PROBASS

IMPROVING PRODUCTION EFFICIENCY OF SEA BASS FARMING BY DEVELOPMENT OF METHODOLOGIES TO ELIMINATE ENVIRONMENTAL ANDROGENESIS (Q5RS-2000-31365)

2001-2004

The Partners

Institute of Aquaculture Torre de la Sal (CSIC), Castellón, Spain. Dr. Silvia Zanuy

Institute of Marine Sciences (CSIC), Barcelona, Spain. Dr. Francesc Piferrer

Institute of Marine Biology of Crete (IMBC), Heraklio, Greece. Dr. Dinos Mylonas

University of Algarve (UALG), Faro, Portugal. Dr. Adelino Canario

National Center for Mariculture (IOLR-NCM), Eilat, Israel. Dr. Amos Tandler

Cardiff University (UWC), Cardiff, UK. Dr. Glen Sweeney

Subcontractor 1: Seafarm Ionian, S.A., Greece
Subcontractor 2: Timar, Lda., Portugal
Subcontractor 3: Ardeg, Ltd. (Red Sea Maricultures), Israel

The Problem

- Second most important marine aquaculture species in S. Europe (81,200 MT in 2005)
- Females grow aprox. 35% more than males
- 3:1 male: female sex ratio in culture
- About 30% of males mature precociously
- 2-year production cycle

The industry would benefit from all-female stocks

Important to know sex determination and differentiation to be able to control these processes

The Objectives

- To investigate key elements of the brain-pituitary-gonad axis for their involvement in regulating sex differentiation in male and female sea bass
- To manipulate some environmental parameters such as temperature and rearing density to control sex ratios
- To use the information gained to develop a reliable methodology to prevent male dominance in sea bass cultivated stocks

The Approach

WP1
Candidate sex determining genes

WP2
Steroidogenic enzymes and sex steroid receptors

WP 3
Brain and pituitary hormones, receptors and growth factors

GOAL 1

Develop tools and methodologies to study sex determination/differentiation

WP7
Analysis of selective grading

WP4
Production of male- and female-dominant stocks by selective size grading

WP 8
Analysis of temperature and density effects

WP5
Temperature experiments

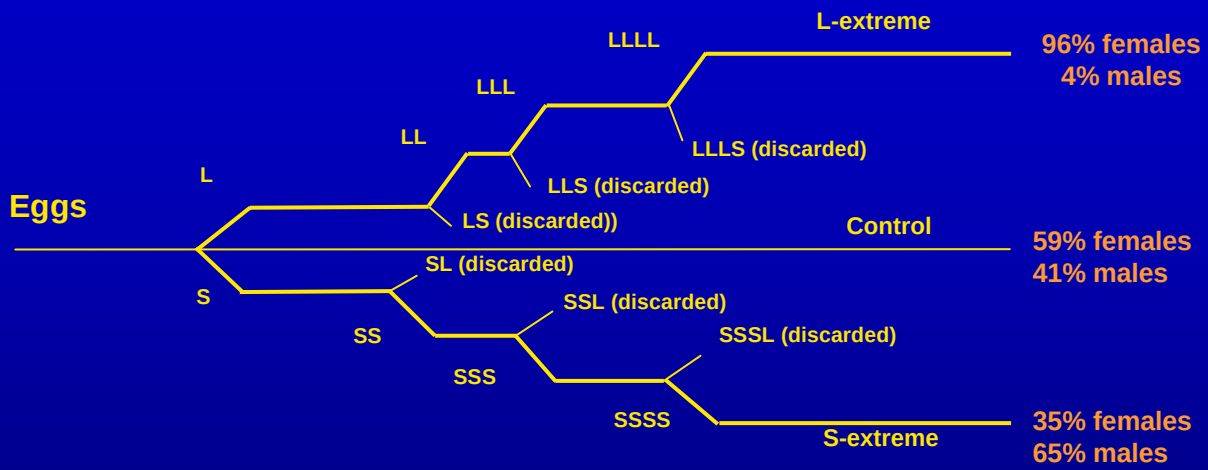
WP6
Density experiments

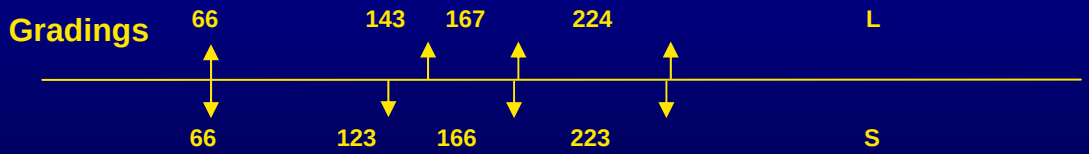
WP9
On-growing female dominant population in commercial aquaculture facilities

GOAL 2
Understanding sex differentiation in males and females

GOAL 3
Method for producing female-dominant stocks/prevent androgenesis

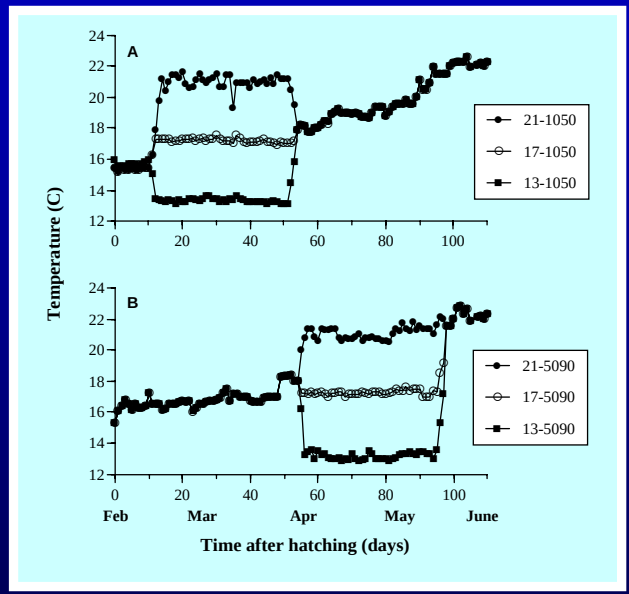
The Methods





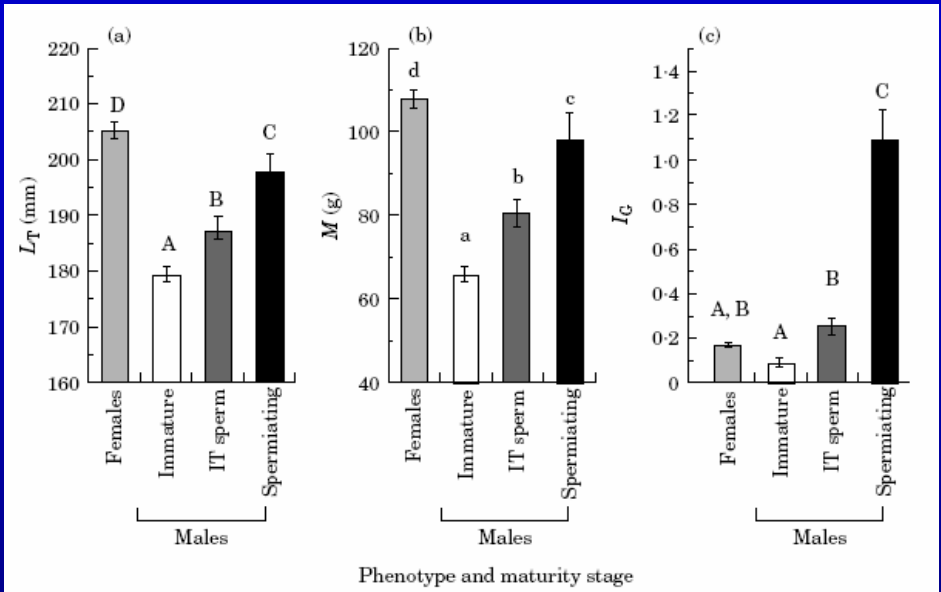
Days post-fertilization

Papadaki et al. (2005) Journal of Fish Biology, 66: 938-956.



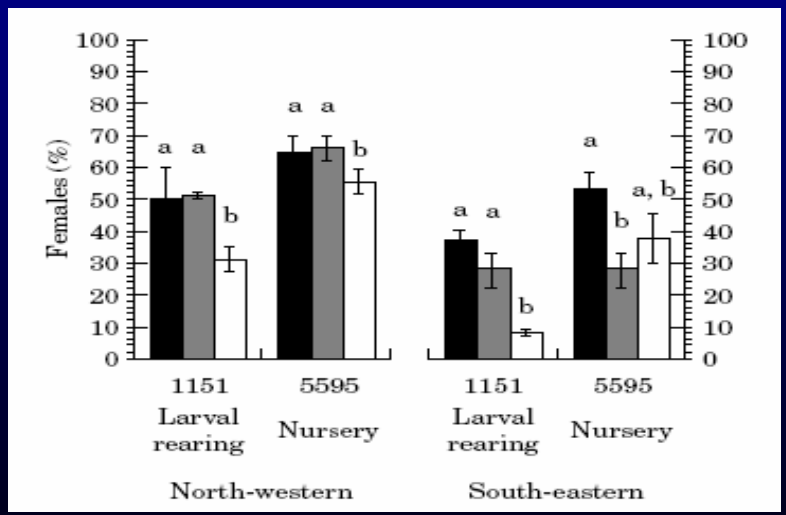
Mylonas et al. (2005) Journal of Fish Biology, 67: 652-668.

The Results: Effects of grading and rearing temperature



Papadaki et al. (2005) Journal of Fish Biology, 66: 938-956

- Usually, larger fish are females and smaller ones males

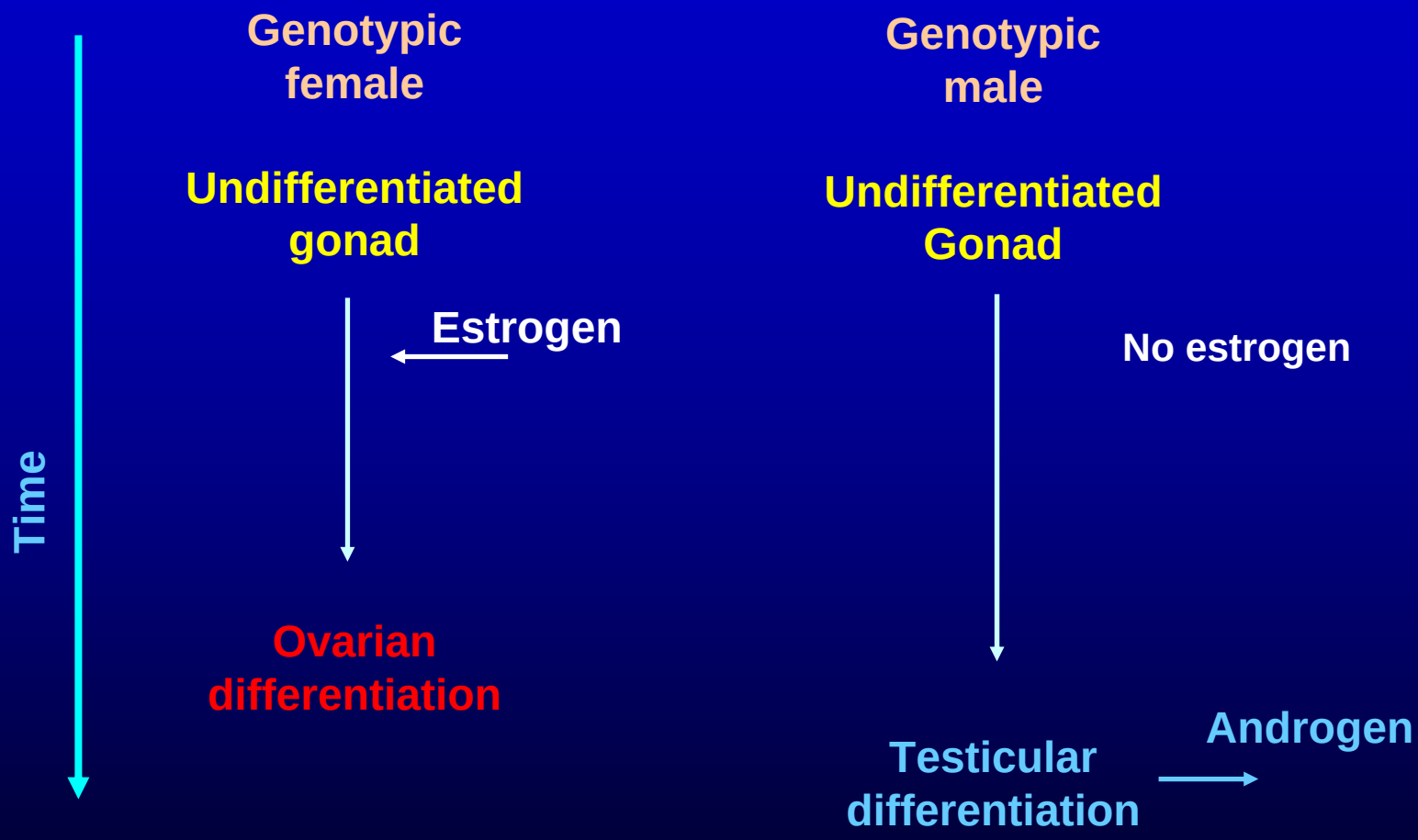


Mylonas et al. (2005) Journal of Fish Biology, 67: 652-668

- The masculinization effect of high rearing temperature is stronger during the larval period

- Maximum number of females of ~70%, suggests that part of the genetic component of sex differentiation is not labile to environmental influences

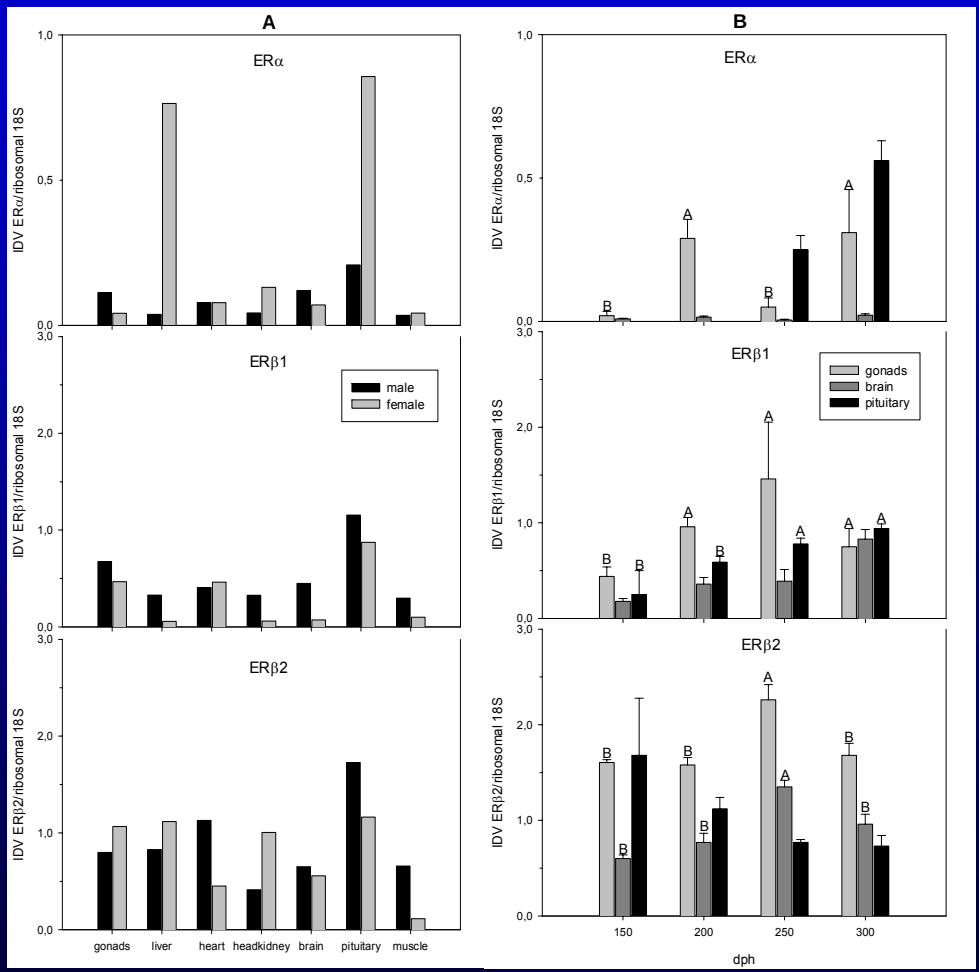
Endocrine Regulation of Sex Differentiation



Blázquez et al.,(1998). Fish Physiol. Biochem., 18:37-47

Papadaki et al. (2005) Journal of Fish Biology, 66: 938-956

Cloning, characterization and expression of three estrogen receptors



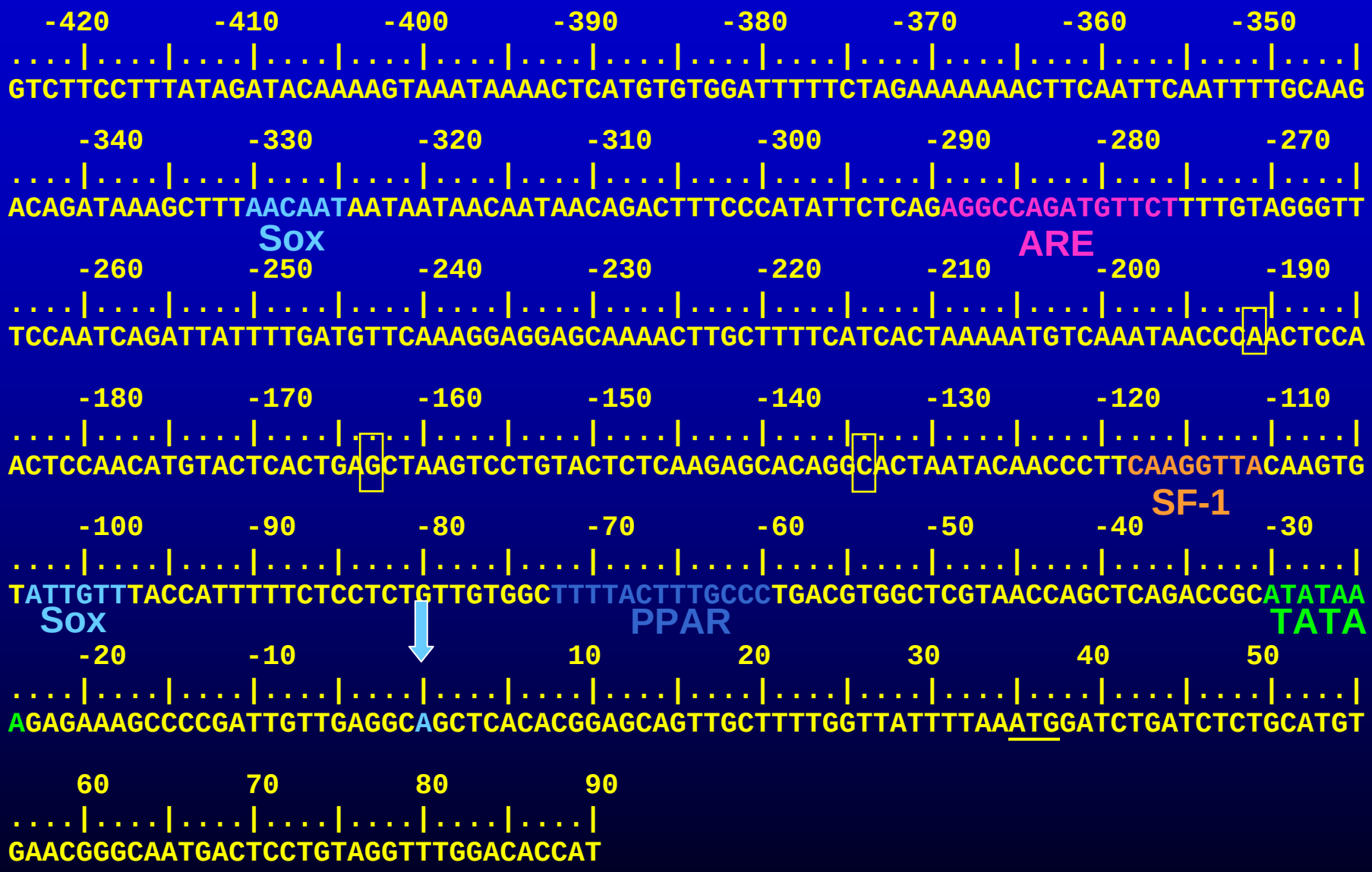
- Southern analysis showed that the three sb-ERs are the products of three distinct genes

- sbERα is predominantly expressed in liver and pituitary, while sb-ERβ1 and sb-ERβ2 are more ubiquitously expressed, with highest expression levels in the pituitary (A)

- sb-ERα expression was elevated in gonads at 200 dph and in pituitary at 250 and 300 dph. sb-ERβ1 and sb-ERβ2 showed highest expression in gonads at 250 dph. sb-ERβ2, expression was also higher in brain at 250 dph (B)

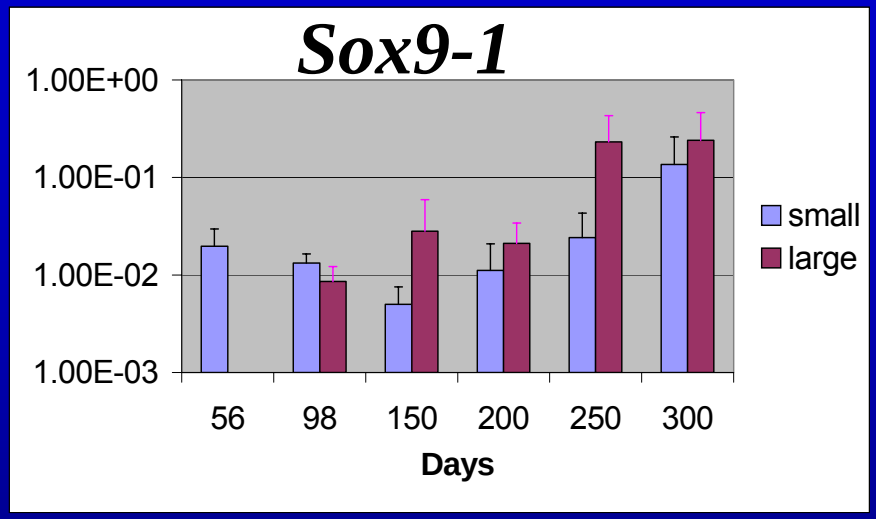
Halm et al., 2004 Molecular and Cellular Endocrinology 223: 63-75

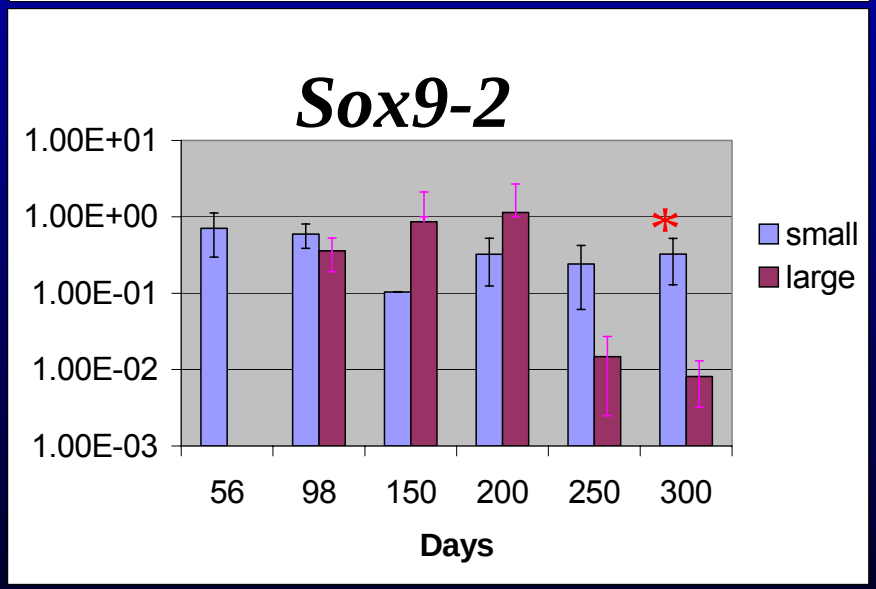
The sea bass ovarian aromatase promoter



Galay-Burgos Grealy C., Navarro L., Piferrer F., Zanuy S., Sweeney GE., (2006). General and Comparative Endocrinology (submitted).

Sox9-2 but not Sox9-1 shows sexually dimorphic expression

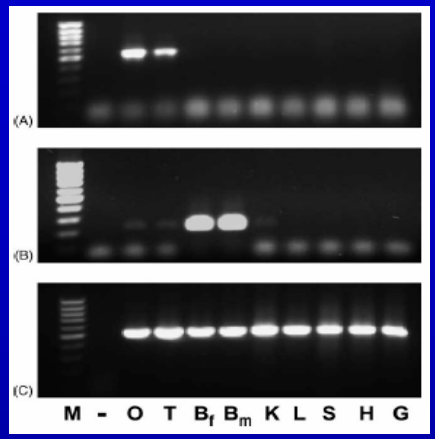




- **Analysis by real time PCR**
- **No clear sex related differences in *Sox9-1* gene expression**
- *Sox9-2* may be related to male sex differentiation

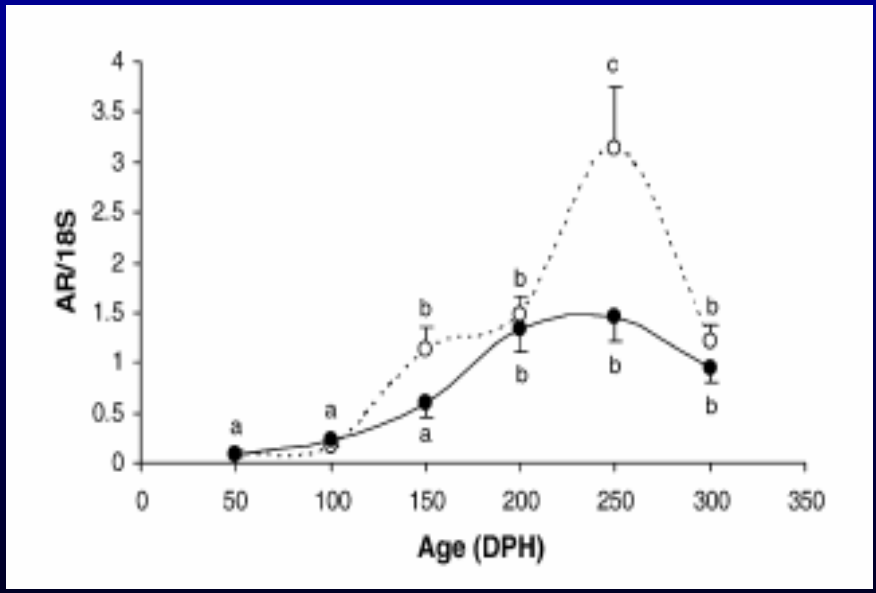
Galay-Burgos et al., 2004. Comparative Biochemistry and Physiology part B 137: 279-284.

Cloning, characterization and expression of aromatase and androgen receptor



- P450aromA is preferentially expressed in gonads and P450aromB in brain.

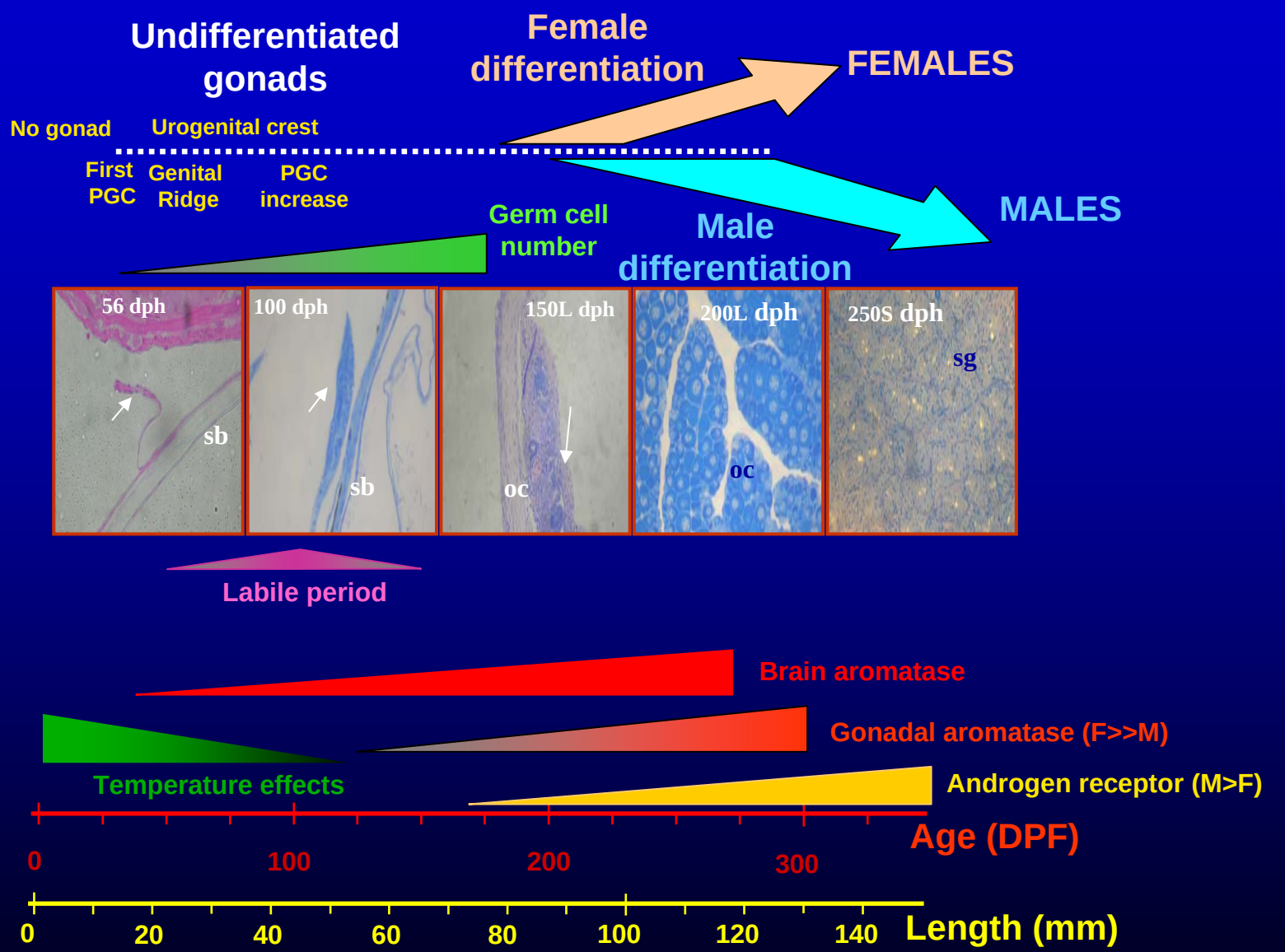
Blázquez and Piferrer (2004). Molecular and Cellular Endocrinology 219: 83-94.



- AR gene expression peaks during male sex differentiation suggesting an important role of AR controlling this process

Blázquez and Piferrer (2005). Molecular and Cellular Endocrinology 237: 37-48.

The Outcome: Age, growth and sex differentiation in the sea bass



Piferrer et al. (2005). General and Comparative endocrinology, 142: 102-110

The Results (summary)

1. Research tools. Cloning, sequence analysis and tissue distribution of:

   Candidate sex determining genes (Sox, Dax, DMRT1)
   Steroidogenic enzymes (aromatase, 11ß-hydroxyase)
   Germ cell markers (vasa)
   Steroid receptors (ER-α, ERß1, ERß2, AR)
   Growth and brain & pituitary factors (GH, IGFs, GnRHs, GnRHR and GtHs)

2. Better understanding of sex differentiation and its control

3. Production of female dominant stocks without the use of hormones

4. Study of genetic and environmental effects:

   Strain effects
   Temperature effects → Management tool
   Density effects

The Prospects

- The sea bass sex differentiation process is well calibrated in relation to age and size and well characterized histologically and molecularly, in a descriptive manner, but the actual mechanisms are still not known
- There are specific assays to measure many genes related to sex differentiation
- Sex differentiation in the sea bass depends on genetic factors that can be influenced by the environment. Sex ratios can be manipulated at will
- Sea bass aquaculture production is steadily increasing. New marketing strategies (e.g., larger fish) are being developed, increasing the need for earlier sex markers and the application of sex control
- Thus, the sea bass is an ideal species where to apply a genomic approach that will benefit from existing information and will help in the identification of new genes involved in sex determination and differentiation.