

Transcriptional expression changes Analysis during Atlantic Salmon (*Salmo salar*) Smoltification process through bioinformatics tools

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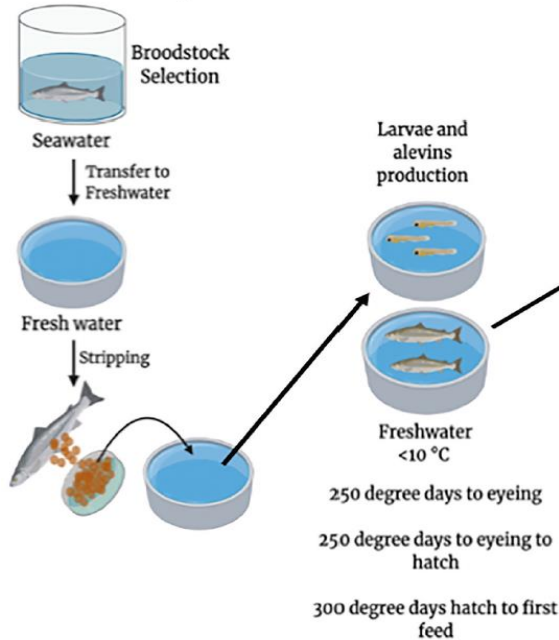
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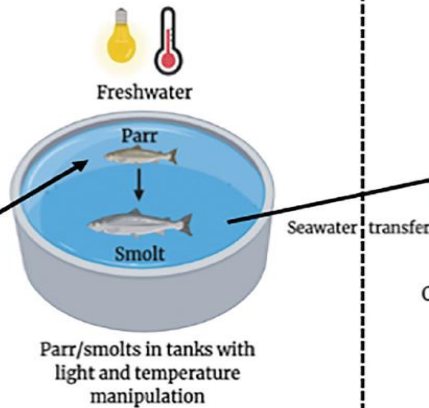
Pharmacology and Morphophysiology Institute,
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Introduction

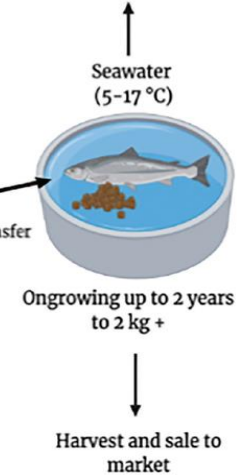
a) Production of brood stock, roe, and fry in freshwater



b) Production of smolts (smoltification)

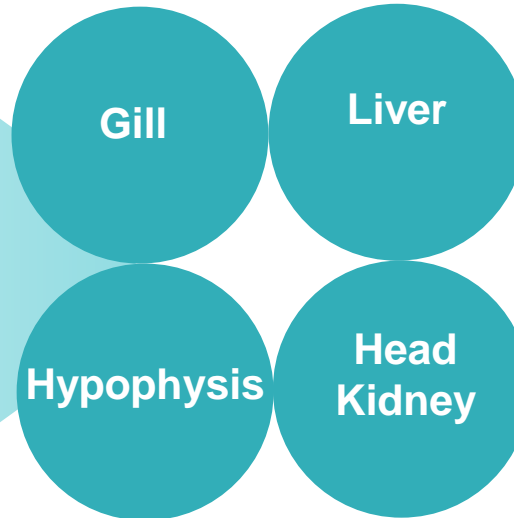
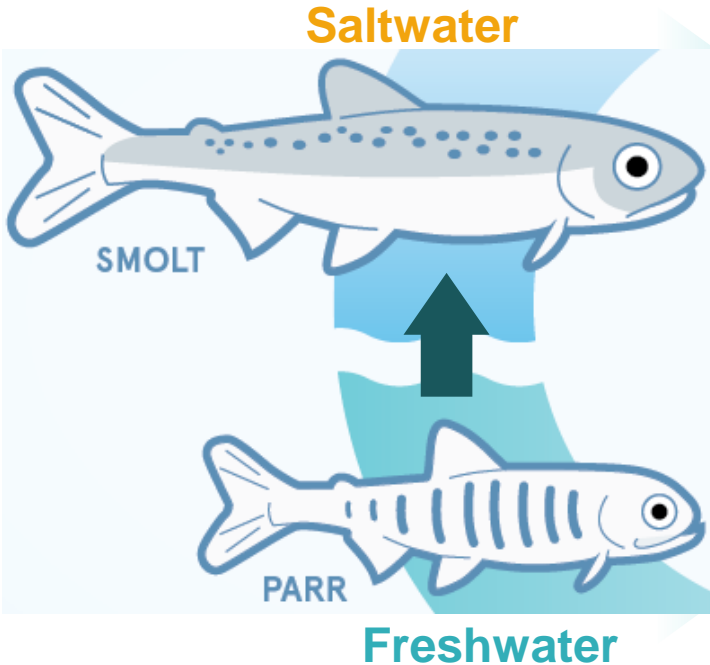


c) Production of farmed fish in seawater Generate new Broodstock



- **Great impact on southern Chile economy**
- **Smoltification understanding can reduce mortality**

Smoltification and Tissues



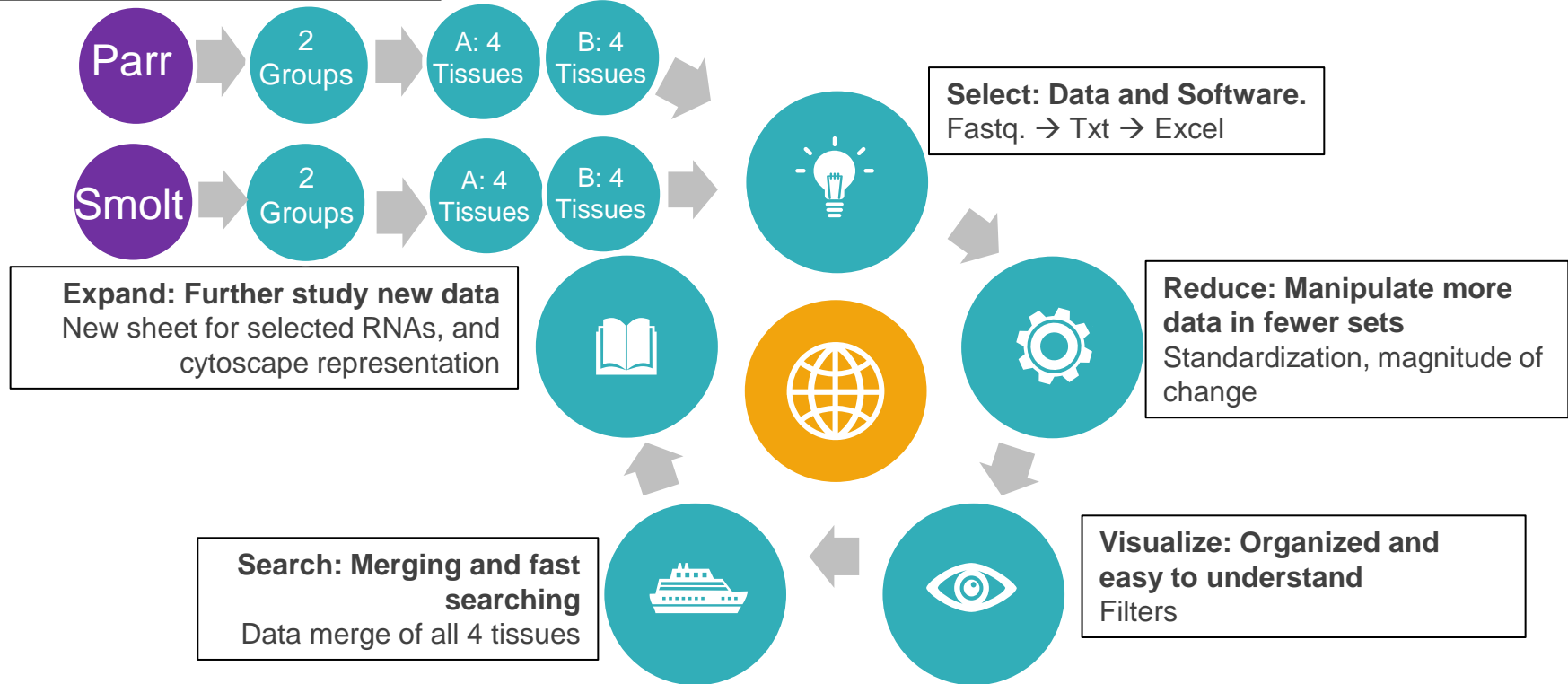
- **Seawater migration**
- **Significant number of morphological, biochemical, physiological and behavior changes**
- **4 different tissues: gill, liver, hypophysis, head kidney**
- **Osmoregulation, metabolism, neuroendocrine control and immunity**
- **Bioinformatics analyzes become increasingly relevant**

**Data from Massive Illumina
RNAseq Australomics**
Gill, liver, hypophysis and head
kidney

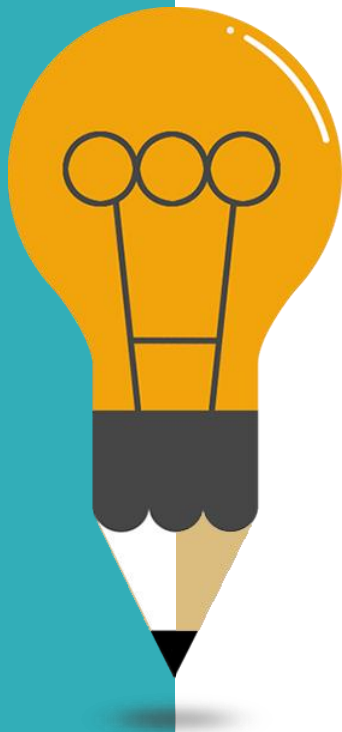
Workflow

Data Treatment

16 Total



Steps



01

Transcriptome data treatment

HISAT2 y Htseq-Count

02

Comparing and Analyzing

Compare both groups in both Parr and Smolt separately

03

Data systematization

Match system

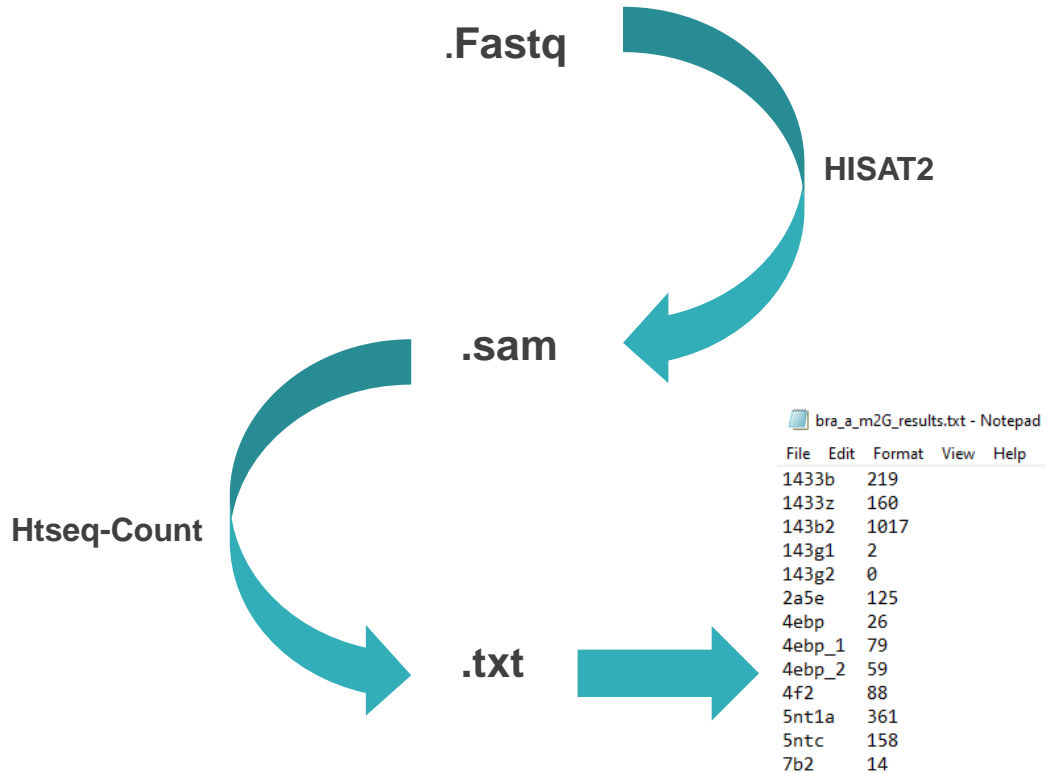
04

Inflammasome pathway data research

Head kidney

Transcriptome data treatment

HISAT2 y Htseq-Count



- **AustralOMICS Massive Illumina RNAseq**
- **2 different stages: Parr and Smolt**
- **2 groups of each**
- **4 tissues. Total of 16 sets of data**
- **HISAT2 alignment software**
- **Htseq-count software**
- **NCBI Data Base**

Comparing and analyzing groups in Parr condition

RNAs Parr	Reads Group A	Reads Group B	A/B Division
1433b	219	965	0.23
1433z	160	194	0.82
143b2	1017	962	1.06
143g1	2	9	0.22
143g2	0	16	0.00
2a5e	125	148	0.84
4ebp	26	41	0.63
4ebp_1	79	103	0.77
4ebp_2	59	64	0.92
4f2	88	65	1.35
5nt1a	361	124	2.91
5ntc	158	78	2.03
7b2	14	4	3.50



Fit Criteria in number and "%"		
47519	Total=79140	
60.04%		

Gill Parr

Fit Criteria in number and "%"		
54325	Total=79140	
68.64%		

Liver Parr

Fit Criteria in number and "%"		
45711	Total=79140	
57.76%		

Hypophysis Parr

Fit Criteria in number and "%"		
50026	Total=79140	
63.21%		

H. Kidney Parr

- Gill example
- 2 different groups (A and B)
- 20% variation

Comparing and analyzing groups in Smolt condition

RNAs Smolt	Reads Group A	Reads Group B	A/B Division
1433b	88	1023	0.09
1433z	106	450	0.24
143b2	668	1600	0.42
143g1	13	20	0.65
143g2	18	23	0.78
2a5e	92	170	0.54
4ebp	191	30	6.37
4ebp_1	128	111	1.15
4ebp_2	152	245	0.62
4f2	504	114	4.42
5nt1a	195	246	0.79
5ntc	88	278	0.32
7b2	14	8	1.75



Fit Criteria in number and "%"		
44313		Total=79140
55.99%		

Gill Smolt

Fit Criteria in number and "%"		
53242		Total=79140
67.28%		

Liver Smolt

Fit Criteria in number and "%"		
45613		Total=79140
57.64%		

Hypophysis Smolt

Fit Criteria in number and "%"		
42874		Total=79140
54.17%		

H. Kidney Smolt

- Gill example
- 2 different groups (A and B)
- 20% variation

Data systematization

Match system

Gill Parr/Smolt

RPM Parr	RPM Smolt	Division A Parr/Smolt	Division B Parr/Smolt
49.30	17.79	0.36	0.91
36.02	21.43	0.59	2.01
228.96	135.05	0.58	1.44
0.45	2.62	5.83	1.92
0.00	3.63	Z	1.24
28.14	18.60	0.66	0.99
5.85	38.61	6.59	0.63
17.78	25.87	1.45	0.93
13.28	30.73	2.31	3.32
19.81	101.89	5.14	1.52
81.27	39.42	0.48	1.72
35.57	17.79	0.50	3.09
3.15	2.83	0.89	1.73

- RPM standardization
- Look for change Smolt divided into Parr
- Z = Gene appeared, 0 = Gene disappeared
- How much each value changed → Ranges
- Match and mismatch system

0	0	Mismatch
A	0	Mismatch
A	0	Mismatch
A	B	Match!
A	B	Match!
0	B	Mismatch

RPM = Reads per million mapped reads



Data systematization

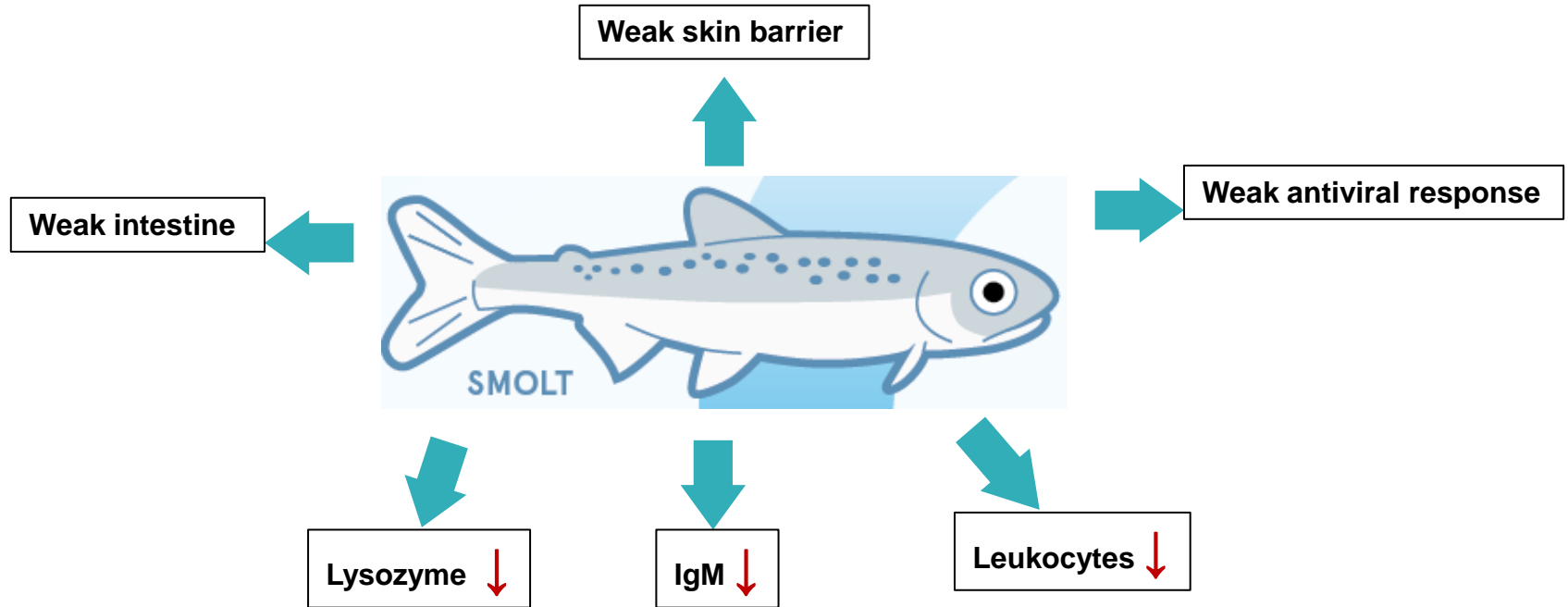
RANGE 1:

Match system

RNAs	Gill	Liver	Hypophysis	Head Kidney
sialic acid synthase-like	0	0	Match	0
Hemoglobin subunit alpha-4 (hemoglobin subunit alpha-4-like)	0	Match	Match	0
heme-binding protein 2-like	0	0	Match	0
A-kinase anchor protein 13-like	0	0	Match	0
E3 ubiquitin-protein ligase HECW2-like	0	0	Match	0
2-phospho-D-glycerate hydro-lyase (EC 4.2.1.11)	0	0	Match	Match
lethal(2) giant larvae protein homolog 1-like	0	0	Match	0
Coronin	0	0	Match	0

- Data merging
- Patterns not only repeat through both groups, but through tissues as well.
- Changes details

Inflammasome pathway data research

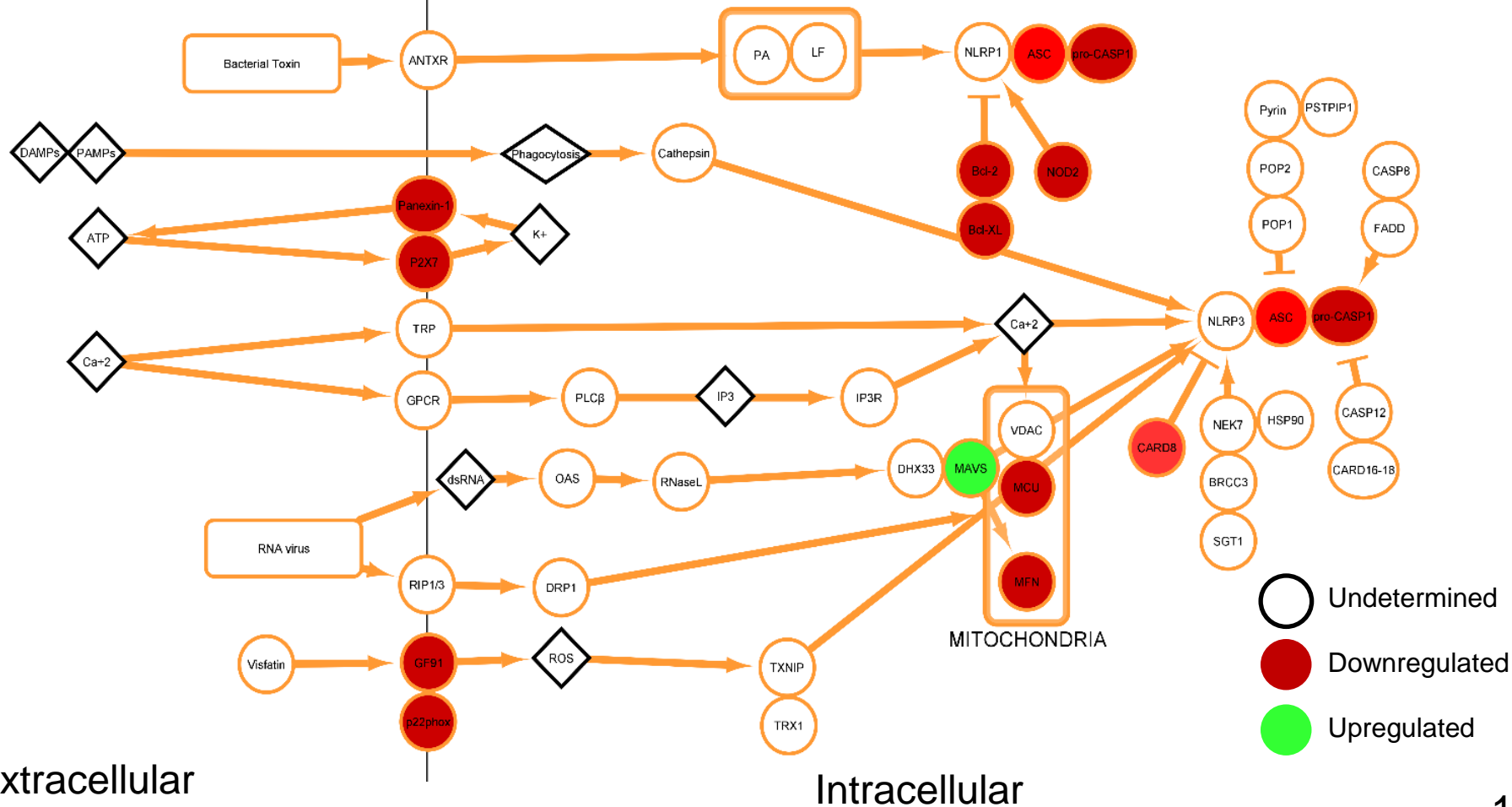


Question arises: Are inflammasome pathway RNAs downregulated during smoltification?

Inflammasome pathway data research

Head Kidney A Parr/Smolt Change	Head Kidney B Parr/Smolt Change	Name of the gene
0.37	0.41	C-X-C motif chemokine 10 (C-X-C motif chemokine 10 precursor)
DownRegulation↓	DownRegulation↓	
0.00	0.18	Apoptosis regulator Bcl-2
0.23	1.41	Apoptosis regulator Bcl-2
0.00	1.07	Apoptosis regulator Bcl-2
0.72	0.34	Apoptosis regulator Bcl-2
DownRegulation↓	DownRegulation↓	
1.00	1.00	Apoptosis-associated speck-like protein containing a CARD
0.79	0.46	Apoptosis-associated speck-like protein containing a CARD
0.74	1.00	Apoptosis-associated speck-like protein containing a CARD
1.09	0.79	Apoptosis-associated speck-like protein containing a CARD
DownRegulation↓	DownRegulation↓	
0.61	0.74	NLR family member X1 isoform X2
DownRegulation↓	DownRegulation↓	
0.42	0.99	caspase recruitment domain-containing protein 8-like
0.34	0.43	caspase recruitment domain-containing protein 8-like
0.00	2.75	caspase recruitment domain-containing protein 8-like
0.00	0.61	caspase recruitment domain-containing protein 8-like
0.00	1.00	caspase recruitment domain-containing protein 8-like
DownRegulation↓	DownRegulation↓	

Adapted from Kegg pathway database. Nod-like receptor signaling pathway (Atlantic Salmon)



Conclusion



01

Data accessibility

We were able to create a system in which data is easy to access and navigate .

02

Shortcut for future studies

Current work tries to get as close as possible to reality, making it a huge step forward for future studies.

03

Inflammasome pathway impact

We have gathered Data that support previously mentioned studies regarding a weakened state after smoltification that may be linked to the inflammasome pathway RNA downregulation.



Thank You