SEX DETERMINISM IN EURASIAN PERCH, *Perca fluviatilis* : EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS

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Introduction. In fish, sex is genetically (GSD) and/or environmentally (ESD) determined. Besides sex chromosomes (generally XX/XY or ZW/ZZ), autosomal genes are also involved in sex determinism mechanism in fish. Several studies have proven that environmental factors, principally temperature, can influence the sex differentiation mechanism towards males or females.

Only 10 % of fish species display heteromorphic sex chromosomes, and despite the recent research, few sex-specific markers exist in fish. In order to study the different genetic mechanisms involved in genetic sex determinism in fish, hormonal sex inversion, crosses with hormonally sex-reversed breeders, gynogenesis (method of chromosome-set manipulation in which offspring inherited all the female set of chromosomes) or interspecific hybridization are currently used (Devlin and Nagahama, 2002).

The aim of this document is to present a synthesis of study genetic sex determinism (using hormonally sex-reversed breeders, gynogenesis, inter-specific hybridization) and environmental sex determinism study (high temperature) in Eurasian perch.

Materials and methods. Exp 1. Hormonally sex-reversed male breeders of Eurasian perch were produced by feeding mixed-sex juveniles (mean body weight (Pm) = 40 to 205 mg) perch populations with diet complemented with exogenous sex steroid 17α-methyltestosterone (40 to 80 mg kg^{-1} food) for 30 to 80 days.

Exp. 2. In order to compare some reproductive characteristics of XY males vs XX males we determined the gonadosomatic index (GSI), sperm concentration, sperm motility and plasma levels sex steroids (testosterone - T, estradiol – E_2 and 11keto-testosterone – 11KT) during the reproductive period (1 April – 15 May) for each male genotype. Gonadosomatic index (100 x gonad weight / total body weight) and sperm concentration (estimated by counting spermatozoa in a hemocytometer) were determined in the middle of the spawning period (25 April). Sperm motility was assessed using computer-assisted sperm analysis (CASA) and expressed by the curvilinear velocity (VCL, µm sec^{-1}), straight line velocity (VSL, µm sec^{-1}), average path velocity (VAP, µm sec^{-1}) and percentage of motile sperm (MOT, %). Plasma T, 11KT and E_2 levels were assessed by radioimmunoassay (RIA) at the beginning (6 April), the middle (25 April) and the end (9 May) of the spawning period.

Exp. 3. Mixed-sex and all-female populations of Eurasian perch were artificially produced by fertilizing eggs with sperm from normal XY male (mixed-sex populations) and hormonally sex-reversed XX male (all-female populations) selected based on gonad morphology (XY males displayed paired testes and XX males displayed a single testis with nodule).

Exp. 4. In order to induce gynogenesis, semen was 10-fold diluted in extender, a solution with Bicine. 10ml of diluted sperm were placed on Petri dishes and placed 5 cm under UV light (254nm, 15 Watts) for 400 seconds. Each spawn was divided into 3 parts and articially fertilized with sperm : one with non-irradiated sperm (control), the second part with UV-irradiated sperm and heat-shocked (gynogens) and the third part with UV-irradiated sperm and not heat-shocked (haploid). A heat shock of 30°C for 25 minutes was applied beginning at 5 minutes post-fertilization on gynogens batches in order to induce the retention of the second polar body (Rougeot *et al.*, 2003). Ploidy levels was assessed by flow cytometry analysis on 2-days old larvae and sex ratio determined by morphological examination of the gonad.

Exp. 5 Hybrid perch were artificially produced by crossing female Eurasian perch and male yellow perch. Each spawn was divided into 2 parts : one part was fertilized with Eurasian perch sperm (control) and the other part was fertilized with Yellow perch sperm (hybrid). After larval rearing, hybrids and their respective control were transferred into a recirculating aquaculture system (23°C, O_2 > 6ppm) for ongrowing during 6 months. When gonads were morphologically differentiated in male and female (6 months old), the sex ratio of the progenies was assessed on 100 random fish from each group.

Exp. 6. In order to test the effect of temperature on sex determinism in Eurasian perch, different batches of undifferentiated juveniles (Pm = 40 mg) were exposed for 30 days to high temperature (29 to 34°C). 33 and 34 °C were lethal in perch (all fish died after one day).

Results. Exp. 1. Complete sex reversal (100 % male progenies) was obtained exclusively when the hormonal treatment was applied to fish initially ranging from 40 to 71 mg, regardless the dose and treatment duration. High initial body weight (> 70 mg), high hormonal doses (>80 mg kg^{-1}) and long duration (80 days) induced variable proportions of males (70 to 97 %), females (0 to 29 %), ovotestis (0 to 18 %) and undevelopped gonads (0 to 27 %) in the resulting populations (Rougeot *et al.*, 2002).

Exp. 2. Gonadosomatic index and sperm concentration were not significantly ($p > 0.05$) different between the two genotypes (Table 1). None of the four motility parameters were significantly ($p > 0.05$) different between XY and XX males. T, 11KT and E_2 levels increased in the middle of the reproductive season (2.70 ± 0.34; 3.93 ± 0.52 and 4.25 ± 0;55 ng ml^{-1}, respectively)

and decreased at the end (1.79 ± 0.27; 1.89 ± 0.26 and 1.18 ± 0.24 ng ml^{-1}) but were not significantly different between the 2 genotypes.

	GSI (%)	Sperm density (10^9 ml^{-1})
XY males	7.6 ± 2.1	32.8 ± 4.3
XX males	7.0 ± 2.4	34.0 ± 3.0

Table 1 : GSI (%) and sperm density (10^9 ml^{-1}) of XY and XX males.

Exp. 3. Sex-ratio of progenies resulting from a cross with normal XY male were not significantly ($p > 0.05$) different from a balanced sex ratio in 7 families and were significantly ($p < 0.05$) skewed towards males or females in two families. Sex ratio of all-female populations is significantly ($p < 0.05$) skewed towards females : 97.0 to 100 % (Table 2).

			Sex ratio (%)		
	N	N of fish sexed	Female	Male	χ^2
XY male	11	49-100	32.7-57.9	42.1-67.3	0.0-3.0
	2	100-469	39.3-65.0	35.0-60.7	4.6-11.0*
XX male	5	56-100	100	0	37.3-66.7*
	3	46-100	97.0-98.5	1.5-3.0	39.8-56.7*

Table 2 : Sex ratio of progenies resulting from crosses with XY males and XX males. Values are minimum and maximum. N = number of batches. *$p < 0.05$.

Exp. 4. Ploidy were 100 % diploid for the 4 control groups and 2 gynogens groups. The two other gynogens batches displayed 6.7 and 10.0 % triploids fish, suggesting that UV-irradiation was not completed. The control displayed a balanced sex ratio and the 4 gynogens batches are all-female (100 %; Fig.1).

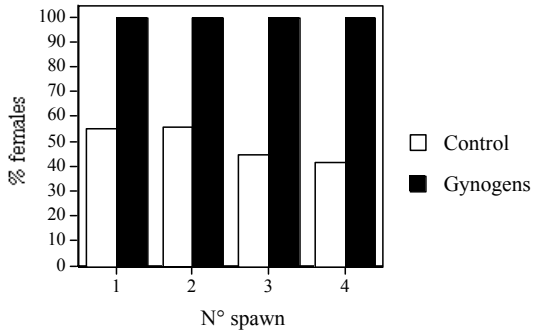

Figure 1. Percentage of females in gynogens group and their respective control.

Exp. 5. Controls displayed a balanced sex ratio and the 2 hybrids batches displayed a significantly ($p < 0.05$) skewed sex ratio towards males (64 and 66%, table 3).

Exp. 6. The sex ratios of all the progenies was not significantly ($p > 0.5$) different from a balanced sex ratio (50 : 50).

	Males (%)	Females (%)	Undeveloped gonads (%)	χ^2
Hybrid 1	64	36	0	3.99*
Control 1	50	50	0	-
Hybrid 2	66	26	8	11.78*
Control 2	50	50	0	-

Table 3 : sex ratio of hybrid progenies between Eurasian perch female and Yellow perch male. * $p < 0.05$.

Discussion . The sex ratio obtained in Exp. 3. suggested that genetic sex determinism in Eurasian perch is controlled by homogametic XX sex chromosomes in females. The significantly skewed sex ratio observed in 2 populations resulting from a cross with a normal XY male and males observed in populations were all-female were expected, suggested the action of other genetic sex-determining factors (e.g., autosomal genes). All-female obtained in gynogens batches confirm that Eurasian perch display a female homogamety XX chromosomic system. Nevertheless, as we did not observe any males in progenies, we can not confirm the hypothesis of an autosomal role. Hybrid's sex ratios also suggested the possible effect of an autosomal gene as was suggested for other species as tilapia or carp (see review by Devlin and Nagahama, 2002). A possible role of environment (temperature) on sex determinism in Eurasian perch was not proven.

Conclusion. In Eurasian perch, sex is primarily determined by sex chromosomes in which female is the homogametic sex (XX). A role of an autosomal sex determining gene is suggested to explain males arising from crosses with XX males, the two skewed sex ratio observed in normal crosses and in hybrid progenies, but this hypothesis is still not confirmed by mitogynogenesis or high inbred gynogenesis..

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References. Devlin, R.H. and Y. Nagahama. 2002. Sex determination and sex differentiation in fish : an overview of genetic, physiological, and environmental influences. Aquaculture 208: 191-364.

Rougeot, C., B. Jacobs, P. Kestemont and C. Mélard. 2002. Sex control and sex determinism study in Eurasian perch, *Perca fluviatilis*, by use of hormonally sex-reversed breeders. Aquaculture 211: 81-89.

Rougeot, C., L. Minet, C. Prignon, A. Vanderplasschen, B. Detry, P.-P. Pastoret and C. Mélard. 2003. Induce triploidy by heat shock in Eurasian perch, *Perca fluviatilis*. Aquatic Living Resources 16: 90-94.