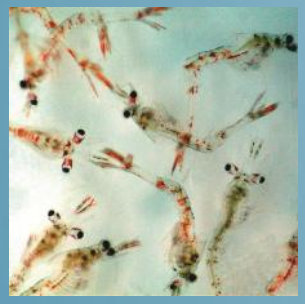
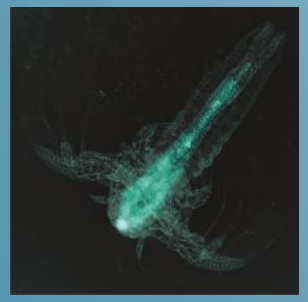
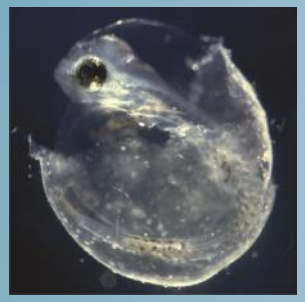
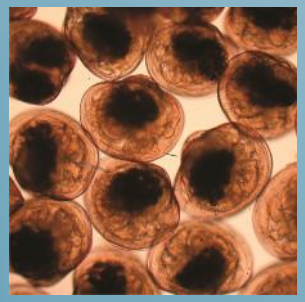




larvi 2013

6th fish & shellfish larviculture symposium

Epigenetic regulation of muscle development and growth in Senegalese sole larvae

Catarina Campos

ghent university, belgium, 2-5 september 2013

EPIGENETIC REGULATION OF MUSCLE DEVELOPMENT AND GROWTH IN SENEGALESE SOLE LARVAE

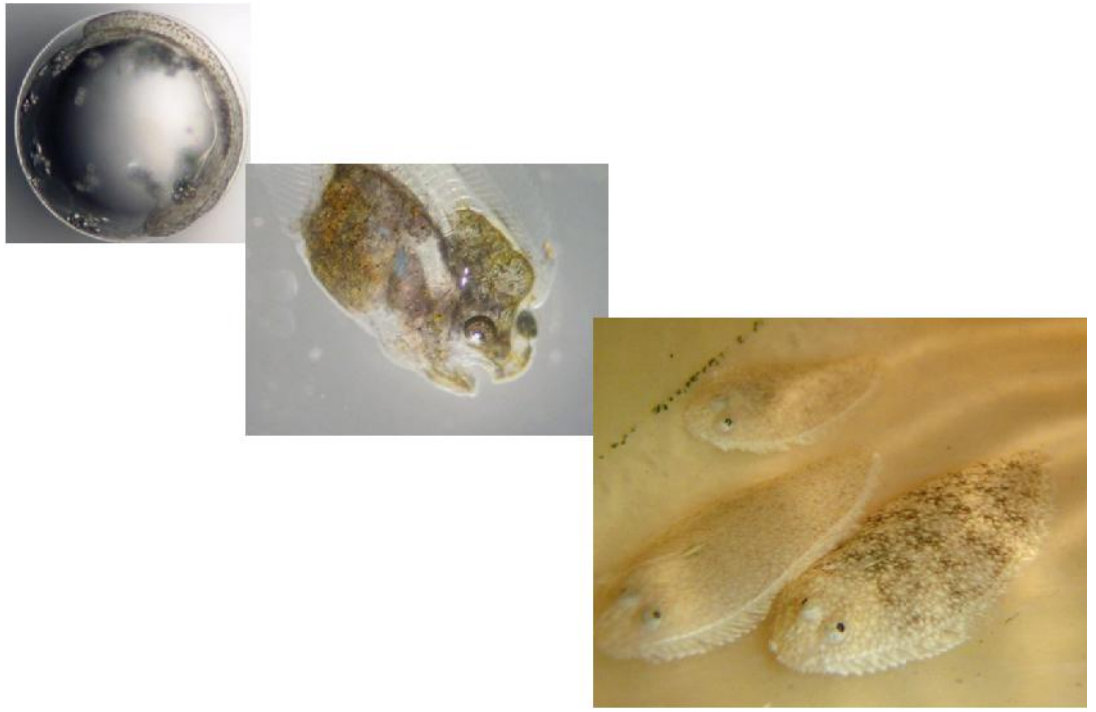
Catarina Campos [a,b,c], Luísa M.P. Valente [a], Luís E.C. Conceição [b,d], Sofia Engrola [b] and Jorge M.O. Fernandes [c]

[a] **CIMAR/CIIMAR & ICBAS**
Centro Interdisciplinar de Investigação Marinha e Ambiental
Univ. Porto,
Porto, Portugal

[b] **CIMAR/CCMAR**
Centro de Ciências do Mar do Algarve,
Univ. Algarve,
Faro, Portugal

[c] **UiN**
Faculty of Biosciences and Aquaculture
University of Nordland,
Bodø, Norway

[d] **SPAROS Lda**,
CRIA - Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.



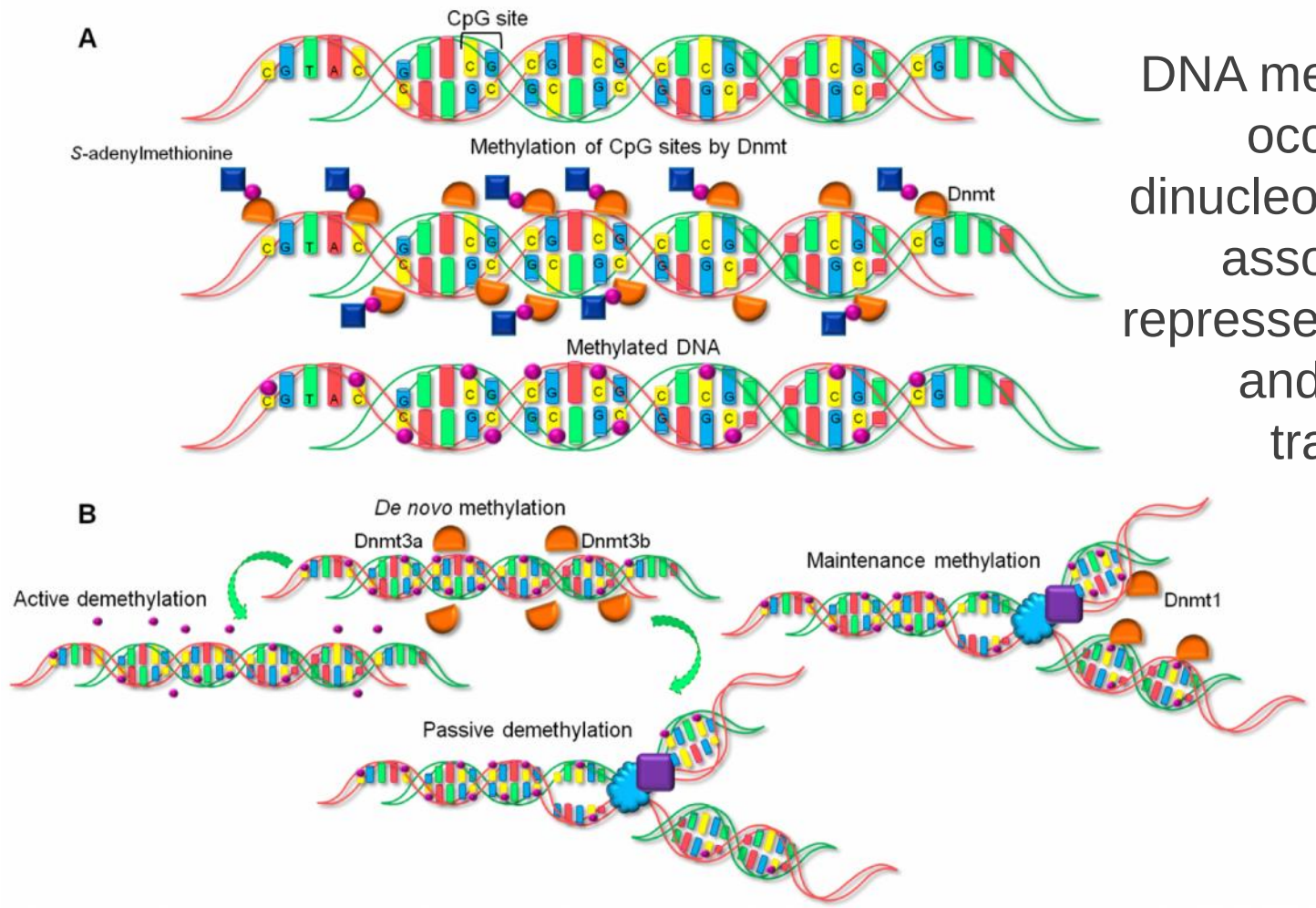
LARVI 2013 – 6th fish & shellfish larviculture symposium

Senegalese sole (*Solea senegalensis*) is a marine flatfish with high potential to the aquaculture industry.

However, great fluctuations of temperature during production are still found, which can contribute to variations on sole's growth and muscle cellularity.

Such thermal plasticity must arise through changes in a multitude of physiological and molecular pathways, in which epigenetic gene regulation plays an essential role → **epigenetics is a bridge between genotype and phenotype**.

DNA methylation

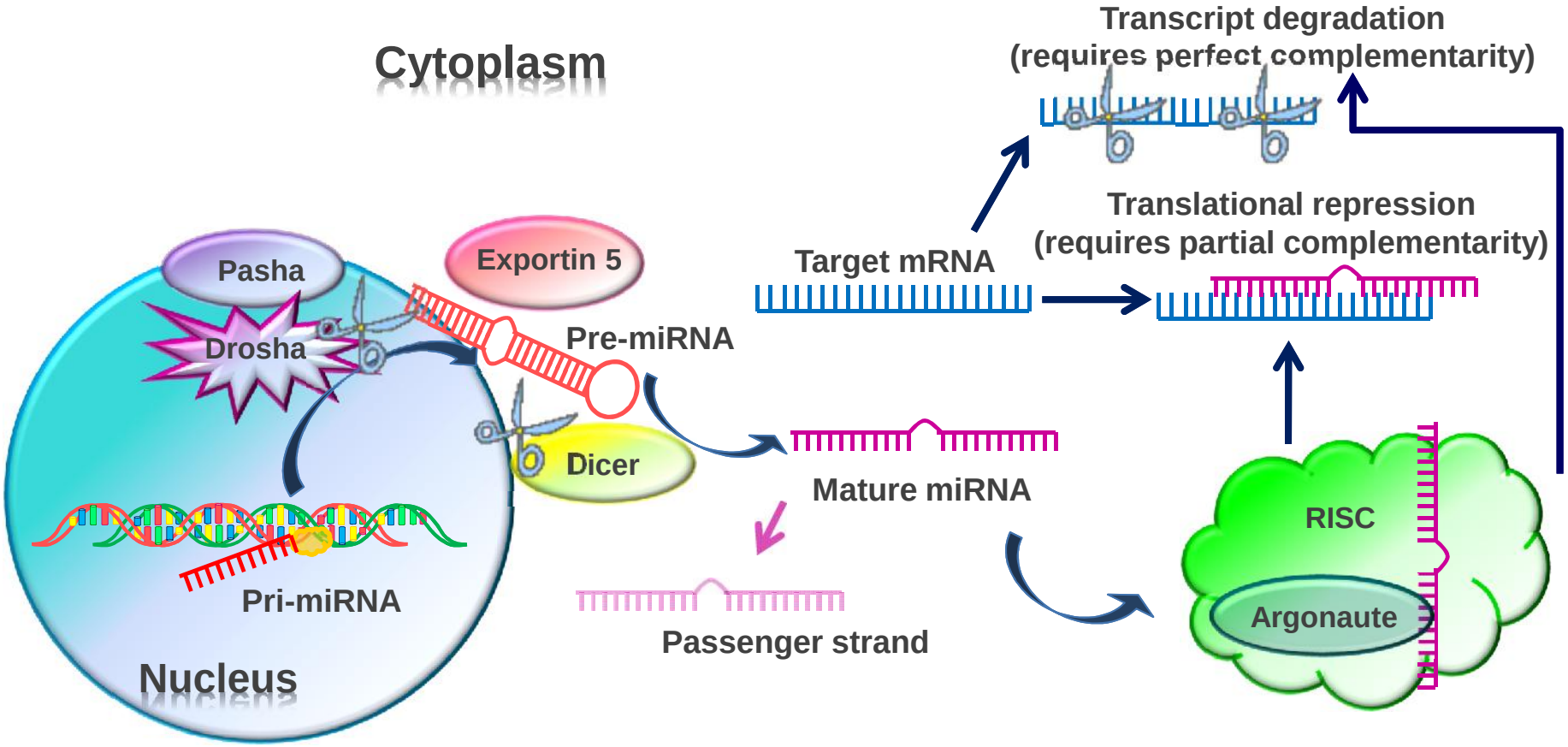


DNA methylation usually occurs on CpG dinucleotides and is often associated with a repressed chromatin state and inhibition of transcription.

The demethylation of regulatory regions in myogenic genes at the beginning of the differentiation program is essential to the commitment of cells towards the muscle lineage.

miRNAs

18–24 nt endogenous non-coding RNAs, that are repressive post-transcriptional regulators of gene expression



Some miRNAs are strongly expressed in muscle and known to interact with the transcriptional networks involved in myogenesis

Experimental approach

Two experiments

Incubation experiment

Three embryonic temperatures:
15, 18 or 21 ºC

One rearing temperature:
21 ºC until 30 dph

Larval rearing experiment

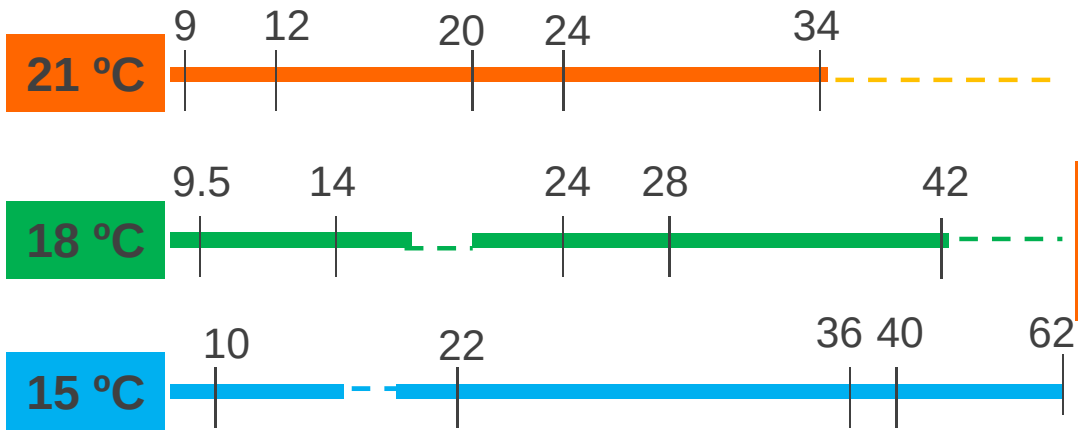
Three rearing temperatures during pelagic phase: 15, 18 or 21 ºC

Transfer to common temperature:
20 ºC until 121 dph

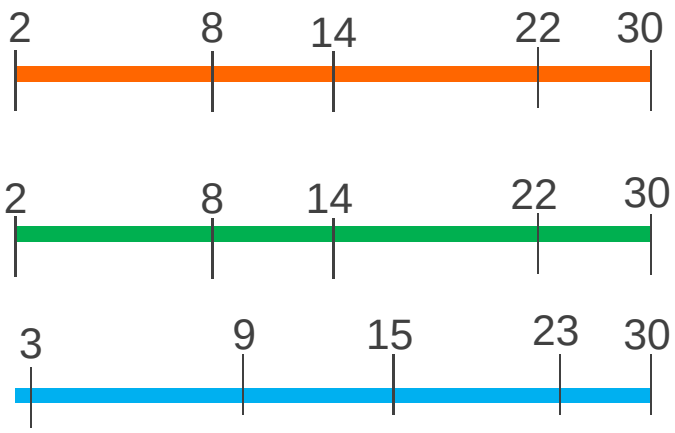
Muscle morphometry, gene expression, DNA methylation levels and protein metabolism were analysed.

Incubation experiment

Hours post-fertilisation



Days post-hatch



Blastula | **Epiboly** | **20 som** | **Final som** | **Hatching** | **Mouth opening** | **Pre-Met** | **Met** | **Post-Met** | **30 dph**

Gene 516 (2013) 209–217

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Incubation temperature induces changes in muscle cellularity and gene expression in Senegalese sole (Solea senegalensis)

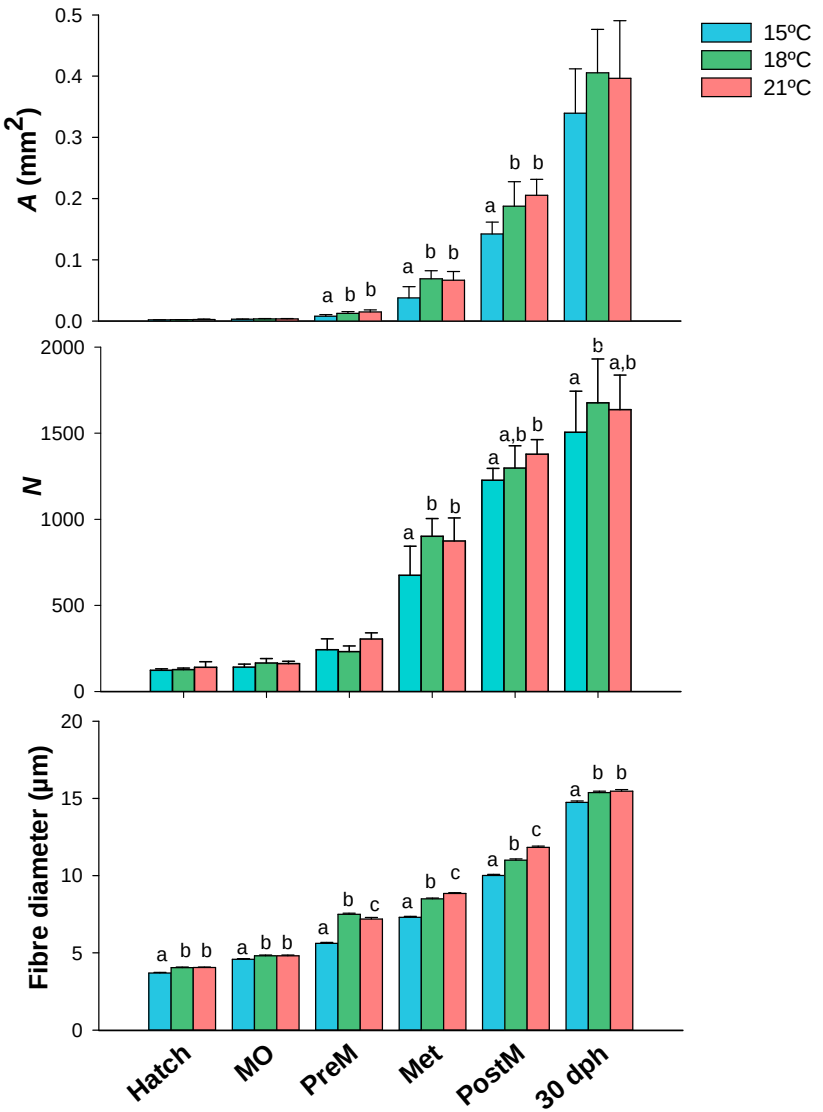
Catarina Campos [a,b,c], Luisa M.P. Valente [a], Luis E.C. Conceição [b], Sofia Engrola [b], Vera Sousa [a], Eduardo Rocha [a], Jorge M.O. Fernandes [c,*]

By 30 dph:

- Weight of 18 and 21 ºC larvae was superior to the 15 ºC ones.

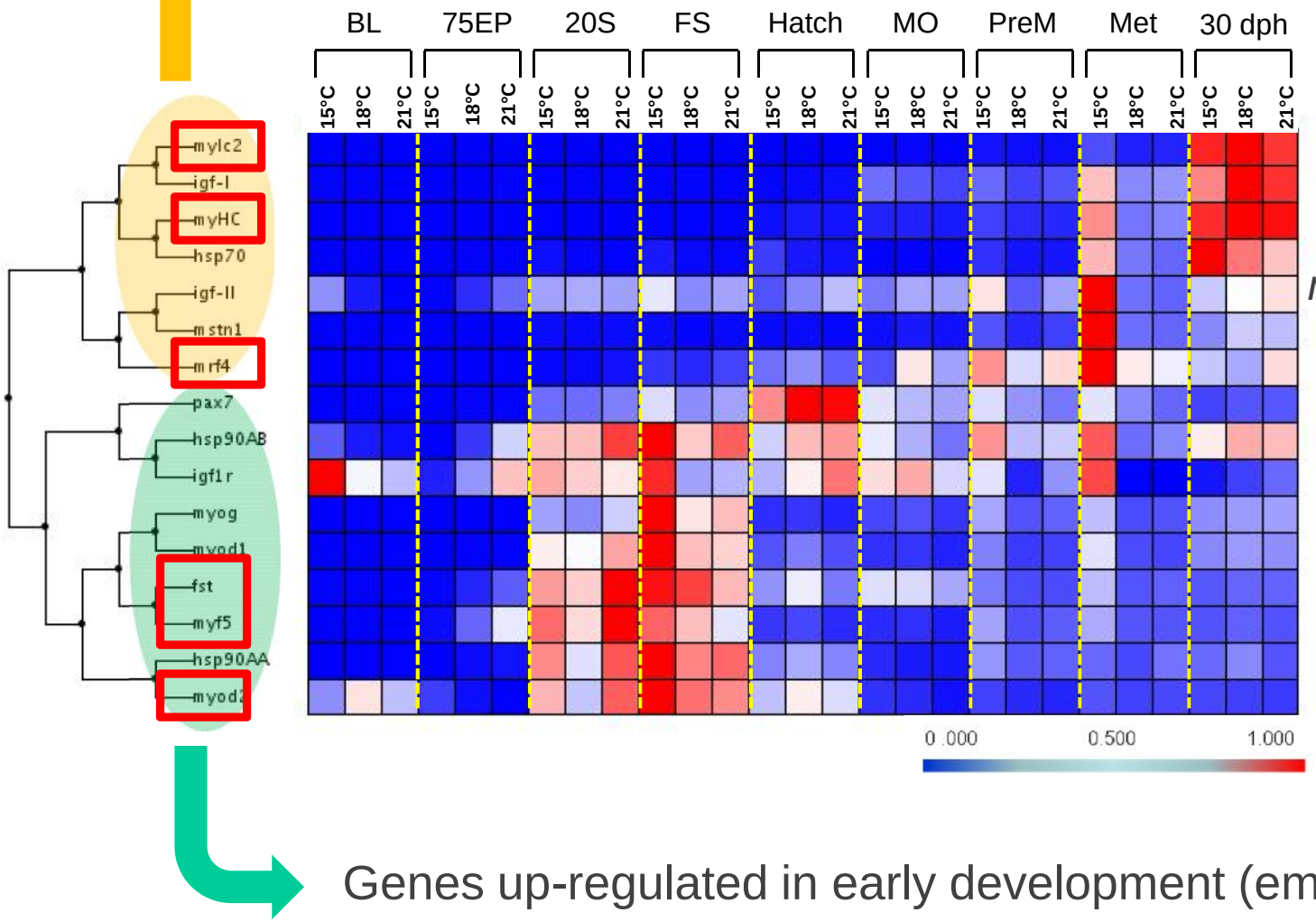
- The 18 ºC group had the highest number of fast fibres, but no differences were found between the 15 and 21 ºC treatments, which instigates the idea that 18 ºC can be an optimal temperature to incubate sole embryos.

- Total muscle area was similar across all treatments, probably due to rearing all larvae at a common temperature.



Transient gene expression during development

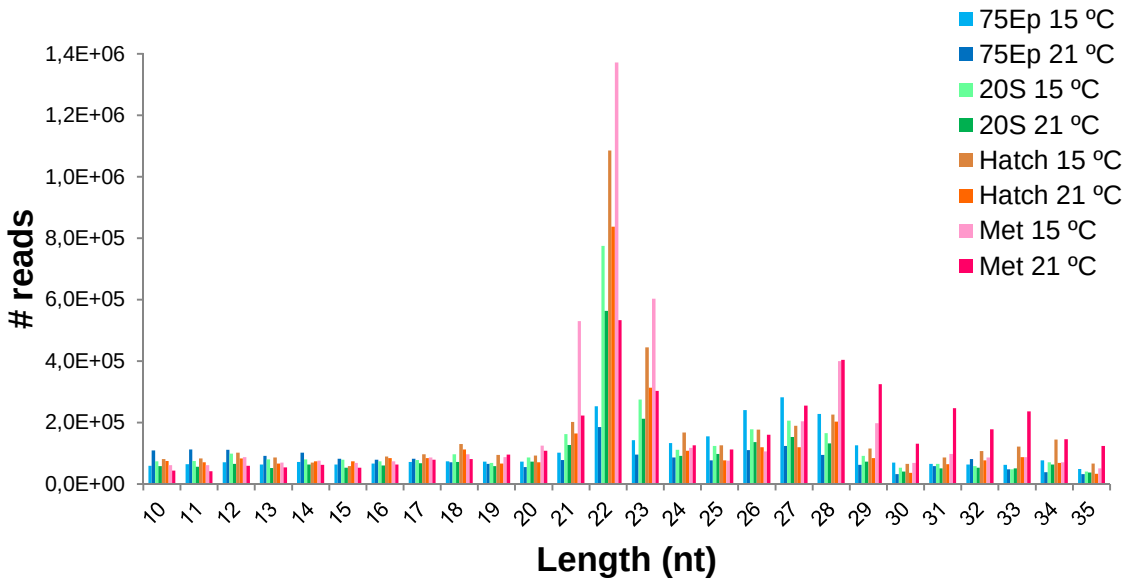
Genes up-regulated later in development (larvae)



myf5, *fst*, *myod2*, *mylc2*, *myHC* and *mrf4* were highest at 18 and/or 21 ºC at specific points, which might have contributed to the general more pronounced growth of these groups

Genes up-regulated in early development (embryo-hatching)

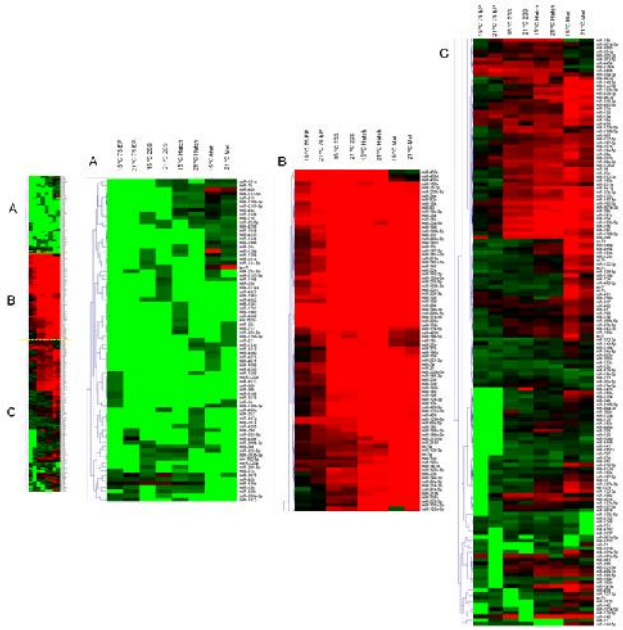
SOLiD sequencing of small RNAs

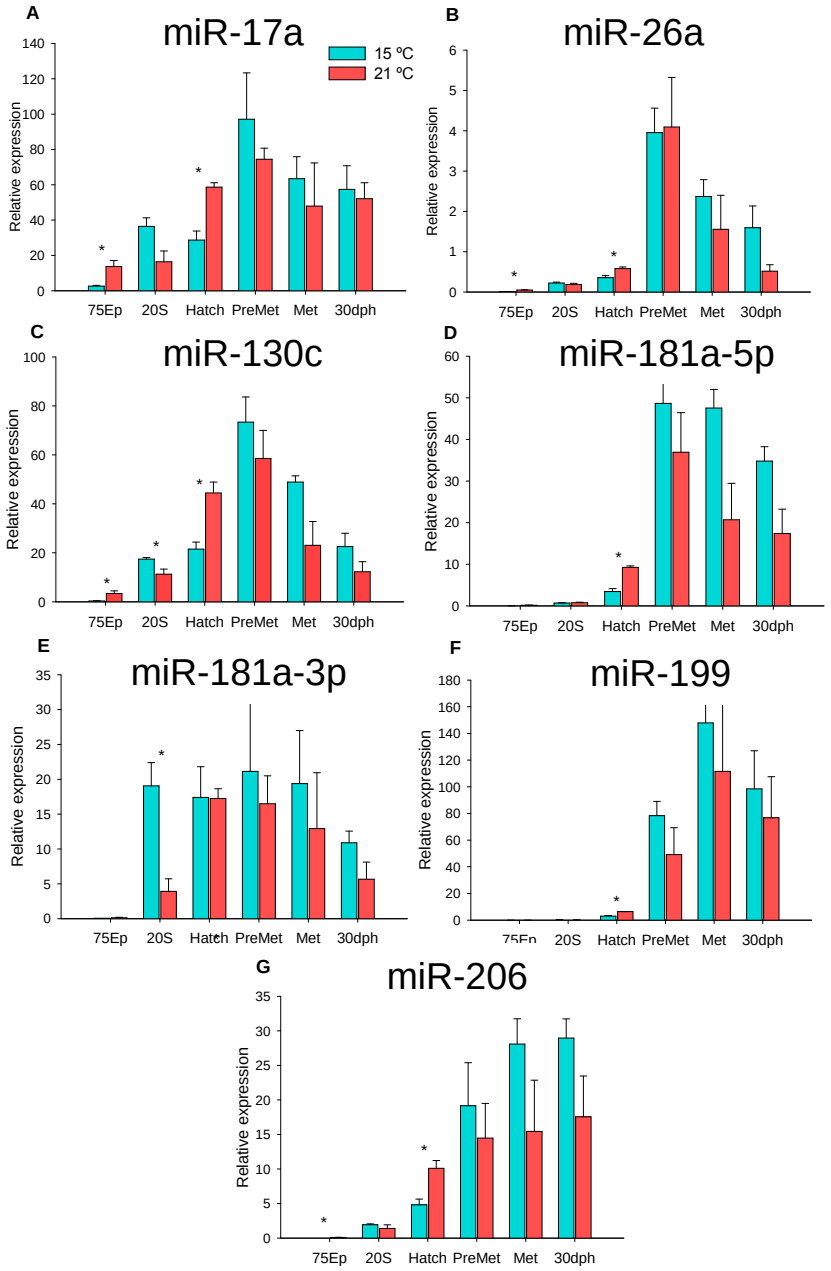


Increasing number and diversity from Epiboly to Metamorphosis

- 320 conserved miRNAs amongst 149 families

Sample	# Total reads	# Trimmed (% Total)	Average length (nt)	# Annotated miRBase (% Total)	# Conserved miRNAs
75Ep 15 ºC	6 642 089	2 802 986 (42.2)	23.5	200 224 (3.0)	232
75Ep 21 ºC	8 842 550	2 169 069 (24.5)	21.1	174 027 (2.0)	231
20S 15 ºC	6 566 636	3 320 266 (50.6)	22.4	993 981 (15.1)	265
20S 21 ºC	6 101 835	2 564 327 (42.0)	22.7	689 954 (11.3)	265
Hatch 15 ºC	7 268 950	4 312 425 (59.3)	23.0	1 467 039 (20.2)	285
Hatch 21 ºC	6 200 277	3 256 433 (52.5)	22.4	1 162 239 (18.7)	281
Met 15 ºC	7 105 697	4 965 849 (69.9)	23.0	2 391 674 (33.7)	288
Met 21 ºC	6 325 932	4 247 722 (67.2)	25.2	804 587 (12.7)	279



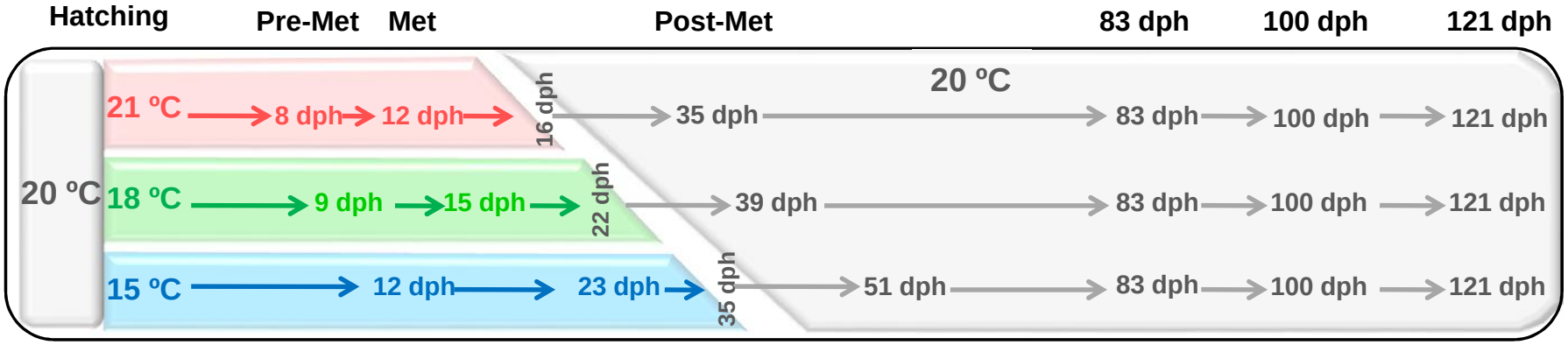


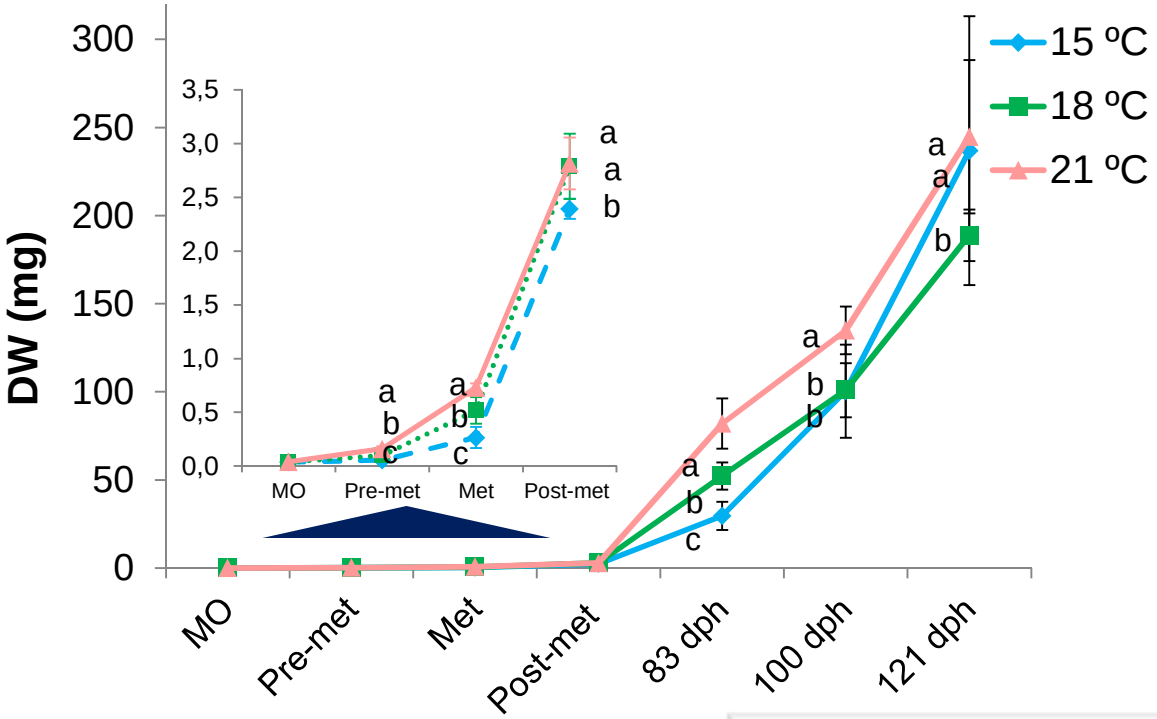
- The very high expression of several miRNAs at a pre-metamorphic stage can be associated with a high growth rate and preparation for the metamorphic process.

- qPCR validation of miR-26a, miR-181a-5p and miR-206 revealed higher expression at 21 ºC than at 15 ºC during embryogenesis and/or at hatching, pointing to a higher activation of the myogenic process at a higher temperature.

Larval rearing experiment

Experimental setup



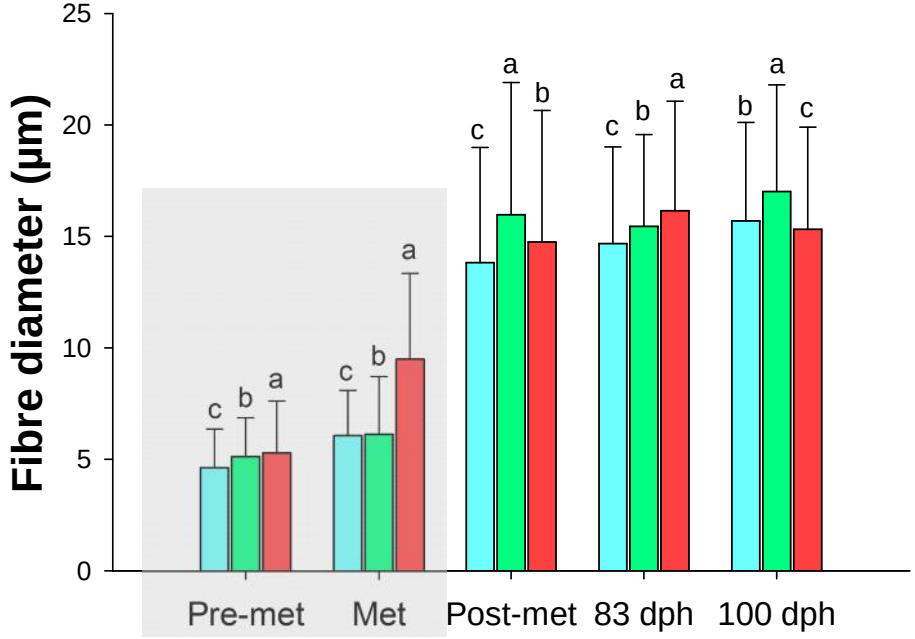


During pelagic phase:

- High mortality and lower growth of the 15 ºC group.

By 121 dph:

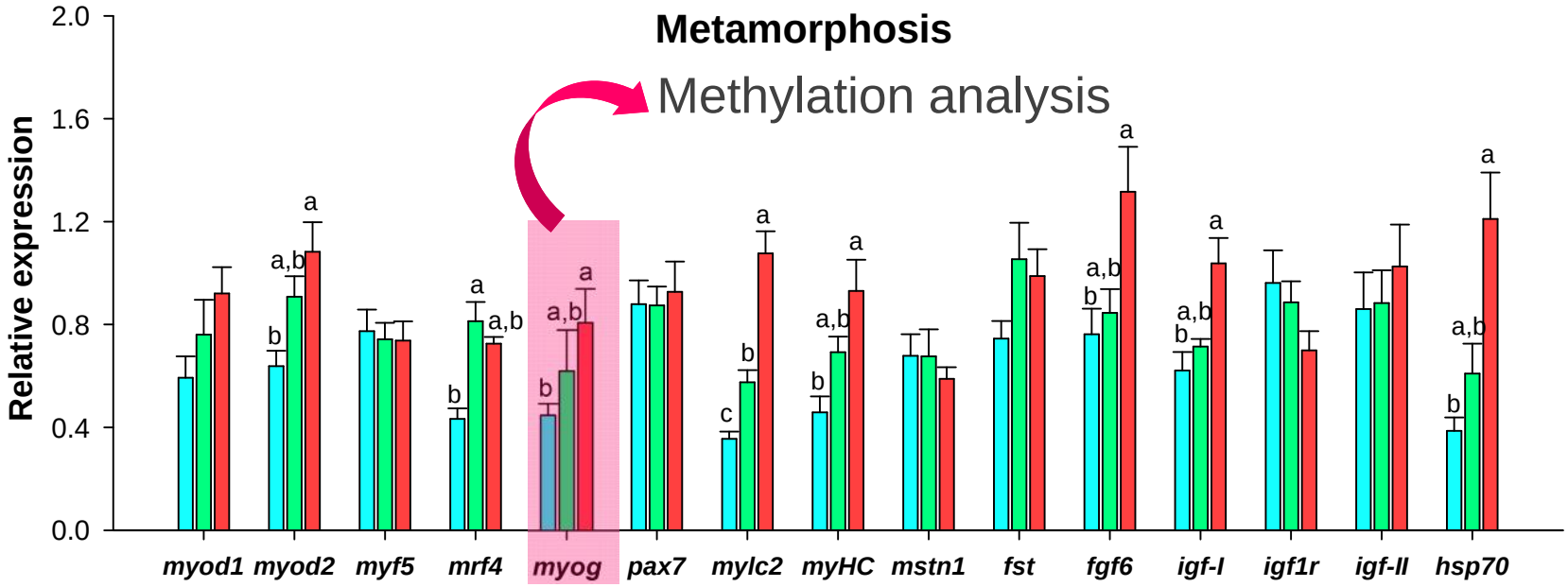
- Weight of juveniles from 15 ºC was similar to 21 ºC, and both were larger than 18 ºC.



During pelagic phase:

- Fibre diameter was larger at 18 and 21 ºC relatively to 15 ºC.

- During pelagic phase, the majority of *MRFs* as well as *myosins*, *fgf6* and *igf-I* were up-regulated at 18 ºC and/or 21 ºC relatively to 15 ºC.



RESEARCH PAPER

Epigenetics 8:4, 1–9; April 2013; © 2013 Landes Bioscience

Temperature affects methylation of the *myogenin* putative promoter, its expression and muscle cellularity in Senegalese sole larvae

Catarina Campos,[1,2,3] Luísa M.P. Valente,[1] Luís E.C. Conceição,[2] Sofia Engrola[2] and Jorge M.O. Fernandes[3,4]

[1]CIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental and ICBAS - Instituto de Ciências Biomédicas de Abel Salazar; Universidade do Porto; Porto, Portugal; [2]CCMAR/CIMAR, Centro de Ciências do Mar; Universidade do Algarve; Campus de Gambelas; Faro, Portugal; [3]Faculty of Biosciences and Aquaculture; University of Nordland; Bodø, Norway

Keywords: *Solea senegalensis*, thermal plasticity, *myogenin*, methylation, epigenetic regulation, myogenesis

Senegalese sole *myogenin* promoter:

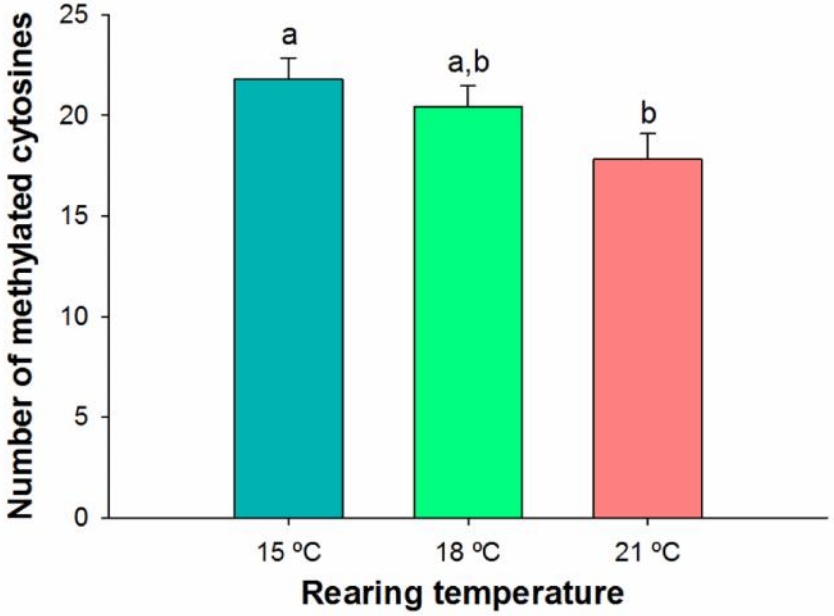
- Highly conserved amongst teleost species.

- Can DNA methylation of *myog* promoter in sole muscle be affected by rearing temperature?

- Higher methylation levels of *myog* at 15 ºC

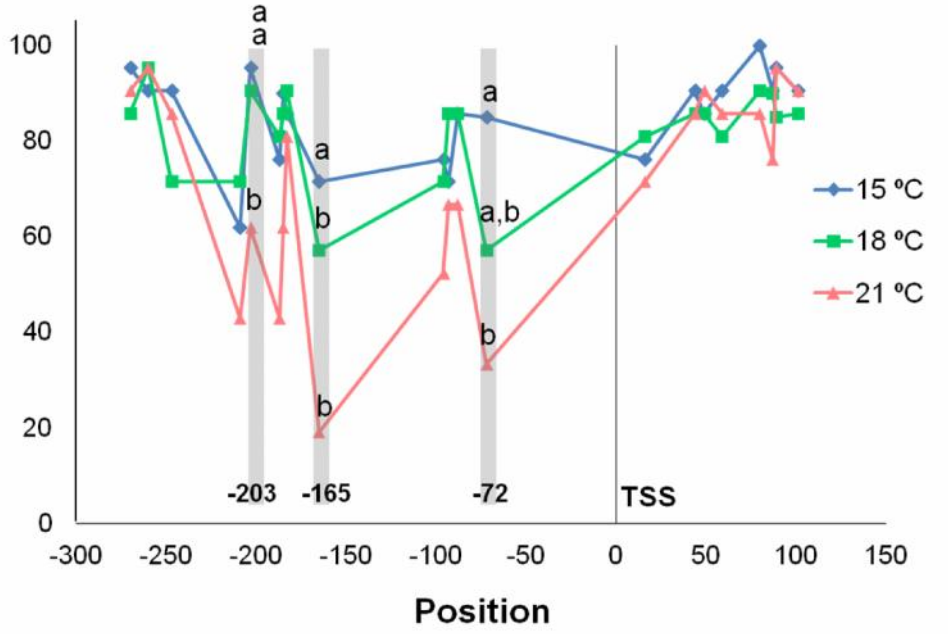
A

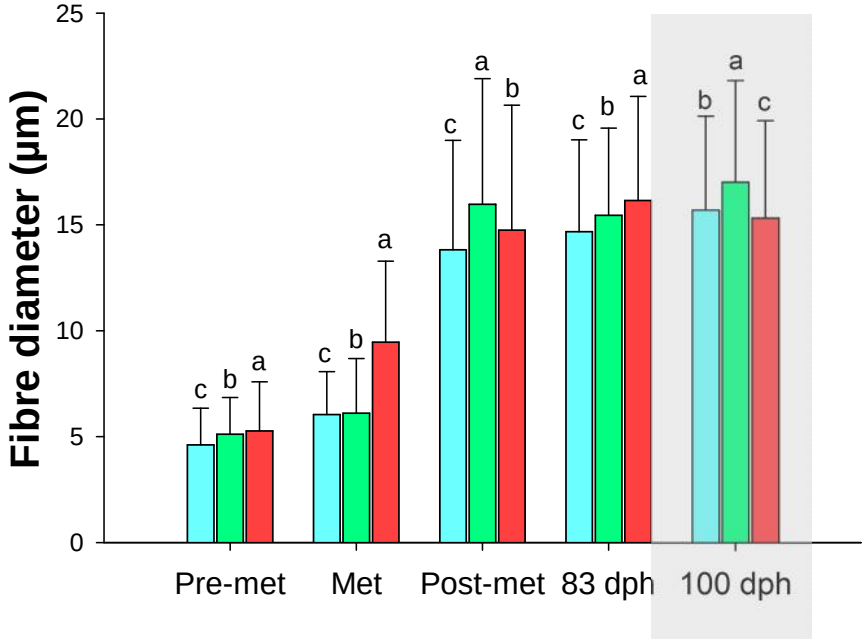
Total number of methylated cytosines



B

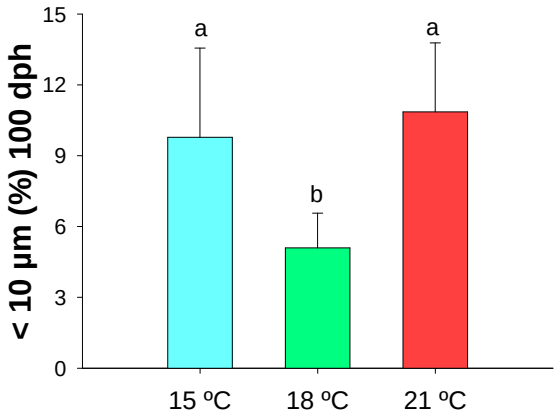
Methylation at CpG sites

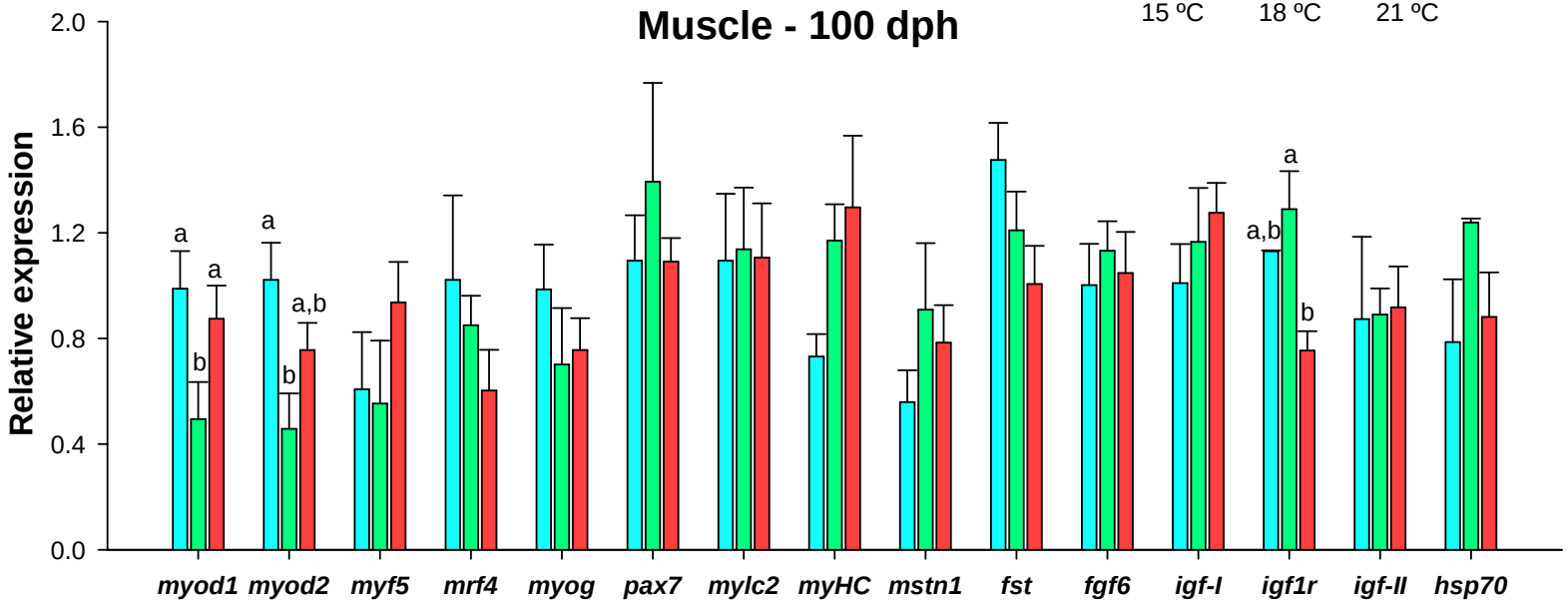




By 100 dph:

- Increased hypertrophy of muscle fibres in the18 ºC group, which might induce a lower growth capacity.



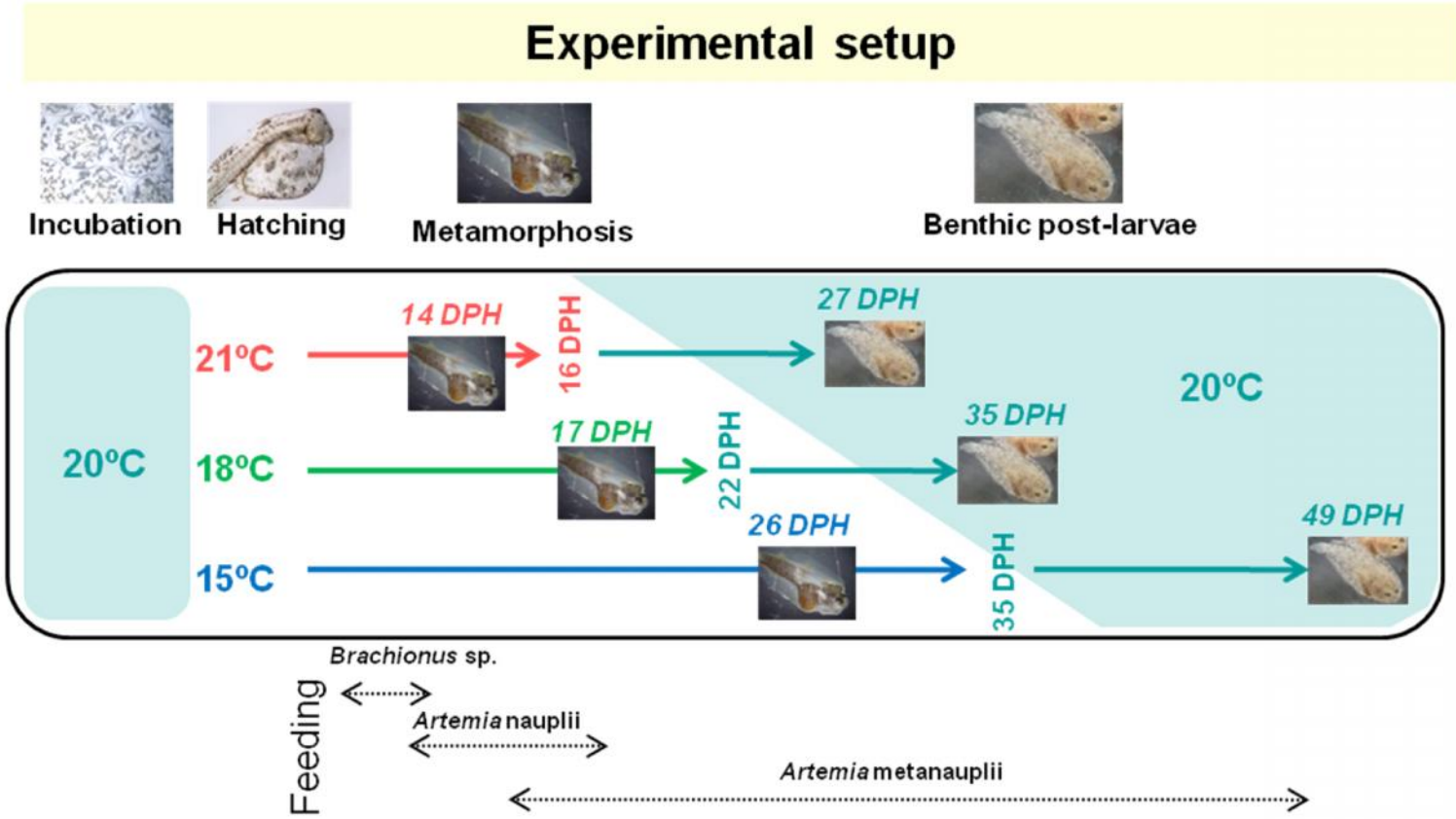


Fish Physiol Biochem
DOI 10.1007/s10695-013-9802-x

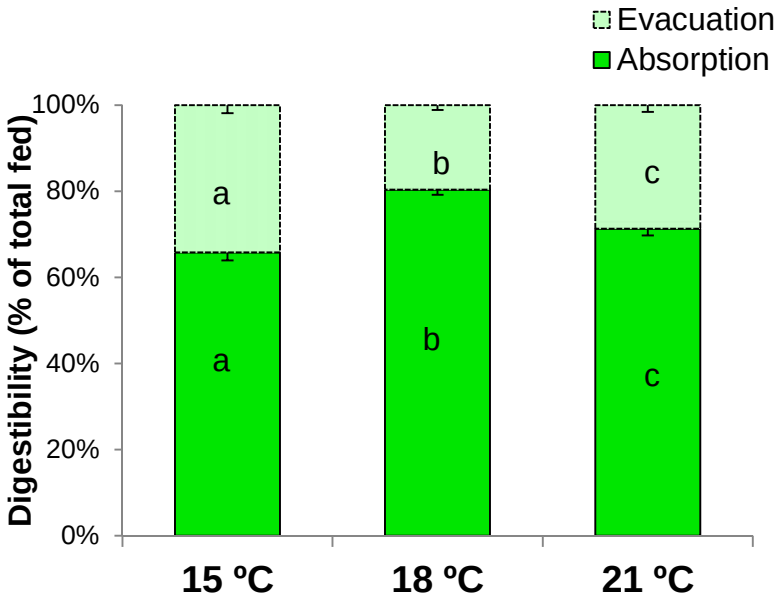
Rearing temperature affects Senegalese sole (*Solea senegalensis*) larvae protein metabolic capacity

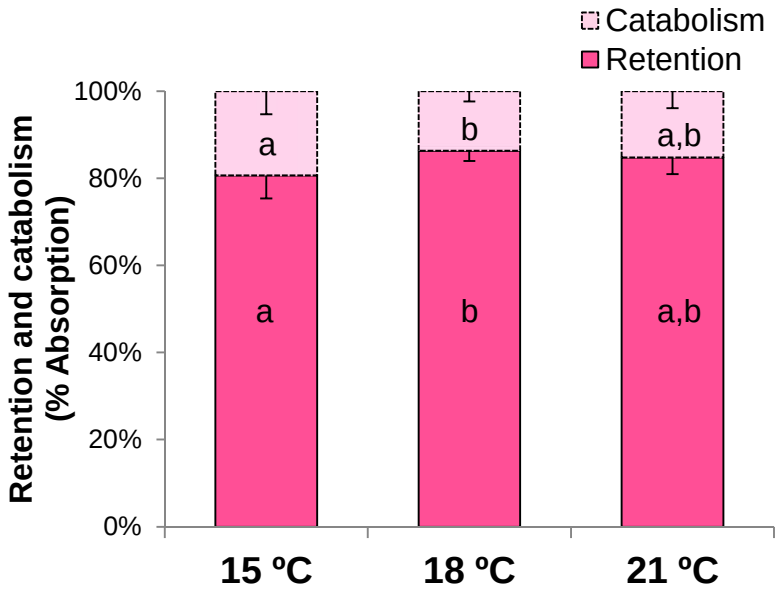
Catarina Campos · M. Filipa Castanheira · Sofia Engrola · Luísa M. P. Valente · Jorge M. O. Fernandes · Luís E. C. Conceição

Artemia radiolabelling feeding trials:
metamorphosis and benthic post-larvae.

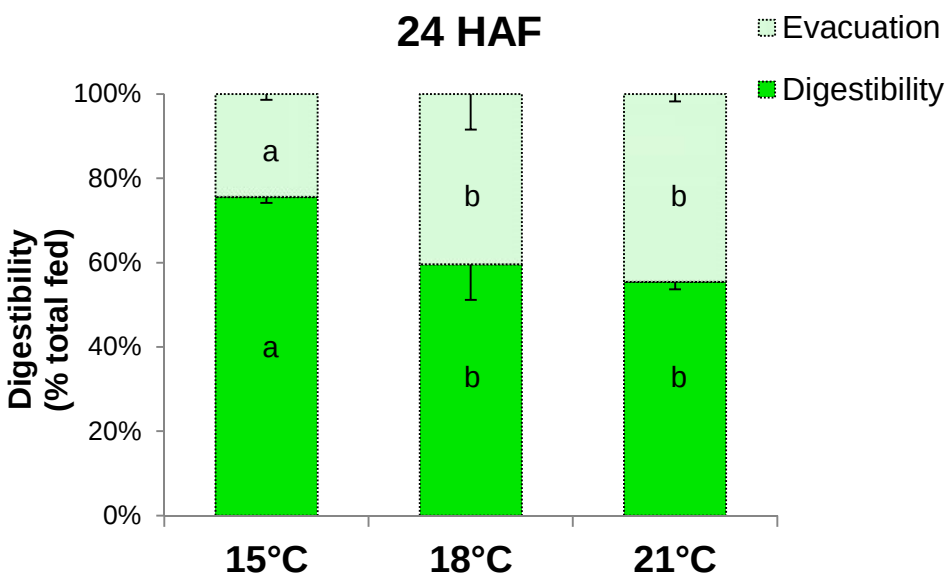


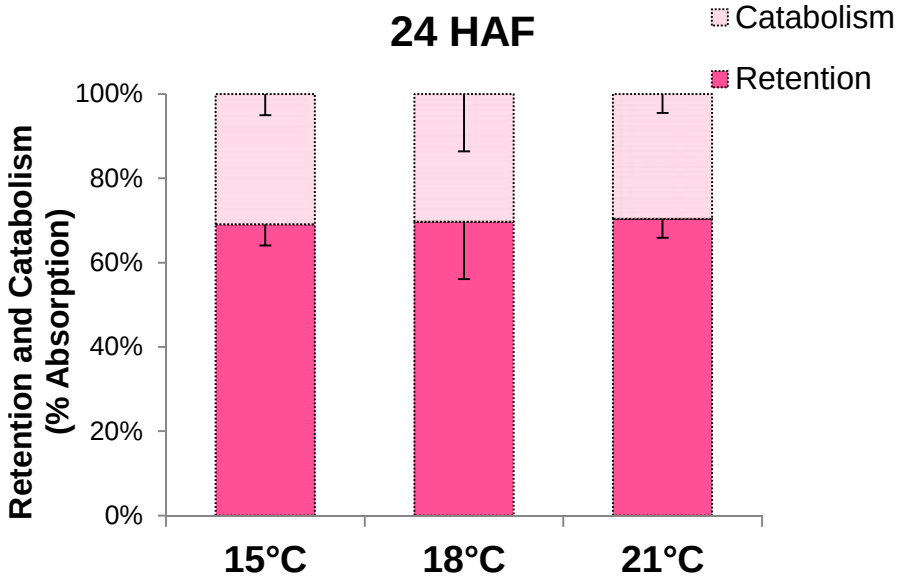
• Protein digestibility and retention is affected by rearing temperature during metamorphosis – lowest at 15 ºC.





• After transfer to a common temperature, post-larvae from 15 ºC showed the highest protein digestibility.





Conclusions

• Senegalese sole pelagic larvae → more susceptible to 15 ºC than embryos.

• Incubation at 18 ºC + larval rearing at 21 ºC → promoted larval muscle hyperplastic growth, which can have positive implications on muscle growth potential.

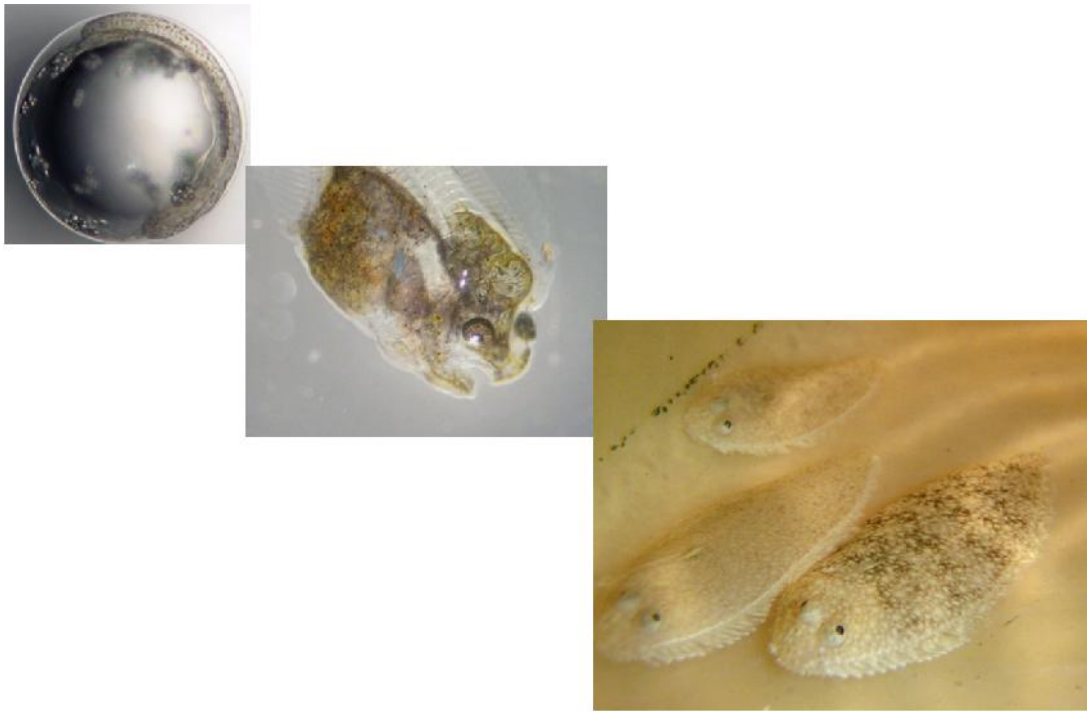
• Thermal plasticity of myogenic genes and miRNAs may be responsible for the observed growth effects during the incubation experiment.

• Rearing pelagic larvae at 15 ºC → greatly decreased growth and survival, decreased protein absorption and retention and increased DNA methylation levels of *myog* promoter in skeletal muscle → however, a mechanism of compensatory growth was later observed in the 15 ºC juveniles.

• Rearing pelagic larvae at 21 ºC → promoted a good growth and survival and therefore is appropriate for Senegalese sole pelagic larvae.

Take home message

• Water temperature during early ontogeny has major effects on Senegalese sole growth potential, both in short and long-term, with implications on muscle cellularity, gene expression, gene regulation and protein utilisation.



Acknowledgements

FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

SFRH / BD / 43633 / 2008

Larva NET

COST Action FA0801

João Sendão
Maria Filipa Castanheira
Paulo Gavaia
Helena Teixeira
Filipa Rocha
Vera Rodrigues
Elsa Cabrita
André Santos

Alessia Giannetto
Arvind Sundaram
Teshome Bizuayehu