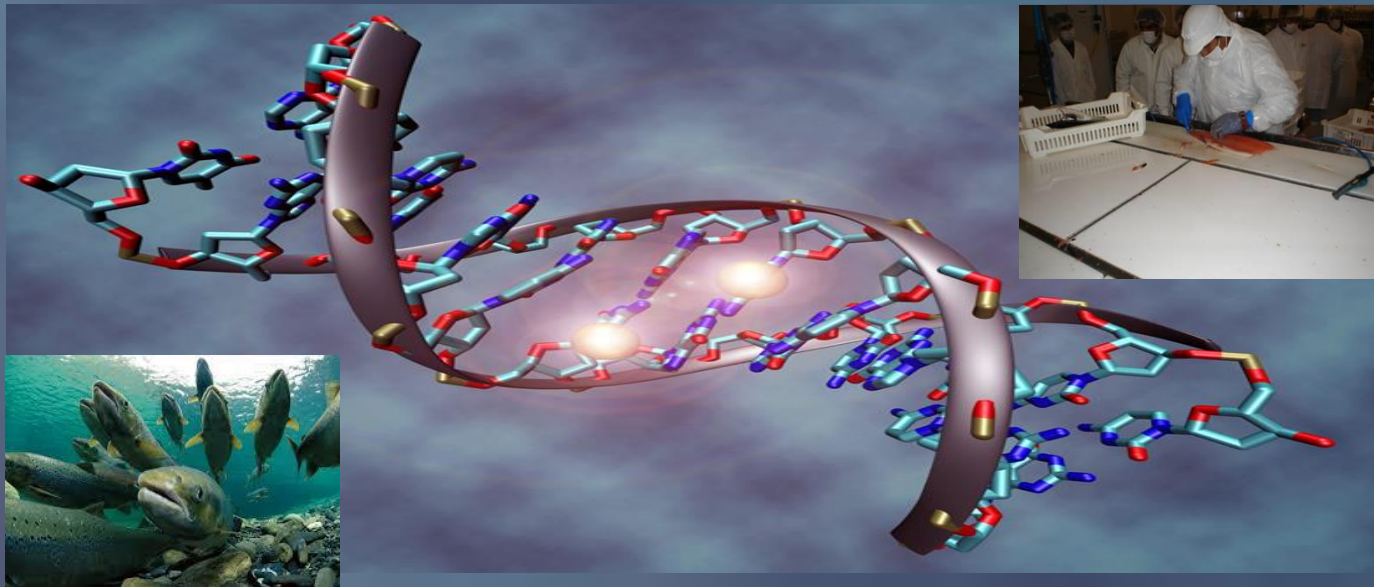


GENOME-WIDE ANALYSIS OF DNA METHYLATION OF ATLANTIC SALMON IN RESPONSE TO STRESS

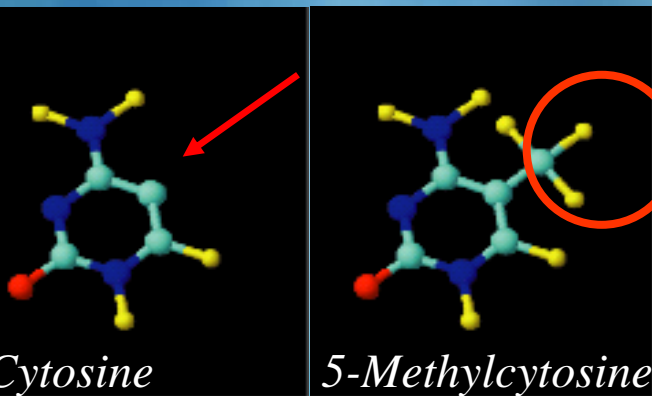


Covelo L, Reyes D., Gonzalez R., Pèrez-Figueroa A., Morán P.,
Deerenberg, R., Vidal, R

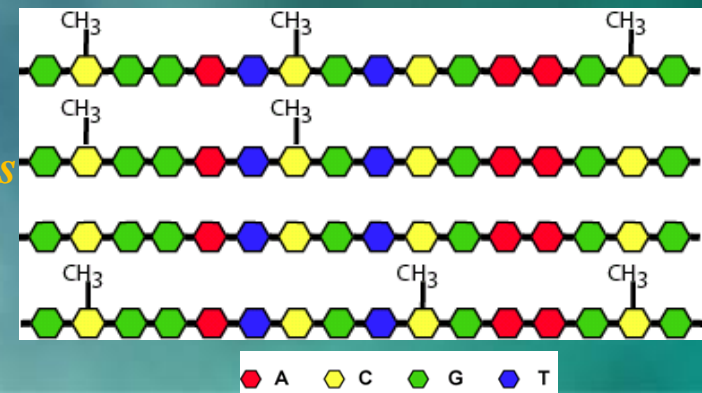
Universidad de Vigo, Faculty of Biology, Spain
Universidad de Santiago, Faculty of Biology, Chile
Marine Harvest, Chile

DNA methylation

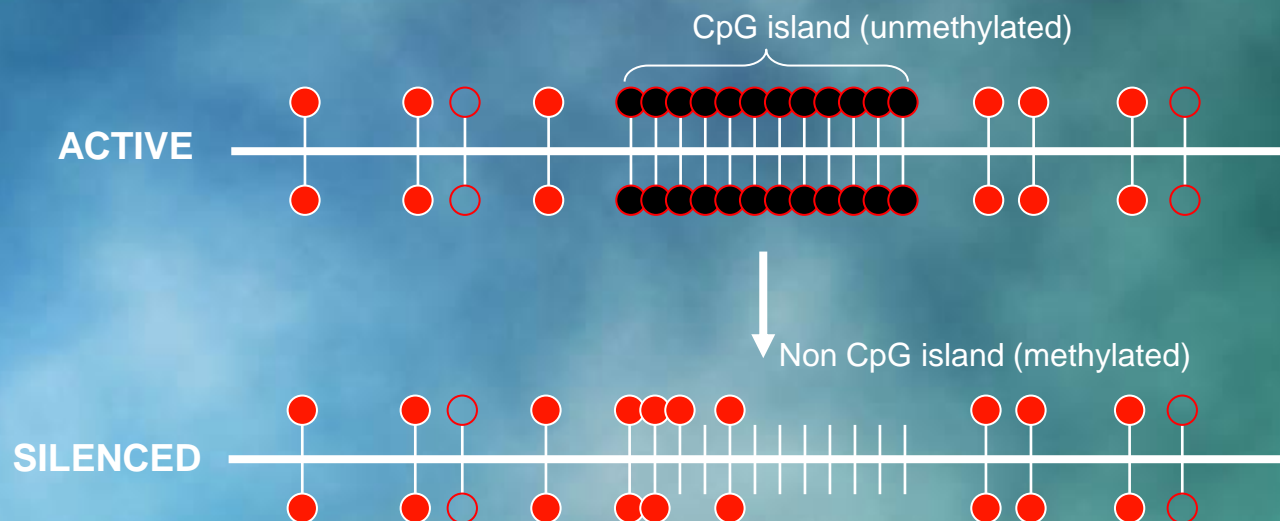
- DNA methylation is one of the most commonly occurring epigenetic events taking place in the genome. The alternative most evaluated correspond to CG – C(CH₃)G
- This change, though heritable, is reversible, making it a therapeutic target.
- DNA methylation is a covalent modification of DNA that does not change the DNA sequence, but has an influence on gene activity



Methylation modifications

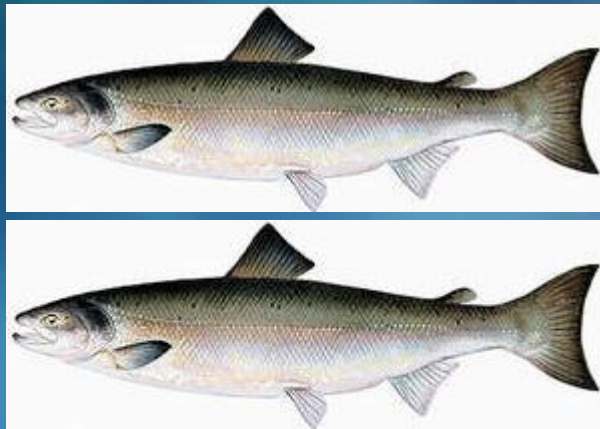


- Over 70% of DNA from mammalian somatic tissues is methylated at 70% of all CpG sites (e.g., satellite DNAs, repetitive elements including transposons, nonrepetitive intergenic DNA, and exons of genes).
- Key exceptions of this global methylation of the mammalian genomes are the CpG islands (regions with high CpG density). Most CpG islands marks the promoters and 5' domains of genes. Approximately 60% of human genes have CpG island promoters.



What Does “Epigenetics” Mean?

- Epigenetics describes phenomenon in which genetically identical cells or organisms express their genomes differently, causing phenotypic differences



Genetically identical cells or individuals

—————→
Different epigenetic modifications leading to different expression patterns

- - - - -→



Different phenotypes

STRESSORS AND STRESS IN SALMON

TOXICOLOGICAL
STRESS

ENVIRONMENTAL
STRESS

HANDLING,
HARVESTING,
STOCKING..etc
STRESS

IMMUNOLOGICAL
STRESS

NUTRITIONAL
STRESS

SOCIAL
STRESS

BBC

Sign in

News

Sport

Weather

Shop

Earth

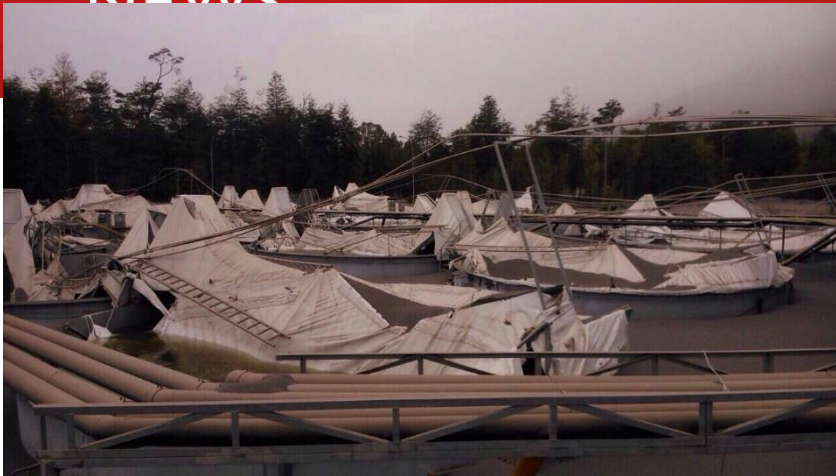
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NEWS



ANTI OXIDATIVE
PROTEINS

CYTOSKELETAL
CHANGES

UNFOLDED
PROTEIN (HSP)

CHANGES GENE
EXPRESSION

IMMUNOSUPPRESSION

ROS
PRODUCTION

DYSFUNCTION
METABOLIC/MITOCHONDRIAL

BEHAVIORAL
MALADAPTATION

APOPTOSIS

NECROSIS

DISTRESS

Epigenetic gene regulations: Two major mechanisms

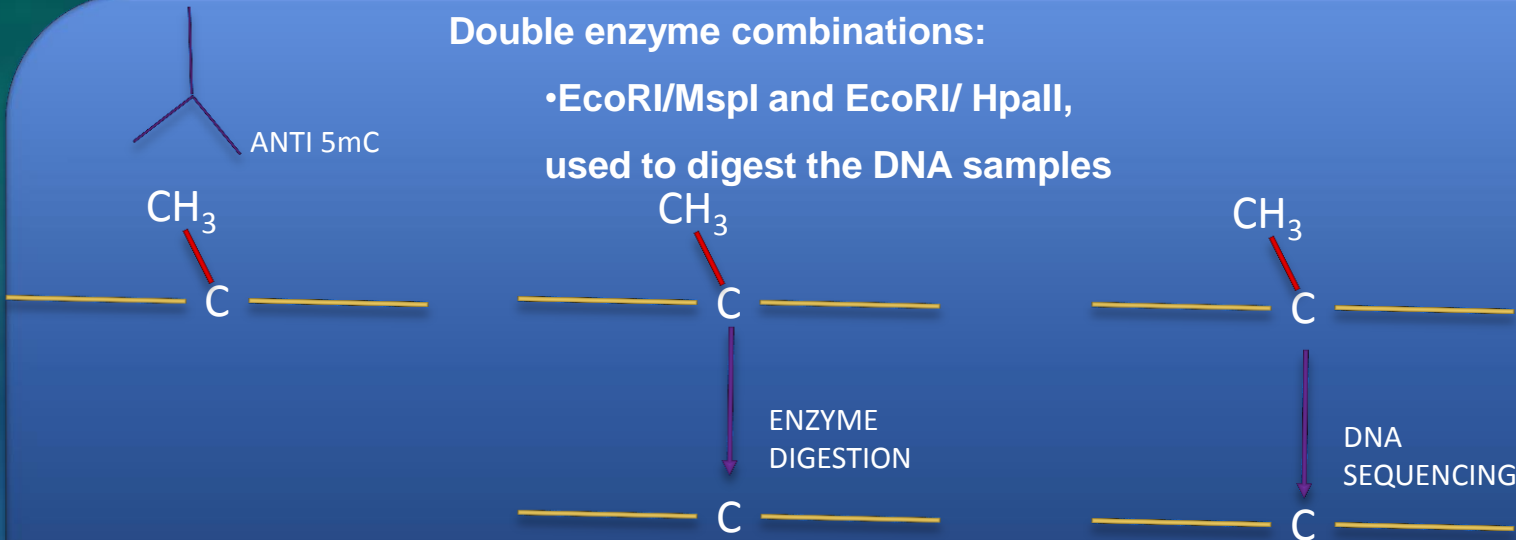
- DNA Methylation
- Histone modification

MATERIALES AND METHODS

STEP 1. DIFFERENTIATION OF UN FROM METHYLATED DNA. GENOME-WIDE

Double enzyme combinations:

•EcoRI/Mspl and EcoRI/ HpaII,
used to digest the DNA samples



PCR is used to
selectively amplify the
DNA fragments

STEP 2 DIFFERENTIATION UN FROM METHYLATED DNA

- MICROARRAYS/BEADCHIPS
- NEXT GENERATION SEQUENCING
- METHYLATION-SENSITIVE AMPLIFIED POLYMORPHISM (MSAP)

MATERIALES AND METHODS

STRESS CHALLENGE MODEL: handling and confinement experimental stress

Pure farmed Atlantic salmon strains,
Gaspe and Laks (Marine Harvest)

unselected, mixed-sex; mean mass \pm SEM: 62.18 ± 1.6 g; mean length \pm SEM: 17.64 ± 0.13

Tissues:

Plasma: Cortisol Analysis



Control

- After adaptation, 10 fishes were sampled and immediately euthanized

Step2

- 10 fishes were sampled and held out of water for 30 s and then transferred to a new tank with a dramatic level of water by 0.5 h

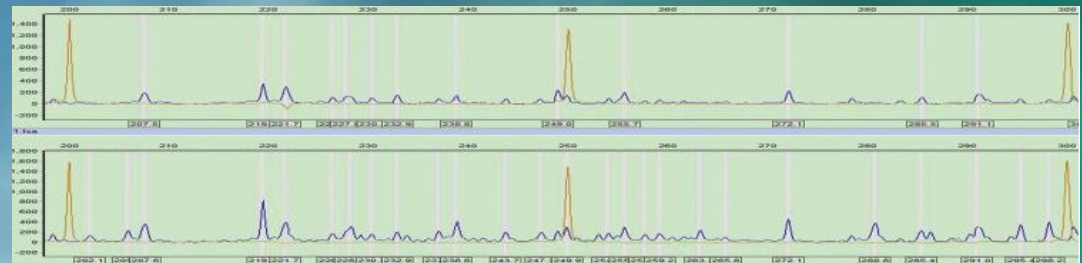
Step3

- 10 fishes were sampled and held out of water for 30 s and then transferred to a new tank with a dramatic level of water by 3.0 h

Step4

- Similar to Step 3, but with the modification that after fishes were returned to normal conditions and sampled after 24 h

KIDNEY, SPLEEN, LIVER AND MUSCLE: Fluorescence - MSAP analysis.



MATERIALS AND METHODS

Amplified DNA fragments

type I	Represents the band presence for both enzyme combinations UNMETHYLATED
type II	Band presence only for EcoRI/HpaII HEMIMETHYLATION
type III	Band presence for EcoRI/MspI FULL METHYLATION
type IV	Represents the band absence for both enzyme combinations

Statistical Analysis (R package msap, Pérez-Figueroa, 2013)

- **AMOVA**
- **PCoA (Principal Component Analysis)**

MATERIALS AND METHODS

Draft assembly of the Atlantic salmon genome (NCBI Assembly)



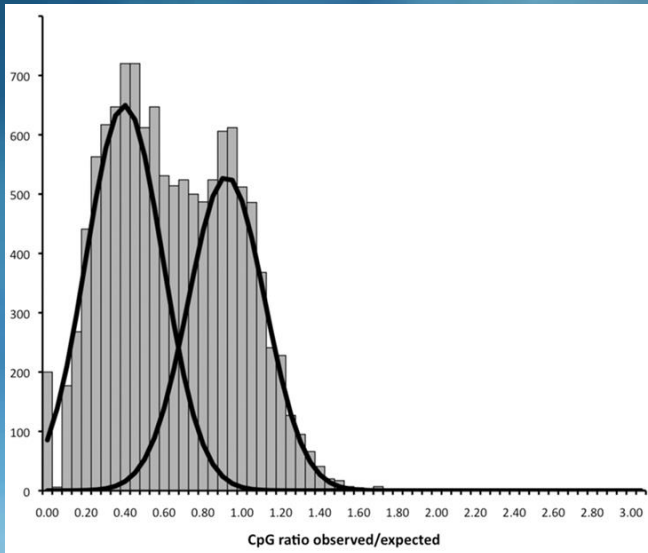
Non-redundant unigenes from public databases

IN SILICO EVALUATION

$CpG_{O/E}$ RATIO (Takai and Jones, 2002)

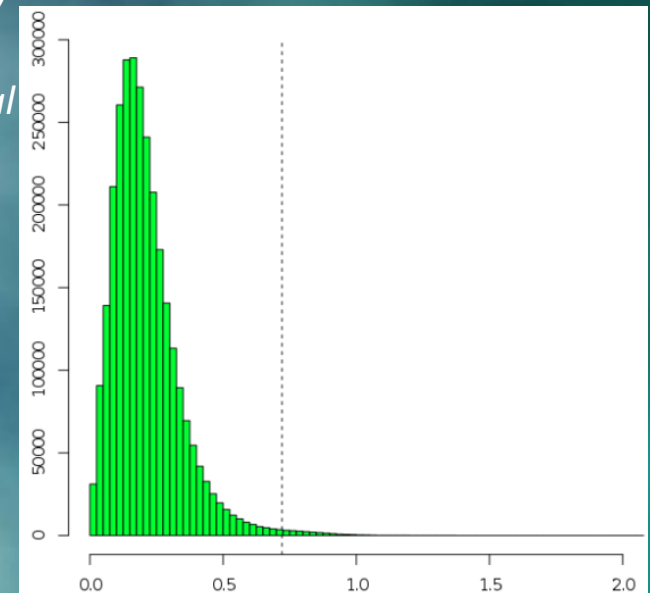
NOCOM program to test whether the gene frequency distribution differed significantly from a unimodal distribution

Invertebrate model



Robinson et al., 2011

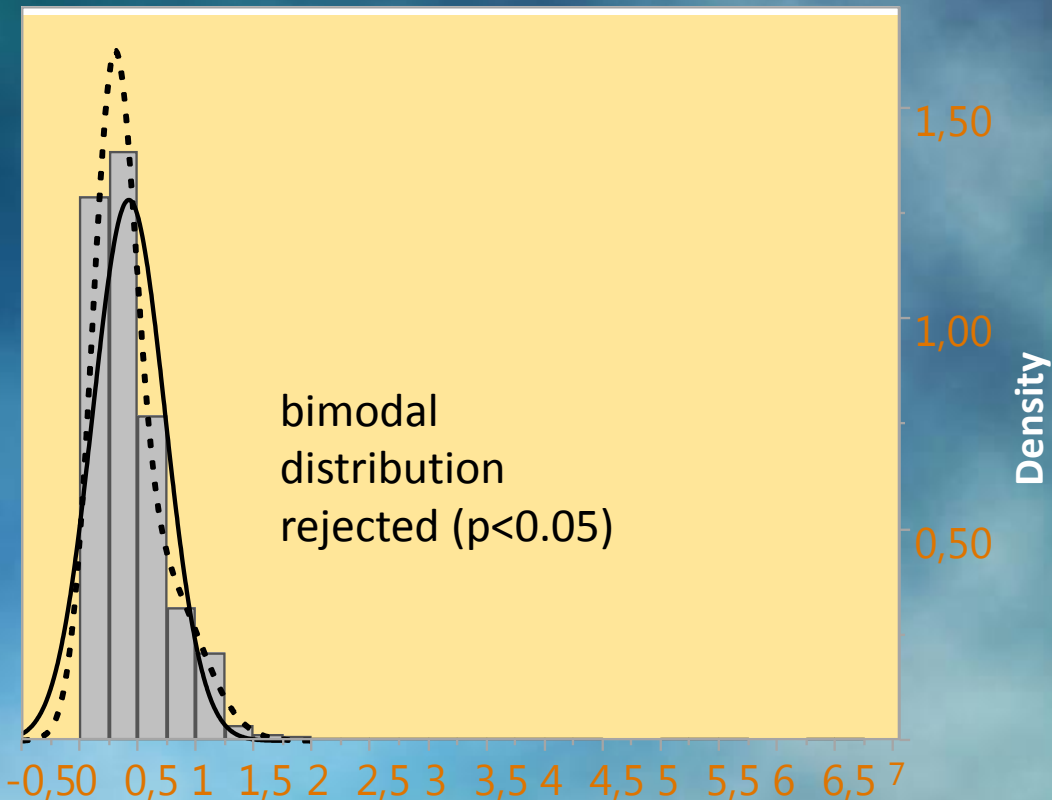
Vertebrate model



Okmura et al., 2011

RESULTS

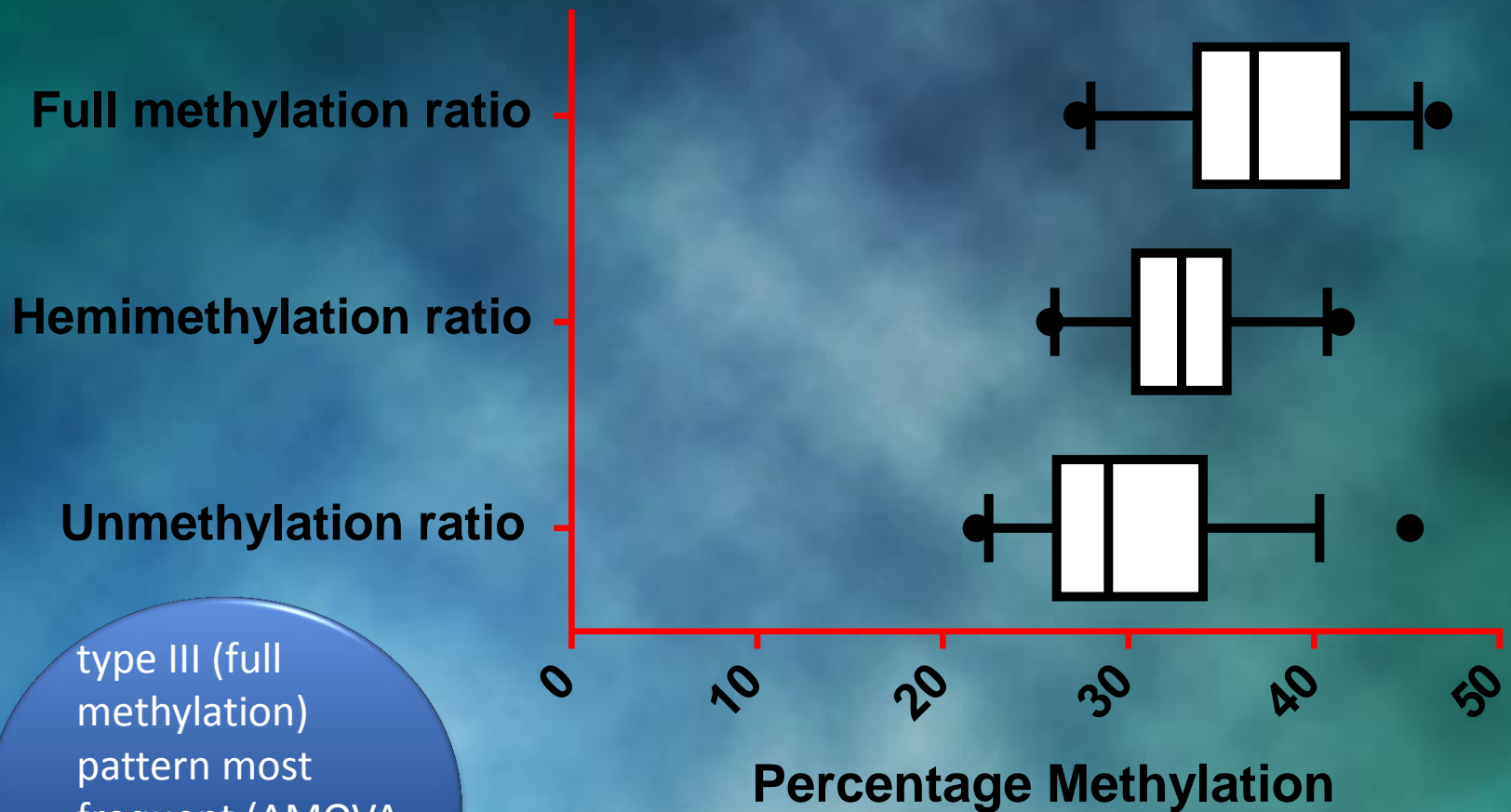
IN SILICO EVALUATION OF CpG RATIOS ATLANTIC SALMON GENOME



- The genomic distribution of CpGo/e ratio were clearly unimodal
- One peak focused at approximately in 0.25 .
- This suggest that Atlantic salmon genome present a global pattern of methylation, characteristic of vertebrates (Where a great percentage of CpG sites are methylated and a reduced CpG sites, probably localized in CpG islands, are unmethylated).

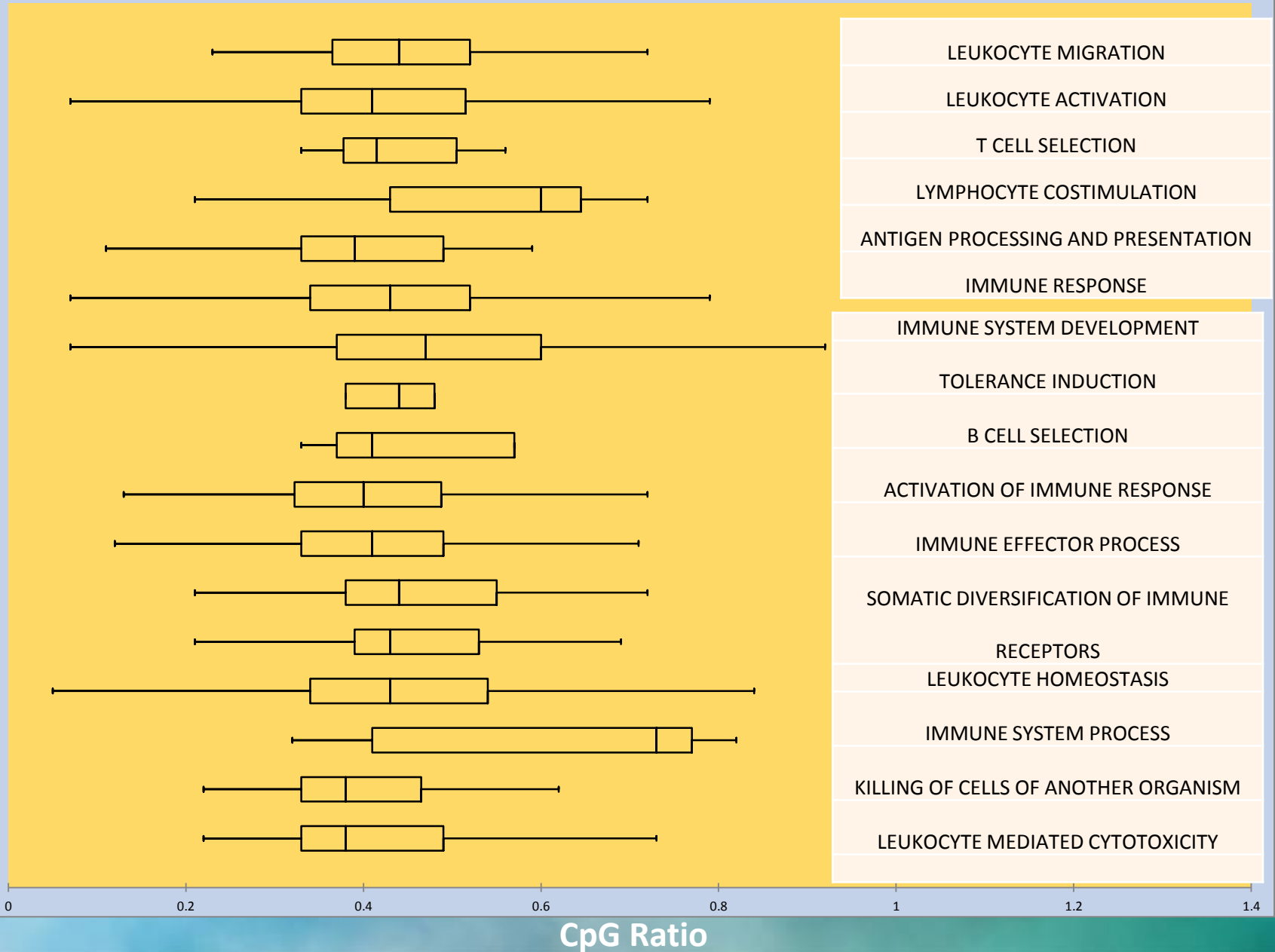
RESULTS

Patterns and levels of DNA methylation in four tissues of Atlantic STRAINS/TISSUE POOLED

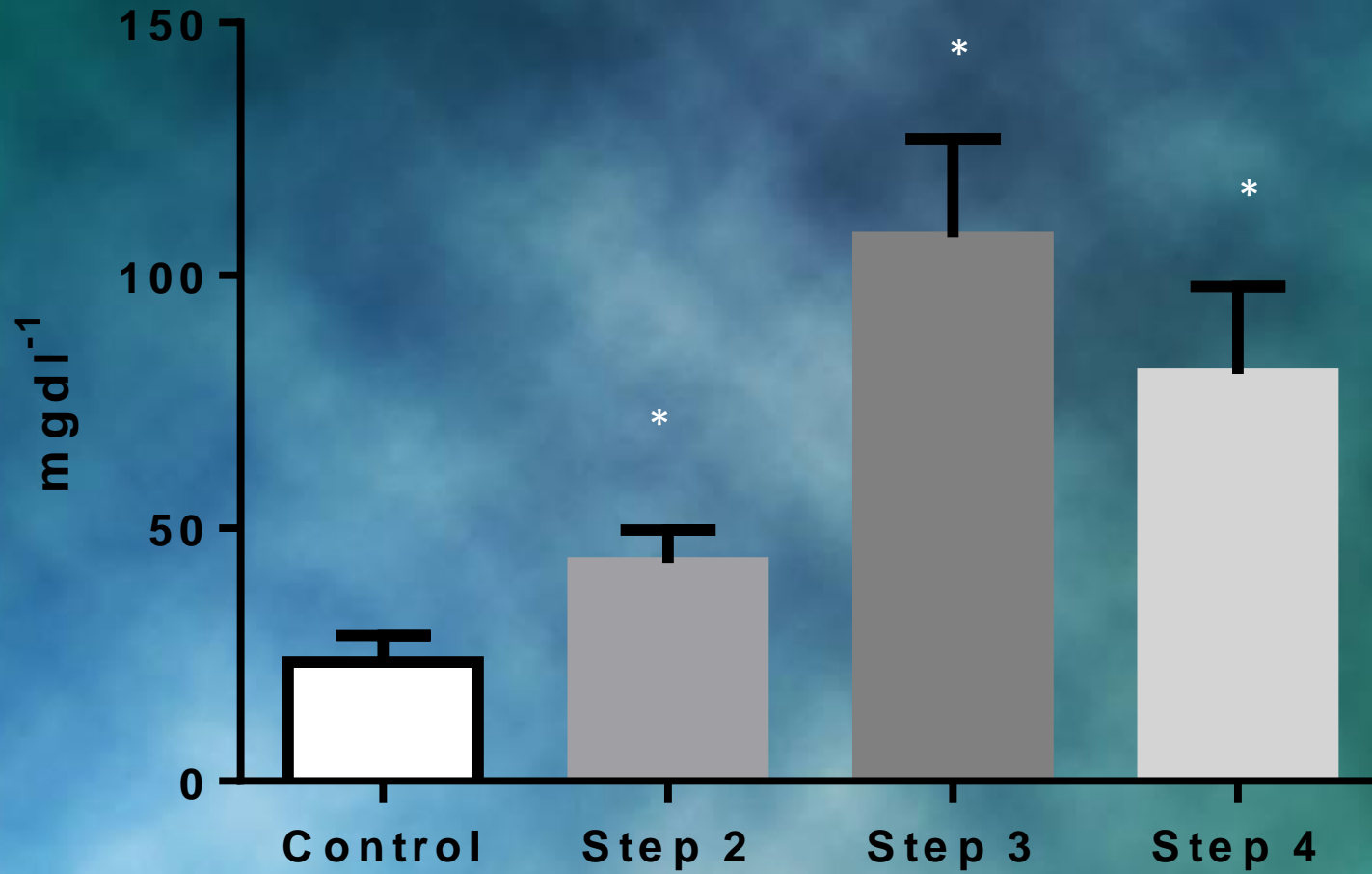


type III (full methylation) pattern most frequent (AMOVA $\Phi_{ST} = 0.0432$, $p < 0.01$),

IN SILICO METHYLATION ANALYSIS – EST AVAILABLE ATLANTIC SALMON



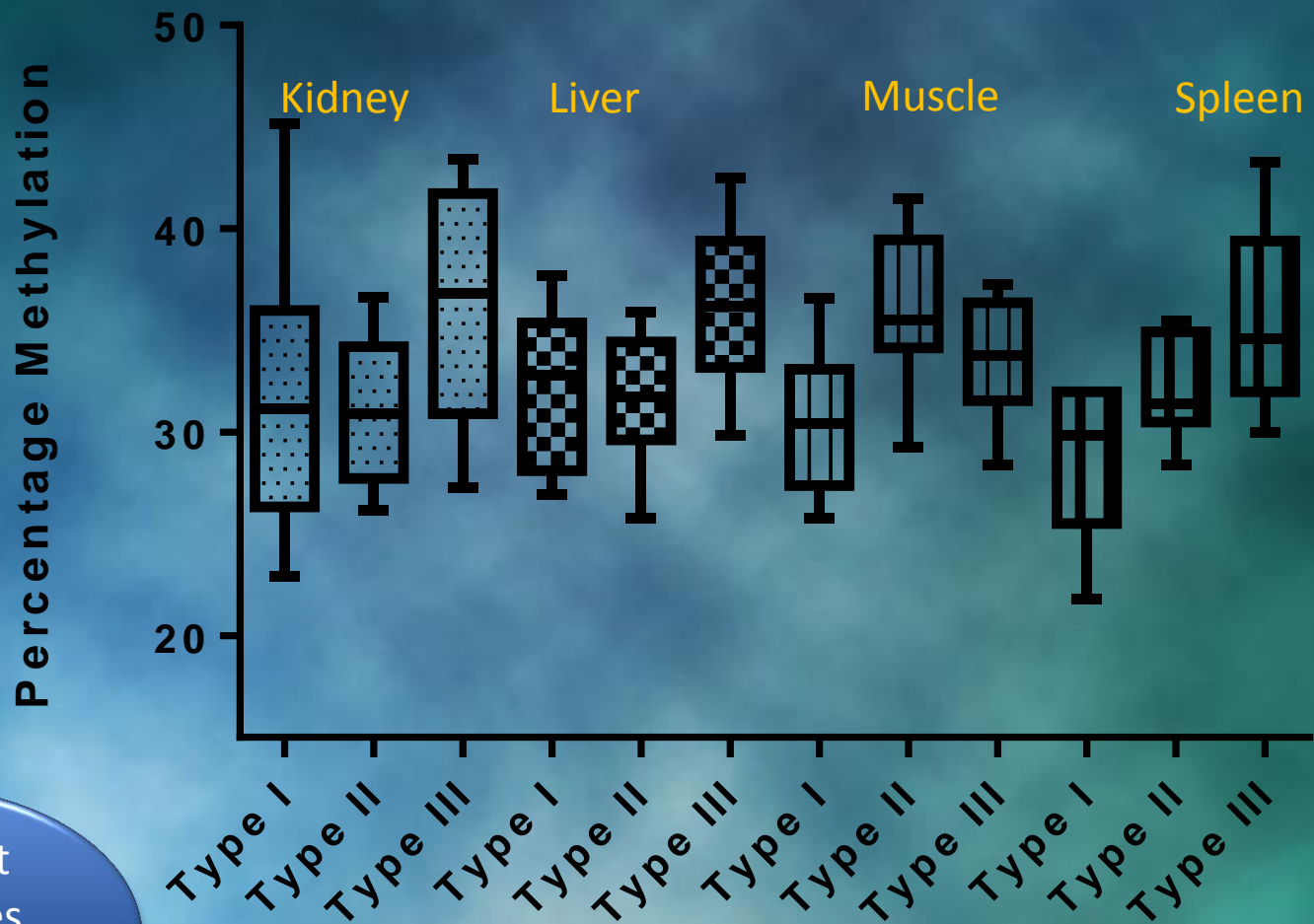
CORTISOL LEVELS



*: p<0.05

RESULTS

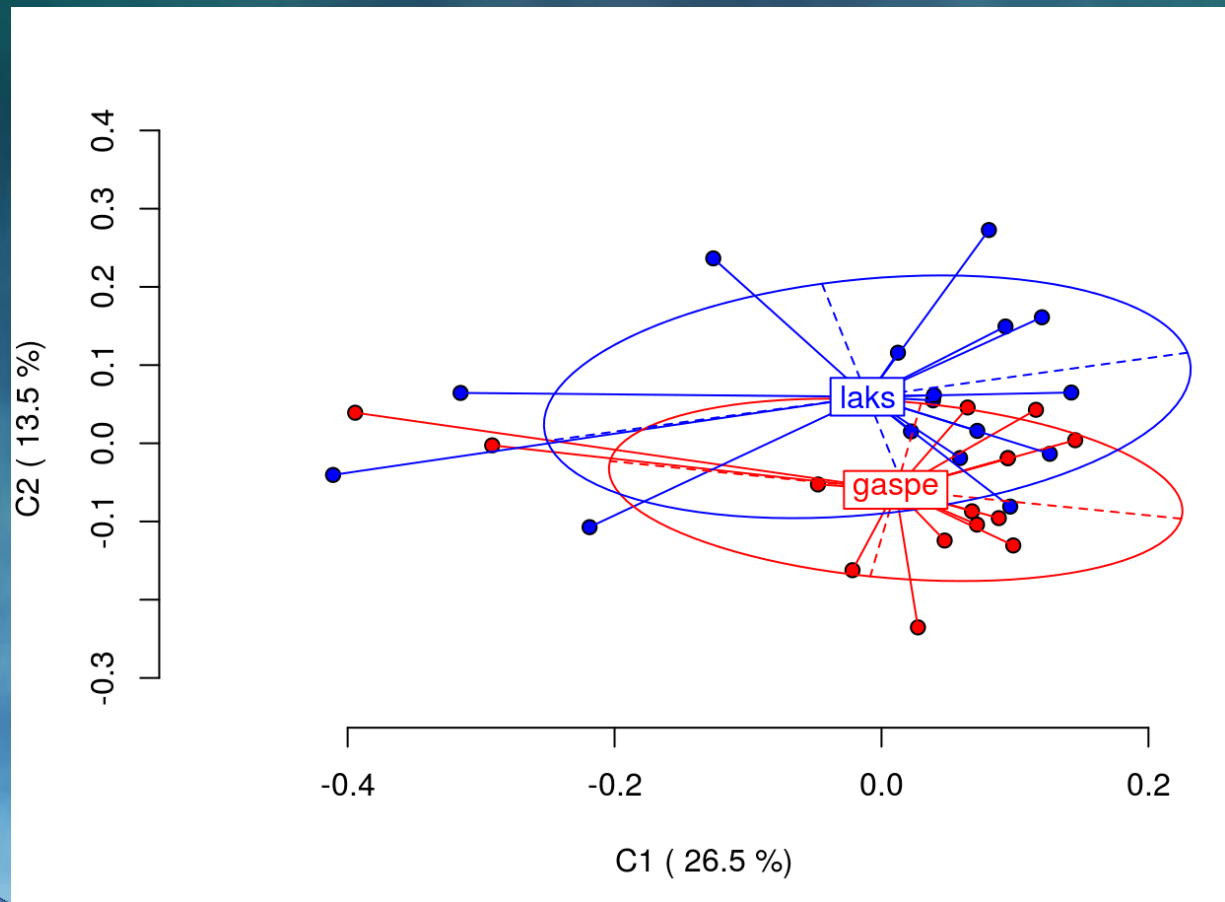
GLOBAL DNA METHYLATION WITHIN AND AMONG TISSUES STRAINS POOLED



No significant
among tissues
(AMOVA $p < 0.01$;
PCoA),

RESULTS

DNA METHYLATION AMONG STRAINS

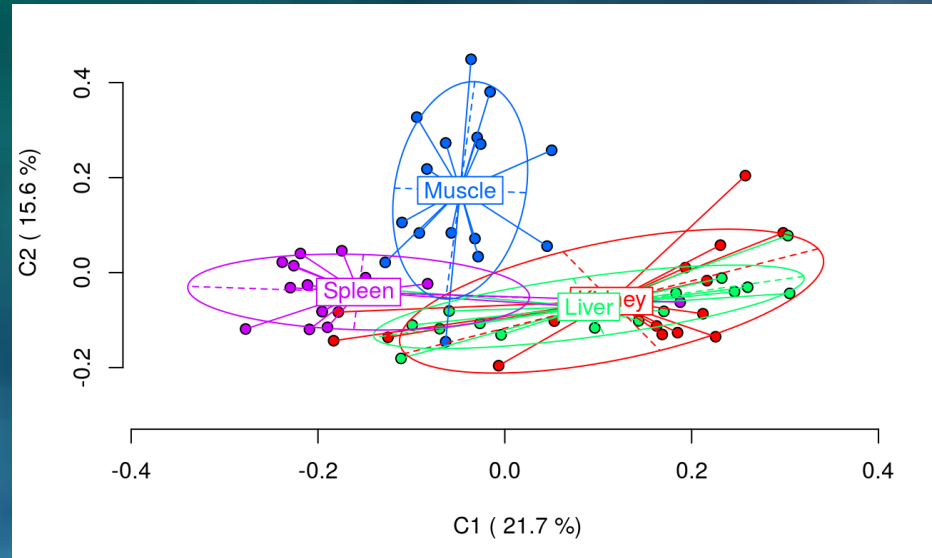


Only SPLEEN showed differences among strains (AMOVA Φ ST=0.0832, $p < 0.01$), PCoA confirm

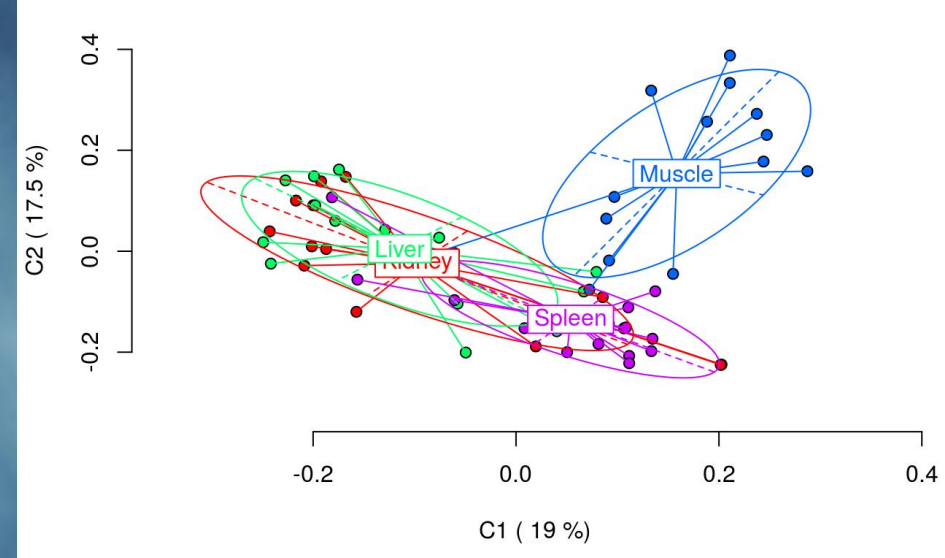
RESULTS

DNA METHYLATION WITHIN STRAINS

GASPE STRAIN



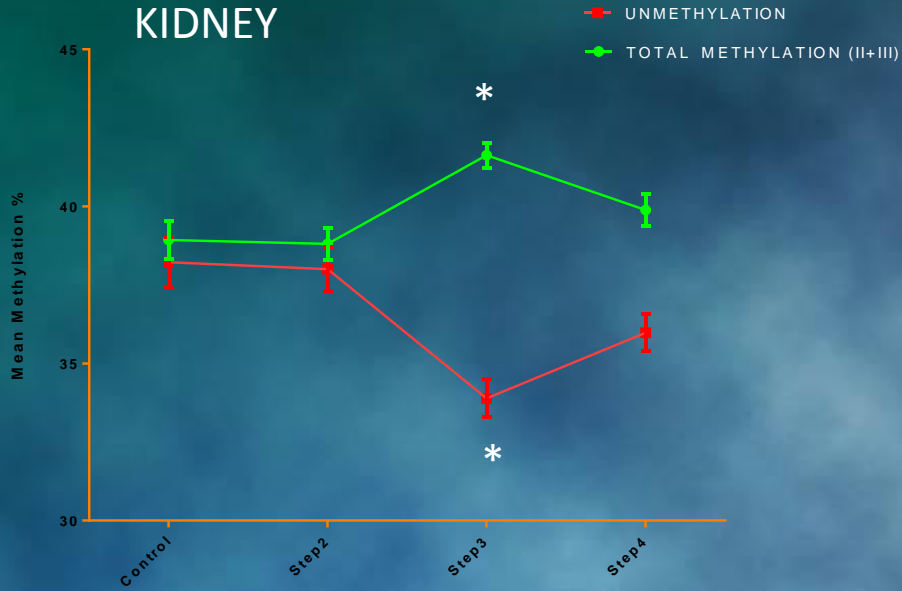
LAKS STRAIN



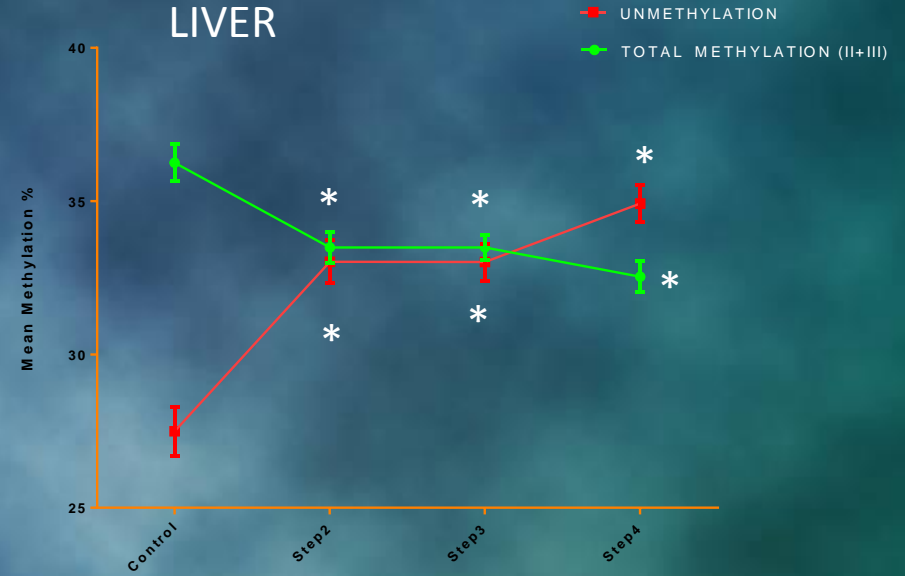
Only THE PAIR
KIDNEY/SPLEEN NO
showed differences
WITHIN strain.

RESULTS: DIVERSITY AND PROFILES EXPRESSION BY TISSUE (STRAINS POOLED) *; p<0.05 - control

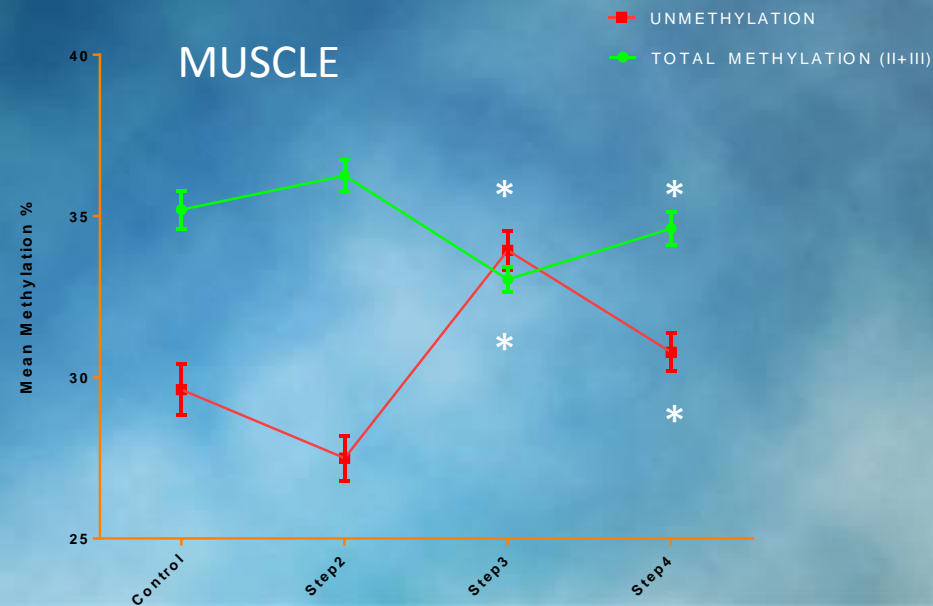
KIDNEY



LIVER



MUSCLE



CONCLUSIONS

ATLANTIC SALMON SHOW A GREAT DIVERSITY OF METHYLATION CHANGES THAT AGREE WITH THE DIVERSITY SHOWED IN RAINBOW TROUT (Blouin et al, 2009).

FULL METHYLATION IS THE STATE MORE FREQUENTLY

SPLEEN WILL BE A IMPORTANT ORGAN TO EVALUATE DIFFERENTE PATTERN OF METHYLATION AMONG STRAIN

STRESS SHOW A CLEAR EFFECT IN THE PATTERNS OF METHYLATION

STUDIES OF GENE EXPRESSION SHOULD INCORPORATED ANALYSIS OF METHYLATION

EPIGENOMICS WILL BE A IMPORTANT ASPECT IN ANIMAL BREEDING BECAUSE IT MAY HELP IDENTIFYING PART OF THE MISSING CAUSALITY AND MISSING HERITABILITY OF COMPLEX TRAITS AND DISEASES

ACKNOWLEDGEMENTS

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THANKS FOR YOUR
ATTENTION