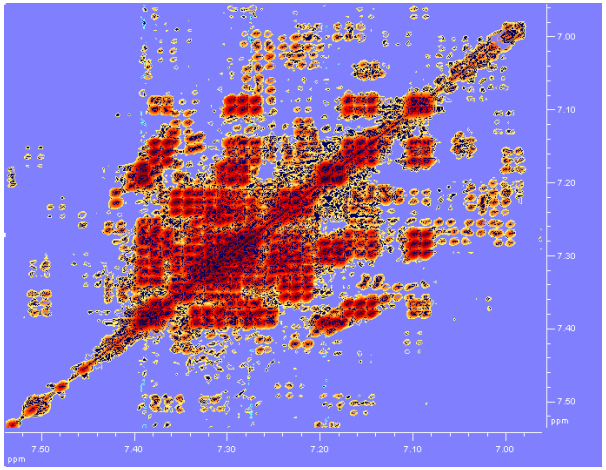
Protocols for assessing quality and traceability of aquaculture products by means of Nuclear Magnetic Resonance

Dr. Roberto Anedda

NMR/MRI facilities

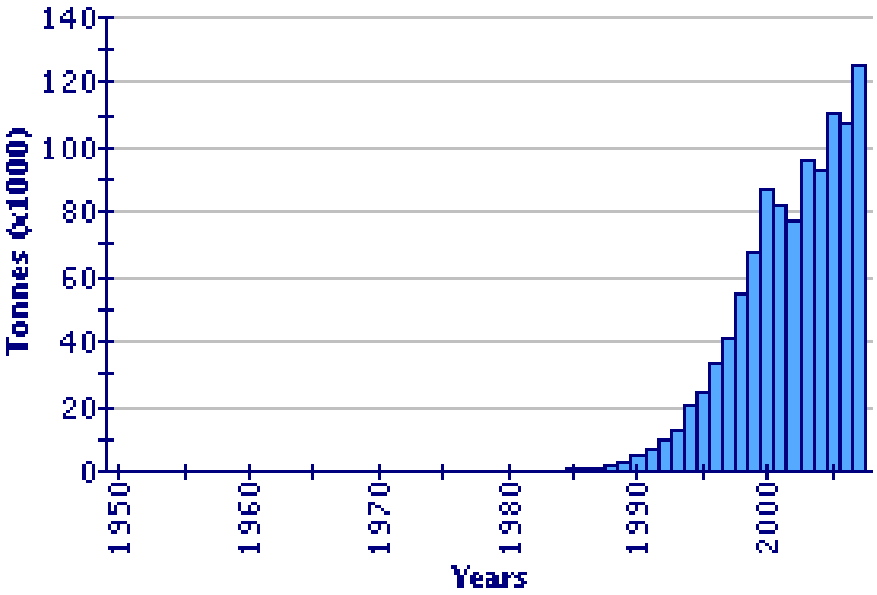
June, 30 2009

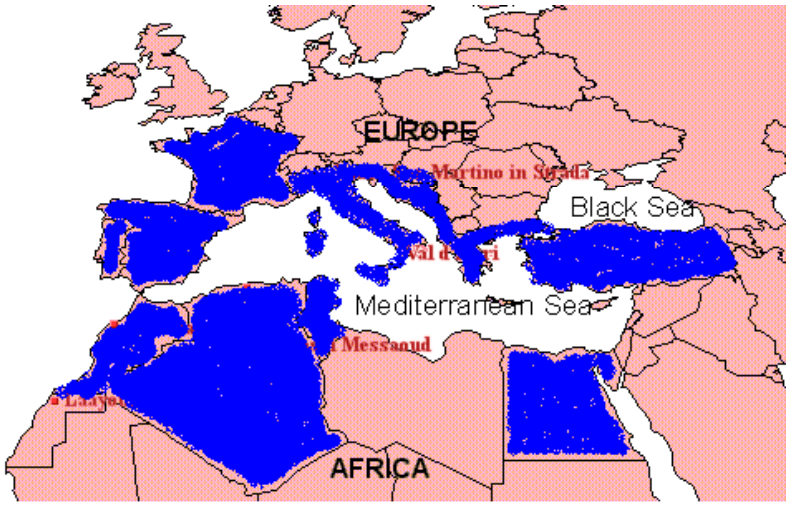


PortoConte Ricerche

Farmed GILTHEAD SEA BREAM: Annual Production in EUROPE

~ 4500 t in 1990 → ~125000 t in 2007





FAO. © 2005-2009. - . Cultured Aquatic Species Information Programme. **Colloca, F.; Cerasi, S.** In: *FAO Fisheries and Aquaculture Department* [online]. Rome.

http://www.fao.org/fishery/culturedspecies/Sparus_aurata/en#tcN90112

RELEVANT ISSUES: developing RELIABLE ANALYTICAL METHODS for assessing

COMPOSITION (METABOLITE PROFILING)

GEOGRAPHICAL ORIGIN

FRESHNESS and **QUALITY**

Molecolar Markers ⟷ **Organoleptic Characteristics**

DISEASES

POLLUTANTS

CONVENTIONAL and CHROMATOGRAPHIC TECHNIQUES FOR METABOLITE PROFILING

LENGHTY SEPARATION STEPS → ARTIFACTS (LABILE METABOLITES)

ENZYMATIC and COLORIMETRIC METHODS → LACK SPECIFICITY
INTERFERENCES

HPLC → CHROMOPHORES, FUNCTIONAL GROUPS
UNEXPECTED, UNKNOWN METABOLITES

GC → VOLATILE SPECIES
DERIVATIZATION

MASS SPECTROMETRY →

- VALID STRUCTURAL ANALISYS
- SENSITIVITY
- GENERALLY DESTRUCTIVE

NMR: a COMPLEMENTARY, COMPREHENSIVE, HIGLY INFORMATIVE TECHNIQUE

NOT LIMITED BY ANY ANALYTE PROPERTIES

MINIMAL SAMPLE PREPARATION

STRUCTURE, CONFORMATION, DYNAMICS

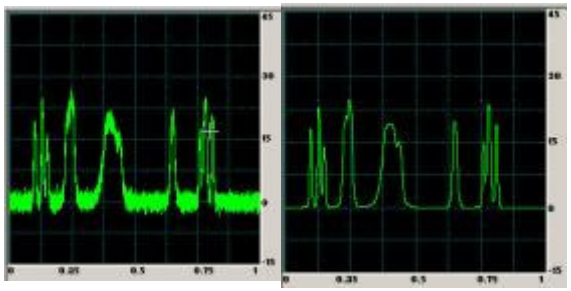
SIMULTANEOUS DETECTION OF ALL ANALYTES

NON DESTRUCTIVE

NUCLEAR MAGNETIC RESONANCE (NMR) has been widely used in metabolomics of complex mixtures

Vast scientific literature concerning NMR-aquaculture

Principal drawbacks of NMR:



Low sensitivity

- Giromagnetic ratio γ,
- Natural abundance
- Magnetic field (instrumentation)

Acquisition times

- Relaxation phenomena - delays
- Sample concentration

High cost

NO ROUTINE, evaluate COSTS-BENEFITS

METABOLOMIC ANALYSIS

"systematic study of the unique chemical fingerprints that specific cellular processes leave behind " *

EXPERIMENTAL

Extraction

NMR 1D e 2D Spectra Acquisition

Interpretation of spectra

STATISTICAL ANALYSIS

Principal Component Analysis

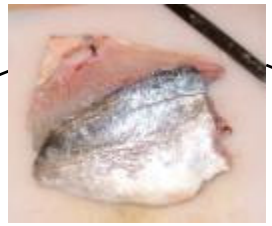
Interpretation

Conclusions

* B. Daviss, "Growing pains for metabolomics," *The Scientist*, 19[8]:25-28, April 25, 2005

EXPERIMENTAL

+ IMPORTED
(Farmed)



Extraction 1
CH_2Cl_2 (stored at -40°C
until analysis)

Extraction 2
EtOH at RT

LIPIDS

POLAR METABOLITES

^{1}H NMR

^{1}H NMR

LIPIDS

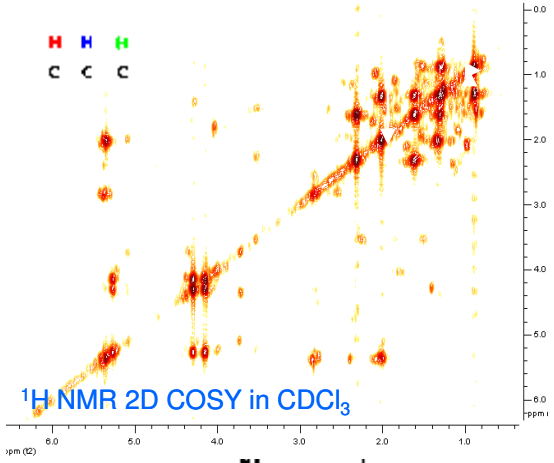
Triacyilglycerols
Diacyilglycerols
Phospholipids (PC,PE,..)

Cholesterol
Cholesterol Esters

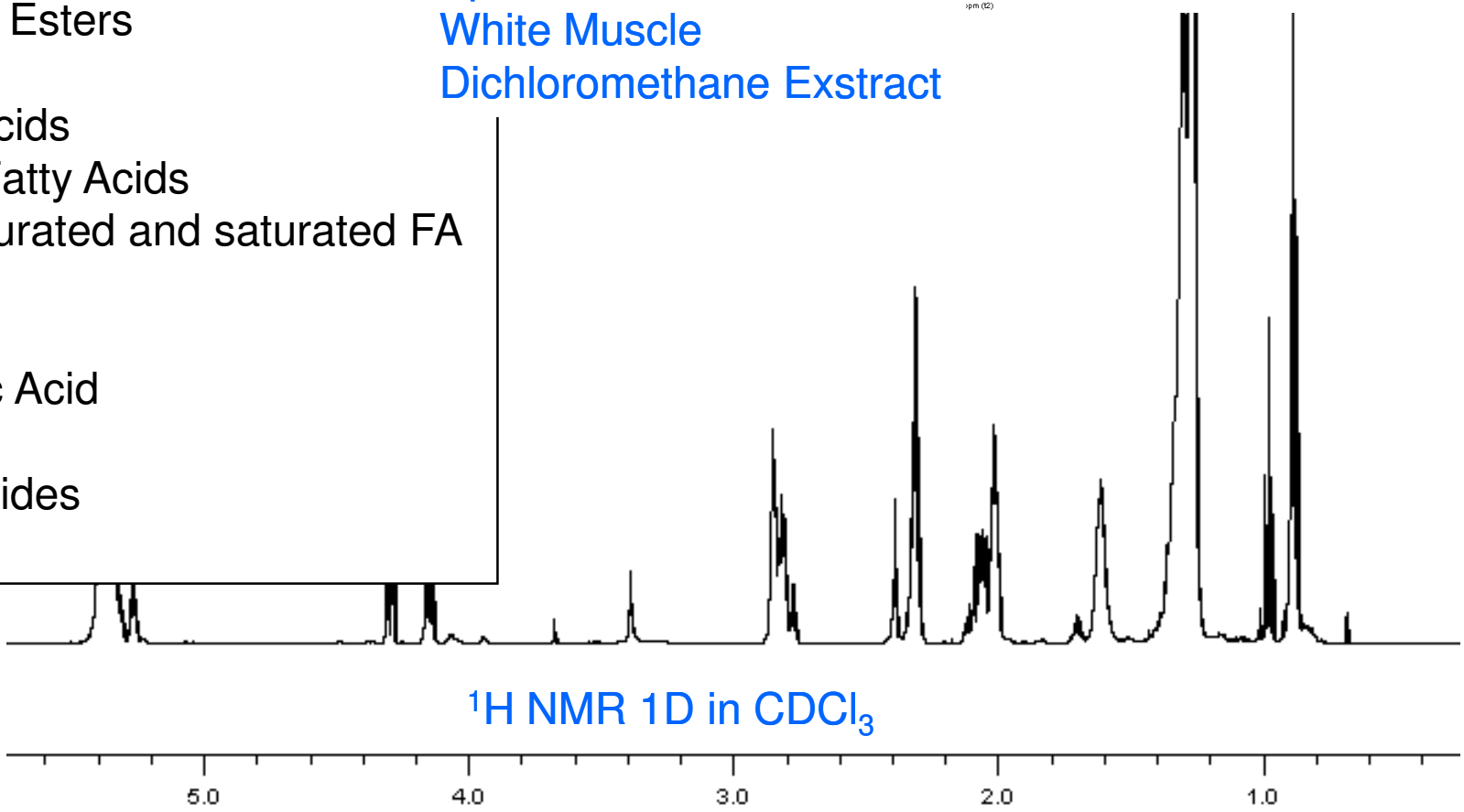
ω-3 Fatty Acids
ω-6 + ω-9 Fatty Acids
Monounsaturated and saturated FA
EPA
DHA
Arachidonic Acid

Hydroperoxides
Aldheydes

Sparus Aurata
White Muscle
Dichloromethane Exstract



^{1}H NMR 2D COSY in $CDCl_3$

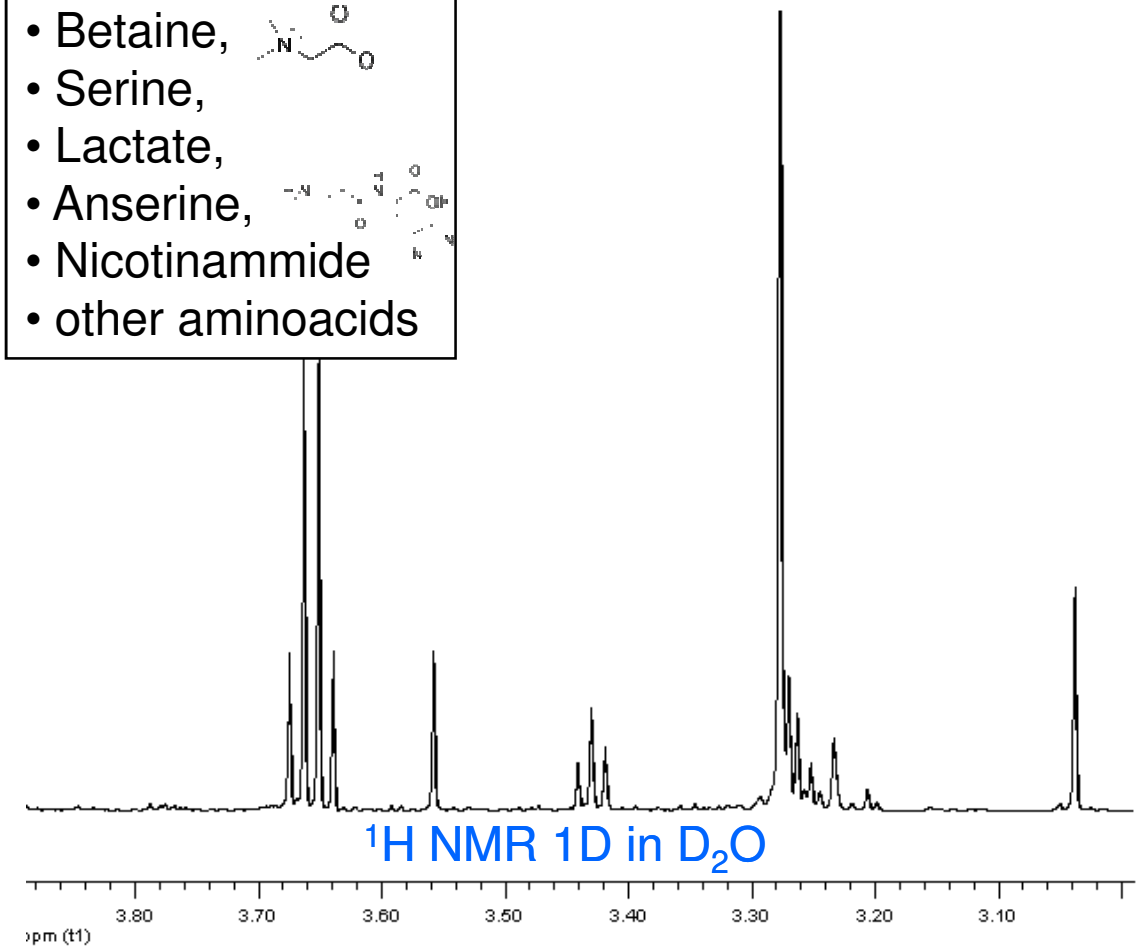


^{1}H NMR 1D in $CDCl_3$

POLAR METABOLITES

- Creatine,
- Taurine,
- TMA,
- TMAO,
- Glicine,
- Betaine,
- Serine,
- Lactate,
- Anserine,
- Nicotinammide
- other aminoacids

Sparus Aurata,
White Muscle
Ethanolic exctract



^{1}H NMR 1D in D_2O

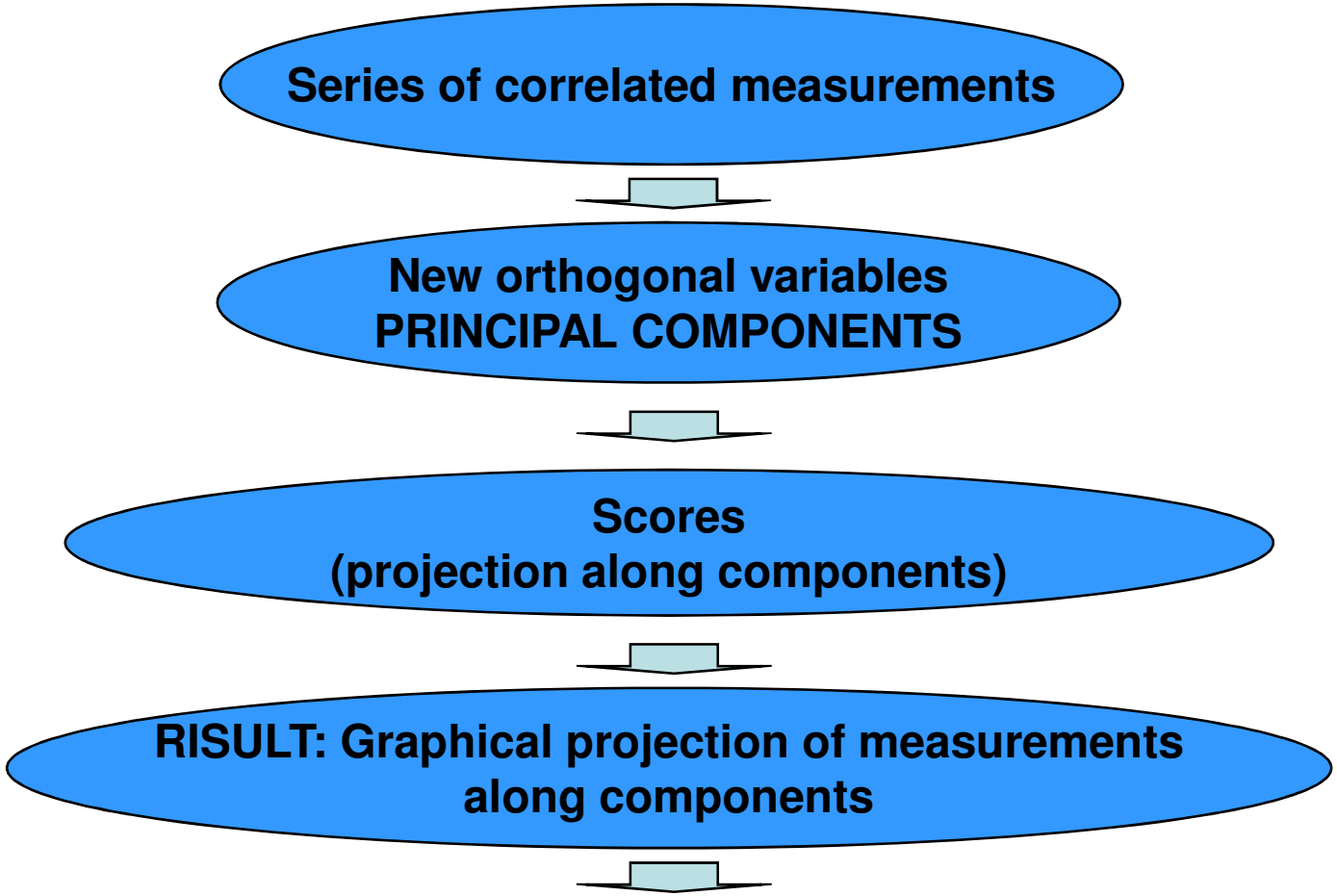
The accumulation of **TMA** in rotting fish as a result of bacterial degradation of choline, as well as the reduction of TMAO to TMA, is responsible for their characteristic 'fishy' odor.

Main Functions of **TAURINE** in Mammals
Bile acid conjugation
Detoxification
Osmoregulation
Membrane Stabilization
Regulation of intracellular Ca^{2+}
Homeostasis

NIACIN (nicotinic acid) and its amide, **NIACINAMMIDE**
High cholesterol
Atherosclerosis
Heart Attacks
Osteoarthritis

ANSERINE
Muscle activity is closely related to the dipeptide concentration. In vigorously contracting muscles, the dipeptide concentration is high.

Multivariate statistical analysis: PCA (Principal Components Analysis):



PCA
***unsupervised* method**
Orthogonal decomposition of variance

GENERAL PROCEDURE

Sample selection

Spectral Bucketing (binning)

Analysis of influence plot

Construction of a Reliable Model

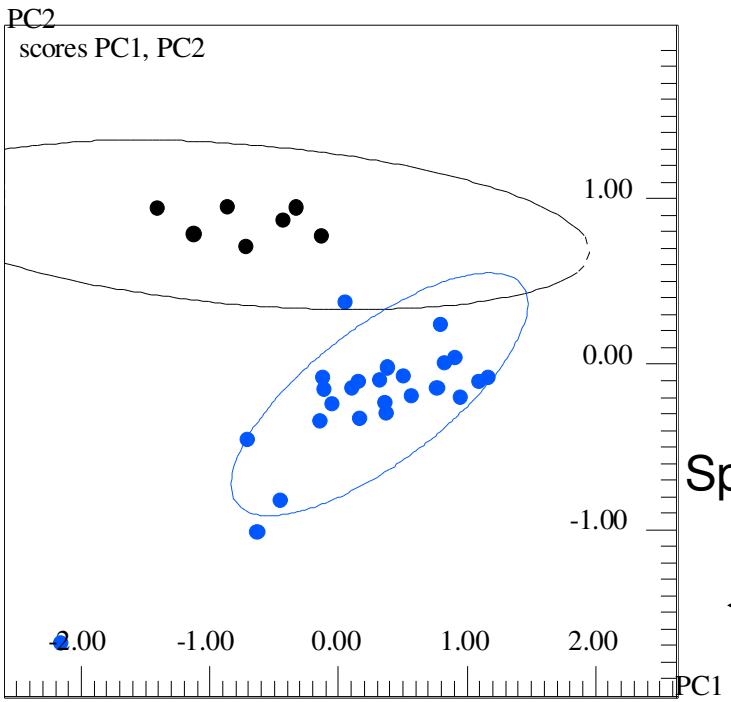
Cross validation

Scores plot, loadings plot

Hotelling's T^2 plot → presence of clusters

Analysis of critical spectra and save model

Scores Plot



FARMED

VS

WILD

~250 gr

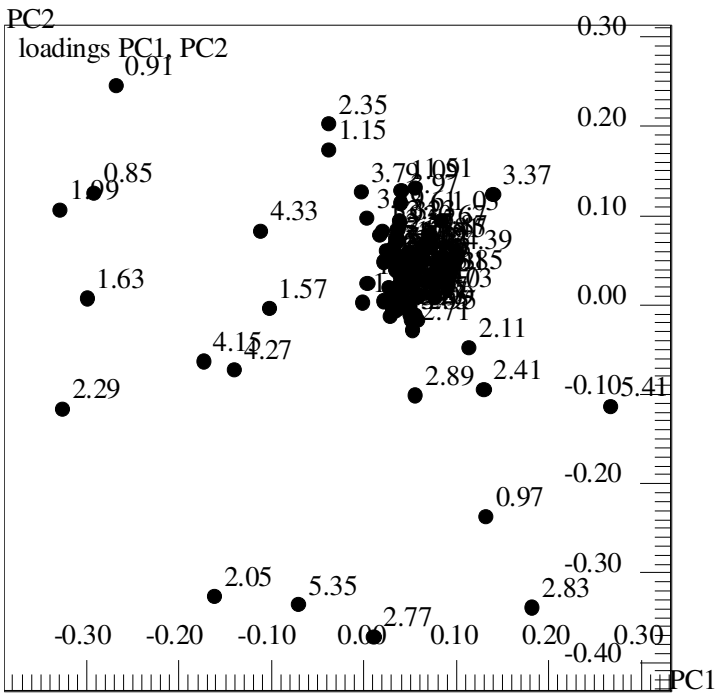
Healthy

(PCA space)

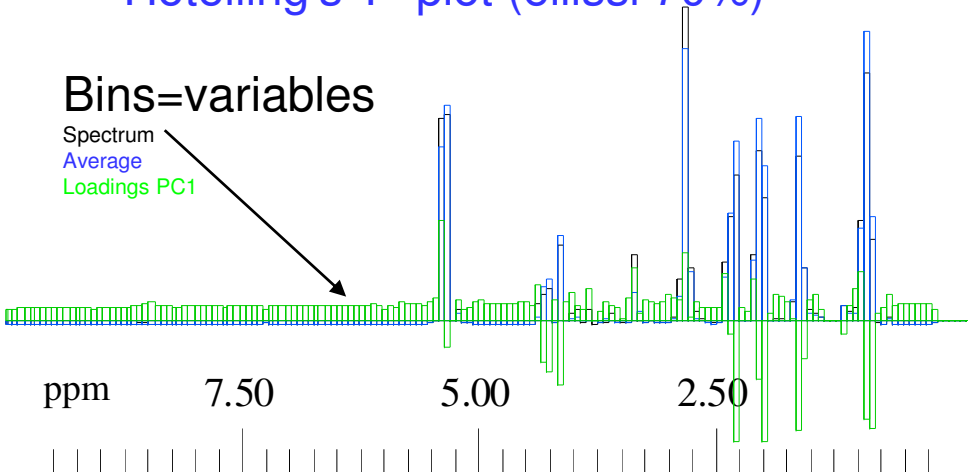
↓

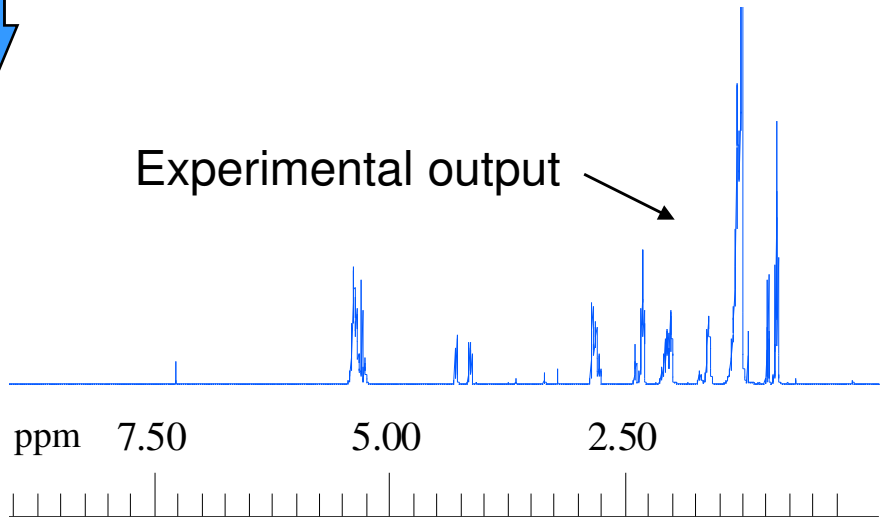
Spectroscopic Data

Loadings Plot

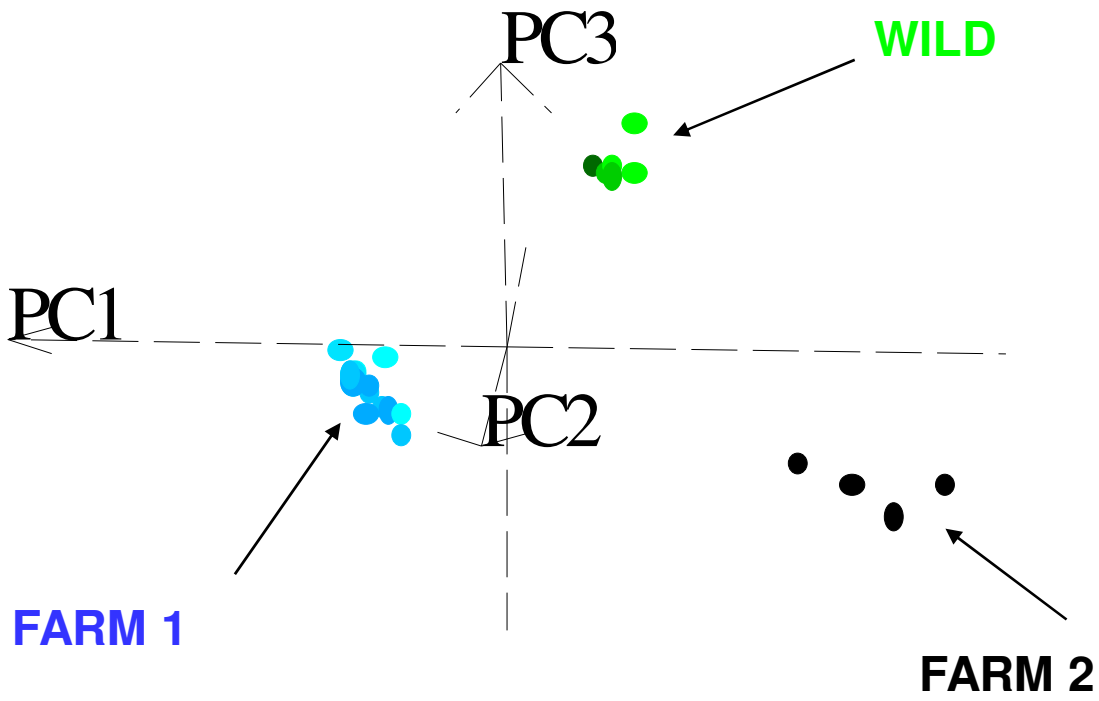


Hotelling's T^2 plot (ellissi 70%)



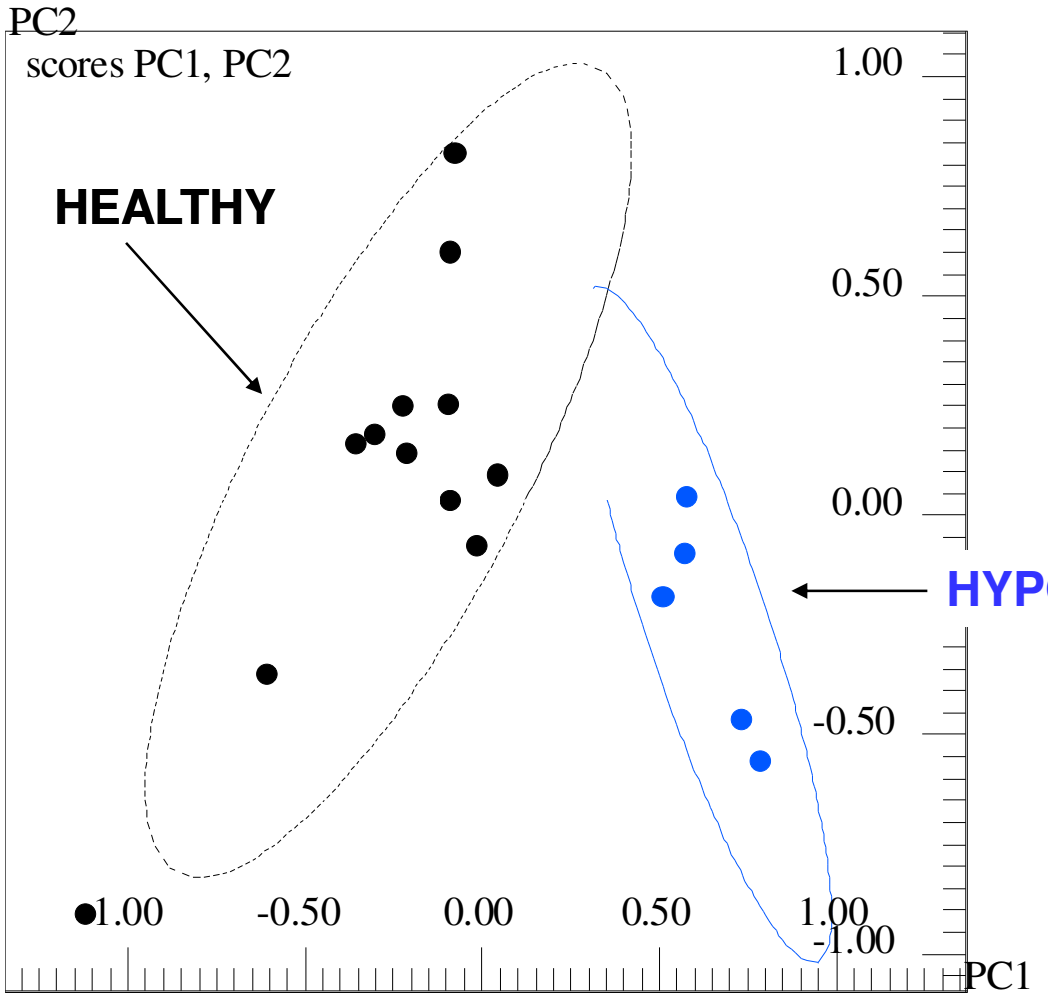


~**150** gr
Healthy
Same sampling period



variance explained by PC1 : 43.60%
variance explained by PC2 : 21.94%
variance explained by PC3 : 11.48%

SAME FARM, SAME SIZE (200-280 gr)

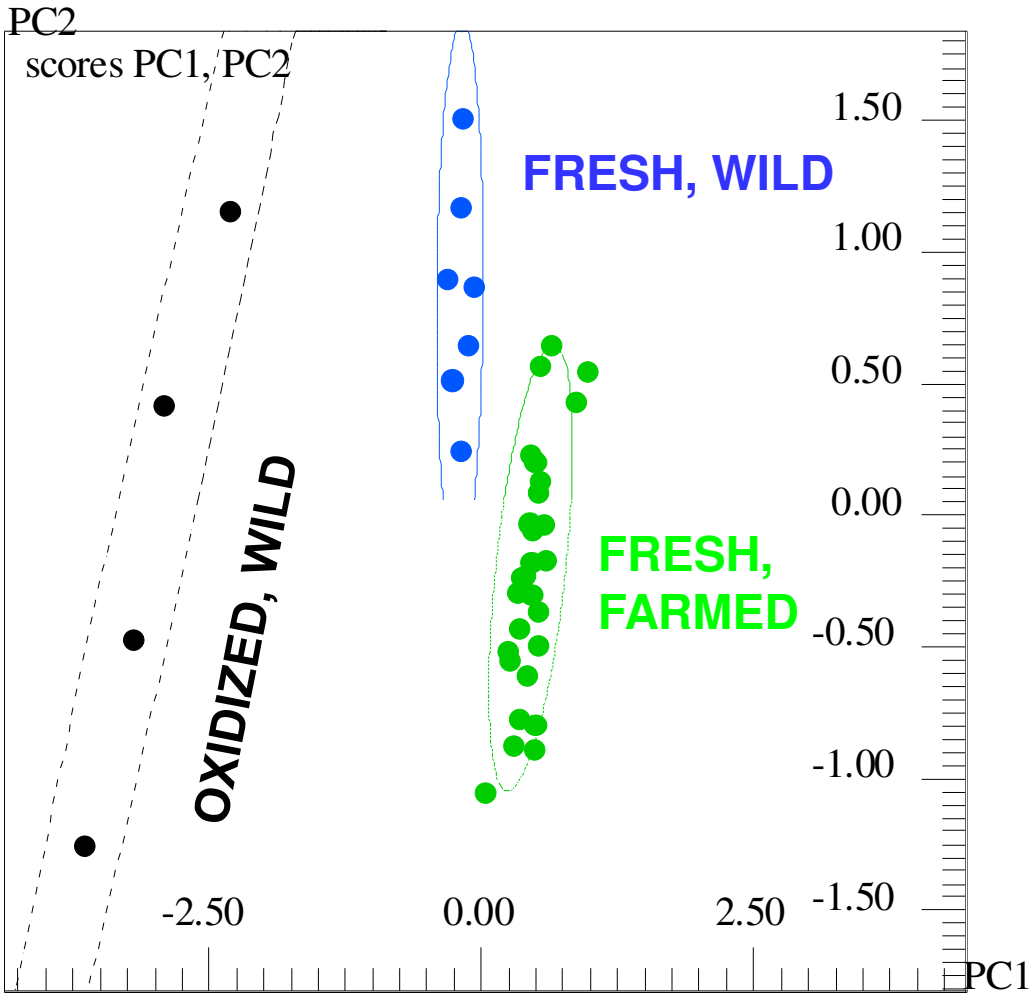


HYPOTROPHIC SPLEEN

ALTERATIONS LIKELY RELATED TO FEEDING STRESS

variance explained by PC1 : 41.55%
variance explained by PC2 : 28.68%
L'intervallo di confidenza relativo all'Hotelling's T2 plot e' dell' 80%.

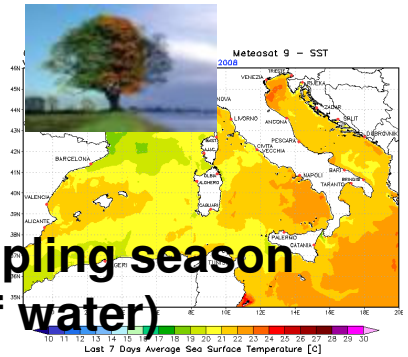
SAME SIZE (200-280 gr). EVALUATING OXIDATION



variance explained by PC1 : 56.83%
variance explained by PC2 : 20.77%

PCA Analysis on ^{1}H NMR measurements of Gilthead Seabream *(Sparus Aurata)*

CH_2Cl_2 extract, related to lipids



Sampling season (T of water)

FACTORS INFLUENCING COMPOSITION

Farmed vs **wild**

Feeding Stress

In conclusion

A Metabolite fingerprinting by means of NMR; has been successfully carried out

Several *nutritionally relevant species* have been identified in white muscle of Gilthead seabream. This lay the foundation for quantitative analysis of potentially interesting compounds

Storage: Aldheydes, peroxides and hydroxy-compounds formed as a consequence of lipid oxidation are clearly observable in NMR spectra. NMR allow indentifying *fish freshness* Also PCA discriminates frsh and oxidized samples.

Studying fish *LIPIDS* by NMR we have successfully discriminated: ***FARMED vs WILD*** fish,

its *geographic origin*,

alterations due to ***feeding stress***

Team

NMR lab:

Dr. Gilberto Mulas

Dr. Maria Grazia Galaffu

Advices on Statistical analysis:

Dr. Roberto Tonelli

PortoConte Ricerche

Sample selection and extraction:

Dr. Viviana Santercole

Dr. Roberto Cappuccinelli

Dr. Monica Madrau

Supervision:

Dr. Tonina Roggio

Prof. Sergio Uzzau

anedda@portocontericerche.it

SARDEGNA RICERCHE

Fondazione Banco di Sardegna