



# Salmon Aquaculture - Case Studies

Using Standard BioTools Microfluidic Technology

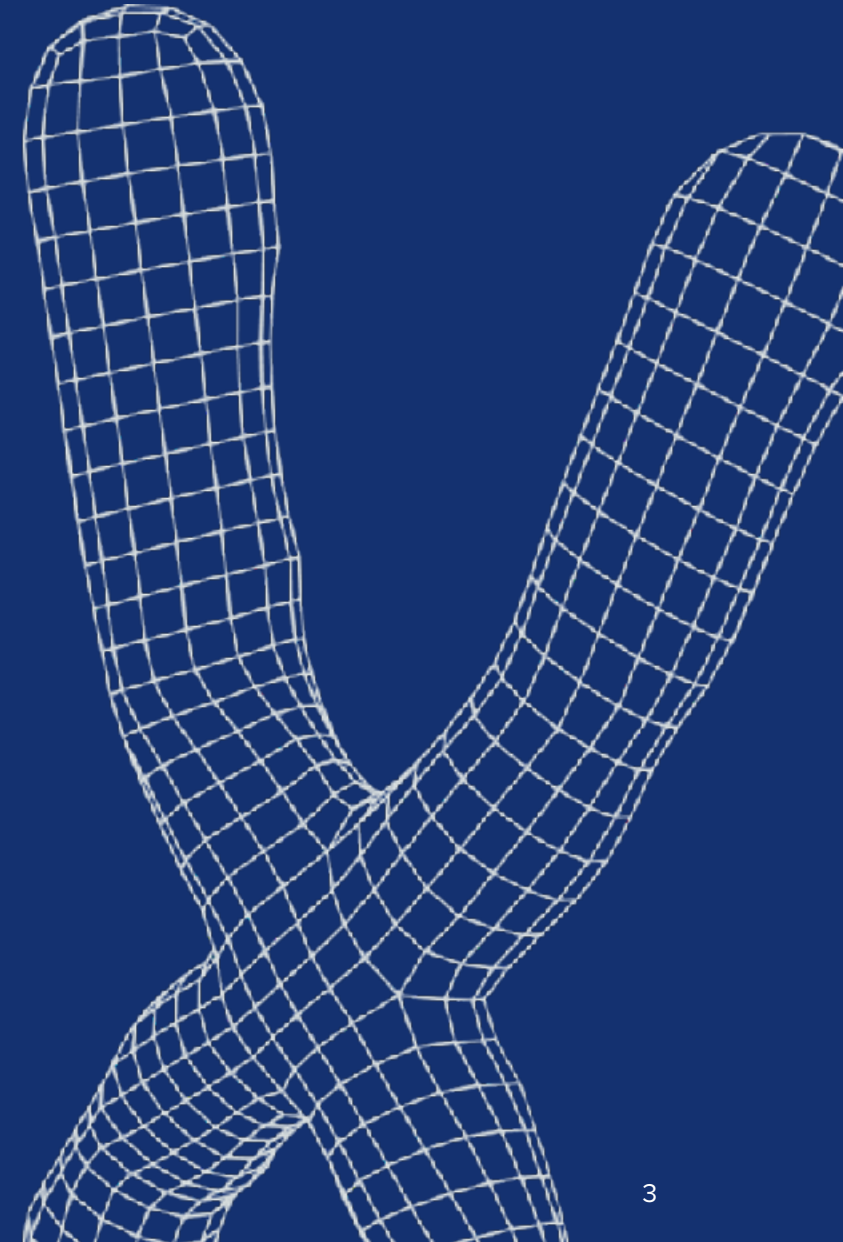
Standard BioTools™  
March 2023

# TABLE OF CONTENTS

1. Gene Expression and Immune Response
2. Gene Expression and Fish Welfare
3. Gene Expression and Epidemiology



# Gene Expression and Fish Welfare



Talk to us

# Gene Expression and Fish Welfare



**Objective:** To examine how moderate hypoxia and incremental temperature increase affects transcript expression in the liver of post-smolts as compared to control conditions

## Utilization of Standard BioTools products:

- Biomark HD System
- 96.96 dynamic arrays

## Conclusions:

- Identified extensive transcriptional changes in stress and immune-related genes in the liver of post-smolt Atlantic salmon when exposed to temperature increase alone, or in combination with moderate hypoxia
- Identified several genes to be used as markers of stress response in post-smolt Atlantic salmon

Beemelmanns, et al. "The Atlantic salmon's stress- and immune-related transcriptional responses to moderate hypoxia, an incremental temperature increase, and these challenges combined". G3 (2021)

# Gene Expression and Fish Welfare

Any further questions?

Talk to us

## Background

- Increasing water temperatures and de-oxygenation of the oceans as a result of global warming may negatively impact the overall health of farmed Atlantic salmon.
- Few studies have looked at the combined effects of hypoxia and incremental temperature change on gene expression in salmonids.
- Authors measured gene expression of various stress and immune markers in the liver, which was selected for its role in stress response, nutrient metabolism, and immunity



Beemelmanns, et al. "The Atlantic salmon's stress- and immune-related transcriptional responses to moderate hypoxia, an incremental temperature increase, and these challenges combined". G3 (2021)

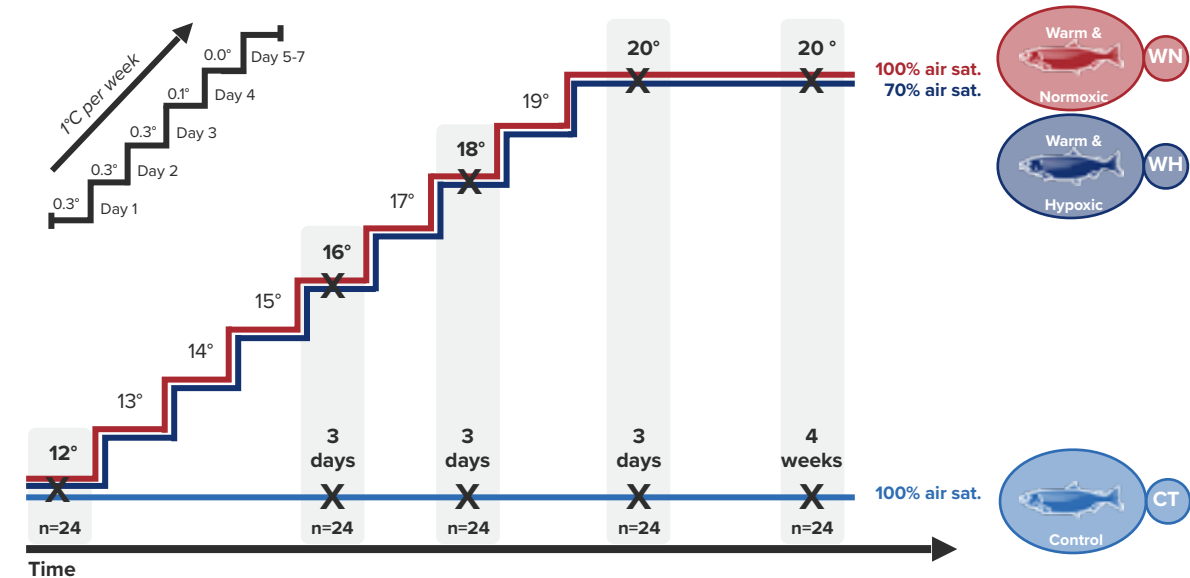
# Gene Expression and Fish Welfare

Any further questions?

Talk to us

## Methods

- Total RNA extracted from flash frozen liver tissue of 360 Atlantic salmon subjected to Standard BioTools reverse transcription, preamplification, and data collection workflows.
- Data collected using the 96.96 GE IFC on the Biomark HD.
- 41 gene target panel included genes related to the following categories:
  - Heat shock response
  - Stress response
  - Oxidative stress response
  - Cellular metabolism
  - Immune response
  - Transcriptional regulation (methylation)



**Figure 1** Schematic diagram of the experimental design. Post-smolt Atlantic salmon were either subjected to: (i) a constant water temperature of 12°C and normoxia (100% air saturation) (Control, CT); (ii) a temperature increase from 12°C to 20°C under normoxia (100% air sat.) (Warm & Normoxic, WN); or (iii) moderate hypoxia (~70% air sat.) and then the incremental increase to 20°C (Warm & Hypoxic, WH). The water temperature was gradually increased by 1°C week<sup>-1</sup> using the following regimen: 1<sup>st</sup> day, +0.3°C; 2<sup>nd</sup> day, +0.3°C; 3<sup>rd</sup> day, +0.3°C; 4<sup>th</sup> day, +0.1°C; 5<sup>th</sup> – 7<sup>th</sup> days no increase in temperature (see upper-left portion of the figure). The sampling of liver tissue was performed initially at 12°C, then 3 days after reaching 16°C, 18°C and 20°C, and 4 weeks after the temperature reached 20°C (20°C-4wks) (n = 8 per treatment/temperature, N = 120 total). The transcriptional responses in the liver of these fish were assessed by measuring the transcript expression of 45 biomarker genes related to the heat shock response, oxidative stress, apoptosis, immunity, metabolism and epigenetics using qPCR (Fluidigm Biomark™ HD).

Beemelmanns, et al. "The Atlantic salmon's stress- and immune-related transcriptional responses to moderate hypoxia, an incremental temperature increase, and these challenges combined". G3 (2021)

# Gene Expression and Fish Welfare

Any further questions?

Talk to us

## Results and conclusions

### Results:

- High temperature initiates immune-related transcript expression changes in the liver
- Moderate hypoxia at 12°C impacts hypoxia and stress related transcript expression, but not immune related genes
- Immune transcript expression at 4 weeks was less impacted as compared to the initial 3-day exposure
- Persistent changes observed in epigenetic markers that facilitate thermal acclimation responses

### Conclusions:

- Identified several genes that can be used as biomarkers to characterize transcriptional stress response

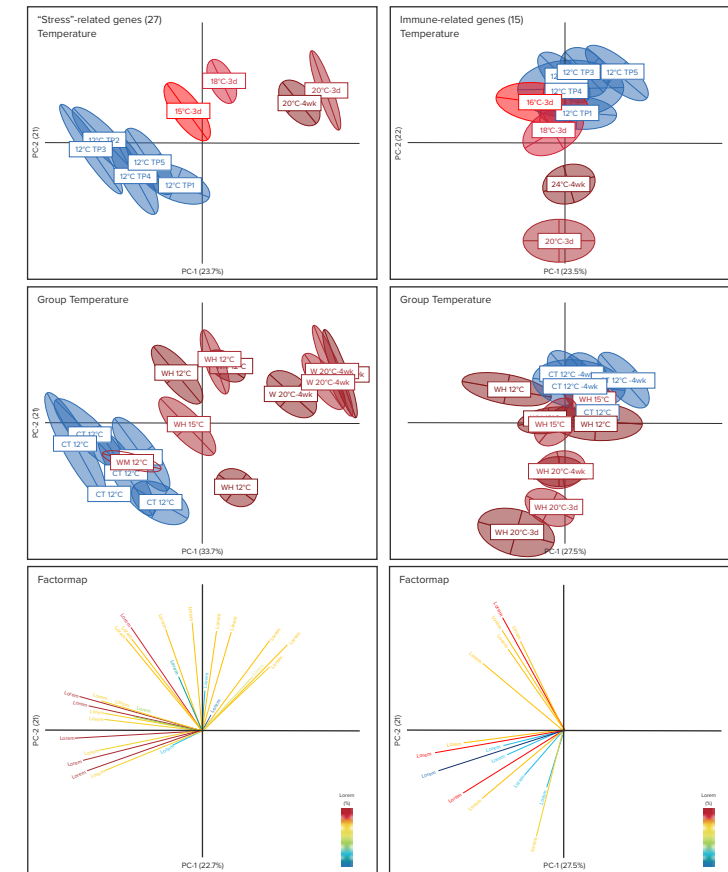
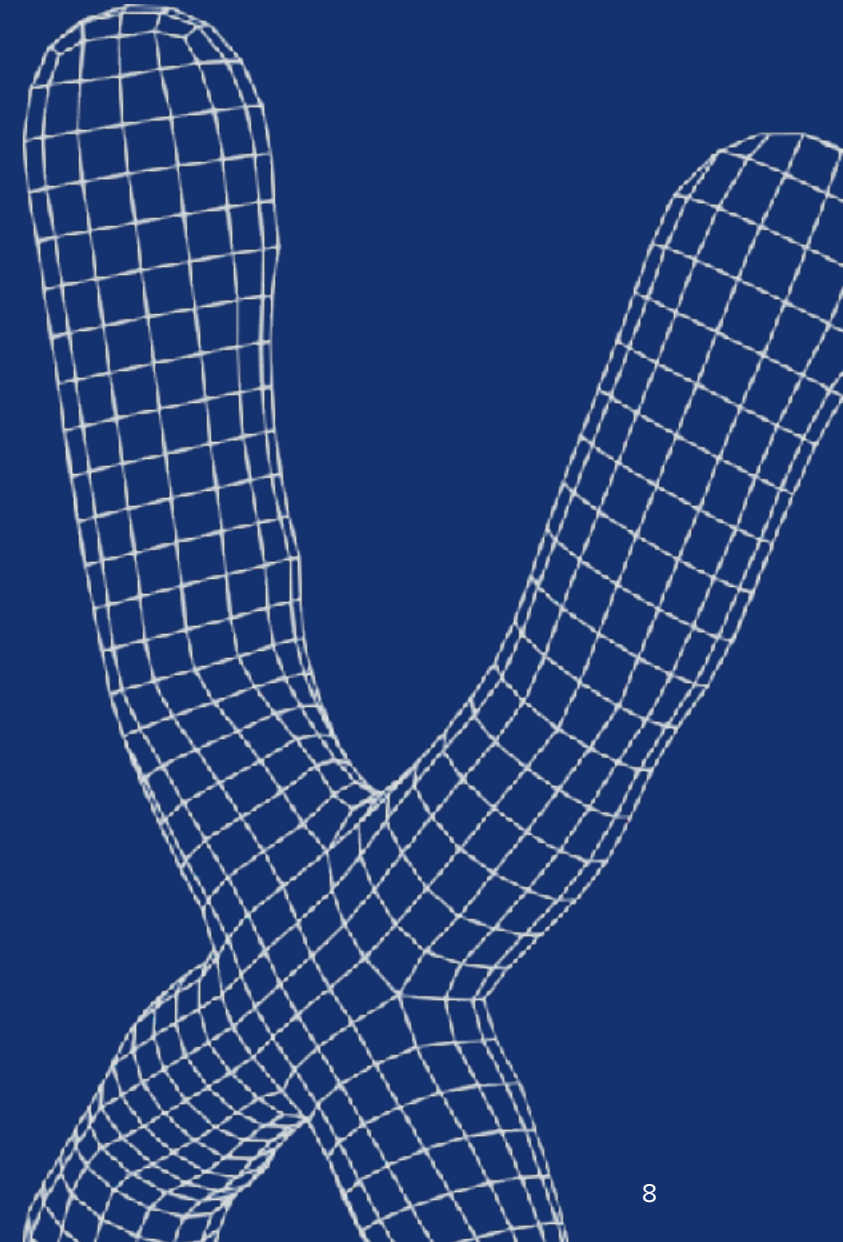


Figure 2: Transcriptional responses of stress- and immune-related genes in Atlantic salmon liver

Beemelmanns, et al. "The Atlantic salmon's stress- and immune-related transcriptional responses to moderate hypoxia, an incremental temperature increase, and these challenges combined". G3 (2021)



# Gene Expression and Immune Response





# Gene Expression and Immune Response



**Objective:** To explore the role of antimicrobial peptides  $\beta$ -defensins and cathelicidins in *N. perurans*, which causes amoebic gill disease

## Utilization of Standard BioTools products:

- Biomark HD System
- 48.48 dynamic arrays

## Conclusions:

- Biomark HD shows  $\beta$ -defensins -3 and -4 and cathelicidin 2 were upregulated in gill of parasite-challenged salmon
- Indicate potential novel roles in innate immune responses to *N. perurans*

McGrath, et al. "Antimicrobial peptide gene expression in Atlantic salmon (*Salmo salar*) seven days post-challenge with *Neoparamoeba perurans*". *Developmental and Comparative Immunology* (2022)

# Gene Expression and Immune Response

Any further questions?

Talk to us

## Background

- Amoebic gill disease (AGD) is an infection of Atlantic salmon caused by *N. perurans*, and is a significant contributing factor to fish mortality
- Antimicrobial peptides (AMP)  $\beta$ -defensins and cathelicidins, mediators of early immune response, have not yet been evaluated in relation to AGD.
- To assess the functional role that these genes play in local and systemic responses to infection, the authors measured gene expression in swim bladder, gill, and intestine from healthy and infected salmon



McGrath, et al. "Antimicrobial peptide gene expression in Atlantic salmon (*Salmo salar*) seven days post-challenge with *Neoparamoeba perurans*". *Developmental and Comparative Immunology* (2022)

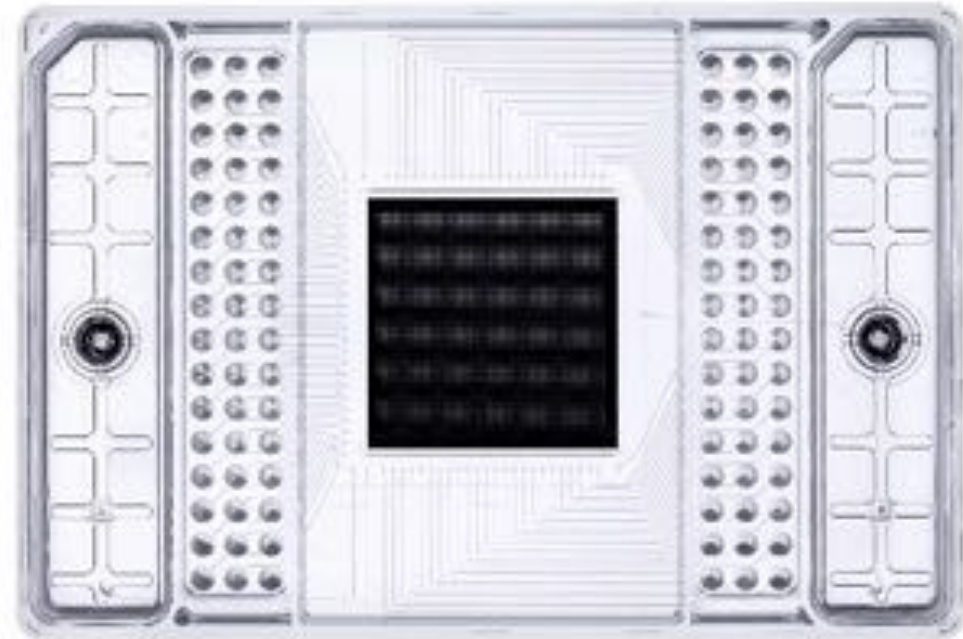
# Gene Expression and Immune Response

Any further questions?

Talk to us

## Methods

- Total RNA was extracted from swim bladder (n=12), whole intestine (n=12), and whole gill (n=12) in control and infected salmon.
- Standard BioTools workflows for reverse transcription, preamplification, and data analysis were followed.
- Data collected using the 48.48 GE IFC on the Biomark HD.
- Housekeeping genes elongation factor 1 $\alpha$  (EF1 $\alpha$ ) and elongation factor 1 $\beta$  (EF1 $\beta$ ) were selected as they are stable across a range of organs in Atlantic salmon.



McGrath, et al. "Antimicrobial peptide gene expression in Atlantic salmon (*Salmo salar*) seven days post-challenge with *Neoparamoeba perurans*". *Developmental and Comparative Immunology* (2022)

# Gene Expression and Immune Response

Any further questions?

Talk to us

## Results and conclusions

### Results:

- In infected salmon,  $\beta$ -defensin and cathelicidin expression was upregulated in the gill in response to early phase of parasitic infection, before clinical presentation was observed
- Upregulation not observed in infected intestine and swim bladder, suggesting there is just a localized immune response in the gill at early stages of infection
- $\beta$ -defensin and cathelicidin expression found in healthy swim bladder for the first time

### Conclusions:

- Atlantic salmon AMP genes play a novel role in early asymptomatic innate immune responses to the parasite *N. perurans*
- $\beta$ -defensin and cathelicidin expression confirmed in both healthy and infected Atlantic salmon
- Swim bladder (whose ancillary functions are largely unexplored) may have a novel immune role

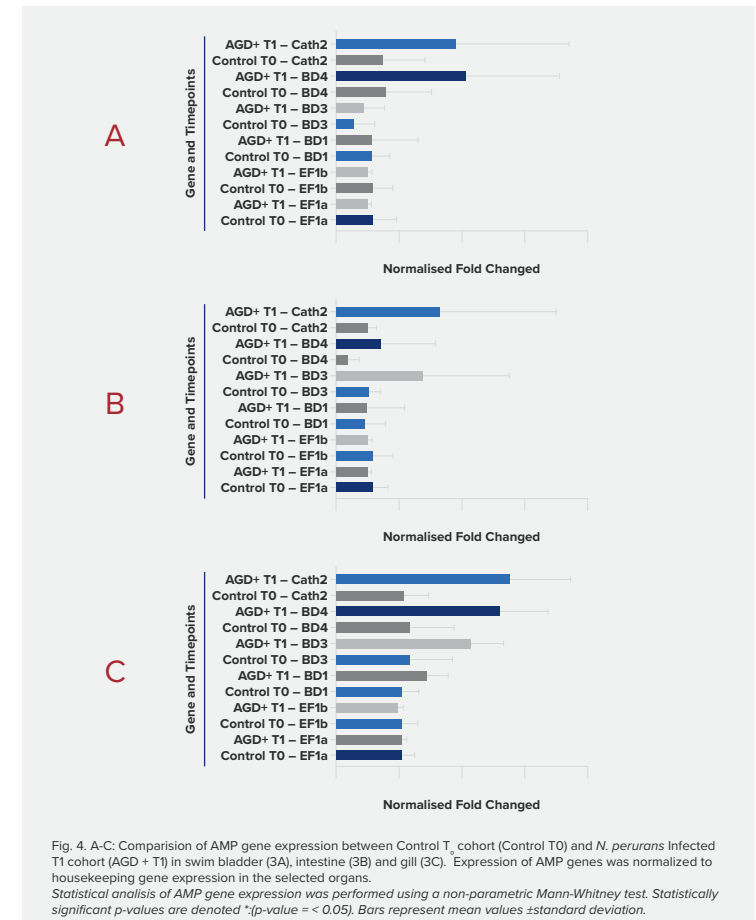
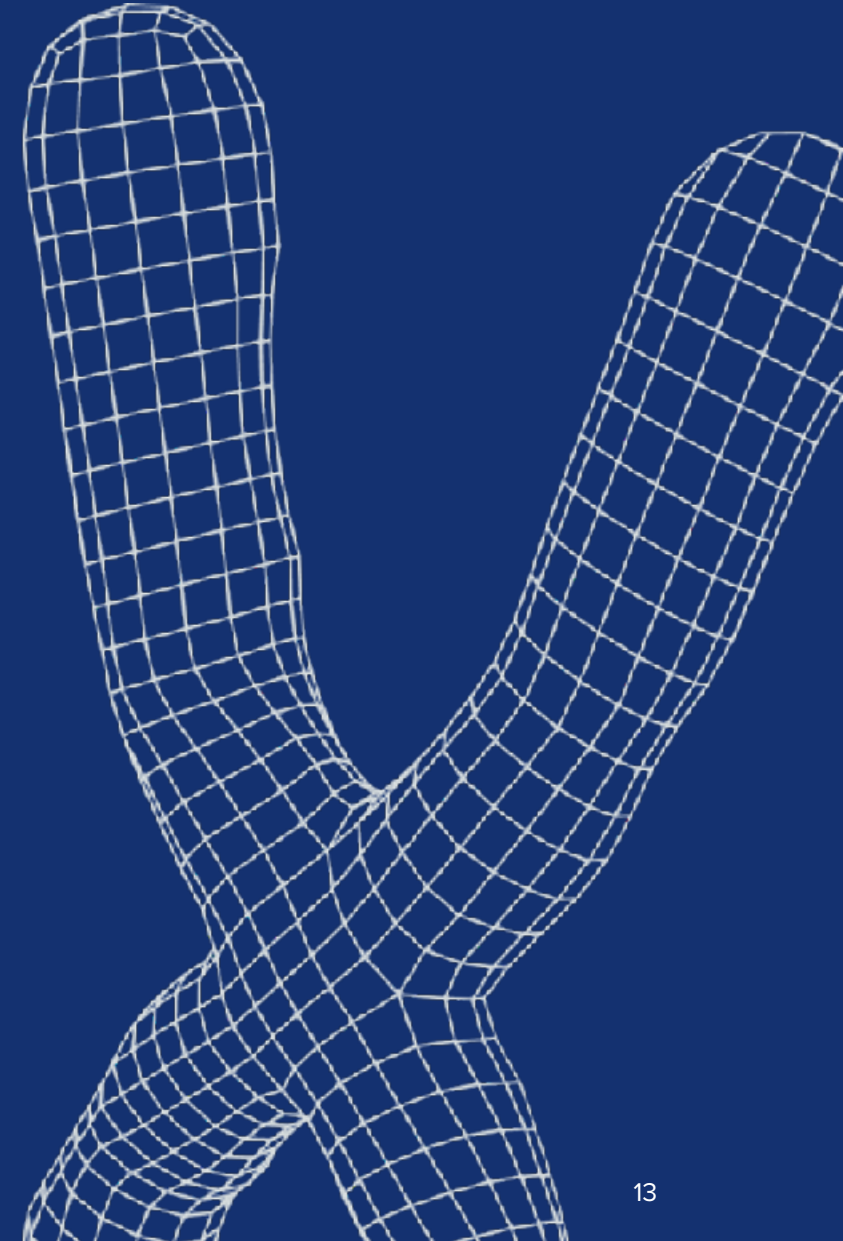


Fig. 4. A-C: Comparison of AMP gene expression between Control T<sub>0</sub> cohort (Control T<sub>0</sub>) and *N. perurans* Infected T<sub>1</sub> cohort (AGD + T<sub>1</sub>) in swim bladder (3A), intestine (3B) and gill (3C). Expression of AMP genes was normalized to housekeeping gene expression in the selected organs. Statistical analysis of AMP gene expression was performed using a non-parametric Mann-Whitney test. Statistically significant p-values are denoted \* (p-value < 0.05). Bars represent mean values  $\pm$  standard deviation.



# Gene Expression and Epidemiology





# Gene Expression and Epidemiology

Any further questions?

Talk to us

## Background

- Piscine orthoreovirus (PRV) causes moderate to severe pathological changes in salmon, endangering wild populations and resulting in downstream effects in the ecosystem.
- The introduction and origin of PRV-1 into wild salmon populations is unclear, thought to potentially be from farmed fish/aquaculture.
- The authors analyzed viral genomes to help determine the evolutionary history of PRV and quantify transmission dynamics between farm and wild salmon populations.



Mordecai, et al. "Aquaculture mediates global transmission of a viral pathogen to wild salmon". *Science Advances* (2021)

# Gene Expression and Epidemiology

Any further questions?

Talk to us

## Methods

- Data collected using the 96.96 GE IFC on the Biomark HD, to quantify the presence and relative load of viral PRV RNA. (Chinook n=6791, coho n=2165, sockeye n=4140)
- Samples were sequenced with NGS
- Phylogenetic analyses were performed to track the evolution of PRV-1 in wild and aquaculture groups
- PRV epidemiological modeling performed to track PRV prevalence in aquaculture and describe the distance between wild salmon vs aquaculture

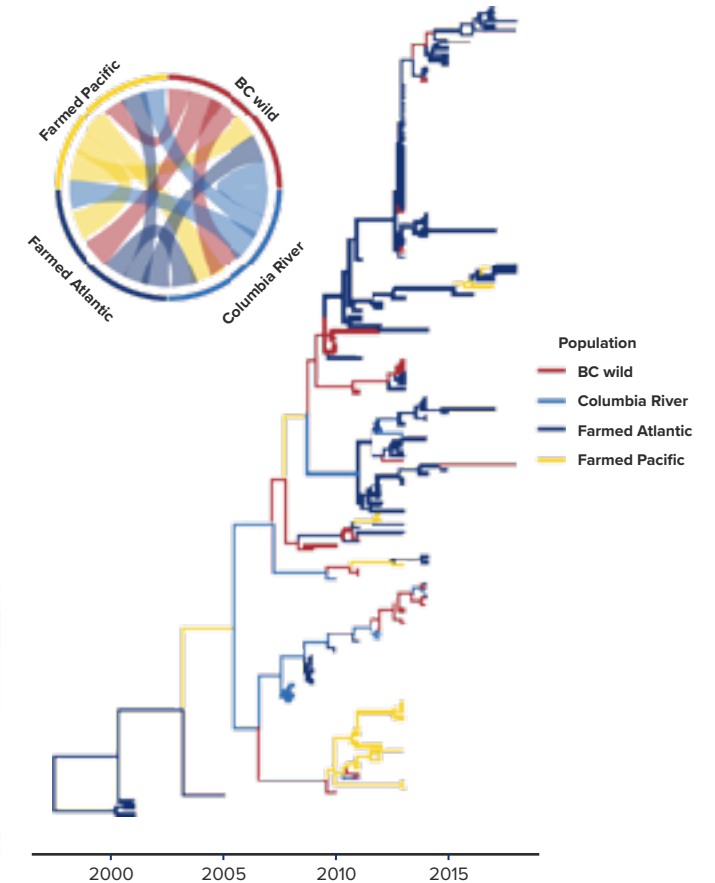
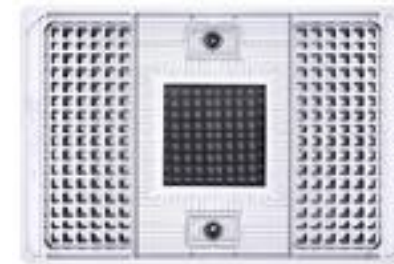


Fig.4. Phylogenetic evidence of transmission between populations. Maximum

Mordecai, et al. "Aquaculture mediates global transmission of a viral pathogen to wild salmon". *Science Advances* (2021)



# Gene Expression and Epidemiology

Any further questions?

Talk to us

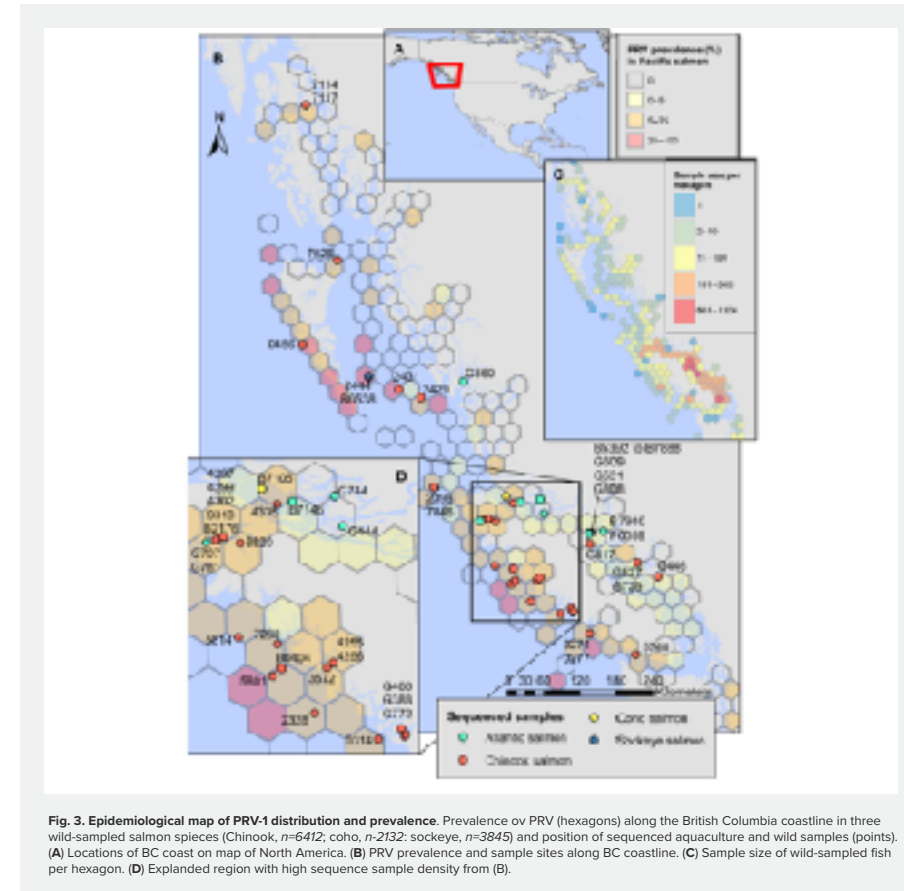
## Results and conclusions

### Results:

- Origin of PRV-1 in the NE Pacific suggested to have diverged from a strain in the Atlantic in 1989
- PRV-1 prevalence in wild Chinook salmon was closely tied to farm proximity, suggesting transmission from farm to wild
- Phylogenetic reconstruction showed that wild and farmed salmon shared clades, indicating PRV-1 transmission

### Conclusions:

- Freshwater hatcheries are a source of PRV transmission to wild salmon populations
- Standard BioTools IFC enabled high-throughput pathogen detection in thousands of samples, providing a fast, cost-effective solution over traditional qPCR and sequencing methods.



Mordecai, et al. "Aquaculture mediates global transmission of a viral pathogen to wild salmon". *Science Advances* (2021)

# Modern aquaculture is powered by X9

Any questions?

**Talk to us**

[education.team@standardbio.com](mailto:education.team@standardbio.com)

[www.standardbiotools.com/x9](http://www.standardbiotools.com/x9)





# Trout Aquaculture - Case Studies


Using Standard BioTools Microfluidic Technology

Standard BioTools™  
March 2023

# TABLE OF CONTENTS

1. SNP Genotyping for parentage
2. Assessment of SNP genotyping for genetic differentiation
3. Gene expression and fish welfare

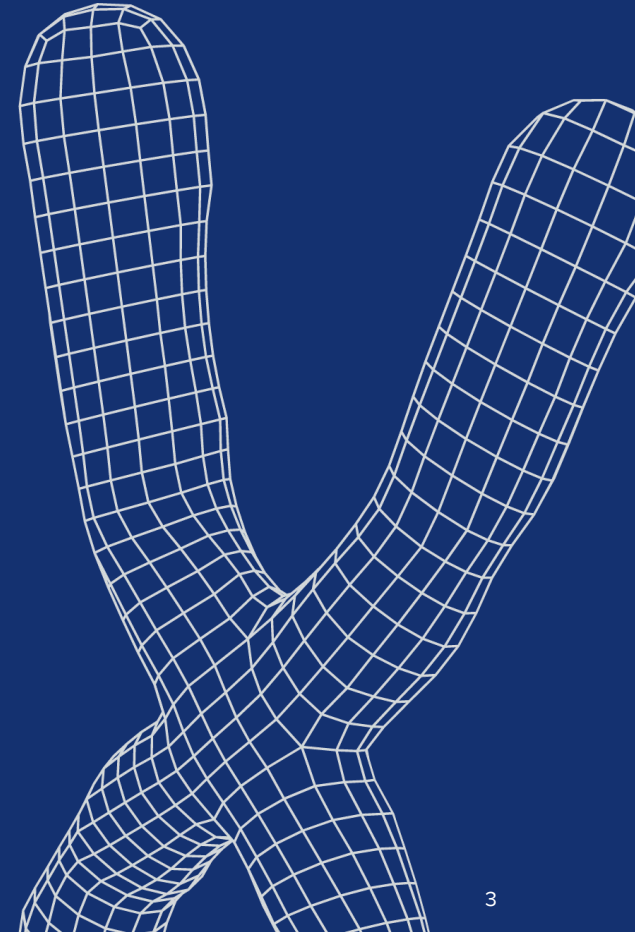




# SNP genotyping for parentage

Any questions?

[Talk to us](#)



# SNP Panel for Parentage of Rainbow Trout

Any further question?

Talk to us

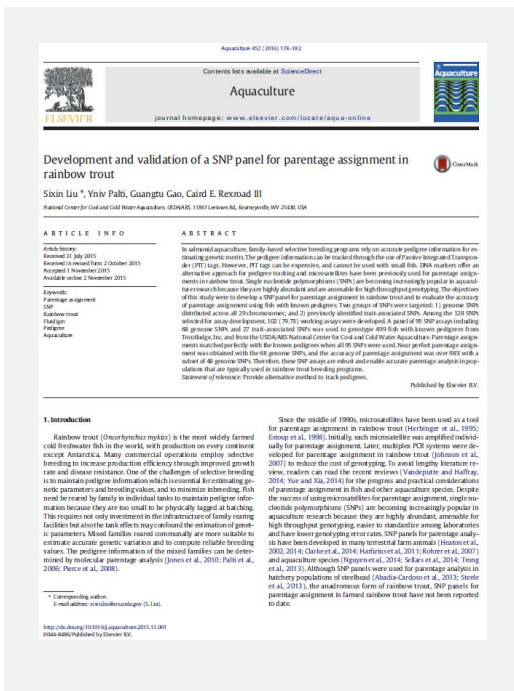
**Objective:** Develop and assess SNP genotyping panel for parentage assignment of rainbow trout.

## Utilization of Standard BioTools products:

- EP1 System
- IFC Controller HX
- 96.96 dynamic arrays

## Conclusion:

The SNP panel has sufficient power for genetic assignment of commercial rainbow trout strains used from Troutlodge in this study.



[Liu, et al. Development and validation of a SNP panel for parentage assignment in rainbow trout Aquaculture \(2016\)](#)

# SNP Panel for Parentage of Rainbow Trout

Any further question?

Talk to us

## Background

Selective breeding programs must maintain pedigree information to help increase production efficiency.

- Estimate genetic parameters & breeding values.
- Minimize inbreeding

## Single Nucleotide Polymorphisms (SNPs) assays:

- High-throughput
- Easy to standardize
- Low error rates
- Proven in terrestrial animals for parentage assignment
- 60-100 SNPs adequate for accurate large scale parentage assignment



[Liu, et al. Development and validation of a SNP panel for parentage assignment in rainbow trout Aquaculture \(2016\)](#)

# SNP Panel for Parentage of Rainbow Trout

Any further question?

Talk to us

## Methods

### Lab:

Fin clip samples

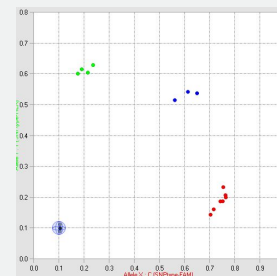
- Offspring previously genotyped on 57K array
- DNA concentration >30ng/uL

### SNP Type assays

- SNPs across 29 chromosomes, MAF > 0.45
- Included additional trait-associated SNPs (stress, growth, disease resistance)
- 128 SNP assay designed, 95 selected for panel

Default SNP Type assay protocol in 96.96 GT Dynamic Array with STA

### Data analysis:



### Genotype calling

- SBIO software

### Parentage analysis

- CERVUS
- SNPPIT

**Table 1**

Rainbow trout with known pedigrees used to evaluate the accuracy of parentage assignment.

Group	No. of families	No. of offspring	No. of dams	No. of sires
NCCCWA	10	60	17	13
Troutlodge	48	192	145	72
Total	58	252	162	85

[Liu, et al. Development and validation of a SNP panel for parentage assignment in rainbow trout Aquaculture \(2016\)](#)



# Results and conclusions

Any further question?

Talk to us

## Offspring to parent matching:

- All 95 SNPs = 100% accuracy
- 48 SNPs = 98% accuracy
- 36 SNPs = 92% accuracy
- Accuracy consistent with prior research

## Conclusion

- SNP assays compatible for parentage assessment, low-cost, high-throughput, flexible assay choice to include trait-associated SNPs for rainbow trout populations studied.

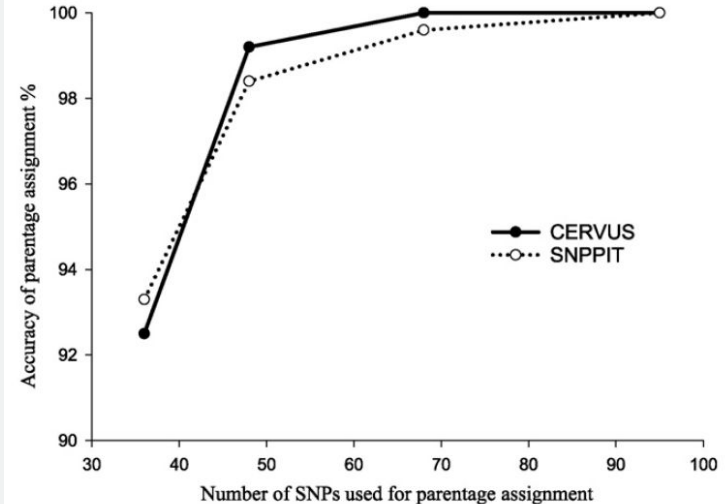


Fig. 1. Accuracy of parentage assignment with different numbers of SNPs.

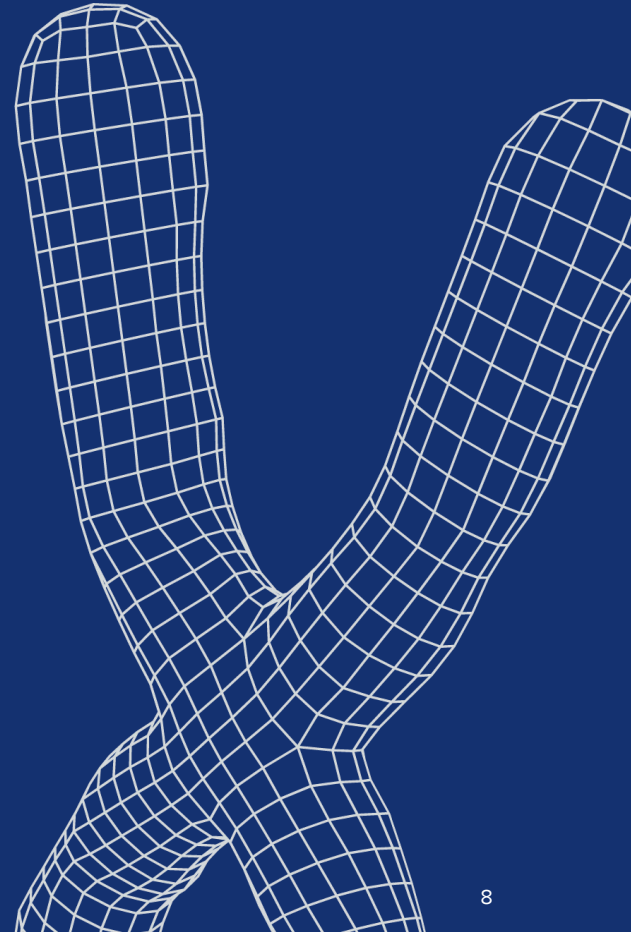
[Liu, et al. Development and validation of a SNP panel for parentage assignment in rainbow trout Aquaculture \(2016\)](#)



# Assessment of SNP genotyping for genetic differentiation

Any questions?

[Talk to us](#)



# Assessment of SNP genotyping for genetic differentiation

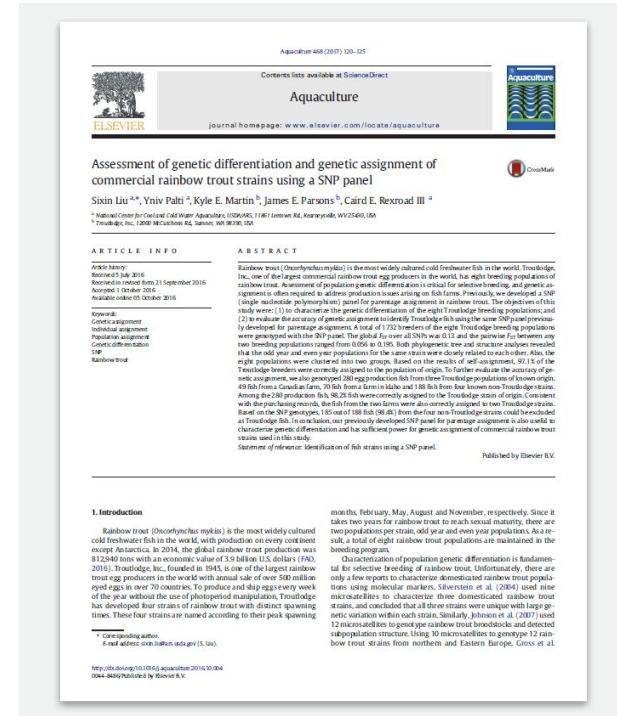
**Objective:** Characterize genetic differentiation of commercial rainbow trout strains in breeders and population fish.

## Utilization of Standard BioTools products:

- EP1 System
- IFC Controller HX
- 96.96 dynamic arrays

## Conclusions:

- 97.1% correct self-assignment to eight breeder populations.
- 98.2% correct assignment of production fish.



[Liu, et al. Assessment of genetic differentiation and genetic assignment of commercial rainbow trout strains using a SNP panel Aquaculture \(2017\)](#)

# Assessment of SNP genotyping for genetic differentiation

Any further question?

Talk to us

## Background and methods

Utilized rainbow trout SNP panel from Liu et al 2016 and EP1 System

### Objectives of the study:

- Characterize eight Troutlodge breeding populations
- Evaluate accuracy of genetic assignment of the eight populations using the SNP panel

2012 Rainbow Trout

**Table 1**

Fish samples used in this study. The strains of Troutlodge breeders and production fish are coded by the last letter of the population names, which are prefixed with two-digit or four-digit of year-class of the populations.

Group	Population	Strain	No. of fish	Source
Breeders	10TLUA	August	216	Troutlodge
	10TLUN	November	234	Troutlodge
	11TLUF	February	216	Troutlodge
	11TLUM	May	217	Troutlodge
	11TLUA	August	213	Troutlodge
	11TLUN	November	234	Troutlodge
	12TLUF	February	196	Troutlodge
	12TLUM	May	206	Troutlodge
Production fish	2012TLUN	November	94	Troutlodge
	2013TLUN	November	92	Troutlodge
	2015TLUF	February	94	Troutlodge
Farmed fish	Farm 1		49	Canada
	Farm 2		70	Idaho, USA
Non-Troutlodge fish	UW		47	NCCCWA
	Shasta		47	NCCCWA
	European 1		47	Europe
	European 2		47	Europe

UW: University of Washington; NCCCWA: National Center for Cool and Cold Water Aquaculture.

[Liu, et al. Assessment of genetic differentiation and genetic assignment of commercial rainbow trout strains using a SNP panel Aquaculture \(2017\)](#)

# Assessment of SNP genotyping for genetic differentiation

Any further question?

Talk to us

Table 3

Results of self-assignment of the eight Troutlodge breeding populations. Numbers in bold represent the number of fish assigned to the correct populations.

Population	11TLUF	12TLUF	11TLUM	12TLUM	10TLUA	11TLUA	10TLUN	11TLUN	Correctly assigned
11TLUF	<b>216</b>								100.0%
12TLUF	6	<b>188</b>	1	1					95.9%
11TLUM	3		<b>200</b>	14					92.2%
12TLUM			1	<b>205</b>					99.5%
10TLUA					215	1			99.5%
11TLUA					5	<b>207</b>	1		97.2%
10TLUN	1						<b>233</b>		99.6%
11TLUN	3				2		12	<b>217</b>	92.7%
Overall									97.1%

## Results and conclusions

- 97.1% correct assignment of breeders to self-assigned populations.
- 98.2% of known origin production fish assigned to correct breeding populations.
- CN farm fish assigned to Troutlodge breeding populations which matched purchasing records.
- 98.4% of non-Troutlodge fish excluded as Troutlodge fish.

**Confirmed SNP panel for parentage assignment and the panel has sufficient power for genetic assignment of commercial rainbow trout strains used.**

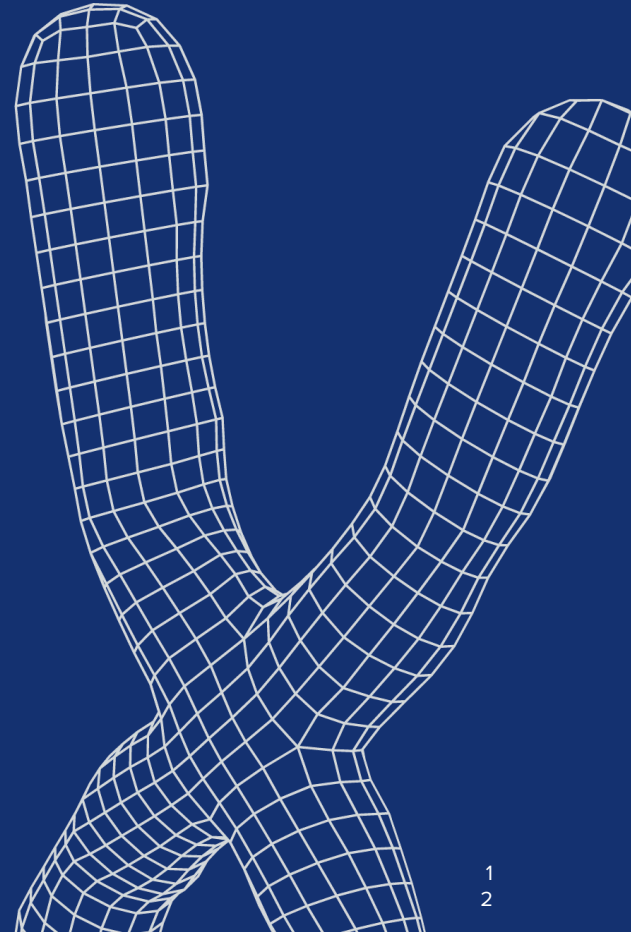
[Liu, et al. Assessment of genetic differentiation and genetic assignment of commercial rainbow trout strains using a SNP panel Aquaculture \(2017\)](#)



# Gene expression and fish welfare

Any questions?

[Talk to us](#)



# Gene Expression Profiling And Fish Welfare

Any further question?

Talk to us

**Objective:** Deeper insight into stress and immune response influenced by different rearing conditions.

## Utilization of Standard BioTools products:

- Biomark HD
- IFC Controller MX
- 48.48 dynamic arrays

## Conclusion:

Stocking density and total water exchange has large impact on gene expression and welfare of fish.



Weirup, et al. "Gene expression profiling supports the welfare evaluation of rainbow trout (*Oncorhynchus mykiss*) reared under different environmental and management conditions in six commercial flow through systems" *Aquaculture* (2022)



Standard BioTools

# Background

Any further question?

Talk to us

**Species-appropriate treatment of fish is important for welfare of fish in farms.**

Welfare measured by stressors in this study:

- Impact health, reproduction, and mortality.
- Elicit specific phenotypic responses and transcript level responses.
- Distinct transcriptional signatures exist for challenging conditions and stress.



[Weirup, et al. "Gene expression profiling supports the welfare evaluation of rainbow trout \(\*Oncorhynchus mykiss\*\) reared under different environmental and management conditions in six commercial flow through systems" Aquaculture \(2022\)](#)



# Methods

Any further question?

Talk to us



## Many environmental and management conditions including:

- Water supply, exchange, temperature, oxygen, pH, bacterial load
- Minerals (ammonium, ammonia, nitrite, nitrate)
- Behavior & external morphological damage

## Gene expression:

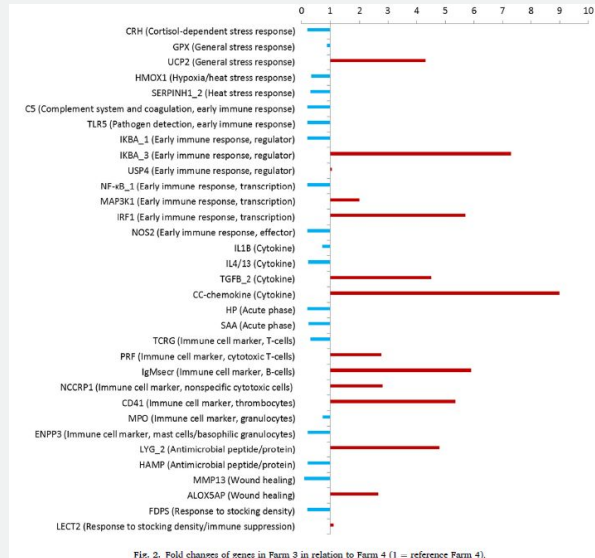
- Blood samples
- 3 HK genes + 91 target genes selected based on prior studies
- MX IFC controller and Biomark HD

## Farms selected:

- Using flow through systems
- Characteristic differences in rearing conditions
- 10 fish per farm

[Weirup, et al. "Gene expression profiling supports the welfare evaluation of rainbow trout \(\*Oncorhynchus mykiss\*\) reared under different environmental and management conditions in six commercial flow through systems" Aquaculture \(2022\)](#)

# Results And Conclusions



Largest differences in fish welfare observed between Farm 3 and Farm 4 (reference) as evidenced by differentially expressed genes.

Significant differences were found in gene expression data between farms which complement the findings on:

- Histology
- Glucose and lactate concentrations
- Organ somatic indices
- External damage
- Water quality
- Environmental and management conditions

# Modern aquaculture is powered by X9

Any questions?

[Talk to us](#)

[education.team@standardbio.com](mailto:education.team@standardbio.com)

[www.standardbiotools.com/x9](http://www.standardbiotools.com/x9)

