Monosexing to Improve Productivity and Quality

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The Future Prospects for Aquaculture Breeding in Europe

Professional and Scientific Workshop
Paris, October 1-3, 2008

Current problems in European fish farming due to skewed sex ratios

- Increased size dispersion and thus more need for size-gradings
- Less produced biomass within a given production unit
- Lower product quality if one sex is more valuable than the other
- Precocious maturation brings several additional problems to fish farming
- Depreciated product when release of sperm

Species for which one sex is more valuable and why

- Trout – maturation, flesh quality
- Sea bass – highly skewed sex ratios, precocious maturation
- Senegalese sole – highly skewed sex ratios
- Turbot – highest sex-related growth differences in favor of females
- Sturgeons – only females for caviar production
- Tilapias – males are usually larger than females
- Trout, Sea bass, Sea bream, etc. – Only female triploids do not develop gonads

Part 1: Summary of the Problem and General Scientific Principles

Sex Ratio:

Relation between the number of males and females in a population. Usually tends to be 1:1 (50% males, 50% females)

In fish farming, sex ratio influences growth patterns before, during and after sexual matration

Sex determination + Sex differentiation = Sex ratio

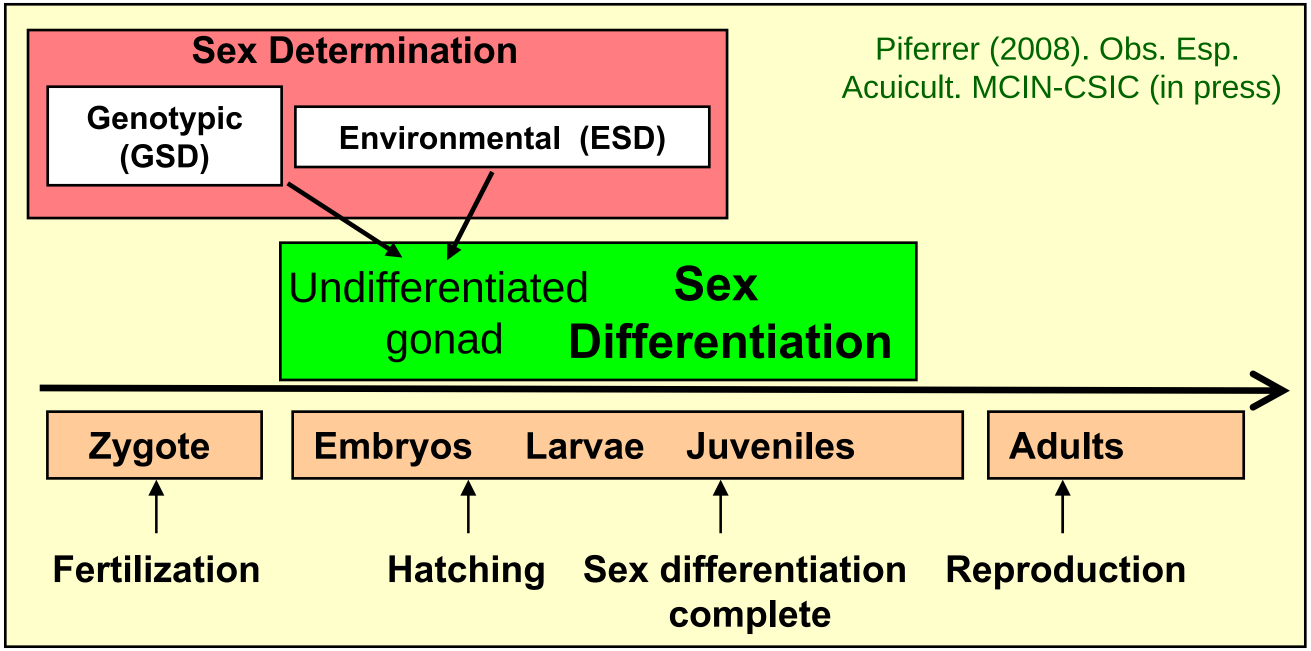
Part 1: Summary of the Problem and General Scientific Principles

Sex Determination (definitions by Bull, 1983)

The genetic or environmental process by which the sex (gender, male or female) of an individual is established.

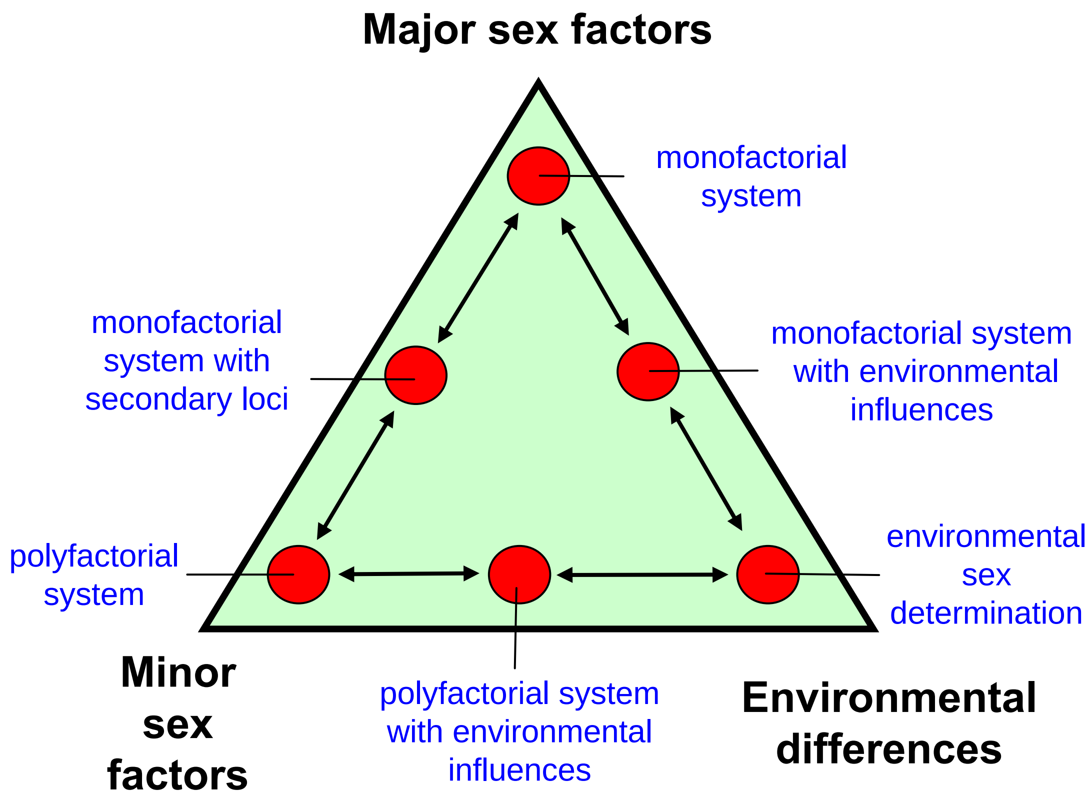
Sex Differentiation

Process by which the various molecular, genetic and physiological processes produce a male or female from a zygote of a given genotype and parents in a given environment



Part 1: Summary of the Problem and General Scientific Principles

What determines sex ratio:



D. Penman & F. Piferrer (2008). Fish Gonadogenesis Part 1. Genetic and Environmental Mechanisms of Sex Determination Rev. Fish Sci., 16 (S1): 14-32.

Part 1: Summary of the Problem and General Scientific Principles

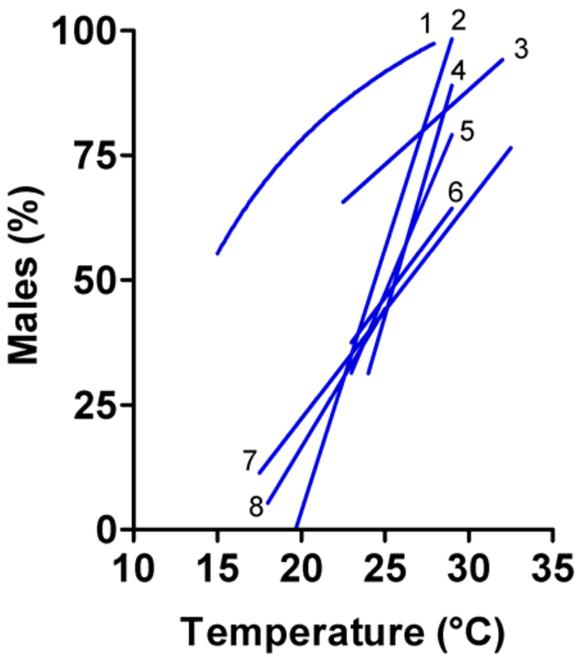
Disentangling Genotypic vs. Temperature-dependent Sex Determination

Sex Determination	Sex Differentiation	Observed Result	Consistent genetic differences?	The genotype determines sex?	Earliest ontogenetic difference	Sex determined at
GSD	No environmental influence	"Pure" GSD	Yes	Yes	Genetic	Fertilization
GSD	Environmental influence	GSD + Temp. effects (GSD+TE)	Yes	Yes	Genetic	Fertilization and thermolabile period
TSD	Environmental influence	TSD	No	No	Environ.	Thermolabile period

A discrete process —produces→ a continuous pattern

Valenzuela et al. (2003). Pattern does not equal process. Exactly when sex is environmentally determined?. Am. Nat. 161: 676-683

Temperature Influences on Sex Ratios



Key:
1 - *Mendia menidia*
2 - *Odontesthes bonariensis*
3 - *Hoplosternum littorale*
4 - *Poeciliopsis lucida*
5 - mean of 33 *Apistogramma* sp.
6 - *Limia melanogaster*
7 - *Menidia peninsulae*
8 - *Odontesthes argentinensis.*

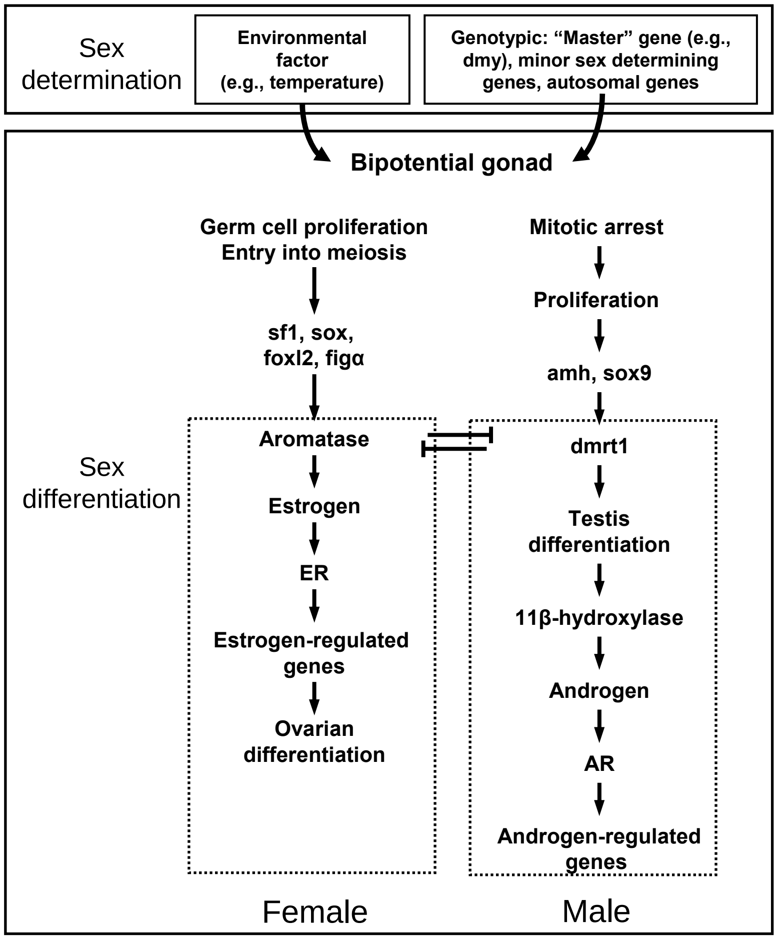
Why is not feasible to obtain all-female stocks with temperature manipulation alone?

Ospina-Álvarez & Piferrer (2008). Temperature-dependent Sex Determination in Fish Revisited: Prevalence, a Single Sex Ratio Response Pattern, and Possible Effects of Climate Change. PLoS ONE 3(7): e2837. doi:10.1371/journal.pone.0002837

Sex ratio response in GSD+TE or TSD species follows a single general pattern: more males with increasing temperatures

↑Temp. → Male sex differentiation → Sex ratio

Hormonal regulation of sex differentiation



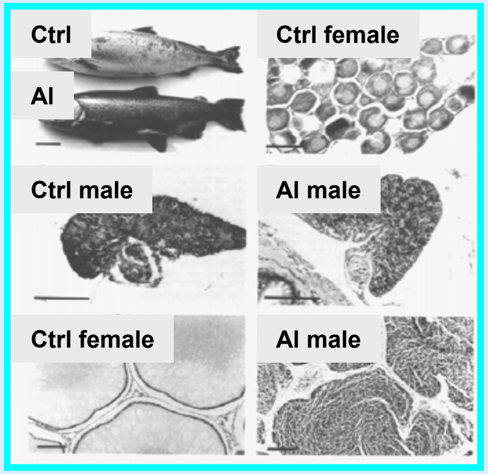
Sex differentatiation involves similar or the same players across vertebrates, with the steroidogenic enzyme aromatase and the transcription factor dmrt1 playing a central role

F. Piferrer & Y. Guiguen (2008). Fish Gonadogenesis. Part 2. Molecular Biology and Genomics of Sex Differentiation. Rev. Fish Sci., 16 (S1): 33-53.

Hormonal regulation of sex differentiation

Gonochoristic fish

Pacific salmon



Aromatase

T → E_2
Ovarian differentiation

T ~~→~~ E_2
Testicular differentiation

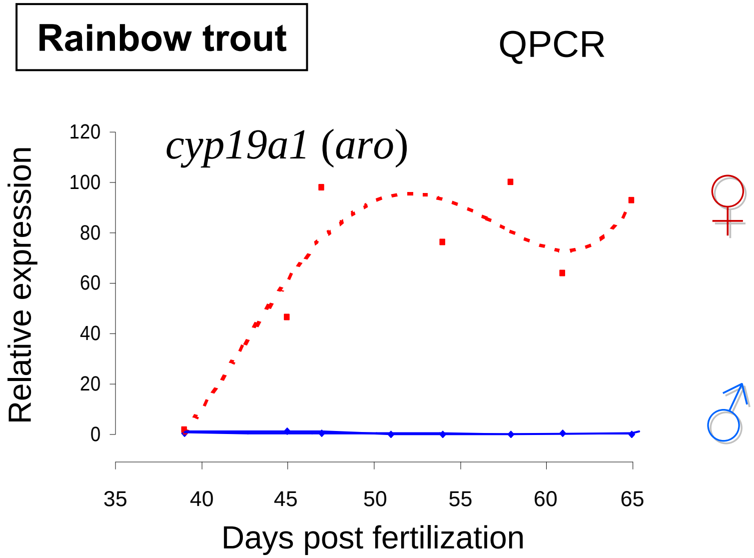
Piferrer et al. 1994. J. Exp. Zool., 270: 255-262.

Aromatase correlates with female sex differentiation, both in gonochoristic and hermaphroditic fish

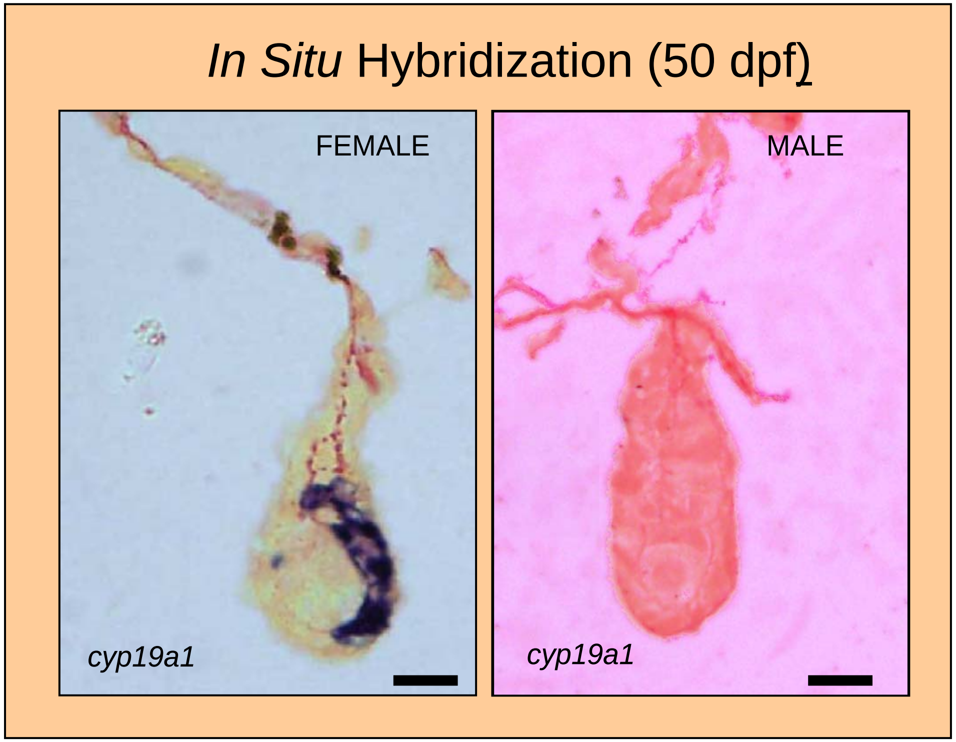
Part 1: Summary of the Problem and General Scientific Principles

Aromatase gene (*cyp19a1*) is expressed only during ovarian differentiation

Aromatase can be used to predict sex



Vizziano et al. (2007). Dev Dyn. 236: 2198-206.



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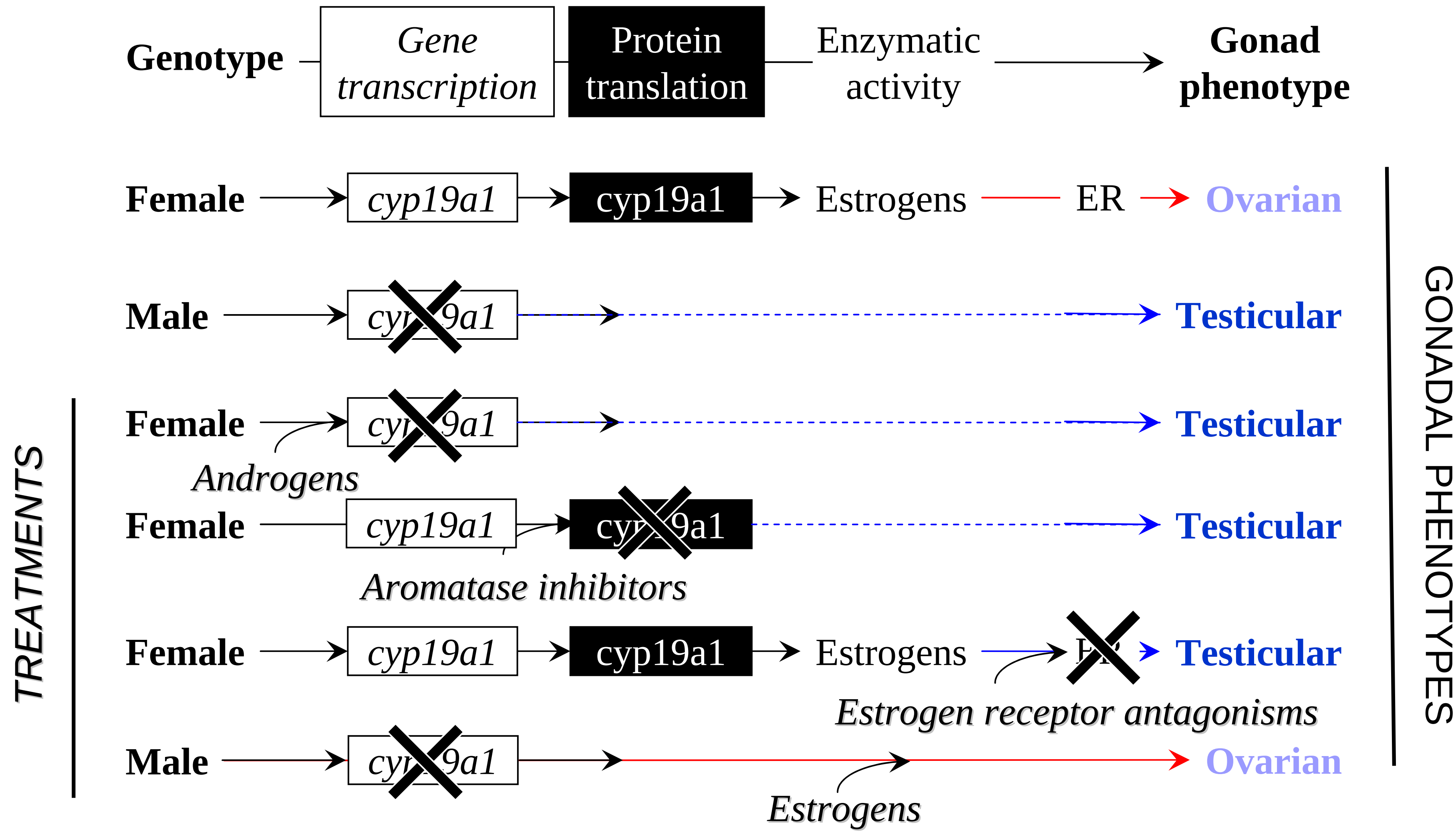
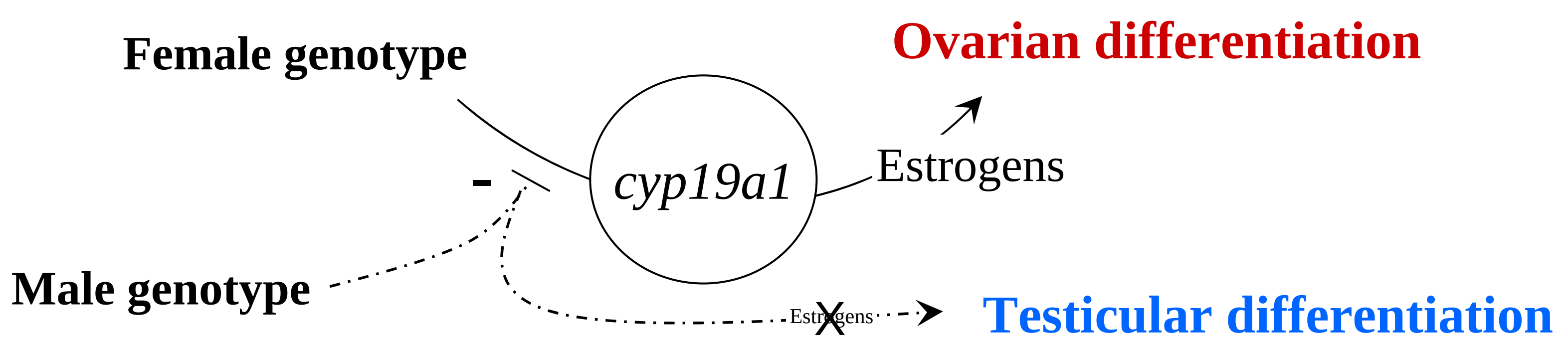


Guiguen et al. (2008). Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish.Gen. Comp. Endocrinol. (in preparation)

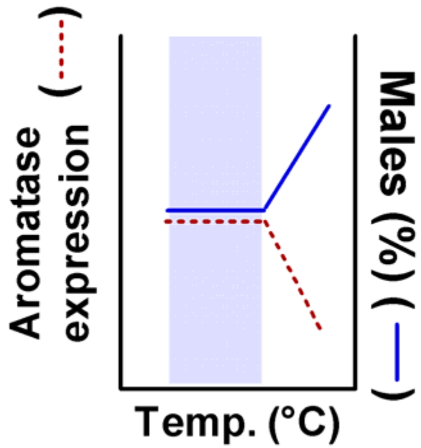
Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish.
Yann GUIGUEN, Alexis FOSTIER,
Francesc PIFERRER, Ching-Fong CHANG.
Gen. Comp. Endocrinol. Special Reprofish review.

Aromatase and estrogens play a pivotal role for fish gonadal differentiation.

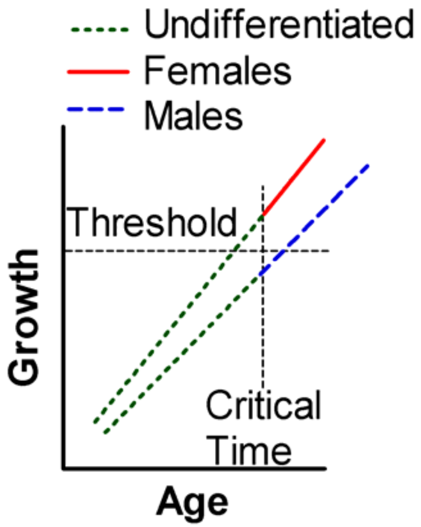


Part 1: Summary of the Problem and General Scientific Principles

Temperature affects sex ratios through changes in aromatase expression



In many species, there is a relationship between growth and sex differentiation



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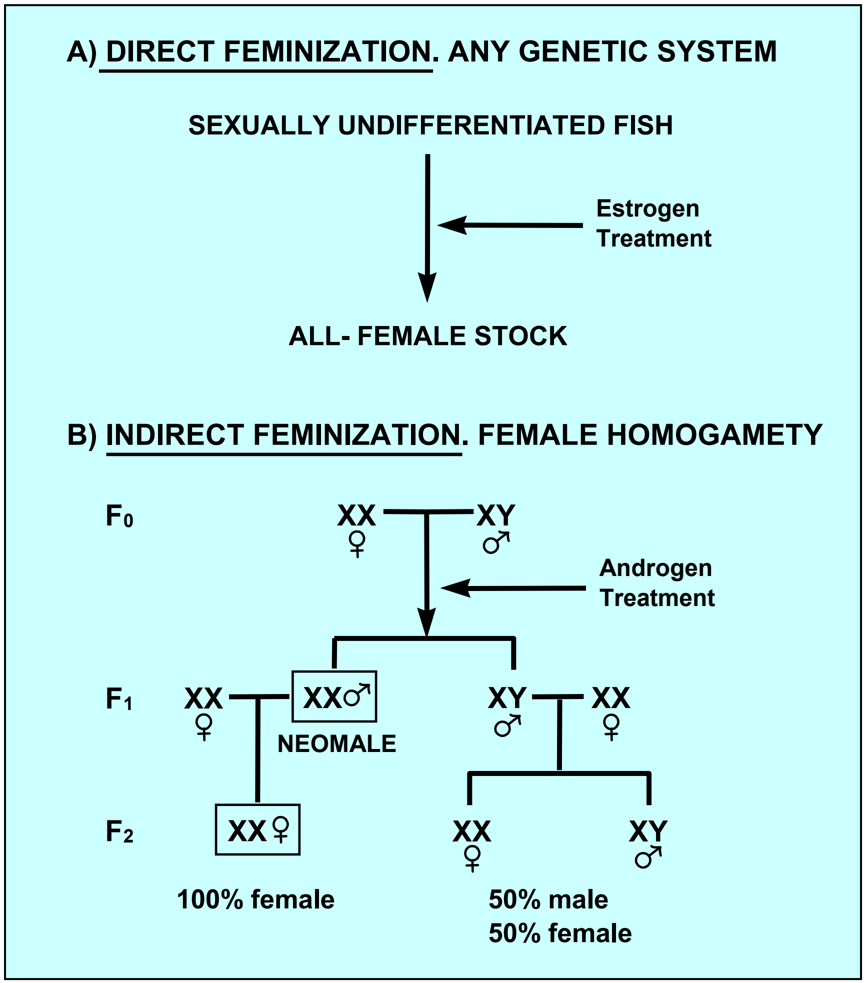
Part 2: State of Application to the Fish Farming Industry

Monosex stocks can be obtained by:

- Direct methods involving treatment with sex steroids or aromatase inhibitors
- Indirect methods involving sex steroids and selection of progeny
- Hormonal methods combined with chromosome set manipulation
- Environmental manipulation (a few cases)

Endocrine Methods to Produce All-Female Stocks in Fish



Piferrer (2001). Endocrine sex control strategies for the feminization of teleost fish. Aquaculture, 197: 229-281.

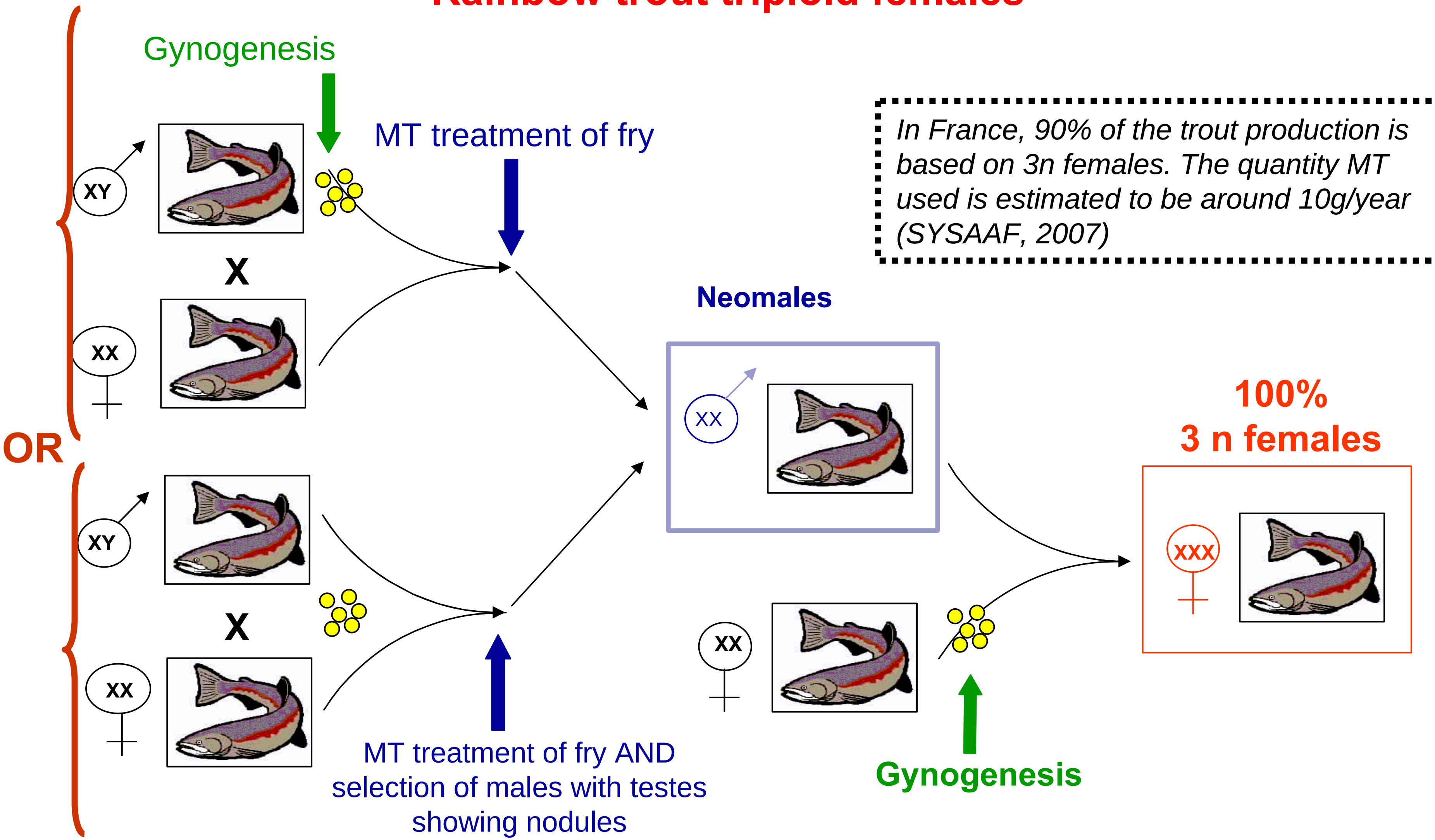
56 species reviewed
Protocols for 28 species

Treatment Parameters

QUALITATIVE	QUANTITATIVE
Type of steroid	Treatment timing
Nature of steroid	Dose
	Treatment duration
	Treatment frequency
	Number of treatments
	Water temperature
	Fish density

Part 2: State of Application to the Fish Farming Industry

Rainbow trout triploid females

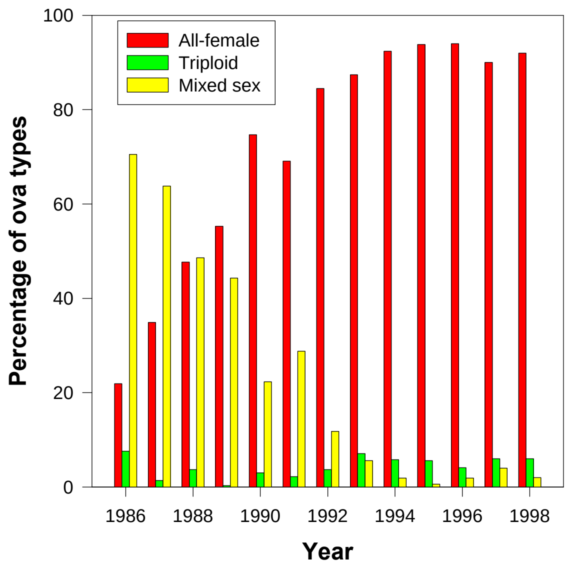


Endocrine Sex Control Involved in Practical Aquaculture

Rainbow Trout (France, Scotland, Japan)
Brown Trout (France)
Atlantic Salmon (Canada)
Coho Salmon (Canada, Japan)
Amago Salmon and Masu Salmon (Japan)
Ayu and Hirame (Japan)
Channel Catfish (USA)
Nile Tilapia (China, Fiji, Philippines, Thailand, USA, Vietnam)
Jordan tilapia (Israel)
Silver Barb (Thailand)

Hulata, G. (2001). Genetica, 111: 155-173.

Scottish Rainbow Trout Production



Information provided by
Dr. B. McAndrew,
Univ. Stirling, Scotland

Part 2: State of Application to the Fish Farming Industry

Food Safety Issues Regarding Hormone-Treated Animals

Fish	Mammals
Legal (96/22/EC of 29 April 1996)	Illegal
To contol reproduction	To enhance growth
Uses a natural hormone (E_2)	Uses a synthetic hormone
Physiological doses	Pharmacological doses
Short term treatment (h-wks)	Long term treatment (months)
Hormone is eliminated (~3 wk)	Hormone residues present
No hormone whatsoever in the marketed animals if the direct method is used	Always in the the marketed animals

Piferrer (2001). Aquaculture, 197: 229-281.

Remaining practical questions in relation to treatments

Use of hormones

- Impacts of MT on the environment are not well known
- From a human health point of view, the use of MT in fish farms is not always well controled
- The use of steroids is not accepted by consumer
- Lack of technical studies on effluents treatments device for hormones

Risks can be reduced by:

- Optimizing protocols to limit amounts used (narrow labile period)
- Proposing efficient efluent treatments
- Searching for alternative methods

Use of environmental factors

- Still very limited
- Today involves only a few species
- Limited information on environmental effects on sex ratios for many species

Part 3: The Known Research Gaps

Remaining practical questions in relation to the species

General

Lack of knowledge on sex determination and of the mechanisms of sex differentiation in many species (both aspects may have impacts on sex control)

New species for aquaculture may not immediatly take advantage of the knowledge gained in other species (e.g., sex detemination is highly variable)

An efficient treatment is not available for complete sex control

For GSD + TE species such as the sea bass

No sex control available for hermaphrodite species

Interest to prevent protandrous sex change in sea bream

Lack of knowledge on the biological regulation of sex inversion

Part 4: The Current and Future Research Needs

The genetic and genomic resources already available for many species (trout, sea bass, salmon, sea bream) need to be integrated in an attempt to contribute to solve production bottlenecks

Lack of sex-specific or sex-linked markers for many species (e.g., early detection and selection of female sturgeon)

The underlying mechanisms responsible for environmental effects on sex ratios in both GSD + TE and TSD species are not known

The relationship between growth and sex differentiation is also still poorly understood

Aquaculture impacts on wild stocks is an issue of increasing concern. Thus, monosexing in combination to triploidy needs to be further explored

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