

## **Supplemental Material**

### **Title**

The Consequences of Feminisation in Breeding Groups of Wild Fish.

### **Authors**

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## **Table of Contents**

**Page 3.** Supplemental Material, Table 1. Relationship of various morphometric, reproductive and genetic parameters with reproductive success of male roach (*Rutilus rutilus*). Full model analyses, excluding sperm quality data.

**Page 4.** Supplemental Material, Table 2. Relationship of various morphometric, reproductive and genetic parameters with reproductive success of male roach (*Rutilus rutilus*). Full model analyses, including sperm quality data.

**Page 5.** Supplemental Material, Table 3. Variables associated with reproductive success of male roach (*Rutilus rutilus*). Stepwise model analyses, including sperm quality data.

**Page 6.** Supplemental Material, Figure 1. The distribution of gonadal disruption in the two rivers examined in this study.

**Page 7.** Supplemental Material, Figure 2. The proportion of reproducing male fish from two breeding studies, illustrated across the range of intersex indices.

**Supplemental Material, Table 1.** Relationship of various morphometric, reproductive and genetic parameters with reproductive success of male roach (*Rutilus rutilus*), as assessed in two competitive breeding studies. Results shown here are those obtained from the full model analysis of the data from each study, plus analysis of the combined data, using Linear Mixed Effect models. *P*-values were calculated using likelihood ratio tests, and are shown for all variables included in the model (both significant and non-significant); \* =  $p \leq 0.05$ .

	Study 1		Study 2		Combined (Studies 1 and 2)	
	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value
Intersex Index	-0.028	0.36	-0.028	0.0001*	-0.027	0.0002*
Ovarian Cavities	-0.12	0.17	0.046	0.061	-0.015	0.35
Length	0.0031	0.048*	0.00037	0.26	0.00080	0.048*
Roughness	0.041	0.16	0.021	0.60	0.028	0.062
Internal Genetic Diversity (HL)	-0.13	0.42	0.143	0.023*	0.069	0.20
Internal Genetic Diversity ( $d^2$ )	0.070	0.86	-0.0097	0.83	-0.039	0.58

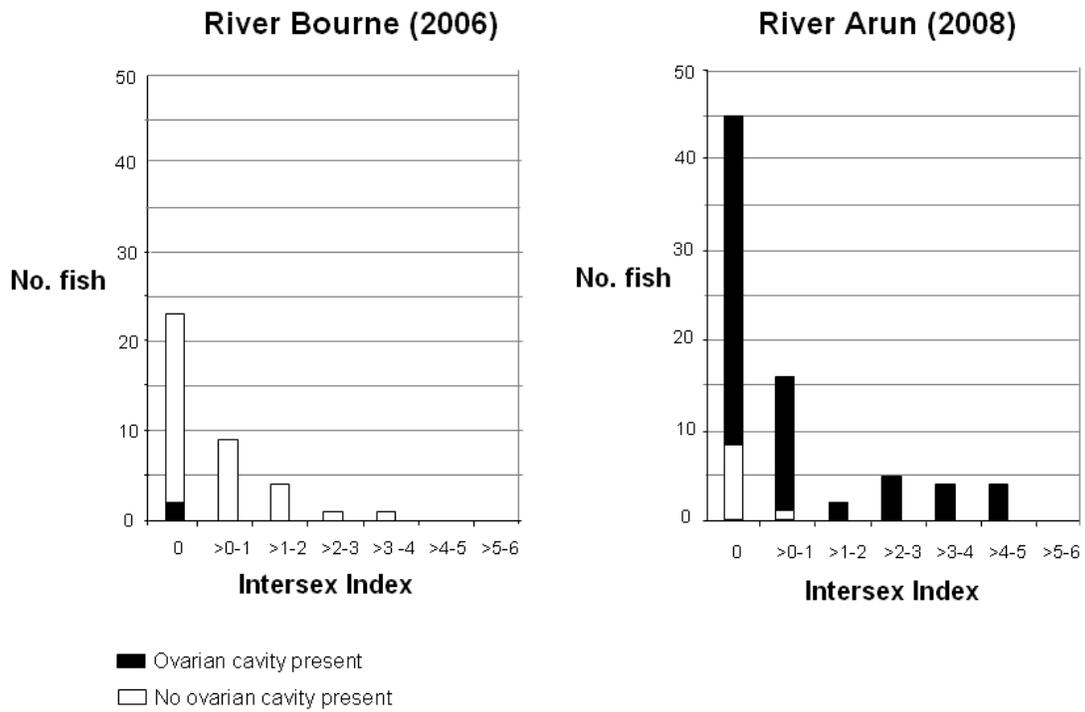
**Supplemental Material, Table 2.** Relationship of various morphometric, reproductive and genetic parameters with reproductive success of male roach (*Rutilus rutilus*). These data show results obtained from full model analysis on all of the parameters of interest in the two studies, that is, those included in Supplemental Material, Table 1 plus sperm viability and sperm density. These sperm parameters were not included in the models shown in Supplemental Material, Table 1, as their inclusion necessitated the removal of certain individuals from the analysis (1 fish from study 1 and 6 fish from study 2), for which no sperm data were available. Hence, two sets of analyses were undertaken; the first in which all fish were included but sperm parameters were excluded (Supplemental Material, Table 1 and Table 1 in the main body of this paper) and the second in which sperm parameters were included but several individual fish were excluded (Supplemental Material, Table 2 shown here and Supplemental Material, Table 3 in which results are shown after stepwise elimination of non-significant variables from the model); \* =  $p \leq 0.05$ .

	Study 1		Study 2		Combined (Studies 1 and 2)	
	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value	Coefficient	<i>p</i> -value
Intersex Index	-0.041	0.22	-0.022	0.016*	-0.022	0.0096*
Ovarian Cavities	-0.153	0.17	0.049	0.050*	-0.030	0.041*
Length	0.0031	0.088	0.00060	0.15	0.00069	0.17
Roughness	0.041	0.10	0.0050	0.63	0.025	0.065
Internal Genetic Diversity (HL)	-0.128	0.46	0.109	0.016*	0.071	0.10
Internal Genetic Diversity ( $d^2$ )	-0.034	0.72	-0.167	0.30	-0.147	0.45
Sperm Density	>0.0001	0.25	>0.0001	0.55	>0.0001	0.32
Sperm Viability	0.0018	0.93	0.0022	0.0015*	0.0024	0.0021*

**Supplemental Material, Table 3.** Variables associated with reproductive success of male roach (*Rutilus rutilus*) in the 2 breeding studies, obtained by Linear Mixed Effect models, after removal of 1 fish from Study 1 and 6 fish from Study 2 for which no sperm data were available. Values shown here are those retained after stepwise elimination of variables with  $p$ -values  $> 0.1$  calculated using Likelihood Ratio Tests (LRTs), hence not all of the variables analysed in these experiments are shown. See Supplemental Material, Table 2 for full model analysis; \* =  $p \leq 0.05$ .

	Number of fish	Number of Tanks	Variable	Coefficient	LRT $p$ -value
Study 1	37	7	Length	0.0037	0.031*
Study 2	69	13	Intersex	-0.014	0.036*
			Genetic Diversity (HL)	0.13	0.0021*
			Sperm Viability	0.0023	0.001*
			Length	0.00031	0.089
Combined Dataset	106	20	Intersex	-0.014	0.037*
			Ovarian Cavities	-0.025	0.092
			Roughness	0.026	0.040*
			Genetic Diversity (HL)	0.12	0.012*
			Sperm Viability	0.0026	0.0007*

**Supplemental Material, Figure 1.** The distribution of gonadal disruption in the two rivers examined in this study.



**Supplemental Material, Figure 2.** The proportion of reproducing male fish from two breeding studies, illustrated across the range of intersex indices. The numbers shown in the bars indicate the number of fish in each of the respective groups. In both studies, the majority of males, including intersex fish of all severities, sired offspring, demonstrating that in the scenarios represented by these experiments, most intersex fish were able to participate in spawning.

