



# International Congress of Comparative Endocrinology



## Mitochondria as key responders of metabolic and hormonal disturbances in fish. Meta-analysis flowchart of transcriptomic data in gilthead sea bream (*Sparus aurata*)

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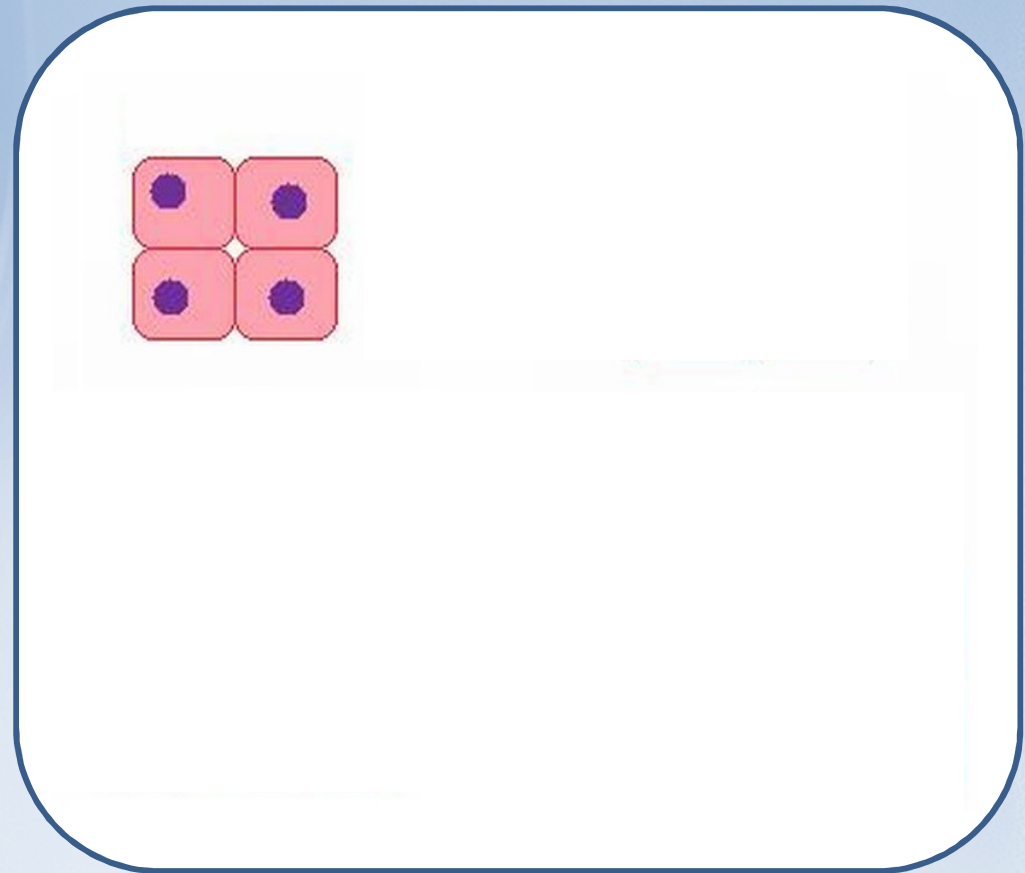
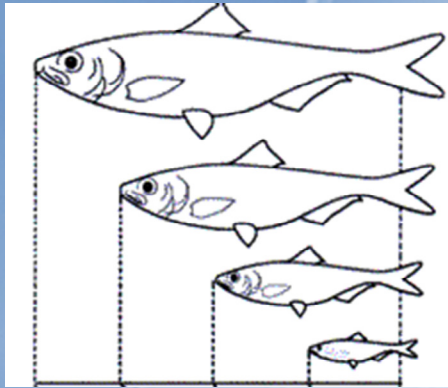
<sup>2</sup>*Laboratoire de Physiologie et Génomique des Poissons (INRA), Rennes, France*



Barcelona, July 2013



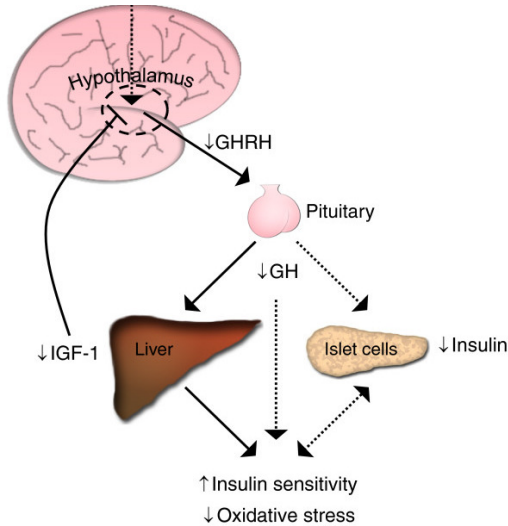
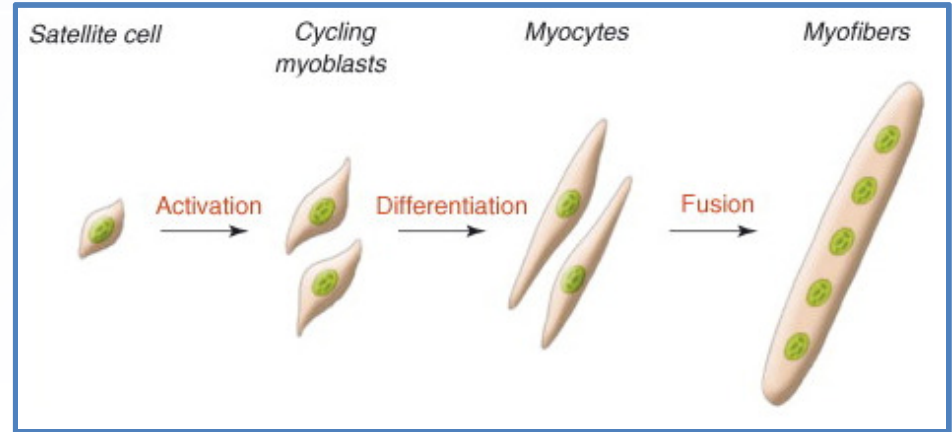
# Muscle Growth



- ✓ Hyperplasia and hypertrophy determine:
  - ✓ Growth potential
  - ✓ Final weight

# Key targeted genes of muscle growth

- Myogenic regulatory factors
  - myoD, mf5, myogenin, MRF4...
- Insulin & somatotropic axes



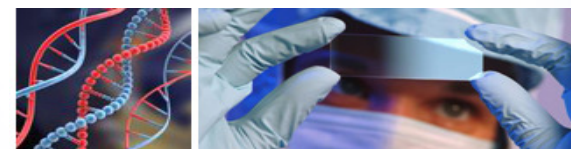
Fast growth phenotype for a given nutritional and developmental condition in sea bream:



- ✓ Low circulating GH levels
- ✓ Enhanced hepatic GHR-I & IGF-I expression
- ✓ High circulating levels of IGF-I
- ✓ Enhanced skeletal muscle expression of IGF-II & GHR-II

Pérez-Sánchez et al., Fish Physiol. Biochem. 2002  
 Saera-Vila et al., J. Endocrinol. 2007  
 Benedito-Palos et al., Aquaculture 2007  
 Saera-Vila et al., Comp. Biochem. Physiol. A 2009

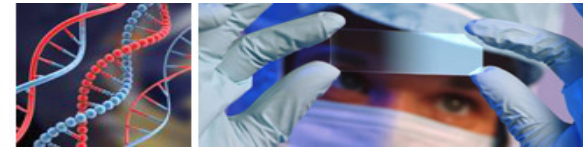
# Genomic tools: new targeted genes and processes



## ▪ Sea bream microarrays

Array type	Target tissue	Challenge	Reference
cDNA microarray	Kidney	Cortisol administration	Sarropoulou et al., <i>Physiol. Genomics</i> 2005
Oligo-array	Liver	Cortisol administration	Teles et al., <i>Mar. Biotechnol.</i> 2013
Oligo-array	Whole larva	Ontogeny	Ferraresso et al., <i>BMC Genomics</i> 2008
Oligo-array	Skin/scales	Tissue damage	Vieira et al., <i>BMC Genomics</i> 2011
cDNA microarray	Liver	Crowding stress	Calduch-Giner et al., <i>BMC Genomics</i> 2010
cDNA microarray	Intestine	Parasite infection	Davey et al., <i>Mol. Immunol.</i> 2011
Oligo-array	Intestine	Parasite x nutritional	Calduch-Giner et al., <i>BMC Genomics</i> 2012

# Genomic tools: new targeted genes and processes




- Next-generation sequencing (sea bream)

NGS	Target tissue	Reference
454	Whole larvae	Yúfera et al., Mar. Biotechnol. 2012
454	<b>Skeletal muscle</b>	García de la Serrana et al., BMC Genomics 2012
454 + SSH	<b>Skeletal muscle</b> , intestine , head kidney, blood, liver, adipose tissue, brain, gills...	Calduch-Giner et al., BMC Genomics 2013

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

High coverage of the codifying sea bream transcriptome

*Sparus aurata*  
transcriptomic database





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**BLAST your query sequence in FASTA format:**

```

CTCATGCTGTCGGTATGTCCTCTGGGCGTCTCTTCAGCCGATCACAGACGGCCAGCGTCTGTTCCCA
TCGCTGTCAGCAGAGTTCAACACCTCCACCTGCTGGCTCAGAGACTCTTCTGACTTTGAGAGCTCTCT
GCAGACGGAGGAGCAGCCGAGCTCAACAAAATCTTCTCGAGGATTTCTGTAAGTGTGATTACATCATC
AGCCCCATCGACAAGCAGCAGAGACGAGCGAGCTCAGTGTGAAAGCTGCTGTATCTCTTATCGATTGG
TCGAGTCTTGGAGTTCCCGAGTCTGTCCTGCTGGCGGTTCTGCTCCGAGGAAACAGATTCACCCAA
ACTGTCTGAGCTGAAGAGCGGATCCATCTCTGATCAGGGCCATGAGAGCGGACAGATCTTCCCT
GATAGTCCGCCCTCCAGCTGGCTCTTATGAAACTACTACCAAAAGTCTGGGCCAGCAGTCCGCTGA
GACGAACC
        
```

**Select database and search:**

Database: Annotated transcriptome

**Options:**

Expect: 10 Matrix: BLOSUM80

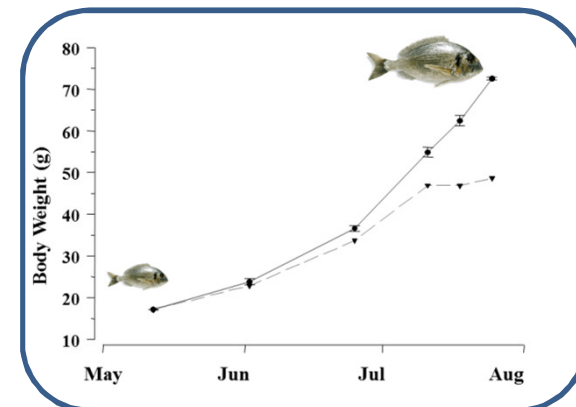


# Gene expression profiling of cardiac and skeletal muscle tissues



## ■ Experimental design & setup:

- Sea bream juveniles (15 g initial BW)
- Restricted nutrient availability (11 wks)
- Two experimental groups:
  - Full ration size (**R100**)
  - 70% full ration + finishing phase at the maintenance ratio (**R70-20**)



# Gene expression profiling of cardiac and skeletal muscle tissues




## ▪ Analytical tools:

### ▪ **Aquagenomics** Oligo-array:

- 4 x 44 K design format
- 15,845 sequences with 2-3 probes each

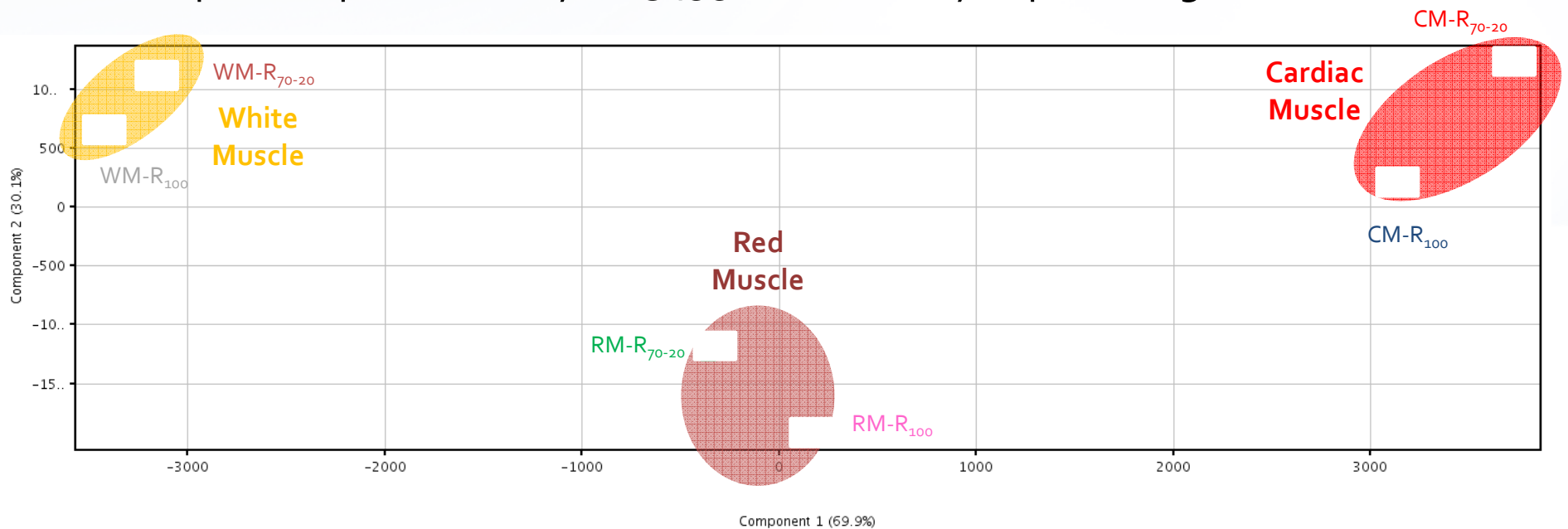
### ▪ **Fish and Chips Tool** (<http://fishandchips.genouest.org>):

- **334** datasets from zebrafish, European Sea bass, Atlantic cod, longjaw mudsucker, rainbow trout and Atlantic salmon microarray experiments
- **21** datasets from **sea bream** microarray experiments 
- Hierarchical gene clustering and **comparison of clusters among datasets**

# Gene expression profiling of cardiac and skeletal muscle tissues



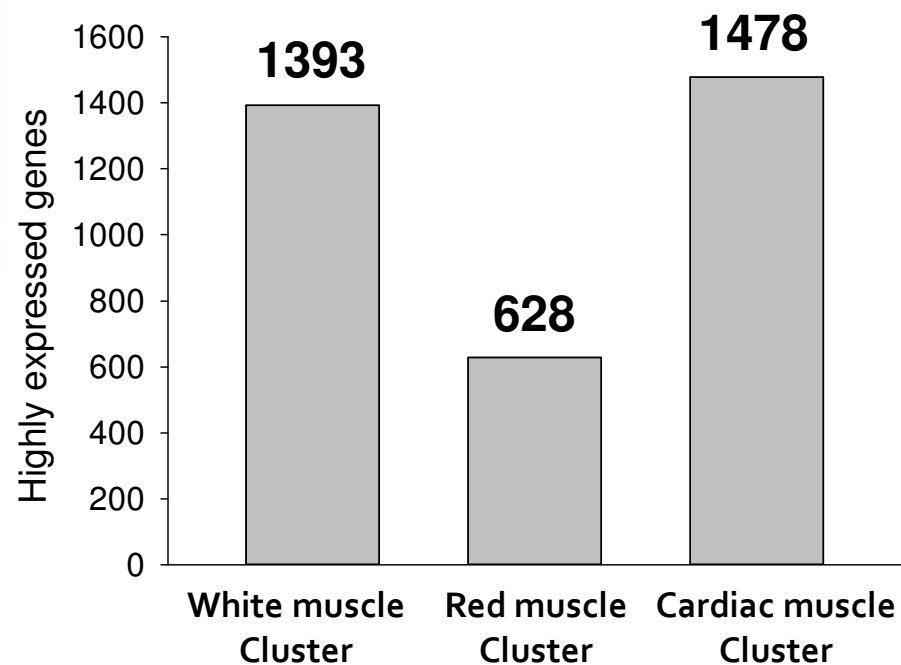
- Principal component analysis (3499 differentially expressed genes)



- ✓ **70% of variation** is explained by the tissue component
- ✓ Tissue distribution along X-axis follows tissue **oxidative capacity: WM < RM < CM**



# Gene expression profiling: K-means clustering

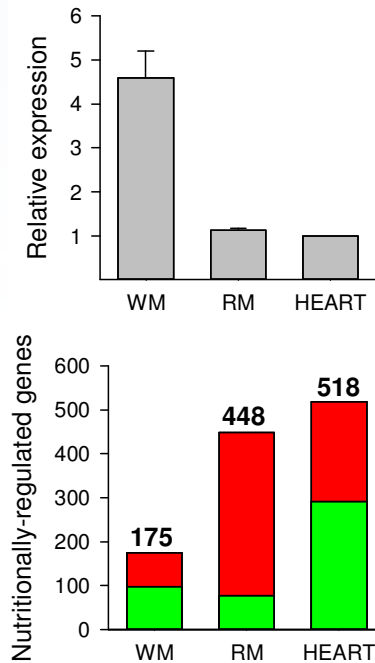


- Definition of **3 clusters** according to **transcript abundance** in a given tissue

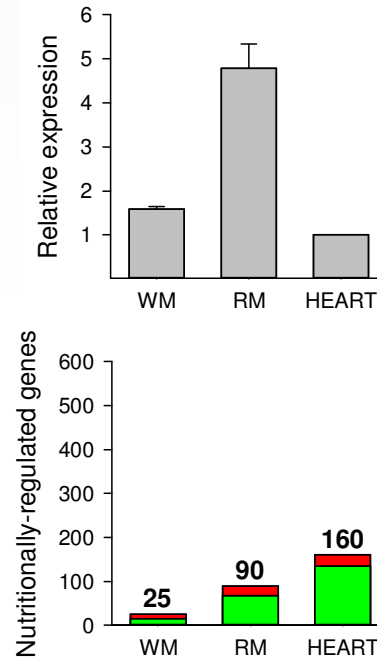
# Cluster phenotyping



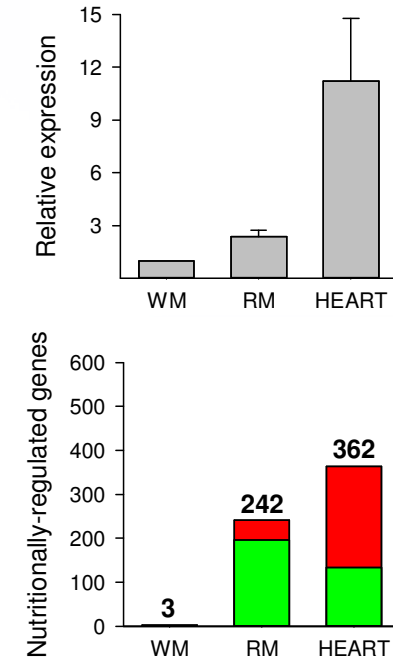
## White muscle cluster



## Red muscle cluster



## Cardiac muscle cluster



- ✓ WM is **poorly** transcriptionally regulated
- ✓ Heart is **highly** transcriptionally regulated

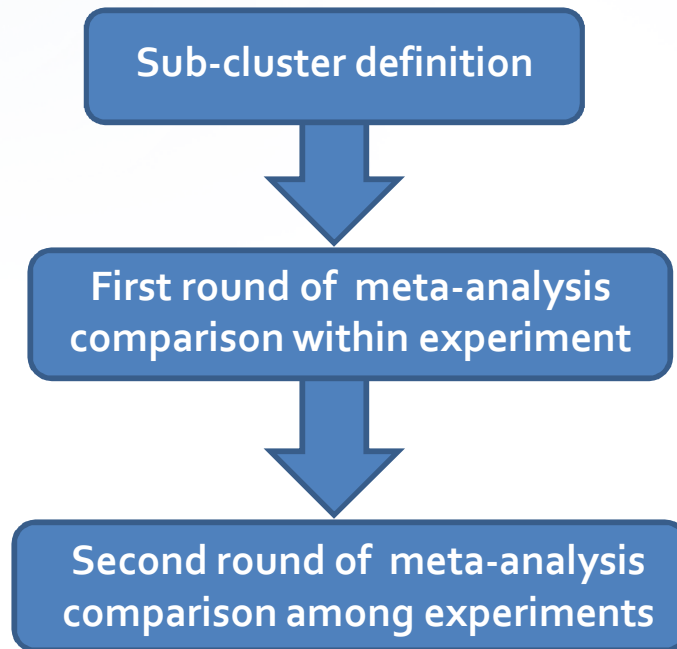
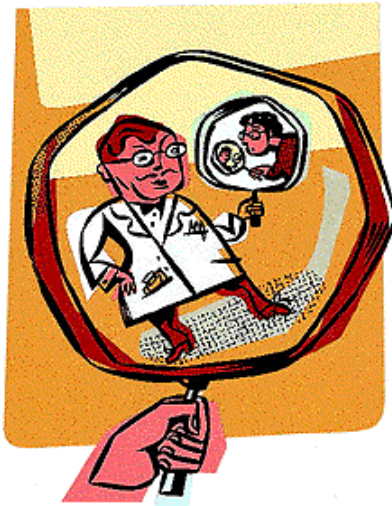
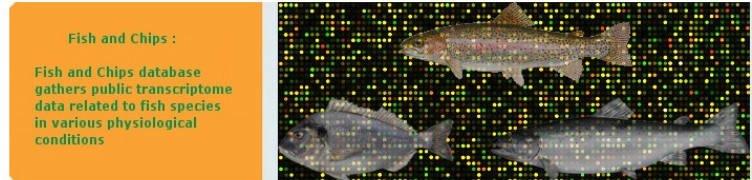


Different metabolic plasticity & survival functions

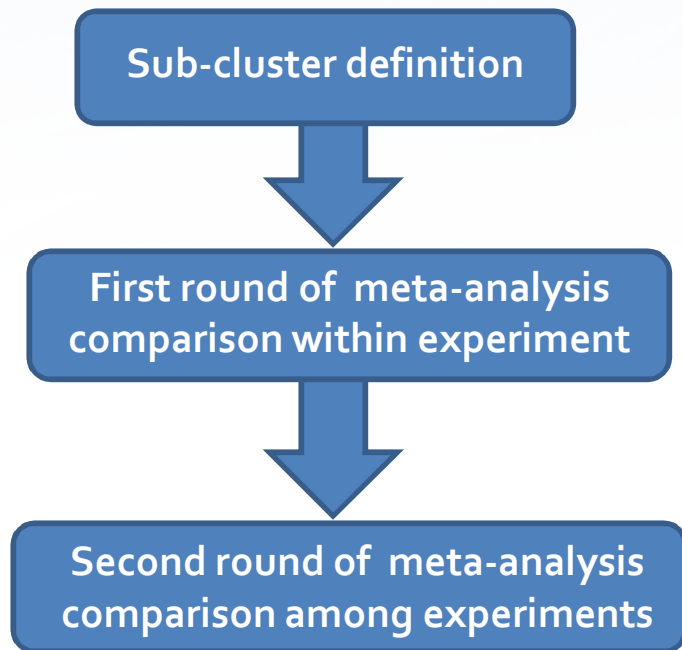
# Meta-analysis flowchart

## Fish and Chips

Fish transcriptome database

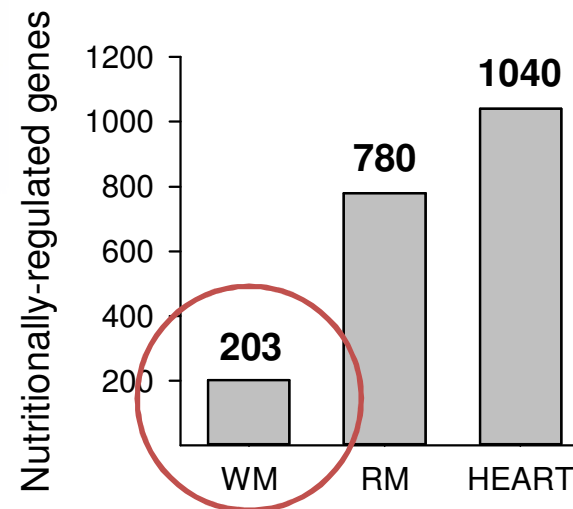
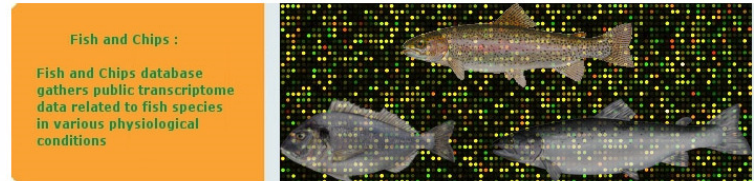


# Meta-analysis flowchart: Sub-cluster definition



## Fish and Chips

Fish transcriptome database



- Selection of a well-defined cluster according to experimental condition

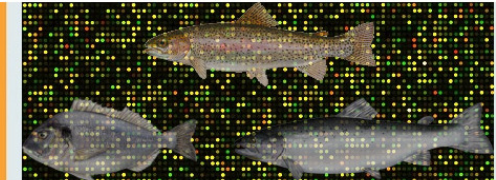
# Meta-analysis flowchart: Sub-cluster definition

## Fish and Chips

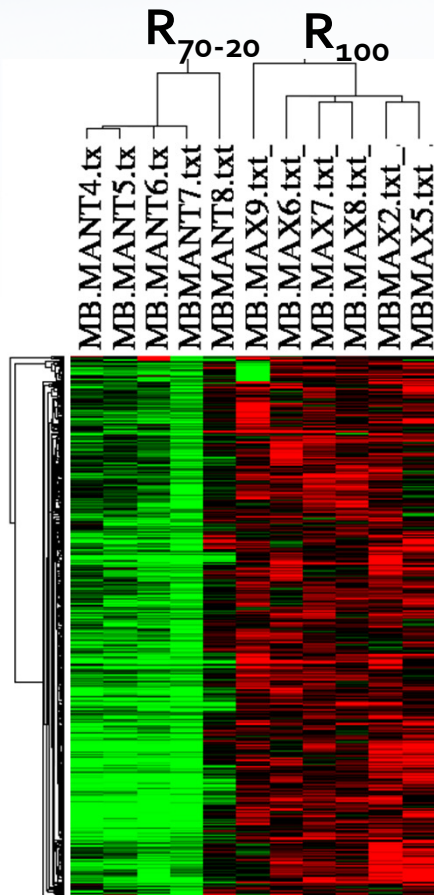
Fish transcriptome database

Fish and Chips :

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



## White muscle cluster 17 (224 genes)



GO term	Number in array	Number in sub-cluster	P-value
proteolysis involved in cellular protein catabolic process	177	22	0.0
negative regulation of ubiquitin-protein ligase activity	36	9	0.0
positive regulation of ubiquitin-protein ligase activity	41	10	0.0
regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	39	10	0.0
regulation of ubiquitin-protein ligase activity	47	11	0.0
mitochondrial membrane	204	24	0.0
ATP synthesis coupled electron transport	37	9	0.0001

# Meta-analysis flowchart within experiment

## White muscle cluster 17 (224 genes)

### Profile comparison among dataset clusters

## Cardiac muscle cluster 17 (501 genes) 61 overlapping genes

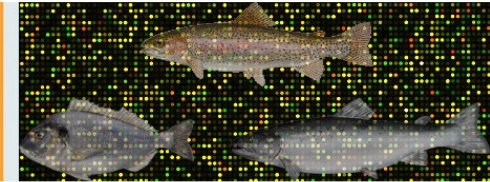
## Red muscle cluster 40 (398 genes) 52 overlapping genes

### 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



#### GO term

proteolysis involved in cellular protein catabolic process  
negative regulation of ubiquitin-protein ligase activity  
positive regulation of ubiquitin-protein ligase activity  
regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle  
regulation of ubiquitin-protein ligase activity  
**mitochondrial membrane**  
**ATP synthesis coupled electron transport**

#### GO term

**cellular respiration**  
cytoplasmic part  
**mitochondrial part**  
**mitochondrial translation**  
organelle envelope

#### GO term

**respiratory chain**  
**oxidation-reduction process**  
**cellular respiration**  
**mitochondrial membrane part**  
cytoplasmic part

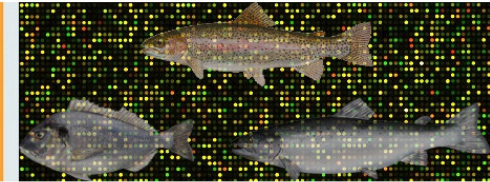
# Meta-analysis flowchart among independent experiments

## 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



Cardiac muscle cluster 17 (501 genes)

Profile comparison among dataset clusters

GO term
cellular respiration
cytoplasmic part
mitochondrial part
mitochondrial translation
organelle envelope

Early development cluster 4 (1464 genes)

146 overlapping genes

GO term
spindle assembly checkpoint
organelle fission
intracellular membrane-bounded organelle
membrane-bounded organelle
mitotic cell cycle spindle assembly checkpoint

Liver – Cortisol administration cluster 40 (139 genes)

41 overlapping genes

GO term
cytosolic part
cytoplasmic part
intracellular membrane-bounded organelle
membrane-bounded organelle
mitochondrion

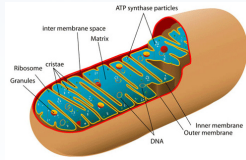
## Concluding remarks



- Sea bream has **divergent muscle transcriptomes**
- Sea bream muscle transcriptomes are **differentially regulated** by nutrient availability
- Highly expressed and nutritionally regulated genes of WM are enriched in genes related to **proteolysis and protein ubiquitination**
- Highly expressed and nutritionally regulated genes of heart and RM are **enriched in the GO term mitochondria**
- Microarray meta-analysis evidences that **mitochondria-related genes are a common link** within and among tissue transcriptomes in challenged fish



# Mitochondria, a subcellular marker of metabolic stress phenotyping



$\approx 1000$  proteins  $\Leftrightarrow$  13 mitochondria-encoded genes  
 $\approx 990$  nuclear-encoded genes

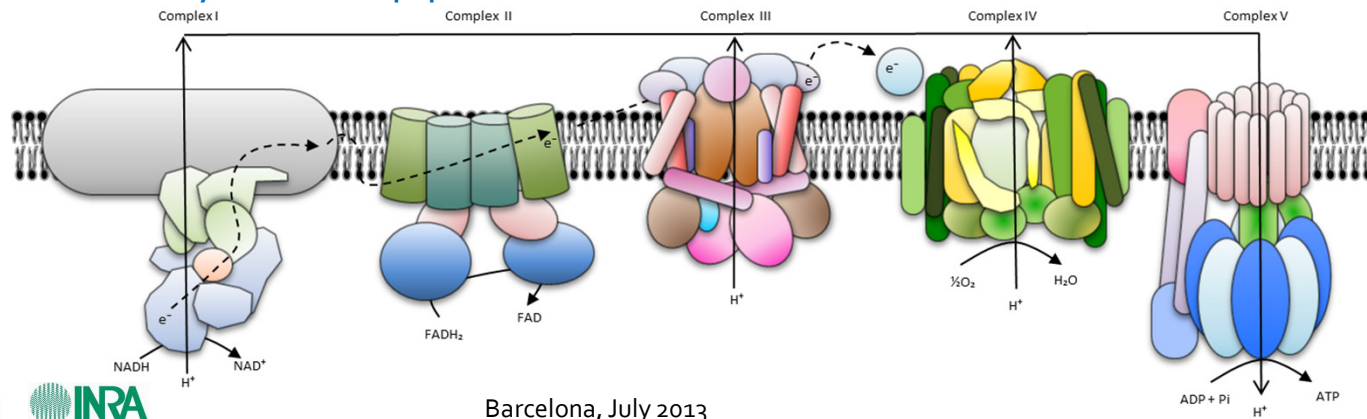
- 926 nuclear-encoded mitochondrial genes are present in the CSIC sea bream reference transcriptome database ([www.nutrigroup-iats.org/seabreamdb](http://www.nutrigroup-iats.org/seabreamdb))
- Development of **two PCR-arrays** for 150 selected mitochondrial markers

## Mito-Chip I (Mitochondria activity, 60 genes)

- Mitochondrial biogenesis
- Antioxidant defence
- Protein import, folding and assembly
- Mitochondrial dynamics and apoptosis

## Mito-Chip II (OXPHOS, 90 genes)

- Complex I-V of mitochondria respiratory chain



# Transcriptional-metabolic phenotyping of gilthead sea bream (*Sparus aurata*) mitochondria

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## HIGHLIGHTS

- ✓ 40 new mitochondrial-related genes were identified
- ✓ The mitochondrion is a key component of the fish stress response against intermittent and repetitive environmental stress
- ✓ The mitochondrion phenotype helps to define the threshold level of stress conditions in fish

The **mitochondria** play a key role in normal growth and cellular homeostasis, housing the oxidative phosphorylation machinery and several biosynthetic activities linking the environment and metabolic condition. However, it remains largely unclear the extent to which mitochondria operate as specialized cellular sentinels that monitor the conditions of the cell to finely adapt its metabolic activity, to coordinate adaptive responses to stress. The aim of the current study was to phenotype the transcriptionally-mediated response of liver mitochondria of gilthead sea bream exposed to three to different **intermittent and repetitive environmental stressors**. A **PCR array** of 60 mitochondria-related genes was used selecting markers of transcriptional regulation, oxidative metabolism, respiration uncoupling, antioxidant defense, protein import/folding/assembly, as well as mitochondrial dynamics and apoptosis. All gene sequences are derived from the recently updated reference transcriptome database of gilthead sea bream (Calduch-Ojeda et al., 2013).

## EXPERIMENTAL DESIGN

**Juvenile** gilthead sea bream of 265 g initial BW were allocated in triplicate groups of 27 fish each (500 L-tanks) at three different environmental stressors:

- Changes in water **temperature (T-ST)**
- Changes in water level & **chasing (C-ST)**
- Multiple** sensory perception stressors (shakes and sounds, window wiper movements, reverse water flow, light flashes) (**M-ST**).

## TISSUE SAMPLING

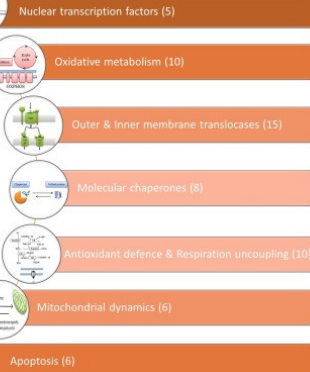
**Blood** was taken for hormone and metabolite analyses.

**Livers** were sampled for gene expression analyses by means of a mitochondrial PCR-array using the EpMotion 5070 Liquid Handling Robot



## MITOCHONDRIAL PCR-ARRAY (60 GENES)

40 NEW SEA BREAM GENES (JX975224-JX975265)

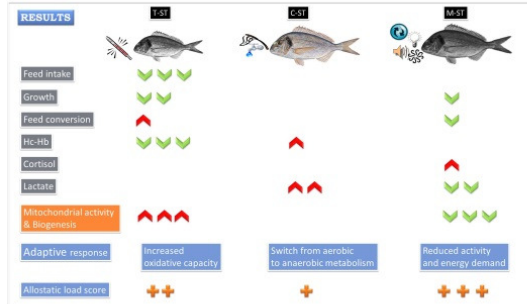


## TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL-RELATED GENES

Corollary of differentially expressed genes. Fold change relative to control. Red: up-regulated genes. Green: down-regulated genes

STRESS GROUP	T-ST	C-ST	M-ST	
Nuclear transcription factors	POU1f6	2.58*	1.24	0.52*
	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 translocases	COX4b	1.28*	0.85	1.03
	Tom70	1.61*	0.95	0.97
	Tom34	1.44*	1.03	0.94
Inner membrane translocases (TIM23 complex)	Tom22	2.11*	1.29	1.42*
	Tim44	1.45*	1.13	0.89
	Tim23	1.38*	1.27	1
Inner membrane translocases (TIM22 complex)	Tim8A	1.04	0.86	0.72*
	Tim10	1.72*	0.83	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*	0.88	0.87*
Antioxidant enzymes	DKR-1	1.35*	1.13	0.88
	GR	1.22*	0.97	1.01
	FRS1	1.32*	1	0.91
Fusion & Fission markers	MFN2	1.42*	0.87	0.68*
	MIF1B	0.92	0.7*	0.73
	MRO2	1.03	1.08	0.83*
Apoptotic markers	AIFM1	1.24*	0.87	1.15
	BAX	1.14	0.84	0.62*
	BCLX1	1.1	0.83	0.68*

ACKNOWLEDGMENTS  
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## CONCLUSIONS

- ✓ The mitochondrion phenotype of T-ST fish improves the aerobic oxidative capacity of fish with a reduced feed intake, haematocrit and circulating haemoglobin content.
- ✓ The mitochondrial response of C-ST fish switches from aerobic to anaerobic metabolism with increased production of lactate and negligible effects on fish performance.
- ✓ The down-regulated mitochondrial response of M-ST fish lowered energy demand with a reduced lactate production and feed efficiency, and the highest allostatic load score.

REFERENCES  
Calduch-Ojeda et al. BMC Omics 2013, 14:176.



Poster 160



# ACKNOWLEDGMENTS



Nutrigenomics and Fish Growth Endocrinology



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et Génomique des Poissons



Patrick Prunet



Yann Echasseriau

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*Thank  
You*

# Thank you for your attention !!

