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Survival, growth and sexual maturation of the triploid hybrid between rainbow trout and arctic char

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Abstract

Triploid hybrids between female rainbow trout (*Oncorhynchus mykiss*) and male arctic char (*Salvelinus alpinus*), together with triploid and diploid rainbow trout controls from the same dams, were tested in freshwater farming from fry stage up to the age of 3 years. When farming was operated in cold water, the survival rate of the hybrids was as good as those of the diploid and triploid rainbow trout. Hybrid growth was noticeably hindered relative to that of diploid controls at the beginning of the juvenile period, but this was partly tempered later on by the effect of diploid sexual maturation; at 3 years of age, the hybrid weighed 20% less than the diploid rainbow trout and did not differentiate significantly from the triploid rainbow trout.The impairment of sexual maturation was very much the same in the hybrid as in the triploid rainbow trout, and dressing traits were therefore quite similar, despite a slight difference in perivisceral fat losses of immature individuals, to the advantage of the hybrid. These results allow one to consider the suitability of this hybrid for large-sized fish production in cold water farming.

Keywords : Salmonidae, hybridization, triploidy, aquaculture.

Survie, croissance et maturation sexuelle de l'hybride triploïde entre la truite arc-en-ciel et l'omble chevalier.

Résumé

Des hybrides triploïdes de truite arc-en-ciel (*Oncorhynchus mykiss*) femelle et omble chevalier (*Salvelinus alpinus*) mâle, ainsi que des truites arc-en-ciel témoins diploïdes et triploïdes de mêmes mères, ont été testés en élevage en eau douce du stade alevin jusqu'à l'âge de 3 ans. Dans un élevage mené en eau froide, la survie des hybrides est aussi bonne que celles des truites diploïdes et triploïdes. La croissance de l'hybride est notablement retardée par rapport à celle du témoin diploïde au début de la période juvénile, mais l'écart est partiellement réduit par la suite, du fait de la maturation sexuelle diploïde; à l'âge de 3 ans, l'hybride pèse 20 % de moins que la truite diploïde et ne s'écarte pas significativement de la truite triploïde. Le blocage de la maturation sexuelle est tout à fait analogue chez l'hybride et chez la truite triploïde, et les caractéristiques corporelles sont par suite très semblables, si ce n'est une légère différence de pertes en graisses périviscérales chez les individus immatures, à l'avantage de l'hybride. Ces résultats permettent d'envisager l'utilisation de cet hybride pour la production d'animaux de grande taille en pisciculture d'eau froide.

Mots-clés : Salmonidés, hybridation, triploïdie, aquaculture.

INTRODUCTION

The production of interspecific hybrids has been practised for a long time in salmonids (Chevassus, 1979), with the aims of obtaining good compromises between qualities and defects of contrastable species and impairment of sexual maturation in the production of large-sized fish. In the past ten years, hybridization gained a renewed interest, due to (1) improvement by artificial triploidization (Chevassus *et al.*, 1983 *a*; Scheerer and Thorgaard, 1983) and (2) evident cases of inherited resistance from parental species to viral diseases (Chevassus and Dorson, 1990). Particularly, the rainbow trout (*Oncorhynchus mykiss*) may be usefully crossed with char (*Salvelinus*) species for protection against viral haemorrhagic septicaemia (Dorson *et al.*, 1991).

However, such interesting hybrids need to be tested for survival and growth performances through farming in order to evaluate their suitability. In that framework, the present work deals with the triploid hybrid between female rainbow trout and male arctic char (*Salvelinus alpinus*), the obtainment of which was first reported by Blanc and Poisson (1988). The fish farming experiments presented here were the continuation, on the same experimental animals, of the studies on incubation, hatching and yolk sac absorption which were the subject of that first report.

MATERIAL AND METHODS

Fertilization and hatchery procedures

Two experiments, hereafter designated as LA and D, were undertaken in two successive breeding seasons at the INRA fish farm of Lées-Athas (Pyrénées-Atlantiques). The material and methods used, the assessment of triploidization efficiency and the results obtained up to the 90th day post-fertilization have been already reported in detail (Blanc and Poisson, 1988).

Both experiments were designed to produce three genotypes, namely:

– diploid rainbow trout control, hereafter designated as R2;

– triploid rainbow trout, hereafter designated as R3;

– rainbow trout female × arctic char male triploid hybrid, hereafter designated as R2C.

The breeders had been raised in the Lées-Athas fish farm and originated from local (rainbow trout) and Swedish (arctic char) domesticated broodstocks. The numbers used were 4 and 6 rainbow trout females, 3 and 6 rainbow trout males and 12 and 20 arctic char males in LA and D experiments respectively. In the LA experiment, the ova were pooled, subsampled and fertilized by the mixtures of the sperm collected within each paternal species. In the D experiment, the gametes were associated according to a factorial design, but the families thus obtained could not be maintained apart beyond the alevin stage and had to be pooled within the three main genotypes. It remains that in both experiments the individual breeders within each sex and species provided balanced contributions to the offsprings produced.

Triploidization was induced by a 26.5°C heat shock lasting 20 minutes and initiated after a 10°C pre-incubation period of 25 minutes post-fertilization, according to Chevassus *et al.* (1983 *b*). Then the eggs were transported to the INRA research station of Saint-Pée-sur-Nivelle (Pyrénées-Atlantiques), either within a few hours post-fertilization (LA experiment) or at eyed stage (D experiment). Survival results obtained until the 90th day post-fertilization (Blanc and Poisson, 1988) were very similar in the two experiments, R3 and R2C averaging respectively 87 and 51% of the corresponding R2 control performance.

Rearing procedures

From that stage onwards, the fry were raised for a few months at the research station of Saint-Pée-sur-Nivelle, up to the age of 175 days (LA experiment) and 233 days (D experiment) post-fertilization. The station facilities were supplied with filtrated water from the Nivelle river, the temperature of which increased progressively from a 9-13°C range (end of February, both experiments) to a 12-16°C range (end of April, LA experiment) and up to a 14-18°C range (end of June, D experiment).

Five-month-old fingerlings from the LA experiment (187 R2, 170 R3 and 585 R2C) were then transported to the fish farm of Lées-Athas, while seven-month-old fingerlings from the D experiment (185 R2, 160 R3 and 380 R2C) were transported to another INRA fish farm, located at Donzacq (Landes). Lées-Athas facilities are provided with mountain spring water at the nearly constant temperature of 8°C (± 1°C seasonal variation). The fish farm of Donzacq is supplied with warmer spring water the temperature of which is about 17°C (± 1°C seasonal variation).

Fishes at Lées-Athas (LA experiment) were raised for three months in separate 1 metre diameter fibreglass tanks (1 for R2, 1 for R3 and 3 for R2C), then marked by fin-clipping and mixed in a 20 m^2 pond. Three months later, they were transferred to a larger (75 m^2) pond, where they were raised until the age of three years. These ponds had a rectangular shape and their bottom was covered with gravel.

Fishes at Donzacq (D experiment) were marked by fin-clipping before their transportation and were mixed in a 10 m^2 concrete raceway with gravel bottom. Six months later, they were transferred to a similar but larger (30 m^2) raceway where they were raised until the age of 18 months.

• In both fish farms, the water depth in ponds and raceways was about 0.5 m; the loading densities were 1 to 5 kg of fish per square metre (2 to 10 kg per cubic metre of water). The minimum flow rates per kilogram of fish were approximately 1 to 2 litres per minute at Lées-Athas and about twice as much at Donzacq.

Food was provided according to the usual fish culture practice and feeding rates were not monitored. However, on one hand fingerlings were fed to near satiation, and on the other hand the mix-up of the fishes later on anyway prevented the evaluation of the actual food intake for each genotype separately.

Study of performances

In both experiments and for each genotype, the number of survivors and their mean weight were regularly recorded, every 6-10 weeks during the juvenile period and every 3-6 months later on. In addition, individual length and weight were measured at the final control of the D experiment.

Sexual maturation was studied through the autopsy of 2 and 3-year-old fishes from the LA experiment, these samples totalling 115 R2, 100 R3 and 268 R2C. Fishes were classified by sex and degree of maturation, and the weights of gonads, remaining viscera and eviscerated body were recorded.

The data were processed through standard statistical methods (Sokal and Rohlf, 1969): comparisons of percentages were achieved through normal approximation, except for small samples where the G-test was used; comparisons of mean weights were run on raw data, after verification of the equality of variances; logarithmic transformation was used for the computation of allometric weight-on-length regression, the comparisons of regression lines being obtained through analysis of covariance.

RESULTS

Survival

The survival curves of the three genotypes in each experiment are reported in *figure* 1. In both cases, R2 had the best survival and R2C the lowest. Fishes in the LA experiment suffered moderate casualties at Saint-Pée-sur-Nivelle prior to transportation; R3 and R2C survival rates did not differ significantly, while R2 rate was significantly superior. In the three groups of fishes, health was readily restored in the Lées-Athas conditions. However, some mortalities occurred at the age of 24-27 months subsequently to the first spawning season; it was observed that both sexes were affected in the R2 group, while female mortalities were less frequent in the R3 and R2C groups. At the end of the experiment, the survival rates of the three groups did not differ significantly from each other.

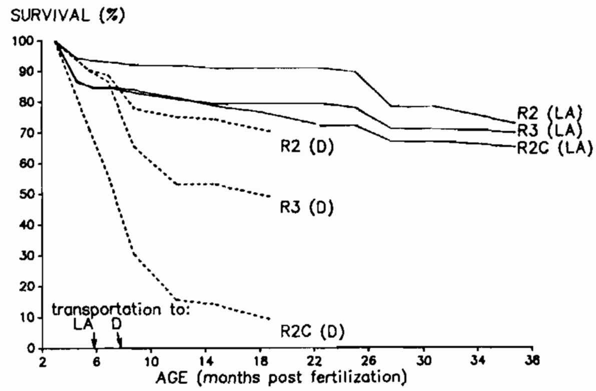

Figure 1. – Survival curves of the three genotypes in LA and D experiments.

Fishes in the D experiment, which were maintained at Saint-Pée-sur-Nivelle until the beginning of summer, suffered important casualties, and their morbidity persisted after transportation to Donzacq, particularly in the R2C group and, to a lesser degree, in the R3 group. Regular antiseptic treatments (chloramine) as well as an antibiotic treatment (flumequine) were inefficient but for a short period of time. Differences in survival between groups were highly significant.

Growth

Initial weights (3 months post-fertilization) and subsequent growth rates are reported in *table* 1. Average weights of diploid rainbow trout in the LA experiment were 7 g at 6 months post-fertilization, 112 g at 12 months, 740 g at 2 years and 1 900 g at 3 years. A regular diminution of growth rate was observed, with a particular impediment from 23 to 28 months of age (first spawning season). Weights were lower in the D experiment, due to a slower embryonic development (lower incubation temperature), but growth rates were slightly better than those of the LA experiment.

In both experiments, triploid rainbow trout and hybrid even more exhibited initial weights inferior to that of R2 control. Subsequent growth rates of the three groups were generally similar to each other. In the LA experiment, the hybrid growth was faster during the first months at Saint-Pée-sur-Nivelle, but a comparison of growth rates in separate fibreglass tanks at Lées-Athas did not show any significant difference among groups, as compared to the variation among replicates within the R2C group.

The relative weights of triploid rainbow trout and hybrid, expressed as percents of respective R2 control groups in each experiment, are reported in *figure* 2. They followed similar trends, and exhibited (in the LA experiment) a temporary increase from 23 to 28 months as a consequence of the above-mentioned

Table 1. – Initial weights (IW, cg) and growth rates in successive periods (GR, % of body weight per day).

	Age (months post-fertilization)	LA experiment R2	LA experiment R3	LA experiment R2C	D experiment R2	D experiment R3	D experiment R2C
IW:	3	46.0	42.8	23.4	16.0	14.5	11.4
GR:	3-6	3.15	3.16	3.71	3.88	3.58	3.73
	6-12	1.54	1.49	1.53	1.57	1.53	1.57
	12-18	0.72	0.63	0.65	0.83	0.94	0.87
	18-23	0.38	0.40	0.39		end of experiment	
	23-28	0.14	0.19	0.25			
	28-36	0.29	0.28	0.26			

R2: diploid rainbow trout.
R3: triploid rainbow trout.
R2C: rainbow trout × arctic char triploid hybrid.

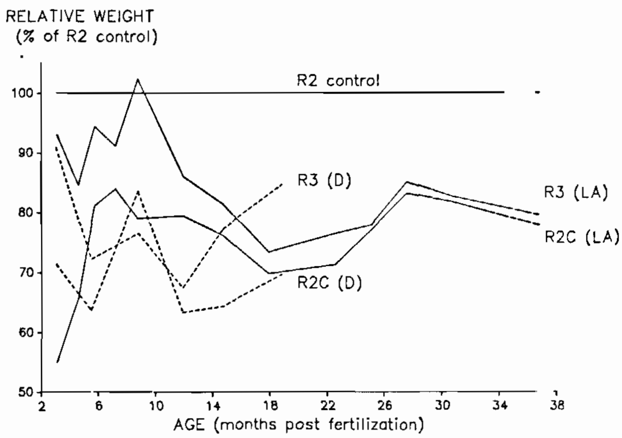

Figure 2. – Evolution of the relative weights of the triploid genotypes in LA and D experiments.

slackening of R2 growth during this period (*table* 1). At three years of age, both triploid rainbow trout and hybrid weighed about 80% of R2 control.

The length and weight records at the end of the D experiment are presented in *table* 2. On average, whatever length or weight was considered alone, R3 was inferior to R2 and R2C was inferior to R3. Within-group individual standard-errors did not differ significantly. The allometric weight-on-length regression had approximately the same slope in the three groups, but the adjusted weight of R2C was inferior to those of R2 and R3, therefore indicating a significantly slimmer body shape of the hybrid as compared to the rainbow trout.

Sexual maturation

The classification of autopsied 2 and 3-year-old fishes according to sex and degree of maturation is presented in *table* 3. Most R2 males already spermiated at 2 years, while widespread maturation in females was not found before 3 years. On contrast, sexual development was limited in both R3 and R2C groups: male gonads remained underdeveloped (except one R2C individual with little, watery sperm), and all females (but two R3's) were hindered at immature stage. Sex ratios were balanced in all samples, with the exception of 3-year-old R2C where females were significantly more numerous, possibly as a consequence of previous male casualties.

Table 2. – Length and weight final control in the D experiment.

		R2	R3	R2C
Sample size		155	111	74
Length (cm)	mean	30.3 ^{a}	28.8 ^{b}	28.1 ^{c}
	ISE	1.9	2.1	2.4
Weight (g)	mean	375 ^{a}	318 ^{b}	261 ^{c}
	ISE	68	63	62
Log W/Log L slope		2.8	2.7	2.8
Mean W (g) adjusted for L		336 ^{a}	326 ^{a}	286 ^{b}

Within each line, estimates with different subscript letters (*a, b, c*) differ significantly from each other at the $p=0.05$ level.
ISE: individual standard error.
R2, R3, R2C: as in table 1.

Table 3. – Sexual maturation: numbers observed among autopsied fishes.

	Age (years)	Males I	Males T	Males S	Females I	Females G	Females O
R2	2	6	1	23	0	12	8
	3	0	8	25	0	1	31
R3	2	11	14	0	22	2	0
	3	1	22	0	28	0	0
R2C	2	10	33	0	57	0	0
	3	5	45	1	117	0	0

I: immature (males and females).
T: incompletely developed testes (no sperm).
S: spermiating.
G: small undeveloped ovocytes.
O: late or completed ovogenesis.
R2, R3, R2C: as in table 1.

Table 4. – Dressing traits in the main groups of autopsied fishes (mean ± individual standard error).

Age (years) and sex	Maturation and genetic group		Sample size	Viscera (% of body weight)		Dressing percentage	Eviscerated weight (g)
				gonads	remainder		
2-males	I	R3	11	0.05 ± 0.01	10.24 ± 1.21	89.71 ± 1.21	572 ± 115
		R2C	10	0.07 ± 0.03	8.43 ± 1.98	91.50 ± 1.98	506 ± 128
	T	R3	14	0.67 ± 0.35	6.26 ± 0.77	93.07 ± 0.72	563 ± 107
		R2C	33	0.72 ± 0.16	6.32 ± 0.98	92.96 ± 1.00	467 ± 116
	T and S	R2	24	1.57 ± 0.54	5.61 ± 0.84	92.83 ± 0.97	684 ± 112
2-females	I	R3	22	0.03 ± 0.01	10.12 ± 1.32	89.85 ± 1.32	525 ± 98
		R2C	57	0.04 ± 0.01	8.88 ± 1.46	91.08 ± 1.46	517 ± 126
	G	R2	12	0.13 ± 0.03	9.62 ± 1.52	90.25 ± 1.52	613 ± 140
	O	R2	5	8.54 ± 2.25	7.87 ± 0.73	83.59 ± 1.75	630 ± 152
3-males	T	R3	21	0.69 ± 0.27	5.25 ± 0.78	94.06 ± 0.81	1 435 ± 418
		R2C	45	0.72 ± 0.20	5.36 ± 0.99	93.92 ± 0.96	1 300 ± 359
	T and S	R2	33	1.98 ± 0.80	5.66 ± 0.90	92.36 ± 1.10	1 652 ± 399
3-females	I	R3	27	0.03 ± 0.03	12.03 ± 2.04	87.94 ± 2.03	1 405 ± 428
		R2C	117	0.04 ± 0.02	9.84 ± 1.83	90.12 ± 1.84	1 419 ± 397
	O	R2	5	10.72 ± 2.11	6.01 ± 2.20	83.28 ± 2.03	1 761 ± 557

I, T, S, G: as in table 3; O: unspawned mature females (ripe females spawned spontaneously in the gravel).
R2, R3, R2C: as in table 1.

Dressing traits measured in the main categories of autopsied fishes are reported in *table* 4. Relative gonadic weights (*i.e.* gonadosomatic indexes) presented obvious differences according to the degrees of sexual development, with close resemblance between R3 and R2C fishes. The relative weights of the remaining viscera, on the other hand, showed noticeable differences which were in part inversely correlated to those recorded in gonadic weights. Deposition of fat along the intestinal tract was observed to be the main cause of gut heaviness and to be impaired by sexual development. As a result, maturing (T) males had finally a better dressing percentage than totally immature (I) males and females. It is also to be noticed that, within immature groups, R2C individuals had less viscera than R3 ones, and therefore better dressing percentages.

DISCUSSION AND CONCLUSION

Effect of environmental conditions

LA and D experiments were respectively performed in different years, with different breeders although from the same stocks, and with different chronological and material designs. It appears however that health problems in the immature period were encountered at Saint-Pée-sur-Nivelle and Donzacq locations where water temperature was much higher than that of Lées-Athas fish farm. Temperature elevation, besides directly influencing fish physiology, is also favourable for the development of bacterial septicemias (De Kinkelin *et al.*, 1985), which probably affected the fishes raised in these locations. However, since the three genotypes were mixed in the same body of water, their respective survival rates can be compared pertinently. Similar conclusions were drawn from a previous work achieved in the same experimental sites (Blanc and Chevassus, 1986).

Chars, and particularly arctic char, are known as rather cold water adapted fishes (Balon, 1980). The very low survival of R2C recorded in the D experiment implies that this hybrid did not inherit much of its rainbow parentage tolerance, despite the doubling of the rainbow genetic material through triploidization. It is also to be pointed out that the triploid rainbow trout itself did not perform well in the D experiment. The usual statement that triploid depression in survival is limited to the hatchery period (reviewed by Ihssen *et al.*, 1990) should be restricted to favourable, cool and healthy rearing environments.

Triploid growth and productivity

The impairment of sexual maturation observed in the R3 group corroborates the assessment of the triploidizing treatment efficiency previously obtained through cytometric measurements (Blanc and Poisson, 1988). In the LA experiment, this triploid sterility resulted in slightly better survival and growth relative to diploids during the first spawning season, but this advantage was not strong enough to compensate for the initially established depression. In disagreement with former reports (Thorgaard, 1986; Quillet *et al.*, 1988), the present work fails to evidence a final superiority of R3 over R2, nor does it show any significant sexual difference in body weight.

A number of economically important aspects, should however be considered, namely: (1) the long-term evolution of survival and growth, through a rearing process for larger sized fish; (2) the food consumption, which could not be measured in our experimental conditions; and (3) the amount of work which would be required to spawn the diploid mature females in intensive fish culture conditions. These aspects, if taken into account, might improve the relative scoring of triploid fishes.

Potential interest of rainbow × char hybrids

Rainbow trout × char diploid hybrids are known to have very low viability (Chevassus, 1979). Artificial triploidization results in a marked improvement of the yield which however remains far beneath that of rainbow trout. Thus, in the rainbow trout female × arctic char male hybridization, the feeding fry obtained amounted to 51% (Blanc and Poisson, 1988) and 48% (Dorson *et al.*, 1991) of the corresponding rainbow control production. These figures are about of the same magnitude as those reported for the rainbow trout female × brook trout (*Salvelinus fontinalis*) male (Chevassus *et al.*, 1983*a*; Quillet *et al.*, 1988; Dorson *et al.*, 1991) and rainbow trout female × lake trout (*Salvelinus namaycush*) male (Dorson *et al.*, 1991) hybrids. These hatchery mortalities, although being of little economical cost, have to be considered to the discredit of the corresponding hybrids with respect to intensive fish culture.

The present work provides, for the first time to our knowledge, a survival and growth test through a 3-year farming of the rainbow trout × arctic char hybrid. It appears that, in cold water, the hybrid performs about as well as the triploid rainbow trout. From a similar study, Quillet *et al.* (1988) reported that the rainbow trout × brook trout hybrid suffered higher mortality rates than those of the triploid rainbow trout, but were about equivalent in weight a few months after the first sexual maturation at 2 years. However, these authors observed a clear discrepancy in weight between hybrid females (scoring as high as the diploid rainbow female control) and males (weighing 36% less than females), while such a sexual dimorphism was not evidenced in the present study.

The classification performed on R3 and R2C fishes according to sex and degree of maturation provided very similar ratios. These results are also analogous to those obtained with other intergeneric hybrids (Suzuki and Fukuda, 1973; Blanc and Chevassus, 1986). Comparison of dressing traits of R3 and R2C groups merely reflects a slight difference in perivisceral fat losses of immature individuals, to the advantage of the hybrid. However, correlated differences might exist in the muscular lipid content, the study of which would therefore be worthwhile. Lastly, the slimmer body shape of the hybrid as compared to rainbow trout could be considered as advantageous from a marketing standpoint, although this might depend upon the fish processing methods.

Conclusively, it appears that the rainbow trout × arctic char triploid hybrid is comparable to the triploid rainbow trout in terms of suitability to cold water fish farming, apart from its specific hatchery mortalities. However, these mortalities might be more than offset by the demonstrated resistance of this hybrid to viral haemorrhagic septicemia, to which rainbow trout is highly sensitive, although this resistance is somewhat lower than that of the rainbow trout × brook trout hybrid (Dorson *et al.*, 1991). Multiple comparative testing of rainbow × char hybrids should be carried out in long-term experiments, along with genetic variability measurements, in order to obtain a more accurate evaluation of the economic potential of these hybrids.

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