



# Farmed fish quality and welfare monitoring using proteomics

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**ALGARVE 21**  
PROGRAMA OPERACIONAL

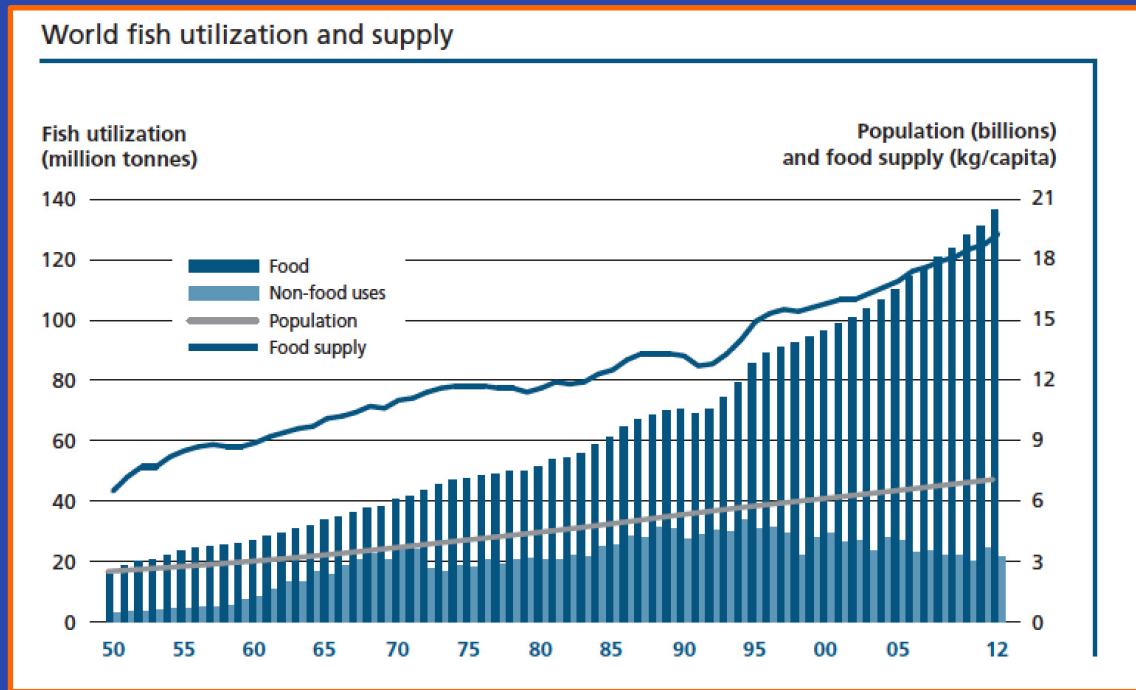


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# Fish Consumption / Aquaculture

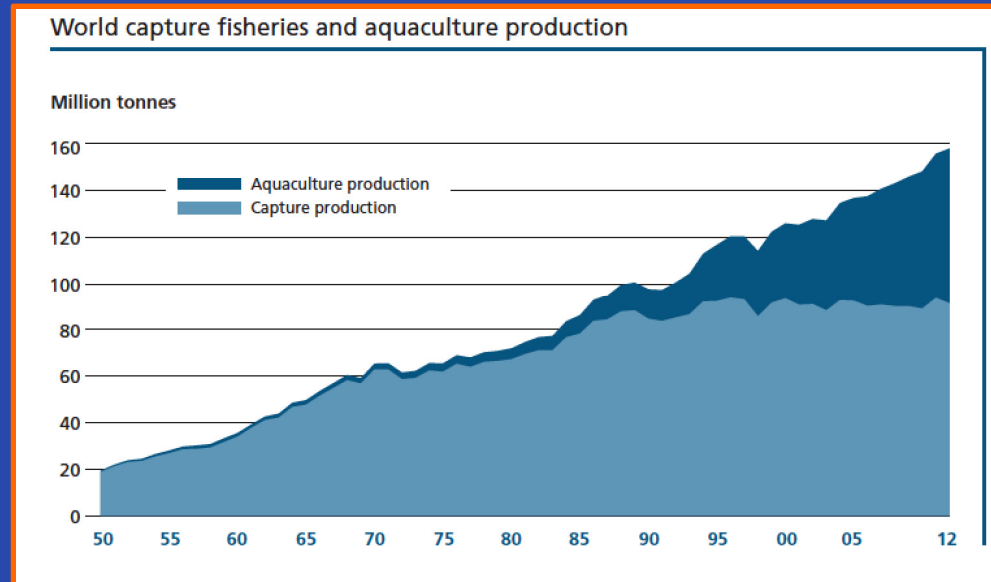
Food Fish supply is increasing at an average annual rate of 3.2%, accounting for almost 17% of the global population's intake of protein.



FAO 2014



# Aquaculture



FAO 2014

Extremely competitive market



# Today's presentation

## Fish Welfare



Fish Welfare is complex definition often related to fish well-being. Depends on good management practice

## Fish quality



Fish quality is a broad and complex concept affected by a genetic basis and management systems





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Aquaculture

journal homepage: [www.elsevier.com/locate/aqua-online](http://www.elsevier.com/locate/aqua-online)

## Metabolic molecular indicators of chronic stress in gilthead seabream (*Sparus aurata*) using comparative proteomics

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Welfare  
Evaluation



Stress response



Fish Welfare

## Dias nummer 5

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**PR1**

biological and physiological responses of an organism to conditions that affect it – The Stress response.

Pedro Rodrigues, 24/08/2014

# Classical approach

Classical parameters  
used as stress indicators

- Cortisol
- Lactate
- Glucose
- Total protein  
in plasma
- Haematocrit  
and  
haemoglobin
- Enzymatic assays

- Fish respond  
differently to stress
- Cortisol levels can  
often return to basal  
due to adaptation  
processes



The proteomics approach



# Experimental



Control  
(CTRL)

2

6x



Regular  
handling  
(HND)

2

6x



High stock  
density  
(HSD)

2

11x

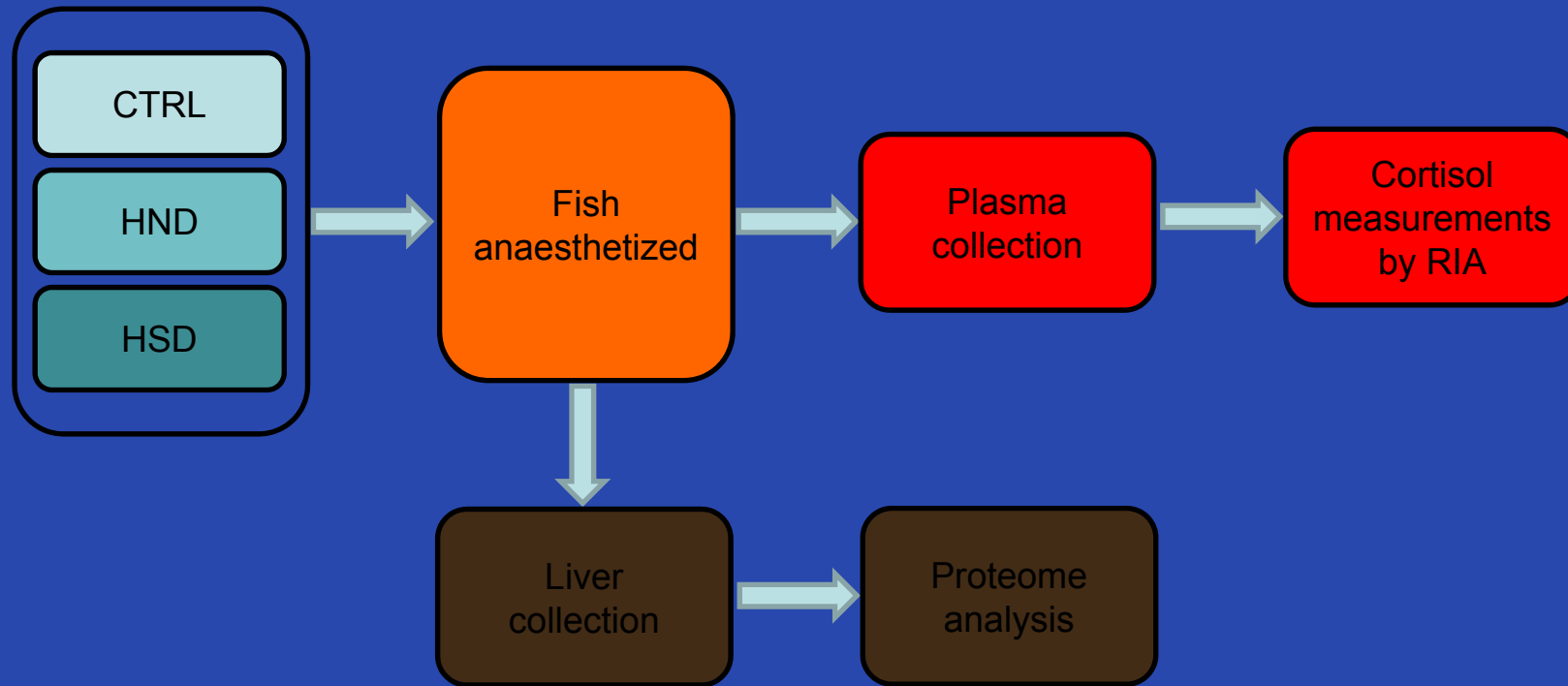


1min , twice/week

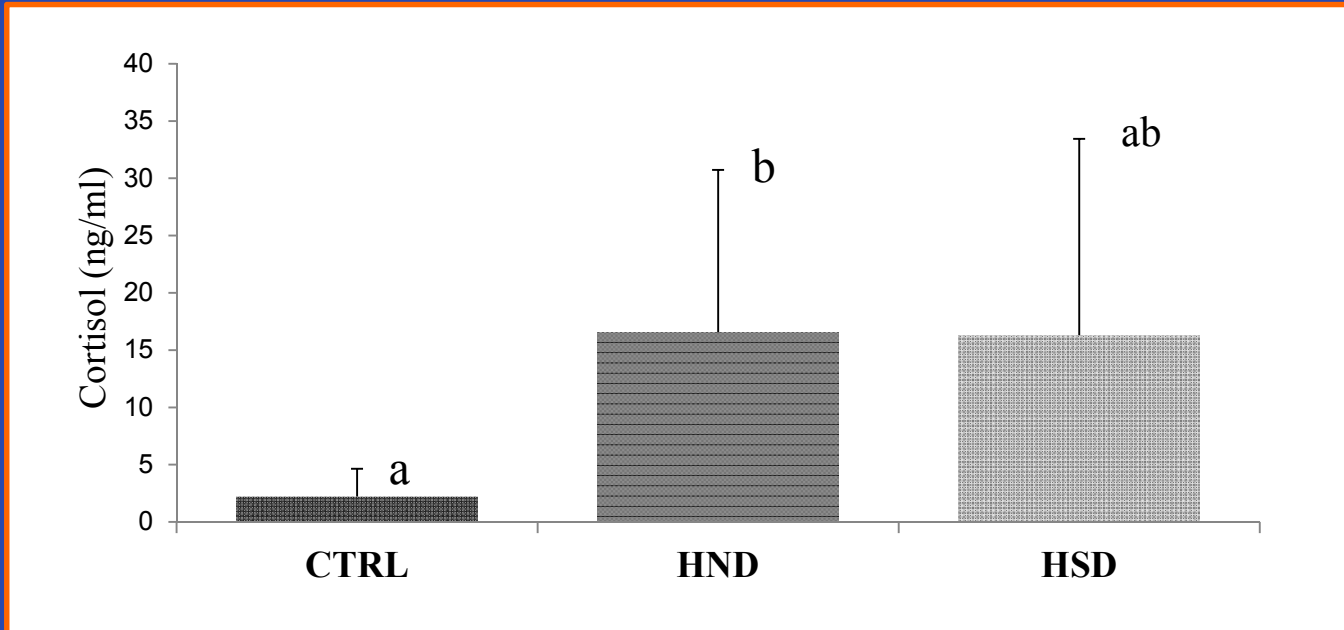




# Experimental



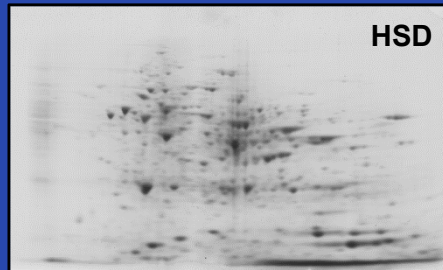
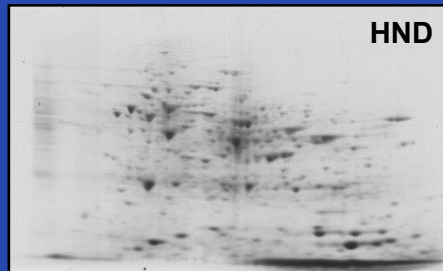
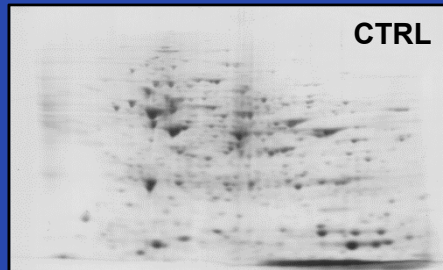
# Results



Mean plasma  
cortisol  
concentration



# Results



pl 3 ← → pl 10

560 spots acquired

299 spots differentially expressed between CTRL and stressed fish (HND and HSD)

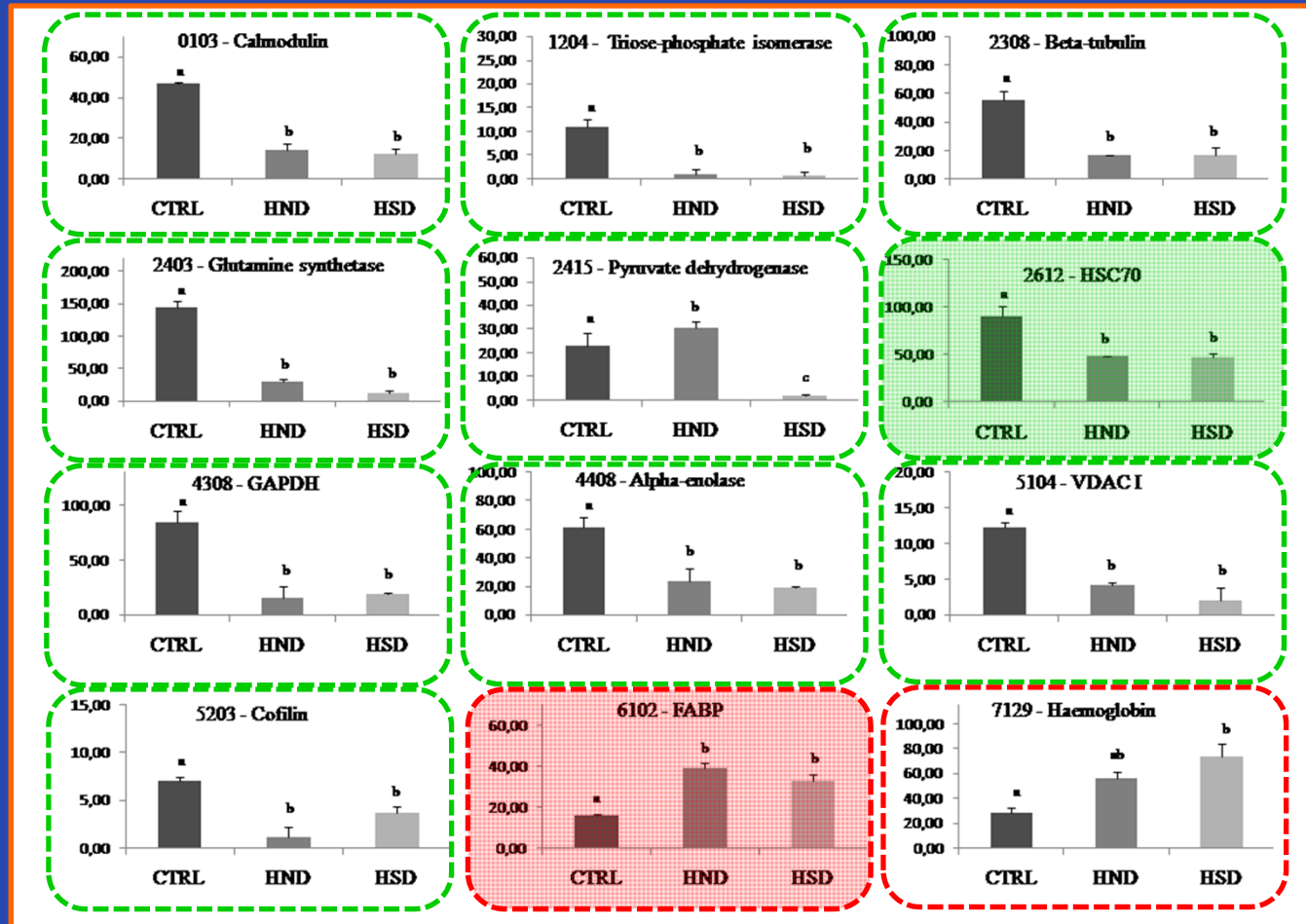
164 relevant spots (greater than 2-fold or lower than 0.5-fold expression)

79 up-regulated spots and 85 down-regulated spots in response to stress

35 spots changed in HND

40 spots changed in HSD





Up-regulated

Down-regulated



# Discussion

**Spot 2612 - Heat shock cognate protein 70**



- Lower gene expression of hsc70 in brain liver of fish subject to high rearing densities (Gornati et al., 2004)

-Immunohistochemical techniques reveal that this protein only accumulates in the (al., 2007)

**Both results suggest that hsc70 is a very good candidate to be used as a biomarker for fish welfare**

-HSPs

- Very conserved

- Chaperones

-Important role in folding

-Regulation, secretion and degradation

ck and  
ed a  
decrease  
of HSPs70  
Vijayan,

2002b)



# Discussion

*Spot 6102 – Fatty acid binding protein (FABP)*



-Increase in the expression in the liver of stressed fish observed in this study can be explained as a mechanism to sustain high rates of lipid usage in liver cells, in order to respond to the increased stress.



- FABPs
- Transport maintenance of
- Uptake of c intest
- Accumulation of fatty acids in the cytosol
- genetic regulation and fatty acid signaling

**FABP is a potential biomarker candidate for fish welfare**

ort and  
Montero  
nd Erol et



# Overall

Proteomics presents itself as a new approach to evaluate chronic stress in gilthead seabream and likely in other species.

## Future work

Validation studies on gene expression (RT-PCR), Immunoassays, and enzymatic activities, as well as in a broader range of chronic stress situations and different species, are required before these biomarkers can be used as chronic stress indicators in fish.



## Effects of Preslaughter Stress Levels on the Post-mortem Sarcoplasmic Proteomic Profile of Gilthead Seabream Muscle

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Pre-  
slaughter  
handling



Muscle cellularity



Fish quality



# Quality Assessment

## *Rigor mortis*

Rigor mortis - the stiffening of the muscles of an animal shortly after death. Greatly affects the quality of fish frozen very soon after capture.



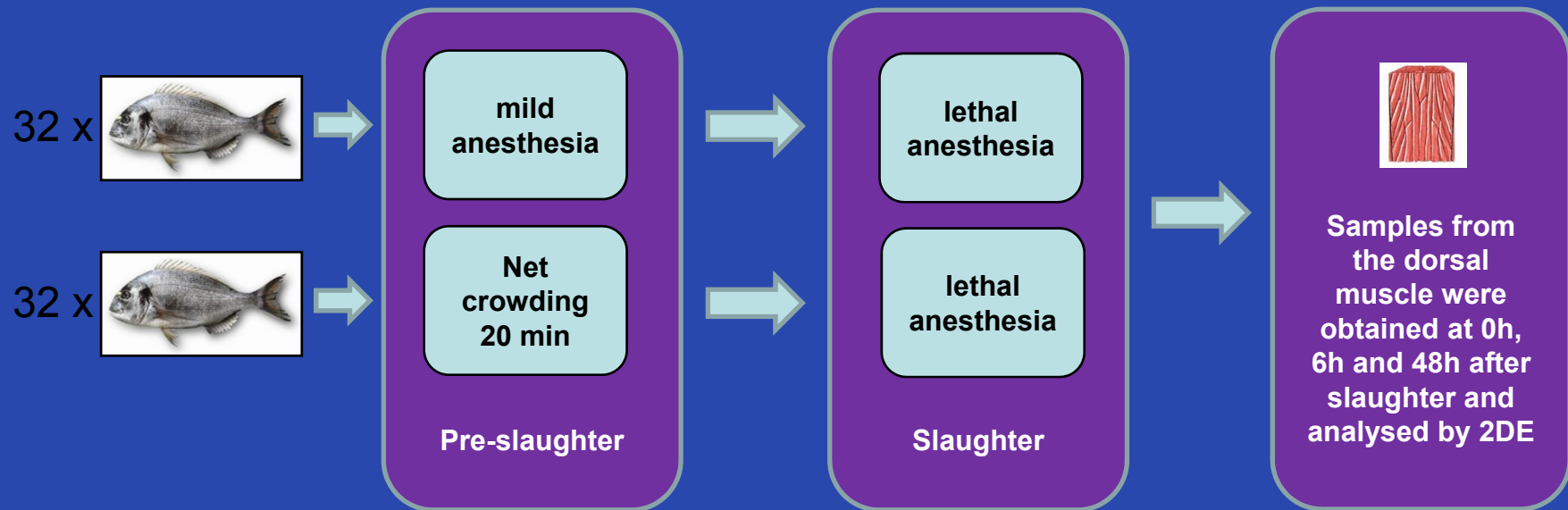
# Industrial c

## Rigor m

Avoid fish filleting due to muscle fiber damaging and consequent less water retention

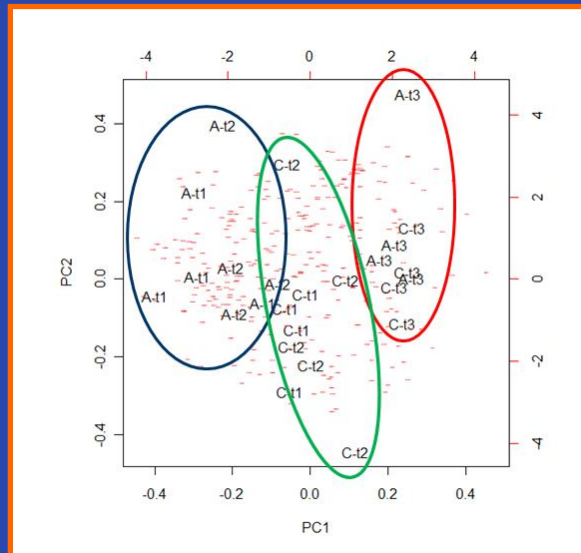


# Experimental design



# Results

## Principal component analysis PCA



311 spots

### mild Anesthesia

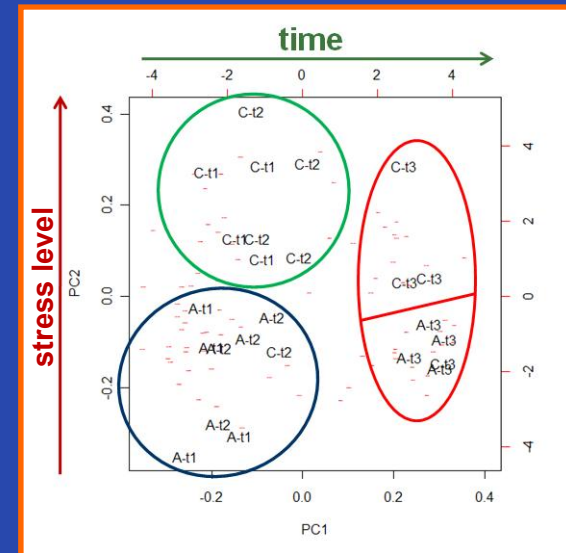
A-t1 (0h after slaughter)  
A-t2 (6h after slaughter)

A-t3 (48h after  
slaughter)

### net Crowding

C-t1 (0h after slaughter)  
C-t2 (6h after slaughter)

C-t3 (48h after  
slaughter)

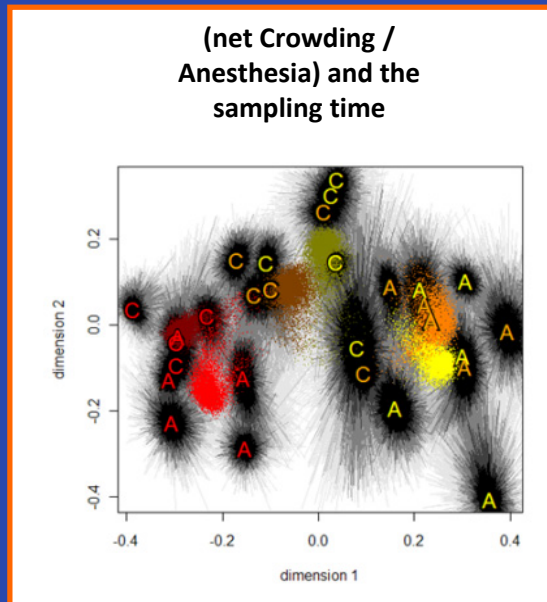


79 spots (after PLSR)



# Results

## Multidimensional scaling



• [0h] • [6h] • [48h]

SameSpots™

-Significant differences between treatments (A/C) are observed especially at 0H and 6H.

-Proteome transformations seem to happen in the first hours post-mortem.

-Significant differences between pre-rigor points at 0H and 6H are observed but C treatment seems to have a closer relation to points at 48H (post-rigor) than A treatment.

-Stress seems to accelerate the process of rigor.



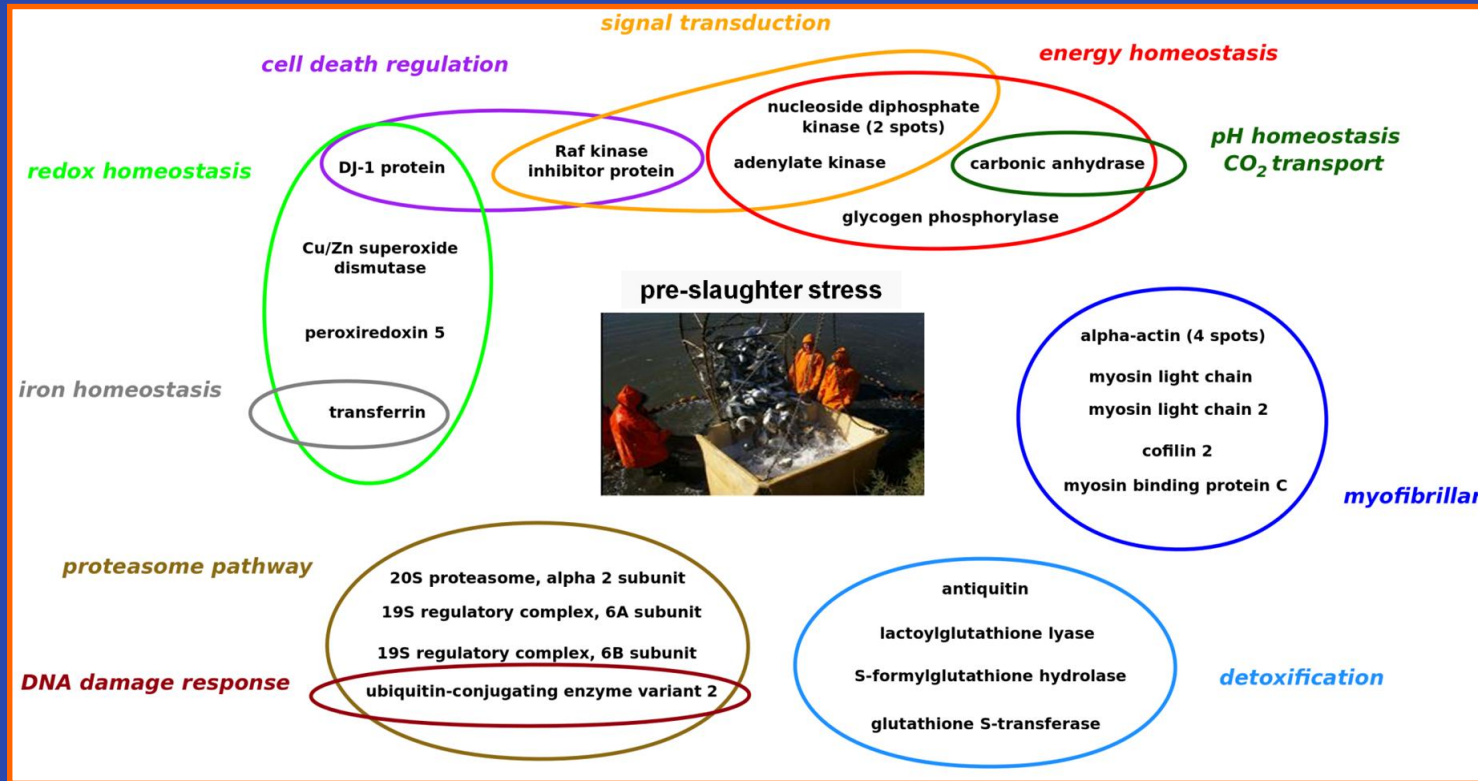
# Results

Relative robustness of gilthead seabream muscle properties to pre-slaughter stress. There were no observable differences between groups in terms of texture properties (namely, hardness and cohesiveness of cooked and raw fillets).



# Results

Affected cellular processes in gilthead seabream muscle



# Overall

The sensitivity of proteomic data in detecting a biological response to certain stimulus, even when no differences are apparent according to macroscopic quality criteria.

## Final Conclusion

**Presented results underline the relevance of proteomics in the context of animal farming, particularly in the field of fish welfare, quality and industrial process optimization.**





# People involved

**Pedro Rodrigues – Assistant Professor at UALG and researcher of the aquaculture group at CCMar**

**Jorge Dias – Researcher of the aquaculture group at CCMar**

**Luís Conceição – Researcher at aquaculture group, CCMar**

**Flemming Jessen, Tune Wulff – DTU Food, Fødevareinstituttet, Danmark Tekniske Universitet, Lyngby, Denmark**

**Tomé Silva – PhD student at University of Algarve (aquaculture group)**

**Odete Cordeiro – Lab. Technician at aquaculture group**

**Elisabete Matos - PhD student at University of Algarve (aquaculture group)**

**Nadège Richard – Pos-Doc student at University of Algarve (aquaculture group)**

**Mahaut de vareilles - PhD student at University of Algarve (aquaculture group)**

**Ricardo alves – Masters student University of Algarve (aquaculture group)**

**Denise Shrama - Lab. Technician at aquaculture group**



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THANK YOU

