BIOMARKERS IN FISH NUTRITION
SEA BREAM & SEA BASS CASE STUDIES

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WP:1 Identification and validation of targeted integrative tools to predict and measure metabolic and health effects

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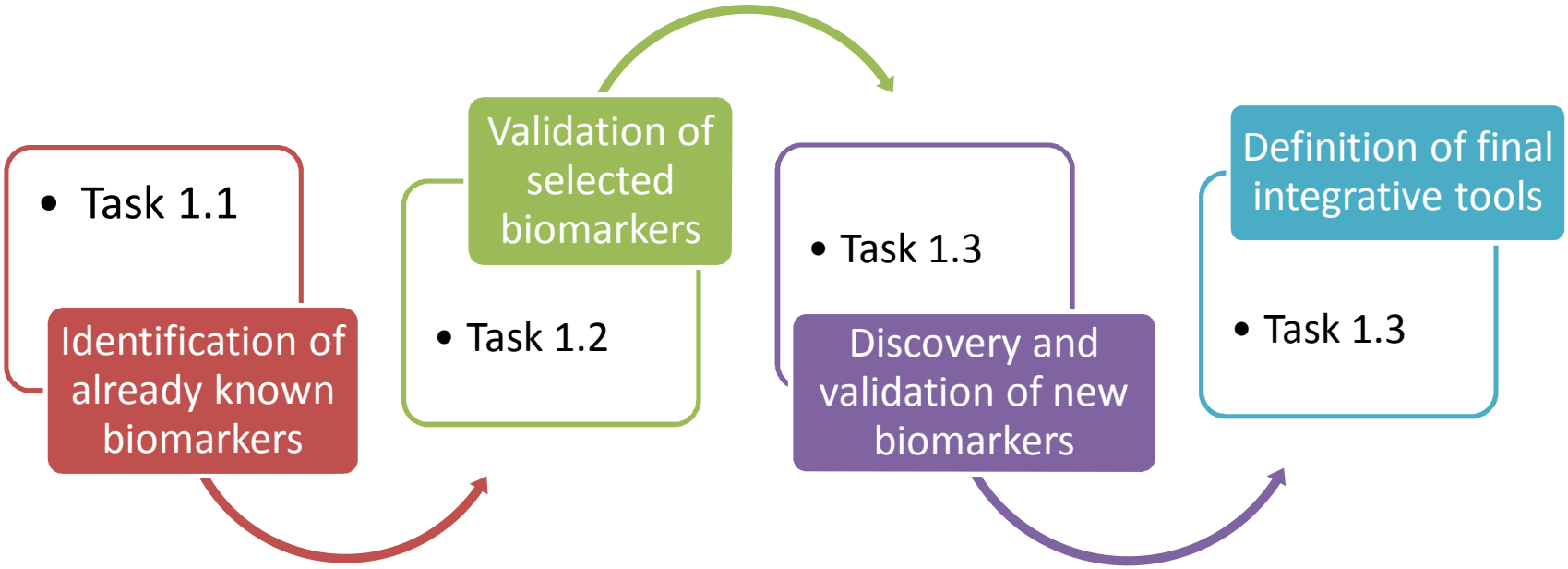
USSEC 2017, Benicàssim

ARRAINA WP1, Step by Step

"Identification and validation of targeted integrative tools to predict and measure metabolic and health effects"



Partner contributors: CSIC, NIFES, INRA, UoS, HAKI, USI, ULPGC, CCMAR

ARRAINA WP1
Fish Species by Partners

Carp
(HAKI)

Sea bass
(CSIC/ULPGC/USI)

Atlantic salmon
(NIFES, UoS)

Trout
(INRA)

Sea bream
(CSIC/CCMAR)

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BIOMARKERS for Metabolic and Functional Phenoyping of Farmed fish

Biomarker Definition

Biomarker evolution

- OMICS-based approaches

Examples of Use

- Larvae rythmicity; Intestine plasticity; Liver, SKM, AT profiling

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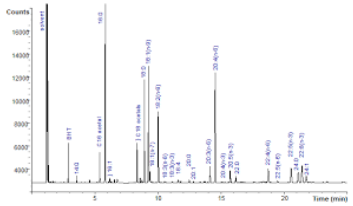
BIOMARKER DEFINITION

Biomarkers **are measurable indicators of health or any other physiological state or productive trait**



Ideal biomarkers:

- Non invasive
- Easy to measure
- Cost efficient
- Highly **sensitive and specific** when used alone or in combination with other biomarkers
- **Consistent across** developmental stage, season, reproductive & nutritional condition, genetic background, etc.

BIOMARKER CLASSIFICATION based on their application

BIOMARKER TYPES

- **DIAGNOSTIC BIOMARKERS** — Indicates if something is wrong
- **PROGNOSTIC BIOMARKERS** — Predicts the course/severity of metabolic /health dysfunctions
- **THERAPEUTIC BIOMARKERS** — Predicts whether intervention will work or is working

BIOMARKER CLASSIFICATION-Type of measure

BIOMETRIC

- HSI & MSI
- Intestine length
- …..

HISTOLOGICAL

- Abundance of Rodlet & Globet cells
- Granulocyte infiltration
- Fatty liver

BIOCHEMICAL

- Hb, Hc, erythrocyte fragility
- Circulating electrolytes, metabolites, hormones…
- Lysozyme & RB activity

MOLECULAR, EPIGENETIC

….

- DNA methylation
- Gene expression
- Protein abundance

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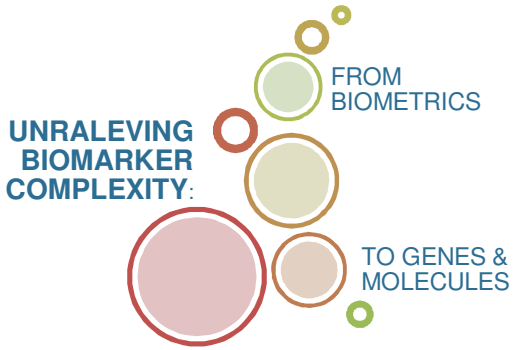
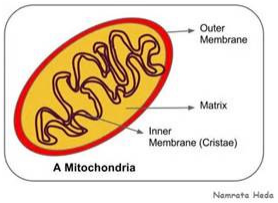
INTEGRATIVE APPROACH





Enzyme activies

- Transaminases
- Antioxidant activity
- OXPOS..

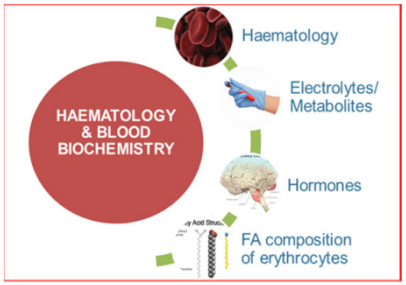
Biometric Indexes

- HSI, MSI, GI…

Histo-pathological scoring of liver & intestine

POPs/Mycotoxyn exposure

- Chemical
- Metabolite



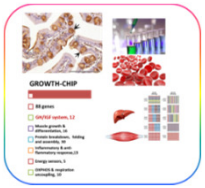
Haematology & Basic blood biochemistry

- Hc, Hb
- Electrolytes
- Metabolites
- Hormones

Assessment of nutrient requirements & nutritional/ metabolic condition

Omic technologies

- Functional genomics
- Metabolomics
- Metagenomics



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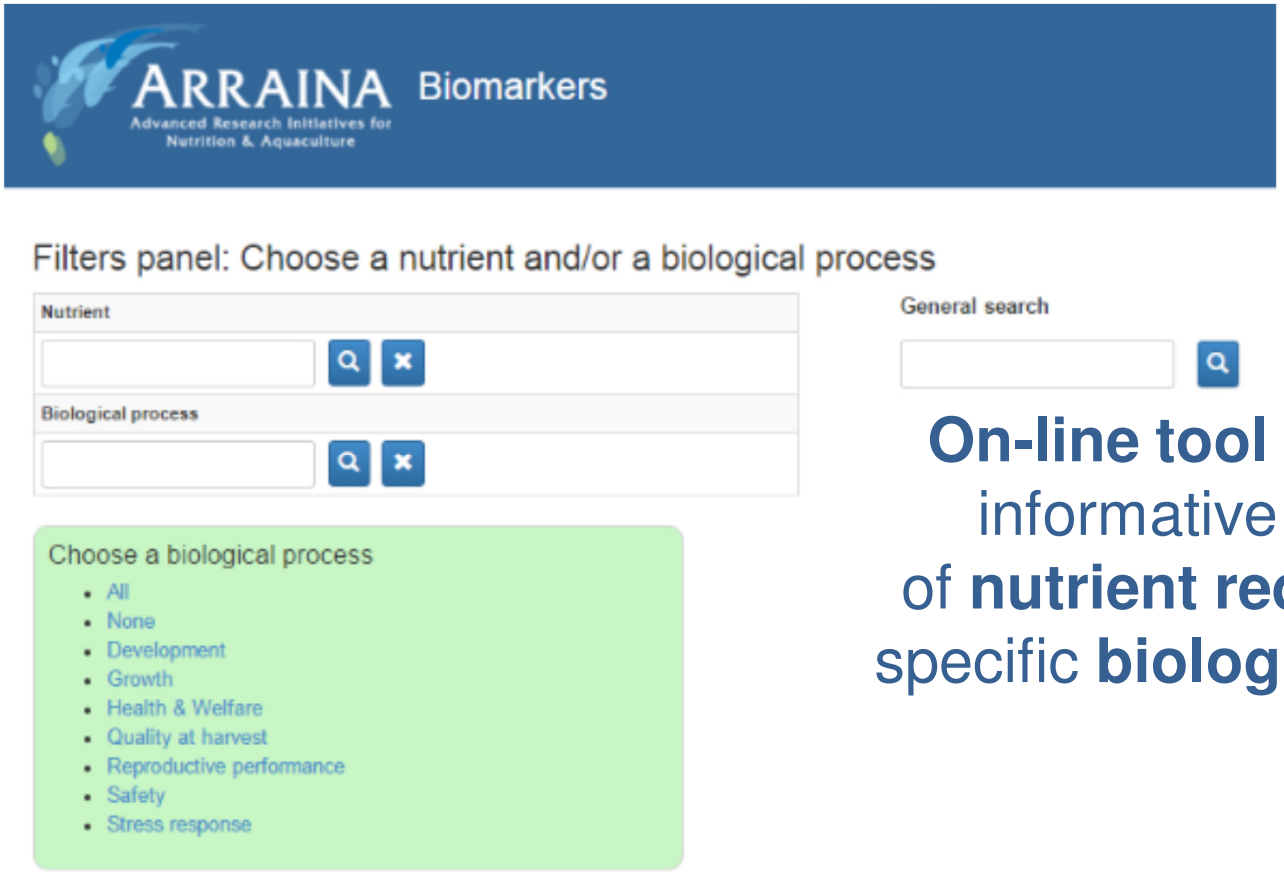
ARRAINA BIOMARKER DATABASE

www.nutrigroup-iats.org/arraina-biomarkers



On-line tool for searching informative biomarkers of **nutrient requirements** or specific **biological processes**

Type of measure:
- Histological
- Haematological
- Biochemical
- Molecular
- ……

OBJECTIVE

HOW DO MOLECULAR APPROACHES CONTRIBUTE TO UNDERLINE RELIABLE CRITERIA TO PREDICT THE PHYSIOLOGICAL CONSEQUENCES OF NEW FISH DIET FORMULATIONS?

- Sea Bream & Sea Bass Case Studies:
  - Circadian rhythmicity of whole larvae
  - Gut transcriptome plasticity
  - Pathway-focused gene expression profiling of liver, SKM & AT

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WIDE-GENE EXPRESSION ANALYSES

www.nutrigroup-iats.org/seabassdb

- 24,906 non-redundant annotated sequences
- 15,367 different gene descriptions

frontiers in Physiology

Calduch-Giner et al., 2016

www.nutrigroup-iats.org/seabreamdb

- 20,565 non-redundant annotated sequences
- 14,535 different gene descriptions

BMC Genomics

Calduch-Giner et al., 2013

- Customized Sea Bream & Sea Bass Microarrays (15,000 unique genes) with a high coverage of the PROTEIN-encoding transcriptome
- RNA-seq (sea bream genome, reference library)

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96-well PCR-arrays (4x20, 2x40, 1x88….)
Automated liquid handling robot

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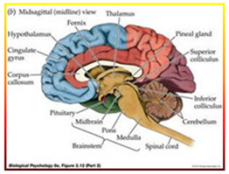
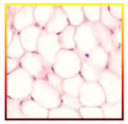
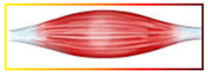
LIPID CHIP	GUT CHIP	GROWTH CHIP
40 genes	86 genes	89 genes
30 new sequences	60 new sequences	49 new sequences
Elongases , 5	Cell differentiation & proliferation (14)	GH/IGF system (12)
Desaturases, 3	Intestine architecture & permeability (19)	Muscle growth & differentiation (16)
PL metabolism, 11	Enterocyte function and epithelia damage (9)	Protein breakdown (20)
Lipases/related genes, 13	ILs, CKs, ILRs, CKRs (21)	Protein folding & assembly (10)
B-oxidation, 4	PRRs (13)	ILs, ILRs, CKs (13)
Transcription factors, 4	Mitochondria function and biogenesis (11)	Energy sensing (5)
		OXPHOS & respiration uncoupling (10)
		Transcription factors (3)



Benedito-Palos et al., 2013; 2014; Rimoldi et al., 2016



Pérez-Sánchez et al., 2013; 2015



Benedito-Palos et al., 2016

Pathway-focused PCR-arrays operated by handling robots for the simultaneous and semi-automated gene expression profiling

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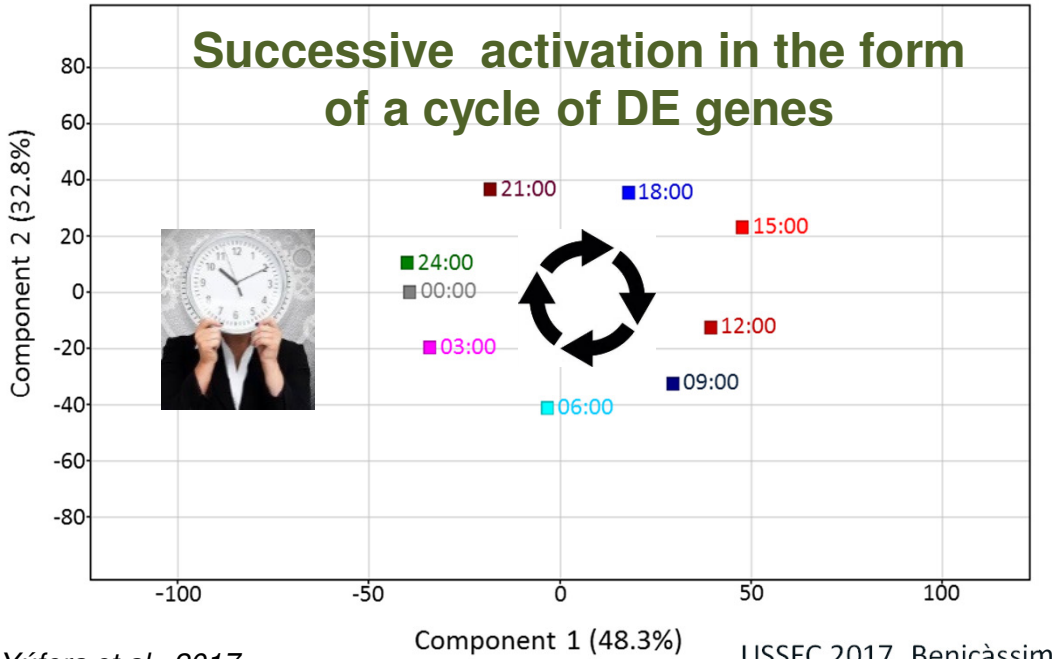
CASE STUDY 1

Biomarker phenotyping at early life stages
-Whole larva microarray gene expression profiling-

Sea bream chronobiology (Spanish clock):

- **3,000 DE genes**
- **The two first components of PCA explain more than 80% of total variance**



Yúfera et al., 2017

Anticipatory food response (onset of lights) → Protein accretion and tissue repair (afternoon) → Phase resetting (night) → Anticipatory food response

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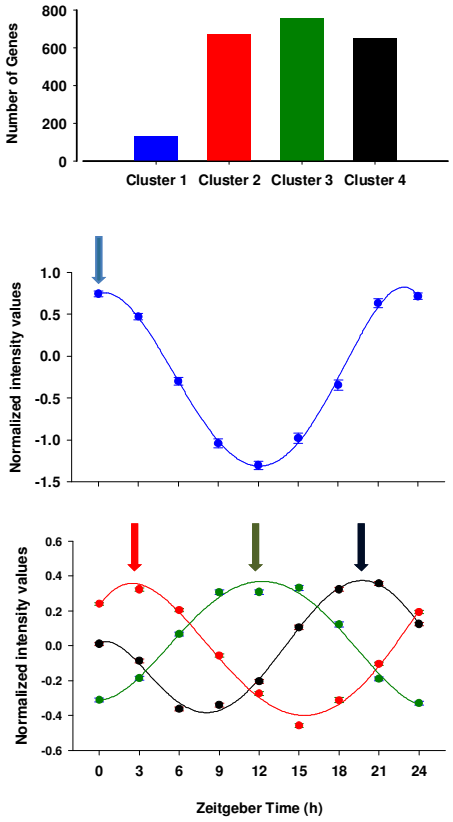
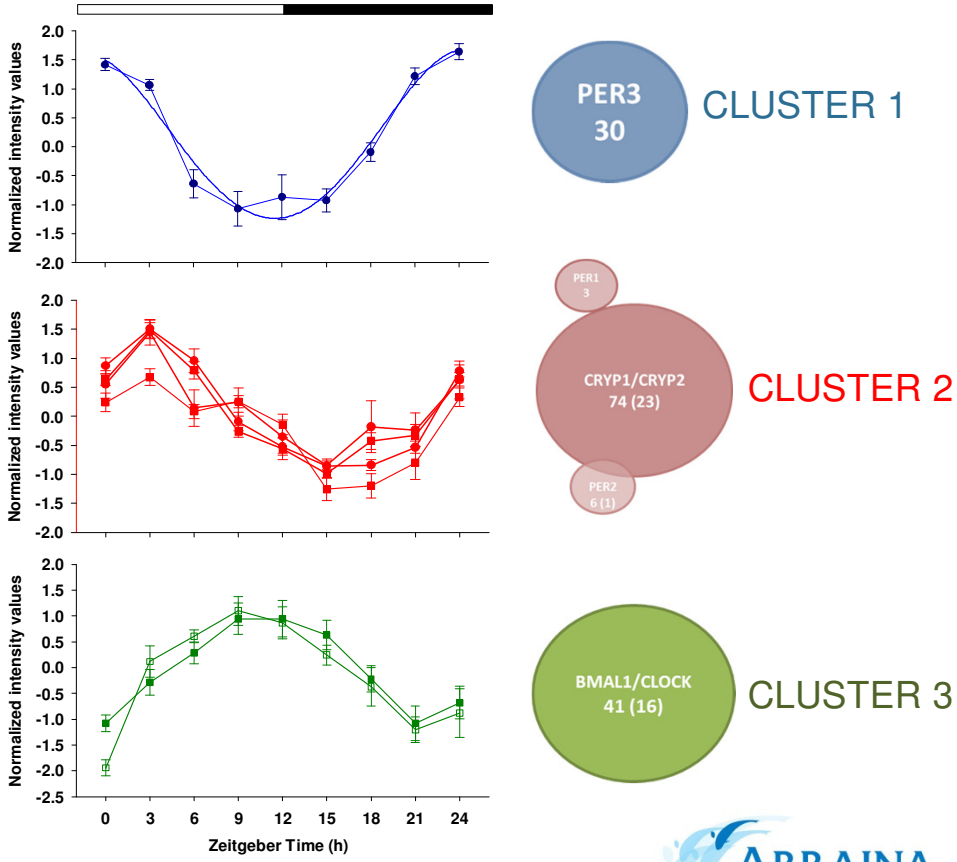
K-means Clustering

Four sequential patterns of gene expression



CLOCK GENES: Correlation analysis

More than 200 genes were identified as high rhythmic genes



Yúfera et al., 2017

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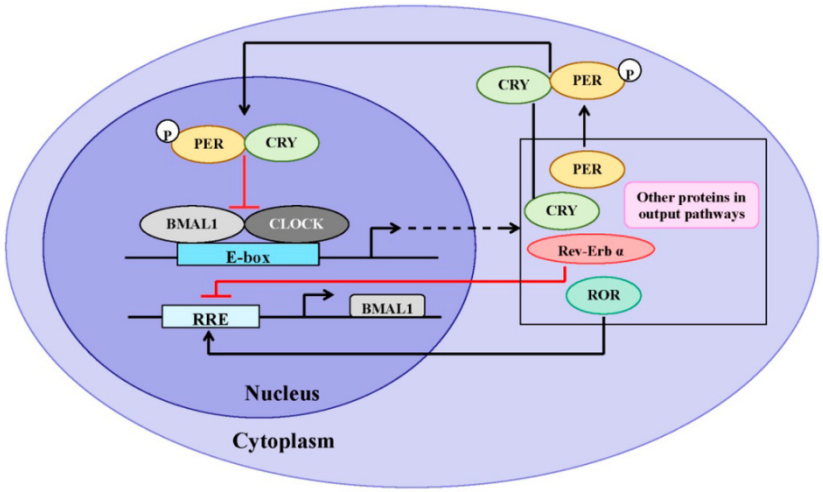
CIRCADIAN CLOCK AT EARLY LIFE STAGES

CASE STUDY 1



ROBUST MARKERS OF CIRCADIAN CLOCK → INTEGRATE METABOLIC STATUS AND INFORMATION FROM THE ENVIRONMENT → EPIGENETIC MARKERS OF FISH QUALITY

Yúfera et al., 2017

GUT Transcriptome Plasticity

CASE STUDY 2

DE genes:18

Ant Int.	D1	D2	D3	D4
D1		2	***12***	2
D2			***9***	1
D3				**10**

Microarray and RNA-seq highlighted minor changes on the intestine transcriptome of fish fed ARRAINA diets

Targeted transcriptomic profiling (gut PCR-array, 86 selected markers) evidenced nutritionally-mediated effects in a dose-dependent manner

BUT supplementation (D4) reversed most of the changes induced by the lowest FM/FO diet (D3))

Estensoro et al., Plos One 2016

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CASE STUDY 2

MICROARRAY INTESTINAL PROFILING

frontiers in Physiology

Calduch-Giner et al., 2016

Standard commercial diet

IP MI AI

Principal Components Analysis

n=8

AI-MI

DE Genes (AI *vs* PI)

5,770 No fold-change cutoff

3,654 Fold-change > 1.5

1,906 Fold-change > 2.0

PI

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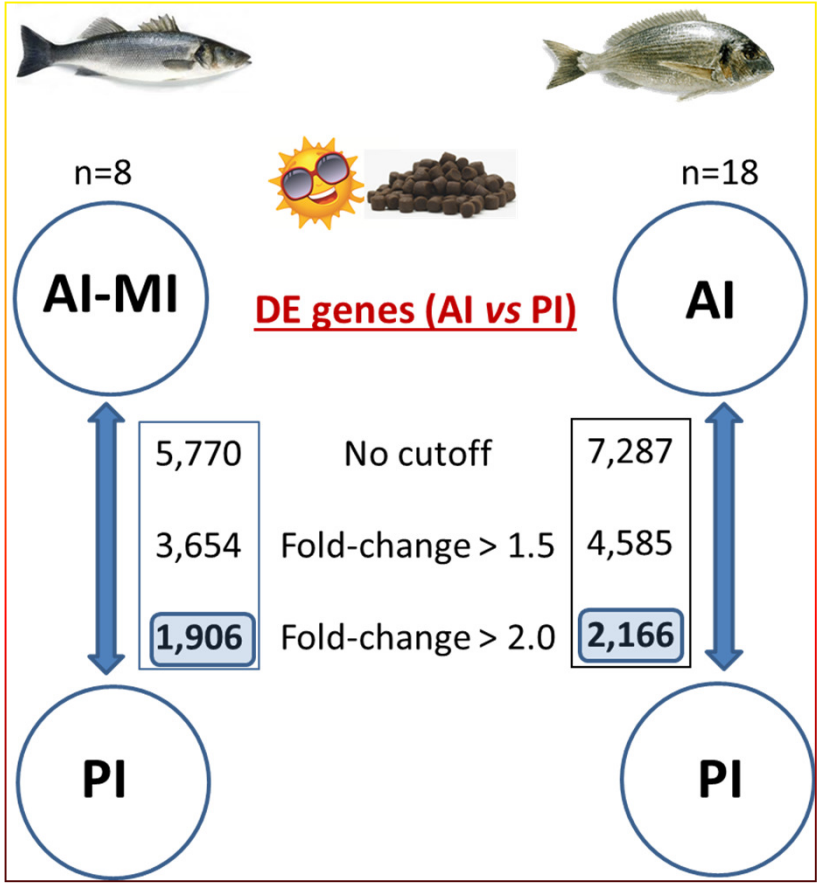
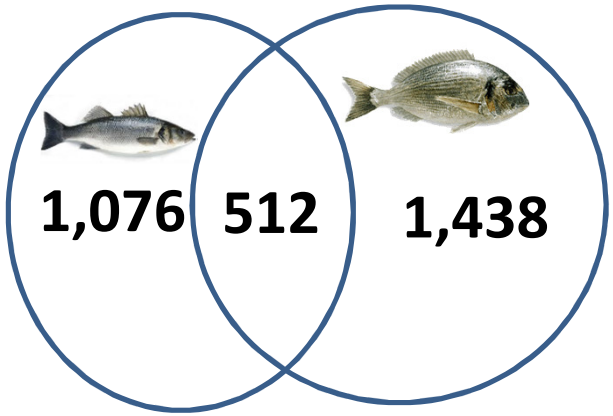
CASE STUDY 2

Spatial Regulation of Gut Transcriptome Sea Bass *vs* Sea Bream



IPA® Interactive pathway analysis of complex 'omics data

3,026 DE genes are mapped with the IPA software



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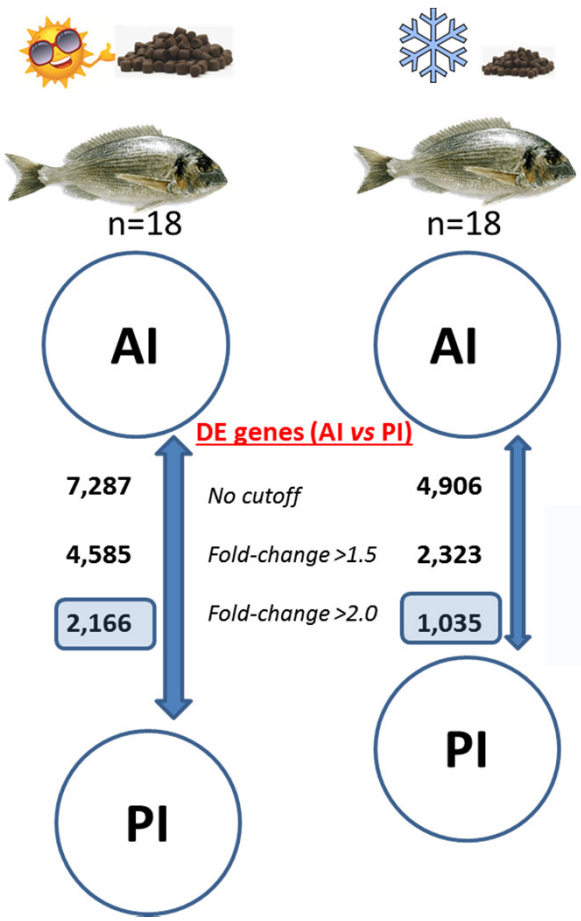
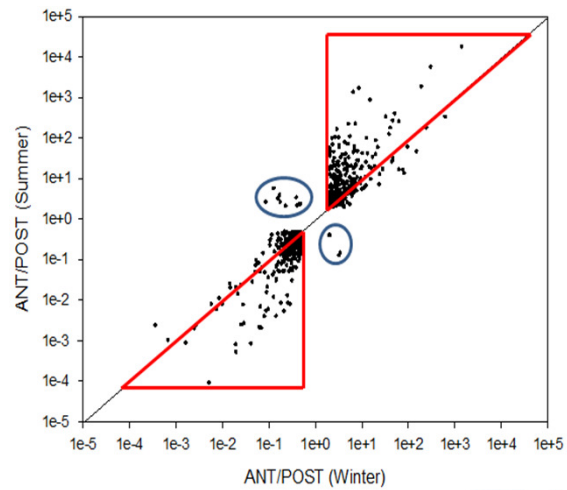
CASE STUDY 2

Spatial Regulation by Season of Sea Bream Gut Transcriptome



- Number of spatially regulated genes: **summer > winter**
- Magnitude of change for common DE genes (675): **summer > winter**



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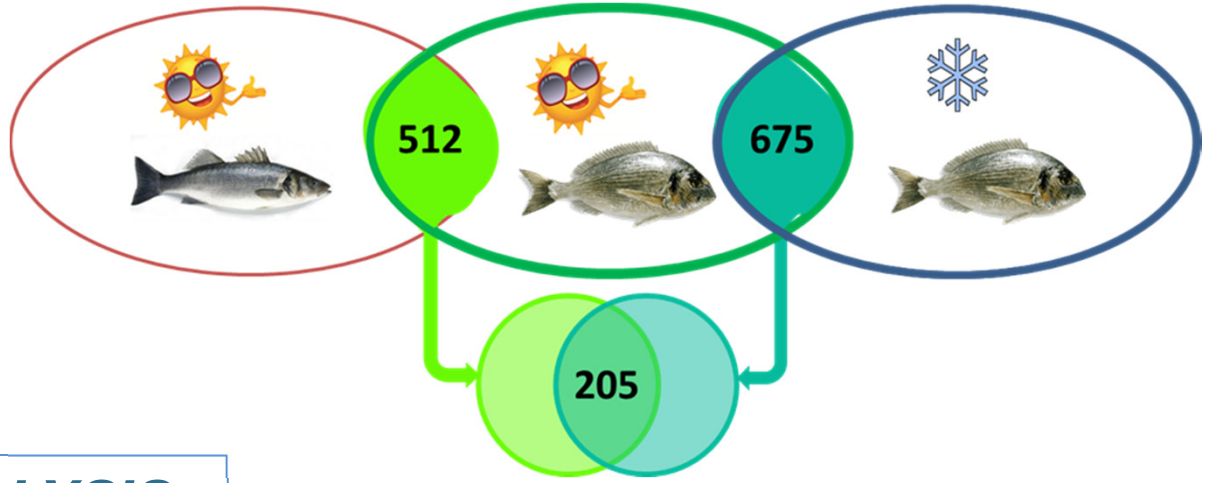

META-ANALYSIS of DE genes across the intestine of Sea bass & Sea bream

84 genes over-represented in AI

78 genes over-represented in PI

Robust multi-species markers of AI & PI segments – Different spatial distribution of nutrient transporters – Candidate markers of high FI & FE

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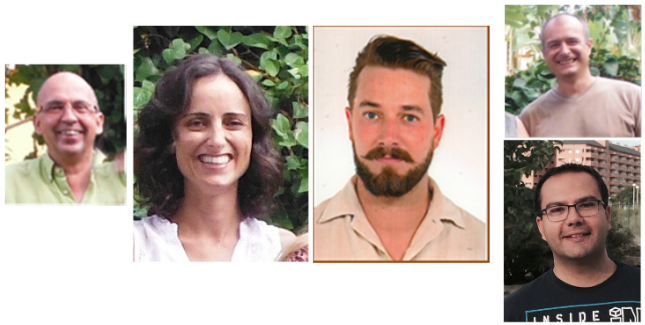
TARGET APPROACH. NUTRIENT SPECIFIC EFFECTS ON GROWTH AND LIPID METABOLISM



CTRL

SAA/PL Min

P/Vit

n-3 LCPUFA

FEEDING TRIALS WITH SEMI-PURIFIED DIETS FORMULATED FOR NUTRIENT DEFICIENCIES

VALIDATE & DISCOVER SPECIFIC BIOMARKERS OF NUTRIENT DEFICIENCIES

DECISSION TREE

Ballester-Lozano et al., 2015

BJN BRITISH JOURNAL OF NUTRITION

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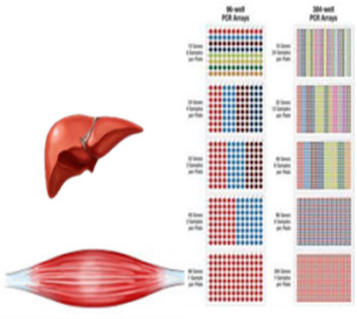
Gene Expression Profiling of Liver/SK Muscle/AT for Selected Markers of Growth & Lipid Metabolism

GROWTH-CHIP

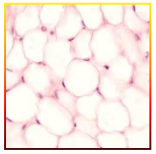
- 88 genes
- GH/IGF system, 12
- Muscle growth & differentiation, 16
- Protein breakdown, folding and assembly, 30
- Inflammatory & anti-flammatory response,13
- Energy sensors, 5
- OXPHOS & respiration uncoupling, 10

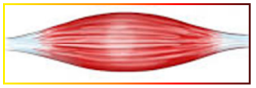


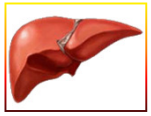
Benedito-Palos et al., 2016

LIPID CHIP

- 43 genes
- Elongases , 4
- Desaturases, 3
- PL metabolism, 10
- Lipase-related genes, 9
- B-oxidation, 4
- Cholesterol & Lipoprotein metabolism, 10
- Transcription factors, 3







Benedito-Palos et al., 2013; 2014; Rimoldi et al., 2016

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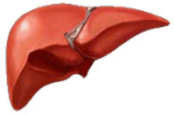
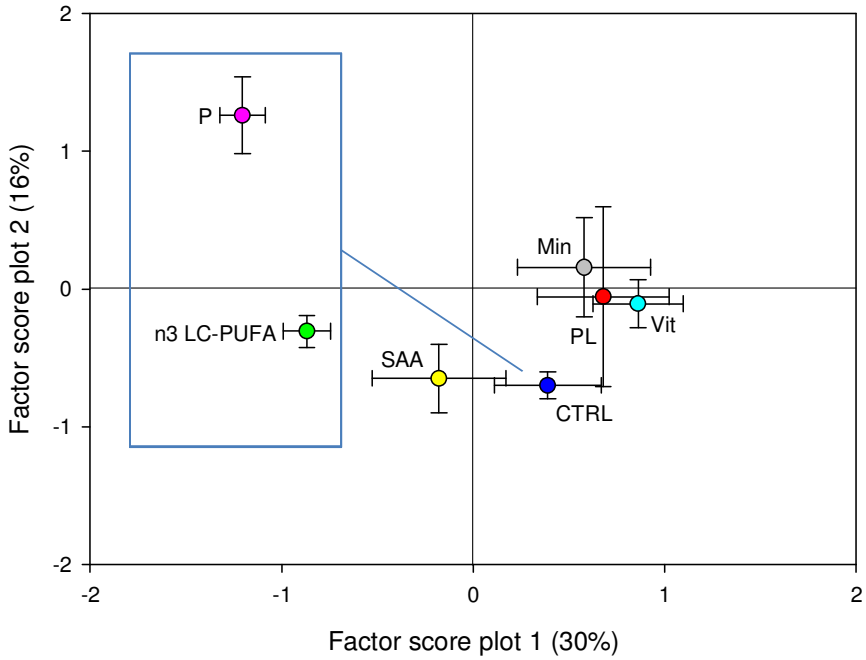
Principal Component Analysis. Growth-chip

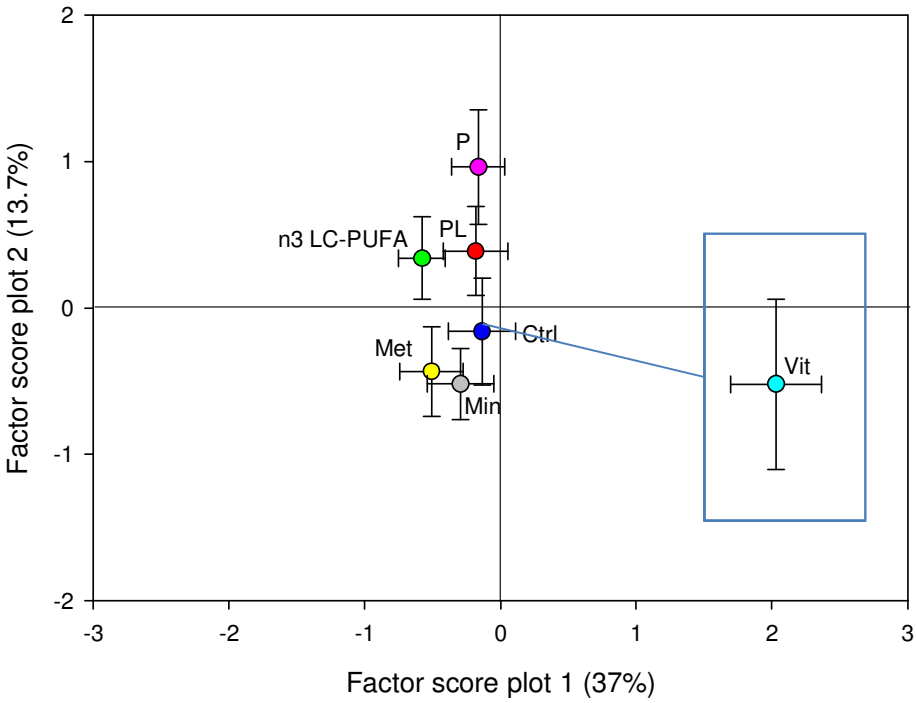
Skeletal muscle is specially sensitive to deficiencies in *Vitamins*



Growth-chip, Liver



Growth-Chip, Skeletal Muscle



Liver is specially sensitive to deficiencies in *EFA & Phosphorous*

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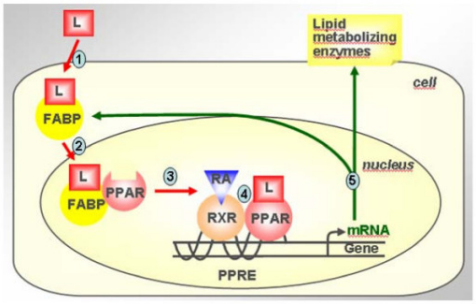
Tissue-specific Markers of FA/PL Deficiencies



LIPID CHIP

- **43 genes**
- **Elongases , 4**
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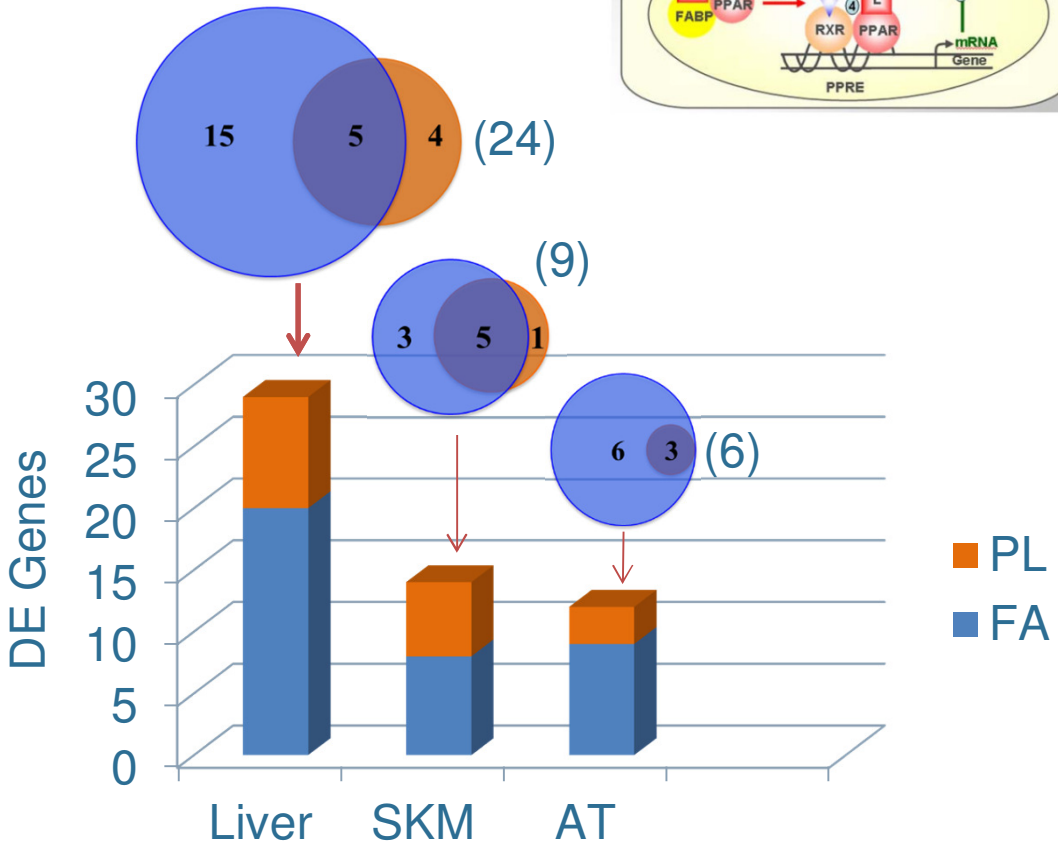
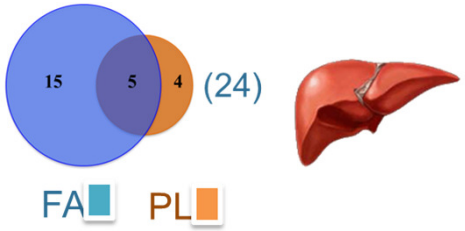
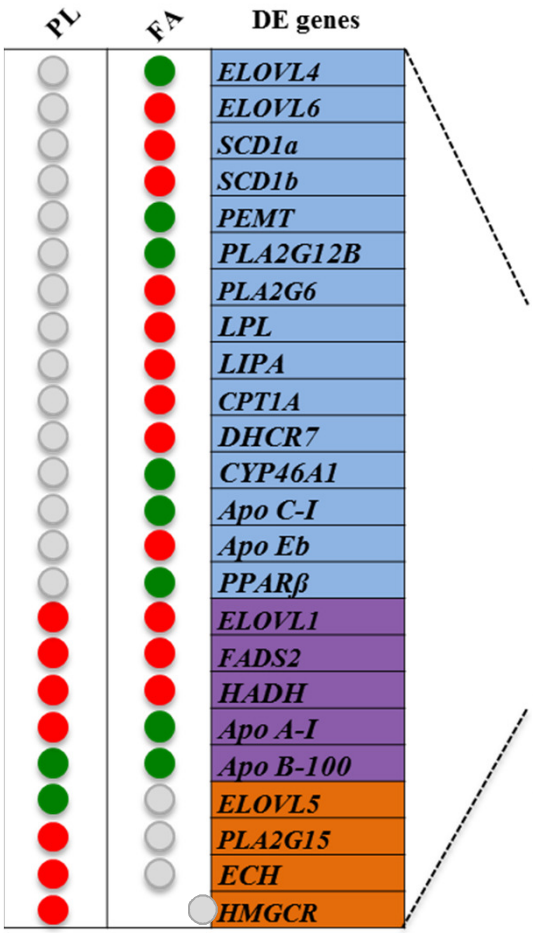
Benedito-Palos et al., 2013; 2014; Rimoldi et al., 2016



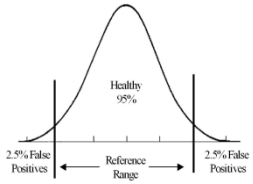


Sequential Filtering by NUTRIENT DEFICIENCY & LOW FM/FO Diets



DISCOVERY of early markers of FA/PL deficiencies

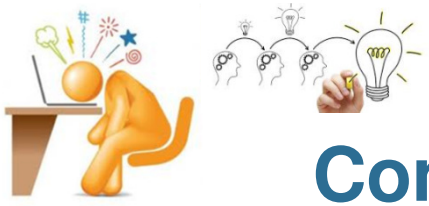
DE genes Low FM/FO
FADS2
SCD1a
SCD1b
ELOVL6
PLA2G6
LPL
DHCR7
CYP46A1
Apo B-100



DOUBLE AIM

DEFINITION of normal reference values

	Summer/ REFERENCE VALUES
ELOVL6	47 (16 - 121)
SCD1a	11 (8 - 43)
SCD1b	81 (41 - 193)
PLA2G6	2.8 (1.8 - 3.7)
LPL	39 (26 - 57)
DHCR7	19 (13 - 40)
CYP46A1	14 (10 - 24)
FADS2	177 (123 - 229)
Apo B-100	0.52 (0.20 - 1.28)



Concluding Remarks and Perspectives

- The magic biomarker (all in one) does not exist
- Wide & targeted transcriptomic approaches considered in a integrative manner contribute to fill the gaps on aquaculture biomarkers
- Circadian clocks are proposed as robust markers of fish quality & nutritional condition at early life stages
- The gut transcriptome is highly regulated on a nutritional, spatial and temporal basis
- Functional specialization across the intestine is by itself a robust biomarker that helps to explain differences in KPIs within and among farmed fish species
- Sequential pathway-focused PCR-arrays lead to define early biomarkers of nutrient deficiencies and their reference values

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This is ALL!!

Thank you for your attention

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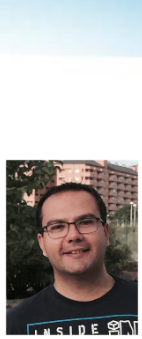

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