%) |
|-------------------|------------------|
| Turbot | 100 |
| Atlantic salmon | 93-95 |
| Rainbow trout | 65-68 |
| Gilthead seabream | 60-66 |
| European seabass | 43-56 |
| Common carp | 0 |



Aquaculture Production in Europe

- ▶ Since 2000, the aquaculture production in the EU seems to have stagnated at 1.3 million tonnes. Over the same period, it has almost **doubled in Asia and America**, and almost **tripled in Africa**.
- ▶ Surprisingly, this lack of growth in the European aquaculture sector contradicts the steadily increasing demand for fish in Europe.
 - lack of growth attributed to a number of factors, such as the shortage of suitable sites, the cumbersome bureaucracy, the competition with other users of marine areas and inland water courses, and the relatively strict environmental protection laws
- ▶ Therefore, **65% of Europe's requirement** for fish and seafood today **is met through imports** since domestic capture fisheries do not meet European requirements for fish.



Pikeperch (*Sander lucioperca*)

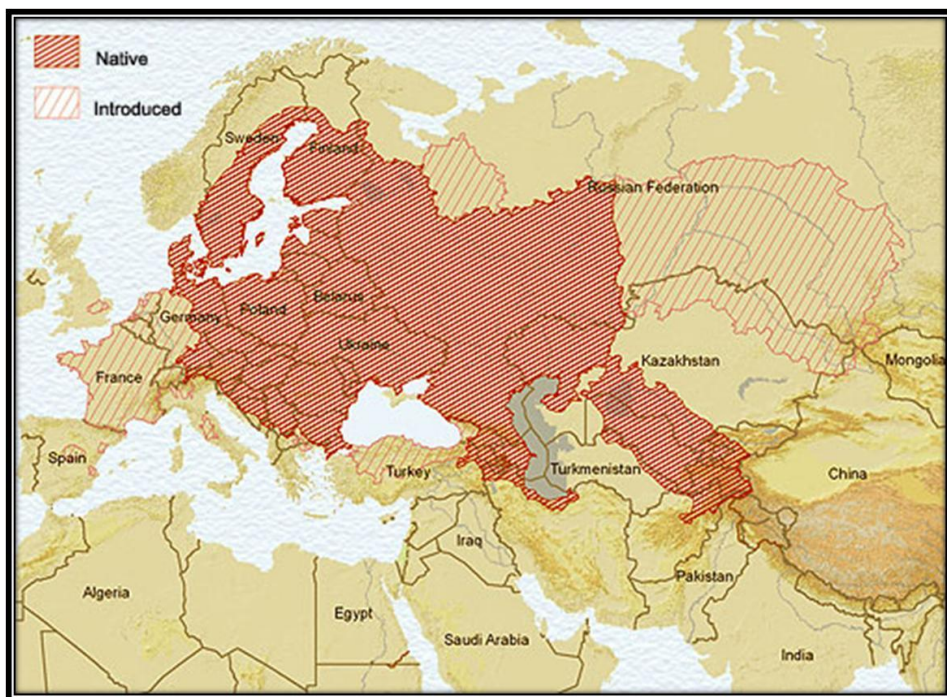
A new species for European aquaculture

- ▶ Several measures to support its sustainable development and the use of **Recirculation Aquaculture Systems (RAS)** that are largely isolated from their surroundings.
- ▶ These systems have been used to grow rainbow trout, salmon, and eel
- ▶ European Percid Fish Culture (EPFC)





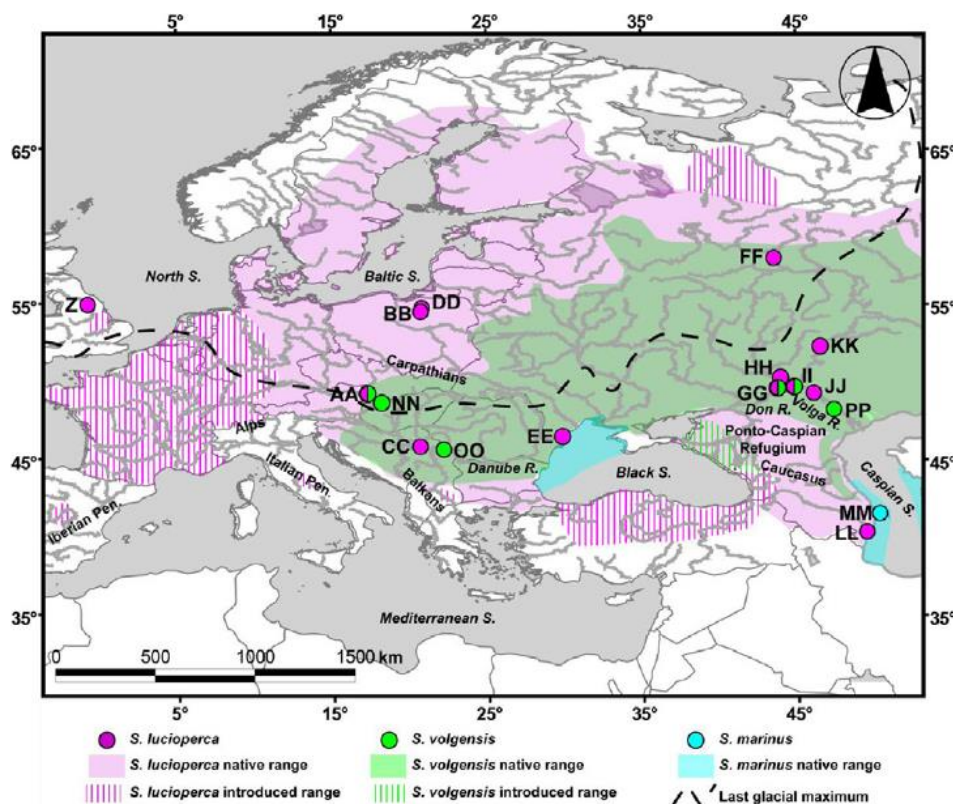
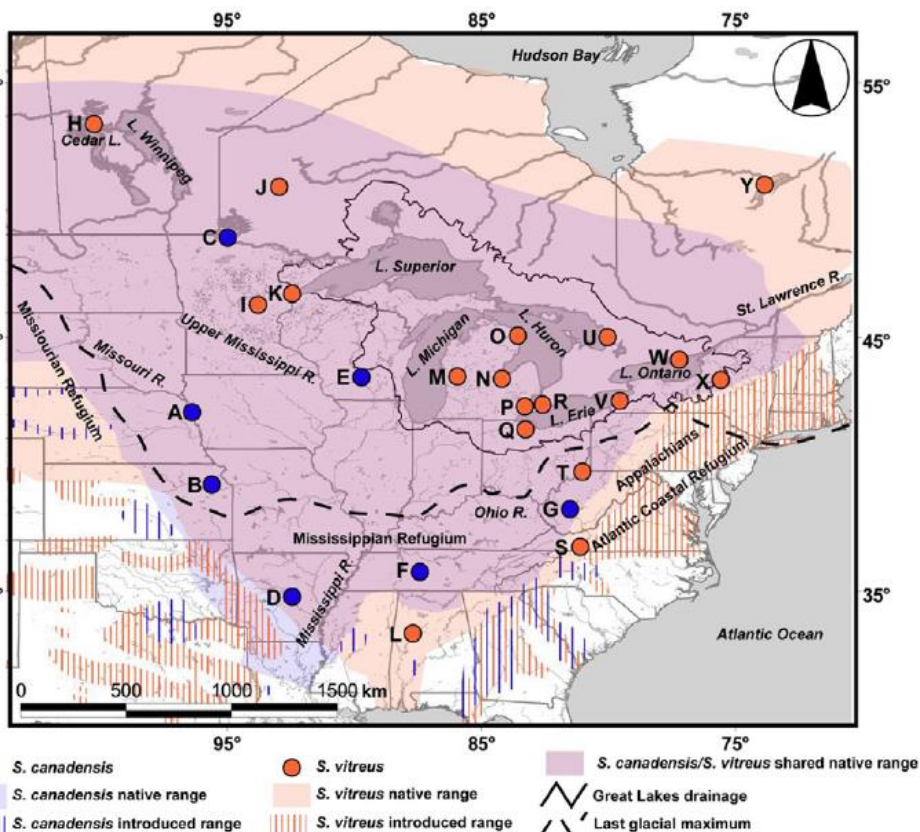
Pikeperch (*Sander lucioperca*).



- ▶ A temperate Eurasian freshwater/brackish water fish species,
- ▶ Wild populations of pikeperch show signs of decline in many areas of its natural range of distribution,
- ▶ Introduced in northern Russia, Italy, Spain, Turkey, the North African countries (from Morocco to Tunisia) and many other regions



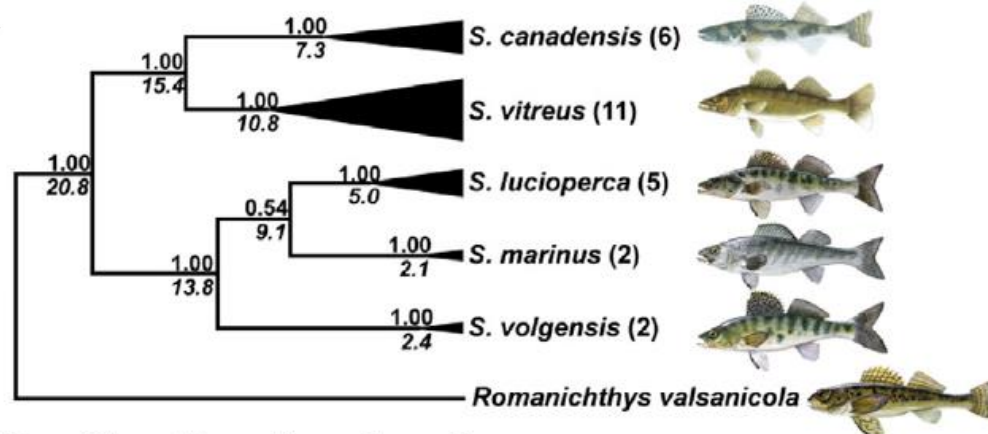
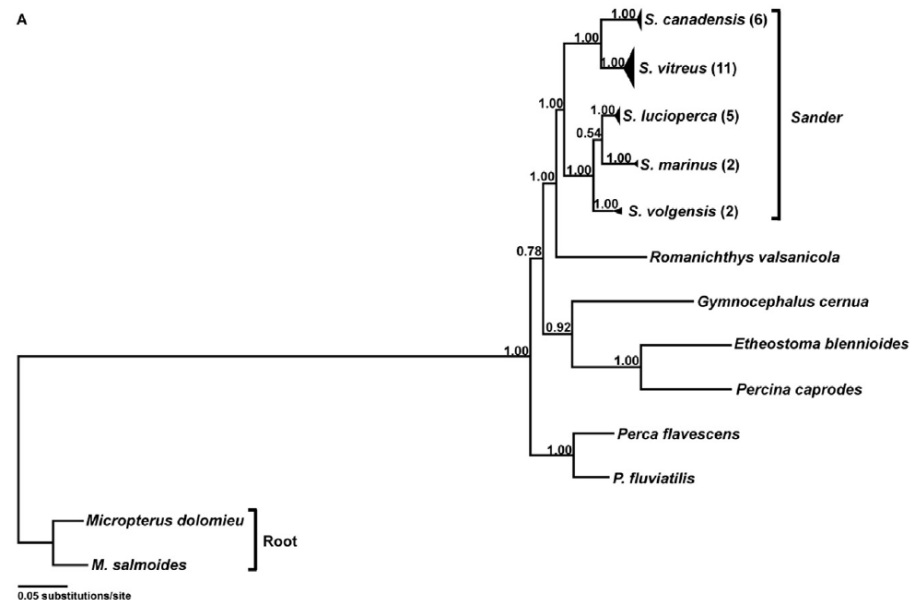
The genus *Sander* in the world



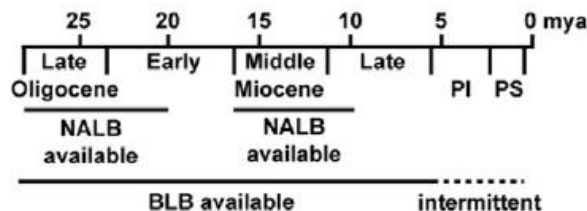
Haponski, A.E., and Stepien, C.A. (2013). Phylogenetic and biogeographical relationships of the *Sander* pikeperches (Percidae: Perciformes): patterns across North America and Eurasia. *Biological Journal of the Linnean Society* 110, 156-179.



Phylogenetic relationships of the genus *Sander* and members of the family Percidae



Haponski, A.E., and Stepien, C.A. (2013)





Pikeperch aquaculture characteristics

- ▶ Pikeperch is considered still a **wild species** for the aquaculture industry,
- ▶ In principle, each pikeperch farm uses its own stock, captured either from the wild or supplied by another farmer
- ▶ Exhibits **cannibalism** and **territorialism**,
- ▶ Spawning **four times a year** in contrast to the wild where **once a year** (April-May) is the norm,
- ▶ Constant high temperatures (24-26°C, only feasible in RAS) to ensure relatively high growth rates and allow high densities of **80-100 kg/m³**.



Pikeperch aquaculture characteristics

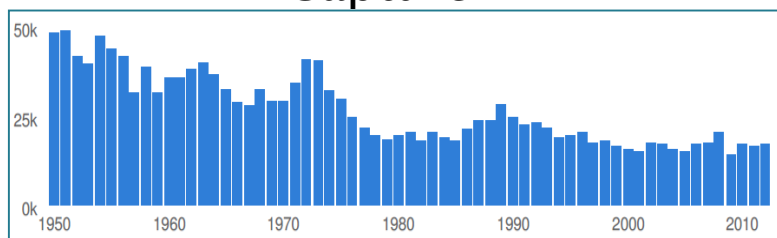
- ▶ Pikeperch flesh quality has a neutral taste
- ▶ It normally takes **15-18 months** to reach a market sized fish of **800 g to 1.2 kg**.
- ▶ The main market product: **whole round fish & fillets**.
- ▶ Pike-perch is still a **niche product**
- ▶ Markets are in Europe and North-America.
- ▶ The market value is at **8-11 €/kg** at farm gate, whole fish.



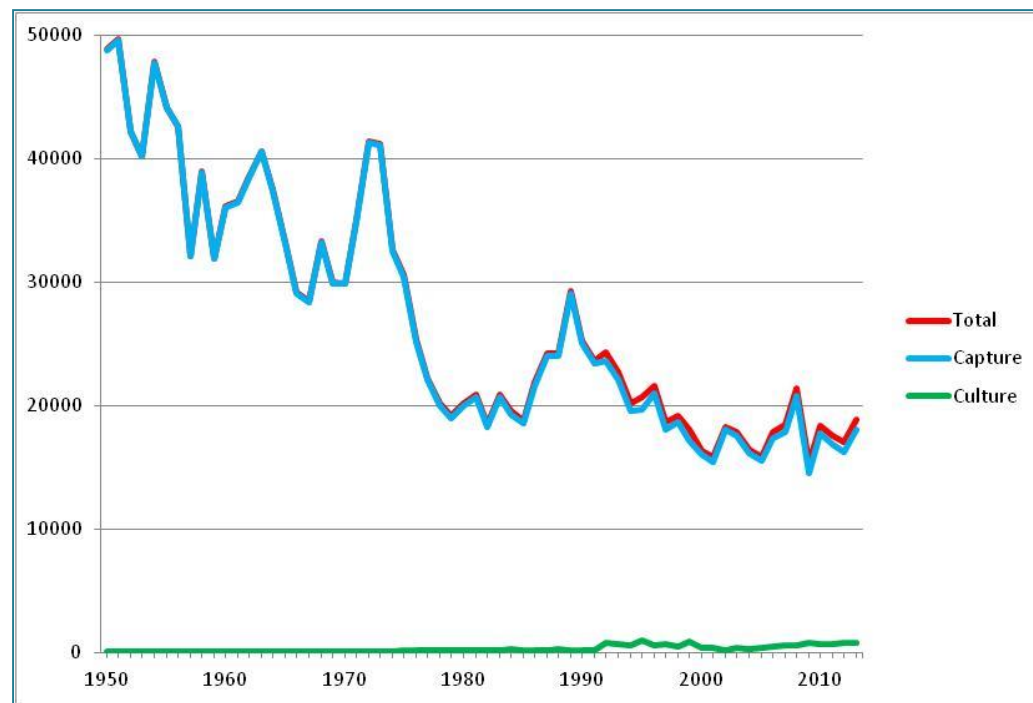
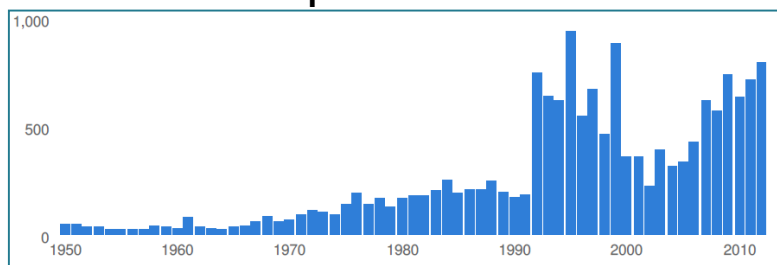
Pikeperch production

Pikeperch demand has been strengthened by **the strong decline** of wild catches from Russia, Finland and Estonia from 50.000 t in the '50s to less than 20.000 t currently (FAO, 2013).

Capture



Aquaculture





Objectives of the current study

- ▶ Assess the genetic variability of captive broodstocks in commercial farms in Europe operating in Recirculating Aquaculture Systems (RAS)
 - Currently, there are no assessments of the genetic diversity of captive pikeperch stocks partly because there are only a few commercial hatcheries (around 10) that produce pikeperch in Europe
- ▶ Assess the genetic variability of wild broodstocks in Europe and compare this variability with that of domesticated pikeperch populations to be applied in future breeding programs of the species



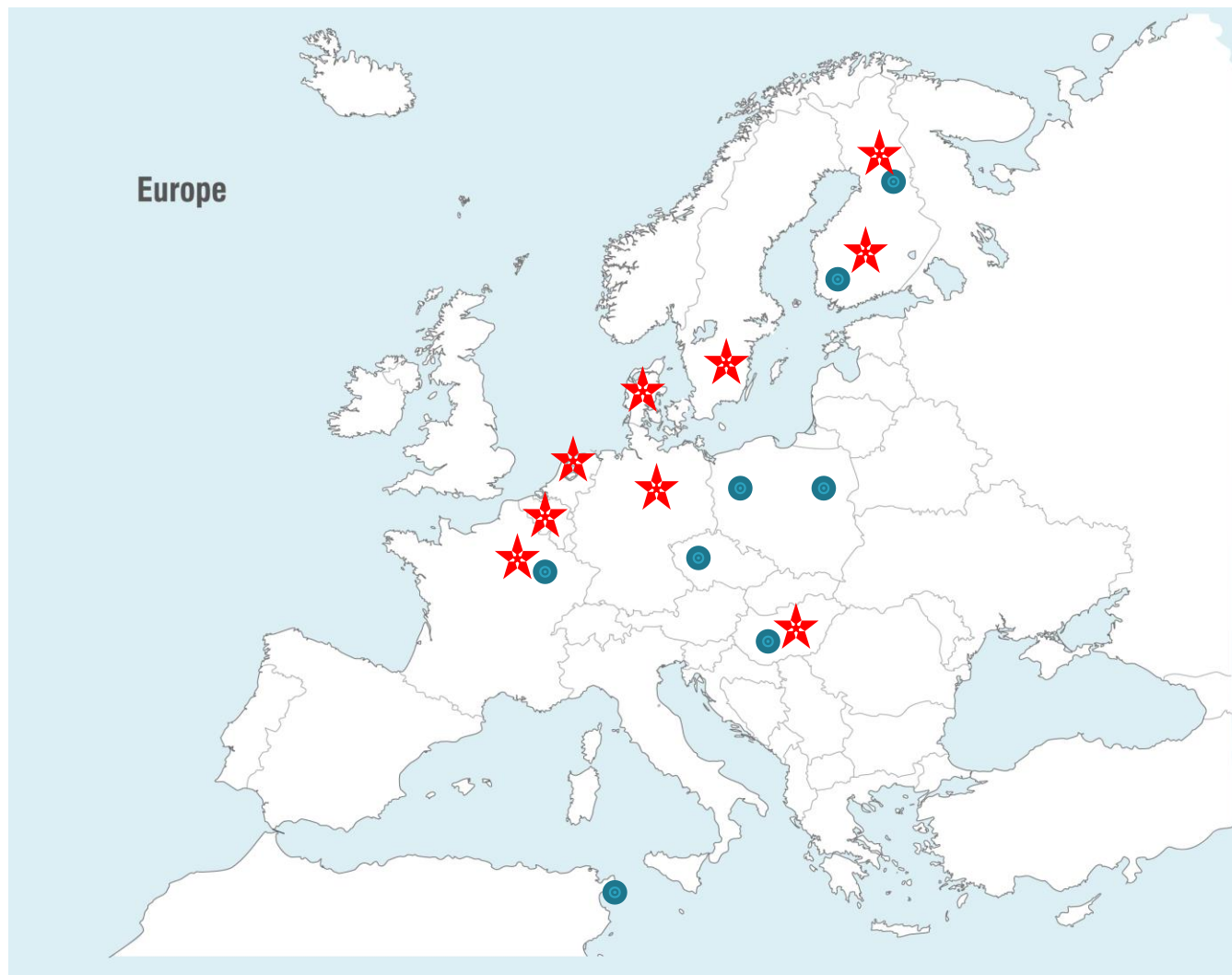
Microsatellite Cross-species amplifications

- ▶ Multiplex optimizations for 23 loci reported in:
 - Leclerc et al. (2000) for the yellow perch (*Perca flavescens*)
 - Borer et al. (1999) and Wirth et al. (1999) in walleye *Stizostedion vitreum*, and
 - Dubut et al (2010) in the Rhone streber (*Zingel asper*)
- ▶ There are currently two 6-plexes used for genotyping and results shown are based on ten loci



Sampling

Populations
8 wild &
13 captive





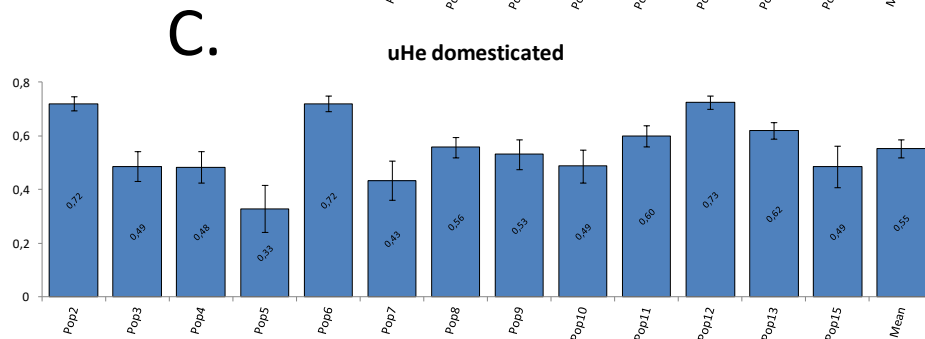
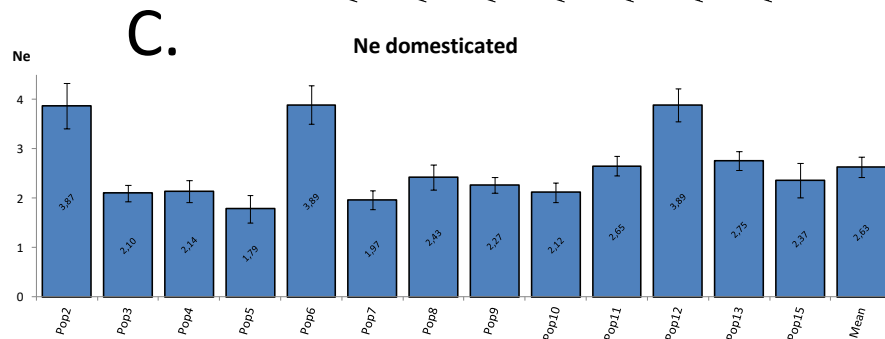
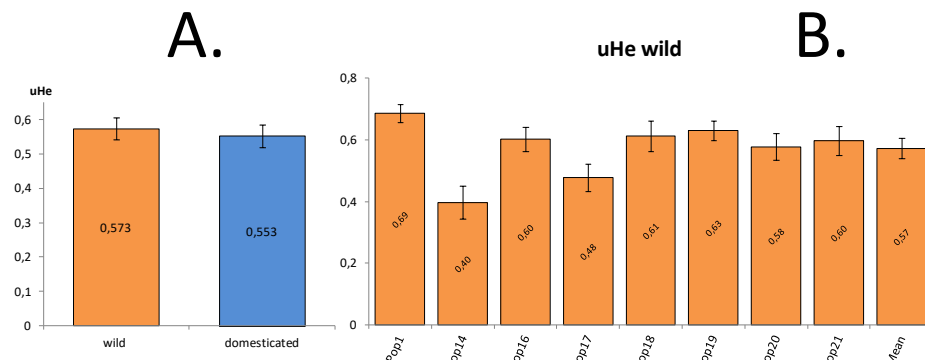
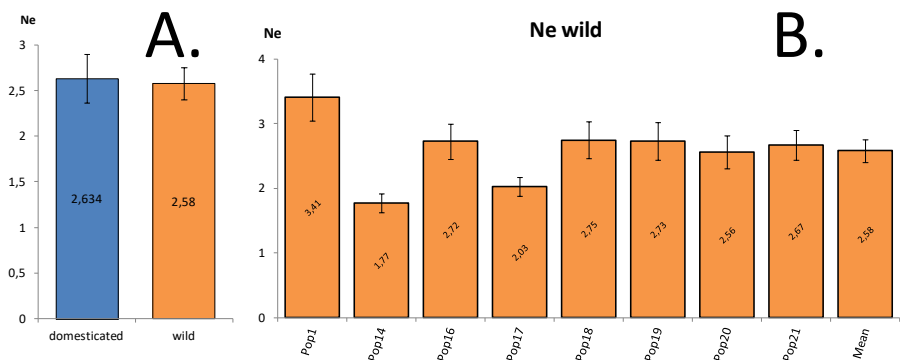
Basic population genetics parameters for all populations analyzed

| | Population | Sample Size | Mean Nb of alleles | H_E | H_O | F_{IS} |
|----|------------|-------------|--------------------|--------|--------|-----------|
| 1 | Hungary-1 | 53 | 6.2 | 0.6826 | 0.7472 | -0.08424 |
| 2 | Hungary-2 | 49 | 7.8 | 0.7182 | 0.6759 | 0.06962* |
| 3 | Denmark-1 | 54 | 2.6 | 0.4675 | 0.6796 | -0.44607 |
| 4 | Denmark-2 | 38 | 3.3 | 0.4616 | 0.4882 | -0.04401* |
| 5 | Denmark-3 | 14 | 2.8 | 0.3408 | 0.4100 | -0.16229 |
| 6 | Denmark-4 | 73 | 8.2 | 0.7194 | 0.7165 | 0.01110* |
| 7 | Denmark-5 | 19 | 3.1 | 0.4169 | 0.3985 | 0.07185* |
| 8 | Germany | 46 | 5.7 | 0.5567 | 0.5502 | 0.02343* |
| 9 | Finland-1 | 31 | 3.7 | 0.5257 | 0.5819 | -0.09055 |
| 10 | Finland-2 | 20 | 2.8 | 0.4743 | 0.6032 | -0.24757 |
| 11 | France-1 | 63 | 5.4 | 0.5940 | 0.5913 | 0.01261 |
| 12 | Belgium-1 | 100 | 7.2 | 0.7224 | 0.8099 | -0.11621* |
| 13 | Belgium-2 | 100 | 4.7 | 0.6156 | 0.6465 | -0.04510 |
| 14 | Tunisia | 59 | 3.7 | 0.4013 | 0.3585 | 0.11512* |
| 15 | Sweden | 30 | 4.4 | 0.5250 | 0.5817 | -0.08989 |
| 16 | France-2 | 51 | 4.6 | 0.5923 | 0.6706 | -0.12237 |
| 17 | Czech Rep. | 70 | 3.8 | 0.4692 | 0.4382 | 0.07357* |
| 18 | Poland-1 | 14 | 4.6 | 0.5763 | 0.5643 | 0.05780* |
| 19 | Poland-2 | 11 | 4.2 | 0.6149 | 0.6764 | -0.05217* |
| 20 | Finland-3 | 32 | 4.8 | 0.5946 | 0.5995 | 0.00787* |
| 21 | Finland-4 | 31 | 4.7 | 0.6034 | 0.5340 | 0.13148* |



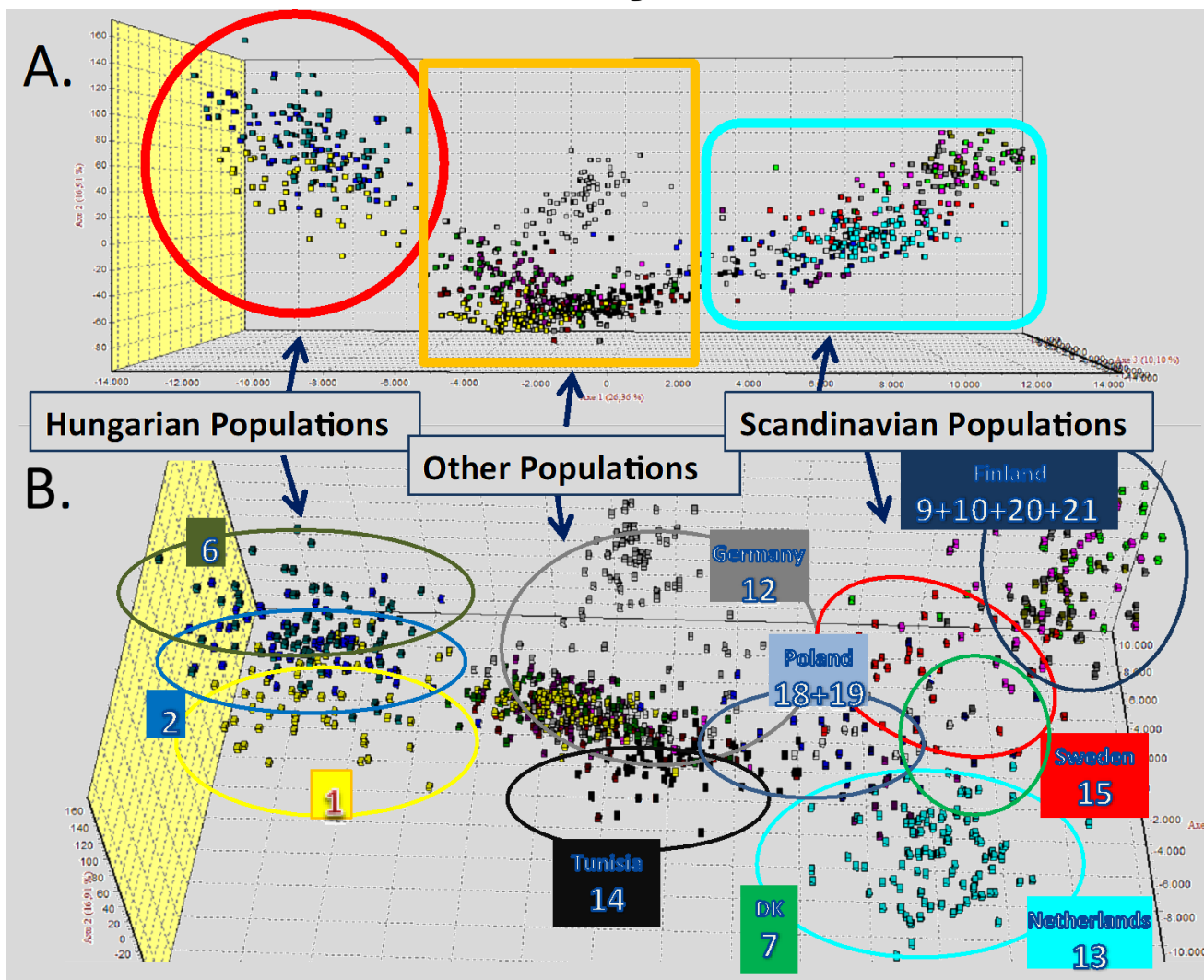
Mean number of alleles for domesticated and wild populations

Estimates of Unbiased Expected Heterozygosity (uHe)



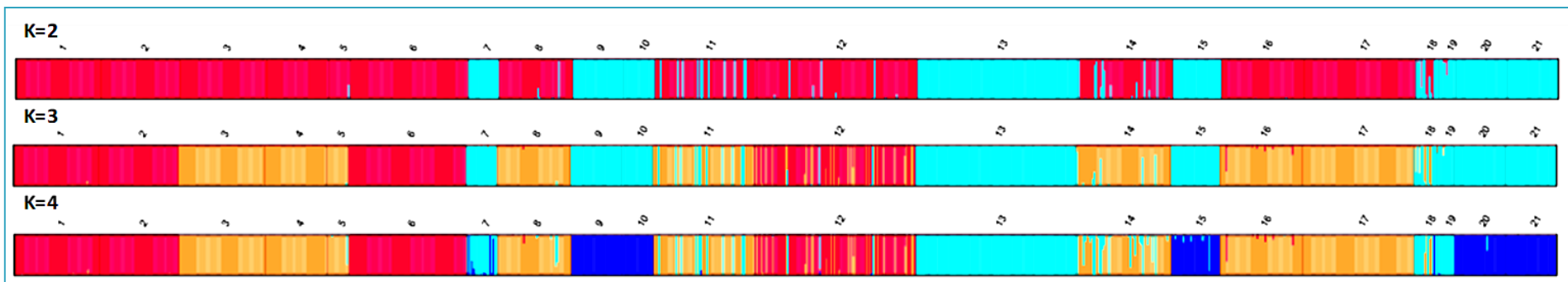
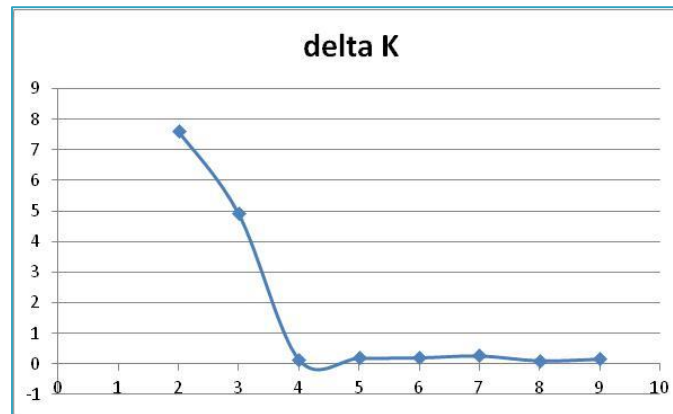
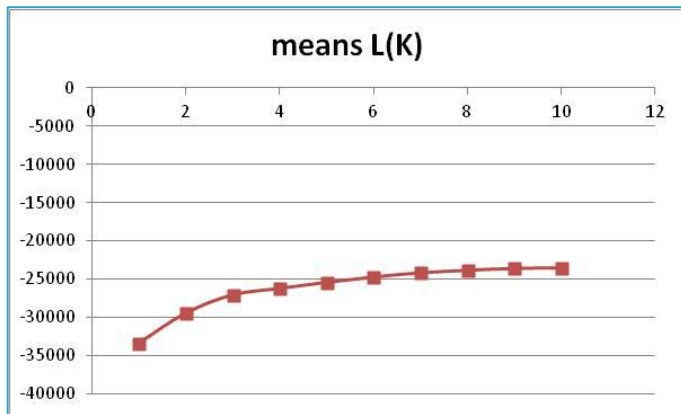


FCA Analysis





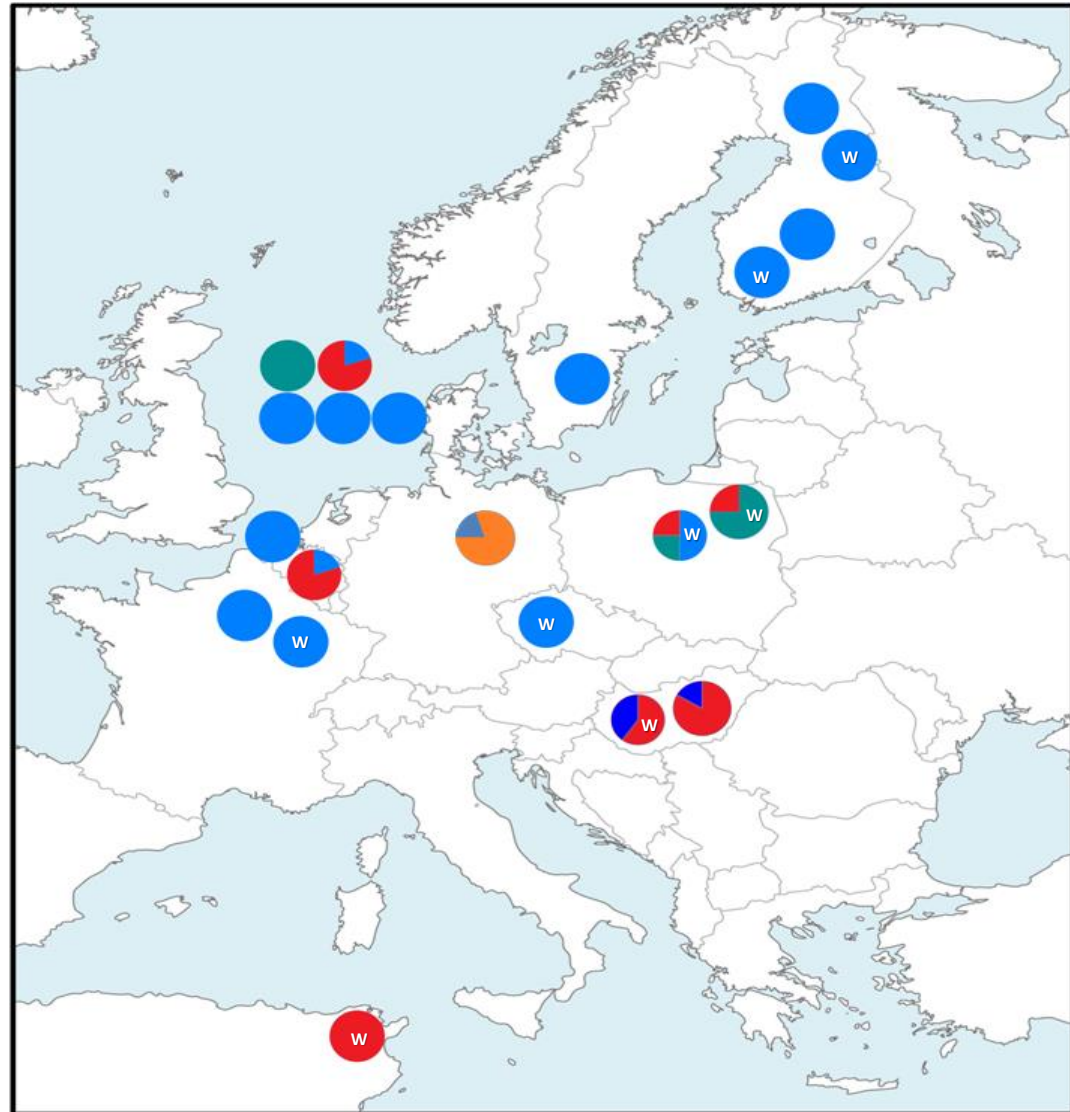
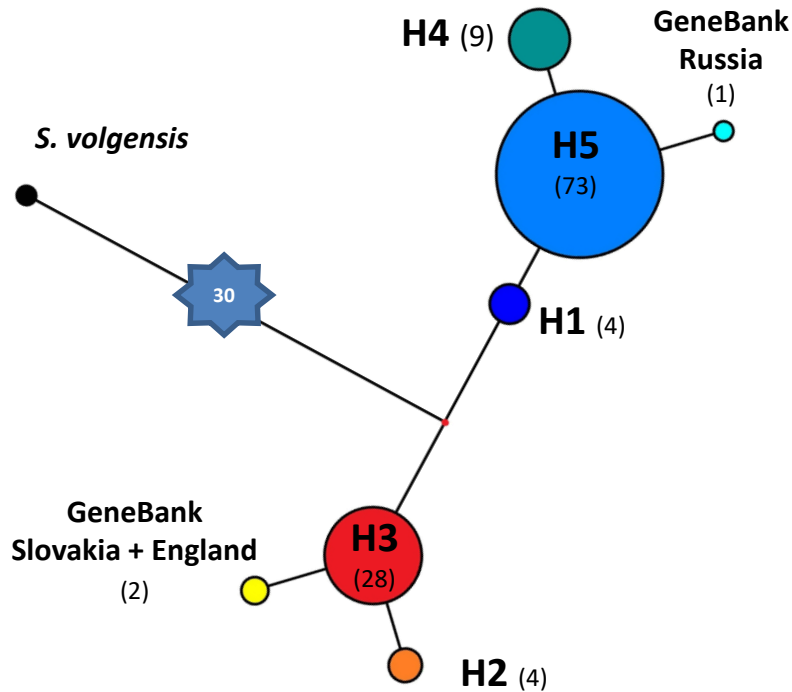
Structure Analysis



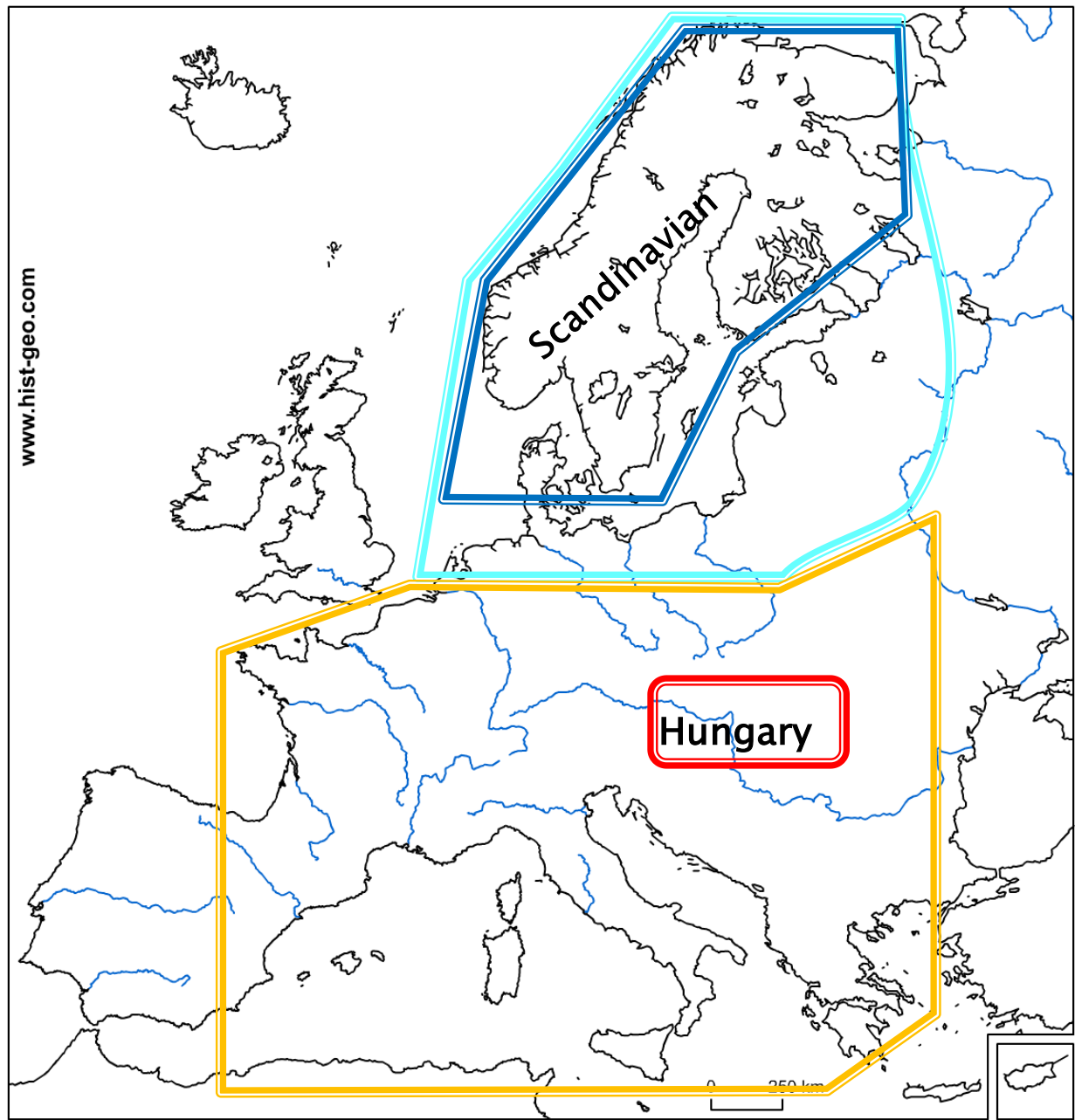
mtDNA phylogeographic Analysis

- ▶ Mitochondrial DNA sequencing:
 - *Control region*: extremely low variability in initial screening (single haplotype)
 - *Cytochrome b* gene: partially sequenced (571bp)
 - In total 106 sequences (4-6 samples/population)
 - Addition of 15 sequences available in Genbank database
 - Results: very few variable sites (7/571) which led to only 7 haplotypes

The same two haplogroups were revealed



A median-joining network of haplotypes was constructed (NETWORK 4.6.1.3) to infer phylogeographic relationships





Conclusions

- ▶ Evidence that pikeperch populations in Europe are part of at least two genetically differentiated groups.
 - The first is found in northern Europe from Netherlands/Denmark to the West and Poland (at least) to the East to the North of Finland. This is the group probably referred also as “Baltic Sea” stock by Björklund et al. (2007) and Poulet et al. (2009).
 - The second group comprises all remaining populations in Central Europe to as south as Tunisia (and probably Spain, Italy and Northern Greece).



Conclusions

- ▶ In the second stock, the Hungarian populations are having a key-position being different from those found geographically near, *e.g.*, from Czech Rep. and Germany.
 - It might be another stock associated with Hungarian lakes, as opposed to all other populations that probably dispersed through the Danube River west-and southwards (see also Kohlmann *et al.*, 2013)
- ▶ Most populations analyzed seemed to contain fish of a single origin with very few exceptions



Conclusions

- ▶ In general, the mean heterozygosity estimates and the count of the number of alleles per population indicate that domesticated samples do not suffer from inbreeding.
- ▶ There are few domesticated populations that either due to their small sample size or their *a priori* known use as 'selected' fish, which indicates the notion of some level of inbreeding
- ▶ Interestingly, the number of alleles in domesticated samples is slightly higher than that in the wild (2.63 vs 2.58), whereas the unbiased heterozygosity is slightly lower (0.553 vs 0.573)



Conclusions

- ▶ Last, we should bear in mind that besides inbreeding that reduces genetic diversity and the effective population size, outbreeding is also a major concern for future breeding programs.
- ▶ Outbreeding is simply the crossing of different stocks, *i.e.*, locally adapted populations/strains with others that are significantly different genetically.
- ▶ The scientists involved should decide whether the benefits from crossing different strains outweigh any later detrimental effects on fitness coming from outbreeding depression.



Co-funded by the
Seventh Framework
Programme
of the European Union



Thank you for your attention

