THE DEVELOPMENT OF TOOLS FOR TRACING AND EVALUATING THE GENETIC IMPACT OF FISH FROM AQUACULTURE: "AQUATRACE"

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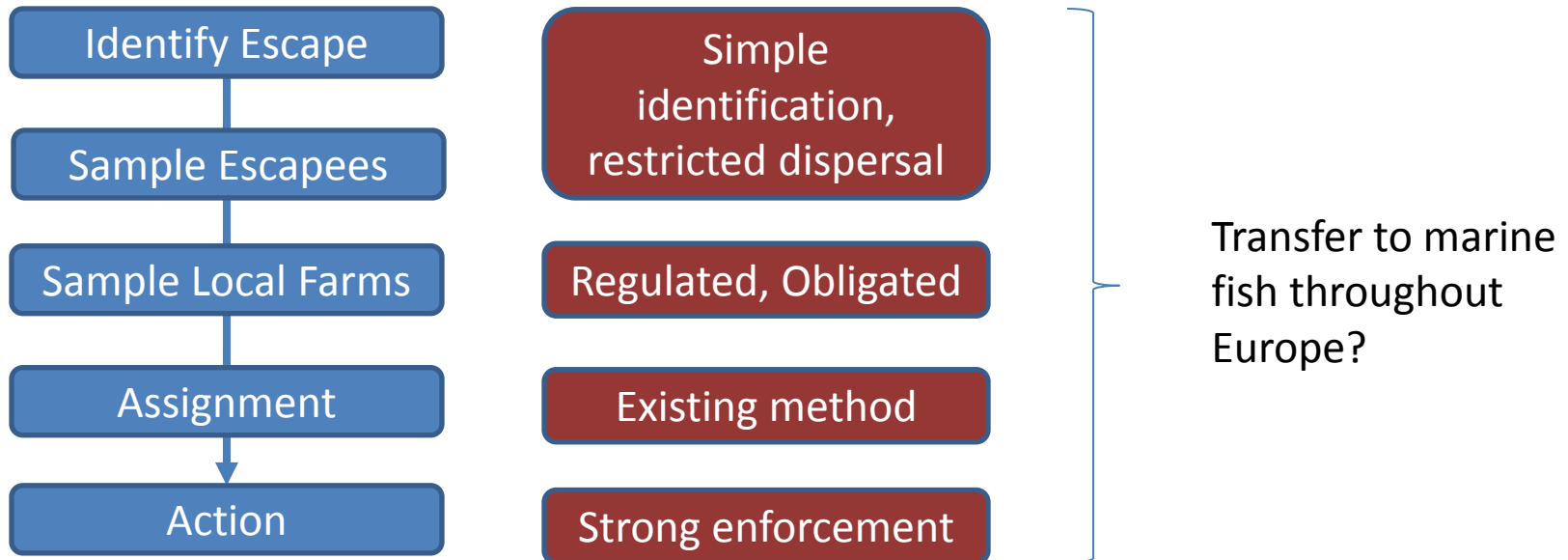
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Background

European Union 'vision' for Aquaculture

- EU should support growth which is:
  - economically viable, environmentally friendly, socially acceptable

- Aquaculture production requires the most advanced research and technology

- Aquaculture fish escapes pose a threat to the integrity and levels of biodiversity
  - through direct competition for resources
  - **through genetic "pollution" of local populations of conspecifics**

- Need to identify methods that allow us to assess and monitor any genetic effects of aquaculture escapees on wild populations

- Assessment of *rates* of introgression, including across spatial and temporal scales

- Assessment of *effects* of introgression on the fitness of local wild populations

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Background Identifying Escapees

Recognised issue throughout the aquaculture industry

Norwegian salmon industry acted to control the problem

Institute Marine Research, Bergen (Kevin Glover)

Identify Escape	Simple identification, restricted dispersal
Sample Escapees	
Sample Local Farms	Regulated, Obligated
Assignment	Existing method
Action	Strong enforcement

Transfer to marine fish throughout Europe?

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Background Effects of Introgression

- Genetic introgression observed in both Atlantic salmon and brown trout in European rivers.
- Level of introgression not only determined by numbers of escaped fish.
- Possible to quantify introgression but not yet understand impact.

Extent and variation of genetic introgression by farmed Atlantic salmon in Norwegian wild river populations after 30 years of aquaculture

After Glover et al. 2012 PLoSone, Glover et al. 2013 BMC Genetics

% introgression from hatchery fish

20 salmon populations on Norwegian rivers
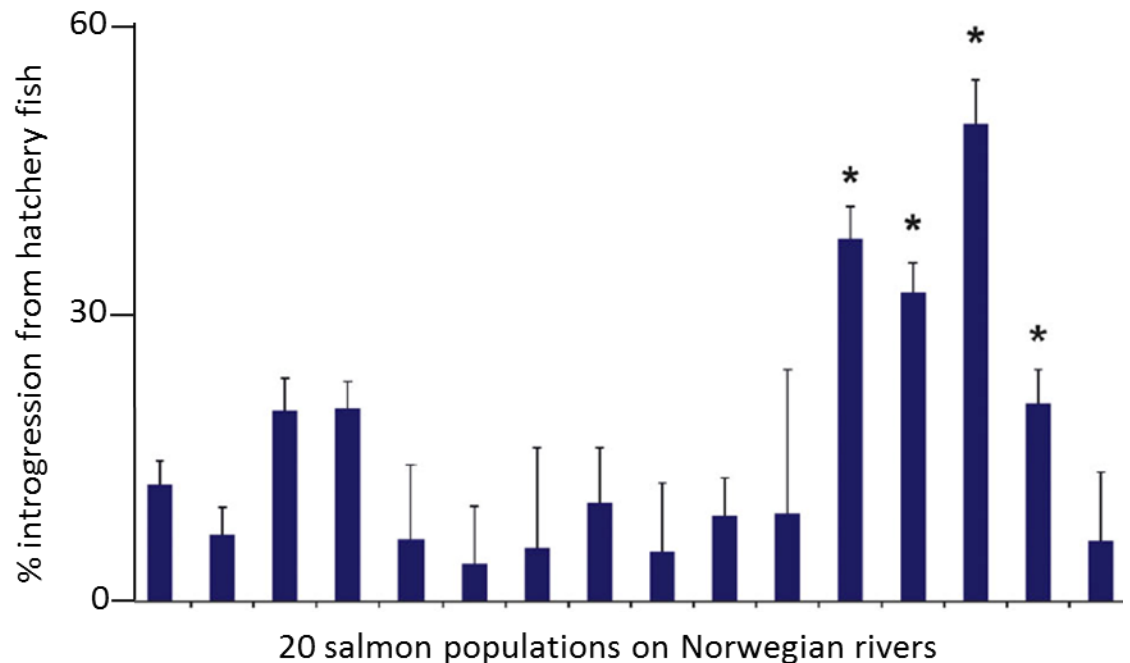
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AquaTrace Scope & Aims

- Development of reliable and cost-effective molecular tools for the identification of the genetic origin of both wild and farmed fish.

- Detection of interbreeding and assessment of genetic introgression.

- Examination of links between key fitness and life-history traits and specific functional genetic variation between wild and farmed fish (common garden).

Marine species:

Gilthead sea bream
(*Sparus aurata*)

European sea bass
(*Dicentrarchus labrax)*

Turbot
(*Scophthalmus maximus*)

Model Species:

Atlantic salmon
(*Salmo salar*)

Brown trout
(*Salmo trutta*)

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AquaTrace Structure

13 Work Packages

- Knowledge integration
- Sampling
- Genomic resources
- Marine species baselines
- Marine species tools
- Common garden experiments
- Trait-genome interactions
- Risk management
- Communication

WP 4. Collection and archiving of samples

WP 1. Industrial survey on aquaculture breeding practices

WP 2. Integration of knowledge and resources from previous projects

WP 3. Website, database and web-based information access platform

WP 6. Development of genomic resources for marine species

WP 7. Genetic and demographic baselines for marine species

WP 8. Development of tools for assessing and monitoring aquaculture impact on marine species

WP 5. Common-garden experiments

WP 9. Linking traits and genetic variation

WP 10. Forensic tool validation and SOP development

WP 11. Risk assessment and management recommendations

WP 12. Communication and dissemination

WP 13. Project coordination and management

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AquaTrace Partners, Budgets, Duration

Partners:
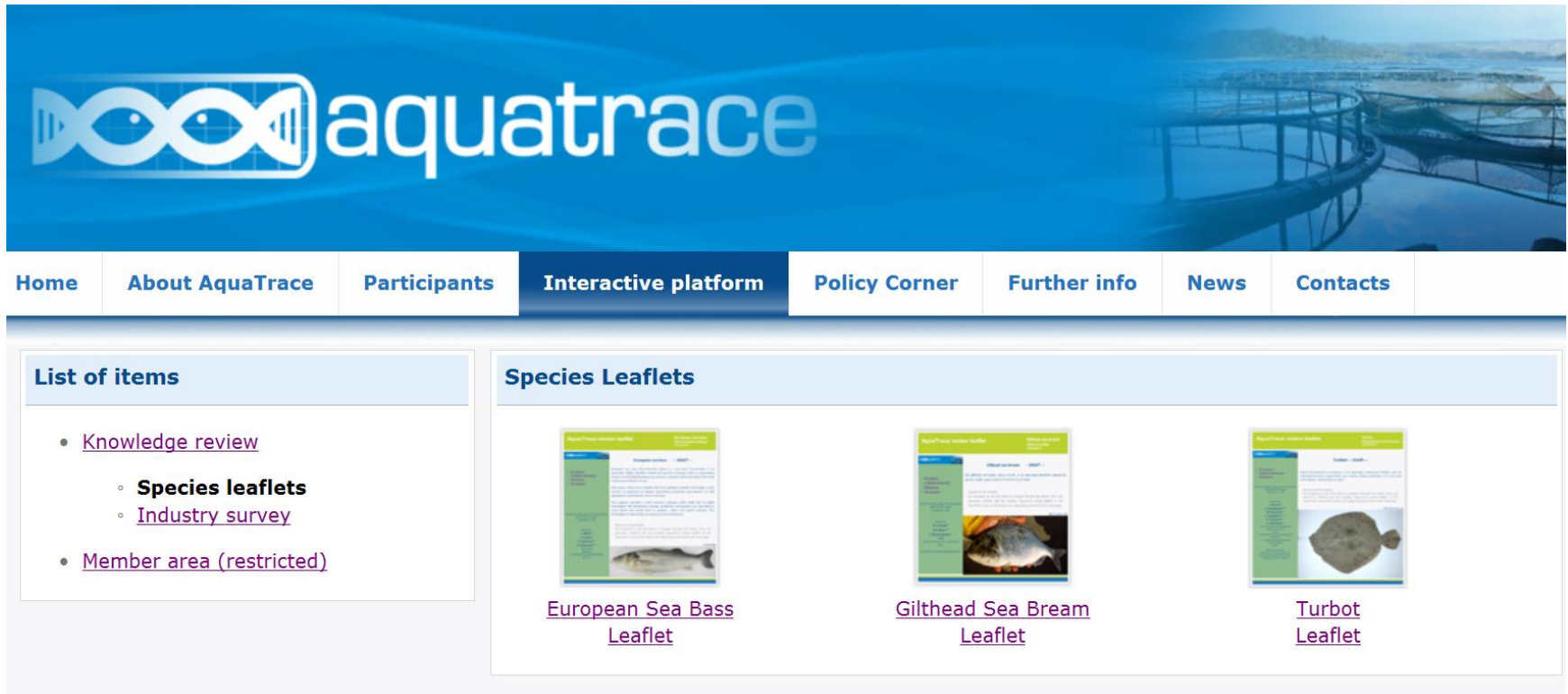
- 22 partners from 11 countries (11 languages!)

- Lead – Danish Technical University (Einar Nielsen)

- 8 Universities

- 5 Research Institutes

- 9 SMEs (Small-Medium sized Enterprises)

Budget:

- 4M Euros over 4 years (2012-2016)

- 3M Euros from European Union 7th Framework Programme

- 1M Euros in-kind contribution from partners.

Integration of knowledge and resources from previous related research projects and other relevant sources

https://aquatrace.eu/species_leaflets
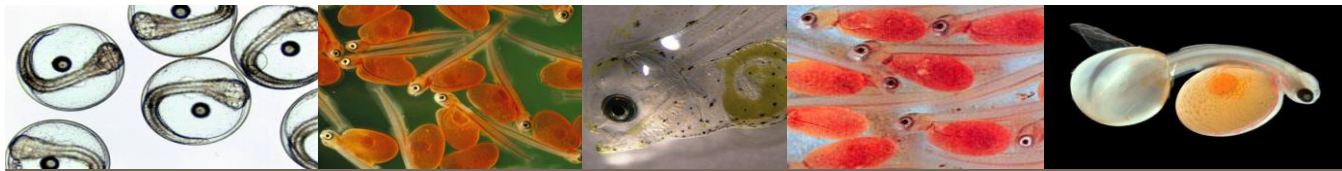
Industrial survey on the breeding practices in the European aquaculture industry

- To gather information on selective breeding programs implemented in Europe on six target species: salmon, rainbow trout, carp, turbot, sea bass, sea bream.

In particular, to provide insight on:
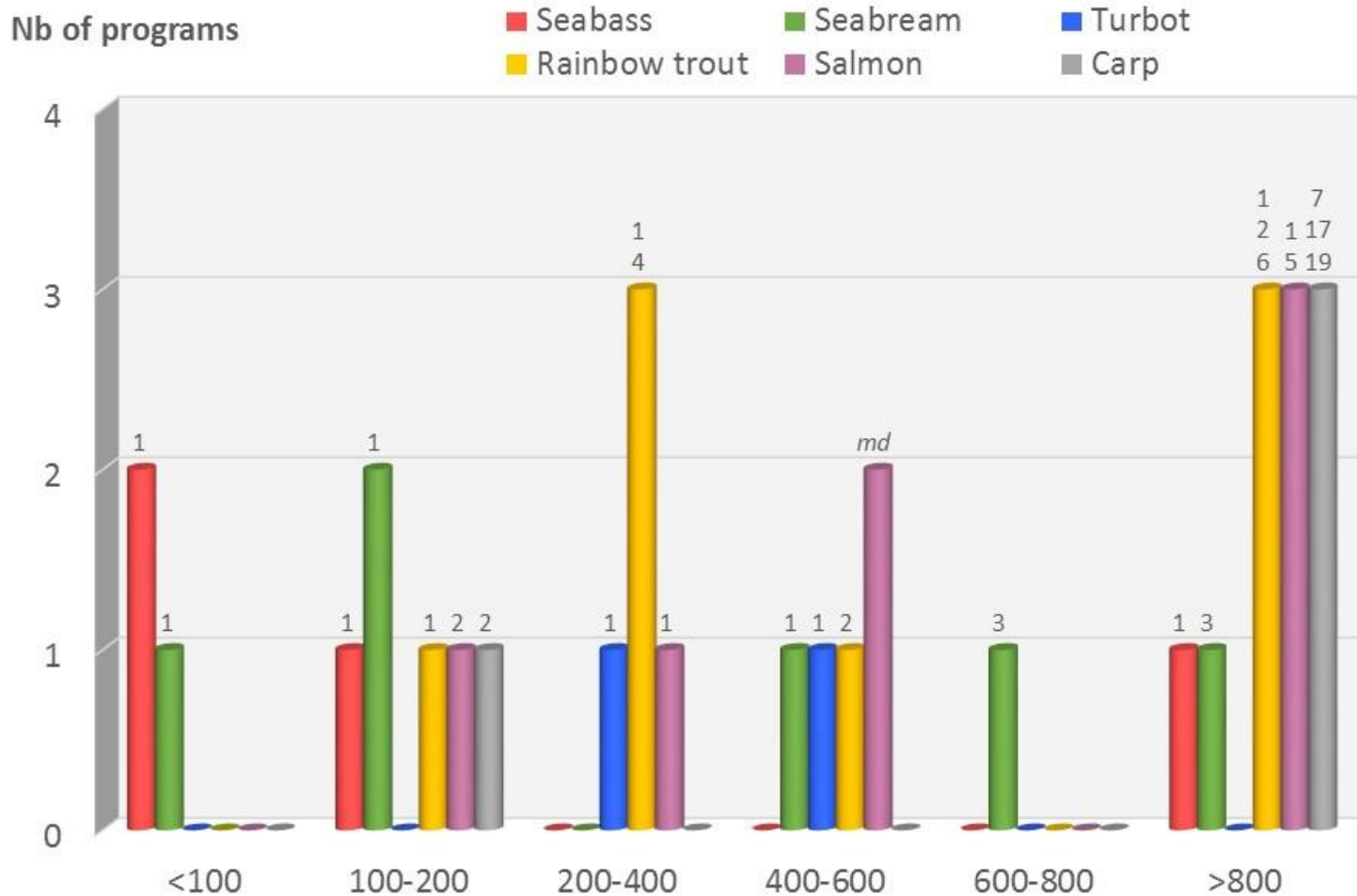
    Inbreeding control
    number of broodfish involved
    selected traits
    volume of seeds produced
    share of selected seeds in the European market

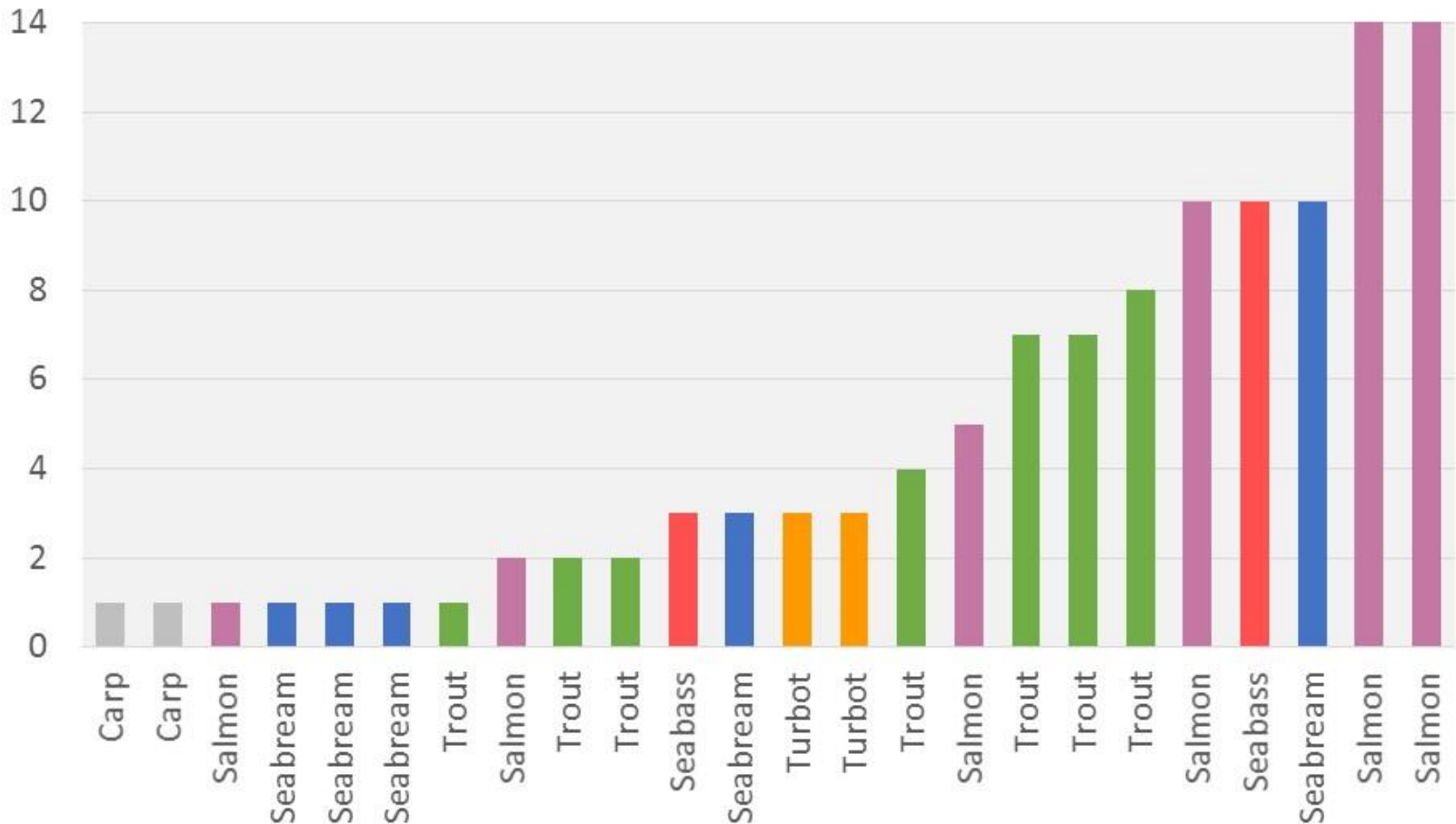- To show the trend of the sector since the last survey (AquaBreeding 2009)
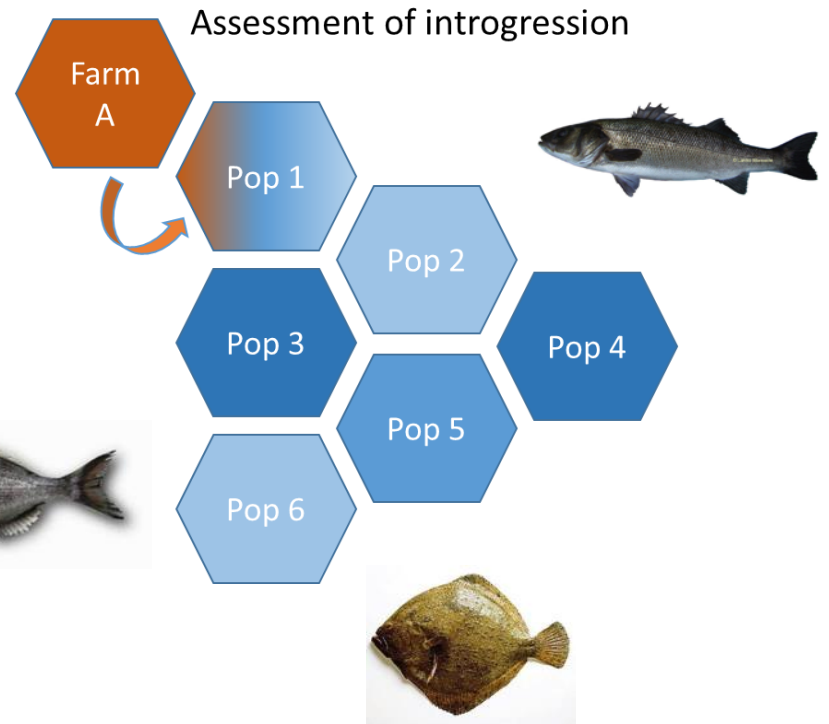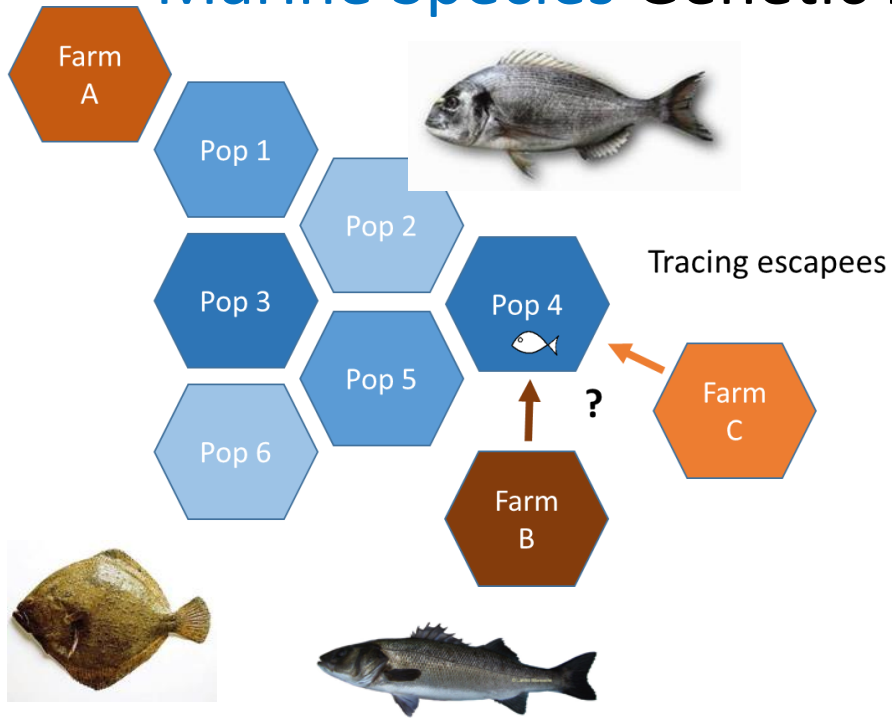
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Broodfish per generation

12 programs managed a unique line, 17 run different lines

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Export countries of selected seeds

Nb of export countries

Marine Species Genetic baseline

Tracing escapees

Assessment of introgression

Marine Species Progress Sampling

3276 samples for sea bream (1740 wild and 1536 farmed specimens)

4394 samples for sea bass (3061 wild and 1334 farmed)

1622 for turbot (1240 wild and 382 farmed)

Location
All

Number of specimens
· 1   ○ 150
○ 50   ○ 223
○ 100

Species
☐ Dicentrarchus labrax
☐ Psetta maxima
☑ Sparus Aurata
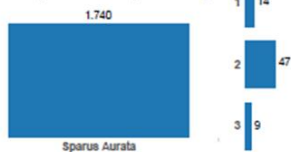
Environment
☐ Farmed
☑ Wild
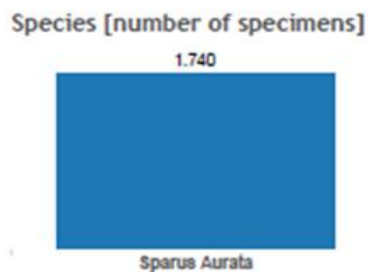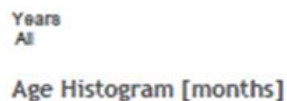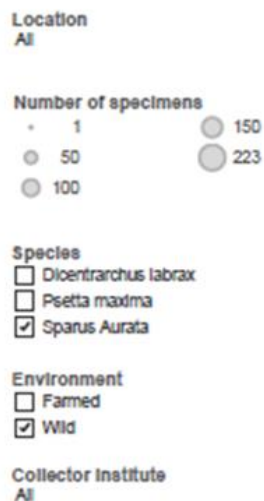
Collector Institute
All

Years
All

Age Histogram [months]

Length [cm]
0 — 369
500 | 26

Weight [Kg]
0 — 355
1 | 14
2 | 47
3 | 9

Species [number of specimens]
1.740
Sparus Aurata

Collector Institute

date (Years)	AUTH	CETGA	ISPRA	MKU	UniPD	USC	Total
2000			20				20
2001			82				82
2002			65				65
2003			25				25
2004	90						90
2005	269		35				304
2006	148						148
2008				22			22
2009						40	40
2012	52				20		72
2013	382		80	30	3	50	545
2014	46	37		26	218		327
Grand Total	987	37	329	56	241	90	1.740

Marine Species Progress Sampling

Marine Species Progress Genomics

Plan	Realisation
Reference genome sequencing	Sea bass existed, Turbot completed, sea bream created
Genome wide SNP discovery via RAD sequencing	ddRAD sequencing: 6155 samples in 42 libraries ~1000 SNP markers per species 2.5×10^{12} bases of data
Wild & Farmed population genotyping (300 markers)	
Mapping DNA markers to genomes sequences	Mapping DNA markers to genomes sequences

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Marine Species Progress Population Analysis

Purpose:

To establish genetic baselines of natural populations and farmed aquaculture stocks

Investigate:

1. Individual and population level diversity (wild and farmed)

2. Pedigree / parentage analysis in hatcheries

3. Spatial and temporal population structure in wild

4. Identification of 'outlier loci', DNA markers possibly under selection

5. Identification of hybrids and introgression

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Model Species Progress Common Garden

Common Garden Experiment

- Phenotype = Genotype x Environment

- Comparison of genetically distinct groups of fish under identical rearing conditions to quantify differences between them for multiple traits.

- Traits: Growth, survival, behavior, reproduction, maturation
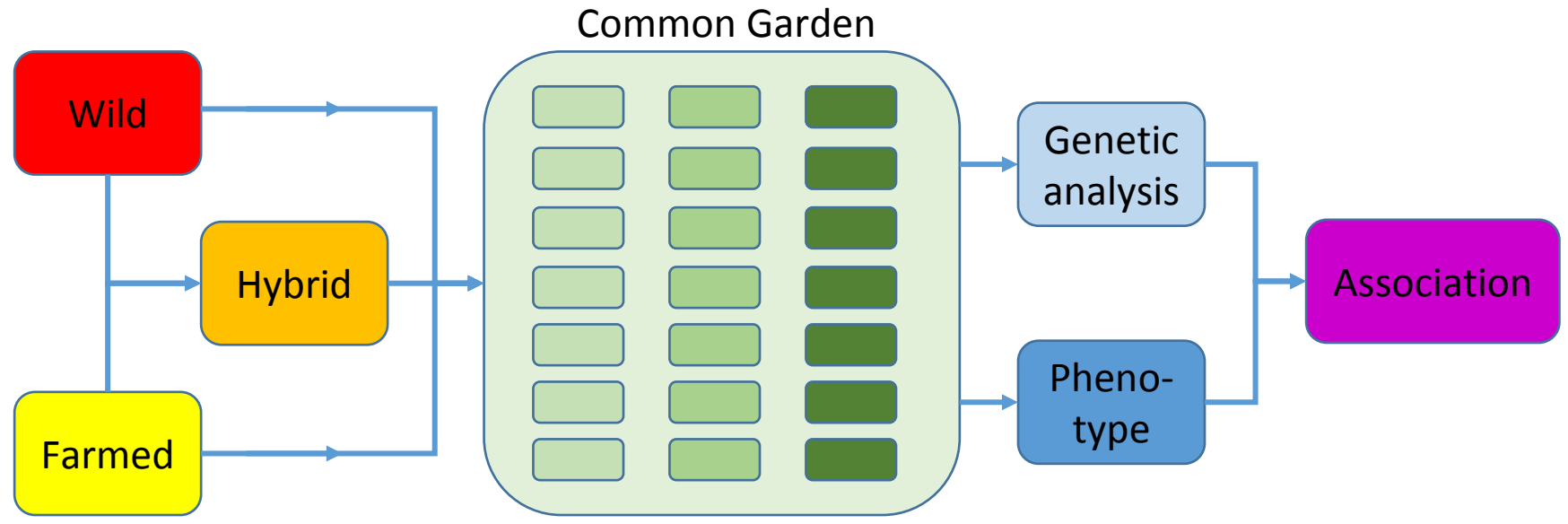
Model Species Progress Common Garden

Fish eggs stripped

Sperm collected

Eggs fertilized

Families mixed

All photos courtesy of K. Glover (IMR)

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Model Species Progress Common Garden

Reared in different environments:

Hatchery (Light, food, temp, stress, density)

'Wild' (Current, food, density, predation)

Caught

Measured and sampled for DNA analysis

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# Model Species Progress Common Garden

Status:

- Common Garden Experiments completed for salmon and brown trout

- Samples being genotypes using existing salmon and trout SNP chip assays

- RNA samples being used for expression analysis

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Risk Assessment

Aim: Assess the environmental risk of aquaculture escapees

- Identify and characterise hazards (e.g. disease, genetic introgression)
  - What is the likely environmental impact (end-point)?
  - What are the processes leading to this end-point?
- Evaluate likelihood of each process to evaluate overall probability
- Assess risk as a combination of harm and probability of occurrence.

Reported releases
Norwegian salmon

*Taranger et al. 2015
ICES J Mar Sci.*
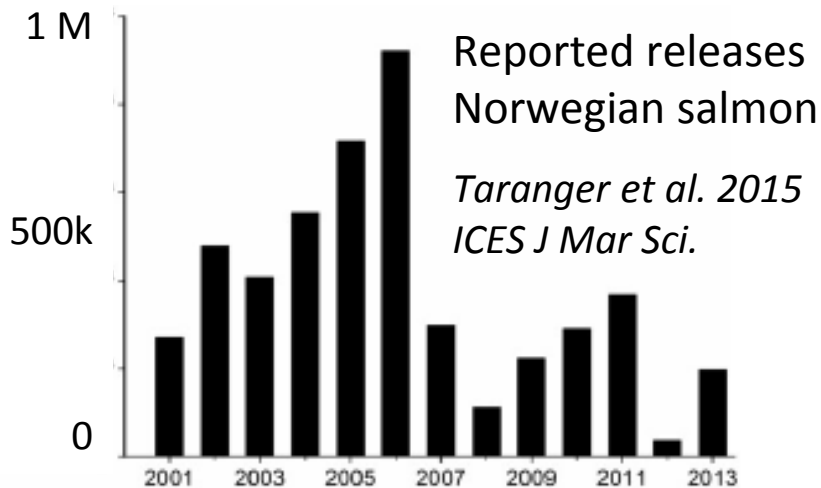
Quantifying Risk

Risk = P(E) x P(H|E)

P(E)   =   probability of exposure,
P(H|E) =   probability of harm given
               that exposure has occurred

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Impact and Application

Outputs beyond fisheries biology

Transfer from Research Project to Application is a key measure of success under EU funding:

- >40% of AquaTrace partners are SMEs, most in Aquaculture

- Transfer of molecular genetic technologies to breeders

- Transfer of traceability tools to regulators

- Transfer / demonstration of risk assessment to policy makers

- Inherent short term costs to monitoring and regulation

- Long-term benefits to aquaculture production and biodiversity conservation should be recognised

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Further Information

Web: https://aquatrace.eu   Email: info@aquatrace.eu

Acknowledgements

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  - Einar Eg Nielsen (DTU), Kevin Glover (IMR), Rob Ogden (Trace)