AQUAculture infrastructures for EXCELLence
in European Fish research

Innovative biomarkers for environmental and nutritional stressors in sea bream

Ariadna Sitjà-Bobadilla & **Jaume Pérez-Sánchez**

AQUAEXCEL INDUSTRY WORKSHOP:

Research Infrastructures: adding value to European aquaculture industry

Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Spain
(AQUAEXCEL partner 4. WP7)

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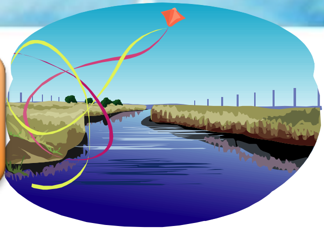
Aquaculture Europe 2014 - San Sebastián
17 October 2014

AQUACULTURE STRESSORS

Nutrition

Environment



BioMar

Nutrition: Requirements

AQUASOJA

Nutrition: Alternative Feed Ingredients I

ARRAINA

Nutrition: Outputs of the EU ARRAINA project

Nutrition: Alternative Feed Ingredients II

Tereos Syral

eas www.easonline.org

EAS/EATiP Mediterranean Performance

European Aquaculture

Advances in Diseases and Welfare I

COPE well

Welfare: Outputs of the EU COPEWELL project

Stress:18 orals + 9 posters

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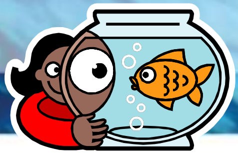
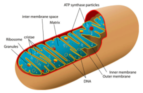
AQUA EXCEL



OBJECTIVES

- To better identify stress effects: there is not a consensus endocrine profile for chronically stressed animals or how to asses it without invoking further stress
- To compare classical measures with molecular and proteomic approaches
- To use non-lethal samples: mucus
- To find alternative biomarkers

Molecular approach:
the mitochondria
why



Mitochondria main cellular function is ATP production

Number & activity of mitochondria reflect cell energy requirements

Mitochondria are highly regulated at transcriptional and transductional level

BACKGROUND

Fish and Chips

Fish transcriptome database

Fish and Chips :

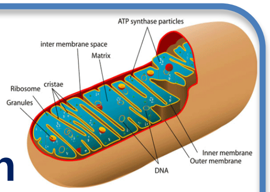
Fish and Chips database gathers public transcriptome data related to fish species in various physiological conditions

http://fishandchips.genouest.org

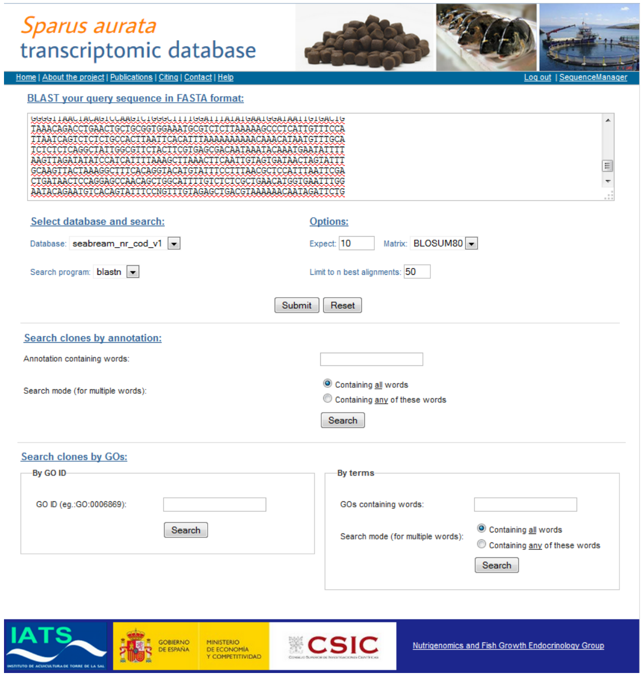
Gathers all public transcriptome data related to fish species in various physiological conditions (> 350 microarrays)

Meta-analysis confirmed that mitochondria play a key role in the adaptive stress response of farmed fish

Calduch-Giner et al., 2014 Mar. Biotechnol.



- **Extensive BLAST searches**
  www.nutrigroup.iats.org/seabreamdb
- **Nucleotide sequence analysis & manual curation**
- **926 mitochondrial-related genes are represented in the Nutrigroup transcriptome database**



Sparus aurata
transcriptomic database

Home | About the project | Publications | Citing | Contact | Help
Log out | SequenceManager

BLAST your query sequence in FASTA format:

```
TAAACAGACCTGAACTGCTGCGGTGGAAATGCGTCTCTTAAAAAGCCCTCATTGTTTCCA
TTAATCAGTCTCTCTGCCACTTAATTCACATTTAAAAAAAAAACAAACATAATGTTTGCA
TCTCTCTCAGGCTATTGGCGTTCTACTTCGTGAGCGACAATAAATACAAATGAATATTTT
AAGTTAGATATATCCATCATTTTAAAGCTTAAACTTCAATTGTAGTGATAACTAGTATTT
GCAAGTTACTAAAGGCTTTCACAGGTACATGTATTTCCTTTAACGCTCCATTTAATTCGA
CTGATAACTCCAGGAGCCAACAGCTGGCATTTTGTCTCTCGCTGAACATGGTGAATTTGG
AATACAGAATGTCACAGTATTTCCNGTTTGTAGAGCTGACGTAAAAAACAATAGATTCTG
```

Select database and search:

Database: seabream_nr_cod_v1

Search program: blastn

Options:

Expect: 10

Matrix: BLOSUM80

Limit to n best alignments: 50

Submit Reset

Search clones by annotation:

Annotation containing words:

Search mode (for multiple words):
● Containing all words
○ Containing any of these words

Search

Search clones by GOs:

By GO ID

GO ID (eg.:GO:0006869):

Search

By terms

GOs containing words:

Search mode (for multiple words):
● Containing all words
○ Containing any of these words

Search

Nutrigenomics and Fish Growth Endocrinology Group

TRANSCRIPTOMIC TOOLS

Two pathway-focused arrays for mitochondrial gene expression profiling to assess the health and metabolic condition of fish exposed to environmental and/or nutritional stressors
www.nutrigroup-iats.org

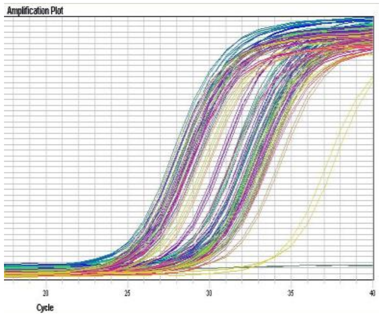
MITO-chip

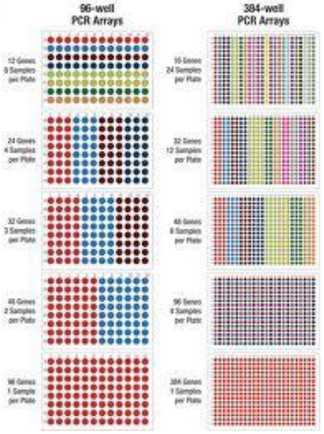
- ☐ 60 genes
- ☐ 52 new sequences
- ☐ Oxidative metabolism & respiration uncoupling, 13
- ☐ Antioxidant defence, 7
- ☐ Protein transport/ folding/assembly, 23
- ☐ Mitochondria dynamics, 6
- ☐ Apoptosis, 5

OXPHOS-chip

- ☐ 88 genes
- ☐ 86 new sequences
- ☐ Complex I, 34
- ☐ Complex II, 6
- ☐ Complex III, 13
- ☐ Complex IV,22
- ☐ Complex V. 13

Figure 1. Automated liquid handling robot





Most components of the OXPHOS pathway have been established for the first time in a farmed fish species

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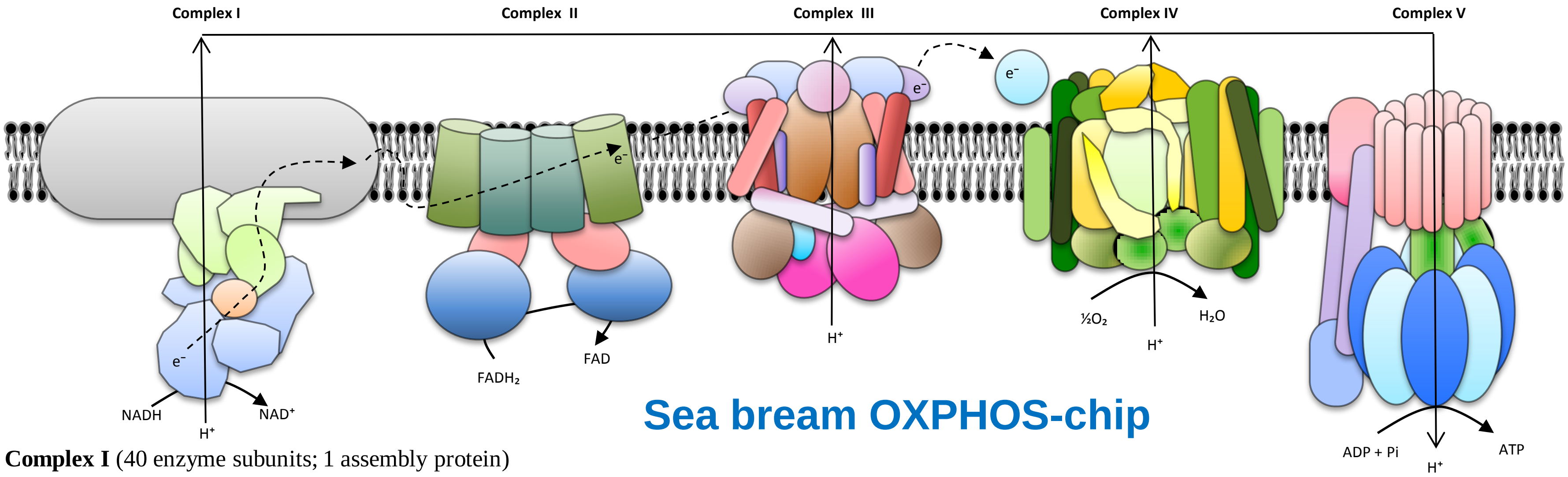
AQUA EXCEL



Complex I (40 enzyme subunits; 1 assembly protein)

ND1 **ND2** **ND5** **ND6**

NDUFA1 NDUFA2 NDUFA3 NDUFA4 NDUFA4-L2 NDUFA5 NDUFA6 NDUFA7 NDUFA8 NDUFA9 NDUFA10 NDUFA11 NDUFA12 NDUFA13

NDUFB1 NDUFB2 NDUFB3 NDUFB4 NDUFB5 NDUFB6 NDUFB7 NDUFB8 NDUFB9 NDUFB10 NDUFB11 NDUFC1 NDUFC2

NDUFS2 NDUFS3 NDUFS4 NDUFS5 NDUFS6 NDUFS7 NDUFV1 NDUFV2 NDUFV3

NDUFAF2

Complex II (4 enzyme subunits; 2 assembly proteins)

SDHA SDHB SDHC SDHD

SDHAF1 *SDHAF2*

Complex III (12 enzyme subunits; 1 assembly protein)

CYB

CYC1 UQCRFS1 UQCRC1 UQCRC2 UQCRH UQCRB UQCRQ UQCR10 UQCR11-A UQCR11-B

CYC

UQCC

Complex IV (20 enzyme subunits; 3 assembly proteins)

COXI **COXII** COXIII

COX4a COX4b COX5a1 COX5a2 COX5b1 COX5b2 COX6a1 COX6a2 COX6b1a COX6b1b COX6c COX7a1 COX7a2

COX7b COX7c COX8a COX8b

COX15 *SURF1* *SCO1*

Complex V (15enzyme subunits; 1 assembly protein)

ATP6 **ATP8**

ATP5A1 ATP5B ATP5C1 ATP5D ATP5E ATP5F1 ATP5G1 ATP5H ATP5I ATP5J2 ATP5L ATP5O OSCP1

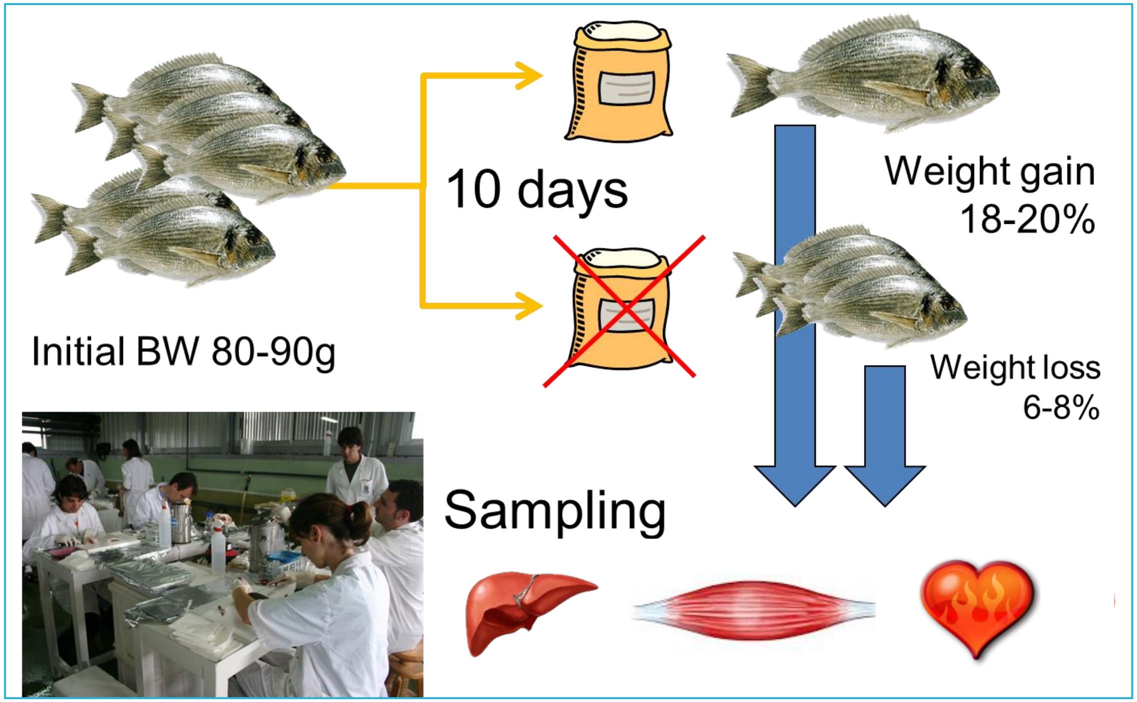
ATPAF2

Experimental approach-1: Nutritional Stressor

Experimental design
“Fasting model”

IATS

INSTITUTO DE ACUICULTURA DE TORRE DE LA SAL



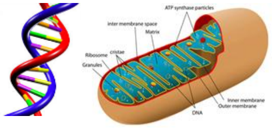
Initial BW 80-90g

10 days

Weight gain
18-20%

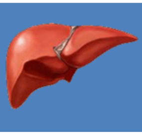
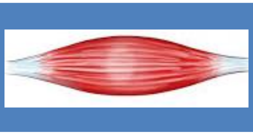
Weight loss
6-8%

Sampling



OXPHOS-CHIP

Nutritional stressor: OXPHOS regulation by fasting “malnutrition/caloric restriction”

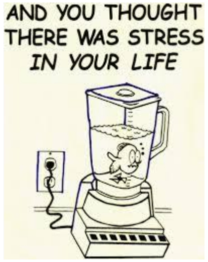
Tissue			
Differentially expressed genes	72	29	10
Type of regulation	⬇⬇	⬆⬆	⬆
Genes involved	Catalytic, regulatory and assembly factors	Regulatory and assembly factors	Catalytic and assembly factors
Gene type	mtDNA/nDNA	nDNA	mtDNA/nDNA

Strong down-regulation of energy–generating processes, with inhibition of lipogenesis

Enhanced oxidative capacity with the reduction of metabolic fuel supply

OXPHOS regulation is driven in a tissue-specific manner by mitochondrial- and nuclear-encoded genes

Experimental approach-2: Chronic environmental stress



Changes in water temperature — **T-ST**

Changes in water level & chasing — **C-ST**

Multiple sensory perception random stressors
(Sound & tank shaking, window wiper in water, water flow reversal, light flashes) — **M-ST**

MITO-chip

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journal homepage: www.elsevier.com/locate/ygcen

Metabolic and transcriptional responses of gilthead sea bream (*Sparus aurata* L.) to environmental stress: New insights in fish mitochondrial phenotyping

Azucena Bermejo-Nogales[a], Marit Nederlof[b], Laura Benedito-Palos[a], Gabriel F. Ballester-Lozano[a], Ole Folkedal[c], Rolf Eric Olsen[c], Ariadna Sitjà-Bobadilla[d], Jaume Pérez-Sánchez[a,*]

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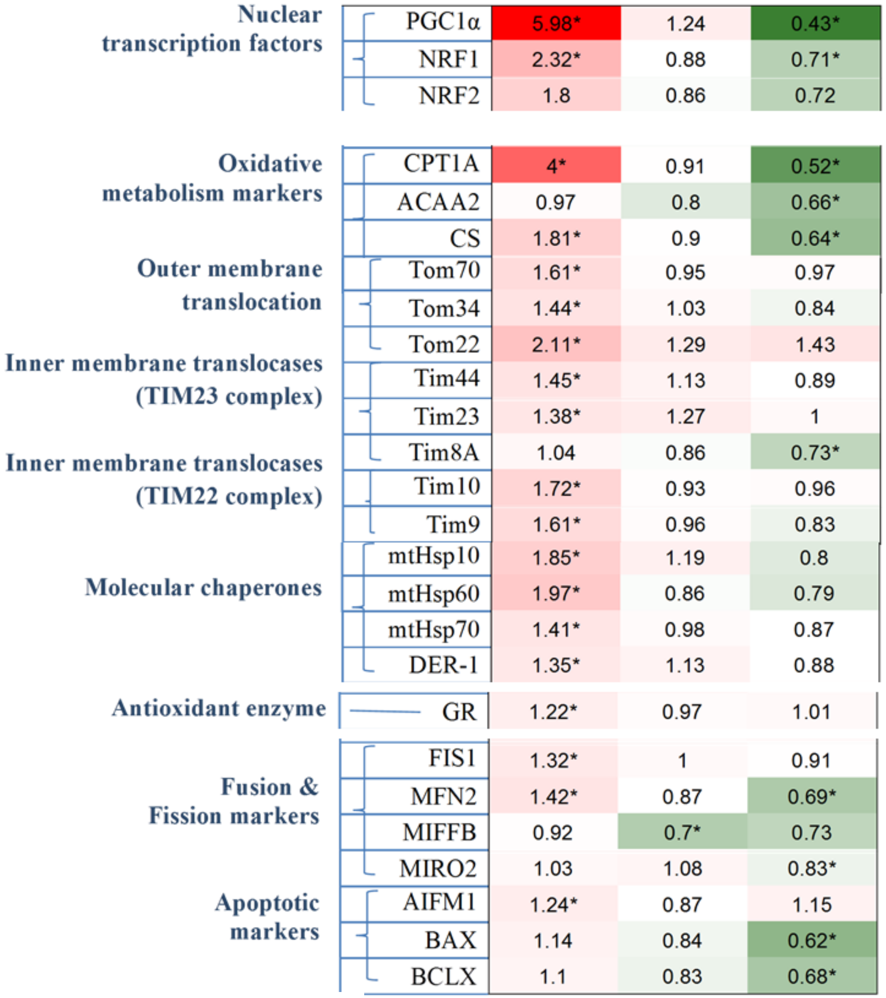
[d] *Fish Pathology Group, Department of Marine Species Biology, Culture and Pathology, Institute of Aquaculture Torre de la Sal, IATS-CSIC, 12595 Ribera de Cabanes s/n, Castellón, Spain*

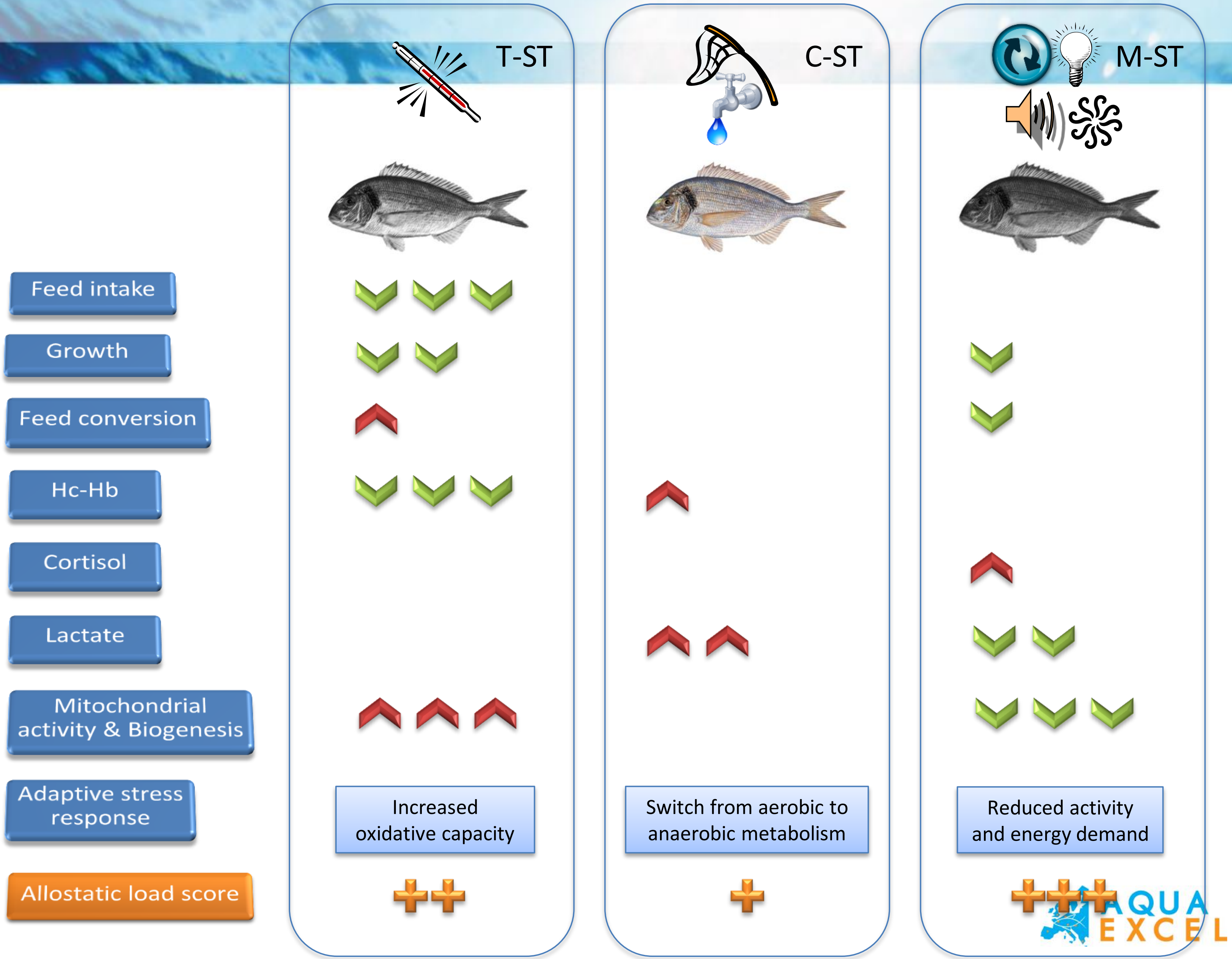
Do not look at the numbers, just the colours



Nuclear transcription factors	PGC1α	5.98*	1.24	0.43*
	NRF1	2.32*	0.88	0.71*
	NRF2	1.8	0.86	0.72
Oxidative metabolism markers	CPT1A	4*	0.91	0.52*
	ACAA2	0.97	0.8	0.66*
	CS	1.81*	0.9	0.64*
Outer membrane translocation	Tom70	1.61*	0.95	0.97
	Tom34	1.44*	1.03	0.84
	Tom22	2.11*	1.29	1.43
Inner membrane translocases (TIM23 complex)	Tim44	1.45*	1.13	0.89
	Tim23	1.38*	1.27	1
	Tim8A	1.04	0.86	0.73*
Inner membrane translocases (TIM22 complex)	Tim10	1.72*	0.93	0.96
	Tim9	1.61*	0.96	0.83
Molecular chaperones	mtHsp10	1.85*	1.19	0.8
	mtHsp60	1.97*	0.86	0.79
	mtHsp70	1.41*	0.98	0.87
	DER-1	1.35*	1.13	0.88
Antioxidant enzyme	GR	1.22*	0.97	1.01
Fusion & Fission markers	FIS1	1.32*	1	0.91
	MFN2	1.42*	0.87	0.69*
	MIFFB	0.92	0.7*	0.73
	MIRO2	1.03	1.08	0.83*
Apoptotic markers	AIFM1	1.24*	0.87	1.15
	BAX	1.14	0.84	0.62*
	BCLX	1.1	0.83	0.68*

The hepatic transcriptome reflects **the type and intensity** of stressor disturbance:

- **T-ST: adaptive response** to cope with the temperature decreased and low basal metabolism
- **C-ST: No response**: fish habituation to stressful condition
- **M-ST: "low power mitochondria"**: low ROS production, adaptive response to an enhanced risk of oxidative stress



	T-ST	C-ST	M-ST
Feed intake	▼▼▼		
Growth	▼▼		▼
Feed conversion	▲		▼
Hc-Hb	▼▼▼	▲	
Cortisol			▲
Lactate		▲▲	▼▼
Mitochondrial activity & Biogenesis	▲▲▲		▼▼▼
Adaptive stress response	Increased oxidative capacity	Switch from aerobic to anaerobic metabolism	Reduced activity and energy demand
Allostatic load score	++	+	+++

AQUA EXCEL

PROTEOMICS

Skin mucus: **non-lethal sample**

1D-Gel electrophoresis

Pooled samples

Trypsin digestion

Liquid Chromatography
Mas Spectrometry
Database search
www.nutrigroup-iats.org/seabreamdb

Mucus proteome mapping

- ✓ **2,062 proteins (score filter > 30)**

SKIN PROTEOME MUCUS MAPPING

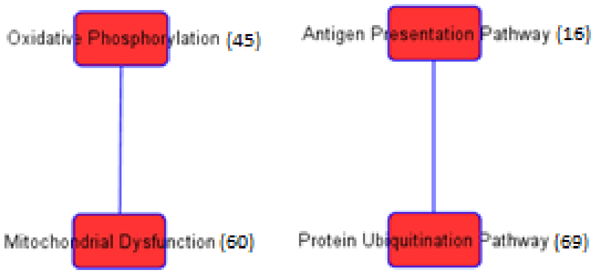
Pathway analysis of proteins with IPA software (www.ingenuity.com)

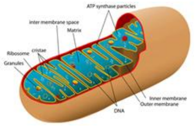
- ✓ 1,848 eligible proteins (89.6 %)
- ✓ 418 IPA pathways (out of 644) Initially represented
- ✓ 17 relevant pathways after filtering for ≥ 3 common proteins among related pathways
- ✓ 3 distinct nodes

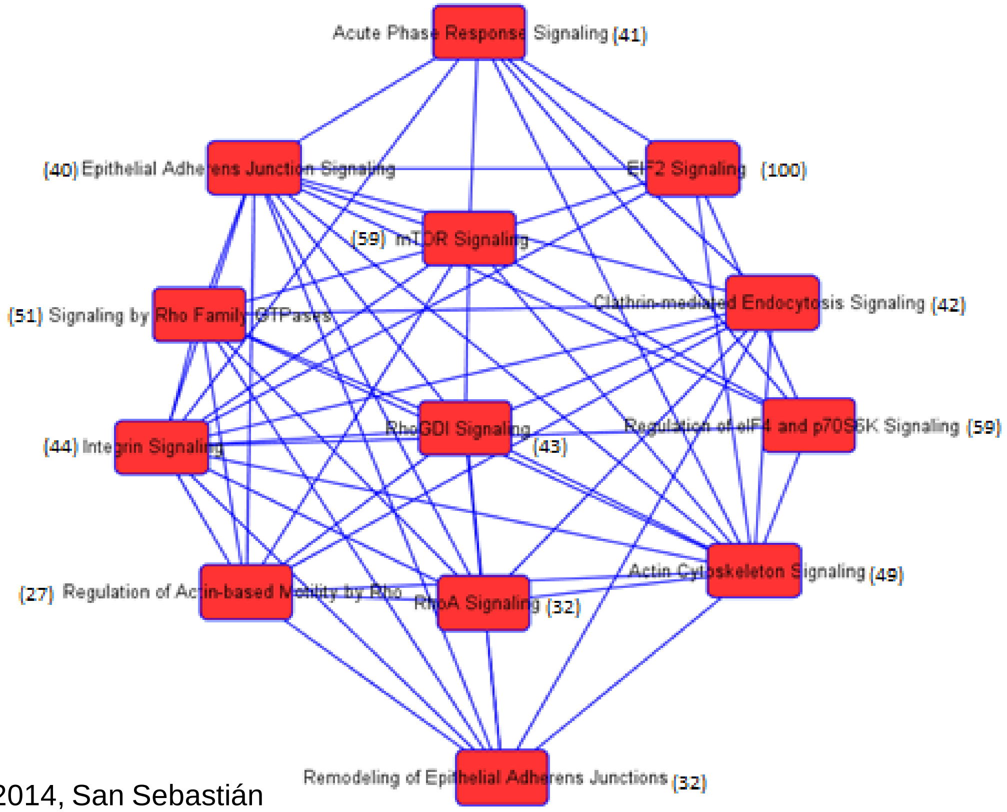
Energy production/apoptosis (60)

Protein degradation/ Th2-immuno response (79)

Protein synthesis/ cellular assembly and organization Th-1 immune response; non-humoral immune response (257)







pathway

IPA® Interactive pathway analysis of complex 'omics data

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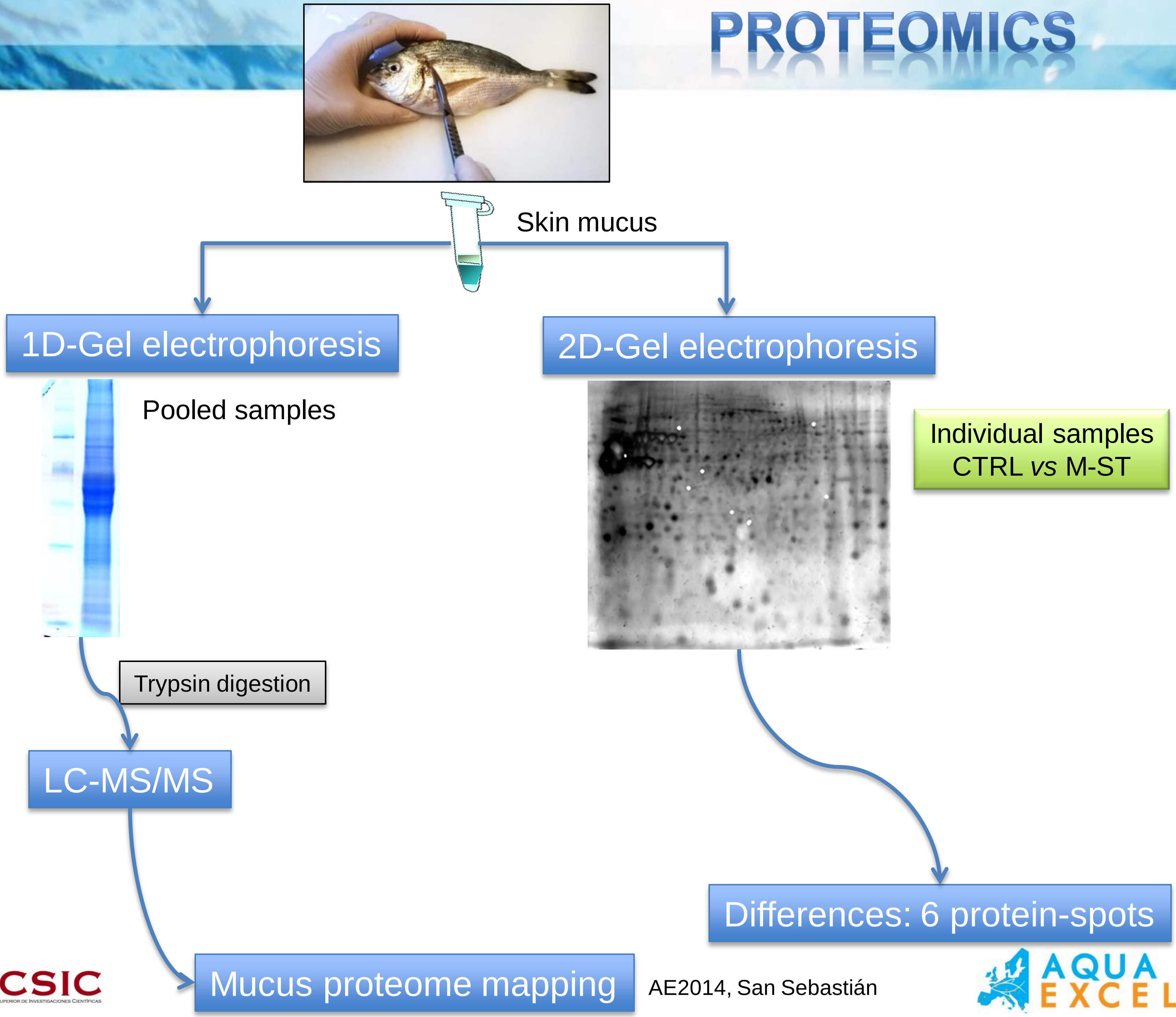
PROTEOMICS



Skin mucus

1D-Gel electrophoresis

Pooled samples

Trypsin digestion

LC-MS/MS

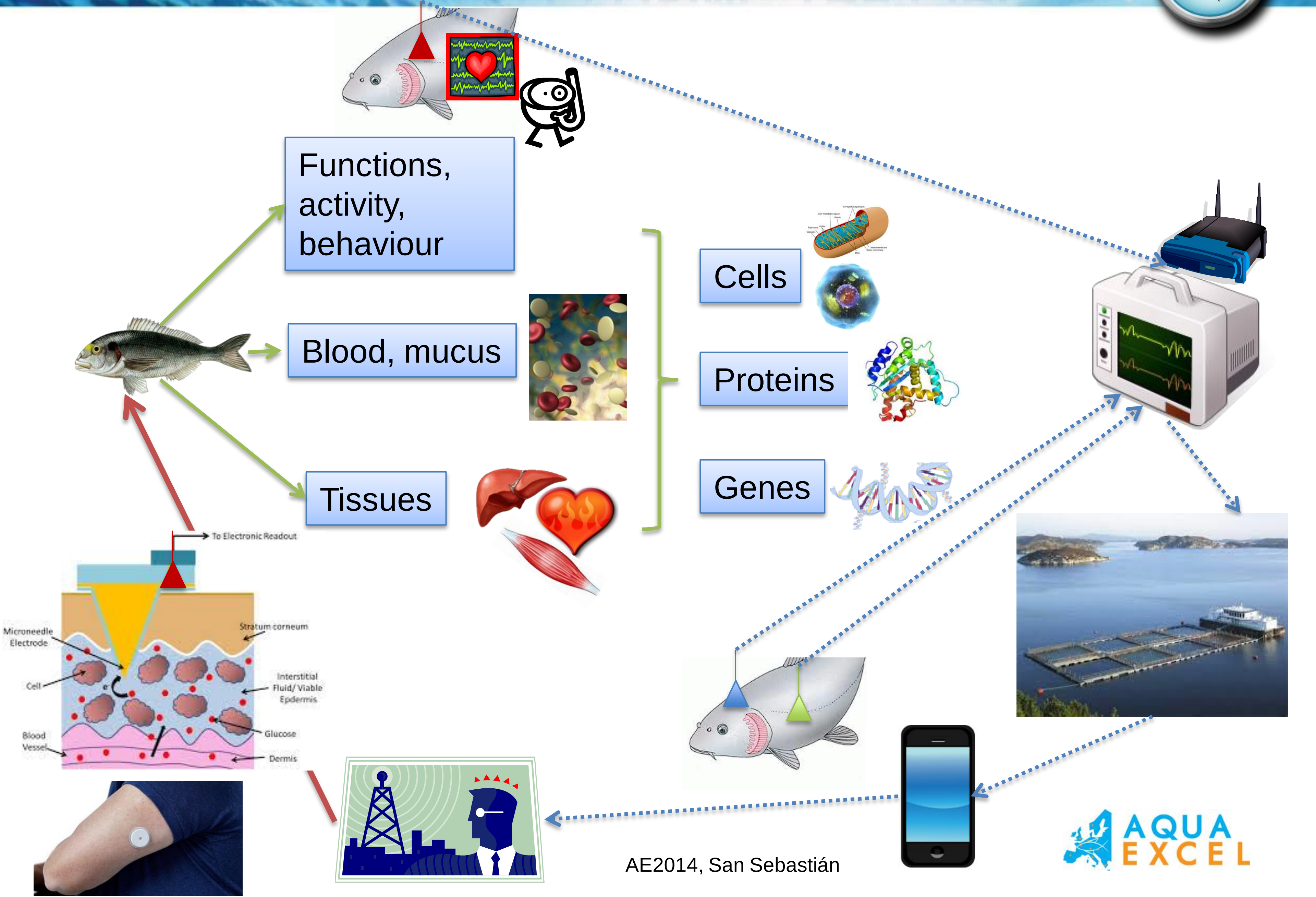
Mucus proteome mapping

2D-Gel electrophoresis

Individual samples
CTRL *vs* M-ST

Differences: 6 protein-spots

FUTURE OUTPUTS: CLOSING THE CIRCLE



Functions, activity, behaviour

Blood, mucus

Tissues

Cells

Proteins

Genes

AE2014, San Sebastián

CONCLUSIONS-TAKE INDUSTRY MESSAGES

- ✓ New genomic and proteomic resources contribute to identify highly informative biomarkers of clinical use in farmed fish

- ✓ We can correlate now the mitochondrial markers with classical measurements and to develop new methods for non-invasive and remote detection
- ✓ We will be able to scrap a fish and to know if it has suffered too much after transport, size sorting or vaccination
- ✓ We will be able to monitor remotely if fish are coping well with the culture conditions
- ✓ We will be able to monitor fish without disturbing them

WHO BENEFITS FROM OUR RESULTS?

- ✓**End- user**: aquaculture industry, scientific community
- ✓**Application**: aquaculture, marine sciences, climate change, environmental management
- ✓**Impact**:
  - ✓**Best monitoring of fish health and welfare**
  - ✓**Provide new insights for unraveling chronic stress in routine aquaculture management**
  - ✓**Help to select for stress resistance fish in broostock selection programs**

AQUA EXCEL

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aquaculture europe 14

Adding Value

Donostia-San Sebastián, Spain
October 14-17, 2014
www.easonline.org

Thank you for your attention

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DISCLAIMER

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