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Genome-wide gene expression analysis during solea sp. embryo-larval development

Xavier Cousin, M. Gonzalo Claros, David Mazurais, Rocío Bautista, Hicham Benzekri, Marie-Laure Bégout, Marian Ponce, Paula Armesto, Jose Luis Zambonino, Josep V Planas, et al.

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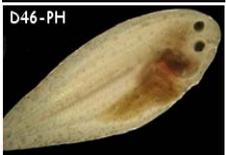
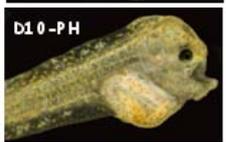
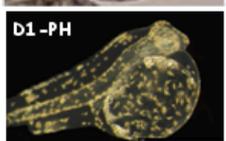
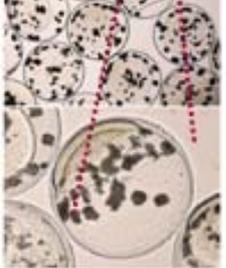
Submitted on 3 Jun 2020

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GENOME-WIDE GENE EXPRESSION ANALYSIS DURING *SOLEA SP.* EMBRYO-LARVAL DEVELOPMENT

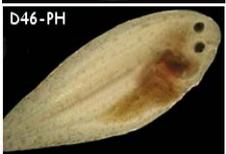
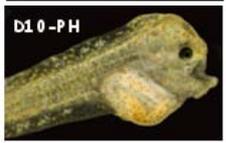
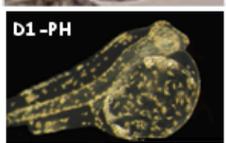
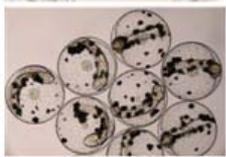
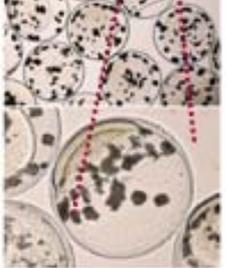
X. Cousin, M.G. Claros, D. Mazurais, R. Bautista, H. Benzekri, M.-L. Bégout, M. Ponce, P. Armesto, J. Zambonino, J.V. Planas and M. Manchado



AQUAGENET network

Ifremer

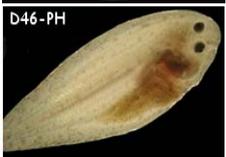
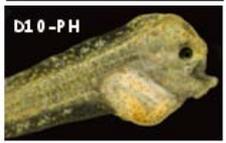
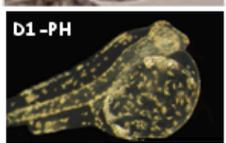
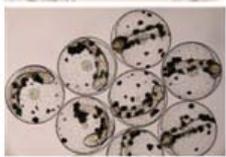
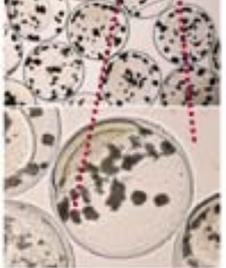
- AQUAGENET is an Interreg IVB funded project
 - IFAPA El Toruño – Manuel Manchado (coordination)
 - Ifremer – Genetics and Pathology Lab (La Tremblade) and Fisheries Lab (La Rochelle)
 - Universidad de Barcelona – Physiology Department
 - CNRS – Evolution sciences Institute (ISEM) UMR 5554 – Sète
 - Universidad de Cadiz – Biomedicine and public health Department
 - IPIMAR – Aquaculture Unit – Olhaõ
- Goal: Set-up of a transnational network aiming at the development of biotechnologies in aquaculture in SUDOE – South Western Europe - area
 - Fish – *Solea senegalensis* and *Solea solea*
 - Bivalves – Oysters, mussel and clam
 - Pathogens of these species



Solea sp. in aquaculture

Ifremer

- *Solea sp.* are high value species in fisheries and aquaculture. *Solea sp.*, in particular *Solea senegalensis*, aquaculture is increasing thanks to advances in husbandry, in particular larval breeding. However production remains low (200+40 MT/y)
- As flatfish *Solea sp.* larvae experiment a dramatic tissue remodeling during metamorphosis
- Impaired metamorphosis could lead to a loss of individual fitness as well as a decrease in commercial value
- Urgent need of tools which could support rise of *Solea sp.* aquaculture and in particular of larval development – understand underlying mechanisms
- At the beginning of the project some data were available for *S. senegalensis* (Cerdeira *et al.*, 2008) and almost nothing for *S. solea*
- Since the start of the project, a publication reported the identification of 22 252 sequences in *S. solea* of which 16 731 were annotated (Ferraresso *et al.*, 2013)

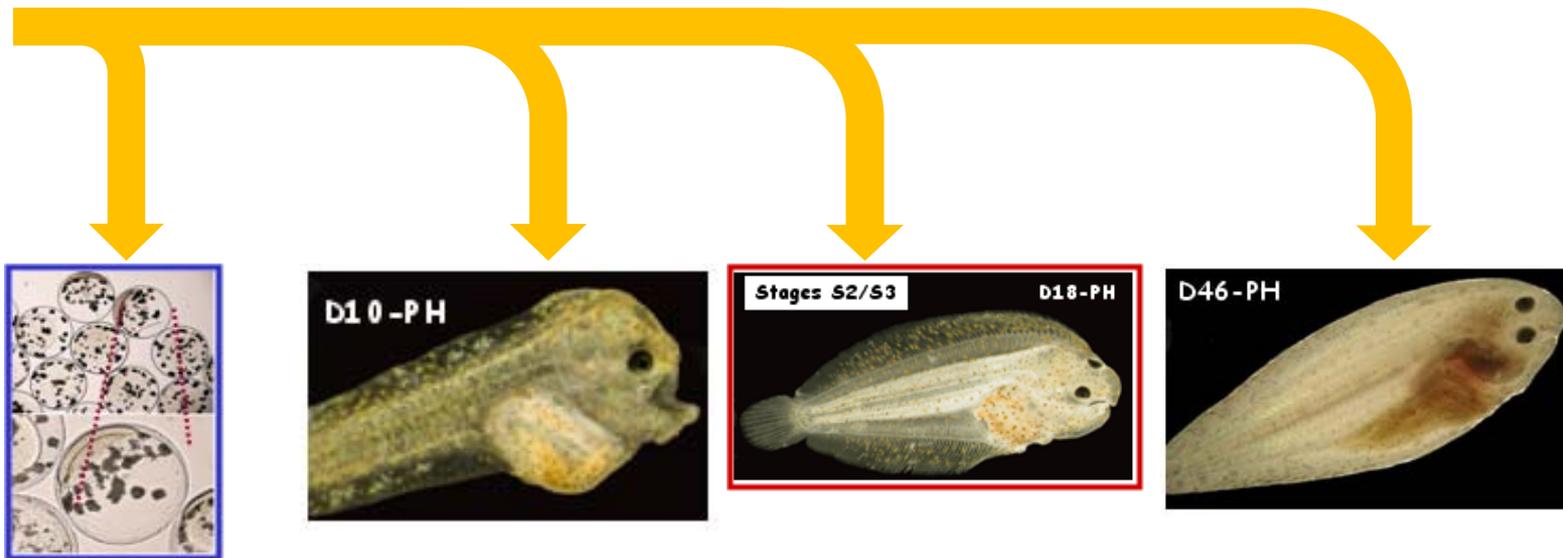


Experimental design

- A similar design was used for sampling in both species and includes embryonic, pre-metamorphic, metamorphic and post-metamorphic stages providing a temporal series.

Hatching

Metamorphosis



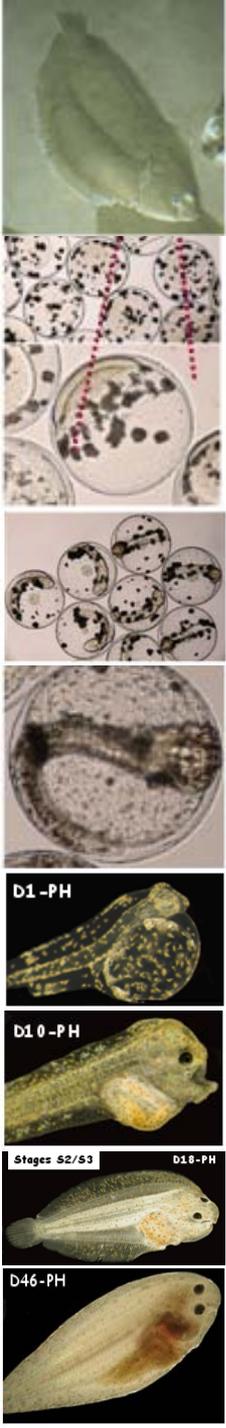
Stages 7/8

D10-PH

Stages S2/S3

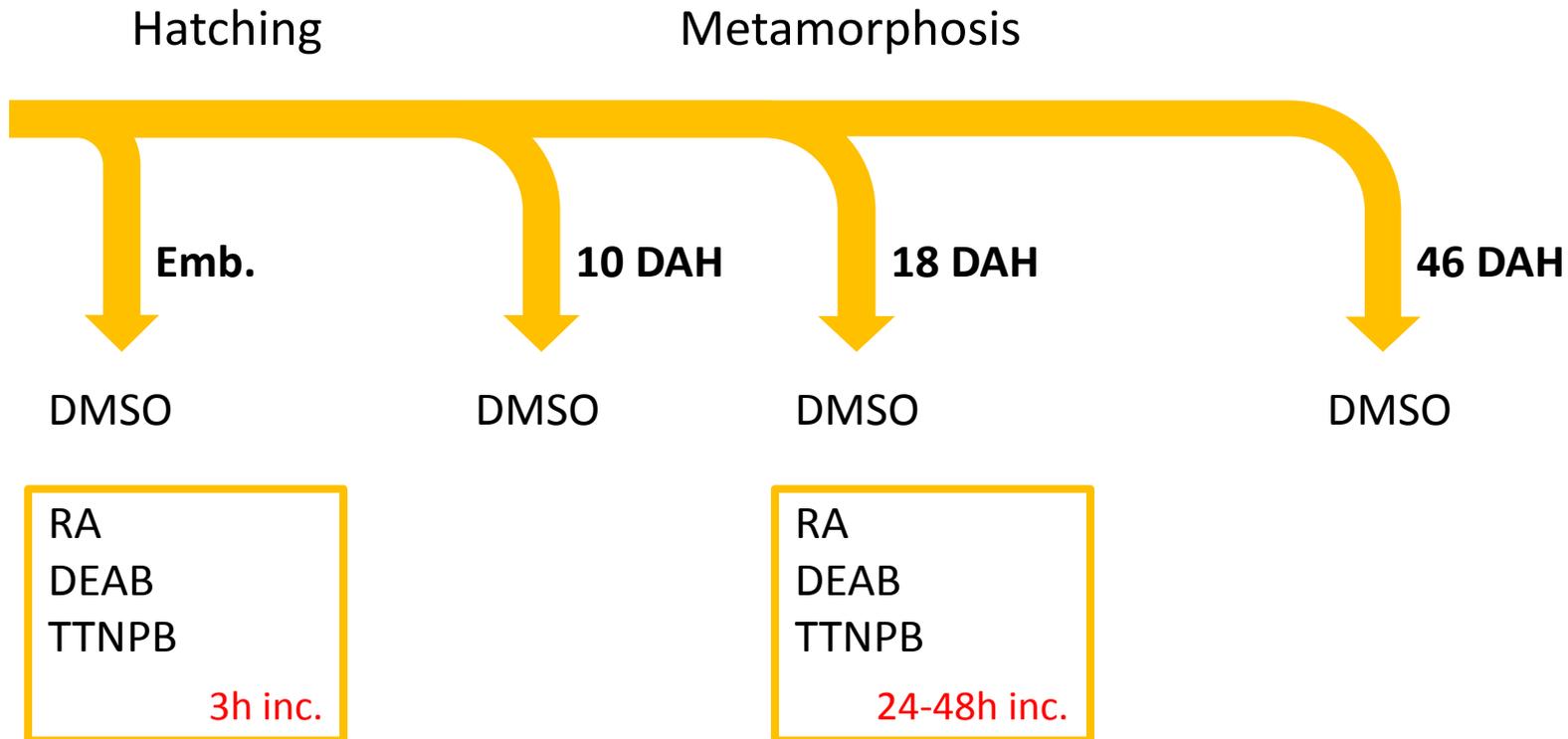
D18-PH

D46-PH



Experimental design

- In order to trigger differentiation processes, we also performed incubation with retinoic acid (RA) pathway modulators at embryonic and metamorphic stages.

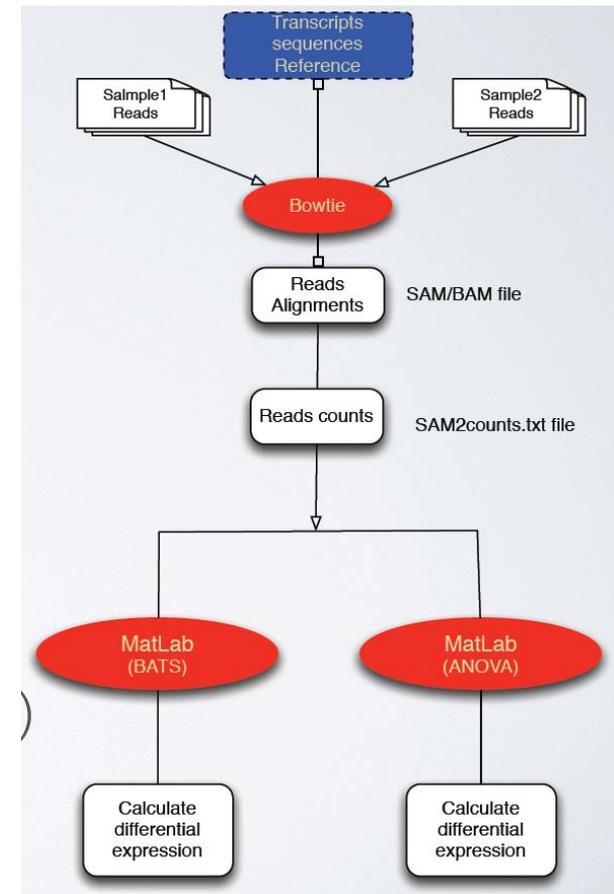


→ ~100 samples for *S. senegalensis*
 → ~50 samples for *S. solea*



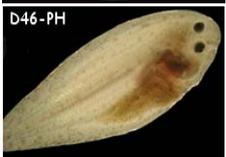
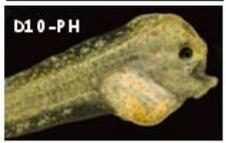
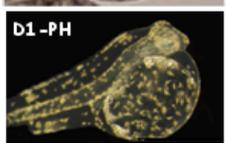
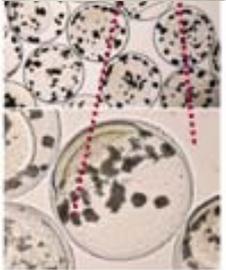
NGS strategy

- Previously (and additional performed on selected tissues) 454 sequencing provided $\sim 250\,000$ *S. senegalensis* unigenes which were used a reference
- Additional 454 sequencing was performed on selected tissues and provided a total of $\sim 5 \cdot 10^6$ reads
- Illumina sequencing was performed on described samples and provided a total of $\sim 4 \cdot 10^9$ reads
- Reads pre-processing was performed using SeqtrimNext
- Reads were mapped to the transcriptome using Bowtie2
- Reads were then counted and differential expression assessed using MatLab routines BATS and ANOVA

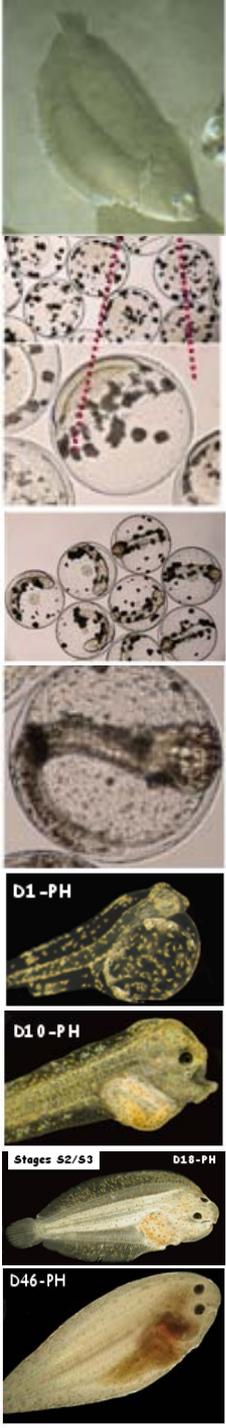


Sequencing data

| | NGS platform | | |
|---------------------------|------------------------|-----------------|------------------------|
| | Illumina | | 454 |
| | <i>S. senegalensis</i> | <i>S. solea</i> | <i>S. senegalensis</i> |
| Total Input Reads | 1,800,249,230 | 2,101,324,072 | 5,663,225 |
| Input Mean length | 76 | 100 | 757 |
| All rejected | N | 237,941,945 | 345,251,849 |
| | % | 13.5 | 17.1 |
| Rejected by contamination | N | 144,247,943 | 226,627,909 |
| | % | 8.2 | 11.2 |
| Useful paired reads | N | 1,503,882,050 | 1,676,160,406 |
| | % | 83.3 | 79.5 |
| Useful single reads | N | 57,534,764 | 70,098,335 |
| | % | 3.2 | 3.3 |
| Output Mean length | 66 | 89 | 184 |



New *Solea sp.* transcriptome **Ifremer**



| | | <i>S. senegalensis</i> | <i>S. solea</i> |
|----------------|--------------------|------------------------|------------------|
| Final assembly | Contigs | 73 932 | 74 812 |
| | Singletons | 623 193 | 448 825 |
| | Unigenes | 697 125 | 523 637 |
| | N seqs > 500: | 154 226 | 160 854 |
| | Mean lengths: | 525 | 799 |
| Unigenes | Unigenes > 500pb | 22.36% | 31.22% |
| | Unigenes > 200pb | 55.18% | 63.89% |
| | Longest unigene | 40 163 | 62 715 |
| | With orthologue | 152 031 (21.68%) | 119 771 (22.62%) |
| | Without orthologue | 549 179 (78.04%) | 409 670 (77.04%) |

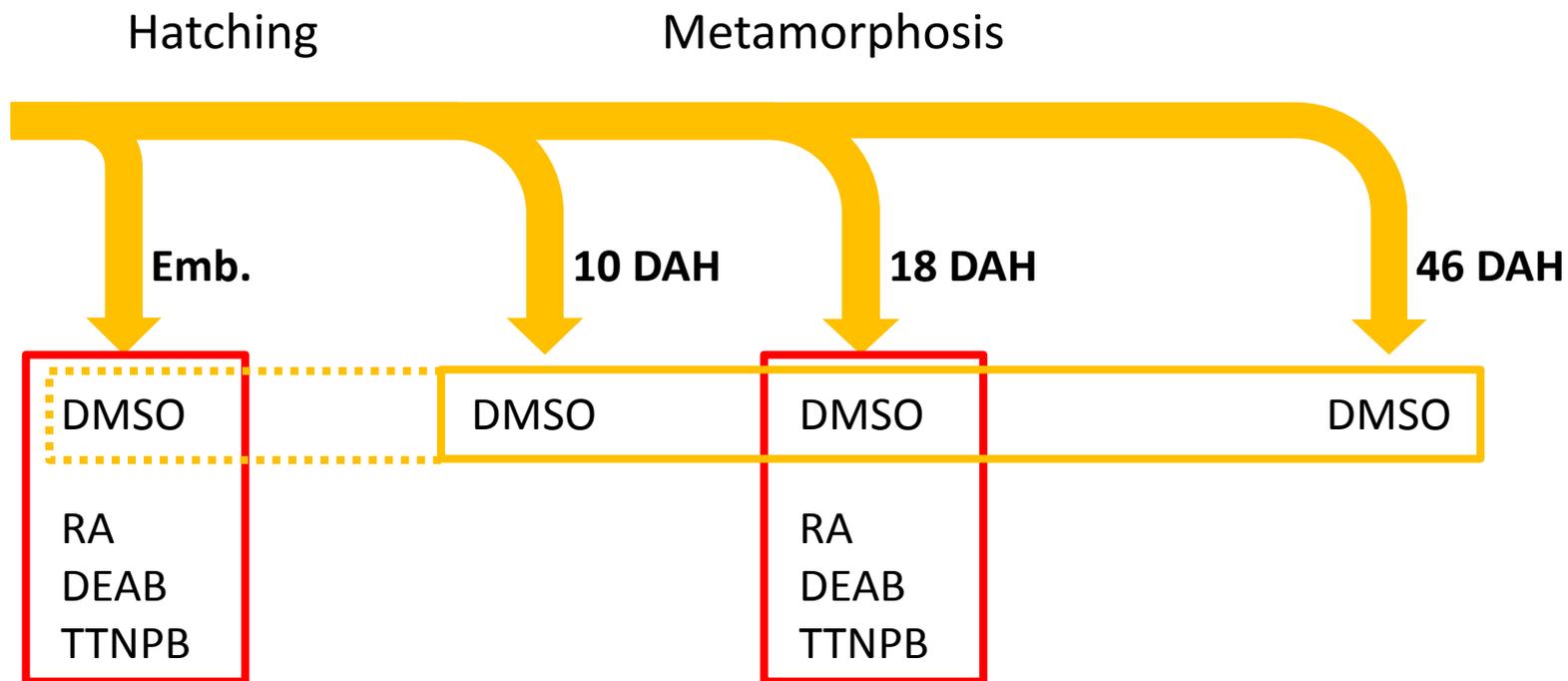
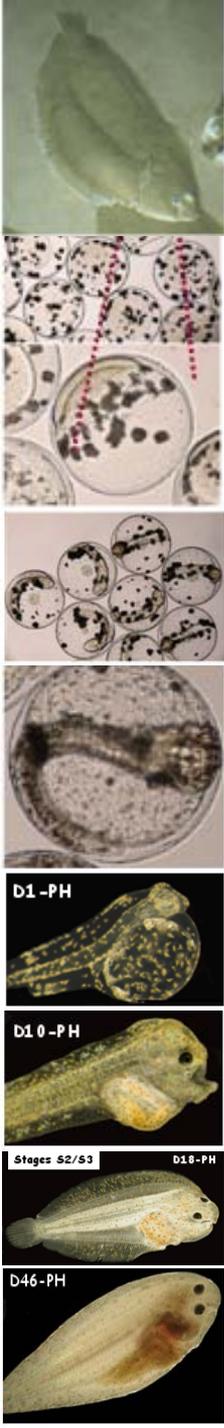
Data publicly available after registration in *Solea-DB*
<http://www.scbi.uma.es/soleadb>



Differential expression

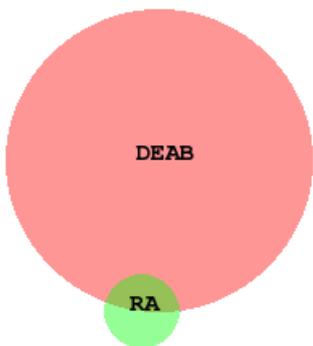
Differential expression (DE) has been analyzed – not yet in full details – between

- treatments at the same stage
- over development

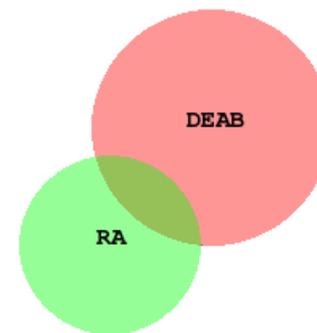


DE / RA pathway larvae

Larvae J18 - 24h treatment Larvae J18 - 48h treatment

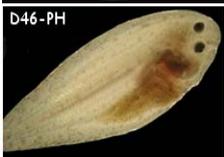
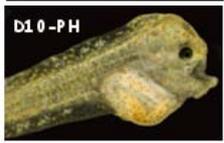
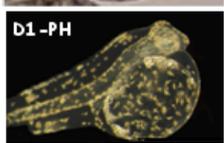
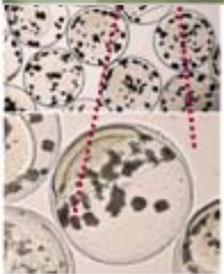


7942 regulated genes

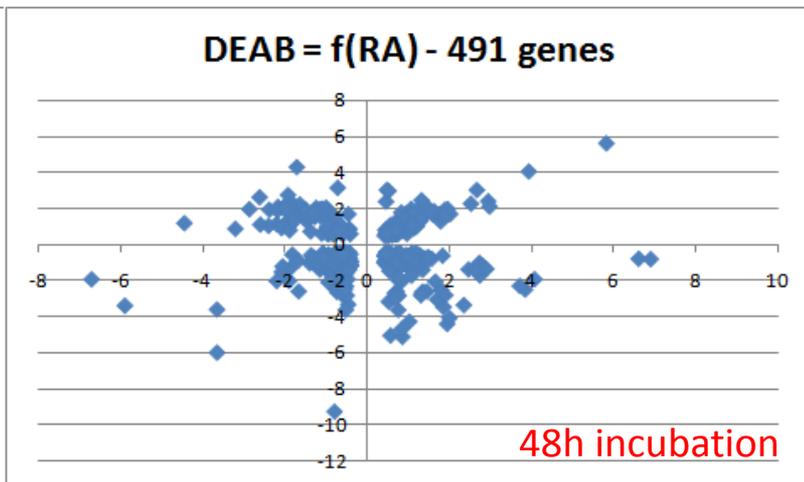
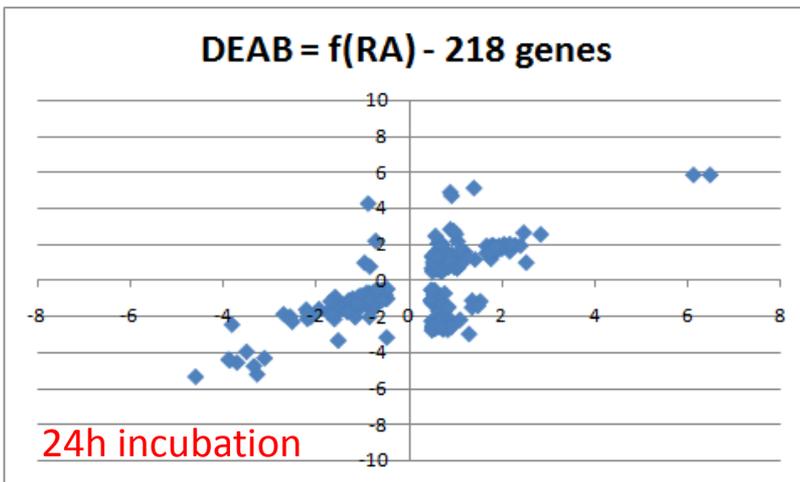
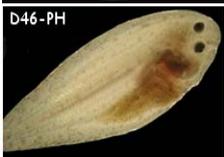
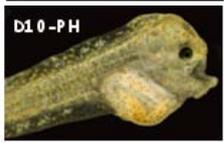
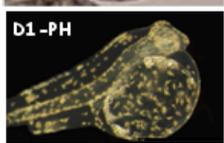
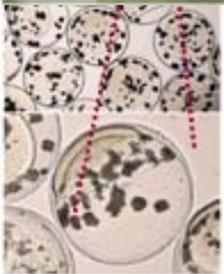


6195 regulated genes

| | | | | | | | | | |
|----------|------|-----------|------|-----|----------|------|-----------|------|-----|
| DEAB | 7704 | DEAB only | 7486 | 97% | DEAB | 4228 | DEAB only | 3737 | 88% |
| RA total | 456 | RA only | 238 | 52% | RA total | 2458 | RA only | 1967 | 80% |
| | | DEAB+RA | 218 | | | | DEAB+RA | 491 | |



DE / RA pathway larvae

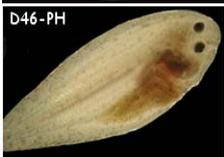
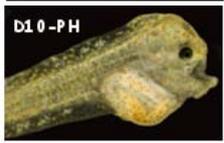
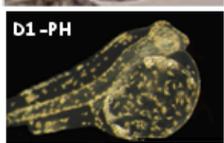
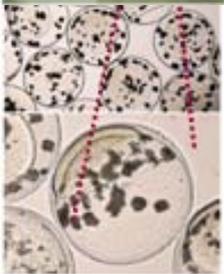


| ↑ RA ↑ DEAB | ↑ RA ↓ DEAB | ↓ RA ↑ DEAB | ↓ RA ↓ DEAB |
|----------------|----------------|----------------|----------------|
| 93 | 40 | 4 | 81 |
| 44 (20%) | | | |

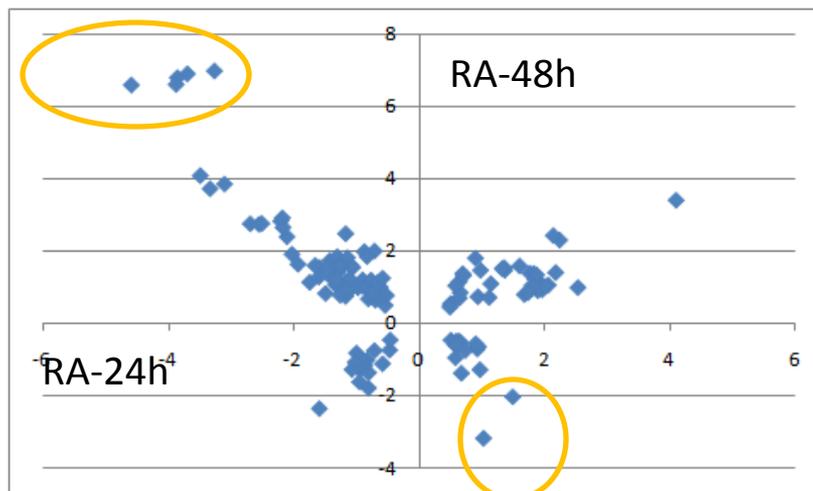
| ↑ RA ↑ DEAB | ↑ RA ↓ DEAB | ↓ RA ↑ DEAB | ↓ RA ↓ DEAB |
|----------------|----------------|----------------|----------------|
| 139 | 129 | 77 | 146 |
| 206 (42%) | | | |



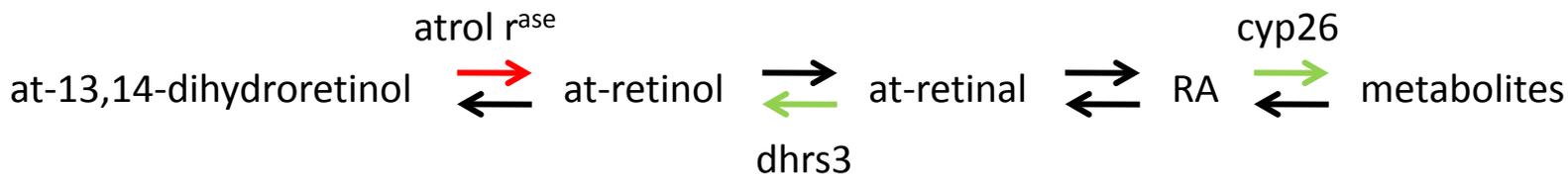
DE / RA pathway larvae



DHRS3
CYP26A1
CYP26B1



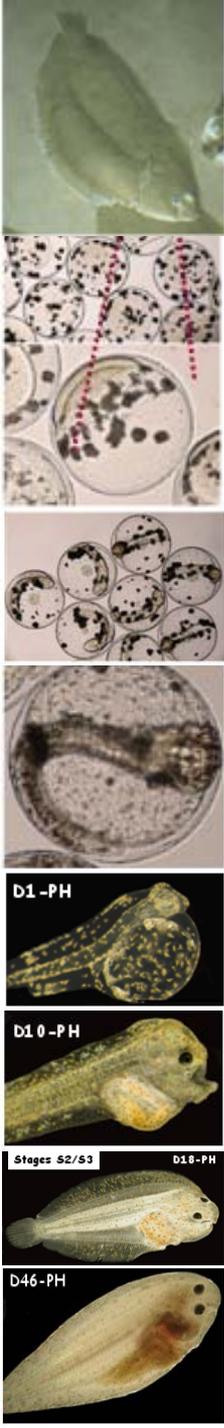
all-trans-retinol 13,14-reductase



The increase in the number of genes regulated by RA likely reflects transcriptional and translational requirement



DE / RA pathway larvae

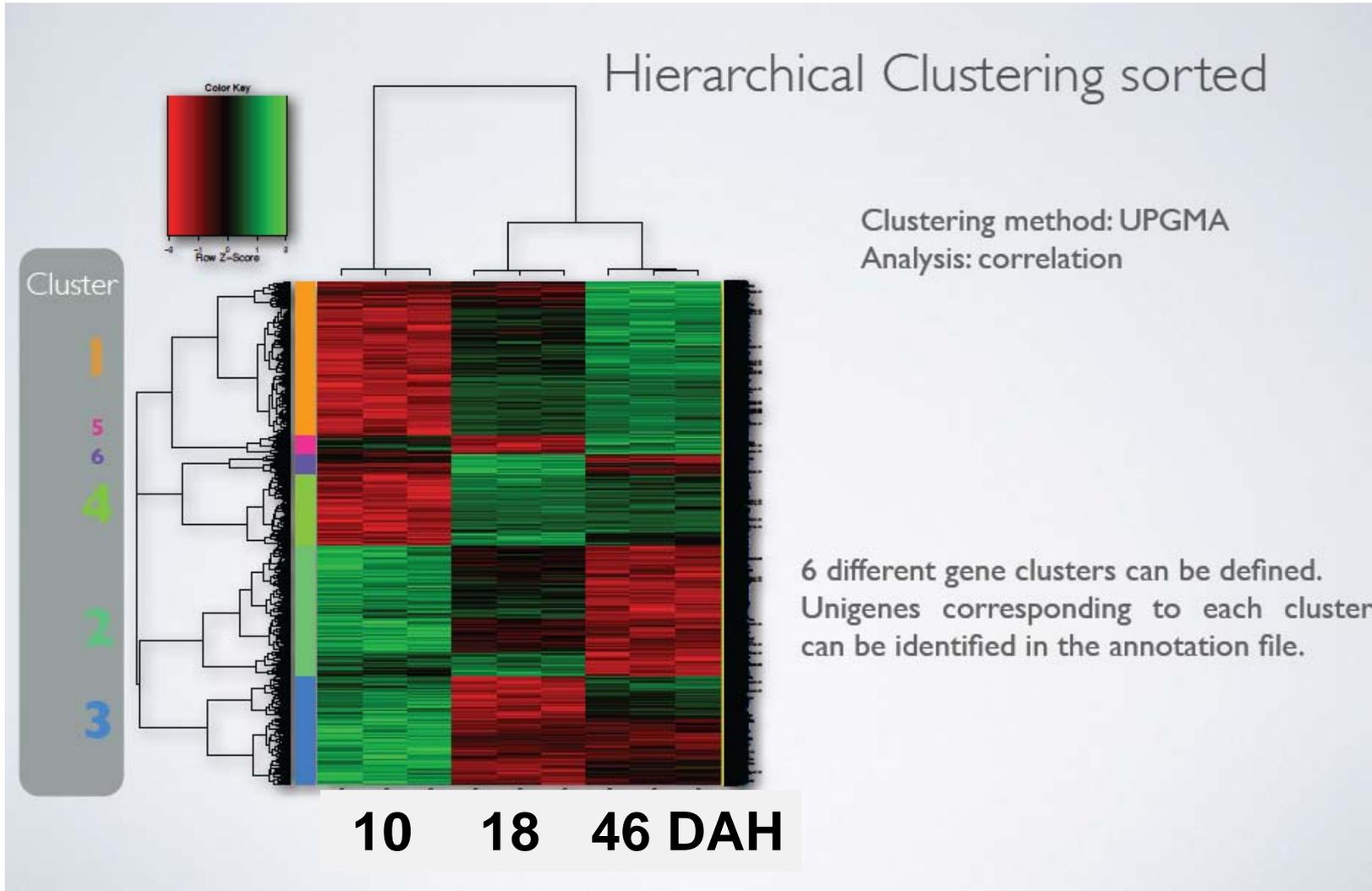
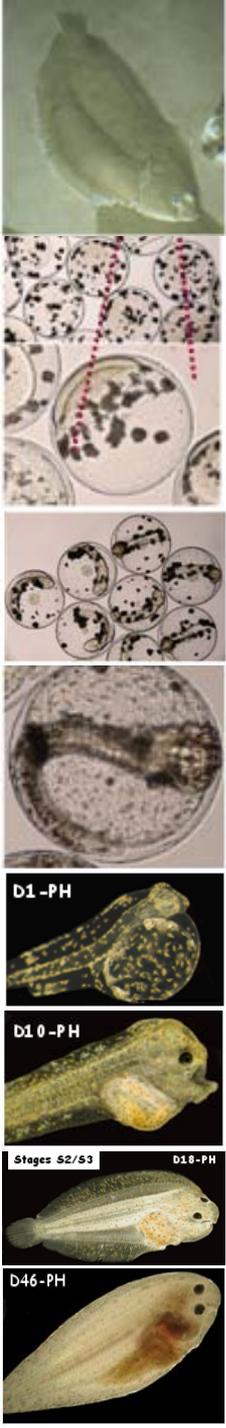


| | RA / TTNPB | | DEAB | |
|---|-------------------------|--------------------------|-------------------------|--------------------------|
| | RNA-seq <i>S. solea</i> | Open array <i>S. sen</i> | RNA-seq <i>S. solea</i> | Open array <i>S. sen</i> |
| <i>Raldh</i> | ↓ | ↓ | = | = |
| <i>Cyp26</i> | ↑ | ↑ | ↓ | = |
| <i>CRBPI_retinol-BP</i> | NA | ↑ | ↓ | ↑ |
| <i>CRBPII_cellular RA-BP</i> | NA | = | NA | ↑ |
| <i>beta-carotene 15,15'-monooxygenase 1</i> | ↓ | = | = | = |
| <i>At retinol dehydrogenase</i> | ↓ | = | = | = |
| <i>RDH1 retinol dehydrogenase</i> | NA | = | NA | = |
| <i>RDH5 retinol dehydrogenase</i> | NA | = | NA | ↑ |
| <i>RDH10A retinol dehydrogenase</i> | ↓ | ↓ | ↑ | ↑ |

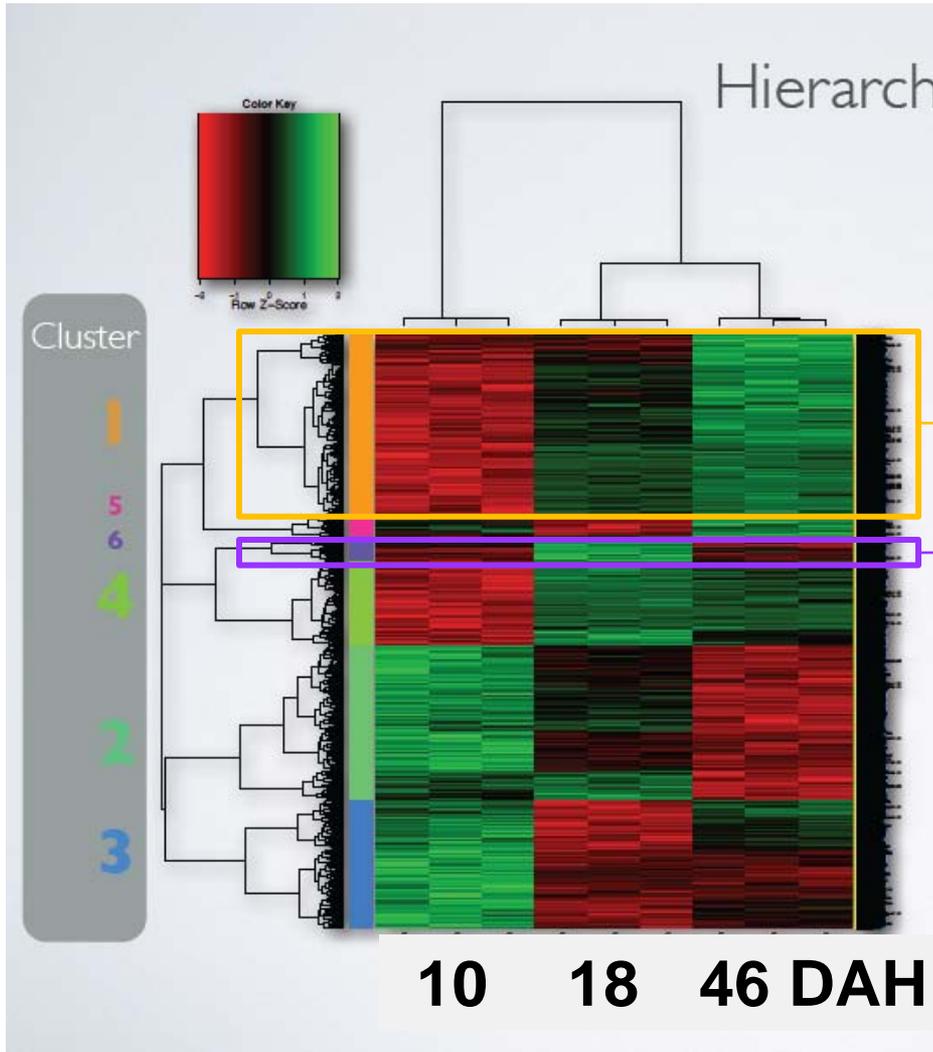
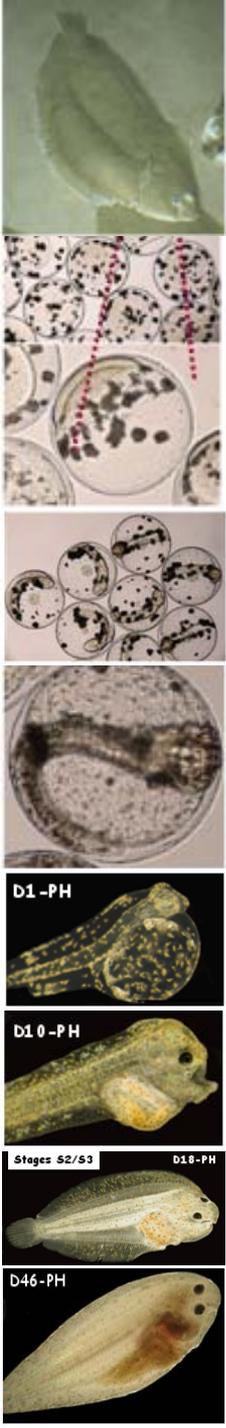
There is a good correspondance between RNA-Seq values from *S. solea* and open-array data from *S. senegalensis*

DE / Development series

Ifremer



DE / Development series



Exemple of clustered genes:

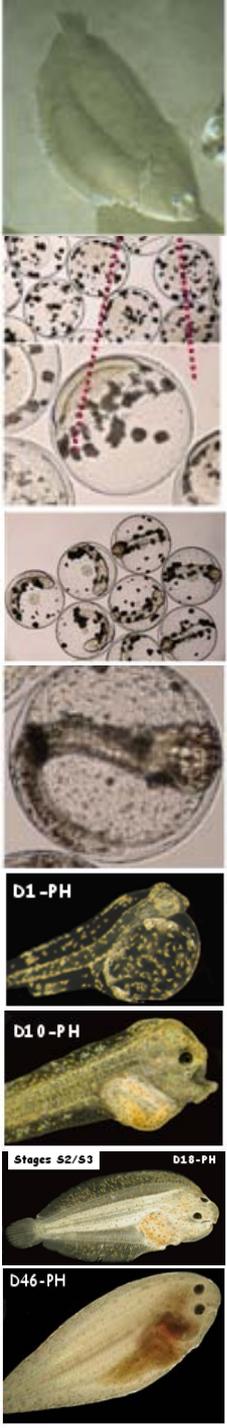
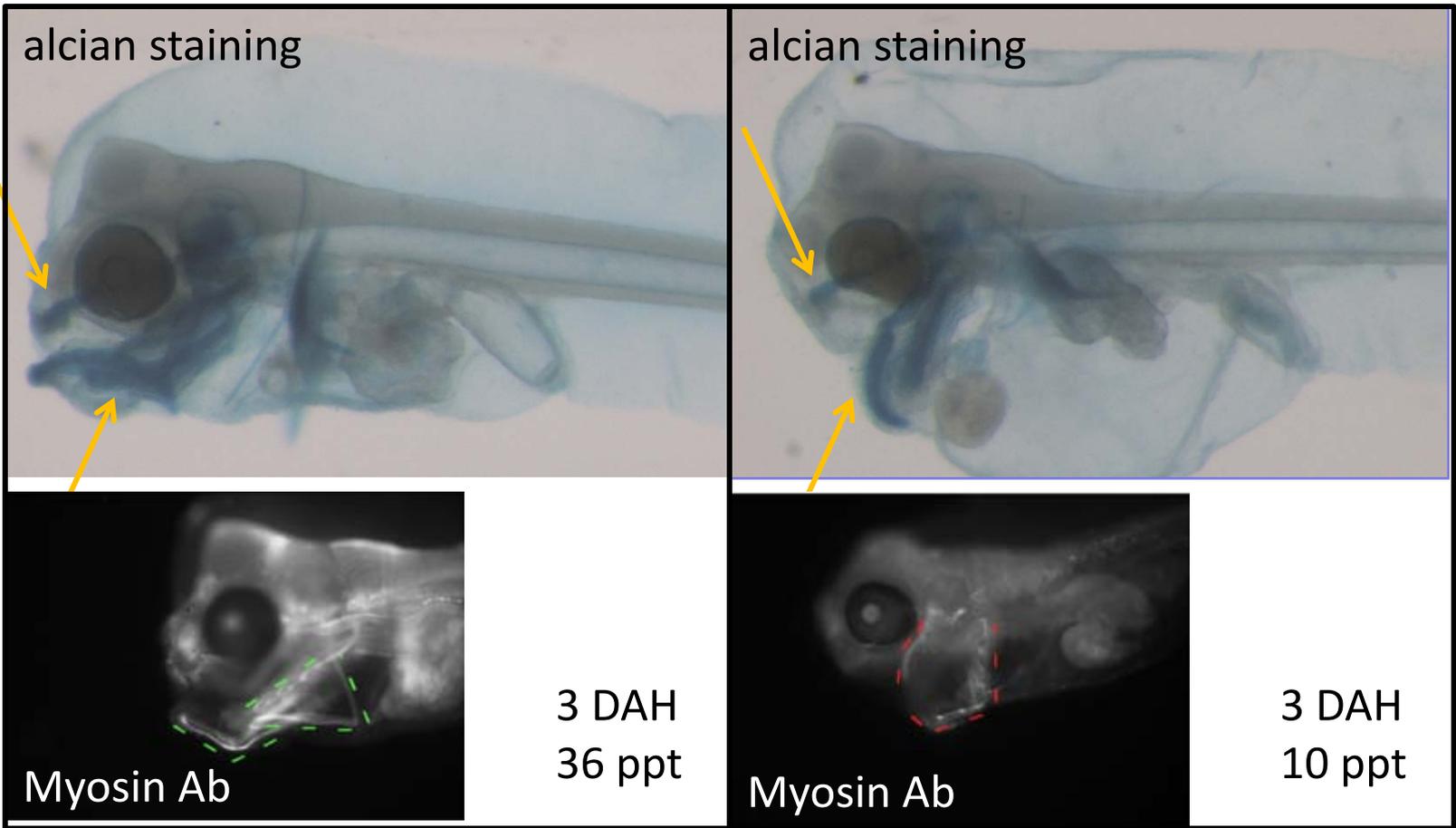
#1 expression increases over time
 immune system genes (*cd4, il15, chemokines*)
 steroid path. (*amh, androgen recept, aroA, estrogen regulated proteins*)
 retinoid (*roldh, raldh, crabb2, tth*)

#6 activated during metamorphosis
 thyroid hormone pathway (*thyroglobulin*)
 retinoid (*rar*)
 KEGG → glycolysis pathway



Larvae rearing at low salinity **Ifremer**

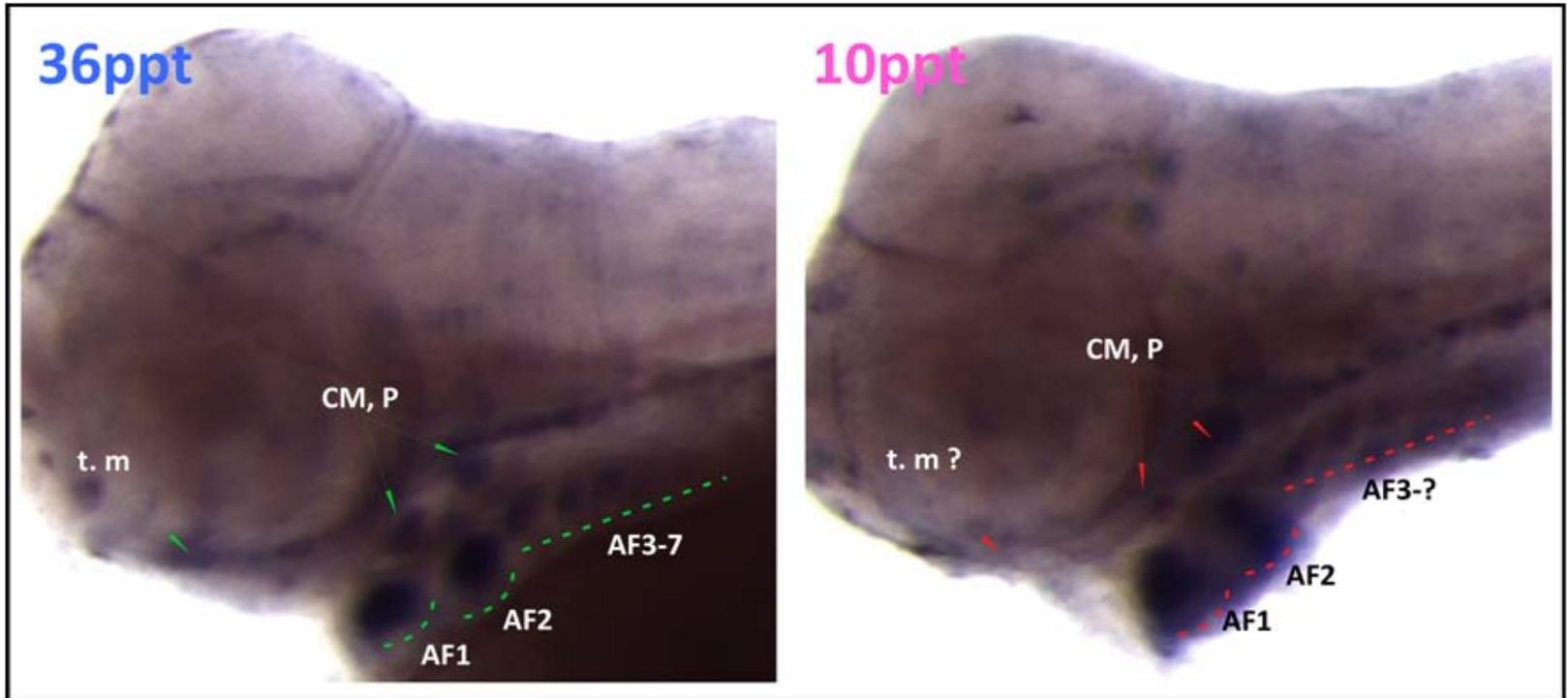
- Low salinity improves larval growth and is more adapted to some rearing practices but generates deformations



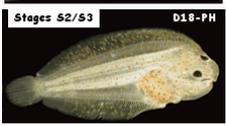
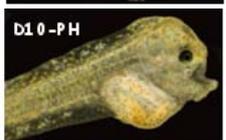
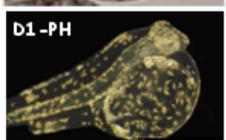
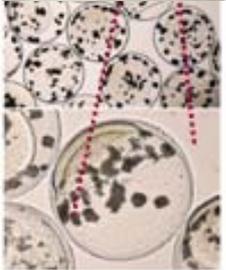
Deciphering mechanisms

Ifremer

sox9a 1DAH

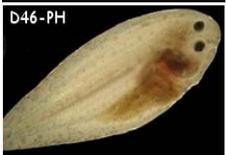
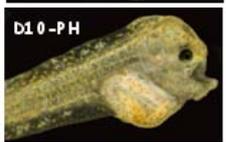
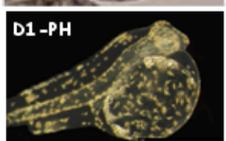
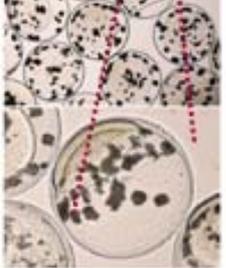


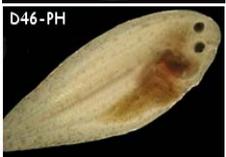
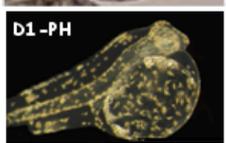
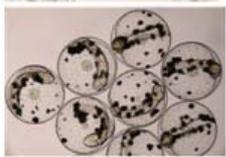
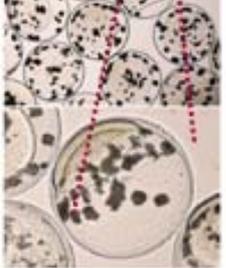
Neural crest cells are not proliferating and/or migrating correctly – specially for precursors of upper jaw



Conclusions

- A combination of 454 and Illumina sequencing has allowed the increase in the knowledge of *Solea sp.* transcriptomes with the identification of ca. 600.000 unigenes for each species
 - ~150.000 have a length >500 bp
 - 150.000/120.000 (22%) have identified orthologues
- This has allowed the development of several tools suitable for genes expression analysis (μ array, openarray) and has made easier the use of classical methods (qPCR, ish)
- The use of several developmental stages and embryos or larvae manipulation (RA/DEAB but also salinity, temperature) will allow the deciphering of particular pathways in response to these factors and will give indications to improve or adapt rearing





Aknowledgments



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