

Egg quality in domesticated and wild seabass (*Dicentrarchus labrax*): a proteomic analysis

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INTRODUCTION:

Fish egg quality is highly variable and can be affected by environmental factors and husbandry practices (Brooks *et al.*, 1997). The improvement and stabilization of quality require a better understanding of the molecular bases of egg developmental competence. Egg components such as maternally-inherited proteins support early development and their abundance in the eggs may be affected by environmental factors, husbandry practices and possibly, domestication. Egg proteome has been studied in zebrafish (Knoll-Gellida *et al.*, 2006) but never analyzed in relationship with the developmental competence of the egg.

This work is an attempt to reveal possible variations of egg proteome,

- 1) due to contrasted conditions of egg origin
- 2) responsible of differential egg developmental performances

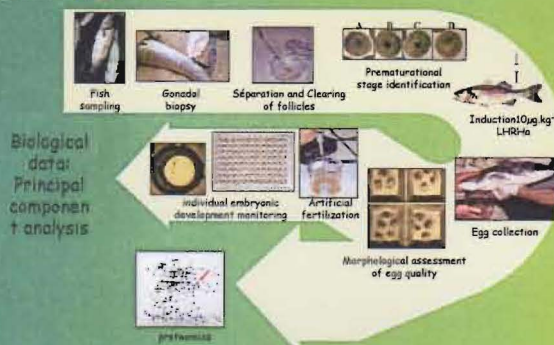
Biological performance study

GENERAL PROTOCOL

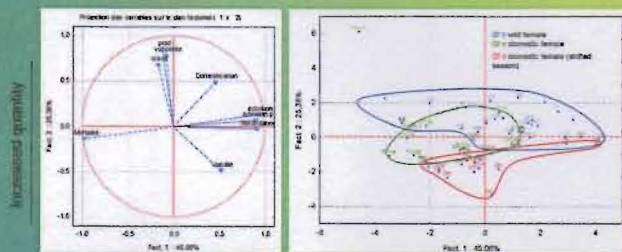
Broodstocks:

- 28 wild females subjected to natural seasonal cycle (2 tanks)
- 14 domestic females (F3) subjected to natural seasonal cycle (2 tanks)
- 16 domestic females (F3) subjected to shifted seasonal cycle (2 tanks)

Experimental procedure



RESULTS

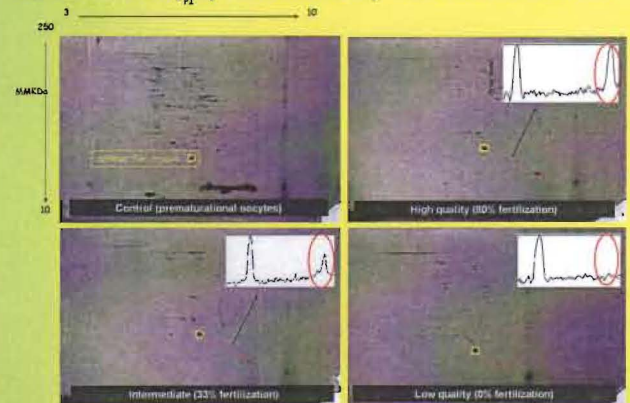


- ◆ Seabass egg quality described by fertilization and hatching is dependant neither on female weight and prematurational stage at stimulation nor volume of spawn
- ◆ Both fertilization rate (62.8%) and hatching rate (32.3%) are significantly higher ($P < 0.001$) in wild population compared to domestic ones (41.4 and 16.1% respectively)
- ◆ Domestication may impact egg quality, however, the different populations being reared individually, uncontrolled environmental effect may also explain the differences.
- ◆ The high intrapopulation variability illustrates the plurifactorial determinism of egg quality
- ◆ The biological experiment provides samples with different characteristics (origin and biological performance) for proteomic analysis

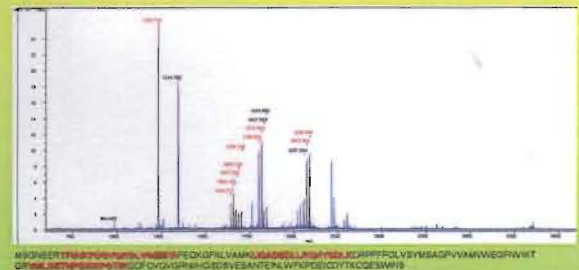
Proteome analysis protocol

- ◆ Egg cytoplasmic proteins were delipidated by a two phase extraction medium (Butanol/Diisopropylether, 4/6).
- ◆ Samples were subjected in triplicates to IEF on 3-10 linear pH gradient 17 cm strip. The second migration was run through 12% SDS polyacrylamide gels.
- ◆ 2D protein expression patterns were analyzed using Image Master2D Platinum after Coomassie blue coloration.
- ◆ Differential spots were analyzed by MALDI TOF/TOF mass spectrometry. Corresponding protein sequences were identified from MSDB database by Mascot software (Matrix science).
- ◆ An antibody directed against a portion of the sequence of a candidate protein, was raised in rabbits and tested by western blot.

Differential display in relationship with fertilization rate

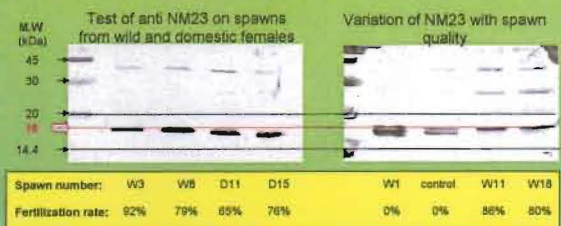


MS-MS analysis



Identification of Nucleoside Diphosphate kinase (NM23) with 36% homology, a highly conserved protein of 18KD.

Validation of the candidate protein



DISCUSSION:

In spite of drastic delipidation necessary to 2D electrophoresis and the use of non sensitive Coomassie blue revealing only around 100 readable spots, it has been possible to identify several differential proteins and particularly the Nucleoside Diphosphate Kinase (NM23) which showed variation in relationship with egg quality. The antibody raised against the fragment allowed confirming its molecular weight in seabass. Moreover, the variation observed on 2Dgels may be linked to a degradation of NM23 featured by the apparition of lower molecular weight bands as the fertilization rate of sample decreases

CONCLUSION:

The use of large scale analytical methodologies such as proteomics to decipher the molecular bases of egg developmental competence is promising. The identification of specific proteins exhibiting a differential abundance in eggs of varying quality will contribute to the identification of the mechanisms triggering egg quality defects. Moreover they can rapidly be used as objective molecular markers to assess the improvements of broodstock management.

REFERENCES

- BROOKS, S., TYLER C.R. & J.P. SUMPTER 1997. Egg quality in fish: What makes a good egg? *Rev. Fish Biol. Fish.*, 7, 387-416
- KNOLL-GELLIDA A., ANDRE M., GATTEGNO T., FORGUE J., ADMON A. & P.J. BABIN, 2006. Molecular phenotype of zebrafish ovarian follicle by serial analysis of gene expression and proteomic profiling and comparison with the transcriptomes of other animals. *BMC Genomics*, 7, 45
- MS identifications were performed by N. Sommerer (INRA - UR 1199 Proteomique, Montpellier France)