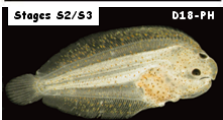
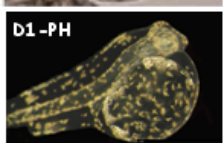
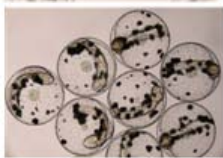
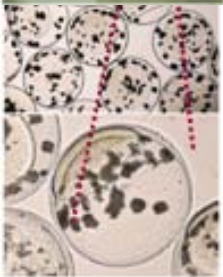


# GENOME-WIDE GENE EXPRESSION ANALYSIS DURING *SOLEA SP.* EMBRYO-LARVAL DEVELOPMENT

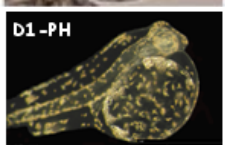
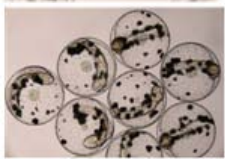
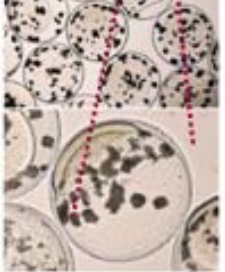
X. Cousin, M.G. Claros, D. Mazurais, R. Bautista, H. Benzekri, M.-L. Bégout, M. Ponce, P. Armesto, J. Zambonino, J.V. Planas and M. Manchado



# AQUAGENET network

Ifremer

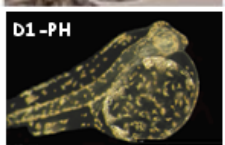
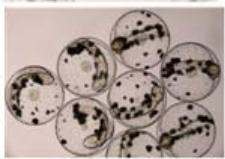
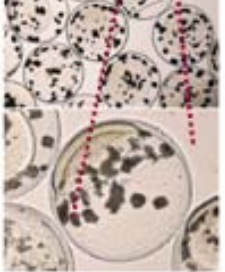
- AQUAGENET is an Interreg IVB funded project
  - IFAPA El Toruño – Manuel Manchado (coordination)
  - Ifremer – Genetics and Pathology Lab (La Tremblade) and Fisheries Lab (La Rochelle)
  - Universidad de Barcelona – Physiology Department
  - CNRS – Evolution sciences Institute (ISEM) UMR 5554 – Sète
  - 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)

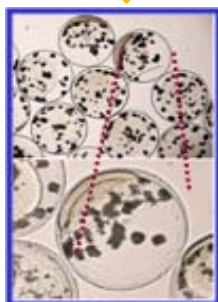
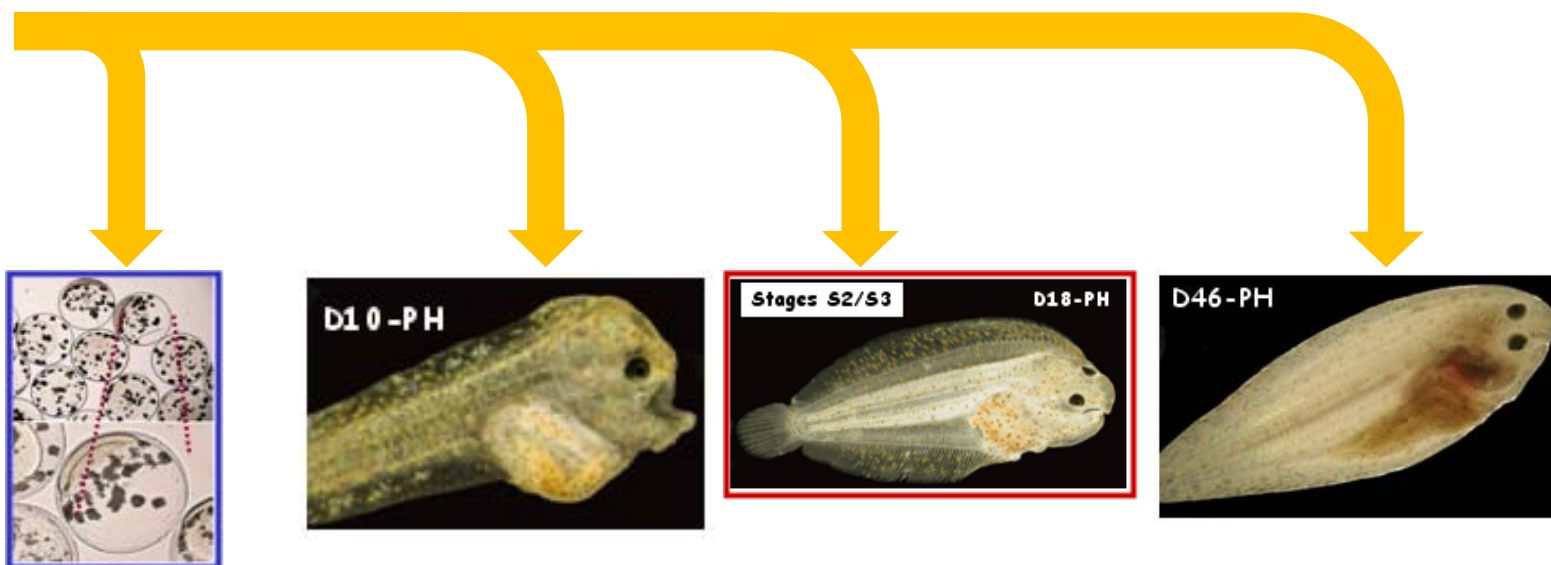


# Experimental design

- 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

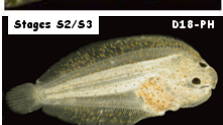
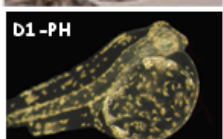
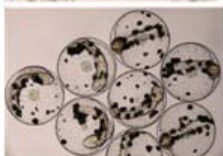
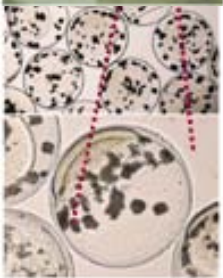


Stages S2/S3

D18-PH

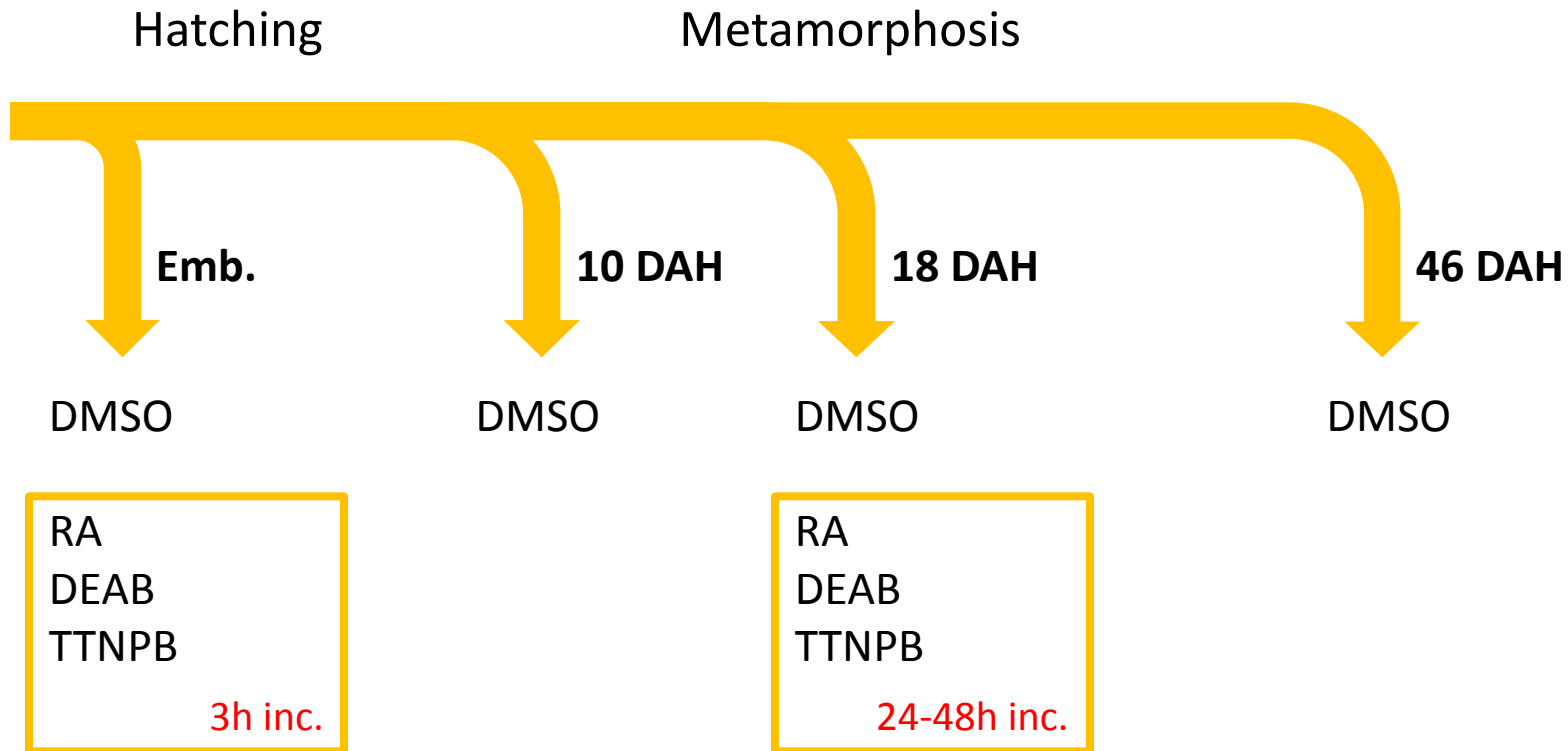


D46-PH



# Experimental design

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

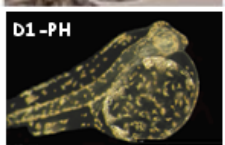
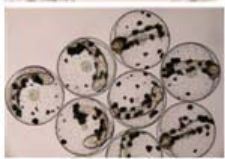
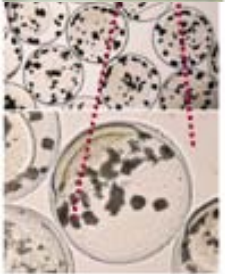
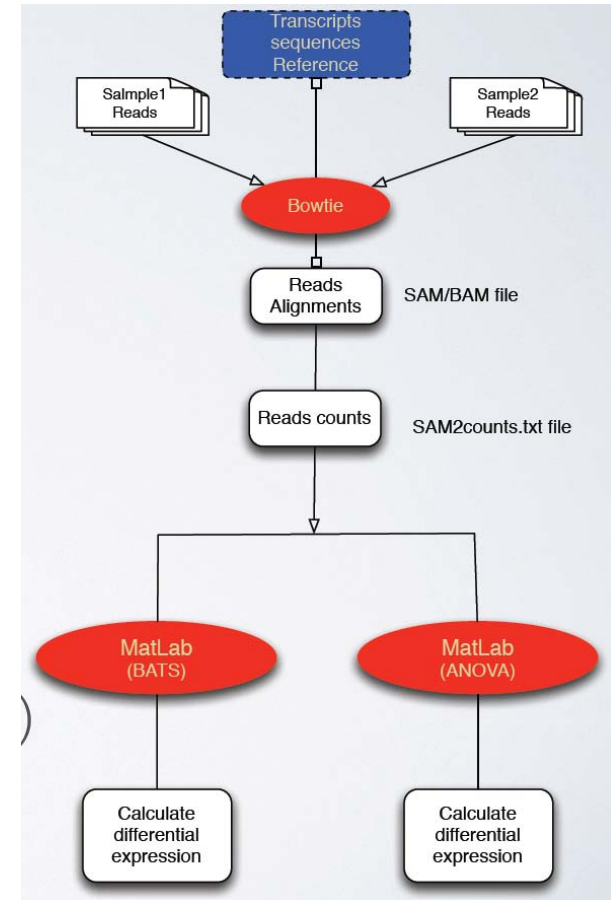


→ ~100 samples for *S. senegalensis*  
 → ~50 samples for *S. solea*



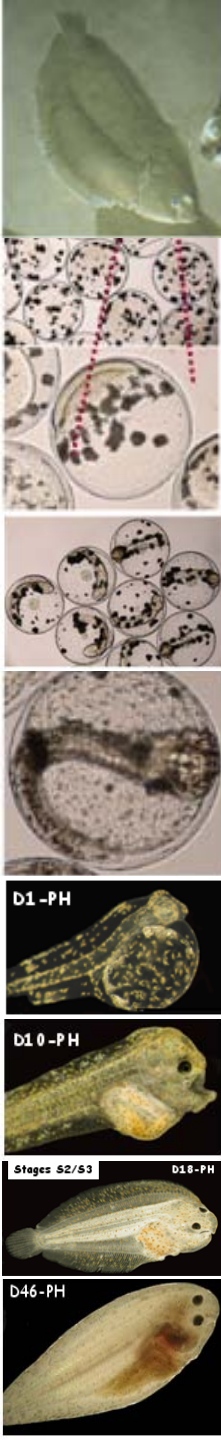
# NGS strategy

- 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

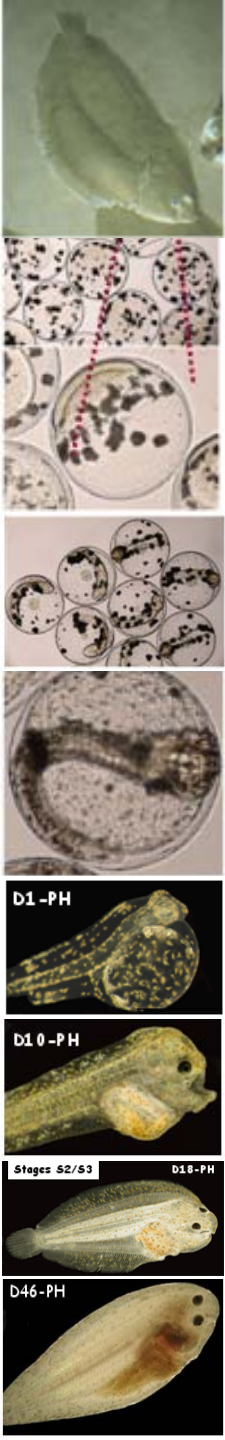


# Sequencing data

	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	<b>76</b>	<b>100</b>	<b>757</b>
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	<b>66</b>	<b>89</b>	<b>184</b>



# 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>

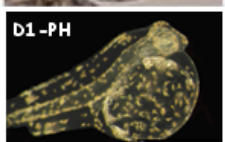
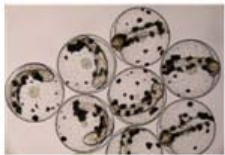
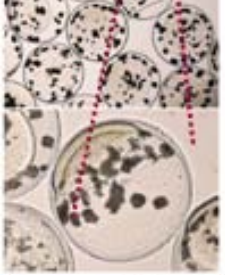
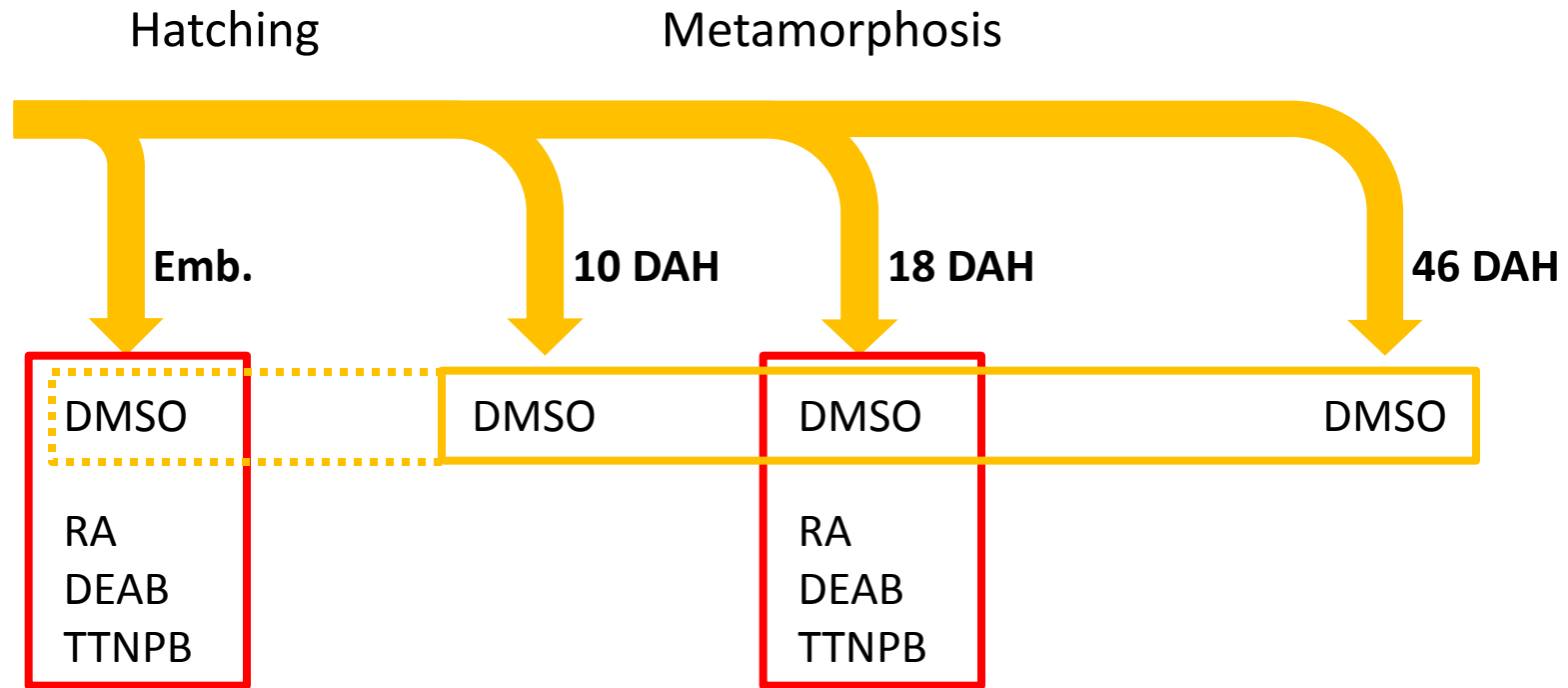




# Differential expression

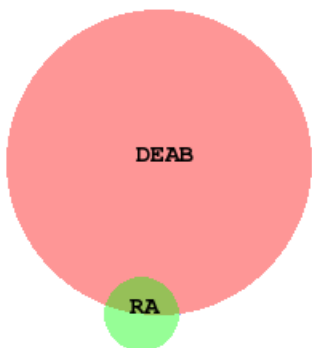
Differential expression (DE) has been analyzed – not yet in full details – between

- treatments at the same stage
- over development

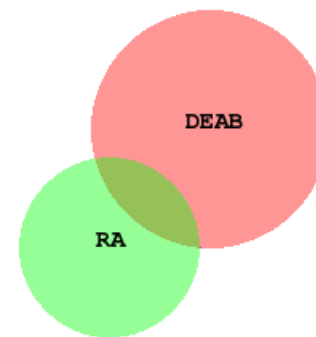


# DE / RA pathway larvae

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

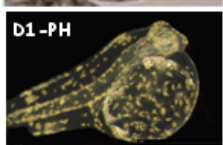
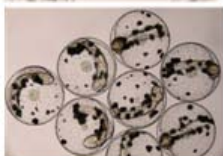
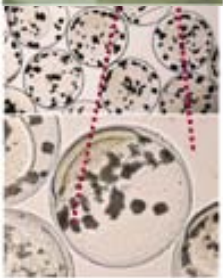


7942 regulated genes

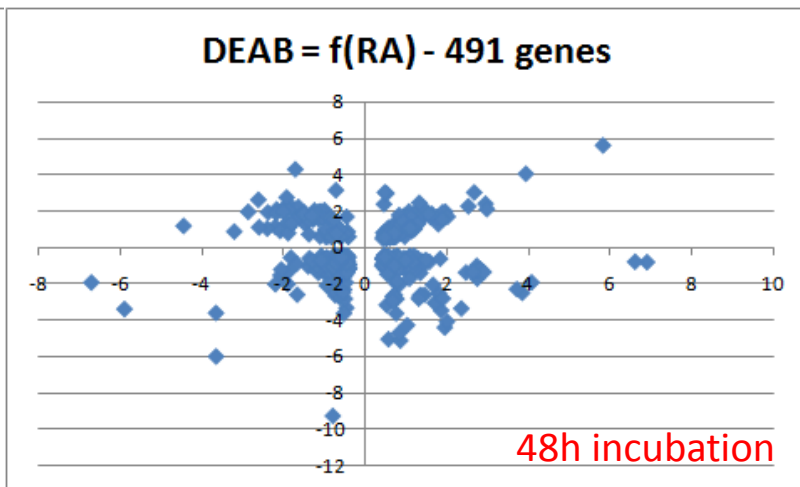
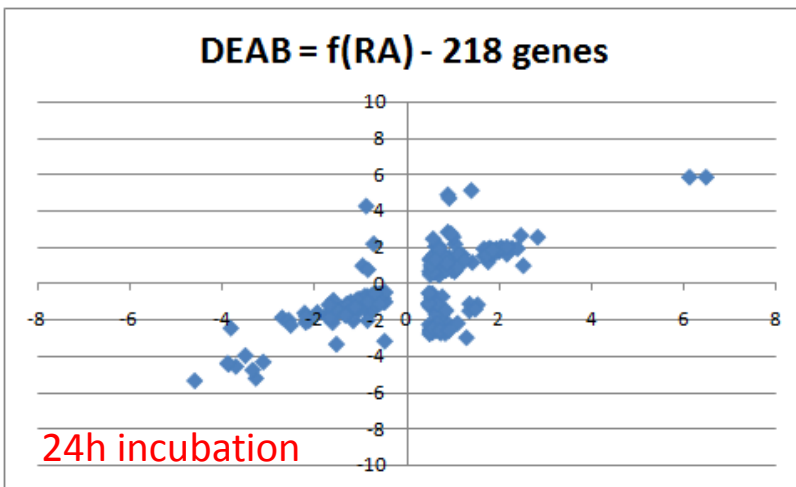
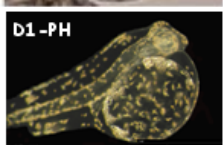
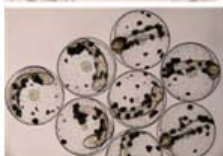
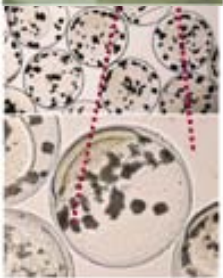


6195 regulated genes

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

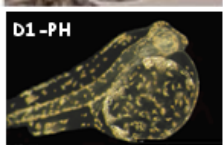
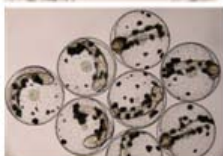
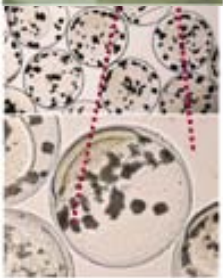


↑ RA ↑ DEAB	↑ RA ↓ DEAB	↓ RA ↑ DEAB	↓ RA ↓ DEAB
93	40	4	81
<b>44 (20%)</b>			

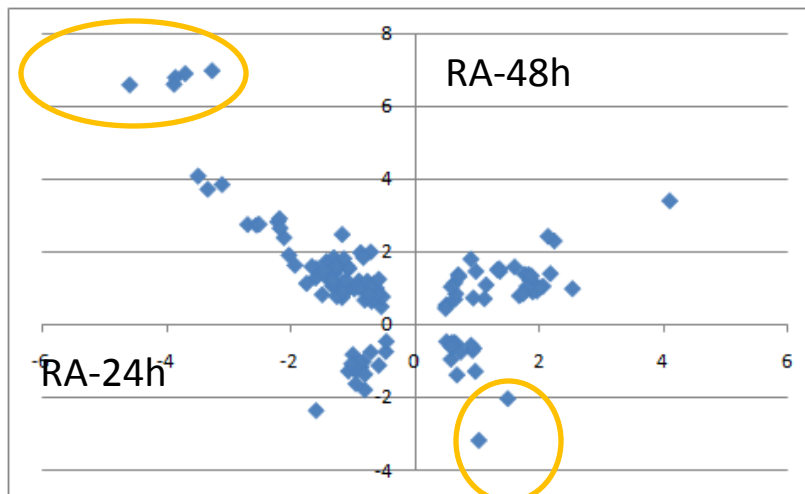
↑ RA ↑ DEAB	↑ RA ↓ DEAB	↓ RA ↑ DEAB	↓ RA ↓ DEAB
139	129	77	146
<b>206 (42%)</b>			



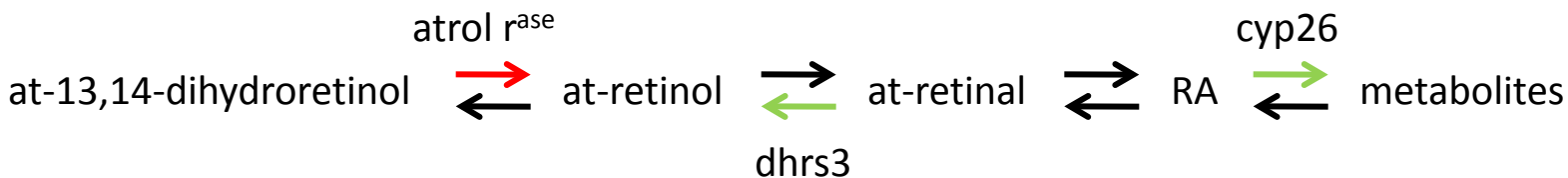
# DE / RA pathway larvae



DHRS3  
CYP26A1  
CYP26B1



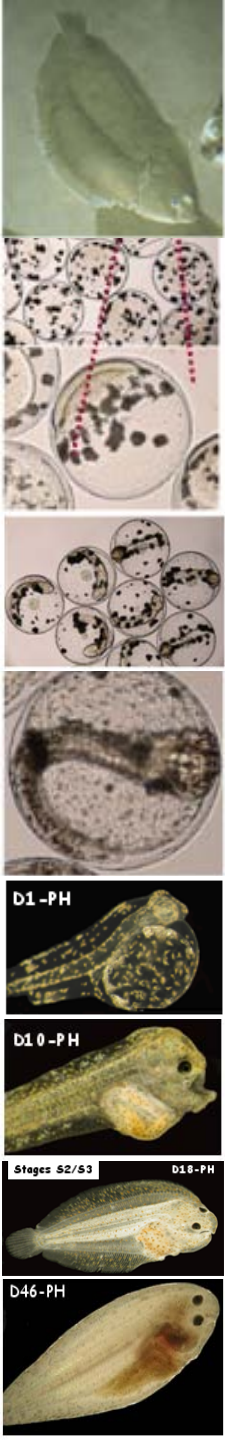
all-trans-retinol 13,14-reductase



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



# DE / RA pathway larvae



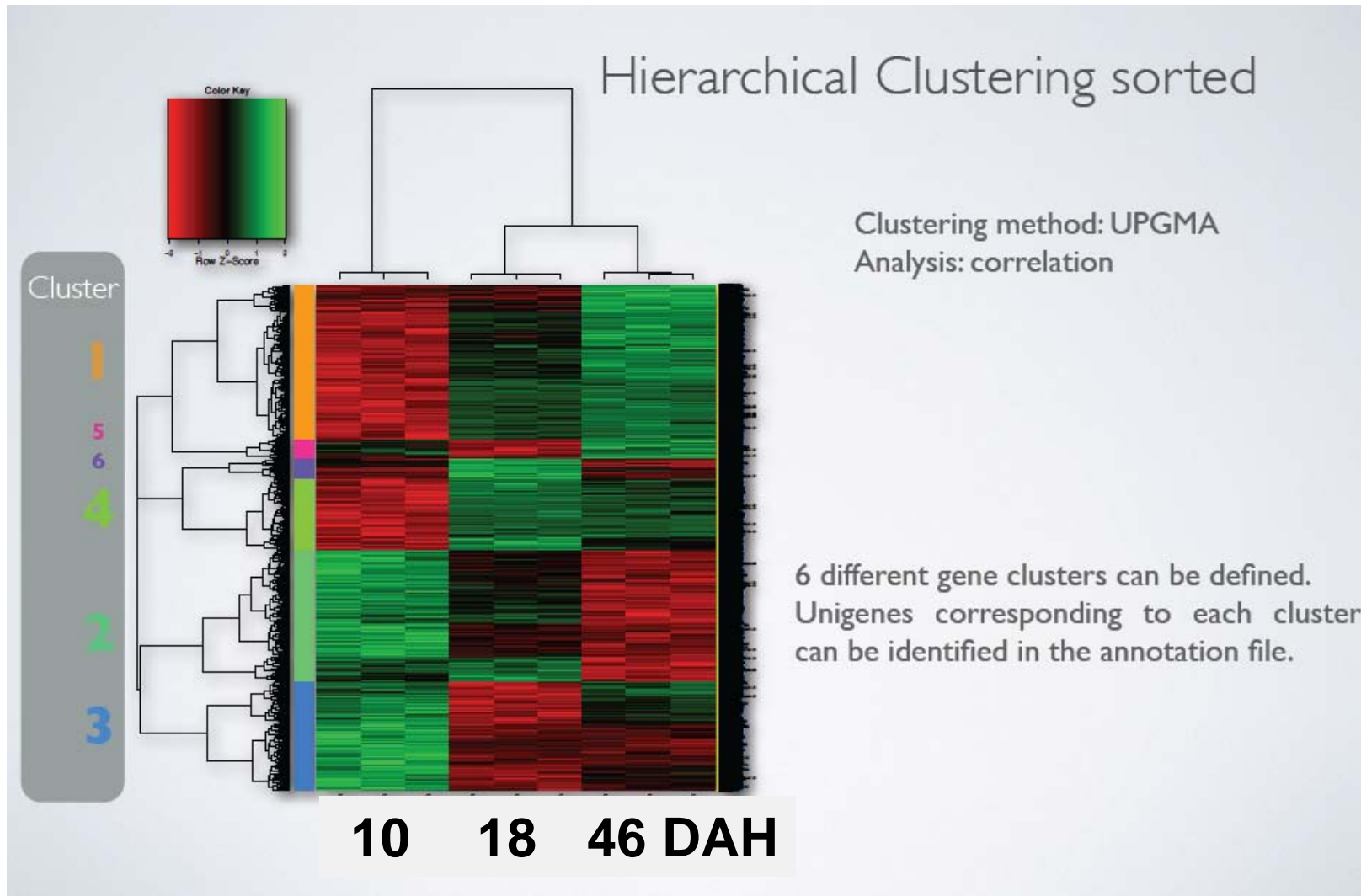
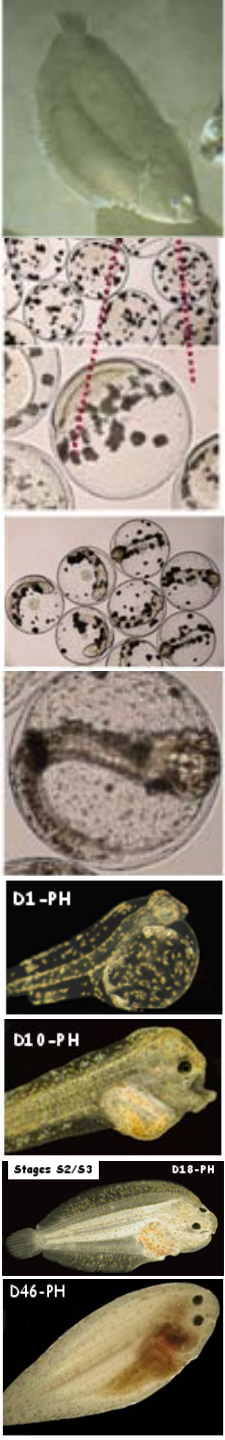
	RA / TTNPB		DEAB	
	RNA-seq <i>S. solea</i>	Open array <i>S. sen</i>	RNA-seq <i>S. solea</i>	Open array <i>S. sen</i>
<i>Raldh</i>	↓	↓	=	=
<i>Cyp26</i>	↑	↑	↓	=
<i>CRBPI_retinol-BP</i>	NA	↑	↓	↑
<i>CRBPII_cellular RA-BP</i>	NA	=	NA	↑
<i>beta-carotene 15,15'-monooxygenase 1</i>	↓	=	=	=
<i>At retinol dehydrogenase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

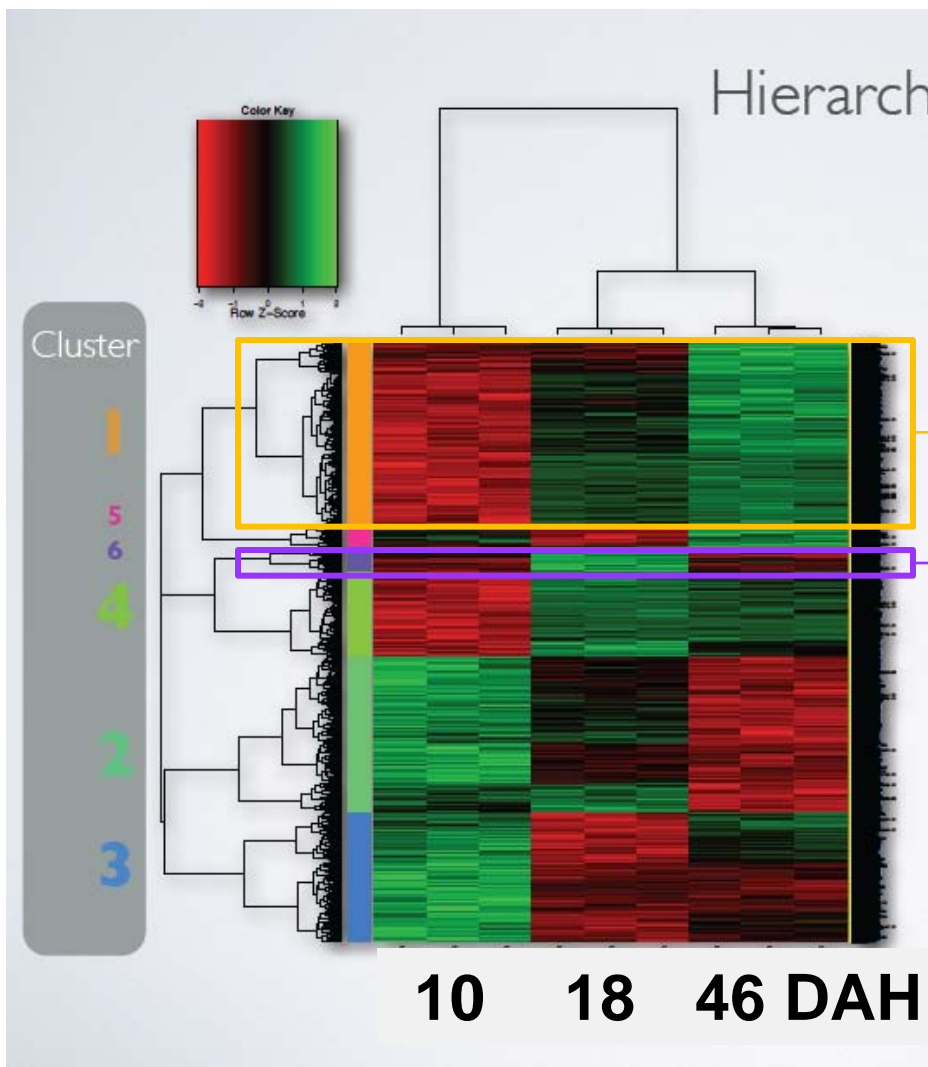
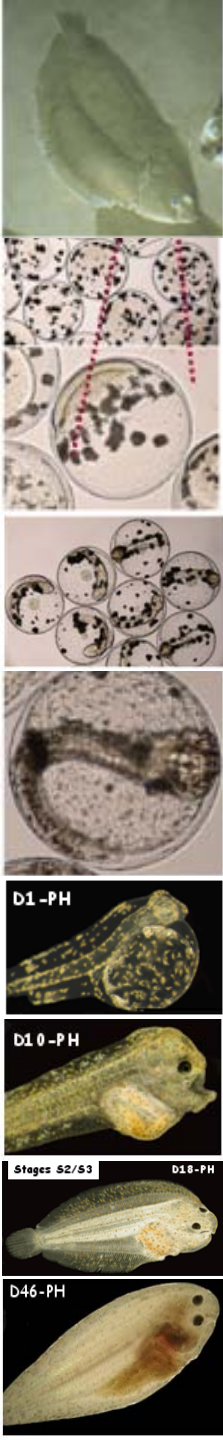


# DE / Development series

Ifremer



# DE / Development series



Exemple of clustered genes:

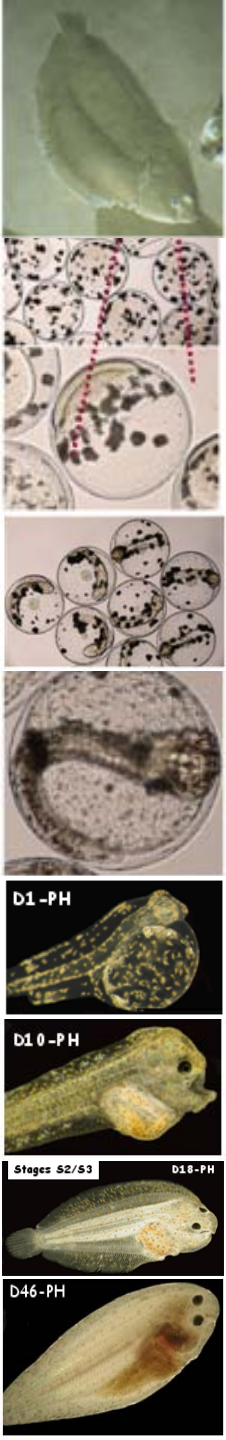
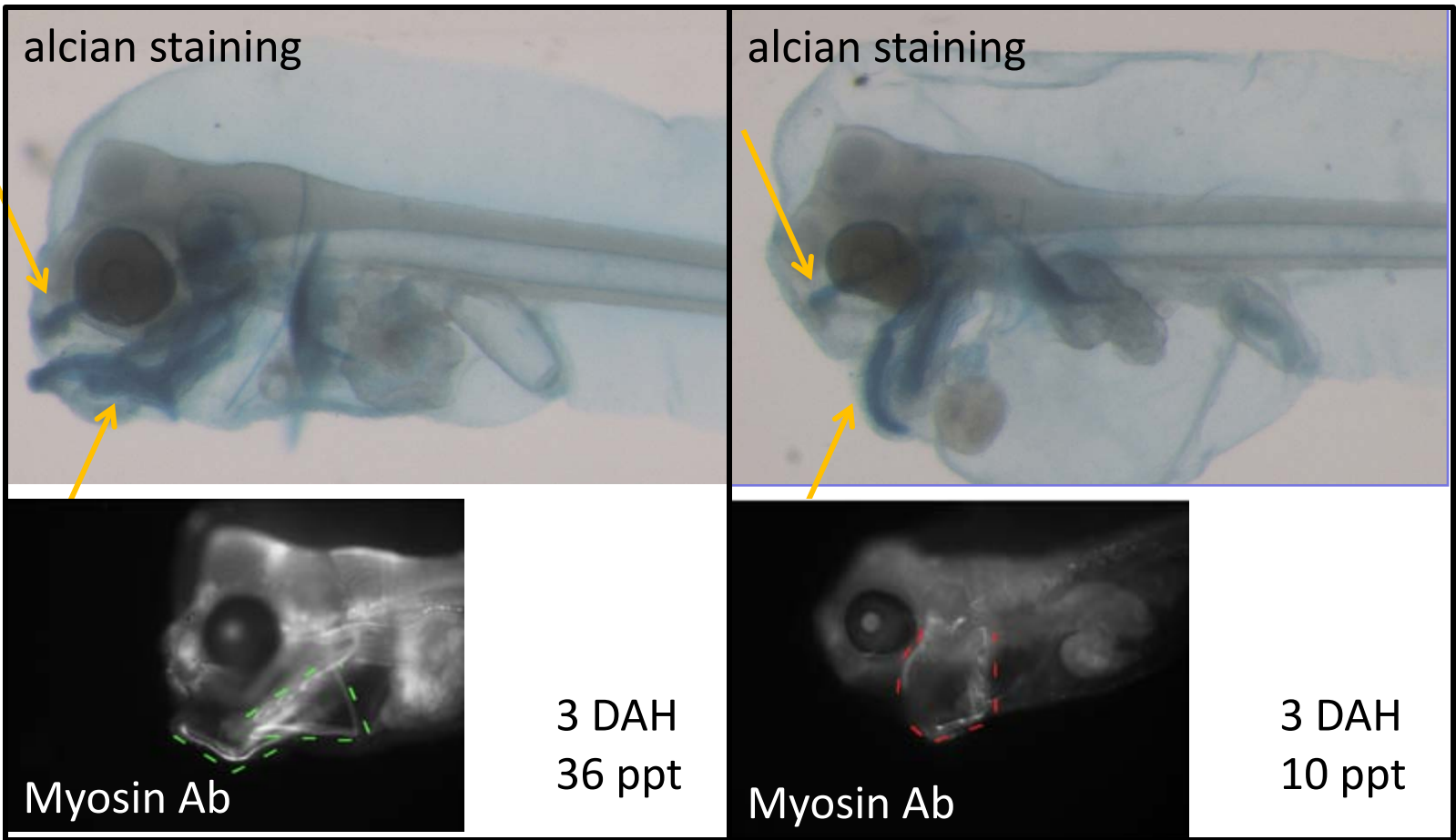
#1 expression increases over time  
 immune system genes (*cd4, il15, chemokines*)  
 steroid path. (*amh, androgen recept, aroA, estrogen regulated proteins*)  
 retinoid (*roldh, raldh, crabp2, tth*)

#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

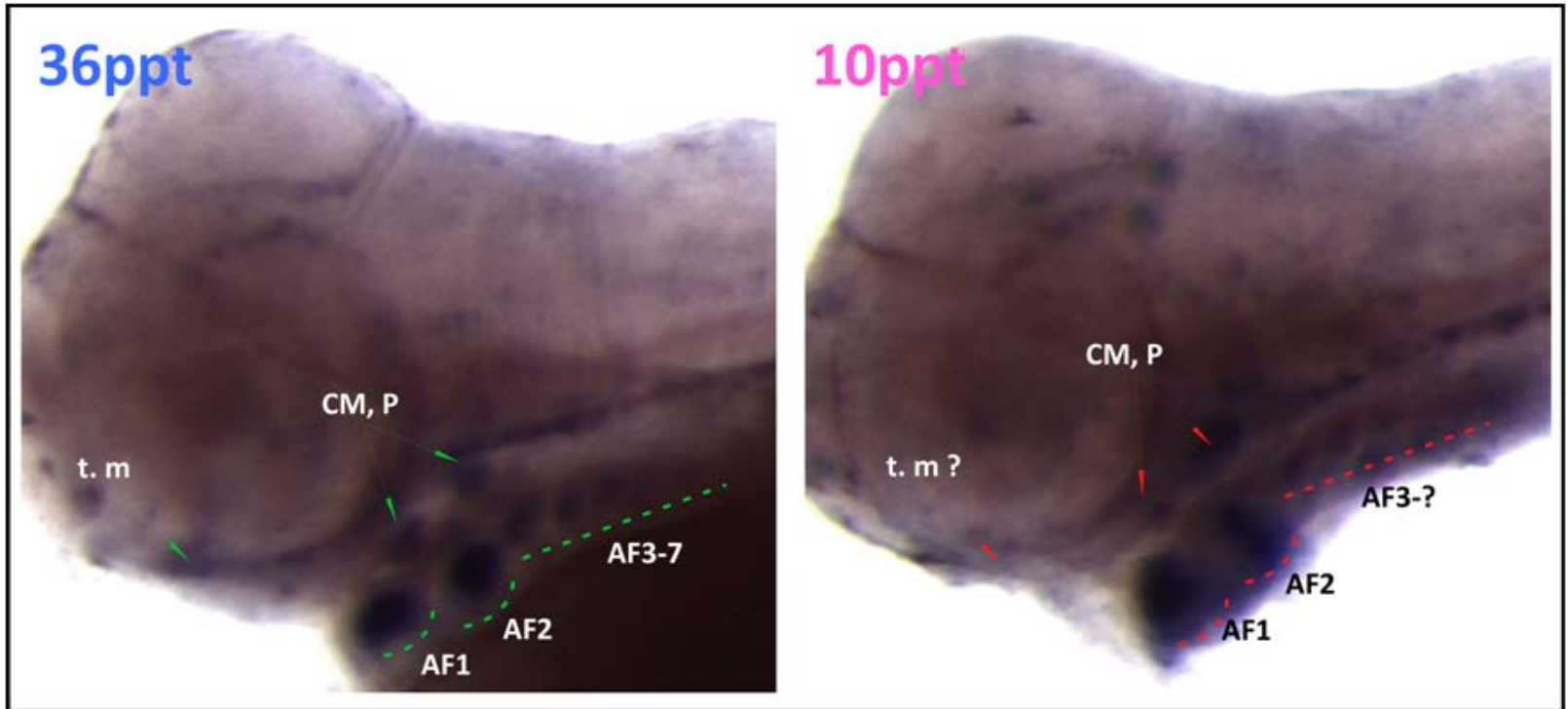




# Deciphering mechanisms

Ifremer

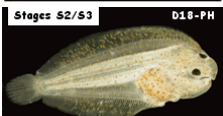
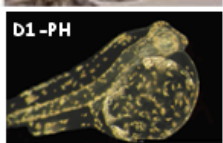
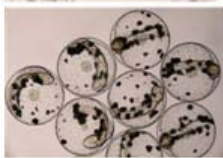
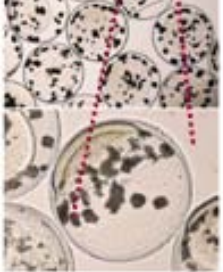
*sox9a* 1DAH

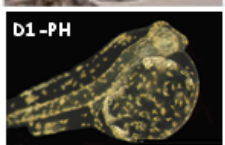
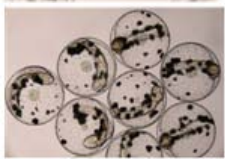
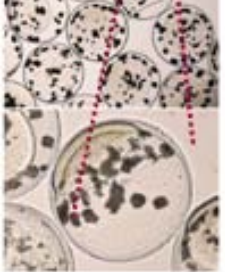


Neural crest cells are not proliferating and/or migrating correctly – specially for precursors of upper jaw

# 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 ( $\mu$ 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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