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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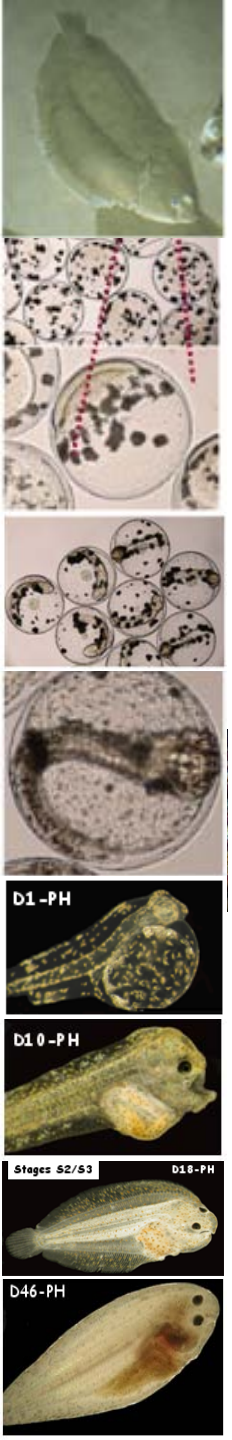

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GENOME-WIDE GENE EXPRESSION ANALYSIS DURING *SOLEA SP*. EMBRYO-LARVAL DEVELOPMENT

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

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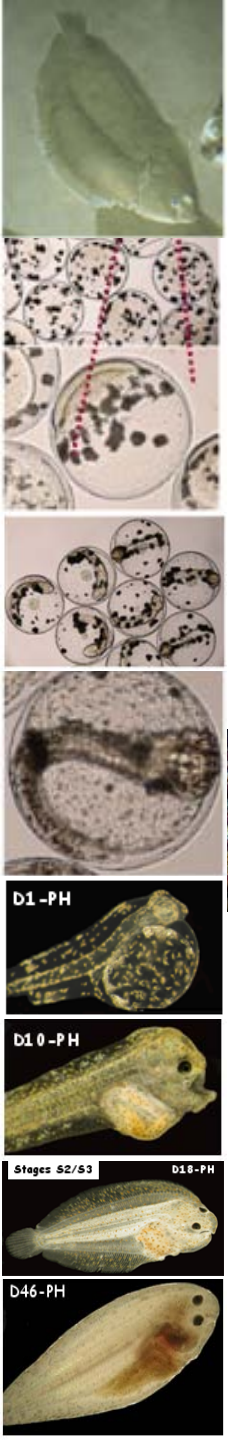

AQUAGENET network

- 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 – Biomedecine 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

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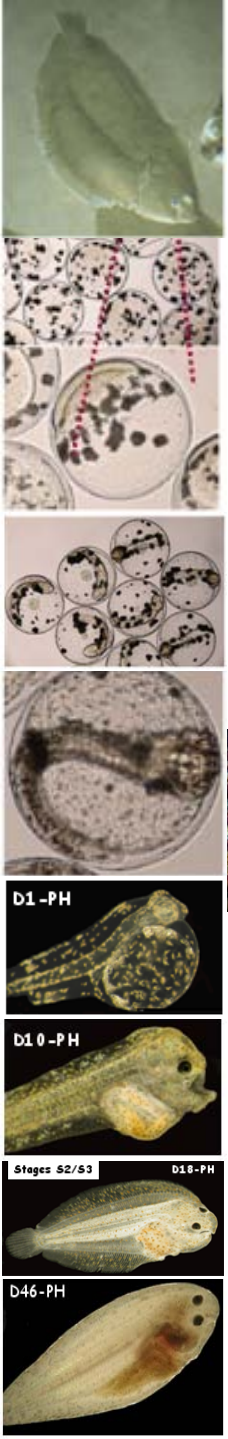

Solea sp. in aquaculture

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- *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* (Cerda *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)

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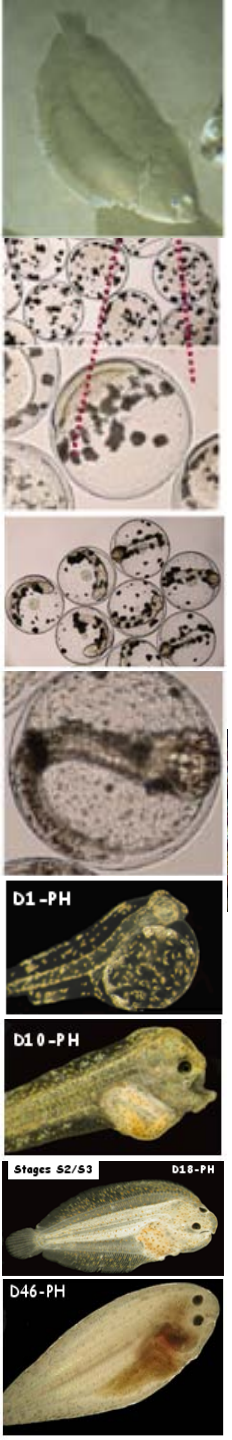
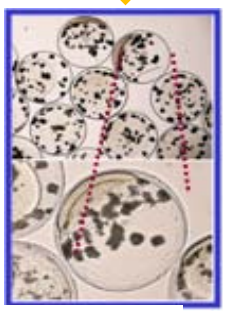


Experimental design

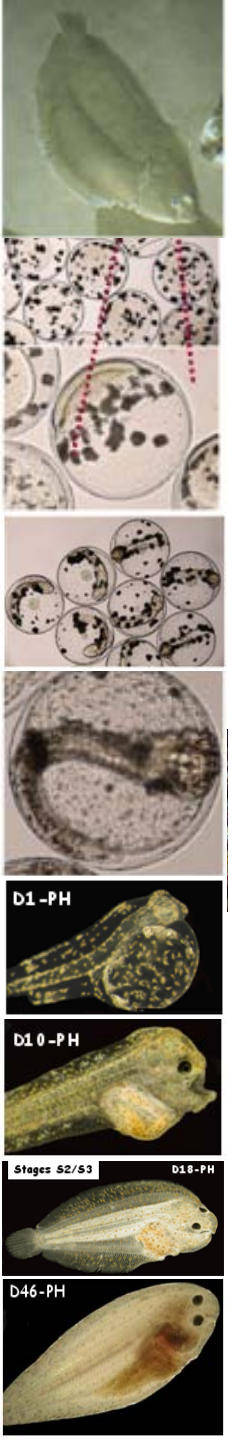
- A similar design was used for sampling in both species and includes embryonic , pre-metamorphic, metamorphic and post-metamorphic stages providing a temporal series.

Hatching

Metamorphosis

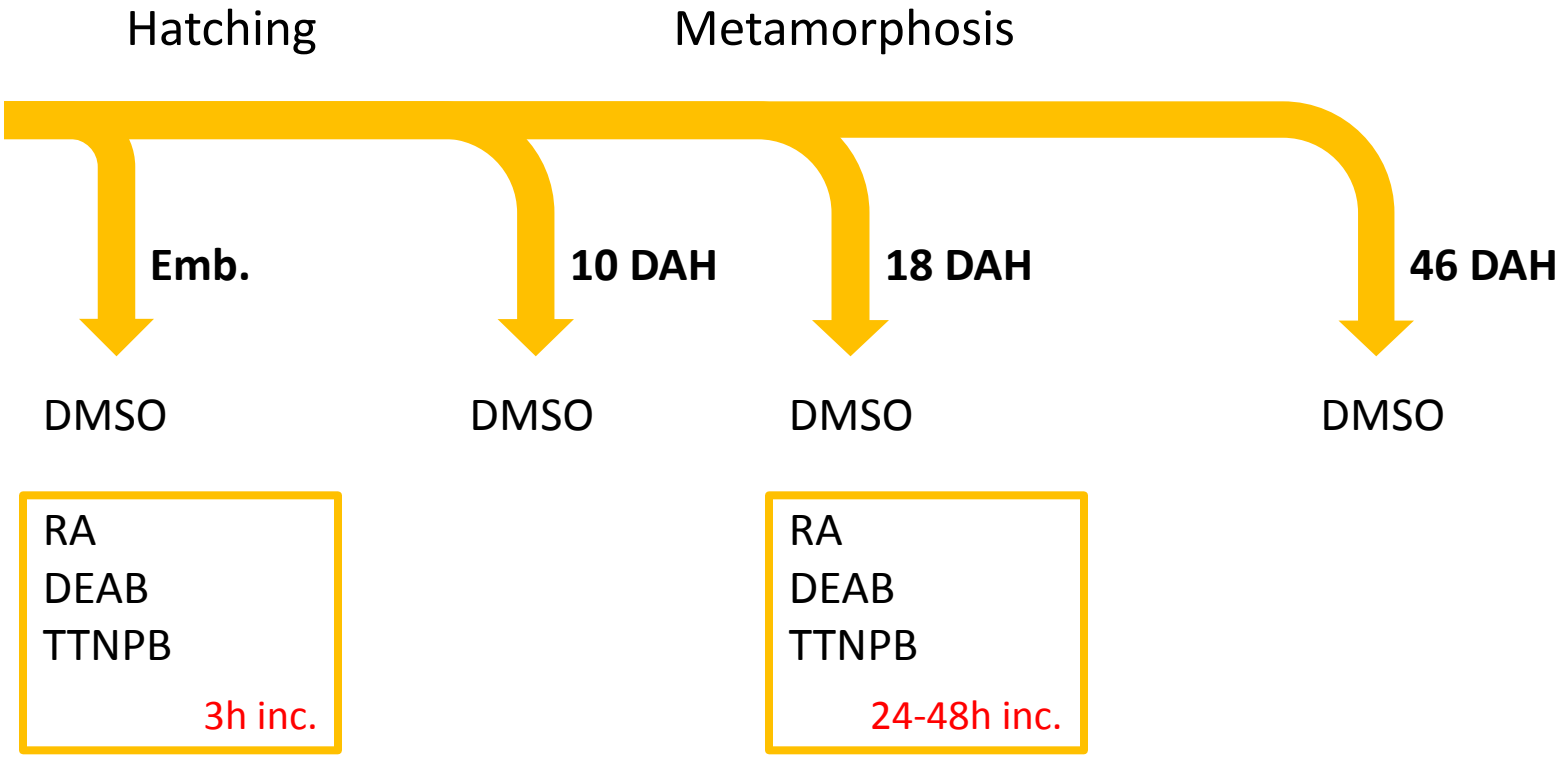


Stages 7/8



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*

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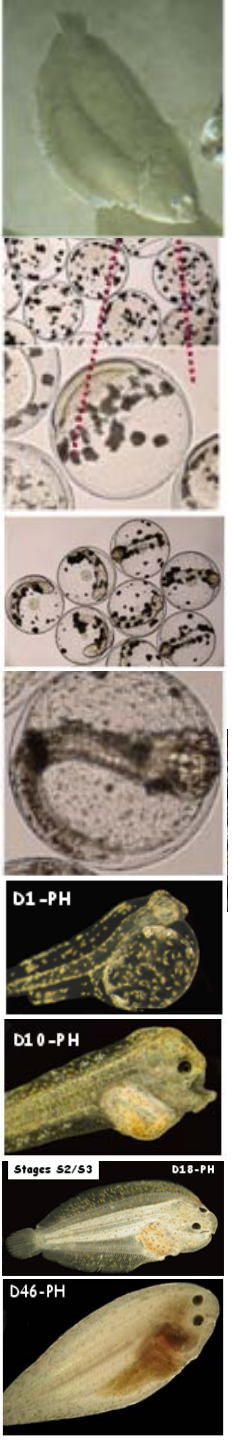
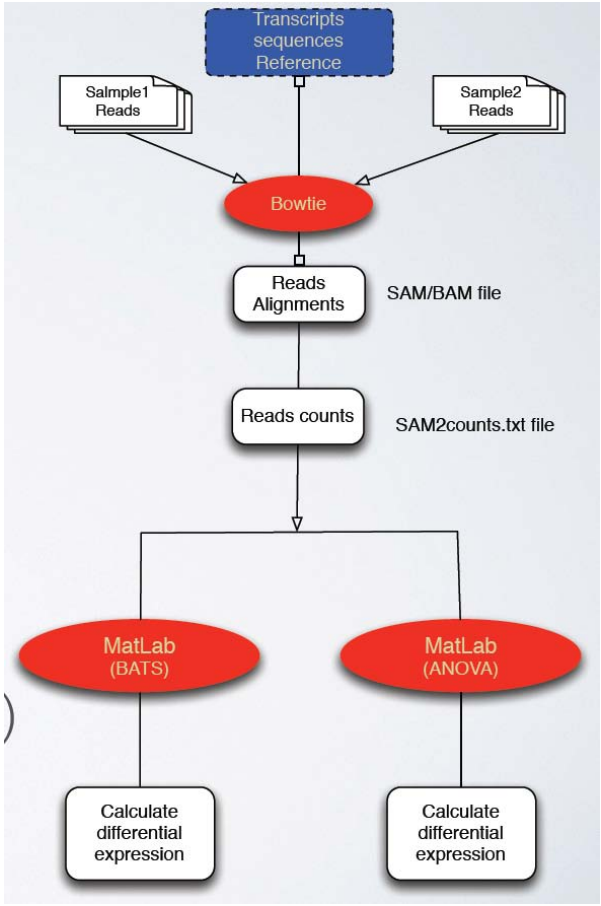


NGS strategy

- Previously (and additional performed on selected tissues) 454 sequencing provided ~250 000 *S. senegalensis* unigenes which were used a reference
- Additional 454 sequencing was performed on selected tissues and provided a total of ~5 10^6 reads
- Illumina sequencing was performed on described samples and provided a total of ~4 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



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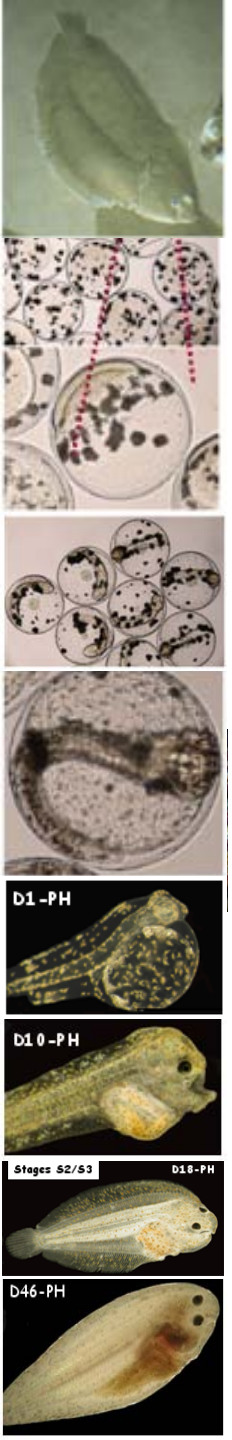

Sequencing data

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		NGS platform		
		Illumina		*454*
		S. senegalensis	*S. solea*	*S. senegalensis*
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	1,562,661
	%	13.5	17.1	26.8
Rejected by contamination	N	144,247,943	226,627,909	156.921
	%	8.2	11.2	3.0
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	3,774,412
	%	3.2	3.3	67.6
Output Mean length		**66**	**89**	**184**

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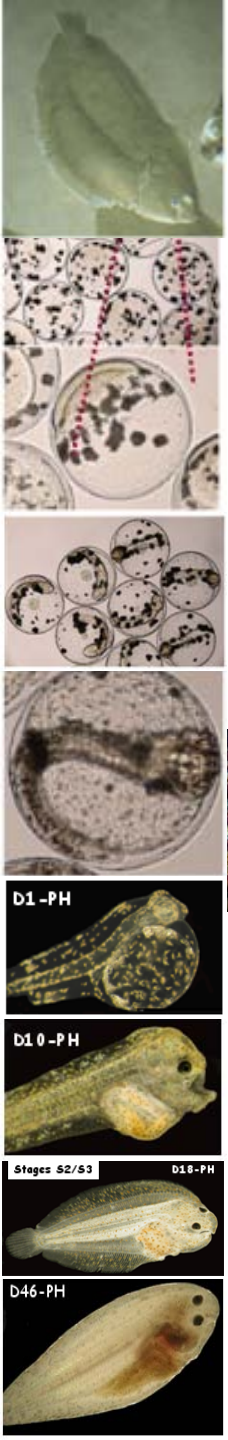

New *Solea sp.* transcriptome

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		S. senegalensis	*S. solea*
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

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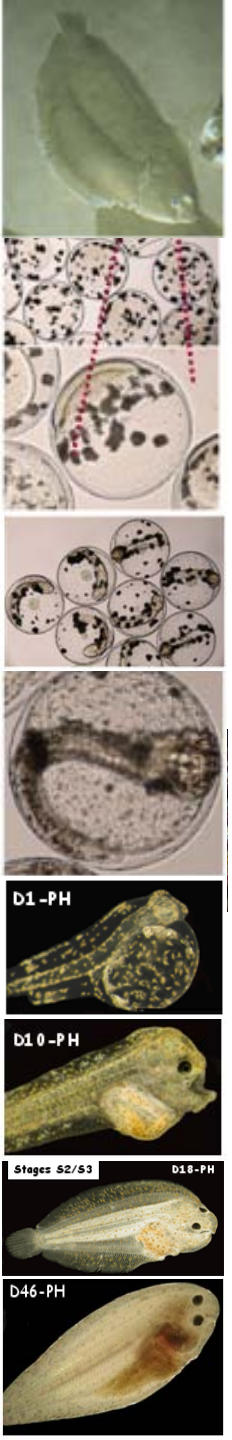

Differential expression

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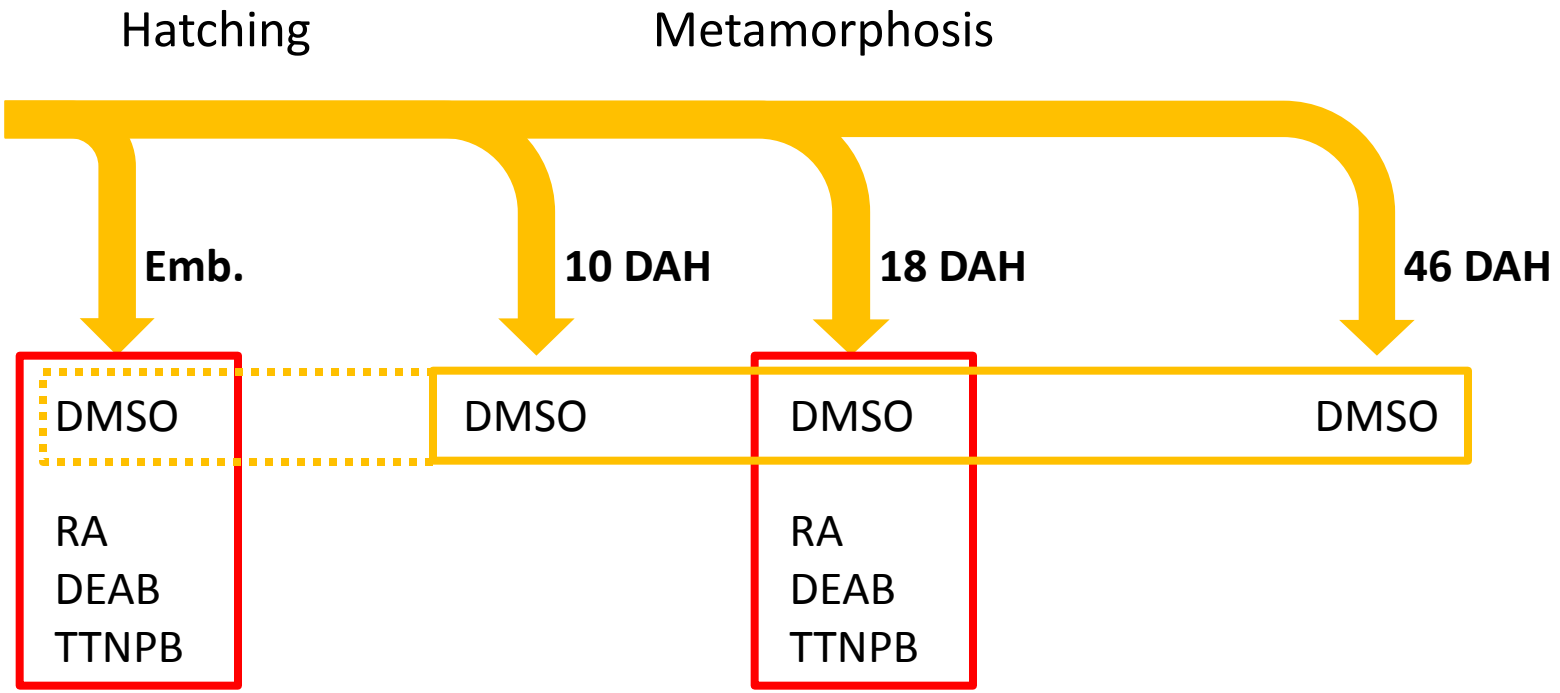
Differential expression (DE) has been analyzed – not yet in full details – between

- treatments at the same stage
- over development



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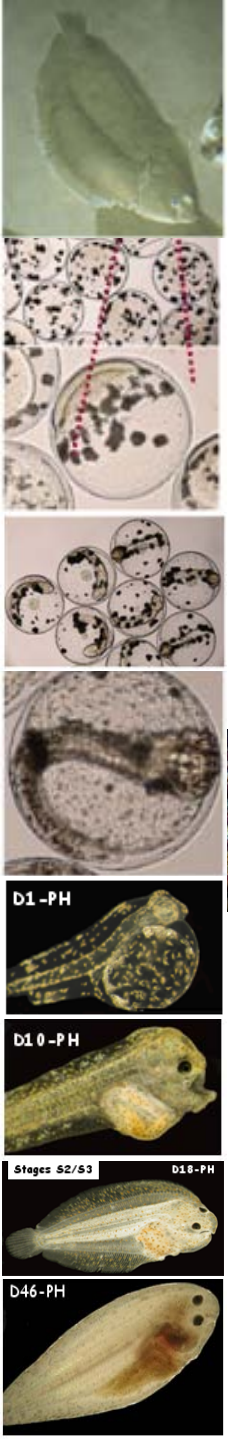
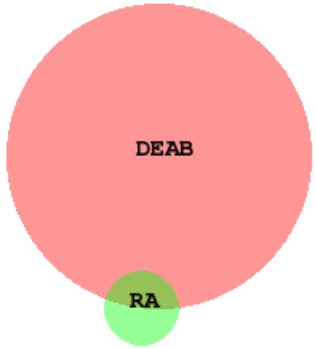


DE / RA pathway larvae

Ifremer

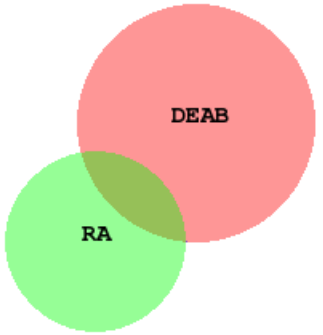
Larvae J18 - 24h treatment



7942 regulated genes

DEAB	7704	DEAB only	7486	97%
RA total	456	RA only	238	52%
		DEAB+RA	218	

Larvae J18 - 48h treatment



6195 regulated genes

DEAB	4228	DEAB only	3737	88%
RA total	2458	RA only	1967	80%
		DEAB+RA	491	

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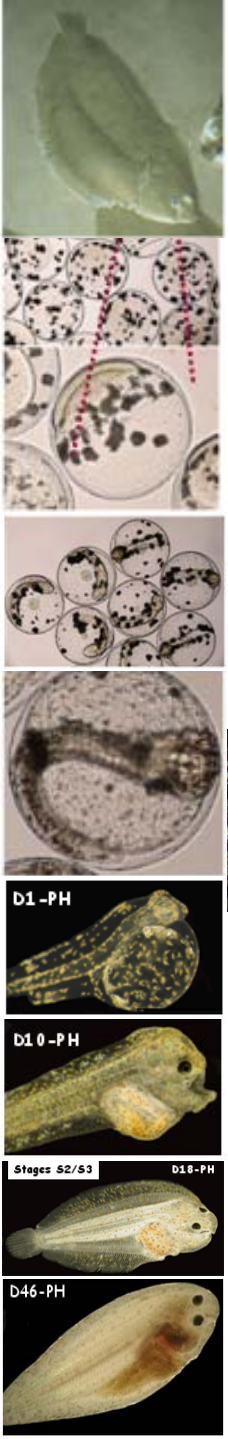

DE / RA pathway larvae

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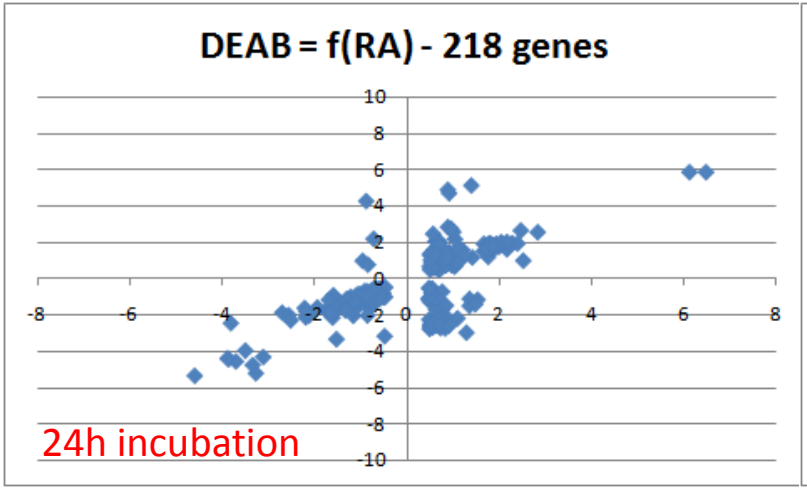

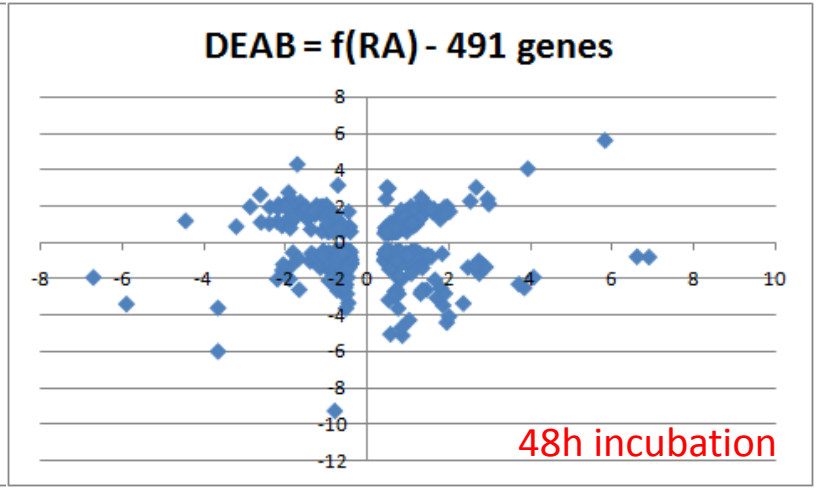

↑ RA ↑ DEAB	↑ RA ↓ DEAB	↓ RA ↑ DEAB	↓ RA ↓ DEAB
93	40	4	81
	44 (20%)		

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

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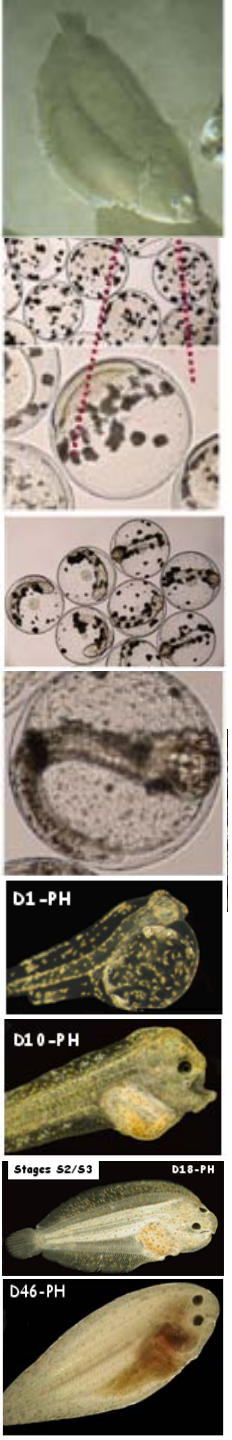
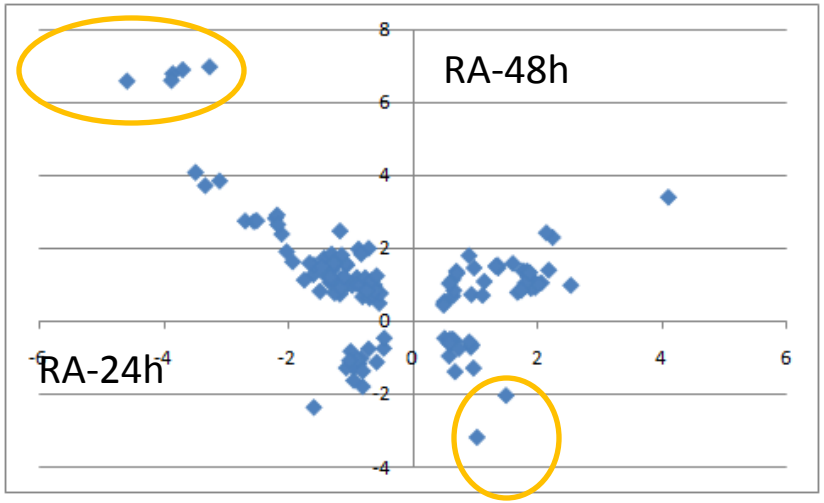


DE / RA pathway larvae

DHRS3
CYP26A1
CYP26B1



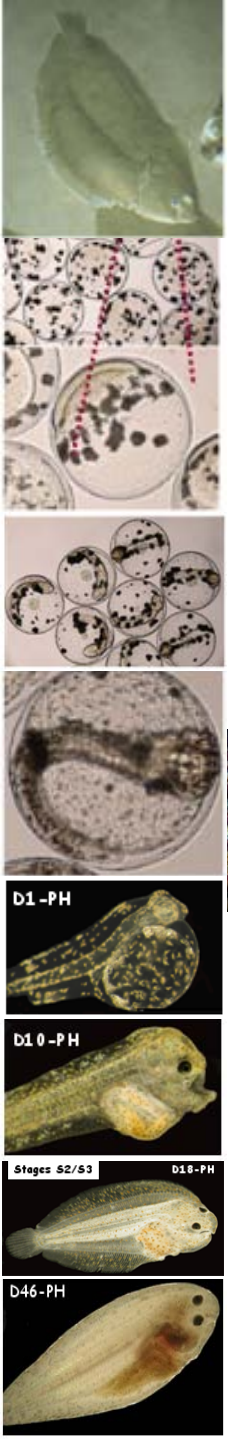
all-trans-retinol 13,14-reductase

atrol r^{ase} cyp26

at-13,14-dihydroretinol ⇄ at-retinol ⇄ at-retinal ⇄ RA ⇄ metabolites

dhrs3

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



DE / RA pathway larvae

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

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

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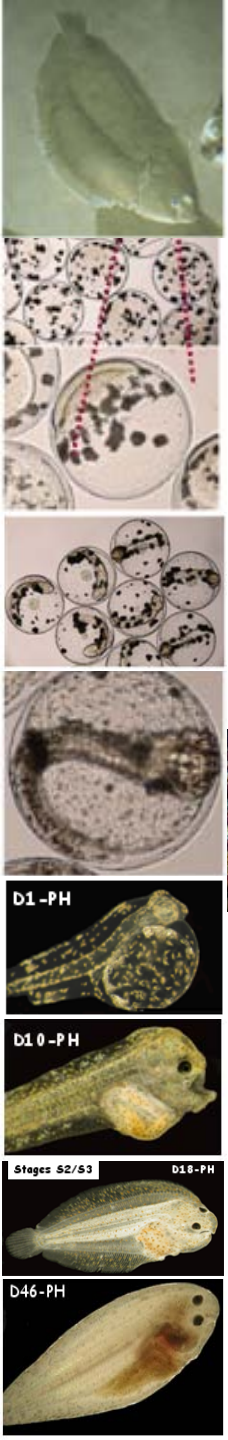

DE / Development series

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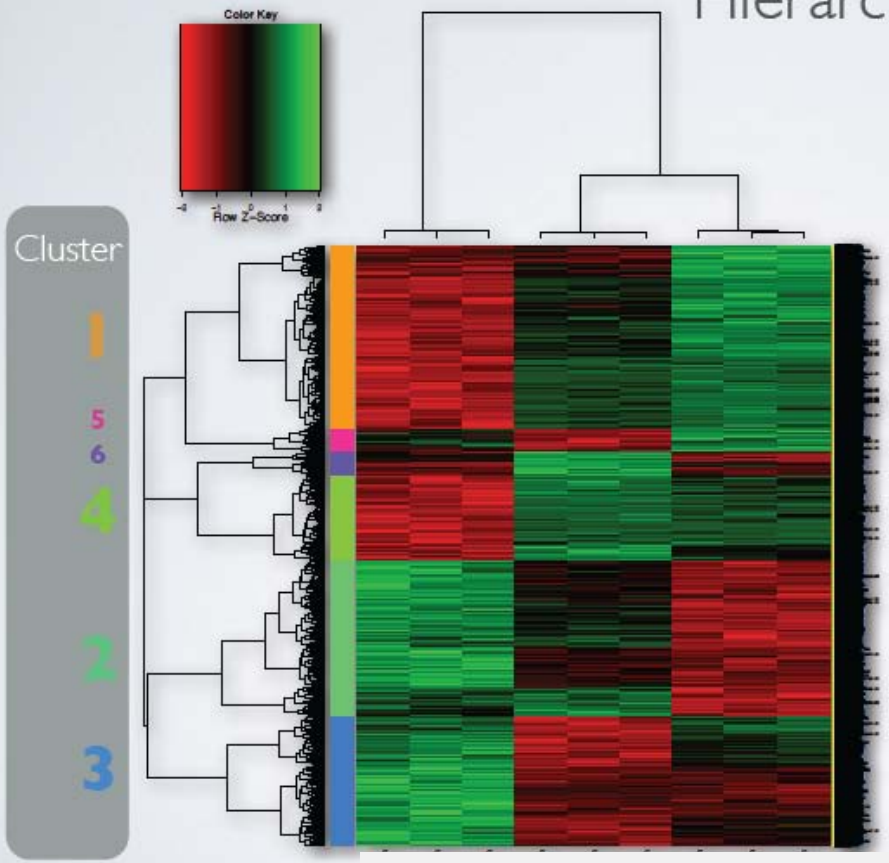
Hierarchical Clustering sorted



10 18 46 DAH

Clustering method: UPGMA
Analysis: correlation

6 different gene clusters can be defined. Unigenes corresponding to each cluster can be identified in the annotation file.

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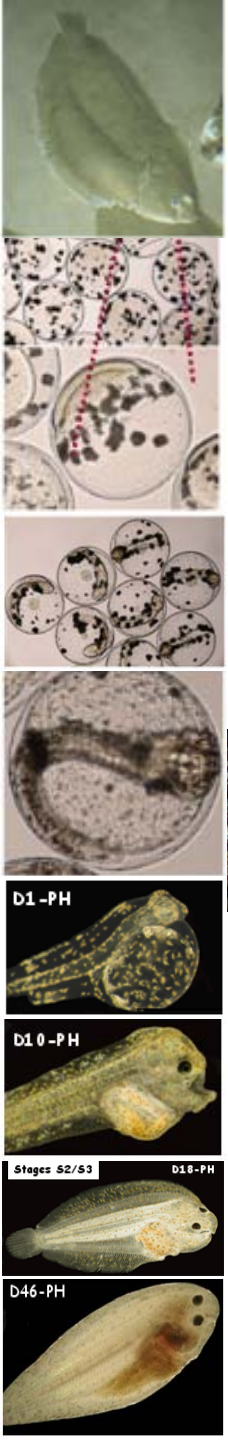

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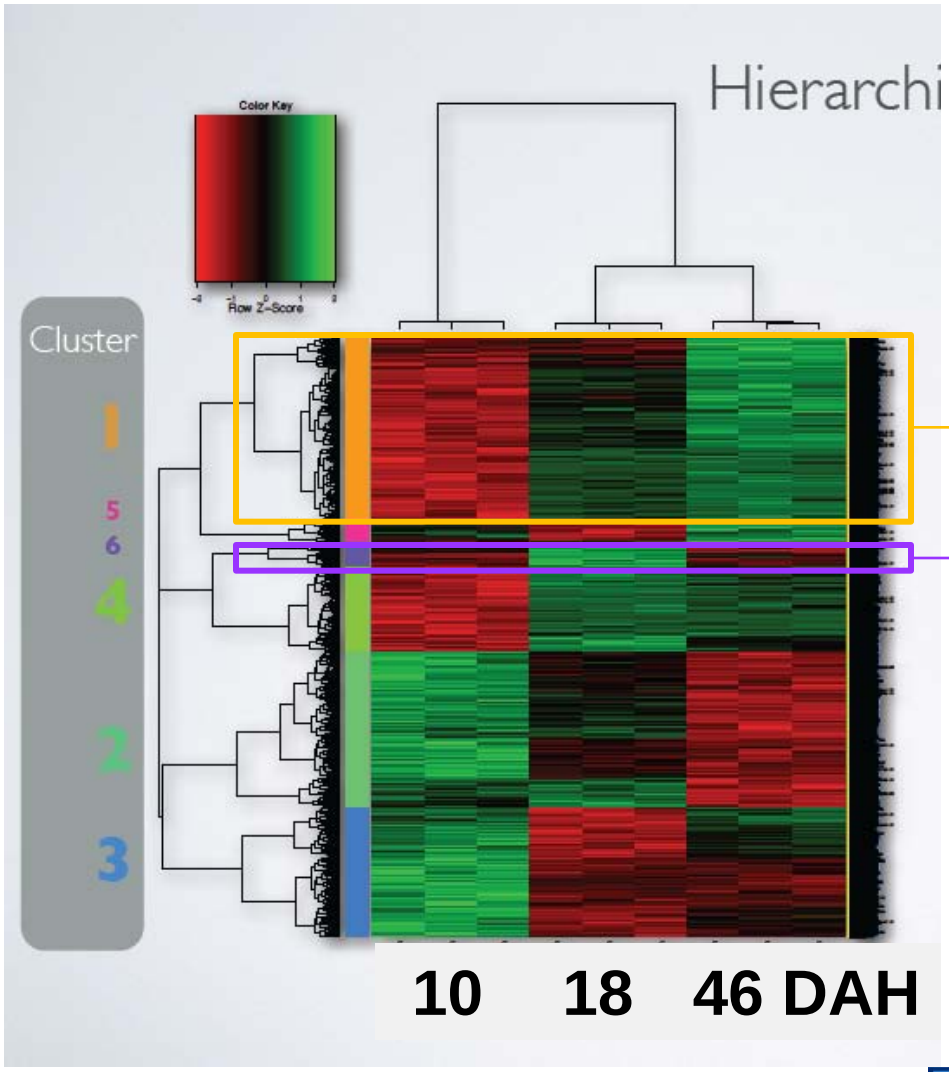

Exemple of clustered genes:

#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

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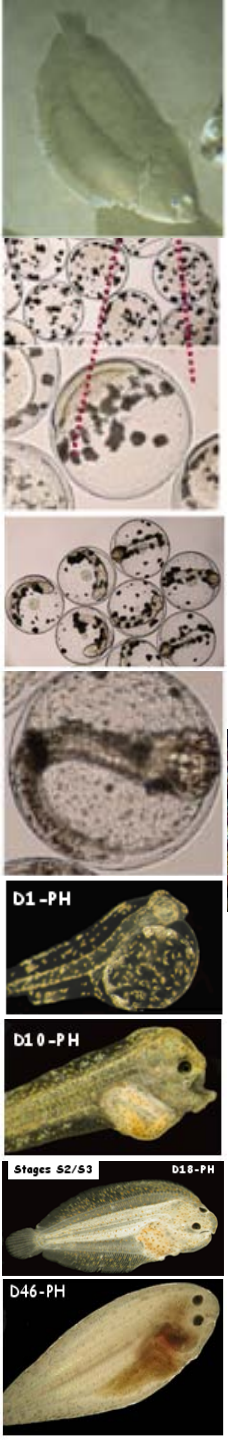

Larvae rearing at low salinity

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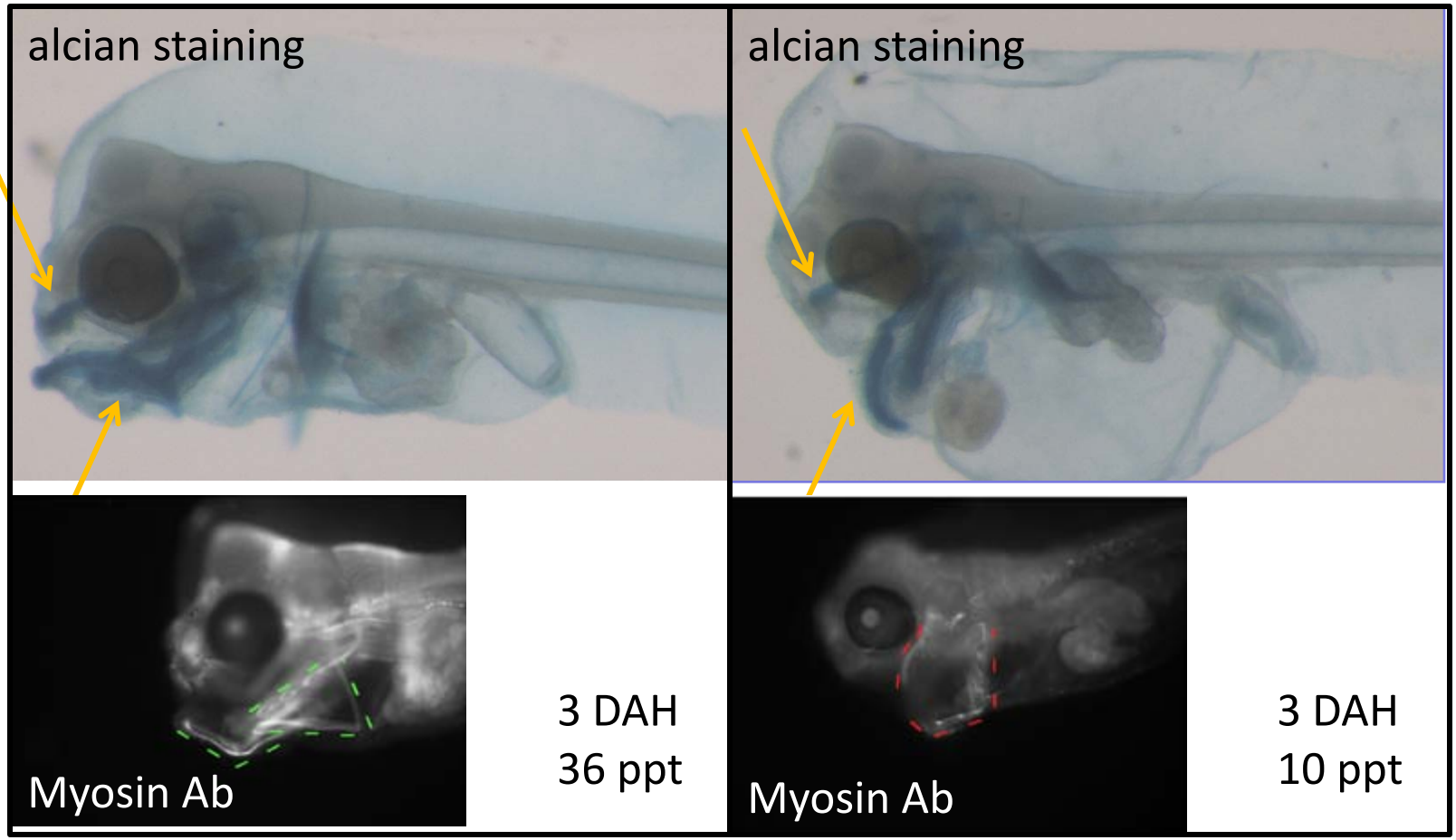
- Low salinity improves larval growth and is more adapted to some rearing practices but generates deformations



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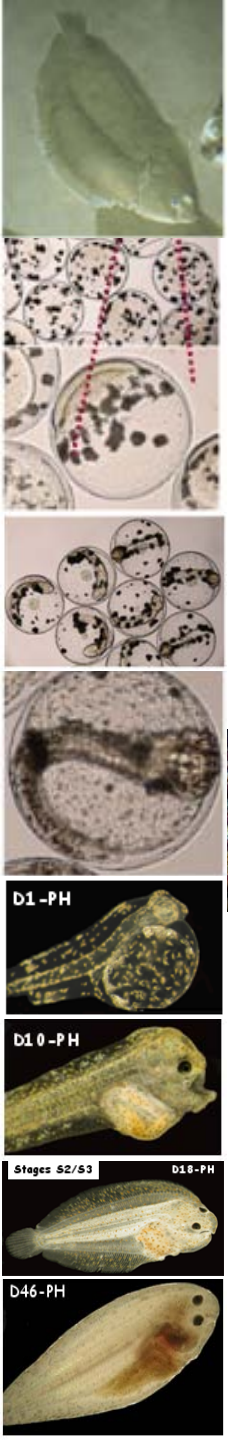

Deciphering mechanisms

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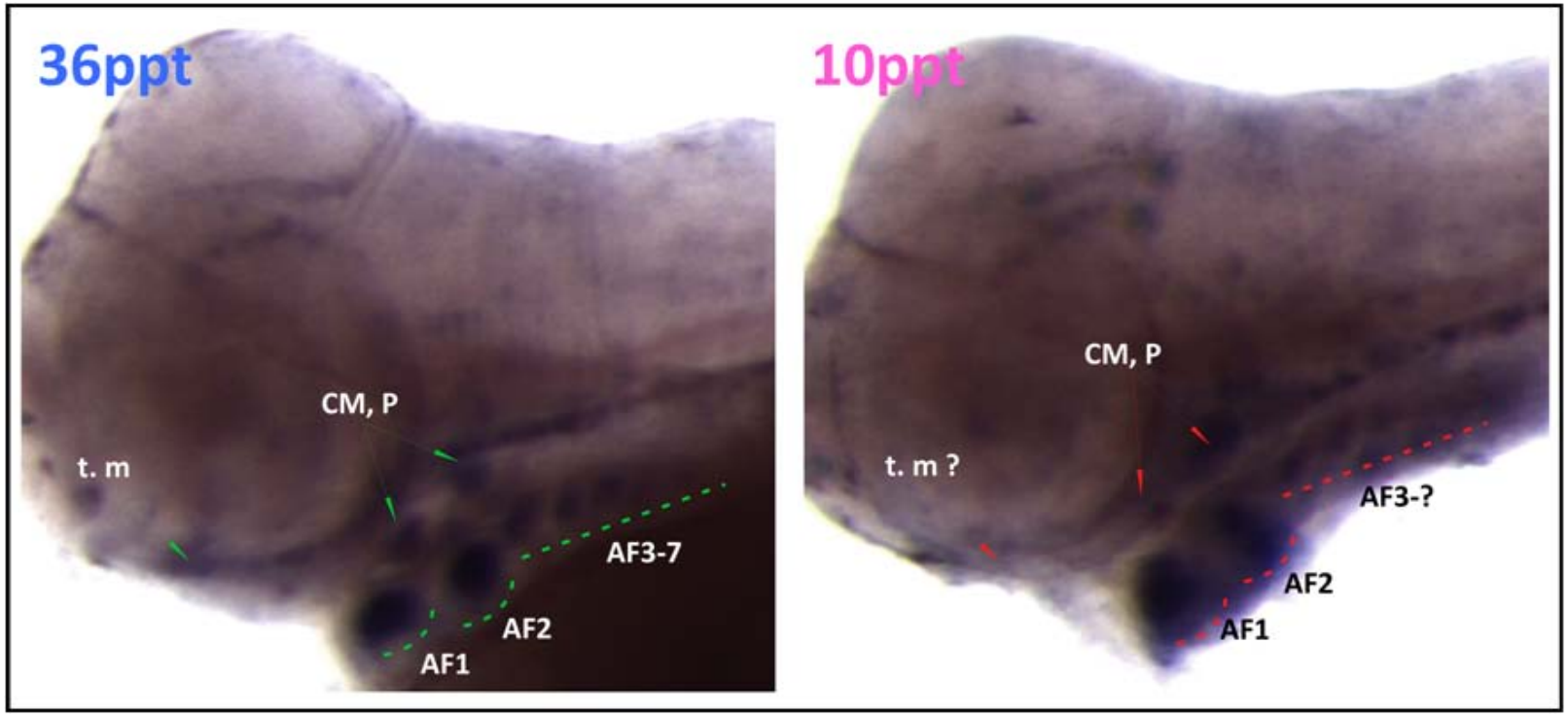
sox9a 1DAH



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

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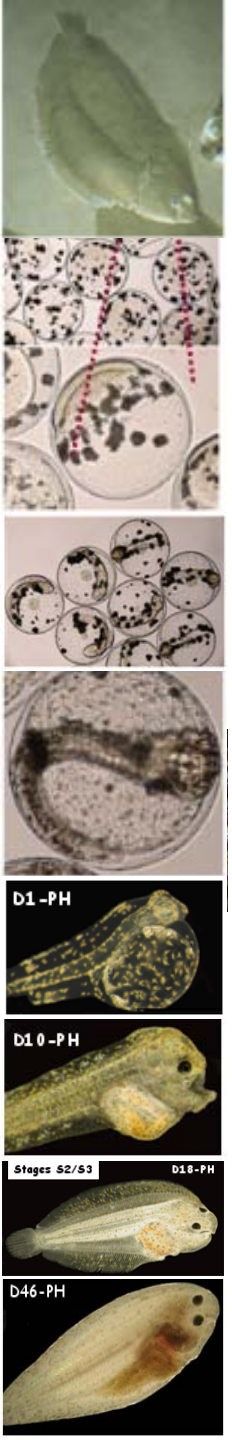

Conclusions

- 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 (µarray, 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

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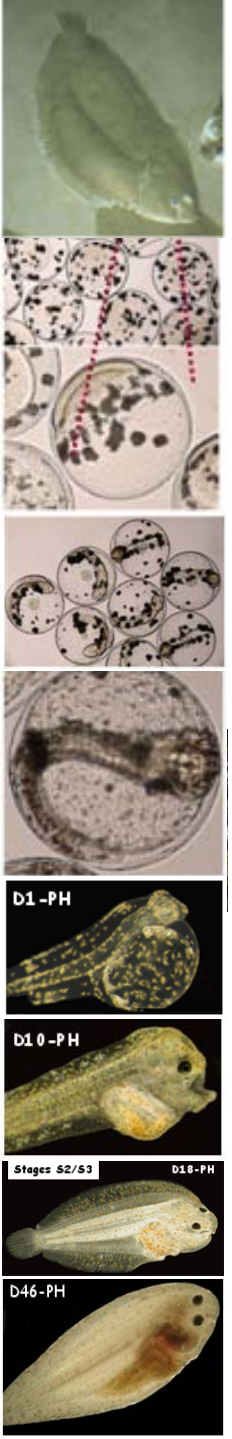

Aknowledgments

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