

# Expression of sex-related genes along the developmental gonad of turbot (*Scophthalmus maximus*) by RT-qPCR

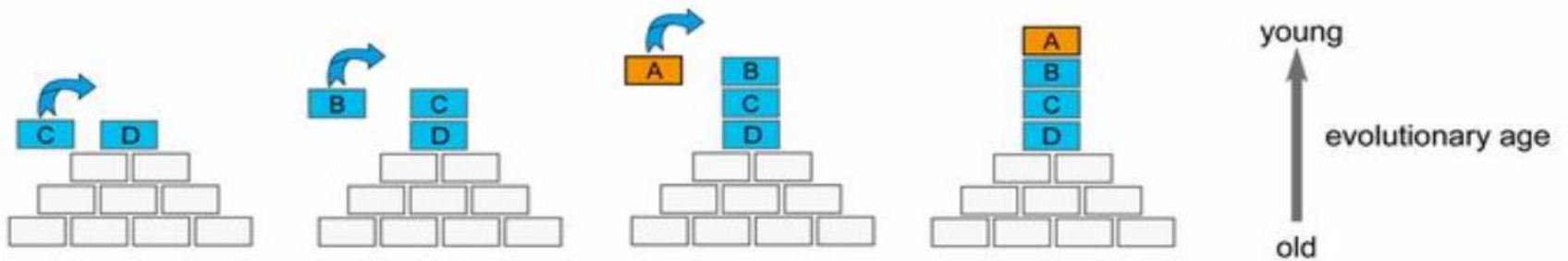
Robledo D, Ribas L, Cal RM, Sánchez L, Piferrer F, Martínez P and Viñas A



An underwater photograph of a coral reef. The water is clear and blue, with sunlight filtering through from the surface, creating a dappled light effect. In the foreground, there are several large, dark, rounded rocks or coral structures. In the background, there are more coral structures and a few small fish swimming. The overall scene is serene and natural.

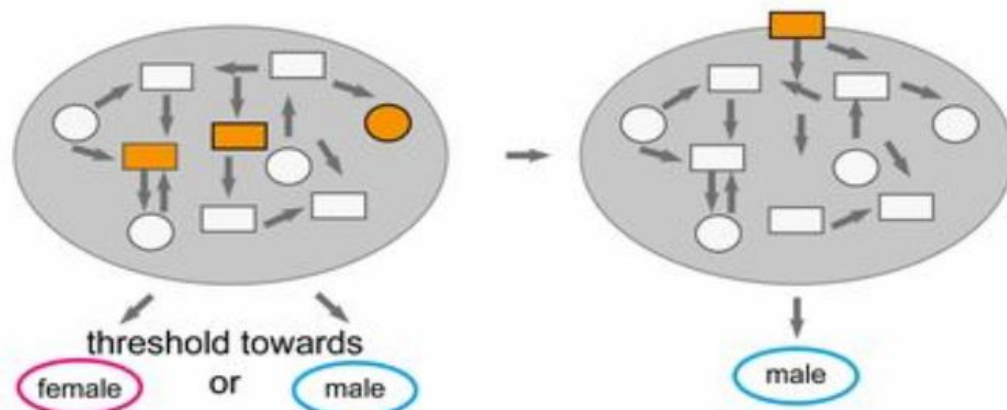
# **Sex Determination AND Sex Differentiation?**

## Bottom-up scenario fitting the classic view: retrograde evolution after Wilkins (1995, 2005)



## Developmental perspective on the evolution of the SD network after (Crews and Bull 2009) and (Uller and Helanterä 2011)

Potential for evolution of a new major effect locus at different levels through natural and sexual selection



Modified from Heule et al. 2014

Genetic (with one major effect locus as possibility), parental or environmental input influencing threshold

# Turbot

(*Scophthalmus maximus*)



- ZZ/ZW sex determination system
- Main sex determining QTL in LG5 + three secondary sex determination QTLs
- Temperature effects on sex ratios have been reported (Haffray et al. 2009)



# Sexual dimorphism



# Experimental design

3 temperatures

23°C 

18°C 

15°C 

6 sampling points

Genetically sexed

Histologically sexed

60

75

90

105

120

135

Fertilization

Days post fertilization

# Experimental design

## 3 temperatures

23°C



18°C



15°C



TOTAL

6 sampling points x 3 temperatures x  
10 samples / sampling point &  
temperature = 180 samples

## 6 sampling points

Genetically sexed

60

75

90

Histologically sexed

105

120

135

Fertilization

Days post fertilization

# Expression analysis: qPCR

## 29 Studied genes:

### - Target genes:

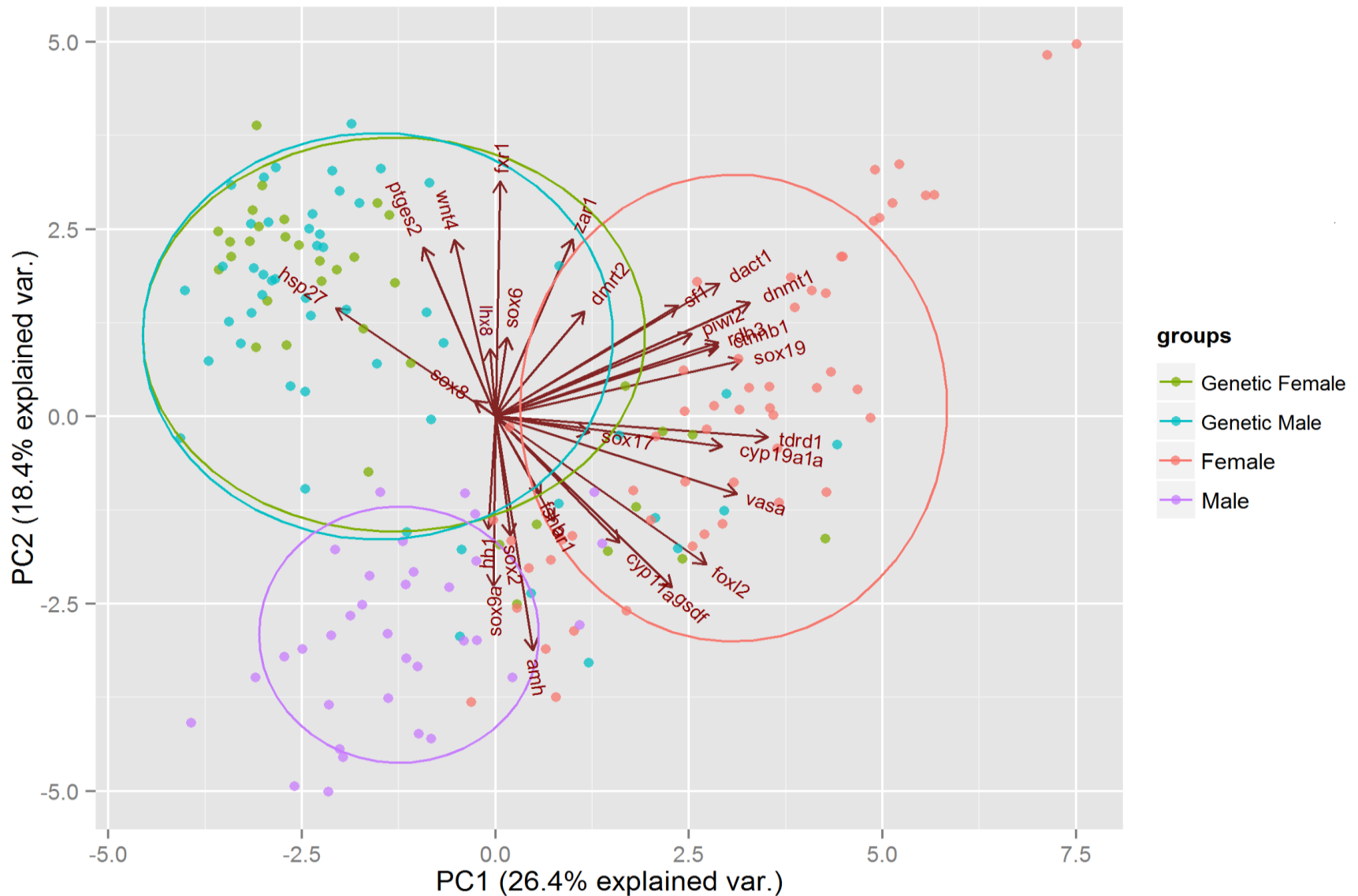
<i>Sox2</i>	<i>Cyp19a1a</i>	<i>Amh</i>	<i>Vasa</i>	<i>Dnmt1</i>
<i>Sox6</i>	<i>Foxl2</i>	<i>Ar1</i>	<i>Tdrd1</i>	<i>Sf1</i>
<i>Sox8</i>	<i>Cttnb1</i>	<i>Dmrt2</i>	<i>Gsdf</i>	<i>Zar1</i>
<i>Sox9a</i>	<i>Wnt4</i>	<i>Cyp11a</i>	<i>Piwil2</i>	<i>Fxr1</i>
<i>Sox17</i>	<i>Fshb</i>	<i>Lhx8</i>	<i>Rdh3</i>	<i>Hsp27</i>
<i>Sox19</i>	<i>Dact1</i>	<i>Hh1</i>	<i>Ptges3</i>	

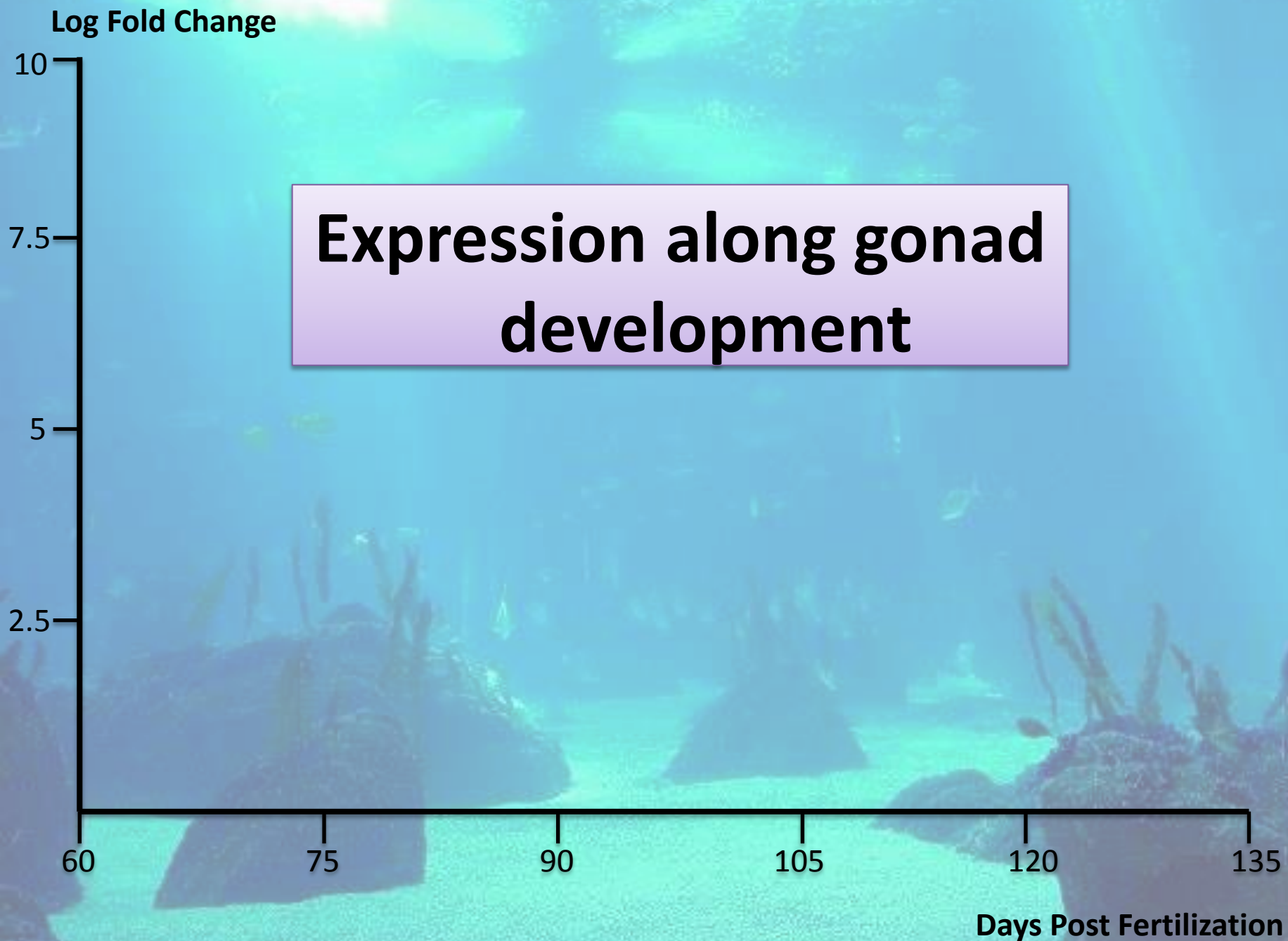
### - Reference genes:

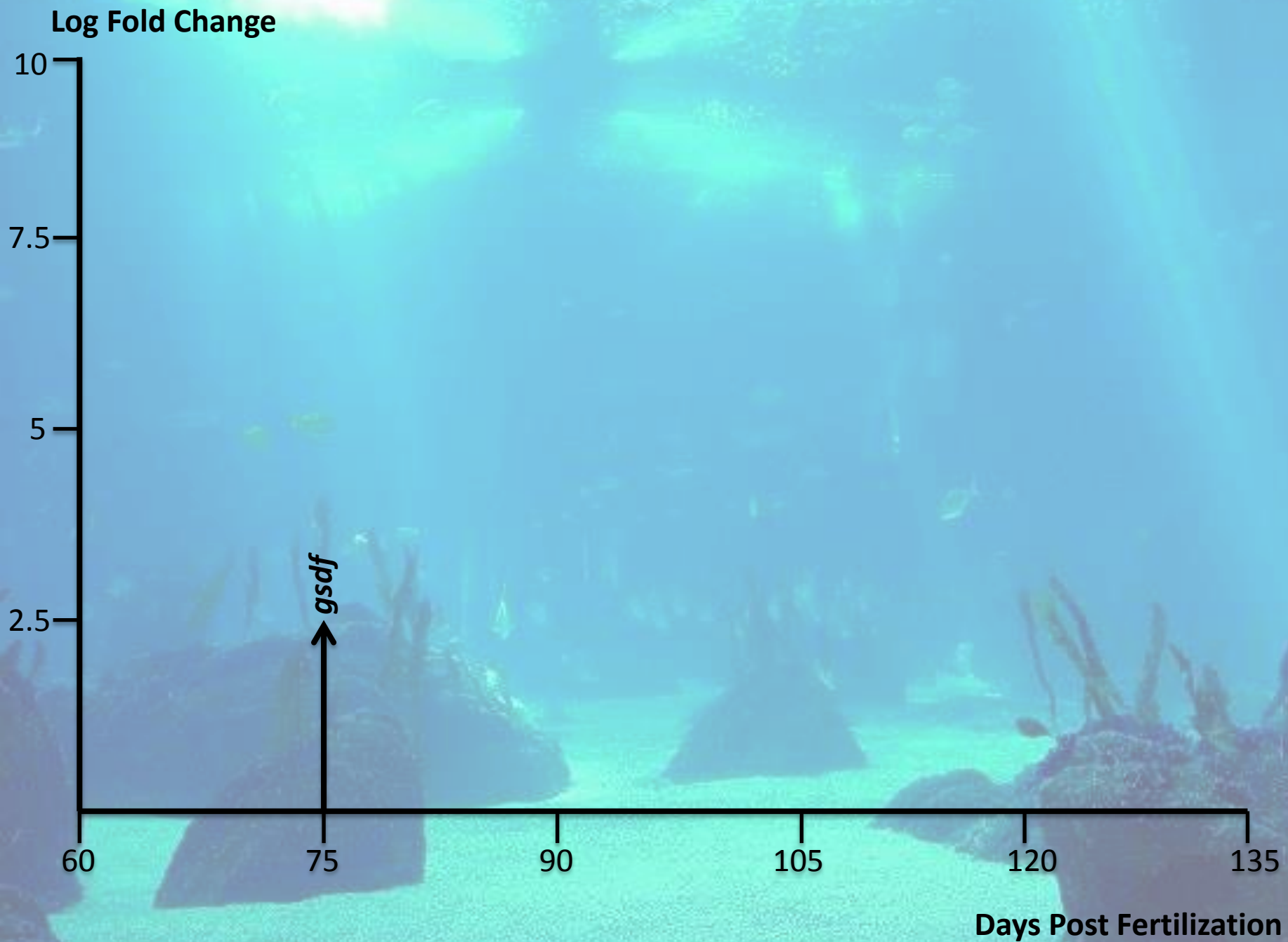
*Rpl17*  
*Rps4*  
*Ubq*

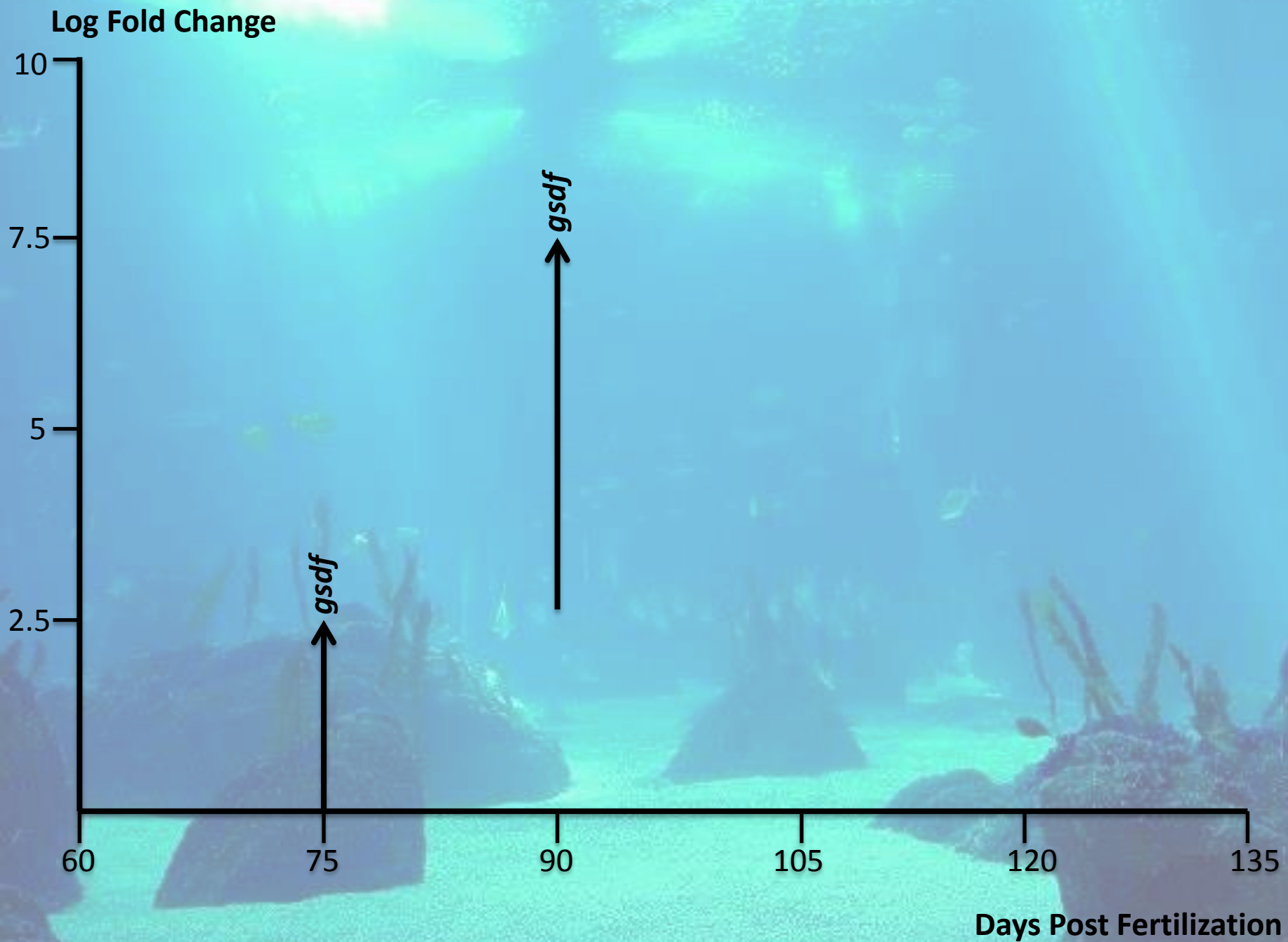


# Principal component analysis

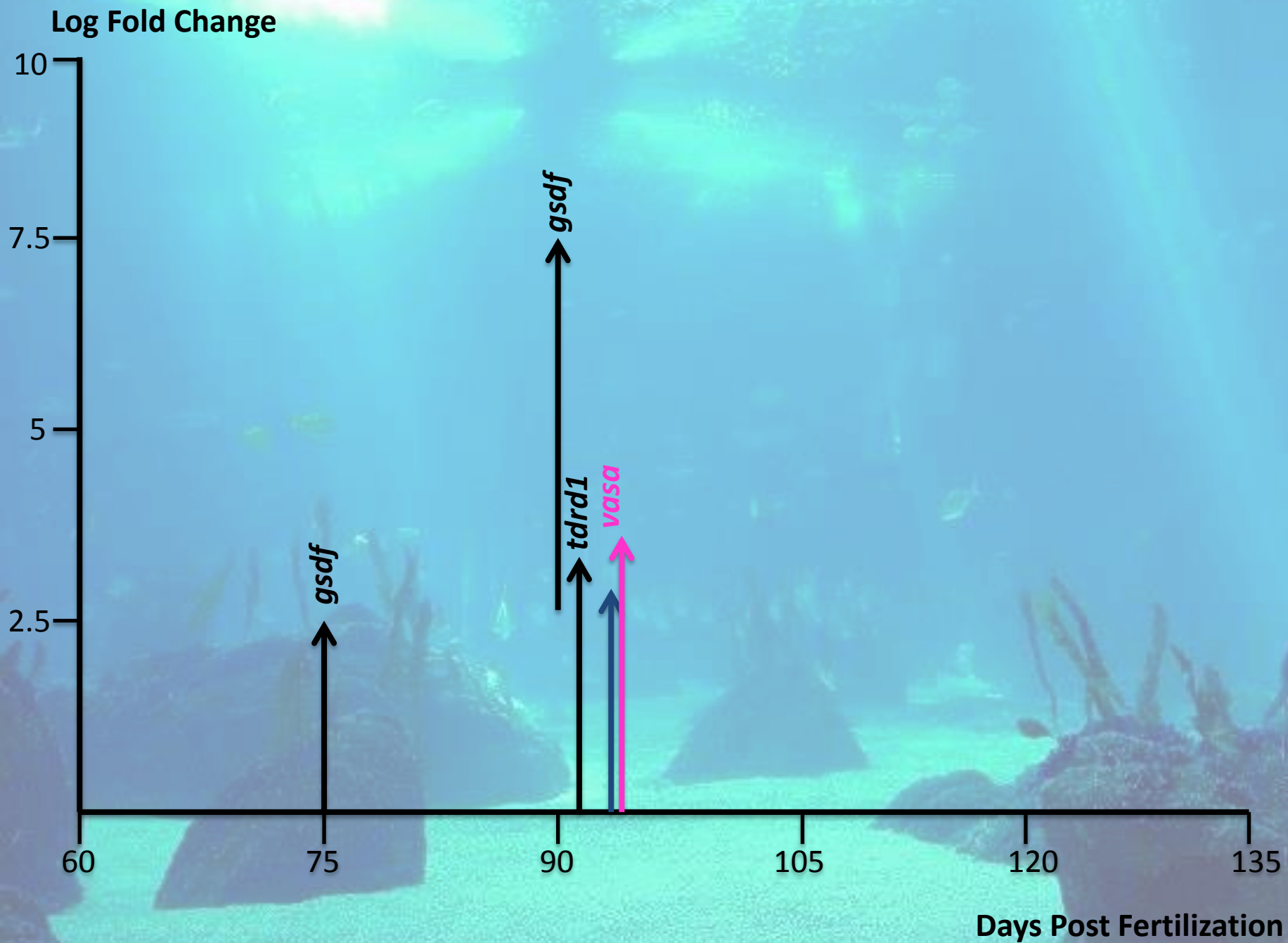


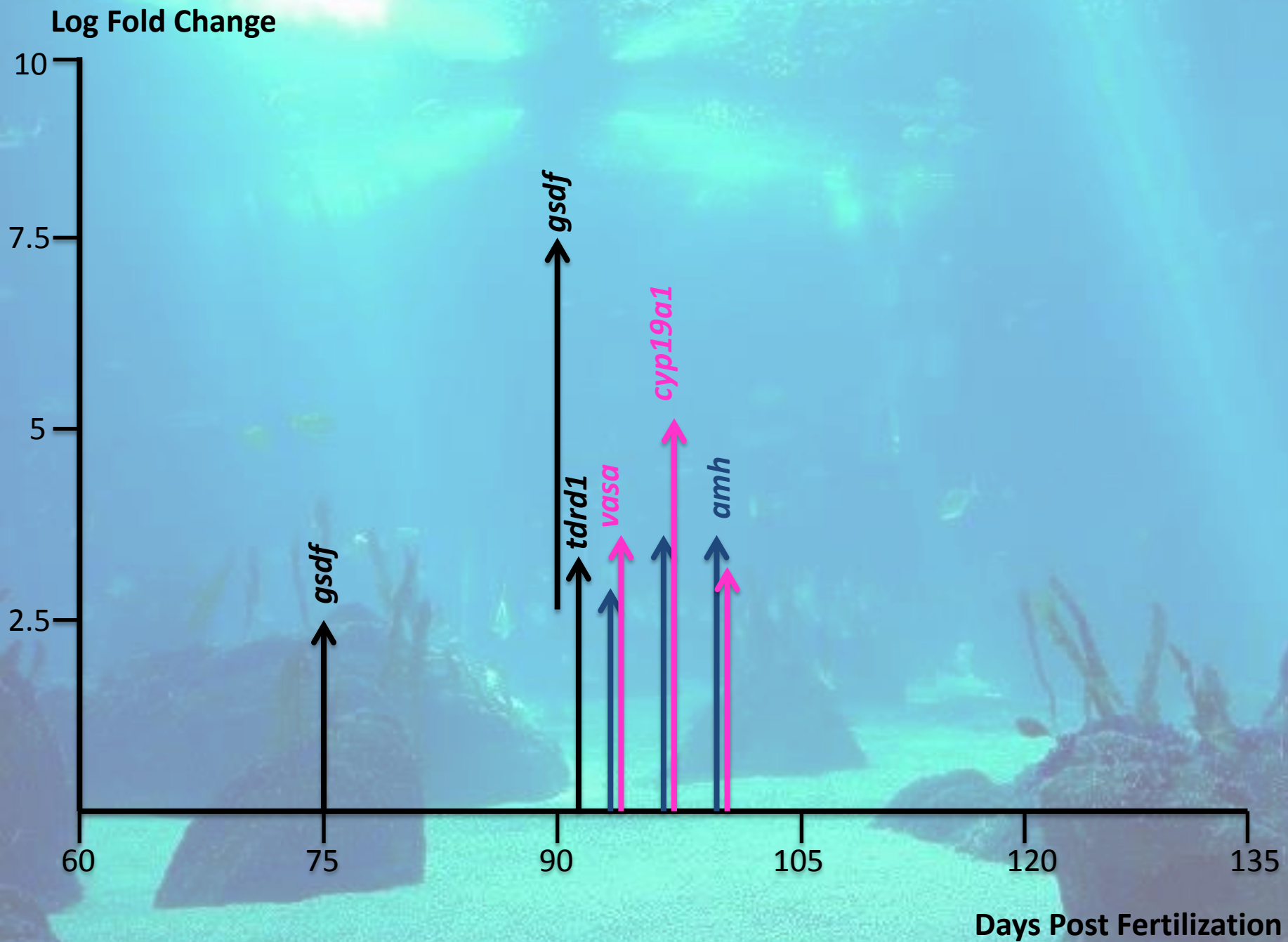




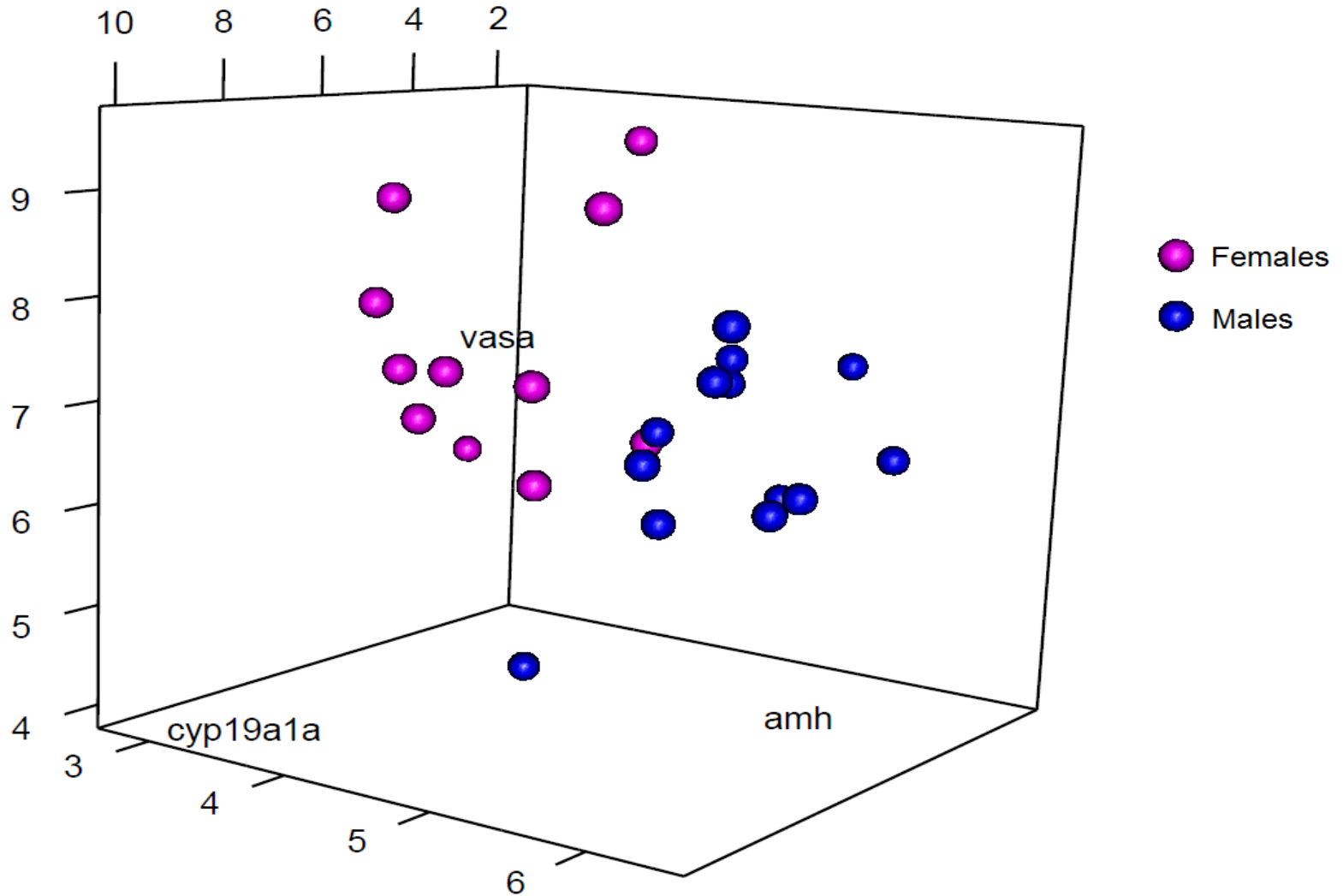




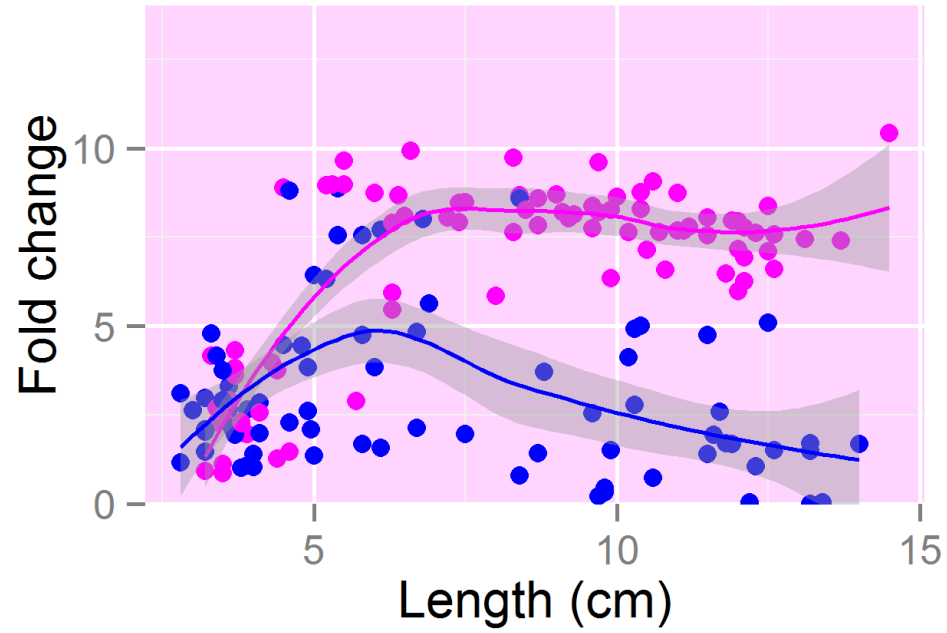




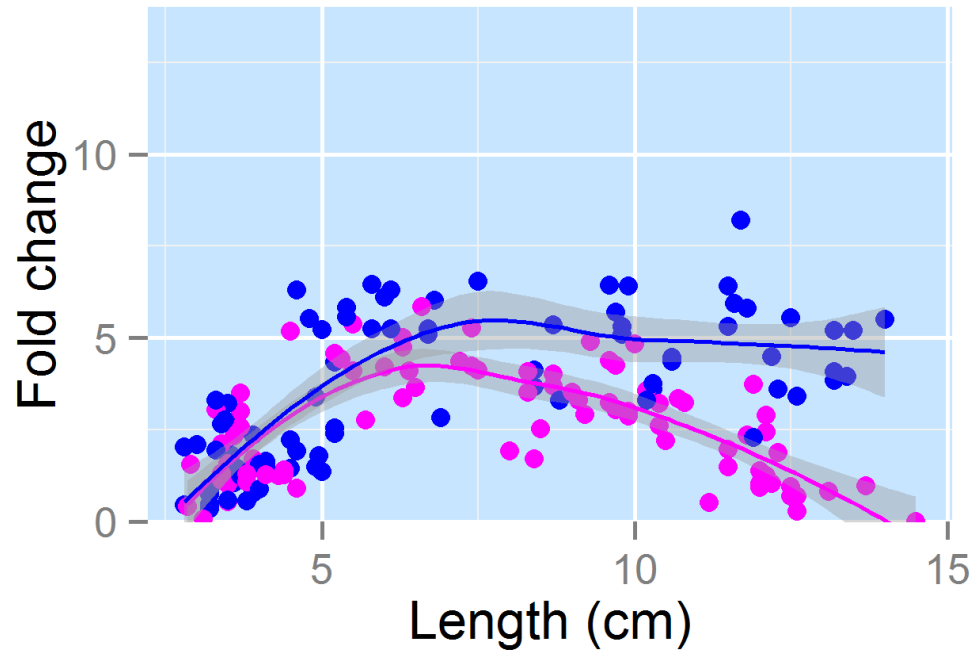
# Discriminant analysis



***cyp19a1a***

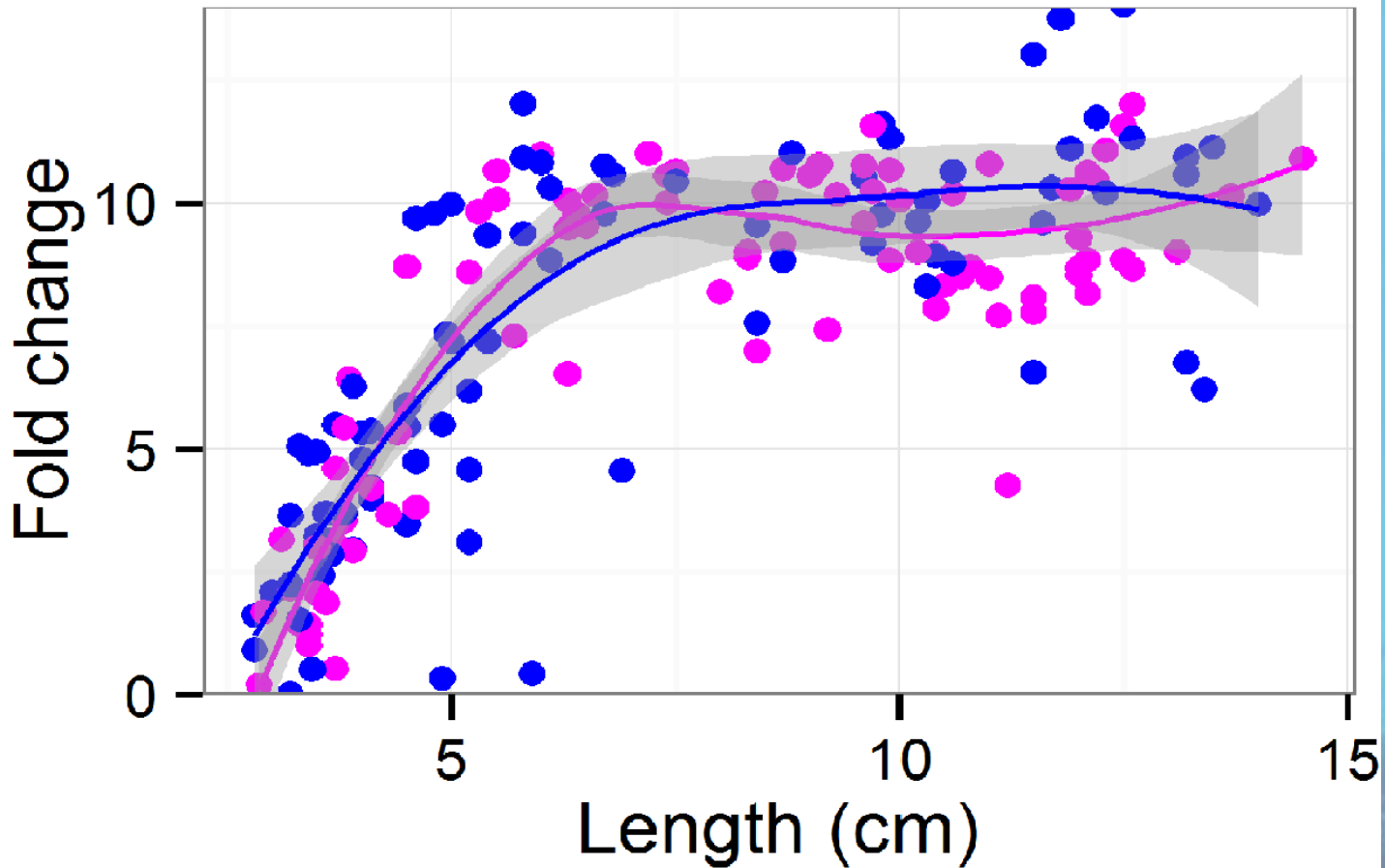


***amh***

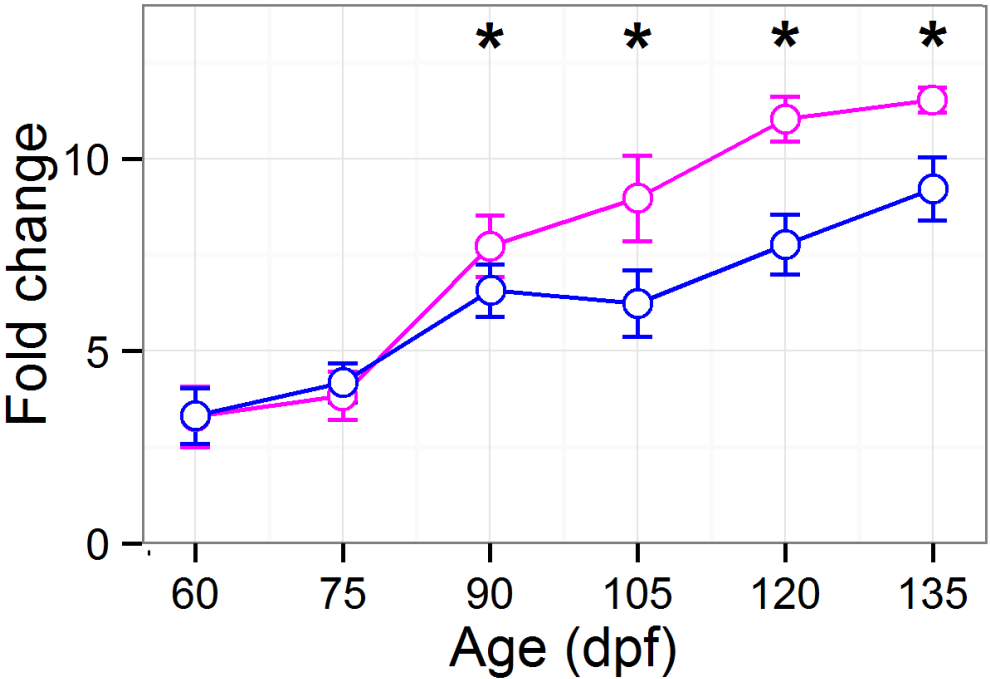




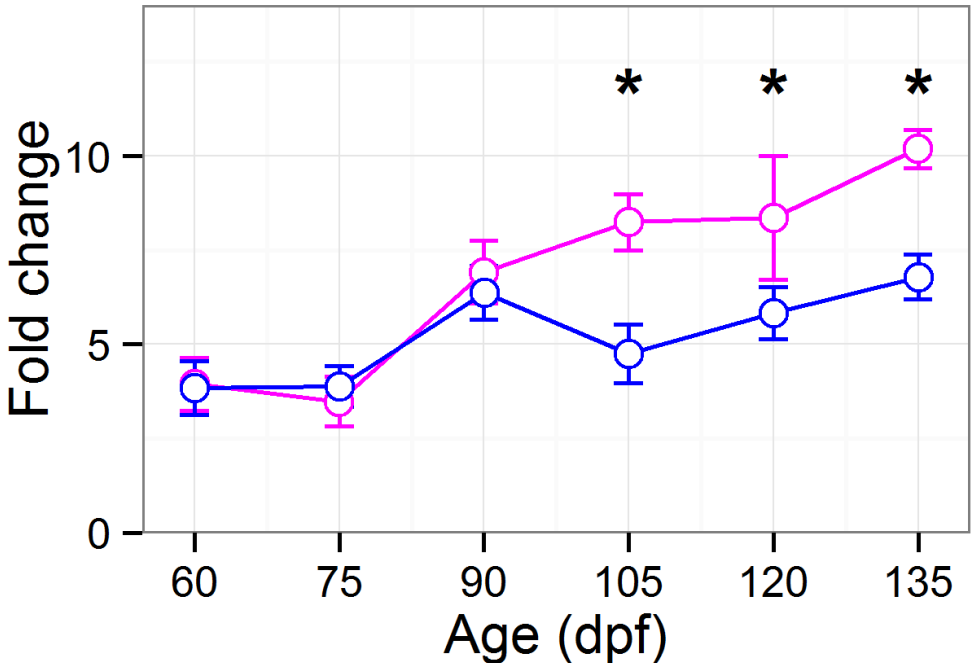
***gsdf***



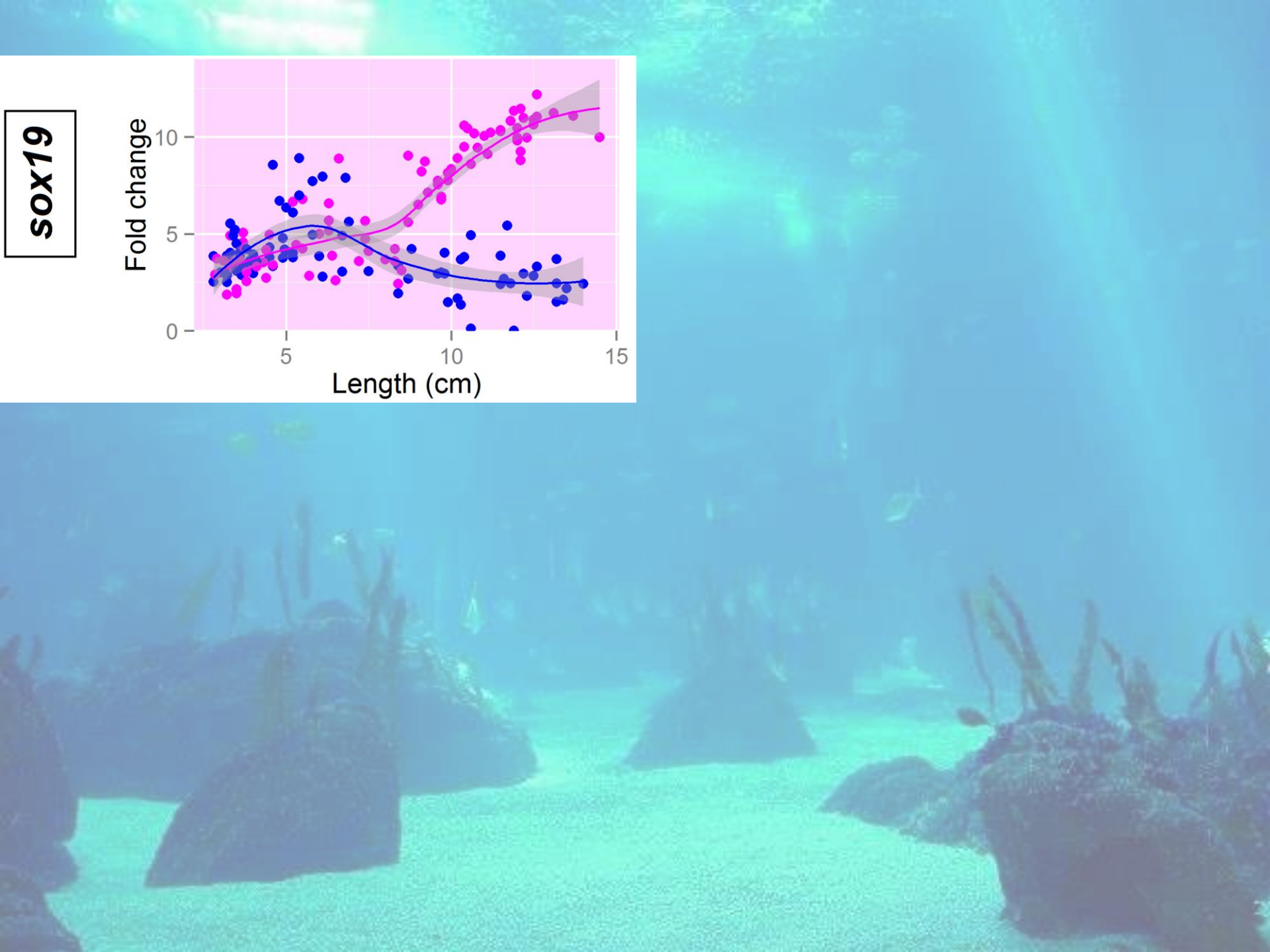
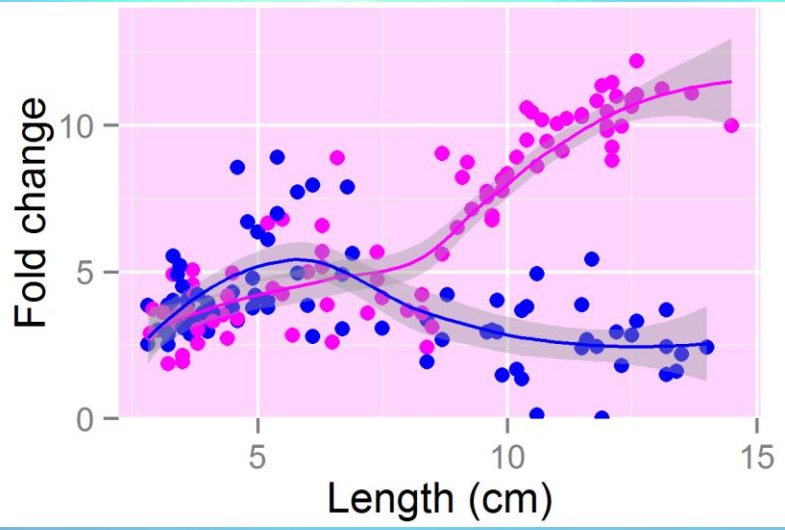
***vasa***



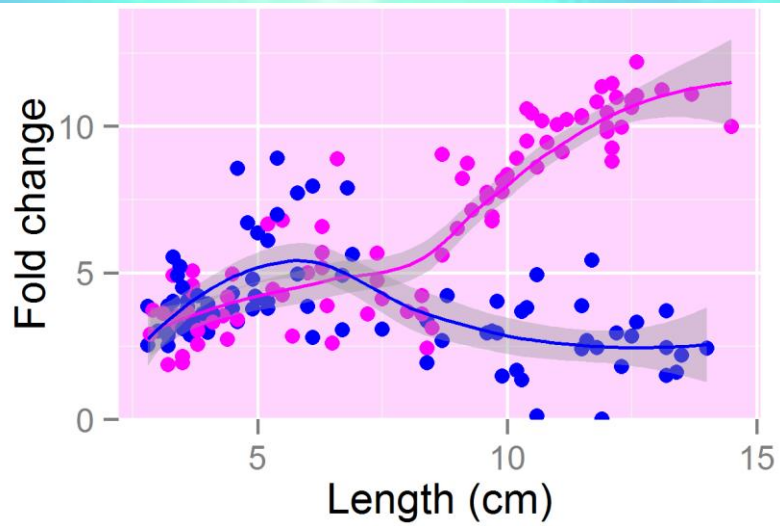
***tldr1***



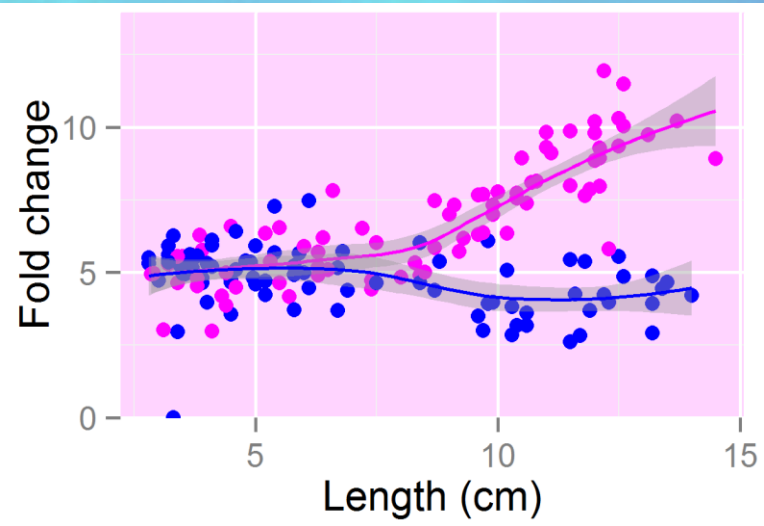
**sox19**



**sox19**

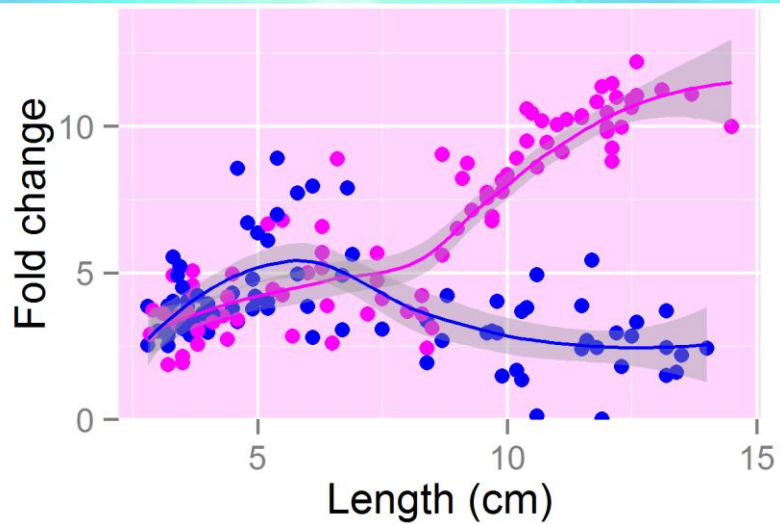


**dnmt1**

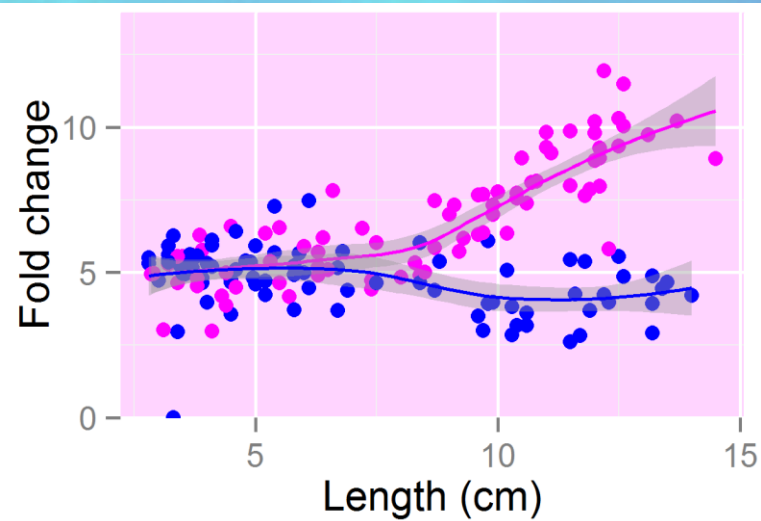




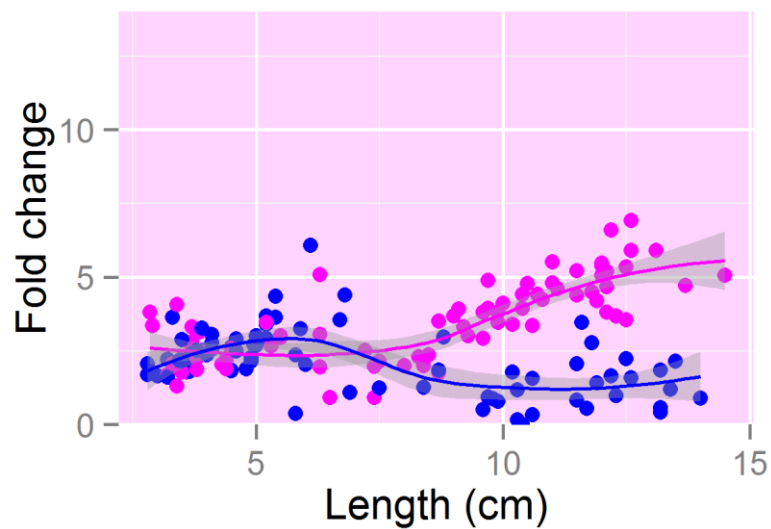
**sox19**



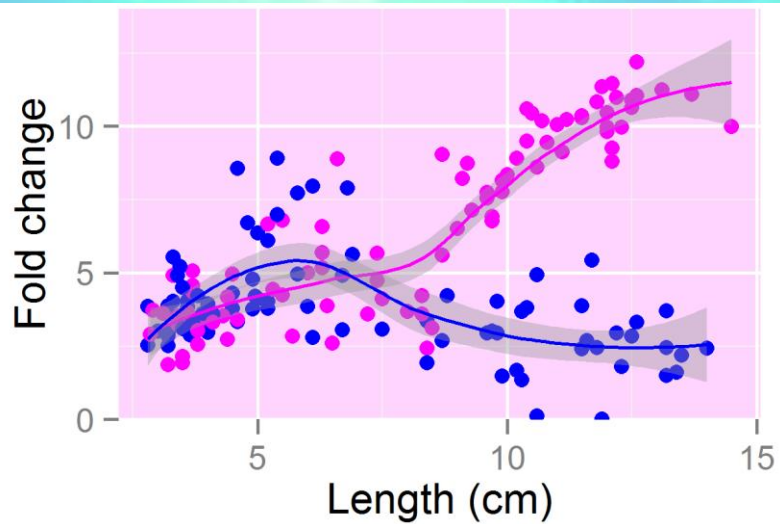
**dnmt1**



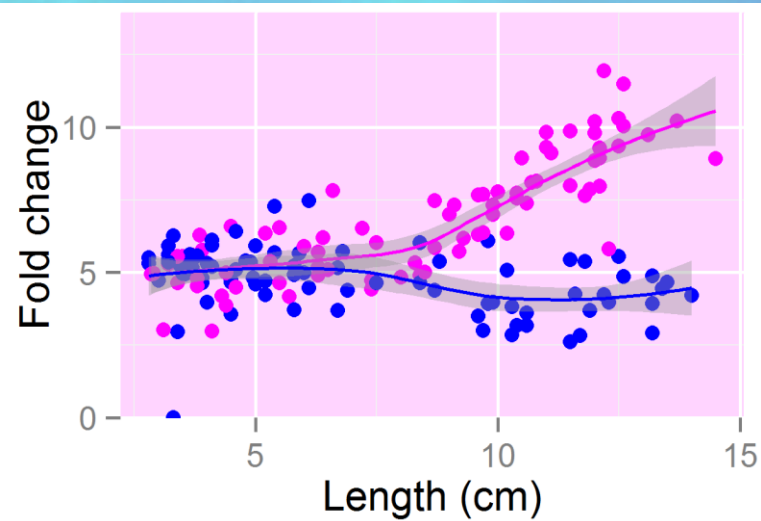
**dact1**



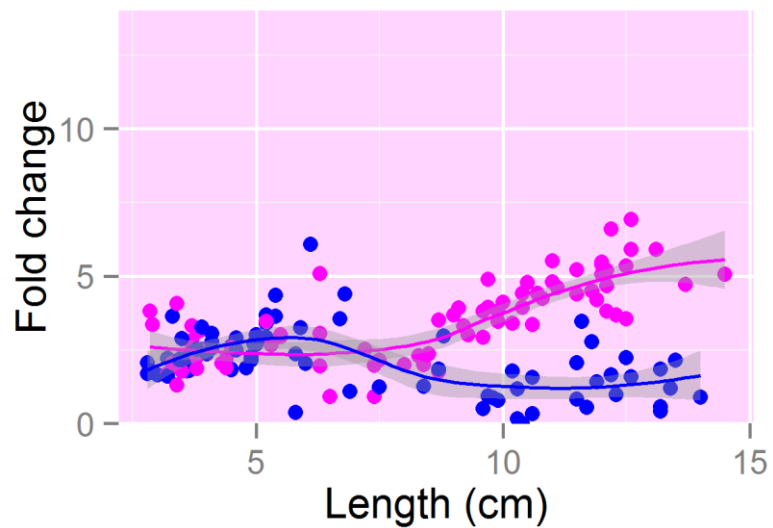
**sox19**



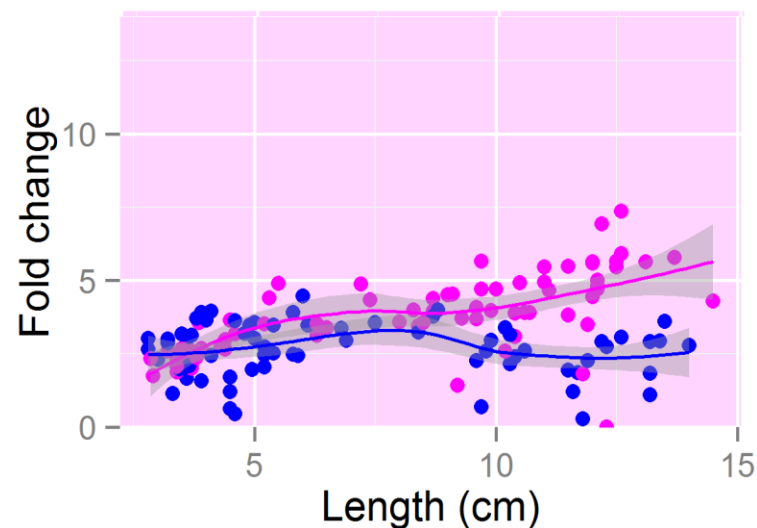
**dnmt1**

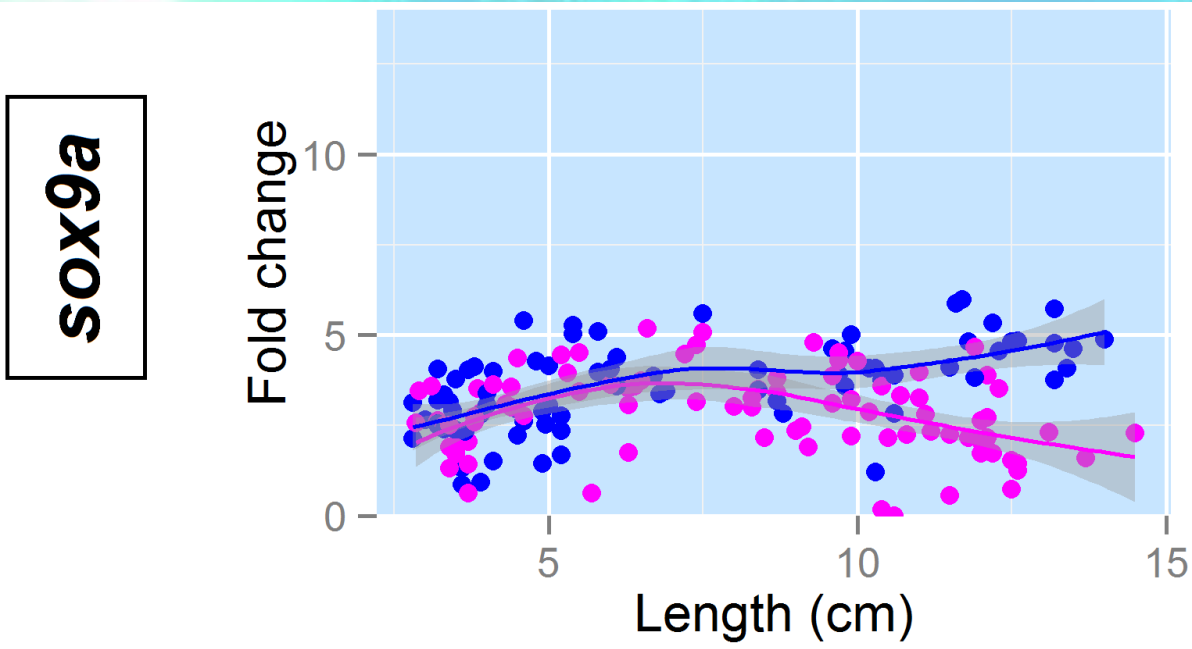


**dact1**

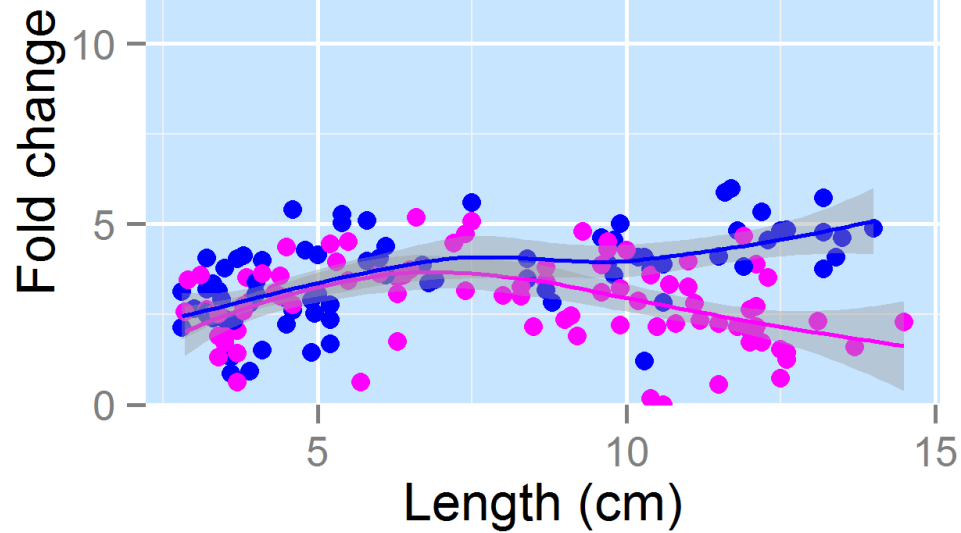


**ctnnb1**

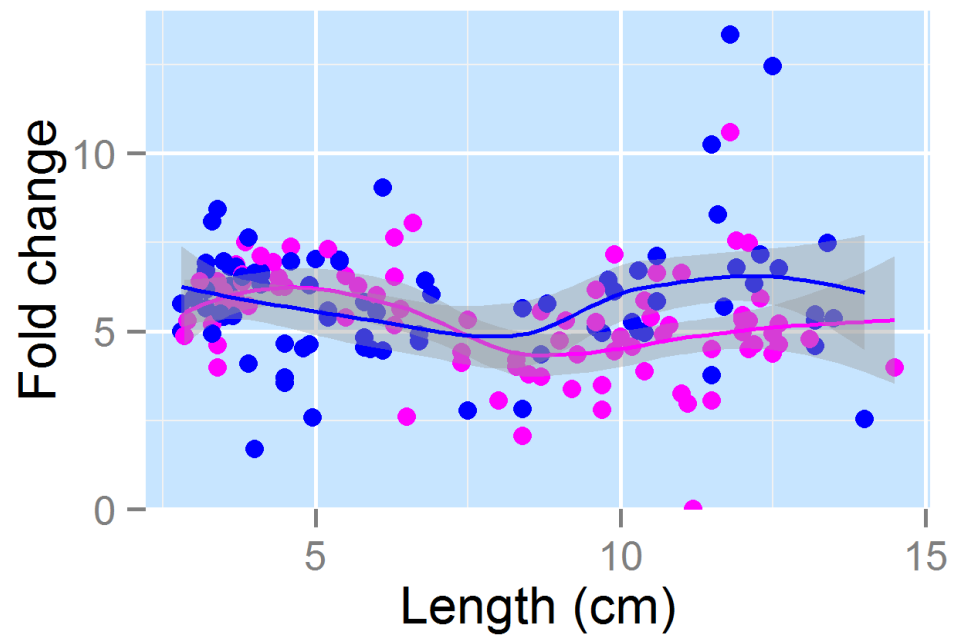




**sox9a**



**sox8**

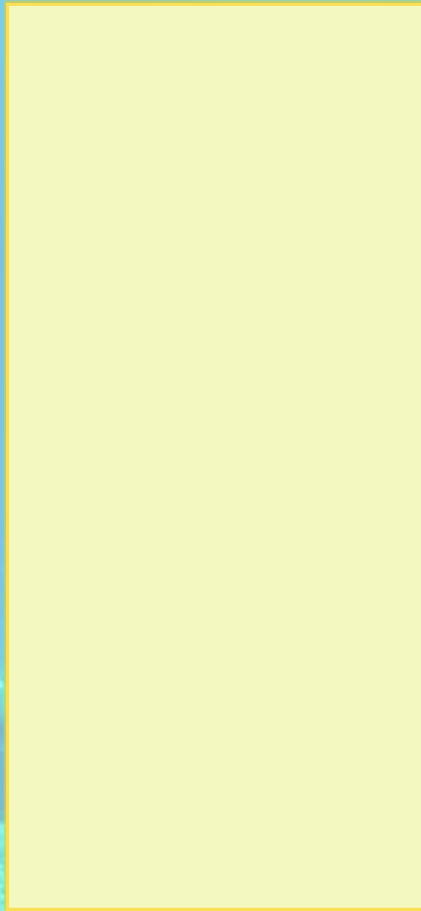
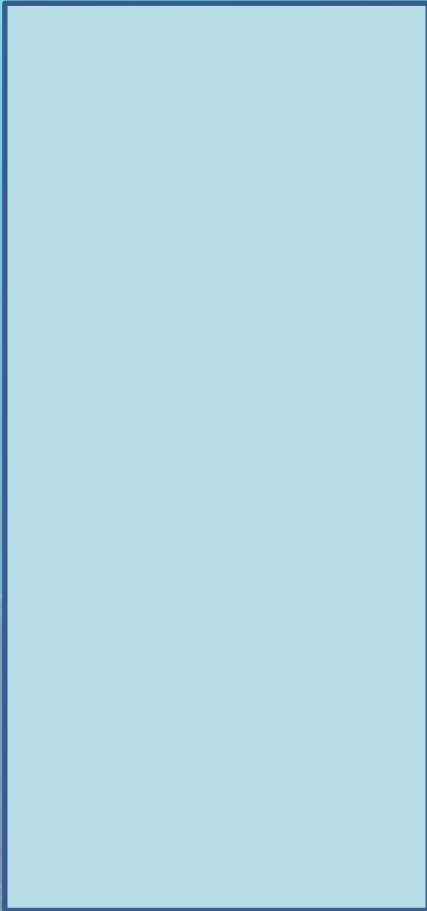




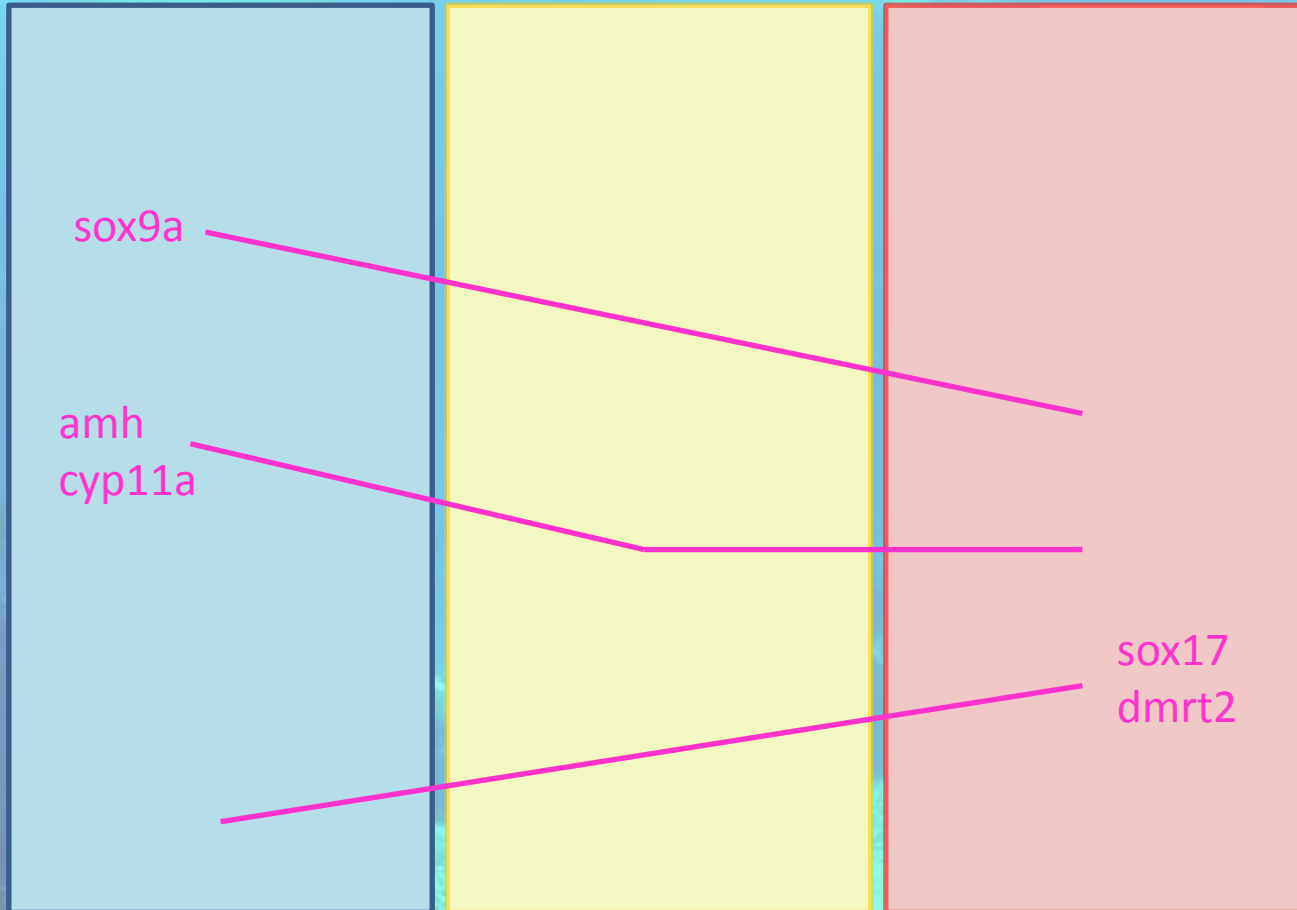
An underwater scene with a sandy bottom, rocks, and seaweed. Sunlight filters through the water from above. A central text box is overlaid on the image.

**Sex determining genes?**

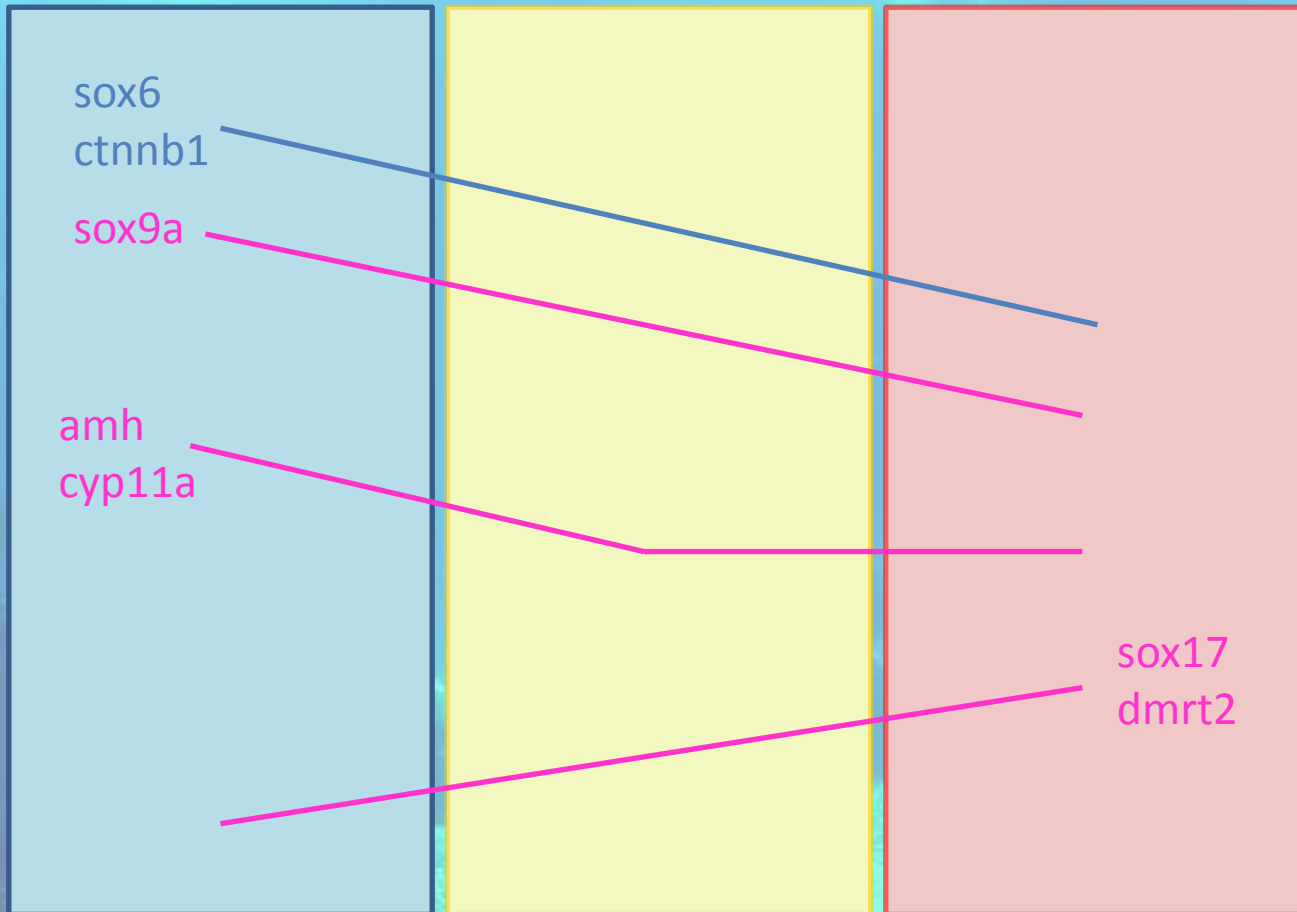
# Temperature effects



# Temperature effects

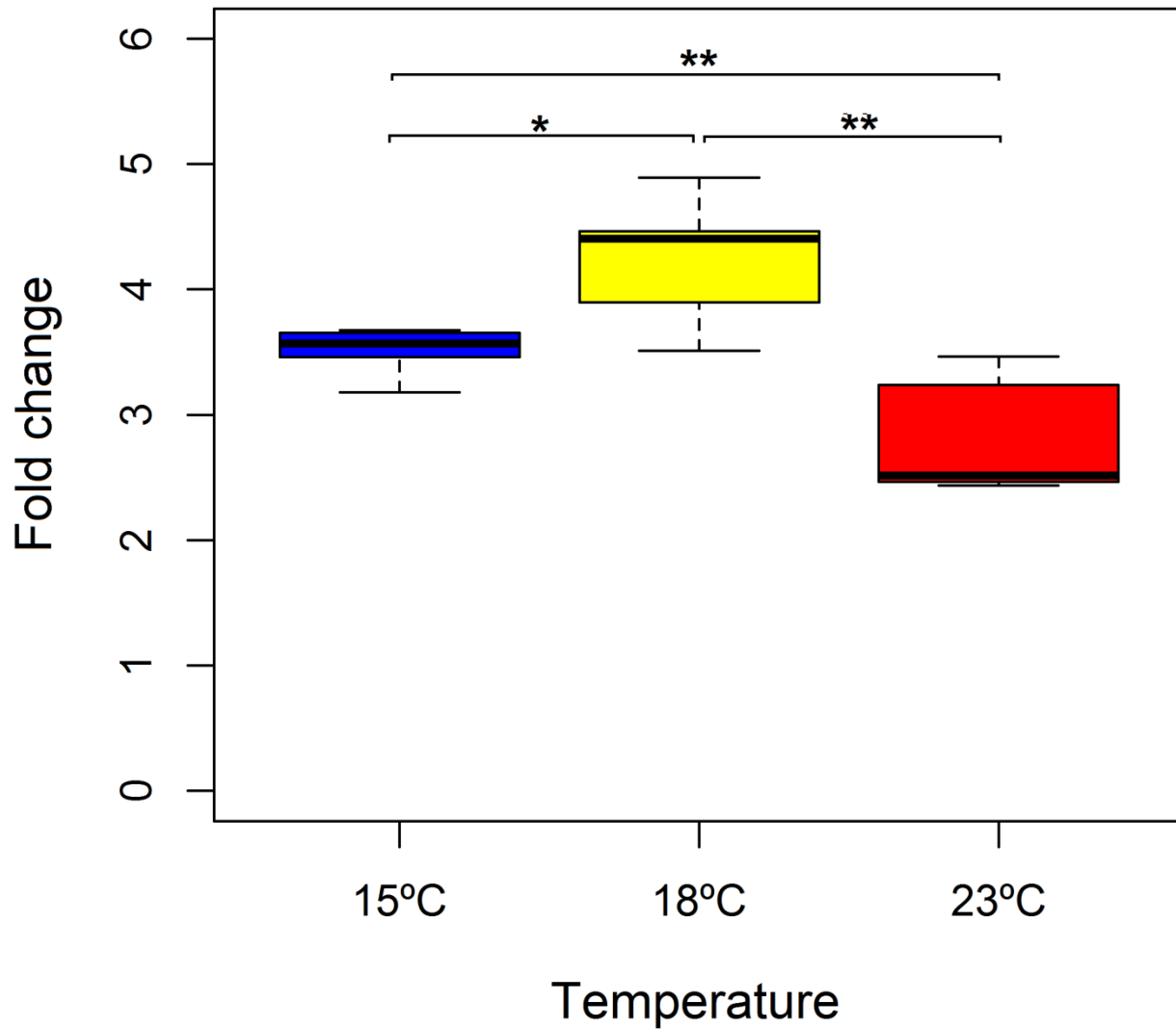


# Temperature effects



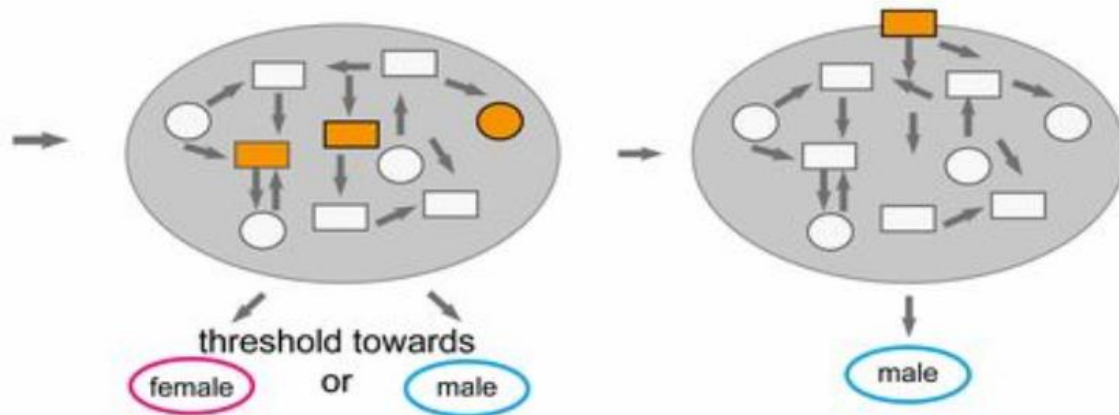


***ctnnb1* at 90dpf**



Developmental perspective on the evolution of the SD network after (Crews and Bull 2009) and (Uller and Helanterä 2011)

Potential for evolution of a new major effect locus at different levels through natural and sexual selection



Genetic (with one major effect locus as possibility), parental or environmental input influencing threshold

# Conclusions

- 1) The first sign of gonad differentiation in turbot is the sex-independent increase of *gsdf* at 75 dpf.
- 2) The onset of sex differentiation takes place at three months of age and is characterized by the increase of *cyp19a1a* and *vasa* in females and *amh* in males.
- 3) Temperature influences in several sex-related genes: *sox9a*, *amh*, *cyp11a*, *sox17*, *dmrt2*, *sox6*, *ctnnb1*.
- 4) *Ctnnb1* exhibited temperature-dependent changes right at the onset of sex differentiation.







Thank you for your attention!!

