

Developmental expression of glucocorticoid receptor during early ontogeny in gilt-head sea bream, *Sparus aurata*, and European sea bass, *Dicentrarchus labrax*

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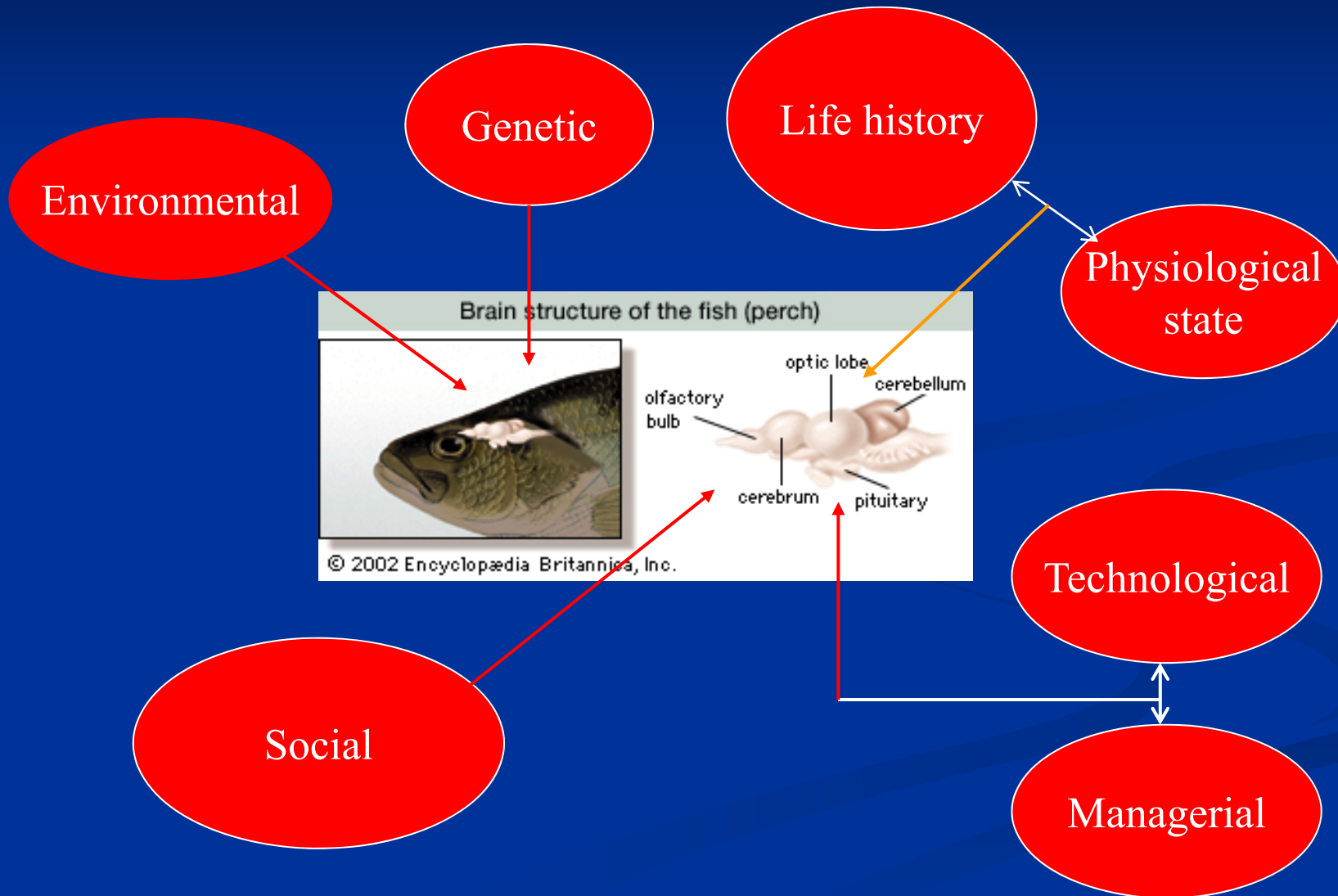
²Institute of Molecular Biology & Biotechnology, FORTH

³Institute of Marine Biology and Genetics, H.C.M.R.

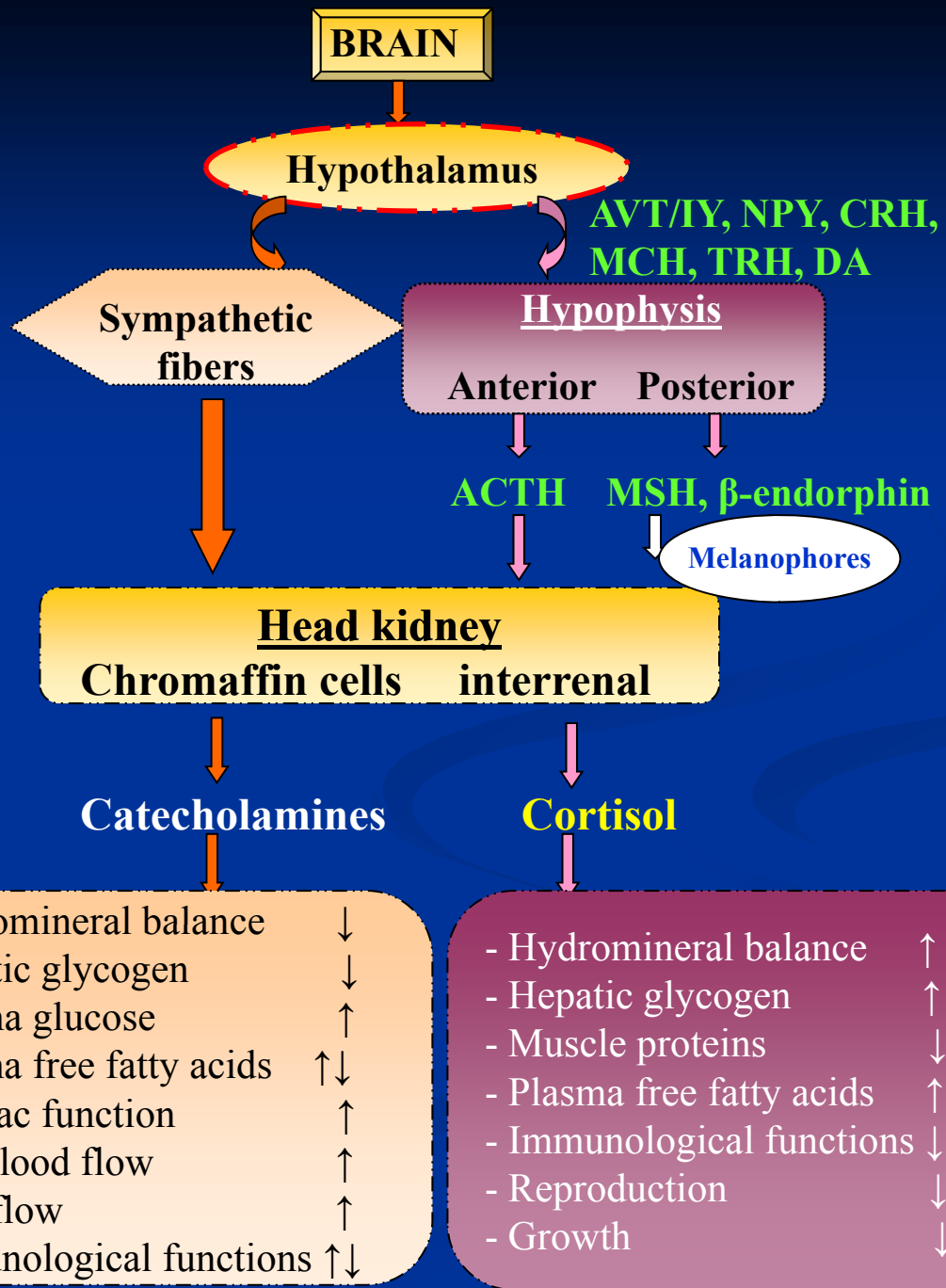
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How fish cope with stressors ?



Physiological Stress Response



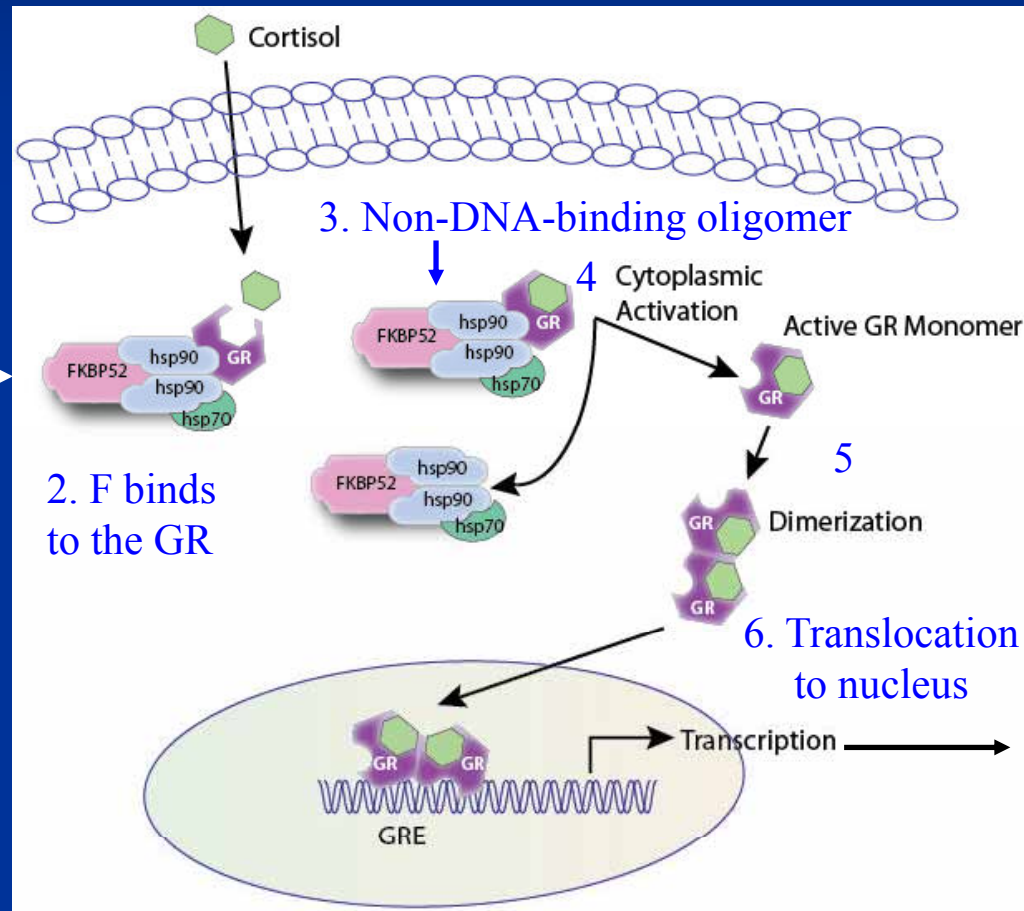
Modified from
S.E. Bonga, 1997

How does Cortisol Act?

1. Cortisol passes through the plasma membrane into the cytoplasm

GR (or NC3C1)

Inactive oligomeric complex

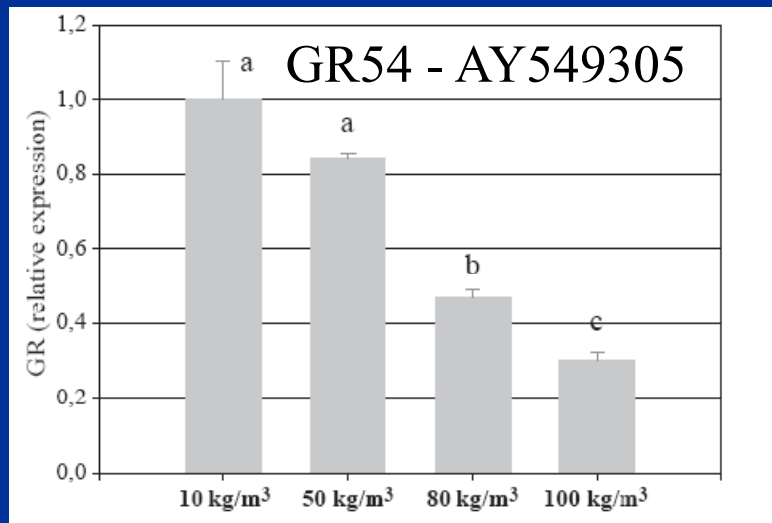


- 1. Transactivation
- 2. Transrepression
- 3. Non-genomic

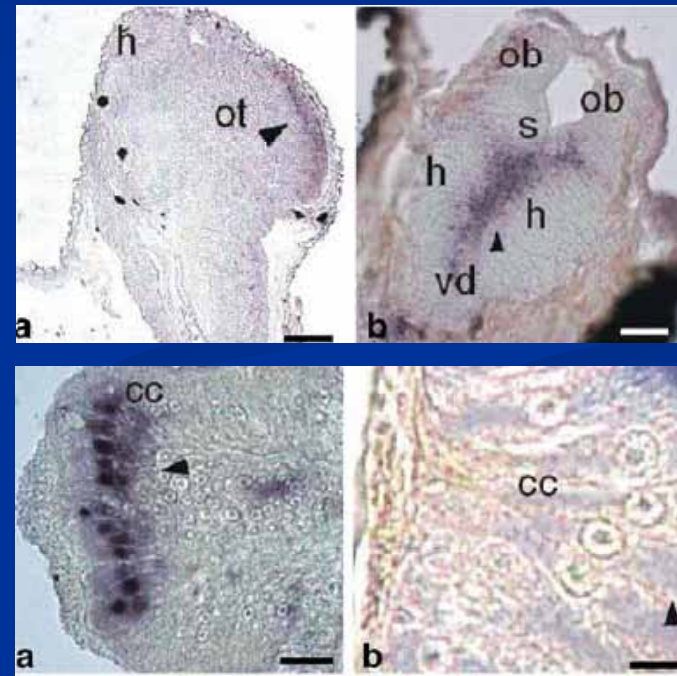
7. Binding to DNA

Glucocorticoid Receptor in European Sea Bass

Two isoforms that differ significantly in their transcriptional activation domain (GR54 & GR61)



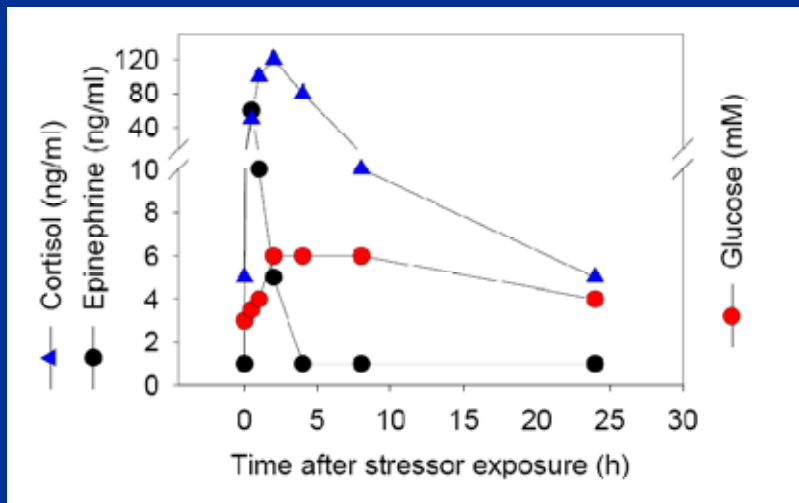
GR expression levels in liver tissue of adult sea bass in response to high rearing densities (Terova *et al.*, 2005)



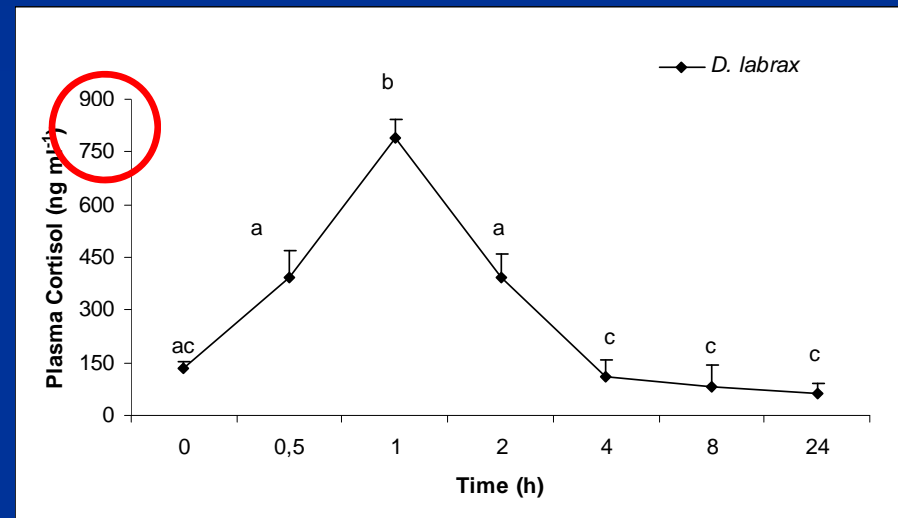
GR61 (AY619996) expression levels in tissues of larval stages of sea bass (Bella *et al.*, 2008)

The cortisol stress response in European Sea Bass

Up to present the stress response has been studied mainly in juvenile and adult fish

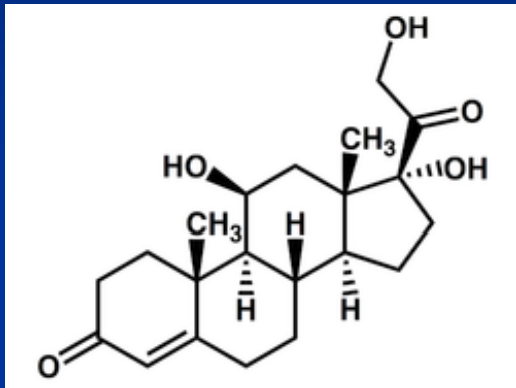


Source: Iwama et al., 2004. AquaNet Workshop on Fish Welfare



Source: Fanouraki, 2008. Fish Physiology Lab. UoC, unpublished data

Recently research has been focused on the



Ontogenesis and molecular regulation of the corticosteroid stress axis during early development

- Gilthead sea bream, *Sparus aurata* (Szisch *et al.*, 2005)
- European sea bass, *Dicentrarchus labrax* (Bella *et al.*, 2008)
- Zebrafish, *Danio rerio* (Alsop & Vijayan, 2008; 2009)
- Red drum, *Sciaenops ocellatus* (Applebaum *et al.*, 2008)

Aims of the Study

- Onset of cortisol production
- Development of cortisol response
- Molecular regulation of the stressor-induced cortisol response

During early life stages in

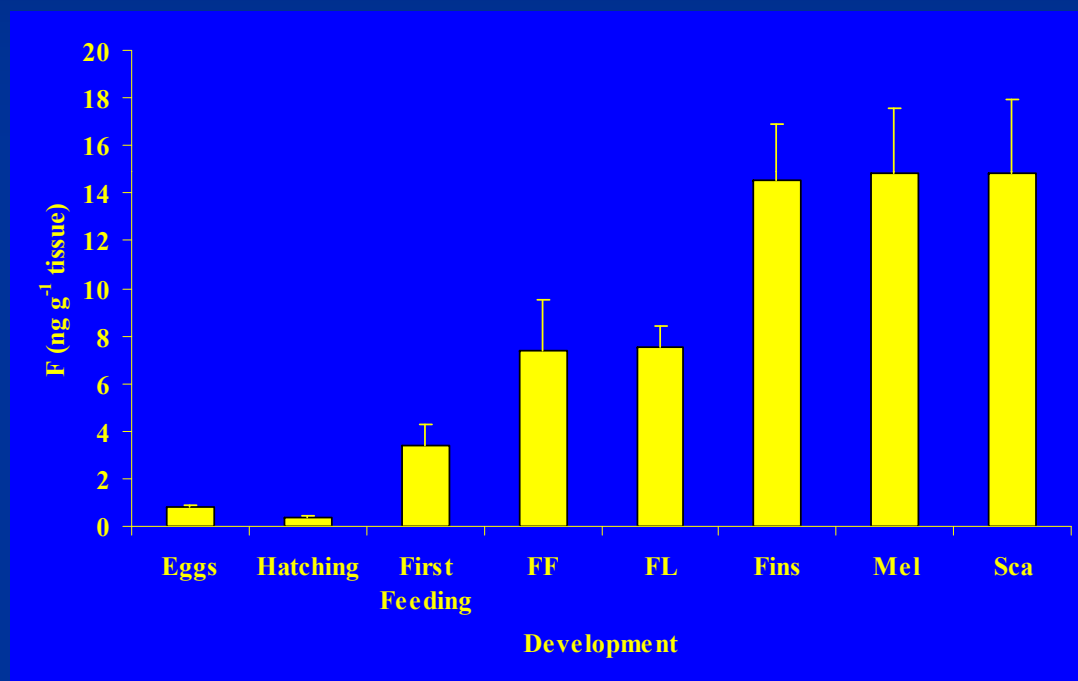


European sea bass



Gilt-head sea bream

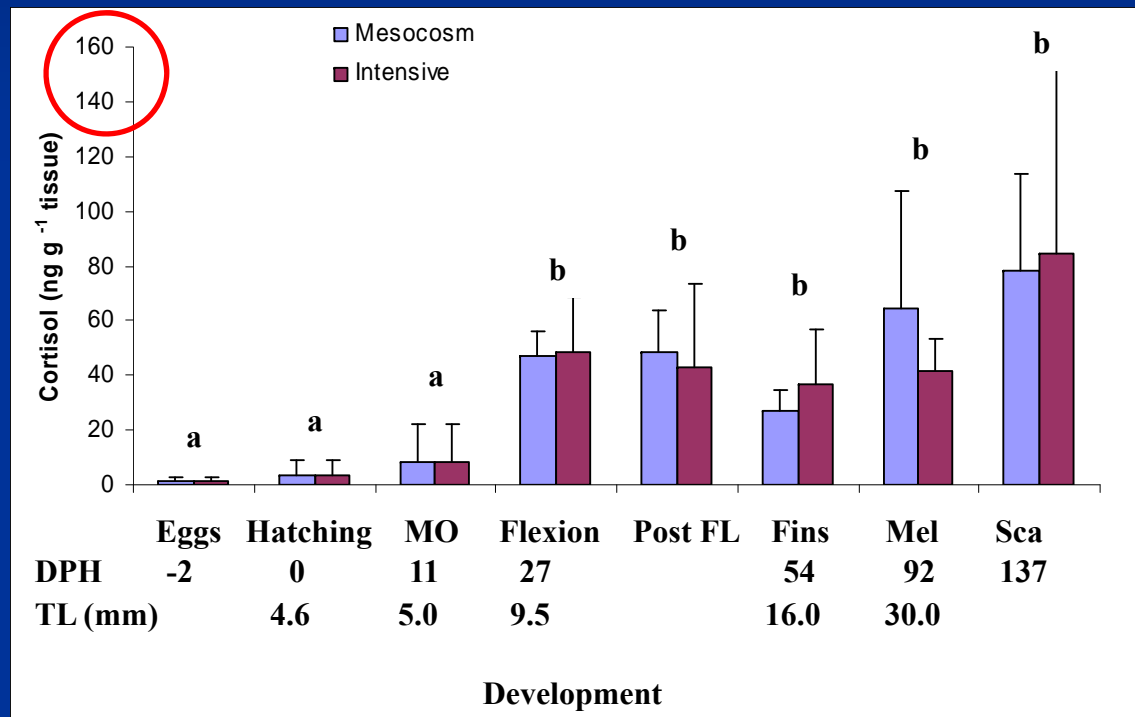
Cortisol whole body concentration during early ontogeny in gilthead sea bream, *Sparus aurata*



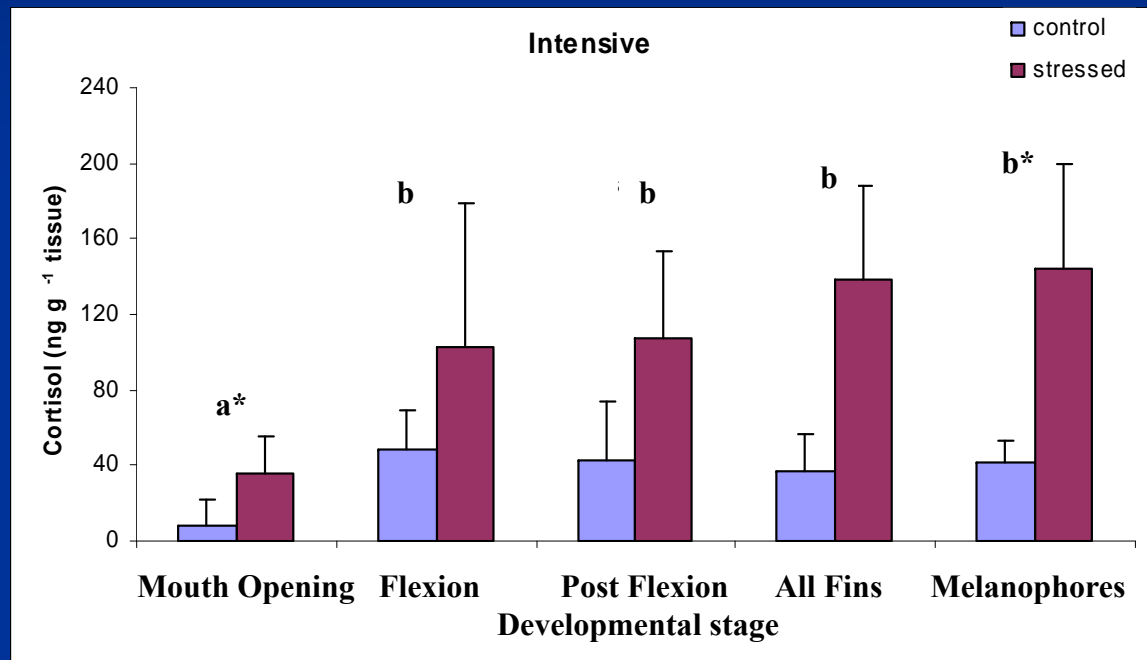
1. Fertilized eggs
2. Hatching
3. First feeding
4. Formation of first fin rays in the tail (**FF**)
5. Flexion (**FL**)
6. Formation of dorsal and ventral fins as in adults (**Fins**)
8. Abundant melanophores all over the body (**Mel**)
9. Development of scales (**Sca**)

Modified from Szisch et al., 2005

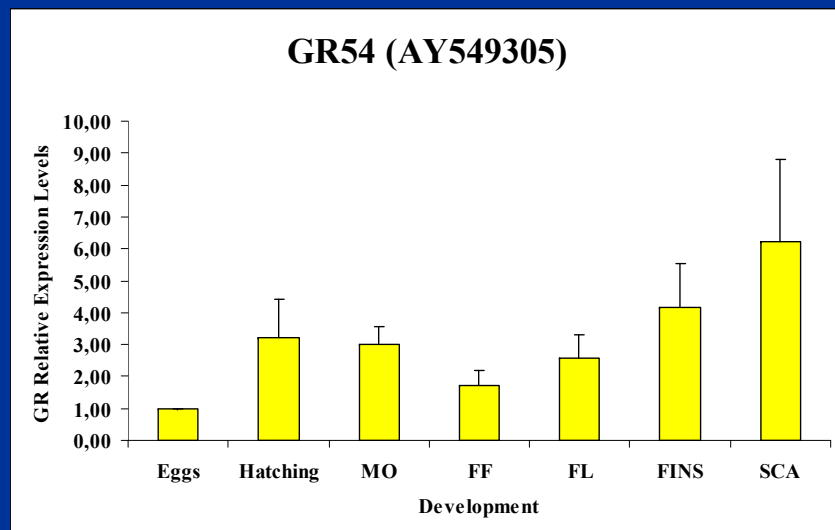
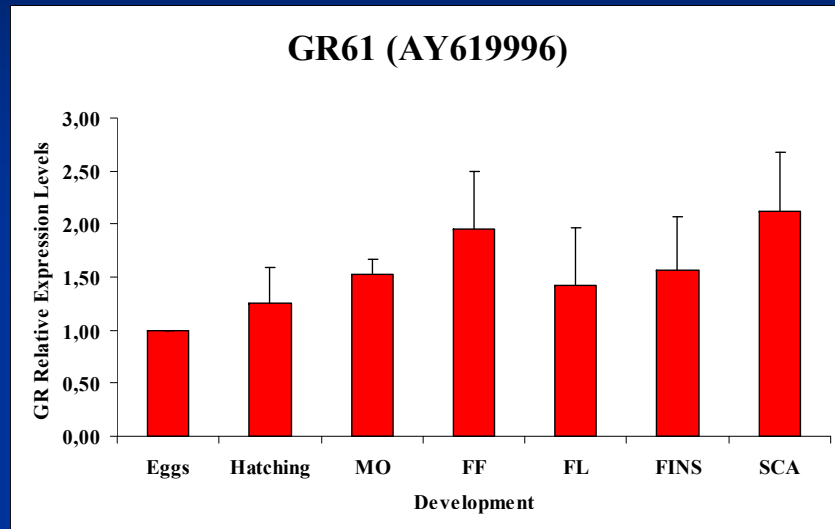
Cortisol whole body concentration during early ontogeny in European sea bass, *D. labrax*



Ontogenesis of the stress response in European sea bass, *D. labrax*

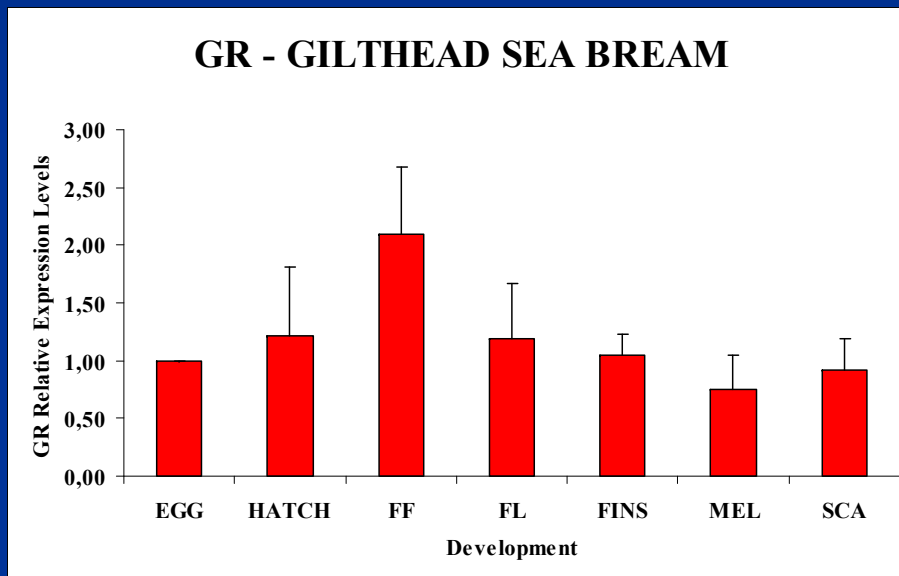


Ontogenesis of the glucocorticoid receptor mRNA in European sea bass, *D. labrax*



1. Fertilized eggs
2. Hatching
3. Mouth opening (**MO**)
4. Formation of first fin rays in the tail (**FF**)
5. Flexion (**FL**)
6. Formation of dorsal and ventral fins as in adults (**Fins**)
8. Development of scales (**Sca**)

Ontogenesis of the glucocorticoid receptor mRNA in gilt-head sea bream, *S. aurata*



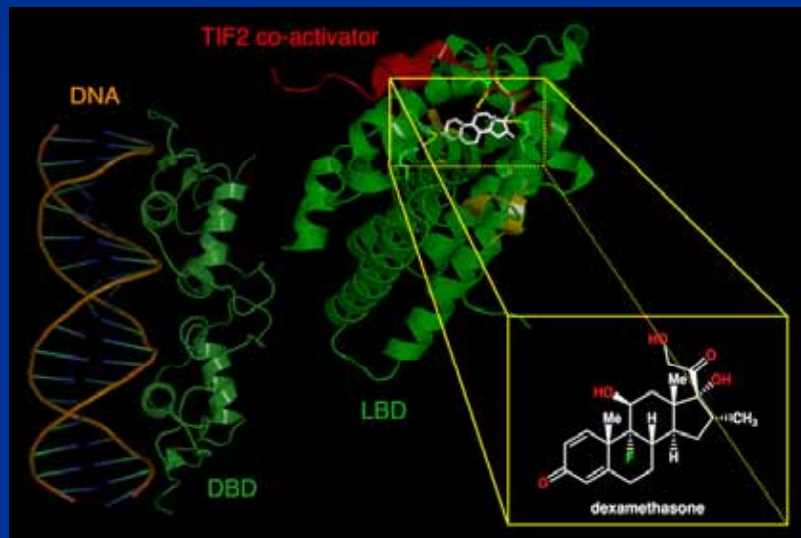
1. Fertilized eggs
2. Hatching
3. Formation of first fin rays
in the tail (**FF**)
4. Flexion (**FL**)
5. Formation of dorsal and ventral
fins as in adults (**Fins**)
6. Melanophores
7. Development of scales (**Sca**)

Conclusions

- 1a. The *ontogenetic pattern* of basal whole body cortisol concentrations in both species examined was *typical* of that observed in other teleosts
- 1b. However, the magnitude of the response was *much higher* in *European sea bass* than that reported in other warm-water teleosts
- 2a. The *presence of cortisol in embryos* is of maternal origin as has been previously observed in other teleosts
- 2b. The *onset of cortisol production* (*de novo* synthesis) occurs near the transition from *endogenous to exogenous feeding*, indicating that cortisol biosynthesis is important for adapting to different nutritional sources for energetic, growth and osmoregulatory purposes

- 3a. Application of the same stressor during early ontogeny elicits a significant increase in whole body cortisol content even at *mouth opening*
- 3b. The dynamics of the response showed changes during the subsequent larval stages to give a *maximum response* at the *melanophores' stage*
- 4a. Expression of the GRs was detected in embryos and in all larvae stages examined
- 4b. Expression of GRs was *different* between the two *species* examined and between the *two isoforms in European sea bass*

8. The *GR61 transcripts* show an *increase* throughout *embryonic* development to reach a *maximum at first feeding and at full formation of scales*



9. The *GR54 transcripts* show an *increase* throughout *embryonic* development till mouth opening to reach a peak at *full formation of scales*

10. The *GR transcripts* in gilt-head sea bream showed a peak at *first feeding* and then a decrease to reach a *minimum at full formation of scales*

Future Perspectives

- Characterization of the GRs in gilt-head sea bream
- Individual functions of the two GRs isoforms in European sea bass ?
- What is the adaptive significance of the differences in the magnitude of the stress response in developing larvae ?

Thank you

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of stress level in farmed fish*