UNIVERSITY OF THESSALY

Department of Biochemistry & Biotechnology

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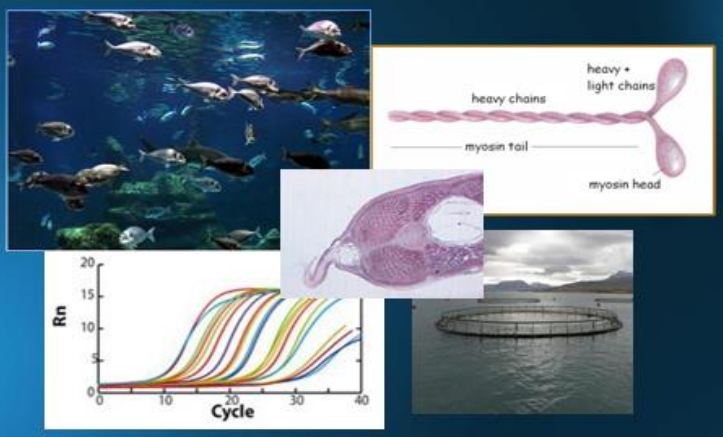
Doctoral thesis

Expression of skeletal myosin light chain 2 in gilthead sea bream (*Sparus aurata, L*): regulation and correlation to growth markers



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- **Zissis Mamuris:** Professor in Animal Population Genetics, Department of Biochemistry & Biotechnology, University of Thessaly, Greece
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Publications

Georgiou, S., Alami-Durante, H., Cluzeaud, M., Bazin, D., Power, D.M., Mamuris, Z., Moutou, K.A. (2011) White muscle growth in gilthead sea bream larvae (*Sparus aurata*); fibers and genes, 381-382, Mediterranean Aquaculture 2020 Proceedings, October 18-21, Rhodes, Greece

Moutou, K.A., Codina, M., **Georgiou, S.,** Gutiérrez, J., Mamuris, Z. (2009) Myosin light chain 2 in gilthead sea bream (*Sparus aurata*): a molecular marker of muscle development and growth, 289-292, Special Publication No. 38, European Aquaculture Society Proceedings, Oostende, Belgium

In submission:

- Myosin light chain 2 isoforms in gilthead sea bream (*Sparus aurata*, L): a paradigm of divergent evolution

  Stella Georgiou, Elena Sarropoulou, Marta Godina, Deborah M. Power, Joaquim Gutierez, Zissis Mamuris, Katerina A. Moutou

- Expression of skeletal myosin light chain 2 (MLC2) in gilthead sea bream (*Sparus aurata*, L): developmental regulation and correlation with growth markers

  Stella Georgiou, Helen Alami-Durante, Deborah M. Power, Zissis Mamuris, Katerina A. Moutou

In preparation:

- Can myosin light chain 2 isoforms serve as growth markers?

  Stella Georgiou, Pavlos Makridis, Dimitris Dimopoulos, Deborah M. Power, Zissis Mamuris, Katerina A. Moutou

Summary

In gilthead sea bream skeletal muscle two isoforms of myosin light chain 2 (MLC2) are expressed, A and B. MLC2A is encoded by three transcripts produced through alternative polyadenylation signal selection differing in the 3' untranslated region. *cis*-acting regulatory elements residing in 3' UTRs were identified using bioinformatic analysis. Functionality of 3' UTRs in interaction with the cell protein machinery was investigated *in vitro* using luciferase reporter constructs. Data indicated that the three transcripts are subject to differential regulation according to 3' UTR length and regulatory interactions between transcripts were detected.

Gene organization of the two isoforms and the syntenic relationships between gilthead sea bream, European sea bass, zebrafish, Atlantic cod, three-spined stickleback, medaka and tetraodon were studied. *mlc2a* and *mlc2b* genes contain 5 and 6 exons respectively in all organisms examined. Exon-intron organization is more similar among evolutionary closer species which is further confirmed by the phylogenetic tree probably indicating different evolutionary trajectories for the two *mlc2* isoforms. Divergent expression patterns during myogenesis in primary muscle cell cultures and in developing larvae, and after administration of growth hormone to juveniles, indicated that the two isoforms of *mlc2* identified in the gilthead sea bream are duplicated genes (paralogues) which have probably undergone subfunctionalization.

White muscle development and growth processes occur through hyperplastic and hypertrophic events driven by several regulatory and hormonal factors. The development of white musculature in gilthead sea bream larvae demonstrated distinct hyperplastic and hypertrophic phases as evident by changes in muscle cellularity. The expression patterns of MLC2 marked the two phases in an isoform-specific manner; MLC2A marked new fiber formation in the germinal zones, appeared early in development and was correlated to myogenic factors; MLC2B was up-regulated only at hypertrophy in a later developmental stage and was correlated to muscle structural genes.

The effect of rearing conditions to *mlc2* isoform plasticity was determined in larvae and juvenile sea bream outcoming from the same egg-batch and raised under

intensive versus mesocosm conditions as well as in juvenile gilthead sea bream reared under commercial aquaculture practices subjected to size-grading with sorters of increasing diameter. In the rearing systems experiment, larvae raised in mesocosm outperformed in growth and *mlc2* isoform expression those raised under intensive. Results from the size-grading experiment suggested that somatic growth of juvenile gilthead sea bream was modestly improved. The ratio of *mlc2a/ mlc2b* expression was significantly correlated to axial growth during the larval and early juvenile stages.

Given the plastic versatility prevailing early development, the present results provide strong evidence of the robustness of *mlc2* isoforms as markers of early growth.