

# Sexual maturity, reproductive behaviour and fertility in the first-generation hybrids of *Blicca bjoerkna* L. x *Abramis brama* L.

by

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**ABSTRACT.** - Sexual maturity, reproductive behaviour and fertility in the artificial first-generation hybrids of silver bream, *Blicca bjoerkna*, and common bream, *Abramis brama*, were investigated in this study. The results revealed that the females and males of hybrids attained their first sexual maturity at the same age. Maturity in F<sub>1</sub> hybrids was observed at the same moment as maturity in the common bream, the latter being the parental species. Sizes of male hybrids at maturity were intermediate to their parents but female hybrids were closer to the highest parental species, the common bream. As for reproductive behaviour, the numbers of spawning and mating behaviours observed were highly similar. Female hybrids mated simultaneously and successively with all types of males, except the common bream male, resulting in fertilised eggs. Male hybrids exhibited aggressiveness and territorial activities as in parental species common bream. Successful survival was observed in all post-F<sub>1</sub> (F<sub>2</sub> and backcross) generations at each developmental stage, proving that these hybrids are fertile.

Key words. - Maturity - Reproductive behaviour - Fertility - Hybrids - Cyprinids.

## Introduction

Silver bream, *Blicca bjoerkna* L., and common bream, *Abramis brama* L., are two common cyprinid fish species in European waters that have the same spawning period and requirements. In rivers, anthropic activities have resulted in the destruction of suitable spawning sites, which has caused an artificial increase in the numbers of these hybrids. Natural hybrids from these species have been observed by several authors (Wheeler, 1969; Swinney and Coles, 1982), but few experiments have been conducted to evaluate their sexual maturity, reproductive behaviour and fertility. This study aims to further examine the effect of artificial hybridisation of silver bream × common bream on these biological features.

## Methods

### *Production of the hybrids and parental species*

The mature silver bream and common bream specimens were captured in the fish pass at the Lixhe dam (Belgian Meuse River, 50°45'N; 5°40'E) during the reproductive migration in spring 2003. They were morphologically identified on the basis of descriptions by Spillman (1961). Four parental fishes (a male and a female of two species) were used to produce two interspecific crosses (male × female: first-generation hybrids, common bream × silver bream = A × B or AB; silver bream × common bream = B × A or BA) and two intraspecific crosses (silver bream × silver bream = B × B; common bream × common bream = A × A). These hybrids and parental species were reared in captivity (at 20°C) at the Tihange aquaculture station in Belgium.

### *Sexual maturity, reproductive behaviour and fertility evaluation*

When the fish reached 20 months of age, maturity was

checked weekly. To verify the presence of sperm, liquid extracted by abdominal pressure was observed under a microscope. The fish was considered as mature when the female was gravid or the male produced milt. Mature fish were noted for sex, age and size.

Reproductive behaviour was studied in two sets of reproduction conditions (at 20°C, 16 L/8 D photoperiod, spawning substrate simulating vegetation, in duplicate) (Tab. I): (i) hybrid reproduction, a female hybrid was placed to reproduce with three corresponding hybrid males and (ii) mixed reproduction, a female hybrid was mixed with a corresponding hybrid male, a male silver bream and a male common bream. Two hybrid types were used in these experiments. The spawning-mating relationship, the numbers of males participating in the mating and aggressive behaviours were analysed over 1 day from 8.00 to 18.00 hours, corresponding to the beginning and end of spawning activity, using a remote-controlled video system. Mating success, expressed as the percentage of eyed embryos 1 day after spawning, was evaluated from two samples of 100 eggs, under a microscope.

To test the fertility of hybrids, ovulation was observed under environmental conditions (20°C, photoperiod 16 L/8 D, spawning substrate and mixture of male and female hybrids) and eggs from the female hybrid were divided into four equal parts and artificially fertilised with the sperm of one of the following: a corresponding hybrid male, a hybrid male from the reciprocal crossbreeding, a male silver bream and a male common bream (Tab. II). Survival rates were estimated at the following developmental stages (in duplicate): eyed embryos (1 day post-fertilisation) from two samples of 100 eggs, viable hatchlings (3 days post-fertilisation)

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Table I. - Experiments and characteristics of fish used. A, common bream; B, silver bream; AB and BA, F<sub>1</sub> hybrids; ♂, male (M); ♀, female (F); range, values of the first and second replications.

Reproduction conditions		Sex	Fork length (mm)	Weight (g)
Hybrid reproduction				
1	3♂AB × 1♀AB	F	150-155	55-52
		M	155-145	57-42
		M	133-142	45-39
		M	128-140	33-40
2	3♂BA × 1♀BA	F	150-152	54-57
		M	136-155	37-53
		M	134-146	35-49
		M	128-142	35-42
Mixed reproduction				
3	1♂AB, 1♂A, 1♂B × 1♀AB	F	144-145	53 - 50
		M	132-140	42 - 40
		M	175-165	96 - 70
		M	128-137	32 - 35
4	1♂BA, 1♂A, 1♂B × 1♀BA	F	150-155	60-68
		M	155-136	61-40
		M	165-165	75-77
		M	137-128	46-36

Table II. - Characteristics of fish used to obtain the post- F<sub>1</sub> generations. A, common bream; B, silver bream; AB and BA, F<sub>1</sub> hybrids; M, male; F, female; range, values of the first and second replications.

Hybrids and species	Sex	Fork length (mm)	Weight (g)
A	M	165-156	75-66
B	M	130-134	39-41
AB	M	146-132	49-41
BA	M	148-135	50-35
AB	F	140-150	51-72
BA	F	155-150	62-58

from two samples of 500 eggs, and larvae to dry food consumption (5 weeks old) from two samples of 50 embryos.

**Statistical analysis**

The size at the first sexual maturity was analysed with Kruskal-Wallis KW-test followed by Mann-Whitney U-test. The percentage of eyed embryos in reproduction experiments and the survival rates in each developmental stage were compared using Fisher’s exact probability FEP-test. For all statistical analyses, null hypotheses were rejected at  $p < 0.05$ .

**Results and discussion**

**Age and size of maturity**

Females and males in each F<sub>1</sub> cross attained their first sexual maturity at the same age. Mature F<sub>1</sub> hybrids were observed at the same moment as mature common bream, the latter being the parental species (990 days old). In silver

bream, this age was much lower (660 days old) (Fig. 1A). For size at maturity, a significant effect of the F<sub>1</sub> cross-breedings was found (KW-test, male/female: d.f. = 3, H = 88.6/33.6 and 84.9/29.1,  $p < 0.0001$  for fork length and weight, respectively) (Figs 1B, 1C). Male hybrids (mean values, 134 mm/37 g and 128 mm/38 g for B × A and A × B, respectively) were intermediate to parents (103 mm/21 g and 156 mm/71 g for silver bream and common bream, respectively). However, female hybrids (144 mm/49 g and 143 mm/56 g for B × A and A × B, respectively) were closer (U-test,  $p > 0.05$ ) to the highest parental species, the common bream (131 mm, 48 g). The smallest maturity size (U-test,  $p < 0.01$ ) was observed in silver bream. No significant size difference (U-test,  $p > 0.05$ ) was found between hybrids.

The age of sexual maturity observed in hybrids was similar to common bream in the Meuse River (Poncin *et al.*, 1996), but sexual maturity was reached at a younger age in English waters (Cowx, 1983). In this study, common bream at this age were larger than those in Tjeukemeer Lake (Goldspink, 1978). The constant conditions of the experimental

environment in terms of high temperature, photoperiod, food availability and mixed sexes could explain this higher growth and maturity for both sexes.

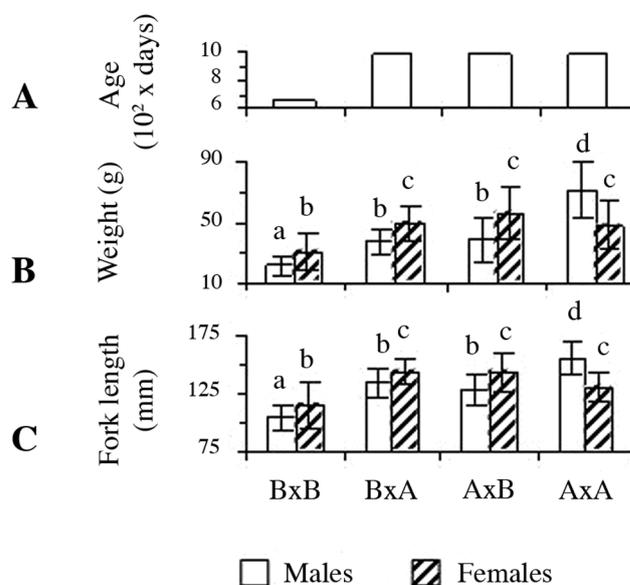
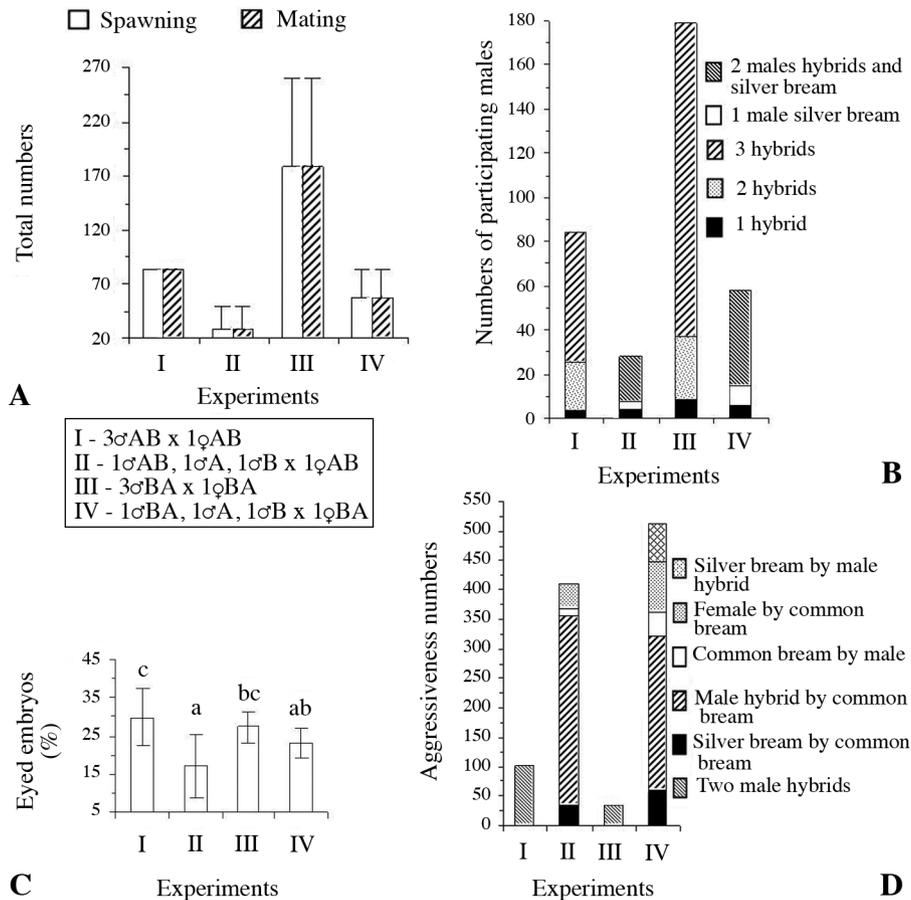


Figure 1. - Age (A) and size (B, C) at first sexual maturity in the F<sub>1</sub> hybrids of silver bream and common bream. A, common bream; B, silver bream; male × female; mean and deviation standard values; values across common bars with different letters differ significantly (U-test,  $p < 0.05$ ). B × B n (male / female) = 42 / 41; B × A n = 37 / 27; A × B n = 28 / 14; A × A n = 26 / 15; n, numbers of fish.

Figure 2. - Spawning-mating relationship (A), males participating in the mating (B), mating success (C) and male aggressiveness (D) in reproduction conditions from 8.00 to 18.00 h. A, common bream; B, silver bream; AB and BA, F1 hybrids;  $\sigma$ , male;  $\varphi$ , female; Arabic numeral, number of fish per sex in experiment; mean values of experimental duplicates; vertical bars, standard deviations of experimental duplicates. Values across common bars with different letters differ significantly (FEP-test,  $p < 0.05$ ).



**Reproductive behaviour**

Highly similar spawning and mating numbers were observed for both types of female hybrid in both reproduction conditions (Fig. 2A). Between reproduction conditions, spawning numbers were lower in mixed reproduction than in hybrid reproduction for both types of female hybrid. The highest spawning activity was observed in BA females.

Each female hybrid mated simultaneously and successively with all types of males, except the common bream male (Fig. 2B). Two major mating combinations were observed most frequently: (i) the female hybrid with its three corresponding male hybrids under hybrid reproduction conditions, accounting for 70% and 79% of the total matings observed in AB and BA, respectively, and (ii) the female hybrid with its corresponding male hybrids associated with the male silver bream under the mixed reproduction conditions, accounting for 75% and 74% of the total matings in AB and BA, respectively.

The mating success assessed 1 day after spawning (Fig. 2C) revealed that the percentage of eyed embryos was lower under mixed reproduction than under hybrid reproduction conditions with both types of female hybrid, but the difference was only significant (FEP-test,  $p < 0.0001$ ) for AB females.

Under hybrid reproduction conditions (Fig. 2D), a male hybrid defended a territory and attacked the two other hybrid males. Under mixed reproduction conditions (Fig. 2D), the male hybrid did not defend a territory but it attacked the common bream and silver bream males. However, in these conditions, a territorial common bream male frequently attacked the male hybrid and this aggressiveness accounted for 79% and 51% of the aggressive behaviours observed in AB and BA, respectively.

The results of reproductive behaviour demonstrate that the level of sexual activity of these hybrids is high. This phenomenon is not only limited to these hybrids, it has already been observed for other hybrids such as artificial roach and silver bream hybrids (Nzau Matondo *et al.*, 2007a) in terms of reproductive success, and *Cyprinidon pecosensis* and *C. variegatus* male hybrids (Rosenfield and Kodric-Brown, 2003) in terms of aggressive behaviour.

**Fertility of hybrids**

Survival curves at the early developmental stages (Fig. 3) revealed that high survival rates (> 20%) were obtained in all post- F<sub>1</sub> crosses. F<sub>2</sub> generations were not significantly different (FEP-test,  $p > 0.05$ ) from the backcrosses in terms of the survival rates of eyed embryos (range, 85%-93% and 59%-94% for F<sub>2</sub> and backcross generations, respectively), viable hatchlings (21%-73% and 11%-76% for F<sub>2</sub> and backcross generations, respectively) and larvae stages (38%-86% and 34%-78% for F<sub>2</sub> and backcross generations, respectively) (Tab. III). These observed successful survival rates have proved that the females and males of hybrids are fertile, which could reveal the total absence of a reproductive isolation mechanism between their parental species. On the contrary, the significantly lower hatchling rates in F<sub>2</sub> generations

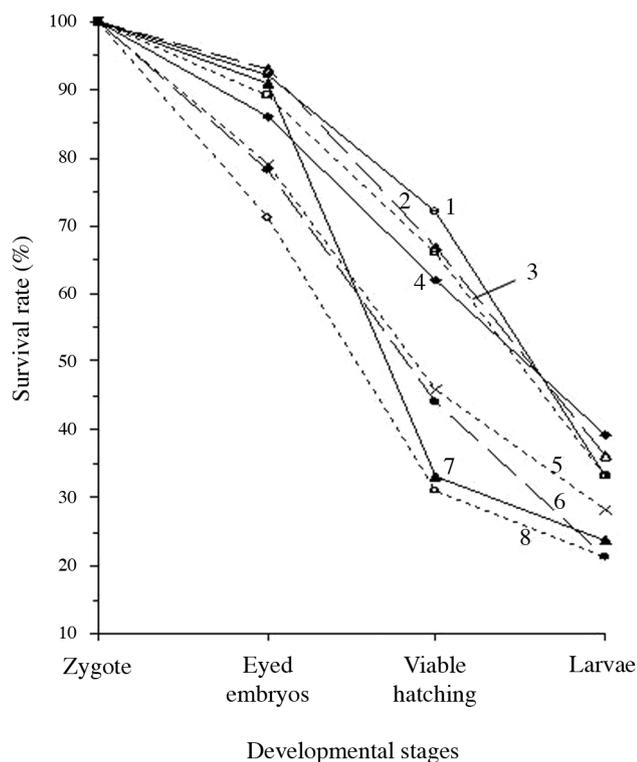


Figure 3. - Survival curves of post-F<sub>1</sub> hybrids. A, common bream; B, silver bream; AB and BA, F<sub>1</sub> hybrids; male × female. 1 - A × AB; 2 - AB × BA; 3 - AB × AB; 4 - BA × AB; 5 - B × AB; 6 - B × BA; 7 - BA × BA; 8 - A × BA.

than those of backcrosses have been reported by Nzau Matondo *et al.* (2007b) for artificial hybrids of roach and silver bream, and by Wood and Jordan (1987) for natural hybrids of roach and common bream.

Table III. - Survival of post-F<sub>1</sub> generations. *n*, number of eggs and hatchlings analysed by observation repeated twice per replication. A, common bream; B, silver bream; AB and BA, F<sub>1</sub> hybrids. Range, extreme values of four observations in two replications in each experiment. Means with a common superscript in the column do not differ significantly (FEP-test, *p* < 0.05).

σ × ♀	Survival (%)					
	Eyed embryos (%) , <i>n</i> = 100 eggs		Viable hatchlings (%) , <i>n</i> = 500 eggs		Larvae: dry food consumption, (%) , <i>n</i> = 50 hatched embryos	
	Mean	Range	Mean	Range	Mean	Range
A × AB	92 <sup>c</sup>	90-94	72 <sup>c</sup>	66-76	46 <sup>a</sup>	40-56
B × AB	79 <sup>ab</sup>	74-84	46 <sup>b</sup>	35-57	61 <sup>abc</sup>	50-68
AB × AB	93 <sup>c</sup>	92-93	67 <sup>e</sup>	63-70	54 <sup>abc</sup>	38-70
BA × AB	86 <sup>bc</sup>	84-89	62 <sup>c</sup>	59-65	63 <sup>abc</sup>	58-76
A × BA	71 <sup>a</sup>	59-83	31 <sup>a</sup>	11-51	69 <sup>bc</sup>	58-78
B × BA	78 <sup>ab</sup>	65-87	44 <sup>b</sup>	34-58	48 <sup>ab</sup>	34-58
BA × BA	91 <sup>bc</sup>	87-93	33 <sup>a</sup>	21-44	72 <sup>c</sup>	66-86
AB × BA	89 <sup>bc</sup>	85-95	66 <sup>d</sup>	61-73	50 <sup>ab</sup>	42-54

**Conclusion**

Our study has demonstrated that these hybrids have the biological capacity to reproduce and to exhibit a high level of sexual activity. The post- F<sub>1</sub> generations produced have proved that these hybrids are fertile. In phylogenetic relationships, it would be better to consider these two species in the same genus, as suggested by some authors. From an ecological point of view, this situation has implications on the genetic integrity of parental species, biodiversity preservation and the population dynamics in natural environments.

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