Aplicaciones de la genética y la genómica en el bienestar animal

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* Proyecto Aquagenomics. Consolider-Ingenio-2010

Transcriptomics in fish biology

Transcriptomics is the branch of molecular biology that deals with the study of messenger RNA molecules produced in an individual or population of a particular cell type *Wikipedia 2010*.

Most "Dark Matter" Transcripts Are Associated With Known Genes.
Bakel et al. (2010) PLoS Biology

Requires strong inter-disciplinary effort

1965- Sequence of the 1st RNA determined
1977- Northern Blot and Sanger sequencing
1980's- PCR revolution
1990's- DD, SSH, EST, SAGE, Microarray

20thC

Sequencers could theoretically give you the entire transcriptomic profile if given enough sequences

Transcript profiles could be quantified at the same time that gene discovery was taking place.

Bridging the Genotype-Phenotype Gap

"for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied."

Krogh's Principle, 1929.

21stC

evolutionary immunology

ibb

Transcriptomics in fish biology

RNA-Seq Microarray

Arrays are only as good as the genes they contain, while sequencers could theoretically give you the entire transcriptomic profile if given enough sequences

The advantage in using sequencing over arrays would be that transcript profiles could be quantified at the same time that gene discovery was taking place.

Functional genomics with microarrays in fish biology and fisheries
Goetz and MacKenzie. (2009) Fish and Fisheries

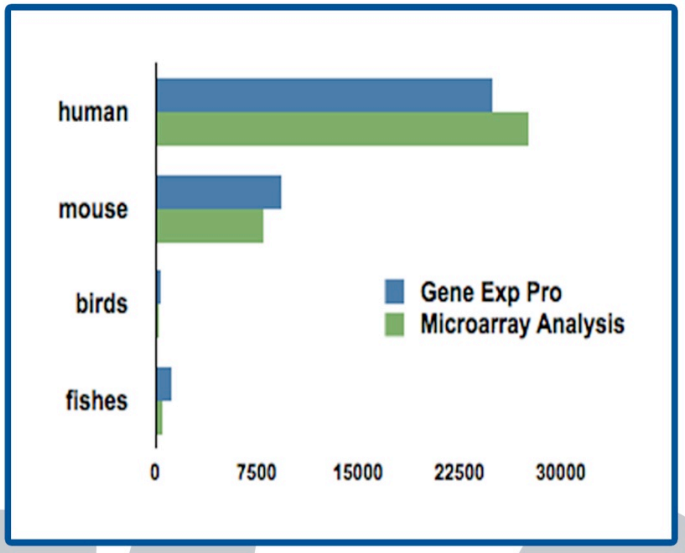
Applications of New Sequencing Technologies for Transcriptome Analysis
Olena Morozova, Martin Hirst, and Marco A. Marra *Annu. Rev. Genomics Hum. Genet. 2009. 10:135–51*

NGS 2nd Generation:

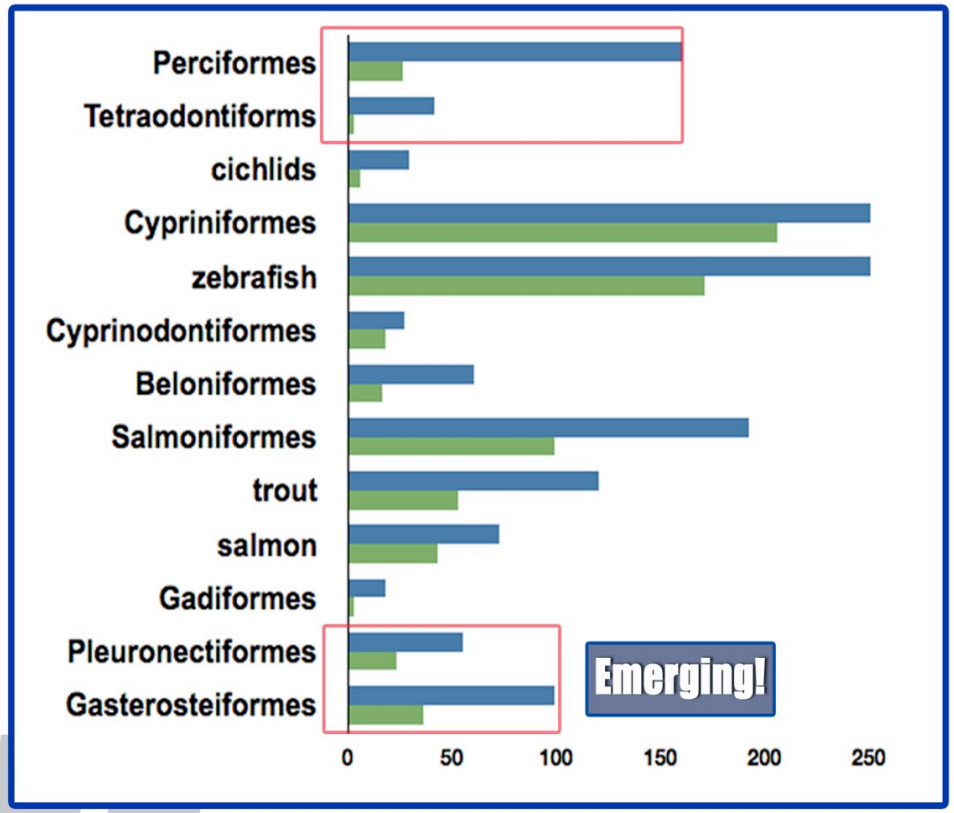
Roche (454) FLX	*200-300bp*	*Emulsion PCR*	*13Mb/h*
Illumina Genome Analyzer	*32-40bp*	*Bridge PCR*	*25Mb/h*
ABI SOLiD 3	*25-35bp*	*Emulsion PCR*	*21-28Mb/h*
Heliscope	*25-35bp*	*NONE(direct)*	*83Mb*

Comparative MeSH analysis using PubMed (2000-10)
Search terms: *Gene Expression Profiling*, *Microarray analysis* and combinations of relevant taxonomic classification.

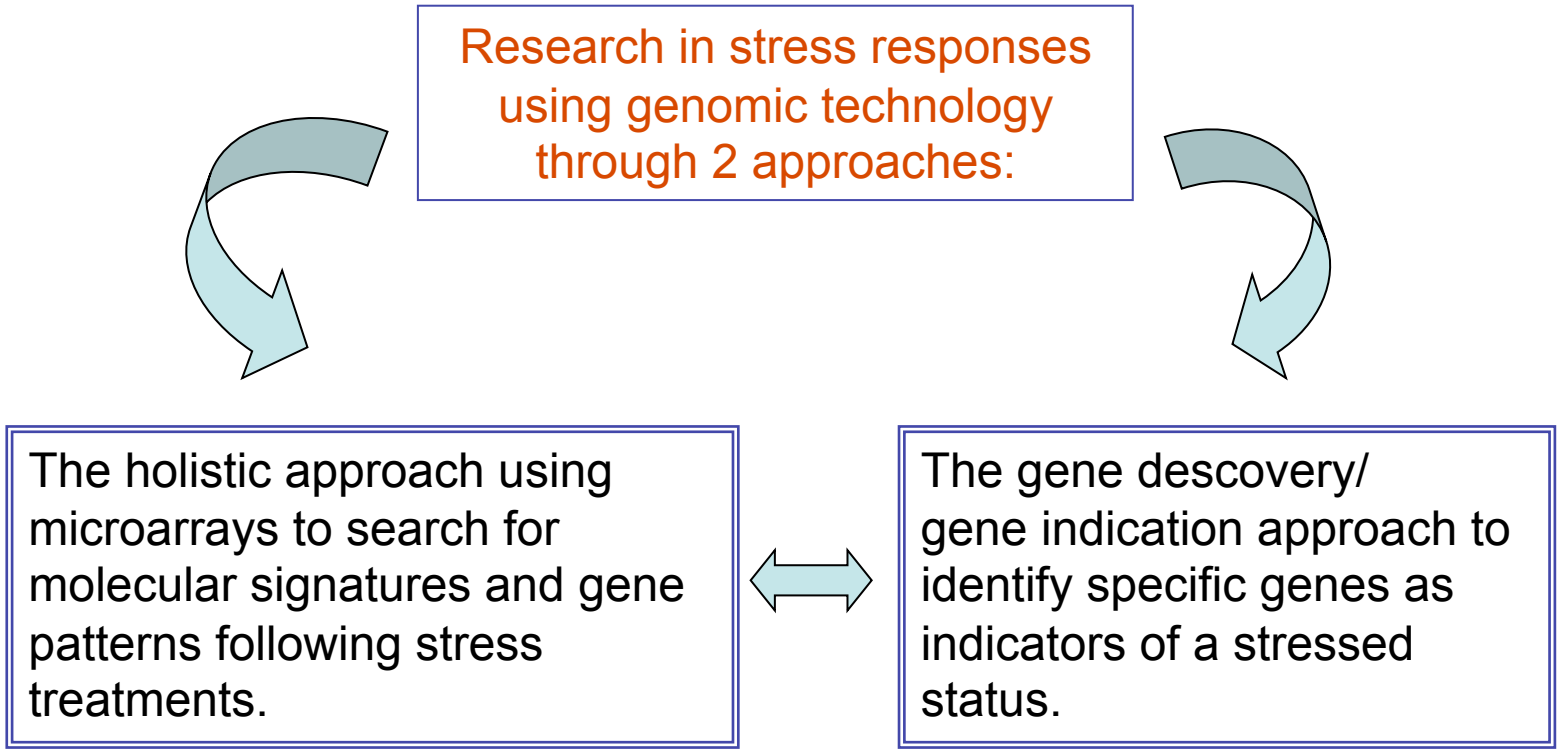
1 Total Citations either Gene Expression Profiling or Microarray Analysis with each organism or group



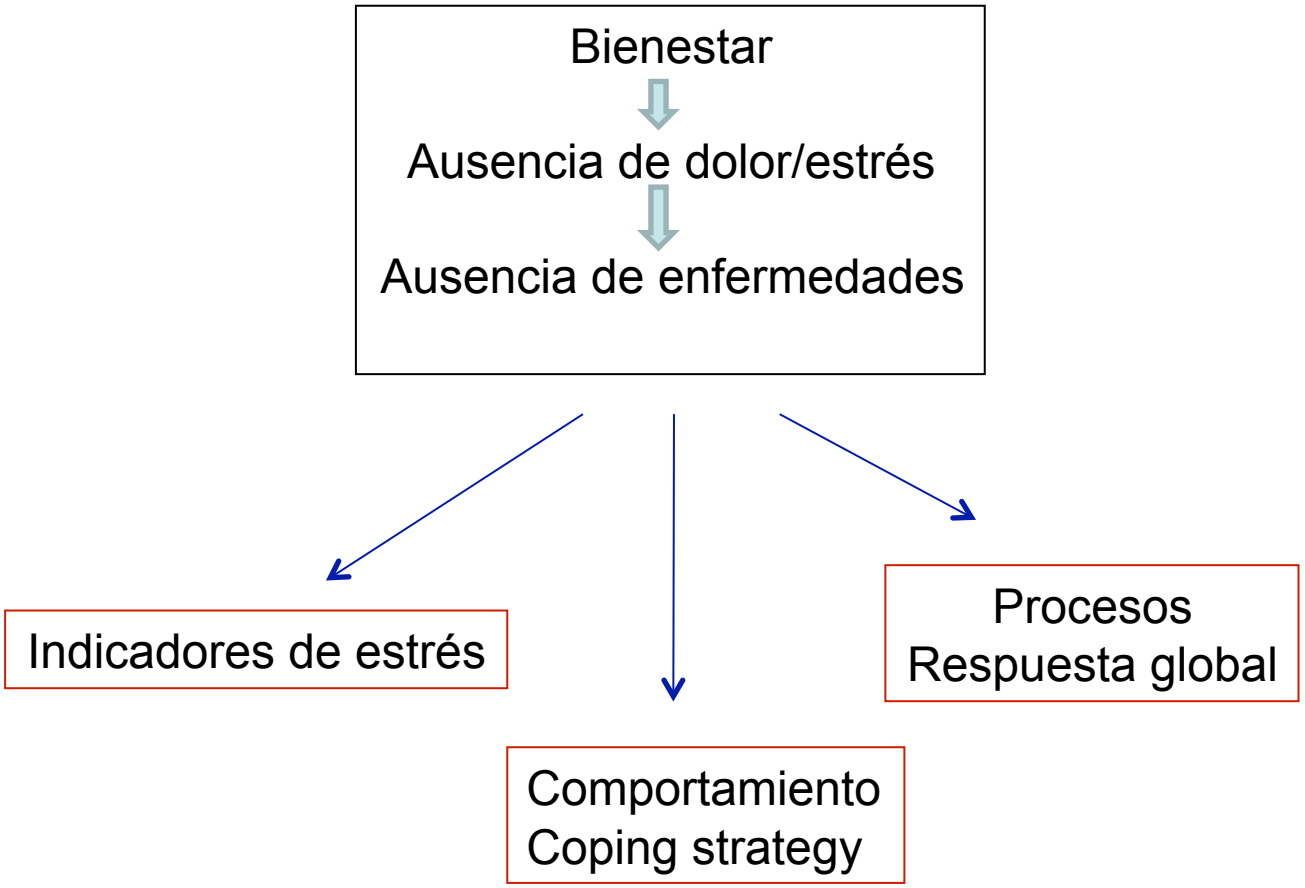
2 Total Citations either GE Profiling or MA within the Fish

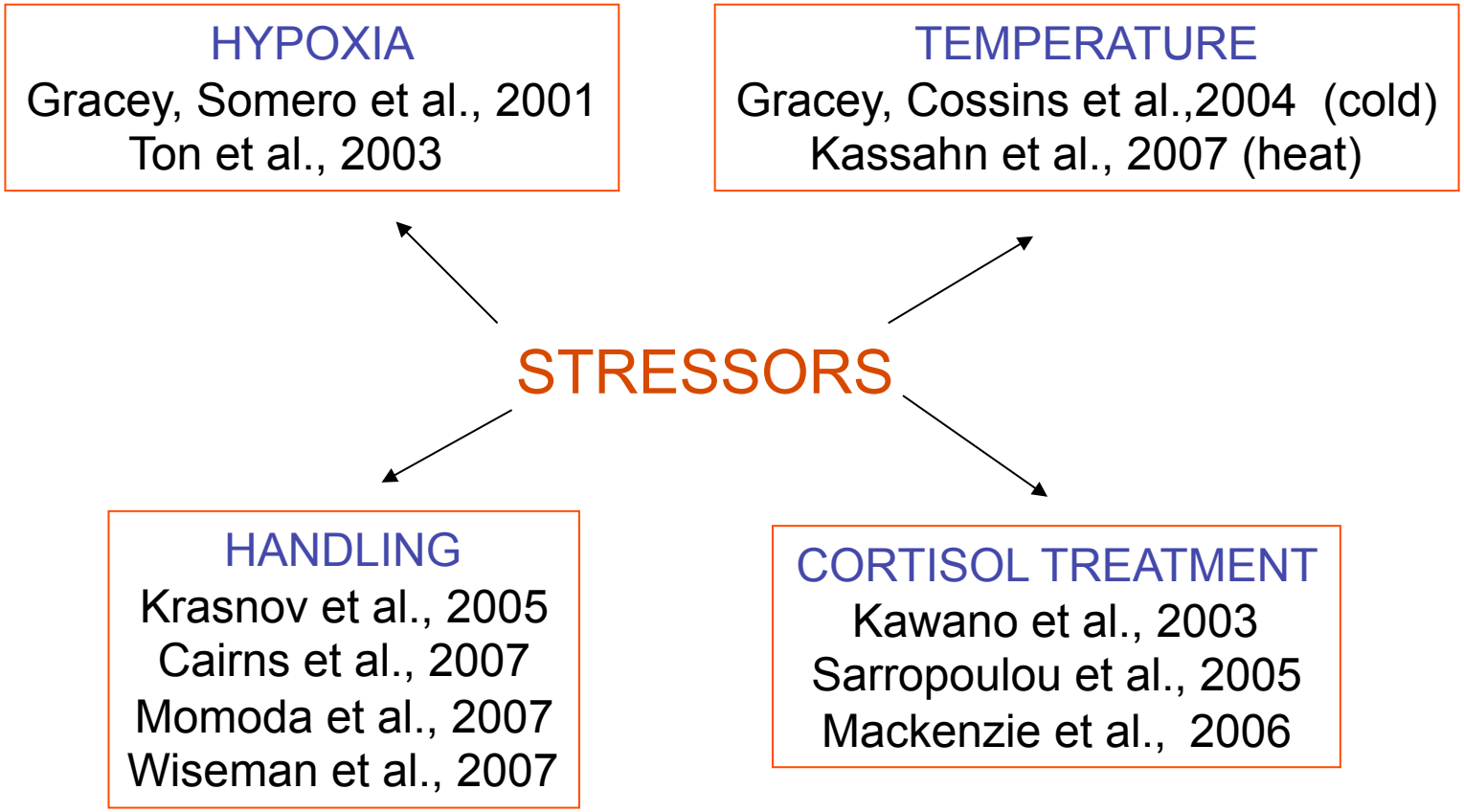


GEP and MA account for 2.5% and 1% of all Citations (21stC) for fish(>32K) in PubMed respectively


Research in stress responses using genomic technology through 2 approaches:
The holistic approach using microarrays to search for molecular signatures and gene patterns following stress treatments.
The gene descovery/ gene indication approach to identify specific genes as indicators of a stressed status.

Estudio del bienestar animal en peces mediante tecnología genómica




HYPOXIA
Gracey, Somero et al., 2001
Ton et al., 2003
TEMPERATURE
Gracey, Cossins et al.,2004 (cold)
Kassahn et al., 2007 (heat)
STRESSORS
HANDLING
Krasnov et al., 2005
Cairns et al., 2007
Momoda et al., 2007
Wiseman et al., 2007
CORTISOL TREATMENT
Kawano et al., 2003
Sarropoulou et al., 2005
Mackenzie et al., 2006

Gene expression in trout after handling stress
Krasnov et al., 2005.
BMC Genomics, 6

- Stressor: acute handling
- Species: trout
- Tissue: brain and kidney
- Timing: 1-24h

Cairns, Pottinger et al., 2007
Assessment of gene expression in trout in response to handling and confinement
Comp. Biochem. Physiol. D

- Stressor: Handling and confinement
- Species: trout
- Tissue: liver
- Timing: early and late: 2h-21 days (Stressgenes)

Momoda, Schreck et al., 2007
Liver gene expression in trout during the stress response to hypoxia and handling
Comp. Biochem. Physiol, D

- Stressor: hypoxia and handling
- Species: trout
- Tissue: liver
- Timing: 0,5-3-24h post stress

Wiseman, Vijayan et al., 2007.
Gene expression during recovery from an acute stressor (handling)
Comp. Biochem. Physiol. D

- Stressor: acute, 3 min handling.
- Species: trout
- Tissue: liver
- Timing: 1-24h

Kawano et al., 2003
Analysis of gene expression in carp treated with cortisol
Comp. Biochem. Physiol. B. 136

- Stressor: Cortisol (1micromolar)
- Species: carp
- Tissue: head kidney macrophages
- Timing: 8h

Sarropoulou et al., 2005
Gene expression profile in seabream during development and detection of stress (cortisol) related genes.
Physiol. Genomics, 23.

- Stressor: cortisol (implant 10mg/kg)
- Species: seabream
- Tissue: embryos, and kidney (juveniles)
- Timing: 72h

Transcriptional analysis in response to LPS treatment and cortisol in trout
Mackenzie et al., 2006
Molecular Immunology, 43

-Stressor: LPS (10ug/mL)
cortisol (600ng/mL)
- Species: trout
- Cells: head kidney macrophages
- Timing: 6-24 h

GENE NAME	LPS	LPS + cortisol
Galectin-1	-1,94	-1,22
Ferritin heavy chain	-2,22	-1,23
Histone H3.3	-1,76	-1,02
Alpha-tubulin 2	-2,20	-1,61
Profilin I.	-2,56	-1,75
Coronin 2	-1,96	-1,25
Cytokeratin 8	-2,96	-1,32
Actin-related protein 2/3 complex subunit 2	-2,41	-1,11
Alpha-actin 1	-3,15	1,10
Beta-actin	-2,53	-1,15
Gamma-actin	-2,22	1,01
Cytochrome c oxidase subunit II.	-1,68	-1,02
Cytochrome c oxidase subunit III	-2,07	1,14
Beta enolase	-2,08	-1,55
NADH dehydrogenase subunit 2	-2,27	-1,62
Transaldolase	-2,80	-1,81
Glucose-6-phosphate isomerase	-1,88	-1,44
Glyceraldehyde 3-phosphate dehydrogenase	-2,65	-1,69
NADH dehydrogenase subunit 5	-2,51	-1,62
Galectin-9	-1,97	-1,27
NADH-ubiquinone oxidoreductase chain 4	-2,11	-1,25
Cytochrome c oxidase polypeptide I	-2,49	-1,31
ATP synthase beta chain, mitochondrial precursor	-2,63	-1,20
ATPase 6	-2,01	-1,18
Cyclophilin A	-3,46	-1,24
Cytochrome B-245 heavy chain	-1,50	-1,21
HLA class II histocompatibility antigen, gamma chain	-2,67	-1,21
Lysozyme C precursor	-2,81	-1,07
Metallothionein-IL	-2,66	-1,45
Peroxiredoxin 1	-2,06	-1,35
Cathepsin S precursor	-3,10	-2,25
Cathepsin C	-1,69	-1,28
Cathepsin Z precursor	-2,66	-1,35
Polyubiquitin UBC	-2,24	-1,03
Heat shock 70 kDa protein	-1,71	-1,34
Guanine nucleotide-binding protein beta subunit-like protein 12.3	-2,21	-1,06
PTD013 similar to CGI-24 protein	-2,11	1,10
Midkine precursor	-2,02	1,12
nitric oxide synthase 2	-2,14	1,18
Nuclease sensitive element binding protein 1	-1,73	1,04
Myelin transcription factor 1	-1,68	-1,20
40S ribosomal protein S9	-2,35	-1,08
40S ribosomal protein S2 (S4)	-2,18	1,21
40S ribosomal protein S3	-1,83	1,24
60S ribosomal protein L6	-1,70	1,05
60S acidic ribosomal protein P0	-1,83	1,11
60S ribosomal protein L15	-1,86	1,17
Elongation factor 1-alpha 1	-2,08	1,06
OK/SW-CL.16	-1,86	-1,08
EST	-3,76	-1,32
Microtubule-associated protein RP/EB family member 3	-1,81	1,36

EXPRESSION SCALE

	< - 3
	-1.4 to -3
	-1.3 to 1.3
	1.3 to 2.7
	> 2.7

A

GENE NAME	LPS	LPS + cortisol
Astrocytic phosphoprotein PEA-15	1,58	-1,19
Alpha-2,8-sialyltransferase 8E	1,39	1,14
FK506-binding protein 3	2,95	-1,21
Secretory granule proteoglycan core protein precursor	2,34	1,04
Tyrosine-protein kinase HCK	1,89	-1,64
Membrane copper amine oxidase	1,64	-1,40
High affinity immunoglobulin gamma Fc receptor I precursor	1,47	1,03
Class I histocompatibility antigen-like protein.	1,63	-1,05
PIntegrin beta-1 precursor	1,76	-1,04
CD39 antigen	1,85	1,40
Macrophage receptor MARCO	2,15	1,50
NF-kappaB inhibitor alpha	2,60	1,60
PEST-containing nuclear protein	1,82	1,40
Heat shock protein HSP 90-alpha	1,62	1,22
KIAA1550 protein	2,28	1,33
MAP kinase p38 delta	4,04	1,85
CCAAT/enhancer binding protein alpha	5,03	1,42
GADD45 alpha	2,08	1,23
Transcription regulator protein BACH1	1,69	1,31
Zinc finger protein 148	1,68	1,01
Similar to RIKEN cDNA 4921505C17 gene.	2,70	-1,02
TFAR15	1,67	1,05

B

GENE NAME	LPS	LPS + cortisol
Heat shock protein HSP 27	1,49	1,65
Matrix metalloproteinase-13	8,09	5,77
Matrix metalloproteinase-9	2,57	1,93
Adenosine deaminase	1,60	1,38
Transcription factor jun-B	1,63	1,33
Leukocyte cell-derived chemotaxin 2 precursor	1,29	2,03
Endoplasmin precursor	-1,28	-2,11
Heat shock protein HSP 90-beta	-1,19	-1,60

C

Heat map

Cortisol acts mainly as antagonist in response to LPS

HANDLING

BRAIN: Increase in metalloproteins and energy driving processes (catabolism)
Decrease of immune defense genes

LIVER: Decrease of HsP
Upregulation of immune related genes
Genes involved in energetics and reprogramming liver machinery
Responsive genes to corticosteroids

CORTISOL

Upregulation of energetic metabolism (enolase, fructose phosphatases)
Upregulation of protein biosynthesis
Upregulation of ion transport (Na-K-ATPases)
Down regulation of cell reorganization and growth (Iron metabolism)

Opposite action on genes regulated by pathogens
-Inhibition of 53% of the genes activated by LPS
-Activation of 78% of the genes suppressed by LPS

Stress and immunosuppression. New stress indicators: Gene discovery

ENOLASE

Glycolisis

2-fosfoglicerate

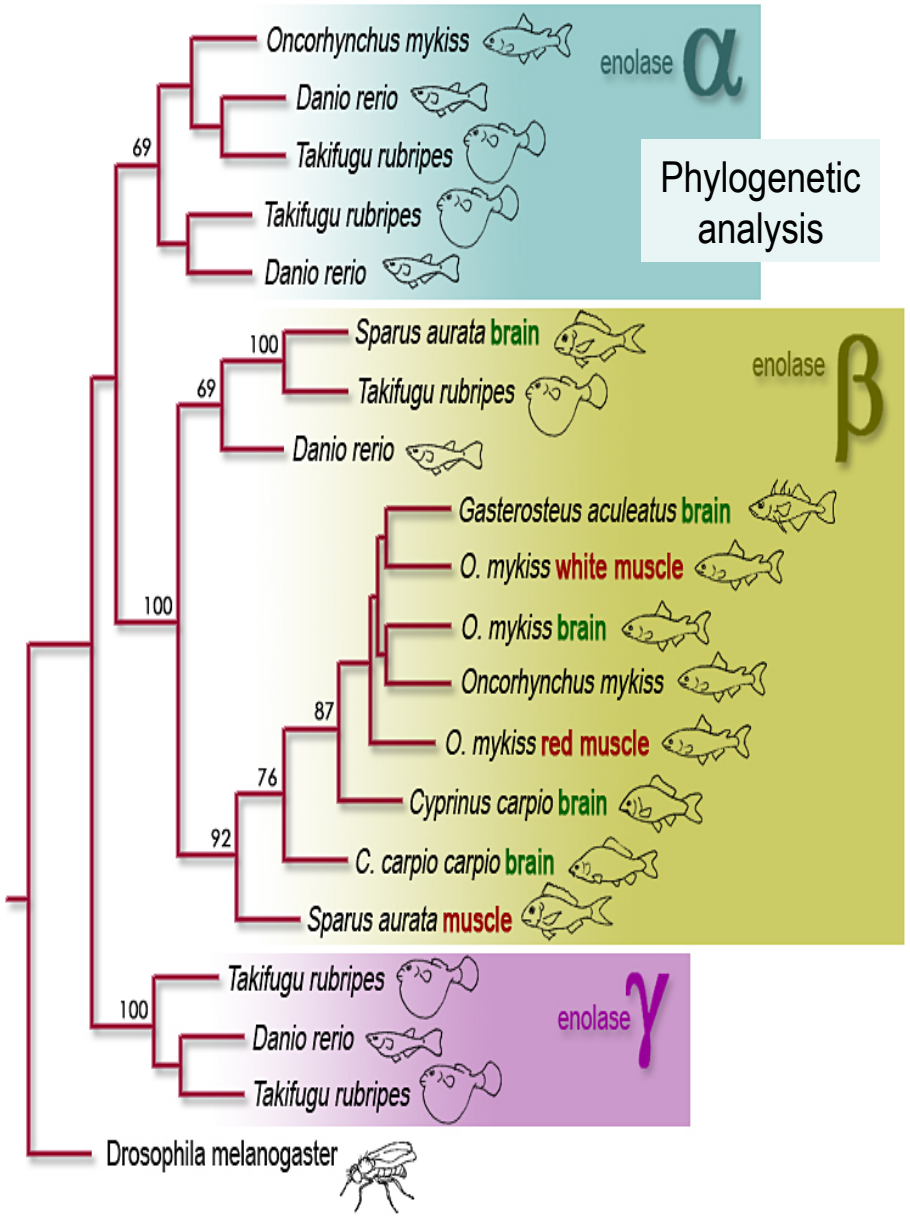
↓ 2-fosfo-D glicerate hidrolase/ENOLASE

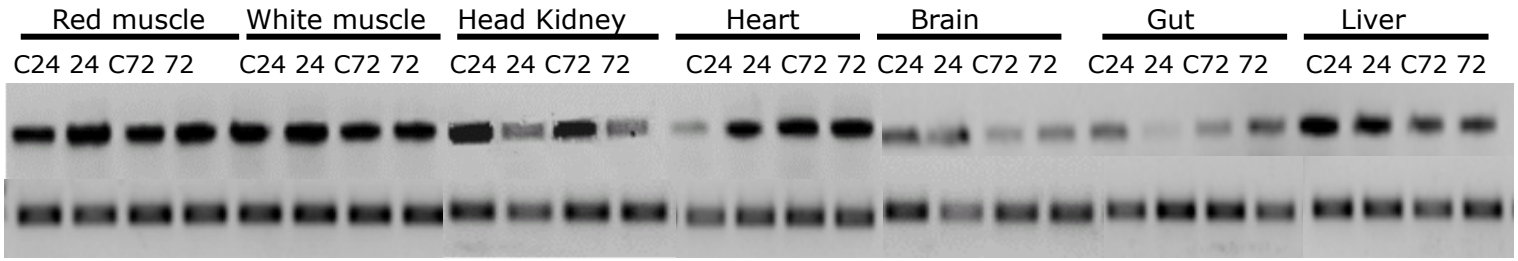
Fosfoenolpiruvate+H_2O

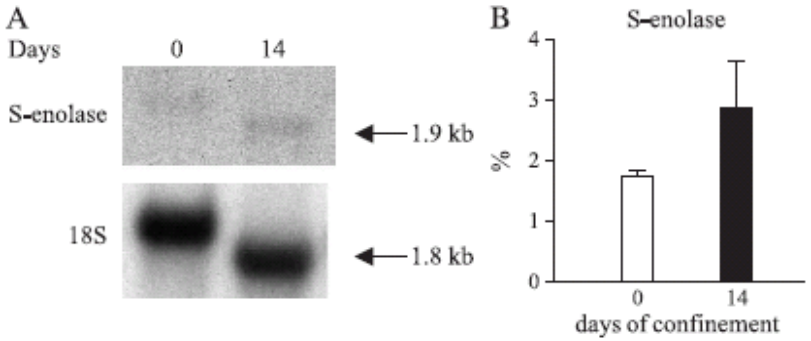
Surface receptor /plasminogen
Miles *et al*., 1991 (Biochem.J)

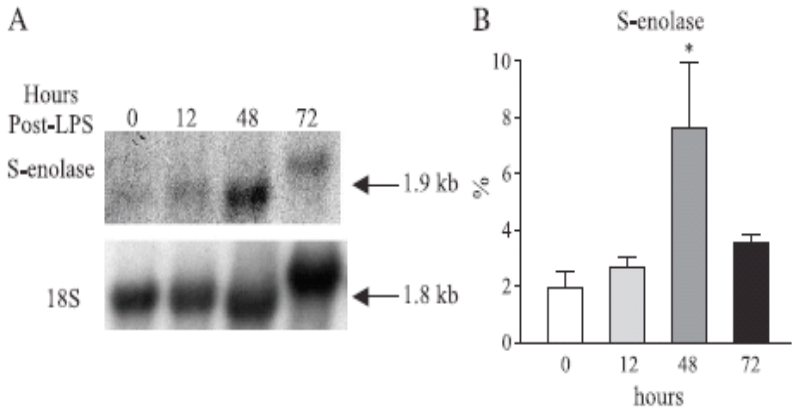
Transcriptional Regulator
Homology with DNA union protein Ray *et al*.1995 (Cancer Res.)

Heat shock protein,
Iida H. *et al*., 1985 (Nature)
Lens t Protein,
Wistow, G.J. *et al*., 1988 (J. Cell. Biol.)
Cancer, Autoimmune disorders, Bacterial diseases









Enolase expression in 34 microarray experiments

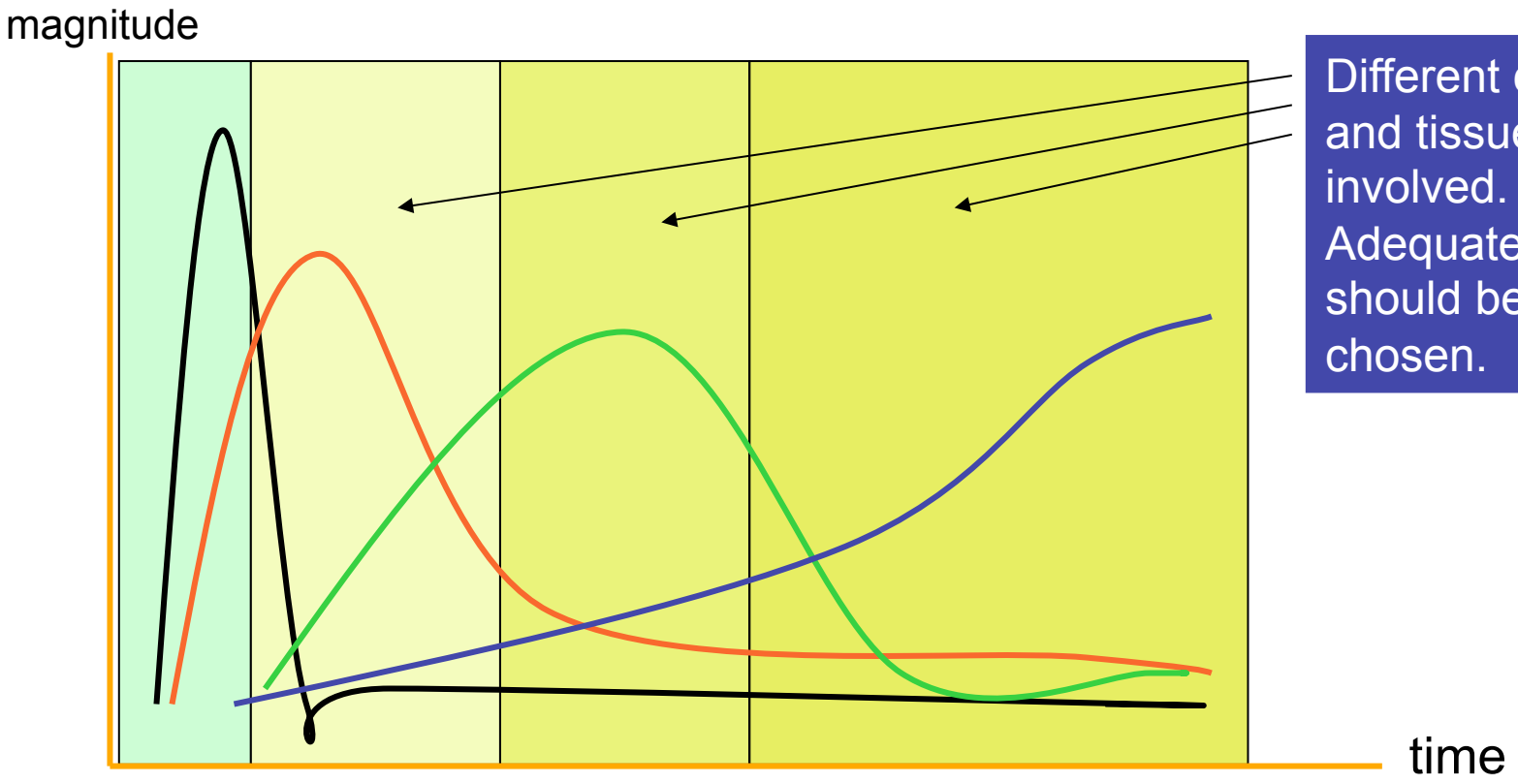
Up-regulated 84%

Down-regulated 16%

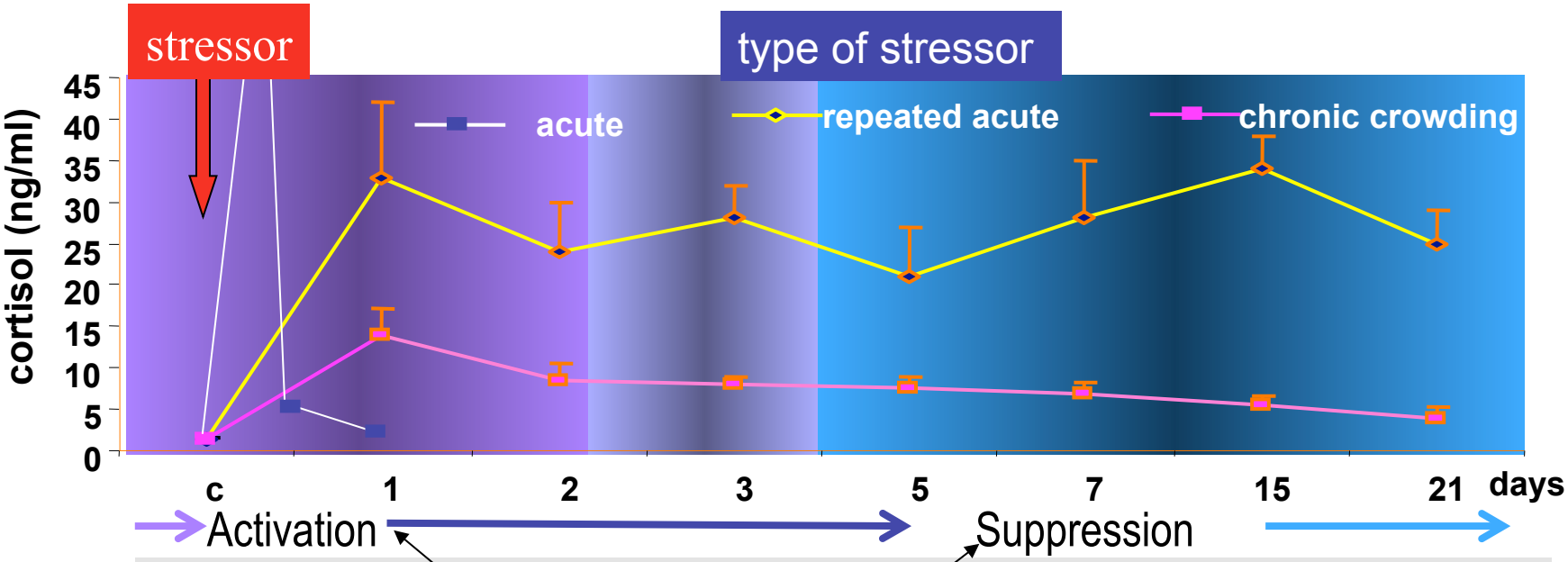
High correlation with GAPDH expression (Glycolitic multifunction)

Enolase expressed differentially after chronic stress or LPS injection (Ribas et al. 2004. Aquaculture)

Still, some relevant issues have to be correctly addressed:



Different cells and tissues involved. Adequate timing should be chosen.



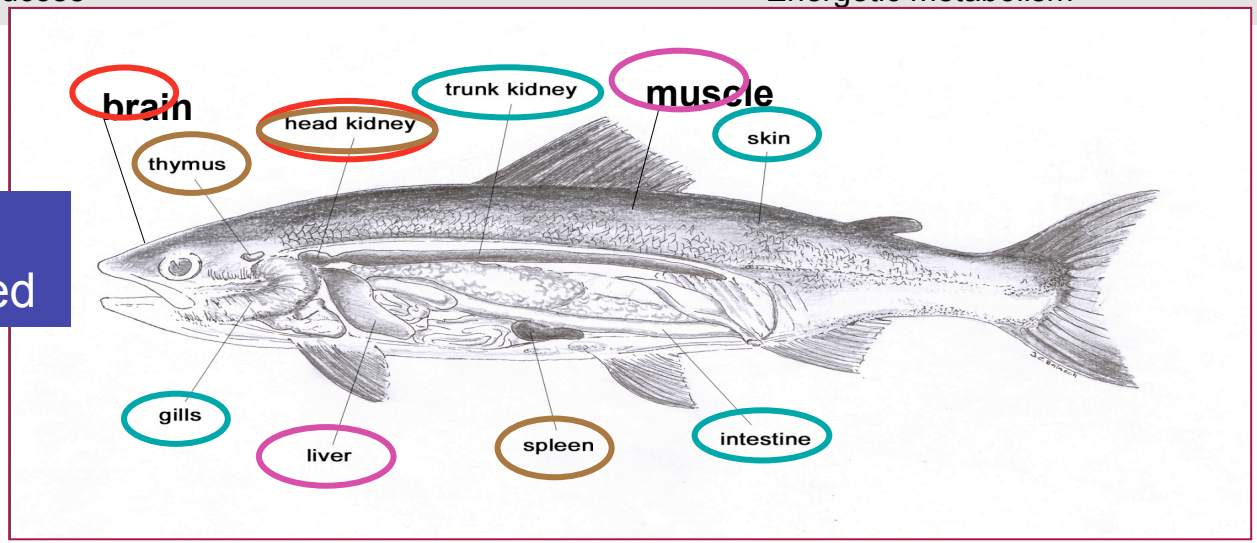
Cytokines (TNF, IL's)
Lysozyme
C3 proteins
Acute phase proteins
Adrenaline, cortisol
Glucose

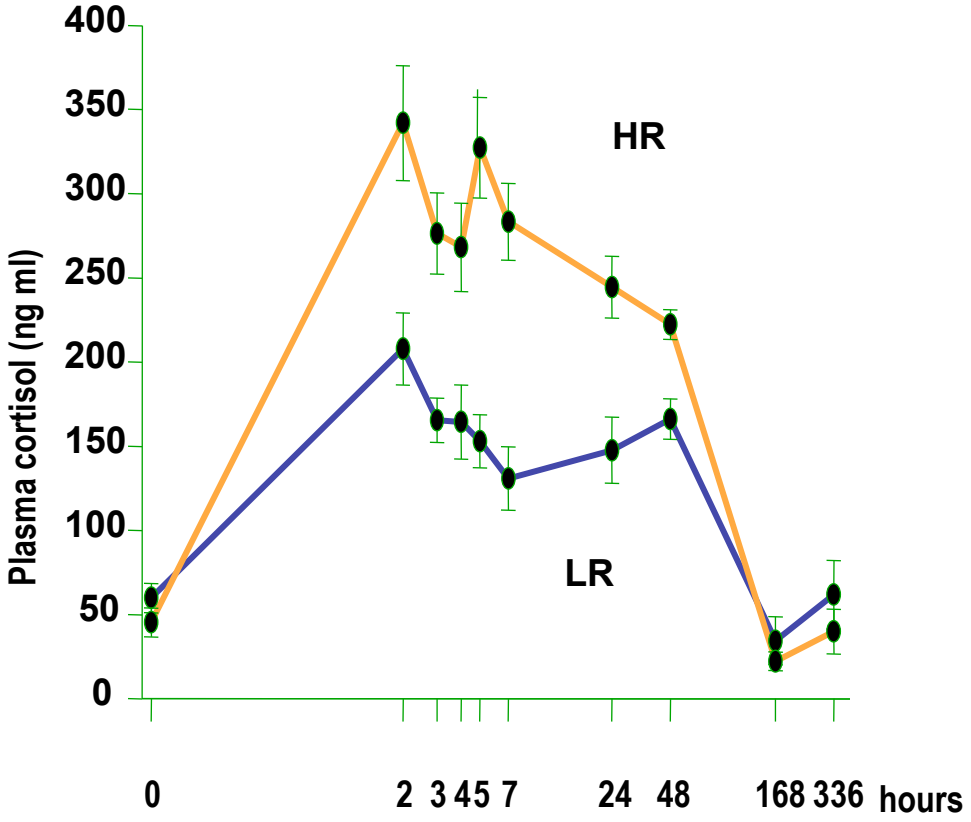
Phase of response

Phagocytosis
Cell numbers and populations
Apoptosis, Lytic activity
GH, MSH
Feed intake, growth
Energetic metabolism

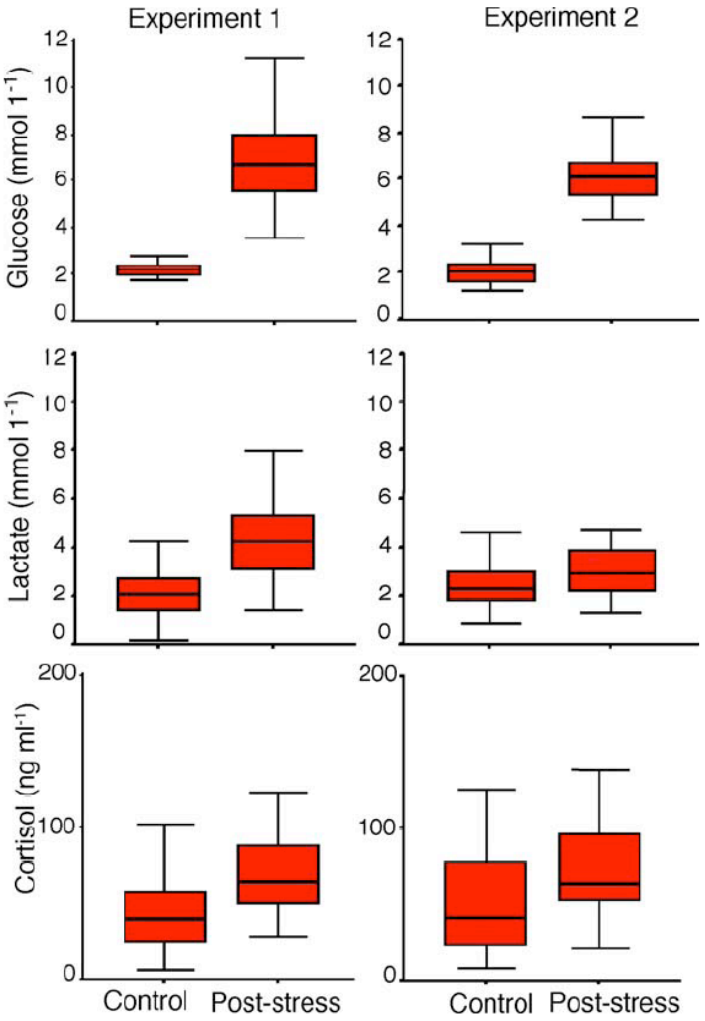
Candidate Markers

Fish species
Tissue involved





Time-course of changes in plasma cortisol level during chronic confinement in LR and HR trout (Pottinger et al., 2002)



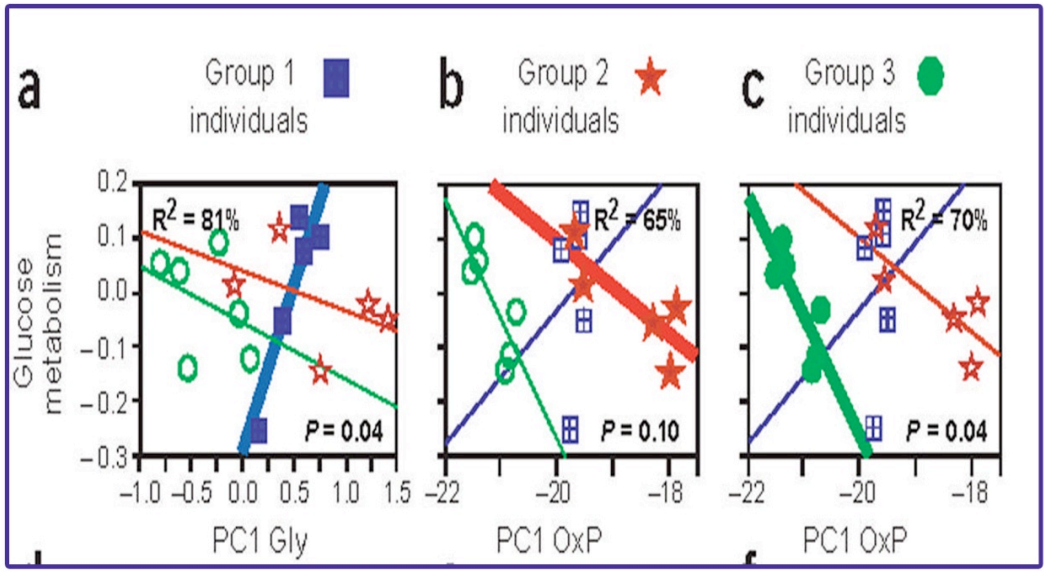
Individual variation in basal and post-stress levels (Martins et al., 2006)

Variability of parameters depending on the individual / group differences

Oleksiak *et al* (2002) Variation in gene expression within and among natural population. *Nat. Genet* 32:261–266.

Oleksiak *et al* (2005) Natural variation in cardiac metabolism and gene expression in Fundulus heteroclitus. *Nat Genet* 37: 67-72.

Crawford DL, Oleksiak MF. (2007) The biological importance of measuring individual variation.*J Exp Biol*.



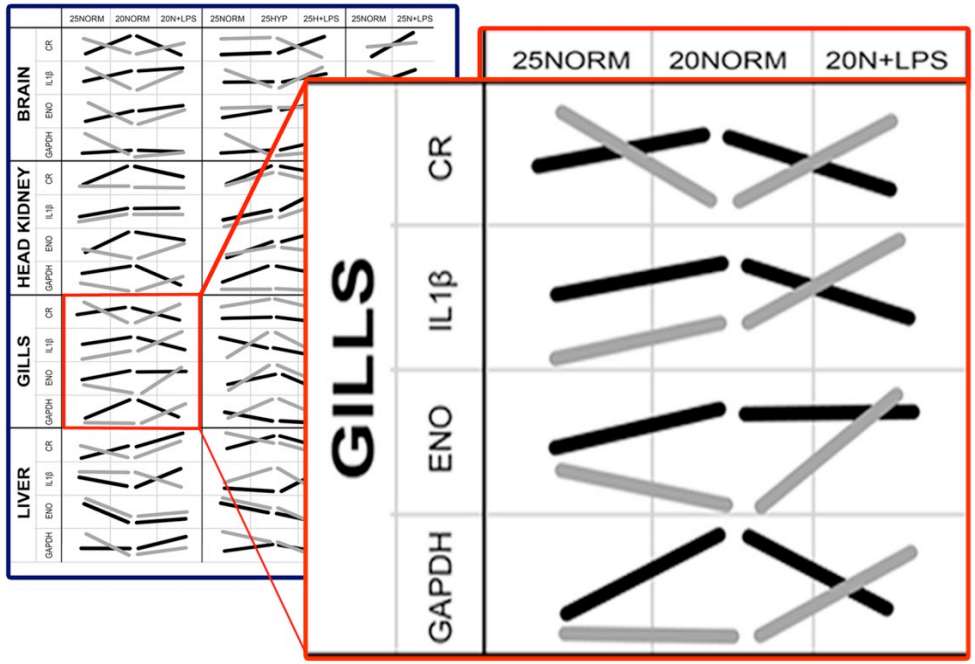
Variation in metabolism-specific genes is higher within a population than between geographically distinct populations

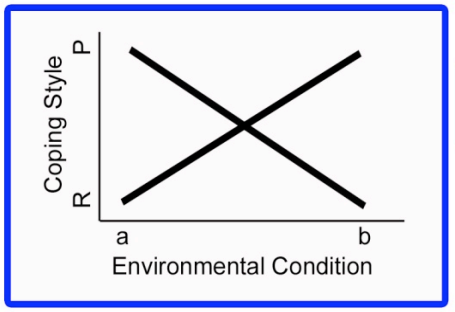
Different individual patterns of gene expression correlate to physiological processes.

Failure to consider this type of biological variation can result in the misidentification of genes that merely represent standing genetic or natural biological variation as 'important'

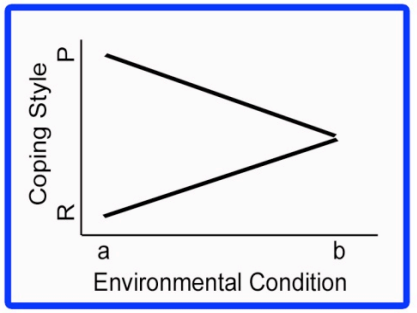
Variation in physiological performance is related to the subtle variation in gene expression and that this relationship differs among individuals

Differential responses in common carp (Cyprinus carpio L.) under environmental challenge highlight the importance of coping style in integrative physiology Morera *et al* 2010 *under review*



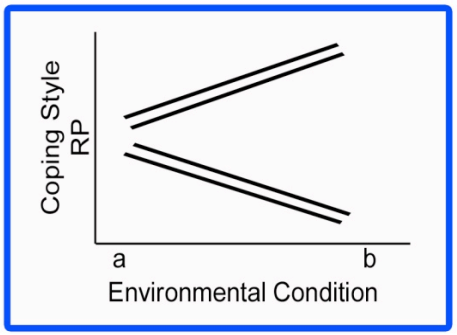


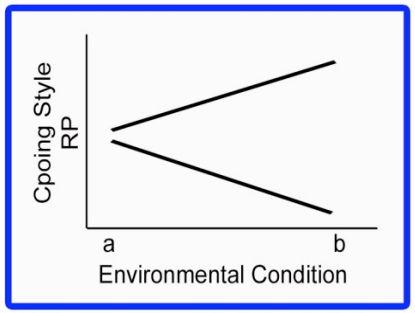
JUXTAPOSE



CONVERGENT

SIMILAR





DIVERGENT

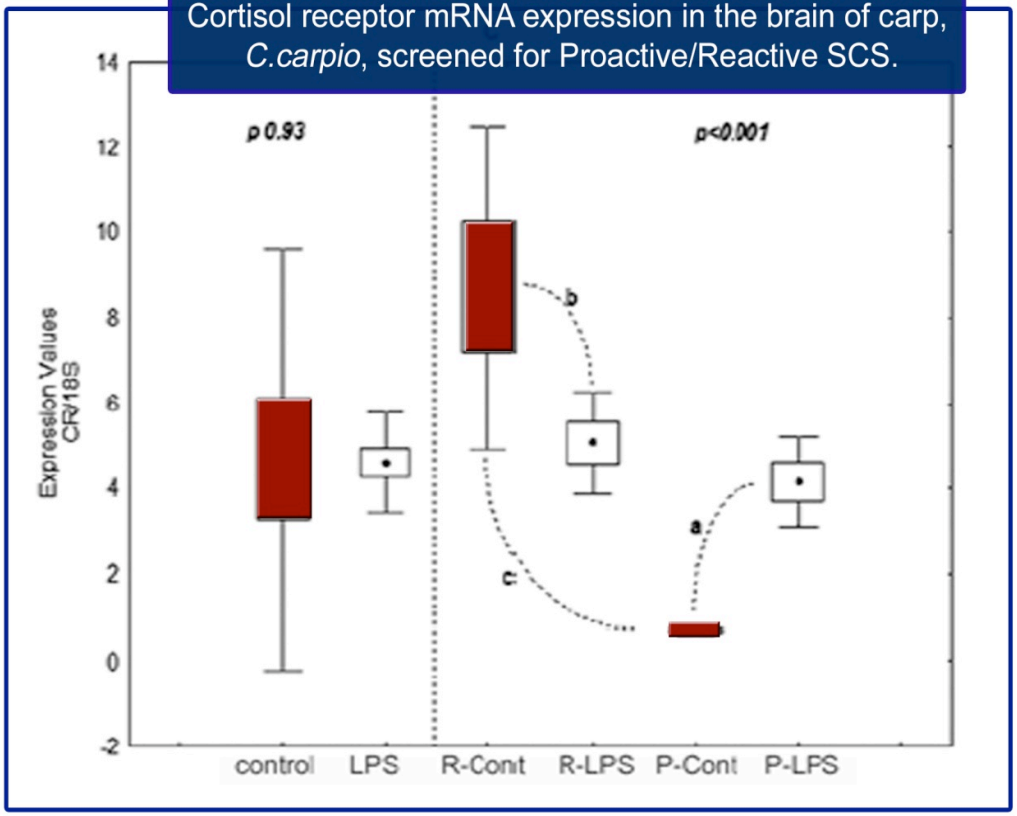
Mackenzie et al (2009) Screening for coping style increases the power of gene expression studies. PLoS ONE, 4: e5314..

Baseline mRNA abundance levels are different between Proactive and Reactive carp.

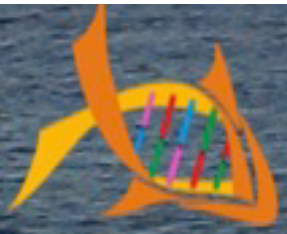
Response to challenge is diametrically opposed.

No screening would have led to a non-significant response.

Cortisol receptor mRNA expression in the brain of carp, *C.carpio*, screened for Proactive/Reactive SCS.



Incorporating coping style as an explanatory variable can account for;
1.unexplained variation that is common to gene expression



Aquagenomics

IMPROVEMENT OF AQUACULTURE PRODUCTION

BY THE USE OF BIOTECHNOLOGICAL TOOLS

Programa Consolider Ingenio 2010

Ministerio Ciencia e Innovación (España)

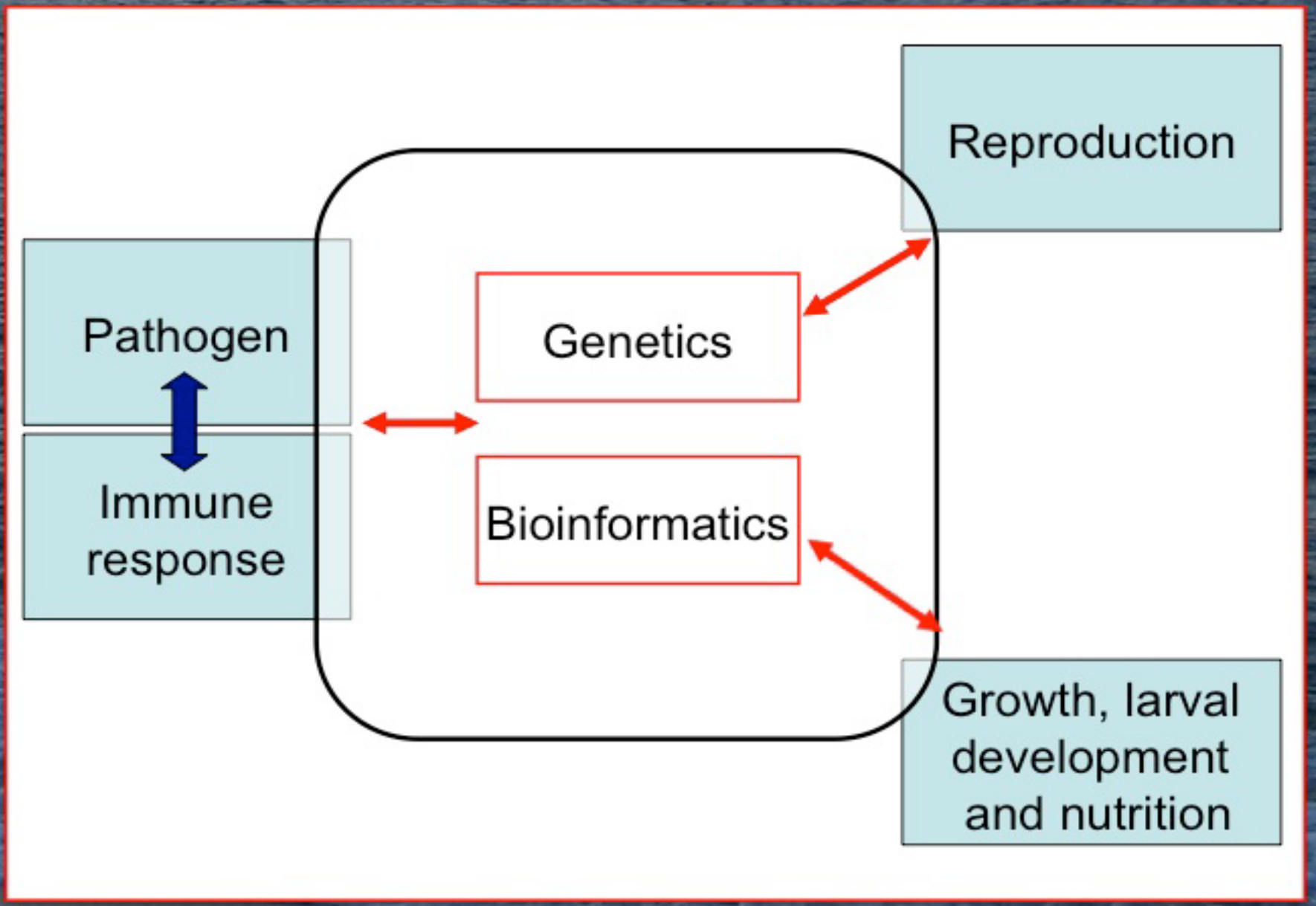
GOBIERNO DE ESPAÑA

MINISTERIO DE CIENCIA E INNOVACIÓN

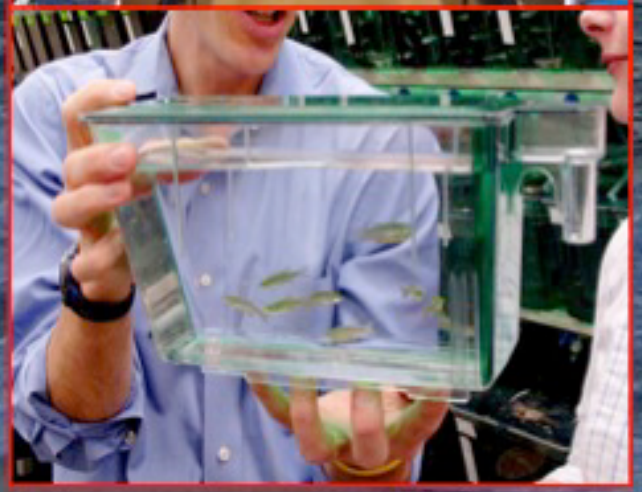
Consolider

PROGRAMA INGENIO 2010

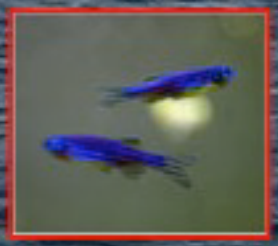
Aquagenomics



Aquagenomics



KNOCKDOWN



OVEREXPRESSION

MICROINJECTION

TRANSGENIC

Aquagenomics

PARTICIPANTS

Universidad de Barcelona
Universidad Autónoma de Barcelona
Consejo Superior de Investigaciones Científicas
- Instituto Investigaciones Marinas
- Instituto Ciencias del Mar de Andalucía
- Instituto Ciencias Marinas
- Instituto Acuicultura Torre la Sal

Universidad de Santiago de Compostela
Universidad de Murcia
Universidad Miguel Hernández
Universidad de Granada
Instituto Nacional de Investigación Agropecuaria
Centro de Investigación en Sanidad Animal

104 researchers

17 groups

80% Doctors

13% PhD Students

7% Technicians

Annual Plennary session

Six months coordination meetings

Bimonthly group and area reports

Microarray platform USC

1- **Update and registration of the turbot database** with around 7500 new EST sequences from challenges with *Enteromyxum* and nodavirus
2- Design and calibration of a new **version 2.0 of the turbot oligo-microarray** for analyze expression profiles
3- Identification of genes and functions regulated in **response to Aeromonas salmonicida**
4- Identification of genes and functions in **response to *Philasterides dicentrarchi***
5- Hybridizations performed of new experiments:
   - **Zebrafish** (48 (format 4x44))
   - **Turbot** (calibration: 32 (format 8x15); filtering: 4 (2x105); VHS: 16 (8x15); Philasterides: 26 (8x15)
   - **TOTAL: 126 hybridizations**

Results to be obtained in the next two years

1- **Update of the EST database with two new 454 runs** from gonad, brain, immune and muscle tissues (February 2011)
2- New version **v3.0 of the turbot oligo-microarray** (May 2011)

International cooperation:
Department of Biology, Research group ecophysiology, biochemistry and toxicology. University of Antwerp. Belgium

Microarray Platform-UAB

Format 4x44K
Gilthead Sea bream
(*S.aurata*)
7285 annotated targets
(x3 oligos/target)
8377 ESTs
(x1 oligos/target)

>160 hybridisations completed

Custom arrays
(Agilent Tech.)
Design and Validation Completed

All data uploaded to INB (Microarray database)

>100 hybridisations completed

Commercial arrays
(Agilent Tech.)

Format 4x44K
Zebrafish
(*D.rerio*)

Sea Bass
(*D.labrax*)
6275 annotated targets
(x3 oligos/target)
6924 ESTs
(x1 oligos/target)

>160 hybridisations completed

Microarray Platform-UAB *INTERNATIONAL IMPACT*

Collaborations are represented as both academic (financed projects) and receptors for microarray services including industry..

School of Freshwater Scienes,
University of Milwaukee-Wisconsin, USA
College of Veterinary Medicine,
Iowa State University, USA

INRA, France

University of Santiago
Chile

CURRENT COLLABORATIONS

Gulbekian Institute
Champalimaud Center
for the Unknown
Portugal

Institute of Biotechnology
and Biomedicine
UAB, Spain

University of Valencia, Spain

Skretting Inc.
Norway

Species-specific arrays under development; Perch (*P.flavescens*), Fathead Minnow (*P.promelas*), Eel (*A.anguilla*), Amphioxus (*B.lanceolatum*), Aquagenomics;Version 2 Sea bass, Sea bream.

Aquagenomics

FORESEEN RESULTS

INDUSTRY

1.- Increase disease resistence.

2.- Control sex differentation, maturation and reproduction.

3.- Improve growth and food conversion rates.

4.- Design and apply selection protocols at production plants.

SCIENTIFIC

1.- Design and improve vaccines and immunostimulants.

2.- Control of sex proportion and gonadal development.

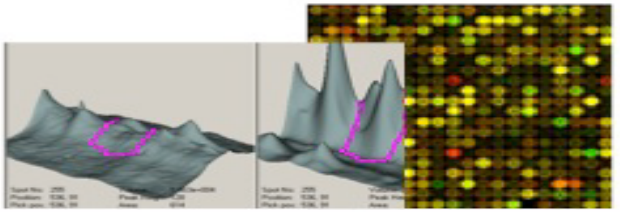
3.- Identify genes that play a critical role in growth, larval development and feeding efficiency

4.- Construct and improve genetic maps of the species of interest.

5.- Develop and maintain data bases with sequences of these species.

6.- Develop, validate and use microarrays for each species.

Increase the efficacy of vaccination and immunostimulation processes



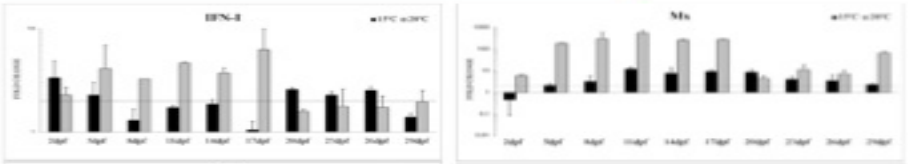
1. Genes related with resistance

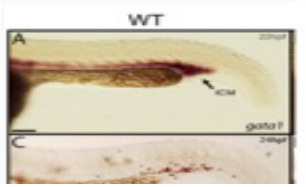
Transcriptomics and proteomics after viral infection: Identification and characterization of genes involved in the immune process (galectin, TLRs, cytokines)

4. Ontogeny of the immune system

How early can we vaccinate or stimulate fish?

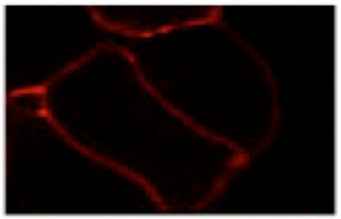
Evolution of the expression of antiviral molecules in





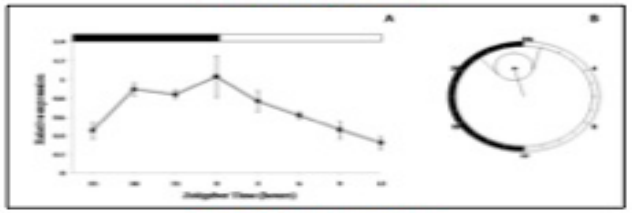
- Identification and characterization of genes regulating food intake, circadian feeding rhythms and the synchronizing role of feeding on digestive function and growth in seabass and seabream.
- Transcriptional analysis by microarray.

Melacortin system



Seabass MCR2 and MRAP coexpressed in HEK293 cells

Clock genes



Seabass *Per1*

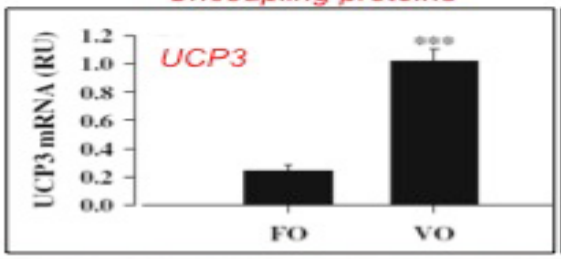
Digestive enzymes

Seabream *Trypsinogen* (15 DAH)

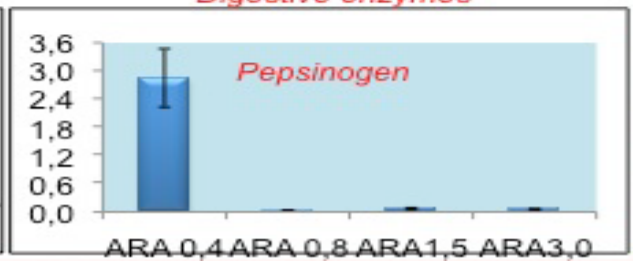
- Transcriptional profiling of the effects of dietary manipulation and stress in relation to feeding in seabass and seabream. Characterization of specific marker genes.

Dietary manipulations:
- Substitution of fish oil for vegetable oil
- Microparticulated diets
- Arachidonic acid (ARA) in diets

Uncoupling proteins



Digestive enzymes



Transcriptomics in fish biology

RNA-Seq Microarray

Arrays are only as good as the genes they contain, while sequencers could theoretically give you the entire transcriptomic profile if given enough sequences

The advantage in using sequencing over arrays would be that transcript profiles could be quantified at the same time that gene discovery was taking place.

Functional genomics with microarrays in fish biology and fisheries
Goetz and MacKenzie. (2009) Fish and Fisheries

Low input - High throughput - No output?
Brenner 2010.Phil.Trans.Royal.Soc.

Available genomic platforms related to stress and welfare in fish

A number of initiatives, under public EU funds, have been undertaken to build genomic know-how and platforms for the last years, most of them including somehow **stress** assessment in fish.

There is a real need to share the information obtained and perhaps to agree in building technological **genomic platforms** to reach efficiency in EU genomic research and development

Bassmap	**Fastfish**
Bridgemap	**Reprofish**
Marine genomics	**Aquabreeding**
Stressgenes	**Eadgene**
Aquagenome	**Wellfish**
Aquafunc	**Pleurogene**
Aquafirst	**Aquagenomics**
Eurocarp	
Wealth	

Genomics-Stress-Physiology-Aquaculture

-The stress response is an evolutive phenomenon that involves all physiological compartments and all regulatory processes.

-Severe/acute stressors may induce molecular signatures that may be identified, but chronic subacute (aquaculture-related) stressors may just modulate processes.

-We probably need to better distinguish between regulatory processes and reactive processes when identifying new stress indicators.

-In aquaculture it will be necessary to address the precise specific models to avoid a bulk of non-relevant genomic information.

-There is a need for a stronger link between genetics and genomics in aquaculture industry to look for specific stress-susceptible / stress-resistant / stress responsive strains.

CONCLUSIONES

-La genómica tiene un potencial importante para identificar genes, procesos y regulación relacionada con el bienestar animal, o por oposición, la ausencia de estrés, enfermedad o malestar.

-Los resultados indican el camino para obtener más posibilidades para obtener:

+ líneas genéticas resistentes al estrés o con mejor índice de bienestar
+ indicadores consistentes sobre bienestar animal en peces.

-La comunidad científica y la administración española han desarrollado unos instrumentos de gran valor, casi únicos en Europa al servicio de la acuicultura.

-Además de producir transferencia al sector, estos esfuerzos deberían resultar en una plataforma de genómica acuática con capacidad para dar servicio a nivel europeo.