



AQUAculture infrastructures for EXCELLence  
in European Fish research

**Molecular characterization and functional  
regulation of mitochondrial respiratory chain  
in gilthead sea bream (*Sparus aurata*)**

**Aquaculture Europe 2014 - San Sebastián  
17 October 2014**

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Instituto de Acuicultura Torre de la Sal (IATS-CSIC), Spain  
(AQUAEXCEL partner 4)  
[www.nutrigroup-iats.org](http://www.nutrigroup-iats.org)

[www.nutrigroup-iats.org/seabreamdb](http://www.nutrigroup-iats.org/seabreamdb)

*Sparus aurata*  
transcriptomic database

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BLAST your query sequence in FASTA format:

```
MSKSLIAPLLEIPLRLLALARRLLVPPSLIILIIIPRRIILAIPLVPRALVLIIPRLV  
FAPAGAGCTGACTCTCTGCGTGGAAATGCTCTCTTAPRAGAGCGCTGATGCTTCA  
TAACTGAGCTCTCTGCGTAACTTACATTAATAAABAAAACAAAGATATGTTTCA  
TCTCTCAGGGTALLGCGTCTTCACTTCTGAGCCAGCAATANAATCAATGATATTT  
AGSTTAGATAATCACTTCAAGCTTAAAGCTTAACTTGTAGTCACTAGTAAATTT  
CGAGTATCAAGAGCTTCTGAGGTCAGATATTTCTTAAAGCTTCTTAAATCA  
CTGATACTCCAGGAGCCACAGCTTCTGCTCTGCTGCTGAGCACTGATTTGG  
AATACAGATGTCACAGTATTCGCTTTGAGAGTGTAGCTGAGCTAATAAACAATGATCTG
```

Select database and search: Database: seabream\_nr\_cod\_v1

Options: Expect: 10 Matrix: BLOSUM80

Search program: blastn Limit to n best alignments: 50

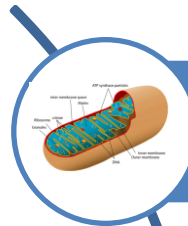
Submit Reset

Search clones by annotation: Annotation containing words: Search mode (for multiple words): Containing all words

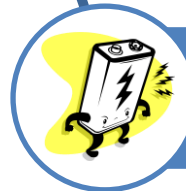
Search clones by GOs: By GO ID: GO ID (eg. GO:0008869): Search

By terms: GOs containing words: Search mode (for multiple words): Containing all words

IATS GOVERNIO DE ESPAÑA MINISTERIO DE ECONOMIA Y COMPETITIVIDAD CSIC Nutrigenomics and Fish Growth Endocrinology Group



Mitochondria main cellular function is ATP production



Number & activity of mitochondria reflect cell energy requirements



Mitochondria is highly regulated at transcriptional and transductional levels



GO term “mitochondrion” (~1100 genes)

926 mitochondrial-related genes are represented in the Nutrigroup transcriptome database

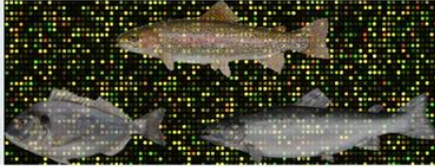
# Sea bream OXPPOS



**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>

Mar Biotechnol (2014) 16:423–435  
DOI 10.1007/s10126-014-9562-3

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

ORIGINAL ARTICLE

**Transcriptional Assessment by Microarray Analysis and Large-Scale Meta-analysis of the Metabolic Capacity of Cardiac and Skeletal Muscle Tissues to Cope With Reduced Nutrient Availability in Gilthead Sea Bream (*Sparus aurata* L.)**

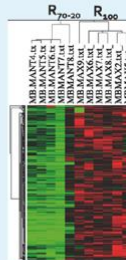
Josep A. Calduch-Giner · Yann Echasserieau · Diego Crespo · Daniel Baron · Josep V. Planas · Patrick Prunet · Jaume Pérez-Sánchez



AQUAEXCEL Partners Present an Online Tool for Meta-Analysis of Fish Genomic Data



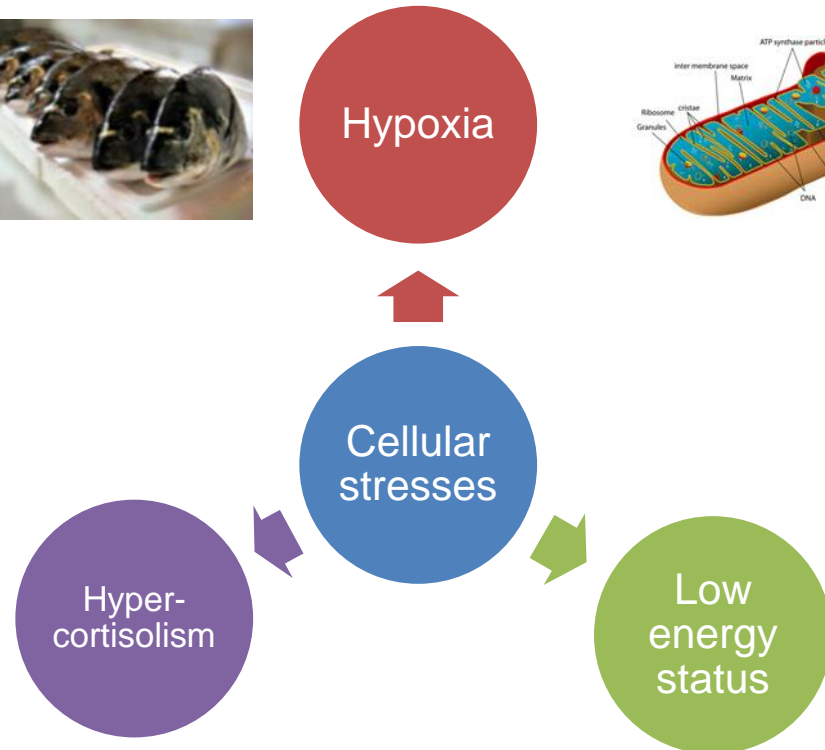
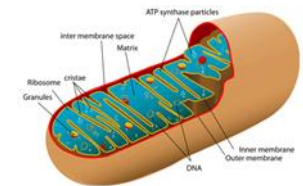
Prof. Jaume Pérez-Sánchez and colleagues at Instituto de Acuicultura Torre de la Sal (IATS-CSIC)



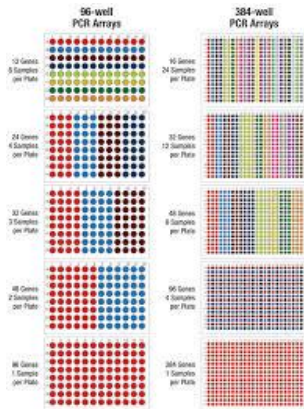
Cluster of genes grouped by Fish and Chips that clearly discriminate experimental groups

**“Fish and Chips”** tool gathers almost all public fish transcriptome data (> 350 microarrays) in a vast array of challenged conditions

This meta-analysis highlights the key role of mitochondria to cope with adaptive cellular stresses in fish and sea bream in particular

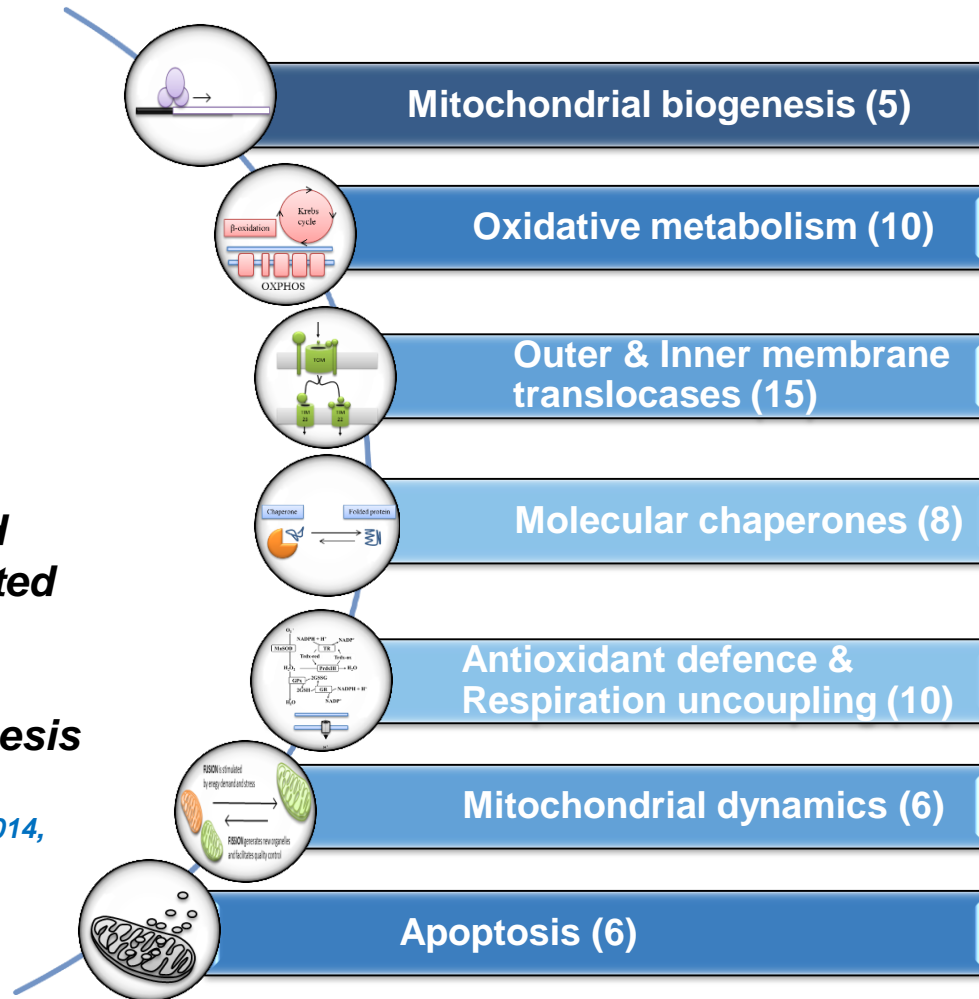


## Sea bream Mito-Chip, 96-well PCR-array



**Pathway-focused array of 60 selected markers of mitochondrial activity & biogenesis**

*Bermejo-Nogales et al., 2014, General and Comparative Endocrinology*



## “Chronic environmental stress”

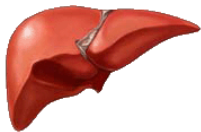
AND YOU THOUGHT  
THERE WAS STRESS  
IN YOUR LIFE



Changes in water temperature



Changes in water level & chasing



Multiple sensory perception random stressors

(Sound & tank shaking, window wiper in water, water flow reversal, light flashes)



INSTITUTE OF MARINE RESEARCH  
HAVFORSKNINGSINSTITUTTET

CSIC  
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

	1	2	3	4	5	6	7	8	9	10	11	12
A	Hsp10	CAT	ACAA2	IDH3B	Tom22	Tim13	MIFFB	PERP			PPC1	PPC1
B	DnaJA3A	GPX4	CPT1A	IDH3G	Tom7	Tim8A	MIRO1A	GABPA			PPC2	PPC2
C	DnaJC20	GR	CPT1B	COX4a	Tom5	Tim22	MIRO2	mtTFA			PPC3	PPC3
D	Hsp60	GST3	ECH	UCP1	Tim44	Tim10	AIFM1	NRF1			PPC4	PPC4
E	Grp-75	PRDX3	HADH	UCP2	Tim23	Tim9	AIFM3	PGC1 $\alpha$			NPC	NPC
F	DER-1	PRDX5	CS	UCP3	Tim17A	FIS1	BAX	PGC1 $\beta$			ACTB	ACTB
G	ERdj3	SOD1	IDH1	Tom70	Tim16	MFN1	BCL2					
H	Grp170	SOD2	IDH3A	Tom34	Tim14	MFN2	BCLX					

## Chronic stress phenotyping



Nuclear transcription factors	PGC1 $\alpha$	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
	mtHsp10	1.85*	1.19	0.8
Molecular chaperones	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:** Phenotype of “low power mitochondria”/low ROS production/ adaptive response to cope with an enhanced risk of oxidative stress

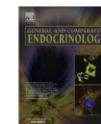
General and Comparative Endocrinology 205 (2014) 305–315



Contents lists available at ScienceDirect

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journal homepage: [www.elsevier.com/locate/ygcen](http://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



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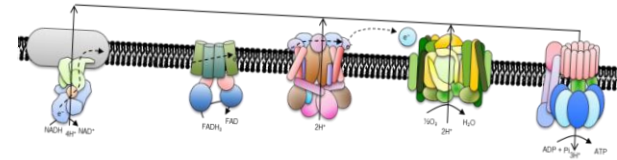
<sup>c</sup> Institute of Marine Research Matre, 5984 Matredal, Norway

<sup>d</sup> 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

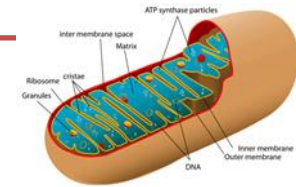


## OBJECTIVES

1. To define the molecular identity of an almost complete set of enzyme subunits of OXPHOS pathway (catalytic, regulatory, assembly factors)
2. To develop a powerful pathway-focused array for simultaneous gene expression profiling of OXPHOS (96-well PCR-array/automated liquid handling robot)
3. To identify the most responsive enzyme subunits of OXPHOS pathway in a tissue-specific manner, “fasting model”



## MOLECULAR IDENTITY

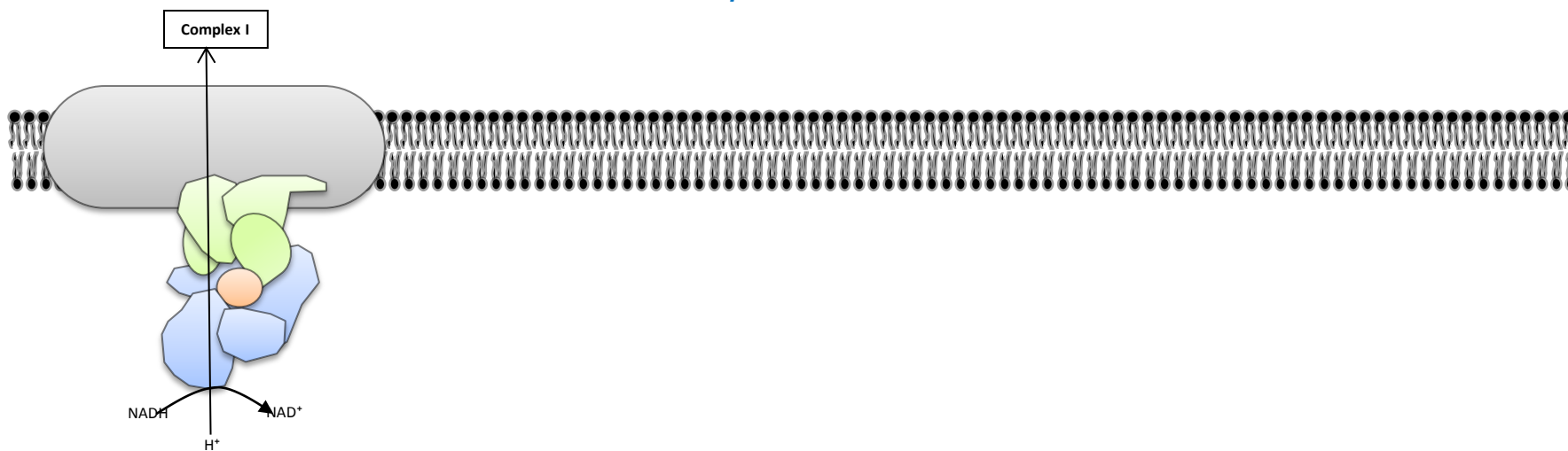


- Extensive BLAST searches  
[www.nutrigroup-iats.org/seabreamdb](http://www.nutrigroup-iats.org/seabreamdb)
- Nucleotide sequence analysis & manual curation
- Unequivocal annotation of an almost complete set of mitochondrial- and nuclear-encoded genes of OXPHOS pathway
  - 97 new sea bream sequences uploaded to GenBank (KC217558-KC217654)



**Complex I** is the largest enzyme complex varying from 14 subunits in prokaryotes to 45 in mammals

*NADH functions as electron donor of Complex I*



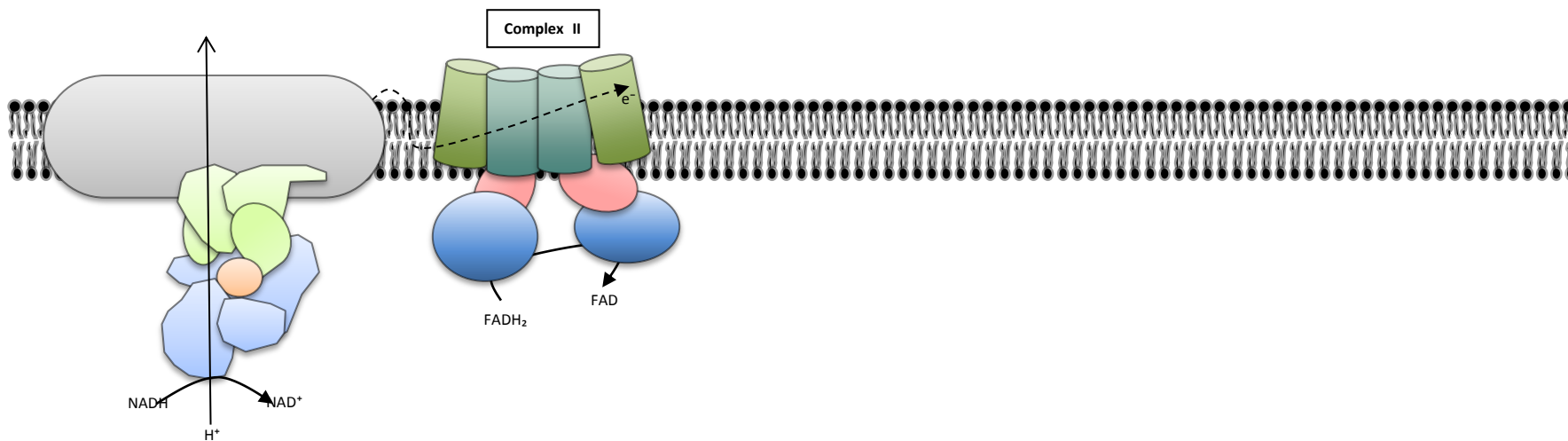
- **Sea bream Complex I**
- 40 enzyme subunits (4 mtDNA+ 37 nDNA)
- 1 assembly protein

**Bold:** catalytic subunits (mtDNA)  
 Regular: catalytic & regulatory subunits (nDNA)  
*Italics:* assembly proteins (nDNA)

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														

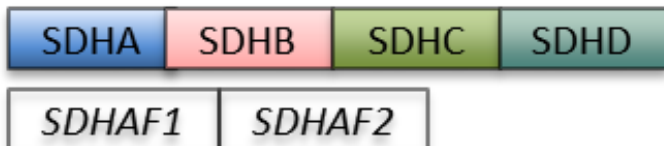
## Complex II is entirely encoded by nDNA

*FADH<sub>2</sub> functions as electron donor of Complex II*



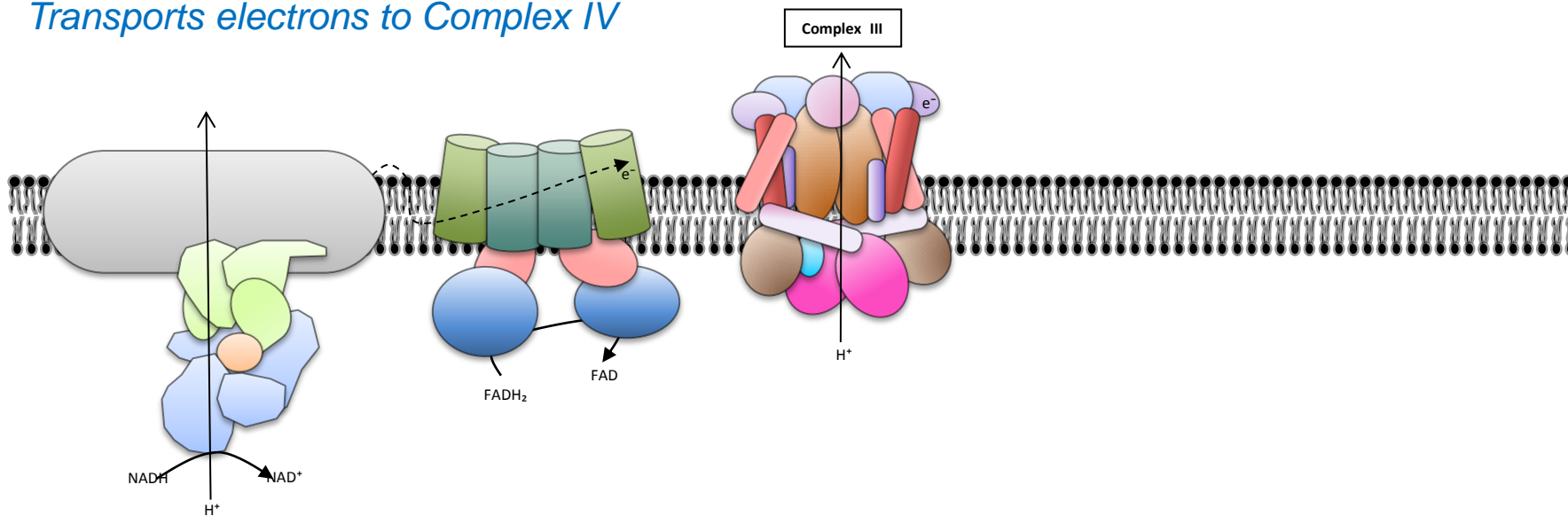
- **Sea bream Complex II**
- 4 enzyme subunits
- 2 assembly proteins

**Bold:** catalytic subunits (mtDNA)  
Regular: catalytic & regulatory subunits (nDNA)  
*Italics:* assembly proteins (nDNA)



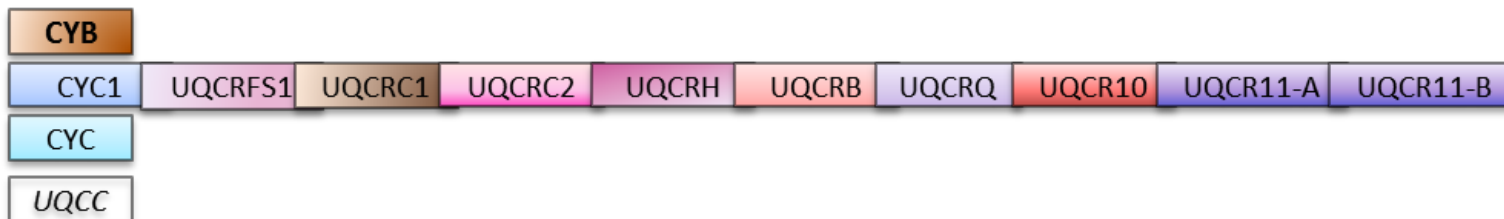
## Complex III is encoded by nDNA with the exception of CYB

*Transports electrons to Complex IV*



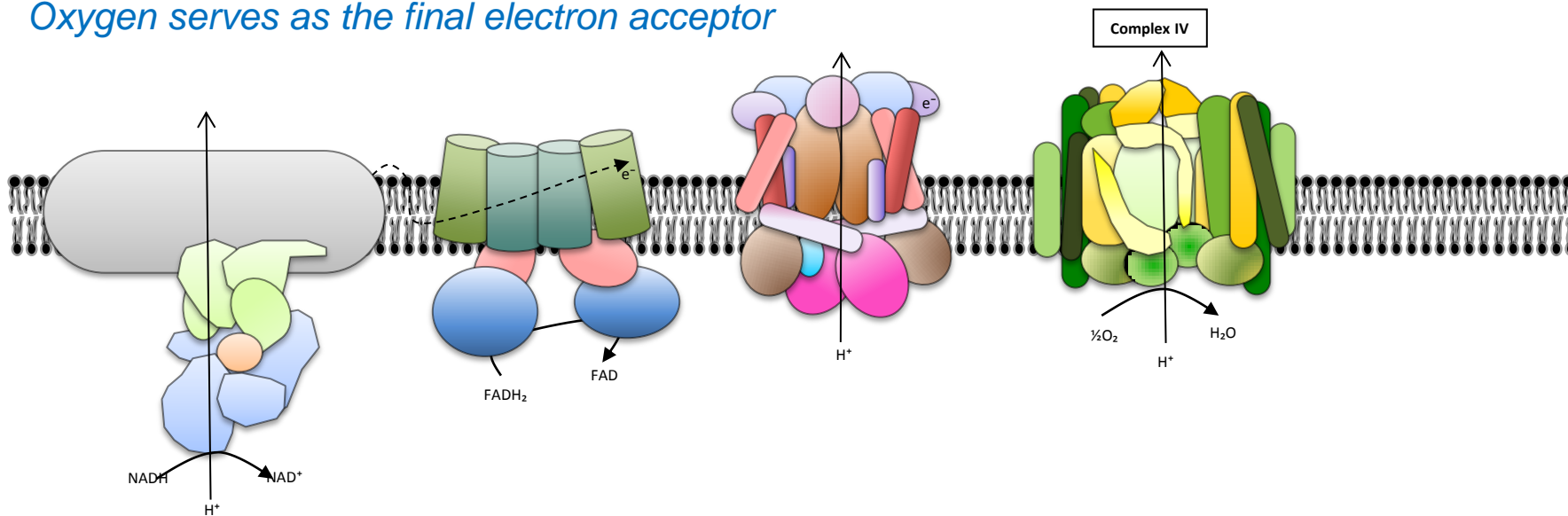
- **Sea bream Complex III**
- 12 enzyme subunits (1mtDNA +11nDNA)
- 1 assembly proteins

**Bold:** catalytic subunits (mtDNA)  
 Regular: catalytic & regulatory subunits (nDNA)  
*Italics:* assembly proteins (nDNA)



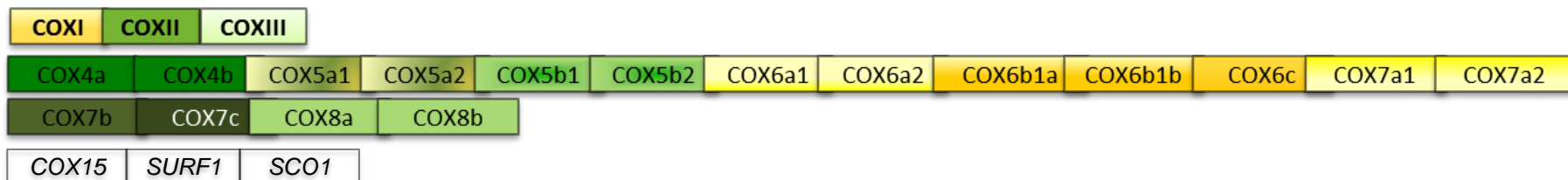
## Complex IV is encoded by mtDNA and nDNA

*Oxygen serves as the final electron acceptor*

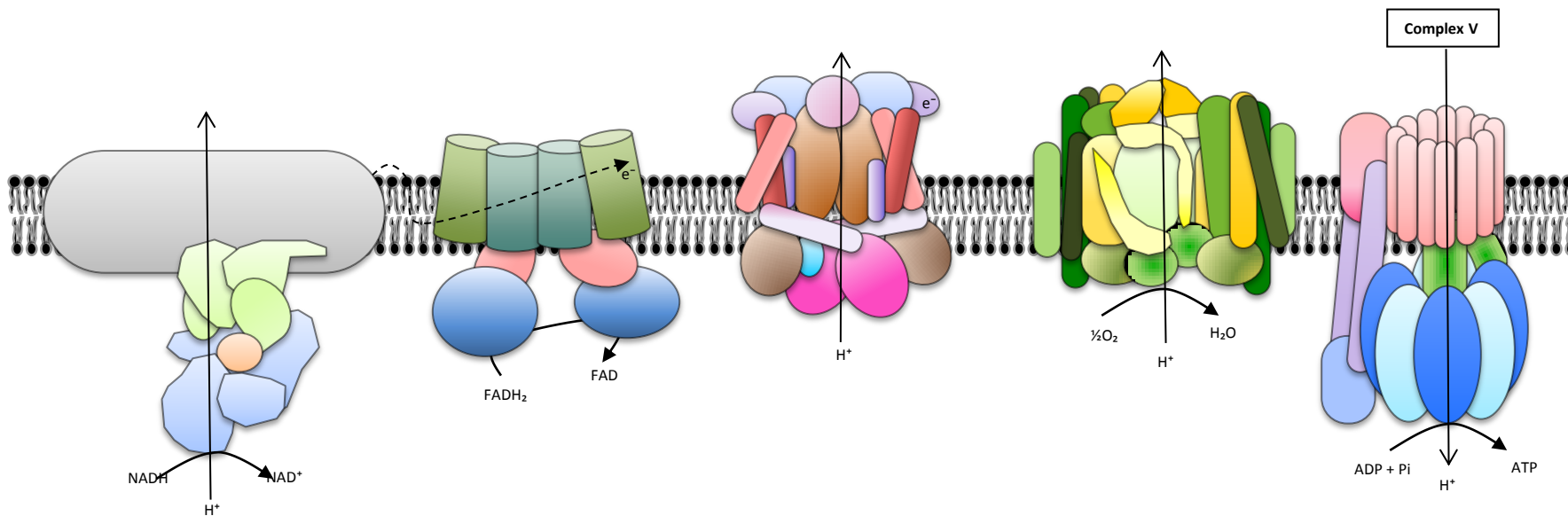


- **Sea bream Complex IV**
- 20 enzyme subunits (3 mtDNA + 17 nDNA)
- 3 assembly proteins

**Bold:** catalytic subunits (mtDNA)  
 Regular: catalytic & regulatory subunits (nDNA)  
*Italics:* assembly proteins (nDNA)



## Complex V couples proton gradient to ATP synthesis



- **Sea bream Complex V**
- 15 enzyme subunits (2 mtDNA + 13 nDNA)
- 1 assembly protein

**Bold:** catalytic subunits (mtDNA)  
**Regular:** catalytic & regulatory subunits (nDNA)  
*Italics:* assembly proteins (nDNA)





## Two pathway-focused arrays for mitochondrial gene expression profiling

[www.nutrigroup-iats.org](http://www.nutrigroup-iats.org)

### MITO-chip



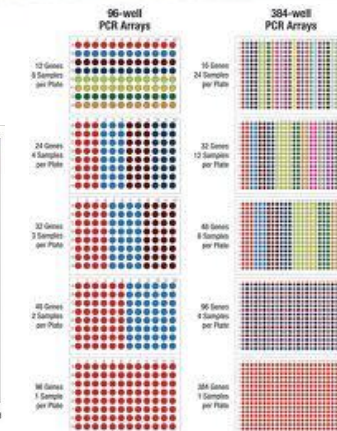
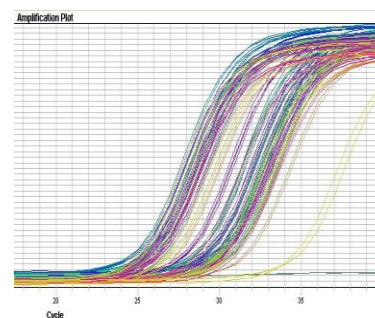
- 60 genes
- 52 new sequences
- Oxidative metabolism & respiration uncoupling, 13
- Antioxidant defence, 7
- Protein transport/ folding/assembly, 23
- Mitochondria dynamics, 6
- Apoptosis, 5

### OXPHOS-chip

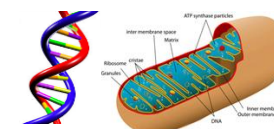
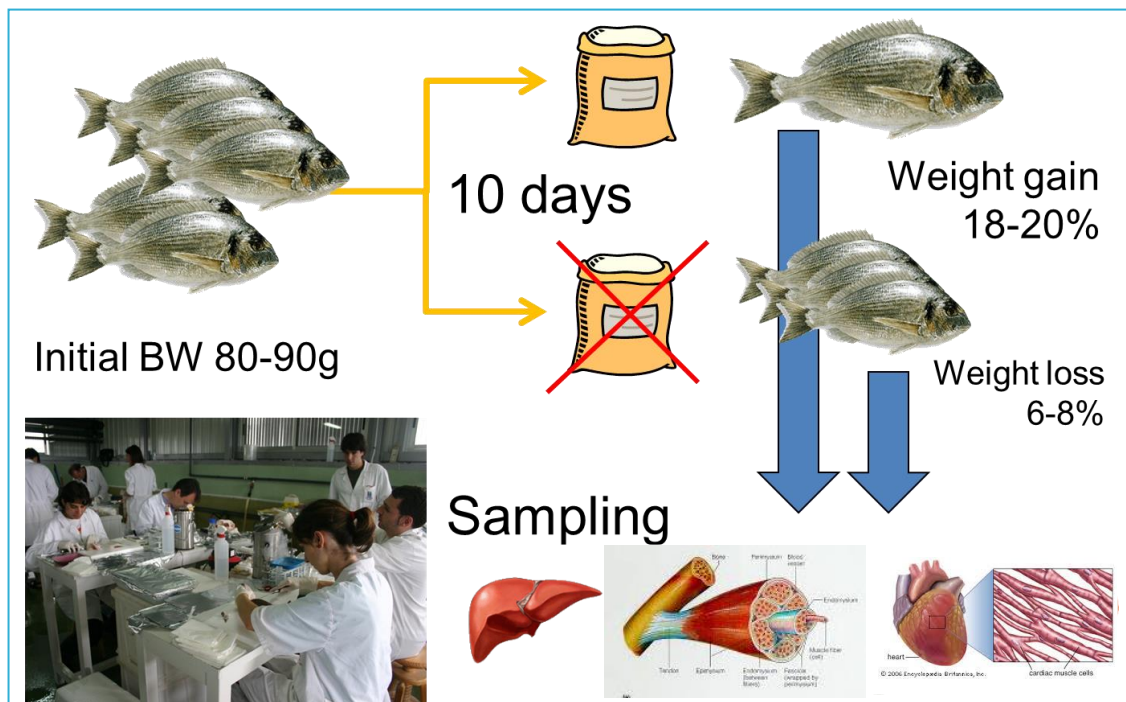


- 88 genes
- 86 new sequences
- Complex I, 34
- Complex II, 6
- Complex III, 13
- Complex IV, 22
- Complex V, 13

Figure 2. Automated liquid handling robot



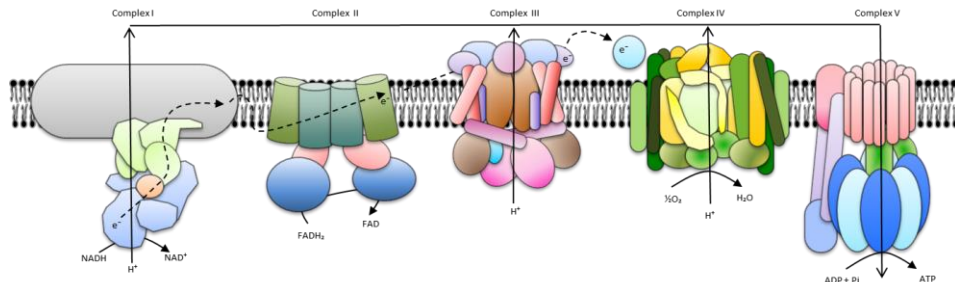
## Experimental design "Fasting model"



**OXPHOS-CHIP**



Liver, fasting down-regulated response  
72 out of 88 are differentially regulated genes



**Red:** catalytic subunits, **mtDNA/nDNA** encoded  
**Black:** regulatory subunits, **nDNA** encoded  
**Blue:** assembly proteins, **nDNA** encoded

## Complex I

<b>ND2</b>	<b>0.37</b>	NDUFB2	0.49
<b>ND5</b>	<b>0.31</b>	NDUFB3	0.70
NDUFA1	0.51	NDUFB4	0.47
NDUFA2	0.57	NDUFB5	0.57
NDUFA3	0.44	NDUFB6	0.54
NDUFA4	0.61	NDUFB9	0.57
NDUFA4-L2	0.50	NDUFC1	0.64
NDUFA5	0.47	<b>NDUFS2</b>	0.36
NDUFA6	0.72	NDUFS4	0.48
NDUFA7	0.69	NDUFS5	0.58
NDUFA8	0.63	<b>NDUFS7</b>	0.49
NDUFA9	0.45	<b>NDUFV1</b>	0.48
NDUFB10	0.52	<b>NDUFV2</b>	0.51
NDUFB11	0.50	NDUFV3	0.53
NDUFA12	0.52	<b>NDUFAF2</b>	0.47

## Complex II

<b>SDHA</b>	0.47
SDHC	0.45
SDHD	0.42
<b>SDHAF2</b>	0.42

## Complex III

<b>CYB</b>	<b>0.53</b>
<b>UQCRFS1</b>	<b>0.57</b>
UQCR1	0.64
UQCR2	0.51
UQCRH	0.67
UQCRB	0.56
UQCRQ	0.50
UQCR10	0.40
UQCR11-A	0.69
UQCR11-B	0.63

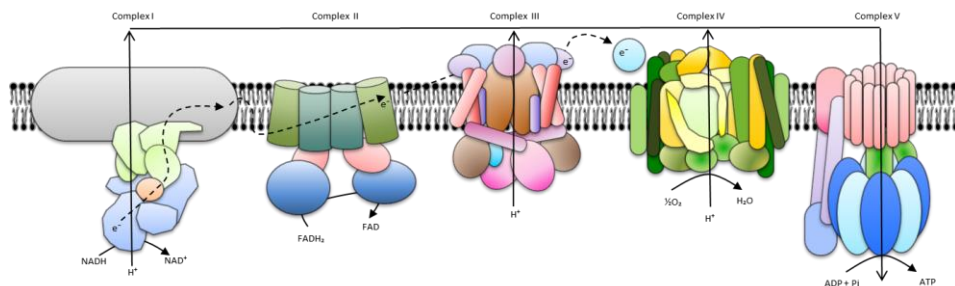
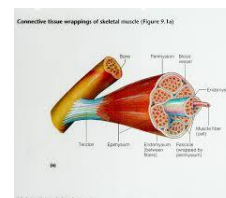
## Complex IV

<b>COXI</b>	<b>0.50</b>
<b>COXII</b>	<b>0.38</b>
<b>COXIII</b>	<b>0.40</b>
COX4a	0.69
COX4b	0.47
COX5a2	0.44
COX5b2	0.44
COX6a2	0.34
COX6b1a	0.56
COX6b1b	0.58
COX6c1	0.39
COX7a1	0.45
COX7a2	0.43
COX7b	0.19
COX7c	0.39
COX8b	0.35
<b>SURF1</b>	0.66

## Complex V

<b>ATP5A1</b>	0.48
<b>ATP5B</b>	0.31
<b>ATP5C1</b>	0.37
<b>ATP5D</b>	0.55
<b>ATP5E</b>	0.40
ATP5F1	0.51
ATP5G1	0.38
ATP5I	0.54
ATP5J2	0.38
ATP5L	0.63
ATP5O	0.35

**WSK (glycolytic muscle), fasting up-regulated response**  
 29 out of 88 are differentially regulated genes



**Red:** catalytic subunits, **mtDNA/nDNA** encoded  
**Black:** regulatory subunits, **nDNA** encoded  
**Blue:** assembly proteins, **nDNA** encoded

### Complex I

NDUFA1	1.35
NDUFA4	1.28
NDUFA6	1.45
NDUFA7	1.72
NDUFA8	1.29
NDUFB5	1.68
NDUFB9	1.46
NDUFB10	1.45
NDUFC2	1.54
NDUFS4	1.43
<b>NDUFS7</b>	1.60
<b>NDUFAF2</b>	2.30

### Complex II

<b>SDHB</b>	1.56
SDHC	1.52
SDHD	1.43

### Complex III

<b>UQCRRS1</b>	<b>1.39</b>
UQCRC1	1.98
UQCRC2	1.95
UQCRH	1.53
UQCRQ	1.46
UQCR10	1.70
<b>UQCC</b>	<b>2.03</b>

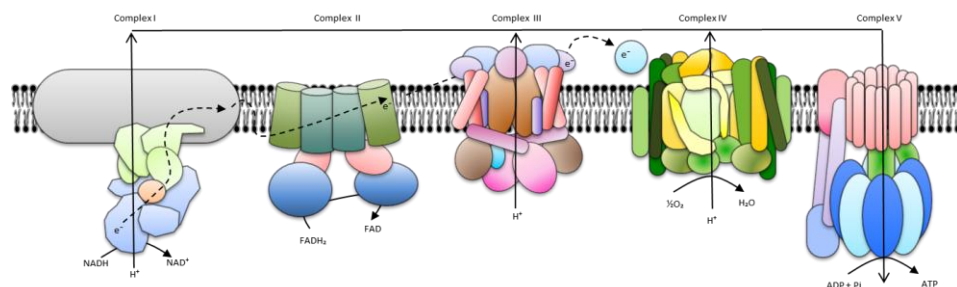
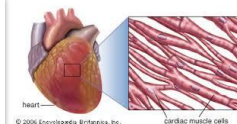
### Complex IV

COX5A1	1.87
COX5A2	1.90
COX8B	1.44
<b>SCO1</b>	<b>2.78</b>
<b>SURF1</b>	<b>1.93</b>

### Complex V

ATP5O	1.56
<b>ATP5A1</b>	<b>1.55</b>

Cardiac muscle (highly aerobic tissue), fasting up-regulated response  
10 out of 88 are differentially regulated genes



**Red:** catalytic subunits, **mtDNA/nDNA** encoded  
**Black:** regulatory subunits, **nDNA** encoded  
**Blue:** assembly proteins, **nDNA** encoded

## Complex I

NDUFA4	1.77
NDUFAF2	1.42

## Complex II

SDHAF1	1.32
SDHAF2	1.31

## Complex III

<b>CYB</b>	<b>1.61</b>
------------	-------------

## Complex IV

<b>COXI</b>	<b>1.78</b>
<b>COXII</b>	<b>1.71</b>
<b>COXIII</b>	<b>1.80</b>
COX6b1a	1.40
<b>SURF1</b>	<b>1.40</b>

## OXPHOS regulation by fasting “malnutrition/caloric restriction”



### LIVER

- Down-regulation
- mtDNA/nDNA
- Catalytic regulatory and assembly factors

Concurrent down-regulation of energy generating & energy demanding (lipogenesis) processes

P105



**WIDE-GENE EXPRESSION ANALYSIS OF LIPID-RELEVANT GENES IN NUTRITIONALLY CHALLENGED GILT HEAD SEA BREAM (*Sparus aurata*)**

LAURA BENEDITO-PALOS, GABRIEL F. BALLESTER-LOZANO, JAUME PEREZ-SÁNCHEZ  
Instituto de Acuicultura Torre de la Sal (IATS-CSIC), 12050 Barris de Calanes, Castellón, Spain  
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**INTRODUCTION**

The use of new tools based on alternative sources to marine raw materials has been successful in several fish species, including gilthead sea bream (1). However, concerted efforts are still needed to undertake in depth the long-term consequences of feeding very low fish meal and fish oil diets and new knowledge is welcome to the gaps in a wide range of proteins and lipid metabolic disorders, closely related with reduced growth and survival. Therefore, there is a strong need for integrative tools that are capable of describing and predicting any metabolic disturbance of lipid metabolism in fish.

**HIGHLIGHTS**

- Forty lipid-relevant genes were simultaneously analyzed by PCR array.
- The expression profile of this gene subset is highly tissue-specific.
- The most responsive genes in nutritionally challenged fish were identified for each tissue.

**OBJECTIVES**

To characterize the transcriptional profile of the tissue-specific regulation of lipid metabolism in nutritionally challenged fish.

To define a state set of highly informative lipid metabolic markers of nutritional condition for a given tissue.

**MATERIALS & METHODS**

- Experimental setup
- Gene expression analysis

**RESULTS**

The top 40 transcriptional genes highlighted the different tissue metabolic capabilities.

**CONCLUDING REMARKS**

- Breeds is highly refractory to changes in nutrient availability.
- Over the first 30 days larvae show changes in OXPHOS, mitochondrial and "lipidic metabolism".
- The sea bass LPL isoforms are key enzymes of tissue FA uptake with a different regulation in adipogenic and muscle tissues.
- This new knowledge will contribute to define new different gene expression patterns integrate with the selection indexes for fast growth and efficient use of alternative diets in intensive fish farming.

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### HEART

- Up-regulation
- mtDNA/nDNA
- Catalytic and assembly factors

### SKELETAL MUSCLE

- Up-regulation
- nDNA
- Regulatory and assembly factors

Enhanced oxidative capacity with the reduction of metabolic fuel supply



## MAIN CONCLUDING REMARKS

1. The molecular identify of almost all the components of the OXPHOS pathway has been established for the first time in a farmed fish species
2. This was the basis for the completion of mitochondria pathway-focused analysis tool to assess the health and metabolic condition of fish exposed to environmental and/or nutritional stressors
3. The OXPHOS regulation by fasting reflects the different metabolic capabilities of liver, glycolytic SKM and the highly oxidative cardiac muscle
4. These metabolic features are regulated in a tissue-specific manner by mitochondrial- and nuclear-encoded genes involving catalytic, regulatory and assembly factors
5. These new insights and genomic resources contribute to identify for a given tissue highly informative biomarkers of clinical use in farmed fish



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## **AQUAEXCEL INDUSTRY WORKSHOP:**

Research Infrastructures: adding value to  
European aquaculture industry

Friday, 17<sup>th</sup> October  
Kicking off at 10.30am  
Room 11 (Exhibition Area)

**See you there!**



# Contact us

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attention**

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