The zebrafish as a model organism for aquaculture research

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Universitat de Barcelona, master class

GBR
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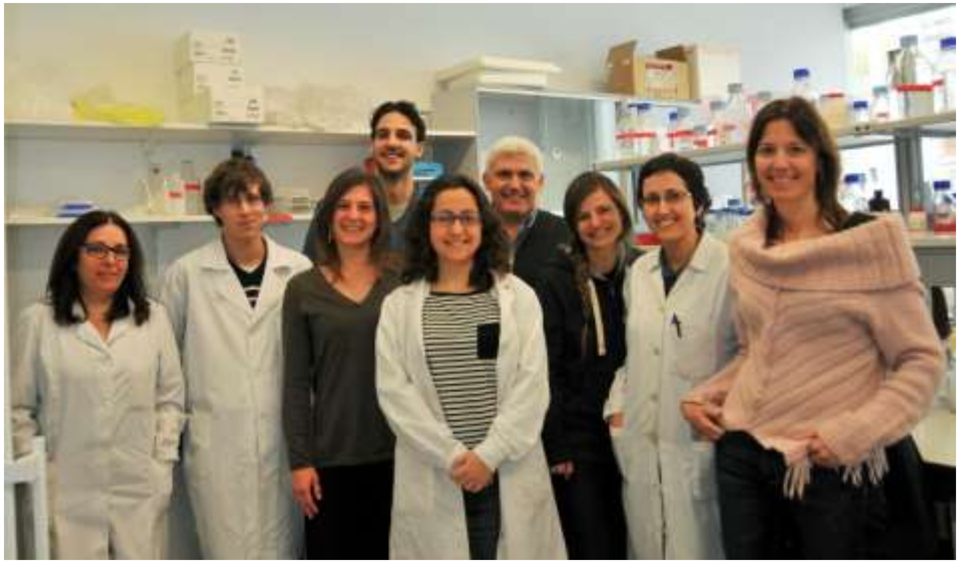

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GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

CSIC

Outline

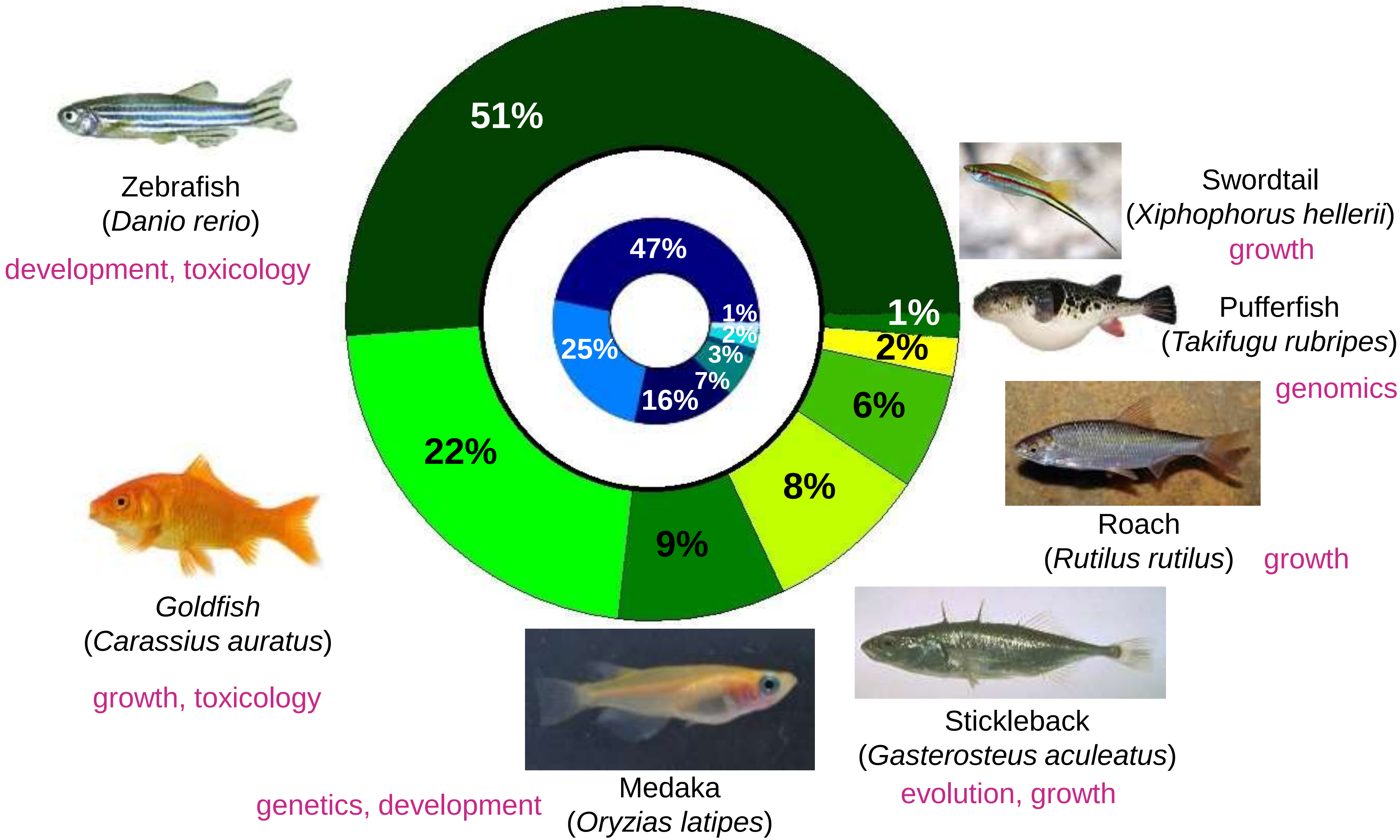
- Laboratory fish models
- Landmarks in zebrafish research
- Requirements for animal model for research
- Zebrafish as a model for finfish aquaculture research?
    - Reproduction-related problems
    - Stress-related problems
    - Nutrition- and growth-related problems
    - Pathology-related problems
    - Toxicology-related problems
- Final considerations of using zebrafish as a model

Laboratory fish models

% entries in the Web of Science

[species] 45,096 (zebrafish)

[species] AND [Aquaculture] 2,719 (zebrafish)



Landmarks in zebrafish research

C. elegans

D. melanogaster

D. rerio

- 1960's
  - 
  - George Streisinger 1927-1984
  - Using mutants in a higher organism
  - Propagate mutations in to homozygous offspring
- 1970's
  - Laale 1977 Journal of Fish Biology 10: 121–173
- 1980's
  - Homozygous diploid zebrafish, Nature 1981
  - 
  -
  - Developmental neurobiology research Dr. Kimmel, Dr. Westerfield
- 1990's
  - 
  - C.Nüsslein-Volhard 1942-
  - 
  -
  - Development vol 123 1996
  - Linkage group map, Genomic resources
- 2000's
  - ZFIN, Genome sequencing by Sanger Institute
- 2010's
  - 30.384 publications

Requirements for animal model for research

Animal model (General)		Fisn model for Aquaculture
Easy and economical to growth in the lab	=	Easy and economical to growth in the lab
Short life cycle	=	Short life cycle
Structural simplicity but with basic cellular process	~	Similar physiological features to cultured species
Relatively small and stable genome Easily amenable to genetic manipulations	≠	Many resources

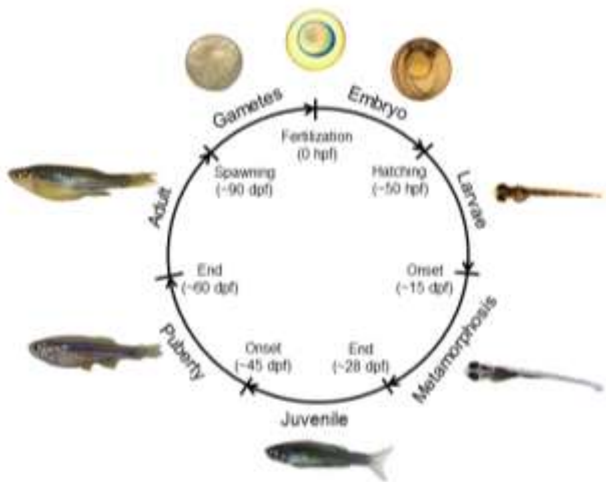
Zebrafish as a model for aquaculture research

Easy and economical to growth in the lab

Short life cycle

Similar physiological features to cultured species

Many resources

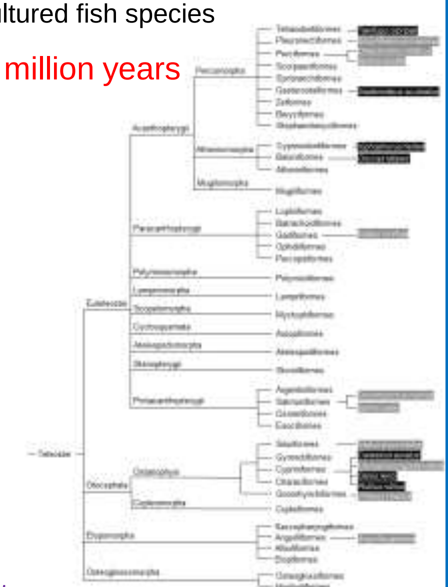


High frequency in fecundity
(~200 eggs/w)

Model fish species

Cultured fish species

<200 million years



Ribas and Piferrer 2014 Rev. Aquaculture

Large genomic resources

Genome and methylome availabilty

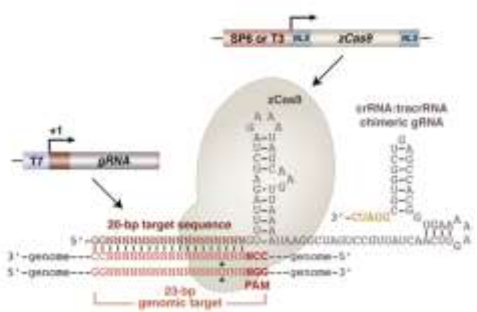
Large number of website

Commercial products

Genetic tractability

Mutagenic lines availability

morpholinos, ZFNs, TALENs, CRIPSR9



Jao et al., 2013 PNAS

Requirements of successful intensive aquaculture in finfish species

- control reproduction	sex ratio bias
- control stress situations	survival is threaten
- control pathologies	disease outbreaks
- control of environment	toxic environments
- control of growth	not reaching to commercial size

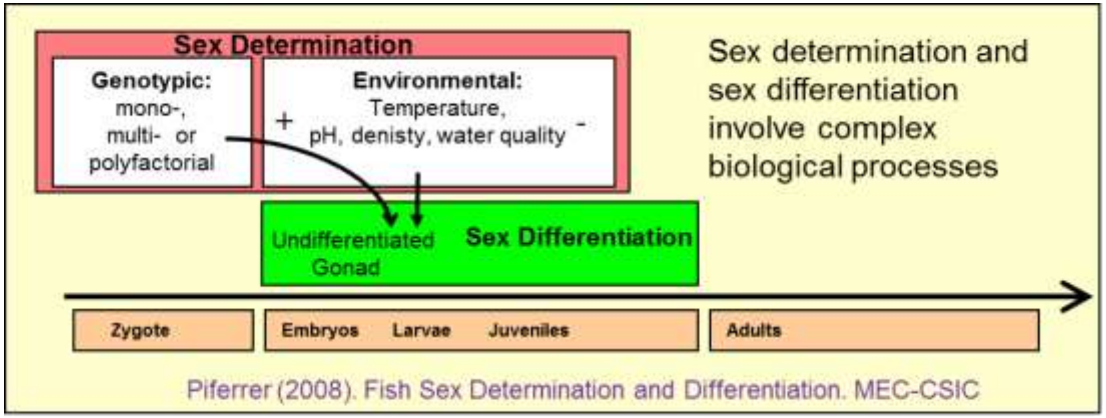
Economic losses

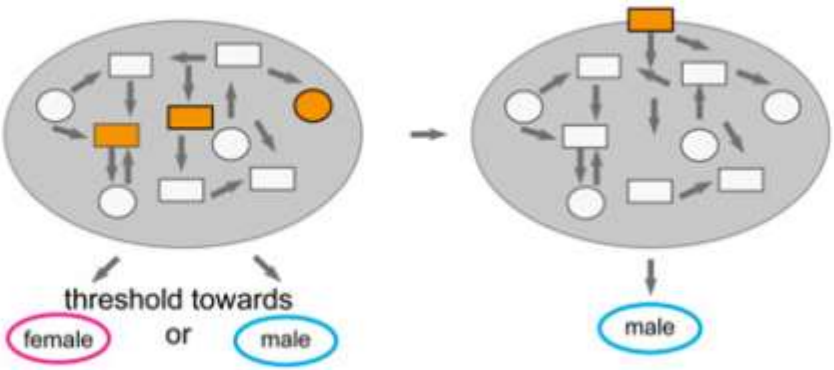
Zebrafish as an animal model to improved aquaculture-related research areas

> 30 years ago but it was not until genomic resources were available for cultured fish species (~15 years) when zebrafish became popular in finfish aquaculture research

Fish reproduction overview

Fish exhibit all types of reproduction known in vertebrates





There is no single genetic cascade, but there is a continuous regulation of environment and heredity

Uller and Helanterä 2011 Q Rev Biol.
Heule et al., 2014 Genetics

Master sex genes

dmy medaka (*Oryzia latipes*) Matsuda et al., 2002 Nature

gsdf medaka (*Oryzia luzonensis*) Myosho et al., 2012 Genetics

sdY trout (*Onchorynchus mykiss*) Yano et al., 2012 Curr Biol

amhr2 fugu (*Takifugu rubripes)* Kamiya et al., 2012 PLoS Genetics

amhy pejerrey (*Odontesthes bonariensis*) Hattori et al., 2012 PNAS

gsdf sablefish (*Anoplopoma fimbria*) Rondeau et al., 2013 BMC Genomics

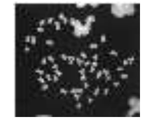
sox3 medaka (*Oryzias dancena*) Takehana et al., 2014 Nature Comm

dmrt1 sole (*Cynoglossus semilaevis*) Chen et al., 2014 Nature Genetics

LG11 Atlantic cod (*Gadus morhua*) Star et al., 2016 Scien Rep

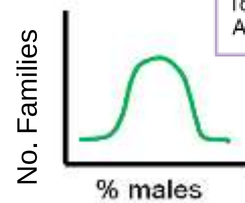
Zebrafish sex determination

Not well understood and in controversy. Heteromorphic sex chromosome system? XX/XY or ZZ/ZW or none?



1. First kariotype: 1964; 25 chromosome pairs Post 1964 Zool Syst Evol
2. Ginogenetic studies: Highly variable sex ratios→ incompatible with either XX/XY or ZZ/ZW Hörstgen et al., 2013 Aquacutlure
3. Genetic studies: SNPs and genetic maps: 5 sex-related regions in different strains
4. Hormonal treatments: ZZ/ZW system Tong et al., 2010 Dev Biol
5. Classical breeding, 62 familias, no dominant sex determining gene: a polygenic sex determination system is contemplated

Cross-type	Chromosome	Reference
INxAB	5, 16	Bradley et al 2011
ABxNA	3, 4	Anderson et al 2012
ABxTu	16	Howe et al 2013
TohxToh ABxAB	N/A	Liew et al 2012



Adapted from Liew et al., 2012 PLoS ONE

6. Temperature treatments
   - GSD+TE (not TSD) Ospina-Álvarez and Piferrer 2008 PLoS ONE
   - Masculinitzation effects of high temperatures (not always). Ushida et al., 2004 Comp Biochem Physiolo A; Abozaid et al., 2011, 2012 Sex Dev; Sfakianakis et al., 2011 J Biol ResThessaloniki

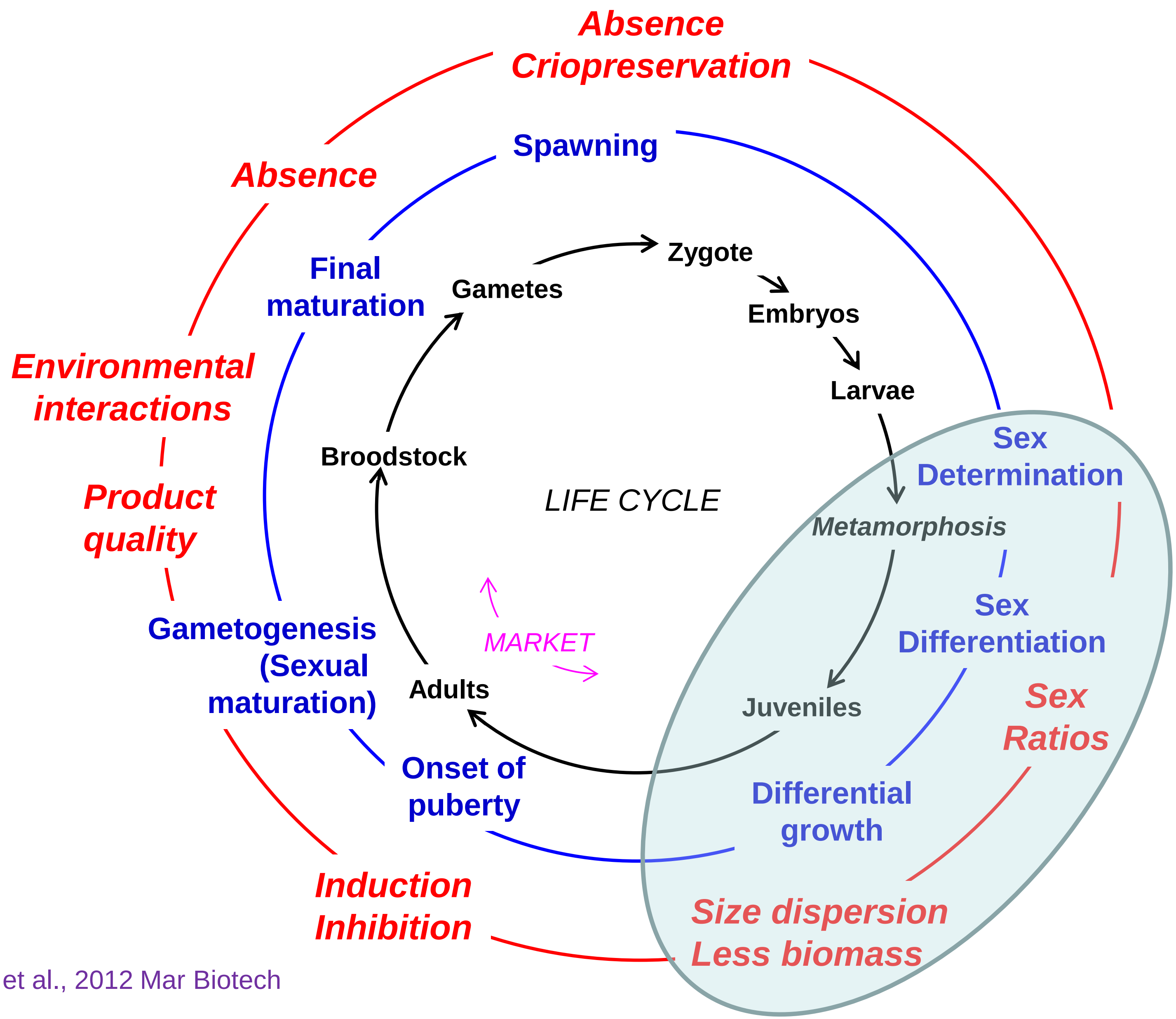
7. Wild zebrafish has a ZZ/ZW with a sex-determining region in the telomer of the chromosome 4 → domesticated zebrafish has lost due to several crosses Wilson et al., 2014 Genetics

Domesticated zebrafish behaves as if having a polygenic system

↓

Good system for studying sex reproduction system in European sea bass

Reproduction-related problems in finfish aquaculture

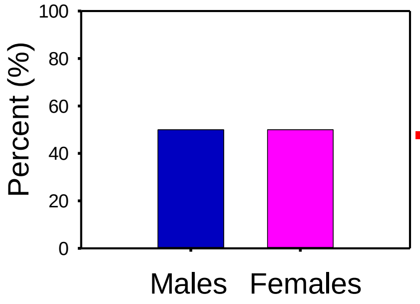


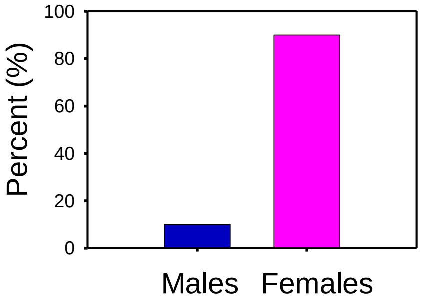
Piferrer et al., 2012 Mar Biotech

Reproduction-related problems in finfish aquaculture

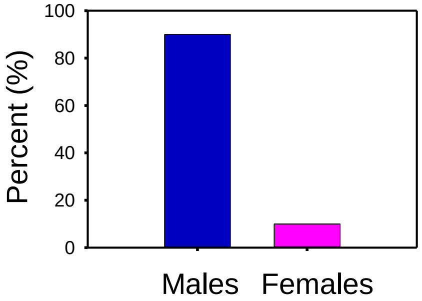
Sexual dimorphism (European sea bass, turbot, etc.) females bigger than males (>20-50%)

Aquaculture is interested in produce female populations





High temperatures increases the number of males (European sea bass)

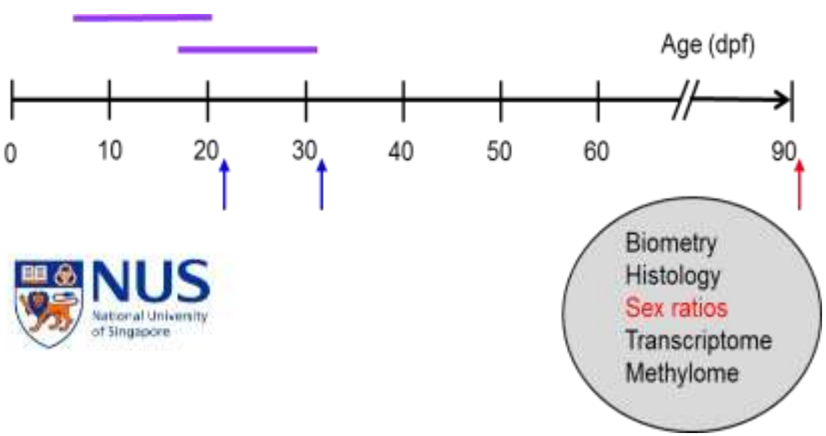


How can zebrafish be useful to better understand this problem?

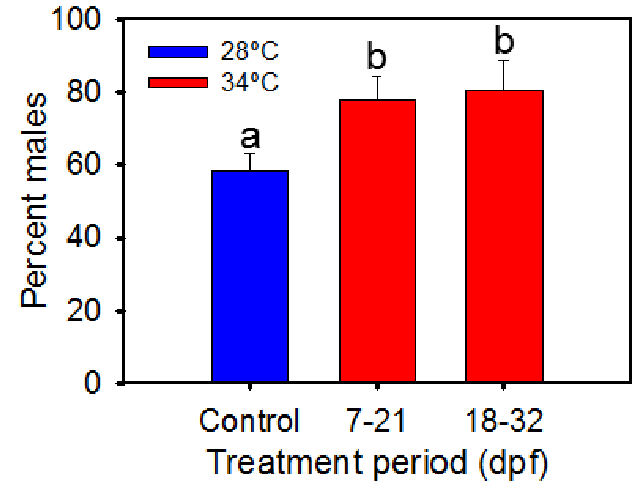
Zebrafish temperature experiment

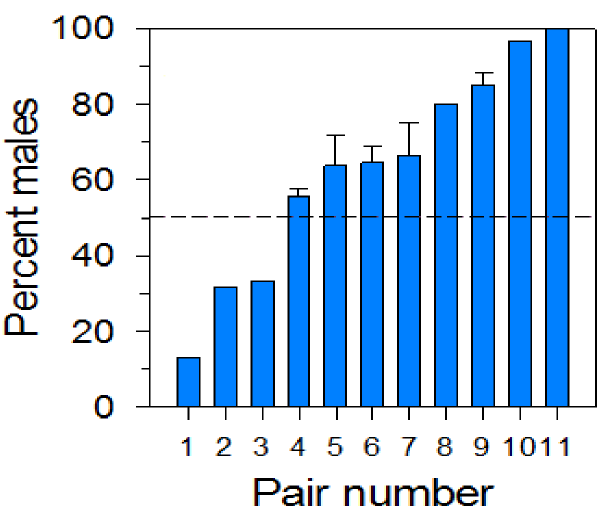
Objective: To determine the thermosensitive period (TSP) in zebrafish and to study the effects of temperature on the gonadal transcriptome and the resulting sex ratios. 10 different families, ~4,000 fish

Experimental design



Sex ratios



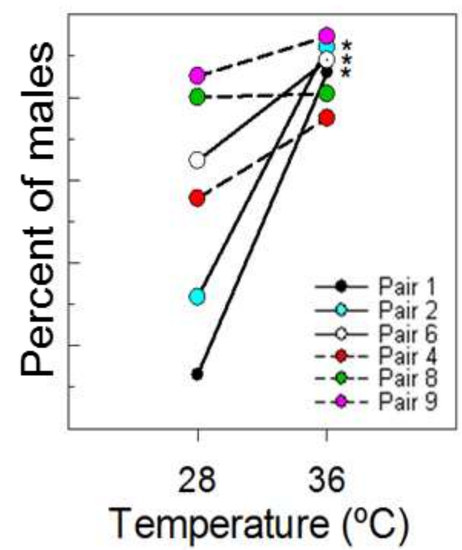


Sex ratio
response is
family dependent

GXE interaction

Intrafamily variation

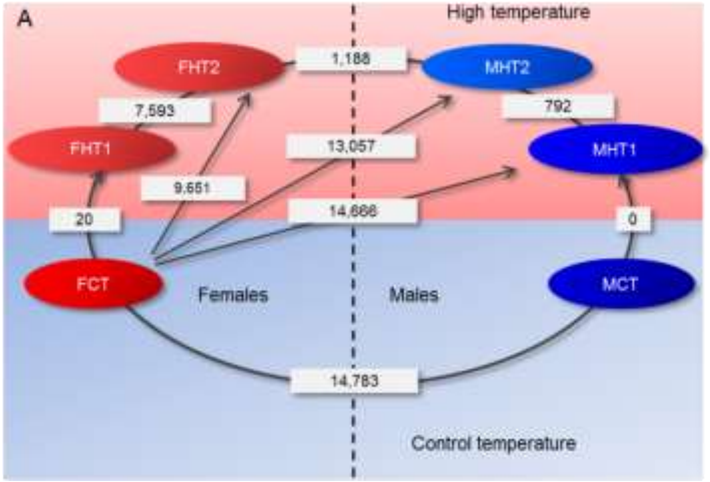
→ polygenic system



Ribas et al., PNAS under revision

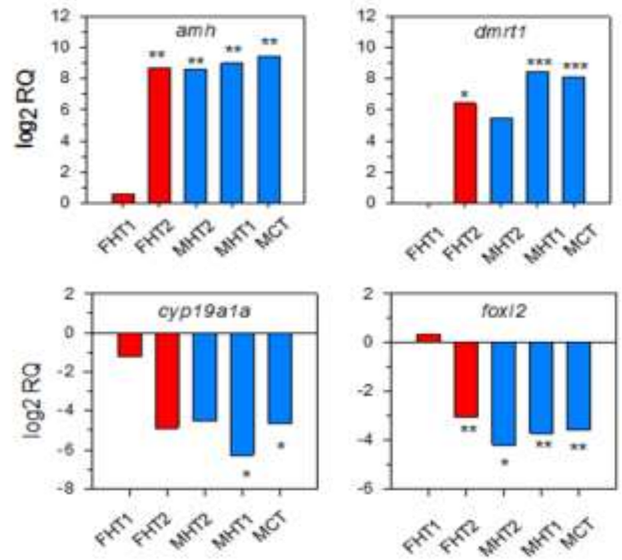
Zebrafish temperature experiment

Gonadal transcriptome



Ovary is very robust
65% of the transcriptome is altered but still ovarian differentiation takes place

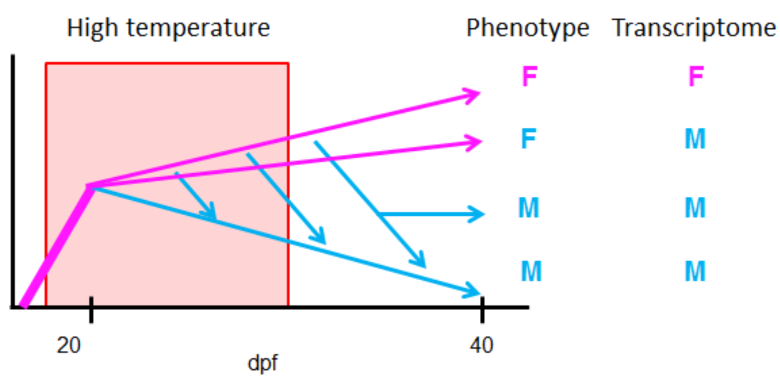
35 canonical reproduction-related genes



Male pathways are upregulated

Female pathways are inhibited

Conclusions

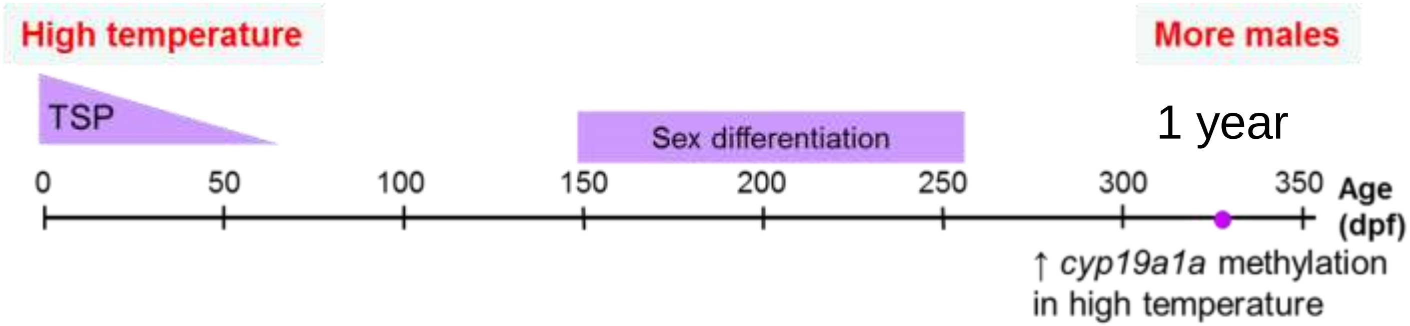


Ribas et al., PNAS under revision

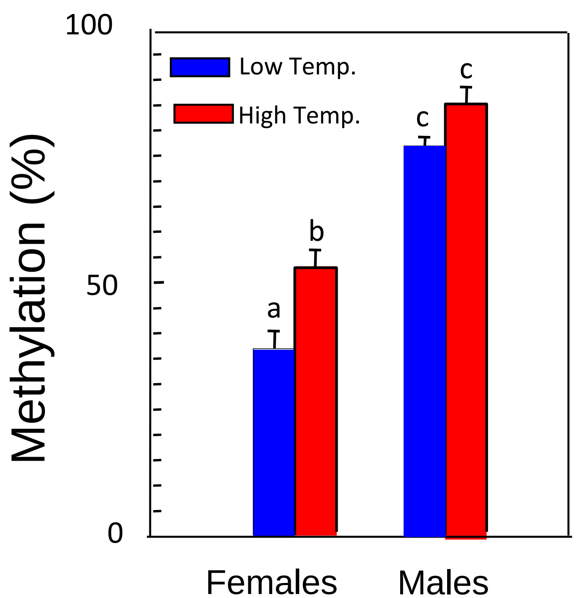
Temperature affects DNA methylation in European sea bass

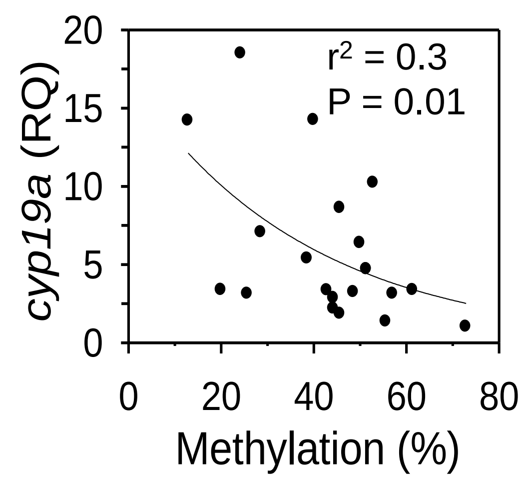
https://youtu.be/F3buIQ5BcUc

Polygenic sex determination with environmental (temperature) influences



Epigenetic mechanism, methylation of the *cyp19a1a* promoter





environment → DNA methylation → gene expression → phenotype

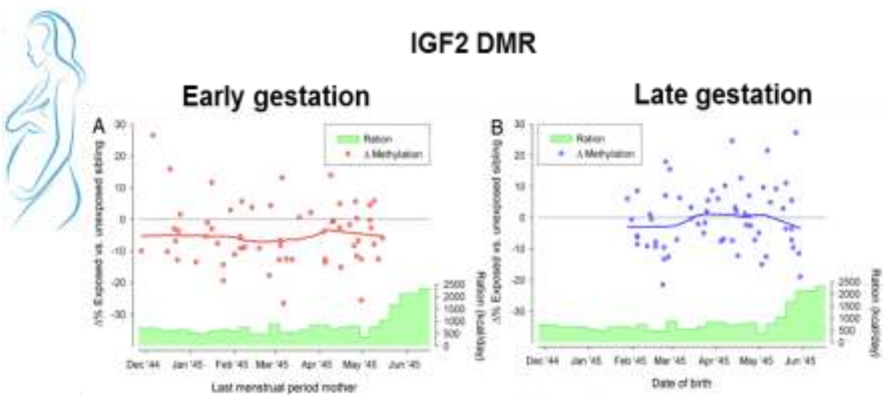
Navarro-Martín et al., 2011 PLoS Genet

https://youtu.be/F3buIQ5BcUc

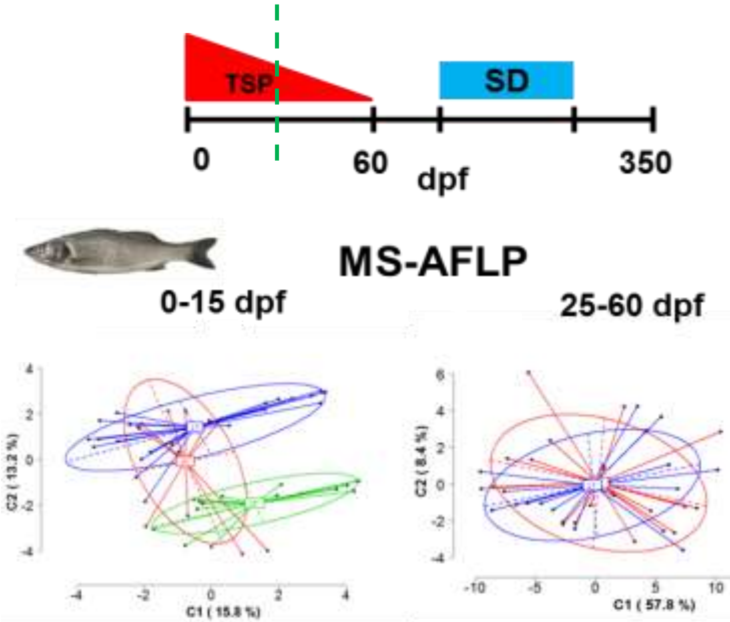
https://www.youtube.com/watch?v=F3buIQ5BcUc

Early environmental influences on phenotype through epigenetics

Early developmental environments may give rise to persistent epigenetic marks which correlate with relevant biological processes later in life



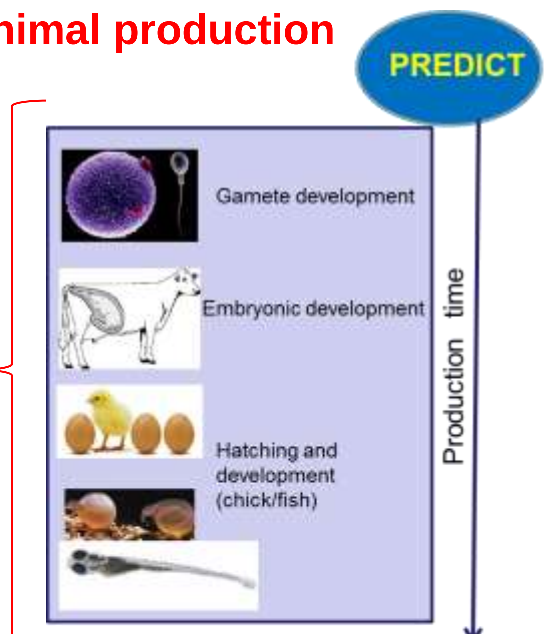
Heijmans et al., 2008 PNAS



Anastasiadi et al., in preparation

Epigenetics and animal production

Artificial environment



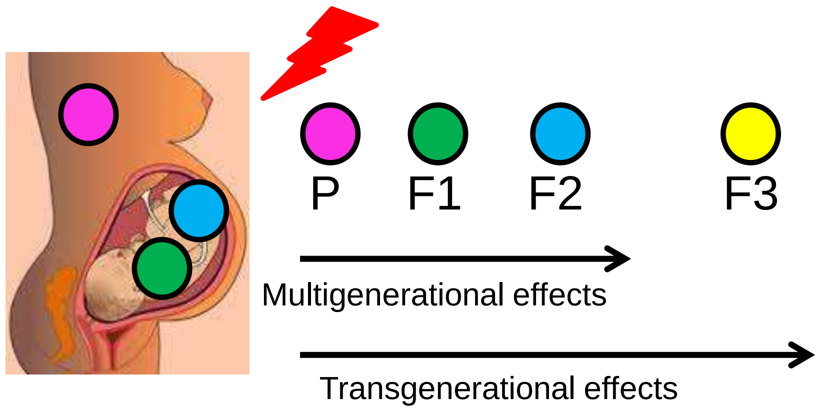
Prognosis markers

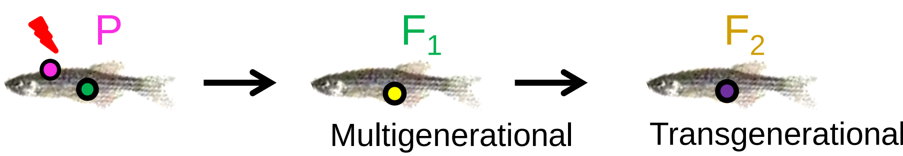
Epigenetic inheritance

- Product quality
- Growth
- Welfare
- Pathology resistance
- Reproduction

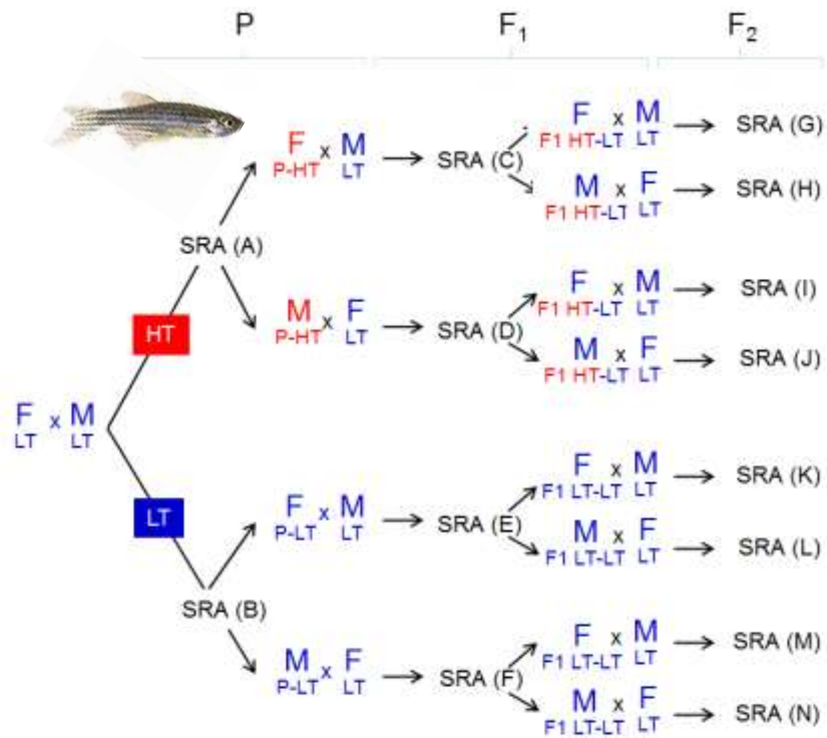
Transgenerational effects

Epigenetic changes are inherited in the offspring through the germ line

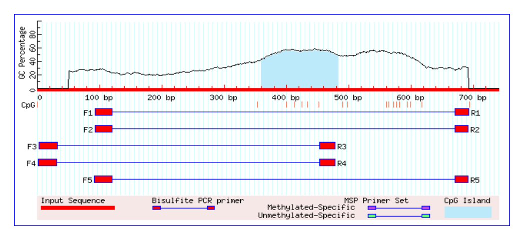




Zebrafish as a model for studying transgenerational effects



Multiplex Bisulfite Sequencing (MBS)

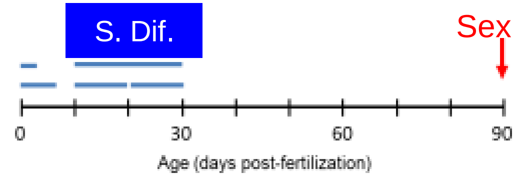


Prognosis tool

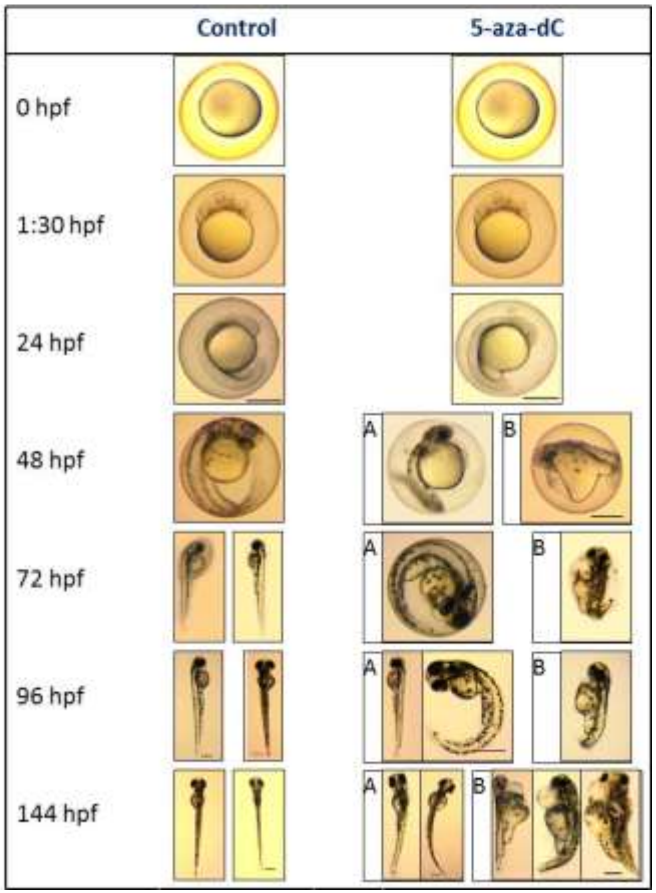
Methylation levels of 20 canonical genes

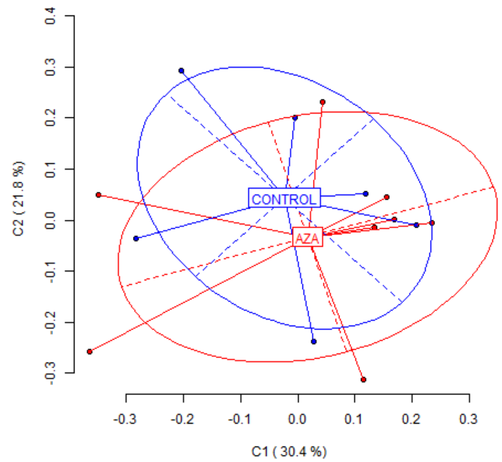
Epimutagens effects on gonad differentiation

Objective: develop a suitable *in vivo* system in which DNA methylation could be altered
- DNA-methyl-transferases inhibitor, 5'-aza-cytidine (aza)



Aza effects during early development

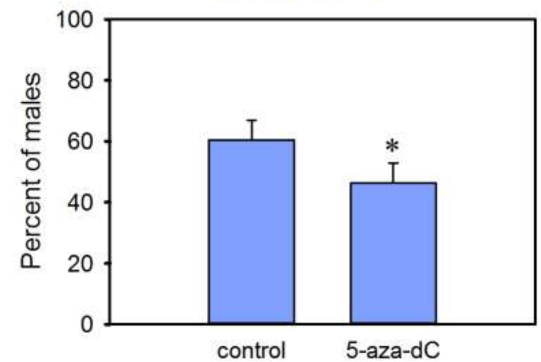




control

aza

Sex ratio



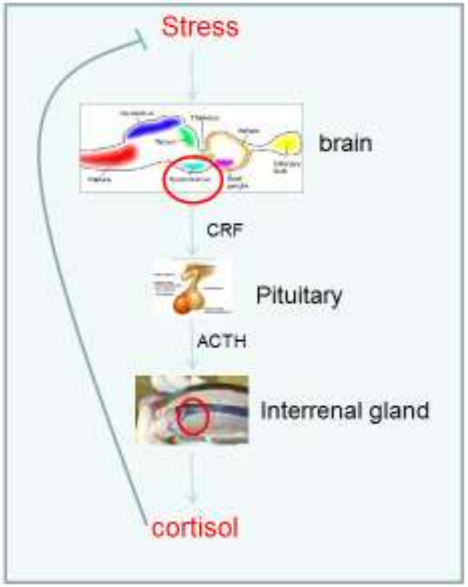
More females

Ribas et al., in preparation

Outline

- Laboratory fish models
- Landmarks in zebrafish research
- Requirements for animal model for research
- Zebrafish as a model for finfish aquaculture research?
    - Reproduction-related problems
    - **Stress-related problems**
    - **Nutrition- and growth-related problems**
    - **Pathology-related problems**
    - **Toxicology-related problems**
- **Final considerations of using zebrafish as a model**

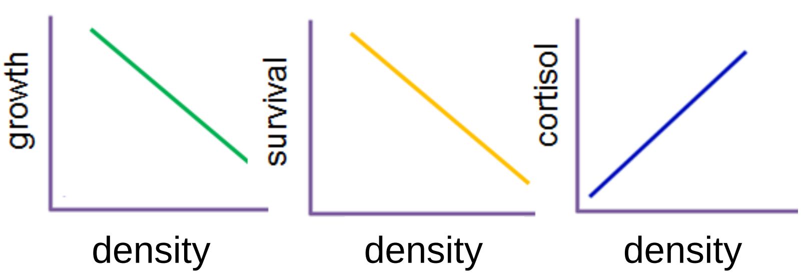
Stress-related problems in finfish aquaculture



Common causes of stress in aquaculture

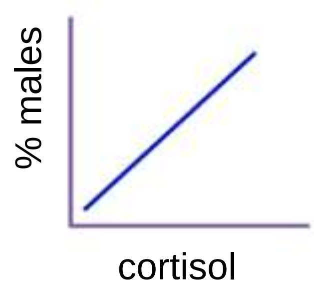
High densities, transport, poor water quality, handling, malnutrition, etc.

Density stress



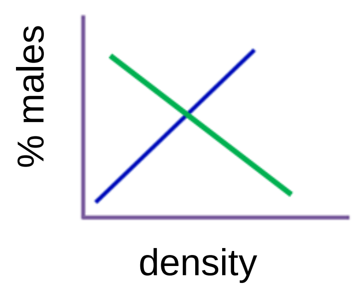
Wendelaar Bonga, 1997 Physiol. Rev.
Barton, et al 2002 Aquaculture

Density effects on sex ratios



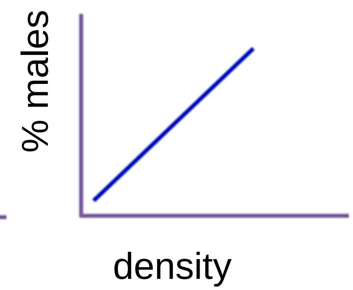
Yamaguchi et al., 2010 Reprod Dev

Pejerrey
(*Odontesthes bonariensis*)



Huertas and Cerdà 2006 Biol Bull; Krueger and Oliveira 1999 Environ Biol Fish

Eel
(*Anguilla anguilla*)

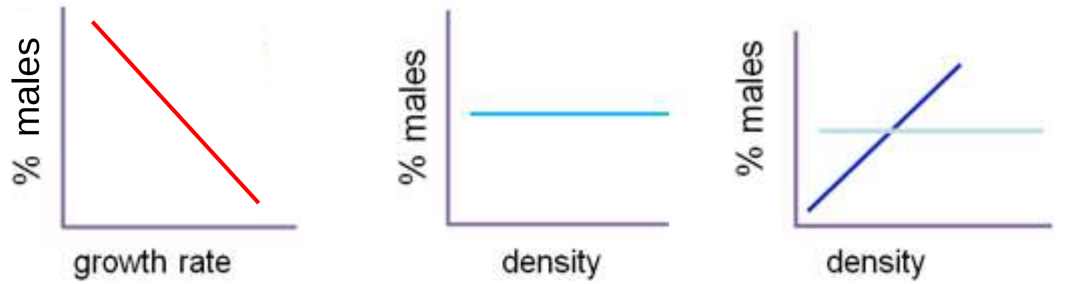


Francis 1984 Behaviour

Paradise fish
(*Macropodos opercularis*)

Usefulness of the zebrafish for stress-related problems in finfish aquaculture

Density affects sex ratios?



Lawrence et al., 2008
Environ Biol Fish

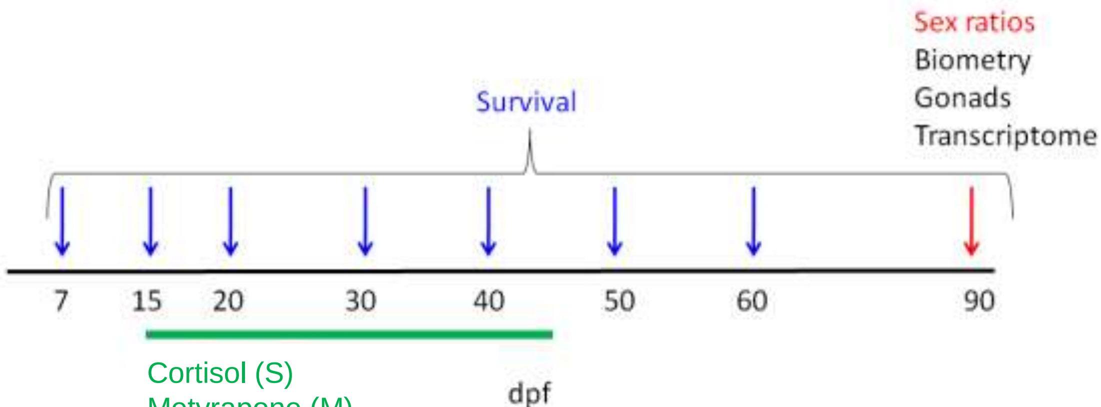
Hazzlerig et al., 2012
PLoS ONE

Liew et al., 2013
PLoS ONE

Drawback in zebrafish husbandry: Which is the density required to not alter sex ratios?

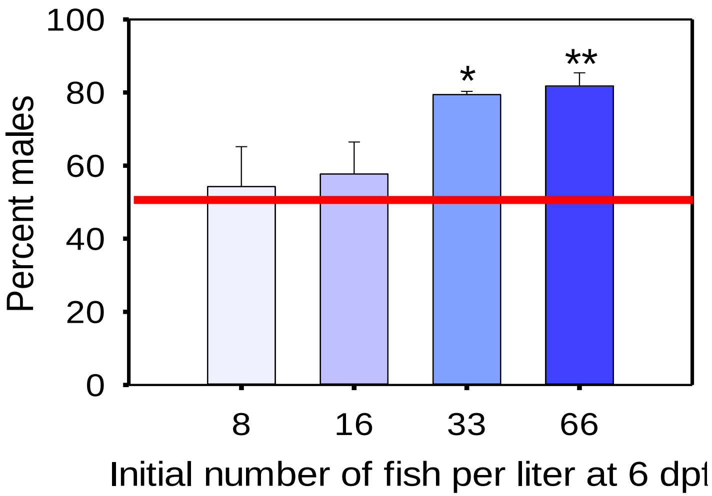
Experiment 1
4 treatment groups (8, 16, 33, 66 f/L)
10 biological replicates (7 different pairs)

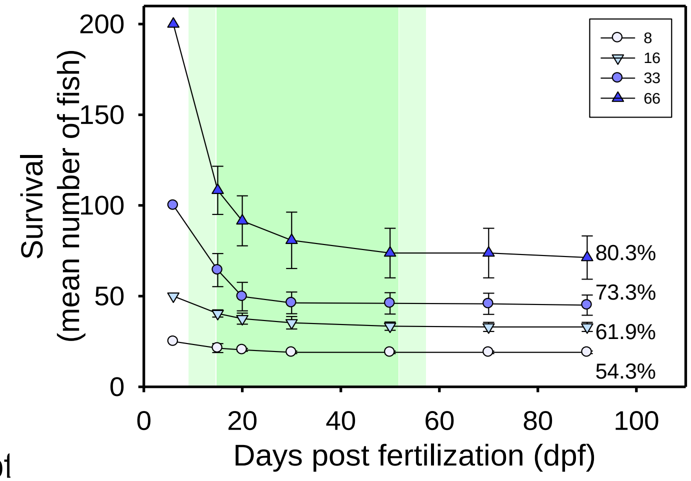
Experiment 2
5 treatment groups at LD
2 biological replicates (6 different pairs)



Cortisol (S)
Metyrapone (M)
M+S
Methyltestosterone (MT)

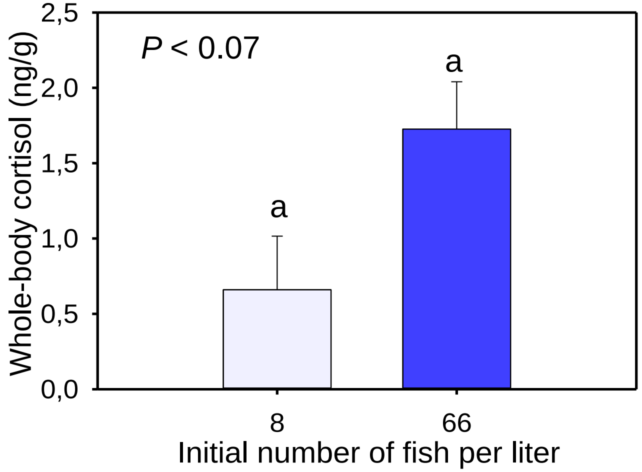
Density effects on sex ratio in zebrafish

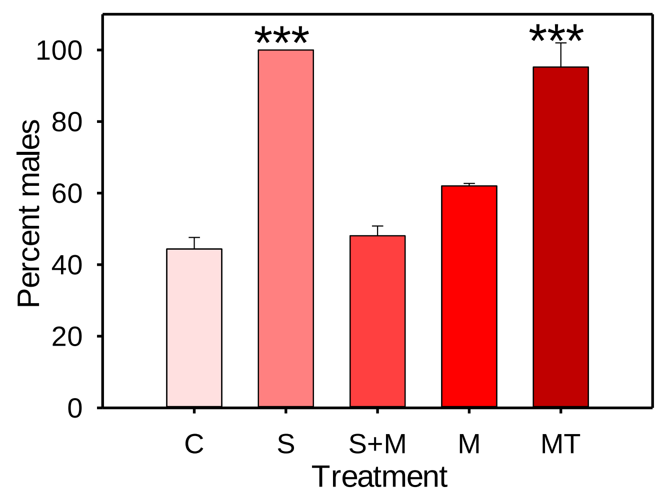




Turbot	15-20 fish/L
Sea bass	30 fish/L

Not exceed 13 zf/L during
sex differentiation process





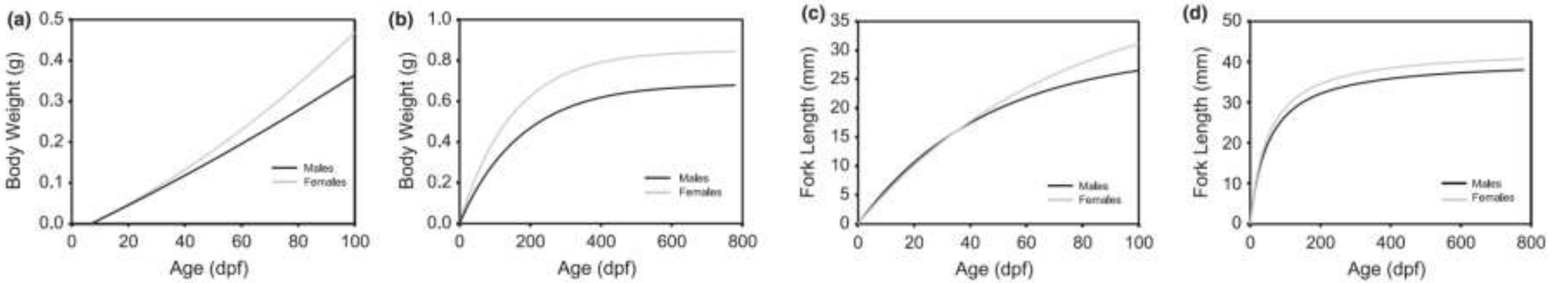
Masculinization through the cortisol pathway

Ribas et al., Exp. Biol. (under revision)

Usefulness of the zebrafish for nutrition- and growth-related problems in finfish aquaculture

1 There are so many **species-specific** differences in many aspects of growth and nutrition

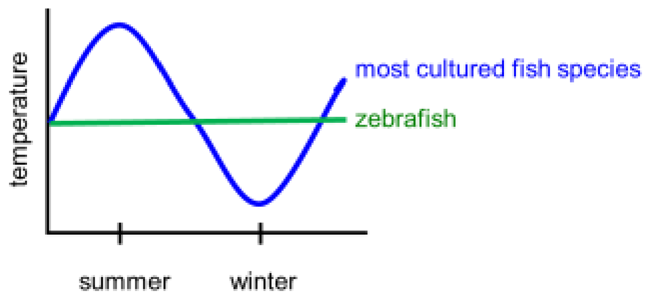
2 **Nutritional requirements** for each developmental stage are not completely established in zebrafish



Ribas and Piferrer 2014 Rev. Aquaculture

3 Zebrafish **growth is defined** (maximal size). Not good for catch up experiments

4 Zebrafish **metabolism** is very different from most cultured species because it lives in constant water temperatures



5 Like other cyprinids, zebrafish are **stomachless**

Zebrafish is a good model ??

Examples of using zebrafish for nutrition- and growth-related problems in aquaculture

Larviculture

- Iodine is required for metamorphosis
- Iodine levels of artemia are smaller than wild-caught zooplankton

Iodine

24–38 days

↑ Survival, better successful in metamorphosis

Hawkyard et al 2011 Aquaculture

Growth performance

Biotin

100 days

X

↑ Sperm quality ↑ Number of eggs

↑ Fertilized eggs
Hatched rate

↑ Larvae survival
Larvae growth

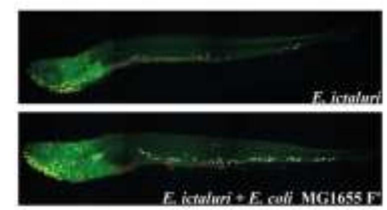
Yossa et al 2015 Rev Aquaculture

Use of probiotics

37 probiotics

4–6 days

Bacterial infection



3 probiotics showed immune protection against bacteria

Rendueles et al 2012 PLoS Pathog

Usefulness of the zebrafish for pathology-related problems in finfish aquaculture

Fish disease outbreaks → high economic losses

Zebrafish considered as a good animal model

Diseases infection models

Virus, bacteria, parasites

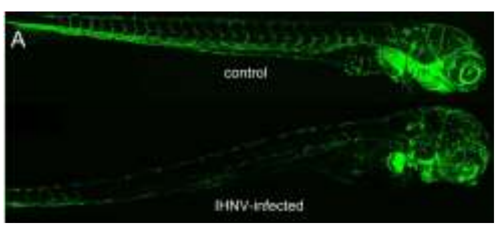
Infectious hematopoietic necrosis virus (IHNv)

Affects salmonid fish

Salmon (2001-2003) 58% died or culled = >10 millones $ (Saksida et al., 2006 Dis Aquat Org)

Detailed analysis of a viral infection in zebrafish

Target of infection= blood vessels (hemorrhages)
Destruction of vascular cells



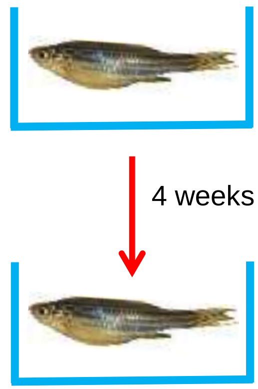
Ludwig et al 2011 PLoS ONE

Vaccine development

Virus, bacteria

Vibrio anguilarum

Bath with attenuated virus



Infections by virus

Vaccinated group

↑ Specific Ab = 90% protection
↑ Specific immune- related genes
No inflammatory response

Zhang et al 2012, 2013 Fish Shellfish Immun; Vet Immunol Immunop

Salmon louse

(*Lepeophtheirus salmonis*)

180 milions € in salmon aquactutlure (Lafferty et al 2015 Rev Adv)

Environment = salt water

Usefulness of the zebrafish for toxicology-related problems in finfish aquaculture

Zebrafish has been a good model for toxicology studies in the last 20 years accepted for the OECD

Water pollution → Alter growth and sex ratios, decrease survival, increase infections,etc.

Immune system

PAH + *Mycobacterium marinum*

>14 days

↑ Inflammation

⇓

↓ Clearance of the pollutant

Prosser et al 2011 Aquat Toxicol

Reproduction system

PFOS

>5 months

↓ Growth

↑ Number of females

F_{PFO} x M 0% survival

M_{PFO} x F 100% survival

Wang et al 2011 Environ Toxicol Chem

Transgenerational effects through diet

benzo[a]pyrene (3 doses)

X

↓	Hatching rate	F1
↑	Deformaties	F2
	-	F3
	-	F4

↓ Global methylation level in embryos (F1)

Corrales et al 2014 Comp Biochem Phys C; Aquat Toxicol

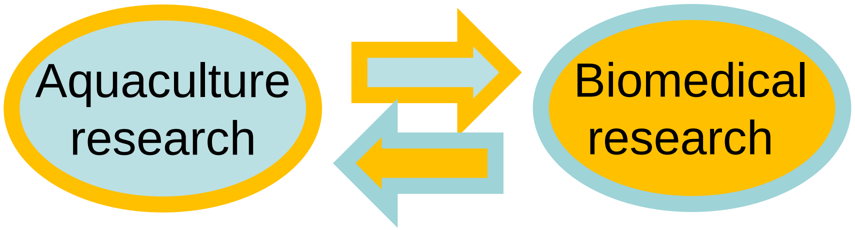
Final considerations of using zebrafish as a model

1. **Small size** → difficult to remove blood (hormone detection in the water)

2. Large **genome size** 1,412 Mb → not convenient for genetic studies (use medaka 800 Mb)

3. Lack of **breeding protocols** → importance to control inbreeding and outbreeding crosses

4. Improved **husbandry protocols** → food intake for each development (nutrients required)
→ health maintenance protocols (disease)



5. The zebrafish is a suitable model for several finfish aquaculture research areas. Some of these areas can benefit more than others, but always cautious is needed when extrapolating results to commercial species