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Hybridization success of three common European cyprinid species, *Rutilus rutilus*, *Blicca bjoerkna* and *Abramis brama* and larval resistance to stress tests

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ABSTRACT: Hybridization success at early developmental stages and larval resistance to osmotic, thermal and fasting tests in roach *Rutilus rutilus*, silver bream *Blicca bjoerkna*, common bream *Abramis brama* and their F1 hybrids were investigated. Results revealed that hybrid survival rates were similar to parents. At the eyed embryo stage, however, a maternal effect was observed as a general trend during hatchling and larval stages. After these stages, hybrids displayed a higher survival rate than their parents. Under stress tests, no survival was observed after 40 min for osmotic and thermal shocks and after 24 days for the prolonged fasting test in these species and their F1 hybrids. The median survivals of hybrids were intermediate between the two parents. For total mortality, hybrids were also affected by a maternal effect but to the advantage of the hybrids.

KEY WORDS: **cyprinids, hybridization success, hybrids, larval resistance.**

INTRODUCTION

Roach *Rutilus rutilus* L., silver bream *Blicca bjoerkna* L. and common bream *Abramis brama* L. are three common cyprinids in European waters. In their distribution area, the three species have overlapping spawning times and have similar spawning requirements.[1–4] Wheeler and Easton[5] have suggested that anthropic activities such as dredging rivers, habitat fragmentation and weed-cutting lead to increased numbers of hybrids in these species by reduction of suitable spawning sites and the resultant increased density of spawners on less available sites.

Hybridization studies of these species have been reported by several authors such as Pitts *et al.*,[6] Lapushkina *et al.*[7] and Andreeva[8] for artificial F1 hybrids of common bream and roach, and Swinney and Coles[4] and Wheeler[9] for natural hybrids of silver bream and roach and natural hybrids of silver bream and common bream. However, few experiments have been conducted to evaluate hybridization efficiency in the survival of hybrids at each developmental stage and larval resistance under lethal conditions. Higher survival rates at each developmental stage and when subjected to stress tests can evidence healthier or better-quality fish.[10–12] Nevertheless, quality or survival of larvae can be modified by nutrition and culture conditions.

From an ecophysiological point of view, investigation of survival success and larval quality in relationships between hybrids and parents is necessary to understand the role of interspecific crosses between the three species of their wild populations. Indeed, the natural environment is characterized by the fluctuating conditions as, for examples, thermal regimes, food availability and wastewater discharges that can vary substantially both seasonally and diurnally and that may stress the fishes and contribute to the decline of the weakest individuals.

The aim of this study is to further examine the effect of artificial hybridization of the three species on survival at early developmental stages and to evaluate larval resistance to stress tests such as osmotic and thermal shocks and prolonged fasting.

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MATERIALS AND METHODS

Intra- and interspecific crosses

During the upstream reproductive migration (March–June), mature roach, silver bream and common bream females and males were collected from natural populations in a fish pass at the Lixhe dam (Belgian Meuse River, 50°45′N; 5°40′E). These parental fishes (Table 1) were identified by general external appearance, as described by Wheeler;[9] Spillman[13] and Maitland.[14] Six parental fishes (a male and a female of each of the three species) were used for producing four interspecific crosses (female × male: common bream × roach = C × R; roach × common bream = R × C; silver bream × roach = S × R; roach × silver bream = R × S) and three intraspecific crosses (roach × roach = R × R; silver bream × silver bream = S × S; common bream × common bream = C × C) in experiment 1. In experiment 2, four parental fishes (a male and a female of two species) were used for producing two interspecific crosses (common bream × silver bream = C × S; silver bream × common bream = S × C) and two intraspecific crosses (silver bream × silver bream = S × S; common bream × common bream = C × C). These crosses were repeated twice (in spring 2002 and 2003).

Egg fertilization

Spawning of producers was induced by injection of Ovaprim, a synthesis hormone as an analog of salmon GnRH and a dopamine inhibitor (Syndel Laboratories Ltd, Vancouver, BC, Canada) at a dose of 0.2 and 0.5 mL per kg body weight in the male and female, respectively. Sperm was individually collected in a syringe by stripping the mature male and was kept on ice until fertilization. Eggs from each female were divided into two parts; one part was mixed with the sperm of the male of the other species (hybrids) and the other with the sperm of the conspecific male (intraspecific crossbreeding). For roach, eggs were divided into three parts which were individually fertilized with sperm from common bream, silver bream and roach. Fertilization was carried out by dry technique at 1 mL of sperm per 100 g of spawn.

Egg incubation and rearing

Fertilized eggs were incubated in 1-l Zoug bottles; larval breeding was done in 0.42 × 0.42 × 0.12-m trays and growth in 1.04 × 1.04 × 0.41-m basins installed in a recirculating system at the Tihange Aquaculture Station in Belgium. The water temperature was recorded hourly and controlled at 18°C and 20°C during embryogenesis and breeding, respectively. The photoperiod was set at 16 h of light and 8 h of darkness, dissolved oxygen (DO) above 6 mg/L, nitrites and ammonium below 0.3 mg/L and pH 7.9 ± 0.8. Fish were fed exclusively with *Artemia* nauplii (50% protein at dry base) for the first 2 weeks after hatching, then with a mixture of artemia and dry food (54% protein) for the following 2 weeks, and thereafter with dry food (52% protein) only. After 7 weeks, daily food was readjusted per fish biomass weekly and was identical in all breeding experiments. The dry food was added to the initial fish feed (Lucky Star, Taiwan) until 7 weeks of age and the Nutra food (Skretting Trow, France) after this age.

In all cross combinations, survival rates were estimated following at the developmental stages: eyed embryos (2 days post-fertilization) from two samples of 100 eggs under microscope; viable hatchlings (5 days post-fertilization) from two samples of 500 eggs; larvae to dry food consumption (7 weeks old) from two samples of 1000 hatchlings and fingerlings (18 months old) from two samples of 300 7-week-old larvae, in each cross experiment repeated twice.

Table 1 Size of parental fishes used for experiments 1 and 2

	♀–♂, first/second replications		
Fish (♀–♂)	*n*	Fork length (mm)	Weight (g)
Experiment 1			
C-C	1–1/1–1	390–430/400–430	1092–1251/1083–1242
R-R	1–1/1–1	196–250/245–225	115–251/262–172
S-S	1–1/1–1	216–230/231–226	226–228/257–191
Experiment 2			
C-C	1–1/1–1	400–455/450–405	1154–1625/1168–1051
S-S	1–1/1–1	252–260/216–219	362–348/226–212

C, common bream; S, silver bream; R, roach; in each replication and parameter, first and second numbers indicate values of female (♀) and male (♂) fish, respectively; *n*, number of parental fish used for cross experiment.

Table 2 Characteristics of roach, common bream, silver bream and their F1 hybrids exposed to osmotic and thermal shocks (9 days after hatching) and prolonged fasting (embryos at hatching)

	First/second replications (mean ± SD)			
	Osmotic and thermal shocks, n = 15 larvae		Prolonged fasting, n = 50 hatched embryos	
F1 crossbreedings (female × male)	Total length (mm)	Weight (0.1 mg)	Total length (mm)	Weight (0.1 mg)
Experiment 1				
C × C	7.4 ± 0.2/7.2 ± 0.4	17.6 ± 0.1/16.6 ± 0.1	5.8 ± 0.4/6.0 ± 0.3	8.0 ± 0.1/8.3 ± 0.1
C × R	7.5 ± 0.4/7.2 ± 0.3	18.0 ± 0.1/17.3 ± 0.2	5.9 ± 0.5/6.2 ± 0.1	9.8 ± 0.1/10.0 ± 0.1
R × C	7.4 ± 0.4/8.2 ± 0.2	16.6 ± 0.1/17.6 ± 0.3	6.5 ± 0.4/6.2 ± 0.2	10.0 ± 0.1/9.5 ± 0.3
R × R	7.7 ± 0.3/8.1 ± 0.4	18.0 ± 0.1/18.5 ± 0.5	6.6 ± 0.4/6.3 ± 0.2	12.0 ± 0.1/11.2 ± 0.2
S × R	7.8 ± 0.3/7.9 ± 0.2	17.2 ± 0.1/17.6 ± 0.1	5.9 ± 0.3/5.8 ± 0.2	7.9 ± 0.3/8.1 ± 0.5
R × S	7.5 ± 0.3/7.7 ± 0.4	17.5 ± 0.1/16.8 ± 0.3	6.1 ± 0.2/6.0 ± 0.1	9.8 ± 0.5/9.5 ± 0.3
S × S	7.4 ± 0.3/7.5 ± 0.2	16.2 ± 0.1/16.6 ± 0.1	5.7 ± 0.4/5.9 ± 0.1	7.8 ± 0.2/8.0 ± 0.3
Experiment 2				
C × C	6.1 ± 0.4/7.1 ± 0.2	11.4 ± 2.8/17.3 ± 0.5	4.6 ± 0.3/4.9 ± 0.2	8.0 ± 0.3/7.8 ± 0.2
S × C	6.5 ± 0.5/7.2 ± 0.3	19.0 ± 3.3/19.7 ± 0.4	4.9 ± 0.3/4.8 ± 0.4	8.9 ± 0.1/8.8 ± 0.4
C × S	7.1 ± 0.5/7.2 ± 0.4	12.7 ± 4.0/17.7 ± 0.4	4.8 ± 0.5/5.0 ± 0.1	7.7 ± 0.1/7.8 ± 0.2
S × S	6.1 ± 0.4/7.0 ± 0.3	18.4 ± 3.0/18.7 ± 0.4	5.0 ± 0.2/4.9 ± 0.3	9.5 ± 0.4/9.2 ± 0.3

SD, standard deviation; n, number of hatchlings or larvae measured by replication.

Larval resistance tests

Larval quality expressed as the time at total mortality and median survival was also examined in all crosses under the conditions of osmotic shock (2% NaCl, 17 ± 0.4°C temperature, 8.3 ± 0.3 mg/L DO), thermal shock (regulated at 33 ± 0.1°C, 5.3 ± 0.4 mg/L DO) and prolonged fasting (17.3 ± 0.9°C, 8.1 ± 0.5 mg/L DO). Osmotic and thermal shocks were conducted using 30 larvae (9 days old) and the fasting test was conducted using 30 embryos at hatching (Table 2), in two replications per cross. Examined larvae were placed in 0.25 × 0.18-m fish-net and immersed in a 1.00 × 0.50 × 0.20-m basin for osmotic shock or in a 0.81 × 0.50 × 0.23-m thermostatic water bath for thermal shock. For the prolonged fasting test, larvae were placed in a 0.15 × 0.13 × 0.10-m experimental nylon basket floating in 0.42 × 0.42 × 0.12-m trays installed in an isolated recirculating system.

Dead larvae were removed and counted every 5 min for osmotic and thermal shock, and daily for the fasting test. These stress tests were also repeated twice (in spring 2002 and 2003).

Statistical analysis

The mean survival rate at early developmental stages and median survival times to stress tests in hybrids and parental species were compared using Fisher's exact probability *FEP*-test and non-parametric Mann–Whitney *U*-test, respectively. For all statistical analysis, null hypotheses were rejected at $P < 0.05$.

RESULTS

Hybridization success

Eyed embryos and hatchling stages

No significant survival difference (Fisher's exact probability *FEP*-test, $P > 0.05$) was observed at the eyed embryo stage between roach (mean value = 91.8%), silver bream (89.8 and 86.5% in experiments 1 and 2, respectively), common bream (89.8 and 85.8% in experiments 1 and 2, respectively) and their hybrids (89.0 and 90.3% for S × R and R × S, 84.8 and 89.3% for C × R and R × C in experiment 1 and 78.8 and 85.3% for C × S and S × C in experiment 2) (Table 3, Fig. 1).

For viable hatchlings, maternal effects of egg quality were found in C × R, R × C, C × S and S × C hybrids. In roach and silver bream hybrids, this effect was only observed in S × R hybrids. The viable hatchlings in R × C hybrids (mean value = 75.5%) and roach (79.5%) were significantly higher (*FEP*-test, $P < 0.001$) than in C × R hybrids (61.0%) and common bream (65.8%). C × S (58.0%) and S × C (61.0%) hybrids and silver bream (63.3%) displayed significantly fewer viable

Table 3 Survival of roach, common bream, silver bream and their F1 hybrids at early developmental stages

F1 crossbreedings (female × male)	Eyed embryos (%), $n = 100$ eggs		Viable hatchlings (%), $n = 500$ eggs		Larvae: dry food consumption, (%), $n = 1000$ hatched embryos		Fingerlings stage, (%), $n = 300$ larvae	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Experiment 1								
C × C	89.8[a]	87.0–92.0	65.8[c]	61.0–69.0	50.0[b]	36.0–65.0	52.4[c]	40.0–64.7
C × R	84.8[a]	80.0–90.0	61.0[c]	56.0–65.0	43.5[a]	35.0–49.0	79.2[d]	73.3–85.0
R × C	89.3[a]	84.0–92.0	75.5[d]	65.0–80.0	56.5[b]	41.0–73.0	71.5[d]	64.0–79.0
R × R	91.8[a]	89.0–93.0	79.5[d]	77.0–82.0	64.5[b]	59.0–71.0	47.2[b]	40.0–54.3
S × R	89.0[a]	87.0–91.0	61.0[c]	56.0–70.0	50.0[b]	45.0–55.0	68.5[d]	59.0–78.0
R × S	90.3[a]	83.0–93.0	58.0[c]	54.0–62.0	53.0[b]	47.0–62.0	75.7[d]	68.3–83.0
S × S	89.8[a]	85.0–92.0	63.3[c]	59.0–68.0	44.3[a]	31.0–52.0	42.9[b]	33.7–52.0
Experiment 2								
C × C	85.8[a]	81.0–89.0	34.5[b]	23.0–56.0	48.8[a]	33.0–64.0	29.0[a]	23.0–35.0
S × C	85.3[a]	80.0–89.0	61.5[c]	46.0–75.0	72.8[c]	60.0–87.0	30.9[a]	29.0–32.7
C × S	78.8[a]	89.0–93.0	20.3[a]	15.0–25.0	62.0[b]	50.0–70.0	34.3[a]	27.3–41.3
S × S	86.5[a]	83.0–91.0	66.5[c]	61.0–73.0	59.8[b]	45.0–67.0	27.0[a]	24.0–30.0

n, number of eggs, hatchlings and larvae analyzed by observation repeated twice per replication. Range indicates extreme values of four observations in two replications in each experiment. Means with a common superscript in the column do not differ significantly (Fisher's exact probability *FEP*-test, $P < 0.05$).

hatchlings (*FEP*-test, $P < 0.0001$) than roach. In the crossbreeding experiment between silver bream and common bream, the difference in viable hatchlings was not significant (*FEP*-test, $P = 0.1136$) between silver bream (66.5%) and S × C hybrids (61.5%). In both silver bream and S × C hybrids, the viable hatchlings were higher (*FEP*-test, $P < 0.0001$) than common bream (34.5%) and C × S hybrids (20.3%). C × S hybrids were significantly fewer in number than common bream.

Larval stage

Survival rates at 7 weeks old revealed that R × C and C × R hybrids were closer to maternal parents than paternal parents. Hybrids of R × S and S × R were intermediate between the two parents. Hybrids of S × C and C × S were superior to parents, with a statistical difference between hybrids and the maternal parent. R × C hybrids (mean value = 56.5%) showed a lower survival rate (*FEP*-test, $P = 0.0003$) than roach (64.5%) and hybrids of C × R (43.5%) had a lower survival rate (*FEP*-test, $P = 0.0041$) than common bream (50.0%). Between these hybrids, survival rates were significantly different (*FEP*-test, $P = 0.0041$). In parental species, survival rates in roach were higher (*FEP*-test, $P < 0.0001$) than in common bream. For the roach and silver bream crossbreeding experiment, the survival rate in roach was higher (*FEP*-test, $P < 0.0001$) than those in C × S hybrids (53.0%). The survival rate in silver bream (42.9%) was significantly lower (*FEP*-test, $P = 0.00121$) than that in S × C hybrids (50.0%). Reciprocal hybrids were not significantly different (*FEP*-test, $P = 0.1944$), but for parental species, the survival rate in roach was significantly higher (*FEP*-test, $P < 0.0001$) than that in silver bream. For the silver bream and common bream crossbreeding experiment, S × C hybrids (72.8%) showed a higher survival rate (*FEP*-test, $P < 0.0001$) than both C × S hybrids (62.0%) and parental species (48.8% and 59.8% for common bream and silver bream, respectively). Common bream showed a significantly lower survival rate (*FEP*-test, $P < 0.0001$) than silver bream.

Fingerling stage

At 18 months of age, hybrids in all crosses showed higher survival rates than parents. In the roach and common bream crossbreeding experiment, the survival rates in C × R hybrids (mean value = 79.2%) were significantly higher (*FEP*-test, $P = 0.0292$) than those in *R* × C hybrids (71.5%). In parental species survival, roach (47.2%) and common bream (52.4) did not differ significantly (*FEP*-test, $P = 0.253$). In hybridization between roach and silver bream, R × S (68.5%) and S × R (75.7%) hybrid survival rates were not significantly different (*FEP*-test, $P = 0.0683$), but they were significantly higher (*FEP*-test, $P < 0.0001$) than those in parental species, silver bream (42.9%) and roach (47.2%). Roach survival rates were not significantly lower (*FEP*-test, $P = 0.3249$) than those in silver

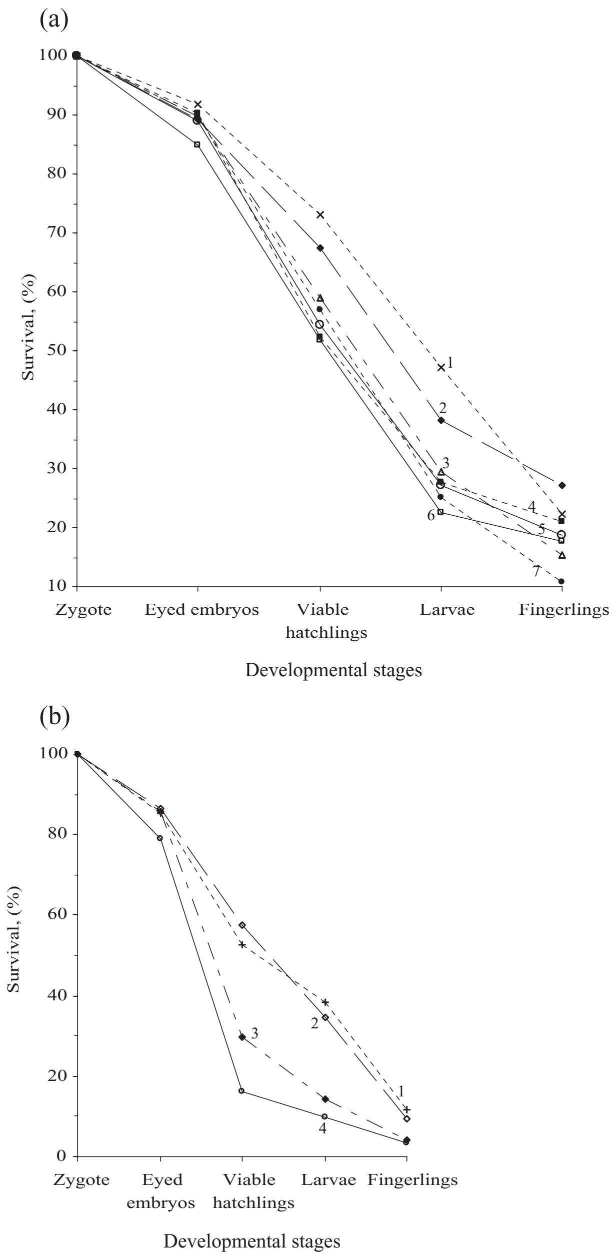

Fig. 1 Survival of roach, common bream, silver bream and their F1 hybrids at early developmental stages. C – common bream; S – silver bream; R – roach. In experiment 1 (a), female × male: 1 – R × R; 2 – R × C; 3 – C × C; 4 – R × S; 5 – S × R; 6 – C × R; 7 – S × S. In experiment 2 (b): 1 – S × C; 2 – S × S; 3 – C × C; 4 – C × S.

bream. In the silver bream and common bream crossbreeding experiment, C × S hybrids (34.3%) showed the highest survival rate, which was not significantly different (*FEP*-test, $P > 0.05$) from those of both S × C hybrids (30.9%) and parents (27.0% and 29.0% for silver bream and common bream, respectively).

Larval resistance tests

Roach and common bream hybrids

Total mortality times in C × R (mean values = 35 min) and R × C (40 min) hybrids during osmotic shock were similar to their maternal parents (35 and 40 min for common bream and roach, respectively) (Fig. 2a). For thermal shock (Fig. 3a) and the fasting test (Fig. 4a), total mortality times of C × R (25 min and 21 days for thermal shock and the fasting test, respectively) and R × C (30 min and 22 days) hybrids were observed after the total mortality times of parental species, common bream (20 min and 19 days) and roach (20 min and 20 days). Median survival times for fish exposed to osmotic shock were much closer (Mann–Whitney *U*-test, $P > 0.9999$) between R × C hybrids (28.7 min) and their maternal parent (28.5 min), whereas this time in C × R hybrids (25.7 min) was significantly later (*U*-test, $P = 0.0209$) than that in roach (19.8 min) (Fig. 5a). Significant differences were found between hybrids (*U*-test, $P = 0.0209$) and between parental species (*U*-test, $P = 0.0209$). For thermal shock, the median survival time for *R* × C hybrids (14.2 min) was longer (*U*-test, $P = 0.0209$) than that for common bream (9.5 min) and it was not significantly different from both C × R hybrids and roach (11.9 and 10.4 min, respectively). In the fasting test, median survival times for hybrids were closer (16.5 and 17.0 days for C × R and R × C hybrids, respectively), while they were significantly longer (*U*-test, $P = 0.0209$) than those for parental species (10.3 and 14.7 days for roach and common bream). The differences were also significant (*U*-test, $P = 0.0209$) between these parental species.

Roach and silver bream hybrids

Concerning total mortality, silver bream (30 min) died before both hybrids and roach (40 min) for osmotic shock (Fig. 2a). For thermal shock, roach (20 min) died before hybrids and silver bream (30 min) (Fig. 3a). In fasting, parents (19 days) died before hybrids (21 days) (Fig. 4a). For median survival times, hybrids were intermediate between parents in all tests (Fig. 5a). Median survival times were significantly shorter (*U*-test, $P = 0.0209$) in roach (10.4 min and 10.3 days for thermal and fasting tests, respectively) than those in hybrids (16.04 min and 16.1 days for R × S hybrids and 17.2 min and 16.6 days for S × R hybrids) and silver bream (21.2 min and 16.9 days) subjected to thermal shock and fasting tests. Those in reciprocal hybrids were not significantly different (*U*-test,

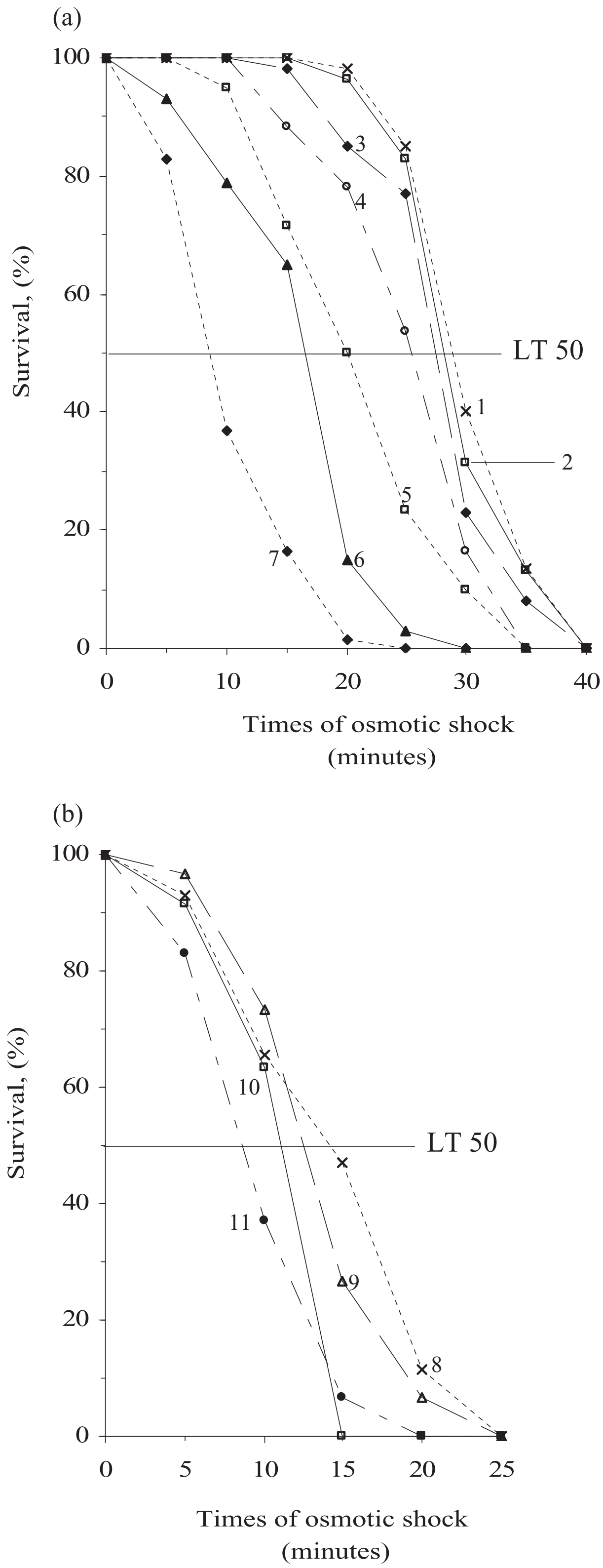

Fig. 2 Survival of roach, common bream, silver bream and their F1 hybrids upon exposure to osmotic shock. LT 50 – median survival; C – common bream; S – silver bream; R – roach. In experiment 1 (a), female × male: 1 – R × C; 2 – R × R; 3 – R × S; 4 – C × R; 5 – C × C; 6 – S × R; 7 – S × S and experiment 2 (b): 8 – S × S; 9 – S × C; 10 – C × S; 11 – C × C.

$P = 0.3865$ and 0.4705 for thermal shock and the fasting test, respectively). For osmotic shock, silver bream (9.4 min) had a lower median survival time (*U*-test, $P = 0.0209$) than that of roach (28.5 min) and hybrids (16.5 and 27.5 min for S × R and R × S hybrids). R × S hybrids differed significantly (*U*-test, $P = 0.0209$) from S × R hybrids, but no statistical difference was found between R × S hybrids and their maternal parent, roach.

Silver bream and common bream hybrids

Concerning the total mortality when exposed to the osmotic test, C × S hybrids (15 min) died before the maternal parent, common bream (20 min), contrary to S × C hybrids (25 min), which died at the same time as the maternal parent, silver bream (Fig. 2b). For thermal shock exposure, parents (25 and 30 min for common bream and silver bream, respectively) died before hybrids (35 and 40 min for S × C and C × S, respectively) (Fig. 3b). In fasting, C × S hybrids (20 days) died at the same time as the maternal parent, silver bream, contrary to S × C hybrids (23 days), which died after the maternal parent, common bream (22 days) (Fig. 4b). The median survival times of hybrids exposed to osmotic shock were intermediate between the parents (Fig. 5b). Median survival times in common bream (8.5 min) were much shorter (*U*-test, $P = 0.0209$) than in silver bream (14.2 min) and hybrids (10.6 and 12.9 min for C × S and S × C hybrids). This survival time in S × C hybrids was longer than that in C × S hybrids, while no significant difference (*U*-test, $P = 0.0606$) was observed between hybrids. Median survival time in silver bream was significantly longer (*U*-test, $P = 0.0209$) than that in C × S hybrids, but it did not differ significantly (*U*-test, $P = 0.3865$) to that in S × C hybrids. For thermal shock treatment, the median survival time of common bream (15.9 min) was also much shorter (*U*-test, $P > 0.05$) than that of silver bream (19.6 min) and hybrids (19.2 and 21.6 min for S × C and C × S hybrids). Median survival in S × C hybrids was closer to that of the maternal parent, but it was significantly (*U*-test, $P = 0.0433$) shorter than C × S hybrids. For the fasting experiment, the lowest median survival

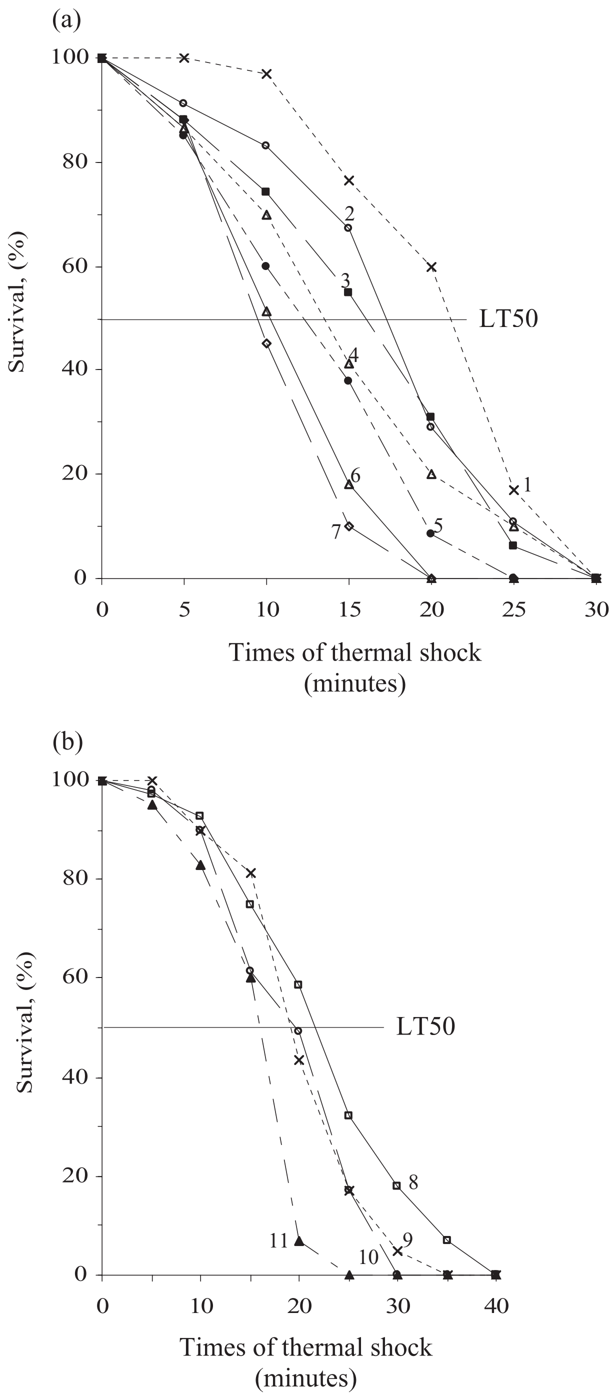

Fig. 3 Survival of roach, common bream, silver bream and their F1 hybrids upon exposure to thermal shock. LT 50 – median survival; C – common bream; S – silver bream; R – roach. In experiment 1 (a), female × male: 1 – S × S; 2 – S × R; 3 – R × S; 4 – R × C; 5 – C × R; 6 – R × R; 7 – C × C and experiment 2 (b): 8 – C × S; 9 – S × C; 10 – S × S; 11 – C × C.

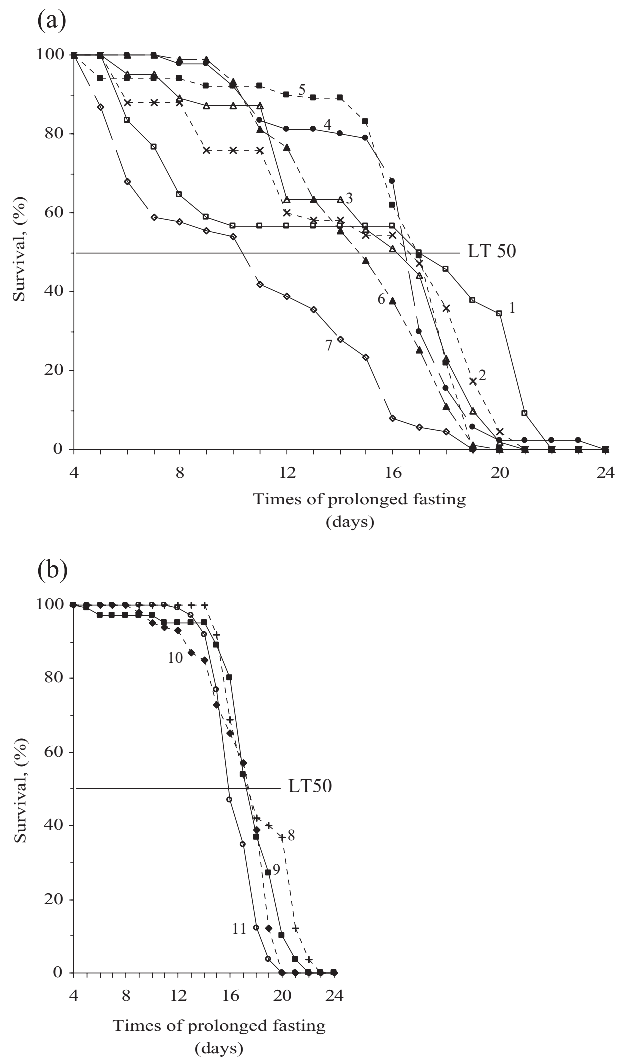

Fig. 4 Survival of roach, common bream, silver bream and their F1 hybrids upon exposure to prolonged fasting. LT 50 – median survival; C – common bream; S – silver bream; R – roach. In experiment 1 (a), female × male: 1 – R × C; 2 – S × R; 3 – R × S; 4 – C × R; 5 – S × S; 6 – C × C; 7 – *R* × R and experiment 2 (b): 8 – C × S; 9 – C × C; 10 – S × S; 11 – S × C.

time was observed in C × S hybrids (15.9 days) and the highest in silver bream (17.4 days), but they were not significantly different (*U*-test, $P = 0.5637$). S × C hybrids (17.3 days) were much nearer to that of the parents, silver bream and common bream (17.2 days).

Between hybrids

Concerning the total mortality, S × C and C × R interspecific crosses died before all other interspecific crosses when subjected to osmotic shock.

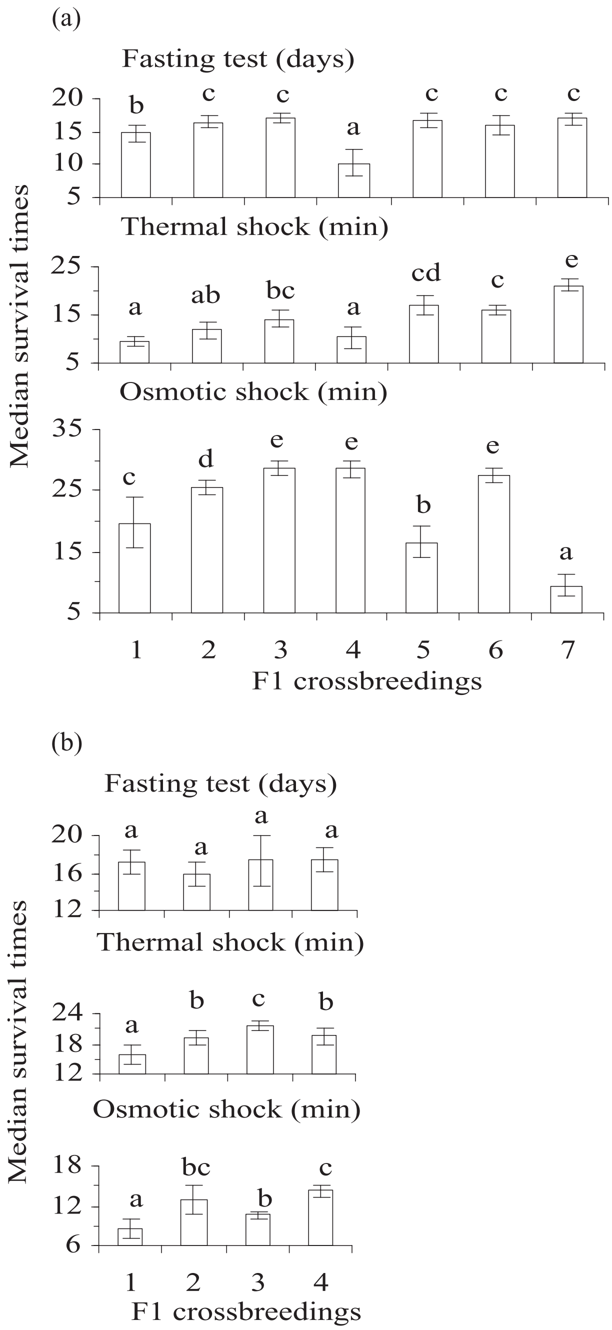

Fig. 5 Median survival times (LT 50) of roach, common bream, silver bream and their F1 hybrids upon exposure to osmotic and thermal shocks (9 days after hatching) and prolonged fasting (embryos at hatching). Means and standard deviations of four observations in two replications of each experiment. Bars sharing at least one common script are not significantly different, whereas other comparisons differ at $P < 0.05$ (Mann–Whitney U-test). C – common bream; S – silver bream; R – roach. In experiment 1 (a), female × male: 1 – C × C; 2 – C × R; 3 – R × C; 4 – R × R; 5 – S × R; 6 – R × S; 7 – S × S and experiment 2 (b): 1 – C × C; 2 – S × C; 3 – C × S; 4 – S × S.

Under thermal shock conditions, roach and silver bream hybrids died after all other hybrids and silver bream and common bream hybrids were the most sensitive. For fasting, C × R hybrids and roach and silver bream hybrids died at the same time, between C × S hybrids (the longest) and S × C (the shortest). Median survival times were much closer with the fasting test. For thermal shock, however, median survival times of roach and common bream hybrids were significantly shorter (*U*-test, $P = 0.0209$) than those of roach and silver bream hybrids in which only S × C hybrids were much shorter (*U*-test, $P = 0.0209$) than hybrids of silver bream and common bream. In the osmotic shock experiment, median survival times in silver bream and common bream hybrids were shorter than those in roach and silver bream hybrids wherein S × C hybrids only were significantly shorter (*U*-test, $P = 0.0209$) than those in roach and common bream hybrids.

Between parental species

Concerning the total mortality, roach died after the two bream species when subjected to osmotic shock. For thermal shock, both roach and common bream died before silver bream. In the fasting experiment, common bream (experiment 2) died after roach and silver bream, whereas only in experiment 1 did roach die after the two bream species, which died at the same time. The longest median survival time was observed in silver bream for thermal and fasting tests and in roach for the osmotic test.

DISCUSSION

The feasibility of reciprocal crossbreeding between roach, silver bream and common bream has been demonstrated in this study. No significant survival difference at the eyed embryo stage was observed between hybrids and parental species. This fact suggests the possibility of the existence of a high potential of hybridization in these species. For viable hatchlings, a maternal effect was found in hybrids because hybrids and their maternal species had been produced from the same female, and therefore the quality of eggs was identical. This phenomenon is not only limited to these hybrids because it was already observed for other species[15] and hybrids.[16] Laine and Rajasilta,[17] McCormick[18] and Steer *et al.*[14] have suggested that maternal nutritional history has a large influence on embryonic development and offspring competency through sequestering and provisioning of yolk resources. The low numbers of viable S × C hybrid hatchlings and their maternal parent, the common bream, may be related to a poor quality of common bream eggs.

In experiments of larval resistance to stress, the higher survival rates observed in silver bream and common bream hybrids (S × C and C × S) and in roach and common bream hybrids (R × C and C × R) subjected to the thermal test may be an indication of their better tolerance to high temperatures. This success could be attributed to parental species such as common bream for roach and common bream hybrids and both silver bream and common bream for hybrids of these two species. Indeed, common bream and silver bream were able to tolerate higher temperatures than roach. For example, the upper lethal temperature of embryonic development is approximately >32°C in the common bream[19,20] versus >26°C in the roach.[21,22] Between the bream species in this study, silver bream was the most tolerant. The median survival times for roach and silver bream hybrids (R × S and S × R) were intermediate between parents but higher than roach and common bream hybrids (R × C and C × R). This could be explained by the reasons mentioned above.

In the prolonged fasting test, total mortality times in hybrids were later than in parents, whereas the weights of their hatched embryos were intermediate. Hybrids could have developed a better adaptability to prolonged fasting. In addition, the best survival rate observed in both hybrids and parental species within the first 10 days in the fasting experiments may be related to yolk resorption. Prolonged fasting was an even greater perturbation in energy status because glucogen stores were completely depleted with extended fasting, thus resulting in larval death.

Under osmotic shock, hybrid survival rates were also strongly influenced by the maternal parent. This could be related to weights of stressed larvae, which were also influenced by a maternal effect. In parental species, the better survival of roach to the osmotic stress than the two bream species could possibly be explained by gill surfaces, which modulate the osmoregulatory capacity in fish. A larger gill surface as in breams could generally increase the surface of ion transport and the functional enzymes (the number of Na^+/K^+-ATPase pumps that are available to exchange ions), perhaps the cause of earlier mortality in breams. Berg showed that of the three species, common bream has the widest gill because its lateral gill rakers are extra long and pointed (Berg CVD, unpubl. data, 1993). Moreover, Lammens reported that the structure of the gill-raker basket in common bream showed the smallest openings (Lammens E, unpubl. data, 1986). Roach has fewer gill rakers than silver bream. For Berg *et al.*,[23] gill raker parameter values in common bream were 20% higher than in silver bream and 40% higher than in roach. In addition, the differences were even more pronounced in the area values. Furthermore, the branchial arches of common bream have more gill rakers than those of silver bream and roach. In all crosses, fast larval mortality could be facilitated by osmoregulatory problems between body fluid and environmental osmotic stress at the cellular level and a lowered level of gill Na/K^+-ATPase activity because the larvae used were young. Consequently, their osmoregulatory organs as gills were not developed.

In each strain, the large differences observed between experiment 1 and experiment 2 in the cross of C × C and S × S may be attributed to individual variations, especially for the species used in this study that were collected from a natural population in which the polygamous[24–26] increases the genetic diversity intraspecies[27] and represents a benefit for the survival of the offspring.

From an ecologic point of view, survival of these hybrids at early developmental stages could translate into a capacity on the part of hybrids to easily thrive in the same living conditions as their parental species in natural habitats where hybrids and their parents live together. Moreover, the resistance of these hybrids to stress tests would make it possible to confirm that hybrids could generally live longer than parents in hostile environments where parental species would have already disappeared.

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