IATS Nutrigenomics Group web tools of practical use in aquaculture

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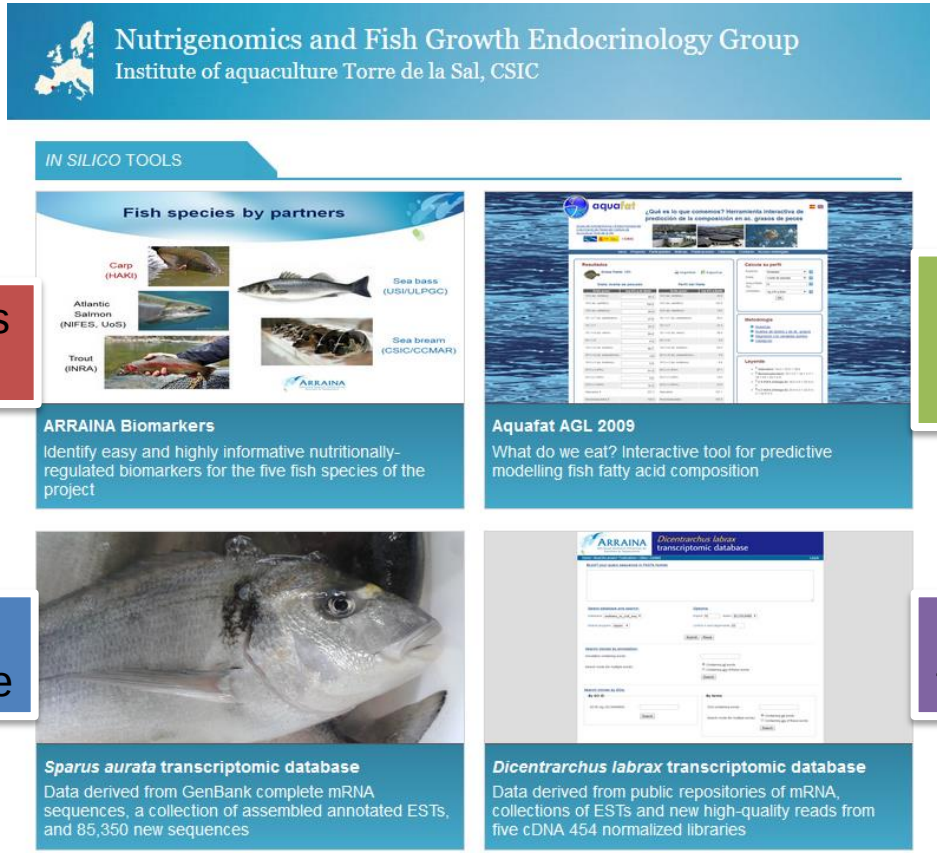


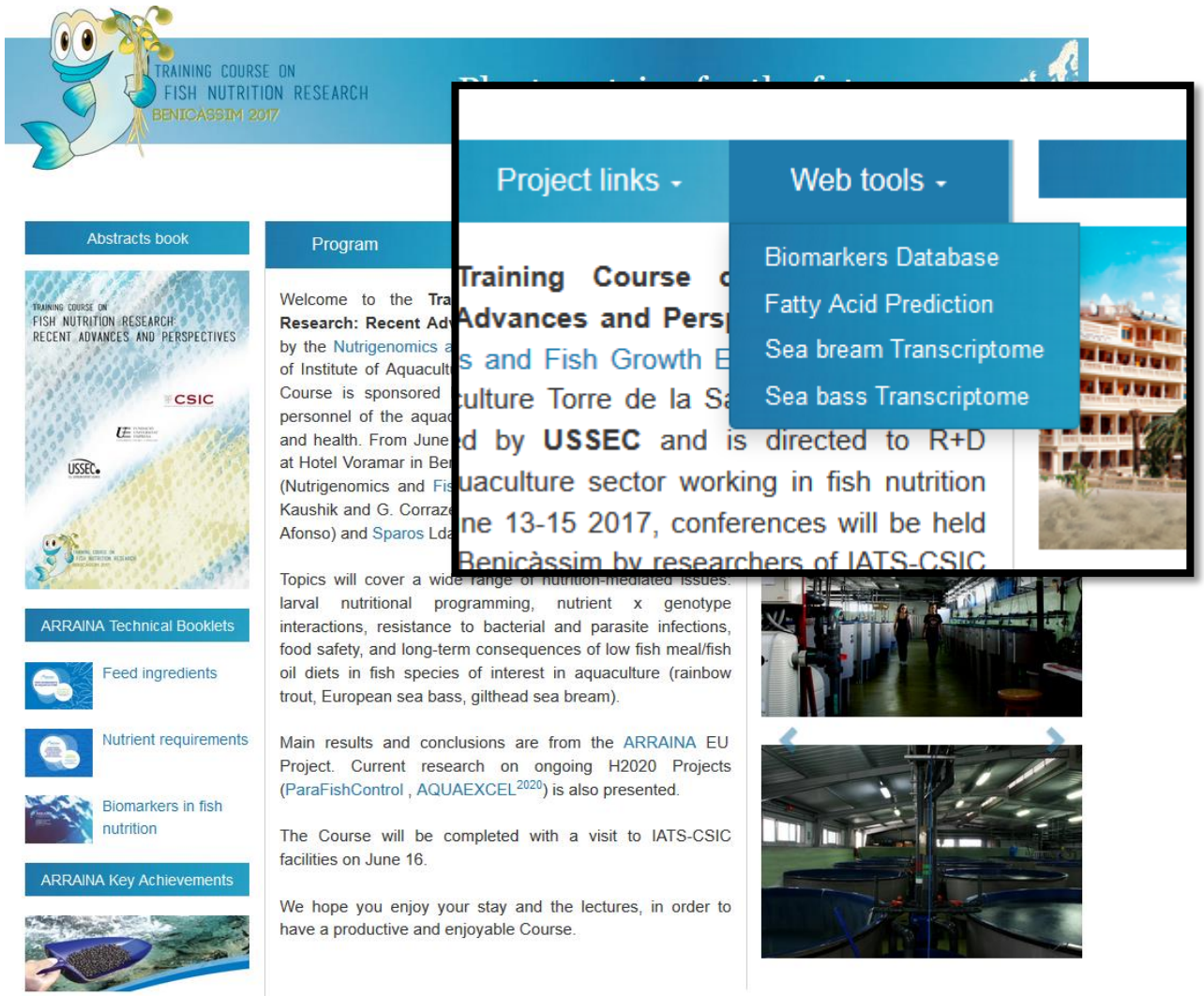
ARRAINA Biomarkers database

AQUAFAT
Fillet fatty acid prediction tool

Sea bream transcriptomic database

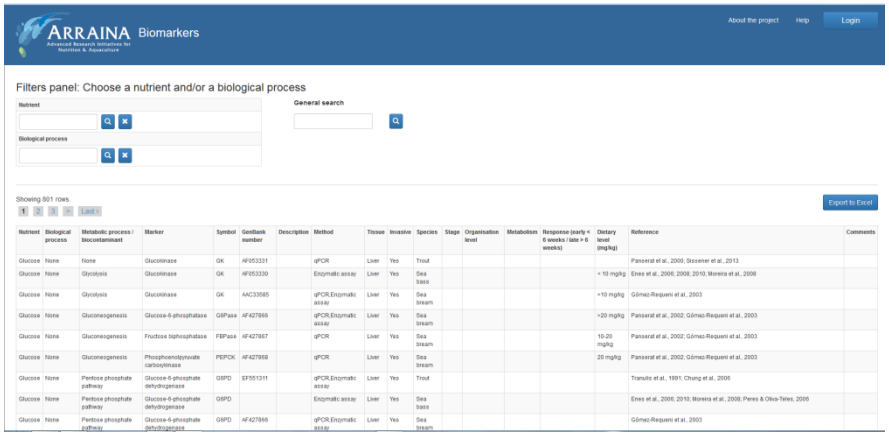
Sea bass transcriptomic database

www.nutrigroup-iats.org/ussec



ARRAINA BIOMARKER DATABASE

www.nutrigroup-iats.org/arraina-biomarkers

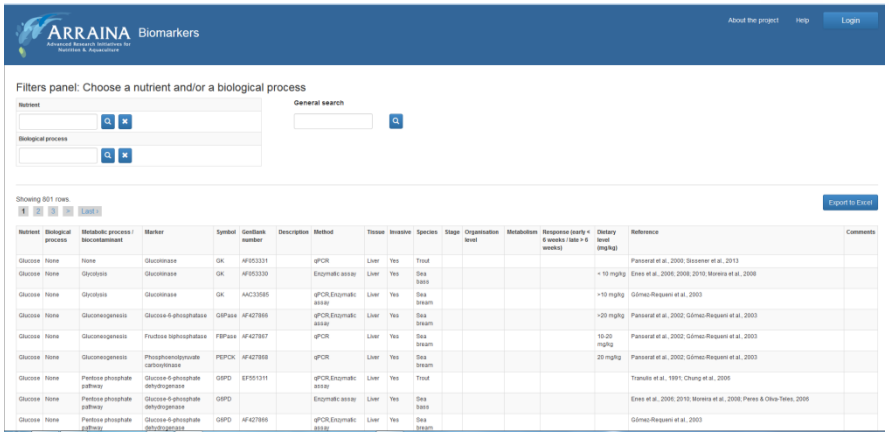


On-line tool for searching informative biomarkers of **nutrient requirements** or specific **biological processes**

- **Interactive & collaborative**: Inputs from INRA, NIFES, UoS, CSIC, ULPGC, HAKI, USI and CCMAR
- More than **800 entries**, **>20 fish species**

ARRAINA BIOMARKER DATABASE

www.nutrigroup-iats.org/arraina-biomarkers

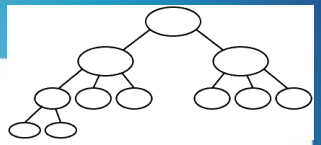


Nutrient	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage	Organisation level	Metabolism	Response (early < 6 weeks / late > 6 weeks)	Dietary level (mg/kg)	Reference	Comments
Glucose	None	None	Glucokinase	GK	AF053331		qPCR	Liver	Yes	Trout						Panserat et al., 2000; Skosener et al., 2013	
Glucose	None	Glycolysis	Glucokinase	GK	AF053330		Enzymatic assay	Liver	Yes	Sea bass					< 10 mg/kg	Enes et al., 2006; 2008; 2010; Moreira et al., 2008	
Glucose	None	Glycolysis	Glucokinase	GK	AAC33585		qPCR,Enzymatic assay	Liver	Yes	Sea bream					> 10 mg/kg	Gómez-Requeni et al., 2003	
Glucose	None	Gluconeogenesis	Glucose-6-phosphatase	G6Pase	AF427866		qPCR,Enzymatic assay	Liver	Yes	Sea bream					>20 mg/kg	Panserat et al., 2002; Gómez-Requeni et al., 2003	
Glucose	None	Gluconeogenesis	Fructose biphosphatase	FBPase	AF427867		qPCR	Liver	Yes	Sea bream					10-20 mg/kg	Panserat et al., 2002; Gómez-Requeni et al., 2003	
Glucose	None	Gluconeogenesis	Phosphoenolpyruvate carboxylinase	PEPCK	AF427868		qPCR	Liver	Yes	Sea bream					20 mg/kg	Panserat et al., 2002; Gómez-Requeni et al., 2003	
Glucose	None	Pentose phosphate pathway	Glucose-6-phosphate dehydrogenase	G6PD	EF551311		qPCR,Enzymatic assay	Liver	Yes	Trout						Tranulis et al., 1991; Chung et al., 2006	
Glucose	None	Pentose phosphate pathway	Glucose-6-phosphate dehydrogenase	G6PD			Enzymatic assay	Liver	Yes	Sea bass						Enes et al., 2006; 2010; Moreira et al., 2008; Peres & Oliva-Teles, 2006	
Glucose	None	Pentose phosphate pathway	Glucose-6-phosphate dehydrogenase	G6PD	AF427866		qPCR,Enzymatic assay	Liver	Yes	Sea bream						Gómez-Requeni et al., 2003	

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

- Filter by **nutrient** and/or **biological process**
- Also includes information for the assessment of **fish safety**
- General search box
- Public users can search and download filtered data

Biological process: “tree” categories



Development	• Bone mineralization • External appearance	• Skeletal development • Skeletal integrity	• Skeletal morphology
Growth	• Digestion • Energy wastage • Feeding behaviour • Growth dynamics	• Muscle growth • Muscle structure • Nutrient sensor	• Performance • Protein turnover • Somatotropic axis
Health & Welfare	• Antioxidant defence • Apoptosis • Cell diferentiation • Cell signalling pathway • Eicosanoid production	• External appearence • Haematology • Inflammatory response • Innate immune response • Internal appeareance	• Iron metabolism • Specific immune response • Tissue homeostasis • Tissue remodelling • Viral response
Quality at harvest	• Fillet FA composition		
Reproductive performance	• Fecundity • Fertility	• Gonad development • Sexual maturation	• Sperm quality • Vitellogenesis
Safety	• Biocontaminants		
Stress response	• Antioxidant defence • Apoptosis • Cell & tissue repair	• Endocrine status • Energy supply • Hypoxia	• Metabolite stress • Nuclear transcription factor • Oxidoreductase activity

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process

Nutrient

Biological process

General search

Showing 805 rows.

1 2 3 > Last ›

Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	S
Glucose		None	None	Glucokinase	GK	AF053331		qPCR	Liver	Yes	Trout	

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process

Nutrient

Biological process

General search

Choose a biological process

- All
- None
- Development
- Growth
- Health & Welfare
- Quality at harvest
- Reproductive performance
- Safety
- Stress response

Showing 805 rows.

1 2 3 > Last ›

Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	T
Glucose		None	None	Glucokinase	GK	AF053331		qPCR	L
Glucose		None	Glycolysis	Glucokinase	GK	AF053330		Enzymatic assay	L
Glucose		None	Glycolysis	Glucokinase	GK	AAC33585		qPCR,Enzymatic assay	L

ARRAINA Biomarkers database

DEMO

Filters panel: Choose a nutrient and/or a biological process

Nutrient

General search

Biological process

Growth

Metabolic process / biocontaminant

Button

Showing 72 rows.

1 2 3 > Last ›

Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage
Amino acid		Growth	Digestion	PepT1		FJ237043		qPCR	Intestine	Yes	Sea bass	
None		Growth	Performance	Specific growth rate	SGR			Weight	Population	No	Trout	
None		Growth	Performance	Specific growth rate	SGR			Weight	Population	No	Carp	
None		Growth	Performance	Specific growth rate	SGR			Weight	Population	No	Sea bass	
None		Growth	Performance	Specific growth rate	SGR			Weight	Population	No	Sea bream	
None		Growth	Performance	Specific growth	SGR			Weight	Population	No	Salmon	

ARRAINA

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process

Nutrient	
Biological process	**Metabolic process / biocontaminant**
Growth	

General search

Choose a metabolic process / biocontaminant

- All
- None
- Digestion
- Energy wastage
- Feeding behaviour
- Growth dynamics
- Muscle growth
- Muscle structure
- Nutrient sensor
- Performance
- Protein turnover
- Somatotropic axis

Species | Refine search

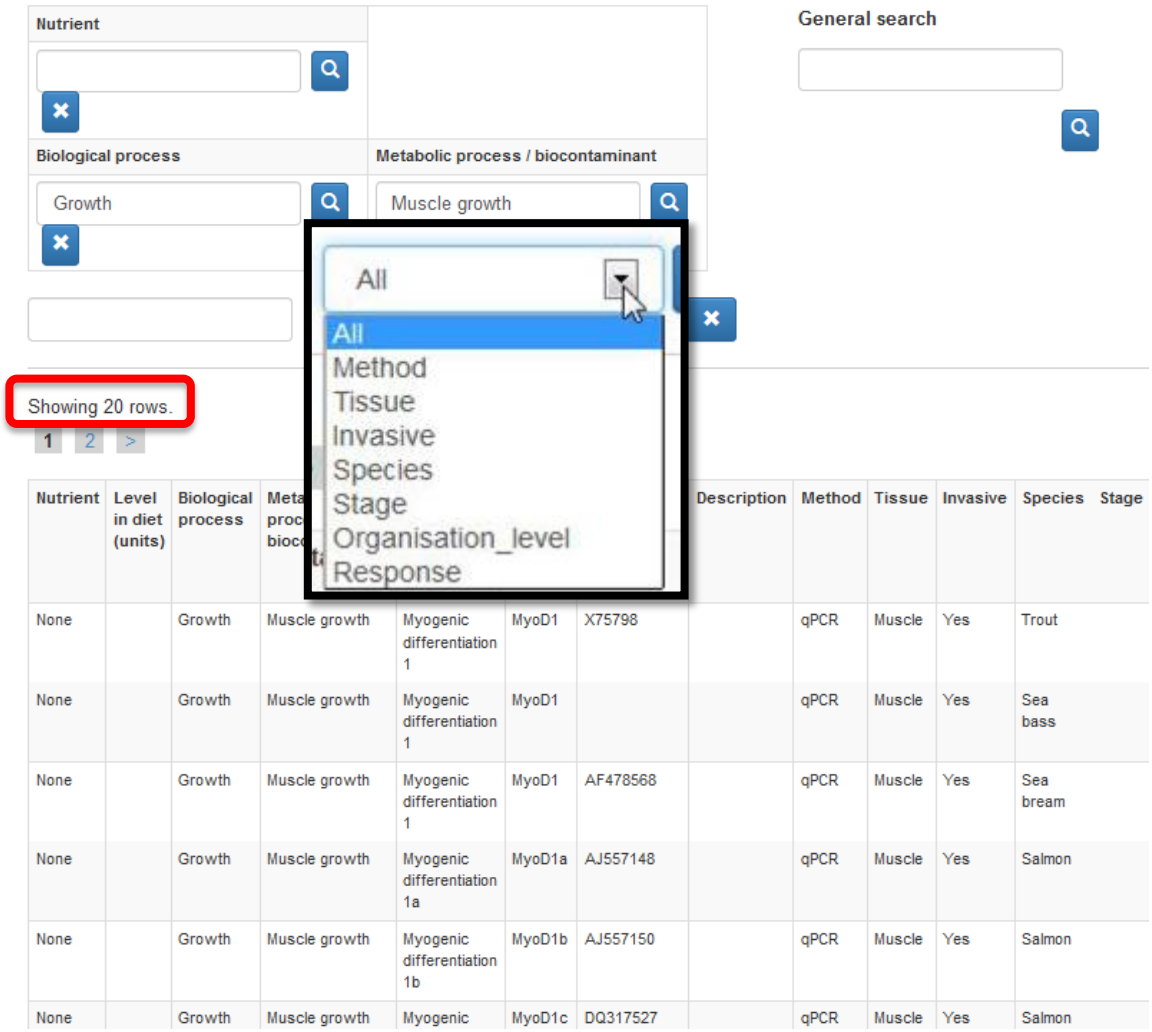
Showing 72 rows.

1 2 3 > Last ›

Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process



DEMO

Button

Refine search

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process

Nutrient

Biological process: Growth

Metabolic process / biocontaminant: Muscle growth

General search

Sea bream | Species | Refine search

Showing 5 rows.

Export to Excel

Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage	Reference
None		Growth	Muscle growth	Myogenic differentiation 1	MyoD1	AF478568		qPCR	Muscle	Yes	Sea bream		Tan & Du, Dev., 2002
None		Growth	Muscle growth	Myogenic differentiation 2	MyoD2	AF478569		qPCR	Muscle	Yes	Sea bream		Tan & Du, 2002
None		Growth	Muscle growth	Myogenic factor 5	Myf5	JN034420		qPCR	Muscle	Yes	Sea bream		García de la Serrana et al., 2014, Comp Biochem Physiol A
None		Growth	Muscle growth	Myogenic factor 6	Myf6/NRF4 /herculin	JN034421		qPCR	Muscle	Yes	Sea bream		García de la Serrana et al., 2014, Comp Biochem Physiol A
None		Growth	Muscle growth	Myogenin	Myog	EF462191		qPCR	Muscle	Yes	Sea bream		Codina et al., 2008

DEMO

Button

Refine search

Export to Excel

ARRAINA

ARRAINA Biomarkers database

Filters panel: Choose a nutrient and/or a biological process

Nutrient

Biological process

General search

Choose a nutrient

- All
- None
- α-Tocopherol
- Amino acid
- Ascorbic acid
- Biotin
- Boron
- Calcium
- Chromium
- Cobalamin
- Cobalt
- Copper
- DHA
- Fluoride
- Folate
- Glucane (Bio-Mos)
- Glucose
- Iodine
- Iron
- Lipids & FA
- Magnesium
- Manganese
- Methionine
- Molybdenum
- Na-butyrate
- Niacin
- Pantothenic acid
- Phosphorous
- Potassium
- Riboflavin
- Selenium
- Taurine
- Thiamine
- Vitamin A
- Vitamin B6
- Vitamin D
- Vitamin K
- Zinc

DEMO

Button

Refine search

Export to Excel

ARRAINA

ARRAINA Biomarkers database

DEMO

Filters panel: Choose a nutrient and/or a biological process

Nutrient

Vitamin A

Biological process

General search

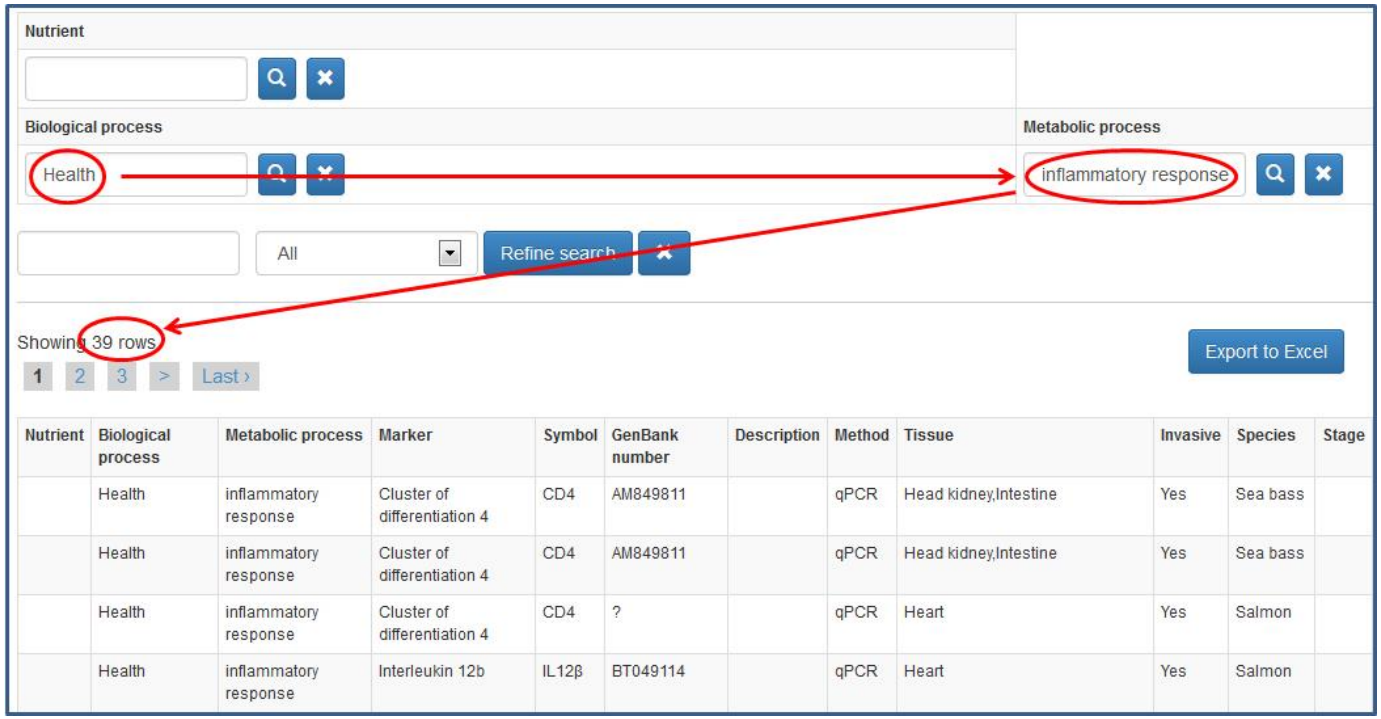
Species

Refine search

Showing 9 rows.

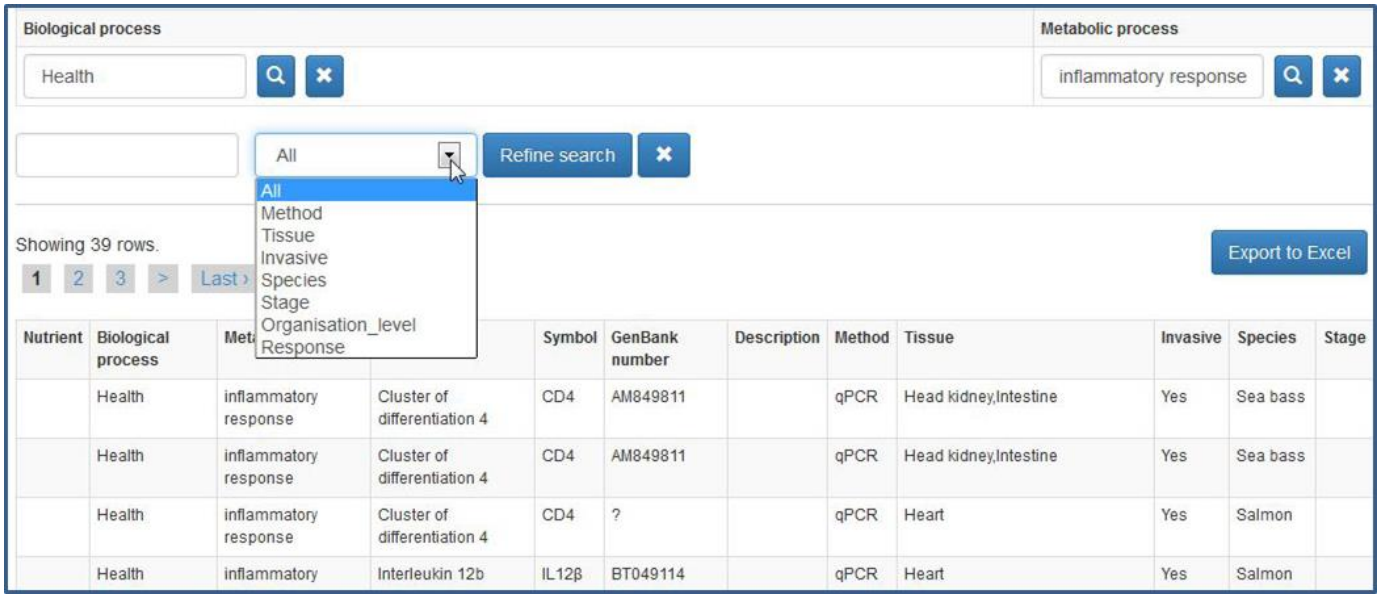
Nutrient	Level in diet (units)	Biological process	Metabolic process / biocontaminant	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species
Vitamin A		None	None	CYP26				qPCR	Liver	Yes	Atlantic salmon
Vitamin A		None	None	Bone deformity				X-ray	Vertebrae	No	Flounder,Rainbow trout
Vitamin A		None	None	Fecundity				Weight	Ovary	No	Atlantic salmon

ARRAINA Biomarkers database



Nutrient	Biological process	Metabolic process	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage
	Health	inflammatory response	Cluster of differentiation 4	CD4	AM849811		qPCR	Head kidney,Intestine	Yes	Sea bass	
	Health	inflammatory response	Cluster of differentiation 4	CD4	AM849811		qPCR	Head kidney,Intestine	Yes	Sea bass	
	Health	inflammatory response	Cluster of differentiation 4	CD4	?		qPCR	Heart	Yes	Salmon	
	Health	inflammatory response	Interleukin 12b	IL12β	BT049114		qPCR	Heart	Yes	Salmon	

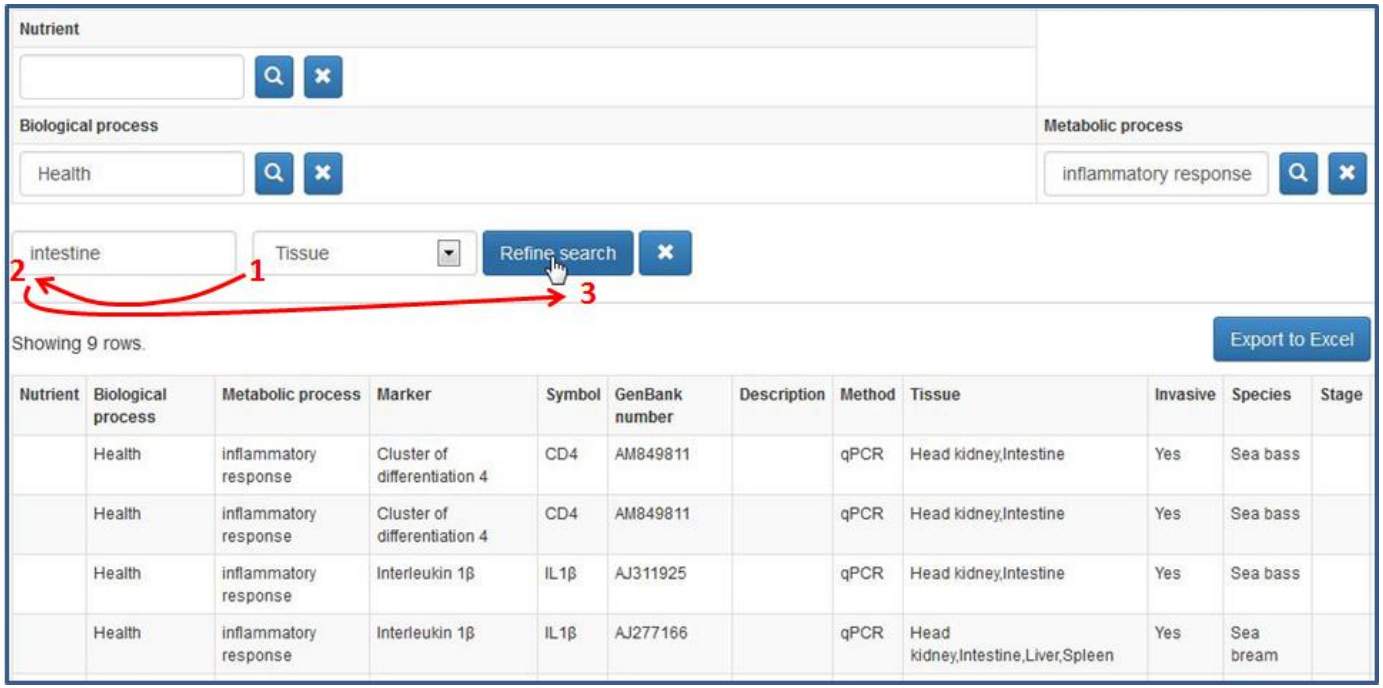
ARRAINA Biomarkers database



Refine search

Export to Excel

ARRAINA Biomarkers database



Nutrient	Biological process	Metabolic process	Marker	Symbol	GenBank number	Description	Method	Tissue	Invasive	Species	Stage
	Health	inflammatory response	Cluster of differentiation 4	CD4	AM849811		qPCR	Head kidney,Intestine	Yes	Sea bass	
	Health	inflammatory response	Cluster of differentiation 4	CD4	AM849811		qPCR	Head kidney,Intestine	Yes	Sea bass	
	Health	inflammatory response	Interleukin 1β	IL1β	AJ311925		qPCR	Head kidney,Intestine	Yes	Sea bass	
	Health	inflammatory response	Interleukin 1β	IL1β	AJ277166		qPCR	Head kidney,Intestine,Liver,Spleen	Yes	Sea bream	

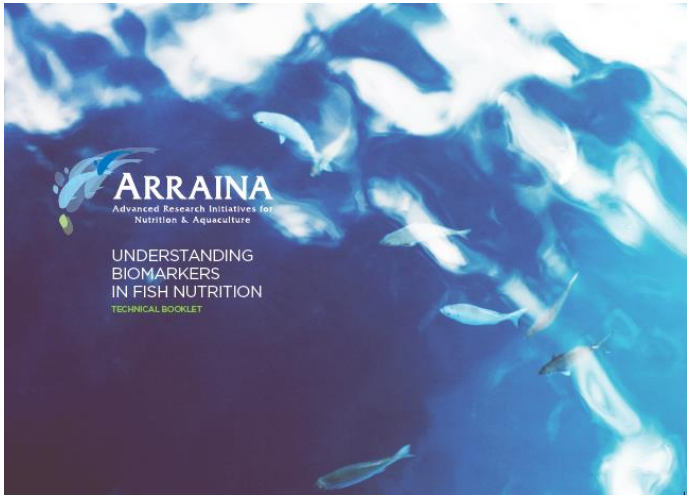
Refine search

Export to Excel

ARRAINA BIOMARKER DATABASE

www.nutrigroup-iats.org/arraina-biomarkers

3rd ARRAINA Technical Booklet

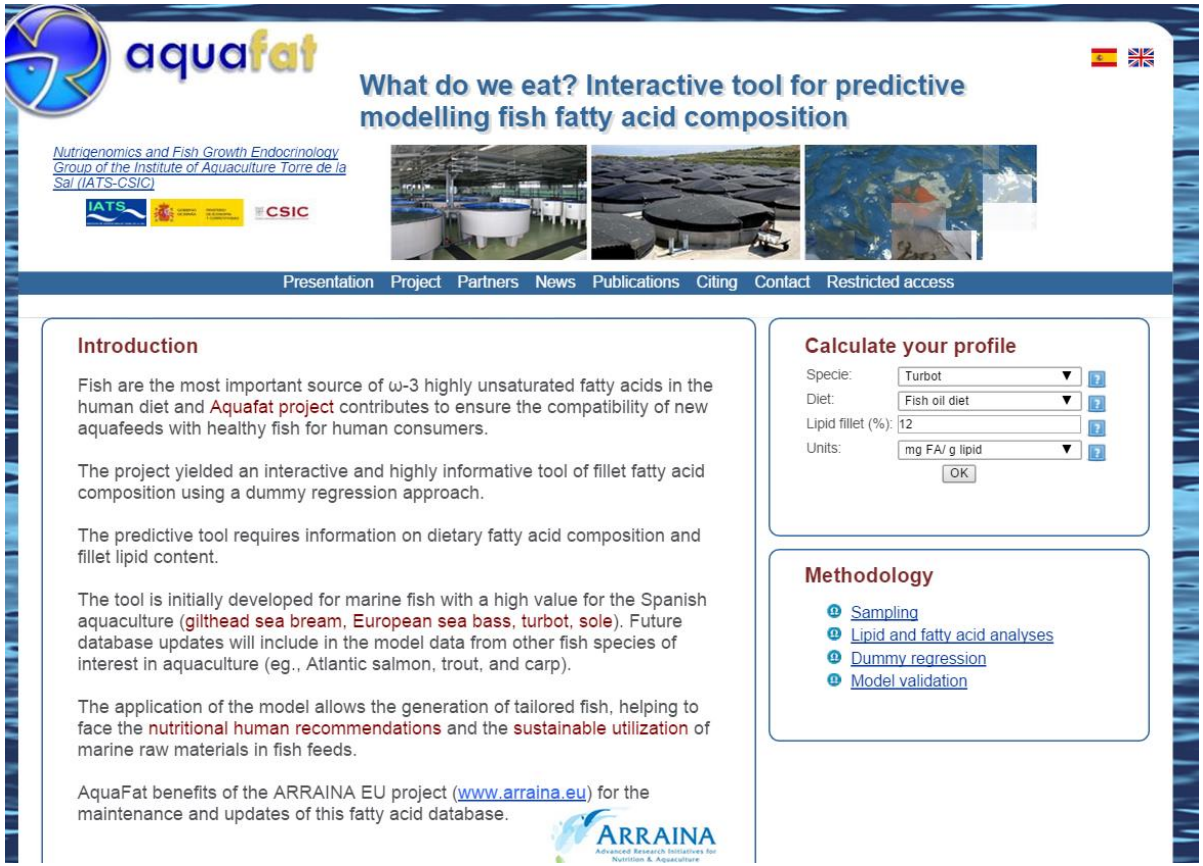


- Biomarkers of Mineral and Vitamin Requirements
- Markers of Methionine Metabolism
- Markers of Lipid metabolism and Growth Performance
- Markers of Intestinal Function and Architecture
- Nutritionally Mediated Effects on the Intestinal Transcriptome
- Potential Presence and Prevalence of Contaminants in Fish Fillets

AQUAFAT: predictive fillet FA composition

www.nutrigroup-iats.org/aquafat

Interactive tool for predictive modelling fish fatty acid composition



Sea bream

Sea bass

turbot

Sole

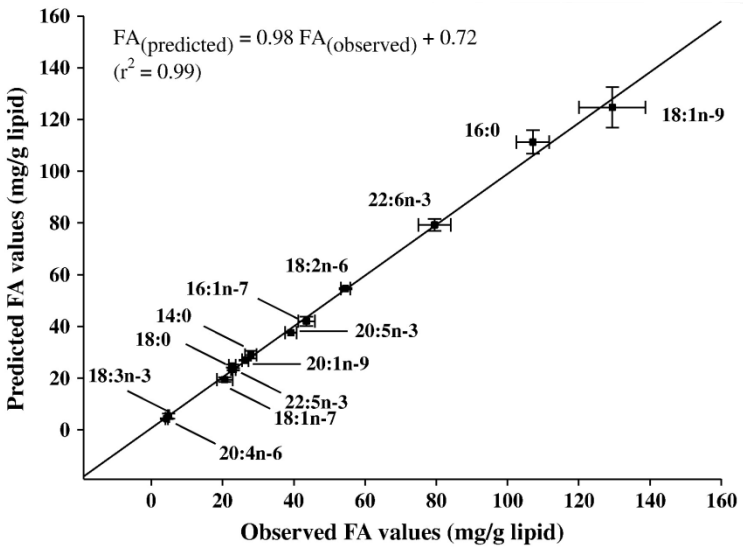
AQUAFAT: predictive fillet FA composition

www.nutrigroup-iats.org/aquafat

STEP 1: Regression modelling in gilthead sea bream

Sea bream

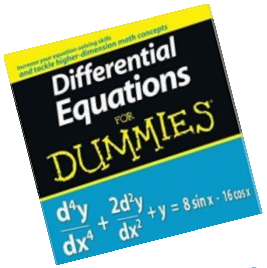
- Sea bream fed commercial **standard diet**
- **2-years** production cycle
- Sampled every 3-4 months:
  - Total lipid levels
  - FA composition
- Fillet FA composition correlated with:
  - Dietary FA composition
  - Fillet lipid level
- **Validation** with a diet based in PP/FO



Ballester-Lozano et al., 2011

AQUAFAT: predictive fillet FA composition

www.nutrigroup-iats.org/aquafat

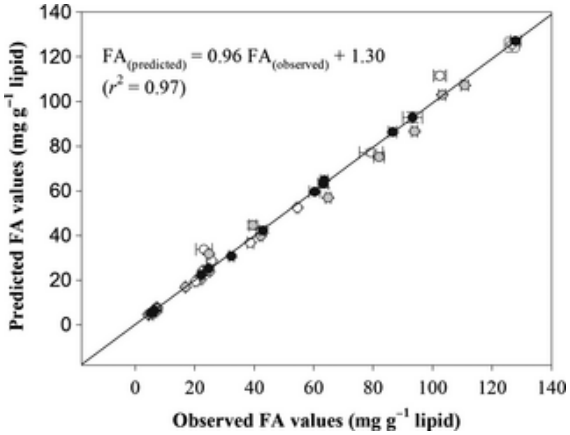


STEP 2: Dummy regression analysis (turbot, sole)

$$y_i = \beta_0 + \beta_1 Z_{1i} + \beta_2 Z_{2i} + \beta_3 Z_{3i} + \beta_4 Z_{4i}$$

Sea bream

- Farmed turbot and sole (North-West Spain) fed commercial diets
- Production cycle (turbot, 24 month; sole, 20 month)
- Sampled at different stages of the production cycle
- **Sea bream** data used as **reference subgroup category**
- **Validation** with data not included in the construction of the model



turbot

Sole

ARRAINA

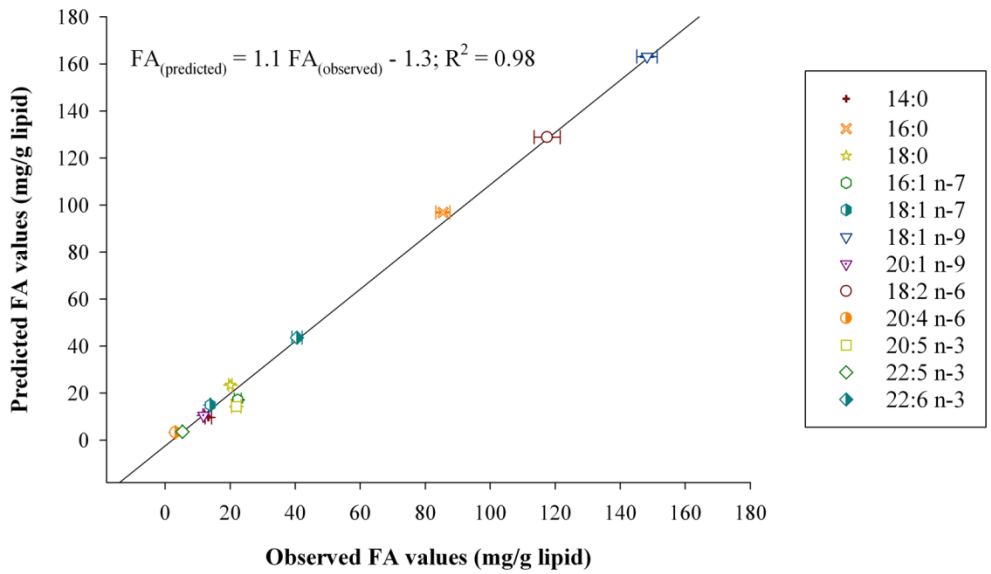
Ballester-Lozano et al., 2014

AQUAFAT: predictive fillet FA composition

www.nutrigroup-iats.org/aquafat

STEP 3: Sea bass up-scaling (farm & experimental scale)

- **Sea bream** data used as **reference subgroup category**
- **Dummy variables:** turbot, sole and sea bass (20 months)
- **Validation: farmed fish** harvested at commercial size



Sea bream

Sea bass

turbot

Sole

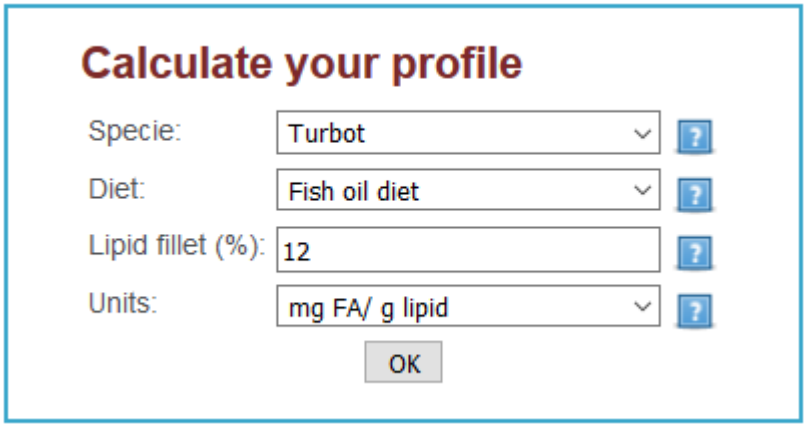
ARRAINA

Ballester-Lozano et al., 2016

AQUAFAT: predictive fillet FA composition

aquafat

What do we eat? Interactive tool for predictive modelling fish fatty acid composition



- Species: turbot, sole, sea bream, sea bass
- Diet: FO, 33VO, 66VO, VO & **customized**
- Unit as mg FA/ g lipid or mg FA/ 150 g fillet

AQUAFAT: predictive fillet FA composition

aquafat

Lipid fillet: 12%

Diet: Customized

Fatty acid	mg FA/ g lipid
14:0 (miristic acid)	0.0
16:0 (palmitic acid)	0.0
18:0 (estearic acid)	0.0
16:1 n-7 (palmitoleic acid)	0.0
18:1 n-7	0.0
18:1 n-9 (oleic acid)	0.0
20:1 n-9	0.0
18:2 n-6 (linolenic acid)	0.0
20:4 n-6 (arachidonic acid)	0.0
18:3 n-3 (linoleic acid)	0.0
20:5 n-3 (EPA)	0.0
22:5 n-3 (DPA)	0.0
22:6 n-3 (DHA)	0.0
Saturated [1]	0.0
Monoenes [2]	0.0
n-6 PUFA (ω-6) [3]	0.0
n-3 HUFA (ω-3) [4]	0.0

Lipid fillet: 12%

Diet: 66% vegetable oil diet

Fatty acid	mg FA/ g lipid
14:0 (miristic acid)	18.2
16:0 (palmitic acid)	124.6
18:0 (estearic acid)	23.9
16:1 n-7 (palmitoleic acid)	17.6
18:1 n-7	0.7
18:1 n-9 (oleic acid)	165.8
20:1 n-9	6.9
18:2 n-6 (linolenic acid)	117.8
20:4 n-6 (arachidonic acid)	1.2
18:3 n-3 (linoleic acid)	116.8
20:5 n-3 (EPA)	29.5
22:5 n-3 (DPA)	1.6
22:6 n-3 (DHA)	12.7
Saturated [1]	166.6
Monoenes [2]	191.0
n-6 PUFA (ω-6) [3]	119.0
n-3 HUFA (ω-3) [4]	43.7

AQUAFAT: predictive fillet FA composition

aquafat

- Fillet FA reflects FA composition of diet
- Fish tailored to consumer needs
- Robustness of the model facilitates inclusion of new species

Results

Lipid fillet: 12%

Print Export

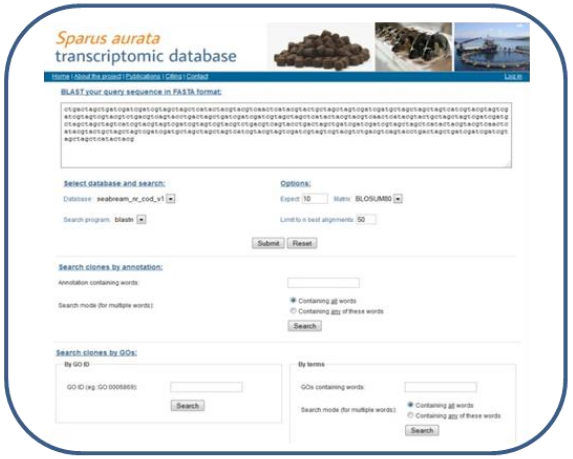
Diet: 66% vegetable oil diet		Fillet profile	
Fatty acid	mg FA/ g lipid	Fatty acid	mg FA/ 150g fillet
14:0 (miristic acid)	18.2	14:0 (miristic acid)	348.0
16:0 (palmitic acid)	124.6	16:0 (palmitic acid)	2,225.6
18:0 (estearic acid)	23.9	18:0 (estearic acid)	454.0
16:1 n-7 (palmitoleic acid)	17.6	16:1 n-7 (palmitoleic acid)	528.3
18:1 n-7	0.7	18:1 n-7	286.0
18:1 n-9 (oleic acid)	165.8	18:1 n-9 (oleic acid)	3,353.6
20:1 n-9	6.9	20:1 n-9	127.2
18:2 n-6 (linolenic acid)	117.8	18:2 n-6 (linolenic acid)	1,989.5
20:4 n-6 (arachidonic acid)	1.2	20:4 n-6 (arachidonic acid)	28.2
18:3 n-3 (linoleic acid)	116.8	18:3 n-3 (linoleic acid)	1,628.5
20:5 n-3 (EPA)	29.5	20:5 n-3 (EPA)	418.5
22:5 n-3 (DPA)	1.6	22:5 n-3 (DPA)	181.6
22:6 n-3 (DHA)	12.7	22:6 n-3 (DHA)	317.0
Saturated [1]	166.6	Saturated	3,021.0
Monoenes [2]	191.0	Monoenes	4,356.8
n-6 PUFA (ω-6) [3]	119.0	n-6 PUFA (ω-6)	1,996.8
n-3 HUFA (ω-3) [4]	43.7	n-3 HUFA (ω-3)	919.2

Recalculate

Searchable transcriptomic databases

www.nutrigroup-iats.org/seabreamdb

www.nutrigroup-iats.org/seabassdb

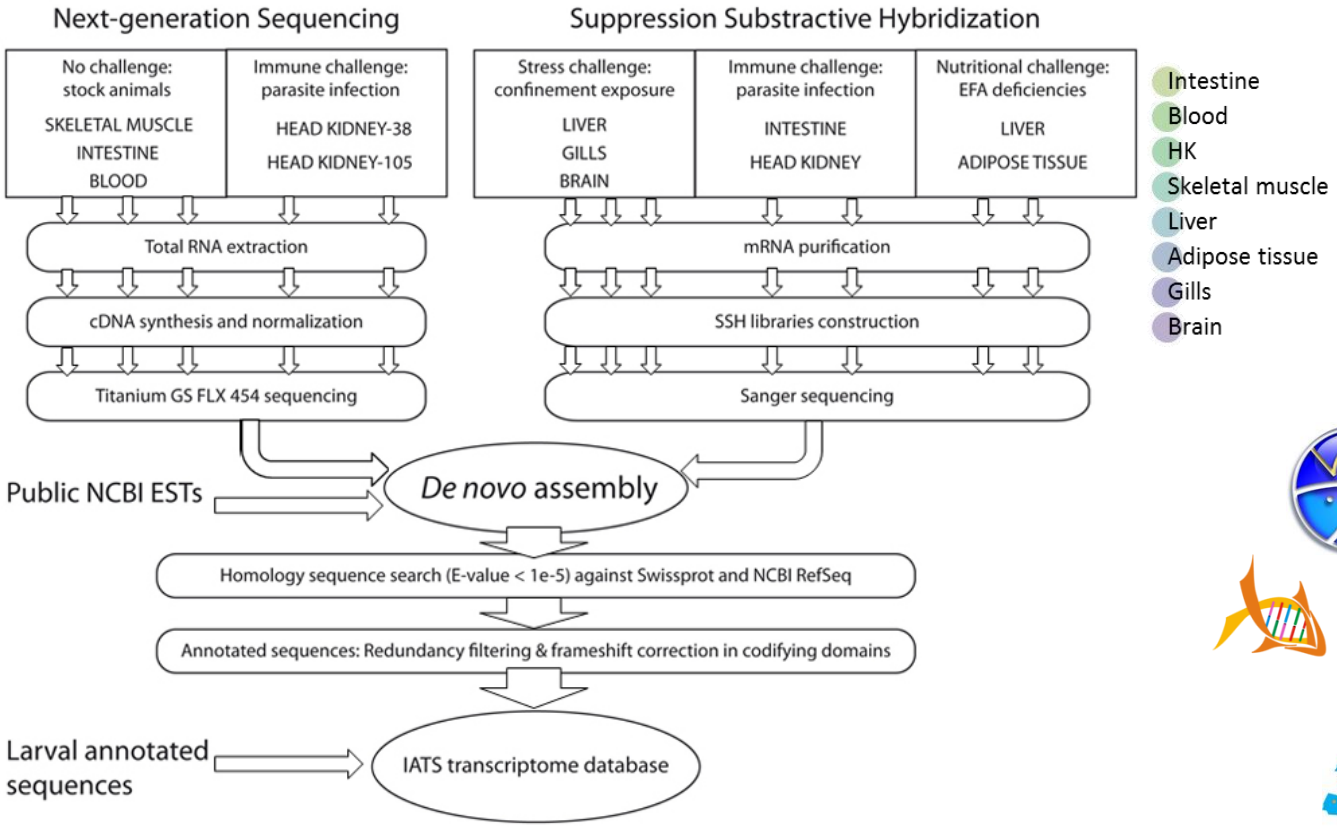


- 20,565 non-redundant annotated sequences
- 14,535 different gene descriptions

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

- BLAST searches (Blastn, tblastn, tblastx)
- Annotation search
- GO ontology search

Sea bream transcriptomic database
www.nutrigroup-iats.org/seabreamdb



- Intestine
- Blood
- HK
- Skeletal muscle
- Liver
- Adipose tissue
- Gills
- Brain

aquafat

Aquagenomics

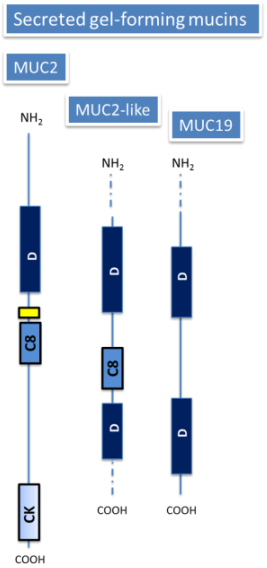
AQUA EXCEL

ARRAINA

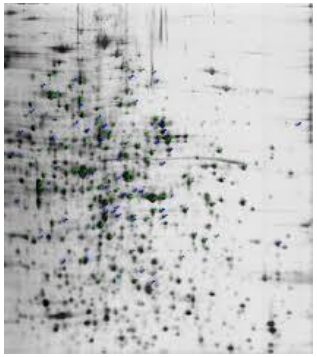
Calduch-Giner et al., 2013

Sea bream transcriptomic database

www.nutrigroup-iats.org/seabreamdb



Definition of molecular identities and discovery of fish-lineage specific genes (mucins, PRDXs, UCPs….)

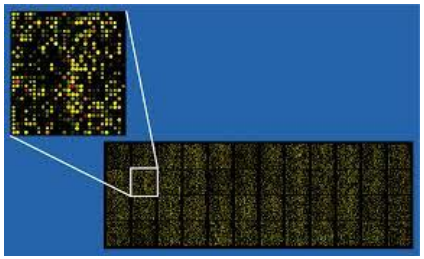


Reference library in proteomic studies

2D-DIGE

iTRAQ

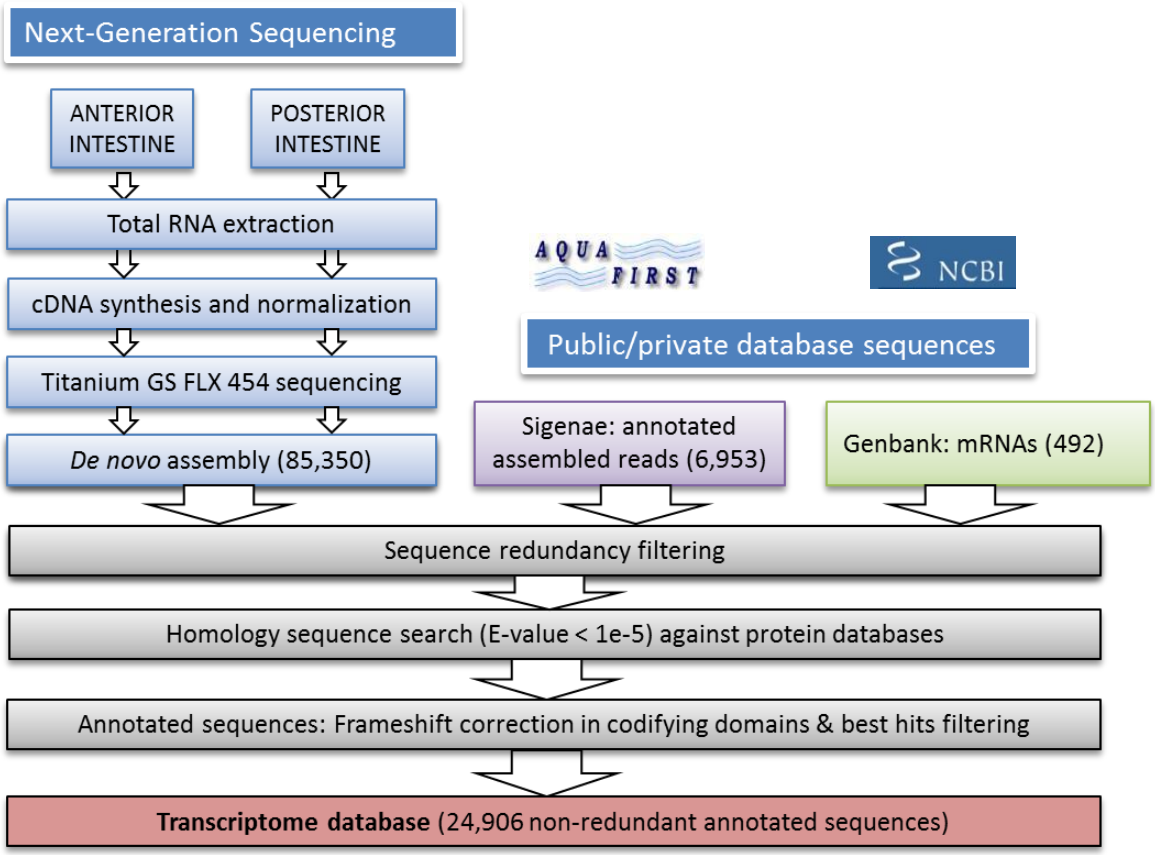
Liver/muscle /intestinal mucus proteomes



Design of microarrays (8 x 15k) and focused PCR-arrays for routine gene expression analyses

Sea bass transcriptomic database

www.nutrigroup-iats.org/seabassdb



Mi2-FISH
Unravelling Metabolic, Intestinal and Immunopathological Fish Status

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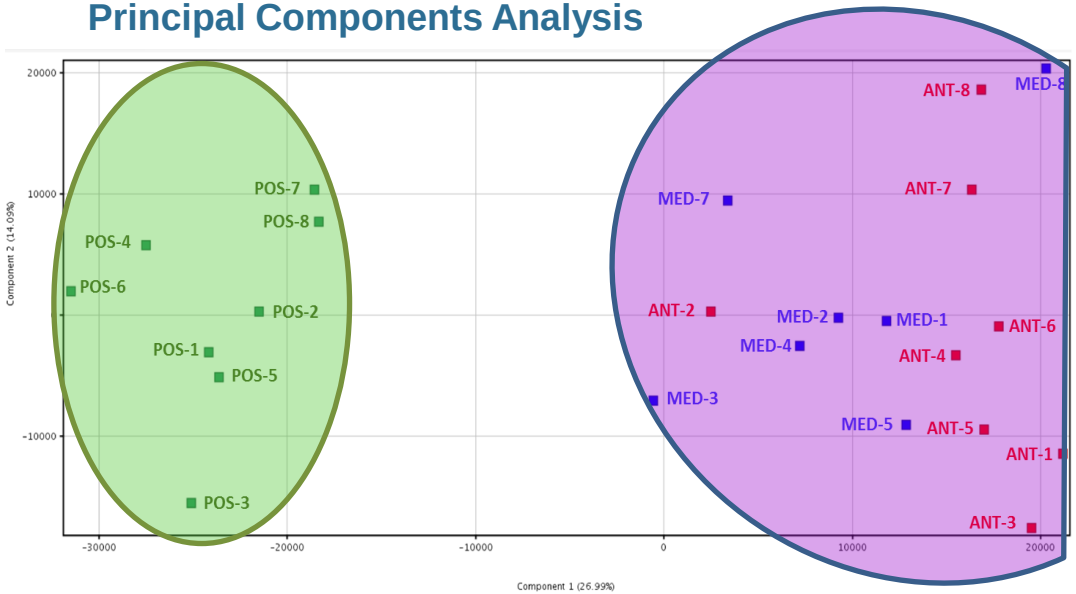
Advanced Research Initiatives for Nutrition & Aquaculture

Calduch-Giner et al., 2016

SEA BASS MICROARRAY V1: Massive gene expression analysis

Principal Components Analysis

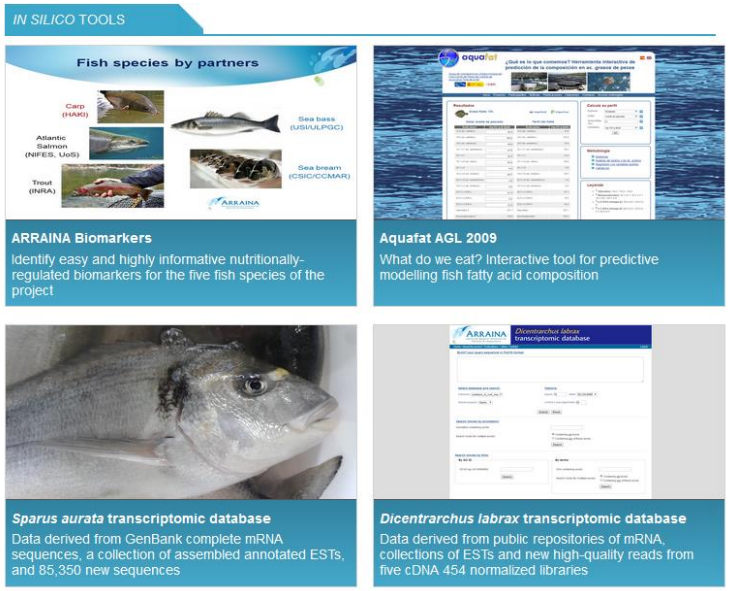


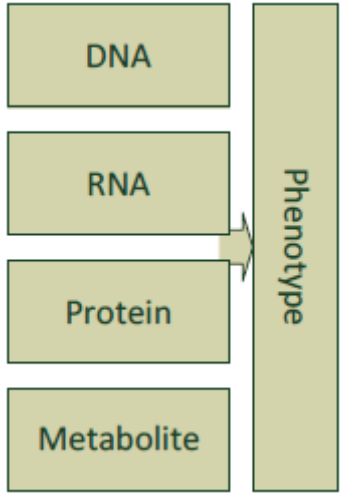
	MID-ANT	POS
MID-ANT	---	960
POS	946	---

MID-ANT vs **POS** comparison (P<0.05, T-test, Benjamini-Hochberg multiple testing correction)

Summary

- Database of biomarkers of traits of interest, nutrient requirements and detection methods of potential contaminants.
- Fillet fatty acid tailoring predictive tool.
- Sea bream and sea bass transcriptomes as the basis of *omic* platforms to phenotype every trait.





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AQUA EXCEL

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BENICÀSSIM 2017

GRACiAS

PLANT PROTEINS ARE GOOD !!