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 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 °C

One rearing temperature: 21 °C until 30 dph

Larval rearing experiment

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

Transfer to common temperature: 20 °C until 121 dph

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

**Hours post-fertilisation**

- **21 °C**
  - 9, 12, 20, 24, 34
- **18 °C**
  - 9.5, 14, 24, 28, 42
- **15 °C**
  - 10, 22, 36, 40, 62

**Days post-hatch**

- **21 °C**
  - 2, 8, 14, 22, 30
- **18 °C**
  - 2, 8, 14, 22, 30
- **15 °C**
  - 3, 9, 15, 23, 30

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

LARVI 2013 – 6th fish & shellfish larviculture symposium
By 30 dph:

• Weight of 18 and 21 °C larvae was superior to the 15 °C ones.

• The 18 °C group had the highest number of fast fibres, but no differences were found between the 15 and 21 °C treatments, which instigates the idea that 18 °C 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 °C 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

![Length (nt) vs # reads](image)

- 320 conserved miRNAs amongst 149 families

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

Increasing number and diversity from Epiboly to Metamorphosis

LARVI 2013 – 6th fish & shellfish larviculture symposium
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 °C than at 15 °C during embryogenesis and/or at hatching, pointing to a higher activation of the myogenic process at a higher temperature.
Larval rearing experiment

During pelagic phase:

- High mortality and lower growth of the 15 °C group.

By 121 dph:

- Weight of juveniles from 15 °C was similar to 21 °C, and both were larger than 18 °C.
During pelagic phase:

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

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

---

**Total number of methylated cytosines**

![Graph showing total number of methylated cytosines](image)

**Methylation at CpG sites**

![Graph showing methylation at CpG sites](image)
By 100 dph:
• Increased hypertrophy of muscle fibres in the 18 °C group, which might induce a lower growth capacity.

LARVI 2013 – 6th fish & shellfish larviculture symposium
Rearing temperature affects Senegalese sole (*Solea senegalensis*) larvae protein metabolic capacity

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

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

**Experimental setup**

- Incubation
- Hatching
- Metamorphosis
- Benthic post-larvae

Temperature Ranges:
- 20°C
- 18°C
- 15°C

Feeding:
- *Brachionus* sp.
- *Artemia* nauplii
- *Artemia* metanauplii
• Protein digestibility and retention is affected by rearing temperature during metamorphosis – lowest at 15 °C.
After transfer to a common temperature, post-larvae from 15 °C showed the highest protein digestibility.
Conclusions

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

• Incubation at 18 °C + larval rearing at 21 °C → 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 °C → 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 °C juveniles.

• Rearing pelagic larvae at 21 °C → 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

COST Action FA0801

FCT

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

Alessia Giannetto
Arvind Sundaram
Teshome Bizuayehu

LARVI 2013 – 6th fish & shellfish larviculture symposium