WP1 - Sustainable breeding of important European aquaculture species
On-line seminar 24.02.2023

Anne Kettunen, Nofima – WP 1 leader

WP1: Sustainable breeding

To **assess**, **validate** and **demonstrate** the level of the ability of the current breeding programs, their breeding goals and methodologies in four of the main European aquaculture species to answer the future challenges of:

1. Increased need for utilization of alternative feed sources in aquaculture feeds.
2. Need for resilience in the face of climate change.
3. Maintained and increased animal welfare through robustness and disease resistance.

Nofima, Avramar (previously Nireus), Benchmark Genetics Norway, Hellenic Centre for Marine Research, Osland Stamfisk

24-02-23

Motivation: climate resilience

- Climate change brings upon several climatic elements:
    - Rise in temperatures, and sea-level
    - Ocean acidification
    - Increased risk of diseases, parasites and harmful algal blooms
    - Changes in rainfall patterns and sea-surface salinity
    - Uncertainty of external input supplies
    - Severe climate events
- Many fish species are poikilothermic
- Rise in temperature may
    - Affect production traits
    - Thermal stress → more susceptible to diseases
    - Increased risk for more opportunistic disease outbreaks

Motivation: feed ingredients

- In the long run, fish farming relying on fish meal and fish oil as feed ingredient is not a sustainable practice.

- Increased need to use alternative raw materials in fish feed is also prompted by regulatory (e.g., fishing quotas), climatic and societal issues.

- How does geographical location/climate and/or the feed formulation affect economically important traits?

    - E.g., growth, survival, disease resistance.

- Genotype-by-environment interaction:

    → discrepancy between expected and realized performance.

    The magnitude of re-ranking of genotypes in different environments and when fed conventional/commercial and innovative diets.
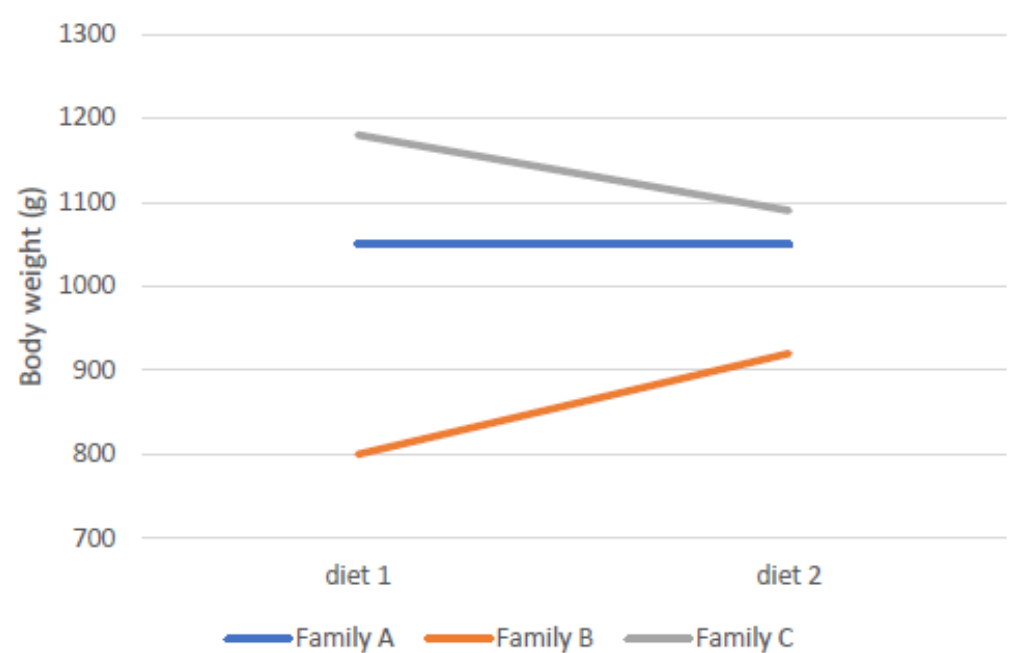
Motivation: feed ingredients

Norwegian salmon feed – change in sources of ingredients over years
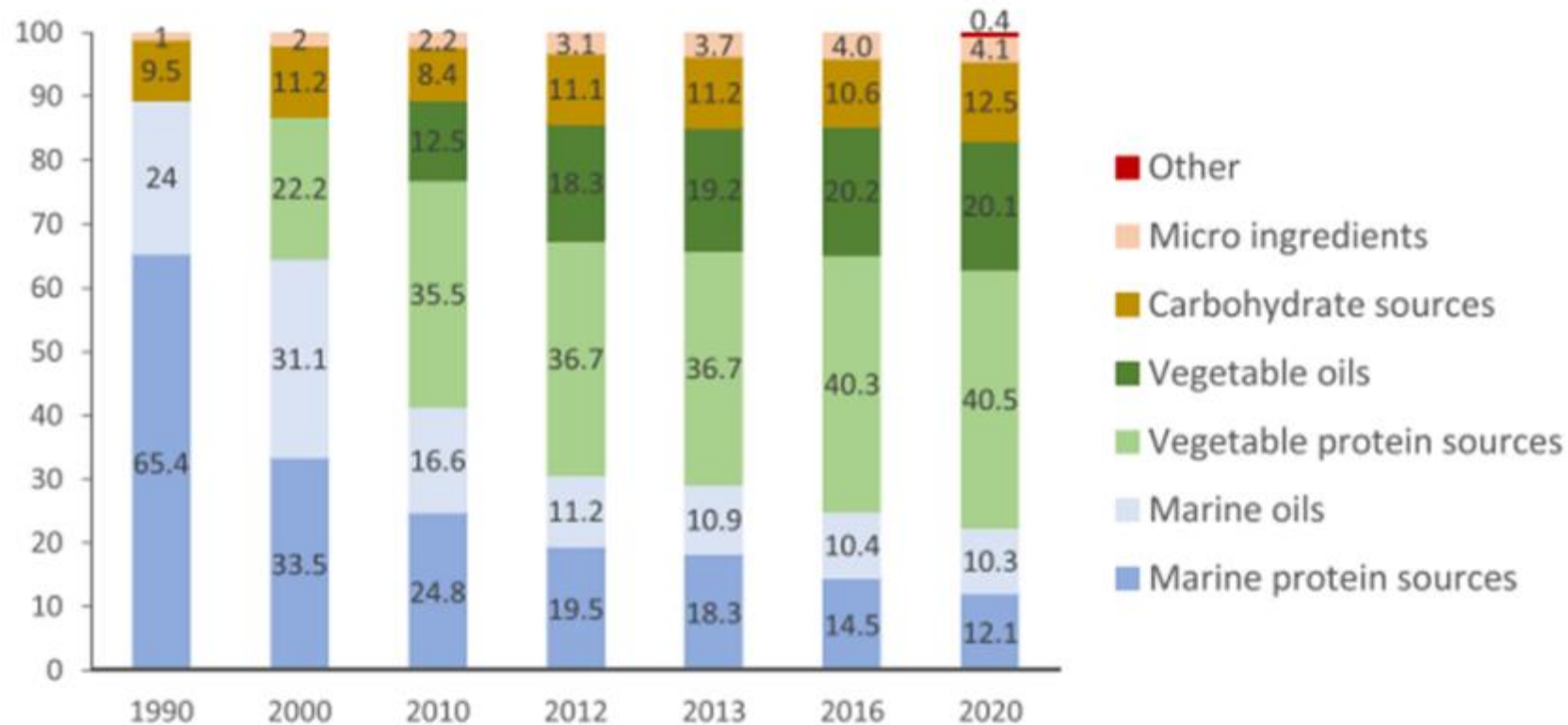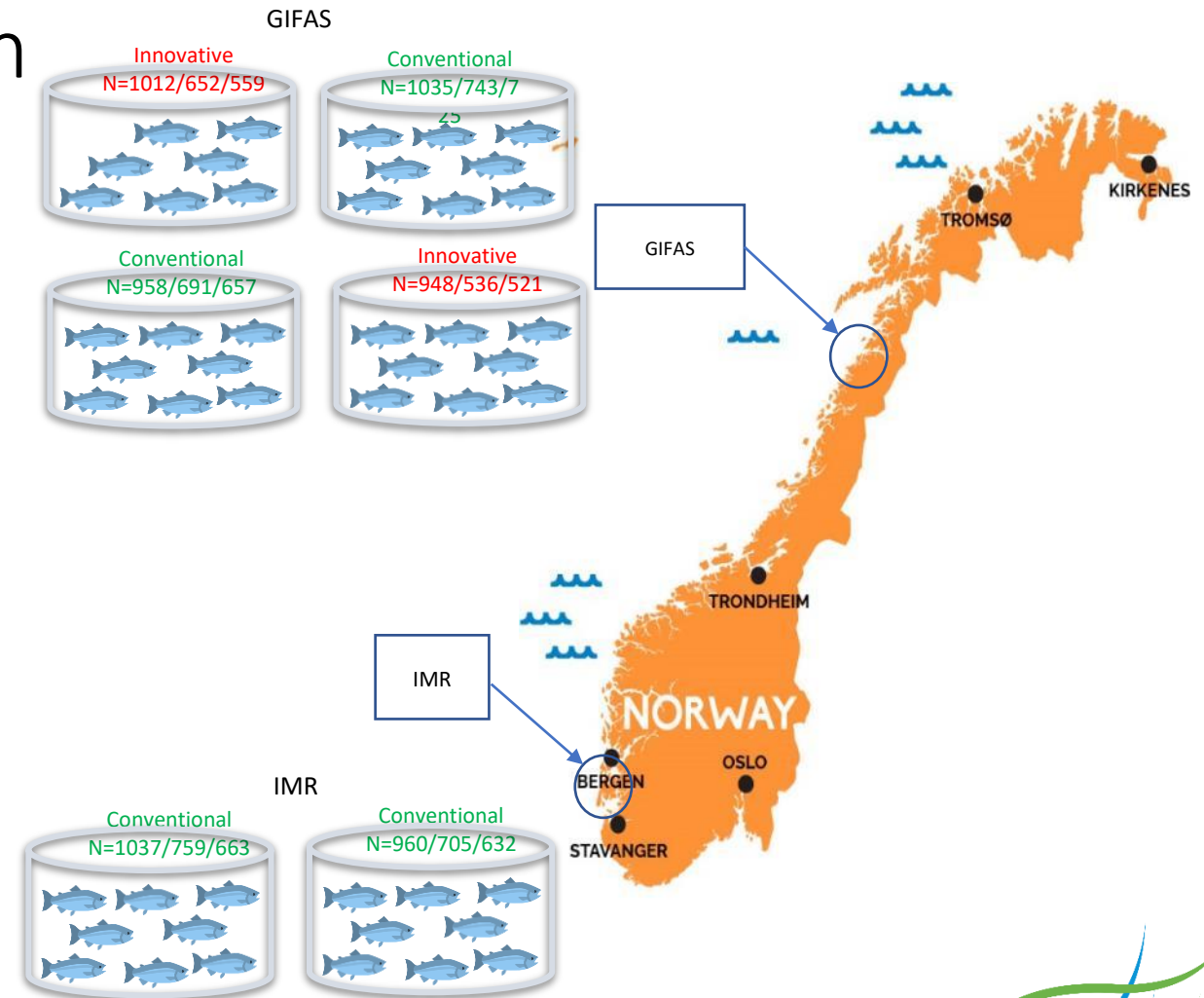

Aas *et al.* 2022

Genotype x Environment

Scaling

Re-ranking

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| Task/species	No of families	N/family/cage or tank	N cages/tanks	N	Experimental period
Task 1.1					
Salmon	75	~13/diet	4	(~4000) 2935	24.10.2019-22.8.2020
Seabass	88	25/cage	5	10958	6.8.2020-19.-29.7.2021
Sea bream	117	25/cage	4	11679	1.11.2021-23.9.2022
Task 1.2					
Salmon	75	~13	4	(~4000) 3231	22.10.2019-19.5.2020
Sea bass	88	20	4	6960	12.5.2020-11.9.2020
Sea bream	114	15	4	6825	19.11.2021– 18.2.2022
Task 1.3					
Sea bass	88	30	1	2606	14.7.2020-30.7.2020
Rainbow trout	98 25	~23 ~24	One for each strain of IPNV	2334 612	26.5.2021-8.7.2021

Experiments: Atlantic salmon

- 67 families from Benchmark Genetics Norway.

- Two geographical locations: NORTH and SOUTH.

- Two isoenergetic diets formulated and produced by Aquafeed Technology Center of Nofima.

  - Innovative: 50 % of the marine oil replaced by algae and 75 % of the fishmeal replaced by insect meal, no soya.

  - Conventional: marine based diet.

GIFAS

Innovative
N=1012/652/559

Conventional
N=1035/743/725

Conventional
N=958/691/657

Innovative
N=948/536/521

GIFAS

IMR

IMR

Conventional
N=1037/759/663

Conventional
N=960/705/632

KIRKENES
TROMSØ
TRONDHEIM
NORWAY
OSLO
BERGEN
STAVANGER

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GxE in Atlantic salmon

Genotype-by-feed

- Innovative 1380 g, conventional 1590 g
- Mortality from tagging to end of the trial:
  - 34.7% innovative vs. 21.1% conventional

- High genetic correlation between growth traits across environments → no significant GxE
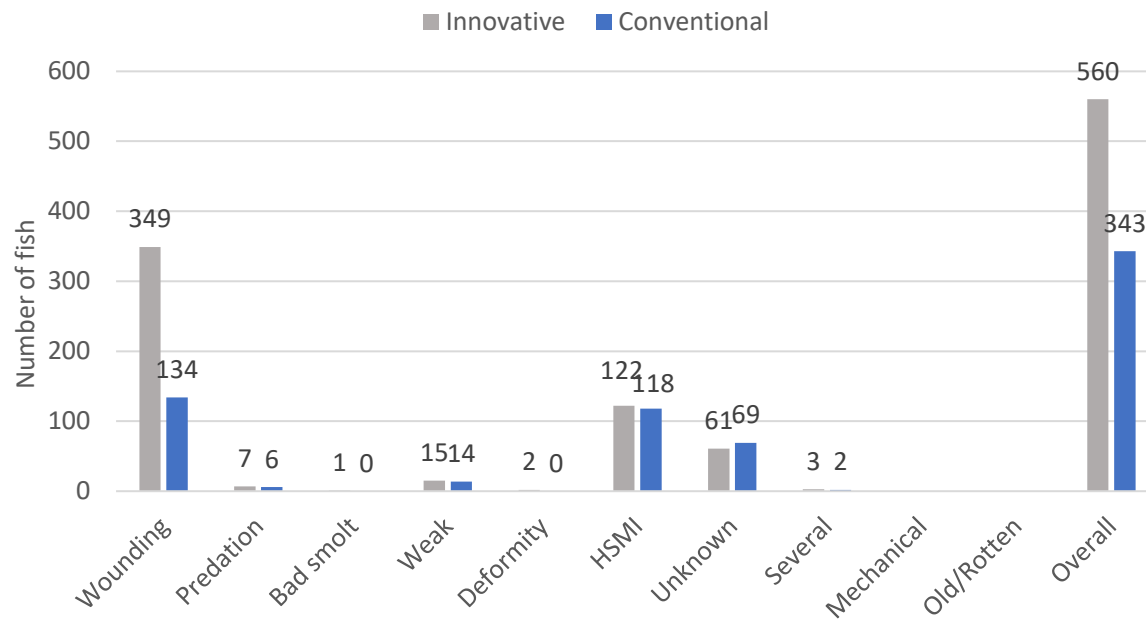
| Traits	End weight		TGC	
	Conv.	Innovative	Conv.	Innovative
Conv.	**0.45±0.00**		**0.44±0.03**	
Innovative	0.99±0.01	**0.44±0.00**	0.96±0.01	**0.41±0.03**

- QTL-study; no statistically significant associations for any of the growth traits were found → support for polygenic inheritance

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GxE in Atlantic salmon

Daily average temperature

Genotype-by-climate

- SOUTH 969 g, NORTH 1590 g (conv.diet only) due to different grow-out period length
- Moderate to high heritability for growth
- High genetic correlation between growth traits across environments → no significant GxE

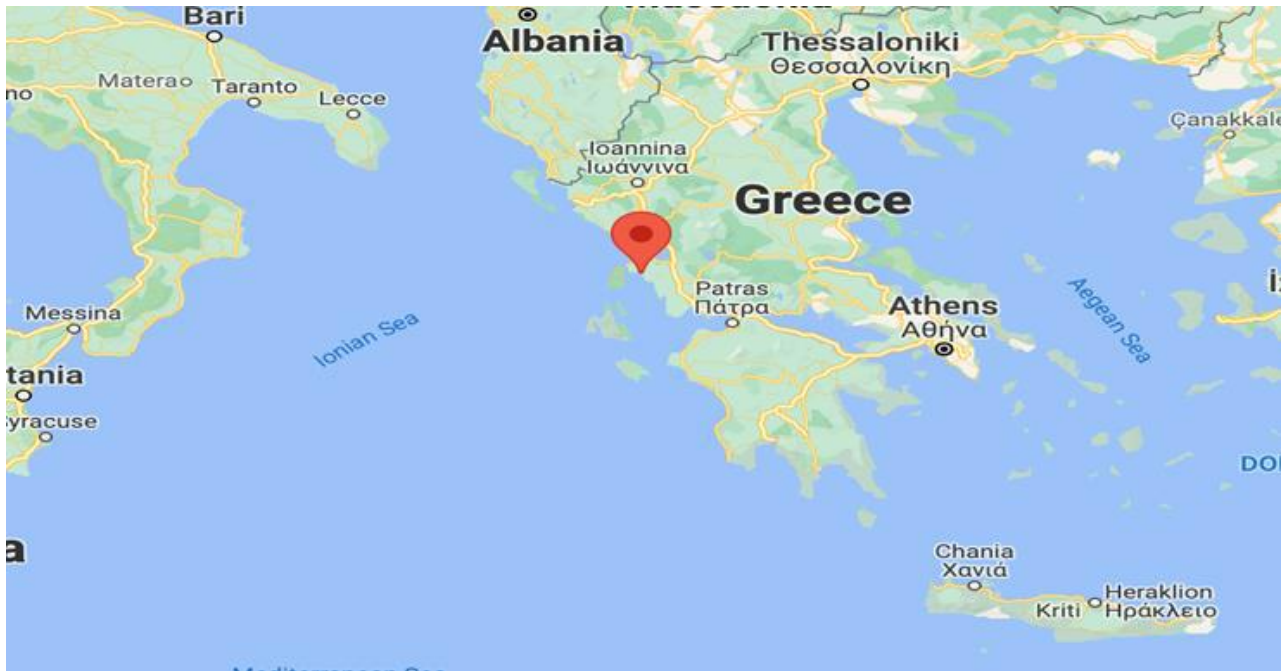| Traits	End weight		TGC	
	SOUTH	NORTH	SOUTH	NORTH
SOUTH	**0.58±0.03**		**0.56±0.04**	
NORTH	0.93±0.06	**0.46±0.02**	0.94±0.08	**0.44±0.03**
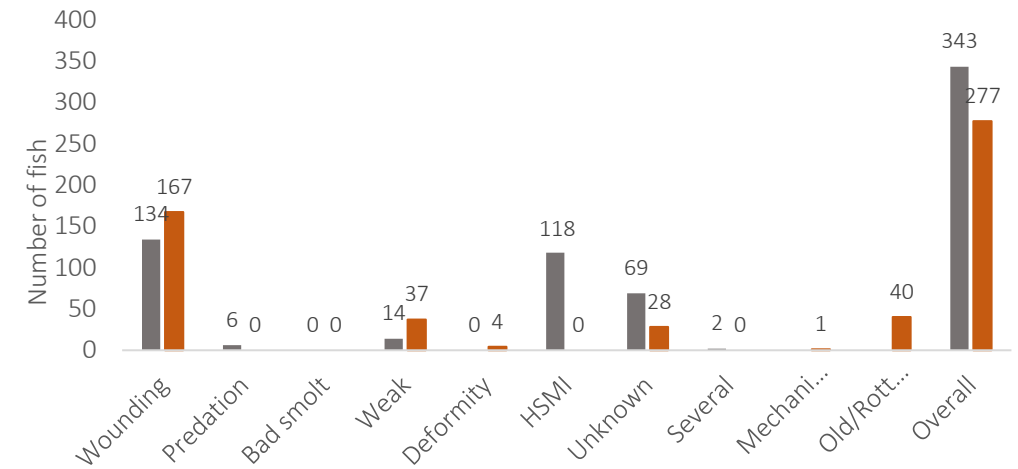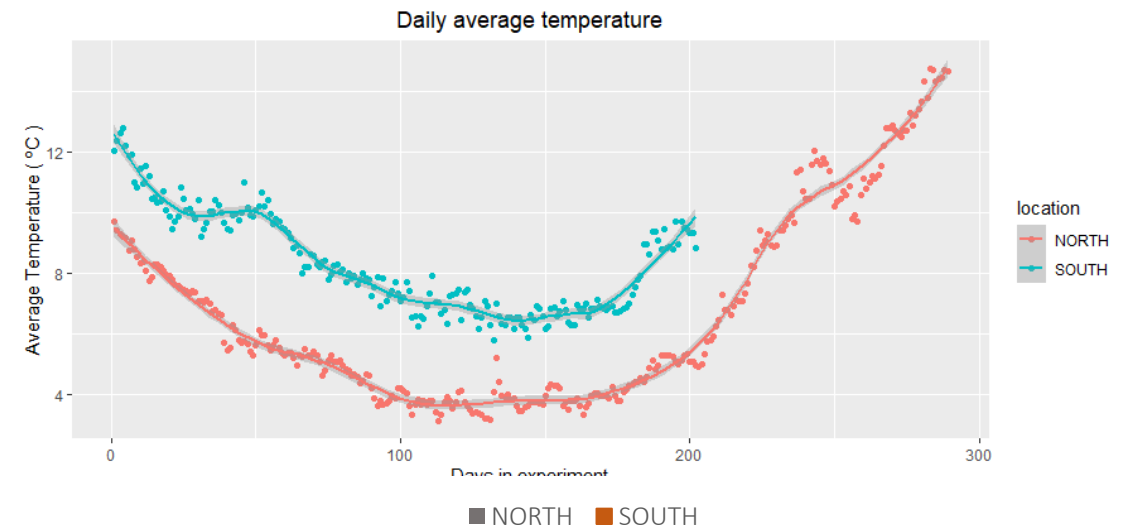
- QTL-study; no statistically significant associations for any of the growth traits were found → support for polygenic inheritance

Experiment: European sea bass

Genotype-by-feed

- N=10958, 88 families

- Commercial vs. plant-based ingredients

- August 2020 – July 2021

LFK1

2196 fish

~25 fish/family feeding on control feed

LFK3

2192 fish

~25 fish/family feeding on control feed

LFK5

2196 fish

~25 fish/family feeding on control feed

LFK4

2184 fish
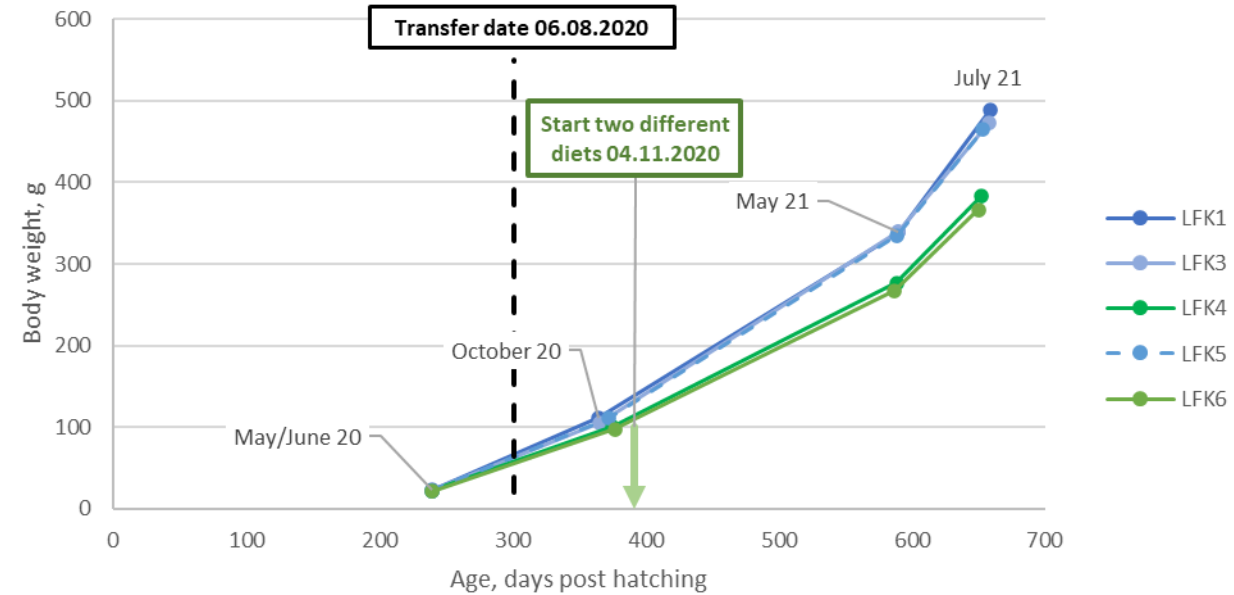
~25 fish/family feeding on experimental feed

LFK6

2190 fish

~25 fish/family feeding on experimental feed
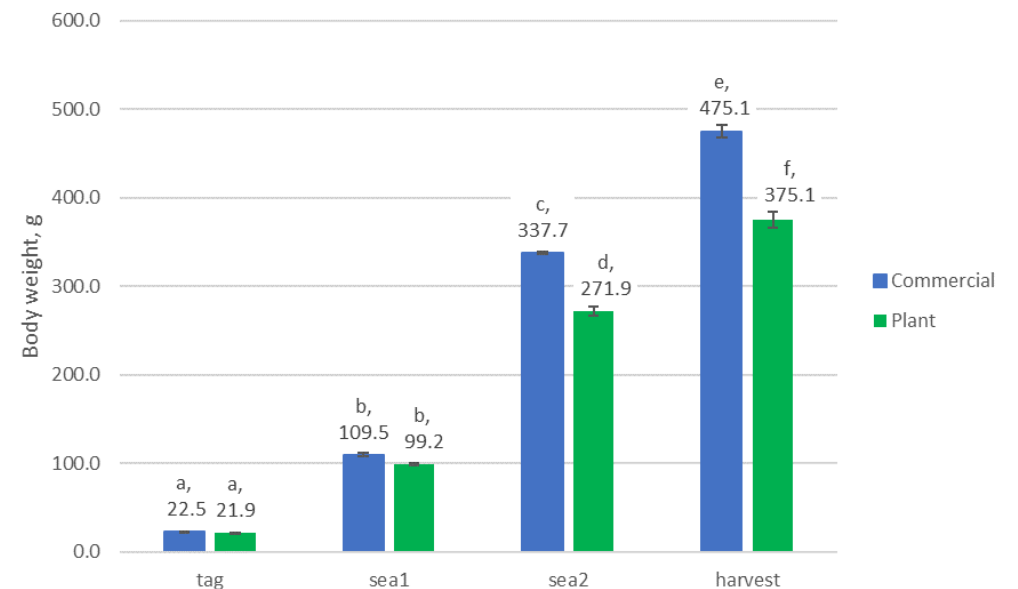
GxE in European sea bass

Genotype-by-feed and genomic analyses of fillet fat

- Commercial diet outperformed the experimental diet in growth: 475 g vs 375 g.
- No differences in survival between the diets.

- QTL-study; no statistically significant associations for any of the growth traits were found → support for polygenic inheritance.

24-02-23

Seabass batch15: Growth at Lefkada

Seabass batch15: Body weight

GxE in European sea bass

Seabass family fillet fat recorded on living candidates fed plant and control diets

R² = 0.511

- Fillet fat → sub-sampling for fatty acid profiles
  - Control diet had higher values of polyunsaturated fatty acids.
  - No difference in fat content between the diets.

- QTL-studies;
  - No conclusive association with fillet fat content or fatty acids.
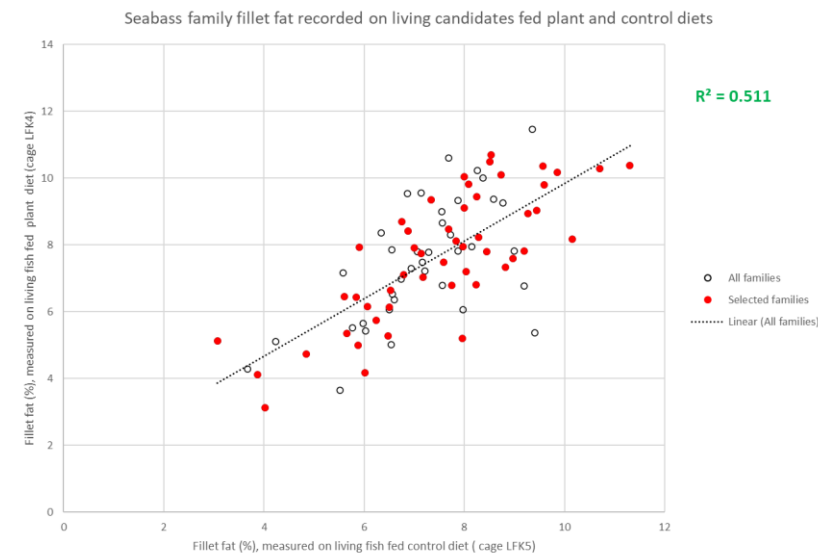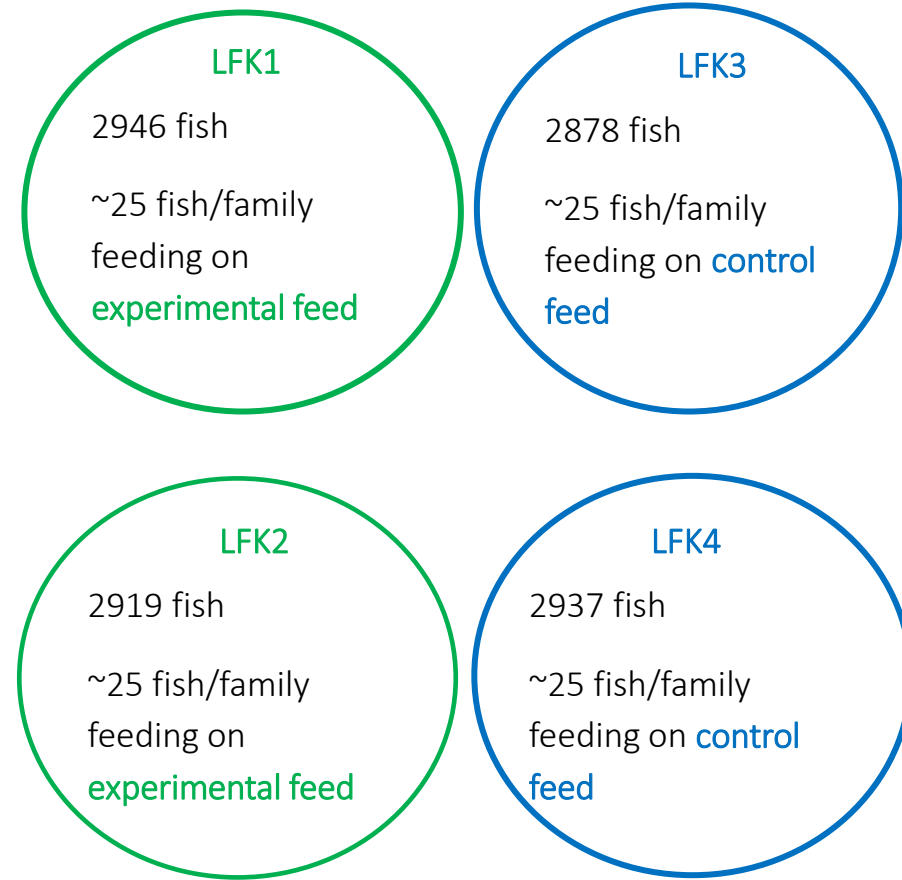
	Body Weight	Fillet fat	Omega-3	EPA	DHA
Body Weight	0.31 (0.08) *	0.76 (0.17) *	0.73 (0.27) *	0.72 (0.41)	0.59 (0.35)
Fillet fat	0.33 (0.04) *	0.36 (0.09) *	0.91 (0.10) *	0.88 (0.11) *	0.80 (0.65)
Omega-3	0.13 (0.05) *	0.77 (0.02) *	0.23 (0.08) *	0.99 (0.25) *	0.96 (0.12) *
EPA	0.12 (0.05) *	0.74 (0.02) *	0.99 (0.03) *	0.22 (0.08) *	0.96 (0.23) *
DHA	0.05 (0.05)	0.65 (0.03) *	0.96 (0.00) *	0.94 (0.01) *	0.19 (0.07) *

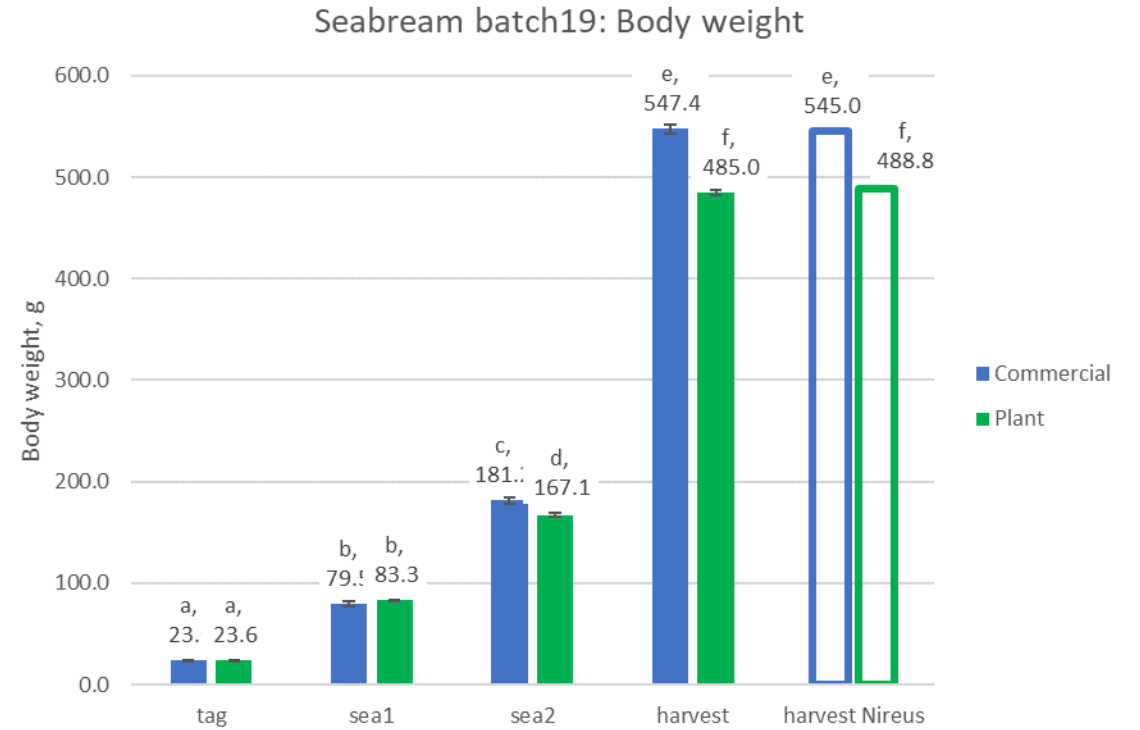*Statistically significant estimation

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Experiment: Gilthead sea bream

Genotype-by-feed

- N=11680, 117 families.

- Same location and diet as for bass.

- November 2021 – September 2022

- Commercial diet outperformed the experimental diet in growth: 547 g vs 485 g.

- No differences in survival between the diets.

LFK1

2946 fish

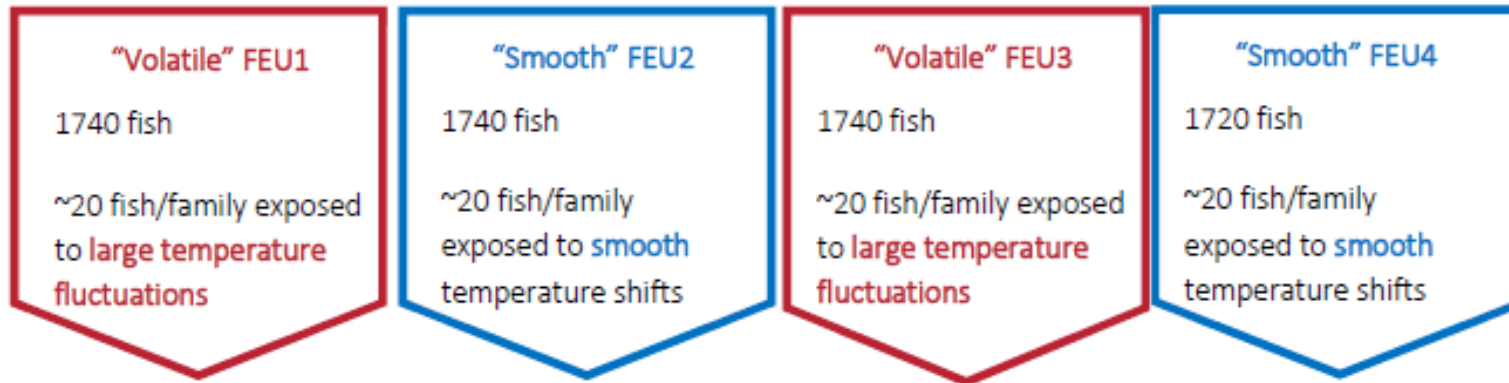~25 fish/family feeding on experimental feed

LFK3

2878 fish

~25 fish/family feeding on control feed

LFK2

2919 fish

~25 fish/family feeding on experimental feed

LFK4

2937 fish

~25 fish/family feeding on control feed

GxE in Gilthead sea bream

Seabream batch19: Growth at Lefkada

Transfer date 01.11.2021

Start two different diets 06.04.2022

September 22
June 22
January 22
Sept/Oct 21

LFK1
LFK2
LFK3
LFK4

Body weight, g

Age, days post hatching

Seabream batch19: Body weight

Body weight, g

	tag	sea1	sea2	harvest	harvest Nireus
Commercial	a, 23.	b, 79.	c, 181.	e, 547.4	e, 545.0
Plant	a, 23.6	b, 83.3	d, 167.1	f, 485.0	f, 488.8

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GxE feed in bass and bream

SEA BASS	Weight plant	Weight commercial
Weight plant	**0.30 ± 0.08**	
Weight commercial	0.92 ± 0.05	**0.35 ± 0.09**

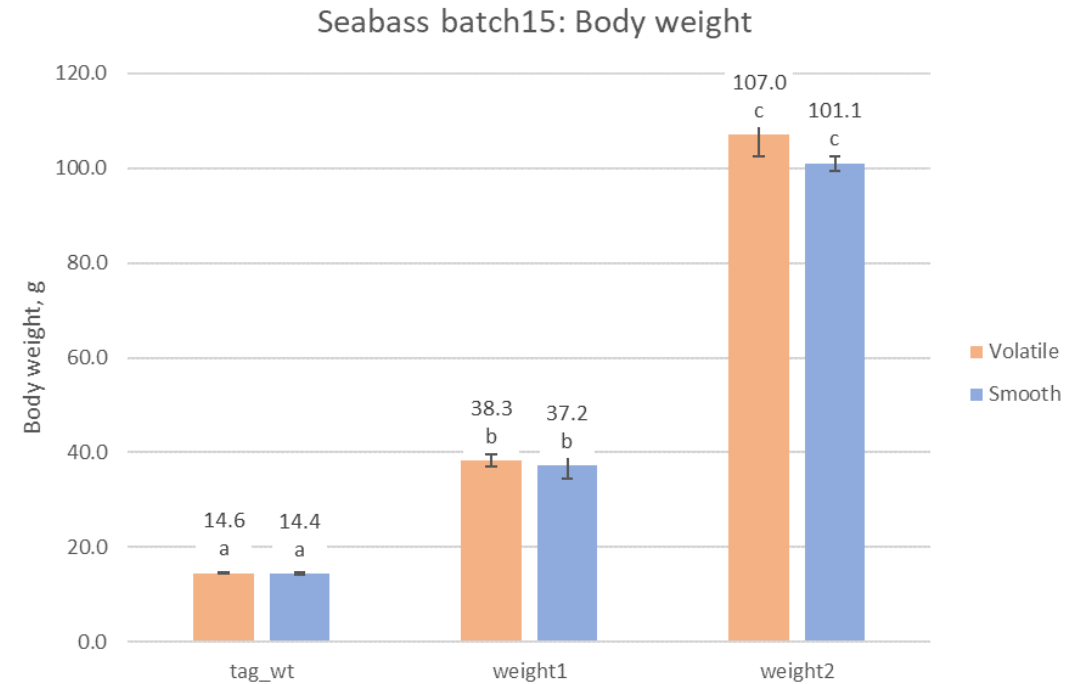| SEA BREAM	Weight plant	Weight commercial
Weigth plant	0.14 ± 0.07 **0.33± 0.11***	
Weight commercial	0.92 ± 0.07 0.96 ± 0.04*	0.16 ± 0.08 **0.34 ± 0.11***

*just Nireus families

Experiment: European sea bass

"Volatile" FEU1	"Smooth" FEU2	"Volatile" FEU3	"Smooth" FEU4
1740 fish	1740 fish	1740 fish	1720 fish
~20 fish/family exposed to large temperature fluctuations	~20 fish/family exposed to smooth temperature shifts	~20 fish/family exposed to large temperature fluctuations	~20 fish/family exposed to smooth temperature shifts

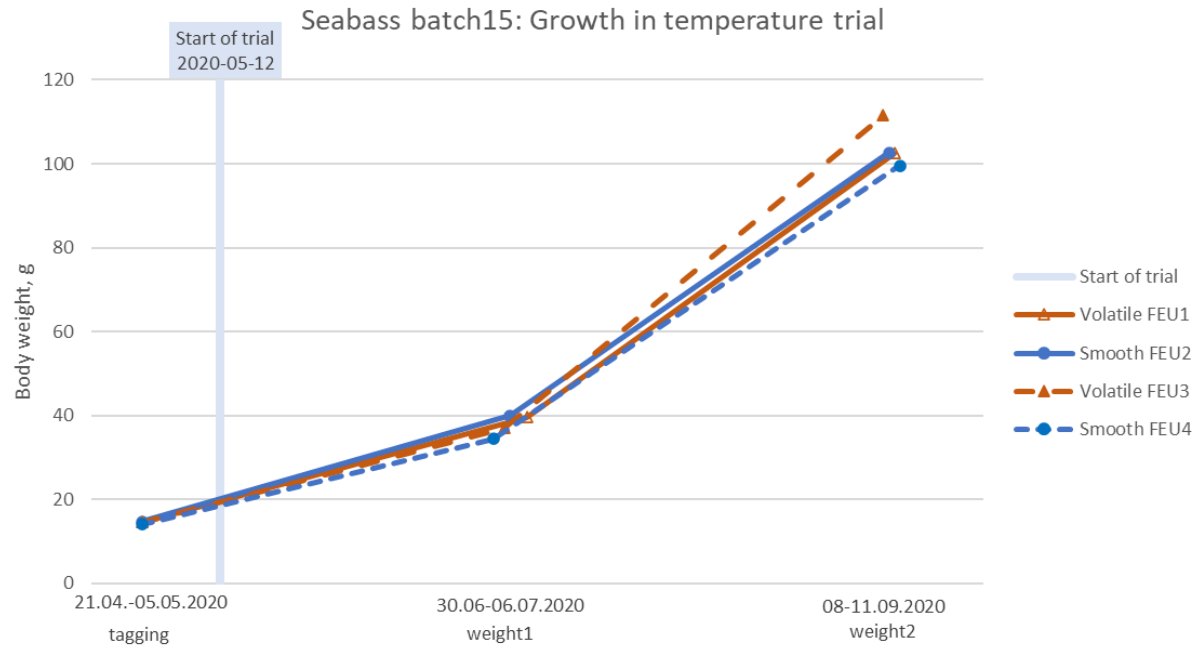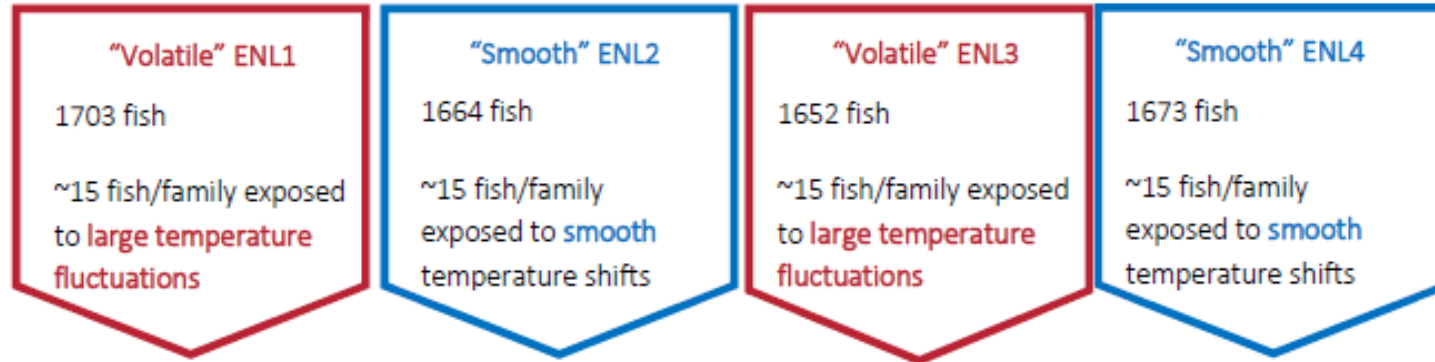*G-by-climate*

- N=6904, 88 families

- 4-month trial:
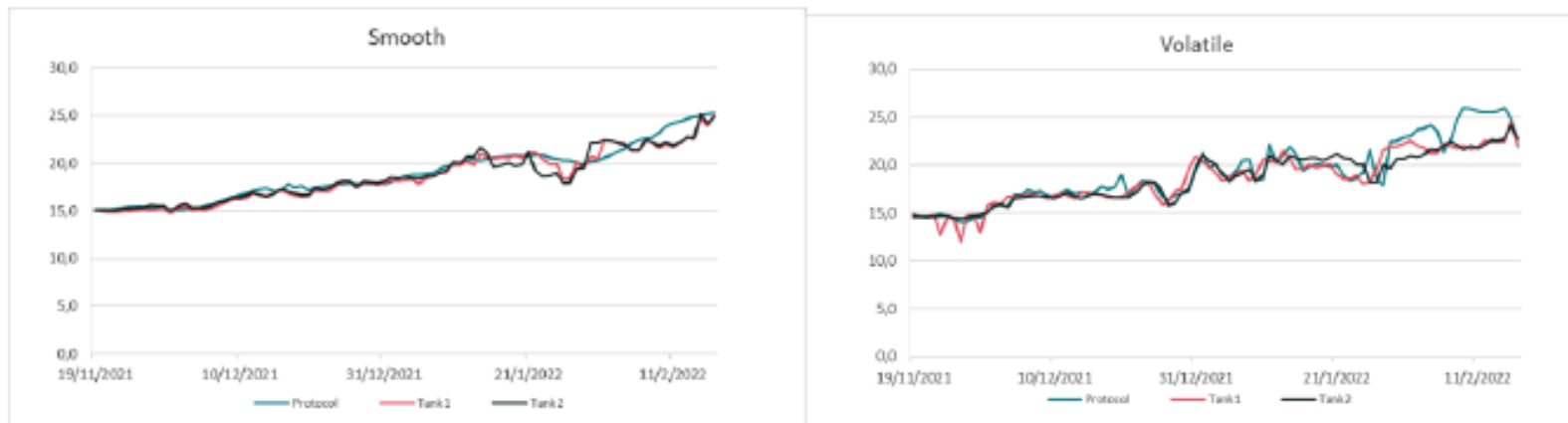 - May-September 2020

GxE in European sea bass

Seabass batch15: Growth in temperature trial

Seabass batch15: Body weight

Experiment: Gilthead sea bream

"Volatile" ENL1

1703 fish

~15 fish/family exposed to large temperature fluctuations

"Smooth" ENL2

1664 fish

~15 fish/family exposed to smooth temperature shifts

"Volatile" ENL3

1652 fish

~15 fish/family exposed to large temperature fluctuations

"Smooth" ENL4

1673 fish

~15 fish/family exposed to smooth temperature shifts
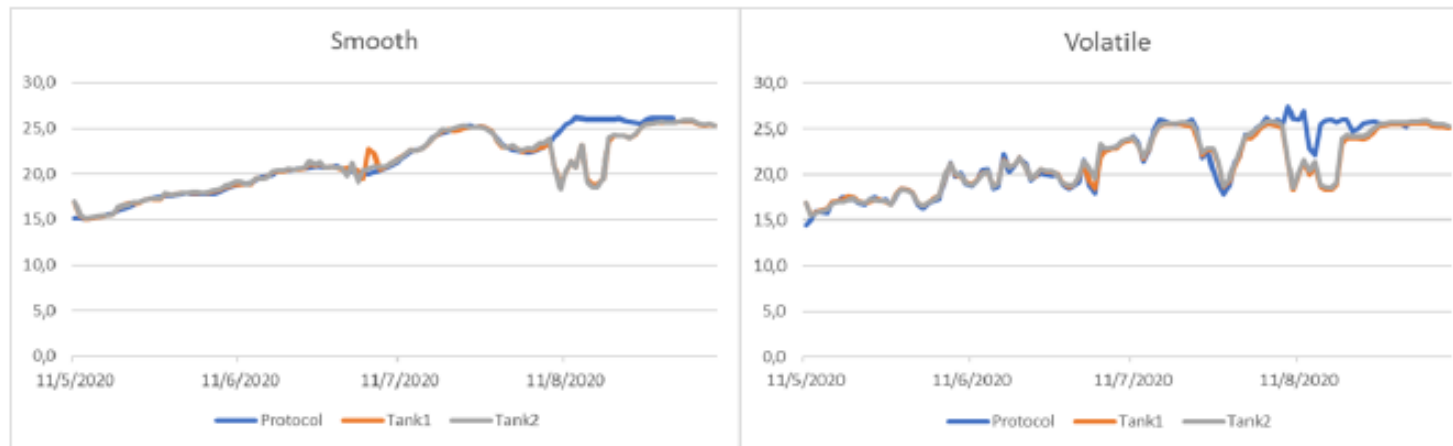
G-by-climate

- N=6692, 114 families
- 3-month trial
  - November 2021-February 2022

GxE in sea bream

Seabream batch19: Growth in temperature trial

Seabream batch19: Body weight
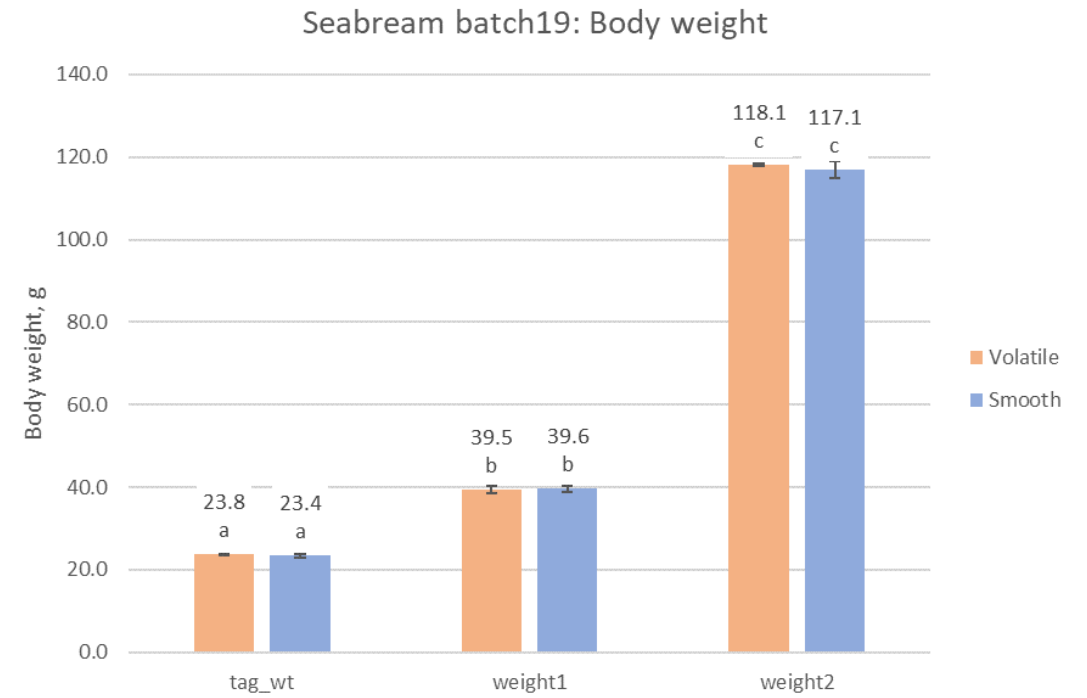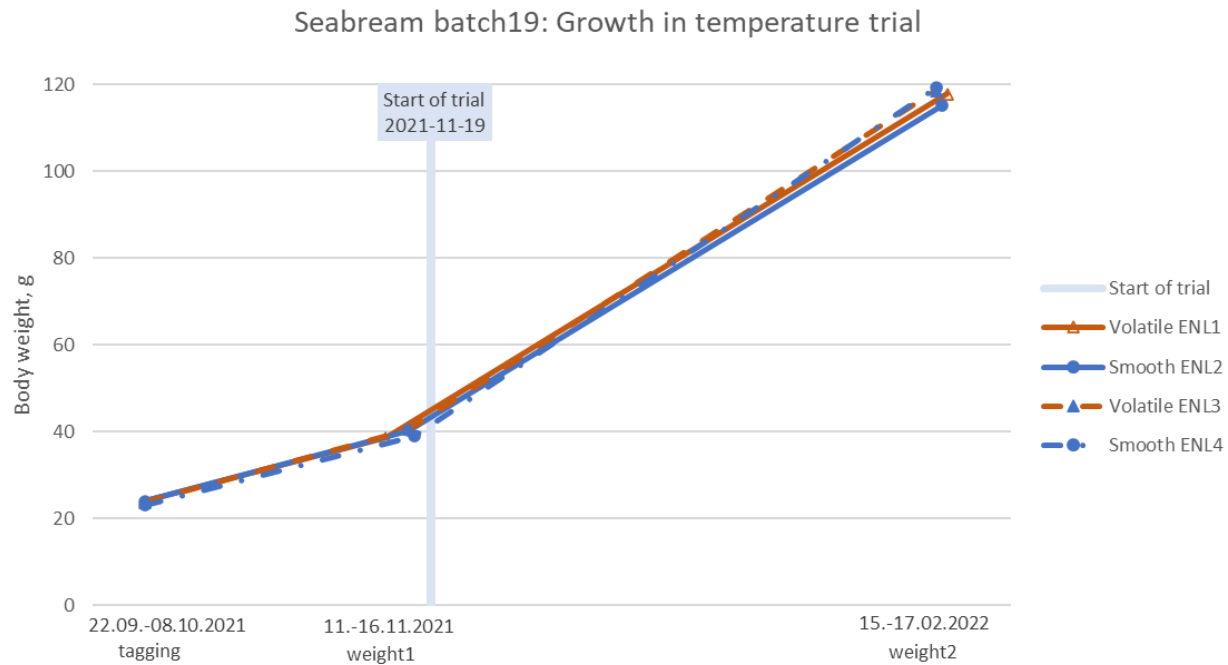
GxE climate in bass and bream

SEA BASS	Weight volatile	Weight smooth
Weight volatile	0.21 ± 0.09	
Weight smooth	0.99 ± 0.04	0.23 ± 0.09

SEA BREAM	Weight volatile	Weight smooth
Weight volatile	0.32 ± 0.10	
Weight smooth	1.01 ± 0.03	0.32 ± 0.09

Validation of selection methods

- Growth selected salmon

- VNN selected seabass

- Growth selected sea bream

- IPN selected rainbow trout

Validate the different selection methods by examining phenotypes of the offspring.

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Growth in salmon: validation

Family average in ENDWT, adjusted for cage, vs prediction parents
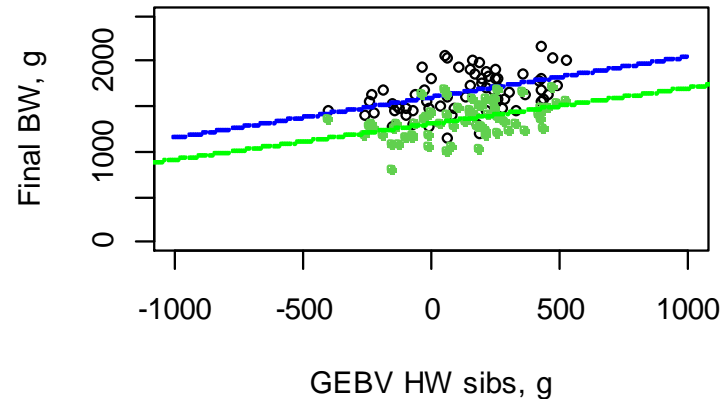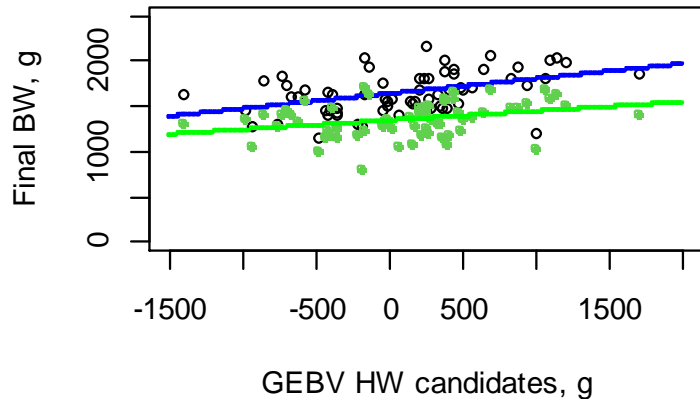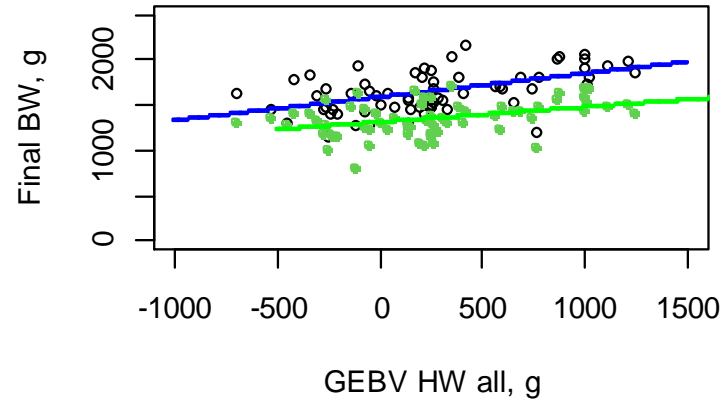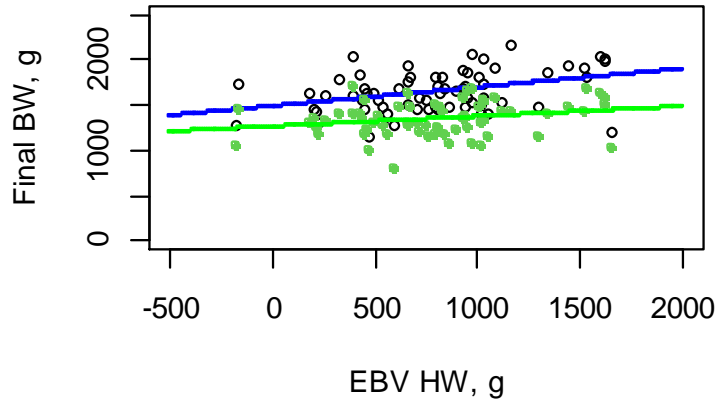
Parents Prediction method	Correlation (r), offspring vs parents	
Feed	**Conv.**	**Innov.**
EBV	0.37**	0.24*
GEBV all	0.52**	0.40**
GEBV cand	0.44**	0.32**
GEBV sibs	0.43**	0.45**

- GEBV prediction gives more accurate ranking of offspring compared to EBV

- GS utilizing information both on candidates and sibs gave most accurate ranking

- More accurate prediction for offspring group for fish fed conventional diet compared to innovative.

* p-value = 0.05
** p-value < 0.01

10% best families

			Conventional feed		Innovative feed	
parents						
Selection criteria	**h² ± se**	**Accuracy***	**7 best fam**	**Rel. to mean**	**7 best fam**	**Rel. to mean**
BLUP EBV (3 sites)	0.27±0.05	0.696	1.78 kg	**+13%**	1.51 kg	**+9%**
GS GEBV_all (3 sites)	0.33±0.03	0.777	1.86 kg	**+18%**	1.59 kg	**+15%**
GS GEBV_cand (1 site)	0.44±0.05	0.768	1.75 kg	**+11%**	1.51 kg	**+9%**
GS GEBV_sibs (1 site)	0.46±0.06	0.519	1.69 kg	**+7%**	1.50 kg	**+9%**

Population mean end weight: **1.58 kg**   **1.38 kg**
Phenotypes 10% best predicted families

- **Realized response growth:**
  - Best with GS utilizing information on both candidates and siblings (totally 3 sites , 4,300 records)
  - GS based on candidates (1 site, ~1,400 rec) and BLUP selection based on total data (3 sites, ~23,800 records) → similar realized response.
  - Lowest for GS based on only siblings (1 site, ~1,300 records)
- Conventional diet gave largest response, except for GS based on sib information, where fish fed innovative diet showed largest response.

VNN in sea bass: validation

- 88 families in challenge test for VNN
  - 2310 individuals (on average 26 individuals/family) with survival data
  - 4 tanks, intramuscular injection of VNN

A: Daily mortality

B: Accumulated mortality
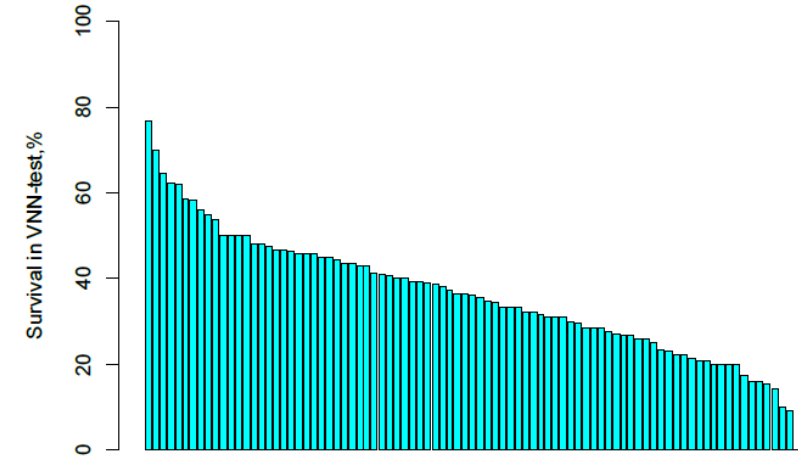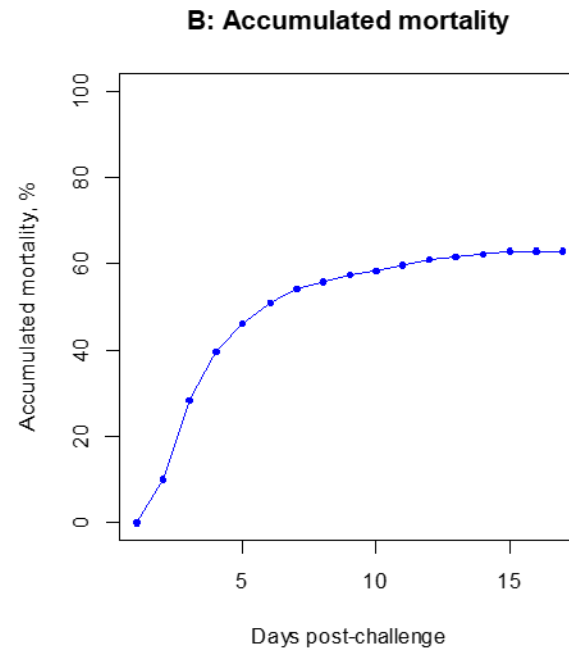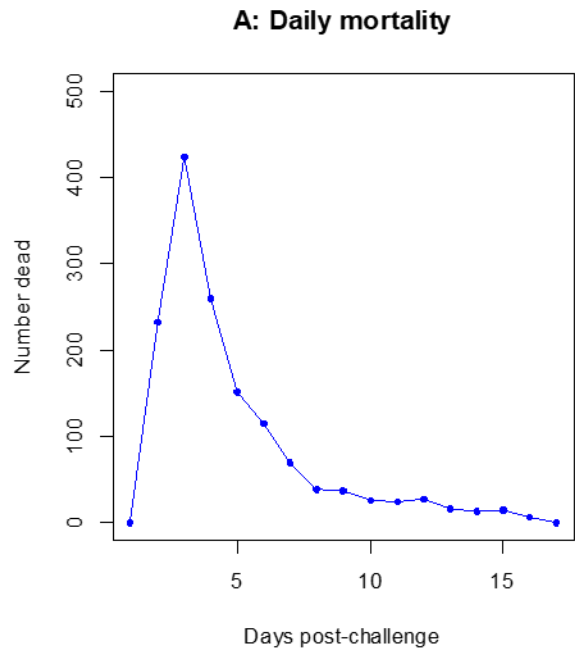
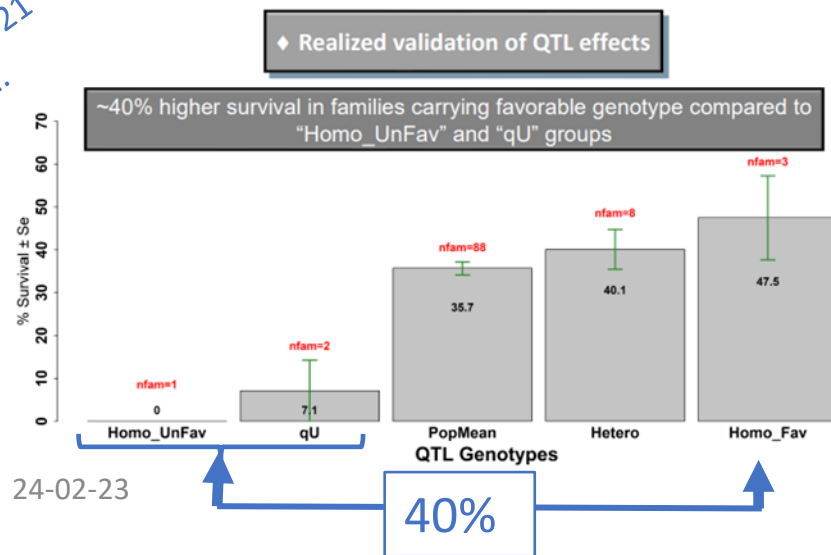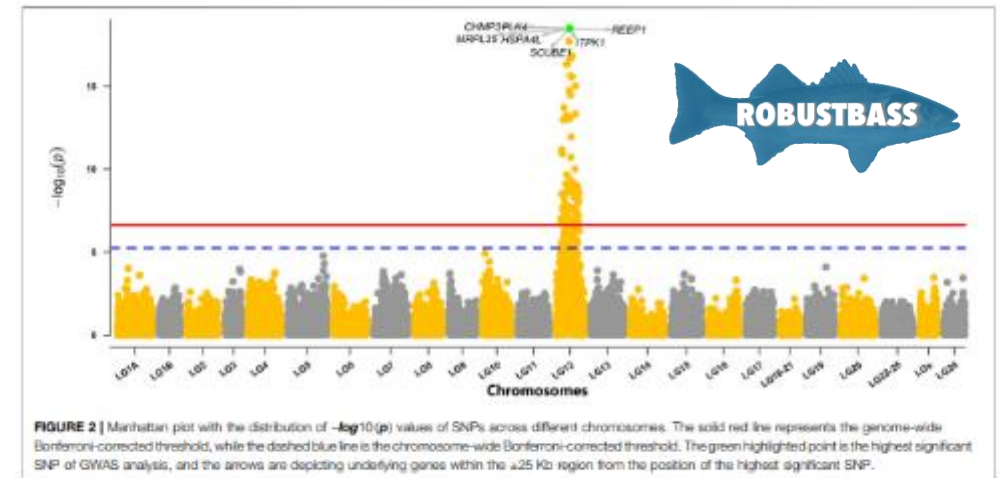|	Genetic parameters	
	h² observed scale	h² liability scale
VNN test: survival, %	0.08 ± 0.03	0.12 ± 0.04

VNN in sea bass: MAS validation

- Pre-selected seabass candidates genotyped on French GeneSea 57K DLabChip array
    - Results from **COFASP** Robustbass used as training-population for VNN **M**arker **A**ssisted **S**election (MAS) and **G**enomic **s**election (GS)
    - MAS: QTL explaining ~33% av genetic variation for VNN resistance detected in Robustbass: 10 SNPs identified (Vela et al. 2022).

- Families with different genotypes produced
    - Offspring results collected from VNN-challenge test batch15.

Genetic Basis for Resistance Against Viral Nervous Necrosis: GWAS and Potential of Genomic Prediction Explored in Farmed European Sea Bass (*Dicentrarchus labrax*)
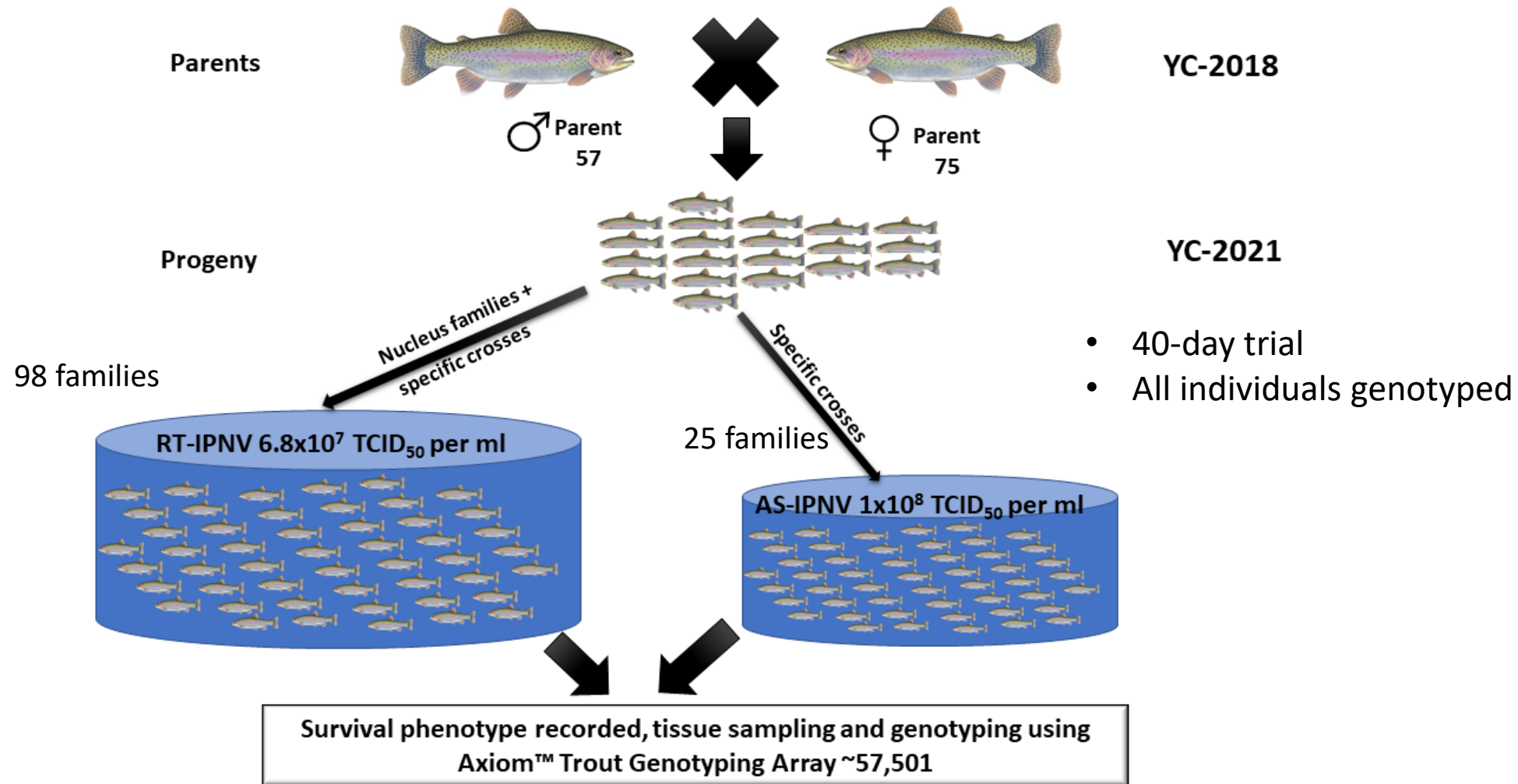
Sergio Vela-Avitúa[1], Ingunn Thorland[1], Vasileios Bakopoulos[2], Kantham Papanna[3], Arkadios Dimitroglou[3], Eleftherios Kottaras[3], Papaharisis Leonidas[3], Bruno Guinand[4], Costas S. Tsigenopoulos[5] and Muhammad L. Aslam[6*]

FIGURE 2 | Manhattan plot with the distribution of –*log*10(*p*) values of SNPs across different chromosomes. The solid red line represents the genome-wide Bonferroni-corrected threshold, while the dashed blue line is the chromosome-wide Bonferroni-corrected threshold. The green highlighted point is the highest significant SNP of GWAS analysis, and the arrows are depicting underlying genes within the ±25 Kb region from the position of the highest significant SNP.
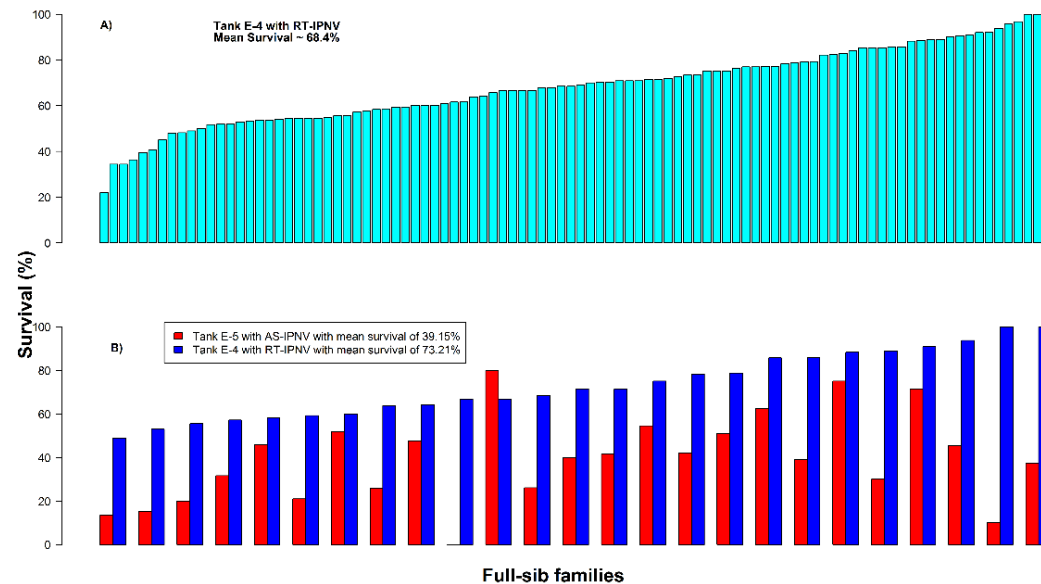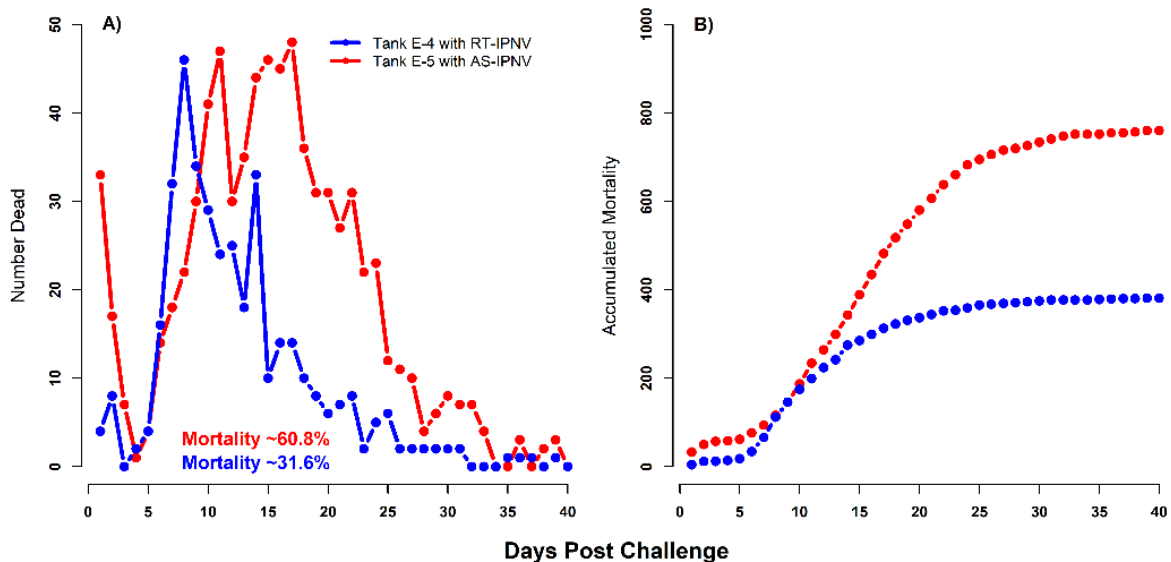

Poster EAS2021 Aslam et al.

Parents re-genotyped with Medaid/Performfish MedFISH 30K SNP-chip
- Offspring from challenge test are genotyped.
- Validation work on going.

24-02-23

IPN in rainbow trout

Parents   **YC-2018**

♂ Parent 57   ♀ Parent 75

Progeny   **YC-2021**

98 families

Nucleus families + specific crosses

RT-IPNV 6.8x10⁷ TCID₅₀ per ml

25 families

Specific crosses

AS-IPNV 1x10⁸ TCID₅₀ per ml

- 40-day trial
- All individuals genotyped

Survival phenotype recorded, tissue sampling and genotyping using Axiom™ Trout Genotyping Array ~57,501

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	Genetic Parameters	
	Observed Scale	Liability Scale
	$h_{sd}^2 \pm se$	$h_{sd}^2 \pm se$
RT-IPNV	0.17 ± 0.04	0.27 ± 0.06
AS-IPNV	0.21 ± 0.09	0.42 ± 0.18

- The genetic correlation between resistance to RT-IPNV and AS-IPNV: 0.96 ± 0.12.

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Main conclusions

- High genetic correlations between growth traits across climates/temperature regimes and across different diets indicate non-significant genotype-by-environment interactions in Atlantic salmon, European sea bass and Gilthead sea bream.
- Independent from the environment/feed growth can be genetically improved by selective breeding in the three species.

- Conventional/commercial diet outperformed the novel diets (insects, algae, plant based) relative to growth of Atlantic salmon, European sea bass and Gilthead sea bream.
- Growth of sea bass or sea bream was not affected by the tested temperature profiles.
- Selection of fatty acid profiles can be implemented in selective breeding for European sea bass.
- VNN resistance in European seabass can be genetically improved using selective breeding.
- Selection for increased growth in European seabass will not have adverse effects on VNN resistance.
- IPN resistance in rainbow trout can be genetically improved using selective breeding.
- Selection for resistance to RT-IPNV (AS-IPNV) will also improve resistance to AS-IPNV (RT-IPNV).
- No conclusive QTLs were detected for growth, fillet fat or fatty acid profiles.
- Progeny performance of bass in VNN challenge comply with the parental genotypes.

Relevance to implementation

- Growth selection in breeding nucleus in the current environmental conditions will be also effective in the changing environment.

- Breeding companies can serve large markets using genetic material from a single breeding program.

- Fish farmers will experience that the expected genetic gain from the nucleus will realize in their production environment.

- Overall resilience regarding climate change and novel feeds is expected to help in resource optimization, and to promote predictable and sustainable aquaculture production, and increased animal welfare in the important European aquaculture species.

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Thank you!