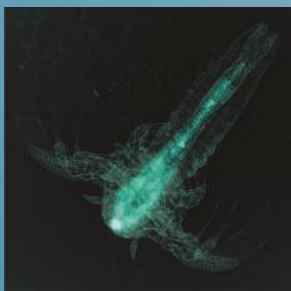
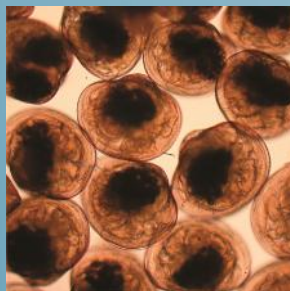
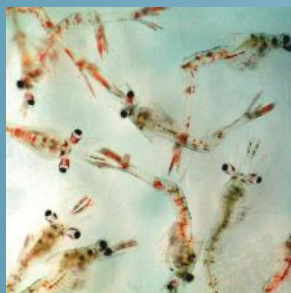
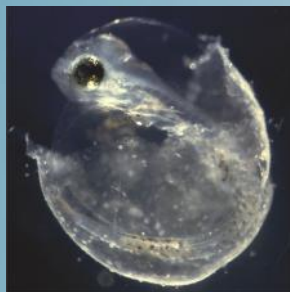


larvi 2013

6th fish & shellfish larviculture symposium

Expression of skeletal myosin light chain 2
in gilthead sea bream (*Sparus aurata*, L)

Styliani Georgiou



ghent university, belgium, 2-5 september 2013

EXPRESSION OF SKELETAL MYOSIN LIGHT CHAIN 2 IN GILTHEAD SEA BREAM (*Sparus aurata*, L): REGULATION AND CORRELATION TO GROWTH MARKERS

Stella Georgiou¹, Elena Sarropoulou², Deborah M. Power³, H  l  ne Alami-Durante⁴, Zissis Mamuris¹, Katerina A. Moutou¹



¹Dept. of Biochemistry & Biotechnology, University of Thessaly, Greece

²Institute of Marine Biology & Genetics, Hellenic Centre for Marine Research, Greece

³Centre of Marine Sciences, University of Algarve, Portugal

⁴Nutrition Aquaculture et G  nomique, INRA, P  le d'Hydrobiologie, St P  e sur Nivelles, France

Prediction of growth is of outmost importance for sustainable fish farming



fry of predictable growth performance

Molecular growth markers of predictable power are expected to:

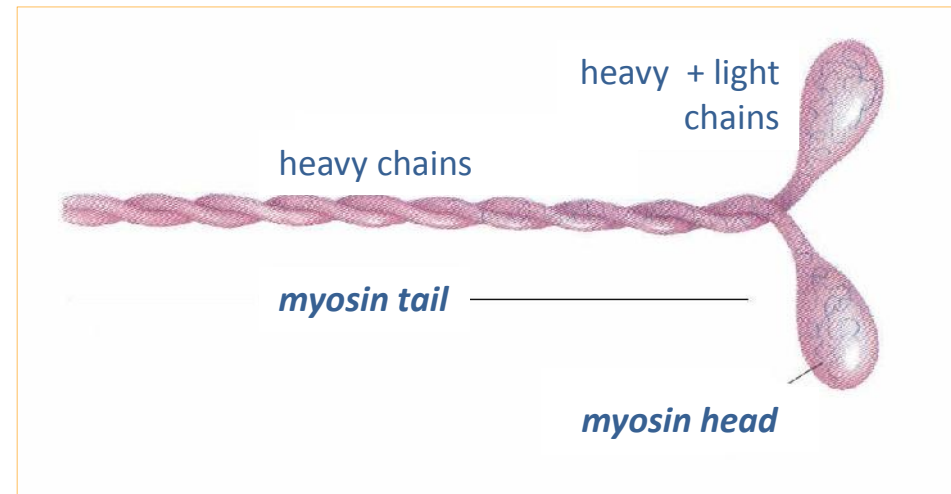
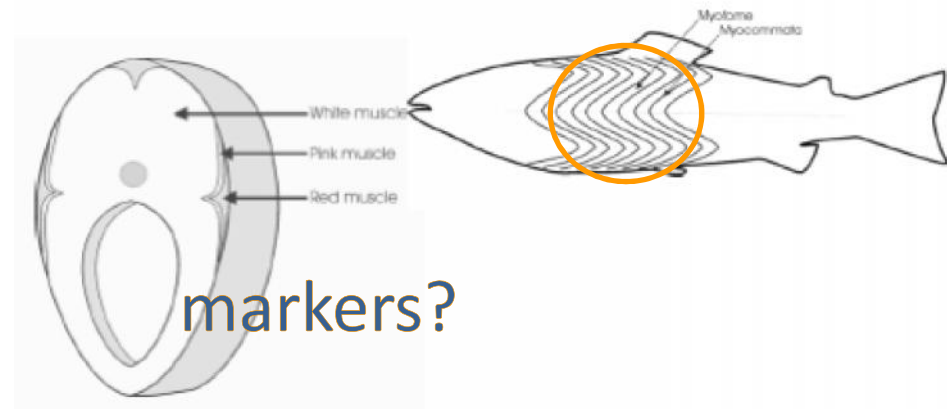


- Correlate with established growth markers
- Regulated by GH-IGF axis
- Age-dependent
- Nutritionally regulated

Muscle is a suitable tissue to look for growth markers



White muscle counts for up to 70% of the fish body mass and is the final product of fish farming



Myosin is a constituent of the final product

Two isoforms of skeletal MLC2 exist in gilthead sea bream

Myosin structure

- 2 heavy chains (MHC)
- 4 light chains (MLC)
 - 2 essential light chains (MLC1, MLC3)
 - 2 regulatory light chains (MLC2)



Ca⁺⁺-binding domain

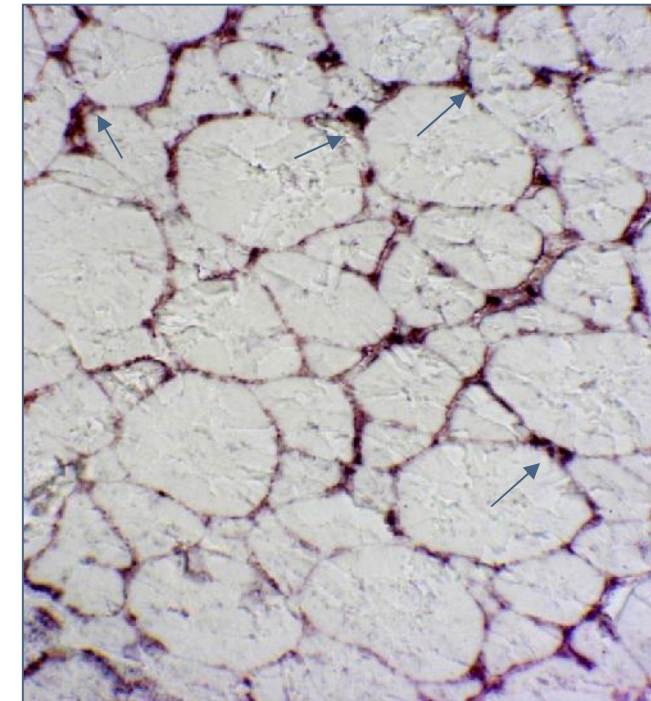
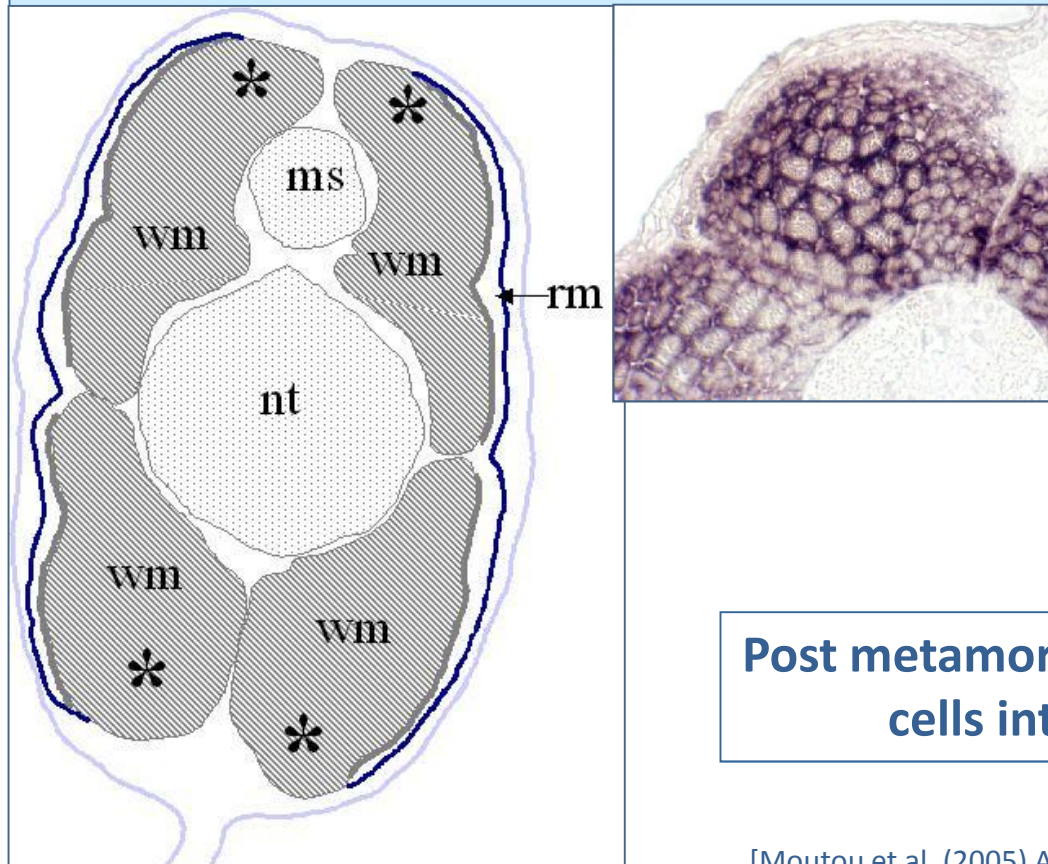
S.aurata	A	MAPKKAKRRQ QQG EGGSSNVF'SMF'EQSQIQEYKEAFTI IDQNRDGIISKDDL RDVLA T MG
S.aurata	B	MAPKKAKRRQ AAGD GGSSNVF'SMF'EQSQIQEYKEAFTI IDQNRDGIISKDDL RDVLA S MG
		***** * *****:*
S.aurata	A	QLNVKNEELEAM V KEASGPINF'TVFLTMFGEK L KGADPEDVI V SAFKVLDPEATGAIKKEF
S.aurata	B	QLNVKNEELEAM I KEASGPINF'TVFLTMFGEK L KGADPEDVI L SAFKVLDPEGTGSIKKEF
		*****:*****:*****:*****.* * *****
S.aurata	A	LEELLTTQCDRFT AEEM TN L WAAFPPDVAGNV DY KNICYVITHGEEKEE
S.aurata	B	LEELLTTQCDRFT KDEIKN MWAAFPPDVAGNV DY KNICYVITHGEEKEE
		*****:*****:*.*:*****

[Moutou et al. (2001) J. Exp. Biol. 204, 3009-3018, Sarropoulou et al. (2006) Arch. Tierz. Dumm. 92-96]

Two isoforms of skeletal MLC2 exist in gilthead sea bream: MLC2A



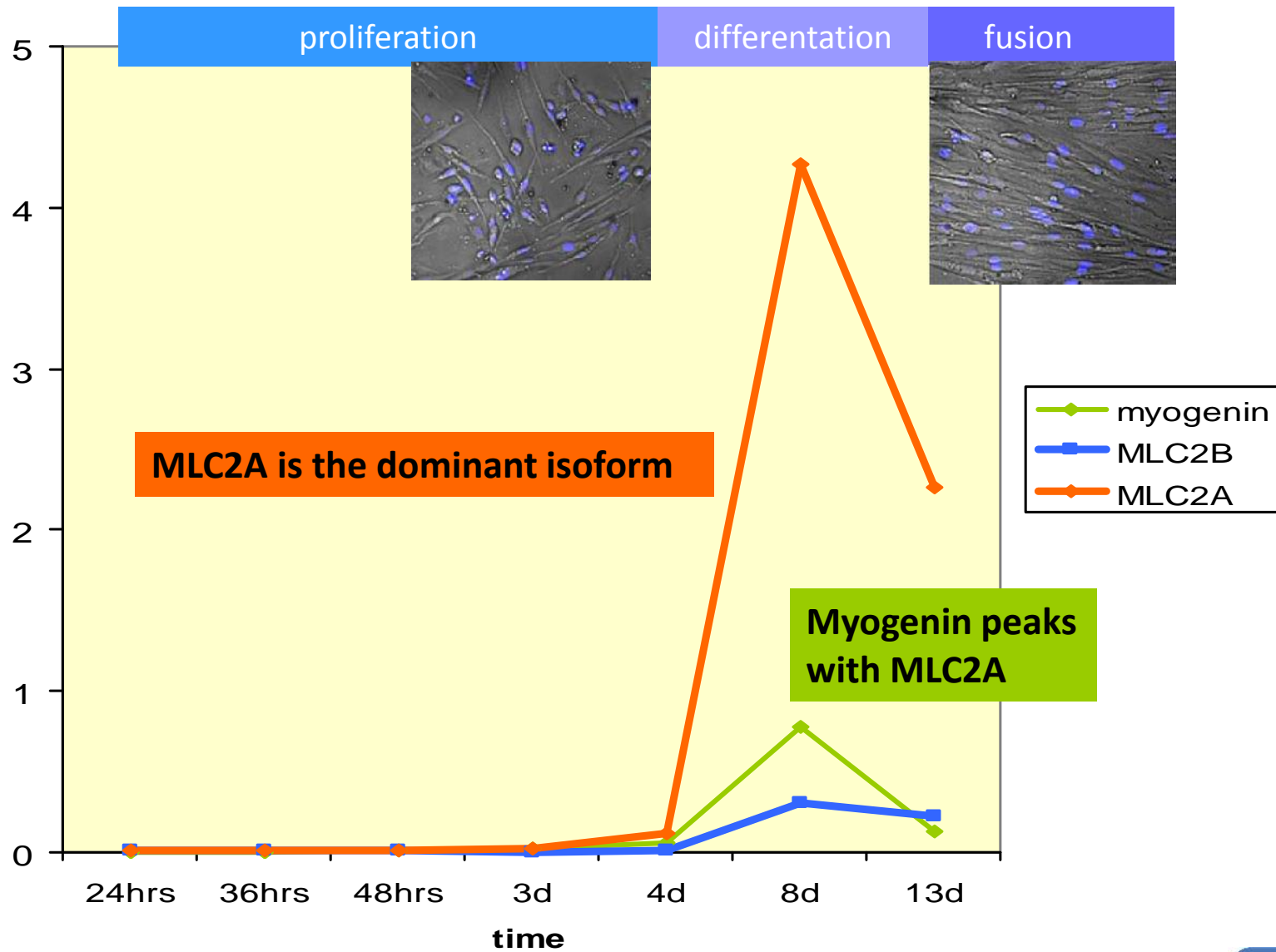
MLC2A expression follows the white muscle development and marks the germinal zones and the newly formed white fibers



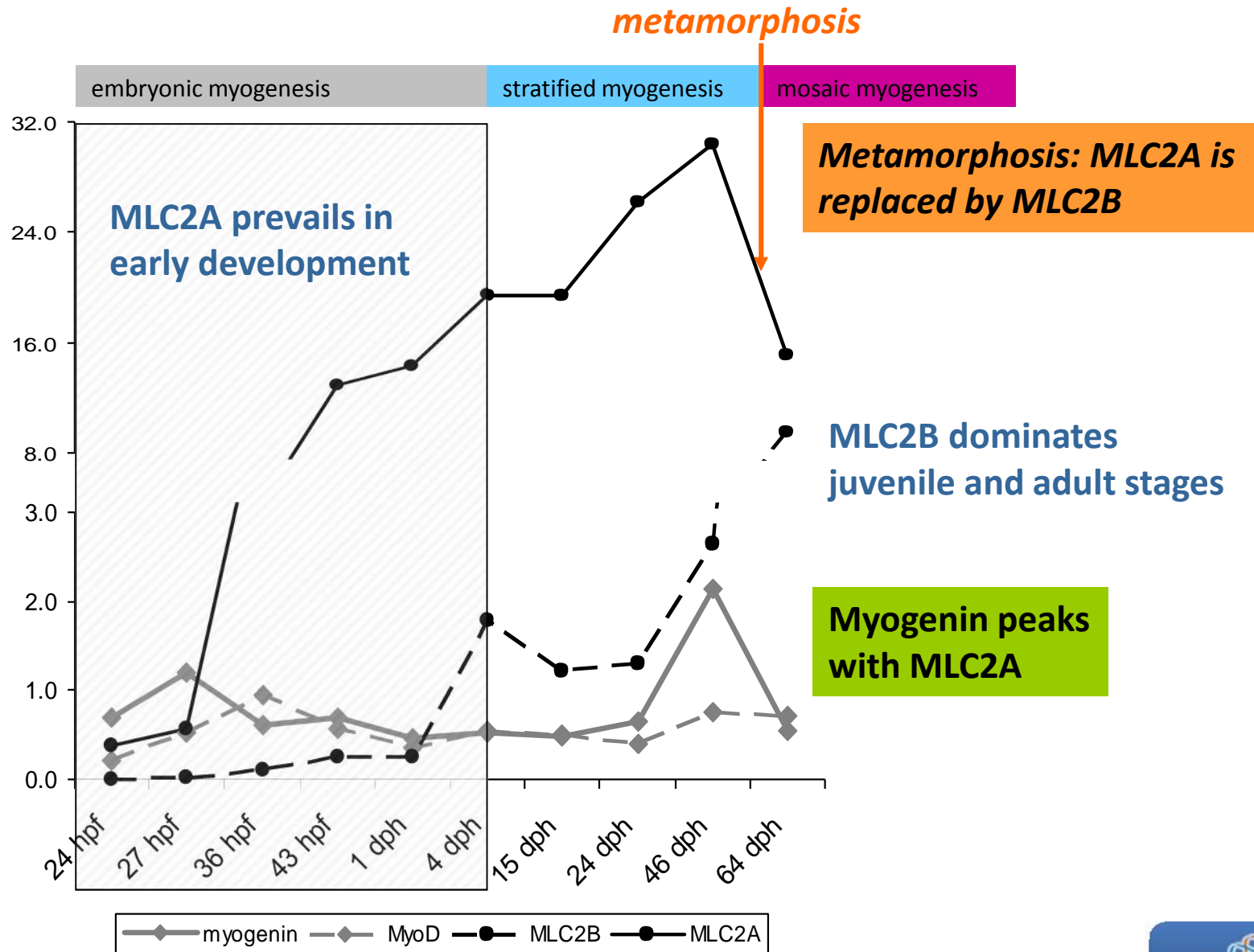
Post metamorphosis, it is expressed in myogenic cells interspersed in the fast white muscle

[Moutou et al. (2005) Arch. Tierz. Dumm. 48]

The two isoforms of skeletal MLC2 in gilthead sea bream are differentially regulated during the myogenic program: *primary myocytes*



The two isoforms of skeletal MLC2 in gilthead sea bream are differentially regulated during the myogenic program: *development*



Objective

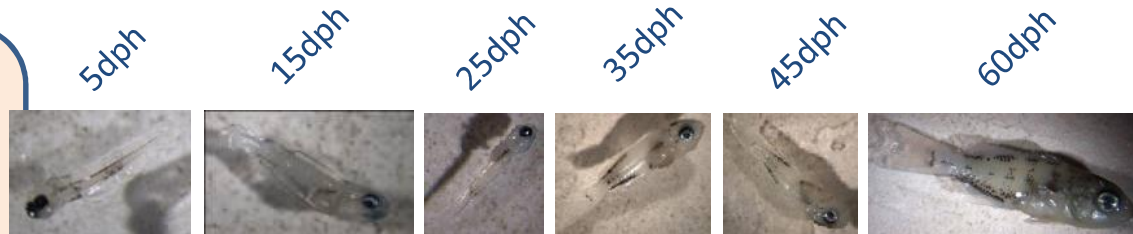
Investigate the potential of MLC2 isoforms as growth markers

- Do they correlate with established growth markers?
- Are they regulated by the GH-IGF axis?
- Do their expression changes with age?

Robust in the field????

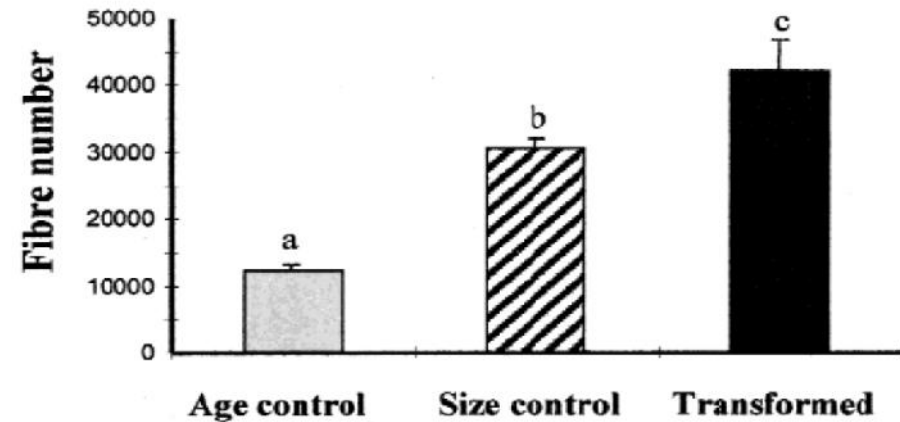
Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?

White muscle cellularity and gene expression profiles were studied in five to sixty days post hatch (dph) gilthead sea bream larvae



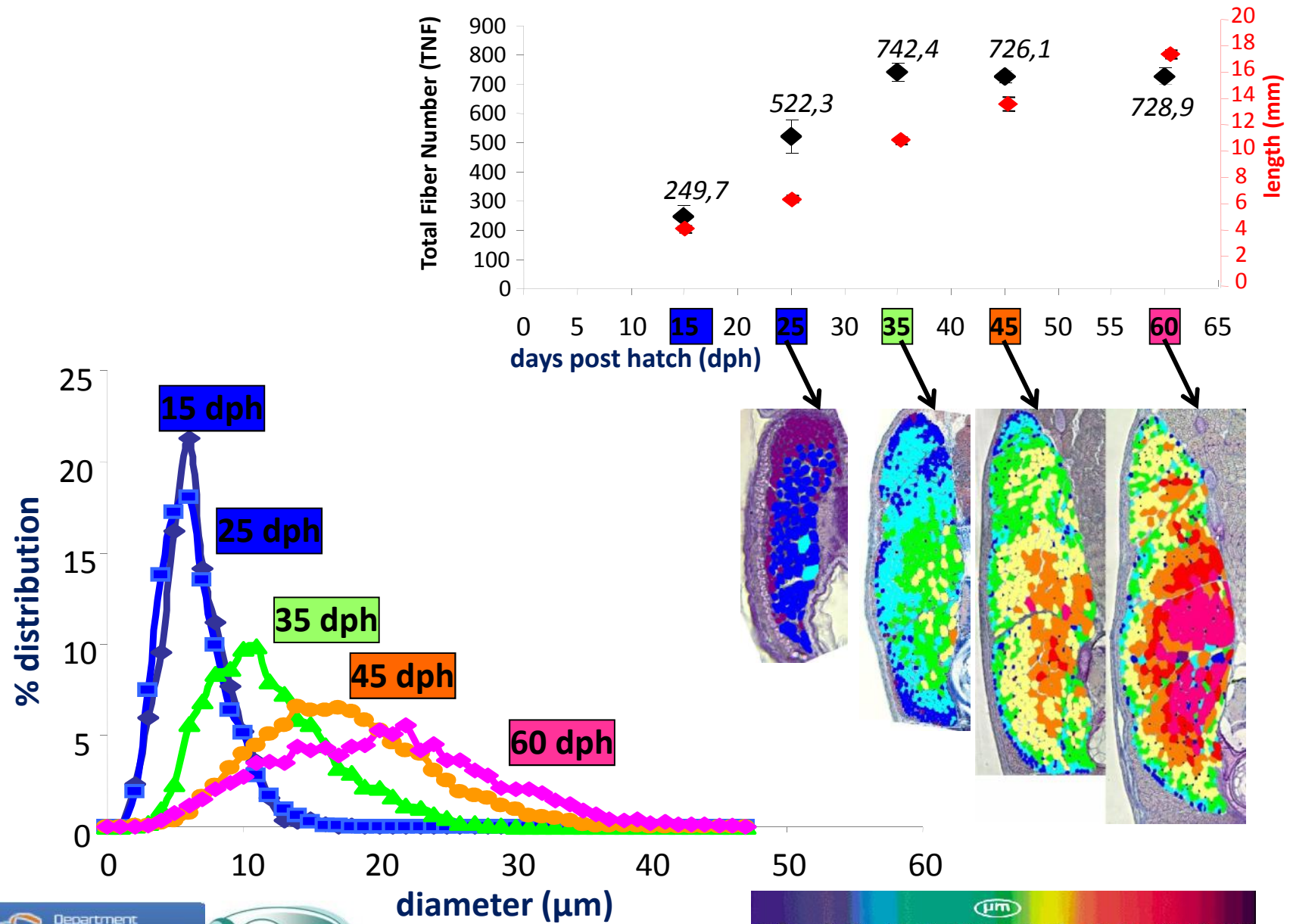
Is there a link between MLC2 isoform expression and established growth markers in gilthead sea bream development?

The ability of fish to grow rapidly depends on maintenance of white muscle fiber recruitment and number of small white fibers

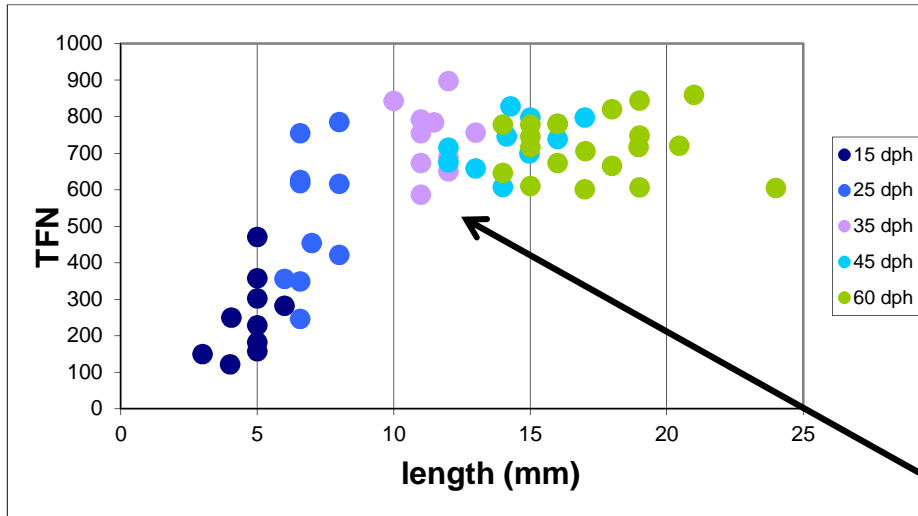


Genetically modified Arctic charr carrying recombinant growth hormone gene
Pitkänen et al. 2001. *Mar Biotech* 3, 188-197

Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?

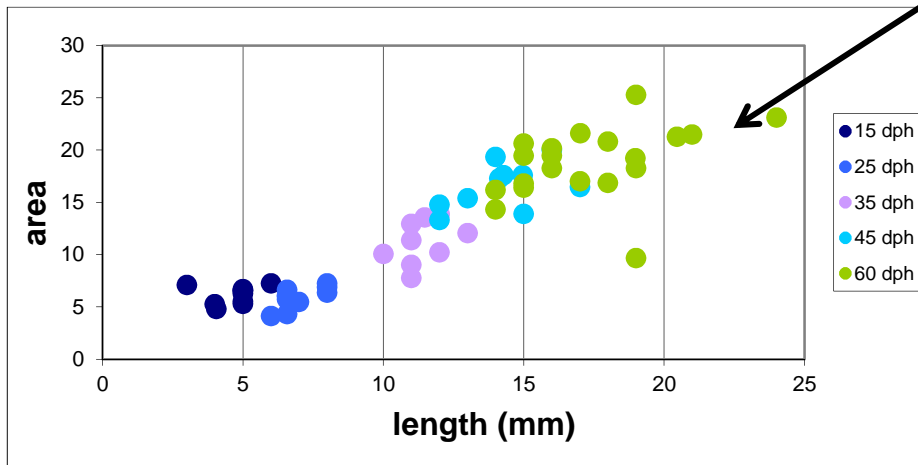


Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?



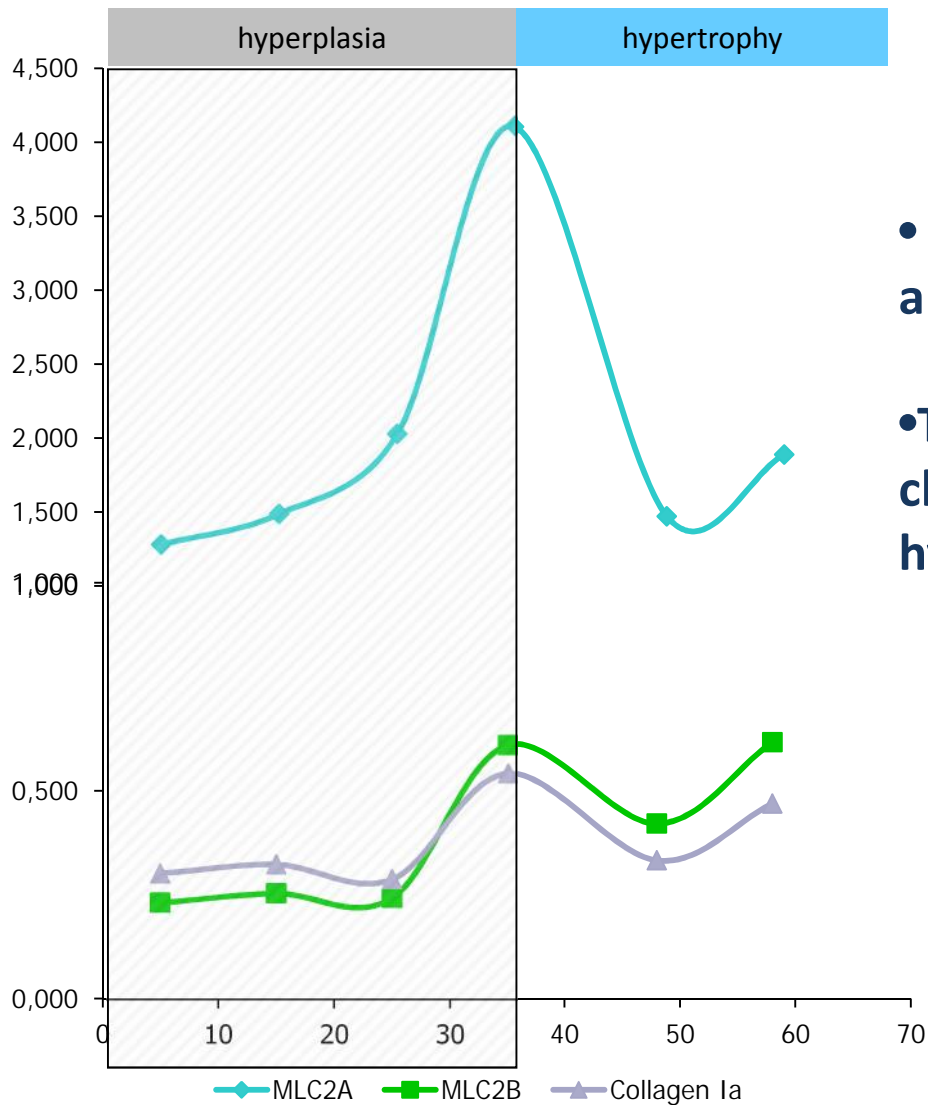
Total fiber number (TNF) indicates the degree of hyperplasia (formation of new fibers).

Hyperplasia slows down whereas **hypertrophy continues.....**



Fiber area indicates the degree of hypertrophy (increase of existing fibers).

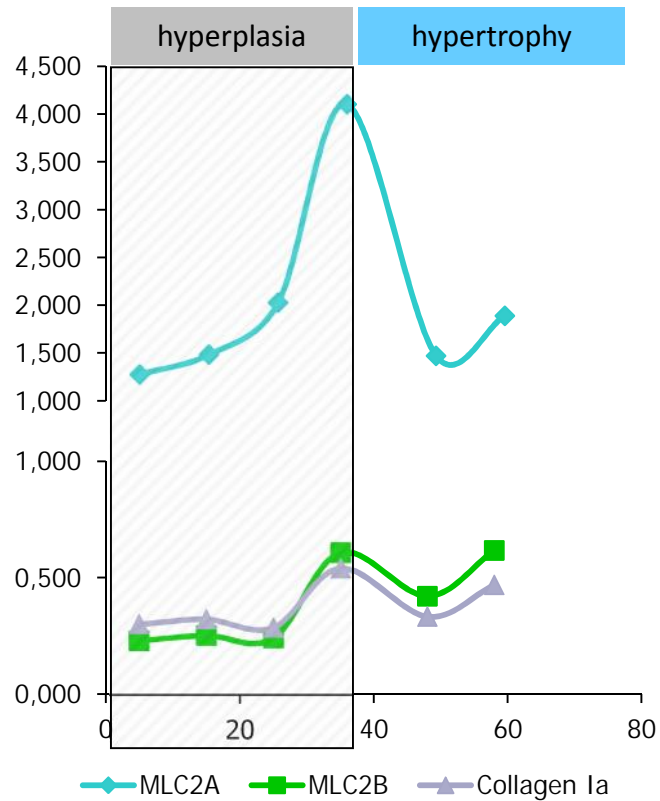
Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?



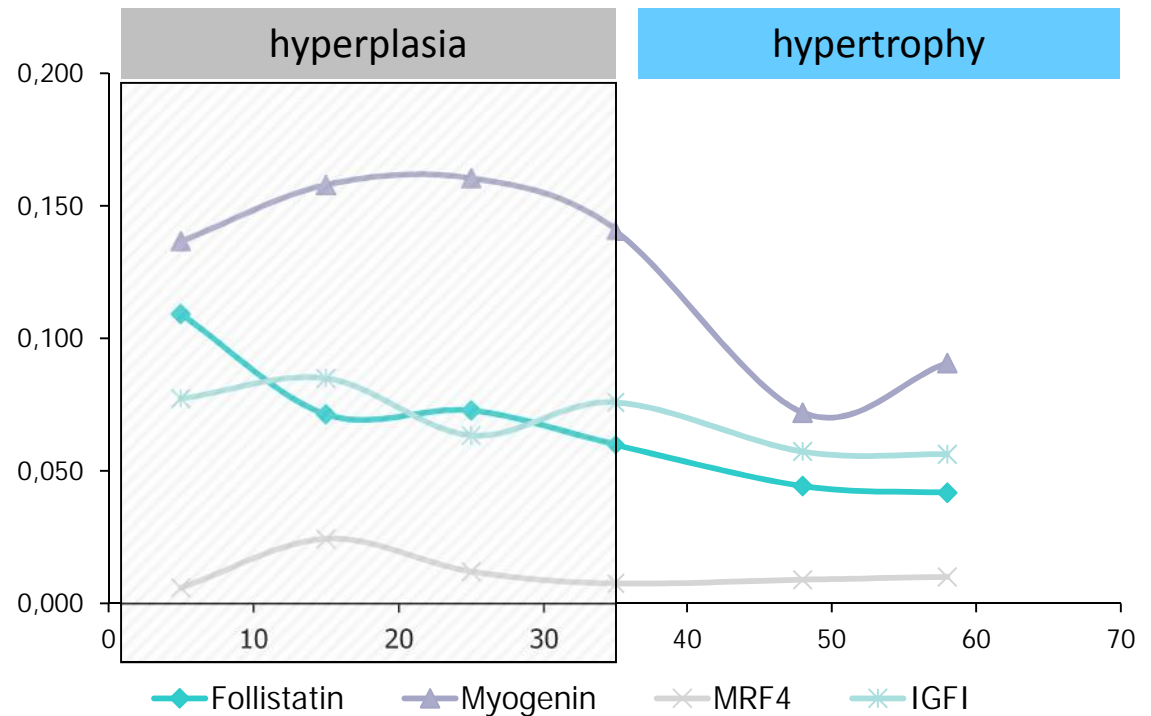
- **MLC2A** elevates during hyperplastic growth and then it is **downregulated**

- The expression of **MLC2B** and Collagen1a changes significantly between the hyperplastic and **hypertrophic phase**

Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?



- Myogenin drives hyperplasia and is downregulated at hypertrophy
- Follistatin decreases with increasing fiber area
- IGF1 remains stable



Is there a link between MLC2 isoform transition and the hyperplastic and hypertrophic growth in gilthead sea bream development?

MLC2 isoform expression tightly follows the development of white muscle gilthead sea bream

MLC2 isoform expression reflects changes in muscle cellularity

MLC2A = hyperplasia VS MLC2B = hypertrophy

MLC2A = embryonic VS MLC2B = adult

Is MLC2 isoform expression regulated by growth hormone?

Is MLC2 isoform expression regulated by growth hormone?

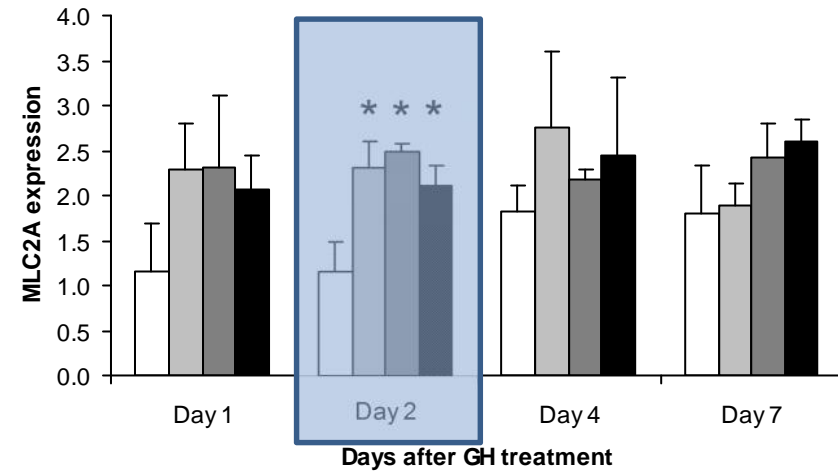
$\mu\text{g oGH/g BW}$
 0
 0.1
 1.0
 10.0



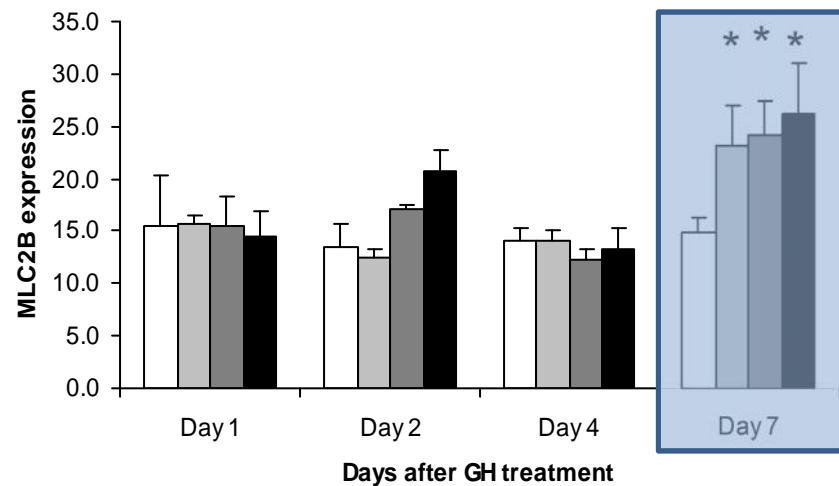
(57.3 g \pm 1.1 g)

GH administration led to a significant non dose-dependent induction of both MLC2 isoform expression

MLC2 isoforms responded differentially to GH administration



□ control □ 0.1 $\mu\text{g oGH/g BW}$ ■ 1.0 $\mu\text{g oGH/g BW}$ ■ 10.0 $\mu\text{g oGH/g BW}$



□ control □ 0.1 $\mu\text{g oGH/g BW}$ ■ 1.0 $\mu\text{g oGH/g BW}$ ■ 10.0 $\mu\text{g oGH/g BW}$

Can myosin light chain 2 isoforms serve as growth markers?



Molecular growth markers of predictable power are expected to:

- Correlate with established growth markers ✓
- Regulated by GH-IGF axis ✓
- Age-dependent ✓



Experiment 1: size-grading of juveniles

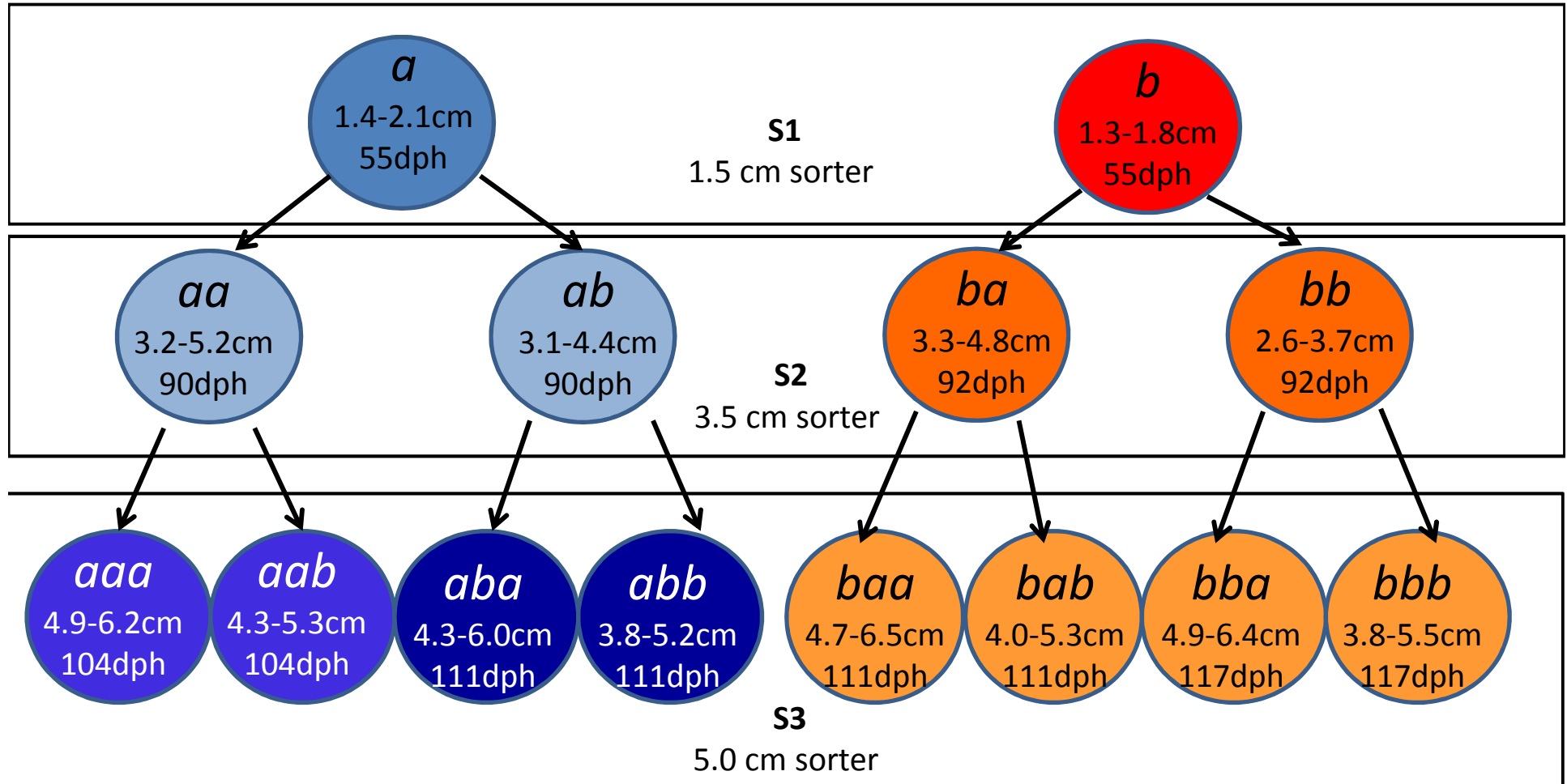
Experiment 2: mesocosm vs intensive larvae rearing



Does MLC2 expression follow axial growth?

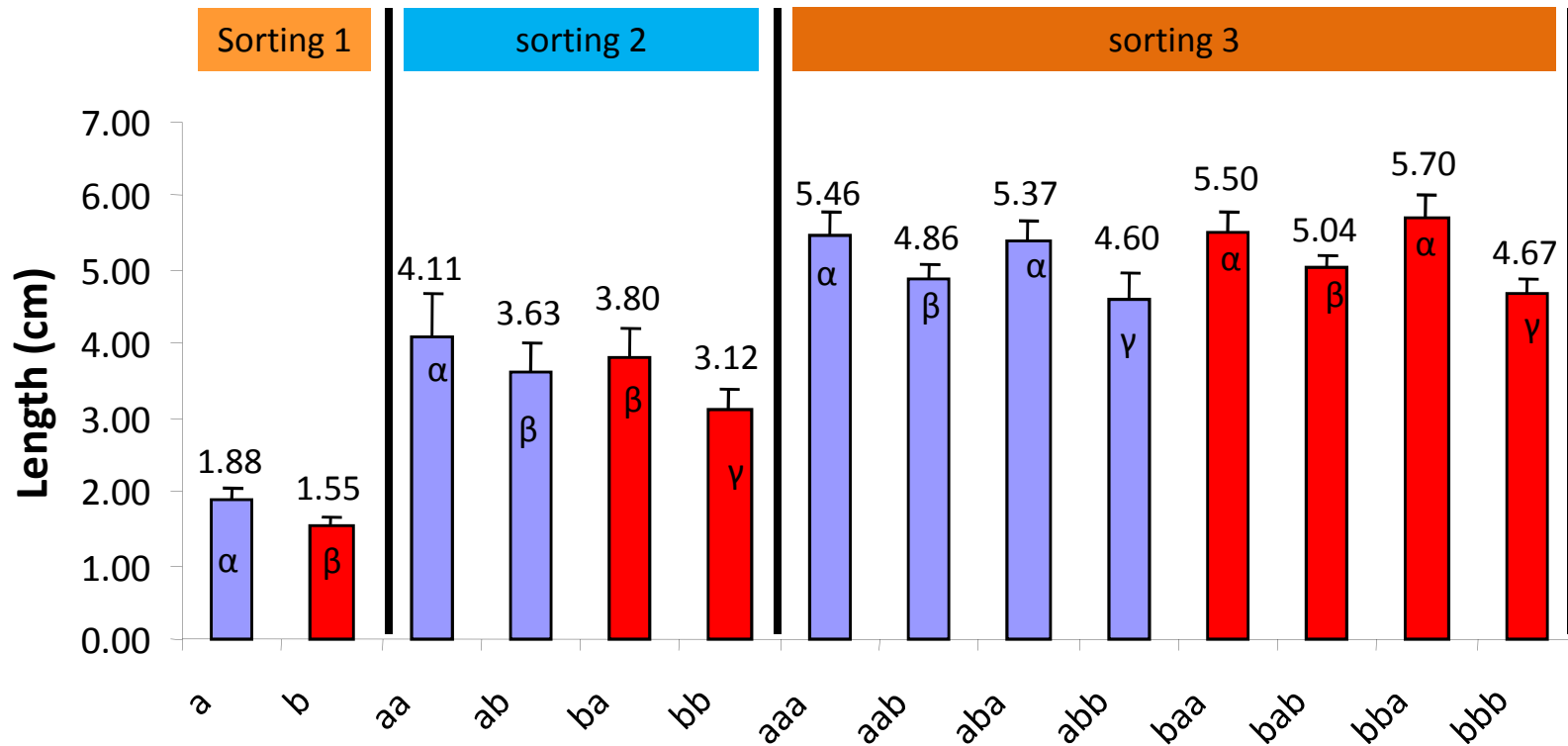
Can myosin light chain 2 isoforms serve as growth markers?

Exp. 1: size-grading



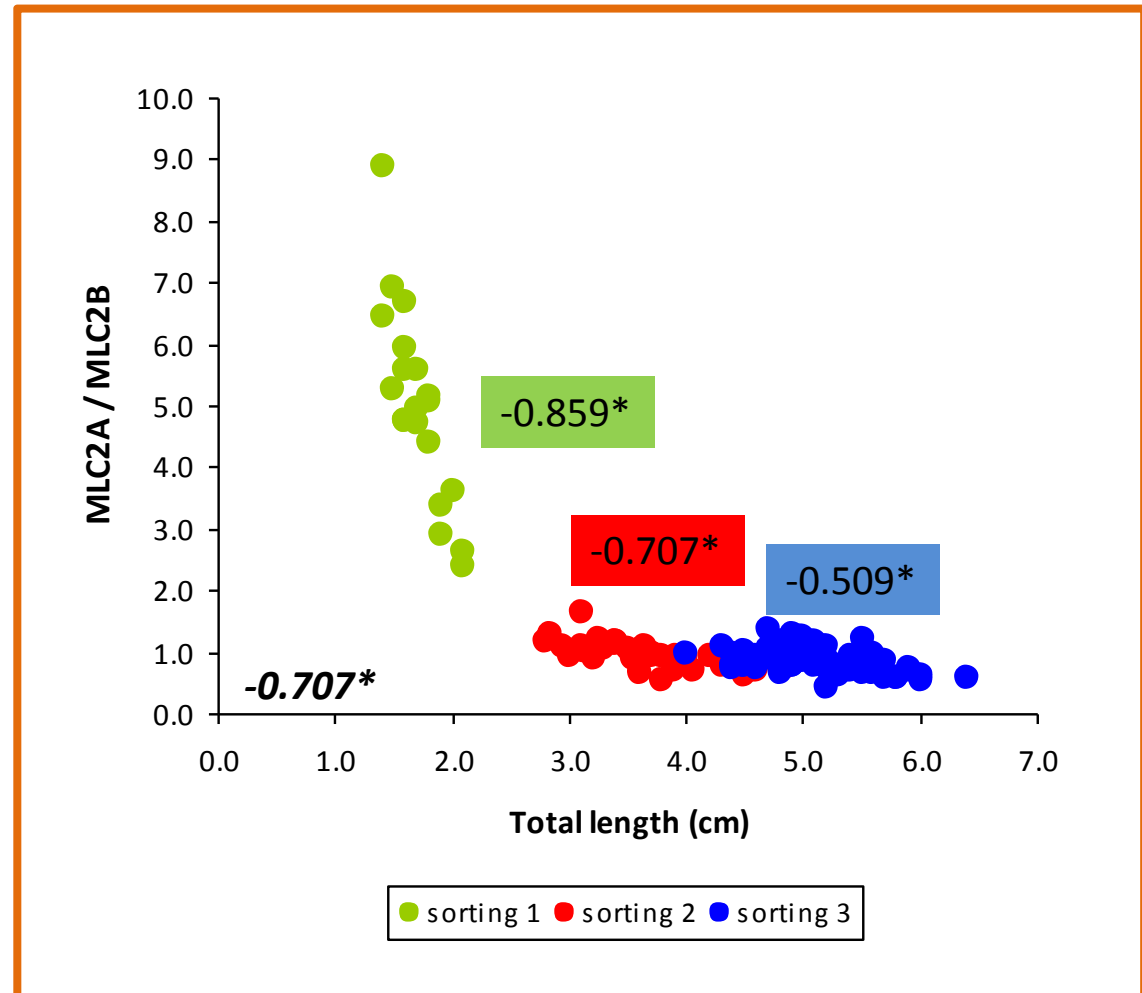
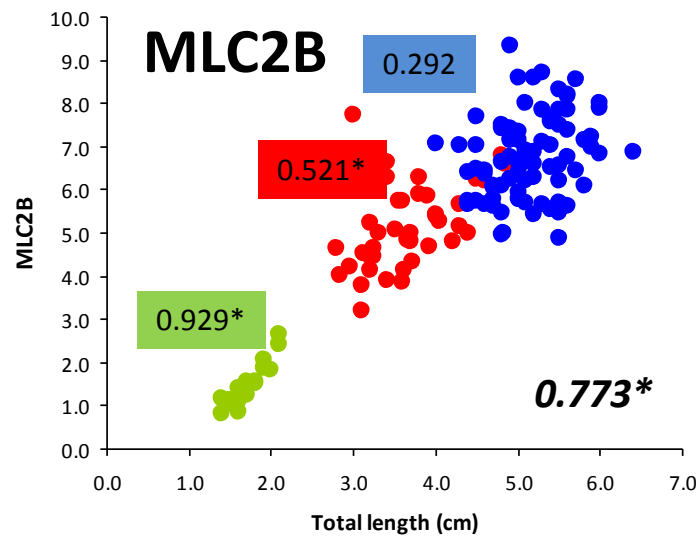
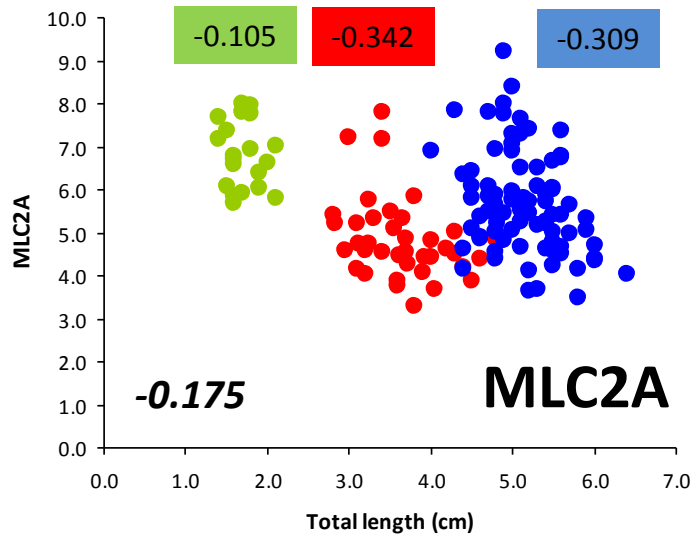
Can myosin light chain 2 isoforms serve as growth markers?

Exp. 1: size-grading



Can myosin light chain 2 isoforms serve as growth markers?

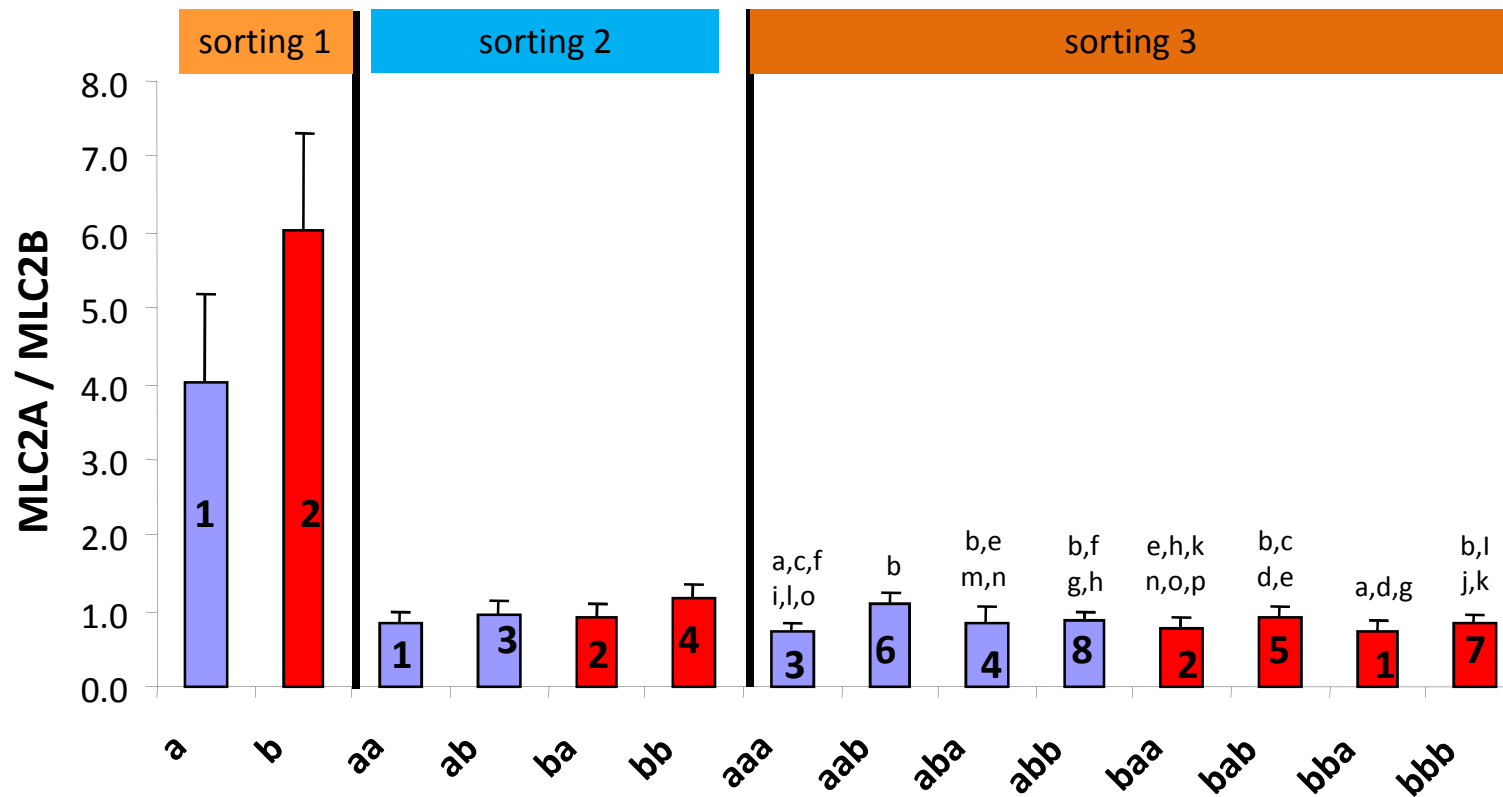
Exp. 1: size-grading



● sorting 1 ● sorting 2 ● sorting 3

Can myosin light chain 2 isoforms serve as growth markers?

Exp. 1: size-grading



*Can myosin light chain 2 isoforms serve as growth markers?
Exp. 1: size-grading*



MLC2A was down-regulated whereas MLC2B was up-regulated
in gilthead sea bream juveniles

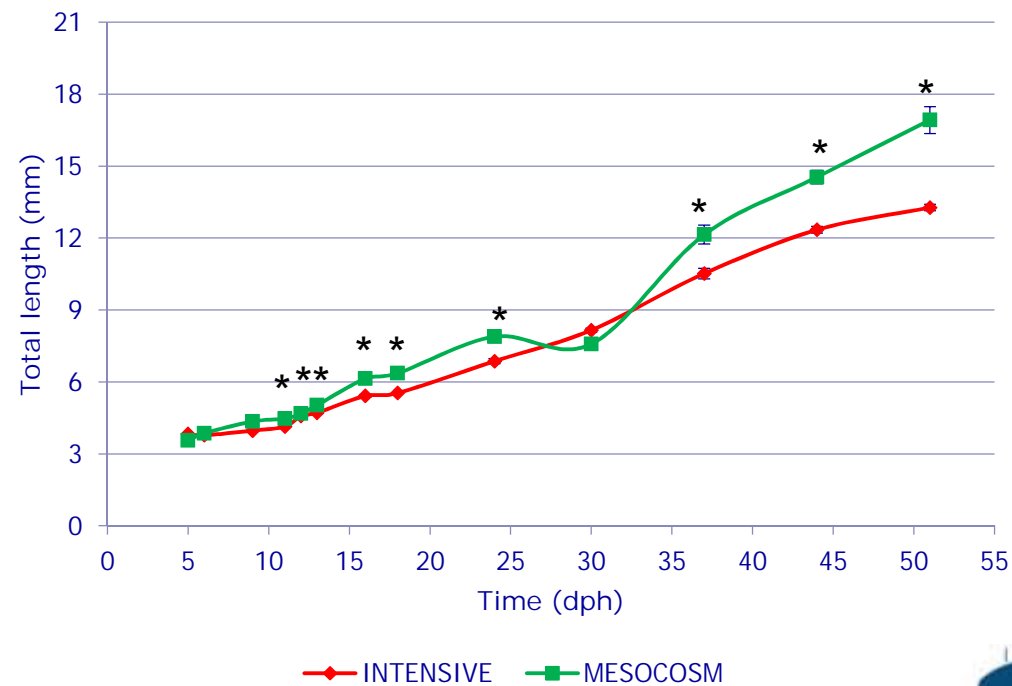
MLC2A/MLC2B expression is highly correlated with axial growth
in gilthead sea bream juveniles

Can myosin light chain 2 isoforms serve as growth markers?

Exp. 2: mesocosm vs intensive

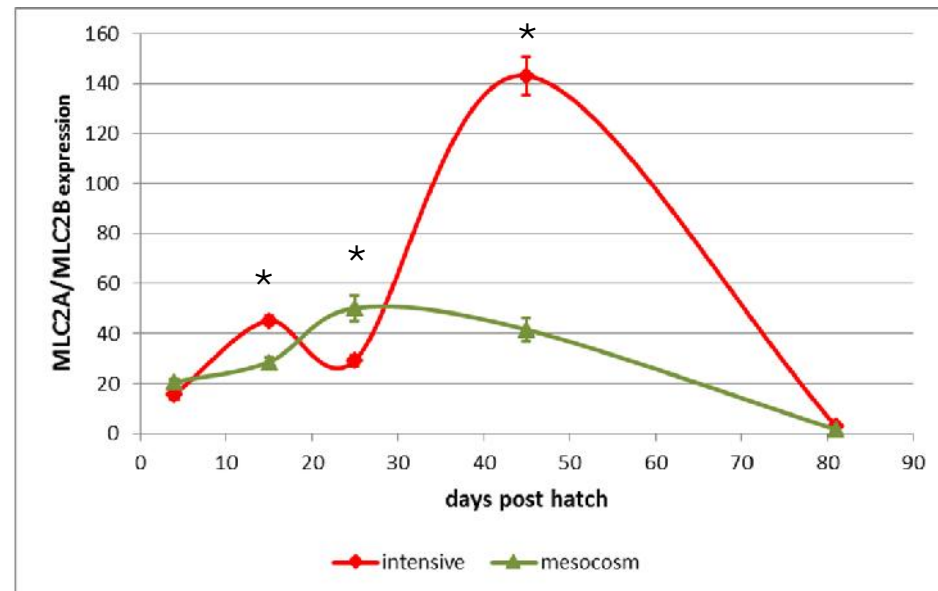
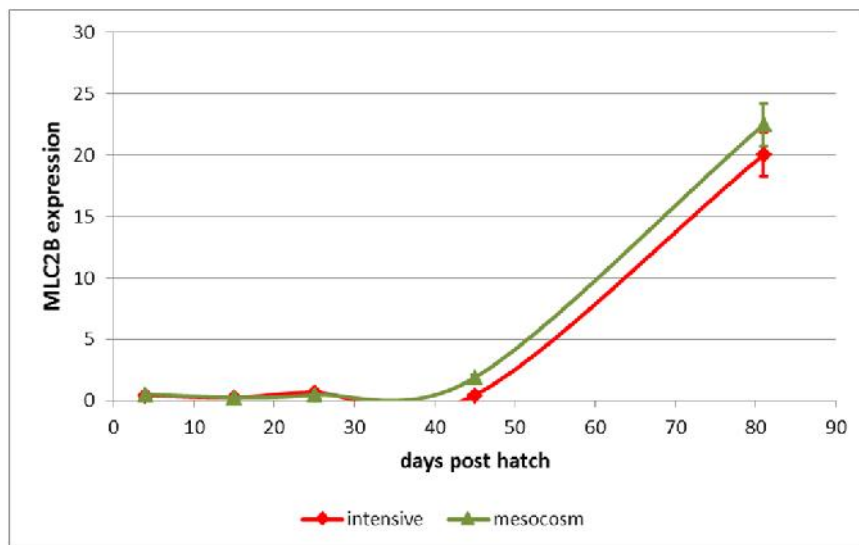
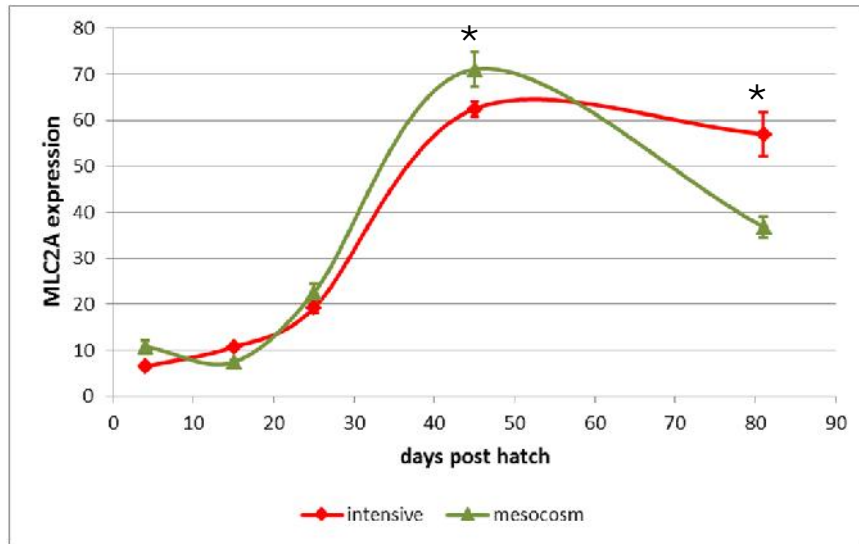


VS



Can myosin light chain 2 isoforms serve as growth markers?

Exp. 2: mesocosm vs intensive



Can myosin light chain 2 isoforms serve as growth markers?

Exp. 2: mesocosm vs intensive



Mesocosm produced larvae of higher growth-performance than the intensive system

Transition from MLC2A to MLC2B at metamorphosis was faster in the mesocosm larvae of higher growth-performance

MLC2A/MLC2B expression is lower in faster growing larvae

Can myosin light chain 2 isoforms serve as growth markers?



MLC2A is replaced by MLC2B at metamorphosis
MLC2B is the dominant isoform in juveniles

The faster transition from MLC2A to MLC2B was associated
with higher growth

MLC2A/MLC2B expression is negatively and significantly
correlated with growth in larvae and juveniles of different
origin and genetic background, reared at different conditions,
different places and at different times

MLC2A/MLC2B exhibited a robust performance and fulfills the
prerequisites to make growth markers

Special Thanks to:

-LarvaNET

-Dimitris Dimopoulos and crew at DIAS Hatchery, Greece

-Pavlos Makridis, Hellenic Centre of Marine Research

Thank you!



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